Pharmaceutical Research, Vol. 25, No. 9, September 2008 (© 2008)
DOI: 10.1007/s11095-008-9593-4

Research Paper

ATR/Raman and Fractal Characterization of HPBCD/Progesterone Complex Solid Particles

Adamo Fini,[1,4] Francesca Ospitali,[2] Giorgio Zoppetti,[3] and Nadia Puppini[3]

Received March 3, 2008; accepted April 4, 2008; published online July 3, 2008

Purpose. Characterization of hydroxypropyl-β-cyclodextrin/progesterone (HPBCD/P) complex solid particles obtained from an aqueous solution, by three different technological processes, with the aim of preparing ready-to-dissolve powders for injectable as well as solid oral formulations in progestinic therapy.
Methods. HPBCD/P complex in the 2:1 molar ratio was prepared in aqueous solution and obtained as dry solid particles by freeze-drying, by spray-drying and by fluid-bed evaporation of the solvent. The particles were characterized by μ-FT-IR, μ-Raman and X-ray spectroscopy, by thermal analysis (differential scanning calorimetry-DSC and thermogravimetry-TGA), by Karl Fischer (KF) titration, by image and fractal analysis and by BET specific surface area analysis. The structure of the complex was also defined by comparison of FT-IR and Raman spectra of progesterone with those of pregnenolone and testosterone, structurally related. Dissolution tests were also performed.
Results. Powders of the complex obtained by the three different methods are different in size and shape. Particles obtained by freeze-drying are flat and angular, irregularly shaped without any relation to known geometrical solid figures. Particles obtained by spray-drying are spherically shaped and display a very small size (5-10 μm), with evident deformations and depression of the external surface, due to the rapid evaporation of the solvent. Particles obtained by fluid bed technique have intermediate sizes, display a tri-dimensional structure and irregular surface, with small and rounded protuberances. Fractal dimension of the particle contour was found close to one unit for the microspheres obtained by spray-drying. FT-IR and Raman spectra confirm the occurrence of the complexation by the shift of representative bands of the two carbonyl groups in positions 3 and 20 of the complexed progesterone. X-ray diffractograms indicate the amorphous nature of all the types of particles, also suggested by the absence of any melting peak of the drug in DSC thermograms. The samples contain different amounts of humidity: particles obtained by fluid-bed method demonstrated non-porous in BET analysis. Dissolution of different types of particles is complete after 3 min and only negligible differences could be appreciated among the three powders.
Conclusions. – μ-FT-IR, μ-Raman and X-ray spectroscopy, and the dissolution test did not reveal defined differences among the three different types of particles, confirming occurrence of the complex in the solid state. The spherical shape, the very small size and the low value of the contour fractal dimension allows better technological performance of the particles obtained by spray-drying: this drying process appears the most promising one to prepare dry particles of the HPBCD/P complex, in view of its formulation in the fast preparation of extemporaneous injectable solutions and solid oral formulations intended for sublingual delivery.

KEY WORDS: dissolution; image analysis; Progesterone/HPBCD complex solid particles; Raman and FT-IR spectra.

INTRODUCTION

The function of cyclodextrins (CD) as solubilizers and stabilizers (1) of hydrophobic drugs is widely recognized: however their practical and commercial applications are at present limited due to the price (mainly for α and γ-cyclodextrin) and low solubility for β-cyclodextrin (BCD).

Unmodified CDs cannot be used in parenteral formulations, mainly due to their hemolitic activity and relatively low solubility, suggesting the possibility of precipitation that restricts usage for practical formulation. This limitation can be overcome by higher aqueous solubility obtained by linking of ionizable groups (e.g. sulfonate moieties) over a broad range of pH, or additional alkyl or hydroxyalkyl groups (e.g. methyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin). The presence of substituents mainly interferes with the BCD intermolecular hydrogen bonds, responsible for a reduced solubility in water as a solvent, but should not affect the formation of an inclusion complex with the drug, whose molecular size fits with that of the ring cavity, even though chemical modification of the molecule may produce distortion of the geometry of the CD torus or capping of the cavity

[1] Dipartimento SMETEC, University of Bologna, Via San Donato 15, 40127, Bologna, Italy.
[2] Dipartimento di Chimica Fisica e Inorganica, University of Bologna, Bologna, Italy.
[3] IBSA Institut Biochimique, Lugano, Switzerland.
[4] To whom correspondence should be addressed. (e-mail: adamo.fini@unibo.it)

opening by steric hindrance of the substituents, potentially compromising the inclusion complexation. It was reported that the binding potential of progesterone with a variety of sulfoalkyl ether of BCD was independent of the degree of substitution (2); and the phase solubility diagram for sulfamethizole presents two overlapping profiles for BCD and HPBCD, even if different complex stoichiometry was found for BCD (1:1) and HPBCD (2:3) (3). Examination of the stability values determined for different testosterone and progesterone complexes with different sulfoalkyl ethers of beta-cyclodextrin, confirms the existence of different K1:1 values, though of the same order of magnitude (4). Differences reported for complexation of testosterone for the binding constants ($1.78.10^4$ M^{-1} for BCD and $1.47.10^4$ M^{-1} for HPBCD) do not appear dramatic (2).

It is interesting to note that the good fit of progesterone is related, as well as to the size of the molecule, also to its shape: the steroid nucleus carries functional groups only at the two ends of the molecule and this fact allows deep insertion of the molecule inside the BCD ring. Supporting this idea is the fact that other steroidal molecules, such as medroxyprogesterone and ursodeoxycholic acid (UDCA) that have functional groups in the middle of the steroid ring system, form an inclusion complex with HPBCD of a lower binding constant and anyway do not offer a similar increase of solubility as that observed for the HPBCD/Progesterone pair. It has been reported that UDCA forms a complex with BCD resulting from the interaction with the small hydrophilic side chain, while complexes involving interaction with the larger hydrophobic end need the larger ring of γ-CD, due to the interfering presence of the 7β-OH (5,6).

The association between P and HPBCD (HPBCD/P) represents an improvement of the steroid hormone solubility, since the sum of a good fit of progesterone and high solubility of the guest molecule means a notable increase of solubility in water of the final complex, that could be formulated as an injectable solution for progestinic therapy. It was reported that water solubility of P (0.011 mg/ml) could be enhanced up to about 360000% in the presence of HPBCD (7).

To prepare the complex, P powder is simply added to an aqueous solution containing HPBCD at 1:2 molar ratio: in a short time P solubilizes and the solution turns clear. After suitable treatment of the solution, the solid complex can be obtained by a variety of techniques. In previous papers we examined the complex particles and demonstrated the existence of the complex also in the solid state by means of μ-Raman and μ-FT-IR spectroscopy (8–10). In this paper we experimented different techniques (freeze-drying-FD, spray-drying-SD and fluid bed-FB) to obtain the complex in the solid form from the solution, and the final powdered material was examined at scanning electron microscope (SEM) for image analysis and contour fractal dimension of the particulate system; X ray diffractometry (XRD) and μ-FT-IR and μ-Raman spectroscopy were used to reveal differences of the three particulates, but also to achieve parameters, able to address the choice of the optimum method of preparation of the powdered material, for commercial application. The objective of present study was also to evaluate and compare particle morphologies produced by the three technologies, SD, FB, FD, in order to assess how morphology impacts the enhancement of the dissolution rate and other technological performances of the final powder.

EXPERIMENTAL PART

Materials

The three free hormones, progesterone (m.p. 127–131° C), testosterone (m.p. 155°C) and pregnenolone (m.p. 193° C), Hydroxypropyl-β-cyclodextrin, as well as samples of the inclusion complexes obtained by the three different techniques, were gifts from IBSA (Lugano, Switzerland) and were all of pharmaceutical grade.

The preparation of the inclusion complex in the form of powder was performed according to different industrial protocols. Anhydro A/S kindly permits divulgation of its Activities Test Report: N° F-6925 (31.10.2006). Trials were conducted with a development approach.

