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The solubility parameter of vehicles as a predictor of relative vehicle effects on the diffusion of 6-mercaptopurine

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Summary

6-Mercaptopurine (6-MP) is active against psoriasis when given systemically but inactive when given topically. In order to optimize the rate of delivery of 6-MP through skin, the fluxes of 6-MP from vehicles and mixtures of vehicles exhibiting solubility parameters (δ_v) from 7.6–23.4 $(cal/cm^3)^{1/2}$ have been measured in diffusion cell experiments using hairless mouse skins as the membranes. For single component vehicles there was a good correlation between experimental permeability coefficients (K_p) obtained from the fluxes divided by the respective solubilities and theoretical partition coefficients (PC) obtained from:

$$\log PC = \left[(\delta_v - \delta_i)^2 - (\delta_s - \delta_i)^2\right] V_i \phi_v^2 / 2.3RT$$

where δ_v, δ_i and δ_s are the solubility parameters of the vehicle, 6-MP and skin, respectively, V_i is the molar volume of 6-MP and ϕ_v is the volume fraction of the vehicle. Thus, log (experimental K_p) was equal to log(theoretical PC) − 3.33. All but one of the exceptions to the correlation were observed for vehicles that were found to have decreased the relative resistance of the skin to topical absorption as determined by the degree of enhanced diffusion obtained from a second application of a solute (theophylline) in propylene glycol — a vehicle that apparently does not appreciably decrease the resistance of the skin barrier. Water as a vehicle did not appear to decrease the resistance of the skin barrier but the corresponding log(experimental K_p) value was much smaller than the log(theoretical PC) value would have predicted. This may be due to the unusual solvent properties of water. Although there was up to a 20-fold increase in 6-MP diffusion from one series of binary vehicles — oleic acid : propylene glycol — there was a clear correlation between increased diffusion and decreased relative resistance of the skin to topical absorption as determined from second application experiments.

Introduction and Background

One of the most important aspects of drug delivery that developers of new topical agents must consider is the formulation in which the new drug is to be tested. Obviously it would be an advantage to the formulator to determine the solubility of the new drug in a few vehicles and be able to predict from that the optimum formulation from which to deliver that drug with a fair degree of certainty. Conversely, the choice of the wrong formulation(s) in which to screen new drugs could lead to the rejection of active agents from further consideration.

The importance of being able to predict at least qualitatively the flux of a drug from knowledge of

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the drug's solubility and partition coefficient has led to the development of a number of predictive models based on the delivery of drug from saturated solutions. Those models have been reviewed recently by Osborne (1986). However, two more models have been developed since the review which specifically deal with the effect of vehicles on the fluxes of a single solute from those vehicles. Dugard and Scott (1986) have shown for the delivery of water through human skin from a wide variety of single, binary and tertiary vehicle systems that the percutaneous absorption rate (J) is equal to the permeability constant multiplied by the applied concentration of water in the vehicle, and that the permeability constant and partition coefficient are inversely proportional to the mole fraction solubilities of water in the vehicles.

Sloan et al. (1986a) have shown for the polar drug theophylline that log(experimental permeability coefficient)(K_p) values can be predicted from log(theoretical partition coefficient)(PC) values plus a constant for the delivery of theophylline from vehicles exhibiting solubility parameters of 12–18 $(cal/cm^3)^{1/2}$:

$$J_i^{s,v} = K_{p,i}^{s,v} C_i^v = (PC_i^{s,v})(D_i^s/h_s)C_i^v \quad (1)$$

$$K_{p,i}^{s,v} = J_i^{s,v}/C_i^v = (PC_i^{s,v})(D_i^s/h_s) \quad (2)$$

where D_i^s is the diffusion coefficient of drug i in the skin, h_s is the thickness of the skin and C_i^v is the concentration of drug i in the vehicle. Then, if D_i^s/h_s is a constant:

$$\log K_{p,i}^{s,v} = \log PC_i^{s,v} + \text{constant} \quad (3)$$

The theoretical PC were calculated from an equation developed by Davis (1970):

$$\log PC = \left[(\delta_v - \delta_i)^2 - (\delta_s - \delta_i)^2\right] V_i \phi_v^2 / 2.3RT \quad (4)$$

where δ_v, δ_i and δ_s are the solubility parameters of the vehicle, drug and skin, respectively, V_i is the molar volume of drug i, and ϕ_v is the volume fraction of the vehicle. Thus, the relationship between experimental K_p for polar drugs and the solubility parameter of the vehicles used to deliver them is predicted to take the form of a parabola with a minimum where $\delta_{v(n)} = \delta_i$ and the solubility of drug i is greatest. Qualitatively, the Dugard and Scott (1986) and the Sloan et al. (1986a) models are similar in that each predicts that increased solubility of drugs in vehicles leads to decreases in permeability coefficients.

6-Mercaptopurine (6-MP) is a polar antimetabolic which has been found to be clinically effective when given systemically to treat psoriasis (Kravetz and Balsam, 1961) but which is clinically ineffective when applied topically (Weinstein et al., 1981; Van Scott and Reinertson, 1959). Water was used as the vehicle to deliver 6-MP topically in the studies. No attempt was made to try to optimize the formulation (vehicle). Since the topical delivery of clinically effective levels of 6-MP would lead to lower systemic levels of 6-MP with the development of fewer concomitant toxic side effects, a formulation of 6-MP that was effective in delivering 6-MP topically would be desirable. In this paper the relative effects of different vehicles on the delivery of 6-MP through hairless mouse skin are examined to determine if any trends exist which would indicate the optimum vehicle from which to maximize the delivery of 6-MP. In addition, an analysis of how well the results fit the solubility parameter based model for predicting percutaneous absorption (Sloan et al., 1986a) is presented.

