Saudi Pharmaceutical Journal 27 (2019) 1096–1106

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Progesterone loaded thermosensitive hydrogel for vaginal application: Formulation and *in vitro* comparison with commercial product

Natalia S. Velázquez, Ludmila N. Turino, Julio A. Luna, Luciano N. Mengatto *

Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Universidad Nacional del Litoral-Consejo Nacional de Investigaciones Científicas y Técnicas (UNL-CONICET), Centro Científico Tecnológico, Colectora Ruta Nacional 168, Paraje El Pozo, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 21 May 2019
Accepted 22 September 2019
Available online 25 September 2019

Keywords:
Progesterone
Methylated-β-cyclodextrin
Inclusion complexes
In vitro diffusion
Drug delivery

ABSTRACT

Progesterone (PGT) is a natural hormone that stimulates and regulates various important functions, such as the preparation of the female body for conception and pregnancy. Due to its low water solubility, it is administered in a micronized form and/or in vehicles with specific solvents requirements. In order to improve the drug solubility, inclusion complexes of PGT and β-cyclodextrins were obtained by the freeze-drying method. Two β-cyclodextrins (native and methylated) in two solvents (water and water: ethanol) and different molar ratio of the reagents were the variables tested for the selection of the best condition for the preparation of the complexes. The PGT/randomly methylated-β-cyclodextrin complexes were incorporated into chitosan thermosensitive hydrogels, as an alternative formulation for the vaginal administration of PGT. Neither the micro and macroscopic characteristics of the gels nor the transition time from solution to gel were modified after the complexes incorporation. In addition, chitosan gels with complexes resisted better the degradation in simulated vaginal fluid in comparison to commercial gel (Crinone®). The chitosan gel with inclusion complexes and Crinone® were tested *in vitro* in a diffusion assay to evaluate the delivery of the hormone and its diffusion through porcine epithelial mucosa obtained from vaginal tissue. Chitosan gel presented sustained diffusion similar to the exhibited by commercial gel. The use of chitosan gels with inclusion complexes based on cyclodextrins would be a viable alternative for vaginal administration of PGT.

1. Introduction

Progesterone (PGT) is a natural steroid hormone involves in the preservation of normal physiology of female reproductive system. In this way, exogenous PGT is used as medication in the prevention of preterm birth, in luteal phase support for *in vitro* fertilization, in hormone replacement therapy and in endometrial hyperplasia and primary endometrial carcinoma treatment (Scavone et al., 2016).

PGT can be administered by different routes: oral, intramuscular and vaginal; having many options for its formulation (Scavone et al., 2016; Almomen et al., 2015; Cometti, 2015). In general, the current commercially available formulations present advantages and drawbacks. For example: capsules for oral administration allow achieving low endometrial concentrations of the drug because of the first pass metabolism by the liver; and intramuscular injections can cause pain and irritation at the injection site reducing the patient's compliance and the therapeutic efficacy. The use of vaginal route can cause vaginal discharge, irritation and mild application site reactions. Nevertheless, it exhibits a preferential absorption, since the drug is transported directly to the uterus (Almomen et al., 2015; Cometti, 2015; Choudhury et al., 2011; Lockwood et al., 2014).

Crinone® is a white soft gel commercially available in boxes containing 6, 15 or 18 applicators. The applicator is designed to deliver a pre-measured dose of the gel directly into the vagina. This gel showed an efficacy comparable to intramuscular formulations, achieving a stable endometrial PGT concentration with low serum levels, while reducing adverse systemic effects (Michnova et al., 2017; Silverberg et al., 2012; Moini et al., 2011; Wang et al., 2015). The carrier vehicle is an oil-in-water emulsion. However,

* Corresponding author.
E-mail address: lmengatto@santafe-conicet.gov.ar (L.N. Mengatto).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2019.09.006
1319-0164/

the PGT is only partially soluble in both phases; the majority of the hormone exists as a suspension in micronized scale. In addition, its main components are cross-linked acrylic acid polymers (carbopol and polycarbophil), which accumulate in the tissue generating vaginal irritation, painful sexual intercourse and other side effects (Check, 2009).

In pharmaceutical technology, the design and development of novel drug delivery systems aim to increase efficiency of drug delivery and safety in the course of administration and treatment, providing more convenience for the patient. During the optimization of PGT formulations, increasing its aqueous solubility is one of the first problems to be overcome. In some dosage forms, it is necessary the use of oils owing to PGT being practically insoluble in water (7 mg/L or 22.26 μM, at 25 °C). Cyclodextrins (CDs) are cyclic oligosaccharides produced by selective enzymatic synthesis from starch. CDs have a three-dimensional structure with a hydrophobic cavity and a hydrophilic exterior, making them useful tools to improve the aqueous solubility of insoluble (or lowly soluble) drugs. Since they are natural products, they have a very low toxic effect and can be used in medicines, food and cosmetics (Messner et al., 2010; Loftsson and Brewster, 2012). In addition to natural CDs, chemically modified CDs are extensively used. In general, CDs consist of 6, 7 or 8 glucopyranose units, linked by α-(1–4) bonds, known as α-, β- and γ-CDs, respectively (Messner et al., 2010; Martin Del Valle, 2004). The hydrophobicity of their internal cavity provides them the capacity to act as a host and form stable structures, called inclusion complexes (ICs), with other molecules of very diverse nature (guest). These complexes exhibit new physicochemical characteristics, since bioavailability, water solubility, stability in light presence, heat or oxidation conditions of the included drug are improved, at the same time that unwanted side effects may decrease (Chaudhary and Patel, 2013; Chordiya and Senthilkumaran, 2012; Sharma and Baldi, 2014). ICs between PGT and natural or modified CDs have been prepared by different methods such as precipitation, freeze-drying and spray-drying, providing remarkable improvement in PGT water-solubility (Scavone et al., 2016; Lahiani-Skiba et al., 2006; Zoppetti et al., 2007b; Cerchiara et al., 2003; Lockwood et al., 2014).

With the advent of *in vitro* fertilization and other assisted reproductive procedures, vaginal formulations of PGT became again the focus of research and one option to administer PGT through this route is gels. CHT is a biodegradable and biocompatible linear polymer which can be used in pharmaceuticals formulations as *in situ* thermosensitive hydrogels (Mengatto et al., 2016). In addition, CHT gels have been proposed for vaginal delivery of different active ingredients. This polymer possesses remarkable features such as good mucoahesion which improve the retention of the formulation inside the vagina, antimicrobial attributes, and suitable mechanical, release and penetration enhancer properties (Caramella et al., 2015; Tuğcu-Demiröz et al., 2015; Cook and Brown, 2018). CHT gels developed for the vaginal delivery of oxybutynin presented the easiest application in comparison to other formulations and histological studies showed that the drug was absorbed without damaging the tissue due to the polymer has preventative effect against cell damage (Tuğcu-Demiröz et al., 2013). Stability and durability studies performed on a CHT based gel for vaginal application of PGT suggested extended residence time due to mucoadhesion and thermosensitive properties of the polymer (Almomen et al., 2015). The aim of our work was the preparation of gels based on CHT and PGT/randomly methylated-β-cyclodextrin complexes. The CHT gel with ICs and Crinone® commercial product were tested in a release experiment to compare the delivery of the hormone and its diffusion performance through porcine epithelial mucosa obtained from vaginal tissue. Therefore, CHT gels containing ICs were studied as an alternative formulation for vaginal application of PGT.

2. Materials and methods

2.1. Materials

PGT (MW = 314.45 g/mol, purity 99.2%) was acquired in Farmabase (Italy). RAMEβ-CD, (MW = 1291.8 g/mol, Degree of substitution = 12) was purchased from Cyclolab (Hungary). β-CD (MW = 1135 g/mol) was donated (Roquette, France). Crinone® 8% gel (Merck-Serono) was purchased at a local supplier. CHT (MW = 600,000 g/mol, Degree of deacetylation = 75–85%) was purchased from China Easter Group (China). β-glycerophosphate disodium salt (GP) was kindly provided by Surfactan S.A. (Argentina). The water was of Milli-Q quality (Millipore, USA). Ethanol (EtOH), acetic acid and lactic acid were analytical grade (Cicarelli, Argentina). Methanol (MeOH) was HPLC grade (Merck, Germany). Isotonic phosphate buffer saline with EtOH (PBS-EtOH, 80:20 v:v, pH = 7) was prepared by mixing 800 mL of PBS with 200 mL of EtOH. PBS was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO4, 1.44 g of $Na_2HPO_4{\cdot}2H_2O$ in 1 L of water. Simulated vaginal fluid (SVF, pH = 4.2) was prepared by dissolving: 3.51 g NaCl, 1.4 g KOH, 0.222 g $Ca(OH)_2$, 0.018 g of bovine serum albumin, 2 g of lactic acid, 1 g of acetic acid, 0.16 g of glycerol, 0.4 g of urea and 5 g of glucose in 1 L of water (Marques et al., 2011). Potassium bromide (KBr) and reagents of PBS and SVF solutions were analytical grade (Anedra, Argentina).

2.2. PGT quantification by HPLC

The concentration of PGT was determined by a HPLC system (Prominence Series 20A, Shimadzu). The chromatographic separation was performed using a Zorbax Eclipse XDB-C18 column (250 × 4.6 mm, 5 μm pore size) (Agilent). The conditions of analysis were: oven temperature 30 °C, mobile phase MeOH:water (95:5, v:v), flow rate 1 mL/min and the wavelength of detection 254 nm (Helbling et al., 2015).

