

STUDY OF THE COMPLEXATION BEHAVIOR OF TENOXICAM WITH CYCLODEXTRINS

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Abstract

Tenoxicam (T) is a lipophilic nonsteroidal anti-inflammatory agent, very slightly soluble in water, often used in the treatment of rheumatic and arthritic diseases for its anti-inflammatory, analgesic and antipyretic properties. In order to increase its solubility in water, inclusion complexes of tenoxicam with α -cyclodextrin (α -CD) and β -cyclodextrin (β -CD) were obtained.

In order to obtain the complexes as particles of small (nanometric) dimensions, complexation by the co-precipitation, kneading and ethanol-water solution methods was used.

The obtained inclusion complexes were characterized through Scanning Electron Microscopy (SEM) for the evaluation of the crystals' morphology and their approximate size and through thermal analysis using the thermogravimetric (TG) method and differential scanning calorimetry (DSC).

The results obtained confirm the inclusion of tenoxicam in the cyclodextrin cavity.

Rezumat

Tenoxicamul (T) este un agent antiinflamator lipofil, foarte puțin solubil în apă, frecvent utilizat în tratamentul bolilor reumatice și artritice pentru proprietățile sale antiinflamatoare, analgezice și antipiretice. Pentru creșterea solubilității acestuia în apă au fost obținuți complecși de incluziune ai tenoxicamului cu α - și β -ciclodextrina (α -, β -CD).

Pentru obținerea complecșilor sub forma unor particule de dimensiuni reduse (nanometrice) s-a apelat la metoda de complexare prin coprecipitare, metoda triturării și prin intermediul metodei în soluție etanol-apă.

Complecșii de incluziune formați au fost caracterizați prin microscopie electronică cu scanare (SEM) pentru evaluarea morfologiei cristalelor și a dimensiunilor aproximative ale

acestora și prin analiză termică utilizând metoda termogravimetrică (TG) și calorimetria dinamică diferențială (DSC).

Rezultatele obținute confirmă includerea tenoxicamului în cavitatea ciclodextrinei.

Keywords: tenoxicam, inclusion complexes, α -cyclodextrin, β -cyclodextrin.

Introduction

Tenoxicam, 4-hydroxy - 2 - methyl -N-2- pyridinyl - 2H - thieno - [2,3e]1,2-thiazine-3-carboxamide-1,1-dioxide (Figure 1a) is a non-steroidal anti-inflammatory drug (NSAID), acting as preferential inhibitor of cyclooxygenase-2 and inhibitor of prostaglandin synthesis. It is very effective as analgesic and anti-inflammatory drug for the systemic treatment of rheumatoid arthritis, osteoarthritis and other joint diseases [1, 2]. However, being a lipophilic drug (Log P=2.4), tenoxicam is sparingly soluble in water (0.076 mg/mL at 25°C) [3, 4], so that its dissolution may be the rate determining step in the absorption process.

α - and β -cyclodextrins are water-soluble cyclic oligosaccharides formed by six, and seven glucose units, respectively (Figure 1b and 1c). Because of their truncated cone structure with a low polarity central void, α - and β - cyclodextrins have been reported in numerous studies in the pharmaceutical field to interact with many drug molecules to form inclusion complexes [1, 5, 6]. The minimum requirement for the formation of such host-guest complexes is a size and shape compatibility between host (α - or β - cyclodextrin) and guest (drug) molecules [7, 8]. These inclusion complexes have been extensively used to improve the solubility of poorly soluble drugs [7, 9], and the biopharmaceutical properties of some oxycam derivatives [1]. The molecular weight of tenoxicam (337.7) and its poor solubility in water are the principal properties which recommend it as a suitable candidate for inclusion complexation with α - and β -cyclodextrins.

