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Improvement of Solubility and Dissolution of 19-Norprogesterone via Inclusion Complexation

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Abstract. In an effort to modify the solubility and dissolution rate of the contraceptive steroid, 19-norprogesterone in order to improve its bioavailability, the cyclodextrin complexation approach was chosen. In solution, the complex formation with β-cyclodextrin (β-CD), hydroxyethyl β-cyclodextrin (HE-β-CD) and hydroxypropyl β-cyclodextrin (HP-β-CD) was confirmed by using solubility, UV, IR and ^{1}H-NMR spectrophotometric techniques. The phase solubility diagrams were categorized as A_L-type. The complexing affinity of the CDs investigated were ranked as follows: β-CD $>$ HP-β-CD $>$ HE-β-CD. The complexation thermodynamic parameters were obtained from the temperature dependence of the dissociation constants. In the solid state, differential scanning calorimetery (DSC) and optical microscopy methods were utilized to characterize the complexes. Dissolution studies showed that such molecularly encapsulated forms offered a marked improvement in the dissolution rate compared to the parent drug.

Key words: Contraceptive steroid, inclusion complexation, β-cyclodextrin, hydroxyethyl β-cyclodextrin, hydroxypropyl β-cyclodextrin, solubility, dissolution.

1. Introduction

The progestin steroid, 19-norprogesterone (16-methylene-17α-acetoxy-19-norpregn-4-ene-3,20-dione, **1**), is a novel synthetic contraceptive drug, hereafter referred to as ST1435. It has no hormonal actions and shows unique properties which make it the most suitable steroid for contraception, even in lactating women, and for the management of postmenopausal disorders [1]. This necessitates its formulation into various dosage forms, such as subdermal implants, vaginal rings, transdermals, nasal and buccal preparations and injections etc. This requires the industrial formulator to modify its solubility, dissolution, stability, permeation, and bioavailability. There has been increasing interest in using cyclodextins (CDs) to confer desirable pharmaceutical properties and improve the bioavailability of drugs intended for use as long- or short-term therapeutic agents. Unfortunately, the parent CDs, especially β-CD, have low aqueous solubility, which restricts their range of application. To solve this problem, chemical modification, including hydroxyalkylation of the hydroxyl group of β-CD, has been used. Hydroxyalkylated CD derivatives are superior in their bioadaptability i.e. low toxicity, low hemolytic action and irritancy compared to the parent CD [2, 3]. The interactions of CDs

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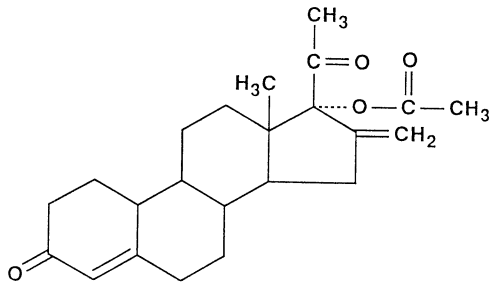

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Formula **1**.

and steroids have been investigated extensively [4–7]. In human trials, Pitha *et al*. [8] found that CD derivatives enabled effective administration of testosterone, estradiol, and progesterone, whereas the improvement obtainable with β-CD was marginal. Loftsson and Bodor [9] reported that HP-β-CD enhanced the solubility and transdermal permeability of 17-β-estradiol. Uekama *et al*. [10] utilized CD: cellulose derivatives combination as a modified release drug carrier for piretanide slow release dosage form. In a previous article [11], HP-β-CD and HE-β-CD showed appreciable stabilizing action towards ST1435 in solution.

The present investigation is concerned with improving the solubility and dissolution rate of ST1435 in aqueous solution in order to modify its bioavailability. This was achieved through the formation of inclusion complexes with HP-β-CD and HE-β-CD. Furthermore, β-CD, the parent host, was utilized for the purpose of comparison. The formation of such complexes was confirmed by a variety of techniques such as solubility, UV spectrophotometery, IR spectroscopy, NMR spectroscopy, differential scanning calorimetery (DSC), and optical microscopy. The work also included the determination of the dissolution profile of the uncomplexed drug compared with its cyclodextrin complexes.

2. Experimental

2.1. Materials

ST1435 is a product of E. Merck Darmstadt, Germany. βCD, HP-β-CD [molar substitution (M.S.): 0.60, moisture content: 2%] and HE-β-CD (M.S.: 0.95, moisture content: 5%) were kindly provided by Pharmatec Inc., Alachua, Florida, USA. Chemicals of analytical grade were purchased from various commercial sources. Deionized double distilled water was used throughout this work.

