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Use of a molecular form technique for the penetration of supersaturated solutions of salicylic acid across silicone membranes and human skin in vitro

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Abstract

Permeation enhancement of salicylic acid (SA) from supersaturated solutions formed using a 'molecular form' technique was investigated. In a conventional cosolvent technique, two solvents are used, one in which the drug is considerably more soluble than the other. Propylene glycol and water have been predominantly used as cosolvents to create supersaturation in skin permeation enhancement. In this paper, we report the use of buffer solutions with different pHs as media for producing different molecular forms.

Supersaturated solutions were prepared using pH 8:pH 2 (80:20 v/v), which gave a nominal pH when mixed of around 5. Model silicone membranes and human skin were used. Hydroxypropyl methyl cellulose (HPMC) was employed to stabilise the supersaturated states. Stability data showed that while the SA supersaturated solutions without HPMC crystallised between 15 min and 46 h depending on the degree of supersaturation, the solutions with HPMC were stable for more than 2 months. The flux of SA increased with the degree of saturation for solutions prepared in a 80:20 buffer pH 8/buffer pH 2 mixture. Although the fluxes of SA with and without HPMC were similar both through silicone membrane and human skin, HPMC was found to be effective in increasing the stability of supersaturated solutions of SA.

Keywords: Supersaturation; pH; Silicone membrane; Skin; Salicylic acid; Permeation enhancement

1. Introduction

The penetration of drugs into the skin is often limited by the barrier function of the stratum corneum. Supersaturation to increase percutaneous absorption has attracted interest, not only because of its low cost but also because it does not perturb the intercellular lipids. This method has demonstrated the quantitative relationship between the degree of saturation and permeation rate. The permeation of drugs such as oestradiol (Megrab et al., 1995), piroxicam (Pellett et al., 1997a), ibuprofen (Iervolino et al., 2000), hydrocortisone acetate (Raghavan et al., 2000) and a lavendustin derivative (Moser et al., 2001a) through artificial membranes and/or skin has been shown to increase with increasing degree of saturation in the vehicle.

However, one of the drawbacks of this method is the thermodynamic instability of the supersaturated solutions leading to crystallisation of the drug and hence a decrease of the drug flux. Nevertheless, it has been shown that addition of certain polymers either inhibits or retards the crystallisation processes and increases the stability of such unstable supersaturated states (Raghavan et al., 2001a). For example, supersaturated solutions of piroxicam were stabilized by hydroxypropyl methyl cellulose (HPMC) (Pellett et al., 1997b). Similar results were reported with different polymers such as methyl cellulose (Raghavan et al., 2000, 2001b).

Supersaturated solutions are usually produced by a cosolvent technique, which involves mixing two solvents where the drug is significantly more soluble in one solvent than the other (Davis and Hadgraft, 1991). In most of the studies, propylene glycol and water have been used as cosolvents. The primary reason for using propylene glycol is the fact that it is commonly used in topical formulations. Propylene glycol is also

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thought to increase the drug solubility in the skin (Moser et al., 2001b).

According to the pH – partition theory, the solubility of an acidic drug increases with the pH. In fact Watkinson et al. (1993) reported the solubility of ibuprofen as a function of pH, which showed an exponential behaviour similar to that obtained with PG–water cosolvent mixtures. This gives rise to the interesting possibility of using buffer solutions with different pHs as 'cosolvents'. The advantage of using such a system is two-fold;

(i) it avoids the use of two different solvents which influence the homogeneity of the solutions and
(ii) suitable 'mixtures can be so chosen that the final mixture produces a pH similar to that of the skin (∼5).

The aim of the present work was to investigate the possibility of preparing stable supersaturated solutions by mixing buffer solutions of different pH. A model drug, salicylic acid (SA), a keratolytic and anti-inflammatory, was chosen for the study. SA has a pK_a of 3 and a log P of 2.1 (calculated using ACD software, Canada). Initial permeation studies were conducted using model silicone membranes, as these are homogenous and facilitate investigation of the effects of formulation and hence interpretation of the data. Permeation studies were subsequently extended to excised human skin.

