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Formation of cyclodextrin inclusion complexes with corticosteroids: their characterization and stability

Renata F.L. Vianna [a], M. Vitória L.B. Bentley [a,*], Gislaine Ribeiro [a], Fernanda S. Carvalho [a], Alberto F. Neto [a], Dionéia C.R. de Oliveira [a], John H. Collett [b]

[a] *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café s/n, 14040-903, Ribeirão Preto, São Paulo, Brazil*

[b] *School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK*

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Abstract

Inclusion complexes of dexamethasone acetate (DMA) with β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and γ-cyclodextrin (γ-CD) in water were characterised by the solubility method, spectroscopy (^{1}H-NMR) and differential scanning calorimetry (DSC). In addition, the influence of complexation on DMA stability was assessed by a thermal stress method. Complexation with CDs increased the DMA aqueous solubility. The stoichiometric ratios of the inclusion complexes were 1:1, 1:2 and 1:1 for β-, γ- and HP-β-CD, respectively. Complexation with β-CD and HP-β-CD increased the DMA stability 5- and 12-fold, respectively. The ^{1}H-NMR studies showed that the DMA 'A' ring was included in the cavity of the CDs. The oil–water partition coefficient values of the DMA decreased significantly when complexes were formed, suggesting that the partition of this drug into lipophilic membranes may be improved. These observations suggest that DMA/CD complexes may be an attractive and practical procedure to modify drug physicochemical properties for use in delivery systems.

Keywords: Inclusion complex; Cyclodextrin; Dexamethasone acetate; Stability study; DSC; ^{1}H-NMR

1. Introduction

The penetration of drugs through the skin is of increasing interest and the penetration through the stratum corneum is a prerequisite for their physiological activity. Nevertheless the relative

* Corresponding author.

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impermeability of the stratum corneum severely limits both the number and the types of drugs which can be administered through the skin (Tsai et al., 1996).

Corticosteroids are often used in topical formulations for their anti-inflammatory action. The esters of corticosteroids are the most commonly used for topical administration, however, their hydrolysis is a significant stability problem. In addition, their low aqueous solubility represent a limiting step in the achievement of therapeutic concentrations.

One potential method of optimizing the efficacy of drug activity is through the use of rationally designed drug carrier materials such as cyclodextrins (CDs). These molecules are cyclic oligosaccharides consisting of 6, 7 or 8 α-1,4-linked glucopyranose units, usually referred to as α-, β- or γ-CD, respectively. The CD cavity exhibits a hydrophobic character, whereas the exterior of the molecule is hydrophilic. The CDs are capable of forming a variety of complexes in which guest molecules are trapped entirely or at least partially by the hydrophobic portion. This inclusion leads to changes in the physicochemical properties of the guest molecules (Lemesle-Lamache et al., 1996), which can improve the molecular stability and bioavailability of several drugs.

In this work, we have prepared and characterized complexes of dexamethasone acetate (DMA) with some CDs, and investigated the influence of the complexation on the chemical stability of this corticosteroid.

2. Material and methods

2.1. Material

β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were obtained from Roquette; γ-cyclodextrin (γ-CD), dexamethasone acetate (DMA), dexamethasone (DM) and desonide were obtained from Sigma (St. Louis, MO). All other reagents were BDH or HPLC reagent grade. The HP-β-CD used in this work had specified degrees of substitution (number of hydroxypropyl groups per β-CD unit) ranging from 3.85 to 4.55.

2.2. HPLC analysis

Analysis of all samples were performed by a Shimatzu Instruments HPLC System, model 5000. UV detector at 254 nm. C_{18} reversed-phase column 125 × 4 mm (5 μm), C_{18} pre-column 4 × 4 mm (5 μm), Intralab 4290 integrator, and 0.01 AUFS. The mobile phase used was methanol:water (60:40), flux of 1 ml/min and the extraction was carried out using chloroform. Desonide in methanolic solution (200 ng/ml) was used as internal standard. The retention time for the degraded product dexamethasone (DM), internal standard and DMA were 4.3, 5.4 and 6.9 min, respectively. The method was linear to a concentration of 50 to 400 ng DMA/ml ($r = 0.99$) and 35 to 300 ng DM/ml ($r = 0.99$).

