Bull. Pharm. Sci., Assiut University, Vol. 20, Part 2, December 1997, pp. 169-179.

IN-VITRO TRANSFER, PERMEABILITY AND INCLUSION COMPLEXATION OF A NOVEL POTENT SYNTHETIC ANDROGEN, METHYL NORTESTOSTERONE ACETATE, COMPARED WITH TESTOSTERONE

Sayed M. Ahmed

Department of Industrial Pharmacy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

تضمن هذا البحث دراسة العقار الجديد ذو الفاعلية العالية المسمى ميثيل نور تيستوستيرون أسيتات بالمقارنة بالتيستوستيرون من ناحية الذوبان ومعدل الأتاحة وسرعة الأنتقال من الاوساط المائية إلى الاوساط الزيتيه.

وتم أيضاً دراسة هذه الصفات لكلا العقارين بعد تحضير متراكب معقد مع الهيدروكسي بروبيل بيتا سيكوديكسترين، وقد أظهرت النتائج أن كلا العقارين يكون متراكبا منغمساً بنسبة ١:١ (عقار: سيكلوديكسترين) عند ثلاث درجات حرارة وهى ٣٠،٣٧،٤٥° م. وقد وجد أن ذوبان الميثيل نور تيستوستيرون قد زاد حوالى ٧٠٠٠ مرة مقارنة بـ ٥٠٠ مرة زيادة فى ذوبان التيستوستيرون نتيجة عمل متراكب منغمس مع الهيدروكسى بروبيل بيتاسيكلوديكسترين. وقد أدى هذا إلى زيادة معدل إتاحة العقار الأول إلى درجة كبيرة بالمقارنة بالعقار الثانى. وقد أدى تكون المتراكب المنغمس لكلا العقارين إلى نقص ملحوظ فى معدل إنتقال العقار من الوسط المائى إلى الوسط الزيتي، وأخيراً وجد أن معدل نفاذ كلا العقارين المحضرين على شكل جيل قد زاد فى وجود الهيدروكسى بروبيل بيتا سيكلوديكسترين.

The solubility, dissolution, in-vitro transfer from aqueous to organic phase, and the in-vitro permeability of a potent nortestosterone ester, methyl nortestosterone acetate (NTA), compared with testosterone (T) in the absence and in the presence of hydroxypropyl-ß-cyclodextrin (HP-ß-CD) were investigated. The phase solubility diagrams of both drugs revealed the formation of 1:1 inclusion complexes with HP-ß-CD of A_L-type at 30°, 37° and 45°C. The solubility of NTA and T were increased approximately 7000 fold and 500 fold, respectively, in the presence of 0.12 M HP-ß-CD at 37°C. NTA interacted more strongly with HP-ß-CD than T, a fact that led to a much better enhancement in the dissolution rate. Such inclusion complexation led to noticeable decrease in the transfer rate of both androgens from the aqueous to the organic phase compared with the drug alone. Finally, it was found that HP-ß-CD enhanced the in-vitro permeability of NTA and T from Klucel gel formulations.

INTRODUCTION

Cyclodextrins (CDs) and their synthetic derivatives are able to form inclusion complexes with various drugs, thus improving their solubility, stability and bioavailability.[1] In addition, they can affect the diffusion and permeability characteristics of the drug molecule.[2,3] The potential uses of CD for the development of formulations of new drugs that are difficult to formulate and deliver are increasing, this is reflected in the increasing numbers of pharmaceutical products being available in the market as CD-based formulations.[4,5] The recent availability of the hydroxyalkylated CD derivatives, particularly hydroxypropyl-ß-cyclodextrin (HP-ß-CD), with low toxicity, low hemolytic activity and irritancy to skeletal muscles and mucous membranes,[5] has created potential interests in extended uses of CDs as novel drug carriers via a variety of routes.

In recent years, it was noticed that much efforts have been directed to the development of

Received in 30/9/1997 & Accepted in 9/12/1997

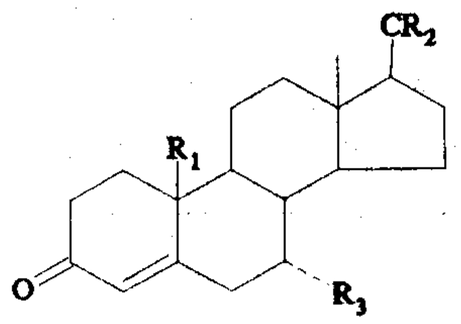
an ideal and safe androgen, to be used as a contraceptive agent for men and for the treatment of hormone deficiency diseases, that can be given by various routes of administration.[6] As a result, scientists created 7α-methyl-19-nortestosterone acetate (NTA) that was found to fulfill the above criteria. It is 4-5 times more potent than testosterone (T) and it has a favourable androgenic to anabolic ratio.[6] Consequently, scientists expected that NTA is going to be an attractive alternative to most of the traditional androgens in the nearest future.

The aim of the current study was to investigate the *in-vitro* interaction between NTA, compared with T, and HP-ß-CD. This interaction was investigated by phase-solubility analysis, ^{1}H-NMR and IR techniques. The dissolution profiles of both steroids were determined. Additionally, in order to mimic *in-vivo* transfer, an *in-vitro* two phase system was used to evaluate the transfer of NTA and T in the absence and presence of HP-ß-CD. Finally, determination and comparison of the *in-vitro* release characteristics of both steroids formulated as gels and the effect of HP-ß-CD on the permeability were among the objectives of the present investigation.

EXPERIMENTAL

Materials

Testosterone (T, **2**) and its synthetic analogue (7α-methyl-19-nortestosterone acetate, NTA, **1**) were obtained from Nakarai Chemicals (Kyoto, Japan). Their purity were checked by differential scanning calorimeter (DSC 50, Shimadzu, Japan). Hydroxypropyl-ß-cyclodextrin (HP-ß-CD) was obtained from Pharmatec Inc. Alachua (Florida, USA). Its molar substitution (M.S.) is 0.6 and moisture content: 2%. Hydroxypropyl-cellulose (Klucel; type 99-HXF NF) was donated by Aqualon (Wilmington, U.S.A.). All other materials were of analytical reagent grade. Deionized double distilled water was used during the study.