Preparation of inclusion complex particulate

a) Freeze dried HPBCD/P (FD) powder (Lampugnani Pharmaceuticals S.p.A. - Italy)

The solution was prepared introducing the total amount of HPBCD in a stainless steel tank 316L (650 litres, with magnetic stirrer) containing a part (24%) of the purified water, applying strong stirring (600 rpm). After 15 minutes, a clear solution was obtained: micronised P was then added, at the same stirring rate for other 30–60 minutes. The final step consisted of the addition of the residual water followed by a gentle mixing procedure (100 rpm for 10 min). The clear final solution (P: 1.92%; HPBCD: 19.22%; water: 78.86% by weight) displayed almost neutral pH, suitable to be freeze dried, after filtration (0.5 μm prefilter and 0.22 μm filter - mixed cellulose ester material) for sterilisation.

Lyophilizer (CRIOFARMA s.a.s, Torino, Italy). This consisted of a cubic lyophilizing chamber with twelve plates (maximum disposable surface of 27 square meters); diathermic fluid permits plate heating and cooling. Freeze-drying cycle: Freezing: –40°C for 12–20 h; Pressure: 165 μbar; Sublimation: -10°C for 4–6 h; Heating: -5°C for 4–6 h; Heating: +2°C for 8–14 h; Heating: +20°C for 12–24 h; Pressure: ambient.

The freeze dried product (FD) obtained was granulated with a VIANI (Trade mark) oscillating granulator with 1 mm (net) sieve. The resulting powder was stored in an aluminium sealed drum.

b) Spray dried HPBCD/P (SD) powder (Anhydro Test Centre - Denmark)

1.923 kg HPBCD was mixed with water using a DIAT mixer running at 700 rpm. After the cyclodextrin was dissolved, 0.1923 kg of micronized P was added together with the rest of the water, so the total amount of feed was 10 kg. Inspection of the plant showed no deposits., After one night's rest, the solution was spray dried, without any problems: the mixer was running at 100 rpm during spray drying.

Pilot Spray drier PSD52. A feed vat was placed above the apparatus, with the flow rate controlled by a clip on the tube. Technical Parameters - Heating system: Electrical air heater; Inlet temperature: 130°C; Atomizer: Two fluid counter-current nozzles (0.7 mm); Outlet temperature: 70°C (±1°C); Drying chamber: Single stage conical drying chamber

with bottom outlet. Outlet air left the chamber through the bottom, dried powders were separated in a cyclone and air finally sent through a bag filter.

c) Fluid bed spray dried HPBCD/P (FB) powder (Anhydro Test Centre - Denmark)

The setup of the Fluid Bed was formed by a ∅ 150 cylinder and a CD1222 distributor plate. The two fluid nozzles were equipped with a 64 ss air cap and 2050 ss fluid cap and sprayed a 20% dry matter solution down towards the bed with an air pressure of 1.5 bar.

The rate of the sprayed solution was approximately 15 g/min. The temperatures of the bed were approximately 65°C inlet air and 30°C outlet air with a pressure drop of 80 mmWG over the bed and powder.

Alternatively the following process parameters were employed without any difference in the final results: 50% dry matter solution, 70 ss air cap and a 2850 ss fluid cap.

Pilot Fluid Bed. The Mini Batch Fluid Bed is a small electrically heated batch fluid bed. It is equipped with instruments for measuring air flow and pressure loss over the distributor plate and powder layer respectively. The rewet agglomeration is performed with two fluid nozzles where water is sprayed through a nozzle with compressed air onto the fluidised powder.

Differential scanning calorimetry (DSC) analysis. Thermal characteristics of the pure materials, the physical mixtures and the HPBCD/P inclusion complexes were determined by an automatic thermal analyser system (Mettler 821^e). The data processing system (Mettler 821^e/500/847 thermo-cryostat) was connected to the thermal analyser. Sealed and holed aluminium pans were used for testing all the samples. Temperature calibrations were made using indium as standard. The thermograms were run at a scanning of 10°C/min, from 30 to 200°C.

Thermogravimetric analysis (TGA). Thermogravimetric analysis was performed with a Mettler Toledo automatic thermal analyser system TGA/SDTA851^e/SF/1100). Open alumina crucibles were used for analysis in the temperature range 30–300°C at 10°C/min. scanning rate under nitrogen stream.

Karl Fisher (KF) titration. KF titration analysis for determining water content was carried out on powdered samples using a KF titrator Mettler-Toledo DL38. The KF reagent (Hydranal Methanol dry Riedel-de-Haen) was standardized using Hydranal composite J. Riedel-de-Haen. All determinations were carried out in triplicate.

X-ray Diffractometric (XRD) Analysis. To perform XRD analysis a Philips PW 3719 diffractometer was used, controlled by a computer. Experimental conditions were as follows: Cu Kα radiation (λ=1.78896 Å); 40 kV and 30 mA. Scanning interval: 5–50° 2θ; Time per step: 1 s; Graphite monochromator on the diffracted beam.

Micro-Raman spectroscopy. Spectra were recorded by means of a Renishaw Raman Invia interfaced to a microscope Leica DMLM (spatial resolution 1–60 μm^2). Experimental details: sources Ar^+ Laser (514.5 nm), Diode Laser (780.0 nm); monochromator: diffraction network (for Ar^+:1800 lines/mm; for diode: 1200 lines/mm); detector: CCD (*Charge-Coupled Device*) cooled at 203 K; spectral resolution: 2 cm^{-1}; power on the sample 0.03–1.5 mW; accumulation time: 10–30 s; scansion number 1–8.

Micro-FTIR. FT-IR (ATR, near-normal reflection-absorption) spectra were recorded by a Nicolet FT-IR Nexus 470 connected to a Nicolet Continuum microscope: Experimental details: source globar (SiC candle); beam splitter m-IR: KBr; detector: MCT (CdTe, doped by Hg) (Hg/Cd); spectral window: 4000–650 cm^{-1}; side resolution: 7–80 μm; spectral resolution: 4 cm^{-1}.

BET specific surface area analysis. Specific surface area was measured using Micromeritics ASAP 2020 system (Micromeritics, Norcross, GA USA) by N_2 absorption porosimetry measurements. A known amount of powder (~1 g) was loaded into a sample cell and degassed for at least 12 h prior to analysis. The nitrogen adsorption was carried out on the powders at room temperature before testing.

Dissolution study. Dissolution studies of P/HPBCD complex particles were carried out using the rotating paddle method with a USP XXII apparatus. Each dissolution test was performed by spreading the powder particles, obtained with different techniques (50 mg, approximately equivalent to 5 mg progesterone (50–100 μm)) over the dissolution medium, consisting of 1 L of distilled and degassed water, in a thermostatic bath at 37°C. Samples of the solution were withdrawn every 15 s and absorbance was monitored at 250 nm for progesterone quantification. The dissolution experiments were carried out in duplicate: the tests were followed for 10 min to obtain a stable final value of the absorbance. Absorbance values were transformed into percentage of dissolved complex and plotted as a function of time. For comparison, a physical mixture of the same composition as the complex was also tested for dissolution, using 5 mg of P and observing its dissolution rate in a dissolution medium containing 45 mg of HPBCD (dissolution profile not reported).

Shape and Surface Morphology. The shape and surface morphology of microparticles were examined using scanning electron microscopy (SEM, XL30; Philips), equipped with a special computer program. The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminium stub. The stubs were then coated with gold to a thickness of about 300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope.

RESULTS AND DISCUSSION

The solubility of drugs containing the steroid ring system is generally low, despite the presence of some hydrophilic groups and this fact introduces difficulty in the preparation of

drugs of this class as liquid dosage forms, such as, for instance, injectable aqueous formulations. Progesterone was recently proposed (8–10) as a 1:2 molar complex with HPBCD: the complex of this composition proved extremely soluble in water and therefore suitable for preparing aqueous solutions.

