Aqueous Solubility and Dissolution Rate Improvement of Etodolac via Inclusion Complexation Technique

Ahmed M. Mohammed, W. Faisal, Khaled I. Saleh and Shaaban K Osman

1Department of Pharmaceutics and Industrial pharmacy, Faculty of Pharmacy, University of Al-Azhar, Assiut, Egypt.

2Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia, 71526, Egypt.

Submission: 5 June 2016
Accepted: 10 June 2016
Published: 25 June 2016

Keywords: Etodolac, Cyclodextrins, Inclusion complexation, Phase diagram

ABSTRACT

Etodolac is a preferential COX-2 inhibitor. It has anti-inflammatory, antipyretic, and analgesic activities. Like other NSAIDs, long-term use of etodolac causes severe gastrointestinal disturbance. The enhancement of its solubility and dissolution profile is expected to significantly improve its bioavailability and consequently, reduce its side effects. In the present study, an attempt was made to improve the solubility and dissolution rate of etodolac by inclusion complexation with different kinds of cyclodextrin (α-CD, β-CD, γ-CD and HP-β-CD). Inclusion complexes were prepared at 1:1 drug: CD molar ratio and the corresponding physical mixtures were also prepared. The formulations were characterized for solubility parameters, drug release studies, and drug-polymer interactions by using different techniques including, dissolution studies, differential scanning calorimetry (DSC), infrared spectroscopy and X-ray diffractometry (XRD) analysis. All the formulations showed marked improvement in the solubility behavior which translated by the increase of dissolution rates of the investigated drug. The extent of solubility enhancement was arranged in a descending order as follows: HP-β-CD > γ-CD > β-CD > α-CD. IR studies showed no interaction between the drug and the carrier. DSC and XRD studies indicate conversion of the drug to an amorphous state. It was concluded that β-CD, γ-CD and HP-β-CD can be well utilized to improve the solubility of poorly soluble drugs.
INTRODUCTION

It is a well-established fact for poorly water-soluble drugs that the rate-limiting step in their absorption process is the dissolution rate in the gastrointestinal fluids rather than the rapidity of their diffusion across the gut wall. Therefore, by improving the dissolution rate profiles of such drugs, it is possible to enhance their bioavailability and reduce their side effects such as gastric irritation, ulceration or bleeding \cite{1}. According to the Biopharmaceutical Classification System (BCS), etodolac (1, 8-diethyl-1, 3, 4, 9- tetrahydropyran [3, 4- 6] indol-1-yl) acetic acid) belongs to class II drugs; a drug that is characterized by low solubility and high permeability, therefore, the enhancement of its solubility and dissolution profile is expected to significantly improve its bioavailability and reduce its side effects \cite{2}.

![Figure 1: Chemical structure of etodolac.](image)

The dissolution enhancement of poorly water-soluble drugs can be achieved by several techniques, such as micronization, recrystallization, cogriding, solid dispersion and inclusion complexation with cyclodextrins \cite{3}. Cyclodextrins (CDs) are cyclic oligosaccharides made up of six (α-CD), seven (β-CD) or eight (γ-CD) dextrose units joined by α-1,4-glycosidic linkages to form a torus-shaped structure \cite{4,5}. The geometry of CDs exhibits a hydrophobic inner cavity and a hydrophilic exterior, which is responsible for their water solubility and their ability to include hydrophobic molecules \cite{6}. Cyclodextrins (CDs) have attracted growing interest in the pharmaceutical industry as complexing agents, because of their low toxicity and ability to produce stable complexes \cite{7}. Cyclodextrins play an important role in formulation of poorly water soluble drugs by acting as hydrophilic carriers which increase wettability of poorly soluble drugs. Reduction of drug crystallinity via complexation with CDs also contribute to increase the drug solubility and dissolution rate \cite{8}.

The aim of the present study was focused on the formation of inclusion complexes of etodolac with different kinds of cyclodextrins (α-CD, β-CD, γ-CD and HP-β-CD). The physicochemical
properties of the modified inclusion complexes were investigated by different analysis, including IR, DSC, and X-ray diffractometry. Moreover, the comparative in-vitro dissolution studies were carried out to explore the most suitable host molecule.

