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Supersaturated solutions evaluated with an in vitro stratum corneum tape stripping technique

M.A. Pellett [a,*], M.S. Roberts [b], J. Hadgraft [a]

[a] *The Welsh School of Pharmacy, University of Wales, Cardiff CFI 3XF, UK*
[b] *Department of Medicine, The University of Queensland, Princess Alexandra Hospital, Brisbane 4102, Australia*

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Abstract

The flux of a compound across a membrane from any formulation, whether it contains penetration enhancers or not, is limited by its saturated solubility in the vehicle. Under such conditions the concentration of the permeant in the outer layers of the stratum corneum is also saturated. Consequently, when the permeation of a drug from a supersaturated solution leads to enhanced penetration, the concentration of the drug in the outer layers of the membrane is also supersaturated. Therefore, the stratum corneum may possess antinucleant properties which inhibit or retard the crystallisation process. In this study, the enhanced in vitro permeation of supersaturated solutions of piroxicam across human skin in diffusion cells was demonstrated. The amount of permeant in the stratum corneum was determined using a tape stripping technique. Supersaturated solutions up to four degrees of saturation were investigated which produced a linear relationship between the degree of saturation and the amount of piroxicam in the stratum corneum ($R^2 = 0.970$). Furthermore, the amount of piroxicam in the viable layers of the skin also increased with increasing degree of saturation. An analysis of the results suggested that enhanced penetration across human skin from supersaturated solutions of piroxicam may occur as a result of the antinucleating ability of the intercellular lipids of the stratum corneum. © 1997 Elsevier Science B.V.

Keywords: Piroxicam; Tape stripping; Supersaturation; Human skin; Intercellular lipids

1. Introduction

1.1. Background

The stratum corneum is widely accepted to be the main barrier to percutaneous absorption, and many techniques have been tried, with varying

* Corresponding author. Tel.: +44 1222 874180; fax: +44 1222 874180; e-mail: pellett@cardiff.ac.uk

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success, to overcome its barrier properties and provide penetration enhancement. Physical techniques such as iontophoresis (Pikal, 1990; Green et al., 1993) have shown that enhanced delivery of both ionised and unionised permeants is possible. Reverse iontophoresis has been used to 'back extract' from the blood to the skin surface (Rao et al., 1993). Application of ultrasound has also shown that enhanced absorption can be obtained in some instances (Benson et al., 1991). These are 'high tech' processes and general use may be precluded on cost grounds. Chemical enhancement has also been extensively studied but the enhancers that cause fluidity in the stratum corneum lipids tend to have structures (polar head groups and long alkyl chains) which can elicit skin toxicity problems. Penetration enhancement through the use of supersaturated systems provides an attractive alternative. This involves raising the activity of a compound in its formulation beyond that at its solubility limit. The main advantage of this method over other techniques is that it is inexpensive and does not perturb the intercellular lipids.

The importance of chemical potential, or thermodynamic activity, in formulations was first recognised by Higuchi (1960) and later tested by Coldman et al. (1969). Supersaturated solutions, by their very nature, are physically unstable, and in many instances, the drug tends to crystallise upon preparation of the solution. However, sometimes this problem can be overcome by the use of antinucleant polymers which either prevent or retard crystallisation. It is anticipated that part of the mechanism of enhancement from these systems is due to an increased thermodynamic activity of the drug in the intercellular lipids of the external layers of the stratum corneum.

In general, the flux of any given compound across a membrane from a saturated solution, irrespective of its concentration, is constant, provided that there are no interactions between the membrane and the components of the formulation (Twist and Zatz, 1986). Therefore, under normal circumstances, the flux of a drug is limited by its solubility which, in turn, can also limit its bioavailability. Consequently, the preparation of stable supersaturated systems not only circumvents some of the regulatory issues that are associated with other mechanisms of enhancement, but it can also lead to increased bioavailability.

In percutaneous drug delivery, there have been essentially three procedures by which supersaturated formulations have been prepared: (i) heating and cooling (Henmi et al., 1994); (ii) evaporation of a volatile component of the vehicle (Kondo and Sugimoto, 1987; Chiang et al., 1989); (iii) mixing of two solvents where the drug is significantly more soluble in one solvent than the other (Davis and Hadgraft, 1991; Megrab et al., 1995).

In this study, the third method was employed, where a solution of the drug in propylene glycol was mixed with an aqueous solution of 2% hydroxypropylmethyl cellulose (HPMC). A more detailed approach to the preparation of supersaturated solutions is provided by Davis and Hadgraft (1991).

