JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1215–1222

Received January 15, 2007
Accepted June 4, 2007
DOI 10.1211/jpp.59.9.0005
ISSN 0022-3573

# Dermal delivery of selected hydrophilic drugs from elastic liposomes: effect of phospholipid formulation and surfactants

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## Abstract

The effect of phospholipid formulation and choice of surfactant on skin permeation of selected hydrophilic drugs from elastic liposomes across human epidermal membrane has been studied. Sodium cholate and various concentrations of phosphatidylcholine were used for the preparation of liposomes namely hydrogenated phosphatidylcholine 90% (Phospholipon 90H), phosphatidylcholine 95% (Phospholipon 90G), phosphatidylcholine 78.6% (Phospholipon 80), and phosphatidylcholine 50% (Phosal PG). To investigate the effect of the surfactant, liposomes were prepared from 95% phosphatidylcholine (Phospholipon 90G) and various surfactants (sodium cholate, sodium deoxycholate, Span 20 (sorbitan monolaurate), Span 40 (sorbitan monopalmitate), Span 60 (sorbitan stearate) and Span 80 (sorbitan monooleate)). The vesicles were prepared by the conventional rotary evaporation technique. The film was hydrated with phosphate-buffered saline (10 mL) containing 9, 2 and 2.5 mg $mL^{-1}$ of methotrexate, idoxuridine and aciclovir, respectively. All formulations contained 7% ethanol. Homogenously-sized liposomes were produced following extrusion through 100-nm polycarbonate filters using Lipex Extruder. Particle size was characterized by transmission electron microscopy. Vertical Franz diffusion cells were used for the study of drug delivery through human epidermal membrane. For the three drugs, the highest transcutaneous fluxes were from elastic liposomes containing 95% phosphatidylcholine. In general, a higher flux value was obtained for liposomes containing sodium cholate compared with sodium deoxycholate. For the liposomes containing sorbitan monoesters, there was no clearly defined trend between alkyl chain length and flux values. Overall, transcutaneous fluxes of liposomal preparations of hydrophilic drugs were comparable with those from saturated aqueous solutions ($P > 0.05$).

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**Funding and acknowledgement:** The authors acknowledge the financial support of IDEA AG, Germany, and the kind donation of phosphatidylcholine by Nattermann Phospholipids, Germany.

## Introduction

The recognition of poor permeability characteristics of the skin has stimulated a great deal of research, aimed at enhancing dermal and systemic drug delivery in a reproducible and reliable manner (Monti et al 2001). Dermal drug delivery may provide convenience, improved patient compliance and elimination of the first-pass effect seen with systemic delivery (Chong & Fung 1989). Although dermal delivery systems have many advantages, most drugs are not amenable to this mode of administration because of the excellent barrier properties of the skin. Molecules must first penetrate the stratum corneum, the outer horny layer of the skin. The molecule then penetrates the viable epidermis before passing into the papillary dermis and through the capillary walls into systemic circulation. The concentrations that can be established in the local environment of the viable epidermis and dermis depend on interplay between the rate of permeation and the local clearance. It is the stratum corneum, a complex structure of compact keratinized cell layers that presents the greatest barrier to absorption of (trans)dermally administered drugs (Valenta et al 2001a).

Methotrexate, aciclovir and idoxuridine (Figure 1) are used in the treatment of basal cell carcinoma as well as herpes simplex and varicella-zoster infections, respectively. These diseases are often encountered in patients suffering from acquired immune deficiency syndrome (AIDS). However, these drugs are hydrophilic exhibiting log P values of −1.2, −1.57 and 0.95, respectively (Chatterjee et al 1997; Kristl & Tukker 1998; Bonina et al 2002).

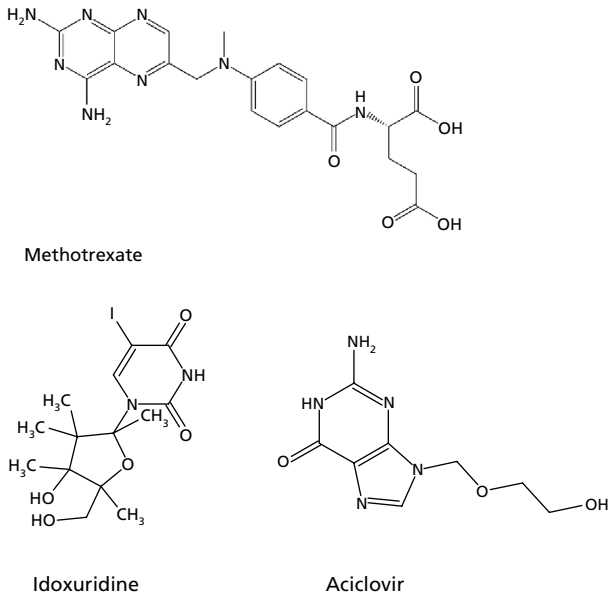


**Figure 1** Chemical structures of methotrexate, idoxuridine and aciclovir.

Since dermal delivery of hydrophilic drugs is challenging (Okumura et al 1989), there is a need to evolve strategies aimed at improving delivery of such compounds.

