International Journal of Pharmaceutics, 76 (1991) 1–8

ADONIS 0378517391003264

IJP 02517

Research Papers

Effect of supersaturation on membrane transport: 1. Hydrocortisone acetate

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(Received 6 March 1991)
(Accepted 10 May 1991)

Key words: Supersaturation; Hydrocortisone acetate; In vitro membrane transport; Solubility; Cosolvent; Topical drug delivery

Summary

In vitro, the transport of hydrocortisone acetate across a model synthetic membrane has been investigated. Subsaturated, saturated and supersaturated solutions of hydrocortisone acetate, formed by mixing appropriate propylene glycol/water cosolvent systems, were studied. Transport was linearly proportional to the degree of saturation over the wide range studied. Supersaturated systems have potential application in topical drug delivery especially when release from saturated solutions is limiting.

Introduction

The importance of the degree, or fraction, of saturated solubility of a drug in a topical formulation on percutaneous absorption was first predicted by Higuchi (1960). Since then, many studies using subsaturated through to saturated systems have demonstrated a correlation between the degree of saturation and in vitro membrane transport, using both synthetic membranes (Poulsen et al., 1968; Flynn and Smith, 1972) and human skin (Ostrenga et al., 1971; Dugard and Scott, 1986), in vivo percutaneous absorption in man (Hadgraft et al., 1973; Woodford and Barry, 1982; Lippold and Schneeman, 1984) and clinical efficacy in man (Malzfeldt et al., 1989).

According to Higuchi (1960), supersaturated and other metastable states will increase transport beyond the limiting value achieved with saturated solutions. Despite early experimental evidence to support this hypothesis (Coldmann et al., 1969) there has, until recently, been little work on supersaturated systems presumably because of concern for their unstable nature.

Recently, however, the remarkable effects of antinucleant polymers in stabilising supersaturated solutions have been utilised to demonstrate marked improvement in percutaneous absorption from supersaturated systems (Kondo et al., 1987; Nitto Electric, 1987). Most previous work has used the effects of volatile additives to produce increasing, thus varying, degrees of supersaturation upon loss of additive.

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In this study hydrocortisone acetate in the mixed cosolvent system propylene glycol/water has been used to generate subsaturated, saturated and physically stable supersaturated solutions to allow comparison of their transport across a model membrane.

Materials and Methods

Materials

Hydrocortisone acetate was purchased from Roussel (U.K.). Polydimethylsiloxane membrane was purchased from Dow Corning (U.K.). All other chemicals used were of at least reagent grade and used without further purification.

Methods

Solubility of hydrocortisone acetate in water-propylene glycol cosolvent system

A series of water-propylene glycol mixtures were prepared from 100% water to 100% propylene glycol in 5% w/w increments. To each was added excess solid hydrocortisone acetate (HA) and the suspensions shaken for 24 h at controlled room temperature (23 ± 1°C). Room temperature was used for convenience, especially as previous work had shown little effect of temperature on saturated solubility of HA in the range 20–30°C. The suspensions were centrifuged and the supernatant solutions carefully sampled. The saturated solutions were assayed, diluted as required, by direct injection onto a suitable high-pressure liquid chromatographic system. Experimental conditions used were: ODS 250 mm × 4.6 mm i.d. column; ultraviolet detection at $\lambda = 240$ nm with methanol/water (65:35% v/v) as the mobile phase at a flow rate of 1.5 ml/min. Quantification was by external standard.

Mixing of cosolvent systems to produce subsaturated, saturated and supersaturated solutions

Depending upon the relative polarities of the solute and the binary cosolvent system, saturated solubility plots will often show an exponential increase with solvent composition as shown in Fig. 1 (curve B′B). A basic property of these systems is that by mixing suitable solute-cosolvent solutions, subsaturated, saturated and supersaturated solutions can be formed. Fig. 1 shows schematically that mixing system A (no solute in 100% solvent a) with system B (saturated solute in 100% solvent b) will result in systems C (subsaturated), D (saturated) and E and F (both supersaturated) depending upon the ratio of A to B used. In practice, systems A and B may themselves be mixed cosolvents and system B need not necessarily be saturated with solute. Thus, for example, in all experiments conducted a common donor solution, as represented by B, 0.08% saturated w/w hydrocortisone acetate in 88% propylene glycol/12% water, has been used with cosolvent systems as represented by A, G and H, to form the required degrees of saturation. Given the composition of B and the final composition required, the compositions of systems represented by G and H were calculated using a simple simultaneous equation. All systems represented by A, G and H contained 0.5% hydroxypropylmethyl cellulose as antinucleant polymer to stabilise the supersaturated solutions.

