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Enhancement of Solubility and Dissolution Rate of Torsemide as Poorly Soluble Loop Diuretic by Inclusion Complexation with both β -Cyclodextrin and Hydroxypropyl- β -Cyclodextrin

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ABSTRACT

Torsemide (1-(1-Methylethyl)-3-[[4-[(3 methyl phenyl) amino] pyridin-3-yl] sulphonyl] urea) is an insoluble loop diuretic drug. Torsemide (TSM) is used for the treatment of oedema associated with heart failure, including pulmonary oedema, and with renal and hepatic disorders. It is also used in the treatment of hypertension, either alone or in combination with other antihypertensive drugs. Complexation of the drug with beta cyclodextrin (β -CD) and hydroxypropyl- β-cyclodextrin (HP- β-CD) was attempted to improve solubility and dissolution rate of torsemide. Inclusion Complexation (IC) is the association between the drug and the used polymer molecules to form a non-covalent complex that has a higher solubility than the used drug itself. The mostly used polymers in the formation of inclusion complexes are cyclodextrins (CDs). CDs comprise a family of water soluble, non-reducing, oligosaccharide which is having the abilities to form inclusions with a hydrophobic drug having low aqueous solubility. The CD molecules are having the versatility nature and have hydrophilic cavities with enough space to incorporate the lipophilic drug as a guest which fits the outer side of the host molecule. Thus the molecular encapsulation of the drug has great improved aqueous solubility and rate of dissolution. The aim of this work was to study the influence of β-CD and HP- β-CD on the dissolution properties of TSM. So, the physicochemical characterization of TSM -β-CD and HP- β-CD binary systems was performed using co-evaporate method for preparation of that systems. Present work includes the preparation of inclusion complex of TSM with ß-CD and HP-β-CD as carriers and to evaluate the formed inclusion complexes for various parameters as drug content, in vitro dissolution study, drug-excipients interaction study using FTIR spectroscopy and DSC method.

1. INTRODUCTION:

Over the last century, cyclodextrin and their derivatives have received considerable importance in the pharmaceutical field due to their ability to form complexes with a variety of drug molecules to increase their dissolution rate [1]. Beta-cyclodextrin (β-CD) is a naturally occurring cyclic oligosaccharide of seven glucose units which is the product of the degradation of starch by the enzyme *Bacillus macerans* [2]. The physiochemical properties of the cyclodextrin are utilized as carriers for lipophilic drugs. Their structures are similar to a cone with a hydrophilic interior and lipophilic exterior. This nature allows the molecular encapsulation of hydrophilic portion of guest molecules (hydrophobic drugs) [3]. Cyclodextrin interacts with some hydrophilic molecules and forms a non-covalent inclusion complex that lowers the chemical potential of the molecule in aqueous solution and thus enhances the solubility of the water-insoluble drugs.

Formation of inclusion complexation with cyclodextrin (CD) has been extensively developed to improve solubility, dissolution rate, absorption, and bioavailability of the poorly water-soluble drugs, and reduce the bitter taste of some drugs [4].

TSM was chosen as a model drug. TSM is a diuretic of pyridine-sulfonylurea class having $t_{1/2}$ of 3-6 hours (figure 1) [5]. By formation of TSM inclusion complexes with β-CD and HP- β-CD we have overcome two problems: the bitter taste and low dissolution rate of TSM.

The actions of TSM can be mediated by several mechanisms operating within the thick, medullary segment of ascending loop of Henle. These include a) interference with $Na^+/K^+/2Cl^-$ co-transporter at the luminal surface; b) $Na^+–K^+$ pump and c) anion exchange. Torsemide selectively blocks the active sodium and chloride reabsorption in the thick ascending loop of Henle promoting rapid excretion of water, sodium, and chloride. This action is a result of binding of the diuretic to a chloride ion-binding site of the transport molecule [6].

