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Ion-pair and complex-coacervate effects on large ion flux through polyamide-6 membrane

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Summary

Using a simple two-compartment diffusion cell it has been demonstrated that the transport of a model large organic ion (sodium cromoglycate), across a polyamide membrane can be altered by the presence of hydrophobic monovalent quaternary ammonium ions added to the bulk donor phase. There is an initial enhancement of ion flux at low quaternary ion concentration, which is directly related to ammonium ion concentration and hydrophobicity. The flux falls markedly at the aqueous solubility product between the two ions (where a complex coacervate forms). Results are consistent with previous findings using in vivo absorption systems.

Introduction

Ionized molecules are generally not well absorbed by biological membranes. A number of physicochemical and chemical approaches to improving ionized molecule delivery have been made including the formation of prodrugs and soft drugs, etc. Additionally, following the suggestion of Schanker (1960) that organic ions might penetrate intestinal tract membranes in the form of less polar complexes formed with materials (sic) normally present in the lumen, a large number of attempts have been made to utilize ion-pair formation for improvement in drug absorption (e.g. Wilson et al., 1981 and references therein). Although the relevance of ion-pair formation to the membrane uptake of ionized molecules has been discussed, unfortunately very little systematic study has been attempted (Ruifrok and Meijer, 1981) to examine how and why ion pairs could be employed in this role. The formation of

ion pairs between ions of opposite electrical charge is both solute- and environment-dependent. Sodium chloride dissociates and does not form ion pairs in water (but does so in liquid ammonia). This is due to the large hydration shell possible with these ions, preventing an electrostatic association. However, large organic ions of opposite electrical charge can form ion pairs in water (Diamond, 1963; Tomlinson et al., 1979a), since such ions generally have some hydrophobic portion, whose coming together, as a result of water structure, can overcome the initial headgroup hydration shell repulsion. If an oil/water two-phase system is considered, then at the interface ion pairs can form between large organic and small inorganic ions, since the dielectric constant of this area is low enough to permit charge attraction.

This suggests that only when large organic ions are used as pairing ions can ionized drugs be better absorbed as the ion pair. Accordingly, we have been studying the effect of pairing-ion structure and concentration on ion-pair formation (Tomlinson et al., 1979a; Tomlinson and Davis, 1980) and liquid–liquid distribution (Tomlinson and Davis, 1980), as well as penetration of in vivo biological membranes (Tomlinson and Davis, 1976; Davis et al., 1978; Wilson et al., 1981). The in vivo findings are that different effects on ion absorption and disposition arise when the pairing ion is at a concentration where only ion pairs are formed, than when it is at a concentration where both ion pairs and complex coacervates form. The possibility that these effects are due to changes in the thermodynamic activity of the ion pair, resulting in alterations in membrane flux has been investigated; this study gives the results of the examination of the effect of pairing-ion hydrophobicity and concentration on the flux of a large model ion through an artificial membrane, (polyamide-6).

A preliminary report on this study has been made (van Dooremalen et al., 1981).

Materials and methods

Materials

Sodium cromoglycate (SCG) and alkylbenzyldimethyl-ammonium chlorides (ABDACs) were as described previously, (Tomlinson and Davis, 1978). Water was double-distilled from an all-glass still. All solvents used for HPLC were of HPLC grade, and were from E. Merck (Darmstadt, F.R.G.). N-Hexadecyl-N,N,N-trimethyl-ammonium bromide and buffer salts were of analytical grade, and were from Merck or Fluka (Switzerland). Polyamide-6 membrane had a thickness of 0.2 mm, and was from Du Pont, NJ, U.S.A. Dimethylpolysiloxane membrane had a thickness of 0.005 in., and was Silastic (non-reinforced) from Dow Corning, Midland, MI, U.S.A.

Methods

(i) Diffusion cell. The rate of transfer of ions across a polymeric membrane, was examined at 60°C (±0.1°C) using a two-compartment diffusion cell, comprised of two water-jacketed parts, (350 cm^3 capacities), with the membrane clamped between the two. The donor and acceptor compartments were filled with 300 cm^3 of an aqueous solution of the ion(s) and 350 cm^3 of water, respectively. The membrane was

presoaked in water for 24 h before use. During ion(s) transfer both donor and acceptor solutions were stirred using 5 cm glass paddles fitted to an overhead electrical stirrer (100 rpm). Samples (5 cm^3) were removed from the acceptor compartment with time, and were analyzed by UV spectroscopy and/or HPLC with UV detection. For all systems studied ABDACs were below their critical micellization concentrations (Mukhayer et al., 1975).

