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ANION DIFFUSION SELECTIVITY IN A PORE MODEL

THE PHOSPHATIDYLCHOLINE-WATER LAMELLAR PHASE

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Summary

The diffusion coefficients $D(cm^2 \cdot s^{-1})$ of the sodium salts of a series of hydrophilic mono- and dicarboxylic acids, have been measured in the hydrophilic layers of phosphatidylcholine-water lamellar phases, as a function of phase hydration. At pH 9.0, the diffusion rates of the anionic ($RCOO^-$) form of the acid exhibit a prominent increase within a narrow range of water content, specific to each anion. This high diffusion rate seems to occur when the Stokes diameter of an anion is equal to the thickness of the aqueous layer between the two planes formed by the quaternary ammonium groups of the choline phosphate dipoles of two facing layers of phosphatidylcholine molecules. This phenomenon demonstrates the importance of the spatial organization of successive binding sites in the rate constant of diffusional processes in hydrophilic channels.

Introduction

In recent years, ion permeation through channels has been analysed in detail by several authors [1—3] on the basis of Eyring's theory. According to this theory, ion movement in a channel is considered as a series of successive jumps from site to site over energy barriers. The specificity of the channel's ionic permeability depends upon the height of the barrier, relative to bulk aqueous solution, given by the product of two terms [4], the change in free energy associated with the transfer of the ion from aqueous solution to the site within the channel (equilibrium binding constant) and the energy necessary to jump from site to site (nonequilibrium rate constant).

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The difficulty of the study of channel selectivity comes, partly, from the fact that experimental measurements of ion permeability cannot yield independant knowledge of the two parameters, but only of their global combination as permeability coefficients.

It has been shown in previous reports [5—7] that it was possible to overcome this difficulty in the study of the diffusion processes in the hydrophilic part of phosphatidylcholine-water lamellar phases. These systems can be considered as good models of the physicochemical conditions in which solute movements occur in channels. The hydrophilic layers of the lamellar phase provide pathways consisting of hydrated choline-phosphate dipoles organized in a very restricted space, a few Angströms wide, in which water molecules are either only in a strongly bound state, or in a state which also has free water [8] depending upon the hydration level of the phase. These systems allow experimental measurement of diffusion of solute introduced into the phase to be made without any interference of partition phenomena between the hydrophilic medium of the phase and the external aqueous solutions. In other words, diffusion measurements in these systems actually represent a direct experimental study of the kinetics of solute movement from site to site within a model of a channel.

In a previous paper [9], the results of the diffusion measurements of monovalent alkali cations in the phosphatidylcholine-water channel have been reported. They show that the kinetics of ionic movements in the channel are, in themselves, selective processes. Moreover, they show that the diffusion selectivity sequences obtained correspond to Eisenman equilibrium binding sequences [10].

In order to interpret these results, it seems necessary to consider that, in the hydrophilic path, ions undergo multiple interactions and that it is the pattern of these interactions which controls the diffusion process by lowering the energy barrier. In other words, the relevant energy parameter would not be the difference in free energy of the ion between the free and bound-to-the-site states, but rather the much lower energy barrier necessary to jump from one site to another equivalent site within the pore. This would mean that the diffusion rate is controlled by the organization of the sites within the pore, which may or may not be favourable for a given ion.

Such an hypothesis finds support in the study presented here, which concerns the selectivity exhibited by the phosphatidylcholine-water hydrophilic path in the case of the diffusion of organic anions.

Results will be presented concerning the diffusion rates of hydrophilic carboxylic acids in ionized ($RCOO^-$) form. It will be shown that at a given hydration of the hydrophilic path the organization of the choline phosphate dipole is such that it selectively favors the diffusion of the anionic form of a given carboxylic acid as compared to all the other acids.

Materials and Methods

Phosphatidylcholine was extracted from egg yolk according to the method of Singleton et al. [11] and checked for purity by thin layer chromatography.

The phases were prepared from phosphatidylcholine and 0.1 M sodium phos-

phate buffers at different pH in the range 4.6—9.0 by mechanical mixing under partial vacuum as described before [7].

The diffusion measurements were carried out at 25°C using a radiotracer technique, fully described elsewhere [12], on a macroscopic scale (i.e., over a distance of centimeters).

