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Penetration enhancement of ibuprofen from supersaturated solutions through human skin

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Abstract

Systematic investigations on the diffusion of ibuprofen (IBU) from supersaturated solutions through human epidermis are reported. Significant flux enhancement was obtained from supersaturated solutions compared to the saturated solution. Hydroxypropyl methylcellulose (HPMC), when used as an additive was found to be effective in maintaining the high activity state at high degrees of saturation (DS). The increase in the flux was proportional to the DS. In the presence of 2-hydroxypropyl-β-cyclodextrin (CD) at DS 2 and 3 a lower flux was observed compared to HPMC. At DS 5 a higher flux enhancement was found suggesting that CD might act as a penetration enhancer at certain CD/drug ratios. Studies on the mechanism of stabilisation of HPMC and CD on IBU crystallisation from supersaturated systems showed that HPMC acts as a growth inhibitor and habit modifier whereas CD does not influence the crystallisation process.

Keywords: Permeation; Human epidermis; Supersaturation; Ibuprofen; Hydroxypropyl methylcellulose; 2-Hydroxypropyl-β-cyclodextrin; Crystallisation

1. Introduction

Supersaturated systems have been used to enhance the penetration of permeants through model silicone membranes as well as human skin (Davis and Hadgraft, 1991; Pellett et al., 1994; Megrab et al., 1995; Pellett et al., 1997a; Lipp, 1998; Schwarb et al., 1999; Iervolino et al., 2000; Raghavan et al., 2000a). This technique offers the advantage of being inexpensive and does not alter the integrity of the stratum corneum. Supersaturation involves increasing the thermodynamic activity beyond the saturation level. The limitation of the technique is the instability of these systems. The drug often rapidly crystallises leading to a decrease in the initial high activity. This problem has been overcome by the use of antinucleant polymers to stabilise the supersaturated systems (Davis and Hadgraft, 1991; Pellett et al., 1994;

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Megrab et al., 1995; Schwarb et al., 1999; Iervolino et al., 2000). Some of the polymers that were found to be effective include hydroxypropyl methylcellulose, methylcellulose and polyvinyl pyrrolidone.

Recently, increased in vitro permeation of ibuprofen (IBU) from supersaturated solutions through model silicone membranes was demonstrated (Iervolino et al., 2000). A cosolvent technique was used to obtain the supersaturated solutions (Davis and Hadgraft, 1991). Propylene glycol (PG) and water were used as the constituent solvents because IBU has a solubility of 301 mg/ml in PG and 0.12 mg/ml in water. Hydroxypropyl methylcellulose (HPMC) and hydroxypropyl-β-cyclodextrin (CD) were used as additives to stabilise the solutions. HPMC is a well-known crystallisation inhibitor and CD has been reported to supersaturate Pancratistatin, an anti cancer drug for parenteral use (Torres-Labandeira et al., 1991), and to inhibit crystallisation of amorphous nifedipine in spray dried powders (Uekama et al., 1992). In addition, the association of CD with IBU has been found to affect the physicochemical properties of the drug (Loftsson et al., 1993; Mura et al., 1998).

The enhancement of IBU permeation using supersaturated solutions through model membranes prompted the extension of the investigations to human skin. The aim of this work was to study the diffusion of IBU from supersaturated solutions through human epidermis. The results were compared with the data obtained with silicone membranes. The influence of HPMC and CD on the penetration enhancement of IBU was systematically evaluated.

The stabilisation of supersaturated systems by additives depends on their ability to inhibit nucleation and subsequent crystal growth. Crystallisation can be controlled using external conditions such as the degree of supersaturation, solvents, impurities, temperature and pH. CD has been proposed to act as a crystallisation inhibitor but its mechanism of action is not known. There are very few reports on the mechanism of crystallisation inhibition by polymers (Simonelli et al., 1970; Ziller and Rupprecht, 1988; Raghavan et al., 2000a). Polymers have been suggested to influence crystallisation by interacting with the drug molecules through hydrogen bonding. The ability of the polymer to inhibit crystallisation is dependent on the nature of the drug and the polymer. It was of interest to understand the crystallisation mechanism of IBU and how these additives can alter the nucleation and growth characteristics of the drug. The mechanisms of stabilisation by the two additives were investigated both by crystal growth studies and infrared spectroscopy and are discussed.

2. Materials and methods

2.1. Materials

Ibuprofen was a generous gift from Whitehall International (Havant, UK). Propylene glycol and HPLC grade acetonitrile were purchased from Fisher Scientific International (UK). Hydroxypropyl methylcellulose grade 65SH viscosity 50cP with the brand name of Metolose was obtained from Shin-Etsu (Japan). 2-Hydroxypropyl-β-CD (molar substitution 0.9) was obtained from Wacker (Walton on Thames, UK).

All other chemicals were purchased from BDH Ltd (Poole, UK) and were used as received. Double distilled de-ionised water was used throughout this study.