In the present diffusion cell arrangement, where the concentration of both solutes in the donor compartment can be approximated as remaining constant during the time of the experiment, and where the acceptor compartment can be regarded as a sink, using the normal flux equations, then ion flux through the membrane, J, can be given by:

$$J = (dA/dt)/S \quad (1)$$

where A is the amount of ion leaving the donor solution in time, t, through a membrane of area, S, and thickness, X, and where under 'steady-state' conditions

$$dA/dt = -DS\,(dC/dX) \quad (2)$$

where D is the diffusivity.

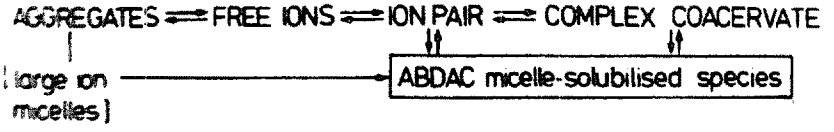
(ii) Analysis. SCG and ABDACs have UV absorbance maxima at 316 nm and 257 nm, respectively. Analysis of ions was by simple spectroscopy or by reversed-phase HPLC using modifications of previously developed (Tomlinson et al., 1979b) ion-pair HPLC methods. For the latter a custom-made system was used with a bonded octyl stationary phase (7 μm Lichrosorb, ex Merck). For SCG the mobile phase was methanol–0.005 mol · dm^{-3} phosphate buffer (pH 6), (40:60 v/v with 0.02 mol · dm^{-3} cetyltrimethylammonium bromide and 0.1 mol · dm^{-3} sodium bromide initially in the water). With detection at 316 nm, SCG had a found capacity factor of 4.3. For ABDACs different mobile phases were used depending upon chain-length; thus for ABDACs with alkyl chain carbon number 8–18, the amount of methanol (by percent volume) in a methanol–water mobile phase ranged from 70 to 92, respectively. Included in the water was 0.025 mol · dm^{-3} tetramethylammonium hydroxide and 0.005 mol · dm^{-3} NaH_2PO_4, and the final mobile phase included 0.1 mol · dm^{-3} $NaClO_4$. Detection was at 206 nm.

(iii) Computing. Three-dimensional model plots were produced using a general plotting program (Dissplа ver 8.0) obtained from ISSCO, San Diego, CA, U.S.A.

Results and discussion

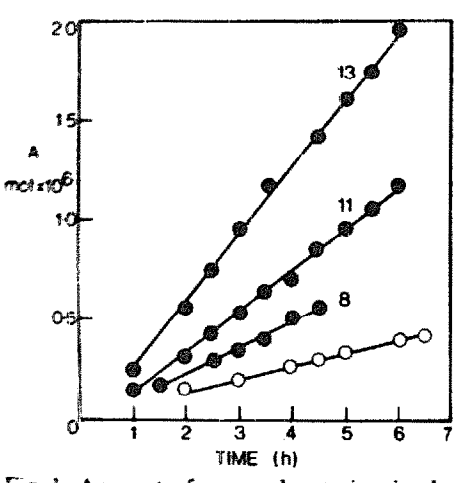
Transport across artificial membranes has been studied for a variety of reasons, including design of ion-selective electrodes, devices for controlled drug release and as models of biological membranes. In the present study polyamide-6 has been chosen since it is formed by peptide bonds between long hydrocarbon chains and hence may have properties similar to cell membranes. Richardson and Meakin

(1973) have studied the sorption of drugs by polyamide (nylon) powder and film, and have concluded that partition takes place, with a definite correlation existing between the slope of the partition isotherm and liquid phase solubility. Importantly, Ågren et al. (1974) have demonstrated that the relatively slow transfer of ions across nylon membrane at 30°C may be enhanced in the presence of counter ions (sic), with an excess of counter ion causing transfer across a concentration gradient. These workers suggested transfer was taking place mainly by partitioning and diffusion and, to a much lesser extent through pores. Additionally, the concept of a carrier incorporated into a membrane that facilitates ion transport has attracted increased attention in recent years (Pefferkorn and Varoqui, 1975), including notably the recent study by Barker and Hadgraft (1981) who have shown that large anion transport across a lipid membrane can be increased by incorporation of pairing ion into the membrane, such that the pairing ion is ionized at only one side of the membrane.