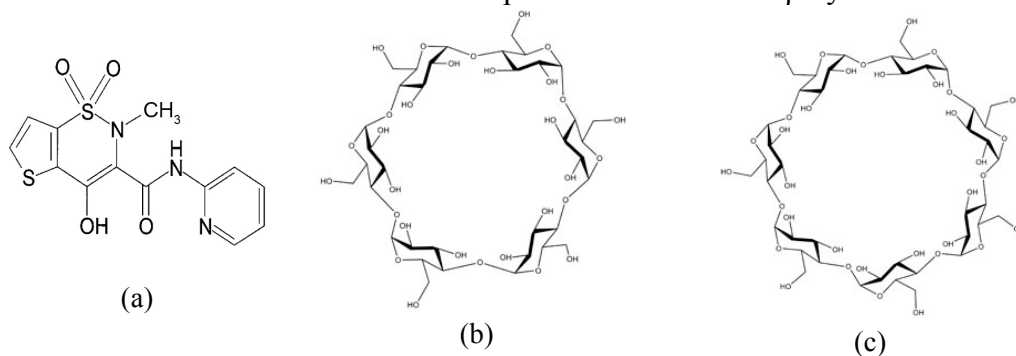


Figure 1
Chemical structure of tenoxicam (a), α -cyclodextrin (b) and β -cyclodextrin (c).

The aim of the present work was to study the formation of inclusion complexes of tenoxicam with α -cyclodextrin and β -cyclodextrin, as a possibility of increasing the solubility of this drug in water [4, 8].

The preparation methods used to obtain complexes of tenoxicam and cyclodextrins were the co-precipitation method, the kneading method and the ethanolic solution method.

The presence of inclusion complexes obtained was detected by microscopical and thermoanalytical studies: Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TG) and Differential Scanning Calorimetry (DSC) [4, 10, 11, 12, 13].

Materials and Methods

Materials

Tenoxicam, with purity >99%, was a generous gift from LaborMed Pharma, (Romania), while α - and β -CD were purchased from Cyclolab (Hungary). Ethanol and other base chemicals of analytical grades were purchased from commercial suppliers and used without further purification.

Methods

Preparation of inclusion complexes

Co-precipitation

The amounts of cyclodextrins and tenoxicam (in a 1:1 molar ratio) were weighed in an ultrasonation reactor, 4 mL of solvent were added and the obtained suspension was ultrasonated by cooling on a water and ice bath, with the following parameter values set for ultrasonation: ultrasonation frequency – 20 kHz; ultrasonation time – 15 minutes; pulse method (30s ultrasonation, 15s pause); amplitude – 80% [4, 10].

Following the completion of an one-hour cold-crystallization (at approx. 4°C), the nanoparticles were separated from solvent by vacuum filtration and washed with 1 mL of 93% ethanol, then dried at 40°C to a constant mass.

Kneading method

Masses of cyclodextrins and tenoxicam (in a 1:1 molar ratio) were weighed, 0.5 mL of solvent were added and the paste obtained was cold-triturated in a mortar for 30 minutes, after which it was dried in the oven at 50°C to a constant mass, while the obtained precipitate was dry-triturated in a mortar, and complexation yields were determined.

Ethanol-water system method

The cyclodextrins were weighed on the analytical balance in a complexation minireactor, the installation was mounted on a mechanical stirring system with the possibility of thermostatisation, and then 4 mL of

distilled water were added. Immediately after the initiation of shaking and heating at 50°C, an ethanolic solution of tenoxicam containing 0.5 mmoles bioactive compounds was added dropwise to the cyclodextrin-water mixture.

Dropwise addition was performed for 15 minutes, after which the system was kept under stirring for 15 minutes at the same temperature. In order to obtain well formed crystals of the complex, the solution was slowly cooled to 25°C for 3 hours, and then the suspension was left in a refrigerator (5°C) overnight (~12 hours) in order to perfect the crystallization. The suspension was then filtered by vacuum filtration, and the complex crystals were washed with 1 mL 93% ethanol and dried at a maximum of 40°C to constant mass. Complexation yields were obtained as the ratio between the complex mass obtained and the sum of cyclodextrin and tenoxicam masses introduced into the process [4, 10].

Apparatus

Sonication of cyclodextrin-tenoxicam mixtures was performed in an immersion ultrasonication system, 500 Watt Ultrasonic Liquid Processor Vibra Cell VC 505.

The surface morphology of binary systems was examined by Scanning Electron Microscopy (Inspect S50, Japan); the pictures were taken at an excitation voltage of 25 kV, a magnitude of 3000-12000 × and focusing from 10 to 14.1 mm.

Thermogravimetric analysis of binary systems was performed with a NETZSCH TG 209 (Germany) model Thermogravimetric Analyzer at a temperature programme of 20-550°C with a heating rate of 10°C/min. Determinations were performed under nitrogen atmosphere.