2.2. Methods

2.2.1. Preparation of solutions at different degrees of saturation

Solutions of IBU from 0.5 to 5 degrees of saturation (DS) in 40:60 (v/v) PG/water mixture in the absence and presence of HPMC and CD were prepared as described previously (Iervolino et al., 2000). Concentrations of IBU ranged from 0.4 to 4 mg/ml corresponding to the different DS. The degree of saturation was calculated by dividing the concentration of the drug in the solution by its saturated solubility. A saturated solution of IBU was also prepared in the presence of excess drug.

2.2.2. Preparation of human epidermis

Excised human skin, obtained from cosmetic surgery and stored at −20°C was thawed overnight. Epidermis was prepared by the heat separation technique (Kligman and Christophers, 1963). After removing the excess fatty and connective tissues, the skin was immersed in a water bath at 60°C for 1 min after which the epidermis was teased away from the dermis. The epidermis was then carefully placed on a filter paper with the stratum corneum facing outwards and allowed to dry before storing it at −20°C. The epidermis was thawed overnight before use.

2.2.3. Diffusion studies

The diffusion of sub-saturated, saturated and supersaturated solutions of IBU across human epidermis was investigated. All glass Franz-type diffusion cells with diffusional areas of approximately 0.95 cm^2 and receptor volumes of approximately 2.7 ml were used. Diffusion experiments were performed with 0.5ml of the drug solution in the donor compartment (for full details, see Iervolino et al., 2000). A total of 0.2 ml of the receptor phase (phosphate buffer, pH 7.4) was removed at predetermined intervals (2, 4, 6, 8, 10, 24 h) which was replaced with an equal volume of pre-thermostated receptor phase. The samples were assayed by HPLC, as previously described (Iervolino et al., 2000).

Flux values were determined, either from the steady state region of the diffusion profiles obtained using linear regression analysis or, where appropriate, they were obtained by using a non-linear curve-fitting method (Pellett et al., 1997a).

2.2.4. Microscopy

The supersaturated solutions were analysed for the presence of crystals by observation using a Vickers microscope at a magnification of 400 ×.

2.2.5. Infrared spectroscopy (IR)

Infrared absorption spectra were recorded in a KBr medium using a Perkin-Elmer 1600 Series FTIR Spectrophotometer. An IBU-HPMC (1:4) solid dispersion was prepared using a solvent evaporation technique. The drug and the polymer were dissolved in a 40:60 (v/v) PG/water cosolvent mixture. The solvents were removed by drying under high vacuum at room temperature for 48 h. Samples of pure IBU and HPMC were prepared using the same procedure.

3. Results and discussion

3.1. Diffusion studies

Fig. 1 shows the flux of IBU through human epidermis from 40:60 (v/v) PG/water solutions in the absence and presence of additives at different degrees of saturation (varying from 0.5 to 5). 1% (w/v) HPMC and CD aqueous solutions were used in the preparation of the supersaturated solutions. These concentrations were chosen based on the previous studies performed with silicone membranes (Iervolino et al., 2000). In these studies, 1% HPMC was found to be sufficient to stabilise the supersaturated solutions and concentrations of CD greater than 1% were found to lower the flux significantly.

3.1.1. Without additives

In the absence of the additives the flux increases proportionally with the DS up to 3 × saturation and decreases at higher DS. The decrease observed at DS 4 and 5 may be attributed to a decrease of the chemical potential due to crystallisation. The instability of the solutions is also reflected in the high standard deviation of the flux

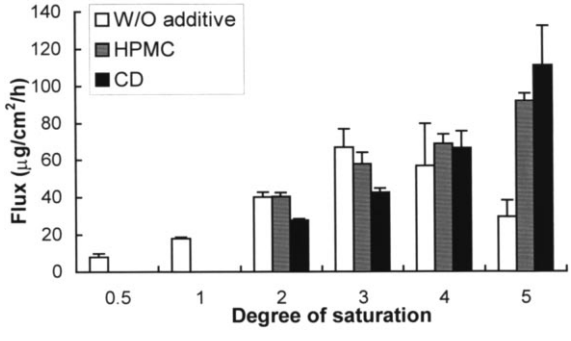

Fig. 1. Flux of ibuprofen (IBU) from solutions at different degrees of saturation (DS) in 40:60 (v/v) propylene glycol (PG)/water cosolvent mixture in the absence and presence of additives ($n \geq 4$; ± S.D.).

values at DS 4 and 5. At 5 × saturation the flux value is between those observed at 1 × and 2 × saturation. At 2 × saturation the solutions were demonstrated to be stable (Iervolino et al., 2000). The flux value at 5 DS suggests that the solutions reach the metastable zone on crystallisation. At this stage no new spontaneous nucleation can occur but the crystals formed can grow over a period of time.

Diffusion experiments were also performed with saturated solutions containing excess drug. The flux value obtained was 41 ± 9 μg/cm^2 per h, which is approximately twice the value obtained without excess drug. This is surprising since both solutions should have the same thermodynamic activity and hence provide the same flux. To establish that this difference is not due to the depletion of the drug in the donor solution without excess drug, a diffusion experiment was performed for a duration of 4 h sampling every 30 min (at the end of 4 h, only 7.5% had permeated). The flux obtained was the same as that obtained from diffusion experiments performed over a period of 24 h.

