Study Regarding the Behaviour of Encapsulated Piroxicam in Liquid Media

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This paper presents the behaviour of piroxicam as active pharmaceutical ingredient vs. piroxicam in or released from inclusion complexes with α-CD and β-CD, respectively, using reversed phase-high performance/ pressure liquid chromatography (RP-HPLC).

Keywords: piroxicam, inclusion complex, cyclodextrin, HPLC

Inclusion complex (IC) formation between a host molecule (HM) and a guest molecule (GM) was intensely studied, especially in the last decades, due to its importance in numerous scientific domains, including pharmaceuticals [1]. Host molecules are represented by the class of water-soluble cyclodextrins (CDs), which are cyclic oligo-saccharides consisting in several D-glucopyranose units that are covalently attached end to end via α-1,4 linkages. In pharmaceutical domain, the most commonly studied cyclodextrins are the ones formed by six, seven and eight glucose units, respectively, which are known as α-CD, β-CD and γ-CD [2], and due to their structure can form entirely or partially encapsulation products, with numerous molecules, including active pharmaceutical ingredients with low solubility and biodisponibility [3-5].

As mentioned in literature references, the formation of CDs inclusion complex can be associated with the presence of intermolecular interactions, such as exclusion of cavity-bounded water, Van der Waals forces, hydrophobic and/or electrostatic interactions, but as well H-bonding [1, 6]. Several instrumental techniques can be employed as main investigation tools in evaluation of complex formation between HM and GM, and the results should be always corroborated with physico-chemical parameters regarding the preparation and storage of the formed IC. It is well known that the formation of the IC depends on parameters such as ratio between concentration of GM and HM, temperature, experimental protocol for complex formation, acidity/alcalinity of the medium, the presence of solvent(s) and co-solvent(s). By the physico-chemical approach, the modification of these parameters leads to the modification of thermodynamic properties of the system, i.e. to the modification of the composition during equilibrium [5].

Piroxicam (PX, $C_{15}H_{13}N_3O_4S$, molar mass 331.35 g/mol) is a non-steroidal anti-inflammatory drug (NSAID) belonging to a class of oxicams, which is used in treat of rheumatoid or osteo-arthritis. The mechanism of action is not known, but it may block some natural substances, called prostaglandins, that cause inflammation, treating only the pain and inflammation symptoms, not the causes of those symptoms [7]. The main side effects of PX include an increased risk of serious digestive complaints like stomach ulcers and bleeding, as well as skin reactions, insomnia, high blood pressure or kidney and liver damage [8]. Piroxicam belongs to the Class II drugs according to Biopharmaceutics Classification System [9], for which the dissolution is usually the rate-limiting step for gastrointestinal absorption. To increase the solubility and oral absorption of those drugs from Class II, the formulation strategies in pharmaceutical field are the synthesis of some inclusion complexes with different cyclodextrins containing the drug as a guest.

This paper presents the results from the research carried by our group, following some already published papers [2, 10, 11], namely the behaviour of pure piroxicam (PX) *vs.* piroxicam released from inclusion complexes with α-CD and β-CD, respectively, using reversed phase-high performance/pressure liquid chromatography (RP-HPLC). The structures of the guest molecule (PX) and the ones for host cyclodextrins (α-CD and β-CD) are presented in figure 1.

Experimental part

Materials and methods

Piroxicam (PX) with pharmaceutical purity was obtained as generous gift from LaborMed Pharma, (Romania). Guest molecules (α-CD and β-CD) were purchased from (Hungary) and used as received, without further purification. Solvents for IC preparation (ethanol and bidistilled water) with analytical purity were used, as well without further purification.

