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Effect of Cyclodextrins on the Chemical Stability of ST1435, a Contraceptive Steroid Progestin, in Aqueous Solution

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Abstract. The influence of cyclodextrins (CDs) on the chemical stability of the contraceptive steroid progestin, ST1435, in aqueous solution has been studied using reversed phase high performance liquid chromatography. The effects of CD structure, temperature, and CD concentration on the rate of degradation were investigated. It was found that the drug degraded to different extents following a pseudo-first order reaction mechanism. The presence of the host molecules affected the degradation rate as a result of complexation which might result in protection of the labile moiety of the drug molecule against degradation. Hydroxypropyl-β-cyclodextrin (HP-β-CD) and hydroxyethyl-β-cyclodextrin (HE-β-CD) retarded the degradation in contrast to β-cyclodextrin (β-CD) which accelerated the steroid degradation. The stabilizing action of HP-β-CD is larger than that of HE-β-CD. The degradation rate increased upon increasing temperature and the Arrhenius equation is valid. Lineweaver-Burk equation analysis indicated that the steroid included inside the CD cavity degraded three times more slowly than did the free ST1435 in solution. This equation further supported the formation of a 1 : 1 inclusion complex between ST1435 and HP-β-CD with a stability constant of 934.5 M^{-1} at 65 °C.

Key words: ST1435, stability, high performance liquid chromatography, hydroxypropyl-β-cyclodextrin, hydroxyethyl-β-cyclodextrin, β-cyclodextrin.

1. Introduction

Recently, considerable attention has been paid to the utilization of CDs and their derivatives in drug formulations in order to ameliorate undesirable pharmaceutical characteristics. Cyclodextrins are capable of forming inclusion complexes with a wide variety of drugs by taking up a whole molecule, or some part of it, into their hydrophobic cavity. Encapsulation of a drug molecule will affect its physicochemical properties, such as aqueous solubility, dissolution and stability [1–3]. A literature survey revealed that the interaction of cyclodextrins and steroids has been extensively investigated [4–7]. The present investigation is concerned with ST1435, a contraceptive progestin steroid, which is a 19-norprogesterone derivative (16-methylene-17α-acetoxy-19-nor-pregn-4-ene-3,20-dione, hereafter referred to as ST1435, Figure 1) and which has been considered one of the best antiovulatory agents currently available. It has unique properties that make it the most suitable

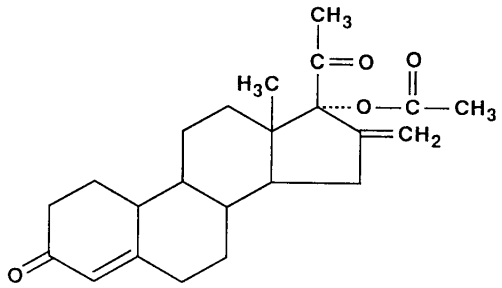

Figure 1. Structural formula of ST1435.

steroid for contraceptive use, even in lactating women [8]. This drug can be developed into various contraceptive dosage forms, such as subdermal implants, vaginal rings, transdermals and injections. In a previous article, the stability of ST1435 was investigated [9] and it was found that the drug is fairly stable in the solid state, while it undergoes a hydrolysis degradation reaction in phosphate buffer solution, pH 7.4, following a pseudo-first order mechanism. The present study is designed to elucidate the effects of HP-β-CD, HE-β-CD, and β-CD on the rate of degradation of ST1435 in aqueous solution. The effect of various factors, e.g. CD structure, storage temperature as well as CD concentration, on the drug degradation are other goals of the present article.

2. Experimental

2.1. MATERIALS

Cyclodextrins were purchased from Pharmatec Inc. Alachua, Florida, USA. The average degrees of substitution (per β-CD molecule) of HP-β-CD and HE-β-CD were found to be 6.4 and 9.5, respectively. These values were calculated based on the mass spectra provided by the manufacturing company according to the following formula [10]: Average degree of substitution = $\sum$ (peak height $\times$ degree of substitution)/$\sum$ (peak heights).

