CHAPTER III

RESULTS

Solubility

The solubility of isosorbide dinitrate in reversed osmosis treated water at 37°C was 0.9306 ± 0.0483 mg/ml. The increase in the solubility of drug was observed on adding of polyethylene glycol 400 as cosolvent to the medium and it was closely related to the amount of cosolvent. The solubility of ISDN in the presence of 20% and 30% polyethylene glycol 400 were 1.3394 ± 0.1953 and 1.5760 ± 0.2416 mg/ml, respectively. The solubility values expressed were the mean ± SD of three determinations.

Film characteristics

Most membrane casting solutions prepared in this work were yellowish, clear viscous solutions except the casting formulation containing corn starch and chitosan which produced yellowish opaque viscous colloidal mixture. All obtained membrane formulations resulted in yellowish and transparent prepared membranes.

Some physical characteristics of chitosan-PVA membrane are listed in Table 7. Membranes obtained from formulations of 1:4 chitosan : PVA could not produce satisfactory films. Membrane A1 plasticized with PEG 1450, unplasticized membrane A4 and membrane A7 plasticized with triacetin could not be recovered intact from the glass plates whereas membrane A10 plasticized with PEG 1450, unplasticized membrane A13 and membrane with

Table 7 Some physical characteristics of crosslinked chitosan : PVA membranes.

Formulation	yellow color	transparency	glossy	sticky	flexibility	integrity	difficulty in preparing
A1	+	+	+	-	-	-	++
A2	+	+	++	-	++	+	++
A3	+	+	++	-	++	+	++
A4	+	+	+	-	-	-	++
A5	+	+	++	-	++	+	++
A6	+	+	++	-	++	+	++
A7	+	+	+	-	-	-	++
A8	+	+	++	-	++	+	++
A9	+	+	++	-	++	+	++
A10	+	+	+	+	++	-	++
A11	+	+	++	-	++	+	++
A12	++	+	++	-	++	+	++
A13	+	+	+	+	++	-	++
A14	+	+	++	-	++	+	++
A15	+	+	++	-	++	+	++
A16	+	+	+	+	++	-	++
A17	+	+	++	-	++	+	++
A18	++	+	++	-	++	+	++

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

triacetin A16 could not form uniformly thin films but produced tough thick membranes with circular shape at the glass plate center. Membranes A1, A4 and A7 were crosslinked by 5% of crosslinking solution based on the formulation while 10% of crosslinking solution based on the formulation was used in the membranes A10, A13 and A16.

All membranes obtained from formulation of 2:3 and 3:2 chitosan : PVA were clear, pliable, integral and isolatable films with highly polished surface. These results indicated that either film-former ratio or concentration of crosslinking agent played as one of the important factor affected on the physical properties of resulting films.

Table 8 shows some physical characteristics of chitosan : PVP K90 membranes. Membranes P7, P8, P9 plasticized with triacetin and obtained from formulations of chitosan : PVP K90 in the proportion of 1:4, 2:3 and 3:2, respectively, at 5% of crosslinking solution based on the formulation were fragile and their integrity during isolation could not be obtained whereas the others which were plasticized with PEG1450 and unplasticized membranes gave perceptibly good film characteristics. However, unplasticized membranes P5 and P6 which were obtained from formulation of chitosan : PVP K90 in the ratio 2:3 and 3:2, respectively, were fragile when cut. At 10% of crosslinking solution based on the formulation, membrane with PEG1450 P10 and unplasticized membrane P13 prepared from formulations of 1:4 chitosan : PVP K90 and unplasticized membrane P14 obtained from formulation of 2:3 chitosan : PVP K90 were also observed to gave the similar results to P7, P8 and P9. However, membrane P12 plasticized with PEG1450 and unplasticized membrane P15 prepared from formulation containing chitosan : PVP K90 in the ratio 3:2 also gave the similar results to P5 and P6.

Table 8 Some physical characteristics of crosslinked chitosan : PVP K90 membranes.

Formulation	yellow color	transparency	glossy	sticky	flexibility	integrity	difficulty in preparing
P1	+	+	+	-	++	+	+
P2	++	+	+	-	++	+	+
P3	++	+	+	-	++	+	+
P4	+	+	+	-	++	+	+
P5	++	+	+	-	+	+	+
P6	++	+	+	-	+	+	+
P7	+	+	+	+	-	-	+
P8	++	+	+	+	-	-	+
P9	++	+	+	+	-	-	+
P10	++	+	+	+	-	-	+
P11	+	+	+	-	++	+	+
P12	++	+	+	-	+	+	+
P13	++	+	+	-	-	-	+
P14	++	+	+	-	-	-	+
P15	++	+	+	-	+	+	+
P16	++	+	+	-	++	+	+
P17	++	+	+	-	++	+	+
P18	++	+	+	-	++	+	+

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

Comparison of membranes P3 and P12 that had the similar polymer ratio and the same plasticizer, it was found that the higher the amount of crosslinking agent, the more brittle the membrane. In addition, greater amount of crosslinking agent produced harder and stronger film which could be isolated from glass plate as observed in membranes P16, P17 and P18, all of which were plasticized with triacetin and obtained from formulation of chitosan : PVP K90 in the ratios 1:4, 2:3 and 3:2, respectively, in comparison with P7, P8 and P9, respectively.

Plasticizers affected the physical characteristics of membranes. The inclusion of PEG1450 into P2 imparted more flexibility of film as compared to membrane P5 whereas incorporation of triacetin as membrane P8 could not be recovered intact from the glass plates. The similar results were observed in membrane P3 plasticized with PEG1450 and P9 plasticized with triacetin in comparison with unplasticized membrane P6.

For membrane characteristics of crosslinked chitosan-HPMC15 and crosslinked chitosan-MC15 as shown in Tables 9 and 11, respectively, it was interesting to note that these membranes were found to form clear, glossy, pliable and isolatable intact films except unplasticized membrane H5 and H14 which were prepared from formulations of chitosan : HPMC15 in the ratio 2:3 at 5% and 10% of crosslinking solution based on formulation, respectively, showed moderate flexibility film characteristic. They were fragile when cut.

Some physical characteristics of crosslinked-corn starch membrane are shown in Table 10. Most membranes obtained from formulations of 2:3 chitosan : corn starch could produce satisfactory film characteristics except unplasticized membrane C14. Membranes C9 and C18 plasticized with triacetin and those were prepared from formulations of 3:2 chitosan : corn

Table 9 Some physical characteristics of crosslinked chitosan- : HPMC15 membranes

Formulation	yellow color	transparency	glossy	sticky	flexibility	integrity	difficulty in preparing
H1	++	+	+	-	++	+	++
H2	++	+	+	-	++	+	++
H3	++	+	+	-	++	+	++
H4	++	+	+	-	++	+	++
H5	++	+	+	-	+	+	++
H6	++	+	+	-	++	+	++
H7	++	+	+	-	++	+	++
H8	++	+	+	-	++	+	++
H9	++	+	+	-	++	+	++
H10	++	+	+	-	++	+	++
H11	++	+	+	-	++	+	++
H12	+++	+	+	-	++	+	++
H13	++	+	+	-	++	+	++
H14	++	+	+	-	+	+	++
H15	+++	+	+	-	++	+	++
H16	++	+	+	-	++	+	++
H17	+++	+	+	-	++	+	++
H18	+++	+	+	-	++	+	++

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

Table 10 Some physical characteristics of crosslinked chitosan-corn starch membranes.

Formulation	**yellow color**	**transparency**	**glossy**	**sticky**	**flexibility**	**integrity**	**difficulty in preparing**
C1	++	+	+	++	-	-	+++
C2	+++	+	+	-	++	+	+++
C3	++	+	+	+	-	-	+++
C4	++	+	+	++	-	-	+++
C5	++	+	+	-	++	+	+++
C6	++	+	+	+	-	-	+++
C7	++	+	+	++	-	-	+++
C8	++	+	+	-	++	+	+++
C9	+++	+	+	-	++	+	+++
C10	++	+	+	++	-	-	+++
C11	++	+	+	-	++	+	+++
C12	+++	+	+	-	-	-	+++
C13	++	+	+	+	-	-	+++
C14	++	+	+	+	-	-	+++
C15	++	+	+	-	-	-	+++
C16	++	+	+	++	-	-	+++
C17	+++	+	+	-	++	+	+++
C18	+++	+	+	-	++	+	+++

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

Table 11 Some physical characteristics of crosslinked chitosan-MC15 membranes.

Formulation	yellow color	transparency	glossy	sticky	flexibility	integrity	difficulty in preparing
M1	++	+	+	-	++	+	++
M2	++	+	+	-	++	+	++
M3	+++	+	+	-	++	+	++
M4	++	+	+	-	++	+	++
M5	++	+	+	-	++	+	++
M6	+++	+	+	-	++	+	++
M7	+	+	+	-	++	+	++
M8	++	+	+	-	++	+	++
M9	+++	+	+	-	++	+	++
M10	++	+	+	-	++	+	++
M11	++	+	+	-	++	+	++
M12	+++	+	+	-	++	+	++
M13	++	+	+	-	++	+	++
M14	++	+	+	-	++	+	++
M15	++	+	+	-	++	+	++
M16	++	+	+	-	++	+	++
M17	+++	+	+	-	++	+	++
M18	+++	+	+	-	++	+	++

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

starch at 5% and 10% of crosslinking solution based on the formulation, repectively, showed the similar results to produce satisfactory clarity, gloss, integrity, flexible and easy isolation of the films. The others and C14 could not produce satisfactory film characteristics. By the meaning, these membranes were fragile and their integrity during isolation could not be obtained due, in part, to associated tackiness.

For overall above description, it could be summarized that free film characteristics were resulted from combined effects of the formulation components including the type and concentration ratio of the polymer blend, type of plasticizer, and the concentration of crosslinking agent, glutaraldehyde. Only favorable formulations could generate desirable membrane characteristics.

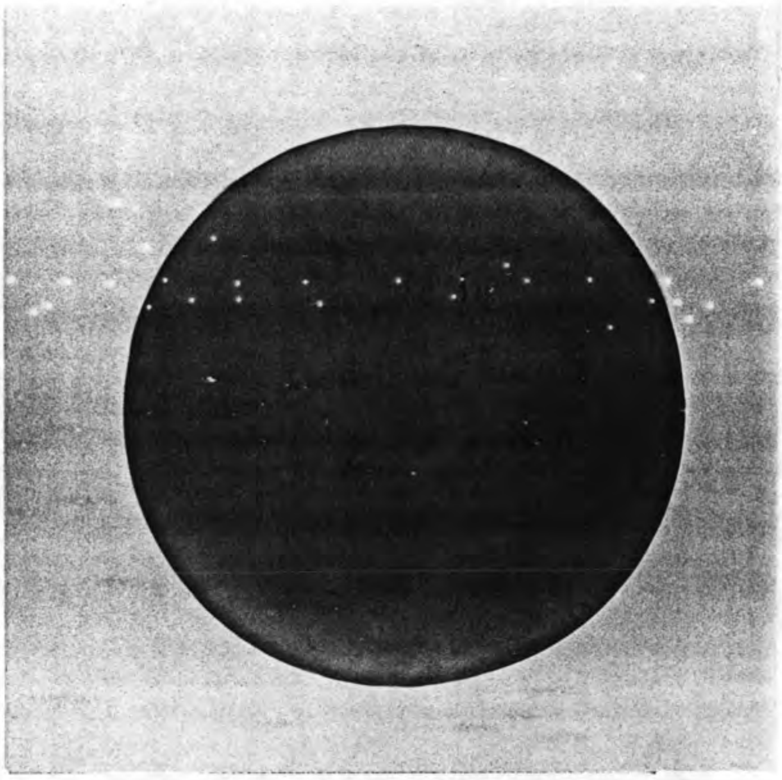


Figure 4 A satisfactory integrity film.

Water sorption

In the course of studying water sorption of membranes, membranes that showed good prerequisite film characteristics based on the ease of intact isolation from glass plates and good flexibility were selected.

After exposure to water, all membranes could be broadly divided into two groups. Those which water sorption ability could be assessed and those which water sorption value could not be evaluated owing to either rupture or unsatisfactory appearance of the membranes. The data are compiled in Table 12 and illustrated in Figure 5. For membranes obtained from formulations of 2:3 chitosan : PVA at 5% of crosslinking solution based on the formulation, membrane A2 plasticized with PEG1450 absorbed more water than unplasticized membrane A5 whereas membrane with triacetin A8 showed lower water sorption. This results were in agreement with the data observed from membrane A3 plasticized with PEG1450 and membrane with triacetin A9 in comparison with unplasticized membrane A6. Membranes A3, A6 and A9 were obtained from formulation of 3:2 chitosan : PVA at 5% of crosslinking solution based on the formulation. It was clearly seen that types of plasticizers affected the water sorption ability of the membranes. Water sorption was increased when adding PEG1450 into the formulation whereas incorporation of triacetin decreased its ability.

For membranes prepared from formulations of 2:3 chitosan : PVA at 10% of crosslinking solution based on the formulation, it was found that membrane with PEG1450 A11 and membrane with triacetin A17 were observed to produce lower water sorption ability in comparison with unplasticized A14. Similarly, water sorption was higher from unplasticized membrane A15 than that observation from membrane containing PEG1450

Table 12 Percent water sorption of various crosslinked chitosan-polymer membranes.

Type	Formulation	Percent water sorption (± SD)
crosslinked chitosan-PVA membranes	A2	25.64 (1.70)
	A3	23.79 (1.19)
	A5	21.54 (2.19)
	A6	20.72 (0.88)
	A8	20.18 (1.15)
	A9	19.33 (2.04)
	A11	11.67 (3.06)
	A12	10.58 (0.89)
	A14	10.97 (0.89)
	A15	11.89 (0.62)
	A17	4.74 (2.91)
	A18	9.06 (0.72)
crosslinked chitosan-PVPK90 membranes	P1	F
	P2	F
	P3	32.92 (1.72)
	P4	F
	P11	F
	P16	F
	P17	U
	P18	13.09 (1.51)
crosslinked chitosan-HPMC15 membranes	H1	F
	H2	F
	H3	36.91 (5.44)
	H4	F
	H6	F
	H7	F
	H8	F
	H9	F
	H10	F
	H11	U
	H12	U
	H13	F
	H15	29.03 (2.24)
	H16	F
	H17	U
	H18	17.77 (0.69)

Table 12 (Cont.)

Type	Formulation	Percent water sorption (± SD)
crosslinked chitosan-corn starch membranes	C2	16.79 (2.23)
	C5	F
	C8	F
	C9	26.28 (0.04)
	C11	U
	C17	22.40 (1.26)
	C18	F
crosslinked chitosan-MC15 membranes	M1	F
	M2	11.56 (1.54)
	M3	21.19 (2.58)
	M4	F
	M5	23.82 (3.95)
	M6	24.35 (2.33)
	M7	F
	M8	U
	M9	20.40 (2.01)
	M10	F
	M11	U
	M12	U
	M13	F
	M14	U
	M15	U
	M16	F
	M17	U
	M18	U

F = membrane became rupture after exposure to water.

U = membrane presented unsatisfactory appearance.

