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Hydroxypropyl methylcellulose microspheres with piroxicam and piroxicam-hydroxypropyl-β-cyclodextrin inclusion complex

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Received October 20, 2003, accepted December 8, 2003

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Pharmazie 59: 686–691 (2004)

Inclusion complexation between piroxicam (PX) and hydroxypropyl-β-cyclodextrin (HPβCD) in the presence of hydroxypropyl methylcellulose (HPMC) was studied in aqueous solution and in the solid state. Phase solubility studies were used to evaluate the HPβCD complexation in the presence of HPMC. Stability constants, K_s, of the complexes were determined. The stability of the inclusion complex was improved in the presence of HPMC. Solid microspheres were obtained by spray drying, and were characterized by differential scanning calorimetry (DSC), regarding drug content, and particle size distribution. Scanning electron microscopy (SEM) was also used to characterize the systems prepared. In the solid system HPMC facilitated to some extent the drug dissolution due to increased solubility. The presence of HPMC and HPβCD in the microspheres promoted dissolution rate. Cyclodextrin complexation increased PX flux through a semipermeable membrane. Presence of HPMC in the system additionally increased the drug flux more than 80%, by increasing the drug solubility and consequently the affinity of the ternary complex for the aqueous diffusion layer in the donor compartment.

1. Introduction

Water soluble polymers are widely used as pharmaceutical excipients. Although they are usually considered to be chemically inert, they are known to form complexes with drugs in aqueous solutions. A notable increase in drug solubility is frequently observed due to formation of water soluble drug-polymer complexes (Loftsson et al. 1996).

Polymers are known to interact with cyclodextrins. It has been shown that at low concentrations, water soluble polymers increase the complexing abilities of cyclodextrins and consequently enhance the availability of drugs in aqueous cyclodextrin solutions (Loftsson 1998; Loftsson and Fridriksdottir 1998; Mura et al. 2001; Valero et al. 2003; Okimoto et al. 1997). The interaction in the formed systems results in physicochemical changes and the solubility of drugs increases (Masson and Loftsson 1999).

The complexation efficiency of a drug by cyclodextrins is rather low and consequently a significant amount of cyclodextrins is needed to solubilise a small quantity of the drug. Therefore it is an imperative to develop methods that can be applied to enhance the complexation efficiency of cyclodextrins, such as addition of a small amounts of water soluble polymers to the formulation (Loftsson 1998). Due to a variety of reasons, including cost, production capabilities and toxicology, the amount of cyclodextrins that can be incorporated into the drug formulations is limited. However, the use of cyclodextrins in solid oral dosage forms is limited to low dose drugs with large stability constants due to the mass limitations of the oral dosage units (Rajewski and Stella 1996).

Most of the studies carried out to analyse the effect of hydrophylic polymers on the complex formation with cyclodextrins were made in solution, not in the solid state. Several papers have been published concerning the improvement of the solubility and bioavailability of drugs through the formation of binary and multicomponent complexes with cyclodextrins (Pose et al. 2002; Cerchiara et al. 2003; Ribeiro and Veiga 2002). Multicomponent complexes made of a sparingly water soluble amino type drug, a cyclodextrin, and a hydroxy carboxylic acid significantly increased the cyclodextrins solubilizing power, and reduced the amount of cyclodextrins necessary for making the targeted formulation (Redenti et al. 2000).

Piroxicam (PX), an established NSAID in the treatment of rheumatic diseases, was used as a model drug. Oral piroxicam administration is characterised by slow absorption due to its poor solubility in water. Formulation of piroxicam as an inclusion complex with β-cyclodextrin has shown a significant increase in its aqueous solubility which resulted in faster onset of action (McEven 2000). Piroxicam has been encapsulated in poly(lactic acid) microspheres by a solvent evaporation method and a spray drying method. The *in vitro* release of the drug from the microspheres prepared by the solvent evaporation method was reported to be rapid with a burst effect (Guizion et al. 1996). On the other hand, microspheres prepared by the spray drying technique showed a very slow release profile

with less than 10% of the incorporated drug being released in 50 h (Wagenaar and Müller 1994).
The aim of the present work was to prepare a solid multicomponent system containing the drug, hydroxypropyl-β-cyclodextrin, and hydroxypropyl methylcellulose. The solid multicomponent microspheres were prepared by spray-drying and characterised by differential scanning calorimetry. Drug dissolution characteristics from microspheres were compared with those of pure piroxicam.
The complexation efficiency and solubilising effect of cyclodextrin by addition of water soluble polymer, hydroxypropyl methylcellulose, might be a useful strategy to decrease the amount of cyclodextrin needed in oral formulations of piroxicam.