At each phase water content (determined by drying until constant weight) diffusion coefficients were determined in triplet or quintuplet for each carboxylic acid. Standard errors of measurements of diffusion coefficients are ±1.25—2.5%.

^{14}C-Labelled carboxylic acids were obtained from the Radiochemical Centre (Amersham, U.K.).

Results and Discussion

The diffusion coefficients $D(cm^2 \cdot s^{-1})$, of the sodium salts of 3 monocarboxylic acids (formate, acetate and propionate), 2 hydroxycarboxylic acids (glycolate and lactate) and 2 dicarboxylic acids (oxalate and malonate), have been measured as a function of phase hydration at pH, 9.0.

The importance of this pH value, for our particular experimental conditions, deserves comment. This pH, 9.0, represents the pH of the 0.1 M phosphate buffer used to make the phase and does not pretend to indicate the pH within the hydrophilic path of the phases. In fact, in any system where most of the water is bound, the very concepts of pH and carboxylic acids' pK values are questionable. This point has already been discussed in detail [7,13]. However, it appears reasonable to consider that, at this pH, the carboxylic acids are completely ionized, since their pK values in bulk solution are low enough (<5.7, Table I).

Moreover, at this pH, the choline-phosphate groups remain in zwitterionic form. It was checked by X-ray diffraction that the phases made up with this phosphate buffer are lamellar in the whole water range with only minor modifications of the spacing distance, as compared to phases made with distilled water [14]. Finally, neither the phase nor solute diffusion rate are significantly affected by the salt concentration itself (up to 0.5 M).

The results obtained are presented in Figs. 1—3. It may be seen that the variation of the diffusion coefficients with the water content (ϕ_w, in g water/g phase) is complex, characterized by two maxima.

The first one occurs at $\phi_w = 0.22$. This maximum is a general feature of the diffusion of all solutes in phosphatidylcholine-water phases. It is obtained at the same water content for water, non-electrolytes, mono- and divalent cations [6,9]. The origin of the first maximum has been fully discussed in previous studies [5,7] and will not be analysed again here. It reflects important changes of the configuration of the choline-phosphate group at the hydration level of 11 water molecules per group.

The properties of the second maximum and its origin appear quite different. In the first place, this second maximum is only obtained with the dissociated, negatively-charged form of carboxylic acids. This was clearly shown by diffusion measurements carried out at pH 4.6. At this pH, it was shown in a previous work [6] that monocarboxylic acids exhibit only the first maximum. The same

TABLE I

STRUCTURAL PARAMETERS OF THE PHSOPHATIDYLCHOLINE-WATER LAMELLAR PHASE AT 25°C

Values were obtained from the regression line given by the cumulated data of Reiss-Husson [20], Bourgès et al. [21], Janiak et al. [22] and ourselves [14].

ϕ_w (g water/g phase)	Spacing distance d (Å)	Thickness of hydrophilic layer d_{aq} (Å)
0.10	49.3	15.49
0.11	49.5	15.93
0.12	49.7	16.37
0.13	49.9	16.81
0.14	50.1	17.26
0.15	50.3	17.72
0.16	50.5	18.18
0.17	50.7	18.63
0.18	50.9	19.08
0.19	51.1	19.56
0.20	51.3	20.02
0.21	51.5	20.49
0.22	51.9	21.05
0.23	52.4	21.65
0.24	52.9	22.26
0.25	53.4	22.88
0.26	53.9	23.51
0.27	54.4	24.13
0.28	54.9	24.78
0.29	55.5	25.47
0.30	56.0	26.12
0.31	56.5	26.80
0.32	57.0	27.47
0.33	57.5	28.14
0.34	58.0	28.82
0.35	58.5	29.52
0.36	59.0	30.22

result is obtained with hydroxycarboxylic acids (Fig. 4). The pK of these acids (Table I) are such that, at pH 4.6, they diffuse undissociated, as RCOONa. Accordingly, the evolution of their diffusion rates with phase water content is the same as for non-electrolytes. On the other hand, the diffusion coefficients of dicarboxylic acids at pH 4.6 exhibit the second maximum. Due to their low pK values, these acids most probably exist in the phase as a mixture of mono- or divalent anions. For this reason it is not surprising to obtain second maxima, but occurring at a different place and with a different magnitude than at pH 9.0. It is difficult to interpret clearly the position and the magnitude of the second maxima, since relative proportions of mono- and divalent anions diffusing are not known. Unfortunately, it is not possible to carry out experiments at a pH lower than 3, since phosphatidylcholine molecules are no longer in zwitterionic form and the lamellar structure no longer exists.

