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Inclusion complex of piroxicam with β-cyclodextrin and incorporation in hexadecyltrimethylammonium bromide based microemulsion

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Abstract

The interaction of piroxicam with β-cyclodextrin (β-CD), hexadecyltrimethylammonium bromide-based microemulsion (ME), and ME in the presence of β-CD aimed at the optimization of topical drug delivery was studied. UV–VIS absorption spectra at pH 5.5 were obtained with and without β-CD and ME. The stability constant (K) values for the piroxicam/β-CD complex in the pH range 4.5–6.0 varied from 87 to 29 M^{-1}. The cationic microemulsion was characterized by pseudo-ternary phase diagram. The association constant (K_s) of piroxicam/ME was determined using the framework of the pseudophase model. The value of K_s obtained for piroxicam at pH 5.5 was 132 M^{-1}. At the same pH, the value of K_s for the incorporation of piroxicam/β-CD complex in the ME was 150 M^{-1}.

Keywords: Piroxicam; β-Cyclodextrin inclusion complex; Microemulsion incorporation

1. Introduction

The interaction of drugs with supramolecular aggregates and β-cyclodextrin (β-CD) is an important feature in the pharmaceutical field since these system can bind drug compounds modifying chemical stability (Andersen and Bundgaard, 1983; Oliveira et al., 1990; Bekers et al., 1991; Oliveira et al., 1991; Oliveira and Chaimovich, 1992; Musson et al., 1993; Loftsson and Brewster, 1996; Oliveira et al., 1997; Stella and Rajewski, 1997; Bakhtiar et al., 1998) and bioavailability (Schiantarelli et al., 1982; Acerbi, 1990; Acerbi et al., 1990a; Arima et al., 1990; Reddy and Udupa, 1993; Reginster and Franchimont, 1993; Ammar et al., 1997).

Piroxicam is a non-steroid anti-inflammatory compound with analgesic and antipyretic effects, used for the treatment of rheumatoid arthritis, osteoarthritis and traumatic contusions. However,

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it has been associated with gastrointestinal side effects. It is possible to minimize these problems by developing drug carriers to prevent the direct contact of drug with gastric mucosal or that allow the topical administration of drug (Schiantarelli et al., 1982; Kageyama, 1987; Babar et al., 1990; Linn et al., 1990; Monteiro-Riviere et al., 1993; Reddy and Udupa, 1993; Marks and Dykes, 1994).

In this way, the interaction of drugs with both cyclodextrins and microemulsions have been described because they can favorably modify the therapeutic activity of different groups of drugs (Otagiri et al., 1983a,b; Frijlink et al., 1989; Lin et al., 1991; Pattarino et al., 1993).

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic inner cavity. Cyclodextrins can increase water solubility of poorly water-soluble drug by taking up the whole molecule or a lipophilic region of the drug into cavity (Duchene and Wouessidjewe, 1992, 1993). Molecular complex of drugs with cyclodextrin can not increase drug solubility, but they can improve therapeutic effects (Acerbi et al., 1990b; Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Ammar et al., 1997; D'Souza et al., 1997; Piel et al., 1997; Stella and Rajewski, 1997; Ammar et al., 1998; Piel et al., 1998).

Microemulsions are isotropic, transparent, thermodynamically stable systems which are usually four-component mixture containing a surfactant, a cosurfactant, an oil and water (Mackay and Hermanski, 1981; Attwood et al., 1992; Oliveira et al., 1997). These structures have been considerably investigated as drug delivery system and also used to dissolve lipophilic drugs in aqueous medium or hydrophilic drugs in lipophilic medium (Attwood et al., 1992; Lawrence, 1994; Constantinides et al., 1996; Ho et al., 1996). Oil in water microemulsions have been described as a reservoir system that can inhibit drug release, increasing the topical effect (Martini et al., 1984; Gasco et al., 1989, 1991; Gallarate et al., 1990; Pattarino et al., 1993).

In this work, we have studied the interaction of the non-steroid anti-inflammatory piroxicam with β-cyclodextrin in the pH range pH 4.5–6.0 and with cationic microemulsion stabilized with HTAB/ethanol. The association of piroxicam/β-CD complex with microemulsion (ME) was also determined.