A possible explanation for the difference in flux between saturated solutions without and with excess drug could be the differential uptake of PG and water by the stratum corneum. Megrab et al. (1995) measured the uptake of PG and water from different aqueous propylene glycol solutions into fully hydrated stratum corneum. They observed a distinct decrease of water content in the stratum corneum with increasing PG. Equilibrating the stratum corneum with a 40:60 PG/water solution they found that PG and water were present in the membrane in ~50:50 ratio. Since the drug has a higher solubility in a 50:50 PG/water system, more drug is needed to saturate the stratum corneum. The excess drug present in the donor solution is used to saturate the drug in the stratum corneum whereas when no excess drug is present, the stratum corneum will be sub-saturated and the thermodynamic activity will be lower than unity. This results in a reduction of the flux.

3.1.2. With HPMC

In the presence of HPMC the flux increases proportionally with the degree of saturation up to 5 × saturation. The stabilising effect of HPMC on the supersaturated state is evident at 4 and 5 DS where the thermodynamic activity is retained for prolonged periods of time. The solutions at 4 and 5 DS appeared translucent on preparation. However crystals could not be observed under the microscope for at least 24 h (the duration of the diffusion experiments). The translucent nature of the solutions was reported earlier for supersaturated solutions of IBU containing HPMC (Iervolino et al., 2000). This phenomenon was related to the occurrence of nucleation and the formation of a stable microsuspension due to the inhibition of the growth by the polymer.

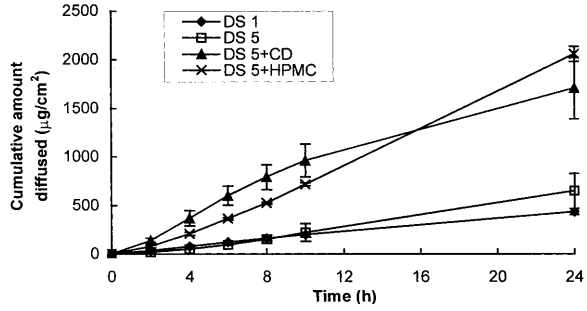

Fig. 2. Diffusion profiles of ibuprofen (IBU) from supersaturated solutions at degrees of saturation (DS) 5 without and with additives. Permeation data from saturated solutions (DS 1) are given in the figure for comparison ($n \geq 4$; ± S.D.).

3.1.3. With CD

When CD was used as the additive, at 2 × and 3 × saturation, the flux values were lower compared to those from solutions containing no additive as well as with HPMC. At 4 × saturation, the flux was comparable to the value obtained in the presence of HPMC, whereas the flux at 5 × saturation was higher. However, a large standard deviation was obtained at the largest DS, attributed to the instability of the system. This is evidenced in Fig. 2 where the cumulative amount diffused as a function of time for DS 5 with and without additives is reported (the diffusion profile at DS 1 is given in the figure for comparison). It can be seen that the diffusion profile of IBU in the presence of CD shows depletion after 8 h due to crystallisation. Crystals in fact could be seen on

the surface of the skin after this period. The depletion is not because of a decrease in concentration due to diffusion as less than 10% had permeated after 6 h.

In order to understand the flux behaviour complexation between IBU and CD was investigated in the cosolvent mixture 40:60 (v/v) PG/water. CD was found to increase the solubility of IBU in 40:60 PG/water (Iervolino et al., 2000). From the solubility data at different CD concentrations a linear phase solubility diagram was constructed (Fig. 3). The apparent 1:1 stability constant of the inclusion complex IBU/CD (K) was calculated from the slope of the diagram, using the Higuchi–Connors equation (Higuchi and Connors, 1965):

$$K = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 is the solubility of IBU in 40:60 PG/water. From the stability constant ($K = 158\ M^{-1}$), the amount of IBU uncomplexed in solution (IBU_{free}) was calculated and shown in Table 1. The actual degrees of saturation based on IBU_{free} were estimated and tabulated. These values were lower than the theoretical DS. As a result the thermodynamic activity in the presence of CD is decreased. The complexation reduces the amount of free drug in solution. As the permeation of drug-CD complexes is insignificantly low (Loftsson et al. 1991), the total flux of the drug is lowered for supersaturated solutions containing CD at DS 2 and 3 (Fig. 1).

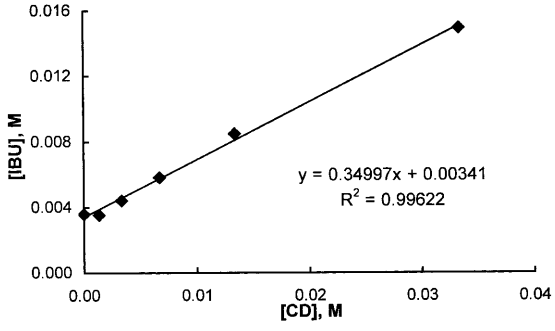

Fig. 3. Phase solubility diagram of ibuprofen (IBU) in 40:60 (v/v) propylene glycol (PG)/water mixture containing cyclodextrin (CD) at 32°C.

