Journal of Pharmaceutical and Biomedical Analysis 63 (2012) 74–79

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Sulfamethoxazole:hydroxypropyl-β-cyclodextrin complex: preparation and characterization

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ARTICLE INFO

Article history:
Received 16 September 2011
Received in revised form
30 December 2011
Accepted 9 January 2012
Available online 2 February 2012

Keywords:
Sulfamethoxazole
Hydroxypropyl-β-cyclodextrin
Aggregation
Conductivity
Solid-state analysis

ABSTRACT

A complex of sulfamethoxazole (SMX) and hydroxypropyl-β-cyclodextrin (HP-β-CD) was developed and characterized in order to investigate their interactions in aqueous solution and the solid state. The SMX solubility was significantly increased upon complexation with HP-β-CD, with the solubility isotherm being an A_N type due to the presence of aggregates and the stability constant calculated for a 1:1 complex being $302 \pm 3\ M^{-1}$. Fourier-transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) experiments were used to compare the freeze-dried system with a physical mixture, and demonstrated the complex formation in the solid state. The differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) showed that the thermal stability of SMX was enhanced in the presence of HP-β-CD.

1. Introduction

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides composed by 6 (α-CD), 7 (β-CD) or 8 (γ-CD) D-(+)-glucopyranose units arranged in a truncated cone-shaped structure [1]. Hydroxypropyl-β-cyclodextrin (HP-β-CD) (Fig. 1), derivative of β-CD, has attracted a growing interest due to its improved complexing ability, high solubility and low toxicity [2,3]. Different molecules can penetrate into the relatively hydrophobic cavity and form non-covalent inclusion complexes modifying their physical, chemical and biological properties [4,5]. CDs are used to improve the solubility and dissolution of poorly soluble drugs in water and provide a way to increase their stability and bioavailability. Recently it has been observed that non-inclusion complexes can also participate in the solubilization of poorly soluble drugs by CDs. In aqueous solutions, CDs and their complexes show a tendency to self-associate to form aggregates with solubilizing properties. Also, the formation of CD complexes can increase the tendency of CDs to form aggregates and can lead to the formation of micellar-type CD aggregates capable of solubilizing poorly soluble compounds [6–8].

Sulfonamides are synthetic agents used in human and veterinary therapy for the prevention and treatment of infections [9]. However, their poor aqueous solubility has hindered their application in the therapy as pharmaceutical formulations. In previous studies, we demonstrated that the CD:sulfonamide complexes improved their solubility in water compared to that of the free drugs [10–12]. In addition, the sulfonamides exhibit interesting solid state properties, among which is the ability to exist in two or more polymorphic forms through the propensity for hydrogen bonding due to the presence of various hydrogen bond donors and acceptors [13]. Sulfamethoxazole (SMX) (Fig. 1) is a sulfonamide agent frequently used in human medicine to treat bronchitis and urinary tract infections. It is widely used in combination with trimethoprim, since this mixture possesses synergistic antibacterial effects [14]. SMX is sparingly soluble in water, and is known to exist in the three polymorphic forms, I, II and III (hemihydrates) [15].

Studies involving the complexation of SMX with β-CD have been reported [16–18]. The formation of a 1:1 complex with HP-β-CD at various pH values was able to improve the aqueous solubility of the drug in trimethoprim/sulfamethoxazole parenteral solutions but could not prevent its precipitation [19]. The chemical stability under oxidation stress of SMX in co-trimoxazole (a 5:1 combination of SMX with trimethoprim) aqueous buffer solutions was increased using HP-β-CD [20]. In addition, we previously developed a method for the simultaneous quantification of trimethoprime and SMX in mixtures using HP-β-CD solutions [21]. However, no reports about the characterization of the new systems SMX:HP-β-CD in solution or in solid state have been published.