2. Materials and methods

2.1. Materials

Salicylic acid (SA) and HPLC methanol grade were purchased from Fisher Scientific International Company (UK). Hydroxypropyl methyl cellulose (HPMC) grade 65SH-viscosity 50cP was obtained from Shin-Etsu Chemical Co. Ltd., Tokyo, Japan. Silicone membrane was purchased from Samco Ltd., St Albans, UK.

Combinations of two buffers were mixed to obtain the required pH values. Phosphate and citrate buffers were prepared using Na_2HPO_4 (0.2 M) and $C_6H_6O_7$ (0.1 M). All reagents were purchased from Fisher Scientific International Company (UK).

2.2. Methods

2.2.1. Solubility studies

Saturated solubility curves of SA (with and without HPMC) as a function of pH (varying from pH 2 to 9) were constructed. Saturated solutions were prepared by adding excess drug to the solution and stirring for 48 h at 32 °C. After this period, the solutions were centrifuged, the supernatant was removed, suitably diluted and assayed using HPLC.

2.2.2. Preparation and stability of supersaturated solutions

The maximum degree of saturation (DS) that could be achieved by mixing a saturated solution of SA in buffer solution at pH 8 (68.3 mg/ml) with buffer solution at pH 2 with or without HPMC (1%) was calculated to be 7.5 degrees of saturation in a 60:40, buffer pH 8/buffer pH 2 mixture (Table 1). As the skin pH is close to 5, a mixture of buffer pH 8/buffer pH 2 (80:20, v/v) was used (final pH 5). The degrees of saturation achievable by mixing a solution of SA in buffer solution at pH 8 (at different concentrations 17.4, 34.7, 52.1, 60.8 and 68.3 mg/ml) with buffer solution (pH 2) with or without HPMC were 2, 3, 3.5 and 4, respectively. Supersaturation was calculated from the 'cosolvent' solubility plot (Fig. 1) by dividing the concentration of the drug in the solution by its saturated solubility in the 'cosolvent' mixture.

Table 1
Degrees of saturation that can be prepared by mixing a solution of salicylic acid (SA) (68 mg/ml) in buffer solution at pH 8 with buffer solution at pH 2 (with or without HPMC (1%)

Solvent composition (% of buffer pH 8/%buffer pH 2)	SA concentrations (mg/ml)	Final pH	Degree of saturation
90:10	61.2	6.5	3.4
80:20	56	5	4
60:40	40.8	3.2	7.5
50:50	34	3	6.5
40:60	27.2	2.8	3.5
20:80	13.6	2.5	2
10:90	6.8	2.4	1.4

2.2.3. HPLC analysis

HPLC analysis of SA was performed using a Spectra Series P100 isocratic pump (Thermo Separation Products, Riviera Beach FL) set at a flow rate of 1 ml/min, with a Spectra Series AS100 autosampler, a Spectra Series UV 100 detector set at 305 nm and a Spectra Series SP 4400 integrator. The stationary phase was an Apex reverse phase ODS 5 μm packed column (250 mm × 4.6 mm). A guard column (ODS, 4 mm × 3 mm) was used in conjunction with the column. The mobile phase was methanol:phosphate buffer, pH ∼3 (60:40). Calibration curves were constructed on the basis of the peak area measurements, using standard solutions of known concentrations. Samples were injected via a 20 μL loop and the retention time was ∼7 min.

