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Characterization of non-linear behaviour of an animal membrane using electro-kinetic studies

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Electro-kinetic studies of water, aqueous solutions of urea, thiourea, glucose, creatinine and urine solution across urinary bladder membranes of goat have been used to explain non-linear behaviour. It has been found that fluxes are non-linearly related with forces. The structure of the membrane is a decisive factor when the range of driving forces is not too high. Stability of the steady state has also been examined and has been found to remain quite stable even in non-linear regions. The results have been examined using the methodology of non-equilibrium thermodynamics.

1. Introduction

The non-linear behaviour of biological systems is quite familiar [1–3]. Experimental studies with membranes analogous to biological membranes [4–10] using electrokinetics show a considerable degree of non-linearity in fluxes and forces. In this paper, by studying electro-kinetics across urinary bladder membranes, the factors responsible for non-linearity have been examined and it has been found that the structural factor of the membrane is primarily responsible for the non-linearity, provided the forces (ΔP and $\Delta\psi$) operating in the system are not too high. The polarizability of the membrane permeant system assumes high values for urinary bladder membranes while for pyrex sinter [11] (G_4) and ion exchange [5] (zeokarb 225 Na^+ form) its values are quite low although they exhibit non-linear behaviour. The concentration gradient inside the membrane matrix leading to acceleration or retardation is also a factor worth considering. Different factors, individually or collectively, may be responsible but it seems that in biological membranes the various factors are collectively responsible for non-linearity.

The stability of the steady state has been investigated and has been found to remain quite unperturbed even in non-linear regions. The results have been examined using the methodology of non-equilibrium thermodynamics.

2. Theoretical

Using the methodology of non-equilibrium thermodynamics, volume flow (J_v) and current flow across a membrane may be expressed as [12,13]

$$\begin{aligned} J_v = {} & L_{11}(\Delta P) + L_{12}(\Delta\psi) + \tfrac{1}{2}L_{111}(\Delta P)^2 \\ & + L_{112}\Delta P\Delta\psi \\ & + \tfrac{1}{2}L_{122}(\Delta\psi)^2 + \tfrac{1}{6}L_{111}(\Delta P)^3 \\ & + \tfrac{1}{2}L_{1112}(\Delta P)^2(\Delta\psi) \\ & + \tfrac{1}{2}L_{1122}(\Delta\psi)^2\Delta P + \tfrac{1}{6}L_{1222}(\Delta\psi)^3 + \dots \qquad (1) \end{aligned}$$

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$$I = L_{21}\Delta P + L_{22}\Delta\psi + \tfrac{1}{2}L_{211}(\Delta P)^2 + L_{212}\Delta P\Delta\psi + \tfrac{1}{2}L_{222}(\Delta\psi)^2 + \tfrac{1}{6}L_{2111}(\Delta P)^3 + \tfrac{1}{2}L_{2112}(\Delta P)^2(\Delta\psi) + \tfrac{1}{2}L_{2122}(\Delta P)(\Delta\psi)^2 + \tfrac{1}{6}L_{2222}(\Delta\psi)^3 + \ldots \tag{2}$$

where L_{ij}, L_{ijk} and L_{ijkl} $(i, j, k, l = 1, 2)$ are the phenomenological coefficients. The pressure difference (ΔP) and electrical potential gradient $(\Delta\psi)$ are the forces operating in the system.

In living systems, the steady states are found to be always stable, since they possess the special control system of homeostasis which corrects minor deviations from the steady state. The stability criterion for dissipative processes makes use of the excess entropy balance equation and the stability condition is determined by the sign of excess entropy production.

For a non-linear phenomenological equation, the steady state will be stable when [14,15]

$$\sum \delta J_v \delta X_i \geqslant 0 \tag{3}$$

where δJ_v and ΔX_i represent the deviations of the thermodynamic flux J_v and force X_i, respectively, from the non-equilibrium steady state. Experimentally, it is found that steady states are stable. In order to examine the stability criterion, let us perturb the steady state under consideration by perturbing the force X_1 and keeping X_2 fixed so that δX_1 is finite and $\delta X_2 = 0$. The corresponding perturbations in the fluxes J_v and I are given by

$$\delta J_v = \left[L_{11} + L_{112}X_2 + L_{1112}X_1X_2 + \tfrac{1}{2}L_{1122}X_2^2 + L_{111}X_1 + \tfrac{1}{2}L_{1111}X_1^2 + \ldots\right]\delta X_1 \tag{4}$$

$$\delta I = \left[L_{21} + L_{212}X_2 + L_{2112}X_1X_2 + \tfrac{1}{2}L_{2122}X_2^2 + L_{211}X_1 + \tfrac{1}{2}L_{2111}X_1^2\right]\delta X_1 \tag{5}$$

where $X_1 = \Delta P$ and $X_2 = \Delta\psi$.

