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Dedicated to Professor Dr. Zeno GÂRBAN, on the occasion of his 70th anniversary

Bioactive compounds (hepatoprotective or anti-inflammatory xenobiotics) / cyclodextrin nanoparticles: a comparative study

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Abstract

This paper presents a comparative study on the complexation of two xenobiotics with natural cyclodextrins. Berberine (a hepatoprotective and antibacterial agent with rigid structure) and piroxicam (an anti-inflammatory compound with more flexible structure, but similar with berberine) were encapsulated in α- and β-cyclodextrin with yields of 83-89% and 71-72%, respectively. The obtained complexes (by using the ethanol-water solution method) were characterized by scanning electron microscopy (the crystal dimensions were in the range of 800 nm to 10 μm), thermogravimetry and differential scanning calorimetry, both analyses revealed the formation of bioactive compound-cyclodextrin complexes.

Keywords: berberine, piroxicam, hepatoprotective, anti-inflammatory, cyclodextrins, nanoparticles, xenobiotics

1. Introduction

Berberine is an alkaloid (Figure 1) which occurs especially in *Berberis* species (barberry) [1-4]. These plants belong to the Berberidaceae family and it is a small shrub which grows in Europe, but also in Africa and Asia, especially to the forest edge, in shining places. Most of bioactive properties of these plants are due to the presence of alkaloids like berberine, which have the capacity to inhibit MAO enzyme and act as antiprotozoal (Leishmania), antimalarial, antibacterial, antidiarrheal, and even hepatoprotective agent [1].

Piroxicam (Figure 1) is a nonsteroidal anti-inflammatory drug [5] used for the treatment of mild to moderate acute and chronic pain and inflammation including musculoskeletal, soft-tissue, and joint disorders like ankylosing spondylitis, chronic polyarthritis, and gout [5-8]. Piroxicam shows a 600-fold selectivity for COX-1 compared to COX-2 in cultured animal cells. Piroxicam is administered orally, rectally, intramuscular, or topically as the free base, as complex with β-cyclodextrine, and as cinnamate or pivalate. After oral application, piroxicam reaches peak plasma concentration after 3 to 5 h, shows a 99 % binding to plasma protein and a long half-life of about 50 h [5].

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The structures of these two xenobiotics are relatively similar, but berberine has a more hydrophilic rigid structure, while piroxicam has a more flexible structure; the behavior of these compounds in the interaction with hydrophobic inner cavity of different matrices, in order to obtain pharmaceutical formulations with controlled release properties, can be different from the geometric and hydrophobic point of view. From the wide range of encapsulation matrices, naturally or chemically modified cyclodextrins are extensively used in medicine and food fields. Naturally occurring cyclodextrins are α-, β-, and γ-cyclodextrin, which are cyclic oligosaccharides with 6, 7, and 8 glucopyranose moieties; they are obtained from starch by using various *Bacillus* species (like *B. macerans*). The specific architecture of the cyclodextrin structure (truncated cone with exterior hydroxyl groups and hydrophobic inner cavity) determine to use them for nanoencapsulation of hydrophobic and geometrically compatible bioactive molecules (like drugs, food additives, odorant and flavoring compounds etc.) in order to obtain powdery formulations with higher water solubility, protecting capacity (against air, light, humidity), and controlled release properties [9-12].

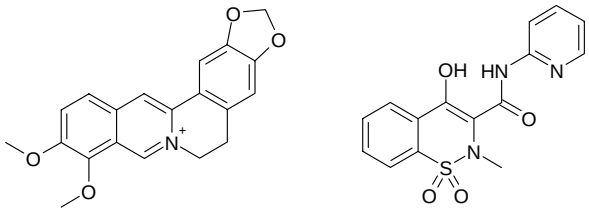

Figure 1. Structures of berberine and piroxicam

In this paper we continue our previous works [13-15] by comparing two xenobiotics with anti-inflammatory or hepatoprotective properties from the possibility of α- and β-cyclodextrin encapsulation point of view in order to find relationships between structures (berberine with rigid structure and more hydrophilic properties, but with hydrophobic moieties which can interact with cyclodextrins, and piroxicam with flexible structure, but relatively similar with the berberine structure).

2. Materials and method

2.1. Materials. Berberine (as hydrochloride), piroxicam, α- and β-cyclodextrin used for nanoencapsulation were purchased from Sigma (>99%, and >98% for cyclodextrins). For complexation of bioactive compounds in cyclodextrins 96% (v/v) ethanol was used (Chimopar, Bucharest).