A tape stripping technique was used to investigate the amounts of drug that were present in the stratum corneum of excised human skin after the application of supersaturated solutions of a model drug, piroxicam, at different degrees of saturation. In addition, the total amounts of piroxicam that diffused across the skin into a receptor phase and the amounts in the epidermis and dermis were also determined. Piroxicam was chosen as the model compound because recent experiments have demonstrated its penetration enhancement from stable supersaturated solutions across both human skin and silicone membranes (Pellett et al., 1996).

The simple technique of tape stripping has been employed in a wide variety of situations (Brehmer-Andersson and Brunk, 1967; Heng et al., 1985; Berardi and Arcangeli, 1992; Pershing et al., 1992; Flamand et al., 1994; Aspe et al., 1995). Due to its mildly invasive nature, it is ideally suited to performing studies in vivo, but these are often expensive to perform and obtaining ethical approval can be difficult. Therefore, in vitro tape stripping can be viewed as an alternative option and is particularly appropriate for preliminary investigations of formulation effects of this kind on the stratum corneum.

1.2. Theory

The steady-state transport of a compound across a membrane is often described by Fick's first law of diffusion:

$$J = \frac{D \cdot A \cdot K}{h}(C_v - C_r) \quad (1)$$

where J is the flux, A is the area of diffusion, K is the membrane-vehicle partition coefficient, D is the diffusion coefficient, C_v is the drug concentration in the vehicle, C_r is the concentration in the receptor phase and h is the diffusional pathlength. C_r is usually very small, and under sink conditions, $(C_v - C_r)$ is generally approximated to C_v.

The likelihood of transport is most appropriately defined by the chemical potential gradient. In supersaturated systems the chemical potential is substantially higher in the vehicle than for saturated solutions. Equilibrium will then require a similar rise in the chemical potential in the outer layers of the stratum corneum. This will only occur if the skin lipids can permit the permeant to exist in a higher activity than would be observed for saturated solutions. A diagrammatic representation of the resulting concentration–distance profile is shown in Fig. 1.

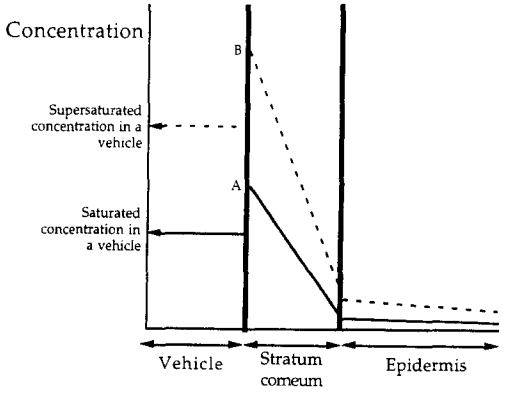

Fig. 1. An adaptation of Higuchi's model of diffusion for saturated and supersaturated solutions (A and B: saturated and supersaturated concentrations in the outer layer of the stratum corneum after application of a saturated and supersaturated solution, respectively).

2. Materials and methods

2.1. Materials

^{3}H-piroxicam and unlabelled piroxicam were gifts from Pfizer, USA. Propylene glycol (PG) was purchased from Sigma (Castle Hill, NSW, Australia). Hydroxypropylmethyl cellulose (HPMC) was obtained from Shinetsu (Tokyo, Japan). Tissue solubiliser, NCS-II, and the liquid scintillation cocktail, OCS (organic counting scintillant) for tissue samples were purchased from Amersham Australia (Sydney). A liquid scintillation cocktail for the aqueous samples, Ultima Gold was purchased from Packard (Canberra, Australia). All other chemicals were of at least reagent grade and were used as supplied. Human skin was obtained from the abdomenoplasty of a 48 year old female patient and frozen until required. 3M Blenderm tape was used to strip the stratum corneum.

2.2. Preparation of the supersaturated solutions

The solubility of piroxicam in a 40:60% v/v PG/water cosolvent mixture has already been determined to be 13.6 mg/100 ml, and supersaturated solutions at 1, 2, 3 and 4 degrees of saturation of piroxicam were prepared as demonstrated in a previous publication (Pellett et al., 1996). From a stock solution of 136 mg/100 ml of piroxicam in PG spiked with ^{3}H-piroxicam, supersaturated solutions of piroxicam were prepared by the mixing of different ratios of this solution with a 2% aqueous solution of HPMC (Table 1).

