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International Journal of Pharmaceutics 219 (2001) 161–176

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Mechanistic study into the enhanced transdermal permeation of a model β-blocker, propranolol, by fatty acids: a melting point depression effect

Paul W. Stott [1], Adrian C. Williams, Brian W. Barry *

Drug Delivery Group, The School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK

Received 7 December 2000; received in revised form 28 February 2001; accepted 5 March 2001

Abstract

Transdermal permeation of propranolol through human skin in the presence of fatty acid (lauric, capric) penetration enhancers has been investigated. Thermal analysis showed that binary mixtures of propranolol with either fatty acid were not simple mechanical mixtures of the two components. Propranolol formed 1:1 molar addition compounds with both lauric and capric acids; the addition compound produced from propranolol and lauric acid (m.p. 79°C) also developed eutectic systems with both propranolol (m.p. 54°C) and lauric acid (m.p. 16°C). Similarly, the addition compound made from propranolol and capric acid (m.p. 97°C) formed eutectic systems with propranolol (m.p. 83°C) and capric acid (m.p. 15°C). Infrared analyses indicated that the addition compounds were fatty acid salts of the β-blocker. The nature of the species permeating through human epidermal membranes from binary mixtures of propranolol with the fatty acids was investigated using a novel attenuated total reflectance Fourier transform infrared method. There was no clear difference in permeation rates of the fatty acids compared with the β-blocker, suggesting that the permeating species was the intact addition compound. The influence of melting point depression of the β-blocker fatty acid systems on transdermal permeation was predicted from a mathematical model; predicted and experimentally determined data correlated well thus providing an alternative explanation as to the mode of action of these permeation enhancers. © 2001 Published by Elsevier Science B.V.

Keywords: Propranolol; Fatty acids; Attenuated total reflectance Fourier transform infrared; Transdermal permeation; Thermal analysis; Eutectic

1. Introduction

Beta-blockers undergo extensive hepatic first pass metabolism and bioavailability estimates as low as 30% have been obtained from oral formulations (Routledge and Shand, 1979). This drawback has led many workers to attempt to deliver β-blockers via the transdermal route (e.g. Touitou

* Corresponding author. Tel.: +44-1274-234760; fax: +44-1274-384769.

E-mail address: bwbarry@bradford.ac.uk (B.W. Barry).

[1] Present address: Bristol-Myers Squibb, Pharmaceutical Research Institute, Moreton, Wirral CH46 1QW, UK.

0378-5173/01/$ - see front matter © 2001 Published by Elsevier Science B.V.

PII: S0378-5173(01)00645-7

et al., 1994; Ahmed et al., 1995). The inherently poor permeability of the human skin (particularly the stratum corneum) has sometimes necessitated the incorporation of penetration enhancers into transdermal formulations and fatty acids have been frequently used for this purpose for many therapeutic classes of drugs, including β-blockers (Bennett and Barry, 1985; Aungst, 1989; Yamashita et al., 1995). Several modes of action of fatty acids in increasing the transdermal permeation of medicaments have been proposed including: increased drug solubility and partitioning (Aungst et al., 1986), disruption of the barrier function of the skin (Aungst et al., 1990), and ion-pair formation (Green and Hadgraft, 1987; Green et al., 1988). No single mechanism has been identified and it would appear that the skin penetration promoting effects of fatty acids are selective for individual drugs and application conditions. For example, oleic acid was shown to increase the permeation of salicylic acid (Cooper, 1984), acyclovir (Cooper et al., 1985) and hydrocortisone (Bennett and Barry, 1985); however, neither lauric acid nor capric acid had any effect on the permeation of salicylic acid (Cooper, 1984) but both were shown to increase the permeation of naloxone (Aungst et al., 1986).

Green and Hadgraft (1987) studied the permeation of four β-blockers across an artificial lipid membrane. A facilitated transport mechanism was proposed when oleic acid and lauric acid were incorporated into the membrane, along with an appropriate pH gradient. The diffusion of the β-blockers was said to be enhanced by an ion-pair mechanism in the presence of the fatty acids. Ogiso and Shintani (1990) investigated the effects of a series of fatty acids on the permeation of propranolol through rabbit skin from a gel formulation. The C_{12} (lauric acid) and the C_{14} (myristic acid) fatty acids produced the largest increases in drug flux. These workers proposed the formation of a 1:1 molar complex ''probably by charge interaction'' between the carbonyl group of the fatty acid and the amino and hydroxyl groups of the β-blocker. The 1:1 molar mixture increased the octanol/water partition coefficient compared to propranolol alone and this was proposed as the mechanism of enhancement. The same explanation was suggested by Ogiso et al. (1991) for the enhanced rectal absorption of propranolol by lauric acid.

Similarly, Elyan et al. (1996) studied the effects of the fatty acids capric, lauric, and myristic acid on the percutaneous absorption of the sympathomimetic agent, metaproterenol sulphate, from a formulation of propylene glycol (40%), ethanol (10%) and water (50%). Metaproterenol sulphate has a similar structure to the β-blockers and both possess an amino group. ^{13}C nuclear magnetic resonance spectroscopy revealed an interaction between the NH group of metaproterenol sulphate and the COOH group of the fatty acids. This interaction increased the lipophilicity and was proposed as a mechanism for enhancing transdermal permeation.

Both the report by Ogiso and Shintani (1990) and that by Elyan et al. (1996) indicate that the C_{12} fatty acid, lauric acid, has the optimum chain length for permeation enhancement. Ogiso and Shintani (1990) suggest that the C_{12} and C_{14} hydrophobic groups have an optimal balance of partition coefficient and affinity for the skin. That is, the short chain fatty acids have insufficient lipophilicity for skin penetration, while long chain fatty acids have a much higher affinity to lipids in the stratum corneum, thereby retarding the penetration of propranolol by hydrophobic interaction.

In light of these reports this paper details an investigation by thermal analysis and Fourier transform infrared (FTIR) into the solid–liquid binary equilibria of the β-blocker, propranolol, with the fatty acids lauric and capric acid and relates the features of the temperature/composition phase diagram to the transdermal permeation characteristics. Also, results of thermal analysis of 1:1 molar mixtures of propranolol with a homologous series of fatty acids from C_6 to C_{18} are presented and these data are used along with predictive models to explain the effects of fatty acid chain length on transdermal permeation of propranolol. FTIR is used to clarify the nature of the complex formed between propranolol and the fatty acids. The nature of the penetrating species from the 1:1 molar mixture is also studied using a novel attenuated total reflectance (ATR) FTIR technique.

2. Materials and methods

Propranolol HCl (purity 99%) was obtained from Sigma Chemical Co. Ltd. Dorset, England and from this propranolol base was extracted (Section 2). A homologous series of saturated fatty acids with the general formula $C_nH_{2n}O_2$ from $n=6$ to $n=18$, and the mono-unsaturated fatty acid oleic acid ($C_{18,1}$) were also obtained from Sigma. The structures of these compounds are shown in Fig. 1.

