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ELECTROKINETIC STUDIES ON CHOLESTEROL–CARBOHYDRATE SYSTEMS

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ABSTRACT

The hydrodynamic and electro-osmotic transport of water and aqueous solutions of D-fructose, D-glucose, sucrose, and urea through cholesterol-coated membrane has been investigated. The results have been examined from the viewpoint of non-equilibrium thermodynamics. Zeta potentials have been estimated in order to characterize the membrane–permeant interface.

INTRODUCTION

Biological membranes consist for the most part of lipids and proteins[1], the lipids present being largely cholesterol and phospholipids[2]. In addition to the lipids and proteins, some carbohydrate is generally found in biomembranes[3]. The carbohydrate is attached to lipoproteins, and is located only at the outer surfaces of the membrane which, in turn, act as binding sites on the membrane surface for various chemical substances.

In view of the complexities of real biological membranes, model membranes artificially produced from lipid or lipoprotein components have been studied extensively in order to permit an understanding of the transport behavior of natural membranes. Attempts have been made by various workers to prepare artificial analogs of the natural membranes[4,5], and it has been found that the model membranes possess certain dimensional, electrical permeability, and excitability characteristics that closely resemble those of biological membranes[5]. The lipids present are mainly cholesterol, which creates some circulatory problems, such as hardening of the arteries and high blood-pressure[6]. For deposition in arteries and as stones in the gall bladder, the first stage is coagulation, which is influenced by the properties of the cholesterol–solution interface.

Hence, a physicochemical study of the interface in different types of "bathing" solutions is needed for an understanding of the precipitation of cholesterol. We now describe electrokinetic studies that have been conducted with a cholesterol membrane, using water and aqueous solutions of D-fructose, D-glucose, sucrose, and urea as "bathing" solutions. The membrane used in the present work was not a model mem-

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brane, but the results obtained with it may be helpful in understanding the electrical nature of the membrane–permeant interface.

EXPERIMENTAL

Cholesterol, obtained from E. Merck AG (Darmstadt, Germany), was used as such. D-Fructose, D-glucose, sucrose, and urea (AR BDH), and double-distilled water (having a specific conductance of the order of 10^{-6} mho.cm^{-1}) were used in these studies. Cholesterol was dissolved in acetone, the solution was coated over a Pyrex-sinter membrane (having G_4 porosity), by evaporation of the acetone, and the membrane was thoroughly cleaned with double-distilled water.

Hydrodynamic and electro-osmotic flux measurements were made by using an experimental technique previously described[7,8]. A potential difference was applied by use of an electronically operated, stabilized power-supply, using coiled, platinum electrodes just touching the surface of the membrane. The volumetric flux induced by the pressure difference and the potential difference was measured by the described procedure[8]. The conductance of the membrane equilibrated with the permeant was measured with an A.C. conductivity bridge (Toshniwal, India) at 50 cycles.s^{-1}. All of the measurements were made on systems placed in an air thermostat maintained at 35°.

RESULTS AND DISCUSSION

The volumetric flux, J_v, through a membrane under the simultaneous action

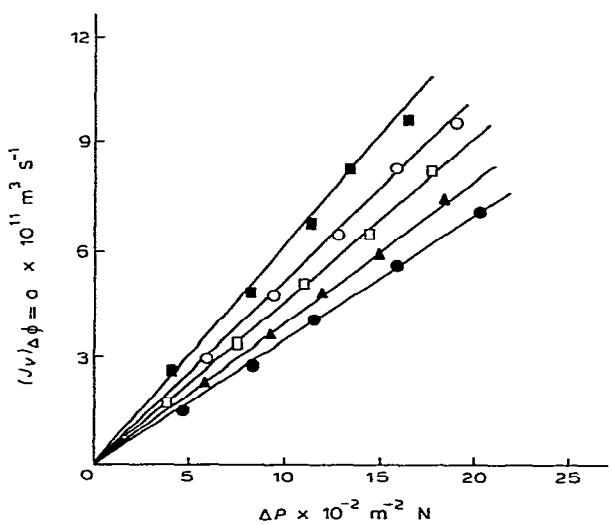

Fig. 1. Dependence of hydrodynamic flux, $(J_v)_{\Delta\phi=0}$, on the applied pressure-difference, ΔP, for cholesterol–carbohydrate systems. [Key: ○ water, □ D-fructose, ▲ D-glucose, ● sucrose, and ■ urea.]

