Enhancement of Solubility and Dissolution of Meloxicam by Cyclodextrin Complexation

S. Charumanee1, A. Titwan1, J. Sirithunyalug1, S. Okonogi1, P. Wolschann2 and H. Viernstein3
1 Faculty of Pharmacy Chiang Mai University, Chiang Mai, Thailand
2 Institute of Theoretical Chemistry, University of Vienna, Vienna, Austria
3 Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Vienna, Austria

Abstract
Complexation between meloxicam, a nonsteroidal anti-inflammatory drug with β-cyclodextrin and γ-cyclodextrin was investigated to improve the solubility and the dissolution of the drug. Phase-solubility studies of the complexes in aqueous solution pH 3 showed that the solubility of meloxicam was ten-fold increased by complexing with γ-cyclodextrin, whereas it was only 5-times in the case of β-cyclodextrin, compared to the pure drug. The equimolar ratio solid complex of the drug and cyclodextrin was prepared by kneading, co-evaporation and co-lyophilization method. The complexes were characterized using differential scanning calorimetry, X-ray diffractometry, infrared spectroscopy and near infrared spectroscopy compared to the physical mixture and the pure drug. The analyses revealed that the inclusion complexes formation between the drug and cyclodextrins was successful by co-evaporation and co-lyophilization techniques. Thus, the in vitro dissolution rate of the drug from the complexes was substantially increased. The dissolution enhancement factors were ranged from co-lyophilized, co-evaporated and kneaded complexes respectively. The dissolution improvement was negligible when the drug and cyclodextrin were physically mixed. The enhancement of the solubility and the dissolution rate of the drug is attributed by the partially inclusion of the drug into cyclodextrin cavity and also the reduction in the crystallinity of the drug during the complex preparation process.

Keywords: cyclodextrins, meloxicam, phase-solubility, solubility, dissolution rate

Introduction
Meloxicam (Mlx) is a non-steroidal anti-inflammatory drug which shows preferential inhibition to cyclo-oxygenase-2-isozymes. It has been introduced and marketed for treatment of osteoarthritis, rheumatoid arthritis and other musculoskeletal disorders. Recently, it’s chemopreventive and chemosuppressive activities on various cell lines have been reported (Banerjee and Sarkar, 2002). This might lead to the role of this drug on cancer treatment. Unfortunately, the drug possesses a very poor aqueous solubility and dissolution rate-limited bioavailability. Although their structures are related (Figure 1), the water solubility of meloxicam is much lower than piroxicam. Due to these intrinsic properties, the steady-state blood concentrations of meloxicam can not achieved for 3-4 days with oral administration (Davies and Skjodt, 1999).

Cyclodextrins, cyclic oligosaccharides derived from starch, have been used successfully to improve solubility and the dissolution rate of poorly water soluble drugs by forming inclusion complexes. This approach has been already applied to piroxicam. The piroxicam-β-cyclodextrin complex is now being marketed to achieve better oral bioavailability. An attempt to improve the dissolution rate of meloxicam using β-cyclodextrin has been reported (Nath and Kumar, 2000).

The aim of this study was to enhance the solubility and the dissolution rate of meloxicam by cyclodextrin complexation, focusing on the application of γ-cyclodextrin, another type of natural cyclodextrin.
Figure 1 Molecular structure of piroxicam (a) and meloxicam (b)

Experimental

Materials
Meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl-2H-1, 2-benzothiazine -3-carboxamide -1, 1-oxide) was kindly provided by Boehringer Ingelheim, Germany and Kwisda Company, Austria. β-cyclodextrin (Cavamax® W7 Pharma), and γ-cyclodextrin (Cavamax® W8 Pharma) were purchased from Wacker Chemie, Germany. Hydrochloric acid and other chemicals were analytical-reagent grade.

Phase-solubility studies
The phase solubility studies were carried out according to Higuchi and Connors (Higuchi and Connors, 1965). An excessive amount of meloxicam was placed in a screw-capped vial, to which 10 mL the acidified water (pH 3.0), containing varying concentrations (0–15 mM) of β-cyclodextrin or γ-cyclodextrin was added. The suspensions were mechanically shaken at 25 ±0.1°C for 5 days. At the end of equilibrium time, the content in each vial was filtered through 0.45 μm membrane filter (Sartorius, Göttingen, Germany). The filtrate was appropriately diluted and analysed for the drug content using absorption spectrophotometry (Spectrophotometer SPEKOL® 1200, Analytik, Jena AG, Germany). The experiment was performed in triplicate.