2.1. Propranolol base extraction method

Approximately 4 g samples of propranolol HCl were dissolved in 200 ml of distilled water to which an excess of a 0.5 M aqueous solution of sodium hydroxide (purity 99.99%, Aldrich) was added to precipitate the base. The precipitate was filtered off and dissolved in chloroform. The chloroform solution was then washed three times with distilled water and the organic layer collected. The chloroform was removed under vacuum to leave propranolol base. The purity of the base was confirmed in each case by differential scanning calorimetry (DSC) since the base has a melting point of 96°C and the hydrochloride melts between 163 and 164°C.

2.2. Preparation of propranolol:fatty acid binary mixtures

Mixtures of propranolol and each fatty acid, accurately weighed into glass vials, were dissolved, by sonication, in chloroform, to allow intimate mixing of the compounds at a molecular level. The chloroform was removed by evaporation under reduced pressure, at room temperature. The resultant binary systems were frozen at −20°C for at least 48 h to crystallize and equilibrate and stored at −20°C until required.

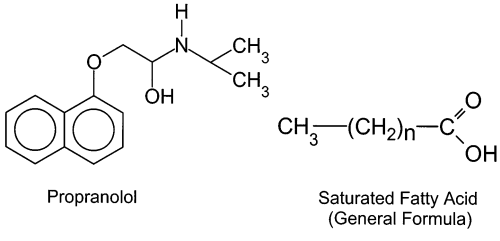

Fig. 1. Structure of propranolol and the general formula for the saturated fatty acids tested as adjuvants to form eutectic systems.

2.3. Differential scanning calorimetry

DSC of the binary mixtures was performed in triplicate on a Perkin–Elmer Series 7 Thermal Analyser System in sealed stainless steel pans under a stream of anhydrous nitrogen gas. The sealed pans prevent any evaporative loses and so composition remains constant, but this means that pressure is an unknown variable. However, pressure has little or no effect on solid–liquid transitions (Mullin, 1993) and so the pressure variable was neglected.

DSC was performed against a reference pan between −30 and 150°C at a heating rate of 1°C/min. The procedure for obtaining transition temperatures from DSC traces has been detailed in the paper by Stott et al. (1998). Briefly, the solidus temperature of the temperature/composition phase diagram, i.e. the temperature below which the system is completely solid, is taken as the onset of the first melting endotherm. The liquidus temperature, above which the system exists as a homogenous liquid, is taken as the peak of the secondary endotherm. Points on a temperature/composition phase diagram enclosed by the solidus and liquidus lines represent two phase systems with the component in excess as a solid in equilibrium with an homogenous liquid mixture.

2.4. ATR FTIR spectroscopy of the propranolol:fatty acid binary mixtures

FTIR analysis of the propranolol:fatty acid binary mixtures were performed on a Matteson Galaxy 6020 Series FTIR spectrometer by ATR using a zinc selenide crystal. Propranolol:fatty acid binary mixtures were dissolved in acetone and added dropwise onto the crystal. Evaporation of the acetone deposited a uniform film onto the crystal, which could then be analysed. This technique allowed analysis of the binary mixtures in their pure state without dilution in a third compo-

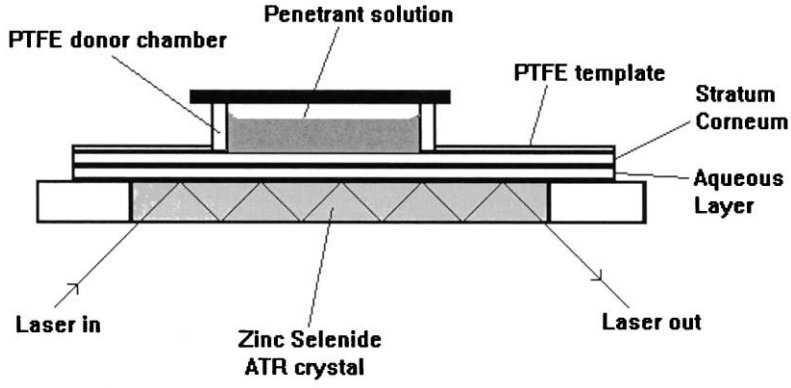

Fig. 2. Schematic representation of the ATR FTIR set-up used for monitoring transdermal permeation of propranolol:fatty acid binary mixtures.

nent, such as KBr, which may have affected the equilibrium of the system.

2.5. Propranolol:fatty acid permeation studies using a novel FTIR technique to determine the nature of the penetrating species

Propranolol:fatty acid mixtures have been used as a means of enhancing the permeation of the drug across skin. However, the nature of the penetrating species is unknown and this was investigated by ATR FTIR. The method used was an adaptation of a technique used by Fieldson and Barbari (1993), Wurster et al. (1993), Farinas et al. (1994) and Buraphacheep et al. (1994) to study penetrant diffusion in polymers and developed by Watkinson et al. (1995) to investigate permeation across the skin. This technique uses ATR FTIR to monitor the appearance of a permeating species on the distal side of a membrane in relation to time by selecting a permeant with an absorbing band in the IR transparent region of the membrane, namely 1800–2500 cm^{-1}. The limitation of this technique is that few drugs absorb in this region and model permeants must be utilised. The method developed in this work uses the same principles as the above technique but instead of the membrane being in direct contact with the ATR crystal, it is floated onto a thin water layer which acts as a reservoir. The set-up is shown in Fig. 2. Since water possesses only two main absorbing regions it is possible to detect a wide range of penetrating species. It should be noted that this technique was used qualitatively to determine the state of the penetrating species and, as yet, has not been developed to provide quantitative flux measurements.

The penetration of the propranolol:lauric acid 1:1 binary mixture and of the individual components was monitored by placing the donor solution in the sealed reservoir and taking scans of the aqueous layer at 30 min intervals thereafter.

3. Results and discussion

3.1. Differential scanning calorimetry of propranolol:fatty acid binary systems

Sample DSC traces for the propranolol:lauric acid binary systems are shown in Fig. 3 and it is evident that these systems do not follow the pattern displayed by a simple eutectic. The trace for pro:lauric 56:44 (% w/w) which represents a 1:1 molar mixture produces a single sharp endotherm indicative of a single compound. Compositions both above and below this produce the double endotherms characteristic of a eutectic mixture. Construction of the phase diagram (Fig. 4) confirms that propranolol and lauric acid interact to form a 1:1 molar addition compound surrounded on either side by eutectic systems, both of which display a degree of solid solubility at the composition extremes. Solid solubility is indicated by bi-

nary mixtures, which, although they produce a depressed melting point compared to the individual components, give only a single broad melting endotherm. This is unlike a simple eutectic system which produces double melting endotherms indicative of the liquidus and solidus melting points (Stott et al., 1998). The lauric acid addition compound system has a eutectic melting point of 15.9°C at a composition of 30% (w/w) propranolol. The addition compound propranolol binary system has a eutectic melting point of 54.1°C at a composition of 72% (w/w) propranolol. The melting characteristics of the propranolol:lauric acid and propranolol:capric acid binary systems are summarised in Table 1.

