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Research Article

PREPARATION AND EVALUATION OF THE INCLUSION COMPLEX OF SILVER SULFADIAZINE WITH CYCLODEXTRIN

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ABSTRACT

The main objective of this study is to enhance the solubility of the poorly water soluble silver sulfadiazine (SSD) and hence enhance its clinical usefulness through studying its complexation with the parent β-CD and its water soluble derivatives, HP-β-CD and CM-β-CD in order to develop a soluble form of the drug as a step towards the development of a highly bioavailable SSD wound healing products. Phase solubility diagrams of SSD with β-CD, HP-β-CD and CM-β-CD in pH4.6 at 25 ± 0.5 °C were constructed. CM-β-CD was selected for formulating equimolar binary systems with SSD using physical mixing, kneading, coevaporation, lyophilization and spray drying techniques. The surface morphology of SSD, CM-β-CD and SSD–CM-β-CD physical mixtures and complexes were examined using Scanning electron microscopy; FTIR; TGA and X-ray diffractometry. The dissolution rate of SSD from different complexes compared to the free drug was assessed. The β-CD system showed a Bi type diagram whereas; HP-β-CD and CM-β-CD showed an A_L-type curves. The solubilizing power of CDs towards the drug followed the order CM-β-CD (0.003637mmole/L) > HP-β-CD (0.000841mmole/L) > β-CD (0.0000mMole/L). All complexes showed drug content range of 100.4 % to 109.6%. The dissolution rate of SSD followed the order: kneaded complex> coevaporated complex> freeze dried complex > free drug. The release efficiency of the kneaded complex was almost 67.215%. Since, the applications of SSD are mainly hampered by its low solubility, inclusion complexation with CM-β-CD should be regarded as an important step towards the enhancement of its efficacy and the broadening of its application.

Keywords: Silver sulfadiazine **–** Cyclodextrin- complexation**-**characterization- *In vitro* release

INTRODUCTION

The main aim of burn management and therapy is wound healing and epithelization as soon as possible in order to prevent infection and to reduce the incidence of burn wound sepsis; a leading cause of mortality and morbidity in these patients [1]. Noble metals antimicrobials, the most prevalent of which is silver [2], are gaining renewed attention to combating the threat of bacterial infection and preventing wound sepsis. Several products have incorporated different silver based components for use as topical antibacterial agents in burn treatment; the gold standard of all was always silver sulfadiazine (SSD). Silver sulfadiazine was introduced by Fox [3] in 1970s as an antibacterial agent for topical treatment of burns and wounds. Silver is mixed with the antibiotic Sulfadiazine producing a complex combining the inhibitory action of the silver with the antibacterial effect of sulfadiazine [4, 5]. This silver complex acts on the bacterial wall in contradistinction to the silver ions which act on the bacterial energy system. All kinds of combinations of sulfa drugs with silver were tested *in vitro*, but silver sulfadiazine appeared to be the most effective [5]. A possible explanation of this effectiveness could be the relatively strong bonding of silver sulfadiazine to bacterial DNA [4] which differs from that of other silver salts [6, 7].

Over the past few years, there has been a rapid increase in the demand of wound healing dressings loaded with accurate doses of SSD. To be pharmacologically active, all drugs must possess some degree of aqueous solubility. The clinical usefulness of insoluble drugs like silver sulfadiazine is hampered by their insolubility in water as any silver dressing efficacy is determined by total available soluble silver, not total silver in dressing [8]. Since there is no point in having a long duration of activity if the lower concentration may result in the development of resistance, maintaining an adequate concentration of soluble silver in a dressing over time is a true challenge.

Cyclodextrin (CD) and its derivatives have been applied to enhance the solubility of poorly water soluble drugs in different areas of drug delivery. The most widely used approach to study inclusion complexation (Fig. 1) is the phase solubility method described by Higuchi and Connors [9, 10] which examines the effect of a solubilizer, *i.e.,* CD or ligand, on the drug being solubilized, *i.e.,* the substrate. Phase solubility diagrams are categorized into A and B types; A type curves indicate the formation of soluble inclusion complexes while B type suggests the formation of inclusion complexes with poor solubility. A B_S type response denotes complexes of limited solubility and a B_I curve indicates insoluble complexes. A-type curves are subdivided into A_L (linear increases of drug solubility as a function of CD concentration), A_P (positively deviating isotherms), and A_N (negatively deviating isotherms) subtypes. β-CD often gives rise to B-type curves due to their poor water solubility whereas the chemically modified CDs like HP-β-CD and CM-β-CD usually produces soluble complexes and thus give A-type systems [11].

Efforts aimed at achieving a perfect wound healing has pushed many researchers into trying various therapeutic options which were thought to aid or accelerate the wound healing process [12]. The main objective of this work is to study the interaction of silver sulfadiazine with the parent β-CD and its water soluble derivatives, HP-β-CD and CM-β-CD in order to explore the solubilization power of different CDs on SSD as a primary step in the development of a water soluble, bioavailable and non-toxic burn product. To our knowledge, literature lacks data about the inclusion behavior of SSD/CD complexes.

