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ORIGINAL ARTICLE

Water soluble complexes of methyl β-cyclodextrin and sulconazole nitrate

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Abstract Complexation between methyl β-cyclodextrin (Me βCD) and sulconazole nitrate (SULC) was realized both under freeze drying and ultrasonication conditions. The process was carried out in solution and in solid state. In solution, the complexation was evaluated using solubility studies, nuclear magnetic resonance spectroscopy (^{1}H-NMR) and UV–Vis absorption studies. In the solid state, differential scanning calorimetry (DSC), and X-ray diffraction studies were used. Solubility studies indicate the existence of inclusion complexes between SULC and Me βCD. ^{1}H-NMR data showed that the inclusion complexes have different structures, according with the method we used for synthesis: for the freeze dried method the complex is obtained by complexation of dichlorobenzene ring of SULC into inner cavity of CD while for ultrasonication method the complex is obtained by complexation of imidazole graph of SULC into the CD molecule. DSC and X-ray studies bring supplementary information concerning the formation of complex Me βCD–SULC. As a result of the inclusion process into Me βCD, the solubility of SULC increase significant, being 10 times more comparative with the pure drug. We anticipate that these modifications will have a significant impact on the biological effects of the drug, making the SULC–Me βCD complex an appropriate candidate for a new drug delivery system.

Keywords Cyclodextrin · Sulconazole · Inclusion complex · Ultrasonication · Freeze drying

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Introduction

Dermatophytoses are of worldwide distribution and constitute a serious health problem in many parts of the world [1]. Dermatophytes are keratinophilic fungi associated with the infections of the skin, hair and nails. Their incidence and their epidemiological characteristics depend on social, geographical and environmental factors, and may change with the passage of time [2–4]. Factors contributing to the rise include the increasing migration of the people, poor living standards and the widespread use of antibiotics, corticosteroids and antineoplastic drugs [5]. That is why the treatment of wide spread dermatophytoses has become difficult in the last decades [6, 7]. In this context the interest in developing new antifungal agents or reducing the dosage, with the resistance reducing has continuously increased [8].

Sulconazole nitrate {(+)-1-[2,4-dichloro-*b*-[(*p*-chlorobenzyl)-thio]-phenethyl]imidazole mononitrate} is an imidazole derivative with significant in vitro antifungal, antibacterial and antiyeast activity [9, 10]. It possesses a broad spectrum of activity in vitro against dermatophytes, yeasts and some Gram-positive bacteria and it is very efficient in the treatment of superficial fungal infections of the skin. The main problem of SULC is their very low water solubility, associated with the need for higher dosage that induces severe side effects (severe allergic reactions) and resistance phenomena [11].

Complexation of SULC with Me βCD, using ultrasonication and freeze drying methods, offers the possibility to improve the aqueous solubility of sulconazole without modification of its original structure. The synthesis and characterization of β-cyclodextrin-sulconazole nitrate and hydroxypropyl β-cyclodextrin–sulconazole nitrate complexes have already been reported [12]. The emphasis of this work is to synthesize methyl β-cyclodextrin–sulconazole nitrate (Me βCD–SULC) inclusion complexes and, to study

its properties, in order to test an improved homogeneous delivery system of sulconazole, meant for increasing its bioavailability.

Ultrasonication and freeze drying methods are environmentally friendly methods useful for synthesizing soluble complexes with cyclodextrins, with various applications in low soluble drugs topic areas. If freeze drying is a common method for inclusion complexes synthesis, ultrasonication is a relative new method that assures a fine mixing of the components in liquid state, an essential step in the precipitation, with a high reaction velocity [13].

Materials and methods

Materials

Sulconazole nitrate (SULC) (Fluka) and Me βCD (Aldrich) were used as received. Double distilled water was used throughout the study.

Methods

Solubility studies

Solubility studies were carried out according to the Higuchi and Connors method [14]. Me βCD solutions of different concentrations (0.33–26.4 $\times$ 10^{-4} M) were added to a supersaturated solution of SULC and shaken at room temperature (22 $\pm$ 1 °C) for 24 h. After reaching equilibrium, the solutions were filtered. The attendance of the equilibrium state can be evidenced by the final aspect of the prepared solutions. Immediately after SULC adding to the CD solution, it is turbid, and after continuous stirring becomes initially semitransparent and in the end completely transparent. The absorbance of solutions containing different mole fraction of the drug and Me βCD was measured by UV at 196 nm and the concentration of SULC in the each solution was determined with reference to a suitably constructed standard curve of the dependence of SULC concentration in distilled water to the absorption of the solution at 196 nm, after 1/100 dilution (Fig. 1).

