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Solubility and in vitro percutaneous absorption of tetracaine from solvents of propylene glycol and saline

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Summary

To gain a better understanding of the factors that govern the percutaneous penetration of topically applied local anesthetics, several properties of a proposed topical formulation of tetracaine were studied. Tetracaine solubility in propylene glycol-saline mixtures is measured for use in developing a topical, local anesthetic formulation. Solubilities of tetracaine free base, tetracaine acid salt, and a 60% free base/40% acid salt mixture are presented as a function of water and propylene glycol content. The solubility of the acid salt remains nearly constant (0.5–0.8 M) as the solvent varies from pure propylene glycol to pure saline. Free base solubility is negligible in saline, but peaks at 2.65 M in 70% propylene glycol (v/v) before falling to 2.17 M in pure propylene glycol. The solubility of the mixture exceeds those of either the free base or acid salt alone at nearly all solvent combinations (maximum 3.00 M in 50% propylene glycol v/v). This increased solubility is attributed to the degree of dissociation and differences in the surface activity of tetracaine free base and tetracaine acid salt. Partitioning data of a 60% free base/40% acid salt (w/w) mixture ($7.3 \leqq pH \leqq 8.4$) into 1-octanol or *n*-octane are presented as a function of water and propylene glycol ratio. The results from the two lipophilic solvents are in general agreement and suggest that partitioning into lipid phases is optimum between 0 and 30% propylene glycol (v/v). The diffusion of tetracaine mixtures (60% free base/40% acid salt w/w) in solvents of propylene glycol and saline was studied through synthetic polycarbonate membranes and hairless-mouse skin. The flux of tetracaine through the synthetic membrane was greatest from an aqueous solution, but also showed a local maximum at 40% propylene glycol. The flux continued to decrease as propylene glycol content increased. Wetting phenomena were assumed to be responsible for the maximum flux at the aqueous limit. The flux of tetracaine through hairless-mouse skin was greatest at 40% propylene glycol, but also showed a high flux at 10% propylene glycol. As with the synthetic membrane, the flux continued to decrease as the propylene glycol content rose to 70% (v/v). The effects of animal age, formulation pH, drug concentration, and formaldehyde (as a preservative) on tetracaine diffusion from solvents of propylene glycol and saline were also studied. The permeability of full-thickness hairless-mouse skin to tetracaine was found to decrease with the age of the mice. Specifically, the skins of mice 6–8 months old are found to have a permeability to tetracaine of only 20% that of mice 6–8 weeks old. The existence of a minimum pH for significant skin permeation of tetracaine is confirmed. A minimum pH value is consistent with the generally accepted idea that the anesthetic free base (favored at higher pH values) is the prominent species diffusing through the skin. Tetracaine concentration does not affect its transdermal flux. This is assumed to be a micellar phenomenon since tetracaine has previously been shown to be capable of forming micelles which are not assumed to contribute significantly to drug diffusion because of their size. Dilute

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solutions of formaldehyde (sometimes used as a preservative for in vitro skin permeation experiments) show a tendency to decrease the skin flux permeation of tetracaine. Furthermore, this inhibition seems to be a result of an interaction between formaldehyde and the skin.

Introduction

Despite the proliferation of new topical drug treatments, an effective, topical, local anesthetic formulation has not yet been developed. While many researchers have worked on this problem for years (Monash, 1957; Campbell and Adriani, 1958; Adriani and Dalili, 1971; Gesztes and Mezei, 1988; McCafferty et al., 1988, 1989; Woolfson et al., 1988, 1990; Kushla and Zatz, 1990), they have been unable to produce an effective nerve block in a reasonable time due to the inability to transport sufficient anesthetic through the stratum corneum.

The advantage of an effective, topical, local anesthetic for the patient is temporary relief of pain during venepuncture and other painful procedures. The advantage for health-care professionals is the reduction of patient trauma associated with these procedures. A new formulation has been developed to provide short-term, topical local-anesthesia. This new formulation is a mixture of tetracaine HCl and tetracaine free base in a solvent of propylene glycol and saline.

A water-miscible system was chosen as a vehicle for the anesthetic to avoid the possibility of phase separation under occlusion. If phase separation occurred as water entered the drug solvent, the drug would have an additional interface to cross (i.e., a water barrier on the skin surface) and drug flux could be decreased.

The solubility of tetracaine was investigated to determine the condition at which the greatest amount of drug could be suspended in the hopes of maximizing the diffusion rate. Lipid-phase partitioning data were assessed to determine the degree to which tetracaine was likely to enter the lipid-rich stratum corneum as a function of propylene glycol content.

Because solubility and lipid-phase partitioning are only two properties related to percutaneous absorption, the effects of propylene glycol as a cosolvent, pH (acid salt:free base ratio), and concentration were also investigated for their direct effects on in vitro diffusion of tetracaine through hairless-mouse skin. Through this method, a multivariate constrained optimum was sought.

Finally, the practice of using low concentrations of formaldehyde (0.1% w/w) as a skin preservative can be detrimental for in vitro transdermal drug diffusion-experiments if the formaldehyde interacts with the skin. Knowledge of the effects of these parameters on the skin permeation of tetracaine through hairless-mouse skin in vitro is crucial to the development of a clinically effective topical anesthetic.