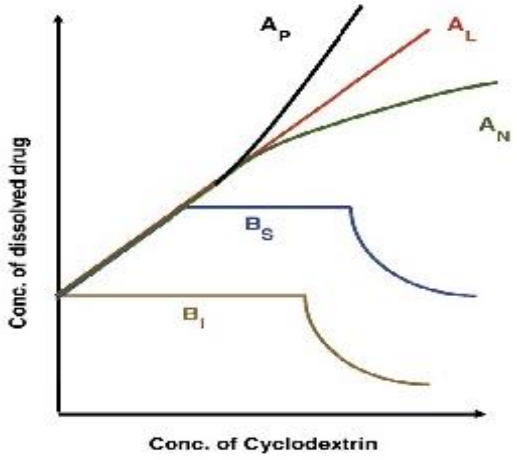


Fig. 1: It shows graphical representations of A and B-type phase–solubility profiles with applicable subtypes (A_P, A_L, A_N and B_S, B_I)

METHODS

Studying the effect of cyclodextrin on the solubility of SSD.

The effect of three different types of the complexing agents; CD [β-cyclodextrin (β-CD), hydroxypropyl β-cyclodextrin (HP-β-CD) and carboxymethyl β-cyclodextrin (CM-β-CD)]; on SSD was extensively studied. Experimentally, an excess of the poorly water-soluble silver sulfadiazine was introduced into several vials having a constant volume of phosphate buffer containing successively larger concentrations of the tested CD. The vials were shaken at room temperature until equilibrium was achieved. The suspensions were then filtered and the total concentration of the drug was determined using high performance liquid chromatography (HPLC) followed by UV detection at 256 nm. The phase solubility diagram was then constructed by assessing the effect of the CD on the apparent solubility of the drug. This experiment was repeated thrice with each of the three different types of CDs.

Formulation of SSD - cyclodextrin systems;

Based on results obtained from the step 2.1, solid binary systems of equimolar ratios of SSD and CM-β-CD were prepared using physical mixing, kneading, coevaporation, lyophilization and spray drying techniques.

Drug – CD physical mixture

Physical binary systems were prepared by 15 min tumble mixing of equimolar amounts of SSD and CM-β-CD in a mortar.

Kneaded complexes;

Complex of SSD with CM-β-CD was prepared by grinding the calculated equimolar amounts of SSD and cyclodextrin in the presence of least amount of water to form a paste. The obtained paste was then dried under vacuum at room temperature in the presence of phosphorus pentaoxide.

Co-evaporated complexes;

Complex of SSD with CM-β-CD was prepared by dissolving the calculated amounts of SSD and CM-β-CD in a suitable solvent (1:1 water and phosphoric acid) using a magnetic stirrer and the obtained clear solution was then evaporated under vacuum in a rotatory evaporator.

Spray – dried complexes;

Complex of SSD with CM-β-CD was prepared by dissolving SSD and the cyclodextrin in distilled water using a magnetic stirrer. The resultant solution was then spray dried using a Butchi spray dryer.

Lyophilized complexes.

Equimolar quantities of CM-β-CD and SSD were dissolved in distilled water and 1:1 water and phosphoric acid, respectively. The resulting solutions were mixed by stirring. The clear monophasic solution was frozen at -80°C and lyophilized in a freeze–dryer (Novalyphe-NL 500; Savant Instruments Corp., USA).

Evaluation of SSD - cyclodextrin complexes

Scanning electron microscopy

The scanning electron microscope was used to detect the changes of the SSD surface before and after complexation for indicating complex formation. The powders to be scanned were fixed on a brass stub using double-sided adhesive tape and then made electrically conductive by coating, in a vacuum, with a thin layer of gold.

Infrared spectroscopy

FT-IR is a useful tool to establish the presence of both host and guest components in the inclusion complex. The FT-IR spectra of SSD, CM-β-CD and their physical mixture as well as binary complexes in the region from 400 to 4000 cm^{-1} were determined. The formation of inclusion complex was identified by the shift and intensity changes on the peaks of inclusion complex compared to those of the individual components.

Thermal measurements

The stability and thermal behavior of SSD, SSD- CM-β-CD physical mixture as well as SSD/CM-β-CD complexes were traced by thermogravimetric technique (TGA). The TGA scan was carried out using a computerized Perkin Elmer TGA series under a dynamic N_2 purging gas atmosphere at a constant rate of 50 cc/min and a heating rate of 5° C/min.

X – Ray diffractometry.

X-ray diffraction patterns of SSD – cyclodextrin physical mixtures and complexes were obtained by using a Diano X-ray diffractometer equipped with Cu Kα radiation. The tube operated at 45 KV, 9 mA. X-ray diffractometry is used to detect the change in degree of SSD crystallinity after complexation.

