PHYSICOCHEMICAL PROPERTIES
OF CARTILAGE IN THE LIGHT OF
ION EXCHANGE THEORY

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ABSTRACT   Ion exchange theory has been applied to articular cartilage. Relationships were derived between permeability, diffusivity, electrical conductivity, and streaming potential. Systematic measurements were undertaken on these properties. Experimental techniques are described and data tabulated. Theoretical correlations were found to hold within the experimental error. The concentration of fixed negatively-charged groups in cartilage was shown to be the most important parameter. Fixed charge density was found to increase with distance from the articular surface and this variation was reflected in the other properties.

INTRODUCTION

The extracellular component of cartilage consists of collagen fibers embedded in a gel of mucopolysaccharides and water, commonly referred to as the matrix. This gel can be considered as a typical ion exchange material, since it consists essentially of water and a network of linear polyanions with fixed negative charges. The water is present partly in the form of solvation shells, and partly as free water with mobile cations and anions in solution.

Since cells occupy a small fraction of the total volume of cartilage, the transport of fluid and solute through cartilage depends on the properties of the matrix (Maroudas, Bullough, Freeman, and Swanson, 1967).

The purpose of the present work is to apply the theory of ion exchange membranes —with its characteristically close interrelationships between different aspects of ionic equilibrium, diffusion, fluid flow, electrical potential, and current—to articular cartilage. It will be shown that the transport properties of cartilage can be satisfactorily explained and even predicted on this basis.

As regards the experimental data, a survey of the literature shows no quantitative data on any of the transport properties of cartilage apart from a few scattered figures on its permeability to liquid. Accordingly, a systematic experimental study of diffu-

sion, permeability, electrical conductivity, and streaming potential was undertaken, and the results obtained to date are presented and correlated on the basis of ion exchange theory.

Theory of Transport Properties in Ion Exchange Membranes

Ion exchange materials are inhomogeneous by their very nature, as they consist of polyelectrolyte networks on the one hand and pore liquid on the other. However, several different scales of inhomogeneity may exist in their structure.

If the polyelectrolyte and the pore liquid are intimately interdispersed on the molecular (or even on the colloidal) scale, the ion exchange material can be considered for the purpose of equilibrium and kinetic studies as a single "quasi-homogeneous phase." Such systems are successfully described in terms of Donnan equilibrium, single diffusion coefficients, and so on.

On the other hand, where inhomogeneities exist on the micron scale, the theoretical treatment is more complex and less satisfactory. Examples are those resins which consist of highly cross-linked islands where most of the fixed ionic groups are concentrated, surrounded by distinct regions of low cross-linking and high water content.

In practice most ion exchange materials fall between these two extremes. It is not possible to predict a priori how successfully the quasi-homogeneous theory outlined below can be applied to cartilage. All one can do is to derive the relationships predicted by theory and to test them by making the appropriate measurements on cartilage.

Donnan Equilibrium

A diagrammatic sketch representing the structure of a cation exchange membrane is shown in Fig. 1.

Since the matrix of cartilage contains negatively-charged groups—the sulfate and the carboxylate groups of chondroitin and keratan sulfates—which are not free to move, one may expect a Donnan potential to be set up across the interface when cartilage is immersed in an electrolyte solution. The distribution of mobile ions between cartilage and the external solution would be expected to obey the Donnan equilibrium equation, viz.,

$$\left(\frac{a_{\text{cation}}}{\bar{a}_{\text{cation}}}\right)^{z_{\text{cation}}} = \left(\frac{\bar{a}_{\text{anion}}}{a_{\text{anion}}}\right)^{z_{\text{anion}}} \tag{1}$$

where a = activity coefficient of the ion in solution, $\bar{a}$ = activity coefficient of the ion in cartilage, z = valency of the ion; and the electroneutrality condition, viz.,

$$z_{\text{cation}} \bar{C}_{\text{cation}} = z_{\text{anion}} \bar{C}_{\text{anion}} + \bar{C}_{\text{immobile anion}} \tag{2}$$
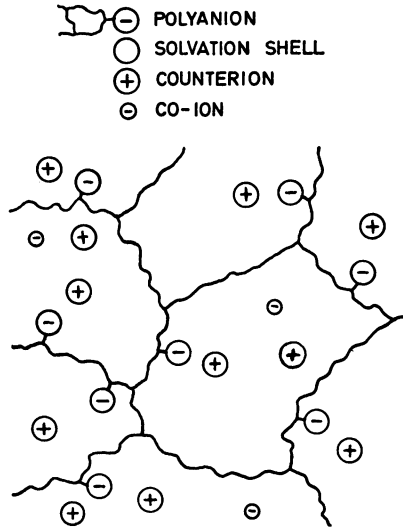
where $\bar{C}$ = molar concentrations in cartilage.

FIGURE 1   Structure of a cation exchange membrane.

If cartilage is immersed in Ringer's solution (0.15 N NaCl with a trace of KCl, CaCl$_2$, and NaHCO$_3$) the main cation (counterion in this case) is Na$^+$ and the main anion (co-ion) is Cl$^-$, so that equations 1 and 2 can be rewritten respectively, as

$$\frac{a_{Na^+}}{\bar{a}_{Na^+}} = \frac{\bar{a}_{Cl^-}}{a_{Cl^-}} \tag{3}$$

and

$$\bar{C}_{Na^+} = \bar{C}_{Cl^-} + \bar{C}_x \tag{4}$$

where $\bar{C}_x$ = concentration of fixed negatively-charged groups in cartilage.

Assuming the activity ratios of both Na$^+$ and Cl$^-$ to be equal to their concentration ratios, one can rewrite equation 3 in the form

$$\frac{C_{Na^+}}{\bar{C}_{Na^+}} = \frac{\bar{C}_{Cl^-}}{C_{Cl^-}} \tag{5}$$

where $\bar{C}$ denotes the concentration of a species in cartilage and C in solution.

If $\bar{C}_x$ is known, it is possible by the use of equations 4 and 5 to calculate both $\bar{C}_{Na^+}$ and $\bar{C}_{Cl^-}$ for a given concentration of the outside solution.

Diffusion Coefficients

A characteristic feature of ion exchange membranes is their difference in permeability to counterions and co-ions as a consequence of the Donnan equilibrium. The

equations for solute transfer across membranes, which will be given below, all embody this feature.

The rate of diffusion can be controlled by the resistance either across the membrane itself or across the stagnant liquid film at the membrane-liquid interface, or it may depend on both. The general steady state equation for self- or tracer-diffusion flux across an ion exchange membrane is (Helfferich, 1962, p. 348)

$$J_A = - \frac{\bar{D}\bar{C}}{d\left(1 + \frac{2\delta\bar{D}\bar{C}}{DCd}\right)} \tag{6}$$

where J_A = flux of solute A, $\bar{D}$ = diffusivity coefficient in the membrane, D = diffusivity coefficient in the solution, d = thickness of the membrane, and δ = thickness of stagnant liquid film.