2. Investigations, results and discussion

2.1. The effect of HPMC on the drug complexation in solution

The intrinsic water solubility of PX is 0.7 μg/ml. The addition of HPMC (0.05 and 0.1%) enhanced aqueous solubility of PX about 6-fold. HPMC possesses a significant solubilising effect which is due to the formation of a water soluble drug-polymer complex. The solubility of PX in aqueous HPβCD solution (4×10^{-2} mol/l) was about 4.2-fold greater than in water. By introducing HPMC into the solution solubility has been improved even further. Solubilising effect of HPβCD was improved about 3-fold when 0.05% HPMC was present in the solution or more than 4-fold in the case of 0.1% HPMC solution. The effect of HPMC on the solubilising effect of PX in the presence of HPβCD is shown in Table 1.
Figure 1 shows the phase solubility diagrams of PX in aqueous HPβCD solution in the presence and absence of HPMC. In all cases the solubility of PX increased linearly as a function of HPβCD concentration. The shape of the solubility diagrams followed an A_L type system (Higuchi and Connors 1965). HPMC enhanced significantly the solubilising effect of HPβCD, but did not alter the type of the phase solubility diagram. So, the presence of HPMC did not change the stoichiometry of the complex formed. The apparent stability constants of PX-HPβCD with and without HPMC were calculated from the slopes of phase solubility diagrams, assuming 1 : 1 stoichiometry (Table 1). The stability constant of the inclusion complex showed an enhancement in the presence of HPMC. The drug-HPβCD binding constant increased as concentration of HPMC increased. The phase solubility study of PX was also performed by 1 h sonication at 70 °C with 3-days equilibration at 25 ± 1 °C. The solubilising effect of HPβCD was unaffected by sonication and heating (Table 1).
In aqueous solutions water soluble polymers increased solubilising effect of cyclodextrin (Loftsson and Fridriksdottir 1998). HPMC increased the efficiency of the complexation by increasing the K_s value. The effect could be explained as a result of a ternary complex formation, which increased the complexation efficiency of HPβCD. The influence of HPMC on the solubilising effect of HPβCD toward PX is not simply additive, accounting for a role of HPMC in the complexation process. HPMC was able to enhance PX incorporation in HPβCD. The effect on drug incorporation was a general effect of the polymers on cyclodextrin complexation. According to Bibby et al. (2000) polymers may interact with drug cyclodextrin complexes forming drug-cyclodextrin-polymer aggregates or a co-complex i.e. a complex between drug-cyclodextrin complex and a polymer chain. This ternary PX-HPβCD-HPMC complex has a higher stability constant than the simple PX-HPβCD complex.

Table 1: Effect of the addition of HPMC to aqueous HPβCD solution on the solubility (S_0, S_p), and apparent stability constants of the complexes (K_s)

HPMC %	S_0*/μg ml^{-1} mean ± SD	S_p**/μg ml^{-1} mean ± SD	K_s/M^{-1} mean ± SD
–	0.76 ± 0.07	3.18 ± 0.09	105.1 ± 7.6 103.7 ± 5.2***
0.05	4.2 ± 0.09	12.93 ± 0.94	299.5 ± 17.4
0.1	4.4 ± 0.14	16.67 ± 0.89	456.9 ± 14.4

(n = 3)
* S_0 – solubility in aqueous solution of HPMC without HPβCD
** S_p – solubility in aqueous solution of HPMC with HPβCD (4×10^{-2} mol l^{-1})
*** – after sonication and heating

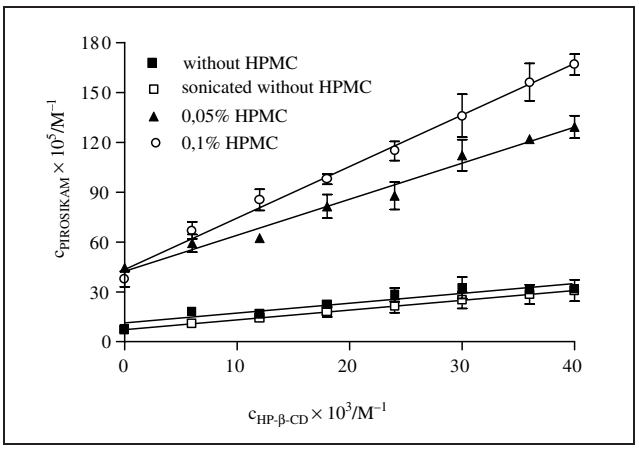

Fig. 1: Phase solubility diagrams of PX-HPβCD system with and without HPMC (mean ± SD, n = 3)

