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Research Article

ENHANCEMENT OF SOLUBILITY AND DISSOLUTION RATE OF A POORLY WATER SOLUBLE DRUG USING SINGLE AND DOUBLE HYDROPHILIZATION APPROACH

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ABSTRACT

The aim of present investigation was to improve the solubility and dissolution rate limited absorption of lornoxicam (LXM) by making solid complexes using single and double hydrophilization approaches. For this, effect of Polyvinylpyrrolidone K-30 (PVP K-30) and Poloxamer-407 (PXM-407) auxiliary substances on complexation of drug with β-cyclodextrin was studied by investigating their interactions in solution and solid state. Phase solubility studies were done to evaluate the solubilizing efficiency of β-CD in association with auxiliary substances to determine stability constant (Ks) and complexation efficiency (CE). Improvement in Ks and CE by double hydrophilization approach evident the additive effect of auxiliary substances. PXM-407 was found to be the promising auxiliary substance in terms of getting optimum results. Solid dispersions by single and double hydrophilization approach were prepared by kneading, co-evaporation, microwave irradiation and lyophilization method. Double hydrophilized solid complexes were superior over single hydrophilized complexes in terms of higher solubility, CE, Ks, *in-vitro* dissolution rate and reduction in formulation bulk. Optimized solid complexes were characterized by SEM, DSC, XRD and FTIR spectroscopy. Lyophilized double hydrophilized complex of LXM-β-CD-PXM-407 indicated a significant improvement in the *in-vitro* dissolution rate as compared to solid complexes prepared by rest of the methods ($p<0.05$) due to porous and amorphous drug particles, reduction in total interfacial tension by PXM407 and formation of inclusion complex by β-CD. In a nut shell it can be concluded that lyophilized double hydrophilized complex was the most prominent approach to enhance the solubility and dissolution rate limited absorption of lornoxicam.

Keywords: β-Cyclodextrin, Dissolution rate, Inclusion complex, Poloxamer-407, PVP K-30.

INTRODUCTION

The complexation of poor water soluble drugs with cyclodextrins has been extensively studied in recent years. But there are many reasons including cost, toxicity, production capabilities, and concentration of cyclodextrins that can be incorporated into drug formulations is limited[1-2]. Even under ideal conditions, cyclodextrin complexation results in a 4-10 fold increase in the formulation bulk which limits their use in solid oral dosage forms[3] and it also improves the stability of drug[4-5]. Figure 1 shows the process of inclusion complex formation where small encircles are water molecules and the oval shaped circles are drug molecules. Molecules of water are repulsed by hydrophobic drug molecules and hydrophobic cavity of the β-cyclodextrin.

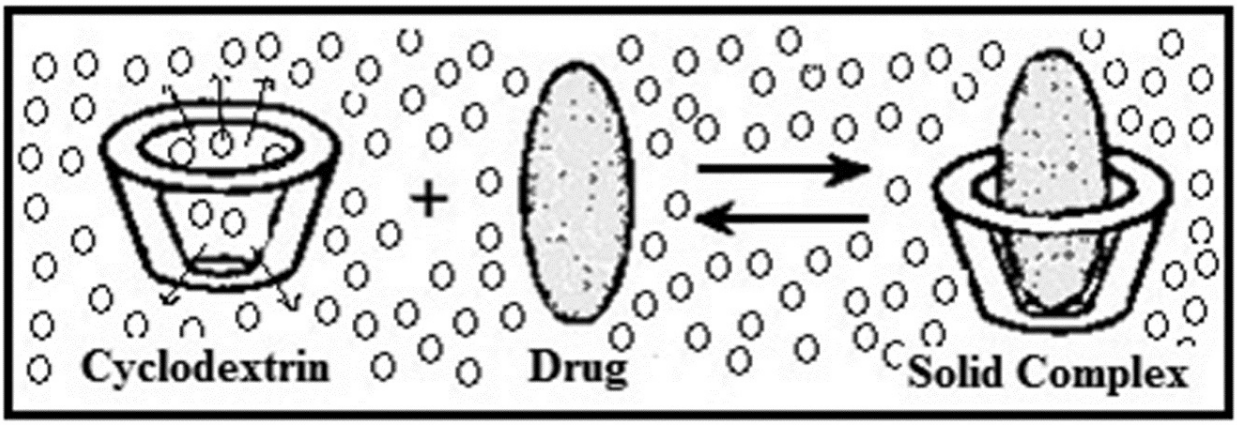

Fig. 1: Schematic representation of host (drug) and guest (cyclodextrin)

For the formation of inclusion complex, substitution of the polar-nonpolar interactions (between the polar water molecules and nonpolar β-CD cavity) by nonpolar-nonpolar interactions (between nonpolar drug molecules and nonpolar β-CD cavity) is essential[6]. The main driving force for complex formation is the release of water molecules from the cavity due to the entrapment of guest molecule[7-8]. The hydrogen bonds, hydrophobic interactions and weak van der waals forces keep the complex formation together. As covalent bonding is not frequent, complexation is considered as replacement of water molecules with drug molecules. Various auxiliary substances such as organic acid[9], amino acid[10], organic base, metal ions, water soluble polymers[11] etc. have been reported in combination with cyclodextrins to enhance the complexation efficiency of β-CDs. In the present investigation attention has been focused on the use of water-soluble polymers and surfactant with cyclodextrin to enhance the efficacy of drug-cyclodextrin complexation and also to reduce the molar amount of β-cyclodextrin[6].

Lornoxicam (6-chloro-4-hydroxy-2-pyridyl-2H-thieno [2, 3-e]-1, 2-thiazine-3-carboxamide-1,1-dioxide) is a non-steroidal anti-inflammatory drug (NSAID) with marked analgesic properties[12-13]. Lornoxicam (LXM) a novel highly selective COX-2 inhibitor is used for a variety of acute and chronic inflammatory diseases[14]. However, it's low aqueous solubility and poor dissolution in upper gastric fluid cause formulation problems and limits its therapeutic application by delaying rate of absorption and finally the onset of action[15].

Another approach utilized for solubility enhancement of LXM is ternary complexation using arginine as auxiliary substances with β-CD[10]. Though enhancement in solubility was reported, limitation associated with arginine such its instability during processing and storage conditions, expensive raw material do not make it a cost effective viable option for production (www.medlinesupplements.mht). Thus the present research was undertaken with the aim to enhance the solubility and dissolution rate limited absorption of poor water soluble drug LXM by making ternary inclusion complex with stable and cost effective auxiliary substances such as PXM407 and PVP K-30. This was done by preparing solid inclusion complexes using different methods such as kneading method (KM), co-evaporation method (CM), microwave irradiation (MWI) and lyophilization technique (LT). Stability study of optimized solid complexes was also performed according to ICH guidelines to check the stability of optimized solid complexes.