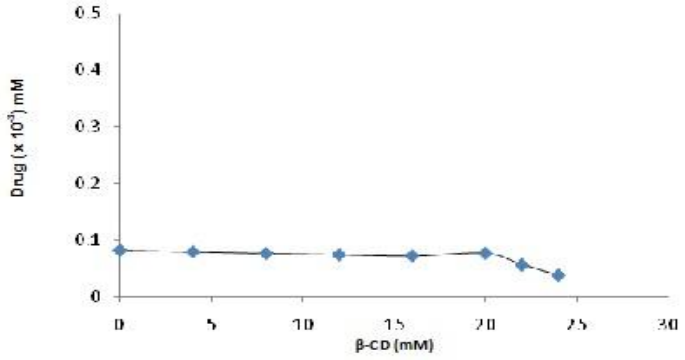
Effect of cyclodextrin on the dissolution of SSD

Dissolution rate and profile of SSD from the prepared complexes were assessed at 32 ± 0.5 °C (skin temperature) in USP Dissolution Tester, Apparatus II, using 900 ml of phosphate buffer (pH = 5.5) as the dissolution medium at a rotation rate of 50 rpm (skin pH). Aliquots, each of 5 ml, from the dissolution medium were withdrawn at different time intervals for six hours and replenished by equal volumes of fresh dissolution medium. SSD content was measured using high performance liquid chromatography (HPLC) followed by UV detection at 256 nm.

RESULTS AND DISCUSSION

Studying the effect of cyclodextrins on the solubility of SSD.

The phase solubility diagrams of SSD with β-CD, HP-β-CD and CM-β-CD in pH4.6 at 25 ± 0.5 °C are shown in Fig. 2a, b and c. Cyclodextrins are known to solubilize lipophilic entities through molecular encapsulation. The parent β-CD system showed a Bi type phase diagram (fig. 2a); this comes into agreement with what was previously reported by Challas *et al.* [11]. Whereas, the solubility of SSD increased as a function of the CDs concentrations supporting the formation of inclusion complexes when the HP-β-CD and CM-β-CD were used [9].



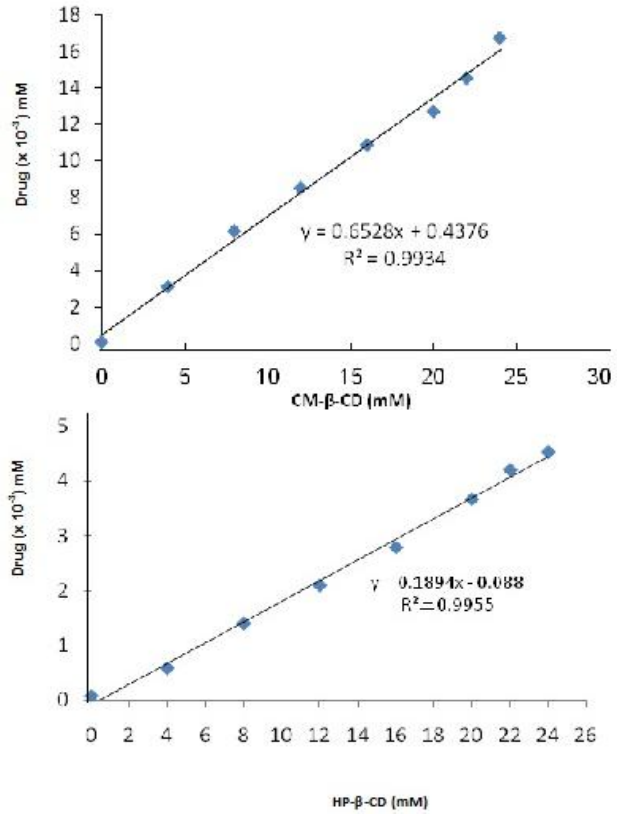


Fig. 2a: **It shows the effect of β-CD on the solubility of SSD, Fig2b. Effect of HP-β-CD on the solubility of SSD, Fig. 2c. Effect of CM-β-CD on the solubility of SSD**

The coefficient of determination (R^2) values of the phase solubility diagrams with HP-β-CD and CM-β-CD were 0.9955 and 0.9934, respectively; therefore, the diagrams were classified as A_L-type curves [13]. A_L profiles indicate a linear increase in solubility as a function of solubilizer concentration (fig. 2b & 2c). The solubilizing power of CDs towards the drug followed the order CM-β-CD (0.003637mmole/L) > HP-β-CD (0.000841mmole/L) > β-CD (0.00001mmole/L). It was evident that the solubility of SSD was increased markedly by complexation with CM-β-CD. The highest solubilizing power exhibited by CM-β-CD could be ascribed to the presence of methyl groups that expand the hydrophobic region of the CD cavity and thus increase its affinity towards SSD [14].

Formulation of SSD - cyclodextrin systems

Based on results obtained from the phase solubility studies; which revealed that among the three tested CDs, SSD solubility increased most through complexation with CM-β-CD; CM-β-CD was selected for further study.