2.2. METHODS

2.2.1. *Determination of ST1435 solubility*

Accurately weighed amounts of ST1435 powder (20 mg) were added to 10 mL of aqueous solutions of CD (2–16 mM βCD, 2–40 mM HP-β-CD or HE-β-CD). The solutions were shaken at a rate of 100 strokes/min in a thermostatically controlled water bath (Gesellschaft fur Labortechnik m.b.H., W. Germany) at three temperatures, viz. 30°, 37°, and 45 $\pm$ 0.5 °C. The solubility experiments were performed according to Higuchi and Conners [12]. After equilibrium was attained (5 days), aliquots were filtered, appropriately diluted with water and assayed spectrophotometrically for their drug content (247 nm, Jasco, Uvedic 320 spectrophotometer, Japan). The spectrophotometric assays were based on the standard curve of the drug (y = 0.05 x + 0.0009, r = 0.9999, n = 5, using linear regression analysis). The solubility of ST1435 in the absence of CDs was determined under the same conditions.

Standard solutions were assayed and calibration curves were constructed before analysis of each sample set to ensure reproducibility of the analysis. Cyclodextrins did not show interference with the assay of the drug in the concentration range used [11]. Each experiment was repeated at least three times.

2.2.2. *Spectrophotometric studies*

The spectrophotometric absorbance (Masco, Uvedic 320 spectrophotometer, Japan) of solutions containing 4×10^{-2} mM of ST1435 and various concentrations of HP-β-CD (0.4–8 mM) dissolved in phosphate buffer (pH 7.4, I = 0.01) were recorded using blanks containing the same concentration of the host.

2.2.3. *Infrared spectrophotometric studies (IR)*

This was performed with a Shimadzu-470 Infrared Spectrophotometer (Shimadzu, Japan). Samples (1 mg) in finely ground KBr (100 mg, Fisher IR grade) were compressed as disks which were scanned over the range 4000–1000 cm^{-1}.

2.2.4. *Nuclear magnetic resonance studies (^{1}H-NMR)*

^{1}H-NMR spectra were recorded and analyzed using a Varian AG 2599 NMR (60 MHz, high resolution) spectrometer, CA, USA. Samples were dissolved in deuterated dimethyl sulphoxide (DMSO-d_6); tetramethyl silane was utilized as standard.

2.2.5. *Preparation of the solid complex*

Three methods were employed for the preparation of the solid complex.

A. *Coevaporation method.* ST1435 and HP-β-CD or HE-β-CD mixture (1 : 1 M) were dissolved in a minimum amount of ethanol and kept in a vacuum oven at 40 °C for 48 h to evaporate the solvent. The residue was dried for another 48 h in a vacuum desiccator to constant weight.

B. *Lyophilization method.* An excess amount of ST1435 was suspended in an aqueous solution of HP-β-CD (0.127 M) and sonicated for 2 h. The suspension was filtered and the filtrate was freeze dried.

C. *Kneading method*. A mixture of ST1435 and HP-β-CD (1 : 1 M) was damped with aqueous ethanol (50%) and vigorously triturated in a glass mortar for 30 min. The paste was dried in a vacuum desiccator to constant weight.

A physical mixture of the drug and HP-β-CD (1 : 1 M) was prepared by simple mixing of the constituents.

The prepared samples were sieved (125–75 μm) and their drug content was confirmed by the UV spectrophotometric assay method detailed previously.

2.2.6. *Differential scanning calorimetric studies (DSC)*

DSC scanning (DSC-50 Shimadzu, Japan) was performed under the following conditions: sample weight 3–5 mg, scanning rate 10 °C/min, N_2 purge (30 mL/min). The instrument was calibrated for temperature and energy with pure indium (99.999%, melting point 156.6 °C, transition energy 28.45 J/g).

2.2.7. *Scanning electron microscopy (SEM)*

This was performed with a JSM-T200 scanning microscope (Jeol, Tokyo. Japan). Samples of ST1435 and its complex with HP-β-CD were mounted onto stubs using double sided adhesive tape and vacuum coated with a gold film of 30 mm thickness.

2.2.8. *Dissolution studies*

Dissolution of ST1435 and its prepared samples with HP-β-CD or HE-β-CD were studied using the USP XXIII paddle method (Erweka, DT-D6, West Germany). Samples (125–75 μm particle size range) equivalent to 10 mg of ST1435 were sprinkled over the surface of the dissolution medium (900 mL, distilled water containing 5% v/v ethanol). The dissolution medium was stirred at a rate of 100 rpm and maintained at a temperature of 37 °C. Aliquots of 5 mL each were withdrawn at intervals and replaced by an equal volume of the dissolution medium kept at 37 °C. The amounts of the drug dissolved were determined spectrophotometrically at 247 nm using the dissolution medium as a blank.

Table I. Mean apparent stability constants ($K_{1:1}$ M^{-1}) and solubility enhancement ratios* for the complexation of ST1435 with cyclodextrins (CDs) at different temperatures in water.

CD	$K_{1:1}$			Solubility enhancement ratios		
	30 °C	37 °C	45 °C	30 °C	37 °C	45 °C
β-CD	4387 ± 278	2958 ± 230	1561 ± 90	40	33	24
HP-β-CD	2817 ± 274	2281 ± 152	1132 ± 36	30	24	19
HE-β-CD	2131 ± 216	1893 ± 173	988 ± 48	18	13	11

* *Solubility Enhancement Ratio* is the ratio between the amount of the steroid dissolved in 0.01 M cyclodextrin to that in water at the same temperature.

