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Effect of supersaturation on membrane transport: 2. Piroxicam

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Abstract

Subsaturated, saturated and supersaturated solutions of piroxicam were prepared in a propylene glycol/water cosolvent system. The diffusion of these solutions across a model membrane of Silastic® was investigated. It was shown that in a single cosolvent system the diffusion of the drug was linear with respect to the degree of saturation. However, the results from the flux of supersaturated solutions in different propylene glycol/water vehicles showed that there was an increase in the flux of piroxicam as the proportion of propylene glycol was increased. This was also true with the diffusion of saturated solutions across Silastic®. Further investigations showed that this effect was most likely due to the enhanced partitioning of piroxicam into the membrane.

Key words: Piroxicam; Propylene glycol; Supersaturation; In vitro membrane diffusion; Silastic®; Partitioning; Vehicle effect

1. Introduction

One of the main objectives in topical drug delivery is to overcome the excellent barrier properties of the stratum corneum. Both physical and chemical methods have been attempted with varying success. Physical systems (e.g., iontophoresis and phonophoresis) require complex and possibly expensive delivery devices. Chemical methods involve penetration enhancers or the manipulation of the physico-chemical properties of the formulation. In general, penetration enhancers alter the barrier properties of the stratum corneum by exerting their effect on the intercellular lipids. Supersaturation avoids the need to disturb the stratum corneum structure and also provides a relatively economical method for achieving penetration enhancement.

The potential benefit of supersaturated solutions was first recognised at least three decades ago (Higuchi, 1960). Since then little work has been carried out in this area, probably partly due to the thermodynamic instability of these solutions. However, with an understanding of antinucleant polymers, supersaturated solutions can be exploited to enhance percutaneous penetration (Simonelli et al., 1970; Sekikawa et al., 1978; Kondo and Sugimoto, 1987; Kondo et al., 1987; Davis and Hadgraft, 1991).

Supersaturated solutions were produced by us-

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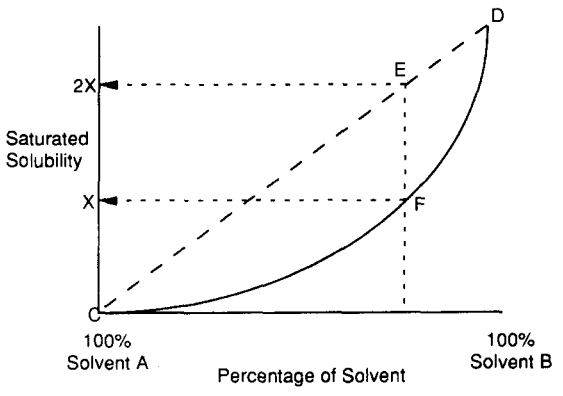

Fig. 1. Solubility of a drug in a binary cosolvent system and the method used to obtain a supersaturated solution.

ing a cosolvent system that has been previously described (Davis and Hadgraft, 1991) and this involves preparing a saturated solubility curve for the drug in a binary cosolvent system (Fig. 1). By mixing a saturated solution of the drug in solvent B with solvent A (which, if required, contains an antinucleant polymer) points are obtained along the line CD. These may be supersaturated solutions and their 'degree of saturation' is calculated by dividing the amount of drug in solution by its saturated solubility in the same cosolvent. For example, a solution (E) that is said to have two degrees of saturation has twice the amount of drug in solution than a saturated solution (F).

This study used the binary cosolvent system of propylene glycol and water to produce subsaturated, saturated and supersaturated solutions of piroxicam. The physical stability of supersaturated solutions of this drug have already been demonstrated (Pellett et al., 1993). The fluxes of these solutions across the model membrane Silastic® were investigated and the effects of propylene glycol on the membrane were studied.

2. Materials and methods

2.1. Materials

Piroxicam was obtained from Pfizer Ltd and propylene glycol and methanol were purchased from Fisons Plc. HPLC grade acetonitrile was purchased from Rathburn Chemicals Ltd and the Silastic® ($8 \times 6 \times 0.005$ inch) was a gift from Dow Corning. Hydroxypropylmethyl cellulose (HPMC) was obtained from Shin-Etsu Chemical Co., Ltd. All other chemicals were purchased from BDH Ltd and were of at least reagent grade and were used as supplied.