The second characteristic of the second maximum is that, at variance with the first maximum which is observed constantly at the same water content, it appears as a very abrupt "burst" of diffusion rate in a very narrow range of water content, which depends upon the acid: at $\phi_w = 0.23$ formate, 0.255 for glycolate, 0.26 for oxalate, 0.27 for acetate, 0.28 for propionate, 0.29 for lactate and 0.305 for malonate. The accuracy with which ϕ_w, is determined for

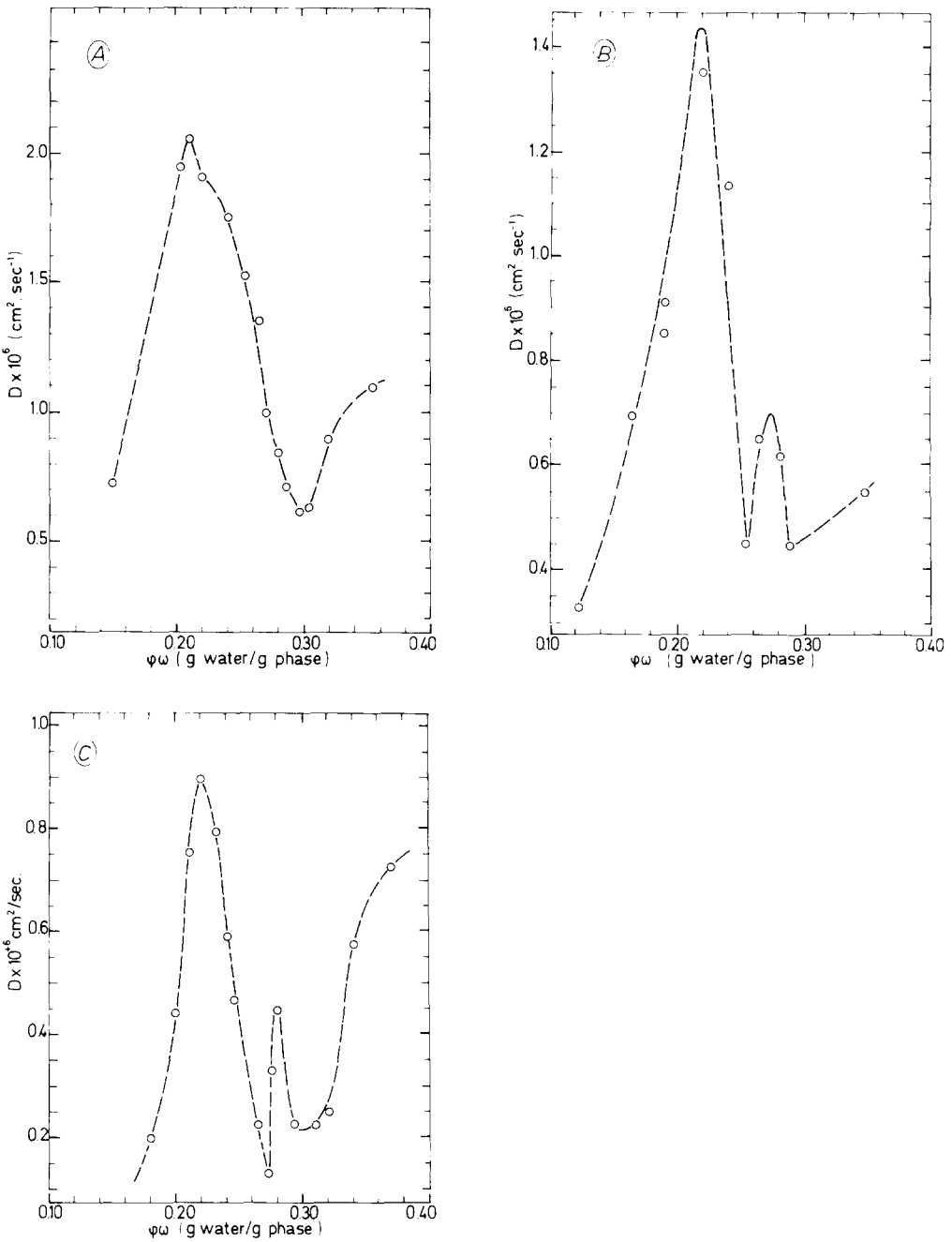

Fig. 1. Diffusion coefficients, $D(cm^2 \cdot s^{-1})$, of formate (A), acetate (B) and propionate (C) as a function of phase hydration, ϕ_w, (in g water/g phase) at pH 9.0. Notice the difference in the ordinate scale in comparing diffusion rates of the three acids.

each maximum, is a function of the spacing of diffusion measurements on the ϕ_w scale. The values given above are within ±0.005.