A stock solution of PGT (300 μg/mL) in MeOH was prepared. In order to verify the linearity of the analytical procedure within a concentration range of 1–100 μg/mL of PGT, six concentration levels of standard solutions were prepared in mobile phase and analyzed in triplicate. The calibration curve (Absorbance as a function of PGT concentration) was fitted to a straight line using linear regression analysis. Experimental samples were diluted in mobile phase as necessary.

2.3. Phase solubility studies

Phase solubility diagrams were obtained following the methodology developed by Higuchi and Connors (1965). A fixed amount of PGT (in excess) and increasing amounts of CD (β-CD: 0–2 mM or RAMEβ-CD: 0–150 mM) were placed in amber glass containers with a specific solvent (water or water:EtOH 50:50, v:v). The containers were stored at 37 °C with orbital shaking (100 rpm) for 1 week. Once the equilibrium was reached, the containers were centrifuged, the supernatants were filtered (0.22 μm) and the concentration of PGT was measured by HPLC. Each experiment was performed in triplicate.

The linear portion of the solubility diagrams was fitted to a straight line with slope and intercept. The Apparent 1:1 Stability Constant ($K_{1:1}$) was calculated according to the Higuchi-Connors equation (Higuchi and Connors, 1965):

$$K_{1:1} = \frac{\text{Slope}}{S_o \cdot (1 - \text{Slope})} \quad (1)$$

where S_o is the intrinsic solubility of PGT in the solvent without CD (y-intercept) and Slope is the slope of the straight line.

In addition, the Complexation Efficiency (CE) was calculated (Eq. (2)). This parameter was proposed by Loftsson and Brewster (2012) and relates the concentration of CD that forms a complex and the concentration of free CD:

$$CE = \frac{Slope}{(1 - Slope)} \quad (2)$$

2.4. Preparation of PGT/CD inclusion complexes

PGT/RAMEβ-CD ICs were obtained by the freeze-drying method. First, PGT was dissolved in EtOH and a proportional amount of RAMEβ-CD was dissolved in water (PGT:RAMEβ-CD molar ratios 1:1; 1:5; 1:10 and 1:20). Both solutions were mixed and magnetically stirred for 15 min. Then, the containers were placed in an ultrasonic bath for 5 min. The organic solvent was removed under reduced pressure with a rotary evaporator and the solution was centrifuged to remove reagents that did not form a complex. The supernatant was frozen at −80 °C and freeze-dried for 24 h at 1 mbar pressure in a laboratory freeze dryer (Cryodos −80, Telstar).

In order to evaluate the inclusion procedure, efficacy and yield were calculated.

The mobile phase used for PGT quantification by HPLC contains MeOH, then it can dissolve the free and the included PGT. In water, only the included PGT will be dissolved (Bouquet et al., 2007). The same amount of sample was dissolved in water and in mobile phase and the solutions were analyzed by HPLC.

The Inclusion Efficacy ($E_{\%}$) was calculated according to the following equation:

$$E_{\%} = \frac{C_{water}}{C_{Mobile\ phase}} \cdot 100 \quad (3)$$

where C_{water} and $C_{Mobile\ phase}$ are the PGT concentrations in the sample dissolved in water and mobile phase, respectively. An $E_{\%}$ value near 100% was considered as a successful interaction and/or formation of complexes between PGT and RAMEβ-CD.

The Inclusion Yield ($Y_{\%}$) was calculated according to the following equation:

$$Y_{\%} = \frac{PGT_{final}}{PGT_{initial}} \cdot 100 \quad (4)$$

where PGT_{final} is the mass of PGT recovered at the end of the freeze-dried procedure in the total lyophilized material and $PGT_{initial}$ is the initial mass of PGT used to prepare the ICs.

2.5. Characterization of PGT/CD inclusion complexes

2.5.1. Differential scanning calorimetry

Approximately 5 mg of the sample were weighed into aluminum capsules and tested on a Mettler DSC821e thermal analyzer (Mettler Toledo). All the assays were carried out under controlled nitrogen atmosphere and with a heating rate of 10 °C/min. The samples were PGT, RAMEβ-CD, and both the physical mixture and the IC in a 1:1 molar ratio.

2.5.2. Nuclear magnetic resonance

Nuclear magnetic resonance spectra were obtained using an AVANCE 300 MHz spectrometer (Bruker). The samples (PGT and the IC in a 1:1 molar ratio) were dissolved in deuterated chloroform ($CDCl_3$) at 25 °C. A chemical shift (δ) of 7.26 ppm for $CDCl_3$ was used as internal reference.

The variation of chemical shift (Δδ) of the protons in the PGT due to the inclusion of the hormone into the cavity of the CD was calculated applying the following equation:

$$\Delta\delta = \delta_{IC} - \delta_{Free} \quad (5)$$

where δ_{IC} is the proton shift of the PGT in the IC and δ_{Free} is the proton shift of the PGT when it is free i.e. not included.

2.5.3. Dynamic light scattering

The hydrodynamic size and size distribution measurements were carried out by dynamic light scattering using a Zetasizer Nano-ZS (Malvern). A sample of the RAMEβ-CD and the IC in a 1:5 molar ratio were dissolved in Milli-Q water and placed in a polystyrene cuvette (dimensions: 1.0 × 1.0 cm). The sample was irradiated with a He-Ne laser (λ = 633 nm) at 25 °C. The refractive index was set at 1.33 and the viscosity at 0.8872 cP. The intensity of the scattered light was detected with a backscattering angle of 90°.

2.5.4. Scanning electron microscopy

Micrographs were obtained by observation with a Phenom ProX Desktop Scanning Electron Microscopy (Thermo Fisher). The observations were made using an acceleration voltage of 15.0 kV. The samples were: PGT, RAMEβ-CD, and both the physical mixture and the IC in a 1:5 molar ratio.

2.6. CHT gels with inclusion complexes

CHT (2% w/w) was dissolved in an acetic acid solution (0.15 M). GP (35% w/w) and the PGT/RAMEβ-CD ICs were dissolved together in water. Both solutions were mixed and allowed to gel at 37.5 °C (Mengatto et al., 2016).

The gels were characterized by Fourier-transform infrared spectroscopy, scanning electron microscopy, stability in SVF and rheological measurements. Infrared spectroscopy studies were performed on a FTIR-8201 PC spectrometer (Shimadzu), in the frequency range of 400–4000 cm^{-1} at a resolution of 8 cm^{-1} and 40 scans per spectrum. A weighed amount of sample was blended with KBr and compressed to obtain disks. The concentration of each sample in the disk was approximately 1% in order to obtain more defined spectra.

Microscopic observations were carried out with a Phenom ProX Desktop Scanning Electron Microscopy (Thermo Fisher). The samples were frozen and freeze-dried before observations.

The stability of the CHT gel with ICs and the commercial gel (Crinone®) in SVF (pH = 4.2) was determined. A weighed amount of the gels was placed in baskets-type containers that were submerged in 20 mL of SVF and were maintained at 37 °C. At different times the baskets with the gels were gently removed and weighed.

Rheological measurements were performed on a rheometer (Haake RheoStress RS80, Haake Instrument Inc.) with parallel plates (35 mm diameter, 2 mm gap). The temperature of the lower plate was maintained at 37 °C with a circulating bath water. Sand paper in the upper plate was used to eliminate slippage (Olivares et al., 2012). The linear viscoelastic region was determined by performing strain sweep tests from 0.01 to 0.1 at 10 Hz. Frequency sweep tests were performed from 0.1 to 10 Hz at strain amplitude of 0.03 (strain deformation within the linear viscoelastic region). The dynamic rheological data obtained included the 2 components of complex shear modulus (G*): the storage modulus (G′) and the loss modulus (G″), and the complex viscosity ($|\eta^*| = |G^*|/\omega$, ω = frequency of oscillation). The samples were CHT gel + IC and Crinone® freshly prepared and after 15 min of immersion in SVF (37 °C). Each experiment was performed in triplicate.

2.7. In vitro release experiments

Release experiments were performed using a vertical Franz diffusion cell (PermeGear Inc., USA), with a diffusion area of 1.77 cm^2 and 12.0 mL of receptor compartment volume.

Porcine vaginal tissue, obtained from females between 5 and 6 months of age, was donated by a local slaughterhouse (Figan, Santa Fe, Argentina). Immediately after the animals were sacrificed, the vaginal tissue was removed and placed in PBS until it arrived at the laboratory.

The epithelial mucosa was carefully separated, fractionated and stored at −80 °C. The day before the experiment, a sample of the tissue was thawed and stabilized in buffer solution. Subsequently, it was placed between both compartments with its luminal face towards the donor compartment. The delivery systems tested in the donor compartment were: PGT solution, ICs solution, CHT gel + ICs and commercial gel. The solutions were prepared in SVF:EtOH 80:20 (v:v). In the case of the gels, 0.25 mL of SVF were placed over them to mimic the vaginal environment conditions and prevent gel dryness. The donor compartment was covered to avoid evaporation. The receptor compartment was continuously stirred and maintained at 37.5 °C. EtOH was used to ensure the solubility of the hormone (Monteiro Machado et al., 2015). The initial concentration of PGT in the four systems was the same. Samples of 200 µL were withdrawn at regular intervals of time and replaced with fresh medium. The amount of PGT was quantified by HPLC. Each assay was performed in triplicate.