MATERIALS AND METHODS

Materials

Lornoxicam (LXM) was obtained as a gift sample from Glen mark Pharmaceutical Ltd. Mumbai, India; β-cyclodextrin (β-CD) and Polyvinylpyrrolidone K-30 (PVP K-30) was procured from International Specialty Product technologies limited, USA; Poloxamer-407 (PXM407) was purchased from BASF Corporation (UK); Acetone and other solvents were used of HPLC grade.

Methods

All experiments were carried out under subdued light to prevent photo degradation of LXM.

Equilibrium / Saturation Solubility Studies

Equilibrium solubility of LXM and hydrophilized solid complex was carried out in 0.1 N HCl buffer, pH 1.2; double distilled water pH 5.8 and phosphate buffer, pH 7.4. For this, an excess amount of LXM was added to three different conical flasks of 50 ml capacity each containing 10 ml of 0.1 N HCl buffer, double distilled water and phosphate buffer pH 7.4 respectively and shaken in digital water bath shaker (HICON, Delhi) for 72 hours at 37±0.5ºC and 100 rpm. After shaking, samples were centrifuged at 4000 rpm (1073g) for 20 minutes, filtered the supernatant through membrane filter (pore size 0.45 µm) and analyzed the drug in the filtrate after proper dilution (if necessary) by UV Spectrophotometer (Shimadzu 1700, Pharmaspec, Kyoto, Japan) at 380nm. The equilibrium solubility data of LXM in the different media was calculated.

Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors[16]. An excess amount of LXM was added to 10 ml of double distilled water, pH 5.8 containing various concentration of β-CD (3-15 mM) taken in a series of 25 ml stoppered conical flasks and the mixtures were shaken in digital water bath shaker (HICON, Delhi) for 72 hours at 37 ± 0.5ºC and 100 rpm. After achieving equilibrium, the solutions were then filtered through a membrane filter (pore size 0.45 µm). The filtered samples were diluted suitably and assayed for content of LXM by UV spectrophotometer at 380 nm against blank. All the solubility experiments were conducted in triplicate. The amount of drug dissolved against moles of carrier (β-CD) was plotted and the stability constant (Ks) and complexation efficiency (CE) was calculated by using following equations[17, 18]:

$$Ks\ (1:1) = slope/S_o\ (1\text{-}slope) \quad \text{Eq. (1)}$$

$$C.E. = slope/\ (1\text{-}slope) \quad \text{Eq. (2)}$$

$$D: CD = 1: (1 + 1/CE) \quad \text{Eq. (3)}$$

Where, S_o is the solubility of LXM in absence of carrier.

Preparation of Solid Inclusion Complexes Using Single and Double Hydrophilization Approach

The hydrophilized solid inclusion complexes of LXM and β-CD in 1:1 molar ratio were prepared with or without auxiliary substances (PVP K-30 and PXM407) in optimized concentration using different methods such as KM, CM, MWI and LT and are reported in Table 1.

Table 1: Preparation of single and double hydrophilized solid inclusion complexes

Method	Code	Ingredients (mg)			
		Lornoxicam	β-CD	PVP K-30	PXM-407
Physical Mixture	PM1	3.71	11.35	_	_
	PM2	3.71	11.35	10	_
	PM3	3.71	11.35	_	40
Kneading Method	KM1	3.71	11.35	_	_
	KM2	3.71	11.35	10	_
	KM3	3.71	11.35	_	40
Co-evaporation Method	CM1	3.71	11.35	_	_
	C2M	3.71	11.35	10	_
	CM3	3.71	11.35	_	40
Microwave Irradiation Method	MWI 1	3.71	11.35	_	_
	MWI 2	3.71	11.35	10	_
	MWI 3	3.71	11.35	_	40
Lyophilization Technique	LT1	3.71	11.35	_	_
	LT2	3.71	11.35	10	_
	LT3	3.71	11.35	_	40

Physical mixture

The physical mixtures were prepared by gently mixing LXM, β-CD with or without optimized concentration of auxiliary substance (PVP K-30 and PXM407), in a mortar with pestle for 10 minute. These mixtures were passed through a sieve no.85 and stored in desiccator till further use.

Kneading method

LXM, β-CD with or without optimized concentration of auxiliary substances (PVP K-30 and PXM407) were triturated in a mortar with a small volume of water. After wetting the physical mixture in a mortar, the thick slurry was kneaded for 45 minute and dried at 320 watts in a microwave oven (Whirlpool, Sweden) for 4 min. The dried mass was pulverized, sieved through sieve no. 85 and stored in desiccator till further use.

Co-evaporation method

Equimolar amount of the LXM, β-CD with or without optimized concentration of different auxiliary substances (PVP K-30 and PXM407) were dissolved in double distilled water, stirred the solution, then 25% v/v ammonia solution was added drop wise. The solvent was removed at reduced pressure in rotary evaporator at 45ºC until paste was obtained. Then it was dried at 45ºC for 3 hours and dried mass was pulverized, sieved through sieve no. 85 and stored in desiccator till further use.

Microwave Irradiation method

Equimolar amount of LXM, β-CD with or without optimized concentration of auxiliary substances (PVP K-30 and PXM 407), was taken in a flask then minimum amount of solvent mixture (acetone: water; 1:1v/v) was added. Then mixture was kept for 1 or 2 minutes at 60 ºC in the microwave oven (Whirlpool, Sweden). After completion of the reaction, an adequate amount of solvent mixture was added to the above reaction mixture to remove residual (uncomplexed free drug and β-CD). The obtained precipitate was separated using membrane filter (pore size 0.45 µm) and dried in vacuum oven (HICON, Delhi) at 40ºc for 48 hrs. The dried mass was pulverized, sieved through sieve no. 85 and stored in desiccator till further use.

Lyophilization Technique

Equimolar amount of LXM, β-CD with or without optimized concentration of auxiliary substances (PVP K-30 and PXM407), were mixed in double distilled water and shaken for 24 hours, then ammonia solution (25% v/v) was added to it drop wise till a clear solutions was obtained. The solution was frozen overnight in petridish at -45ºC and then lyophilized in a freeze dryer (Macflow Engineering, Delhi) at -45ºC for 48 hours. Secondary drying was carried out at room temperature.

DETERMINATION OF DRUG CONTENT

Accurately weighed 10 mg sample(s) of each single and double hydrophilized solid inclusion complexes were dissolved in 10 ml of 0.1N HCl buffer, pH 1.2. The samples were diluted suitably with 0.1 N HCl buffer and assayed spectrophotometrically for LXM at 380 nm. The experiment was performed in triplicate to get average drug content ± S.D.

***IN-VITRO* DISSOLUTION**

The *in-vitro* dissolution of prepared solid inclusion complexes was carried out using powder dispersion method USP type II apparatus at 100 rpm[19].The dissolution study was conducted in 900 ml of 0.1 N HCl buffer pH 1.2 at 37 ± 0.5°C and 100 rpm. Solid complexes containing equivalent of 8mg of LXM were suspended in 900 ml of 0.1N HCl buffer, pH 1.2 and aliquots of 5 ml sample were withdrawn at specified time intervals and analyzed spectrophotometrically at 380 nm. The dissolution studies were carried out in triplicate and the mean ± SD values were calculated.

