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Effect of chitosan on progesterone release from hydroxypropyl-β-cyclodextrin complexes

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Abstract

An inclusion complex composed of progesterone (Prog) and hydroxypropyl-β-cyclodextrin (HPβCD) was prepared by the spray-drying and freeze-drying methods. Prog alone and its inclusion complex with HPβCD were incorporated into chitosan by spray-drying and freeze-drying. The inclusion complex was characterized by IR and DSC. The inclusion complex was investigated in solution by phase solubility diagrams and stability constant was determined at pH 7.4 and at different temperatures (10, 25 and 37 °C) to obtain the thermodynamic parameters of inclusion. The results indicate that the Prog–HPβCD inclusion complex is more water soluble than Prog alone. Release data from all samples showed significant improvement of the dissolution rate of Prog and a controlled release is obtained in the presence of chitosan.

Keywords: Progesterone; Spray-drying; Freeze-drying; Chitosan; Hydroxypropyl-β-cyclodextrin

1. Introduction

Progesterone (Prog) is a lipophilic drug used to control reproductive function and as postmenopausal therapy (Buntner et al., 1998; Latha et al., 2000). The oral delivery of Prog is limited since it is not tolerated in higher doses, but its biological half-life is short. One of the possibilities of a controlled delivery of Prog involves the application of a biodegradable drug containing microspheres incorporated into the body by injection (Buntner et al., 1998). Sidman et al. (1977) prepared injectable biodegradable drug reservoirs from glutamic acid/leucine copolymers in the form of tubes and solid rods to provide controlled release of Prog. Lee et al. (1981) incorporated Prog into glutaraldehyde cross-linked serum albumin microspheres and showed that an extended release of 1–2 ng/h/ml of serum was possible for about 20 days. Recently, albumin microcapsules and microspheres cross-linked with formaldehyde, 2,3-butanedione and glutaraldehyde were investigated for Prog delivery by Orienti and Zecchi (1993). In a recent study, Jameela et al. (1998) showed that cross-linked chitosan microspheres loaded with Prog, injected intramuscularly in rabbits, could sustain a plasma concentration of 1–2 ng/ml of the steroid for about 5 months.

The main aim of this study was to investigate the effect of chitosan on the complexation of 2-hydroxypropyl-β-cyclodextrin (HPβCD) with Prog. Cyclodextrins are used as complexing agents to improve the aqueous solubility of non-polar drugs, and consequently their bioavailability, or to modify some physico-chemical characteristics of drugs by means of inclusion into the hydrophobic cavity of the

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cyclodextrin (Duchene, 1987). 2-HPβCD has a higher water solubility than β-cyclodextrin which decreases its renal toxicity allowing parenteral administration (Irie et al., 1992; Irie and Uekama, 1997).

Chitosan is a cationic polysaccharide derived by deacetylation of chitin, and has been widely used for drug carrying devices in controlled drug delivery systems (Paul and Sharma, 2000; Ravi Kumar, 2000; Filipovic-Grcic et al., 2000). It is a hydrophilic, biocompatible and biodegradable polymer of low toxicity (Sawayanagi et al., 1983; Hirano et al., 1990; Vila et al., 2002).

2. Materials and methods

2.1. Materials

Prog, 2-HPβCD (with an average MS value of 0.6), high-molecular weight chitosan (MW: 600,000, viscosity: 400 mPa s (1% solution in 1% acetic acid), degree of deacetylation: 80%) was purchased from Fluka (Milan, Italy).

2.2. Preparation of Prog–HPβCD inclusion complex

Prog–HPβCD inclusion complex (Prog–HPβCD) was prepared by the spray-drying (Prog–HPβCD_s) and the freeze-drying (Prog–HPβCD_f) techniques.

For Prog–HPβCD_s, a suspension of Prog and HPβCD was prepared in the molar ratio 1:1. Briefly, Prog (0.31 g) was dispersed in 50 ml of water containing 1.38 g of HPβCD and mixed for 7 days at room temperature. The suspension was filtered through a 0.22-μm filter (Albet-Jacs, Valencia, Spain). The filtrate was subjected to spray-drying using a Mini Spray Dryer (Büchi, Milan, Italy). The drying conditions were as follows: inlet temperature, 105 °C; outlet temperature 44 °C and air flow rate, 600 N l/h.

