CHAPTER IV
RESULTS

1) Evaluation of diclofenac sodium powders

1.1) Morphology of diclofenac sodium powders

The DS powders were examined by a scanning electron microscope (SEM) at different magnifications. The magnifications of 1000 and 5000 were used to investigate the shape and surface topography of powders as presented in Figure 10. The powders were of irregular shape. Various sizes and rough surface could also be seen at both low and high magnifications.

(a) x 1,000 **(b) x 5,000**

Figure 10 SEM photomicrographs of untreated diclofenac sodium powder

1.2) Preliminary investigation on compressibility of diclofenac sodium powders

Table 11 summarizes the processes and results from the investigation to determine the compressibility for the microtabletting process of different diclofenac sodium powders after compression of microtablets.

Table 11 The parameters for indicating the compressible properties after compression of microtablet.

Process	Result		
	Compressibility	Hardness (Kp) (n=10)	
		Mean	SD
1) Untreated diclofenac sodium powder	+	2.92	0.127
2) Diclofenac sodium powders by milling with mortar and pestle for 3 minutes	+	2.96	0.159
3) Sieved diclofenac sodium powders through #30 mesh	+	2.98	0.133

O : could not compression + : could compression

It was seen that all three investigated DS powders could be compressed into microtablets. Their hardness were not different. The compressibility of all DS powder were also not different. Therefore, the untreated diclofenac sodium powder was chosen for preparing microtablet, because of low cost and ease to production.

2) Preliminary investigation on flowability of suitable diluent for the preparation of diclofenac sodium microtablets

The flowability and compressibility of suitable diluents for diclofenac sodium microtablets were investigated. The results of suitable diluent investigated by various parameters, which supported for flowability and compressibility were presented in Table 12. Both Ludipress and lactose in the powder mixtures were suitable to be diluent for diclofenac sodium microtablets in this study.

Table 12 Summary of the results of physical properties of preliminary diclofenac sodium preparations containing various diluent.

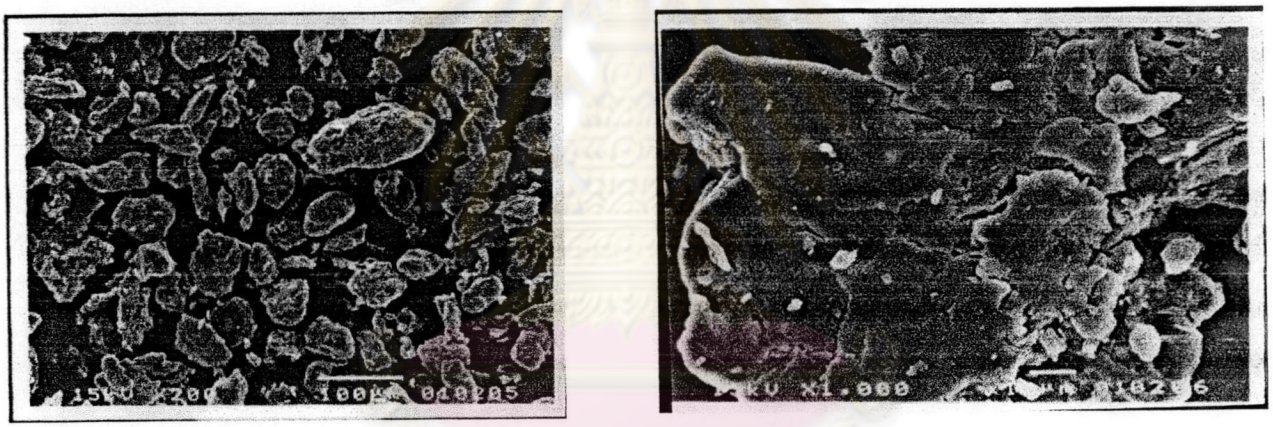
Formulation	Tablet		Powder	
	Hardness (Kp) Mean (SD)	%F	Flow Rate (g/s) (SD)	Angle of Repose (°) (SD)
D1	2.86 (0.140)	0.538 %	13.30 (0.125)	20.27° (0.112)
D2	UNCOMPRESSIBLE	-	No flow	55.67° (0.354)
D3	2.92 (0.196)	0.552 %	18.67 (0.108)	15.28° (0.173)
D4	UNCOMPRESSIBLE	-	No flow	55.64° (0.225)
D5	UNCOMPRESSIBLE	-	No flow	54.54° (0.279)

The diluent used in formulation D1 and D3 were lactose and Ludipress, respectively. They had good compressibility and could be compressed into the microtablets that could be confirmed with hardness and the percentage of friability. Moreover, it was observed that the angles of repose of the powder mixtures with both diluents were less than 30° and the flow rates were high. Therefore, it indicated that Ludipress and lactose had free-flowing property. Comparison to lactose, it appeared that Ludipress showed better flowability. The other diluents could not be compressed into microtablets. Their flowability was very poor that could be confirmed by flow rate and the angles of repose. It was observed that their angles of repose were more than 30° and their flow rate could not be detected.

3) Evaluation of the powder mixture and granules of diclofenac sodium

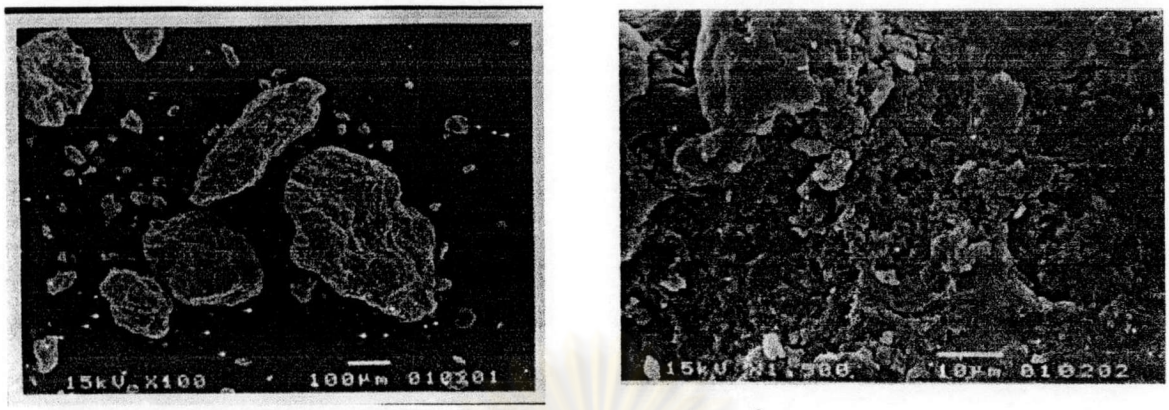
3.1) Morphology of diclofenac sodium powders and cellulose derivative powders

DS, HPMC, and EC were examined using scanning electron microscope (SEM) at different magnifications for investigating the shape and surface topography of powders. The magnifications of 1000 and 1500 of diclofenac sodium, the magnifications of 2000 and 1000 of HPMC, and the magnifications of 100 and 1500 of EC are shown in Figure 10 (a, b), 11 (a, b), and 12 (a, b), respectively. It was observed that HPMC was of fibrous or cylindrical shape and had smooth surface whereas EC exhibited rods and round shape with porous surface.



(a) x 200 **(b) x 1,000**

Figure 11 Photomicrographs of hydroxypropylmethylcellulose (Methocel E4M)



(a) x 100 **(b) x 1,500**

Figure 12 Photomicrographs of ethylcellulose (Ethocel 10 cps.)

3.2) Moisture content

The moisture content of diclofenac sodium was 1.56 % as shown in Table 13. When diclofenac sodium were mixed with different amount of HPMC and EC by using direct compression and wet granulation methods. Their moisture contents were not much different and were in a range of 1.0 – 3.0 % as present in Tables 13 – 15.

3.3) Flow rates and angles of repose

3.3.1) The diclofenac sodium powder mixtures and granules formulation at dose 4.2 mg per microtablet.

The flow rate of powder mixtures in all direct compression formulations that mixed with various amount of HPMC and EC and by direct compression could not flow pass through the hold of funnel whereas the granule from wet granulation formulations could be detected. The flow rate of granules in formulation WGHCL, WGHCH were 18.35 and 15.26 g/sec respectively as presented in Table 14. It was found that the flow rate was decreased when the amount of HPMC was increased. Moreover, the flow rate of granules in the formulation WGEC that was mixed with EC and by wet

granulation method was 25.21 g/sec. Comparison to HPMC formulation, it appeared that EC formulation showed faster flow rate.

The angles of repose of powder mixtures in all direct compression formulations are reported in Table 14. It was found that their angles of repose were within a range of 37° – 39° which indicated of fair flowability (Gordon et al., 1990) whereas those of granules in all wet granulation formulations were within a range of 25° – 30° which indicated good flowability (Gordon et al., 1990) as shown in Table 33, in Appendix B. It was observed that the angle of repose of formulation contained with EC was lower than formulation contained with HPMC. Therefore, the flowability of formulation contained EC was better than the formulation contained HPMC. It could be investigated that the results of angles of repose and flow rate were related in all formulations. The angle of repose was high and flow rate was low indicated that poor flowability whereas the angle of repose was low and flow rate was high indicated that good flowability.

3.3.2) The diclofenac powder mixtures and granules formulation at dose 3 mg per microtablet.

The flow rate of powder mixtures that contained HPMC and by direct compression method was 11.92 g/sec (formulation DHC) whereas formulation contained with EC was 13.87 g/sec (formulation DEC) as presented in Table 15. It was showed that the flow rate of formulation contained EC was faster than formulation contained HPMC.

The flow rates of granules of formulation WHCL, WHCH that were mixed with various amounts of HPMC were 17.56 and 14.37 g/sec, respectively. It was found that the flow rate decreased when the amount of HPMC was increased. The flow rates of formulation WECL, WECH that were mixed with EC and by the wet granulation method were 25.21 and 23.46 g/sec respectively. It was observed that the flow rate decreased when the amount of EC was increased. When comparing the flow rates between the granules of HPMC formulations and EC formulations, the results indicated that slower flow rate was obtained from the granules of HPMC formulations.

The angles of repose of powder mixtures in direct compression formulations are reported in Table 15. It was found that their angle of repose were within a range of 32° – 34°

which indicated of fair flowability (Gordon et al., 1990) whereas those of granules in all wet granulation formulations were within a range of 22° – 29° which indicated of good flowability (Gordon et al., 1990) as shown in Table 33, in Appendix B. It was found that the angles of repose of the formulation contained EC was lower than the formulation contained HPMC in the both direct compression and wet granulation method.

The results of flow rate of formulation at dose 3 mg were higher than those of formulation at dose 4.2 mg and the results of angles of repose correlated to the flow rate results. As a result, flowability of 3 mg formulations were better than those of 4.2 mg formulations.

Table 13 The physical properties of DS powder, HPMC, and EC

Physical properties	Mean values (SD)		
	DS	HPMC	EC
Moisture content (%)	1.56	2.28	1.94
Flow rate (g/sec) (n=6)	No flow	15.32 (0.057)	24.17 (0.025)
Angle of repose (x ^{0}) (n=3)	34.22 (0.141)	30.09 (0.042)	22.26 (0.09)
Bulk density (g/ml) (n=3)	0.42 (0.012)	0.46 (0.095)	0.38 (0.043)
Tapped density (g/ml) (n=3)	0.50 (0.020)	0.56 (0.034)	0.44 (0.068)
Carr's index (%) (n=3)	16.60 (0.015)	18.17 (0.023)	11.49 (0.081)

Table 14 The physical properties of powder mixtures and granules at dose 4.2 mg per microtablet

Formulation	Moisture content (%)	Flow rate (g/sec) (SD) n=6	Angle of repose (x °) (SD) n=3	Bulk density (g/ml) (SD) n=3	Tapped density (g/ml)(SD) n=3	Carr's Index (%) (SD) n=3
DLHC	1.80	No flow	38.21 (0.011)	0.32 (0.058)	0.36 (0.038)	9.52 (0.029)
DLEC	1.75	No flow	37.12 (0.095)	0.38 (0.085)	0.42 (0.046)	7.91 (0.039)
DSHC	2.00	No flow	39.08 (0.042)	0.33 (0.049)	0.38 (0.055)	14.28 (0.061)
DSEC	1.90	No flow	38.26 (0.068)	0.33 (0.111)	0.37 (0.095)	10 (0.049)
WGHCL	2.14	18.24 (0.093)	27.12 (0.106)	0.28 (0.108)	0.32 (0.098)	12.5 (0.104)
WGHCH	2.18	15.41 (0.092)	30.02 (0.106)	0.30 (0.095)	0.35 (0.093)	13.28 (0.108)
WGEC	2.09	25.21 (0.103)	25.18 (0.100)	0.30 (0.105)	0.33 (0.109)	9.09 (0.077)

Table 15 The physical properties of powder mixtures and granules at dose 3 mg per microtablet

Formulation	Moisture content (%)	Flow rate (g/sec) (SD) n=6	Angle of repose ($\hat{x}$) (SD) n=3	Bulk density (g/ml) (SD) n=3	Tapped density (g/ml) (SD) n=3	Carr's index (%) (SD) n=3
DHC	2.08	11.72 (0.102)	34.01 (0.055)	0.36 (0.082)	0.38 (0.059)	7.03 (0.072)
DEC	2.02	13.49 (0.086)	32.14 (0.102)	0.33 (0.106)	0.36 (0.090)	6.72 (0.120)
WHCL	2.24	17.65 (0.095)	26.64 (0.117)	0.50 (0.104)	0.63 (0.068)	19.12 (0.247)
WHCH	2.35	14.37 (0.125)	29.75 (0.222)	0.49 (0.107)	0.57 (0.035)	20.00 (0.059)
WECL	2.13	25.21 (0.091)	22.45 (0.071)	0.52 (0.109)	0.65 (0.086)	13.49 (0.105)
WECH	2.17	23.46 (0.101)	24.78 (0.099)	0.49 (0.105)	0.56 (0.097)	16.64 0.130)

3.4) Bulk densities, tapped densities and carr's compressibilities

The bulk density and tapped density of DS powders, hydroxypropylmethycellulose (Methocel E4M), ethylcellulose (Ethocel 10 cps.) were shown in Table 13. For the powder mixtures and granules of DS at the different dose, which were 4.2 mg and 3 mg per microtablet are presented in Table 14 and 15, respectively. It was observed that the bulk and tapped densities of products that contained with EC and contained with HPMC were different.

The percentage of compressibility of DS powder, HPMC and EC are shown in Table 13 and those of all powder mixtures and granules at the different dose are shown in Table 14 and 15, respectively. The percentage of compressibility was another parameter to indicate the flowability of powder. The low percentage of compressibility indicated free-flowing property (Gordon et al., 1990).

For the product prepared by direct compression method, the percentage of compressibility of formulation contained with Ludipress® was lower than formulation contained with lactose. Moreover, the types and the amounts of cellulose derivatives were major factor affecting to percent compressibility. It was found that the percentage of compressibility of product contained EC was lower than product contained HPMC in both direct compression and wet granulation method.

3.5) Particle size distribution

The particle size distribution of the powder mixtures and granules was shown in Table 16 and depicted in Figures 13 – 25. It was observed that particle size of all direct compression formulations were relatively larger than those of granules of all wet granulation formulations. Higher percentages of fine powders were attained from the formulation with HPMC that prepared by wet granulation method. Moreover, it was observed that broad size distribution was obtained from the formulation with EC that prepared by wet granulation method.

Table 16 Particle size distribution of powder mixtures and granules

Formulation	% Weight Retained on Sieve Size*(SD)					
	250 μm	**180 μm**	**150 μm**	**125 μm**	**106 μm**	**pan**
DLHC	9.24(0.012)	86.89 (0.019)	1.68 (0.025)	0.73 (0.035)	0.39 (0.024)	1.12 (0.019)
DLEC	23.56 (0.102)	72.77 (0.109)	1.09 (0.207)	0.76 (0.103)	0.56 (0.022)	0.79 (0.030)
DSHC	2.48 (0.057)	77.12 (0.018)	5.45 (0.115)	11.62 (0.224)	0.73 (0.109)	1.48 (0.225)
DSEC	13.86 (0.112)	60.86 (0.111)	11.91 (0.016)	7.46 (0.060)	1.85 (0.097)	1.88 (0.119)
WGHCL	0.74 (0.017)	2.40 (0.014)	17.28 (0.103)	21.02 (0.089)	10.22 (0.115)	45.94 (0.097)
WGHCH	0.82 (0.057)	8.15 (0.060)	7.38 (0.207)	7.51 (0.030)	6.73 (0.112)	72.57 (0.014)
WGEC	27.04 (0.079)	21.04 (0.056)	37.16 (0.055)	16.22 0.101)	0.46 (0.203)	0.44 (0.111)
DHC	10.52 (0.029)	72.73 (0.096)	11.36 (0.102)	1.79 (0.065)	1.57 (0.088)	1.86 (0.101)
DEC	10.56 (0.076)	64.67 (0.025)	18.88 (0.158)	3.13 (0.054)	0.39 (0.072)	1.03 (0.063)
WHCL	4.58 (0.021)	23.11 (0.091)	37.14 (0.086)	19.33 (0.114)	16.56 (0.107)	0.17 (0.027)
WHCH	18.88 (0.096)	14.92 (0.054)	10.68 (0.102)	7.74 (0.072)	8.52 (0.158)	42.46 (0.101)
WECL	27.68 (0.133)	21.24 (0.102)	44.42 (0.092)	5.18 (0.058)	0.60 (0.092)	0.96 (0.014)
WECH	22.98 (0.117)	24.58 (0.023)	31.66 (0.079)	10.72 (0.028)	0.34 (0.012)	0.60 (0.096)