The apparent stability constant was calculated from the phase solubility diagram using the Eq. 1 [6].

$$K1{:}1 = \text{slope}/\text{So}\ (1 - \text{slope}) \quad (1)$$

where So is the solubility of SULC in the absence of Me βCD and slope is the slope of the experimental phase solubility diagram for Me βCD–SULC system.

Preparation of the solid complex

The inclusion complexes (C_s) were prepared by freeze drying and ultrasonication methods. For freeze drying, an aqueous solution containing SULC and Me βCD in a 1:1 molar ratio, was frozen by immersion in liquid nitrogen and freeze-dried in a Martin Christ, ALPHA 1-2LD Freeze-Dryer. The aqueous solution was obtained by dissolving 4.34 $\times$ 10^{-4} mol SULC and 4.34 $\times$ 10^{-4} mol cyclodextrin in 25 mL distilled water, and stirred at room temperature for 48 h. For ultrasonication an aqueous solution containing the two components in the same molar ration was ultrasoned for 15 min, at 100% power of a HD 3200 Sonoplus reactor, with 200 W power. The liquid mixture obtained was maintained at 4 °C for 24 h, and the white, granular and glass adherent precipitate formed was isolated by centrifugation. The aqueous solution was obtained by 5 $\times$ 10^{-4} mol Me βCD and 5 $\times$ 10^{-4} mol SULC, dissolved in 50 mL distilled water. We used different concentrations, because we intended to obtain a higher quantity of ultrasoned complex, and we recalculated the initial recipe for higher quantities of CD and SULC.

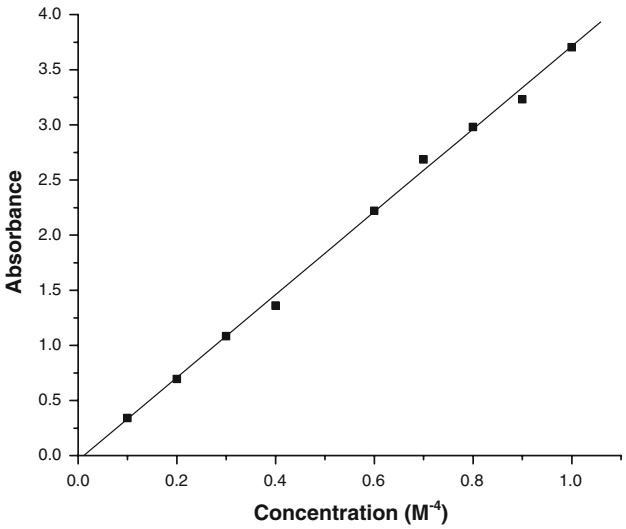

Fig. 1 SULC etalon curve in distilled water

Preparation of the physical mixture (Ph.M) was performed by mixing the powders in a 1:1 molar ratio in an agate mortar.

Complex characterization

UV measurements were performed on an Analytik Jena Specord 200 Spectrophotometer, using pure and complexed SULC water solutions with the same concentration 0.6 $\times$ 10^{-4} M SULC and 1.12 $\times$ 10^{-1} M complex (containing 0.6 $\times$ 10^{-4} M SULC).

Differential scanning calorimetry (DSC) DSC data were obtained using a Perkin Elmer-Diamond device. Each sample (2−6 mg) was exactly weighted in an aluminum pan and was heated at a rate of 10 °C/min from 30 to 330 °C, under nitrogen gas flow.

Nuclear magnetic resonance (^{1}H-NMR) Nuclear magnetic resonance studies were performed in tetrahydrofurane on a Bruker Advance DRX 400 device.

X-ray diffraction (XRD) XRD patterns were obtained on a Bruker AXS D8 Advance RX diffractometer.

Results and discussion

Phase solubility method

As we previously stated, SULC has a very low water solubility, 2.17×10^{-4} M at 20 °C [4]. According with the literature data, a feasible method to increase the water solubility of drugs is their complexation with CD [6]. This is why, we decide to study the Me βCD–SULC inclusion complexes. In Fig. 2 it is presented the solubility curves obtained for SULC as a function of CD concentration.

