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The permeability of hydrophobic membranes to ^{22}Na salts and $^{14}CO_2$ in low dielectric media

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The one-way fluxes of $^{14}CO_2$ and a series of ^{22}Na (Cl, Br, HCO_3, ClO_4, I) salts across *n*-hexadecane-impregnated solid-support liquid membranes have been measured in water and low dielectric media (50–90 vol% dioxane/water). One-way fluxes for $^{14}CO_2$ ($J_{^{14}CO_2}$) were 0.84 and 1.03×10^{-9} mol cm^{-2} s^{-1} in 75% dioxane (aq.) and water, respectively, across both impregnated cellulose and teflon membranes. ^{22}Na fluxes across impregnated cellulose membranes in 75% dioxane (aq.) ranged from 1.8 to 11.4×10^{-10} mol cm^{-2} s^{-1} and had the order NaCl < NaBr < $NaHCO_3$ < $NaClO_4$ < NaI. ^{22}Na fluxes across impregnated teflon membranes were slightly smaller, $1.5–7.1 \times 10^{-10}$ mol cm^{-2} s^{-1}, but had the same order for the anions tested. No measurable ^{22}Na fluxes were observed in aqueous media. For NaI and $NaClO_4$ there was a 3–6-fold enhancement of fluxes in 90% dioxane (aq.) compared to 75% dioxane (aq.). The corresponding enhancement for fluxes of $NaHCO_3$, NaBr and NaCl was 1.5-fold. The results are discussed in terms of ion-paired salt transport in low dielectric media.

1. Introduction

There has been some speculation in recent years about the possible role that inorganic ion pairs play in normal physiological processes such as the transport of ions across cell membranes [1]. There is indirect evidence of ion pair transport from physiological studies such as the augmentation of passive Li^+ and Na^+ fluxes across the human red cell membrane by divalent carbonate and sulfite anions [2–4]. Other work concerning the effects of bicarbonate acid-extrusion rates in barnacle muscle fibers [5], squid axon [6] and snail neuron [7] is consistent with exchange of extracellular $NaCO_3^-$ with intracellular Cl^- in a process known as an anionic cation transport.

Physiologically relevant cations (Na^+, K^+, Ca^{2+}) and anions (HCO_3^-, Cl^-, PO_4^{3-}) are normally assumed to be in a fully dissociated or solvated form in water solution. According to Bjerrum's theory, the association constant (K_A) for the formation of neutral ion pairs (ref. 8, pp. 251–261),

$$M^+ + A^- = IP^0 \qquad (1)$$

varies as the inverse cube of the dielectric constant (ϵ) of the medium and is given by the following relationship:

$$K_A = \frac{4\pi N_A}{1000} \left(\frac{Z_+ Z_- e^2}{\epsilon k T} \right)^3 \int_2^b e y^{-4} \, \mathrm{d}y \qquad (2)$$

where $k = 1.381 \times 10^{-16}$ erg/K, $N_A = 6.02 \times 10^{23}$ mol^{-1} and $Z_+ e_0$, $Z_- e_0$ are the electrostatic charges of the respective ions. In addition, if r represents the distance separating the ions, then $y = 2q/r$ where q is the upper limit of separation

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past which there is no pairing and $b = 2q/a$ where 'a' (the ion size parameter) is the distance of closest approach of the two ions. Two limiting types of neutral ion pairs are generally recognized. Retention of ionic hydration shells by paired ions due to strong solvent-ion interactions gives rise to solvent-separated ion pairs:

$$(\mathrm{M}\cdot\mathrm{S}_N)^+ + (\mathrm{A}\cdot\mathrm{S}_M)^- = \left[(\mathrm{M}\cdot\mathrm{S}_N)^+(\mathrm{A}\cdot\mathrm{S}_M)^-\right]^0 \tag{3}$$

where $(\mathrm{M}\cdot\mathrm{S}_N)^+$ denotes the cation and its associated waters of hydration and $(\mathrm{A}\cdot\mathrm{S}_M)^-$ has the same meaning for the anion. In weakly electrophilic and/or nucleophilic solvents a more intimate pairing is possible, giving rise to the contact ion pair:

$$(\mathrm{M}\cdot\mathrm{S}_N)^+ + (\mathrm{A}\cdot\mathrm{S}_M)^- = \left[(\mathrm{M}^+\mathrm{A}^-)\cdot\mathrm{S}_{N+M-X}\right]^0 + \mathrm{S}_X \tag{4}$$