MATERIALS AND METHODS

Materials

Etodolac was kindly gifted by Pharco Pharmaceutical Co. (Alexandria, Egypt). β-cyclodextrin (β-CD) was provided by Acros Organics Co. (New Jersey, United States). Other cyclodextrins (α-CD, γ-CD and HP-β-CD) were supplied by Sigma-Aldrich Co. (St. Louis, USA). Ethanol was purchased from El-Nasr Pharm. Chem. Co., (Cairo Egypt). Other materials and solvents were of analytical grade and used without further purification.

Construction of phase solubility diagram

Solubility of etodolac in distilled water, in the presence of different concentrations of cyclodextrins (α-CD, β-CD, γ-CD and HP β-CD) were performed according to the method reported by Higuchi and Connors [9]. An excess amount of etodolac (20 mg) was added to 20-ml stoppard glass tubes containing 10 ml of carrier solutions. The tubes were sonicated for 1 hr then transferred to a water bath, previously adjusted at required temperatures 25°C ± 1. After 48 hours (equilibrium time), aliquots were withdrawn, filtered using a 0.45 μm membrane disc filter and assayed spectrophotometrically at λmax 280 nm after appropriate dilution employing the same concentration of the cyclodextrin solution as a blank. The results are the mean values of three determinations ± SD.

Preparation of physical mixtures

Etodolac and the selected cyclodextrins (α-CD, β-CD, γ-CD and HP-β-CD) at equimolar ratio were physically mixed and sieved through a sieve no. of 60 (250 μm pore size).

Preparation of etodolac inclusion complexes

Etodolac/CDs inclusion complexes and physical mixtures were prepared in 1:1 molar ratio. An equimolar ratio of etodolac /β-CD was prepared by adding the aqueous β-CD solution to the
ethanolic solution of etodolac. The obtained mixture was stirred for one hour and allowed to evaporate at 40°C in a vacuum oven until complete dryness \[^{10}\]. The inclusion complexes of etodolac with \(\alpha\)-CD, \(\gamma\)-CD and HP-\(\beta\)-CD were prepared by dissolving an equimolar ratio of etodolac and CDs in ethanol and following the same above mentioned procedure. Then, the residue was placed in a desiccator containing anhydrous calcium chloride for further 24 hrs. The obtained solid mass was ground and passed through sieve no. 60 (250 \(\mu\)m pore size).

**Characterization of the prepared inclusion complexes**

**Differential ultraviolet absorption study**

Scanning of 1% solution of the selected cyclodextrins was carried out in the presence and absence of etodolac in order to investigate the effect of CDs on the drug maximum absorbance \[^{11}\].

**Measurement of drug content**

Known amounts of the prepared mixtures were dissolved in ethanol and then the drug concentration was evaluated spectrophotometrically at 280 nm. Percent drug content was calculated for each sample by using the following formula \[^{12}\]:

\[
\% \text{ Drug content} = \frac{\text{Actual amount of the drug in the formula}}{\text{Theoretical amount of the drug in the formula}} \times 100
\]

**In-vitro dissolution studies**

All measurements were performed using 6 paddles USA Hanson dissolution tester. An accurately weighed amount (20 mg) of pure drug or equivalent amount of physical mixtures or inclusion complexes with cyclodextrins were dispersed over 500 ml distilled water which is immediately stirred at the speed of 50 rpm and temperature of \((37 \, ^{\circ}\text{C} \pm 0.1)\). At different time intervals, 5 ml samples were withdrawn and filtered through a membrane filter (0.45\(\mu\)m), and the corresponding concentrations of etodolac were analyzed by measuring their absorbances at 280 nm. An equal volume of pre-warmed fresh dissolution medium was added to the cells so as to keep the volume of the dissolution medium constant. The results are the average of three independent experiments ± SD.
Infrared spectroscopy

In order to investigate the existence of any interaction of drug with the investigated cyclodextrins, the IR spectra of pure drug, cyclodextrins, physical mixtures and inclusion complexes (drug/CDs) of equimolar (1:1) ratio were recorded using Shimadzu IR-476 spectrophotometer, Japan, at a range of 4000- 400 cm⁻¹ using KBr disk method. The samples were mixed with KBr and compressed into discs using IR compression machine \(^{[13]}\).