*** Average from two determinations**

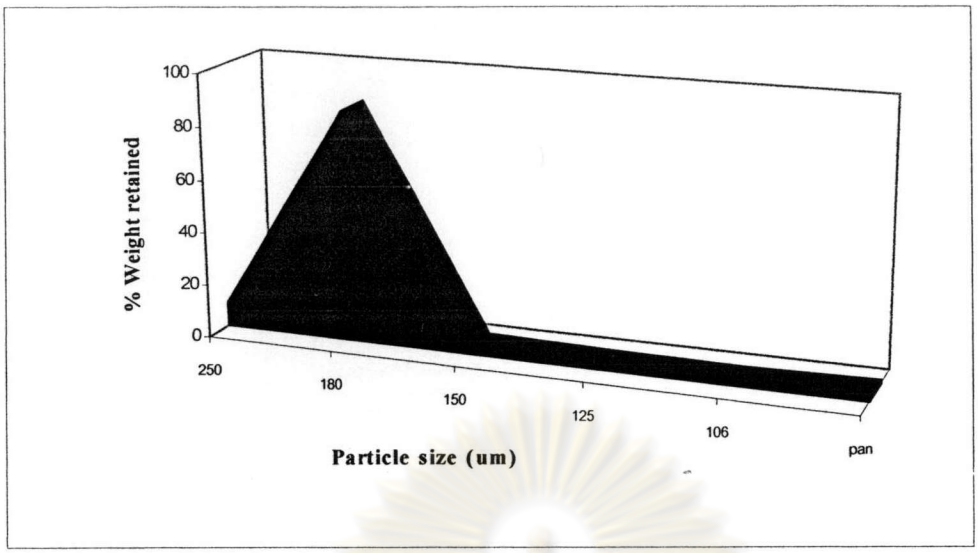


Figure 13 Particle size distribution of powder mixtures of formulation DLHC

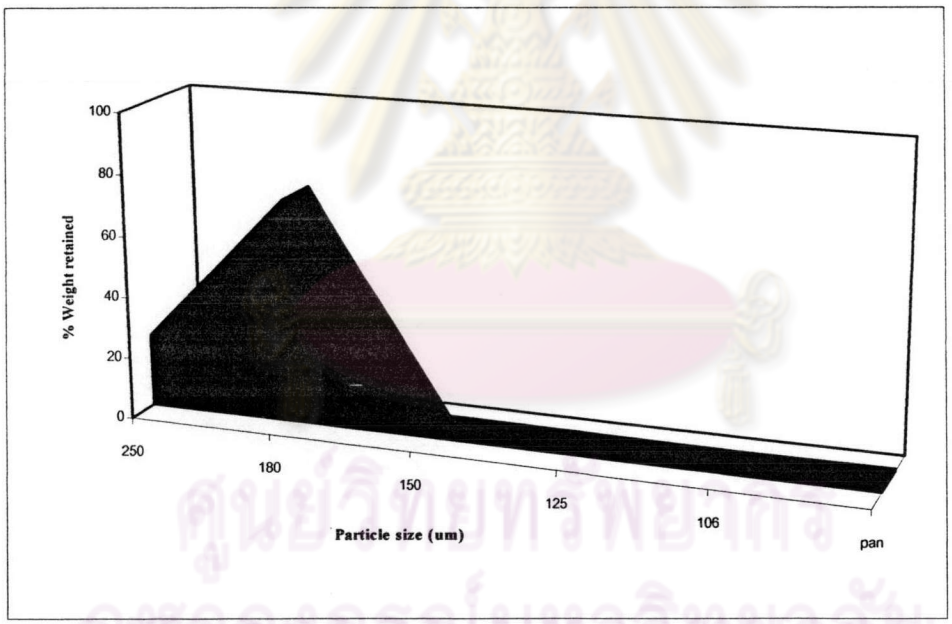


Figure 14 Particle size distribution of powder mixtures of formulation DLEC

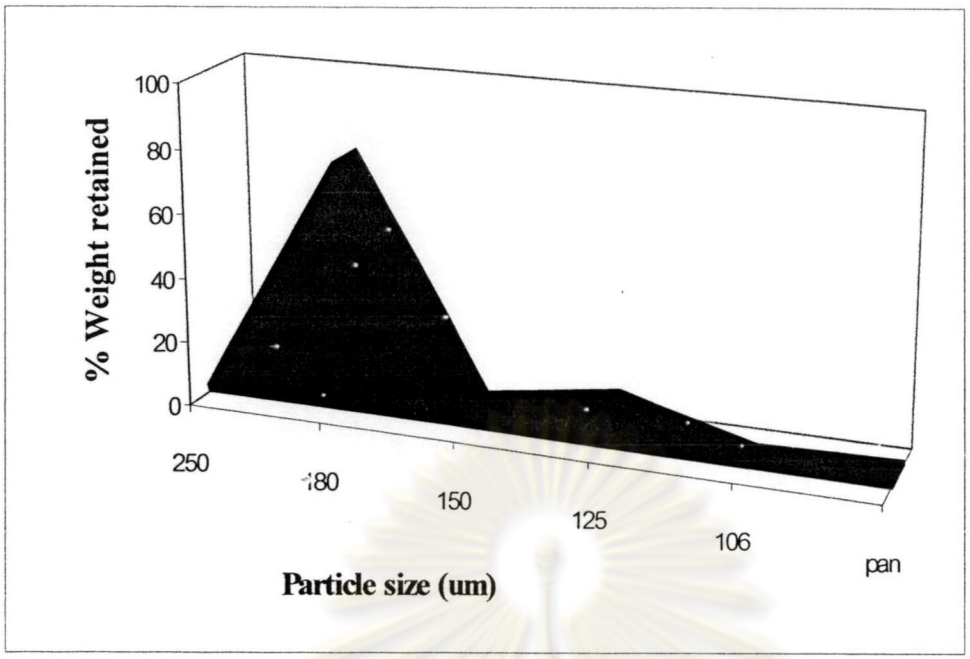


Figure 15 Particle size distribution of powder mixtures of formulation DSHC

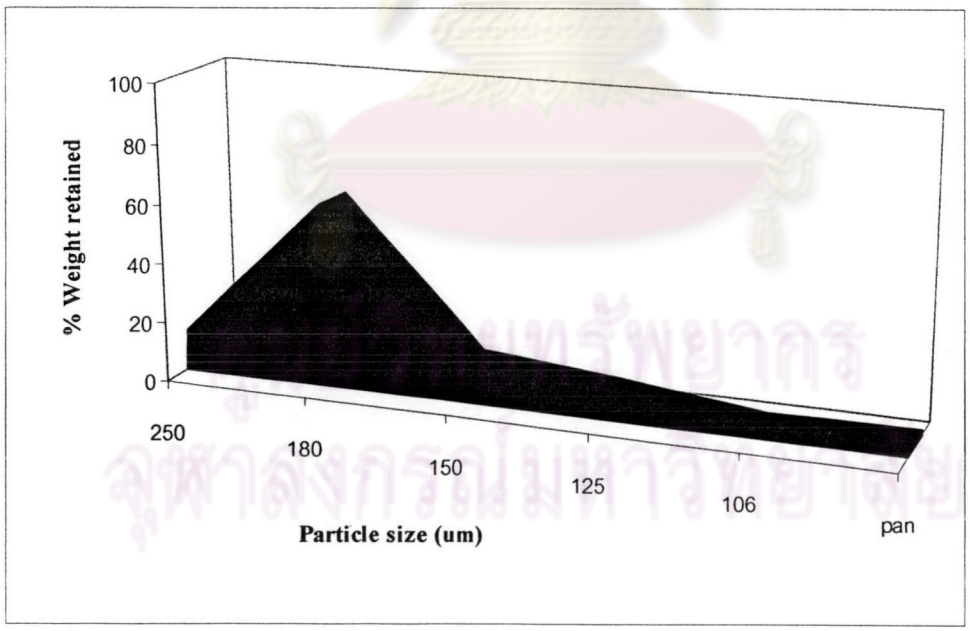


Figure 16 Particle size distribution of powder mixtures of formulation DSEC

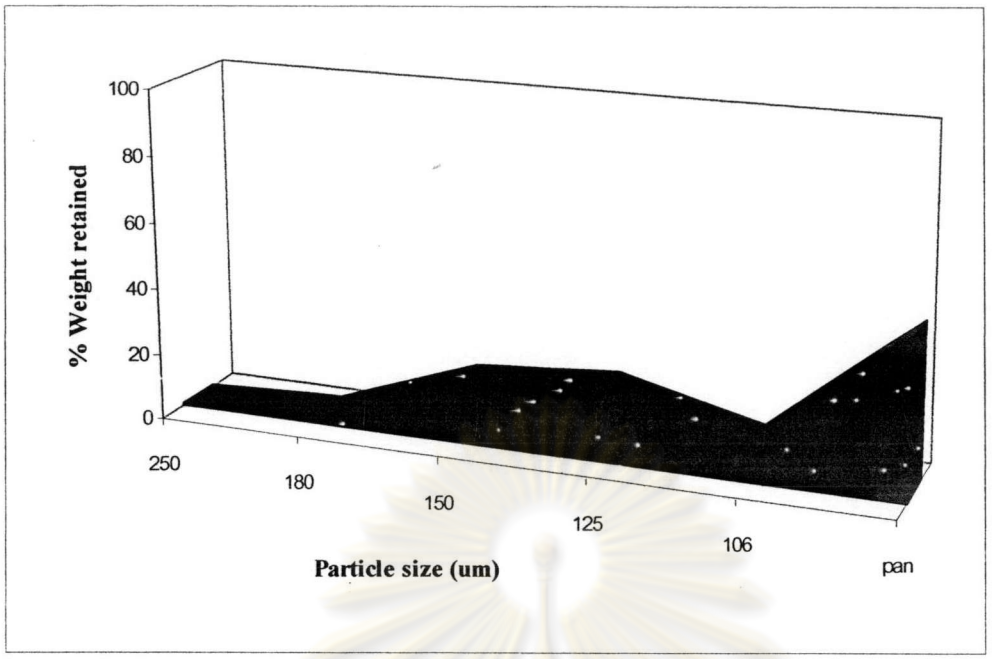


Figure 17 Particle size distribution of granules of formulation WGHCL

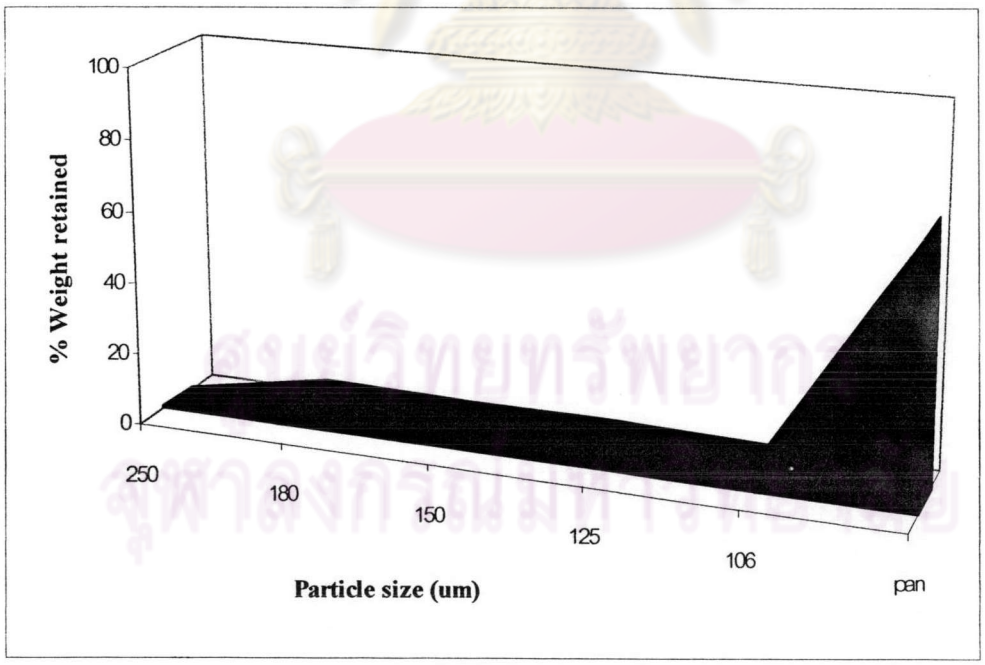


Figure 18 Particle size distribution of granules of formulation WGHCH

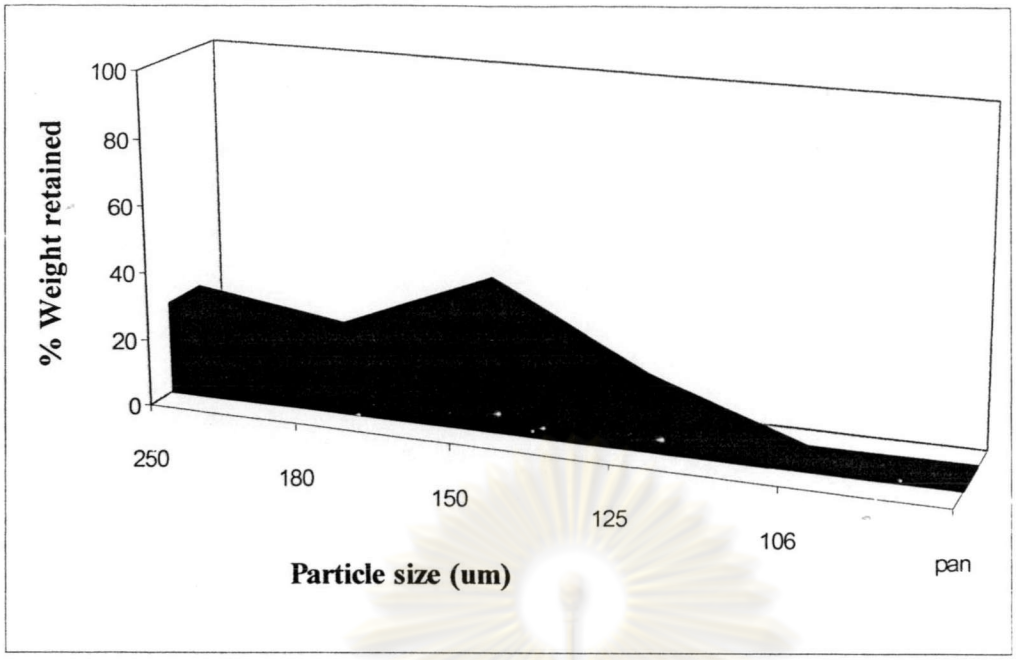


Figure 19 Particle size distribution of granules of formulation WGEC

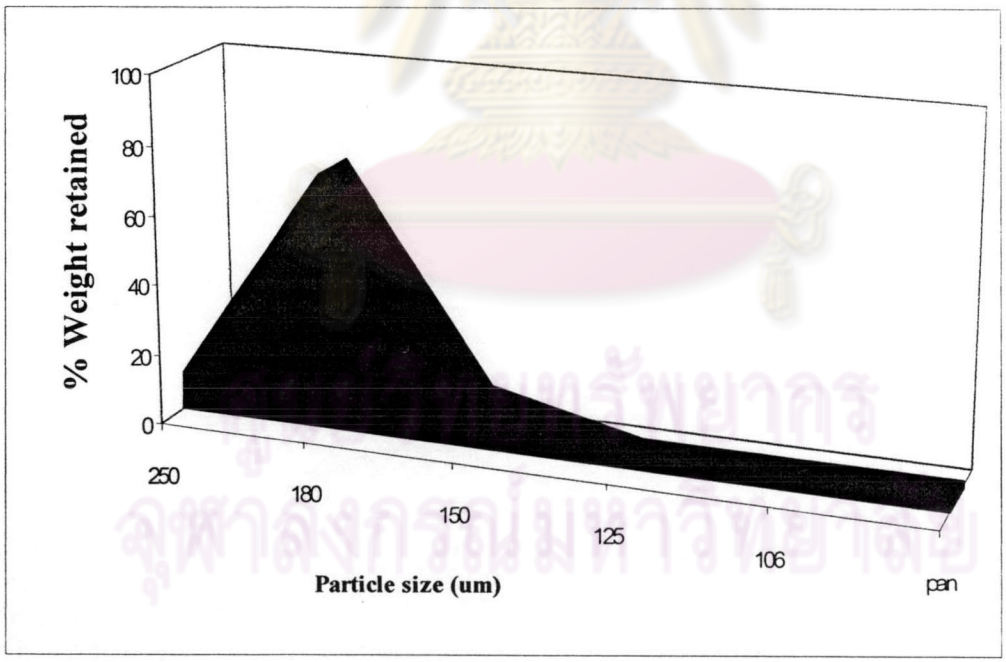


Figure 20 Particle size distribution of powder mixtures of formulation DHC

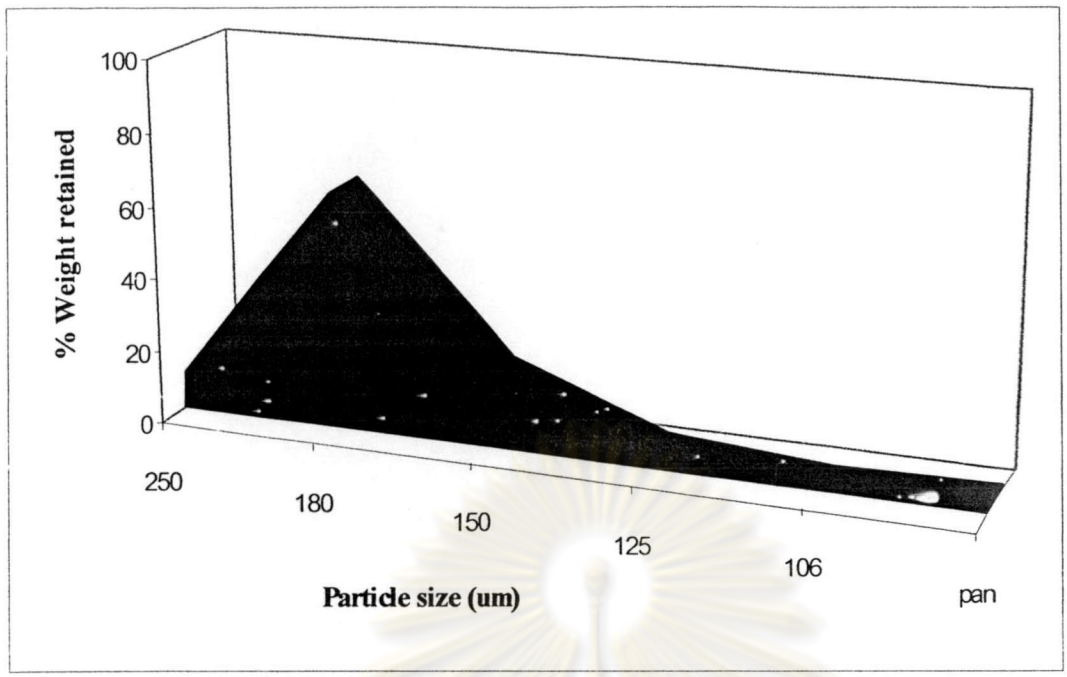


Figure 21 Particle size distribution of powder mixtures of formulation DEC

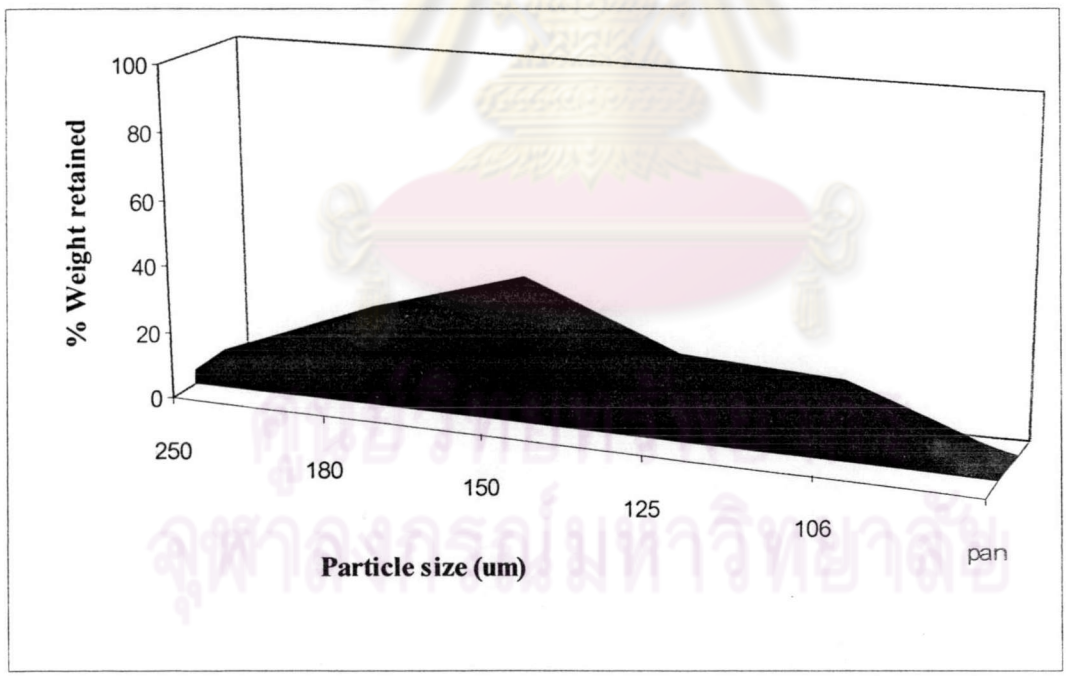


Figure 22 Particle size distribution of granules of formulation WHCL

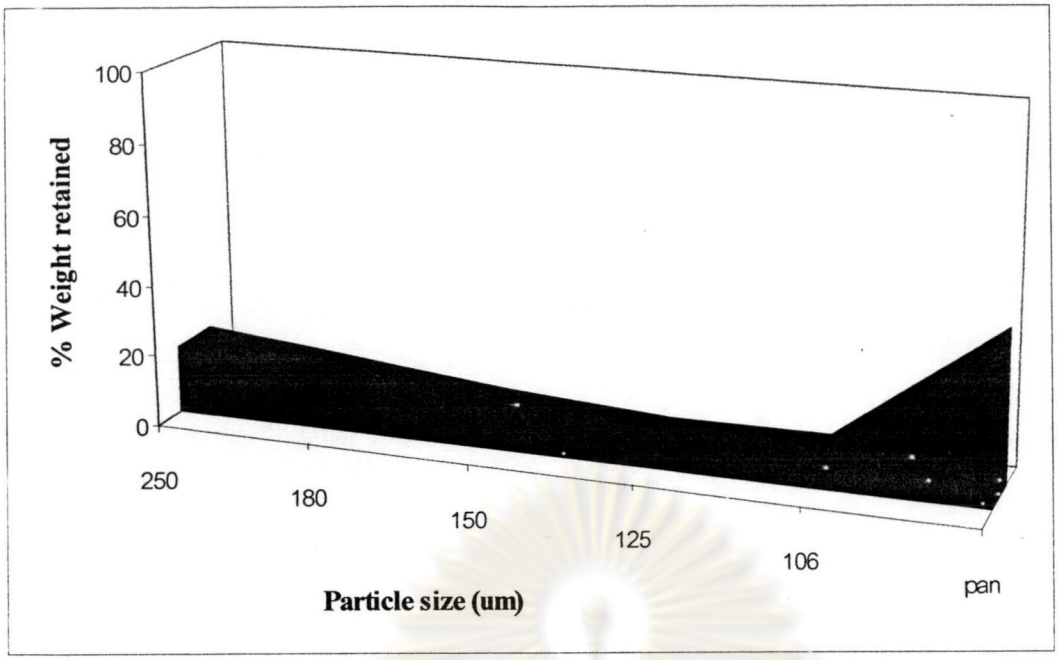


Figure 23 Particle size distribution of granules of formulation WHCH

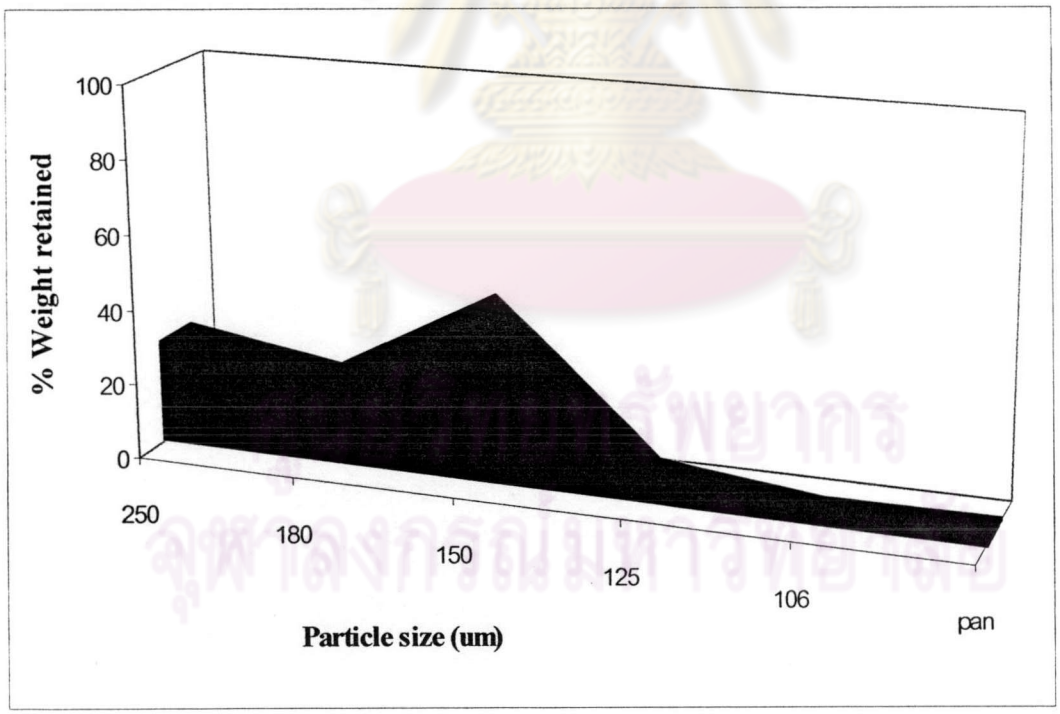


Figure 24 Particle size distribution of granule of formulation WECL

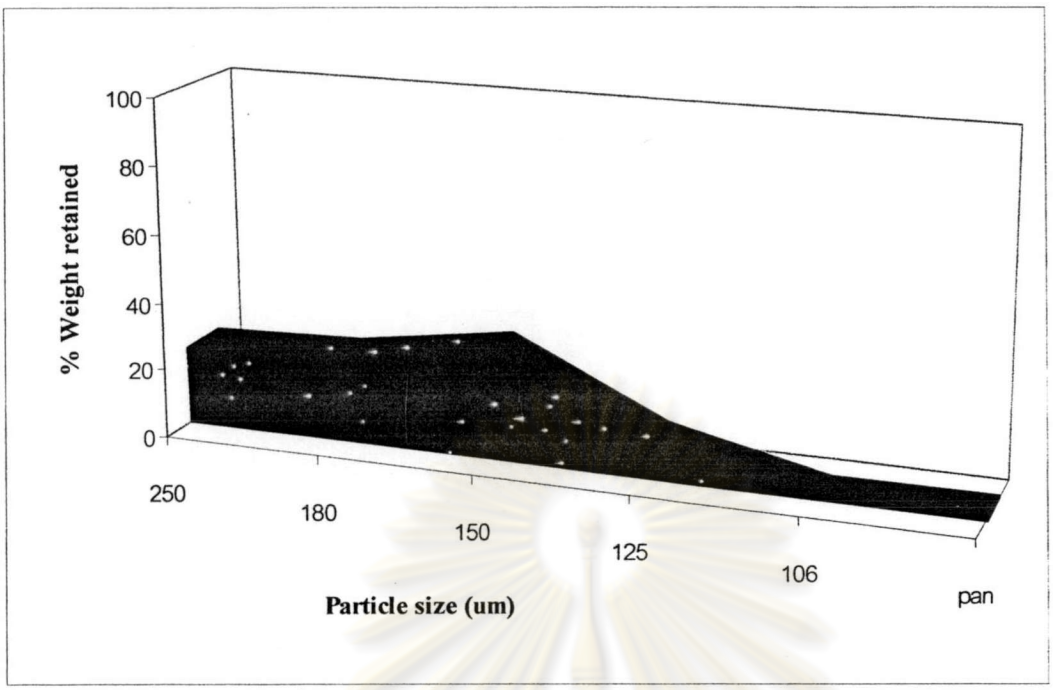


Figure 25 Particle size distribution of granule of formulation WECH

3.6) Infrared spectrometry

The IR spectrum of untreated diclofenac sodium is shown in Figure 26. The principal peaks were observed at wave numbers of 747, 767, 1284, 1306, 1506, and 1575 cm^{-1}. The peaks at 747 and 767 cm^{-1} were resulted from C-H out of plane blending. The IR absorption band at 1284 and 1308 cm^{-1} were resulted from C-N stretching. The peaks at 1506 and 1575 cm^{-1} were resulted from C=C stretching (Mofflat et al., 1986). Moreover, the IR spectra of HPMC, EC, Ludipress and lactose are depicted in Figures 27 - 30.

