

THE COMBINED INCLUSION COMPLEXATION OF ACETONE AND FLUNITRAZEPAM WITH DIMETHYL β -CYCLODEXTRIN

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ABSTRACT: Upon recrystallization from acetone, dimethyl β -cyclodextrin (DM- β -CyD) has shown physicochemical properties changes which were investigated by X-ray diffractometry, differential scanning calorimetry (DSC) and infrared (IR) spectroscopy. X-Ray diffraction analysis showed new crystalline diffraction pattern different from that of intact DM- β -CyD. An endothermic peak was recorded on the DSC thermogram of the recrystallized sample which was attributed to the release of acetone molecules included into DM- β -CyD cavities. Furthermore, the IR spectra of the recrystallized sample showed carbonyl stretching band at 1725 cm^{-1} , although DM- β -CyD has no carbonyl group, which proved the presence of included acetone molecules into DM- β -CyD cavities. The molar ratio of the included acetone molecules into DM- β -CyD molecules was determined by thermogravimetric analysis (TGA) and was found to be 0.73. In addition, when flunitrazepam and DM- β -CyD were recrystallized from acetone, it was found that flunitrazepam forms crystalline inclusion complex with DM- β -CyD in 1:3 molar ratio showing the same X-ray diffraction pattern of recrystallized DM- β -CyD alone from acetone. This means that the inclusion of acetone molecules into DM- β -CyD took place in addition to the formation of inclusion complex of flunitrazepam with DM- β -CyD.

INTRODUCTION

The ability of cyclodextrins to form crystalline insoluble complexes with many liquids as hydrocarbons and halogenated hydrocarbons facilitates their separation from crude starch digests¹. Takeo and Kuge reported that α -, β - and γ -CyDs formed inclusion

complexes with a number of organic compounds²⁻⁵. They inferred the formation of ternary complexes, which means that molecule (alone) do not form crystalline stable complexes with CyDs may be incorporated into the CyD cavity when accompanied by another molecule⁵. For instance, indomethacin forms no well-defined crystalline complex with β -CyD, but in the presence of some organic solvents (e.g. dimethylformamide) it gives a ternary complex of regular structure as supported by X-ray diffraction⁶.

The aim of this work is to investigate the inclusion complex formation of acetone with DM- β -CyD upon recrystallization at -15°C . Another goal was to shed light upon the possibility of incorporation of flunitrazepam molecule into DM- β -CyD cavity accompanied by acetone molecule under the same conditions.

EXPIRIMENTAL

MATERIALS

Heptakis-(2,6-di-O-methyl)- β -cyclodextrin (DM- β -CyD) was purchased from Toshin Chemicals CO., Japan and was used as recieved. Flunitrazepam, 1,3-dihydro-7-nitro-5-(2-fluoro)-phenyl-2H-1,4-benzodiazepin-2-one was kindly donated from Roche CO., Ltd., Japan and was used without further purification. Acetone was of analytical grade.

Equipement

X-Ray diffractometer (Rigaku Geigerflex 2204, Rigaku Denki Co., Ltd, Japan).

Differential scanning calorimeter (Perkin Elmer, USA).

Infrared spectrophotometer (Hitachi, Japan).

Thermogravimeter (Dupont company, USA).

Karl Fischer apparatus (Hiranuma AQ-3c, Japan).

Methods

Preparation of recrystallized DM- β -CyD sample

Recrystallized DM- β -CyD was prepared from acetone by dissolving 532.4 mg in 50 ml acetone (50 ml conical flask) and incubated in ethylene glycol bath at -15°C for 97 hours. The recrystallized DM- β -CyD was filtered and dried in air, the residue obtained was 150 mg.

Crystallization of flunitrazepam and DM- β -CyD from acetone

Accurate weights of flunitrazepam and DM- β -CyD (125.3 : 532.4 mg), equivalent to 1:1 molar ratio, were dissolved in 50 ml acetone and incubated in ethylene glycol bath at -15°C for 97 hours. The recrystallized sample was filtered using filter paper and left to dry in air until constant weight (167 mg). The flunitrazepam content in the recrystallized sample was determined spectrophotometrically at 253 nm.

Powder X-ray Diffraction

The powder X-ray diffraction patterns of recrystallized samples were measured under the following conditions; target Cu, filter Ni, voltage 30 kV, current 5 mA, time constant 0.5 s, scanning speed $4^{\circ}/\text{min}$, chart speed 40 mm/min and count range 1000 or 2000 cps.

Differential Scanning Calorimetry (DSC)

DSC curves of the samples were obtained using a Perkin Elmer DSC-2 which was operated at a scanning speed of $10^{\circ}\text{C}/\text{min}$, range 5 mcal/s, under a nitrogen stream, using a sample pan for liquids. The sample weight was about 3-5 mg.