2.2. The effect of HPMC on the drug complexation in solid state

Solid inclusion complexes were obtained by spray-drying which is a common pharmaceutical preparation technique, known to be simple, reproducible, and easy to scale up. The PX-HPβCD ratio in the solid products was equimolar, as found in the phase solubility study, and HPMC concentration was 0.1%. Differential scanning calorimetry was used to characterise the systems prepared.
DSC thermograms are shown in Fig. 2. The DSC curve of PX showed an endothermic event as a melting peak with onset temperature at 200.8 °C indicating a cubic crystal polymorph form (polymorphic form 1) (Vrecer et al. 2003). The DSC thermogram of HPMC showed a wide endothermic peak from 35 to 110 °C which was correlated to a loss of adsorbed moisture or solvent from the molecule. In the region from 120 to 216 °C a small exothermic event indicated phase transition. DSC thermogram of HPβCD showed the exit of adsorbed moisture or solvent seen as the wide endothermic peak (35 to 114 °C). In the region from 166 to 225 °C a small exothermic event was noticed, indicating phase transition. The Thermogram of the PX-HPMC complex showed a small endothermic peak at 73 °C (exit of solvent), followed by an exothermic peak at 113 °C which indicated some crystallisation process. At 198 °C the endothermic (melting) peak of PX was observed. The position and shape of the peak indicated the interaction of PX and HPMC, and partial transformation of PX polymorph I (cubic form) to polymorph II (needle form). The PX-HPβCD complex in the region from 168.8