The association constant given by eq. 2, therefore, clearly relates to both types of ion pair which are in equilibrium with each other in solution. Therefore, in regions of low solution dielectric, such as at the interface of natural membranes ($\epsilon \simeq 12$) or near the mouths of certain ion channels, electrostatic attraction between inorganic cations and anions is possible with the resultant formation of transitory ion pairs [9]. The lipophilic nature of neutral ion pairs clearly offers an alternative means of mass transport across membranes for inorganic ions normally found in physiological fluids.

To date, a number of studies of ion-paired drug transport through synthetic membranes in both water and low dielectric media have demonstrated fluxes in the range 10^{-11}–10^{-12} mol cm^{-2} s^{-1}. Lee et al. [10] reported the permeation of silicone rubber membranes by sodium salicylate (M_r 160) and warfarin (M_r 330) in dioxane/DMSO ($\epsilon \sim 2$) and isopropanol ($\epsilon = 18.3$), respectively. In addition, Tomlinson et al. [11] have observed permeation of polyamide-6 membranes by a series of alkylbenzyldimethylammonium cromoglycates ($M_r > 600$) in aqueous media. For sufficiently large organic ions, ion pairing is possible even in high dielectric media due to the weakly held hydration shell surrounding the ions [12]. Similarly, Davis et al. [13] have demonstrated the enhancement of chloramphenicol succinate (M_r 464) transport across impregnated membranes in the presence of phosphonium ions. Duffey [14] measured both ionic and ion pair transport of tetrabutyl- and tetraethylammonium bromides across n-heptyl cyanide impregnated cellulose, Celgard and Mitex membranes in aqueous and low dielectric media and obtained ion pair fluxes in the range 10^{-10} mol cm^{-2} s^{-1}. Certain related studies in which one of the pairing ions is incorporated into the membrane matrix itself have also achieved impressive rates of ion flux even in high dielectric media [15].

The present study was undertaken to determine the extent to which the physiologically relevant ions (Na^+, Cl^-, HCO_3^-) were capable of ion pairing and membrane permeation in both high and low dielectric media. In addition, CO_2 as well as three large inorganic anions not normally found in physiological milieu (Br^-, I^-, ClO_4^-) were also studied for purposes of comparison.

2. Experimental

2.1. Materials

Reagent grade NaI, NaBr and NaHCO_3 were purchased from Sigma and were used without further purification; n-hexadecane (99%) was purchased from Sigma and purified by distillation in an all-glass apparatus. Reagent grade 1,4-dioxane and NaClO_4 were purchased from Fisher Scientific and were used without further purification. Cellulose lens paper (Fisher) and teflon PTFE membranes (Millipore, Bedford, MA) were used as membrane solid-support material.

$\mathrm{NaH^{14}CO_3}$ (600 μCi/ml) and ^{22}NaCl (250 μCi/ml) were purchased from New England Nuclear.

2.2. Methods

Radiotracer fluxes of $^{14}\mathrm{CO}_2$ and a series of ^{22}Na salts across hexadecane-impregnated solid-support liquid membranes were measured in water ($\epsilon = 78$) and in 50 ($\epsilon = 35.9$), 75 ($\epsilon \sim 15$) and 90 ($\epsilon \sim 6$) vol% dioxane-water mixtures, correspond-

