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Solubility and Dissolution Rate of Progesterone-Cyclodextrin-Polymer Systems

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ABSTRACT This contribution focused on the solubility improvement of the poorly water-soluble steroid hormone progesterone which, in its natural state, presents a reduced oral bioavailability. In the first part of this study, two simple, reproducible methods that were candidates for use in the preparation of inclusion complexes with cyclodextrins were investigated. Solubility capacities of the progesterone complex with hydroxypropyl-β-CD (HPβ-CD), hydoxypropyl-γ-CD (HPγ-CD), permethyl-β-CD (PMβ-CD), and sulfobutylether-β-CD (SBEβ-CD), prepared by the freeze-drying and precipitation methods, were evaluated by Higuchi phase solubility studies. The results showed that HPβ-CD and PMβ-CD were the most efficient among the four cyclodextrins for the solubilization of progesterone, with the highest apparent stability constants. Therefore, dissolution studies were conducted on these latest progesterone/cyclodextrin complexes and physical mixtures. Two additional natural cyclodextrins, β-CD and γ-CD, were taken as references. Hence, the influence of more highly soluble derivatives of β-CD (HPβ-CD, PMβ-CD) on the progesterone dissolution rate, in comparison to pristine β-CD, alongside an increase in the cavity width for γ-CD versus β-CD, were investigated. The dissolution kinetics of progesterone dissolved from HPβ-CD, PMβ-CD, and γ-CD revealed higher constant rates in comparison to β-CD. Therefore, the aim of the second part of this study was to investigate the possibility of improving the dissolution rate of progesterone/β-CD binary systems upon formation of ternary complexes with the hydrophilic polymer, PEG 6000, as β-CD had the smallest progesterone solubility and dissolution capacity among the four cyclodextrins studied (β-CD, HPβ-CD, HPγ-CD and PMβ-CD). The results indicated that dissolution constant rates were considerably enhanced for the 5% and 10% progesterone/β-CD complexes in PEG 6000.

The interaction of progesterone with the cyclodextrins of interest on the form of the binary physical mixtures, complexes, or ternary complexes were investigated by differential scanning calorimetry (DSC) and Fourier transformed-infrared spectroscopy (FT-IR). The results proved that progesterone was diffused into the cyclodextrin cavity, replacing the water molecules and, in case of ternary systems, that the progesterone β-cyclodextrin was well

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dispersed into PEG, thus improving progesterone bioavailability for subsequent oral delivery in the same way as derivatized cyclodextrins. The present work proves that ternary complexes are promising systems for drug encapsulation.

KEYWORDS Progesterone, Cyclodextrins, PEG 6000, Ternary complex

INTRODUCTION

The steroid hormone progesterone is a lipophilic drug used in menopausal hormone replacement therapy and reproductive control function. However, its oral delivery is limited due to its poor aqueous solubility (3.79×10^{-5} M) and its low tolerance when administered in high doses. To overcome these undesirable properties, suitable carriers were developed to facilitate the drug administration specifically to the target site, optimizing delivery amount over a predetermined period.

The complexation approach has been frequently used to improve the aqueous solubility, wettability, and bioavailability of various drugs (Shimpi et al., 2005). Complexation has also been used to decrease the toxicity of drugs, or to modify some of their physicochemical features. In particular, cyclodextrins have been extensively used to enhance the oral bioavailability and stability of a great number of pharmaceuticals. A thorough review of pristine and derivatized cyclodextrins synthesized for drug delivery was presented by Challa et al. (2005), and the methods of preparation and characterization of drug complexes with cyclodextrins were reviewed by Astakhova and Demina (Astakhova & Demina, 2004). Among the cyclodextrins investigated in the above-mentioned reviews, the fully per-o-methylated β-CD (PMβ-CD), hydroxypropyl-β-CD (HPβ-CD), and the anionic sulfobutyl ether-β-CD (SBEβ-CD), which have higher aqueous solubility levels than the parent β-CD, could be good candidates for oral delivery (Loftsson & Brewster, 1996; Stella & Rajewski, 1997). β-CD is effectively limited in its pharmaceutical application by its low aqueous solubility (18.5 mg/mL) at 25°C (Szetjli, 1988) and its demonstrated nephrotoxicity when administered parenterally, which limit its usage to oral forms. For instance, Pitha and coworkers (Pitha et al.,1985; Pitha et al., 1986) and Shimpi et al. (2005) have shown, respectively, that progesterone and testosterone could be solubilized with HPβ-CD and effectively absorbed after a sublingual/buccal administration, thus avoiding the hepatic first-pass metabolism of the drug. X-ray powder diffractometry and thermal analysis undertaken by Lin et al. (1996) proved the formation of inclusion complexes between progesterone and β- and γ-CD. Consequently, the ability of the various derivatives to solubilize progesterone through complexation was studied in this work, and the results were compared to those of β-CD.

Alternative systems for progesterone delivery have been presented by several authors. Inclusion complexes of progesterone with cyclodextrins have been studied when they were incorporated in various ternary systems: progesterone/HPβ-CD into chitosan by spray-drying or freeze-drying methods (Cerchiara et al., 2003), into liposomes (McCormack & Gregoriadis, 1994), solid lipid nanospheres (Cavalli et al., 1999), or bovine serum albumine nanospheres (Luppi et al., 2005).

New strategies need to be developed for decreasing the amount of cyclodextrins necessary in oral dosage forms, so that both the cost and the toxic effects of drugs containing cyclodextrins can be curtailed. This study was devoted to the preparation of a ternary complex with a suitable auxiliary substance: PEG 6000. PEG 6000 is a relatively low cost and highly water-soluble polymer of ethylene glycol, and it has been approved by the Food and Drug Administration (FDA) for internal use in humans (Harris, 1985). As stated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), pure polyethylene glycols have toxicity levels that are inversely proportional to their molecular weights (JECFA, 1980). Therefore, we selected a linear, long-chain polymer with a limited rate of absorption by the gastrointestinal tract. PEG 6000 crystallizes, forming lamellae with chains either fully extended or folded once or twice, thus allowing the penetration and incorporation of drug and inclusion complexes (Verheyen et al., 2001). The strategy, consisting of preparing ternary complexes with hydrophilic polymers, has been studied by several authors. Drug (indomethacin and griseofluvin) α-, β-, γ-cyclodextrin inclusion complexes in PEG 6000 were studied by Wulff and Aldén (Wulff & Aldén, 1999), nicardipine/

β-CD in PEG 2000 by Quaglia et al. (2001), and glimepiride/β-CD, HPβ-CD in PEG 4000 and PEG 6000 by Ammar et al. (2006). Nandi et al. (2003) put in evidence the synergistic effect of PEG 400 and Trappsol® HPB (hydroxypropyl beta CD) on the enhancement of progesterone solubility. Bibby et al. (2000) presented a review of works that studied the effects of polymer on the drug releases from inclusion complexes incorporated in various polymer matrices.

Herein, we investigated the possibility of enhancing the solubility and the dissolution rate of progesterone by two methods: through the formation of inclusion complexes with derivatized cyclodextrins and by combining the synergistic effect of cyclodextrins and PEG 6000. The latter tentative was led through the formulation of ternary complexes of progesterone/β-CD complexes in the hydrophilic polymer. The first part of the study was devoted to the preparation of progesterone inclusion complexes, and to the study of the influence of more soluble derivatives of β-CD (HPβ-CD and PMβ-CD were chosen as host molecules) or of derivatives with higher cavity width (γ-CD) on the dissolution rate of the poorly water-soluble sex-hormone progesterone, in comparison to the influence of β-CD. In the second part of the study, the possibility of improving the dissolution rate of progesterone included in the β-CD, through the formation of ternary systems with PEG 6000, was investigated, as β-CD had the smallest progesterone solubility and dissolution capacity among the four cyclodextrins studied (β-CD, HPβ-CD, HPγ-CD and PMβ-CD).

