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A STUDY OF THE INCLUSION COMPLEX OF AMOBARBITAL WITH HEPTAKIS (2,6-DI-O-METHYL)-ß-CYCLODEXTRIN

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ABSTRACT

The inclusion complex of amobarbital, a hypnotic and sedative agent, with heptakis (2,6-di-O-methyl)-ß-cyclodextrin (DMCD) in aqueous solution and in the solid phase was studied by the solubility method, UV spectroscopy, infrared spectroscopy (IR), differential scanning calorimetry (DSC) and X-ray diffractometry. The inclusion complex was confirmed to be 1:1. The solid complex of amobarbital with DMCD in 1:1 molar ratio was prepared by coprecipitation method. The solubility and dissolution characteristics of the solid complex in water at 37±1°C were examined. The complex formation constant, K_c, was calculated from the slope and intercept of the A_L solubility diagram (950 M^{-1}). The dissolution rate of amobarbital was significantly enhanced by the complexation with DMCD specially in the initial period of dissolution. The hypnotic effect of amobarbital and its complex given orally to mice was evaluated. The inclusion complex, showed a decrease in the onset of action and an increase in the duration of hypnotic effect.

INTRODUCTION

Amobarbital is a slightly water soluble hypnotic and sedative medicament. The inability to formulate poorly soluble drugs into appropriate aqueous dosage forms has severely limited the application of many potentialy drugs. Several approaches have been proposed to overcome this problem including the use of organic co-solvents, emulsions, liposomes and micelles. Unfortunately, all of the above mentioned techniques have their associated problems [1]. An attractive alternative to these methods is the use of cyclodextrins and their derivatives.

Cyclodextrins have been used successfully to form inclusion complexes, in which the drug molecules are included in the relatively hydrophobic cavity of cyclodextrins and lead to improve solubility, dissolution, stability and oral bioavailability of various poorly soluble drugs [2-4]. Complexation process of cyclodextrins and their derivatives can be accessed in solution and in solid states, but the widespread uses of ß-cyclodextrin as a drug carrier, are sometimes restricted by its low aqueous solubility (1.85 g/100 ml at 25°C) [5,6]. The complexing capacities of alkylated and hydroxyalkylated cyclodextrins for improvement of the physicochemical properties of slightly soluble drugs is more effective than their parent cyclodextrins. Recently, the methylated cyclodextrins have received considerable attention because their physicochemical properties and inclusion behaviours are different from their parent cyclodextrins [7-9].

The aim of this study was to investigate and evaluate the amobarbital-DMCD inclusion complexation and its effect on the solubility and dissolution characteristics and bioavailability of amobarbital.

EXPERIMENTAL

Materials and Methods

Materials

Amobarbital, Sigma Chemical Co. (USA), Heptakis (2,6-di-O-methyl)-ß-cyclodextrin (DMCD), Toshin Chemical Co. (Japan). All other materials and solvents used were of analytical reagent grade.

Phase-solubility Studies

Solubility measurements were carried out according to the method of Higuchi and Conners [10]. Excess amount (34 mg) of amobarbital of particle size pass through 200 um sieve, were added to equal volumes (10 ml) of aqueous solutions containing various concentrations of DMCD (0-1.6×10^{-2} M). The previous solutions were shaken at constant temperature (37±1°C) by using water-bath shaker operating at 50 rpm. At equilibrium, after 3 days, different solutions were centrifuged and 1 ml of each solution was pipetted through a cotton plug and the filtered aliquots were diluted and analyzed spectrophotometrically in alkaline medium (1.0 M NaOH). The change in absorbance of amobarbital by the addition of various concentrations of DMCD was measured at 254 nm using double beam spectrophotometer Shimadzu UV-150-02 (Japan). Complex formation constant, K_c, was calculated from the A_L phase solubility diagram according to the equation of Higuchi and Conners [10].

$$K_c = \frac{\text{slop}}{\text{intercept (1-slope)}}$$

Preparation of Amobarbital-DMCD Solid Complex

By using conditions derived from the solubility diagram (Figure 1), the solid complex of amobarbital with DMCD in 1:1 molar ratio was prepared by coprecipitation method[11] by dissolving equimolar amounts of amobarbital and DMCD into suitable volume of acetone and evaporating the solvent, under stirring.

Preparation of the Physical Mixture

The calculated and exactly weighed amounts in 1:1 molar ratio of amobarbital and DMCD were pulverized in a porcelain mortar and carefully mixed.