A criterion indicating the nature of the rate-determining step is given by Helfferich (p. 348): membrane diffusion is controlling and film resistance is negligible when

$$\frac{DCd}{\bar{D}\bar{C}\delta} \gg 2 \tag{7}$$

when equation 7 holds it is possible to use a simpler equation for the flux, viz.,

$$J_A = -\frac{\bar{D}\bar{C}}{d} . \tag{8}$$

Equation 7 has been used by the present author to estimate the conditions for articular cartilage membranes under which film resistance can be neglected; equation 8 can be used in the calculation of diffusion coefficients.

From the results given in Table II,

$$\frac{D}{\bar{D}} \approx 3, \qquad \left(\frac{C}{\bar{C}}\right)_{\text{cation}} \approx \frac{1}{2}, \qquad \left(\frac{C}{\bar{C}}\right)_{\text{anion}} \approx 2.$$

In the presence of adequate stirring, $\delta = 3 \times 10^{-3}$ cm (Hellferich, p. 348). Table I gives the approximate values of the ratio $DCd/\bar{D}\delta$ as a function of d for cations, anions, and nonelectrolytes.

Examination of Table I shows that for cartilage slices more than 1 mm thick, film resistance can be neglected. Equation 8 may then be used to calculate the diffusion coefficients without introducing an appreciable error. However, in the case of 200 μ slices, the use of equation 8 would result in an error of up to 20%.

It can be seen from equations 6 and 8 that $\bar{D}$ can be calculated from the experimentally determined value of the steady-state flux J_A only if the equilibrium concentration, $\bar{C}$, of the given solute in the membrane is known. For nonelectrolytes,

TABLE I

dcm	$DCd/\bar{D}\bar{C}\delta$ (from equation 7)			% error in $\bar{D}$ resulting from use of equation 8		
	Cations	Anions	Nonelectrolytes	Cation	Anions	Nonelectrolytes
2×10^{-2}	10	40	20	20	5	10
10^{-1}	50	200	100	4	1	2
2×10^{-1}	100	400	200	2	0.5	1

$\bar{C} = C$, the concentration of the substance in the external solution. For cations and anions, however, $\bar{C} \neq C$. $\bar{C}$ can be calculated by means of equations 4 and 5 provided $\bar{C}_x$ is known. If $\bar{C}_x$ is not known, steady-state diffusion experiments can only provide the values of the permeability coefficient $\bar{P}$ which is defined by the equation

$$J_A = -\frac{\bar{P}\triangle C}{d}. \tag{9}$$

Comparison of equations 8 and 9 gives the following relation between $\bar{P}$ and $\bar{D}$:

$$\bar{D} = \bar{P}\frac{\bar{C}}{C}. \tag{10}$$

By contrast, unsteady-state diffusion experiments can yield directly the value of the diffusion coefficient $\bar{D}$, the knowledge of $\bar{C}$ being unnecessary. The method of calculation is described in detail by Crank (1956).

The actual diffusion coefficients depend both on the diffusing solute and on the free cross-sectional area available to diffusion within the membrane.

Conductivity

In an ion exchange material, the specific conductivity is given by Helfferich (p. 331)

$$\kappa = F(\sum_i z_i^2 \bar{u}_i \bar{C}_i + \bar{u}_0 \bar{C}_x) \tag{11}$$

where κ = over-all specific conductivity of the material, F = Faraday's constant, z = valency of the ionic species, $\bar{u}_i$ = electrochemical mobility, and $\bar{u}_0$ = mobility parameter of pore liquid.

The first term in equation 11 represents the ionic conductivity while the second term is due to convection conductivity. The latter depends on the specific flow resistance of the material. Because specific flow resistance in cartilage is very high, the convection conductivity term is several orders of magnitude lower than the ionic conductivity and can be neglected. Thus, for cartilage, equation 11 reduces to

$$\kappa = F(\bar{u}_{Na^+}\bar{C}_{Na^+} + \bar{u}_{Cl^-}\bar{C}_{Cl^-}). \tag{12}$$

The ionic mobility $\bar{u}$ depends, as does the diffusion coefficient, on the ionic species involved and the porosity of the medium. In fact, the well-known Planck-Einstein relation between u and D, viz.,

$$u = \frac{F}{RT} D \qquad (13)$$

holds particularly well in ion exchange materials.

In accordance with the Donnan equilibrium, the concentration of the co-ion in cartilage immersed in a very dilute external solution should become negligible. Thus, for cartilage equilibrated, for instance, in distilled water, equation 12 can be reduced to

$$\bar{\kappa}_{\text{dilute}} = F(\bar{u}_{\text{Na}^+}\bar{C}_{\text{Na}^+}). \qquad (14)$$

Since $\bar{C}_{\text{Na}^+} \approx \bar{C}_x$, equation 14 can be further simplified to

$$\bar{\kappa}_{\text{dilute}} = F(\bar{u}_{\text{Na}^+}\bar{C}_x). \qquad (15)$$

Fluid Flow

Cartilage can be thought of as consisting of two phases: a dense phase of immobilized mucopolysaccharides, collagen, and bound water, and an aqueous phase with mobile counterions and co-ions. The two phases are intimately interdispersed and effectively give rise to a porous structure. Fluid flow through cartilage can thus be described by the Darcy Equation for porous media

$$V = \frac{Q}{A} = K \frac{\triangle p}{d} \qquad (16)$$

where V = velocity of approach, Q = volumetric flow rate, A = cross-sectional area of membrane, K = permeability coefficient, $\triangle p$ = pressure drop across membrane, and d = membrane thickness.

The permeability coefficient K, depends on the viscosity of the fluid and the porosity of the medium.

In ion exchange membranes, the porosity of the medium depends on the free water content and the degree of cross-linking of the resin. The free water content depends largely on the fixed charge density in the membrane, increasing as the latter decreases.

Streaming Potential

An impcrtant consequence of fluid flow through an ion exchange membrane is the development of an electrical potential called streaming potential (Helfferich, p. 393). There is a simple relation between the streaming potential, $\triangle \psi$, and the ap-

plied pressure, $\triangle p$:

$$\frac{\triangle \psi}{\triangle p} = \frac{FK\bar{C}_x}{\bar{\kappa}} \tag{17}$$

where $\triangle \psi$ = streaming potential. Two main assumptions are involved in the derivation of equation 17.

Firstly, the ion exchanger is considered a two-phase system in which there is a fixed network containing immobilized charged groups and a uniformly distributed pore space through which liquid flow takes place. Secondly, the distribution of mobile species within the pores themselves is uniform. The ratio $\triangle \psi / \triangle p$ depends on the permeability to flow, the fixed charge density, and the electrical conductivity of the membrane.