Various approaches have been developed to overcome the skin barrier and to increase drug transport across the skin. One method to increase the penetration of a drug across the skin is encapsulation or association of the drug in or on lipid vesicles (Van Kuijk-Meuwissen et al 1998). Encapsulation of drugs in elastic liposomes has been reported for diclofenac (Boinpally et al 2003), insulin (Cevc et al 1998; Guo et al 2000), estradiol (El-Maghraby et al 1999) and 5-fluorouracil (El-Maghraby et al 2001). Boinpally et al (2003) demonstrated enhanced flux of diclofenac from lecithin vesicles containing sodium cholate and 10% ethanol. Even large hydrophilic compounds such as insulin were shown to achieve high delivery rates from ultraflexible liposomes (Cevc et al 1998; Guo et al 2000). El Maghraby et al (1999) also studied the skin delivery of estradiol from ultradeformable vesicles and reported a 17-fold flux enhancement. However, the same authors did not observe any statistically significant differences in the skin permeation of a hydrophilic drug, 5-fluorouracil, from these ultradeformable vesicles (El-Maghraby et al 2001).

Elastic liposomes are usually prepared from phospholipids with the addition of "edge activators" such as sodium cholate. Phospholipids are the major structural components of biological membranes. The most common phospholipids are phosphatidylcholine (PC) molecules—amphipathic molecules in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains with a hydrophilic polar headgroup, phosphocholine. In water, molecules of PC align themselves closely in planar bilayer sheets to minimize the unfavourable interactions between the bulk aqueous phase and the long hydrocarbon fatty acid chains. These interactions are completely eliminated when the sheets fold on themselves to form closed sealed vesicles (Styer 1995).

Even though elastic liposomes have been shown to increase drug transport in some cases, the mechanism is still poorly understood. Few studies have specifically investigated the effects of different phospholipid formulations on the percutaneous permeation of hydrophilic drugs from these vesicles.

Surfactants are considered to be the major constituents that impart ultradeformability to liposomes. Sodium cholate, sodium deoxycholate, Tween 80 and Span 80 have been used to prepare these vesicles (Cevc et al 1998; El-Maghraby et al 1999; Guo et al 2000). The elasticity of liposomal bilayers and their transdermal delivery efficiency can be altered by surface-active substances (Cevc 1996). However, no systematic studies have been carried out to determine the effect of surfactant type on (trans)dermal delivery of hydrophilic drugs from elastic liposomes.

In this study, the influence of various commercial phospholipid preparations as well as the effect of bile salts (sodium cholate and sodium deoxycholate) and sorbitan monoesters (sorbitan monolaurate, palmitate, stearate and monooleate) on transcutaneous flux of methotrexate, aciclovir and idoxuridine have been investigated. This should provide an insight into the relationship between surfactant structure and transdermal delivery of hydrophilic drugs from deformable liposomes.

## Materials and Methods

### Materials

Methotrexate, aciclovir, idoxuridine, sodium cholate, sodium deoxycholate, Span 20 (sorbitan monolaurate), Span 40 (sorbitan monopalmitate), Span 60 (sorbitan stearate) and Span 80 (sorbitan monooleate) were purchased from Sigma-Aldrich Corporation (Johannesburg, South Africa). Phosphoric acid and HPLC grade methanol were supplied by Merck (Johannesburg, South Africa). Dipotassium hydrogen orthophosphate, absolute ethanol (99.9%), sodium chloride (NaCl), anhydrous disodium hydrogen orthophosphate ($Na_2HPO_4$) and sodium dihydrogen orthophosphate hydrate ($NaH_2PO_4.H_2O$) were purchased from Saarchem (Johannesburg, South Africa). Double distilled deionized water was prepared using a Milli-Q water purification system (Millipore, Milford, USA). Phospholipon 90H, Phospholipon 90G, Phospholipon 80, and Phosal PG were kindly donated by Nattermann Phospholipids (Germany).

