CHAPTER III

RESULTS

Physicochemical properties

Membranes characteristics

Crosslinked chitosan - polyvinyl alcohol membranes

The characteristics of membrane prepared from casting solution technique were yellow, transparent, glossy, integrity, easy to prepare and peel off from the glass petri dish. All obtained membrane formulations resulted in flexible membranes. Therefore the using of plasticizer was not necessary. Some characteristics of crosslinked chitosan - PVA membranes were shown in Tables 11 to 13.

Higher amount of chitosan produced dark yellowish membrane. Increasing the concentration of crosslinking agent led to yellower membrane especially in the high ratio of chitosan formulations. All obtained membranes of 1 : 4 and 1 : 9 crosslinked chitosan : PVA were clear, glossy and yellowish.

With the same molecular weight of PVA, high ratio of PVA tended to produce flexible membrane which was easy to peel off from the glass petiri dish. Moreover, increasing the degree of crosslinking caused

Table 11. Some Characteristics of Crosslinked Chitosan - PVA 30,000 Membranes

Formulations	yellow color	transparency	glossy	flexibility	integrity	easy to peel off
CPL_4	+++	++++	+	++	+	++
CPL_5	++++	++++	+	+	+	++
CPL_9	+++	++++	+	++	+	++
CPL_{10}	++++	++++	+	+	+	++
CPL_{14}	++	++++	+	++	+	++
CPL_{15}	+++	++++	+	+	+	++
CPL_{19}	++	++++	+	++	+	++
CPL_{20}	+++	++++	+	++	+	++
CPL_{24}	++	++++	+	++	+	+++
CPL_{25}	+++	++++	+	++	+	+++
CPL_{29}	++	++++	+	+++	+	+++
CPL_{30}	++	++++	+	+++	+	+++
CPL_{34}	++	++++	+	+++	+	+++
CPL_{35}	++	++++	+	+++	+	+++
CPL_{39}	+	++++	+	++++	+	+++
CPL_{40}	+	++++	+	++++	+	+++
CPL_{44}	+	++++	+	++++	+	+++
CPL_{45}	+	++++	+	++++	+	+++

The symbols of (+) and (-) showed the appearance and no appearance, respectively. The number of the symbol of (+) showed a degree of the appearance.

Table 12. Some Characteristics of Crosslinked Chitosan - PVA 70,000 Membranes

Formulations	yellow color	transparency	glossy	flexibility	integrity	easy to peel off
CPM_4	+++	++++	+	++	+	++
CPM_5	++++	++++	+	+	+	++
CPM_9	+++	++++	+	++	+	++
CPM_{10}	++++	++++	+	+	+	++
CPM_{14}	++	++++	+	++	+	++
CPM_{15}	+++	++++	+	+	+	++
CPM_{19}	++	++++	+	++	+	++
CPM_{20}	+++	++++	+	++	+	++
CPM_{24}	++	++++	+	++	+	+++
CPM_{25}	+++	++++	+	++	+	+++
CPM_{29}	++	++++	+	+++	+	+++
CPM_{30}	++	++++	+	+++	+	+++
CPM_{34}	++	++++	+	+++	+	+++
CPM_{35}	++	++++	+	+++	+	+++
CPM_{39}	+	++++	+	++++	+	+++
CPM_{40}	+	++++	+	++++	+	+++
CPM_{44}	+	++++	+	++++	+	+++
CPM_{45}	+	++++	+	++++	+	+++

Table 13. Some Characteristics of Crosslinked Chitosan - PVA 100,000 Membranes

Formulations	yellow color	transparency	glossy	flexibility	integrity	easy to peel off
CPH_4	+++	++++	+	++	+	++
CPH_5	++++	++++	+	+	+	++
CPH_9	+++	++++	+	++	+	++
CPH_{10}	++++	++++	+	+	+	++
CPH_{14}	++	++++	+	++	+	++
CPH_{15}	+++	++++	+	+	+	++
CPH_{19}	++	++++	+	++	+	++
CPH_{20}	+++	++++	+	++	+	++
CPH_{24}	++	++++	+	++	+	+++
CPH_{25}	+++	++++	+	++	+	+++
CPH_{29}	++	++++	+	+++	+	+++
CPH_{30}	++	++++	+	+++	+	+++
CPH_{34}	++	++++	+	+++	+	+++
CPH_{35}	++	++++	+	+++	+	+++
CPH_{39}	+	++++	+	++++	+	+++
CPH_{40}	+	++++	+	++++	+	+++
CPH_{44}	+	++++	+	++++	+	+++
CPH_{45}	+	++++	+	++++	+	+++

stronger membrane. However higher concentrations of crosslinking agent could not be incorporated because the obtained membranes became too brittle.

Varying the molecular weight of PVA showed some effect on membrane characteristics. The membranes prepared form crosslinked chitosan-PVA 70,000 and 100,000 formulations were stronger than the membranes prepared from crosslinked chitosan-PVA 30,000 formulations.

Some problems occurred during the preparation of casting solution of crosslinked chitosan-PVA 70,000 and 100,000 membrane formulations. PVA powder tended to aggregation, therefore the time for preparing clear solution was quite long. Increasing temperature and speed of agitating could solve this problem but the forming of air bubbles was to be beware.

The viscosity of the casting solution was interesting because it affected filtration and disappearance of air bubbles. The high concentration of chitosan and crosslinking agent and the use of high molecular weight of PVA produced solution of high viscosity. Therefore the filtration of casting solution and the disappearance of air bubbles were difficult.

Crosslinked chitosan - starch membranes.

The casting solutions prepared in this experiment were yellow opaque viscous colloidal mixture. The resultant membranes were less transparent and glossy than the membranes prepared form crosslinked chitosan - PVA membranes. The transparency of prepared crosslinked chitosan-starch membranes could be ranked in the following manner : crosslinked chitosan - tapioca starch membranes < crosslinked chitosan - corn starch membranes < crosslinked chitosan - potato starch membranes. The effect of degree of chitosan and crosslinked on the color of membrane was the same as previously noted. In the preparing of crosslinked chitosan - starch casting solutions, only 0.4 g of glutaraldehyde solution (5% w/w of total polymer) was used as the highest concentration of crosslinking agent while the preparing of chitosan - PVA casting solutions, the highest concentration of crosslinking solution was 0.8 g (10% w/w of total polymer). Higher amount of glutaraldehyde solution led to gelatinous substance. In crosslinked chitosan - starch membrane formulation, ratio of chitosan : starch 1 : 9, 1 : 4 and 3 : 7 could not produced integrity membranes because the films tightly attached with the glass petri dish.

Adding plasticizer could resulted in stronger membranes. Besides, plasticized membranes were easier to peel off than unplasticized membranes. For all crosslinked chitosan - starch membranes, increasing the ratio of starch underwent to the brittle membranes.

The same as in crosslinked chitosan - PVA membranes, increasing the amount of crosslinking agent tended to stronger crosslinked chitosan - starch membranes. Some characteristics of crosslinked chitosan - starch membranes were demonstrated in Tables 14 to 16.

Table 14. Some Characteristics of Crosslinked Chitosan - Corn Starch Membranes

Formulations	yellow color	transparency	glossy	flexibility	integrity	easy to peel off
CC_7	++++	++	+	++	+	+
CC_8	++++	++	+	++	+	++
CC_{15}	++++	++	+	++	+	+
CC_{16}	++++	++	+	++	+	++
CC_{23}	++++	++	+	++	+	+
CC_{24}	++++	++	+	++	+	++
CC_{31}	+++	++	+	+	+	-
CC_{32}	+++	++	+	+	+	+
CC_{39}	++	++	+	-	+	-
CC_{40}	++	++	+	+	+	-
CC_{47}	++	++	+	-	+	-
CC_{48}	++	++	+	+	+	-

Table 15. Some Characteristics of Crosslinked Chitosan - Potato Starch Membranes

Formulations	yellow color	transparency	glossy	flexibility	integrity	easy to peel off
CP_7	++++	+++	+	++	+	+
CP_8	++++	+++	+	++	+	++
CP_{15}	++++	+++	+	++	+	+
CP_{16}	++++	+++	+	++	+	++
CP_{23}	++++	+++	+	++	+	+
CP_{24}	++++	+++	+	++	+	++
CP_{31}	+++	+++	+	+	+	-
CP_{32}	+++	+++	+	+	+	+
CP_{39}	++	+++	+	-	+	-
CP_{40}	++	+++	+	+	+	-
CP_{47}	++	+++	+	-	+	-
CP_{48}	++	+++	+	+	+	-

Table 16. Some Characteristics of Crosslinked Chitosan - Tapioca Starch Membranes

Formulations	yellow color	transparency	glossy	flexibility	integrity	easy to peel off
CT_7	++++	+	+	+	+	+
CT_8	++++	+	+	+	+	+
CT_{15}	++++	+	+	++	+	+
CT_{16}	++++	+	+	++	+	+
CT_{23}	+++	+	+	++	+	+
CT_{24}	+++	+	+	++	+	+
CT_{31}	+++	+	+	++	+	-
CT_{32}	+++	+	+	++	+	+
CT_{39}	++	+	+	-	+	-
CT_{40}	++	+	+	+	+	+
CT_{47}	++	+	+	-	+	+
CT_{48}	++	+	+	+	+	++

Figure 7. An integrity membrane

Water sorption

Selected crosslinked chitosan - polymer membranes which showed good characteristics ; favorable color, glossy, integrity, flexibility and ease to isolate from the glass petri dish, were further studied. After exposed to the water, these membranes expanded. Moreover, some membrane formulations, especially crosslinked chitosan - starch membranes, broke and could mot be evaluated. The results of water sorption were presented in Table 17 and Figure 8.

The results indicated that, the variation of molecular weight of PVA did not affect the water absorption of membranes. But ratio of blending polymer was the important factor to this phenomenon. In the ratio of chitosan : PVA 1 : 4 (CPL_{40}, CPM_{40}, CPH_{40}) the degree of absorption was low.

For crosslinked chitosan - starch membrane, plasticized membrane showed lower absorption than unplasticized membrane. The type of starch affected on percent water sorption and the strength of membrane after the membrane contacted to water. Crosslinked chitosan - potato starch gave higher water sorption than the other crosslinked chitosan - starch membranes (CP_7 comparison with CC_7 and CP_{15} comparison with CC_{15}). The effect of crosslinking agent on degree of water absorption of membrane could be summarized that, increasing the amount of crosslinking agent led to decreasing of water sorption.

Table 17. Percent Water Sorption of Various Crosslinked Chitosan - Polymer Membranes

Type of membranes	Formulations	% Water sorption (± SD)
crosslinked chitosan - PVA 30,000	CPL_{40}	32.5642 (2.67)
	CPL_{43}	48.5724 (2.65)
	CPL_{44}	45.1753 (3.94)
	CPL_{45}	35.2751 (1.28)
crosslinked chitosan - PVA 70,000	CPM_{29}	62.7034 (2.21)
	CPM_{30}	26.2516 (2.16)
	CPM_{34}	56.9940 (1.98)
	CPM_{35}	32.3925 (2.67)
	CPM_{39}	50.2288 (1.90)
	CPM_{40}	27.6199 (3.60)
	CPM_{44}	64.7493 (7.84)
	CPM_{45}	41.6579 (7.28)
crosslinked chitosan - PVA 100,000	CPH_{33}	67.8009 (2.68)
	CPH_{34}	56.4005 (0.29)
	CPH_{38}	69.6616 (4.44)
	CPH_{39}	52.7221 (1.05)
	CPH_{40}	31.2630 (1.61)
	CPH_{44}	62.2707 (2.23)
	CPH_{45}	49.8981 (4.16)
crosslinked chitosan - corn starch	CC_7	44.9345 (2.39)
	CC_8	39.3323 (1.22)
	CC_{15}	44.5650 (1.54)
	CC_{16}	36.0886 (0.34)
crosslinked chitosan - potato starch	CP_7	48.3505 (3.05)
	CP_8	37.6398 (1.15)
	CP_{15}	45.9382 (0.55)
	CP_{16}	36.9409 (1.92)
crosslinked chitosan - tapioca starch	CT_{47}	27.7284 (2.02)
	CT_{48}	26.1743 (2.06)

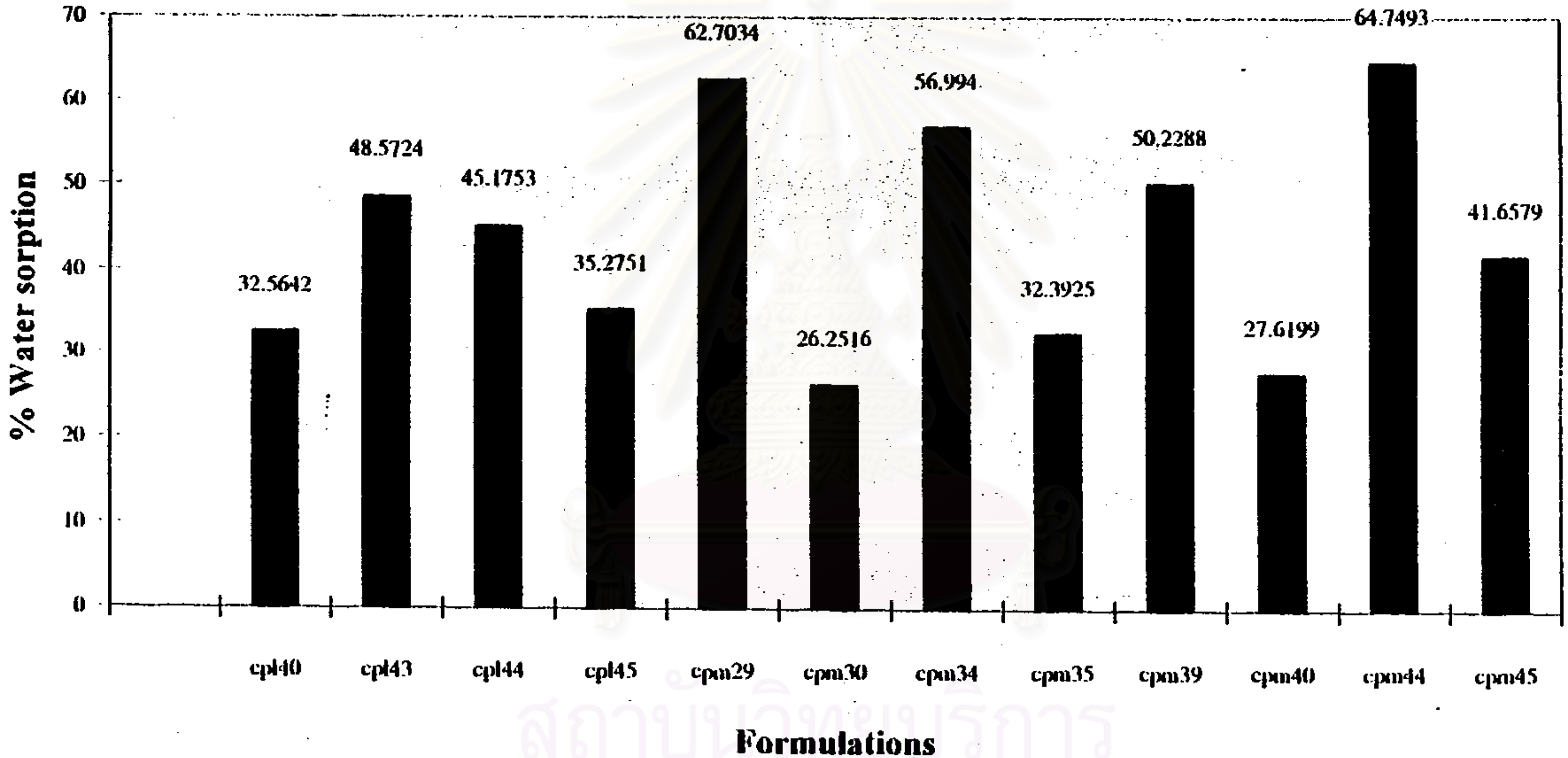

Figure 8. Percent water sorption of various crosslinked chitosan-polymer membranes

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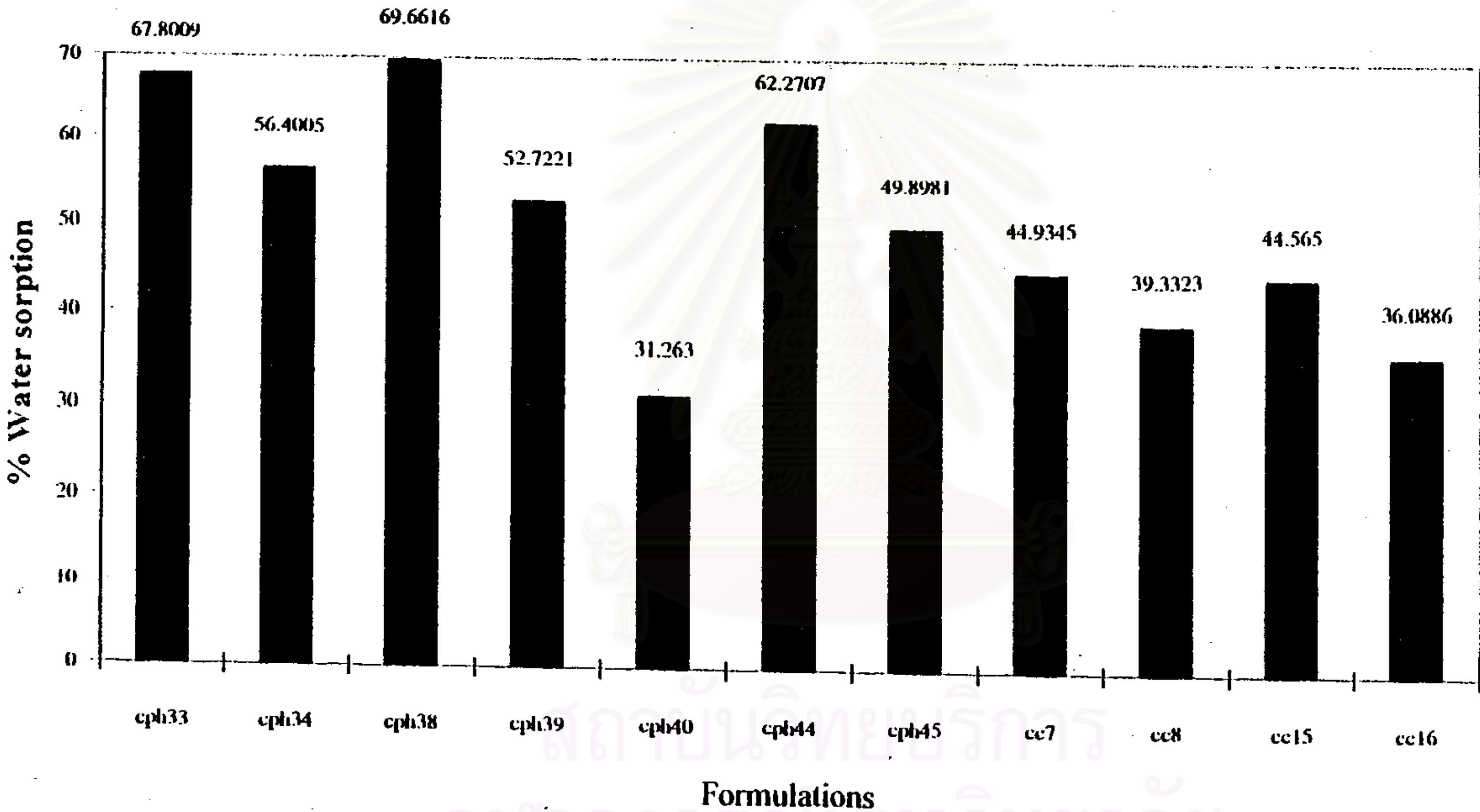

Figure 8. (cont.) Percent water sorption of various crosslinked chitosan-polymer membranes

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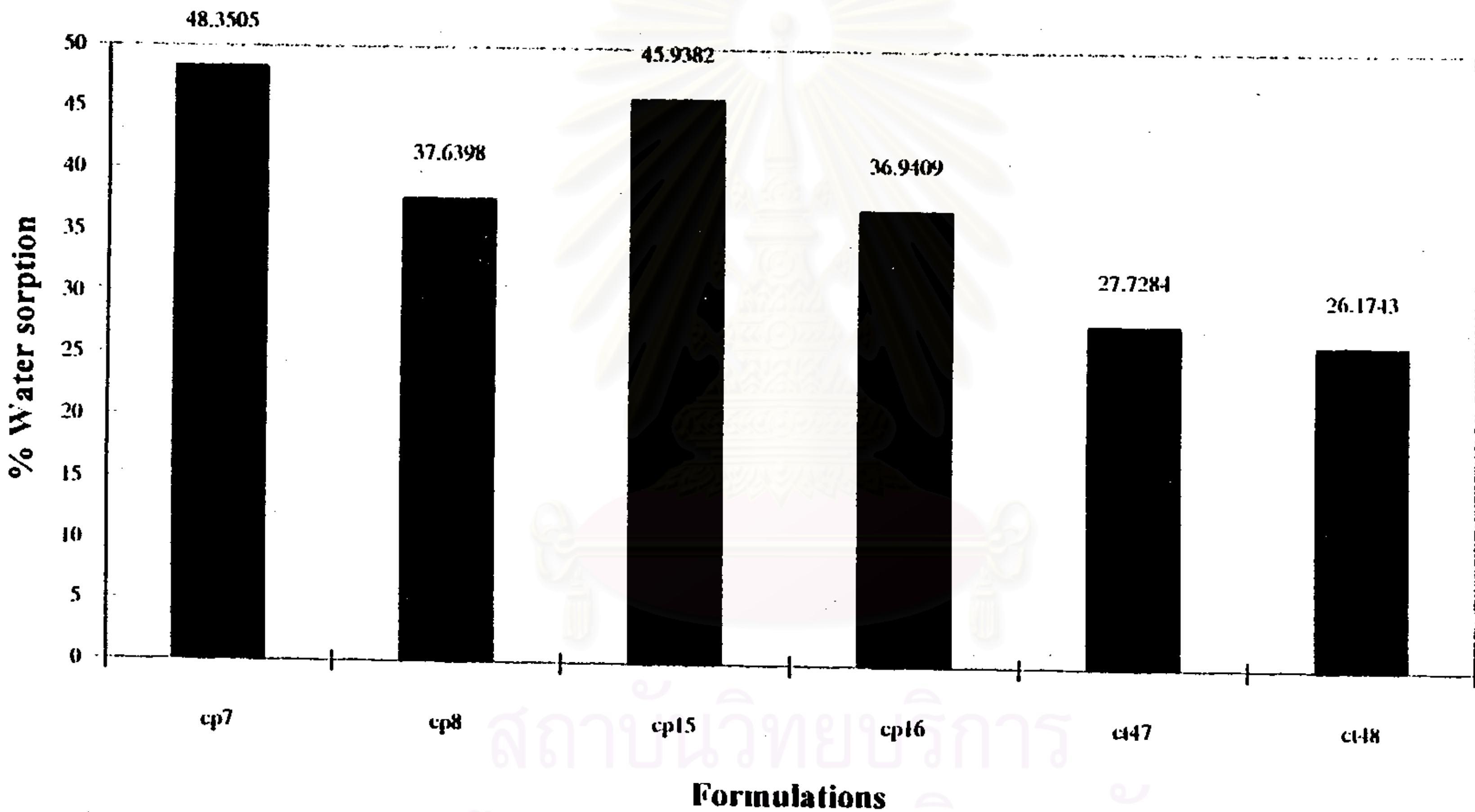

Figure 8. (cont.) Percent water sorption of various crosslinked chitosan-polymer membranes

Mechanical properties

Ultimate tensile strength and percent elongation at break.

In the evaluation, only crosslinked chitosan - polymer membranes, which water sorption was not excess 50%, were test. Overall data including ultimate tensile strength and percent elongation at break were presented in Table 18 and Figures 9 and 10.

For crosslinked chitosan - PVA membranes, higher molecular weight of PVA underwent to increasing ultimate tensile strength and percent elongation at break. In the formulation which composed of the same molecular weight of PVA, the increasing amount of PVA led to high ultimate tensile strength and elongation at break. The difference of percent elongation at break was obviously indicated in high molecular weight of PVA (CPH_{40}, Chitosan : PVA 100,000 1 : 4 comparison with CPH_{45}, chitosan : PVA 100,000 1 : 9).

