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Binary inclusion complexes of diflunisal with β-cyclodextrin and hydroxypropyl-β-cyclodextrin: Preparation and characterization

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Abstract: Low aqueous solubility and bioavailability is the limiting factor to achieve desired therapeutic efficacy for variety of new and existing drug moieties. The goal of the present study was to explore the effects of β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) on the solubility and dissolution profile of diflunisal (DIF) prepared by using two different methods (physical mixing and solvent evaporation) at DIF-cyclodextrins weight ratios of 1:1, 1:2 and 1:4. The phase solubility studies demonstrated that DIF solubility increased proportionally with an increase in βCD and HPβCD concentration. The inclusion complexes were subjected to characterization of scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). Solvent evaporation yielded higher DIF solubility than physical mixing method. HPβCD-DIF inclusion complexes yielded higher dissolution profile than βCD complexes when prepared under same experimental design. FTIR, DSC and XRD confirmed the successful inclusion of DIF into cyclodextrin (βCD/HPβCD) by both preparation methods with enhanced water solubility and drug release in comparison with pure drug.

Keywords: Cyclodextrins, diflunisal, dissolution, inclusion complexes, solubility.

INTRODUCTION

There has been a tremendous increase in aqueous solubility of poorly water soluble drugs by using high-throughput screening (HTS) and combinatorial chemistry in the field of drug discovery (Lipinski, *et al*., 1997). These drugs pose great problem in drug research and development because the aqueous solubility is considered as rate limiting step in the drug dissolution. The poor dissolution profile ultimately results in low drug bioavailability (Horter and Dressman, 2001). Under such circumstances dose escalation is mandatory to maintain therapeutic drug concentration in the blood which can cause toxicity and ultimately leads to patient non-compliance. For the development of pharmaceutical product, it is advisable to have complete understanding of biopharmaceutical and physicochemical properties of individual drug moiety. From a biopharmaceutical point of view, Biopharmaceutical classification system (BCS) is considered as a valuable tool during formulation development (Amidon, *et al*., 1995).

BCS class I drugs exhibit high solubility and high permeability, whereas BCS class II drugs have high permeability with poor solubility. Drugs with high poor permeability and high solubility represent BCS class III and lastly the drugs with poor permeability and poor solubility can be found in BCS class IV (Zoeller, *et al*., 2012).

Cyclodextrins (CD) are basically torus shaped cyclic oligosaccharides having ring structure of α (1→4)-linked glucose units, with hydrophobic central cavity and hydrophilic external surface (Fulop, *et al*., 2014; Jambhekar and Breen, 2016). They have been used extensively as important pharmaceutical excipients, owing to its ability to form inclusion complexes, where a hydrophobic drug moiety (guest molecule) is incorporated in the central lipophilic core (Loftssona and Jarvinen, 1999) through some non-covalent interactions including electronic effects, hydrophobic interactions, steric factors, van der Waals forces and hydrogen bonding (Mura, 2015; Mendes, *et al*., 2016). Inclusion complexation of hydrophobic moiety will not only result in improved physicochemical properties but also enhanced solubility and dissolution profile that ultimately leads to improved drug bioavailability and stability (Wang *et al*., 2014; Vickers, 2017; Bashir M *et al*., 2020). Cyclodextrins also has been associated with reduced cytotoxicity (Tang, *et al*., 2015). β-cyclodextrin (β-CD) being cost effective, is widely utilized cyclodextrin, but its low aqueous solubility poses problem. To address the solubility

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problem associated with β-CD, a 2-hydroxypropyl-β-CD (HP-β-CD) was developed by substituting the hydroxyl groups in the ring structure of molecule which will lead to improved toxicological and solubility profile (Sergey and Thorsteinn 2013).

Diflunisal (DIF), a BCS class II drug, which is categorized as non steroidal anti-inflammatory drug (NSAID) is basically salicylic acid derivative (developed by Merck Sharp and Dohme research laboratories). It exhibits anti-inflammatory, antipyretic and analgesic properties. The pain associated with inflammatory conditions (osteoarthritis, rheumatoid arthritis and post-episiotomy pain etc) can be better addressed by this drug. Till now only the film coated tablets for oral administration of DIF are available commercially. Poor aqueous solubility and limited dissolution profile is considered as major barrier for drug bioavailability in gastric fluid (Kaur, *et al*., 2017). Hence, the formulation of inclusion complexes could be a better option to improve the solubility and dissolution profile of DIF. The available literature on diflunisal is related to its analytical characterization such as ion electrode potentiometry and nuclear magnetic resonance (Lincoln *et al*., 1988; Sideris *et al*., 1999). The concept of binary system was utilized by zugasti *et al* in 2009 by using coevaporation technique, but the used only single ratio. The reported work regarding solubility enhancement of diflunisal included solid dispersions of polyethylene glycol and eudragit (Rs100 and RL 100), coprecipitates with polyvinyl pyrolidine and pyrazinamide-nicotinamide co-crystals (Najib and Suleiman, 1989; Pignatello *et al*., 2001; Rodriguez *et al*., 1998; Evora *et al*., 2011; Wang *et al*., 2013). Till now no other significant work was performed for solubility enhancement of diflunisal.

In the present work, our aim was to develop and characterize the binary complexes of DIF by using βCD and its derivative HPβCD by physical mixing and solvent evaporation method and to present a comprehensive data for the researchers and scientists working on the solubility and dissolution enhancement of DIF.