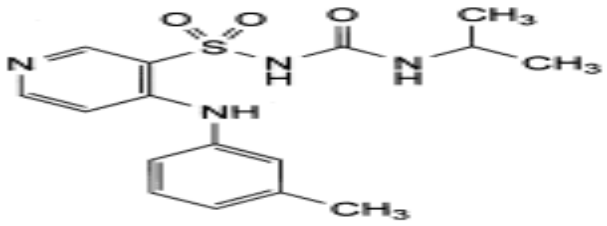

Figure 1: Chemical structure of torsemide.

1.1. Cyclodextrins structure and properties

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. Cyclodextrin molecules consist of (α-1, 4-)-linked α-D-glucopyranose units with a lipophilic cavity as illustrated in figure 2.

In fact, the aqueous solubility of the natural cyclodextrins (β-CD) is much lower than that of comparable acyclic saccharides. This is may be due to relatively strong intermolecular hydrogen bonding in the crystal state. Substitution of any of the hydrogen bond forming hydroxyl groups resulting in an improvement in their aqueous solubility. Cyclodextrin derivatives of pharmaceutical interest include the hydroxypropyl derivatives of β-cyclodextrin [7].

Studies involving inclusion complexation of active pharmaceutical substances into CDs are important due to the improvement of solubility, stability of the drug and due to the possibility of controlled drug release application [8].

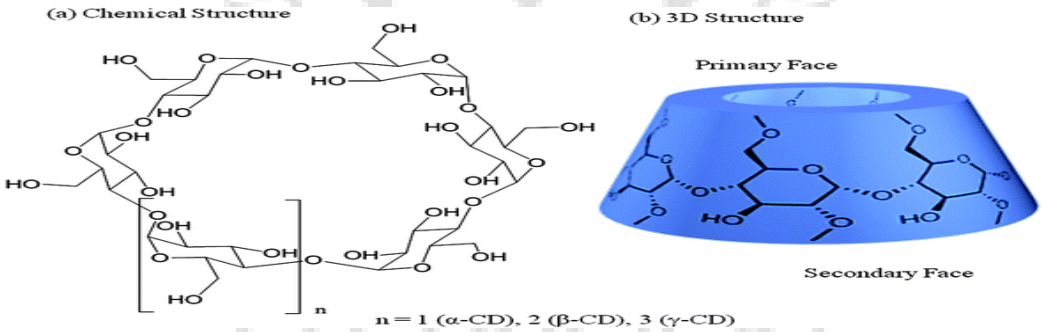

Figure 2: The chemical structure and the conical shape of the β-CD molecule.

Due to the chair formation of the glucopyranose units, the molecules of CD are shaped like cones with hydroxyl groups extending from the wider edge and the hydroxyl groups from the narrow edge.

CD molecules have a molecular weight ranging from almost 1000 to > 2000 Da with a large number of hydrogen donors and acceptors and are poorly absorbed through biological membranes. α-CD and β-CD, unlike γ-CD, cannot be hydrolyzed by human salivary and pancreatic amylases, but they are subjected to fermentation by the intestinal microflora.

CDs are non-toxic at low to moderate oral dosages. The natural CDs and their derivatives are used in topical and oral formulations, but only α-CD and the hydrophilic derivatives of β- and γ-CD can be used in the preparation of parenteral formulations [9].

2. MATERIALS AND METHODS

2.1. Materials

TSM was kindly provided by Global Napi Pharmaceuticals, Egypt; Methyl alcohol was supplied from ELNASR Pharmaceutical chemicals co., Egypt; Potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate were supplied from NICE Chemicals (p) LTD, India; β-cyclodextrin and hydroxypropyl- β-cyclodextrin were supplied from SIGMA-ALDRICH, Germany; all other materials and solvents were of analytical grade.

2.2. Equipment:

- Digital sensitive electric balance (RADWAG, Poland).
- Double beam spectrophotometer, UV-1601 (Shimadzu Co., Japan).
- Dissolution test apparatus, SR II, 6 flasks (Hanson Research Co., USA).
- Ultrasonic water bath (ultrasonic cleaner set, Korea).
- Vacuum oven drier, SPT-200 (Zeamil Horyzont Co., Poland).
- X-ray diffractometer (Philips Co., 1710 Netherland).
- Infrared spectrophotometer, IR-476 (Shimadzu Co., Japan).
- Differential scanning calorimeter, DSC-50, (Shimadzu Co., Japan).
- Desiccator (MPW, Med. Industries. Poland).