2.2. Analysis

The HPLC analysis of piroxicam was performed using a Milton Roy LDC Constametric IIIG pump set at a flow rate of 1.5 ml/min, with a Rheodyne 7125 injector, a Spectromonitor III variable wavelength UV detector set at 365 nm and a CI4100 computing integrator. The stationary phase was a Zorbax reverse phase ODS 5 μm column. For the saturated solubility and partitioning work, 20 μl samples were injected using a mobile phase of acetonitrile/water/acetic acid (65:31:4) and, for the diffusion work, samples were injected using a 400 μl loop and a mobile phase of acetonitrile/water/acetic acid (55:41:4). Calibration curves were constructed on the basis of peak area measurements.

2.3. Determination of saturated solubilities

An excess of the drug was added to the solvent and the mixtures were agitated with a teflon-coated magnetic bar and left in a water bath at 25°C for 48 h. The solutions were then centrifuged and a sample of the supernatant was assayed by HPLC. Saturated solubility curves were constructed for piroxicam in two separate cosolvent systems of propylene glycol/water and propylene glycol/phosphate citrate buffer pH 4.2. A buffered cosolvent system was chosen so that the degree of ionisation of the drug could be controlled and the flux of piroxicam from a buffered vehicle could be compared with that from an unbuffered vehicle.

2.4. Diffusion studies

Diffusion experiments were conducted using Franz-type diffusion cells that have a receptor

volume of 5 ml and a diffusional surface area of about 1.6 cm^2. The receptor chambers had side arms through which samples could be taken. Phosphate-buffered saline pH 7.4 (PBS) was filtered through a Whatman 0.45 μm cellulose nitrate membrane filter and used as the receptor phase. The Silastic® was cut to the appropriate size and allowed to soak in PBS overnight. White soft paraffin was used to produce a leak proof seal between the flanges of the two halves of the cell and the Silastic®. The two halves of the cell were held together with a pinch clamp. Samples of 1 ml of the test solutions were introduced to the donor compartments and the cells were maintained at 25°C in a water bath. Teflon coated magnets were used to agitate the receptor compartments, and Nescofilm® was used to cover the sampling arms and the opening of the donor compartments to prevent evaporation and change in the vehicle. At designated intervals, 1 ml of the receptor phase was removed and replaced with an equal volume of pre-thermostated PBS. The samples were assayed by HPLC.

Supersaturated solutions were prepared by mixing 140 mg/100 ml of piroxicam (93% of the drug's saturated solubility) in propylene glycol with a 2% solution of HPMC in water.

The following series of experiments were performed:

(i) Diffusion of piroxicam from a saturated solution of 60:40% v/v propylene glycol/water;
(ii) Flux of piroxicam from different degrees of subsaturation and supersaturation in the single cosolvent vehicle of 60:40% v/v propylene glycol/water;
(iii) Flux of piroxicam from the supersaturated solutions that are formed from mixing different ratios of 140 mg/100 ml of piroxicam in propylene glycol with a 2% solution of HPMC in water;
(iv) Flux of piroxicam from saturated solutions of different propylene glycol/water vehicles;
(v) Flux of piroxicam from saturated solutions of different propylene glycol/phosphate citrate buffer pH 4.2 vehicles;

The diffusion experiments were conducted over a period of at least 6 h and the flux was calculated from the linear part of the curve.

2.5. Determination of partition coefficients

The partition coefficient of piroxicam between various vehicles and Silastic® was determined using a modified method after Jetzer et al. (1986). A known volume of Silastic® (based on a density of 1.45 g/cm^3) was added to 1 ml of piroxicam solution and agitated overnight at 25°C with a teflon-coated magnet. The next day the pieces of Silastic® were quickly washed with water and dried. Then 0.5 ml of methanol was added to extract the piroxicam from the Silastic® and, after further agitation for at least 2 h, a sample of the methanol was assayed. Previous work had indicated that equilibrium was reached within 1 day and that one extraction with methanol was sufficient. With the knowledge of the initial vehicle concentration of piroxicam, the final vehicle concentration after partitioning can be calculated by difference. The partition coefficient was calculated using the following simplified equation:

$$K = \mathrm{Mc}/[\mathrm{Sv}(2C_i - \mathrm{Mc})]$$

where K is the partition coefficient, Sv denotes the Silastic® volume, Mc is the methanol concentration, and C_i represents the initial concentration of piroxicam in the vehicle.