As it can be seen from Fig. 1, SULC water solubility presents a linear increase for the studied systems, SULC solubility increasing more than 10 times when it is solubilized in a CD solution having 26.4×10^{-4} M concentration. The obtained curve can be classified as A_L type (linear positive isotherm) [14]. Supposing a 1:1 stoichiometric ratio between components, the apparent solubility constant, K1:1, was calculated from the slope of the diagram, according to Eq. 1 [14]:

$$K1{:}1 = \frac{\text{Slope}}{\text{So} \cdot (1 - \text{Slope})} \quad (1)$$

where So is the solubility of SULC in the absence of CDs and slope is the slope of the experimental curves, and it was equal to 8,775 M^{-1}; this prove a great solubility of SULC in Me βCD.

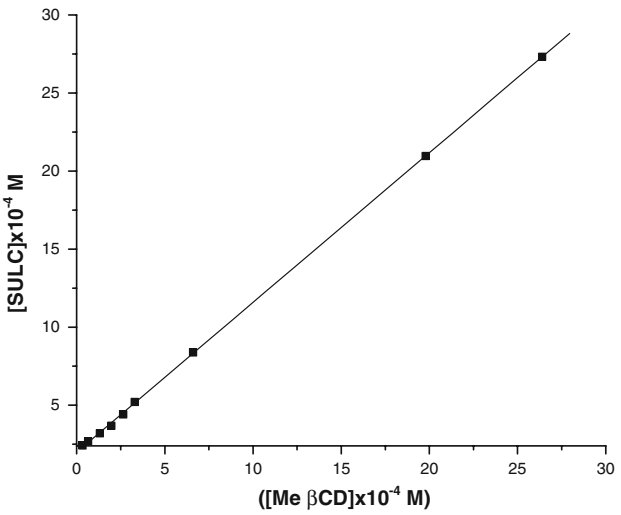

Fig. 2 Phase solubility diagram for the Me βCD–SULC system

Differential scanning calorimetry

DSC reveals useful information on solid-state interactions between drug and cyclodextrin in the inclusion complexes. The DSC thermograms of pure components, physical mixture and of the Me βCD–SULC inclusion complexes (obtained by both methods, freeze drying and ultrasonication) are presented in Fig. 3. A typical DSC curve for a

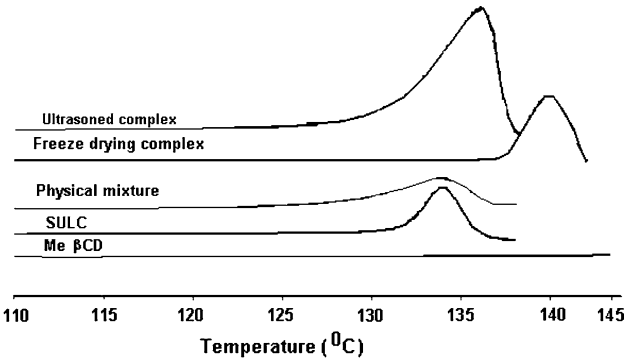

Fig. 3 DSC thermograms for Me βCD, SULC, physical mixture and complex obtained by freeze drying and ultrasonication

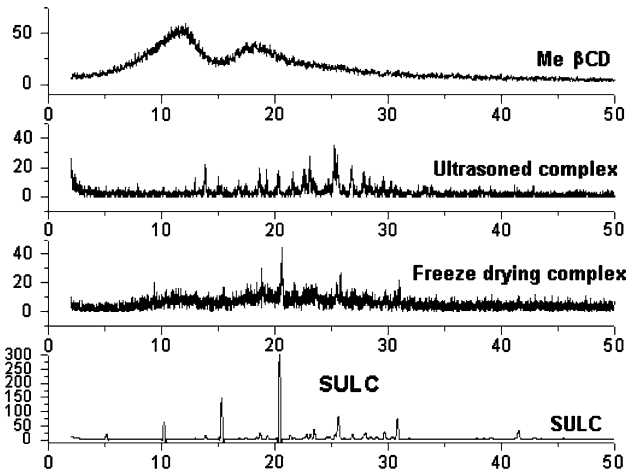

Fig. 4 X-ray Diffraction patterns of: SULC, Me β-CD, freeze dried and ultrasoned complex

Table 1 Interplanar distances calculated from X-ray pattern

Sample code	*d* (Å)
Me β-CD	7.64, 4.99.
SULC	8.56, 4.34, 2.91, 2.40, 2.20, 2.11, 1.77, 1.70, 1.63, 1.55, 1.50, 1.16.
Freeze dried SULC–Me β-CD	9.43, 7.92, 6.86, 5.72, 4.99, 4.67, 4.29, 4.08, 3.72, 3.48, 2.86.
Ultrasoned SULC–Me β-CD	11.04, 6.86, 6.27, 5.89, 5.304, 4.74, 4.56, 4.35, 4.11, 3.84, 3.505, 3.32, 3.18, 3.13, 3.01, 2.92.

