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FACILITATED PERCUTANEOUS ABSORPTION: A MODEL SYSTEM

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SUMMARY

The transport of a model anionic drug, methyl orange, across a lipid membrane has been studied. Various N-substituted di-isopropanolamines have been shown to facilitate the rate of transfer. Data are presented to show how the structure of the carrier molecules influences overall permeation.

A mechanism is proposed by which anionic drug molecules may be effectively transported across the stratum corneum.

INTRODUCTION

Human skin, since it is largely lipoidal in nature, does not allow the easy transport of inorganic ions (Tregear, 1966a) and highly polar substances (Rothman, 1954). For this reason little emphasis has been placed on the formulation of topical preparations containing this type of drug. However, some drugs within this category may have considerable therapeutic potential. Sodium cromoglycate may be useful in the topical therapy of eczema (Haider, 1979), although as no rational attempts have been made to promote its percutaneous absorption, Zachariae et al. (1979) report no advantage over placebo. One possible way of accelerating the transport of ionic drugs is by facilitated transport. Since the stratum corneum consists primarily of inert keratinized cells, drug absorption by enzyme-mediated active transport is not possible (Tregear, 1966b). Horita and Weber (1964) and Poulsen (1973) have reported that many substances may accelerate drug absorption although the mechanisms by which they act are largely unknown. Allenby et al. (1969) have reported that aliphatic amines may promote the percutaneous penetration of tri-*n*-propyl phosphate and Jacob et al. (1964) have suggested that one way in which dimethyl sulphoxide may potentiate absorption is by acting as a weak base which complexes with the penetrating drug.

Lee et al. (1978), Cussler (1979), Thelander et al. (1980) and Babcock et al. (1980)

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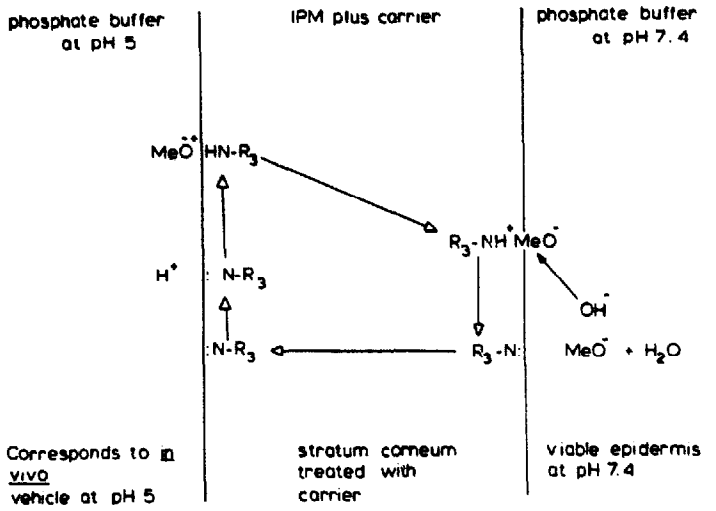


Fig. 1. Schematic diagram of the facilitated transport of MeO.

have been able to utilize a pH gradient to effect the facilitated transport of inorganic ions from an aqueous compartment across a non-polar organic phase to a receptor aqueous phase against the ion concentration gradient. This principle has been developed to attempt to facilitate the transport of anionic drugs across the stratum corneum. Since the epidermal surface is slightly acidic, pH 4.2–5.6 (Katz, 1973), a topical formulation of pH 5 is well tolerated by the skin. As the dermal pH is 7.3–7.4, the physiological pH, an in vivo pH gradient across the epidermis may be utilized to provide the free energy for facilitated transfer of an anionic drug. This is illustrated schematically in Fig. 1.