	R_1	R_2	R_3
1 Nortestosterone acetate (NTA)	H	$-\overset{O}{\overset{\|}{C}}-CH_3$	CH_3
2 Testosterone (T)	CH_3	H	H

Methods

Solubility studies

The solubility of NTA and T in water was determined by adding an excess amount of the drug (10 mg) to 10 mL of water contained in 20 mL screw capped scintillation vials. The vials were shaken (100 strokes/min) horizontally in a thermostatically controlled water bath (Gesellschaft fur Labortchnik mbH, Germany) at 30°, 37° and 45±0.5°C. For construction of the phase-solubility diagrams of both drugs in aqueous HP-ß-CD solution, excess amounts of each drug were added to 10 mL of water containing various concentrations of HP-ß-CD (0.008-0.2 M). The solutions contained in 20 mL screw capped vials were shaken at a rate of 100 strokes/min in the water bath kept at 30°, 37° and 45±0.5°C. After equilibrium for 3 days, aliquots were filtered through 0.45 μm membrane filters (Millex-HV, Millipore, MA, U.S.A.), diluted with water and analysed for their drug content using U.V. spectrophotometer (Uvedic 320, Japan) at 248 nm. The spectrophotometric assay adopted was based on standard calibration curves whose equations were as follows: $y = 0.0455x + 0.003$, $r = 0.9998$ for NTA and $y = 0.0554x + 0.00007$, $r = 0.9999$ for T. HP-ß-CD did not show any interference with the spectrophotometric assay of both drugs. Calibration curves were checked before analysis of each sample set to ensure reproducibility. Each experiment was repeated three times at least and data were averaged.

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

Nuclear magnetic resonance spectra were recorded and analysed 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.

Preparation of the solid complexes

A- Coevaporation method

Accurately weighed amount of each drug was mixed with HP-ß-CD in a molar ratio of 1:1. The mixture was dissolved in a minimum volume of ethanol. The solvent was driven off by keeping the solution in a vacuum oven for 4 days at room temperature. The solid obtained was dried for another 2 days in a desiccator untill constant weight.

B- Lyophilization method

Excess amount of the steroid was suspended in aqueous solution of HP-ß-CD (0.127 M) and sonicated for 2 hr. The filtrate was freeze dried. Physical mixture of each drug with HP-ß-CD (1:1 M) was prepared by intimate mixing of them in a glass bottle. The prepared samples were sieved (125-75 μm) and were assayed for their drug content using U.V. spectrophotometer.

Infrared studies (IR)

These were performed using a Shimadzu-470 infrared spectrophotometer (Shimadzu, Japan). Samples (1 mg) in finely ground potassium bromide (100 mg, Fisher IR grade) were compressed as disks which were scanned over the range 4000-600 cm^{-1}.

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). Thermal analysis data were obtained using TA 50I PC system with Shimadzu software programs. Those programs allow the highest levels of calorimetric accuracy and reproducibility of heat flux DSC (1% and 0.1%, respectively).

Dissolution studies

Dissolution studies were conducted for NTA, T, physical mixtures of each drug with HP-ß-CD and their inclusion complexes, that were prepared by the coevaporation method, using USP XXIII paddle method (SRII 6-flask dissolution test station, Hanson Research Co., CA, U.S.A.), using the rotation speed of the paddles of 100±2 rpm. The dissolution medium (900 mL) was low ionic strength phosphate buffer (I= 0.01, pH 7.4). The bath was maintained at 37±0.2°C. Samples having particle size range of 125-75 μm equivalent to 7 mg of NTA or 9 mg of T were sprinkled over the surface of the dissolution medium. Aliquots of 10 mL each were collected at 5, 10, 15, 20, 30, 60 and 120 minutes using filter pipette. Each aliquot was replaced by an equal volume of the buffer at the same temperature. The filtrate was analysed spectrophotometrically at 248 nm using the buffer as a blank. It is worthy to note that there were no significant differences between the spectrophotometric assay standard curves of both steroids in water and in the buffer solution.

Determination of transfer rate using a two phase-transfer system

Transfer experiments for NTA or T in the absence or in the presence of HP-ß-CD (1:10 and 1:25, drug:CD) were carried out in a simple two phase system very similar to that described by Frijlink *et al.*.[7] Solution of the drug [150 mL, $6x10^{-5}$ M] in phosphate buffer pH 7.4 (I= 0.01), in the absence or in the presence of HP-ß-CD, was charged into 450 mL conical flask. To the aqueous layer, 0.5 mL aliquot of n-octanol, previously saturated with the buffer, was added so as not to disturb their interface. The conical flask was housed in a controlled temperature shaking water bath (20 strokes/min) maintained at 37±0.5°C. Samples of 10 mL were obtained from the aqueous phase through a glass tube deeply immersed in the aqueous layer, at appropriate time intervals, within 1 hr. Concentration of the steroid (free and complexed) in the aqueous phase was determined spectrophotometrically at 248 nm using blank

containing the same concentration of HP-ß-CD. Upon plotting the logarithm of the percentage of the drug remaining in the aqueous phase versus time, the first order transfer rate constant (K_{obs}) and the half live ($T_{½}$) of the phenomenon were obtained. The validity of such system in determination of transfer rate constants and their correlation with drug activity and absorption has been extensively discussed.[7] However, in the present study, the relative transfer rate constants rather than their absolute values were of interest. It should be noted that the aqueous phase was not saturated with octanol before the experiment, because octanol could interfere with the complexation of the drugs with CD.[7]

Preparation of klucel gels

In a glass container, a weighed amount of NTA or T (1% w/w) (and HP-ß-CD, 1:1 M) was dissolved in ethanol. An appropriate amount of hydroxypropylcellulose (Klucel) solution in water (2% w/v) was added to the drug solution while maintaining constant agitation. The gels were left overnight in a refrigerator (4-5°C). One hour before every permeation experiment, the gels were taken out of the fridge and allowed to return to room temperature.

