Journal of Inclusion Phenomena and Macrocyclic Chemistry (2005) 51: 219–224
DOI 10.1007/s10847-004-6957-1

Preparation and Characterisation of Meloxicam Hydroxy Propyl β-Cyclodextrin Inclusion Complex

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(Received: 17 March 2004; in final form: 30 November 2004)

Key words: meloxicam, HPβ-CD, inclusion complexation, phase diagrams, aqueous solubility, dissolution profile

Abstract

Meloxicam is a non steroidal anti inflammatory drug, used in the treatment of rheumatoid and osteoarthiritis. It is practically insoluble in water and its prolonged use is associated with the incidence of side effects like gastro intestinal perforations, ulcerations and bleeding. Therefore, an attempt has been made to improve the aqueous solubility of the drug by making an inclusion complex using hydroxy propyl β cyclodextrin(HPβ-CD). The complexes were prepared by physical mixture and freeze drying method. The different methods employed for evaluation such as DSC, XRD, SEM and FT-IR studies indicated complete formation of the complex by freeze drying method in a molar ratio of 1:2. The prepared complexes showed improved *in-vitro* dissolution profile as compared to the pure drug.

Introduction

Meloxicam is a non steroidal anti inflammatory drug prescribed for the long term treatment of musculo skeletal complaints. The major drawback to NSAID drug use is the preponderance of gastrointestinal (GI) side effects. These are generally recognized to be due to interference of the drug with the biosynthesis of prostaglandins and other arachidonic acid metabolites in the gastric mucosa. These side effects can reduce patient compliance and discourage physician from prescribing them. The most common GI adverse effects include upper GI perforations, ulcerations and bleeding which may require hospitalization. There is therefore, a need for a delivery system for NSAIDs with improved GI tolerability which retains its efficacy.

Cyclodextrins especially hydroxy propyl β cyclodextrin (HPβ-CD) are widely used in the pharmaceutical field owing to their high aqueous solubility and ability to stabilize insoluble drug molecules. They are known for their ability to molecularly encapsulate a wide variety of drugs into their hydrophobic cavity without the formation of any covalent bonds [1–4]. Over the past few years several papers have been published concerning the improvement of solubility and bioavailability using hydroxy propyl β cyclodextrin [5–10].

Meloxicam is a preferential COX-2 inhibitor with strong anti inflammatory activity. It is practically insoluble in water and has been implicated in causing gastro intestinal ulceration by remaining in contact with stomach mucosa for a longer duration of time, resulting in dangerously high concentration. The present study is an attempt to form an inclusion complex of meloxicam with hydroxy propyl β-cyclodextrin (HPβ-CD) to improve the aqueous solubility of the drug, thus enhancing its dissolution rate, thereby showing a faster onset of action and less GI mucosal toxicity.

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Materials and methods

Materials

Meloxicam was obtained as a gift sample from Sun Pharmaceuticals (India) Ltd. HPβ-CD was purchased from Sigma Aldrich (USA). Other reagents and chemicals were of analytical reagent grade.

Solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors [11] in water. An excess amount of meloxicam was added to the aqueous solution of HPβ-CD solution (M.W. = 1380) at various concentrations (2–10 mM/L). The contents were stirred for 72 h at 30 ± 1 °C. After equilibrium, the samples were filtered and absorbance read at 362 nm.

The apparent stability constant of meloxicam: HPβ-CD complex was calculated from the initial straight portion of the phase solubility diagrams.

Preparation of inclusion complexes

Meloxicam and HPβ-CD complex was prepared in two molar ratios viz. 1:1 and 1:2.

Physical mixtures (PMs): The ground components were mixed in a mortar and sieved through a 180 μm mesh B.S. sieve.

Freeze dried products (FDs): Meloxicam/25% aqueous ammonia solution and CD/water solutions were mixed and stirred to obtain a clear solution. The FDs products were obtained by using a Heterodry winner freeze dryer (Germany) which was connected to a rotary vane vacuum pump.