Differential scanning calorimetry

Differential Scanning Calorimetric (DSC) analysis of the pure drug, CDs, physical mixtures and inclusion complexes was performed using Shimadzu-thermal analyzer DSC-T50, Japan, calibrated with indium. Samples of about 4-5 mg were heated under nitrogen atmosphere on a sealed aluminum pans at a rate of 10°C/minute over the temperature range of 30 - 200 °C. Thermal analysis was carried out using TA 50 PC system with Shimadzu software program \(^{[14]}\).

X-ray diffractometry

To investigate the effect of cyclodextrins on the crystallographic properties of etodolac, the X-ray diffraction patterns of the selected samples were recorded using Philips PW1710 diffractometer, USA. The samples were irradiated with CuKα radiation with a wavelength of 1.5418 Å at 40 kV and 40 mA, then analyzed between 2θ angles of 4-60° at a scan rate of 0.06°/ min \(^{[14]}\).

RESULTS AND DISCUSSION

Phase solubility diagram

Phase solubility analysis (the effect of complexing agents on the drug solubility) is considered as a traditional approach to determine not only the value of the stability constant but also to give an insight about the stoichiometry of the equilibrium. The results showed that the solubility of etodolac increases linearly as a function of β-CD, γ-CD and HP-β-CD concentration at the selected temperature (Figure 2) since the phase solubility diagrams of etodolac follow A\(_L\)-type profile, defined by Higuchi and Connors. This finding proved that etodolac formed soluble inclusion complexes (of 1:1 ratio) in a solution. In contrast, the assessment of etodolac solubility
as a function of α-CD concentration showed a nonlinear profile (B-type). This result may be attributed to the formation of an insoluble complex or may be due to the smaller cavity size of α-CD that is insufficient for many drugs\textsuperscript{15}. Data obtained from phase solubility diagram was used to determine stoichiometric ratio by plotting drug concentration against the concentrations of different cyclodextrins\textsuperscript{16}. The stability constant $K_s$ for the complexes were determined from the graph using following Higuchi and Connors equation\textsuperscript{9}:

$$K_s = \frac{\text{Slope}}{S_0(1-\text{slope})}$$

Where the slope is obtained from the graph and $S_0$ is the equilibrium solubility of etodolac in water. The intrinsic solubility ($S_0$) of etodolac in water was found to be 0.00032 M/L. Subsequently, the calculated apparent stability constant at 25 °C was 60, 57.4, 100.3 and 93.8 M$^{-1}$ for α-CD, β-CD, γ-CD, and HP-β-CD, respectively, confirming highly stable complex formation. It was reported by Higuchi and Connors that complexes showing $K_{1:1}$ less than 50 M$^{-1}$ are generally very labile exhibiting premature drug liberation profile or instead, they are very stable ($K_{1:1}$ more than 2,000 M$^{-1}$) displaying an incomplete or very slow drug release rate. Therefore, the $K_{1:1}$ values assessed in this work fell within the practical range\textsuperscript{17,18}.

![Figure 2: Phase solubility diagrams of ETD with different types of CDs at 25 °C, solubility expressed in (mole x 10$^{-4}$).](image)

\textit{Citation: Shaaban K Osman et al. Ijprr.Human, 2016; Vol. 6 (3): 304-318.}
Characterization of the prepared inclusion complexes

**Differential ultraviolet absorption study**

Absorption spectrum of etodolac in the presence of β-CD, γ-CD and HP-β-CD show a little change in the UV spectrum. The insertion of the etodolac molecule into the macrocyclic cavity was accompanied by changing its environment upon inclusion, thus causing some changes in peak intensity and bathochromic shift.

**Drug content measurement**

The drug content in all the formulations was estimated spectrophotometrically at 280 nm (UV-1601 (Shimadzu Co., Japan). The drug content of the prepared inclusion complexes was found to be in the range of 95 % to 104.8% indicating the uniform distribution of drug in the formulation.