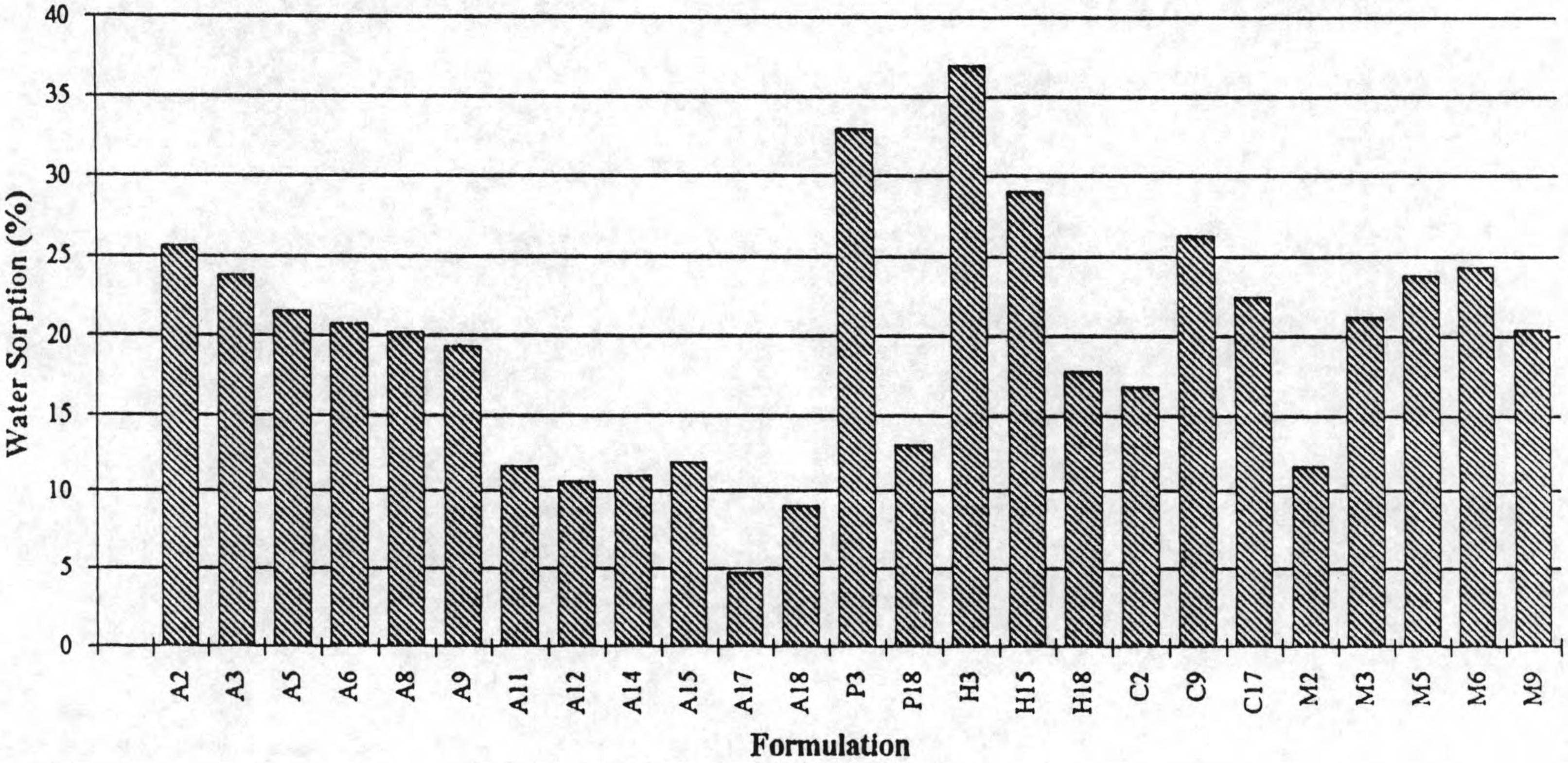


Figure 5 Percent water sorption of various crosslinked chitosan-polymer membranes.A=crosslinked chitosan-PVA membrane, P=crosslinked chitosan-PVPK90 membrane, H=crosslinked chitosan-HPMC15 membrane, C=crosslinked chitosan-corn starch membrane, M=crosslinked chitosan-MC15 membrane.

and triacetin, A12 and A18, respectively. Membranes A12, A15 and A18 were obtained from formulations of 3:2 chitosan : PVA.

It was also interesting to note that concentration of crosslinking agent affected the water sorption capacity of the membranes. Water sorption was decreased with increasing the concentration of the crosslinking agent. These results could be observed in water sorption ability of membrane A2, A3, A5, A6, A8, A9 compared with membrane A11, A12, A14, A15, A17, A18, respectively.

For water sorption of crosslinked chitosan : PVPK90 membrane, only two membranes could be evaluated. The percent water sorption of membrane with PEG1450 P3 and membrane P18 plasticized with triacetin were 32.92 and 13.09, respectively.

Considering the water sorption of crosslinked chitosan-HPMC15 membrane, it was found that water sorption of unplasticized membrane H15 was higher than membrane plasticized with triacetin H18. Those membranes were obtained from formulations containing chitosan : HPMC15 in the ratio 3:2 at 10% of crosslinking solution based on the formulation. This results also indicated the effect of triacetin on water sorption of the membrane.

Focused on water sorption of crosslinked chitosan-MC15, membrane with PEG1450 M2 presented much lower value as compared to unplasticized membrane M5. These membranes were obtained from formulations of chitosan - MC15 2:3 at 5% crosslinking solution based on the formulation. Similarly, water sorption of unplasticized membrane M6 was higher than membranes M3 and M9 plasticized with PEG1450 and triacetin, respectively. Membranes M3, M6 and M9 were prepared from formulations containing chitosan : MC15 in the ratio 3:2 at 5% of crosslinking solution based on the formulation. It was noted that type and ratio of film-former, type of plasticizer and concentration of crosslinking agent directly affected water sorption of the membrane, resulting in different water sorption characteristic.

Mechanical Properties

For mechanical property evaluation, twenty-five membrane formulations were selected on the basis of water sorption determination. The overall data including tensile strength and percent elongation at break are presented in Table 13 and illustrated in Figures 6 and 7.

Tensile strength and percent elongation at break

For 2:3 chitosan : PVA membrane with 5% crosslinking agent, membrane A2 plasticized with PEG1450 was observed to show higher ultimate tensile strength and lower percent elongation as compared to unplasticized membrane A5. On the other hand, membrane with triacetin A8 resulted in notable decreasing in both ultimate tensile strength and elongation at break in comparison with unplasticized membrane A5.

For 3:2 chitosan : PVA membrane with 5% crosslinking agent, membrane A3 plasticized with PEG1450 showed an increase in both the ultimate tensile strength and percent elongation at break in comparison with unplasticized membrane A6 whereas membrane A9 plasticized with triacetin was observed to produce lower ultimate tensile strength than that obtained with unplasticized membrane A6 while their ultimate tensile strengths were not greatly different.

With 10% crosslinking agent in both 2:3 and 3:2 chitosan : PVA membrane, it was found that incorporation of PEG1450 increased the ultimate tensile strength of the membranes whereas the addition of triacetin decreased it.

Table 13 Mechanical properties of various membranes.

Formulation	Mean ultimate tensile strength (kg/mm^2) (± SD)	Mean percent elongation (± SD)
A2^a	0.5193 (0.1624)	100.00 (12.75)
A3^a	0.7434 (0.2571)	67.00 (6.71)
A5^a	0.4317 (0.0915)	104.00 (5.48)
A6^a	0.5992 (0.2377)	66.00 (4.18)
A8^a	0.1697 (0.0521)	71.00 (11.94)
A9	0.4413 (0.1136)	66.67 (7.64)
A11	0.5314 (0.1545)	58.33 (7.64)
A12^a	0.7875 (0.2897)	33.00 (6.71)
A14^a	0.4670 (0.1871)	38.00 (7.58)
A15^a	0.6112 (0.1767)	24.00 (4.18)
A17^a	0.2613 (0.0281)	41.00 (2.24)
A18^a	0.4851 (0.1293)	31.00 (4.18)
P3^a	0.6639 (0.2339)	24.00 (9.62)
P18^b	0.5976 (0.2139)	20.00 (6.32)
H3^a	0.5954 (0.2578)	34.00 (6.52)
H15^a	0.7317 (0.2720)	18.00 (2.74)
H18^a	0.4802 (0.1460)	13.00 (5.70)
C2	0.3132 (0.0956)	25.00 (5.00)
C9^a	0.3208 (0.1001)	29.00 (4.18)
C17	0.1659 (0.0629)	13.33 (2.89)
M2^a	0.4062 (0.0896)	30.00 (3.54)
M3^c	0.5617 (0.2153)	24.29 (8.38)
M5^a	0.5081 (0.1865)	28.00 (9.08)
M6^b	0.5642 (0.1494)	23.33 (5.16)
M9^a	0.4308 (0.1069)	28.00 (4.47)

^{a}n = 5, ^{b}n = 6, ^{c}n = 7

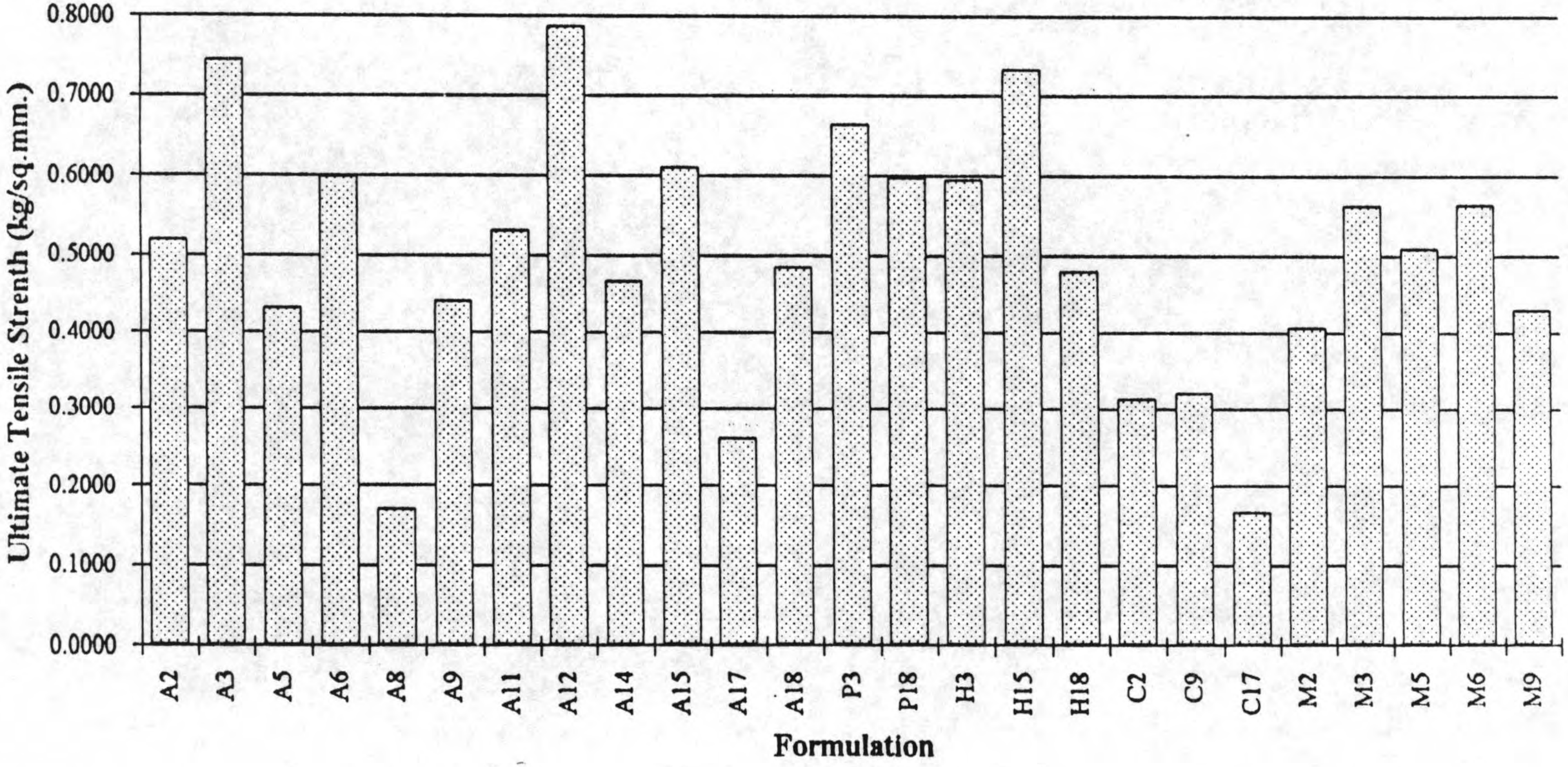


Figure 6 Ultimate tensile strength of various crosslinked chitosan-polymer membranes. A=crosslinked chitosan-PVA membrane, P=crosslinked chitosan-PVPK90 membrane, H=crosslinked chitosan-HPMC15 membrane, C=crosslinked chitosan-corn starch membrane, M=crosslinked chitosan-MC15 membrane.

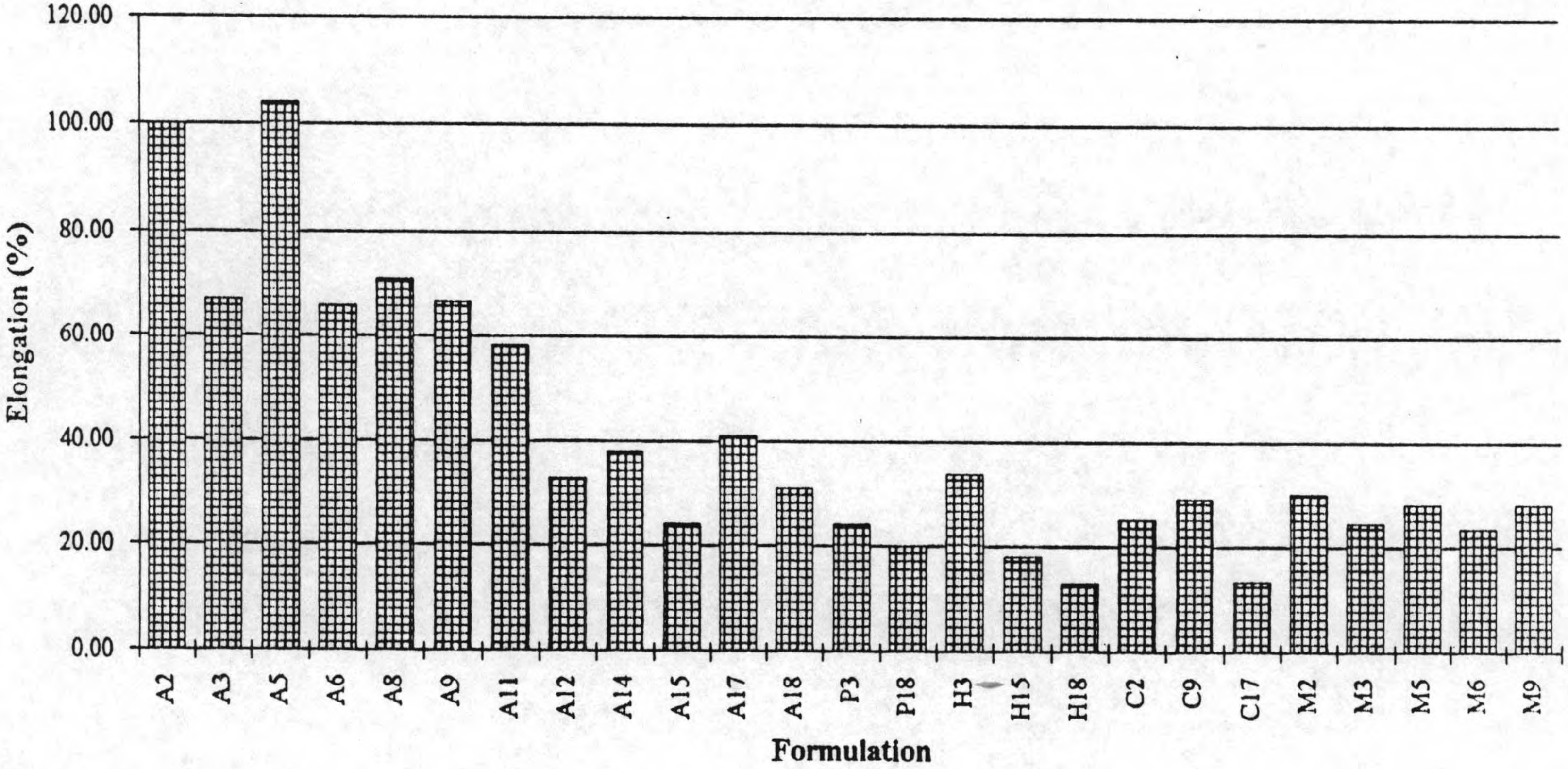


Figure 7 Percent elongation of various crosslinked chitosan-polymer membranes. A=crosslinked chitosan-PVA membrane, P=crosslinked chitosan-PVPK90 membrane, H=crosslinked chitosan-HPMC15 membrane, C=crosslinked chitosan-corn starch membrane, M=crosslinked chitosan-MC15 membrane.

Apparently, both plasticizers, PEG1450 and triacetin, were observed to increase the elongation at break of the membranes as compared to unplasticized membranes. These results could be observed in membranes with PEG1450 and triacetin, A11 and A17, respectively, as compared to unplasticized membrane A14 and membranes containing PEG1450 and triacetin, A12 and A18, respectively, in comparison with unplasticized membrane A15. In addition, it was noted that increasing in the concentration of crosslinking agent caused an increase in the ultimate tensile strength and a decrease in the elongation at break as shown in membranes with PEG1450, A11 and A12, unplasticized membranes A14 and A17, and membranes plasticized with triacetin, A17 and A18, in comparison with membranes plasticized with PEG1450, A2 and A3, unplasticized membranes A5 and A6, and membranes plasticized with triacetin A8 and A9, respectively. These observations strongly pointed out the influence of the ratio of polymer blend, type of plasticizer as well as the concentration of crosslinking agent on the mechanical properties of the membranes.

Comparison the unplasticized membrane H15 with membrane H18 plasticized with triacetin indicated that incorporation of triacetin into membrane caused a decrease in both the ultimate tensile strength and percent elongation at break.

For membrane obtained from concentrations of 2:3 chitosan : MC15, membrane with PEG1450, M2 not only exhibited lower ultimate tensile strength but also showed longer elongation at break in comparison with unplasticized membrane M5. Similarly, for 3:2 chitosan : MC15 membrane, membranes with PEG1450 and triacetin, M3 and M6, respectively, were observed to give lower ultimate tensile strength and higher elongation at break in comparison with unplasticized membrane A6. However, the ultimate tensile strengths of membrane with PEG1450 M3 and unplasticized membrane M6 were not greatly different.