UV Absorption Study

The UV absorption changes of amobarbital (2.2×10^{-3} M) in the absence and the presence of various concentrations of DMCD (0-10×10^{-3} M) were measured at the range of 200-400 nm using an Unicam SP 1750 ultraviolet spectrophotometer.

Differential Scanning Calorimetry (DSC)

The DSC patterns were recorded on Perkin-Elmer Model DSC-1B. The measurements were done using the sample pan for the liquid sample, at a scanning speed 10°C/min under nitrogen stream as purgin gas from 320 to 480 K. Sample weight was about 4 mg.

Infrared Absorption Spectroscopy (IR)

The IR spectra of amobarbital and its solid complex with DMCD were carried out with Hitachi 295 infrared spectrophotometer using KBr disks. For comparison, the IR spectra of physical mixture were carried out using the same procedure.

X-ray Diffractometry (powder method)

The X-ray powder diffraction patterns were measured using Rigaku-denki 2027 Diffractometer under the following conditions: Target, Cu; Filter, Ni; Voltage, 30 kv; Current, 5 mA; Time constant, 0.5 s; Scanning speed, 2°/min; Count range, 2000 cps.

Dissolution Rate Measurement

The dissolution rate studies from tested samples were done in aqueous solution (250 ml) at 37±1°C. At appropriate intervals, 2 ml of solution was pipetted through a cotton plug, diluted and assayed spectrophotometrically. Equivalent volume (2 ml) of fresh aqueous medium, preheated at 37°C, was added. A correction was applied for the cumulative dilution caused by replacement of the sample by equal volume of the original medium.

Evaluation of the Hypnotic Effect in Mice

Both the drug and its inclusion complex were dissolved in water (2 mg/ml) and were given orally to mice by means of a stomach tube in a dose of 80 mg/kg into groups of 10 mice. The onset as well as the duration of hypnotic effect were determined.

RESULTS AND DISCUSSION

The phase solubility diagram obtained for amobarbital with DMCD is shown in Figure 1, which illustrates that a linear relationship exists between the amount of the drug solubilized and the concentration of DMCD in solution. This indicates the formation of a soluble complex of the A_L type. Based on Figure 1, the stoichiometry of the inclusion complex is 1:1. The observed rate constant for the formation of the complex (K_c) was calculated according to the equation of Higuchi and Connors and was found to be 950 M^{-1}.

In the presence of DMCD, the UV absorption spectra of amobarbital were changed as indicated in Figure 2, where there was decreasing in molar absorptivity. However there was no significant peak shifting to longer wavelength in UV spectra. These observations suggested that the drug molecules were interacted with DMCD to form inclusion complex.

The DSC spectra of DMCD, amobarbital, the physical mixture and the inclusion complex are presented in Figure 3. The DSC trace of amobarbital shows one endothermic peak at 432°K, corresponding to its melting point, meanwhile, the DSC trace of DMCD shows no melting peak. On the other hand, the DSC curve of the physical mixture shows one endothermic peak at 410°K. The change of the peak of the physical mixture can be attributed to the weak interaction between the components. However, the DSC curve of the inclusion complex lacks the endothermic peak characteristic of pure amobarbital. The disappearance of the endothermic peak provides a further indication of the formation of an inclusion complex between amobarbital and DMCD.

Figure 4 shows the IR spectra of DMCD, amobarbital, physical mixture and amobarbital-DMCD inclusion complex. As shown in the figure, DMCD has no absorption band in the region of carbonyl stretching vibration (1770-1700 cm^{-1}) but the spectrum of amobarbital (curve B) showed three absorption bands at 1765, 1730 and 1700 cm^{-1}. Curve C shows the spectrum of physical mixture, in which there is no change in the above three hydrogen-bonded carbonyl stretching bands. In case of IR spectrum of the inclusion complex (curve D) the carbonyl bands at 1730 and 1700 cm^{-1} were shifted to higher frequencies at 1735 and 1715 cm^{-1} respectively. The IR spectra support the DSC results and confirm the formation of amobarbital-DMCD inclusion complex by coprecipitation method.

The powder X-ray diffraction patterns of the amobarbital-DMCD systems are shown in Figure 5. The diffraction pattern of the physical mixture was simply a superposition of each component, while that of the inclusion complex was apparently

different from each component and constituted a new solid phase. The crystallinity pattern of the powder was declined in the complex system as evidenced by fewer and broader peaks of lower intensity.