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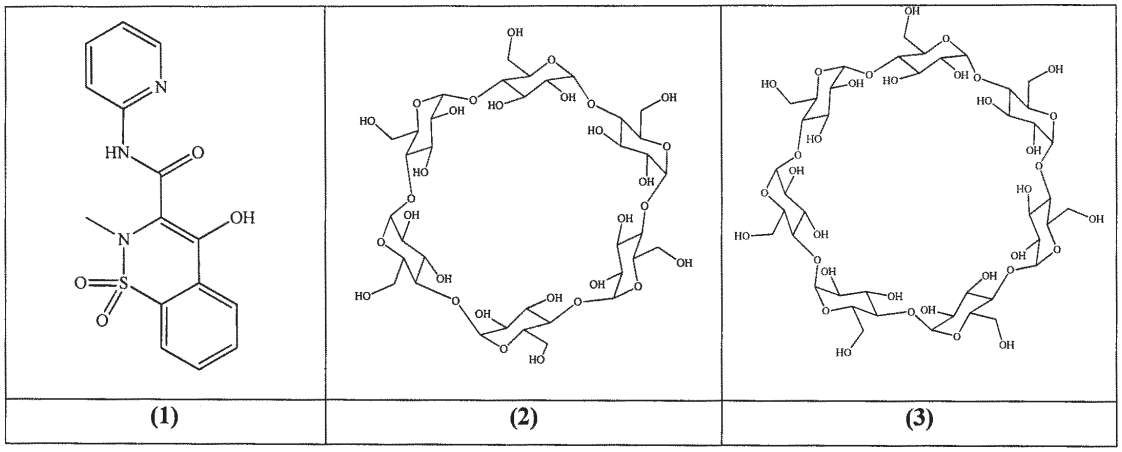

Fig. 1. Structure of guest molecule - piroxicam (1) and host cyclodextrins: α-CD (2) and β-CD (3)

For HPLC analysis, methanol (HPLC grade, Fluka Chemie AG) and buffer solution of *p*H=2.5 (citric acid, Chimopar Bucureşti and disodium phosphate, Merck& Co., Inc) were used. The buffer was prepared by mixing 945 mL solution of citric acid 0.1 M and 55 mL solution of Na_2HPO_4 0.2 M.

IC of PX with α-CD and β-CD were prepared according to a protocol previously established and described by our group [12], as follows: the selected host molecule, α-CD and β-CD (62.5 mmol), respectively, was introduced in bidistilled water (5.0 mL), inside the reaction vessel (heated minireactor), equipped with controlled monitor of temperature, condenser, dropping funnel, and placed on a controllable magnetic stirrer. The minireactor was heated up to 50°C, then the equimolar ratio of piroxicam (62.5 mmol) dissolved in ethanol was added dropwise to the cyclodextrin solution (dropping time 20 min). Following this, the mixture was kept under heating and stirring for another 20 min, then slowly cooled to room temperature and later sealed and refrigerated at +5 °C for 24h. The solid product that crystallized was filtered under reduced pressure, washed with ethanol and dried at +25 °C. The yield of complexation was estimated to be 90.2% for α-CD-IC and 72.6% for β-CD-IC, which are similar to the ones previously reported [12], proving the reproducibility of the experimental procedure.

The chromatographic analysis RP-HPLC for pure piroxicam (PX) and the one released from inclusion complexes α-CD-ICRPX and β-CD-ICRPX was carried out using a Jasco HPLC system with a PU-2080 Plus Pump, LG-2080-04 Quaternary Gradient unit and DG-2080-54 degasser. The detection was realized using UV-Vis spectrophotometry (UV-2070 Plus Intelligent UV/Vis Detector). The acquired data were processed using the JASCO ChromPass Chromatography Data System, Version 1.7.403.1. The analysis protocol was set as follows: Nucleosil 100 C18 column with 5 μm particle size; temperature 25°C, flow 1.0 mL/min, analyzed volume: 20 μL, detection wavelength: 361 nm. As mobile phase, a mixture of methanol:buffer solution of *p*H=2.5 in ratio 9:11 was used.

Results and discussions

For the evaluation of controlled release of PX from encapsulation complexes, the quantitative analysis was carried out for the compound from solution/suspension of complex in aqueous or aqueous-ethanol medium, for an established period of time (min). The quantitative estimation was achieved using the RP-HPLC protocol previously described, using a calibration curve *Area = f(concentration)* for the pure PX.