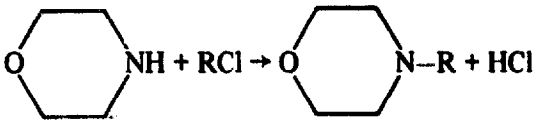
A model system has been used to design some carrier molecules and test their effectiveness in transporting anions. In order to simulate the epidermal barrier a Millipore filter impregnated with isopropyl myristate, a liquid representative of skin lipids (Poulsen et al., 1968), was used in a rotating diffusion cell (Albery et al., 1976 and 1979). To effect the interfacial transfer process described in Fig. 1, the ideal carrier should have a pK_a between 5 and 7.4, have a suitably high partition coefficient and have surfactant properties. Preferably the compound should be non-volatile, non-toxic, non-sensitizing and inert to the different skin constituents. Di-isopropanolamine (Poulsen et al., 1968), and morpholines (Quack, 1976) have been reported as topical formulatory adjuvants and a variety of similar amines are considered to be well tolerated by the skin (Harry, 1963). With these criteria in mind, a series of substituted amines have been synthesized and tested for their capacity to transport anionic drugs.

METHODS AND MATERIALS

A series of N-substituted bis-(2-hydroxypropyl)-amines (di-isopropanolamines) were prepared by the method proposed by Boivin (1958).

$$RNH_2 + 2\ CH_3 \cdot CH \cdot CH_2 \rightarrow RN(CH_2CH(OH)CH_3)_2$$
$$\qquad\qquad\quad \backslash O /$$

Their distillation temperatures at reduced pressure agreed with the reported values. N,N-Didecyl isopropanolamine was also prepared by a similar method ($B.P._{0.5mmHg}$, 194°C). N-Alkyl morpholines were synthesized according to the method given by Niederl et al. (1948) and their boiling points agreed with published values (Niederl and McCreal, 1952).



All reagents were of technical grade supplied by Aldrich Chemicals and the products were confirmed by IR and NMR spectroscopy.

Ethomeen S15 is a tertiary polyethoxylated amine derived from soyabean oil and was supplied by Akzo Chemie, U.K. Didodecylamine was supplied by Eastman Kodak, and diphenyl ether from BDH Chemicals.

The different amines that were synthesized are given along with their formulae, pK_a and B.P. in Table 1.

Methyl orange (MeO) was supplied by BDH and was recrystallized from water. Its molar extinction coefficient in aqueous solution for pH > 5 is 2.06×10^4 dm^3 mol^{-1} cm^{-1} at 464 nm.

TABLE 1

THE STRUCTURAL FORMULAE OF THE SYNTHESIZED AMINES

		B.P. °C (mmHg)	pK_a (Boivin, 1958)
$C_{12}H_{25}$-N-(CH_2-CH(OH)-CH_3)$_2$	N-dodecyldi-isopropanolamine (DIP)	186 (2.0)	6.2
$C_{14}H_{29}$-N-(CH_2-CH(OH)-CH_3)$_2$	N-tetradecyldi-isopropanolamine (TIP)	177 (0.8)	5.6
$C_{16}H_{33}$-N-(CH_2-CH(OH)-CH_3)$_2$	N-hexadecyldi-isopropanolamine (HIP)	190 (0.5)	4.8
$C_{18}H_{37}$-N-(CH_2-CH(OH)-CH_3)$_2$	N-octadecyldi-isopropanolamine (OIP)	211 (0.5)	4.6
$C_{10}H_{21}$, $C_{10}H_{21}$>N-CH_2-CH(OH)-CH_3	N, N-didecylisopropanolamine (DDP)	194 (0.5)	
$C_{12}H_{25}$-N⟨O⟩	N-dodecylmorpholine (DM)	141 (2.0)	
$C_{16}H_{33}$-N⟨O⟩	N-hexadecylmorpholine (HM)	167 (2.0)	
C_{18}-H_{37}-N⟨O⟩	N-octadecylmorpholine (OM)	173 (1.0)	

Isopropyl myristate (IPM) was supplied by Croda Chemicals and had a refractive index of 1.4346 at 25°C.