MATERIALS AND METHODS

Materials

Progesterone, in micronized powder form, was purchased from Sigma (MW: 314.45 g/mol). β-CD was obtained from Roquette Frères (Lestrem, France) (1135 g/mol, with an aqueous solubility of 18.5 mg/mL); γ-CD (1297 g/mol, 232 mg/mL), hydroxypropyl β-CD (HPβ-CD, 1488 g/mol, 400 mg/mL), and hydroxypropyl γ-CD (HPγ-CD, 1713 g/mol, 600 mg/mL) were provided by Wacker Chemie Gmbh (Munich, Germany). Permethylated β-CD (PMβ-CD, 1330 g/mol, 800 mg/mL) was received from Orsan company (Pointet Girard, France). Captisol® (Sulfobutyl ether β-CD, SBE-CD, 2163 g/mol, 2100 mg/mL) was supplied by Cydex, Inc. (Kansas City, KS, USA). PEG used in this study was Lutrol® E 6000 commercialized by BASF. Injectable preparation water was obtained from Fresenius Kabi (Sévres Cedex, France). Methanol was purchased from Prolabo. All other reagents were of analytical grade.

Phase Solubility Studies

The phase solubility studies were performed according to the method developed by Higuchi and Connors (Higuchi & Connors, 1965). Excess amounts of progesterone were added to 5-mL tubes containing aqueous solutions of increasing concentrations of PMβ-CD (0–300 mM), SBEβ-CD, or Captisol® (0–100 mM), HPγ-CD (0–300 mM), HPβ–CD (0–60 mM), and then shaken at 37°C under a constant agitation rate.

Aliquots were withdrawn at the equilibrium after one week. The aliquots were subsequently centrifuged, supernatant filtered on 0.22 μm cellulose nitrate membrane, and spectrophotometrically assayed for drug content at 250 nm using a UV-Vis Beckman UV 24 double beam spectrophotometer equipped with a dissolution system (SAFAS, Monaco). Each experiment was carried out in duplicate. The apparent binding capacities were calculated from the straight line portion of the phase solubility diagram according to the Higuchi-Connors equation, given below in Eq. (1):

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

Preparation of Solid Inclusion Complexes

The following inclusion complexes containing cyclodextrins were prepared:

- progesterone/β-CD complex: molar ratio 1:2
- progesterone/γ-CD complex: molar ratio 2:3
- progesterone/HPβ-CD complex: molar ratio 2:5
- progesterone/PMβ-CD complex: molar ratio 1:1

The optimal molar ratios for the formation of inclusion complexes were taken from Lin et al.

(1992) for progesterone/β-CD and progesterone/γ-CD, resulting in a maximum preparation yield defined by $\frac{precipitate\,(w)}{drug\,(w)+CD\,(w)}\times 100$. In the present work, similar experiments were performed on progesterone/PMβ-CD complexes and progesterone/HPβ-CD complexes.

Preparation of Physical Mixtures

The physical mixtures of progesterone/cyclodextrin, progesterone/cyclodextrin/PEG 6000, and progesterone/cyclodextrin complex/PEG 6000 were obtained by thoroughly mixing the various components together with a spatula. The following physical mixtures were prepared by this method:

- progesterone/cyclodextrin:
  - progesterone/β-CD: molar ratio 1:2
  - progesterone/γ-CD: molar ratio 2:3
  - progesterone/HPβ-CD: molar ratio 2:5
  - progesterone/PMβ-CD: molar ratio 1:1
- progesterone/cyclodextrin/PEG 6000
  - 10%, 50% of physical mixture progesterone/β-CD dispersed into PEG 6000.
- progesterone/cyclodextrin complex dispersed into PEG 6000
  - 5%, 10%, and 50% of progesterone/β-CD complex dispersed into PEG 6000.

These samples were allocated to the dissolution studies, and the DSC and IR analyses. For comparison purposes, molar ratios for the physical mixtures were maintained at a level identical to those used for solid inclusion complexes.

Precipitation Method

Inclusion progesterone/β-cyclodextrin and progesterone/γ-cyclodextrin complexes were prepared by the precipitation method. β-CD and γ-CD were dissolved in distilled water. Progesterone was added to these solutions in the carefully predetermined molar ratio (progesterone over cyclodextrin) mentioned above. The entire solutions were stirred with a magnetic stirrer for two weeks in a hood at 37°C ± 2°C. The resultant solutions were evaporated to dryness. After solidification, the products were ground in a mortar. These samples were assigned to dissolution studies or, solely in the case of the progesterone/β-cyclodextrin complex, melted for the preparation of ternary systems. They were subsequently characterized by DSC techniques.

Freeze-Drying Method

Inclusion progesterone/HPβ-CD cyclodextrin and progesterone/PMβ-cyclodextrin were prepared by the freeze-drying method. PMβ-CD and HPβ-CD were first dissolved in distilled water. Progesterone was added to these solutions in the carefully predetermined molar ratio (progesterone over cyclodextrin) mentioned above. The entire solutions were stirred with a magnetic stirrer for one week in a hood at 37°C ± 2°C. The solutions were then filtered and freeze-dried. The freezing phase was carried out on a shelf at −50°C and this phase was continued for at least three hours. Lyophilization parameters, validated by preliminary experiments, were as follows: vacuum $<$ 200 mTorr, condenser $<$ −40°C, shelf at +30°C. Vials containing the freeze-dried products were plugged immediately after removal from the freeze dryer. These samples were allocated to dissolution studies and DSC analysis.

Melting Method: Preparation of Progesterone-Cyclodextrin-Polymer Ternary Systems

A physical mixture consisting of progesterone, cyclodextrin, and PEG 6000, or ternary systems consisting of solid dispersions of the progesterone complex were prepared by the melting method.

Progesterone and excipients (β-CD and PEG) were accurately weighed to obtain a total mass of 5 g and they were mixed with a Turbula stirrer (Prolabo) in a glass tank immersed in an oil bath regulated at a temperature of 130°C, which corresponds to the melting point of progesterone. As soon as a homogeneous liquid was obtained, heating was discontinued and the solution was allowed to cool slowly (at an average rate of 1°C/min). After solidification at room temperature, the product was introduced in a desiccator for 48 h. The samples were then crushed, sifted, and stored in brown glass tubes.

Dissolution Studies

Dissolution studies of pure drugs and binary or ternary systems were carried out using the rotating

paddle method with a USP XXII apparatus 2 (Erweka DT6R, Heustenstamm, Germany), coupled with a measurement device from SAFAS (Prolabo) and a double beam spectrophotometer from Beckman (UV 24). Each dissolution test was performed by spreading the powder samples, equivalent to 5 mg of progesterone, over the dissolution medium consisting of 1 L of distilled and degassed water, in a thermostatic bath at 37°C, and with a pH value near 7.0. Aqueous solutions were filtered and continuously pumped with a peristaltic pump to the flow cell of the spectrophotometer. Absorbance was monitored at 250 nm for progesterone quantification. The dissolution experiments were conducted in triplicate. The dissolution tests were carried out for 60 min, and the results were subsequently computed with a standard calibration curve of the drug. A correction was made for volume loss after each sample was discarded.

Physicochemical Characterizations

DSC

Thermal analysis was performed using a PerkinElmer differential scanning calorimeter (DSC-4), which was equipped with a compensated power system. All samples were weighed (4 mg) and heated at a scanning rate of 10°C/min to a temperature level between 30°C and 250°C under a nitrogen gas flow. Aluminum pans and lids were used for all samples. Energy calibrations were done with a known mass of indium with 99.99% purity, and a melting point of 156.6°C. Temperature calibrations were performed with indium and benzoic acid.