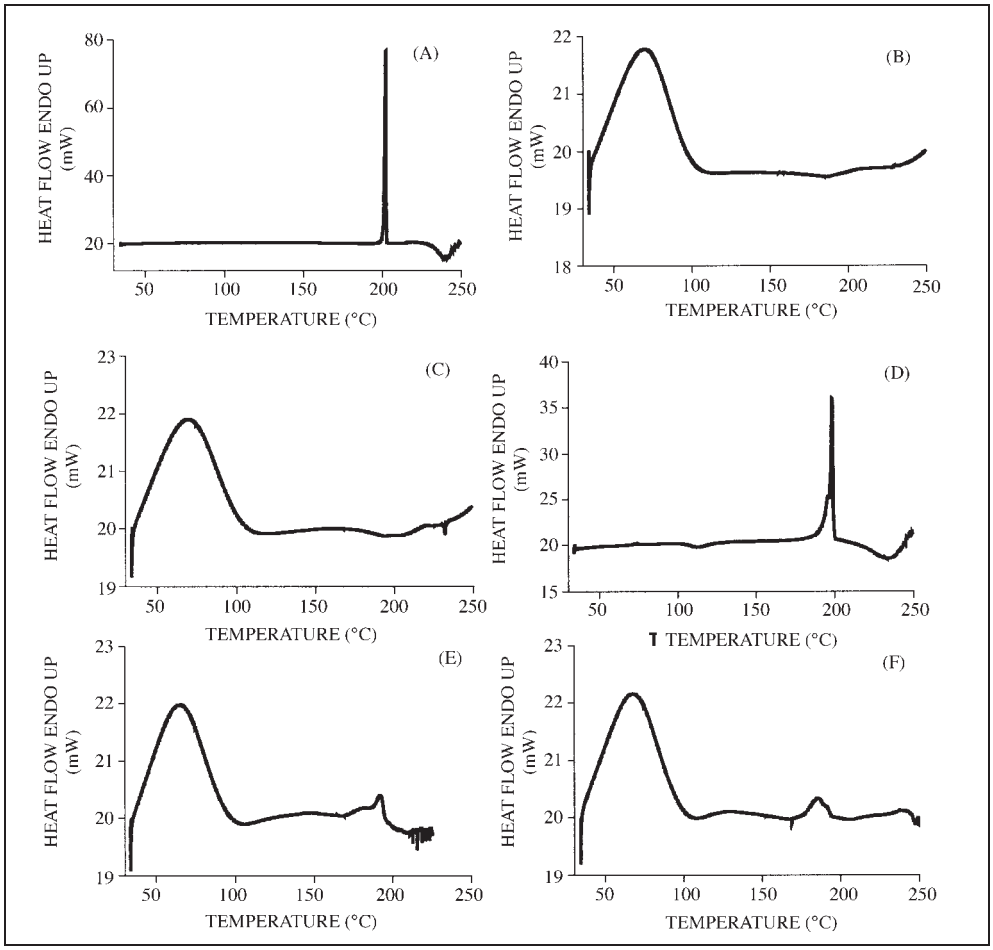

Fig. 2:
DSC thermogram of PX (A), DSC thermograms HPMC (B), HPβCD (C), PX-HPMC (D), PX-HPβCD (E), PX-HPβCD-HPMC (F)

to 200 °C showed an endothermic event with a peak at 192 °C ($\Delta H = 20.14\ J\ g^{-1}$), assigned to melting of the piroxicam molecule which was partially incorporated into cyclodextrin. The DSC curve of PX-HPβCD-HPMC complex showed the solvent exit from HPβCD molecules (35 to 106 °C). In the region from 170 to 200 °C there was another endothermic event with peak at 176 °C ($\Delta H = 13.08\ J\ g^{-1}$). Shifting of the melting peak of the PX molecule to lower temperatures in the presence of HPMC confirms the interaction between PX, HPβCD and

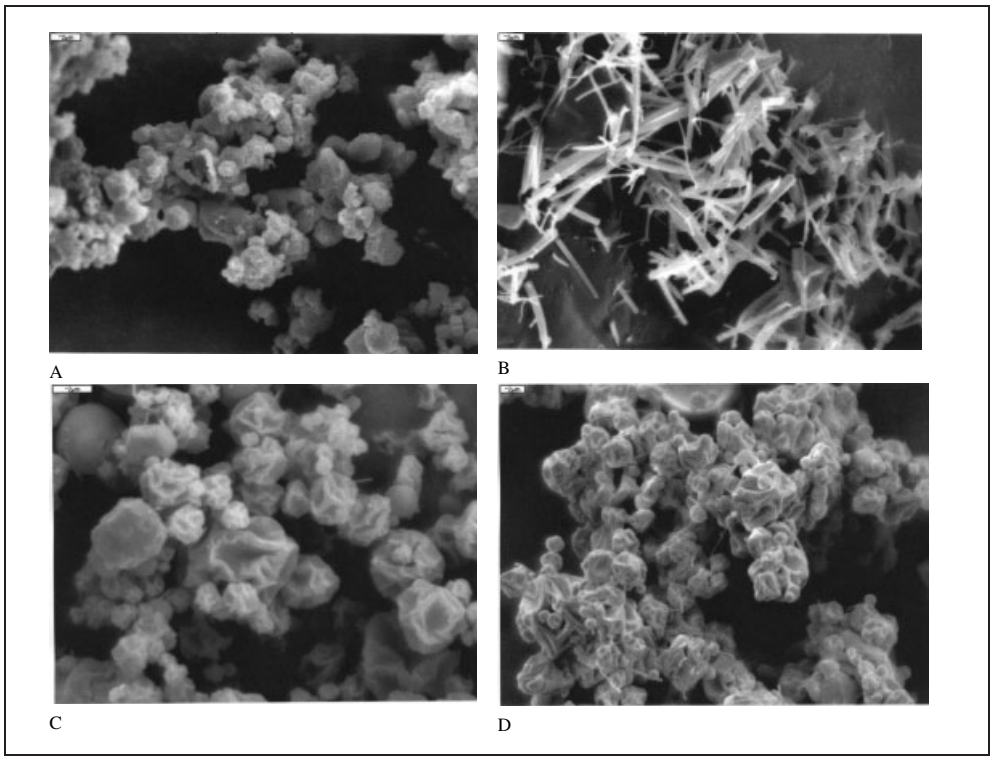

Fig. 3:
Scanning electron microscopic photographs of PX (A), PX-HPMC (B), PX-HPβCD (C), PX-HPβCD-HPMC (D) microspheres

HPMC. Partial incorporation of PX into HPβCD was also observed. Concerning the PX-HPβCD and PX-HPβCD-HPMC complexes, the melting peak of PX was shifted to lower temperatures with lower enthalpy.