2.3. Methods

2.3.1. Solubility study

The inclusion complex of torsemide with β-CD and HP-β-CD in phosphate buffer pH 7.4 was studied by solubility method at 37^0C which employs a saturated solution of the material, obtained by shaking an excess of the material in the solvent for a prolonged period until equilibrium is achieved.

2.3.2. Preparation of inclusion complexes

2.3.2. 1. Inclusion complex preparation

Inclusion complex of TSM was prepared by using two different polymers: β-cyclodextrin (β-CD) and HP-β-cyclodextrin (HP-β-CD) with co-evaporation technique. 1:1 molar ratio drug: polymer inclusion complex was prepared.

Co-evaporates of TSM with β-CD and HP-β-CD (1:1) molar ratio were prepared by dissolving calculated amount of torsemide in methanol and the equivalent amount of β-CD and HP-β-CD in distilled water and mixed together then sonicated till clear solution is resulted. The solvents were removed by keeping the mixture at room temperature till complete drying. The obtained co-evaporates were sieved to obtain a particle size range of (125-250) μm and stored in a desiccator till further studying.

2.3.2. 2. Physical mixtures of torsemide with different polymers

Physical mixtures of TSM with CDs were prepared by blending torsemide and CDs at 1:1 molar ratio using a mortar and pestle. The obtained mixtures were sieved and then stored in a desiccator till further studying.

2.3.3. Physical characterization of inclusion complex

2.3.3. 1. Drug content estimation

Accurately weighed the amount of IC and its physical mixtures equivalent to 20 mg of TSM was dissolved in phosphate buffer pH 7.4 using Sonicator, and the concentration of TSM was determined spectrophotometrically at 285.5 nm. Only those samples containing 100± 15% of the claimed amount was used for further studies.

2.3.3. 2. Dissolution rate determination

A dissolution study was performed for different ICs of TSM with different polymers using USP dissolution apparatus type II. By weighing amounts of each formulation equivalent to 20 mg TSM was sprinkled over the surface of the dissolution medium. The dissolution medium was 900

ml phosphate buffer of pH 7.4 at 37^0C ±1^0C and 50 rpm. Samples were taken at 5, 10, 15, 20, 25, 30, 45, and 60 min. The amount of torsemide dissolved was determined spectrophotometrically at 285.5 nm using the dissolution medium as a blank [10].

2.3.3. 3. FTIR- spectroscopy characterization

FTIR spectra of TSM, β-CD, HP-β-CD and physical mixtures and their binary ICs were recorded using FTIR spectrophotometer, Jasco, Japan. Samples were mixed with potassium bromide (Spectroscopic grade) and compressed into disks using a hydraulic press and scanned from 4000 to 400 cm^{-1}[11].

2.3.3. 4. Differential scanning calorimetry (DSC)

Thermograms of the samples (TSM, polymers, binary physical mixtures and ICs) were recorded using a differential scanning calorimetry (DSC) (DSC-60, Shimadzu, Kyoto, Japan). Samples equivalent to 20 mg of the drug were loaded into aluminum pans and the lids were crimped. The thermal behavior of samples was investigated under nitrogen with a heating rate of 10^0C/min and temperature ranges of 25–200^0C [12].

3. RESULTS AND DISCUSSION

3.1. Solubility study

The inclusion complex of torsemide with β-CD and HP-β-CD in phosphate buffer pH 7.4 was studied by solubility method at 37^0C. The phase solubility diagrams of torsemide with β- CD and HP-β-D are presented in Table (1) and illustrated in Figure (3). The results showed that the solubility of torsemide decreased as a function of cyclodextrin concentration. Thus, the phase solubility diagrams of torsemide can be classified as A_N-type [13]. Type A_N- solubility phase diagram can be explained as the solubilizer (polymer) is altering the bulk properties of the media by changing its viscosity, surface tension or conductivity and/or self-association of the solubilized at high concentrations. The results also showed that the effect of β-CD on solubility was more effective than HP-β- CD.