Preparation of the physical mixtures
The 1:1 molar ratio of drug and cyclodextrins previously sieved (180 μm/150 μm) were thoroughly mixed using vortex mixer.

Preparation of inclusion complexes
Kneading method
The 1:1 molar ratio of the drug and cyclodextrin was mixed by geometric dilution fashion. A small portion on 1:1 (v/v) ethyl alcohol and water mixture was added to dry mixture. It was vigorously kneaded using mortar and pestle to obtain a smooth thick mass, and then dried at 45°C overnight. The dry mass was pulverized, sieved and kept in dried place for further study.

Co-evaporation method
The required amount of the drug was added to the solution of cyclodextrin while stirring. To aid the complete dissolution of the drug, the pH of the suspension was adjusted to approximately 9.0 by addition of ammonium hydroxide (32% w/v) solution dropwise. The solution was evaporated under vacuum at 50°C using rotary evaporator (Heidolph VV2011, Germany). The solid residue was further dried at 45°C overnight.

Co-lyophilization method
The clear solution of the drug in cyclodextrin solution as previously described was frozen at -80°C in a deep freezer then lyophilized for 48 hours (Freeze dryer, Christ® Beta 1-8K, Christ GmbH, Germany). The dry mass was pulverized, sieved and kept in dried place for further investigation.
Characterization of the inclusion complexes

Dissolution studies

The in vitro dissolution studies of the pure drug, physical mixtures and the inclusion complexes were performed at 37±0.5°C using USPII, paddle method (Dissolution Tester Type PTWS3C, PharmaTest, Germany). 900 Milliliters of simulated gastric fluid without enzymes (pH 1.2, USP27) maintained at 37°C was used as the dissolution medium. The stirring speed of the paddle was 100 rpm/minute. The powder sample (180 µm/150 µm fraction) equivalent to 15 mg of the drug was spread over the dissolution medium. A 5-ml aliquot of the dissolution medium was taken through a filter-fitted Teflon tube at the appropriate time interval to 90 minute and then replaced by fresh portion of the dissolution medium. The collected samples were analyzed spectrophotometrically. The experiment was performed in triplicate. The dissolution profiles of the drug from the physical mixture and the inclusion complexes were compared using the percentage of dissolution efficiency up to 30 minutes, %DE30 (Khan, 1975).

Differential scanning calorimetry (DSC)

DSC thermograms of the samples were recorded using differential scanning calorimeter (Perkin-Elmer, DSC7, USA). The accurately weighed sample was placed on an aluminum pan with holes and scanned at the heating rate of 10°C/minute over the temperature range of 50-280°C under nitrogen gas flow (flow rate 20 mL/minute).

X-ray powdered diffractometry (XPD)

The X-ray diffraction patterns of all samples were obtained from diffractometer (Semem-D500, Germany). The experiment was performed at room temperature according to the following conditions: voltage 20 kV; current 20 mA; time constant 0.5 seconds at scanning speed of 4°/minute over the scattering angle (2θ) range of 10-60.

Fourier transform infrared spectroscopy

The IR spectra of the drug and the complexes were recorded using Infrared spectrophotometer (Hitachi 295, Japan). The samples were prepared as potassium bromide disks and scanned over the absorbance of 4000-600 cm⁻¹.

Near infrared spectroscopy (NIR)

The NIR spectra of the samples were obtained using NIRVIS/1004 (Bühler, Switzerland). The data were processed using software NIRCal version 4.21, build 389.

