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# The effects of complexation with hydrogenated phospholipid on the transport of salicylic acid, diclofenac and indomethacin across snake stratum corneum

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## Summary

Hydrogenated phospholipid (HPL) has been found to form complexes with three non-steroidal anti-inflammatory drugs: salicylic acid, diclofenac and indomethacin. The solubility of the salicylic acid complex in squalane was less than that of the plain drug, but the solubilities of the diclofenac and indomethacin complex increased upon complexation. The permeability coefficients of salicylic acid and diclofenac across shed snake skin are not affected significantly by the presence of hydrogenated phospholipid. However, the permeability coefficient for the indomethacin-HPL complex is about double that for either plain indomethacin or an indomethacin-HPL physical mixture.

## Introduction

Transdermal drug delivery systems are gaining increasing popularity and several drugs have been successfully delivered by this route for both local and systemic action. The bioavailability of transdermally administered drugs is largely a function of their permeability across the stratum corneum. Several approaches have been used to increase drug permeability. These include: (a) the use of metastable forms of drugs (Campigli et al., 1986), (b) the use of penetration enhancers (Walters, 1989), (c) facilitated transport of drugs (Hadgraft and Wotton, 1985) and (d) chemical modification of drugs (Hadgraft, 1985). Scheuplein and Bland (1971) have reported that for optimum permeability it is desirable that the drug have (i) adequate solubility in the formulation vehicle and (ii) a favorable partition coefficient from the formulation into the stratum corneum.

This work reports on the effect of complexation of three non-steroidal anti-inflammatory drugs (NSAID) with hydrogenated phospholipids on the transdermal penetration of the drugs.

The stratum corneum forms the major barrier

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to transdermal penetration of drugs due to its highly ordered and rigid structure. The use of phospholipids in topical formulations is expected to enhance the penetration of drugs by temporarily altering the properties of the stratum corneum. It has been reported that anionic drugs interact with phospholipids to form fairly stable complexes which have much greater chloroform-water partition coefficients than the ionized forms of the drugs themselves (Horton and McLure, 1971; Furusawa et al., 1972). They have also shown that these complexes are transported across model biological membranes to a much greater extent than the ionized forms of the drugs.

Phospholipids are an important component of biological membranes. Although they have several useful properties, their use in pharmaceutical formulations is restricted by their susceptibility to oxidative and thermal degradation. Hydrogenated phospholipids, on the other hand, are remarkably stable and have been successfully employed in sustained release granules, suppositories, topical gels, etc. (Nishihata et al., 1986, 1987; Hirotani et al., 1987). We report here the preparation, physicochemical characterization and in vitro evaluation of the effect of complexation with hydrogenated phospholipid on the transport of three drugs across snake stratum corneum. This tissue was chosen as a model membrane for evaluation based on the reports by several workers (Kligman, 1984; Ibuki, 1985; Akazawa et al., 1989; Itoh et al., 1990) that it has many properties similar to those of the human stratum corneum, and is comparable to some other membranes that have been used for in vitro evaluation. It is also easy to obtain, store and use.

## Experimental

### *Materials*

Hydrogenated phospholipid (Nikkol) was supplied by Nikko Chemical Co., Tokyo, Japan. Salicylic acid, diclofenac and indomethacin were obtained from Sigma Chemical Co., St. Louis, MO. All solvents used were of HPLC grade. Skin from the black rat snake, *Elaphe obsoleta* was obtained from the Animal Care Unit of the University of Kansas.

TABLE 1

*Drug-HPL combinations used to prepare complexes for in vitro diffusion study*

| | Drug (mg/10 ml [a]) | HPL (mg/10 ml [a]) |
|---|---|---|
| Salicylic acid | 50 | 50 |
| Diclofenac | 50 | 100 |
| Indomethacin | 50 | 50 |

[a] Chloroform.

### *Methods*

#### *Complex preparation*

Complexes were prepared by combining each of the three drugs, salicylic acid, diclofenac and indomethacin in varying ratios with hydrogenated phospholipids (HPL) in 10 ml of chloroform and mixing using a magnetic stirrer for several hours (Table 1). At a drug-HPL ratio specific to each drug, the chloroform turned completely clear. At all other ratios, the preparations contained some uncomplexed HPL which remained as a turbid dispersion (see Table 2).

