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Development and evaluation using hairless mouse skin of a transdermal timolol product

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Summary

Timolol base was prepared and characterised for purity, partition coefficient and pK_a prior to formulation into a complex transdermal system designed to avoid unwanted first-pass metabolism, variable plasma levels and multiple daily administration associated with conventional forms of the maleate salt. Various components of a transdermal device were evaluated using a modified diffusion cell. The effect of different rate-controlling membranes such as microporous polypropylene, silastic and polyethylene vinylacetate on drug permeability was studied. Also the influence of hydrophilic drug reservoirs containing sodium carboxymethylcellulose (NaCMC) or Carbopol 934 and hydrophobic drug reservoirs containing Aerosil-liquid paraffin or Plastibase were investigated. The permeability of hairless mouse skin to timolol base in a range of drug reservoirs with or without membrane was studied. This showed the overall permeability of these systems to be mainly skin-controlled. However, addition of a range of suitable adhesives applied to the outer surface of the membrane made the composite system mainly device-controlled. The average drug flux from a NaCMC–microporous polypropylene membrane–basic adhesive system across hairless mouse skin was 0.08 $mg \cdot cm^{-2} \cdot h^{-1}$ and indicated the potential to deliver an adequate zero-order dose of timolol across a conveniently small area of skin.

Introduction

Complex transdermal delivery systems have been developed for drugs such as clonidine, fentanyl, glyceryl trinitrate, hyoscine, nicotine, oestradiol, progesterone, salbutamol and testosterone. Such products are capable of delivering a uniform effective flux of drug through the skin over an extended period of time, whose rate is device-controlled principally by the membrane, but also by the drug reservoir and contact adhesive employed. Advantages of such transdermal systems over conventional therapy include avoidance of the variables associated with gastrointestinal absorption, reduction of first-pass metabolism in the liver and improved management of the disease state by absence of pulsed delivery. However, there are relatively few suitable candidates for the approach because of the poor permeability of human skin to most drugs necessitating application of a device with an unacceptably large area or because of local irritation provoked by contact with the drug.

There have been a large number of publications on transdermal therapeutic systems including several excellent reviews (Chien, 1987; Guy and Hadgraft, 1985). Functional elements of such systems have been examined such as possible rate-controlling membranes (Touitou and Abed, 1985) and the clinical assessment of final products have been frequently reported. However, with the exception of disclosures in the patent literature,

the systematic development of complex transdermal drug delivery systems has not been described. The objectives of this initial study were to: (a) modify and evaluate the physicochemical properties of a suitable candidate drug; (b) formulate it into a complex transdermal system which is device-controlled; and (c) evaluate the product using hairless mouse skin prior to studies in humans.

Timolol was chosen as the candidate drug because it is a potent non-cardioselective β-adrenoceptor blocking agent used orally to treat hypertension, angina and myocardial infarction. Conventional therapy is associated with moderate first-pass metabolism (Johnsson and Regardh, 1976) and multiple daily administration for long periods with the associated lack of patient compliance. Vlasses et al. (1985), Cargill et al. (1986) and Guy and Hadgraft (1986) have shown that timolol base applied from a variety of simple formulations has potential for transdermal delivery. This study describes the development of more elegant transdermal systems for the free base and their preliminary evaluation using hairless mouse skin. Such skin has been shown by Durrheim et al. (1980) to be a good model for human skin. To facilitate the evaluation, a special diffusion cell was constructed.