ST1435 was obtained from E. Merck Darmstadt, Germany. All were used as received. HPLC solvents were purchased from J. T. Baker Chemicals Co., Philipsburg, NJ, 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. *HPLC Method*

Samples of the progestin steroid ST1435 were analyzed using reversed-phase high performance liquid chromatography (HPLC) with a Hewlett-Packard 1090 instrument controlled by a 310 Chemstation equipped with a Diode Array UV

detector set at 247 nm and a Rheodyne model 7125 injector. The samples were eluted at a flow rate of 1.1 mL/min. at room temperature, under isocratic conditions, on an octadecylsilyl column (Spherosorb ODS2 column, 15 $\times$ 0.46 cm, particle size 3 μm, Phase Separation Inc., Norwalk, CT, USA), fitted with an RP-ODS2 precolumn. The mobile phase consisted of 56% aqueous acetonitrile (v/v). Standard solutions containing 2–20 μg/mL of ST1435 were prepared by diluting an ethanolic stock solution of the steroid with 0.2 M phosphate buffer, pH 7.4 [11]. Calibration curves, based on the average of the peak area of different concentrations of ST1435, were employed to evaluate the amount of the undecomposed drug in the treated samples. Standard solutions were assayed and calibration curves were constructed before analysis of each sample set to ensure reproducibility of the analysis.

2.2.2. *Kinetic studies*

2.2.2.1. *Effect of cyclodextrin structure*. Solutions of ST1435 (1 mL, 10 μg/mL) in 0.2 M phosphate buffer, pH 7.4, containing 0.01 M of either HP-β-CD, HE-β-CD, or β-CD were placed in 5 mL Type I brown glass vials covered with Teflon lined screw caps and stored at 65°C in a thermostatically controlled oven (Isotemp oven, Fisher Scientific Co., NJ, USA). At timed intervals, samples were taken out of the oven, frozen immediately in a dry ice-acetone bath and kept in the freezer (-20°C) pending analysis by HPLC. The samples to be analyzed were removed from the freezer and equilibrated to room temperature just before injection into the column.

2.2.2.2. *Effect of temperature*. Solutions of ST1435 (1 mL, 10 μg/mL) in 0.2 M phosphate buffer, pH 7.4, containing 0.01 M HP-β-CD, were placed in the brown glass vials and stored at 37°, 50°, 65°, or 80°C in thermostatically controlled ovens. The same procedure was continued and samples were collected, handled and analyzed as described before.

2.2.2.3. *Effect of HP-β-CD concentration.* The effect of HP-β-CD concentration on the degradation rate of ST1435 was carried out at a constant temperature of 65°C. Samples containing 10 μg/mL of ST1435 in phosphate buffer, pH 7.4, containing 0.01, 0.025, 0.035 or 0.05 M of the host, were charged into brown glass vials and stored in the oven at 65°C. The same procedure was continued as before and treated samples were analyzed by the HPLC method for the amount of the drug remaining after predetermined time intervals.

3. Results and Discussion

3.1. HPLC METHOD

The reversed phase HPLC method adopted in this article enabled the achievement of satisfactory quantitative analysis of ST1435 within the concentration range of 2–20 μg/mL. Figure 2A shows a typical chromatogram for the intact drug