MATERIALS AND METHODS

Materials

Diflunisal (CAS: 22494-42-4) was provided by AK scientific, whereas Roquette (Lestrem, France) provided both beta-cyclodextrin (βCD) (Batch: E 1089) and its derivative HPβCD (Batch E0347). All materials were utilized as received.

Phase solubility study

Higuchi and Connors methodology (1965) was adopted for phase solubility study. Excess quantity of DIF was added into 10 mL distilled water having various concentrations of β-CD and HPβ-CD. The flasks were maintained in shaking bath maintained at 37 ± 2°C for next 72 hours by keeping the speed of 100 rpm to reach equilibrium. Samples of 3 mL were withdrawn and centrifuged at 6000 rpm for 30 minutes and filtered through a 0.45 µm membrane syringe filter. The filtrate was analyzed spectrophotometrically at 251.5 nm wavelength after suitable dilution. Each measurement was repeated in triplicate. The complexation efficiency, Gibbs free energy and stability constant from the phase solubility diagram were calculated by using the following equations.

$$Ks = \frac{slope}{So(1-slope)} \quad (1)$$

Where So is the equilibrium solubility of DIF in water.

$$C.E = \frac{slope}{1-slope} \quad (2)$$

$$\Delta G°tr = -2.303RTlog(S°/Ss) \quad (3)$$

Whereas, So/Ss is the ratio of the molar solubility of DIF in aqueous solution of CDs to that of the pure water.

DIF/CDs binary inclusion complexes

The inclusion complexes of DIF/CD were prepared by using βCD and its derivative HPβCD with 1:1, 1:2 and 1:4 weight ratios by using two different methodologies including physical mixing (trituration) and solvent evaporation.

Physical mixing involved proper mixing of weighed quantities of drug and cyclodextrin in mortar and pestle up to one hour to ensure final consistent mixture. The final mixture was then passed through sieve number 60 and kept in a sealed container.

In solvent evaporation technique, cyclodextrins (βCD or HPβCD) were dissolved in 25ml water and DIF in ethanol followed by through mixing of these two solutions by magnetic stirrer at heating temperature of 50 °C at which ethanol was evaporated. Rotary evaporator was used to remove residual water. The dried mass was then ground to powder and stored in tight container after sieving.

Solubility studies

The surplus amount of the solid binary complexes was added in 20 mL of distilled water followed by vortex mixing for 3 minute. The flasks were then agitated for 72 hours at 100 rounds per minute at 30°C. Appropriate quantity of samples were taken and filtered through membrane filter (0.45 µm). The filtrate was analyzed by UV spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) at 251.5 nm after suitable dilution. Each measurement was taken three times.

Drug contents determination

Drug content of DIF inclusion complex was determined by adding complex equivalent to 10 mg of pure drug in 10 ml methanol followed by vortex mixing and centrifugation to separate the supernatant. The absorbance of supernatant was taken after suitable dilution and found the percent drug content by the following equation

$$\%D.C = conc.of drug in \frac{sample}{actual} * 100 \quad (4)$$

In-vitro dissolution studies

USP dissolution apparatus II (PTWS 3CE, Pharmatest, Hainburg, Germany) paddle method (USP XXXII, 2009) was used for *in-vitro* dissolution study for both pure DIF and its binary complexes. Distilled water (900 ml) was used as dissolution medium with a paddle speed of 100 rpm at 37 ± 0.5°C .The powder weight equal to 10 mg of pure DIF was weighed precisely and added in all the vessels. Sampling was done at predetermined time points of 2.5, 5, 10, 15, 30, 45 and 60 minutes and analyzed spectrophotometrically at 251.5 nm.

The dissolution efficiency was determined by applying the following equation,

$$DE(\%) = \frac{\int_0^t yXdt}{y\ 100\ Xt} X\ 100\% \quad (5)$$

The dissolution half life ($T_{50\%}$) was calculated for all binary complexes and pure DIF.

Scanning electron microscopy (SEM)

Scanning electron microscope (JSM-6480, Tokyo, Japan) was used to elucidate surface morphology of samples.

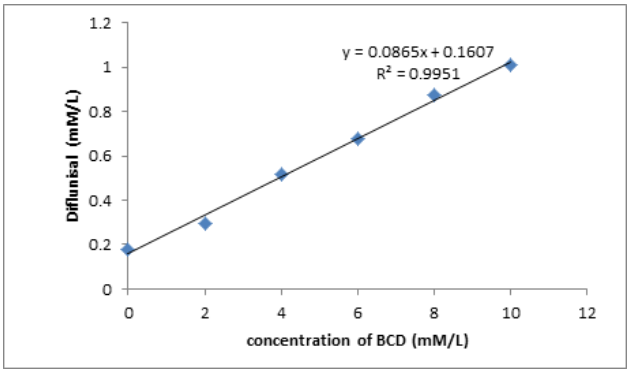

Fig. 1(A): DIF phase solubility diagram in aqueous solution of βCD. Mean ± SD, N=3

Fourier transform infrared spectroscopy (FTIR)

Potassium bromide (KBr) disk method was adopted to obtain FTIR spectrum of samples. FTIR (BRUKER-Tensor II-Alpha, Berlin, Germany) was used for analysis and samples were scanned over 400 to 4000 cm^{-1} spectral range.