Thermograms of binary systems were recorded on a Netzsch DSC 204 (Germany) model Differential Scanning Calorimeter at a temperature programme from 20°C to 400°C with a heating rate of 4°C/min, cooling being performed with liquid nitrogen. Data acquisition was carried out using the Netzsch DSC 204-Acquisition Soft/2000 specific programme; the processing of data was performed with the 4.0/2000 version of the Netzsch Proteus-Thermal Analysis programme [4, 10].

Results and Discussion

Regarding the efficiency of inclusion complexes preparation method, the kneading method proved to be the best, as it was carried out with relatively high complexation yields (73.17% for α -cyclodextrin, 82.5% for β -cyclodextrin), compared with the co-precipitation method, where complexation yields were low (38.52% for α -cyclodextrin).

Scanning Electron Microscopy (SEM) studies

The particles morphologies of the solid products obtained from raw materials (tenoxicam, α - and β -cyclodextrins) by co-precipitation/evaporation, as well as their approximate size, were evaluated by SEM photographs.

In the case of samples obtained by *co-precipitation* method, the particles, although agglomerated, were of reduced dimensions (in hundreds of nanometers range) and showed regular rhomboidal forms (Figure 2a).

Although they showed a certain uniformity, the particles obtained by the *kneading* method did not have a clearly defined shape. These particles are also agglomerated, but their dimensions range in a relatively narrow domain, between micrometres and tens of micrometres (Figure 2b), both for T/ α -CD, and for T/ β -CD.

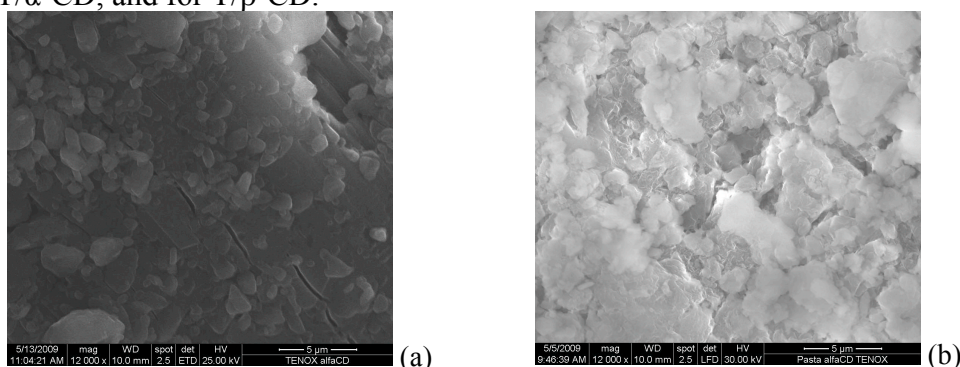


Figure 2

SEM images of T/ α -CD complexes obtained by the co-precipitation (a) and kneading method (b)

The particles of samples obtained by *the solution method*, did not have a uniform distribution of dimensions, which have varied from units to tens of micrometers; also, the crystals of β -CD products were prismatic rhombohedral in shape (Figure 3).

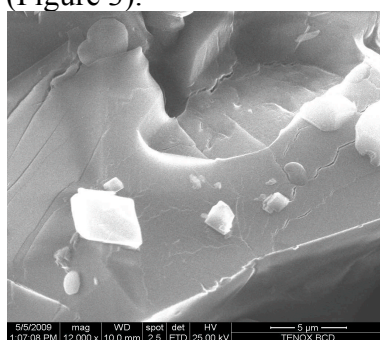


Figure 3

SEM images of T/ β -CD complexes obtained by the solution method.

Thermogravimetric analysis

Thermogravimetric analysis (TG) clearly indicated the formation of inclusion complexes by all used methods, but the best results were observed in the case of β -CD products obtained by co-precipitation and kneading. Figures 4 and 5 show the TG curves obtained for tenoxicam and pure α - and β -cyclodextrin, figures 6, 7 and 8 show the TG curves recorded for T/ β -CD complexes obtained by co-precipitation, crystallization from solution and kneading, and for T/ α -CD complex obtained by crystallization from solution and kneading.

