Study on increasing the solubility and dissolution rate of sulfamethoxazole by cyclodextrins

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The aim of this study was to enhance the solubility and dissolution rate of sulfamethoxazole (SMZ) by preparing the inclusion complexes with β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HPβ-CD) and γ-cyclodextrin (γ-CD). The effect of the type of cyclodextrins used for the preparation of complexes, on the in vitro dissolution profiles and solubilities in different mediums (pH 4.5 and 7.0) was also evaluated. The interaction between SMZ and cyclodextrins in solution was studied by the phase-solubility method. Inclusion complexation was confirmed by the results from the studies of infrared spectoroscopy (IR) and differential scanning calorimetry (DSC). The effect of water-soluble polymers, that is, polyethylene glycols 4000, 10000, 20000 and non-ionic surfactants, that is, polysorbate 20, 40 and 60 on the complexation of SMZ with CD₄ were also investigated by the same methods. The rates of release of the active material from the complexes were determined from dissolution studies using USP XXII paddle method. As a result of this study, it was found that the solubility of SMZ was significantly enhanced by inclusion of β-CD, especially when the water soluble additives are added (from 0.086 to 0.377 mg/ml with SMZ:β-CD:PEG20000).

Key words: Cyclodextrins, sulfamethoxazole, solubility, dissolution rate.

INTRODUCTION

Sulfonamides are bacteriostatic agents which are systematically used in the treatment of bacterial infections. SMZ, a derivative of sulfonamide, inhibits the synthesis of folic acid, which is an important metabolite of bacteria’s DNA synthesis (Martindale, 1993; Raja et al., 2009). SMZ is absorbed from gastro-intestinal area; however, its absorption and bioavailability are limited with its dissolution rate, due to its low solubility like all the other sulfonamide groups. The aim of the study was to prepare the inclusion complexes of SMZ using CD₄ to enhance the solubility, dissolution rate and oral bioavailability.

CD₄ which are made up of 6D (+) glucopyranose units at minimum, with α 1-4 glycoside bonds and formed as a result of degradation of starch by the help of glucosyltransferase (CGT) enzyme, are cyclic oligosaccharides with hydrophobic cavity and hydrophilic outer surface (1).CD₄ can be defined as enzymatically modified starch made of glucopyranose units (Szejtli, 1991a). The commonly available CD₄ are α-, β- and γ-
cycloextrim, which consist of six, seven and eight glycopyranose units, respectively (Gabriel et al., 2016; Uekama and Otagiri, 1987). The more the glucose units, the bigger the dimensions of CD₆, CD₇ are molecules with a polar hydrophilic outside, and an apolar hydrophobic cavity, which provides a guest-host relation to hydrophobic drugs in hydophilic medium which is called “inclusion complex” (Brewster and Loftsson, 2007; Jambhekar and Breen, 2016).

Some CD₅ contain 9 and more glucose units, but they are not used since it is difficult to obtain them in pure form and they have low complex forming capacity. Those containing less than 6 units of glucose, on the other hand, cannot be prepared for steric reasons (Bekers et al., 1991; Fromning and Szejtli, 1994).

In these complexes, the drug (guest molecule) is entrapped in the cavity of the cycloextrim (CD) (host). After the complexation, some physical properties of the guest molecule can be changed. For instance, the strength against oxidation, hydrolysis and photochemical reactions can increase, the evaporation speed of volatile substances can significantly decrease. Also when poor soluble drugs are in complex form, the solubility increases. For this reason, in order to enhance the water solubility of poor soluble substances, complex forming with CD’s are widely used (Duchene and Woussidjewe, 1990; Sharma and Baldi, 2016; Sinha et al., 2007; Szejtli, 1988).

The stoichiometries of active material: cycloextrim complexes and the numerical values of their stability or binding constants are frequently obtained from the plots of drug solubility against cycloextrim concentration. This phase-solubility technique was first developed by Higuchi and Connors (1965). Phase-solubility diagrams are categorized into A and B types. A type phase-solubility profiles are obtained when the solubility of the active material increases with an increase in cycloextrim concentration. The A type profile is further subdivided into three profiles. The A₁ profile indicates that there is a linear increase in solubility as a function of cycloextrim concentration; the A₂ profile indicates an isotherm, wherein the curve deviates from linearity in a positive manner, suggesting that a ligand or a solubilizer is proportionally more effective at higher concentrations. Conversely, the A₃ type relation indicates a negative deviation from linearity, which means that the cycloextrim is proportionally less effective at higher concentration (Jambhekar and Breen, 2016).