Materials and Methods

The 6-mercaptopurine hydrate was obtained from Sigma. The isopropyl myristate was obtained from Givaudan Corp., Clifton, NJ. The oleic acid was obtained from Fisher. The remaining vehicles were obtained from Aldrich; they were all > 99% pure and were used as received. The diffusion cells were Franz type cells (2.5 cm in diameter, 4.9 cm^2 = area, 20 ml receptor phase volume) which were obtained from Crown Glass of Somerville, NJ. The mice were female hairless mice (Skh:hr-1) obtained from Temple University Skin and Cancer Hospital and weighed 20–30 g.

Solubility determination

The solubility of 6-mercaptopurine in each vehicle was determined in triplicate (S.D. ± 3%) by

stirring an excess of 6-mercaptopurine hydrate (200–400 mg) in the vehicle (3 ml) with a magnetic stirrer for 48 h at room temperature (23 ± 1° C) in a sealed flask, which was thermally insulated from the stirrer (Table 1). The 48 h was sufficient to ensure that the solution was saturated. The suspension was filtered through a 0.45 μm nylon membrane filter, and a portion of the filtrate was immediately diluted with methanol. The concentration of 6-mercaptopurine in the methanol solution was determined by measuring the UV absorption at 329 nm ($\varepsilon = 1.91 \times 10^4$ l/mol). Subsequently, the residue was washed twice with one ml of tetrahydrofuran and dried in vacuo at room temperature before it was analyzed by NMR spectroscopy.

Calculated solubility parameters

The calculated solubility parameters were obtained using the method of Fedors (1974) as illustrated by Martin et al. (1985). The results for 6-mercaptopurine are given in Table 2.

TABLE 1

Solubilities of 6-mercaptopurine (6-MP)

Vehicle	$\delta_{v(n)}$ [a]	Solubility (mg/ml solution)	Mole fraction solubility
Single component systems			
(1) Oleic acid	7.6	0.0030	0.0000062
(2) Isopropyl myristate	8.5 [b]	0.0034	0.0000071
(3) 1-Octanol	10.3	0.23	0.00024
(4) Dimethylformamide	12.1	14.5	0.0074
(5) Dimethylsulfoxide	13.0	34.8	0.016
(6) Propylene glycol (PG)	14.8	6.2	0.0030
(7) Ethylene glycol	16.1	3.0	0.0011
(8) Formamide	17.9	9.1	0.0024
(9) Water	23.4	0.17	0.00020
Binary component systems [c] (mole ratio)			
(10) 1-Octanol : PG (1 : 1)	11.7	1.58	0.0012
(11) Oleic acid : PG (3 : 1)	8.1	0.087	0.00015
(12) Oleic acid : PG (1 : 1)	9.3	0.39	0.00050
(13) Oleic acid : PG (1 : 3)	10.6	1.23	0.00109
(14) Oleic acid : PG (1 : 5)	12.0	2.25	0.0017
(15) Oleic acid : PG (1 : 14.5)	13.5	4.35	0.0026

[a] $\delta_{v(n)}$ were obtained from Barton (1975).
[b] Calculated from Fedors (1974).
[c] $\delta_{v(n)}$ for binary systems were calculated according to Chertkoff and Martin (1960). Densities were measured at 23° C.

Diffusion determination

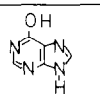
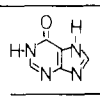
The mice were sacrificed by cervical dislocation. Whole thickness intact skin was removed using blunt dissection to separate the skin from the underlying fascia. The dorsal portion of the mouse skin was immediately placed in the diffusion cell in contact with the receptor phase, which was pH 7.3 (at 32° C) phosphate buffer (0.05 M, ionic strength = 0.11) containing 0.1% v/v of 36% aqueous formaldehyde as a preservative. The skin was held in place with a rubber O-ring and the two halves of the cells were held together with a clamp. The receptor phase of the diffusion cell was maintained at 32° C — the approximate temperature of the skin — with a circulating water bath during the entire experiment. The room temperature was 23 ± 1° C during diffusion determinations. The mouse skin was kept in contact with the receptor phase for 48 h to allow any water-soluble UV-absorbing materials to leach from the skin; the receptor phase was changed 3 times during this preapplication leach period.

A suspension of 6-mercaptopurine was prepared by stirring an excess (51–255 mg) of 6-mercaptopurine hydrate in 3 ml of vehicle for 48 h at room temperature. After 48 h, a 0.5 ml aliquot of a 6-mercaptopurine suspension in a particular vehicle was applied to the donor side of each of 3 skins using an Eppendorf digital pipetter. The suspension was kept well stirred while each aliquot was withdrawn.

Samples of the receptor phases (3 ml) for UV analysis (321 nm, $\varepsilon = 1.94 \times 10^4$ l/mol) were usually taken at 3, 6, 9, 12, 18, 21, 24, 30, 36 and 48 h after each suspension was applied. In some cases, samples were taken at 1, 2, 4, 6, 8, 12, ... h where there was not much lag time in development of steady-state flux or where the vehicle rapidly changed the skin and allowed back diffusion of water from the receptor phase into the donor phase. The receptor phase was replenished with 3 ml of fresh pH 7.3 phosphate buffer after each sample was taken. Thus, the analysis of each subsequent sample had to be corrected for all the previous samples that had been removed for analysis. This was accomplished by adding 15% of the amount of 6-mercaptopurine in each previous sample to the amount of 6-mercaptopurine in the sample being analyzed.

