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ORIGINAL ARTICLE

Dissolution properties, solid-state transformation and polymorphic crystallization: progesterone case study

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Abstract

Progesterone is a natural steroid hormone and a poor soluble drug which presents two polymorphs (forms 1 and 2). Different methods to obtain form 2 were tested and a complete solid-state characterization of both polymorphs (forms 1 and 2) was conducted. X-ray powder diffraction, hot stage microscopy, Fourier transform infrared, dispersive Raman, ^{13}C solid-state nuclear magnetic resonance spectroscopy, thermal analysis, scanning electron microscopy techniques and intrinsic dissolution rates (IDR) were applied to investigate physical–chemical and dissolution properties of these two polymorphs. Form 2 was obtained from diluted solutions and from melting after cooling at room temperature. Form 1 was obtained from concentrated solutions and, a mixture of both polymorphs was crystallized from intermediate solutions. The crystal habit was not a distinctive characteristic of each polymorph. The effect of mechanical stress was evaluated in the metastable polymorph (form 2). We observed that grinding form 2 produced seeds of form 1 that induced the transformation of form 2 into form 1 at high temperature. The polymorphic quantification from XRD patterns of ground samples were carried out by the Rietveld method. After grinding and at room temperature conditions (~25 °C), it was observed the transformation of 17% of form 2 into form 1 in 10 days.

Keywords

Intrinsic dissolution rate, phase transformation, polymorphism, progesterone, solid-state characterization

History

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Introduction

Pharmaceutical solids can exist in more than one crystalline phases, called polymorphs, that generally present differences between them in solubility, dissolution rate, stability and bioavailability[1,2]. Polymorphs can appear during the standard manufacturing process, affecting the quality, safety and efficacy of a solid dosage form if not properly controlled[3,4].

Progesterone, pregn-4-ene-3,20-dione (Figure 1), is a natural steroid hormone that has six chiral centers[5,6]. It is secreted by the ovary as part of the menstrual cycle, is involved in pregnancy and embryogenesis of humans and another species. In humans it is used in birth control pills, in menopausal hormone replacement therapies and polycystic ovary syndromes[7]. On the other hand, in animals, it is used for artificial insemination programs. Progesterone is known to exists in two polymorphic forms: form 1 (α-form) and form 2 (β-form)[8] and its physical–chemical properties has been deeply studied[9,10]. Form 2 is considered a ''disappearing'' polymorph[6]; nevertheless, Heredia et al.[11] and Tripathi et al.[12] reported the presence of this polymorph in their experiments and established relation between its presence with improvements in dissolution characteristics. Several advantages could be obtained if the pharmaceutical and/or veterinary products would be formulated with form 2. However, to date, the Intrinsic Dissolution Rate (IDR) of progesterone polymorphs and the polymorphic stability of form 2 against grinding process have not been determined.

In this article, the IDR of both progesterone polymorphs was determined. Forms 1 and 2 were previously characterized and the solid-state transformation of form 2 into form 1 with quantification by the Rietveld method was performed. Finally, as form 2 is more soluble than form 1, solvent evaporation crystallizations were conducted in order to obtain form 2.

Materials and methods

Samples preparation and crystallization experiments

Form 1, micronized progesterone (purity > 99%) was purchased from Pharmanostra (Rio do Janeiro, Brazil) imported from China. Form 2 was obtained from the molten sample of form 1 at 140 °C by slow cooling. It guaranteed to obtain form 2 as a pure form[9].

For the polymorphic stability of form 2, ~1 g of sample was placed in a porcelain mortar and ground for different time ranges from 5 to 25 min. Recrystallizations were conducted in chloroform and in acetone by solvent evaporation at room temperature

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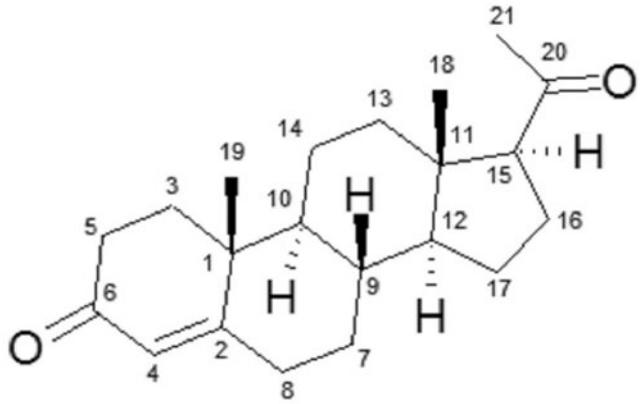

Figure 1. Chemical structure of pregn-4-ene-3,20-dione (progesterone).

(~25 °C) at an evaporation rate of ~533 and 318 mg s^{-1}/m^2, respectively. The solvents were selected because of the high solubility of progesterone in them and precisely to cover two different evaporation rates. Progesterone solutions of 0.5, 10 and 40 mg/mL in both solvents were prepared and 2.00 mL each of these solutions were added onto glass substrates.

Analytical methodology

X-ray powder diffraction

X-ray diffraction patterns were collected in reflection mode from a PANanytical X'PERT Multipurpose diffractometer equipped with a Cu Kα source ($\lambda = 1.5418$°A) operated at 45 kV and 40 mA, step size 0.016°, step time 20 s and 2θ angular range between 5° and 50°. The ground samples were sieved in 325 mesh sieves and supported on a zero background Si holder that spins at 2 s/revolution to minimize preferential orientation, particle size distribution and flat surface imperfections effects in XRD data.

Polymorphic quantification methodology – Rietveld refinements

The polymorphic quantifications from XRD patterns of the ''milled sample'' as a function of ageing were carried out by the Rietveld method using the graphical interface of the General Structure Analysis System (GSAS + EXPGUI)[13,14] and the published single crystal structure data[6]. The average crystallite size and microstrain of each phase were also obtained using a pseudo-Voigt function modified by P. Stephens[15] and instrumental line broadening provided by the refinements of a standard sample (Y_2O_3).

In each case, background parameters, phase fractions, peak shape parameters, cell parameters and sample position shift were refined before variation of further structural parameters. Isotropic dislocation parameters were refined for all the C and O atoms.

Fourier transform infrared spectroscopy

Spectra from 1% solid dispersions in KBr were recorded in a FT-IR Shimadzu IR Prestige-21 in the range of 4000–600 cm^{-1}. The polymorphs were placed into the holder directly without compressing.