3. Results and Discussion

3.1. SOLUTION STATE STUDIES

The solubility of ST1435 in the absence of CDs was determined and found to be 3.84 ± 0.144 × 10^{-5} M, 5.49 ± 0.130 × 10^{-5} M and 7.17 ± 0.227 × 10^{-5} M (Mean ± SD, n = 5) at 30°, 37°, and 45 °C, respectively. Figure 1 shows the equilibrium phase solubility diagrams for ST1435 progestin with β-CD, HP-β-CD, and HE- β-CD in water at three temperatures. The three cyclodextrins effectively increased the drug solubility. This increase is linearly proportional ($r > 0.99$) to the concentration of the solubilizer, indicating the formation of soluble inclusion complexes of A_L type. The apparent stability constant values, $K_{1:1}$, are listed in Table I. These values were calculated from the straight lines of the phase solubility diagrams [12]. β-CD showed a higher complexing affinity, as demonstrated by the higher $K_{1:1}$ and solubility enhancement parameters (Table I) than HP-β-CD, followed by HE-β-CD. Similarly, Yamamoto *et al.* [13] stated that β-CD exhibited higher complexing affinity than its derivatives. This decrease in the complexing ability of the cyclodextrin derivatives could be attributed to the steric hindrance caused by the introduction of hydroxyalkyl groups [13]. Although the steric hindrance of the hydroxypropyl moiety, of HP-β-CD, is stronger than that of the hydroxyethyl moiety, of HE-β-CD, the former exhibited higher complexing affinity than the latter. In this respect, the role of the molar substitution (M.S.) level should be considered. Consequently, the higher complexing affinity exhibited by HP-β-CD towards ST1435 might be explained on the basis of its lower M.S. value compared to that of HE-β-CD (M.S. = 0.60 and 0.95 for HP-β-CD and HE-β-CD, respectively). Pitha *et al.* [8], Yoshida *et al.* [14], and Loftsson and Johannson [15] found that the complexing ability of cyclodextrin derivatives towards various guest molecules decreased with increasing M.S. In a recent article [11] dealing with the stability of ST1435 in the presence of hydroxyalkylated derivatives, it was found that HP-β-CD displayed better stabilizing action than HE-β-CD.

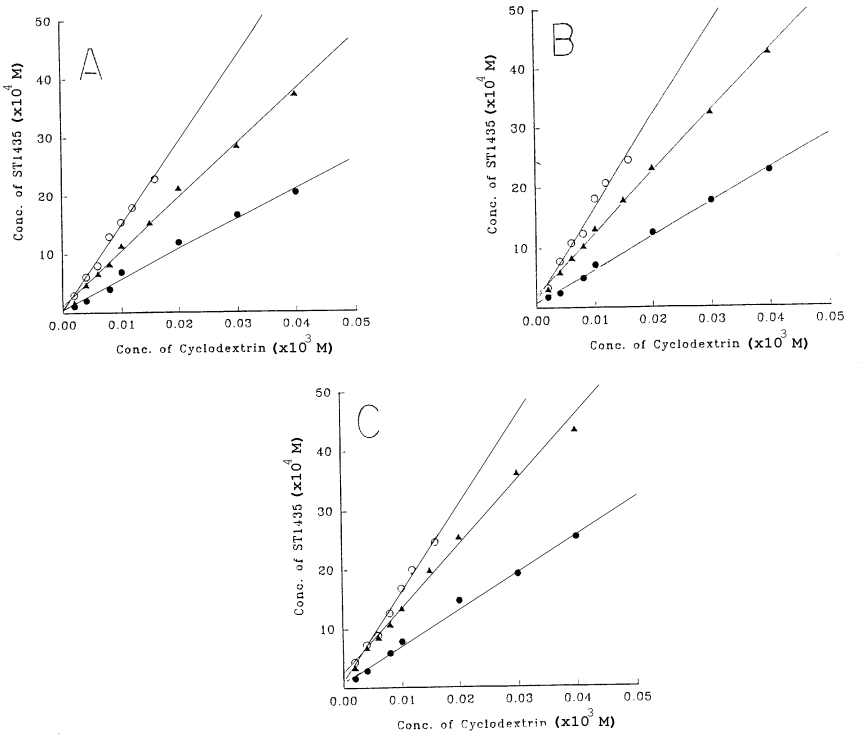

Figure 1. Phase diagrams of ST1435-cyclodextrin systems in water at 30 °C (A), 37 °C (B), and 45 °C (C), ○, β-CD; ▲, HP-β-CD; ●, HE-β-CD.