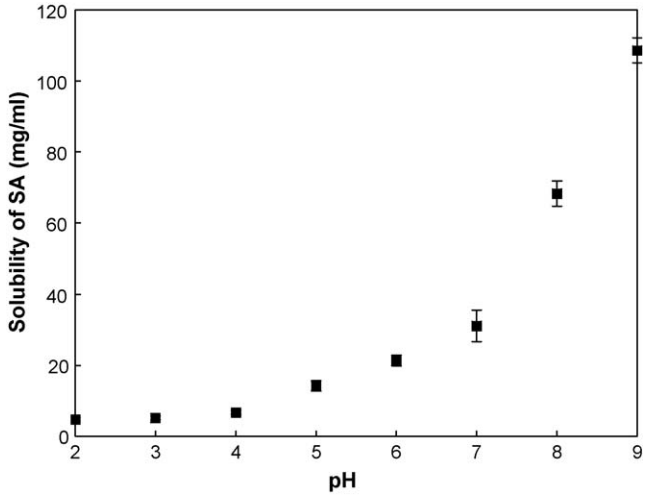

Fig. 1. Solubility of SA as a function of pH (mean ± S.D., $n = 3$).

2.2.4. Microscopy

The solutions were analysed for the presence of crystals by observation on microscope slides using a Leica Galen microscope (Switzerland).

2.2.5. Diffusion studies

The diffusion of saturated and supersaturated solutions across 300 μm thick silicone membranes and human epidermis were investigated. Human epidermis was prepared from full thickness skin using the heat separation technique (Kligman and Christophers, 1963). Diffusion experiments were conducted using Franz cells with a receptor volume of 3.9 ml and a diffusional surface area of about 0.95 cm^2. The receptor chambers had side arms through which samples could be taken. Phosphate buffered saline, pH 7.4, was used as a receptor phase. Silicone grease was used to produce leak-proof seals between the membranes and the two compartments of the diffusion cells. A magnetic bar stirred the receptor phase, and the diffusion cells were placed in a water bath at 37 °C. A volume of 1 ml of SA solution was placed in the donor compartment. During the experiment, the donor compartments and sampling arms were occluded to prevent evaporation. At predetermined intervals, every 2 h during 12 h, 0.4 ml of the receptor phase was removed and replaced with an equal volume of pre-thermostatted receptor phase. The samples were assayed by HPLC.

The following series of experiments were performed:

1. Diffusion of SA from saturated solutions in different pH solutions (pH 2, 5, and 8).
2. Diffusion of SA from saturated solutions in different pH solutions with 1% HPMC (pH 2, 5, and 8).
3. Diffusion of SA from supersaturated solutions with different degrees of supersaturation in a cosolvent vehicle of pH 8/2 (80:20, v/v).
4. Diffusion of SA from supersaturated solutions with different degrees of supersaturation in a cosolvent vehicle of pH 8/2 with 1% HPMC (80:20, v/v).

At least three replicates were conducted.

3. Results and discussion

3.1. Solubility studies

The solubility of SA in buffer solutions increased exponentially with an increase in pH (Fig. 1). HPMC (1%) did not influence the solubility of SA. The solubility of SA in buffer solution at pH 8 is almost 14-fold higher than at pH 2. This is in good agreement with the pH – solubility theory: solubility increases with pH as the degree of ionisation increases with increasing pH (Hadgraft and Valenta, 2000).

As the skin surface pH is near 5, a mixture of buffer at pH 8 and buffer at pH 2 (80:20, v/v) (Table 1) was used in the following investigations. The DS achievable by mixing a solution of SA in buffer (pH 8) at 17.3, 34.7, 52.1, 60.8 and 68.3 mg/ml of drug with buffer solution at pH 2 (80:20, v/v) are 1, 2, 3, 3.5 and 4, respectively.

Table 2
Physical stability data at 32 °C for supersaturated solution of varying DS of SA with and without HPMC in buffer pH 8/buffer pH 2 (80:20, v/v)

SA concentration in buffer pH 8 (mg/ml)	DS	Stability without HPMC (h)	Stability with HPMC (months)
17.3	1	–	–
34.7	2	<45	>2
52.1	3	<45	>2
60.8	3.5	<45	>2
68.3	4	<46	>2

3.2. Physical stability

The physical stability of supersaturated solutions of SA with different DS was studied. The stability data were obtained by observing the first appearance of crystals under the microscope. Table 2 shows the stability at different degrees of saturation of supersaturated and saturated solutions in 80:20 buffer pH 8/buffer pH 2 mixtures with and without HPMC (1%). The solutions at 2, 3 and 4 DS appeared to be stable for the duration of the diffusion experiment. However, these solutions remained opaque between 20–46 h depending on the DS and did not show observable growth. When HPMC (1%) was introduced to the mixtures as an antinucleant polymer, the supersaturated solutions were transparent for up to 2 months.