The dissolution profiles of amobarbital, physical mixture and inclusion complex in water at 37°C are shown in Figure 6. From the inclusion complex and physical mixture amobarbital dissolved rapidly about 13 and 8 times respectively faster than the amobarbital alone at 5 min. Physical mixture showed significant increase in the dissolution rate in comparison with amobarbital crystals. This can be explained by partial interaction between amobarbital and DMCD in the physical mixture and formation of the complex. Irrigular points on the curves B and C can be explained by the recrystallization and again redissolving of amobarbital from the physical mixture and the inclusion complex.

Results in Figure 7, revealed a significant decrease in the onset of action of the inclusion complex than that of the drug. Also, the duration of hypnotic effect was found to be more prolonged for the inclusion complex.

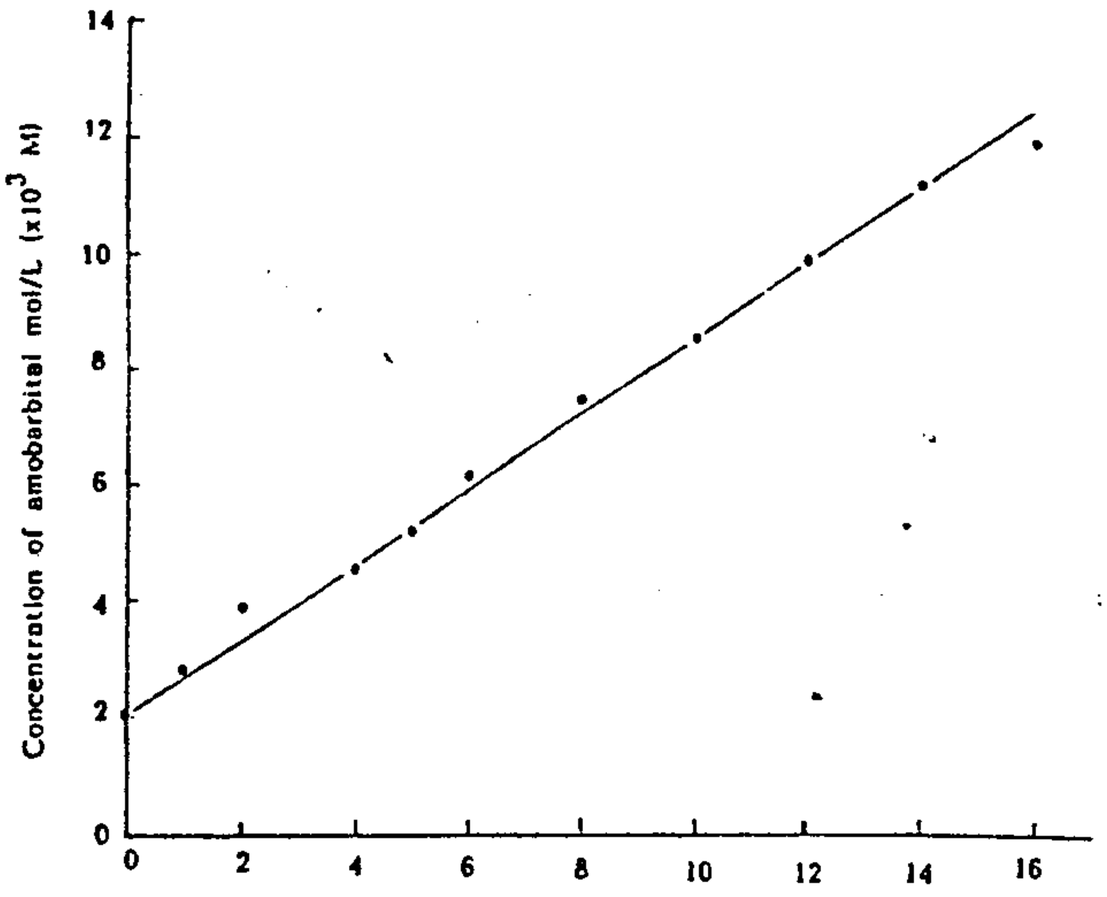

Fig. 1: Phase Solubility Diagram of Amobarbital and DMCD in Water at 37°C.