The effect of temperature on the solubilizing capacity of the three cyclodextrins were studied and the thermodynamic parameters of such interactions were computed. Although the apparent solubility of ST1435 increased upon increasing the temperature from 30° to 45 °C, the stability constant values ($K_{1:1}$) decreased (Table I). This was not unexpected since cyclodextrin complexes usually dissociate upon increasing the temperature of the solution [16]. This may also be attributed to the negative enthalpy change occurring as a result of inclusion complexation [17]. Undoubtedly, the increase in the apparent solubility of ST1435 arises from increasing its intrinsic solubility with increasing temperature $\{3.84 \pm 0.144 \times 10^{-5}$ M, $5.49 \pm 0.130 \times 10^{-5}$ M and $7.17 \pm 0.227 \times 10^{-5}$ M (Mean ± SD, n = 5) at 30°, 37°, and 45 °C, respectively$\}$. As an increase in temperature results in an increase in the solubility of the different CDs, it was expected that they include more drug even if it is of lower stability constant. Interestingly, Van't Hoff plots of ln $K_{1:1}$ versus $1/T$ were constructed as shown in Figure 2. Such treatment provided further illustration of the effect of temperature on the stability constant of the steroid-CD complexes. The linearity of the three Van't Hoff profiles (r = 0.9978 ± 0.0011) proved that the apparent enthalpy change, ΔH°, and entropy change, ΔS°, are

Table II. Apparent thermodynamic parameters for the interaction of ST1435 with cyclodextrins.

CD	ΔH° k J mol^{-1}	ΔS° k J mol^{-1} K^{-1}	ΔG° k J mol^{-1}
β-CD	−40	−62	−21
HP-β-CD	−24	−13	−20
HE-β-CD	−25	−22	−18

Where ΔH°, ΔS°, ΔG° represent the apparent enthalpy, entropy and free energy changes, respectively.

constant over the temperature range investigated. ΔG°, the apparent standard free energy change, was also calculated. It is obvious that the reaction between ST1435 and cyclodextrin is exothermic as negative enthalpies were obtained in all cases (Table II). It was reported that most reactions involving host–guest interactions are exothermic [15]. A contribution to the enthalpy term may be the release of water molecules from the CD cavity into the solution. This also suggests the possible involvement of dipole and/or hydrogen bonding in the complex formation. On the other hand, it was noticed that the inclusion reaction is unfavourable with respect to ΔS° (Table II). An unfavourable entropy change can result from the ordering effect of solvent reorganization as well as the reduced degrees of freedom of ST1435 when it is confined in and around the CD cavity. The favourable enthalpy change and unfavourable entropy change resulted in a negative free energy change, ΔG°. Extrapolation of the Van't Hoff plot (Figure 2) gives the value of the complex stability constant at other temperatures: e.g. the HP-β-CD plot gave the stability constant of its complex with ST1435 at 65 °C: $K_{1:1}$ = 937.2 M^{-1}. Interestingly, this value is in good agreement with that calculated ($K_{1:1}$ = 935.4 M^{-1} at 65 °C) in the previous article using the Lineweave–Burk relation [11]. The linearity of this relationship provides further supporting evidence for the formation of a 1 : 1 type inclusion complex between the drug and HP-β-CD.

Further supporting evidence for the complex formation was assessed from the decrease in the intensity of maximum absorbance (A) of the steroid at 247 nm upon increasing the concentration of HP-β-CD. On plotting $1/\Delta A$ vs. 1/[HP-β-CD] according to the Benesi and Hildebrand relation [18], a linear relationship ($y = 0.0287x + 7.636$, r = 0.9999) was obtained, which further confirmed the formation of a 1 : 1 type inclusion complex. Such spectral changes might be due to perturbation of the electronic energy levels of the guest molecule, caused by direct interaction with CD or by exclusion of the solvating water molecules or by a combination of these two effects. Unfortunately, reliable values of $K_{1:1}$ could not be obtained with this method since the absorbance differences were too small, the solubility of the drug is very low and the medium was different from that used in solubility studies.