Permeation studies through cellophane membrane

The release characteristics of the gels containing either NTA or T alone or in the presence of HP-ß-CD (1:1 M) were studied using standard cellophane membrane (Viskin Co., type 36/32).[8] An accurately weighed 0.5 g sample was placed on the membrane which was soaked in phosphate buffer for 24 hr before the experiment. The membrane was stretched on one end of an open glass tube (2.8 cm in diameter). The tube was immersed in a 400 mL beaker containing 200 mL phosphate buffer pH 7.4 (I= 0.01) at 32±0.2°C. The medium was stirred by a magnetic stirrer rotating at 30 r.p.m.. At suitable time intervals, 10 mL samples were taken and replaced by buffer. Samples were assayed spectrophotometrically for their drug content at 248 nm. All release experiments were carried out in triplicate. As the results were reproducible (SD < 3%), only the average values were reported. Drug release data were treated according to Higuchi equation 1.[9]

$$Q = 2C_o\sqrt{D_{app}.\frac{t}{\pi}} \qquad \text{Eq. 1}$$

where Q is the amount of the drug released per unit area, C_o is the initial drug concentration in the donor compartment. D_{app} is the apparent diffusion coefficient, and t is the time. D_{app} was calculated from the slope (B) of the linear plot of Q versus $(t)^{½}$ as shown by equation 2:

$$D_{app} = (\frac{B}{2C_o})^{½}.\pi \qquad \text{Eq. 2}$$

RESULTS AND DISCUSSION

Interactions in solution

Figure 1 represents the phase-solubility diagrams of NTA and T as a function of HP-ß-CD concentration at three temperatures. It is apparent that the solubility of both steroids increases linearly ($r > 0.99$) upon increasing the concentration of the host, HP-ß-CD. This confirms that both drugs form inclusion complexes in solution of A_L-type.[10]

A great differences in the affinity of NTA and T towards HP-ß-CD was observed where the former exhibited higher complexing ability than the latter. This was demonstrated by the significantly higher values of the stability constant, $K_{1:1}$ (Table I), which was calculated using the following equation,[11]

$$D_t = D_o + \frac{D_o K_{1:1}}{1 + D_o K_{1:1}} C_t \qquad \text{Eq. 3}$$

Where D_o is the solubility of the drug in water, D_t is the solubility of the drug in a solution containing HP-ß-CD at a concentration of C_t. The reason for such great difference in the complexing affinity of both drugs might arise from the differences between their molecular structures that led to expected variations in water solubility and hydrophobicity. Table I shows that the water solubility of NTA is much less than that of T. Additionally, log P value for NTA is found to be 4.38±0.27 (calculated using ACD log P program, Toronto, Canda) compared with

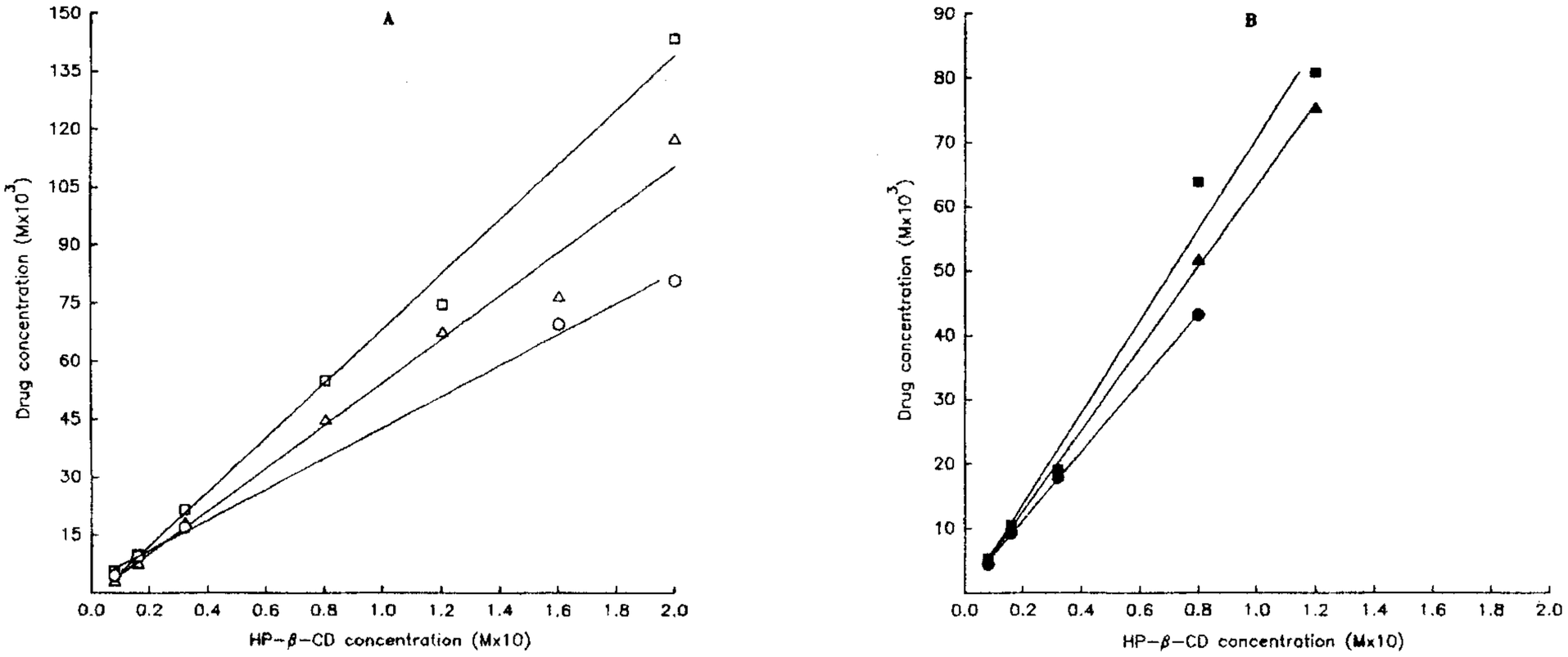


Fig. 1: Effect of HP-ß-CD on the solubility of methyl nortestosterone acetate (A) and testosterone (B) at 30° (○); 37° (Δ) and 45°C (□).

Table I: Mean apparent stability constants ($K_{1:1}$ M^{-1}) and solubility enhancement factor for the complexes of methyl nortestosterone acetate and testosterone with HP-ß-CD at different temperatures in water.