Scheme I.

Scheme I gives the physicochemical equilibria possible between SCG and ABDACs in this study. Previously (Tomlinson and Davis, 1978, 1980), these equilibria were examined quantitatively and were shown to be highly dependent upon both the relative concentrations of both large ions and ABDAC hydrophobicity. Below the



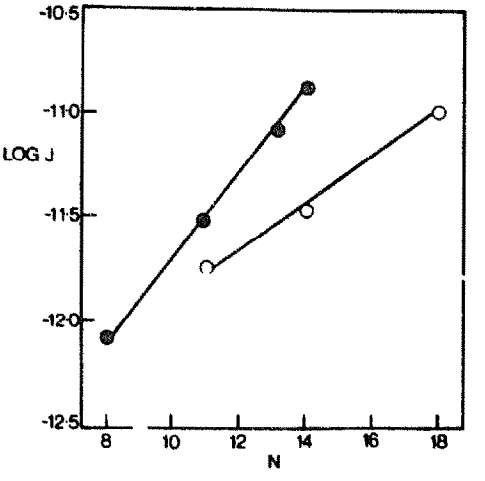


Fig. 1. Amount of cromoglycate ion in the acceptor compartment, (A), with time in the absence (open datum points) and presence of ABDACs of differing alkyl chain-length. Initial donor compartment concentrations, SCG 5×10^{-4} mol·dm^{-3}, ABDAC 1.5×10^{-4} mol·dm^{-3}. (ABDAC homologue number given next to appropriate data.)

Fig. 2. Relationship between ABDAC flux, J, (mol·s^{-1}·cm^{-2}), at 60°C and alkyl chain-length, N, in the absence (open datum points) and presence of ion pair-forming amounts of cromoglycate ion. (Initial acceptor compartment concentrations as for Fig. 1. Drawn lines are regression lines according to Eqns. 3 and 4.)

aqueous solubility product, (K_s), SCG and ABDAC exist as water soluble 1:1 ion pairs (Tomlinson and Davis, 1980a) in equilibrium with their free ions, with the interaction being due to electrostatic attraction and water-structure-enforced solvophobic effects (Tomlinson and Davis, 1980b). However, in the presence of an oil phase, 2:1 ion pairs form which then transfer into the non-polar phase. At and above the aqueous solubility product a 2:1 complex coacervate is formed which results in effect in a two-phase system of coacervate-rich and coacervate-poor phases.

Since the free-energies of ion-pair formation and transfer (between immiscible solvents) are additive and constitutive molecular properties (Tomlinson and Davis, 1980a), then, if ion-pair formation can alter cromoglycate ion flux, these properties should be reflected in the findings. Indeed this was found to be the case, such that in the absence of pairing ion, the flux of SCG at 60°C through polyamide-6 membrane is 5.36×10^{-13} mol · s^{-1} · cm^{-2} (initial concentration 5×10^{-4} mol · dm^{-3}), whereas in the presence of ABDAC (1.5×10^{-4} mol · dm^{-3}) there is a marked increase in cromoglycate flux (Fig. 1) which is dependent upon the length of the ABDAC alkyl chain. Due to their large hydrophobic character ABDACs penetrate polyamide-6 at this concentration in the absence of cromoglycate ion (Fig. 2), with the found relationship between flux (J) and quaternary ammonium alkyl chain length N being

$$\log J_A = 0.108N - 12.9 \quad n = 3, r = 0.995 \tag{3}$$

where n and r are the number of data points and the correlation coefficient, respectively, and where subscript A refers to ABDAC flux. In the presence of cromoglycate ion this relationship alters (Fig. 2) to give:

$$\log J_A = 0.216N - 13.9 \quad n = 4, r = 0.999 \tag{4}$$

The doubling in the slope coefficient indicates a 2:1 stoichiometry of interaction