Materials and Methods

Materials

The local anesthetic tetracaine was chosen because of its relative potency and favorable lipid partitioning characteristics (De Jong, 1977). Two forms of tetracaine were used in the experiments, tetracaine free base (a hydrophobic ester) and tetracaine HCl (a hydrophilic salt of the free base).

Preliminary experiments in our laboratory indicated that tetracaine free base decomposes at room temperature with a half-life ($t_{1/2}$) of 2–3 weeks. At physiological temperature (35°C), tetracaine free base decomposes more rapidly ($t_{1/2} \approx 1$ week). Potential decomposition is evaluated for subsequent interpretation of experimental data, although decomposition during the experiments was not measured directly.

Fig. 1 is a schematic representation of tetracaine. Both forms of tetracaine were used as received (Sigma). The pK_a for tetracaine is reported to be 8.5 (De Jong, 1977) and was confirmed in our laboratory.

The solvents used to dissolve tetracaine were

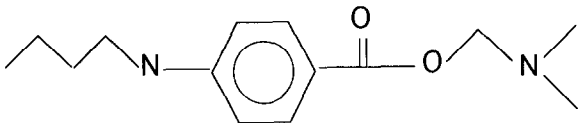

Fig. 1. Molecular structure of tetracaine.

mixtures of distilled, deionized water or 0.9% (w/w) saline (NaCl) solution (made from distilled, deionized water and biological grade NaCl from Fisher Scientific) and USP-grade propylene glycol (1,2-propanediol) also from Fisher Scientific. These solvents are completely miscible and their ratio was varied primarily to control the solubility of tetracaine.

Two solvents were used to simulate the partitioning behavior of skin: 1-octanol and *n*-octane (both ACS Fisher Scientific). Octanol was preferred for estimating stratum corneum partitioning, but the octanol/propylene glycol/water system is single-phase above 60% propylene glycol. Therefore, partition coefficients for systems containing more than 60% propylene glycol could not be evaluated using octanol.

The receptor phase for the diffusion studies was phosphate-buffered saline or PBS (pH = 7.2 at 25°C). Diffusion experiments were performed using synthetic polycarbonate membranes of 0.45 μm nominal pore diameter (Type HA Millipore) or the fresh, full-thickness skin of female, hairless mice (Strain SKH-HR-1 Temple).

Methods

Solubility was determined by allowing a solution of propylene glycol and saline to equilibrate with excess drug at least 24 h at room temperature. A sample was then filtered and the total drug concentration in solution was determined by HPLC.

Solubility studies were conducted at room temperature for the sake of simplicity. Drug solubility was expected to follow the same trend at room temperature as skin temperature. Therefore, the solvent yielding the highest solubility at 25°C should also yield the highest solubility at 32°C. Determining the solubility at elevated temperature was beyond the scope of the present study. After 3 days, approx. 10% of the tetracaine free base could be expected to decompose (25°C), however, solubility was never measured unless excess (solid) drug was present. Furthermore, small amounts of decomposition products were assumed to have a negligible effect on overall tetracaine solubility. Finally, the analysis of samples by HPLC was performed immediately after sampling to eliminate the possibility of further decomposition so mathematical correction for tetracaine decomposition was not required.

To simulate the environment encountered by the drug when placed in contact with the skin, the anesthetic preparation was placed in contact with a hydrophobic organic phase. No account was made for hydrophobic-phase solubility in the vehicle or vehicle solubility in the hydrophobic phase and tetracaine decomposition was not considered a factor for reasons described above. Vehicle formulations in contact with an equal volume of the hydrophobic organic phase were rotated at 4 rpm for at least 18 h. The samples were then allowed to separate into hydrophilic and hydrophobic phases. A sample of each phase was filtered and analyzed by HPLC to determine the total drug concentration. The ratio of drug concentrations in the hydrophilic vehicle and the hydrophobic solvent at equilibrium was taken as the partition coefficient (K_p) for the formulation (i.e., $K_p = C_{solvent}/C_{vehicle}$).

In vitro skin permeation * was measured using flow-through type Franz diffusion cells (FDC-200 25, Crown Glass, Somerville, NJ) over a period of 8 h. The cell body was modified by inserting a magnetically driven stirring tee that greatly increased mixing efficiency and reduced the tendency to form a stagnant boundary layer next to the skin's inner surface. The receptor compartment initially contained 15 ml of buffered 0.9% (w/w) saline. The cell cap contained 2 ml of the donor phase.

* Transdermal diffusion was measured as opposed to tetracaine concentration in the skin. The primary reason for this was experimental simplicity and the assumption that a formulation that promotes rapid diffusion through the stratum corneum would also promote rapid diffusion through full-thickness skin in vitro.