Table 1: Phase solubility studies of torsemide in presence of β-CD and HP-β-CD in phosphate buffer pH 7.4:

Molar concentration of polymers	Concentration of solubilized torsemide (M) with	
	β- cyclodextrin	HP-β-cyclodextrin
0	2.561	2.561
0.01	4.528	4.166
0.02	3.973	3.865
0.03	3.549	3.456
0.05	3.487	3.348

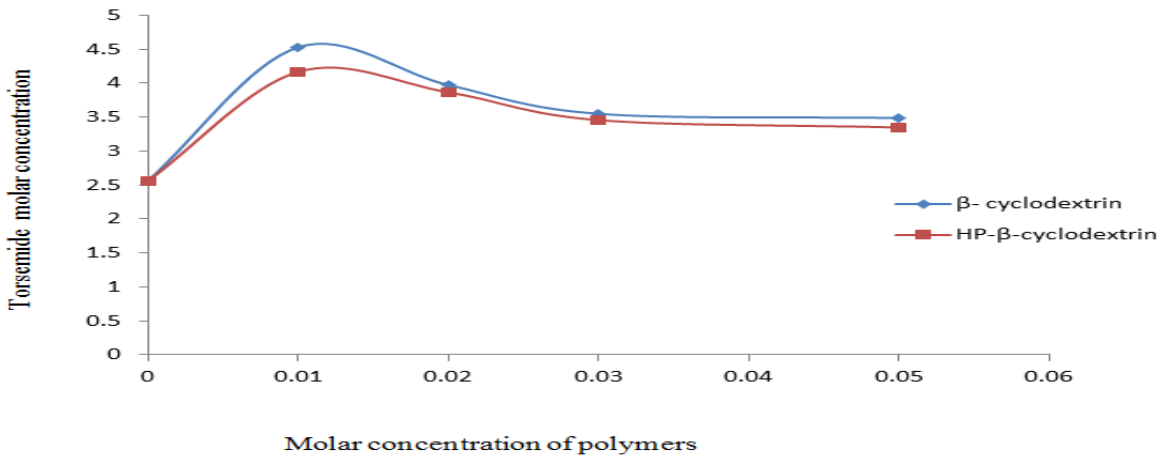

Figure 3: Solubility studies of torsemide in presence of different concentrations of β-cyclodextrin and HP-β-cyclodextrin in phosphate buffer pH 7.4

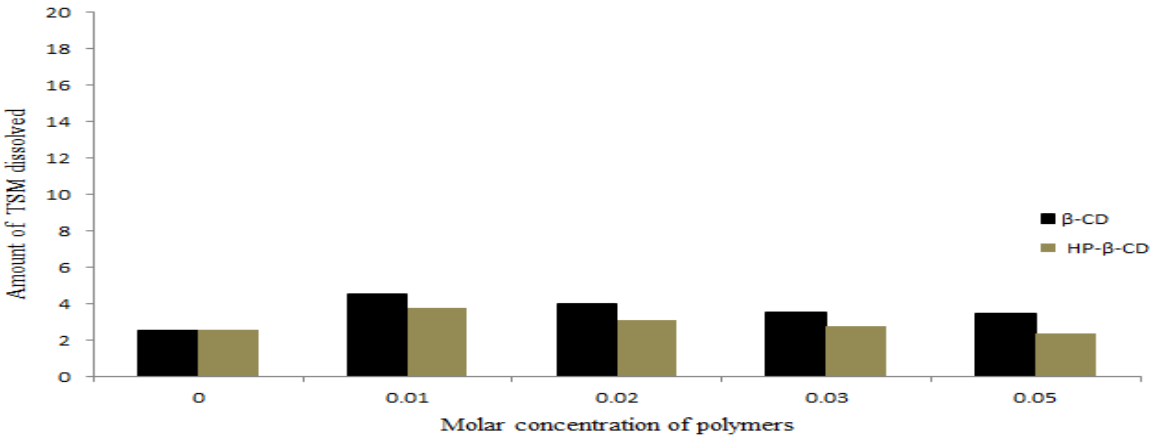

Figure 4: Bar graph illustrates the solubility study of torsemide with different molar concentrations of β-CD and HP-β-CD.