Equilibrium extraction coefficients were measured by shaking an equal volume of 0.1 M 'carrier' solution in IPM with 20 μM MeO in sodium phosphate buffer. The solutions were equilibrated over an 8-h period at 32°C which was chosen to reflect the temperature of the stratum corneum. The ionic strength of the buffer was adjusted to 0.2 M with sodium chloride. After equilibration the immiscible phases were separated and further clarified by centrifugation. The aqueous phase was assayed by visible spectrophotometry relative to the equilibrium mixture without carrier.

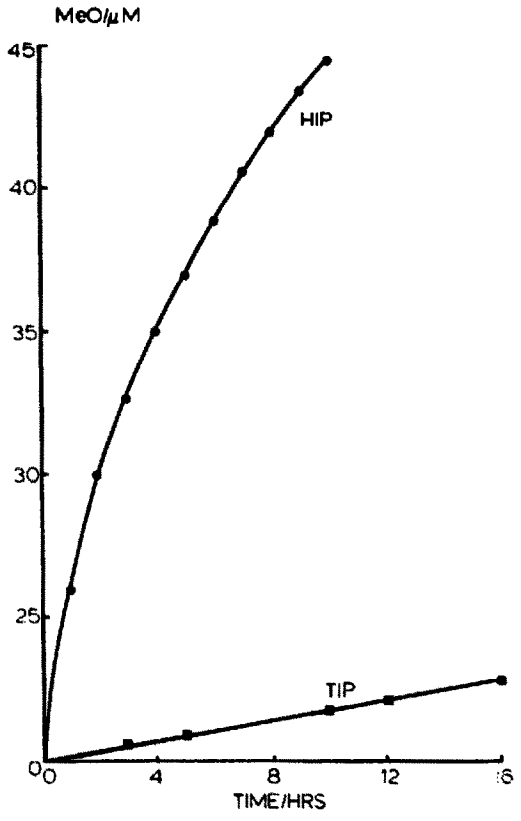
In this study the rate of transfer of MeO across an IPM-impregnated Millipore filter was studied in a rotating diffusion cell (Albery et al., 1976). This cell uses the hydrodynamics of the rotating disc system to impose a known pattern of convective flow on either side of the Millipore filter. The rotation of the cell produces stagnant diffusion layers of known thickness on both sides of the filter. It is thus possible to examine whether diffusion across these layers is a rate-determining step. In all experiments 1.2 μm pore size mixed cellulose ester filters were used which were impregnated with carrier solution in IPM by saturating the filter and carefully removing any excess solution with a tissue.

The donor and receptor compartments were filled with 250 cm^3 and 30 cm^3 of 20 μM MeO, respectively. The pH in both compartments was maintained by using constant ionic strength sodium phosphate buffer. The rate of appearance of MeO was monitored continuously using a flow-through cell in a spectrophotometer.

RESULTS AND DISCUSSION

In preliminary studies didodecylamine which has a pK_a of 10.99 at 25°C (Hoerr et al., 1943) was used as a carrier in diphenyl ether to check that the system was functioning correctly. With this compound it was possible to transport MeO against its own concentration gradient provided a pH gradient of 5.0–13.0 was established across the membrane. For these conditions a MeO flux of 3.4×10^{-9} mol mm^{-2} h^{-1} was obtained.

In all the following transport experiments the pH gradient established across the membrane was pH 5 to pH 7.4, achieved using phosphate buffer of constant ionic strength. The ionic strength was maintained constant since in preliminary experiments a decrease of MeO flux was observed as the ionic strength was increased. At high ionic strengths it is possible that there is competition between the other anions and MeO for 'transfer sites' at the interface. The synthesized carriers transported MeO by two distinct processes which appear to be a function of chemical structure and carrier concentration. Fig. 2 shows the two extreme types of behaviour when the MeO concentration in the receptor compartment was monitored as a function of time. Efficient carriers (OIP (>0.02 M), HIP (>0.05 M), DDP (>0.05 M)) give high rates of transfer and the build up of MeO in the receptor compartment is not linear with time. The experiments also indicate for this type of profile that there is some contribution to the overall transport process from the stagnant diffusion layers on either side of the filter. In the other type of behaviour there is no contribution from the aqueous stagnant diffusion layers, and transport rates are governed by diffusion through the membrane. Those carriers that are inefficient (Ethomeen, SI5, DIP,