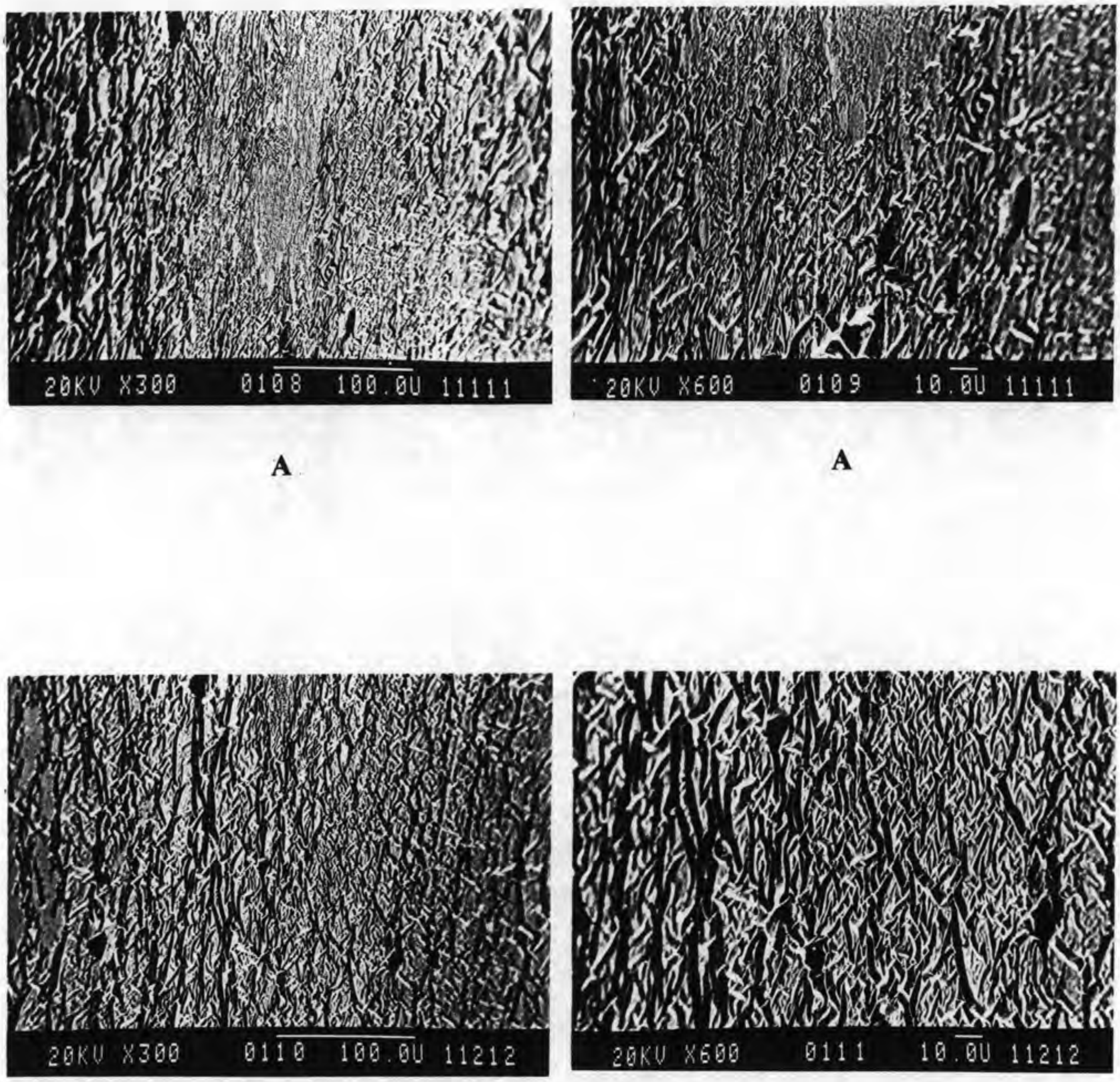
Overall, for the mechanical data stated above, it was obvious that the type and ratio of polymer blend, the concentration of crosslinking agent and the type of plasticizer had an influence on the mechanical properties of prepared membranes.

From preliminary in-vitro skin permeation studies, it was found that water sorption ability of the membrane was the critical factor in this study. Obviously, the preliminary results indicated that membranes which exhibited lower water sorption ability were good candidate to act as rate-controlling membrane in transdermal patch. Apparently, these membranes could preserve their integrity in transdermal patch throughout the permeation study without membrane rupture. Thereby, ten membrane formulations including A11, A12, A14, A15, A17, A18, P18, H18, C2 and M2 were selected based on their water sorption ability for in-vitro permeation study and furthur examinations.

Surface morphology

The surface morphology of free films, including membranes plasticized with PEG1450 A11, A12 C2, M2 unplasticized membranes A14, A15 and membranes plasticized with triacetin A17, A18, were manifested at 300 and 600 times. All photomicrographs showed that these free films possessed pore structure in their polymer networks.

For crosslinked chitosan : PVA membranes, Figure 8 shows surface characteristic of membranes with PEG1450 A11 and A12. These photomicrographs indicated that membrane A11 and A12 presented the wrinkled rough surface. In addition, the surface photomicrographs of



A A

B B

Figure 8 Surface photomicrographs of crosslinked chitosan-PVA membranes with PEG 1450 at manification of 300X and 600X, A = 2:3 chitosan : PVA membrane A11, B = 3:2 chitosan : PVA membrane A12.

membrane A11 also exhibited some macroporous structure and loosely medium pore-size distributions whereas membrane A12 showed only loosely medium pore-size distributions.

Surface characteristics of unplasticized membrane A14 and A15 are presented in Figures 9 and 10. Obviously, membrane A14 showed high porous structure and seemed to have smooth surface. Not only did it present more medium to large pore size but also highly compact pore distributions as well. Similarly, the surface photomicrographs of membrane A15 also exhibited smooth surface, high porosity and dense pore distributions. However, the pore size seemed to range from small to medium.

Photomicrographs of membranes containing triacetin A17 and A18 as illustrated in Figure 11 showed that membrane A17 possessed moderately compact of small to medium pore-size distributions whereas membrane A18 had some macroporous and narrower pore-size distributions. In addition, these two membranes were quite different in degree of smoothness of the membrane surfaces. Membrane A18 exhibited smooth surface while membrane A17 seemed to have rough surface with rodlike assemblies.

The photomicrographs presented in Figure 12 showed the surface morphology of crosslinked chitosan - PVP K90 membrane P18 plasticized with triacetin and crosslinked chitosan - HPMC15 membrane H18 plasticized with triacetin. It was apparent that membrane P18 had highly dense of small to medium pore-size distributions in its polymer network whereas the surface morphology of membrane H18 indicated that this membrane not only presented less porosity but also much smaller pore sizes. The photomicrographs also indicated that membrane P18 seemed to have both rough surface regions and smooth surface regions while membrane H18

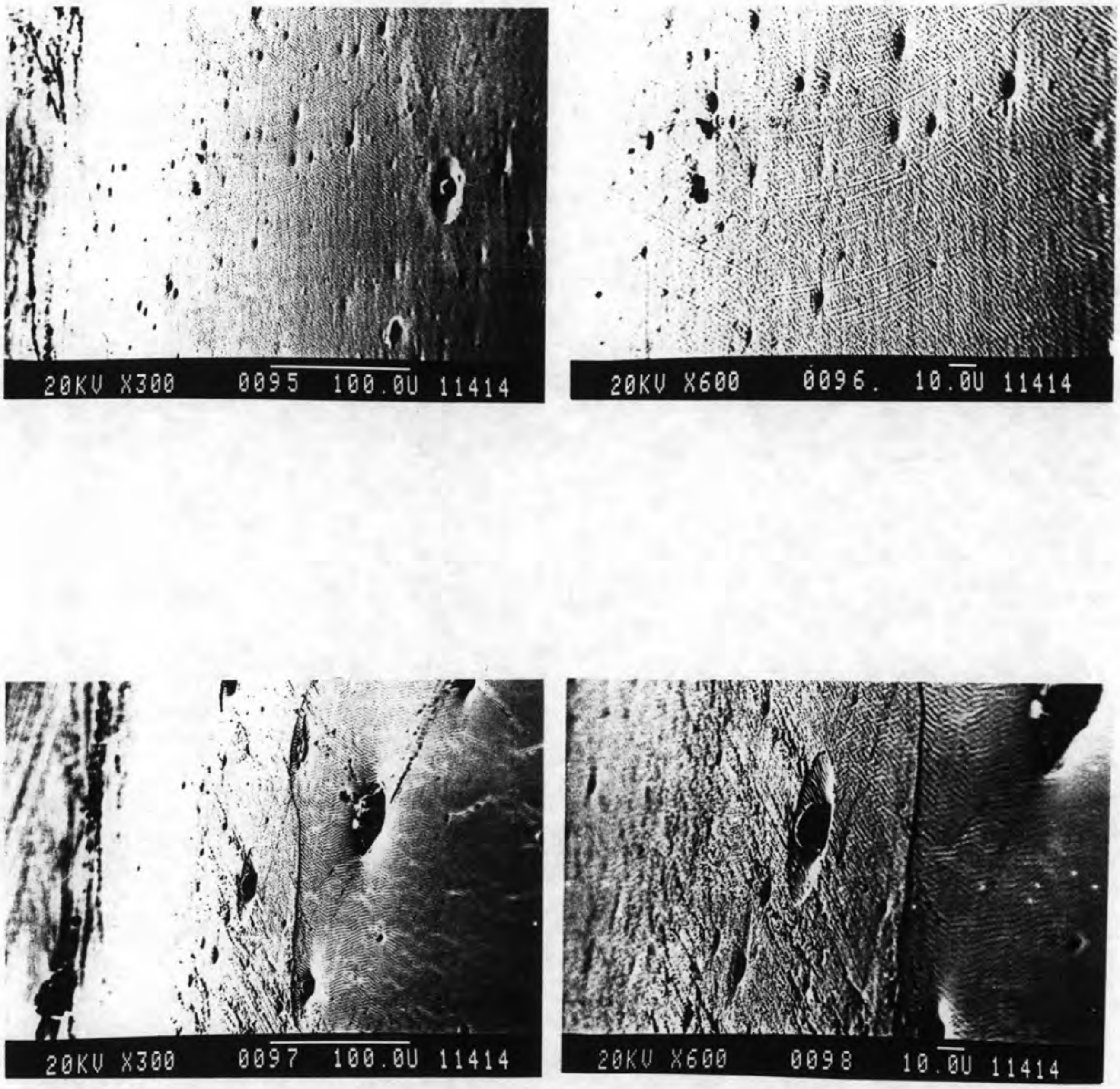


Figure 9 Surface photomicrographs of unplasticized crosslinked 2:3 chitosan : PVA membrane A14 at manification of 300X and 600X.

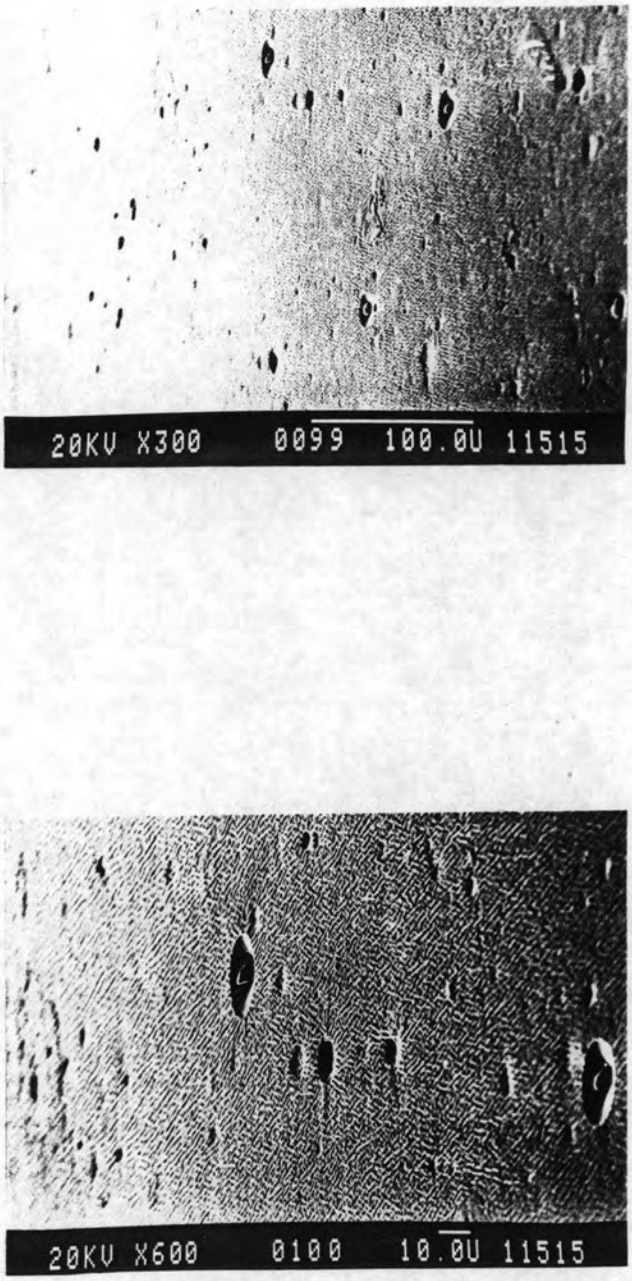


Figure 10 Surface photomicrographs of unplasticized crosslinked 3:2 chitosan : PVA membrane A15 at manification of 300X and 600X.

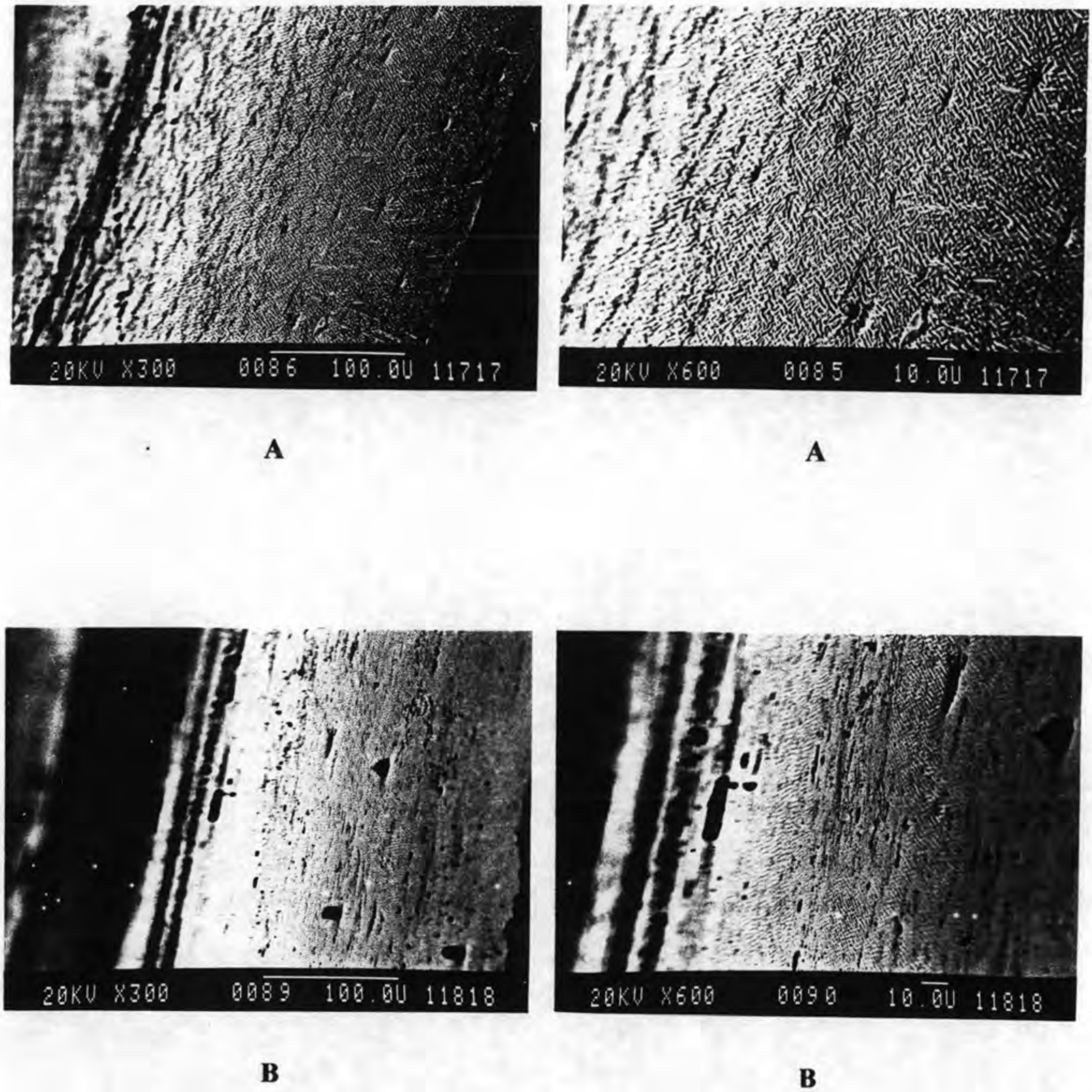


Figure 11 Surface photomicrographs of crosslinked chitosan-PVA membranes with triacetin at manification of 300X and 600X, A = 2:3 chitosan : PVA membrane A17, B = 3:2 chitosan : PVA membrane A18.