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doi:10.1016/j.jpba.2012.01.011

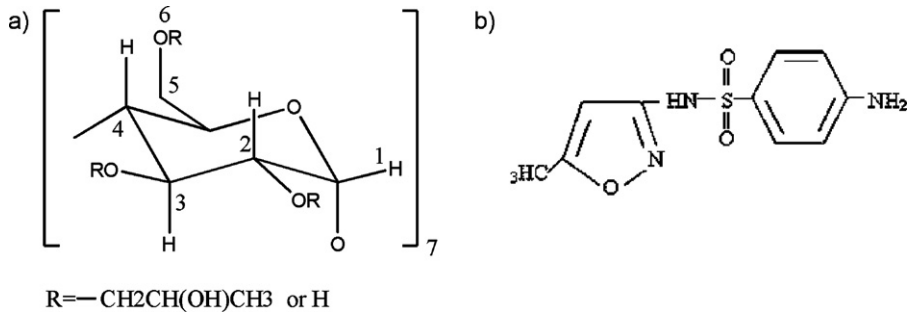

Fig. 1. Chemical structure of (a) HP-β-CD and (b) SMX.

Taking into account these previous studies, in the present work we prepared and characterized a complex between the commercial SMX active ingredient and HP-β-CD.

2. Materials and methods

2.1. Chemicals and reagents

All the experiments were performed with analytical grade chemicals and solvents. HP-β-CD, with a degree of substitution of 0.63, was kindly supplied by Ferromet agent of Roquette (France). Millipore Milli Q Water Purification System was used to generate the water used in these studies.

2.2. Phase solubility studies

The solubility measurements were performed according to the method of Higuchi and Connors [22] in aqueous solutions containing different concentrations of HP-β-CD, ranging from 14.3 mM to 143.2 mM.

2.3. Conductivity measurements

The conductance measurements were taken in HP-β-CD solutions, in the range 1.65–161.55 mg/ml, in the presence of a constant SMX concentration throughout the experiment. The critical concentration for the aggregate formation was determined by measuring the specific conductivity change as a function of concentration, using a Malvern Zetasizer 3000 (Malvern Instruments Inc., London, UK). All measurements were recorded at 25 °C, with the values shown being the mean of 20 conductance measurements.

2.4. Solid sample preparation

The preparation of a solid complex SMX:HP-β-CD with a 1:1 molar ratio was carried out using the freeze-dry method [23]. Physical mixtures were prepared by mixing the SMX and HP-β-CD powders or the corresponding freeze-dried components, with a 1:1 molar ratio uniformly in a mortar.

2.5. Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR spectra (potassium bromide disks) were recorded on a Nicolet 5 SXC FT-IR Spectrophotometer (Madison, WI, USA).

2.6. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

The DSC curves of the samples were produced using a DSC TA 2920, and the TGA curves were recorded on a TG TA 2920. The samples were placed in aluminum hermetic pans, with the experiments being carried out under a nitrogen gas flow, at a heating rate of 10 °C/min, and over a temperature range of 25–400 °C.

2.7. Scanning electron microscopy (SEM)

Microscopic morphological structures of the solid samples were investigated and photographed using a scanning electron microscope LEO Model EVO 40XVP. To improve the conductivity, samples were gold-coated under vacuum employing a sputter coater PELCO Model 3.

3. Results and discussion

3.1. Phase solubility analysis

The phase solubility diagram of SMX and HP-β-CD, obtained by plotting the changes in guest solubility as a function of HP-β-CD concentration (Fig. 2) can be classified as an A_N type, with the negative deviation from linearity at higher concentrations. This curve form may originate from both an alteration in the effective nature of the solvent in the presence of large concentrations of HP-β-CD and a self-association of HP-β-CD at higher concentrations [22,24].

The initial ascending portion of the diagram had a slope less than 1, which indicated that a soluble inclusion complex with a 1:1 molar ratio was formed at low HP-β-CD concentrations. The apparent stability constant (K_C) value of $302 \pm 3\ M^{-1}$ was estimated from the slope of the initial linear portion of the diagram and the

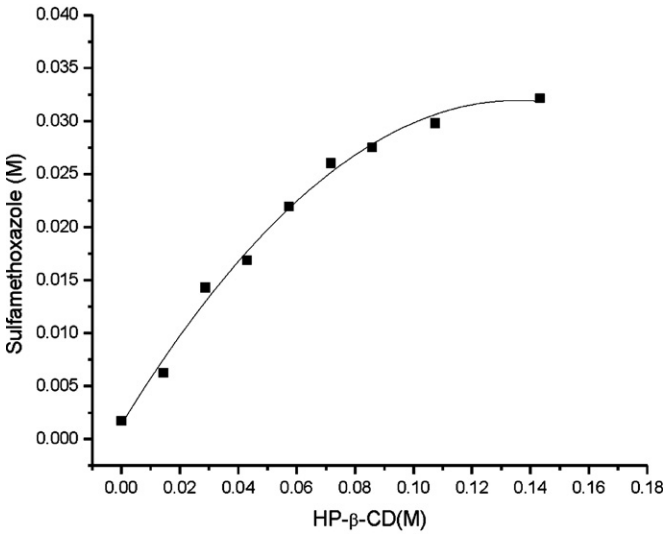

Fig. 2. Effect of HP-β-CD on the solubility of SMX in an aqueous solution at 25.0 °C.