Focused on ultimate tensile strength of crosslinked chitosan starch membranes, ingenerally the resulted crosslinked chitosan - starch membranes showed lower tensile strength than the crosslinked chitosan - PVA membranes as well as percent elongation at break. For crosslinked chitosan - starch membranes, the results indicated that, the plasticizer was important factor to mechanical properties of membranes whereas the effect of crosslinking agent was not obvious. The data showed that,

Table 18. Mechanical Properties of Various Crosslinked Chitosan - Polymer Membranes

Type of membranes	Formulations	Ultimate tensile strength (kg/mm^2) ($\pm$ SD)	% Elongation at break ($\pm$ SD)
crosslinked chitosan - PVA 30,000	CPL_{40}	2.1935 (008)	21.67 (2.89)
	CPL_{43}	1.9961 (0.07)	31.67 (4.71)
	CPL_{44}	2.2814 (0.16)	30.00 (5)
	CPL_{45}	3.4872 (0.11)	16.67 (2.89)
crosslinked chitosan - PVA 70,000	CPM_{30}	1.2806 (0.13)	15.00 (5)
	CPM_{35}	1.3022 (0.22)	18.33 (2.89)
	CPM_{39}	2.3243 (0.18)	31.67 (5.77)
	CPM_{40}	3.5232 (0.02)	35.00 (5)
	CPM_{45}	4.7451 (0.08)	30.00 (8.66)
crosslinked chitosan - PVA 100,000	CPH_{40}	4.3563 (0.21)	38.33 (2.89)
	CPH_{45}	4.9696 (0.19)	41.67 (2.89)
crosslinked chitosan - corn starch	CC_7	0.6318 (0.04)	8.33 (2.89)
	CC_8	1.3815 (0.06)	12.50 (3.54)
	CC_{15}	0.5850 (0.04)	6.67 (1.44)
	CC_{16}	1.0597 (0.04)	11.67 (2.89)
crosslinked chitosan - potato starch	CP_7	0.8255 (0.02)	9.17 (1.44)
	CP_8	1.4697 (0.11)	15.00 (5)
	CP_{15}	0.5295 (0.05)	11.67 (2.89)
	CP_{16}	1.0457 (0.06)	13.33 (2.89)
crosslinked chitosan - tapioca starch	CT_{47}	1.3412 (0.03)	16.67 (2.89)
	CT_{48}	1.6237 (0.17)	21.67 (2.89)

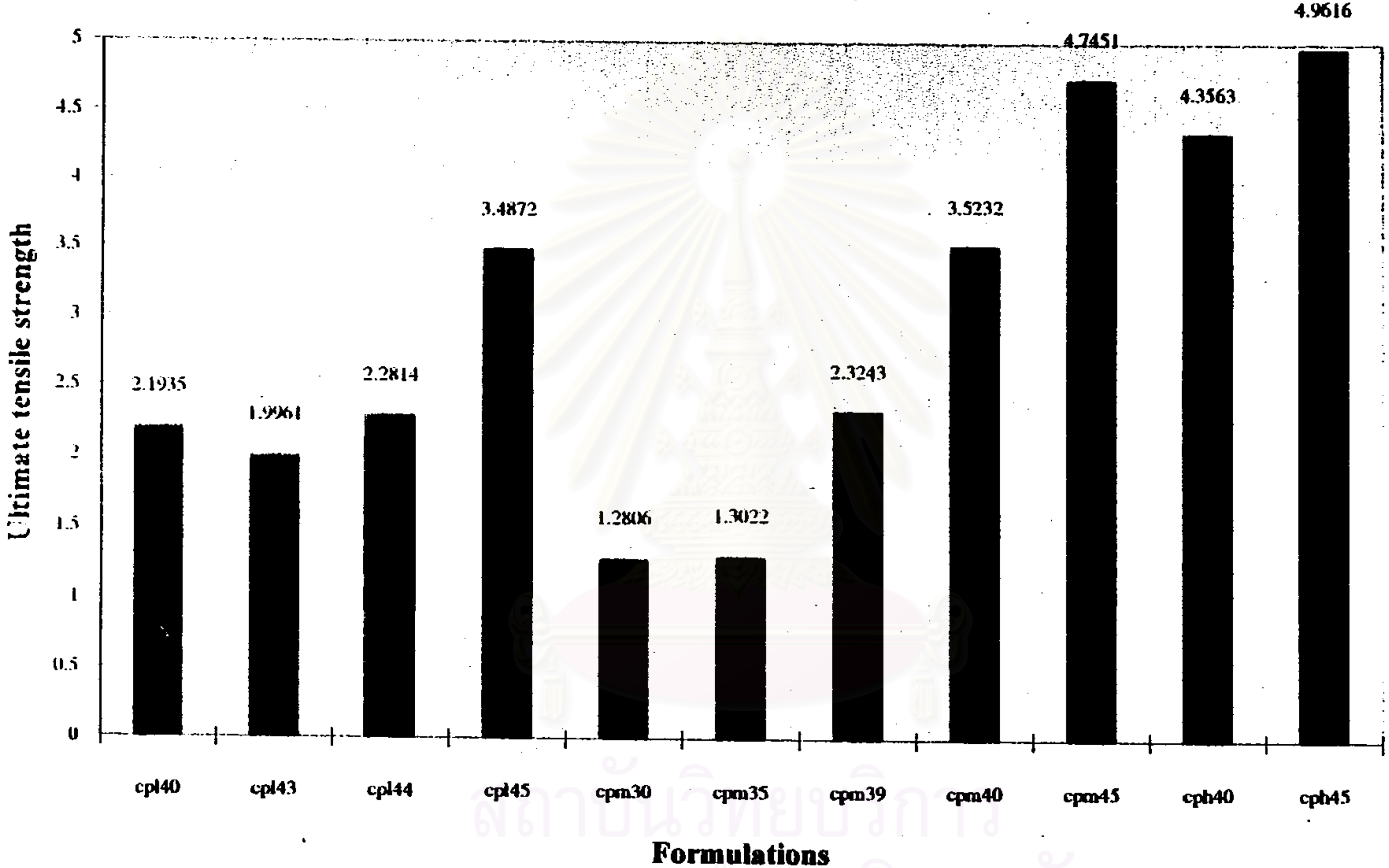

Figure 9. Ultimate tensile strength of various crosslinked chitosan-polymer membranes

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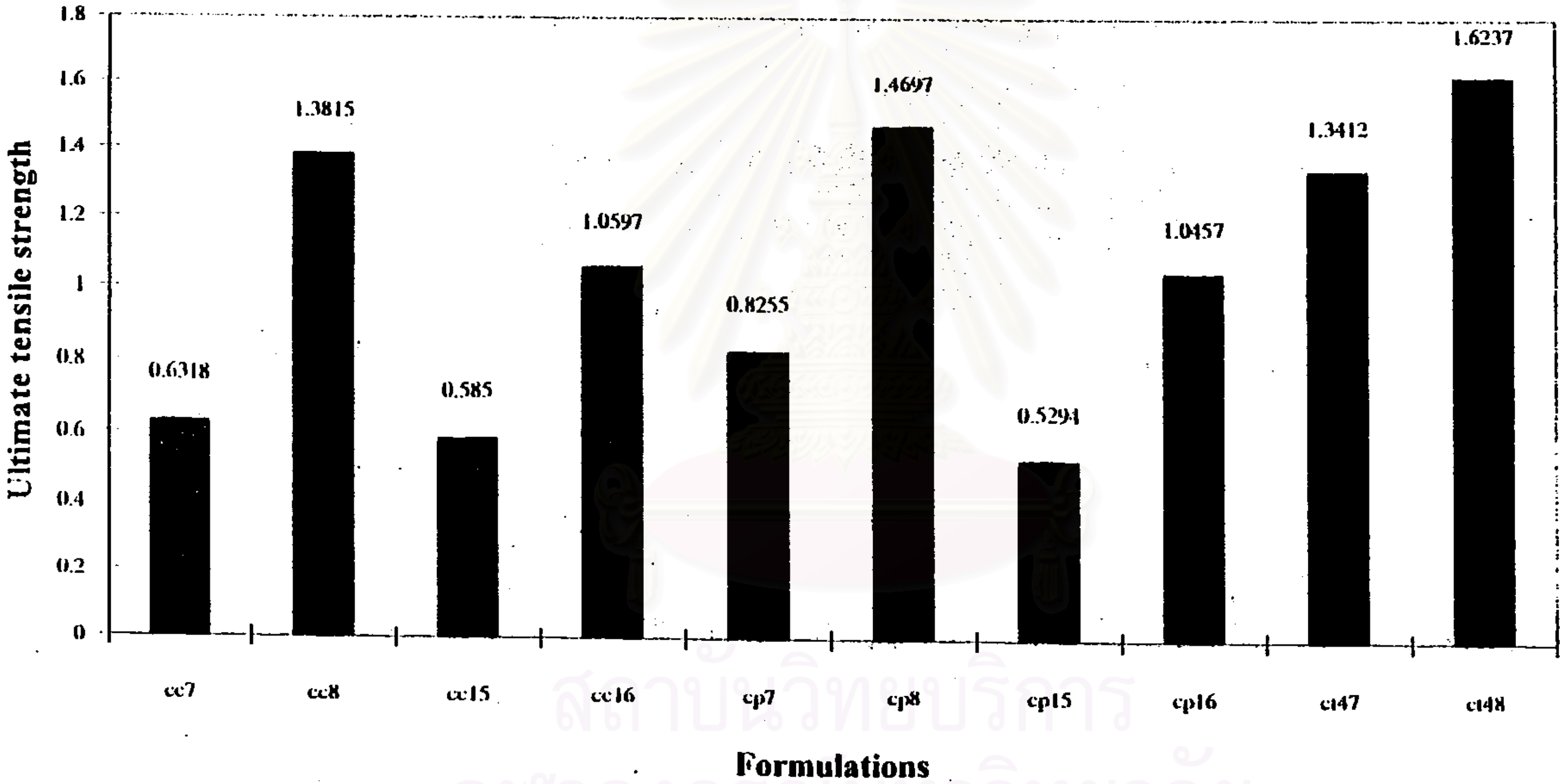

Figure 9. (cont.) Ultimate tensile strength of various crosslinked chitosan-polymer membranes

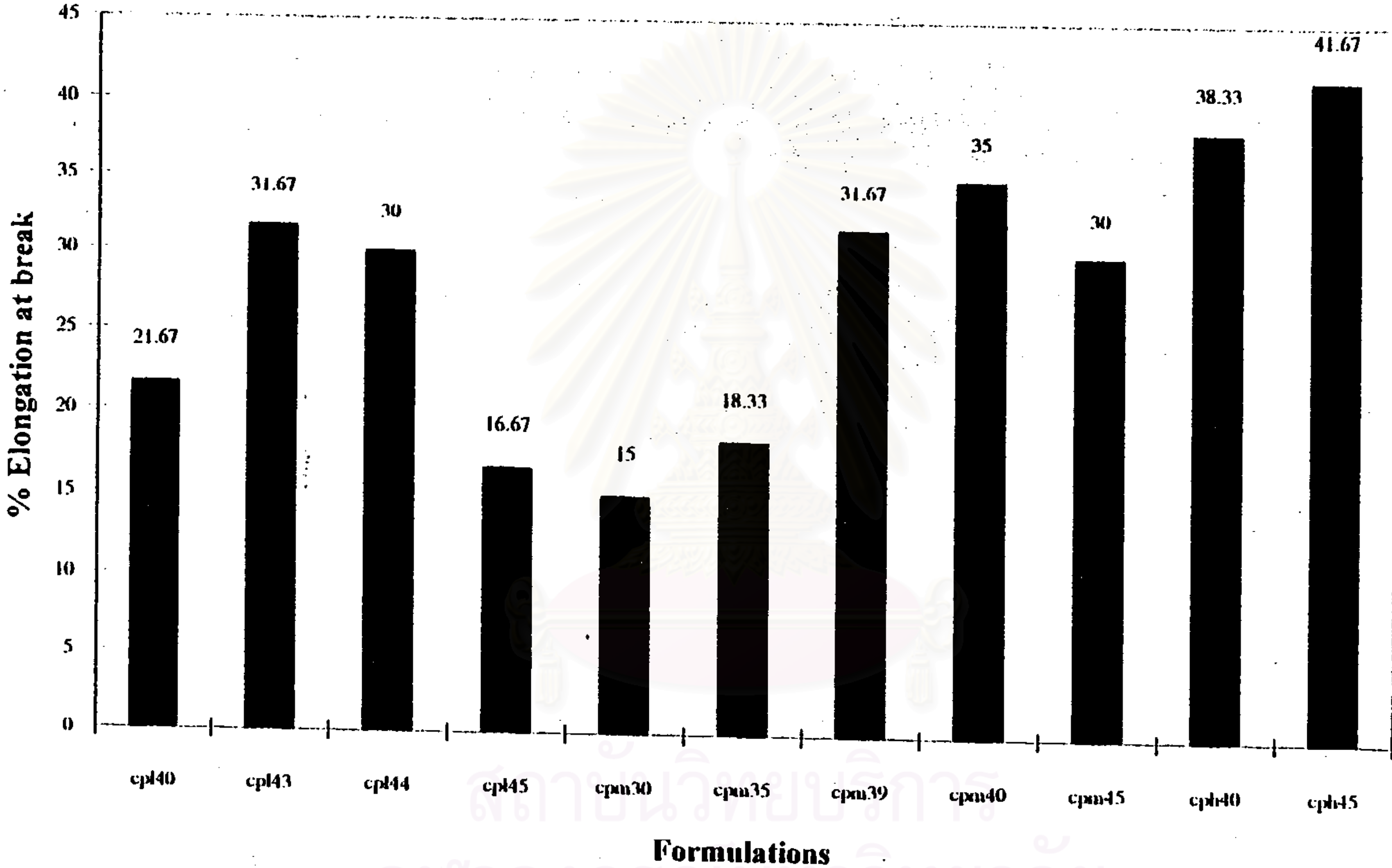

Figure 10. Percent elongation at break of various crosslinked chitosan-polymer membranes

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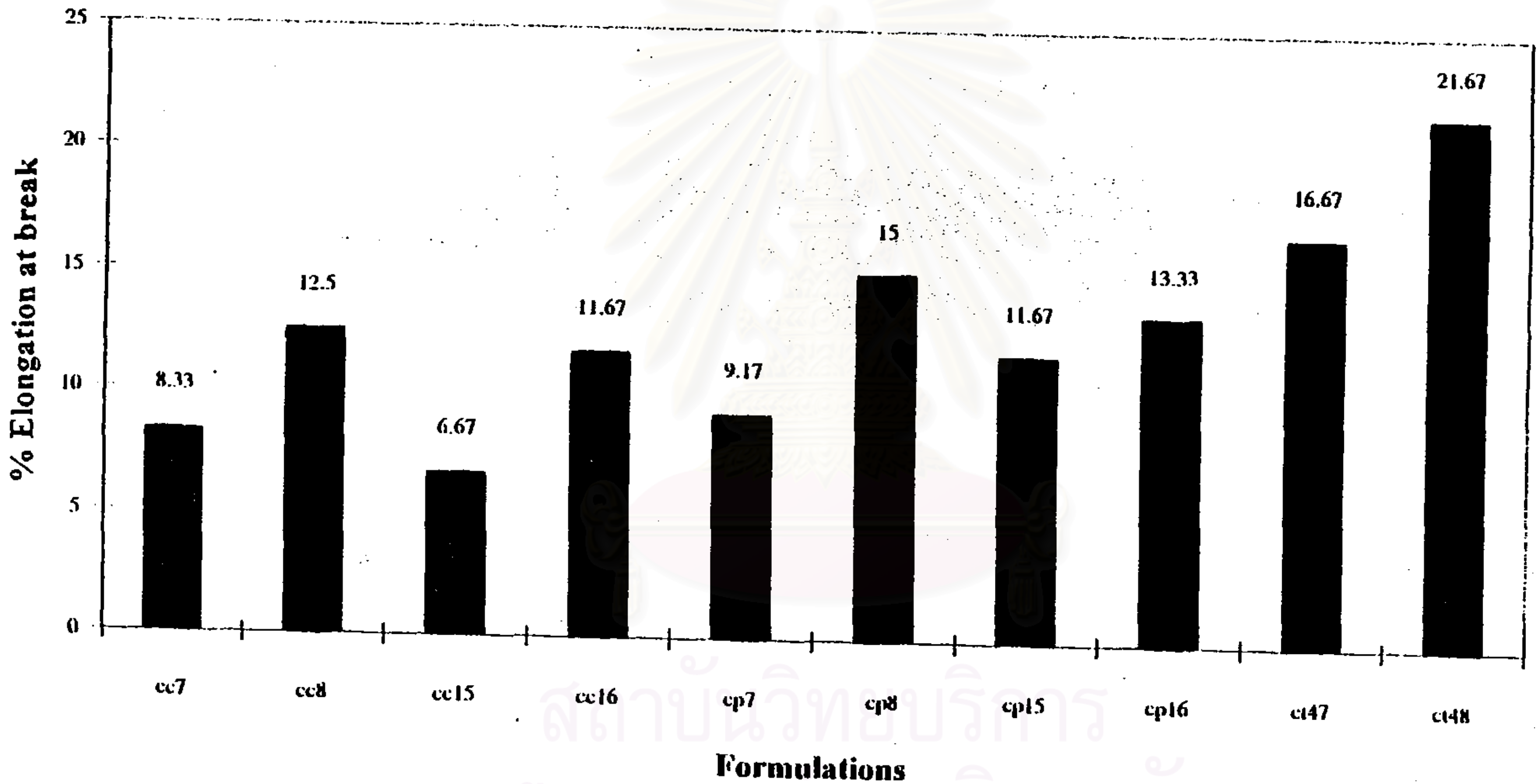

Figure 10. (cont.) Percent elongation at break of various crosslinked chitosan-polymer membranes

plasticized crosslinked chitosan - starch membranes had higher ultimate tensile strength than unplasticized crosslinked chitosan - starch membranes.

Considering the effect of crosslinking agent on mechanical properties of membrane, high concentration of crosslinking agent led to high ultimate tensile strength. The result was be obvious in crosslinked chitosan - PVA membranes.

Stability of membranes

The crosslinked chitosan - polymer membranes which had appropriate water sorption (not more than 50%) and a good mechanical properties (tensile strength not less than 1) were selected for stability test. After membranes were kept in controlled condition for one week, they were evaluated for their physical characteristics, water sorption and mechanical properties. The results were exhibited in Tables 19 to 21 and Figures 11 to 13.

For physical characteristics, most test crosslinked chitosan - polymer membranes were yellow color, transparence, integrity, glossy and flexible. In the formulations which composed of high concentration of chitosan, the membrane color was rather deep yellow. Considering water absorption data, the degree of water sorption of crosslinked chitosan - polymer membranes was closed the crosslinked chitosan - polymer membranes before stability test. For mechanical properties, most

Table 19. Some Physical Characteristics of Crosslinked Chitosan - Polymer Membranes after Stability Test

Formulation	Yellow color	Transparency	Glossy	Flexibility	Integrity
CPL_{40}	++	+	+	+++	+
CPL_{43}	++	+	+	+++	+
CPL_{44}	++	+	+	+++	+
CPL_{45}	++	+	+	+++	+
CPM_{30}	+++	+	+	+++	+
CPM_{35}	+++	+	+	+++	+
CPM_{39}	++	+	+	++++	+
CPM_{40}	++	+	+	++++	+
CPM_{45}	++	+	+	++++	+
CPH_{40}	++	+	+	++++	+
CPH_{45}	++	+	+	++++	+
CC_{7}	+++++	+	+	+++	+
CC_{8}	+++++	+	+	++++	+
CC_{15}	+++++	+	+	++++	+
CC_{16}	+++++	+	+	++++	+
CP_{7}	+++++	+	+	+++	+
CP_{8}	+++++	+	+	+++	+
CP_{15}	+++++	+	+	+++	+
CP_{16}	++++	+	+	+++	+
CT_{47}	+++	+	+	++	+
CT_{48}	+++	+	+	++	+

Table 20. Percent Water Sorption of Various Crosslinked Chitosan - Polymer Membranes after Stability Test

Type of membranes	Formulations	% Water sorption (± SD)
crosslinked chitosan - PVA 30,000	CPL_{40}	31.5355 (2.03)
	CPL_{43}	49.9026 (2.06)
	CPL_{44}	39.2144 (2.17)
	CPL_{45}	35.8259 (0.69)
crosslinked chitosan - PVA 70,000	CPM_{30}	22.5271 (0.83)
	CPM_{35}	28.6738 (2.87)
	CPM_{39}	42.8608 (2.38)
	CPM_{40}	25.8976 (3.08)
	CPM_{45}	34.9327 (2.44)
crosslinked chitosan - PVA 100,000	CPH_{40}	29.5020 (1.42)
	CPH_{45}	49.3047 (1.71)
crosslinked chitosan - corn starch	CC_{7}	44.5830 (2.11)
	CC_{8}	34.3494 (1.32)
	CC_{15}	39.1048 (0.45)
	CC_{16}	30.8353 (1.08)
crosslinked chitosan - potato starch	CP_{7}	42.5924 (0.48)
	CP_{8}	35.0652 (2.20)
	CP_{15}	43.7023 (0.31)
	CP_{16}	37.3742 (3.16)
crosslinked chitosan - tapioca starch	CT_{47}	24.2228 (2.22)
	CT_{48}	23.0191 (0.86)

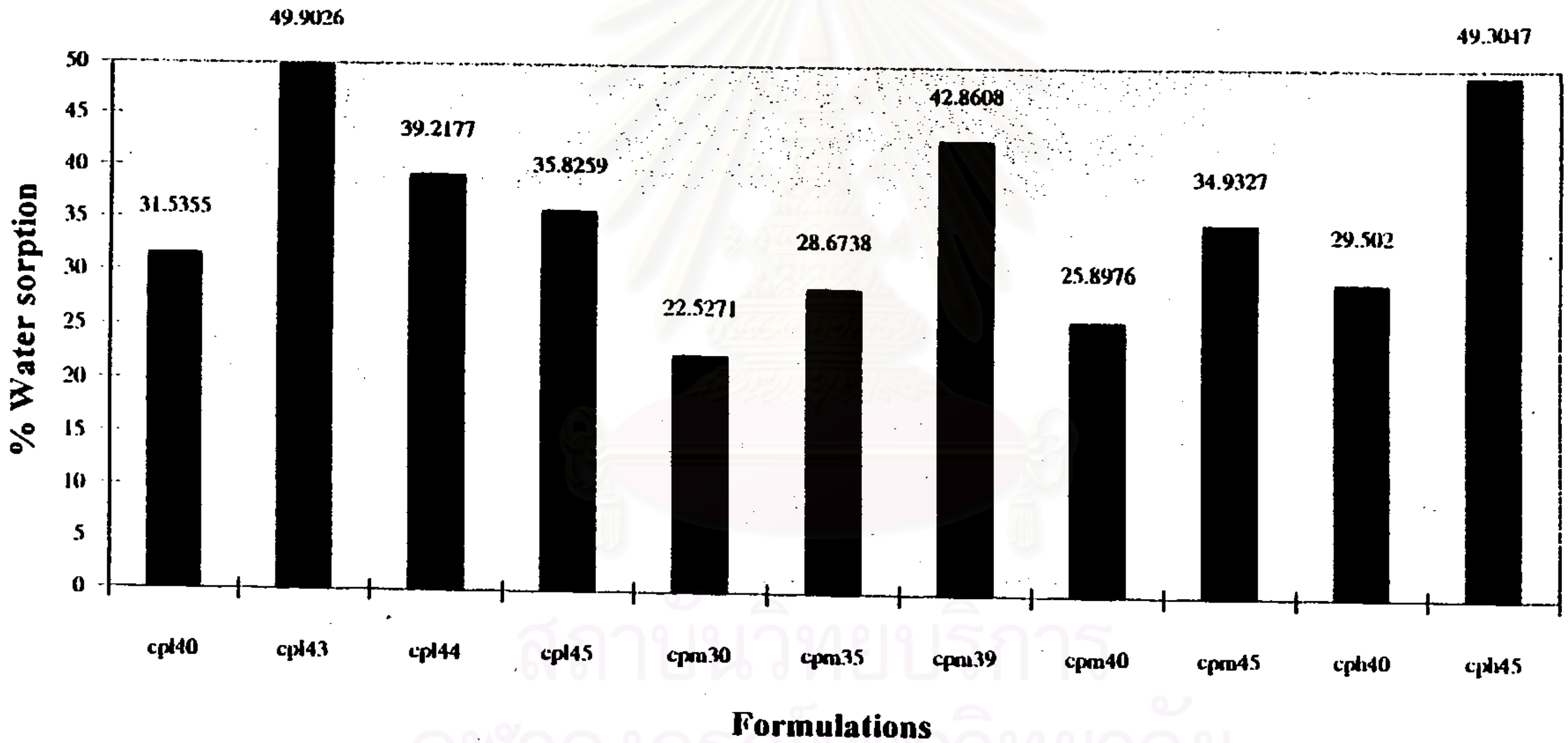

Figure 11. Percent water sorption of various crosslinked chitosan-polymer membranes after stability test

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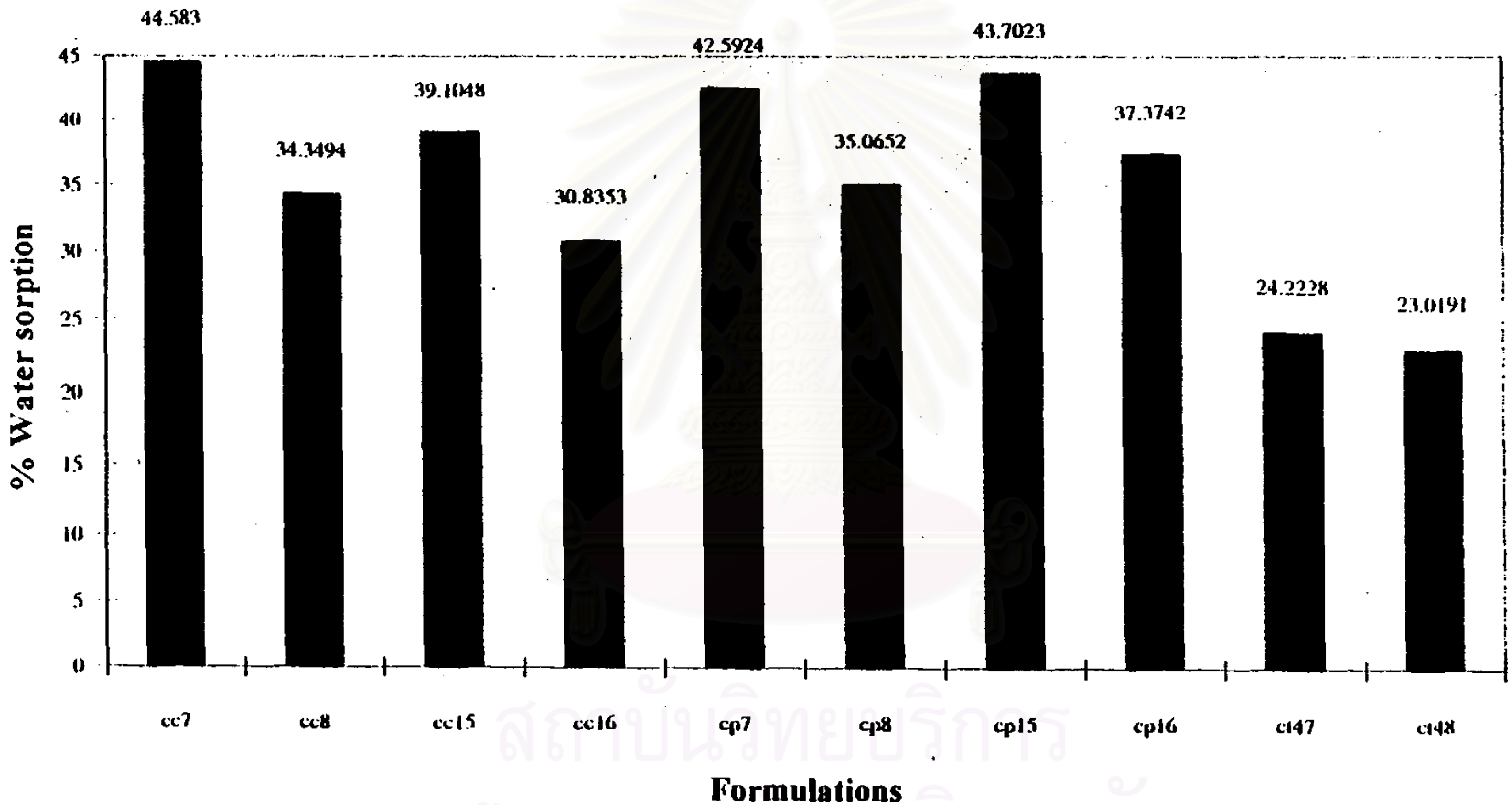

Figure 11. (cont.) Percent water sorption of various crosslinked chitosan-polymer membranes after stability test

Table 21. Ultimate Tensile Strength and Percent Elongation at Break of Various Crosslinked Chitosan - Polymer Membranes after Stability Test