TABLE 2

Theoretical calculated solubility parameters [a] for 6-MP, hypoxanthine and their tautomers

Functional group or atom	 I		
	Number of groups	Δe_i (cal/mol)	Δv_i (cm^3/mol)
-HC=	2	1030 × 2 = 2060	13.5 × 2 = 27.0
C=	3	1030 × 3 = 3090	−5.5 × 3 = −16.5
-N=	3	2800 × 3 = 8400	5.0 × 3 = 15.0
NH	1	2000	4.5
SH	1	3450	28.0
ring closure	2	250 × 2 = 500	16.0 × 2 = 32.0
double bond	4	400 × 4 = 1600	−2.2 × 4 = −8.8
		$\Sigma\Delta e_i$ = 21100	$\Sigma\Delta v_i$ = 81.2
		$\delta_i = (\Sigma\Delta e_i/\Sigma\Delta v_i)^{1/2} = 16.1$ (cal/cm^3)$^{1/2}$	
Functional group or atom	 II		
	Number of groups	Δe_i (cal/mol)	Δv_i (cm^3/mol)
OH	1	7120	10.0
-SH	−1	−3450	−28.0
I	1	21100	81.2
		$\Sigma\Delta e_i$ = 24770	$\Sigma\Delta v_i$ = 63.2
		$\delta_i = (\Sigma\Delta e_i/\Sigma\Delta v_i)^{1/2} = 19.8$ (cal/cm^3)$^{1/2}$	
Functional group or atom	 III		
	Number of groups	Δe_i (cal/mol)	Δv_i (cm^3/mol)
HC=	2	1030 × 2 = 2060	13.5 × 2 = 27.0
C=	2	1030 × 2 = 2060	−5.5 × 2 = −11.0
N=	2	2800 × 2 = 5600	5.0 × 2 = 10.0
N-H	1	2000	4.5
CONH	1	8000	9.5
ring closure	2	250 × 2 = 500	16 × 2 = 32.0
double bond	3	400 × 3 = 1200	−2.2 × 3 = −6.6
		$\Sigma\Delta e_i$ = 21420	$\Sigma\Delta v_i$ = 65.4
		$\delta_i = (\Sigma\Delta e_i/\Sigma\Delta v_i)^{1/2} = 18.1$ (cal/cm^3)$^{1/2}$	

[a] Calculated according to Fedors (1974).

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At the end of this second 48 h period the donor phase was washed 3 times with 10 ml of methanol taking care to remove all solid particles containing 6-mercaptopurine and to keep the time of contact between the skin and the methanol to a minimum (< 3 min total). The methanol washes from each cell were combined and analyzed by UV spectroscopy to determine the amount of 6-MP that remained in the donor phase for each cell. The receptor phase was changed and the skin was then kept in contact with fresh buffer for an additional 23 h to allow any residual 6-mercaptopurine in the skin to leach out. At the end of that time period, a sample (3 ml) was removed from the receptor and was analyzed by UV spectroscopy. The receptor phase was changed again. The total amount of 6-mercaptopurine that diffused, that was washed off the donor phase, and that leached from the skin during the last 23 h period accounted for $86 + 7\%$ of 6-mercaptopurine that had been originally applied. After another one hour of contact between the skin and the fresh receptor phase, the receptor phase was analyzed by UV spectroscopy to assure that no more 6-mercaptopurine was leaching from the skin into the receptor phase.

In order to assess the degree of any irreversible effects of 6-mercaptopurine in the various vehicles on permeability, an aliquot (0.5 ml) of a theophylline suspension in propylene glycol (400 mg/6 ml) was applied to each cell and 3 ml samples were withdrawn from the receptor phase, usually after 3, 6, 12, 24 and 48 h, for analysis by UV spectroscopy (270 nm, $\varepsilon = 1.02 \times 10^4$ 1/mol).

In order to assess the effect of the length of time the skin was in contact with the buffer before application of the donor phase, control experiments were run in which the mouse skins were kept in contact with the pH 7.3 phosphate buffer receptor phase for 2, 24 or 120 h before being treated with 0.5 ml aliquots of a theophylline suspension in propylene glycol (400 mg/6 ml).

In order to determine the effect of a methanol wash, a control experiment was run in which there was a 96 h pre-application leach period and 23 h leach period after a methanol wash before theophylline/PG was applied.

The flux in all cases was obtained by plotting the cumulative mg of 6-mercaptopurine or theophylline measured in the receptor phase against

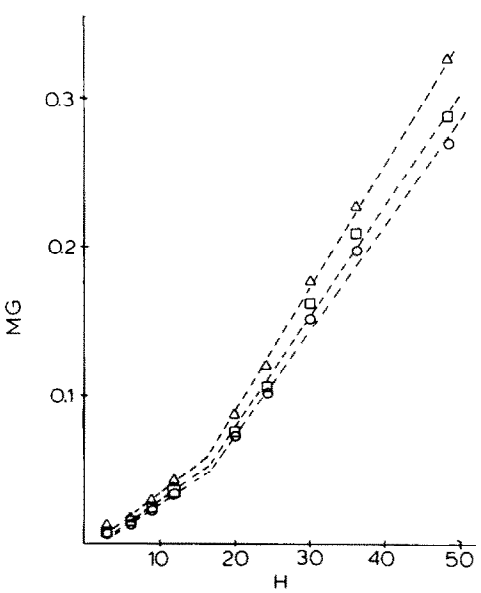

Fig. 1. Plots of cumulative mg of 6-mercaptopurine diffused through hairless mouse skins from oleic acid-propylene glycol (1:5) suspensions versus time. The dashed lines and open symbols represent the data from individual diffusion cells.