FT-IR Analysis

The FT-IR spectra acquired were taken from dried samples. An FT-IR machine from PerkinElmer equipped with an ATR (Ge crystal) from Pike Technologies was used for the analysis in the frequency range between 4000 cm^{-1} and 700 cm^{-1}, at a resolution of 8 cm^{-1}.

UV Analysis

UV measurements were taken with a double beam Beckman UV 24 spectrophotometer that was equipped with a dissolution system from SAFAS. Calibration curves were obtained with progesterone diluted in methanol. Progesterone dosage was set at 250 nm.

RESULTS AND DISCUSSION

Influence of Initial Molar Ratio of the Raw Materials

Preparation yield was evaluated for each molar ratio. The results indicated that the best molar ratios were 1/2 for progesterone/βCD and 2/3 for progesterone/γ-CD complexes, corresponding to preparation yields equal to 91.2 ± 1.3% and 93.7 ± 0.9%, respectively. Other molar ratios corresponded to lower preparation yields. These results were in accordance with the findings of Liu et al. (1990), who demonstrated that the solubility approximated the solubility level of the pure 1:2 steroid complex for β-CD concentrations above 20 mM.

Solubility Diagrams

Generally, drugs are incorporated into cyclodextrins and they form inclusion complexes in drug-CD ratios of 1:1 or 1:2 (Uekama et al., 1998). Interaction studies between progesterone and the various cyclodextrins provided solubility diagrams of various types (Fig. 1).

The PMβ-CD and HPβ-CD phase solubility diagrams obtained in this study exhibited typical A_L curves corresponding to progesterone/cyclodextrin 1:1 molar ratio, confirming, for the latter, the results obtained by Brewster (1991) and Cerchiara et al. (2003). The

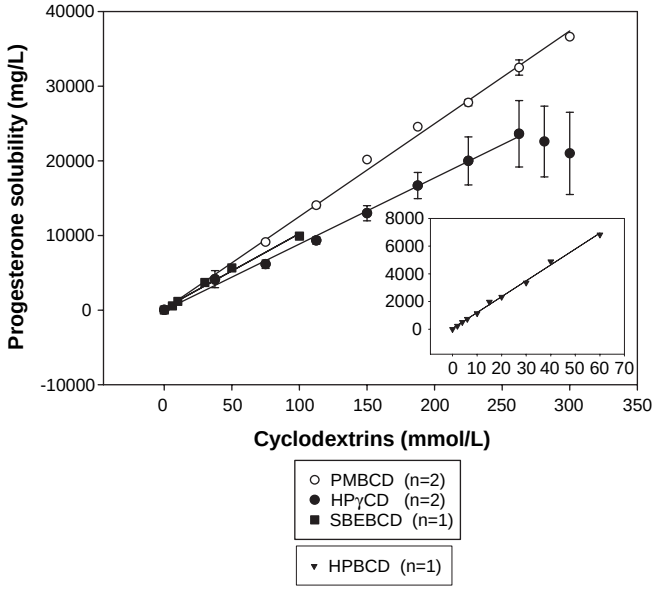

FIGURE 1 **Phase-Solubility Digrams for Progesterone in the Presence of PMβ-CD, HPγ-CD, SBEβ-CD, and HPβ-CD in Water at 37°C.**

A_L-type diagram is typical for the formation of a soluble complex between progesterone and cyclodextrin, corresponding to an apparent progesterone solubility level above 30 mg/mL. The inclusion compounds formed are entirely soluble in water in equimolar ratios. The B_S-type solubility diagrams for β-CD (Uekama et al., 1982), γ-CD (Lin et al., 1992) and HPγ-CD (present study) correspond to the formation of insoluble complexes. The linear portion reflects the increase of apparent progesterone solubility at 37°C to 0.04 mg/mL, 0.45 mg/mL, and 23.6 mg/mL, respectively. The increase of the apparent progesterone solubility reaches a plateau, which represents the limit of complex solubility, respectively, before starting to decrease when the concentration of cyclodextrins increases due to inclusion complex precipitation. SBEβ-CD solubility diagram of A_N type was typical for a soluble complex formation. Progesterone solubility decreased when cyclodextrin concentration was decreased. The apparent solubility of progesterone with SBEβ-CD was calculated to be 9.9 mg/mL. From these results, it is evident that the apparent water solubility of progesterone (without cyclodextrins, 0.011 mg/mL) was increased significantly through the formation of inclusion complexes. The initial solubility enhancement of progesterone was 400% for β-CD, 4000% for γ-CD, 90000% for SBEβ-CD, 200000% for HPγ-CD, 300000% for PMβ-CD, and 360000% for HPβ-CD.

The apparent solubilities of PMβ-CD, HPγ-CD, and SBEβ-CD in each complex were determined to be 300 mmol/L (399 mg/mL), 260 mmol/L (445 mg/mL), and 100 mmol/L (216.3 mg/mL), respectively. These thresholds were lower than the corresponding thresholds for the cyclodextrins alone (800, 600, 2100 mg/mL, respectively), and the complexes were also limited by an noticeable increase in viscosity in the presence of progesterone.

From DSC measurements, Zannou et al. (2001) have confirmed that β-CD was more rigid than γ-CD due to intermolecular hydrogen bonding occurring inside the cavity. This fact could be attributable to the lower aqueous solubility of β-CD (18.5 mg/mL) and the lower apparent progesterone aqueous solubility in the β-CD/progesterone complex (0.04 mg/mL), compared to the corresponding values for γ-CD (232 mg/mL and 0.45 mg/mL, respectively). Nevertheless, in that study, the authors highlighted a reverse flexibility when the pristine cyclodextrins were derivatized. Zannou and coworkers concluded that the cause of the reverse flexibility was the disruption of hydrogen bonding due to the substitution of some hydrogen atoms by hydroxypropyl and sulfobutylether groups. This observation may be in agreement with the higher aqueous solubility of HPγ-CD (600 mg/mL) compared to γ-CD (232 mg/mL), and also with the better progesterone apparent solubility in the HPγ-CD/progesterone complex (23.6 mg/mL) compared to the apparent solubility observed in the γ-CD/progesterone complex (0.45 mg/mL). The same conclusion can also be reached by comparing the solubility of β-CD (18.5 mg/mL) with the solubility levels of HPβ-CD (400 mg/mL), PMβ-CD (800 mg/mL), and SBEβ-CD (2100 mg/mL). Moreover, derivatized β-CD was found to improve the apparent solubility of progesterone in the respective complexes (> 40, 36.6, 9.9 mg/mL). The general increase in the solubility of derivatized cyclodextrins, which is related to the presence of hydroxypropyl or methylated groups substituting for external hydroxyl groups, has been studied by González-Gaitano et al. (2002). These authors demonstrated that the aggregation displayed by CDs with partial substitution of the hydroxyl groups was also much weaker, thus confirming the implication of the hydrophilic rims of the pristine CDs in the process through hydrogen bonding. Similar results were obtained by Coleman et al. (1992) and Szente et al. (1998), in studies in which the solubility levels of PMβ-CD and β-CD were compared. This aggregation phenomenon would be responsible for the limited aqueous solubility of the pristine CDs. Furthermore, this result is in agreement with the increase in the apparent solubility of progesterone for derivatized cyclodextrin/progesterone complexes observed in our study.

With regard to the significant progesterone aqueous solubility observed in the sulfobutylether-cyclodextrin complex, our results indicate that that solubility could be attributed to the butyl micellar arms that extend into the depths of the hydrophobic cavities of the CDs, as has been stated by Ashwinkumar and Moji. (Ashwinkumar & Moji, 2001).

On the assumption that the complexes obtained were of 1:1 stoichiometry, the apparent stability constants (K_c) of the inclusion complexes can be calculated from the straight line portion of the curves according to the following equation:

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

where K_c is the apparent stability constant (M^{-1}). The intercept represents the progesterone solubility value in the absence of cyclodextrin (M), and the slope is given by the curve of progesterone solubility (M) in the presence of cyclodextrin (M) = f (cyclodextrin).