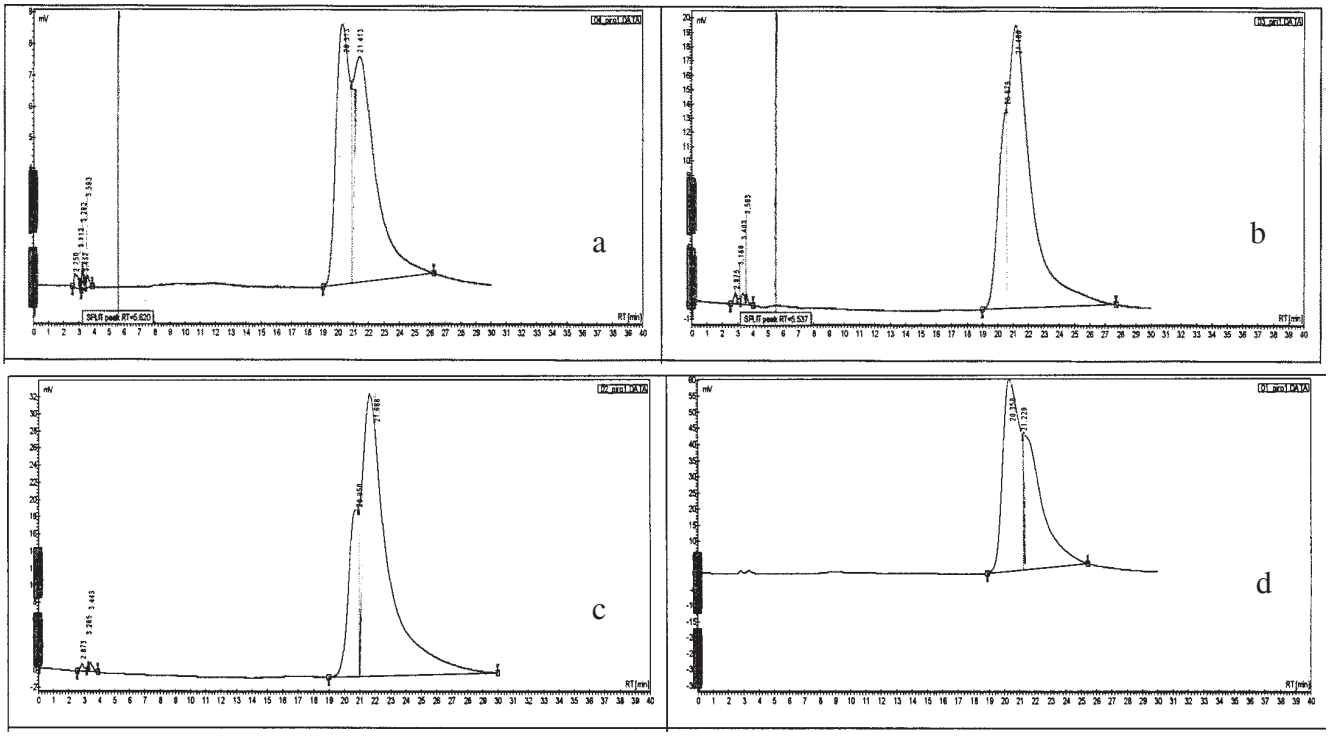

Fig. 2. The HPLC chromatograms recorded for pure piroxicam (PX) of concentrations (per 100 mL solution): (a): 1.38 mg; (b): 2.75 mg; (c): 5.5 mg; (d): 11 mg

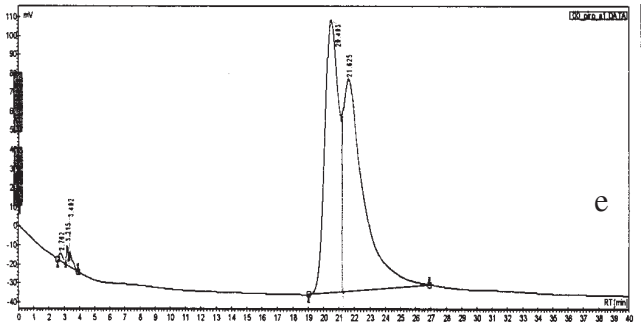

Fig. 2 (cont).The HPLC chromatograms recorded for pure piroxicam (PX) of concentrations (per 100 mL solution): (e): 22 mg

According to this, ethanol solutions (93%) were prepared, with concentrations of 1.38-22 mg/100 mL for PX, with a characteristic peak at retention time near 21 min (20.5-21.6 min). The chromatograms from HPLC analysis are presented in figure 2.