The preparation of the complex was accompanied by a few problems. First, the presence of unsubstituted BCD, as a side product of HPBCD, caused the formation of a poorly soluble complex, that was eliminated by filtration after suitable aging of the mother solution. Second, to ensure complete complexation of progesterone different ratios HPBCD/P were tested and the 2:1 ratio was chosen as suitable also for the complete solubilization of the steroid drug. Third, since the complex was prepared as an aqueous solution, different methods to eliminate the solvent from the final solution originated particles of different shape, morphology and technological properties. Particles obtained by freeze-drying (FD), by spray-drying (SD) and by fluid bed evaporation (FB) were examined to define the method most suitable for the formulation under examination. Lyophilization or freeze-drying process is the most common method to produce solid and amorphous powders. Other important methods include spray drying and fluid bed.

SEM images. Powders obtained by the different techniques were examined by SEM for shape and surface morphology. Micrographs of isolated particles are shown in Fig. 1. These particles are dissimilar in size and also have quite different morphologies. FD particles are flat and angular, irregularly shaped without any relation to known geometrical figures (Fig. 1A, B). FB particles have intermediate sizes, display a tri-dimensional structure and irregular surface with small and rounded protuberances (Fig. 1C, D). Particles are well separated, but single particles appear formed by close agglomeration of smaller portions. SD particles display a very small size, are spherically shaped, with evident deformations and depression of the external surface, due to the rapid evaporation of the solvent that leaves the microspheres similar to a bowling ball (Fig. 1F). This is due to the rapidity of the spray-drying process that generates rapid nucleation of the dissolved substance and restrict growth of the final particle, that occurs after the fine droplets are formed. Due to their small size, these particles tend to abundantly agglomerate (Fig. 1E) and this appearance is well documented by the image analysis of the powder. SD particles should display flowability precisely because of their spheroid shape: however this aspect is cancelled by the very small size of the particles (angle of repose for the powder: 41±2° FD; 42±3° FB; 48±2° SD).

Image analysis. The intention of image analysis is to more accurately extract and determine the information contained in the microscopic sample, by means of digital image processing analysis and presentation of the shape and size parameters of the particles. Unless the sample on the SEM plate was previously sieved and a very narrow dimensional fraction is examined, the machine "sees" a range of particles of different sizes. In order to "homogenize" the final results we choose to take into account single particles having the same surface area in order to exclude from the examination particles resulting from agglomeration that could generate false mean values. Among many parameters that can be used in characterizing the shape of particles, some (length and width) derive from direct measures and some other shape descriptors of individual particles (area, perimeter, aspect ratio) are calculated. No single shape descriptor is suitable for all applications. The following three parameters, which are all normalised (defined to have values lying in the range 0 – 1) are frequently used to quantify different aspects of particle shape: shape factor, elongation, and fractal dimension and were derived for this study.

Shape factor of particle ranges between 0 and 1, with 1 representing a perfect circle. It is determined from the measured area A and the perimeter P, using the equation $4\pi A/P^2$. A perfect circle has a shape factor or sphericity of 1 while a very 'spiky' or irregular object has circularity closer to 0. FB particles display a sphericity value of 0.58; a lower value (0.35) was found for FD particles; while SD particles displayed a mean value of 0.93, as a consequence of their spherical shape.

Elongation is a measure of the length/width ratio: it is defined as 1-[width/length]. Shapes symmetrical in all axes such as circles or squares have similar length and width (elongation close to 0): the present particles show quite low and similar elongation values, indicating a similar symmetry, independently of the size and external shape.

Fractal analysis of a particle contour was carried out by means of SEM, endowed with suitable software. Well-isolated SD particles were selected for analysis, excluding those resulting from agglomeration: in fact the SD sample contained particles with a mean size of 1–5 micron and almost regular spherical shape, but forming large agglomerates (Fig. 1E). In this case fractal dimension could be different for an agglomerate or for single particles: these appeared as smooth and regularly shaped microspheres and the value of the fractal dimension was low and close to 1 (1.086). The case of the FB sample was different: in this case the particles had an irregular shape and perimeter, but could be seen as isolated, of a larger (≈100 μm) and apparently uniform size (the sample was not previously sieved). A higher value of contour fractal dimension (1.196) agrees with what can be observed in the micrographs of Fig. 1C, that is with an irregular contour and, with reasonable extrapolation, the surface can also be considered uneven and more suited to prompt dissolution than the spray-dried smoother particles; an almost similar value (1.187) could be determined for FD samples. Since powders of the HPBCD/P complex are designed for an extemporary preparation of an injectable solution, this parameter represents an important pre-requisite and fractal analysis proved useful to discriminate the optimum technological process to prepare the powder.

The specific surface area of the three different samples determined by BET analysis was very low and ranged from 0.7 m^2/g for the FD sample to 1.9 m^2/g for the SD sample. FB particles, which appeared at SEM as an aggregation of smaller particles, showed a surface area that could not be measured by gas absorption. The rapidity of the processes generates a rapid nucleation rate of the dissolved substance: this does not restrict growth of the final particles that occurs after the fine droplets are formed, but prevents the formation of a porous matrix structure. The values obtained by BET analysis are consistent with a poorly porous structure of the

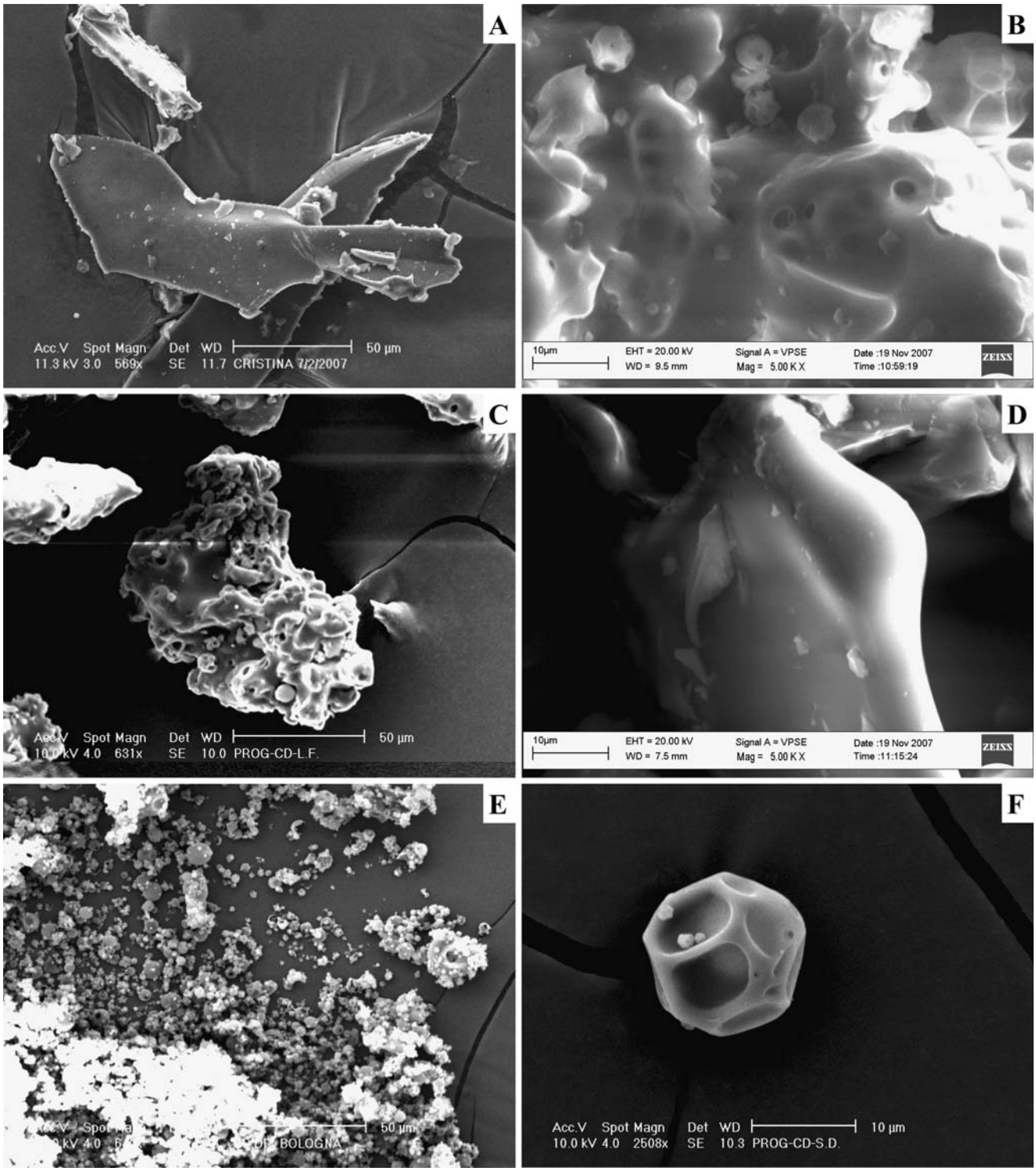

Fig. 1. SEM images. A and B–FD particles at different magnifications; C and D–FB particle at different magnifications; E and F–SD particles at different magnifications.

particles, as was also documented by the SEM micrographs taken at higher magnification (Fig. 1B and D).