Dispersive Raman spectroscopy (Raman)

Raman spectra were collected in backscattering geometry using a PeakSeeker 785 (RAM – PRO – 785) Raman system operating with a diode laser of 785 nm and 300 mW at the source. The collected Raman radiation was dispersed with a grating and focused on a Peltier-cooled charge-coupled device CCD detector obtaining a spectral resolution of 6 cm^{-1}. The laser was focused on the sample by 20× objective lens of a microscope given spot of ~2 μm in diameter. All spectra were recorded in the spectral window 200–1800 cm^{-1} with the same acquisition time (10 s). The sample powders were analyzed in glass blades at room temperature (~25 °C).

Solid-state nuclear magnetic resonance

High-resolution solid-state ^{13}C cross polarization/magic angle spinning (CP/MAS) spectra for progesterone 1 and 2 were recorded using a Bruker Avance II-300 spectrometer (300.13 MHz for ^{1}H and 75.46 MHz for ^{13}C). The samples were packed into a 4-mm rotor and spun with a rate of 10 kHz. The CP/MAS spectra were recorded employing a variable amplitude CP (2 ms contact time)[16]. The SPINAL64 sequence was used for heteronuclear decoupling during acquisition with a proton field H_{1H} satisfying $\omega_{1H}/2\pi = \omega_H H_{1H} = 62.5$ kHz[17]. The recycling time was 5 s and 4096 scans were recorded for each compound in order to obtain an adequate signal to noise ratio. The quaternary carbon edition spectra were recorded for all the samples. These spectra were acquired with the non-quaternary suppression (NQS) sequence, in which the ^{1}H and ^{13}C r.f. fields are removed during 40 μs after CP and before acquisition[16]. This experiment allowed us to identify quaternary carbon signals and methyl groups, and to perform the assignments in the solid state. All the solid-state NMR experiments were performed at room temperature (~25 °C).

Thermal analysis

Differential scanning calorimetry (DSC) curves were obtained on a Shimadzu DSC-60 cell using aluminum pans, under a dynamic nitrogen atmosphere (50 mL/min) and a heating rate of 5 °C/min in a temperature range from 40 to 150 °C. In order to prevent an uncontrolled variation in pressure, the samples were placed on pans which were sealed and subsequently was made a small perforation[18].

Hot-stage microscopy

Thermal events were observed on Olympus BX50 microscope equipped with a Mettler Toledo FP-82 hot stage. The sample was placed on a microscope slide, covered with a cover slip and heated at a rate of 5 °C min^{-1}.

Scanning electron microscopy (SEM)

Microphotographs were obtained from a JEOL JSM-6390LV scanning electron microscope operated at 15 kV. The samples were mounted on a metal stub with double-sided adhesive tape and coated under vacuum with gold in an argon atmosphere.

Intrinsic dissolution rate test

Intrinsic dissolution assays were conducted in a Varian VK 7000 dissolution testing station, a rotating disk system of 0.8 mm in diameter (surface area 0.5 cm^2) according to USP 31[19]. Then, 100 mg of pure progesterone forms 1 and 2 were compressed at 2845 psi in an ASTA hydraulic press.

Given that progesterone is practically insoluble in water, hydroalcoholic solutions were reported by Taghizadeh et al.[20] for *in vitro* controlled released experiments. In order to evaluate the dissolution performance of both polymorphs, the conditions of IDR test were 200.0 mL of ethanol/water (50/50, v/v) as dissolution medium at 25 ± 1 °C for 20 min at 100 rpm. Aliquots of 5.0 mL were withdrawn every 2 min. The dissolution medium was replaced after every sampling. The concentration of

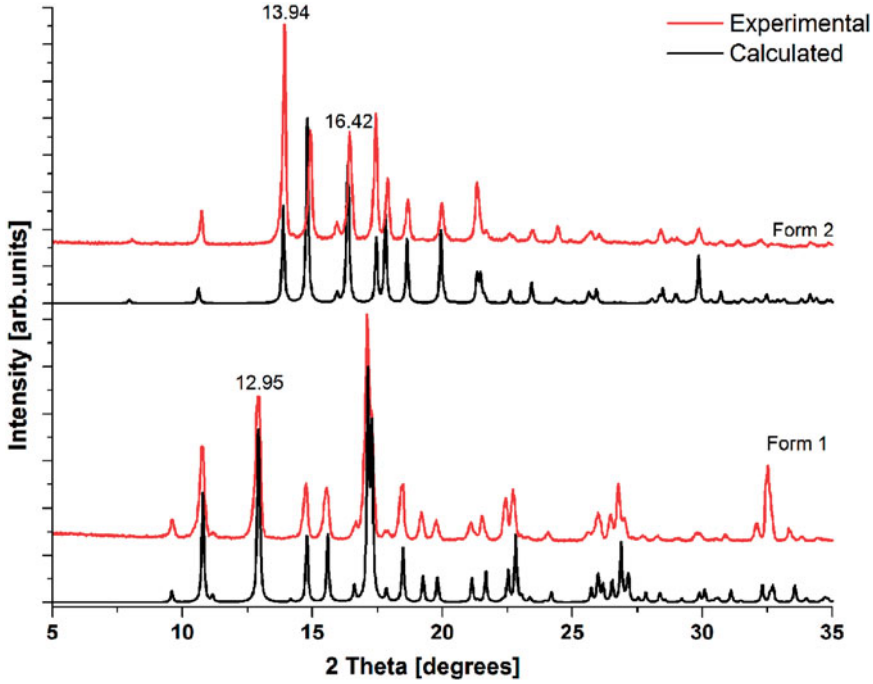

Figure 2. Experimental and calculated XRPD patterns of progesterone polymorphs. The XRPD patterns for forms 1 and 2 were calculated from CSD data: PROGST12 and PROGST13, respectively[6].