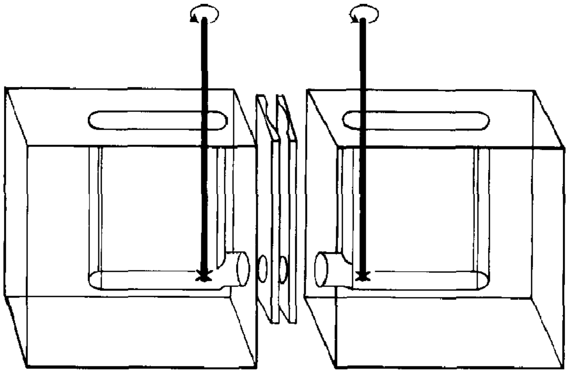

Fig. 1. Lucite flux chamber used for study of ^{22}Na salt and $^{14}CO_2$ fluxes across impregnated membranes.

ing to 17.4, 38.8 and 65.5 mol% dioxane. Whereas hexadecane and dioxane are miscible in all proportions, hexadecane has a very low solubility in dioxane-water mixtures. The solubility of hexadecane in 75% dioxane (aq.) was estimated by shaking 5-μl aliquots with 100 ml of the cosolvent mixture and after settling inspecting for the presence of two phases. By this means it was determined that the solubility of hexadecane in 75% dioxane (aq.) is not greater than 20 mM. All flux measurements were made in a lucite flux chamber (fig. 1) consisting of two half chambers of 10 ml volume each. Each chamber was stirred from above by means of paddle stirrers attached to small d.c. electric motors. Before impregnation with hexadecane strips of membrane support material (12 × 40 mm) were first sandwiched between the halves of a special gasket fabricated from two 16 × 50 mm sheets of 3 mm thick black rubber. Cellulose supports consisted of two sheets of lens paper (combined thickness 0.0127 cm), whereas teflon (Mitex) supports consisted of a single sheet (thickness 0.0150 cm). Approximately equal thickness was assumed for the two systems, however, due to the sandwiched layer of hexadecane in the cellulose system. The support material was centered over two matched 5.6 mm diameter holes bored 5 mm from the bottom of each sheet. Two 10 mm wide, 1.5 mm deep troughs were carved from the inner surface of each sheet extending from the top of the gasket to within 4 mm of each hole. With the membrane support in place, the two halves of the gasket were bonded together at the edges with cyanoacrylate resin, thus creating an inner reservoir. The level of hexadecane in this reservoir was kept above the fluid level in each chamber during the course of an experiment. The gasket assembly was then mounted between the two halves of the flux chamber.

The measurement of $^{14}CO_2$ fluxes was performed by the simultaneous addition of 3.5 ml water or cosolvent mixture to each side of the flux chamber after the appropriate membrane support had been mounted and impregnated with hexadecane. A 5 mm thick layer of hexadecane was floated on the surface of the liquid in each chamber to help minimize the loss of CO_2 during the experiment. A run was initiated by the addition of 100 μl of 1 M $NaHCO_3$, 25 μl of stock $NaH^{14}CO_3$ and 100 μl of 1 M HCl (in dioxane) to the radioactive (hot) chamber followed by the addition of 225 μl of solvent to the nonradioactive (cold) chamber. 50-μl aliquots were removed from both chambers at zero time and periodically thereafter for 90 min. These samples were shaken with 0.5 ml of 0.1 N NaOH prior to counting in 15 ml Aquasol scintillation cocktail in a Beckmann LS 7000 counter.

Flux determinations for various ^{22}Na salts in water and dioxane-water mixtures were performed in an analogous manner using 3.5 ml solvent in both the hot and cold chambers. At zero time, 120 μl of solvent was added to the cold chamber followed by addition of 100 μl of 1 M cold sodium salt and 20 μl of stock $^{22}NaCl$ to the hot chamber. 50-μl aliquots were removed from both chambers periodically and cold chamber samples were first added to 0.5 ml water before counting in 15 ml scintillation cocktail. The one-way radiotracer fluxes were calculated from the relationship:

$$J = \frac{\text{cpm}^F}{tA\text{SA}^R} \tag{5}$$

where cpm^F denotes the total amount of tracer entering the cold compartment during the time interval t (s), A the surface area of the membrane (0.246 cm^2) and SA^R the specific activity of the tracer in the hot compartment (cpm/mol). Each flux determination is the average of at least three experiments. The porosity of Mitex membranes was given by the manufacturer as 80% which was close to literature values for the typical porosity of cellulose membranes. The effective area for both types of membrane, therefore, was taken as 0.246 $cm^2 \times 0.8 = 0.197\ cm^2$.