At regular intervals (1 h), a 0.2 ml sample was withdrawn from the receptor phase through the upper sample port using a long, thin needle and a 1 ml syringe. This sample was sealed in an autosampler vial for later analysis by HPLC (there was no accounting for metabolites as only tetracaine itself could be detected by the HPLC). The sample volume taken from the Franz cell was replaced by fresh, buffered saline. The samples were stored at room temperature for no longer than 8 h before HPLC analysis. Therefore, tetracaine decomposition is estimated to be less than 4% during the course of the diffusion experiment (8 h at 35°C) and an additional 2% during storage awaiting analysis (8 h at 25°C). This translates to a total loss of tetracaine of no more than 6% (96% × 98% = 94%) from the beginning of the experiment to the end of HPLC analysis and is well within the intraspecies variation so no correction for tetracaine decomposition was necessary.

The concentrations obtained from HPLC were used to calculate the total mass transferred through the skin. The following mass balance accounts for the sampling process:

$$M(t_n) = C(t_n)V + V_s\left[\sum_{x=0}^{n-1} C(t_x)\right]$$

where $M(t_n)$ is the total mass transferred at time t_n, $C(t_n)$ represents the measured concentration at time t_n, V is the volume of the receptor compartment ($\simeq$ 15 ml), V_s denotes the sampled volume (0.2 ml), x is a summation index and n is the number of samples.

The total mass obtained from this equation can then be converted to a corrected concentration by dividing by the receptor compartment volume (V) or to a flux by dividing by the mass transfer area (diameter = 25 mm) and sampling interval.

The effect of subject age on the diffusion of tetracaine from solvents of propylene glycol and saline was determined by comparing the cumulative flux of tetracaine through the full-thickness skins of hairless mice 6–8 weeks old and 6–8 months old.

Formulation pH was varied by manipulating the bulk ratio of tetracaine free base to tetracaine acid salt (0–100%) in a solvent of 70% propylene glycol and 30% saline (v/v). In this case, a tetracaine salt solution of 0.36 M in 70% propylene glycol and 30% saline (v/v) corresponded to an apparent pH of 4.71 and a tetracaine base solution of 0.36 M in 70% propylene glycol and 30% saline (v/v) corresponded to an apparent pH of 12.20.

Formaldehyde, in low concentrations, is sometimes used to preserve the skin for long-term in vitro experiments (Sloan et al., 1991). The amount and location of formaldehyde in the experimental system were varied to determine the effects of the preservative on the diffusion of tetracaine through hairless-mouse skin (formaldehyde was not used in any of the previously described experiments).

Results

Solubility

Three independent solubility studies were performed in solvents of propylene glycol and saline: tetracaine free base, tetracaine acid salt, and a 60% free base/40% acid salt mixture (w/w). Mixing the free base and its acid salt in solution is equivalent to adding HCl acid to a solution containing only the free base (or adding NaOH to a preparation containing only the acid salt) (De Jong, 1977). Thus, the ratio of acid salt to free base (in solution) can be calculated from the pK_a. *

Fig. 2 shows solubilities of tetracaine salt, tetracaine base, and the 60% free base/40% acid salt mixture (w/w) in propylene glycol-water solvents. Tetracaine base is sparingly soluble in aqueous solution. Adding propylene glycol greatly increases the solubility of the base which peaks at 2.65 M in 70% (v/v) propylene glycol then falls to 2.17 M in pure propylene glycol. The solubility of tetracaine salt decreases slightly as the propylene

* The apparent ionization constant is a function of the solvent (Miller et al., 1993).

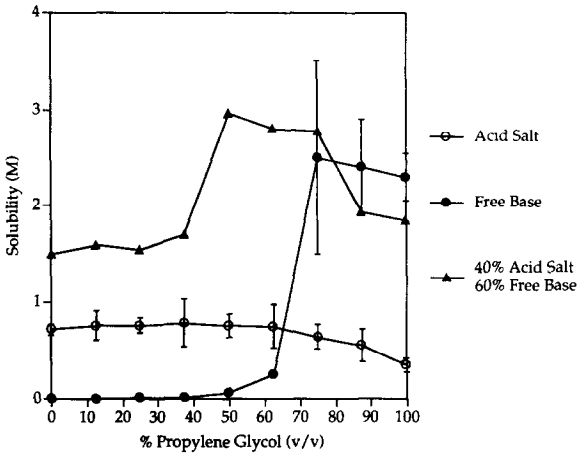

Fig. 2. Tetracaine solubility in propylene glycol and saline.

glycol fraction increases, but changes little overall ($0.5\ M \leqq C_{sat} \leqq 0.8\ M$). The solubility of a 40% acid salt/60% free base mixture peaks at 3.00 M in 50% propylene glycol. The solubility of the mixture in 50% propylene glycol is far greater than the sum of the salt and base solubilities. This non-additivity near 50% propylene glycol shows that HCl acid can enhance the solubility above what would be expected from the pure component solubility curves.

Lipid-phase partitioning

Partitioning into 1-octanol The partitioning of a 60% tetracaine free base, 40% tetracaine acid salt mixture between propylene glycol-water solutions and 1-octanol ($H_3C\text{-}(CH_2)_7\text{-}OH$) declines as the organic content of the vehicle increases (Fig. 3 and Table 1). This may indicate a decreasing tendency for the drug to partition into the hydrophobic phase or it may be a result of the increasing mutual solubility of the hydrophilic and hydrophobic phases. Above 60% propylene glycol, the system of 1-octanol/saline/propylene glycol no longer develops an interface. Therefore, at high propylene glycol concentrations, partitioning behavior cannot be assessed. To simulate the partitioning behavior of tetracaine into a lipid phase at higher propylene glycol concentrations, a more hydrophobic solvent is required.