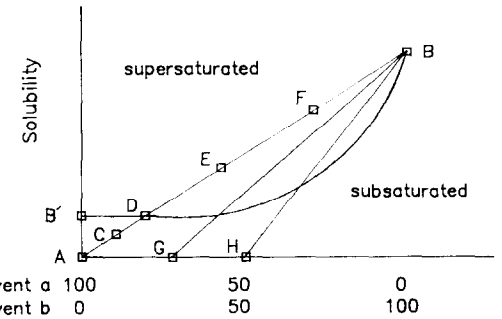

Fig. 1. Saturated solubility of a solute in a binary cosolvent system. Effects of mixing: B′B is the saturated solubility curve of solute in binary cosolvent system ab. System B (saturated solute in solvent b) is mixed with system A (no solute in solvent a) or systems G or H (no solute in cosolvent ab) to produce subsaturated, saturated or supersaturated solutions exemplified by C, D and E, F, respectively.

The degree of saturation of experimental solutions of varied composition was calculated by dividing the resulting concentration after mixing with the experimentally determined value of the saturated solubility at that resulting composition.

In vitro membrane transport cell methodology

A simple cylindrical glass cell was used. The receptor compartment (approx. 100 ml) and the donor compartment were clamped together with the membrane between flat flanges. The membrane surface area was approx. 20 cm^2. A port in the receptor compartment was used for sampling. Polydimethylsiloxane membrane (0.005 inch) was washed in water, dried, and then soaked in isopropyl myristate for 1 h and wiped with tissue to remove surface liquid. The receptor fluid used was propylene glycol/water (25:75% v/v) which was stirred at 100 rpm using a magnetic flea. The studies were conducted at controlled room temperature (23 ± 1°C). In previous experiments it was demonstrated that the addition of antinucleant polymer to supersaturated systems produced a (pseudo) stable state showing no measurable changes in transport when studied over several days after mixing. However, all solutions were studied within 1 h after mixing. 10 g samples of the solutions under study, an infinite dose, were placed on the donor side of the membrane. 100 μl of receptor was sampled at appropriate times. Analysis was as described under Solubility studies.

In vitro membrane transport studies

Three series of experiments were conducted (a–c).

(a) Transport from 0.02% w/w saturated hydrocortisone acetate: In order to confirm that the cell and membrane system chosen could be described by Fick's First Law of diffusion, transport of hydrocortisone acetate was studied from a 0.02% w/w saturated solution (propylene glycol/water, 56:44% w/w) over 8 h.

(b) Transport from subsaturated and saturated solutions: Poulsen (1972) has analysed several models of percutaneous absorption. In the simplest case, where the vehicle has no effect on the rate-limiting membrane, the partition coefficient between the vehicle and the membrane (P_c) is a reciprocal function of drug saturated solubility in the vehicle (C_v).

$$P_c = \text{constant}\frac{1}{C_v(\text{saturated})} \quad (1)$$

In these studies, P_c was not determined experimentally but was expressed as $1/C_v$(saturated) from Eqn 1.

In three experiments, the effects of concentration and partition coefficient (expressed as reciprocal saturated solubility in the vehicle) were studied. (1) A classical concentration response was studied from 0.005, 0.0067, 0.008, 0.10, 0.013 and 0.02% w/w hydrocortisone acetate all in a fixed vehicle of propylene glycol/water (56:44% w/w). These correspond to the following fractional degrees of saturation, 0.25, 0.33, 0.40, 0.50, 0.67 and 1. (2) A response to the partition coefficient was studied using 0.02% w/w hydrocortisone acetate in a range of vehicles chosen to correspond to the following fractional degrees of saturation, 0.25, 0.33, 0.40, 0.50, 0.67 and 1. (3) In Expts 1 and 2, either partition coefficient (expressed as reciprocal saturated solubility in the vehicle) or concentration was fixed with the other being varied. In this study, saturated solutions of 0.005–0.08% w/w were investigated in which both concentration and partition coefficient vary but in relation to each other so that their product is a constant. As transport is proportional to P_cC_v transport from these saturated systems was expected to be similar (Poulsen, 1972). Experiments were conducted over a 3 h period and reported as amount transported per h.