#### *Infrared spectroscopy*

The infrared spectra of the drugs, HPL, and drug-HPL complexes were measured using KBr pellets of the materials on a Perkin Elmer 1420 Ratio Recording IR spectrophotometer.

#### *Differential scanning calorimetry*

The melting characteristics of the drugs and their HPL complexes were studied by differential

TABLE 2

*Drug-HPL ratios which give a clear solution in chloroform (drug concentration 50 mg / 10 ml)*

| Drug | Ratio (drug:HPL) | |
|---|---|---|
| | Weight | Molar [a] |
| Salicylic acid | 1:1 | 5.5:1 |
| Diclofenac | 1:2 | 1.4:1 |
| Indomethacin | 1:1 | 2.2:1 |

[a] HPL is a mixture of several components; its average molecular weight is about 789.

TABLE 3

*HPLC conditions for the drugs used in the experiment*

| Drug | Mobile phase | Flow rate (ml/min) | Detection wavelength (nm) |
|---|---|---|---|
| Salicylic acid | 55:80 $CH_3OH$:pH 2.5 0.01 M phosphate buffer | 1 | 280 |
| Diclofenac | 74:41 $CH_3OH$:pH 3 0.01 M phosphate buffer | 0.8 | 234 |
| Indomethacin | 60:50 $CH_3CN$:pH 3 0.1 M phosphate buffer | 0.8 | 260 |

Note: injection volume was 20 $\mu$l in each case.

scanning calorimetry (DSC) using a Perkin Elmer 4 differential scanning calorimeter.

*Solubility in squalane*

The solubilities of the drugs, viz. salicylic acid, diclofenac and indomethacin in plain squalane, and of drug-HPL physical mixtures and complexes in squalane were investigated. Saturated squalane suspensions of the drugs were prepared and analyzed as follows. 200-$\mu$l samples of the suspensions were centrifuged at 3000 rpm for 10 min in an IEC HN-SII centrifuge. 100 $\mu$l of the clear supernatant was pipetted out and extracted with 25 ml of 0.001 N NaOH solution. 1 ml of the aqueous layer was diluted to 10 ml with 0.005 M pH 7.2 isotonic phosphate buffer solution. The solution was filtered and analysed by HPLC. The HPLC conditions for the drugs are given in Table 3. Four determinations were made for each substance or mixture.

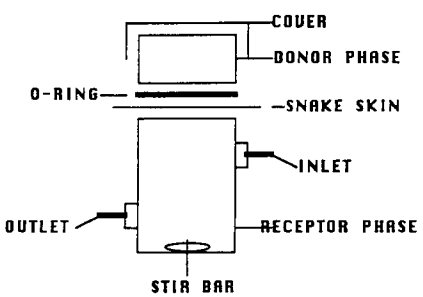


Fig. 1. Schematic diagram of modified Franz cell.

*In vitro diffusion of the drugs*

For the diffusion studies, a modified form of the Franz cell was used. Fig. 1 shows a diagram of the cell. The donor and receptor phases were separated by the snake skin. The receptor phase contained 0.005 M isotonic phosphate-buffered saline at pH 7.2. The buffer was degassed prior to use. The cell was assembled, the formulation applied on the skin using a syringe, and the whole unit placed in a 37 °C water bath with the receptor phase being stirred by a magnetic stirrer.

The receptor phase was sampled by collecting 10 drops (after discarding the first five drops) into vials for analysis by HPLC. As the samples were collected, the receptor phase was replenished by fresh, degassed buffer. Sampling was performed at 2-h intervals for the first 12 h and then a 24 h sample was collected. With indomethacin, however, 48 and 72 h samples were also collected. Four determinations were made for each formulation.

The different formulations that were studied were as follows:

(1) All three drugs, salicylic acid, diclofenac and indomethacin were formulated as suspensions containing (i) plain drug, (ii) drug-HPL physical mixture and (iii) drug-HPL complex. Each formulation contained the equivalent of 1% w/w of drug as suspensions in squalane. They were used to study the effect of complexation on the permeability coefficients of the drugs.

(2) Using salicylic acid as a model drug, the effect of HPL present in physical mixtures was studied using formulations containing 5, 10 and 15% HPL in combination with 10% drug in squalane.

(3) The effect of formulation on the penetration of the drug and its complex was studied using aqueous formulations, and triglyceride gel formulations (see Table 4) of diclofenac.