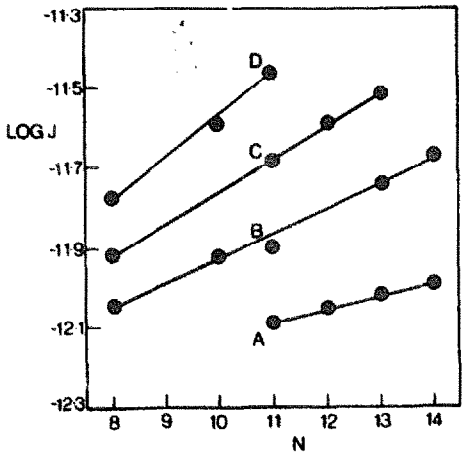


Fig. 3. Relation between ABDAC alkyl chain-length, N, and SGC flux, J, (mol·s^{-1}·cm^{-2}), at 60°C, for differing initial donor compartment ion pair-forming concentrations of quaternary ammonium ion. Initial SCG concentrations 5×10^{-4} mol·dm^{-3}. (Key: lines A–D are for ABDAC compartment concentrations of 2×10^{-5}, 5×10^{-5}, 1.5×10^{-4} and 5.0×10^{-4} mol·dm^{-3}, respectively).

TABLE 1

FLUX OF CROMOGLYCATE ION THROUGH POLYAMIDE-6 MEMBRANE AT 60°C IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF A SERIES OF ALKYLBENZYLDIMETHYLAMMONIUM CHLORIDES

ABDAC concentration ($mol \cdot dm^{-3}$)	Flux of cromoglycate [a] ($mol \cdot s^{-1} \cdot cm^{-2}$)					
	ABDAC alkyl chain carbon number:					
	8	10	11	12	13	14
1.0×10^{-5}	5.8×10^{-13}	6.4×10^{-12}	6.5×10^{-13}	6.5×10^{-13}	6.9×10^{-13}	6.9×10^{-13}
2.0×10^{-5}			8.1×10^{-13}	8.6×10^{-13}	9.3×10^{-13}	1.05×10^{-12}
5.0×10^{-5}	9.0×10^{-13}	1.15×10^{-12}	1.25×10^{-12}		1.81×10^{-12}	2.05×10^{-12}
1.0×10^{-4}		1.50×10^{-12}		2.11×10^{-12}	2.64×10^{-12}	3.30×10^{-12}
1.5×10^{-4}	1.20×10^{-12}		2.08×10^{-12}	2.56×10^{-12}	3.00×10^{-12}	3.30×10^{-12}
5.0×10^{-4}	1.65×10^{-12}	2.58×10^{-12}	3.41×10^{-12}	3.49×10^{-12}	2.78×10^{-12}	1.55×10^{-12}
1.0×10^{-3}			3.55×10^{-12}	3.32×10^{-12}		9.2×10^{-13}
2.0×10^{-3}	2.47×10^{-12}	3.81×10^{-12}	3.74×10^{-12}	1.99×10^{-12}	1.06×10^{-12}	5.3×10^{-13}
5.0×10^{-3}		4.03×10^{-12}	2.41×10^{-12}	9.8×10^{-13}	5.2×10^{-13}	
1.0×10^{-2}	3.16×10^{-12}	3.56×10^{-12}	1.48×10^{-12}			
SCG:ABDAC solubility product [b] at 60°C ($(mol \cdot dm^{-3})^3$)	1.1×10^{-5}	7.9×10^{-8}	5.5×10^{-9}	5.0×10^{-10}	4.9×10^{-11}	3.4×10^{-12}

[a] Initial sodium cromoglycate concentration 5×10^{-4} $mol \cdot dm^{-3}$.

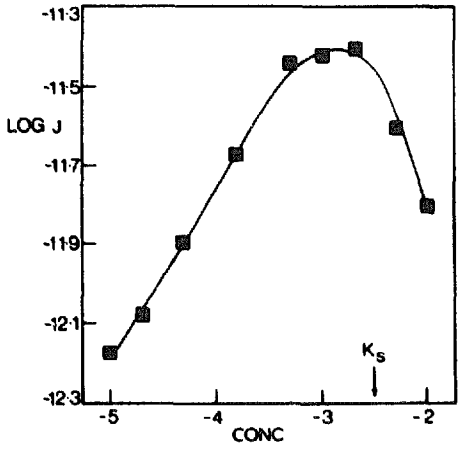
[b] Tomlinson and Davis (1980b).

