

CHAPTER III

RESULTS

3.1 Analysis of fixolide

In this study, UV-spectrophotometry and gas chromatography (GC) were used for the determination of fixolide. In the experiments on quantitation of fixolide content in the fixolide-CD complexes and fixolide stability studies, GC was performed because of its specificity. While in dissolution studies, spectrophotometry was used for quantitation of fixolide because it was convenient and rapid.

3.1.1 Spectrophotometric Method

3.1.1.1 The maximum absorption of fixolide and fixolide-CD complex

A free fixolide and fixolide- β CD soluble complex were prepared in 50 % (v/v) aqueous ethanolic solution. The complex was formed by mixing fixolide with β CD. The absorption spectrum in the range of 200-400 nm were analyzed. Spectrum of β CD was determined as a control. UV spectra of these compounds were compared. β CD gave no UV spectrum because of its structure. For fixolide, the maximum absorption at 216 and 258 nm were observed (Figure 9). And UV spectra of fixolide did not change when it was in the form of fixolide- β CD soluble complex.

3.1.1.2 Calibration curve of fixolide

The calibration curve of fixolide was performed at 258 nm as shown in Figure 10. The regression coefficient of the linear relationship was highly significant ($R^2 = 0.9999$).

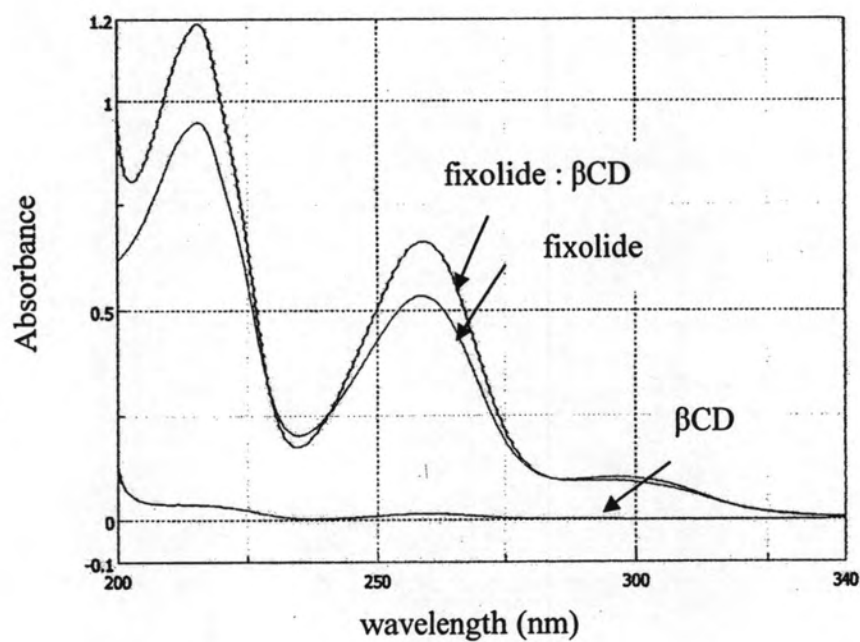


Figure 9. The UV spectrum of fixolide and fixolide-βCD complex

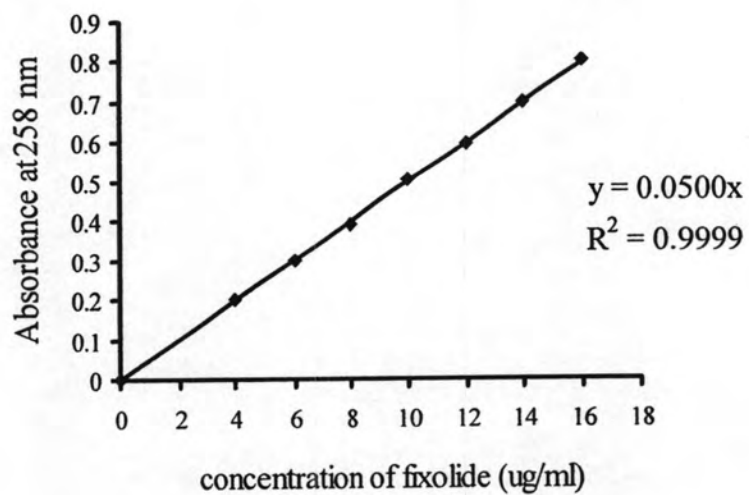


Figure 10. Calibration curve of fixolide by spectrophotometric method

3.1.2 GC Method

3.1.2.1 Chromatogram of fixolide

In Figure 11, the chromatogram of standard fixolide and naphthalene (internal standard) was presented. The naphthalene and fixolide peaks were distinctly resolved, with the retention times of 4.8 and 12.2 min, respectively.

3.1.2.2 Standard curve of fixolide

The relationships between peak area ratio and fixolide concentration was linear with a regression coefficient (R^2) of 0.9983 (Figure 12). The standard curve was performed in the milligram range of fixolide since this was the range used in complex formation with CDs, though usual quantitative analysis by GC method could go down to the nanogram level.

3.2 Phase solubility studies

The phase solubility diagrams of fixolide in the presence of CDs are shown in Figure 13. In the absence of CDs, the concentration of fixolide found in solution was in the range of 9.96-10.05 mM, which is the solubility of fixolide in 50 % (v/v) aqueous ethanolic solution at 30°C. The diagram shows that the solubility of the fixolide increased linearly as increasing concentration of methyl- β CD and HP- β CD (Figure 13b and c). It is clearly observed that the phase solubility diagram can be generally classified as A-type according to Higuchi and Connors (1965). The solubility enhancement can be attributed to the formation of a soluble inclusion complex. Methyl- β CD and HP- β CD at 100 mM concentration made fixolide solubility increased by 2.2 and 1.7 times, respectively. Methyl- β CD gave the A_L -type solubility diagram while for HP- β CD, the A_N -type was observed, solubility increase in the initial range of CD (0-20 mM) was more pronounced than at higher concentration (40-100 mM). In the case of β CD (Figure 13a), an insoluble microcrystalline complex was formed at high β CD concentrations. The pattern of phase solubility can be classified

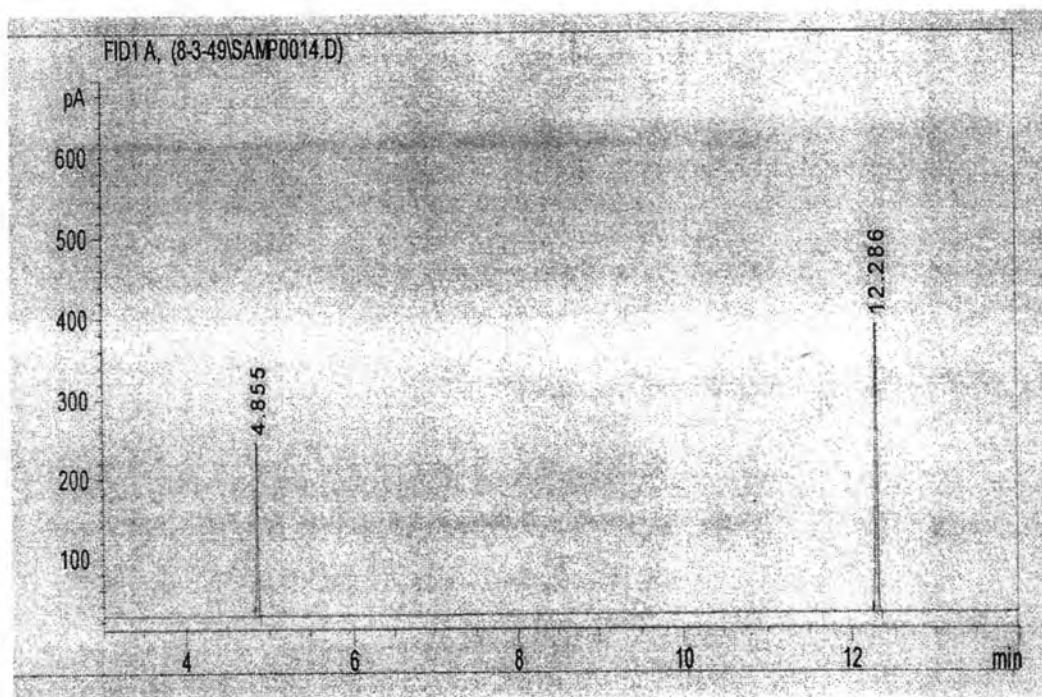


Figure 11. GC chromatogram of fixolide and naphthalene in n-hexane; retention time of naphthalene and fixolide were at 4.8 and 12.2 min, respectively

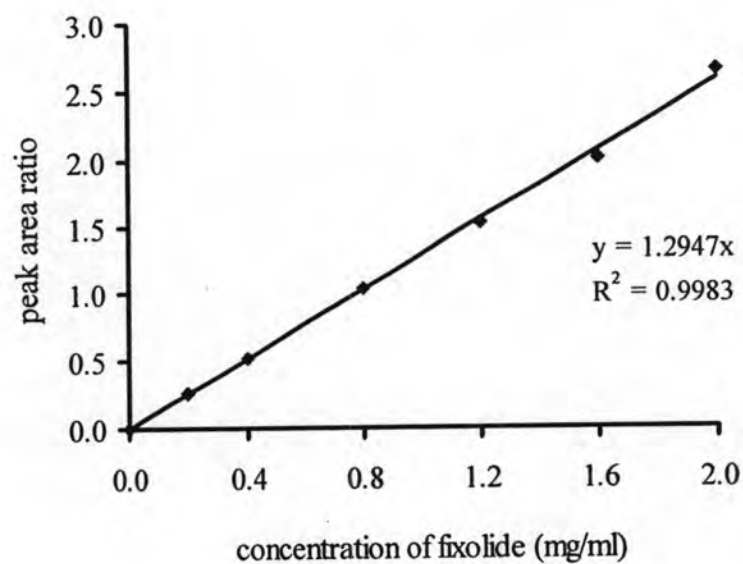
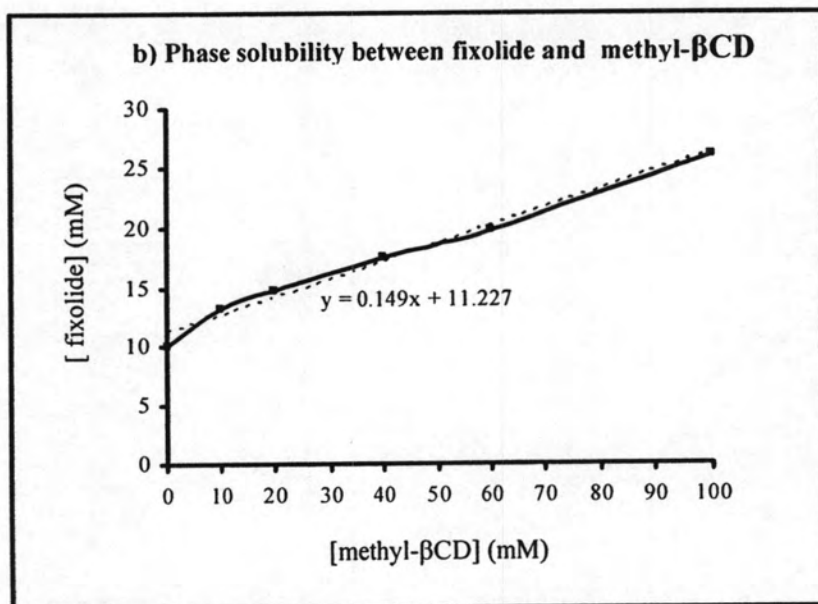
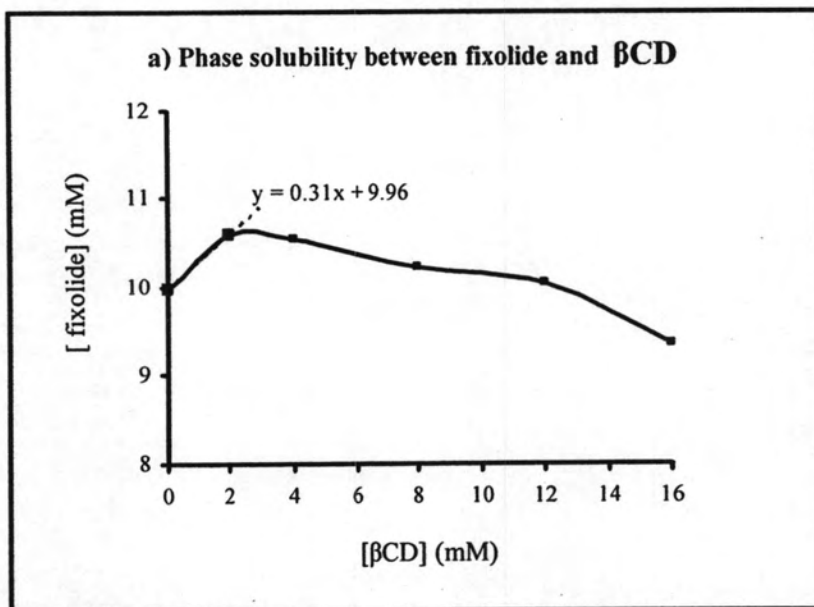