According to the stability criterion (eq. 3) we have

$$\delta J_v \delta X_1 + \delta I \delta X_2 \geqslant 0 \tag{6}$$

Since $\Delta X_2 = 0$, the above stability condition becomes

$$\delta J_v \delta X_1 \geqslant 0 \tag{7}$$

Substituting these values into eq. 7 we obtain the following condition

$$\left[L_{11} + L_{112}X_2 + L_{1112}X_1X_2 + \tfrac{1}{2}L_{1112}X_2^2\right] = A > 0 \tag{8}$$

In the case of Poiseuille's flow where the range of driving force (X_1) is not too high, the coefficients L_{111} and L_{1111} automatically vanish. A positive value of A suggests that the steady state is stable.

3. Results and discussion

The membrane chosen for characterization of non-linear behaviour is the urinary bladder of goat which is known to respond to some of the living characteristics [8,9] so far as passive behaviour is concerned. Studies of the electrokinetics across the membrane will be used to analyse the non-linear behaviour. In order to specify the role of interfaces, electrokinetics across pyrex sinter (G_4) and ion-exchange membrane (Zeokarb 225 Na^+ form) have been used as supporting data.

Urinary transport may be defined [3] as the process of development, sustenance and finally release of pressure to the basal tonic pressure of the bladder. Development of pressure across the bladder gives rise to a streaming potential which in turn produces streaming current. As the phenomenon itself [3] is non-linear, electrokinetic studies acquire great significance. The volume excreted [16] is also non-linearly related with pressure. The results of studies [8–10] on electrokinetics across urinary bladder may be summarised as follows:

(i) J_v is non-linearly related with pressure and electrical potential gradients.
(ii) Streaming potential and current (streaming) are also non-linear
(iii) In the case of urine as permeant, volume flow is non-linearly related with pressure and electrical potential gradient even when their values are quite low.

Non-linearity can arise on account of the following factors [17]

(i) Appearance of velocity gradients in the systems as can occur during coupling of mass flux and heat flux [18].

(ii) Appearance of higher-order terms in the expansion of forces as in chemical reactions [19].
(iii) Dependence of phenomenological coefficients on intensive properties [20] as in coupling of mass flux and heat flux [21].
(iv) Dependence of phenomenological coefficients on the characteristics of the systems which depend on forces such as in electro-osmosis [22].

As far as the present investigation is concerned, factors i–iii are out of the question. It is of interest to ascertain how factor iv can explain non-linearity.

There are three types of non-linear situations [1]:
(i) Assumption of local equilibrium may be invalid, i.e., Onsager's relation is not fulfilled.
(ii) Local equilibrium may be preserved but the properties of the systems change continuously with increasing distance from equilibrium. In such situations, the system retains some of the properties of linear systems.
(iii) There appears a dynamic order, new types of organization of the substance in space and time which are displaced only by open, far-from-equilibrium systems which are called dissipative structures.

In the case of urinary bladder membranes, it has been found [9] that first-order symmetry ($L_{12} = L_{21}$) holds good while higher-order phenomenological coefficients are not symmetrically related. Thus, situation ii seems to fit our data.

Fluxes (volume flow J_v and current flow I) in the non-linear range can be written as a function of forces X_1, X_2 along with the structural factor of the membrane

$$J_i = f(X_1,\ X_2 \ldots G)$$

where

$$X_1 = \Delta P,\ X_2 = \Delta\psi \tag{9}$$

In terms of phenomenological coefficients, fluxes (J_i) may be expressed as

$$J_i = \sum_j L_{ij} X_j + \tfrac{1}{2} \sum_j \sum_k L_{ijk} X_j X_k + \ldots \tag{10}$$

where L_{ij}, L_{ijk} are phenomenological coefficients.

Let us now discuss individual contribution towards nonlinearity.

3.1. Dependence of J_v vs. ΔP

In situations where Poiseuille's law holds good, chances of non-linearity do not appear to be distinct. This is evident from studies carried out across membrane [11,23,24]. In other words, the appearance of higher-order phenomenological coefficients such as L_{111}, L_{1111}, etc., in the flux equation are ruled out.