The IR spectra of DS granules with HPMC and with EC at various amounts of the cellulose derivatives that prepared from wet granulation method are illustrated in Figures 31 and 32. As the concentration of both HPMC and EC increased, the prominent peaks of spectra of diclofenac sodium did not shift but the peak intensity was slightly weaker. These indicated that the interaction between diclofenac sodium and both cellulose derivatives was scarce.

Table 17 IR peak of spectra of diclofenac sodium and of diclofenac sodium granules contained with various amount and type of cellulose derivatives

Formulation	Principle Peak (cm^{-1})					
DS	747	767	1284	1306	1506	1575
WHCL	748	773	1282	1306	1508	1578
WHCH	747	767	1283	1306	1507	1577
WECL	747	769	1282	1305	1508	1578
WECH	746	767	1282	1305	1509	1578

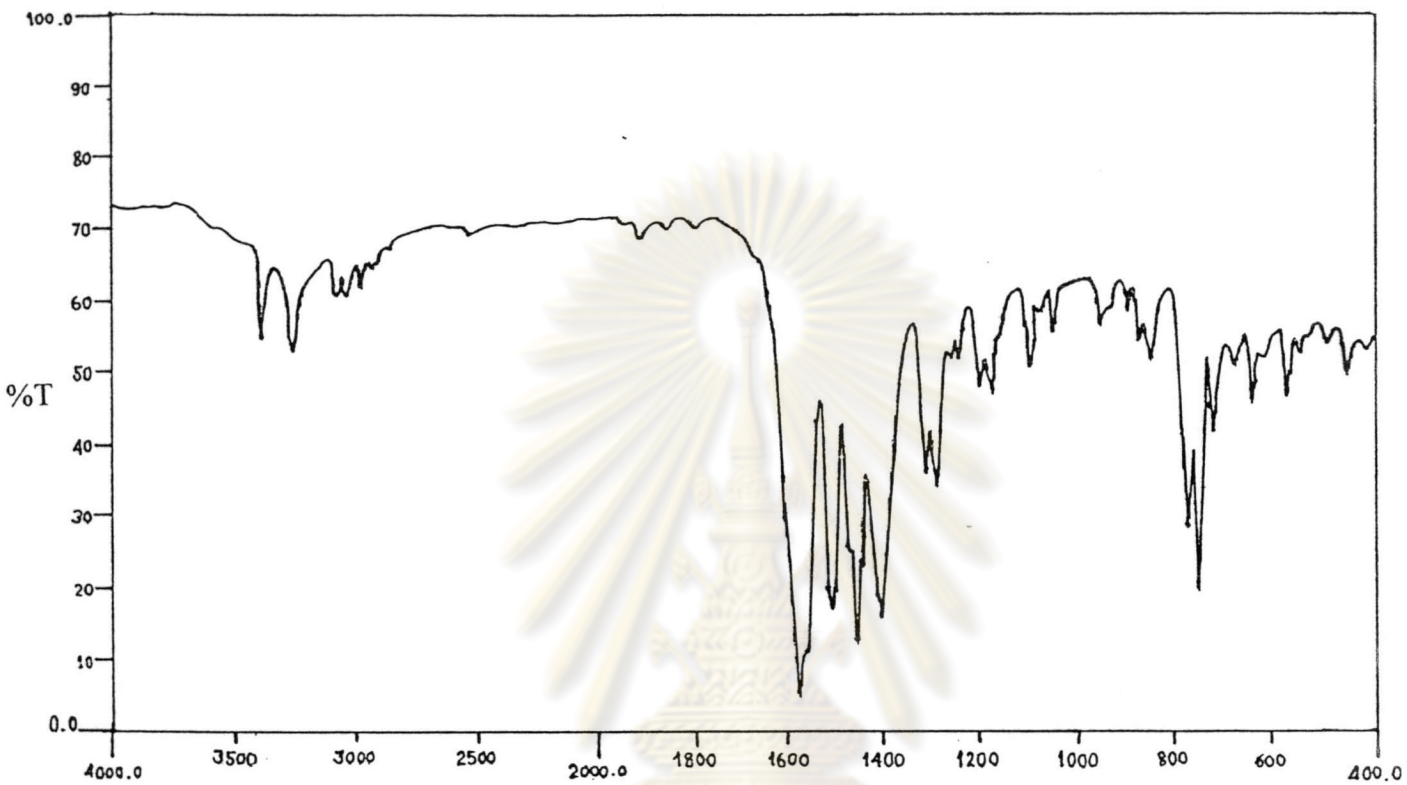


Figure 26 Infrared spectrum of diclofenac sodium

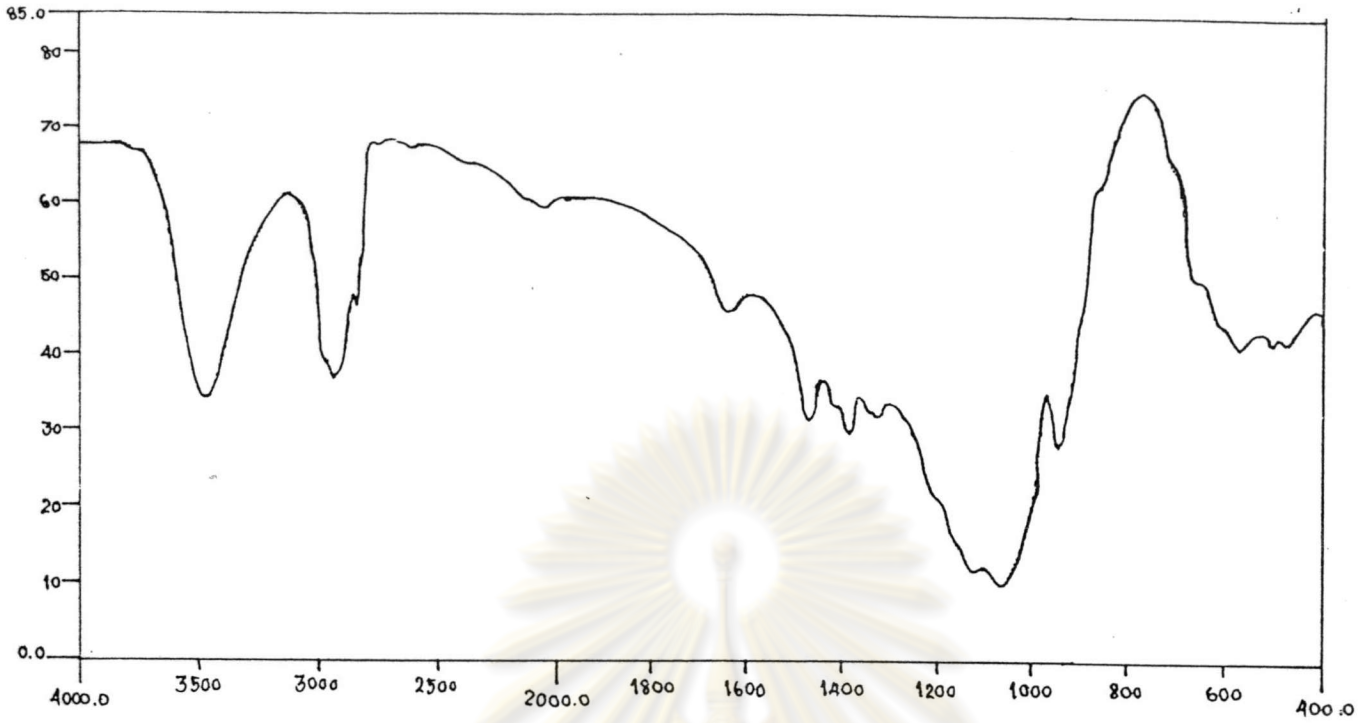


Figure 27 Infrared spectrum of hydroxypropylmethycellulose (Methocel E4M)

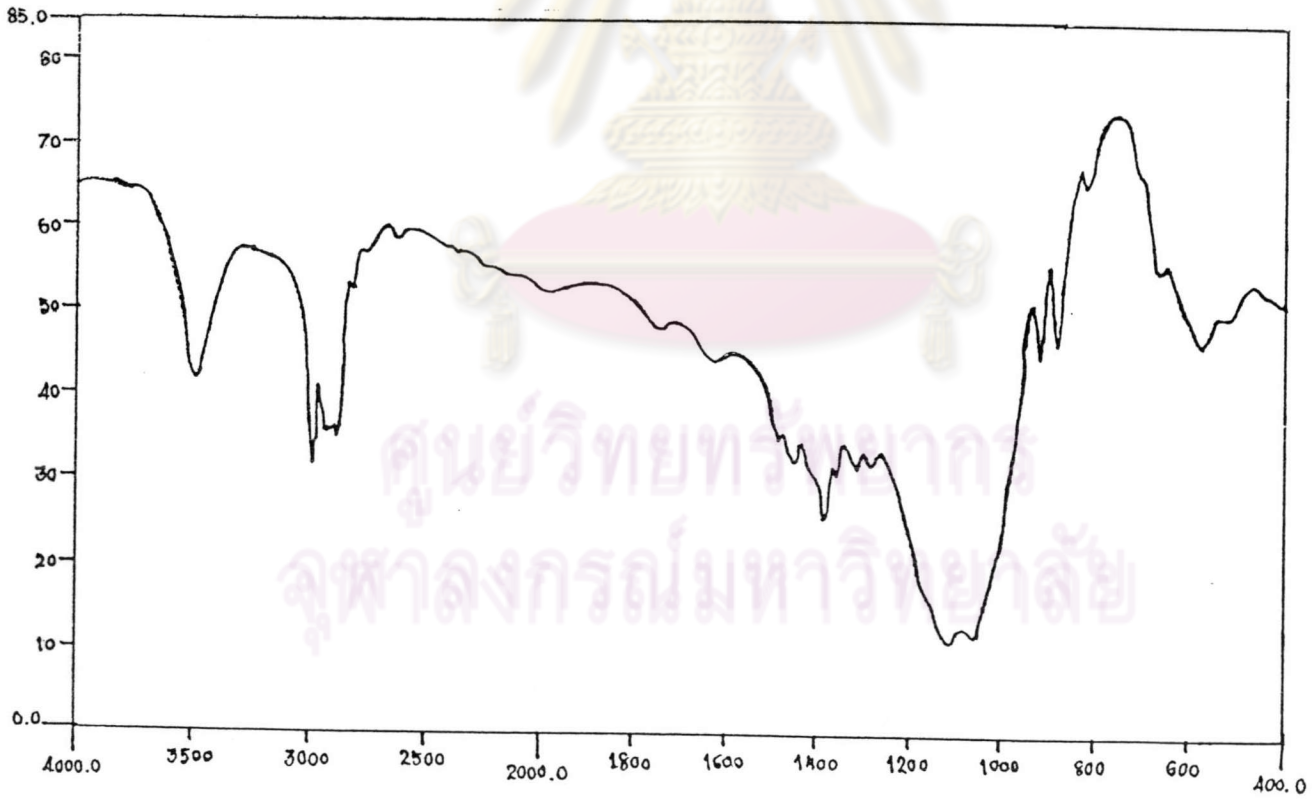


Figure 28 Infrared spectrum of ethylcellulose (Ethocel 10 cps.)

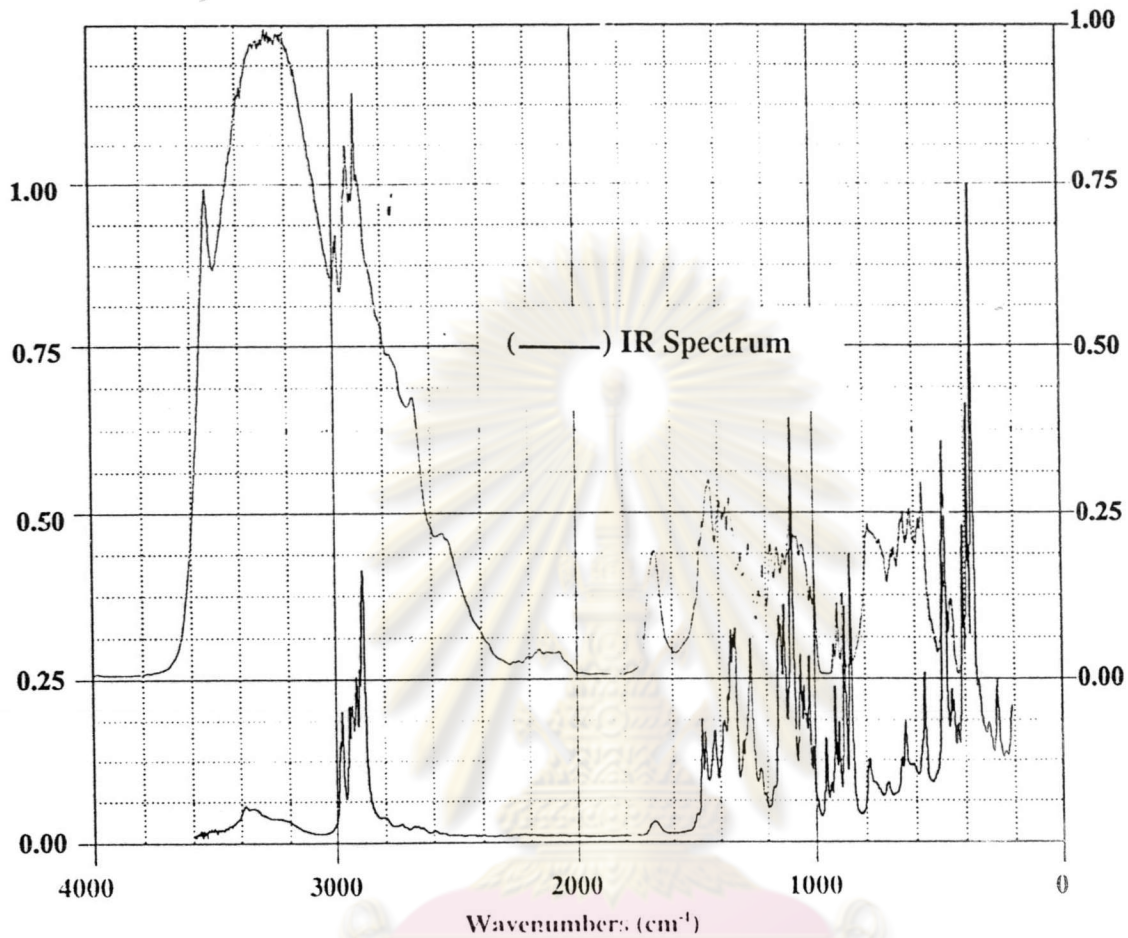


Figure 29 Infrared spectrum of ludipress

Reference from Drug and the pharmaceutical sciences

(David E. Bugay and W. Paul Findlay, 1992)

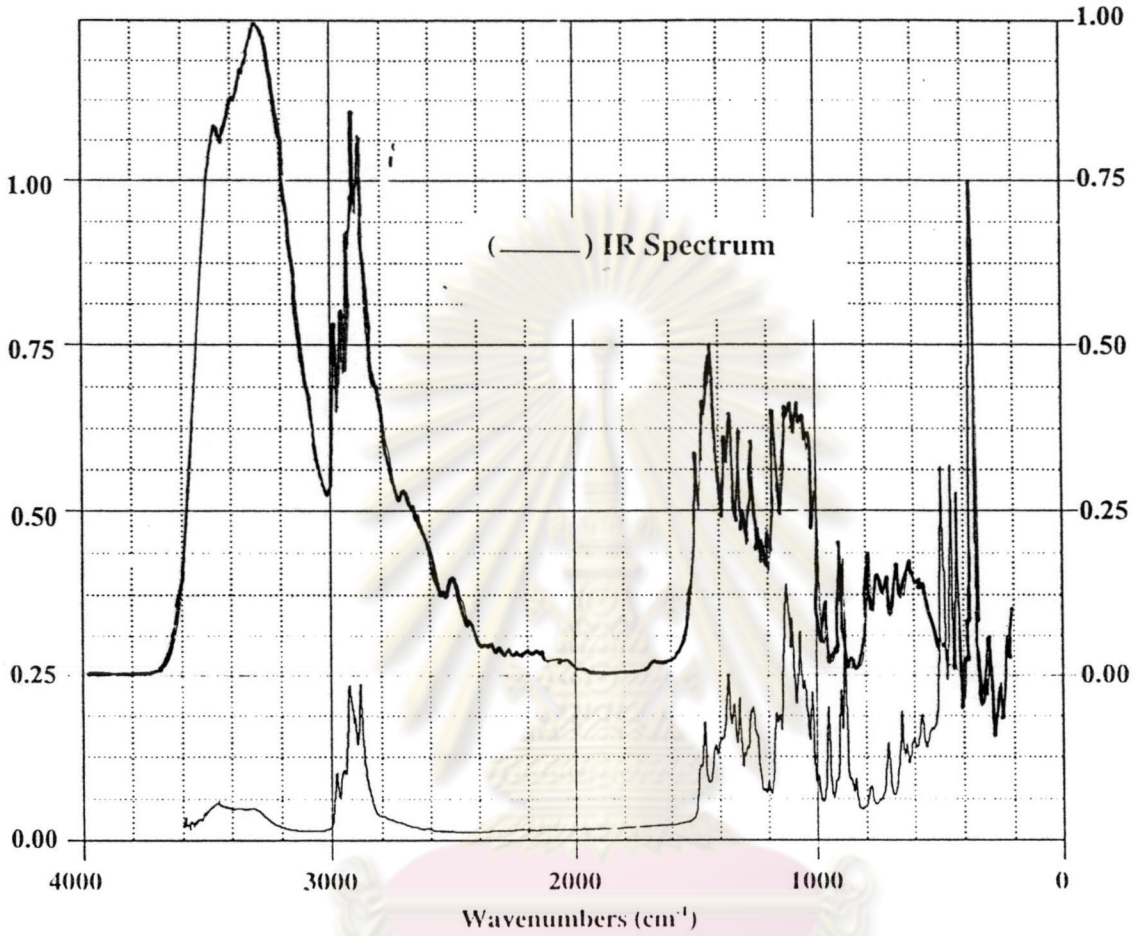


Figure 30 Infrared spectrum of lactose

Reference from Drug and the pharmaceutical sciences

(David E. Bugay and W. Paul Findlay, 1992)