The permeability of *n*-hexadecane-impregnated membranes to CO_2 or ^{22}Na salts in both water and low dielectric media was determined from the relationship:

$$P_M = \frac{K_P D^M}{\Delta X^M} \tag{6}$$

where K_P represents the partition coefficient for CO_2 or Na salts between *n*-hexadecane and solvent, D^M the respective diffusion coefficient in *n*-hexadecane, and ΔX^M the membrane thickness.

Partition coefficients were determined by shaking 1.9 ml water or 75% dioxane (aq.), containing 5 μl of 1 M $NaHCO_3$, 5 μl of 1 M HCl and 10 μl stock $NaH^{14}CO_3$ or 50 μl of 0.01 M ^{22}Na salt (in dioxane) and 10 μl label with an equal volume of *n*-hexadecane for 10 min in stoppered 4-ml test tubes. The sealed solutions were allowed to stand overnight before counting. K_P values were determined directly from the ratio of counts/ml in the upper (hexadecane) phase to counts/ml in the lower (H_2O, dioxane) phase. Hexadecane to be partitioned against 75% dioxane/H_2O was modified by the addition of approx. 15–18 vol% dioxane to avoid depletion of the lower phase.

3. Results and discussion

A preliminary study was made of the permeation rates of $^{14}CO_2$ across hexadecane-impregnated cellulose and teflon membranes in both high and low dielectric media. This was done to provide a means of comparing the rates of sodium salt ion pair transport in the system with the rates of a better studied neutral, lipophilic diffusant. The permeation rates of $^{14}CO_2$ across *n*-hexadecane-impregnated cellulose membranes are shown in fig. 2 in both 75% dioxane (aq.) and water. Permeation rates were linear in both cases over the time course of the study and gave mean fluxes of 0.84 ± 0.04 and $1.03 \pm 0.07 \times 10^{-9}$ mol cm^{-2} s^{-1}, respectively. The slopes of the regression lines were significantly different at $P < 0.05$, corresponding to 0.0103 μmol min^{-1} in 75% dioxane and 0.0124 μmol min^{-1} in water. There was a gradual and continual loss of $^{14}CO_2$ from the stirred solutions in both water and 75% dioxane, despite the maintenance of a floating layer of hexadecane over the solutions in both chambers and the use of concentrations below the solubility limit (of CO_2 in water) of approx. 36 mM. Both curves shown in fig. 2, however, correspond to a 30% loss of $^{14}CO_2$ from the front chamber during the 90 min of observation, thus the data have been corrected for CO_2 loss. The true J_{CO_2} values in both media are, therefore, somewhat higher.

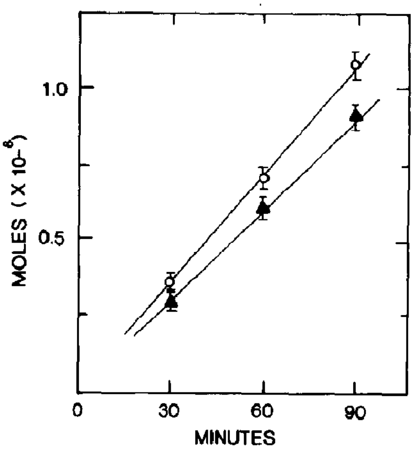

Fig. 2. Permeation rates for $^{14}CO_2$ across *n*-hexadecane-impregnated cellulose membranes in 75% dioxane (▲) and water (○). Initial CO_2 concentration 28 mM.

Essentially identical results were obtained with impregnated single sheets of Mitex teflon of the same thickness as the cellulose membranes. Assuming a $D_M^{CO_2}$ for CO_2 in hexadecane of 5×10^{-8} cm^2 s^{-1}, equivalent to the observed diffusion coefficient of methanol in the human red cell membrane [16], the corresponding permeabilities (P_M) for CO_2 in 75% dioxane and water, respectively, of impregnated cellulose and Mitex membranes using the K_P values given in table 1 and from eq. 6 are 2.1 and 3.3×10^{-6} cm s^{-1}.

