

Electrokinetic energy conversion studies of alkaline solutions of uric acid, oxalic acid, L-cystine and L-tyrosine across urinary bladder membranes

P C SHUKLA and J P MISRA

Biophysical Laboratory, St. Andrew's PG College, Gorakhpur 273 001, India

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Abstract. Electrokinetic studies of alkaline solutions of oxalic acid, cystine and tyrosine across urinary bladder membranes have been made. Data have been analysed in the light of non-equilibrium thermodynamics. Maximum energy conversion efficiency (η_{\max}), kinetic energy term (α_1) and polarization term (α_2) have been computed. It has been found that η_{\max} , α_1 , and α_2 , etc., are maximum for uric acid among the permeants used. Since η_{\max} , and α_1 and α_2 are related with membrane interface, such studies are relevant in understanding the comparative effect of permeants on bladder interface.

Keywords. Electrokinetic energy; alkaline solutions; urinary bladder membranes.

1. Introduction

The variation of hydrostatic pressure (Guyton 1981) and electrical potentials (Schultz 1972) in biological systems are very important for the occurrence of life processes. Spontaneous activity, commonly observed (Sibley 1984; Creed 1985; Van Duyl 1985) in smooth muscles strip of urinary bladder is a collective property of pressure and electrical potential gradients respectively. Proper expulsion of urine means proper functioning of the bladder. Development, sustenance and final release of pressure is the most obvious definition of urination. Pressure develops electrical potential and potential is responsible for the generation of streaming current, probably micturition waves and thus the process of urination is a collective effect of pressure and electrical potential gradient (Shukla and Misra 1987, 1989) respectively. Due to the change in these gradients, there will be a change in kinetic energy term (α_1), polarization term (α_2) and maximum energy conversion efficiency (η_{\max}) *i.e.*, in membrane interface and thus computation of α_1 , α_2 and η_{\max} acquires importance because these are related with membrane phase.

Inefficient functioning of bladder may be due to infection or due to loss of distention power. Since in urination electrical energy is possibly converted into mechanical action, energy conversion studies acquire importance. Data have been analysed in the light of non-equilibrium thermodynamics. Electrokinetic energy conversion studies show that η_{\max} for uric acid is highest among the permeants used. All the permeants used, are the stone forming materials and if function of bladder is inefficient, all of these have tendency to precipitate from supersaturated urine. Since the various constituents of urine affect the bladder to varying degree depending upon their nature, comparative electrokinetic studies of stone forming acids are of significance. It has already been stated that inefficient functioning of

the bladder leads to precipitation of crystal from supersaturated urine leading to the growth of urinary calculi (Anderson 1981) in the bladder besides the chances of infection and thus interfacial behaviour of urine supersaturated with the permeants used, with urinary bladder interface deserves special attention. Thus the present study is an attempt to fill this gap.

2. Theoretical

Using non-equilibrium thermodynamics, non-linear volume flow (J_v) and current flow (I) (Lakshminarayanaiah 1984; Lorimer 1985) may be expressed as

$$J_v = L_{11} \Delta P + L_{12} \Delta \psi + \frac{1}{2} L_{111} (\Delta P)^2 + L_{112} \Delta P \Delta \psi + \frac{1}{2} L_{122} (\Delta \psi)^2 + \frac{1}{6} L_{111} (\Delta P)^3 + \frac{1}{2} L_{1112} (\Delta P)^2 \Delta \psi + \frac{1}{2} L_{1122} (\Delta \psi)^2 \Delta P + \frac{1}{6} L_{1222} (\Delta \psi)^3 + \dots \quad (1)$$

and

$$I = L_{21} \Delta P + L_{22} \Delta \psi + \frac{1}{2} L_{211} \Delta P^2 + L_{212} \Delta P \Delta \psi + \frac{1}{2} L_{222} (\Delta \psi)^2 + \frac{1}{6} L_{2111} (\Delta P)^3 + \frac{1}{2} L_{2112} (\Delta P)^2 \Delta \psi + \frac{1}{2} L_{2122} (\Delta P) (\Delta \psi)^2 + \frac{1}{6} L_{2222} (\Delta \psi)^3 + \dots, \quad (2)$$

L_{11} and L_{12} denotes filtration and electro-osmotic coefficients respectively while L_{21} represents ultrafiltration and L_{22} , the membrane permeant conductance. L_{111} , L_{112} , L_{122} , L_{211} , L_{212} , L_{222} , L_{1111} , L_{1112} , L_{1122} and L_{2222} are the higher order phenomenological coefficients.