The partitioning of piroxicam between 0:100, 20:80, 40:60, 80:20, and 100:0% v/v propylene glycol/water and Silastic® was investigated.

3. Results and discussion

Fig. 2 and 3 show that as the percentage of the propylene glycol is increased the saturated solubility of the drug increases. The pK_a of piroxicam is 5.3 (Herzfeldt and Kümmel, 1983) so at pH 4.2 more than 90% of the drug is in its non-ionised form. At percentages below 70:30% v/v propylene glycol/water the saturated solubility is slightly greater than those in propylene glycol/phosphate citrate buffer pH 4.2 but the reverse is true at percentages above 70:30% v/v propylene glycol/water. These differences are explained by the degree of ionisation of the drug in the polar solvent (water) and the solubility of the

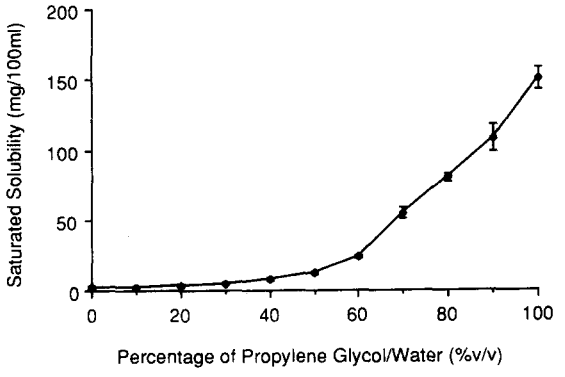

Fig. 2. Solubility of piroxicam in a propylene glycol/water cosolvent system at 25°C ($n = 3$; ± SD).

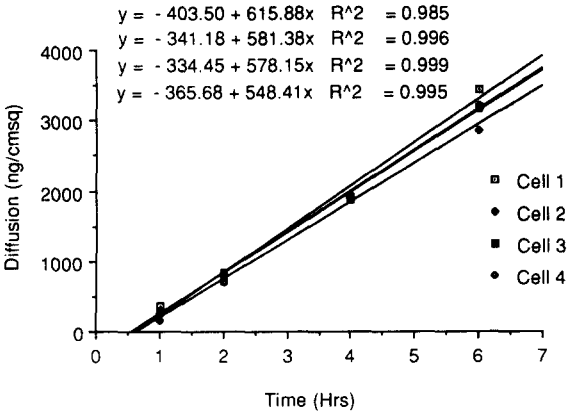

Fig. 4. Determination of flux of piroxicam across Silastic® from a saturated solution of 60:40% v/v propylene glycol/water.

increased proportion of the molecular species in the organic solvent (propylene glycol).

The flux of piroxicam from a vehicle was determined by plotting the amount of drug diffused against time for each cell and then calculating the average and standard deviation of the slopes at the linear part of the curve. Fig. 4 demonstrates the amount of piroxicam diffused against time for each cell when 1 ml of a saturated solution of 60:40% v/v propylene glycol/water is introduced to the donor compartment. The average flux was 581 ± 28 ng/cm^2 per h. The diffusion was shown to be linear for a further six hours after which it started to tail off probably due to depletion of the donor phase. Approx. 11 μg of piroxicam was delivered during the linear phase from a total loading dose of 236 μg per cell.

Fig. 5 depicts the linear response from plotting flux against the degree of both subsaturated and supersaturated solutions in the single cosolvent vehicle of 60:40% v/v propylene glycol/water.

