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The effect of β-cyclodextrins on the permeation of diclofenac from supersaturated solutions

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Abstract

Supersaturation is a very useful method of enhancing the permeation of drugs across membranes such as skin, because unlike other methods, it does not interfere with the ultrastructure of the stratum corneum. Many drugs are able to form inclusion complexes with β-cyclodextrins (β-CDs) and this study investigates the anti-nucleating effects of these compounds on supersaturated solutions of diclofenac. The ability of various βCDs to form inclusion complexes with diclofenac was assessed by measuring their saturated solubilities. Solutions containing hydroxypropyl β-cyclodextrin (HPβ-CD, with a molar substitution of 0.9) produced a 7.5-fold increase in the solubility of diclofenac, which suggested that a strong complex was formed between the two compounds. This association was characterized using differential scanning calorimetry. Permeation across silicone membranes of these saturated solutions of diclofenac in the presence of the different βCDs produced similar flux values suggesting that the overall activity was also similar. The effect of different molar ratios of HPβ-CD and diclofenac, and the anti-nucleating effect of HPβ-CD (both on its own and in combination with a known anti-nucleant, hydroxypropylmethyl cellulose (HPMC)) on the diffusion of diclofenac across silicone membranes was investigated. HPβ-CD appears to have a stabilizing effect on supersaturated solutions of diclofenac as a co-ingredient with HPMC.

Keywords: β-Cyclodextrins; Diclofenac; Hydroxypropyl methyl cellulose; Supersaturation; Permeation; Silicone membranes

1. Introduction

Poor absorption of drugs by the skin is due to its excellent barrier properties and necessitates novel ways of improving the delivery of drugs through this route. Many methods using physical and chemical enhancement techniques have been investigated but most of them rely on perturbing the integrity of the main barrier, the stratum corneum. Supersaturated systems have been successful at enhancing skin permeation. The technique involves increasing the thermodynamic activity beyond saturated solubility concentrations and as flux is proportional to thermodynamic activity, an increase in the latter can lead to an increase in flux. The major advantage of this technique is its non-interference with the barrier properties of the stratum corneum. However, supersaturated systems are thermodynamically unstable

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and tend to crystallize either on preparation or during storage.

Some polymers act as anti-nucleating agents and can control the crystallization process and hence enhance permeation of a number of drugs (Davis and Hadgraft, 1991; Raghavan et al., 2000, 2001a; Iervolino et al., 2000, 2001; Pellett et al., 1994, 1997; Megrab et al., 1995). The inhibition of crystallization by these polymers has been rarely discussed in the past (Simonelli et al., 1970; Ziller and Rupprecht, 1988) but more recently a mechanism was proposed based on the adsorption of polymers onto the crystal surface through hydrogen bonding (Raghavan et al., 2001b). Infrared spectroscopic and differential scanning calorimetric studies have demonstrated the existence of such interactions (Taylor and Zografi, 1997; Tantishaiyakul et al., 1999; Iervolino et al., 2001; Raghavan et al., 2001a).

Iervolino et al. (2000, 2001) explored the possibility of using hydroxypropyl β-cyclodextrin (HPβ-CD) to stabilize supersaturated systems of ibuprofen by investigating the alterations in the physicochemical properties of the drug through the formation of an inclusion complex with HPβ-CD. The use of CD to supersaturate Pancratistatin, an anticancer drug for parenteral use (Torres-Labandeira et al., 1990) and to inhibit crystallization of amorphous nifedipine in spray dried powders (Uekama et al., 1992) has also been reported.

β-Cyclodextrins (β-CDs) are cyclic oligosaccharides, containing seven α-1,4 linked glucopyranose units in a truncated cone shape. The exterior surface of the molecule is hydrophilic whereas the internal cavity is hydrophobic. Therefore, in aqueous solutions, hydrophobic molecules have a higher affinity for the cavity than hydrophilic molecules and if a molecule fits entirely or at least partially into the centre of the structure, an inclusion complex is formed. The inclusion complex relies on weak associations such as hydrogen bonding and van der Waal's interactions. Drugs complexed with β-CDs are therefore easily dissociated and the free molecules are in fast equilibrium with bound molecules within the cavity (Loftsson et al., 1991).