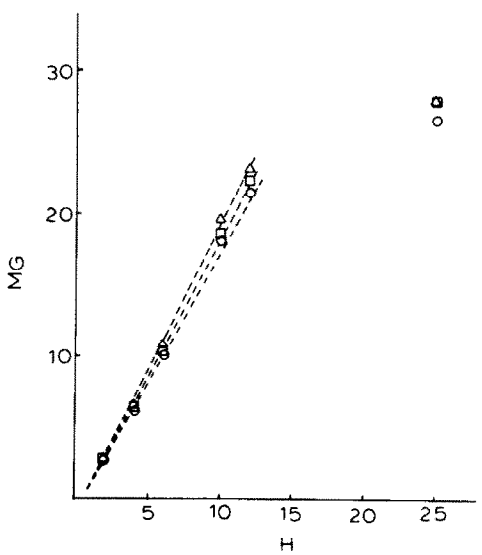

Fig. 2. Plots of cumulative mg of theophylline diffused through hairless mouse skins from propylene glycol suspensions versus time after the skins had been pretreated with 6-mercaptopurine/oleic acid–propylene glycol (1:5). The dashed lines and open symbols represent the data from individual diffusion cells.

time in h (for example, see Fig. 1). The flux for the first phase of the diffusion of 6-mercaptopurine from the various vehicles was obtained over a period which ranged from 0–3 up to 3–24 h. The flux for the second phase was then obtained over the remaining time. For the application of theophylline, only one diffusion phase was observed (for example, see Fig. 2). In all cases linear regression analysis was used to obtain the slope for the plot of cumulative mg against h. Correlation coefficients were at least $r = 0.99$. Flux was obtained by dividing the above slope by the area of the diffusion cell (4.9 cm^2), and the permeability coefficient was then obtained by dividing the flux (mg/cm^2 h) by the corresponding solubility of 6-MP in that solvent (mg/cm^3 of solution from Table 1) (see Tables 4, 5 and 6).

Results and Discussion

Solubilities, solubility parameters and theoretical partition coefficients

The solubilities of 6-mercaptopurine in the various solvents (vehicles) that were used in this study are given in Table 1. The specific solvents and mixtures of solvents were chosen to represent as wide a range of solubility parameters as possible [from oleic acid $\delta_v = 7.6$ $(cal/cm^3)^{1/2}$ to water $\delta_v = 23.4$ $(cal/cm^3)^{1/2}$] and to represent possible components of topical formulations. For example, isopropyl myristate and propylene glycol are common components of topical formulations while dimethylformamide (DMF) (Munro and Stoughton, 1965), dimethylsulfoxide (DMSO) (Stoughton and Fritsch, 1964) and mixtures of oleic acid in propylene glycol (Cooper, 1984; Cooper et al., 1985) are all considered to be penetration enhancers.

There was a gradual increase in mole fraction solubility of 6-mercaptopurine from the solvent exhibiting the lowest solubility parameter—oleic acid—up to DMSO. Then the solubility decreased as the solubility parameter of the solvent was increased further. Usually the solubility parameter of the solute (drug) is considered to be equal to the solubility parameter of the solvent for that solvent in which the solute exhibits its maximum mole fraction solubility, i.e. $\delta_i = \delta_v$ (Barton, 1984). This is one way that δ_i can be determined experimentally. However, this peak solubility method should only be used as a measure of δ_i in those cases where the solvent does not solvate or complex drug i (Martin et al., 1985). In the case of 6-MP, solvates were isolated from suspensions of 6-MP hydrate in DMF and DMSO (see below). If the mole fraction solubilities of 6-MP in DMF and DMSO are excluded, it can be seen (Table 1) that the maximum mole fraction solubility of 6-MP hydrate is in a solvent or mixture of solvents that exhibits a solubility parameter of about 13.5–14.8 $(cal/cm^3)^{1/2}$.

The 6-mercaptopurine that was used in each solubility determination was initially the commercially available 6-MP hydrate which has a distinctive yellow color. In some cases the residues from the solubility determinations lost their yellow color. In those cases it seemed possible that the solubility that was being determined was not that of 6-MP hydrate but that of a 6-MP solvate or an anhydrous form. In order to identify the residues, one residue from each solubility determination was washed twice with 1 ml of tetrahydrofuran to remove any occluded solvent. The washed residues were then dried in vacuo at room temperature for 48 h before they were analyzed by ^{1}H-NMR spectroscopy. Inspection of the ^{1}H-NMR spectra (dry DMSO-d_6) showed that the solid residues in equilibrium with 6-MP in solution were the solvates only in the cases of DMF (1 : 1) and DMSO (1 : 1). It was also clear from ^{1}H-NMR spectroscopy that there was no water associated with those residues.

On the other hand, inspection of the ^{1}H-NMR spectra of the residues recovered from 1-octanol, isopropyl myristate, oleic acid, propylene glycol and ethylene glycol showed that those residues were 6-MP hydrate. However, the residue recovered from formamide appeared to be anhydrous by ^{1}H-NMR spectroscopy. In each case where the hydrate was recovered the residue was yellow, while in the cases where a different solvate or the anhydrous form was isolated, the residue was white to off-white.

The occurrence of different hydration/solvation states of 6-MP in the residues, however, is not necessarily indicative of the form of 6-MP in

solution. Thus, the solubilities reported in Table 1 are normalized to give solubilities only in terms of mg/ml of 6-MP and not in terms of mg/ml of 6-MP hydrate, 6-MP · DMF or 6-MP · DMSO solvate.

It is not clear a priori what effect the formation of solvates will have on the amount of 6-MP in solution. In the cases of DMF and DMSO it seems clear that the solvates are less soluble than the hydrate. For instance, up to 50 mg of 6-MP hydrate will dissolve in 1 ml of DMF, but after a few minutes at room temperature with stirring, the 6-MP · DMF solvate precipitated and the amount of 6-MP in solution amounted to 14.5 mg/ml.