Materials and Methods

Materials

Timolol maleate (Leo Laboratories), backing membrane Scotchpack no. 1006, EVA membrane 717 (91%:9% polyethylene/vinylactate), acrylate based-Lewis base contact adhesive on release liner MSX 582, acrylate based-Lewis acid contact adhesive on release liner MSX 583, silicone based-neutral adhesive on release liner MSX 584 (3M Corp.) Celgard 2400, 2402 and 2412 microporous polypropylene membranes (Celanese Corp.), Silastic sheeting 500-1 (Dow Corning), sodium carboxymethylcellulose (Courlose grade F1000P – Courtaulds), Aerosil (type 200 – Degussa), Plastibase (grade 50W – Squibb), liquid paraffin B.P., silicone fluid (type F11/1000 – Lennox), ammonia, chloroform, diethyl ether, hexane, hydrochloric acid, magnesium sulphate, methanol, *n*-octanol, sodium hydrogen phosphate, sodium hydroxide, sodium phosphate dodecahydrate, toluene (GPR – Riedel de Haen) and distilled water were used.

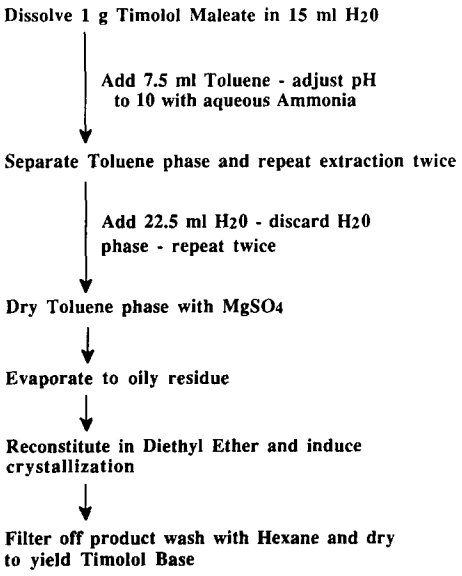

Fig. 1. Flow chart for the production of timolol base.

Methods

Preparation of timolol free-base

Timolol free-base was prepared from timolol maleate by treatment with aqueous ammonia, extraction with toluene and crystallization from diethyl ether. Fig. 1 shows a flow diagram of the process. The purity of the base was confirmed by melting point (61°C) and thin-layer chromatography using silica gel GF 254 plates (Merck) and a mobile phase of chloroform/methanol/water (25:24:1). A reference standard of purified base was supplied by Leo Laboratories.

Determination of pK_a

The ionization constant of timolol base (0.01 M) was determined by potentiometric tritration with 0.1 N HCl at 20°C, using a glass electrode as described by Albert and Serjeant (1971).

Determination of partition coefficient

The partition coefficient of timolol maleate and timolol base was determined between *n*-octanol

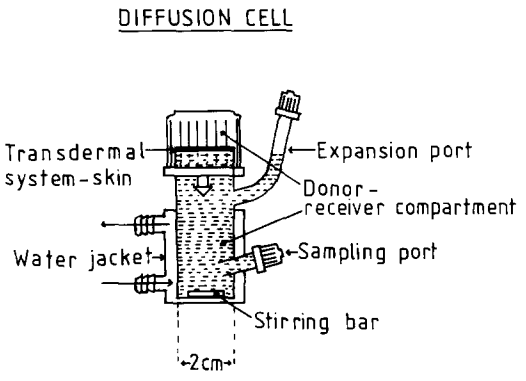

Fig. 2. Diffusion cell for transdermal studies.

and McIlvaine buffer pH 6.6. A 0.01% solution of drug was prepared in the organic phase and 10 ml aliquots pipetted into each of six 250 ml conical flasks. 40 ml of buffer pH 6.6 was added to each flask which were sealed and agitated at 37 °C for 6 h. After separation, the concentration of drug in the aqueous phase was determined spectrophotometrically at 294 nm. The entire procedure was repeated after 7 h and 8 h of agitation, and from these results it was apparent that equilibrium concentrations of drug in both phases were reached by 6 h.