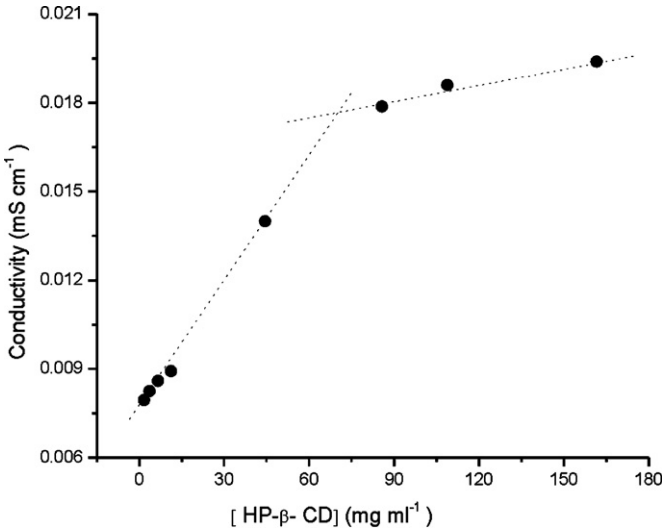

Fig. 3. Conductivity measurements as a function of HP-β-CD concentration for aqueous solutions of SMX:HP-β-CD at 25 °C.

solubility of SMX in water (S_0), according to the following equation:

$$K_c = \frac{\text{Slope}}{S_0(1 - \text{Slope})}$$

SMX (pK_a 5.6) mainly existed in its unionized form in aqueous solutions of pH 4.7, which was the reason why the interaction with HP-β-CD occurred efficiently, and consequently, an important increase in solubility was observed as a function of HP-β-CD concentration. The aqueous solubility of SMX in water was 0.4 mg/ml. This increased approximately 10.5 times (4.6 mg/ml) in 57.3 mM HP-β-CD solution, while 143.2 mM HP-β-CD caused a 20.2-fold rise in the drug solubility (8.7 mg/ml). Therefore, these results showed the large solubilizing effect of HP-β-CD. It seems that our results are in good agreement with those obtained previously by Loftsson et al. [25]. They have described the phase solubility studies of SMX with HP-β-CD and reporting K_C value of 370 M^{-1}.

3.2. Aggregation behavior of SMX:HP-β-CD complex

The formation of aggregates in aqueous solution and the critical concentration for the SMX:HP-β-CD complex was studied, by conductivity, to interpret the phase solubility diagrams observed. In Fig. 3, a plot of conductivity is shown as a function of the HP-β-CD concentration for aqueous solutions of SMX:HP-β-CD at 25 °C. The results revealed a significant increase in the conductance of the solutions caused by SMX:HP-β-CD system compared to pure HP-β-CD (with this latter value has already been determined in our previous work, [26]), demonstrating that the complex SMX:HP-β-CD was more effective as charge carrier than the free macromolecule in aqueous solution, because the conductivity of water is dependent on the concentration of the conducting species present. A break point on the slope of the plot demonstrates that at high concentrations of HP-β-CD was favored the formation of aggregates. The critical concentration, defined as the point corresponding to the maximum change in gradient of a physical property of solution against ligand concentration, was determined from the intersection point of the linear segments, corresponding to the monomeric and aggregate forms, and the best correlation coefficient was chosen. A value of was 70.99 mg/ml (about 50.8 mM) was determined, and its similarity with the value of 69.3 mg/ml reported for free HP-β-CD [26] suggests that the complex formation did not affect the balance of the intermolecular forces that held the HP-β-CD molecules together in the aggregates. These experimental results indicated that the self-association between SMX:HP-β-CD complexes as well as that between free HP-β-CD molecules and the complexes may explain why the observed solubilization phenomena had a negative deviation from linearity at higher host concentrations (Fig. 2).