Consideration of the transport properties of ion exchange membranes shows that fixed charge density is a very important parameter. In synthetic cation exchange materials, fixed charge density is usually determined by converting the resin into its hydrogen form and titrating against standard alkali. This method cannot be used for cartilage because, apart from its fixed anionic groups, cartilage also possesses fixed cationic groups owing to its proteins. At the physiological pH the degree of dissociation of the proteins is small, but if one were to convert the matrix into the hydrogen form by treatment with acid the degree of dissociation of the proteins would be considerably increased and cartilage might be converted from a cation to an anion exchange material.

Accordingly, the author has made equation 17 the basis of a method for determining fixed charge density in cartilage from the measurement of $\triangle \psi$, K, and $\bar{\kappa}$ at the physiological pH (Maroudas, 1967).

The equations derived in this section predict a number of relationships between different properties of cartilage. In order to test the applicability of these equations, it was necessary to make systematic measurements on cartilage. The experimental results are presented, and the agreement with theory examined, in the following sections.

APPARATUS AND EXPERIMENTAL PROCEDURE

Human postmortem cartilage taken from the femoral condyles was used in all experiments. The specimens were stored at $-20°C$ and were kept at room temperature only for the duration of the experiments. No mucopolysaccharides were leached out under these conditions. It has been previously shown (Maroudas et al., 1967) that storage at $-20°C$ for a period of several weeks does not alter the transport properties of cartilage.

All measurements were taken at $20°C$. Unless otherwise stated, cartilage was equilibrated in Ringer's solution prior to use.

Diffusion of Solutes through Cartilage

The diffusion cell is shown in Fig. 2. Cartilage slices cut to the required thickness (usually about 1 mm) on a sledge microtome were clamped between the two compartments of the cell.
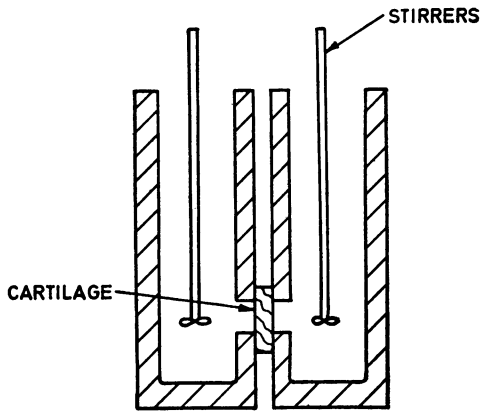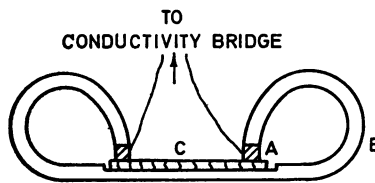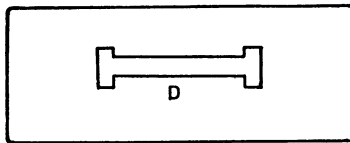
FIGURE 2 Sectional side view of diffusion cell.

A: PLATINUM CONTACTS
B: PERSPEX STRIP
C: CARTILAGE
D: RECESS 1cm x 2mm x 0·5 mm

TO
CONDUCTIVITY BRIDGE

SIDE VIEW SHOWING CURVED ARMS
TO PRESS DOWN ON CARTILAGE

PLAN SHOWING RECESS TO HOLD
STRIP OF CARTILAGE

FIGURE 3 Conductivity cell.

Both compartments contained Ringer's solution and the liquid was vigorously stirred. The solute whose rate of diffusion was to be investigated was introduced into the solution on one side of the cartilage membrane, so as to achieve a concentration which corresponded to its level in normal synovial fluid. The solute gradually diffused across the membrane into the second compartment, the contents of which were withdrawn at regular time intervals for analysis and completely replaced each time by pure Ringer's solution.

The solutes used were tagged with radioactive isotopes (^{22}Na, ^{40}K, ^{36}Cl, ^{35}S, ^{14}C). The solute concentration in the samples was determined by measuring the amount of radioactivity. A scintillation counter was used. Where γ-radiation was emitted (^{22}Na and ^{40}K), a solid potassium bromide crystal was used as scintillator, while for β-radiation a water-miscible liquid scintillator was employed. Standard counting techniques were applied.

Electrical Conductivity

The conductivity apparatus is shown in Fig. 3. The cartilage strip is placed in the recess D. Curved Perspex arms (B) with flat platinum contacts (A) cemented at the ends press down on the cartilage. In this way, good electrical contact is ensured. The distance between the electrodes is always the same.

Cartilage strips 0.2 cm in width, 4×10^{-2} cm in thickness, and about 1 cm long were used. Before the strips were inserted into the cell, surface electrolyte was removed from them by gentle wiping with absorbent paper. To minimize the evaporation of water from the cartilage during the measurements, the cell was placed in a plastic box in which the air was kept saturated with water vapor.

The platinum electrodes were connected to a direct reading Conductivity Bridge (range 3 μ mho-0.3 mho full-scale deflection).

A : PRECISION BORE GLASS CAPILLARY
B : PERSPEX FLANGE
C : CARTILAGE SLICE
D : SINTERED P.T.F.E. DISC
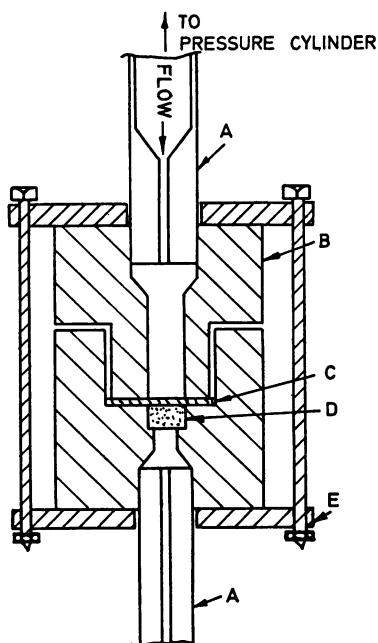E : BRASS BOLTS AND COLLARS

FIGURE 4 Permeability tube.

Fluid Flow

The apparatus for measuring the permeability of cartilage to fluid flow is shown in Fig. 4.

The cartilage slice, about 0.8 cm in diameter and 2-4 $\times$ 10^{-2} cm in thickness is placed in the recess as shown and rests on a porous P.T.F.E. disc. The disc prevents the cartilage from bulging and thinning out when pressure is applied, without interfering with the flow. The rate of flow of liquid through cartilage under a given applied pressure is measured by the rate of rise of liquid in the capillaries. To detect leakage, two capillaries were used, one on the upstream and one on the downstream side of the membrane. A vernier microscope was used to measure fluid displacement in the capillaries. In all tests, the applied pressure was equal to 10^6 dynes per cm^2.