Type of membranes	Formulations	Ultimate tensile strength (kg/mm^2) ($\pm$ SD)	% Elongation at break ($\pm$ SD)
crosslinked chitosan - PVA 30,000	CPL_{40}	1.4065 (0.11)	13.33 (5.77)
	CPL_{43}	2.6370 (0.03)	40.00 (8.66)
	CPL_{44}	2.6226 (0.27)	36.67 (5.77)
	CPL_{45}	3.1605 (0.16)	33.33 (7.63)
crosslinked chitosan - PVA 70,000	CPM_{30}	1.2129 (0.54)	23.33 (5.77)
	CPM_{35}	1.5736 (0.11)	20.00 (5.00)
	CPM_{39}	2.2680 (0.24)	43.33 (7.64)
	CPM_{40}	2.8393 (0.32)	36.67 (2.89)
	CPM_{45}	4.3036 (0.08)	36.67 (2.89)
crosslinked chitosan - PVA 100,000	CPH_{40}	4.0838 (0.07)	35.00 (5.00)
	CPH_{45}	4.8360 (0.41)	31.67 (2.89)
crosslinked chitosan - corn starch	CC_7	0.7157 (0.04)	6.67 (2.89)
	CC_8	1.4786 (0.20)	16.67 (2.89)
	CC_{15}	0.6717 (0.11)	10.00 (1.73)
	CC_{16}	1.1954 (0.20)	11.67 (2.89)
crosslinked chitosan - potato starch	CP_7	0.8240 (0.24)	15.00 (5.00)
	CP_8	1.5381 (0.06)	16.67 (2.89)
	CP_{15}	0.6282 (0.26)	13.33 (2.89)
	CP_{16}	1.2015 (0.12)	16.67 (2.89)
crosslinked chitosan - tapioca starch	CT_{47}	1.3696 (0.07)	13.33 (5.77)
	CT_{48}	1.8161 (0.26)	23.33 (7.64)

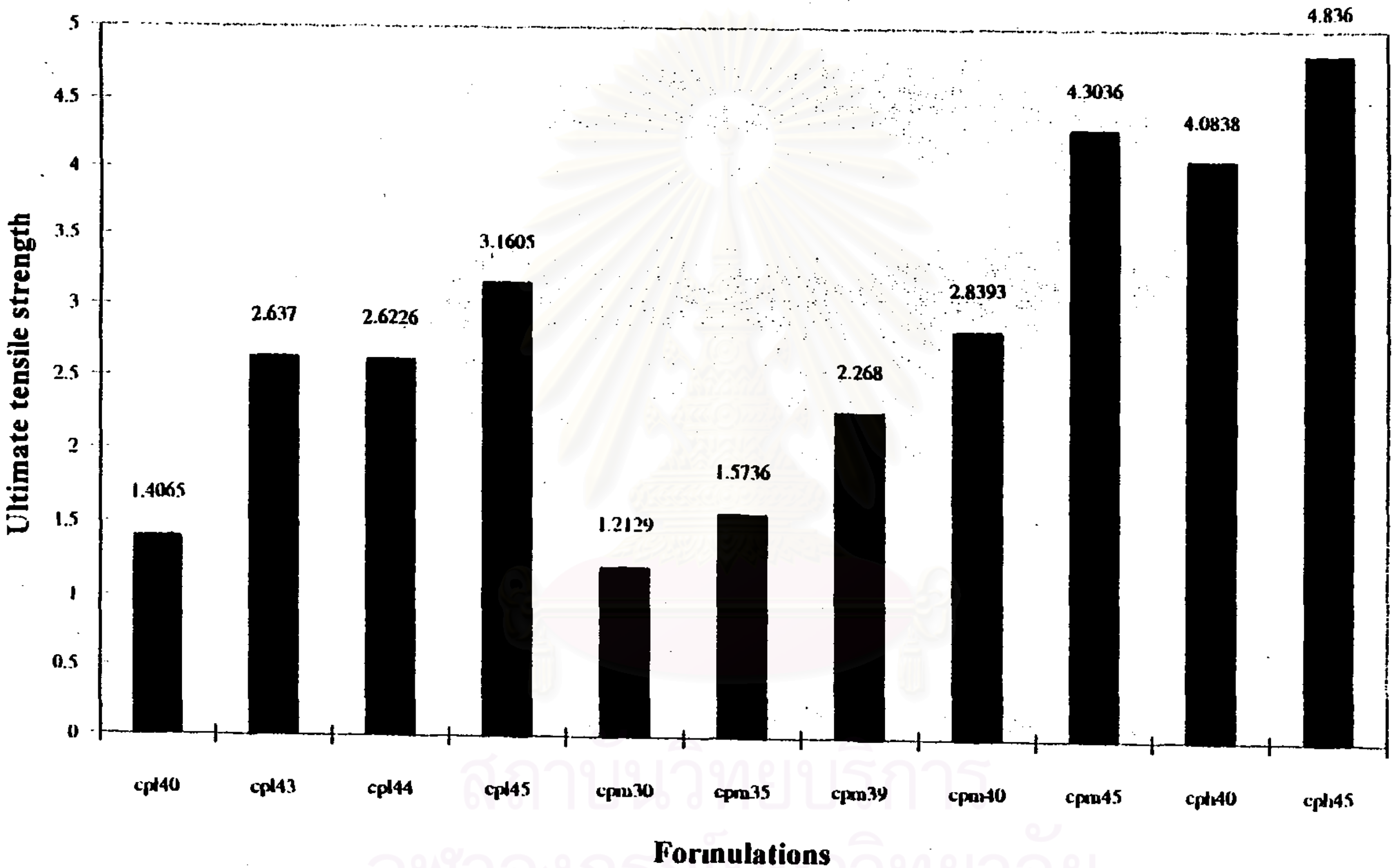

Figure 12. Ultimate tensile strength of various crosslinked chitosan-polymer membranes after stability test

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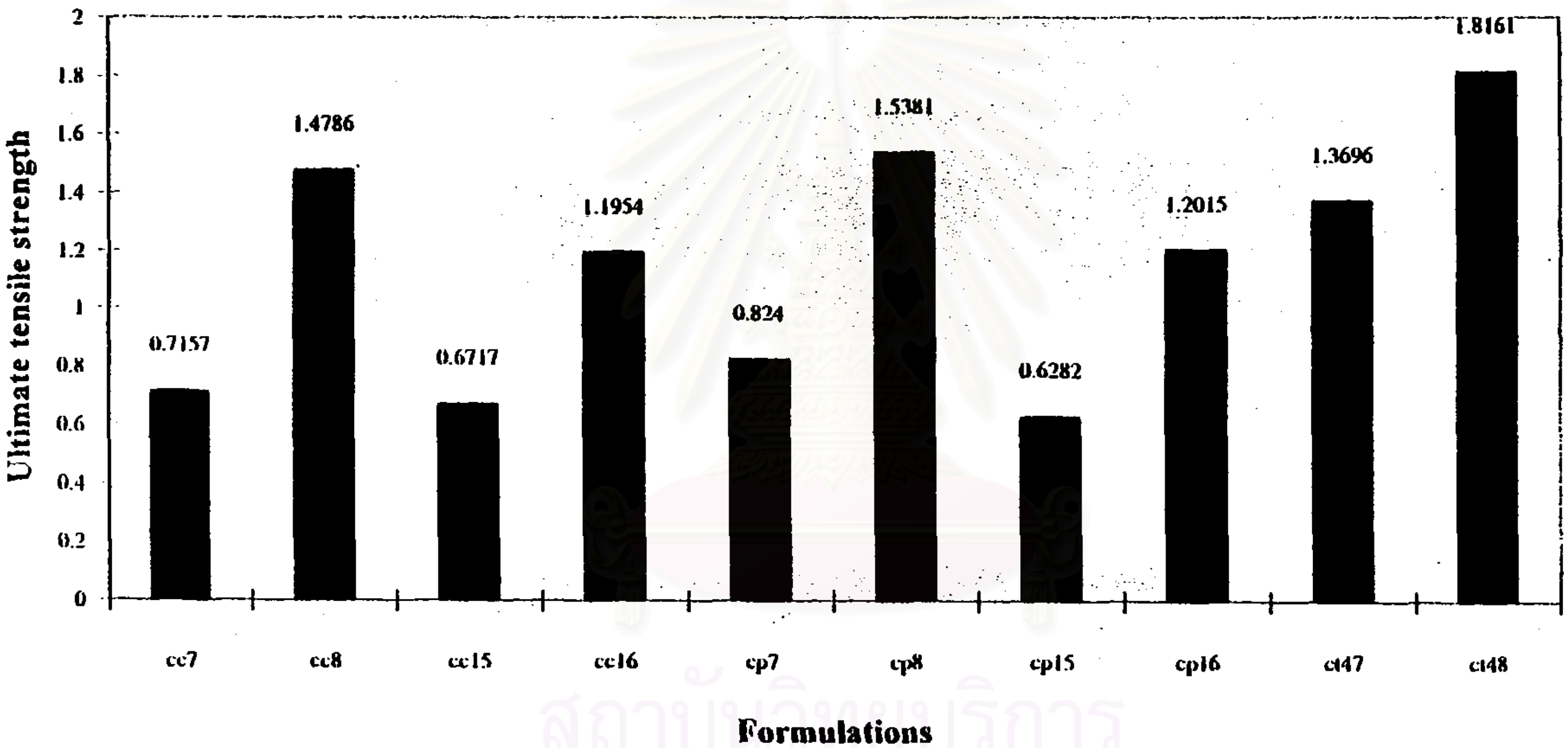

Figure 12.(cont.) Ultimate tensile strength of various crosslinked chitosan-polymer membranes after stability test

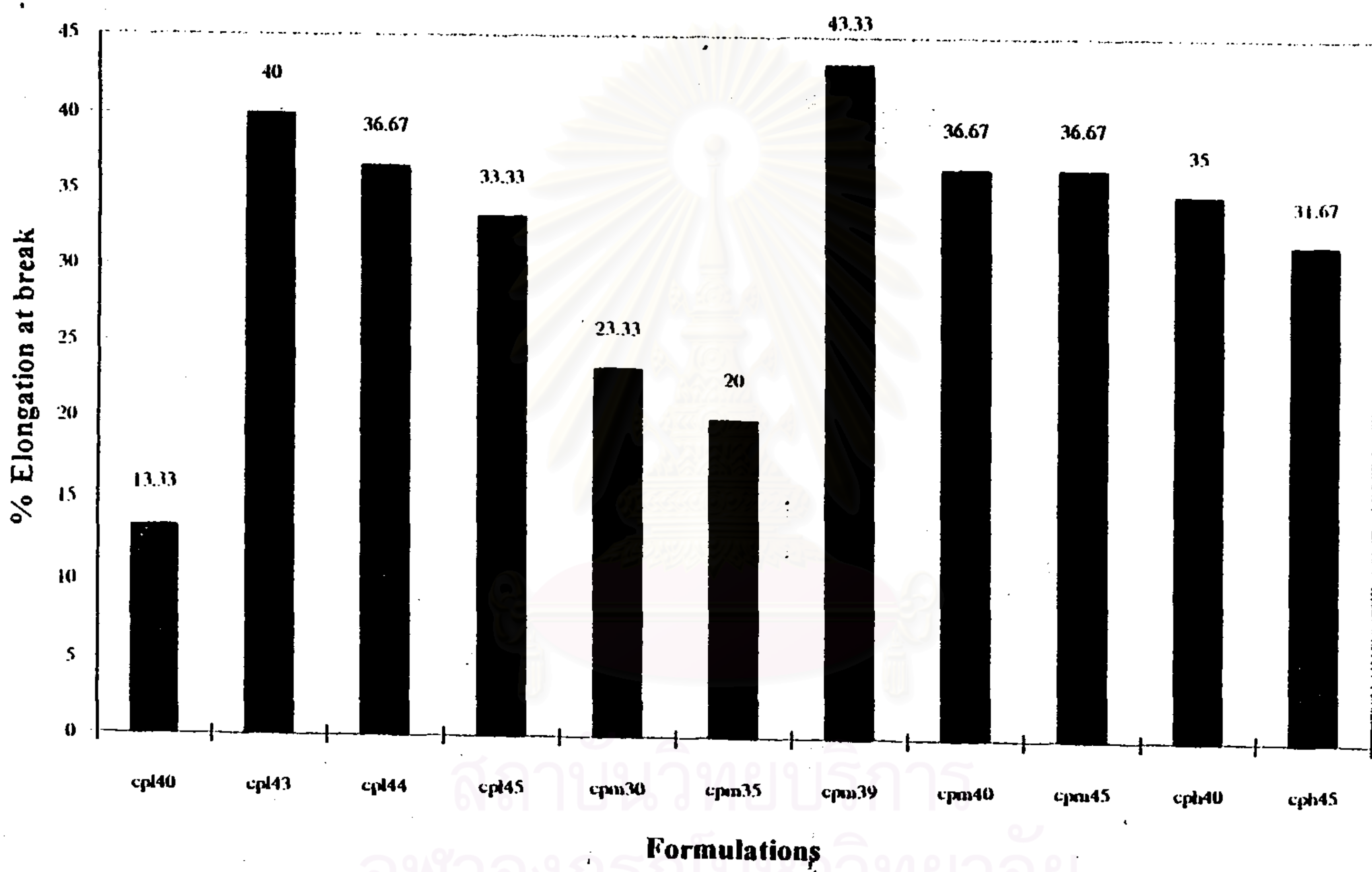

Figure 13. Percent elongation at break of various crosslinked chitosan-polymer membranes after stability test

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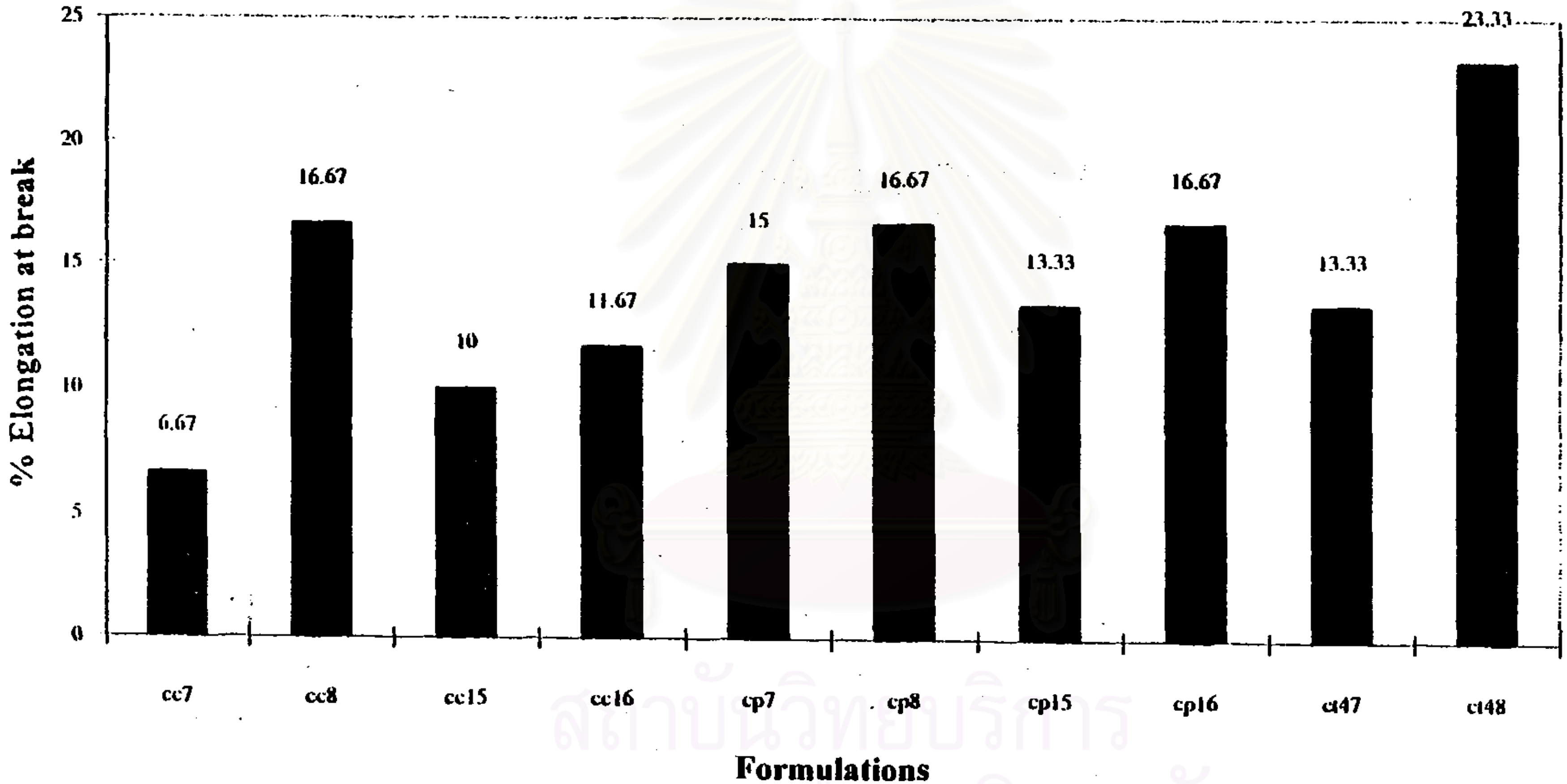


Figure 13. (cont.) Percent elongation at break of various crosslinked chitosan-polymer membranes after stability test

membranes were rather soft which could observe from the slight increasing of percent elongation at break.

From data noted above it was concluded that, the prepared crosslinked chitosan - polymer membranes were rather stable in test condition.

For overall studies, it was found that the water sorption ability of crosslinked chitosan - polymer membrane was the critical factor for this in vitro - permeation study. Then the membranes which exhibited low water sorption were the good candidate to act as rate - controlling membrane in transdermal patch because high water sorption ability of membrane might be led to breakable membrane between in vitro permeation study. Therefore, 11 membrane formulations including CPL_{40}, CPL_{45}, CPM_{30}, CPM_{35}, CPM_{40}, CPH_{40}, CC_{16}, CT_{47}, CT_{48}, CP_8 and CP_{16} were selected. Moreover, these selected crosslinked chitosan - polymer membranes were also rather good mechanical properties and stability.

Surface morphology

The surface morphology of crosslinked chitosan - polymer membranes for isosorbide dinitrate transdermal patch, were manifested at 350 and 750 times. All photomicrographs were shown in Figures 14 to 23.

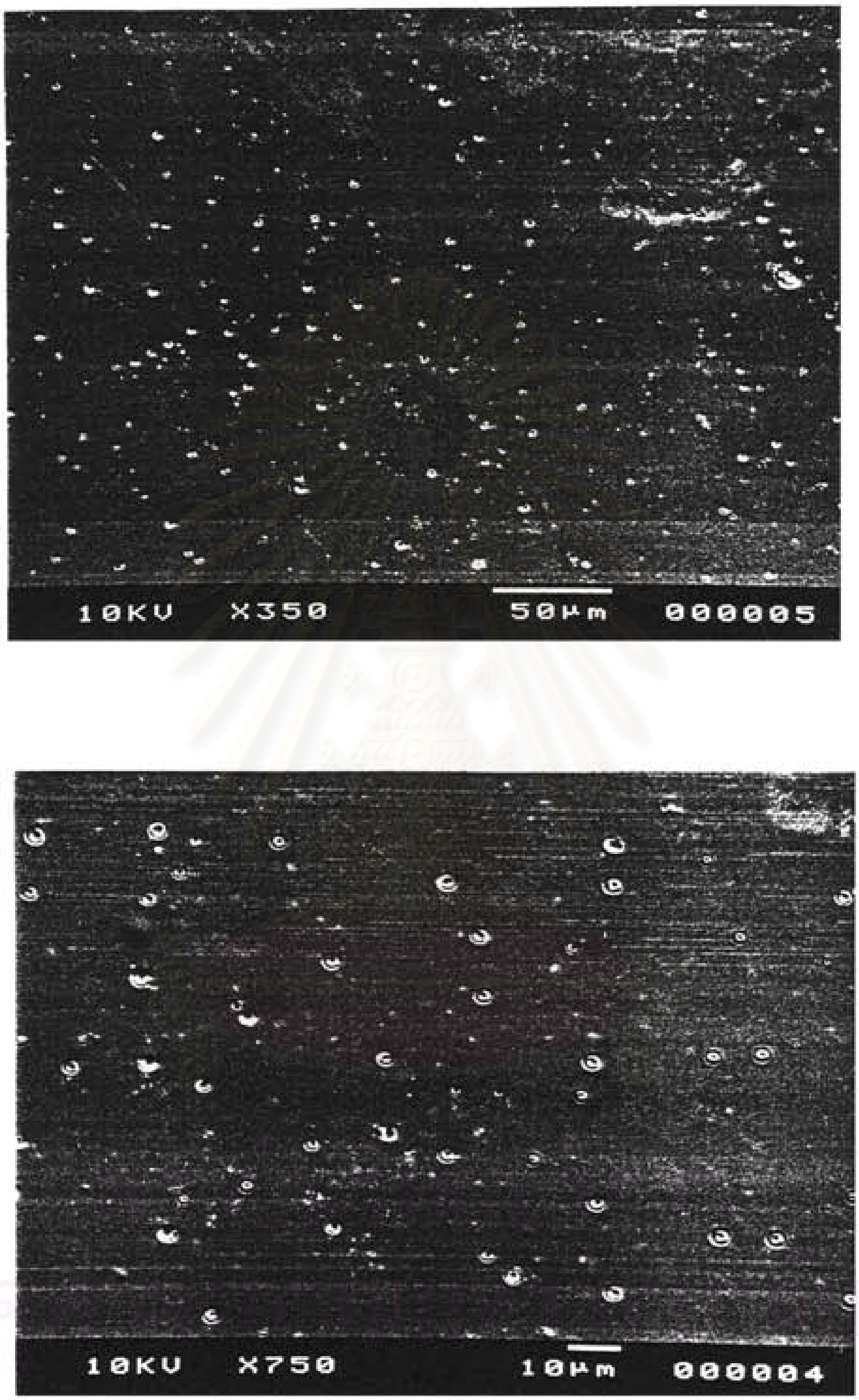


Figure 14. Surface photomicrographs of crosslinked chitosan - PVA 30,000 membrane at magnification of 350 x and 750 x (CPL$_{40}$; chitosan : PVA 1 : 4)

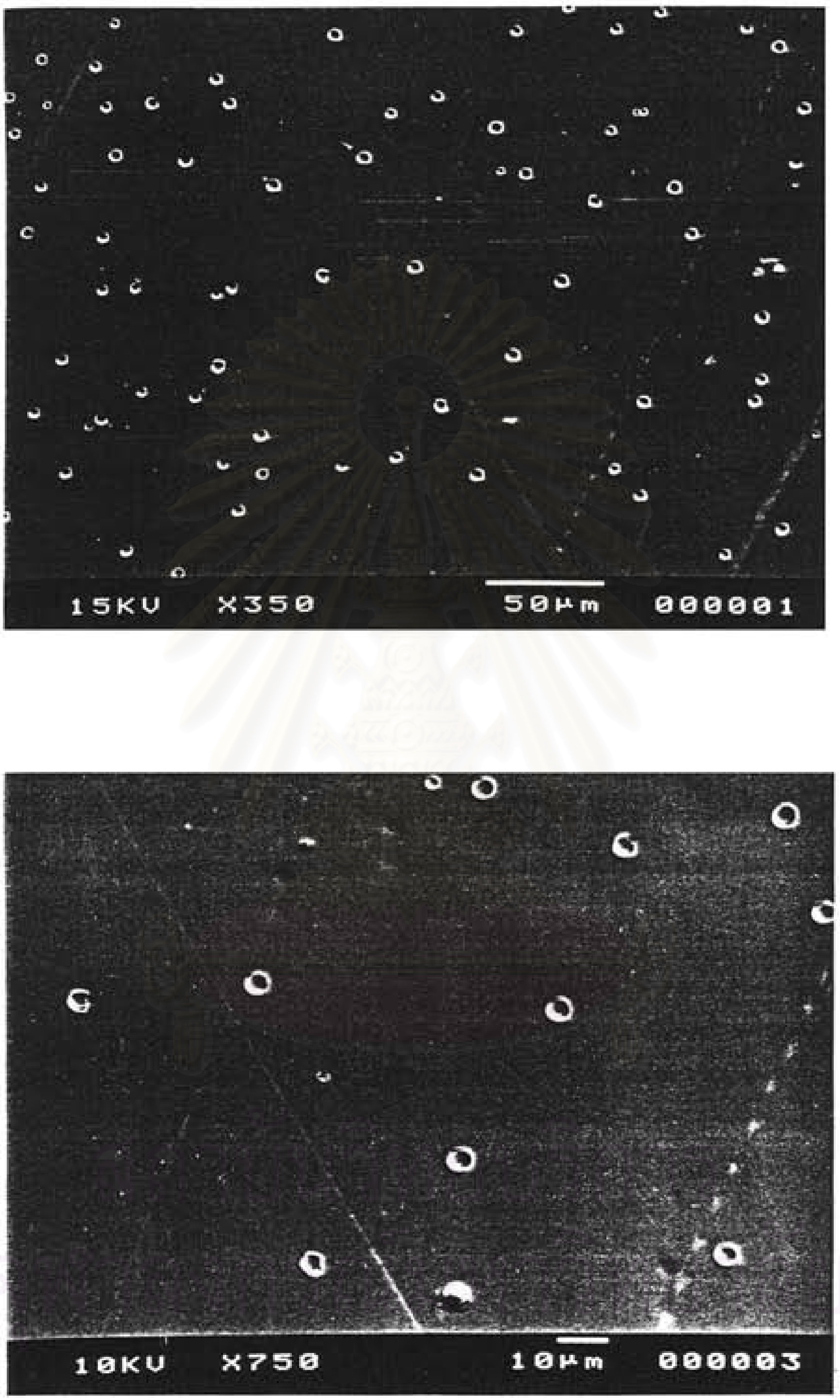
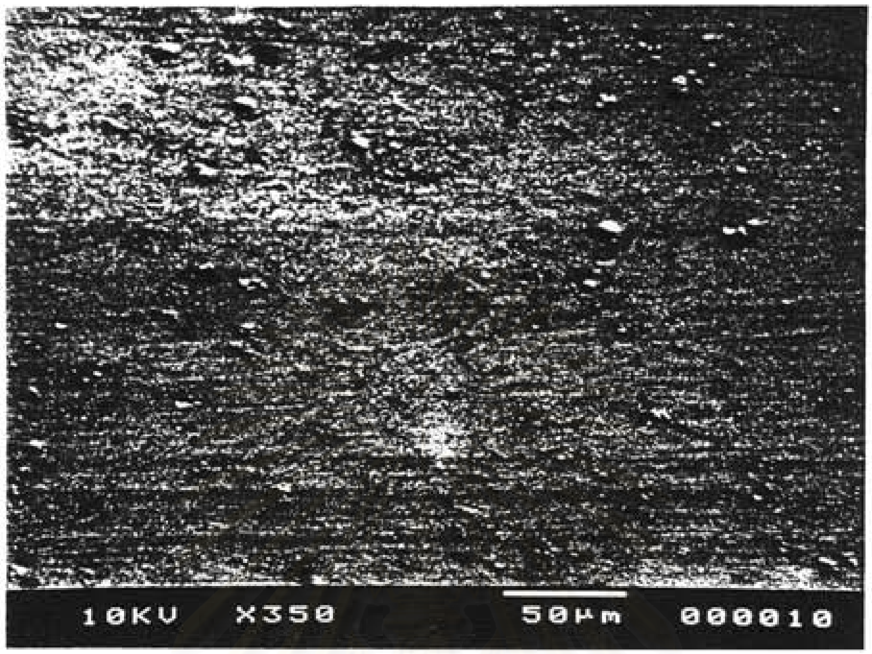