Homogenously-sized liposomes were produced following extrusion through 100-nm polycarbonate filters (Nucleopore) using Lipex Extruder (Northern Lipids, Canada).

### High-pressure liquid chromatography (HPLC) method

The HPLC analyses of methotrexate, aciclovir and idoxuridine were performed on an HPLC system (Hewlett Packard 1100) equipped with a G1310A isocratic pump, G1313 autosampler, G1314 variable wavelength detector and Chemstation version 8.0x for control and data analysis. A Luna 5-$\mu$m ($C_{18}$) column (250×4.60 mm) from Phenomenex was used.

The mobile phase for methotrexate and idoxuridine consisted of methanol and 40 mM dipotassium phosphate pH 7.4 buffer (30:70) and the flow rate was 1 mL $min^{-1}$. The eluent was monitored at 300 nm. The mobile phase for aciclovir consisted of methanol and 40 mM dibasic potassium phosphate pH 7.4 buffer (10:90) and the flow rate was 1 mL $min^{-1}$. The eluent was monitored at 254 nm. The retention times for methotrexate, idoxuridine and aciclovir were ~6.9, 5.2 and 7.1 min, respectively. The limit of detection was 10 ng $mL^{-1}$ for the three compounds. Intra-day and inter-day variations were less than 5%.

### Solubility determination

The solubility of methotrexate, idoxuridine and aciclovir was measured in phosphate-buffered saline (PBS) pH 7.4. Excess amounts of the drugs were added to PBS and the mixture incubated in a water bath with a constant temperature of 25°C. Stirring with Teflon-coated magnetic bars was maintained for 24 h until there was no further change in concentration. Excess of solute was always present in the slurries. The solutions were then filtered through Millipore filters (0.45 $\mu$m). Each filtrate was appropriately diluted with PBS before its assay by HPLC. All experiments were conducted at least six times.

### Preparation of elastic liposomes

Elastic liposomes were prepared using a conventional rotary evaporation technique. Phospholipid 440 mg and 60 mg surfactant were dissolved in 10 mL of methanol/chloroform (1:2). The organic solvent was evaporated using a rotary evaporator and solvent traces removed by drying under vacuum overnight. The film was hydrated with 10 mL PBS (pH 7.4) containing methotrexate 9 mg $mL^{-1}$, idoxuridine 2 mg $mL^{-1}$ and aciclovir 2.5 mg $mL^{-1}$. Liposomes containing Phospholipon 90H were annealed at 60°C (above the phase transition temperature). Homogenously-sized liposomes were then produced following extrusion through 100-nm polycarbonate filters (Nucleopore) using a Lipex Extruder (Northern Lipids, Canada).

### Characterization

Particle size determination was carried out with a Phillips CM-10 Transmission electron microscope (Ganesan et al 1984; Guo et al 2000) five times for each formulation. Phosphotungstic acid was used for negative staining before microscopy.

### Skin preparation

The experiments were carried out under the approval of the Ethics Committee of North-West University, Potchefstroom Campus, South Africa. Female human abdominal skin tissue from cosmetic surgery was used. Full thickness skin was thawed overnight, adipose tissue removed by blunt dissection and the skin immersed in water at 60°C for 1 min. The epidermis was carefully peeled away from the dermis (Du Plessis et al 2002).

### Skin permeation method

In-vitro permeation studies were conducted with vertical Franz diffusion cells with a 2.3-mL capacity receptor compartment and a 1.075-$cm^{-2}$ diffusion area. The receptor phase was sonicated for 15 min to remove air bubbles and to avoid build-up of air pockets. A loading dose of 5 $\mu$L liposome formulation containing methotrexate 9 mg $mL^{-1}$, aciclovir 2.5 mg $mL^{-1}$ and idoxuridine 2 mg $mL^{-1}$ was used. Loading doses were placed in the donor compartments non-occlusively to simulate a transepidermal hydration gradient (Cevc et al 1998). The cells were mounted on a magnetic stirring bed in a water bath at 37°C. Magnetic bars (2-mm) stirred the receptor compartments continuously at 500 rev $min^{-1}$. At 2, 4, 6, 8, 10 and 12 h, the receptor phase was removed and replaced by PBS pH 7.4 to maintain sink conditions. Samples were assayed by HPLC. Saturated solutions (5 $\mu$L) of methotrexate (9 mg $mL^{-1}$), aciclovir (2.5 mg $mL^{-1}$) and idoxuridine (2 mg $mL^{-1}$) in PBS served as controls. All permeation experiments were carried out six times.