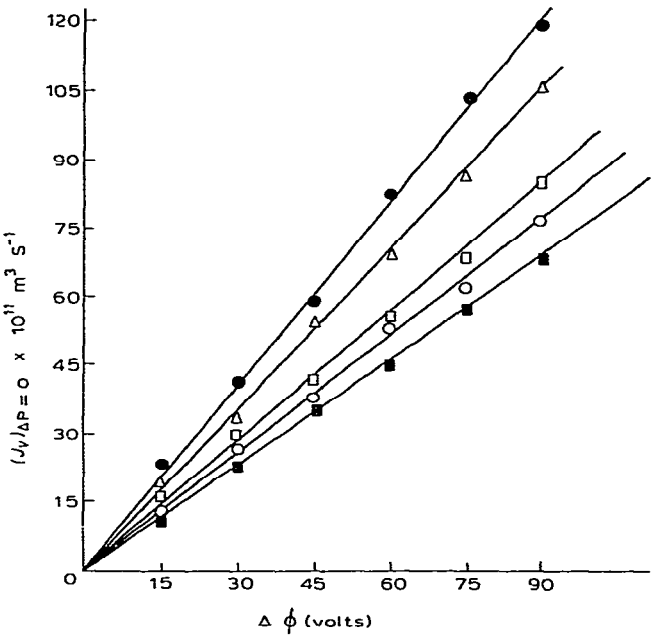

Fig. 2. Dependence of electro-osmotic flux, $(J_v)_{\Delta P=0}$, on the applied potential-difference, $\Delta\phi$, for cholesterol–carbohydrate systems. [Key: ○ water, □ D-fructose, △ D-glucose, ● sucrose, and ■ urea.]

TABLE I

MEMBRANE PARAMETERS

System	$(L_{11}/T) \times 10^{14}$ $(m^5N^{-1}s^{-1})$	$(L_{22}/T) \times 10^{6}$ (ohm^{-1})	$r \times 10^{7}$ (m)	$n \times 10^{-6}$
Cholesterol–water	5.45	2.90	5.65	4.6
Cholesterol–D-fructose	5.00	2.65	5.88	3.6
Cholesterol–D-glucose	4.28	3.75	4.78	7.1
Cholesterol–sucrose	3.75	3.50	4.68	6.7
Cholesterol–urea	6.66	4.50	4.86	10.3

TABLE II

ELECTRO-OSMOTIC COEFFICIENT AND ESTIMATED ZETA POTENTIALS

System	$(L_{12}/T) \times 10^{12}$ $(m^3s^{-1}V^{-1})$	$\xi \times 10^{3}$ (V)
Cholesterol–water	8.83	9.37
Cholesterol–D-fructose	10.00	12.55
Cholesterol–D-glucose	11.84	11.47
Cholesterol–sucrose	14.16	10.22
Cholesterol–urea	7.75	4.99

of a pressure difference, ΔP, and a potential difference, $\Delta\phi$, is, on the basis of non-equilibrium thermodynamics, expressed by a linear, phenomenological equation[9] as

$$J_v = L_{11}(\Delta P/T) + L_{12}(\Delta\phi/T), \tag{1}$$

where L_{11} and L_{12} are phenomenological coefficients, ΔP and $\Delta\phi$ are the pressure difference and the potential difference, respectively, and T is the absolute temperature. Eq. *1* predicts a linear dependence between (*i*) hydrodynamic flux, $(J_v)_{\Delta\phi=0}$ and ΔP, and (*ii*) electro-osmotic flux, $(J_v)_{\Delta P=0}$ and $\Delta\phi$. The plots of $(J_v)_{\Delta\phi=0}$ *versus* ΔP and $(J_v)_{\Delta P=0}$ *versus* $\Delta\phi$ are linear, as shown in Figs. 1 and 2, confirming the validity of Eq. *1*. The values of the phenomenological coefficients L_{11}/T and L_{12}/T obtained from the slopes of Figs. 1 and 2 are included in Tables I and II, respectively.