Figure 15. Surface photomicrographs of crosslinked chitosan - PVA 30,000 membrane at magnification of 350 x and 750 x (CPL_{45} ; chitosan : PVA 1 : 9)



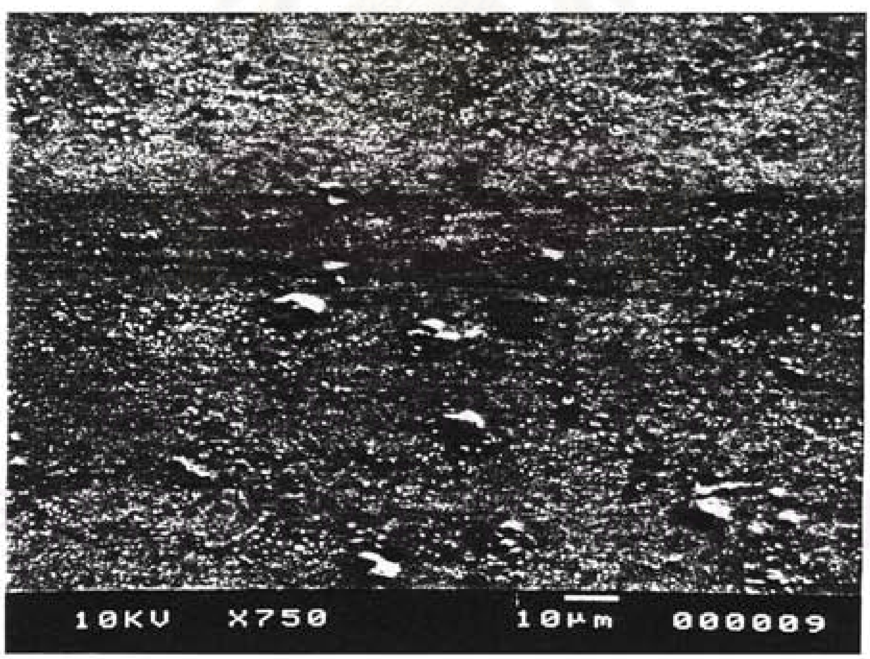
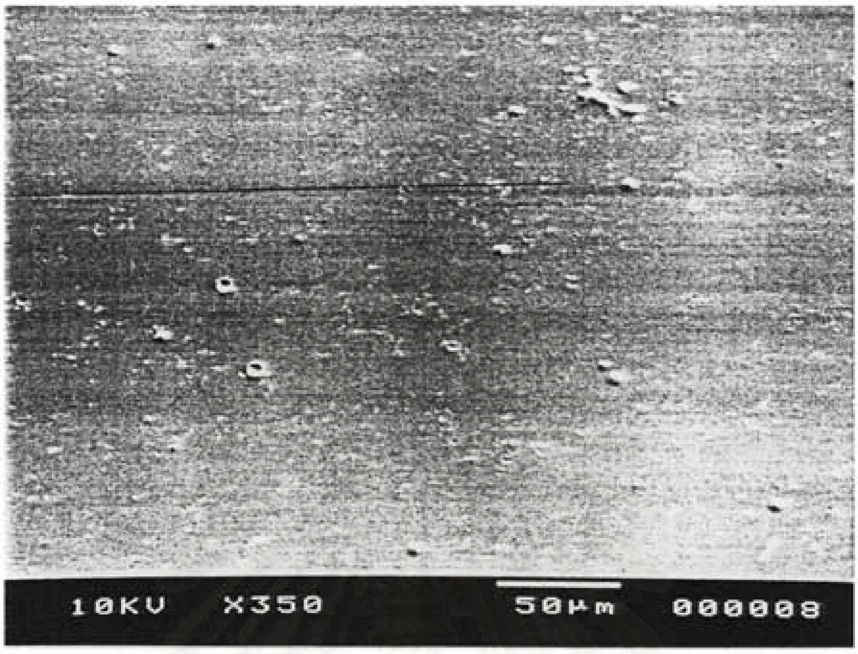


Figure 16. Surface photomicrographs of crosslinked chitosan - PVA 70,000 membrane at magnification of 350 x and 750 x (CPM_{30} ; chitosan : PVA 2 : 3)



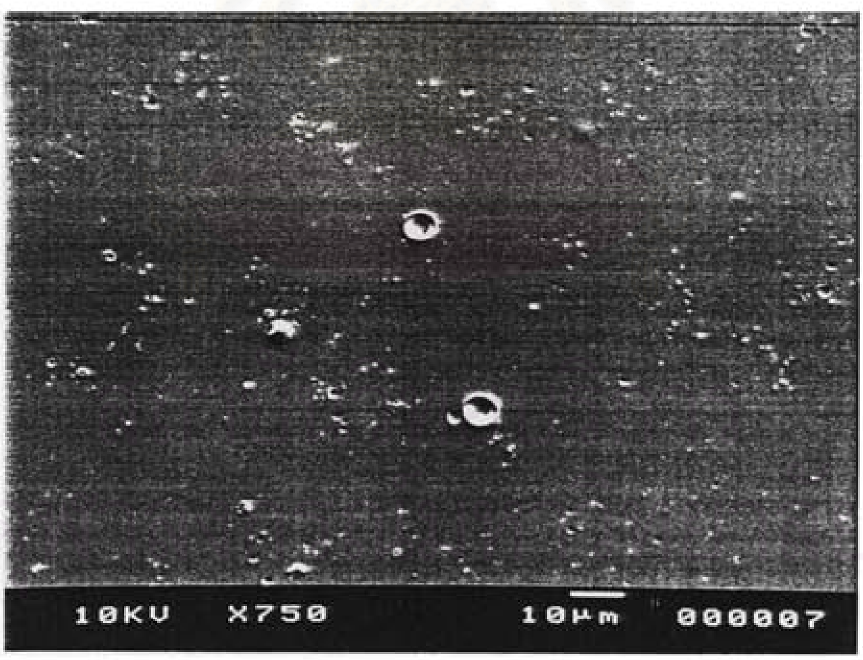


Figure 17. Surface photomicrographs of crosslinked chitosan - PVA 70,000 membrane at magnification of 350 x and 750 x (CPM_{35} ; chitosan : PVA 3 : 7)

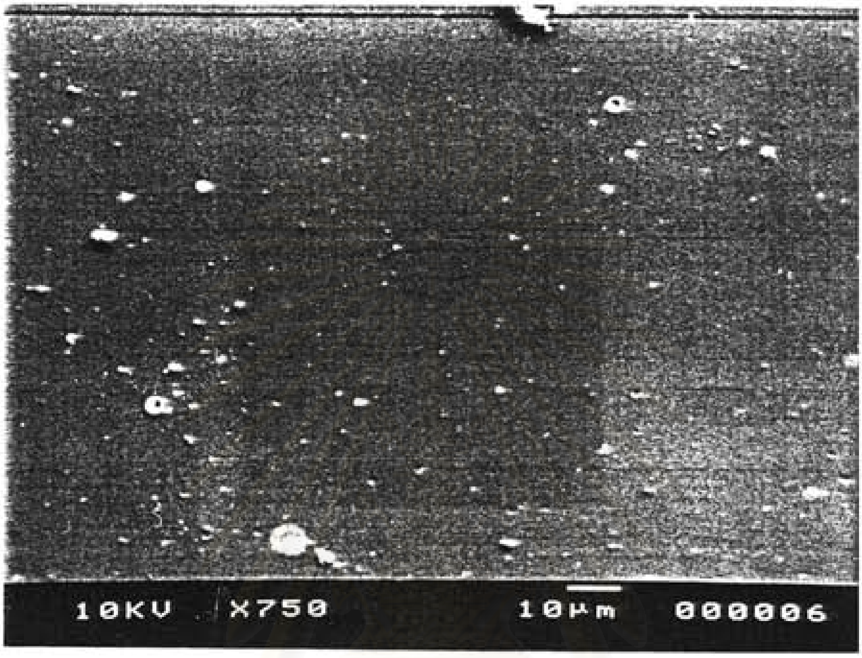
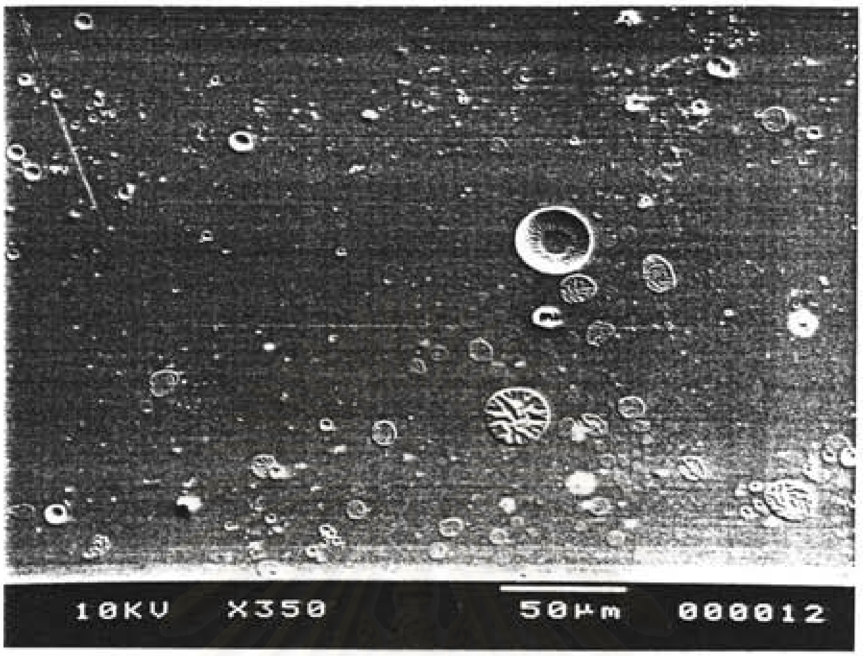


Figure 18. Surface photomicrographs of crosslinked chitosan - PVA 70,000 membrane at magnification of 750 x (CPM$_{40}$; chitosan : PVA 1 : 4)



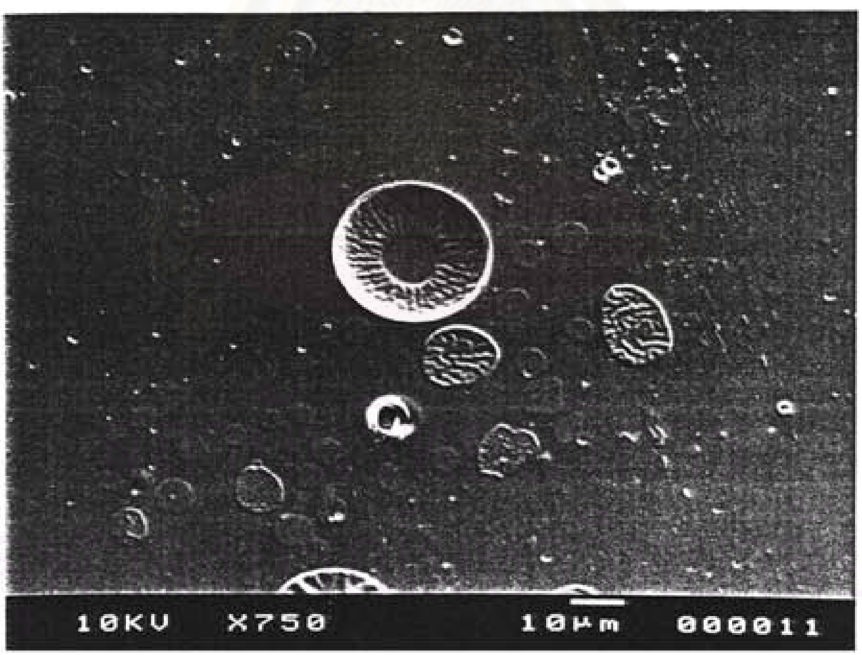


Figure 19. Surface photomicrographs of crosslinked chitosan - PVA 100,000 membrane at magnification of 350 x and 750 x. (CPH_{40} ; chitosan : PVA 1 : 4)

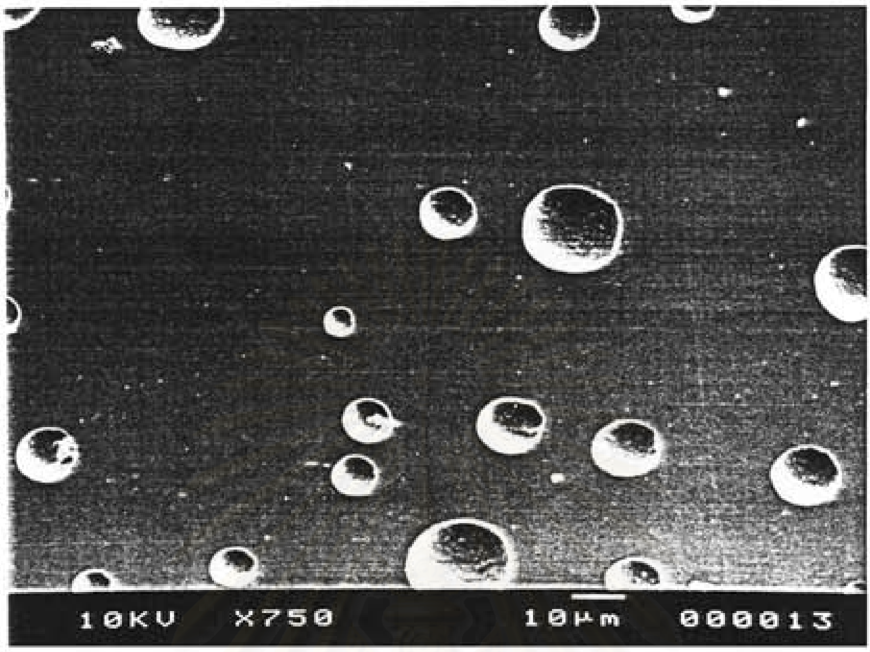
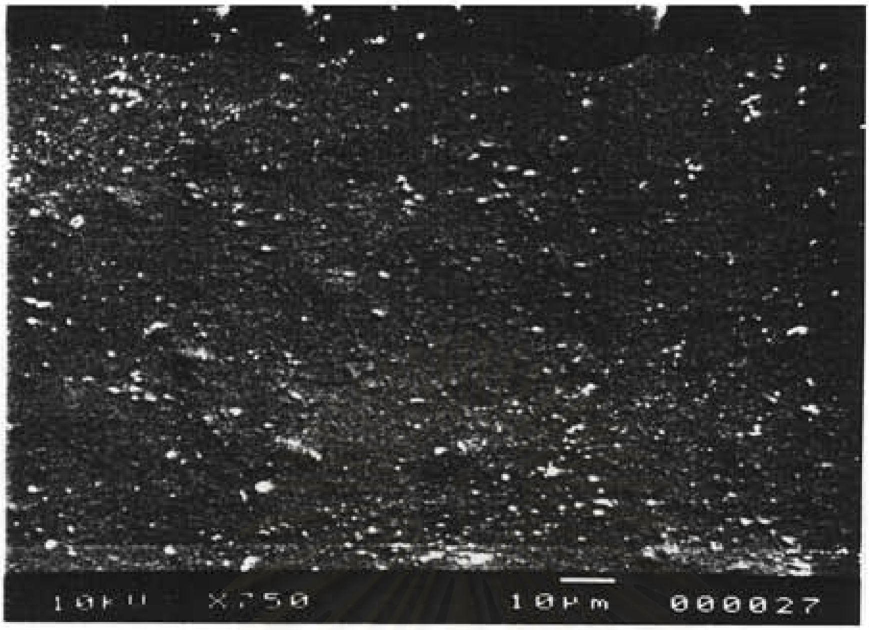
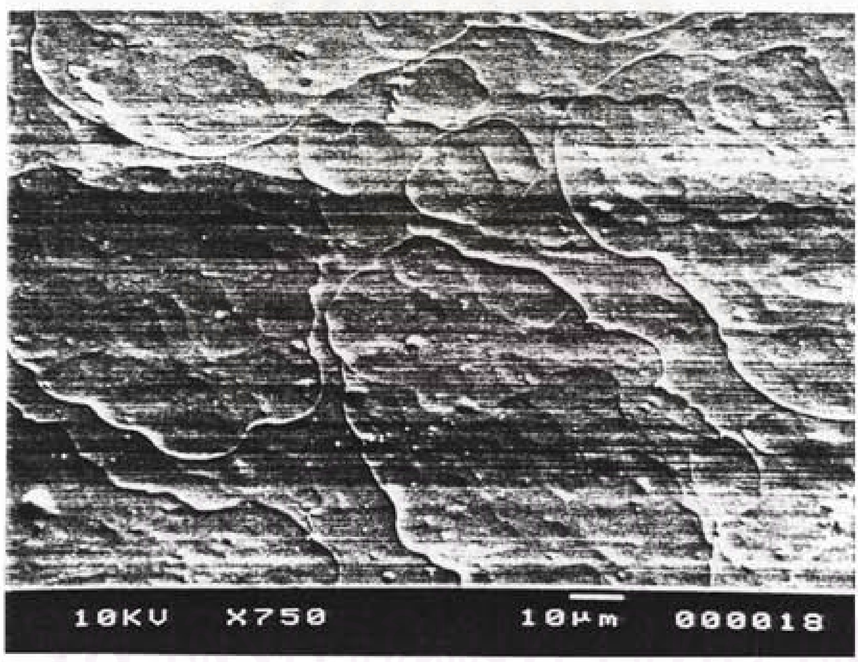


Figure 20. Surface photomicrographs of plasticized crosslinked chitosan - corn starch membrane at magnification of 750 x (CC_{16} ; chitosan : corn starch 4 : 1)



a



b

Figure 21. Surface photomicrographs of plasticized crosslinked chitosan - potato starch membrane at magnification 750 x

(a) CP_8 ; chitosan : potato starch 9 : 1)

(b) CP_{16} ; chitosan : potato starch 4 : 1)

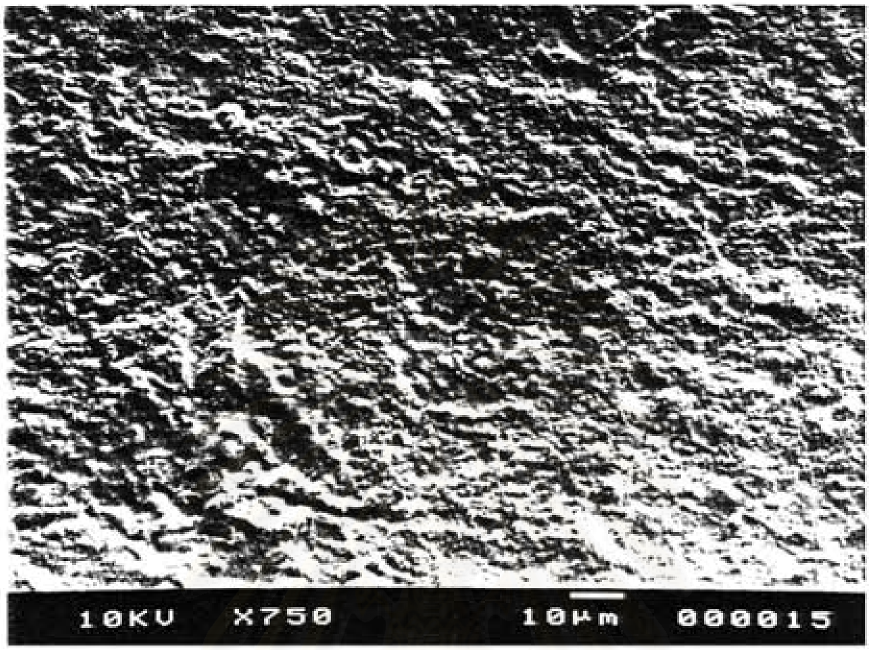


Figure 22. Surface photomicrographs of plasticized crosslinked chitosan - tapioca starch membrane at magnification 750 x (CT_{47} ; chitosan : tapioca starch 2 : 3)

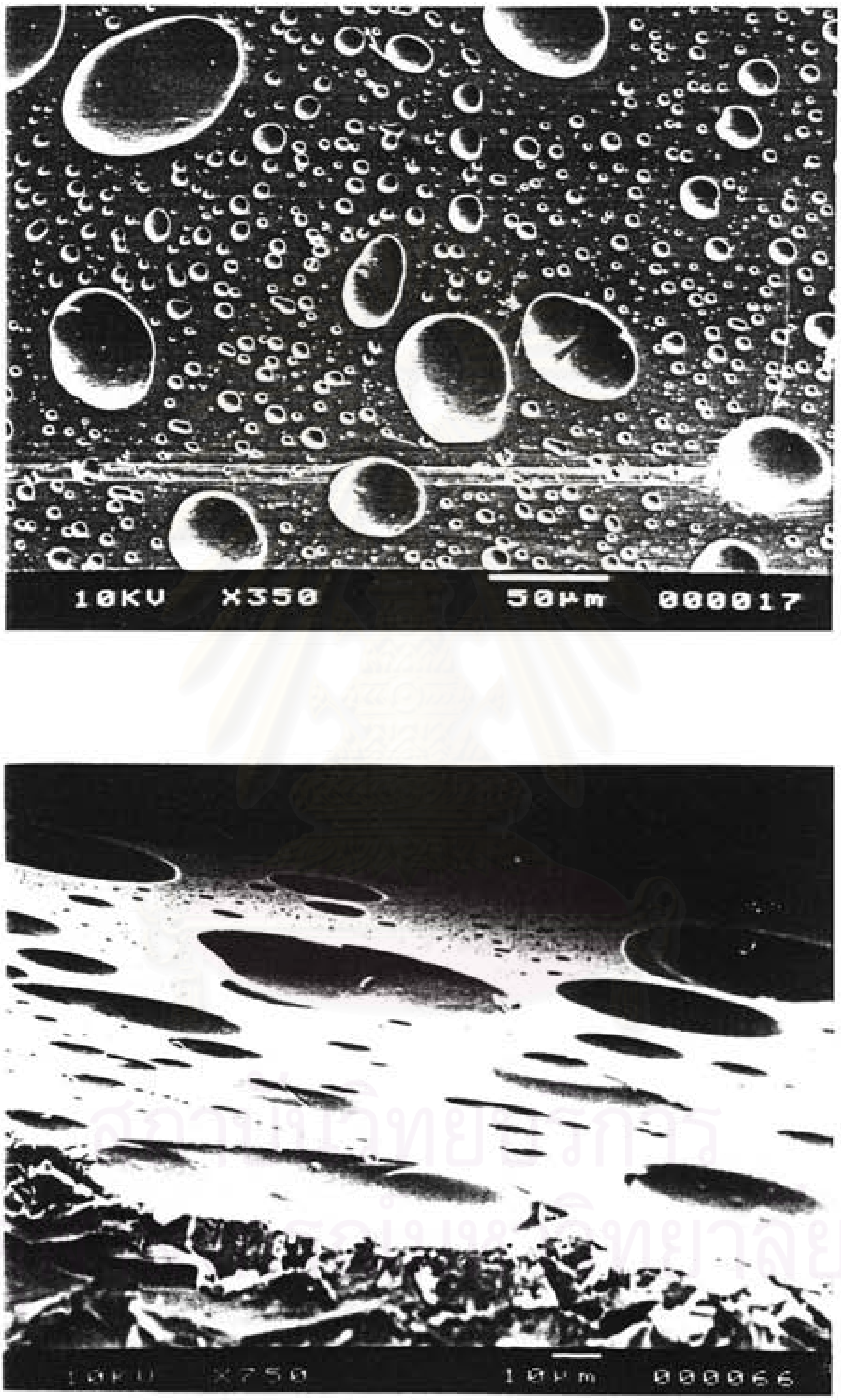


Figure 23. Surface photomicrographs of plasticized crosslinked chitosan - tapioca starch membrane at magnification 350 x and 750 x (CT_{48} ; chitosan : tapioca starch 2 : 3)

Focused on crosslinked chitosan - PVA 30,000 membranes, pores structure were found in their polymer networks. These uniformly dispersed in the free film. Comparison CPL_{40} membrane formulation (1 : 4 chitosan : PVA) with CPL_{45} membrane formulation (1 : 9 chitosan : PVA), the porosity and pore size of CPL_{45} membrane formulation was higher and larger than those of CPL_{40}. For crosslinked chitosan - PVA 70,000 (CPM_{30}, CPM_{35}, CPM_{40}) and 100,000 (CPH_{40}) membranes, the photomicrographs showed rather smooth surface and low porosity. From the photomicrographs, it could be concluded that, molecular weight of PVA affected the surface morphology of crosslinked chitosan - PVA membranes. Low molecular weight and high concentration of PVA led to high porosity of membrane.

Plasticized crosslinked chitosan - corn starch membrane (CC_{16}) showed rough membrane surface with numerous pits. The same as plasticized crosslinked chitosan - tapioca starch membrane (CT_{48}), the surface characteristic was very rough irregular pit size. The surface morphology of unplasticized crosslinked chitosan - tapioca starch membrane (CT_{47}) was nonpitted membrane. The surface of plasticized crosslinked chitosan - potato starch membranes (CP_8, CP_{16}) were also nonpitted. Varying concentration of potato starch in the formulation showed no effect on the surface characteristics.

Surface area

The surface area of some crosslinked chitosan - polymer films used as controlled release membranes for isosorbide dinitrate transdermal system were measured. The data was listed in Table 22.

Table 22. Surface Area Data of Selected Crosslinked Chitosan - Polymer Membranes.

Formulations	Surface area (m^2 / g)
CPL_{40}	0.38
CPL_{45}	0.66
CPM_{40}	0.22
CPH_{40}	0.14
CC_{16}	0.39

The difference in surface area of these membranes was due to the different characteristics of the surface. The porous or rough membrane was likely to have higher surface area than the smooth membrane. Therefore the surface area measurement could present surface morphology of membrane and elucidate the controlled release mechanism.

Infrared spectra

Infrared spectra of pure substances and selected crosslinked chitosan - polymer membranes were exhibited in Figures 24 to 29. In general IR spectra of chitosan was divided into 3 zones ; between 3600 - 3200 cm^{-1} indication stretching - vibration of free O-H group (3650-3580 cm^{-1}), of bonding O-H group (3550-3200 cm^{-1}) and of N-H group (3520-3400 cm^{-1}) ; between 1694-1515 cm^{-1} indicating stretching - vibration of C = O group (1694-1650 cm^{-1}) and bending N-H group (1650-1515 cm^{-1}); between 1170-1114 cm^{-1} indicating C-O-C stretching (Ritthidej et al., 1994). IR spectra of all molecular weight of polyvinyl alcohol showed O-H stretching-vibration of intermolecular hydrogen bonding at 3500-3200 cm^{-1} and C-H stretching-vibration at 3000-2800 cm^{-1}. In higher molecular weight of polyvinyl alcohol, the peak at 3500-3200 cm^{-1} region was longer and sharper. IR spectra of all type of starches showed O-H stretching - vibration at 3500-3200 cm^{-1} too (Silverstein Bassler and Morrill, 1981).