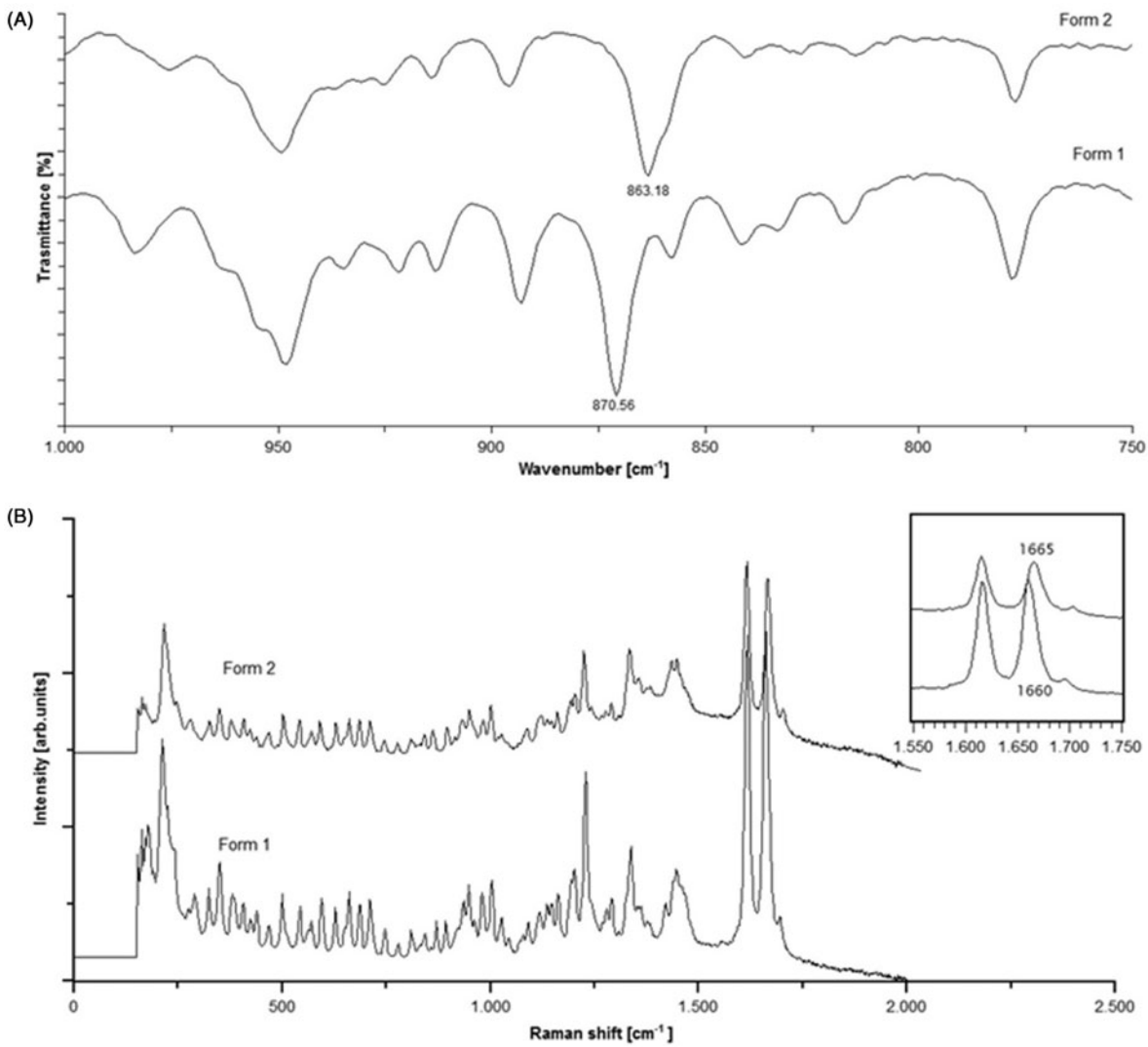

Figure 3. (A) FT-IR Spectra and (B) of Raman spectra of solid-state progesterone polymorphs.

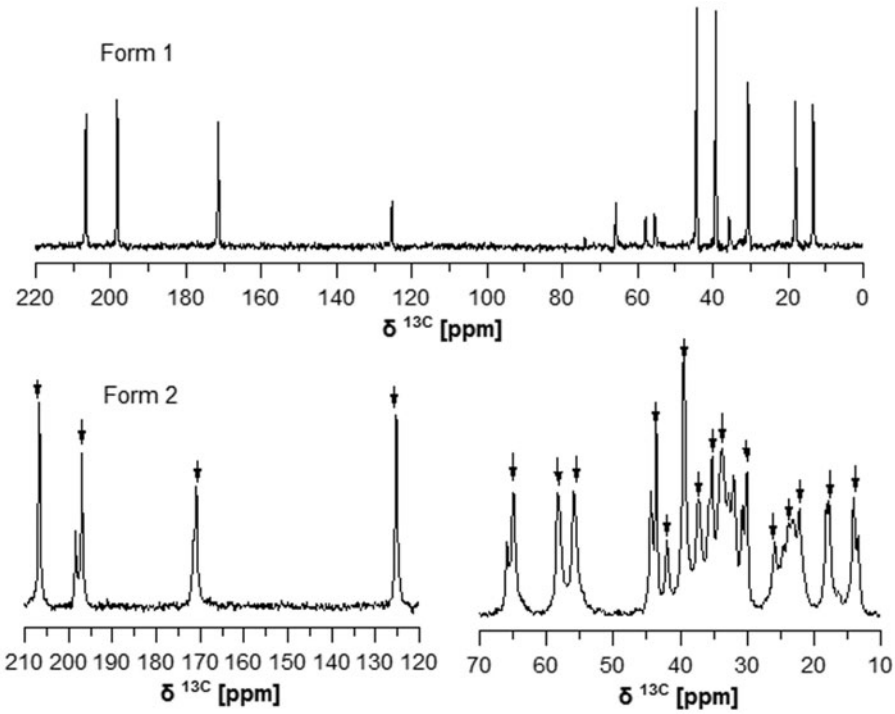

Figure 4. ^{13}C CP/MAS ssNMR spectra of progesterone forms 1 and 2.

progesterone in the solution was measured by UV spectrophotometer Varian Cary 50 Bio at a wavelength of 240 nm[21]. The sink conditions were maintained during the entire dissolution experiment.

Results and discussion

Progesterone polymorphs identification

X-ray powder diffraction

Forms 1 and 2 are pure enantiomers and its crystal structure has been re-determined recently by Lancaster et al.[6] with considerable improvements of previous crystal structure determinations. Both polymorphs are orthorhombic; form 1 ($P2_12_12_1$; $a = 10.2496(7)$ Å, $b = 12.4830(9)$ Å, $c = 13.6406(9)$ Å; $Z' = 1$; 150 K; $R = 3.9\%$) and form 2 ($P2_12_12_1$; $a = 6.2089(6)$ Å, $b = 12.5804(12)$ Å, $c = 22.188(2)$ Å; $Z' = 1$; 150 K; $R = 4.5\%$)[6].