Water-soluble polymers, such as hydroxypropylmethyl cellulose (HPMC) and polyvinyl pyrrolidone, have anti-nucleant properties and have also been shown to increase complexation of β-CDs with hydrocortisone and dexamethasone (Loftsson et al., 1993; Loftsson and Sigurdardottir, 1994).

In the present study the effects of using mixtures of HPMC and HPβ-CD as anti-nucleant systems to sustain supersaturated solutions of diclofenac are investigated. Silicone membranes were chosen as model membranes for the diffusion studies because β-CDs, such as methyl β-CD and hydroxypropyl β-CD may modify the skin barrier and act as chemical enhancers (Vollmer et al., 1993; Loftsson and Sigurdardottir, 1994), and this interference would make it difficult to study the formulation effects. Apart from the diffusion studies, the formation of an inclusion complex was studied using differential scanning calorimetry.

2. Materials and methods

2.1. Materials

Diclofenac free acid was obtained from diclofenac sodium salt (Secifarma, s.p.a, Milan) by acidification of its aqueous solution with HCl until pH 2–3 was reached. After filtration and drying at room temperature under vacuum, the melting point was determined to confirm the presence of the pure diclofenac acid. Propylene glycol (PG) was purchased from Fisons Plc., Loughborough, UK; HPLC grade acetonitrile was obtained from Rathburn Chemicals, Walkerburn, UK; hydroxypropylmethyl cellulose (HPMC) was obtained from Shin-Etsu Chemical Co. Ltd., Tokyo, Japan. Sheets of silicone membrane with a measured thickness of 275 μm were purchased from Samco, St. Albans, UK.

Methyl β-cyclodextrin (Mβ-CD) (partly substituted, 1.7–1.9) and hydroxypropyl β-CD (molar substitution of 0.9) (HPβ-CD) were obtained from Wacker Chemicals Ltd., Walton-on-Thames, UK; HPβ-CD (molar substitution 0.39) was obtained from Janssen, Olen, Belgium and β-CD from Roquette, Lestrem, France. All other chemicals were purchased from BDH Ltd., Poole, UK and were used as received.

2.2. Methods

2.2.1. Solubility studies

The calculated pK_a (ACD Software, Toronto, Canada) of diclofenac is 4.2, hence an aqueous phase

with pH 3.0 was chosen to suppress ionization. A known excess of diclofenac was added to a 50/50 (% v/v) cosolvent mixture of PG/buffer pH 3.0 with a 10-fold excess of each β-CD (w/v). The mixtures were stirred overnight at 32 °C. After filtration at 32 °C (the temperature chosen to represent the skin surface), the filtrate was suitably diluted and assayed using high performance liquid chromatography (HPLC). Solubility studies were performed in triplicate and mean values calculated.

2.2.2. Differential scanning calorimetry (DSC)

A solid complex of equimolar concentrations of diclofenac and HPβ-CD (with a molar substitution of 0.9) was prepared by dissolving the two compounds in ethanol and freeze-drying for 48 h. A physical mixture was also prepared by pulverizing the compounds in a mortar. DSC thermograms of the complex and the physical mixtures were recorded using a Perkin-Elmer DSC-7 (Cabral-Marques et al., 1990).

2.2.3. Diffusion studies

Diffusion experiments of diclofenac from supersaturated and saturated solutions in 50:50 (% v/v) PG/buffer pH 3.0 vehicles across silicone membranes were performed using Franz-type diffusion cells with a diffusional surface area of approximately 2 cm^2. Receptor compartments were filled with approximately 6 ml of phosphate buffered saline (PBS) pH 7.4, which had been previously degassed by filtration through a Whatman 0.45 μm membrane filter. Donor phase volumes of 1 ml were used, and the diffusion cells were placed on a submersible magnetic stirring bed in a water bath set at 37 °C with magnetic followers to stir the receptor phase. Evaporation from the donor compartments and sampling arms were prevented by using microscope cover slips and aluminium lids, respectively. At designated time points, 400 μl samples were removed from the receptor phase and replaced with an equal volume of pre-thermostatted PBS.