When the flow converges, diverges or follows a curved path, a linear dependence of volume flow on pressure difference is ruled out. The urinary bladder membrane is an example of a continuous membrane phase and the concept of pores as such may be misleading. Pores are nothing but a pathway of permeation. These pathways of permeation may lead to either viscous flow, diffusive flow or a mixture of both. The permeability behaviour of aqueous solutions of non-electrolytes and electrolytes shows [25] that flow is neither viscous nor diffusive but is of the slip flow type. A creeping tendency of the permeants provides an indication of the molecular size of the pores. Irmay [26] developed an equation of motion assigning importance to inertial terms and it was derived by averaging the local hydrodynamic equation of motion over a volume of the interior of the membrane sufficiently large to be considered as homogeneous and isotropic but small enough that the overall properties do not vary greatly within it. Mickulecky and Caplan [27] showed that average viscous contribution to the dissipation function were negligible and it has been shown [28] that neglecting the viscous contribution leads to hydrodynamic creeping motion.

In situations where the permeant experiences acceleration or retardation inside the membrane, the kinetic energy [13] of the centre of mass (α_1), may be expressed as [10]

$$\alpha_1 = \frac{L_{111} A_r^2}{\rho L_{11}^2} \tag{11}$$

where ρ is the density, A_r the effective cross-sectional area of the membrane and α_1 a constant whose value depends upon the structure of the membrane. For a well-developed (Poiseuille's) flow in a uniform capillary, the kinetic energy term is

Table 1

Values of α_1, α_2 and A for different permeants across urinary bladder membranes

Serial no.	Permeant	α_1 ($m^{-1}\ N\ s^2\ kg^{-1}$)	α_2 ($m^{-3}\ V^{-2}\ J$)	A ($m^5\ s^{-1}\ N^{-1}$)
(1)	Creatinone (0.01 M)	-0.16×10^{10}	26.18	18.9×10^{-13}
(2)	Thiourea (0.01 M)	-0.13×10^{10}	17.13	18.5×10^{-13}
(3)	Glucose (0.01 M)	-0.20×10^{10}	23.19	10.5×10^{-13}
(4)	Urea (0.01 M)	-0.48×10^{10}	27.04	12.0×10^{-13}
(5)	Water	-1.5×10^{10}	5.12	–
(6)	Urine (goat urine)	-4.3×10^{10}	2772.00	–

Table 2

Values of α_1, α_2 and A for different sets across pyrex G_4 for acetone

Serial no. (sets)	Permeant	α_1 [a] ($m^{-1}\ N\ s^2\ kg^{-1}$)	α_2 [a] ($m^{-3}\ V^{-2}\ J$)	A ($m^5\ s^{-1}\ N^{-1}$) ($x_1 = 50$ cm acetone, $x_2 = 300$ V)
I	Acetone	-0.016×10^{10}	0.0843	0.97×10^{-11}
II		-0.0104×10^{10}	0.0404	1.26×10^{-11}
III		-0.024×10^{10}	0.0561	0.97×10^{-11}
IV		-0.0087×10^{10}	0.0750	1.11×10^{-11}
V		-0.00708×10^{10}	0.1228	1.35×10^{-11}

[a] Values are calculated assuming second-order symmetry.

zero [28]. However, where the flow converges, diverges or follows a curved path, this is no longer the case. The effect of the kinetic energy term is equivalent to a velocity head which decreases the effective pressure across the membrane. Values of α_1 for different permeants in the case of urinary bladder are listed in table 1, while for pyrex sinter (G_4) and Zeokarb 225 (Na^+ form) the values are given in tables 2 and 3.

Development of hydrostatic pressure across a membrane gives rise to a streaming potential. Since α_1 is a measure of the net pressure, the values of the streaming potential are also expected to be non-linearly related, higher values of the streaming potential having been obtained for the inner to outer side and lower values having been measured for the outer to inner side. This indicates that the pore geometry is different on the two sides of the membrane.

3.2. Dependence of J_i vs. $\Delta\psi$

Migration of a fluid across a membrane under the action of an electrical potential may be ascribed

Table 3

Values of α_1, α_2 and A for different alcohol/water systems across Zeokarb 225 (Na^+ form) membrane

Serial no.	Permeant	α_1 [a] ($m^{-1}\ N\ s^2\ kg^{-1}$)	α_2 [a] ($m^{-3}\ V^{-2}\ J$)	A ($m^5\ s^{-1}\ N^{-1}$) ($x_1 = 50$ cm, $x_2 = 200$ V)
1	Methanol	1.159×10^{10}	-0.0506	2.7×10^{-12}
2	0.60 mole fraction of water methanol	9.46×10^{10}	-0.0092	0.75×10^{-12}

[a] Values are calculated assuming second-order symmetry.

to the following factors:

(i) Existence of an electrical double layer formed as a result of the preferential adsorption of ions by the matrix of the membrane and subsequent balancing of the ions dispersed in solution.

(ii) Unequal distribution of ions in solution contained within the pores of the membrane.

The phenomenon of electro-osmosis in uncharged membranes is due to the former process while in charged membranes the latter factor is responsible. In the case of urinary bladder, factor i is responsible.