Infrared Spectroscopy

IR measurements of the prepared samples were done using KBr disk method.

Thermogravimetric Analysis

TGA of the prepared samples were carried out under the following conditions, heating rate of $10^{\circ}\text{C}/\text{min}$ over a temperature range of $25-300^{\circ}\text{C}$ under nitrogen gas stream. The sample weight was about 12-20 mg. TG curve of recrystallized DM- β -CyD sample represents the percent of total weight loss of both water and

acetone contents. Subtraction of water content of sample determined by Karl Fischer, from the total weight loss of TG curve, gave the weight loss of evaporated acetone content. The combining molar ratio of the acetone to DM- β -CyD was calculated from the weight loss of acetone and its molecular weight.

RESULTS AND DISCUSSION

Recrystallized DM- β -CyD From Acetone

The powder X-ray diffraction patterns of intact and recrystallized DM- β -CyD samples are shown in Fig.1. The powder X-ray diffraction pattern of recrystallized DM- β -CyD sample (curve B) showed new crystalline X-ray diffraction peaks at $2\theta = 9.0^{\circ}$, 11.4° , 11.6° , 15.4° and 18.6° which were quite different from the diffraction peaks of intact DM- β -CyD (curve A). As the acetone molecules produce no diffraction pattern at all and the diffractogram of recrystallized DM- β -CyD from acetone differs significantly from that of uncomplexed (intact) DM- β -CyD, the confirmation of complex formation of DM- β -CyD with acetone can be established⁶.

