ORIGINAL ARTICLE

Water soluble progesterone–hydroxypropyl-β-cyclodextrin complex for injectable formulations

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Abstract The use of cyclodextrin to increase the water solubility of progesterone (P) was described by Pitha as a complex with β-cyclodextrin and derivates to obtain a water soluble formulation (Pitha, J.: US patent n. 4,727,064). Hydroxypropyl-β-cyclodextrin (HPBCD) has a high water solubility which allows the solubilization of high quantity of P. Considering a 1:2 guess/host complex stoichiometry it is possible to obtain up to 50 mg/ml of P concentration, which is a considerable dosage for drug development in the progesterone therapy. In our drug development the P/HPBCD complex in water showed the formation of a light precipitate during stability ICH conditions. A precipitate formation was described already by Choi (J. Korean Pharm. Sci. **31**(3), 151, 2001) and also by Pitha (US patent n. 4,727,064) but the chemical structure was not elucidated. In our case the precipitate was purified and it turned out to contain progesterone and residual unmodified β-cyclodextrin. We have developed a production process in which the residual unreacted β-cyclodextrin is separated from the HPBCD by the formation of the insoluble inclusion complex (Zoppetti et al.: European Patent deposit n. 05108494.5). The resulting P/HPBCD contains up to 0.1% of residual β-cyclodextrin and does not produce precipitate during the stability study. The complex stoichiometry and the complex constant were calculated by the phase solubility study according to Higuchi and Connors (Adv. Anal. Chem. Instrum. **4**, 117, 1965) and the presence of the inclusion complex was demonstrated by DSC, NMR, X-ray, FTIR. The formulation prepared at pilot scale as injectable form compared with the commercial oil formulation demonstrated a favourable kinetic in humans.

Keywords Progesterone · Cyclodextrin · Hydroxypropyl-β-cyclodextrin · Phase solubility study · Raman · NMR · X-ray · FTIR · DSC

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Introduction

Progesterone (P) is a natural hormone utilized as a drug to control reproductive function and for postmenopausal therapy. The current administration routes are oral by soft gel caspules, vaginal by soft gel capsules or cream and intramuscular oily solution or water suspension injections. Oral and vaginal routes have low bioavailability and, in addition, oral route has a limited usefulness because of its short half-life and extensive degradation after absorption. Therefore the injection route should be the preferred choice, but the oily solution and the water suspension allow only intramuscular administration with a considerable pain

during the treatment. This encourages the research of new injectable pharmaceutical forms to increase patient compliance. A water soluble P could be a good solution because it could be administered also by subcutaneous route, therefore allowing the auto medication and less pain during the treatment is expected. The use of cyclodextrin to increase the water solubility of progesterone (P) was described by Pitha as a complex with β-cyclodextrin and derivatives to obtain a water soluble formulation [1]. The evidence of the inclusion complex formation of progesterone with β-cyclodextrin and derivatives is described by other authors using different physico-chemical methods [2–4]. Among the cyclodextrins tested hydroxypropyl-β-cyclodextrin (HPBCD) is the preferred choice for a formulation with therapeutic amount of P. The therapeutic dosages of P by injection vary from 5 to 200 mg. in function of the specific pathology [5]. HPBCD has a high water solubility (48% P/V), which allows the solubilization of high quantity of P. Considering a 1:2 guess/host complex stoichiometry it is possible to obtain up to 50 mg/ml of P concentration, which is a considerable dosage for drug development in the progesterone therapy. In our drug development the P/HPBCD complex in water showed the formation of a light precipitate under stability ICH conditions. A precipitate formation was already noticed by Jeong [4] and also by Pitha [1] but the chemical structure was not elucidated. This work describes a method to obtain a P/HPBCD without insoluble particles and the physico-chemical characterization of the purified inclusion complex.

Experimental

Materials

Progesterone was provided by Diosynth (The Netherlands) and hydroxypropyl-β-cyclodextrin (HPBCD) by Roquette Freres (Lestrem Cedex, France).