The K_c values derived by using the solubility method should be interpreted carefully and cautiously because the calculations may imply assumptions that are not always fulfilled (Djedaïni & Perly, 1991). In any case, the present data suggest the formation of stable complexes and a highly efficient complexation procedure.

Increases were observed in the apparent stability constants between progesterone and the various cyclodextrins studied. The increases were respectively from HPγ-CD (11,221 M^{-1}, SBEβ-CD (13,322 M^{-1} in this study, 18,300 in Cydex, 2003), HPβ-CD (16,500 M^{-1} this study, 11,200 in Cydex, 2003) to PMβ-CD (18,654 M^{-1}). Consequently, the results of this study indicate that β- and γ-derivatives are thermodynamically favorable to progesterone inclusion in their hydrophobic cavity, indeed increasing the progesterone apparent solubility. β-CD was also very effective in stabilizing the complex β-CD/progesterone. Preiss et al. (1994) showed that the molar ratio of β-cyclodextrin complexes with the steroid progesterone, studied by phase solubility, was 1:2 progesterone:β-cyclodextrin, with a stability constant equal to 13,300 M^{-1} (Uekama et al., 1982). Liu et al. (1990) determined the two equilibrium association constants, K_1 and K_2, respectively, for a two-step 1:2 association process, enabling a study of the molecular associations between β-CD and progesterone. The K_1 value for the 1:1 complex between β-CD and progesterone was estimated to be 24,705 M^{-1}, where as the corresponding value was only 686 M^{-1} for the 1:2 complex. The 1:1 formation is apparently the preferred process for the binding of the 1:1 complex with the second molecule of β-CD. In these two cases, the authors highlighted the high affinity of progesterone for β-CD. This strong affinity is certainly due to the high hydrophobicity of progesterone, as would be expected from its partition coefficient in an octanol–water system, P_{oct} = 7410 (Tomida et al., 1978).

Dissolution Studies

As the initial solubility enhancement of progesterone was less important in the case of SBEβ-CD than for the other derivatized cyclodextrins, and because the apparent stability constant for HPγ-CD was the lowest of the four cyclodextrins studied, we chose to focus dissolution and ternary system studies on HPβ-CD and PMβ-CD, using β-CD and γ-CD as references.

Progesterone/Cyclodextrins Complexes and Physical Mixtures

Dissolution of progesterone/PMβ-CD, γ-CD, and HPβ-CD complexes were instantaneous (Fig. 2 The entire progesterone that was included in the cyclodextrins was released in less than 3 min. Rate constants were evaluated by a 1-component exponential decay model using the kinetic results given in Table 1. The results indicated that the rate constants were equal to 1.555, 1.450, and 0.789 min^{-1}, with half-lives equal to 0.446, 0.478, and 0.878 min, respectively. This improvement was less pronounced in the case of β-CD. Effectively, its kinetic constant rate was equal only to 0.034 min^{-1}, with a half-life equal to 20 min.

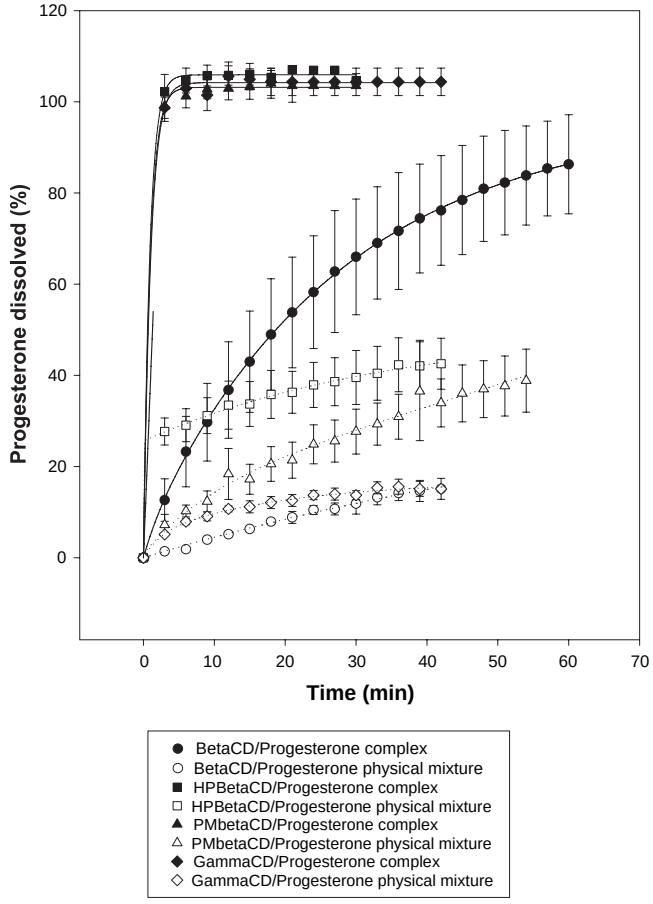

FIGURE 2 **Dissolution Curves of Progesterone from Binary Systems.**

50% and 80% of the progesterone included in β-CD was released after 20 min and 45 min, respectively. Consequently, we can conclude that the PMβ-CD, γ-CD, and HPβ-CD inclusion complexes present good dissolution features, improving the apparent solubility of progesterone (see Phase Solubility Studies) and its dissolution kinetics in water. Due to the amorphous nature of the complexes (see DSC) and an increase in water solubility following complexation, the progesterone complexes with cyclodextrins presented higher dissolution releases compared to the dissolution release of the corresponding pure drug. Nevertheless, kinetics were more rapid for progesterone included in β-CD than for the corresponding physical mixture ($k = 0.004$ min^{-1}).

Analyses of PMβ-CD, γ-CD, and HPβ-CD/progesterone physical mixture kinetics were performed with a 2-component exponential model and interpreted in terms of the short-lived and long-lived loss components. Short-lived loss progesterone represented 8.68, 9.88, and 27.83% of the total progesterone, with half-lives equal to 3.425, 3.693, and 0.722 min^{-1}, respectively. Long-lived loss progesterone represented 91.03, 89.97, and 72.16% of the total progesterone, with half-lives equal to 90, 433, and 130 min, respectively. Consequently, we concluded that the physical mixtures were essentially characterized by slow kinetics.

As the progesterone/β-CD complex did not present an optimal dissolution profile, it seemed interesting and worthwhile to us to study a preparation based on a new process, consisting of the incorporation of inclusion complexes into a hydrophilic polymer, using a melting-solidification method. For this study, the progesterone/β-CD inclusion complexes were incorporated in a carrier, PEG 6000, which had already been used by some authors (Nandi et al., 2003; Wulff & Aldén, 1999). This study is the subject of the following section.

Ternary Systems

Ternary systems prepared from progesterone/β-CD exhibit a significant improvement in their drug dissolution kinetics. This improvement was one of the criteria used for the selection of ternary systems preparation.

The results showed better dissolution levels for systems containing progesterone included in β-cyclodextrin Fig. 3 than for systems containing progesterone alone, as noted by Duclos (1989). Effectively, ternary systems incorporating 50% of progesterone/β-CD in PEG exhibited a dissolution level of 95% of progesterone in the first 30 min, but only 30% in the case of systems containing 50% progesterone, not included in β-CD, in PEG 6000.

Moreover, ternary systems containing the progesterone/β-CD complex significantly increase the hormone dissolution compared to the hormone dissolution of the progesterone/β-CD complex alone. For 10% and 50% progesterone/β-CD complexes, short-lived loss progesterone was estimated to represent 81.63% and 81.07% of the complexes, with half-lives of 1.558 min and 2.53 min, respectively, compared to progesterone dissolved from β-CD complex whose half-life was estimated at 20 min with a 1-component exponential model. Long-lived loss progesterone represented 18.19% and 18.18%, with half-lives equal to 72 min and 55 min, respectively (Table 1).