The percentage of accumulated PGT (%) was represented as a function of time (t).

The permeation profiles were compared using the difference (f_1) and similarity (f_2) factors (Cascone, 2017). These factors were calculated by the following equations:

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \cdot 100 \quad (6)$$

$$f_2 = 50 \cdot \log\left\{ \left[1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} |R_t - T_t|^2 \right]^{-0,5} \cdot 100 \right\} \quad (7)$$

where n is the number of samples, R_t and T_t are the percentages of drug released from the reference product and the system to be evaluated, respectively, for each time t. Two profiles were considered equivalent when the value of f_1 is less than 15 and f_2 is in the range 50–100 (Cascone, 2017).

2.8. Statistical analysis

ANOVA and Fisher test were used to compare two or more means, respectively.

3. Results and discussion

3.1. Phase solubility studies

Solubility diagrams for the β-CD and the RAMEβ-CD are presented in Fig. 1A and B, respectively. Although the study do not verify the formation of the ICs, they describe how the drug solubility increases when CD concentration increases (Messner et al., 2010), providing valuable information about their interaction. Solubility diagrams of the PGT with β-CD in water (Fig. 1A) showed a behavior of the B_s type, according to the classification of Higuchi and Connors (1965). This suggests the formation of complexes of limited solubility in water (Uekama et al., 1982; Lahiani-Skiba et al., 2006). Solubility diagrams of the PGT with RAMEβ-CD (Fig. 1B) presented typical curves of the A_L type. The diagrams showed that the PGT solubility increases linearly with the concentration of RAMEβ-CD throughout the range of CD concentrations studied, due to the formation of soluble complexes. Lahiani-Skiba et al. (2006) evaluated the behavior of PGT with a trimethylated β-CD and Luppi et al. (2005) with a dimethylated β-CD. These authors reported A_L type profiles. The PGT solubility in the absence of RAMEβ-CD (S_o) was greater in the solution with EtOH in comparison to water. The addition of an organic cosolvent renders the mixture more favorable for the dissolution of a hydrophobic solute such as PGT (Loftsson and Brewster, 2012).

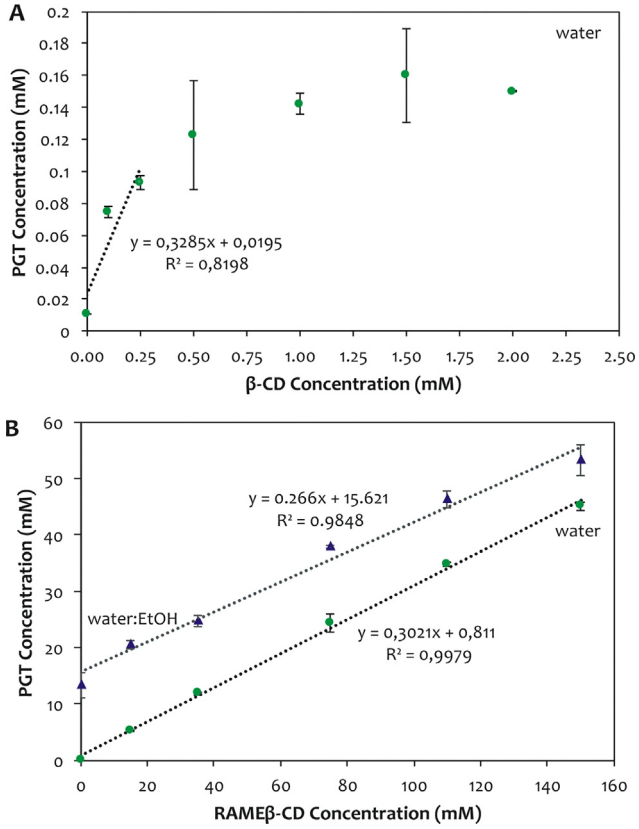

Fig. 1. Phase solubility diagrams of PGT (A) with β-CD in water and (B) with RAMEβ-CD in water and in water:EtOH 50:50 v/v.

Table 1 shows the values of S_o (y-intercept), $K_{1:1}$ and CE, calculated with Eqs. (1) and (2), respectively. $K_{1:1}$ value for the RAMEβ-CD in water indicated a strong affinity of the CD for the PGT (Luppi et al., 2005; Lahiani-Skiba et al., 2006; Ma et al., 2011). This behavior is due to the high hydrophobicity of the PGT (partition coefficient in an octanol-water system: P_{oct} = 7410; Tomida et al., 1978). Also, the $K_{1:1}$ obtained in water (74526.09 M^{-1}) was higher than that obtained for the mixture water:EtOH (27.13 M^{-1}). As the organic portion of the medium increases, the apparent constant decreases due to a reduction of the polarity of the medium (Loftsson and Brewster, 2012).

The value of the constant for the β-CD (46256.79 M^{-1}) indicated that the CD is also very effective in forming stable complexes. The difference in the PGT interaction with natural CD and its methylated derivative could be explained taking into account the differences in aqueous solubility (Popielec and Loftsson, 2017). In the RAMEβ-CD, the methyl groups, increase the hydrophobicity of the cavity and facilitate drug binding (Cirri et al., 2005).

The estimation of $K_{1:1}$ is affected by the value of the solubility. Therefore, Loftsson and Brewster (2012) proposed the calculation of CE. This parameter is independent of the solubility and it is more suitable for the determination of the solubilizing effect of CDs. The CE values agreed with typical values reported for aqueous media

Table 1
Solubility (S_o), Apparent Stability Constants ($K_{1:1}$) and Complexation Efficiencies (CE) values.

CD	Solvent (v:v)		S_o (mM)	$K_{1:1}$ (M^{-1})	CE
	Water	EtOH			
β-CD	100	–	0.0106 ± 0.0001	46256.79 ± 3614.41	0.489 ± 0.035[a]
RAMEβ-CD	100	–	0.0058 ± 0.0041	74526.09 ± 3326.72	0.433 ± 0.004[a]
	50	50	13.36 ± 2.24	27.13 ± 8.16	0.362 ± 0.036[b]

[a,b]Different superscript letters in a column indicate statistically significant differences between groups (analysis of variance and Fisher test, $p < 0.05$).

($CE_{average} \sim 0.3$) (Loftsson and Brewster, 2012). The CE obtained for the CDs in water (β-CD = 0.489 and RAMEβ-CD = 0.433) were slightly higher ($p < 0.05$) than that obtained for the mixture water:EtOH (0.362). Therefore, in water, 1 of each 3 CD molecules is assumed to form a water soluble complex. For RAMEβ-CD in water:EtOH, 1 of each 4 CD molecules formed a water soluble complex (Loftsson et al., 2007). Both CDs can form stable complex with PGT. Nevertheless, RAMEβ-CD was selected for the preparation of the ICs based on its excellent aqueous solubility and due to the obtained complexes also being soluble.

3.2. Preparation of PGT/CD inclusion complexes

The preparation of the complexes was carried out using water and water:EtOH (50:50, v:v) as solvents. Although most of the studies report 1:1 or 1:2 molar ratios (PGT:CD), experiments with higher ratios were done in order to evaluate their effect on the ICs formation. Inclusion Efficacy ($E_{\%}$) and Inclusion Yield ($Y_{\%}$) were calculated with Eqs. (3) and (4), respectively. In general, these parameters are not reported in the bibliography; however, they are very useful to evaluate the inclusion method. The ICs prepared in water presented $E_{\%}$ values greater than 90%, but $Y_{\%}$ values were lower than 40%. For this reason, water:EtOH was the solvent selected for the ICs preparation throughout the work.

In Table 2, $E_{\%}$ and $Y_{\%}$ for ICs prepared in water:EtOH are presented. $E_{\%}$ was greater than 95% when RAMEβ-CD concentration increased up to a molar ratio of PGT:RAMEβ-CD 1:10. Any further increase in CD concentration (1:20) decreased $E_{\%}$, probably due to CD precipitation (Frömming and Szejtli, 1993a; Frömming and Szejtli, 1993b). Regarding $Y_{\%}$, this value was in the range of 77–91 % for molar ratios equal or higher than 1:5. For the molar ratio 1:1, $Y_{\%}$ was the lowest ($p < 0.05$). When higher CD concentrations are used, the CD + PGT ↔ IC equilibrium is displaced to the complexes formation. In addition, other structures between the PGT and the RAMEβ-CD, such as non-inclusion complexes or aggregates can coexist with the ICs (Shakalisava and Regan, 2006; Loftsson et al., 2007). Also, there is an increase in variability with an increase in the concentration of CD. The molar ratio 1:5 was selected for the preparation of ICs for the *in vitro* release experiments due to $E_{\%}$ and $Y_{\%}$ presented the best values with the less CD concentration.

3.3. Characterization of PGT/CD inclusion complexes

In order to verify the formation of the ICs, thermal analysis, proton nuclear magnetic resonance, dynamic light scattering and scanning electron microscopy experiments were carried out. The thermal behavior of PGT, RAMEβ-CD, and both the physical mixture and the lyophilized IC in a 1:1 molar ratio was studied (Fig. 2). PGT thermogram showed the characteristic melting peak of the drug at 130.9 °C (Lahiani-Skiba et al., 2006; Zoppetti et al., 2007a; Li et al., 2018). In the physical mixture thermogram, a shift of the PGT endothermic peak to a slightly lower temperature (127.9 °C) was observed. According to Lahiani-Skiba et al. (2006), the explanation could be the existence of a very weak interaction at high temperatures between the PGT and the CD. In the IC thermogram, the absence of the melting peak of the PGT supported the formation of the complex (Lahiani-Skiba et al., 2006; Cerchiara et al., 2003). RAMEβ-CD did not produce any peaks of interest in the studied temperature range.