Methods

P/HPBCD powder for injection

The P/HPBCD powder for injection was prepared according to the Eurpean patent deposit n.05108494.5 [6].

Phase solubility study

The stoichiometry and the overall complex formation constant K_3 were measured according to Higuchi and Connors [7] (experiment A). In addition the determination of the single complex formation constants (K_1 and K_2) were measured by a modified phase solubility (experiment B):

(A) Experiment with different concentrations of HPBCD Solutions with different concentrations of HPBCD were added to fixed aliquots of about 811 mg of P. The final weight of solutions was 20.0 g. The resulting suspensions (Fig 1a) were stirred for 120 min at TA then kept to 5 °C for 24 h, then filtered by 0.45 μm hydrophilic filter. From the clear solutions (Fig. 1b) aliquots were weighed and diluted with ethanol and read at 241 nm by a a Jasco UV/Vis V550 spectrophotometer.

(B) Measurement of K_1/K_2 ratio Same solutions were obtained as indicated in experiment A. The resulting suspensions (Fig. 1a) were stirred for 120 min at TA then kept to 5 °C for 24 h. From the resulting turbid supernatant (Fig. 1c) aliquots were weighed and diluted with weighted amounts of 48% HPBCD solution to reach a clear solution (Fig. 1d). Aliquots of the clear solution were diluted with ethanol and read at 241 nm by a Jasco UV/Vis V550 spectrophotometer. K_1 and K_2 were calculated by mathematic system from K_3 and from K_1/K_2 ratio obtained by experiment B.

Physico-chemical methods

Differential scanning calorimetry DSC thermograms were obtained by a Mettler Four DSC30 Logiciel STARe v. 8.10. Acquisition conditions: 10–250 °C, with rate of 10 °C/min, running fluid nitrogen 20 mL/min.
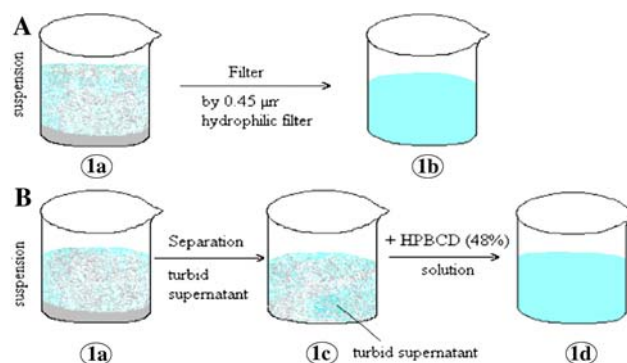

Fig. 1 Experiment A and B

X-ray analysis X-ray experiment was made by X'Pert MPD Panalytical equipped with proportional Detector, a Tube with Cobalt anode, acquisition system X'Pert Data Collector v. 2.0e, treatment X'Pert Highscore v2.0a-Panalytical BV. Acquisition conditions: Scan mode continuous, 4–34°, Step-size 0.01°, Time per step 1.2 (s), speed 0.008333°/s, Step numbers 3000.

FT-Raman spectroscopy Spectra were recorded by means of a Renishaw Raman System RM1000 interfaced to a microscope Leica DMLM (spatial resolution 1–60 μm^2). Experimental details: source Laser Ar$^+$ (514.5 nm), Diode Laser (780.0 nm); monochromator: diffraction network; detector: Charge-Coupled Device (CCD) (cooled at 203 K); spectral resolution: 2 cm^{-1}; power on the sample 0.03 e 3 mW; accumulation time: 10 s; scansion number >10.

NMR spectroscopy 1D and 2D ^{1}H experiments were performed at 500 MHz, on a Bruker Avance 500 spectrometer equipped with a TXI 5 mm probe at a temperature of 313 K. A proton NMR spectrum of the progesterone sample was recorded from a saturated D_2O solution. The HPBCD and P/HPBCD were characterized by 2D-NMR homonuclear (COSY and TOCSY) and heteronuclear (HSQC and HMBC) experiments, from a 7.0 mM D_2O solution.