The permeation rates for a series of ^{22}Na salts in media of variable dielectric constant are shown

Table 1

Partition coefficients and membrane permeabilities of $^{14}CO_2$ and ^{22}Na salts between *n*-hexadecane and 75% dioxane/H_2O

Molecule	Initial concentration (mM)	cpm/ml		K_P	P_M [a] ($\times 10^6$) (cm s^{-1})
		Hexadecane phase	Lower phase		
$^{14}CO_2$	2.63	1.992×10^5	1.980×10^5 (H_2O)	1.006	3.33
$^{14}CO_2$	2.63	1.500×10^5	2.486×10^5 (dioxane/H_2O)	0.640	2.13
^{22}NaI	0.26	74	4.560×10^3 (dioxane/H_2O)	0.016	0.05
$^{22}NaClO_4$	0.26	96	3.712×10^3 (dioxane/H_2O)	0.026	0.09
$^{22}NaBr$	0.26	72	7.280×10^3 (dioxane/H_2O)	0.010	0.03
$^{22}NaHCO_3$	0.26	~ 25	3.559×10^3 (dioxane/H_2O)	0.007	0.02
$^{22}NaCl$	0.26	< 10	4.771×10^3 (dioxane/H_2O)	< 0.002	< 0.006

[a] Calculated from eq. 6.

in figs. 3 and 4 for hexadecane-impregnated cellulose membranes. Whereas no measurable permeation was detected in aqueous media for any of the salts tested, significant rates were observed in 50% dioxane/water mixtures. The rates, furthermore, continued to increase as the dielectric constant of the medium was lowered from 38.8 (50% dioxane) to 6 (90% dioxane). The time course for observation in 90% dioxane (aq.) was limited due to solubilization of the chamber material and consequent fouling of the membrane.

$J_{^{22}Na}$ values calculated from eq. 5 are listed in table 2. The ordering of flux values for ^{22}Na salts in low dielectric media conforms to a lyotropic series in which the highest rates are associated with salts possessing the largest cationic radii. The extent of ion association predicted from eqs. 1 and 2 correlates positively with both low dielectric constant and with small ionic radii, which for the latter leads to a small value for a and to a large value for the upper limit b of the integral. Such an analysis, however, ignores noncoulombic contributions to ion pair membrane transport such as solvation and the polarizability of ions. In a previous study of ion paired transport through hydrophobic membranes in low dielectric media, ion pair fluxes similar to those shown in table 2 have been reported. Duffey [14] obtained fluxes of 4 and 18×10^{-10} mol cm^{-2} s^{-1} for the permeation of *n*-heptyl cyanide-impregnated cellulose and Celgard membranes by tetrabutylammonium chloride in methanol. Lee et al. [10] reported fluxes 10^{-2}–10^{-3}-fold lower (0.074 and 0.285×10^{-11} mol cm^{-2} s^{-1}) for the permeation of silicone rubber membranes by sodium salicylate and sodium warfarin in dioxane/DMSO and isopropanol, respectively.

Ion pair association constants are shown in table 3 for the various ^{22}Na salts in both water and low dielectric media. The constants were calculated using eq. 2 assuming a constant value for the ion size parameter (a) in the various media [18]. With the exception of $NaHCO_3$ the ion size parameters correspond to values given by Della Monica et al. [19] for solvated cation and anion radii in methanol. In the case of NaCl excellent agreement was obtained between calculated K_A values and those observed in dioxane-water mixtures [20].