Supersaturated solutions were prepared by mixing equal volumes of diclofenac in PG with either 0.127% HPβ-CD (with a molar substitution of 0.9) in phosphate citrate buffer pH 3.0 (w/v), or a solution of 0.127% HPβ-CD (with a molar substitution of 0.9) (w/v) and 1% HPMC in phosphate citrate buffer pH 3.0 (w/v). This produced a solution where the degree of saturation was calculated with reference to the concentration of a saturated solution of diclofenac without the presence of any additives. A more detailed approach of this methodology has been described previously (Pellett et al., 1994). All solutions were freshly prepared and maintained at 32 °C before placing in the donor compartments. Flux values were determined from the linear (steady-state) section of the diffusion profiles. The lag times in these experiments were very small and steady state diffusion had been established by the first sample point.

2.2.4. HPLC analysis of diclofenac

Quantitative determination of diclofenac was performed using an HPLC system consisting of a Milton Roy LDC Constametric IIIG pump set at a flow rate of 1 ml/min, a Spectromonitor III variable wavelength UV detector set at 280 nm and a CI4100 computer integrator. The stationary phase was an Apex reverse phase ODS 5 μm column (25 cm × 4.6 mm), and a mobile phase of 60% acetonitrile and 40% water adjusted to pH 3 with acetic acid was used. Samples were injected via a 20 μl loop using a Spark Holland Marathon autosampler. Retention times were ∼7 min, and calibration curves were constructed on the basis of integrated peak areas obtained for the standards.

3. Results and discussion

3.1. Characterization of the inclusion complex

3.1.1. Solubility

The determined solubilities of diclofenac in the PG/buffer mixtures in the absence as well as the presence of the different β-CDs are shown in Table 1.

Table 1
Saturated solubilities of diclofenac in 50:50 (% v/v) PG/phosphate citrate buffer (pH 3.0) mixtures in the presence of various CDs

Type of CD	Saturated solubility (μg/ml)	Ratio of solubility to control	Mean flux ± S.E., $n = 3$ (μg/cm^2 h)
Control (no additives)	138	1	2.87 ± 0.12
β-CD	113	0.8	2.27 ± 0.54
Mβ-CD	506	3.7	2.7 ± 0.32
HPβ-CD (0.39)	226	1.6	2.48 ± 0.34
HPβ-CD (0.9)	1030	7.5	2.90 ± 0.17

Without any CD present, the solubility was 138 μg/ml and this value increased in the presence of all the CDs except β-CD for which a slight decrease was observed (113 μg/ml). The highest increase (7.5-fold) was found for HPβ-CD with a molar substitution of 0.9 indicating that this oligosaccharide complexed more of the drug than the others.

3.1.2. Differential scanning calorimetry

The thermal behaviour of a diclofenac–HPβ-CD complex was studied using DSC. When guest molecules are incorporated into a β-CD cavity, their melting, boiling or sublimation points usually shift to a different temperature, or disappear within the temperature range where the β-CDs decompose (Cabral-Marques et al., 1990). The thermographs in the present study (Fig. 1) showed an endothermic peak at 179 °C for pure diclofenac (Fig. 1a), and 156 °C for the diclofenac–HPβ-CD physical mixture (Fig. 1c). This shift of 24 °C indicated that there was an interaction between the two compounds. Evidence that the freeze-dried mixture was a true inclusion complex and not a simple physical mixture was based upon the disappearance of an endothermic peak in thermograph (Fig. 1b).

3.2. Diffusion

3.2.1. Saturated solutions

The steady state fluxes obtained, over a period of 12 h, from the diffusion of diclofenac across silicone membranes from saturated solutions in the presence of various β-CDs are given in Table 1. Fig. 2 also shows the cumulative amount of diclofenac transported from solutions containing no oligosaccharide, 0.39 and 0.9 M substituted HPβ-CDs. The flux values were similar within the standard deviation for all the samples despite the large differences in the drug