The formulations mentioned above were prepared for diffusion studies as follows. The complex was isolated by evaporating off the chloroform from the clear solutions and collecting the dry residue. The squalane formulations were prepared by making uniform dispersions of the drug, complex or the drug-HPL physical mixture in squalane and stirring them for several hours us-

TABLE 4

*Triglyceride gel formulations*

| Ingredient | % w/w |
|---|---|
| Sodium diclofenac [a] | 1.0 |
| Hydrogenated phospholipid | 7.0 |
| Triglyceride (Witepsol H32) [b] | 3.5 |
| pH 7.2, 0.01 M isotonic phosphate buffer | q.s. to 100 |

[a] Formulation no. 3 had diclofenac-HPL complex which gave the equivalent of 1% w/w diclofenac in the formulation.
[b] Formulation no. 1 had 3.5% triglyceride, nos 2 and 3 had 7.0% triglyceride.

ing magnetic stirrers. Aqueous gels were prepared by heating a 7% dispersion of HPL in the drug solution to 65 °–70 ° C with constant stirring. The gels were then cooled gradually with continued stirring. The triglyceride gel formulations are described in Table 4. These were prepared by dissolving sodium diclofenac in the buffer and heating the solution to 80 ° C. The hydrogenated phospholipid was added to the triglyceride, also at 80 ° C. The aqueous solution was added to the lipid phase, with brisk and continuous stirring. The preparation was cooled gradually with stirring for about 2 h.

## Results

Infrared spectroscopy showed that there were several distinct differences in the absorption characteristics of the drug-HPL physical mixture and the complex. Sample spectra are shown in Figs 2–4. Marked differences are seen in the bands at 3000–3500, 1600–1700, and 1400–1600 $cm^{-1}$ in the spectra shown.

The results obtained by DSC are listed in Table 5. Samples scans are shown in Figs 5–7. It is observed that the melting points of the complexes are substantially lower than those of the plain drugs and that all the complexes melt with endotherms. Furthermore, the endotherms were quite sharp indicating a distinct complex, rather than the broadening one would expect from an intimate mixture.

The solubility of the salicylic acid complex in squalane was less than that of the plain drug, but solubilities of the diclofenac and indomethacin complex improved upon complexation. The results are shown in Table 6. While the solubility of salicylic acid and diclofenac in drug-HPL physical mixtures seems to be somewhat reduced, that of indomethacin is improved. This suggests that indomethacin probably undergoes complexation in squalane.

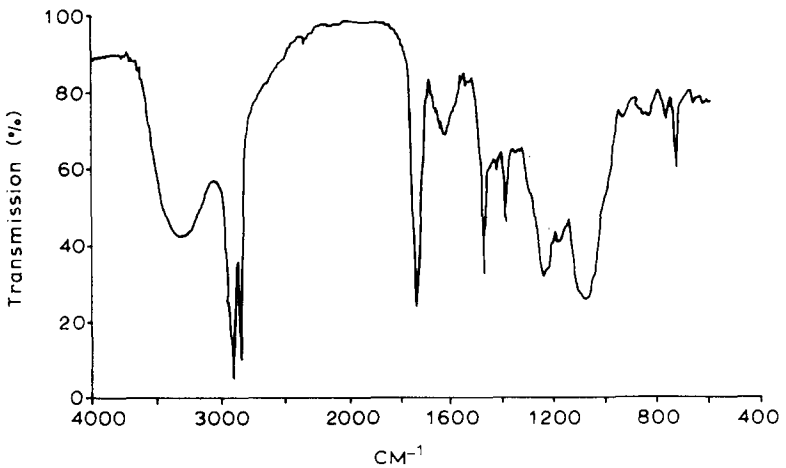


Fig. 2. Infrared spectrum of hydrogenated phospholipid.