Fig. 6 shows the effect of varying the ratio of the mixed phases on the flux, and also the degree of saturation predicted from the saturated solubility plots. Having already shown in Fig. 5 that a plot of flux against degree of saturation is linear for a single vehicle, it might have been expected that degree of saturation could be used to predict

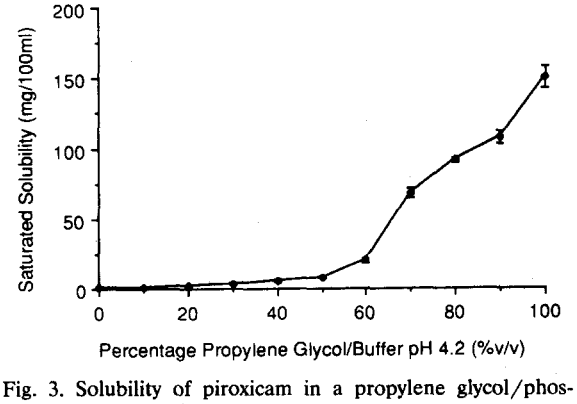

Fig. 3. Solubility of piroxicam in a propylene glycol/phosphate citrate buffer pH 4.2 cosolvent system at 25°C ($n = 3$; ± SD).

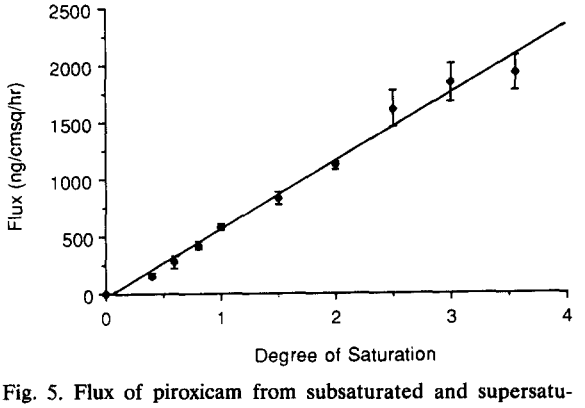

Fig. 5. Flux of piroxicam from subsaturated and supersaturated solutions of piroxicam in 60:40% v/v propylene glycol/water ($n \geq 3$; ± SD; $R^2 = 0.985$)

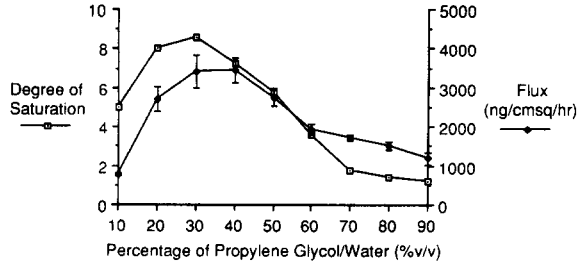

Fig. 6. A graph showing the maximum degrees of saturation from mixing different ratios of 140 mg/100 ml of piroxicam in propylene glycol with 2% HPMC solution and their corresponding fluxes ($n \geq 3$; $\pm$SD).

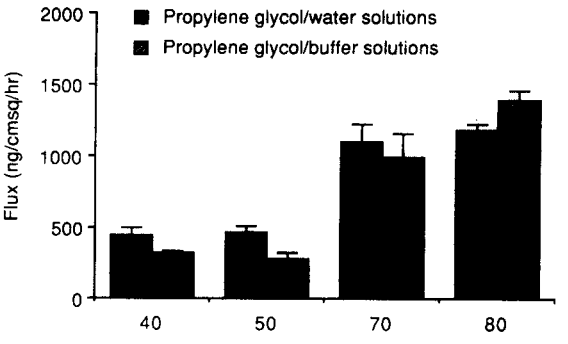

Fig. 8. Flux of piroxicam from saturated solutions of propylene glycol/water compared with fluxes from saturated solutions of propylene glycol/phophate citrate buffer pH 4.2 ($n \geq 3$; $\pm$SD).

flux. However, at high propylene glycol concentrations the flux is greater than expected whereas at low propylene glycol concentrations the flux is less than expected. This was further investigated by monitoring the flux from saturated solutions. As shown in Fig. 7, with high proportions of water the flux was less than that from solutions containing high proportions of propylene glycol. In an ideal situation, the product of the partition coefficient and the saturated solubility are constant giving a constant flux for all saturated solutions studied whereas non-ideal behaviour, i.e., varied flux from saturated solutions, is often attributed to vehicle-membrane interactions (Poulsen, 1972; Barry, 1983; Twist and Zatz, 1986). The results in Fig. 7 do not demonstrate ideal behaviour; two explanations were considered to account for this: (a) effect of ionisation and (b) vehicle-membrane interactions.