### Data analysis

Transcutaneous flux was calculated by linear regression from the steady-state portion of the cumulative amount vs time curves. The permeation data for methotrexate, aciclovir and idoxuridine was fitted using Easyplot (Cherwell Scientific, USA) to equation 1 (Diez-Sales et al 1996):

$$Q(t) = AKhC\left[\frac{Dt}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \quad (1)$$

where Q(t) is the amount that passes through the membrane and reaches the receptor solution at a given time (t), *A* is the surface diffusion area, K is the partition coefficient of the permeant between the membrane and the donor vehicle, h represents the thickness of the membrane, D is the diffusion coefficient of the permeant across the membrane and C represents the concentration of the permeant in the donor solution, which in this study is the solubility of the drug in the vehicle.

From equation 1, $\alpha$ and $\beta$ values were estimated:

$$\alpha = K^{*}h \quad (2)$$

$$\beta = D/h^2 \quad (3)$$

The effect of the various formulations on the flux values and the permeation parameters was evaluated using a one-way analysis of variance with a post-hoc test (Dunnett's multiple comparison test) with significance levels set at $P < 0.05$ to denote significance in all cases.

## Results and Discussion

### Characterization

Transmission electron micrographs of representative liposomes are illustrated in Figure 2. Mean liposome size as determined by transmission electron microscopy was

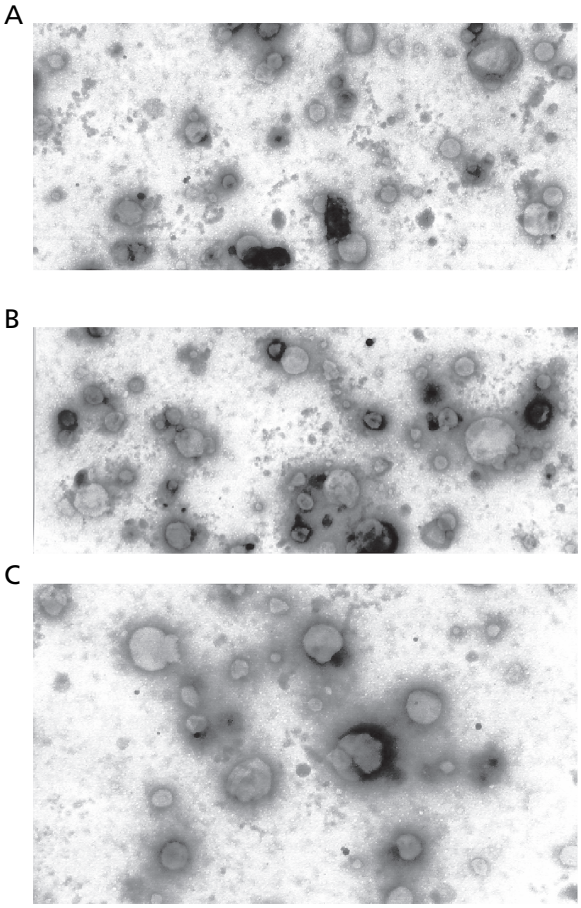


**Figure 2** Transmission electron micrograph of elastic liposomes loaded with (A) methotrexate, (B) aciclovir and (C) idoxuridine.

120 ± 8 nm for all formulations with a similar size range observed for all formulations.

### Influence of phospholipid mixtures

Commercially available mixtures containing different concentrations of phosphatidylcholine (PC) were used to prepare elastic liposomes. All formulations contained sodium cholate and 7% ethanol. Our aim was to investigate the influence of these formulations on transdermal flux of methotrexate, aciclovir or idoxuridine from elastic liposomes. The aqueous solubility values (mg $mL^{-1}$) ± s.d. (n = 6) of methotrexate, aciclovir and idoxuridine in phosphate-buffered saline (pH 7.4) were determined at 25°C and are listed in Table 1. Bar plots of the mean steady-state flux of methotrexate, aciclovir and idoxuridine from control formulations, and from elastic liposomes containing sodium cholate and different phospholipids are shown in Figure 3. Permeation parameters obtained from curve-fitting on Easyplot for Windows for different phospholipids are shown in Table 2. Flux values for methotrexate, aciclovir and idoxuridine from saturated (control) solutions were determined as 7.43 ± 1.35, 18.34 ± 3.52 and 33.80 ± 6.35 ng $cm^{-2}$ $h^{-1}$, respectively.