This phenomenon was also observed in the dissolution curves of the physical mixtures (Progesterone/β-CD complex 10% or 50% + PEG), which were slightly improved in the presence of PEG compared to the mixtures without PEG.

An explanation for these results could be that the progesterone/β-CD complex was dispersed in a hydrosoluble carrier, i.e., PEG 6000. In the presence of water, this linear polymer of ethylene glycol supports the wettability of particles to dissolve. This results in the acceleration of the dissolution rate with an increase in the amount of progesterone liberated *in vitro*. Similar results have already been observed by Ruan and coworkers. (Ruan et al., 2005). In that study, the authors studied the effect of PEG on the solubility of ampelopsin by ternary systems with PEG and inclusion complexes with β-cyclodextrin. They proposed several mechanisms to explain the increase of drugs' dissolution kinetics in ternary systems. Decreased crystallinity, increased wettability, and reduction of drug particle size were considered to be the predominant factors (Craig, 2002).

The dissolution profiles of the ternary systems containing 5% and 10% progesterone/β-CD complex were similar and could be interpreted with a 1-component exponential model. Nevertheless, a ternary system consisting of 50% progesterone/β-CD was better fitted with a 2-component exponential model. Therefore, we concluded that a cyclodextrin high-loading system released progesterone in two distinct mechanisms. It is highly probable that, in the present case, some fractions of progesterone were included in the cavity and that

TABLE 1 **Parameters and Statistics of Equations Describing Progesterone Loss Kinetics from the Cyclodextrins Studied**

Regression type	*a*	*b*	*c*	*d*	y_0	R^2
βCD/Progesterone physical mixture	$y = a \exp(-bx)$	99.7600 ± 0.2493	0.0041 ± 0.0001			0.9877
βCD/Progesterone complex	$y = a \exp(-bx)$	96.7059 ± 0.7235	0.0344 ± 0.0004			0.9979
PMβCD/Progesterone physical mixture	$y = a \exp(-bx) + c \exp(-dx)$	8.6772 ± 2.0627	0.2024 ± 0.0996	91.0338 ± 1.8107	0.0077 ± 0.0005	0.9879
PMβCD/Progesterone complex	$y = a \exp(-bx)$	100.0002 ± 4.8211	1.5552 ± 0.0452			0.9787
γCD/progesterone physical mixture	$y = a \exp(-bx) + c \exp(-dx)$	9.8833 ± 0.8550	0.1877 ± 0.0323	89.9697 ± 0.8170	0.0016 ± 0.0003	0.9907
γCD/progesterone complex	$y = a \exp(-bx)$	100.00 ± 0.0046	1.4503 ± 0.0012			0.9999
HPβCD/progesterone physical mixture	$y = a \exp(-bx) + c \exp(-dx)$	27.8331 ± 0.8380	0.9607 ± 0.1726	72.1631 ± 0.5013	0.0053 ± 0.0003	0.9967
HPβCD/progesterone complex	$y = a \exp(-bx)$	99.9704 ± 1.1443	0.7894 ± 0.0401			0.9999
Dispersion 5% (Progesterone/βCD/PEG 6000 5%)	$y = y_0 + a \exp(-bx)$	96.9617 ± 0.1025	0.94 ± 0.0059		3.0377 ± 0.0231	0.9999
Dispersion 10% (Progesterone/βCD/PEG 6000 10%)	$y = a \exp(-bx)$	100.0013 ± 0.0569	0.8695 ± 0.0026			0.9999
Dispersion 50% (Progesterone/βCD/PEG 6000 50%)	$y = a \exp(-bx) + c \exp(-dx)$	95.1254 ± 0.8651	0.2522 ±0.0052	5.2623 ± 0.5741	0.0010 ± 0.0027	0.9991
Progesterone/βCD physical mixture 10%/PEG 90%	$y = a \exp(-bx) + c \exp(-dx)$	24.1393 ± 0.4473	0.1342 ± 0.0050	75.7058 ± 0.4329	0.0026 ± 0.0001	0.9992
Progesterone/βCD physical mixture 50%/PEG 50%	$y = a \exp(-bx) + c \exp(-dx)$	15.7173 ± 0.8280	0.1369 ± 0.014	84.6984 ± 0.8123	0.0052 ± 0.0002	0.9982
Progesterone/βCD complex 10%/PEG 90%	$y = a \exp(-bx) + c \exp(-dx)$	81.6285 ± 1.2906	0.4449 ± 0.0186	18.1857 ± 0.7914	0.0096 ± 0.0013	0.9975
Progesterone/βCD complex 50%/PEG 50%	$y = a \exp(-bx) + c \exp(-dx)$	81.0729 ± 1.7474	0.2739 ± 0.013	18.1797 ± 1.3460	0.0126 ± 0.0021	0.9960
Solid inclusion Progesterone/βCD complex/PEG 10%	$y = a \exp(-bx)$	100.0013 ± 0.0569	0.8695 ± 0.026			0.9999
Solid inclusion Progesterone/βCD complex/PEG 50%	$y = a \exp(-bx) + c \exp(-dx)$	95.1254 ± 0.8651	0.2522 ± 0.0052	5.2623 ± 0.5741	0.0010 ± 0.0027	0.9991

A 1-component model was used: $y = a \exp(-bt)$ where y is the remaining progesterone in the cyclodextrin at time t (min), a is the initial percentage of progesterone at time zero, R^2 is the determination coefficient, and b is the rate constant (min^{-1}).

TABLE 2 DSC Peak Positions Relative to Figure 4 and Figure 5

Sample	T_{peak} (°C)
Progesterone	132.43
β-CD	122.22, 151.19, 169.12, 181.72
β-CD/Progesterone physical mixture	115.86, 158.19, 183.28
β-CD/Progesterone complex	117.54, 156.86, 193.43
PMβ-CD	157.23, 190.52
PMβ-CD/Progesterone physical mixture	112.19, 194.54, 246.51
PMβ-CD/Progesterone complex	63.56, 194.55, 237.86
γ-CD	127.57, 156.87, 170.55,180.43
γ-CD/Progesterone physical mixture	118.18, 166.52, 169.52, 184.08
γ-CD/Progesterone complex	148.16, 159.34
HPβ-CD	138.57, 162.56, 178.87
HPβ-CD/Progesterone physical mixture	118.54, 139.88, 159.55, 173.87, 176.44
HPβ-CD/Progesterone complex	64.56, 108.55, 161.20, 178.42
Dispersion 5% (Progesterone/β-CD/PEG 6000 5%)	64
Dispersion 10% (Progesterone/β-CD/PEG 6000 10%)	64.71
Dispersion 50% (Progesterone/β-CD/PEG 6000 50%)	58.81, 222.85, 241.21
Progesterone/β-CD physical mixture 10%/PEG 90%	63.25, 232.16
Progesterone/β-CD physical mixture 50%/PEG 50%	61.11, 175.19, 186.98
Progesterone/β-CD complex 10%/PEG 90%	62.97
Progesterone/β-CD complex 50%/PEG 50%	60.45, 187.55, 214.53

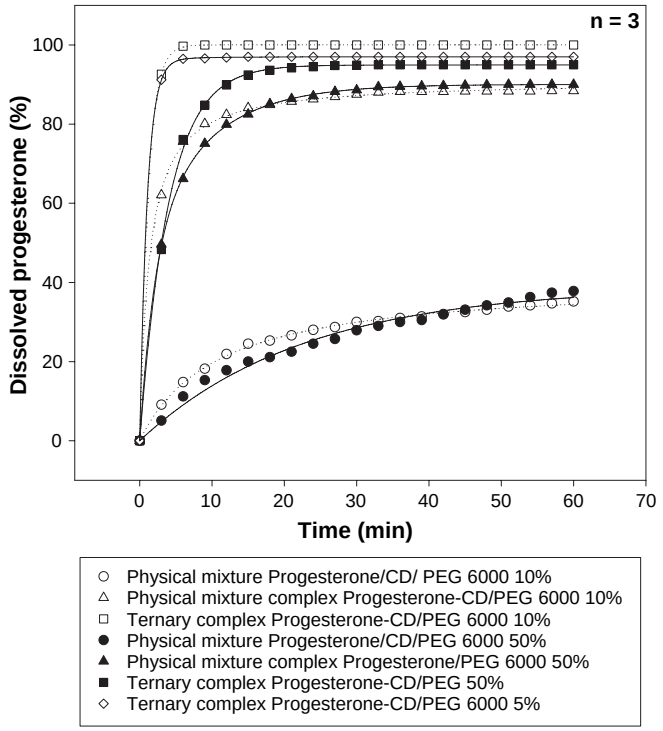

FIGURE 3 Dissolution Curves of Progesterone from Ternary Systems.

others were adsorbed on the cyclodextrin hydrophilic external face, resulting in two different release rate constants: 95.12% with a half-life of 2.75 min and 5.2% with a half-life of 693 min.