In general, the water-soluble CD₅ form A-type phase solubility profiles, whereas the less soluble natural CD₆ generally form B-type profiles. Type B phase-solubility profiles indicate the formation of a complex with naturally occurring CD₅, in particular β-CD (Brewster and Loftsson, 2007).

It is accepted that water soluble polymers or non-ionic surfactants have significant effects on the formation of the inclusion compounds (Hirlekar et al., 2009; Loftsson et al., 1991, 1994, 1996). The effects of commonly used water soluble polymers, polyethylene glycol 4000 (PEG4000), polyethylene glycol 10000 (PEG10000), polyethylene glycol 20000 (PEG20000), hydroxypropyl methylcellulose (HPMC) and the non-ionic surfactants polysorbate 20 (PS20), polysorbate 40 (PS40), and polysorbate 60 (PS60) on the dissolution rate and solubility of SMZ from the inclusion complexes were also investigated.

In this study, the solid dispersions were prepared by coprecipitation method (Dhanaraju et al., 1998; Elgindy et al., 2011; Ozdemir and Erkin, 2012; Polonosamy and KNom, 2011; Patel and Rajput, 2009). The rates of release of the active material from the complexes were determined from dissolution studies using USP XXII paddle method (USP XXII, 1990).

MATERIALS AND METHODS

Sulfamethoxazole (SMZ) was obtained from Fako Medical (Turkey); in addition, the following were used in the study: β-cycloextrim (β-CD), hydroxypropyl-β-cycloextrim (HPB-CD), γ-cycloextrim (γ-CD) (Fluka), polyethylene glycol 4000 (PEG4000), polyethylene glycol 10000 (PEG10000), polyethylene glycol 20000 (PEG20000), hydroxypropyl methylcellulose (HPMC) polysorbate 20 (PS20), polysorbate 40 (PS40) and polysorbate 60 (PS60) (Merck). All other chemicals were of analytical grade.

Apparatus

UV spectrophotometer (Shimadzu UV 1202, Japan), IR spectrophotometer (Jasco FT/IR 420, Japan), differential scanning calorimetry (DSC) (Netzsch Geratebau DSC 204, Germany) and dissolution rate apparatus (Aymes, Turkey) were used.

Phase-solubility studies

Solubility measurements were performed by the method of Higuchi and Connors (1965). Solubility studies containing various concentrations of CD₅, ranging from 2 × 10⁻³ to 15 × 10⁻³ M (the solubility of β-CD is 16.3 × 10⁻³ M) were shaken with 100 mg of SMZ in sealed flasks in a thermostat water bath at a constant temperature of 37°C (Loftsson and Duchene, 2007). After an equilibrium was attained (approximately after three days), aliquots were withdrawn and filtered through 0.45 μm filters. A portion of the filtrate was then diluted with water and analyzed spectrophotometrically. The solubility constant and the ratio of SMZ:CD₂ in the complexes were calculated from the phase solubility diagram (Connors and Mollica, 1986; Higuchi and Connors, 1965).

The solubilizing effect of water-soluble polymers and non-ionic surfactants was also investigated with phase-solubility studies. For this purpose, an 100 mg active material was added to aqueous solutions containing 0 to 15% of CD₅ and 0.50% (w/v) of water soluble polymers (PEG4000, PEG10000, PEG20000 and HPMC) and non-ionic surfactants (PS20, PS40 and PS60). After equilibration, which took at least three days took place at room temperature, the suspensions were filtered through 0.45 μm membrane filter and analyzed by UV spectrophotometer. The type of complexation and the apparent stability constants (K₀) of the drug : cycloextrim complexes were calculated from the slope of the phase solubility diagrams (Loftsson et al., 1991, 1994, 1996).
Solubility studies

The cationic form of SMZ is the dominating form at pH below 1.5, while the nonionized form dominates between pH 2.0 and 6.0 and the anionic form dominates pH above 7.0. Although CD, are able to solubilize both nonionized and the anionic forms of the drug, they have a small solubilizing effect on the cationic form. After taking these results into consideration, the pH of the mediums to be used for solubility and the dissolution studies were adopted to be either pH 4.5 or 7.0.

Excess amount of active material (more than it can be dissolved) was added to a closed flask with either pH 4.5 or 7.0 and then mixed in a magnetic mixer at 37°C. Thereafter, the liquid phase was filtered through 0.45 μm filters and the amount of active material in the solution was determined. Solubility of the active material was calculated by the point measured during the formation of the equilibrium status.

Preparation of inclusion compounds

Coprecipitation method given below was used to prepare SMZ’s inclusion compounds with CD.