As a result, at any given water content of the phase, beyond $\phi_w = 0.22$, the

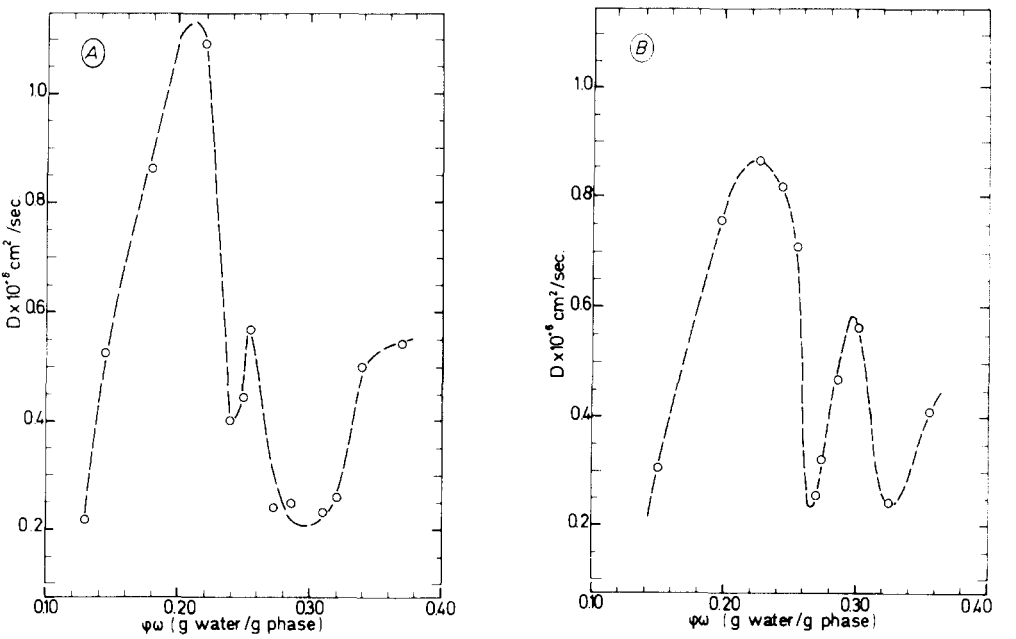

Fig. 2. Diffusion coefficients, $D(cm^2 \cdot s^{-1})$, of glycolate (A) and lactate (B) as a function of phase hydration, ϕ_w, (in g water/g phase) at pH 9.0.

hydrophilic path exhibits a different selectivity pattern, i.e., one of the acids diffuses much faster than all the others:

at $\phi_w = 0.22$: formate > accetate >> glycolate > propionate, oxalate, lactate > malonate

at $\phi_w = 0.24$: formate > acetate > lactate > malonate, propionate > glycolate > oxalate

at $\phi_w = 0.26$: formate > oxalate >> acetate > glycolate, lactate, malonate > propionate

at $\phi_w = 0.28$: formate > malonate > acetate > glycolate > lactate > oxalate > propionate

at $\phi_w = 0.30$: malonate >> formate, lactate > acetate > oxalate > propionate, glycolate.