Parameters	Nortestosterone acetate			Testosterone		
	30°	37°	45°	30°	37°	45°
Solubillity in water (10^{-5} M)	0.83	0.98	2.20	8.84	11.82	19.37
$K_{1:1}$ (10^3 M^{-1})[a]	165	157	94	14	11	10
Solubility enhancement factor[b]	-	7705	-	-	572	-
Thermodynamic parameters[c]						
ΔH (kJ/mol)	-62.64			-35.56		
ΔS (kJ/mol.K)	-30.46			-24.34		
ΔG (kJ/mol)	-103.68			-36.17		

(a) The average probable error is ± 1.7x10^3.

(b) Solubility enhancement ratio is the ratio between the amount of the steroid dissolved in HP-ß-CD solution (0.12 M) to that in water at the same temperature.

(c) These values were determined by the least square fitting of the Van't Hoff's equation. ΔH°, ΔS°, ΔG° are the apparent enthalpy, entropy and free energy changes, respectively.

that of T (3.31 is the reported value and 3.40 ± 0.28 is the calculated one). From these observations, along with other studies[12-14] concerned with the dependence of the complexing affinity towards CD on the structural variation of the guest molecule, it has become increasingly clear that the less soluble the drug in water and the higher the hydrophobicity, the stronger the interaction with CD i.e. larger $K_{1:1}$ values.[2] It was noticed that the values of the stability constant, $K_{1:1}$, of both drugs decreased with increasing temperature, although the apparent solubility of them increased (Table I). This may be due to dissociation of the complexes and the negative enthalpy change resulted upon increasing temperature from 30 to 45°C.[11,15] Furthermore, application of Van't Hoff's plots of Ln $K_{1:1}$ versus 1/T (T is the absolute temperature) helped calculating the complexation thermodynamic parameters, e.g. $\Delta H°$, $\Delta S°$ and $\Delta G°$ (apparent enthalpy, entropy and free energy changes, respectively). It is possible that the release of water molecules from the HP-ß-CD cavity and the possible involvement of dipole and/or hydrogen bonding during complex formation may led to the negative enthalpy change.[15] However, the unfavourable entropy change can result from the reduced degrees of freedom of NTA or T molecules upon complexation. Generally, from the present investigation it could be concluded that the extent of interaction as well as the thermodynamic parameters values of the steroid complexation with HP-ß-CD are very much dependent on the steroid structure.

Figure 2 shows the ^{1}H-NMR spectral changes of HP-ß-CD exhibited upon complexation with either NTA or T. The spectrum of HP-ß-CD (Fig. 2A) shows a large peak at 4.5 ppm due to water, while the $C\underline{H}_3$ and $C\underline{H}_2$ protons appear at 1.0 and 3.55 ppm respectively. Upon complexation with either NTA (Fig. 2C) or T (Fig. 2E), it was noticed that these peaks were significantly shifted. The $C\underline{H}_3$ doublet protons were shifted to 1.15 ppm as well as the $C\underline{H}_2$ multiplet protons were shifted to 3.75 ppm. Additionally, in the spectra of the steroids the $C\underline{H}_3$ peaks, appear at 0.8 and 0.7 ppm in NTA and that at 0.8 ppm in T spectrum, also experienced slight shifts.

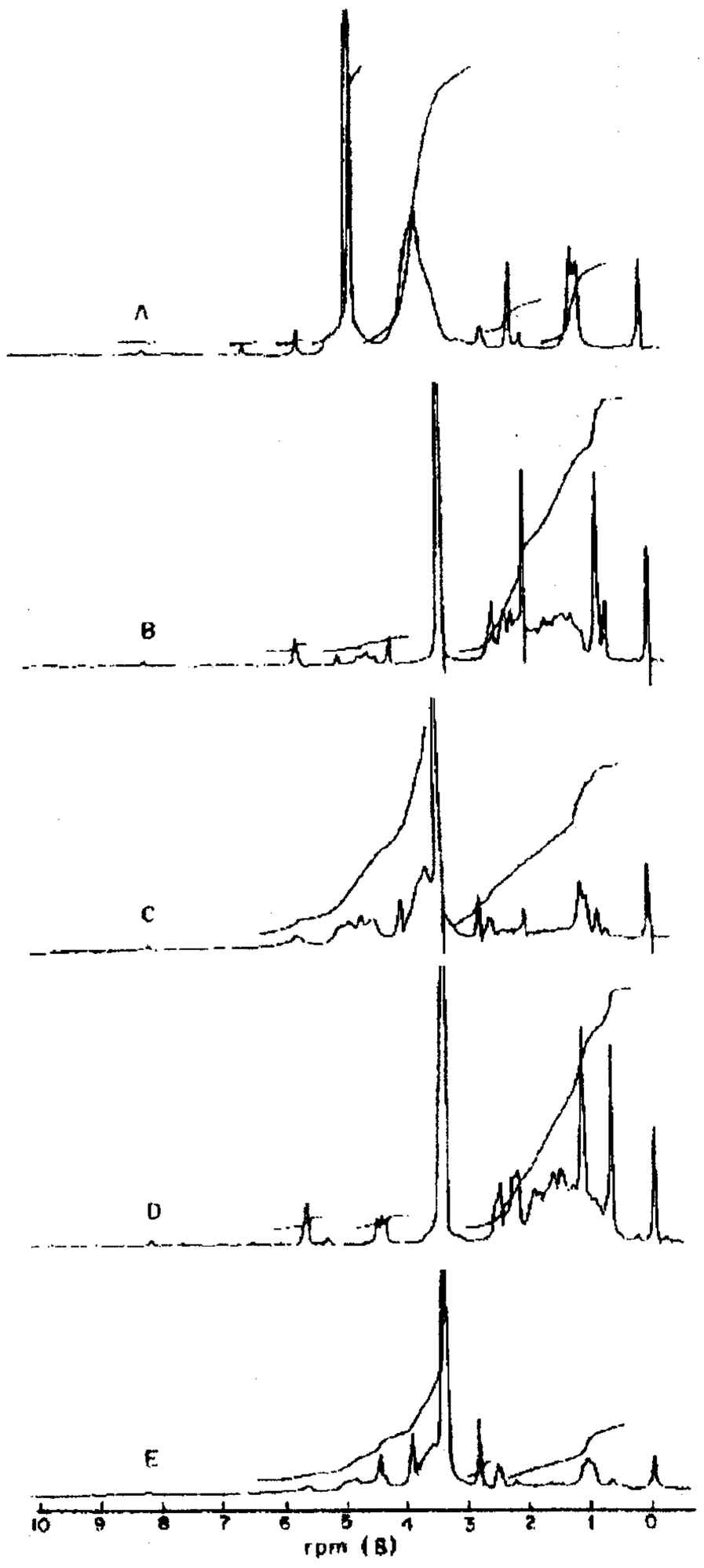


Fig. 2: ^{1}H-NMR spectra of HP-ß-CD (A), nortestosterone aetate (B), nortestosterone acetate/HP-ß-CD complex (C), testosterone (D) and testosterone/HP-ß-CD complex (E).