The association constants are related to the concentrations of ion pairs and free ions in solution by the relationship:

$$K_A = [IP]/[M^+][A^-]. \qquad (7)$$

The lowest K_A value given in table 3 is 2.6 M^{-1} for $NaHCO_3$ in 50% dioxane/water. Allowing x to represent the concentration of Na^+ or HCO_3^- in equilibrium with $NaHCO_3^0$ under these conditions, it follows that $[IP - x]/x^2 = 2.6$ M^{-1}. By substituting 28.6 mM for IP and solving the quadratic equation for x, one may determine that of the 28.6 mM $NaHCO_3$ present initially, 25.5 mM or 89% of the salt is in the ion paired form in 50% dioxane. For the other salts tested in 50% dioxane and for all salts in 75 and 90% dioxane (aq.) the extent of ion association would be even

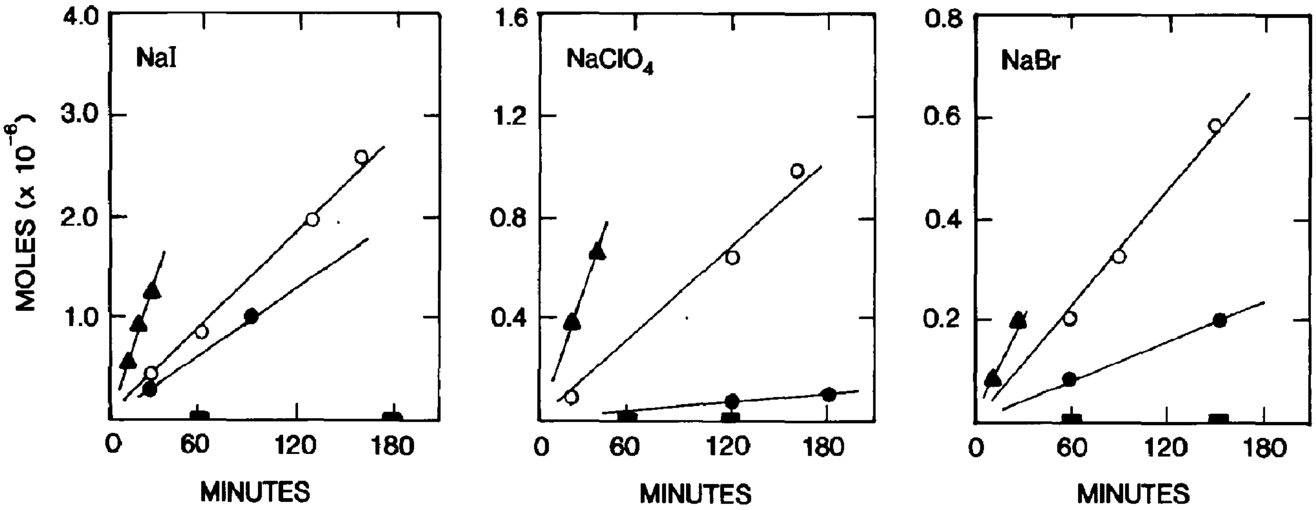

Fig. 3. Permeation rates for ^{22}Na salts in water and low dielectric media. (■) H_2O, (●) 50% dioxane, (○) 75% dioxane, (▲) 90% dioxane. Initial salt concentration 28 mM.

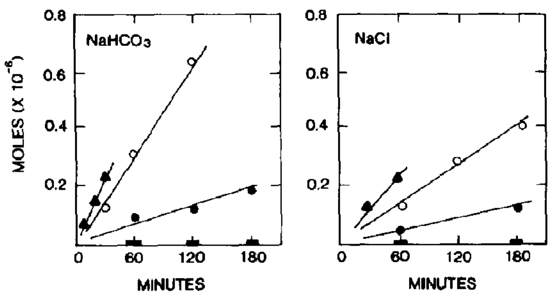

Fig. 4. Permeation rates for ^{22}Na salts in water and low dielectric media. (■) H_2O, (●) 50% dioxane, (○) 75% dioxane, (▲) 90% dioxane. Initial salt concentrations 28 mM.

greater. Since the K_A values given in table 3 refer to both solvent-separated and contact ion pairs (eqs. 3 and 4), it seems likely that the enhanced fluxes of ^{22}Na salts observed upon going from 50 to 90% dioxane relate to a shift in the equilibrium between the two types of ion pairs favoring the production of desolvated contact ion pairs. Transport of simple inorganic salt ion pairs through the extremely low dielectric environment of the membrane interface, therefore, is probably dependent upon complete loss of the hydration shell surrounding the paired ions giving rise to lipophilic and unionized species.