The characteristic of pure chitosan spectrum differed from both unplasticized and plasticized crosslinked chitosan - polymer membrane spectra. The important peak in IR spectrum of crosslinked chitosan - polymer membrane was the appearing of a double peak between 1660 and 1590 cm^{-1} which was the characteristics of the C=N group. This result was closed to the result which observed by Thacharodi and Rao (1993). The other difference between spectrum of pure chitosan and crosslinked chitosan - polymer membrane was the disappearance of the crystallization

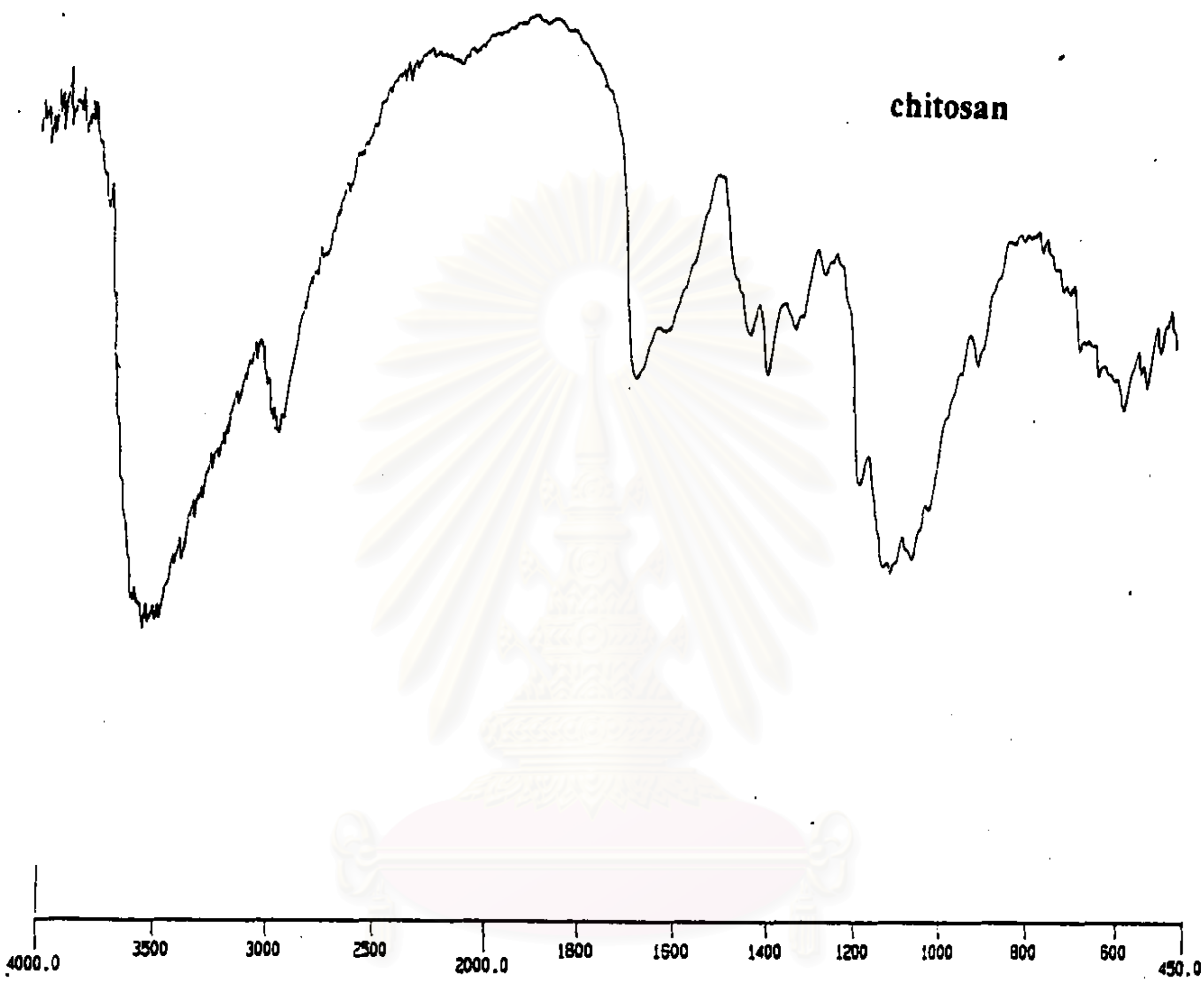


Figure 24 IR spectrum of chitosan

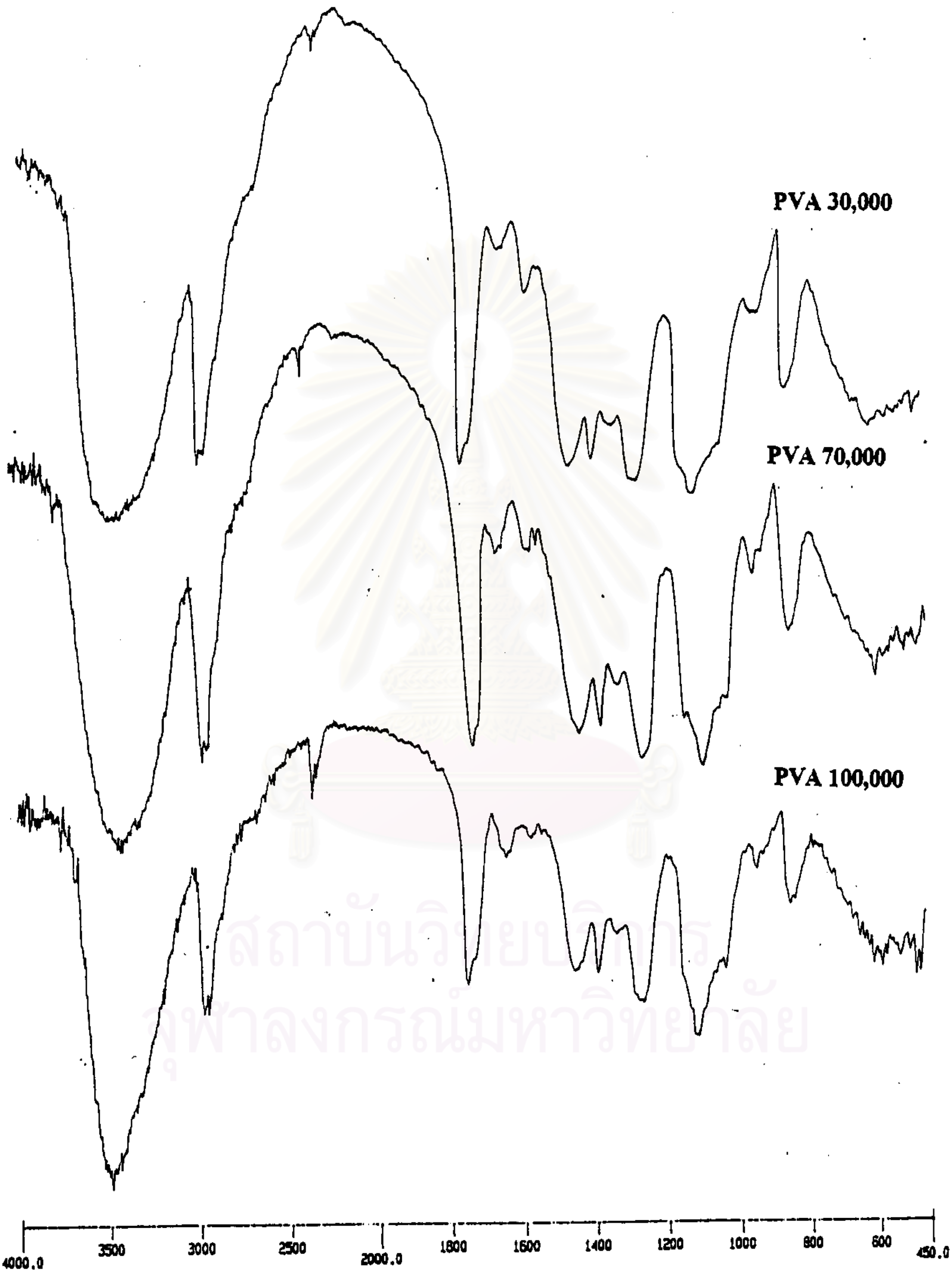


Figure 25. IR spectra of PVA 30,000 , PVA 70,000 and PVA 100,000

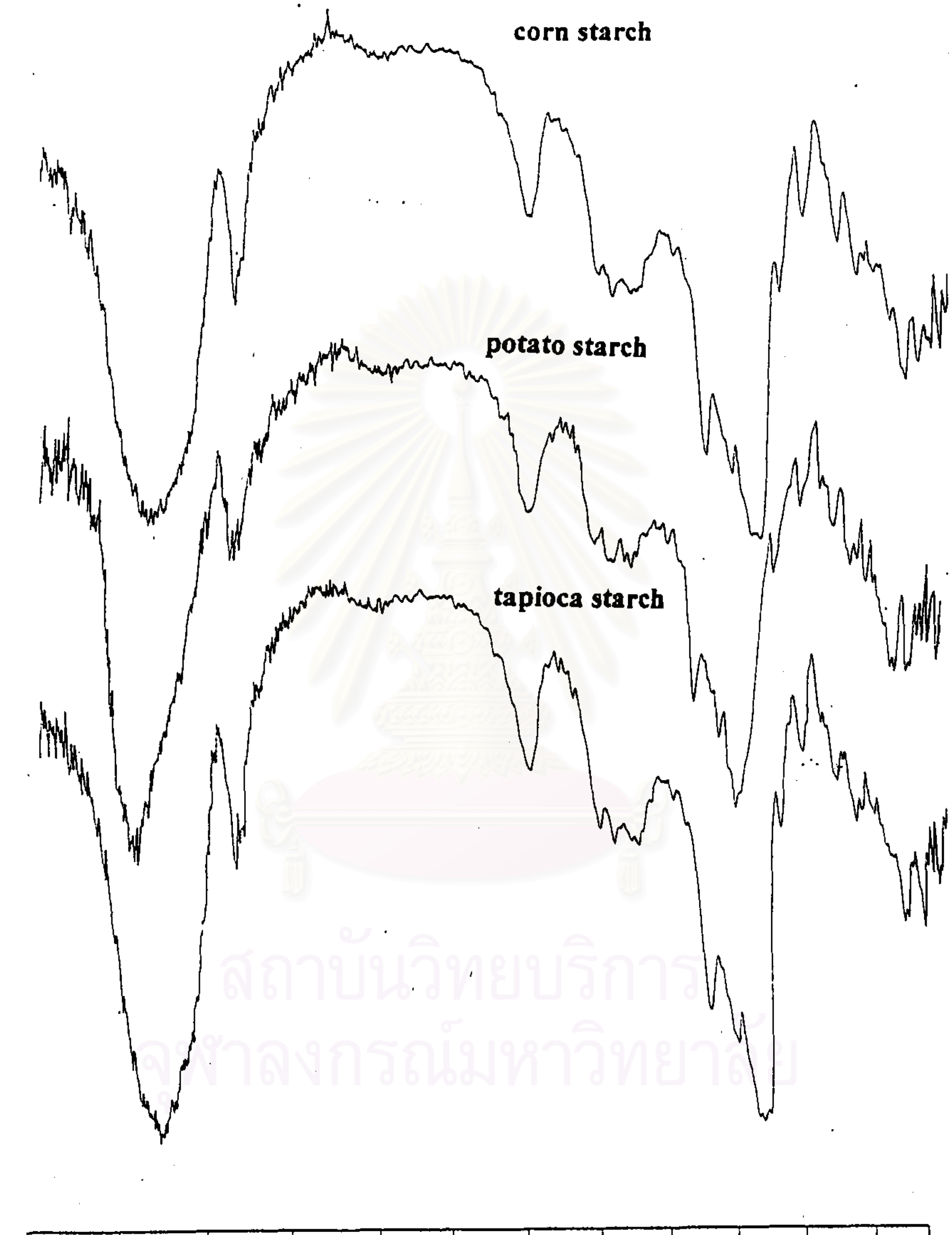


Figure 26. IR spectra of corn , potato and tapioca starch

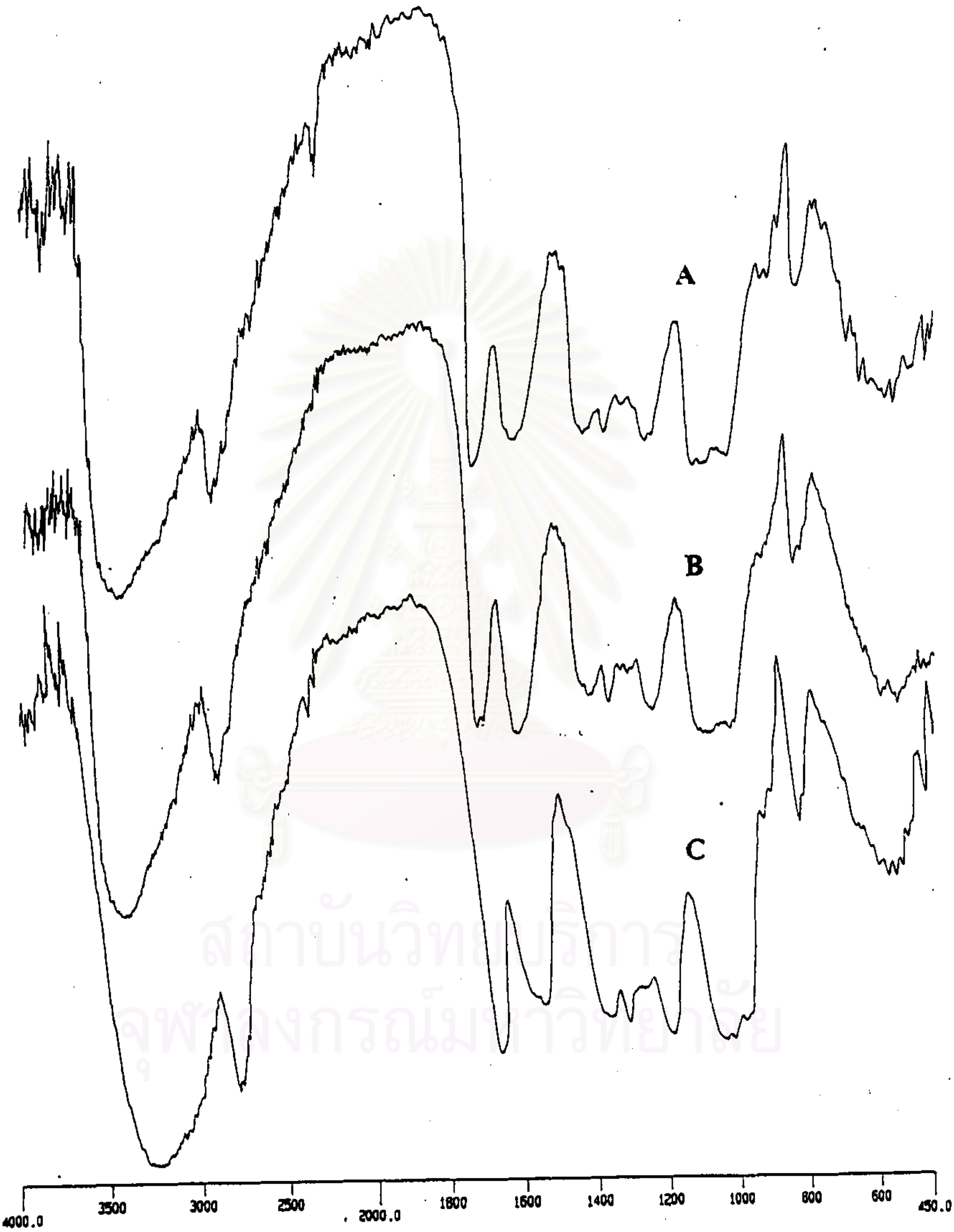


Figure 27. IR spectra of crosslinked chitosan : PVA 30,000 1: 9 (**A**), crosslinked chitosan : PVA 70,000 1: 9 (**B**) and crosslinked chitosan : PVA 100,000 1: 9 (**C**) membrane

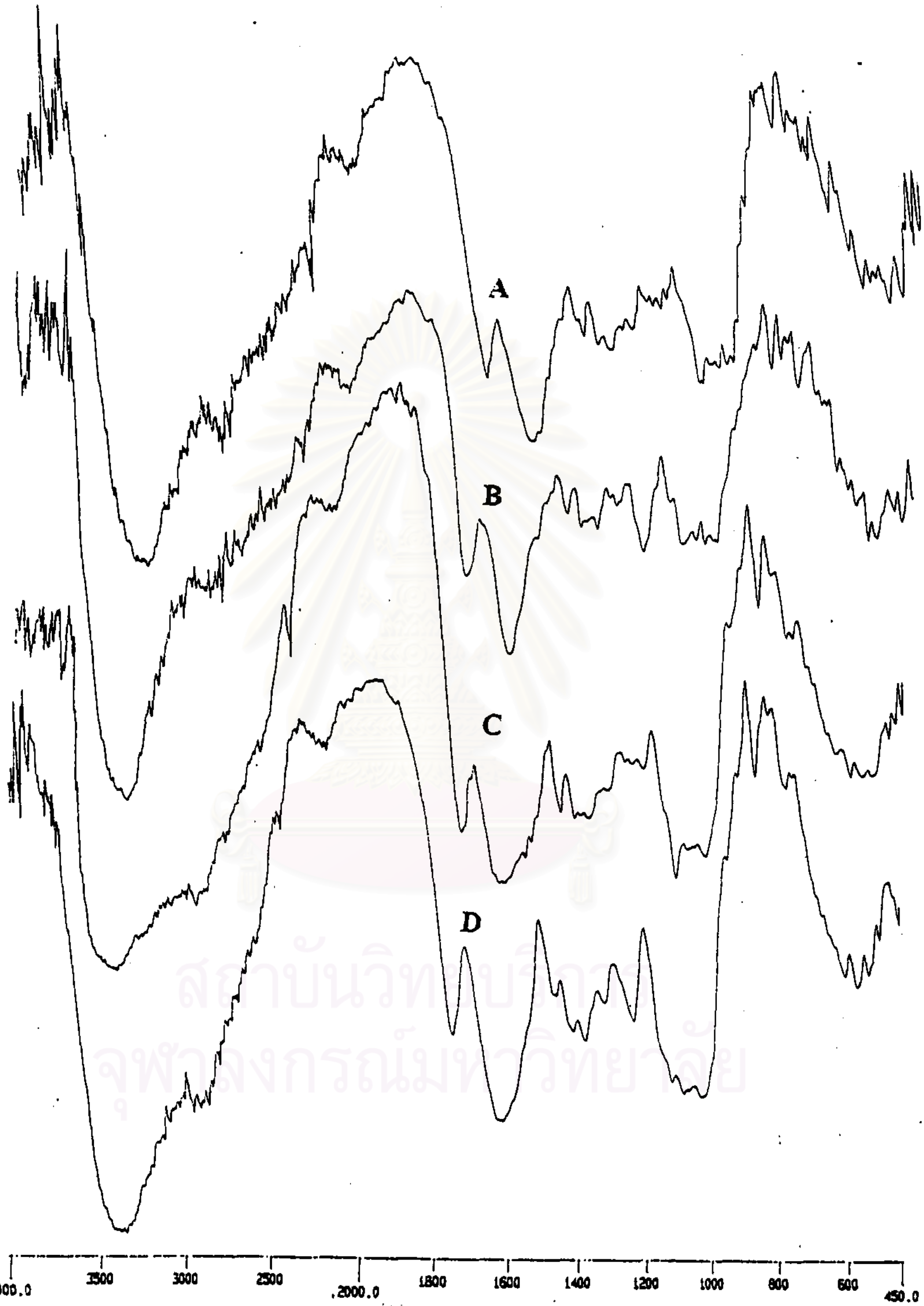


Figure 28. IR spectra of crosslinked chitosan : corn starch 4 :1 (**A**), plasticized crosslinked chitosan : corn starch 4 :1 (**B**), crosslinked chitosan : potato starch 4 :1 (**C**) and plasticized crosslinked chitosan : potato starch 4 :1 (**D**) membrane

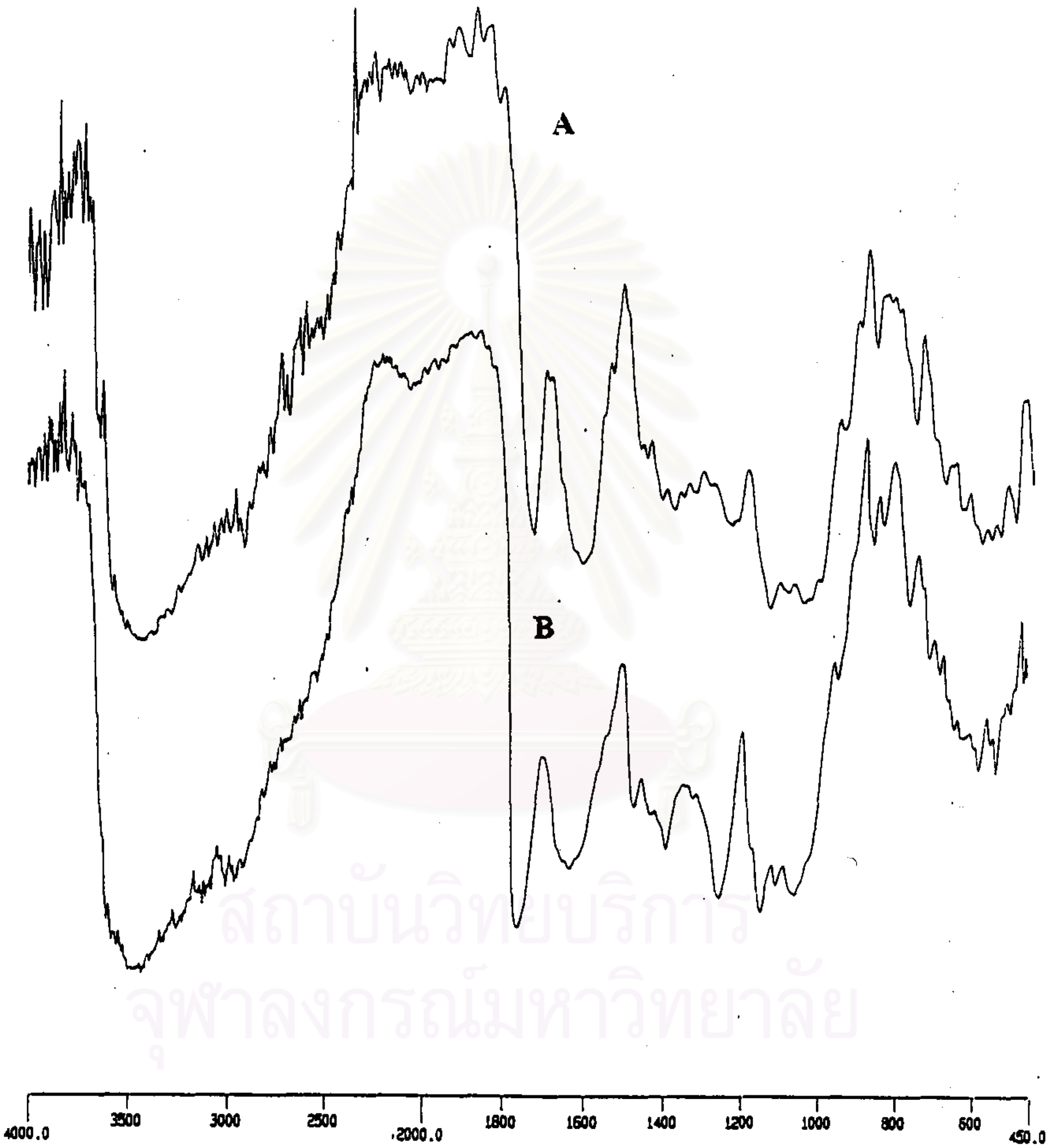


Figure 29. IR spectra of crosslinked chitosan : tapioca starch 2 :3 (**A**) and plasticized crosslinked chitosan : tapioca starch 2 : 3 (**B**) membrane

peak at 3451 cm^{-1}, 1598 cm^{-1} and 1091 cm^{-1} in both unplasticized and plasticized crosslinked blend membrane (Kim et al., 1992). In addition, the band of pure chitosan at 3600-3200 cm^{-1} indicating stretching-vibration of O-H group also changed and shifted to the lower frequency.

Differential scanning calorimetry

The DSC thermograms of pure polymer substance and selected crosslinked chitosan - polymer membrane were demonstrated in Figures 30 to 36 and summarized in Table 23.

The DSC thermograms of pure polymer substance, chitosan showed melting endotherm at 138.7°C. For PVA, high molecular weight of PVA tended to have high melting endotherm. For the melting endotherm of starch, potato starch gave highest melting point.

All investigated crosslinked chitosan - polymer membranes did not show separation peak of pure polymer substance. The DSC thermograms of all membranes were different in pattern and endothermic peak temperature. In crosslinked chitosan - PVA membranes, the endothermic melting temperature was rather high and characteristic of endothermic peak was sharp.