Figure 12. Standard curve of fixolide by GC method



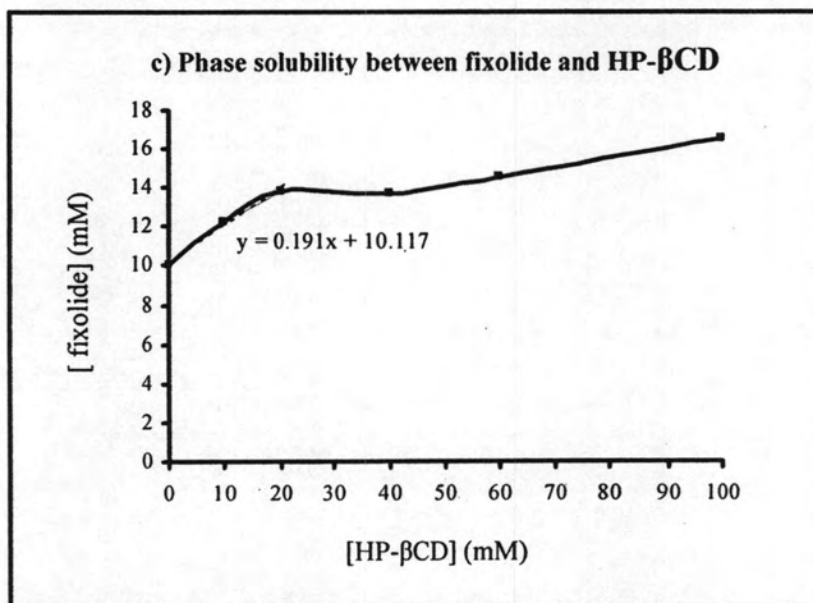


Figure 13. Phase solubility diagrams between fixolide and CDs in 50 % (v/v) aqueous ethanolic solution at 30 °C: a) fixolide and β CD, b) fixolide and methyl- β CD and c) fixolide and HP- β CD

as B_S-type. The diagram shows an initial rise of solubility in the range of 0-2 mM β CD concentration and follows by a plateau region, finally the concentration of fixolide decreased when the solid complex was precipitated.

The formation constant (K_c) of 1:1 complex was calculated from the initial portion of the phase solubility diagram according to equation of Higuchi and Connors, 1965, as mentioned in Methods. The observed constant of the formation of the complex (K_c) calculated for β CD, methyl- β CD and HP- β CD were 45.11, 15.60 and 23.34 M⁻¹, respectively.

3.3 Preparation of fixolide : CD (β CD , Methyl- β CD and HP- β CD) solid complexes

The solid complexes of fixolide-CDs were prepared by various methods. The appearance of fixolide is white, strong sweet fruity musk odor. β CD is white, non-hygroscopic, crystalline powder. Methyl- β CD and HP- β CD are white, fluffy, non-hygroscopic powder.

In co-precipitation method, solid complexes were prepared by stirring the solution containing fixolide and β CD at the mole ratios of 1:1, 1:2 and 1:3 for 24 hours (as described in 2.3.3.1). The precipitate of complexes were obtained from all ratios, then they were filtered and dried at 40 °C for 24 hours. The precipitate had a white microcrystalline powder. On the other hand, when prepared the solid complexes with methyl- β CD and HP- β CD as host molecules, there was no precipitation after stirring for 24 hours. This result suggests that co-precipitation method was failed for preparation of complexes with methyl- β CD and HP- β CD host.

For freeze-drying method, the mixture solutions between fixolide and CDs were clear and homogeneous before it was operated in the freeze dryer. The dried products were obtained for 1:2 and 1:3 mole ratios of fixolide to CDs. While, when prepared the solid complexes with β CD and HP- β CD hosts of 1:1 mole ratio, there were precipitates in the solutions. Thus, this ratio was failed in freeze-drying method.

In case of freeze-drying, the product obtained by β CD as the host was white fluffy and more dense than the products prepared by methyl- β CD and HP- β CD as the hosts.

For kneading method, the complexes were easy to prepare. During preparation of kneaded products for all three CDs, the kneaded mixtures were white homogeneous paste, sticky viscous mass. The products were white finer and denser after they had been dried. Therefore, the products were oven-dried without sieve-screening.

In order to confirm the solid complex formation between fixolide and three CDs, all products by different methods and three mole ratios of fixolide to CDs were subjected to DSC and FTIR analyses (except for the product prepared by co-precipitation method of methyl- β CD and HP- β CD hosts since precipitation was not formed).

3.4 Detection of Fixolide : Cyclodextrin (β CD, Methyl- β CD, HP- β CD) solid complexes

3.4.1 Differential Scanning Calorimetry (DSC)

This technique can be applied if the guest molecule has a melting or boiling temperature below the temperature at which the CDs decompose, i.e., about 300 °C. In this case, no energy absorption is observed at the melting temperature of the guest molecule when it is complexed.

Fixolide : β CD system : DSC thermograms of fixolide, β CD, physical mixture and fixolide: β CD products prepared by co-precipitation, kneading and freeze-drying methods are shown in Figure 14-16, respectively.

The DSC thermogram of fixolide gave a sharp endothermic peak at 57.5 °C which could be referred to its melting point. The thermogram of β CD displayed a broad endothermic peak around 121.6 °C, corresponding to a dehydration process, followed by an irreversible solid-phase transition at 220 °C. The thermogram of

physical mixture of 1:2 showed a broad endothermic peak around 121.6 °C and a prominent peak at 57.5 °C which were the peaks of β CD and fixolide, respectively. A complete disappearance of melting endotherm of fixolide was observed in the co-precipitation and kneading at a mole ratios of 1:2 and 1:3 and also in the freeze-drying at a mole ratio of 1:3. This results were consistent with inclusion complex formation. On the contrary, the co-precipitation and kneading of 1:1 and freeze-drying of 1:2 mole ratio showed a small peak of fixolide which suggest the complex formation was incomplete.

Fixolide : Methyl- β CD system: DSC thermograms of fixolide, methyl- β CD, and fixolide : methyl- β CD products prepared by kneading and freeze-drying are shown in Figure 17-18, respectively.

The DSC thermogram of methyl- β CD showed a broad endothermic peak around 79.2 °C which could be referred to a dehydration process. The physical mixture of 1:2 displayed a small endothermic peak at 57.3 °C and a broad peak around 83.6 °C. On the contrary, a complete disappearance of the melting endotherm of fixolide was observed for all solid complexes except for the product prepared by kneading method at a mole ratio of 1:1 which showed a small peak of fixolide at 56.3 °C.

Fixolide : HP- β CD system: DSC thermograms of fixolide, HP- β CD, and fixolide : HP- β CD products prepared by kneading and freeze-drying are shown in Figure 19-20, respectively.

The DSC curve for HP- β CD displayed a broad endothermic peak around 97 °C, corresponding to a dehydration process. The physical mixture of 1:2 showed a small endothermic peak at 57.3 °C and a broad peak at 85-85.3 °C. In the kneading and freeze-drying of 1:3 mole ratio, the characteristic endothermic melting peak at 57.5 °C which was the major peak of fixolide was absent. This result suggested the true inclusion complex formation. While the kneading mixtures of 1:1 and 1:2, and freeze-

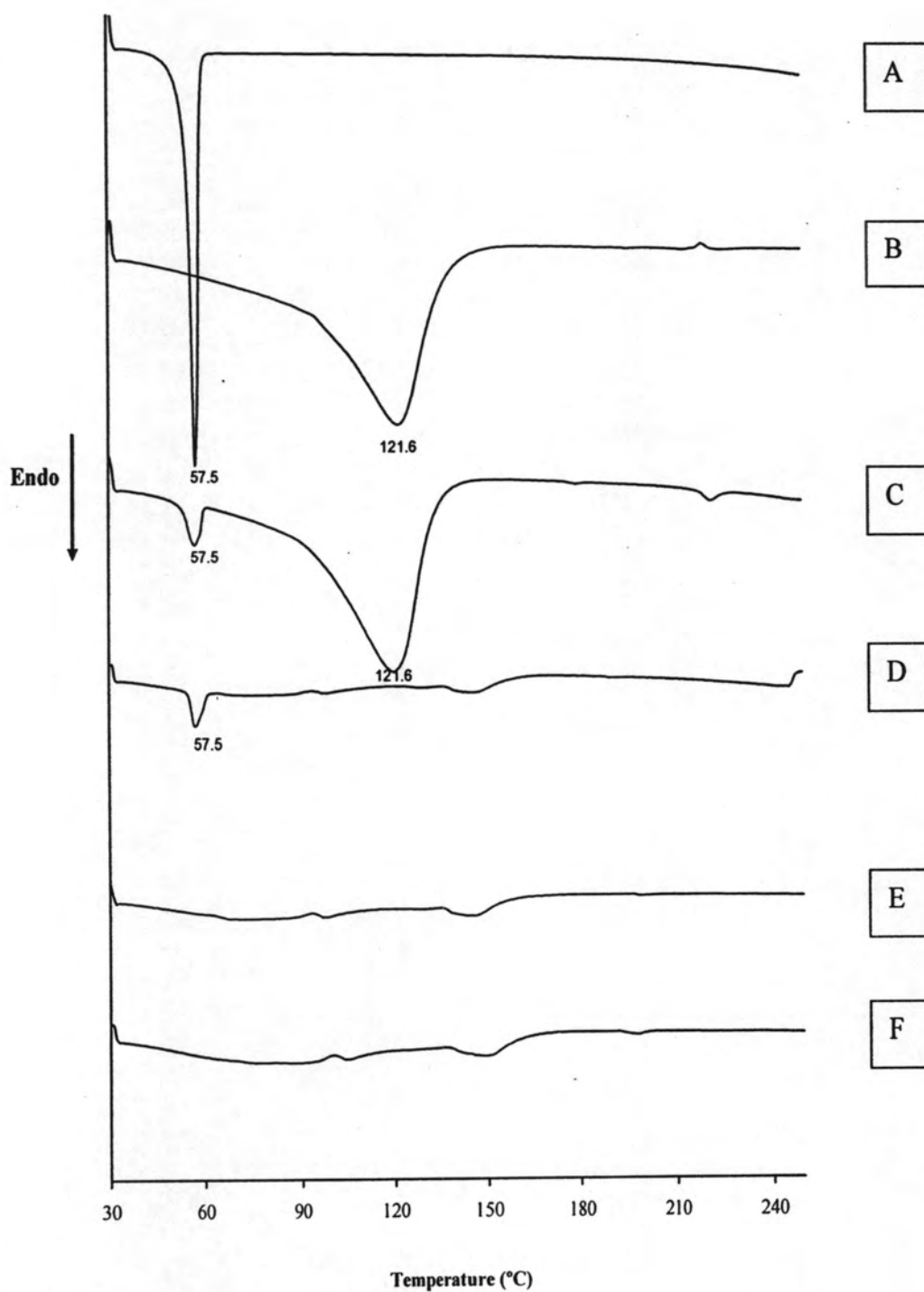


Figure 14. DSC thermograms of fixolide : β CD complex by co-precipitation method at mixing time of 24 hours: (A) fixolide, (B) β CD, (C) physical mixture of 1:2 mole ratio, (D) mole ratio 1:1 (E) mole ratio 1:2 and (F) mole ratio 1:3