Drug / CM-β-CD binary systems were prepared in an equimolar ratio. All prepared binary complexes gave a SSD/CM-β-CD yield of more than 95% except for the spray drying technique where the yield was about 30%, therefore, complexes resulting from this technique were not further investigated.

Complexes formed with all complexation techniques showed almost complete inclusion, with drug content ranging from 100.4 % to 109.6%. The driving forces between CDs and drugs which have been proposed to justify the complex formation are hydrogen bonds, van der Waals forces, hydrophobic interactions and the release of "high-energy water" molecules from the cavity [15].

Evaluation of SSD - cyclodextrin complexes

Scanning electron microscopy

Scanning electron microscopy was used to study the structural aspects of raw materials, *i.e.,* the CD and the drug as well as the products obtained by different methods of preparation, such as physical mixing, kneading, coevaporation and others to confirm the process of complexation [16].

Scanning electron microscopy demonstrated CM-β-CD as large crystalline particles with no definite shape (fig. 3a) and SSD as needle shaped crystals (fig. 3b). SSD/CM-β-CD physical mixture appeared as unmodified particles of CM-β-CD covered by adhering small crystals of the drug (fig. 3c). On the other hand, a drastic change in the original morphology and shape of both SSD and CM-β-CD particles was observed in all complexes and it was impossible to differentiate crystals of both components indicating interaction of drug particles with CM-β-CD (fig. 3d-3f). In complexes formed using the kneading technique, round edged particles (no sharp edges) were seen due to the grinding process applied in the complex formation (fig. 3d), whereas when the coevaporation technique was used, new sharp edged particles were observed (fig. 3e). Freeze drying inclusion complex showed small sized particles with a tendency to aggregation, suggesting the existence of an amorphous product with the presence of a single component in the complex. This indicated a maximum or complete complex formation. Generally, the reported changes, in complex morphology is indicative of the presence of a new solid phase that could simply be a consequence of a crystalline habit change in the formed complex systems supporting the evidence of a new single phase formation (SSD- CM-β-CD complex) [17].

Infrared spectroscopy

In the IR spectrum of SSD (fig. 4a), absorption bands ranging from 1110-1200 cm^{-1} generally indicate SO_2 groups. It was assumed that the bands at 3387.35 cm^{-1} and 3340.1 cm^{-1} were related to NH groups. C-S bands were found in the range of 570-730 cm^{-1}.

In CM-β-CD IR spectrum (fig. 4b), the vibration of free OH group was presented in the range of 3100–3400 cm^{-1} and CO band was found at 1094.4 cm^{-1}. The vibration of –CH and $–CH_2$ groups appeared in the region 2950–2800 cm^{-1}.

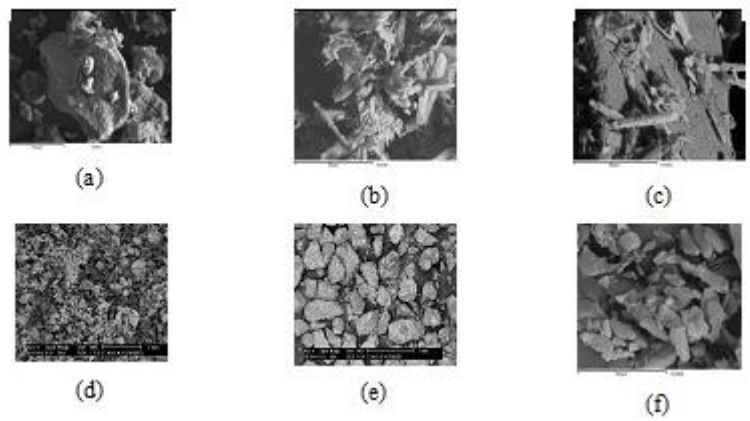
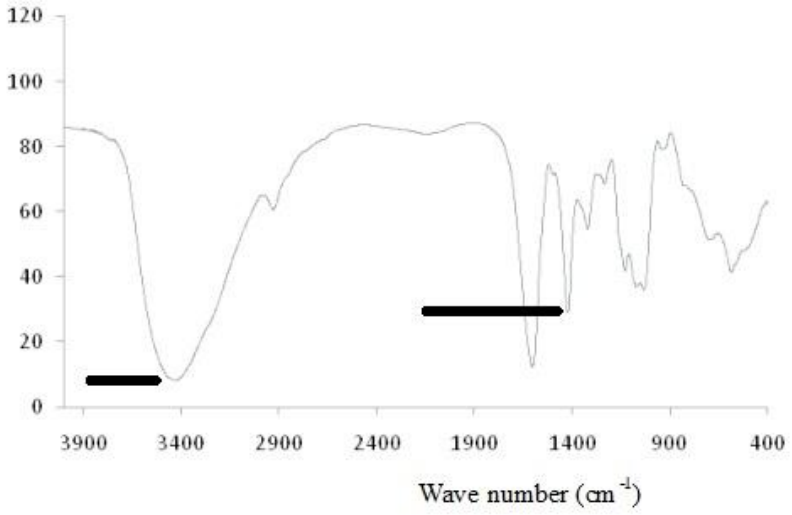