Scanning electron microscopy photographs of the spray dried products are shown in Fig. 3. Spray drying of solubilised PX yielded a product of amorphous appearance with spherical particles. The particles were distinguished by the formation of aggregates (A). The SEM photographs of PX-HPMC solid system (B) showed a transformation of amorphous PX to polymorph II (needle form) in the presence of HPMC. This was also evident from DSC. Amorphous piroxicam rapidly converts to its crystalline form (Redenti et al. 1996). The SEM photographs of spray dried PX-HPβCD complex (C) showed spherical and folded surface, while SEM micrographs of spray dried PX-HPβCD-HPMC complex (D) showed spherical folded agglomerates with a continous membrane around the dispersed spheres. It was no longer possible to differentiate the components.

Fig. 4 presents the size distribution of the microspheres. Particle size analysis indicated a narrow logarithmic-normal distribution for all samples with about 70% of particles having a spherical diameter ranging from 1–3 μm. Table 2 shows the mean spherical diameters of the microspheres prepared. This indicated that the presence of HPMC and HPβCD complexation did not substantially influence the particle size.

PX release profiles from different solid systems are shown in Fig. 5. The dissolution profiles of the prepared complexes were compared with spray-dried PX. The dissolution of PX-HPβCD solid complex was very fast, and completed within about 10 min, reflecting the high aqueous solubility of the drug. The ability of forming a complex with HPβCD leads to an increase in the apparent drug solubility and a marked increase in the dissolution rate of PX from the solid system was observed.

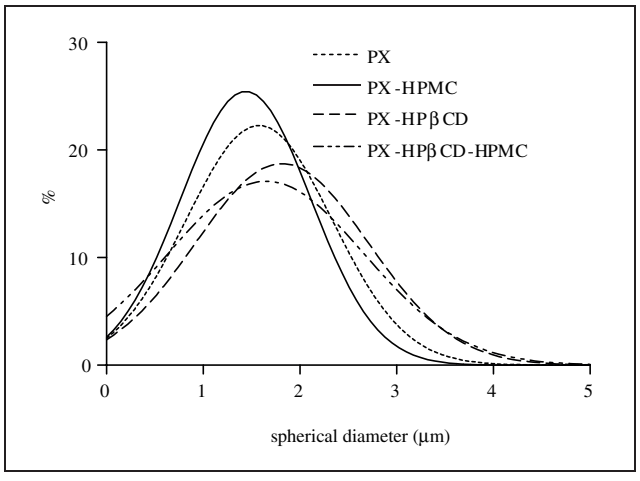

Fig. 4: Particle size distribution of spray dried microspheres

Table 2: Formulation characteristics, and PX flux (J) through semipermeable membrane of the microspheres prepared (n = 3)

Microspheres	Drug loading %	Mean diameter μm	J/μg h^{-1} cm^{-2} mean ± SD
PX	–	1.97	9.64 ± 1.05
PX-HPMC	86.7	1.78	20.34 ± 1.19
PX-HPβCD	20.6	2.28	52.59 ± 7.35
PX- HPβCD-HPMC	18.4	2.20	98.31 ± 6.81

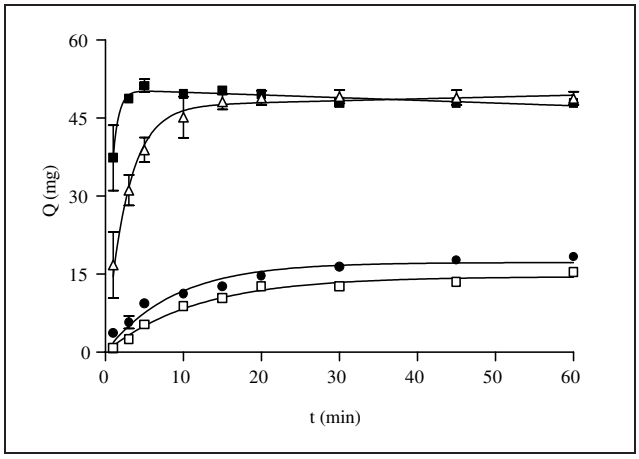

Fig. 5: *In vitro* release of PX in water from spray-dried microspheres (mean ± SD, n = 3); □ PX, ● PX-HPMC, △ PX-HPβCD, ■ PX-HPβCD-HPMC

HPMC in the solid system facilitated to some extent the drug dissolution due to the increased solubility in the microenvironment on the hydrodinamic layer surrounding the drug particle. HPMC dissolution created a local surfactant concentration in the boundary layer surrounding the drug particles, providing a lower energy pathway for drug dissolution.