2.3. Phase solubility studies

Solubility measurements were determined according to a modification of the method of Higuchi and Connors (1965). Excess amounts of DMA were added to aqueous solutions containing various concentration of CDs, ranging from 1.6 to 53.0 mM for HP-β-CD and γ-CD, 1.6 to

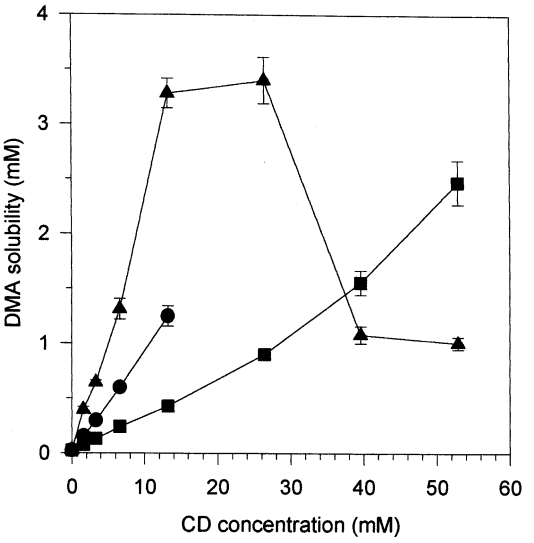

Fig. 1. Solubility diagram of the DMA in the presence of different concentration of cyclodextrins: (▲) γ-CD; (●) β-CD and (■) HP-β-CD.

Table 1
Solubility and stoichiometry of DMA/CDs complexes

Compound	Solubility curve type[a]	K_C (1:1)[b]	Increase of DMA solubility[c]	Stoichiometric ratio (DMA:CD)[d]
DMA/β-CD	A_L	2.48	48	1:1
DMA/HP-β-CD	A_L	1.60	88	1:1
DMA/γCD	B_S	1.40	121	1:2

[a] According to Higuchi and Connors (1965).
[b] K_C: apparent stability constant.
[c] Ratio between the concentration of DMA solubilized in presence and absence of CD.
[d] Determined from the differencial UV spectrum of DMA in presence of CD.

13.2 mM for β-CD. The suspensions were shaken at 25°C for 11 days. After equilibration, the suspensions were filtered through 0.45 μm membrane filters, appropriately diluted with the mobile phase and the total concentration of the DMA in the filtrate was analysed by HPLC.

An apparent stability constant ($K_{1:1}$) was calculated from the initial straight line portion of the phase solubility diagrams.

2.4. Solid complexes

The solid complexes were obtained from saturated DMA solutions in the presence of CDs. The supernatant was removed from the aqueous DMA/CD complex solution by freeze-drying or precipitation, depending on the physical state of the DMA complex, and were assayed by HPLC. The stoichiometries of DMA/β-CD and DMA/HP-β-CD complexes were obtained from the differential UV spectrum ($\lambda = 242$ nm) of DMA in presence of different concentrations of CDs, according to the procedure of Ammar and El-Nahhas (1995).

2.5. Partition coefficient ($K_{IPM/water}$) determination

Partition coefficients of DMA were determined between isopropylmyristate (IPM) and water using the shake-flask method. Aqueous solutions of DMA or complexes (DMA/β-CD, DMA/HP-β-CD and DMA/γ-CD) were prepared containing 14 μg DMA/ml. Equal volume of IPM and each DMA solution were shaken for 30 min. After allowing to stand for 5 min, the supernatant was removed and the residue centrifuged for 10 min at 2000 rpm. The aqueous phase was assayed by HPLC at time zero (C_{in}) and after shaking to ensure partition (C_w). The partition coefficient was $K_{IPM/water} = (C_{in} - C_w)/C_w$.