The XRPD patterns of pure samples of progesterone forms 1 and 2 were analyzed and compared with Cambridge Structural Database (CSD) crystallographic data (PROGST12: form 1 and PROGST13: form 2)[6]. Subsequently, these patterns were used as references to analyze the samples obtained in the crystallization screening study, presented in the following session. Figure 2 shows the calculated and experimental patterns of both progesterone polymorphs. Taking into account that the XRPD patterns of both polymorphs presented several superimposed reflections, we selected 12.95° to identify form 1 and 13.94° and 16.42° to distinguish form 2. These reflections are intense and they are not overlapped.

Fourier transform infrared spectroscopy

The FT-IR spectra of progesterone forms 1 and 2 show a distinctive band due to the out-of-plane bending of hydrogen bonded to a sp^2 carbon (C2–C4) at 870.56 cm^{-1} in form 1 that it is shifted to 863.18 cm^{-1} in form 2 at 870.56 cm^{-1} in form 1 that it is shifted to 863.18 cm^{-1} in form 2 (Figure 3A). These results were in agreement with previously reported in the literature.

Table 1. Solid State ^{13}C CP/MAS NMR peaks of progesterone forms 1 and 2.

	^{13}C-chemical shift (ppm)	
Carbon number	Form 1	Form 2
18, 19	13.3, 18.1	14.0, 17.7
14, 16, 17	22.9, 23.2, 24.6	22.2, 20.8, 25.9
21	30.6	30.1
3, 5, 7, 8, 9	31.9, 32.7, 33.3, 35.7, 37.0	31.9, 32.7, 33.8, 35.2, 37.3
13	39.0	39.5
1	39.2	42.0
11	44.3	43.6
10	55.5	55.9
12	58.0	58.3
15	65.9	64.9
4	125.3	125.3
2	171.4	170.8
6	198.4	196.9
20	206.7	206.7

Raman spectroscopy

Figure 3(B) shows the Raman spectra of both progesterone polymorphs. The selected region has been previously described in the literature[22] and a peak shift from 1660 to 1665 cm^{-1} corresponds from form 1 to form 2 is observed.

Solid-state nuclear magnetic resonance

The ^{13}C ssNMR spectra for both progesterone forms are shown in Figure 4. The assignments for the ^{13}C spectra (see carbon numbering in Figure 1) were carried out taking into account the quaternary carbon spectra and by comparing with the simulations of solution spectra resulting from commercial software. The ^{13}C ssNMR chemical shifts for both forms 1 and 2 are displayed in Table 1. The greatest chemical shift was observed in C6 and C16. The other carbons presented similar chemical shifts. Chemical shift of C3, C5, C7, C8 and C9 between 31.9 and 37.0 cannot be assigned to a specific carbon. This suggests small differences

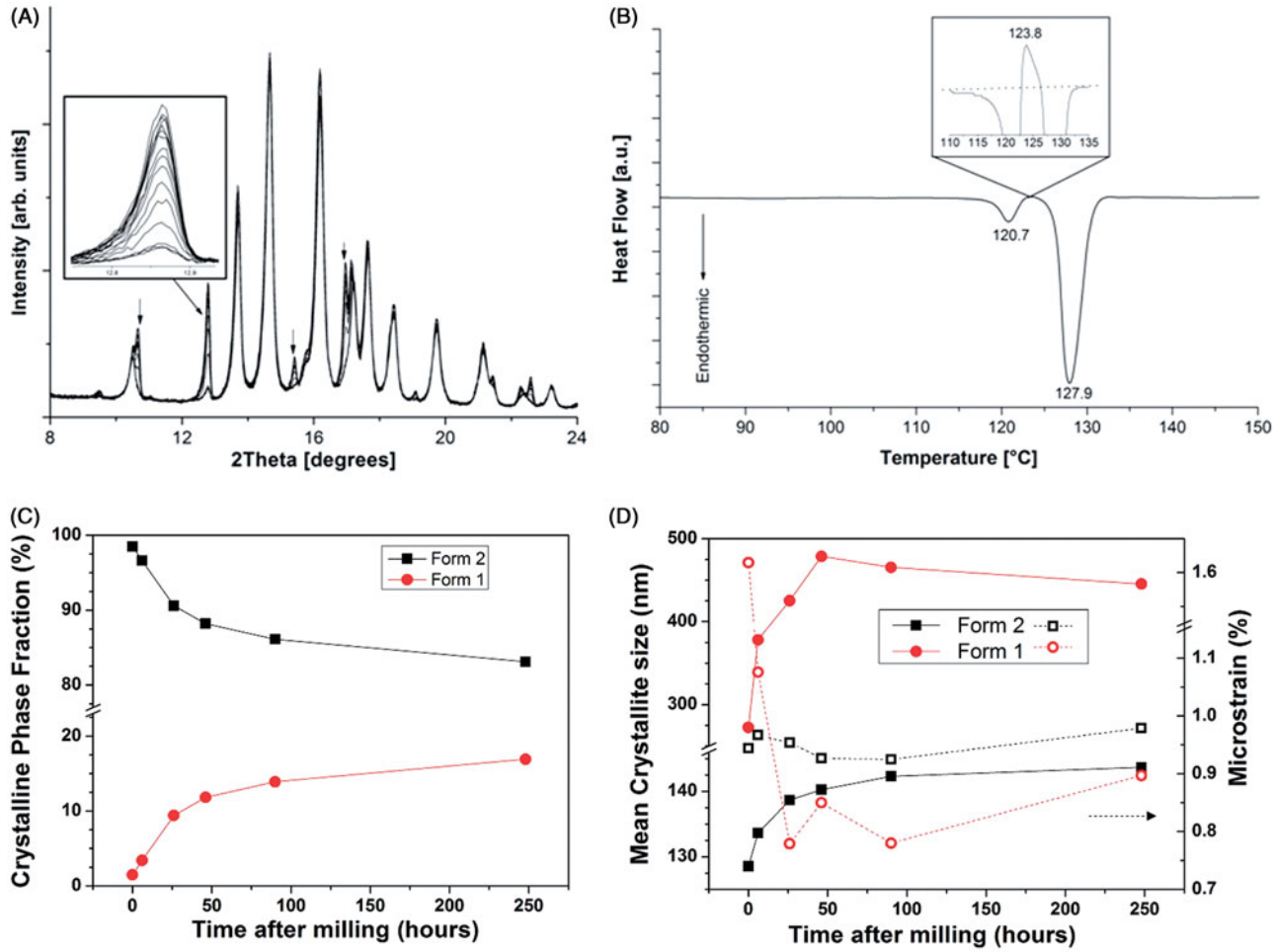

Figure 5. (A) XRPD patterns of progesterone form 2 ground for 15 min as a function of ageing time up to 258 hours. (B) DSC curve of progesterone form 2 obtained immediately after grinding. (C) Phase fractions, (D) mean crystallite sizes and microstrains obtained from Rietveld refinements (R from 8% to 7%) of XRPD pattern measured as a function of ageing.