3.2. Drug content estimation

The drug content of the prepared ICs was in the acceptable range (table 2). The drug content values were in the range of 98.6– 102% w/w, excluding any segregation of the drug or polymer during ICs preparation. The solid state characterization involved thermal analysis using the DSC, FTIR, and dissolution rate studies.

Table 2: Drug content estimation of TSM in the β-CD and HP-β-CD inclusion complexes and its corresponding physical mixtures:

Formulation	% Drug Content ± SD
1:1 β-CD inclusion complex	99.78 ± 0.534
1:1 β-CD physical mixture	102 ± 0.397
1:1 HP-β-CD inclusion complex	98.60 ± 0.743
1:1 HP-β-CD physical mixture	100.24± 0.444

Results ±SD (n=3)

3.3. Dissolution rate determination

The dissolution study of ICs and physical mixtures of TSM with β-CD and HP-β-CD (1:1 molar ratio) in comparison with pure TSM are illustrated in Figure 4. The resulted showed that the

inclusion complex of TSM with β - CD exhibited higher dissolution rate than inclusion complex with HP-β-CD, physical mixture, and pure torsemide. Also, results showed that the dissolution rate of pure TSM was higher than the physical mixture of the drug with β-CD and HP-β-CD in 1:1 molar ratio. TSM solubility enhancement may be due to the transformation of it from crystal form to amorphous form; also, the wetting effect of polymers may be attributed to the enhancement of solubility. The release profile of TSM from ICs and from physical mixtures is illustrated in table 3 and figure 4.

Table 3: *In-vitro* TSM dissolution profile of different formulations in phosphate buffer pH 7.4:

Time(minute)	% Cumulative amount of TSM dissolved				
	Pure TSM	1:1 β-CD IC	1:1β-CD Ph. Mix.	1:1 HP-β-CD IC	1:1HP-β-CD Ph. Mix
0	0	0	0	0	0
2	7.98	58.54	3.38	57.09	3.26
4	10.80	65.28	4.85	73.01	4.61
6	16.51	71.24	14.54	74.19	5.95
8	20.04	76.72	23.66	75.16	9.34
10	24.87	80.74	26.37	76.61	13.59
15	31.34	85.11	29.78	77.59	18.94
20	38.39	88.04	34.27	77.84	29.86
25	44.23	89.26	36.96	78.08	34.51
30	47.29	90.12	43.38	78.32	39.13
45	57.83	90.73	50.19	78.45	47.63
60	64.30	91.09	57.00	78.81	57.95

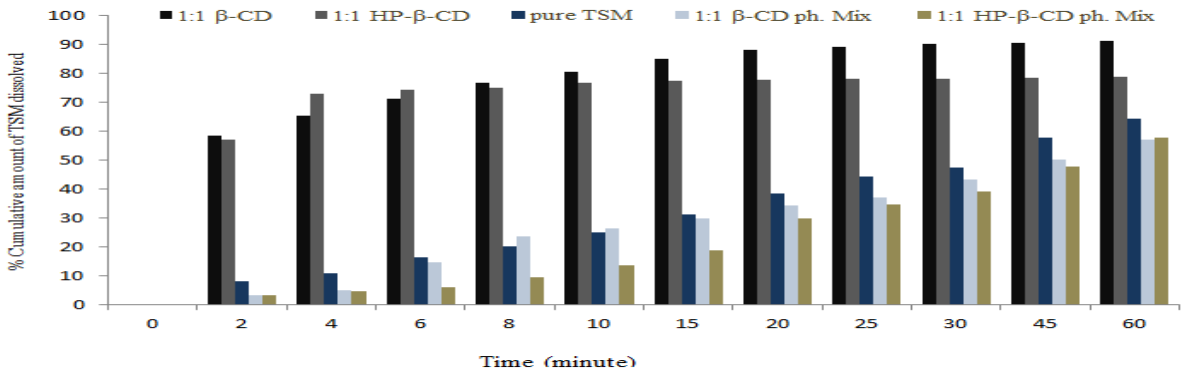

Figure 5: Dissolution profile of pure TSM and inclusion complexes of it with β-CD and HP-β-CD in 1:1 molar ratio and its corresponding physical mixtures.