Diffusion cell studies

A diffusion cell was constructed for transdermal studies as shown in Fig. 2 which was based on the design of Keshary and Chien (1984), but had a superior sampling port. The surface area exposed to the eluent (21 ml isotonic phosphate buffer pH 7.4 replaced as necessary to maintain adequate sink conditions) in the receiver compartment was 3.1 cm^2. The timolol base (15%) was mixed with the following reservoirs: (i) 4% NaCMC in purified water; (ii) 1% Carbopol 934 in purified water with pH adjustment to 7.0 using sodium hydroxide solution; (iii) 4% Aerosil 200 in silicone fluid; and (iv) Plastibase. Each drug reservoir was equilibrated overnight at 37 °C in a sealed container prior to use of 1 g aliquots.

The permeability of the following 5 rate-controlling membranes was examined; (i) Celgard 2400; (ii) Celgard 2402; (iii) Celgard 2412; (iv) EVA; and (v) Silastic. Celgard membranes are composed of microporous polypropylene with the 2400 grade having a nominal thickness of 2.5×10^{-2} mm. The 2402 and 2412 grades have a nominal thickness of 5×10^{-2} mm, but differ in that the 2402 grade is 2-ply and the 2412 grade is 1-ply. Each membrane was pretreated by passage of eluent containing 0.05% polysorbate 80 under positive pressure to displace air and wet. The membrane was rinsed well and stored in eluent prior to use.

Male hairless mice, 5–7 weeks old, were sacrificed by dislocating the cervical part of the spinal cord. The entire skin was excised from the abdominal region and its dermal side was cleaned of any adhering subcutaneous tissue. The skin sample was mounted on the diffusion cell with the stratum corneum facing the donor compartment.

Contact adhesive type MSX 582, MSX 583 or MSX 584 were examined by application to surface-dried rate-controlling membrane and removal of the release liner prior to application to the stratum corneum of excised skin. As required, backing membrane was secured over the drug matrix in the donor compartment, which was routinely covered with Parafilm. Samples of eluent for determination of drug content were periodically withdrawn from the receptor compartment with a hypodermic needle and syringe through the rubber septum in the sampling port. Plots of amount penetrated versus time were constructed and the data fitted by least-squares regression, from which the drug flux was determined.

Results and Discussion

Ionization constant (pK_a)

The pK_a value obtained for timolol base was 9.03. This value is in good agreement with the literature value of 9.2 and is within the range of related β-blockers: atenolol – 9.6, labetolol – 9.4 and propranol – 9.5 as reported by Albert and Serjeant (1971). As the value for timolol is high it can be concluded that the compound is a relatively strong base. In McIlvaine's buffer pH 6.6 and pH 7.4, timolol base is 99.6% and 97.7% ionized, respectively, at 20 °C. Poulson et al. (1968) reported that the surface of human skin is slightly