Dissolution Efficiency

The dissolution efficiency is, area under the dissolution curve between time points t_1 and t_2 which expressed as a percentage of the curve at maximum dissolution, 100, over the same time period or the area under the dissolution curve up to a certain time, t, which expressed as a percentage of the area of rectangle which described by 100% dissolution in the same time.

The dissolution efficiency (DE) of the solid complexes was calculated by following equation[18]:

$$\% \text{ Dissolution Efficiency (DE\%)} = \frac{\int_0^t y\,dt}{y_{100}\,(t2-t1)} \times 100 \quad \text{Eq. (4)}$$

Statistical test

All data are reported as mean ± SEM, and the differences between the groups were tested using Student's t-test, at the level of $p<0.05$. More than two groups were compared using ANOVA and differences greater at $p<0.05$ were considered significant.

Characterization of Hydrophilized Solid Complexes

Scanning Electron Microscopy (SEM)

The surface morphology of pure drug, physical mixture (PM3) and double hydrophilized solid complexes prepared by various methods was examined by Scanning Electron Microscope (Zeiss EVO® 50, UK). In this study the samples were fixed on a brass stub using double-sided tape and then gold coated in vacuum by a sputter coater. The pictures were then taken at an excitation voltage of 15 kV.

Differential Scanning Calorimetry (DSC)

The thermal behavior or LXM, β-CD, PXM407, PM3 and lyophilized double hydrophilized solid inclusion complex were recorded on the Perkin-Elmer (Pyris Diamong UK) model differential scanning calorimeter. In this study, about 10 mg of samples was sealed in aluminum pans and an empty aluminum pan was used as a reference. This experiment was carried out under nitrogen flow (20 ml/min) at scanning rate of 10ºC/min in the range from (30-250ºC).

X-Ray Powder Diffractometry (XRPD)

Diffraction patterns of pure LXM, β-CD, PXM407, PM1, PM3, and lyophilized single and double hydrophilized solid complexes were recorded with X' Pert PRO, Netherland. A voltage of 40 kV and a current of 30 mA for the generator were used with Cu as the tube anode material. The solids were exposed to Cu-Kα radiation (α1 = 1.54060 Å andα2 = 1.54439Å, with a α1/ α2 ratio of 0.5), over a range of 2θ angles from 10ºC to 30ºC, at an angular speed of 1º (2) per minute.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform Infrared spectra were obtained using JASCO FT-761 spectrometer (Tokyo, Japan). The sample of pure drug, β-CD, auxiliary substances, PM3 and lyophilized double hydrophilized solid inclusion complex were previously grounded and thoroughly mixed with KBr and formed an infrared transparent solid disk. The KBr disks were prepared by compressing the powder blend. Three scans were executed at a resolution of 1 cm^{-1} (from 4000-400 cm^{-1}).

Stability Studies

The stability study of optimized LXM-β-CD-PXM407 complex was performed by storing the samples in sealed vials at 40ºC for one month, 40ºC and 75% relative humidity for one month and 60ºC for 15 days. Any change in physical appearance and *in-vitro* drug release profile as a result of storage was documented.

RESULTS AND DISCUSSION

Selection of optimized concentration of auxiliary substances

For the selection of optimized concentration of auxiliary substance, equilibrium solubility studies of LXM were performed in the presence of auxiliary substance(s) PVP K-30 and PXM-407, in the concentration range of (0.04-0.8%w/v). Solubility of LXM increased with increasing concentration of auxiliary substance and reached maximum solubility at 0.1% for PVP K-30 and 0.4% for PXM-407 and thereafter the solubility of the LXM decreased (Table 2).

Table 2: Comparative table showing the results of phase solubility studies for single and double hydrophilization solid complexes

S. No.	Solid Complex	Complexation Efficiency	Stability Constant Ks (min^{-1})	Drug: β-CD ratio
1.	LXM–β-CD	0.050	283	1:21
2.	LXM–β-CD–PVP K-30	0.110	349	1:10
3.	LXM–β-CD–PXM-407	0.194	368	1:6

At lower concentrations, both PVP K-30 and PXM-407, showed solubilizing effect on LXM, this could be attributed to the weak polymeric drug interactions. At higher concentration of auxiliary substance, reduction in solubility can be attributed to the formation of electrostatic bond between auxiliary substances which decreases their ability to form complex[20, 21]. Thus optimal polymer concentration selected was 0.1% for PVP K-30 and 0.4% for PXM-407, since further increase in concentration of auxiliary substances

not showed any increment in drug solubility. On further analysis of solubility data it was observed that enhancement in solubility of LXM was more pronounced in the presence of PXM-407 as compared to PVP K-30. The polyoxyethylene segment of tri-co-block polymer which is relatively hydrophilic dissolved rapidly in the dissolution medium and created a microenvironment that played major role in solubility enhancement[22].

Phase solubility studies

The phase solubility study plots of LXM obtained with β-CD and β-CD with optimized concentration of auxiliary substances PVP K-30 and PXM407 are shown in (Figure 2A & 2B).

The equilibrium phase solubility diagram for both LXM with β-CD and LXM-β-CD with auxiliary substances showed that the solubility of LXM increased linearly as a function of β-CD concentration and the soluble complexes were formed without occurrence of precipitation. The phase solubility curve can be classified as AL type according to Higuchi and Connors. The increment in LXM solubility seems to be related to the inclusion ability of the β-CD molecules in water.

The slope values in all diagrams were <1, suggesting the formation of 1:1 M ratio complexes in solution. Similar results have been reported for other oxicam derivatives[19, 23, 24].

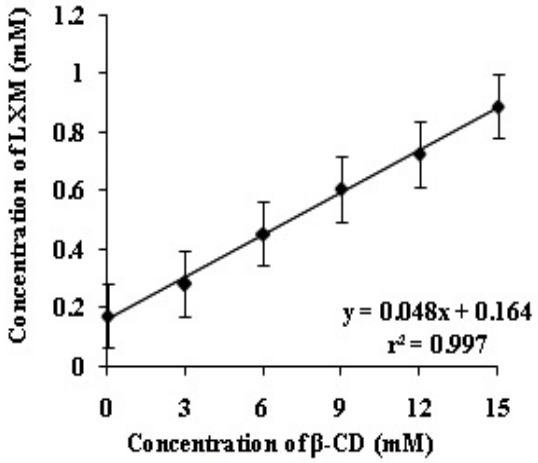

Figure 2 (A)