There are two main clues for interpreting the results: the first clearly appears to be the fact that only negatively-charged solutes exhibit the second maximum; mono- and divalent cations do not [9]. This indicates that the phenomenon depends upon ion-dipole interactions which control finally the localization of the anion within the hydrophilic path, as has been shown previously [7]. The structure of the hydrophilic path beyond $\phi_w = 0.22$ can be described in the following way. There is convincing evidence that choline-phosphate dipoles are in an extended configuration [6] that is roughly perpendicular to the plane of the bilayers. Therefore, the positively-charged quaternary ammonium groups, $N^+(CH_3)_3$, are grouped in the middle of the hydrophilic layers (Fig. 5). As the amount of water increases in the phase, each new water molecule is intercalated between the two layers of $N^+(CH_3)_3$ groups facing each other. As shown by X-ray measurement, beyond 11 water molecules per phos-

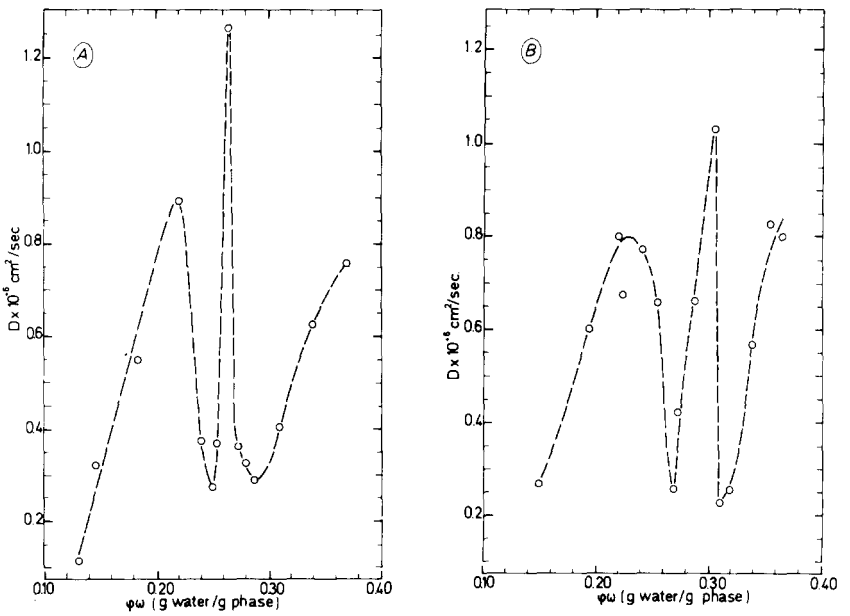

Fig. 3. Diffusion coefficients, $D(cm^2 \cdot s^{-1})$, of oxalate (A) and malonate (B) as a function of hydration, ϕ_w, (in g water/g phase) at pH 9.0.

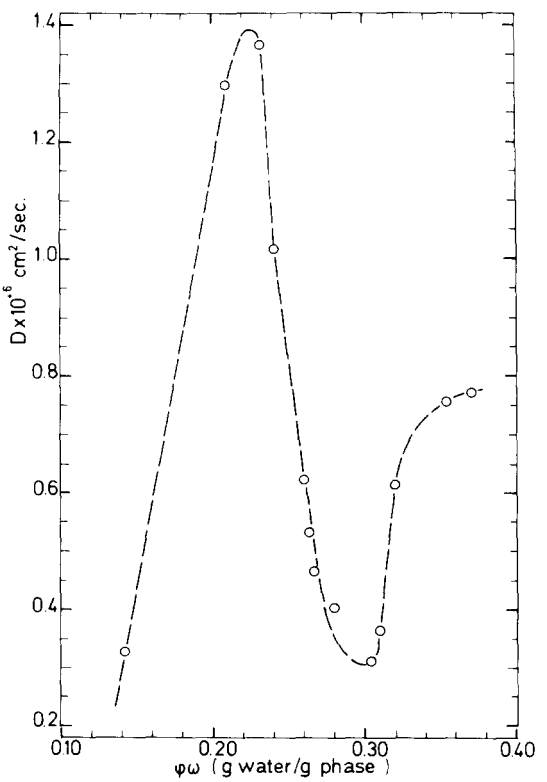

Fig. 4. Diffusion coefficients, $D(cm^2 \cdot s^{-1})$, of glycolate as a function of hydration, ϕ_w, (in g water/g phase) at pH 4.6.