TABLE 1

Composition of supersaturated solutions produced by mixing B:A in various proportions

Ratio of B:A			Concentration (% w/w)	Degree of saturation
Vehicle No.	B	A		
1	1	0	0.08	1
2	1	1	0.04	4
3	1	2	0.027	6.9
4	1	3	0.02	8.33
5	1	7	0.07	6.67
6	1	15	0.005	3.84

B: 0.08% w/w saturated hydrocortisone acetate (vehicle propylene glycol/water, 88:12 w/w); C: 100% water containing 0.5% antinucleant polymer. Degree of saturation was calculated by reference to Fig. 1.

(c) Transport from supersaturated solutions: Two experiments were conducted. (1) At a fixed concentration of 0.02% w/w hydrocortisone acetate, transport from times 1, 2, 4, 5, 6 and 8 degrees of saturation were studied. Vehicle compositions were calculated by reference to Fig. 1. (2) The effect of mixing systems equivalent to A and B in Fig. 1 in varied ratios was investigated. In this experiment, concentration, degree of saturation and their product was varied. Table 1 lists the ratios used and the resulting concentrations and degrees of saturation. Experiments were run over 1 h and reported as amount transported per h.

Results

Fig. 2 shows the saturated solubility of hydrocortisone acetate in propylene glycol/water cosolvent system. As the percent propylene glycol increases, the saturated solubility of hydrocortisone acetate also increases but in an approx. exponential manner. This exponential increase has been observed with other solutes in propylene glycol/water cosolvent systems and these results are very similar to values reported previously (Yalkowsky and Roseman, 1981).

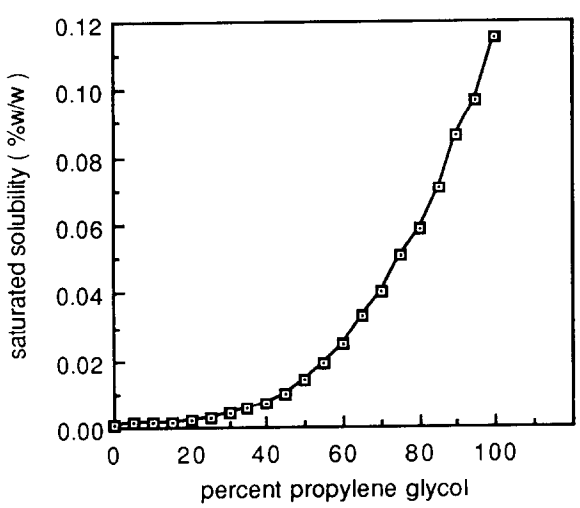

Fig. 2. Saturated solubility of hydrocortisone acetate in water-propylene glycol cosolvent system at 23°C. Mean, $n = 3$.

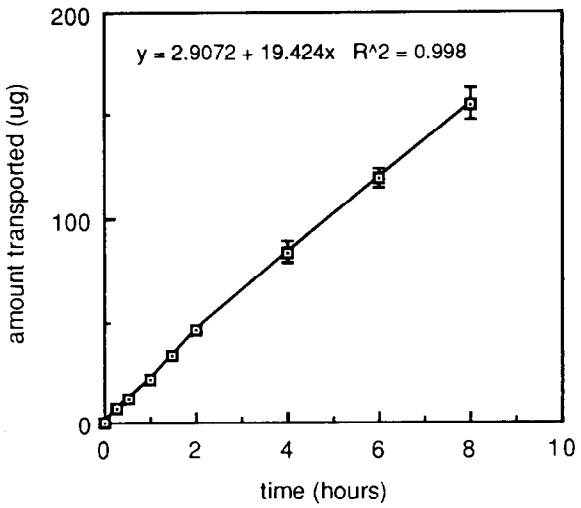

Fig. 3. Transport of hydrocortisone acetate from a saturated 0.02% w/w solution over 8 h (vehicle propylene glycol/water, 56:44% w/w). Demonstration of Fickian diffusion. Mean, $n = 3$, ±S.E.