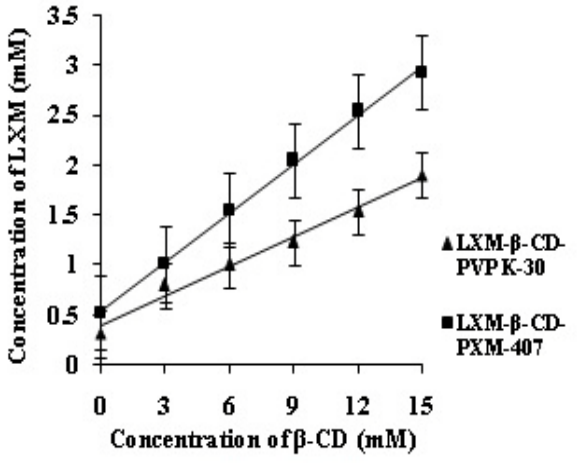

Figure 2 (B)

Fig. 2: Phase solubility plots of (A) LXM with β-CD and (B) LXM with β-CD in presence of auxiliary substance

The apparent stability constant (Ks), complexation efficiency (CE) of the complex obtained from the slope of the linear phase solubility diagram were calculated and are shown in Table 2. The higher value of the Ks indicated that the complex of LXM- β-CD-PXM407 was more stable than other complexes. The increase in CE of β-CD signifies the importance of auxiliary substance PXM 407 over PVP K30; in addition PXM 407 also decreased the drug: β-CD ratio to a significant extent.

A synergistic effect on LXM solubility was observed in the presence of auxiliary substances with β-CD, since the solubility values achieved in the presence of both β-CD and auxiliary substances were higher than the sum of individual solubility values obtained with the β-CD and auxiliary substances solution. The addition of auxiliary substances to the β-CD solution did not change the type of phase solubility diagrams (A_L) obtained for double hydrophilized complexes and always resulted in an increase in the Ks and CE values. A low stability constant Ks value of 283 M^{-1} for single hydrophilized complex (LXM-β-CD) indicated that interaction was weak and unstable between the components. While in case of double hydrophilized complexes, the auxiliary substances increased the wettability and dispersibility, enhance formation of hydrogen bonds between drug and carrier, and also decreased crystallinity of the product; therefore, it was possible to attain overall solubilization[15, 25]. The absolute solubility enhancement was related to increase in complexation efficiency[26], and it was higher in case of PXM407 than PVP K-30 with β-CD.

Determination of drug content

Percentage drug content for all the solid complexes prepared by different methods was found to be in the range of 80.19 ± 0.16% to 99.80±0.13%, while for physical mixture it was found to be 80.19± 0.16% . The highest drug content was found to be 99.8±0.1% for lyophilized LXM-*β*-CD-PXM-407solid complex, which indicating preparation of solid complexes with better content uniformity.

***In-vitro* dissolution studies**

The comparative *in-vitro* dissolution profiles of LXM, PM and all single hydrophilized solid complexes prepared by different methods and double hydrophilized solid complexes consisting of PVP K30 and PXM407 in 0.1 N HCl buffer, pH 1.2 are shown in Fig. 3A, 3B and 3C respectively. It is clearly evident from that percent LXM dissoluted from pure drug sample was 32.76%, while 39.16% from PM1 and 64.48% from PM3 after 120 minutes. It was interesting to note that in case of lyophilized double hydrophilized solid complex (LT2 and LT3); the dissolution was more than 90% after 20 minutes. As is apparent from the figure, double hydrophilization approach improved the dissolution rate of LXM to a great extent. This is also evident from the percentage of drug dissolved in 20 min (DE_{20}%) and time taken for 50% and 90% dissolution ($t_{50\%}$ and $t_{90\%}$) values that were recorded in Table 3.

Both the double hydrophilized complexes LXM-β-CD-PXM-407 and LXM-β-CD-PVP K-30 showed higher dissolution rates than the single hydrophilized complexes. A marked increase in the release of drug from double hydrophilized complexes was seen when compared with single hydrophilized complexes due to formation of water-soluble drug polymer complexes since polymers mainly interact with drug molecules via electrostatic bonds, i.e., ion-to-dipole and dipole-to-dipole bonds and other forces such as vanderwals forces and hydrogen bridges[27, 28].

Table 3: *In-vitro* dissolution parameters for various optimized solid complexes of Lornoxicam prepared by different methods

METHOD	% Drug Content ± SD	%Cumulative drug release (after 120 min)	$t_{50\%}$ (min)	$t_{90\%}$ (min)	DE_{20} (%)
LXM	100	32.76	>120	>120	7.32
LXM-β-CD (PM)	80.19 ± 0.16	39.16	>120	>120	76.77
LXM-β-CD-PXM-407 (PM)	90.42 ± 0.18	64.48	43.36	>120	87.05
LXM-β-CD-PXM-407 (MWI)	95.73 ± 0.4	72.9	20.00	>120	88.31
LXM-β-CD-PXM-407 (KM)	96.70 ± 0.2	89.98	17.63	>120	89.91
LXM-β-CD-PXM-407 (CE)	99.07 ± 0.4	94.94	13.31	57.85	91.99
LXM-β-CD-PXM-407 (LT)	99.80 ± 0.1	99.38	10.00	19.13	95.98

The other possible mechanisms of marked increase in dissolution rate of LXM from double hydrophilized complexes may be due to solubilization effect of carriers, improved wettability and dispersibility[29], dissolution in hydrophilic carriers, reduction in particle size of drug, absence of aggregation of drug crystallites due to the conversion of the LXM to the amorphous state which leads to increase in dissolution rate of poorly water soluble LXM[30, 31]. From the dissolution profiles of double hydrophilized complexes it was clear that LXM-β-CD-PXM-407 complex showed higher dissolution than the LXM-β-CD-PVP K-30, which may be due to one or more characteristics of PXM-407 such as formation of hydrophilic amorphous complex, the polyoxyethylene segment of tri-co-block polymer which is relatively hydrophilic, dissolved rapidly in the dissolution medium and creating a microenvironment that played major role in solubility enhancement, the polymeric surfactant allows formation of a bond between hydrophobic and hydrophilic materials as concluded in Figure 2B and it also provides an effective surface area for dissolution that results in higher dissolution[32, 33].

The dissolution rate of LXM-β-CD-PXM-407 complexes was significantly higher (*p<0.05*) than the LXM-β-CD-PVP K-30 complexes prepared by all methods[30] but among all, it was the LXM-β-CD-PXM-407 complex prepared by LT that displayed highest dissolution; Figure 3 (B and C), this may be due to decrease in LXM crystallinity as a consequence of the specific interaction produced by the method of preparation in order to get a porous, amorphous powder with high degree of interaction between LXM and β-CD. Lyophilized LXM-β-CD-PXM-407 complexes also displayed highest $DE_{20\%}$ 87.61, least $t_{50\%}$ and $t_{90\%}$ 8.88 and 19.13 respectively, when compared with pure drug and all the complexes prepared by the other methods (Table 3). This was also evidenced by the physicochemical characterization viz. FTIR, DSC, XRD and SEM analysis of the lyophilized LXM-β-CD-PXM-407 complex[15, 34].