**In-vitro dissolution studies**

The cumulative amount of dissolved etodolac from the inclusion complexes as well as the corresponding physical mixture after 15 minutes was obtained in Table 1. It is observed that solid inclusion complexes of γ-CD and HP-β-CD exhibited higher dissolution rates of 77% (6.1-fold) and 71.2 (5.6-fold), respectively compared with the pure drug. These results could be attributed to the improved solubility and/or reduction in crystallinity of etodolac. The results, also, indicated that the dissolution rate of the drug from inclusion complexes is higher than physical mixtures and both are higher than that of plain drug (see figures 3 and 4).
Table 1: Relative amounts of etodolac dissolved after 15 minutes from inclusion complexes and physical mixtures with different cyclodextrins.

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Formula</th>
<th>R.A.D after 15 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CD (1:1)</td>
<td>Inclusion complex</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>Physical mixture</td>
<td>2.04</td>
</tr>
<tr>
<td>β-CD (1:1)</td>
<td>Inclusion complex</td>
<td>3.99</td>
</tr>
<tr>
<td></td>
<td>Physical mixture</td>
<td>3.86</td>
</tr>
<tr>
<td>γ-CD (1:1)</td>
<td>Inclusion complex</td>
<td>6.06</td>
</tr>
<tr>
<td></td>
<td>Physical mixture</td>
<td>5.78</td>
</tr>
<tr>
<td>HP-β-CD (1:1)</td>
<td>Inclusion complex</td>
<td>5.60</td>
</tr>
<tr>
<td></td>
<td>Physical mixture</td>
<td>5.75</td>
</tr>
</tbody>
</table>

\[ R.A.D = \frac{\text{Amount of the drug dissolved in presence of carrier}}{\text{Amount of the drug dissolved alone}} \]

Noteworthy, the increase in the dissolution of etodolac when it is physically mixed with CDs may be due to an improvement in the wettability and solubility of the drug in the aqueous CDs solutions resulting from the coexistence in the dissolution medium and the formation of inclusion complexes in solution \[^3\]. The impact of different CDs on the dissolution of drug was arranged in a following descending order:

\[ γ-CD > HP-β-CD > β-CD > α-CD. \]
Figure 3: Dissolution profiles of etodolac from inclusion complex with α-CD, β-CD, γ-CD and HP-β-CD in (1:1) molar ratios.

Figure 4: Dissolution profiles of etodolac from physical mixture with α-CD, β-CD, γ-CD and HP-β-CD in (1:1) molar ratios.

Infrared spectroscopy

The IR spectra were carried out using FTIR spectrophotometer in order to investigate the possible interaction between the drug and the host molecules in the solid state. The spectrum of the drug (figure 5, trace A) showed characteristic bands at wave number 1746 cm\(^{-1}\) corresponding to (C=O) stretching vibration of the carboxylic group, 3344 cm\(^{-1}\) due to single -NH stretching.
vibration of amine group and 2971 cm\(^{-1}\) corresponding to C-H stretching. These data are in a good accordance with the previously reported data\(^ {19}\).

**Figure 5: IR spectra of etodolac (A), CD (B), physical mixtures (C) and inclusion complexes (D)**

The CDs spectra (figure 5, trace B) showed mainly abroad vibrational band of free OH between 3300-3700 cm\(^{-1}\) or more specifically at 3383; 3383; 3385 and 3402 cm\(^{-1}\) for of α-CD, β-CD, γ-CD and HP-β-CD, respectively. The IR spectra of the physical mixture of the investigated cyclodextrins (figure 5, trace C) showed peaks of both etodolac and cyclodextrins with decrease in the peak intensity, indicating a little interaction between etodolac and cyclodextrins in the physical mixture. In the case of solid complexes (figure 5, trace D), there are small shifts in the position of some peaks about (2-3 cm\(^{-1}\)) as well as reduction of the intensity of the peaks. For
example, the characteristic –NH stretching band at 3344 cm\(^{-1}\) appeared as a single broad peak at 3346; 3344; 3347 and 3347 cm\(^{-1}\) for α-CD, β-CD, γ-CD and HP-β-CD complexes, respectively. Moreover, the intensity of C=O band (at 1746 cm\(^{-1}\)) is clearly decreased in all prepared complexes. This broadening of the peak and the decreased intensity of C=O bands in complexes indicated the breakdown of intramolecular hydrogen bonds of the drug molecules and formation of intermolecular hydrogen bonds between the drug and CD molecules. Also, the results proved the monomeric dispersion of a drug molecules and its entrapment in the hydrophobic cavity of cyclodextrins.