Taken together, the results suggest that the increase in the solubility of SMX, at low concentrations of HP-β-CD, was the result of the formation of an inclusion complex. However, at ligand concentrations above the critical concentration, water-soluble aggregates were formed, which possess the capacity to solubilize SMX by forming non-inclusion complexes [6].

3.3. Solid-state studies

The pure materials, the freeze-dried system SMX:HP-β-CD and the corresponding physical mixture were examined.

3.3.1. Fourier-transform infrared (FT-IR) spectroscopy

In order to identify the polymorph form of SMX in the commercial sample, the characteristic FT-IR bands in the region 3500–3100 cm^{-1} were examined. As shown in Fig. 4a, bands were found at 3467.3 and 3375.7 cm^{-1} (NH_2), 3300 cm^{-1} (NH) and 3143.7 cm^{-1} (CH), which are in agreement with the FT-IR spectrum corresponding to the SMX form I reported by Takasuka et al. [15]. Also, other bands were located at 1620.9 cm^{-1} (a combination of NH_2 and isoxazole ring CN), 1596.9 and 1504 cm^{-1} (phenyl ring C=C), 1473.4 and 1380.8 cm^{-1} (isoxazole ring), 1307.5 and 1145.8 cm^{-1} (SO_2), 926.2 cm^{-1} (SN), 884.3 cm^{-1} (isoxazole ring CH) and 829.4 cm^{-1} (benzene ring CH). SMX showed a large variation in the hydrogen-bond pattern between polymorphs, and the polymorphic conversion resulted in a change in the hydrogen-bond pattern of the amido proton. It is also noteworthy that Form I appeared to have the weakest hydrogen bonds [13,27].

The FT-IR spectrum of the freeze-dried system (Fig. 4b) did not reveal any new bands, although if SMX and HP-β-CD form a solid inclusion complex, the non-covalent interactions between them such as hydrophobic interactions, Van der Waals interactions and hydrogen bonds lower the energy of the included part of SMX, thereby reducing the intensity of the corresponding absorption bands. Based on these considerations, the differences between the spectra of SMX, freeze-dried system and the physical mixture were analyzed to obtain supporting evidence of complexation. We can see an absence of SMX bands in the region 3700–3000 cm^{-1}, demonstrating that the aniline NH_2, and the sulphonamide NH and the CH of the isoxazole ring of the drug were involved in the interaction process. In addition, the band assigned to the combination of the NH_2 group and the CN isoxazole ring was broader and shifted to 1619.3 cm^{-1} with intensity reduction, indicating a host restriction of vibration within the cavity of the HP-β-CD. The isoxazole ring bands became broadened and shifted to lower frequencies of 1465.3 and 1376.2 cm^{-1}, respectively, with the bands assigned to SO_2 group being broader and shifted to higher frequencies of 1325.9 and 1154.9 cm^{-1} respectively, suggesting that the sulphonamide group of SMX interacts with the groups of the host during inclusion complexation. The characteristic band assigned to CH of the isoxazole ring disappeared, and the band of the aromatic protons was broader and shifted to a higher frequency of 838.6 cm^{-1}. According to these observations, there seemed to be formation of an inclusion complex between SMX and HP-β-CD in the solid state. In contrast, the FT-IR spectra of the physical mixture (Fig. 4c) there were no changes since they were derived from the superposition of the spectra of the single components, suggesting the absence of interactions.