For crosslinked chitosan - starch membranes, the plasticized membranes showed lower endothermic melting point than the unplasticized membranes. However, the phenomenon of the opposition of

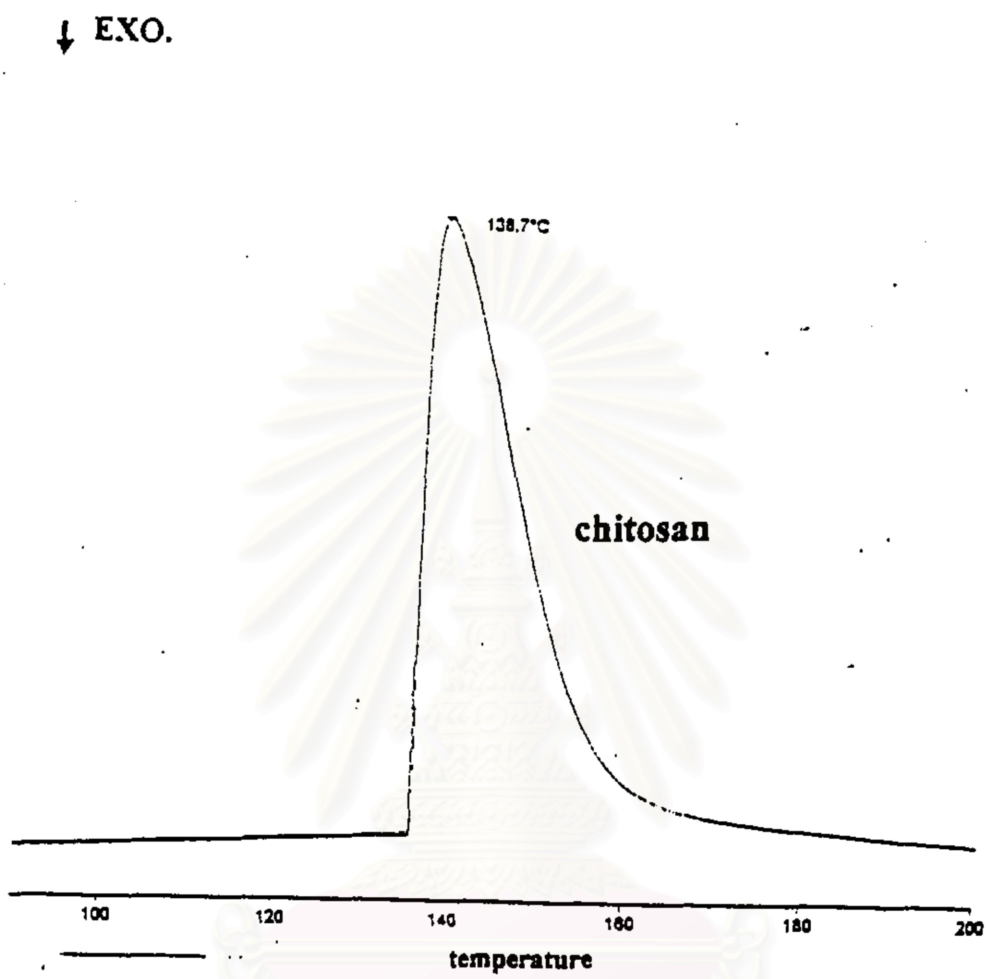


Figure 30 DSC thermogram of chitosan

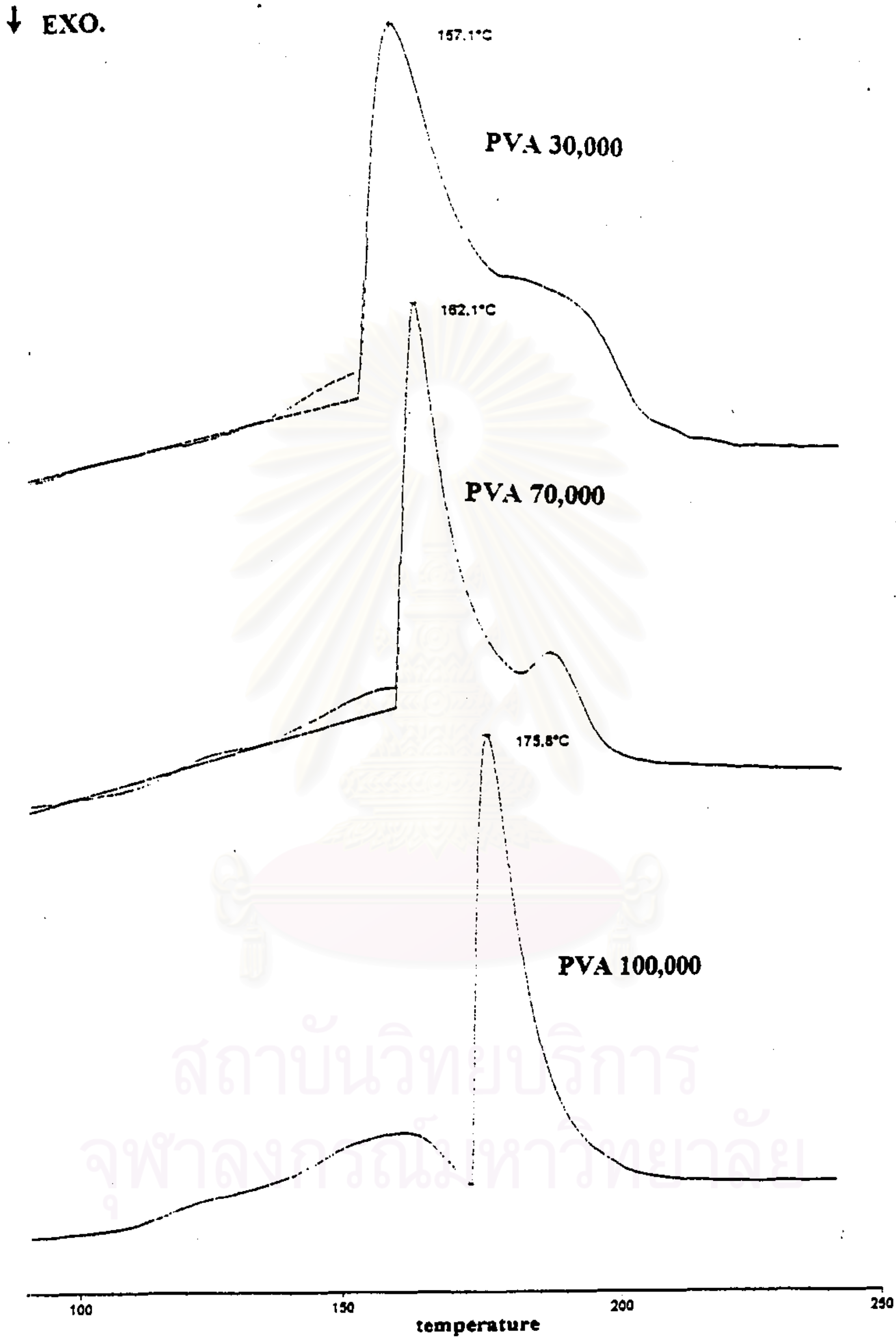


Figure 31.DSC thermograms of PVA 30,000, PVA 70,000 and PVA 100,00

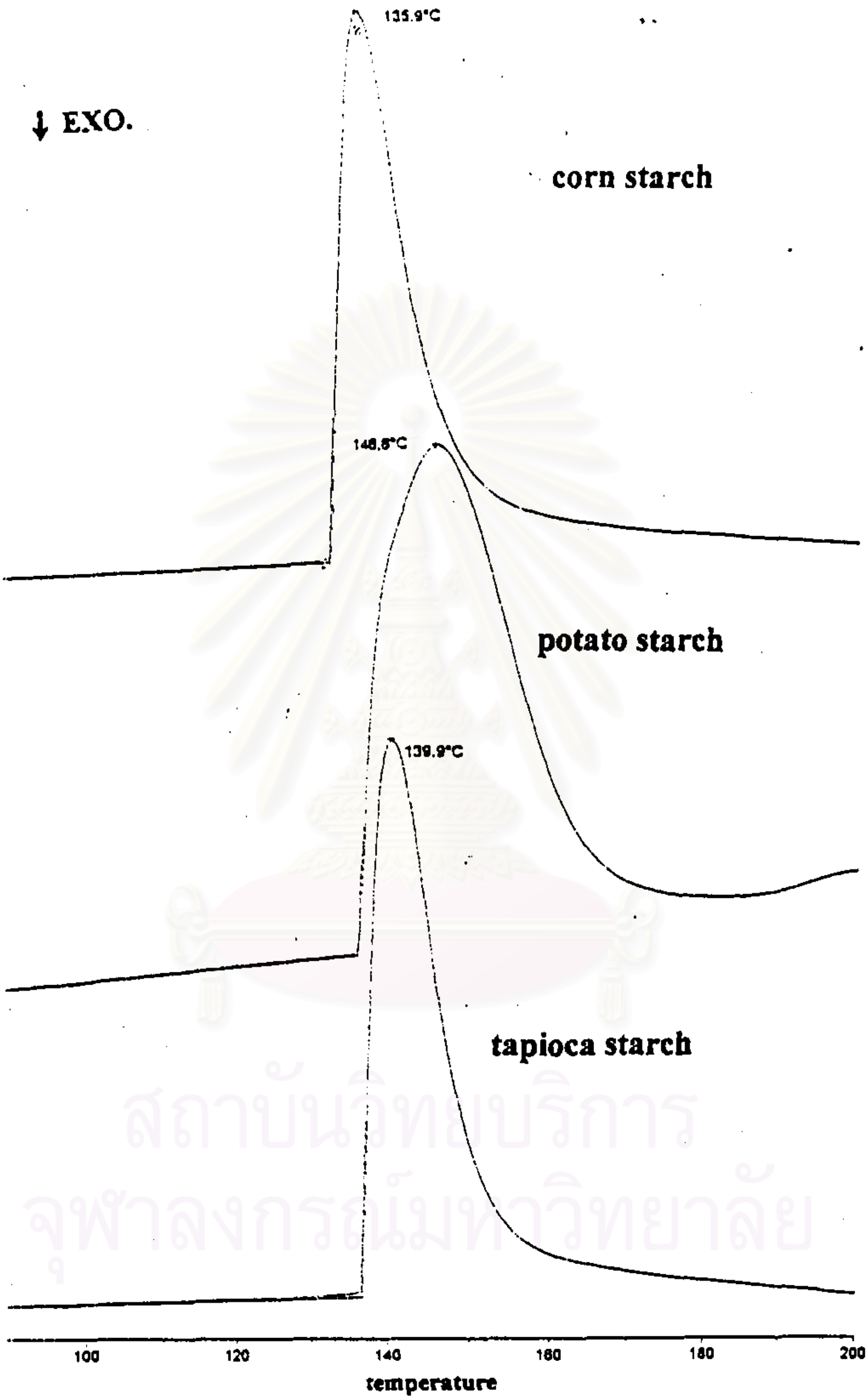


Figure 32.DSC thermograms of corn, potato and tapioca starch

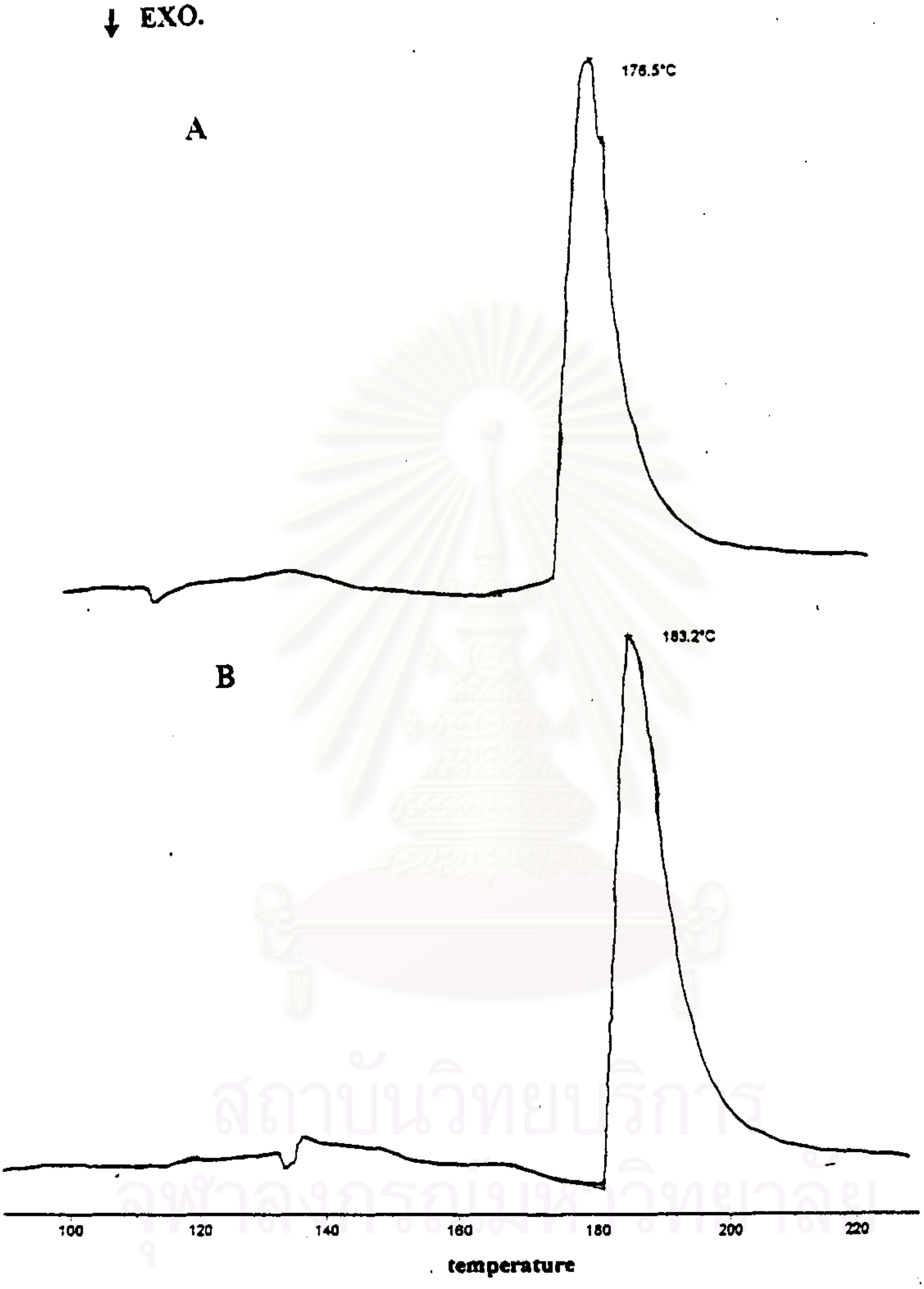


Figure 33. DSC thermograms of crosslinked chitosan : PVA 30,000 1: 9 (**A**), crosslinked chitosan : PVA 70,000 1: 9 (**B**) and crosslinked chitosan : PVA 100,000 1: 9 (**C**) membrane

(cont.)

↓ EXO.

C

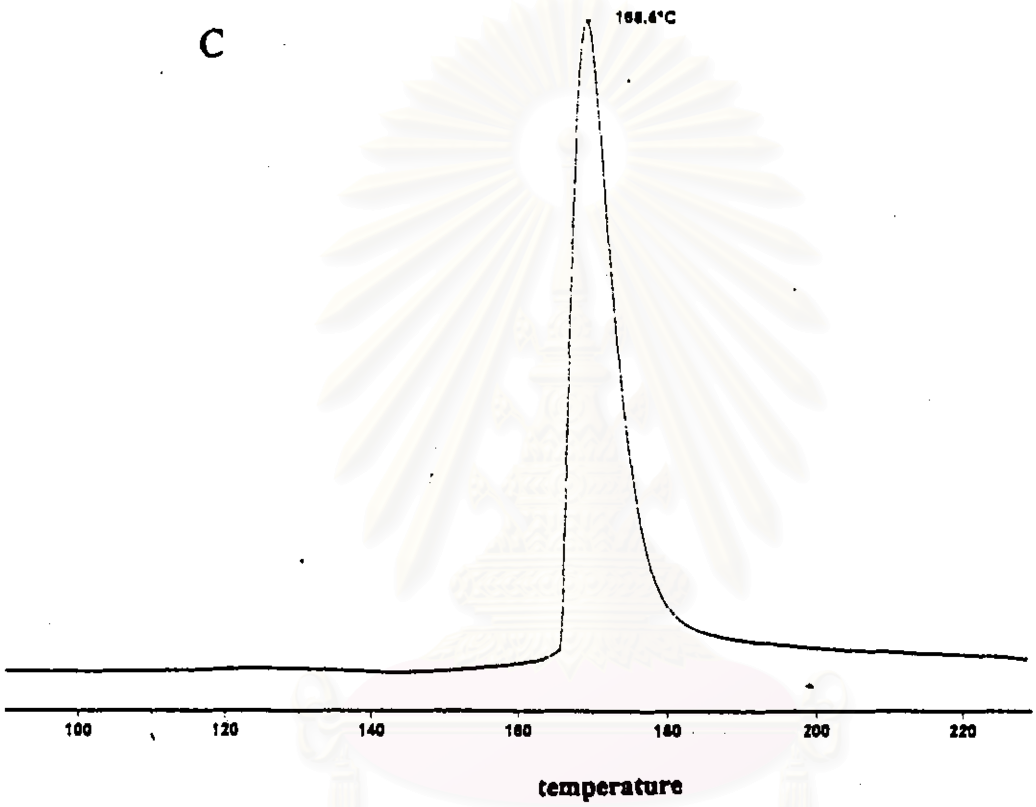


Figure 33. (cont.) DSC thermograms of crosslinked chitosan : PVA 30,000 1: 9 (A), crosslinked chitosan : PVA 70,000 1: 9 (B) and crosslinked chitosan : PVA 100,000 1: 9 (C) membrane

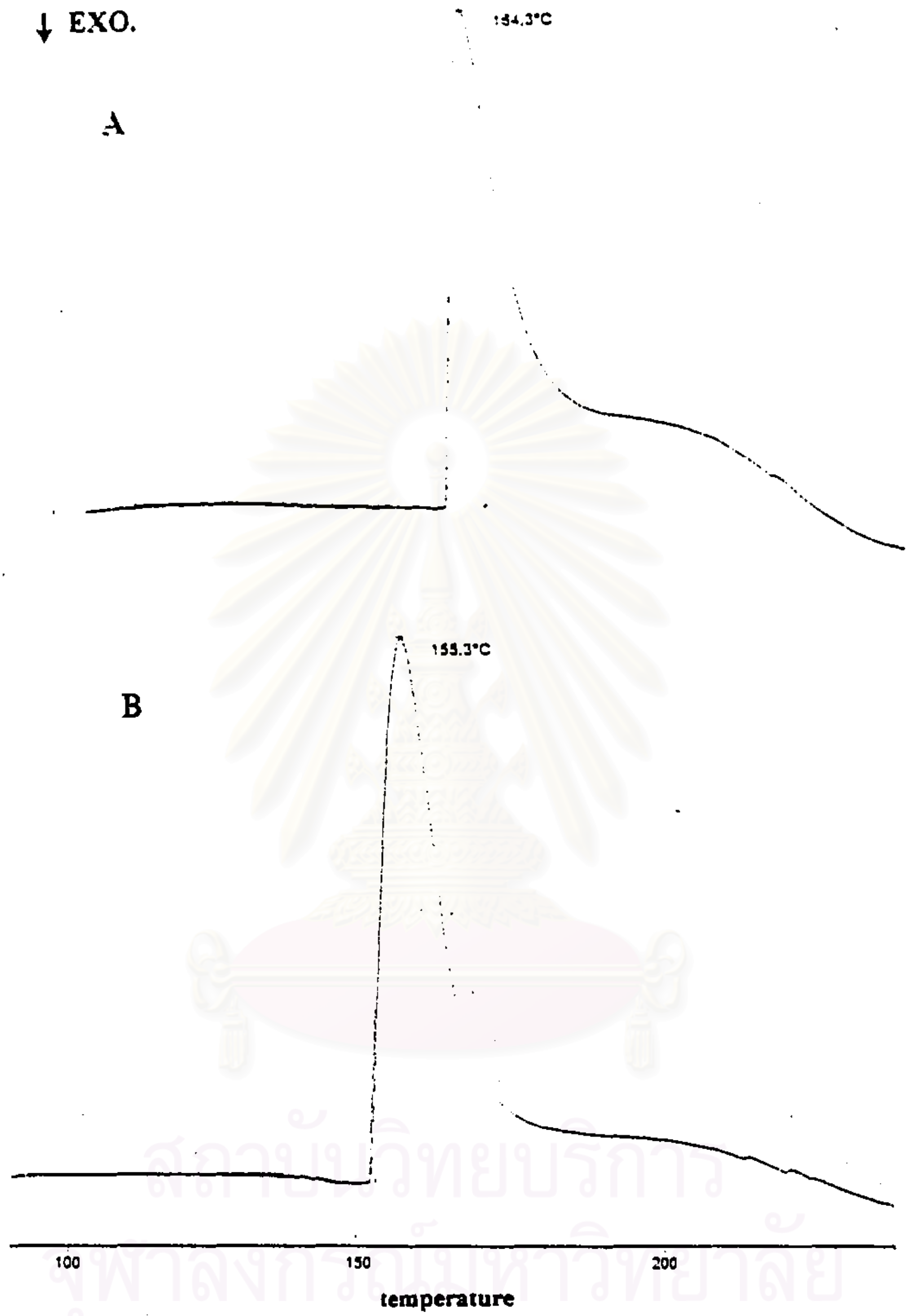


Figure 34. DSC thermograms of crosslinked chitosan : corn starch 4 :1 (**A**) and plasticized crosslinked chitosan : corn starch 4 :1 (**B**) membrane

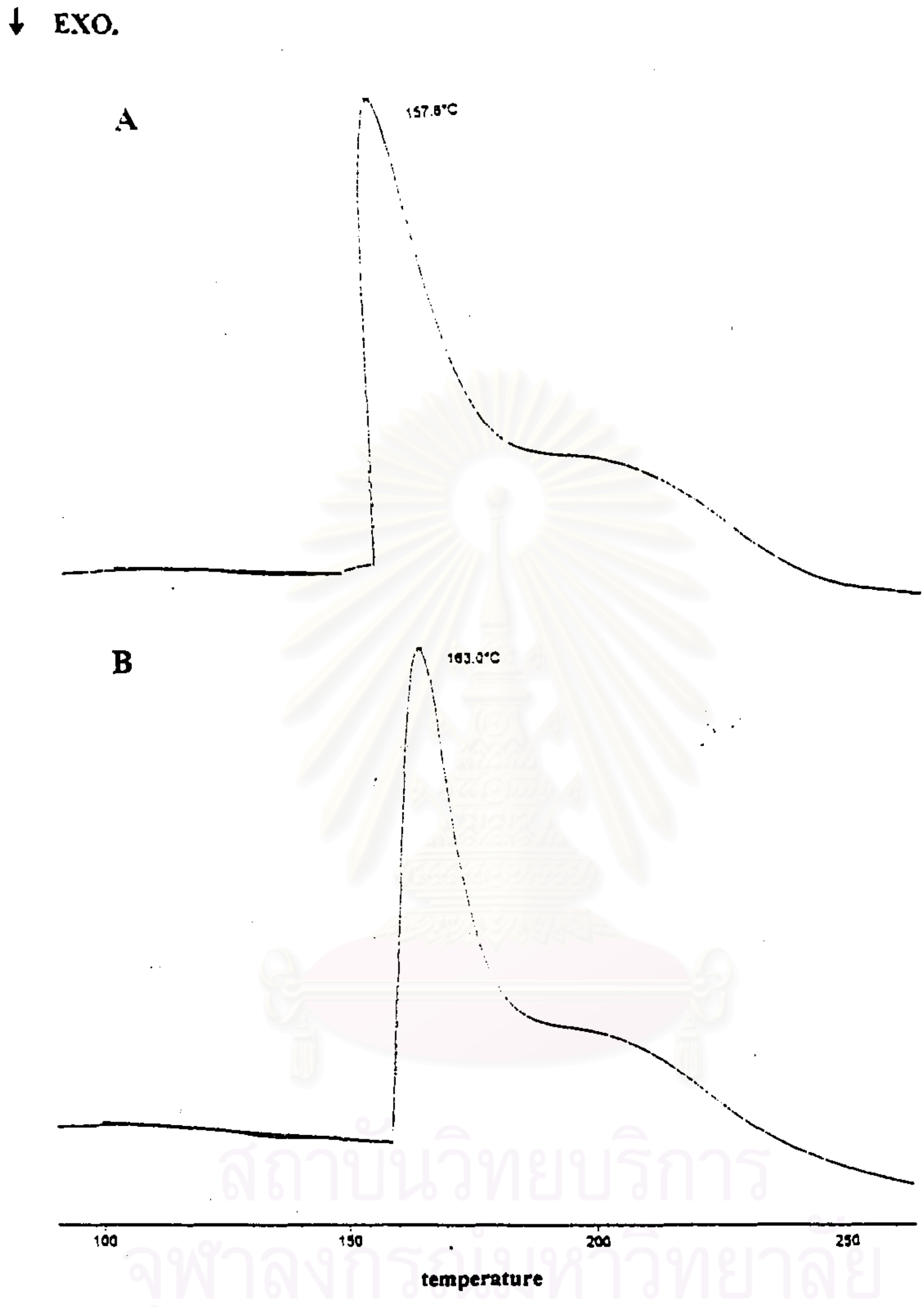


Figure 35. DSC thermograms of crosslinked chitosan : potato starch 4 :1 (**A**) and plasticized crosslinked chitosan : potato starch 4 :1 (**B**) membrane

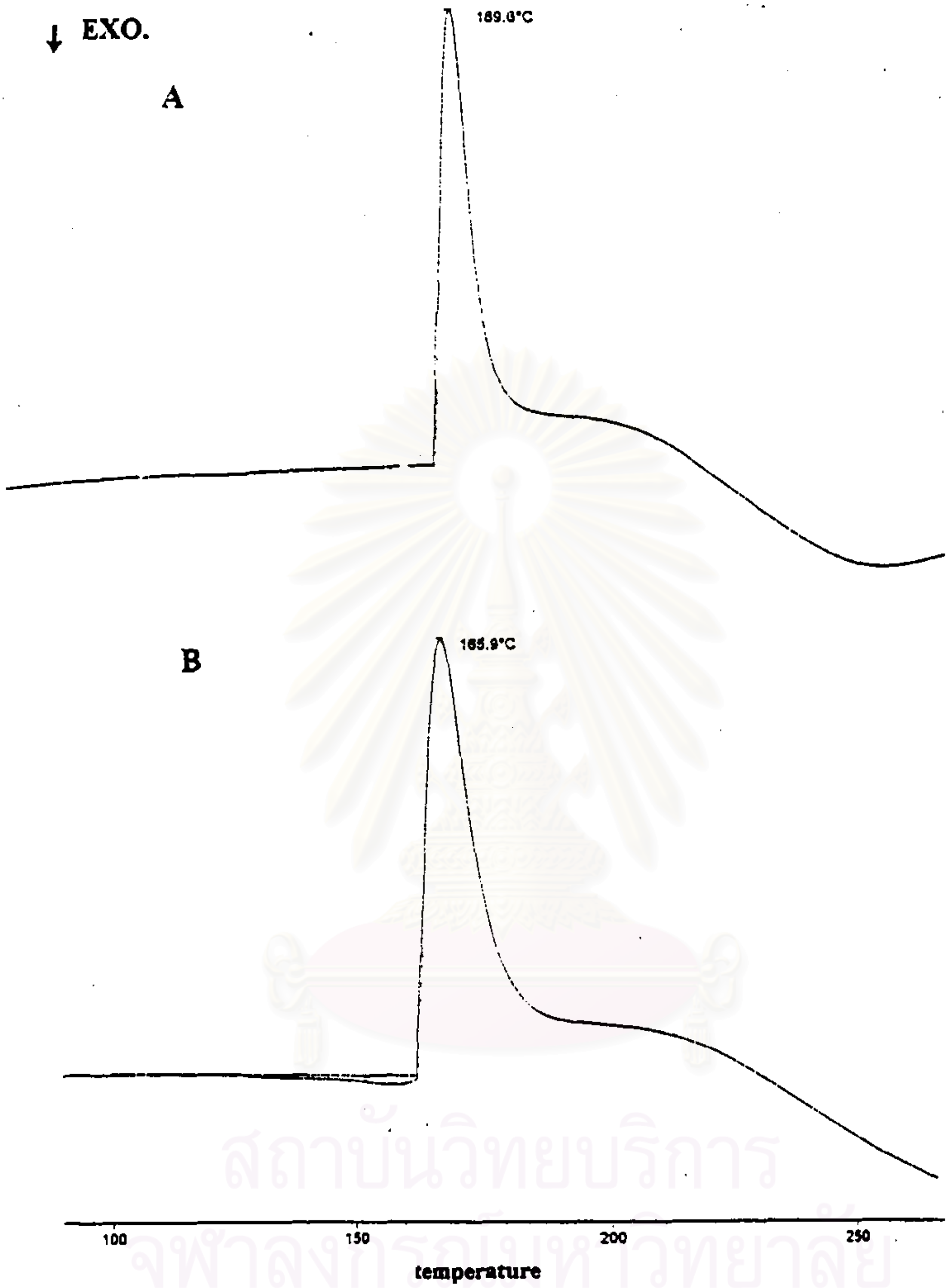


Figure 36. DSC thermograms of crosslinked chitosan : tapioca starch 2 :3 (**A**) and plasticized crosslinked chitosan : tapioca starch 2 : 3 (**B**) membrane

result between plasticized and unplasticized membranes occurred in crosslinked chitosan - potato starch membrane formulations.