Characterization of solid complexes

The complexes were characterized and evaluated by:

- Differential Scanning Calorimetry (DSC):
  Thermal behaviour of meloxicam, HPβ-CD and each inclusion complex was examined by using a DUPONT model 910 (USA) Thermal Analyser Argon was used as carrier gas and the DSC analysis was carried out at a heating rate of 10 °C/min and an argon flow rate of 35 cc/min. The sample size was in the range of 1–3 mg and examinations were made in the temperature intervals between 50–500 °C.
- Powder X-Ray Diffraction Studies (XRD):
  XRD of the samples was performed using high power X-ray diffractometer RU-200B from M/s Riguao Japan. The scanning speed was 4 °/min. The voltage/current used was 40 kV/50 mA and the target/filter (monochromator) was Cu.
- FT-IR spectral analysis:
  FT-IR spectral studies were carried on FT-IR Magma IR 750 by nicolet series II instrument using KBr disc technique. Scanning was done from 4000 to 500 cm^{-1}.
- Scanning Electron Microscopy (SEM):
  SEM of samples were performed using Jeol scanning Microscope JSM-840 with a 10 kV accelerating voltage. The surface of the samples for SEM were previously made electrically conductive in a sputtering apparatus (Fine coat ion sputter JFC-1100) by evaporation of gold. A magnification of 1500 was used.

Aqueous solubility studies

The aqueous solubility of compounds, i.e., pure meloxicam drug powder and meloxicam HPβ-CD (FD, 1:2) inclusion complex was determined at 37 ± 0.5 °C in pH 1.2 and pH 7.4. Solubility was measured by shaking a well powdered or well dispersed solute in excess (40 mg) with water (100 ml) until the equilibrium was attained. Solute and solvent were placed in stoppered conical flasks immersed in thermostatted water bath and agitated continuously for 24 h. The temperature during agitation was kept at 37 ± 0.5 °C. After 24 h, the solution was filtered through a Millipore filter (0.22 μm). It was diluted sufficiently with water and absorbance was recorded at 362 nm. By using the calibration curve, aqueous solubility was determined.

In-vitro dissolution rate studies

Dissolution studies were carried for pure meloxicam and for inclusion complex using USP paddle type dissolution apparatus at 37 ± 1 °C at 100 rpm. The dissolution medium used was 900 ml of simulated gastric fluid pH 1.2 without pepsin (SGF) and phosphate buffer pH 7.4. The drug and the inclusion complex were filled in hard gelatin capsule shell so as to contain 15 mg meloxicam/capsule. Sampling was performed after 5, 15, 30 45, 60, 90 and 120 min. The meloxicam content was determined spectrophotometrically at 362 nm (Spectronic 21 UV/Vis spectrometer). All studies were carried out in triplicate.

Results and discussions

Solubility studies

The most common and widely used method to evaluate the ability of the CD to complex a drug is the phase solubility studies. Higuchi and Connors (1965) have classified the various solubility behaviours seen during complex formation as A-type (a soluble inclusion complex is formed) or B-type (an inclusion compound of finite solubility is formed). The equilibrium binding or association constant (k) for the 1:1 complex can be determined from the slope of linear portion using the following relationship, where S_0 is the intrinsic solubility of the drug under the conditions studied.

$$K_{1:1} = \frac{\text{Slope}}{S_0(1 - \text{slope})}.$$

The phase solubility diagram for Meloxicam-HPβ-CD system in water is shown in Figure 1. The solubility of meloxicam did not increase linearly as a function of HPβ-CD concentration and thus the solubility curve was classified as Bs type phase solubility curve indicating the formation of a 1:2 complex. The apparent 1:1 stability constant was calculated from the straight line of phase solubility diagram and was found to be 1648 M^{-1}.