Table 2
Inclusion Efficacy (E%) and Inclusion Yield (Y%) for PGT/RAMEβ-CD inclusion complexes.

COMPLEX	PGT:RAMEβ-CD (mol:mol)	E (%)	Y (%)
IC_1	1:1	95.23 ± 4.22[a]	47.95 ± 7.72[b]
IC_2	1:5	98.25 ± 2.74[a]	82.12 ± 11.04[a]
IC_3	1:10	99.56 ± 0.76[a]	77.50 ± 19.64[a]
IC_4	1:20	86.74 ± 2.57[b]	91.33 ± 12.80[a]

[a,b]Different superscript letters in a column indicate statistically significant differences between groups (analysis of variance and Fisher test, $p < 0.05$).

The chemical structure with numbering of protons for each molecule is shown in Fig. S1. The nuclear magnetic resonance spectra obtained for the PGT and the IC are presented in Fig. 3. Inner protons of the RAMEβ-CD (H3 and H5), and protons H4, H18, H19 and H21 of the PGT (Fig. S1) are the most affected during inclusion of the drug into the cavity of the CD (Frömming and Szejtli, 1993a; Salústio et al., 2009; Zoppetti, 2011). A well-resolved nuclear magnetic resonance spectrum for the RAMEβ-CD was not obtained, possibly due to the presence of residual β-CD content. As a result, only some of the signals could be identified unambiguously. Signals of H3, H5 and H6 produced wide peaks caused by overlapped signals. For this reason, IC formation between PGT and RAMEβ-CD was studied only on the basis of the chemical changes of the drug (Jablan et al., 2011; García et al., 2014). The variation of chemical shift (Δδ) of the protons in the PGT due to the inclusion was calculated (Table 3). The greatest change was observed in H4, indicating that A ring may be the part of PGT involved in IC formation (Uekama et al., 1982).

IC and RAMEβ-CD were characterized by dynamic light scattering. Hydrodynamic diameter based on number (Dn) and

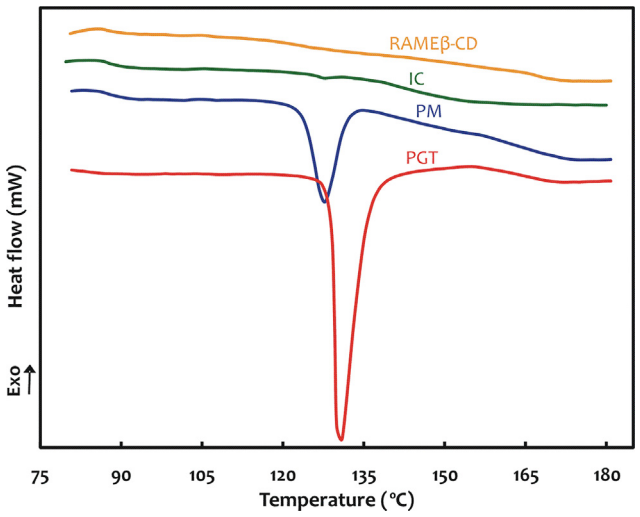

Fig. 2. Thermal transitions of PGT (red), RAMEβ-CD (yellow), 1:1 PGT/RAMEβ CD physical mixture (blue) and 1:1 PGT/RAMEβ-CD IC (green).

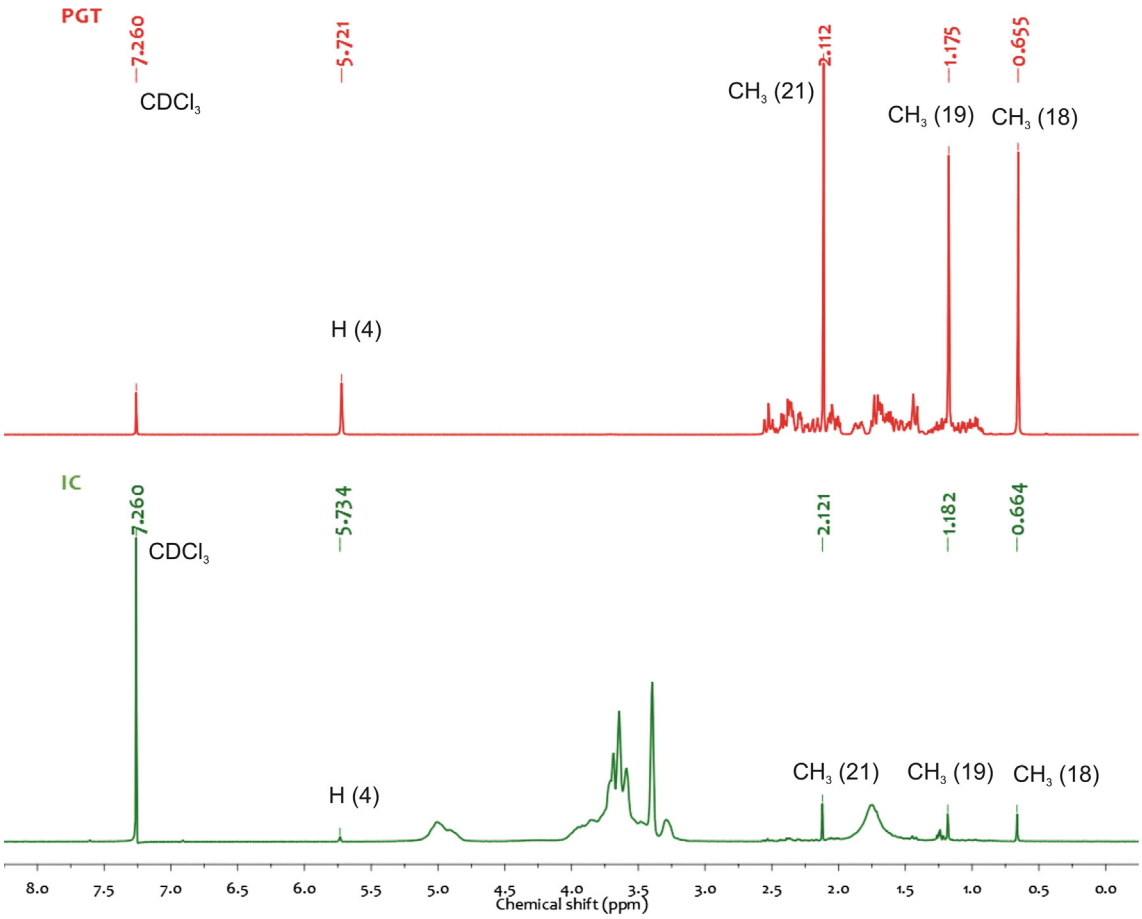

Fig. 3. Proton nuclear magnetic resonance spectra of PGT (red) and the IC (green).

polydispersity index (PDI) of the samples were obtained. One population was observed in the RAMEβ-CD sample, corresponding to monomeric CD (Dn: 1.1 ± 0.3 nm) with PDI value of 0.567 ± 0.014, which would indicate the presence of some CDs aggregates. The sample with the complexes also displays one population (Dn: 142.8 ± 6.8 nm, PDI: 0.199 ± 0.011) that presented a larger size than the monomeric CD. CDs and ICs can form aggregates, but in most cases they are small (Loftsson et al., 2007). In our case, the aggregates of the PGT/RAMEβ-CD ICs did not affect the optical properties of the solutions since they remained transparent.

Electron micrographs of PGT, RAMEβ-CD, physical mixture and IC were obtained (Fig. 4). PGT (A) presented a form of irregular granules and RAMEβ-CD (B) displayed hollow spherical particles and porous fragments. The physical mixture (C) showed a mixture of PTG and RAMEβ-CD structures. IC (D) morphology, by contrast, was observed like plates/plane structures with regular edges. The comparison of the images reveals that the ICs are structurally distinct from the CD and the physical mixture, which supports the PGT inclusion into the RAMEβ-CD cavity.

Table 3
Chemical shift values (δ) of protons of the free and included PGT and values of the chemical shift differences (Δδ).