The maximal observed rate of $NaHCO_3$ flux in 90% dioxane (fig. 4) approaches the rate observed for CO_2 in an identical membrane system (fig. 2). Furthermore, since the dielectric constant of the medium ($\epsilon = 6$) is similar to that found at the interface of natural membranes, it seems possible that a 'silent' ion pair flux of Na^+ and HCO_3^- may contribute to the known fluxes of these ions in physiological systems. The transient formation and subsequent transport of $NaHCO_3^0$ across secretory membranes would involve a shedding of the anionic and cationic waters of hydration in the low dielectric environment of the membrane interface. Once beyond the interface of the opposing side of the membrane the ions would rehydrate giving rise to an imbalance in the chemical potential of the system leading to the overall transport of water in same direction as ion movement. The presence of the enzyme carbonic anhydrase at the secretory site in the presence of suitable buffers would provide a continuous and plentiful supply

Table 2

One-way radiotracer fluxes across *n*-hexadecane-impregnated cellulose solid-support liquid membranes in low dielectric media

Solvent	$J_{^{22}Na}$ ($\times 10^{-10}$) (mol cm^{-2} s^{-1})				
	NaCl	NaBr	$NaHCO_3$	$NaClO_4$	NaI
H_2O (78) [a]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
50% dioxane/water (35.9) [a]	0.6 ± 0.1	1.8 ± 0.3	2.6 ± 0.5	3.1 ± 0.5	4.2 ± 0.7
75% dioxane/water (15) [a]	1.8 ± 0.2	3.8 ± 0.8	4.3 ± 0.3	5.3 ± 1.3	11.4 ± 2.2
90% dioxane/water (6) [a]	3.9 ± 0.3	6.2 ± 1.2	6.8 ± 1.5	31.7 ± 3.0	35.4 ± 4.2

[a] Dielectric constant of medium.

Table 3

Calculated ion pair association constants (K_A, M^{-1}) [a] in water and low dielectric media

Solvent	$NaClO_4$ ($a = 5.43$ Å)	NaI ($a = 5.71$ Å)	NaBr ($a = 5.97$ Å)	NaCl ($a = 6.18$ Å)	$NaHCO_3$ ($a = 7.04$ Å)
H_2O ($\epsilon = 78$)	0(0.2) [b]	0	0	0	0(0.7) [c]
50% dioxane ($\epsilon = 35.9$)	8.46	7.37	6.39	5.58	2.61
75% dioxane ($\epsilon = 15$)	5.73×10^2	4.82×10^2	4.46×10^2	4.34×10^2	3.37×10^2
90% dioxane ($\epsilon = 6$)	19.55×10^5	11.73×10^5	6.48×10^5	6.19×10^5	4.93×10^5

[a] Determined from eq. 2.

[b] From ref. 21.

[c] From ref. 22.

of the pairing anion (HCO_3^-). Translocation of Na^+ and HCO_3^- as the contact ion pair would be expected to give rise to the movement of an absolute minimum of approx. 10 mol water ($S_N + S_M = S_X$, eq. 4) per mol ion pairs transported, yielding a concentration of $NaHCO_3$ in newly formed fluid of approx. 5 M. Thus, the movement of water would have to be 25–100-times as great to account for the concentration of $NaHCO_3$ in nascent fluid which defines a concentration range of 50–200 mM among the secretions of aqueous humor, CSF, pancreas and alkaline gland [23].

The 2–4-fold greater ^{22}Na fluxes in the presence of HCO_3^- compared to Cl^- seen in this study also seem to be in qualitative agreement with the fact that the accession rate of HCO_3^- contributes at least 40% to the Na^+ movement into pancreatic juice, aqueous humor and CSF [23]. Further studies are underway to elucidate possible roles that carbonic anhydrase may play with respect to ion paired salt transport as this contributes to fluid secretion.

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