Comparison of Progesterone/Cyclodextrin Complexes, Physical Mixtures, and Ternary Systems

The results shown in Figs. 2 and 3, combined with the discussion in the previous two paragraphs, prove that progesterone dissolution was better for ternary complexes, and even for ternary physical mixtures, than for the binary system in the form of an inclusion complex, with regard to β-CD. Moreover, the progesterone dissolution from ternary complexes was similar to the progesterone dissolution from the derivatized β-CD that was studied. From this study, one can conclude that ternary systems show promise for use with poorly soluble drug dissolutions such as progesterone.

Differential Scanning Calorimetry

Inclusion Complexes–Progesterone/Cyclodextrin Physical Mixtures

DSC curves obtained for pure materials, inclusion complexes, and their corresponding physical mixtures are shown in Fig. 4.

The progesterone differential scanning calorimetry thermogram presents a single characteristic endothermic melting peak at 132.43°C. Hence, no polymorphs of progesterone could be found by DSC in this study.

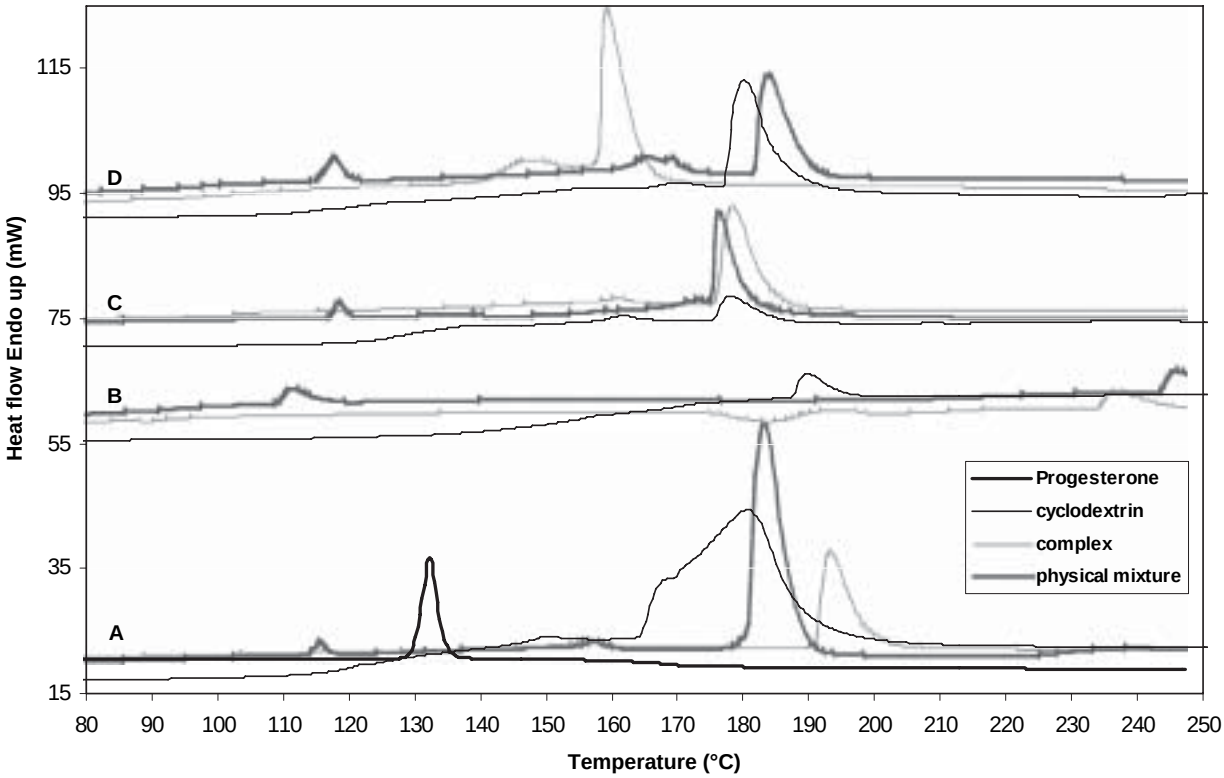

FIGURE 4 **DSC Curves of Binary Systems: (a) Progesterone/β-CD, (b) Progesterone/PMβ-CD, (c) Progesterone/HPβ-CD, And (d) Progesterone/γ-CD.**

Endothermic peaks between 164°C and 200°C appeared at 181.72°C, 190.52°C, 178.87°C, and 180.43°C, in the thermograms for β-cyclodextrin, PMβ-CD, HPβ-CD, and γ-CD, respectively. These endothermic peaks were attributed to the loss of the water molecules inside the cavity of the cyclodextrins. Thermal decomposition could be observed in the case of PMβ-CD at 246.51°C.

With regard to the complexes, the disappearance of the endothermic peak of progesterone was observed in all the cyclodextrins thermograms. The disappearance of the thermal feature of the drug indicates that one of two events had occurred. One possibility was that progesterone had penetrated into the cyclodextrin cavity and replaced the water molecules, confirming inclusion complex formation. The other possibility was that progesterone was no longer crystalline in free form, and that it had been converted into an amorphous state. In the case of the β-CD complex with progesterone, the peak that was attributed to the loss of water molecules exhibited a reduced area in the DSC thermogram of the inclusion complex and it also displayed a higher melting temperature (193.43°C, instead of 181.72°C for the pristine β-CD). This observation is consistent with the substitution of some water molecules by progesterone, with regard to the work presented by Forgo and Göndös (Forgo & Göndös, 2002). In that study, the authors proved, using rotating frame overhauser spectroscopy, that was immersed deeply in the cavity as dipolar interactions between the protons of the progesterone and H-5 and H-3 of the β-cyclodextrin were observed. This result is also consistent with the shift of the endothermic peak from β-CD when the guest molecule enters the β-CD cavity as the energy of the remaining water changes when some of the cavity water is lost. The same reasoning applies to γ-CD. The melting point of the complex exhibited a sharp melting behavior at 159.34°C, which suggests that the complex is a new phase that is different from the guest or the host, confirming that the complex is formed. For PMβ-CD and HPβ-CD, the complex peaks were nearly superimposed with the endotherms from the cyclodextrin.

However, in case of the physical mixtures, the progesterone peaks were shifted to slightly lower temperatures. The progesterone peaks were observed at 115.86°C, 112.19°C, 118.54°C, and 118.18°C for β-CD, PMβ-CD, HPβ-CD, and γ-CD, respectively.

These results may be explained by the existence of a very weak interaction between progesterone and the cyclodextrins studied at high temperatures.

Ternary Systems

In the thermogram of PEG 6000, a sharp peak at 65.4°C (not shown) was associated with the melting endotherm of PEG, which has also been shown by Ruan et al. (2005).