There are three types of non linear situations (Shukla and Misra 1989, 1992).

- (i) Assumption of local equilibrium may be invalid *i.e.* Onsager's reciprocal relationship is not fulfilled.
- (ii) Local equilibrium may be preserved but the properties of 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 type of organization of 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, first order symmetry holds good while higher order phenomenological coefficients are not symmetrically related. Thus situation (ii) seems to fit our data.

Reciprocal relations in non linear regime are taken to mean symmetry of λ , coefficients in the pseudo-linear expression where λ coefficients are functions of forces. Sauer (1973) has shown that it is always possible to find at least one set of values which will ensure that $L_{ij} = L_{ji}$, to the second order in X , S . Similar arguments can be applied to the higher order terms.

The nonlinear flow may be related with kinetic energy term (α_1) and polarizability (α_2) as follows (Lorimer 1985):

$$\alpha_1 = \frac{L_{1111} A^2}{L_{11}^3} = - \left[\frac{L_{1111} A^4}{3 \rho^2 L_{11}^5} \right]^{1/2} \quad (3)$$

and

$$\begin{aligned}\alpha_2 &= \frac{L_{1122} A^2}{\alpha_1 \rho L_{11}^3} - \frac{3 \alpha_1 \rho L_{12}^2}{A^2} \\ &= -\frac{L_{1222} A^2}{3 \rho L_{12} L_{11}^2 \alpha_1} - \frac{\alpha_1 \rho L_{12}^2}{2A^2},\end{aligned}\quad (4)$$

where p and A represents density of the bathing solution and cross-sectional area of the membrane respectively.

Maximum conversion efficiency η_{\max} (Kedem and Caplan 1965; Morrison and Osterle 1968; Jain *et al* 1987; P C Shukia and J P sisra, unpublished results) may be given as follows

$$\eta_{\max} = \frac{(1 + \beta_{io})^{1/2} - 1}{(1 + \beta_{io})^{1/2} + 1}, \quad (5)$$

where

$$\beta_{io} = \left(\frac{L_{ii} L_{oo}}{L_{io}^2} - 1 \right)^{-1}. \quad (6)$$

In equations (5) and (6), β_{io} , $\beta_{io} \cdot L_{io}$, and L_o , have been used in general sense, however, they stand for-

$\beta_{10} = \beta_{12}$ Figure of merit for electro-osmotic mode of conversion.

$\beta_{01} = \beta_{21}$ Figure of merit for streaming potential mode of conversion.

$L_{io} = L_{12}$ Phenomenological coefficient for electro-osmotic.

$L_{oi} = L_{21}$ Phenomenological coefficient for streaming potential.

Owing to Onsager's reciprocity relation ($L_{10} = L_{01}$), it is obvious that

$$\beta_{io} = \beta_{oi} \quad (7)$$

With complete coupling $q = 1$ and with incomplete coupling $0 < q < 1$. The coupled flow of two fluxes leads to their definition of degree of coupling q which is a dimensionless parameter and in terms of η_{\max} it may be represented as

$$\eta_{\max} = \frac{q^2}{(1 + \sqrt{1 - q^2})} \quad \text{or} \quad q = \frac{2 \sqrt{\eta_{\max}}}{1 + \eta_{\max}}. \quad (8)$$

3. Experimental

3.1 Membrane

Urinary bladder membrane of goat is used as membrane due to its easy availability and to withstand high pressure. It was preserved for experimental purpose, using formaline-alcohol solution (Shukla and Misra 1987, 1989).

3.2 *Permeants*

Alkaline solutions of uric acid, oxalic acid, cystine- and L-tyrosine were used as permeants. The permeants were of AR grade in quality and were used as such without any further purification.

The output amount of oxalic acid, uric acid, cystine, and tyrosine is of great clinical importance because they are known to form urinary calculi depending upon the clinical condition of the body. The abnormal quantity of uric acid has been reported in various diseases such as gout, leukemia and other diseases associated with liver (West and Todd 1979). Oxalic acid output in normal urine is 20 to 50 mg per 24 h. An increased amount of oxalic acid is observed in certain liver diseases and diabetes mellitus.

The L-tyrosine and L-cystine are found in urine in traces, but under certain diseases such as acute yellow antropy (Oser 1979) of the liver, in severe typhoid fever and smallpox etc., these occur in measurable quantities.