Results and discussion

Precipitate formation

The precipitate was purified from a pilot scale production and was the 0.055% of the total amount product. The structural analysis by NMR indicates the presence of P and β-cyclodextrin, but not HPBCD (Fig. 2).

The formation of an insoluble complex P/β-cyclodextrin is confirmed by Forgo [2]. Author concluded that progesterone molecule is deeply included in the cyclodextrin cavity, therefore a strong inclusion complex is formed with low solubility in water. Although in our experiment we cannot confirm the complex between P and β-cyclodextrin it is evident that our original process eliminates most of the theoretical P/β-cyclodextrin complex (produced by the 0.8% unreacted β-cyclodextrin in the commercial HPBCD) leading to a product with residual β-cyclodextrin below 0.1%. This low amount of residual β-cyclodextrin does not form precipitate under ICH stability study. The
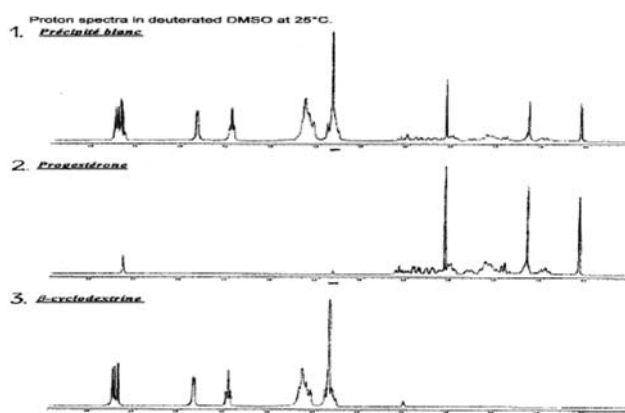

Fig. 2 Proton NMR spectra of: 1. precipitate, 2. Progesterone and 3. β-cyclodextrin

resulting P/HPBCD complex allows a stable injectable formulation. Nevertheless we have selected a powder form to be reconstituted before the use to reach longer shelf life.

This formulation was tested in human against the commercial oily formulation to compare the bioavailability by intramuscular injection route (Fig. 3). The new formulation shows a better bioavailability than the oily formulation suggesting the possibility to reduce the dosage in specific therapies.

Phase solubility studies

The stoichiometry of the complex and the complex formation constant were calculated by phase solubility experiment. The resulting diagram is type A curve (Fig. 4) with a 1:2 guest:host complex stoichiometry.

The overall constant (K_3), resulting from combination of two complex formation constants K_1 and K_2, was calculated by the phase solubility experiment (A)
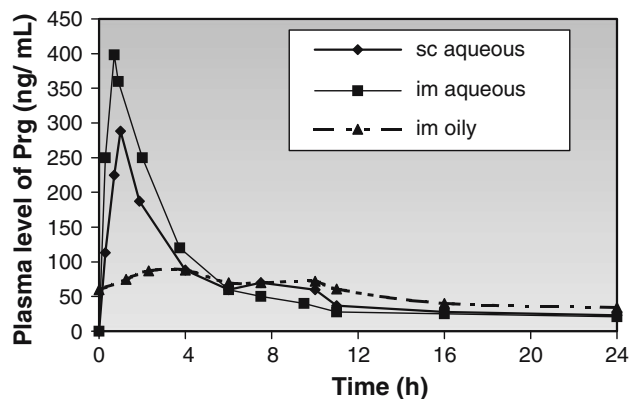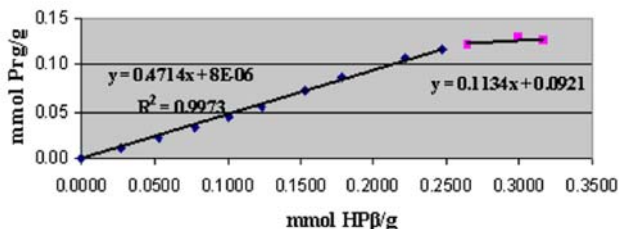