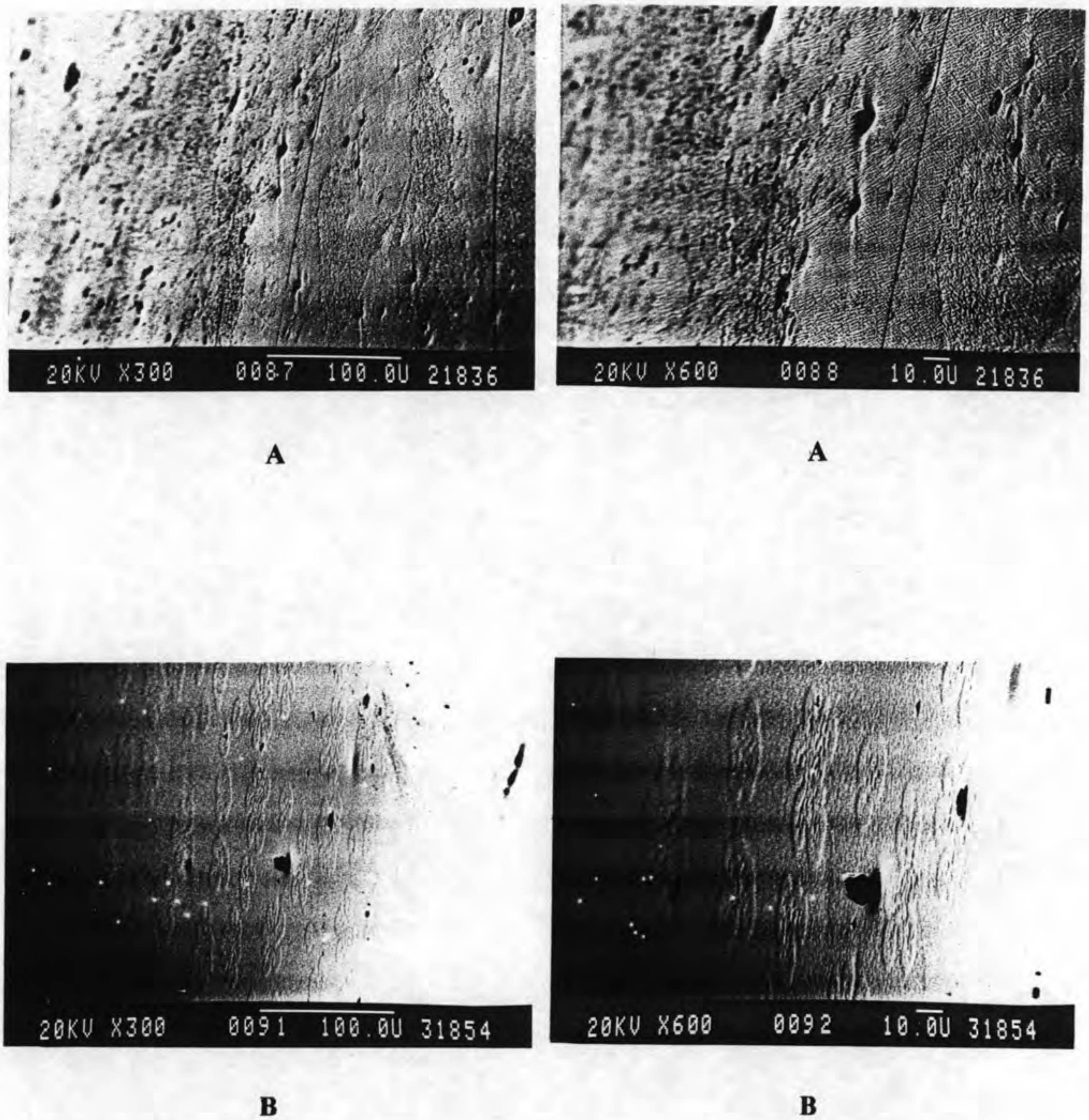


Figure 12 Surface photomicrographs of crosslinked chitosan-PVPK90 membrane with triacetin P18 (A) and crosslinked chitosan-HPMC15 membrane with triacetin H18 (B) at manification of 300X and 600X.

seemed to present smooth surface with moldlike colonies spreading over the membrane surface.

Figure 13 illustrates the surface characteristics of crosslinked chitosan - corn starch membrane C2 plasticized with PEG1450 and crosslinked chitosan - MC15 membrane M2 plasticized with PEG1450. The photomicrographs of membrane C2 not only presented rough surface but also indicated high porosity with various pore-size distributions as well whereas the surface photomicrographs of membrane M2 revealed that this membrane exhibited smooth surface and high porosity showing highly dense pore distributions of small to medium pore-size.

Infrared spectrometry

Figures 14 to 17 show infrared spectra of the pure components and of selected crosslinked chitosan - polymer membranes. IR spectrum of chitosan could be broadly divided into three zones, around 3600-3200 cm^{-1} indicating O-H stretching vibrations of free hydroxy group (3650-3584 cm^{-1}), O-H stretching vibrations of intermolecular hydrogen bonding (3550-3200 cm^{-1}), and N-H stretching vibrations (3520-3400 cm^{-1}), between 1650-1515 cm^{-1} indicating N-H bending vibrations and between 1170-1114 cm^{-1} indicating C-O-C stretching band. (Silverstein, Bassler, and Morrill, 1991, Garnpimol Ritthidej et al., 1994) In addition, the chitosan spectrum also showed the crystallization-sensitive bands at 3451-, 1094- and 665 cm^{-1}. HPMC 15 and MC15 had similar infrared spectra but their spectra mainly differed from that of chitosan in that the band around 3520-3400 cm^{-1}. The IR spectrum of PVA indicated the characteristic peak for crystalline region at 1145 cm^{-1}. These crystallization-sensitive bands of chitosan and PVA were fairly close in frequency with the observation of Miya and Iwamoto, (1984), and Kim et al., (1992).

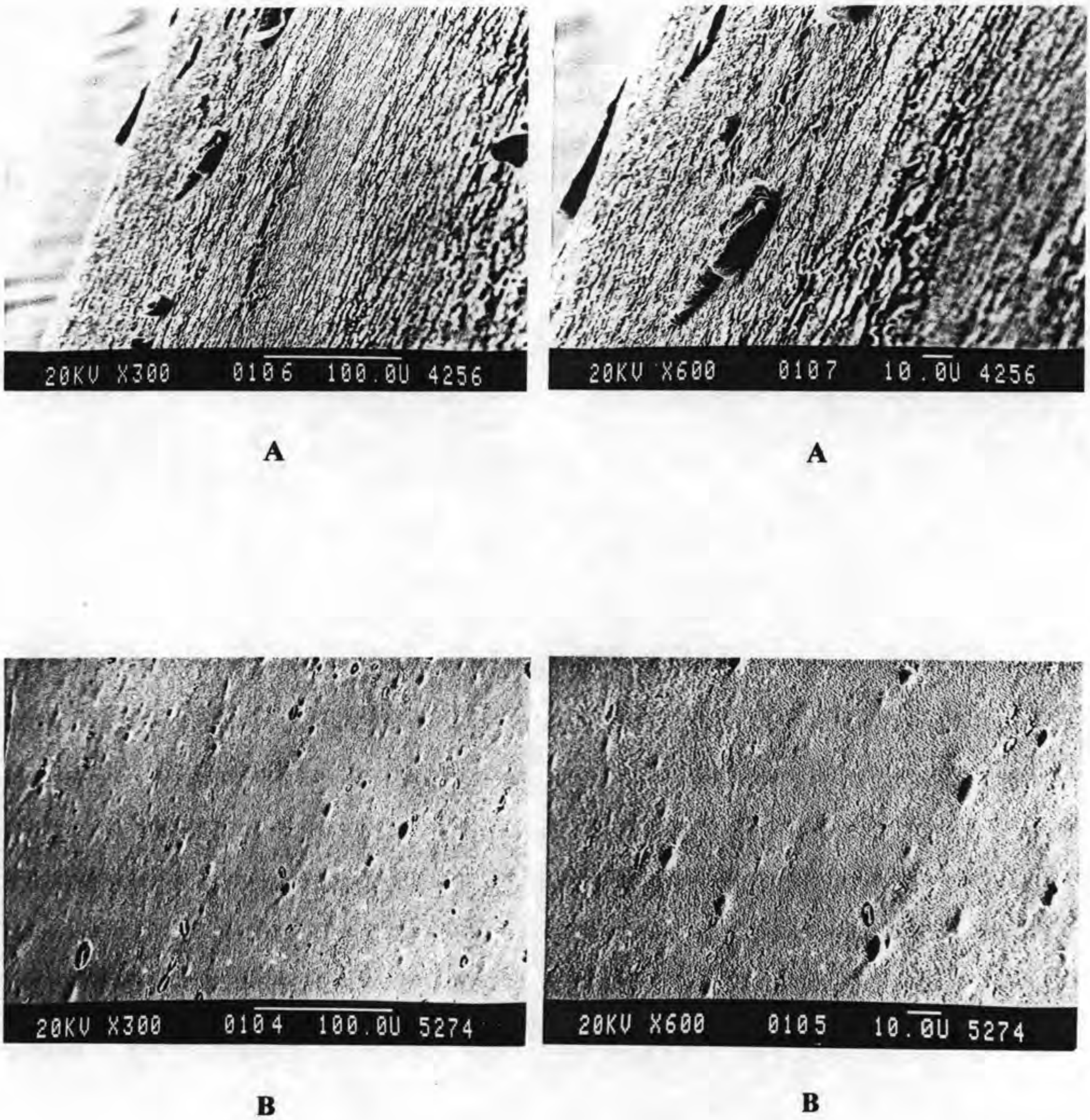


Figure 13 Surface photomicrographs of crosslinked chitosan-corn starch membrane plasticized with PEG 1450 C2 (A) and crosslinked chitosan-MC15 membrane with PEG 1450 M2 (B) at manification of 300X and 600X.