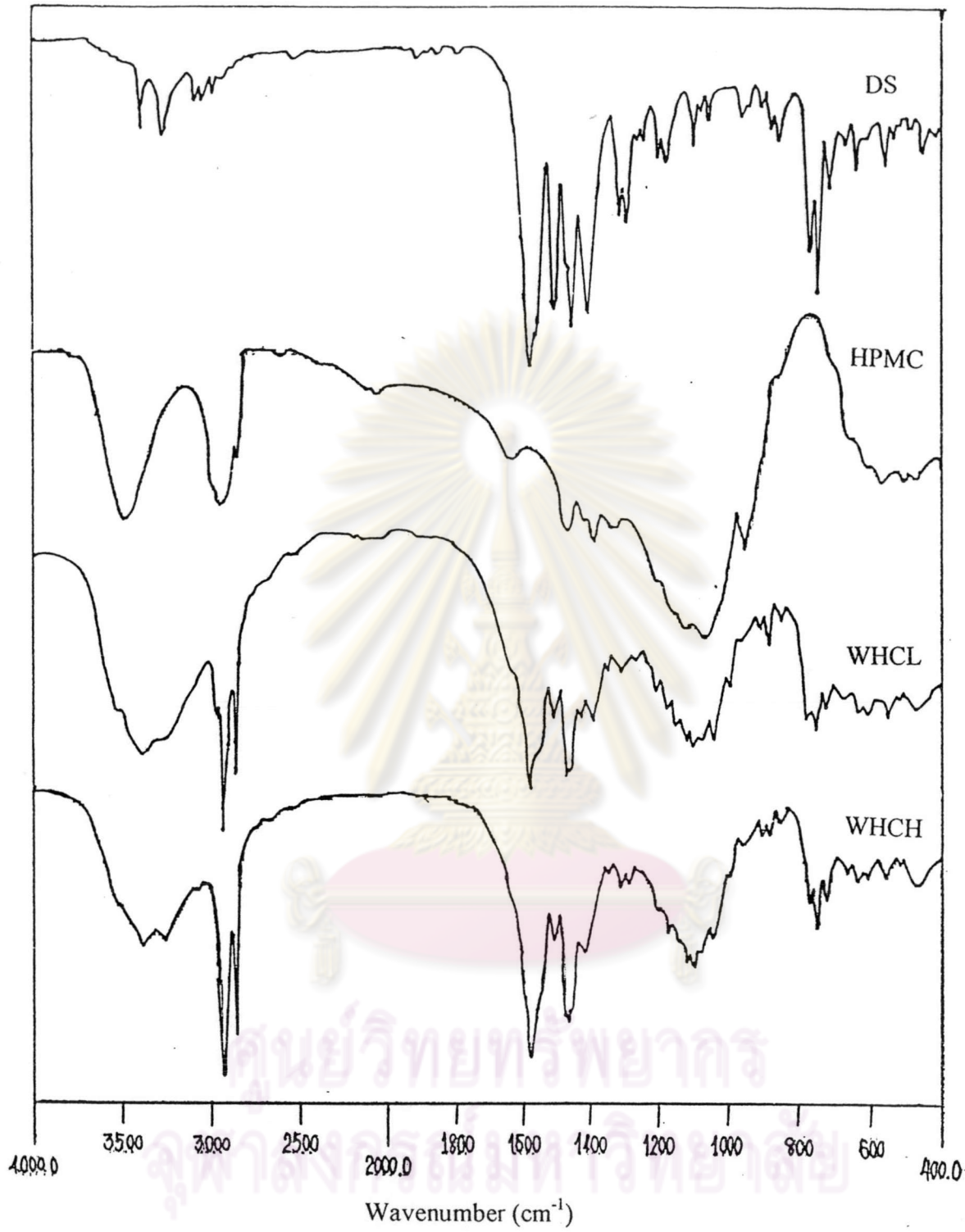


Figure 31 IR Spectra of diclofenac sodium-hydroxypropylmethycellulose granules

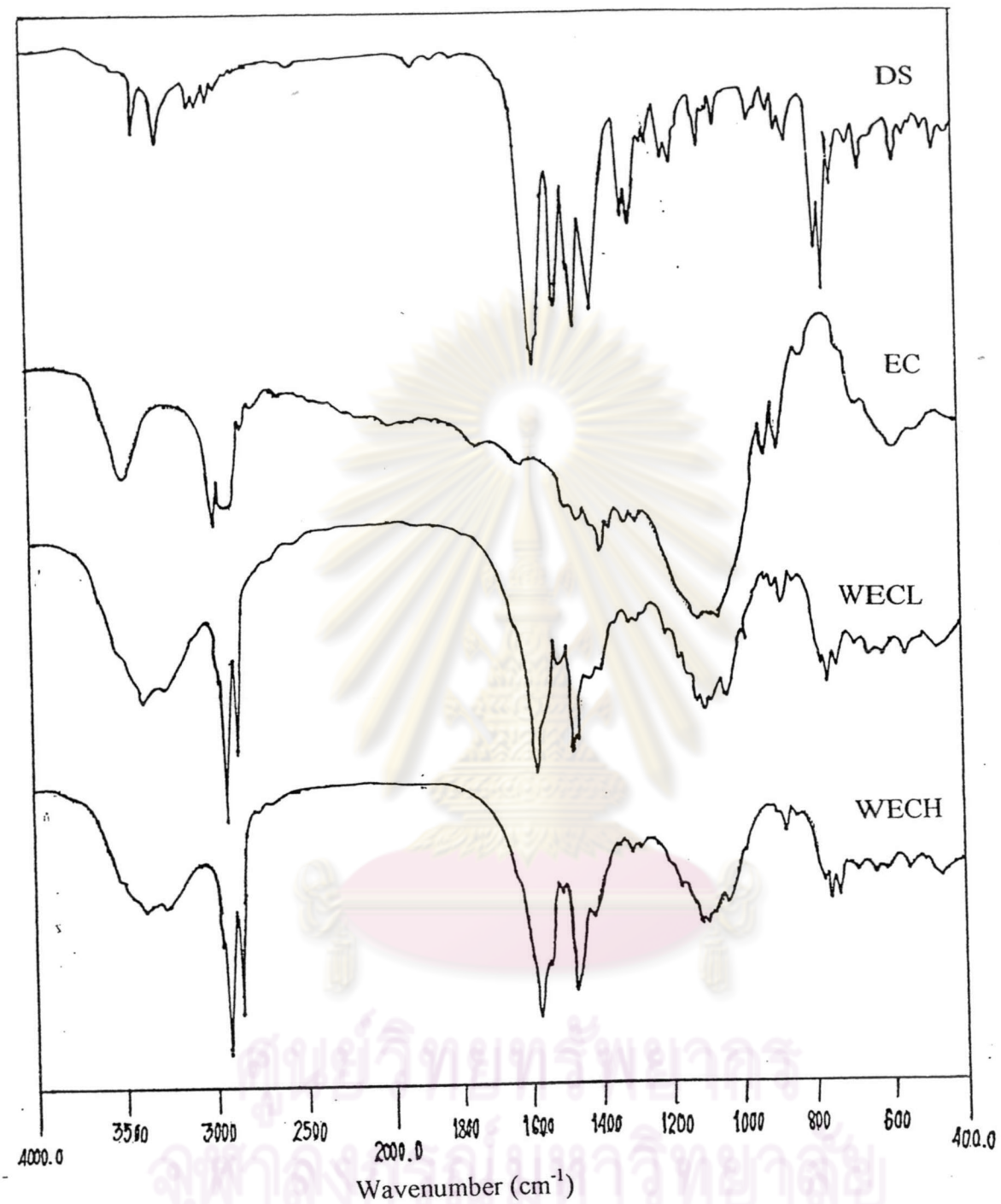


Figure 32 IR spectra of diclofenac sodium-ethycellulose granules

3.7) X – ray diffraction

The X – ray diffraction patterns of diclofenac sodium, lactose and diclofenac sodium granules with different cellulose derivatives are illustrated in Figures 33 - 36.The most characteristics of the X – ray diffraction peaks of untreated diclofenac sodium were observed and also showed the heap of sharp peaks at the diffraction angle between 20 – 30°.

The X – ray diffraction patterns of diclofenac sodium granules with various amount of HPMC are depicted in Figure 35. It was observed that the both formulations contained with low and high content of HPMC (Formulation WHCL and WHCH) exhibited absence of some prominent pure diclofenac sodium peaks. In contrast, it exhibited the new peaks that were different from the pure DS peaks. Moreover, remarkably fewer intense peaks and a slightly higher baseline were detected. All detail data of the X-ray diffraction peaks of granules are shown in Table 18 It is indicated that these products were still crystalline form but the characteristic of crystals may be changed. Some crystals converted to an amorphous form.

The X – ray diffraction patterns of diclofenac sodium granules with various amount of EC are displayed in Figure 36. It was found that the peak and intensities of both formulation contained with EC (Formulation WECL and WECH) were differ from pure DS peaks. The diffraction peaks showed some peaks disappeared and new peaks occurred. Moreover, the intensities of peak were reduced and baseline was a slightly high. All detail data showed the different peak of granules as presented in Table 18. Therefore, the almost of diclofenac sodium was still crystalline form but the characteristics of crystal may be changed and some crystals converted to an amorphous form.

Table 18 The peak of X-ray diffraction of Diclofenac sodium, additives, and granules of formulations

Peak NO.	Diclofenac sodium (°)	Magnesium stearate (°)	Lactose* (°)	WGHCL (°)	WGHCH (°)	WGECL (°)	WGECH (°)
1	6.76	5.44	6.81	5.62	5.42	5.56	5.44
2	8.64	7.28	9.97	6.52	6.64	6.73	6.80
3	11.36	8.92	10.42	8.57	8.45	8.72	8.68
4	12.76	9.32	12.39	12.44	12.52	12.64	12.64
5	15.32	14.24	13.19	16.36	15.28	15.32	15.36
6	16.24	14.92	15.50	19.08	16.40	16.52	16.48
7	17.28	16.08	16.30	19.56	19.12	19.24	18.92
8	17.92	16.92	17.55	20.00	19.56	19.68	19.24
9	20.00	17.84	18.97	20.84	20.00	20.12	19.68
10	23.64	18.84	19.43	21.24	21.24	20.96	20.08
11	23.96	19.64	19.97	21.84	21.88	21.36	20.92
12	25.16	21.32	20.46	23.80	22.60	21.96	21.36
13	26.04	21.92	20.86	26.12	23.52	22.92	21.96
14	27.24	22.56	21.49	37.86	37.76	23.92	22.68
15	28.04	23.56	22.23			25.92	37.01
16		25.36	22.71			37.72	37.74
17		30.12	23.52				
18		37.80	23.88				
19		39.00	24.52				
20		40.04	24.84				
21		42.00	25.42				
22		43.50	26.64				
23		45.96	27.38				
24			27.93				
25			28.96				
26			30.10				
27			30.75				
28			31.35				
29			31.62				

* Referrence from Analytical profiles of drug substances (Harry G.B. et al., 1990)

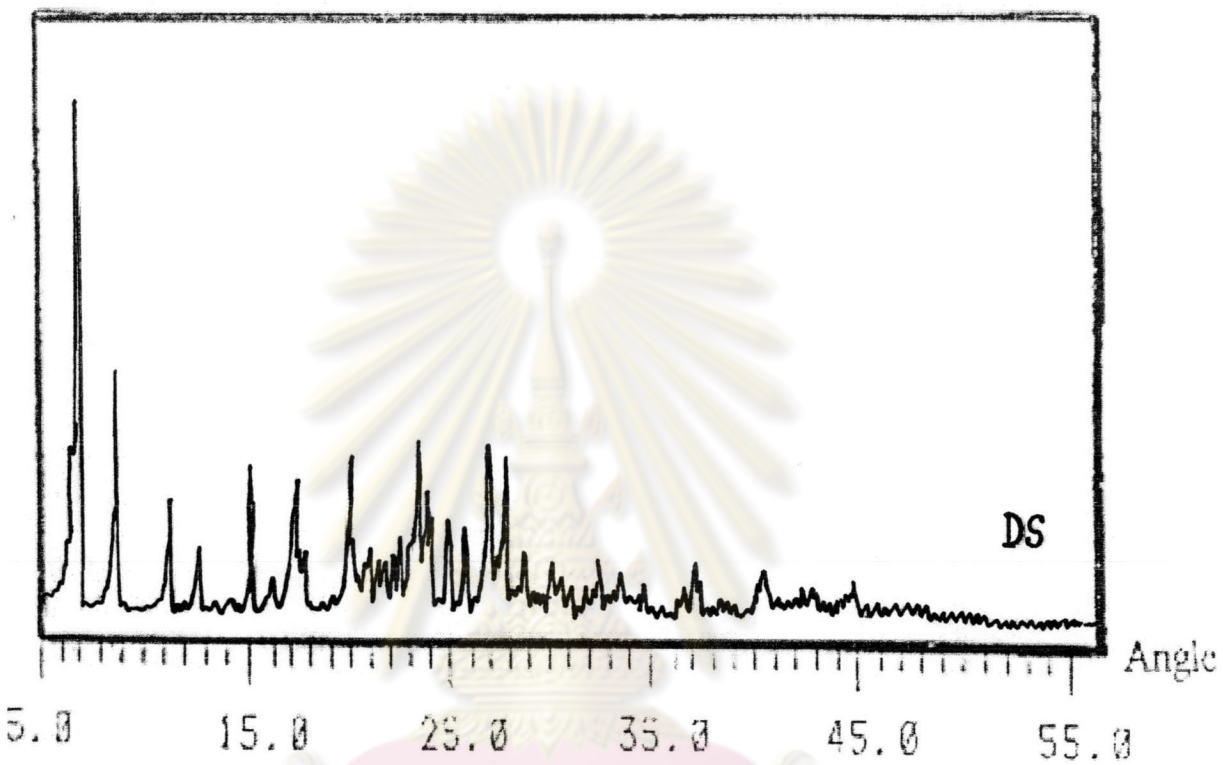


Figure 33 X-ray diffraction spectra of diclofenac sodium

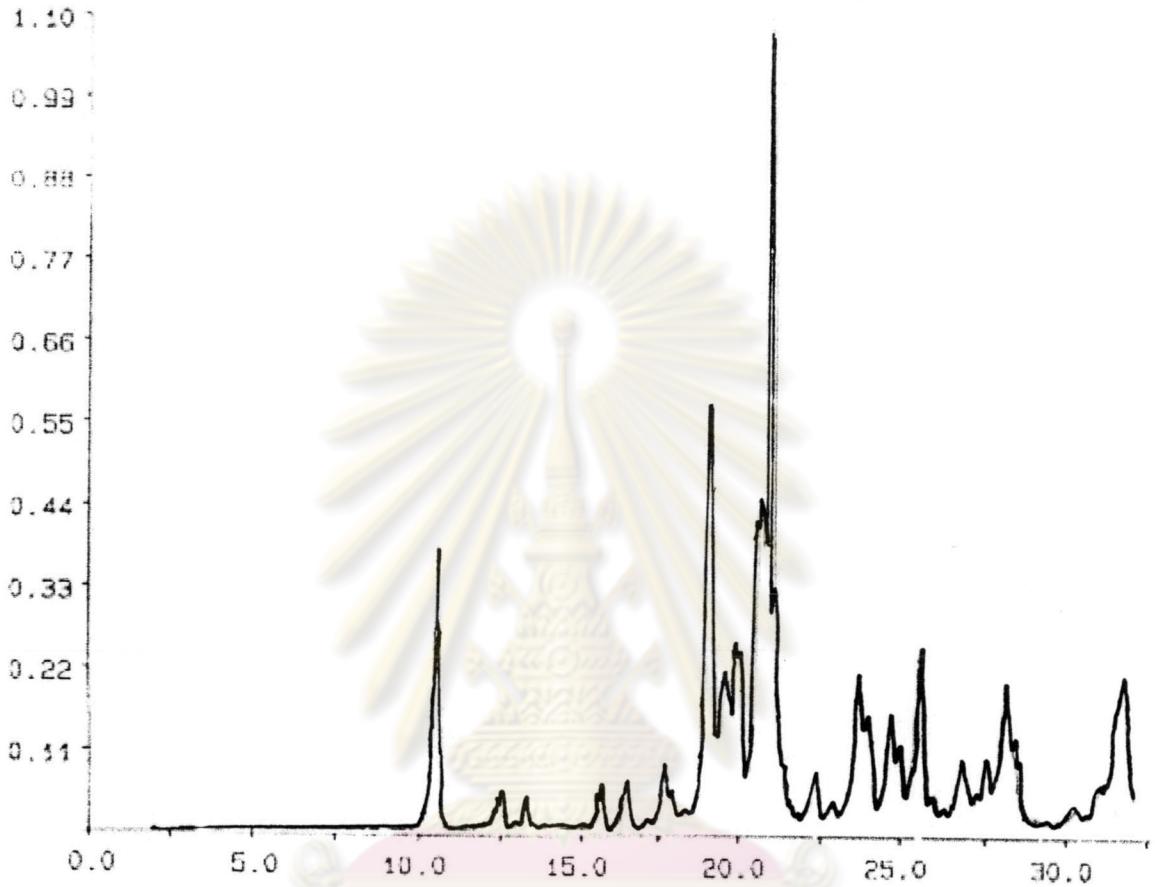


Figure 34 X-ray diffraction spectra of lactose anhydrous

* Referrence from Analytical profiles of drug substances (Harry G.B. et al., 1990)

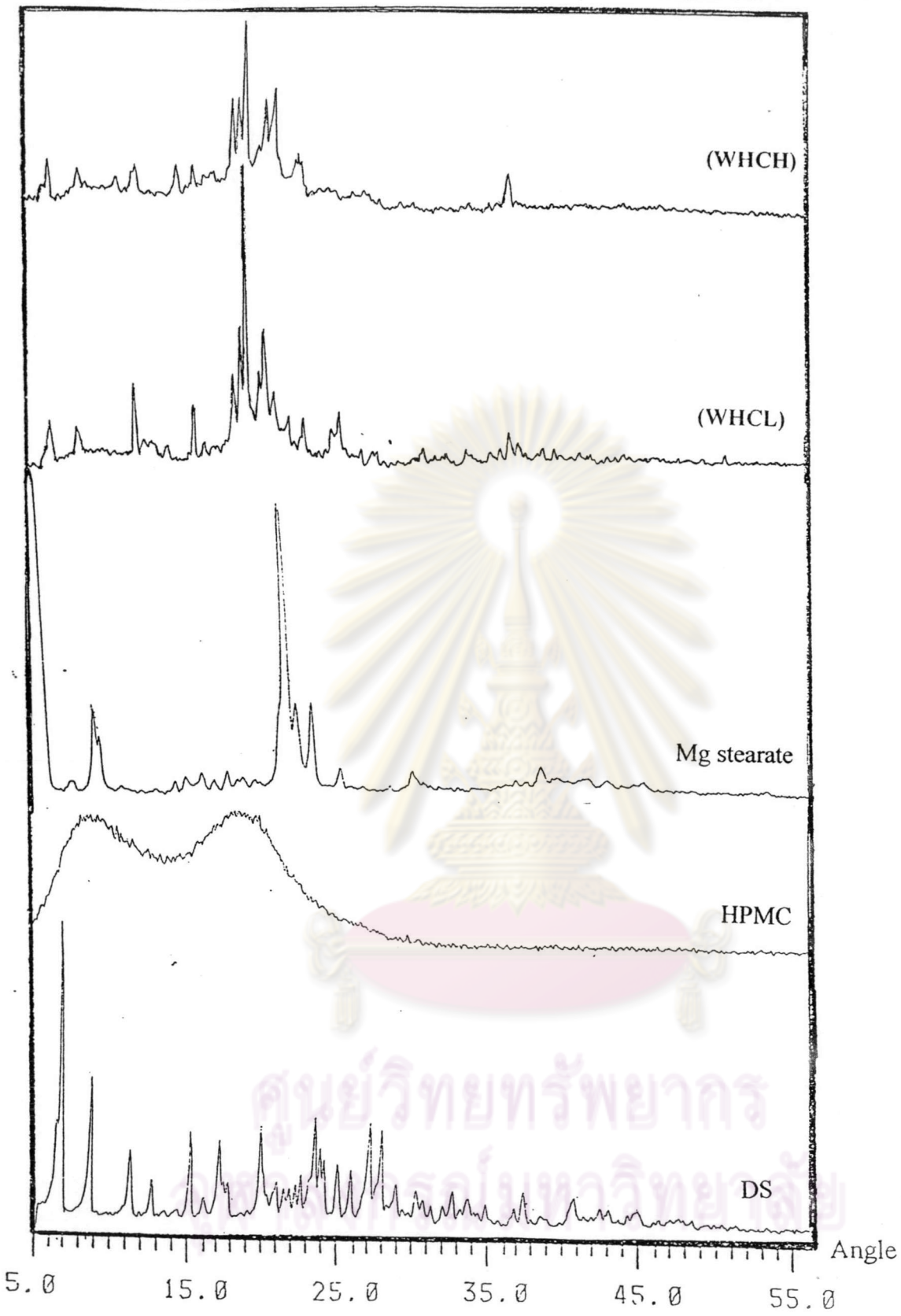


Figure 35 **X-ray diffraction spectra of diclofenac sodium-hydroxypropylmethycellulose granules**

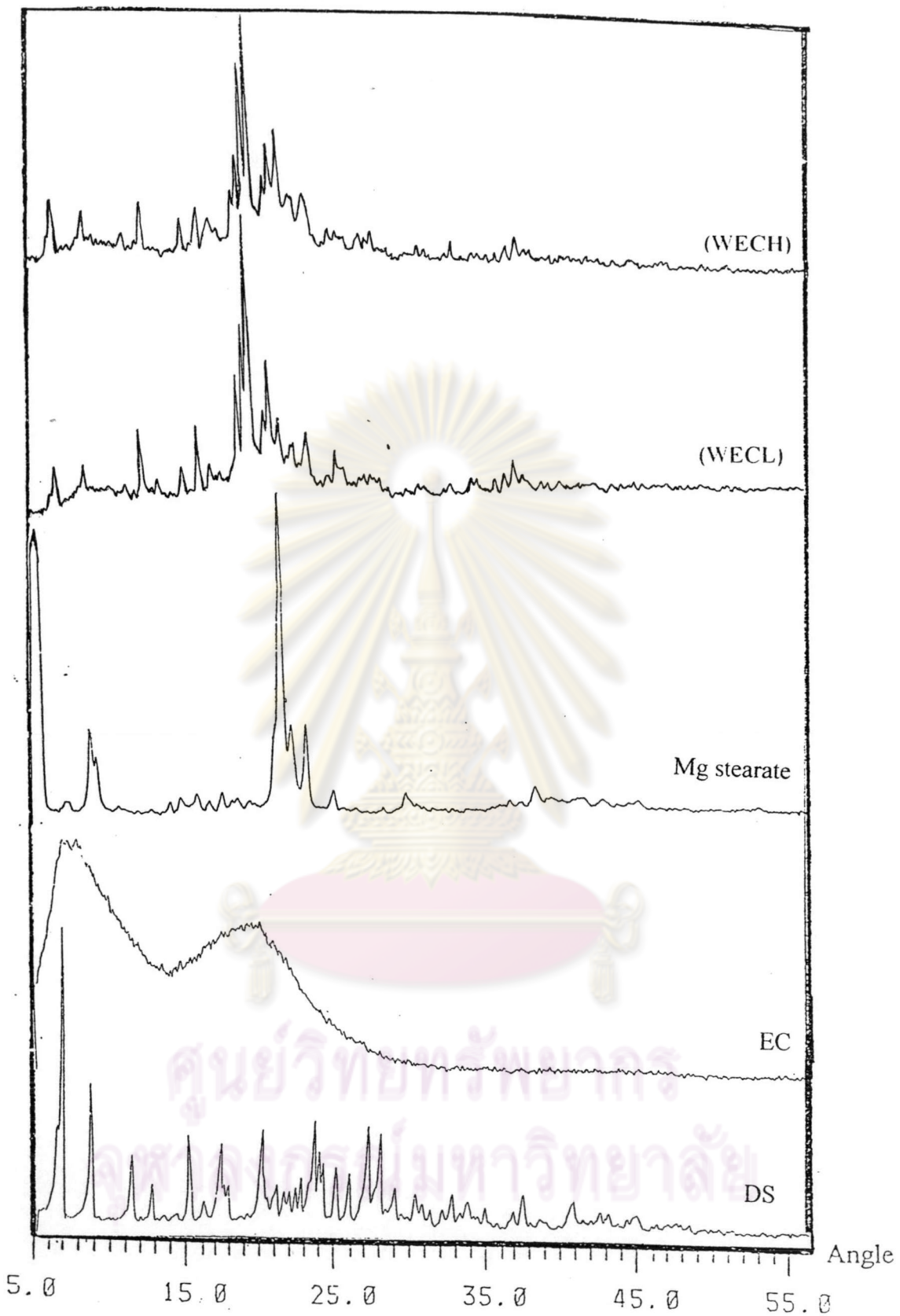


Figure 36 X-ray diffraction spectra of diclofenac sodium-ethycellulose granules

3.8) Differential scanning calorimetry (DSC)

The DSC thermograms of diclofenac sodium and of diclofenac sodium granules with various type and amount of the cellulose derivatives are shown in Figures 37 - 41. The thermogram of pure diclofenac sodium exhibited the characteristic melting endotherm at 285.0^{0}C and the peak of 54.2^{0}C, 60.2^{0}C exhibited vapourization of moisture content into diclofenac sodium powders (Fini et al., 1999). The DSC peak temperatures of diclofenac sodium granules contained both HPMC and EC were disappeared in duplicate determinations. But it could detect the heap of endothermic peaks at 100^0 - 150^0 in all formulations.