TABLE 1

Tetracaine (60% free base, 40% acid salt w/w) equilibrium concentrations and partitioning into 1-octanol

% propylene glycol	$C_{vehicle}$ (M)	$C_{octanol}$ (M)	K_p
0	3.59×10^{-3}	2.29×10^{-2}	6.37
20	3.99×10^{-3}	2.22×10^{-2}	5.55
40	3.87×10^{-3}	2.05×10^{-2}	5.30
50	3.99×10^{-3}	1.99×10^{-2}	4.99
60	3.57×10^{-3}	1.70×10^{-2}	4.75

Partitioning into n-octane The partitioning of tetracaine between propylene glycol-water solutions and *n*-octane ($H_3C\text{-}(CH_2)_6\text{-}CH_3$) is constant at about 0.004 up to 30% propylene glycol (Fig. 4 and Table 2). This plateau indicates that

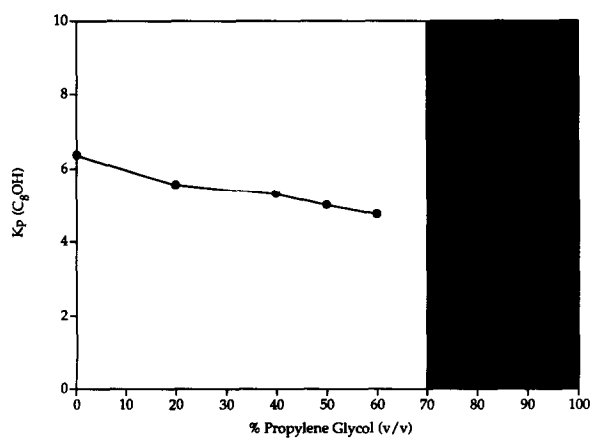

Fig. 3. Tetracaine (60% free base, 40% acid salt) partitioning from propylene glycol and saline into 1-octanol.

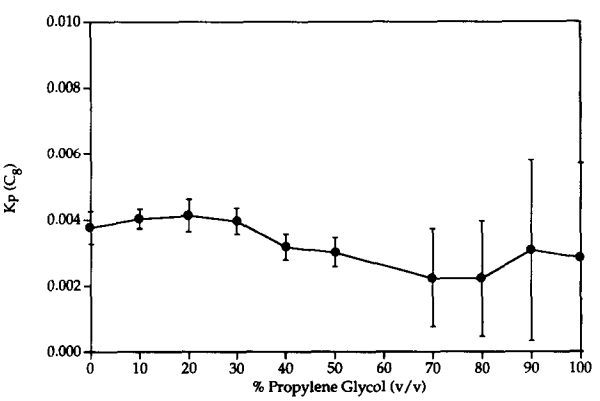

Fig. 4. Tetracaine (60% free base, 40% acid salt) partitioning from propylene glycol and saline into *n*-octane.

TABLE 2

Tetracaine (60% free base, 40% acid salt w/w) equilibrium concentrations and partitioning into n-octane

% propylene glycol	$C_{vehicle}$ (M)	C_{octane} (M)	K_p
0	9.38×10^{-3}	3.53×10^{-5}	3.77×10^{-3}
10	1.08×10^{-2}	4.37×10^{-5}	4.03×10^{-3}
20	9.56×10^{-3}	3.95×10^{-5}	4.13×10^{-3}
30	9.73×10^{-3}	3.86×10^{-5}	3.97×10^{-3}
40	1.05×10^{-2}	3.34×10^{-5}	3.17×10^{-3}
50	1.23×10^{-2}	3.73×10^{-5}	3.03×10^{-3}
60	9.07×10^{-3}	3.04×10^{-5}	3.35×10^{-3}
70	1.73×10^{-2}	3.86×10^{-5}	2.23×10^{-3}
80	1.97×10^{-2}	4.34×10^{-5}	2.21×10^{-3}
90	1.19×10^{-2}	3.66×10^{-5}	3.08×10^{-3}
100	1.21×10^{-2}	3.43×10^{-5}	2.85×10^{-3}

the partitioning of the drug into the lipophilic phase is unaffected by the presence of low concentrations of propylene glycol. The partition coefficient then appears to decline steadily with increasing propylene glycol content to 70% propylene glycol. The partitioning data show a decreasing tendency for the drug to partition into octane from 30 to 60% propylene glycol (much like that in octanol). Above 80%, however; partitioning into the oil phase seems to increase. It is inferred from these data that a minimum partition coefficient (≈ 0.0022) may exist between 70 and 80% propylene glycol and oil-phase partitioning increases above 80% propylene glycol. The apparent minimum between 70 and 80% propylene glycol and the apparent rise in partition coefficient above 80% propylene glycol may not have physical meaning due to excessive scatter caused by the low concentrations of tetracaine in octane. If such features truly exist, then the least drug partitions into the lipophilic phase between 70 and 80% propylene glycol and addition of propylene glycol above 80% enhances drug partitioning into the lipophilic phase.