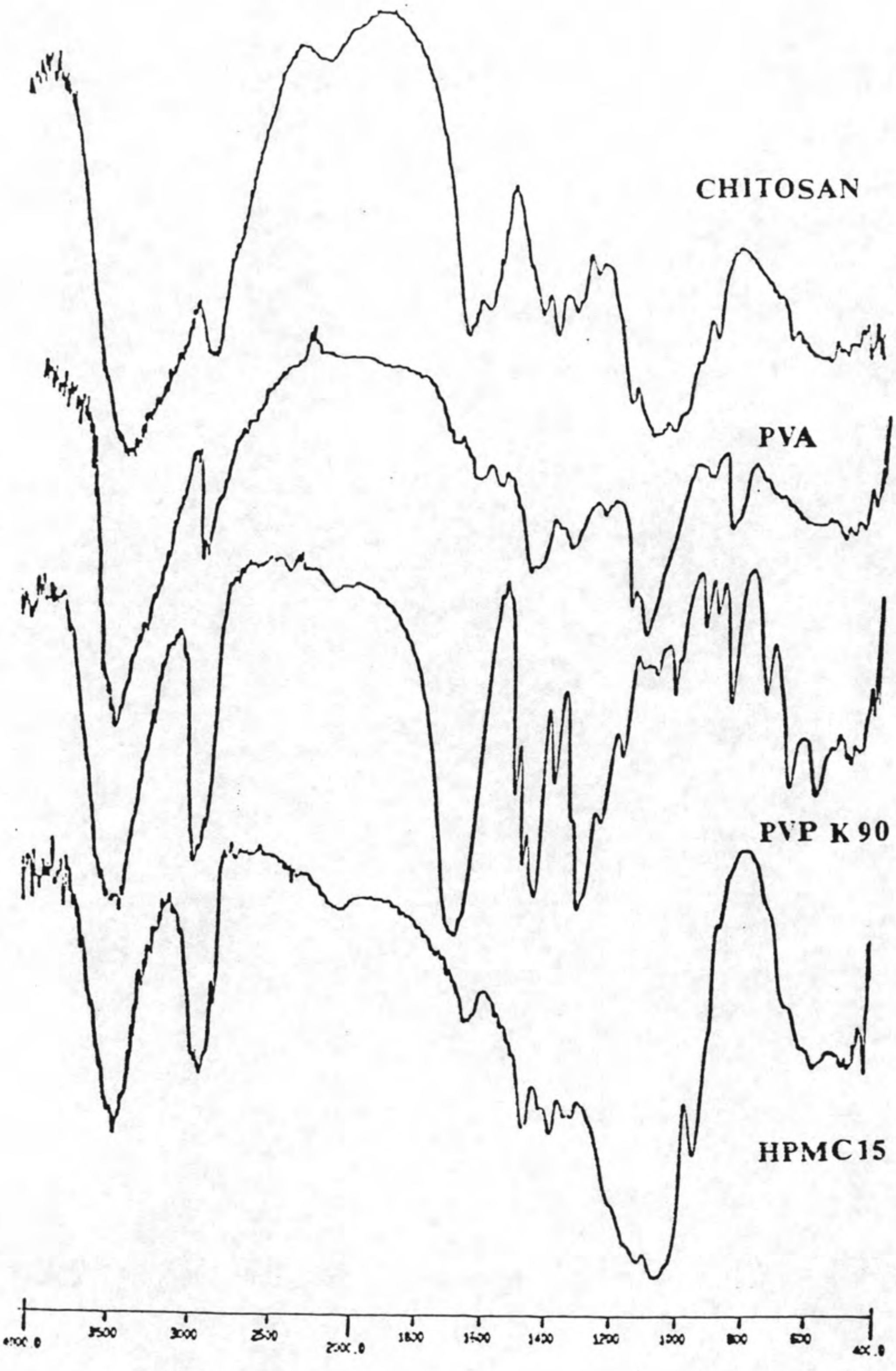


Figure 14 IR spectra of chitosan, PVA , PVPK90 , and HPMC-15

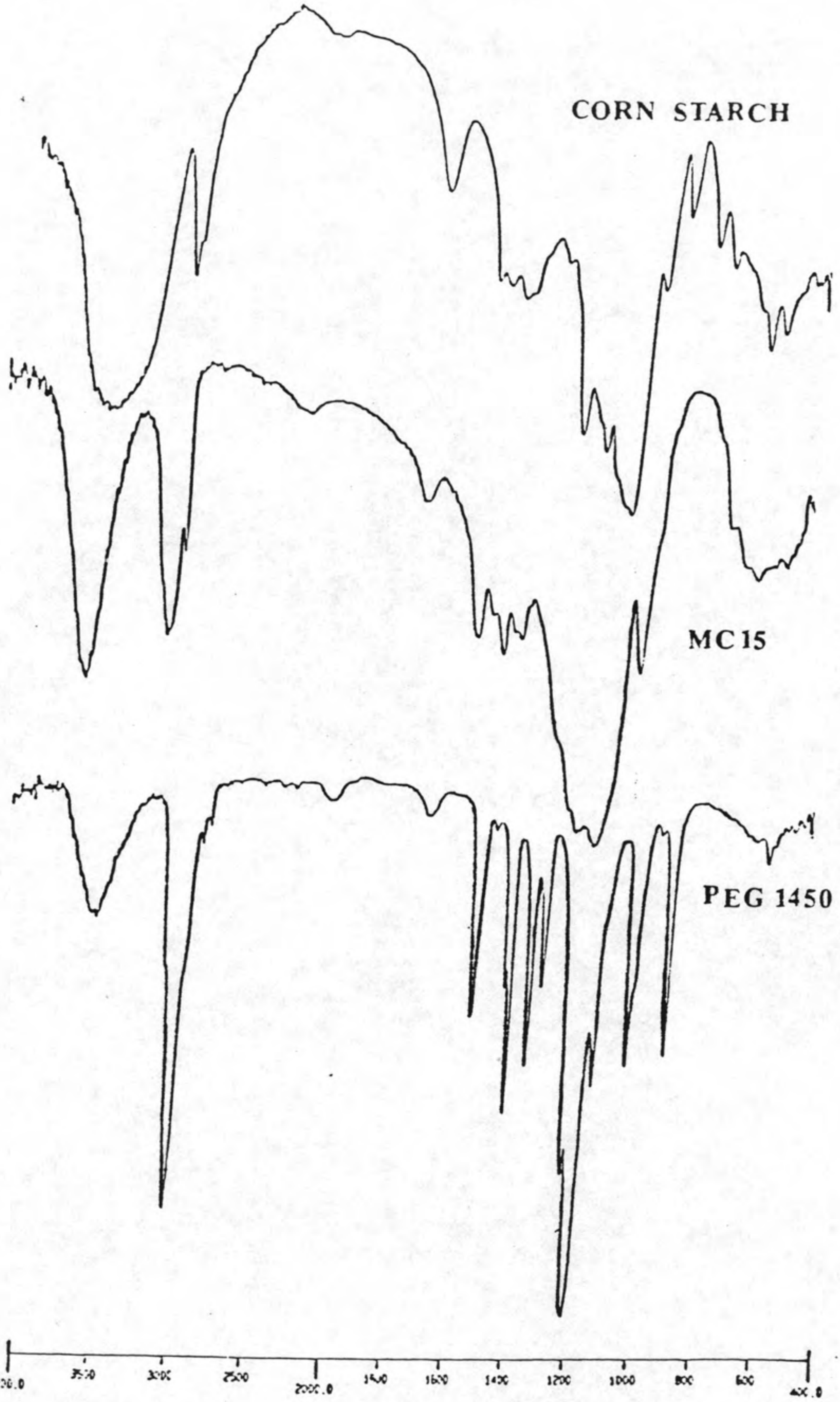


Figure 15 IR spectra of corn starch , MC15 , and PEG 1450

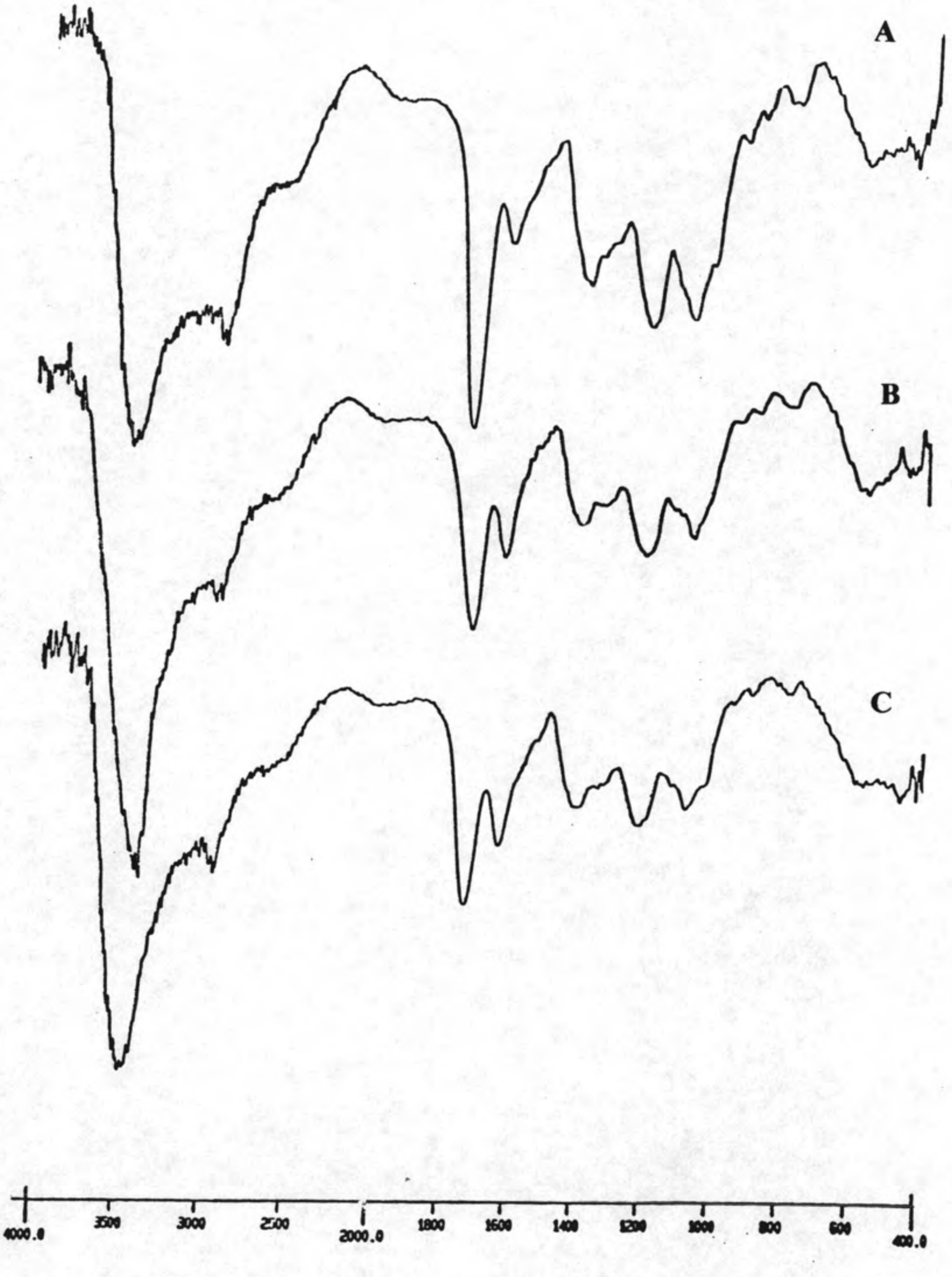


Figure 16 IR spectra of crosslinked chitosan-PVA membranes, A = membrane plasticized with PEG 1450, B = unplasticized membrane, and C = membrane plasticized with triacetin.

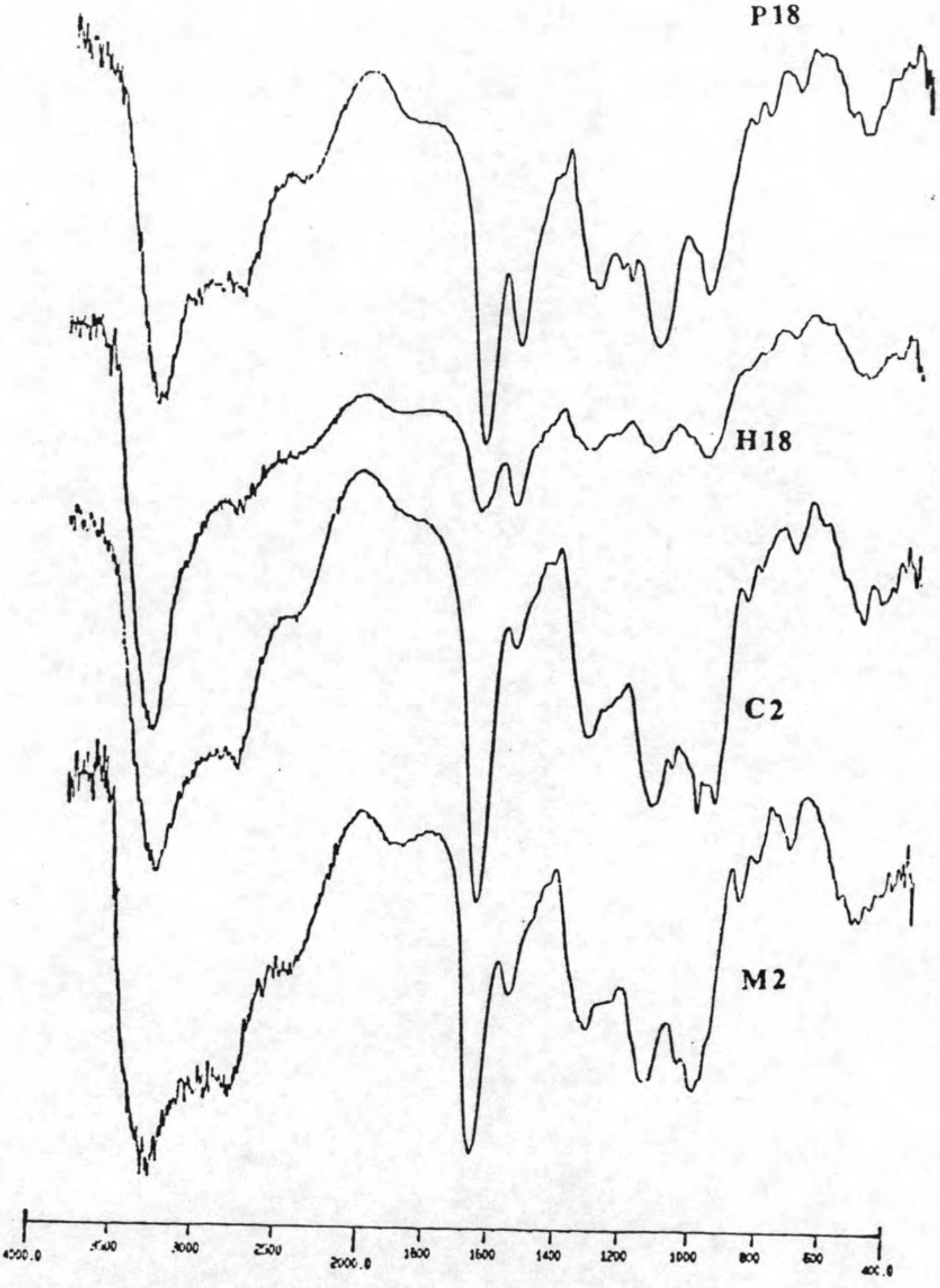


Figure 17 IR spectra of crosslinked chitosan-PVPK90 membrane with triacetin P18, crosslinked chitosan-HPMC15 membrane with triacetin H18, crosslinked chitosan-corn starch membrane with PEG 1450 C2, and crosslinked chitosan-MC15 membrane with PEG 1450 M2.

There were differences between the infrared spectra of chitosan in the pure state and in the crosslinked chitosan - polymer membranes. The first was the feature in the 3600-3200 cm^{-1} region which was concerned with O-H and N-H stretching vibrations. The crystallization-sensitive peak at 3451 cm^{-1} (Miya and Iwamoto, 1984) in pure chitosan did not appear in the crosslinked chitosan-polymer membranes, and not only did the characteristic shape of the spectrum of chitosan change in the crosslinked chitosan - polymer membranes but the band also shifts to a lower frequency at 3447-3448 cm^{-1}, 3452 cm^{-1}, 3430 cm^{-1}, 3456 cm^{-1} and 3461 cm^{-1} for crosslinked chitosan - PVA membranes either with or without plasticizer, crosslinked chitosan - PVPK90 membrane plasticized with triacetin P18, crosslinked chitosan - HPMC15 membrane with triacetin H18, crosslinked chitosan - corn starch membrane with PEG1450 C2 and crosslinked chitosan - MC15 membrane containing PEG1450 M2, respectively. The second characteristic peak for crystalline region at 1094 cm^{-1} (Miya and Iwamoto, 1984, Kim et al., 1992) prominent in pure chitosan did not exhibit in the crosslinked chitosan - polymer membranes. The third crystallization sensitive band at 665 cm^{-1} (Miya and Iwamoto, 1984) in pure chitosan became much less distinct in the crosslinked chitosan - polymer membranes. Finally, the band at 1598 cm^{-1} in pure chitosan indicating the bending vibrations of amino group (Silverstein, Bassler, and Morrill, 1991) did not appear in the crosslinked chitosan - polymer membranes, whereas, all crosslinked chitosan - polymer membranes exhibited a new band at 1689-1471 cm^{-1} region which was characteristic of the C=N stretching vibrations (Silverstein, Bassler, and Morrill, 1991).

Differential scanning calorimetry

The normalized DSC thermograms of all test samples are depicted in Figures 18-23 and DSC peak temperatures are summarized in Table 14. The DSC thermogram of pure chitosan gave the characteristic melting endotherm at 155°C. Citric acid not only showed the different DSC peak temperature but its DSC pattern also differed from those of the pure polymers. The homogenous PVA exhibited the first melting endotherm with a sharp peak at 175.7°C and the subsequent broad peak endotherm at 226.2°C while PVP K90 showed the single melting endotherm at 158.5°C. However, the melting termperatures of chitosan and PVP K90 were higher than the reported values of Qurashi et al. (1992) whereas PVA showed the lower endothermic peak temperature than the value reported by Kim et al.,(1992) . The melting temperatures of chitosan and PVP K90 reported by Qurashi et al. (1992) were 142.47 °C and 149.39 °C, respectively. Kim et al. (1992) reported the single endothermic peak of PVA around 230 °C.

All crosslinked chitosan - polymer membranes, which were investigated, exhibited similar pattern of DSC thermograms. Their DSC thermograms were different in endothermic peak temperature and different in both DSC pattern and peak temperature from those of pure substances. The DSC thermograms of all test membranes did not indicate separation peak of pure polymer substances. The unplasticized crosslinked chitosan - PVA membrane A14 exhibited the highest melting endothermic peak temperature at 164°C whereas the lowest endothermic peak value (151.7°C) was observed in crosslinked chitosan - PVP K90 membrane plasticized with triacetin P18.

For crosslinked chitosan - PVA membranes, the addition of plasticizers reduced the crystalline melting point. The melting endothermic

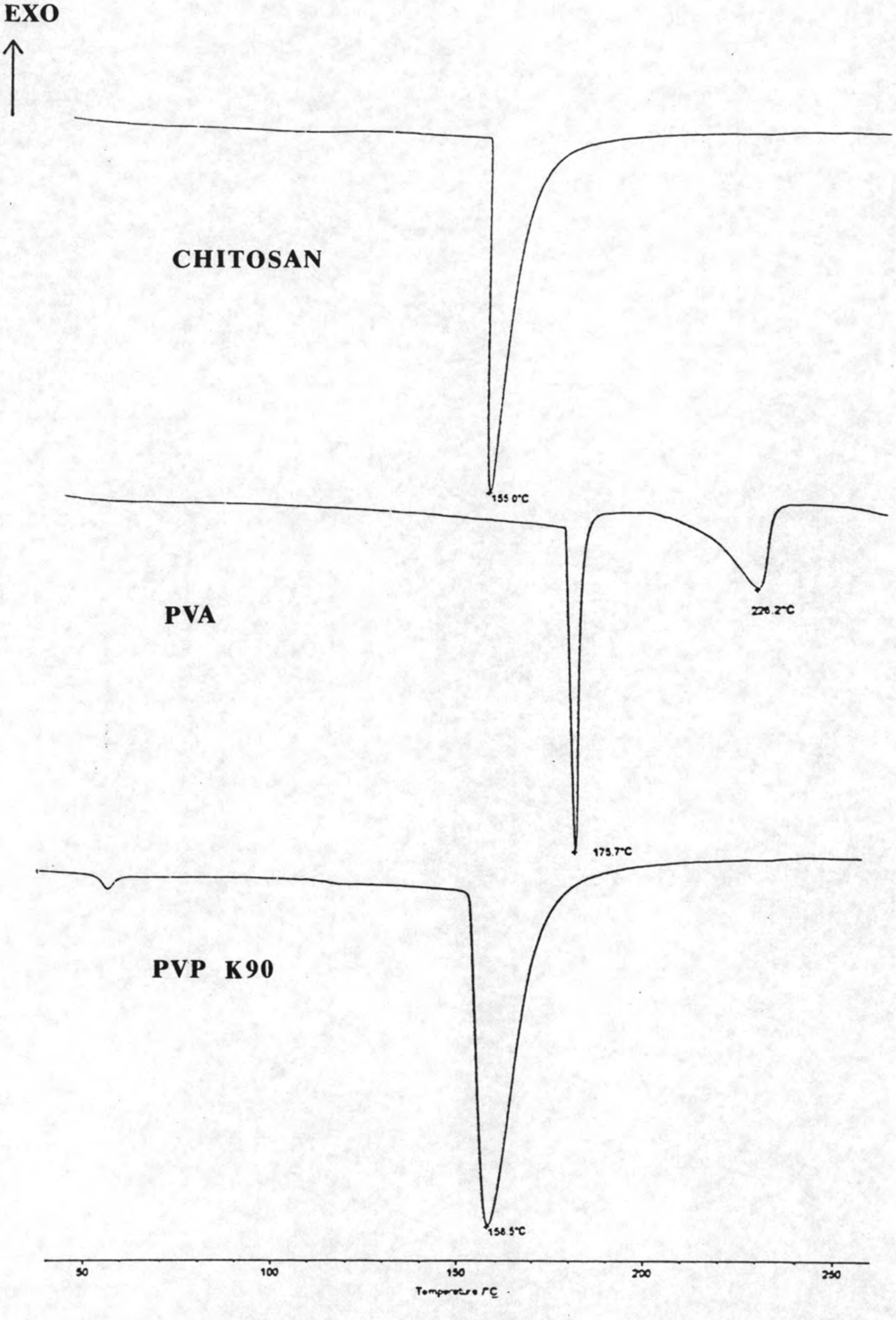


Figure 18 DSC thermograms of chitosan, PVA, and PVP K90.