**Table 1** Solubility of methotrexate, aciclovir and idoxuridine in phosphate-buffered saline (PBS)

| Drug | Solubility in PBS (7.4) (mg $mL^{-1}$) at 25°C |
|---|---|
| Methotrexate | 9.02 ± 0.05 |
| Aciclovir | 2.51 ± 0.03 |
| Idoxuridine | 2.06 ± 0.01 |

Values are mean ± s.d.; n = 6.

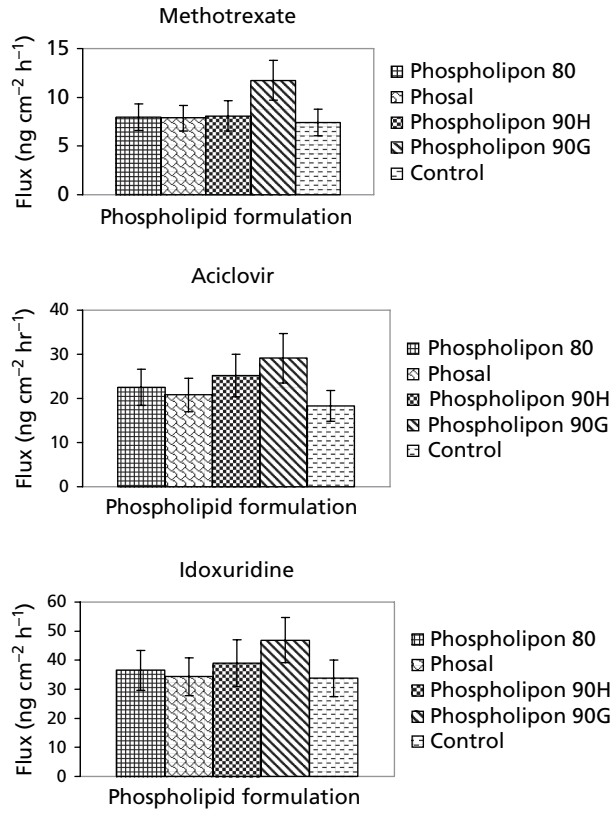


**Figure 3** Effect of phospholipid concentration on the flux of methotrexate, aciclovir and idoxuridine.

For the three drugs, transcutaneous flux values were significantly increased ($P < 0.05$) for the liposomes prepared using Phospholipon 90G relative to values for control formulations (Figure 3). Phosphal PG contains approximately 50% propylene glycol which might have been expected to influence the flux values of idoxuridine relative to Phospholipon 90G (95% phosphatidylcholine, 4% lysophosphatidylcholine) because of possible cosolvent or enhancement effects of propylene glycol, however this was not the case. The presence of 7% ethanol in all liposomal formulations did not appear to contribute to any enhancement in flux values.

These results are in agreement with the findings of Valenta & Janisch (2003). The authors investigated the permeation of cyproterone acetate through pig skin from different liposomal formulations (Valenta & Janisch 2003). The highest cyproterone amount was released by the formulation

**Table 2** Permeation parameters obtained from curve-fitting on Easyplot for Windows

| Phospholipid | $\alpha \times 10^5 \pm$ s.d. | $\beta \times 10^4 \pm$ s.d. | $k_p$ (cm$^2$ h$^{-1}$) $\times 10^7 \pm$ s.d. | $J$ (ng cm$^{-2}$ h$^{-1}$) $\pm$ s.d. |
|---|---|---|---|---|
| Methotrexate | | | | |
| Phospholipon 80 | 2.40 (0.49) | 384 (70) | 9.21 (1.49) | 8.28 (1.35) |
| Phosal PG | 2.19 (0.35) | 413 (67) | 9.04 (1.52) | 8.13 (1.28) |
| Phospholipon 90H | 2.33 (0.48) | 421 (73) | 9.80 (1.78) | 8.82 (1.54) |
| Phospholipon 90G | 2.72 (0.41) | 504 (88) | 13.70 (1.65) | 12.33 (2.03) |
| Control | 2.35 (0.47) | 363 (59) | 0.85 (0.17) | 7.67 (1.35) |
| Aciclovir | | | | |
| Phospholipon 80 | 1.21 (0.24) | 760 (135) | 9.19 (1.61) | 22.97 (4.13) |
| Phosal PG | 1.18 (0.37) | 695 (128) | 8.20 (1.55) | 20.50 (3.75) |
| Phospholipon 90H | 2.07 (0.31) | 519 (95) | 10.74 (1.97) | 26.85 (4.82) |
| Phospholipon 90G | 5.83 (1.18)* | 202 (41) | 11.77 (2.21) | 29.44 (5.60) |
| Control | 0.92 (0.17) | 942 (157) | 8.68 (1.51) | 21.71 (3.52) |
| Idoxuridine | | | | |
| Phospholipon 80 | 4.57 (0.88) | 409 (77) | 18.69 (3.41) | 37.38 (6.87) |
| Phosal PG | 3.43 (0.61) | 506 (93) | 17.35 (3.27) | 34.70 (6.53) |
| Phospholipon 90H | 4.88 (0.99) | 417 (88) | 20.34 (4.05) | 40.68 (8.04) |
| Phospholipon 90G | 5.10 (0.85) | 475 (72) | 24.22 (3.81) | 48.45 (7.80) |
| Control | 4.24 (0.70) | 410 (78) | 17.38 (2.18) | 34.76 (6.35) |