The 1:1 addition compound was found to have a melting point of 79°C and an enthalpy of fusion of 85.9 J/g compared to propranolol base with a melting point of 91.7°C and an enthalpy of fusion of 149.4 J/g. As has been shown previously (Touitou et al., 1994), the melting characteristics of a drug can have a profound effect on its permeation across a membrane and these data will be considered in later sections.

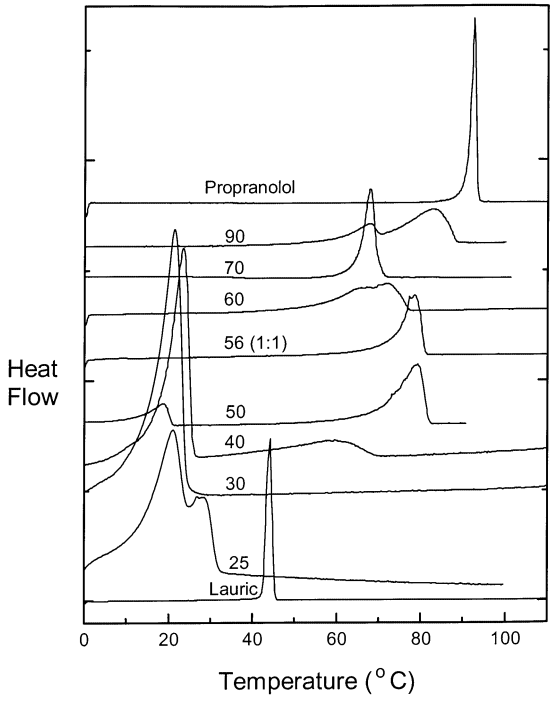

Fig. 3. Sample DSC traces of propranolol:lauric acid binary mixtures. The labels represent the percentage (w/w) of propranolol in each binary mixture. Fifty-six percent (w/w) propranolol is the equimolar mixture.

The propranolol:capric acid binary system was found to be similar to the propranolol:lauric acid system with the 1:1 molar mixture (60% propranolol by weight) producing a single sharp endotherm in DSC analysis (Fig. 5). The phase diagram in Fig. 6 shows two simple eutectic systems either side of the propranolol:capric acid 1:1 addition compound. In this case there is less evidence of solid solubility, since each composition tested produced double endotherms, although the existence of some degree of solid solubility at very low concentrations of each component cannot be dismissed. The capric acid addition compound eutectic has a melting point of 15.4°C and a composition of 25% (w/w) propranolol. The propranolol addition compound eutectic has a melting point of 82.8°C and a composition of 85% (w/w) propranolol.

The propranolol:capric acid 1:1 addition compound has a melting point of 97.1°C, which is an increase in melting point over propranolol base, and has an enthalpy of fusion of 104.8 J/g.

This type of interaction between binary mixtures of organic compounds is not rare and in a review of the literature regarding solid–liquid binary equilibria, Matsuoka (1991) suggested that up to 25% of binary systems produced this type of interaction compared to up to 55% which form the simple eutectic of the type produced by ibuprofen:terpene eutectic systems (Stott et al., 1998).

Rai and George (1992) and Rai and George (1994) described the solid/liquid phase equilibria of benzidine with α-napthol, *p*-nitrophenol, *m*-aminophenol and resorcinol. The phase diagrams of each system exhibited a double simple eutectic and showed the formation of one addition compound. These systems are similar to the propranolol:fatty acid system in that one component contains a hydroxyl group and the other has an amino group allowing possible association by hydrogen bonding.

Other examples of binary systems which produce stable complexes in stochiometric propor-

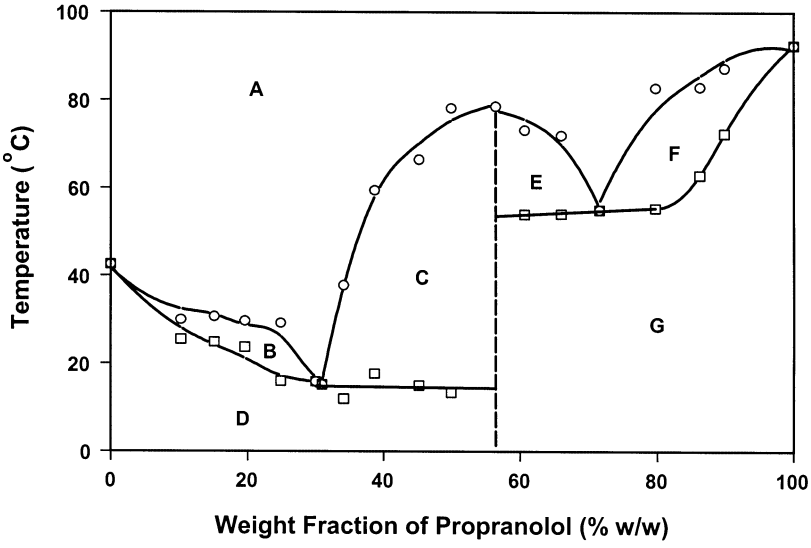

Fig. 4. The temperature/composition phase diagram of the propranolol:lauric acid binary mixture showing the formation of a new 1:1 (mol:mol) addition compound (add. comp.), as determined by DSC. The dashed line represents the 1:1 molar addition compound. A – homogenous liquid, B – liquid + solid lauric acid, C – liquid + solid add. comp., D – solid add. comp. + lauric acid, E – liquid + solid add. comp., F – liquid + solid propranolol, G – solid add. comp. + propranolol.

tions include *p*-toluidine:phenol and polyethylene oxide:*p*-nitrophenol (Point and Damman, 1992) which both form 1:1 intermolecular compounds, and griseofulvin (G):phenobarbitone (P) which forms two complexes of the formulae PG_3 and P_3G (Grant and Abougela, 1982). More complicated systems can arise when considering the binary interactions of compounds with water, for example, the sulphuric acid:water system produces three intermolecular complexes, the hydrate, the dihydrate, and the trihydrate, revealing four separate eutectic points on the temperature/composition phase diagram (Walas, 1984).