Differential scanning calorimetry (DSC)

Differential scanning calorimeter (Universal V4.2E TA Instruments, Newcastle, USA) was used to record thermograms of samples. Sample of 5 mg was placed in aluminium pan and heated in an atmosphere of nitrogen (20 ml/min) at a rate of 10 °C/min over a temperature range of (25-400 °C).

X-ray diffractometry (XRD)

X-ray diffractograms of all samples were recorded to estimate the physical state of DIF. After slight grinding, the samples were packed in aluminium pan. The X-ray tube was drived at 30 mA current with a potential of 30 kV. The scanning range was from 5-60°C at 0.02°C increment.

STATISTICAL ANALYSIS

All the results were analyzed statistically by using SPSS (version 20) and expressed in term of mean ± SD. One-way analysis of variance (ANOVA) was adopted for the analysis of solubility data. The results of $T_{50\%}$ and DE (dissolution efficiency) were elucidated through statistical analysis. The data was subjected to post-hoc Tukey-HSD in case of statistically significant difference. Statistically significant difference was considered in case of $p<0.05$.

RESULTS

Phase solubility study

DIF solubility in both cyclodextrins was analyzed by phase solubility study. Fig. 1(A and B) indicated the proportional increase in DIF solubility with the gradual increase in βCD and HPβCD concentration, with relatively higher solubility in HPβCD that could be justified by analogously superior solubilisation efficiency of HPβCD.

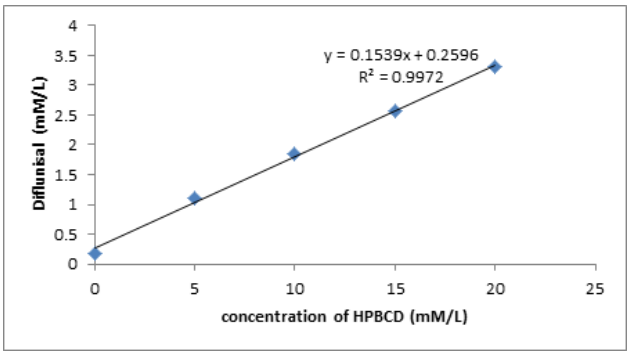

Fig. 1(B): DIF phase solubility diagram in aqueous solution of HPβCD. Mean ± SD, N=3

Solubility studies

The solubility data of DIF along with its binary inclusion complexes with βCD and HPβCD are given in table 2. Solubility was enhanced irrespective of the preparation methods both with βCD and HPβCD but with varied extent of solubility.

Evaluation for drug content

All binary complexes were analyzed for percent drug entrapment which was almost more than 96% for all formulations indicative of uniform drug distribution.

Table 1: Gibbs free energy of DIF-βCD/HPβCD complexes in water

βCD concentration (mM)	Gibb's free energy (J/mole)	HPβCD concentration (mM)	Gibb's free energy (J/mole)
2	-1154	5	-4134.8
4	-2433	10	-5315
6	-3046	15	-6052
8	-3610	20	-6628
10	-3941		

Table 2: Solubility profile of DIF and DIF-βCD and DIF-HPβCD. Mean ± SD, N=3

Code	Diflunisal: Cyclodextrin (Ratio)	Solubility (µg/ml)
βCD0 (Diflunisal)	Diflunisal: No Cyclodextrin	44.606 ± 0.023
βCD 1:1 (PT)	Diflunisal: βCD (1.0: 1.0)	152.907 ± 0.617*
βCD 1:2 (PT)	Diflunisal: βCD (1.0: 2.0)	154.333 ± 0.577*
βCD 1:4 (PT)	Diflunisal: βCD (1.0: 4.0)	155.107 ± 0.845*
HPβCD 1:1 (PT)	Diflunisal: HPβCD (1.0: 1.0)	309.013 ± 0.520*
HPβCD 1:2 (PT)	Diflunisal: HPβCD (1.0: 2.0)	310.000 ± 1.000*
HPβCD 1:4 (PT)	Diflunisal: HPβCD (1.0: 4.0)	310.888 ± 0.831*
βCD 1:1 (SE)	Diflunisal: βCD (1.0: 1.0)	173.873 ± 0.328*#
βCD 1:2 (SE)	Diflunisal: βCD (1.0: 2.0)	175.333 ± 0.577*#
βCD 1:4 (SE)	Diflunisal: βCD (1.0: 4.0)	175.853 ± 0.790*#
HPβCD 1:1 (SE)	Diflunisal: HPβCD (1.0: 1.0)	366.267 ± 0.642*#
HPβCD 1:2 (SE)	Diflunisal: HPβCD (1.0: 2.0)	485.000 ± 1.000*#
HPβCD 1:4 (SE)	Diflunisal: HPβCD (1.0: 4.0)	486.333 ± 0.577*#

*reflects $p<0.05$ vs Pure drug (DIF), # reflects $p<0.05$ vs PT

Table 3: The dissolution parameters of DIF, DIF/βCD, DIF/HPβCD (using two different methods). Mean ± SD, N=3

Code	DE60 (%)	$T_{50\%}$(min)
βCD0(Diflunisal)	24.238 ± 0.278	57.858 ± 0.207
βCD1:2(PT)	56.509 ± 0.091	14.963 ± 0.032
βCD1:2(SE)	58.311 ± 0.064	13.949 ± 0.028
HPβCD1:2(PT)	57.213 ± 0.014	13.469 ± 0.026
HPβCD1:2(SE)	63.063 ± 0.066	11.877 ± 0.035

In-vitro dissolution study

Fig. 2(A and B) indicated the %age dissolution of pure DIF along with its binary complexes prepared with both CDs at weight ratio of 1:2 by physical trituration and solvent evaporation method.