PGT			
Proton	δ_{Free} (ppm)	δ_{IC} (ppm)	Δδ (ppm)
H (4)	5.721	5.734	0.013
CH_3 (18)	0.655	0.664	0.009
CH_3 (19)	1.175	1.182	0.007
CH_3 (21)	2.112	2.121	0.009

3.4. CHT gels with inclusion complexes

Fig. 5 shows infrared spectra of CHT gel, IC, CHT gel + IC, Crinone® and PGT. CHT gel + IC spectrum presented the characteristic peaks of their constituent components, with some variations. The band at 3400 cm^{-1} corresponding to the superposition of the —OH and —NH stretching vibrations shifted towards 3465 cm^{-1}. These groups are involved in the formation of inter and/or intramolecular hydrogen bonds (Islam et al., 2013), therefore the IC could present this type of interaction with the CHT molecule. The vibration of the —CH and —CH_2 groups of the CD in the region between 2800 and 3000 cm^{-1} (2920 cm^{-1}) and the peak corresponding to the group —OCH_3 (2852 cm^{-1}) were observed in the CHT gel + IC spectrum. The intensity of the band at 1650 cm^{-1} corresponding to C=O stretching vibration in amide I, changed after the addition of the IC to the gel. Similar changes were present in the peaks at 1458, 1419 and 1325 cm^{-1} corresponding to —OH and —CH bending and C—O stretching of the CHT molecule. In addition, there were significant changes in the spectral shape from 900 to 1250 cm^{-1}. The intensity of the bands at 1080 and 980 cm^{-1} decreased and a clear peak appeared at 1222 cm^{-1}. The IR spectrum obtained of the PGT agreed with the results reported by other authors (Zoppetti et al., 2007b; Lahiani-Skiba et al., 2006; Cerchiara et al. 2003; Liu et al., 2007; Li et al., 2018). The peaks corresponding

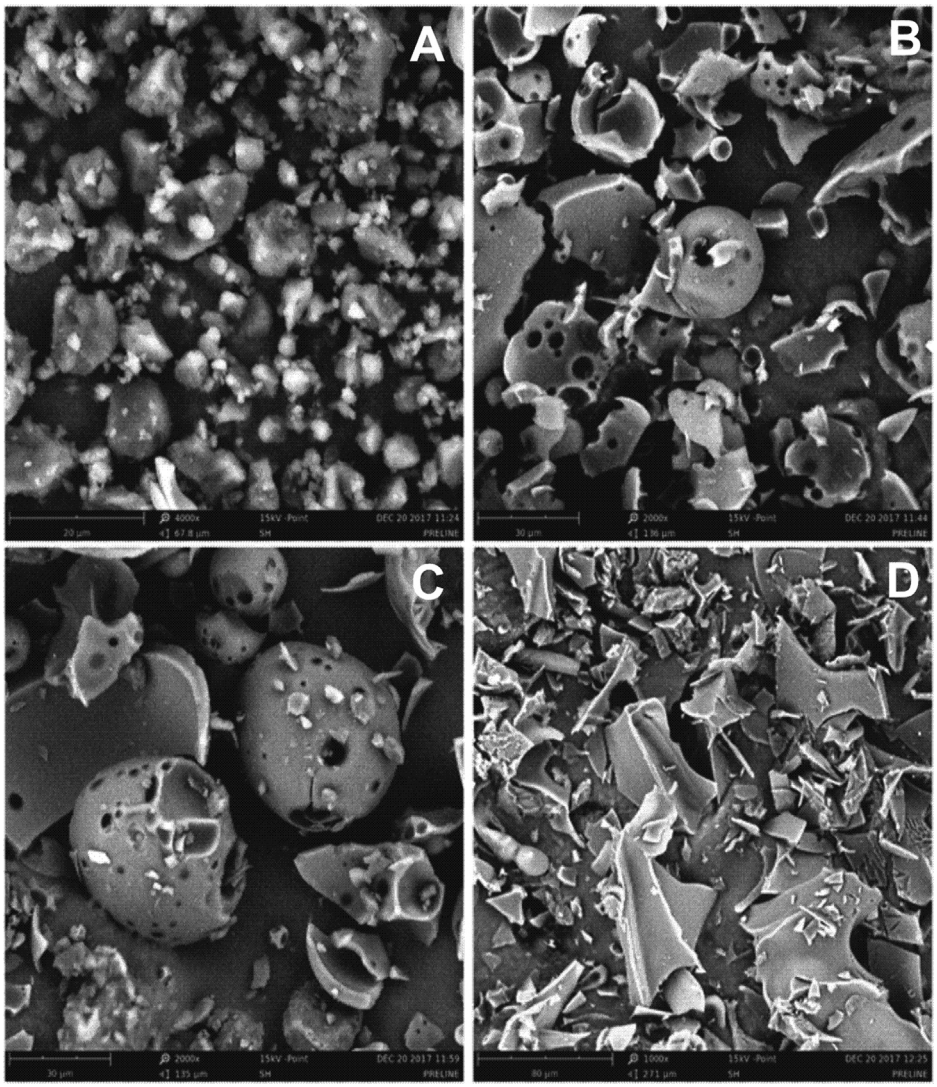

Fig. 4. Electron micrographs of (A) PGT (4000×), (B) RAMEβ-CD (2000×), (C) 1:1 PGT/RAMEβ-CD physical mixture (2000×) and (D) 1:1 PGT/RAMEβ-CD IC (1000×).

to the stretching of the C—H bond of CH_2 and CH_3 groups were observed in the region 2950–2850 cm^{-1}. The spectrum also showed the peaks at 1699 and 1662 cm^{-1} of the stretching of the C═O. In addition, the peak corresponding to the stretching of the C═C bond was noticed at 1614 cm^{-1}. Some of the peaks of the PGT appeared in the spectrum of Crinone® which possesses an overly complex matrix to make thorough analysis by this technique.

CHT gel, CHT gel + IC and Crinone® electron micrographs were obtained (Fig. 6). CHT gel (A) presented an open structure characterized by interconnected pores (Goycoolea et al., 2011; Ho et al., 2004). On the other hand, the CHT gel + IC (B) presented a microstructure similar to interconnected sheets with bigger pores. The structure of gels did not present important changes with the incorporation of the IC; they showed a good macroscopic consistency and the formation times were not modified in comparison to those of the blank gel. Crinone® (C) presented a smooth morphology with visible PGT crystals embedded throughout the material and absence of pores.

In order to study the stability of the CHT gel + IC and commercial gel in an acid medium (pH = 4.2) similar to *in vivo* condition, gels were placed in simulated vaginal fluid (SVF) at 37 °C. After 30 min, the commercial gel was completely dissolved, while the CHT gel + IC showed greater resistance to degradation at the same time (Fig. S2). Due to the lack of stability of the commercial gel it was not possible to obtain the variation of the weight over time.

The value of the strain amplitude was set at 0.03 (3%) after checked that all measurements were carried out within the linear viscoelastic region, where G* is independent of the strain amplitude (Fig. S3). Fig. 7(A and B) shows typical frequency dependence of the storage and loss moduli for CHT gel + IC and Crinone® freshly prepared and after 15 min of immersion into SVF (37 °C). The time of immersion was fixed in 15 min taking into consideration that during stability study the commercial gel was completely dissolved after 30 min. All samples showed values of G′ higher than G″ without exhibiting crossing point throughout the frequency range. Both gels can be classified as strong or true gels, because the molecular rearrangements within the network are reduced over the time scales analyzed and G′ is almost independent of the frequency (Rao, 1999). Freshly prepared CHT gel + IC and Crinone® samples show similar viscoelastic characteristics, i.e., the samples presented similar values of G′ and G″. After the immersion into SVF, some differences in the viscoelastic characteristics were noted. While the values of the moduli (G′ and G″) of Crinone® decreased, the values of CHT gel + IC slightly increased. These results suggest that CHT gel + IC better preserves its structure

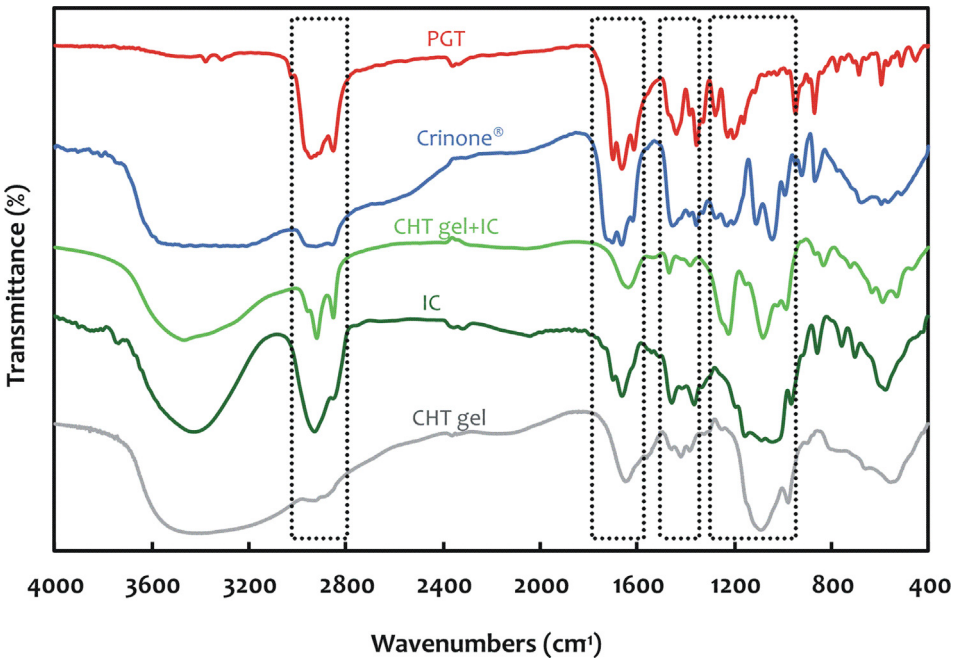

Fig. 5. Infrared spectra of PGT (red), Crinone® (blue), CHT gel + IC (light green), IC (green), and CHT gel (grey).