Interactions in the solid state

With respect to the method of preparation of NTA and T solid complexes with HP-ß-CD, the coevaporation and lyophilization methods

were selected as they were found to be the best methods as stated in the previous article[15] also Hoshio *et al.*,[11] stated that the coevaporation method gave a true inclusion complex of T/HP-ß-CD.

Infrared and DSC techniques were utilized to prove the existence of steroid complexes in the solid state. IR spectra of NTA [Fig. 3-I (A)] is characterized by three peaks at 1735, 1665 and 1600 cm^{-1} due to its -C=O stretching vibrations. In the mean time, testosterone spectrum [Fig. 3-II (A)] displayed two peaks at 1654 and 1609 cm^{-1} due to -C=O stretching vibrations. Similarly, the spectra of the physical mixtures of both drugs with HP-ß-CD [Fig. 3-I & II (C)] show the same characteristic peaks without any changes. Upon complexation of NTA with HP-ß-CD [Fig. 3-I (D&E)], the first two vibrations were shifted to 1726 and 1652 cm^{-1} while the third one disappeared. In case of T/HP-ß-CD complex [Fig. 3-II (D&E)] the changes were less obvious than those of NTA where the first peak did not show any change while the second one was shifted to 1618 cm^{-1}. These changes proved that NTA strongly interacted with HP-ß-CD more than T.

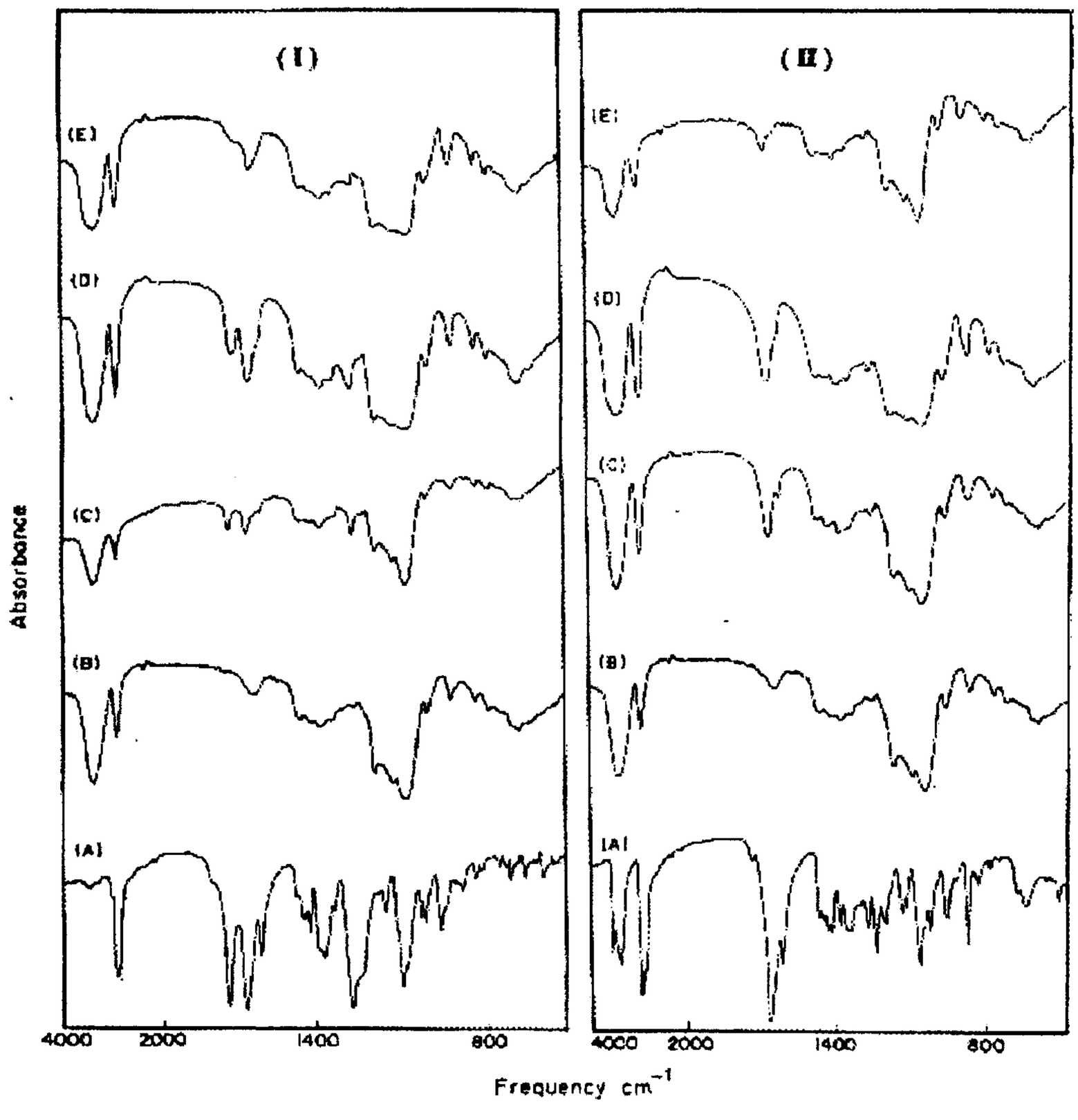


Fig. 3: Infrared spectra of HP-ß-CD, steroids, steroid:HP-ß-CD physical mixtures and complexes.
(I) Nortestosterone acetate, (II) Testosterone.
(A), Drug; (B), HP-ß-CD; (C), physical mixture (1:1); (D), coevaporation product; (E), Lyophilization product.