Streaming Potential

The apparatus for measuring the streaming potential of cartilage (Fig. 5) has been described previously (Maroudas, 1967). It is a simple extension of the permeability apparatus, in which Ag–AgCl electrodes have been placed on either side of the membrane. The electrodes are connected to an electrometer capable of measuring accurately voltages down to 10^{-4} v.

Cartilage slices about 4 $\times$ 10^{-2} cm in thickness were used and as in the permeability tests, the applied pressure was 10^6 dynes per cm^2.
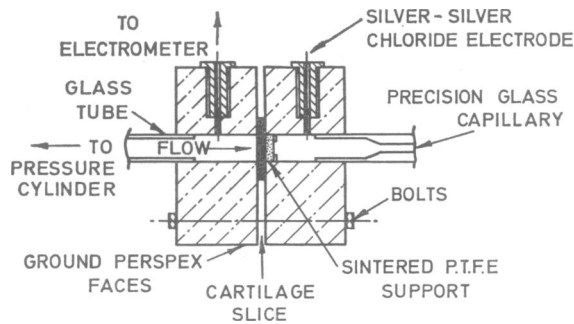

FIGURE 5   Streaming potential apparatus.

Fixed Charge Density

Fixed charge density in cartilage has been calculated by means of equation 17 from the measurements of streaming potential, specific conductivity, and permeability to fluid flow carried out on the given specimen by the methods outlined above.

RESULTS AND DISCUSSION

Preliminary experiments by the present author as well as data found in the literature (McCutchen, 1962) suggested that there was a variation in the properties of cartilage with distance from the articular surface. Accordingly, whenever possible an attempt has been made to study these properties as a function of depth.

However, no measurements were made on the region of cartilage within approximately 2 $\times$ 10^{-2} cm of the articular surface because of the experimental difficulties

involved in dealing with slices of small cross-sectional area obtained near the surface. These difficulties have now been overcome and a study on the properties of the surface layers is in progress.

Considerations presented in the section on theory show that most of the properties of fluid and solute transport through ion exchange membranes depend very strongly on fixed charge density. Thus, in order to discuss and correlate the experimental data on transport properties of cartilage in the light of ion exchange theory, the results on the distribution of fixed charge density in cartilage are presented first.

Fixed Charge Density in Articular Cartilage

Fig. 6 shows typical curves of $\bar{C}_x$ vs. depth. The values of $\bar{C}_x$ were calculated from the values of $\triangle\psi$, K, and $\bar{\kappa}$ given in the Appendix.

The concentration of fixed negatively-charged groups in cartilage appears to increase from the articular surface to the subchondral bone. These results are consistent with those of Stockwell (1967) on the distribution of chondroitin and keratan sulfates in articular cartilage. According to Stockwell the chondroitin sulfate content is low near the articular surface, reaches a maximum in the middle zone,
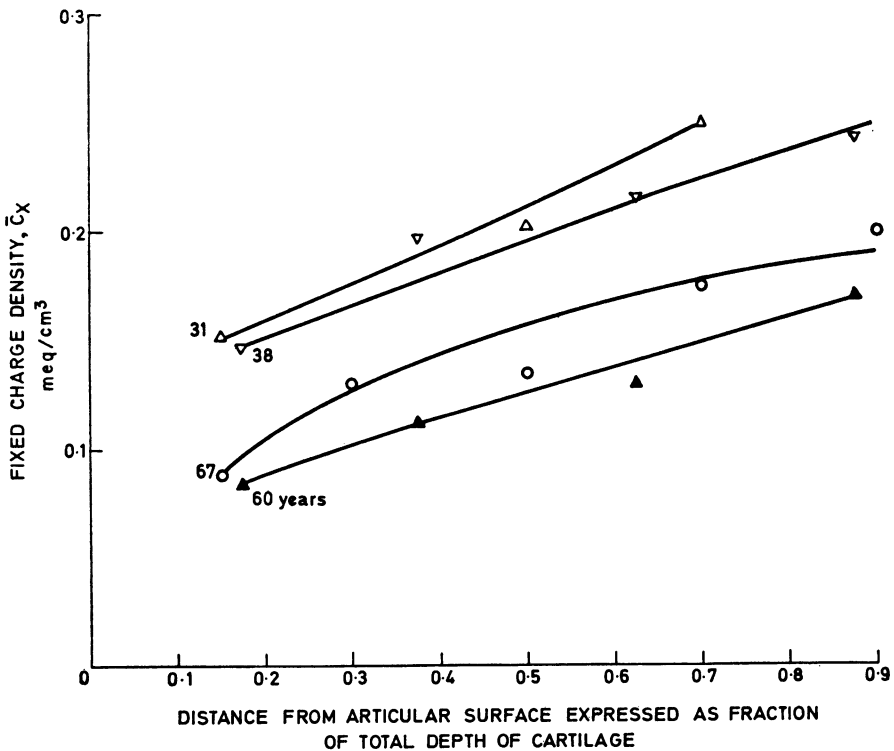

FIGURE 6   Variation of fixed charge density with distance from articular surface.

and decreases again in the deep zone. The over-all mucopolysaccharide content shows a net increase with depth from the articular surface to the beginning of the deep zone (i.e., to within approximately 0.2 mm from the bone). The pattern of variation of fixed charge density in cartilage thus appears to agree with the variation in total mucopolysaccharide content as determined by Stockwell.

Quantitative comparison has been carried out between fixed charge density as determined by the streaming potential method and as calculated from the muco-polysaccharide content determined on the same samples by chemical methods; close agreement has been found.[1]

No results are available in the literature on the fixed charge concentration in articular cartilage. However, Gersh and Catchpole (1960) have measured, using a different electrometric technique, the fixed charge density in other connective tissues. It varies from 0.02 eq/ml of tissue water for synovial fluid to 0.17 for sternal cartilage and symphysis pubis. The value quoted for sternal cartilage lies in the range of values obtained in the present work for articular cartilage.

Diffusion Coefficients

Self-Diffusion Coefficients of Na^+ and Cl^-. Permeability coefficients for Na^+ and Cl^- were obtained from steady-state flux measurements.

Fixed charge density was determined for the same specimens and the diffusion coefficients ($\bar{D}$ indirect) were then calculated by means of equations 4, 5, and 10. In a few cases the diffusion coefficients were also obtained directly ($\bar{D}$ direct) from the comparison of unsteady- and steady-state flux measurements.

The value of $\bar{P}$, $\bar{C}_x$, $\bar{D}$ (indirect), and $\bar{D}$ (direct) for Na^+ and Cl^- are given in Table II. The values of the ratio $\bar{D}_{Cl^-}/\bar{D}_{Na^+}$ are also tabulated.