This plot was used as shown in Fig. 1 to design formulations of varying concentration and degree of saturation produced by mixing appropriate cosolvent-solute systems. Degree of saturation was determined by dividing actual concentration achieved after mixing by the value for saturated solubility, from Fig. 2, for the vehicle composition after mixing.

Fig. 3 shows transport from a 0.02% w/w saturated hydrocortisone acetate formulation over 8 h. Transport is almost linear up to 8 h with approx. 160 μg total release. Slight deviation at later times is probably due to depletion effects from the total loading of 2000 μg per cell. Linearity of transport demonstrates that the membrane is rate limiting.

Fig. 4a–d shows transport from subsaturated to saturated solutions. Fig. 4a shows the classical linear response between transport and concentration for a single vehicle and thus fixed partition coefficient. Less well recognised, Fig. 4b shows the linear response between transport and partition coefficient for a fixed concentration of 0.02% w/w. Fig. 4c redraws these data using degree of saturation as the common abscissa.

Fig. 4d shows transport from saturated solutions from 0.005 to 0.08% w/w hydrocortisone

acetate in which both concentration and partition coefficient vary but such that the product of these two parameters is a constant. Release is similar from all saturated solutions studied.

Fig. 5 shows transport from 0.02% w/w hydrocortisone acetate at times 1 to times 8 degrees of saturation. A clear linear response between transport and degree of saturation is seen.

Fig. 6 shows transport from solutions of different concentrations and degrees of saturation in the supersaturated range as formed from mixed type A and B cosolvent systems in varying ratios,