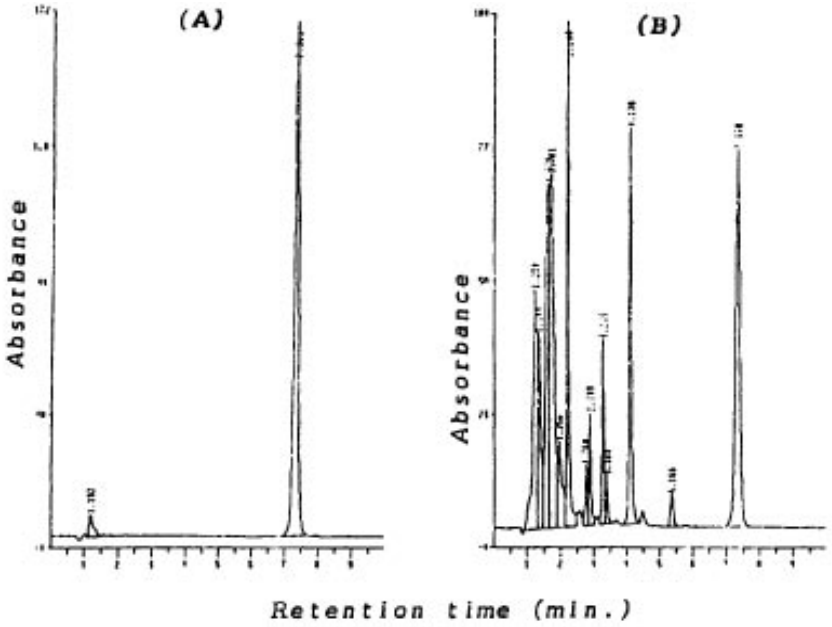

Figure 2. Typical HPLC chromatogram of ST1435 in phosphate buffer (0.2 M, pH 7.4): (A), Untreated [Control]; (B), after storage for 14 days at 65 °C in the presence of HP-β-CD. The retention time of the parent intact compound in all cases is 7.368 $\pm$ 0.015 (n = 7) min.

dissolved in phosphate buffer pH 7.4 (Retention time = 7.368 $\pm$ 0.015, min, n = 7). Standard curves were linear with a correlation coefficient of 0.9995 $\pm$ 0.0003 (n = 7). Replicate injections of standard solution containing 20 μg/mL of the drug in phosphate buffer were performed. The peak area value obtained was 2947 Au with standard deviation (SD) of 55.5 Au, RSD% = 1.88 (interday measurements). This confirmed the reproducibility, accuracy and precision of the analytical method used throughout this article. Moreover, this method is suitable to quantitate the amount remaining of the steroid subjected to different storage conditions without interference of the decomposition products and additives. This is because the decomposition products are less polar than the intact drug and hence eluted earlier as shown in Figure 2B.

3.2. EFFECT OF CYCLODEXTRIN STRUCTURE

In a previous article [9] it was reported that ST1435 dissolved in phosphate buffer pH 7.4, degrades through a hydrolytic rather than oxidative mechanism, following a pseudo-first order reaction. The major degradation product, 16-methylene-17α-hydroxy-19-nor-pregn-4-ene-3,20-dione, was separated and identified by mass spectrometry. The present work was designed to study the effects of two CD derivatives viz. HP-β-CD and HE-β-CD compared with the parent β-CD in the

hope of improving the stability of ST1435 in solution by inclusion complexation and, choosing the best stabilizer. For that purpose, CD concentrations were maintained at 0.01 M and the initial concentration of ST1435 at 10 μg/mL in phosphate buffer pH 7.4. The storage temperature was kept at 65°C for the purpose of comparison. This temperature was chosen for the convenience of kinetic measurements, because the degradation of the progestin steroid was rather slow at room temperature [9]. In all cases, it was found that the degradation of the contraceptive steroid follows a pseudo-first order reaction mechanism based on the linearity between log (percentage drug remaining) and time (Figure 3). This also confirmed that the kinetic behaviour of the drug was not changed as a result of the interaction with the three CDs selected. In the meantime, it was noticed that the degradation rate constant decreased considerably in the presence of HP-β-CD and HE-β-CD, and consequently $T_{1/2}$ and $T_{90\%}$ were increased, as reflected in Table I. These results proved that the addition of both hydrophilic cyclodextrin derivatives, hydroxypropyl or hydroxyethyl-β-CD, led to stabilization of ST1435 against hydrolytic degradation in solution. HP-β-CD displayed higher stabilizing activity than HE-β-CD. It was interesting to look for the reasons that led to enhancing the stabilizing action of HP-β-CD rather than HE-β-CD although they have similar features. Undoubtedly, this may be due to differences in their complexing ability towards ST1435. In a recent article [12] it was found, using various techniques, that ST1435 forms inclusion complexes with HP-β-CD and HE-β-CD in solution as well as in the solid state. Based on the solubility studies, the apparent stability constants, $K_{1:1}$, for the drug-CD complex with HP-β-CD (2817 $\pm$ 274, 2281 $\pm$ 152, 1132 $\pm$ 36 M^{-1}) are higher than those with HE-β-CD (2131 $\pm$ 216, 1893 $\pm$ 173, 988 $\pm$ 48 M^{-1}) at 30°C, 37°C, and 45°C, respectively. A least squares fit linear regression line for the above data, in accordance with Van't Hoff's plot of ln $K_{1:1}$ versus 1/T [12], was used for the projections of stability constants at 65°C. Stability constant values of 937.2 M^{-1} and 551.7 M^{-1} for ST1435 complexes with HP-β-CD and HE-β-CD at 65°C, respectively, were calculated. These data revealed that HP-β-CD had a higher solubilizing and complexing power than HE-β-CD within the temperature range investigated as well as at 65°C. Similarly, Yoshida *et al.* [13] stated that the solubilizing power of HP-β-CD towards various drugs is often higher than that of HE-β-CD. This order supported the existence of good correlation between the solubilizing power, which directly reflects the complexing ability, and the stabilizing potential of cyclodextrins. Accordingly, it could be assumed that the degradation rate may be dependent on the hydrolysis of the free drug molecules in solution which resulted from dissociation of the complex. This may be the reason for the relatively higher degradation rate of the steroid complexed with HE-β-CD due to its lower complexing affinity and consequently the presence of a larger amount of free drug molecules in solution. Furthermore, since the average degree of substitution of HP-β-CD is lower than that HE-β-CD (see experimental section) it was not unexpected that its solubilizing power, and consequently its stabilizing potential, are higher than those of HE-β-CD. This may