3.3. Diffusion studies

3.3.1. Influence of pH

Diffusion studies on silicone membrane at pH 2, 5 and 8 were performed using Franz-cell type diffusion cells as described. The highest steady state flux was determined at pH 2 when SA is unionised ($pK_a = 3.1$) (Table 3). The results indicated that the SA flux significantly decreased with the amount of ionised species. The fraction ionised (f_{ion}) can be calculated according to the following equation:

$$f_{ion} = \frac{1}{1 + 10^{(pK_a - pH)}}$$

Under these conditions, permeation followed the pH partition theory, the transmembrane permeation essentially resulted from the unionised species and the contribution of the ionised species was negligible. These results were in good agreement with those previously reported (Leveque et al., 2004; Smith and Irwin, 2000).

In order to see if similar results could be obtained using human skin, permeation experiments were performed with epi-

Table 3
Flux of SA across silicone membrane from a saturated solution in buffer pH 8/buffer pH 2 (80:20, v/v) in the absence and presence of HPMC ($n = 4$; mean ± S.D.)

pH	Fraction ionised	Flux without HPMC (μg/cm^2/h)	Flux with HPMC (μg/cm^2/h)
2	0.0736	92.1 ± 5.8	90.4 ± 4.4
5	0.9876	51.6 ± 6.2	54.1 ± 3.2
8	0.9999	37.9 ± 2.4	40.4 ± 2.5

Table 4
Flux of SA across human skin from saturated solution in buffer pH 8/buffer pH 2 (80:20, v/v) in the absence and presence of HPMC ($n > 3$; mean ± S.D.)

pH	Flux without HPMC (μg/cm^2/h)	Flux with HPMC (μg/cm^2/h)
2	162.6 ± 10.2	156.2 ± 11
5	108.4 ± 6.8	107.7 ± 8.8
8	55.8 ± 3.9	51 ± 6.0

dermal membranes. Saturated donor phases at pH 2, 5 and 8 were used. Similar results were obtained with skin compared with silicone membrane (Tables 3 and 4). As pH increases, the solubility increases with the degree of ionisation, but the permeability coefficient decreases. In the case of skin, the flux of SA was higher than with silicone membrane. This could be explained by the modulation of the barrier function as a result of the keratolytic effect of SA at these relatively high concentrations. This would not occur in the silicone membrane.

3.3.2. *Diffusion of saturated and supersaturated solutions of SA across silicone membrane*

For the preparation of supersaturated solutions, a mixture of 80:20 buffer pH 8/buffer pH 2 (v/v) was chosen. This mixture gave a final pH of 5. As mentioned earlier, this pH was deliberately chosen to match the pH of the skin surface. Supersaturated solutions at different DS were prepared using different SA concentrations in buffer solution at pH 8 (from 17.3 to 68.3 mg/ml). Transport measurements were performed for the supersaturated solutions in the absence and presence of HPMC. A fixed HPMC concentration of 1% was used in the preparation of the solutions.

Diffusion of SA across silicone membranes increased with increasing DS (Figs. 2 and 3). It appears that this system has long lag times and true steady-state diffusion was not reached during the time course of the experiment. Therefore, steady state flux values cannot be determined directly using solutions to Fick's first law. The permeation profiles were also analysed using non-linear curve fitting to the following equation:

$$u = c_{\text{app}}\alpha\left(\beta t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)}{n^2}\exp(-n^2\pi\beta t)\right)$$

where u is the concentration in the receptor phase at time t, c_{app} the applied concentration, α and β are the two unknowns

$$\alpha = Kh$$

and

$$\beta = \frac{D}{h^2}$$

K is the partition coefficient between the skin (of thickness h) and the applied formulation and D is the diffusion coefficient in the skin. The product $\alpha\beta$ is equal to the permeability coefficient k_{p} and therefore the predicted steady state flux is $c_{\text{app}}\alpha\beta$ The flux of SA was calculated and plotted as function of DS (Fig. 4). The flux increases proportionally to the DS indicating that the