Results and Discussion

Phase-solubility studies

The phase-solubility of meloxicam in aqueous solution of β-cyclodextrin and γ-cyclodextrin were A₁ type as described by Higuchi and Connors (Figure 2). This curve type is characterized by linearly increase in the drug solubility with the increasing cyclodextrin concentrations. The linear relationship associated with the slope of the straight line less than one could suggest the formation of 1:1 inclusion complex. The association constant, K of 1:1 inclusion complex can be calculated from the intrinsic solubility of the drug and the slope of the solubility curve, the K values were illustrated in Table 1. The higher K value obtained from meloxicam-γ-cyclodextrin complex is attributed by the stronger binding between the drug and cyclodextrin. As a consequence, the solubility of meloxicam was markedly increased when the drug formed complex with γ-cyclodextrin compared to β-cyclodextrin.
Figure 2  Phase solubility diagrams of meloxicam in aqueous solution of cyclodextrins pH 3.0 at 25°C. β-Cyclodextrin, solid symbol; γ- Cyclodextrin, open symbol

Table 1  Stability constants of meloxicam-cyclodextrin inclusion complexes together with meloxicam solubility at 25°C in aqueous solution at pH 3.0

<table>
<thead>
<tr>
<th></th>
<th>K (M⁻¹)</th>
<th>Solubility (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam, intact</td>
<td>-</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>Meloxicam in β-CD</td>
<td>295.4 ± 6.5</td>
<td>2.44 ± 0.18</td>
</tr>
<tr>
<td>Meloxicam in γ-CD</td>
<td>540.2 ± 0.4</td>
<td>4.22 ± 0.14</td>
</tr>
</tbody>
</table>

Dissolution studies

The dissolution profiles of meloxicam from physical mixtures and the complexes were shown in Figure 3, in addition to the dissolution efficiency illustrated in Table 2, it is clearly demonstrated that the dissolution of meloxicam was significantly improved by complex formation. The method of complex preparation played an important role on the extent of the dissolution enhancement. For meloxicam-β-cyclodextrin complexes, co-lyophilization method showed the greatest effectiveness. However, in the case of meloxicam-β-cyclodextrin complex, there was not significantly different between the co-evaporation and co-lyophilization method. The presence of cyclodextrin in physical mixture, when the complex formation unlikely occurred showed no solubilizing effect.

Table 2  Dissolution efficiency of meloxicam (% DE30) in simulated gastric fluid from 1:1 meloxicam-cyclodextrin inclusion complexes prepared by different methods

<table>
<thead>
<tr>
<th>Complexes</th>
<th>%DE30 β-cyclodextrin</th>
<th>%DE30 γ-cyclodextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam, intact</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Physical mixture</td>
<td>0.02 ± 0.001</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td>Kneaded complex</td>
<td>7.84 ± 0.55</td>
<td>9.62 ± 0.67</td>
</tr>
<tr>
<td>Co-evaporated complex</td>
<td>12.09 ± 0.61</td>
<td>29.20 ± 0.60</td>
</tr>
<tr>
<td>Co-lyophilized complex</td>
<td>37.80 ± 0.45</td>
<td>28.42 ± 0.73</td>
</tr>
</tbody>
</table>
The 3rd Conference on Starch Technology

Figure 3 Dissolution profiles of meolxicam from intact drug (Mlx), Mlx:β-cyclodextrin (left); Mlx: γ-cyclodextrin (right) PM, physical mixture; KN, COE, and COL the inclusion complexes prepared by kneading, co-evaporation, and co-lyophilization methods.

**Differential scanning calorimetry (DSC)**

Figure 5 shows the DSC thermograms of meloxicam inclusion complexes with β-cyclodextrin and γ-cyclodextrin prepared by different methods. The melting peak of the drug exhibited at 253.5°C. The peak intensity was reduced and shifted to lower temperature in the cases of kneaded and co-evaporated complexes, indicating an interaction between the drug and cyclodextrins. The melting peak of the drug was nearly or completely disappeared in co-lyophilized complex for both cyclodextrins suggesting the inclusion of the drug into cyclodextrin cavity (Babu and Pandit, 2004; Jug and Becirevic-Lacan, 2004) and the successive reduction in crystallinity of the complexes (Fernandes et al., 2002).

Figure 4 DSC thermograms of meloxicam, (Mlx); BCD, physical mixture, PM; the inclusion complexes prepared by kneading (KN); co-evaporation (COE) and co-lyophilization method (COL); Right, β-cyclodextrin; left, γ-cyclodextrin.