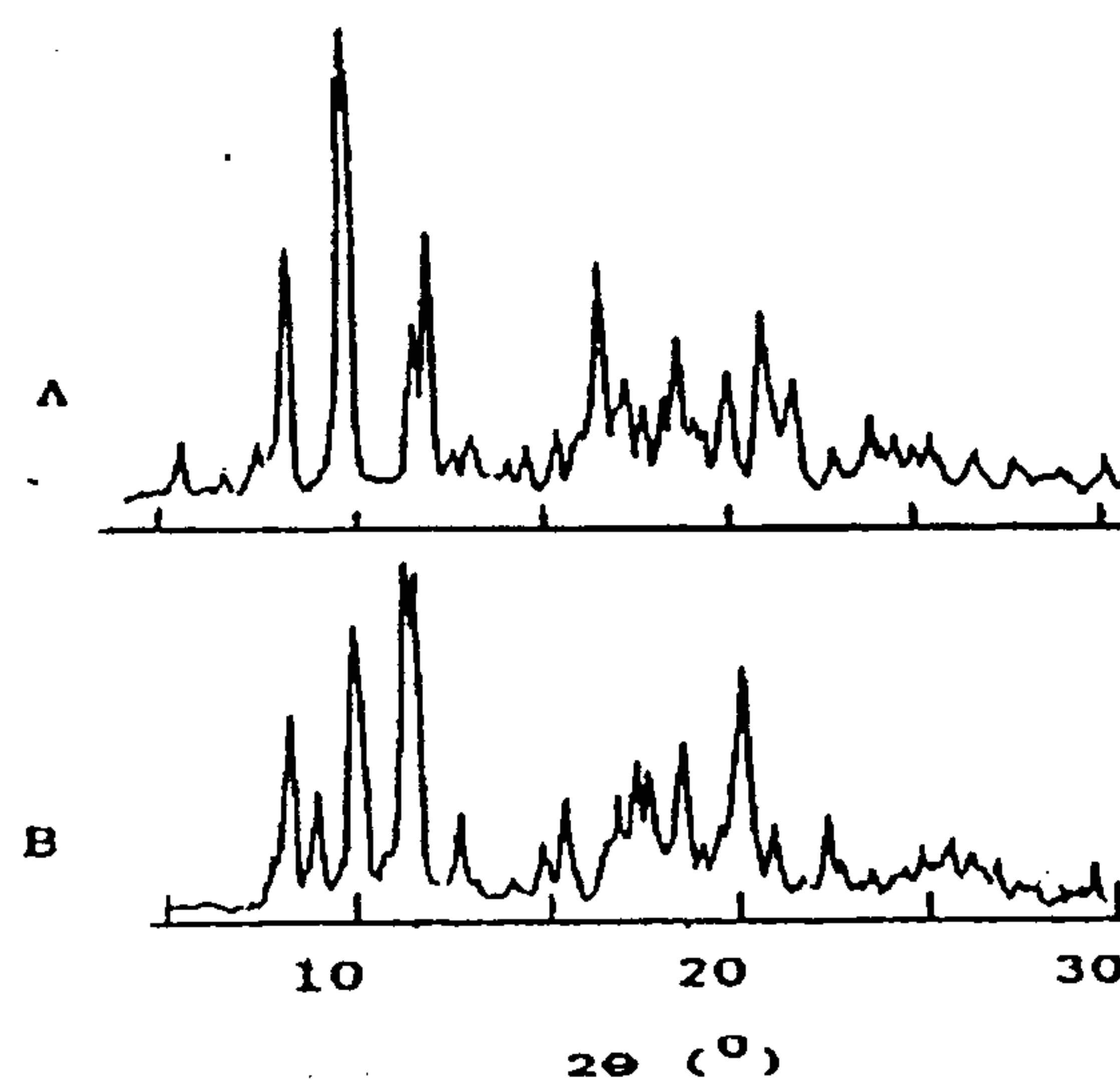


Fig. 1: Powder X-ray diffraction patterns of intact and recrystallized DM- β -CyD samples.
A-intact DM- β -CyD B-recrystallized DM- β -CyD.

Fig. 2 shows DSC curves of intact and recrystallized DM- β -CyD samples. DSC (curve A) of intact DM- β -CyD shows no endothermic

peak while that of recrystallized DM- β -CyD (curve B) shows a sharp endothermic peak at 436 K. This peak could be attributed to the release or evaporation of acetone molecules from DM- β -CyD cavities. These results confirm the inclusion complexation of acetone into DM- β -CyD cavities, since the boiling point of acetone is 56.5°C while the included acetone evaporated at 436 K (163°C). Furthermore, TG curve of recrystallized DM- β -CyD showed weight loss of 5.51 % (Fig. 3 curve B)