Fig. 3a: It shows SEM of CM-β-CD, Fig.3b. SEM of SSD, Fig. 3c. SEM of SSD- CM-β-CD physical mixture, Fig. 3d. SEM of SSD- CM-β-CD kneaded mixture, Fig. 3e. SEM of SSD- CM-β-CD co-evaporated mixture, Fig.3f. SEM of SSD- CM-β-CD lyophilized mixture

The IR spectrum of the inclusion complex showed that some bands (fig. 4c - fig. 4f) of the host and guest are affected by the complexation process, resulting in a change of position and relative intensities. In this study the characteristic –NH stretching band of SSD was masked in all the prepared systems by the broad intense band corresponding to the free –OH vibration of CD. Also, there was an overlap between the SO_2 stretching of the drug in the range of 1100-1200 cm^{-1} and the band corresponding to the CO within CD molecules found at 1094.4 cm^{-1} confirming complex formation.

a



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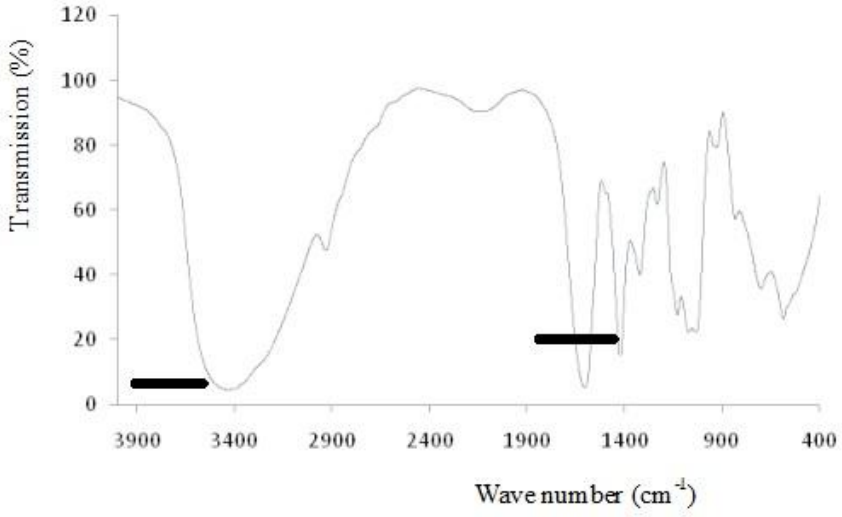