3.2. The mode of interaction of propranolol:fatty acid mixtures determined from heat of fusion data of the eutectics and addition compounds

Rai and Shekhar (1991) and Rai and George (1992) used heat of fusion data on pure compounds, eutectics and molecular complexes of binary organic systems to gain information on the nature of the interaction. If a eutectic is a simple mechanical mixture of the two components involving no heat of mixing or any type of association in the melt, the heat of fusion can simply be given by the mixture law (Rai et al., 1983; Eq. (1))

$$\Delta Hf_{\mathrm{m}} = x_1 \Delta Hf_1 + x_2 \Delta Hf_2, \quad (1)$$

where ΔHf_{m} is the enthalpy of fusion of the mixture and x and ΔHf are the mole fraction and heat of fusion, respectively, of the component given by the subscript. Table 2 presents the experimental and calculated heat of fusion data for the propranolol:lauric acid and propranolol:capric acid systems. The experimentally determined enthalpy of fusion data for the addition compounds and the eutectics are lower than the corresponding values calculated using the mixture law (Eq. (1)). It can be inferred from the values of enthalpy of fusion given in Table 2 that the propranolol:fatty acid systems are not simple mechanical mixtures of the two components. The binary mixtures clearly form equimolar addition compounds surrounded by two eutectics (Figs. 4 and 5). The violation of the mixture law in both systems would suggest that the eutectics are formed due to an associative interaction between the addition compound and each of the pure compounds, possibly by hydrogen bonding.

Table 1
Melting characteristics of the propranolol (Pro):lauric acid and propranolol:capric acid binary systems from thermal analysis data

	Pro:Lauric		Pro:Capric	
	Composition % (w/w) pro	Melting point (°C)	Composition % (w/w) pro	Melting point (°C)
Propranolol alone		91.7		91.7
Fatty acid alone		44.2		31.4
[a]Eutectic 1	30	15.9	35	15.4
Addition compound	54	79.0	60	97.1
[b]Eutectic 2	72	54.1	85	82.8

[a] The eutectic formed between the fatty acid and the addition compound.
[b] The eutectic formed between the addition compound and propranolol.

The heat of mixing (ΔH_{mix}), which is the difference between the experimental and calculated values of heat of fusion, is given by (Rai and Shekhar, 1991):

$$\Delta H_{mix} = \Delta Hf_{exp} - \Delta Hf_{calc}, \quad (2)$$

where ΔHf_{exp} and ΔHf_{calc} are the experimentally determined and calculated heats of fusion, respectively. It is evident from Table 2 that the heats of mixing of the propranolol:fatty acid eutectics and addition compounds are all negative. Thermochemical studies (Singh et al., 1985) suggest that the structure of a eutectic melt depends on the sign of the enthalpy of mixing. Three types of structure are proposed: quasi-eutectic for $\Delta H_{mix} > 0$, clustering of molecules for $\Delta H_{mix} < 0$, and a molecular solution for $\Delta H_{mix} = 0$. The large negative values of ΔH_{mix} suggest clustering of molecules in the propranolol:fatty acid systems.

Rai and George (1992) proposed a similar mechanism for the benzidine:α-napthol system with the formation of clusters in the eutectic melt, since the heat liberated during cluster formation (i.e. the heat of mixing) may lower the actual value of the heat of fusion. It is probable that the same mechanisms occur in the propranolol:fatty acid systems as in the benzidine:α-napthol system since both have the same potential for intermolecular hydrogen bonding. The mode of interaction of these systems is investigated further by spectroscopic techniques in subsequent sections.

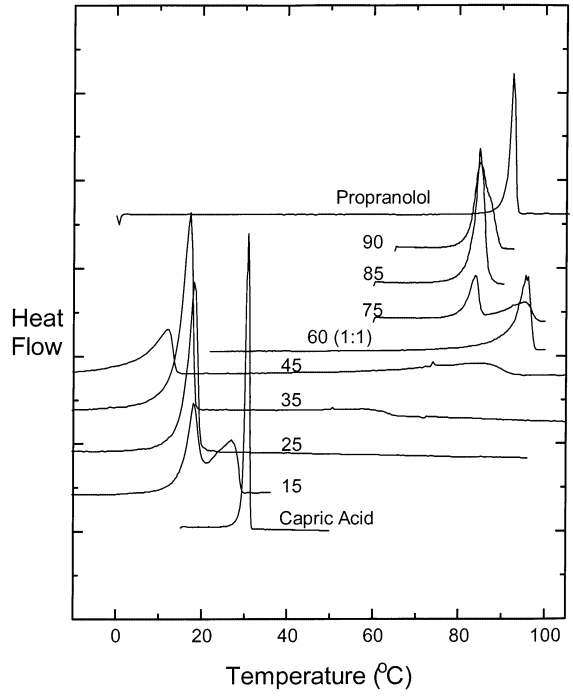

Fig. 5. Sample DSC traces of propranolol:capric acid binary mixtures. The labels represent the percentage (w/w) of propranolol in each binary mixture. Sixty percent (w/w) propranolol is the equimolar mixture.

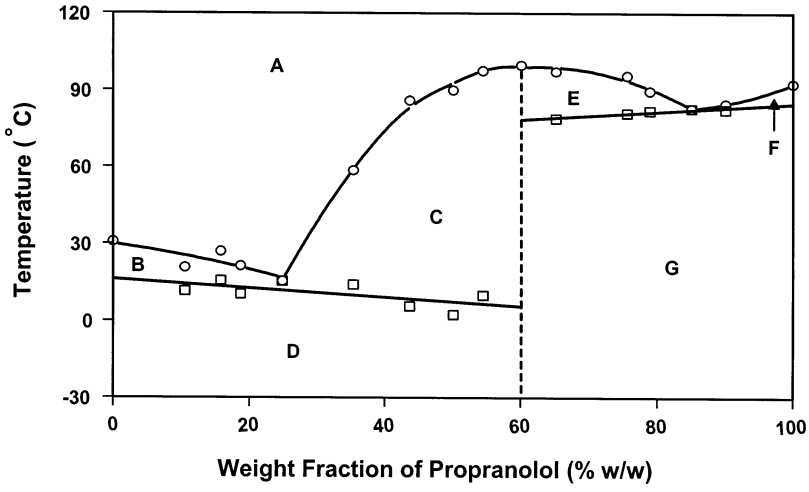

Fig. 6. The temperature/composition phase diagram of the propranolol:capric acid binary mixture showing the formation of a new 1:1 (mol:mol) addition compound (add. comp.), as determined by DSC. The dashed line represents the 1:1 molar addition compound. A – homogenous liquid, B – liquid + solid capric acid, C – liquid + solid add. comp., D – solid add. comp. + capric acid, E – liquid + solid add. comp., F – liquid + solid propranolol, G – solid add. comp. + propranolol.