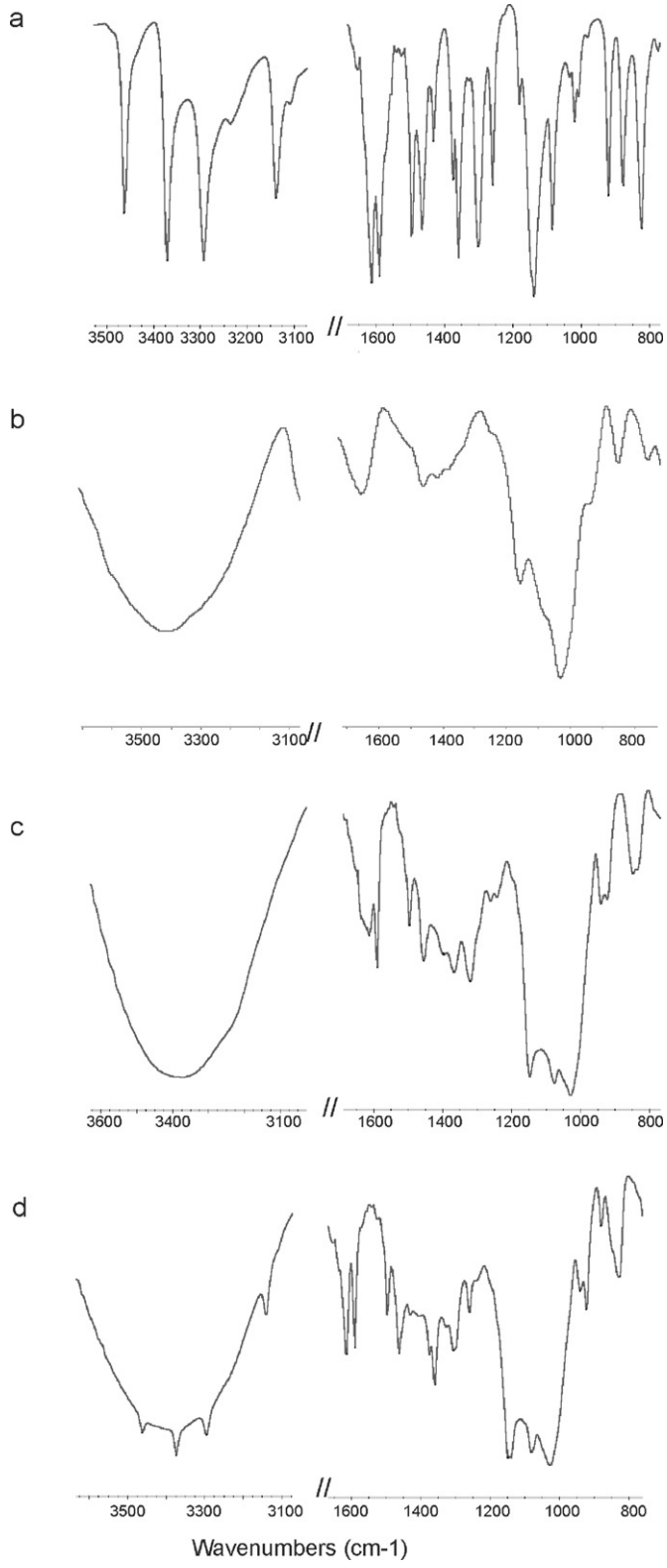

Fig. 4. FT-IR spectra of: (a) SMX, (b) HP-β-CD, (c) SMX:HP-β-CD freeze-dried, (d) SMX:HP-β-CD physical mixture.

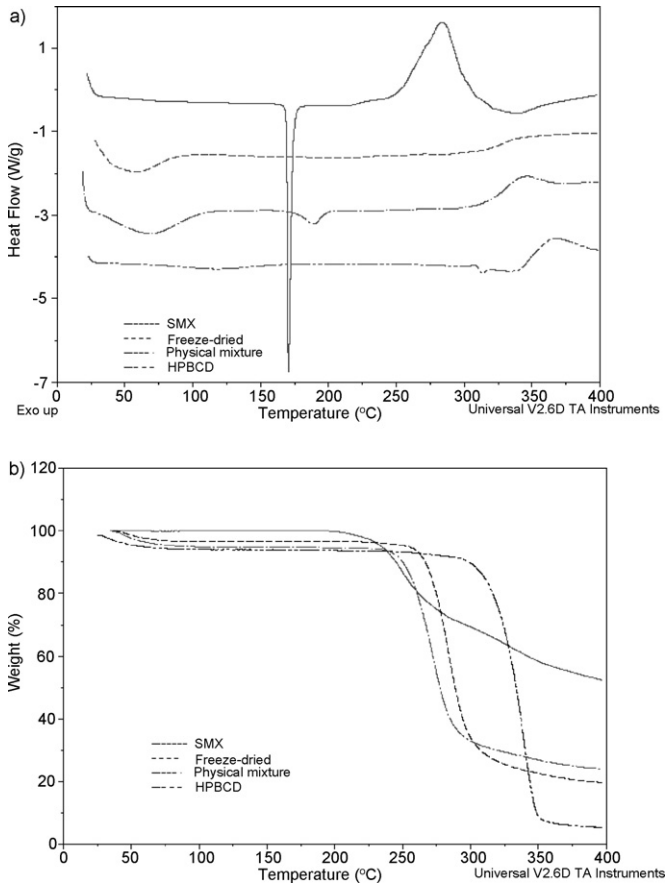

Fig. 5. (a) DSC curves of SMX (solid line), SMX:HP-β-CD freeze-dried (short dash) SMX:HP-β-CD physical mixture (dash dot line) and HP-β-CD (broken double dash line). (b) TGA curves of of SMX (solid line), SMX:HP-β-CD freeze-dried (short dash) SMX:HP-β-CD physical mixture (dash dot line) and HP-β-CD (broken double dash line).