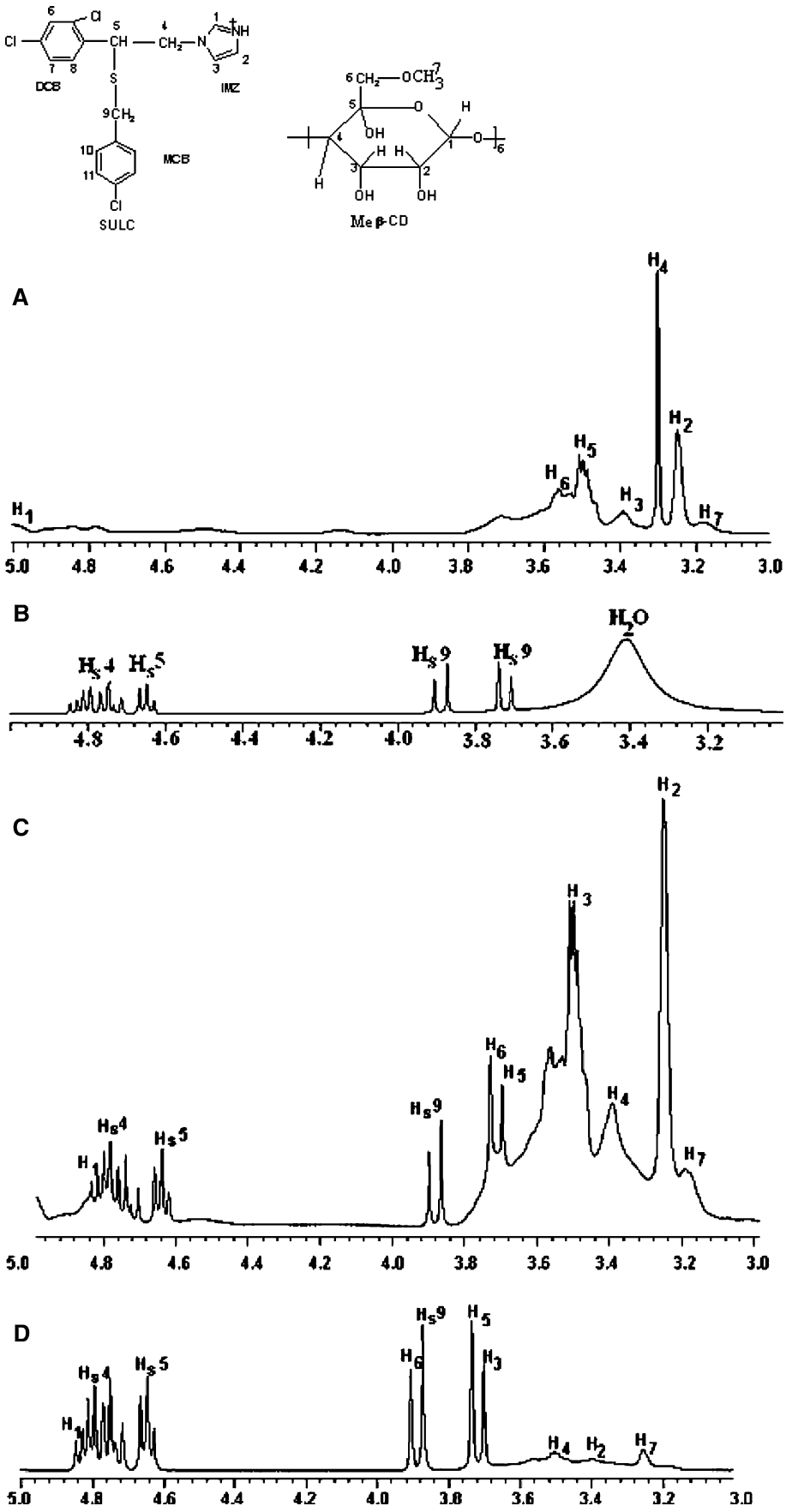

Fig. 5 ^{1}H-NMR in 3.0–5.0 ppm for Me βCD (**a**), SULC (**b**), freeze dried complex (**c**), ultrasoned complex (**d**)