Ternary Systems of Progesterone/β-CD Complex 5%, 10%, 50% Dispersed in PEG 6000

Ternary systems of 5%, 10%, and 50% progesterone/β-CD complexes in PEG 6000 are shown in Fig. 5. First mention of Fig. 5 These ternary systems presented endothermic peaks at 64°C, 64.71°C, and 58.81°C, respectively, whereas the progesterone/β-CD complex melted at 193.43°C (Fig. 4). In the case of 50% dispersion, thermal decomposition occurred at 222.85°C. At the same time, ΔH decreased significantly as long as the concentration in progesterone/β-CD complex continued to increase. When guest molecules, such as progesterone used in this study, are incorporated into the CD cavity or the crystal lattice, their melting, boiling, and sublimation points usually shift to a different temperature or disappear completely within the particular range (Cabral Marques et al., 1990). These facts indicate that progesterone may have formed a new system, in which the inclusion complexes might be dissolved in the PEG carrier.

Physical Mixtures of Progesterone/β-CD 10%, 50% Dispersed in PEG 6000

Physical mixtures of 10% and 50% progesterone/β-CD in PEG 6000 are shown in Fig. 5. These physical mixtures presented the superposition of endothermic melting peaks from β-CD and PEG at 186.98°C and 60–65°C, respectively. When the concentration of the progesterone/β-CD physical mixture was increased to 50%, a peak appeared at 186.98°C, while the enthalpy value for the 60–65°C peak decreased. This outcome could be explained by a simple dilution effect of the progesterone/β-CD mixture into the PEG matrix since the progesterone/β-CD physical mixture (Fig. 4) showed an endothermic peak at 183.28°C.

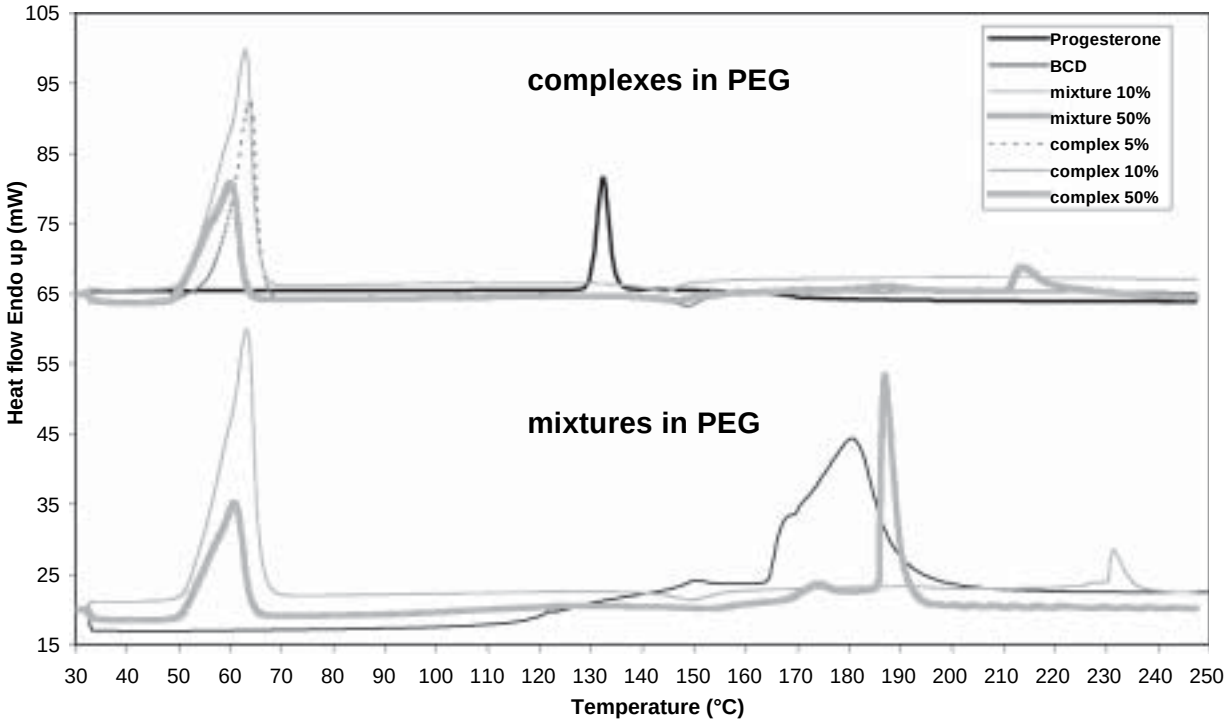

FIGURE 5 **DSC Curves of Ternary Systems. (Percentages Given Are Relative to the Amount of Progesterone/β-CD Complex or Physical Mixture in PEG.)**

Complex of 10% Progesterone/βCD and Complex of 50%Progesterone/βCD Dispersed in PEG 6000

A peak was observed at 62.97°C for the 10% progesterone/β-CD complex and at 60.45°C for the 50% progesterone/β-CD complex (Fig. 5). A slight additional peak near 187.55°C for the 50% progesterone/β-CD complex could be attributed to the β-CD diluted into the PEG matrix. Disappearance of the progesterone endotherm was also observed.

Physical Mixture 10% and Complex of Progesterone/β-CD 10% Dispersed in PEG, Physical Mixture 50% and Complex of Progesterone/βCD 50% Dispersed in PEG

Comparison of a 10% physical mixture to a 10% complex of progesterone/β-CD in PEG did not indicate any significant differences (Fig. 5). On the other hand, in the comparison of a 50% physical mixture against a 50% complex of progesterone/β-CD in PEG, the thermograms illustrated a peak at 186.98°C for the physical mixture, which could be attributed to the melting endotherm from β-CD already observed at 183.28°C in the case of the progesterone/β-CD physical mixture (Fig. 4); whereas this peak disappeared completely in the case of the complex dispersion into PEG. A peak appears at 214.53°C in the case of the incorporation of progesterone/β-CD complex into PEG. This suggests that the inclusion complex was fully incorporated into the PEG matrix in the case of complex ternary systems, forming a new system; whereas it was simply incorporated into the PEG as distinct phases in the case of the physical mixture.

IR Analysis

Fig. 6 shows the spectra of pure materials and respective progesterone-carrier systems. The spectrum of progesterone is shown in Fig. 6(c). This spectrum illustrated the presence of the carbonyl stretching bands at 1661 cm^{-1} and at 1699 cm^{-1}. Cerchiara et al. (2003) assigned these peaks to C_3=O d and C_{20}=O progesterone, respectively.

The spectra of pure β-CD presented a broad band between 3100 cm^{-1} and 3800 cm^{-1} attributed to free OH from primary and secondary OH groups, between 2800 cm^{-1} and 3100 cm^{-1} corresponding to bound OH, at 1626 cm^{-1} due to water molecules present in the cavity, and a large band that displayed distinct peaks between 900 cm^{-1} and 1200 cm^{-1}, responsible for C-O vibrations.

PEG displayed a band at 2875 cm^{-1} and a pattern of highly distinct peaks situated between 800 cm^{-1} and 1500 cm^{-1}.

The IR spectra of the 10% and 50% physical mixtures of progesterone, β-CD, and PEG are shown in Figs. 6(d) and 6(e). In these spectra, bands of the cyclodextrin and PEG overlapped with progesterone main characteristic peaks. This was not the case when progesterone/β-CD was analyzed on its own. Furthermore, no such overlap was observed in the case of the inclusions or the progesterone/β-CD complex dispersion in PEG, for which peaks assigned to progesterone disappeared. These spectral changes could be explained either by the progesterone dilution in the excipient or by the dissociation of the intermolecular hydrogen bonds of progesterone through inclusion complexation, a mechanism that has been observed and explained by several authors.