Table 2
Chemical shifts (ppm) for the protons of β- and HP-β-CyD in the free state and complexed with DMA

Proton	$\delta_{\beta\text{-CD}}$	$\delta_{DMA/\beta\text{-CD}}$	$\Delta(\delta_{DMA/\beta\text{-CD}}$ and $\delta_{\beta\text{-CD}})$*	$\delta_{HP\text{-}\beta\text{-CD}}$	$\Delta_{DMA/HP\text{-}\beta\text{-CD}}$	$\Delta(\delta_{DMA/HP\text{-}\beta\text{-CD}}$ and $\delta_{HP\text{-}\beta\text{-CD}})$*
H-1	5.03	5.10	0.07	5.041	5.109	0.068
H-2	3.62	3.68	0.06	3.651	3.730	0.079
H-3	3.94	3.99	0.05	3.986	4.066	0.080
H-4	3.55	3.61	0.06	3.505	3.577	0.072
H-5	3.82	3.86	0.04	3.798	3.849	0.051
H-6	3.85	3.90	0.05	3.826	3.913	0.087
H-7	—	—	—	1.096	1.196	0.100
H-8	—	—	—	5.203	5.283	0.080

* Chemical shifts difference between CD complexed with DMA and CD free.

Table 3
Chemical shifts (ppm) for the protons of DMA and for protons of DMA complexed with CDs

Proton	δ_{DMA}	$\delta_{DMA/\beta\text{-CD}}$	$\Delta(\delta_{DMA/\beta\text{-CD}}$ and $\delta_{DMA})$*	$\delta_{DMA/HP\text{-}\beta\text{-CD}}$	$\Delta(\delta_{DMA/HP\text{-}\beta\text{-CD}}$ and $\delta_{DMA})$*
H-1	7.548	7.586	0.038	—	—
H-4	6.204	6.233	0.029	6.239	0.035
H-18	1.033	1.086	0.053	1.098	0.065
H-22	0.907	0.945	0.038	0.957	0.050
H-24	2.227	2.219	−0.008	2.257	0.030

* Chemical shifts difference between DMA complexed with CD and DMA free.

2.6. H-NMR measurements

NMR was carried out using a BRUKER DPX spectrometer operating at 300 MHz. The concentrations of DMA and DMA/CD complexes in D_2O/deuterated methanol (50:50) were 2.0 mg/ml of DMA. The conditions for Fourier transform measurements were: acquisition time, 3 s; pulse angle, 30°; delay time, 2 s; number of spectra, 32. The chemical shift at 4.8 ppm due to residual solvents (H_2O and HDO) was used as internal reference.

2.7. DSC measurements

DSC measurements were performed using a Seiko 2400 (Seiko Instruments, Tokyo, Japan) linked to a Seiko 2100 Data Analysis station. Samples were hermetically sealed in steel pans and scanned over the temperature range of 25–270°C at a heating rate of 10°C/min.

2.8. Kinetic stability measurements

The stability of DMA/β-CD and HP-β-CD in methanolic solution (20% v/v) was studied in the presence of β-CD (1% w/v) and HP-β-CD (10% w/v), respectively. Methanol was used as the solvent due to the poor aqueous solubility of the DMA in the absence of CD. Solutions were prepared by dissolving the complexes at 80 μg/ml in the respective CD solution, stored in amber glass ampoules, placed in a constant temperature (37, 42 and 50°C) and removed at the appropriate intervals. The remaining DMA was assayed according to the peak height measured by the HPLC method described previously. The first-order rate constants (K_{obs}) and the time for 10% DMA degradation (t_{90}) for the overall degradation of DMA were determined from the slopes of the linear semilogarithmic plots of the remaining DMA versus time. The degradation parameters at 25°C were determined using Arrhenius plots.

2.9. Molecular model study

The most probable structure of the DMA/β-CD inclusion complex was determined using the Chem 3D molecular modelling program, β versions, 3.5.1. and 4.0.1. Serial number, 99999. The use of these β versions was authorized by Cambridge Software Corporation.