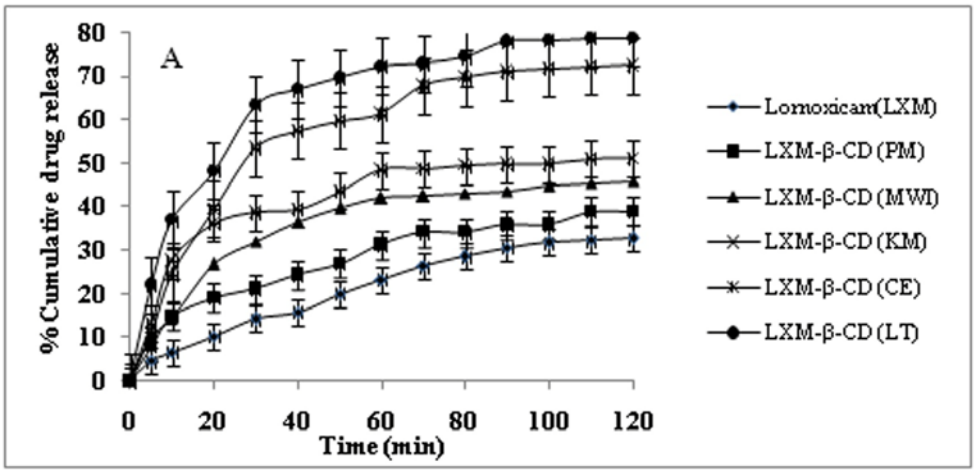

Fig. 3(A): Dissolution profiles of single hydrophilized solid complexes prepared by different methods

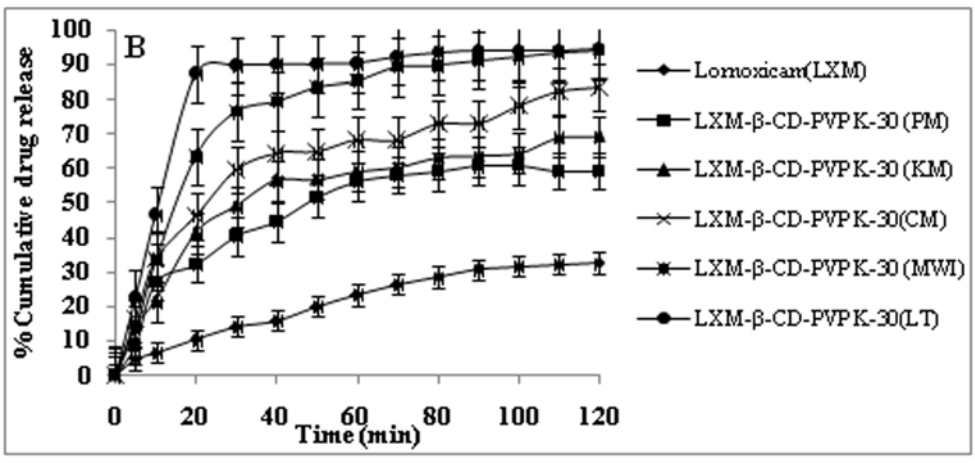

Fig. 3(B): Dissolution profiles of double hydrophilized solid complexes of PVP K-30 prepared by different methods

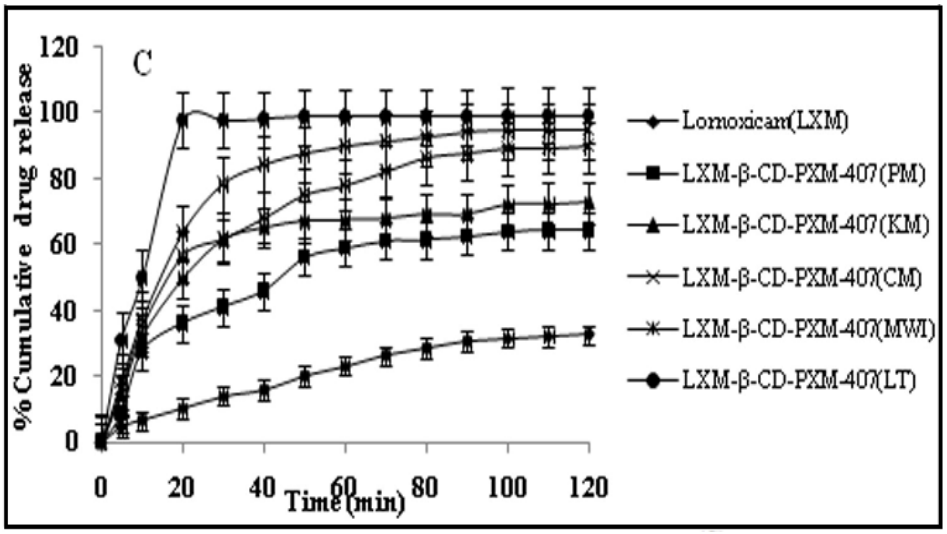

Fig. 3 (C): Dissolution profile of double hydrophilized solid complexes with PXM-407 prepared by different methods

Comparative Equilibrium Solubility Analysis of Lornoxicam and Lyophilized Double Hydrophilized Complex

When the equilibrium solubility data of LXM was compared with equilibrium solubility data of optimized solid complex i.e. lyophilized LXM-β-CD-PXM-407, the increment in solubility of LXM by LXM-β-CD-PXM-407 in the mediums such as 0.1 N HCl buffer pH 1.2, DD water pH 5.8 and phosphate buffer pH 7.4 was found to increased by 14.42 folds, 26.47 folds and 12.93 folds respectively than the pure drug which was confirming the enhancement in solubility of LXM by the lyophilized double hydrophilized solid complex as shown in Figure 4.

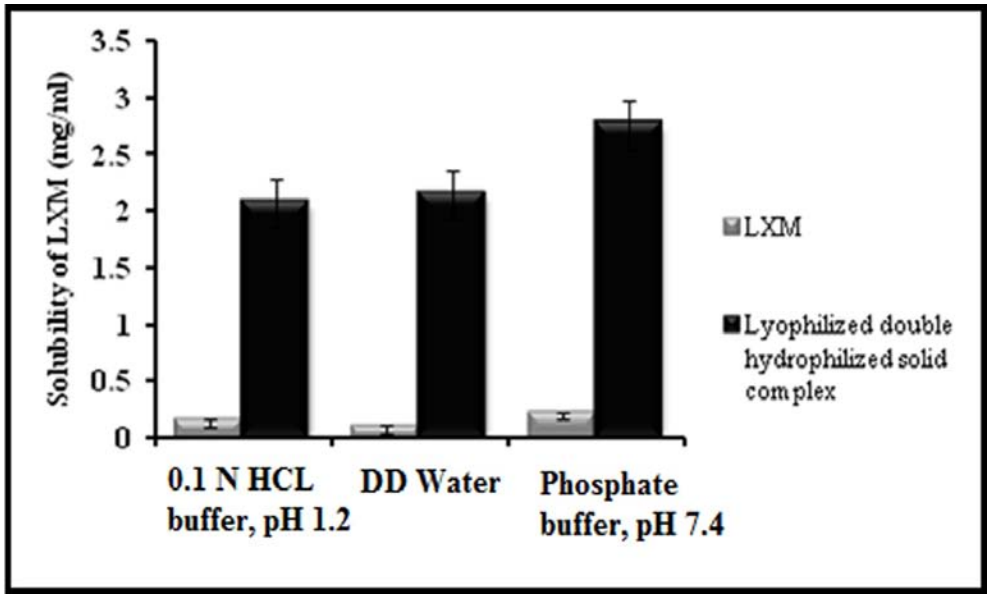

Fig. 4: Comparative equilibrium solubility profiles of LXM and Lyophilized double hydrophilized solid complex in different media