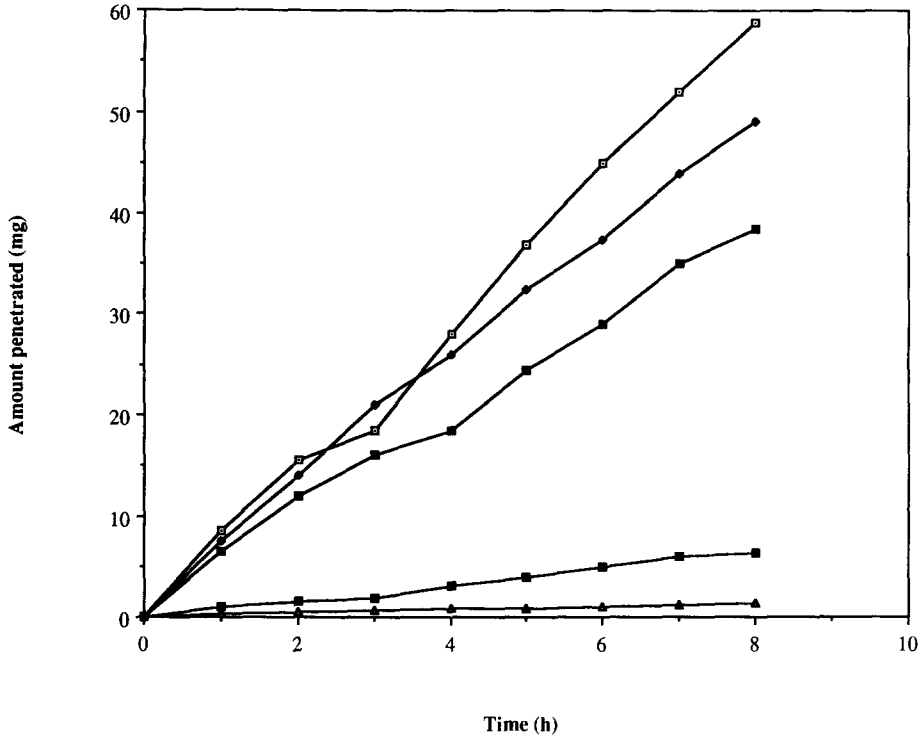

Fig. 3. Effect of various rate-controlling membranes on the permeability of timolol base from a 4% NaCMC-containing reservoir: Celgard 2400 (⊡), 2412 (◆), 2402 (■), Silastic (⊞), EVA (△).

acidic with a pH of 4.2–5.6, under which conditions timolol base will be over 99.9% ionized. The lower layers of the skin have a pH of 7.4. These results indicate that at all physiological pHs likely to be encountered on application to the skin, timolol base is likely to be almost completely ionized. Thus passage through polar regions and aqueous-filled pores in the biomembrane should be an important route of skin penetration.

Partition coefficient (P)

The observed partition coefficient of timolol maleate and base between *n*-octanol and McIlvaine's buffer pH 6.6 was determined, from which the true partition coefficient of the unionized species was calculated as 0.45 and 1.72, respectively. These values indicate the greater lipophilicity of timolol base. Due to the complex multilayered nature of skin it is difficult to define an optimum partition coefficient for transdermal penetration, In general an *n*-octanol/water partition coefficient of just over unity is considered desirable for good skin penetration. Compounds with higher *P* values are so lipid-soluble that they tend to remain dissolved in the stratum corneum and do not partition well into the underlying water-rich dermal tissue.

Effect of different rate-controlling membranes and drug reservoirs

Preliminary studies showed that unwetted membranes exhibited an initial lag in drug permeability which could be eliminated by the pretreatment without affecting the steady-state flux achieveable. Fig. 3 shows the effect of various rate-controlling membranes on the permeability of timolol base from a 4% NaCMC-containing hydrophilic reservoir. The profiles are zero-order with drug flux from Celgard 2400 and 2402 being 2.36 and 1.54 $mg \cdot cm^{-2} \cdot h^{-1}$, respectively. Both these membranes are microporous in structure wherein pore penetration is likely to predominate over