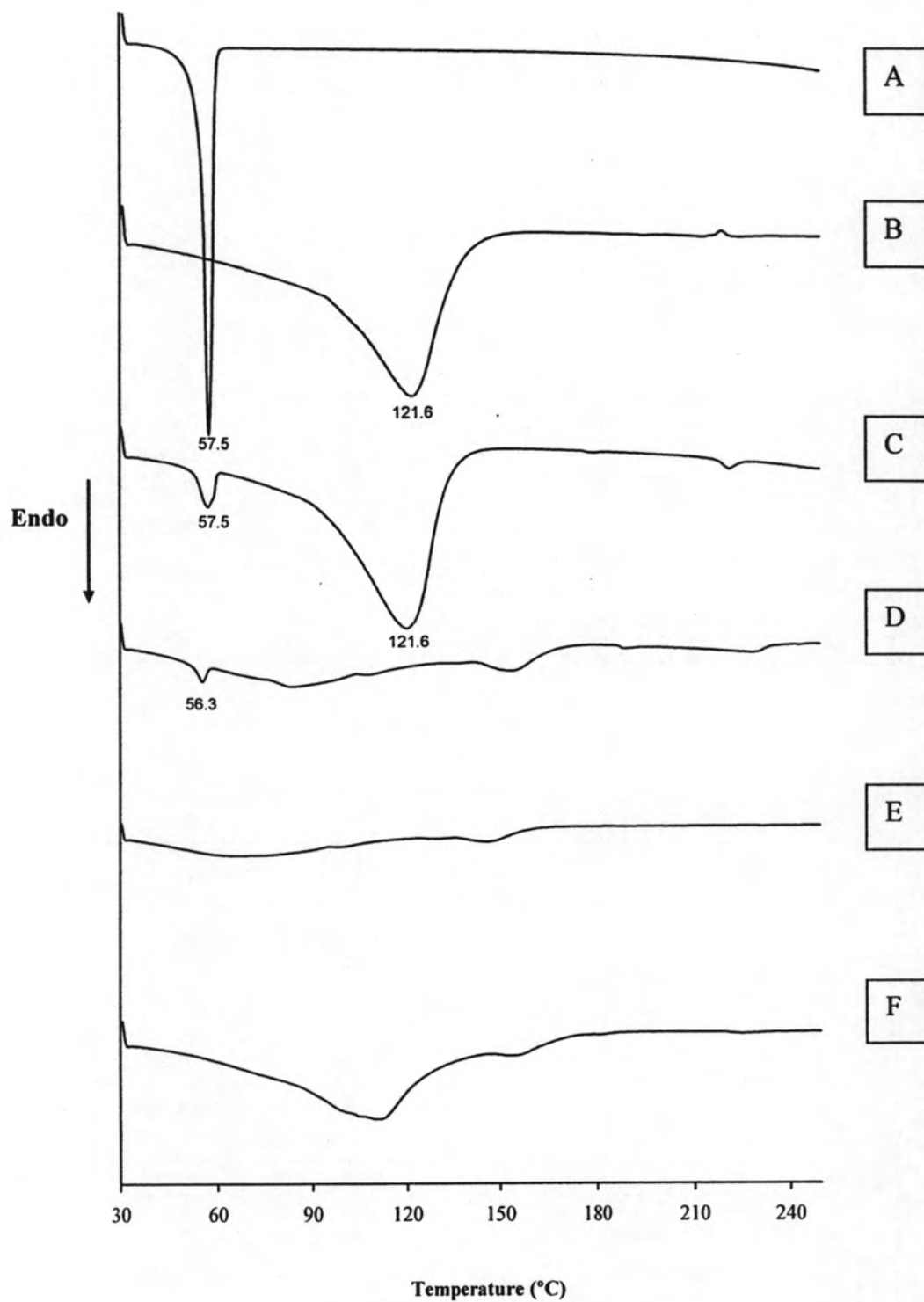


Figure 15. DSC thermograms of fixolide : β CD complex by kneading method :
(A) fixolide, (B) β CD, (C) physical mixture of 1:2 mole ratio, (D) mole ratio 1:1 ,
(E) mole ratio 1:2 and (F) mole ratio 1:3

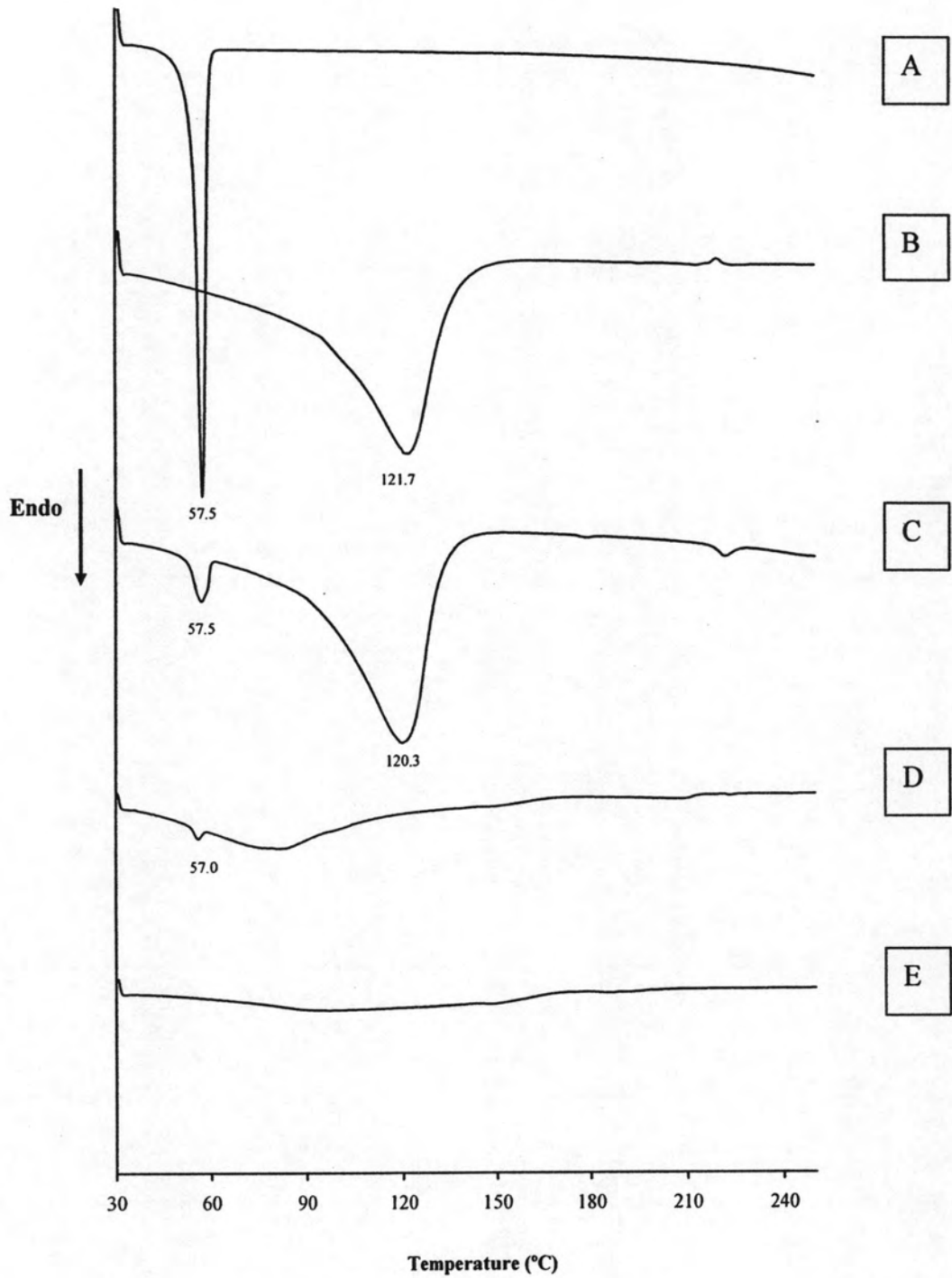


Figure 16. DSC thermograms of fixolide : β CD complex by freeze-drying method: (A) fixolide, (B) β CD, (C) physical mixture of 1:2 mole ratio, (D) mole ratio 1:1 (E) mole ratio 1:2 and (F) mole ratio 1:3

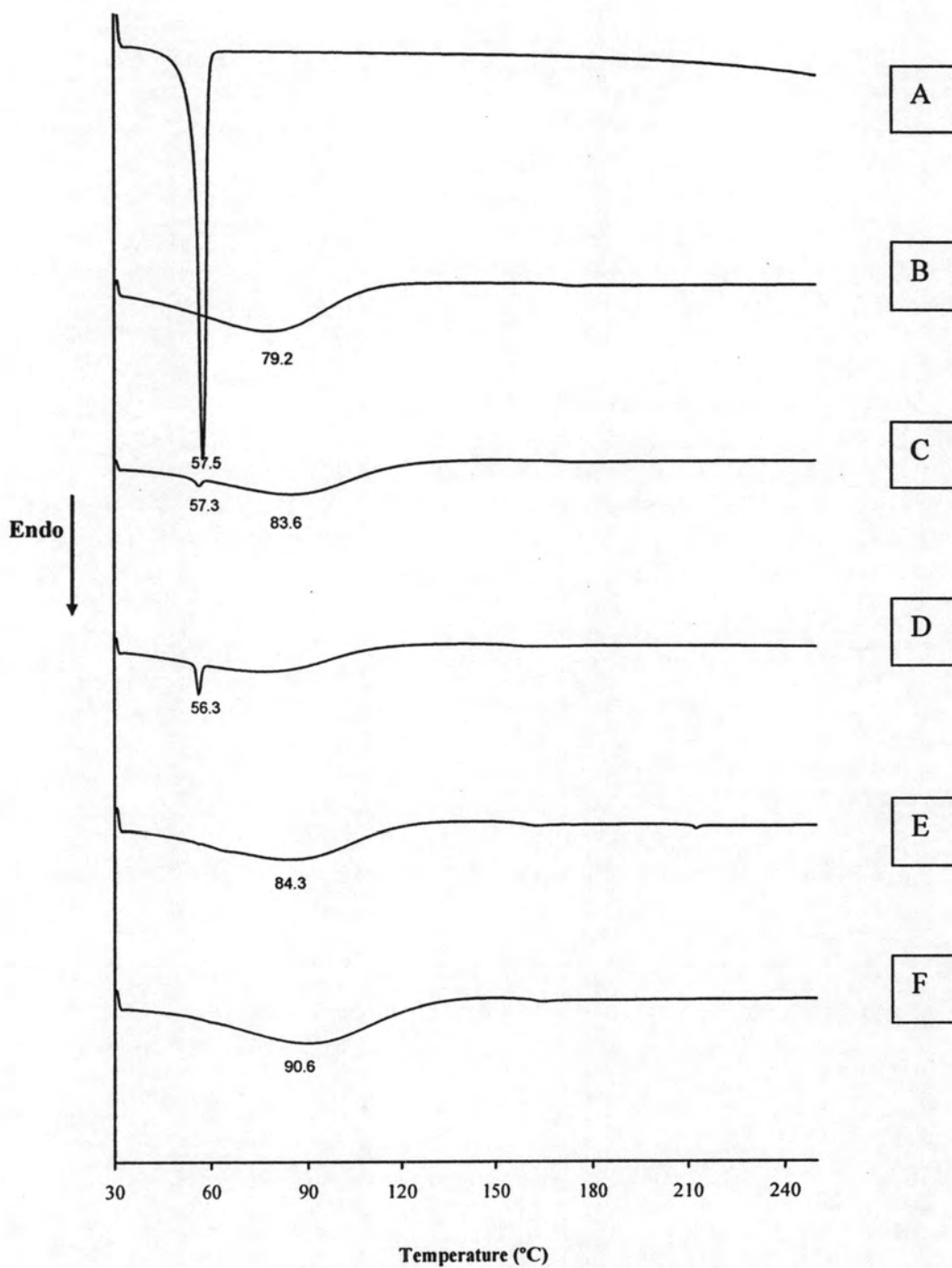


Figure 17. DSC thermograms of fixolide : methyl- β CD complex by kneading method: (A) fixolide, (B) methyl- β CD, (C) physical mixture of 1:2 mole ratio, (D) mole ratio 1:1 (E) mole ratio 1:2 and (F) mole ratio 1:3