The linear correlation *Area = f(concentration)* is represented by a coefficient of determination that indicate a good fitting of the statistical model (r^2=0.99), with the regression equation:

$$Area(mV \cdot min) = -2.45 + 14.3871 \cdot c(mg / 100\ mL) \quad (fig.\ 3)$$

The evaluation of solubilization for the α-CD-PXIC and β-CD-PXIC and/or controlled release of PX from complexes was realized by the measuring the concentrations for complex or PX in aqueous (for both complexes) or ethanolic medium (solely for the β-CD-PXIC, which showed a limited solubility in water).

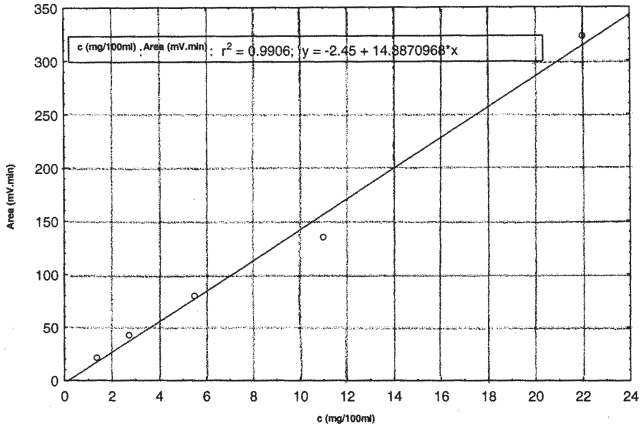

Fig. 3. The calibration curve for HPLC analysis of PX

The analysis of α -CD-PXIC

The HPLC analysis of aqueous supernatant from the suspension that contain 0.1 g α-CD-PXIC in 14 mL of distilled water revealed an increasing of concentration of bioactive substance (most probably in complex, due to the decreased retention time comparative to pure PX) in the first 15 min of stirring, followed by a slight decreasing after 30 min and later almost constant (fig. 4).

The analysis of β - CD-PXIC

The considerable lower solubility of β-cyclodextrin and of the analyzed inclusion complex comparative to the case of α-cyclodextrin is also sustained by the results obtained through HPLC analysis. It was evidenced a concentration up to 10 times lower of complex/bioactive compound in the supernatant obtained after suspension of 0.1 g β-CD-PXIC in the same amount of distilled water as previously (14 mL) (fig.5)

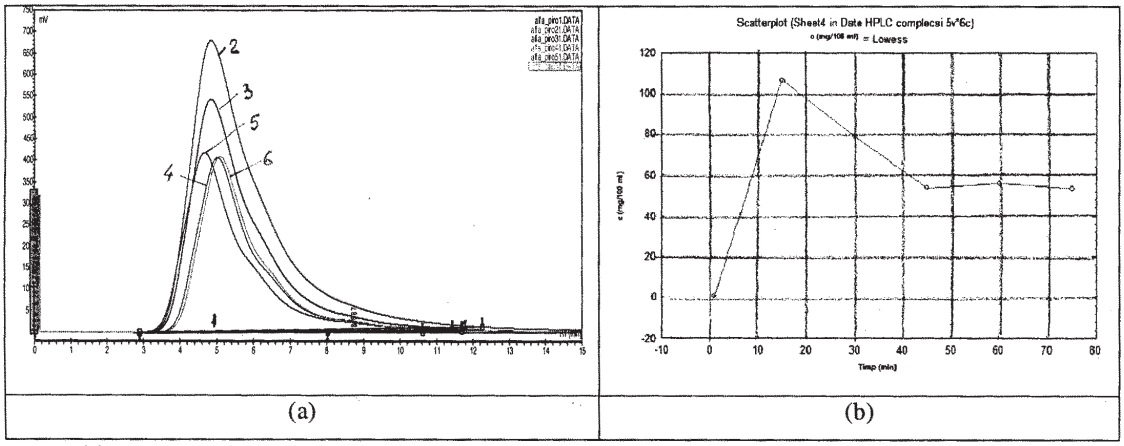

Fig. 4. (a) HPLC chromatograms for supernatant samples from aqueous suspensions of α-CD-PXIC obtained on 0-75 min time interval; (b) the variation of concentration for α-CD-PXIC in supernatant *vs.* time