Diffusion studies

Propylene glycol content The effect of propylene glycol on the diffusion of tetracaine mixtures is complex. Thus, diffusion of tetracaine mixtures (40% acid salt, 60% free base w/w, 0.36 M overall) in solvents of propylene glycol and 0.9% saline was investigated in two systems. The flux of tetracaine through polycarbonate synthetic membranes was studied to determine the effect of propylene glycol on a simple, well-characterized membrane. Identical formulations were also applied to mounted hairless-mouse skin from mice 6–8 weeks old. Fig. 5 is an example of the raw data obtained from these diffusion studies.

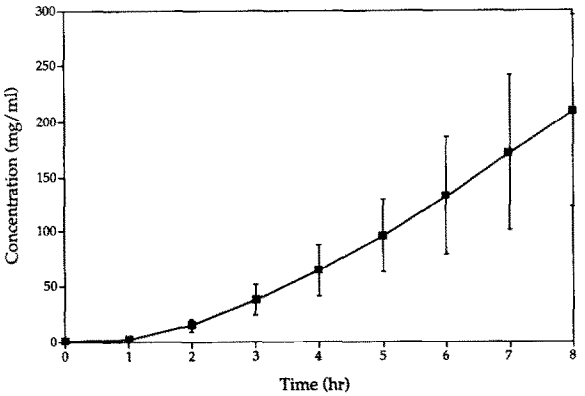

Fig. 5. Raw data of receptor-phase tetracaine concentration as a function of time.

The cumulative flux (flux integrated with respect to time) of these tetracaine formulations through synthetic polycarbonate membranes (nominal pore diameter of 0.45 μm) after 5 min is shown in Fig. 6. The highest flux through the membrane results from the fully aqueous formu-

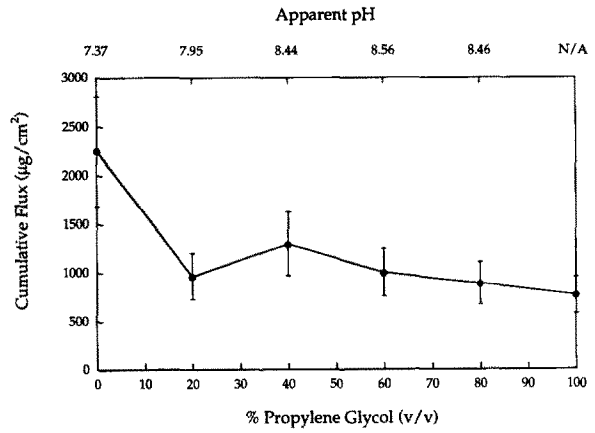

Fig. 6. Cumulative flux of tetracaine through synthetic polycarbonate membrane (60% free base, 40% acid salt w/w, 0.36 M overall, 5 min).

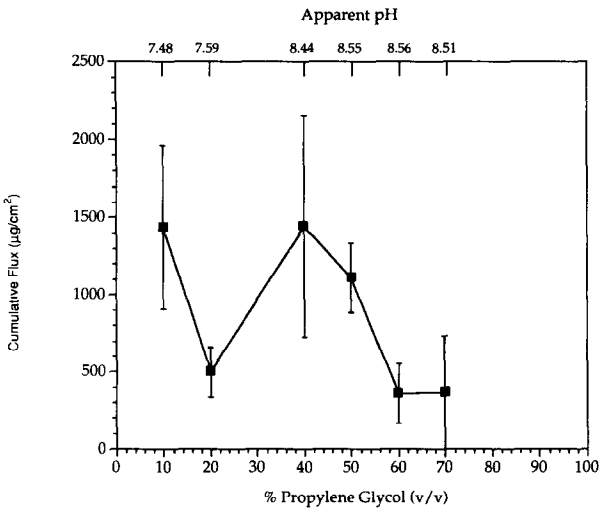

Fig. 7. Cumulative flux of tetracaine through 'young', full-thickness, hairless-mouse skin (60% free base, 40% acid salt w/w, 0.36 M overall, 8 h).

lation because the hydrophilic membrane is most easily wetted by the aqueous formulation. * The flux of tetracaine decreases at higher propylene glycol concentrations because the viscosity and hydrophobicity increase. The apparent local maximum in flux at 40% propylene glycol is not so easily explained, but this feature recurs in experiments using hairless-mouse skin as described below.

Fig. 7 shows the cumulative flux of tetracaine (60% free base, 40% acid salt w/w, 0.36 M overall) through 'young' hairless-mouse skin over 8 h (6–8 weeks old). At 10% propylene glycol, the flux of tetracaine is relatively large (similar to the behavior of the synthetic membrane). The flux of tetracaine decreases as the propylene glycol content increases to 20% and then rises to a maximum at 40% propylene glycol and 60% saline (v/v). Above 40% propylene glycol, the flux decreases as the propylene glycol fraction increases leveling off at 60% propylene glycol. The general trend of decreasing tetracaine flux with increasing propylene glycol content can be explained (as above) by the increasing viscosity, tetracaine base solubility, and hydrophobicity.