between the large organic ions, but since ion-pairing reduces the amount of free ion, one cannot calculate from these experiments whether this indicates that formed ion pairs as such traverse the membrane, or whether ion association between the large ions simply increases the concentration of each ion at the hydrocarbonaceous membrane surface. Notwithstanding the mechanism of this behaviour, in the concentration region (Table 1) where only ion pairs and free ions exist, an increase in ABDAC chain-length results in an increase in SCG flux (Fig. 3). It can be seen that the relationship between improved cromoglycate flux and ABDAC chain-length is dependent upon the initial pairing-ion concentration. At an initial ABDAC concentration corresponding to Eqn. 4 the relationship is given by:

$$\log J_S = 0.080N - 12.6 \quad n = 4, r = 0.999 \tag{5}$$

where subscript S refers to SCG flux. Thus at ABDAC concentrations below their micellization concentrations (Mukhayer et al., 1975), an increase in pairing-ion hydrophobicity (which results in the formation of a more hydrophobic ion pair) increases the flux of cromoglycate.

It would be attractive to consider the ABDAC ions as ideal for effecting the enhanced transfer of SCG (and similar ions) across membranes (whether biological or analytical); however, as discussed previously, at higher concentrations these ions and SCG form a hindered precipitate (complex coacervate) whose properties are very different to those of the ion pair. Levine et al. (1955) have shown that the absorption of organic compounds through biological membranes is reduced by the formation of stable salt complexes. Thus it is to be expected that the formation of



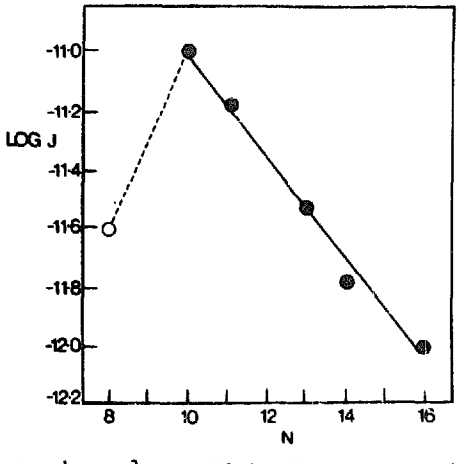


Fig. 4. Flux of cromoglycate ion through polyamide-6 ($mol \cdot s^{-1} \cdot cm^{-2}$) at 60° in the presence of concentrations ($mol \cdot dm^{-3}$) of C_{11}BDAC below and above the 2:1 aqueous solubility product, (K_s), between both large organic ions. (Initial SCG concentration 5×10^{-4} $mol \cdot dm^{-3}$.)

Fig. 5. Flux of cromoglycate ion through polyamide-6 ($mol \cdot s^{-1} \cdot cm^{-2}$), at 60°C in the presence of 5×10^{-3} $mol \cdot dm^{-3}$ ABDACs, such that only the C_8BDAC:SCG system is below the 2:1 solubility product. The unbroken line is the regression line according to the correlation given by Eqn. 6. (Initial SCG donor compartment concentration 5×10^{-4} $mol \cdot dm^{-3}$.)

complex coacervates between large hydrophobic ions would a priori reduce in vitro membrane flux. This is indeed found. Fig. 4 gives the effect of C_{11}BDAC on SCG flux at concentrations of pairing ion below and above those causing coacervation. It is clearly demonstrated that the increase in ABDAC concentration in the donor compartment is limited when the combined concentrations of ABDAC and SCG reach their 2:1 aqueous solubility product (Table 1). Since further increases in amounts of C_{11}BDAC complex more cromoglycate ion, there is a demonstrated fall in SCG flux.

As complex coacervation between these ions is increased as the ABDAC becomes larger, (Tomlinson and Davis, 1978), in the complex coacervate concentration region an increase in ABDAC size should reduce SCG flux. Fig. 5 shows this to be the case, such that

$$\log J_S = -0.172N - 9.30 \quad n = 5, r = 0.993 \tag{6}$$

It should be realized that the initial SCG and ABDAC concentrations (both 5×10^{-3} mol · dm^{-3}) mean that only the sign of the slope coefficient in Eqn. 4 can be compared with that of Eqn. 3, and not the regression coefficients themselves.

Table 1 gives the combined results of SCG flux through a polyamide-6 membrane in the presence of ABDACs of varying hydrophobicities and at concentrations above and below the aqueous solubility products. These data show a complex pattern of behaviour (which has been modelled using a three-dimensional plotting procedure).