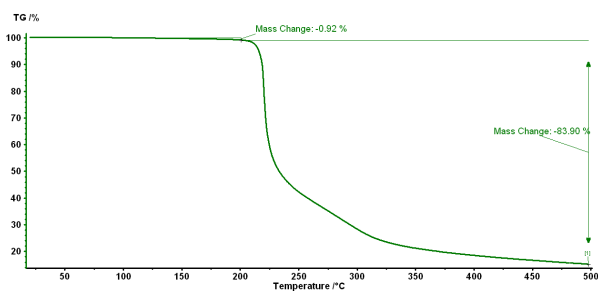


Figure 4
TG curve of tenoxicam (50-500°C)

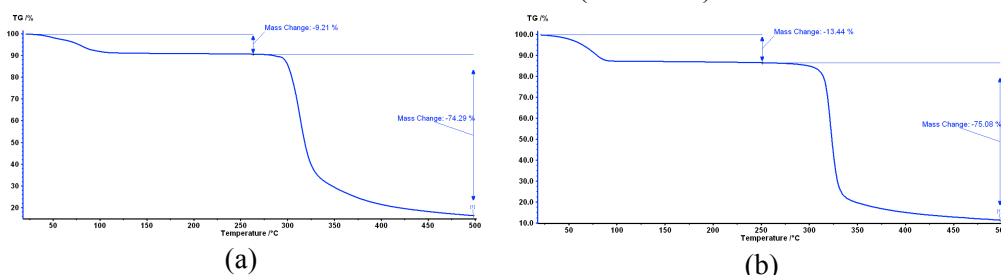


Figure 5
TG curve of pure α -cyclodextrin (a) and β -cyclodextrin (b) (50-500°C)

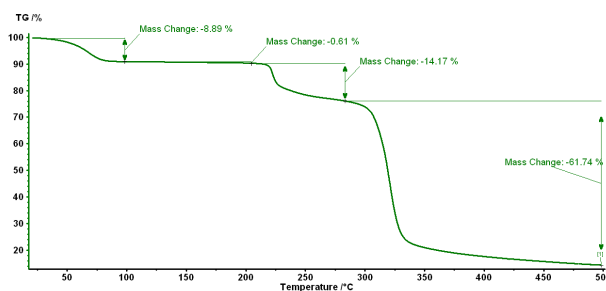
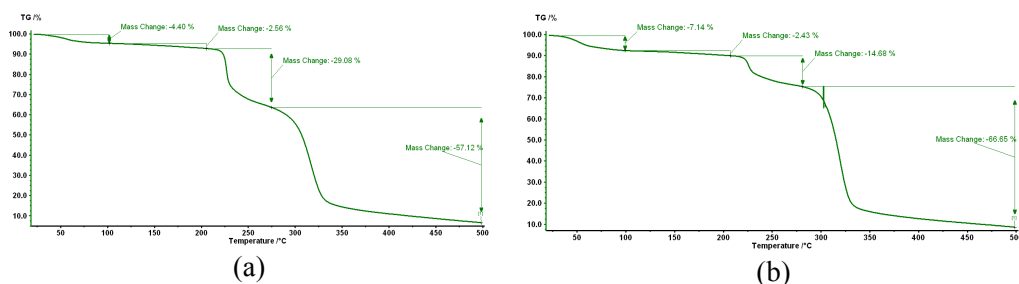
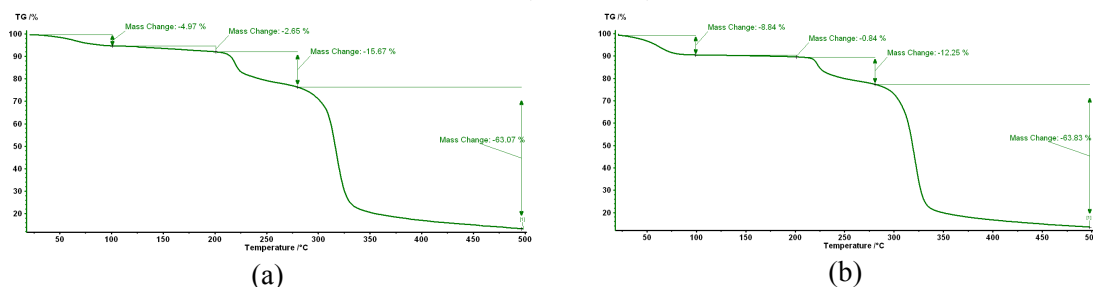


Figure 6
TG curve of T/ β -CD complex obtained by co-precipitation (50-500°C)

**Figure 7**

TG curve of T/α-CD (a) and T/β-CD (b) complex obtained by crystallization from solution (50-500°C)

**Figure 8**

TG curve of T/α-CD (a) and T/β-CD (b) complex obtained by kneading (50-500°C)

Differential Scanning Calorimetry analysis

Samples subjected to analysis were binary mixtures of tenoxicam with α- and β- cyclodextrin respectively.