Table 1
Actual degrees of saturation (DS) for supersaturated solutions of ibuprofen (IBU) in 40:60 (v/v) propylene glycol (PG)/water mixture containing cyclodextrin (CD)[a]

Theoretical DS	IBU free (M)	Actual DS[b]
2	0.0054	1.39
3	0.0093	2.39
4	0.0132	3.38
5	0.0171	4.37

[a] A saturated solution contains 0.83 mg/ml.
[b] DS calculated using the concentration of the IBU free.

3.2. Penetration enhancement

The enhancement effect of supersaturated solutions on the permeation of IBU was evaluated by plotting the flux ratio (flux at $n\times$ saturation/flux at $1\times$ saturation, $n = 0.5$, 1, 2, 3, 4 and 5) as a function of DS. In the case of CD the actual DS were used for the analysis.

The decrease of flux ratio can be seen clearly at high DS for solutions without any polymer (Fig. 4). The flux ratio for solutions containing HPMC showed a linear correlation with DS. The proportionality constant is close to unity (0.985) confirming that the flux enhancement is equal to the increase in the DS.

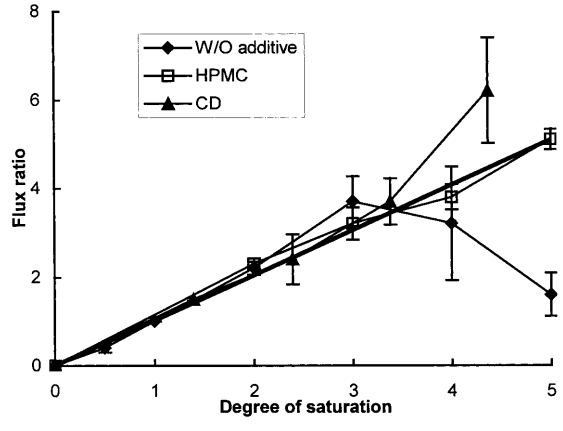

Fig. 4. Flux ratio of ibuprofen (IBU) as a function of degrees of saturation (DS) from solutions without and with additives ($n \geq 4$; $\pm$S.D.). The thick solid line represents the expected flux ratio behaviour of IBU at different DS.

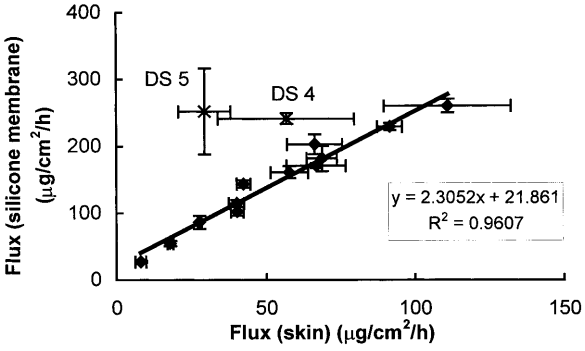

Fig. 5. Flux of ibuprofen (IBU) through silicone membrane vs. flux of IBU through human epidermis (degrees of saturation, DS, 4 and 5 were not included in the regression analysis) ($n \geq 4$; $\pm$ S.D.).

With CD the flux ratio for DS 2, 3 and 4 corresponds well with the actual DS. This implies that the thermodynamic activity is related to the amount of free drug in the solution and not to the total amount of IBU. At DS 5, the flux ratio is higher than the value expected for that DS. A similar effect was found for permeation of IBU through silicone membrane (Iervolino et al., 2000). This increase may be attributed to an additional contribution of CD to the drug permeation through the epidermis. CDs have been suggested to act as a penetration enhancer of poorly soluble drugs through biological membranes, depending on the CD/drug ratio (Másson et al., 1999).

The diffusion studies of IBU through human epidermis demonstrate that supersaturated systems can be effectively used to enhance permeation of IBU through human skin. HPMC is capable of maintaining thermodynamic activity at high degrees of saturation for prolonged periods of time. CD influences the flux through complexation.

3.3. Comparison of flux through silicone membrane and skin

It is of interest to compare the present flux results of IBU using human epidermis with those for silicone membranes. The flux values through silicone membranes (Iervolino et al., 2000) are plotted as a function of the flux through epidermis (Fig. 5) in order to determine if, under these conditions, silicone membranes can represent skin as model membranes. A good correlation is obtained with a regression coefficient of 0.96. It should be noted that the values for DS 4 and 5 without any additive were not included in the regression. The flux in skin is significantly lower than expected due to instability of these systems. Since the rest of the conditions are the same, the decrease may be caused by the induction of crystallisation by the rough surface of the stratum corneum. These could act as nucleation centres (Pellett et al., 1997b). As a result, secondary nucleation occurs and reduces the effective concentration of the drug. Moreover, the amount of drug diffusing through the skin (20–100 μg/cm^2 per h) is much lower than the amount diffusing through the silicone membrane (50–260 μg/cm^2 per h) and high DS are retained for longer times in the donor phase. These solutions were opaque when prepared indicating that crystal nuclei were present at the beginning of the experiment. At high DS, these nuclei can grow eventually causing a reduction in the DS.