2.3. Diffusion study

The skin was allowed to thaw overnight before removing excess fat by blunt dissection. The full-thickness skin was then cut into suitable sizes and mounted in Franz-type upright diffusion cells. The cells provided an average diffusional surface area of 1.25 cm^2 and a receptor compartment filled with $\approx$3 ml of phosphate buffered saline pH 7.4. Three cells were used for each degree of saturation, and white soft paraffin was used to help produce a seal between the skin and the two compartments. In order to prevent evaporation of

Table 1
Preparation of supersaturated solutions of piroxicam in a 40:60% v/v PG/water cosolvent mixture using a stock solution of 136 mg/100 ml of piroxicam in PG

Degree of saturation	Vol. of stock solution (μl)	Vol. of PG (μl)	Vol. of 2% HPMC (μl)	Final conc. (mg/100 ml)
1	50	150	300	13.6
2	100	100	300	27.2
3	150	50	300	40.8
4	200	0	300	54.4

the volatile component of the vehicle (i.e. water), 140 μl aliquots of the formulations were placed onto the skin surface and occluded using O-rings and a silicone membrane. The diffusion study was performed over a 24 h period. Previous experiments have shown that the supersaturated solutions prepared at four degrees of saturation were only stable for a period of 16 h, and therefore, all donor phases were replaced with freshly prepared solutions after 12 h. Furthermore, the entire contents of the receptor phase was assayed and replaced with an equal volume of pre-thermostated phosphate buffered saline pH 7.4 after each 12 h period. Prior to the replacement of the fresh phases and after 24 h, the donor and receptor compartments were washed three times with phosphate buffered saline pH 7.4. The washings were added to their respective donor or receptor phases before analysing for ^3H.

2.4. Tape stripping

At 24 h, after the removal of the donor and receptor phases, and after completing the washing procedures, the diffusion cells were carefully dismantled, and the skin was pinned on a piece of Parafilm™ which was stapled to a solid surface. The imprints of the flanges from the diffusion cells (≈ 5 mm width) clearly marked the exposed diffusional area (≈ 1.25 cm^2). The skin was dabbed dry with tissue, and pieces of tape were cut so that they covered the diffusional area but did not overlap the areas outside of the flange imprints. The first tape strip was discarded and the following 15 tape strips were placed in a vial. It is normal practice to discard this first strip as it is considered to be part of the cleaning procedure and it may be contaminated with drug from the formulation. Complete removal of the stratum corneum was indicated by the glistening of the viable epidermal layer after 15 strips. Excess skin was trimmed away from the flange imprints and the remaining viable epidermis and dermis were placed in separate vials. The contents of the individual vials were wetted with 0.5 ml of water and then ≈ 3 ml of tissue solubiliser, NCS-II, was added. The samples were then incubated at 50°C for 24 h prior to analysis.

2.5. Analysis

Organic counting scintillant was used for the NCS-II-treated samples and Ultima Gold was used for the aqueous samples. Background counts were taken into consideration from cells using unlabelled compounds, and all samples were counted for ^3H on a 1600TR Liquid Scintillation Analyser (Packard, Canberra, Australia).

3. Results and discussion

The cumulative amount of drug diffused across full-thickness human skin into the receptor compartments from the different supersaturated solutions after 12 and 24 h is shown in Fig. 2. The amounts of piroxicam that penetrated across the membrane increased with increasing degree of saturation, and this re-confirms the trend obtained in a recent publication (Pellett et al., 1996). Calculations based on the amounts penetrated at 24 h showed that not more than 5% of the donor phase concentrations penetrated into the receptor phase, and furthermore, the solubility in this

phase did not exceed 0.2% demonstrating that sink conditions were maintained throughout the experiment.

A mechanism of action for the antinucleant polymer, HPMC, on its stabilisation of supersaturated solutions of piroxicam has already been proposed (Pellett et al., 1996). In brief, piroxicam is capable of forming a hydrate, and this hydrated formation is associated with a change in the colour of its solutions from pale yellow for the anhydrous to bright yellow for the hydrate form. In the presence of HPMC, the solutions were pale yellow indicating the anhydrous form of the drug, whereas in the absence of HPMC, the solutions were bright yellow indicating the hydrate form. Solvate forms of different drugs are reported to have different solubilities and dissolution rates compared to their non-solvate forms (Shefter and Higuchi, 1963). Therefore, in the supersaturated systems prepared by the biphasic mixing of PG and water, it was proposed that HPMC prevented the formation of a hydrate form of the drug. Although the saturated solubility of a solution containing HPMC was only five times greater than those without HPMC (a maximum of 5.8 degrees of saturation could have been achieved in the system used in that study), it appears that this only partly explains the mechanism of action and it is likely that other mechanisms also contribute to the stabilising effect.