Due to these above facts, oxalic acid, uric acid, L-tyrosine and L-cystine as permeants acquire significance.

3.3 *Measurements*

Electrokinetic phenomena include hydrodynamic permeability, electro-osmotic permeability, streaming potential and streaming current respectively, hence electrokinetic measurements will be the measurement of these. The liquid whose permeability was to be measured, was filled into the apparatus 6–8 h before starting the experiments in order to familiarize the membrane with permeating material.

Hydrodynamic permeability (Shukla and Misra 1987, 1989) was measured by applying a pressure difference (ΔP) across one side of the membrane and noting the advancement of liquid level in the horizontal capillary tube. During this measurement the potential difference ($\Delta \Psi$) was maintained equal to zero by short circuiting the electrodes. For electro-osmotic permeability measurement a potential difference was applied by an electronically operated power supply and advancement of liquid level in the horizontal capillary tube was noted. Care was taken to see that there were no evolution of bubbles occurring at both the electrodes and applied potential difference was not for more than two min in order to avoid deformation of the membrane system. In this measurement, pressure difference (ΔP) was maintained equal to zero.

Streaming potential was measured by applying a pressures difference across one side of the membrane and noting the resulting potential using a Mill's-2165 digital multimeter with a comparator function with an impedance of the order of $10^{13} \Omega$, and a current sensitivity of the order of 10^{-7}A . Streaming current was calculated using streaming potential and resistance data. Conductance was determined by applying a certain voltage across the membrane and noting the resulting current.

Electrokinetic energy conversion (Kedem and Caplan 1965; Morrison and Osterle 1968) measurements were made as described earlier.

The horizontal capillary tube was steamed for about an hour before being used. The experiments were carried out in a liquid thermostat with an accuracy of 0.1°C at 35°C .

4. Results and discussion

Transport behaviour of urine depends upon the constituents of urine. The various constituents of urine effects the bladder membrane interface and thus produce changes in the behaviour of bladder membrane surface. Continuous presence of acidic urine in the bladder ruptures the bladder mucosa layer and increases the adherence of uric acid crystals (Person *et al* 1985), if present in the urine and thus leads to ineffective functioning of the bladder. Sluggish flushing action of bladder may allow time for precipitation of crystals from supersaturated urine besides the chances of infection. The sluggish flushing action of bladder may be due to (i) obstruction to urinary flow, (ii) infection and (iii) the formation of stones. Since the transport behaviour of each constituent of urine is quite different from each other, studies of transport behaviour of oxalic acid, uric acid, L-cystine and L-tyrosine are of significance. The urinary transport may be related with the electrokinetic phenomena (Shukla and Misra 1987), results of electrokinetic measurements prove their importance. The results of these measurements may be summarized as:

- (i) Hydrodynamic permeability of L-tyrosine is the highest while that of uric acid is the lowest (figure 1a).
- (ii) Hydrodynamic permeability of the inner side to the outer side of the membrane is higher than that of outer side to the inner side of the membrane. The term, inner side, means the side which was initially in touch with urine.
- (iii) Streaming potential appears with following trend (figure 1b):

Uric acid < L-cystine < oxalic acid < L-tyrosine.

- (iv) Electro-osmosis takes place from the outside of the membrane to the inner side of the membrane. Electro-osmotic permeability of uric acid is highest (table 1) while that of L-tyrosine is lowest (figure 2a) amongst the permeants used.
- (v) Membrane-permeant conductance has following trend (figure 2b):

L-tyrosine < oxalic acid < L-cystine < uric acid.

Since, during urination electrical energy is converted into mechanical action, maximum conversion efficiency, η_{\max} , appears with following trend (figure 3).

Uric acid > L-cystine > oxalic acid > L-tyrosine.

The kinetic energy term (α_1) and polarization term (α_2) have the following trend (table 2).

Uric acid > L-cystine > oxalic acid > tyrosine.

The above experimental findings may be explained as follows.

In the bladder, urine is collected through kidney by ureters. When there is no urine in the bladder, intravesical pressure is almost zero. As urine collects in the bladder there is a development of pressure which stretches the bladder. Development of pressure across bladder give rise to streaming potential which in turn produces streaming current (Shukla and Misra 1987). The streaming current is probably the micturition waves which empty the bladder. Proper functioning of the bladder means proper generation of micturition waves, *i.e.*, proper development of streaming potential.