3.3.2. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

The DSC and TGA profiles in Fig. 5 SMX showed a sharp endothermic peak at 170.5 °C due to drug melting. The exothermic peak at 283.8 °C ascribed to the oxidation of evolved products [28] was associated with a loss in the mass fraction of 30%, between 200 and 300 °C in the TGA curve. HP-β-CD exhibited a typical broad endothermic peak between 50 and 175 °C, which resulted from a dehydration process (6.5% mass loss) that corresponded to a loss of about 4.5 water molecules per HP-β-CD molecule. Also, at temperature higher than 300 °C, the CD decomposition began to appear.

The DCS curve of the binary freeze-dried system showed complete disappearance of the SMX melting peak, indicating molecular encapsulation of the drug inside the CD cavity. Interestingly, the TGA curve for the system showed that the dehydration stage contained only 3.3% of water compared to the 5.0% present in the physical mixture, which indicates that most of the water molecules in the HP-β-CD cavity were replaced by SMX during the inclusion process. In addition, in the physical mixture, the characteristic events observed for the individual curves of SMX and HP-β-CD were also found.

Finally, the TGA curves showed mass losses indicating the following order of thermal stability: freeze-dried system > physical mixture > SMX, with the drug being thermally stable up to 243, 234 and 200 °C, respectively. Furthermore, the mass loss observed for the freeze-dried system and physical mixture occurred through a fast process whereas the mass loss for SMX began as a slow process. The considerably higher temperature needed for degrading the freeze-dried system compared with the pure drug, suggests a substantial enhancement of the thermal stability of SMX in solid state through the formation of the inclusion complex.