*Significant enhancement ($P < 0.05$).

**Table 3** Permeation parameters obtained from curve-fitting of flux data on Easyplot for Windows

| | $\alpha$ (cm) $\times 10^5 \pm$ s.d. | $\beta$ (h$^{-1}$) $\times 10^3 \pm$ s.d. | kp (cm$^2$ h$^{-1}$) $\times 10^6 \pm$ s.d. | $J$ (ng cm$^{-2}$ h$^{-1}$) $\times$ s.d. |
|---|---|---|---|---|
| Methotrexate | | | | |
| Sodium deoxycholate | 1.67 (0.37) | 568 (107) | 0.95 (0.17) | 8.53 (1.64) |
| Span 20 | 2.20 (0.31) | 405 (61) | 0.89 (0.13) | 8.01 (1.27) |
| Span 80 | 2.80 (0.48) | 450 (75) | 0.13 (0.03) | 11.34 (1.80) |
| Span 60 | 2.28 (0.51) | 471 (87) | 0.11 (0.01) | 9.66 (1.73) |
| Sodium cholate | 2.72 (0.42) | 504 (83) | 0.14 (0.03) | 12.33 (2.03) |
| Span 40 | 1.93 (0.24) | 636 (195) | 0.12 (0.04) | 11.04 (1.60) |
| Control | 2.35 (0.47) | 363 (59) | 0.85 (0.17) | 7.67 (1.35) |
| Aciclovir | | | | |
| Sodium deoxycholate | 1.83 (0.32) | 597 (114) | 10.92 (2.06) | 27.31 (5.14) |
| Span 20 | 1.99 (0.48) | 489 (91) | 9.73 (1.79) | 24.32 (4.70) |
| Span 80 | 1.19 (0.26) | 757 (125) | 9.00 (1.62) | 22.52 (3.81) |
| Span 60 | 1.41 (0.33) | 847 (176) | 11.94 (2.48) | 29.85 (6.04) |
| Sodium cholate | 5.83 (1.18) | 202 (34) | 11.77 (2.27) | 29.44 (5.60) |
| Span 40 | 1.07 (0.21) | 863 (148) | 9.23 (1.58) | 23.08 (3.84) |
| Control | 0.92 (0.17) | 942 (157) | 8.68 (1.51) | 21.71 (3.52) |
| Idoxuridine | | | | |
| Sodium deoxycholate | 3.28 (0.63) | 701 (135) | 22.99 (4.24) | 45.98 (8.54) |
| Span 20 | 2.79 (0.58) | 696 (143) | 19.41 (3.76) | 38.13 (7.31) |
| Span 80 | 17.20 (3.21) | 108 (24) | 18.57 (3.67) | 37.15 (7.02) |
| Span 60 | 2.46 (0.43) | 880 (169) | 21.64 (4.01) | 43.29 (8.15) |
| Sodium cholate | 5.10 (0.85) | 475 (81) | 24.22 (3.97) | 48.45 (7.80) |
| Span 40 | 3.17 (0.63) | 585 (116) | 18.54 (3.40) | 37.08 (6.94) |
| Control | 4.24 (0.70) | 410 (78) | 17.38 (2.18) | 34.76 (6.35) |

with the highest lipid content (140 mg mL$^{-1}$). It was suggested that the high lipid content could be responsible for increased flux via direct interaction of the lipid with the lipid bilayer structure of the skin.