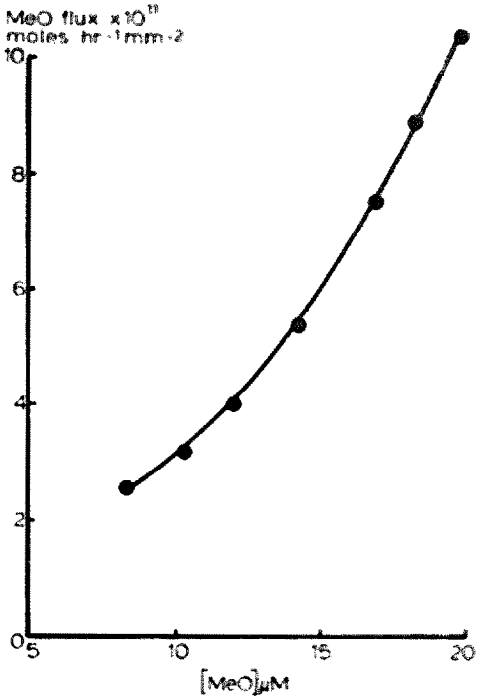


Fig. 2. The two distinct types of transport observed for the increase in MeO concentration in the receptor phase.

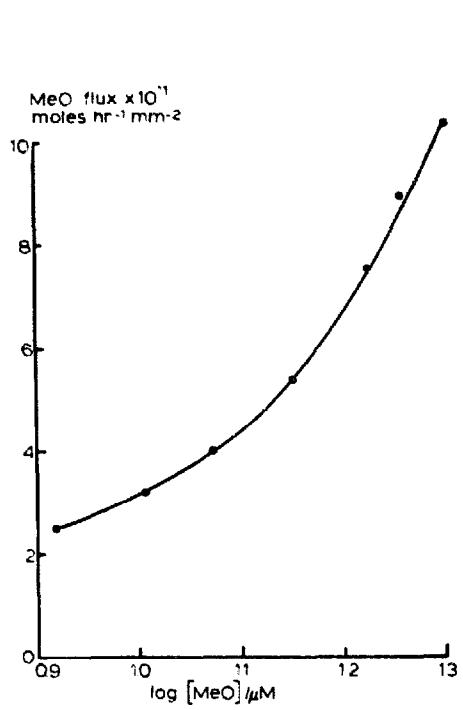
Fig. 3. Relationship between MeO flux and MeO concentration in the donor compartment. The carrier is 0.1 M HIP in IPM.

DM, OM) produce a MeO flux that is independent of the donor compartment concentration and some form of 'saturation' kinetics must be occurring. Some of the carriers exhibited properties between these two extremes (OIP (<0.02 M), HIP (<0.05 M), DDP (<0.05 M), TIP) but the results obtained were reproducible.

Figs. 3 and 4 show that for the efficient carrier HIP there is no simple relationship between the flux of MeO and its concentration in the donor compartment. This suggests that the transfer kinetics are complex with no individual process being rate limiting.

In order to compare the different carriers, experiments were allowed to run for at least 10 h. After a 6-h period the flux was estimated and it was assumed that by this time 'pseudo-steady-state' conditions had been achieved. Fig. 5 shows the MeO flux after 6 h as a function of the di-isopropanolamine concentration. Each point represents the mean of 3 determinations and the maximum standard deviation is 3% of the mean value. The reproducibility tended to be better for the lower fluxes.