be explained on the basis of decreased steric hindrance around the HP-β-CD cavity. Similarly, Loftsson and Johannesson found that decreasing the degree of substitution of CDs led to increasing their stabilizing effects as a result of increasing their complexing ability [3]. The possible explanation for the stabilizing effect of the two cyclodextrin derivatives investigated is that the labile ester moiety, 17 α-acyl group in ST1435, might be completely, or at least partly, enclosed inside the CD cavity and hence shielded against hydrolysis attack. Although there are many essential advantages of CD derivatives in stabilizing and solubilizing drugs [14], it was interesting to investigate the effect of the parent host molecule, β-CD, on the stability of our candidate progestin, for comparison with HP-β-CD and HE-β-CD, the most pharmaceutically recommended derivatives, to demonstrate their excellence over β-CD, and to assess the effect of structural variations on their stabilizing activity. Further examination of Figure 3 and Table I reveals that β-CD exhibited a destabilizing action towards ST1435. Such a catalytic effect may be primarily attributed to the presence of the active center of the drug strictly fixed in close proximity to the free hydroxyl groups of β-CD and it may possibly proceed through nucleophilic attack on the drug active center [14]. In this respect, Uekama *et al.* [15] showed that methylated β-CD in which hydroxyl groups are blocked has a much greater retardation effect for the hydrolysis of prostacycline in comparison with that of the parent β-CD. Hirayama *et al.* [16] found that β-CD had a negative effect on the stability of prostaglandins while methylated β-CD displayed a positive effect. Loftsson and Johannesson [3] reported that the degradation of β-lactam antibiotics was accelerated in the presence of CDs. Anderson and Bundgaard [17] noticed that the rate of degradation of hydrocortisone was accelerated in the presence of β-CD and such a rate-accelerating effect approached a maximum value as the host concentration was increased. Recently, Stankovicova *et al.* [18] revealed that the hydrolysis of a new antiarrhythmic ester drug was accelerated in the presence of β-CD. In reality, most of the attempts to stabilize drugs with natural CD were disappointing because of their positive catalytic effects on the reactions. Generally, it should be emphasized that favourable results are not always obtained when one tries to use cyclodextrins as stabilizing agents, since such stabilization depends on many factors. Some of the factors reported in the literature are the nature of the guest molecule as well as its orientation within the host cavity, the type and degree of substitution of the CD, and the reaction medium. So one can expect that inclusion complexation may have an accelerative, decelerative or even no effect on the stability of the target drug [1,3,14–19].