Analysis of the permeation parameters confirmed that there were no significant differences for any of the formulations studied on the values of $\alpha$ and $\beta$ for methotrexate or idoxuridine relative to the control values (Table 2, $P < 0.05$). For aciclovir, Phospholipon 90G formulations significantly enhanced $\alpha$ relative to the other phospholipid formulations and control, while no increase of the $\beta$ parameter was observed for any of the formulations relative to the control values (Tables 2, $P > 0.05$).

The mechanism of stratum corneum-liposome interaction is a subject of many investigations using confocal laser scanning microscopy (Betz et al 2001b), freeze-fracture electron microscopy (Hofland et al 1995) and differential scanning calorimetry (Valenta et al 2001b). Hofland et al (1995)

observed that the degree of interaction between vesicular dispersions and the skin depended on the physicochemical properties of the liposomal components. The authors investigated three types of liposomes containing various amounts of phosphatidylcholine (PC). The formulation containing the highest amount of PC (85% PC) was found to have the greatest effect on the ultrastructure of the stratum corneum compared with the other two formulations (10% PC and 28% PC). It was concluded that liposomal constituents could penetrate into the stratum corneum (SC), mix with the SC lipids and induce a penetration-enhancing effect by producing ultrastructural changes in the intercellular lamellae.

Betz et al (2001a) studied the penetration behaviour of heparin across human skin from liposomes prepared from Phospholipon 80. The results showed enhanced penetration into the epidermal membrane from the liposomes while no penetration was found with a purely aqueous solution. The authors also studied the interaction of Phospholipon 80 and sphingomyelin liposomes containing heparin with the human skin using confocal laser scanning microscopy and observed a strong, and in some respects, composition-dependent interaction of the phospholipids with the skin (Betz et al 2001b).

## Effect of surfactant

For this study, elastic liposomes were prepared from 95% phosphatidylcholine (Phospholipon 90G) and various surfactants. Sodium cholate, sodium deoxycholate and a series of sorbitan esters were investigated. The esters were chosen to study the effect of different alkyl chain length on skin permeation. The vesicles were formulated with methotrexate, aciclovir or idoxuridine and flux values relative to saturated solutions (control) of methotrexate, idoxuridine and aciclovir are shown in Figure 4.

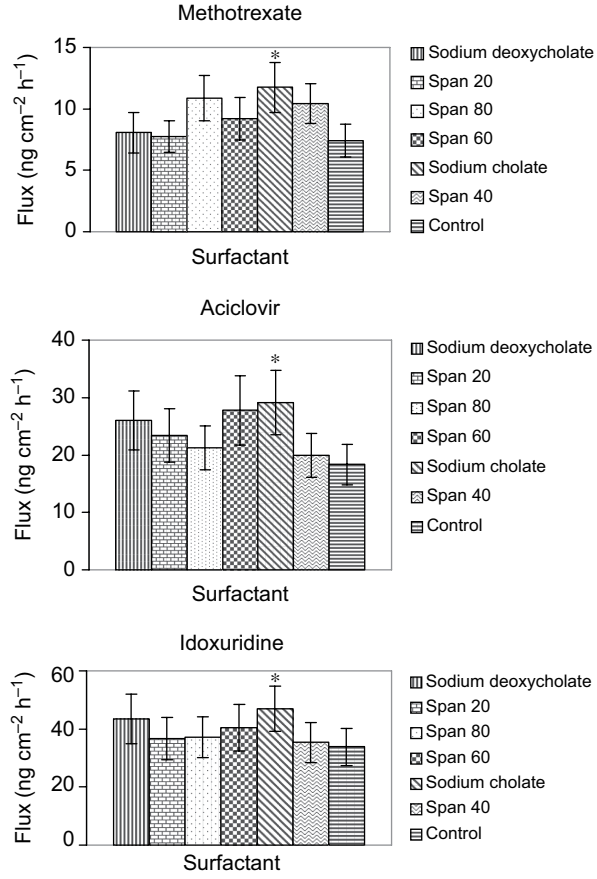


**Figure 4** Effect of surfactants on permeation of methotrexate, aciclovir and idoxuridine from elastic liposomes across human epidermal membrane. *Significant enhancement ($P < 0.05$).