**Differential scanning calorimetry**

Further supporting evidence for the formation of inclusion complexes between the drug and CDs was obtained from the DSC thermograms (see figures 6). The DSC thermogram of untreated drug (trace A in all CD) showed an endothermic peak at 152.3 °C corresponding to the melting point of the drug. Regarding the DSC curves of CDs, it was observed that they are characterized by broad endothermic peaks at the range of 104 °C, 114 °C, 95 °C and 81 °C for α-CD, β-CD, γ-CD and HP-β-CD, respectively, due to the release of water molecules which were entrapped inside the cavity or those existing as residual humidity as reported by many authors (trace B)\(^{[20]}\). Other observed peaks indicate the degradation of cyclodextrins. DSC thermograms of etodolac/CDs physical mixtures showed the presence of endothermic peak of drug which confirms its crystallinity. These observations may be attributed to little interaction between the pure components in the physical mixture. Inclusion complexes with α-CD, β-CD, and γ-CD (Figures 6, trace C) showed similar results to that obtained by the corresponding physical mixture.

Regarding the DSC thermogram of drug/HP-β-CD complex, complete disappearance of drug endothermic peak is observed (Figure 12, trace D) which may be attributed to the complete transformation of drug from crystalline to amorphous state\(^{[21]}\). So, the considerable increase in the dissolution rate may be attributed to both complex formation and conversion of drug to amorphous state.
Figure 6: DSC thermograms of etodolac (A), CD (B), physical mixtures (C) and inclusion complexes (D)

X-ray diffractometry

To get further evidence on the solid state changes, x-ray diffraction spectra were carried out (Figures 7). The presence of numerous distinct peaks in the x-ray diffraction spectrum of etodolac indicates that the drug is present as a crystalline form with characteristic diffraction peak appearing at diffraction angles of 2θ at 9.16°, 13.6°, 14.38°, 18.58°, 22.9° and 27.28° with relative intensities of 44, 54, 100, 35, 65, and 30, respectively.
X-ray diffraction patterns of β-CD, as shown in Figure 13 (trace B) displays diffraction peaks, reflecting the crystallinity of this excipient. While the spectrum of HP-β-CD are characterized by the complete absence of any diffraction peaks, indicating its amorphous nature (Figure 7, trace B). Most of the principal peaks of etodolac are present in the diffraction patterns of physical mixtures with β-CD and HP-β-CD (Figure 7, trace C). These results indicated that there is a partial interaction between the drug and these CDs in the case of physical mixtures. In contrast, inclusion complexes of etodolac and β-CD (Figure 7, trace D) showed a decrease in the intensity of some peaks corresponding to both etodolac and β-CD, but it is no longer possible to distinguish the characteristic crystallinity peaks of pure drug or of β-CD. Many peaks of pure etodolac are not present but several new peaks are also observed in the x-ray diffraction pattern of complex, indicating the formation of complex. This finding is in accordance with that obtained and reported previously by Sinha et al[22].

![Figure 7: X-ray diffraction patterns of etodolac (A), CD (B), physical mixtures (C) and inclusion complexes (D).](image)

In the case of the inclusion complex based on HP-β-CD, the small crystalline spikes in XRD may correspond to the crystalline form of the unprocessed drug substance, which occurred on the amorphous halo in the X-ray diffraction pattern. Correspondingly, DSC investigation of ETD complexed with HP-β-CD confirmed that there was no sign of melting.
CONCLUSION

Etodolac/CDs inclusion complexes were formed using co-evaporation technique which is subsequently confirmed by IR, DSC, and x-ray diffraction analysis. The prepared inclusion complexes showed the highest increase in the dissolution rate as compared to either plain drug or other corresponding physical mixtures. HP-β-CD and γ-CD showed the highest effect on the enhancement of the dissolution rate of drug which was explained by the hydrophilicity and high amorphous nature of these CDs.

REFERENCES


Citation: Shaaban K Osman et al. Ijprr.Human, 2016; Vol. 6 (3): 304-318.