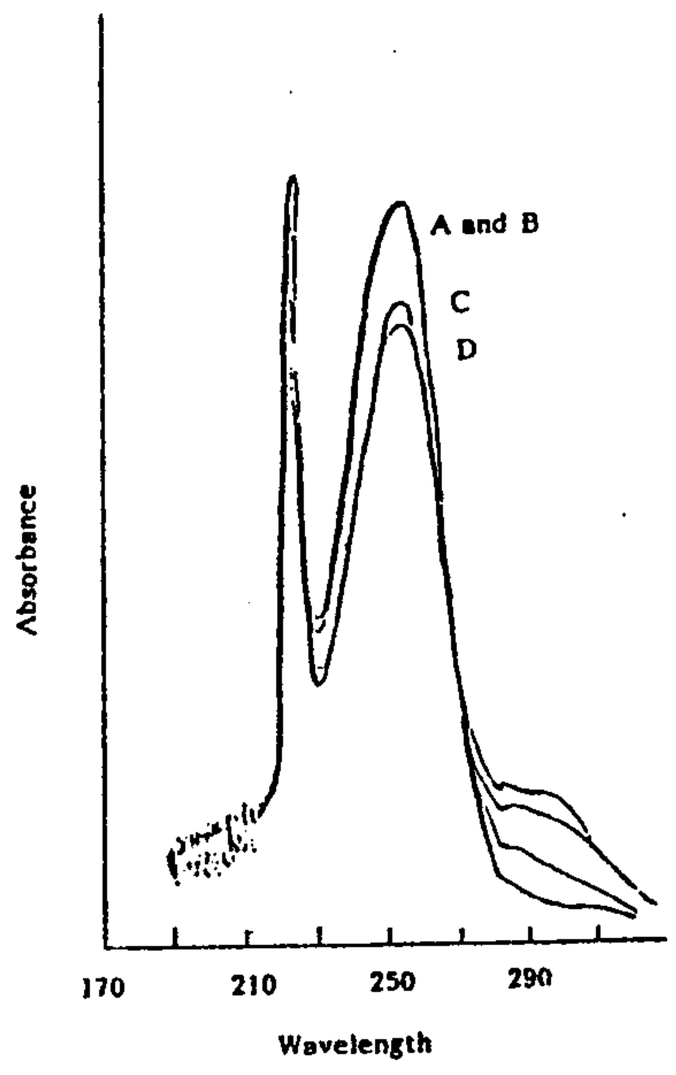

Fig. 2: The UV Absorption Spectra of Amobarbital (2.2x10^{-3} M) in the Absence and the Presence of Various Concentrations of DMCD: (A) 0.0M (B) 2.5 x 10^{-3}M (C) 5.0 x 10^{-3} (D) 10.0x10^{-3}M