Liu et al. (1990) registered the IR spectrum of β-CD complex with progesterone. In that study, the authors showed a shift of the conjugated carbonyl group wave number at the 3 position of 7 cm^{-1} for the 1651 cm^{-1} peak; 7 cm^{-1} being the difference between the pure complex and the physical mixture. Uekama et al. (1982) suggested from ^{1}H-NMR, that the A-ring of the steroid molecule was predominantly included in the cavity of CDs. These results were emphasized by Le Questel et al. (2000). Le Questel and coworkers performed semiempirical or ab initio calculations on progesterone, hydrogen-bonded to water, in vacuo and they proved that the electrostatic potential was more negative around the conjugated carbonyl (O_3 in progesterone). They concluded, consequently, that it would favor the hydrogen-bond donors' fixation to the O_3 site. Forgo and Göndös studied β-cyclodextrin inclusion complexes with progesterone through complex formation monitored by intermolecular dipolar interactions between ^{1}H signals in the hydrophobic cavity (H-3 and H-5 of the α-glucose units) and the steroid moiety, in DMSO solvent and ROESY spectra (Forgo & Göndös, 2002). The data revealed that progesterone was fully immersed in the β-CD. Combining these findings with the results of our own study, we can conclude that progesterone was partially

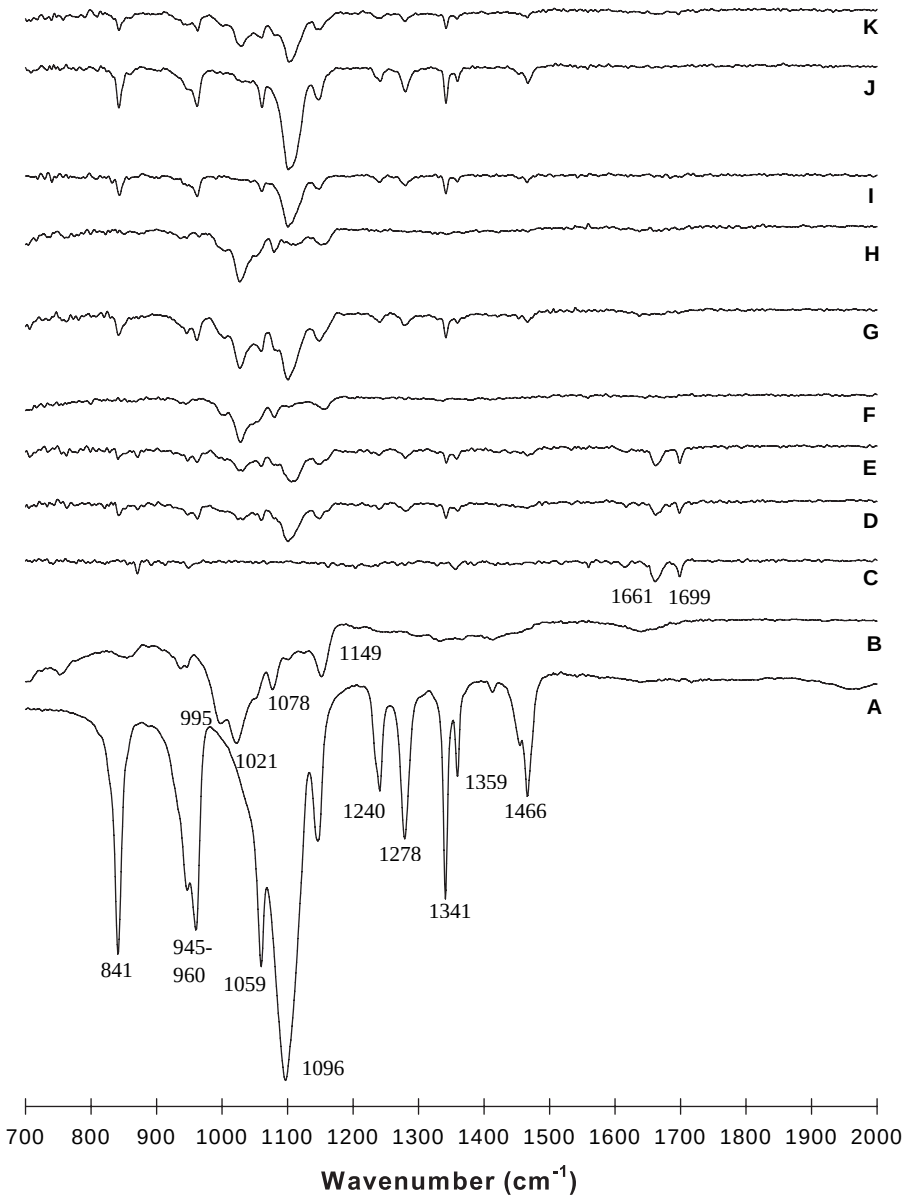

FIGURE 6 **Infra-Red Spectra: (a) PEG, (b) β-CD, (c) Progesterone, (d) Progesterone/β-CD/PEG 6000 Physical Mixture 10%, (e) Progesterone/β-CD/PEG 6000 Physical Mixture 50%, (f) Progesterone-β-CD Complex, (g) Progesterone-β-CD Complex/PEG 6000 Physical Mixture 10%, (h) Progesterone-β-CD Complex /PEG 6000 Physical Mixture 50%, (i) Ternary Complex 5% in PEG, (j) Ternary Complex 10% in PEG, (k) Ternary Complex 50% in PEG.**

or totally included in the β-CD in the case of the progesterone/β-CD complex, contrary to the results obtained with the physical mixture. These results are also in accordance with the results attained in the DSC experiments.

The progesterone/β-CD complex, 10% dispersed in PEG in the form of a physical mixture, is seen in Fig. 6(g). This complex was similar to β-CD and PEG pattern. On the other hand, the progesterone/β-CD complex 50% dispersed in PEG, seen in Fig. 6(h), highlighted the intensity increase of the β-CD bands, as the PEG peaks were partially masked by those from the cyclodextrin. The same observation could be made for the ternary systems. The β-CD peaks observed in the 50% ternary systems (Fig. 6(k)) were more significant compared to the corresponding peaks in the 10% ternary systems (Fig. 6(j)). These results were expected due to the dilution of the complex into PEG.

CONCLUSIONS

The present paper was devoted to the improvement of the solubility and the dissolution rate of progesterone. These improvements were achieved either by forming inclusion complexes with derivatized cyclodextrins or by the formulation of ternary complexes of progesterone/β-cyclodextrins inclusions with PEG 6000.

HPβ-CD and PMβ-CD were determined to be the most efficient among the four cyclodextrins studied (hydroxypropyl-β-CD (HPβ-CD), hydoxypropyl-γ-CD (HPγ-CD), permethyl-β-CD (PMβ-CD), and sulfobutylether-β-CD (SBEβ-CD)), in terms of solubilizing progesterone.

The dissolution kinetics of progesterone dissolved from HPβ-CD, PMβ-CD, and γ-CD complexes revealed high constant rates in comparison to β-CD. Nevertheless, dissolution constant rates were considerably enhanced for 5% and 10% progesterone/β-CD complexes when incorporated into PEG, compared to the dissolution constant rate of progesterone alone in aqueous media.

Physicochemical characterizations (DSC, FT-IR) proved that progesterone penetrates into the cyclodextrin cavity and replaces the water molecules, and that it can form ternary systems of progesterone/β-cyclodextrin well dispersed in PEG. Dissolution profiles of progesterone incorporated in derivatized cyclodextrins and in ternary complexes were similar. Therefore, we conclude that progesterone incorporated in ternary complexes could be very useful under circumstances in which the use of a smaller amount of cyclodextrin is desirable. This could be true, for instance, when lower cost and reduced toxicity are sought

This demonstrates the value and significance of ternary complex preparations with hydrophilic polymers when solubility and dissolution enhancement of poorly soluble drugs, such as progesterone, are required.

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