Thermal analysis. As previously reported (8) for FD samples, also SD and FB particles do not show any melting of the included drug. DSC thermograms (Fig. 2) show only a broad and rounded endotherm in the range 40–130°C that corresponds to a loss of weight in TGA profile. KF titration confirms that water is contained inside the samples, due to hydrophilicity of HPBCD. In fact a varying number of water molecules is always associated with the CD molecule: those present inside the cavity, whose replacement is one important mechanism of the formation of the inclusion complex, are particularly important. By KF titration it was found that 9–

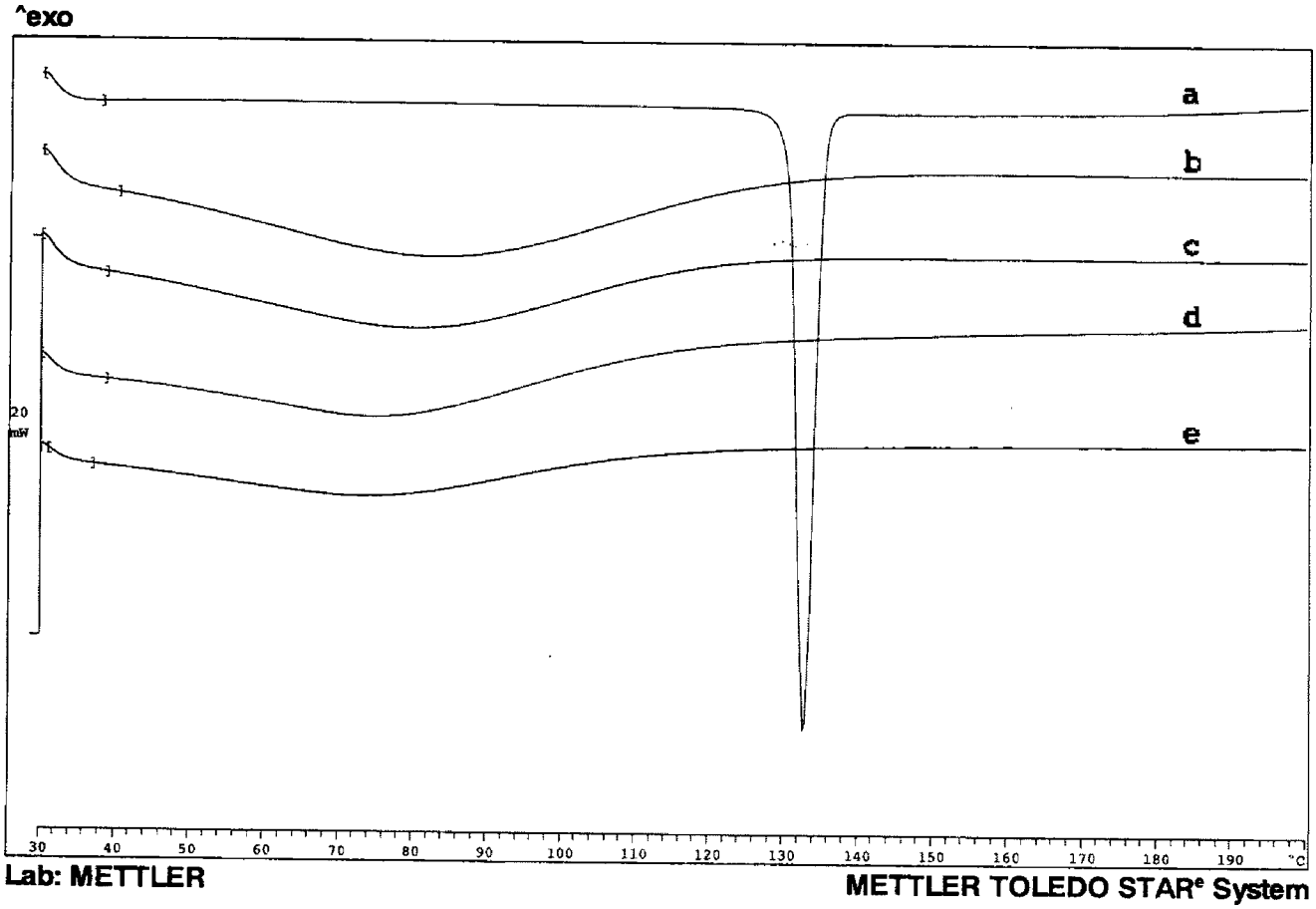

Fig. 2. DSC thermograms profiles. A–progesterone; B–HPBCD; C–FD complex; D–FB complex; E–SD complex.

10% of water that corresponds to 7–8 water molecules is mainly associated with HPBCD. After dehydration over silica gel for one night the water content falls to 5–5.5%, corresponding to a loss of about 3 molecules of water: these are rapidly re-absorbed when the sample is placed in a 70% RH box. The easiness of dehydration, even partial at room temperature, and the large endotherm of dehydration in DSC thermogram profiles suggests that water molecules associated with HPBCD behave differently and are not all bound with the same bond strength and can be freed in a large range of temperatures (Fig. 2). Also the three particle samples examined were shown to contain different water percentages after their preparation and the loss was higher than that observed for HPBCD, kept in the same experimental conditions (see Table 1). This amount almost halved when SD and FB samples were kept in a desiccator over silica gel for one night. KF titration confirmed the TGA results but represents a more reliable method of measurement for these complexes. In fact DSC thermograms reveal that dehydration of the complexes occurs in a large temperature range, likewise for HPBCD, around 100°C. Dehydration endotherm starts at room temperature and terminates at about 130°C and, as a consequence, definition of the onset for the thermal event is approximate. After dehydration endset, slightly above 130°C, the complex demonstrated weight stability up to about 200°C in air; afterwards loss of weight occurs as a consequence of thermal decomposition. The FD material contains about 5% w/w water: this amount practically does not change after one night in a 75% RH box. These results can be important when calculating the dosage of the active agent and the conditions of storage and selecting the best particle type.

Micro-Spectroscopy Raman. Progesterone (4-pregnen-3,20-dione) is characterized by the presence of two carbonyl groups: the cyclic one in position 3 of the A ring of the steroid system; and a methyl-keto group present in position 20 of the other end of the molecule. Two reference molecules were chosen having only one of the two carbonyl groups. Testosterone and pregnenolone contain only one carbonyl group, in position 3 or 20 respectively: from the comparison of the characteristic band frequencies in these compounds, the exact attribution of the Raman and IR bands of progesterone, alone and in the complex, can be obtained.