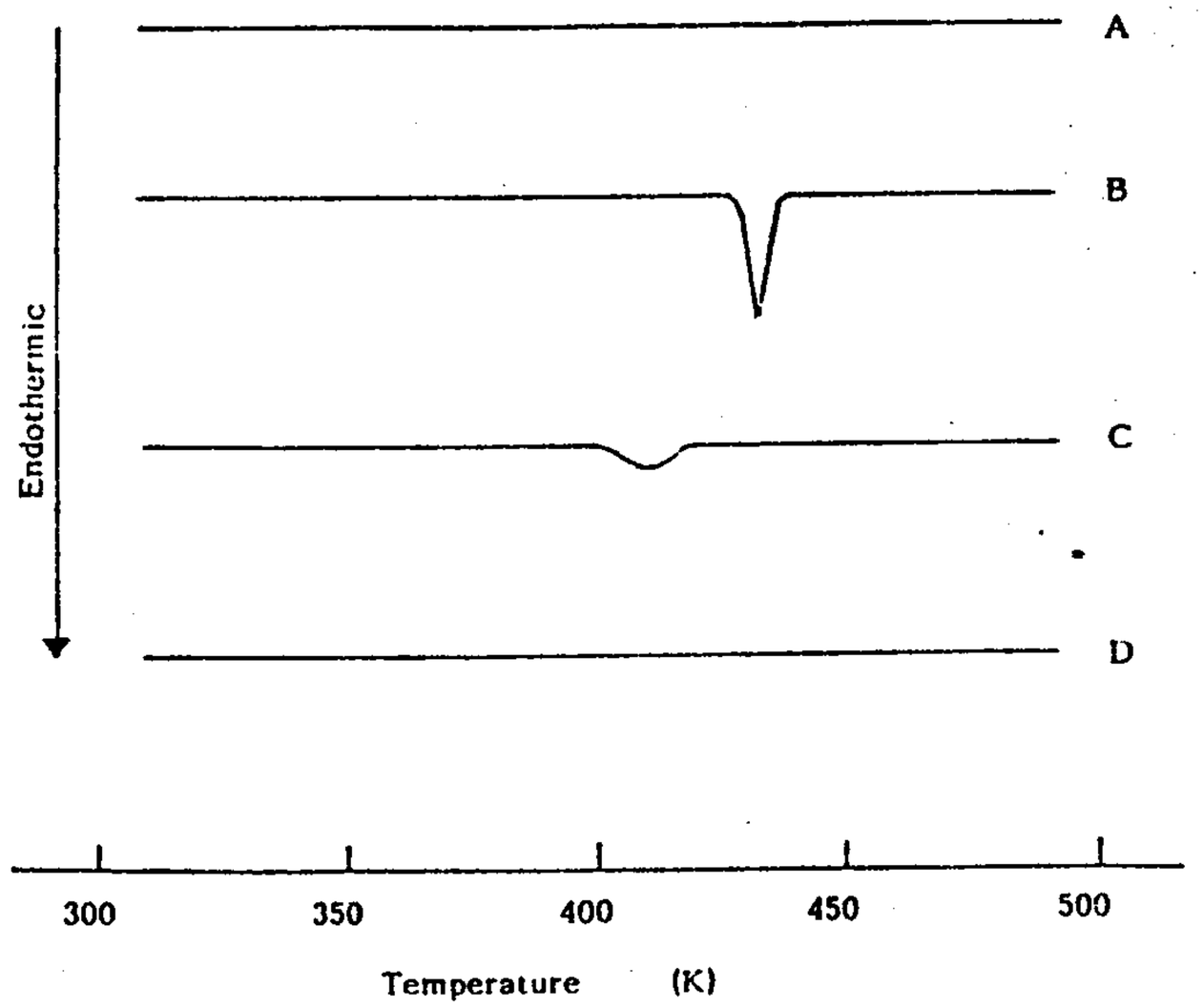

Fig. 3: DSC Curves of Amobarbital-DMCD Systems: (A) DMCD (B) Amobarbital (C) Physical Mixture (D) Inclusion complex.

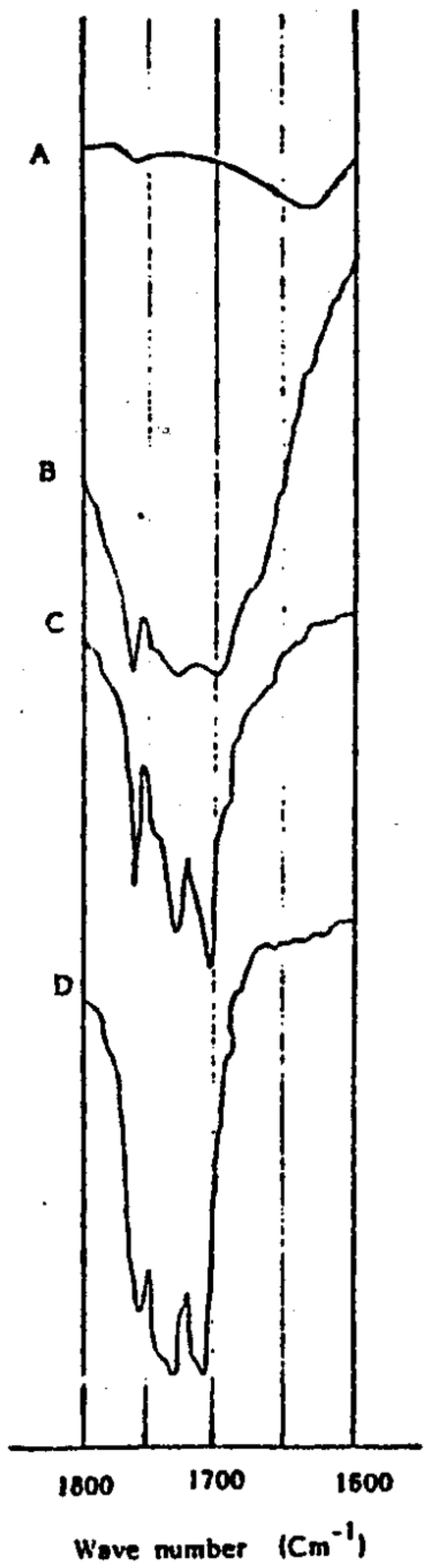

Fig. 4: IR Spectra of Amobarbital-DMCD Systems: (A) DMCD (B) Amobarbital (C) Physical Mixture (D) Inclusion Complex.