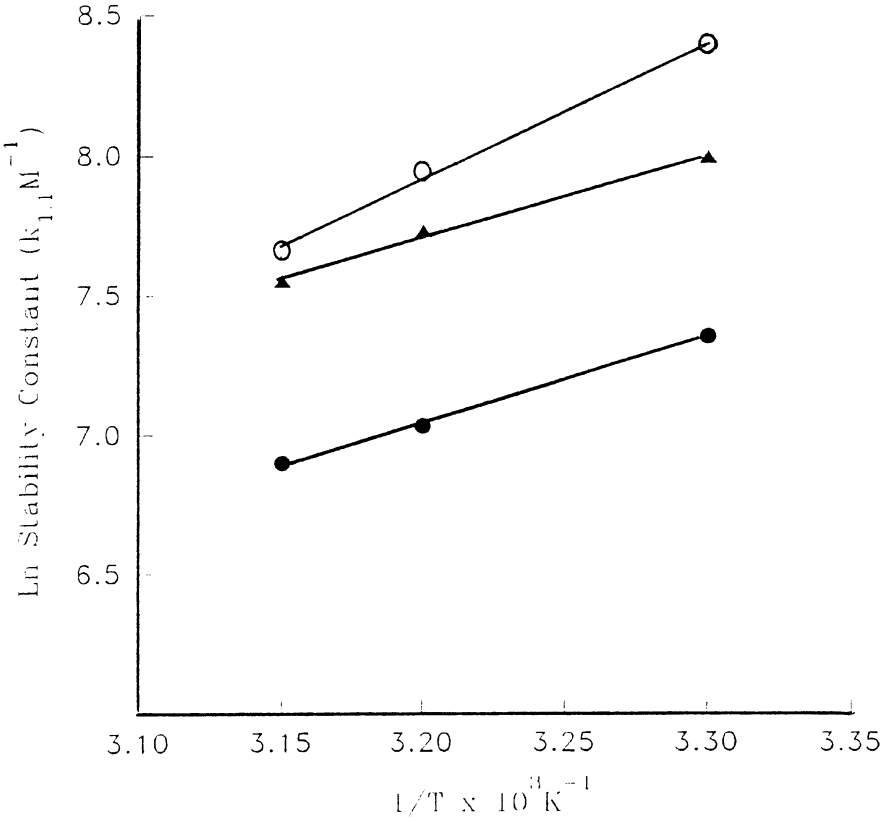

Figure 2. Temperature dependence of the equilibrium constant of ST1435-cyclodextrin complexes in solution (Van't Hoff Plot). ○, β-CD; ▲, HP-β-CD; ●, HE-β-CD.

Figure 3 shows the IR spectra of ST1435, HP-β-CD, their 1 : 1 M physical mixture and coevaporated product. ST1435 is characterized in particular by three peaks between 1600 and 1750 cm^{-1}, corresponding to its three carbonyl groups stretching vibrations (Figure 3A). For HP-β-CD, the spectrum (Figure 3B) shows only the vibrations of free OH between 3100 and 3700 cm^{-1} and those of bound OH at 2970 cm^{-1}. The spectrum of the physical mixture (Figure 3C) is a superposition of the spectra of the pure compounds. For the complex there are small displacements of the carbonyl stretching bands and changes in their shapes (Figure 3D). Such changes are not indicative of hydrogen bond formation as a result of complexation. This indicates that the carbonyl groups of the steroid lies some distance from the secondary hydroxyl groups of HP-β-CD, and thus the formation of hydrogen bonds between these groups is unlikely [19].

^{1}H-NMR spectra of HP-β-CD and the HP-β-CD/ST1435 inclusion complex in DMSO-d_6 are shown in Figure 4. The HP-β-CD spectrum (Figure 4A) reveals a large peak at approximately 4.3 ppm due to the presence of small amounts of

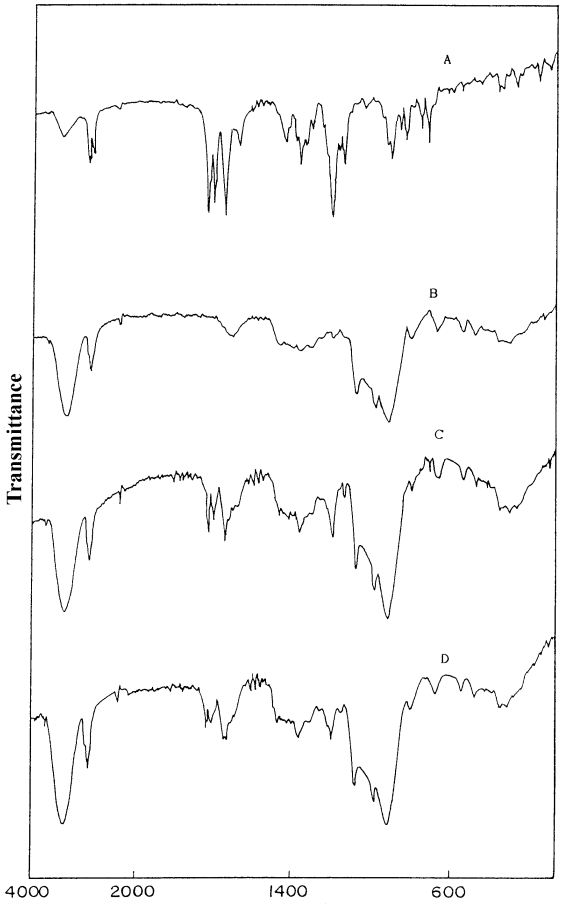

Figure 3. IR spectra of pure ST1435 (A), HP-β-CD (B), 1 : 1 M physical mixture (C) and solid inclusion complex (D).

water. The C$\underline{H}_3$ protons appear as a doublet at 1.12 ppm, while the C$\underline{H}_2$ protons at 3.75 ppm as a multiplet. In the presence of ST1435, the two groups of protons experienced shifts as a result of complexation. In the spectrum of the complex (Figure 4B) the C$\underline{H}_3$ doublet was shifted to 1.14 ppm while the C$\underline{H}_2$ multiplet was shifted to 3.55 ppm. The peak at 0.77 ppm is related to the C$\underline{H}_3$ peak of ST1435, while the others peak at 2.6, 2.2, 3.2 ppm are due to DMSO-d_6. It appears that most of the protons are experiencing shifts, suggesting that a portion of the steroid in the inclusion compound is proximate to HP-β-CD protons.