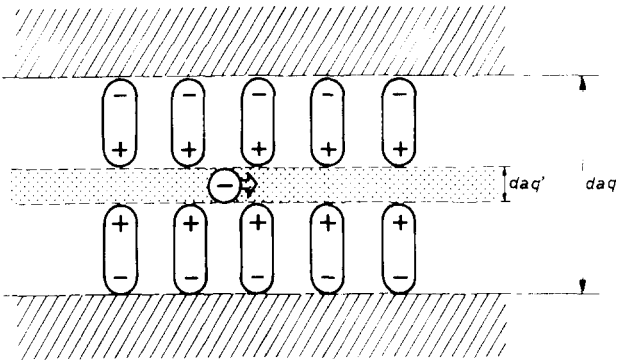

Fig. 5. Schematic representation of the hydrophilic layer of the lamellar phase.

phatidylcholine molecule ($\phi_w = 0.22$), the area per molecule has practically reached its maximum and remains constant. At this point, there is no space for more water molecules between the adjacent dipoles of the same layer. Then a median aqueous layer, limited by two planes of positive sites is formed. the thickness of which increases with phase hydration. The negatively charged PO_4^- groups of the dipole form two symetrical planes some 4—5 Å further apart, near the hydrophilic-lipophilic interface. The binding sites can be considered immobile as compared to the diffusing anions, since the lateral diffusion rate of phosphatidylcholine molecules is two orders of magnitude lower ($\simeq 10^{-8}$ $cm^2 \cdot s^{-1}$) [7].

One may consider that, due to their own negative charge, hydrophilic anions are repelled from the external zone of this electrical sandwich and are restricted to the middle aqueous layer in contact with the positive $N^+(CH)_3$ groups where they diffuse.

The second clue is that there is an obvious relationship between the size of the anion and the localization of its second maximum of diffusion. This indicates that the spatial configuration of the interaction between anion and site is important. In order to check this hypothesis, the relationship between the size of the hydrophilic channels and the size of the anion has been analysed in the following way.

The total thickness of the hydrophilic layers of the lamellar phase, d_{aq}, (including both choline-phosphate groups and water) is known from X-ray diffraction measurements (see Table I). The length of the choline-phosphate group in a fully extended configuration, as measured on molecular models, is approx. 10 Å. On this basis, it is possible to estimate the thickness, d_{aq}', of the middle aqueous layer included between the positively-charged groups as a function of phase water content. The values of d_{aq} and d_{aq}' corresponding to each diffusion maximum are reported in columns 5 and 6 of Table II.

On the other hand, the diameters of the different anions have been computed as their Stokes diameter, using the limiting ionic conductance at infinite dilution at 25°C [15]. Although questionable, the choice of this size parameter is justified by the fact that the small hydrophilic acids involved are roughly spherical in shape and that it may be reasonably assumed that in this range of water content they are fully hydrated.

TABLE II

Carboxylic acid parameters			Phase parameters		
	pK (25°C)	Stokes radius (Å)	d_{aq}' (Å ± 0.3 Å)	d_{aq} (Å)	Hydration corresponding to 2nd maximum at pH 9.0 (ϕ_w ± 0.005)
Formate	3.67	1.68	1.65	21.65	0.23
Acetate	4.75	2.23	4.19	24.13	0.27
Propionate	4.87	2.57	4.78	24.78	0.28
Glycolate	3.83	1.82	3.20	23.20	0.255
Lactate	2.86	2.59	5.47	25.47	0.29
Oxalate	1.23 4.19	1.91	3.51	23.51	0.26
Malonate	2.83 5.69	3.43	6.46	26.46	0.305

In Fig. 6, the values of d_{aq}' at which second maxima occur have been plotted as a function of anion Stokes diameters. A straight line is obtained, the slope of which indicates clearly an identity relationship between the two parameters. All the mono- and divalent anions tested, except formate, fall with a good approximation on this identity line.