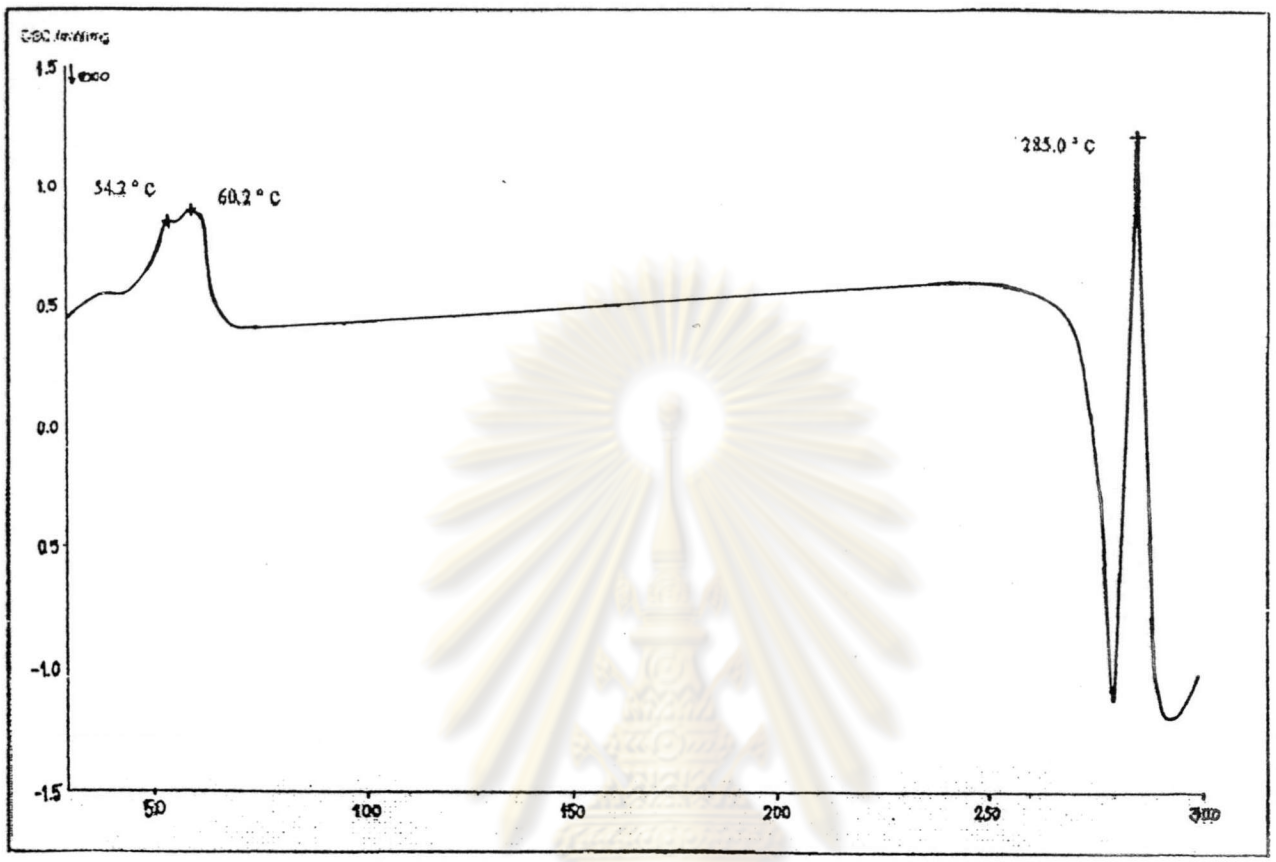


Figure 37 DSC thermagram of diclofenac sodium

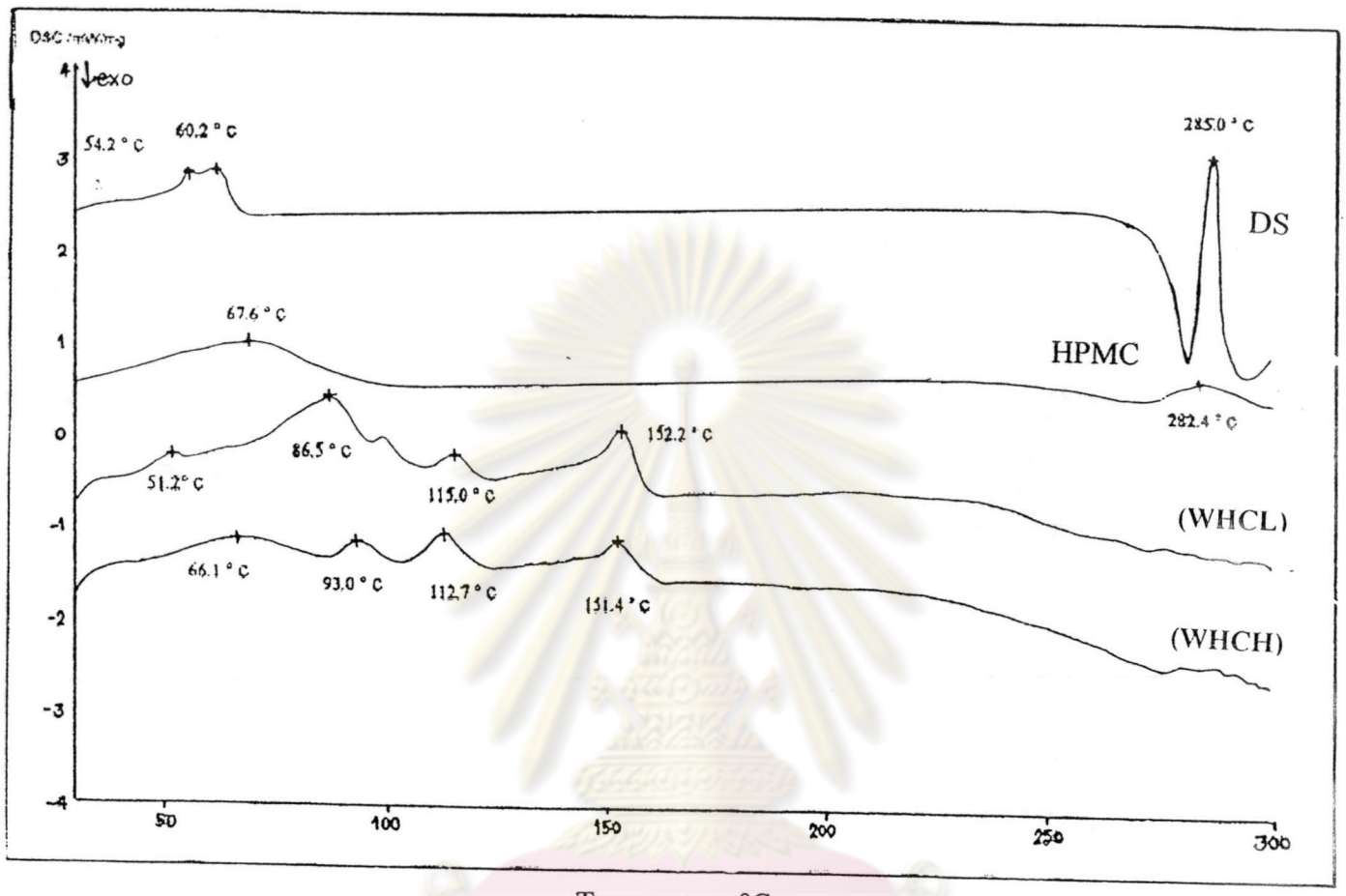


Figure 38 DSC thermagrams of diclofenac sodium-hydroxypropylmethycellulose granules in first condition

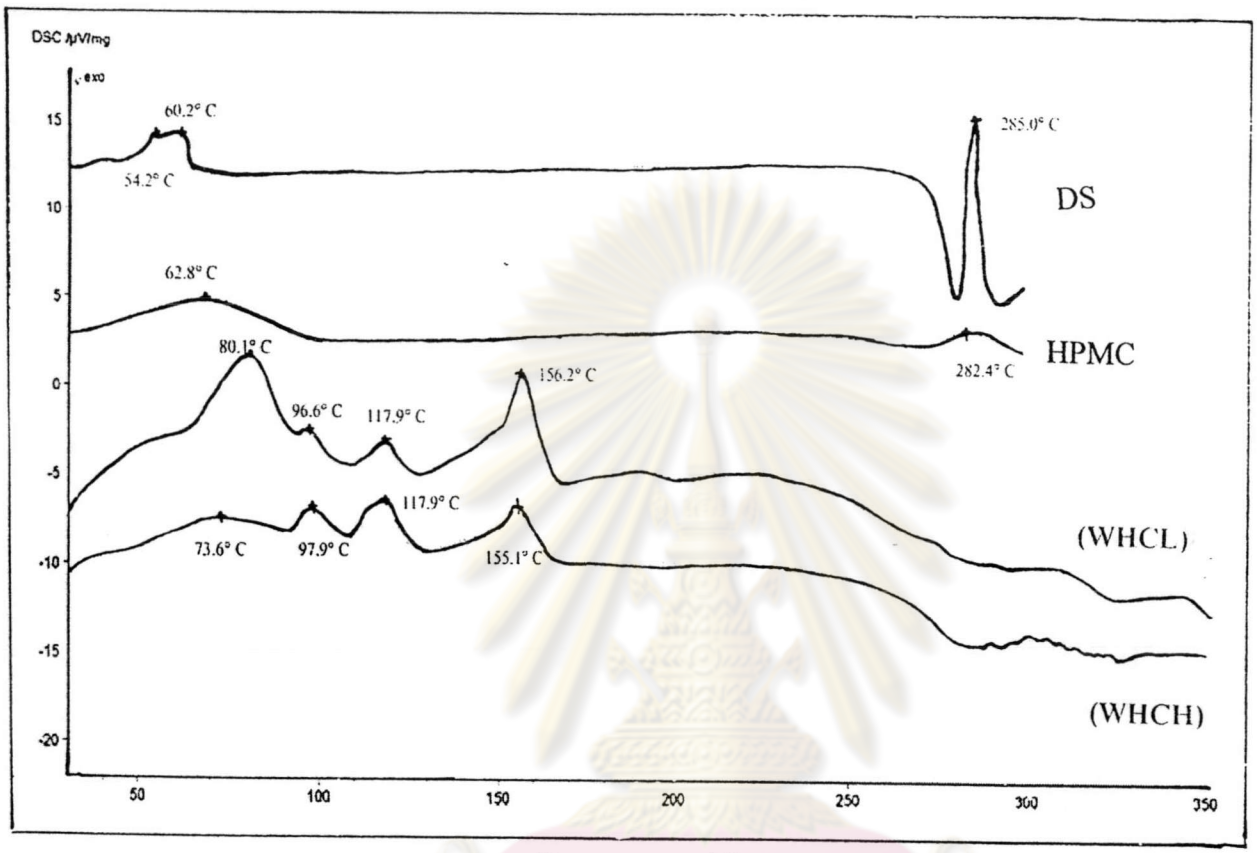


Figure 39 DSC thermagrams of diclofenac sodium-hydroxypropylmethycellulose granules in second conditon.

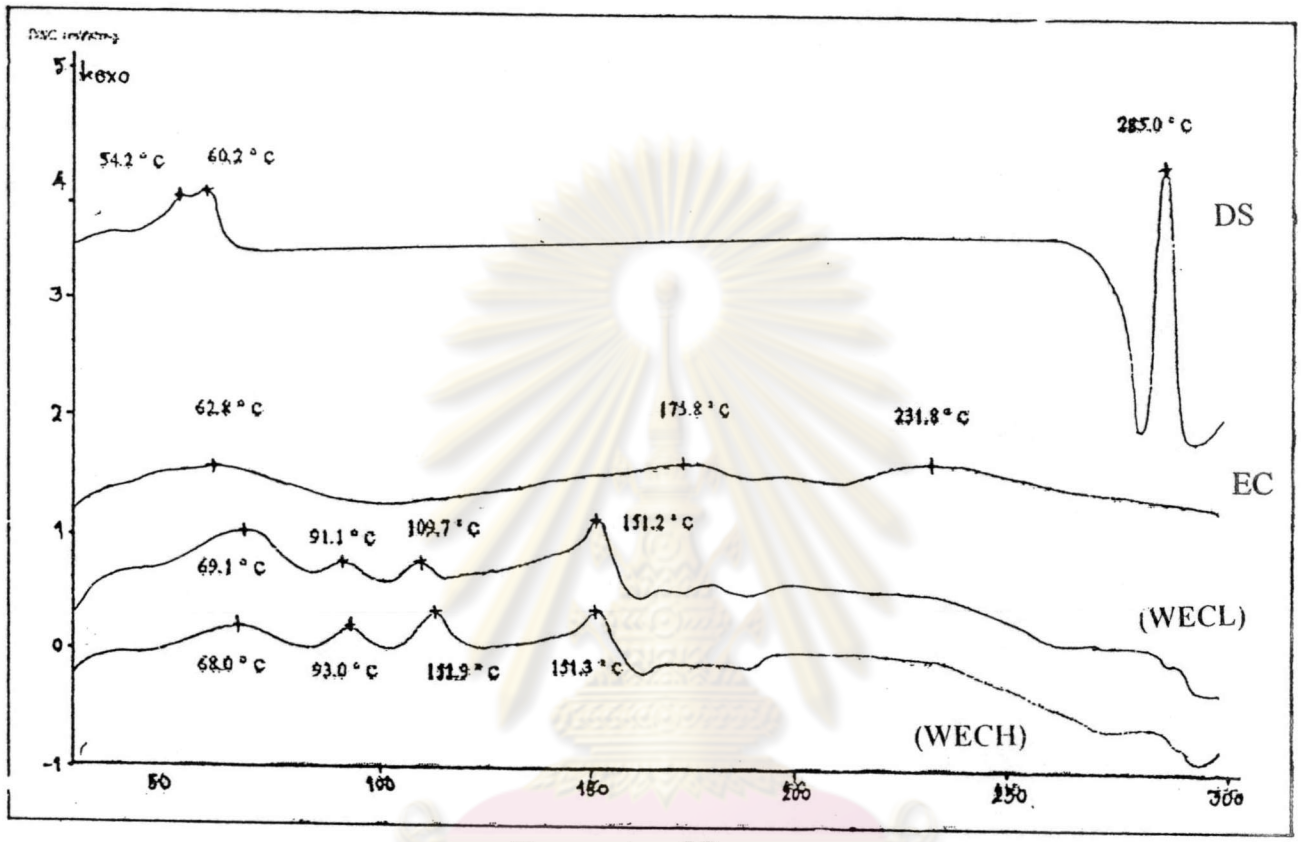


Figure 40 DSC thermograms of diclofenac sodium-ethycellulose granules in first condition

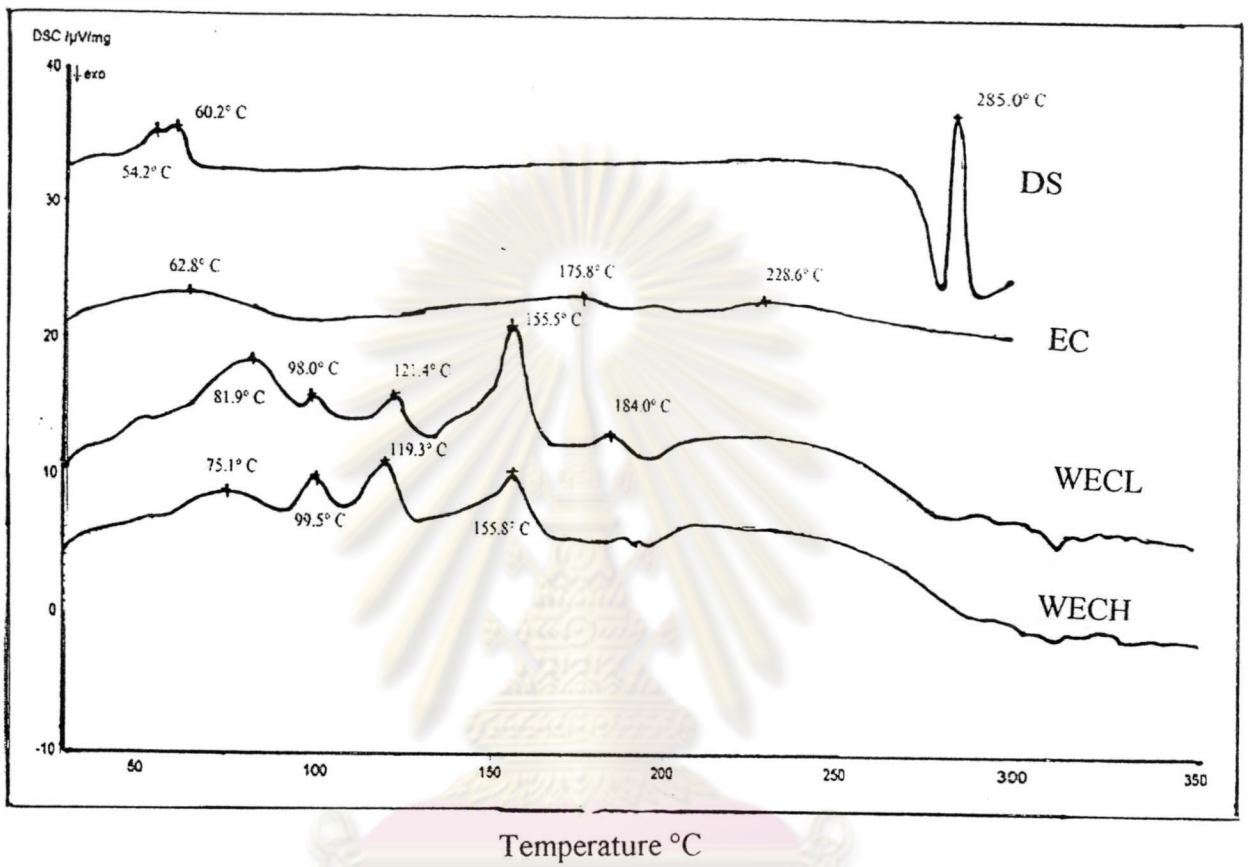


Figure 41 DSC thermograms of diclofenac sodium-ethycellulose granules in second condition

4) The evaluation of the microtablets

4.1) Hardness

The mean and standard deviation of hardness of microtablet are displayed in Table 19. All direct compression formulations at dose of 4.2 mg per microtablet and containing high amount of both HPMC and EC could not be directly compressed. But all formulations that prepared by wet granulation method could be compressed. It was found that microtablets from formulation with EC were harder than that of HPMC.

Most formulations at dose of 3 mg per microtablet could be compressed to microtablets. It was observed that the increase in the amount of cellulose derivatives caused decrease in hardness values especially of HPMC. Moreover, the microtablets from the formulation contained EC were harder than that of HPMC in both direct compression and wet granulation method.

4.2) Friability study

The percentage friability of the formulations that could be compressed to microtablets were in a range of 0.3% – 0.5% that less than 0.6%. Therefore, the percentage friability passed the specification in official standard USP XXIV. It was observed that the formulation with HPMC had more friability than formulation with EC. All detail data are present in the Table 19.

4.3) Weight variation of microtablets

The mean and standard deviation in weight variation are displayed in Table 19 It was found that the weight variation of the formulations that could be compressed the microtablets were not different.

Table 19 Physical properties of diclofenac sodium microtablets

Formulation	Hardness (Kp) (Mean)(SD) n=10	Thickness (mm) (Mean)(SD) n=10	Friability (%)	Weight variation (mg/tab) (Mean)(SD) n=10
DLHC	UNCOMPRESSIBLE			
DLEC	UNCOMPRESSIBLE			
DSHC	UNCOMPRESSIBLE			
DSEC	UNCOMPRESSIBLE			
WGHCL	2.18 (0.13)	3.50 (0.11)	0.446	10.4 (0.04)
WGHCH	2.06 (0.16)	3.48 (0.15)	0.507	10.3 (0.02)
WGEC	2.44 (0.11)	3.50 (0.11)	0.347	10.2 (0.01)
DHC	1.82 (0.27)	3.49 (0.16)	0.528	10.5 (0.04)
DEC	2.18 (0.20)	3.50 (0.20)	0.369	10.3 (0.01)
WHCL	2.18 (0.12)	3.50 (0.13)	0.499	10.5 (0.02)
WHCH	2.02 (0.30)	3.42 (0.28)	0.503	10.4 (0.03)
WECL	2.31 (0.10)	3.50 (0.12)	0.409	10.3 (0.01)
WECH	2.30 (0.11)	3.50 (0.11)	0.389	10.4 (0.01)

5) Drug content of diclofenac sodium miocrotablets

The percentages of drug contents of the DS microtablets prepared from various formulations are shown in Table 20. All direct compression formulations at dose 4.2 mg were not examined because they could not be compressed to microtablet. But all wet granulation formulations could be compressed. It was observed that the percentage of drug contents of these formulation did not pass the specification in official standard USP XXIV that the percentage drug content for delay – release DS tablet was not less than 90.0 percent and not more than 110.0 percent.

All preparation of the formulations that had 3 mg per microtablet passed the specification in official standard USP XXIV.

Table 20 The percentage drug content of diclofenac sodium microtablets

Formulation	% Drug Content	% Coefficient Variation(%CV) (n=5)
DLHC	UNCOMPRESSIBLE	UNCOMPRESSIBLE
DLEC	UNCOMPRESSIBLE	UNCOMPRESSIBLE
DSHC	UNCOMPRESSIBLE	UNCOMPRESSIBLE
DSEC	UNCOMPRESSIBLE	UNCOMPRESSIBLE
WGHCL	114.71 %	4.19 %
WGHCH	82.21 %	4.81 %
WGEC	141.17 %	3.66 %
DHC	99.26 %	4.06 %
DEC	109.12 %	3.79 %
WHCL	104.38 %	0.02 %
WHCH	97.89 %	0.01 %
WECL	106.96 %	0.01%
WECH	92.39 %	0.01 %

* The percentage drug content did not pass the specification of official standard for USPXXIV

6) The content uniformity of diclofenac sodium microtablets

The formulation, which passed the specification of drug content must be examined the uniformity of content. The content uniformity was determined by the percentages of coefficient variation (%CV) that are shown in Table 20. The percentage coefficient variation were less than 3%, it would be passed the specification official standard USP XXIV. The percentages of coefficient variation of direct compression formulations were more than 3 percent that indicates non-uniformity of content. But all wet granulation formulations had

the percentage of coefficient variation less than 3 percent that indicated uniformity of content.

7) Morphology of diclofenac sodium microtablets

The observation of size, shape, the surface and cross – sectioned morphology was done by scanning electron microscopy. The scanning electron photomicrographs of the microtablets prepared from various amounts of the cellulose derivatives are shown in Figures 42 - 43.

7.1) The microtablets prepared with hydroxypropylmethylcellulose (HPMC)

The photomicrographs of DS microtablets produced at various amount of HPMC (Formulation WHCL and WHCH) are shown in Figures 42A and 42D at low magnifications. The microtablets of both formulations that contained with HPMC had regular shape with smooth surface. The microscopic images of surface morphology in high magnifications are shown in Figures 42B and 42E. It was observed that both of the formulations with HPMC had rough surface and had plenty fine particles spreaded to the surface of the microtablet. Comparison to the lower amount of HPMC, it appeared that the microtablet with higher amount demonstrated rougher surface and more fine particle spreaded to the surface. Moreover, the cross–sectioned morphology of microtablet demonstrated that microtablet contained with higher HPMC content exhibited less porous and more condensed than that of microtablet contained with lower HPMC content as shown in Figures 42C and 42F.