2.2. Obtaining of bioactive compounds/ cyclodextrin nanoparticles. Protecting and controlled release of xenobiotics like bioactive compounds were realized by cyclodextrin micro/nanoencapsulation. The cyclodextrin was dissolved in 4 ml distilled water in a thermally controlled minireactor, equipped with reflux condenser, dropping funnel, and magnetic stirrer; the cyclodextrin solution/suspension was heated to 50ºC and berberine or piroxicam (an ethanolic solution with the concentration of bioactive compound corresponding to the biocompound : cyclodextrin ratio of 1 : 1) was added to the cyclodextrin solution for 15 minutes. The complex solution/suspension was stirred for another 15 minutes. The complexation mass was slowly cooled, the complex crystallized and the suspension was put in a refrigerator for completing the crystallization. The biocompound:cyclodextrin micro/nanoparticles were filtered, washed with ethanol and dried at environmental temperature. The complexation conditions and yields are presented in Table 1. The obtained micro/nanoparticles were analyzed by scanning electron microscopy (SEM), thermogravimetry (TG), and differential scanning calorimetry (DSC).

Table 1. Complexation conditions and yields for obtaining bioactive compounds/cyclodextrin nanoparticles

No	Code	$m_{biocompd.}$ (g)	m_{CD} (g)	$m_{complex}$ (g)	Yield (%)
1	Ber_aCD	0.169	0.540	0.589	83.1
2	Ber_bCD	0.168	0.672	0.599	71.2
3	Pir_aCD	0.166	0.543	0.631	89.1
4	Pir_bCD	0.175	0.671	0.607	71.7

2.3. Scanning electron microscopy (SEM). Morphological and dimensional analysis of the biocompound/cyclodextrin particles were performed by using an INSPECT S SEM apparatus, voltage of 25 kV, 6000× magnitude level, focusing 9.9-10.1 mm.

2.4. Thermogravimetry (TG). Thermogravimetric analysis of the biocompound / cyclodextrin nanoparticles was realized by using a TG 209 Netzsch apparatus, a program temperature of 20-550ºC with a heating rate of 4ºC/min. All determinations were conducted under nitrogen atmosphere. Data acquisition was performed with the TG Netzsch 209-Acquisition Soft/2000 and the data analysis was realized with the Netzsch Proteus-Thermal Analysis ver. 4.0/2000 soft.

2.5. Differential scanning calorimetry (DSC). The DSC analysis of the nanoparticles was performed by using a DSC Netzsch 204 apparatus. Aluminum oxide dishes were used for weighting and analysis of the samples (7±2 mg sample). The DSC conditions were: temperature program 20-400ºC, with a heating rate of 4ºC/min, cooling of the sample was achieved with liquid nitrogen. Data acquisition was performed by using the DSC Netzsch 204-Acquisition soft/2000 and data handling was realized with the same program as for TG analysis (Netzsch Proteus-Thermal Analysis ver. 4.0 / 2000).

3. Results and Discussion

The complexation of berberine or piroxicam with α-cyclodextrin seems to be better than in the case of β-cyclodextrin complexation, in the first case the yield being 83-89%, while in the last one the yield being in the range of 71-72%. This is possible due to the higher water solubility of α-cyclodextrin and a better complexation with berberine (especially the hydrophobic moiety) or with piroxicam (a more flexible structure related to berberine), in comparison with the corresponding β-cyclodextrin complexes.

The SEM analysis revealed that the berberine/α-cyclodextrin complex has rhombohedral crystals, with main dimensions of ~10 μm, while the berberine/ β-cyclodextrin complex has icosahedral crystals, with lower dimensions from hundred of nm to μm (Figure 2). The corresponding complexes with piroxicam have crystals with the same morphology, but with lower dimensions in both cases, <10 μm (Figure 2).