For the three drugs studied the flux values for sodium cholate formulations were significantly higher than for control formulations ($P < 0.05$). Flux values for sodium deoxycholate formulations were significantly higher for aciclovir and idoxuridine vs control values ($P < 0.05$) but not for methotrexate. Sorbitan monolaurate, monopalmitate and stearate are saturated and sorbitan monooleate has an unsaturated alkyl chain. Saturated sorbitan monoesters differ from each other in the length of the hydrocarbon chain. The hydrocarbon chain length of sorbitan monolaurate, monopalmitate and stearate is 12, 16 and 18 carbon atoms, respectively. The unsaturated sorbitan monooleate has the same carbon chain length but differs from the sorbitan stearate with a double bond in the hydrocarbon chain. The double bond in the sorbitan monooleate molecule is thought to be responsible for the reduction of hydrophobic chain–chain interactions (Korhonen et al 2004).

In this series, sorbitan monolaurate has the shortest hydrophobic hydrocarbon chain, which makes it the most hydrophilic surfactant. Differences in surface activity of sorbitan monoesters are primarily derived from the alkyl group. The increasing chain length promotes the tendency towards the formation of condensed monolayers. The more condensed packing of sorbitan stearate and sorbitan monopalmitate as compared with the sorbitan monolaurate did not significantly affect transdermal delivery efficiency ($P > 0.05$). The differences in flux values of methotrexate, aciclovir and idoxuridine from liposomes containing different sorbitan monoesters were not statistically significant as determined by analysis of variance ($P > 0.05$) and there was no clearly defined trend between alkyl chain length and flux values. Despite the differences in chemical structures, the flux values from formulations prepared with sodium cholate and sodium deoxycholate did not differ significantly for the formulations prepared with the non-ionic Span surfactants ($P > 0.05$). The effect of elastic liposomes on the partition parameter ($\alpha$) was not significant for methotrexate ($P > 0.05$), however sodium deoxycholate and Span 40 enhanced the magnitude of the diffusion parameter $\beta$ ($P < 0.05$) (Table 3). For aciclovir, significant effects on the partition parameter ($P < 0.05$), but not the diffusion parameter, were observed for the bile salts and Span 20. The Span 80 formulations appeared to significantly enhance the partition parameter $\alpha$ of idoxuridine ($P < 0.05$), while sodium deoxycholate, Span 20, and Span 60 formulations significantly enhanced the diffusion parameter ($\beta$) ($P < 0.05$). In general, the surfactants may have been expected to intercalate into the skin lipids and affect their diffusional parameters. This would have been seen in significant increases in the $\beta$ values, however this was not observed for the majority of the formulations investigated. This might arise because of low

concentrations of free surfactant available where the bulk is incorporated into the liposome structure. Methotrexate, aciclovir and idoxuridine had low aqueous solubility as shown in Table 1. Due to their poor solubility and low partition coefficients, their permeation across the human stratum corneum was low. It was interesting that the experimental partition behaviour of the three compounds into the stratum corneum was very similar, as seen by comparing the $\alpha$ values for the control experiments. All three compounds were hydrophilic with experimentally-determined log P values of −1.2, −1.57 and 0.95 for methotrexate, aciclovir and idoxuridine, respectively (Chatterjee et al 1997; Kristl & Tukker 1998; Bonina et al 2002). Calculated values (ACD Software, Toronto, Canada) were −0.28, −1.76 and −0.49. The skin appeared to attenuate any difference seen for the octanol partition values. There was little difference in the diffusional properties for the control experiments suggesting that the three permeants diffused through the stratum corneum at a similar rate.

## Conclusions

The physicochemical properties of the hydrophilic compounds methotrexate, aciclovir and idoxuridine combined with the barrier properties of the stratum corneum suggested that transport of these drugs from simple vehicles may not result in therapeutic drug concentrations either in the skin or in local or distal tissues. Many studies performed in the last decade have shown significantly higher absorption rates as well as greater pharmacological effects for drugs applied to the skin entrapped in ultradeformable liposomal vehicles as compared with conventional topical formulations. In this study ultradeformable formulations of methotrexate, aciclovir and idoxuridine were developed, which varied in the concentration of phospholipid and nature of surfactant. In-vitro penetration studies into human skin showed that the highest flux values for the three compounds were observed from elastic liposomes containing 95% phosphatidylcholine. Higher flux values were also observed for liposomes containing sodium cholate compared with sodium deoxycholate. However, for the sorbitan esters studied there was no clearly defined trend between alkyl chain length and flux values. While phospholipid concentration appeared to be a critical consideration for formulation of hydrophilic materials in ultradeformable liposomes, the flux values observed for the drugs from elastic liposomes were comparable with those from saturated aqueous solutions. This contrasts with the influence of such vehicles on the permeation of more lipophilic compounds where significant flux enhancement has been shown.

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