**X-ray powdered diffractometry (XPD)**

More useful evidence supports the inclusion complex formation between the drug and cyclodextrins is X-ray powdered diffractometry (Figure not shown). The diffused diffraction XPD peaks exhibited by the kneaded complexes and co-evaporated complexes is responsible to the partial inclusion of the drug into the cavity of cyclodextrins. The halo type diffractionogram shown by the co-lyophilized complexes can be ascribed to the complete inclusion of the drug into cyclodextrin cavity (Scalia et al., 1998)
**Fourier transform infrared spectroscopy (FTIR)**

FTIR and NIR spectra also provided evidences of the complex formation between the drug and cyclodextrins (Figure not shown). The disappearance of the absorption amide peak of the drug at 1616 cm\(^{-1}\), in addition to the significant shift of the second amide peak from 1546 cm\(^{-1}\) to 1520 cm\(^{-1}\) in FTIR spectra suggesting the interaction between the drug and cyclodextrins by intermolecular hydrogen bond formation (Dollo et al., 1996). NIR spectra of the complexes also showed significant changes from the spectra of the drug and cyclodextrins thus could suggest the interaction and the inclusion of the drug into cyclodextrin cavity (Viernstein, 1994).

**Conclusion**

The complexation of meloxicam and the two natural cyclodextrins was evidenced by DSC, XPD, FTIR and NIR spectroscopy investigations. The solid inclusion complexes showed an increase in drug solubility and the dissolution rate. This enhancement offers the promised improvement of meloxicam bioavailability. \(\gamma\)-Cyclodextrin showed higher effectiveness than \(\beta\)-cyclodextrin, especially when the complexes are prepared by simple kneading method and co-evaporation method. \(\gamma\)-Cyclodextrin is recommended as an alternative cyclodextrin for improving the bioavailability of meloxicam.

**Acknowledgements**

The authors thank the Graduate School, Chiang Mai University for partial financial support. Boehringer Ingelheim Pharma GmbH&Co., Germany and Kwisda GmbH&Co., Austria is acknowledged for kindly donated meloxicam for the study.

**References**

Figure Captions
Figure 1 Molecular structure of piroxicam (a) and meloxicam (b).

Figure 2 Phase solubility diagrams of meloxicam in aqueous solution of cyclodextrins pH 3.0 at 25°C. β-Cyclodextrin, solid symbol; γ- Cyclodextrin, open symbol.

Figure 3 Dissolution profiles of meloxicam from intact drug (Mlx), Mlx:β-cyclodextrin (a); Mlx: γ-cyclodextrin (b) PM, physical mixture; KN, COE, and COL the inclusion complexes prepared by kneading, co-evaporation, and co-lyophilization methods respectively.

Figure 4 DSC thermograms of meloxicam, (Mlx); BCD, physical mixture, PM; the inclusion complexes prepared by kneading, KN; co-evaporation, COE and co-lyophilization method, COL.

Tables
Table 1 Stability constants of meloxicam-cyclodextrin inclusion complexes together with meloxicam solubility at 25°C in aqueous solution at pH 3.0

<table>
<thead>
<tr>
<th>Complexes</th>
<th>K (M⁻¹)</th>
<th>Solubility (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam, intact</td>
<td>-</td>
<td>0.47 ±0.04</td>
</tr>
<tr>
<td>Meloxicam in β-CD</td>
<td>295.4 ±6.5</td>
<td>2.44 ±0.18</td>
</tr>
<tr>
<td>Meloxicam in γ-CD</td>
<td>540.2 ±0.4</td>
<td>4.22 ±0.14</td>
</tr>
</tbody>
</table>

Table 2 Dissolution efficiency of meloxicam (%DE30) in simulated gastric fluid from 1:1 meloxicam-cyclodextrin inclusion complexes prepared by different methods

<table>
<thead>
<tr>
<th>Complexes</th>
<th>% DE30</th>
<th>β-cyclodextrin</th>
<th>γ-cyclodextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meloxicam, intact</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Physical mixture</td>
<td>0.02 ±0.001</td>
<td>0.02 ±0.001</td>
<td></td>
</tr>
<tr>
<td>Kneaded complex</td>
<td>7.84 ±0.55</td>
<td>9.62 ±0.67</td>
<td></td>
</tr>
<tr>
<td>Co-evaporated complex</td>
<td>12.09 ±0.61</td>
<td>29.20 ±0.60</td>
<td></td>
</tr>
<tr>
<td>Co-lyophilized complex</td>
<td>37.80 ±0.45</td>
<td>28.42 ±0.73</td>
<td></td>
</tr>
</tbody>
</table>