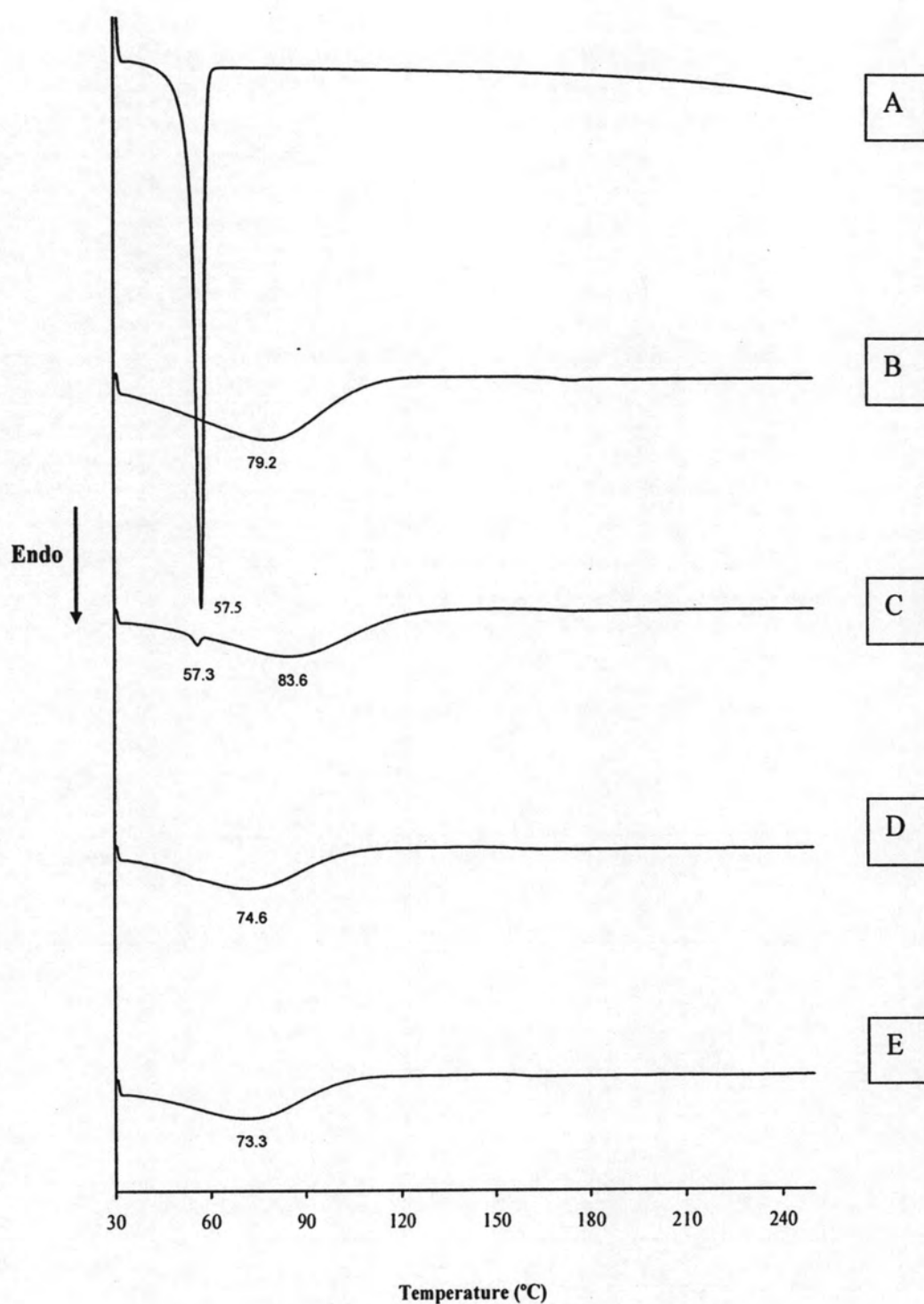


Figure 18. DSC thermograms of fixolide : methyl-βCD complex by freeze-drying method : (A) fixolide, (B) methyl-βCD, (C) physical mixture of 1:2 mole ratio, (D) mole ratio 1:2 and (E) mole ratio 1:3

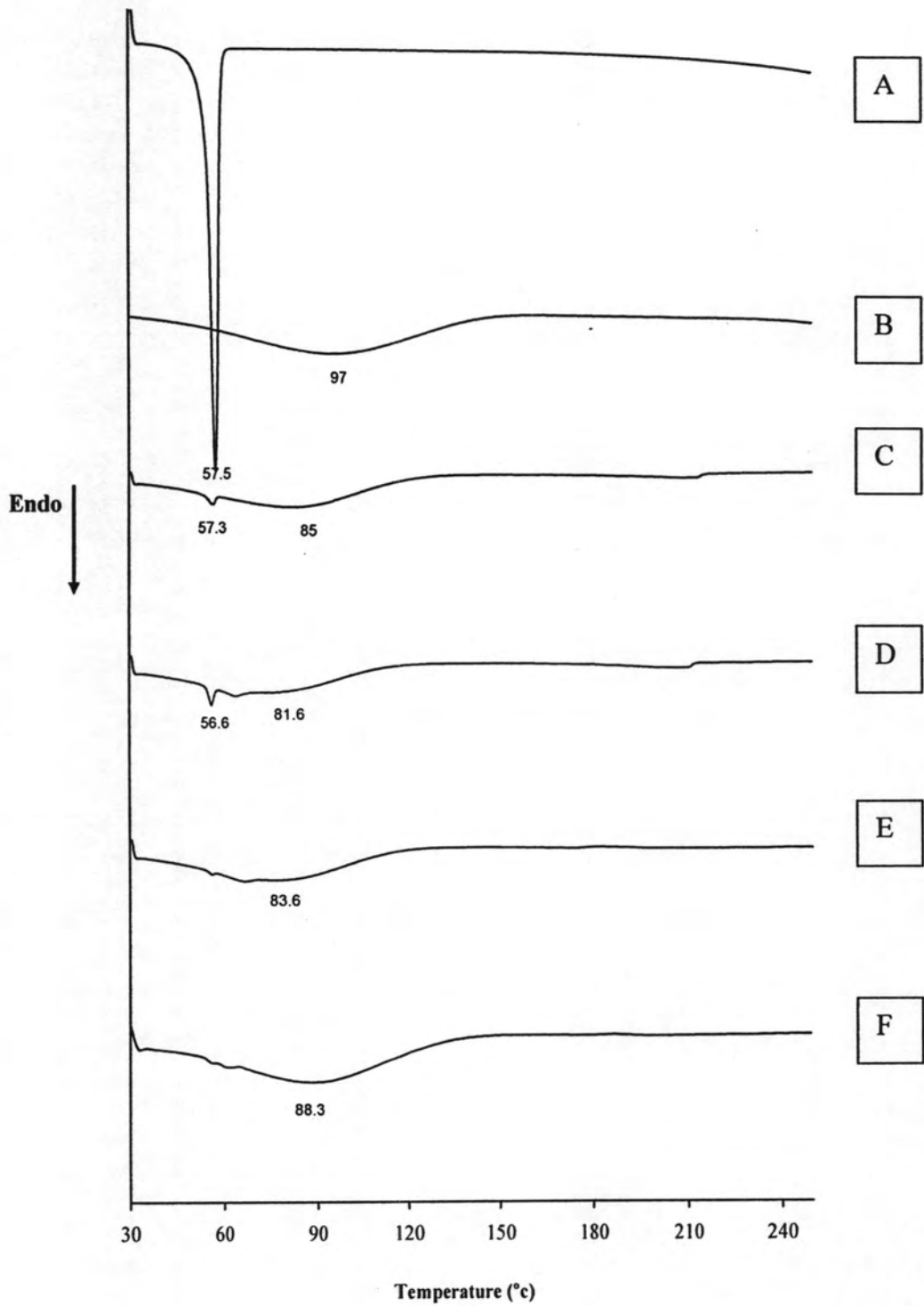


Figure 19. DSC thermograms of fixolide : HP- β CD complex by kneading method: (A) fixolide, (B) HP- β CD, (C) physical mixture of 1:2 mole ratio, (D) mole ratio 1:1, (E) mole ratio 1:2 and (F) mole ratio 1:3

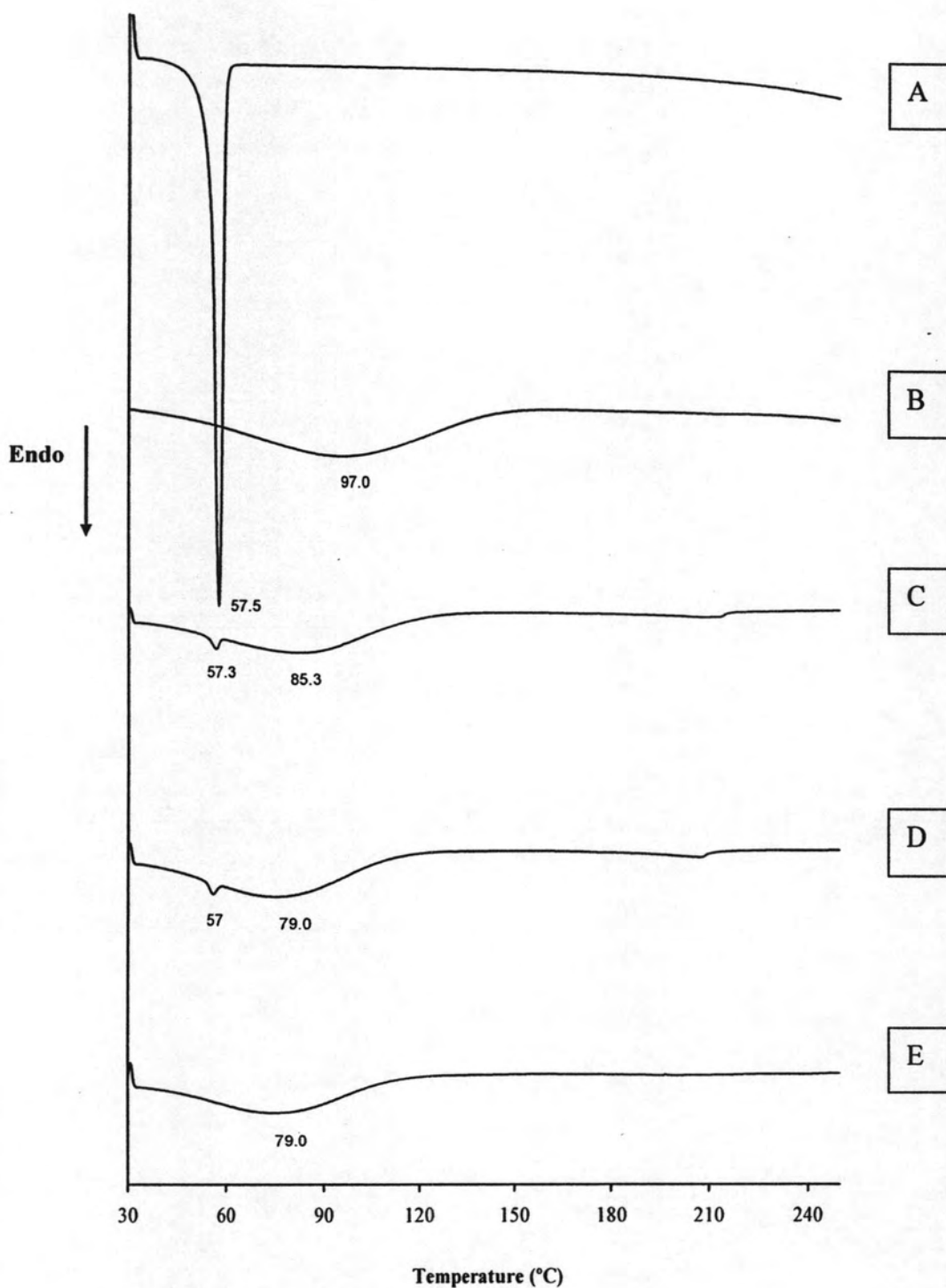


Figure 20. DSC thermograms of fixolide : HP- β CD complex by freeze-drying method: (A) fixolide, (B) HP- β CD, (C) physical mixture of 1:2 mole ratio, (D) mole ratio 1:2 and (E) mole ratio 1:3

drying mixture of 1:2 mole ratios displayed a small endothermic peak at the temperature range of 56-57 °C which indicated the incomplete complex formation.

3.4.2 Fourier Transform Infrared Spectrometry (FTIR)

FTIR spectroscopy is one of the useful technique for analysis of complexes. Upon complexation of the guest, shifts or changes in the spectrum occur. The IR spectra of fixolide : CD systems are presented in Figure 21-23. Since the DSC thermograms indicated that the mole ratio of 1:1 for all systems resulted in incomplete complex formation, the mole ratio of 1:2 was used for true complex formation through analysis of FTIR spectrum.