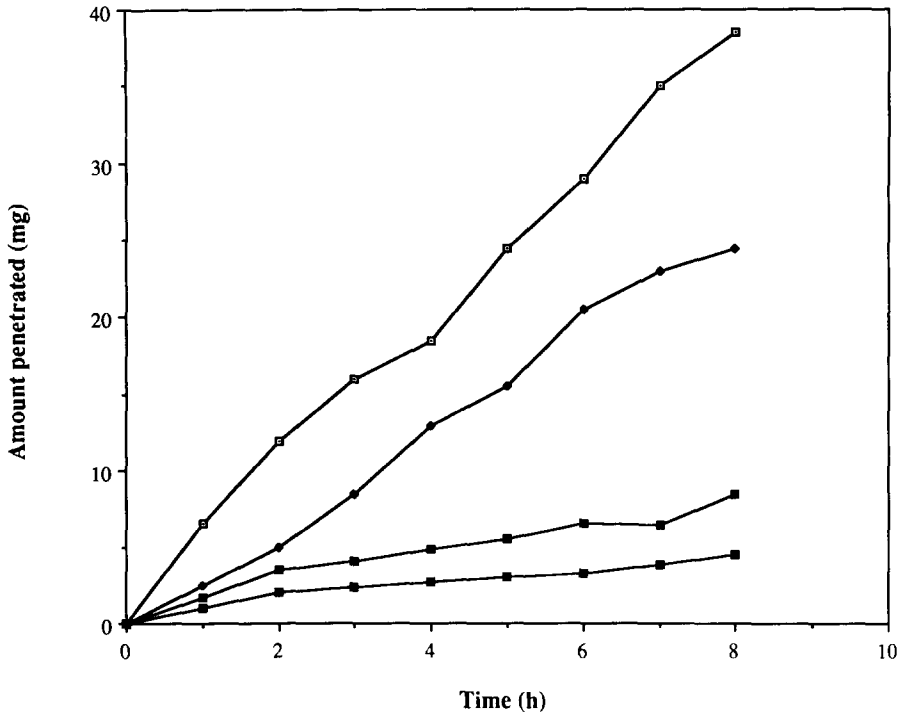

Fig. 4. Effect of various drug reservoirs on the permeability of timolol base through Celgard 2402: NaCMC (⊡), Carbopol (◆), Aerosil (■), Plastibase (⊞).

penetration of the continuum. The reduced rate of penetration associated with the 2402 grade is due to its greater thickness and its laminated structure. Care is necessary in handling this range of membranes as they tear quite easily, though this is unlikely to be a serious problem in patch fabrication. Celgard 2402 being a 2-ply form of Celgard 2400 provides improved handling characteristics and superior strength. However, the laminated structure decreases permeability in comparison to a single-ply membrane of the same thickness (Fig. 3, Celgard 2412 – drug flux 1.94 $mg \cdot cm^{-2} \cdot h^{-1}$), presumably by entrapping air pockets between the plys. Celgard films display inherent hydrophobic characteristics of polypropylene, and high surface tension liquids such as water do not readily penetrate it. Surfactant pretreatment as employed reduces this problem which might be expected with a hydrophilic drug matrix.

Silastic membrane, which is an elastomer, has a drug flux of 0.28 $mg \cdot cm^{-2} \cdot h^{-1}$. Drug permeability should be primarily by diffusion through the polymer matrix. Similar means of penetration would be expected through EVA membrane, which is a polyethylene internally plasticized with vinylacetate, that had the lowest observed drug flux of 0.03 $mg \cdot cm^{-2} \cdot h^{-1}$. An EVA copolymer with 16% vinylacetate was reported by O'Neill (1980) to be less permeable to progesterone than Silastic, the effect being attributed to a greater degree of cross-linking within the EVA polymer matrix. It was considered unlikely that either of these membranes would be adequately permeable in a composite transdermal device and were not investigated further.

Fig. 4 shows the effect of various drug reservoirs on the permeability of timolol base through Celgard 2402 membrane. The range of differences in release rate from the 4 reservoirs assessed shows that vehicle composition is an important factor. The flux from the hydrophilic vehicles, NaCMC and Carbopol (1.00 $mg \cdot cm^{-2} \cdot h^{-1}$), is much greater than from the hydrophobic vehicles, Aerosil in silicone fluid and Plastibase