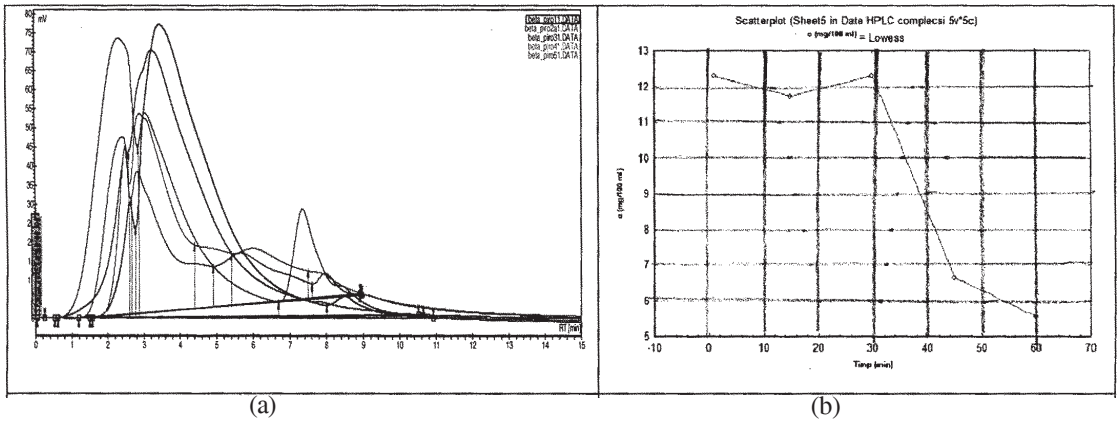

Fig. 5. (a) HPLC chromatograms for supernatant samples from ethanol (93%) suspensions of β-CD-PXIC obtained on 0-60 min time interval; (b) the variation of concentration for β-CD-PXIC in supernatant *vs.* time

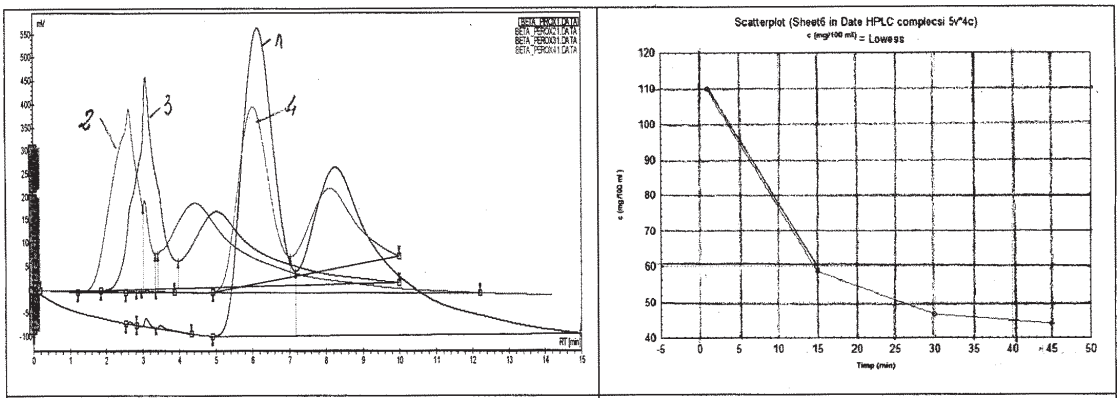

Fig. 6. (a) HPLC chromatograms for supernatant samples from ethanol (93%) suspensions of β-CD-PXIC obtained on 0-45 min time interval; (b) the variation of concentration for β-CD-PXIC in supernatant *vs.* time

When using the solubilization/controlled release in ethanol 93%, for the β-CD-PXIC, a maximum concentration was observed after one minute of stirring, followed by a drastically decreasing of concentration for bioactive compound beyond half of initial value (fig.6.). Also, it was noticed that two chromatographic peaks appear at close retention time values, which can be associated with the appearance of another encapsulation complex, or to partial dissociation of the complex in the presence of ethanol.

Conclusions

This study presented the behaviour in condensed phase (in two polar solvents) of two inclusion complexes of α-cyclodextrin and β-cyclodextrin with piroxicam, which is a non-steroidal anti-inflammatory with low water-solubility.

For the evaluation of controlled release of piroxicam from encapsulation complexes, the quantitative analysis was carried out for the compound from solution/ suspension of complex in aqueous or aqueous-ethanol medium. The quantitative estimation was achieved using the RP-HPLC protocol.

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