3. Results and discussion

Fig. 1 shows the aqueous phase-solubility of DMA in different concentrations of the CDs. The differences in the solubility curves are clearly noted. The solubility of DMA increased with increasing β-CD, γ-CD and HP-β-CD concentrations. The solubility studies indicated that the DMA probably formed complexes with the three CDs. The solubility plot for DMA/β-CD and DMA/HP-β-CD showed an A_L type solubility curve. This type of diagram indicates that the solubility of DMA increased linearity with the increase of the CD concentration, depending on the aqueous solubility of the CD (Higuchi and Connors, 1965). If concentrations of β-CD greater than 13.2 mM could be used then an A_N type solubility curve may be well be obtained, which would be associated with an alteration in the effective nature of the solvent in the presence

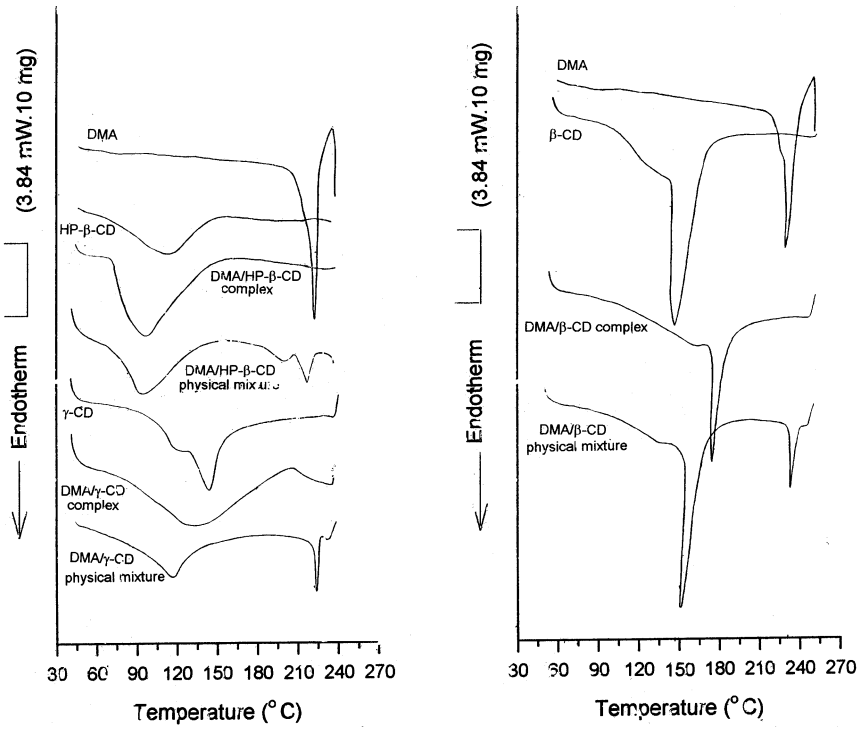

Fig. 2. DSC thermograms of DMA, CDs, DMA/CDs complexes and their respective physical mixtures.

of large amounts of CD, thus leading to a change in the complex formation constant. An alternative explanation for the A_L type curve is self-association of β-CD molecules at higher concentrations. In the B_S diagram of DMA/γ-CD complex, the initial rising portion is followed by a plateau region followed by decrease in DMA concentration, with precipitation of a microcrystaline complex at high γ-CD concentration (39.6 mM).

The solubility of DMA in the presence of CDs are presented in Table 1. DMA solubility increased 48- and 88-fold when β-CD and HP-β-CD were used as complexing agents, respectively. This difference in the performance of the CDs can be related to the K_C of the complex which is an empirical parameter that describes the increase in apparent solubility of DMA in the presence of CD. Thus, assuming that a 1:1 complex is initially formed, the values of K_C increased in the order γ-CD > β-CD > HP-β-CD. The results suggested that the spatial relationship between host and guest molecules (steric and hydrophobic factors) was responsible for their interaction. The γ-CD solubilized the DMA better than β-CD probably because it has the largest cavity size and the highest aqueous solubility among the non-substituted CDs. In contrast, with DMA/CD complexes in solution, solid state complexes exist as static species with a constant stoichiometry. This increase in the DMA solubility may be attributed to complex formation. It is difficult to determine the exact stoichiometry of the β-CD and HP-β-CD complexes because of their high aqueous solubility. Thus, these interactions were investigated utilizing a spectrophotometric method. The difference between the intensities of UV absorbance of DMA alone and in presence of CDs showed an abrupt change in slope at mole fraction of 0.5 DMA/0.5C. This change indicates that DMA forms 1:1 complexes with β-CD and HP-β-CD. The plots were highly symmetrical indicating that no other complex is present (Ammar and El-Nahhas, 1995). DMA formed an 1:2 complex with γ-CD, that is a characteristic stoichiometric

ratio for complexes obtained from precipitation (Fig. 1).