The IR spectrum of β CD, methyl- β CD and HP- β CD were not significantly different. They showed strong broad peak of OH stretching at about 3500-3300 cm^{-1} and a major peak of OH bending of β CD, methyl- β CD and HP- β CD at 1644, 1647 and 1655 cm^{-1} , respectively. Other characteristics were shown in Table 5-7. The IR spectrum of fixolide showed the major peak at 1683 cm^{-1} of the C=O stretching of the carbonyl group. Other important peaks are the peak at 1544 cm^{-1} which was due to C=C stretching of aromatic ring and the peak at 2964 cm^{-1} which indicated the C-H stretching (Table 8).

From the IR spectrum of fixolide : β CD products, the characteristic peak of C=O group of the fixolide in physical mixture showed no change. While the shifts of the major peak from 1683 to 1677 cm^{-1} in co-precipitated and freeze-dried products, and to 1672 cm^{-1} in kneaded product were observed (Figure 21).

In the IR spectrum of fixolide : methyl- β CD products, the major peak of the carbonyl group appeared unchanged in physical mixture. Whereas, in the IR spectrum of the complexes which were prepared by kneading and freeze-drying methods, the carbonyl band was changed from 1683 to 1679 and 1678 cm^{-1} , respectively (Figure 22). When the spectrum of fixolide : HP- β CD were analyzed, there were not any change of the carbonyl peak of the fixolide in all products prepared (Figure 23).

In the spectrum which showed a shift of C=O peak, a modification of electronic environment of C=O group was suggested which means inclusion complexes could be formed in solid state. In the case of IR spectrum with C=O peak unchanged, it suggests that inclusion complex was not formed in this system.

From the above results, the assignment of each peak in each spectrum was suggested and summarized in Table 9-11.

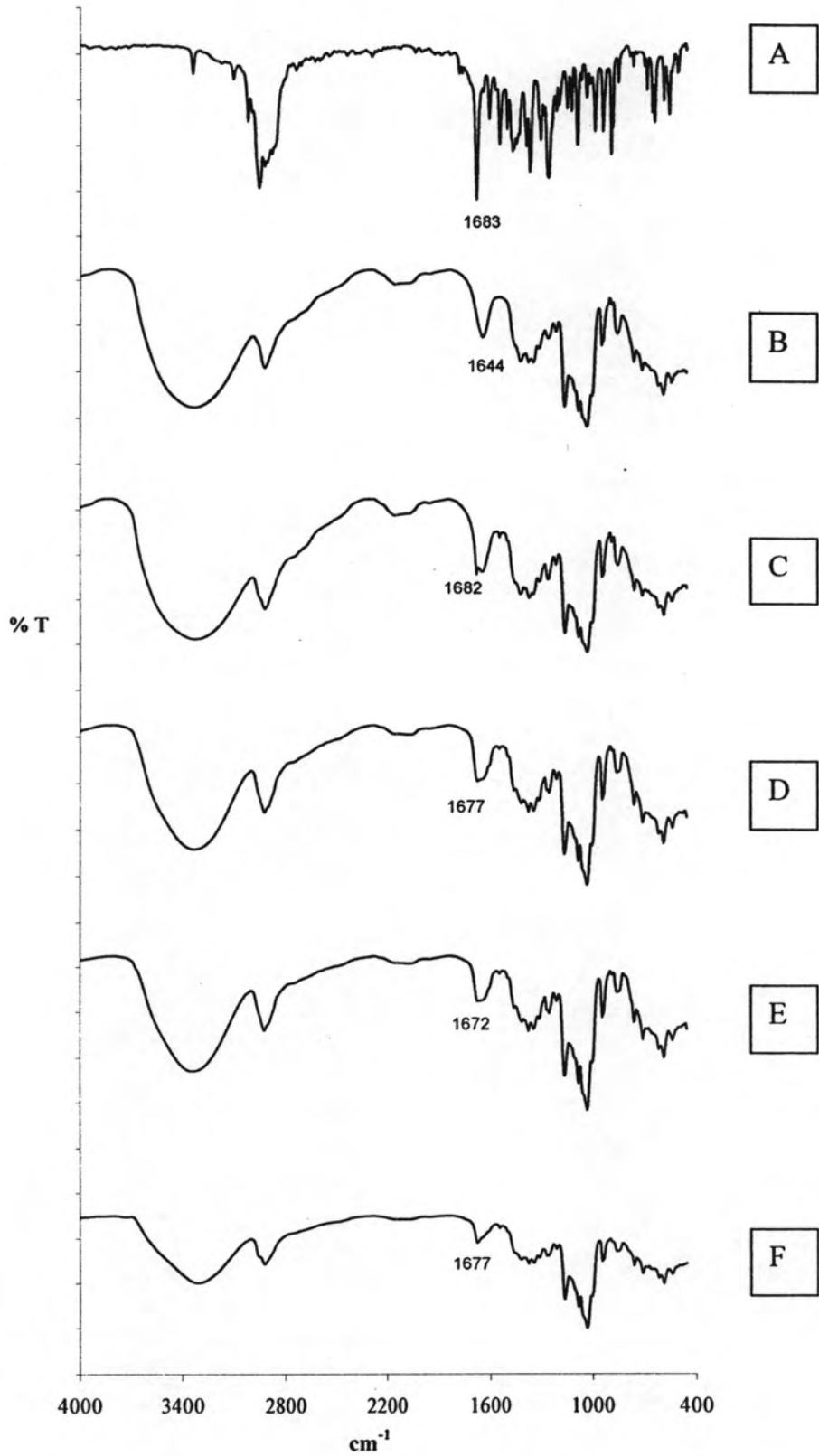


Figure 21. Infrared spectra of fixolide : β CD complex : (A) fixolide , (B) β CD , (C) physical mixture , (D) co-precipitation mixture , (E) kneading mixture and (F) freeze-drying mixture. All mixtures were of the 1:2 mole ratio.

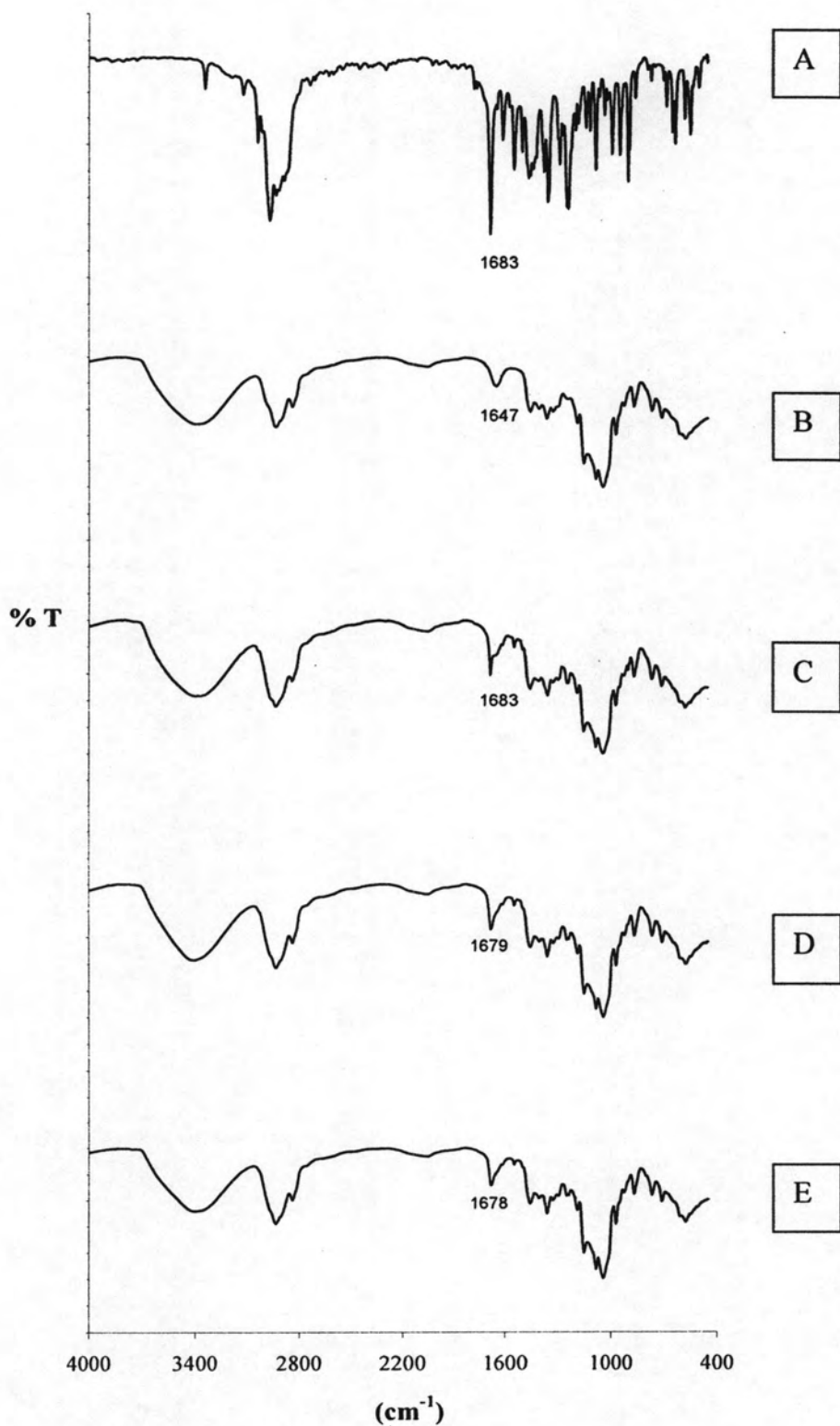


Figure 22. Infrared spectra of fixolide : methyl-βCD complex : (A) fixolide , (B) methyl-βCD , (C) physical mixture , (D) kneading mixture and (E) freeze-drying mixture. All mixtures were of the 1:2 mole ratio.

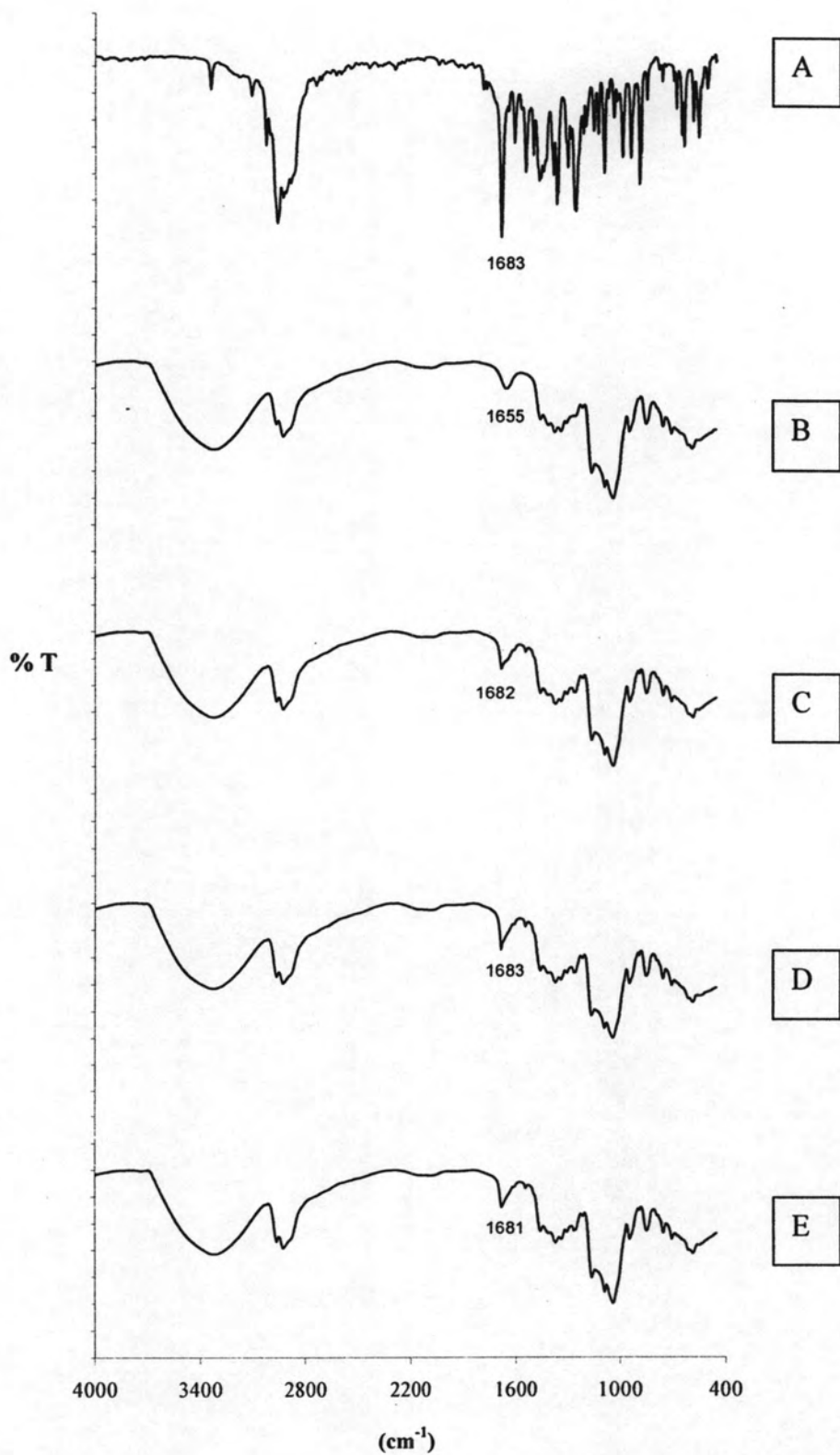


Figure 23. Infrared spectra of fixolide : HP-βCD complex : (A) fixolide , (B) HP-βCD , (C) physical mixture , (D) kneading mixture and (E) freeze-drying mixture. All mixtures were of the 1:2 mole ratio.