(a) It is well known that the molecular species of a drug will diffuse more readily than its ionised form (Swarbrick et al., 1984; Irwin et al., 1990). Therefore, to show how much of an effect this has on the flux of piroxicam across the membrane the diffusion of saturated solutions of propylene glycol/phosphate citrate buffer pH 4.2 were monitored. Fig. 8 compares the flux of piroxicam from saturated solutions of propylene glycol/water and propylene glycol/phophate citrate buffer pH 4.2. Statistical analysis of these data failed to show a significant difference ($p = 0.70$).

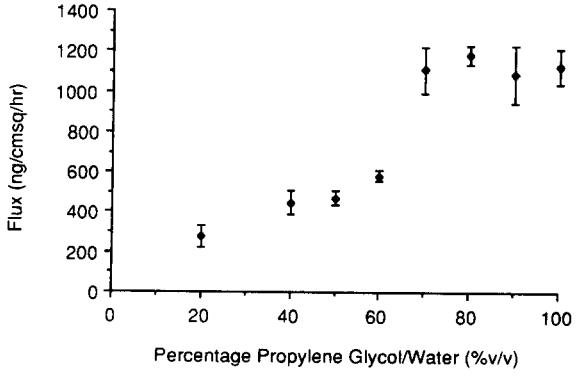

Fig. 7. Flux of piroxicam from saturated solutions of propylene glycol/water cosolvent systems at 25°C ($n \geq 3$; $\pm$SD).

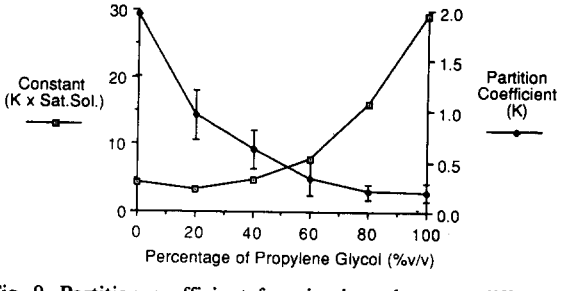

Fig. 9. Partition coefficient for piroxicam between different cosolvent vehicles and Silastic® ($n \geq 14$; $\pm$SD), and a plot of the product of the partition coefficient and saturated solubility (Sat. Sol.) against percentage of propylene glycol/water.

(b) Fig. 9 shows the partition coefficient for piroxicam between Silastic® and different vehicles, and also a plot of the product of the partition coefficient and saturated solubility against the respective cosolvent. In an ideal system, a plot of these two variables would be expected to give a straight line, but instead the product gradually increases with increasing propylene glycol content. Assuming that water has a relatively minimal effect on the membrane, these results suggest that the propylene glycol enhances the partitioning of the drug into the membrane. This may either simply be due to absorption of the propylene glycol or due to a more complex interaction between the components of the membrane and propylene glycol. Further investigations in this field are continuing.

4. Conclusions

This study has shown that in a single cosolvent vehicle of propylene glycol/water flux is directly proportional to degree of saturation. However, in a range of vehicles, if the proportion of propylene glycol is increased, flux is also increased but, if the proportion of water in the vehicle is increased, flux is decreased. It was shown by comparing the fluxes of various saturated solutions in propylene glycol/water with propylene glycol/phosphate citrate buffer pH 4.2 that the proportions of the molecular species to the ionised species were not responsible for the gradual increases in flux. Investigations into the partitioning of the drug between various vehicles and Silastic® showed that with increasing proportion of propylene glycol the partitioning of the drug into the membrane also increased. This work has shown that within these systems both the degree of saturation and vehicle-membrane interactions are important in controlling flux.

Acknowledgements

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