The ^{1}H-chemical shifts of CDs in presence of DMA are summarized in Table 2. It can be observed that a paramagnetic shift of the CDs protons occurred when they were complexed with DMA. Table 3 summarizes the effects of β-CD and HP-β-CD on some ^{1}H-chemical shifts of DMA. In the ^{1}H-NMR spectra all CDs protons were deshielded, indicating that the DMA molecule created paramagnetic anisotropy effects in the interior of the cavity due to weak interactions (van der Waals forces) with the internal hydrogen atoms (H-3 and H-5). Similar observations were reported by Nishijo and Nagai (1991) when 8-anilinonaphthalene-1-sulfonate was complexed with β-CD. The DMA molecule can be totally or partially enclosed in the CD cavity. There may be an interaction between the A ring of the DMA inside the CD because the H-1 and H-4 protons are deshielded. The H-24 (methyl group) showed only a slight downfield shift, due to the freely rotating ester group. However, it was observed that H-18 and H-22, which are near to the ester group, deshielded, suggesting that this part of the DMA molecule could be accommodated. The magnitude of the shifts presented by DMAM/HP-β-CD complex was stronger than DMA/β-CD complex, probably due to the internal hydroxypropyl groups of that CD.

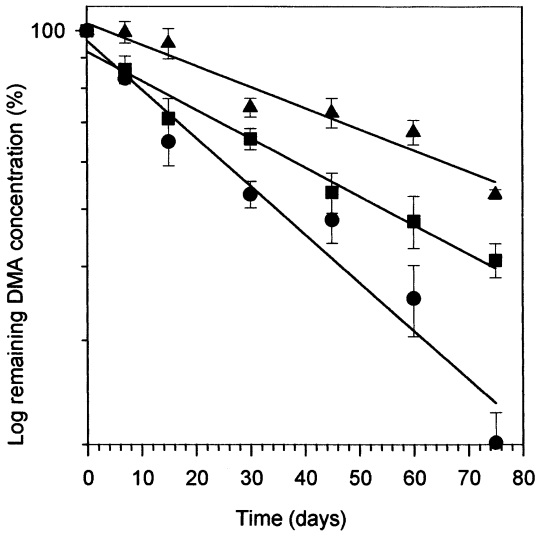

Fig. 3. Semilogarithmic representation of the degradation at 37°C: (●) DMA; (■) DMA/β-CD and (▲) DMA/HP-β-CD.

Table 4
Degradation constants (K_{obs}) and time for 10% degradation of DMA (t_{90} at 25°C)

Compound	K_{obs} 10^{-3} (days^{-1})[a]	t_{90} at 25°C (days)
DMA	13.90	7.5
DMA/β-CD[b]	2.60	40.8
DMA/HP-β-CD[c]	1.13	92.9

[a] Obtained from Arrhenius equation to 25°C.
[b,c] Incorporated in methanolic solutions of β-CD (1.0% w/v) and HP-β-CD (10.0% w/v), respectively.