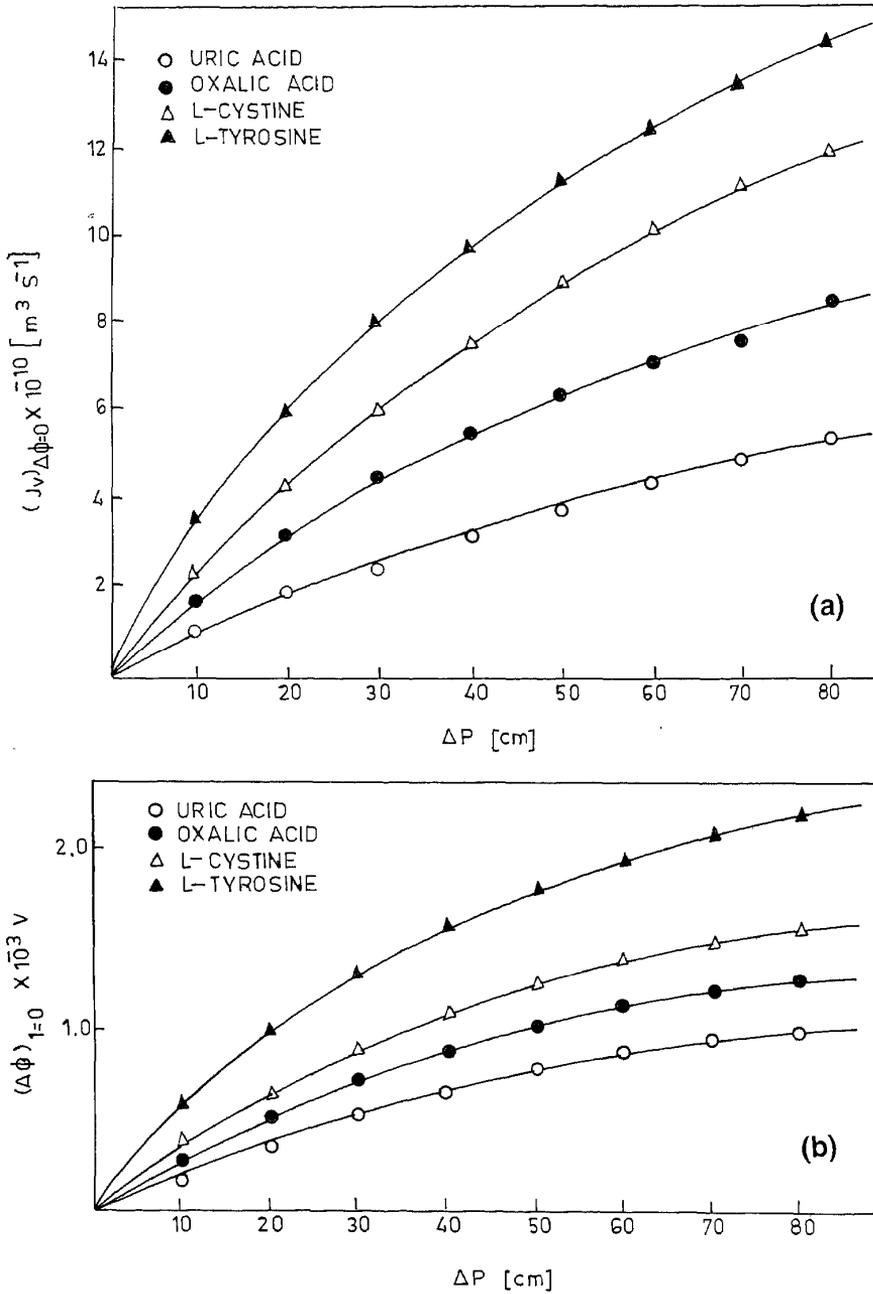


Figure 1. Hydrodynamic permeability (a) and streaming potential $\Delta\phi$ (b) vs pressure difference ΔP permeation from outer to inner side.