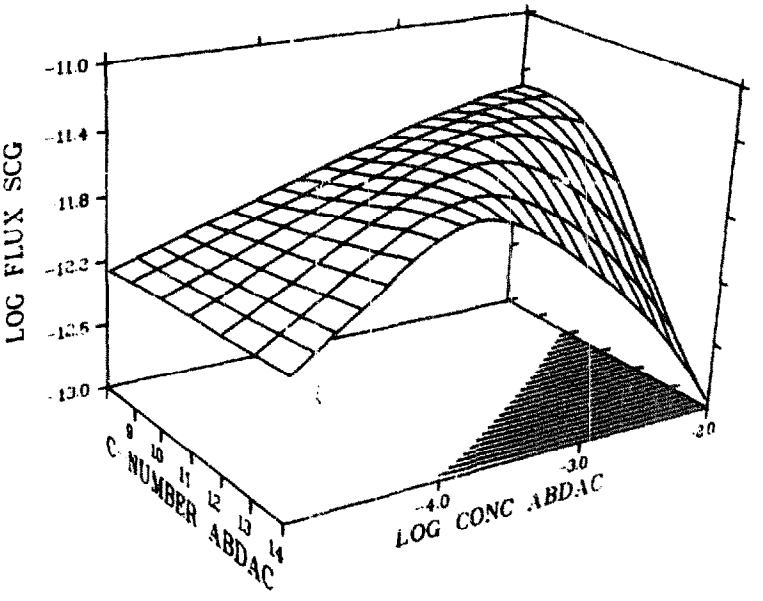


Fig. 6. Three-dimensional model plot of the interrelationship between cromoglycate ion flux (mol·s^{-1}·cm^{-2}), at 60°C and ABDAC alkyl chain-length, and ABDAC donor compartment concentration (mol·dm^{-3}). (Initial donor compartment concentration of SCG 5×10^{-4} mol·dm^{-3}). The shaded area in the x–z plane indicates the area of complex coacervation between SCG and the ABDACs with this initial SCG donor compartment concentration.

and the total shape of this complex behaviour is given in Fig. 6. A number of features can be identified. First, at low ABDAC chain-length an increase in pairing-ion concentration results in an increase in SCG flux. Second, a raising of ABDAC hydrophobicity increases the rate of SCG flux increase which, third, limits at an ever decreasing ABDAC concentration level. Fourth, above the limiting flux value for any ABDAC an increase in its concentration results in a sharp decrease in SCG flux, with, at very high ABDAC concentrations SCG flux falling below that found in the absence of ABDAC, which is due to the fact that nearly all the SCG has been complexed. A further feature of the shape of SCG flux behaviour in the presence of ABDACs is that with this initial SCG concentration level of 5×10^{-4} mol · dm^{-3} the maximal flux obtained is approximately the same (4×10^{-12} mol · sec^{-1} · cm^{-2}) irrespective of the ABDAC used, and finally the ridge of maximal flux corresponds to the position of the 2:1 aqueous solubility products between SCG and the ABDACs.

Conclusions

This study clearly shows that the penetration of a hydrocarbonaceous membrane by a large organic ion can be greatly altered by the presence of a large organic ion of opposite electrical charge. It is demonstrated that, for any one pairing ion, an increase in cromoglycate ion flux through polyamide-6 membrane is increased in a region of ion-pair formation, that this increase is limited by the formation of complex coacervate, and that further increases in quaternary ammonium ion concentration produce a marked reduction in cromoglycate ion flux. It is postulated that the increase in flux is due to the properties of formed ion pairs, and that decrease in flux above the solubility product is due to a decrease in the thermodynamic activities of both large ions. It is not possible to conclude from these experiments whether the formed ion pairs cross the membranes in the pore network or via a membrane solution process or both. An experiment performed using dimethylpolysiloxane membrane showed that neither free cromoglycate nor SCG in the presence of ion-pair forming amounts of ABDAC transported across this pore-free membrane.

Ahmed et al. (1980) have concluded that ion pairing between phenothiazines and bile salts does not increase the interfacial transport rate of phenothiazines across an oil-impregnated membrane. Conversely it has been shown recently (Kinkel et al., 1981) that the interfacial transport of chloramphenicol succinate across such membranes is increased in the presence of phosphonium ion. These current contradictions indicate that further work on ion-pair systems could be involved in understanding more about the nature of ion-pair formation, particularly with respect to pairing-ion structure and surface activity.

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