Differential scanning calorimetry

The DSC graph of pure meloxicam drug powder showed a sharp endotherm near 262 °C, which was indicative of its melting temperature, followed by an exotherm, which signified that after melting meloxicam decomposes. In the thermogram of HPβ-CD, two endothermic peaks were observed. In the temperature range between 160–190 °C, loss of water occurs and near 350 °C, the

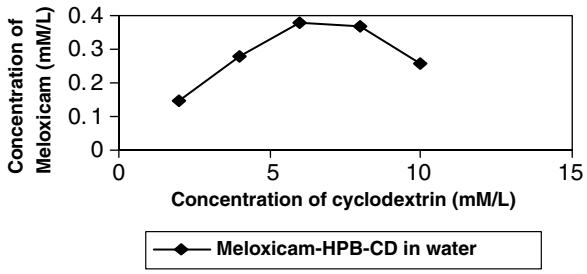

Figure 1. Phase solubility diagram of meloxicam with HPβ-CD in water.

endothermic peak corresponding to HPβ-CD, fusion is observed.

The DSC pattern of meloxicam-HPβ-CD inclusion complexes (1:1 and 1:2) prepared by physical mixture showed the presence of peaks of both pure compounds except for a difference that the intensity of meloxicam melting endotherm had decreased. In the 1:2 molar ratio complex, the endothermic peak of HPβ-CD near 320 °C broadened and a split was also observed indicating partial complex formation.

Thermogram of meloxicam HPβ-CD complexes prepared by freeze drying method (1:2) showed complete disappearance of the endothermic peaks characteristic of cyclodextrin and meloxicam, thus suggesting maximal/complete complex formation. In the 1:1 ratio, the melting endothermic peak of meloxicam was observed but its intensity was very much diminished (Figure 2).

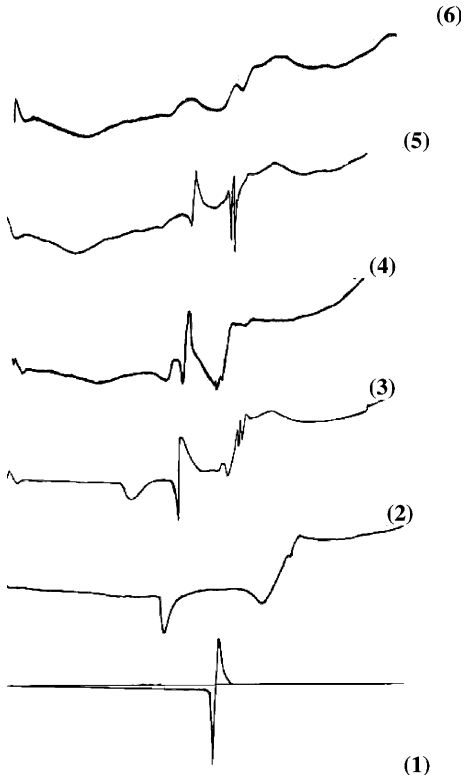

Figure 2. Differential scanning thermograms of Meloxicam (1); HPβ-CD (2); and complexes prepared by Physical mixture(1:1) (3);Physical mixture(1:2) (4); Freeze Drying (1:1) (5) and Freeze Drying (1:2) (6) methods.

Powder X-ray diffraction

The XRD pattern of meloxicam showed peaks which were intense and sharp indicating its crystalline nature. Inclusion complexes of meloxicam with HPβ-CD physical mixture in a molar ratio of 1:1 and 1:2 showed undefined, broad, diffused peaks with low intensities. Though this signifies amorphous nature but a few sharp peaks having less intensities of meloxicam are also present. Minimum intensity of peaks was observed with 1:1 ratio.

The Inclusion complexes of meloxicam with HPβ-CD prepared by freeze drying method in a molar ratio of 1:1 and 1:2 showed peaks of diminished intensity suggesting almost complete amorphization of the complex. In a 1:2 complex, meloxicam peaks was also not visible indicating complete complex formation (Figure 3).