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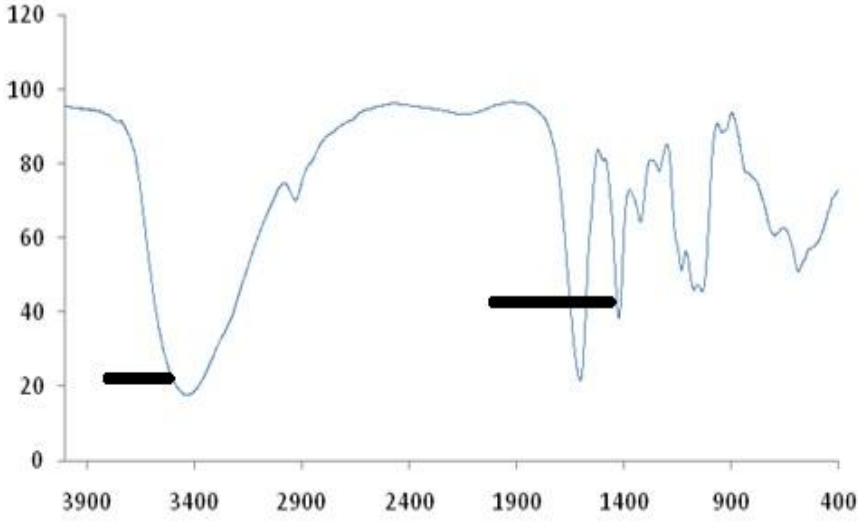
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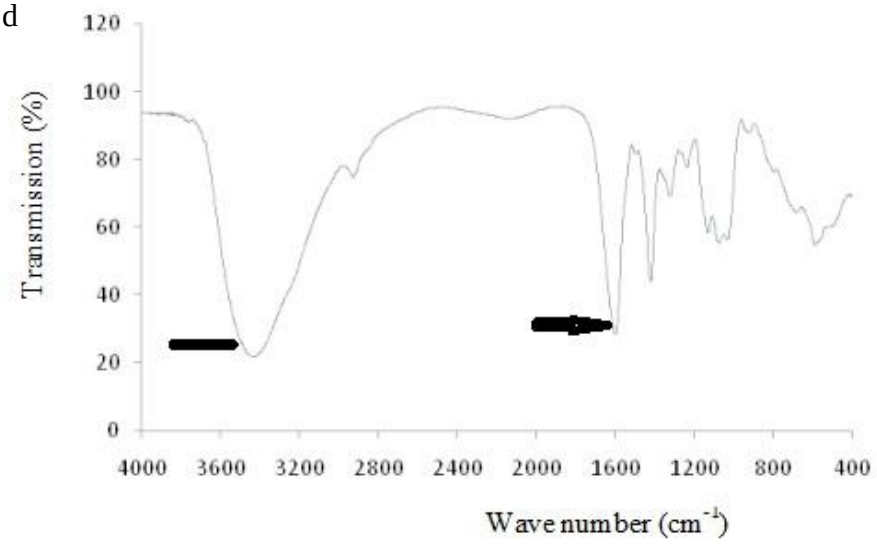
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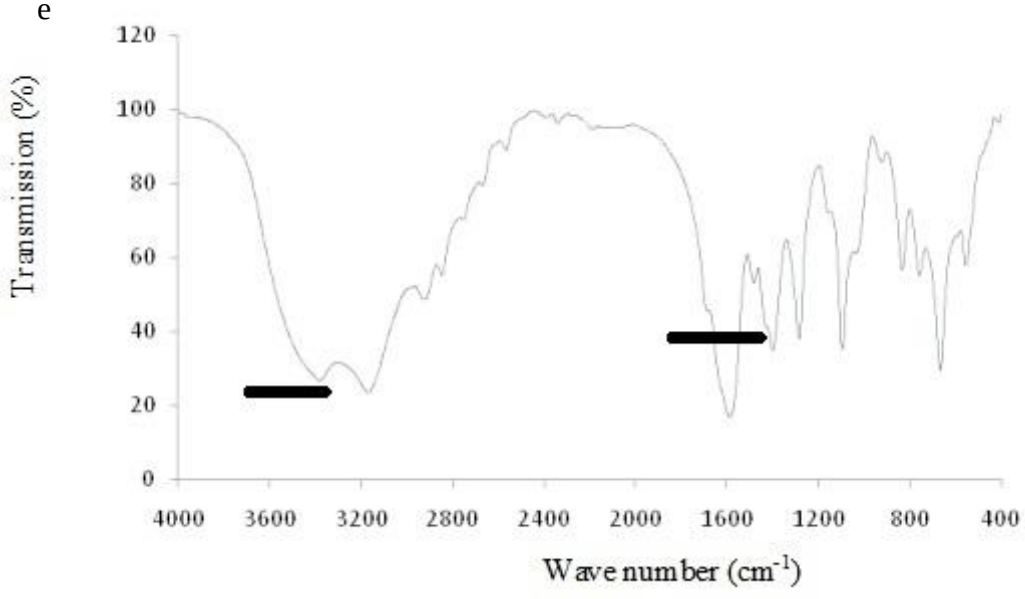
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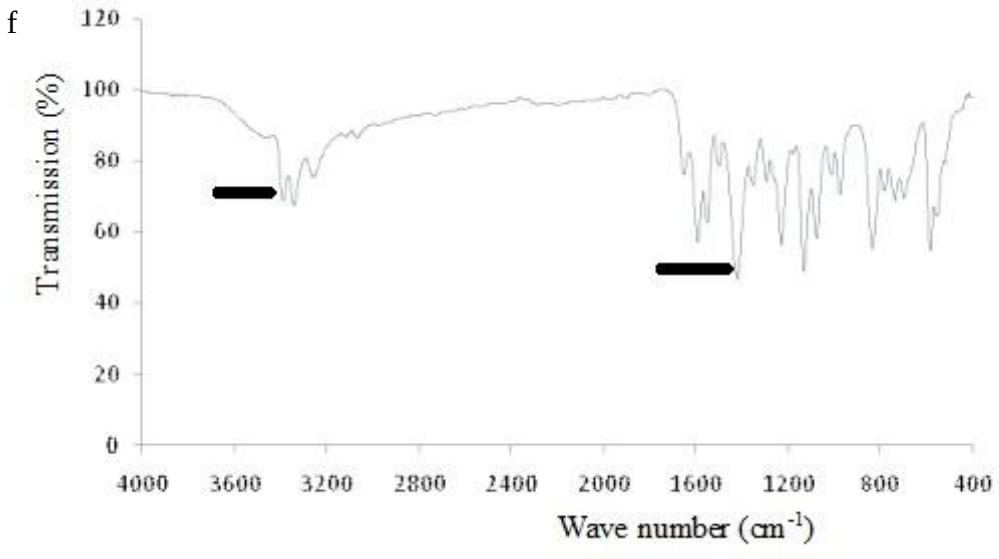

Fig. 4a: It shows IR spectra of SSD, 4b. IR spectra of CM-β-CD, 4c. IR spectra of SSD/CM-β-CD physical mixture, 4d. IR spectra of SSD/CM-β-CD kneaded mixture, 4e. IR spectra of SSD/CM-β-CD co-evaporated mixture, 4f. IR spectra of SSD/CM-β-CD lyophilized mixture

Thermal measurements

The stability and thermal behavior of SSD, SSD- CM-β-CD physical mixture as well as SSD/CM-β-CD complexes were traced by thermogravimetric technique (TGA). TGA showed that the complexes of SSD with CM-β-CD contain less water compared to that of the physical mixture (fig. 5a-5f). Water is present within the cavity of the cyclodextrin molecule to stabilize the ring structure [18]. The low water contents of the complexes (table 1), compared to that of the physical mixture, may result from SSD occupying the position of some of the water molecules associated with torus of the cyclodextrin [19].