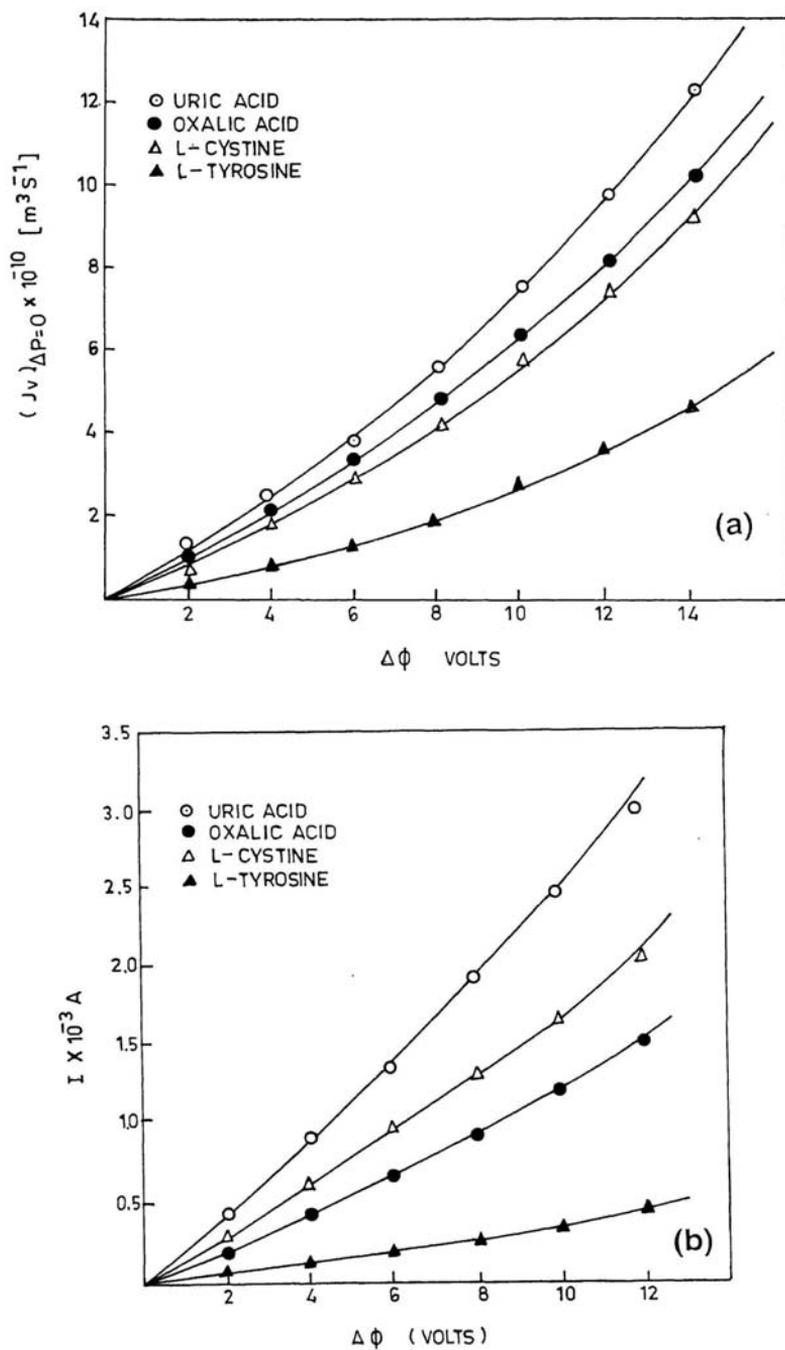


Figure 2. Electro-osmotic permeability (a) and current flux (b) vs potential difference $\Delta\phi$ permeation from outer to inner side.

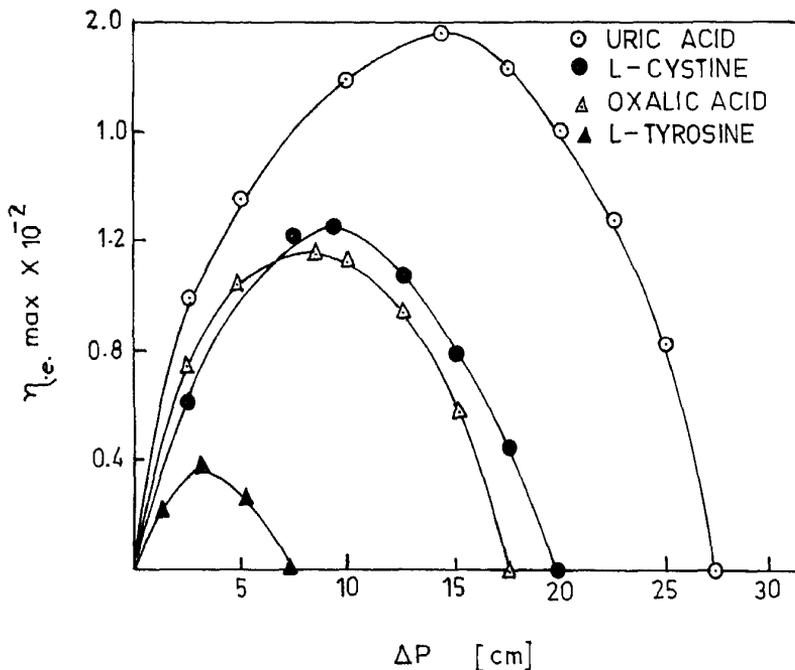
Table 1. Phenomenological coefficients for acids.