Table 5. FTIR data of β CD

Wave number (cm^{-1})	Assignment
3500-3300	Broad -OH stretching
2927	-CH stretching
1645	-OH bending
1412 , 1366 , 1336	CH_2 , CH_3
1155 , 1077 , 1031	C-O stretching

Table 6. FTIR data of methyl- β CD

Wave number (cm^{-1})	Assignment
3500-3300	Broad -OH stretching
2931	-CH stretching
2837	- OCH_3
1647	-OH bending
1455 , 1408	CH_2
1366	CH_3
1194 , 1157 , 1086 , 1044	C-O stretching

Table 7. FTIR data of HP- β CD

Wave number (cm^{-1})	Assignment
3500-3300	Broad -OH stretching
2928	-CH stretching
1655	-OH bending
1456 , 1408	CH_2
1371 , 1332	CH_3
1154 , 1082 , 1036	C-O stretching

Table 8. FTIR data of fixolide

Wave number (cm^{-1})	Assignment
2964	=C-H stretching
1683	C=O
1544	C=C ring stretching
1499 , 1380	CH_2 , CH_3
1359	C-CO-C bending
987 , 940 , 892	=C-H out of plane bending
850 , 677	aromatic ring

Table 9. FTIR data of fixolide- β CD inclusion complex prepared by different methods

Wave number (cm ⁻¹)				Assignment
Physical mixing	Co-precipitation	Kneading	Freeze-drying	
3500-3300	3500-3300	3500-3300	3500-3300	Broad -OH stretching
2927	2929	2930	2929	-CH stretching
1682*	1677*	1672*	1677*	C=O
1648	-	1648	-	-OH bending
1544	1544	1544	1544	C=C ring stretching
1414 , 1366	1407 , 1366 , 1334	1407 , 1365 , 1334	1409 , 1366 , 1333	CH ₂ , CH ₃
1155 , 1079	1156 , 1080	1156 , 1080	1156 , 1080	C-O stretching

* carbonyl peak

Table 10. FTIR data of fixolide-methyl- β CD inclusion complex prepared by different methods

Wave number (cm^{-1})			Assignment
Physical mixing	Kneading	Freeze-drying	
3500-3300	3500-3300	3500-3300	Broad -OH stretching
2930	2930	2930	-CH stretching
1683*	1679*	1678*	C=O
1544	1544	1544	C=C ring stretching
1456 , 1360	1456 , 1364	1456 , 1364	CH ₂ , CH ₃
1156 , 1086 ,1044	1156 , 1086 ,1043	1156 , 1086 ,1043	C-O stretching

* carbonyl peak

Table 11. FTIR data of fixolide-HP- β CD inclusion complex prepared by different methods

Wave number (cm ⁻¹)			Assignment
Physical mixing	Kneading	Freeze-drying	
3500-3300	3500-3300	3500-3300	Broad -OH stretching
2929	2929	2929	-CH stretching
1682*	1683*	1681*	C=O
1544	1544	1544	C=C ring stretching
1456 , 1365	1456 , 1365	1456 , 1365	CH ₂ , CH ₃
1154 , 1082 , 1036	1154 , 1082 , 1036	1154 , 1082 , 1036	C-O stretching

* carbonyl peak

3.5 Determination of Fixolide in the Solid Complexes

The extraction efficiency and the content of fixolide in cyclodextrin complexes were determined by solvent extraction and analyzed by GC method. Triplicate samples of the complexes were extracted.

In preliminary study, the extraction method was carried out according to the method of Reineccius (2004). In each extraction, n-hexane : water in the ratio of 4:30 (v:v) was used. β -cyclodextrin could be dissolved in this amount of water used (solubility in water at room temperature of β CD is 1.8 g/100 ml), while fixolide was effectively partitioning into the hexane phase. When number of extractions were determined in the complex of β CD host, it was found that one extraction gave the least content of fixolide, while results from two and three extractions were not different (Figure 24). Thus, two extractions were sufficient for total recovery of fixolide in the solvent extraction method.

Recovery of fixolide was evaluated and shown in Table 12, good recoveries from extraction of 1:2 and 1:3 mole ratios were obtained (100.78 and 97.92 %, respectively). However, recovery of fixolide from extraction of 1:1 mole ratio with β CD host was less efficient (81.32 %). Based on these findings, determination of fixolide in CD was carried out using solvent extraction procedure.

In this method, the solid complexes prepared by co-precipitation at the stirring time of 3, 6, 12, 18, and 24 hours, kneading and freeze-drying methods at 1:1, 1:2 and 1:3 mole ratios of fixolide : CDs were extracted as described in Method (section 2.3.6). The amounts of fixolide included in CDs and the percent of complex yield were shown in Table 13-15. From the results, it was found that the complexes at the mole ratio of 1:1 in all formulations gave the highest fixolide content. However, this mole ratio showed the residual free fixolide when investigated by DSC and showed no change in the carbonyl peak of the fixolide by FTIR method.

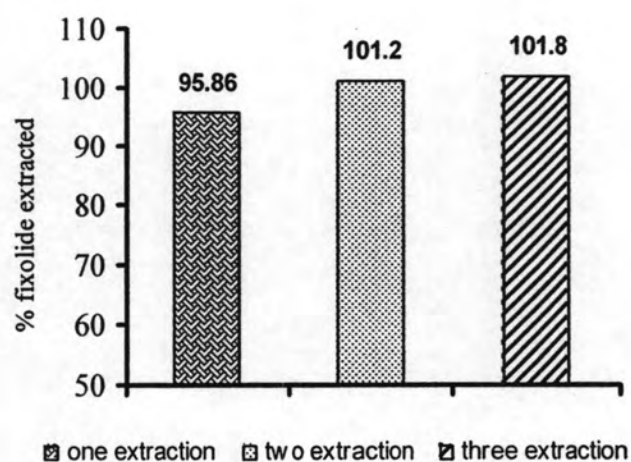


Figure 24. Extraction of fixolide using n-hexane (the values are average from duplicate experiments).

Table 12. Recovery of fixolide

Fixolide : β CD (mole ratio)	Recovery (%) ^a
1:1	81.32 \pm 0.76
1:2	100.78 \pm 0.89
1:3	97.92 \pm 1.76

^a mean \pm SD , n= 3

Table 13. Complex yield and fixolide included in β CD by different methods of complex formation

Method	Fixolide : β CD Ratio (^a)	Complex yield (%)	Fixolide included (mg/g complex) ^b
co-precipitation	1:1 (24)	71.70	152.25 \pm 8.85
	1:2 (3)	45.34	88.64 \pm 0.23
	1:2 (6)	69.60	68.48 \pm 2.87
	1:2 (12)	75.43	63.73 \pm 1.16
	1:2 (18)	75.44	69.69 \pm 1.06
	1:2 (24)	73.63	96.93 \pm 1.35
	1:3 (24)	67.86	62.66 \pm 3.14
kneading	1:1	61.93	127.37 \pm 4.86
	1:2	75.43	59.62 \pm 4.33
	1:3	68.79	37.48 \pm 4.36
freeze-drying	1:2	96.62	56.66 \pm 4.31
	1:3	93.51	25.48 \pm 1.24

^a stirring time at various hours

^b mean \pm SD , n = 3

$$\text{Complex yield (\%)} = \frac{\text{total weight of dried complex}}{\text{weight of CD + weight of fixolide added}} \times 100$$

Table 14. Complex yield and fixolide included in methyl- β CD by different methods of complex formation

Method	Fixolide : methyl- β CD Ratio	Complex yield (%)	Fixolide included (mg/g complex) ^a
kneading	1:1	73.77	147.03 \pm 0.69
	1:2	83.53	81.72 \pm 2.71
	1:3	76.35	56.36 \pm 2.43
freeze-drying	1:2	85.38	73.05 \pm 2.41
	1:3	84.75	58.42 \pm 2.26

^a mean \pm SD , n = 3

Table 15. Complex yield and fixolide included in HP- β CD by different methods of complex formation

Method	Fixolide : HP- β CD Ratio	Complex yield (%)	Fixolide included (mg/g complex) ^a
kneading	1:1	72.85	141.06 \pm 2.18
	1:2	65.94	78.99 \pm 0.65
	1:3	71.59	53.14 \pm 0.47
freeze-drying	1:2	82.64	46.91 \pm 3.83
	1:3	70.99	35.36 \pm 0.71

^a mean \pm SD , n = 3

Of all the fixolide : CD complexes which gave complete complex formation as analyzed from DSC and FTIR results, the complex of fixolide : β CD of 1:2 mole ratio prepared by co-precipitation method at the stirring time of 24 hours possessed the highest fixolide content of 96.93 mg/g complex with the acceptable complex yield of 74 %. The fixolide : methyl- β CD complex of 1:2 mole ratio prepared by kneading method was nearly as good, that it has the fixolide content of 81.72 mg/g complex with 84 % yield. Owing to the cost of methyl- β CD which is higher than β CD, thus the complex of fixolide : β CD at the mole ratio of 1:2 prepared by co-precipitation was the most appropriate to be prepared for use in the properties study in the next section.

3.6 Determination of Properties of the Solid Complexes

3.6.1 Thermal stability test

Fixolide and fixolide : β CD complex (1:2 mole ratio) prepared by co-precipitation were subjected to stability study at 35 °C for the long-term testing and 50 °C and 80 °C for the accelerated testing. Table A.1-A.3 in Appendix A and Figure 25-27 demonstrate percent remaining of fixolide in the complex form and its free form kept at various temperatures.

Results from temperature stability test show that free fixolide was more stable at 35 °C than at higher temperature, 50 and 80 °C. And fixolide in the complex form with β CD was more stable than its free form. After storage at 35 °C for 120 days, percent remaining of fixolide were 75 % and 90 % for the free form and the complex form, respectively. At 50 °C after two weeks, free fixolide lost 17 % while in the complex form, fixolide was not significantly lost. When incubated at 80 °C for a week, the loss of fixolide in the free form was significantly higher than in its complex form (27 % versus 6 %).