The same protocol was applied to the preparation of Prog–HPβCD_f. The filtrate was frozen and then freeze-dried (Christ, Milan, Italy).

2.3. Solubility studies

Solubility studies were performed according to the method reported by Higuchi and Connors (1965). Prog in amounts that exceeded its solubility (50 mg) was carefully weighed into a 50-ml Erlenmeyer flask to which 20 ml of aqueous solutions containing HPβCD at various concentrations (0–0.016 mol/l) were added. The flasks were sealed and equilibrated by shaking at 10, 25 and 37 °C and at pH 7.4. When equilibrium had been reached (5 days), the samples were filtered through a 0.22 μm filter (Albet-Jacs, Valencia, Spain) and the concentration of Prog was measured spectrophotometrically at 244 nm (UV-VIS, Shimadzu, Kyoto, Japan).

The apparent 1:1 stability constants, K_s, were calculated from the initial straight line portion of the phase solubility diagram according to the equation (Ashwinkumar and Moji, 2001):

$$K_s = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the saturation concentration of the drug measured without cyclodextrin (intercept).

The change in enthalpy (ΔH°) on complexation was determined using the Van't Hoff equation

$$\ln\left(\frac{K_2}{K_1}\right) = \Delta H^\circ \frac{T_2 - T_1}{RT_2T_1} \quad (2)$$

The Gibbs free energy changes (ΔG°) were determined using the following equation:

$$\Delta G^\circ = -RT \ln K \quad (3)$$

and the change in entropy (ΔS°) upon complexation was determined by the equation:

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \quad (4)$$

2.4. Preparation of spray-dried chitosan–Prog–HPβCD complex

0.25 g of CH (1.55 mmol of glucosamine) was dissolved in 50 ml of HCl 0.1 N. Spray-dried Prog–HPβCD complex (0.49 g) was dispersed in this solution. The solution was sonicated for 10 min to produce a clear solution and subjected to spray-drying. The drying conditions were as follows: inlet temperature, 105 °C; outlet temperature, 44 °C; and air flow rate, 600 N l/h. The experimental yield for the chitosan–Prog–HPβCD complex was 0.67 g. The percentage of Prog in the spray-dried chitosan–Prog–HPβCD complex, determined from

release studies (see Section 2.8) at time infinite, was 17%.

2.5. Preparation of lyophilized chitosan–Prog–HPβCD complex

0.25 g of CH (1.55 mmol of glucosamine) was dissolved in 50 ml of HCl 0.1 N. Lyophilized Prog–HPβCD complex (0.49 g) was dispersed in this solution. The solution was frozen and then freeze-dried. The experimental yield for the chitosan–Prog–HPβCD complex was 0.70 g. The percentage of Prog in the lyophilized chitosan–Prog–HPβCD complex was 17%.

2.6. FTIR

Infrared (IR) spectra were recorded with a Jasco FTIR-410 spectrophotometer. The samples were prepared by processing compressed KBr disks.

2.7. Differential scanning calorimetry (DSC)

Differential scanning calorimetric analysis were performed using a Perkin-Elmer DSC 6. All samples (7–10 mg) were heated in crimped aluminium pans at a scanning rate of 10 °C/min in the temperature range 80–160 °C.

2.8. In vitro release studies

In vitro release profiles of the Prog, Prog–HPβCD$_s$, Prog–HPβCD$_f$ were examined in phosphate buffer, pH 7.4. The drug (50 mg) and the complexes (50 mg) were introduced into a donor cell containing 3 ml of pH 7.4 separated by a dialysis membrane (cellulose acetate, MW cut off = 10,000, Delchimica Scientific Glassware) from a receiving compartment containing 10 ml of the same aqueous buffer, which was replaced after time intervals suitable to guarantee sink conditions throughout the runs. The studies were performed at 37 °C and the drug was spectrophotometrically detected in the receiving phase. The release of Prog was also evaluated from chitosan–Prog–HPβCD$_s$ and chitosan–Prog–HPβCD$_f$. All experiments were carried out in triplicate and average values were plotted.

2.9. Statistics

All data are presented as arithmetic mean values ± S.D. Level of significance was analyzed using Student's *t*-test.