Characterization of Single and Double Hydrophilized Solid Complexes

Scanning Electron Microscopy (SEM)

SEM images of the LXM, PM3 and double hydrophilized solid complexes prepared by various methods are represented in the Figure 5. SEM is used to study the microscopic aspects of the raw materials like pure drug, β-CD and the products obtained from different methods of preparation. Even if there is a clear difference in crystallization state of the raw materials and the products, this study is clearly evident to inclusion complexation but nevertheless helps to assess the existence of a single component in the preparations obtained. From SEM analysis it can be seen that pure LXM (Fig. 5A) particles appeared as needle shaped with smooth surfaces.

The microscopic examination of the physical mixture PM3 (Fig. 5 B) showed the presence of LXM crystals adhered and mixed on the surface of the β-CD spherical particles, revealing no apparent interaction between species in solid state of the complexes and a drastic change in the original morphology and shape of both LXM particles were observed in double hydrophilized complexes which may be attributed to the preparation methods adopted[35]. In kneaded, co-evaporated and microwave irradiated (Fig. 5 C,D & E) and lyophilized double hydrophilized solid complexes, it was still possible to distinguish few LXM crystals as agglomerates on the surface of β-CD that had lost their original shapes.

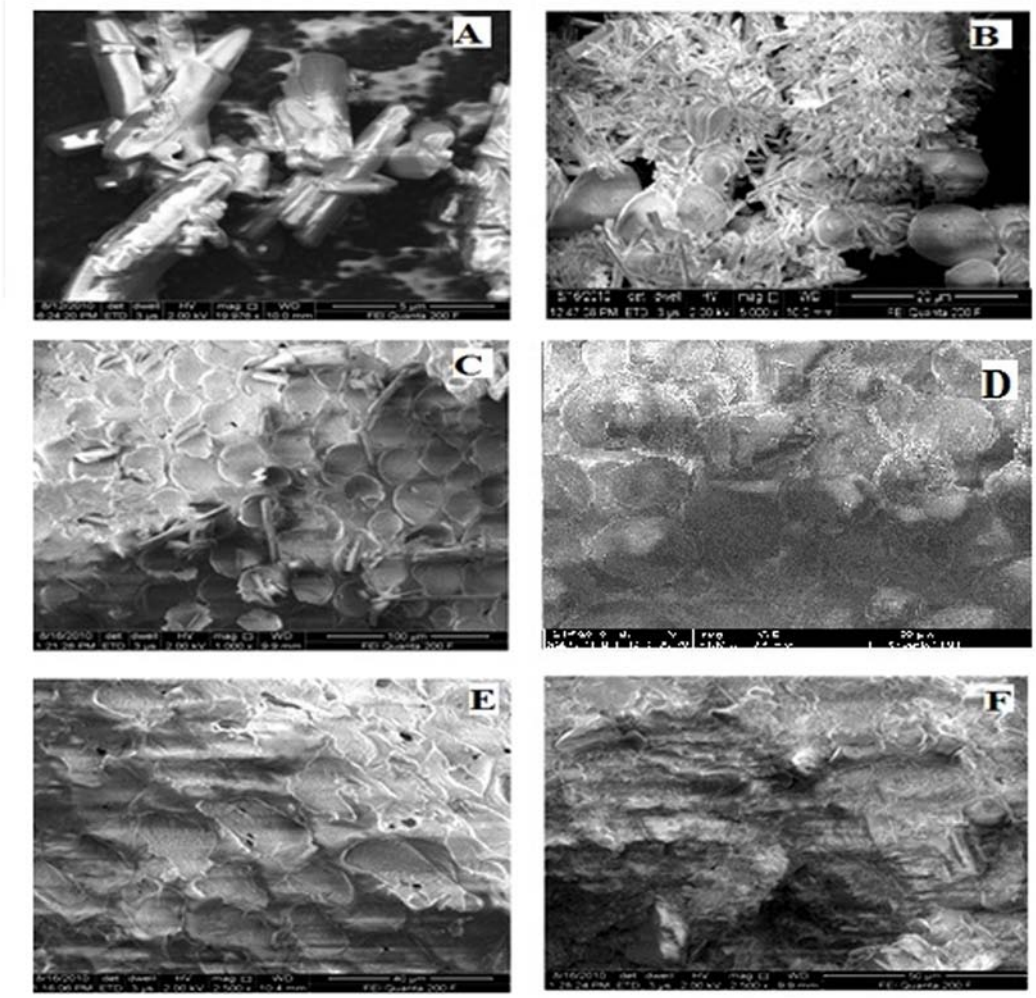

Figure 5: SEM Photomicrographs of (A) LXM and Double hydrophilized solid complex with (B) (PM), (C) MWI, (D) KM, (E) CE, (F) LT.

Finally, the lyophilization technique showed the inclusion and amorphous particles of irregular size with lamellate form of double hydrophilized solid complex which could be distinguished as a single component (Fig. 5 F). The drastic change in particle shape in aspect to LXM-β-CD-PXM-407 lyophilized complex indicated the presence of new solid phase, which could be due to a consequence of a crystalline habitus change in the solid complex, or it may support the evidence of existence of single phase[25, 36].

Differential Scanning Calorimetry

The thermal behavior of LXM, β-CD, auxiliary substances, physical mixtures and LXM-β-CD-PXM-407 complex is shown in Figure 6. The DSC thermograms of LXM (Fig.6A) showed a typical behavior of an anhydrous crystalline drug with a well defined endothermic peak at 230ºC corresponding to its melting point in optimized solid complex. The DSC thermogram of β-CD (Fig. 6B) showed an endothermic peak at about 135ºC due to the liberation of water of crystalline, whereas broader endothermic peak at 56ºC was associated with water loss from amorphous PXM-407 (Fig. 6C). Both the characteristic peak of LXM and β-CD was clearly distinguishable in the physical mixture, PM3 (Fig. 6D). Despite of the lower size and shift to lower temperature, the small peak correspondent to the melting of free LXM suggested that in PM3 there was no inclusion complex formed in either system even though LXM-β-CD. The DSC curves of LXM-β-CD-PXM407 solid complex exhibited a complete disappearance of the endothermic melting peak of LXM (Fig. 6F) and this may be attributed to amorphization of LXM and due to formation of inclusion complex. Disappearance of endothermic melting peak of LXM showed that only lyophilized LXM-β-CD-PXM407 solid complex formed a true inclusion complex which was differing from physical mixture, PM3[19, 25].