Using the electrical double layer model, it has been shown [22] that deviation from linearity is determined by the total number of absorbed dipoles, the dipole moment and the angle which the dipole moment makes with electrical field vector. It may, therefore, be inferred that deviation from linearity under the influence of $\Delta\psi$ will exclusively be determined by alterations in the orientations of adsorbed dipoles.

The polarization term (α_2) arising due to oriented solvent at a planar solid/solution interface inside the membrane is given as [13]

$$\alpha_2 = \frac{-L_{1122}A_r^2}{\alpha_1 \rho L_{11}^3} - \frac{3\alpha_1 \rho L_{12}^2}{A_r^2} \tag{12}$$

From the values of α_2, it appears that polarizability has a role to play in non-linear behaviour. The higher the value of α_2, the greater is the non-linearity. In situations where α_2 is at a minimum, the orientation of the dipoles gives rise to non-linearity as is evident from the values of α_2 in tables 1–3.

3.3. Dependence of J_i on ΔP and $\Delta\psi$

The forces ΔP and $\Delta\psi$ may act individually or collectively so as to exhibit non-linear behaviour. In the case of urinary bladder, it appears that both forces act collectively and, therefore, phenomenological coefficients containing terms in ΔP and $\Delta\psi$ have definite meanings. For example, the second-order phenomenological coefficient [22], L_{122}, has a vectorial character and has been shown to be related to the dipole moment vector associated with the permanent dipole of the molecule.

The values of A for the three types of membranes turn out to be positive although their characteristics are quite different. The three membranes exhibit non-linear behaviour although the driving forces appear to be different. Positivity of A has also been verified [29] for pyrex/acetone, pyrex/methanol and pyrex/ethyl methyl ketone [30] and Dowex-1 (Cl^- form)/methanol systems [31]. A may take negative values if the range of driving forces (ΔP and $\Delta\psi$) is quite low. Transition from a negative to positive value depends upon the relative magnitude of forces (ΔP and $\Delta\psi$) which may act collectively or separately. Thus, it may be inferred that a stable steady state prefers definite values of ΔP and $\Delta\psi$.

3.4. Viscoelasticity theories

According to the theory of viscoelasticity [32], the electrical field of a diffuse double layer is considered to be the cause of the non-linear behaviour of the interface. It has been observed that non-linearity can be detected only if the range of driving forces is very large or if the capillary has a very high surface potential. Like most biological membranes, urinary bladder membranes are elastic in nature. They are constructed in such a way as to permit stretching. The role of permeant is of particular significance. The membrane-permeant system as a whole acquires viscoelastic behaviour. This is evident from electrokinetic studies of water, aqueous urea and urine solution [10]. The polarizability factor (α_2) and kinetic energy of the centre of mass (α_1) are exceptionally high for urine which shows that urine alone is capable of producing the necessary changes in the bladder when it develops a voiding tendency. Since the filling and emptying of the bladder are connected with micturition waves, which arise from the development of a streaming current [9] across the bladder, the surface potential acquires great significance.

3.5. Membrane structure and non-linearity

The structure of a membrane is a decisive factor in deciding non-linearity. Urinary bladder is an example of a transitional epithelial tissue [33] consisting of several layers. The task of identifying the elements which make up a single functional structure is quite difficult. Complex membranes [34] may be built up in an infinite number of ways as regards the nature of the elements, the fraction of area covered and the geometrical array. A parallel array of elements expresses additive properties of the component membranes and deviation from additivity reflects the phenomenon of circulation among the membrane elements while in the case of a series array of elements, the continuity of potentials across all boundaries appears to be of prime importance. The continuity of an electrochemical potential does not imply continuity of the concentration, pressure or electrical potential. It is very likely that each term may undergo a drastic jump on passing from one phase to another.

Studies [35] across urinary bladder membranes suggest that cells are essentially coupled in series and that the relation between cell length and bladder radius deviates from linearity for very small bladder volumes. Experimental studies across urinary bladder membranes show that they are anisotropic in character. Hydrodynamic permeabilities, streaming potentials and electro-osmotic permeabilities have different values on the two sides of a membrane. As regards electro-osmosis, it is from the outer to inner side of the membrane only [9,36]. Thus, it may be inferred that urinary bladder is an example of a series array of elements and not a parallel array as the latter gives rise to isotropic properties [11].

There exist two pathways [37] for permeation, i.e., cellular and extracellular. In the case of leaky epithelia, fluxes occur through junctions and lateral intercellular spaces in the case of urinary bladder, which is an example of light epithelia, fluxes through junctions are ruled out. Lateral intercellular spaces represent a common pathway for permeation, the detailed knowledge of which would lead to a better model and greater understanding of the mechanism of transport.

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