3.2. SOLID STATE STUDIES

DSC was utilized for characterizing the progestin steroid and its complexes in the solid state and for obtaining further supporting evidence about complex formation.

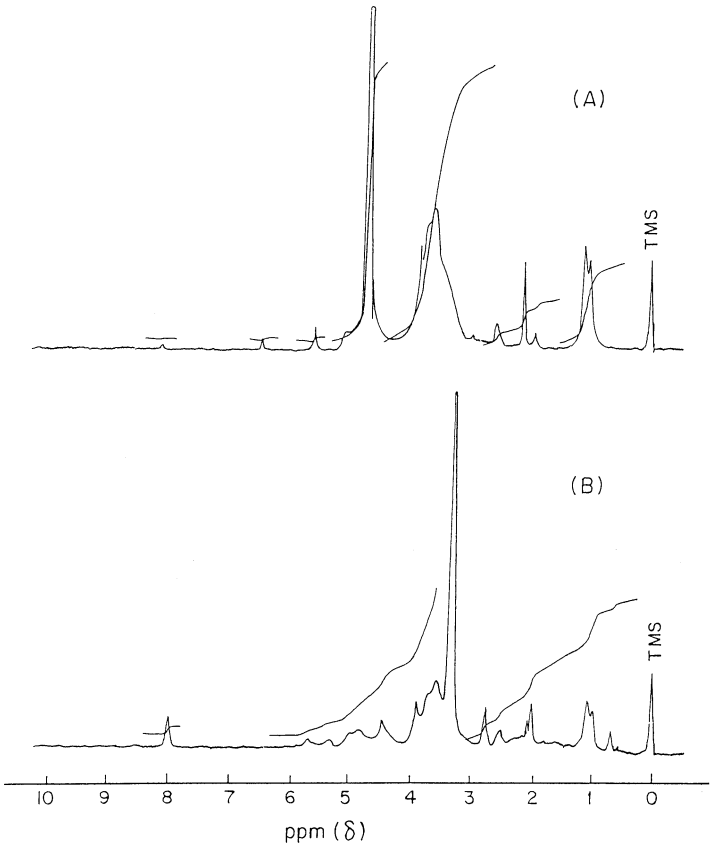

Figure 4. ^{1}H-NMR spectra of HP-β-CD (A) and the ST1435/HP-β-CD inclusion complex (B) in dimethylsulphoxide.

The DSC curve of ST1435 shows an endothermic peak at 178.5 °C corresponding to its fusion (Figure 5, curve I). The DSC curve of HP-β-CD shows a shallow and broad endothermic peak which was attributed to the release of water molecules entrapped inside the cavity (curve II). On the other hand, melting of the physical mixture (1 : 1 M) gave rise to two endothermic peaks characteristic of the pure components (curve III). The DSC thermogram of the steroid/HP-β-CD systems shows that the drug melting endotherm either completely disappeared in the case of lyophilized products (Figure 5, curve IV) or appeared at a lower temperature, 175 °C, with a substantial reduction in peak area in the case of kneaded or coevaporated products (curves V and VI, respectively). This implies that the molecular arrangement of the steroid in the solid complex is different from that in their own crystal, indeed, this can at least indicate the reduction of the drug crystallinity or probably that it is partially dispersed at a molecular level in the solid products. Furthermore, in both cases it was noticed that the endothermic peak attributed to the host molecule was

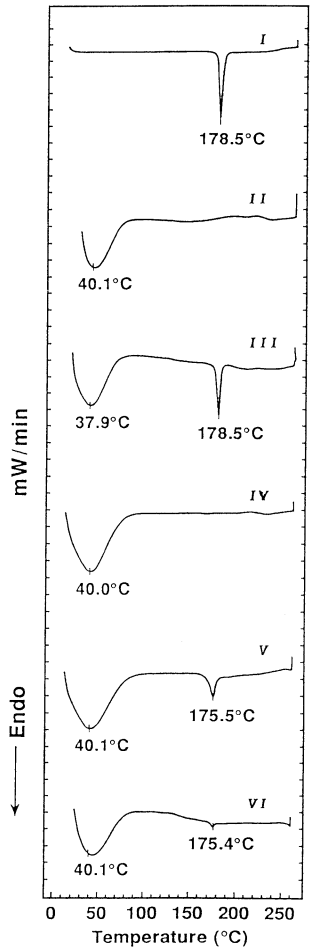

Figure 5. DSC thermograms of ST1435/HP-β-CD systems. ST1435(I), HP-β-CD (II), ST1435: HP-β-CD physical mixture (III), ST1435/HP-β-CD complex {lyophilized product} (IV), ST1435/HP-β-CD complex {kneaded product} (V), ST1435/HP-β-CD complex {coevaporated product} (VI).

slightly distorted. This might be due to exchange of water molecules, entrapped inside the CD cavity, by the drug molecules as a result of complex formation.