2. Experimental section

2.1. Materials

Piroxicam (lot f 301) was kindly donated by (ANSA, Milano, Italy). Stock solutions of piroxicam were prepared daily, maintained at 4°C, and discarded after use. Analytical grade *N*-hexadecyl-*N*,*N*,*N*-trimethylammonium bromide (HTAB) (Merck S.A., Brazil), citric acid (Merck S.A., Brazil), phosphoric acid (Merck S.A., Brazil), sodium hydroxide (Merck S.A., Brazil), ethanol (Merck S.A., Brazil), sodium chloride (Merck S.A., Brazil), *N*,*N*-dimethylformamide (Merck S.A., Brazil), β-cyclodextrin (Roquette, France), isopropyl miristate (Aquatec S.A, Brazil) were used as received.

2.2. Methods

2.2.1. Determination of stability constant of piroxicam/β-CD inclusion complex

The stability constants (K) were determined by adding an excess of piroxicam in the β-CD solutions buffered at pH's 4.5, 5.0, 5.5 and 6.0. In assay, increasing volumes of β-CD 0.016 M solution and variable volumes of citrate buffer were added in order to obtain concentrations between 0–0.016 M and a final volume of 8 ml. In the quantitative determination, 4 ml of each dilution was used as a control. Approximately 10 μl of 0.2 M of piroxicam solution in dimethylformamide, sufficient for the saturation of the medium, were added and constantly stirred in a thermostatic chamber at 25 ± 0.1°C until equilibrated. Following this, the material was filtered through 0.22 μm membrane diluted and the absorbance was determined at 360 nm. The solubility diagram was plotted, relating the absorbance as a function of concentration of the dissolved piroxicam. The constant (K) were determined by the correspondent phase solubility diagram (Higuchi and Connors, 1965).

2.2.2. Microemulsion preparation

Selected weights of ethanol were added to solid HTAB and after isopropyl miristate and aqueous buffer. This addition sequence enabled the solubilization of the surfactant with gentle stirring. The mixture was left for 10 min in thermostatic chamber at 25 ± 0.1°C.

2.2.3. Phase diagram determination

An identical weight of ethanol was added to a suitable weight of solid HTAB, To the semisolid mixture of HTAB/ethanol (S), isopropyl miristate (O), was added. The S/O weight ratios were used to obtain the phase diagram ranged from 1:9 to 9:1. To the constantly stirred semisolid mixture (1.0 g), containing HTAB, ethanol and isopropyl miristate, 0.01 M citrate buffer pH 5.5 (W) was slowly added with a precision burette. The transitions from semisolid mixture to optically clear microemulsion and from microemulsion to opaque dispersion (emulsion) were sharp and reproducible with 0.05 ml of W. A similar procedure was carried out employing S/W mixtures, and adding isopropyl miristate.

The volume fraction of the microemulsion droplets (ϕ) (Mackay and Hermanski, 1981) was defined by:

$$\phi = 1 - \omega \times \delta \quad (1)$$

Where ω is the ratio of buffer weight to the total weight of the microemulsion and δ is the density of the bulk microemulsion divided by the buffer density.

The HTAB concentration in stock microemulsion was 0.99 M. The stoichiometric HTAB concentrations at any volume fraction can be obtained from the following expression:

$$[\text{HTAB}] = \phi \times 1.4348 \quad (2)$$

The correction factor was obtained by dividing the molar concentration of HTAB in stock microemulsion (0.99 M) by the ϕ of the stock solution (0.69).

2.2.4. Spectral determination

An excess of piroxicam was added to 4 ml of a citrate buffer pH 5.5 to a buffered 15 mM β-CD solution, to a microemulsion at $\phi = 0.69$ and to a microemulsion containing 15 mM of β-CD. These dispersions were constantly stirred for 24 h in a thermostatic chamber at 25 ± 0.1°C. After this, the amorphous material was filtered through a 0.22 μm membrane and the UV–VIS spectra of saturated solutions was registered in the range 200–500 nm.

2.2.5. Determination of incorporation constant (K_s) of piroxicam in the microemulsion

Homogeneous mixtures of microemulsion with citrate buffer pH 5.5 were prepared in order to obtain the total volume of 8 ml containing ϕ varying from zero to 0.69. Of each dilution, 4 ml were used as a control in quantitative determination was separated. An excess of piroxicam was added and the material was constantly stirred for 24 h in a thermostatic room at 25 ± 0.1°C for equilibrium. Following this, the amorphous material was filtered through a 0.22 μm membrane, diluted properly and the absorbance was determined at 350 nm. The data was plotted relating the absorbance with the volume fraction of microemulsion. The K_s value was calculated through the framework of the pseudo-phase model (Sepulveda et al., 1986; Oliveira et al., 1991).