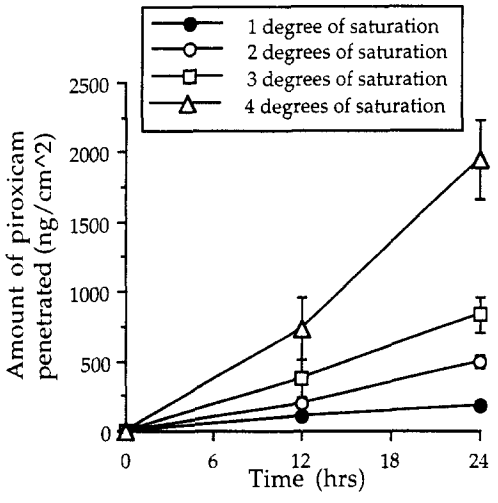

Fig. 2. Cumulative amounts of labelled piroxicam diffused across full-thickness skin from different supersaturated solutions (Mean ± S.E., $n = 3$).

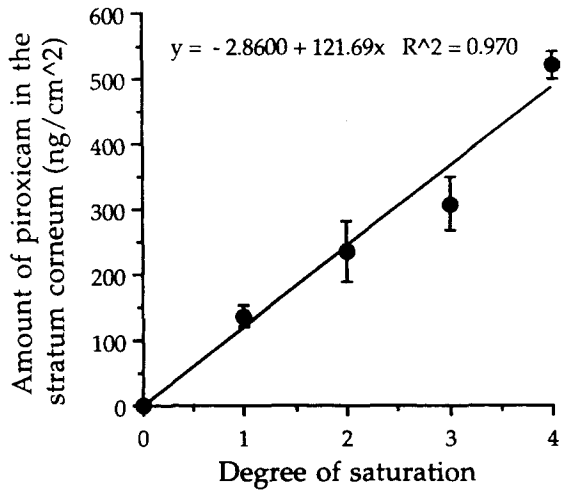

Fig. 3. Amount of piroxicam in the stratum corneum after the application of different supersaturated solutions for 24 h (Mean ± S.E., $n = 3$).

The amount of labelled piroxicam in the stratum corneum after 24 h is shown in Fig. 3. Using linear regression analysis, a good correlation ($R^2 = 0.970$) between the degree of saturation and the amount of piroxicam in the stratum corneum was demonstrated, and therefore, it appears that the stratum corneum is capable of supporting supersaturated states of piroxicam. The outer layer of the skin is composed of corneocytes packed with keratin which are surrounded by a unique combination of lipids. It is thought that diffusing molecules take the tortuous route through the intercellular lipids and around the corneocytes. Therefore, it can be proposed that any antinucleating ability of the stratum corneum probably lies within these lipids.

Using a PG and water cosolvent system, the maximum activity which was stable for long enough periods for these type of diffusion experiments was four degrees of saturation, but if higher degrees of saturation were used, the antinucleating ability of the stratum corneum may have been exceeded. It appears that this was the experi-

ence of Megrab et al. (1995) who prepared supersaturated solutions of oestradiol with 18 degrees of saturation, and demonstrated an 18-fold increase in uptake into the stratum corneum but only a 13-fold increase in flux.

The success of this tape stripping technique means that the effect of vehicles and penetration enhancers on partition coefficients can also be investigated in vitro. This is an important consideration for determining mechanisms of penetration enhancement, particularly for xenobiotics that are irritant or are not approved for application to skin. Trebilcock et al. (1994) have recently demonstrated that this type of in vitro stripping provides a good model for the in vivo situation.

After the stratum corneum was tape stripped, the amount of piroxicam in the remaining epidermis and dermis was determined and is shown in Fig. 4. These values represent ≈31%, 46% and 58% of the total amount of piroxicam in the skin (stratum corneum, viable epidermis and dermis) for 1, 2 and 3 degrees of saturation respectively. However, taking into account the relative thicknesses of the stratum corneum and the combined thickness of the viable epidermis and dermis, the concentration of piroxicam in the stratum corneum was ≈100-fold higher (assuming the