Fourier transform infrared studies

Since FTIR is a highly sensitive method of analysis, all spectra of complexes show some or other changes from

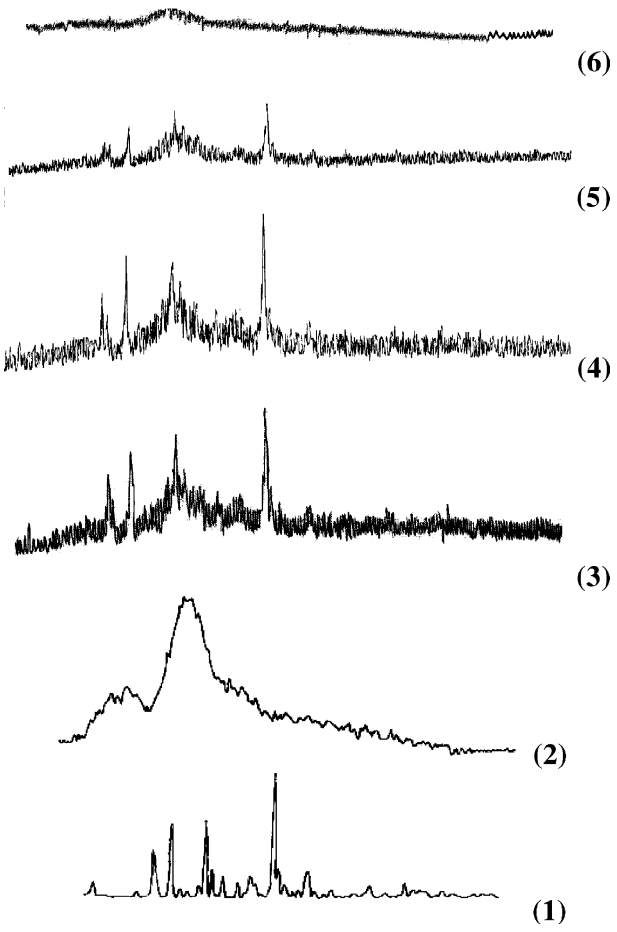

Figure 3. X-Ray diffraction patterns of meloxicam (1); HPβ-CD (2); and complexes prepared by Physical mixture(1:1) (3);Physical mixture(1:2) (4); Freeze Drying (1:1) (5) and Freeze Drying (1:2) (6) methods.

parent spectra i.e. pure drug and cyclodextrins. Some complex formation could thus be assigned to every method and every ratio. The IR spectra of meloxicam showed the presence of the following peaks: 3291 cm^{-1} (secondary CONH), 1625 cm^{-1} (CONH) and 1550 cm^{-1}, 1529 cm^{-1} (N-H bending). The IR spectra of HPβ-CD showed prominent absorption bands at 3414 cm^{-1} (for O–H stretching vibrations), 2933 cm^{-1} (for C–H stretching vibrations) and 1164, 1083 and 1083 cm^{-1} (C–H, C–O stretching vibration). In the IR spectrum of meloxicam-HPβ-CD physical mixture in a molar ratio of 1:1 the secondary amide band appeared at 3290 cm^{-1} along with the hydroxyl group band at 3414 cm^{-1} suggesting partial or little interaction of the drug with HPβ-CD molecule. The IR-spectrum of meloxicam-HPβ-CD physical mixture (PM) in a molar ratio of 1:2 displayed absorption bands at 3404 cm^{-1} (–OH), 3290 (–CONH), 1620 (–CONH) 1550, 1530, 1346, 1161 and 1082 cm^{-1} indicating a mixture containing drug along with the complexing agent i.e. HPβ-CD. Slight shifting of –OH stretching vibration from 3414 cm^{-1} in the complexing agent i.e. HPβ-CD to 3404 cm^{-1} in the physical mixture (1:2) inclusion complex suggested interaction of the drug i.e. meloxicam with the hydroxyl groups of HPβ-CD due to formation of weak hydrogen bondings.

However, in meloxicam-HPβ-CD freeze dried complex in a molar ratio of 1:1 the amide N–H stretching vibration at 3290 cm^{-1} could not be detected indicating strong interaction between meloxicam and the hydroxyl groups of HPβ-CD.