Typical DSC thermograms for NTA and T samples are shown in Figure 4(I&II). In case of NTA, the steroid exhibited a single melting endothermic peak (thaw temperature: 94.1°C, peak temperature: 110.0°C, heat of fusion, ΔH: -63.4 J/g). Similarly, testosterone displayed a single melting endotherm (thaw temperature: 144.9°C, peak temperature: 154.5°C and heat of fusion, ΔH: -12.02 J/g). The DSC curve of HP-ß-CD (Fig. 4, B) is characterized by a broad endotherm due to release of water molecules entrapped inside the CD cavity. In the mean time, the curves of the steroid: HP-ß-CD (1:1 M) physical mixtures have the features of both components. Interestingly, upon melting of the steroid complexes prepared by coevaporation (Fig. 4, D) and lyophilization (Fig. 4, E), the characteristic melting endotherms of the steroids, NTA and T, were completely disappeared. This confirmed the formation of amorphous inclusion complexes of the steroids where the molecular arrangements are completely different from their own crystal habit.

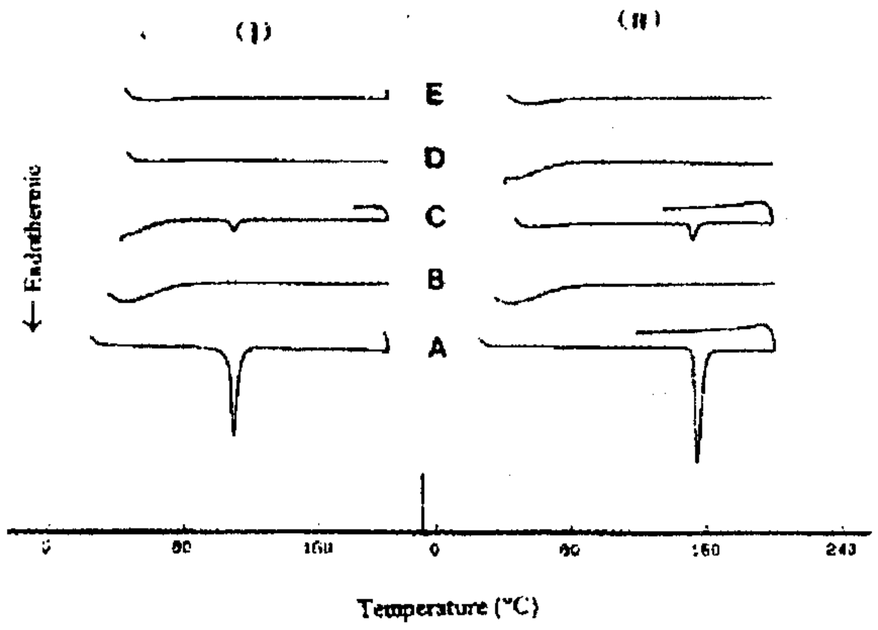


Fig. 4: DSC thermograms of nortestosterone acetate and testosterone/HP-ß-Cd systems.
(I) Nortestosterone acetate, (II) Testosterone.
(A), Drug; (B), HP-ß-CD; (C), physical mixture (1:1); (D), coevaporation product; (E), lyophilization product.

Dissolution studies

Figure 5 shows the dissolution profiles of the steroids, their physical mixtures and complexes with HP-ß-CD. It is obvious that both drugs exhibited a relatively slow dissolution rate ($T_{50\%}$ > 120 min. for NTA, and 85 min. for T). Meanwhile, the physical mixtures exhibited a slight improvement in the release rates which may be due to the increased wettability and solubility of the steroids as a result of the coexistence of HP-ß-CD in the dissolution medium. With respect to the dissolution of the complexes, it was found that the dissolution of each drug was significantly enhanced ($T_{50\%} \leq 2$ min) compared with the untreated drug and physical mixtures as demonstrated by the distinguished drop in $T_{50\%}$ and the rise in the RDR values (Table II). This dissolution enhancement may be mainly due to partial or total entrappment of the steroid inside the CD torus that led to increasing its hydrophilicity and hence, increase its solubility and wettability. Moreover, the marked reduction in crystallinity of both steroids, proved by DSC, seems to be one of the reasons that led to the achieved enhancement in the dissolution rate of the complexes.

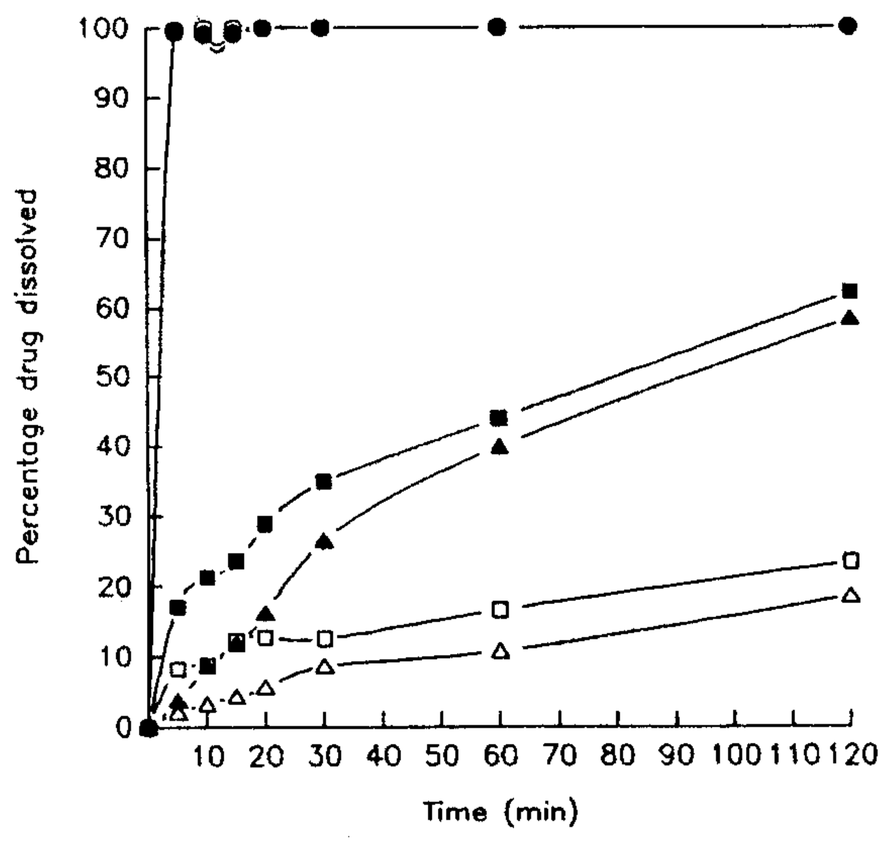


Fig. 5: Dissolution profiles of nortestosterone acetate (open symbols) and testosterone (closed symbols)/HP-ß-CD systems in phosphate buffer (pH 7.4, I= 0.01) at 37°C.
Δ, Drug alone, □, Physical mixture (1:1 M); ○, Solid complex.