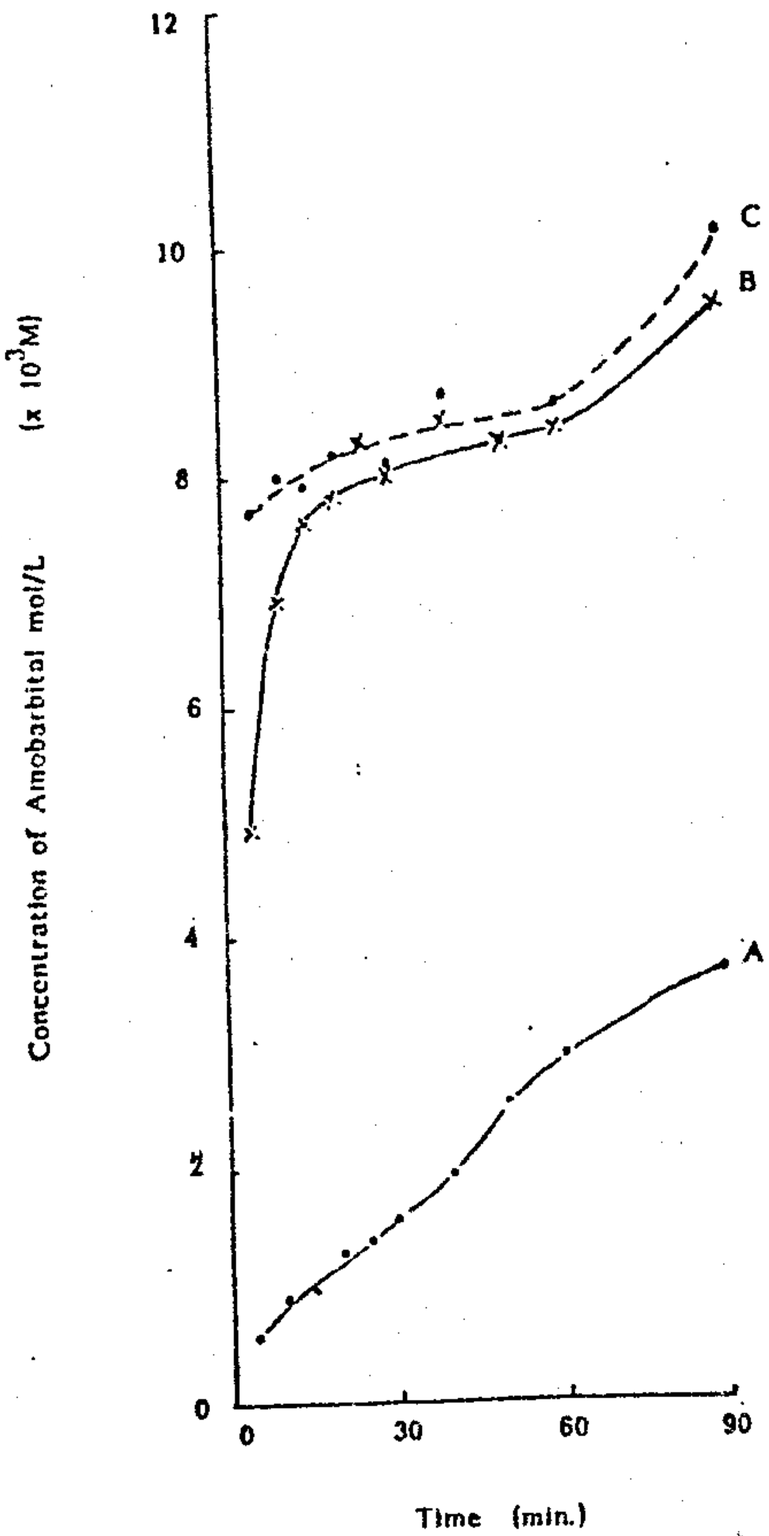

Fig. 6: Dissolution Profiles of Amobarbital from Amobarbital-DMCD Systems at 37°C: (A) Amobarbital (B) Physical Mixture (C) Inclusion complex.