Age Tetracaine formulations were also tested on the skins of 'old' mice (6–8 months). This was done to determine how age affects the skin permeation of tetracaine from these formulations. Fig. 8 shows the cumulative flux of tetracaine (60% free base, 40% acid salt w/w, 0.36 M overall) through old skin as a function of propylene glycol content after 8 h. The maximum flux occurs at 40% propylene glycol, but there is no corresponding high flux near the aqueous limit (as with the synthetic membrane and younger mouse skin) indicating that age does not affect the maximum at 40% propylene glycol, but may affect the maximum at the aqueous limit.

The measured fluxes through synthetic membranes and young hairless-mouse skin (Figs 6 and 7) both show high fluxes near the aqueous limit, but old hairless-mouse skin does not. All three of these barriers show relatively high fluxes at 40% propylene glycol. Thus, the optimum vehicles for in vitro transdermal delivery of the tetracaine

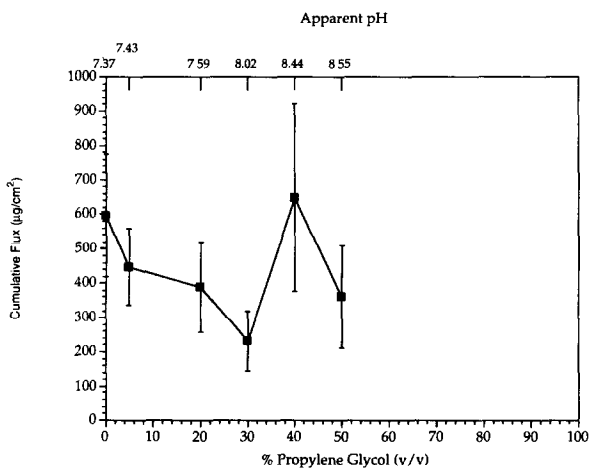

Fig. 8. Cumulative flux of tetracaine through 'old', full-thickness, hairless-mouse skin (60% free base, 40% acid salt w/w, 0.36 M overall, 8 h).

* Synthetic polycarbonate membranes will adopt a negative charge in aqueous media. This negative charge would have a tendency to bind tetracaine cations to the surface of the membrane pores. However, the saline concentration is relatively high (0.9% w/w) and it is likely that Na^+ would adsorb preferentially to the pore walls. Furthermore, the pores are sufficiently large (0.45 μm) that even a layer of adsorbed drug molecules would not constrict the pores enough the hinder diffusion. Consequently, the effect of charged pores on drug diffusion is assumed to be negligible.

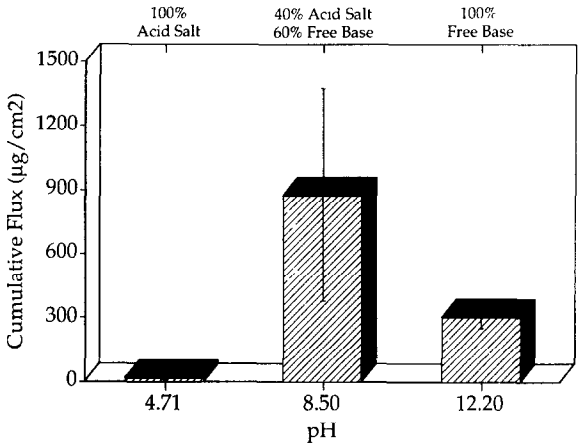

Fig. 9. Cumulative flux of tetracaine through full-thickness, hairless-mouse skin (0.36 M, 8 h, pH effect).

mixture is 40% propylene glycol and 60% saline (v/v).

Mixture ratio or pH To determine the effect of pH, three solutions (identical except for their pH) were allowed to diffuse through skin in vitro. One solution contained tetracaine free base, another, tetracaine acid salt which, in solution, corresponds in bulk to equal parts of tetracaine base and HCl acid; and the third, a 40% acid salt-60% free base (w/w) mixture. A suitable propylene glycol-saline ratio had to be found for which the three systems were single phase. Tetracaine base is not sufficiently soluble in the optimal 40% propylene glycol solution (solubility had to be at least 0.36 M). Consequently, a higher propylene glycol content of 70% was used. Fig. 9 shows the cumulative, in vitro, transdermal flux of tetracaine from 70% propylene glycol at (pH) * = 4.71, 8.50, and 12.20 with an overall drug concentration of 0.36 M through hairless-mouse skin. Fig. 9 shows that the flux of drug is significant in the range $8.5 \leqq (pH) \leqq 12.2$ and negligible for $(pH) \leqq 4.71$. Below some minimum value of pH, therefore, the flux of tetracaine through skin becomes negligible. This is probably caused by the decreasing amount of tetracaine free base available in solution. Tetracaine free base has been found to penetrate the skin more readily than the acid salt (Monash, 1957).

Concentration Fig. 10 shows how donor phase drug concentration affects the flux of tetracaine (40% acid salt, 60% free base w/w, 0.36 M overall) through hairless-mouse skin. If the mechanism of diffusion is strictly Fickian, then the flux should be proportional to the donor-phase concentration. The nearly constant transdermal flux, despite doubling the donor phase concentration, indicates that the driving force for diffusion remains constant. A constant driving force suggests that the topical formulation is not a simple molecular solution and that it probably contains micellar aggregates of tetracaine. This is also suggested by the fact that tetracaine (as well as other similar local anesthetics) is a surface active molecule capable of forming micelles (De Jong, 1977).