The significant increase in solubility together with enhanced dissolution rate suggest that the complexed forms of both drugs might improve their bioavailability. Similarly, Uekama

et al. found that the bioavailability of diazepam was improved upon complexation with CD.[16]

Transfer and permeability studies

It was of interest to investigate the transfer rate of both steroids in the absence and in the presence of HP-ß-CD. For that purpose, the two phase transfer system was utilized to simulate the membrane transfer process and the transport from GIT to the circulation. Primarily, it was stated that the effect of CD on drug transfer depends on the complex stability constant, diffusion and partition coefficient of the free and complexed form.[17] Based on those factors, complexation may not change, may reduce or increase the transfer rate of drugs.

Table III shows the transfer rate data of the steroids from the aqueous to oil phase. It is obvious that NTA exhibited a faster transfer rate than T which may be due to its higher hydrophobicity.[18] Addition of HP-ß-CD led to a marked decrease in the transfer rate of both drugs (Fig. 6). The reason for this may be due to the complex formation with HP-ß-CD; a factor that led to retaining of the drug in the aqueous phase. It was noticed that NTA was retained (28%) to a higher extent in the aqueous phase than T (19%) as it forms stronger complex with HP-ß-CD. Frijlink *et al.*,[7] explained this phenomenon by means of displacement of the drug from the complex by the oil (n-octanol).

Table II: Relative dissolution rate and dissolution half lives for the dissolution of nortestosterone acetate and testosterone and their complexes with HP-ß-CD in phosphate buffer (pH 7.4) at 37°C.

Parameters	Nortestosterone acetate		
	Drug alone	Physical mixture	Complex
R.D.R. (min.) at			
10	1	2.7	30.6
30	1	1.4	11.5
60	1	1.5	9.3
$T_{50\%}$ (min.)	>120	>120	≤2
	Testosterone		
R.D.R. (min.) at			
10	1	2.5	11.5
30	1	1.3	3.8
60	1	1.1	2.5
$T_{50\%}$ (min.)	85	75	≤2

- Relative dissolution rate (R.D.R.) represetns the ratio between the amount of the drug released from the physical mixture or the complex to that from the drug alone at the same time interval.
- Dissolution half live ($T_{50\%}$) is the time in minutes needed fot 50% of the steroid to be dissolved.

Table III: Effect of HP-ß-CD on the transfer of nortestosterone acetate (NTA) and testosterone (T) from aqueous to n-octanol layer at 37°C and on the release of both drugs from Klulcel gel at 32°C.

Compound	Transfer rate			Release characteristics from klucel gel	
	K_{obs} ($x10^3$ min^{-1})	$T_{½}$ (min)	r	Diffusion coefficient D_{app} (10^{-6} cm^2/sec)	Release rate (mg/cm^2/hr)
NTA alone	37.2	18.6	0.982	50.8	1.2
NTA/HB-ß-CD	26.8 (1:10)*	25.9	0.991	86.5 (1:1)*	4.0
	15.1 (1:25)*	46.0	0.998	-	-
T alone	30.0	22.9	0.994	53.5	1.5
T/HP-ß-CD	24.2 (1:10)*	28.7	0.985	89.8 (1:1)*	4.2
	14.0 (1:25)*	50.3	0.980	-	-

* Molar ratio of the drug to HP-ß-CD at the begining of the experiment.

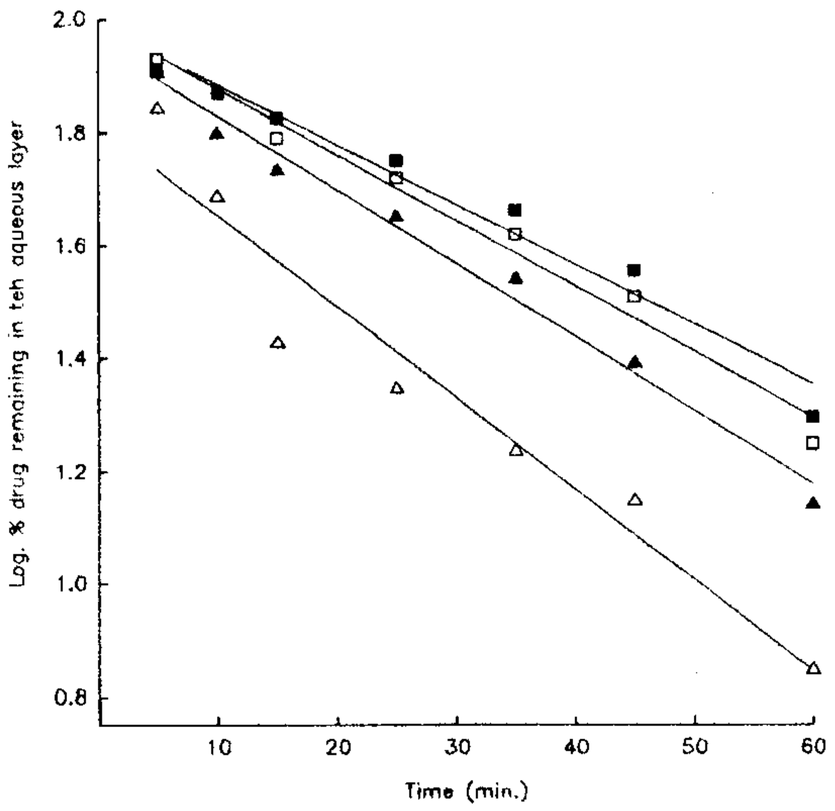


Fig. 6: Effect of HP-ß-CD on the transfer of nortestosterone aetate (open symbols) and testosterone (closed symbols) from phosphate buffer to octanol, as a function of time.
Δ, Drug alone; □, Drug/HP-ß-CD (1:1 M).