Characterization of the membrane has been achieved in terms of the average, pore radius and the number of capillary channels of the membrane. The pore radius (r) has been calculated by using the relation[10,11]

$$r = [(8\eta(L_{11}/T)k/(L_{22}/T)]^{\frac{1}{2}}. \tag{2}$$

Furthermore, the number (n) of capillary channels could be estimated by using the relation[10,11]

$$n = 8\eta l(L_{11}/T)/(\pi r^4). \tag{3}$$

The calculated values of the average, pore radius, r, and number of capillary channels, n, are included in Table I.

An examination of Table I reveals that the magnitude of L_{11}/T decreases in the following sequence.

$$(L_{11}/T)_{\text{urea}} > (L_{11}/T)_{H_2O} > (L_{11}/T)_{\text{D-Fru}} > (L_{11}/T)_{\text{D-Glc}} > (L_{11}/T)_{\text{Suc}}$$

This trend may be explained on the basis of the structure-making properties of D-fructose, D-glucose, and sucrose, as well as the structure-breaking property of urea. The results confirm the findings previously reported[11]. It may be inferred that the structure-making tendency decreases in the order sucrose > D-glucose > D-fructose.

Electro-osmotic flux occurs towards the negative electrode for all of the cases studied. All of the values of the zeta potentials are negative; this may be explained on the basis of an electrical double-layer formed at the interface cholesterol–aqueous solution of carbohydrate, due to adsorption of ions, particularly of anions, to cholesterol[12]. From the schematic representation of an electrical double-layer shown in Fig. 3, it is evident that the diffuse part of the double layer contains an excess of positive ions which move towards the negative electrode when the potential difference, $\Delta\phi$, is applied across the membrane.

Electro-osmosis occurs in porous membranes because of the formation of an electrical double-layer endowed with an electrical potential, ζ. The rate of volumetric flux due to the application of electrical potential-difference alone is given[13] by Eq. 4.

$$(J_v)_{\Delta P=0} = nr^2\zeta\varepsilon.\Delta\phi/4\eta l, \tag{4}$$

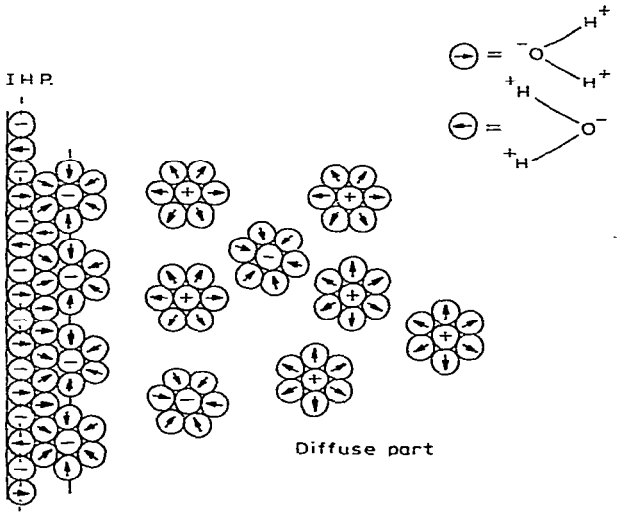

Fig. 3. Structure of the electrical double-layer for cholesterol–carbohydrate systems.

where ε denotes the dielectric constant of the medium. Eq. *4* can be compared with Eq. *1*, within the linear range, so that

$$L_{12}/T = nr^2\zeta\varepsilon/4\eta l. \tag{5}$$

ζ can be estimated by combining the membrane-conductance data and the electro-osmotic-flux data. The conductance of a membrane equilibrated with the permeant may be expressed by Eq. *6*.

$$L_{22}/T = n\pi r^2 k/l \tag{6}$$

Using Eqs. *6* and *5*, it is found that

$$\zeta = 4\pi\eta k/(\varepsilon L_{22}/T).(L_{12}/T) \qquad \text{e.s.u.,} \tag{7}$$

or

$$\zeta = [4\pi\eta k/(L_{22}/T)\varepsilon].(L_{12}/T) = 90 \text{ kV}. \tag{8}$$

The calculated values of zeta potentials using Eq. *8* are given in Table II. It is evident from Table II that the value of the zeta potential is greater than that of water for aqueous solutions of D-fructose, D-glucose, and sucrose, and lower than that of water in the case of aqueous solutions of urea. This behavior can be explained on the basis of an altered structure of water[14], which is likely to affect the electro-osmotic behavior marginally.

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