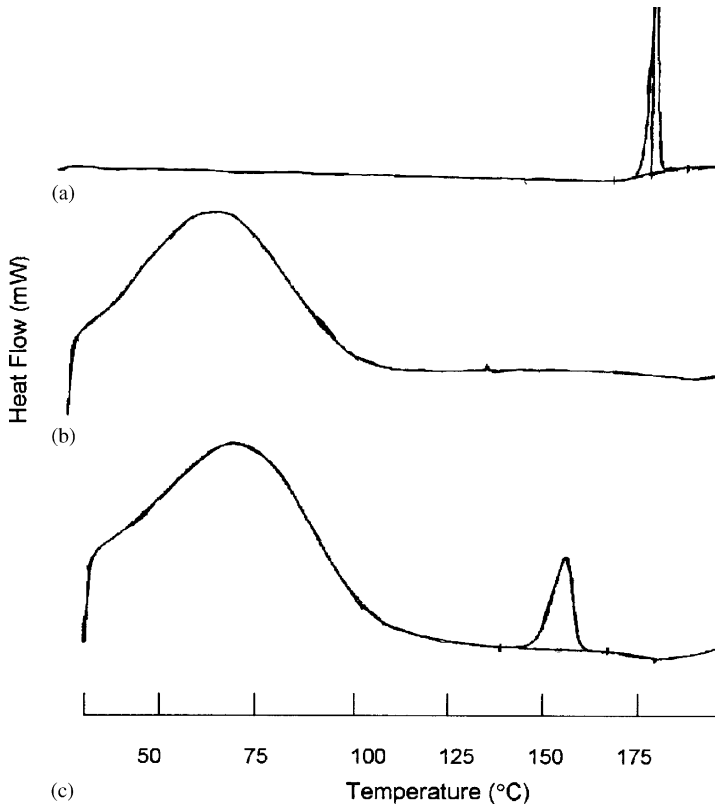

Fig. 1. DSC thermograms of (a) diclofenac, (b) diclofenac–CD inclusion complex and (c) complex diclofenac–CD physical mixture.

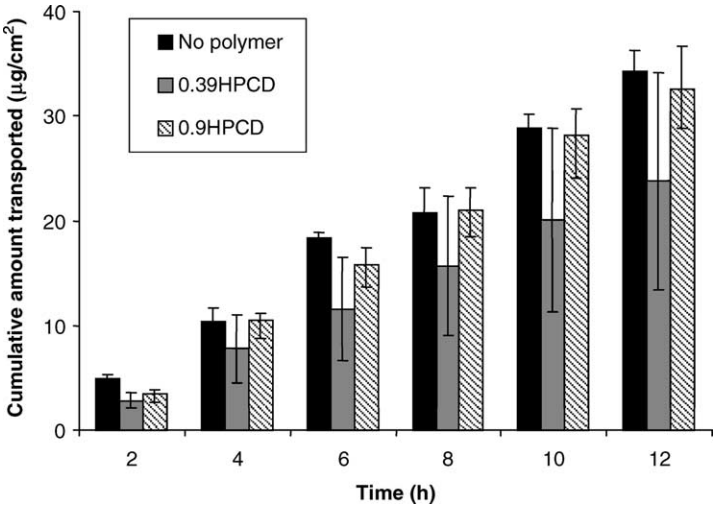

Fig. 2. Cumulative amount of diclofenac transported from saturated solutions containing no oligosaccharide and containing HPβ-CD (0.39 and 0.9 substituted).

concentrations (see Table 1). For saturated solutions, the thermodynamic activity is constant irrespective of the amount of drug present in the solution. Any deviation from this behaviour is a result of the interaction of the formulation components with the membrane. In the present studies, the only variable is the presence of different β-CDs. The similar fluxes indicate that the β-CDs do not interact significantly with the membrane. This is not surprising since the β-CD molecules are large in size. It should also be noted that only the free diclofenac molecules are capable of permeating across the membrane and hence the activity of the drug is related to the free molecules.

Fig. 3 shows the effect of different molar ratios (0.9 M substitution) of HPβ-CD on the diffusion of diclofenac from saturated solutions. Rather surprisingly, the fluxes increased with increasing HPβ-CD loading up to 2:1 ratio (CD:diclofenac) and decreased at very high amounts of HPβ-CD. One would expect the flux to be similar since the activity is expected to be the same. However, as mentioned earlier, the flux is due to the diclofenac molecules, which are not complexed with HPβ-CD. With increasing HPβ-CD, the associated diclofenac increases so that the dissociated molecules available for diffusion decrease. This affects the flux especially at high HPβ-CD concentrations (>10 times HPβ-CD) where a decrease of the permeation rate is observed. This behaviour needs to be addressed further by performing detailed studies on the kinetics of complexation of this system. The data however suggest that there is an optimum molar ratio at which maximum flux can be obtained. Loftsson and Masson (2001) reported on the interactions of CDs with skin and how it affects topical drug delivery. In this study they suggest that CDs enhance topical drug delivery by increasing the availability of drug at the barrier surface. They also suggest that the permeation is both diffusion-controlled as well as membrane-controlled. The present study deals with silicone membrane which is a very homogenous membrane and it is unlikely that same type of interactions can exist. This suggests that the effect of CD on the permeation of diclofenac is due to the physico-chemical characteristics of the formulation and not due to interactions of the formulation components with the membrane, which is possibly the case reported by Loftsson and Masson.