Thermal analysis has been reported as a method to characterize CD complexes (Pedersen et al., 1993; Sharma et al., 1995). Fig. 2 illustrates the DSC profiles of DMA, CDs, physical mixtures and complexes. In the DMA thermogram, an endothermic transition was observed at about 230.0°C, corresponding to its fusion peak. β-CD, HP-β-CD and γ-CD exhibit transitions at about 148.8, 105.1 and 151.0°C, respectively. The transitions attributed to CDs can be extended due to the release of water from the molecules. Sharp and broad endothermic transitions approximating to the DMA and CD transitions, respectively, were seen in the physical mixture. A new transition at about 205.1°C can be observed in the physical mixture of DMA and HP-β-CD. This transition could be due a possible complex formation during the physical mixture procedure, Abdel-Rahman et al. (1994). New characteristic peaks (different than drug and CD transitions) at about 176.0, 121.2 and 149.9°C for the DMA/β-CD, DMA/HP-β-CD and DMA/γ-CD complexes, respectively. These thermal behaviour changes may result from the formation of a new compound through weak interactions between the DMA and CDs.

Several solvent systems have been used to relate partition coefficients to percutaneous absorption. IPM is a suitable system, since its polar and non-polar nature mimics the complex nature of the skin (Barry, 1993). In our studies, $K_{IPM/water}$