between structures of forms 1 and 2, caused by the limited flexibility of the progesterone molecule. Additionally, progesterone has no conventional hydrogen bond donors[8].

The spectrum of form 1 showed a set of narrow resonances, ~30 Hz FWHM, indicating a high crystalline polymorph. Almost each carbon atom in the molecule was resolved, except for two broader peaks at 23 and 39 ppm, corresponding each to a couple of resonances not completely resolved. We can conclude that there is only one molecule in the asymmetric unit of form 1. The ^{13}C spectrum of form 2 clearly showed the presence of resonances corresponding to form 1. In order to obtain a fine powder for the CP/MAS NMR experiment sample was ground, it probably transforms part of the form 2 sample into form 1. In this case, the resonances were broader than in form 1, due to crystal stress produced during the cooling down from the melt of form 1. Resonances of form 2 are indicated by arrows. The number of resonances indicated that there is also only one molecule in the asymmetric unit of form 2. These results were in agreement with crystallographic data of progesterone forms 1 and 2.

Thermal analysis

DSC curves show an endothermic event at 128.2 °C for form 1 and at 121.8 °C for form 2, both in agreement with previously reported values[18]. The system was evaluated in four different heating rates: 1, 2, 5 and 10 °C/min without significant changes in the peak shape and melting point temperature. Then 5 °C/min was selected as the appropriate heating rate with adequate resolution of the endothermic events.

Solid-state transformation of form 2 into form 1

The transformation of progesterone form 2 into form 1 by grinding process was earlier observed by the ^{13}C ssNMR measurements, previously mentioned. Grinding and compacting are routine parts of the drug processing, therefore, it is important to study the effect on polymorphic transitions. In addition to that, when a metastable form is being used an investigation of effects induced by mechanical stress is essential[23].

In sample preparation for the IDR determination, the solid form undergoes mechanical stress[24]. Hence, the polymorphic stability of progesterone form 2 was previously evaluated. Immediately after the grinding process of the sample, DSC curve showed two exothermic events ~118 °C and 123 °C, corresponding to the melting point of forms 2 and 1, respectively (Figure 5B). Since these two polymorphs are monotropically related[25], three scenarios are possible: first each polymorphs melts a defined temperature; second, form 2 transforms into form 1 and third, form 2 melts followed by recrystallization and then melts as form 1[26]. In the particular case of progesterone, each polymorphs presents a defined endothermic event when is presented in its pure form, as observed in Figure 5(B). Therefore, the solid-state transformation of form 2 into form 1 induced by heating was not expected; we deduced that a mixture

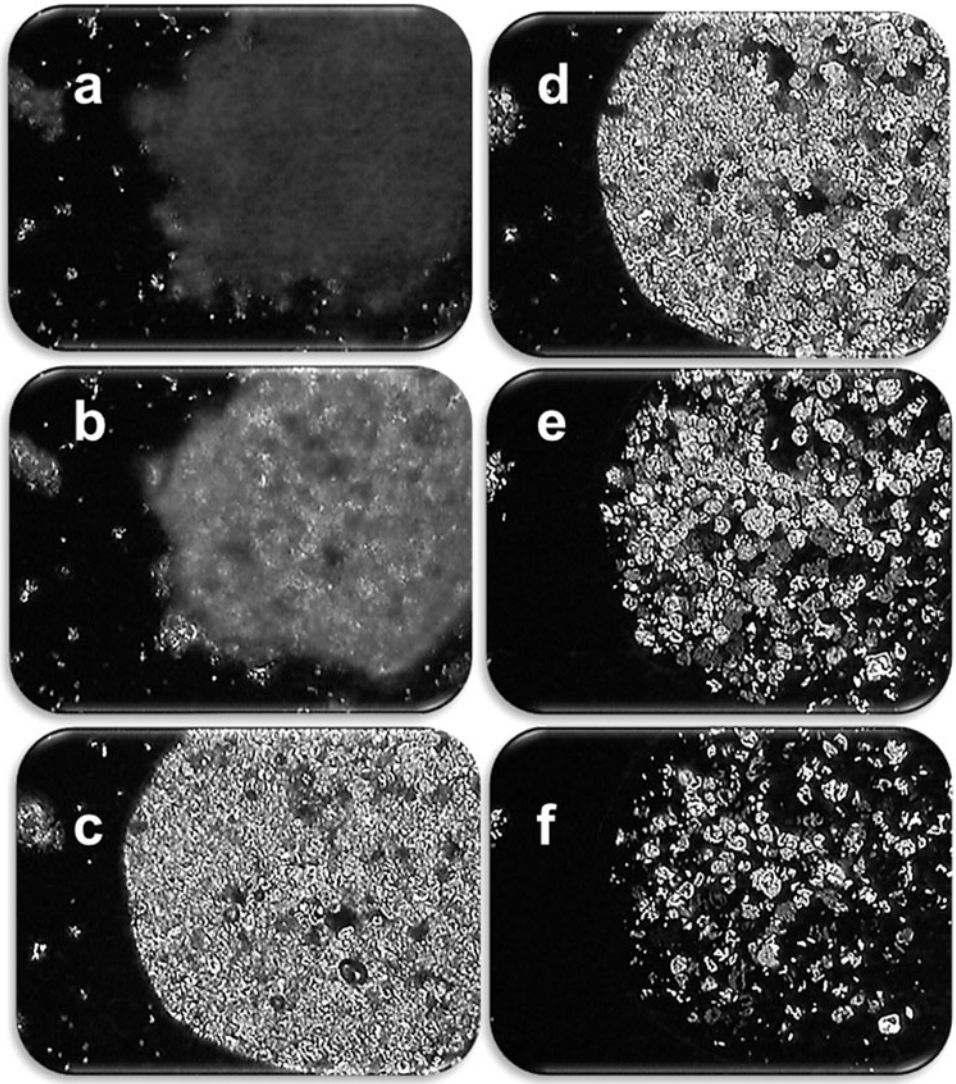

Figure 6. Hot stage microscopy of progesterone form 2 grinding by 15 minutes: (a) 118 °C, (b) 122 °C, (c) 123 °C, (d–f) 130 °C.