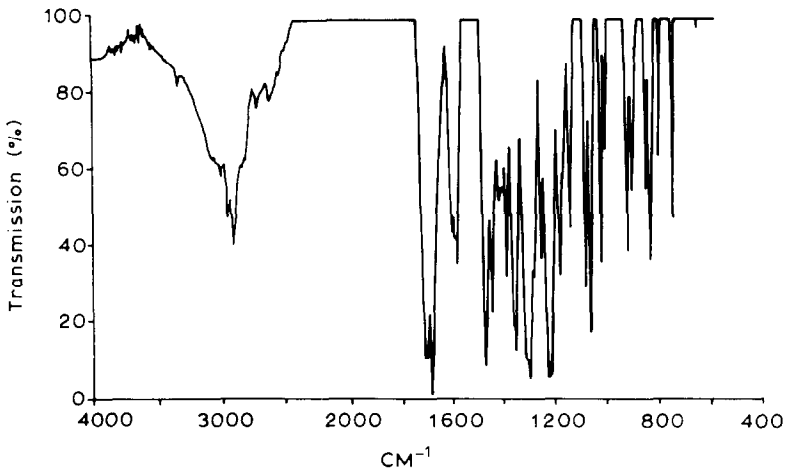


Fig. 3. Infrared spectrum of indomethacin.

TABLE 5

*Effect of complexation on the melting points of the drugs and hydrogenated phospholipid*

| Compound | Melting point (°C); exo/endotherm | |
|---|---|---|
| | Plain compound | Complex |
| Hydrogenated phospholipid | 80.7 (endo) | |
| Salicylic acid | 158 (exo) | 147.4 (endo) |
| Diclofenac | 167 (exo) | 139.22 (endo) |
| Indomethacin | 163 (endo) | 69.51 (endo) |

*In vitro diffusion studies*

The results of the in vitro diffusion studies are illustrated in Figs 8–13.

It is seen from Figs 8 and 9 that the penetration of salicylic acid across snake skin is not affected to any significant extent by the presence of HPL either in the physical mixture or in the complex.

With diclofenac, complexation does not seem to have any significant effect on permeation from aqueous solutions (Fig. 10). However, in triglyc-

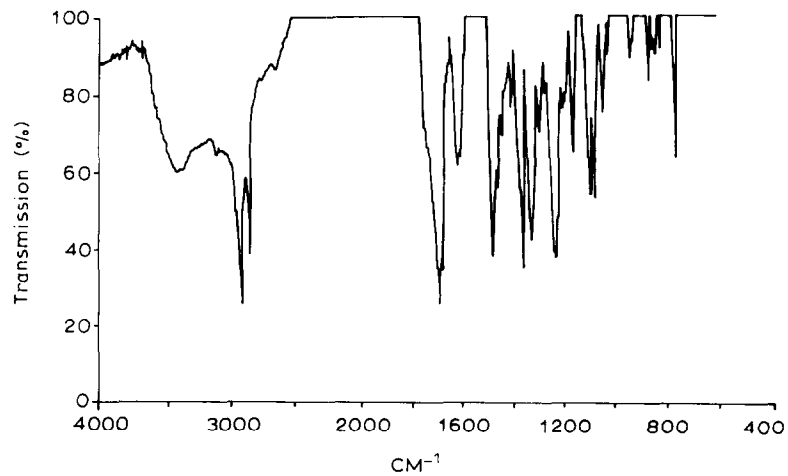


Fig. 4. Infrared spectrum of indomethacin-HPL complex.

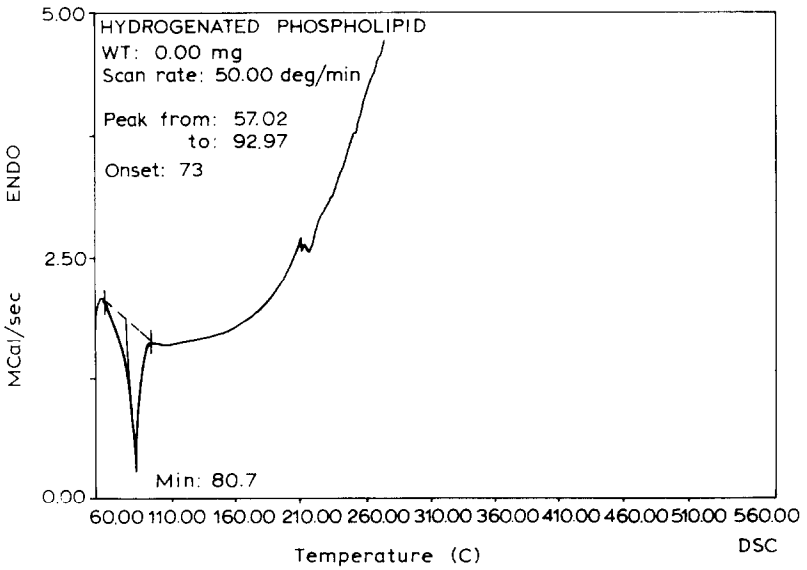


Fig. 5. DSC scan of hydrogenated phospholipid.

eride gel formulations (Fig. 12), the penetration of diclofenac was slightly better with the complex than with the plain drug. Studies with squalane suspensions of diclofenac (Fig. 11), indomethacin (Fig. 13) and their HPL complexes showed that the complex improved the drug permeability significantly.