The self-diffusion coefficients of Na^+ lie in the range 4.3–4.9 $\times$ 10^{-6} cm^2 per sec and those for Cl^- in the range 6.6–7.9 $\times$ 10^{-6} cm^2 per sec; i.e., both of them are approximately 40% of their value in aqueous solution. The ratio $\bar{D}_{Cl^-}/\bar{D}_{Na^+}$ varies from 1.44–1.65; i.e., it is close to the value of 1.5 in aqueous solution.

For cartilage equilibrated in Ringer's solution, the difference between $\bar{D}$ (direct) and $\bar{D}$ (indirect) does not appear to be significant. This suggests that the use of a mean value of $\bar{C}_x$ as obtained from the measurement of streaming potential is justified and that equations 4, 5, and 10 which were used to calculate $\bar{D}$ (indirect) from the value of $\bar{P}$ hold well for cartilage in Ringer's solution. In particular, it may be concluded that the distribution of Na^+ and Cl^- between cartilage and Ringer's solution obeys the Donnan equilibrium.

In order to see whether the diffusion coefficients of Na^+ and Cl^- remain the same when a dilute external solution is used and whether the Donnan equilibrium equa-

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[1] H. Muir and A. Maroudas. Paper in preparation.

TABLE II
SELF-DIFFUSION COEFFICIENTS OF Na+ AND Cl-

Cartilage equilibrated in	Age	$\bar{C}_x$	Na+			Cl-			$\bar{D}_{Cl^-}/\bar{D}_{Na^+}$	
			$\bar{P} \times 10^6$	$\bar{D} \times 10^6$ indirect	$\bar{D} \times 10^6$ direct	$\bar{P} \times 10^6$	$\bar{D} \times 10^6$ indirect	$\bar{D} \times 10^6$ direct		
	yr	meq/cm³	cm²sec⁻¹	cm²sec⁻¹	cm²sec⁻¹	cm²sec⁻¹	cm²sec⁻¹	cm²sec⁻¹		
Ringer's solution	53	0.20	8.15	4.35	—	3.5	6.6	—	1.54	
Ringer's solution	58	0.14	7.0	4.55	—	4.8	7.5	—	1.65	
Ringer's solution	30	0.18	8.2	4.4	4.3	4.0	7.2	6.9	1.64	1.60 direct
1:10 Ringer's solution	30	0.18	0.45	4.2	—	1.3	17	7.1	—	
Ringer's solution	55	0.15	7.9	4.8	4.9	4.25	7.0	7.5	1.44	1.53 direct
1:10 Ringer's solution	55	0.15	0.39	4.65	—	2.21	22.1	7.8	—	

tion can be applied with equal accuracy, two specimens were studied in a 1:10 Ringer's solution. The results show that the value of $\bar{D}$ (direct) for Cl- is close to that obtained for cartilage equilibrated in Ringer's solution.

However, the value of $\bar{D}$ (indirect) for Cl- is very much higher. This must mean that the value of $\bar{C}_{Cl^-}$ used in equation 10 to calculate $\bar{D}$ (indirect) from the measured value of $\bar{P}_{Cl^-}$ is incorrect.

In fact, if the procedure is reversed and $\bar{C}_{Cl^-}$ is calculated by substituting the experimentally determined values of $\bar{P}$ and $\bar{D}$ (direct) into equation 10, it is found that for the 30 year-old specimen $\bar{C}_{Cl^-} = 0.0026$ meq per cm³ instead of the Donnan equilibrium value of 0.0011 meq per cm³, and for the 55 year-old specimen $\bar{C}_{Cl^-} = 0.0043$ meq per cm³ instead of the Donnan equilibrium value of 0.0015 meq per cm³.

This result is consistent with the reported behavior of other ion exchange materials (Helfferich, pp. 143–145). Thus additional non-Donnan type sorption may be due to two factors: firstly, the presence of very small amounts of impurities which may bind certain ions stoichiometrically; secondly, the possible existence of some highly cross-linked "islands" embedded in swollen regions of low cross-linking and low concentration of fixed charges. The latter regions exert little Donnan exclusion and thus can sorb electrolytes even from very dilute solutions. Such a situation could exist in the matrix of cartilage, as has in fact been suggested by Gersh and Catchpole.

It was not possible to calculate accurately the value of the diffusion coefficient of Na+ in cartilage immersed in a dilute solution because the film resistance is no

longer negligible in this case. However, using equation 6 and substituting as before, the value $\delta = 3 \times 10^{-3}$ cm, the diffusion coefficient for Na$^+$ is 4.5×10^{-6} cm^2 per sec, i.e. not very different from that obtained for cartilage in Ringer's solution.

Diffusion Coefficients of K$^+$, SO$_4^=$, and Glucose. Some preliminary values of the permeability and the diffusion coefficients for K$^+$, SO$_4^=$, and glucose are listed in Table III.

Because the distribution coefficients for K$^+$ or SO$_4$- between Ringer's solution and cartilage are not known, it was not possible to calculate the values of $\bar{D}$ from the values of $\bar{P}$ for these ions. It is hoped to determine the distribution coefficients in a future investigation.

The values of $\bar{D}_{K^+}$ and $\bar{D}_{SO_4}{}^-$ determined by the direct method are close to the value of $\bar{D}_{Cl^-}$, which is again consistent with the behavior of these ions in aqueous solution.

In the case of glucose, the diffusion coefficients determined by the direct and the indirect method agree, which indicates that there is no specific interaction between glucose and cartilage. The value of $\bar{D}_{glucose}$ is approximately one-third its value in water.

The ratio of the diffusion coefficient in cartilage to the diffusion coefficient in aqueous solution is lower for glucose than for Na$^+$, Cl$^-$, K$^+$, and SO$_4^=$. It is possible that this is due to the glucose molecule being relatively larger in size, and hence relatively more subject to frictional drag in the narrow pores of cartilage.

A preliminary study has also been made of the variation of the diffusion coefficients with fixed charge density. The results so far obtained indicate a slight decrease in the diffusion coefficients with increase in fixed charge density. The corresponding decrease in the diffusion coefficients with depth from the articular surface has also been observed.

Where thick slices were used, permeability coefficients and fixed charge density were measured in two directions, viz., articular surface → deep zone and deep zone → articular surface. No significant difference was observed and it was concluded that in both cases, average values were obtained.

TABLE III

DIFFUSION COEFFICIENTS OF K$^+$ + SO$_4^-$ AND GLUCOSE

Age	K$^+$			SO$_4^-$			Glucose		
	$\bar{P} \times 10^6$	$\bar{D} \times 10^6$ indirect	$\bar{D} \times 10^6$ direct	$\bar{P} \times 10$	$\bar{D} \times 10^6$ indirect	$\bar{D} \times 10^6$ direct	$\bar{P} \times 10^6$	$\bar{D} \times 10^6$ indirect	$\bar{D} \times 10^6$ direct
yr	*cm²sec⁻¹*			*cm²sec⁻¹*			*cm²sec⁻¹*		
55	11.5		7.4						
58				1.12		6.8			
19							2.2	2.2	2.3
24							1.4	1.4	1.45
52							2.0	2.0	—

Some typical results of conductivity measurements are shown in Table IV. These measurements were carried out on cartilage equilibrated in Ringer's solution, in Ringer's solution diluted 1:10, and in distilled water. More values of $\bar{\kappa}_{0.15\,N}$ can be found in the Appendix.