The presence of HPMC and HPβCD in the solid product led to a faster dissolution rate. In the ternary solid system the molecules of the PX-HPβCD complex were supposed to be present in an intimate dispersed state within the HPMC matrix, through interaction between the exterior of the complex and HPMC. This state could be responsible for the higher dissolution rate with respect to the PX-HPβCD inclusion complex.

The increase in the dissolution rate could be ascribed to the ability of HPβCD to form a complex with PX, which eventually led to an increase in the apparent PX solubility. The solubility of HPβCD itself resulted in an increase of the water uptake in the matrix that could favour drug solubilisation. These findings suggested that HPβCD incorporated with HPMC in the solid system could improve the hydration of the microspheres.

The permeation test was intended to investigate the drug passage through a semipermeable membrane. The profiles of PX permeation from the microspheres are shown in Fig. 6. Flux values were determined from the steady state region of the diffusion profiles (Table 2). The differences in the flux values of PX from the microspheres could be attributed to the cyclodextrin-polymer complexation. When PX molecules diffused from an aqueous donor phase through a semipermeable membrane to a receptor phase, two processes were occured: PX diffusion in the aqueous donor phase and the diffusion through the membrane. Both of the processes contributed to the overall diffusion rate. PX diffusion in the aqueous diffusion layer (donor phase) was increased by improving the diffusible form of the drug species through complexation. Though the complex did not penetrate the membrane, the drug in the complex was in rapid dynamic equilibrium with the "free" drug, thus continuously supplying PX molecules to the membrane in a diffusible form. HPβCD enhanced PX delivery through the membrane by increasing the drug availability at the surface where PX molecules partitioned from the HPβCD cavity into the membrane.

Such cyclodextrin complexation increased the drug concentration gradient over the membrane which resulted in the increased PX flux.

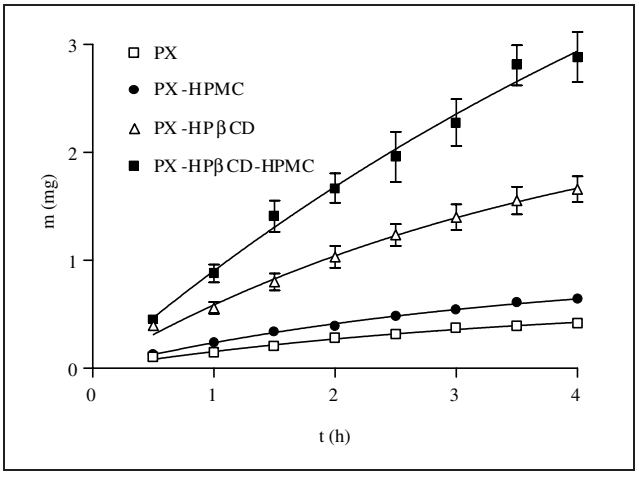

Fig. 6: Cumulative amount of diffused PX across semipermeable membrane from the microspheres as a function of time (mean ± SD, n = 3)

Addition of HPMC to the system increased the drug flux through the membrane. This flux was more than 80% higher than from microspheres prepared without HPMC. HPMC increased the complexation efficiency of HPβCD, by forming a ternary complex with higher stability constant compared to PX-HPβCD complex. As HPMC enhanced HPβCD complexation of PX resulting in higher drug flux through semipermeable membrane. As HPMC and HPβCD cannot penetrate the semipermeable membrane, it would be expected to affect the flux by increasing the drug solubility and concequently the affinity of PX-HPβCD-HPMC complex for the aqueous diffusion layer in the donor compartment.

3. Experimental

3.1. Materials

Piroxicam (PX) was kindly donated by Belupo (Koprivnica, Croatia). Hydroxypropyl-β-cyclodextrin (HPβCD) with an average substitution degree per anhydroglucose unit of 0.9 was used as received (Wacker, Chemie GmbH, Munich, Germany). Hydropropyl methylcellulose (HPMC), K4M, 4000 mPa s, (2% aqueous solution) was obtained from Shin-Etsu Chemical Co., Ltd. Japan. All other materials and solvents used were of analytical reagent grade.