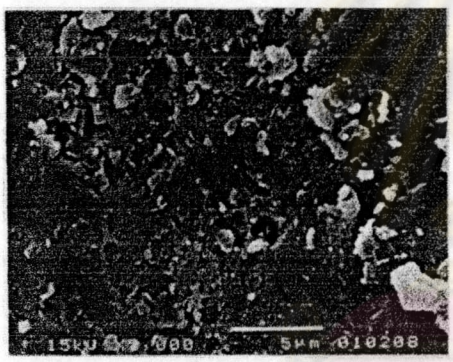
7.2) The microtablets prepared with ethylcellulose (EC)

The photomicrographs of DS microtablets produced with various amounts of EC (Formulation WECL and WECH) are displayed in Figures 43A and 43D in low magnifications. The surface of microtablets that prepared with lower EC content (Formulation WECL) had smoother than that of microtablets prepared with higher EC content (Formulation WECH). The microscopic images of surface morphology in high magnifications are shown in Figures 43B and 43E. It was observed that both of the formulations with EC had rough surface with aggregated particles that spreaded on the

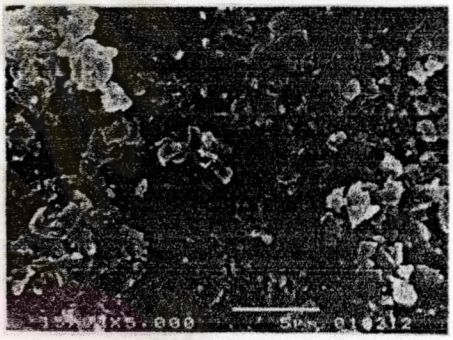
surface of the microtablet. Comparison to the lower amount of EC, it appeared that the microtablet with higher amount of EC demonstrated rougher surface characteristics and more aggregated particles spreaded to the surface. Moreover, the cross–sectioned morphology of microtablet was demonstrated that the microtablet contained with higher EC content exhibited less porous and more condensed than that of microtablet contained with lower EC content as shown in Figures 43C and 43F.

(A)

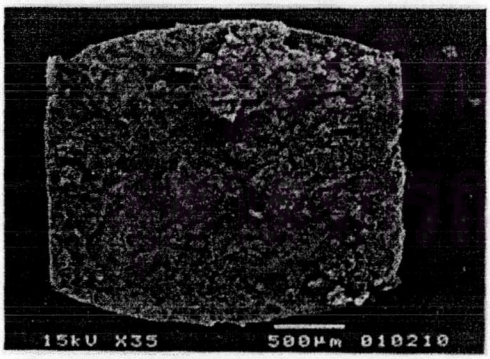
(D)



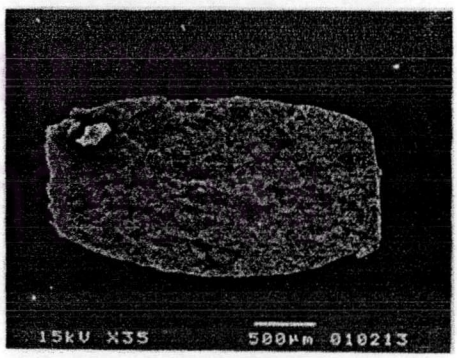
(B)



(E)

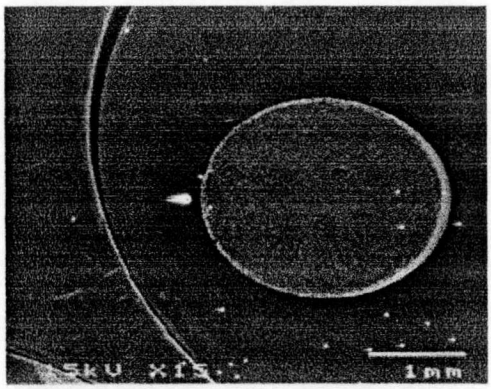


(C)

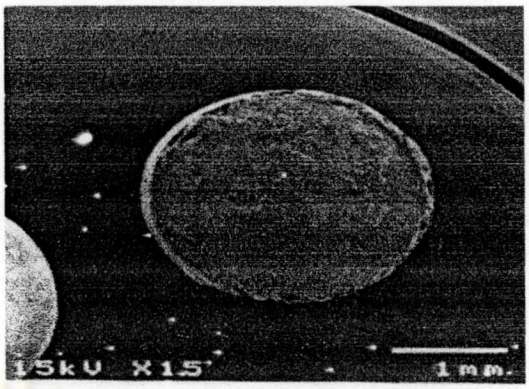


(F)

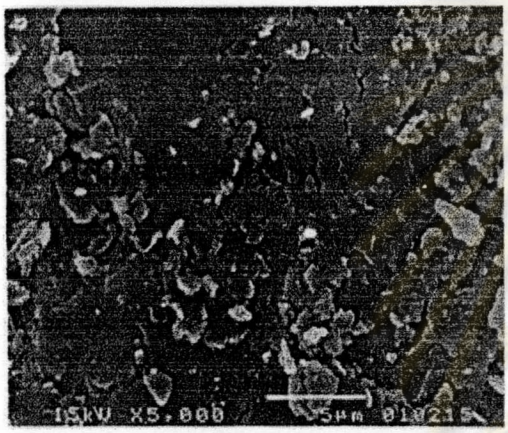
Figure 42 Scanning electron photomicrograph of diclofenac sodium microtablet (Formulation WHCL and WHCH) (A, D : microtablet × 15; B, E : the surface of microtablet × 5,000; C, F : cross-section × 35)



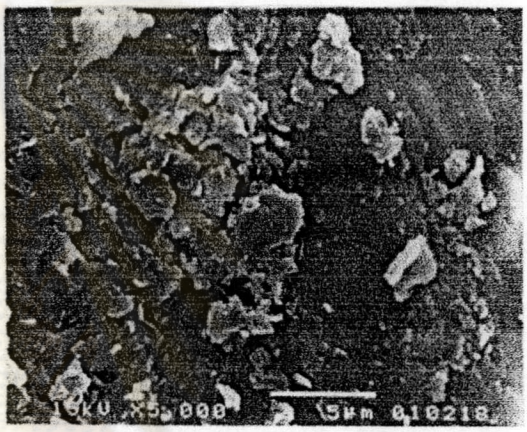
(A)



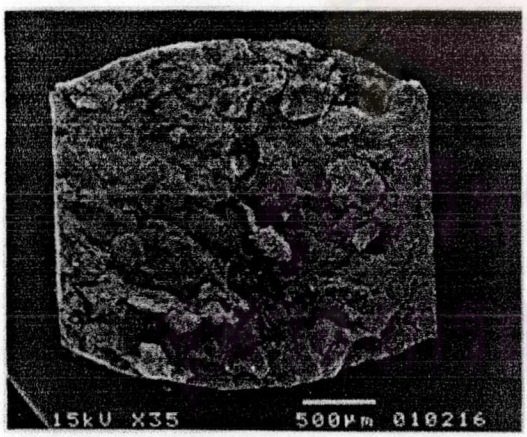
(D)



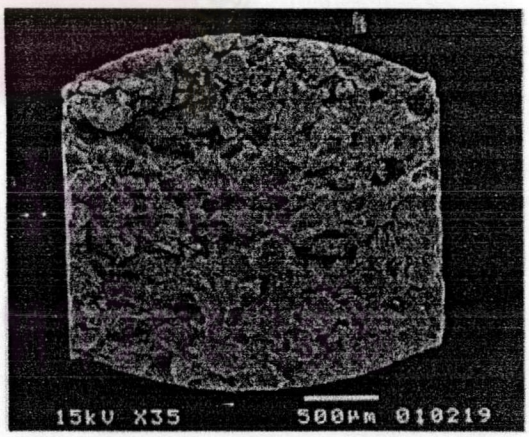
(B)



(E)



(C)



(F)

Figure 44 Scanning electron photomicrograph of diclofenac sodium microtablet (Formulation WECL and WECH) (A, D : microtablet × 15; B, E : the surface of microtablet × 5,000; C, F : cross – section × 35)

8) Specific surface area diclofenac sodium microtablets

The surface area and the total pore volume of DS microtablets were measured by BET method. The specific surface area and the total pore volume of DS microtablet contained with various amount of the cellulose derivatives are displayed in Table 21. Increasing the proportion of polymer in all formulations increased specific surface area but decreased total pore volume. It could be seen that the amount of cellulose derivatives might affect the specific surface area and total pore volume of DS microtablet.

The microtablets from the formulations that contained with HPMC exhibited higher specific surface area and total pore volume than those of the formulation contained with EC at the same quantity.

Table 21 Specific surface area and pore volume of diclofenac sodium microtablets.

Formulation	Content of cellulose derivatives	Specific Surface Area ($m^2/g \pm SD.$)	Total Pore Volume (cm^3/g)
WHCL	Low content of HPMC	1.31 ± 0.02	0.76
WHCH	High content of HPMC	1.43 ± 0.05	0.40
WECL	Low content of EC	1.27 ± 0.02	0.43
WECH	High content of EC	1.44 ± 0.01	0.29

9) Infrared spectrometry

The IR spectra of DS microtablets with HPMC and DS microtablets with EC at various amounts of the both cellulose derivatives that prepared by wet granulation method are illustrated in Figures 44 and 45, respectively. It was found that the concentration of both HPMC and EC were increased, the prominent peaks of spectra of diclofenac sodium did not shift but the peak intensity was slightly weaker. These revealed that no interaction between drug and both cellulose derivatives occurred

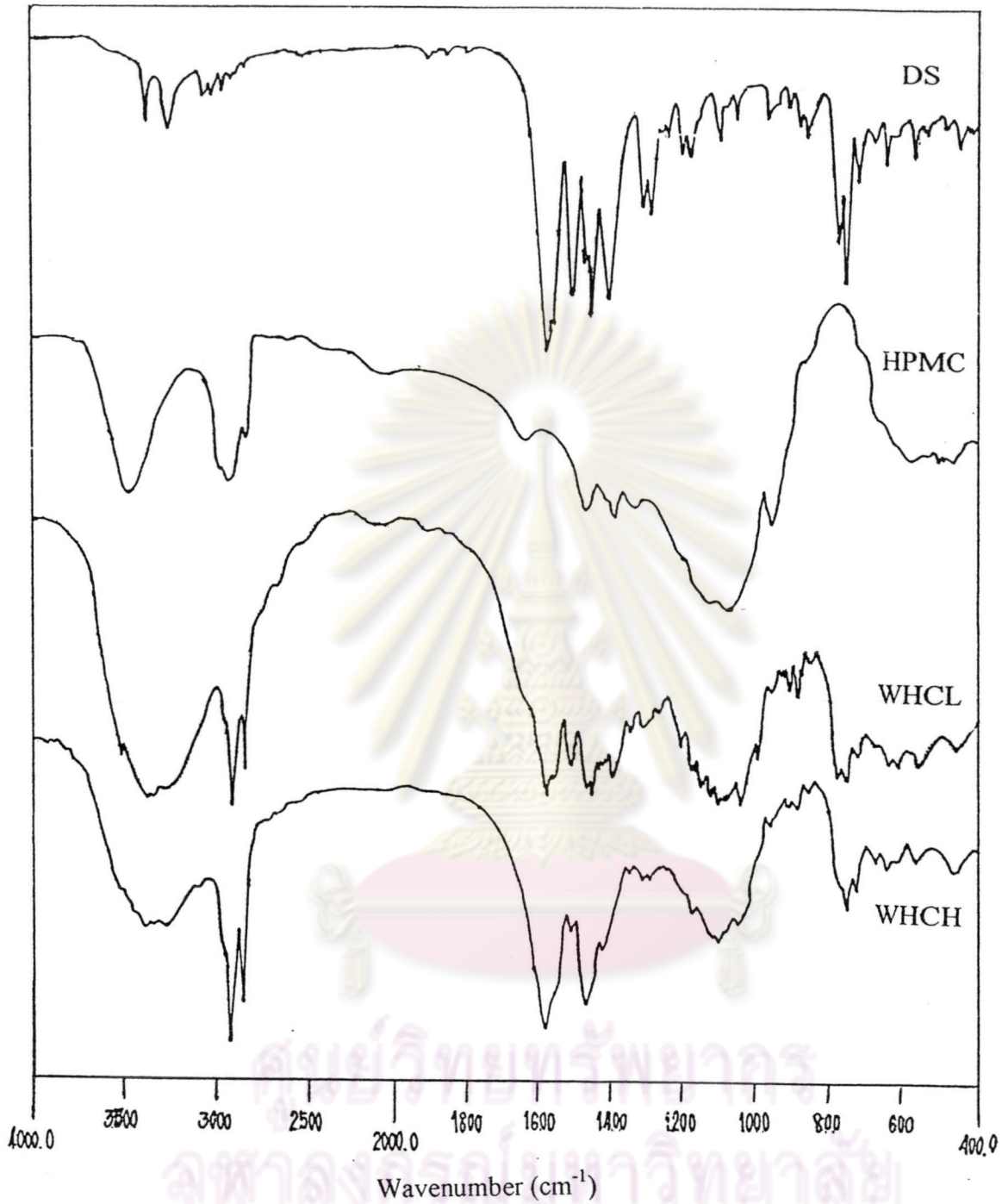


Figure 44 IR spectra of diclofenac sodium-hydroxypropylmethycellulose microtablets

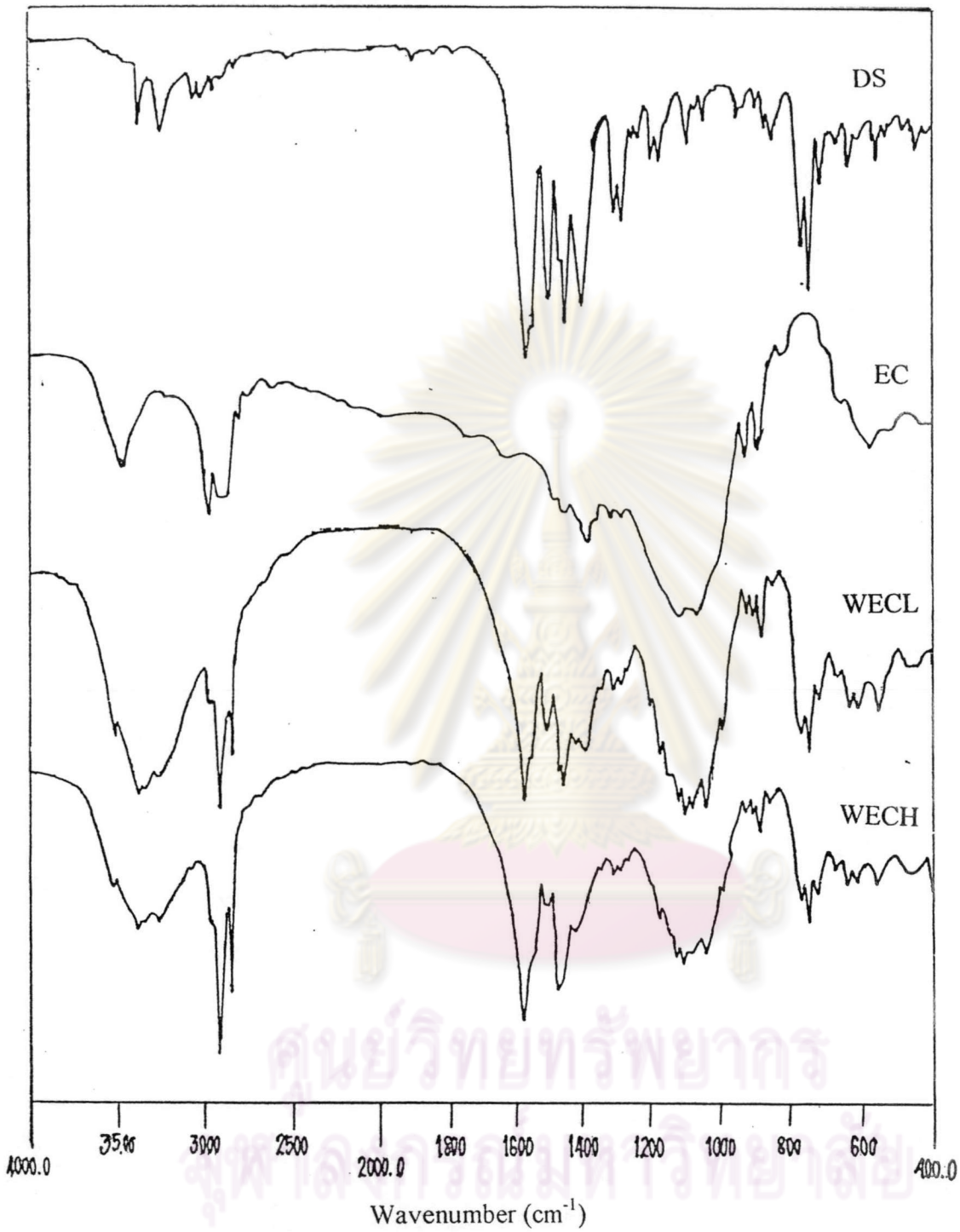


Figure 45 IR spectra of diclofenac sodium-ethycellulose microtablets

Table 22 IR peak of spectra of diclofenac sodium and of diclofenac sodium granule.

Formulation	Principle Peak (cm^{-1})					
DS	747	767	1284	1306	1506	1575
WHCL	749	774	1282	1306	1508	1577
WHCH	747	767	1283	1305	1506	1579
WECL	747	769	1282	1306	1508	1577
WECH	746	767	1282	1305	1508	1577

10) X – Ray diffraction

The X - ray diffraction patterns of diclofenac sodium and diclofenac sodium microtablets with types and various amount of the cellulose derivatives are illustrated in Figures 46-47.

The X – ray diffraction pattern of DS microtablets with various amounts of HPMC are displayed in Figure 46. It was observed that both of microtablets that contained with low and high content of HPMC (Formulation WHCL, WHCH) exhibited different pattern from DS. Their pattern showed absence of some eminent peaks. In contrast, they had new peaks in the pattern. All detail data of the X-ray diffraction peak are shown in Table 23. Moreover, remarkably fewer intense peaks, and a slightly higher baseline was detected. It is indicated that their microtablets were still crystalline from but the characteristics of crystal may be changed and some crystals may be converted to an amorphous form. .

The X – ray diffraction pattern of DS microtablets with various amount of EC are displayed in Figure 47. It was found that the both formulations that contained with both low and high content of EC (Formulation WECL and WECH) compared with pure DS that showed different peak and intensities. Some eminent diffraction peaks of DS crystals in their microtablets were absented. And the new peaks were occurred in the pattern. Moreover, the peak intensities were reduced and base was slightly high. All data that compared the X-ray diffraction peak of microtablets are shown in Table 23. Therefore, the

almost of diclofenac sodium was still crystalline form but the characteristics of crystal may be changed and some crystals may be changed to amorphous form.

Table 23 The peak of X-ray diffraction of diclofenac sodium, additives, and microtablets.

Peak NO.	Diclofenac sodium (°)	Magnesium stearate(°)	Lactose* (°)	WHCL (°)	WHCH (°)	WECL (°)	WECH (°)
1	6.76	5.44	6.81	5.48	5.56	5.61	5.56
2	8.64	7.28	9.97	6.79	6.68	6.54	6.62
3	11.36	8.92	10.42	8.42	8.56	8.63	8.49
4	12.76	9.32	12.39	12.48	12.56	12.52	12.48
5	15.32	14.24	13.19	16.40	15.28	16.40	16.40
6	16.24	14.92	15.50	19.12	19.12	19.12	19.16
7	17.28	16.08	16.30	19.52	19.60	19.56	19.60
8	17.92	16.92	17.55	19.98	20.04	20.04	20.00
9	20.00	17.84	18.97	20.80	21.32	21.28	20.88
10	23.64	18.84	19.43	21.28	21.88	21.88	22.64
11	23.96	19.64	19.97	21.84	22.68	23.80	2352
12	25.16	21.32	20.46	23.76	23.52	25.60	25.64
13	26.04	21.92	20.86	25.60	25.71	26.16	26.12
14	27.24	22.56	21.49	26.00	27.01	37.60	37.56
15	28.04	23.56	22.23	37.60	37.41		43.12
16		25.36	22.71				
17		30.12	23.52				
18		37.80	23.88				
19		39.00	24.52				
20		40.04	24.84				
21		42.00	25.42				
22		43.50	26.64				
23		45.96	27.38				
24			27.93				
25			28.96				
26			30.10				
27			30.75				
28			31.35				
29			31.62				

* Referrence from Analytical profiles of drug substances (Harry G.B. et al., 1990)

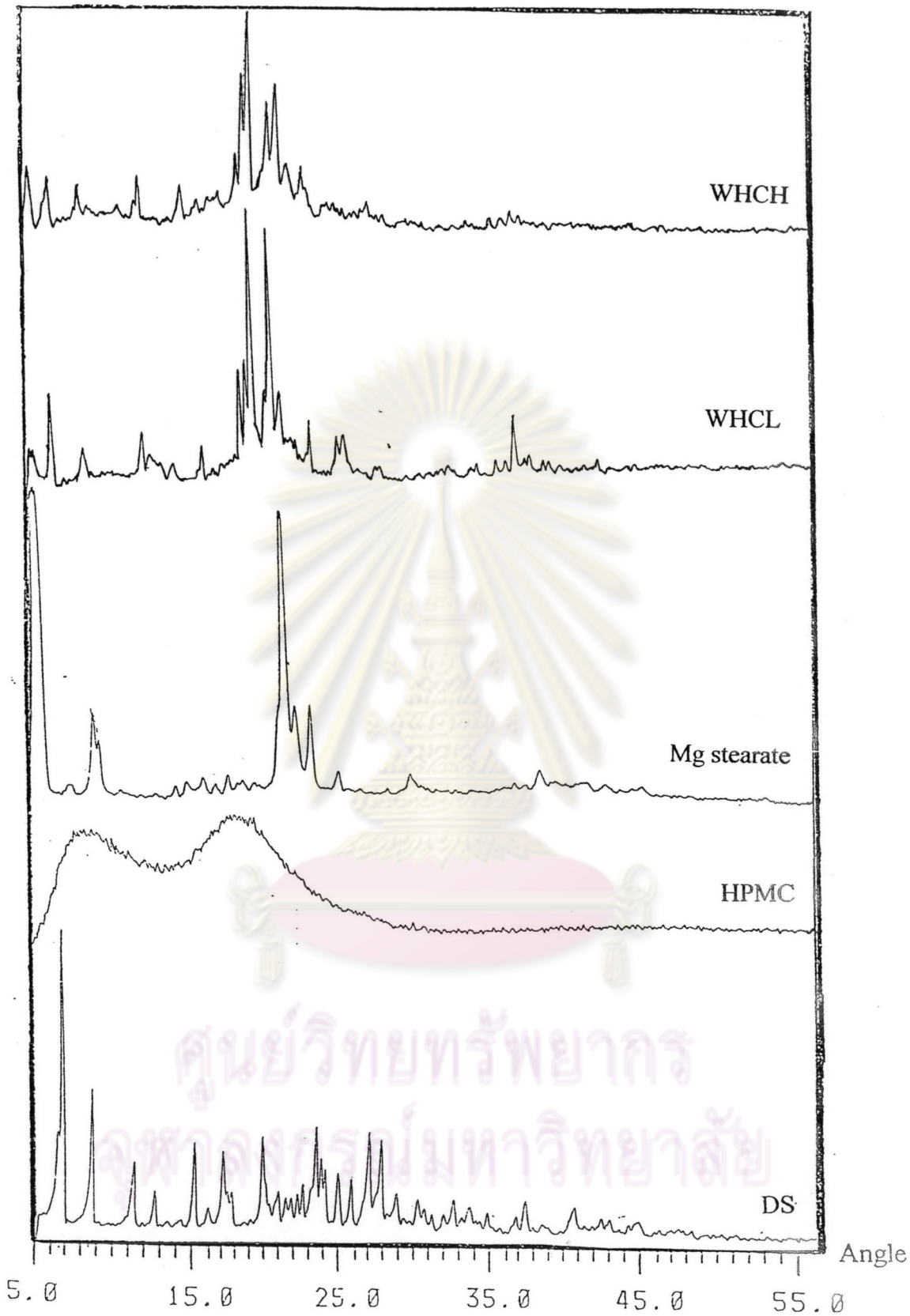


Figure 46 X-ray diffraction spectra of diclofenac sodium-hydroxypropylmethycellulose microtablets