The flux through silicone membrane is found to be 2.3 times the flux through the skin. The linear relationship in Fig. 5 confirms that silicone membranes can be used to investigate the properties of this type of formulation, in this case, supersaturation.

3.4. Mechanism of stabilisation

Stabilisation of thermodynamically unstable supersaturated states is crucial in the permeation enhancement of drug molecules from supersaturated solutions. Instability often leads to nucleation and subsequent crystal growth. In order to stabilise supersaturated solutions, both these processes need to be controlled. Crystallisation can be influenced by a number of external parameters such as solvents, supersaturation, impurities, temperature, pH and hydrodynamics. One or more of these parameters are often used to control the crystallisation process. The nucleation time (induction time), the time required to form nuclei of critical size before the growth can take place, decreases with increasing degree of saturation

(Mullin, 1993). This is clearly observed for IBU. In a previous study, Iervolino et al. (2000) have reported that stable supersaturated solutions can be obtained with low DS whereas at higher DS, the solutions were translucent or opaque, indicating the presence of IBU micro-crystals. Similarly, impurities (or additives), either present inherently or added deliberately, are often used to inhibit as well as accelerate the nucleation processes. Small amounts of fatty acids used as additives alter the physicochemical properties of adipic acid significantly by incorporating into the crystals (Fairbrother and Grant, 1978, 1979; Chow et al., 1984). Tailor-made additives are also known to inhibit nucleation and growth. Para-acetoxy acetanilide has been found to inhibit the growth of paracetamol (Chow et al., 1985).

In order to understand the influence of the HPMC and CD on the crystallisation of IBU, crystals were grown both in the absence and presence of the additives. Fig. 6a–c show IBU crystals grown over a period of 48 h in 5 × saturated solutions containing no additive, 1% CD and 1% HPMC, respectively. Crystals grown from solutions containing HPMC were, in general, smaller compared to those grown in the presence of CD and without any additive. Moreover, the habit was modified in the presence of HPMC. IBU crystals grown in the presence of CD were similar to those grown without additive. The results suggest that the mechanisms of stabilisation are different for CD and HPMC.

The growth inhibition was analysed in a quantitative way by measuring the aspect ratio defined as the length (a) to width (b) ratio. It was not possible to consider the thickness for the aspect ratio measurements since the thickness dimension was not measurable. The values of a, b and a/b for the crystals grown without and with HPMC and CD are tabulated (Table 2). The values of a and b are the average of dimensions measured on six crystals for each sample. The aspect ratios for crystals grown without and in the presence of CD are very similar within the standard deviation confirming that CD does not influence the growth of IBU. Although CD does not inhibit crystallisation of IBU, it has been shown that CD increases the solubility of IBU by formation of inclusion complex. The increase in solubility leads to a decrease of the DS. Consequently the crystals grow at a lower degrees of supersaturation.

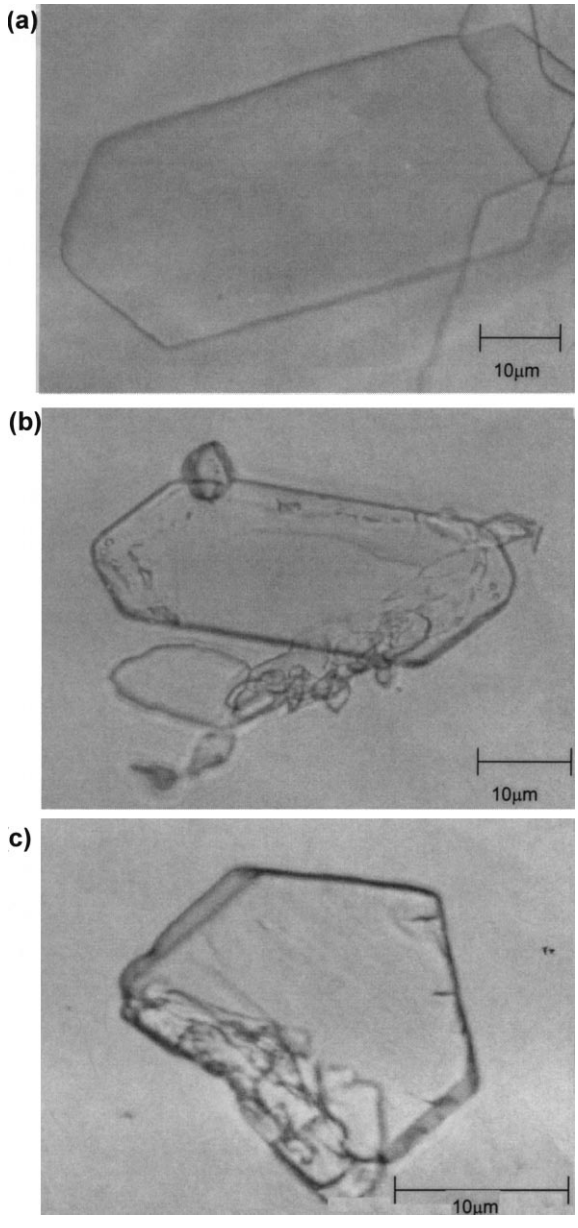

Fig. 6. Ibuprofen (IBU) crystals grown from solutions at degrees of saturation (DS) 5 containing (a) no additive, (b) cyclodextrin (CD) and (c) hydroxypropyl methylcellulose (HPMC) over a period of 48 h.