Fig. 3 Plasma levels of progesterone after intramuscular and subcutaneous administration

Phase solubility diagram of HPB/Prg complex

$$R = \frac{\text{mmol HPB}}{\text{mmol Prg}} =$$

$$= \frac{(\text{mmol HPB/g}) \text{ to the break point}}{(\text{mmol Prg/g}) \text{ to the brek point} - (\text{mmol Prg/g}) \text{ to So point}} = 2.12$$

Fig. 4 Type A Phase solubility diagram of HPBCD/progesterone 2:1 complex

considering the slope of the resulting curve (Fig. 4) and the formula below reported (Eq. 1):

$$K_3 = \frac{\text{slope}}{S_0(1 - \text{slope})} \tag{1}$$

S_0 refers to the solubility of P without HPBCD.

Applying the above formula the overall constant K_3 is:

$$K_3 = 111473.70 \text{ m}^{-1}$$

To understand the mechanism of molecular association also the complex formation constant K_1 and K_2 were calculated from a modified phase solubility experiment (B). K_1 constant is presumed to be the constant of the initial 1:1 complex and K_2 constant is related to the second entering cyclodextrin molecule to form the final 1:2 complex (Fig. 5).

In this scheme K_3 is the overall constant resulting from the two complex formation constants K_1 and K_2.

In the experiment B after 24 h at 5 °C, the stirred samples still showed a thick turbidity in the supernatant (Fig. 1c), which became clear after addition of a water solution containing HPBCD (Fig. 1d). The 1:2 complex has a good solubility in water, and we speculate that 1:1 complex has only a partial solubility due to the partial complexation of P by one molecule of HPBCD. Therefore the sustained turbidity present in the supernatant could be referred to 1:1 complex. The two complexes can be both present in condition of supersaturated quantity of P and are regulated by the two complex formation constant K_1 and K_2.

The single ratio K_1/K_2, obtained from each solution, was calculated according to Eq. 2:
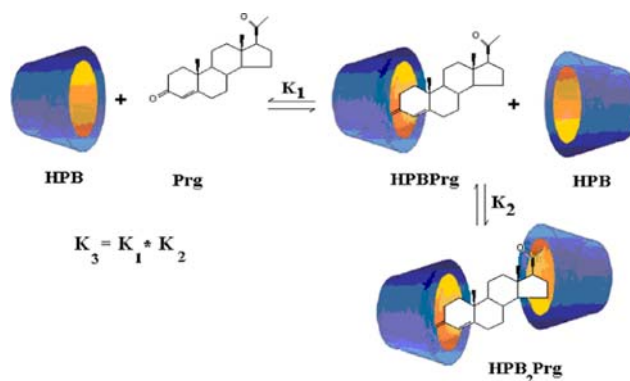

Fig. 5 Mechanism of molecular association of the complex

$$\frac{K_1}{K_2} = \frac{(\text{HPBCDP}) * (\text{HPBCDP})}{(\text{P}) * (\text{HPBCD}_2\text{P})} \tag{2}$$

Where

$$(\text{HPBCDP}) = (\text{HPBCD}_2\text{P} + \text{HPBCDP}) - (\text{HPBCD}_2\text{P}) \tag{3}$$

(HPBCD_2P) concentration was measured from experiment A while $(\text{HPBCD}_2\text{P} + \text{HPBCDP})$ concentration was measured from experiment B.

In order to calculate preliminarily the magnitude of K_1 and K_2 constants we have assumed that the actual K_1/K_2 ratio is the mean value calculated from the single ratio obtained.