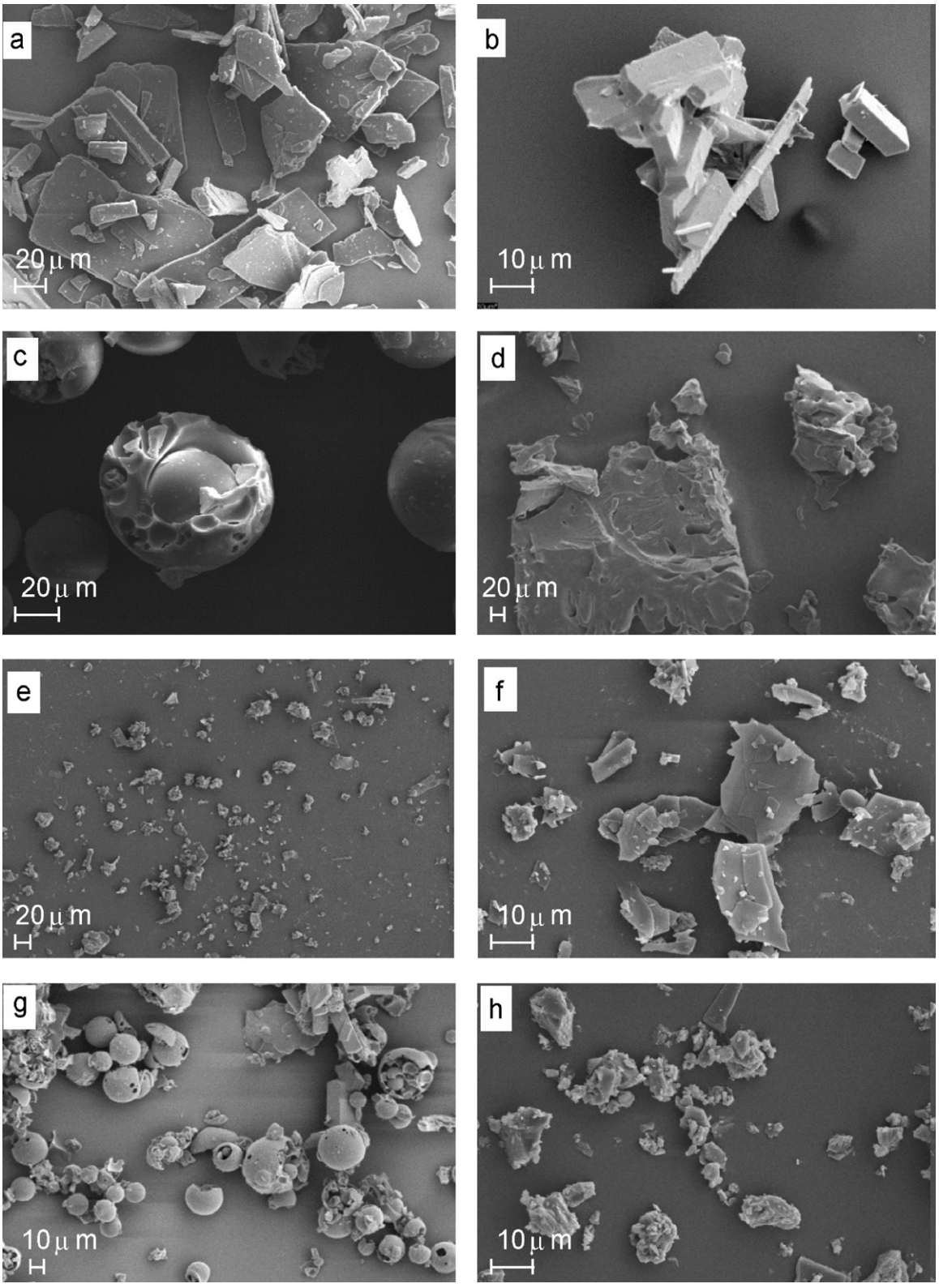

Fig. 6. Scanning electron microphotographs of: SMX (a) powder and (b) freeze-dried, HP-β-CD (c) powder and (d) freeze-dried; SMX:HP-β-CD freeze-dried (e and f); SMX:HP-β-CD physical mixture prepared with (g) pure powders and (h) freeze-dried components.

3.3.3. Scanning electron microscopy (SEM)

Supporting evidence for complexation of SMX with HP-β-CD was also obtained from SEM microphotographs (Fig. 6). Samples of SMX and HP-β-CD freeze-dried powders were included in the assays to evaluate the effect of the lyophilization process on the morphology of the solids used to prepare the solid systems. SMX showed a plate like habit crystal, compact structures can be observed with irregular shapes and different sizes,

characterized by a smooth surface. Their morphology was not affected by the lyophilization process. On the other hand, the HP-β-CD microphotographs reveal hollow spherical particles with a broad size distribution (10–50 μm). In addition, large particles were detected containing smaller particles, which may be assumed to be an aggregation of HP-β-CD in the solid powder. However, the HP-β-CD freeze-dried appeared in the form of irregular particles in which the original morphology disappeared.

The images of the freeze-dried system showed a less crystalline structure. By image magnification, it was possible to visualize laminated structures of irregular size and shape, with smooth surfaces and a fragile aspect. By contrast, both physical mixtures, prepared with pure powders or freeze-dried components, showed the characteristic crystals of SMX mixed with particles of HP-β-CD, confirming the presence of crystalline drug and revealing the absence of interaction in the solid system.

Considering the drastic change in the shape of particles obtained by the freeze-dried method in which the original morphology of both components disappeared, together with their differences with the systems obtained by physical mixing, reveals a solid-state interaction and constitutes clear evidence of a new solid phase formation resulting from the molecular complexation of SMX in the cavity of HP-β-CD.

4. Conclusions

This study clearly evidence that the complexation with HP-β-CD is an effective strategy to increase the solubility of SMX form I. Furthermore, the results show that the ligand is capable of producing binary complexes in solution and solid state.

Acknowledgments

Financial support from Fondo para la Investigación Científica y Tecnológica (FONCYT) Préstamo BID 1728/OC-AR PICT 1376, the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT-UNC), are greatly acknowledged. We also thank Ferromet S.A. (agent of Roquette in Argentina) for their donation of hydroxypropyl-β-cyclodextrin. Finally, we would like to thank Dr. Paul Hobson, native speaker, for revision of the manuscript.

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