In the IR-spectrum of meloxicam-HPβ-CD freeze dried complex (1:2), the amide N–H stretching vibration at 3291 cm^{-1} could not be detected which might be due to co-occurrence of N–H band with the O–H intensified band at 3400 cm^{-1}. This indicated a strong interaction and complete complex formation of meloxicam with HPβ-CD in a molar ratio of 1:2. The intensities of the bands appearing at 1623, 1523, 1460, 1349 and 1323 cm^{-1} were also affected due to such type of interaction (Figure 4).

Scanning electron microscopy

Pure meloxicam is characterized by the presence of crystalline particle of regular size. Pure HPβ-CD also appears as crystalline particles without a definite shape. The inclusion complexes of meloxicam/HPβ-CD physical mixture showed the crystalline structure of both meloxicam and HPβ-CD. Crystals of meloxicam mixed with CD crystals were seen adhering to their surfaces.

The photomicrographs of freeze dried samples showed the typical morphology of preparations generally obtained by this method, that is small size particles tending to aggregation, suggesting the existence of an amorphous product with the presence of a single component in the complex, thus suggesting maximum or complete complex formation (Figure 5).

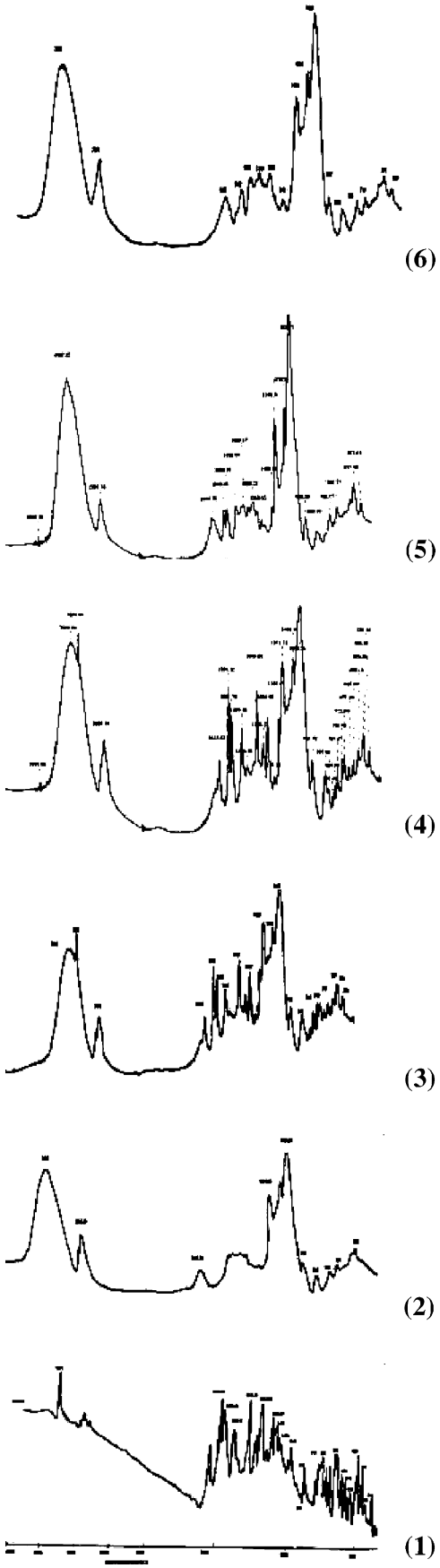

Figure 4. FT-IR spectra of Meloxicam (1); HPβ-CD (2); and complexes prepared by Physical mixture(1:1) (3);Physical mixture(1:2) (4); Freeze Drying (1:1) (5) and Freeze Drying (1:2) (6) methods.