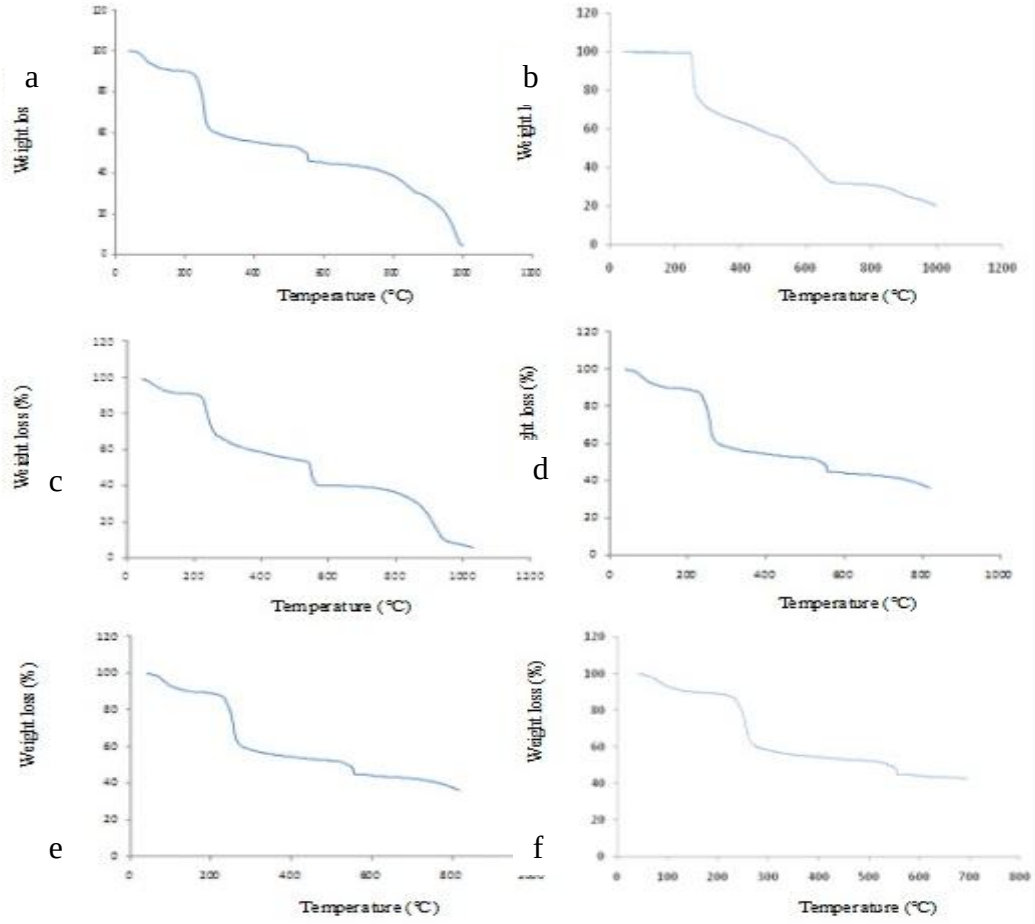

Fig. 5a: It showsTGA of SSD, Fig.5b. TGA of CM-β-CD, Fig.5c. TGA of SSD/CM-β- CD physical mixture, Fig.5d TGA of SSD/CM-β-CD kneaded mixture, Fig.5e.TGA of SSD/CM-β-CD co-evaporated mixture, Fig.5f. TGA of SSD/CM-β-CD lyophilized mixture

Table 1: It shows data from TGA diagrams of SSD, CM-β-CD and SSD/CM-β-CD systems

System	Water loss (% w/w)
SSD	6.44
Carboxymethyl-β-cyclodextrin	0.038
SSD/CM-β-CD physical mixture	4.589
SSD/CM-β-CD Kneaded complex	6.446
SSD/CM-β-CD coevaporated complex	6.738
SSD/CM-β-CD freeze dried complex	6.325