3. Results and discussion

The characterization of the drug inclusion complex with cyclodextrins in solution can be achieved using several methods. Among them, the solubilization method associated with spectral changes can be used to measure the transference of the drug from the aqueous phase to the apolar cavity of cyclodextrin or the ME. Particularly in the solubility method, if the drugs have limited water solubility, the concentration of solubilized drug can be increased by the inclusion in cyclodextrin or ME. However, in the case of the β-CD inclusion complex, if some co-solvent is present in the medium, the drug solubility can be affected. In this way, we have determined the influence of dimethylformamide in the solubility of piroxicam in the presence of β-CD since DMF was used as a drug solvent in the solubility diagram obtainment. No difference was observed

when piroxicam was added dissolved in DMF (10 μl of 0.2 M piroxicam) or directly as a powder. In fact, the DMF added represents only 0.25% in the solvent composition and in both cases generates a saturated solution.

Fig. 1 shows the linear responses between the concentrations of piroxicam solubilized as a function of the concentration of β-CD. This profile of the solubility diagram suggests stoichiometry 1:1 for the piroxicam/β-CD complex in all ranges of pH studied (Fig. 1).

These results can be analyzed quantitatively through the framework of the Higuchi and Connors method (Higuchi and Connors, 1965) from the following equilibrium

$$P_f + \beta - CD \overset{K}{\leftrightarrows} P_b \quad (3)$$

where the subscripts f and b refer to free and bound piroxicam, respectively.

The stability constant (K) can be calculated through the following expression

$$K = \frac{\alpha}{So \times (1-\alpha)} \quad (4)$$

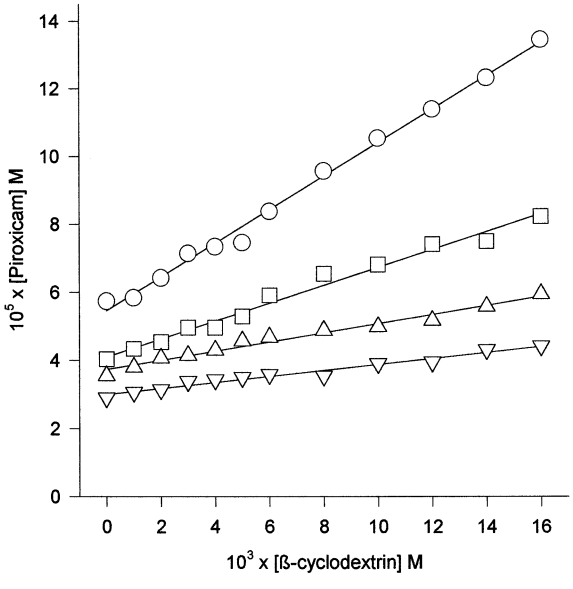

Fig. 1. Effect of β-cyclodextrin on the solubilization of piroxicam. Citrate buffer 0.01 M, ionic strength 0.02 M, pH's (○) 4.5; (□) 5.0; (△) 5.5; and (▽) 6.0.

Table 1
Stability constants obtained for piroxicam at various pHs[a]

pH	K (M^{-1})
4.5	87.68
5.0	61.94
5.5	30.45
6.0	28.79

[a] Data calculated from Eq. (4).

where So represents the solubilized piroxicam without cyclodextrin and α represents the slope of the straight line.

The results of K obtained (Table 1) decrease with an increase in pH. This phenomena is reasonable since the inclusion complex formation is dependent on the hydrophobic interactions between the drug and the apolar cavity of β-CD. Thus, this interaction has a more significant influence when the non-dissociated species of piroxicam are present in the medium.

For microemulsion, it is necessary to determine the phase diagram, that describes the experimental conditions in which it is possible to obtain optically transparent systems, essential for spectrophotometric UV-VIS experiments. The phase diagram (Fig. 2) shows the wide possibilities to obtain the clear microemulsion. It can be seen that it is possible to add great volume of aqueous buffer and significant volumes of oil phase, maintaining the thermodynamic stability of system. The phase diagram for HTAB/ethanol/isopropylmiristate, and aqueous buffer (Fig. 2) was comparable to others described previously (Mackay and Hermanski, 1981; Oliveira et al., 1997) and is presented here to illustrate the microemulsion region used in this study.