3.2.2. Supersaturated solutions

Two different degrees of potential supersaturation, 5× and 10× the concentration of saturated solution, were chosen for the study. Fig. 4 shows the diffusion profiles of diclofenac from 50:50 (% v/v) PG/buffer pH 3.0 solutions containing 0.127% of 0.9 M substituted HPβ-CD (w/v). For comparison, Fig. 4 also contains data for a control solution (saturated) without any

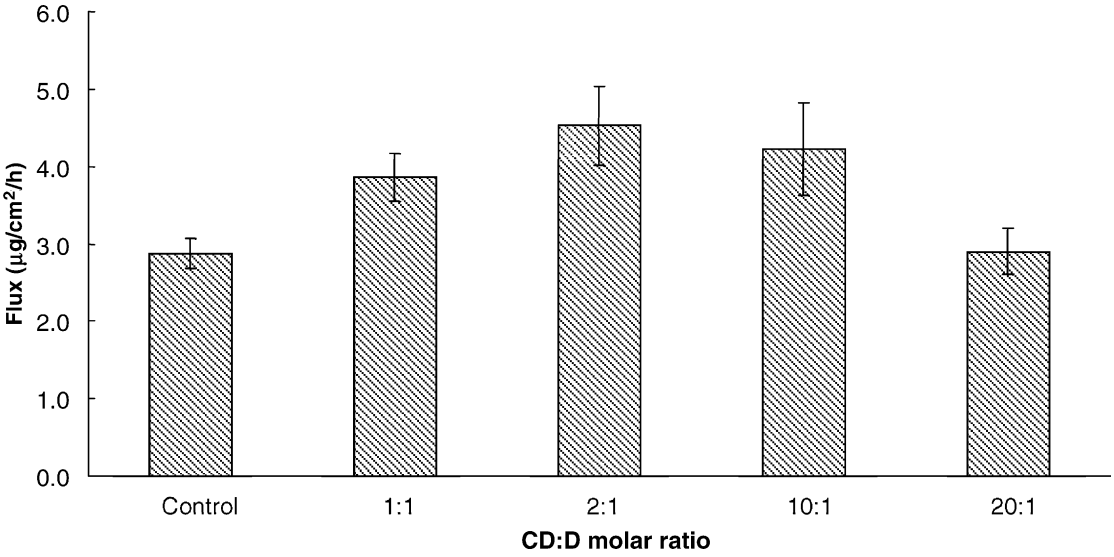

Fig. 3. Cumulative amount of diclofenac transported from saturated solutions containing different molar ratios of 0.9 substituted HPβ-CD.

additives. Permeation enhancement was observed for the supersaturated solutions. According to Fick's law, the flux values should be proportional to the chemical potential gradient, or in other words, the degree of saturation. An analysis of the flux values however reveals that under these conditions this is not observed. The values are in fact lower than what would be expected from the theory. The discrepancy could arise from either of the following two factors or both. Firstly, HPβ-CD is not able to stabilize the supersaturated system and hence precipitation occurs leading to a decrease of the activity and secondly, in the presence of HPβ-CD, the solubility increases due to the formation of an inclusion complex and the actual thermodynamic activity is lower than the value estimated from the saturated solubility values of diclofenac without the additive present. Similar results were recently obtained by Iervolino et al. (2000, 2001) for ibuprofen,