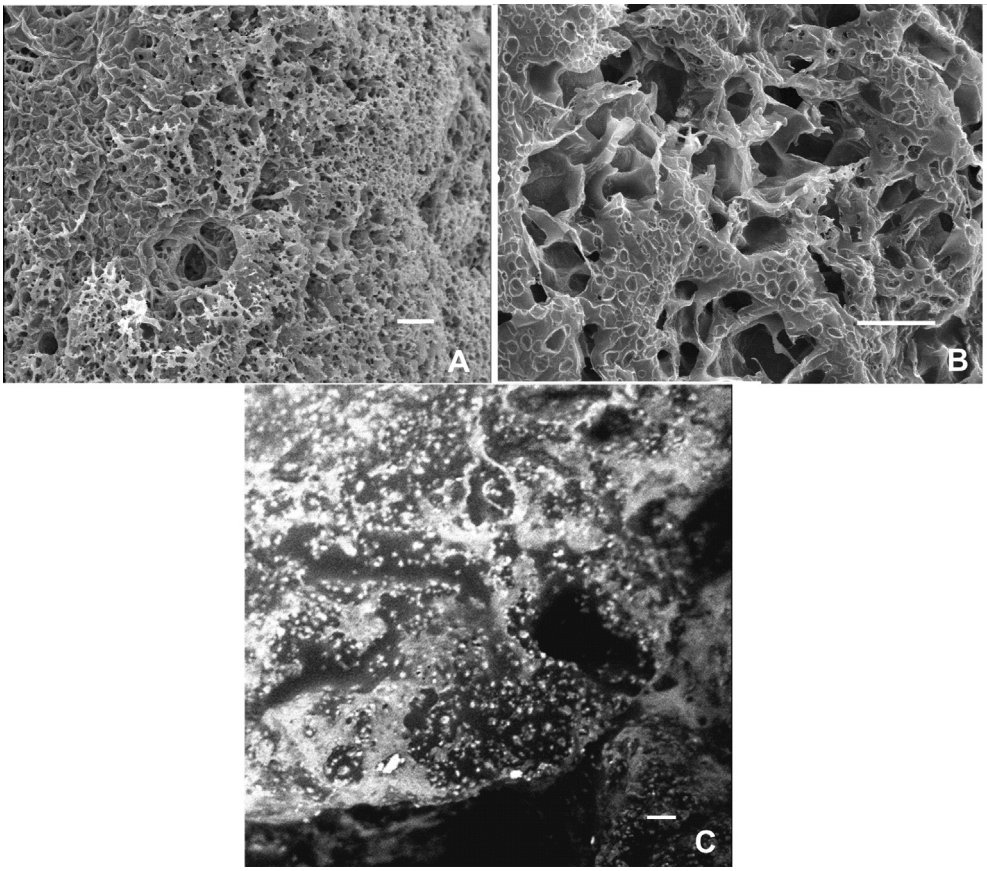

Fig. 6. Electron micrographs of (A) CHT gel (400×), (B) CHT gel + IC (400×) and (C) Crinone® (800×), scale = 20 μm.

and its strong gel characteristic after being exposed to SVF conditions than Crinone®. Fig. 7 (C and D) presents typical curves of complex viscosity as function of frequency. This material function determined under oscillatory shear testing allows the analysis of viscosity behavior of gel-type samples since it is a test that minimally disturbs the material avoiding the gel fracture. It is observed that all samples are shear thinning. However, while Crinone® decreased its viscosity and shear thinning character after the

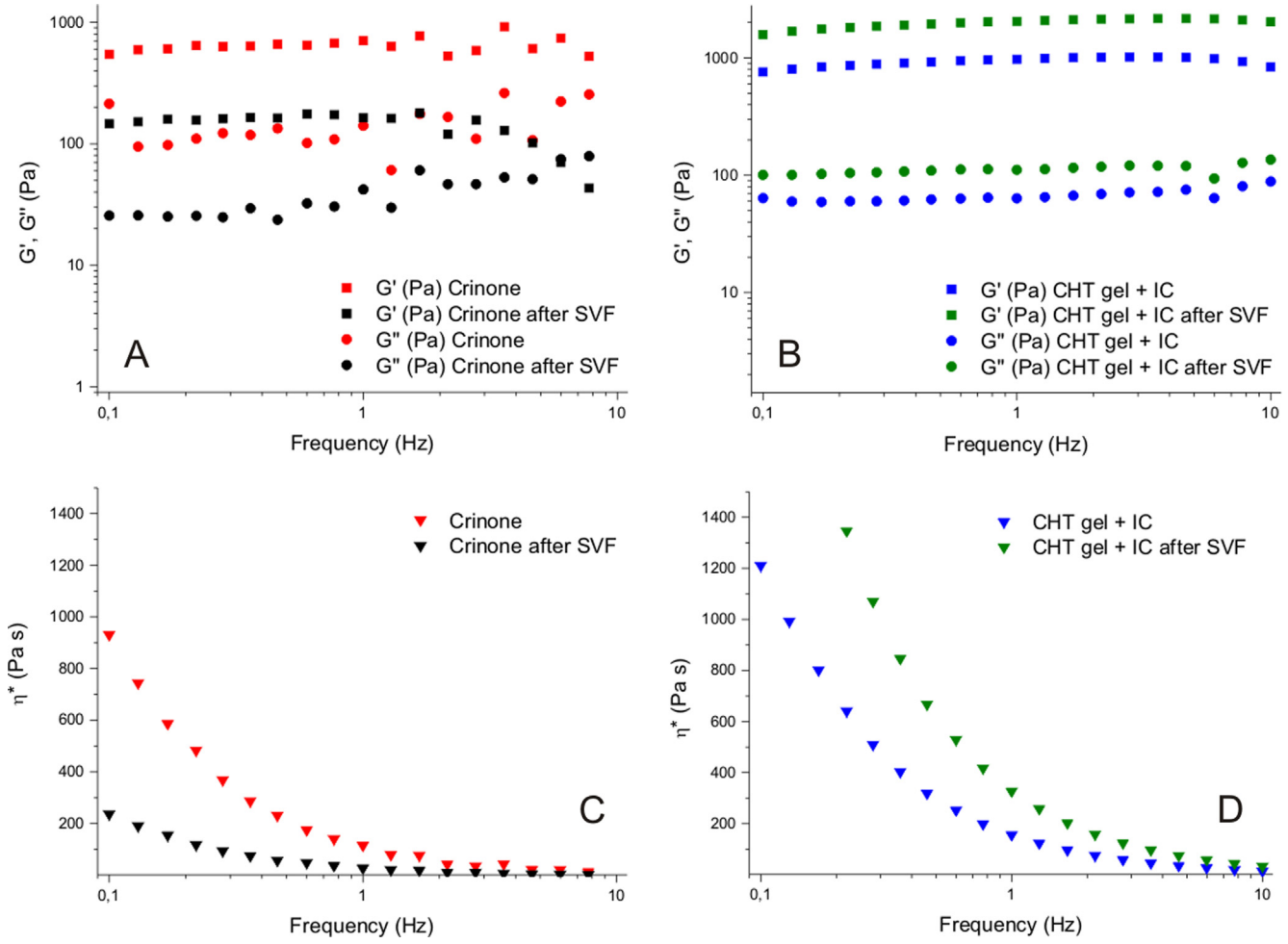

Fig. 7. Frequency dependence of the storage (G′) and loss moduli (G″) for Crinone® (A) and CHT gel + IC (B). Curves of complex viscosity as function of frequency for Crinone® (C) and CHT gel + IC (D).

immersion into SVF, the viscosity and shear thinning character of CHT gel + IC increased. These results may indicate that the CHT gel + IC sample with higher viscosity may stay longer inside the vaginal canal.

3.5. In vitro release experiments

Release studies through porcine epithelial mucosa obtained from vaginal tissue were performed on Franz diffusion cells with four different systems in the donor compartment: PGT solution, ICs solution, CHT gel + IC and Crinone®. The accumulated PGT (%) as a function of time (h) was plotted (Fig. 8). The permeation rate of the PGT in solution was much greater than the rate of the other systems (A).

For PGT solution, 49% and 100% of the initial amount of PGT permeated at 24 h and 60 h, respectively. For ICs solution, at 24 h, 15% of the drug was accumulated in the receptor compartment and at the end of the assay (170 h) it reached 89% of PGT. The profiles of PGT and ICs solutions were compared according to a model independent approach utilizing a difference factor (f1) and a similarity factor (f2) using Eqs. (6) and (7), respectively. The profiles were found to be different because f1 was greater than 15 and f2 less than 50 (f1 = 59.6 and f2 = 19.9). It was reported that hydrated CD molecules and ICs are able to penetrate into the lipophilic biological barriers with considerable difficulty (Loftsson and Másson, 2001). At the surface of the biological membrane, the drug is released from the IC; therefore, the CD + Drug ↔ IC equilibrium moves to the left as the drug penetrates the membranes (Stella et al., 1999; Shimpi et al., 2005). The partition of the drug from the cavity into the lipophilic barrier could explain the delay in the PGT permeation from ICs solution, with respect to the PGT solution. This result agrees with the consideration that ICs can retard the release of guest molecules, which is a suitable alternative for controlling release.