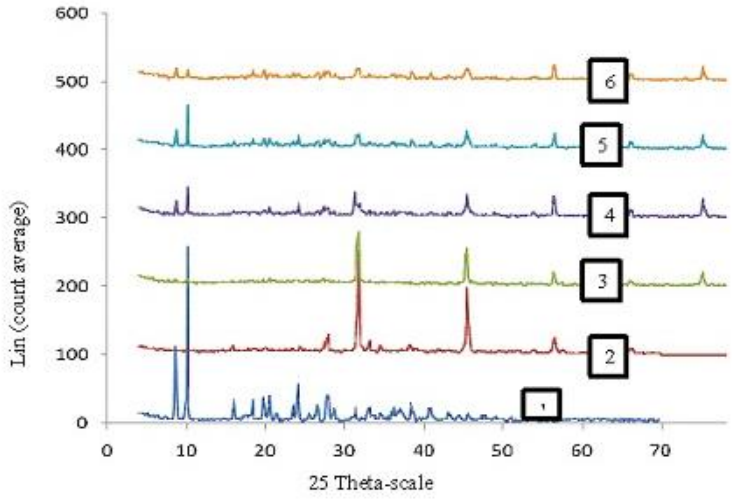

Fig. 6: It shows X-ray diffraction patterns of SSD, CM-β-CD and SSD/CM-β-CD systems; 1. SSD, 2. CM-β-CD, 3. SSD/CM-β-CD physical mixture, 4. SSD/CM-β-CD kneaded complex, 5. SSD/CM-β-CD co-evaporated complex, 6. SSD/CM-β-CD lyophilized complex.

X – Ray diffractometry.

The crystalline nature of cyclodextrin was clearly demonstrated by its characteristic powder X-ray diffraction (PXRD) pattern containing well-defined peaks (fig. 6). Based on the method used, it is verified that SSD forms inclusion complexes in solid phase with CM-β- CD. The diffraction patterns of formed complexes showed that most of the crystalline diffraction peaks of CM-β-CD disappeared after complexation with SSD (fig. 6-4 to 6-6) indicating complex formation [20].

Effect of cyclodextrin on the dissolution of SSD

Fig. 7 shows the dissolution profile of SSD from SSD and both the physical mixture and complexes with CM-β-CD. It was evident that the complex exhibited higher dissolution rates compared to the physical mixture and of course to the free drug, reflecting the improved aqueous solubility of the drug. The increase of dissolution rate of SSD by complexation with CM-β-CD followed the order: kneaded complex> coevaporated complex> freeze dried complex>physical mixture>free drug. The dissolution efficiency of the kneaded complex was almost 67.22% in 24 hrs. In sharp contrast the dissolution efficiency of the free drug was 4.67% in 24 hrs.

Improvement of SSD solubility obtained with the physical mixture over the free drug (dissolution efficiency of 53.90% *vs* 4.67%) can be attributed to the local solubilizing effect of CD, operating in the microenvironment, on the hydrodynamic layer surrounding the drug particles, which improves SSD wettability and/or solubility. Also, the *in situ* formation of a readily soluble complex in the dissolution medium additionally contributes to the drug release from the prepared physical mixture. However, the increase of the SSD dissolution rate in case of inclusion complexes might be due to several factors such as the reduced particle size and the high energetic amorphous state obtained following complexation as confirmed by XRD studies. This is a consequence of an increase in the drug-carrier contact surface [16].

It is worth noting that a rapidly dissolving form of SSD can be achieved simply thorough kneading *i.e.,* by grinding the drug with the hydrophilic carrier. This result is of utmost importance since grinding is a simple low-cost process, with interesting perspectives for industrial applications.

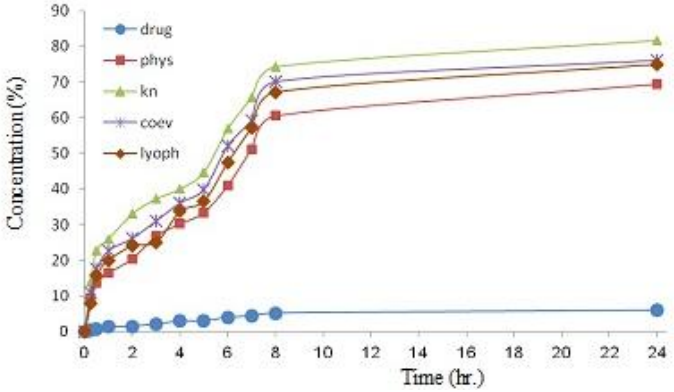

Fig. 7: It shows the effect of CM-β-CD on the dissolution rate of SSD

CONCLUSION

The inclusion complexation behavior of SSD with native β-cyclodextrin and its derivative hydroxypropyl-β-cyclodextrin (HP-β-CD) and carboxymethyl-β-cyclodextrin (CM-β-CD) was assessed. Based on the results of the phase solubility study complexation of SSD with CM-β-CD using different complexation techniques was investigated. The results showed that CM-β-CD-SSD kneaded complex can enhance the water solubility of SSD. Given the shortage of applications for SSD due to its low solubility this inclusion complexation should be regarded as an important step towards the enhancement of its antimicrobial efficacy.

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