The first term of the monocarboxylic series, formate, is obviously not on the line. However, it must be noted that the determination of its second maximum at $\phi_w = 23.5$ is dubious, since it only appears as a shoulder of the first maxi-

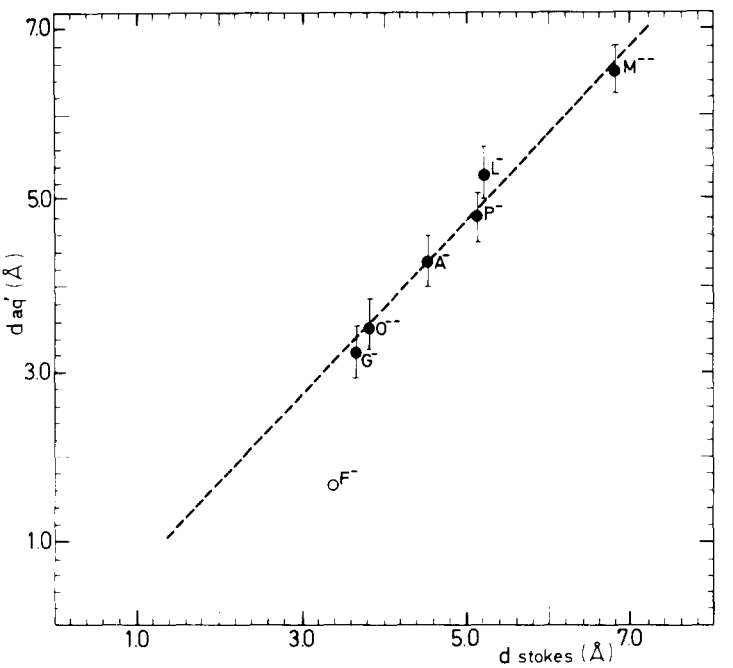

Fig. 6. Identity relationship between anion Stokes diameter and thickness of the middle aqueous layer, d_{aq}', (for explanation see text). F, formate; G, glycolate; O, oxalate; A, acetate; P, proprionate; L, lactate; M, malonate.

mum (Fig. 1). If taken at face value, this second maximum corresponds to a Stokes diameter of 1.60 Å, i.e., half the value derived from limiting conductance, which, if compared with other size estimation, appears possibly as the diameter of a dimeric form. It must be noted that from the relationship between size and d_{aq}' it is clear why an inorganic anion such as Cl^- does not exhibit a second maximum of diffusion: this second maximum would be located at a water content that places it practically on the first maximum of diffusion so that it cannot be seen.

From this analysis, it is clear that the diffusion rate of an anion reaches its maximum when its diameter becomes equal to the distance between the two planes of positively-charged quaternary ammonium groups, which seems to be a very special situation. To account for this phenomenon, it is important to consider that in this particular condition, the diffusion process is no longer the classical three-dimensional random walk from site to site, but a more ordered two-dimensional movement. What is more, the sandwich must by symmetrical, so that the center of the potential profile is located exactly in the center of the space between the polar heads. The results indicate that the maximum of diffusion is very sensitive to geometrical parameters and that very small departures from the exact fit between d_{aq}' and Stokes diameters lead to an abrupt fall of the diffusion rate on both sides: where d_{aq}' is smaller than Stokes diameter, steric hindrance becomes predominant and slows down the diffusion; on the other side, if d_{aq}' is greater than the Stokes diameter, the anion rapidly begins to undergo the three-dimensional random walk from site to site which results in a much slower rate of diffusion.

Conclusions

The present data may be analysed in the same way as the permeation through channels [3,16]. However, at variance with classical permeability measurements, the equilibrium parameter of ion partition between bulk solution and site within the channel is dissected out in the present case, since only movements of ions already within the channel are measured. Therefore, diffusion rates have to be related exclusively to the energy profile within the channel, i.e., to the height of the energy barriers between successive and identical energy minima of binding sites. It appears that if this energy profile is uniform and smooth, which apparently results from the optimal spacing of the site relative to a given ion, the diffusion rate is high. If, on the other hand, the energy profile is more irregular, which apparently results from a greater than optimal spacing of the sites, the diffusion rate will be low.

It is noteworthy that the maximal diffusion rate in the channel is obtained (at a specific hydration) only for the anionic forms of acids, which bind to the choline-phosphate site much more strongly than the undissociated form. This result is akin to the results obtained with monovalent alkali cation in the same system: their diffusion rate sequences are Eisenman equilibrium binding sequences. One is lead to the idea that this type of strong binding may actually promote specific high mobility in a channel. This is similar to the fact that the mobility of counter ions is higher in the direction parallel to a polyelectrolyte backbone than in the perpendicular direction [17]. This line of reasoning may

be relevant in the current discussions concerning the mechanism of selectivity of biological channels [18,19].

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