In the case of tenoxicam-α-cyclodextrin binary mixtures, encapsulation appears to be more important for the kneading method. Thus, the DSC plot of T/α-CD mixtures obtained by the kneading method (Figure 9) shows endothermic effects, especially in the range 135-185°C. By contrast, the DSC plot of T/α-CD mixtures prepared by solution method (Figure 10a) indicates that the dehydration process occurs in a temperature range of up to ~135°C (where decomplexation of biologically active compounds may also occur) and at ~215°C (tenoxicam) where melting/decomposition of biocompounds occurs.

The DSC analysis of the T/β-CD complexes obtained by all the three methods (crystallization from solution, kneading and co-precipitation), indicated the dehydration/dissociation of the inclusion complex, melting/decomposition of the drug and decomposition process of cyclodextrin. Thus, the thermogravimetric plots of T/β-CD complex prepared by crystallization from solution evidenced a relatively low complexation, with decomposition at ~210°C (Figure 10b). The inclusion complex obtained by kneading is much better formed, as shown by the DSC

curve presented in figure 9b, indicating that the dissociation process occurs at 150°C.

In the case of samples obtained by the co-precipitation method, the results indicate an advanced encapsulation ability, but it is likely that the structures involved to be partially decomposed.

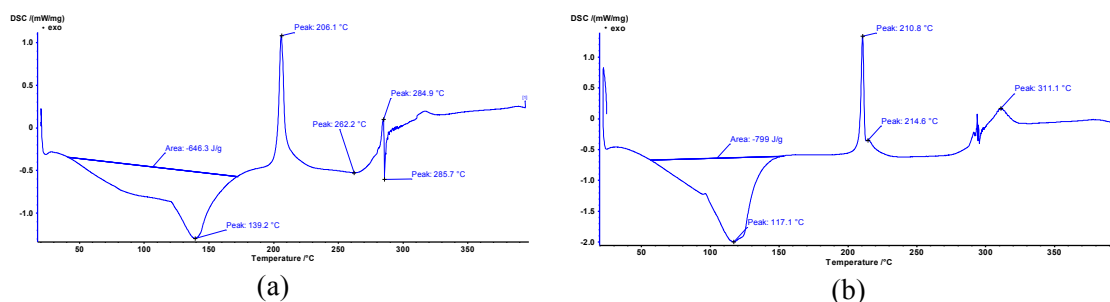


Figure 9
DSC thermograms of T/α-CD (a) and T/β-CD (b) complex obtained by the kneading method

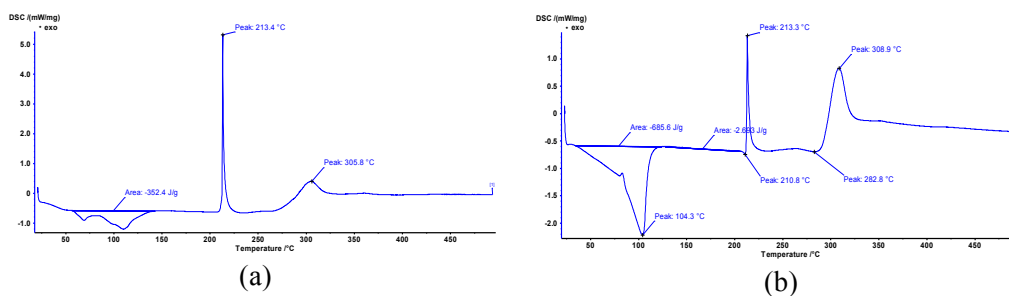


Figure 10
DSC thermograms of T/α-CD (a) and T/β-CD (b) complex obtained by the crystallization from solution

Conclusions

Inclusion complexes of tenoxicam/α-CD and tenoxicam/β-CD were prepared successfully by crystallization from solution, kneading and co-precipitation methods in a molar ratio of 1:1. Complexes formation has been studied. The thermogravimetric analysis (TG) clearly indicated the formation of complexes by all methods used to obtain the inclusion complexes, but the best results were obtained especially for tenoxicam complexation with β-cyclodextrin by co-precipitation and kneading.

The DSC analysis of T/ β -CD inclusion complexes highlighted that the complex obtained by the kneading method is much better formed than those obtained by co-precipitation and crystallization from solution.

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