It should be mentioned here that with all the formulations, the donor phase drug concentration was maintained constant at 1% w/w in order to eliminate permeation differences due to varying concentration gradients. The donor phase was saturated with drug and it is possible that some undissolved drug particles may have settled down

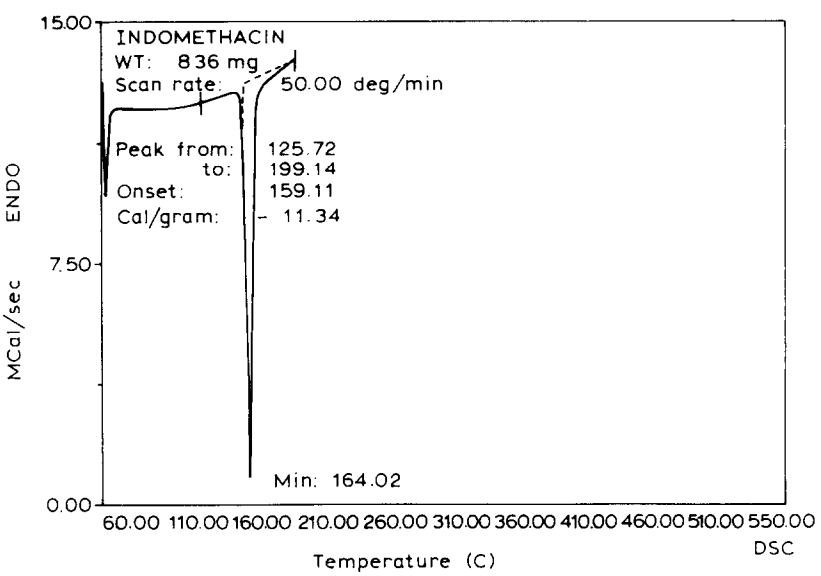


Fig. 6. DSC scan of indomethacin.

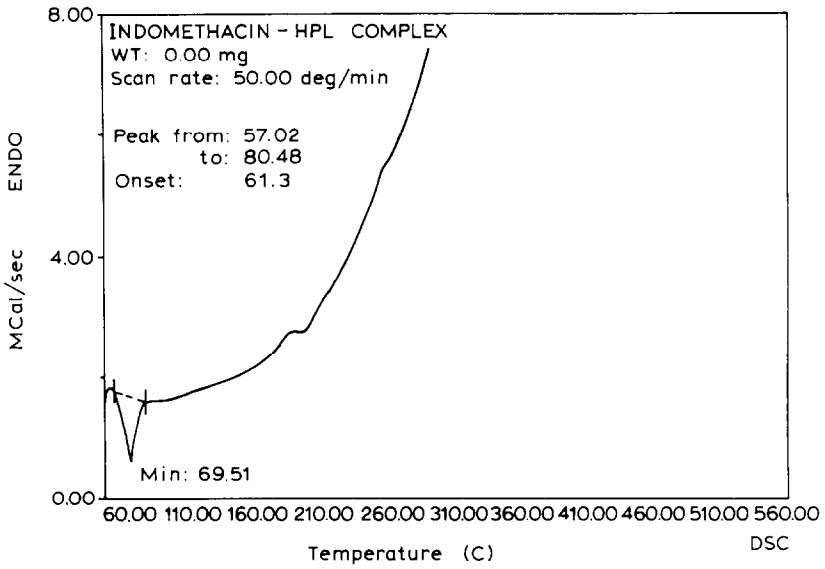


Fig. 7. DSC scan of indomethacin-HPL complex.

TABLE 6

*Solubilities of drugs, drug-HPL physical mixtures and complexes in squalane (mg ml$^{-1}$)*

| Drug | Plain | Physical mixture | Complex |
|---|---|---|---|
| Salicylic acid | 0.55504 | 0.38501 | 0.30802 |
| Diclofenac | 0.02004 | 0.01303 | 0.07009 |
| Indomethacin | 0.00016 | 0.00030 | 0.00025 |

on the surface of the snake skin. It is assumed that these undissolved particles affected drug permeation from all formulations to the same extent.