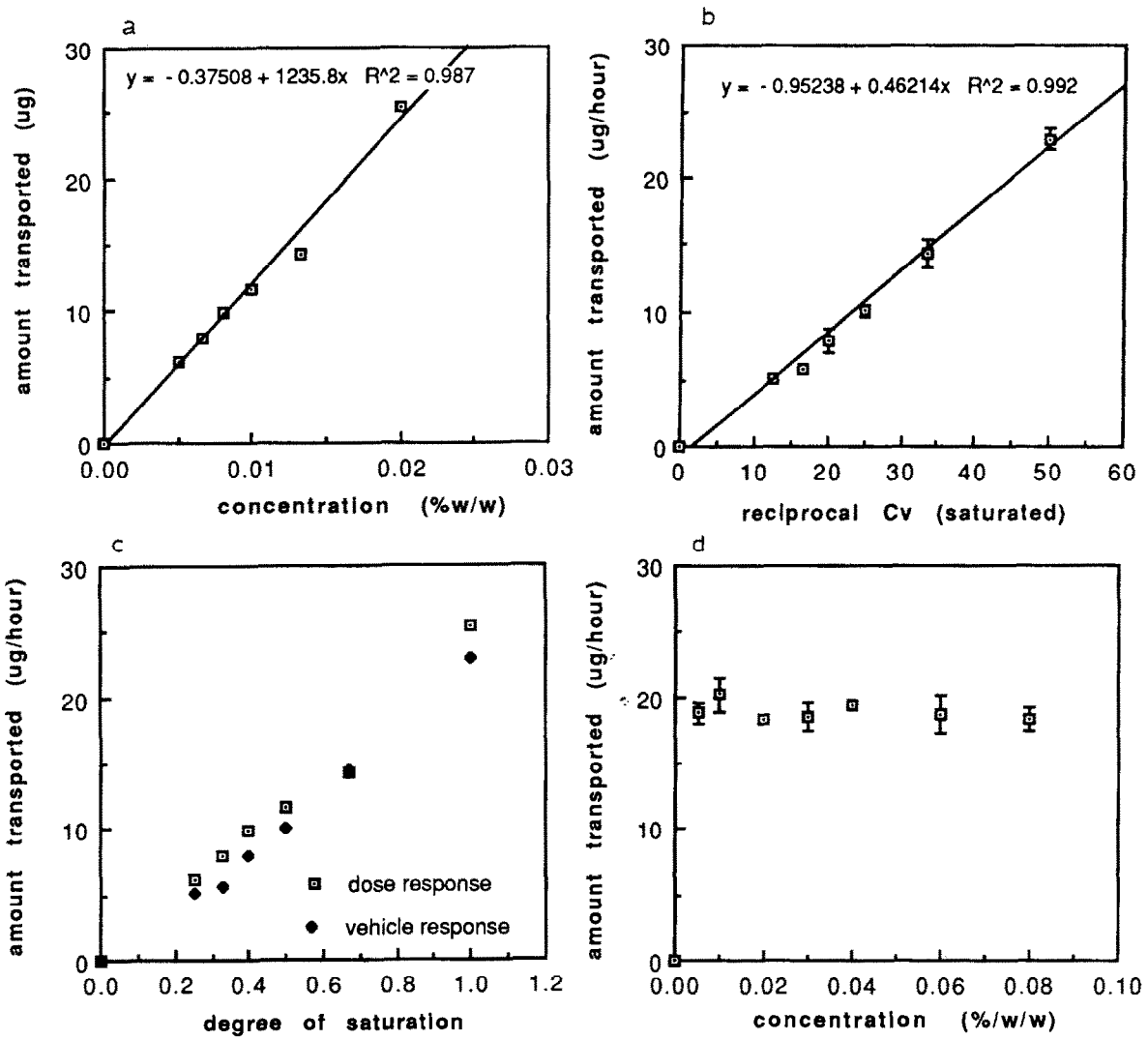

Fig. 4 (a). Transport of hydrocortisone acetate from 0.005 to 0.02% w/w in a single vehicle (propylene glycol/water, 56:44% w/w). Demonstration of response to dose. Mean, $n = 3$, $\pm$S.E. (b) Transport of hydrocortisone acetate, 0.02% w/w from different propylene glycol/water vehicles. Demonstration of response to partition coefficient expressed as reciprocal saturated solubility. Mean, $n = 3$, $\pm$S.E. (c) Comparison of response of concentration and vehicle partition coefficient, expressed as degree of saturation, to transport of hydrocortisone acetate (data as in Panels a and b). (d) Transport of hydrocortisone acetate from 0.005 to 0.08% w/w saturated solution. Increase in concentration is offset by decrease in partition coefficient. Mean, $n = 3$, $\pm$S.E.

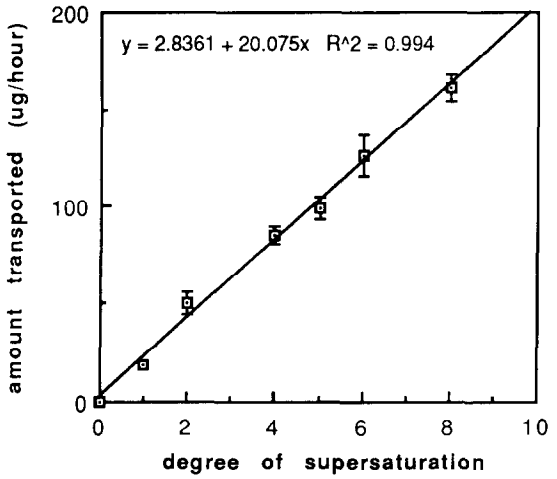

Fig. 5. Transport of hydrocortisone acetate, 0.02% w/w from supersaturated vehicles. Demonstration of response to degree of supersaturation. Mean, $n = 6 \pm$S.E.

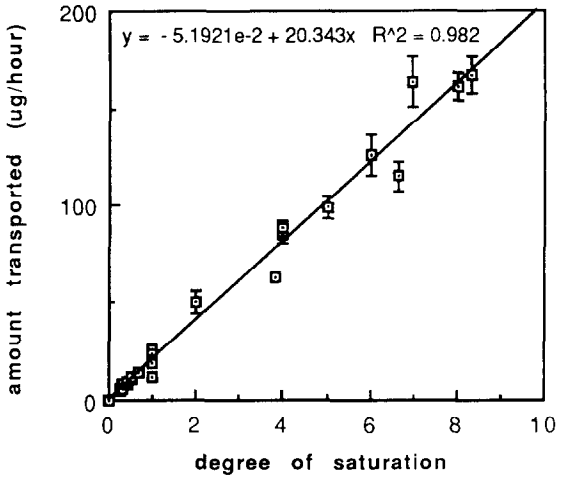

Fig. 7. Linear relationship between transport of hydrocortisone acetate and degree of saturation over the range of subsaturated to supersaturated systems. Combined data from Figs. 4–6. Mean ($n = 3$ or 6) $\pm$S.E.

as shown schematically in Fig. 1. Transport again is proportional to degree of saturation. An interesting property of this system is the maximum value of release corresponding to a ratio of A : B = 3 : 1 at approx. 0.02% w/w times 8.3 degrees of saturation.