Scanning electron microscopy (SEM)

Fig. 3 showed scanning electron micrographs of DIF, βCD, HPβCD, DIF/ βCD and DIF /HPβCD binary inclusion complexes prepared by two different methodologies. Commercial DIF appeared as a crystalline matter seemed as bars of varied length and width (fig. 3a). βCD appeared as large rhomboidal crystals with rough surface(fig. 3b), while HPβCD exhibited oval shaped glassy smooth particles of distinct size having concave depression. The smaller particles were tending to attached with larger particles (fig. 3c).

Fourier transformation-infrared spectroscopy (FTIR)

Fig. 4 showed the FTIR spectra of DIF, CDs and DIF inclusion complexes. In the DIF spectrum, due to aromatic -C-H stretching and OH association and stretching, a distinctive broad band appeared at 3152 cm^{-1}. Peaks at 1678 cm^{-1}, 1664 cm^{-1} and 1269 cm^{-1} were correlated with -C=O stretching, stretching in phenyl nucleus and CF stretch (fig. 4a). In case of βCD and HPβCD, a strong and broad band appeared between 3300-3400cm^{-1} which is corresponded to stretching vibrations of -OH groups relative to intermolecular hydrogen bonds, the peak at 2920cm^{-1} was assigned to stretching vibration of aliphatic C-H group, whereas a band at 1600 cm^{-1} is suggestive of deformation vibration of OH group and C-O stretching vibration sin ester and bands located at 1030 and 1150 cm^{-1} correspond to C-O-C stretching vibration (Das and Subuddhi U, 2013) (fig. 4b & c).

Differential scanning calorimetry (DSC)

Fig. 5 showed the DSC thermograms of DIF, βCD, HPβCD, DIF/ βCD and DIF/ HPβCD binary inclusion complexes prepared by two different methods. Pure diflunisal manifested an endothermic peak around 210°C (fig. 5a). βCD and HPβCD showed broad endothermic peaks at 92.41°C and 61.79°C specifying loss of water due to dehydration process and melting peaks at 325.18°C and 340.22°C respectively (fig. 5b & c).

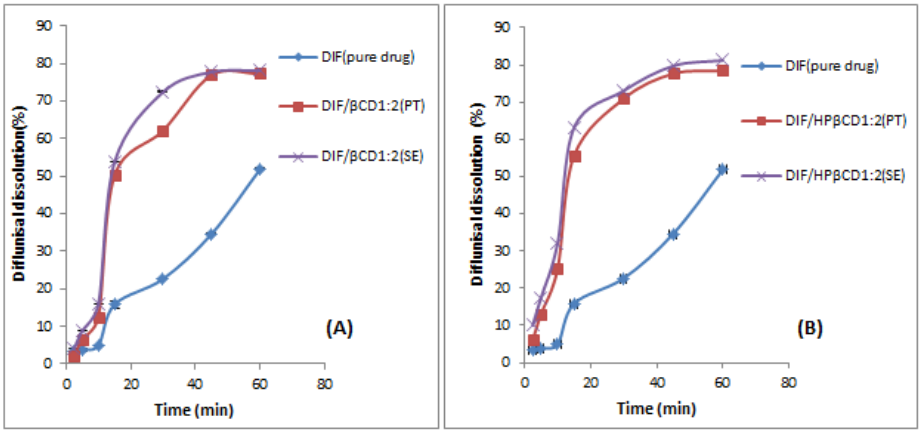

Fig. 2: Percent release profiles of DIF and binary complexes with βCD (A) and HPβCD (B). Mean ± SD, N=3.

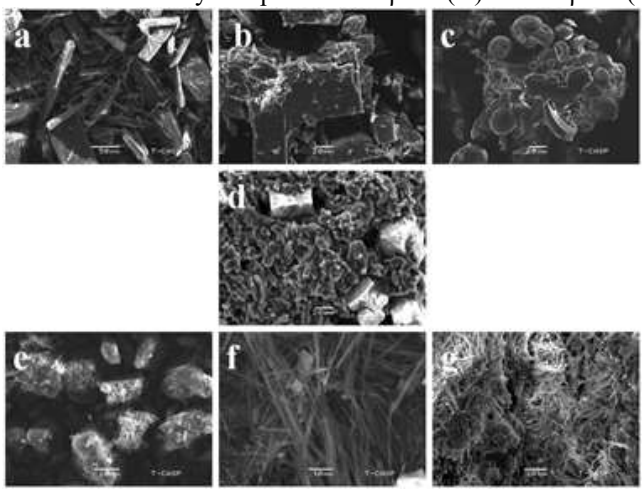

Fig. 3: Scanning electron micrographs of (a) DIF; (b) βCD; (c) HPβCD; (d) DIF/βCD(1:2), prepared by physical mixing; (e) DIF/HPβCD(1:2), prepared by physical mixing; (f) DIF/βCD(1:2), prepared by solvent evaporation method (g) DIF/HPβCD(1:2), prepared by solvent evaporation method