3. Results and discussion

3.1. Prog–HPβCD complexation

The phase solubility diagram for complex formation between Prog and HPβCD is presented in Fig. 1. The plot shows a typical A_L-type solubility curve classified by Higuchi and Connors (1965), showing that soluble complex was formed. The solubility of Prog increased in a linear fashion as a function of HPβCD concentration. Table 1 lists the 1:1 apparent stability constants of the inclusion complex (K_s). The stability constant decreased with increasing temperature, probably due to the decrease in the interaction forces, such as Van der Waals and hydrophobic forces (Ashwinkumar and Moji, 2001). The thermodynamic parameters of complexation are reported in Table 1.

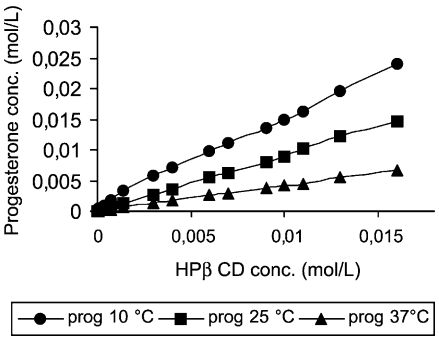

Fig. 1. Phase-solubility diagrams for progesterone in the presence of HPβCD at 10, 25 and 37 ± 1 °C.

Table 1
Thermodynamic parameters of the inclusion process of progesterone in HPβCD

pH	*T* (°C)	K_a (M^{-1})	Δ*G* (J/mol)	Δ*H* (J/mol)	ΔS (J/mol K)
7.4	10	94930.0	−26965.9	−69967.1	−151.9
	25	10974.1	−23049.6		
	37	7115.3	−22861.0		

In general, changes in thermodynamic parameters during complexation may be attributed to changes in Van der Waals interaction energy, hydrogen bonding and hydrophobic interaction between the guest molecule and cyclodextrin (Ashwinkumar and Moji, 2001). The negative enthalpy indicates that the complexation reaction is exothermic, i.e. there was release of energy that favored formation of the complex. Also, the entropy change is negative indicating that complexation of Prog with HPβCD resulted in an increase in the order of the system.

3.2. FTIR

Fig. 2 shows the IR spectra of Prog and Prog–HPβCD complexes. The peaks at 1661 and 1698 cm^{-1} are assigned to carbonyl-stretching bands of C_3 and C_{20} in Prog, respectively. In the spectrum of the spray-dried and freeze-dried complexes, the stretching bands of the carbonyl group disappeared. These spectral changes can be explained by the dissociation of the intermolecular hydrogen bonds of Prog through inclusion complexation (Uekama et al., 1982).

3.3. DSC

DSC was performed on the raw materials and on the inclusion complexes. The thermogram of Prog shows the characteristic endothermic peak at 125.3 °C, corresponding to drug melting. The thermal behaviour of the other components used for complex formation, i.e. HPβCD show no phenomena in the same temperature interval (Fig. 3B).

The spray-dried and freeze-dried Prog–HPβCD showed a disappearance of the endothermal peak of Prog, indicating that the drug penetrates into the cyclodextrin cavity replacing the water molecules (Echezarreta-Lopez et al., 2000) confirming drug amorphization and/or inclusion complex formation (Fig. 3C and D).