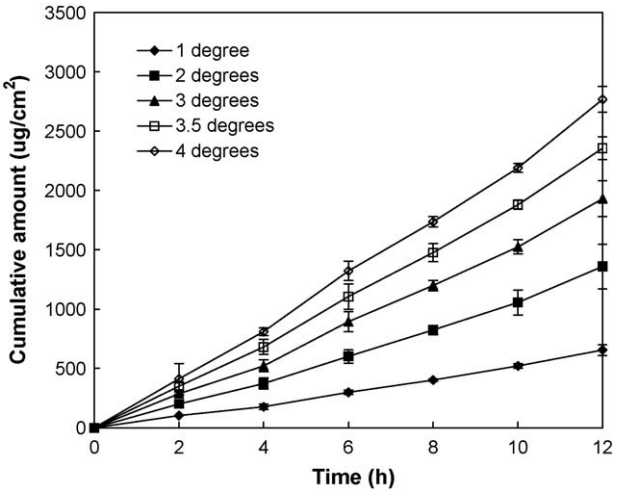

Fig. 3. Diffusion of SA from saturated and supersaturated solutions across silicone membrane in the presence of HPMC (mean ± S.D., $n \geq 3$).

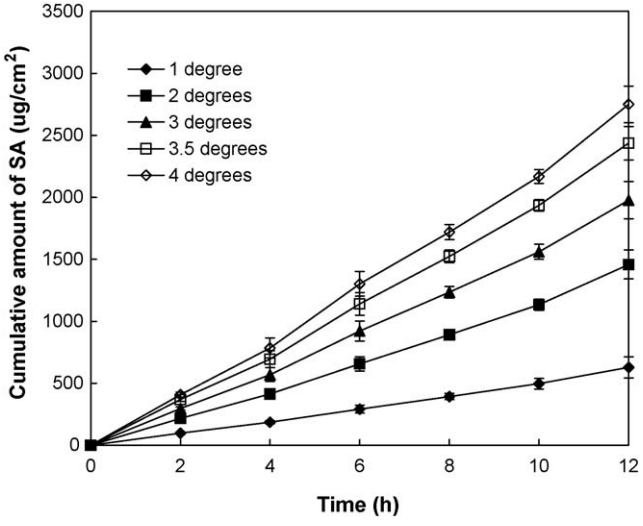

Fig. 2. Diffusion of SA from saturated and supersaturated solutions across silicone membrane in the absence of HPMC (mean ± S.D., $n \geq 3$).

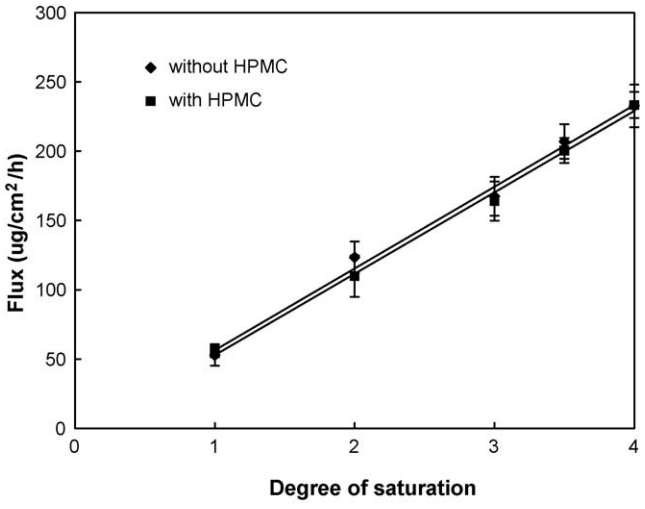

Fig. 4. Flux of SA from supersaturated solutions across silicone membranes without and with HPMC (mean ± S.D., $n \geq 3$).