3.4. Differential scanning calorimetry (DSC)

Further supporting evidence for the formation of solid inclusion complexes was obtained from the DSC thermograms of TSM with the used CDs. These thermograms are illustrated in Figure (5).

DSC studies were performed to detect the solid state changes of TSM with the used carriers; it conducted on the pure drug, pure polymers, IC of the drug with the selected polymers in (1:1) molar ratio and its corresponding physical mixtures. The DSC thermogram of the untreated drug shows an endothermic peak at 164 ˚C at a scanning rate of 12^0C/min. The endothermic peak of the drug is related to its melting point.

The DSC curves of CDs are characterized by broad endothermic peaks due to the release of water molecules entrapped inside the cavity (Figure 5, trace B and E).

DSC thermograms of TSM /CDs physical mixtures (which are illustrated in trace C and F of Figure 5) show the presence of an endothermic peak of the drug but with little shift and reduction in the intensity. Regarding the DSC thermogram of TSM /β-CD co-evaporate (Figure 5, trace G) and TSM /HP-β-CD co-evaporate (Figure 5, trace D) show the disappearance of any endothermic peak is observed which may attribute to the complete transformation of drug from crystalline to amorphous state, and so the considerable increase in the dissolution rate may be attributed to both complex formation and conversion of drug to amorphous state.

From the previous results, we can conclude that the extent of conversion of TSM from crystalline to the amorphous state has been attained by usingβ-CDand HP-β-CD. This confirms the results obtained from dissolution studies.

3.5. FTIR Spectroscopy

The FTIR spectra were conducted in order to detect the interaction between TSM and the polymeric carrier in the ICs and its corresponding physical mixture. Figure (6) demonstrate the IR spectra of pure TSM, pure polymers (β-CD, HP-β-CD), and ICs of TSM with these polymers (1:1) molar ratio, and its corresponding physical mixtures. The spectrum of TSM showed characteristic bands, at wave number 1579 cm^{-1} corresponding to carbonyl group (C=O), 2850 cm^{-1} corresponding to aromatic (C=C) stretching, 3385 cm^{-1} corresponding to tertiary amine group(N-H), 1384 cm^{-1} corresponding to sulphone group(S=O) and other peak at 1466 cm^{-1} refer to aromatic group. The characteristic IR bands of TSM can be seen in the physical mixtures of TSM /CDs at the same position. In the case of solid complexes, there is a small shift in the position of some peaks about 2-3 cm^{-1}) as well as reduction of the intensity of the peaks. The observed changes in the drug IR spectrum indicate that no chemical interaction has been occurred between drug and CDs and these changes may attribute to the inclusion of the drug within the CD cavity, dissociation of the intramolecular hydrogen bonds associated with the drug molecules and formation of new intermolecular hydrogen bonds between the drug and CD molecules. Finally, it could be concluded that, the co-evaporate products of TSM with β-CD and HP-β-CD can be considered as solid complexes and there is no any chemical interaction between TSM and the used polymers.