Table 23. DSC Peak Temperature.

Samples	DSC peak temperature (°C)
chitosan	138.7
polyvinyl alcohol	
molecular weight 30,000	157.1
70,000	162.1
100,000	175.8
corn starch	135.9
potato starch	146.6
tapioca starch	139.9
CPL_{45} membrane	176.5
CPM_{45} membrane	183.2
CPH_{45} membrane	168.6
CC_{15}	164.3
CC_{16}	155.3
CP_{15}	157.6
CP_{16}	163
CT_{47}	169.6
CT_{48}	165.9

Membrane thickness

The mean membrane thickness of 11 formulations used to prepare isosorbide dinitrate transdermal patch were shown in Table 24. It was varied in the range of 100 $\pm$ 10 μm.

Table 24. Thickness of Various Controlled Release Membranes

Formulations	Mean thickness of sample (μm)		
CPL_{40}	109	104	107
CPL_{45}	109	104	109
CPM_{30}	105	109	110
CPM_{35}	106	107	110
CPM_{40}	106	107	109
CPH_{40}	98	108	108
CC_{16}	96	110	96
CP_{8}	110	109	110
CP_{16}	108	104	110
CT_{47}	93	94	93
CT_{48}	91	90	97

Permeation

Permeability of isosorbide dinitrate from saturated solution through shed snake skin

Cumulative amount of isosorbide dinitrate permeated through shed snake skin was exhibited in Table 25 and Figure 37.

According to the correlation coefficient of the permeation profile was 0.9860, the permeation profile of isosorbide dinitrate was rather linear. The obtained result indicated that the permeation of isosorbide dinitrate through shed snake skin was good. Therefore the efficiency of controlled release of isosorbide dinitrate transdermal system affected from only prepared crosslinked chitosan - polymer membranes.

Permeability of isosorbide dinitrate from transdermal patch

In this study, prepared isosorbide dinitrate transdermal patches were stored in the room temperature about 12 hours prior to in vitro permeation study. In addition, commercial isosorbide dinitrate transdermal drug delivery system was also investigated for its permeation profile. The cumulative permeated isosorbide dinitrate through shed snake skin of various transdermal systems was shown in Table 26 and Figures 38 to 42 showed cumulative permeated drug against time.

From the result, is could be seen that CPL_{45} (crosslinked chitosan - PVA 30,000) and CC_{16} (plasticized crosslinked chitosan - corn starch) gave the highest cumulative drug permeated through shed snake skin and CP_8, CP_{16} (plasticized crosslinked chitosan - potato starch), CT_{47} (unplasticized crosslinked chitosan - tapioca starch) and CT_{48} (plasticized crosslinked chitosan - tapioca starch) exhibited rather low cumulative amount of drug. For crosslinked chitosan - PVA membrane, the result of using high concentration of PVA led to increasing cumulative permeated drug. Moreover, using low molecular weight of PVA in crosslinked chitosan - PVA membrane formulations exhibited rather high cumulative amount of permeating drug. In crosslinked chitosan - tapioca starch, adding plasticizer in the formulation did not affect to the cumulative permeated drug.

Correlation coefficient and regression equation of the relationship between cumulative permeated isosorbide dinitrate versus time and the relationship between cumulative permeated isosorbide dinitrate versus square root time were shown in Tables 27 and 28. The permeation profile of cumulative permeated drug versus square root time also were showed in Figures 43 to 47. From all correlation coefficient, it could lead to the controlled release pattern. The correlation coefficient of relationship between cumulative amount of permeating drug against time was higher than the correlation coefficient of relationship between cumulative amount of permeating drug against square root time except in CPM_{30}, CPM_{35} and CPM_{40} membranes (crosslinked chitosan - PVA 70,000 membrane formulations). The obtained result from the statistical test indicated that, the kinetic pattern of these controlled release membrane (Table 29) was likely to be zero order except in CPM_{30} and CPM_{35} membranes which kinetic pattern might be zero order or Higuchi's model and CPM_{40} which kinetic pattern was likely to be Higuchi's model.

From preliminary study about surface morphology and the acquired data from permeation study, it might estimate that, the controlled release mechanism of these prepared membranes was likely to be pore and partition mechanism.

Table 25. Cumulative Amount of ISDN Permeated Through Shed Snake Skin

Time (hrs)	Cumulative drug permeated (mcg)
0	0
0.5	108.5917
1	155.4717
1.5	197.1927
2	237.4176
3	312.1986
4	503.9975
6	565.3617
8	762.3363
10	910.7846

Correlation coefficient = 0.9860

Regression equation $y = 84.9522x + 77.2316$

where :

x = time (hrs)

y = cumulative amount of isosorbide dinitrate (mcg)

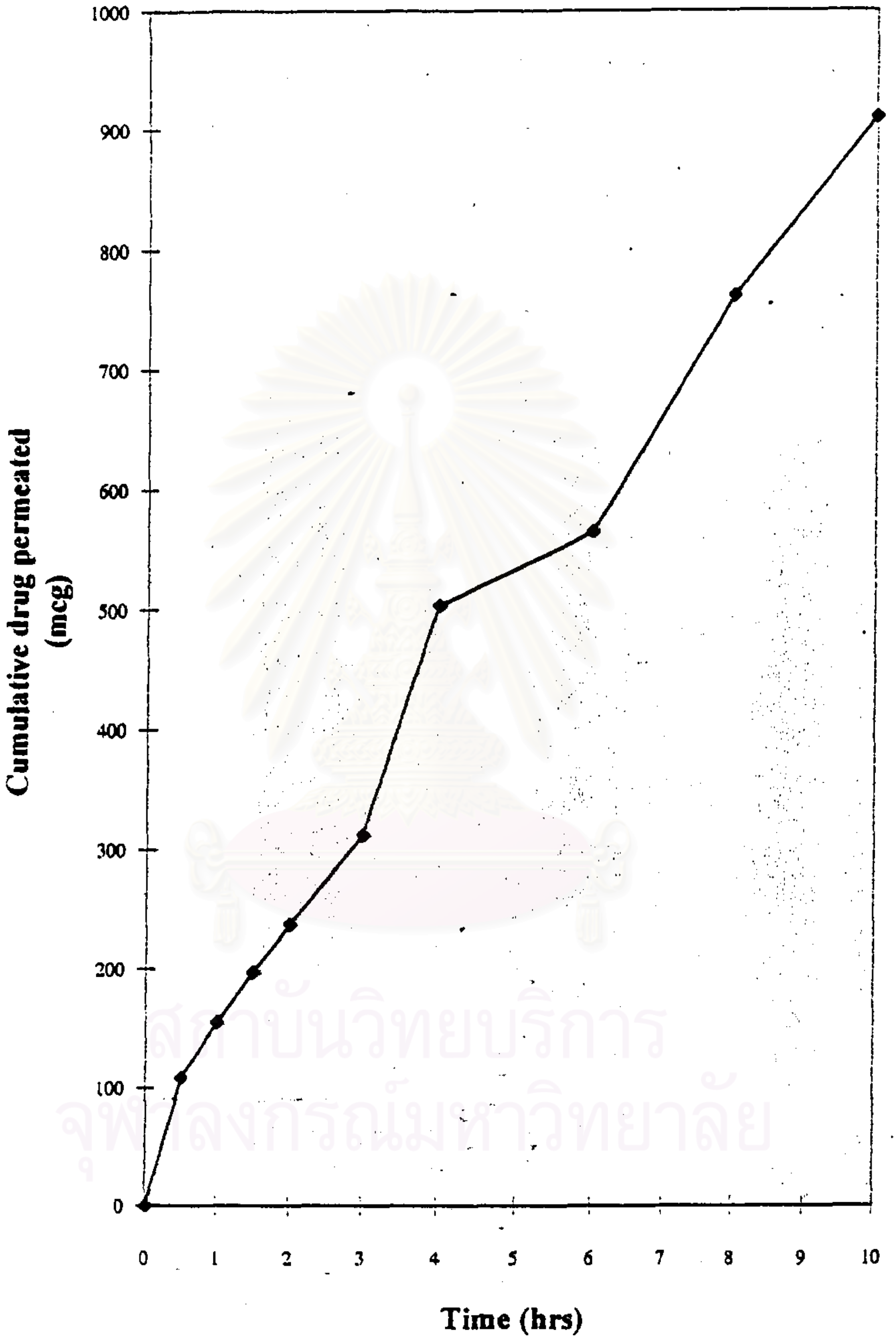


Figure 37. Cumulative amount of ISDN permeated through shed snake skin from ISDN saturated solution

Table 26. Cumulative Amount of Commercial ISDN TDDS and ISDN Transdermal Patch Which Composed of Various Crosslinked Chitosan - Polymer Membranes

Times (hrs)	Cumulative drug permeated (mg)											
	Commercial TDDS	CPL_{40}	CPL_{45}	CPM_{30}	CPM_{35}	CPM_{40}	CPH_{40}	CC_{16}	CP_8	CP_{16}	CT_{47}	CT_{48}
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0.2853	0.5493	1.6111	0.5538	0.4166	0.3947	0.5470	1.4942	0.0399	0.0392	0	0.0712
2	0.1362	0.5237	2.6668	0.5597	1.0890	1.2195	0.2231	2.4849	0.0667	0.0648	0.0512	0.1003
4	0.5979	1.4333	3.2109	1.2441	1.2198	1.8763	0.4010	2.4637	0.1455	0.1856	0.1209	0.1086
6	1.7549	2.4161	4.4653	1.4999	1.6152	2.9691	1.0275	3.4158	0.2066	0.2501	0.2098	0.1414
8	2.2148	2.5768	5.4233	1.9664	2.3291	2.9325	1.3004	4.7031	0.2935	0.3678	0.2491	0.2219
12	6.9551	2.9211	5.8514	2.1854	2.4316	3.5226	2.3205	6.3389	0.4544	0.4928	0.4176	0.3949
16	7.8848	3.1855	9.3002	2.8397	2.7224	3.7560	3.5575	10.9499	0.6128	0.7918	0.6077	0.6702
20	11.0311	5.3610	13.5745	3.3035	3.3829	4.5531	4.0994	13.1065	0.7570	1.0108	0.7902	0.7622
24	11.3580	7.2005	14.5670	3.4508	4.1028	5.3673	7.7700	14.7160	0.9064	1.1826	0.9648	0.8969

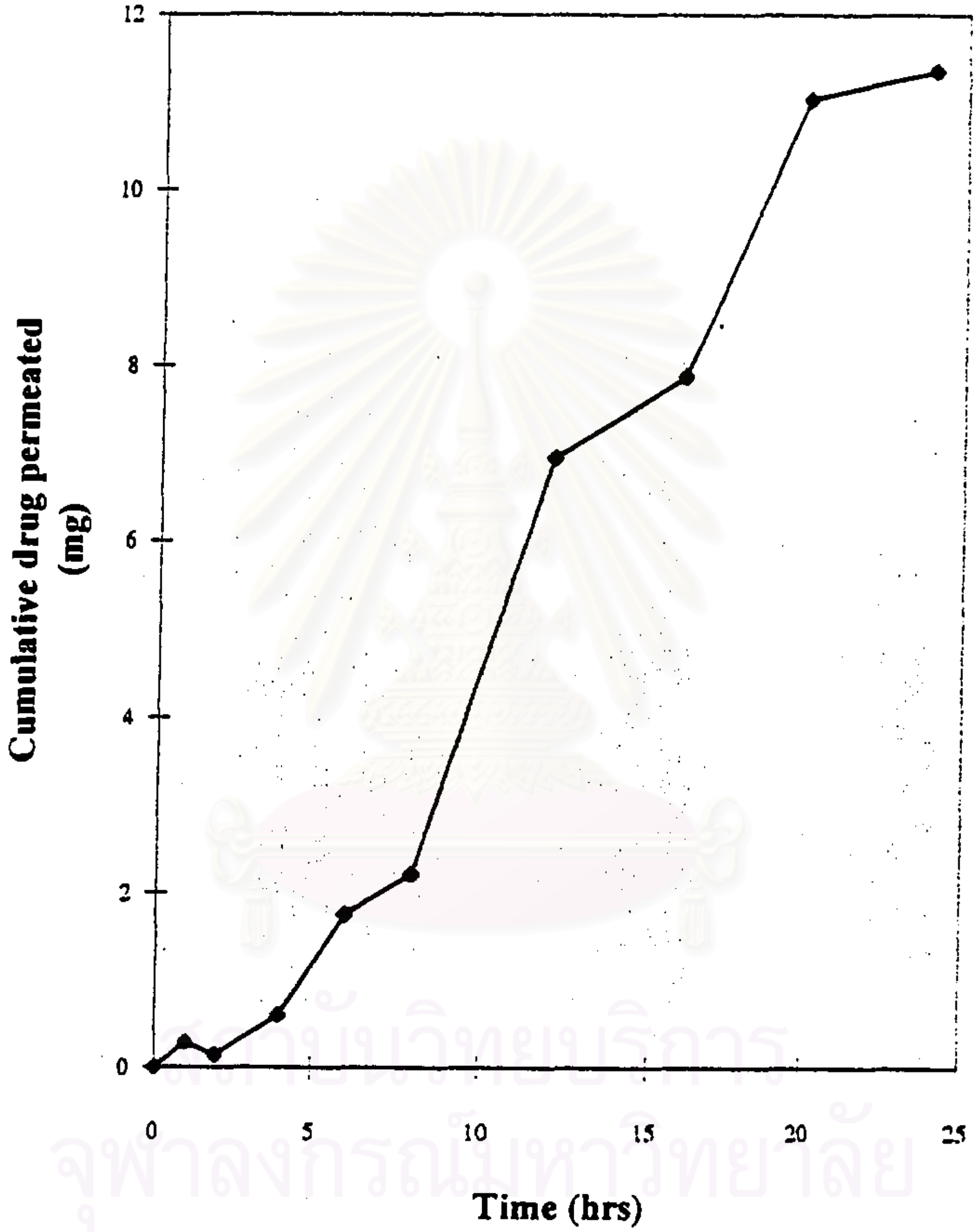


Figure 38. Cumulative drug permeated profile of commercial TDDS

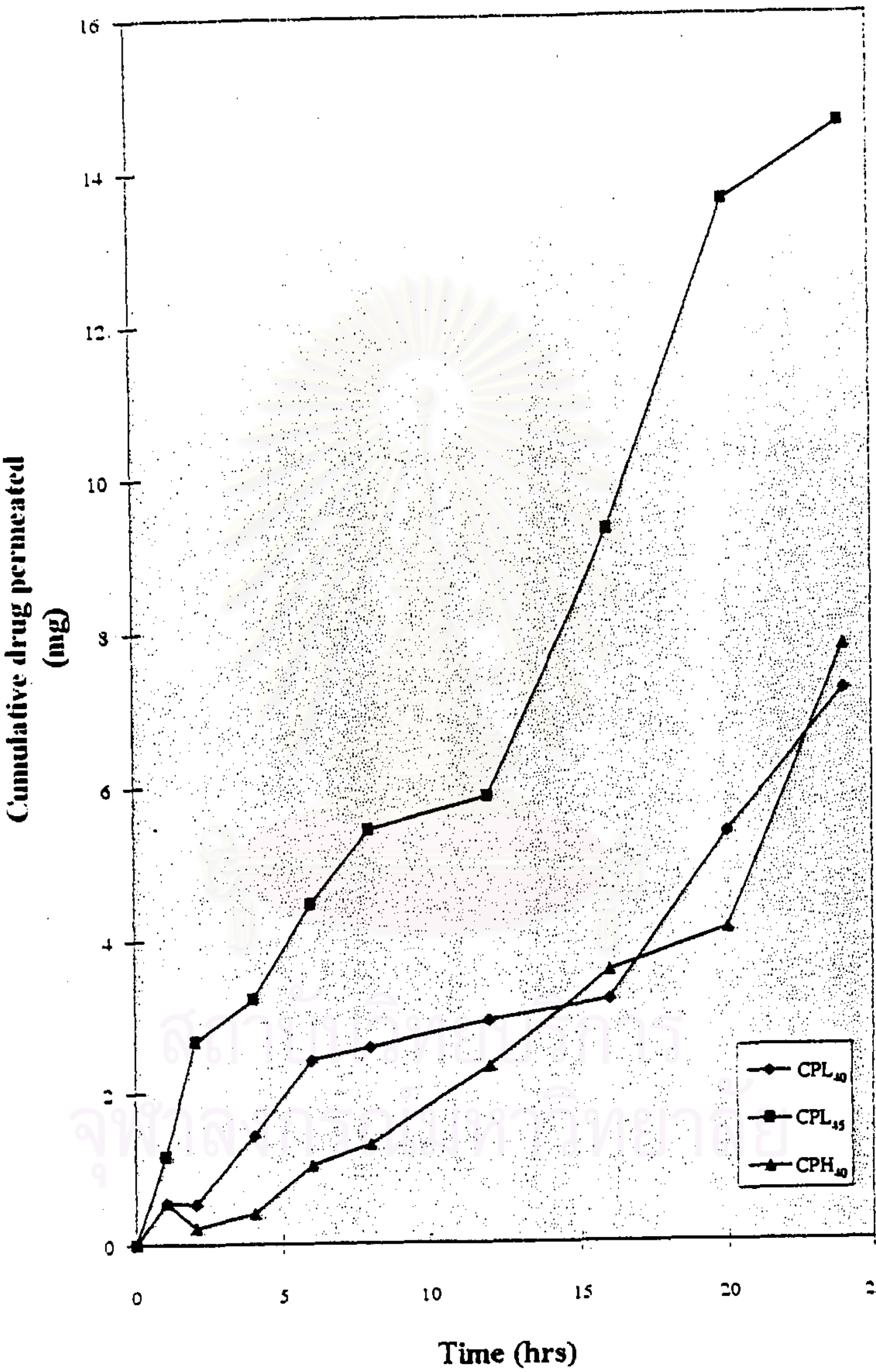


Figure 39. The permeation profiles of ISDN transdermal patch using crosslinked chitosan : PVA 30,000 1 : 4 (CPL_{40}), 1 : 9 (CPL_{45})and crosslinked chitosan : PVA 100,000 1 : 4 (CPH_{40}) as rate-controlling membrane

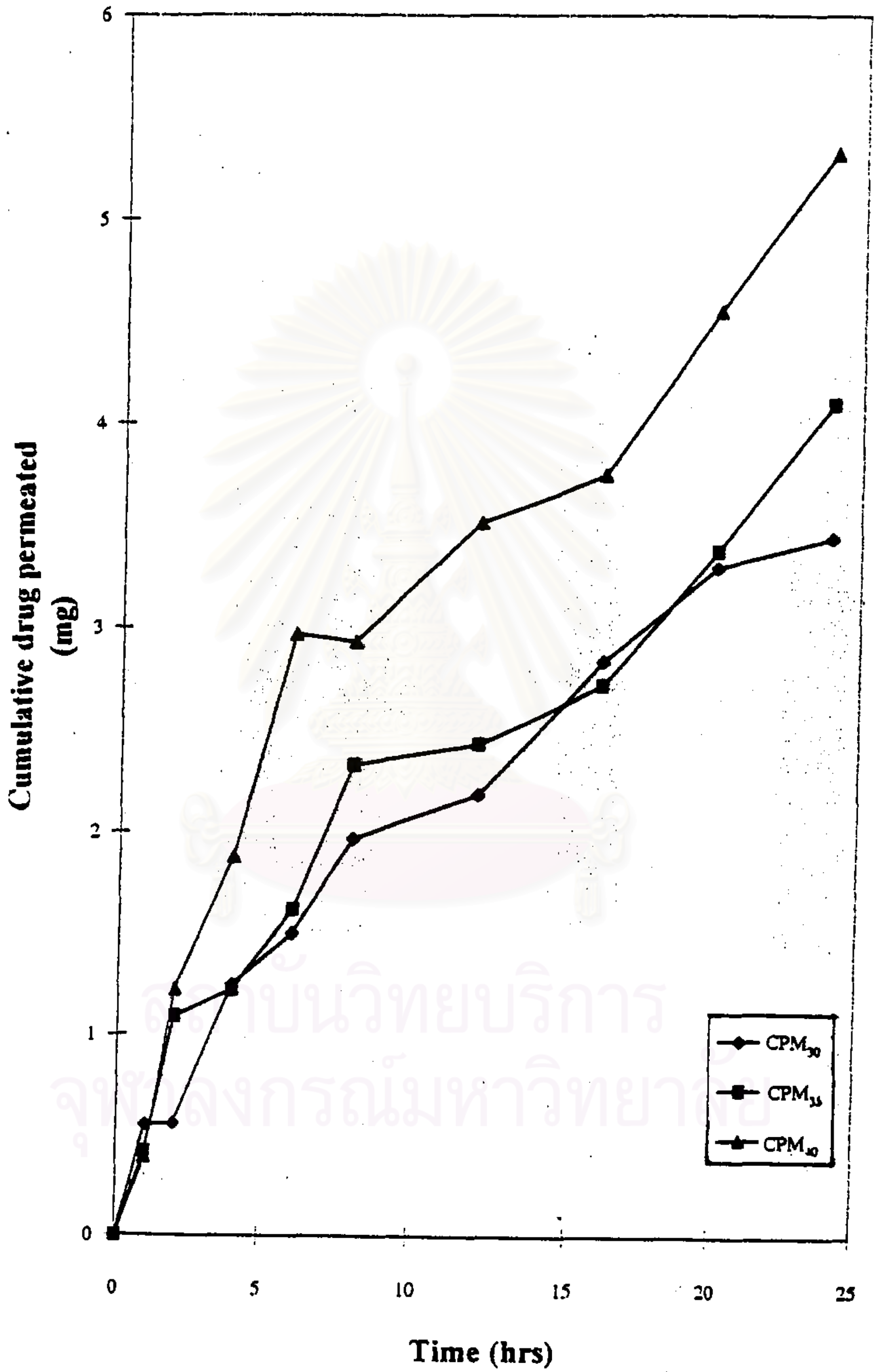


Figure 40. The permeation profiles of ISDN transdermal patch using crosslinked chitosan : PVA 70,000 2 : 3 (CPM_{30}), 3 : 7 (CPM_{35}) and 1 : 4 (CPM_{40}) as rate-controlling membrane

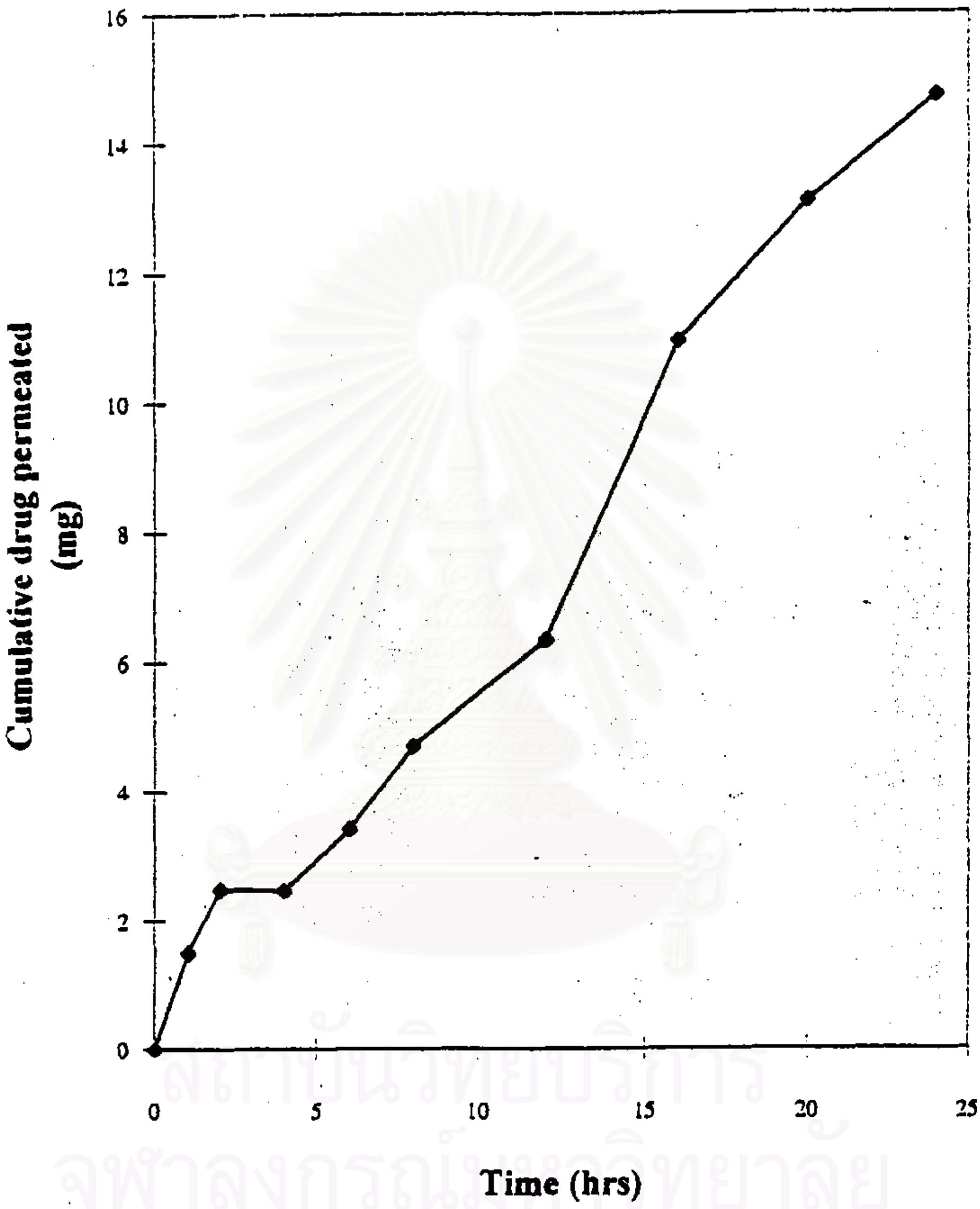


Figure 41. The permeation profile of ISDN transdermal patch using plasticized crosslinked chitosan : corn starch 4 : 1 (CC_{16}) as rate-controlling membrane