Formaldehyde The effect of low receptor-phase concentrations (0.1% w/w) of the preservative formaldehyde on tetracaine diffusion through the skin is illustrated in Fig. 11. Fig. 11 shows the flux of tetracaine (40% acid salt, 60% free base w/w, 0.36 M tetracaine overall) from 40% propylene glycol through mounted mouse skin into buffered saline or buffered saline with 0.1% formaldehyde (w/w). In the presence of formaldehyde, the transdermal flux of tetracaine

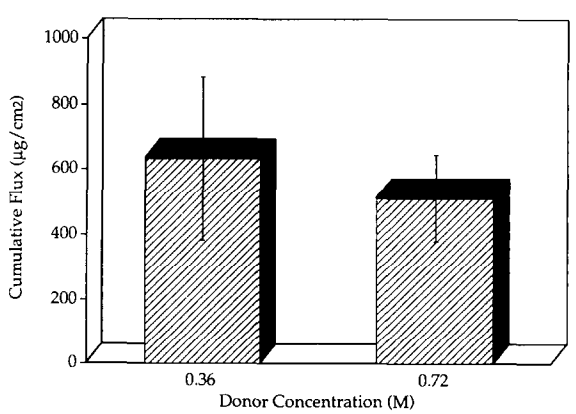

Fig. 10. Cumulative flux of tetracaine through full-thickness, hairless-mouse skin (60% free base, 40% acid salt w/w, 8 h, concentration effect).

* The term (pH) indicates apparent pH because the solutions this value refers to are not fully aqueous and hence, the value indicated by the pH electrode (pH) is not the true pH.

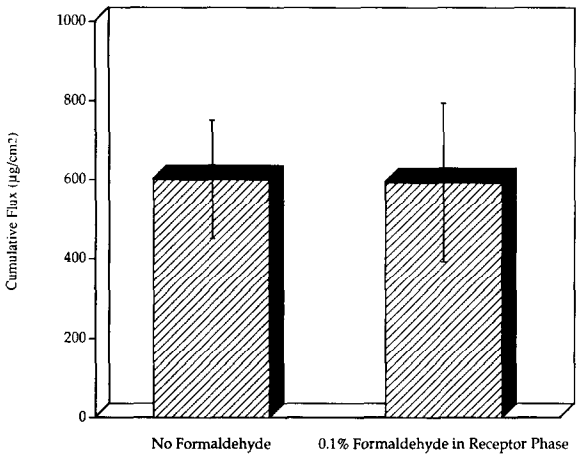

Fig. 11. Cumulative flux of tetracaine through full-thickness, hairless-mouse skin (60% free base, 40% acid salt w/w, 8 h, receptor-phase formaldehyde effect).

decreases by an average of 8% over an 8 h period. This result may be due to either a chemical change in the skin structure brought on by formaldehyde or a decrease in drug flux caused by the counter-diffusion of formaldehyde through the skin.

To determine whether counter-diffusion plays a role in formaldehyde's apparent inhibition of tetracaine diffusion, an identical system was prepared with formaldehyde in equal concentration on both sides of the skin. This configuration eliminates the formaldehyde concentration differences. If the effect of formaldehyde is solely through counter-diffusion, this system should behave like the system with no formaldehyde. If formaldehyde reduces the permeability of the skin, then the increase in formaldehyde content should reduce permeability below even that found with formaldehyde only in the downstream reservoir.

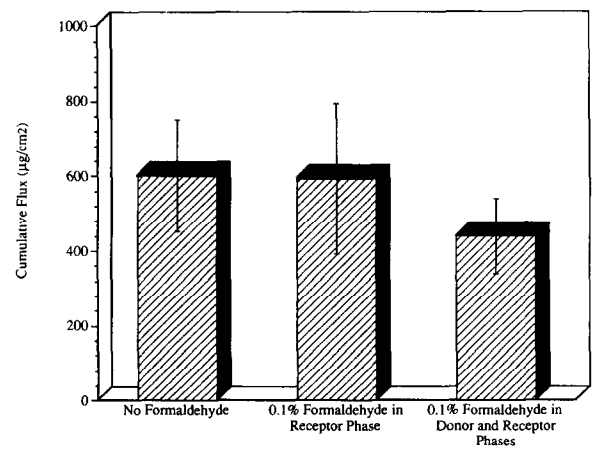

Fig. 12. Cumulative flux of tetracaine through full-thickness, hairless-mouse skin (60% free base, 40% acid salt w/w, 8 h, overall formaldehyde effect).

The results show that the total amount of formaldehyde in the system is the key (Fig. 12). The low formaldehyde concentration (0.1% w/w) probably does not affect the drug solubility or partitioning. Although Fig. 12 does not show a great difference between the three systems, higher formaldehyde content seems to cause lower fluxes. If so, formaldehyde increases the resistance to diffusion through interaction with the skin.