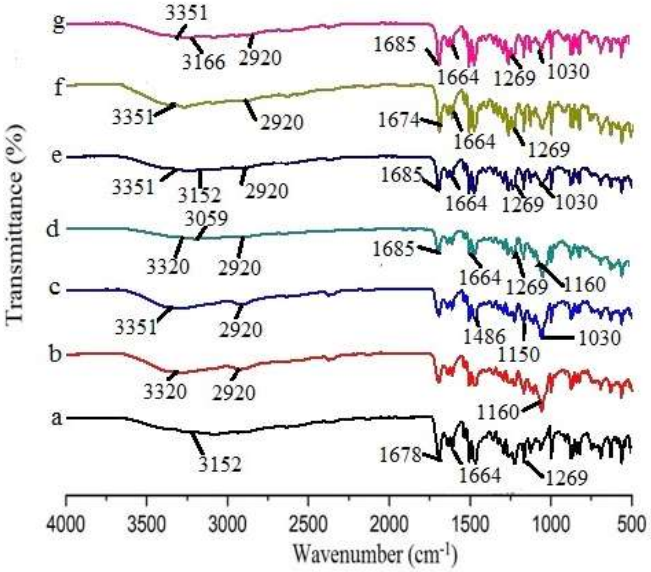

Fig. 4; FTIR spectra of (a) DIF; (b) βCD; (c) HPβCD; (d) DIF/βCD(1:2), prepared by physical mixing; (e) DIF/HPβCD(1:2), prepared by physical mixing; (f) DIF/βCD(1:2), prepared by solvent evaporation method (g) DIF/HPβCD(1:2), prepared by solvent evaporation method

An endothermic peak at 210°C corresponding to DIF melting point was displayed by all binary complexes of either cyclodextrins formulated by physical trituration, so it is evident that this method was not suitable method to integrate drug molecule inside the cyclodextrin aperture (fig. 5d & e). Binary complexes prepared by solvent evaporation indicated the peak shifting to 211.75°C (for DIF/βCD) (fig. 5f) and 212.12°C (for DIF/HPβCD) (fig. 5g) with considerable reduction in peak intensity as compared to physical trituration technique. From the DSC data of binary complexes it is clear that solvent evaporation is quite suitable for preparation of inclusion complex of DIF whereas HPβCD produced better complexation than that of βCD.

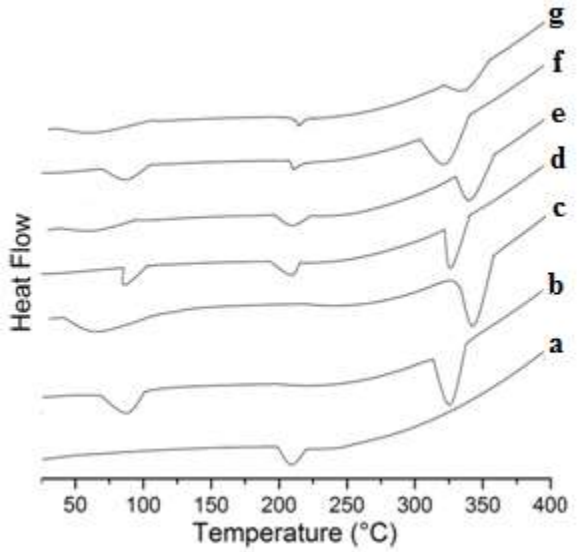

Fig. 5: DSC thermograms of (A) DIF; (B) βCD; (C) HPβCD; (D) DIF/βCD(1:2), prepared by physical mixing; (E) DIF/HPβCD(1:2), prepared by physical mixing; (F) DIF/βCD(1:2), prepared by solvent evaporation method (G) DIF/HPβCD(1:2), prepared by solvent evaporation method

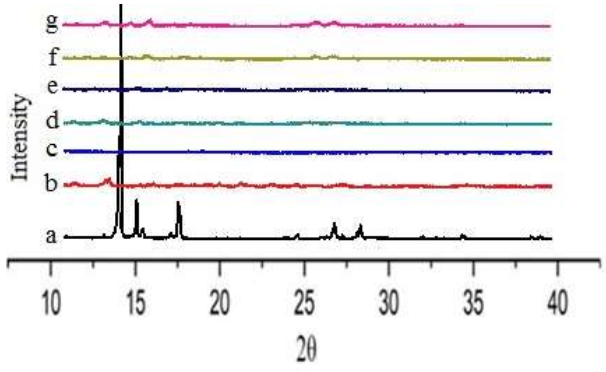

Fig. 6: XRD diffractograms of (A) DIF; (B) βCD; (C) HPβCD; (D) DIF/βCD(1:2), prepared by physical mixing; (E) DIF/HPβCD(1:2), prepared by physical mixing; (F) DIF/βCD(1:2), prepared by solvent evaporation method (G) DIF/HPβCD(1:2), prepared by solvent evaporation method

X-ray diffractometry (XRD)

Fig. 6 showed the powder XRD patterns for DIF and its inclusion complexes. DIF exhibited crystalline nature and manifested characteristic peaks at 2θ values of 13.49°, 14.43°, 16.99° and two less intense peaks at 26.50° and 27.90° (fig. 6a). βCD exhibited crystalline features (fig. 6b) while HPβCD seemed to be in amorphous state (fig. 6c).