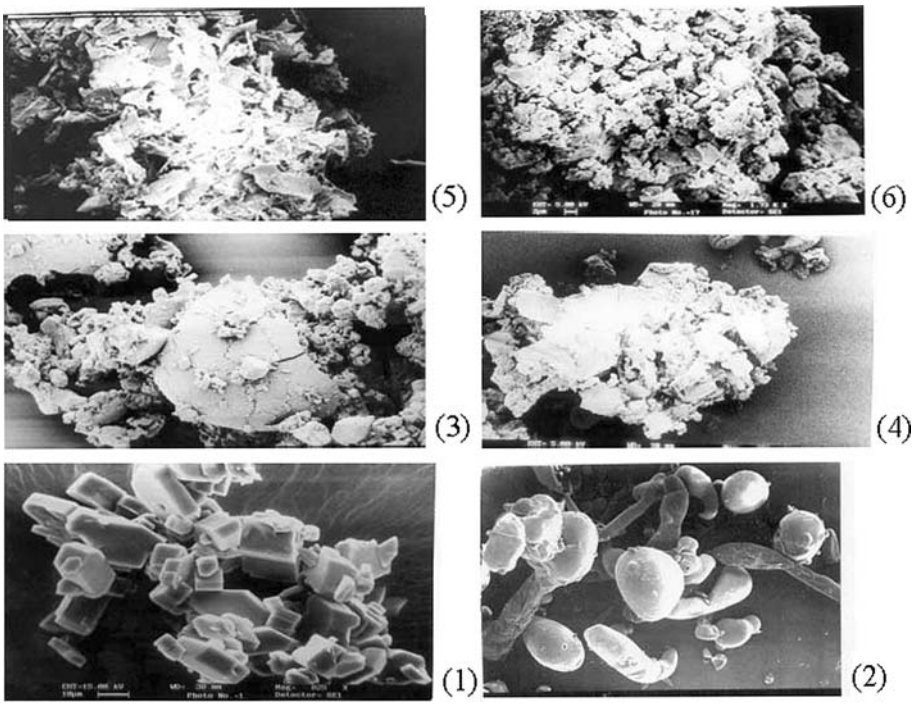

Figure 5. Scanning Electron Micrographs of Meloxicam (1); HPβ -CD (2); and complexes prepared by Physical mixture(1:1) (3); Physical mixture(1:2) (4); Freeze Drying (1:1) (5) and Freeze Drying (1:2) (6) methods.

Aqueous solubility studies

The aqueous solubility of the optimized inclusion complexes i.e. meloxicam HPβ-CD was more than the pure drug i.e. meloxicam both in pH 1.2 and pH 7.4, (Table 1) indicating that a considerable portion of meloxicam will be present in the non ionized form in the acidic gastric juice of the stomach. These non-ionized molecules can easily penetrate through lipid membranes into the mucosal cells of the stomach wall, where the higher intracellular pH leads to ionization which results in direct local damage to the gastric mucosa.

Dissolution rate studies

The comparative release studies (Figure 6 and 7) indicated that the release of active material was strongly affected by the method of formulation. The dissolution profiles of the complexes were studied in different dissolution media with the aim of differentiating between

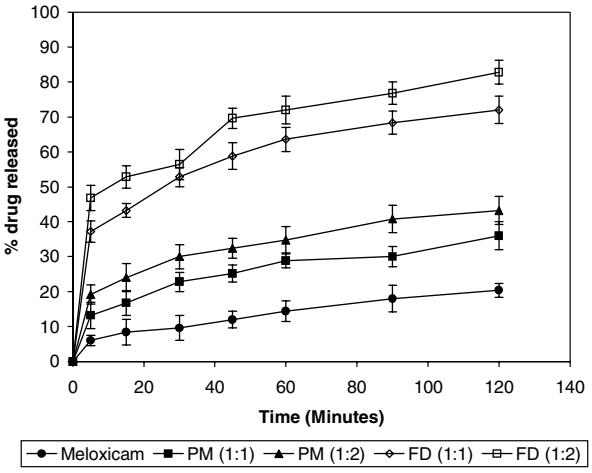

Figure 6. Dissolution profile of (●) meloxicam, (■) physical mixture 1:1, (▲) physical mixture 1:2, (◇) freeze dried mixture 1:1, (□) freeze dried mixture 1:2, in Simulated gastric fluid pH 1.2.