Figure 7. Intrinsic dissolution profile of progesterone polymorphs.

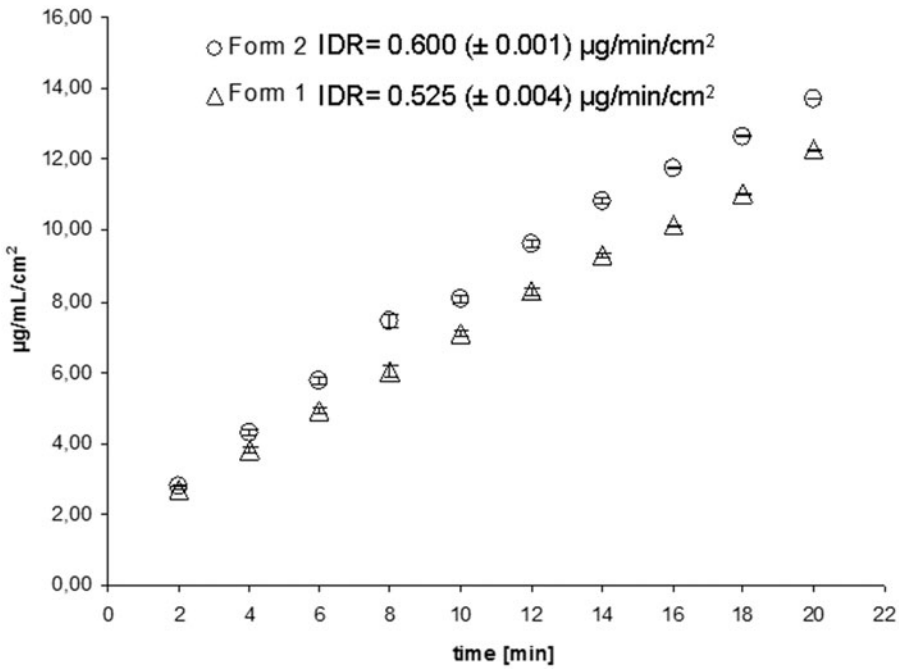

Table 2. Progesterone crystal form obtained from acetone and chloroform at the three concentrations.

	Polymorph		
	Progesterone concentration (mg/mL)		
Solvent	40	10	0.5
Acetone	Form 1	Mixture	Form 2
Chloroform	Form 1	Mixture	Form 2

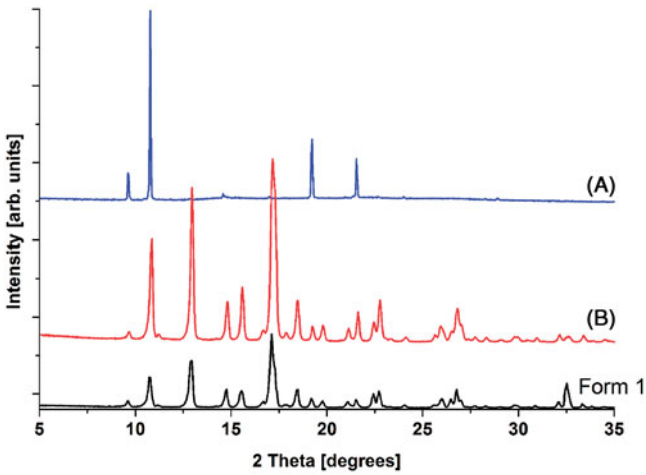

Figure 8. Experimental XRPD of progesterone forms 1 and progesterone crystallized from 40 mg/mL solutions in: (A) acetone and (B) chloroform.

of forms 1 and 2 was present in the sample. This expectation was mainly because the enthalpy of the endothermic event of form 1 is significantly higher than the one of form 2. However, this assumption was not confirmed by XRPD analyses in the samples ground. It was a surprise that no reflection of form 1 was observed in the samples ground for 5 and 10 min. In the sample, ground for 15 min with an endotherm of form 1, as shown in Figure 5(B), only a very low reflection of the highest reflection of form 1 (12.95°) was detected. The latter sample was monitored from 0 to 258 h and Figure 5(A) shows the arising of this reflection of form 1, evidencing the phase transformation of form 2 to form 1. Figure 5(C) shows that the phase fractions evolution is not linear with time after milling (ageing) and that after ~10 days (258 h) the sample, originally of form 2, which was ground for 15 min, have ~17% of form 1. Furthermore, Figure 5(D) shows that the mean crystallite size of both phases increased with ageing while only microstrains of form 1 have changed (decreasing as its contribution to the sample volume increases). This is further evidence that the grinding process caused the formation of seeds of form 1 (which would explain the DSC results) and that these seeds grow with time (ageing) by atomic diffusion mechanisms driven by the energy stored in defects due milling process. The effect of milling on samples generating crystal nuclei and the subsequently thermally-induced crystallization event was also observed in amorphous and crystalline griseofulvin samples[27].