DISCUSSION

Phase solubility study

The ligand-host binding power could be better evaluated by determining the stability constant (K_S) values (Tommasini *et al*., 2004). In case of weaker ligand-host interaction, the K_S will be small which in turn specifies relatively larger amount of free ligand., while higher stability constant values denotes formulation of stable inclusion complexes. There is increase in stability constant from 528.601M^{-1} (with βCD) to 1014.800 M^{-1}(with HPβCD). In literature, reported value for stability constant ranged from 200 to 5000M^{-1} (Gururaj, *et al*., 2002) for improved drug stability. The value of complexation efficiency (C.E) for βCD was 0.094 and 0.180 for HPβCD. As C.E is least sensitive to errors related to determination of intrinsic solubility so it is quite favorable to compare C.E of inclusion complexes rather than their stability constants (Brewster and Loftsson 2007). The change in Gibbs free energy could be used to measure free energy that is available to perform practical work. At constant temperature and pressure, it provides a measure about spontaneity of the process (JS, *et al*., 2010). The reaction becomes more favorable when ΔG° is more negative. With both types of CDs, ΔG° values were negative and keep on increasing by increasing concentration of either cyclodextrins (table 1). Hence it was suggested that in comparison with pure water, the solution of CDs is quite favorable for DIF.

Solubility studies

It is evident from table 2 that both βCD and HPβCD increased the solubility of DIF with both physical trituration and solvent evaporation method, but to different extent. The binary complexes of βCD prepared by physical trituration method has increased solubility up to 152.90 µg/ml at 1:1 weight ratio and 154.33 µg/ml at ratio of 1:2. Similarly, with HPβCD binary complexes prepared by physical trituration, the observed solubility values were 309.013µg/ml (at 1:1) and 310.0 µg/ml (at 1:2). Further increase in concentration of both cyclodextrins caused minimal change in solubility.

The solubility values of βCD complexes prepared with solvent evaporation technique were 173.873µg/ml (at 1:1) and 175.333µg/ml (at 1:2), whereas the observed solubility values for HPβCD binary complexes prepared by same technique were 366.267µg/ml (at 1:1) and

485.000µg/ml (at 1:2). Further increment in cyclodextrins concentration did not affect solubility considerably.

Seven different subsets were designed by Post-hoc Tukey-HSD of DIF and various ratios of DIF/CDs. Hence it is clear from the solubility data that solvent evaporation method produced statistically significant ($p<0.05$) increase in solubility as compared to physical trituration and that the optimum ratio for preparation of binary complexes of DIF is 1:2 and was selected further for dissolution and characterization.

In-vitro dissolution study

Due to limited aqueous solubility, DIF manifested minimal dissolution than all other complexes. Only 50% release of pure DIF was observed even after 60 minutes. According to graphical results it could be concluded that both βCD and HPβCD complexes has increased the percent release of DIF. The percent drug release from all binary inclusion complexes (irrespective of method of preparation) was considerably higher than that of pure diflunisal. Improved dissolution of binary complexes prepared by physical trituration could be better related to enhanced wetting properties of complexed drug (Dua *et al*., 2007). Higher values of in vitro release with solvent evaporation system might be associated with various factors such as; drug amorphization, particle size reduction, formation of inclusion complexes, enhanced solubility and wettability (Veiga *et al*., 2001). The dissolution efficiency (DE) and time for 50% of drug release (T_{50}) results are mentioned in table 3. The results for T_{50} and DE60 of inclusion complexes (with both CD) were analyzed statistically and found to be significant ($p<0.05$) as compared to free drug. Free drug and its binary complexes were categorized to five subsets for T50 and DE60 according to Post-hoc Tukey-HSD. This difference could be possibly explained on the basis of rapid drug release in a short time period. The longest T_{50} was observed in case of pure DIF as compared to its binary complexes. Hence, it was clear from the *in-vitro* dissolution data that improved solubility and dissolution could be obtained by all solid inclusion complexes, which may be possibly described by the decreased drug crystallinity of final inclusion complexes (Bashir M *et al*., 2020).

Scanning electron microscopy (SEM)

In the binary complexes of both CDs prepared by physical trituration method, although the original shape of drug and carrier was lost due to particle aggregation and appeared as amorphous complex but still the crystalline features of drug were evident on the photomicrographs (fig. 3d & e). As far as the binary complexes prepared by solvent evaporation were concerned, they appeared as elongated fibers shaped structure in case of DIF/βCD (fig. 3f) and dense clusters in case of DIF/HPβCD binary complex, retaining distinct shape of original drug particles, while the porous shape of HPβCD was lost due to inclusion complexation with DIF (fig. 3g). These morphological changes could be better assigned to the mixing procedure. The presence of uneven and amorphous particles with asymmetrical size and shape indicated the successful formation of new solid phase. These modifications are thought to be responsible for the favorable changes in the solubility and in vitro dissolution profile of final inclusion complexes.

Fourier transformation-infrared spectroscopy (FTIR)

The successful formation of inclusion complexes was better indicated through intensity changes and shifting of peaks/bands. There was no major change in the IR spectrum of DIF, which specified the absence of chemical incompatibility among the formulation components (Bashir M *et al*., 2020). There was slight difference among binary system with two kinds of carriers (βCD and HPβCD). The change in shift and intensity of inclusion complex relative to individual components gave indication of inclusion complexation. The physical mixture of DIF/βCD or HPβCD showed bands identical to host and guest. While the peak at $1678cm^{-1}$ has undergone slight modification in all binary complexes (fig. 4d & e). While in case of solvent evaporation method, the drug peak at $3152cm^{-1}$ is shifted towards $3166cm^{-1}$ while retaining most of original peaks of drug. As the intensity of peaks was also reduced by the solvent evaporation so this method can be successfully used for the preparing binary inclusion complexes of DIF.