The kinetic principle is always of great importance in stability programs. Two parameters indicating the kinetics of chemical reactions are order and rate of reactions. The order of reaction determines the shape of the concentration-time profile, while the

degradation rate constant is determined by its initial slope that is best fit to the reaction. The rate constants of free and complex forms of fixolide at different temperature are shown in Table 16. It was found that the degradation rate constants of free fixolide were higher than fixolide in the complex form at all temperatures studied. After storage at 80, 50 and 35 °C, the rate constant of degradation of fixolide in the complex with β CD was about 4-5 times slower than that of free fixolide. Complexation of fixolide with β CD showed dramatic increase in the values of half-life time ($t_{1/2}$) (Table 16). For the order of reaction, it was assessed by a plot of concentration of compound remaining versus time. If a plot of concentration versus time is linear, it means zero-order reaction. When a plot of $\ln(\text{concentration})$ against time is a straight line, it is first-order reaction. Linear regression was used to estimate the regression coefficient (R^2) of each plot. The order of reaction was decided on which plot gave the higher regression coefficient. Table 17 illustrates the regression coefficient of fixolide and fixolide : β CD formulations at different temperatures. The result shows that higher regression coefficient was obtained from plotting of $\ln(\text{concentration})$ versus time at all temperatures studied. Thus the degradation of all formulations followed first-order kinetics. The results of thermal stability suggest that the degradation mechanism of fixolide by temperature was still the same even when complexed with β CD.

The most factor stimulated degradation of compounds is the heat. The relationship of the degradation rate constant with temperature was exhibited by Arrhenius equation as follow:

$$\ln k = \ln A - \left(\frac{E_a}{R}\right) \frac{1}{T}$$

where, k - the rate constant, T - the temperature in Kelvins,
 A - the frequency factor, R - the molar gas constant
 E_a - the activation energy,

Figure 28 depicts the Arrhenius plot, i.e. $\ln(\text{degradation rate constant})$ versus the reciprocal of temperature, of the degradation rates in the absence and presence of βCD at three temperatures (35, 50 and 80 °C). The Arrhenius equations were obtained graphically from Arrhenius plot. Arrhenius equations of free fixolide and fixolide inclusion complexed with βCD were presented in equation (1) and (2), respectively.

$$\ln k = 12.334 - 5354.3 (1/T) \quad (1)$$

$$\ln k = 10.896 - 5401.8 (1/T) \quad (2)$$

The activation energy (E_a) was calculated from the slope of the regression line, for fixolide formulation, the value was 10.64 Kcal/mole. When complexed with βCD , E_a was increased to 10.73 Kcal/mole. From the data, the higher activation energy of the complex indicated that higher energy was needed for the complex to be activated to the excited state. Thus, the degradation of fixolide in the presence of βCD was more difficult than that of fixolide alone.

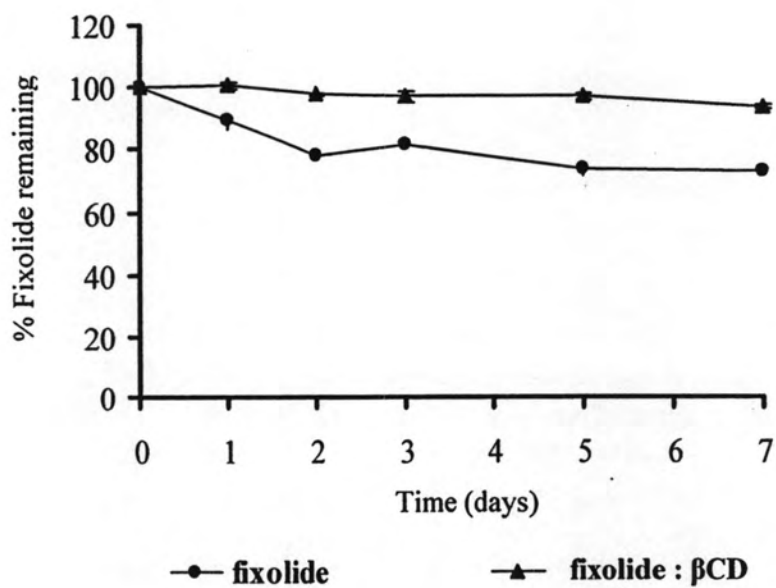


Figure 25. Percent remaining of fixolide and fixolide : β CD complex kept at 80 °C

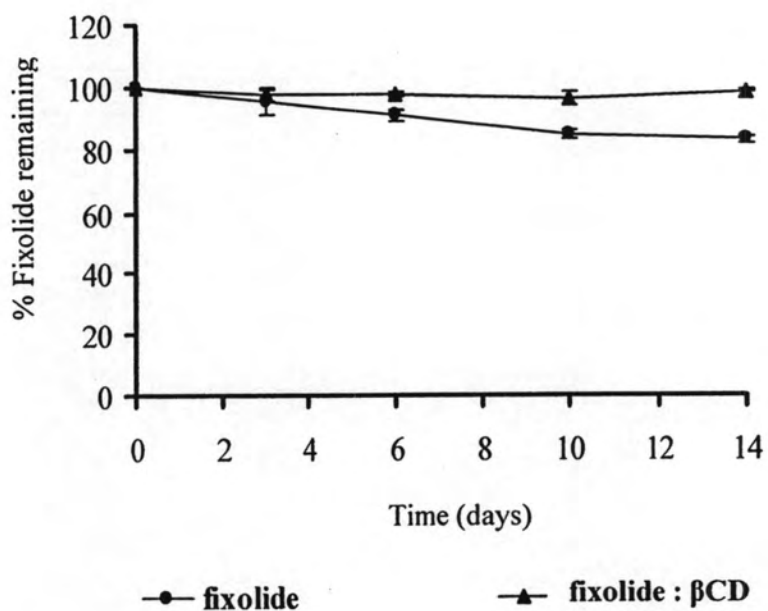


Figure 26. Percent remaining of fixolide and fixolide : β CD complex kept at 50 °C

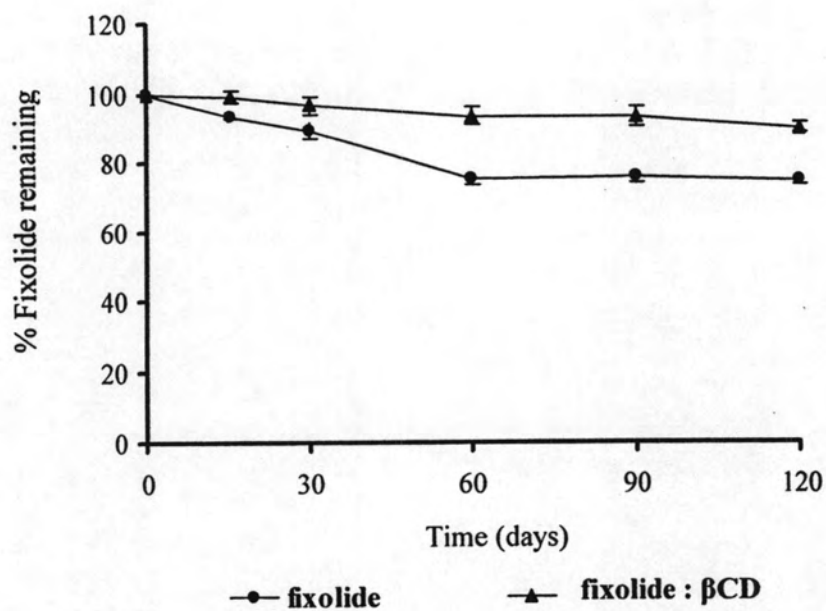


Figure 27. Percent remaining of fixolide and fixolide : β CD complex kept at 35 °C

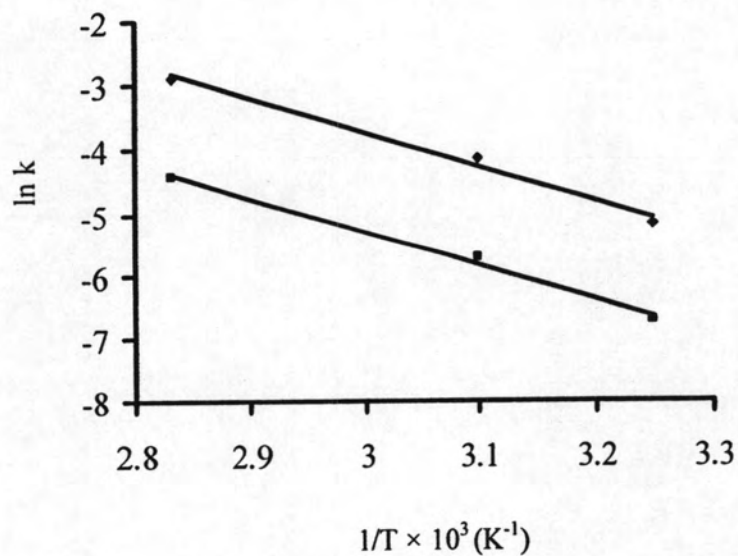


Figure 28. Arrhenius plot of fixolide (●) and fixolide : β CD (■) formulations

Table 16. The degradation rate constant of fixolide kept at various temperatures

Formula	Temperature (°C)	Degradation rate constant (day ⁻¹)	t _{1/2} (day)
Fixolide	80	5.66 x 10 ⁻²	12.2
	50	1.63 x 10 ⁻²	42.5
	35	4.70 x 10 ⁻³	147.4
Fixolide : βCD	80	1.19 x 10 ⁻²	58.2
	50	3.20 x 10 ⁻³	216.6
	35	1.20 x 10 ⁻³	577.5

Table 17. Regression coefficients of zero and first order reactions for fixolide with/without βCD formulations

Formula	Temperature (°C)	Regression coefficient (R ²)	
		Zero order	First order
Fixolide	80	0.7995	0.8183
	50	0.9967	0.9988
	35	0.9858	0.9914
Fixolide : βCD	80	0.7621	0.7644
	50	0.9344	0.9361
	35	0.9807	0.9812

3.6.2 UV stability test

The stability data of fixolide in both free and complex form with β CD under UV light is depicted in Figure 29 and Table A.4 (Appendix A). The amount of fixolide in the complex form remained as high as 97 %, while 84 % of the free form remained after exposure to UV light for 24 hours. The result indicates that fixolide was rather stable to the condition used in this UV exposure study.

The degradation rate constants for both formulations were calculated and displayed in Table 18. It was found that the rate constant of fixolide in the complex form with β CD was ten times slower than free fixolide.

The order of reaction of free fixolide and fixolide complex were determined by the plot of $\ln(\text{concentration})$ versus time which gave higher regression coefficient (Table 19), therefore the photodegradation kinetics of both formulations was concluded to be first-order. The results suggest that the presence of β CD did not affect this kinetic behaviour.

3.6.3 Dissolution study of inclusion complexes

The solubility data and dissolution profiles of fixolide : β CD co-precipitated product and fixolide : methyl- β CD kneaded product, both at the mole ratio of 1:2 are presented in Figure 30-31 and Table B.1 (Appendix B), Table C.1 (Appendix C). The free fixolide was also studied to compare with inclusion complex form. The dissolution profiles are plotted between the percent of fixolide dissolved against time.

Figure 30-31 show the dissolution rate profiles of fixolide alone and inclusion complexes. It was evident that both the complexes exhibited a faster dissolution rate than the free fixolide especially in the initial phase. After 5 minutes, the dissolved amount of fixolide alone was 28 %, while the amounts were 89 % and 93 % when fixolide was complexed with β CD and methyl- β CD, respectively.