Table 2
The length (a), width (b) and the aspect ratio (a/b) of ibuprofen (IBU) crystals grown in 5× saturated 40:60 (v/v) propylene glycol (PG)/water solutions without and with additives

Sample	$A \pm$ S.D. (μm)	$B \pm$ S.D. (μm)	$a/b \pm$ S.D.
W/o	53 ± 30	27 ± 13	1.98 ± 0.29
CD	54 ± 10	20 ± 3	2.81 ± 0.59
HPMC	13 ± 4	16 ± 4	0.85 ± 0.09

The a and b dimensions and the aspect ratio of IBU crystals grown in the presence of HPMC were smaller compared to the dimensions of the crystals grown without the additive. The change in size of IBU crystals grown in the presence of HPMC is a clear indication of significant interaction between IBU and HPMC. Growth inhibition of crystals by polymers has been reported in the literature (Simonelli et al., 1970; Ziller and Rupprecht, 1988; Raghavan et al., 2000a). Simonelli et al. (1970) proposed that the polymer forms a net-like structure around the growing crystal and allows the drug to grow out in finger like protrusions. According to Ziller and Rupprecht (1988), the polymer occupies the adsorption sites and forms a mechanical barrier against crystallisation thus inhibiting growth. More recently, Raghavan et al. (2000b) proposed a model in which the growth inhibition occurs by adsorption of polymer on the crystal surface through hydrogen bonding as well as by the hydrodynamic boundary layer surrounding the growing crystal. The carboxylic acid group of IBU has hydrogen bonding functional groups, which can interact with the hydrogen bonding functional groups of the polymer and block the growth sites (Fig. 7). The polymer molecules cannot however become included into the crystal due to their incompatibility in size. This was confirmed in the X-ray crystallographic studies where the crystal structure was determined for crystals grown without and in the presence of HPMC. If the HPMC was incorporated into the crystal, it would significantly distort the crystal lattice and alter the lattice parameters. The lattice parameters were quite similar in both cases confirming the absence of significant amounts of HPMC.

The interactions between IBU and HPMC were examined using IR spectroscopy. IR spectra were recorded on the IBU-HPMC solid dispersion and on the pure components. Fig. 8 shows the IR spectra of pure IBU and IBU-HPMC solid disper-

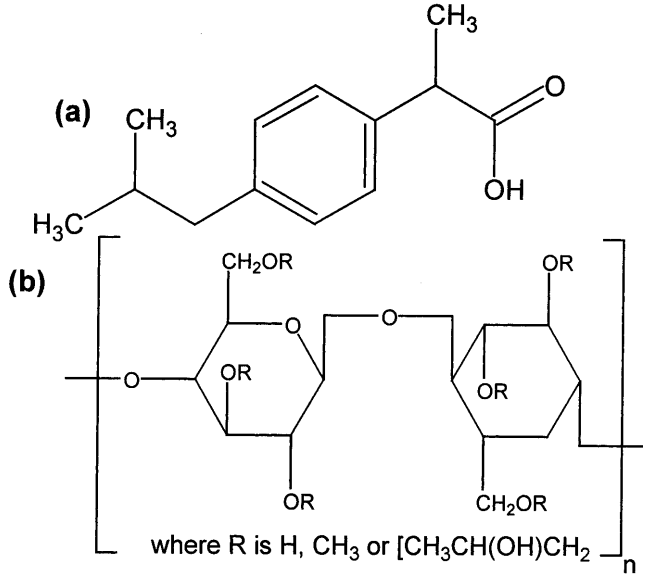

Fig. 7. Molecular structures of (a) ibuprofen (IBU) and (b) hydroxypropyl methylcellulose (HPMC).