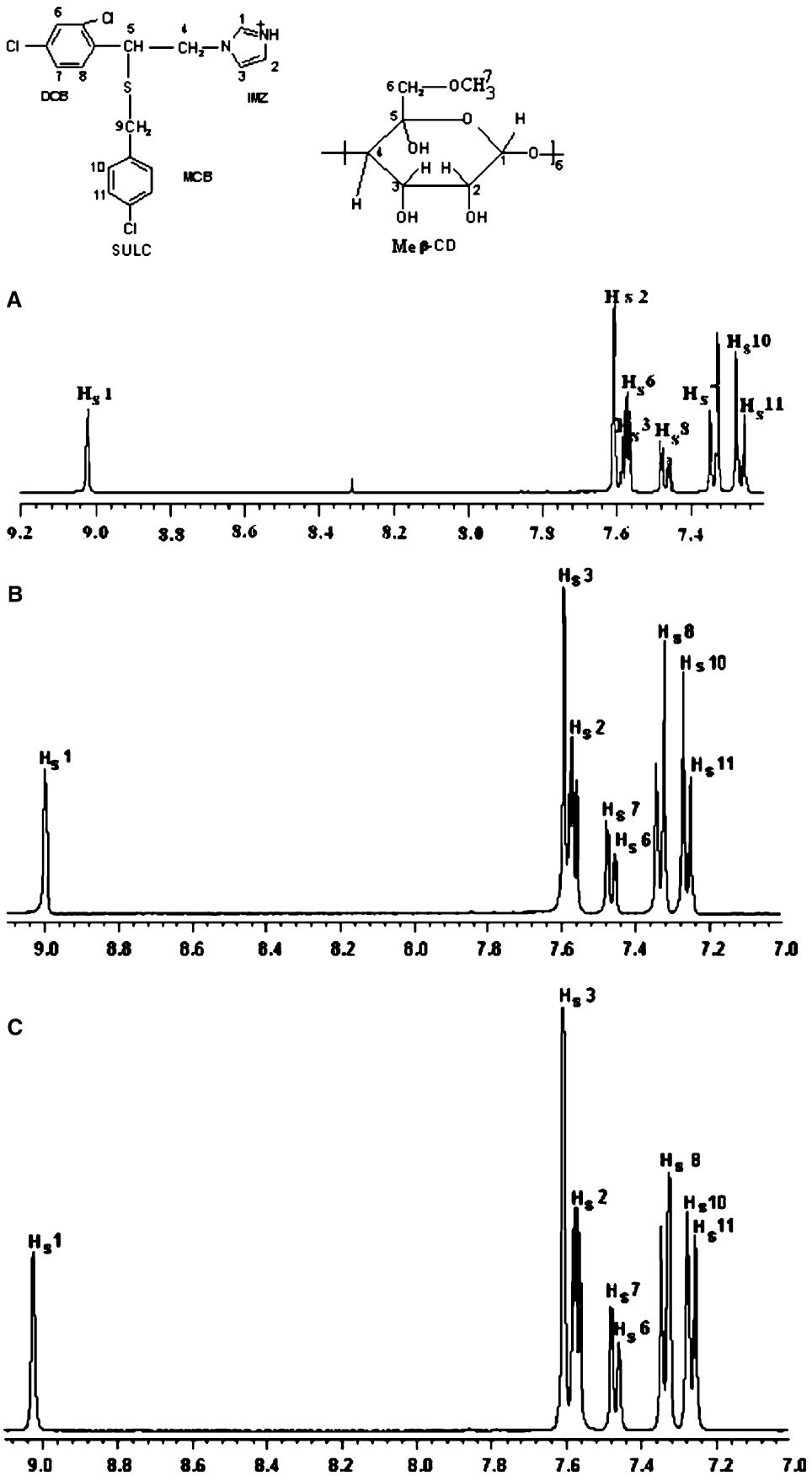

Fig. 6 ^{1}H-NMR in 7.0–9.1 ppm for SULC (**a**), freeze dried complex (**b**), ultrasoned complex (**c**)

crystalline anhydrous substance, with a sharp fusion endotherm (T peak = 135 °C), was obtained for SULC and for physical mixture. For the inclusion complexes, which are solid, the characteristic thermal peak is shifted to 136.5 °C, in the case of ultrasoned complex, and to 140 °C, for the freeze dried one; these results argue in the favor of inclusion. The differences between the shifts for the two complexes are determined by the inclusion site position (at the dichlorobenzene ring for the freeze dried complex and imidazole ring for the ultrasoned complex).