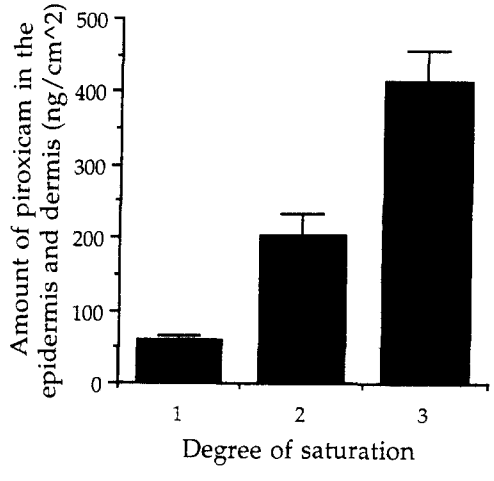

Fig. 4. Amount of piroxicam in the remaining epidermis and dermis (Mean ± S.E., $n = 3$, a value for 4 degrees of saturation was not obtained).

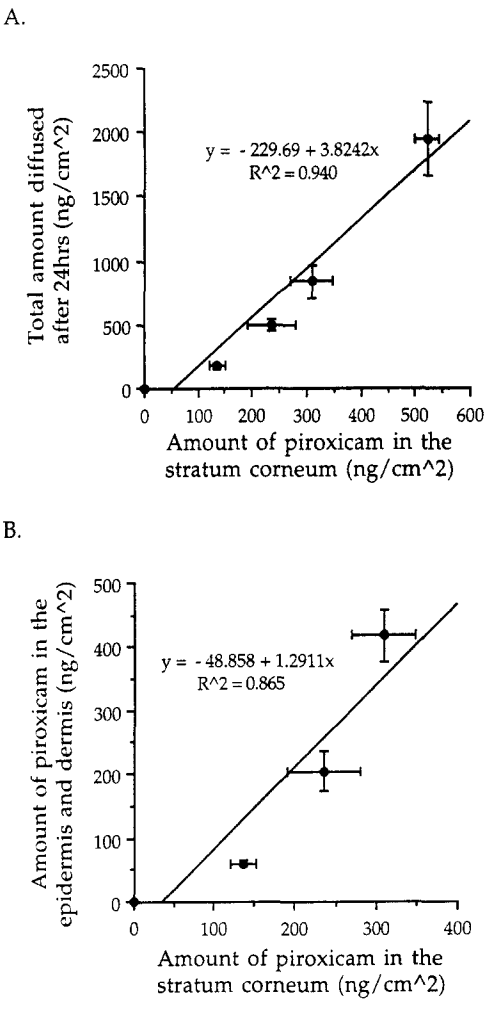

Fig. 5. Plots of the amount of piroxicam in the stratum corneum against A, total amount of drug diffused into the receptor phase after 24 h; and B, the amount in the viable epidermis and dermis. (Mean ± S.E., $n = 3$).

stratum corneum thickness to be 20 μm, and the epidermis and dermis to be 2000 μm) than in the other two layers.

The reservoir effect of the stratum corneum is an established phenomenon (Rougier et al., 1985), and in this study, after a 24 h application period ≈0.8% of the dose applied was found in the stratum corneum.

Fig. 5 shows the relationships between the amount of piroxicam in the stratum corneum and the amounts that diffused into the receptor phase,

and the amounts in the stratum corneum with the amounts in the viable epidermis and dermis. Using linear regression analysis, correlation coefficients of $R^2 = 0.940$ and 0.865 were obtained for the two plots respectively. It is also noticeable that both plots have a large x-axis intercept, which may be caused by lateral diffusion of the drug in the stratum corneum, and inadvertant removal of the membrane during the tape stripping process from the flange imprints of the donor compartment of the diffusion cell.

Although these studies have examined the benefits of supersaturation on enhancing the percutaneous penetration of a drug that is likely to be delivered locally, the method could equally be used to enhance the penetration of drugs for systemic use. Therefore, these results have demonstrated that supersaturation can be added to the growing number of techniques that are now available for providing enhanced penetration of drugs across skin.

4. Conclusions

The preparation of supersaturated solutions of piroxicam and their diffusion across full thickness human skin in diffusion cells was shown to increase with increasing degree of saturation. Furthermore, the amount of drug that diffused into the stratum corneum also increased. The results were consistent with the hypothesis that penetration enhancement was dependent on the ability of the intercellular lipids of the outer layers of the stratum corneum to maintain a supersaturated state of the diffusant within their tightly packed structure. The amount of piroxicam in the viable epidermis and dermis also increased with increasing degree of saturation. Investigations on supersaturation and delivery to local subcutaneous structures are continuing in our laboratories.

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