The above results showed clearly that HP-β-CD is a good stabilizer for ST1435 in addition to the extensive studies reported in the literature about its lower toxicity, higher biological compatibility c.f other cyclodextrins, as well as a lack of detrimental effects on the kidney, c.f β-CD [14]. As a result, the following two parts of the present article study the effects of temperature and concentration on the stabilizing potential of HP-β-CD towards ST1435.

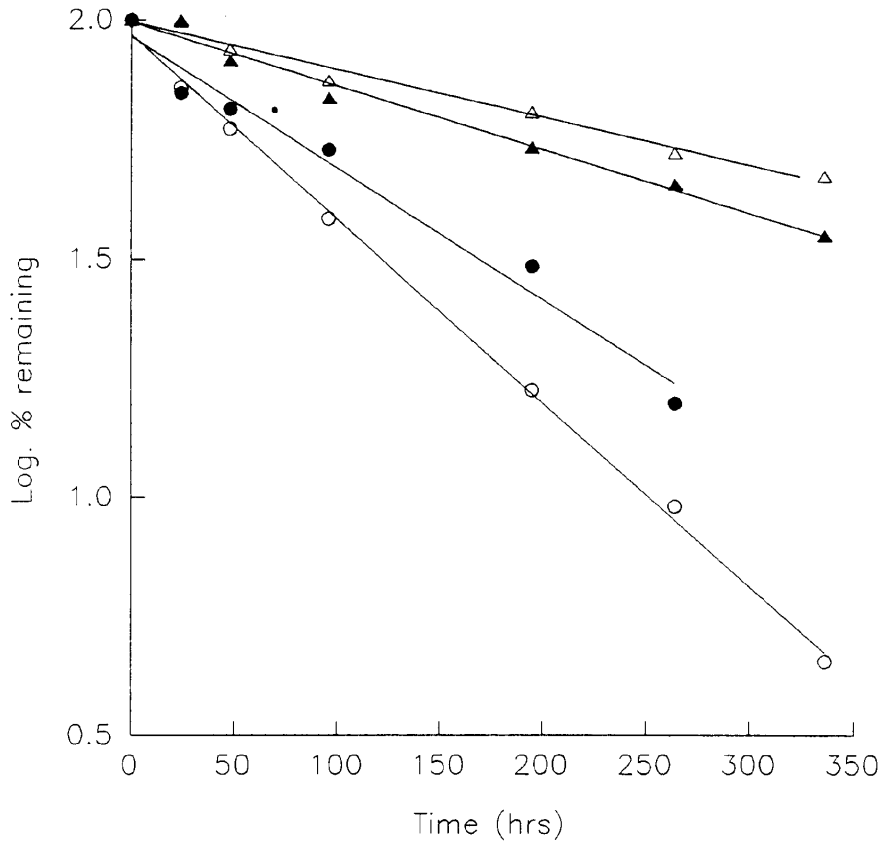

Figure 3. Effect of cyclodextrin structure (0.01 M) on the degradation of ST1435 in phosphate buffer pH 7.4 stored for different time intervals at 65 °C: ●, ST1435 alone; ○, with β-CD; △, with HP-β-CD; ▲, with HE-β-CD.

Table I. First order rate constants (k_{obs}), half lives ($T_{1/2}$), shelf lives ($T_{90\%}$), correlation coefficients (r) and relative enhancement for the degradation of ST1435 in the absence and presence of cyclodextrins (0.01 M) at pH 7.4 and 65°C.

CD	k_{obs} (10^{-4}, h^{-1})	$T_{1/2}$ (h)	$T_{90\%}$ (h)	(r)	Relative Enhancement
None	63.5	109.2	16.6	0.988	1.0
HP-β-CD	23.0	301.2	45.6	0.991	2.8
HE-β-CD	30.7	226.1	34.3	0.993	2.1
β-CD	88.8	78.0	11.8	0.999	0.7

Relative Enhancement = the ratio between k_{obs} in the absence to k_{obs} and in the presence of cyclodextrin at the same temperature.
The kinetic parameters for the degradation of ST1435 in the absence of cyclodextrins were obtained from Ref. [9].

Table II. Influence of temperature and HP-β-CD concentration on the first order rate constants (k_{obs}), half lives ($T_{1/2}$), shelf lives ($T_{90\%}$) for the degradation of ST1435 in phosphate buffer pH 7.4.