A calculated value for the solubility parameter of 6-MP was determined according to a modification of the method of Fedors (1974). Since the thione tautomer IV (see Table 2) is the main tautomer of 6-MP according to Chenon et al. (1975), and there was no group contribution to the energy of vaporization and molar volume for NH–C=S given by Fedors; δ_i and V_i values for IV were extrapolated from δ_i and V_i values for the N=C–SH tautomer I and the corresponding oxygen analogues of IV and I, i.e. III and II, respectively. The difference in δ_i between the hydroxy tautomer of hypoxanthine (II) and the carbonyl tautomer III was 1.7 $(cal/cm^3)^{1/2}$ while there was very little difference in the V_i values. Therefore, it has been assumed that a similar difference in δ_i between the thiol tautomer of 6-MP (I) and the thione tautomer IV exists and that no difference in the respective values for V_i exists. Thus, δ_i for IV was estimated to be about $16.1 - 1.7 = 14.4$ $(cal/cm^3)^{1/2}$ — a value in the range suggested by the solubilities that were measured — while V_i was estimated to be about 81.2 cm^3/mol.

It should be noted that the δ_i so determined for 6-MP, which is then used to calculate the theoretical *PC*, does not include a molecule of solvent in the calculation. In the present experiments it was not possible to determine whether 6-MP itself or its solvates were actually partitioning between the solvents and skin. Generally, it has been assumed that the solvated solute is desolvated before it is transferred from one phase to another in the partitioning process (Vilallonga and Phillips, 1980).

The calculated values for δ_i and V_i were used to calculate the log(theoretical partition coefficients (*PC*) for each of the single component vehicles in Table 3 using Eqn. 4. The mathematical form of the relationship between these theoretical *PC* and the solubility parameters of the vehicles is a parabola which is shown in Fig. 3. The minimum in the parabola for log (theoretical *PC*) exists where $\delta_i = \delta_{v(n)}$, i.e. for that vehicle in which 6-MP hydrate exhibits its maximum solubility. Since it has previously been shown that the permeability coefficient ($K_{p,i}^{s,v(n)}$) is directly proportional to *PC* under certain conditions (Sloan et al., 1986a; Dugard and Scott, 1986) and that K_p is inversely related to mole fraction solubility (Dugard and Scott, 1986), a minimum in a log(experimental *PC*) curve would be expected for the partitioning of 6-MP between skin and the vehicle in which 6-MP was most soluble.

Diffusion experiments

The results for the diffusion of 6-MP from the various single and binary solvent systems are given

TABLE 3

Log(theoretical PC) and log(theoretical K_p) values compared with log(experimental K_p) values

Vehicle	log(theoretical *PC*) [a]	log(experimental K_p) [b]	log(theoretical K_p) [c]
(1) Oleic acid	+1.57	−1.84	−1.76
(2) Isopropyl myristate	+0.90	−0.73	−2.43
(3) 1-Octanol	−0.15	−1.09	−3.43
(4) Dimethylformamide	−0.82	−3.58	−4.15
(5) Dimethylsulfoxide	−1.02	−4.23	−4.35
(6) Propylene glycol	−1.12	−4.82	−4.45
(7) Ethylene glycol	−0.96	−4.47	−4.29
(8) Formamide	−0.42	−3.79	−3.75
(9) Water	+3.76	−2.68	+0.42

[a] Calculated from $\log PC = [(\delta_{v(n)} - \delta_i)^2 - (\delta_s - \delta_i)^2]V_i\phi_{v(n)}^2/2.3RT$, according to Davis (1970), where $\delta_i = 14.4$ $(cal/cm^3)^{1/2}$ and $V_i = 81.2$ cm^3/mol from Table 2, R = gas law constant (1.98 cal/degree mol), $T = 305°K$, $\delta_{v(n)}$ are obtained from Table 1, $\phi_{v(n)}^2$ is assumed to approach a value of one and $\delta_s = 10$ $(cal/cm^3)^{1/2}$ (Liron and Cohen, 1984).

[b] From Table 5.

[c] From log(theoretical *PC*) − Σ[log(theoretical *PC*) − log(experimental K_p)]/6 = log(theoretical K_p). Calculation of Σ[log(theoretical *PC*) − log(experimental K_p)]/6 = 3.33 excludes data for vehicles 2, 3 and 9; however, log(theoretical K_p) values are still given for those vehicles.

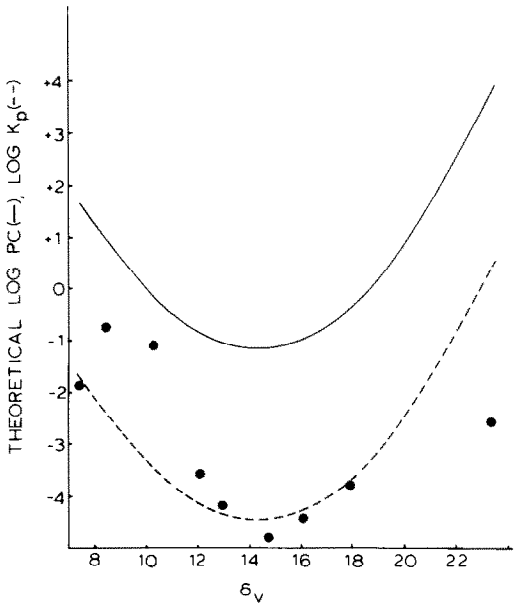

Fig. 3. A plot of log(theoretical $PC_i^{s,v(n)}$) (———) from Table 3 versus $\delta_{v(n)}$ and log(theoretical $K_{p,i}^{s,v(n)}$) (log $PC_i^{s,v(n)} - 3.33$) (— — —) versus $\delta_{v(n)}$, including the values for the steady-state second phase (●) of the experimentally determined log $K_{p,i}^{s,v(n)}$ for the single component vehicles.