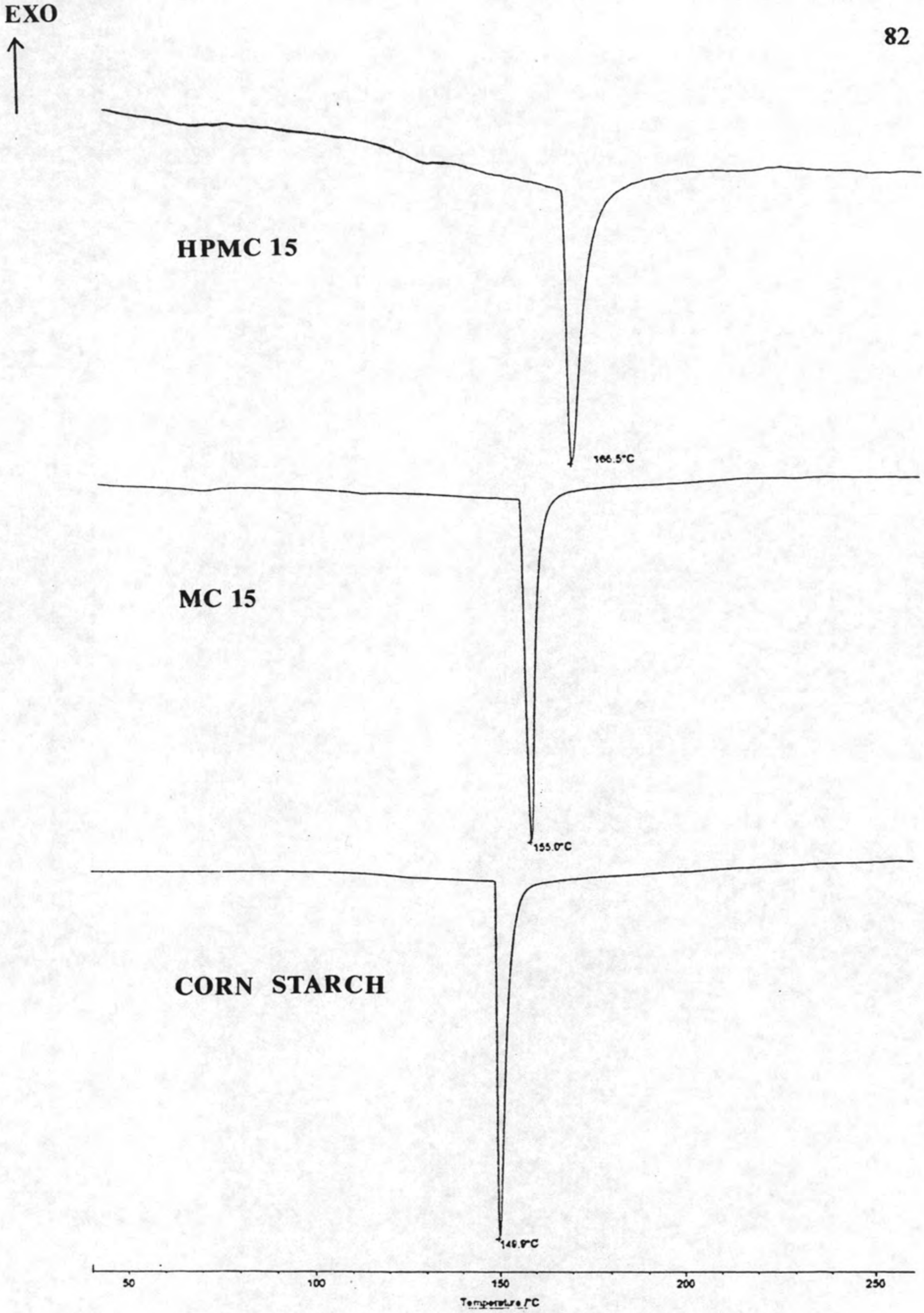


Figure 19 DSC thermograms of HPMC15, MC15, and corn starch.

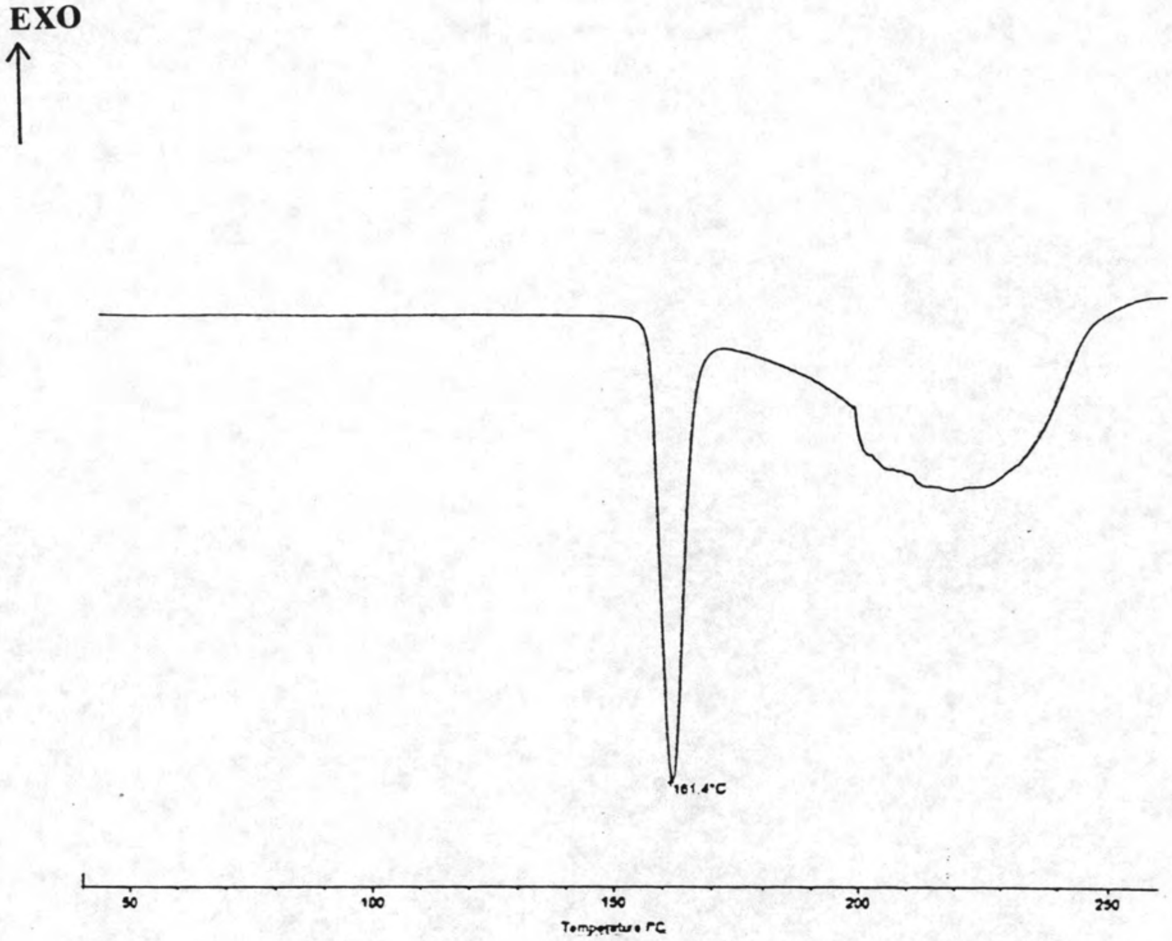


Figure 20 DSC thermogram of citric acid.

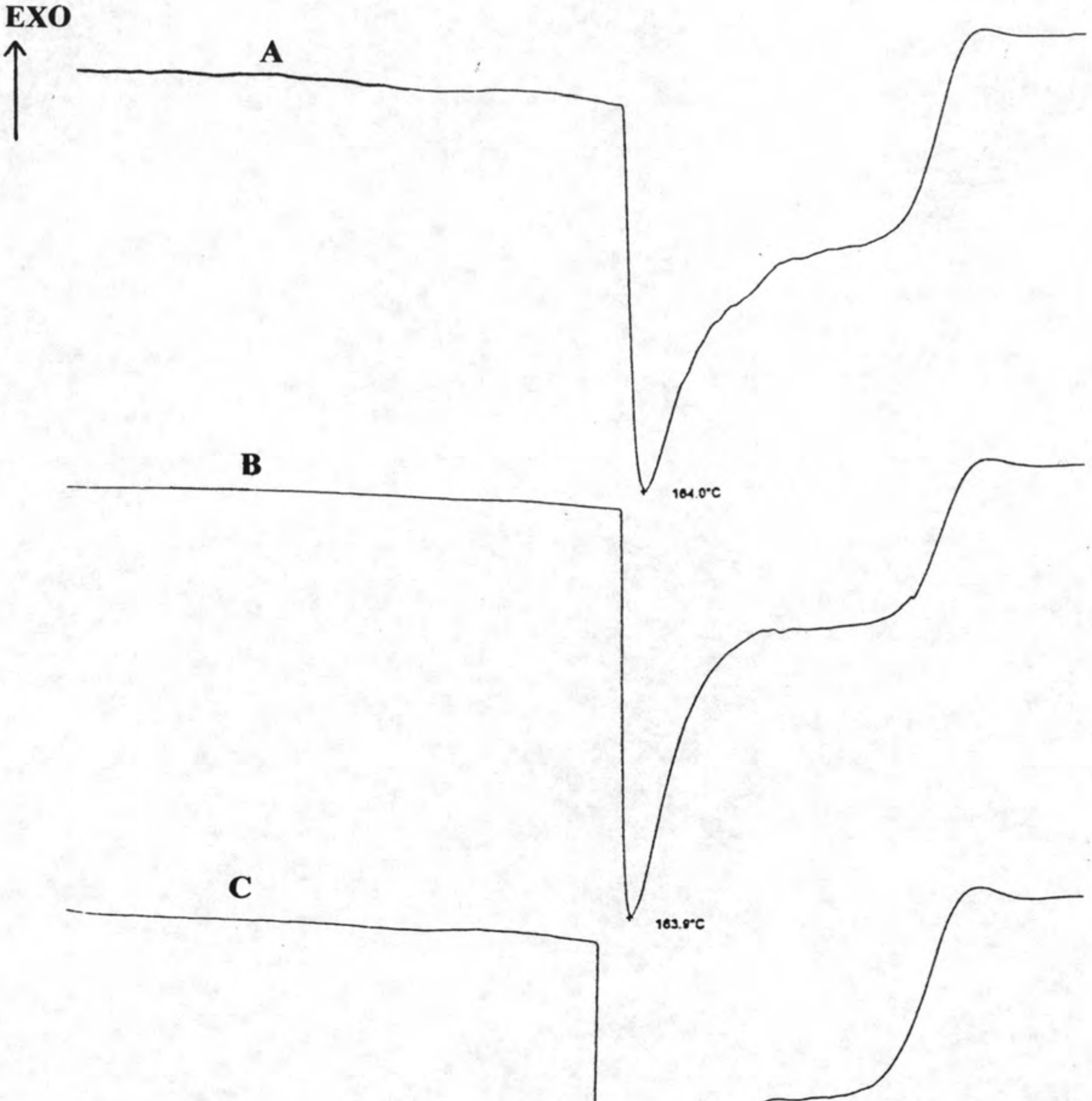


Figure 21 DSC thermograms of crosslinked chitosan-PVA membranes, A = unplasticized membrane A14, B = membrane plasticized with PEG 1450 A11, and C = membrane plasticized with triacetin A17.

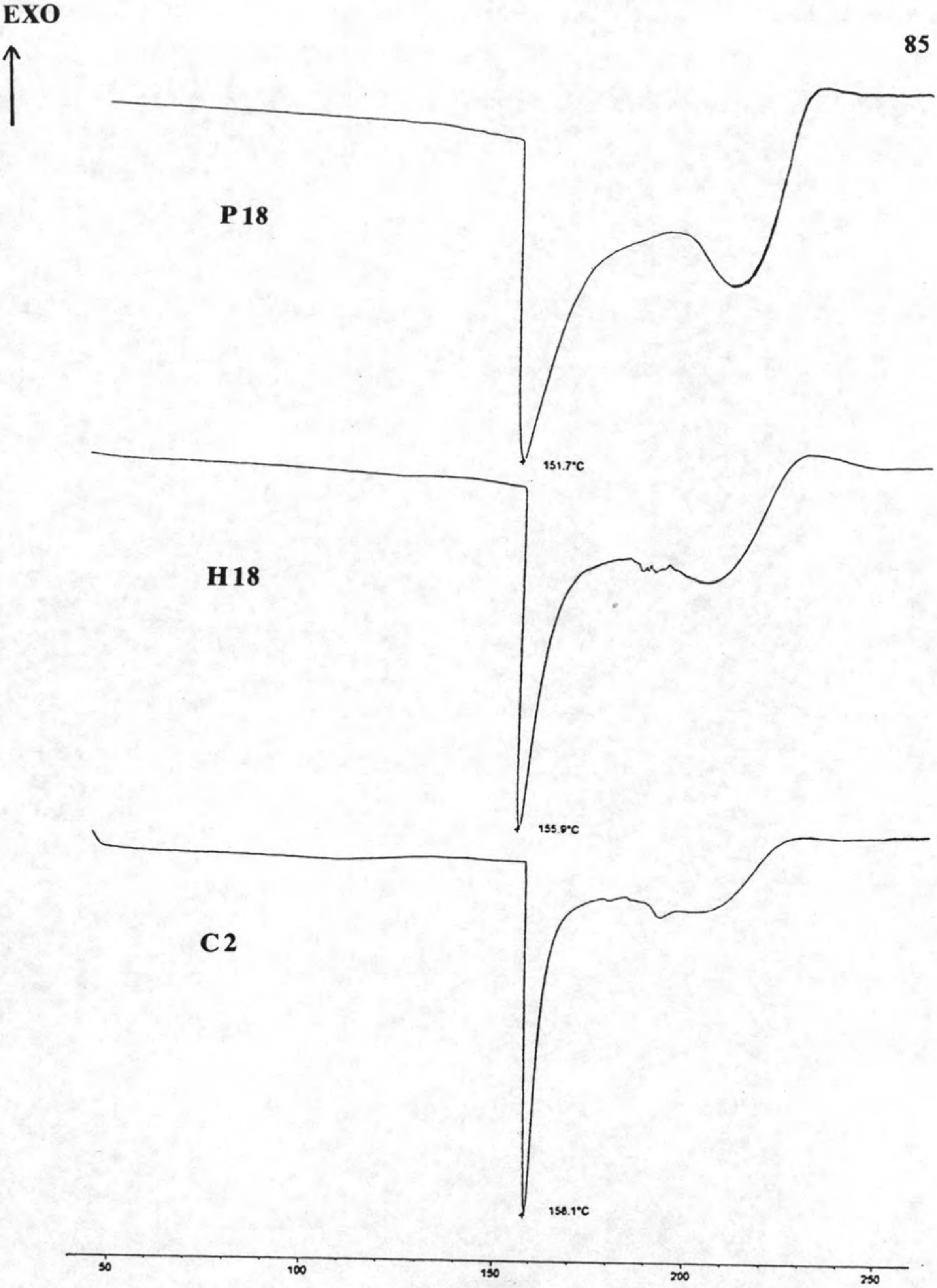


Figure 22 DSC thermograms of crosslinked chitosan-PVPK90 membrane with triacetin P18, crosslinked chitosan-HPMC15 membrane with triacetin H18, and crosslinked chitosan-corn starch membrane with PEG 1450 C2.