3.3. Differential scanning calorimetry of propranolol:fatty acid 1:1 addition compounds

The phase diagrams of the propranolol:lauric and propranolol:capric acid binary mixtures both indicated the formation of an addition compound at equimolar compositions, and so one may reasonably expect other fatty acids to interact in the same way. Therefore, equimolar binary mixtures of propranolol with a homologous series (C_6 to C_{18}) of fatty acids, formed by dissolution in chloroform followed by evaporation of the solvent, were analysed by DSC. The DSC traces for each addition compound (propranolol caproate to propranolol stearate) are shown in Fig. 7. Each equimolar mixture produced a single, sharp endothermic peak indicative of a single compound. The chain length of the fatty acid affected both the melting point and the enthalpy of fusion of the resultant compound and these data for each system are represented in Table 3. It has previously been shown that melting point and enthalpy of fusion of a drug play a major role in determining drug flux (Touitou et al., 1994) and the data generated by DSC have been used to predict which propranolol:fatty acid addition compound would give the highest transdermal flux.

3.4. ATR FTIR spectroscopy of the propranolol:fatty acid binary mixtures

The propranolol:lauric acid and propranolol:capric acid binary mixtures were analysed

Table 2
Experimental and calculated heat of fusion data for the propranolol:lauric acid and propranolol:capric acid binary systems

Compound	Enthalpy of fusion (kJ/mol)	
	Pro:Lauric	Pro:Capric
Propranolol alone	38.7	38.7
Fatty acid alone	35.4	26.9
Eutectic 1		
Experimental	15.8	22.4
Calculated	36.4	29.9
Heat of mixing (kJ/mol)	−20.6	−7.5
Eutectic 2		
Experimental	23.0	28.3
Calculated	37.7	36.9
Heat of mixing (kJ/mol)	−14.7	−8.6
Addition compound		
Experimental	19.7	22.6
Calculated	37.1	32.8
Heat of mixing (kJ/mol)	−17.4	−10.2

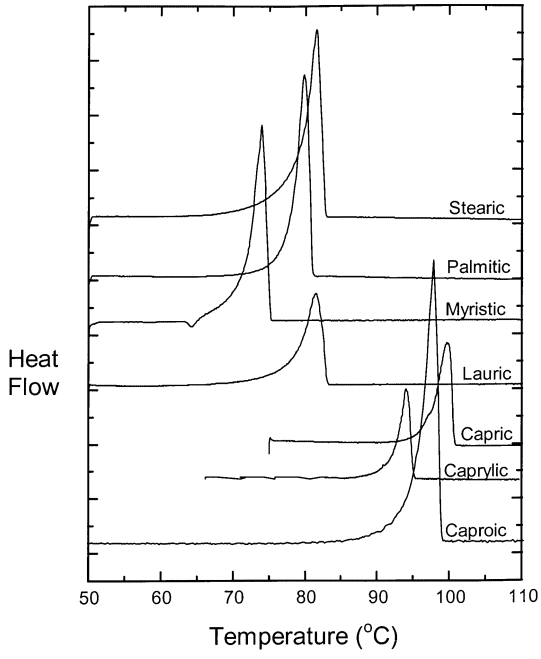

Fig. 7. DSC traces of equimolar binary mixtures of propranolol with a homologous series of fatty acids.

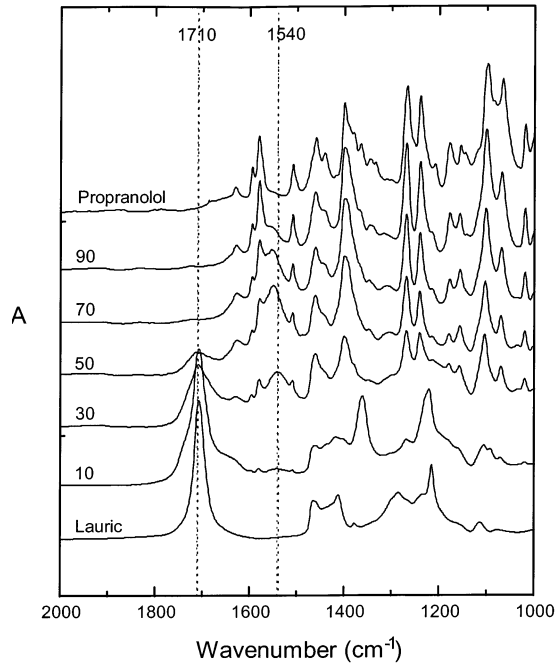

Fig. 8. Sample FTIR traces of propranolol:lauric acid binary mixtures taken at room temperature. The figures indicate the composition (% w/w) of propranolol.

by FTIR spectroscopy using an ATR module. Figs. 8 and 9 show sample spectra for the propranolol:lauric acid and propranolol:capric acid binary mixtures, respectively. Both lauric and capric acid produce a strong absorbance peak around 1710 cm^{-1} corresponding to the C=O antisymmetrical stretching of a hydrogen bonded carboxylic acid (Colthup et al., 1990). Upon addition of increasing amounts of propranolol to either fatty acid the intensity of this peak decreases and it is completely absent at compositions of greater than equimolar amounts of propranolol (54% (w/w) for pro:lauric and 60% (w/w) for pro:capric).

Propranolol produces a sharp peak at 1590 cm^{-1} due to N–H stretching of the amino group.

Table 3
Melt characteristics of equimolar binary mixtures of propranolol with a homologous series of fatty acids determined from thermal analysis data

Fatty acid	Carbon number	Molecular weight[a]	Melting point (°C)	ΔHf^{b} (J/g)	ΔHf^{b} (kJ/mol)
Caproic	6	375.5	95.5	122.2	45.9
Caprylic	8	403.5	92.8	119.6	48.2
Capric	10	431.6	97.1	104.8	45.2
Lauric	12	459.6	79.0	85.9	39.4
Myristic	14	487.7	71.7	97.0	47.2
Palmitic	16	515.7	77.9	117.0	60.3
Stearic	18	543.8	79.1	113.1	61.4

[a] The combined molecular weights of propranolol and each fatty acid, i.e. assuming no losses on formation of the addition compound.
[b] The enthalpy of fusion.

Again, upon addition of increasing amounts of either fatty acid the intensity of the peak due to the amino group decreases and is absent at less than equimolar compositions of propranolol. The peak due to the C=O group of the carboxylic acid (1710 cm^{-1}) and that due to the N–H group of propranolol (1590 cm^{-1}) are replaced by a broad peak at 1540 cm^{-1}. These findings suggest that an interaction takes place between the carbonyl group of the fatty acids and the amino group of the β-blocker.

The plots in Fig. 10 show the effects of composition of the binary mixtures on the normalised peak area of the 1710 and 1540 cm^{-1} peaks for the pro:lauric and pro:capric systems, respectively. The 1710 cm^{-1} peak was normalised against the peak at 2850 cm^{-1}, and the peak at 1540 cm^{-1} was normalised against the peak at 1020 cm^{-1}. In each case the maximum area of the peak at 1540 cm^{-1} is obtained from an equimolar composition. The area of the carboxylic acid peak (1710 cm^{-1}) decreases as the composition of propranolol increases and approaches zero at a 1:1 molar ratio.