Table I. Content of Humidity Before and After One Night in Desiccator Over Silica Gel

Sample	Original (%)	After 1 night over silica gel (%)
SD	7.26	3.97
FD	5.23	5.28
FB	8.58	3.59
HPBCD	9.19	5.67

Raman spectra of P and HPBCD are shown in Fig. 3A and D. Both compounds show bands associated with CH stretching at about 3000 cm^{-1}: the patterns of peaks in P are more complex and intense than those of HPBCD. The spectrum of this last compound shows a very broad and relatively weak band between 3000 and 3500 cm^{-1}: both the shape and position of this band are characteristic of hydrogen bonded hydroxy groups. In the P spectrum, two sharp and intense bands at 1614 and 1660 cm^{-1} are present in the region where C=C and carbonyl stretchings are usually observed and can be attributed to the double bonds present in the progesterone. At 1694 cm^{-1} a band of lower intensity can also be observed. In this range of frequencies no peak is present in the HPBCD spectrum and this fact offers a suitable tool to investigate the structure of the complex in the solid state.

From the examination of the two reference compounds it was possible to obtain the exact attribution of the bands in the 1500–1700 cm^{-1} range for P (Table 2).

The Raman spectrum of pregnenolone (Fig. 3C) shows two bands very close together at 1683 and 1670 cm^{-1} that can be attributed, respectively, to the carbonyl stretching in position 20 and to the trisubstituted alkene: they appear of lower intensity with respect to similar bands of progesterone and testosterone. In the spectrum of pure P the band attributed to the C-20 carbonyl can be observed as a weak shoulder of the two symmetric bands of much higher intensity. As a consequence this band is no longer appreciable in any of the complex particles considered. The C=C stretching in pure P is shifted towards lower wavenumbers, because of the conjugation effect.

Table II. IR and Raman Frequencies Suitable for the Attribution of the Carbonyl Bands

Steroid	IR frequency (cm^{-1})	Raman frequency (cm^{-1})
Pregnenolone	1699 (sh), 1680	1683, 1670
Testosterone	1656 (s), 1613 (m)	1656, 1614
Progesterone	1694 (s) 1664 (s), 1615 (m)	1694 (sh), 1660, 1616
HPBCD/P	1691 (m), 1657 (s), 1613 (m)	1694 (sh), 1653, 1608
HPBCD	1641	/

Sh=shoulder; s=sharp; m=mean

Similarly, the testosterone spectrum (Fig. 3B) offers two bands of stronger intensity, associated with the vinyl keto group at 1615 cm^{-1} (conjugated C=C) and 1656 cm^{-1} (conjugated C=O). The band centred at 1656 cm^{-1} is a

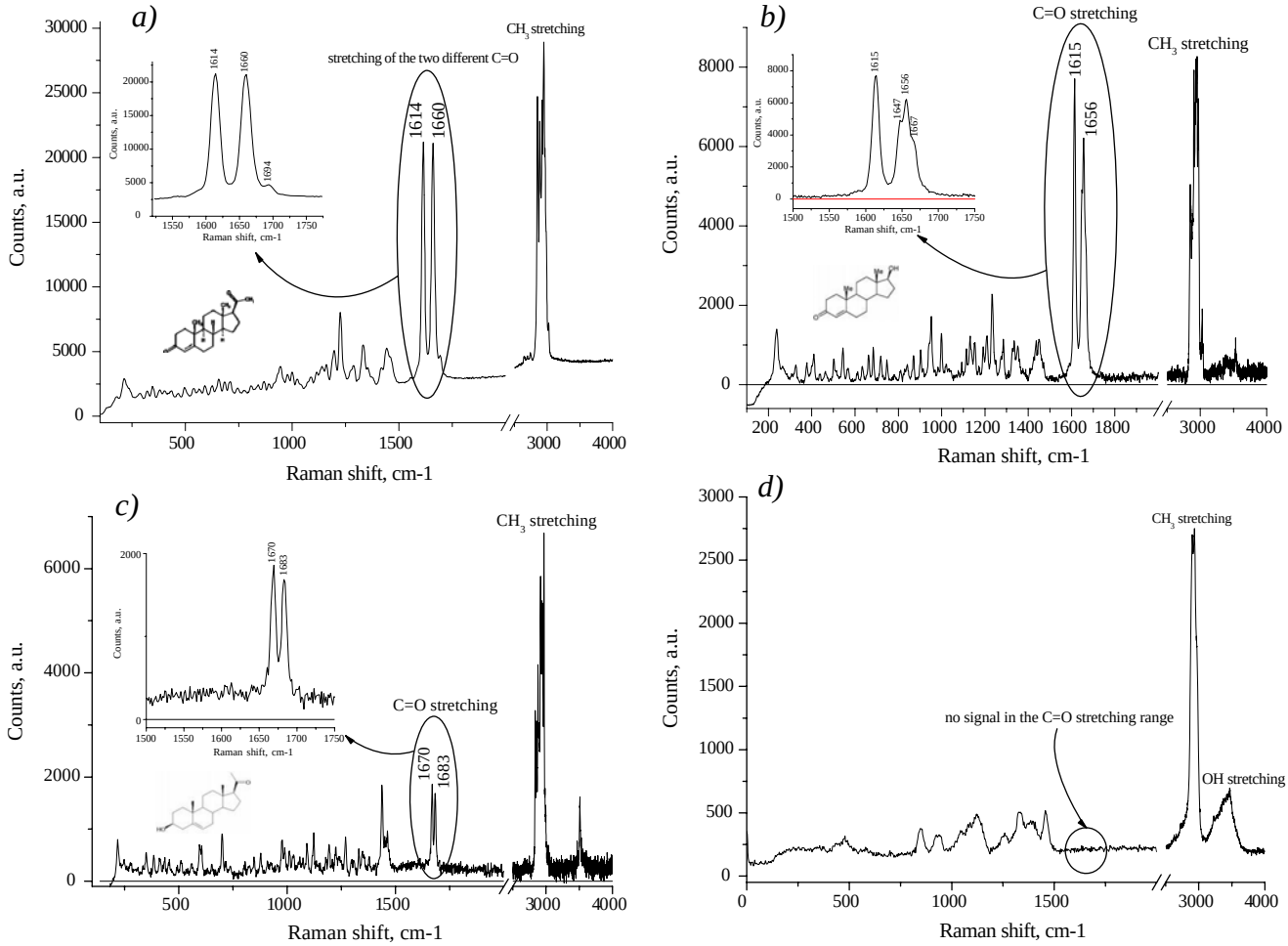

Fig. 3. Raman spectra of pure compounds. A–Progesterone; B–Testosterone; C–Pregnenolone; D–HPBCD. In the windows: molecular formula and details concerning representative peaks.

multiplet, showing shoulders at 1647 and 1667 cm^{-1}, as can be seen in the small window inside Fig. 3B.

In pure progesterone the two bands are practically similar to those of the vinyl keto group in testosterone for intensity and shape (without shoulders). In the complex these bands are weaker, asymmetric and shifted respectively to 1609 and 1653 cm^{-1}.

From the examination of the Raman spectra of the three samples (Fig. 4) two aspects can be highlighted. The first one is that the spectrum of the complex resembles that of HPBCD more than that of P (with the exclusion, obviously, of the two bands of the carbonyls and of the double bond) and this fact can be interpreted as due to the deep insertion of the P molecule into the HPBCD cavity: the cyclodextrin rings, that represent the external surface of the complex, hinder the laser beam to reach the underlying P molecule and this originates the second aspect. The second aspect is that the bands characteristic of P now show a very poor intensity and are no longer symmetric, probably related to the different mode of insertion of the two ends of the P molecule inside the cavity that causes conformational restriction and reduction of movements of the inserted molecule. The bands of CH stretching around 3000 cm^{-1} are also of lower intensity, when the complex is considered with respect to pure P, suggesting a restricted situation of the methyl groups of the progesterone molecule. This reduction cannot be attributed only to the stoichiometry of the complex, where P is at 1:2 molar ratio with respect to HPBCD and represents evident proof of the formation of an inclusion complex. These changes with respect to pure P could be observed in the spectra of the three samples, obtained from aqueous solution by means of different techniques. Figure 5 shows Raman spectra of the three samples in the 1550–1750 cm^{-1} spectral range. By comparison with the position of the bands of the pure P, the greatest shift could be observed for the FD sample, while in the other cases differences are lower. This comparison could not be carried out for the stretching of the C=O in 20, because of its weakness.