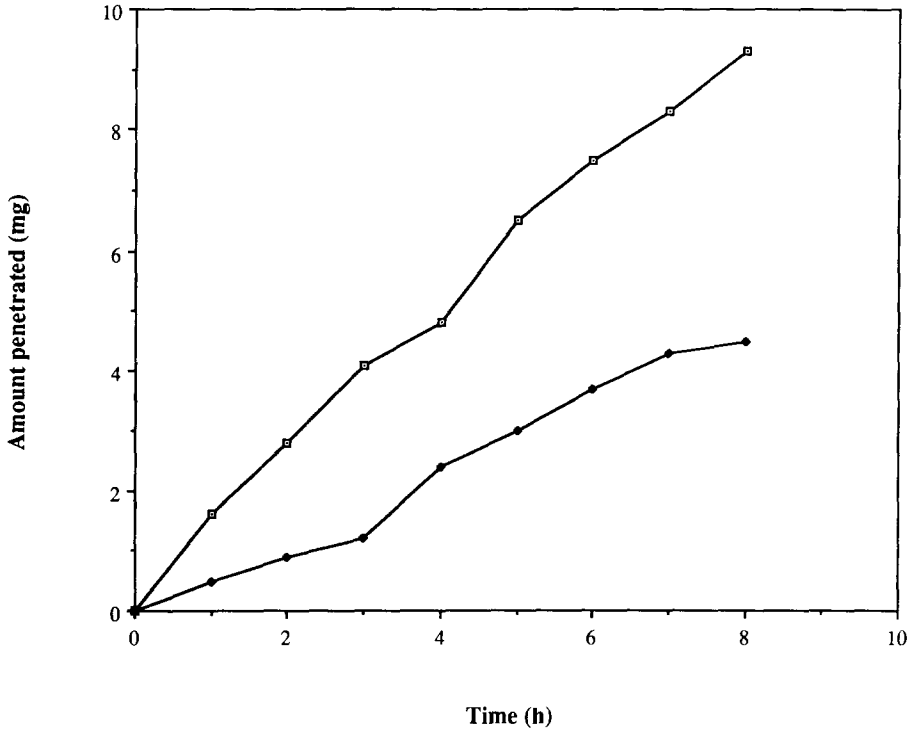

Fig. 5. Effect of NaCMC (□) and Plastibase (◆) reservoirs on the permeability of timolol base through hairless mouse skin.

(0.30 and 0.21 mg · cm^{-2} · h^{-1}, respectively). With the NaCMC reservoir, reduction in polymer concentration from 4% to 2% resulted in a 95% increase in drug flux presumably due to less binding of the ionized drug to the polyanion and reduced hinderance to diffusing drug molecules in the less viscous vehicle. Due to the porous nature of Celgard membranes, drug penetration is likely to be mainly by diffusion through fluids entrapped in the membrane. Thus vehicle characteristics are critical in determining drug partition rates unlike non-porous membranes where partitioning and diffusion through the continuum are likely to be more important.

Permeability of hairless mouse skin

The permeability of timolol base through hairless mouse skin from two different vehicles is shown in Fig. 5. The drug flux from NaCMC and Plastibase reservoirs were 0.38 and 0.21 mg · cm^{-2} · h^{-1}, respectively, indicating the importance of vehicle composition. The drug is less soluble in water (8 mg · ml^{-1} at 20 °C) than in liquid paraffin (12 mg · ml^{-1} at 20 °C), but does not appear to partition as readily from Plastibase into the lipophilic layers of the stratum corneum. Also the occlusive properties of Plastibase may somewhat increase the degree of hydration of the stratum corneum thus further decreasing the partitioning of timolol base. The mouse skin gives a linear plot for cumulative amount penetrated versus time with no initial lag indicating that the drug partitions rapidly into the skin. The flux of timolol maleate from a 4% NaCMC-containing reservoir through hairless mouse skin was found to be only 0.039 mg · cm^{-2} · h^{-1}, confirming its lack of transdermal permeability.