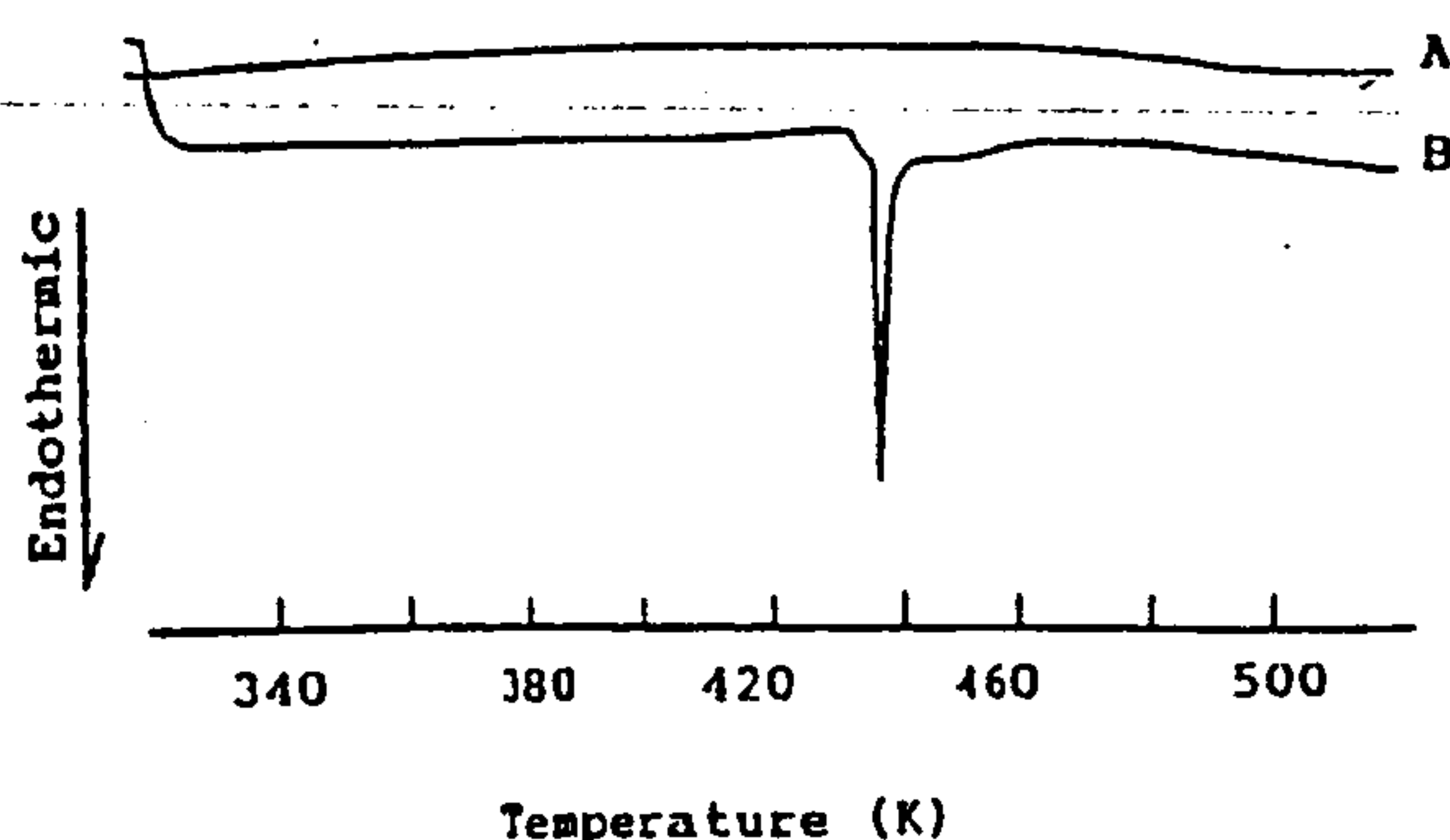


Fig. 2: DSC curves of intact and recrystallized DM- β -CyD samples.
A-intact DM- β -CyD B-recrystallized DM- β -CyD