UV–VIS spectra, (Fig. 3) carried out with free piroxicam (curve D) in the presence of β-CD 15 mM (curve C), microemulsion $\varnothing = 0.69$ (curve B) and with ME containing 15 mM of β-CD (curve A), shows that the solubility of piroxicam measured at 360 nm increased by 6, 105 and 110 fold, respectively. Piroxicam solubility was not significantly increased by the incorporation of the inclusion complex in the microemulsion.

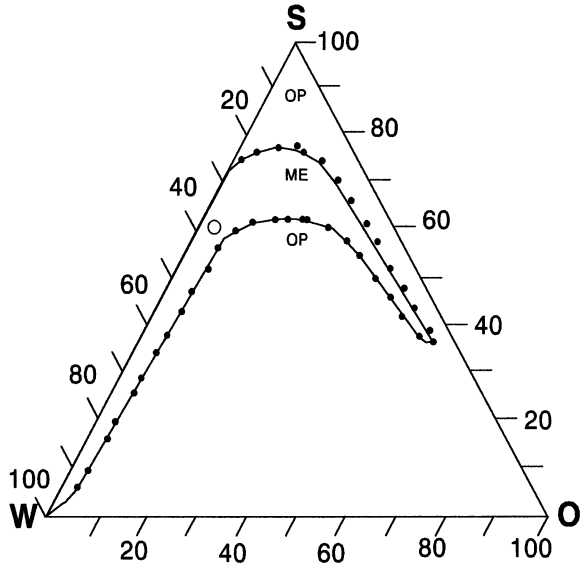

Fig. 2. Phase diagram of HTAB/ethanol (1:5 moles) (S), isopropyl miristate (O) and aqueous buffer (W). Key: ●, transitions from opaque dispersion to optically transparent microemulsion system; ME, region of optically transparent microemulsion; OP, areas of opaque dispersion of the components; ○, microemulsion with $\phi = 0.69$. Citrate buffer 0.01 M, ionic strength 0.02 M, pH 5.5 (see text).

The association of piroxicam to microemulsion was characterized by the solubility diagram at pH 5.5 (Fig. 4).

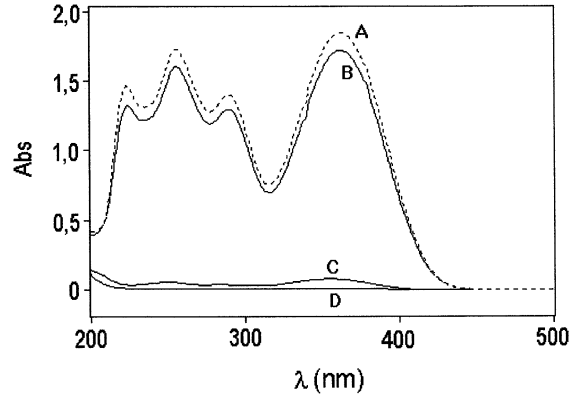

Fig. 3. UV–VIS spectra of saturated solutions of piroxicam in citrate buffer 0.01 M, ionic strength 0.02 M, pH 5.5: (A) Microemulsion with $\phi = 0.69$, containing 15 mM of β-CD; (B) Microemulsion with $\phi = 0.69$; (C) β-CD 15 mM; (D) Buffered piroxicam solution. Spectra obtained against controls of the respective solutions.