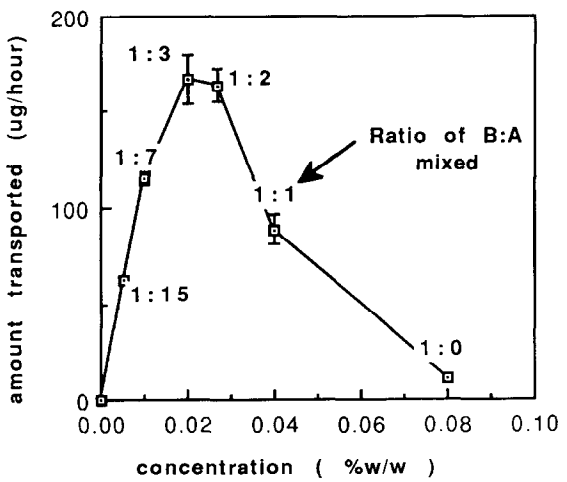

Fig. 6. Transport of hydrocortisone acetate from supersaturated solutions produced by mixing various ratios of B : A (as in Fig. 1). A plateau of transport is seen at around 0.02% w/w times 8 degrees of saturation. See Table 1 for details of compositions.

Finally, Fig. 7 shows the linear relationship between transport and degree of saturation over the range from subsaturated to supersaturated for all data shown in Figs 4–6.

Discussion

The in vitro transport of hydrocortisone acetate from solutions of varying concentration and partition coefficient as expressed by reciprocal saturated solubility has been shown to be proportional to degree of saturation. Transport is linear from subsaturated through to saturated solutions, as would be anticipated from the literature, (Poulsen et al., 1968; Flynn and Smith, 1972) but this work confirms the observations of Theeuwes et al. (1976), that supersaturated systems result in linear transport proportional to their degree of saturation and beyond the limiting transport value from saturated systems.

The driving force for diffusion across the stratum corneum is the concentration of diffusant within the outermost layer of the skin. This concentration is a mathematical product of the concentration within the vehicle times the partition

coefficient between the vehicle and the stratum corneum (Higuchi, 1960; Poulsen, 1972).

The effects of concentration on response are clearly established, for example, dose responses are the basis of pharmacological and toxicological investigations. The response, of transport, shown in Fig. 4a is a further example of this. Less well established is the linear response to vehicle partition coefficient as depicted in Fig. 4b. Fig. 4c shows that both parameters can be expressed as degree of saturation which, as a single variable, is proportional to transport. Fig. 4d shows that transport from all saturated systems is within experimental limits, as found previously (Hadgraft et al., 1973; Theeuwes et al., 1976; Woodford and Barry, 1982; Dugard and Scott, 1986).

Thus, Fig. 4 shows the response of the in vitro model to both concentration and partition coefficient. Synthetic membrane systems such as used here are often criticised as not being representative of human skin. Mainly this is due to vehicle/membrane interactions which are specific to the skin or membrane being studied and are not easily simulated one by the other. Fig. 4d demonstrates that as release from all saturated systems is similar, vehicle/membrane interactions in the present study are likely to be insignificant. Thus, models similar to that used here may be useful in predicting the drug/vehicle interaction component only of transport across human skin.

Fig. 5 shows that transport from supersaturated systems is linearly proportional to degree of supersaturation at a fixed concentration and Fig. 7 demonstrates that all data reported here from 0.25 times subsaturated to 8 times supersaturated fit a single linear relationship between transport and degree of saturation.

The ability of supersaturated systems to increase transport beyond the limiting value imposed by saturated systems is clearly of interest in topical drug delivery. Fig. 6 shows data to evaluate the potential of mixed cosolvent systems in topical drug delivery. By suitable selection of the ratio and design of the cosolvent system, large increases in degree of saturation can be achieved. Fig. 6 shows that by suitable selection of ratios of B : A, a plateau response can be achieved whereby slight variation in change of ratio as might occur for various reasons in practical pharmaceutical products will not significantly alter degree of saturation or transport.

Further work is currently in progress to evaluate the drug delivery potential of these systems.

Acknowledgement

The authors thank Mr G. James for his contribution to this work.

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