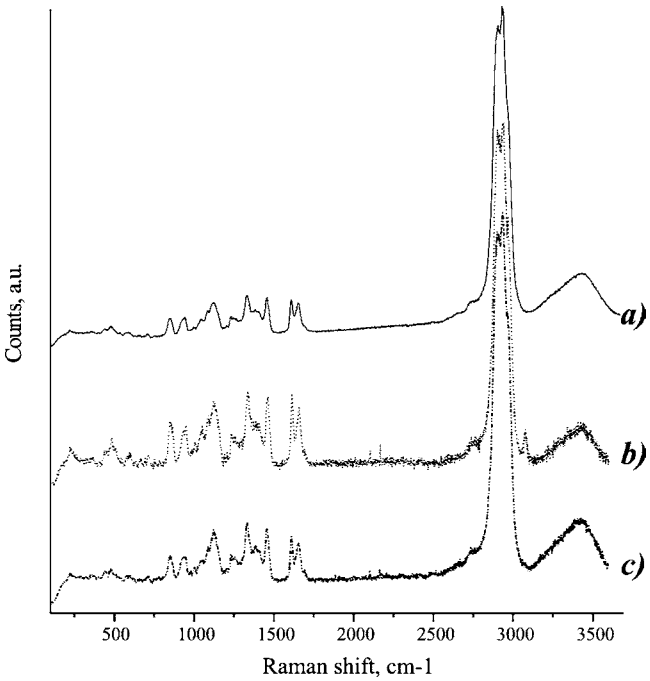

Fig. 4. Raman spectra in the spectral ranges 100–2000 cm^{-1} and 2750–3700 cm^{-1} of HPBCD/P complexes. A–FD complex; B–SD complex; C–FB complex. *Experimental set-up: Ar^+ laser, 514.5 nm, t=30 s, n=1, P=1.5 mW.*

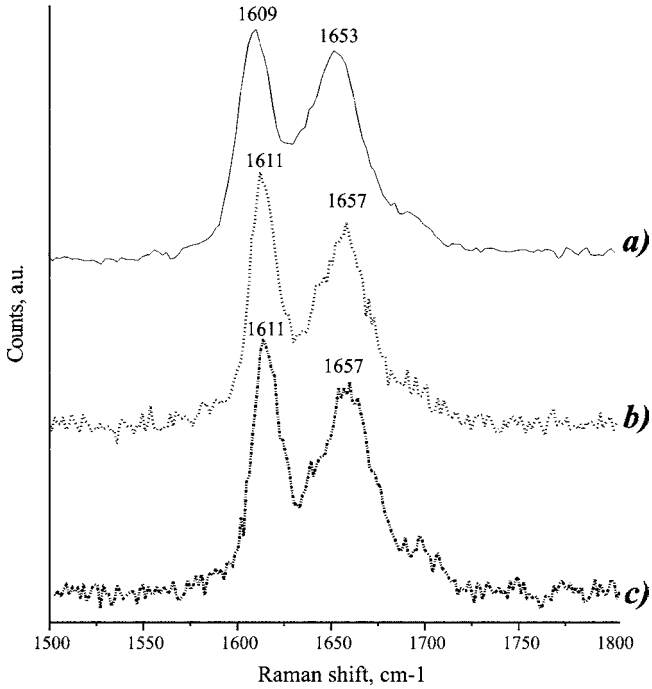

Fig. 5. Raman spectra in the spectral range 1500–1800 cm^{-1} of HPBCD/P complexes. A–FD complex; B–SD complex; C–FB complex. *Experimental set-up: Ar^+ laser, 514.5 nm, t=30 s, n=1, P=1.5 mW.*

The differences within the Raman spectra of the three samples are very small and probably meaningless, and are however difficult to interpret: the band at higher wavenumber presents the largest differences, especially concerning the shape. These results allow the conclusion that the different ways of eliminating the solvent to obtain the solid material did not cause differences concerning the formation and the structure of the final complex, where the host/guest organization appears similar in each case. This supports the idea that the rapidity of the processes allows preservation of the structure of the complex existing in the solution.

Micro-Spectroscopy FT-IR/ATR. In this case too, the region in the frequency range 1500–1700 cm^{-1} is useful for investigation: in this range three bands at 1616, 1664 and 1699 cm^{-1} are visible for P (Fig. 6A and B and Table 2): the peaks appear well-defined, sharp and symmetric and can be assigned to the presence of vinyl and carbonyl groups.

By comparison with the FT-IR spectrum of testosterone it emerges that the cyclic carbonyl in position 3 vibrates at 1664 (conjugated C=O) and 1616 (conjugated C=C) cm^{-1}. The testosterone spectrum in fact shows two bands: one at 1612 cm^{-1} and the second one appears as the sum of several poorly resolved bands, peaking at 1657 cm^{-1}. This last band, attributed to the conjugated carbonyl, is more intense and this difference of intensity is maintained in the P sample. The pregnenolone spectrum shows only one very narrow band at 1684 cm^{-1} concerning the methyl group stretching present on

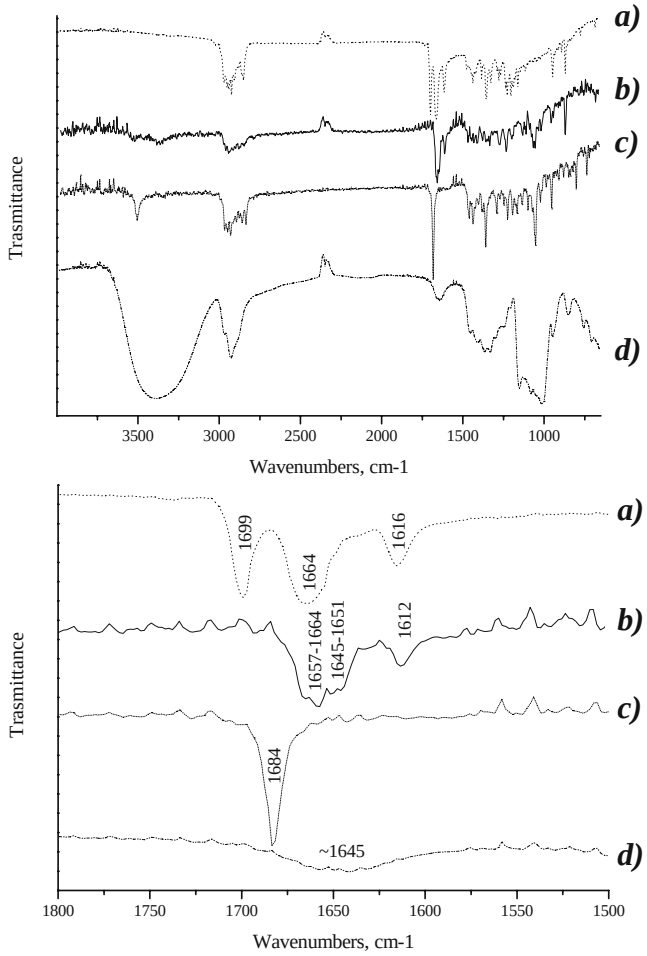

Fig. 6. FT-IR total spectrum up to 4000 cm^{-1} (upper); and spectrum between 1500 to 1800 cm^{-1} (below). A–Progesterone; B–Testosterone; C–Pregnenolone; D–HPBCD.

the pentaatomic ring of the steroid. This allows the three bands of P to be identified.

In the same region, at 1645 cm^{-1}, HPBCD presents a broad, weak and complex band. The FT-IR spectra of the three samples practically overlap and only a broad poorly resolved band is present in the same frequency range as those observed for pure P. The bands of progesterone appear shifted to lower frequencies and attenuated in their intensity. The greatest change could be observed concerning the 1689 cm^{-1} band of methyl-keto group that almost disappeared in the spectrum of the complex, suggesting a strong interaction between this terminal of the molecule and the cyclodextrin ring, also favoured by the length, mobility and small size of the side aliphatic chain. Two other reference bands of progesterone in the complex cannot be distinguished from those of HPBCD, suggesting a strong interaction of the A ring of the steroid molecule with the CD cavity in this case too, according also to the view of Uekama *et al.*, who concluded the same using ^1H NMR (11).