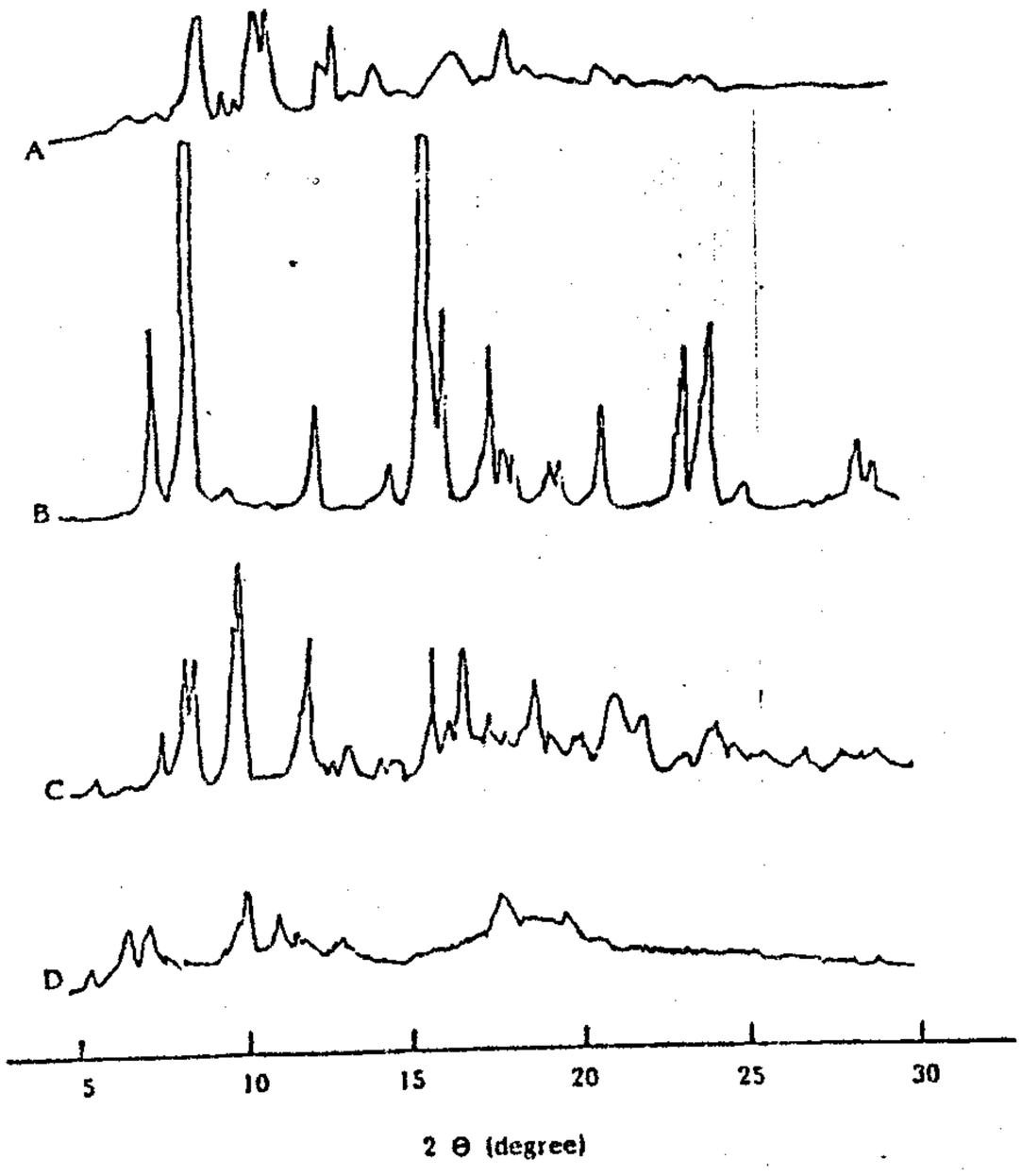

Fig. 5: Powder X-ray Diffraction Patterns of Amobarbital-DMCD systems: (A) DMCD (B) Amobarbital (C) Physical Mixture (D) Inclusion Complex.