Coprecipitation method

SMZ was dissolved in acetone and added to the aqueous solution of CD at 1:1 molar ratio. The solvent was allowed to evaporate and then dried under vacuum at 40°C for 24 h and sieved to obtain the optimum particle size (Dhanaraju et al., 1998).

The solid complexes including solubility enhancer such as water-soluble polymers (PEG4000, PEG10000, PEG20000, HPMC) and non-ionic surfactants (PS20, PS40, PS60) were also prepared with the same method.

Characterization of complexes

The infrared spectroscopy (IR) of SMZ, β-CD and the inclusion complexes were measured with KBr discs at 400 to 4000 cm\(^{-1}\). The amount of active material in each sample was kept constant in each measurement. Differential scanning calorimetry (DSC) was performed using a scanning rate of 10°C/min on a Netzsch Geratebau DSC 204. Samples were heated in a sealed aluminum pans from 50 to 300°C.

Dissolution rate studies

Active material release from all the inclusion compounds prepared were performed by using the USP XXII paddle method at 50 rpm in 37°C for three hours (USP XXII, 1990). It was done in 900 ml and at pH 4.5 and 7.0 mediums. Samples taken at certain intervals were measured spectrophotometrically.

RESULTS AND DISCUSSION

Phase solubility diagram

Data obtained from the phase solubility studies were used to calculate the apparent stability constant (Kc) according to the following equation:

\[
    K_c = \frac{S_t - S_o}{S_o (L_i - (S_t + S_o))}
\]

\[S_t = \text{total concentration of dissolved SMZ}; S_o = \text{molar solubility of SMZ in the presence of cyclodextrin}; L_i = \text{total cyclodextrin concentration}.
\]

A B type solubility graph was obtained with β-CD. The stoichiometric ratio of the complex determined from the descending part of the diagram was found to be 1:1 (SMZ:β-CD) and the stability constant was found as 122.3 M\(^{-1}\) (Figure 1). A type phase-solubility profiles are obtained when the solubility of the active material increases with an increase in cyclodextrin concentration. A type solubility graph was obtained by chemically modified HPβ-CD and the stability constant was too low (39 M\(^{-1}\)) (Figure 2). No significant increase in solubility of SMZ was observed with γ-CD and it was concluded that the inclusion complex was not formed (Figure 3). However, high stability constants of the complexes indicate high stability, very high values complicate the solubility of the drug. Comparing the stability constants, it was concluded that the β-CD was the best complex producing CD for SMZ. Thus, in all the remaining studies, only β-CD was used as host molecule (Duchene and Woussidjewe, 1990).

Solubility studies

At pH 4.5 (Figures 4 and 5) and pH 7.0 (Figures 6 and 7), the solubility of the active material was enhanced in the presence of water soluble polymers (PEG4000, PEG10000 and PEG20000) and non-ionic surfactants (PS20, PS40 and PS60) (Table 1). No significant increase in the solubility of active material with HPMC was observed. Hence, this result is not given in Table 1. As can be seen in Figure 5, a higher increase in solubility was observed when PS20 was used rather than PS40 and PS60.

The solubility of SMZ was significantly increased with the addition of β-CD from 0.086 to 0.175 mg/ml at pH 4.5 and 0.188 mg/ml at pH 7.0, respectively. When PEG4000, PEG10000 and PEG20000 were used with SMZ:β-CD, the solubility increase were greater than the SMZ:β-CD alone (Figures 4 and 6). When the effects of polymers and surfactants on the solubility of active material were examined, it was found that the solubility of SMZ was increased by 4.4 times with the formulation including SMZ:β-CD:PEG20000 (from 0.086 to 0.377 mg/ml at pH 4.5 and to 0.406 mg/ml at pH 7.0) as compared to pure SMZ. The higher solubility (0.377 mg/ml at pH 4.5 and 0.406 mg/ml at pH 7.0) were obtained with PEG20000 than the other PEGs because of higher hydrophilic nature. The solubility values of SMZ from SMZ:β-CD complexes including PEG10000 or PEG4000 were 0.287 mg/ml at pH 4.5 and 0.193 mg/ml at pH 7.0 for PEG10000 and 0.241 mg/ml at pH 4.5 and 0.100 mg/ml at pH 7.0 for PEG4000, respectively. Furthermore besides the solubility increase in both pH 7.0 and 4.5, there was found, a slight difference between
two mediums and the higher solubility observed in the pH 7.0 medium was attributed to the presence of SMZ in anionic form. The results are agreement with the results published by other authors (Jambhekar and Breen, 2016).