The results show that the facilitated transport of MeO varies linearly with the carrier concentration except in the case of OIP which was the most efficient of the carriers syn-



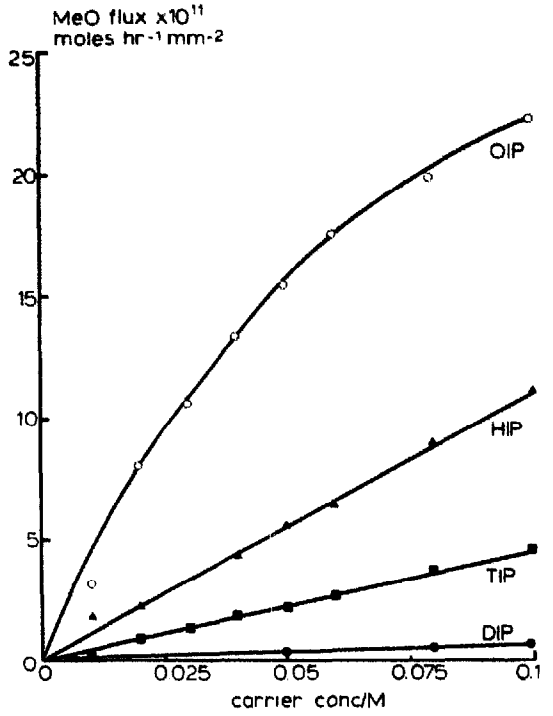


Fig. 4. Relationship between MeO flux and log MeO concentration in the donor compartment. The carrier is 0.1 M HIP in IPM.

Fig. 5. Relationship between MeO flux and carrier concentration for the di-isopropanolamines.

thesized. The relative gradients in Fig. 5 give some indication of the carrier efficiencies and these are listed in Table 2. The flux of the least efficient carrier DIP was studied at amine concentrations up to 1.0 M and the linear relationship still applied.

It would be expected that the efficiency of transport will be related to the stability of the MeO carrier ion pair complex in the organic phase. With increasing carbon chain-length, lipophilicity increases and a more stable ion pair would be expected. Fig. 6 shows the relationship between log relative carrier efficiency and carbon chain-length (or expected lipophilicity). The linear relationship indicates that carrier lipophilicity is of prime importance in determining the efficiency of these carrier systems.

TABLE 2

RELATIVE CARRIER EFFICIENCY OF THE DI-ISOPROPANOLAMINES

Alkyl chain-length	Gradients from Fig. 5	Relative efficiency
18	3.88×10^{-6}	100
16	1.10×10^{-6}	28.4
14	4.57×10^{-7}	11.8
12	6.15×10^{-8}	1.6