TABLE IV
ELECTRICAL CONDUCTIVITY OF ARTICULAR CARTILAGE

Age	Distance from articular surface as a fraction of total depth	$\bar{C}_x$	$\bar{\kappa}_{0.15\,N} \times 10^3$	$\bar{\kappa}_{0.15\,N} \times 10^3$	$\bar{\kappa}_{H_2O} \times 10^3$	$\bar{\kappa}_{dilute} \times 10^3$ computed from the value of $\bar{D}$
yr		meq/cm²	mho/cm	mho/cm	mho/cm	mho/cm
30	0.175	0.13	8.1	2.7	—	
	0.375	0.16	7.5	3.0	2.7	3.0
	0.625	0.18	7.0	3.1	2.9	(mean value)
	0.875	0.21	6.8	3.6	2.3	
55	0.375	0.13	7.5	2.6	2.4	
	0.625	0.14	7.5	2.7	2.4	2.8
	0.875	0.16	7.05	2.9	2.5	(mean value)
38	0.175	0.19	7.8	3.7	—	
	0.375	0.20	7.7	3.9	3.5	
	0.625	0.20	7.6	3.9	3.9	
	0.875	0.25	7.0	4.1	3.8	
31	0.15	0.15	8.0	—	—	
	0.300	0.115	7.5	3.5	—	
	0.500	0.204	6.9	4.3	3.9	
	0.700	0.25	6.6	4.2	3.8	
41	0.375	0.18	8.3	3.8	3.6	
	0.625	0.19	7.7	3.7	3.3	
	0.875	0.24	7.55	4.3	3.7	

The following points emerge from an examination of the results.

(a) The specific conductivity of cartilage equilibrated in Ringer's solution, $\bar{\kappa}_{0.15}$ lies in the range 6.5×10^{-3}–8.5×10^{-3} mho per cm.

There is a general trend for $\bar{\kappa}_{0.15\,N}$ to decrease slightly with increase in $\bar{C}_x$. In particular, for each individual, the results indicate a small but consistent decrease in $\bar{\kappa}_{0.15\,N}$ with distance from the articular surface.

It will be of interest to examine how these results can be related to theory. On the basis of equation 12 ($\bar{\kappa}_{0.15\,N} = F(\bar{u}_{Na^+}\bar{C}_{Na^+} + \bar{u}_{Cl^-}\bar{C}_{Cl^-})$), it can be seen that $\bar{\kappa}_{0.15\,N}$ is a function of the concentrations of Na^+ and Cl^- in cartilage and of the mobilities of these ions. It has been shown in the present work that, as in water,

$\bar{D}_{Cl^-}/\bar{D}_{Na^+} = 1.5$; it may hence be inferred that $\bar{u}_{Cl^-}/\bar{u}_{Na^+} = 1.5$. Thus equation 12 can be rewritten as

$$\bar{\kappa}_{0.15\ N} = (F\bar{u}_{Na^+})(\bar{C}_{Na^+} + 1.5\ \bar{C}_{Cl^-}). \qquad (18)$$

The dependence of the terms ($\bar{C}_{Na^+} + 1.5\ \bar{C}_{Cl^-}$ and $\bar{u}_{Na^+}$, respectively, on $\bar{C}_x$ will be examined.

According to the Donnan equilibrium equation, an increase in $\bar{C}_x$ results in an increase in $\bar{C}_{Na^+}$ and a simultaneous decrease in $\bar{C}_{Cl^-}$. Hence the over-all effect of the variation of $\bar{C}_x$ on the term ($\bar{C}_{Na^+} + 1.5\ \bar{C}_{Cl^-}$) is small. For example, for an increase in $\bar{C}_x$ from 0.1–0.25 meq per cm^3 (a 150% increase) the value of ($\bar{C}_{Na^+} + 1.5$ $\bar{C}_{Cl^-}$) increases only from 0.3875–0.425 meq per cm^3 (a 10% increase).

The term $\bar{u}_{Na^+}$, like the diffusivity, depends on the effective porosity of cartilage, i.e. on its free water content, and will thus be expected to show a decrease with increase in $\bar{C}_x$.

It follows that the experimentally observed decrease in $\bar{\kappa}_{0.15\ N}$ with increase in $\bar{C}_x$ can be attributed directly to the corresponding reduction in the ionic mobility $\bar{u}_{Na^+}$.

(b) The value of the specific conductivity of cartilage equilibratea in 1:10 Ringer's solution or in water, generally shows a very slight increase with depth.

According to equation 13, the specific conductivity of cartilage equilibrated in a dilute solute (such that the presence of the co-ion can be neglected) is given by $\bar{\kappa}_{dilute} = \bar{u}_{Na^+}\bar{C}_x$, i.e., $\bar{\kappa}_{dilute}$ should be proportional to the product of the fixed charge density and the mobility of Na$^+$. Since $\bar{C}_x$ increases from the articular surface to the deep zone, while $\bar{u}$ slightly decreases, the two effects should compensate and not much variation in $\bar{\kappa}_{0.015\ N}$ or $\bar{\kappa}_{H_2O}$ should result. The slight increase in $\bar{\kappa}_{0.015\ N}$ and $\bar{\kappa}_{H_2O}$ actually observed is consistent with the author's finding that in cartilage the increase in $\bar{C}_x$ with depth is more pronounced than the corresponding decrease in $\bar{D}$.

(c) A comparison of the values of $\bar{\kappa}_{0.015\ N}$ and $\bar{\kappa}_{H_2O}$ with each other shows that the latter can be up to 10% lower than the former. If the Donnan equilibrium equations were perfectly obeyed, the difference between the two sets of values should never exceed 2.5%. The fact that the discrepancy is higher points to a small additional non-Donnan type sorption in dilute solution and is consistent with the conclusions drawn from the results of the diffusion experiments.

(d) The values of the specific conductivity calculated by means of the Planck-Einstein relation (equation 13) and equation 18 agree within about 10% with the experimentally determined values of $\bar{\kappa}_{H_2O}$.