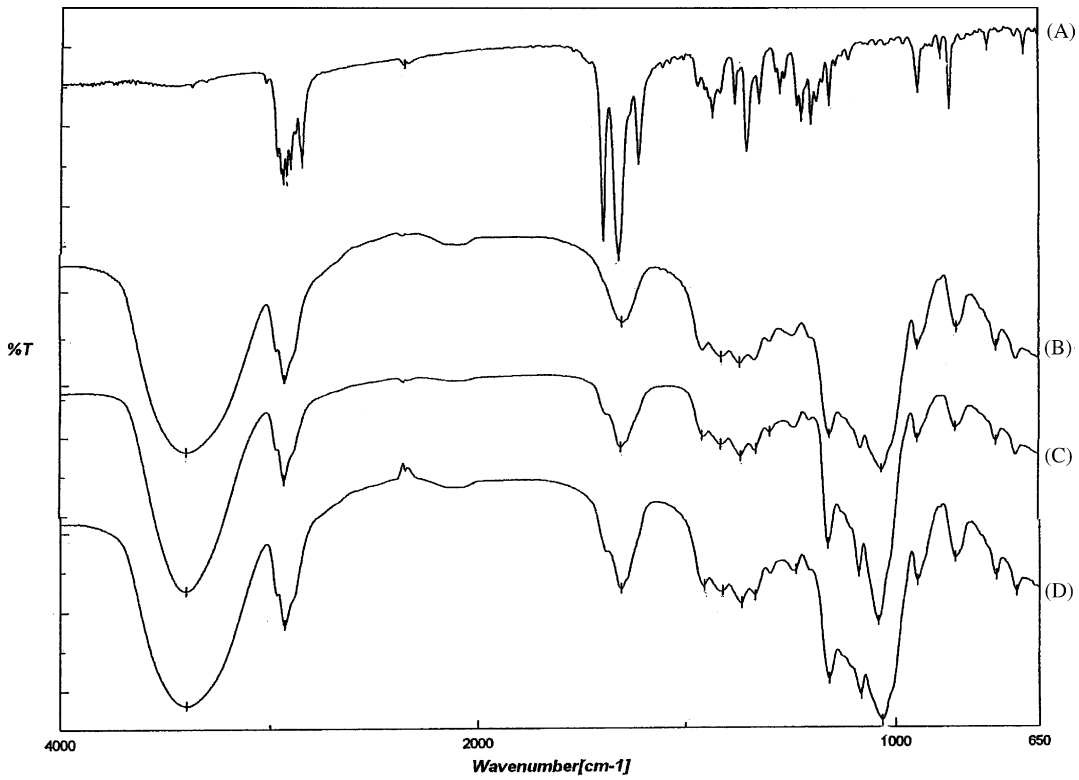

Fig. 2. IR spectra of progesterone (Prog) and HPβCD: (A) Prog; (B) HPβCD; (C) spray-drying; (D) freeze-drying.