Conclusions

Solubility

The increased solubility of 60% free base/40% acid salt (w/w) mixtures cannot arise solely from the protonation of the tetracaine molecule since an even larger fraction of molecules is protonated in tetracaine acid salt. Some interaction between the acid salt and free base forms, each stabilizing the other, appears to occur. Since local anesthetics are, as a class, surface active (Vold and Vold, 1983), it appears that altering the ratio of tetracaine free base and acid salt effects the micellization of the drug in solution thereby increasing solubility. Also, since the acid salt and the free base are in equilibrium, it is not strictly correct to consider them two different species. They are more likely assuming some intermediate structure when they associate (partial charge). These intermediates must be more soluble in propylene glycol/saline solutions than either the acid salt or the free base.

Lipid-phase partitioning

Partition coefficients do influence percutaneous absorption. If partitioning completely determined permeability, the maximum transdermal flux would have the largest partition coefficient

and the optimum vehicle would be the one associated with the greatest partition coefficient. For 1-octanol, the maximum partition coefficient corresponds to the aqueous vehicle. For *n*-octane, the maximum partition coefficient corresponds to a range of 0–30% propylene glycol. The partitioning of tetracaine (60% free base, 40% acid salt w/w) from vehicles of propylene glycol and saline into either 1-octanol or *n*-octane suggests that the best topical formulation would contain between 0 and 30% propylene glycol (v/v). Since the in vitro diffusion studies indicate that the optimum propylene glycol content is 40% (v/v), partitioning into the stratum corneum is not the overriding constraint.

Diffusion studies

Propylene glycol content The flux of a tetracaine mixture (60% free base, 40% acid salt w/w, 0.36 M overall) through a synthetic polycarbonate membrane (nominal pore diameter 0.45 μm) is greatest from an aqueous solution. This may be a wetting phenomenon related to the hydrophilic membrane. Tetracaine flux through the synthetic membrane decreases as propylene glycol content increases from 40 to 100%. This general trend can be explained by the increases in the vehicle viscosity and hydrophobicity. A local maximum in tetracaine flux at 40% propylene glycol corresponds to the overall maximum in tetracaine flux observed through hairless-mouse skin for the same formulations. Therefore, the optimum formulation for the skin permeation of tetracaine from solvents of propylene glycol and saline in vitro is 40% propylene glycol and 60% saline (v/v).

The existence of a flux maximum at a given concentration indicates maximum thermodynamic activity of the drug in 40% propylene glycol and 60% saline (v/v). Gummer (1985) has previously concluded that the solute generally has the greatest rate of diffusion or the highest activity (at a given concentration) in the poorest solvent (lowest saturation concentration), but the solubility of the tetracaine mixture is near its maximum in this optimum solvent. There are two possible explanations for this behavior; either the solution is micellar and the overall concentration and solubility of tetracaine are irrelevant to the thermodynamic activity of tetracaine monomers or the solvent activity is maximum at 40% propylene glycol and 60% saline (creating a significant solvent velocity to carry the drug through the membrane).

It has been suggested that a solvent of 40% propylene glycol would be irritating to the skin, however unpublished human trials displayed no significant irritation when the formulation contacted the skin for up to 2 h. Another concern expressed is the possibility of systemic toxicity from tetracaine. Again, human trials indicate that systemic toxicity from tetracaine is not a concern.

Age The cumulative, in vitro, transdermal flux of tetracaine (60% free base, 40% acid salt w/w, 0.36 M overall) through old hairless-mouse skin (6–8 months) over 8 h averaged approx. 20% that through young skin (6–8 weeks). The general trend of the transdermal flux with propylene glycol content was unchanged by subject age.

Mixture ratio or pH The cumulative, in vitro, transdermal flux of tetracaine (0.36 M) through hairless-mouse skin over 8 h is significant in the range $8.50 \leqq (\text{pH}) \leqq 12.20$ and negligible for $(\text{pH}) \leqq 4.71$. This indicates that below some minimum value of pH, tetracaine ceases to diffuse appreciably through hairless-mouse skin in vitro. This may be a result of a lack of tetracaine free base in solution since tetracaine base has been shown to diffuse through skin more readily than tetracaine salt (Monash, 1970).

Concentration Doubling the tetracaine concentration in an established topical anesthetic formulation failed to produce a significant change in the in vitro transdermal flux. This behavior indicates that the driving force for diffusion remains constant. Since tetracaine is surface active (De Jong, 1970), it may be forming micellar aggregates in solution. These aggregates could account for the observed behavior since increasing the concentration would increase the number of micelles in solution, but not the molecularly dispersed drug concentration. If the micelles do not contribute significantly to the diffusion of tetracaine, then increasing the concentration of tetracaine would not affect the transdermal flux.

Formaldehyde By varying the location of dilute formaldehyde solutions in the in vitro system,

the effect of formaldehyde on the skin permeation of tetracaine (60% free base, 40% acid salt w/w, 0.36 M overall) from 70% propylene glycol and 30% saline (v/v) has been determined. Formaldehyde (0.1% w/w) seems to inhibit tetracaine diffusion through its interaction with the skin. The influence of counter diffusion due to unequal concentrations of formaldehyde across the skin has been eliminated as a cause of the decrease in flux. Based upon these studies, caution should be exercised in the use of formaldehyde as a preservative for in vitro skin permeation studies.

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