in Tables 4 and 5. In about 60% of the experiments there was a well-defined first phase to the diffusion process. The fluxes, permeability coefficients and lag times (intercept, h) for the first phases are given in Table 4 while those for the steady-state phases are given in Table 5. The fluxes from the steady-state portions of the graphs of cumulative mg versus time were in most cases 2–3 times greater than those from the initial phases of the diffusion processes but a few were only 30% greater. A plot of cumulative mg of 6-MP delivered from a typical vehicle versus time (h) which exhibits two distinct phases is shown in Fig. 1.

Comparison of the values for the flux of 6-MP from water with the fluxes of 6-MP from the other solvents shows that while water is not that good a solvent (vehicle), it was still roughly 4 times better than those from propylene glycol or ethylene glycol which are commonly used as vehicles for polar drugs. The best vehicle by far was 1-octanol (≈ 50 times better than water) or a mixture of octanol and propylene glycol (≈ 40 times better than water). Of the known penetration enhancers, DMSO and DMF were only 6 and 10 times better than water, respectively, and the mixture of oleic

TABLE 4

Experimentally determined fluxes (J_i) and permeability coefficients ($K_{p,i}$) for the first phase of the diffusion of 6-MP through hairless mouse skin from the vehicles

Vehicle	Flux (J_i) $\pm$S.D. $\times 10^3$ (mg/cm^2 · h)	$K_{p,i}$ $\pm$S.D. $\times 10^3$ (cm/h)	Intercept (h)
(1) Oleic acid	0.013 ± 0.012	4.3 ± 4.0	9.5
(2) Isopropyl myristate	0.18 ± 0.07	53.0 ± 21.0	8.0
(3) 1-Octanol	–	–	–
(4) Dimethylformamide	–	–	–
(5) Dimethylsulfoxide	–	–	–
(6) Propylene glycol (PG)	0.056 ± 0.006	0.0090 ± 0.00097	6.0
(7) Ethylene glycol	–	–	–
(8) Formamide	1.2 ± 0.6	0.13 ± 0.066	2.3
(9) Water	0.27 ± 0.14	1.6 ± 0.82	0.0
(10) 1-Octanol : PG (1 : 1) [a]	–	–	–
(11) Oleic acid : PG (3 : 1) [a]	–	–	–
(12) Oleic acid : PG (1 : 1) [a]	0.51 ± 0.05	1.3 ± 0.13	1.7
(13) Oleic acid : PG (1 : 3) [a]	0.66 ± 0.072	0.54 ± 0.059	2.2
(14) Oleic acid : PG (1 : 5) [a]	0.77 ± 0.076	0.34 ± 0.034	1.9
(15) Oleic acid : PG (1 : 14.5) [a]	1.2 ± 0.13	0.28 ± 0.030	2.8

[a] Mole ratios.

TABLE 5

Experimentally determined fluxes (J_i) and permeability coefficients ($K_{p,i}$) for the steady-state (second) phase of the diffusion of 6-MP through hairless mouse skin from the vehicles

Vehicle	Flux (J_i) $\pm$S.D. $\times 10^3$ (mg/cm^2 · h)	$K_{p,i}$ $\pm$S.D. $\times 10^3$ (cm/h)	Intercept (h)
(1) Oleic acid	0.043 ± 0.021	14.3 ± 7.0	20
(2) Isopropyl myristate	0.60 ± 0.30	176.0 ± 88.0	19.8
(3) 1-Octanol	18.6 ± 1.6	81.0 ± 7.0	3.3
(4) Dimethylformamide	3.8 ± 0.71	0.26 ± 0.044	0.3
(5) Dimethylsulfoxide	2.1 ± 0.16	0.059 ± 0.0046	0.4
(6) Propylene glycol (PG)	0.093 ± 0.006	0.0015 ± 0.00097	13.5
(7) Ethylene glycol	0.10 ± 0.010	0.033 ± 0.0033	5.1
(8) Formamide	1.5 ± 0.10	0.16 ± 0.011	5.1
(9) Water	0.36 ± 0.21	2.1 ± 1.2	3.8
(10) 1-Octanol : PG (1 : 1) [a]	13.6 ± 0.070	8.6 ± 0.044	2.0
(11) Oleic acid : PG (3 : 1) [a]	0.44 ± 0.021	5.1 ± 0.24	2.9
(12) Oleic acid : PG (1 : 1) [a]	0.95 ± 0.13	2.4 ± 0.33	8.6
(13) Oleic acid : PG (1 : 3) [a]	1.6 ± 0.19	1.3 ± 0.15	9.4
(14) Oleic acid : PG (1 : 5) [a]	1.6 ± 0.15	0.71 ± 0.067	9.5
(15) Oleic acid : PG (1 : 14.5) [a]	1.9 ± 0.17	0.44 ± 0.039	5.8

[a] Mole ratios.

acid and propylene glycol that gave the greatest flux was only about 5 times better than using water as the vehicle. It was also interesting that the flux of 6-MP from isopropyl myristate was about twice that from water yet 6-MP was about 50 times more soluble in water.