Figure 6 shows the scanning electron photomicrographs of ST1435 as received and that coevaporated with HP-β-CD. The parent drug is composed of agglomerated crystals with irregular shape (Figure 6A) while the coevaporated product crystals are transparent glassy crystals with a smooth surface (Figure 6B). The transformation of the drug crystals into the glassy state might be one of the reasons that led to the observed enhancement in dissolution.

Figure 7 shows the dissolution profiles of ST1435, the ST1435 : HP-β-CD physical mixture and its complexes with HP-β-CD and HE-β-CD. The physical mixture

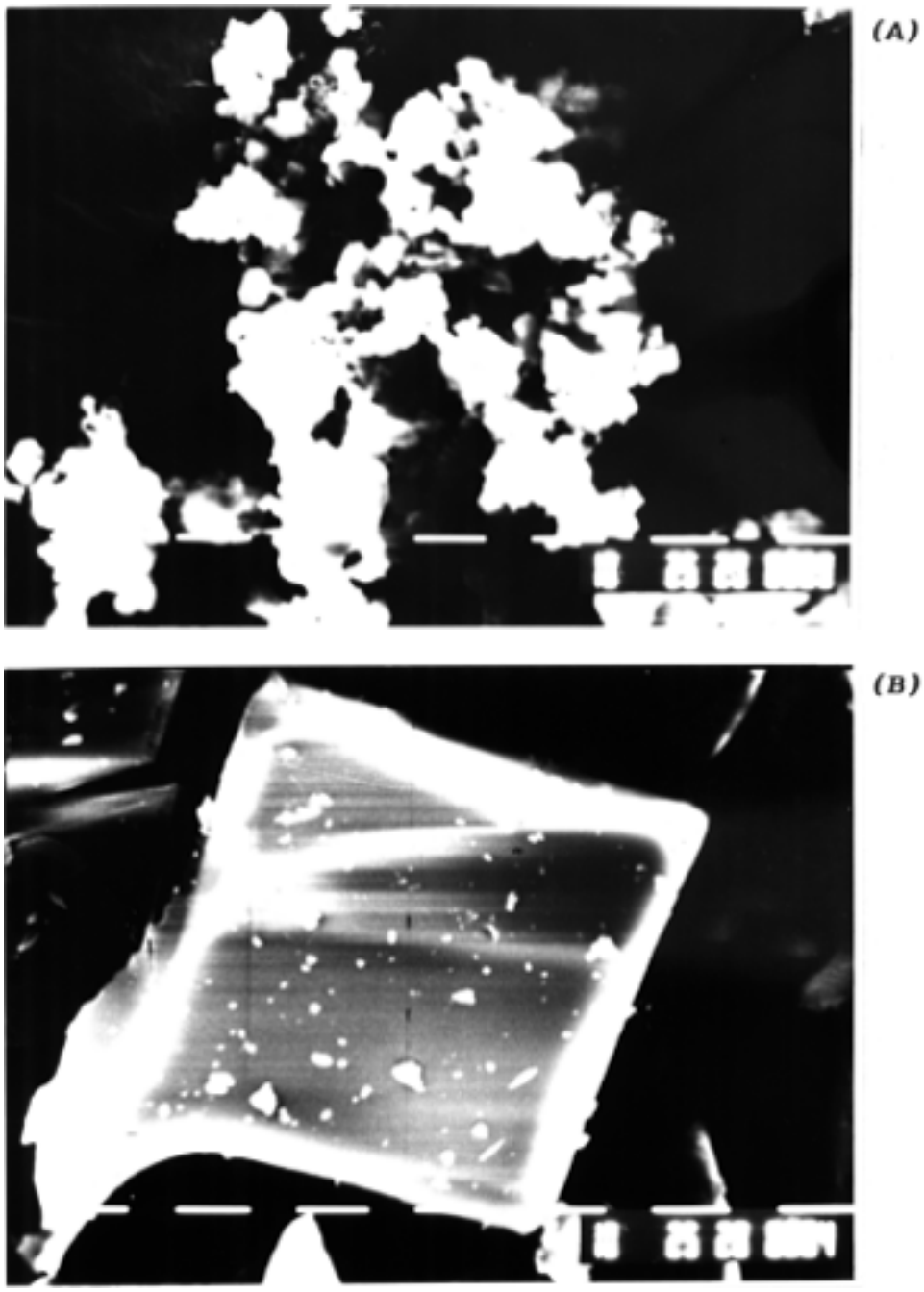

Figure 6. Scanning electron micrographs of ST1435 (A) and its complex with HP-β-CD (B).