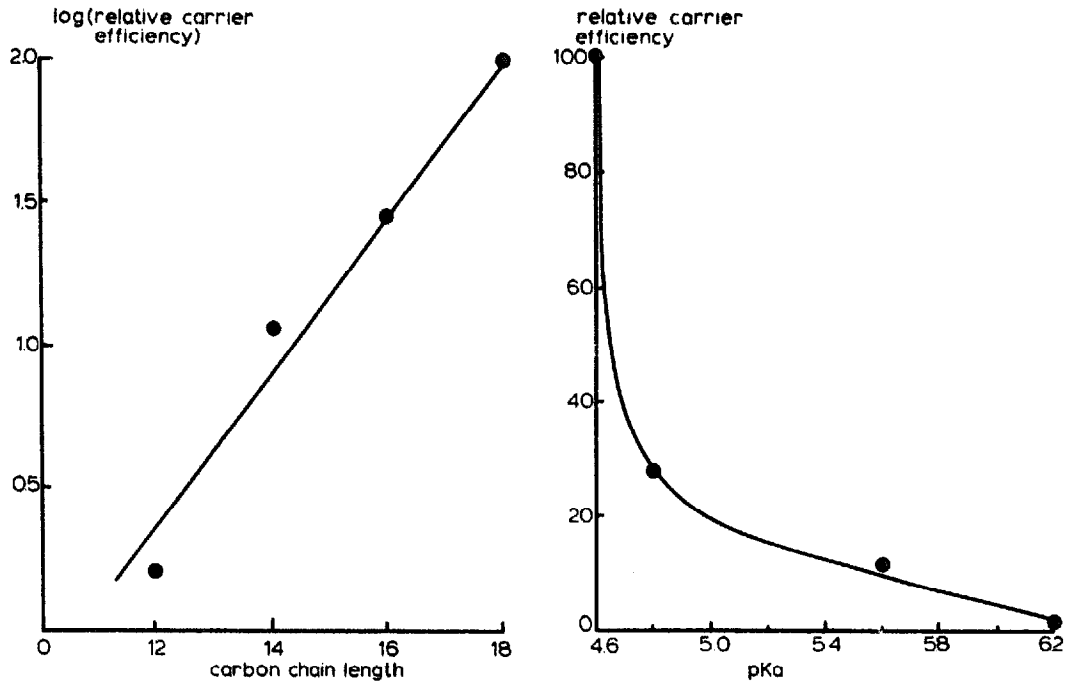


Fig. 6. Relationship between the carrier efficiency and carbon chain-length for the di-isopropanolamines.

Fig. 7. Relationship between the carrier efficiency and pK_a of the different di-isopropanolamines.

The pK_a values of these di-isopropanolamines tend towards a limiting value of ~4.6 as the carbon chain-length is increased (Boivin, 1958). Fig. 7 shows how the efficiency of the carrier varies with pK_a for this series of compounds. It appears from this figure that the carrier efficiency increases very rapidly for a carbon chain-length of 18 and greater. However, it is probable that other factors, such as stagnant diffusion layers and saturation interfacial kinetics will limit the overall transport efficiency for carriers with carbon chain-lengths greater than 20. At the other extreme it is seen that for compounds with alkyl chains smaller than DIP the carrier efficiency is very small.

Equilibrium extraction coefficients were also measured for the different amines. These are shown as a function of pH in Fig. 8. OIP extracts MeO very favourably at pH 5 and unfavourably at pH 8. The large difference in extraction coefficients reflects the potential carrier capabilities of the amine. From the results shown it appears that the maximum extraction coefficient occurs at or around the pK_a of the carrier. This confirms the proposal given by Babcock et al. (1980) that the optimum solution pH is that equivalent to the carrier pK_a. The unusual profile exhibited by TIP may be explained because this amine had a suitable HLB at low pH to stabilize emulsion formation, thus rendering it unavailable for ion pairing. Alternatively, at low pH values the TIP may be selectively removing buffer phosphate ions rather than MeO (Babcock et al., 1980). The technique of measuring extraction coefficients at the pH of the donor and receptor phases is useful for screening substrates for their potential as anion carriers.