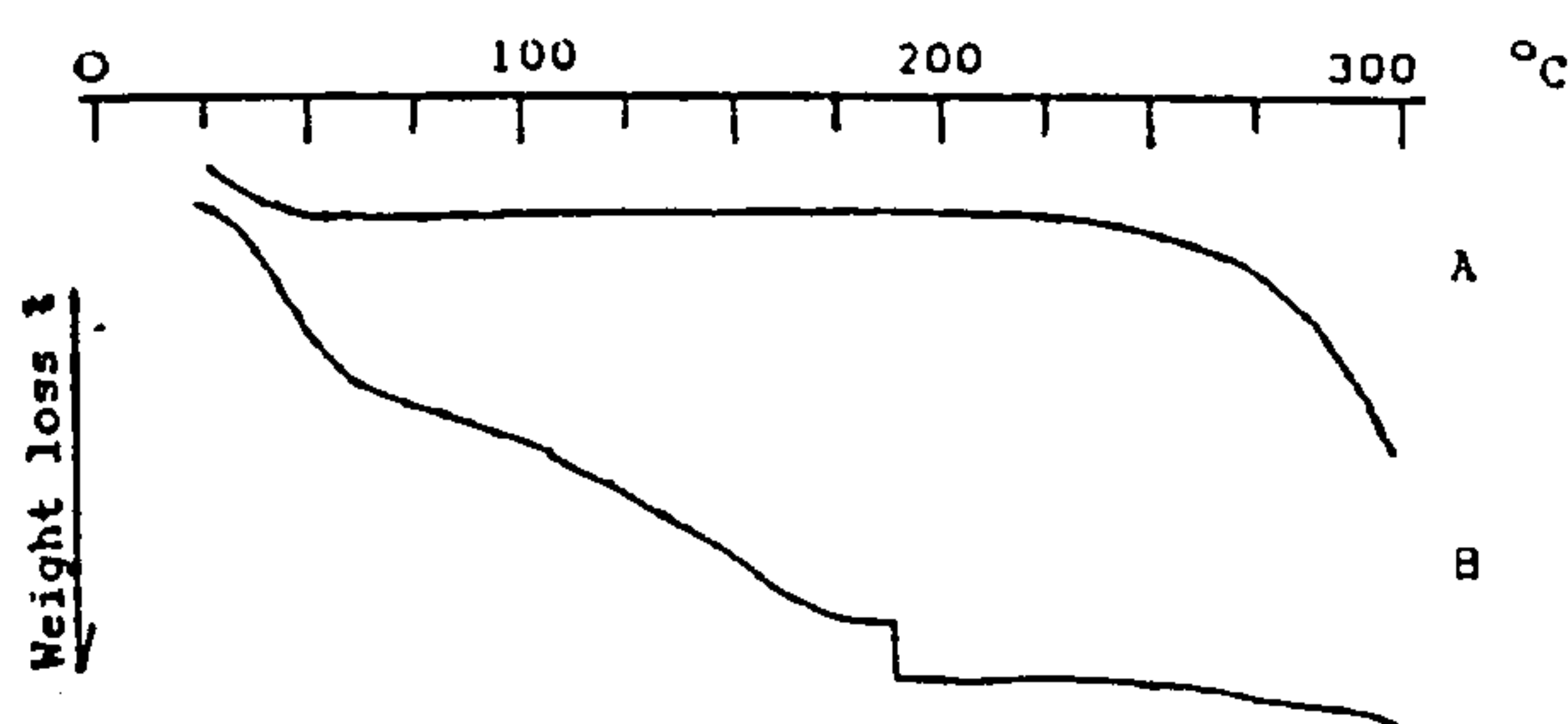


Fig. 3: TG curves of intact and recrystallized DM- β -CyD samples.
A-intact DM- β -CyD B-recrystallized DM- β -CyD