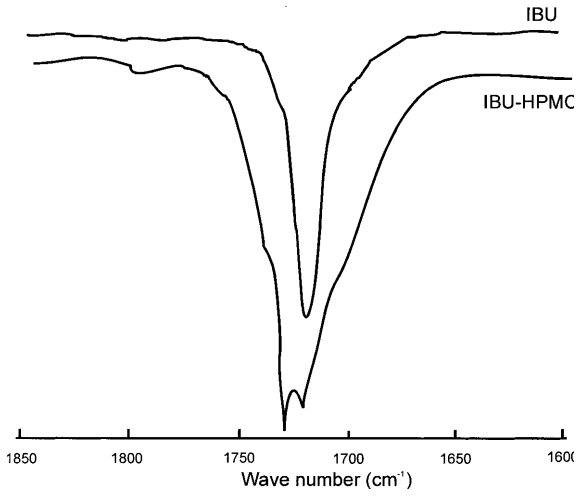

Fig. 8. IR spectra of pure ibuprofen (IBU) and IBU-hydroxypropyl methylcellulose (HPMC) solid dispersion in the C=O stretching region.

sion in the C=O stretching region of IBU. HPMC does not have any absorption in this region. The characteristic C=O band of IBU was observed at 1721 cm^{-1} as previously reported (Mura et al., 1998). For the solid dispersion, apart from the carbonyl peak of IBU, a new peak appeared at 1731 cm^{-1}. This suggests the formation of hydrogen bonds between IBU and HPMC by breaking part of the intermolecular hydrogen bonds of IBU crystals. A similar effect was observed with IBU and polyethylene glycol by Shakhtshneider et al. (1996).

Similar drug-polymer interactions are reported in literature. Taylor and Zografi (1997), for example, have studied the nature of interactions between indomethacin and polyvinyl pyrrolidone (PVP) using IR. They established the breaking of the indomethacin dimers by PVP and formation of an indomethacin-PVP hydrogen bond by examining the changes in the carboxyl band of the drug similar to the present study.

3.4.1. Morphology

The morphology or the habit of a crystal depends on the relative growth rates of the different crystallographic faces. According to Raghavan et al. (2000b), if the adsorption of the additives on the crystal surface is similar on all the faces, then the growth inhibition would be uniform and will not modify the morphology of the crystal. However, the adsorption is dependent on the hydrogen bonding functional groups that end at each crystal face, which in turn is dependent on the crystallographic structure of a given material.

From Fig. 6a and b, it can be seen that the morphology of the as grown crystals and crystals grown in the presence of CD are elongated needles. The morphology of IBU crystals has been reported in literature (Bunyan et al., 1991; Cano et al., 1999) but there is a disagreement on the assignment of the various $\{hkl\}$ values of the grown crystallographic faces between the two studies. The slowest growing faces determine the morphology of a crystal. These faces are referred to as the morphologically important faces (MI). For IBU, these faces have been assigned as (001), (100) and (110) by Bunyan et al. (1991) and as (100), (002) and (011) by Cano et al. (1999) in the order of morphological importance. The $\{hkl\}$ values are assigned on the basis of a reference system. The difference in the assignment of $\{hkl\}$ values reported arises due to the different reference systems chosen by the two groups.

IBU crystals grown in the presence of HPMC had an asymmetric prismatic morphology (Fig. 6c) compared to a symmetric thin needle-like morphology for the crystals grown without the additive. HPMC acts both as a growth inhibitor and a habit modifier, a feature earlier observed in the crystallisation of hydrocortisone acetate (Raghavan et al., 2000b). Observation of the morphology of the crystals shows that HPMC inhibits growth in the side faces. These side faces have hydrogen bonding functional groups of IBU capable of associating with the polymer molecules. In order to confirm the hydrogen bonding ability of these faces, one would have to consider the molecular structure of the various faces and determine the molecular groups that end at each face. This was not possible in view of the differences in the reported $\{hkl\}$ values. Detailed work on the identification of the crystallographic faces corresponding to the various habit faces is currently in progress.

4. Conclusions

Penetration enhancement of IBU from supersaturated solutions through human epidermis was investigated. In the absence of additives, the flux was lower than expected for DS 4 and 5. With HPMC the flux was proportional to the DS due to the stabilisation of the supersaturated solutions. With CD, the flux was lower than for solutions without any additive because of decrease in the thermodynamic activity, a consequence of the formation of IBU/CD inclusion complex. In general the flux of IBU through human skin was found to have a linear correlation with the flux through silicone membrane. Deviations from linearity were found to occur for systems at high DS without any additive. The anti-nucleating effect of HPMC is crucial for dermal delivery applications using supersaturated systems of IBU. The IBU crystals grown without any additive had a long needle like morphology, which did not change when grown in the presence of CD. Growth inhibition and habit modification were observed for IBU crystals grown in the presence of HPMC indicating the strong interaction between IBU and HPMC.

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References

Bunyan, J.M.E., Shankland, N., Sheen, D.B., 1991. Solvent effects on the morphology of ibuprofen. J. Am. Inst. Chem. Eng. Symp. Ser. 87, 44–57.

Cano, H., Costeseque, P., Gabas, N. and Canselier, J.P., 1999. Solvent effect on ibuprofen crystal morphology: experimental study and molecular modelling. Proc. 14th Int. Symp. Industrial Crystallisation, p. 36.

Chow, K.Y., Go, J., Mehdizadeh, M., Grant, D.J.W., 1984. Modification of adipic acid crystals: influence of growth in the presence of fatty acid additives on crystal properties. Int. J. Pharm. 20, 3–34.