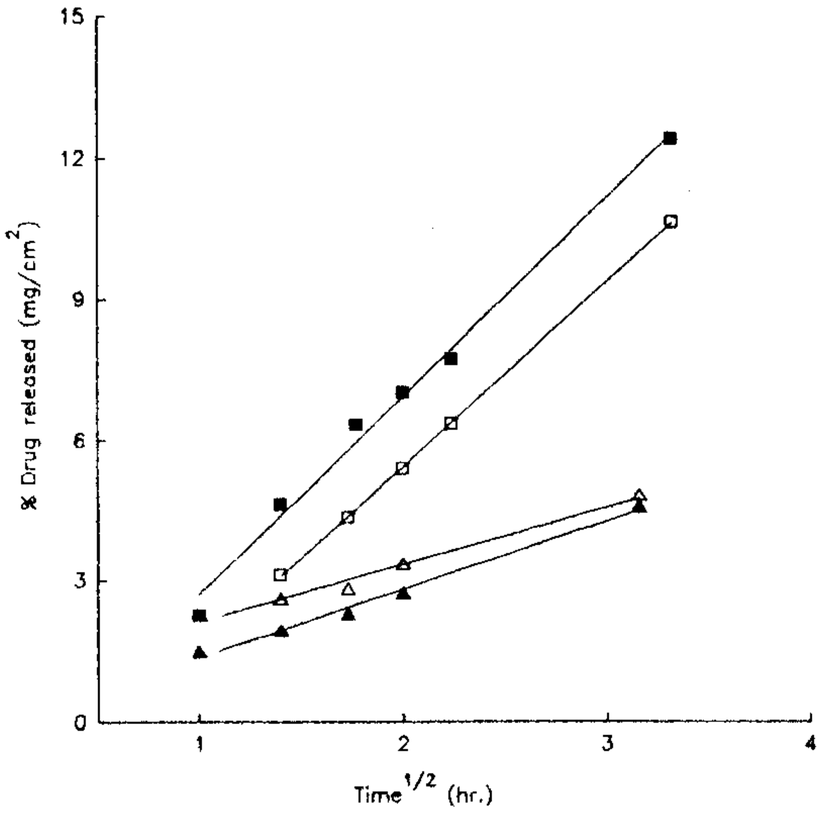


Fig. 7: Effect of HP-ß-CD on the release of nortestosterone acetate (open symbols) and testosterone (closed symbols) from Klucel gel. Δ, Druge alone; □, Drug/ HP-ß-CD (1:1 M). Dissolution media: Phosphate buffer pH 7.4; temperature: 32±0.5°C; surface area: 6.15 cm^2.

Finally, the permeability of NTA and T incorporated into Klucel gel was investigated and the effect of HP-ß-CD was assessed. In this respect, it was found that the *in-vitro* permeability of both drugs followed Higuchi model[9] as linear relationship (r: > 0.99) were obtained upon plotting percentage drug released against square root of time (Fig. 7). This indicates a direct dependence of the release rate on the diffusion coefficient. Table III shows that the release of NTA and T from Klucel gel were enhanced in the presence of HP-ß-CD (1:1 M) following the order: NTA < T < NTA/HP-ß-

CD < T/HP-ß-CD. The enhancement in the release rate of the steroids in the presence of HP-ß-CD could only be explained by the higher hydrophilicity and solubility associated with molecular complex formation. Similarly, Chen *et al.*[18] found that the presence of HP-ß-CD improved the permeability of alkannin from cream and gel preparations.

In conclusion, this study demonstrated that molecular complexation with CD, particularly HP-ß-CD, could be used successfully to modifiy the aqueous solubility, dissolution, transfer and permeability of NTA as well as T. Such complexation reaction is highly dependent on the steroid structure. This kind of knowledge provide a rational basis for formulation design of steroids and a means of enhancing their bioavailability.

REFERENCES

1- K. Frömming, J. Szejtli, Cyclodextrins in Pharmacy, Kluwer Academic, Dordrecht/The Netherlands (1994).
2- K. Uekama and M. Otagiri, CRC Crit. Rev. Ther. Drug. Carrier. Sys. 3, 1 (1987).
3- H. Arima, H. Adachi, T. Irie, K. Uekama and J. Pitha, Pharm. Res. 7, 1152 (1990).
4- T. Loftsson, M.E. Brewster, J. Pharm. Sci. 85, 1017 (1996).
5- I. Tetsumi, K. Uekama, ibid. 86, 147 (1997).
6- K. Sundaram, N. Kumar, C. Bardin, Recent Progress in Hormone Research 49, 373 (1994).
7- H.W. Frijlink, A.M. Schoonen and C.F. Lerk, Int. J. Pharm. 49, 91 (1989).
8- S. Miyazakis, S. Takeuchi, C. Yokouchi and M. Takada, Chem. Pharm. Bull. 32, 4205 (1984).
9- W.I. Higuchi, J. Pharm. Sci. 51, 802 (1962).
10- T. Higuchi, K.A. Connors, Advances in Analytical Chemistry and Instrumentation, Ed. C.N. Reilley, Vol. 4, p. 117, Interscience (1965).
11- T. Hoshino, K. Uekama and J. Pitha, Int. J. Pharm. 98, 239 (1993).
12- M. Otagiri, K. Uekama and K. Ikeda, Chem. Pharm. Bull. 23, 188 (1975).
13- N.S. Sosnowska, J. Incl. Phenom. 27, 31 (1997).
14- N.S. Sosnowska, Eur. J. Pharm. 3, 1 (1995).
15- S.M. Ahmed, J. Incl. Phenom. 1997 (In press).
16- K. Uekama, S. Narisama, F. Hirayama and M. Otagiri, Int. J. pharm. 16, 327 (1983).
17- G. Flynn, S. Yalkowsky and T. Roseman, J. Pharm. Sci. 63, 479 (1974).
18- C.Y. Chen, F.A. Chen, A. Wu, H. Hsu and J. Kang, Int. J. Pharm. 141, 171 (1996).