Temperature (°C)	[HP-β-CD] (M)	k_{obs} (10^{-4}, h^{-1})	$T_{1/2}$ (h)	$T_{90\%}$ (h)	(r)	Relative Enhancement
37	0.000	3.5	1978	300	0.904	1.00
	0.010	1.4	4950	750	0.928	2.50
50	0.000	14.8	470	72	0.997	1.00
	0.010	5.0	1386	210	0.995	2.95
65	0.000	63.5	109	17	0.988	1.00
	0.010	23.0	301	46	0.991	2.76
	0.025	21.8	318	48	0.993	2.91
	0.035	20.8	333	50	0.995	3.16
	0.050	20.3	342	52	0.983	3.13
80	0.000	278.0	24	5	0.999	1.00
	0.010	210.8	33	5	0.994	1.32

(r) is the correlation coefficient.
Relative Enhancement = the ratio between k_{obs} in the absence to k_{obs} and in the presence of cyclodextrin at the same temperature.
The kinetic parameters for the degradation of ST1435 in the absence of HP-β-CD (0.00 M) were obtained from Ref. [9].

3.3. Effect of temperature on the stabilizing activity of HP-β-CyD

As an integral part of this project it was interesting to study the effect of temperature on the stabilizing efficiency of HP-β-CD. Kinetic stress tests at four temperatures viz. 37°, 50°, 65° and 80°C in the presence of 0.01 M HP-β-CD in buffer solution, pH 7.4, were conducted (Table II). It was found that the decomposition of the steroid follows a pseudo-first order mechanism, within the temperature range investigated, based on the linearity between log (percentage drug remaining) and time (Table II). Moreover, a linear relationship between K_{obs} and the reciprocal of absolute temperatures was obtained, as displayed in Figure 4, according to the Arrhenius Equation (1):

$$\ln K_{obs} = \ln A - E_a/RT \tag{1}$$

were A represents the frequency factor, E_a is the activation energy, R is the gas constant and T is the absolute temperature. These findings confirmed that the Arrhenius relation is valid and consequently the pattern of breakdown at any temperature may represent that at any other temperature, despite the difference in rates. The value of the activation energy (E_a) for ST1435 degradation calculated from the gradient of the Arrhenius regression line, increased from 93.3 kJ mol^{-1} in the absence of [9] to 101.7 kJ mol^{-1} in the presence of 0.01 M HP-β-CD. It is