X-Ray Powder Diffractometry (XRPD)

The XRPD patterns of pure LXM, β-CD, PXM407 and their corresponding single and double hydrophilized solid complexes with LXM is shown in Figure 7. It is a useful method for the detection of complexation in powder of microcrystalline states. The diffractogram of LXM showed sharp and intense peaks which indicating its crystalline nature (Fig. 7A). The XRPD pattern of pure β-CD showed crystallinity which was determined by comparing representative peak heights in the diffraction patterns of the single hydrophilized solid complex with those of a reference (Fig. 7B).

The lowered intensity of the diffraction peaks and overlapping of some LXM peaks with the peaks of β-CD was observed in the PM1 (Fig. 7D). It was attributed to reduction in particle size during the preparation of solid complexes by physical mixing. The diffraction patterns of the lyophilized single and double hydrophilized solid complexes showed crystalline state, but in the comparison with the diffractogram of the correspondent to the physical mixture, it was possible to observe disappearance of some diffraction peaks of β-CD and LXM. Furthermore, for lyophilized single and double hydrophilized solid complexes obtained XRPD patterns were diffused (Fig. 7F & 7G) indicating the amorphous state reached by the lyophilization technique. SEM and DSC studies also supported the same hypothesis, which was confirmed by X-ray diffractometry[37].

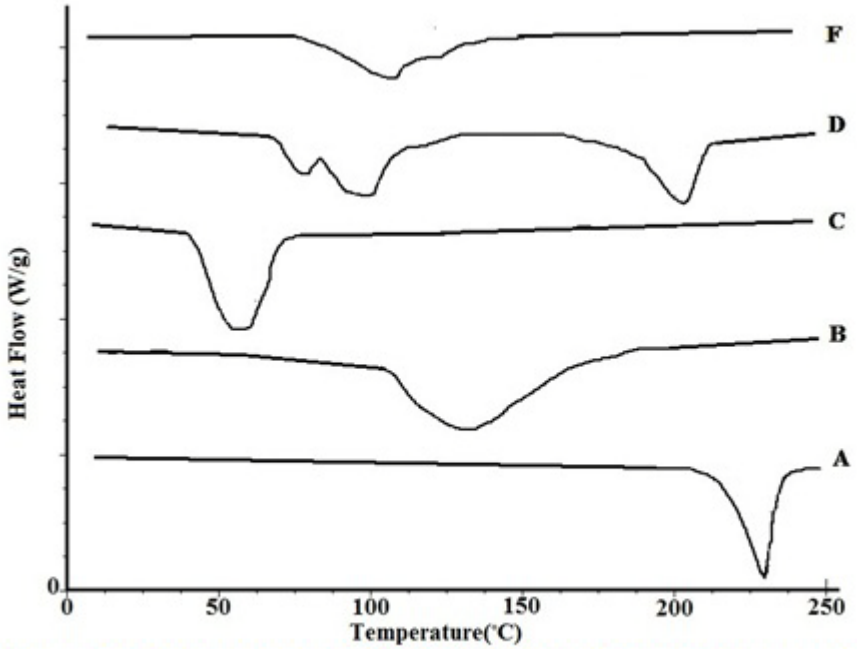

Figure 6: DSC thermograms of (A) Pure LXM, (B) β-CD, (C) PXM-407, (D) Physical mixture LXM-β-CD-PXM-407,(E) Lyophilized LXM-β-CD-PXM-407

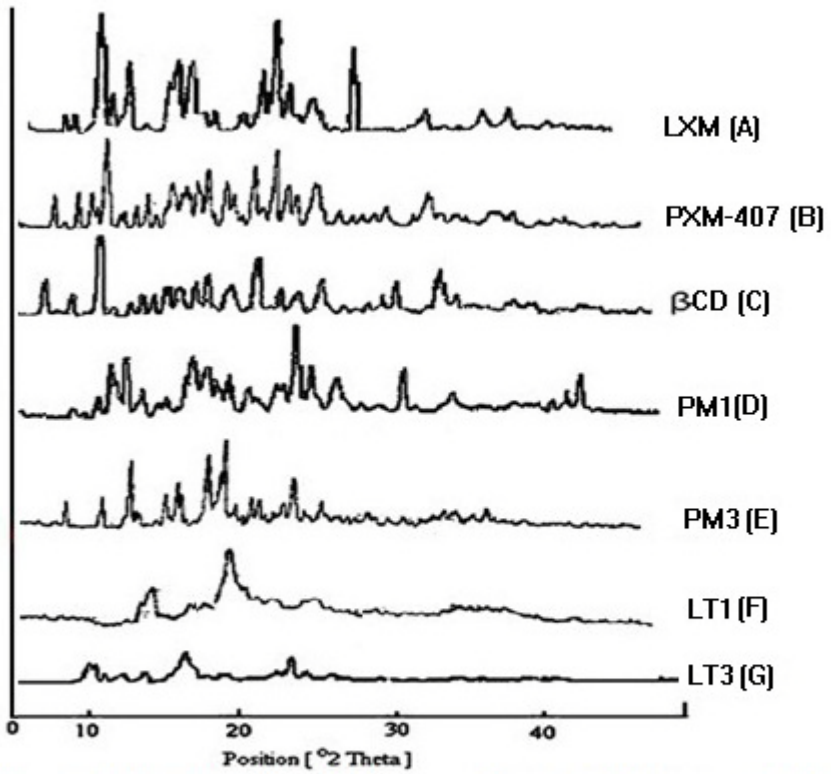

Figure 7: Powder X-ray Diffraction Patterns of Drug, β-CD, Poloxamer 407, PM1 & LT1 (single hydrophilized solid complexes) and PM3 & LT3 (double hydrophilized complexes)

Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of the LXM, β-CD, physical mixtures PM1 & PM3and lyophilized single and double hydrophilized solid complexes are shown in Figure 8. The IR spectroscopy has also been used to assess the interaction between cyclodextrin and guest molecules in the solid state, since upon the complexation, shifts or changes in the absorption spectrum occur[25].