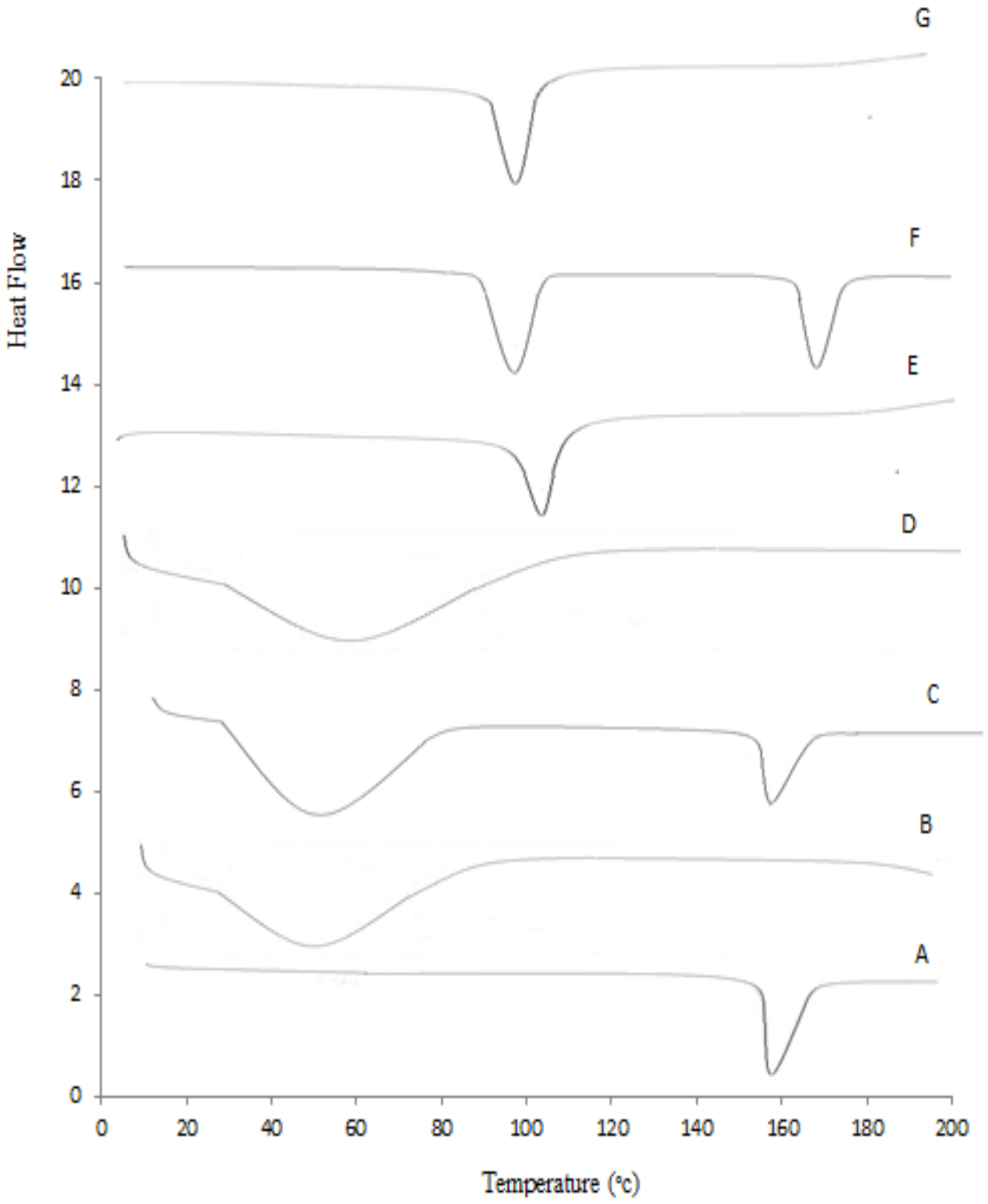

Figure 5: DSC thermograms of: A- Pure TSM, B- HP-β-CD, C- (1:1) Physical mixture with HP-β-CD, D- (1:1) IC with HP-β-CD, E- β-CD, F- (1:1) Physical mixture with β-CD, G- (1:1) IC with β-CD.