Powder X-ray diffraction

X-ray powder, diffraction patterns of pure SULC and Me βCD, and corresponding solid inclusion complexes with CD, are shown in Fig. 4. In the X-ray diffractograms of SULC powder, sharp diffraction peaks are present, indicating its crystalline state. Me βCD pure presents only two large diffraction peaks, due to its reduced crystalline state. By contrast the X-ray diffraction pattern of freeze dried Me βCD–SULC system was characterized by sharp diffraction peaks, in which it is no longer possible to distinguish the characteristic crystallinity peaks of pure drug or of Me βCD. The ultrasoned Me βCD–SULC system presented sharper diffraction peaks than the initial components. The X-ray diffraction results indicate that both complexes exist in the crystalline state.

The interplanar distances for the investigated samples are presented in Table 1.

The higher crystalline state of the two types of complexes compared with the pure compounds, suggest that the inclusion process of SULC into Me βCD took place.

^{1}H-NMR spectroscopy

H^1-NMR studies reveal useful information on the nature of the interactions between drug and cyclodextrin in inclusion complexes.

Analyzing the chemical structure of SULC, we may presume the formation of three type of inclusion complexes, according on which part of SULC interact with Me βCD (Fig. 4): the dichlorobenzene group (DCB), chlorobenzene–CH_2–S– group (MCB) and protonated imidazole group (IMZ); the resulting complexes are respectively notated DCB–Me βCD, MCB–Me βCD, and IMZ Me βCD.

The ^{1}H-NMR spectra reveal clear differences for the freeze dried and ultrasoned complex compared to that of individual components (Figs. 5 and 6; Tables 2 and 3).

In the freeze dried complex, the protons H_s7, H_s8 and H_s9 are substantially shifted compared to pure SULC. The chemical shift differences between these protons from SULC and complex are: Hs8 (dδ (ppm) = −0.16) > Hs9 (0.143–0.168) > Hs7 (0.105) > Hs6 (−0.091) > Hs2

Table 2 Chemical shift data (δ in ppm) of H–C protons in SULC and Me β CD in free-state and in the freeze dried complex state

Code	δ_{free}	$\delta_{complex}$	$d\delta$* (ppm)
SULC			
Hs1	9.021	8.998	−0.023
Hs2	7.604	7.570	−0.034
Hs3	7.609	7.591	−0.018
Hs4	4.740	4.738–4.749	−0.002–0.009
Hs5	4.648	4.637–4.657	−0.011–0.009
Hs6	7.57	7.479	−0.091
Hs7	7.347	7.452	0.105
Hs8	7.482	7.322	−0.16
Hs9	3.7215	3.865–3.890	0.143–0.168
Hs10	7.277	7.271	−0.006
Hs11	7.256	7.250	−0.006
Me-β-CD			
H1	4.793–4.827	4.833	0.04–0.006
H2	3.395	3.250	−0.145
H3	3.701	3.489–3.498	−0.212:−0.203
H4	3.511	3.391	−0.12
H5	3.734	3.504	−0.23
H6	3.905	3.696–3.729	−0.209:−0.176
H_7	3.253	3.171	−0.082

Table 3 Chemical shift data (δ in ppm) of H–C protons in free SULC and Me β CD in the ultrasoned complex

Code	δ_{free}	$\delta_{complex}$	$d\delta$* (ppm)
SULC			
Hs1	9.021	9.024	0.003
Hs2	7.604	7.578	−0.026
Hs3	7.609	7.609	0
Hs4	4.740	4.714–4.748	−0.026–0.008
Hs5	4.648	4.626–4.664	−0.022–0.016
Hs6	7.57	7.563	−0.007
Hs7	7.347	7.347	0
Hs8	7.482	7.461	−0.021
Hs9	3.7215	3.872	0.1505
Hs10	7.277	7.276	−0.001
Hs11	7.256	7.255	−0.001
Me-β-CD			
H1	4.793–4.827	4.848–4.991	0.055–0.164
H2	3.395	3.247	−0.148
H3	3.701	3.463–3.487	–0.238–0.214
H4	3.511	3.300	–0.211
H5	3.734	3.497–3.507	–0.237–0.227
H6	3.905	3.821	–0.124
H7	3.253	3.173	–0.08