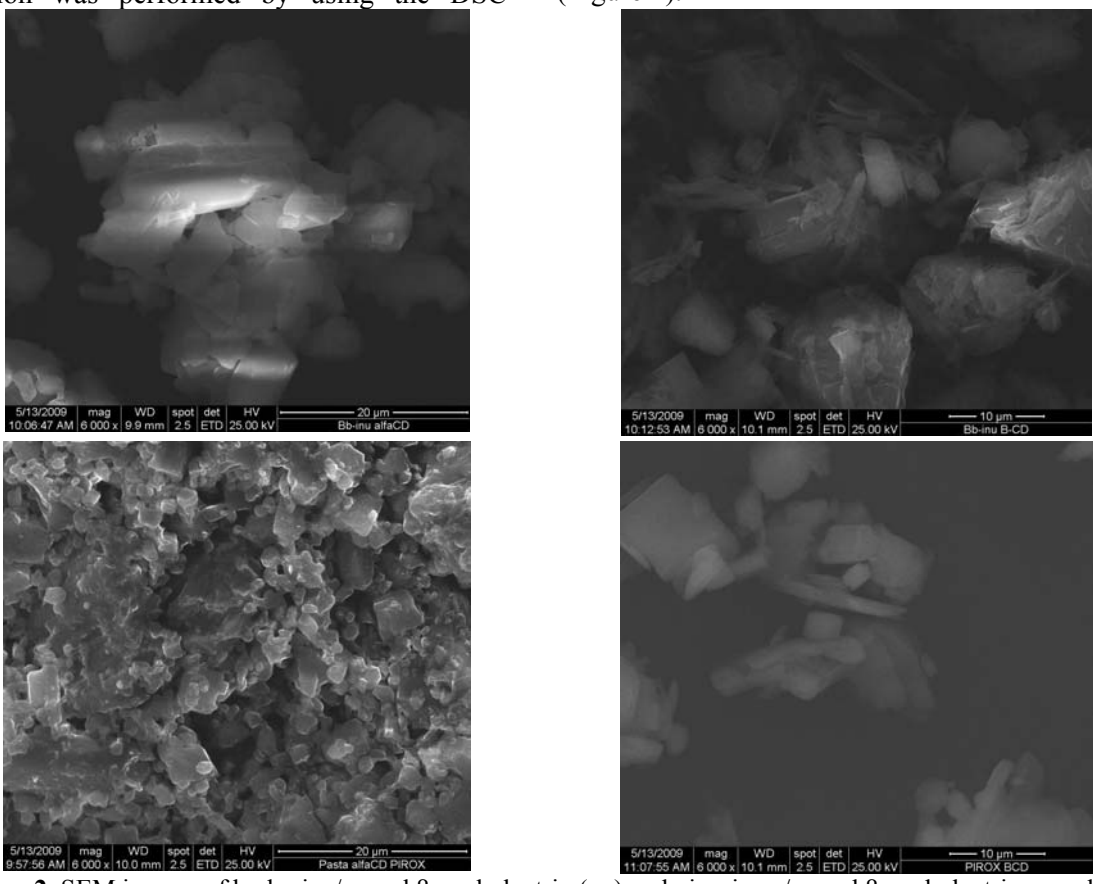

Figure 2. SEM images of berberine/α- and β-cyclodextrin (up) and piroxicam/α- and β-cyclodextrin complexes

Thermogravimetric analysis as well as differential scanning calorimetry analysis revealed the formation of complexes of berberine and piroxicam with α- and β-cyclodextrin. Thus, the mass loss in the case of berberine/β-cyclodextrin was 2.9% in the range of 100-275ºC, where the mass loss of β-cyclodextrin was lower than 1%. In all cases the water release appears up to 110ºC, the mass loss being 12.5-13% for β-cyclodextrin and 9.6% for berberine/β-cyclodextrin complex (Figure 3). The same result was obtained in the case of α-cyclodextrin, the mass loss up to 100ºC being ~9% and the mass loss in the case of the berberine complex was ~2% in the range corresponding to the biocompounds release.

In the case of piroxicam/α- and β-cyclodextrin complexes, the complex formation is more clearly, especially for β-cyclodextrin complex; thus, the mass loss up to 100ºC is ~5.7% for α-cyclodextrin complex (Figure 4) and ~7% for β-cyclodextrin complex (Figure 5), while the mass loss corresponding to the release of biocompounds was 1.4% and 3.2%, respectively. In the last case the piroxicam release is more evident (from 120ºC; Figure 5).

The same results were obtained by DSC analyses of berberine and piroxicam/cyclodextrin complexes. Thus, in all cases the water release up to 100-120ºC and cyclodextrin decomposition at ~300ºC is observed. The endothermal peak corresponding to the water release from α-cyclodextrin complex was ~500 J/g, while in the case of β-cyclodextrin complex ~900 J/g. The biocompound release can be observed in the range of 100-275ºC for berberine/β-cyclodextrin complex (142 J/g; Figure 6) and between 180-220ºC for piroxicam/α-cyclodextrin (Figure 7) and piroxicam/β-cyclodextrin (Figure 8).