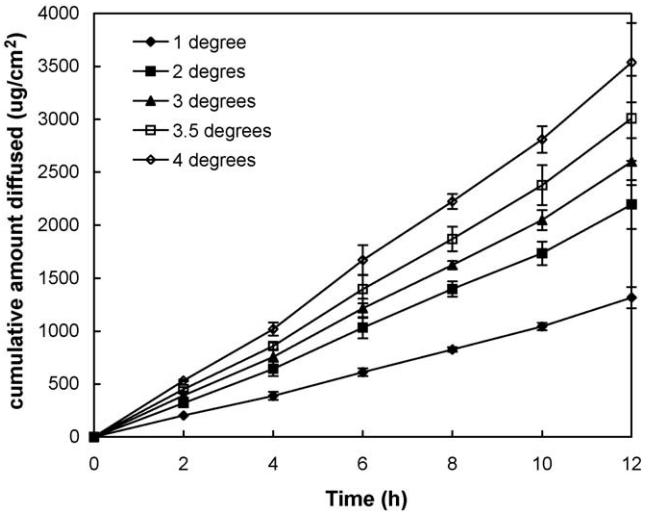

Fig. 5. Diffusion of SA from saturated and supersaturated solutions across human skin in the absence of HPMC (mean ± S.D., $n \geq 3$).

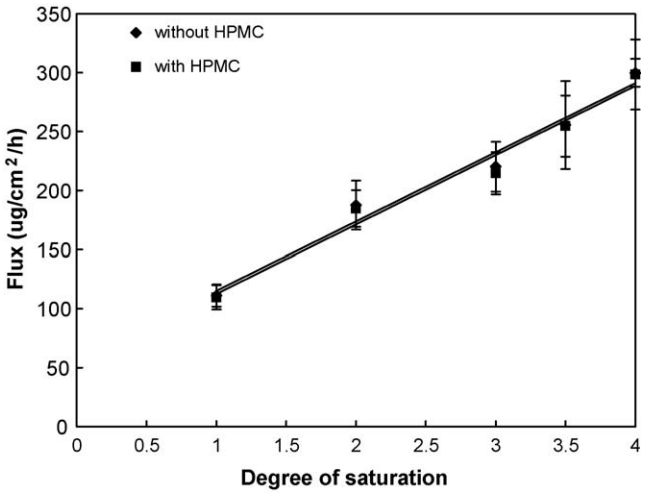

Fig. 7. Flux of solutions of SA across human skin without and with HPMC (mean ± S.D., $n \geq 3$).

supersaturated systems are stable for the duration of the experiment. In fact, linear regression analysis of the data shows that the degrees of correlation (R^2) between the flux and DS in the absence and presence of HPMC are 0.995 and 0.993, respectively. These results may seem to negate the need for the use of polymer. However, in formulating supersaturated systems, one needs to consider the stability of these systems on storage for lengthy periods of time. The stability data given in Table 2 shows that supersaturated systems, which did not contain any HPMC, were not stable for more than 46 h depending on the DS chosen; solutions containing HPMC were stable for more than two months. Polymers are known to inhibit nucleation as well as crystal growth (Simonelli et al., 1970; Sekikawa et al., 1978; Ziller and Rupprecht, 1988; Raghavan et al., 2001b) and HPMC has been found to be an effective crystal growth inhibitor for a number of pharmaceutical compounds (Iervolino et al., 2000; Raghavan et al., 2001a; Pellett et al., 1997b).