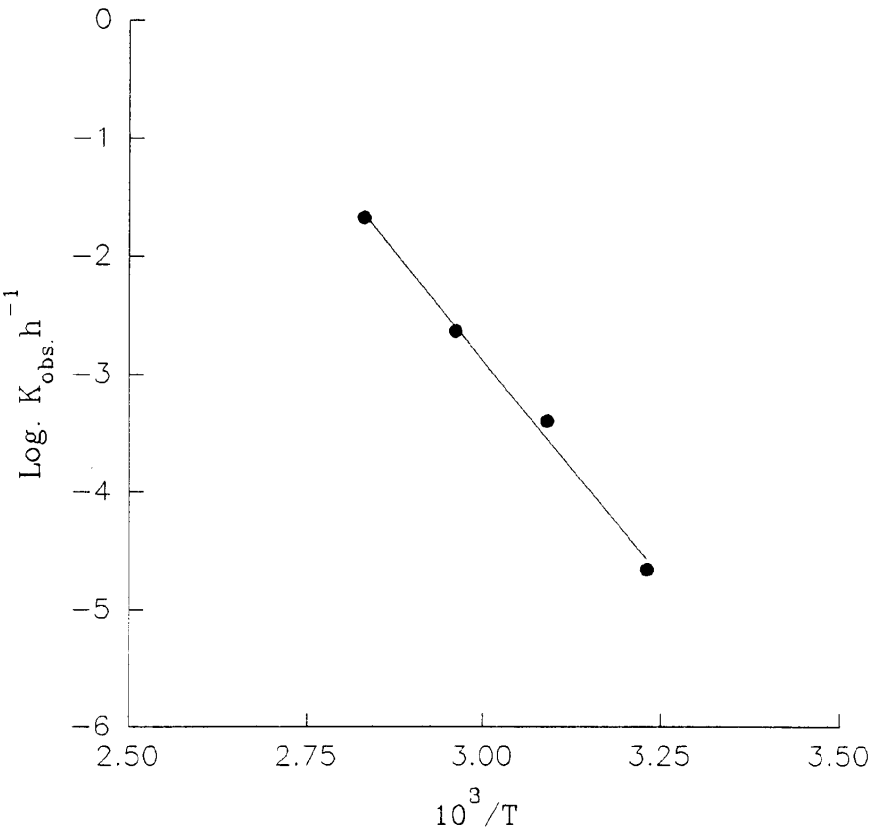

Figure 4. Arrhenius plot of logarithm of the degradation rate vs. $1/T$ of ST1435 in the presence of 0.01 M HP-β-CD in phosphate buffer pH 7.4.

worth noting that these two values lie within the usual range of activation energy values characteristic of hydrolytic degradation reactions (58–102 kJ mol^{-1} [19]).

In fact, it is well known that E_a is a measure of a 'barrier' which prevents the reactants from immediately becoming products [19]. Such an increase in that 'barrier' can be attributed to protecting the drug against degradation as a result of complexation. Besides, it provides further supporting evidence about the stabilizing action of HP-β-CD and suggested that some sort of protection was achieved against the influence of heat on the degradation. Furthermore, based on the Arrhenius relationship and extrapolation to 25 °C (room temperature), the predicted k_{obs}, $T_{1/2}$ and $T_{90\%}$ were found to be 2.5×10^{-5} h^{-1}, 3.2 year and 0.48 year, respectively, in the presence of 0.01 M HP-β-CD. Comparison of such data with those obtained in the absence of CD ($k_{obs} = 8.2 \times 10^{-5}$ h^{-1}, $T_{1/2}$ = 0.96 and $T_{90\%}$ = 0.15 year, respectively, reported in the previous article [9]) supported the positive stabilizing action of the host as demonstrated by the decreasing value of k_{obs} and consequently

$$ST1435 + HP-\beta-CD \xleftrightarrow{K_{1:1}} [ST1435.HP-\beta-CD]$$

$$\downarrow k_o \qquad\qquad \downarrow k_c$$

Degradation products — **Degradation products + HP-β-CD**

$$\left(\frac{[HP-\beta-C\ D]}{k_o - k_{obs}} = \frac{[HP-\beta-C\ D]}{k_o - k_c} + \frac{1}{K_{1:1}(k_o - k_c)}\right)$$

Scheme 1. Lineweaver-Burk analysis for the decomposition of ST1435 in the presence and absence of HP-β-CD. k_0 and k_{obs} represent the degradation rate constant in the absence and presence of [HP-β-CD], respectively. $K_{1:1}$ and k_c represent the complex stability constant and the degradation rate constant for the steroid incorporated inside the HP-β-CD cavity, respectively.