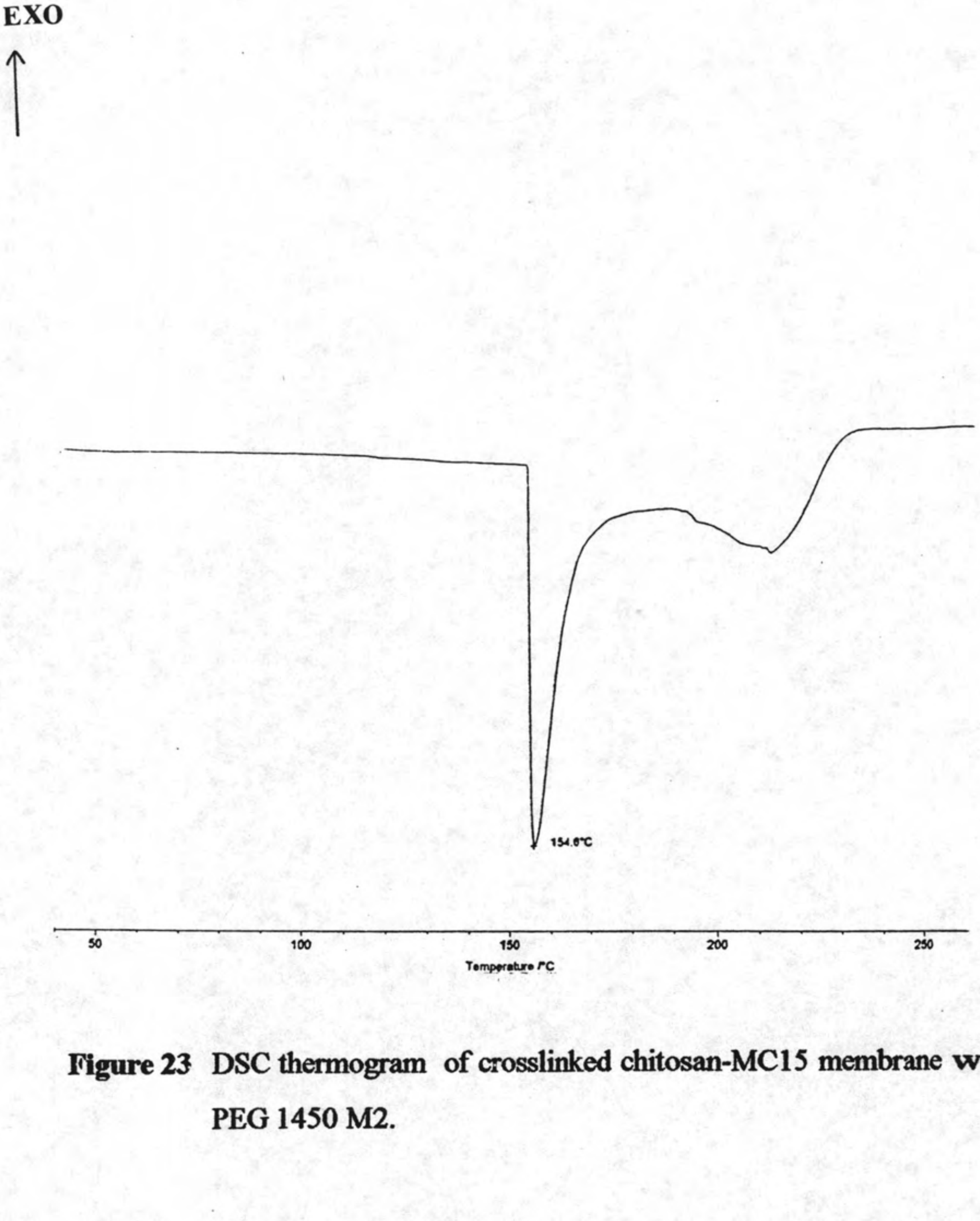


Figure 23 DSC thermogram of crosslinked chitosan-MC15 membrane with PEG 1450 M2.

Table 14 DSC peak temperatures

Sample	Order of DSC peak temperature (^{o}C)	
	1	2
Chitosan	155.0	-
PVA	175.7	226.2
PVPK90	158.5	-
HPMC15	166.5	-
Corn starch	149.9	-
MC15	155.0	-
Citric acid	161.4	-
Membrane A11	163.9	-
Membrane A14	164.0	-
Membrane A17	159.2	-
Membrane P18	151.7	-
Membrane H18	155.9	-
Membrane C2	158.1	-
Membrane M2	154.6	-

peaks of unplasticized membrane A14 and membrane with PEG1450 A11 were not substantially different whereas membrane with triacetin A17 showed lower endothermic peak temperature as compared to membranes A11 and A14. The influence of plasticizers on the DSC results appeared to agree with the study of Lim and Wan (1994).

Membrane thickness

The mean membrane thickness of the ten formulations used in transdermal patch were included in Table 15 and were varied within 100 ± 20 μm of this criteria limitation.

Table 15 Thickness of various permeated test membranes.

Formulation	Mean Thickness of Samples (mcm)		
	1	2	3
A11	110.8	111.4	114.0
A12	110.2	111.0	116.6
A14	112.6	109.0	111.4
A15	110.2	114.6	104.4
A17	108.4	107.2	116.6
A18	111.2	115.2	105.6
P18	102.6	103.0	103.2
H18	111.0	106.8	111.0
C2	109.6	105.8	111.0
M2	99.4	103.6	116.4

Permeation

For in-vitro skin permeation studies, the results as shown in Tables 16-17 were noted that a 2:3 chitosan : PVA unplasticized membrane A14 transdermal system provided the highest cumulative drug permeated through shed snake skin whereas the lowest cumulative value was observed with a 3:2 chitosan : HPMC 15 membrane plasticized with triacetin H18, transdermal patch. Comparing the permeation amount of ISDN from the patch containing membrane obtained from formulations of 2:3 chitosan : PVA as rate-controlling membrane suggested that the permeation amount of ISDN generated from unplasticized membrane A14 patch was about twice as great as that from the patch containing membranes A11, A17 plasticized with PEG1450 and triacetin, respectively, as rate-controlling membrane. Similarly, for 3:2 chitosan : PVA membrane, the unplasticized membrane A15 patch showed two fold greater cumulative amount permeated of ISDN than that obtained with the patch containing membrane A18 plasticized with triacetin as rate-controlling membrane. However, the cumulative amount permeated of ISDN delivered by membrane with PEG1450 A12 patch was found to be about 50% lower than the cumulative amount achieved by unplasticized membrane A15 patch. The total ISDN delivered through shed snake skin after 24 hours by transdermal patch containing unplasticized membrane A14 and membrane with PEG1450 C2 as rate-controlling membrane, were fairly close in magnitude.

In order to point out the permeation pattern, the permeation data were calculated and reported based on the generally described permeation mechanisms of solutes in a polymeric membranes, the partition and pore mechanisms (Nakatsuka and Andrady, 1992, Thacharodi and Rao, 1993).

Table 16 Cumulative ISDN skin permeated from various transdermal systems through shed snake skin.

Time	Cumulative Drug Permeated (mcg)								
(hrs.)	A11	A12	A14	A15	A17	A18	P18	H18	C2
0	0	0	0	0	0	0	0	0	0
0.5	0	14.6088	8.2058	23.4184	15.4422	18.5884	12.5340	0	9.4303
1	0	12.1703	6.1493	26.1062	12.8026	7.7675	25.7345	15.7908	20.1243
2	10.3061	15.4472	23.5200	33.0288	15.4819	14.9585	28.9895	16.9690	15.6443
4	45.3153	25.9642	32.2226	34.8817	13.6247	0.9659	28.1753	28.3282	18.9430
6	40.5977	31.3510	20.8996	40.7403	10.5003	68.4612	32.1468	26.3295	25.3837
8	7.6626	37.4905	38.6831	46.4740	26.1048	50.2067	35.4244	16.5220	34.0014
10	11.2444	50.8605	62.3330	58.0462	13.2333	35.1669	18.4999	26.5242	56.3156
12	11.5501	60.3049	121.7334	92.0143	13.7377	58.6141	35.1982	37.1662	87.8288
16	64.3221	98.3584	208.4352	173.3146	80.0641	103.2221	52.3482	37.1567	179.9316
20	178.0092	171.4426	395.8701	305.2341	200.9479	147.7980	77.9879	62.3615	310.3292
24	273.8649	228.9448	549.2224	440.9888	282.6771	209.5014	160.9537	80.3245	547.5985

Table 17 Cumulative ISDN skin permeated from membrane M2 transdermal patch through shed snake skin.

Time (hrs.)	Cumulative drug permeated (mcg)
0	0
0.5	42.6361
1	32.7566
4	25.6284
8	37.7993
12	65.0955
18	128.7066
24	210.1368

The shed snake skin permeation profiles of ISDN delivered from the patch are shown in Figures 24 to 29 and the correlation coefficients of the relationship between cumulative drug permeated versus time and versus square root time are presented in Table 18.

Apparently, the permeation of ISDN through the shed snake skin, following the release from the transdermal system, seemed to result in a constant rate profile in all cases. Thus, the permeation profile was rather following a zero-order kinetic than that followed Higuchi's model. This trend of kinetic pattern could be better described by the higher correlation coefficients in the relationship between the cumulative ISDN permeation versus time. Even though the cumulative drug permeated might be substantially different, all patches were capable of providing a sustained pattern throughout the period of 24 hours.

In consideration, each cumulative permeated profile of the transdermal patch could be divided into two phases of different permeation. The first regime was observed to show ISDN permeation of lower permeation rate over a ten-hour period. The second regime (10-24 hours) was a region of increasing permeation rate. The permeation rate and the linear correlation coefficient of each stage of each transdermal system are shown in Table 19.

Table 18 Correlation coefficient of the relationship between cumulative ISDN permeated versus time (A), cumulative ISDN permeated versus square root time (B) and kinetic pattern.

Formulation	A			B			Kinetic pattern
	correlation coefficient	Y-intercept	x-coefficient	correlation coefficient	Y-intercept	x-coefficient	
A11	0.7165	-24.4693	9.0483	0.5350	-48.5483	40.3153	Zero-order
A12	0.9093	-10.3403	8.4157	0.7416	-37.0134	39.1853	Zero-order
A14	0.8637	-55.0306	20.5569	0.6662	-113.5250	93.0881	Zero-order
A15	0.8544	-28.6584	15.6343	0.6642	-73.8428	71.0721	Zero-order
A17	0.7441	-26.8580	9.7286	0.5389	-51.0861	42.6905	Zero-order
A18	0.8895	-6.3147	7.6428	0.7459	-31.8025	36.0855	Zero-order
P18	0.7274	3.5865	4.4923	0.5865	-10.3499	20.7980	Zero-order
H18	0.8801	5.2439	2.7492	0.8133	-5.5610	13.6266	Zero-order
C2	0.8162	-52.0727	18.6512	0.6141	-102.4980	83.4136	Zero-order
M2	0.9373	6.8000	7.2350	0.8535	-11.9187	33.3352	Zero-order

Table 19 Permeation rate (slope) and correlation coefficient of the first and the second phase of the cumulative drug permeated profiles

Formulation	The first phase		The second phase	
	permeation rate (mcg/hr)	correlation coefficient	permeation rate (mcg/hr)	correlation coefficient
A11	1.7064	0.1212	19.6166	0.9529
A12	4.2279	0.9462	13.1630	0.9753
A14	5.0230	0.8439	34.9184	0.9834
A15	4.1734	0.8062	27.4746	0.9834
A17	0.9062	0.2228	20.5381	0.9661
A18	4.5539	0.4725	12.1953	0.9949
P18	1.5355	0.2373	9.2210	0.8920
H18	2.1059	0.4889	3.7105	0.9288
C2	4.2300	0.8584	33.9946	0.9476
M2 *	1.8787	0.1409	10.8612	0.9850

* For the formulation M2, the first phase was the first 8 hours and the second phase was the later 8 - 24 hours.

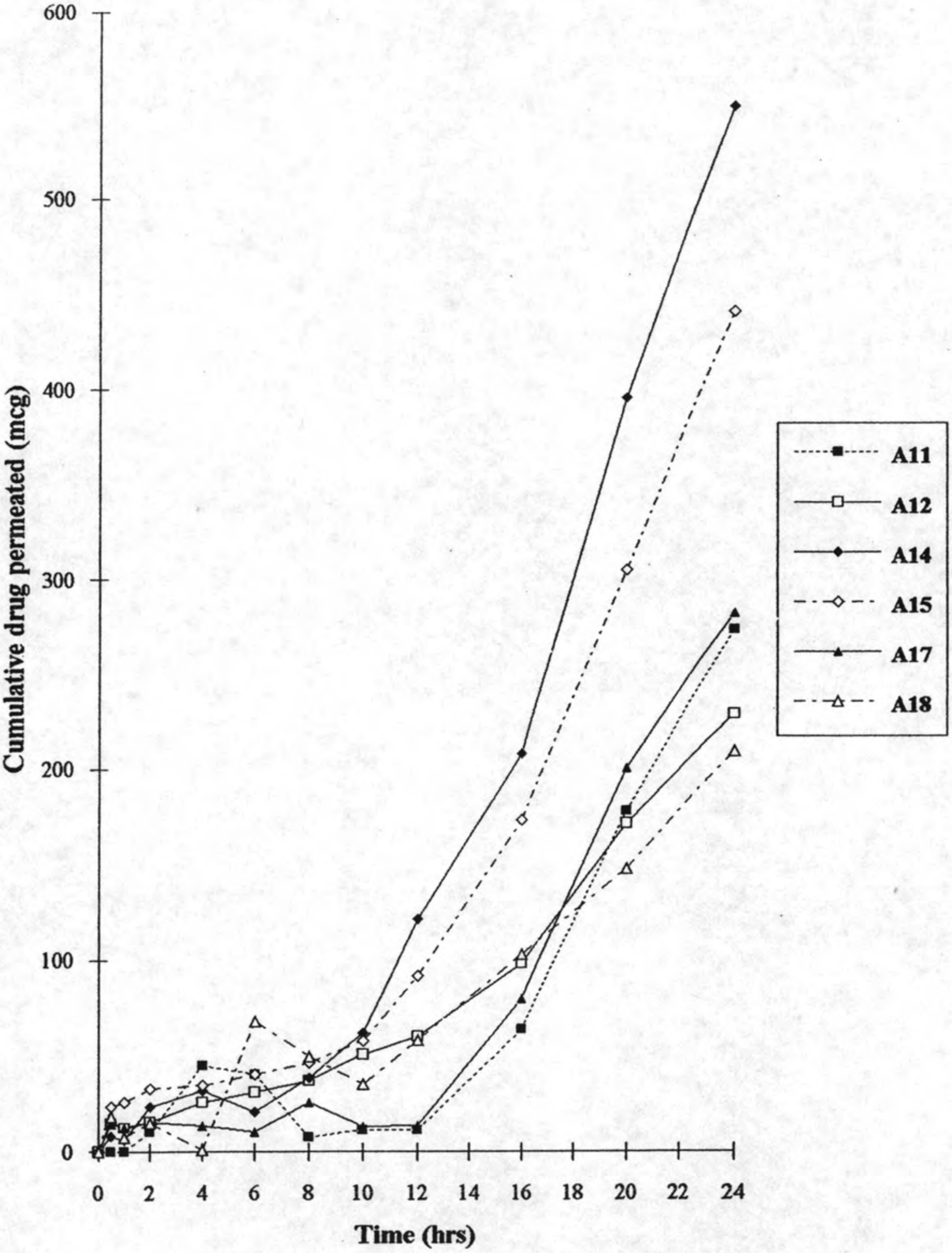


Figure 24 Cumulative ISDN permeated profiles of transdermal patches using crosslinked chitosan-PVA membranes as release rate-controlling membranes.

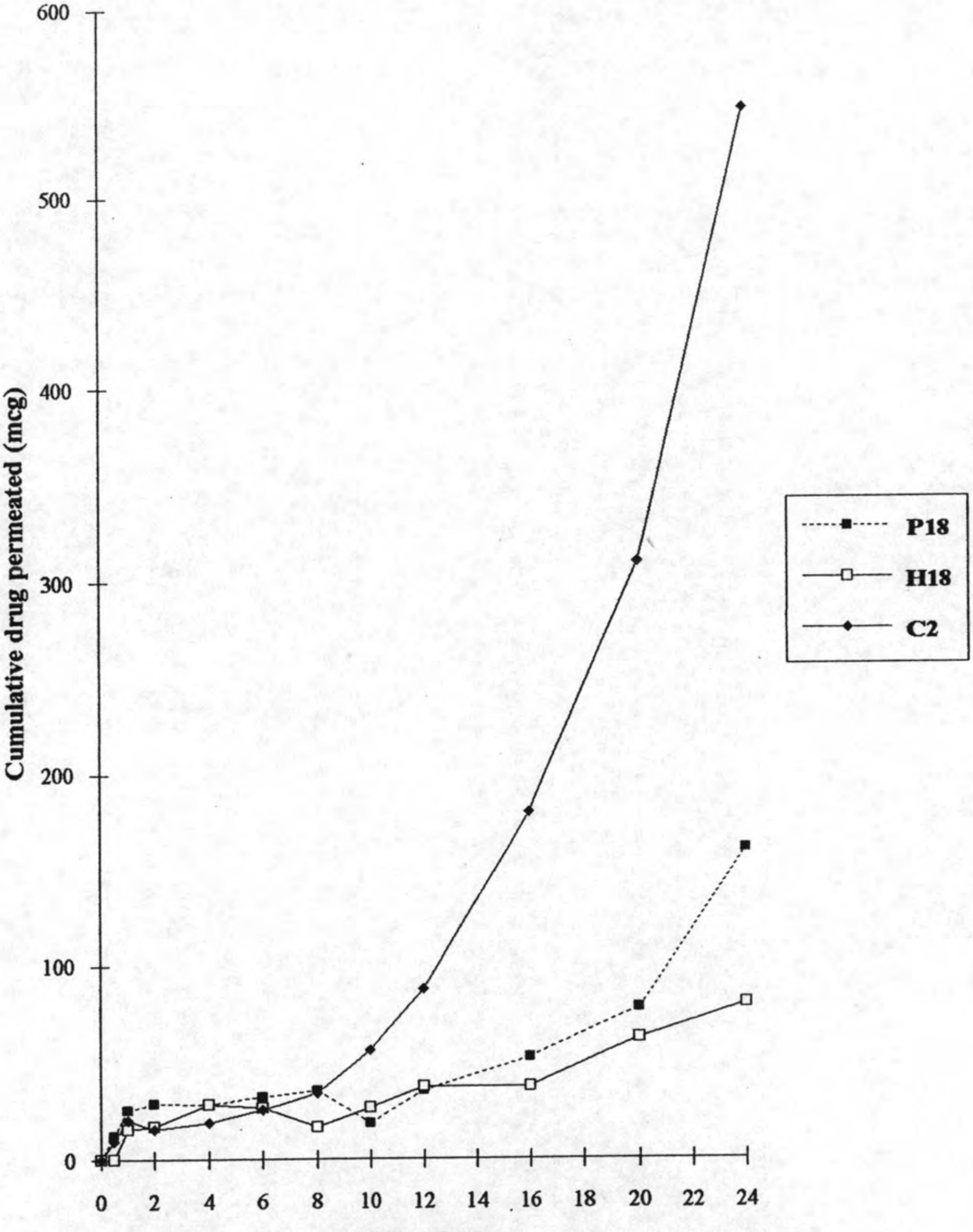


Figure 25 Cumulative ISDN permeated profiles of transdermal patches using various crosslinked chitosan-polymer membranes as release rate-controlling membranes. P = crosslinked chitosan-PVPK90 membrane, H = crosslinked chitosan-HPMC15 membrane and C = crosslinked chitosan-corn starch membrane.