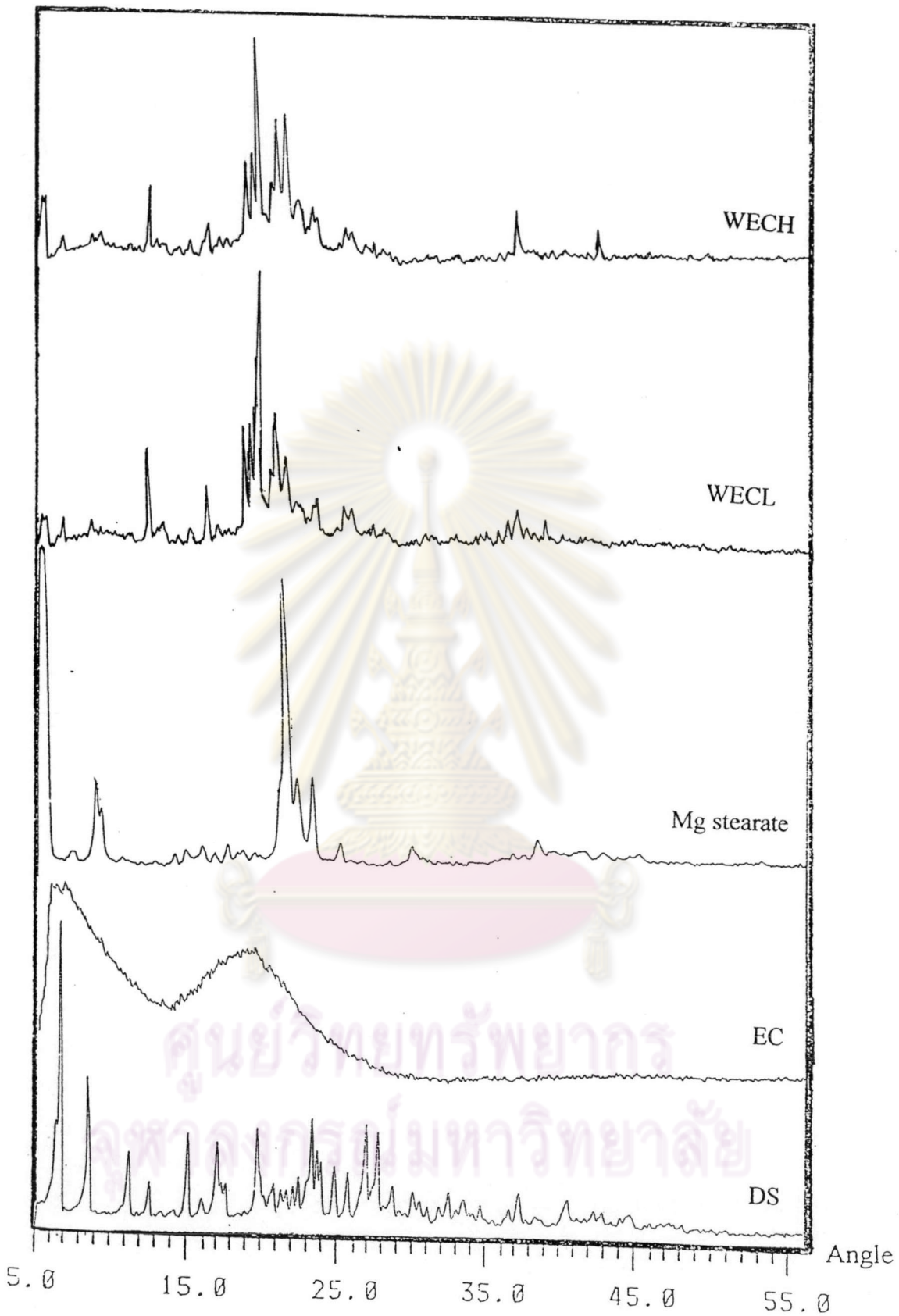


Figure 47 X-ray diffraction spectra of diclofenac sodium-ethycellulose microtablets

11) Differential scanning calorimetry (DSC)

The DSC thermograms of DS and of DS microtablets with various amounts of the cellulose derivatives are shown in Figures 48 - 51. The thermogram of pure diclofenac sodium exhibited the characteristic melting endotherm at 285°C and the peak of 54.2^{0}C, 60.2^{0}C exhibited vapourization of moisture content into diclofenac sodium powders (Fini et al., 1999). The DSC peak temperatures of diciofenac sodium microtablets contained with both HPMC and EC were disappeared in duplicate determinations. But it could detect the heap of endothermic peaks at 100° –150° in all formulations.

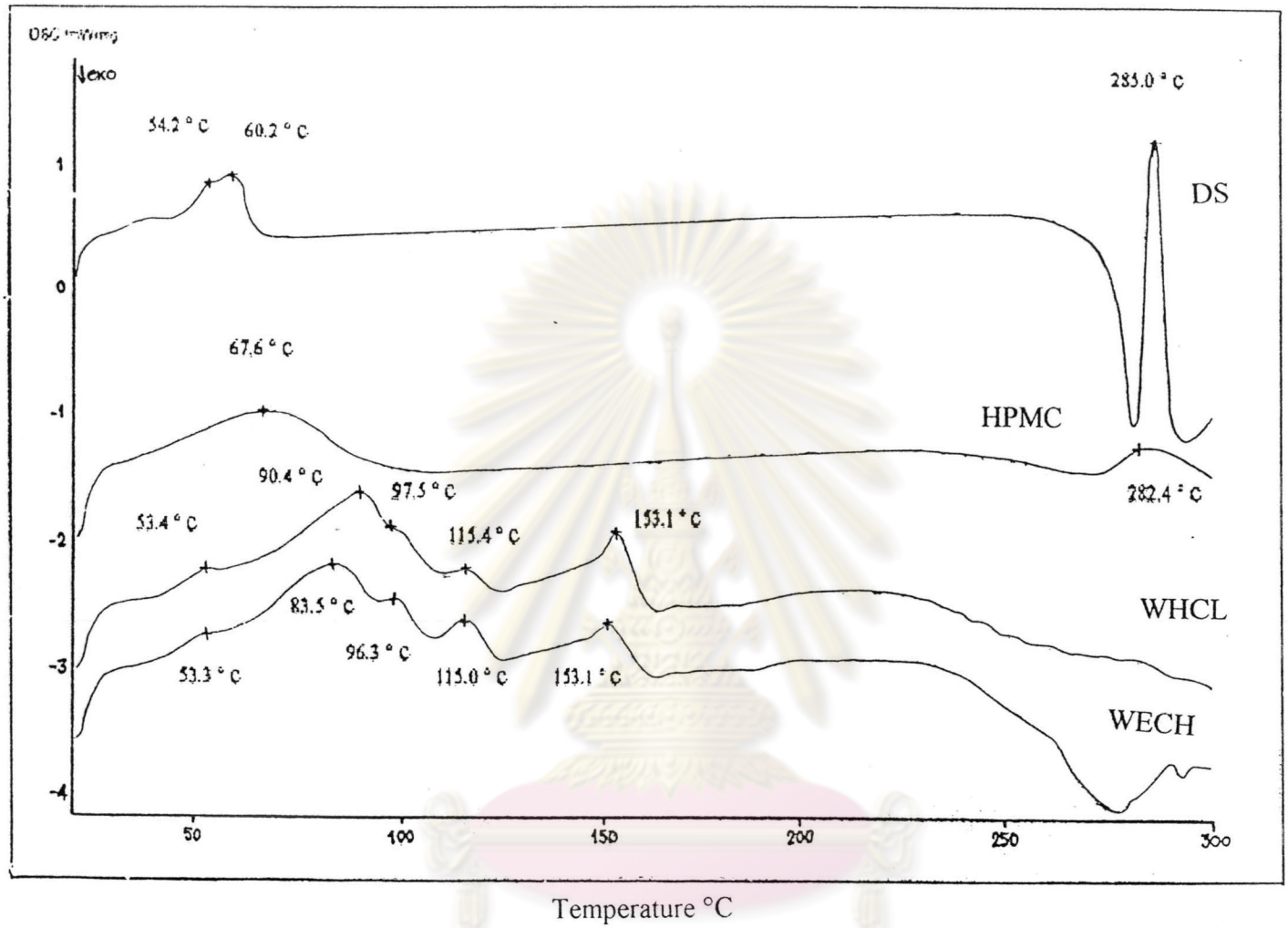


Figure 48 DSC thermograms of diclofenac sodium-hydroxypropylmethycellulose microtablets in first condition

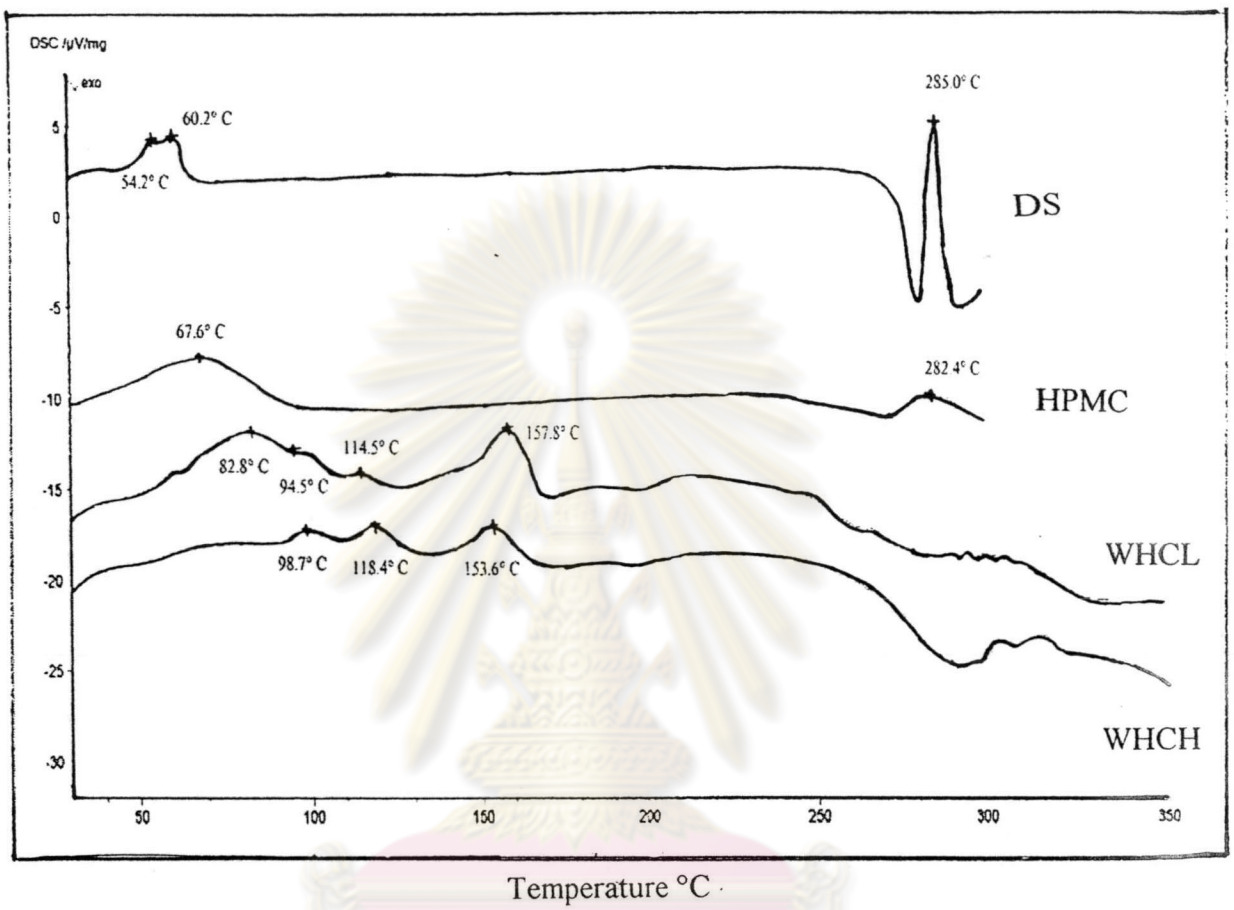


Figure 49 DSC thermograms of diclofenac sodium-hydroxypropylmethycellulose microtablets in second condition

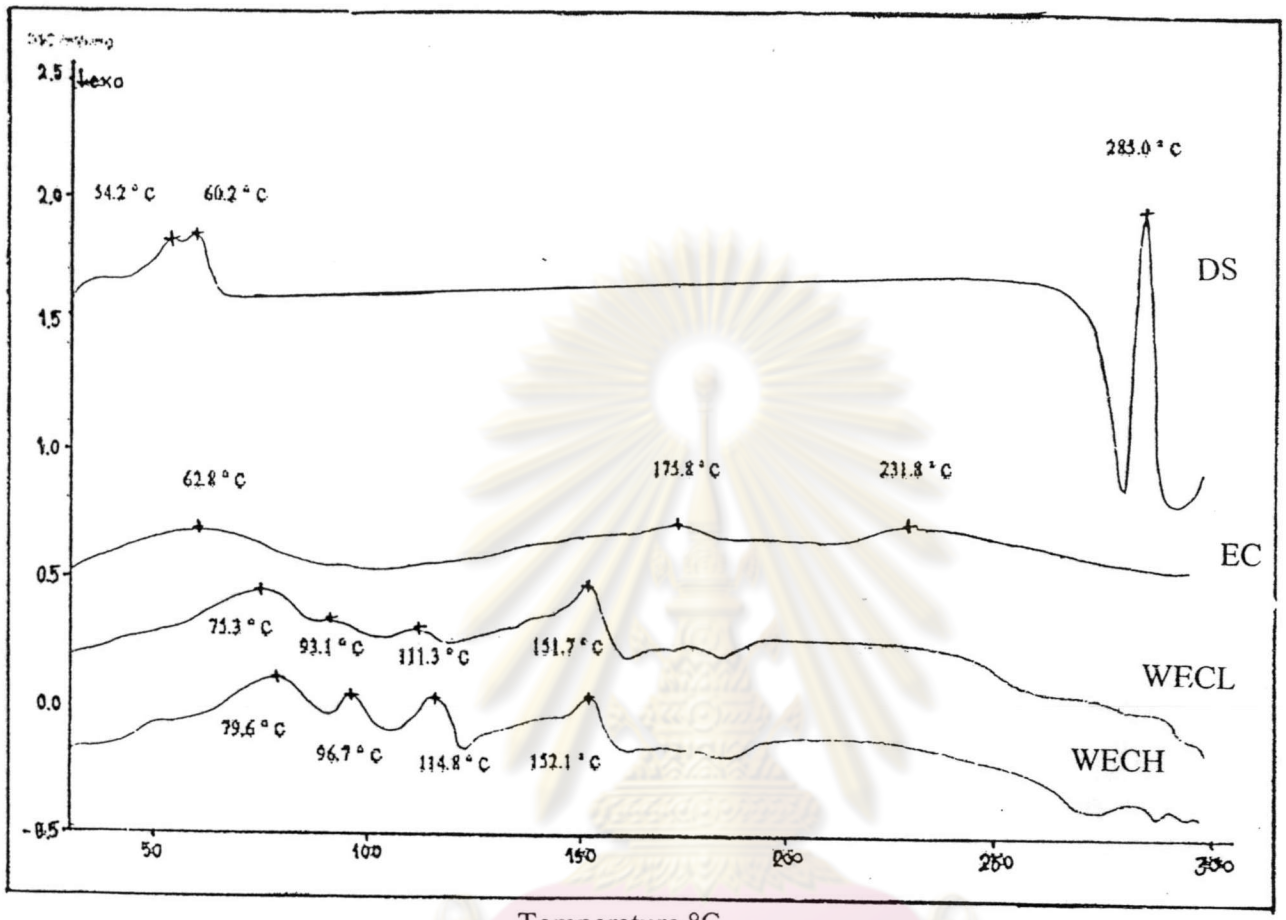


Figure 50 DSC thermograms of diclofenac sodium-ethycellulose microtablets in first condition

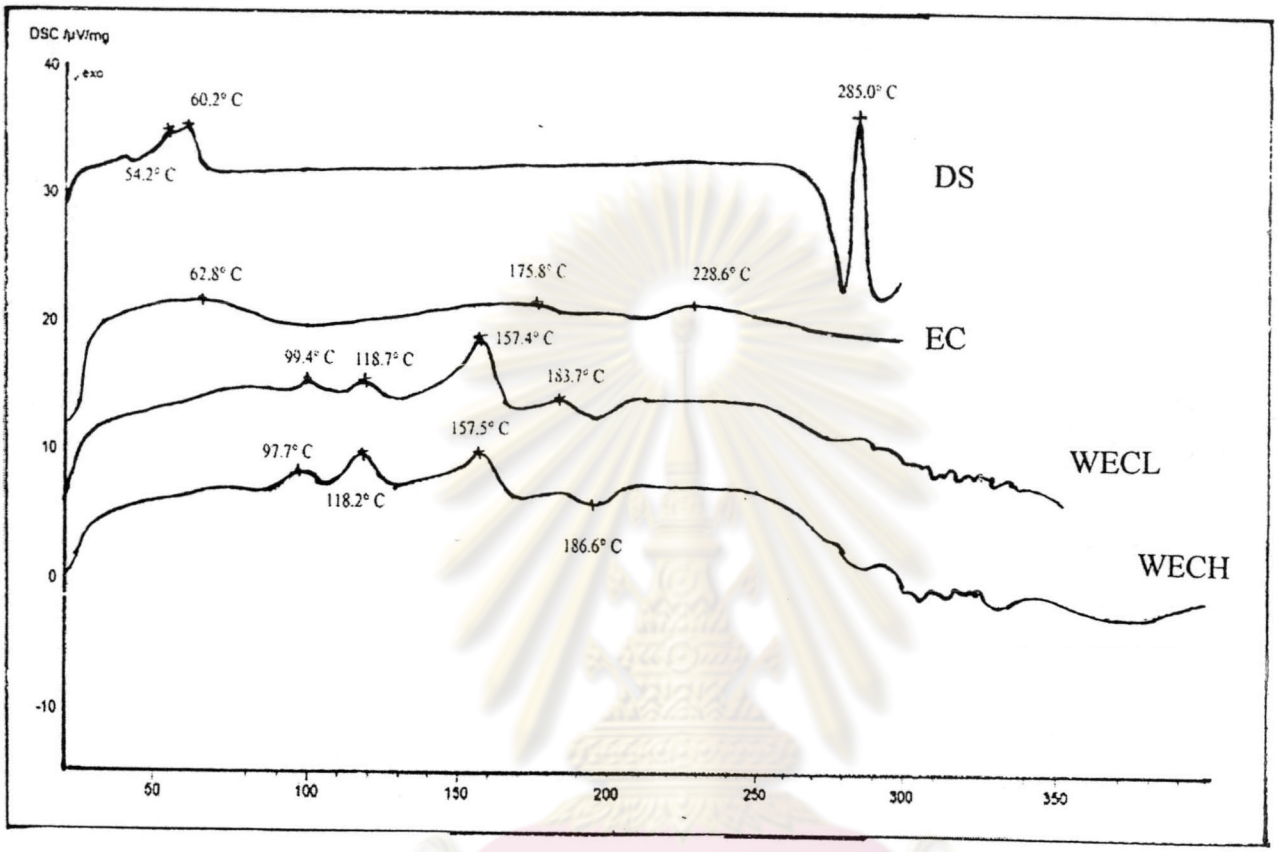


Figure 51 DSC thermograms of diclofenac sodium-ethycellulose microtablets in second condition

12) Dissolution Study

12.1) Dissolution profiles and release rate profiles

From the experimental data, the dissolution or the release profiles could be plotted between the amount of drug release against time. Then, the change of release rate profiles was constructed from the dissolution profiles to elucidate the release rate at various time intervals during the course of drug dissolution from the microtablets. The dissolution and release rate data of each formulation are described in Table 35 – 43 (Appendix C).

The release rate was calculated by dividing the difference of percent drug release at various time interval with the time utilized to release that certain amount of the drug (see data in Table 41 – 43 (Appendix C). The rate, then was plotted with the midpoint of the time interval. It was shown that the rate of release decreased with the time.

All formulations of sustained release DS microtablets that filled into the capsule were evaluated in pH-change dissolution system. In pH-change system, these capsules were tested in acid stage (0.1 N HCl, pH 1.2) for 2 hours, the percentages of drug release from all formulations were less than 3.5%. Then the pH of dissolution medium was changed to 6.8 by using phosphate buffer pH 6.8, the capsules were continuously tested until 24 hours. For phosphate buffer stage, the percentage of drug release at the first 2 hours was more than 6.5% and continuously increased until 24 hours.

12.1.1) The voltaren SR tablet

The amount of drug release at any time interval are presented in Table 35.(Appendix C). The release of diclofenac sodium from Voltaren SR tablet was affected by dissolution medium as illustrated in Figure 52. In pH – change system, the percentage of drug release from Voltaren SR tablet at the first 2 hours (acid stage) was less than 2%. Whereas, the percentage of drug release at the first 2 hours (buffer stage) was more than 3.5% and continuously increased until 24 hours in phosphate buffer pH 6.8 stage. The release rate of these tablets decreased as the time increased as shown in Figure 53. The amount of drug that released in 24 hours was 75.22%.