3.3.3. *Diffusion of saturated and supersaturated solutions of SA across human skin*

The cumulative amounts diffused across human skin are shown in Fig. 5 (without HPMC) and Fig. 6 (with HPMC). As with the silicone membrane there was an increase in the total amount of drug diffused with increasing DS. There was no statistical difference between the flux of SA without and with HPMC. Although the flux at 2 × DS was twice that for the saturated solutions, the flux at higher DS was reduced. Such a phenomenon was however not observed with silicone membrane. The reduced flux across skin suggests a decrease of thermodynamic activity; Iervolino et al. (2001) found a similar behaviour with ibuprofen. They reported that the flux in skin was significantly lower than that was expected for the supersaturated systems. They explained the behaviour as resulting from the rough surface of the stratum corneum inducing secondary nucleation which causes a decrease in the concentration and hence the thermodynamic activity of the drug. Just as in the case of flux from saturated solutions, the flux of SA through skin was slightly higher than that through silicone membrane (Fig. 7).

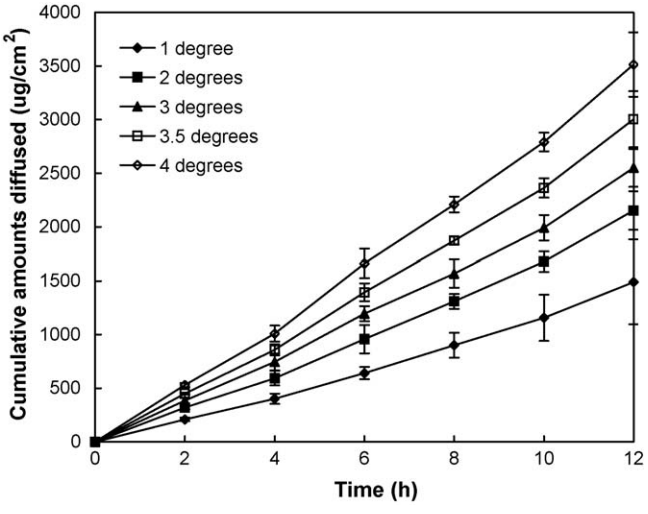

Fig. 6. Diffusion of SA from saturated and supersaturated solutions across human skin in the presence of HPMC (mean ± S.D., $n \geq 3$).

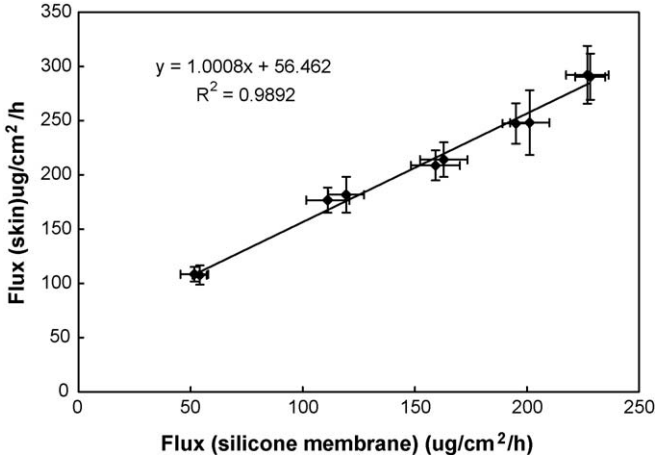

Fig. 8. Flux of SA from supersaturated solutions through human epidermis as a function of flux of SA from supersaturated solutions through silicone membrane.

Fig. 8 shows the relation between the flux of SA from supersaturated solutions through skin and through silicone membrane. The data show a good correlation. Similar results were earlier reported for ibuprofen (Iervolino et al., 2001). Such a good correlation supports the view that silicone membranes can be used as an effective model membrane to study the formulation effects provided permeation enhancers which interact with skin lipids are not present.

4. Conclusions

Importantly, this study shows that a 'molecular form' pH technique can be used with solutions to produce supersaturated solutions. The results demonstrate that permeation of SA through silicone membrane and human skin at different pH conforms to the pH partition theory. This study has also investigated the feasibility of using supersaturated solutions of SA for enhancing the permeation across silicone membrane as well as human skin. The permeation of SA through silicone membrane and human skin increased with increasing degree of saturation. The permeation of SA in the absence or presence of HPMC was not statistically different with silicone membrane or human skin. Addition of HPMC (1%) to the supersaturated solution increased the stability for more than two months indicating that HPMC plays a vital role in the stability of supersaturated systems.

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