As expected (Sloan et al., 1968a and b), all of the fluxes (except for the comparison between ethylene glycol and propylene glycol) and permeability coefficients from the steady-state portions of the diffusion processes for those solvents where there was a good correlation between K_p and PC were significantly different from each other ($P < 0.05$) even though the diffusion experiments were all run with suspensions of 6-MP in the solvents to maintain saturated conditions. Thus, thermodynamic control (Flynn and Smith, 1972) would not have predicted these results. Also as predicted (Sloan et al., 1986a and b; Dugard and Scott, 1986), there was a clear trend towards lower permeability coefficients for the delivery of 6-MP from vehicle(s) in which 6-MP exhibited greater solubility. This trend existed not only for the single solvent systems but also for the binary solvent systems where there was a regular decrease in K_p from oleic acid : propylene glycol (3 : 1) to (1 : 14.5) while the mole fraction solubility of 6-MP increased from 1.5×10^{-4} to 2.6×10^{-3}.

When the log(experimental K_p) values for the single component vehicles were plotted against $\delta_{v(n)}$ (Fig. 3) the points corresponded approximately to a parabola of the same shape as that described by the plot of log(theoretical PC) versus $\delta_{v(n)}$ if the values for 1-octanol, isopropyl myristate and water were excluded (the basis for this exclusion is explained below). A parabola corresponding to log(theoretical K_p) versus $\delta_{v(n)}$ was constructed from values of log(theoretical K_p) = log(theoretical PC) − 3.33 (see Table 3). The log(experimental K_p) values considered in this treatment of the data fit the parabola fairly well (S.D. = ±0.32) so that it should be possible to predict experimental K_p values and fluxes for other vehicles that exhibit solubility parameters in the range of 12–18 (cal/cm^3)$^{1/2}$. It should be noted that the log(experimental K_p) for oleic acid also closely approximates the log(theoretical K_p) curve versus $\delta_{v(n)}$ so that K_p and hence fluxes from vehicles exhibiting δ_v around 7–8 (cal/cm^3)$^{1/2}$ may also be predicted from the same log(theoretical K_p) versus $\delta_{v(n)}$ curve.

A plot of log (experiment K_p) values for the

binary component vehicles (oleic acid–propylene glycol) versus $\delta_{v(n)}$ is shown in Fig. 4, along with the parabola described by log (theoretical K_p) versus $\delta_{v(n)}$ from Fig. 3 for comparison. There is no correlation between the experimental K_p and the theoretical curve. The reason for this follows.

In order to assess the relative extent of changes in the barrier function of the skin caused by the treatment of 6-MP in the various vehicles, a second application of a standard drug/vehicle combination (theophylline/PG) was made. It has been shown previously that the application of theophylline in PG does not cause much obvious decrease in the resistance of the hairless mouse skin to subsequent topical absorption in diffusion cell experiments (Sloan et al., 1986a). Thus, theophylline in PG was applied to each diffusion cell after all of the initial donor suspension had been removed and the 6-MP remaining in the skin had been given 23 h to leach out. The flux of the theophylline was determined and the results are given in Table 6 along with data from experiments in which the initial applications were theophylline

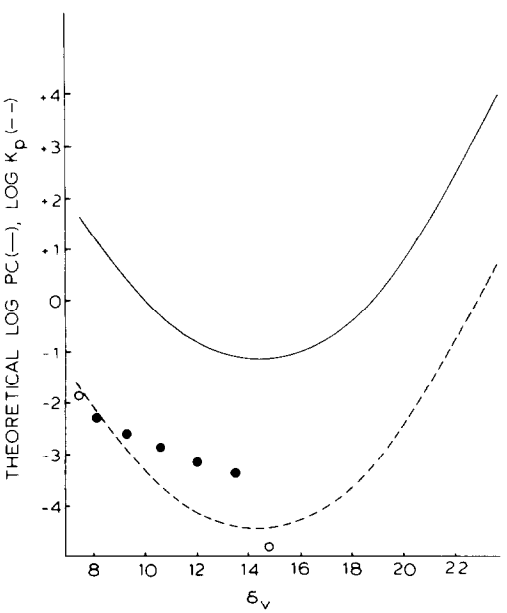

Fig. 4. A plot of log(experimental $K_{p,i}^{\delta,v(n)}$) values for the steady-state phase of the delivery of 6-mercaptopurine from the binary component vehicles (oleic acid–propylene glycol) versus $\delta_{v(n)}$ (●) including values for oleic acid and propylene glycol versus $\delta_{v(n)}$ (○).

suspensions in various vehicles (Sloan et al., 1986a). It was assumed that any increase in the flux of theophylline (from the theophilline/PG second application) represented, at least qualitatively, a measure of the decrease in the relative resistance of the skin towards topical absorption. This apparent damage to the barrier function of the skin may or may not correlate with any functional or structural changes in the skin. The higher the value for the flux of theophylline observed from the second application, the greater the apparent damage to the skin resulting from the initial application of the 6-MP in various vehicles.

Control experiments were run to assess the relative amounts of apparent damage to the skin as a result of varying amounts of time the skin was in contact with the receptor phase prior to application of the initial drug–vehicle combination. Pre-application leach periods of 2, 24 and 120 h resulted in fluxes of theophylline from a theophylline–PG suspension (400 mg/6 ml) of $1.29 \pm 0.64 \times 10^{-3}$, $1.54 \pm 0.31 \times 10^{-3}$ and $1.41 \pm 0.09 \times 10^{-3}$ mg/cm^2 · h, respectively, which are not significantly different from each other ($\alpha = 0.05$). In addition, an experiment was run to assess the effect of a methanol wash of the skin after a 96 h pre-application leach period. This methanol wash was followed by a 23 h leach period and resulted in a flux of theophylline from a theophylline/PG suspension (400 mg/6 ml) of $2.4 \pm 0.4 \times 10^{-3}$ mg/cm^2 · h.