Mean $K_1/K_2 = 108.512$

K_1/K_2 ratio value and the value of the apparent complex formation constant (K_3) are used in the following mathematic system to calculate the specific K_1 and K_2 values

$$\begin{cases} K_3 = K_1 * K_2 = 111473.7041 \\ K_1/K_2 = 108.512 \end{cases}$$

$$\begin{cases} K_1 * K_2 = 111473.7041 \\ K_1 = 108.512\, K2 \end{cases} \begin{cases} K_1 * K_2 = 111473.7041 \\ K_1 = 108.512\, K2 \end{cases}$$

$$\begin{cases} K_2 = \sqrt{\dfrac{111473.7041}{108.52}} \\ K_1 = 108.512\, K2 \end{cases}$$

$$\begin{cases} K_2 = 32.051 \text{ m}^{-1} \\ K_1 = 108.512 * 32.051 \end{cases} \begin{cases} K_2 = 32.051 \text{ m}^{-1} \\ K_1 = 3477.96 \text{ m}^{-1} \end{cases}$$

$K_1 = 3477.96 \; K_2 = 32.051 \; K_3 = 111473.7041$ (from the phase solubility exp.)

In the presence of oversaturated quantity of P, the first complex 1:1 is regulated by a relatively low constant (K_1) with a magnitude of 10^3. This complex is quickly converted to a 1:2 complex by a weak constant (K_2) with a magnitude of 10^1. The mechanism of complex formation is in favour of the scheme reported in Fig. 5, a sequential formation of the 1:1 followed by the 1:2 complex.

Physico-chemical methods

The DSC (Fig. 6) and X-ray (Fig. 7) analysis confirm the published data indicating the absence of crystal structures in the powder.

The structural evaluation by spectroscopic analysis (Raman, FTIR and NMR) confirm the presence of an inclusion complex with a stoichiometry guest/host 1:2.

In the Raman spectra of P/HPBCD complex (Fig. 8A), the intensity of the corresponding peaks of pure Progesterone (Fig. 8B), are reduced and shift towards lower cm^{-1} values ($\Delta = 4$–6 and 6–8 cm^{-1}, according to the different points of the sample tested). Moreover a broadening of these bands (stretching
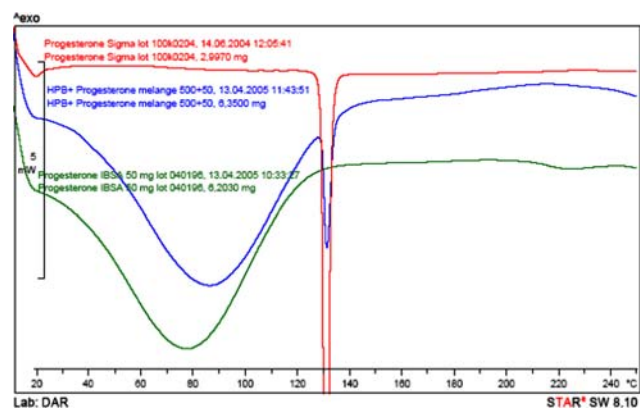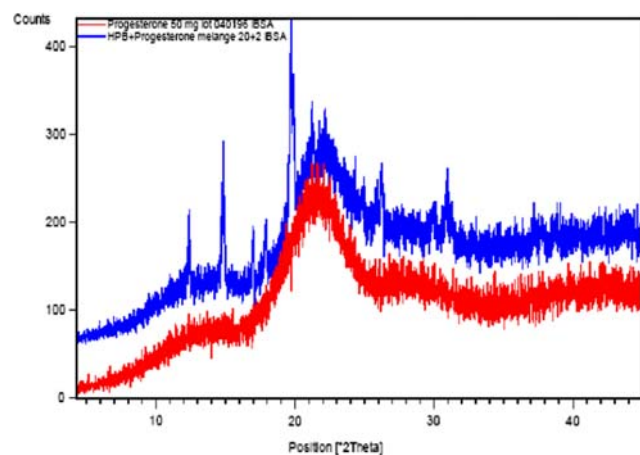