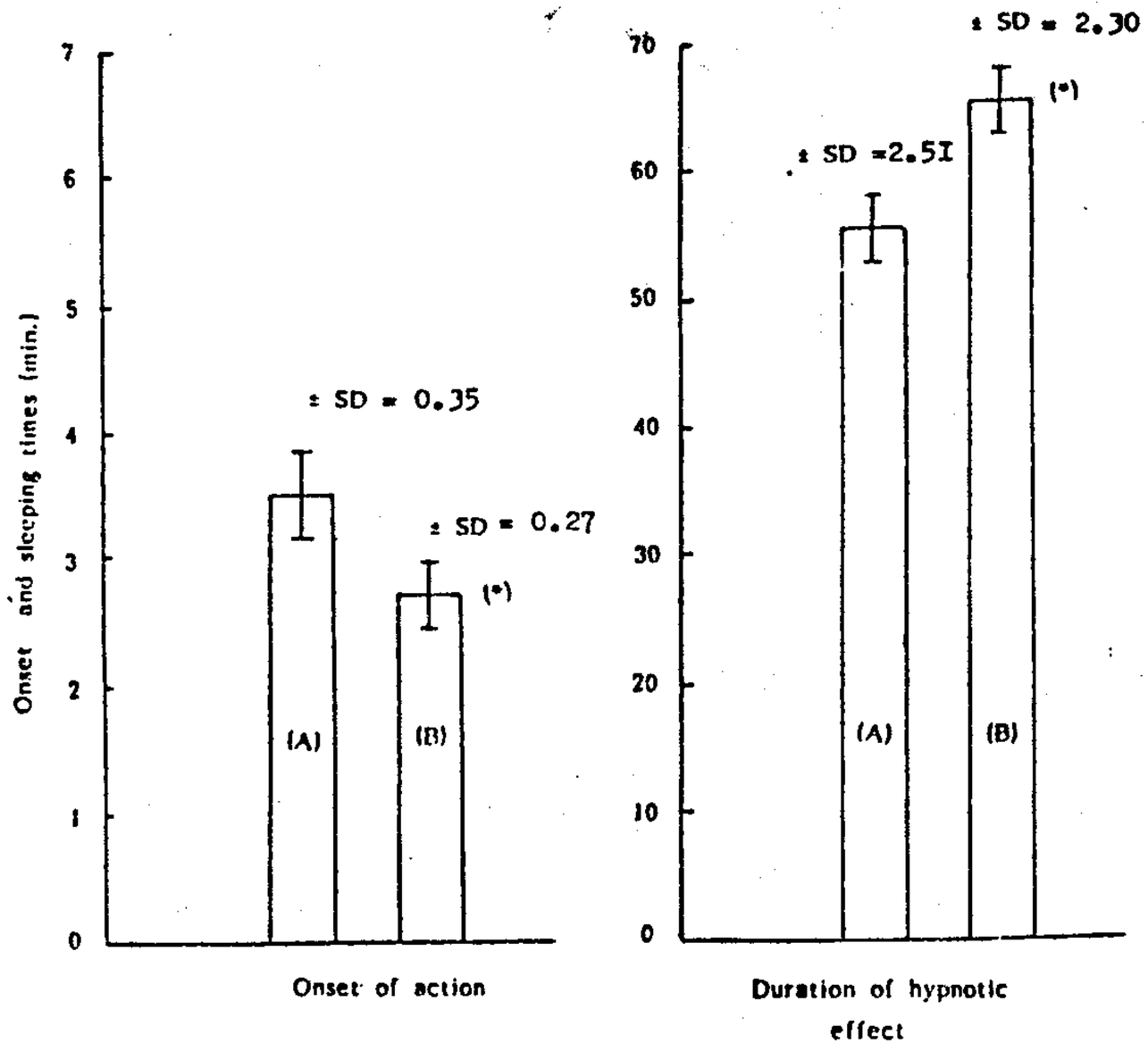

Fig. 7: Onset and Duration of Hypnotic Effect of Amobarital (A) and Inclusion Complex (B) Given Orally to Mice in a Dose of 80 mg/kg
- Values represent the mean of 5 experiments ± SD
- (*) Significant at P<0.05

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دراسة المتراكب التداخلى لعقار الاموباربيتال مع هيبتاكز (٦ر٢ - داى - اورثو - داى ميثيل بيتا - سيكلوديكسترين

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تمت دراسة المتراكب التداخلى لعقار الاموباربيتال الذى يستخدم كمسكن ومنوم مع احد مشتقات بيتاسيكلوديكسترين "هيبتاكز (٦ر٢ - داى - اورثو - داى ميثيل) بيتا - سيكلوديكسترين" فى المحلول المائى وفى الحالة الصلبة وقد تم دراسة تكون المتراكب التداخلى والتعرف على نوعه بواسطة الطرق المختلفة الاتية : طريقة الذوبان والتحليل الطيفى بواسطة الاشعة الفوق بنفسجية والاشعة تحت الحمراء والتحلل الحرارى والانكسار بواسطة اشعة اكس . وقد ثبت ان النسبة الجزيئية للمتراكب ، الذى تم تحضيره بطريقة التراسب ، تساوى ١ : ١ ، وتم حساب ثابت تكوين المتراكب وقد وجد انه يساوى ٩٥٠ مول $^{-1}$.

وبدراسة معدل الذوبان والانطلاق الحزيئى فى المحلول المائى من المتراكب التداخلى ومن العقار (الاموباربيتال) وجد انه زاد بدرجة كبيرة وملموسة بالمقارنة بالعقار . وتمت دراسة التأثير المنوم للعقار والمتراكب التداخلى والتى اوضحت سرعة ظهور التأثير المنوم للمتراكب التداخلى وامتداد مفعوله على الفئران المختبرة بدرجة اكبر مقارنة بالعقار .

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