**The characterization of inclusion compound formation**

Infrared spectrophotometers (IR) and differential scanning calorimetry (DSC) were used to prove the formation of inclusion complexes between the active material and β-CD. The characteristic bonds of SMZ at 1599 (-SO₂-NH) and 1306 (-SO₂) which were observed by IR, seemed to disappear in the inclusion compounds or become modified significantly (Figure 8).

The observed decreases in the intensities of the characteristic bonds of SMZ may be due to its restriction within the β-CD cavity.

Figure 9 shows the thermogram of SMZ, β-CD and the
Table 1. The solubility values of sulfamethoxazole from the inclusion complexes at 37°C.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Solubility (mg/ml)</th>
<th>pH 4.5</th>
<th>pH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazole</td>
<td>0.086</td>
<td>0.124</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole:β-CD (1:1)</td>
<td>0.175</td>
<td>0.188</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole:β-CD:PEG20000 (1:1)</td>
<td>0.377</td>
<td>0.406</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole:β-CD:PS20 (1:1)</td>
<td>0.349</td>
<td>0.386</td>
<td></td>
</tr>
</tbody>
</table>

Studies on dissolution rate

The studies conducted at pH 4.5 conditions, in which active material exists in non-ionic form, demonstrated an increase in dissolution rates of complexes as compared to pure active drug rates. On the other hand, adding water soluble polymers (PEG4000, PEG10000 and PEG20000) to the formulation by 0.50% (w/v) increased the drug release as compared to the pure drug. 4.7% of pure SMZ were dissolved within 30 min, whereas the measured values for the formulations including water soluble polymers coded as β-CD:SMZ, β-CD:SMZ:PEG4000, β-CD:SMZ:PEG10000 and β-
Figure 4. Effect of water soluble polymers (PEG4000, PEG10000, PEG20000 and HPMC) (0.5% w/v), on the solubility of SMZ at pH 4.5.

Figure 5. Effect of non-ionic surfactants (PS20, PS40 and PS60) (0.5% w/v), on the solubility of SMZ at pH 4.5.
CD:SMZ:PEG20000 were 38.0, 22.5, 38.0 and 41.7%, respectively (Figure 10). It was found that, as the PEG molecule weight increases, so does the active material release. The effect of non-ionic surfactants (PS20, PS40
Figure 8. The IR spectra of, A) Sulfamethoxazole, B) β-Cyclodextrin, C) SMZ:β-CD (1:1) coprecipitated inclusion complex, D) SMZ:β-CD:PEG20000 (1:1) coprecipitated inclusion complex, E) SMZ:β-CD:PS20 (1:1) coprecipitated inclusion complex.
Figure 9. The DSC thermograms of, A) Sulfamethoxazole, B) β-Cyclodextrin, C) SMZ:β-CD (1:1) coprecipitated inclusion complex, D) SMZ:β-CD:PEG20000 (1:1) coprecipitated inclusion complex, E) SMZ:β-CD:PS20 (1:1) coprecipitated inclusion complex.
and PS60) with the concentration of 0.5% (w/v) were also examined with dissolution tests. The active material released at the end of the three hours were 39.1, 42.9, 44.1%, and 37.3% from β-CD: SMZ, β-CD:SMZ:PS20, β-CD:SMZ:PS40, and β-CD:SMZ:PS60 respectively. To conclude, the presence of these substances was observed to have a somehow significant effect on the active material release (Figure 11).

A significant increase in drug release was observed in the complexes at pH 7.0 as compared to the pure drug alone. At the end of 30 min, the SMZ release rates were 9.9, 25.5 and 17.1% from pure drug alone, β-CD:SMZ
and β-CD:SMZ:PEG20000, respectively. The release of active material from the formulations including water soluble polymers (PEG4000, PEG10000 and PEG20000) and nonionic surfactants (PS20, PS40 and PS60) were lower than 20% within 3 h. These results point out to the fact that additives had no further effect on the active material release rate at pH 7.0, where the drug substance is in anionic form, as compared to the β-CD:SMZ complex (Figures 12 and 13). The in vivo studies also showed that β-CD:SMZ:PEG20000: coded formulation,
which achieved the highest increase in the active material solubility and dissolution rate at pH 4.5 when compared with the pure SMZ, β-CD:SMZ coded complexes, increased the bioavailability among healthy volunteer participants (Özdemir and Erkin, 2012).

Conclusion

As a result of this study, it may be concluded that the solubility and the dissolution rate of SMZ was significantly enhanced by the complex formation with β-CD. The water soluble polymers and non-ionic surface active substances added to the solid dispersions increased the solubility and dissolution rate of active material-CD complexes at either pH 4.5 or 7.0.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

REFERENCES