Table 1. Aqueous solubility studies

Compound	Aqueous solubility (μg/ml) $n = 3$	
	pH 1.2	pH 7.4
Pure Meloxicam	3.6 ± 2.54	780 ± 2.87
Meloxicam – HPβ-CD (PM) complex (1:2)	124 ± 2.44	1200 ± 1.28
Meloxicam – HPβ-CD (FD) complex (1:2)	720 ± 1.47	1920 ± 3.43

the dissolution behaviour of the complexes. The dissolution characteristics of the complexes in simulated gastric fluid (SGF) without pepsin pH 1.2 was studied, to gain information about the dissolution of the drug in the acidic conditions of the stomach, which would have an influence on the ulcerogenic potential of the drug. The freeze dried product (meloxicam-HPβ-CD) in a molar ratio of 1:2 exhibited the best dissolution properties followed by freeze dried product (1:1), and then physical mixture (1:2 and 1:1) respectively. The dissolution studies revealed that all the formulations showed an increased rate and was more in alkaline medium which

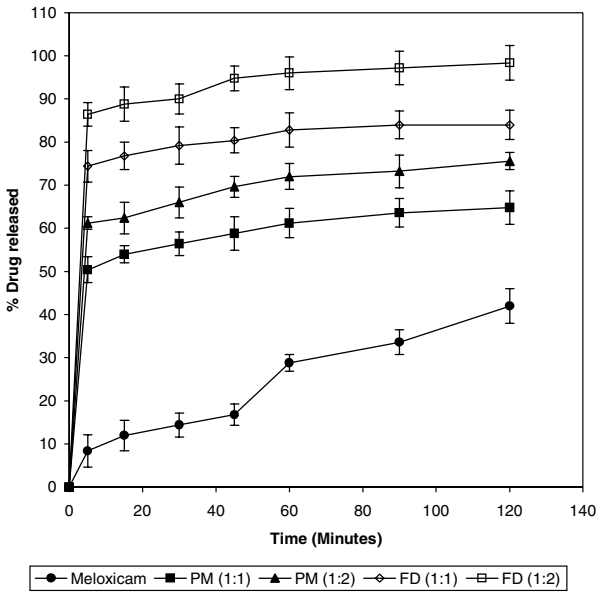

Figure 7. Dissolution profile of (●) meloxicam, (■) physical mixture 1:1, (▲) physical mixture 1:2, (◇) freeze dried mixture 1:1, (□) freeze dried mixture 1:2, in Phosphate buffer pH 7.4.

may be due to the ionization of the drug as it is a weak acid. In case of SGF after 5 min only 6% of pure meloxicam was dissolved and after 2 h only 20.4% of the drug went into solution whereas in case of meloxicam-HPβ-CD inclusion complex prepared by freeze drying method in a molar ratio of 1:2, 37.2% of drug was released after 5 min and after 2 h 82.8% drug release was obtained. In case of phosphate buffer, almost all the active material of freeze dried complex (1:2) was released within 60 min (99.6%) as compared to 42.0% of the pure meloxicam at the end of two hours. The improvement in the dissolution rate of the drug/cyclodextrin systems may be attributed to the degree of crystallinity of the active material, together with the increase in both the wettability and the solubility of the drug.

Conclusions

- A complex of meloxicam was successfully prepared with HPβ-CD by freeze drying method in a molar ratio of 1:2.
- The dissolution behaviour of the freeze dried products were higher compared to physical mixtures in both media.
- Solubility profile of complexes of meloxicam prepared using HPβ-CD as complexing agent in a molar ratio of 1:2 by freeze drying method in pH 1.2 and pH 7.4 indicated that the acid solubility of meloxicam was enhanced considerably by formation of an inclusion complex with hydroxy propyl β-cyclodextrin.

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