3.2. Phase solubililty studies

Phase solubility studies were performed by the method reported by Higuchi and Connors (1965). Briefly, an excess amount of PX (0.05 g) was added to 20 ml of aqueous solutions containing various concentrations of HPβCD (0 to 4×10^{-2} mol l^{-1}). The suspensions were vigorously shaken at 25 ± 1 °C for 3 days. After equililbrium was attained the samples were filtered through a 0.45 μm Millipore membrane filter and suitably diluted with 0.01 mol l^{-1} methanol hydrochloric acid. The PX concentration was determined spectrophotometrically, λ = 242 nm (Ultrospec Plus, LKB, Pharmacia, Sweden). The 3-days equilibrium was considered sufficient. To establish the effect of the polymer on the solubility diagram, HPMC was added (0.05 and 0.1%) to the suspensions. The suspensions were sonicated in an ultrasonic bath for 1 h, at 70 °C, and than allowed to equilibrate at 25 ± 1 °C for 3 days. The apparent 1 : 1 stability constants, K_s, were calculated from the phase solubility diagrams using eq. (1):

$$K_s = \text{slope}/S_0\,(1 - \text{slope}) \quad (1)$$

where S_0 is PX solubility in water in absence of HPβCD or polymer.

3.3. Preparation of the inclusion complex

PX or equimolar amounts of PX (1.32 g) and HPβCD (6 g) were dissolved in water (400 ml) by addition of 25% ammonium hydroxide solution. The solution was stirred the next 24 h at ambient temperature, to obtain complexation equilibrium, and was subjected to spray-drying. The drying conditions were as follows: flow rate 0.25 l h^{-1}, inlet temperature 110 °C, outlet temperature 80 °C and air flow rate 700 Nl h^{-1}.

3.4. Preparation of spray-dried HPMC microspheres

PX or PX-HPβCD inclusion complex was dispersed in 0.1% HPMC solution. The dispersion was sonicated for 10 min to produce a clear solution, and subjected to spray drying. The drying conditions were described in section 3.3. The percentage of PX in spray dried systems was determined spectrophotometrically after solubilisation of the product in 0.01 mol l^{-1} methanol hydrochloric acid.

3.5. Differential scanning calorimetry (DSC)

DSC thermograms of the drug, polymer, cyclodextrin, and the complexes prepared were recorded on a Perkin Elmer Pyris 1 instrument. The instrument was calibrated with indium and zinc before analysing the samples. All accurately weighed samples (2–5 mg) were sealed into aluminium pans and scanned at a heating rate of 10 °C min^{-1} over the temperature range 30–250 °C under a dry nitrogen (35 ml/min).

3.6. Scanning electron microscopy (SEM)

SEM analysis was carried out using JSM 5800 (JEOL, Tokyo, Japan) scanning electron microscope. The samples were previously gold sputtered using Edwards S150 sputter coater under argon atmosphere to render them electrically conductive. Images were analysed using software package Link ISIS, Series 300, Version 3.35.

3.7. Size distribution of the systems

A microscopical imaging analysis technique for determination of size distribution was applied. The size and distribution were determined with an Olympus BH-2 microscope, equipped with a computer controlled image analysis system (Optomax V, Cambridge, UK).

3.8. In vitro dissolution studies

The dissolution studies were performed according to the dispersed amount method by adding the solid systems, equivalent to 50 mg of PX, to 500 ml of water thermostated at 37 °C ± 0.5 °C, and stirred at 50 rpm. At fixed time intervals samples were withdrawn with a filter-syringe (0.45 μm) and assayed spectrophotometrically for drug content.

3.9. In vitro permeation study

The effect of the HPMC and HPβCD on the diffusion of PX through a cellophane membrane (Medicell Dialysis Tubing MW CO 600) was investigated using a Franz diffusion cell (Perme Gear, USA) with a diffusion area of 10.18 cm^2, and an acceptor compartment volume of 100 ml. The dispersion acceptor compartment was continuously stirred at 600 rpm using a magnetic stirrer. The samples (equivalent to 50 mg of PX in 10 ml of water) were placed into the donor compartment thermostated at 37 °C.

The amount of the drug permeated through the membrane was determined by removing aliquots at fixed time intervals from the acceptor compartment. The PX concentration was determined spectrophotometrically as in the phase solubility studies. PX flux through the membrane was calculated using eq. (2):

$$J = dQ/A\,dt \quad (2)$$

where J is the steady-state flux and A is the diffusion area.

Acknowledgement: The authors would like to thank Dr. M. Tudja (PLIVA, Croatia) for his valuable help with SEM analysis.

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