while TG curve A of intact DM- β -CyD showed only 0.667% weight loss which represents its water content. The weight loss % of acetone in the recrystallized DM- β -CyD sample was calculated by subtracting the water content (2.35%) of the sample from the total weight loss (5.51%) of its TG curve to give 3.16%. The molar ratio of acetone to DM- β -CyD was found

to be 0.73.

Fig. 4 shows IR spectra of both intact and recrystallized DM- β -CyD in the carbonyl stretching region 1800-1600 cm^{-1} . The intact DM- β -CyD crystals have no carbonyl stretching band in this region as seen in curve A. On the other hand, the recrystallized DM- β -CyD sample (curve B) shows a carbonyl stretching band at 1725 cm^{-1} indicating the presence of acetone molecules within DM- β -CyD cavities.

From the results of X-ray diffraction, DSC, TG and IR, it could be concluded that an inclusion complex of acetone with DM- β -CyD was formed during the process of recrystallization.

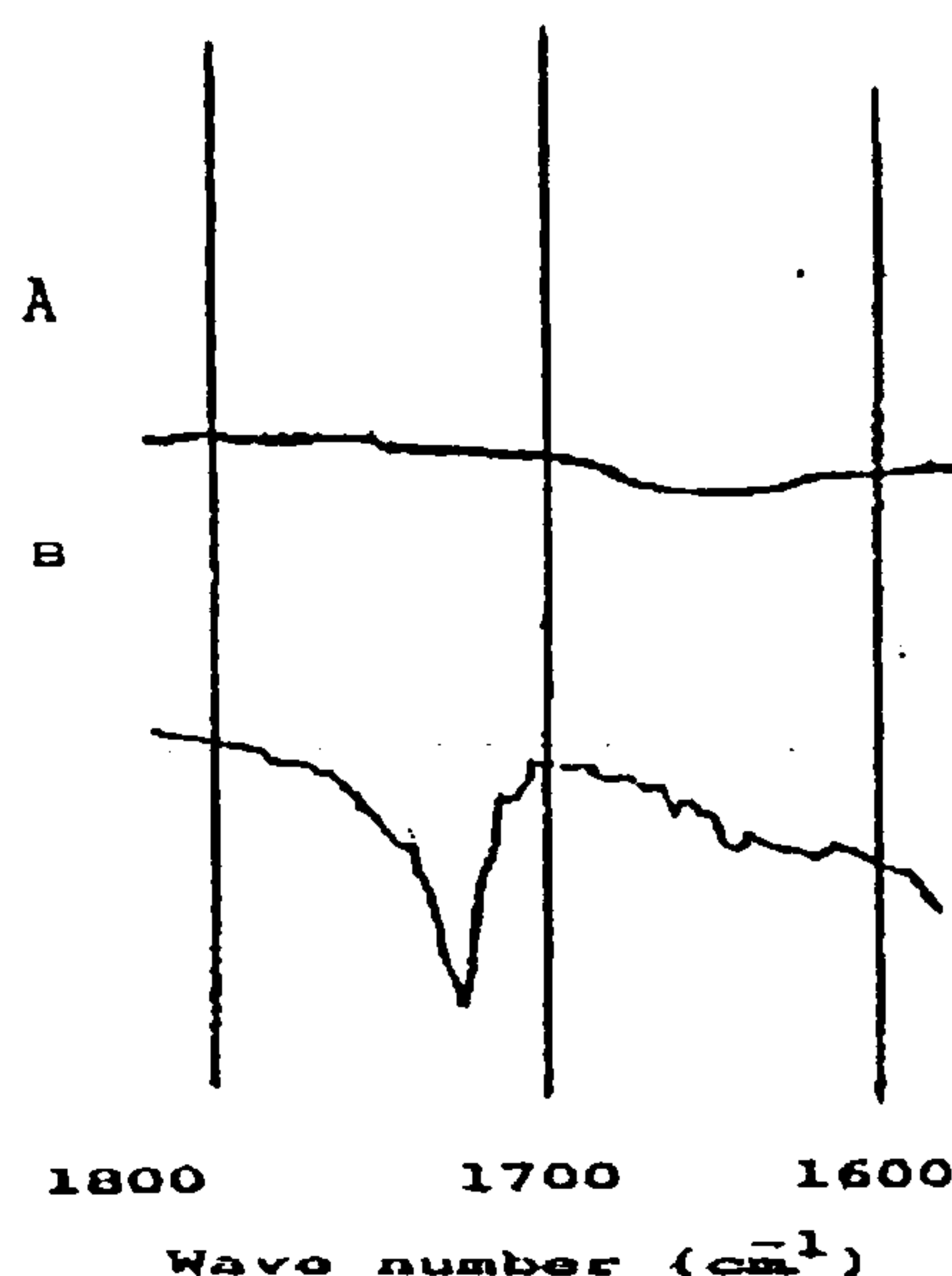


Fig. 4: IR spectra of intact and recrystallized DM- β -CyD sample in the carbonyl stretching region using KBr disk method.

A-intact DM- β -CyD B-recrystallized DM- β -CyD

Fig. 5 shows powder X-ray diffraction patterns of flunitrazepam with DM- β -CyD systems. Flunitrazepam crystals (curve A) show crystalline diffraction pattern with characteristic peak at $2\theta = 14.0^\circ$. The powder X-ray diffraction pattern of coprecipitate of flunitrazepam with DM- β -CyD in 1:1 molar ratio (curve C) shows new crystalline peaks at $2\theta = 9^\circ, 11.2^\circ, 11.5^\circ$ and 18.5° different from the diffraction peaks of both intact components, but with presence of characteristic peak of flunitrazepam at $2\theta = 14^\circ$ indicating the presence of excess flunitrazepam. On the other