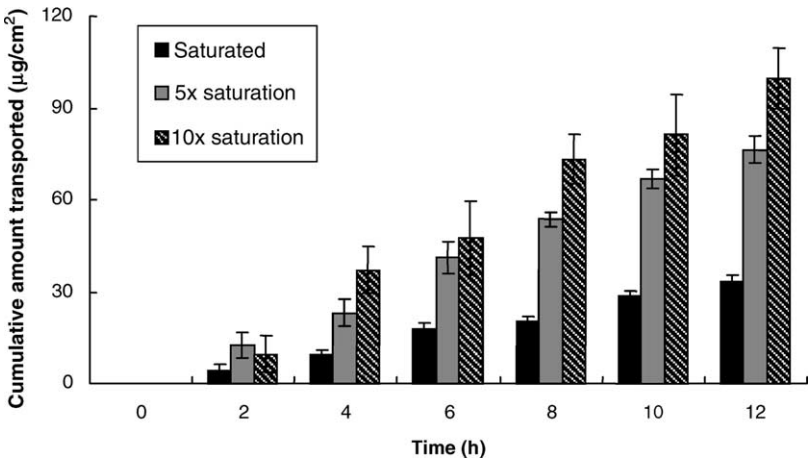

Fig. 4. Cumulative amount of diclofenac transported from 5× to 10× supersaturated solutions ($n = 4$; ±S.D.).

where they found that the decrease in flux is due to the increase in solubility caused by the formation of the inclusion complex. Furthermore, degrees of saturation are calculated on the total amount of the drug in solution and takes into account both molecules, which are not associated, and those, which are associated. The permeation of the associated proportion of the drug is negligible and therefore insignificant. The amount of un-associated drug that contributes to the degree of saturation is lower which leads to a lower