A plot of cumulative mg of theophylline delivered from propylene glycol after pretreatment with oleic acid : propylene glycol (1 : 5) is shown in Fig. 2 as an example of the results obtained for the flux of theophylline as a second application. This particular example also shows for those cases where the resistance of the skin has apparently been significantly decreased that a steady flux of theophylline is maintained only up to the point where the donor phase is no longer a saturated solution, i.e. all the suspended theophylline has dissolved and diffused. As soon as the point of non-saturation of the solute in the donor phase is reached the flux decreases dramatically.

The data in Table 6 suggest that except for water and dimethylsulfoxide, those vehicles that do not appear to cause much apparent damage to

TABLE 6

The effect of 6-MP / vehicle on hairless mouse skins as measured by the diffusion of theophylline from propylene glycol

Vehicle	Flux (J) ± S.D. × 10^3 (mg/cm^2 · h)		Intercept (h)	$\delta_{v(n)}$
(1) Oleic acid	42.0 ± 18.0		0.6	7.6
(2) Isopropyl myristate	160.0 ± 20.0	(110.0) [b]	2.7	8.5
(3) 1-Octanol	710.0 ± 40.0	(470.0) [b]	0.0	10.3
(4) Dimethylformamide	21.0 ± 16.0	(4.6) [b]	2.5	12.1
(5) Dimethylsulfoxide	120.0 ± 82.0		1.6	13.0
(6) Propylene glycol (PG)	3.0 ± 0.30	(5.8) [b]	3.3	14.8
(7) Ethylene glycol	5.2 ± 1.5	(5.1) [b]	4.7	16.1
(8) Formamide	6.0 ± 0.20	(8.1) [b]	2.2	17.9
(9) Water	4.4 ± 0.04		1.7	23.4
(10) 1-Octanol : PG (1 : 1) [a]	820.0 ± 60.0		0.0	11.7
(11) Oleic acid : PG (3 : 1) [a]	151.0 ± 31.0		1.3	8.1
(12) Oleic acid : PG (1 : 1) [a]	390.0 ± 40.0		0.1	9.3
(13) Oleic acid : PG (1 : 3) [a]	300.0 ± 99.0		1.2	10.6
(14) Oleic acid : PG (1 : 5) [a]	409.0 ± 16.0		0.7	12.0
(15) Oleic acid : PG (1 : 14.5) [a]	464.0 ± 61.0		0.5	13.5

[a] Mole ratios.

[b] Values for the flux of theophylline from propylene glycol in Sloan et al. (1986a).

mouse skin are also the vehicles for which there is good agreement between experimental K_p and theoretical K_p values. Thus, the delivery of 6-MP from propylene glycol, formamide and ethylene glycol caused the least amount of apparent damage followed by dimethylformamide, oleic acid and dimethylsulfoxide while isopropyl myristate and oleic acid : PG (3 : 1) caused somewhat more apparent damage than dimethylsulfoxide. 1-Octanol or 1-octanol : PG (1 : 1) were by far the worst vehicles in terms of decreasing the resistance of the skin to a second topical application while mixtures of oleic acid and propylene glycol were intermediate in the amount of apparent damage they caused. The latter result was somewhat surprising in view of the fact that neither oleic acid nor propylene glycol by themselves caused as much apparent damage to the skin as the mixtures. The relative fluxes of 6-MP from oleic acid : PG mixtures reflect the relative amounts of apparent damage caused by oleic acid : PG mixtures, i.e. increased flux from the initial application resulted in decreased resistance to a second application.

The reason that the log(experimental K_p) value obtained from the flux of 6-MP from DMSO fits the parabola describing the theoretical K_p values, even though DMSO seems to cause moderate apparent damage to the skin, is that the value for the flux was calculated from only the first 4–6 h after application of 6-MP in DMSO to the cells. After 6 h it was quite obvious that changes in the skins had occurred since there was diffusion of water from the receptor phases into the donor phases causing the donor phases to increase from 0.5 ml to 1–1.25 ml. On the other hand, water does not seem to cause much apparent damage to the skin yet its log(experimental K_p) value is much lower than its expected log(theoretical K_p) value. However, water exhibits very special solvent properties because of the very strong mutual interaction of water molecules (i.e. water is both a strong hydrogen bond donor and acceptor) and mutually compensating changes in the entropy and enthalpy of aqueous solutions can occur with little or no change in the free energy of the system (Jencks, 1969). Thus, both non-polar molecules and moderately polar molecules, such as purines, aggregate in water with the driving force in the later case appearing as a favorable enthalpy of aggregation (Jencks, 1969). Since partitioning is dependent on the free (non-aggregated) drug concentration, a

solvent such as water that causes aggregation will cause a decrease in free drug concentration and hence flux (Barry, 1983) compared to other solvents exhibiting less cohesive energy density (i.e. lower $\delta_{v(n)}$ value).

Conclusions

The results from the in vitro delivery of 6-MP from various solvents (vehicles) through hairless mouse skin and the results from the second application experiments using theophylline in propylene glycol as a measure of apparent damage to the skin show that little improvement in the delivery of 6-MP can be expected using a formulation approach without also expecting some significant changes in the resistance of the skin to subsequent topical applications. For those vehicles that did not cause much apparent damage to the mouse skins (dimethylformamide, propylene glycol, ethylene glycol, formamide, oleic acid), there was a good correlation between theoretical partition coefficients and permeability coefficients similar to that observed for theophylline (Sloan et al., 1986a) and salicylic acid (Sloan et al., 1986b).

It should be noted that these results describe the relative effects of the vehicles on the delivery of 6-MP through hairless mouse skin. The clinical situation presents quite different conditions so that the absolute quantities of 6-MP delivered into and through human skin may be quite different.

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