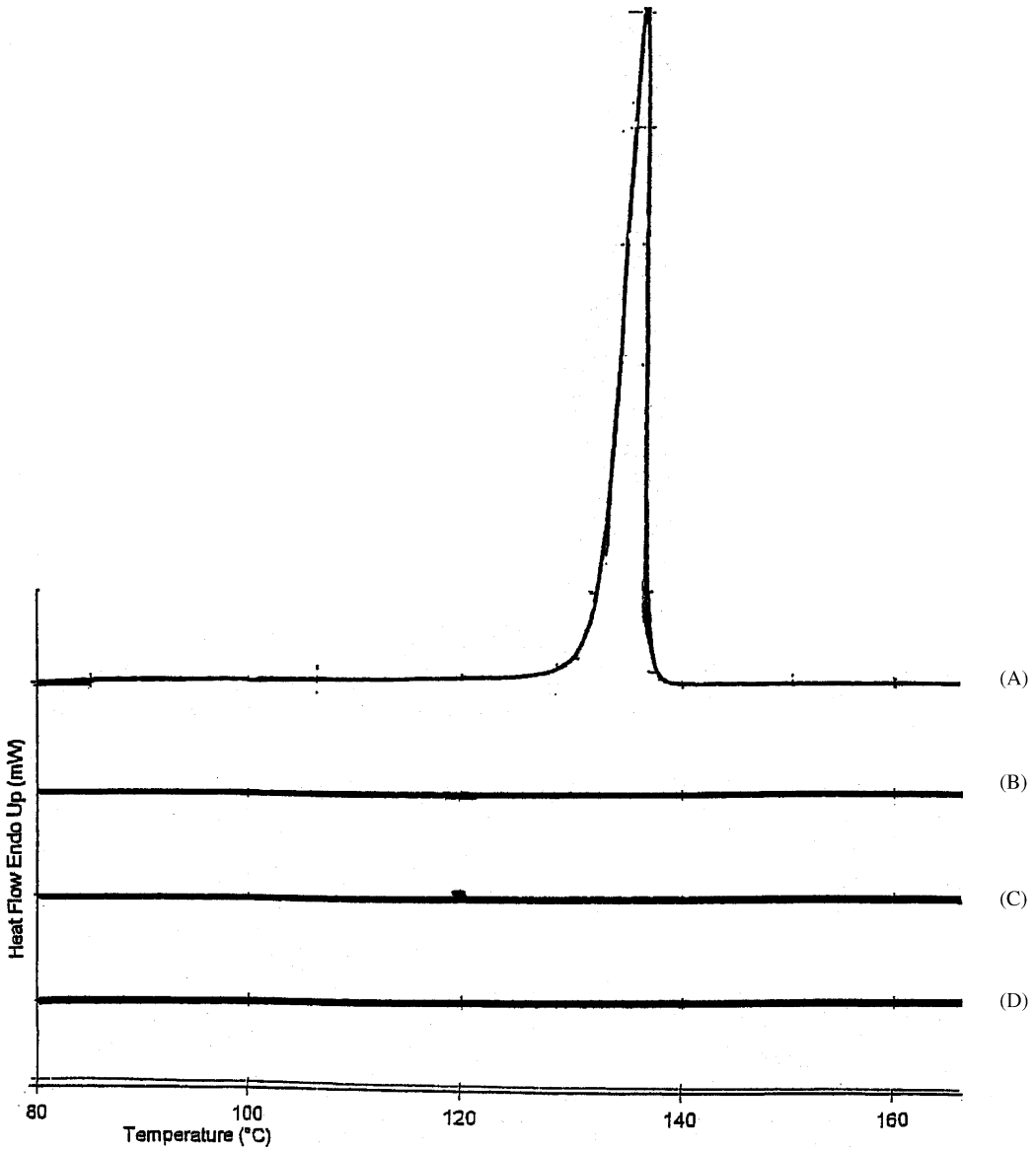

Fig. 3. DSC thermograms of progesterone (Prog) and HPβCD: (A) Prog; (B) HPβCD; (C) Prog–HPβCD$_s$; (D) Prog–HPβCD$_f$.

3.4. *In vitro release studies*

The in vitro release profiles of Prog and Prog–HPβCD complexes are shown in Fig. 4. The dissolution profiles of the complexes were compared with pure drug. Due to the amorphous nature (DSC data confirming) and an increase in water solubility following complexation, the Prog–HPβCD$_s$ and Prog–HPβCD$_f$ complexes showed significantly higher dissolution releases compared to the corresponding pure drug.

The presence of chitosan decreased the release at 24 h due to its low dissolution at alkaline pH (7.4). The retardant effect of chitosan can be explained by the slow diffusion of Prog through the more hydrophilic chitosan/cyclodextrin matrix layer around

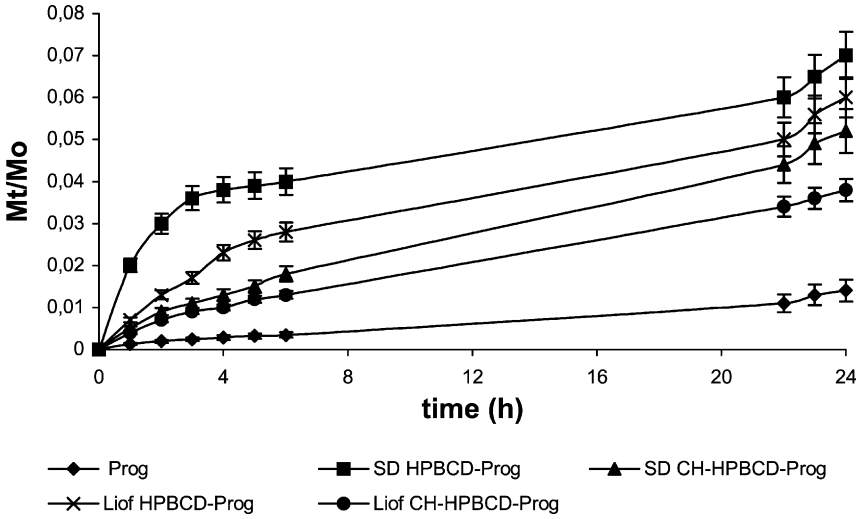

Fig. 4. In vitro release profiles of Prog, Prog–HPβCD$_s$, Prog–HPβCD$_f$. All data are means ± S.D. ($n = 3$).

the lypophilic drug. Rates at which a drug is released from chitosan formulations also depend on the character of the drug. Substances with low solubilities in water are the most slowly released (Akbuga, 1993).

4. Conclusions

The solubility of Prog can be increased by inclusion complexation with HPβCD. The Prog–HPβCD$_s$ and Prog–HPβCD$_f$ complexes were characterized by enhanced dissolution rates with respect to the drug. The presence of chitosan significantly decreased the dissolution rate of Prog–HPβCD$_s$ and Prog–HPβCD$_f$ complexes.

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