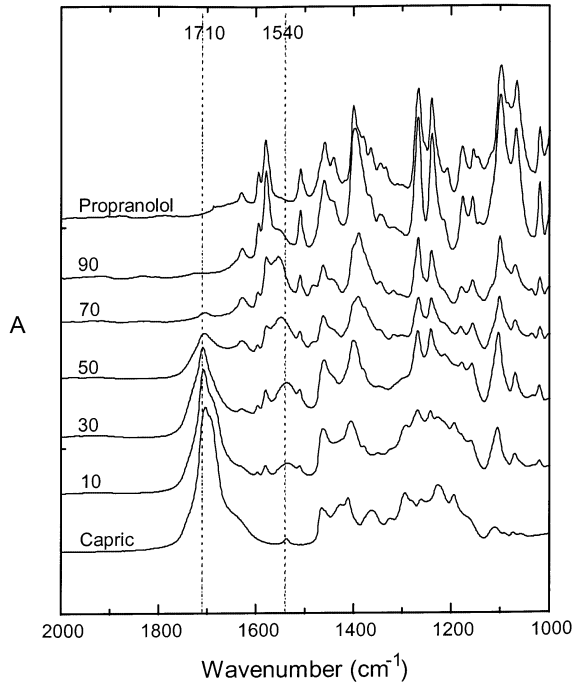

Fig. 9. Sample FTIR traces of propranolol:capric acid binary mixtures taken at room temperature. The figures indicate the composition (% w/w) of propranolol.

The fatty acids exist, in the condensed state, as hydrogen bonded dimers which give rise to the carbonyl-stretching mode at 1710 cm^{-1}. The structure of the dimers is given in Fig. 11(a) (the dashed lines represent hydrogen bonds).

The FTIR traces of the propranolol:fatty acid binary mixtures indicate that the addition of propranolol disrupts the fatty acid dimers and the dimerised species does not exist in greater than equimolar compositions of propranolol. The peak at 1710 cm^{-1} is replaced by a relatively broad peak at 1540 cm^{-1}. The 1:1 molar complex could possibly be formed by a hydrogen bonding interaction between the carboxylic acid group of the fatty acid and the hydroxyl and amino groups of the β-blocker to produce the structure represented in Fig. 11(b).

This structure would explain the loss of the peak due to the dimerised carboxylic acid but appears to be sterically less favourable than the dimerised form. An alternative explanation would be the formation of a salt by protonation of the amine group on propranolol from the hydroxyl of the fatty acid (Fig. 11(c)).

In order to confirm the presence of the salt form it was necessary to assign the absorbance peak found at 1540 cm^{-1} in the equimolar binary mixtures. Fig. 12 shows the FTIR trace of the sodium salt of lauric acid (sodium laurate) analysed under the same conditions as the binary mixtures. The sodium salt contains the COO^- group and as can be seen from the trace produces no absorbance at 1710 cm^{-1} but gives a strong absorbance at 1540 cm^{-1}. This confirms the existence of a COO^- group in the addition compound and strongly suggests that the addition compound is the fatty acid salt of propranolol (propranolol laurate and propranolol caprate) formed by an ionic interaction, and not the hydrogen bonded structure.

3.5. Determination of the permeating species from a propranolol:lauric acid binary mixture using a novel ATR FTIR technique

FTIR analysis of the binary propranolol:fatty acid mixtures has indicated the formation of a salt between the two species. These would exist independently, in the ionised form, in aqueous solution

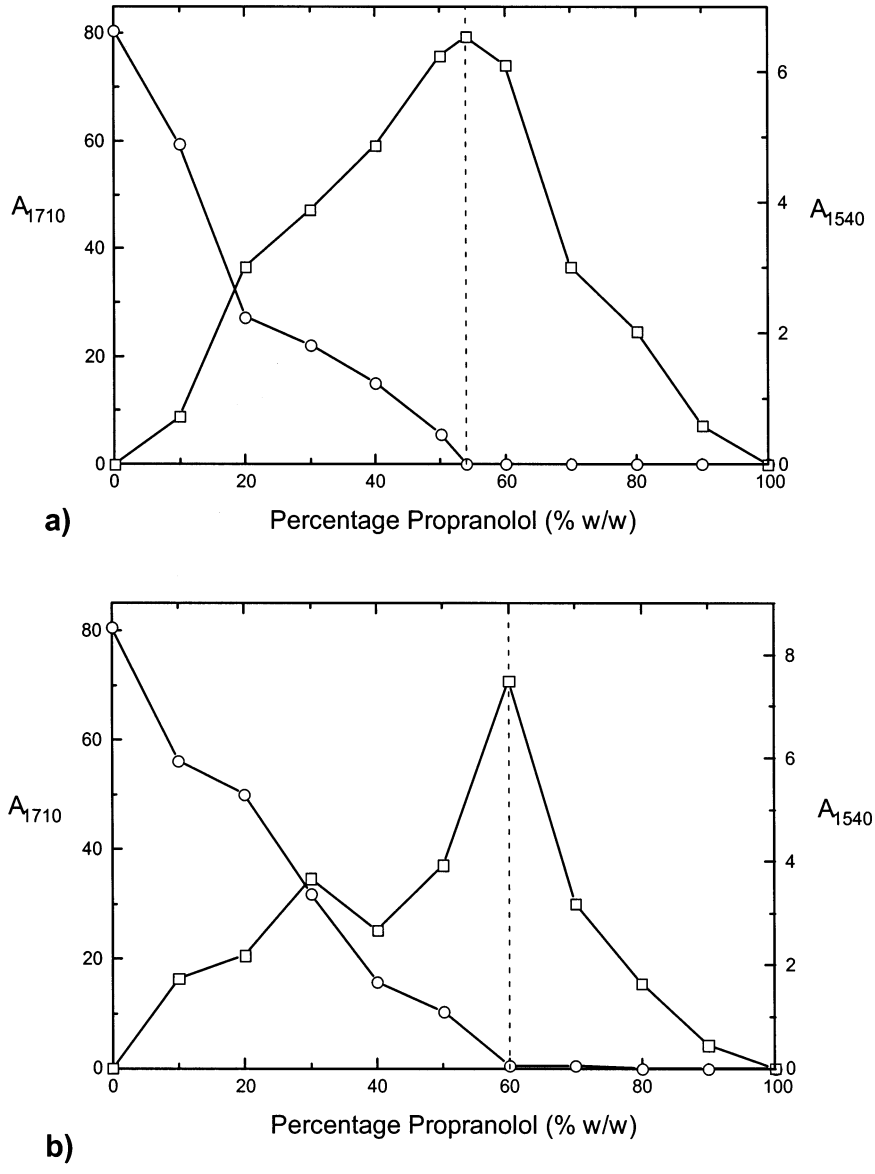

Fig. 10. Plots of normalised peak area for the carboxylic acid peak (circle; 1710 cm^{-1}) and the peak at 1540 cm^{-1} (square) versus composition for (a) the propranolol:lauric acid, and (b) the propranolol:capric acid binary systems. The vertical lines represent the equimolar compositions.