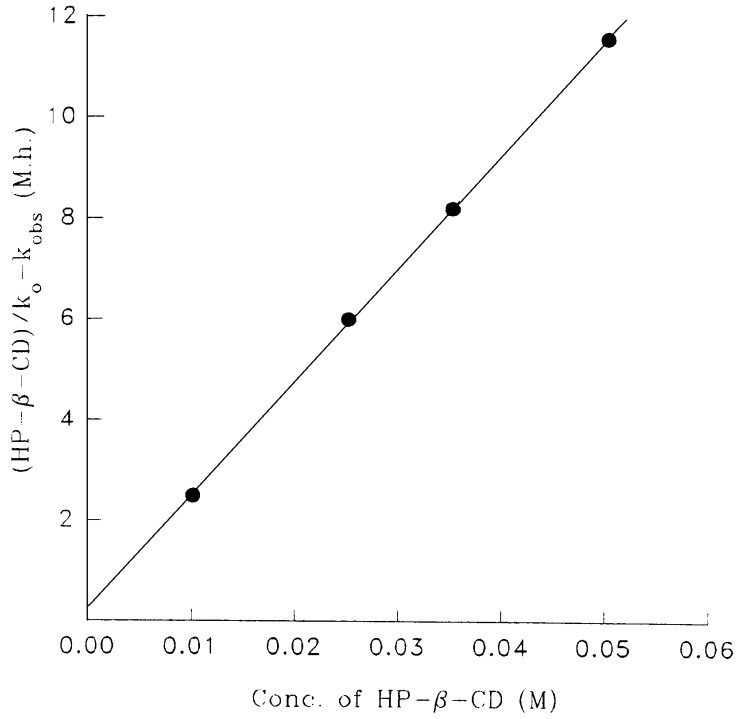

Figure 5. Lineweaver-Burk plot for the degradation of ST1435 in the presence HP-β-CD.

increasing $T_{1/2}$ and $T_{90\%}$ as a result of complexation. It is worth mentioning that the exact mechanism of degradation of free ST1435 has still not been exactly explored. Further studies are necessary, which are out of the scope of the present investigation, in order to establish the exact mechanism of degradation of the drug in the free form and consequently the exact effect of CD on its degradation mechanism can be explained.

3.4. EFFECT OF HP-β-CD CONCENTRATION

Table II illustrates the effect of the concentration of HP-β-CD on the degradation rate of ST1435 at a constant temperature of 65°C. It is evident that increasing the concentration from 0.01 to 0.05 M has increased the stabilization potential of the host towards the steroid as evidenced by the decrease in the k_{obs} and increase in $T_{1/2}$ and $T_{90\%}$ as a function of HP-β-CD concentration. This observation can be explained by assuming that the addition of a sufficient ligand concentration almost led to capturing more of the guest molecules. Interestingly, the dependency of k_{obs} on the concentration of HP-β-CD was quantitatively treated according to the reaction equations shown in Scheme 1. The Lineweaver-Burk relation [20] was valid and a linear plot having the equation: $y = 227.2x + 0.243$ ($r = 0.9999$) was obtained upon plotting [HP-β-CD]/($k_{\text{o}} - k_{\text{obs}}$) vs. [HP-$\beta$-CD], where [HP-$\beta$-CD] represents the total host concentration, k_{o} and k_{obs} denote the degradation rate constants for the drug in the absence and in presence of the complexing agent, respectively (Figure 5). The linearity of this relationship provided further supporting evidence for the formation of a 1 : 1 type inclusion complex between the drug and CD with a stability constant, $K_{1:1}$, of 934.5 M^{-1} at 65°C, calculated from the intercept of the Lineweaver-Burk plot. This value is in good agreement with that obtained upon extrapolating Van't Hoff's linear plot to 65°C. This plot was constructed based on the solubility data performed at other temperatures as mentioned in the previous article ($K_{1:1}$ = 937.2 M^{-1} [9]). Furthermore, the rate constant of the degradation of the guest molecule included inside the CD cavity (k_{c}) was found to be 1.95 $\times$ 10^{-3} h^{-1}, as obtained from the slope of the Lineweaver-Burk plot, compared to that of the free drug (6.35 $\times$ 10^{-3} h^{-1}, Table I) at 65°C. This clearly indicates that the degradation rate has been reduced by more than three times upon incorporation of the steroid molecule inside the cyclodextin torus. This could also be explained on the basis of complexation of the drug which results in shielding of the labile moiety of the molecule against hydrolysis.

4. Conclusion

In summary, cyclodextrin derivatives including HE-β-CD and HP-β-CD were found to have a stabilizing potential towards ST1435 to varying extents in aqueous solution as a result of inclusion complexation. HP-β-CD appeared to be more effective than HE-β-CD. Additionally, it was proved that the drug forms a 1 : 1

inclusion complex with HB-β-CD. Therefore, HB-β-CD appeared to be suitable for stabilization of ST1435 and for altering its solubility in favour of solution isotherms of the A_L-type. The use of natural β-CD is not recommended as it led to acceleration of the drug's degradation in addition to its poor aqueous solubility and toxic manifestations on the kidney.

The current investigations will be extended to the enhancing effect of cyclodextrins on the absorption and bioavailability of ST1435 as well as other steroids.

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