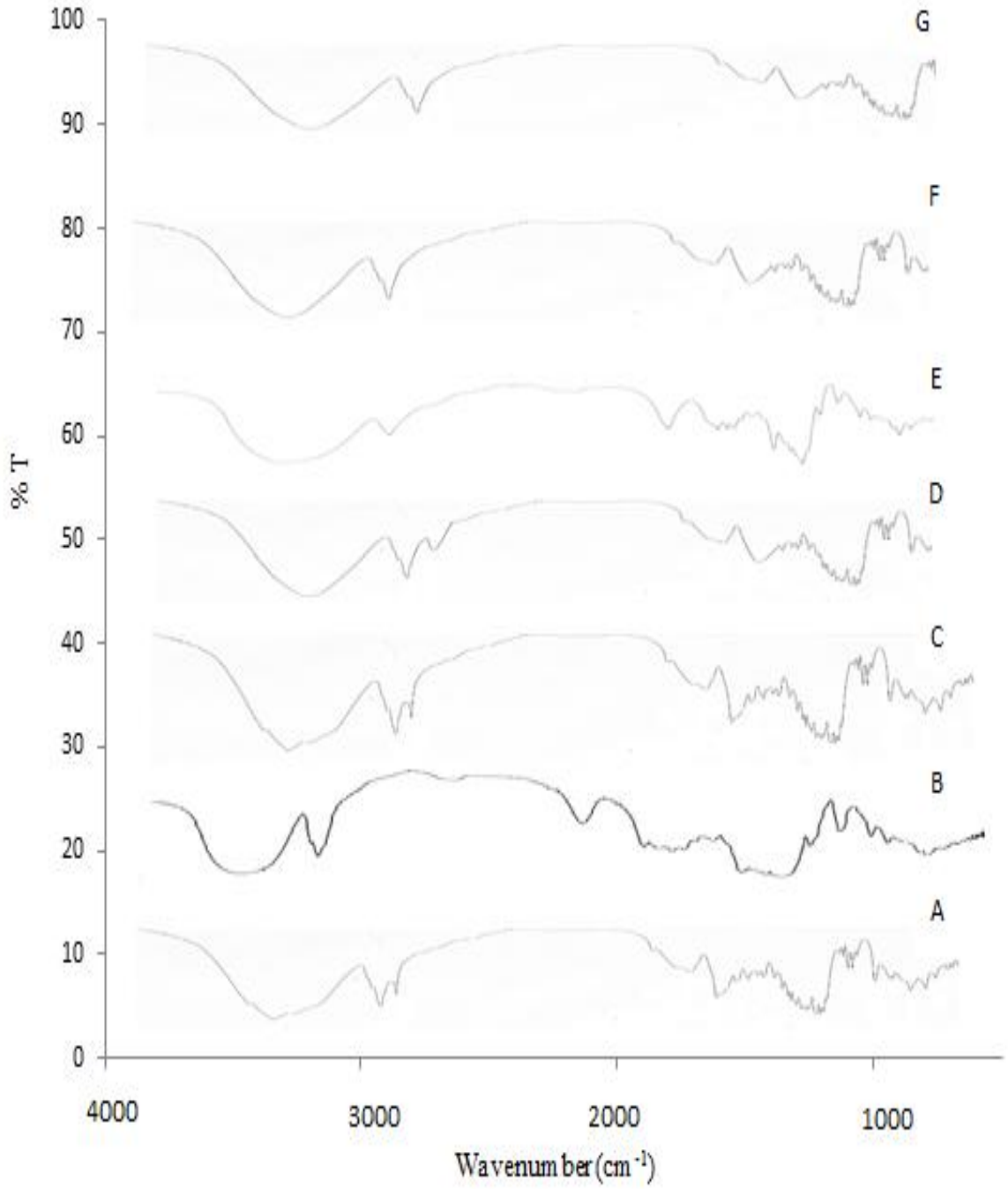

Figure 6: The FTIR absorption spectra of TSM ICs with β-CD and HP-β-CD in a drug / polymer molar ratio (1:1) where: A- Pure TSM, B- HP-β-CD, C- (1:1) Physical mixture with HP-β-CD, D- (1:1) IC with HP-β-CD, E- β-CD, F- (1:1) Physical mixture with β-CD, G- (1:1) IC with β-CD.

4. CONCLUSION

Cyclodextrins like β -CD and HP- β -CD can be used to prepare inclusion complexes of TSM with an improved solubility of the drug. TSM formed inclusion complexes with β -CD and HP- β -CD in 1:1 molar ratio. All inclusion complexes showed an increase in dissolution rate than pure drug. The inclusion complexes prepared with β-CD by co-evaporate method showed the highest enhancement in dissolution profile. DSC and FTIR analysis of the prepared inclusion complexes showed that no interaction between the drug and the polymers.

Acknowledgments

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