but may associate to permeate through the lipid regions of the stratum corneum, i.e. by an ion-pair mechanism. If the two species permeate by this mechanism one would expect them to produce approximately equimolar flux values across the membrane. Ogiso and Shintani (1990) showed that the flux of lauric acid through rabbit skin was approximately twice that of propranolol when applied individually from the same formulation. Therefore, if the equimolar addition compound was applied to the skin and the ion-pairing mechanism did not take place one would expect the

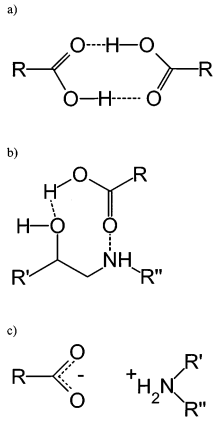

Fig. 11. Representation of the possible interactions in propranolol and the fatty acids, (a) hydrogen bonded fatty acid dimers, (b) hydrogen bonded fatty acid:propranolol complex, (c) propranolol; fatty acid salt.

individual components to appear at the opposite side of the membrane in an approximately 2:1 lauric acid:propranolol molar ratio. The excess fatty acid in the receptor, not involved in the equimolar interaction, would then form hydrogen bonded dimers, which would absorb at 1710 cm^{-1} in the IR spectrum. If, however, the species permeate as an ion-pair there would be no excess of the fatty acid and so the complex in the receptor solution would only absorb at 1540 cm^{-1}.

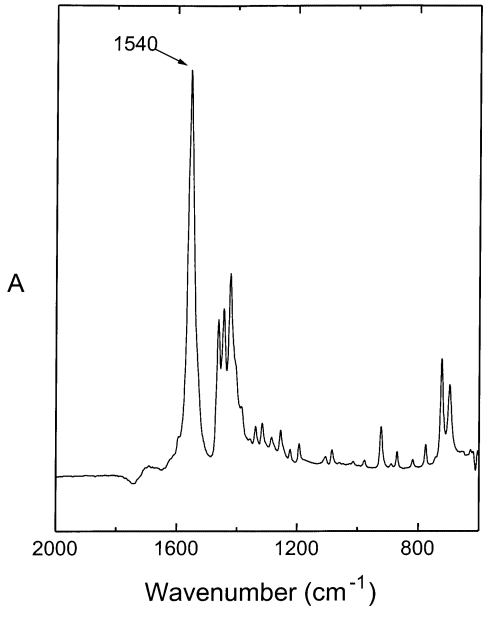

Fig. 12. FTIR trace of sodium laurate solid taken at room temperature on an ATR module. The peak at 1540 cm^{-1} is assigned to the COO^- group of the fatty acid salt (A – absorbance).

The mode of action by which fatty acids aid the permeation of the β-blocker, propranolol, was investigated by monitoring the appearance of the permeant through a barrier by ATR FTIR. The set-up of the membrane and the ATR crystal is shown in Fig. 2. The method has been used qualitatively here to monitor the permeating species from a propranolol:lauric acid 1:1 addition compound. Subsequent work has developed this technique further to provide quantitative data for the diffusion of a number of permeation enhancers (Edge et al., 1996).

To establish if one could differentiate between the dimerised carboxylic acid and the COO^- group of the propranolol laurate salt it was first necessary to characterise the permeation of lauric acid alone. A saturated solution of lauric acid in propylene glycol was applied to the stratum corneum of human epidermal membrane and the appearance of the permeant in the aqueous receptor phase was monitored by FTIR. The traces in Fig. 13 clearly show the appearance of an absorption peak at 1710 cm^{-1}, characteristic of the dimerised carboxylic acid groups of the fatty acid. Although there is some overlap between the carbonyl peak and that from the water, one can clearly see that the relative intensity of the peak at 1710 cm^{-1} increases with time.

The propranolol:lauric acid 1:1 molar addition compound does not possess the peak at 1710 cm^{-1} due to the hydrogen bonded dimers, and this is replaced by the absorbance peak at 1540 cm^{-1} due to the COO^- group of the salt. Compositions with excess lauric acid, i.e. greater than equimolar amounts of the fatty acid, produce absorbance peaks at both 1540 and 1710 cm^{-1}. Fig. 14 shows the FTIR scans of the aqueous receptor following the permeation of the addition compound across human epidermal membrane from a saturated solution in propylene glycol. The intensity of the COO^- peak increases with time and there is no evidence of absorption due to excess dimerised lauric acid at 1710 cm^{-1}. This indicates that the

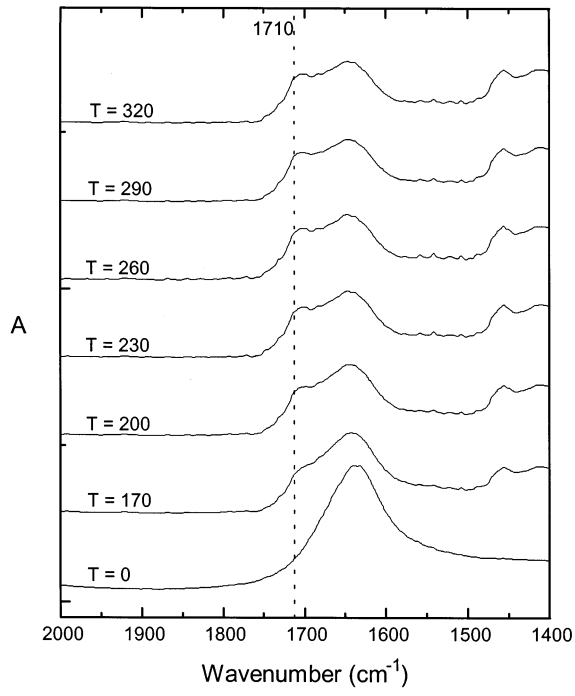

Fig. 13. ATR FTIR spectra of the aqueous receptor phase showing increasing intensity of the lauric acid COOH group (at 1710 cm^{-1}) with time after permeation across human epidermal membrane from a saturated solution in propylene glycol. The time after application of the donor for each scan is given in minutes.

lauric acid does not permeate at a faster rate than propranolol when applied to the membrane together, in an equimolar ratio. This would be consistent with the two species permeating via an ion-pairing mechanism.