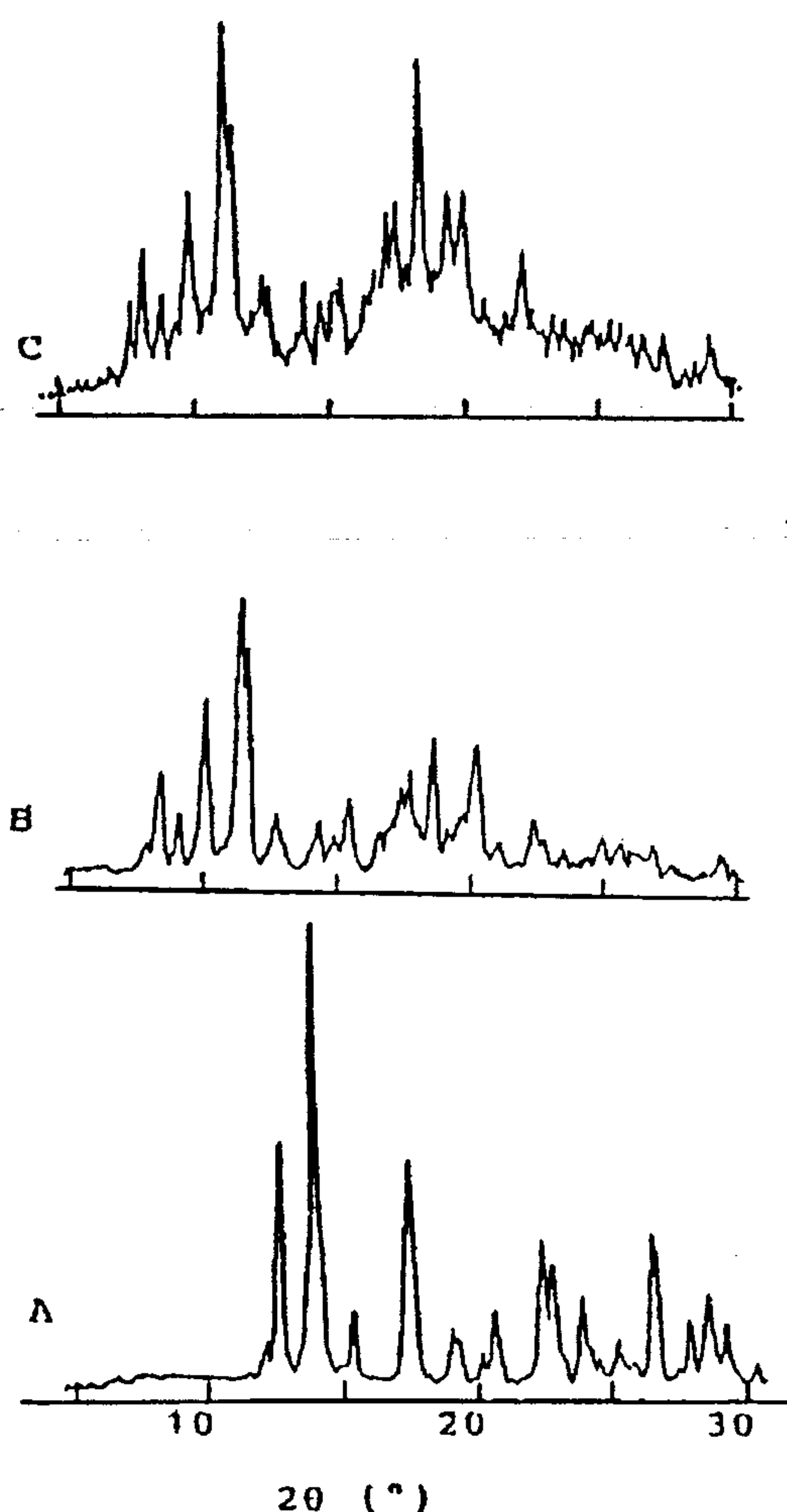


Fig. 5: Powder X-ray diffraction patterns of flunitrazepam with DM- β -CyD systems.

A-flunitrazepam crystals.

B-recrystallized sample of flunitrazepam with DM- β -CyD.

C-coprecipitate of flunitrazepam with DM- β -CyD (1:1).

hand, the powder X-ray diffraction pattern of recrystallized sample of flunitrazepam with DM- β -CyD (curve B) shows new crystalline diffraction peaks at $2\theta = 9.0^\circ, 11.5^\circ, 11.7^\circ$ and 18.6° similar to that of recrystallized DM- β -CyD alone (Fig. 1 curve B) and at the same time different from each component flunitrazepam or DM- β -CyD. The flunitrazepam content in this sample was found to be 0.65 mg per 10 mg equivalent to 0.34 mole, i.e. the molar ratio of flunitrazepam to DM- β -CyD is 1:3 in this recrystallized sample. From powder X-ray diffraction data of recrystallized sample of flunitrazepam with DM- β -CyD, we could infer the formation of a ternary inclusion complex

between DM- β -CyD, acetone and flunitrazepam. From the solubility study, the solubility of flunitrazepam increased linearly by increasing the concentration of DM- β -CyD showing A_L -type phase solubility diagram⁷. This means that an insoluble inclusion complex can not be obtained from aqueous solution⁸. Therefore, the alternative to prepare an inclusion complex of flunitrazepam with DM- β -CyD was by the recrystallization from acetone.

Fig. 6 shows DSC curves of flunitrazepam with DM- β -CyD systems. Flunitrazepam crystals (curve A) show an endothermic peak at 441K which is due to the melting of flunitrazepam. DSC (curve B) of the physical mixture of flunitrazepam with DM- β -CyD in 1:1 molar ratio shows also an endothermic peak at 431K due to the melting of flunitrazepam. On the other hand, the coprecipitate of flunitrazepam with DM- β -CyD 1:1 (curve C) shows three peaks; one exothermic peak at 370K and two endothermic peaks at 416 and 435K respectively.