Coefficients	Permeants (0.001 M)			
	L-Tyrosine	Oxalic acid	L-Cystine	Uric acid
$L_{11} \times 10^{-13} \text{ m}^2 \text{ sec}^{-1} \text{ N}^{-1}$	4.35	2.90	2.40	1.45
$L_{12} \times 10^{-11} \text{ m}^3 \text{ sec}^{-1} \text{ V}^{-1}$	1.30	3.90	4.40	5.20
$L_{21} \times 10^{-11} \text{ m}^3 \text{ sec}^{-1} \text{ V}^{-1}$	1.40	3.80	4.45	5.15
$L_{22} \times 10^{-4} \text{ A V}^{-1}$	0.25	1.07	1.58	2.34
$L_{111} \times 10^{-16} \text{ m}^7 \text{ sec}^{-1} \text{ N}^{-2}$	-1.53	-1.14	-1.02	-0.72
$L_{1122} \times 10^{-14} \text{ m}^3 \text{ sec}^{-1} \text{ V}^{-2} \text{ N}$	1.40	1.00	0.75	2.40

Membrane thickness = $0.16 \times 10^{-2} \text{ m}$.

Membrane cross sectional area = $1.95 \times 10^{-4} \text{ m}^2$.

Temperature of experiment = $35 \pm 01 \text{ }^\circ\text{C}$.

**Figure 3.** $\eta_e \text{ max}$ vs pressure difference ΔP .

The proper functioning of the bladder depends upon the interaction of constituents of urine with urinary bladder. Continued presence of supersaturated urine with materials such as oxalic acid, L-cystine, uric acid, L-tyrosine, etc. leads to its adsorption on the bladder membrane interfaces. As a result of which it loses its distention power. Since expulsion of urine is related with the conversion of electrical energy into mechanical work, more energy (Shukla and Misra 1990) is required to expel urine supersaturated with stone forming materials. From experimental findings,

Table 2 kinetic energy term (α_1) and polarization term (α_2) for acids

Permeants (0.001 M)	Coefficients	
	$\alpha_1 \times 10^{11}$ ($\text{m}^{-1} \text{N sec}^{-2} \text{Kg}^{-1}$)	α_2 ($\text{m}^3 \text{V}^{-2} \text{J}$) from (L ₁₁₂₂)
L-Tyrosine	- 0.70	99.2701
Oxalic acid	- 1.77	109.3250
L-cystine	- 2.80	116.4404
Uric acid	- 8.98	524.9230

Membrane thickness = 0.16×10^{-2} m.

Membrane cross sectional area = 1.95×10^{-4} m².

Temperature of experiment = $35 \pm 01^\circ\text{C}$.

it is clear that the adsorption capacity of uric acid with urinary bladder interface is maximum in comparison with rest of other materials (table 3). This was further supported by the fact that continuous acid treatment of bladder (Person *et al* 1985) increases the adherence of uric acid crystals. The highest polarizability (α_2) suggests that uric acid crystals will adhere with bladder membranes much higher than oxalic acid and L-cystine. The value of α_1 also supports the above view with highest value in negative sign for uric acid. The negative sign indicates retardation in membrane phase and positive sign acceleration in membrane phase. It has been well established that cystine crystals are more common than tyrosine crystals in urine. It is recognized that the two elements in stone are the matrix or scaffold and the fill of crystalline lattice (Blandy 1985). The interaction of the molecules at the urinary bladder membrane is important in this context perhaps the higher hydrophobicity of L-cystine compared to L-tyrosine is responsible for its differential interaction with the membrane. The α_1 , and α_2 , values of L-cystine and L-tyrosine given in table 2 support this view.