3.6. Prediction of propranolol permeation from binary mixtures using mathematical models incorporating melting point and heat of fusion data

Ogiso and Shintani (1990) presented a study of the enhancing effects of a homologous series of fatty acids towards propranolol and found the C_{12} (lauric acid) and the C_{14} (myristic acid) to have the optimum chain lengths. The reason for this finding was proposed as the C_{12} and C_{14} hydrophobic regions had an optimum balance of partition coefficient and affinity to the skin. The shorter chain fatty acids were said to have an insufficiently high lipophilicity to partition adequately into the skin, whilst the longer chain fatty acids were considered to have a much higher affinity for the lipids in the stratum corneum, thereby retarding the penetration of propranolol due to a "hydrophobic interaction". The systems tested in the paper by Ogiso and Shintani (1990) were equimolar mixtures of propranolol with each fatty acid delivered in a gel formulation. However, the previous work in this paper has shown that equimolar mixtures of propranolol and fatty acids form 1:1 addition compounds (or salts), and that the individual charged species permeate the skin via an ion-pair mechanism.

DSC data of the propranolol:fatty acid addition compounds are presented in previous sections and these have been entered into the melting temperature/membrane transport (MTMT) model of permeation by Touitou et al. (1994), in an attempt to

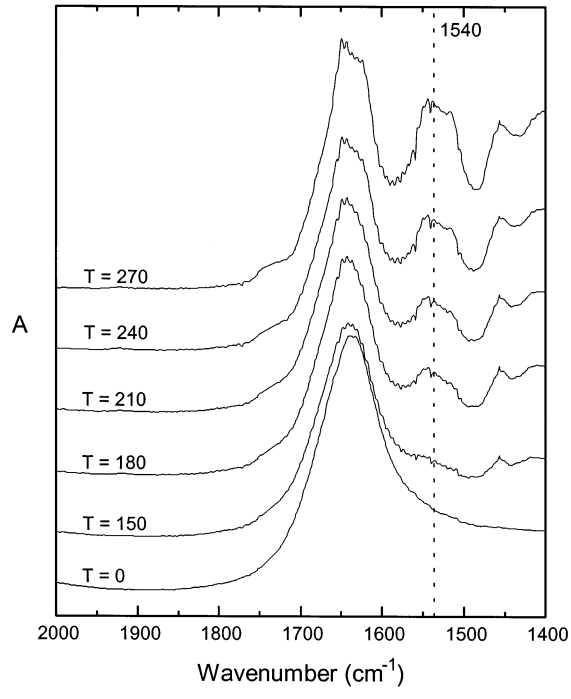

Fig. 14. ATR FTIR spectra of the aqueous receptor phase showing increasing intensity of the peak at 1540 cm^{-1} with time after permeation of the propranolol:lauric acid 1:1 addition compound across human epidermal membrane from a saturated solution in propylene glycol. The time after application of the donor for each scan is given in minutes.

Table 4
Transdermal permeation ERs of equimolar mixtures of propranolol with a homologous series of fatty acids calculated by the MTMT model (Touitou et al., 1994) and determined experimentally by Ogiso and Shintani (1990)

Fatty acid	Carbon number	Calculated ER[a]	Normalised[c] ER[a]	Ogiso and Shintani ER[b]	Normalised[c] ER[b]
Caproic	6	0.54	0.36	4.35	0.29
Caprylic	8	0.52	0.34	4.33	0.29
Capric	10	0.53	0.35	9.06	0.60
Lauric	12	1.53	1	15.16	1
Myristic	14	1.43	0.93	12.06	0.80
Palmitic	16	0.54	0.36	5.87	0.39

[a] Values calculated using the MTMT model of permeation (Touitou et al., 1994).
[b] Values taken from Ogiso and Shintani (1990).
[c] Normalised values are the fraction of the corresponding value for lauric acid.

correlate the melting characteristics with the enhancing effects seen by Ogiso and Shintani (1990).

Although the addition compound has been shown to be a salt, which would dissociate in aqueous solution, the individual species must reassociate to form the ion-pair, which aids permeation. Therefore, the thermal characteristics of the associated, pure solid are relevant to the permeation of the 1:1 molar complex.

The equation derived for the MTMT model is shown below (Eq. (3)) using a propranolol:lauric acid system as an example

$$\ln\frac{J_{PR:LA}}{J_{PRO}} = \frac{\Delta H_{PRO}(Tm_{PRO} - T)}{RTm_{PRO}T} - \frac{\Delta H_{PR:LA}(Tm_{PR:LA} - T)}{RTm_{PR:LA}T}, \quad (3)$$

where J is the flux, ΔH is the enthalpy of fusion (J/mol), Tm is the melting point (Kelvin) and T is the temperature of the membrane (Kelvin) and where the subscripts PRO and PR:LA relate to propranolol and the propranolol:lauric acid equimolar mixture, respectively. The predicted enhancement ratio (ER) is 1.53.

Similar treatments were performed on the full range of propranolol:fatty acid addition compounds and the results are summarised in Table 4.

The predicted ERs and those determined by Ogiso and Shintani (1990) for each propranolol:fatty acid mixture over propranolol alone is presented in Table 4. In order to provide a meaningful comparison both sets of ER values have been normalised against the corresponding value for lauric acid. Therefore, the ERs for each fatty acid are expressed as a fraction of the value obtained with lauric acid. This was necessary since the MTMT model is based on melting characteristics only and does not include variables such as molecular size and permeant lipophilicity, both of which are altered when propranolol interacts with a fatty acid.

The normalised predicted values of permeation enhancement show good correlation with the experimental values obtained by Ogiso and Shintani (1990). This indicates that the melting characteristics of the propranolol:fatty acid 1:1 molar addition compounds have a significant bearing on the permeation of the drug from these equimolar mixtures. This feature provides an alternative explanation as to why the C_{12} and C_{14} fatty acids produce the largest increase in permeation.

4. Conclusions

Binary mixtures of propranolol and fatty acids have been shown, by thermal analysis, to produce equimolar addition compounds, bracketed on either side of the temperature/composition phase diagram by two eutectic points. The addition compounds are formed by interaction between the carbonyl group of the fatty acid and the amino group of the β-blocker, to form a salt. The oppositely charged species of the salt have been shown to permeate the human epidermal membrane by an ion-pair mechanism. This is in agreement with

the work by Green and Hadgraft (1987) which suggested the formation of ion-pairs between propranolol and fatty acids, although in that case the anionic fatty acids were loaded in the membrane and the process was driven by a pH gradient.

Thermal analysis data from the addition compounds formed between propranolol and a homologous series of fatty acids have been entered into the predictive model of permeation by Touitou et al. (1994). The predictive flux values showed good correlation with the experimental values obtained by Ogiso and Shintani (1990). This shows that the effects of fatty acid chain length on propranolol flux can be explained by the melting characteristics of the addition compound formed.

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