The amount of PGT permeated from the gels increased slowly along time in a sustained manner (B). However, the diffusion rate from the commercial gel was slightly higher. At 24 h, 8% and 5% of PGT was accumulated from Crinone® and CHT gel + IC, respectively. At the end of the assay, the percentages were 33% and 29% for Crinone® and CHT gel + IC, respectively. f1 and f2 values were 12.0 and 69.5, and indicated equivalence between both gels profiles. In addition, the gels presented a diffusion rate lower than the PGT and ICs solutions, as a result of the presence of the polymeric matrix. There is not only an increase in the pathway that the drug has to migrate to reach the surface of the epithelial mucosa but also two partition equilibriums. In the case of CHT gel + IC, a partition from the CD cavity to the gel and then from the gel to the epithelium. For Crinone®, the first step involves the dissolution of crystals, and then PGT is partitioned into the gel and diffuses to reach the epithelium.

It is noteworthy, that a CHT solution containing complexes between PGT and RAMEβ-CD can form a gel *in situ*, which presents

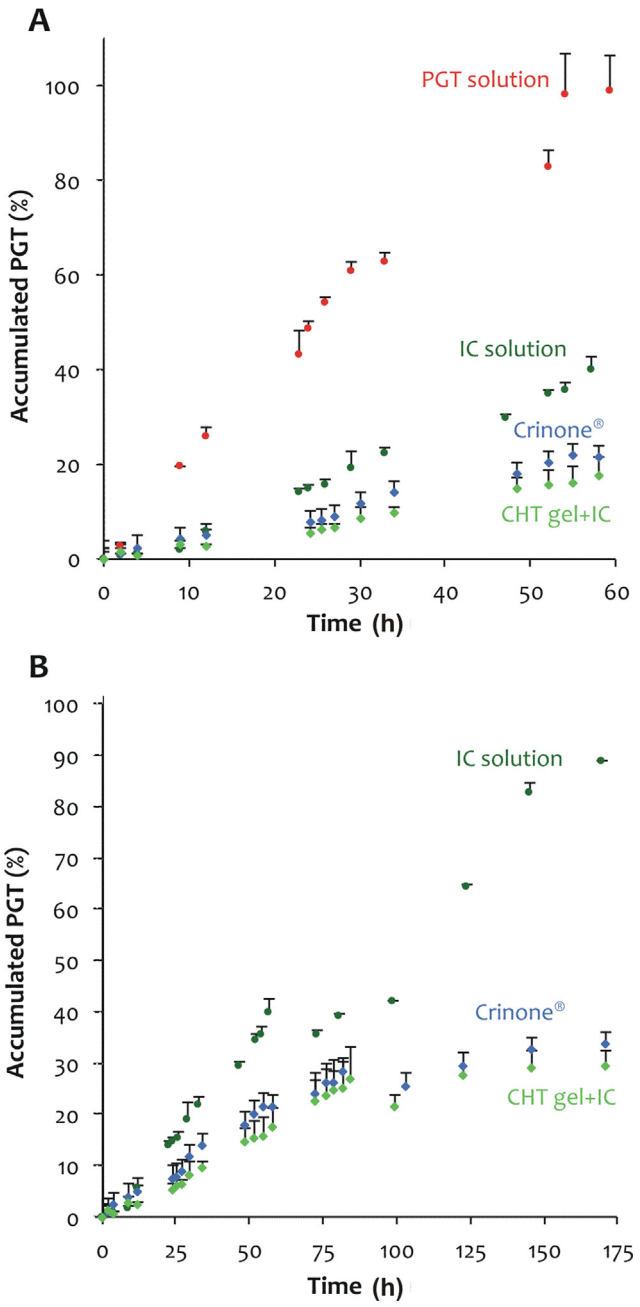

Fig. 8. Percentage of accumulated PGT (%) as a function of time (t) for PGT solution, IC solution, Crinone® and CHT gel + IC.

a similar release profile than a commercial formulation. The ICs were well incorporated in the GP solution generating a clear mixture. In the commercial gel the direct incorporation of the hydrophobic hormone leads to the formation of drug crystals (Fig. 6C) even with the presence of oil components in the formulation. Therefore, the CHT gel + IC shown to be superior in respect to the simplicity of preparation. The ability of CHT to interact with mucus indicates that the residence time of the CHT gel + IC at the vaginal site would be enough to provide localized sustained release of PGT. In addition, vaginal insert based on CHT showed better mucoadhesion than inserts based on carbopol which is one of the main components of Crinone® (Darwesh et al., 2018). Vaginal irritation and other undesirable effects at the site of application were reported for Crinone®. These effects would be less significant with the CHT gel + IC due to the formulation has fewer inactive ingredients and the biocompatibility of the polymer was showed in vaginal formulations based on the lack of toxicity and absence of inflammatory reactions (Tuğcu-Demiröz et al., 2013; Darwesh et al., 2018; Cook and Brown, 2018). Crinone® is applied as a gel, while the low viscosity of the thermosensitive CHT formulation ensures the capacity of covering the required surface of the tissue resulting in the *in situ* formation of a gel mucoadhesive layer at the physiological temperature.

4. Conclusions

Inclusion complexes of progesterone and randomly methylated β-cyclodextrin were obtained by the freeze-drying method. These complexes were included into the glycerophosphate solution during the preparation of chitosan thermosensitive hydrogels. The chitosan gel with inclusion complexes and Crinone®, which is a vaginal gel used to supplement progesterone in women who have luteal phase defect, were tested *in vitro* in a diffusion assay. This experiment was carried out to evaluate the delivery of the hormone and its diffusion through porcine epithelial mucosa obtained from vaginal tissue. Chitosan gel presented sustained diffusion similar to the exhibited by commercial gel. The use of chitosan gels containing inclusion complexes prepared with progesterone and randomly methylated β-cyclodextrin is proposed as a viable alternative for vaginal hormone delivery. The use of a natural polymer as chitosan and a pharmaceutical ingredient as cyclodextrins could help to avoid side effects associated with the commercial gel.

Declaration of Competing Interest

None.

Acknowledgments

The authors thank Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina), Consejo Interuniversitario Nacional (Argentina) (Project PDTS N° 453) and Universidad Nacional del Litoral (Argentina) (Project CAI+D 50020150100013 LI) for the financial support.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2019.09.006.

References

Almomen, A., Cho, S., Yang, C.-H., Li, Z., Jarboe, E.A., Peterson, C.M., Huh, K.M., Janát-Amsbury, M.M., 2015. Thermosensitive progesterone hydrogel: a safe and effective new formulation for vaginal application. Pharm. Res. 32, 2266–2279.
Bouquet, W., Ceelen, W., Fritzinger, B., Pattyn, P., Peeters, M., Remon, J.P., Vervaet, C., 2007. Paclitaxel/β-cyclodextrin complexes for hyperthermic peritoneal perfusion-Formulation and stability. Eur. J. Pharm. Biopharm. 66, 391–397.
Caramella, C., Rossi, S., Ferrari, F., Bonferoni, M.C., Sandri, G., 2015. Mucoadhesive and thermogelling systems for vaginal drug delivery. Adv. Drug Deliv. Rev. 92, 39–52.
Cascone, S., 2017. Modeling and comparison of release profiles: effect of the dissolution method. Eur. J. Pharm. Sci. 106, 352–361.
Cerchiara, T., Luppi, B., Bigucci, F., Zecchi, V., 2003. Effect of chitosan on progesterone release from hydroxypropyl-β-cyclodextrin complexes. Int. J. Pharm. 258, 209–215.
Chaudhary, V.B., Patel, J.K., 2013. Cyclodextrin inclusion complex to enhance solubility of poorly water soluble drugs: a review. Int. J. Pharm. Sci. Res. 4, 68–76.
Check, J.H., 2009. Lacteal phase support in assisted reproductive technology treatment: focus on Endometrin (R) (progesterone) vaginal insert. Ther. Clin. Risk Manag. 5, 403–407.
Chordiya, M.A., Senthilkumaran, K., 2012. Cyclodextrin in drug delivery: a review. Res. Rev. J. Pharm. Pharm. Sci. 1, 19–29.