(−0.034) > Hs1 (−0.023) > Hs3 (−0.018) > Hs4, H_s5 (0.009) > and Hs10, Hs11 (∼−0.006). These are good evidences that in this case SULC penetrates the Me βCD cavity with the dichlorobenzene fragment, the DCB–Me βCD complex being formed (Fig. 4). Supplementary evidence in this respect, are furnished by analyzing the chemical shift of protons H_5, H_3, H_6, H_2 and H_4, from Me βCD in the pure compound and complex. Thus, H_5 (dδ (ppm) = −0.23) and H_3 (dδ (ppm) = −0.203) (located inside the cavity [10]), suffer a significant displacement, while for H_6, H_2, H_4 these modification are not so big, but still can be observed, which indicate the entrance of the drug molecule inside the CD cavity.

In the ultrasoned complex, the protons H_s8, H_s9, H_s10 and H_s11 are also shifted comparative with pure SULC. The chemical shift differences between these protons from SULC and complex are: H_s9 (dδ (ppm) = 0.15) > H_s2, H_s4 (−0.026) > H_s5 (−0.022) > H_s8 (−0.021) > H_s1 (0.003) > H_s10, H_s11 (−0.001). These are good evidences that in this case SULC penetrates the Me βCD cavity with the imidazole fragment, the IMZ Me βCD complex being formed (Fig. 4). Moreover, the signals of protons H_3, H_4, H_5, and H_6, from Me βCD in the pure compound and complex, are more significantly shifted to each other. Thus, H_3 (dδ (ppm) = −0.238), H_5 (dδ (ppm) = −0.237), H_4 (−0.211), H_2 (−0.148) and H_6 (−0.124), suffer a significant displacement, which indicates the entrance of the drug molecule inside the CD cavity.

UV–Vis spectroscopy

UV–Vis absorption spectra for the pure drug and the inclusion complexes have been analyzed in distilled water. As it can be seen from Fig. 7, UV–Vis spectra of inclusion complexes reveal the disappearance of the absorption band at 214–220 nm, and also the absorption band from 196 nm are decreasing strongly in intensity and it is more broadened. The disappearance of the band indicates that one part of the SULC molecule is covered by the cyclodextrin, due to drug inclusion inside it's cavity.

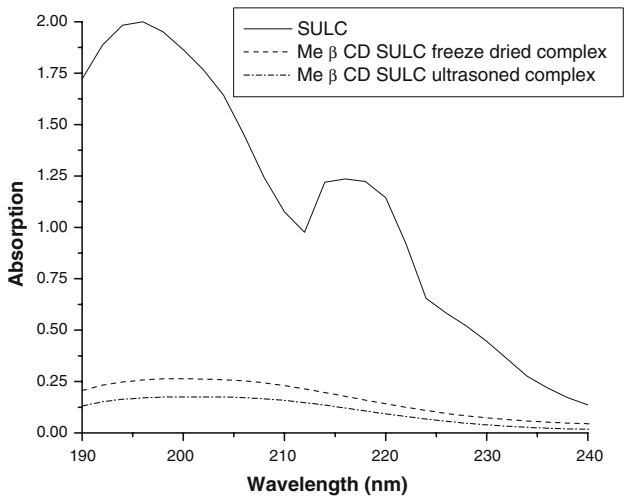

Fig. 7 UV–Vis spectra for pure SULC and freeze dried and ultrasoned complex

Conclusions

DSC, XRD, UV–Vis and ^{1}H-NMR spectroscopy have been used to study the inclusion complexes between SULC and Me βCD. Significant changes were detected in all characterization methods, indicating the formation of binary inclusion complexes. As a result of the inclusion process into Me βCD, the solubility of SULC increase significantly, being ten times higher than pure drug. We anticipate that these modifications will have a significant impact on the biological effects of the drug, making the SULC–Me βCD complex an appropriate candidate for a new drug delivery system.

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