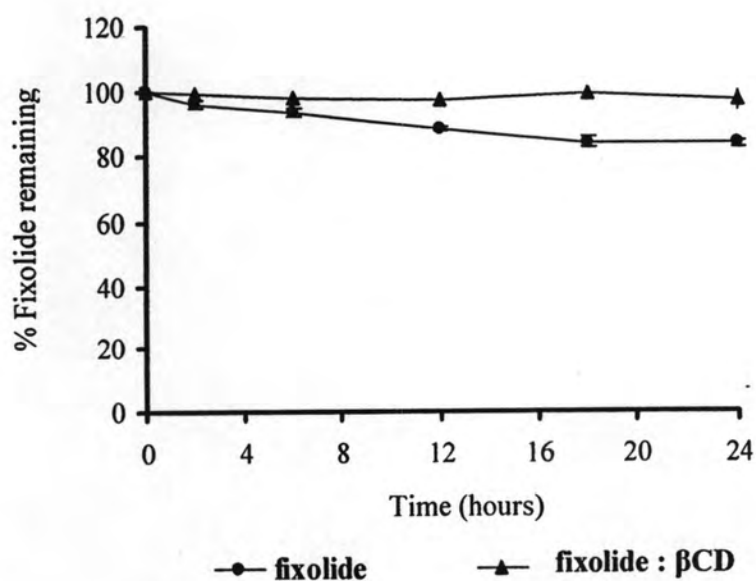


Figure 29. Percent remaining of fixolide and fixolide : βCD complex under UV light

Table 18. The degradation rate constants of fixolide under UV light

Formulation	Degradation rate constant (day ⁻¹)	t _{1/2} (day)
Fixolide	7.54 x 10 ⁻³	91.9
Fixolide : βCD	8.27 x 10 ⁻⁴	837.9

Table 19. Regression coefficients of zero and first order reactions for fixolide with/without βCD formulation under UV light

Formulation	Regression coefficient (R ²)	
	Zero order	First order
Fixolide	0.9708	0.9721
Fixolide : βCD	0.5791	0.6431

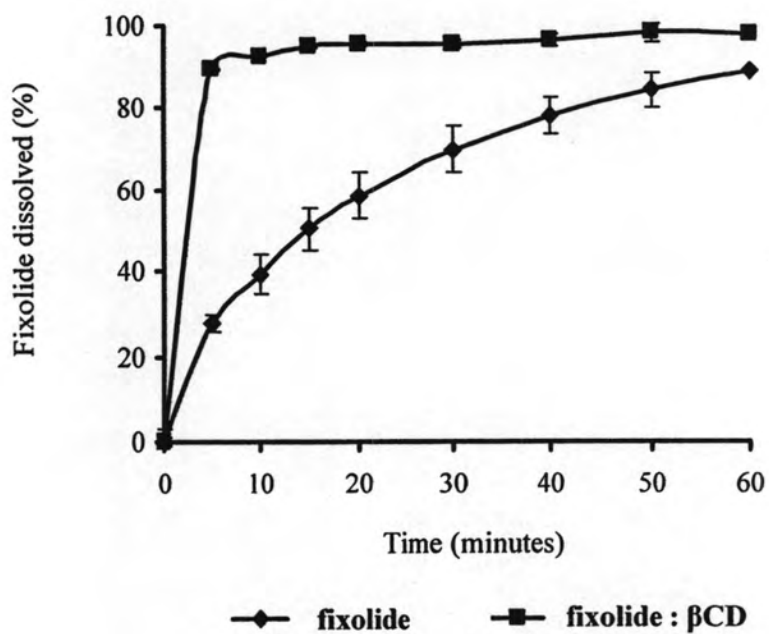


Figure 30. Dissolution profiles of fixolide and its β CD complex in 50% (v/v) ethanolic aqueous solution

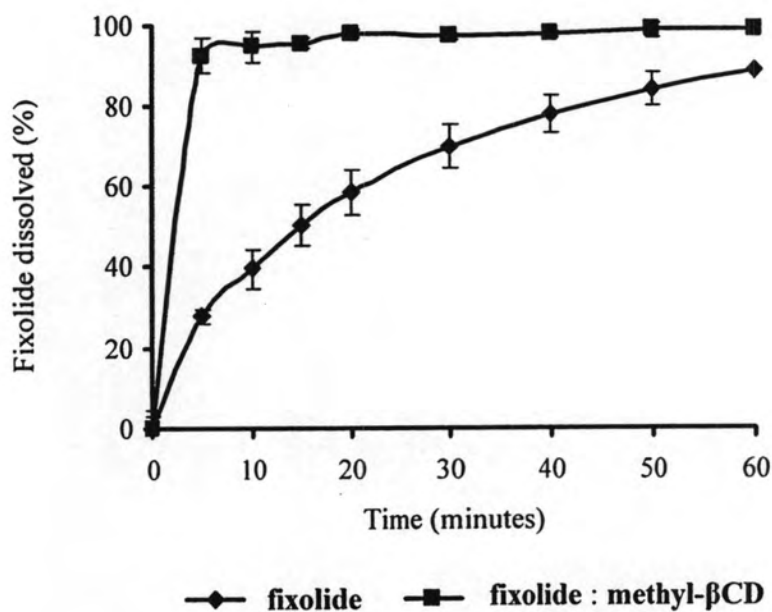


Figure 31. Dissolution profiles of fixolide and its methyl- β CD complex in 50% (v/v) ethanolic aqueous solution

3.7 Application of the Inclusion Complex in Fabric Softener

This study was designed to compare the stability of fixolide in free form and in complex form with β CD when the fragrance was added in a formulation of fabric softener. The fabric softener was prepared in aqueous media, having ditallow dimethyl ammonium chloride (DTDMAC) as the main ingredient of the formulation. The fixolide or the fixolide inclusion complex with β CD of 1:2 mole ratios by coprecipitation method was dissolved in fabric softener solution, then the stability under different storage conditions at 50 °C, and 80 °C for accelerated condition and under UV light exposure similar to the experiment in section 3.6 were investigated. The fixolide remained at time intervals were determined by solvent extraction and GC analysis. Figure 32-34 and Table D.1-D.3 in Appendix D show percent remaining of fixolide in the complex and its free form.

The result of temperature stability test at 80 °C for a week shows that percent remained of fixolide were 50 % and 87 % for free form and in the complex form with β CD, respectively. After storage at 50 °C for two weeks, 47 % of free fixolide remained, while up to 92 % was left if fixolide was in the complex form. When exposed to UV light for 24 hours, the loss of fixolide was 17 % and 11 % from the free and inclusion complex form, respectively.

The degradation rate constants of fixolide in the free and inclusion complex forms were determined by slope of the plot between fixolide remained versus time. Table 20-21 show degradation rate constants and the values of half-life time ($t_{1/2}$) of fixolide at 80, 50 °C and under UV light. It was found that degradation rate constants of fixolide in the complex form with β CD were four and ten times slower than free fixolide when incubated at 80 and 50 °C, respectively. When exposure to UV light for 24 hours, degradation rate constant of fixolide in the presence of β CD and fixolide alone were slightly different. Degradation by UV exposure might need longer time. The result from thermal stability clearly indicates that inclusion complexation with β CD could extend the half-life ($t_{1/2}$) of fixolide in fabric softener.

The order of reaction of free fixolide and fixolide complex with β CD was determined by plotting of concentration or $\ln(\text{concentration})$ versus time. The regression coefficients were estimated for each plot by using a linear regression. Table 22-23 displays the regression coefficients of fixolide and fixolide inclusion complex with β CD at 80 °C, 50 °C and under UV light. When the formulations were incubated at all conditions, higher regression coefficients were obtained from plotting of $\ln(\text{concentration})$ versus time. Thus, the degradation of free fixolide and fixolide inclusion complex followed first-order kinetics. It was observed that the presence of β CD in the formulation did not affect this kinetic behaviour.

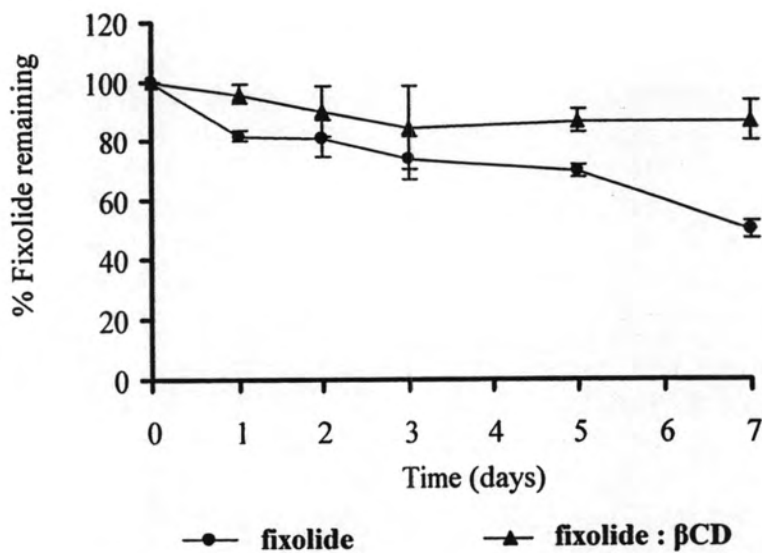


Figure 32. Percent remaining of fixolide and fixolide : β CD complex in fabric softener kept at 80 °C.

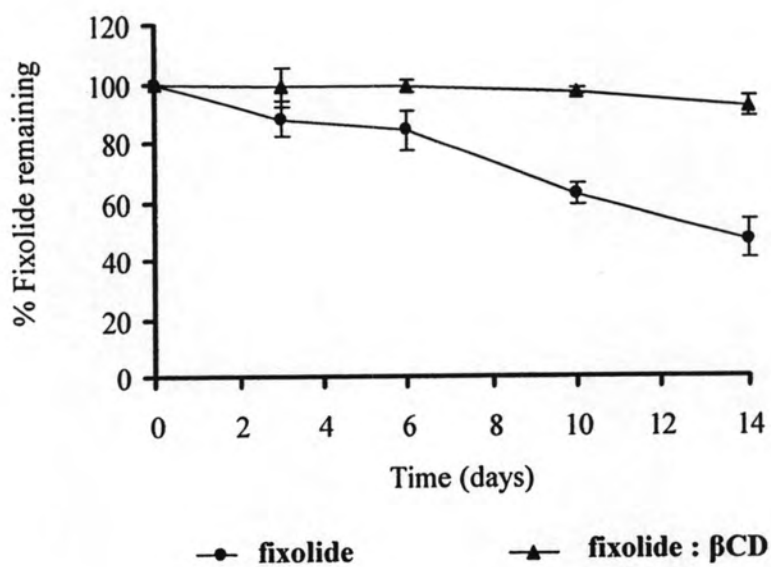


Figure 33. Percent remaining of fixolide and fixolide : β CD complex in fabric softener kept at 50 °C

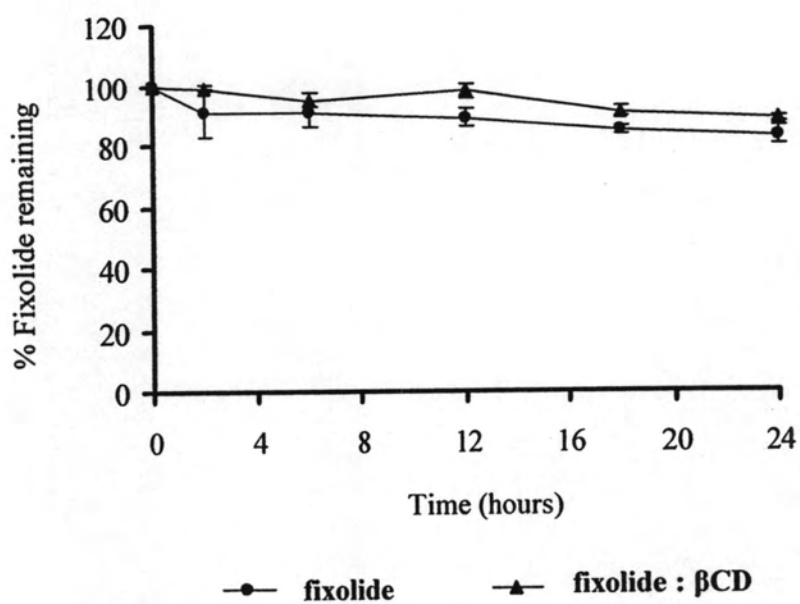


Figure 34. Percent remaining of fixolide and fixolide:βCD complex in fabric softener under UV light

Table 20. The degradation rate constants of fixolide in fabric softener kept at various temperatures

Formula	Temperature (°C)	Degradation rate constant (day ⁻¹)	t _{1/2} (day)
Fixolide	80	8.55 x 10 ⁻²	8.1
	50	5.34 x 10 ⁻²	12.9
Fixolide : βCD	80	1.97 x 10 ⁻²	35.2
	50	5.50 x 10 ⁻³	126

Table 21. The degradation rate constants of fixolide in fabric softener under UV light

Formulation	Degradation rate constant (day ⁻¹)	t _{1/2} (day)
Fixolide	6.40 x 10 ⁻³	108.3
Fixolide : βCD	5.20 x 10 ⁻³	133.3

Table 22. Regression coefficients of zero and first order reactions for fixolide with/without β CD formulations in fabric softener

Formula	Temperature ($^{\circ}$ C)	Regression coefficient (R^2)	
		Zero order	First order
Fixolide	80	0.9154	0.9186
	50	0.9609	0.9803
Fixolide : β CD	80	0.5952	0.5987
	50	0.8215	0.8279

Table 23. Regression coefficients of zero and first order reactions for fixolide with/without β CD formulation in fabric softener under UV light

Formulation	Regression coefficient (R^2)	
	Zero order	First order
Fixolide	0.8916	0.9004
Fixolide : β CD	0.9167	0.9433