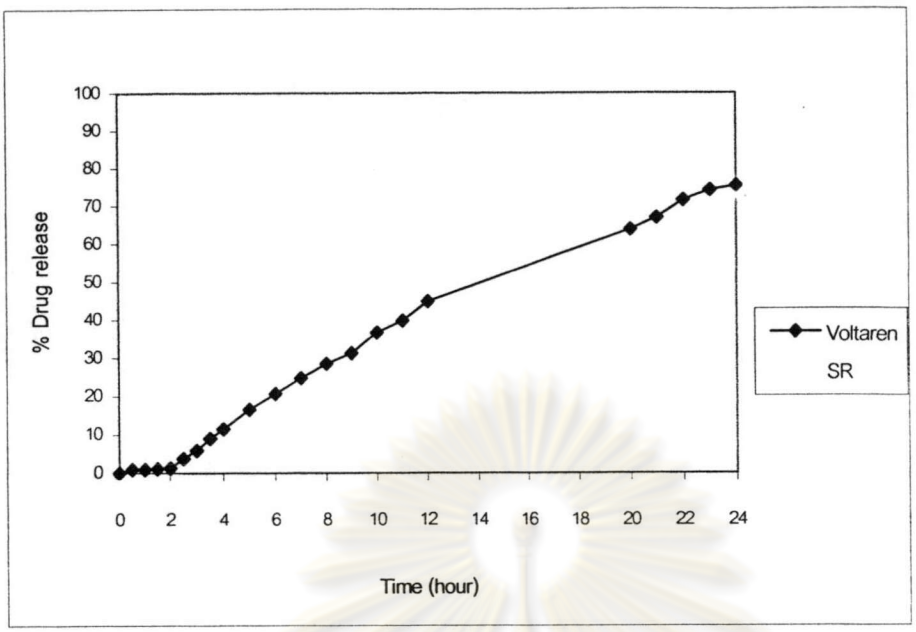


Figure 52 The release profiles of commercial product in pH change system (Voltaren SR 75 mg)

+

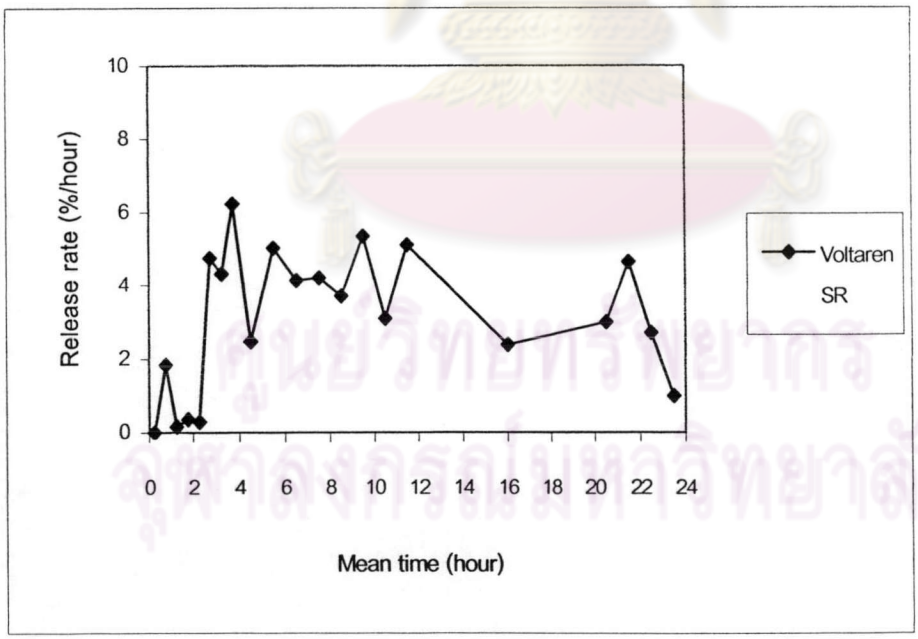


Figure 53 The release rate profiles of commercial product in pH change system (Voltaren SR 75 mg)

12.1.2) The formulation contained with various amount of hydroxypropylmethylcellulose (HPMC)

The dissolution profile of diclofenac sodium microtablets with various amount of HPMC into pH – change system are shown in Figure 54 and 55, respectively (Table 36, 37, Appendix C). Each point represents the average value obtained from three determinations at the given sampling time. The release rate was decreased with time as shown in Figure 55. The release pattern as strongly dependent of pH of the dissolution medium.

In acid stage (0.1N HCl) for 2 hours, the percentages of drug release from these formulations were less than 1%. The both formulations that contained with low and high content of HPMC gave the drug release of less than 80% of total capacity at the 24th hour. The initial gradually release of drug in the first 9 hours followed by slower release until 24 hours was observed. For this study, increasing the amount of HPMC resulted in a corresponding decrease of the dissolution rate. The percentage of drug release at 24 hours for the formulation WHCL and WHCH were 66.26% and 60.72%, respectively. Their percentage of drug release at 24 hours were less than 75% that did not passed the specification in official standard USP XXIV.

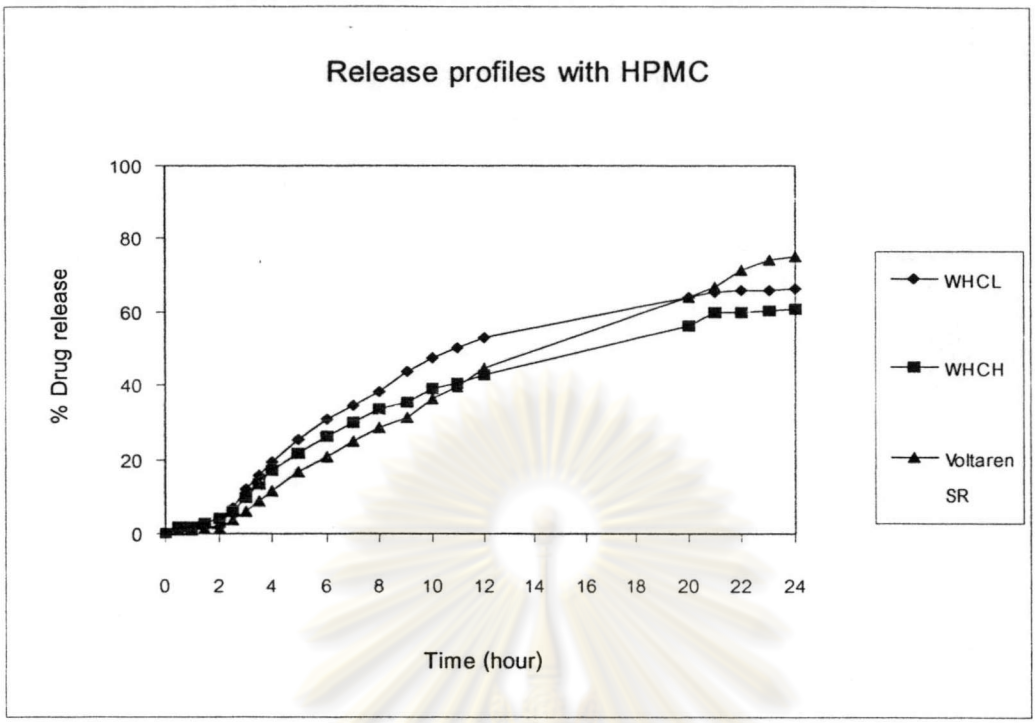


Figure 54 The release profiles of diclofenac sodium of HPMC microtablets by pH - change system

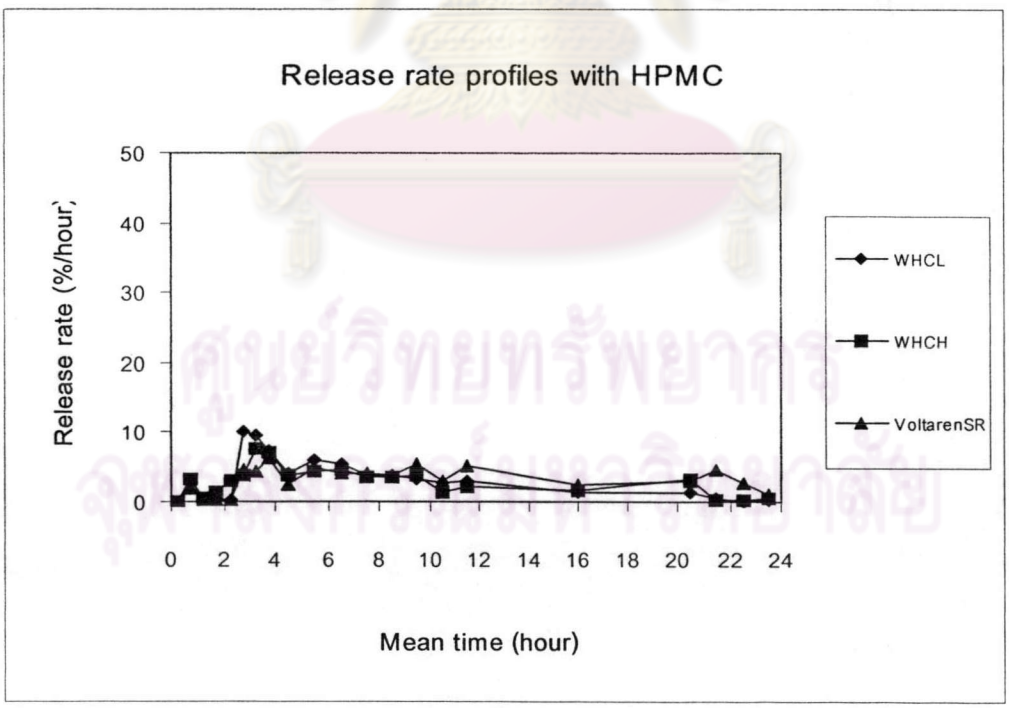


Figure 55 The release rate profiles of diclofenac sodium with HPMC microtablets by pH-change system

12.1.3) The formulation contained with various amount of ethylcellulose (EC)

The dissolution profiles of diclofenac sodium microtablets with various amount of EC into pH – change system are shown in Figure 56 and 57 (Table 38, 39, Appendix C). The release rate was decreased with time as shown in Figure 57. After the drug was release in 24 hours, the cellulose derivatives remained undissolved.

Controlled release of drug was observed in the pH – change system. A very low percentage of drugs release that less than 3.2% was remarked in the first 2 hours (acid stage, 0.1N HCl). The releases of diclofenac sodium decreased with the increasing of the amount of ethylcellulose in formulation as expected. The percentage of diclofenac sodium release at 24 hours for formulation WECL and WECH were 94.10% and 89.19%, respectively. Their percentages of drug release were more than 75% that passed the specification in official standard USP XXIV.

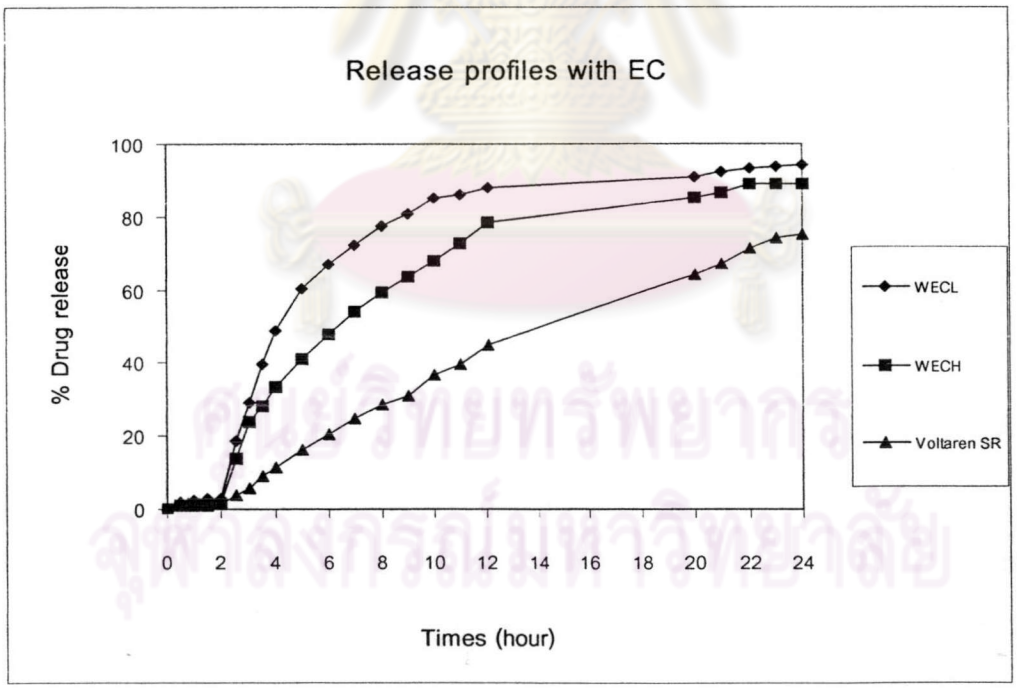


Figure 56 The release profiles of diclofenac sodium with EC microtablets in pH-change system

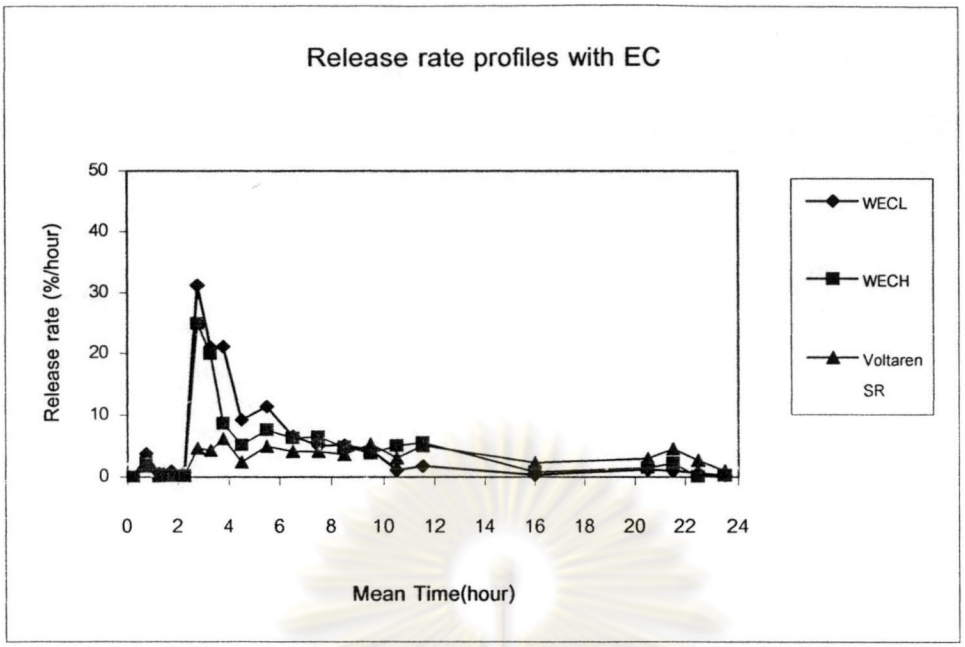


Figure 57 The release rate profiles of diclofenac sodium with EC microtablets in pH-change system

12.1.4) Comparison between the microtablets produced with both cellulose derivatives and a commercial product diclofenac sodium (Voltaren SR 75 mg).

Comparison of drug release pattern between each formulation of the microtablets and a commercial product of sustained – release DS tablet (Voltaren SR 75 mg) were examined. It was observed that the drug release profile of formulation contained with low content of HPMC (Formulation WHCL) was similar to the release profile of Voltaren SR tablet. The amount of drug that releases in 24 hours were 66.26% and 75.22% for formulation WHCL and Voltaren SR tablet, respectively. It was observed that the amount of drug release in 24 hours of formulation WHCL did not pass the specification in official standard USP XXIV. The dissolution profiles are demonstrated in Figure 58 and 59. (Table 40, Appendix C).

It is interesting that the comparison between the microtablets contained with low content of HPMC (Formulation WHCL) and Voltaren SR tablet. But the capsule size

that filled the microtablets was changed to be a bigger size (formulation WHCL1). It was found that the drug release profile of formulation WHCL1 was similar to the drug release profile of Voltaren SR tablet. The amount of drug that released in 24 hours were 75.84% and 75.22% for formulation WHCL1 and Voltaren SR tablet, respectively. The amount of drug release of both formulations passed the specification in official stadard USP XXIV. Whereas, the t-test showed statistically significant difference ($p > 0.05$) in acid stage and statistically non - significant difference in buffer stage of the release pattern (Table 47, Appendix D).

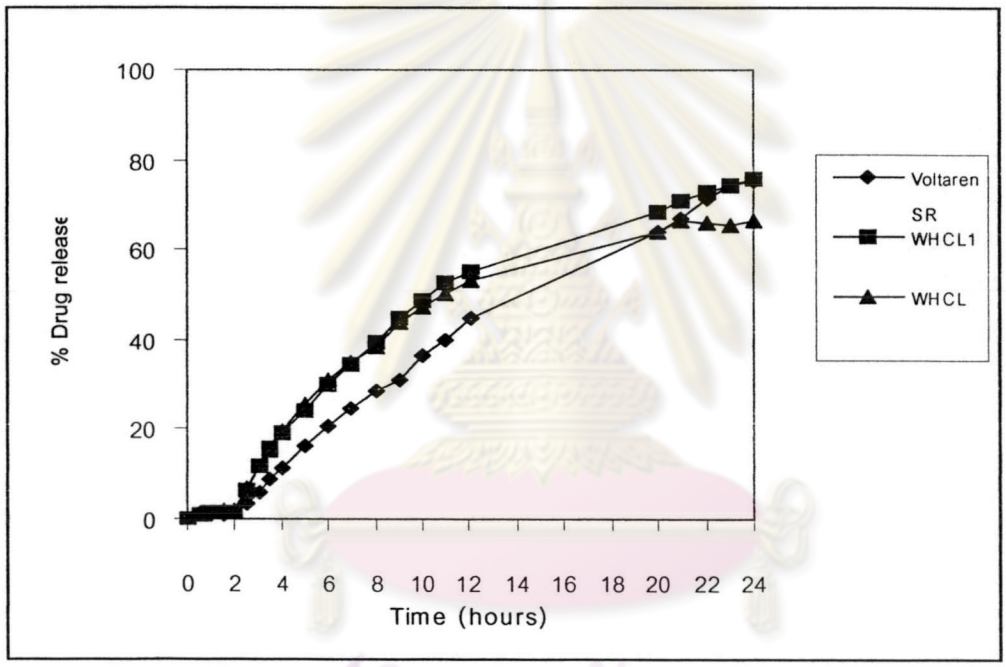


Figure 58 The release profiles of diclofenac sodium with HPMC microtablets in various capsule size by pH-change system

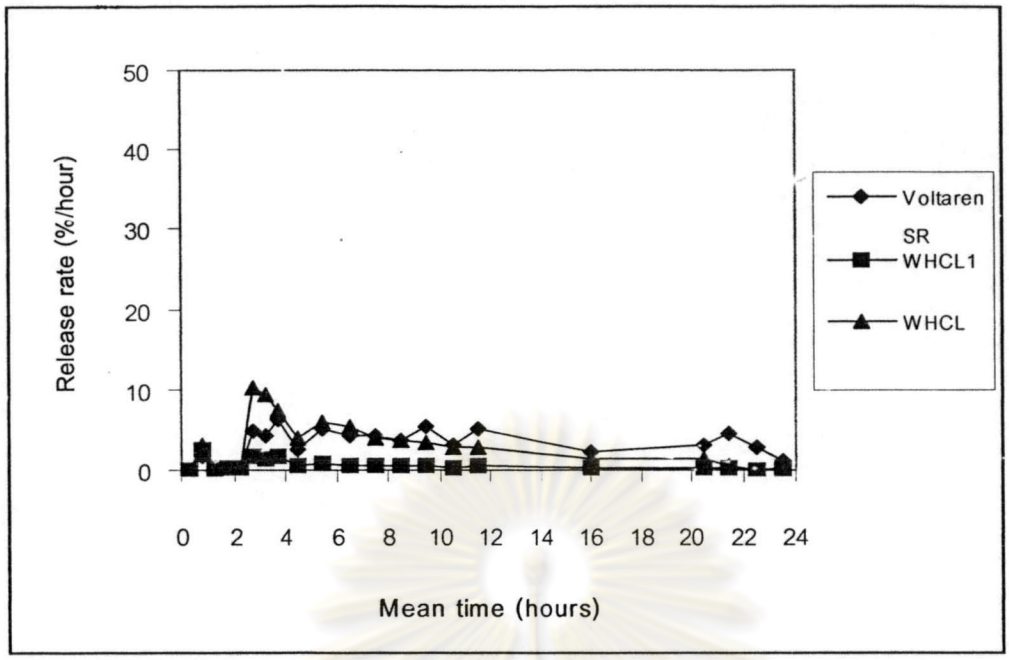


Figure 59 The release rate profiles of diclofenac sodium with HPMC microtablets in various capsule size by pH changing system

13) Elucidation of Drug Release Model

In general, the release kinetics of sustained – release preparations could be described by the use of one or more than three kinetics models comprising the zero order equation, the first order equation and the Higuchi square root equation. The analysis of all dissolution data was carried out to elucidate the most fitted model. The plot between percentage of drug released against time (zero- order), log percent of drug remained versus time (first-order) and percentage of drug released versus square root of time (Higuchi model) were, therefore, constructed and determined the one which was the most linear as the accepted model of drug release. Whenever, the determination coefficient of Higuchi and first-order relationships did not allow unambiguous distinction to made between the two kinetics. The further treatment was base upon the use of the different forms of the first – order and Higuchi equations. The plots of rate of release versus 1/Q were linear when the release was fitted with the Higuchi model. If the plots of rate of release versus Q were linear, they indicated that the first- order model was operative. The correlation coefficients of rate

of release against reciprocal amount (1/Q) and amount (Q) of diclofenac sodium release from the microtablets are shown in Table 24.

Table 24 Comparison of linearity between plots of rate of release against reciprocal amount (1/Q) and amount (Q) of diclofenac sodium released from the microtablets in pH – change system.

Formulation	Correlation coefficient of rate dQ/dt	
	Versus Q	Versus 1/Q
Voltaren SR	0.1319	0.1070
WHCL	0.1089	0.0350
WHCH	0.1046	0.0001
WECL	0.0775	0.0303
WECH	0.3824	0.0130
WHCL1	0.4013	0.0141

Table 25 Correlation coefficient ($r^{2)}$ of the relationships between percentage drug released versus time (A), log percentage drug remained versus time (B), and percentage drug released versus square root time (C).

Formulation	Dissolution Study		
	pH Change Method		
	A	B	C
Voltaren	0.9687	0.9890	0.9468
WHCL	0.8994	0.9629	0.9540
WHCH	0.9375	0.9824	0.9696
WECL	0.7054	0.9195	0.8533
WECH	0.8391	0.9757	0.9330
WHCL1	0.9320	0.9900	0.9619

13.1) The Voltaren SR Tablet

The highest correlation coefficient of Voltaren SR 75 mg tablet was 0.9890 obtained from the first-order plot. And, the correlation coefficients of rate of release versus Q were higher than those of rate versus 1/Q as shown in Table 24. The first –order model would possibly be operative.

13.2) The formulation contained various amounts of hydrohydroxypropylmethylcellulose

These formulations showed similar release in pH-change system. The highest correlation coefficient values of formulation WHCL, WHCH, and WHCL1 were 0.9629, 0.9824, and 0.9900, respectively. The result indicated that the release data might have followed the first –order model. And, the correlation coefficient of rate of release versus Q were higher than the correlation coefficient of rate of release versus 1/Q. In conclusion, the formulation WHCL, WHCH, and WHCL1 might have followed the first-order model.

13.3) The formulation contained various amount of ethylcellulose

All these formulations gave similar release model in pH-change system. The highest correlation coefficient values of formulation WECL and WECH were 0.9195 and 0.9757, respectively, obtained from the first - order plots. For the correlation coefficient of release rate of the first – order plot was higher than other models. Therefore, the release profiles of both formulations might follow the first – order model.