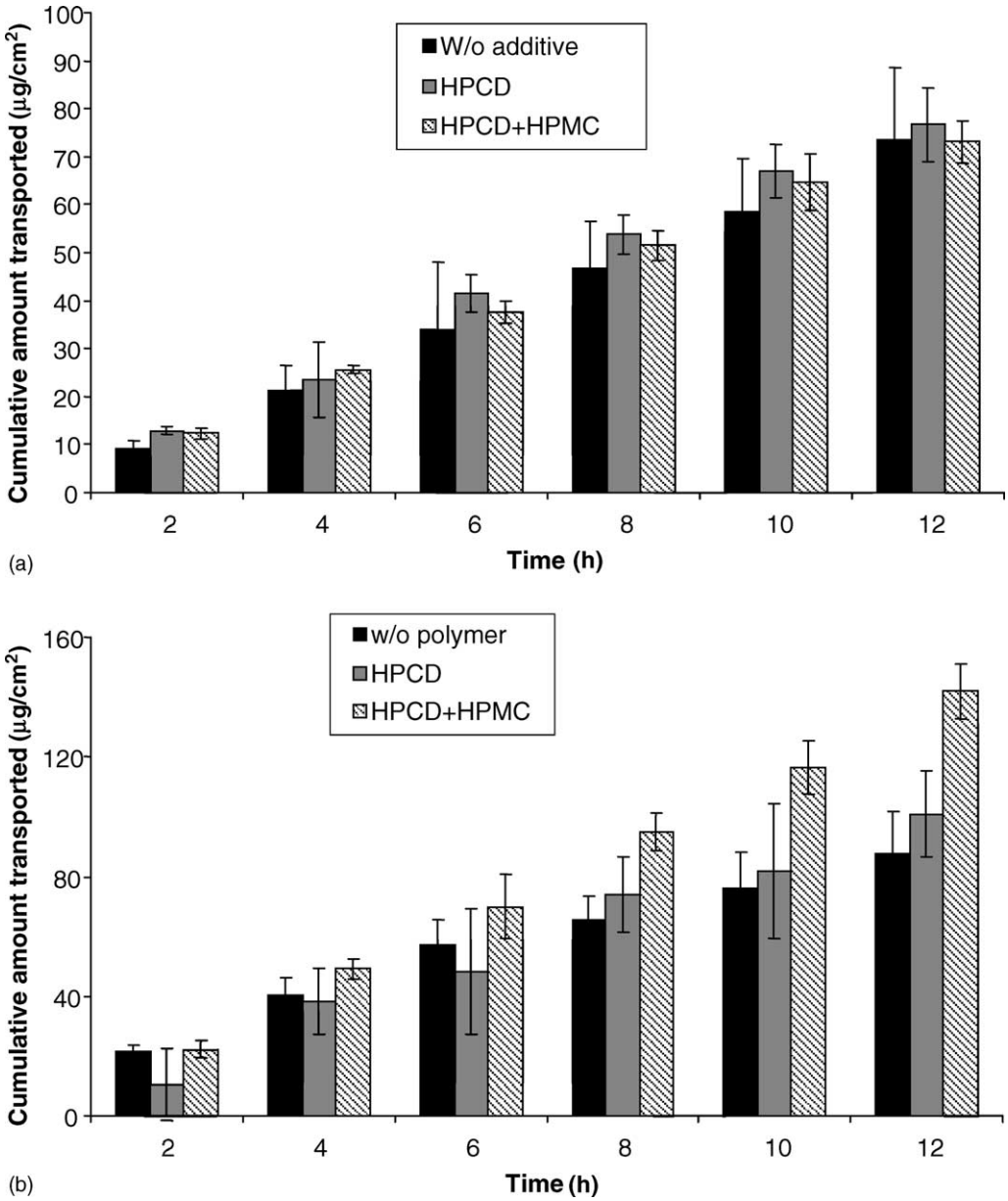

Fig. 5. Cumulative amount of diclofenac transported from (a) 5× and (b) 10× supersaturated solutions containing no additive, containing HPβ-CD and HPβ-CD with HPMC ($n = 4$; ±S.D.).

thermodynamic activity. This might be contributory to the lower flux observed. Moreover, at high degrees of saturation, the standard deviations were quite high in their as well as our studies indicating probable instability in the systems. Instability leads to partial crystallization and hence lower thermodynamic activity. On comparison of the results, lowering of the activity caused by both physical instability and increase in solubility due to complexation can be contributory factors for the lower flux observed for diclofenac, the latter especially for the system, which contained 10× the concentration of a saturated solution.

HPMC has been shown to be an effective antinucleant polymer for a number of pharmaceutical drugs (Davis and Hadgraft, 1991; Raghavan et al., 2000, 2001a; Iervolino et al., 2000, 2001; Pellett et al., 1994, 1997). If instability was a factor in supersaturated systems of diclofenac containing HPβ-CD then addition of HPMC can prevent crystallization and give rise to further enhancement in the flux. In order to assess its feasibility, a 1% HPMC solution was used instead of water to prepare the supersaturated solutions. Fig. 5a and b show the amount of diclofenac transported across silicone membranes without any additive and with the addition of HPβ-CD and in the presence of HPMC at 5× and 10× saturated concentration, respectively.

An enhancement in the flux can be seen at these two degrees of supersaturation even when additives were not used. However the standard deviations were quite high indicating that these systems are relatively unstable. In the presence of HPβ-CD alone, the behaviour was similar but in the presence of HPMC, the standard deviation was lower indicating that the supersaturated systems were relatively more stable. This can be especially seen at 10× the concentration of a saturated solution. The results suggest that even though permeation enhancement can be achieved without or with HPβ-CD, a polymer such as HPMC might be required to inhibit the crystallization process. Moreover, in most instances, the formulations need to be stable for long periods of time after preparation and such problems of storage have already been reported in the literature (Lipp, 1998; Ma et al., 1996). Polymers can be used as additives in such cases to prevent crystallization and increase stability of transdermal patches over a long period of time.

4. Conclusions

The ability of CDs to stabilize supersaturation and aid in permeation enhancement of diclofenac was investigated using silicone as a model membrane. CDs were found to form an inclusion complex with diclofenac, confirmed by solubility and DSC studies. Despite largely differing solubilities, the fluxes obtained from saturated solutions containing the different CDs were similar. This is expected as the flux is determined by the activity of the drug in the solution rather than the actual amount of the drug present. The fluxes also increased when using 5× and 10× saturated concentrations although the increase was not proportional to that from a saturated solution indicating the solutions are not stable. CD by itself was not sufficient to stabilize and sustain a supersaturated state but the presence of HPMC increased the stability of the supersaturated solutions. The studies suggest that CDs can be used to produce supersaturated solutions by changing the solubility but they have a limited stabilising effect on the supersaturated systems. Recent work by Shaker et al. (2003) on the influence of HPβ-CD and polyvinyl pyrroliodone on the permeation of corticosterone through semi-permeable cellulose membrane and hairless mouse skin support the results obtained in the current study.

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