Table 3. Figure of merit (β), maximum conversion efficiency (η_{max}) and degree of coupling for acids.

Permeants (0.001 M)	Coefficients					
	$B_e \times 10^{-5}$	$B_s \times 10^{-5}$	$\eta_e \cdot \text{max} \cdot 10^{-5}$	$\eta_s \cdot \text{max} \cdot 10^{-5}$	$q_e \times 10^{-2}$	$q_s \times 10^{-2}$
L-Tyrosine	1.51	1.76	0.37	0.43	0.38	0.41
Oxalic acid	4.90	4.65	1.21	1.15	0.69	0.67
L-cystine	5.10	5.22	1.26	1.29	0.70	0.71
Uric acid	7.97	7.81	1.98	1.94	0.88	0.88

Membrane thickness = 0.16×10^{-2} m.

Membrane cross sectional area = 1.95×10^{-4} m².

Temperature of experiment = $35 \pm 01^\circ\text{C}$.

The above studies conclude that inefficient functioning of bladder will be more with uric acid followed by L-cystine, oxalic acid and L-tyrosine.

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References

- Anderson J R 1981 *Murir's textbook of pathology* 11 th edition (London: ELBS and Edward Arnold)
- Blandy J 1985 *Lecture notes on urology* (Singapore: PG Publishing Pvt. Ltd.)
- Creed K E 1985 Effect of ions and drugs on the smooth muscle cell membrane of guinea-pig urinary bladder; *Pflug: Arch.* **326** 127
- Guyton A C 1981 *Textbook of medical physiology* (Philadelphia: W B Saunders)
- Jain A K, Tewari R K and Srivastava Rajesh Kr 1987 Electrokinetic energy conversion across liquid membranes generated by cholesterol; *J. Membr. Sci.* **31** 195-208
- Kedem O and Caplan S R 1965 Degree of coupling and its relations to efficiency of energy conversion; *Trans. Faraday Soc.* **61** 1897
- Lakshminarayanaiah W 1984 *Equations of membrane biophysics* (Orlando: Academic Press)
- Lorimer J W 1985 Viscous flow and non linear phenomena in non equilibrium thermodynamics of membrane transport; *J. Membr. Sci.* **25** 211-221
- Morrison F A and Osterle J F 1968 Electrokinetic energy conversion in ultrafine capillaries; *J. Chem. Phys.* **43** 2111
- Oser B L 1979 *Hawk's physiological chemistry* (New Delhi: Tata McGraw Hill)
- Person C L, Danielson B G and Fellstroem B 1985 *Protschr. Urol. Nephrol.* **23** 35-37
- Sauer F 1973 Renal physiology; in *Handbook of physiology* (eds) J Orloff and R W Berliner (Washington D C: Am. Physiological Soc.)
- Schultz S G 1972 Electrical potential difference and electromotive forces in epithelial tissues; *J. Gen Physiol.* **59** 794
- Shukla P C and Misra G 1987 Electrokinetic studies of aqueous solutions of urea, thiourea, glucose and creatinine across urinary bladder membranes; *J. Membr. Sci.* **31** 157-176
- Shukla P C, Misra G and Misra J P 1989 Characterization of nonlinear behaviour of an animal membrane using electrokinetic studies; *Biophys. Chem.* **33** 31-37
- Shukla P C and Misra J P 1990 Electrokinetic energy conversion by, aqueous oxalic acid, citric acid, ascorbic acid, hipuric acid and acetylsalicylic acid across urinary bladder membrane; *Indian J. Biophys.* **27** 179-182
- Shukla P C and Misra G 1992 Studies on concentration dependence of higher order phenomenological coefficients using electrokinetic studies across an animal membrane; *Langmuir*- **8** 1149-1153
- Shukla P C, Misra G and Misra J P 1989 Electrokinetic studies of aqueous solutions of urea across urinary bladder membranes; *J. Colloid Interface Sci.* **129** 53
- Sibley G N A 1984 A comparison of spontaneous and nerve mediated activity in bladder muscle from man, pig and rabbit; *J. Physiol.* **354** 431
- Van Duyl W A 1985 Spontaneous activity in urinary bladder smooth muscle; *Neural. Urol. Urodynamic* **4** 301
- West W S and Todd W R 1974 *Textbook of biochemistry* (New Delhi: Oxford and IBH)