Differential scanning calorimetry (DSC)

An endothermic peak at 210°C corresponding to DIF melting point was displayed by all binary complexes of either cyclodextrins formulated by physical trituration, so it is evident that this method was not suitable method to integrate drug molecule inside the cyclodextrin aperture (fig. 5d & e). Binary complexes prepared by solvent evaporation indicated the peak shifting to 211.75°C (for DIF/βCD) (fig. 5f) and 212.12°C (for DIF/HPβCD) (fig. 5g) with considerable reduction in peak intensity as compared to physical trituration technique. From the DSC data of binary complexes it is clear that solvent evaporation is quite suitable for preparation of inclusion complex of DIF whereas HPβCD produced better complexation than that of βCD.

X-ray diffractometry (XRD)

Although the binary complexes (with either cyclodextrins) prepared by physical trituration showed crystalline peaks of DIF and cyclodextrins but their intensity is reduced and complexes appeared as amorphous (fig. 6d & e). Solvent evaporation tends to improve further amorphousness. This amorphousness is greater in binary complexes prepared with HPβCD as compared to βCD. It is clear from the diffractograms of binary complexes that higher inclusion complexation

efficiency could be achieved by using HPβCD and solvent evaporation method (fig. 6g). The XRD plots showed the typical crystalline peaks of DIF (with less number and reduced intensity). In the X-ray diffractogram, the nanosized particles of inclusion complexes lead to inadequate diffraction centers that lead to reduction in peaks intensity and ultimately cause peak broadening phenomenon (Pathak *et al*., 2010). A relationship between peak height and degree of crystallinity could be described according to Hodge *et al* criterion (1996). These findings suggest the presence of little crystallinity of DIF in the inclusion complexes but final formulations appear to be more amorphous. HPβCD binary complexes produced better solubility than that of βCD.

CONCLUSION

The solubility and dissolution of DIF was enhanced by physical mixing of DIF with βCD and HPβCD. The solubility of DIF was increased about 3.46 times with βCD and 6.95 times with HPβCD. The crystallinity of drug was not reduced significantly by physical trituration as indicated in XRD studies. In case of solvent evaporation method, the observed increase in solubility of DIF was about 3.93 times with βCD and 10.87 times with HPβCD. Advance characterization of prepared binary inclusion complexes such as SEM, FTIR, DSC and XRD has established successful inclusion complexation of DIF without any significant change. In future, these inclusion complexes prepared by solvent evaporation method could be effectively used to address solubility related problems of other hydrophobic drugs.

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REFERENCES

Amidon GL, Lennernas H, Shah VP and Crison JR (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and *in vivo* bioavailability. *Pharm. Res.*, **12(--- issue numbers---)**: 413-420.

Bashir M, Syed HK, Asghar S, Irfan M, Almalki WH, Menshawi SA, Khan IU, Shah PA, Khalid I, Ahmad J and Gohar UF (2020). Effect of Hydrophilic Polymers on Complexation Efficiency of Cyclodextrins in Enhancing Solubility and Release of Diflunisal. *Polymers*., **12**: 1564.

Brewster ME and Loftsson T (2007). Cyclodextrins as pharmaceutical solubilizers. *Adv. Drug Deliv. Rev.*, **59**: 645-666.

Das S and Subuddhi U (2013). Cyclodextrin mediated controlled release of naproxen from pH-sensitive chitosan/poly (vinyl alcohol) hydrogels for colon targeted delivery. *Ind. Eng. Chem*., **52**: 14192-14200.

Dua K, Ramana MV, Singh Sara UV, Himaja M, Agrawal A, Garg V and Pabreja K (2007). Investigation of enhancement of solubility of norfloxacin β-cyclodextrin in presence of acidic solubilizing additives. *Curr. Drug Deliv*., **4**: 21-25.

Evora AO, Castro RA, Maria TM, Rosado MT, Ramos Silva M, Matos Beja A, Canotilho J and Eusébio ME (2011). Pyrazinamide-diflunisal: A new dual-drug co-crystal (2011). *Cryst. growth des*. **11**: 4780-4788.

Fulop Z, Saokham P and Loftsson T (2014). Sulfobutylether-β-cyclodextrin/chitosan nano-and microparticles and their physicochemical characteristics. *Int. J. Pharm.,* **472**: 282-287.

Gururaj AE, Belakavadi M, Venkatesh DA, Marme D and Salimath BP (2002). Molecular mechanisms of anti-angiogenic effect of curcumin. *Biochem. Biophys., Res.,* **297**: 934-942.

Higuchi T and Connoras KA (1965). A phase solubility technique. Adv. Anal. Chem. Instrum., **4**: 117-211.

Hodge RM, Edward GH and Simon GP (1996). Water absorption and states of water in semicrystalline poly (vinyl alcohol) films (1996). *Polymer*., **37**: 1371-1376.

Horter D and Dressman JB (2001). Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract *Adv. Drug Deliv. Rev.,* **46**: 75-87.