In any case, the FT-IR spectra also confirm occurrence of the complexation and since the spectra of the three samples are practically superimposable, no differences could be seen related to technological processes which therefore have a greater effect on the physical aspect of the solid particle rather than the chemical organization of the inclusion complex (Fig. 7).

Dissolution rate. The dissolution profiles shown in Fig. 8 illustrate the enhanced dissolution rate of progesterone achieved through complexation with HPBCD. Dissolution of the complex in the form of different particles was almost instantaneous. The dissolution reaction could be followed for 3 min, but after 1 min more than 80% of the FB and FD samples were already dissolved. Within the first 3 min, the dissolution of each sample was nearly 100%. The samples employed for dissolution studies consisted of particles with a narrow and small size range, since otherwise it could have been difficult to obtain a different particle size, common to the three samples: the spray-drying technique only allowed the preparation of very small particles. Due to the amorphous nature of the complex (Fig. 9) and improvement of water solubility, the complex presented a higher dissolution release compared to that of the corresponding physical mixture, of the same composition. It was previously reported (7) that progesterone included in BCD dissolved more rapidly than the corresponding physical mixture, however it was also pointed out that this complex did not present an optimal dissolution profile, thus suggesting a ternary system with the incorporation of the complex into a hydrophilic polymer, such as PEG 6000. This could represent a demonstration of the superior performance to dissolution of the complex with HPBCD at the proposed molar ratio; and also of the presence of the inclusion complex in the solid state.

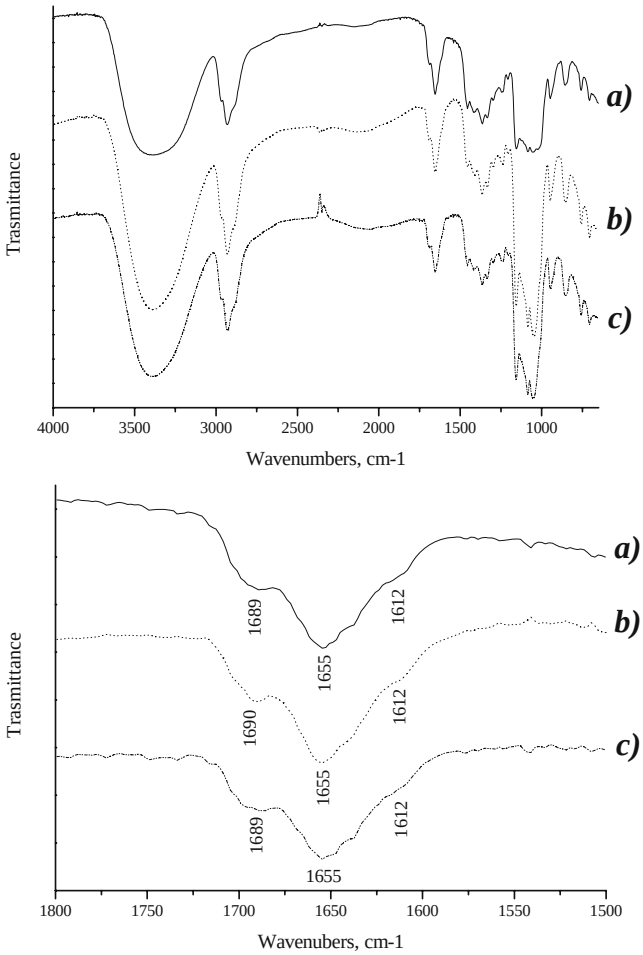

Fig. 7. FT-IR total spectrum up to 4000 cm^{-1} (upper); and spectrum between 1500 to 1800 cm^{-1} (below) of HPBCD/P complexes. A–FD complex; B–SD complex; C–FB complex.

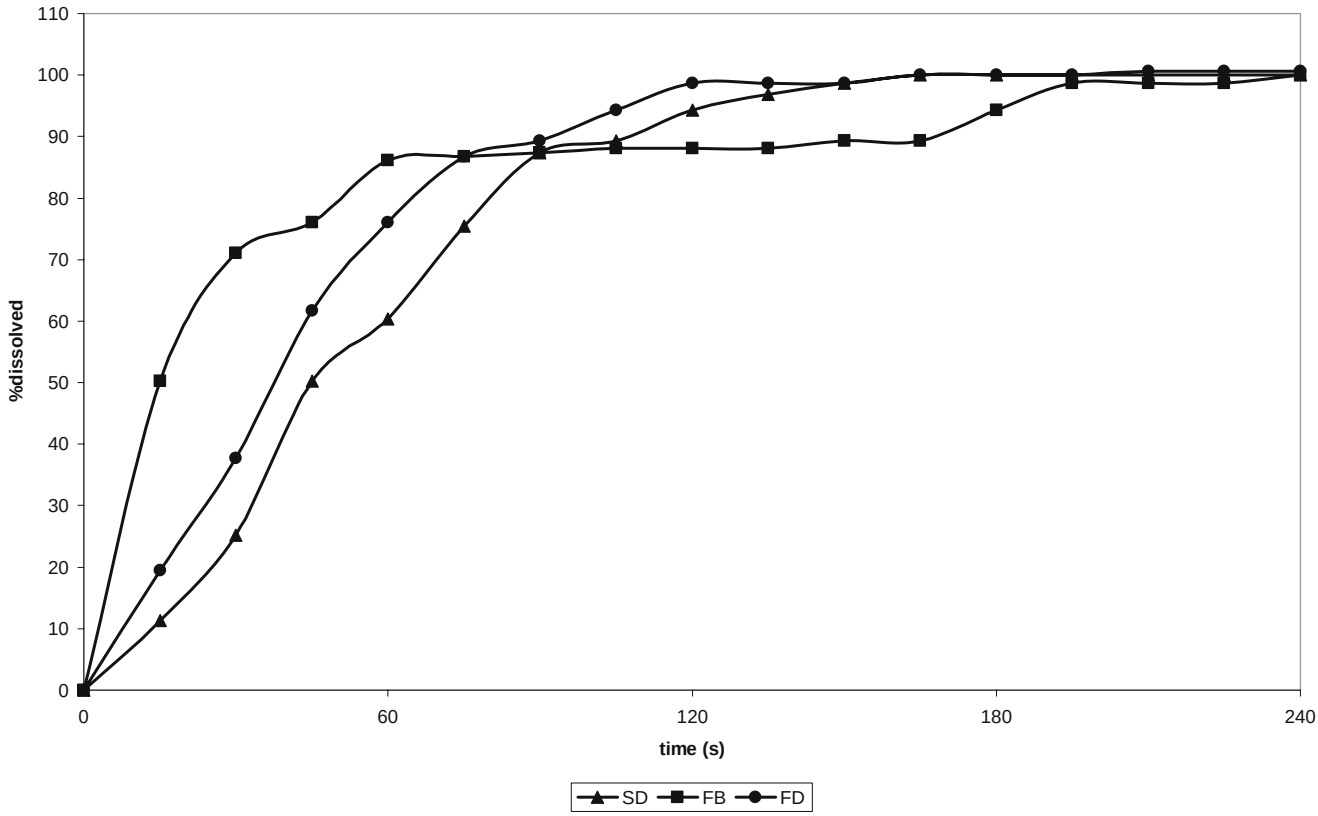

Fig. 8. Dissolution profiles of HPBCD/P complexes. ▲–SD complex; ●–FD complex; ▪–FB complex.