Table 7 lists the permeability coefficients of the drugs, drug-HPL mixtures and the complexes. The permeability coefficient was calculated using the formula: slope/$C_d A$ cm h$^{-1}$, where slope

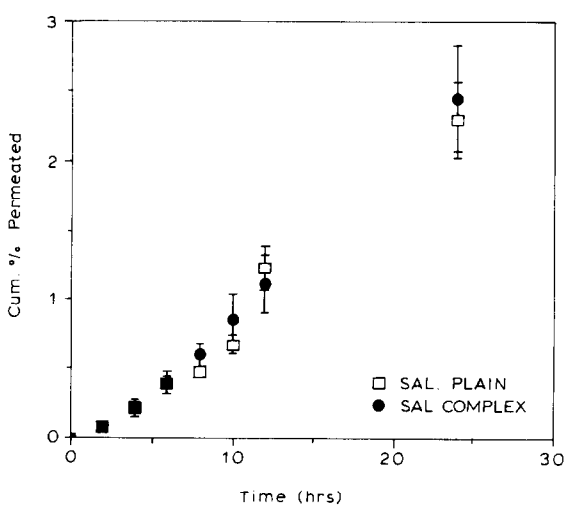


Fig. 8. Penetration of salicylic acid and its complex from squalane formulations.

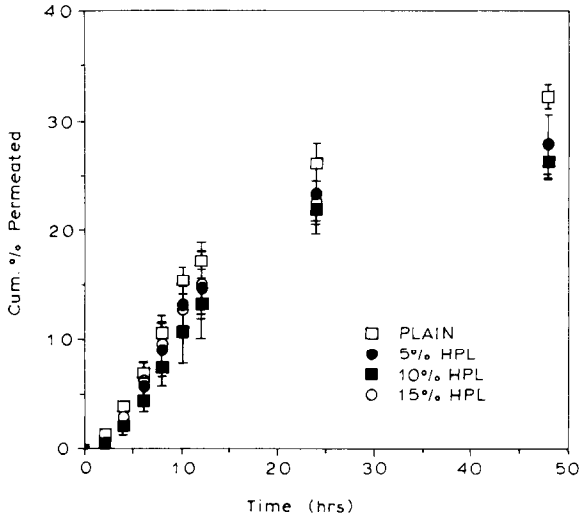


Fig. 9. Penetration of salicylic acid from formulations containing plain drug and its physical mixtures with HPL.

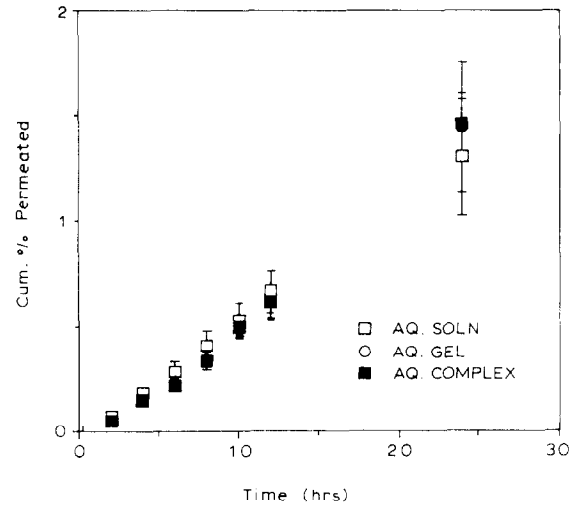


Fig. 10. Penetration of diclofenac from aqueous gel formulations.