Choudhury, A., Das, S., Kar, M., 2011. A review on novelty and potentiality of vaginal drug delivery. Int. J. Pharmtech Res. 3, 1033–1044.
Cirri, M., Maestrelli, F., Orlandini, S., Furlanetto, S., Pinzauti, S., Mura, P., 2005. Determination of stability constant values of flurbiprofen–cyclodextrin complexes using different techniques. J. Pharm. Biomed. Anal. 37, 995–1002.
Cometti, B., 2015. Pharmaceutical and clinical development of a novel progesterone formulation. Acta Obstet. Gynecol. Scand. 94 (S161), 28–37.
Cook, M.T., Brown, M.B., 2018. Polymeric gels for intravaginal drug delivery. J. Control. Release 270, 145–157.
Darwesh, B., Aldawsari, H.M., Badr-Eldin, S.M., 2018. Optimized chitosan/anion polyelectrolyte complex based inserts for vaginal delivery of fluconazole vitro/in vivo evaluation. Pharmaceutics 10, 227.
Frömming, K.H., Szejtli, J., 1993a. Cyclodextrin Inclusion Complexes. In: Davies, J.E.D. (Ed.), Cyclodextrins in Pharmacy. Springer Science & Business Media, Dordrecht, pp. 45–82.
Frömming, K.H., Szejtli, J., 1993b. Preparation and characterization of Cyclodextrin complexes. In: Davies, J.E.D. (Ed.), Cyclodextrins in Pharmacy. Springer Science & Business Media, Dordrecht, pp. 83–104.
García, A., Leonardi, D., Salazar, M.O., Lamas, M.C., 2014. Modified β-cyclodextrin inclusion complex to improve the physicochemical properties of albendazole. Complete in vitro evaluation and characterization. PLoS One 9, e88234.
Goycoolea, F.M., Fernández-Valle, M.E., Aranaz, I., Heras, A., 2011. pH- and temperature-sensitive chitosan hydrogels: swelling and MRI studies. Macromol. Chem. Phys. 212, 887–895.
Helbling, I.M., Ibarra, J.C.D., Luna, J.A., 2015. Evaluation and optimization of progesterone release from intravaginal rings using response surface methodology. J. Drug Deliv. Sci. Technol. 29, 218–225.
Higuchi, T., Connors, A., 1965. Phase-solubility techniques. Adv. Anal. Chemi. Instrum. 4, 117–212.
Ho, M.H., Kuo, P.Y., Hsieh, H.J., Hsien, T.Y., Hou, L.T., Lai, J.Y., Wang, D.M., 2004. Preparation of porous scaffolds by using freeze-extraction and freeze-gelation methods. Biomaterials 25, 129–138.
Islam, A., Riaz, M., Yasin, T., 2013. Structural and viscoelastic properties of chitosan-based hydrogel and its drug delivery application. Int. J. Biol. Macromol. 59, 119–124.
Jablan, J., Weitner, T., Gabričević, M., Jug, M., 2011. Stability and structure of inclusion complexes of zaleplon with natural and modified cyclodextrins. Croat. Chem. Acta 84, 169–178.
Lahiani-Skiba, M., Barbot, C., Bounoure, F., Joudieh, S., Skiba, M., 2006. Solubility and dissolution rate of progesterone-cyclodextrin-polymer systems. Drug Dev. Ind. Pharm. 32, 1043–1058.
Li, L., Li, W., Sun, J., Zhang, H., Gao, J., Guo, F., Yang, X., Zhang, X., Li, Y., Zheng, A., 2018. Preparation and evaluation of progesterone nanocrystals to decrease muscle irritation and improve bioavailability. AAPS Pharm. Sci. Tech. 19, 1254–1263.
Liu, Q., Wang, X.Y., Zhang, H., 2007. Solvent effects on infrared spectra of progesterone in CHCl3/cyclo-C6H12 binary solvent systems. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 66, 202–207.
Lockwood, G., Griesinger, G., Cometti, B., European Centers, 2014. Subcutaneous progesterone versus vaginal progesterone gel for luteal phase support in in vitro fertilization: a noninferiority randomized controlled study. Fertil. Steril. 101, 112–119.
Loftsson, T., Brewster, M.E., 2012. Cyclodextrins as functional excipients: methods to enhance complexation efficiency. J. Pharm. Sci. 101, 3019–3032.
Loftsson, T., Hreinsdóttir, D., Másson, M., 2007. The complexation efficiency. J. Incl. Phenom. Macrocycl. Chem. 57, 545–552.
Loftsson, T., Másson, M., 2001. Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm. 225, 15–30.
Luppi, B., Cerchiara, T., Bigucci, F., Caponio, D., Zecchi, V., 2005. Bovine serum albumin nanospheres carrying progesterone inclusion complexes. Drug Deliv. 12, 281–287.
Ma, B., Shen, Y., Fan, Z., Zheng, Y., Sun, H., Luo, J., Wang, M., 2011. Characterization of the inclusion complex of 16, 17α-epoxyprogesterone with randomly methylated β-cyclodextrin in aqueous solution and in the solid state. J. Incl. Phenom. Macrocycl. Chem. 69, 273–280.
Marques, M.R.C., Loebenberg, R., Almukainzi, M., 2011. Simulated biological fluids with possible application in dissolution testing. Dissolut. Technol. 18, 15–28.
Martin Del Valle, E.M., 2004. Cyclodextrins and their uses: a review. Process Biochem. 39, 1033–1046.
Mengatto, L.N., Pesoa, J.I., Velázquez, N.S., Luna, J.A., 2016. Application of simultaneous multiple response optimization in the preparation of thermosensitive chitosan/glycerophosphate hydrogels. Iran. Polym. J. 25, 897–906.
Messner, M., Kurkov, S.V., Jansook, P., Loftsson, T., 2010. Self-assembled cyclodextrin aggregates and nanoparticles. Int. J. Pharm. 387, 199–208.
Michnova, L., Dostal, J., Kudela, M., Hamal, P., Langova, K., 2017. Vaginal use of micronized progesterone for luteal support. A randomized study comparing Utrogestan® and Crinone® 8%. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc 161, 86–91.
Moini, A., Zafarani, F., Eslami, B., Sadeghi, M., Kamyabi, Z., Jahangiri, N., 2011. Comparing intramuscular progesterone, vaginal progesterone and 17-hydroxyprogestrone caproate in IVF and ICSI cycle. Iran. J. Reprod. Med. 9, 119–124.
Monteiro Machado, R., Palmeira-de-Oliveira, A., Gaspar, C., Martinez-de-Oliveira, J., Palmeira-de-Oliveira, R., 2015. Studies and methodologies on vaginal drug permeation. Adv. Drug Del. Rev. 92, 14–26.
Olivares, M.L., Sihufe, G.G., Capra, M.L., Rubiolo, A.C., Zorrilla, S.E., 2012. Effect of protective atmospheres on physicochemical, microbiological and rheological characteristics of sliced Mozzarella cheese. LWT-Food Sci. Technol. 47, 465–470.
Popielec, A., Loftsson, T., 2017. Effects of cyclodextrins on the chemical stability of drugs. Int. J. Pharm. 531, 532–542.
Rao, M.A., 1999. Rheology of Fluids and Semisolid Foods. Principles and Applications. Aspen Publishers, Gaithersburg.
Salústio, P.J., Feio, G., Figueirinhas, J.L., Pinto, J.F., Cabral Marques, H.M., 2009. The influence of the preparation methods on the inclusion of model drugs in a β-cyclodextrin cavity. Eur. J. Pharm. Biopharm. 71, 377–386.
Scavone, C., Bonagura, A.C., Fiorentino, S., Cimmaruta, D., Cenami, R., Torella, M., Fossati, T., Rossi, F., 2016. Efficacy and safety profile of diclofenac/cyclodextrin and progesterone/cyclodextrin formulations: a review of the literature data. Drugs R&D 16, 129–140.
Shakalisava, Y., Regan, F., 2006. Determination of association constants of inclusion complexes of steroid hormones and cyclodextrins from their electrophoretic mobility. Electrophoresis 27, 3048–3056.
Sharma, N., Baldi, A., 2014. Exploring versatile applications of cyclodextrins: an overview. Drug Deliv. 23, 739–757.
Shimpi, S., Chauhan, B., Shimpi, P., 2005. Cyclodextrins: application in different routes of drug administration. Acta Pharm. 55, 139–156.
Silverberg, K.M., Vaughn, T.C., Hansard, L.J., Burger, N.Z., Minter, T., 2012. Vaginal (Crinone 8%) gel vs. intramuscular progesterone in oil for luteal phase support in in vitro fertilization: a large prospective trial. Fertil. Steril. 97, 344–348.
Stella, V.J., Rao, V.M., Zannou, E.A., Zia, V.V., 1999. Mechanisms of drug release from cyclodextrin complexes. Adv. Drug. Del. Rev. 36, 3–16.
Tomida, H., Yotsuyanagi, T., Ikeda, K., 1978. Solubilization of steroid hormones by polyoxyethylene lauryl ether. Chem. Pharm. Bull. 26, 2832–2837.
Tuğcu-Demiröz, F., Acartürk, F., Erdoğan, D., 2013. Development of long-acting bioadhesive vaginal gels of oxybutynin: formulation, in vitro and in vivo evaluations. Int. J. Pharm. 457, 25–39.
Tuğcu-Demiröz, F., Acartürk, F., Özkul, A., 2015. Preparation and characterization of bioadhesive controlled-release gels of cidofovir for vaginal delivery. J. Biomater. Sci. Polym. Ed. 26, 1237–1255.
Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Yamasaki, M., 1982. Inclusion complexations of steroid hormones with cyclodextrins in water and in solid phase. Int. J. Pharm. 10, 1–15.
Wang, Y., He, Y., Zhao, X., Ji, X., Hong, Y., Wang, Y., Zhu, Q., Xu, B., Sun, Y., 2015. Crinone gel for luteal phase support in frozen-thawed embryo transfer cycles: a prospective randomized clinical trial in the Chinese population. PLoS One 10, e0133027.
Zoppetti, G., Puppini, N., Ospitali, F., Fini, A., 2007a. Solid state characterization of progesterone in a freeze dried 1: 2 progesterone/HPBCD mixture. J. Pharm. Sci. 96 (1729), 1736.
Zoppetti, Giorgio, 2011. Evaluation of host/guest cyclodextrin complex by NMR in drug product development. In: Rahman, A.U., Choudhary, M.I. (Eds.), Structure-Activity Relationship Studies in Drug Development by NMR Spectroscopy. Bentham Science Publishers, pp. 156–183. https://doi.org/10.2174/978160805164910156.
Zoppetti, G., Puppini, N., Pizzutti, M., Fini, A., Giovani, T., Comini, S., 2007b. Water soluble progesterone-hydroxypropyl-β-cyclodextrin complex for injectable formulations. J. Incl. Phenom. Macrocycl. Chem. 57, 283–288.