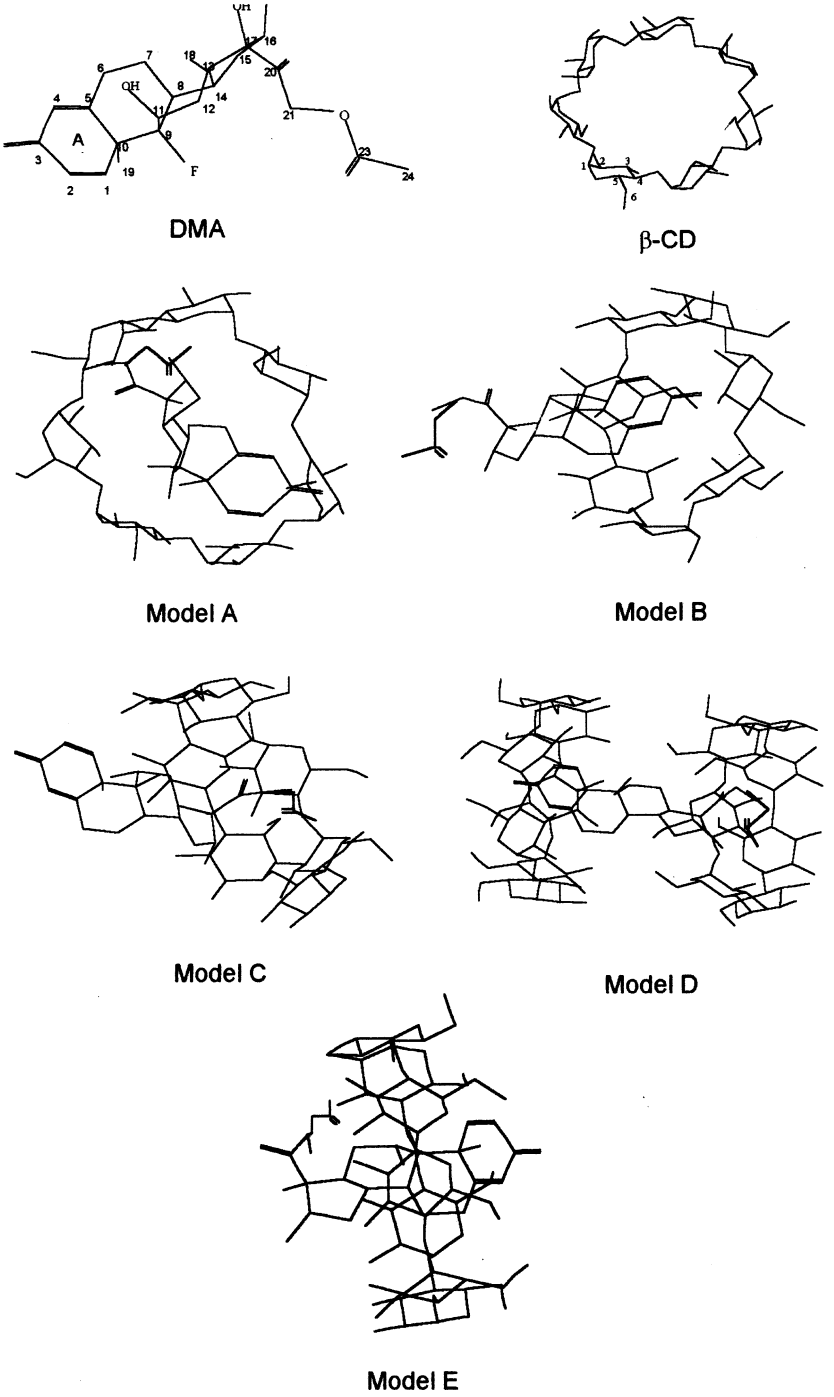

Fig. 4. Molecular models of DMA/β-CD complex: model A, the DMA molecule is whole enclosed into CD cavity; model B, only the A ring of DMA is interacted inside the CD; model C, only the ester group of DMA is enclosed; model D, one DMA molecule is interacted with two CD molecules; model E, model A of DMA/β-CD complex at 70°C.

values of the complexes ($K_{IPM/water} \cong 14$) were approximately half that of DMA ($K_{IPM/water} \cong 28$), showing that the lipophilic characteristics of DMA decreased when it was complexed with CD. Increased aqueous solubility in the presence of CD was not directly related to the change in lipophilicity.

In addition to improving the aqueous solubility of DMA, its complexation with β-CD and HP-β-CD was effective in enhancing the stability of the drug. The effect of these CDs on the stability of DMA was studied as a function of temperature. Fig. 3 illustrates the first order plots for degradation of DMA in the presence and absence of β-CD and HP-β-CD at 37°C. Kinetic stress test indicated a linear relationship between the $\log K_{obs}$ and the reciprocal absolute temperature. The degradation constants (K_{obs}) and the time at which 10% DMA was degraded (t_{90}) were calculated by first order kinetic equations.

The results of stability studies show that the degradation of DMA in methanolic solutions (20% v/v) decreased dramatically when the drug is complexed with β-CD and HP-β-CD (Table 4). Complexation with these CDs enhanced the stability of DMA by 5.4 and 12.4 times more for the β-CD and HP-β-CD, respectively. One possible explanation for the protective effect is that the ester group is at least partly enclosed and hence shielded against the hydrolytic attack of the water.

The Chem 3D modelling program gave four possible models (Fig. 4) for the most probable structure of the DMA/β-CD inclusion complex based on the energetic behaviour of the molecules. Model D was the less probable because the structure was energetically unfavorable. In addition, the stoichiometry 1:2 disagrees with the spectrophotometric study. The models B and C do not concur with the stability and ^{1}H-NMR results, respectively. The most probable structure seems to be the model A where the ester group is protected against hydrolytic attack and the carbonyl group of the A ring is enclosed, confirming the stability and ^{1}H-NMR studies, respectively. The suitability of model A was also considered as a function of temperature. At temperatures higher than 60°C, the DMA molecule fits in the CD in a different manner. The ester group and the A ring being out of the cavity (model E). This behaviour concurs with the results of stability studies at 70°C, where the DMA/CD complexes were less stable than the free DMA.

The results presented show that the inclusion of DMA in β-CD, HP-β-CD and γ-CD in the solution state, results in an increase in solubility of DMA. The partition coefficient ($K_{IPM/water}$) was decreased by the complexation with CD, suggesting that the partition of DMA into or through a lipid membrane may be improved. In addition, the stability of DMA was greatly enhanced when this drug was complexed with β-CD and HP-β-CD. The ester group probably is enclosed into the cavity of the CDs, resulting in protection against hydrolysis.

It is interesting to consider that the pre-treatment of the skin with CDs can induce some changes in the stratum corneum. Our previous work (Bentley et al., 1997) suggested that the HP-β-CD may cause extraction of lipid from the stratum corneum and thus can increase the permeation of drugs through the skin. However, in the case of pre-treatment with CD, the cutaneous permeability of a drug is only increased if it does not form an inclusion complex (Legendre et al., 1995). In the present work, the inclusion complexes with CDs can be considered as a promising way to enhance the physicochemical properties of the drugs for the use in topical and–or transdermal delivery system since their aqueous solubility is increased.

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