Permeability to Fluid Flow

Permeability coefficients were determined for cartilage specimens of different individuals and corresponding to different depths from the articular surface (Appendix). Fig. 7 shows the variation of K with $\bar{C}_x$. The results, which are pooled in this figure, show a strong over-all correlation between permeability and fixed charge
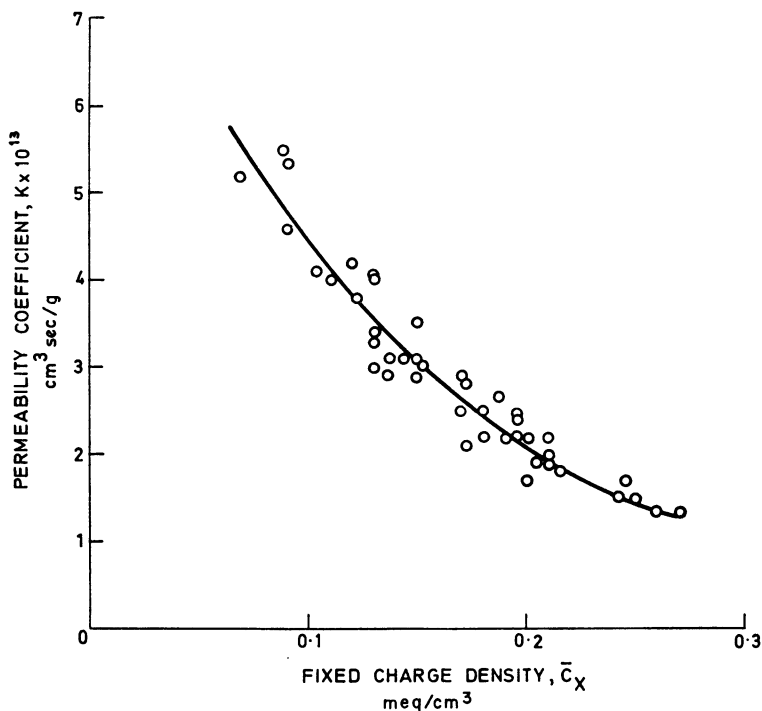
FIGURE 7   Variation of permeability coefficient with fixed charge density.

density. As fixed charge density increases, permeability to flow decreases. The scatter is most probably due to the fact that, apart from being dependent on fixed charge density, the permeability coefficient depends to some extent on other factors which may vary from individual to individual. This is shown more clearly in Fig. 8, where permeability vs. fixed charge density is plotted for separate individuals.

The decrease in the permeability coefficient with increase in fixed charge density can be explained as follows. The higher the fixed charge density, the lower the proportion of free water in the matrix of cartilage and the lower the effective porosity. Since in a porous medium permeability increases sharply with increase in porosity, it must decrease correspondingly with decrease in fixed charge density.

It is interesting to observe that diffusion appears to be much less sensitive than permeability to variations in fixed charge density. This is in accordance with the fact that diffusion is much less sensitive to changes in porosity than is fluid flow.

A study on the variation of the permeability coefficient with age will be reported elsewhere.

As for the variation of K with distance from the articular surface, some typical curves are shown in Fig. 9. Permeability decreases significantly as distance from the articular surface increases. In view of what has been said above on the relation between K and $\bar{C}_x$, this result is completely consistent with the observed increase in $\bar{C}_x$ with depth (see Fig. 6).
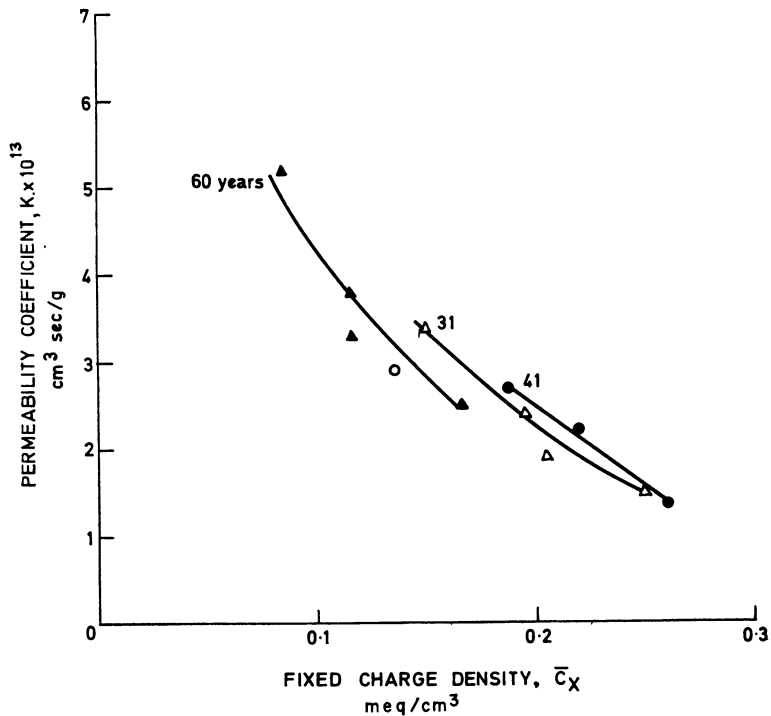
FIGURE 8 Typical curves of permeability coefficient vs. fixed charge density for different individuals.
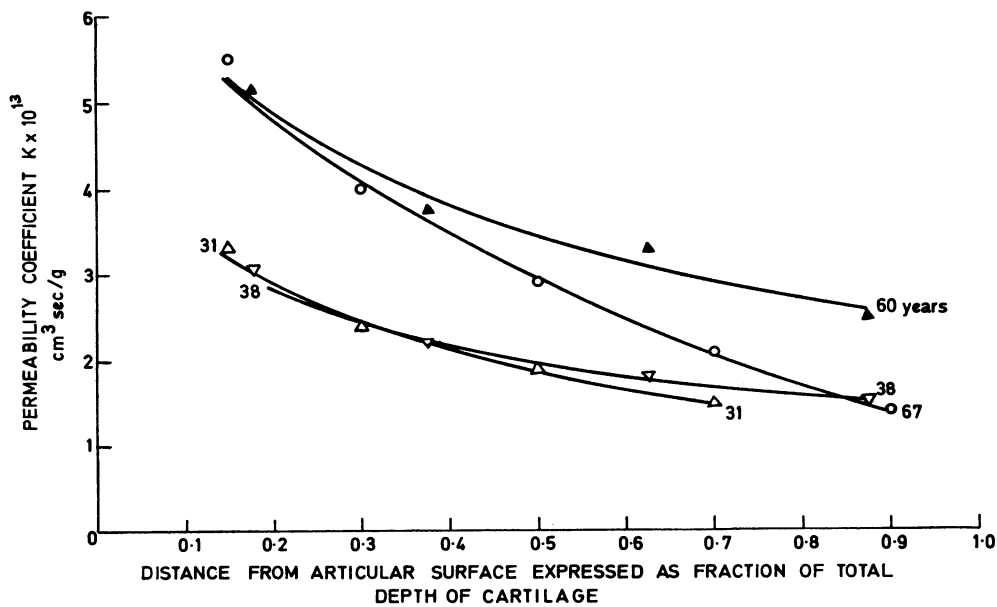

FIGURE 9 Variation of permeability coefficient with distance from articular surface.