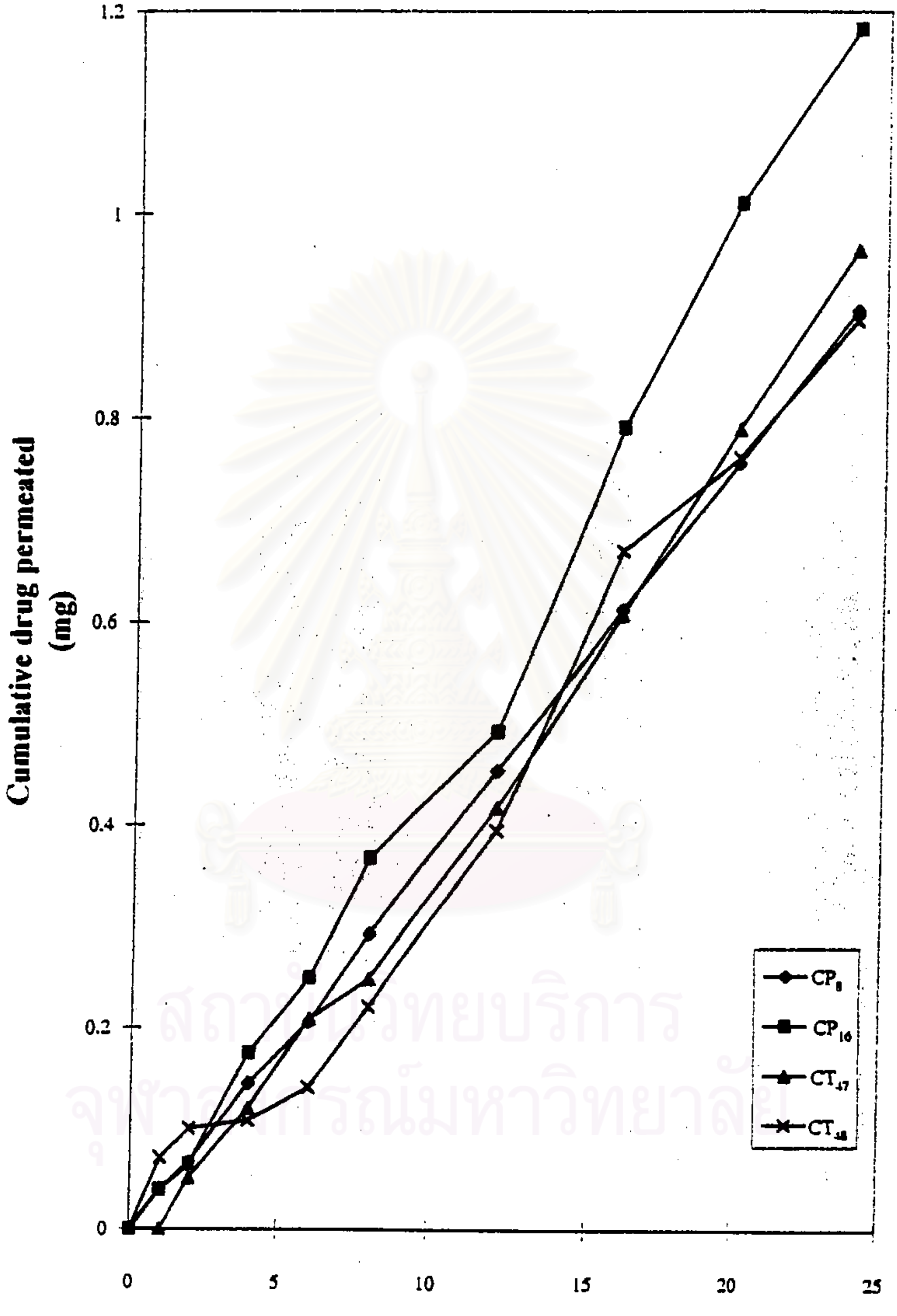


Figure 42. The permeation profile of ISDN transdermal patch using plasticized crosslinked chitosan : potato starch 9 : 1 (CP_8), 4 : 1 (CP_{16}), unplasticized crosslinked chitosan : tapioca starch 2 : 3 (CT_{47}) and plasticized crosslinked chitosan : tapioca starch 2 : 3 (CT_{48}) as rate-controlling membrane

Table 27. Correlation Coefficient and Regression Equation of the Relationship Between Cumulative Permeated Isosorbide Dinitrate Against time

Formulations	Correlation coefficient	Regression Equation
Commercial TDDS	0.9633	y = 0.5556x - 1.0498
CPL_{40}	0.9291	y = 0.2576x + 0.2452
CPL_{45}	0.9655	y = 0.5656x + 0.8967
CPM_{30}	0.9616	y = 0.1306x + 0.6059
CPM_{35}	0.9545	y = 0.1414x + 0.6059
CPM_{40}	0.9128	y = 0.1849x + 1.0444
CPH_{40}	0.9021	y = 0.2852x - 0.5861
CC_{16}	0.9765	y = 0.6052x + 0.3764
CP_{8}	0.9994	y = 0.0383x - 0.0083
CP_{16}	0.9938	y = 0.0509x - 0.0398
CT_{47}	0.9968	y = 0.0416x - 0.0505
CT_{48}	0.9730	y = 0.0387 - 0.0261

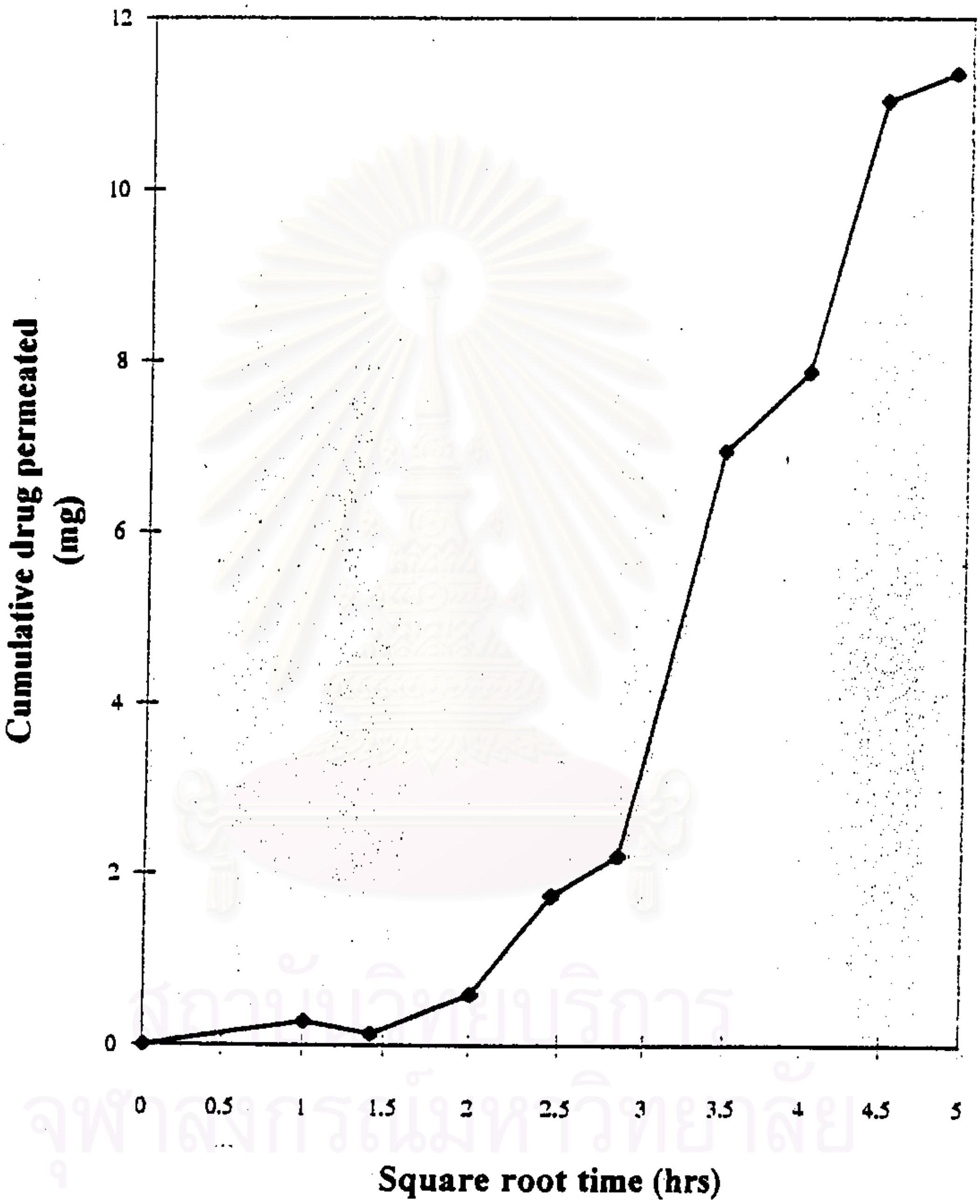


Figure 43. Cumulative drug permeated profile of commercial TDDS

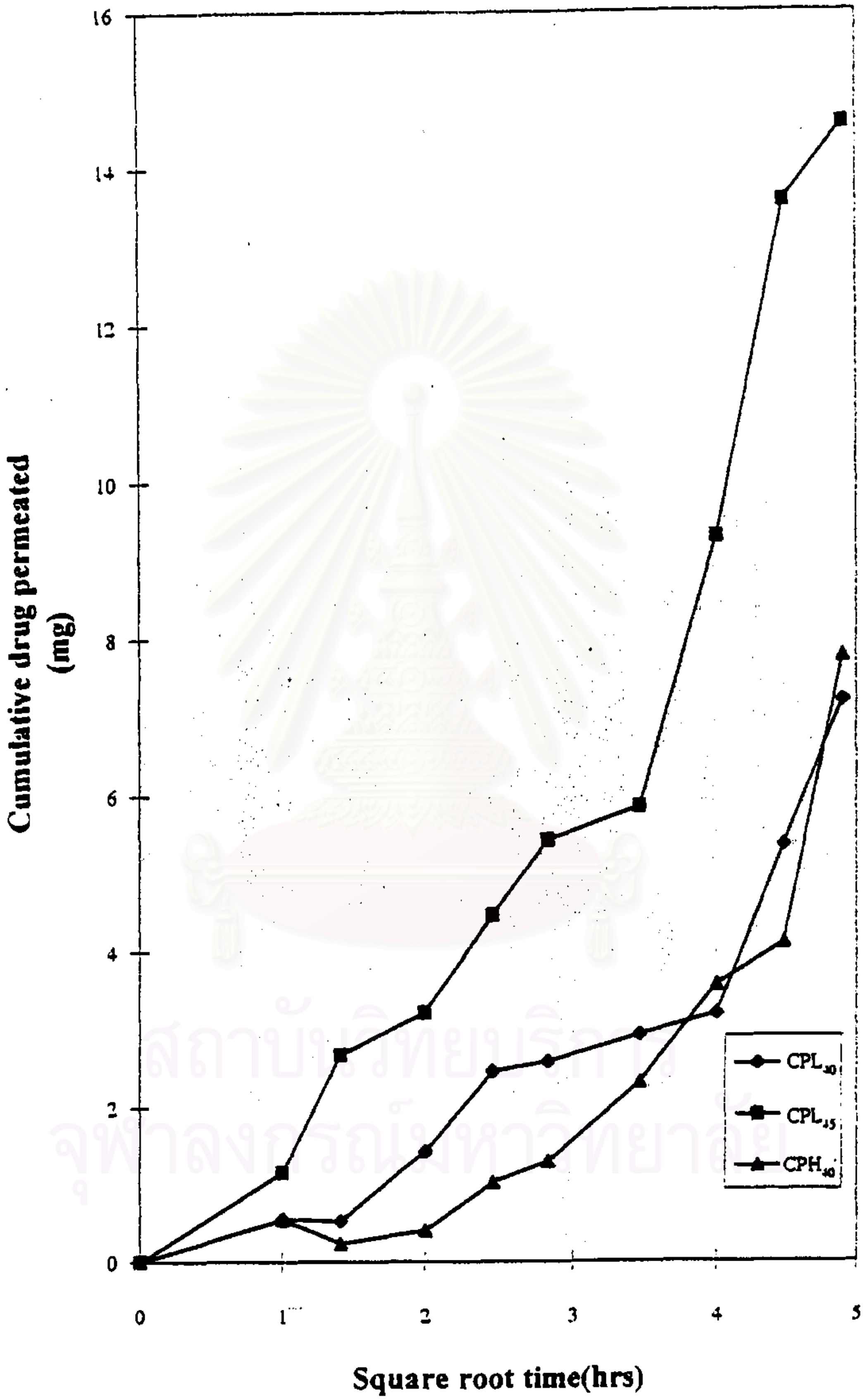


Figure 44. The permeation profiles of ISDN transdermal patch using crosslinked chitosan : PVA 30,000 1 : 4 (CPL_{40}), 1 : 9 (CPL_{45})and crosslinked chitosan : PVA 100,000 1 : 4 (CPH_{40}) as rate-controlling membrane

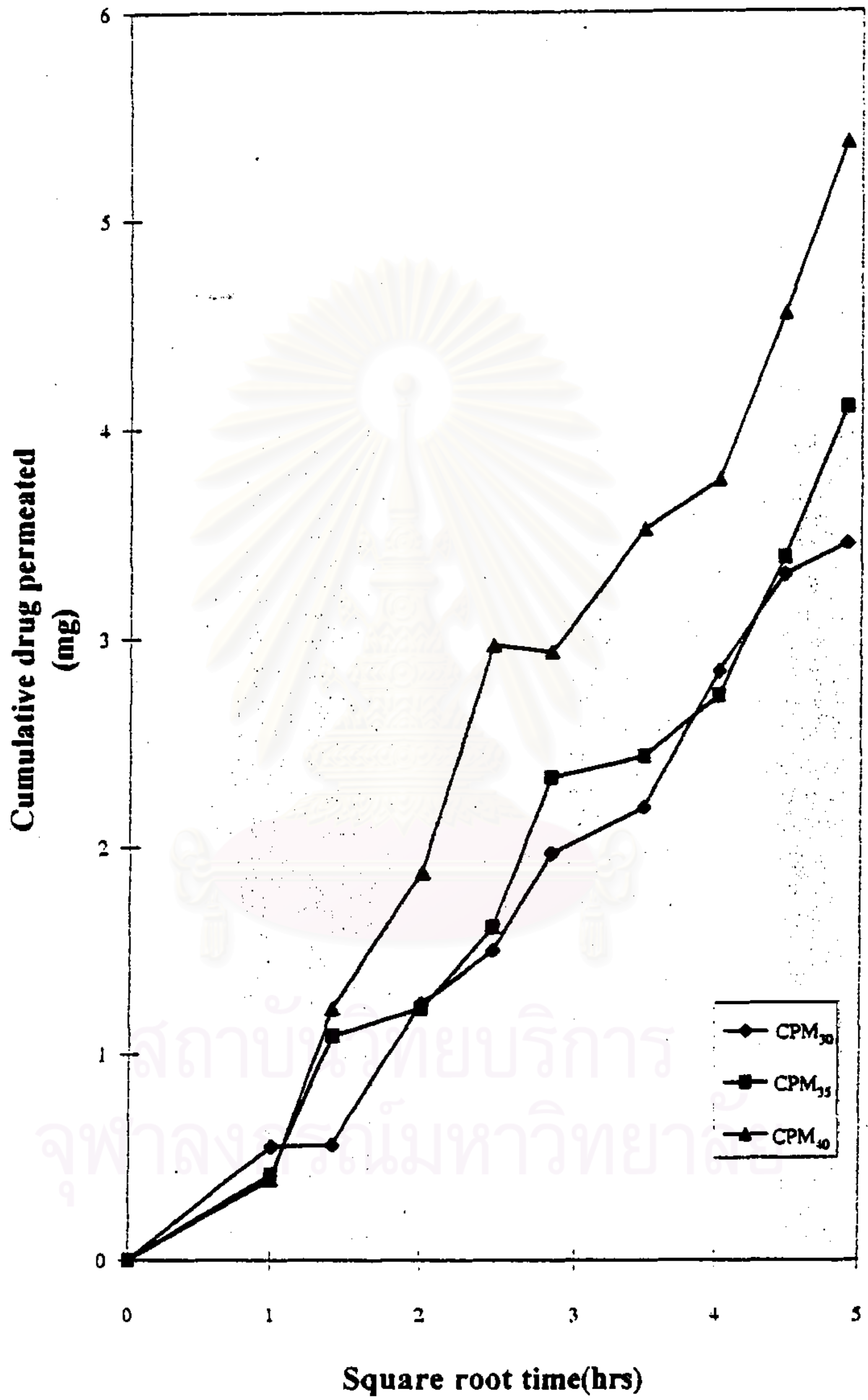


Figure 45. The permeation profiles of ISDN transdermal patch using crosslinked chitosan : PVA 70,000 2 : 3 (CPM_{30}), 3 : 7 (CPM_{35}) and 1 : 4 (CPM_{40}) as rate-controlling membrane

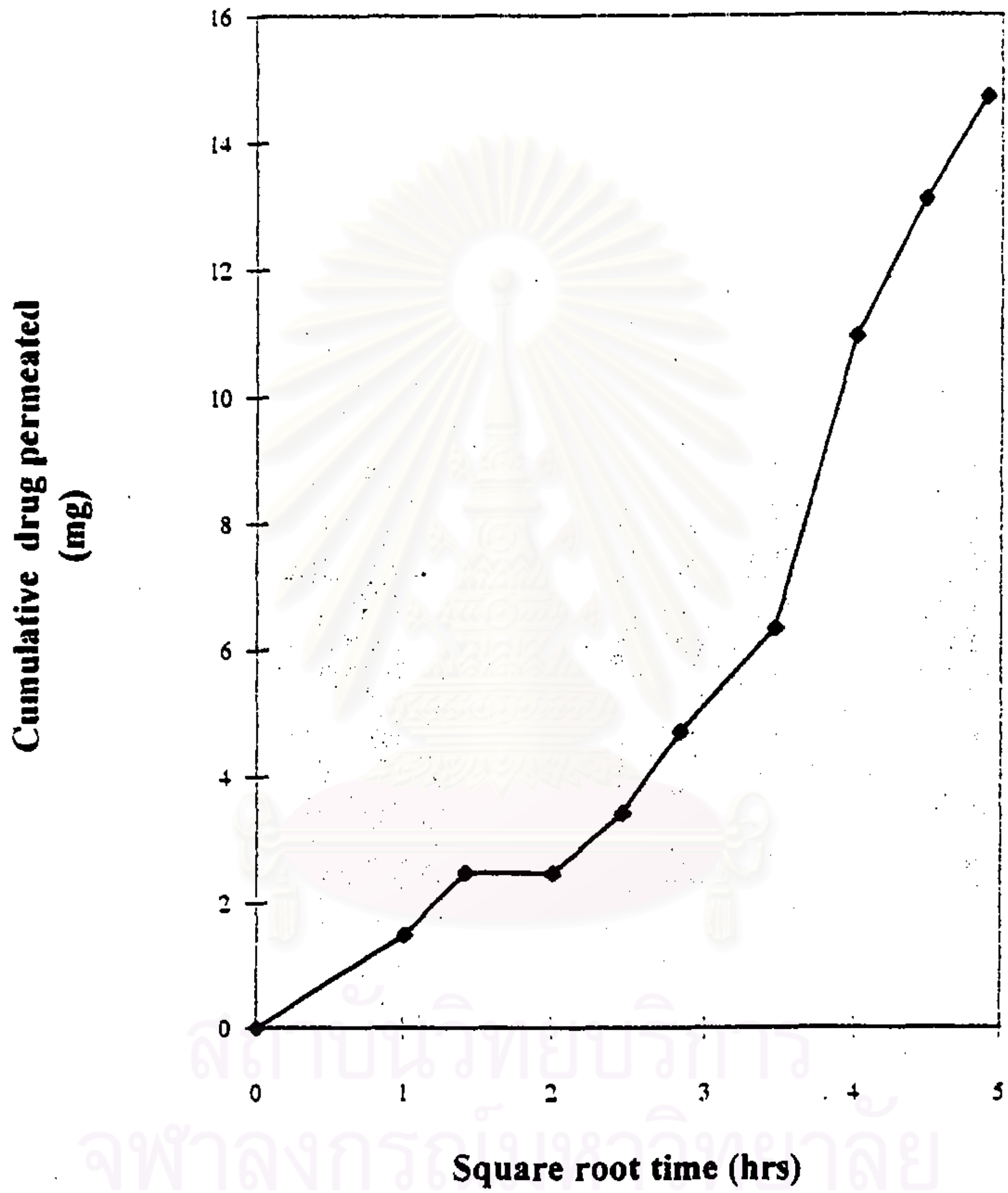


Figure 46. The permeation profile of ISDN transdermal patch using plasticized crosslinked chitosan : corn starch 4 : 1 (CC_{16}) as rate-controlling membrane

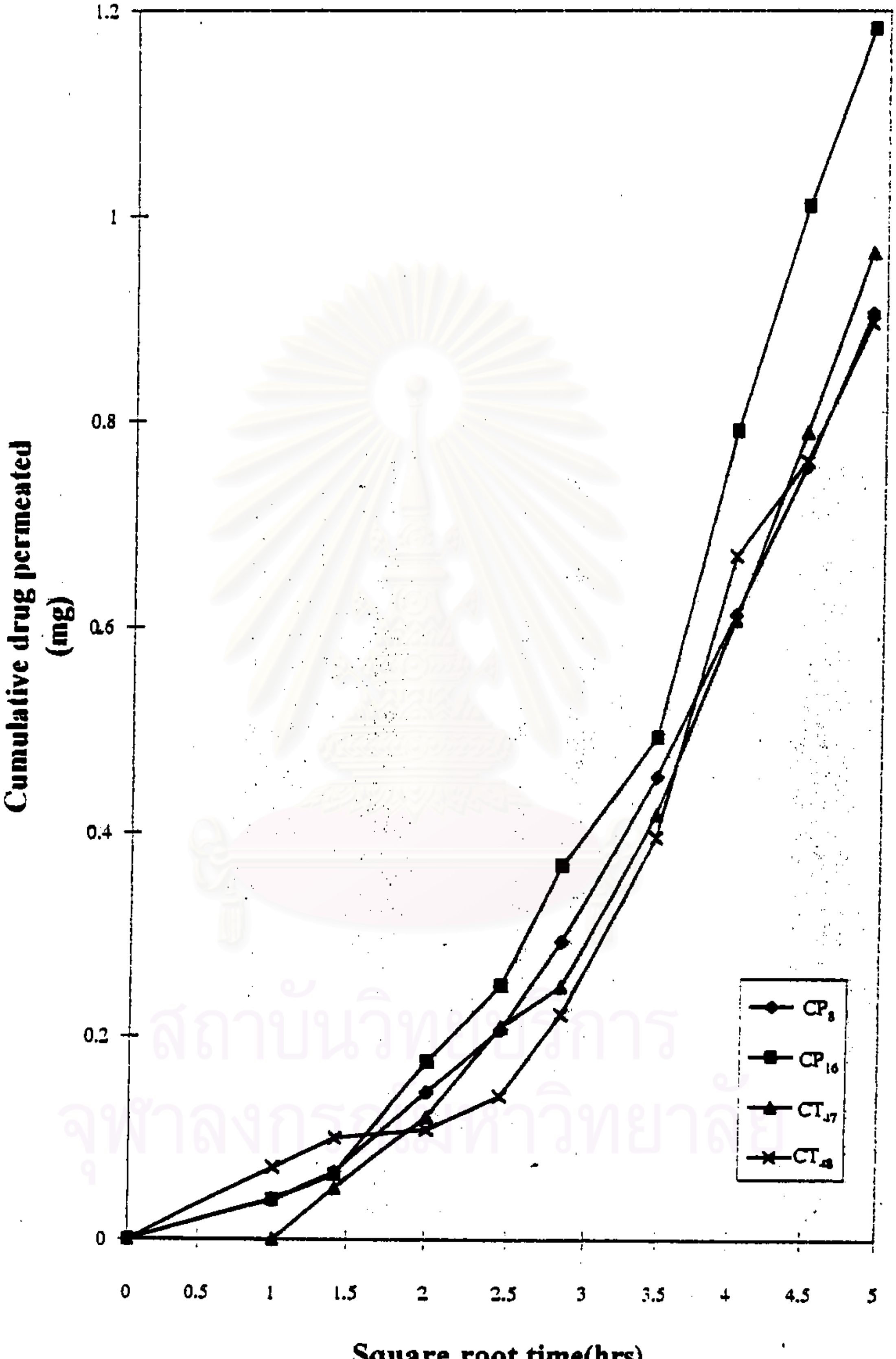


Figure 47. The permeation profile of ISDN transdermal patch using plasticized crosslinked chitosan : potato starch 9 : 1 (CP_8), 4 : 1 (CP_{16}), unplasticized crosslinked chitosan : tapioca starch 2 : 3 (CT_{47}) and plasticized crosslinked chitosan : tapioca starch 2 : 3 (CT_{48}) as rate-controlling membrane

Table 28. Correlation Coefficient and Regression Equation of the Relationship Between Cumulative Permeated Isosorbide Dinitrate Against Square Root Time

Formulations	Correlation coefficient	Regression Equation
Commercial TDDS	0.9218	y = 3.2683x - 4.942
CPL_{40}	0.8804	y = 1.588x - 1.5395
CPL_{45}	0.9136	y = 3.3538x - 3.1941
CPM_{30}	0.9869	y = 0.7962x - 0.3908
CPM_{35}	0.9645	y = 0.8483x - 0.3546
CPM_{40}	0.9661	y = 1.1441x - 0.4176
CPH_{40}	0.7983	y = 1.6138x - 3.7548
CC_{16}	0.9147	y = 0.2260x - 0.2791
CP_8	0.9637	y = 0.2988x - 0.3947
CP_{16}	0.9467	y = 0.2453x - 0.3459
CT_{47}	0.8491	y = 0.2453x - 0.3459
CT_{48}	0.9056	y = 0.2252x - 0.2893

Table 29. Kinetic pattern.

Formulations	Kinetic pattern
Commercial TDDS	Zero order
CPL_{40}	Zero order
CPL_{45}	Zero order
CPM_{30}	Zero order / Higuchi's model
CPM_{35}	Zero order / Higuchi's model
CPM_{40}	Higuchi's model
CPH_{40}	Zero order
CC_{16}	Zero order
CP_{8}	Zero order
CP_{16}	Zero order
CT_{47}	Zero order
CT_{48}	Zero order