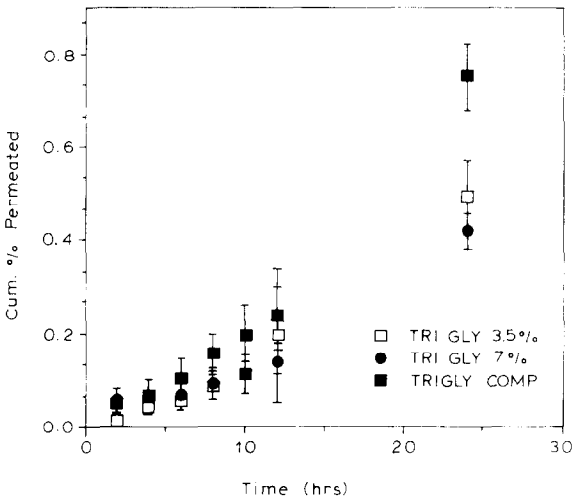


Fig. 12. Penetration of diclofenac from triglyceride gel formulations.

refers to the slope of the drug permeation graph, $C_d$ is the concentration of the drug in the donor compartment and $A$ represents the area (in $cm^2$) of the diffusion membrane, i.e. the stratum corneum. It can be seen that complexation has significantly increased the permeability coefficient in the case of indomethacin. For salicylic acid and diclofenac, however, the permeability coefficients are unaffected.

The decreased melting points and increased squalane solubilities of the drugs suggest that dissolution of the drug in the vehicle and subsequent release from the vehicle are generally improved by complexation. Also, since phospholipids are known to have an affinity for the proteins of biological membranes, it is possible that the complex partitions readily into the stratum corneum. It is known that the HPL alone does not penetrate the intact stratum corneum (in sample analysis, the HPL peak is observed in the HPLC as a major peak appearing a few seconds

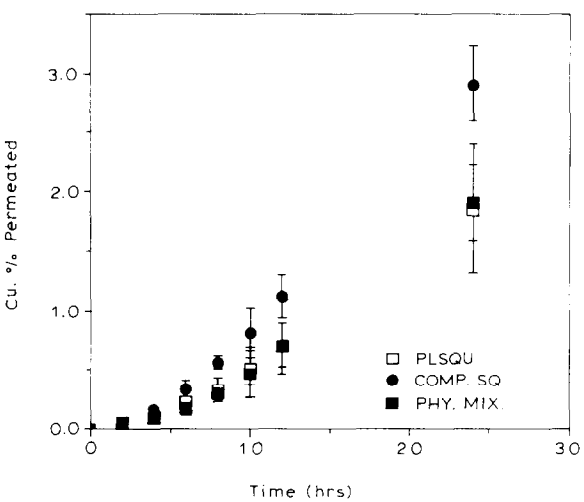


Fig. 11. Penetration of diclofenac from squalane formulations.

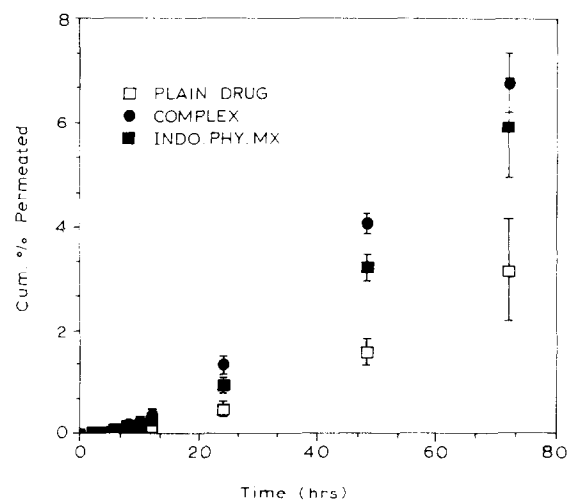


Fig. 13. Penetration of indomethacin from squalane formulations.

TABLE 7

*Permeability coefficients of drugs, complexes and physical mixtures from squalane*

| Formulation | Permeability coefficient ($cm\ h^{-1}$) |
|---|---|
| Salicylic acid | $0.026 \times 10^{-3}$ |
| Salicylic acid-HPL complex | $0.020 \times 10^{-3}$ |
| Salicylic acid-HPL physical mixture | $0.023 \times 10^{-3}$ |
| Diclofenac | $0.12 \times 10^{-3}$ |
| Diclofenac-HPL complex | $0.15 \times 10^{-3}$ |
| Diclofenac-HPL physical mixture | $0.12 \times 10^{-3}$ |
| Indomethacin | $0.57 \times 10^{-3}$ |
| Indomethacin-HPL complex | $1.19 \times 10^{-3}$ |
| Indomethacin-HPL physical mixture | $0.52 \times 10^{-3}$ |

after the solvent front only when there is a leak in the skin). It is therefore possible that the complex partitions into the skin, the HPL preferentially binds to the skin, and the drug is released for absorption.

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