The presence of seeds of form 1 and the crystallization of form 1 from molten sample of form 2 was clearly observed by Hot stage microscopy (see Video 1). Initially, the crystalline sample was identified with polarized light as a wide gray area (Figure 6a). At 122 °C, some dark areas were started to arise (Figure 6b) corresponding to fusion of form 2 and simultaneously the seeds of form 1 were distinguished. The shiny crystals of form 1 were very well detectable using polarized light. Afterward, a rapid crystallization ~123 °C occurred (Figure 6c) and finally, the fusion of

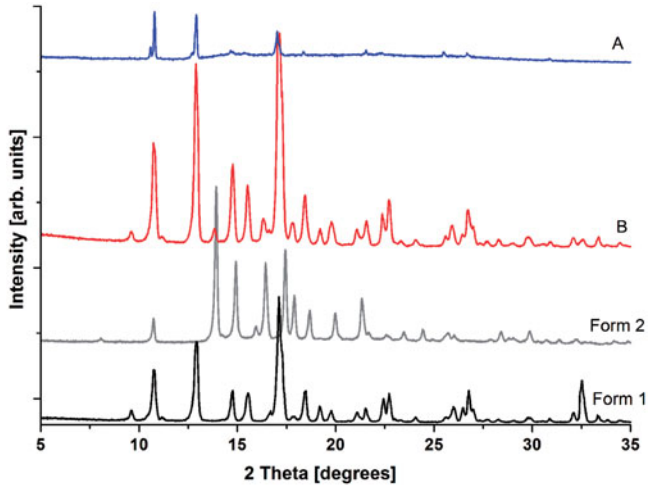

Figure 9. Experimental XRPD of progesterone forms 1 and 2 and progesterone crystallized from 10 mg/mL solutions in: (A) acetone and (B) chloroform.

form 1 was detected when the crystallized area was transformed completely dark at 130 °C (Figure 6d–f). This result confirmed that seeds of form 1 were nuclei present in molten form 2 that induced the crystallization of form 1.

Intrinsic dissolution rate

Muramatsu et al.[28] determined the aqueous solubility for single crystals of both progesterone polymorphs. However, the IDR value could be considered more useful than solubility to correlate the *in vivo* drug dissolution dynamics[24]. According to Šehić et al.[29], values lower than 0.001 μg/min/cm^2 indicate dissolution rate-limiting absorption. Under sink conditions the IDR for two polymorphs of a drug must be proportional to the respective solubilities[1]. In the previous section, the polymorphic instability of form 2 was evaluated against mechanical stress and was determined that with less of 5 min of grinding the sample and performing the analysis in the subsequent 2 h the sample is kept as pure form 2 only with low quantity of seeds of form 1. Polymorphs did not change at the pressure used in this.

Figure 7 shows a linear behavior in the intrinsic dissolution profile of both progesterone polymorphs. The IDR of form 2 was (0.600 ± 0.001) μg/min/cm^2 and (0.525 ± 0.004) μg/min/cm^2 to form 1, indicating that form 2 is more soluble than form 1.

Crystallization experiments

The study of progesterone crystallization using acetone and chloroform solvents and from three different concentrations was analyzed. Table 2 shows that form 1 was obtained when progesterone was crystallized from concentrated solutions whereas form 2 was obtained when crystallization was performed starting from diluted solutions and a mixture of the two polymorphs from intermediate concentrations.

In order to identify the polymorphism in different preparations, XRPD patterns of the sample and the calculated powder pattern of single crystal data were compared. The XRPD pattern for crystals obtained from 40 mg/mL solutions in chloroform and acetone confirmed the presence of progesterone form 1 (Figure 8). In addition, crystallization from acetone presented four intense reflections, indicating high preferred orientation (Figure 8A). The XRPD pattern for crystals obtained from intermediate solutions, in chloroform, matched with form 1 and presented a reflection of form 2 at 13.94 ° (Figure 9B), thus confirming the presence of a mixture of progesterone polymorphs. In the case of crystals

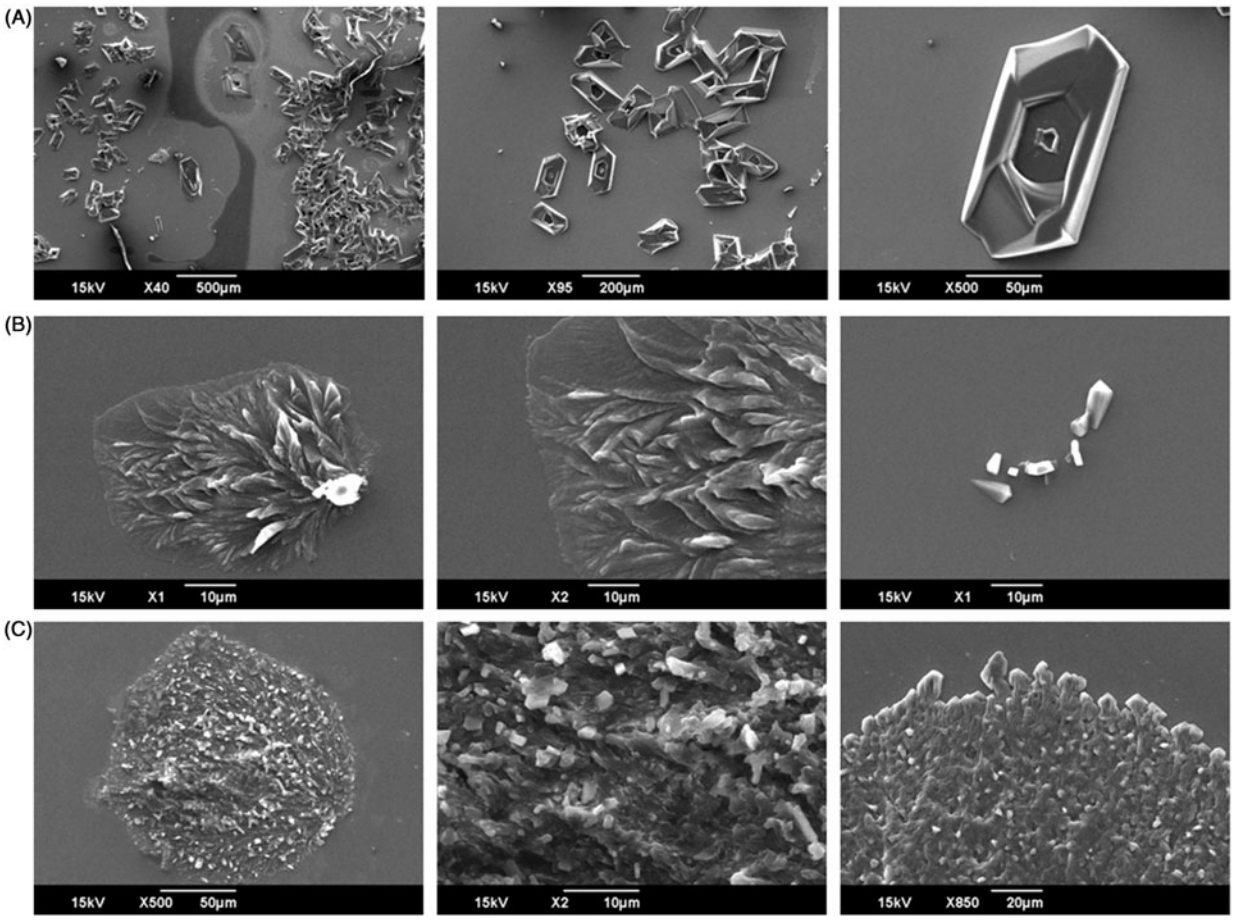

Figure 10. Microphotographs of progesterone crystallized from chloroform: (A) 40 mg/mL, (B) 10 mg/mL and (C) 0.5 mg/mL.