Streaming Potential

The values of the streaming potential for cartilage equilibrated in Ringer's solution lie generally in the range 4.8–6.0 $\times$ 10^{-4} v/atm (Appendix). For any given specimen the variation of streaming potential with depth is very small.

As can be seen from equation 17, $\triangle\psi \propto K\bar{C}_x/\bar{\kappa}$. Since K decreases as $\bar{C}_x$ increases, the numerator in the above expression would not be expected to vary much. The conductivity $\bar{\kappa}$, itself, does not show much variation. It is thus not surprising to find little variation in the values of $\triangle\psi$.

CONCLUSIONS

Although some deviations from the Donnan equilibrium were observed in dilute solution, with respect to most of its properties, cartilage may be described in quantitative terms by means of ion exchange theory. Hence fixed charge density is the fundamental property, a knowledge of which makes it possible to predict the concentration of ionic species in cartilage. When this is known, such basic properties of ions in cartilage as diffusivity and ionic mobility can be calculated from the steady-state diffusion or conductivity measurements. Permeability to fluid flow and streaming potential can also be correlated directly with fixed charge density.

In particular, the following observations have been made in the course of the experimental study.

(*a*) Fixed charge density in cartilage increases considerably with distance from the articular surface.

(*b*) The values of the diffusion coefficients of Na$^+$, Cl$^-$, K$^+$, and SO$_4^=$ in cartilage have been found to be approximately 40 % of their values in aqueous solution, while that of glucose is about 30 %. The ratios of the ionic diffusivities of Cl$^-$, K$^+$, Na$^+$, and SO$_4^=$ are the same as in water. Hence it is reasonable to suppose that the diffusivities of other ions in cartilage can be deduced on this basis. However, it must be borne in mind that their fluxes may be different because of differences in the distribution coefficients. This aspect is being investigated.

Preliminary results indicate a small decrease in the values of the diffusion coefficients with depth from the articular surface, i.e. with increase in fixed charge density.

(*c*) The specific conductivity of cartilage equilibrated in Ringer's solution lies in the range 6–8 $\times$ 10^{-3} mho per cm and decreases very slightly with distance from the articular surface. The Planck-Einstein equation between the diffusion coefficient and the specific conductivity holds very well in cartilage.

(*d*) The permeability to fluid flow has a strong inverse correlation with fixed charge density and increasing as the latter decreases.

(*e*) The streaming potential of cartilage for fluid flowing under a differential pressure of 1 atm lies in the range 4.8–6.0 $\times$ 10^{-4} v and shows very little variation with depth.

A direct study of the equilibrium concentrations of various solutes in cartilage is at present in progress.

It is hoped that the approach to the study of physicochemical properties of articular cartilage proposed in this paper will in due course lead to a better understanding of its physiology, in particular its nutrition and its function in joint lubrication.

LIST OF SYMBOLS

A cross-sectional area of membrane
a activity coefficient
C molar concentration
C_x concentration of fixed negatively-charged groups in cartilage
d thickness of cartilage slice
D diffusion coefficient
e porosity
F Faraday's constant
K permeability to fluid flow in cartilage
P permeability to solutes
$\triangle p$ pressure drop
Q volumetric flow rate
R universal gas constant
T absolute temperature
u electrochemical mobility
u_0 mobility parameter of pore liquid
V approach velocity
y distance from articular surface expressed as fraction of total depth of cartilage
z electrochemical valence
δ thickness of stagnant liquid films at the cartilage-liquid interface
$\bar{\kappa}$ specific electric conductivity of cartilage
$\bar{\kappa}_0$ convection conductivity in cartilage
$\triangle \psi$ streaming potential in cartilage
Subscripts Na^+, Cl^-, i, etc. refer to the species named.
Subscripts 0.15 N and 0.015 N refer to the approximate concentrations of the NaCl buffer solution in which cartilage is equilibrated.
Superscripts $+$ and $-$ are used for the cation and anion, respectively.
Symbols with overbars are used for the interior of the ion exchange material.

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Age	Distance from articular surface as a fraction of total depth	$K \times 10^{13}$	$\triangle\psi/\triangle p \times 10^4$	$\bar{\kappa} \times 10^3$	$\bar{C}_x$
yrs		$cm^2\ sec/g$	v/atm	mho/cm	meq/cm^3
30	0.175	3.4	5.3	8.1	0.13
	0.375	2.9	6.3	7.5	0.17
	0.625	2.5	6.2	7.0	0.18
	0.875	1.9	5.6	6.8	0.21
31	0.15	3.4	6.2	8.0	0.15
	0.3	2.4	6.0	7.5	0.195
	0.5	1.9	5.4	6.9	0.204
	0.7	1.5	5.4	6.6	0.250
38	0.175	3.1	5.5	7.8	0.143
	0.375	2.2	5.4	7.7	0.195
	0.625	1.8	4.9	7.6	0.215
	0.875	1.5	5.0	7.0	0.242
41	0.35	2.66	6.5	8.3	0.187
	0.59	2.2	6.5	7.6	0.21
	0.825	1.35	5.0	7.5	0.26
46	0.15	3.1	5.3	7.7	0.137
	0.3	2.8	6.0	7.75	0.172
	0.5	2.2	5.8	7.65	0.200
	0.7	1.7	5.7	7.05	0.245
	0.9	1.35	5.5	6.5	0.27
48	0.175	4.6	4.5	8.9	0.09
	0.375	4.1	4.7	8.9	0.103
	0.625	2.9	5.0	8.6	0.153
59	0.33	5.35	5.5	8.5	0.09
	0.50	4.0	5.4	8.0	0.11
	0.90	3.0	5.0	7.5	0.13
60	0.175	5.2	4.8	8.8	0.084
	0.375	3.8	4.8	8.6	0.113
	0.625	3.3	5.0	8.4	0.13
	0.875	2.5	5.0	8.1	0.17
63	0.175	4.2	6.2	7.8	0.12
	0.375	4.1	6.7	7.6	0.13
	0.625	3.0	6.5	7.0	0.155
	0.875	2.2	5.6	6.8	0.180
67	0.15	5.5	5.5	8.5	0.088
	0.3	4.0	5.9	8.4	0.13
	0.5	2.9	4.9	7.7	0.135
	0.7	2.1	4.8	7.4	0.175
	0.9	1.7	4.8	7.1	0.200
78	0.28	3.1	5.5	8.1	0.15
	0.475	2.4	5.9	7.8	0.195
	0.67	2.35	5.8	7.8	0.193
	0.86	2.2	5.4	7.4	0.190
	0.95	2.0	5.5	7.2	0.21