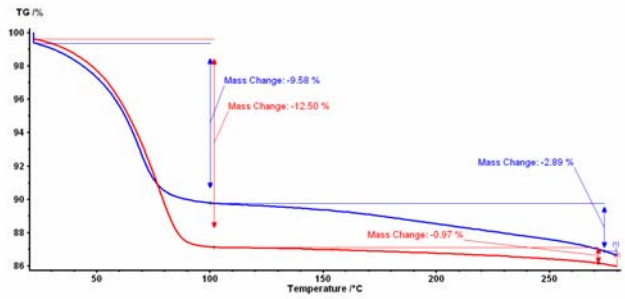

Figure 3. Thermogravimetric analysis for β-cyclodextrin (red) and for the berberine/β-cyclodextrin (blue)

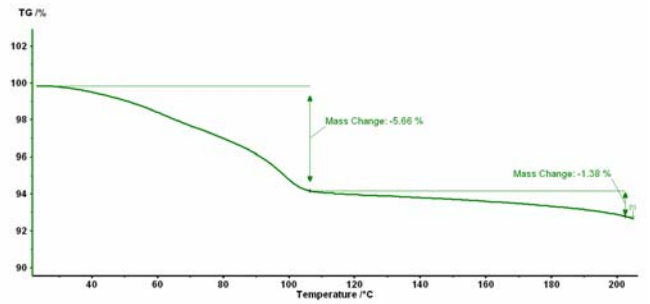

Figure 4. Thermogravimetric analysis for the piroxicam/α-cyclodextrin

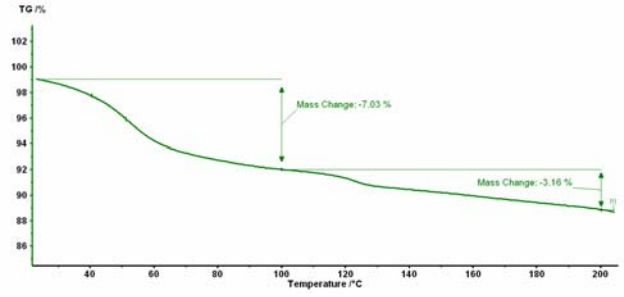

Figure 5. Thermogravimetric analysis for the piroxicam/β-cyclodextrin

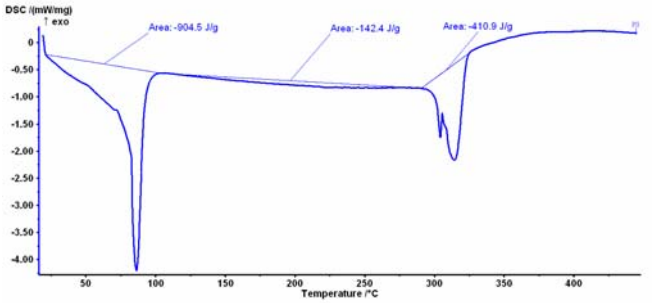

Figure 6. DSC analysis for the berberine/β-cyclodextrin complex

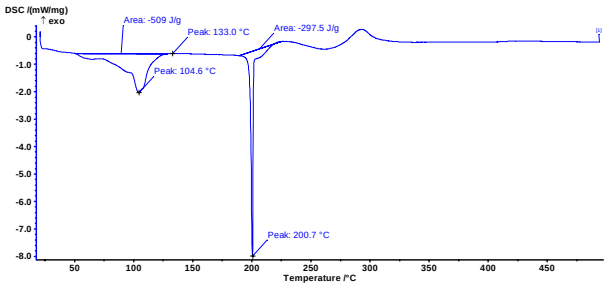

Figure 7. DSC analysis for the piroxicam/α-cyclodextrin complex

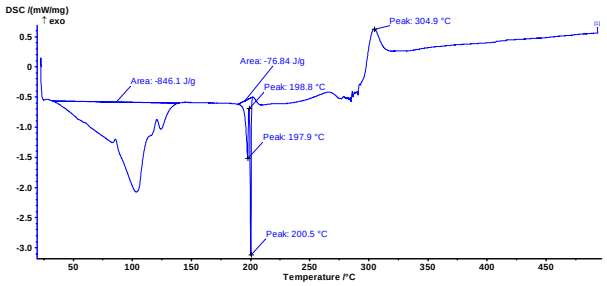

Figure 8. DSC analysis for the piroxicam/β-cyclodextrin complex

4. Conclusions

The following conclusion among the complexation of berberine and piroxicam in α- and β-cyclodextrin can be drawn: (1) the complexation yield was higher in the case of α-cyclodextrin than in the case of β-cyclodextrin, probably due to the better water solubility of the first cyclodextrin and formation of a higher quantity of complex (which has lower water solubility); (2) the β-cyclodextrin complexes have higher concentrations of bioactive compounds (~3%), comparatively with the α-cyclodextrin complexes, as is revealed by thermogravimetry, probably due to a better accommodation of hydrophobic moieties of xenobiotics in β-cyclodextrin; (3) the piroxicam complexes are more probable well formed in comparison with berberine complexes, as is revealed by differential scanning calorimetry.

As an overall conclusion, both berberine (a hepatoprotective and anti-bacterial xenobiotic) and piroxicam (an anti-inflammatory xenobiotic) interact with cyclodextrin cavity in the same manner, but the release of bioactive compounds is more clearly in the case of piroxicam.

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