Fig. 6 Differential scanning calorymetry (DSC)
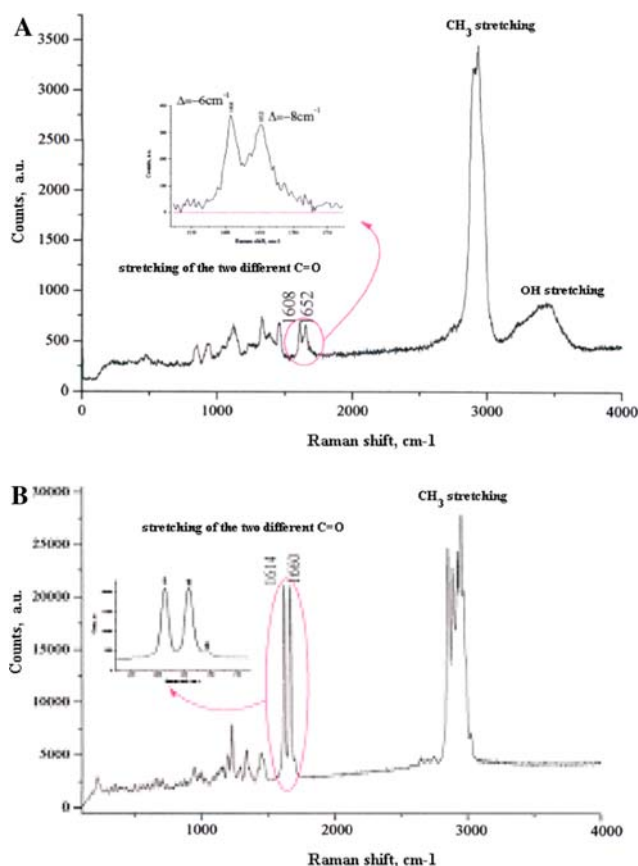

Fig. 7 X-ray analysis

Fig. 8 (A) Raman spectra of P/HPBCD complex, (B) Raman spectra of P complex

vibration of the C=O groups) is also observed in the Raman spectrum of the complex. The increase of the bandwidths, that means the decrease of the vibrational relaxation time, confirms the interaction between both C=O Groups with the HPBCD.
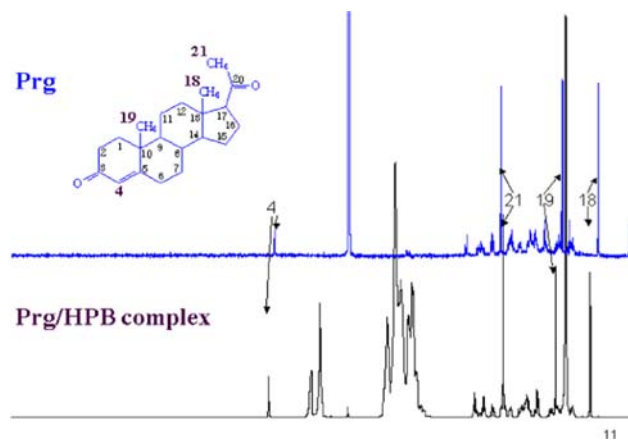

Fig. 9 Proton NMR spectra in deuterated water of progesterone (upper line) and P/HPBCD 1:2 complex (lower line). Number and arrow above the progesterone spectrum indicate the chemical shift induced by HPBCD inclusion

NMR experiment (Fig. 9) shows shifts of signals attributed to P protons 4, 18,19 and 21 indicating a involvement of rings A and D in the inclusion complex.

From the measure of the area of P proton 4 and anomeric proton of HPBCD, the 1:2 guest/host molecular ratio is confirmed.

Conclusions

The inclusion complex in study (P-HPBCD) has a 1:2 type guest/host complex with a medium apparent complex formation constant (K_3) of 111473.70 m^{-1}. This constant is a product of two primary formation constants (K_1 and K_2) having values of 3477.96 m^{-1} and 32.051 m^{-1} respectively. This inclusion complex is demonstrated by Raman, NMR, DSC and X-ray techniques.

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