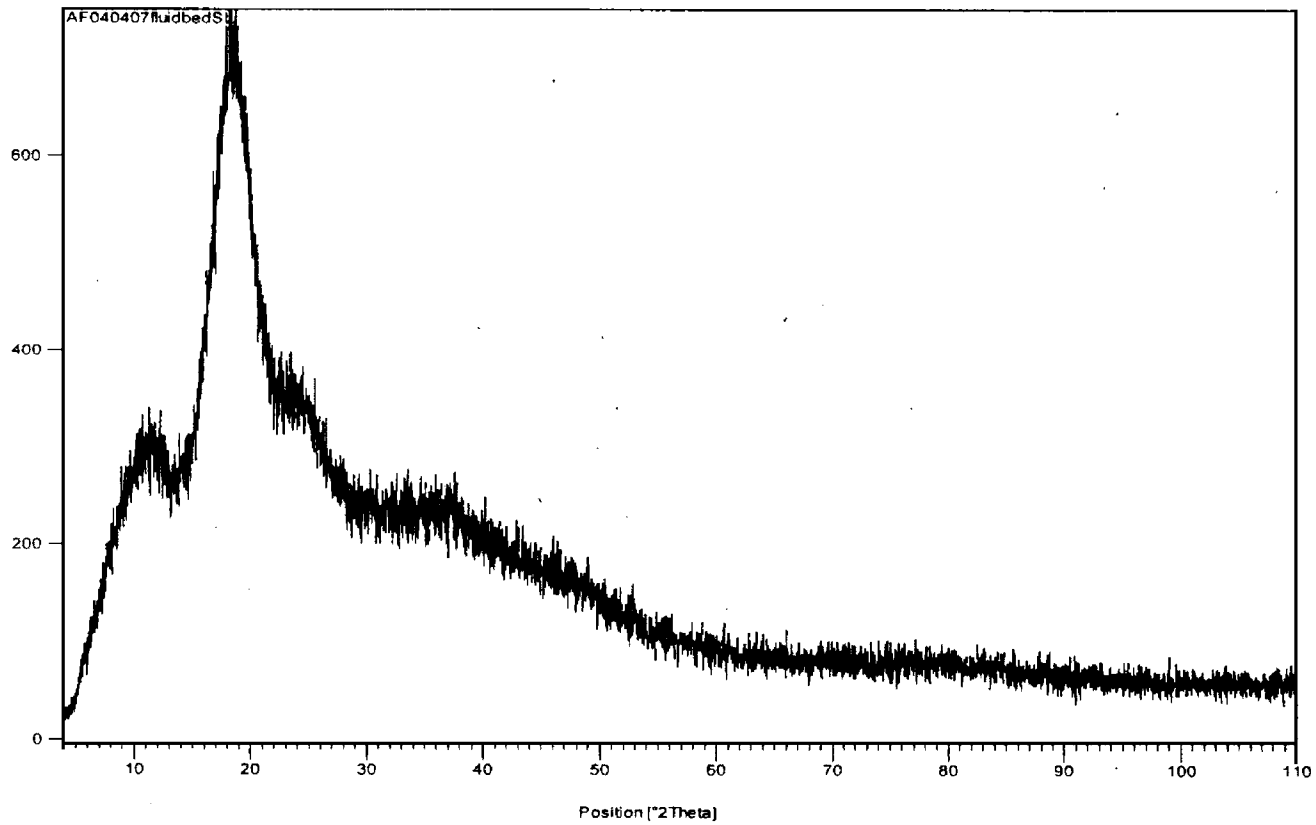

Fig. 9. XR diffractogram of the FB complex.

Small dissolution profile differences agree with those of fractal dimension values for the particle contour: this last parameter increases as irregularity of the particle contour increases and, as a simple extension, this rank order could also reflect surface irregularity and thus higher reactivity to dissolution. Finally, comparison of these parameters with those of the physical mixture suggest the importance of the preliminary preparation of the complex (whatever the method) for improving dissolution. Since the HPBCD/P complex is designed to prepare extemporary injectable solutions, the dissolution rate represents an important parameter in selecting the optimum material for the prefixed purpose: in this case an improvement of the dissolution rate of progesterone could be obtained by a simpler method than those previously reported (12,13).

CONCLUSIONS

μ-FT-IR, μ-Raman and X-ray spectroscopy, and the dissolution test did not reveal defined differences among the three different types of particles, confirming occurrence of the complex in the solid state, by comparison of band shift with structurally related compounds. More information was obtained by SEM micrographs and image analysis of the particles obtained by the different methods. The spherical shape and the very small size of the particles obtained by spray-drying process and the low value of the contour fractal dimension could allow better technological performance. However the repose angle does not suggest differences and, moreover, the very small size of these particles favours aggregation. On the contrary FB particles have a more irregular contour, do not aggregate and display a better dissolution profile; the fluid bed drying process therefore appears the most promising one to prepare dry particle of the HPBCD/P complex, in view of its formulation in the preparation of extemporaneous injectable solutions.

ACKNOWLEDGMENTS

We thank Dr. S. Beninati for BET analysis. The text was revised by an English translator.

REFERENCES

1. T. Loftsson, J. Baldvinsdottir, and A. M. Sigurdardottir. The effect of cyclodextrins on the solubility and stability of medroxyprogesterone acetate and megestrol acetate in aqueous solution. *Int. J. Pharm.* **98**:225–30 (1993).
2. V. Zia, R. A. Rajewski, E. R. Bornancini, E. A. Luna, and V. J. Stella. Effects of alkyl chain length and degree of substitution on the complexation of sulfoalkyl ether beta-cyclodextrin with steroids. *J. Pharm. Sci.* **86**:200–24 (1997).
3. B. Pose-Vilarnovo, I. Perdomo-Lopez, M. Echexarreta-Lopez, P. Schroth-Pardo, E. Estrada, and J. J. Torres-Labandeira. Improvement of water solubility of sulfamethizole through its complexation with beta- and hydroxypropyl-beta-cyclodextrin. Characterizaton of the interaction in solution and in the solid state. *Eur. J. Pharm. Sci.* **13**:325–31 (2001).
4. M. L. Lahiani, C. Barbot, F. Bounoure, S. Joudich, and M. Skiba. Solubility and dissolution rate of progesterone cyclodextrin-polymer system. *Drug Dev. Ind. Pharm.* **32**:1043–58 (2006).
5. X. Tan, and S. Lindenbaum. Studies on complexation between BCD and bile salts. *Int. J. Pharm.* **74**:127–35 (1991).
6. E. Redenti, L. Szente, and J. Szejtli. Cyxlodextrin complex of salts of acidic drug. Thermodynamic properties, structural features and pharmaceutical applications. *J. Pharm. Sci.* **90**:979–86 (2001).
7. M. Lahiani-Skiba, C. Barbot, F. Bounoure, S. Joudieh, and M. Skiba. Solubility and dissolution rate of progesterone-cyclodextrin-polymer systems. *Drug Dev. Ind. Pharm.* **32**:1043–58 (2006).
8. G. Zoppetti, N. Puppini, F. Ospitali, and A. Fini. Solid state characterization of progesterone in a freeze dried 1:2 progesterone/HPBCD mixture. *J. Pharm. Sci.* **96**:1729–36 (2007).
9. G. Torri, S. Bertini, T. Giavana, M. Guerrini, N. Puppini, and G. Zoppetti. Inclusion complex characterization between progesterone and hydroxypropyl-b-cyxlodextrin in aqueous solution by NMR study. *J. Incl. Phenom. Macrocycl. Chem.* **57**:317–21 (2007).
10. G. Zoppetti, N. Puppini, M. Pizzuti, A. Fini, T. Giovani, and S. Comini. Water soluble progesterone-hydroxypropyl-b-cyxlodextrin complex for injectable formulations. *J. Incl. Phenom. Macrocycl. Chem.* **57**:283–88 (2007).
11. K. Uekama, F. Hirayama, and T. Irie. Cyclodextrin drug carrier systems. *Chem. Rev.* **98**:2045–76 (1998).
12. P. Potluri, and G. V. Betageri. Mixed-micellar proliposomal systems for enhanced oral delivery of progesterone. *Drug Deliv.* **13**:227–32 (2006).
13. H. I. Chang, M. R. Williamson, Y. Perrie, and A. G. Coombes. Precipitation casting of drug-loaded microporous PCL matrices: incorporation of progesterone by co-dissolution. *J. Control. Release.* **106**:263–72 (2005).