The IR spectrum of the LXM showed an absorption band at 3,400 cm^{-1} due to an O-H stretching vibration; the broadness of this band is indicative of hydrogen bonding. The absorption band at 3,090 cm^{-1} due to N-H stretching; aromatic C-H stretching at 2,927 cm^{-1} and an absorption band located at 766 cm^{-1} is due to the stretching vibration of C –Cl[38]. The IR spectra of the β-CD had an intense bands at 3,300-3,500 cm^{-1} due to O-H stretching vibrations and vibration band located at 2,800-3,000 cm^{-1} due to the –CH and CH_2 groups. The

PXM-407 exhibits characteristic peaks at 3503, 2884, and 1114 cm^{-1} due to stretching of O-H, C-H, and C-O groups' respectively. The

IR spectra of double hydrophilized solid complex was compared with physical mixtures and LXM, there was no significant change in the characteristics stretching band of LXM were observed in physical mixtures, the above bands are unchanged for position and intensity with respect to the IR spectra of LXM alone in physical mixture. The O-H band of LXM and C-O stretching band of PXM-407 shifted towards shorter wavelength in spectra of LXM- β-CD-PXM407 which confirmed the existence of interaction of the drug, β-CD and these spectral changes due to dissociation of intermolecular hydrogen bonds of pure drug through inclusion complexation, similarly the C-O stretching band was highly diminished and interact with β-CD and shifted to lower frequencies in all spectral patterns of solid complexes prepared by KM, CM and MWI. It was clearly verified that the magnitude of alteration of the original C-O stretching band was clearly influenced by the method of preparation. This result confirmed the existence of strong interactions between LXM, β-CD, and PXM-407. In IR spectra of inclusion complex of LXM- β-CD-PXM-407 lyophilized double hydrophilized solid complex, absorption band at 1078 cm^{-1} could not be detected which might be due to co-occurrence of C-O band with other band and this existence indicated a strong interaction and complete formation of the solid complex with β-CD and PXM-407. Inclusion complexes showed absence of the above-mentioned peaks, which indicates entrapment of LXM into the cavity and confirmed the complex formation[25, 39].

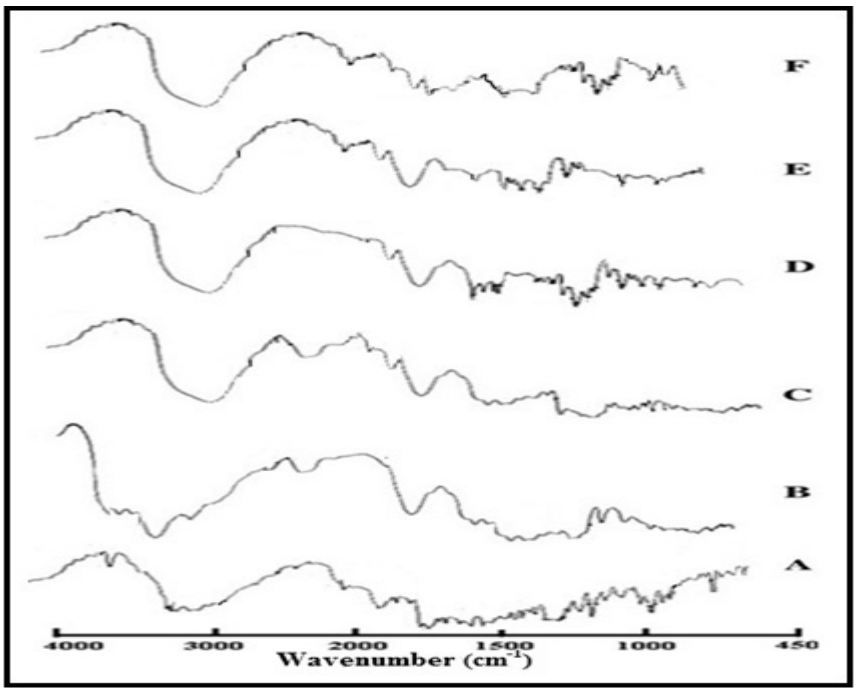

Fig. 8: FTIR spectra of (A) LXM, (B) β-CD, (C) PM1, (D) LXM- β-CD lyophilized complex, (E) PM3, (F) LXM- β-CD-PXM-407 lyophilized complex

Stability Studies of Optimized Solid Complex

Stability studies of optimized solid complex were conducted in order to determine any physicochemical changes such as change in color, texture, physical state and appearance; and to predict any changes in *in-vitro* dissolution profile of LXM-β-CD-PXM-407 as a result of storage, a stability study of optimized double hydrophilized solid complex LXM-β-CD-PXM-407 which had the maximum dissolution release, was conducted 40ºC for one month, 40ºC and 75% relative humidity (40ºC/75%RH) for one month, and at 60ºC for 15 days. As shown in Figure 9, under these conditions no significant changes in physical appearance and LXM release profile from LXM-β-CD-PXM-407 solid complex was observed[22].

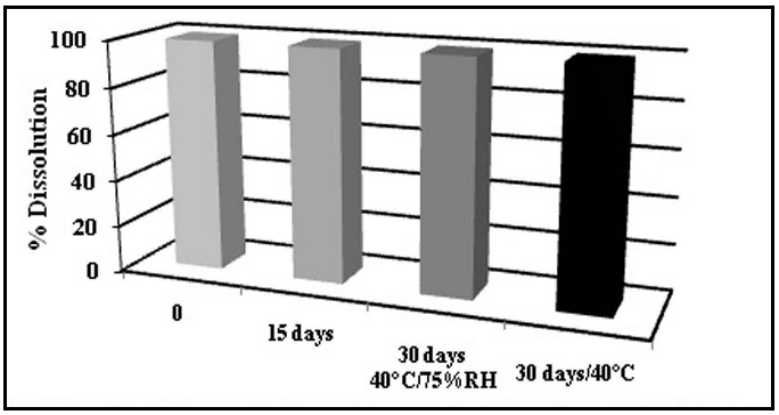

Fig. 9: % Dissolution of optimized solid complex after different time of storage conditions

CONCLUSION

This study confirmed that LXM can interact with β-CD, forming an inclusion complex in aqueous phase. The formation of an inclusion complex is dependent on β-CD, different preparation methods, pH variance, and incorporation of auxiliary substances. Double hydrophilized solid complex of LXM-β-CD-PXM-407 prepared by lyophilization technique was found to be optimum in comparison with the single hydrophilized complex of LXM- β-CD. The study determined greater Ks values for double hydrophilized complexes than single hydrophilized complex, which suggests significant improvement in the CE between pure drug and β-CD in addition of small amount of auxiliary substances. The results obtained justified the possibility of significantly improving the dissolution rate limited absorption of lornoxicam using double hydrophilization approach. Among the two auxiliary substances, PXM-407 was an appropriate choice to more give CE, stability constant and to enhance the aqueous solubility and dissolution rate of LXM. FTIR, DSC, XRD and SEM studies of the lyophilized double hydrophilized solid complex of LXM -β-CD-PXM407 justified the significance of the approach used. Further product development with prepared solid complexes and their *in-vivo* studies should be investigated to achieve clinical significance of the developed systems.

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