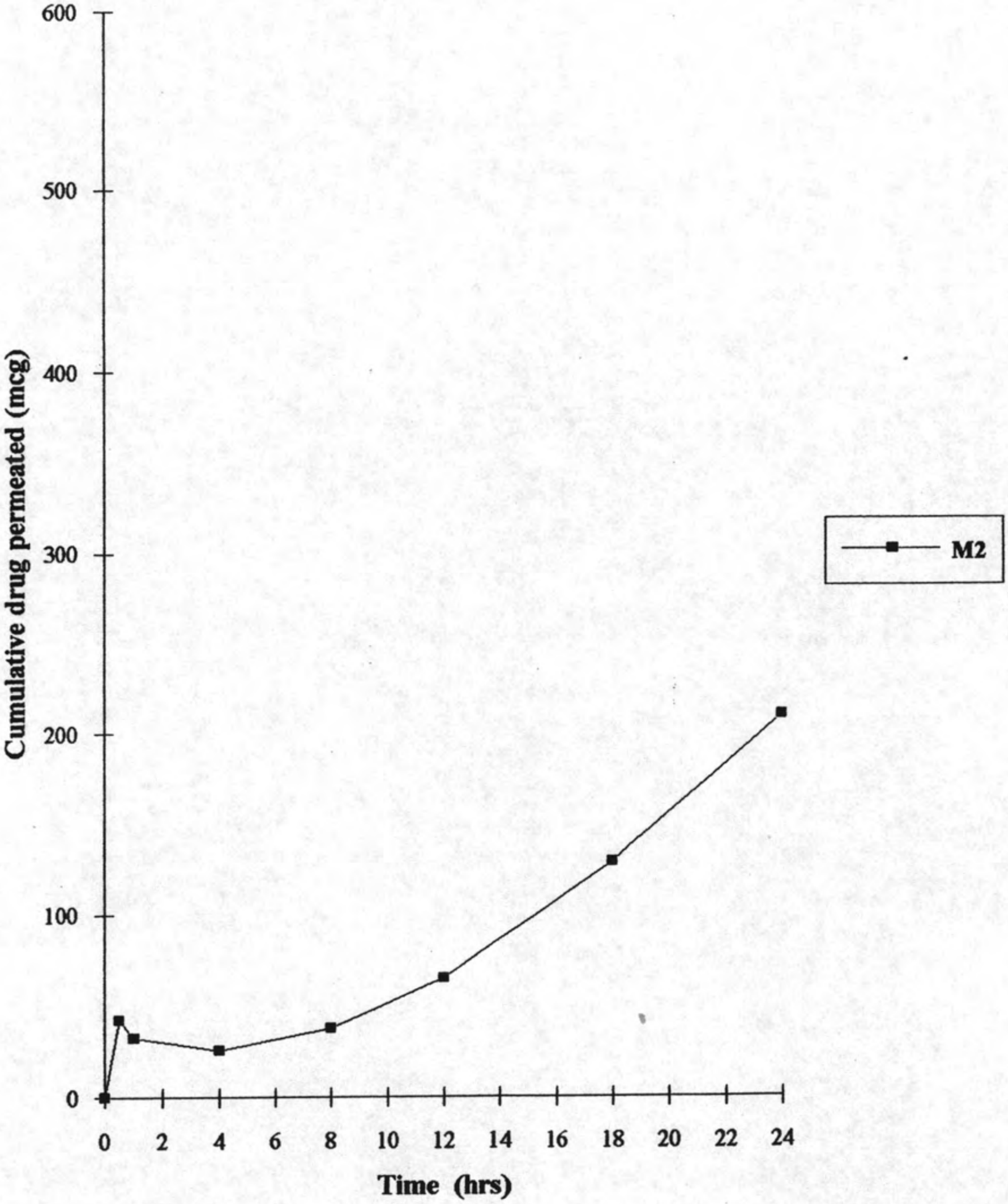


Figure 26 Cumulative ISDN permeated profile of transdermal patch using crosslinked chitosan-MC15 membrane with PEG1450 M2 as release rate-controlling membrane.

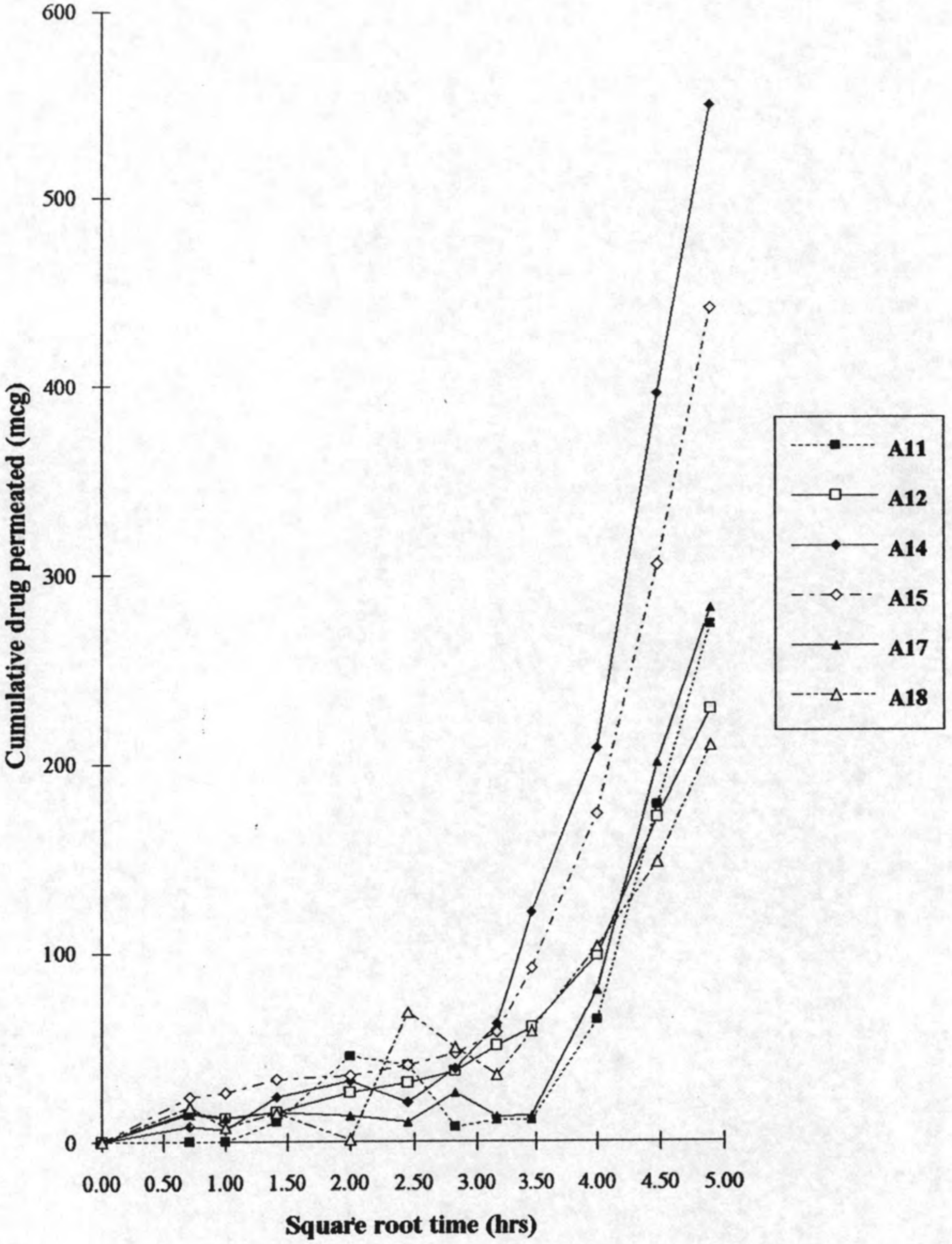


Figure 27 Cumulative ISDN permeated profiles of transdermal patches using crosslinked chitosan-PVA membranes as release rate-controlling membranes.

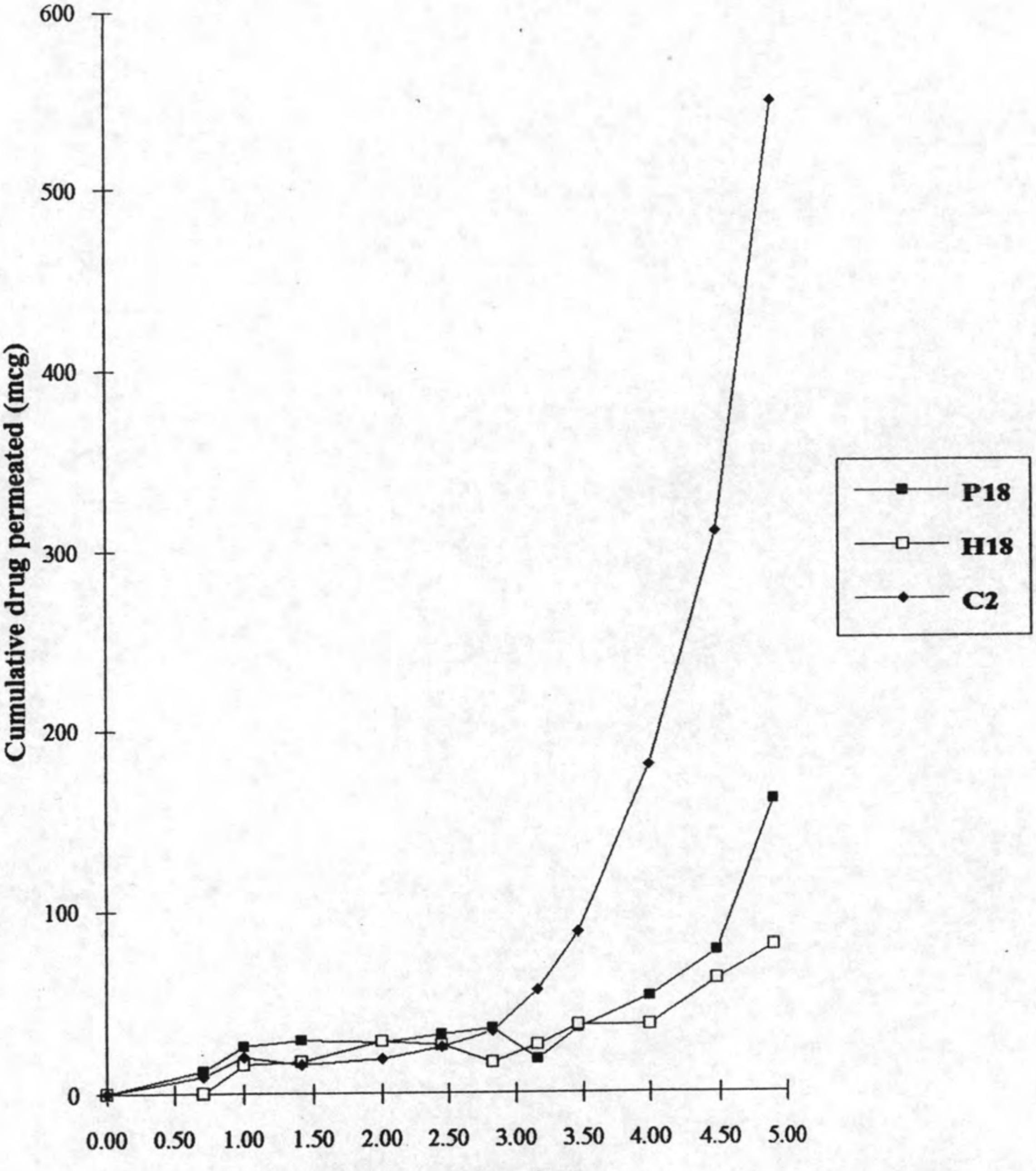


Figure 28 Cumulative ISDN permeated profiles of transdermal patches using crosslinked chitosan-polymer membranes as release rate-controlling membranes. P = crosslinked chitosan-PVPK90 membrane, H = crosslinked chitosan-HPMC15 membrane and C = crosslinked chitosan-corn starch membrane.

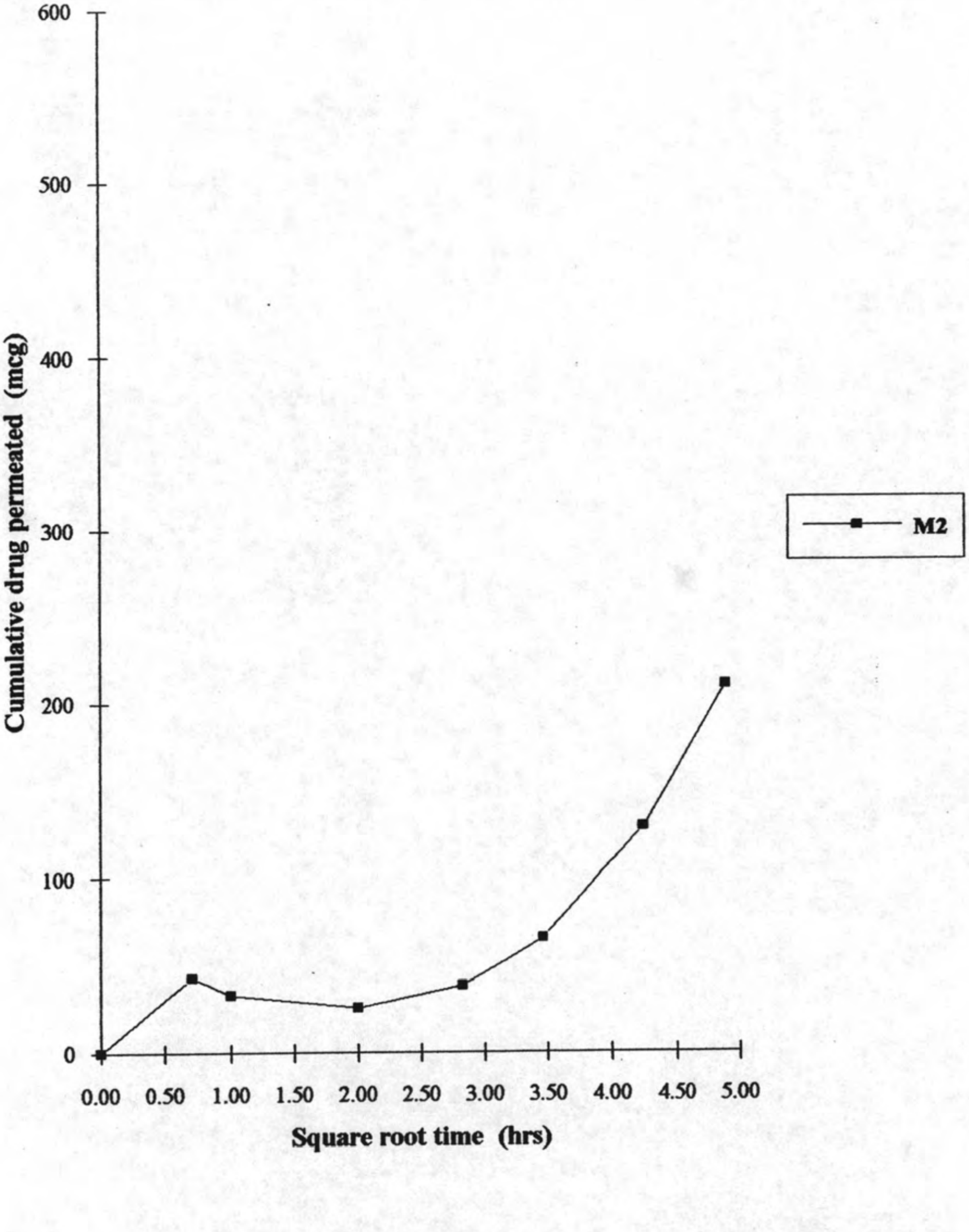


Figure 29 Cumulative ISDN permeated profile of transdermal patch using crosslinked chitosan-MC15 membrane with PEG 1450 M2 as release rate-controlling membrane.