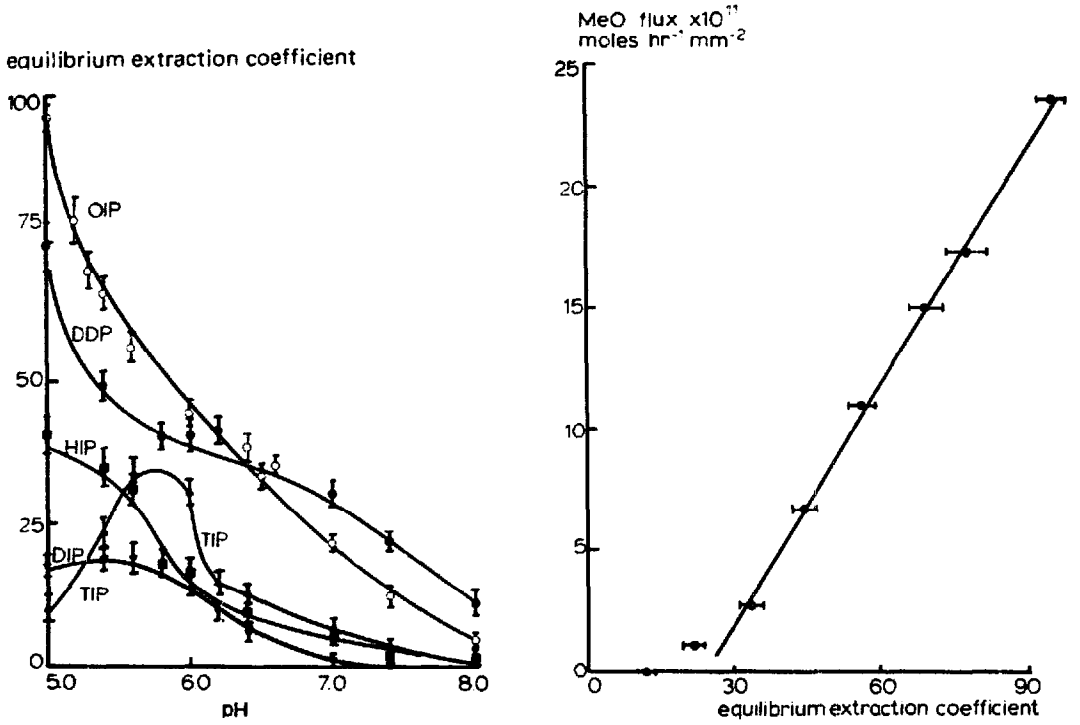


Fig. 8. Relationship between the equilibrium extraction coefficient and pH for OIP, DDP, HIP, TIP and DIP. The standard deviations for the determinations are shown.

Fig. 9. Relationship between MeO flux and equilibrium extraction coefficient for 0.1 M OIP in IPM.

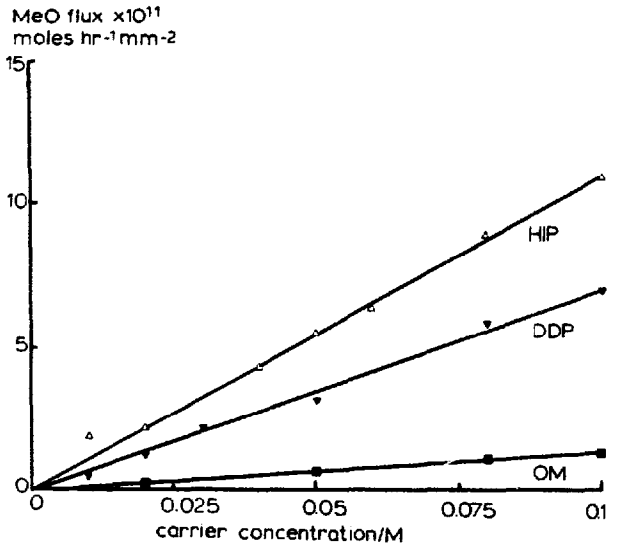


Fig. 10. Relationship between MeO flux and carrier concentration for DDP and OM; HIP is included for comparison.

Fig. 9 shows the relationship between MeO flux and donor pH equilibrium extraction coefficient for DIP. A linear profile is observed between pH 5 and pH 6.5. The variation in flux with donor pH shows that the free energy for transporting MeO is derived from the pH difference (or H^+ ion gradient). It also indicates the necessity for buffering the system even though this may cause some problems when buffer ions are preferentially transported.

In addition to the di-isopropanolamines, other carriers were synthesized and tested. These are DDP, DM, HM, OM and Ethomeen S15. DDP was shown to be quite an efficient carrier whereas the morpholines were less effective (Fig. 10). The reason for DM and HM being virtually ineffective and OM being of only limited efficiency is probably due to steric hindrance of the N-centre. Ethomeen S15 was also shown to transport MeO but was of low efficiency.

Transport of a model anionic drug across a lipid membrane has been achieved by making use of a pH gradient. The pH gradient utilized is that normally expected across human stratum corneum. It may therefore be possible to use this gradient to produce transport of anionic drugs across the stratum corneum at levels which will be of therapeutic benefit.

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