Chow, A.H.-L., Chow, P.K.K., Zhongshan, W., Grant, D.J.W., 1985. Modification of acetaminophen crystals: influence of growth in aqueous solutions containing p-acetoxyacetanilide on crystal properties. Int. J. Pharm. 24, 239–258.

Davis, A.F., Hadgraft, J., 1991. Effect of supersaturation on membrane transport: 1. Hydrocortisone acetate. Int. J. Pharm. 76, 1–8.

Fairbrother, J.E., Grant, D.J.W., 1978. The crystal habit modification of a tablet lubricant, adipic acid. J. Pharm. Pharmacol. 30 (Suppl.), 19.

Fairbrother, J.E., Grant, D.J.W., 1979. Crystal engineering studies with an excipient material (adipic acid). J. Pharm. Pharmacol. 31 (Suppl.), 27.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instrum. 4, 117–212.

Iervolino, M., Raghavan, S.L., Hadgraft, J., 2000. Membrane penetration enhancement of ibuprofen using supersaturation. Int. J. Pharm. 198, 229–238.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88, 70–73.

Lipp, R., 1998. Selection and use of crystallisation inhibitors for matrix-type transdermal drug-delivery systems containing sex steroids. J. Pharm. Pharmacol. 50, 1343–1349.

Loftsson, T., Olafsdottir, B.J., Bodor, N., 1991. The effects of cyclodextrin on transdermal delivery of drugs. Eur. J. Pharm. Biopharm. 37 (1), 30–33.

Loftsson, T., Olafsdottir, B.J., Fridriksdottir, H., Jonsdottir, S., 1993. Cyclodextrin complexation of NSAIDs: physicochemical characteristics. Eur. J. Pharm. Sci. 1, 95–101.

Másson, M., Loftsson, T., Másson, G., Stefánsson, E., 1999. Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. J. Control. Release 59, 107–118.

Megrab, N.A., Williams, A.C., Barry, B.W., 1995. Oestradiol permeation through human skin and silastic membrane: effects of propylene glycol and supersaturation. J. Control. Release 36, 277–294.

Mullin, J.W., 1993. Crystallisation. Butterworth-Heinemann, Oxford.

Mura, P., Bettinetti, G.P., Manderioli, A., Faucci, M.T., Bramanti, G., Sorrenti, M., 1998. Interactions of ketoprofen and ibuprofen with β-cyclodextrins in solution and solid state. Int. J. Pharm. 166, 189–203.

Pellett, M.A., Davis, A.F., Hadgraft, J., 1994. Effect of supersaturation on membrane transport: 2. Piroxicam. Int. J. Pharm. 111, 1–6.

Pellett, M.A., Castellano, S., Hadgraft, J., Davis, A.F., 1997a. The penetration of supersaturated solutions of piroxicam across silicone membranes and human skin in vitro. J. Control. Release 46, 205–214.

Pellett, M.A., Davis, A.F., Hadgraft, J., 1997b. Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique. Int. J. Pharm. 151, 91–98.

Raghavan, S.L., Trividic, A., Davis, A.F., Hadgraft, J., 2000a. Influence of cellulose polymers on the supersaturation and permeation of hydrocortisone acetate. Int. J. Pharm. 193, 231–237.

Raghavan, S.L., Trividic, A., Davis, A.F., Hadgraft, J., 2000b Influence of polymers on the crystallisation of Hydrocortisone Acetate. Int. J. Pharm. (in press).

Schwarb, F.P., Imanidis, G., Smith, E.W., Haigh, J.M., Surber, C., 1999. Effect of concentration and saturation of topical Fluocinonide formulations on in vitro membrane transport and In vivo availability on human skin. Pharm. Res. 16, 917–923.

Shakhtshneider, T.P., Vasiltchenko, M.A., Politov, A.A., Boldyrev, V.V., 1996. The mechanochemical preparation of solid disperse systems of ibuprofen-polyethylene glycol. Int. J. Pharm. 130, 25–32.

Simonelli, A.P., Mehta, S.C., Higuchi, W.I., 1970. Inhibition of sulfathiazole crystal growth by polyvinylpyrrolidone. J. Pharm. Sci. (1970) 633–638.

Taylor, L.S., Zografi, G., 1997. Spectroscopic characterisation of interactions between PVP and indomethacin in amorphous molecular dispersion. Pharm. Res. 14, 1691–1698.

Torres-Labandeira, J.J., Davignon, P., Pitha, J., 1991. Oversaturated solutions of drug in hydroxypropylcyclodextrins-parenteral preparation of pancratistatin. J. Pharm. Sci. 80, 384–386.

Uekama, K., Ikegami, K., Wang, Z., Horiuchi, Y., Hirayama, F., 1992. Inhibitory effect of 2-hydroxypropyl-β-cyclodextrin on crystal growth of nifedipine during storage: superior dissolution and oral bioavailability compared with polyvinylpyrrolidone K-30. J. Pharm. Pharmacol. 44, 73–78.

Ziller, K.H., Rupprecht, H., 1988. Control of crystal growth in drug suspensions. Drug Dev. Ind. Pharm. 14, 2341–2370.