Cargill et al. (1986) reported a maximum drug flux for timolol base of 0.1 mg · cm^{-2} · h^{-1} using fuzzy rat skin. It is not known which skin type is the best model for human skin using this drug. However, the drug flux from the slowest releasing vehicle, Plastibase, through Celgard 2402 or hairless mouse skin is identical and was found to be

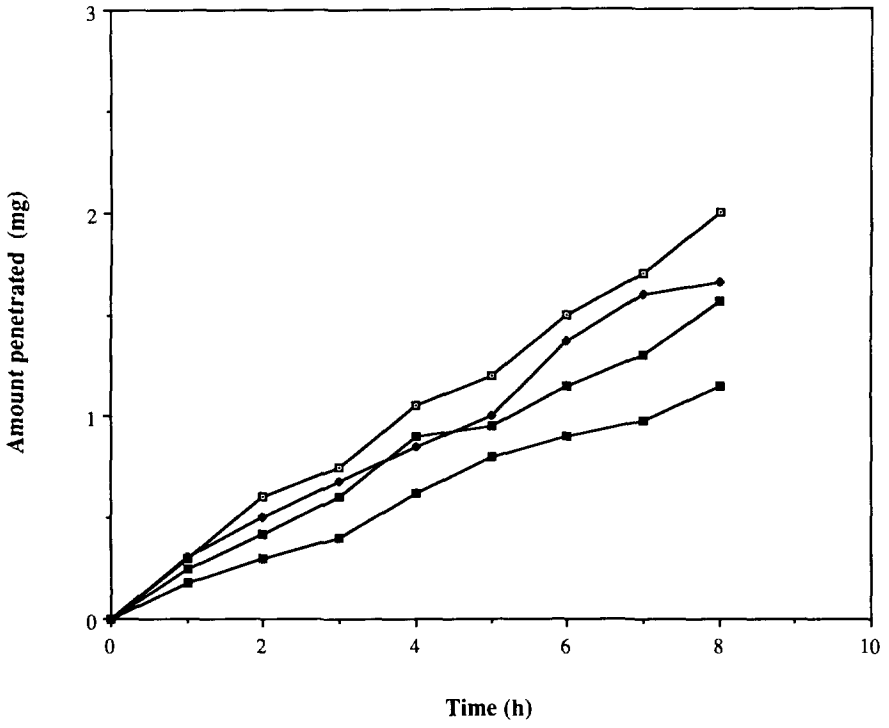

Fig. 6. Effect of transfer adhesive on permeability of timolol base from a NaCMC (⊡) or Plastibase (◆, ■, ⊞) reservoir through Celgard 2402 and hairless mouse skin bonded with basic (⊡, ◆), acidic (■) or silicone (⊞) types.

unaffected when both were used in parallel. Therefore it is unlikely that a transdermal system including these components would be adequately device-controlled unless the contact adhesive required further reduced release or the skin was made more permeable by use of a penetration enhancer.

Effect of transfer adhesive and backing membrane

Fig. 6 shows the effect on permeability of timolol base from a NaCMC or Plastibase reservoir through Celgard 2402 and hairless mouse skin bonded together with various transfer adhesives. The drug fluxes for basic, acidic and silicone adhesives were 0.07, 0.066 and 0.052 $mg \cdot cm^{-2} \cdot h^{-1}$, respectively, using the Plastibase reservoir and 0.08 $mg \cdot cm^{-2} \cdot h^{-1}$ for the basic adhesive–NaCMC system. In all cases the flux is greatly decreased by the addition of the adhesive indicating that this component layer offers the greatest resistance to drug transfer. The basic acrylate adhesive has the least effect, presumably as it exhibits the minimum interference with diffusion of the basic drug. There is no evidence of initial lag in the plot of cumulative amount penetrated with time even though there was no priming dose used in the adhesive. Products were normally tested within 7 days of preparation, indicating that the drug rapidly partitions into the adhesive layer during storage.

The release from these systems does show potential for device control. The largest dose of 14.6 mg base equivalent to 20 mg timolol maleate daily would require an active contact area of only 7.6 cm^2 for the most permeable system examined. The addition of a backing membrane of Scotchpack no. 1006 (metalised polyvinylfluoridene film) had no effect on the rate of penetration. Studies are in progress to evaluate a composite transdermal device of similar composition in humans and to investigate the use of penetration enhancers for reduction in the area of the product.

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