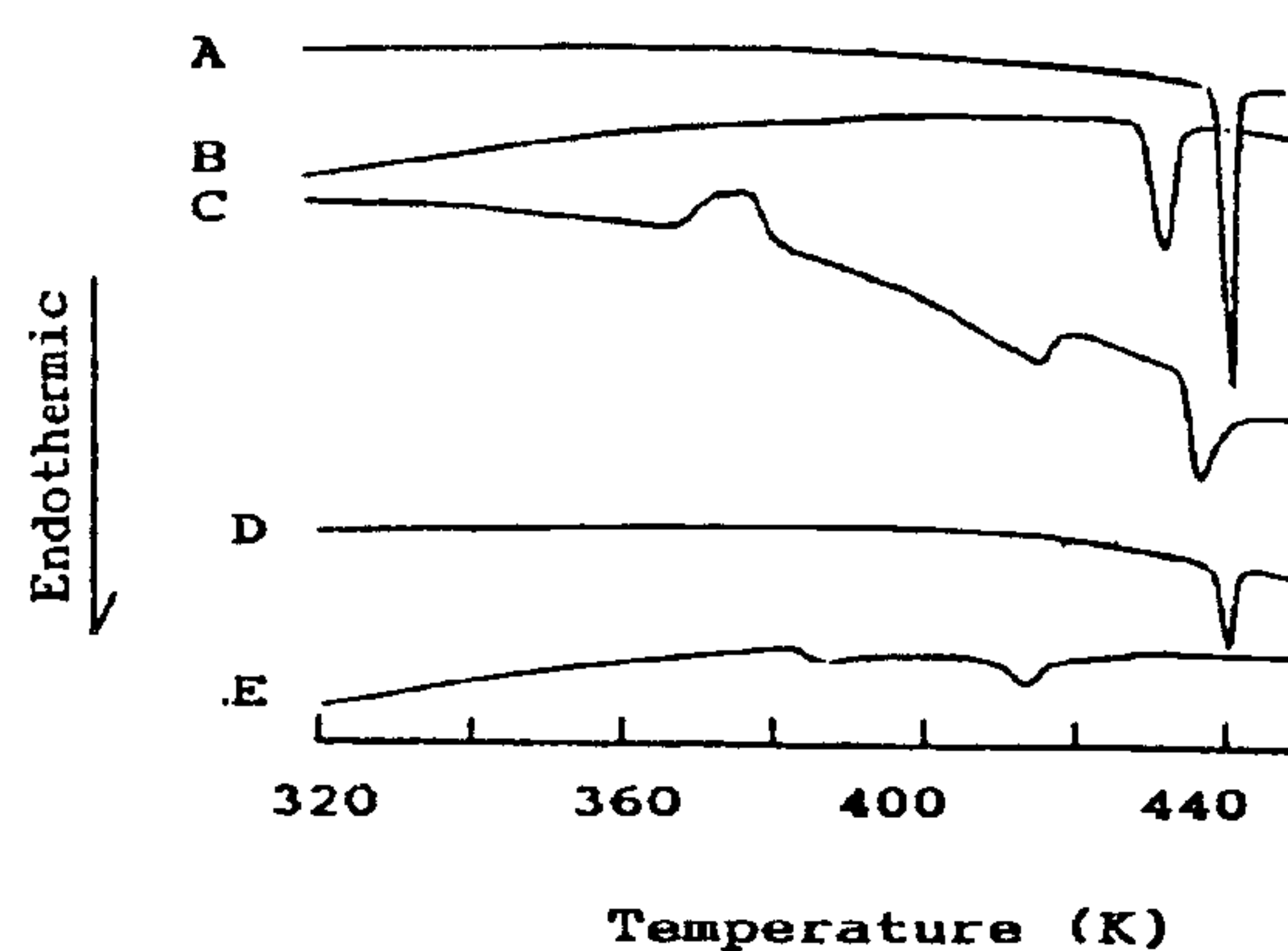


Fig. 6: DSC curves of flunitrazepam with DM- β -CyD systems.

A-flunitrazepam crystals.

B-physical mixture of flunitrazepam with DM- β -CyD (1:1).

C-coprecipitate of flunitrazepam with DM- β -CyD (1:1).

D-physical mixture of flunitrazepam with DM- β -CyD (1:3).

E-recrystallized sample of flunitrazepam with DM- β -CyD.

The exothermic peak at 370K may be due to recrystallization of the complex of flunitrazepam with DM- β -CyD⁷. The first endothermic peak at 416K may be due to release of the included acetone and the second endothermic one at 435K may be due to the fusion of excess flunitrazepam. DSC (curve E) of the recrystallized flun-

itrazepam with DM- β -CyD (1:3) shows two small consequent peaks at 386 and 409 K respectively, and no peak in the melting region of flunitrazepam. The disappearance of melting peak of flunitrazepam on DSC curve indicates the formation of an inclusion complex of flunitrazepam with DM- β -CyD upon recrystallization from acetone; and the two endothermic peaks at 386 and 409K may be due to release of the included acetone molecules. These DSC results revealed also the formation of the combined inclusion of acetone and flunitrazepam molecules into DM- β -CyD cavities.

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تكوين المتراكب المنغمس المشترك من الاسيتون والفلونيترازيام

فى ثنائى ميثيل بيتاسيكلوديكسترين

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تم فى هذا البحث اثبات تكوين المتراكب المنغمس للاسيٲون فى ثنائى ميثيل بيتاسيكلوديكسترين وذلك أثناء اعادة بلورة الأخير من الأسيتون عند درجة حرارة ١٥ م تحت الصفر وكذا تم تعيين النسبة الجزيئية للمتراكب المنغمس من الاسيتون فى ثنائى ميثيل بيتاسيكلوديكسترين باستخدام التحليل الحرارى الوزنى .

ومن ناحية أخرى تم اثبات تكوين متراكب منغمس مشترك من الاسيتون والفلونيترازيام فى ثنائى ميثيل بيتاسيكلوديكسترين عند اعادة بلورة الفلونيترازيام وثنائى ميثيل بيتاسيكلوديكسترين من الاسيتون تحت نفس الظروف .

وقد استخدمت طرق عديدة فى اثبات تكوين هذه المتراكبات مثل طريقة التشتت وأشعة اكس وطريقة التحليل السعري التفاضلى وطريقة التحليل بواسطة الأشعة تحت الحمراء .

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