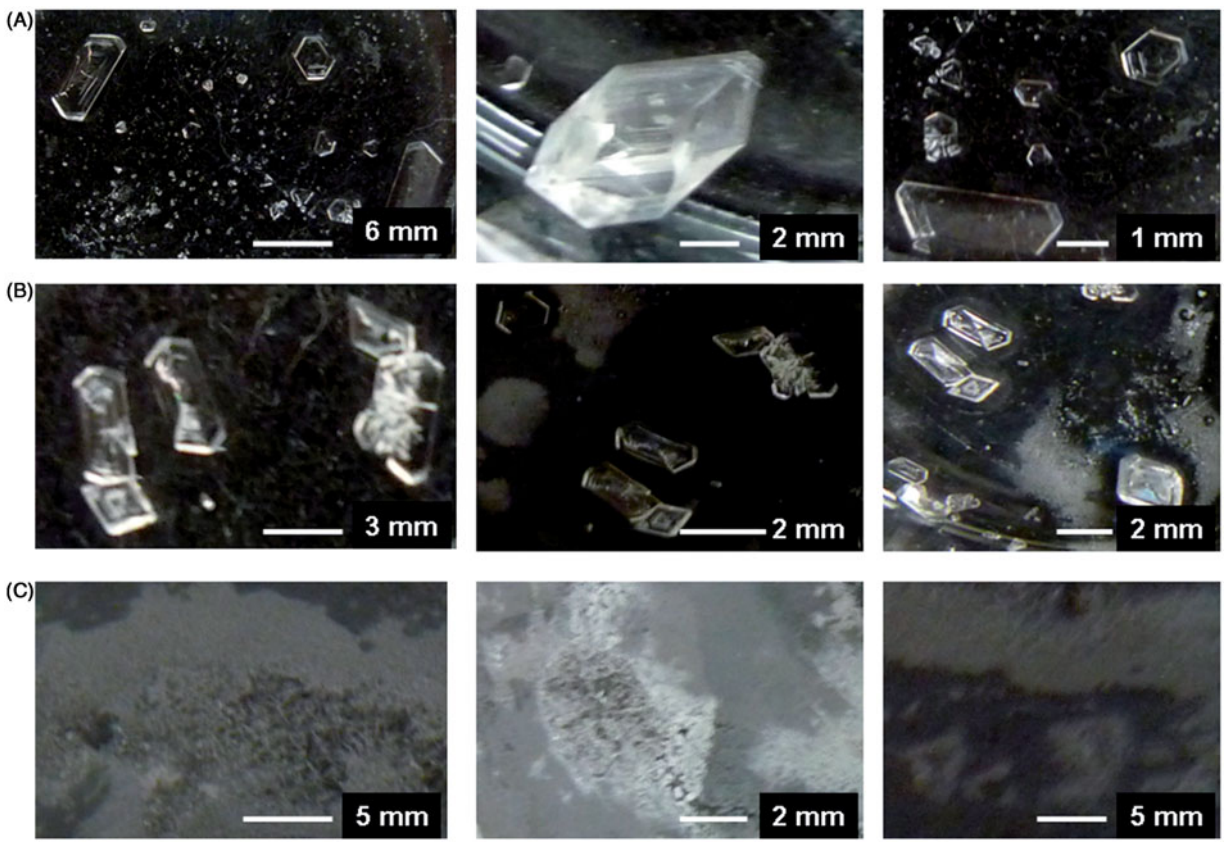

Figure 11. Photographs of progesterone crystallized from acetone: (A) 40 mg/mL, (B) 10 mg/mL and (C) 0.5 mg/mL.

obtained from acetone, presenting preferred orientation again, the reflections observed, were coincident in both polymorphs. For samples obtained from diluted solutions, a few reflections corresponding to form 2 were. The presence of form 2 was confirmed by DSC analyses.

Lancaster et al.[6] reported progesterone form 2 as a ''disappearing polymorph'' since the metastable form was exceptionally difficult to obtain. They argued that when form 2 was initially reported, it was stabilized by impurities of synthesis in the sample used. In a recent work, the same authors analyzed a 50-year-old sample of progesterone, showing the role of impurities in form 2 stabilization[30]. However, we could obtain the metastable form (form 2) using a highly pure raw material, using two different approaches: crystallization from dilute solutions and melting. Finally, as it was concluded in a preceding review by Dunitz and Bernstein, in our view the case of progesterone polymorphs may be the result of incomplete experimental conditions reported that turn difficult to reproduce the polymorph crystallizations[31].

Morphology of crystals

The crystals obtained from 40 and 10 mg/mL solutions prepared from acetone solutions, were greater than those obtained from chloroform solutions at the same starting concentration (Figures 10 and 11A, B). However, the crystal habit presented by form 1 crystallized from both solvents were similar (Figures 10 and 11B). On the other hand, form 2 presented a different crystal habit than form 1. However, we did not observe different morphologies when progesterone was crystallized as a mixture of polymorphs; disabling differentiation between polymorphs by their morphology.

Conclusions

The IDR experiments demonstrated that progesterone form 2 is more soluble than form 1 and DSC, XRPD, ssNMR and HSM measurements permitted the determination of the phase transformation of form 2 into form 1 by grinding. Progesterone forms 1 (stable) and 2 (mestastable) were obtained as pure samples and a mixture of forms depending on the preparation conditions. Form 2 was crystallized from molten sample and from acetone and chloroform diluted solutions. Form 1 was crystallized from concentrated solutions, whereas a mixture of the two polymorphs was obtained from intermediate solutions concentration. The morphology was not a distinctive characteristic of each polymorph.

Finally, the results reported herein suggest that a good strategy to prepare formulations containing progesterone in form 2 could be to crystallize it on different matrices or any other dosage form that avoid grinding process.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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