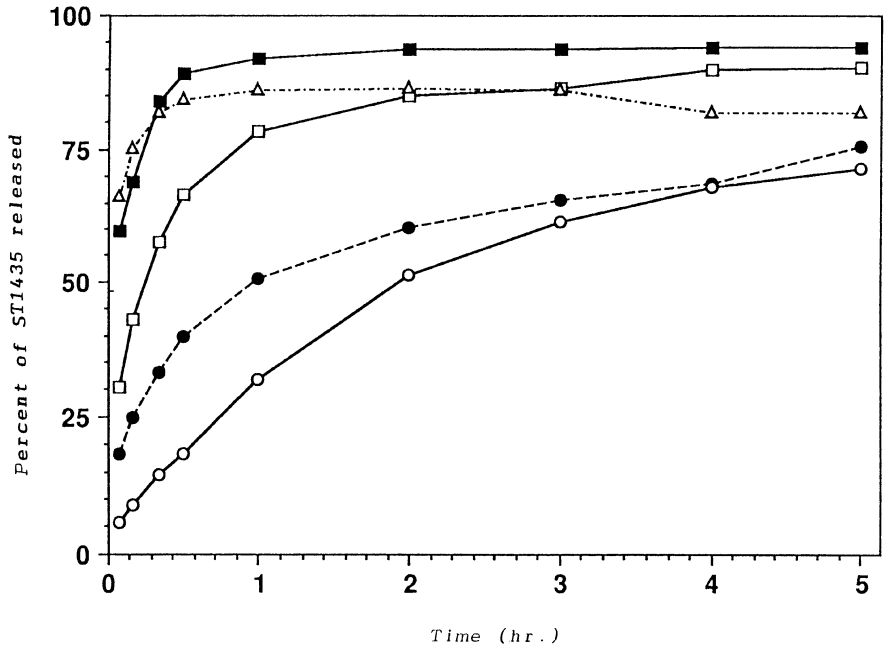

Figure 7. Dissolution profiles of ST1435-cyclodextrin systems. ○, ST1435; ●, ST1435 : HP-β-CD {physical mixture}; ■, ST1435/HP-β-CD complex {coevaporated product}; Δ, ST1435/HP-β-CD {kneaded product}; □, ST1435/HE-β-CD {coevaporated product}.

of ST1435 : HP-β-CD showed a slight improvement in dissolution over the free drug but had a much slower initial rate and lower apparent solubility as compared to its respective complex. This might be due to improvement of the wettability and solubility of the drug resulting from the coexistence of the CD in the dissolution medium. However the dissolution rate of the complexed drug was significantly enhanced compared to the free drug as demonstrated by the drop in $T_{50\%}$ and the rise in the R.D.R. values listed in Table III. It was also noticed that the positive enhancing effect of HP-β-CD is higher than that of HE-β-CD. This may be mainly attributed to its higher solubilizing power and lower M.S. Additionally, it was expected that the method of preparation of the complex might have an effect on its dissolution rate. The higher dissolution of the complex prepared by the coevaporation method compared to that prepared by the kneading method proved this expectation. Generally, the increase in dissolution rate of the steroid progestin may be attributed to partial or total entrapment of the drug molecule inside the CD torus which imparts it hydrophilic character and hence, increase its solubility and wettability. Additionally, it seemed that reduction in the drug crystallinity, proved by DSC, as well as changing of the drug from crystalline agglomerates to glassy state, as seen by optical microscope, are other factors that led to dissolution enhancement.

In a previous article [1], it was stated that ST1435 administered orally is biologically ineffective even in large doses. This was attributed either to poor absorption

Table III. Half lives ($T_{50\%}$) and relative dissolution rates (R.D.R.) for the release of ST1435 and its cyclodextrin complexes in water containing 5% v/v ethanol at 37 °C.

Test preparations	$T_{50\%}$ (min)	R.D.R. 5 min	30 min	120 min
ST1435 alone	120	1.0	1.0	1.0
ST1435/HE-β-CD complex*	15	5.1	3.5	1.7
ST1435/HP-β-CD complex*	3	9.9	4.7	1.8
ST1435/HP-β-CD complex**	3	11.0	4.4	1.7

Half life ($T_{50\%}$) is the time in minutes needed for 50% of the steroid to be dissolved.
Relative dissolution rate (R.D.R.) represents the ratio between the amount of the drug dissolved from the complex to that of the drug alone at the same time interval.
* Denotes that the complex was prepared by the coevaporation method.
** Denotes that the complex was prepared by the kneading method.

of the drug or to its metabolism in the intestinal tissue or the liver. This indicated that ST1435 has a bad bioavailability problem that needs to be solved. In the present investigation, ST1435 was found, by a variety of techniques, to form inclusion complexes with cyclodextrin derivatives. This led to appreciable improvement in its solubility and dissolution rate compared with the pure drug which has a very poor aqueous solubility. These results are of great importance to improve the bioavailability of the guest molecule and help formulating it in suitable dosage forms. The impacts of these effects on the bioavailability of ST1435 from different dosage forms, are of great potential for future application.

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