Jambhekar SS and Breen P (2016). Cyclodextrins in pharmaceutical formulations I: structure and physicochemical properties, formation of complexes, and types of complex. *Drug Discov. Today,* **21**: 356-362.

Patil JS, Kadam DV, Marapur SC and Kamalapur MV (2010). Inclusion complex system; a novel technique to improve the solubility and bioavailability of poorly soluble drugs: A review. *Int. J. Pharm. Sci. Rev. Res.,* **2**: 29-34.

Kaur A, Bhoop BS, Chhibber S, Sharma G, Gondil VS and Katare OP (2017). Supramolecular nano-engineered lipidic carriers based on diflunisal-phospholipid complex for transdermal delivery: QbD based optimization, characterization and preclinical investigations for management of rheumatoid arthritis. *Int. J. Pharm.,* **533**: 206-224.

Lincoln SF, Hounslow AM, Coates JH, Villani RP and Schiller RL (1988). The inclusion of diflunisal by γ-cyclodextrin and permethylatedβ-cyclodextrin. A UV-visible and 19 F nuclear magnetic resonance spectroscopic study. *J. Inc. Phenom*., **6**: 183-191.

Lipinski CA, Lombardo F, Dominy BW and Feeney PJ (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.,* **23**: 3-25.

Loftssona T and Jarvinen T (1999). Cyclodextrins in ophthalmic drug delivery. *Adv. Drug Deliv. Rev.*, **36**: 59-79.

Mendes C, Buttchevitz A, Kruger JH, Kratz JM, Simoes CM, de Oliveira Benedet P, Oliveira PR and Silva MA (2016). Inclusion complexes of hydrochlorothiazide and β-cyclodextrin: Physicochemical characteristics, in vitro and *in vivo* studies. *Eur. J. Pharm. Sci.,* **83**: 71-78.

Mura P (2015). Analytical techniques for characterization of cyclodextrin complexes in the solid state: A review. *J. Pharm. Biomed.,* **113**: 226-238.

Najib NM and Suleiman MS (1989). Characterization of a diflunisal polyethylene glycol solid dispersion system (1989). *Int. J. Pharm.,* **51**: 225-232.

Pathak TK, Vasoya NH, Lakhani VK and Modi KB (2010). Structural and magnetic phase evolution study on needle-shaped nanoparticles of magnesium ferrite. *Ceram. Int*., **36**:275-281.

Pignatello R, Ferro M, De Guidi G, Salemi G, Vandelli MA, Guccione S, Geppi M, Forte C and Puglisi G (2001). Preparation, characterisation and photosensitivity studies of solid dispersions of diflunisal and Eudragit RS100® and RL100®. *Int. J. Pharm*., **218**: 27-42.

Rodriguez-Espinosa C, Martinez-Oharriz MC, Martin C, Goni MM, Velaz I and Sanchez M (1998). Dissolution kinetics for coprecipitates of diflunisal with PVP K30. *Eur. J Drug Metab. Ph*., **23**: 109-112.

Sergey VK and Thorsteinn L (2013). Cyclodextrins. *Int. J. Pharm.,* **453**: 167-180.

Sideris EE, Valsami GN, Koupparis MA and Macheras PE (1999). Studies on the interaction of diflunisal ion with cyclodextrins using ion-selective electrode potentiometry (1999). *Eur. J.Pharma. Sci*., **7**: 271-278.

Tang P, Li S, Wang L, Yang H, Yan J and Li H (2015). Inclusion complexes of chlorzoxazone with β-and hydroxypropyl-β-cyclodextrin: Characterization, dissolution and cytotoxicity. *Carbohydr. Polym.,* **131**: 297-305.

Tommasini S, Raneri D, Ficarra R, Calabrò ML, Stancanelli R and Ficarra P (2004). Improvement in solubility and dissolution rate of flavonoids by complexation with β-cyclodextrin (2004). *J. Pharm. Biomed*., **35**: 379-387.

Veiga F, Fernandes C and Maincent P (2001). Influence of the preparation method on the physicochemical properties of tolbutamide/cyclodextrin binary systems (2001). *Drug Dev. Ind. Pharm*., **27**: 523-532.

Vickers NJ (2017). Animal Communication: When I'm Calling You, Will You Answer Too?. *Curr. Biol.,* **27**: R7113-15.

Wang L, Tan B, Zhang H and Deng Z (2013). Pharmaceutical cocrystals of diflunisal with nicotinamide or isonicotinamide. *Org. Process Res. Dev*., **17**: 1413-1418.

Wang X, Luo Z and Xiao Z (2014). Preparation, characterization and thermal stability of β-cyclodextrin/soybean lecithin inclusion complex. *Carbohydr. Polym*.. **101**: 1027-1032.

Zoeller T, Dressman JB and Klein S (2012). Application of a ternary HP-β-CD-complex approach to improve the dissolution performance of a poorly soluble weak acid under biorelevant conditions. *Int. J. Pharm.,* **430**: 176-183.

Zugasti ME, Zornoza A, del Mar Goñi M, Isasi JR, Vélaz I, Martín C, Sánchez M and Martínez-Ohárriz MC (2009). Influence of soluble and insoluble cyclodextrin polymers on drug release from hydroxypropyl methylcellulose tablets. *Drug Dev. Ind. Pharm.,* **35**: 1264-1270.