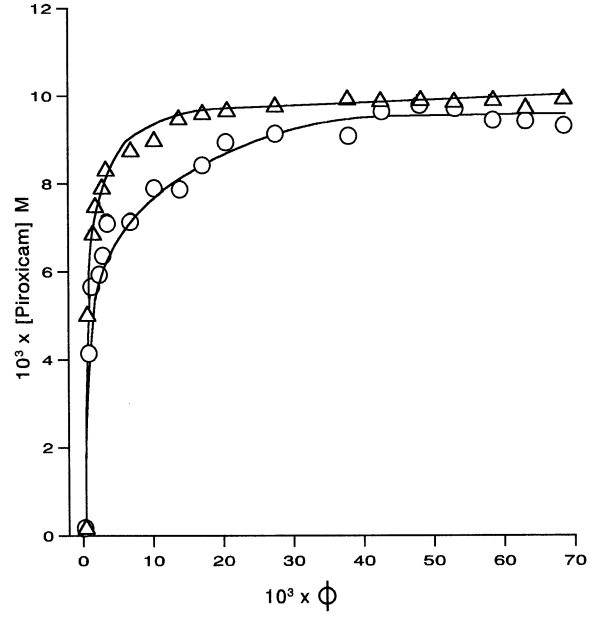

Fig. 4. Incorporation of piroxicam in O/A microemulsion. Citrate buffer 0.01 M, ionic strength 0.02 M, pH 5.5. Key: ○, ME; Δ, ME containing 15 mM of β-CD. Data plotted according to Eq. (5).

The concentration of solubilized piroxicam in both phases (P_s) was remarkably increased by the addition of microemulsion. The P_s increases with increasing ϕ up to a plateau (Fig. 4). This rate P_s-ϕ profile is typical of drug incorporation on supramolecular aggregate as micelles and microemulsions and can be analyzed quantitatively by the framework of pseudo-phase model (Sepulveda et al., 1986; Oliveira et al., 1991, 1997) using the expression:

$$P_s = \frac{P_f + P_b \times K_s \times \phi}{1 + K_s \times \phi} \quad (5)$$

The distribution of piroxicam between the microemulsion and the aqueous continuous phase can be expressed by the piroxicam–microemulsion association constant (K_s):

$$K_s = P_b / P_f \times \phi \quad (6)$$

where the subscripts f and b refer to the concentrations of free and bound piroxicam, respectively.

K_s can be obtained from the linearized expression by plotting the $(1/P_s - P_f)$ variation with $1/\phi$, according to Eq. (7) (Fig. 5):

$$(1/P_s - P_f) = 1(P_b - P_f) + [1/(P_b - P_f) \times Ks \times 1/\phi] \quad (7)$$

The piroxicam K_s value obtained for cationic microemulsion at pH 5.5 was 131 M^{-1} and $\sim$ 150 M^{-1} for ME in the presence of β-cyclodextrin. Since the pK_1 and pK_2 of piroxicam were found to be 1.86 and 6.3 (Fini et al., 1992), respectively, at this pH region the piroxicam is an amphoteric compound and can interact with microemulsion aggregates.

The results described show that the inclusion of piroxicam in β-cyclodextrin is pH dependent and is more effective at low values. At the same pH region, the non-steroid anti-inflammatory piroxicam interacts strongly with microemulsions aggregates. Results show (Figs. 4 and 5) that complex entrapment into ME enhanced slight the K_s value obtained. The aqueous solubility of piroxicam was significantly increased via complexation with β-CD and association to cationic ME. Both, cyclodextrin and microemulsion offer many promising features for their possible widespread use as vehicles for piroxicam delivery.

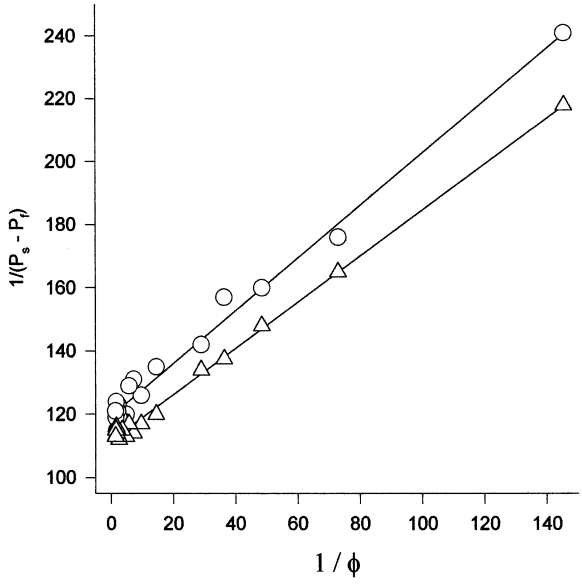

Fig. 5. Determination of the association constant of piroxicam in cationic microemulsion. Citrate buffer 0.01 M, ionic strength 0.02 M, pH 5.5. Data plotted according to Eq. (7). Key: ○, ME; Δ, ME containing 15 mM of β-CD.

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