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Electrophoresis of Progesterone Particles Dispersed in Aqueous Solutions of D-Glucose and Urea

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Electrophoretic mobilities of progesterone particles dispersed in aqueous solutions of D-glucose and urea, in the concentration range 10^{-3}—10^{4} mol l^{-1} have been measured to investigate electrical properties of the interface. Zeta potentials have been determined for this purpose. The dependence of zeta potentials on concentration has also been examined. Theory of the electrical double layer has been used to explain the results.

Electrophoresis is a subject of interest in many branches of pure and applied science, playing an important role in matters as diverse as clinical diagnosis and study of colloid stability. It plays significant roles in clay technology, water purification, detergency, ore floatation, electrodeposition and is extensively used for the fractionation and analysis of proteins, nucleic acids, polysaccharides and other complex substances of biological interest and activity.[1–3] In our earlier papers[4–6] we have reported results on the electrophoresis of testosterone (a male sex hormone) particles dispersed in aqueous solutions of electrolytes (e.g. NaCl, KCl, and $MgCl_2$), nonelectrolyte (e.g. urea) and carbohydrate (e.g. D-glucose) to characterize the electrified testosterone-permeant interface. Such studies with other hormones are highly desirable. Progesterone, a female sex hormone, responsible for normal development of female sex organs and for the maintenance of secondary sex characteristics[7] has been chosen for this purpose. The characteristic biochemical and physiological effects of this hormone depends to a large extent on the properties of hormone/solution interface. A comprehensive physicochemical study with respect to progesterone interface in different type of nonelectrolytic solutions is thus needed for an understanding of the various facets of its functions.

Electroosmotic studies on progesterone–carbohydrate and progesterone–urea systems have been reported quite recently.[8] In order to supplement these studies, the electrophoresis of progesterone particles dispersed in aqueous solutions of D-glucose and urea which are of great metabolic relevane and medicinal utility[9] is being reported in the present communication

Experimental

(a) Materials and Suspensions. Progesterone obtained from Sigma Chemical Company (Saint Louis, U.S.A.) was used. AR grade samples of D-glucose and urea obtained from BDH (India) were employed as such without further purification.

Solutions of D-glucose and urea were prepared in doubly distilled water, redistilled over $KMnO_4$, having specific conductance of the order of 10^{-6} mho cm^{-1}. Suspensions were prepared by homogenizing finely grounded particles of progesterone in these solutions.

(b) Measurement of Electrophoretic Velocity. Measurements of electrophoretic velocities were made using an experimental cell shown in Fig. 1. Progesterone particles were pulverized and suspensions of these particles were prepared in the various solutions. Care was taken to obtain a fairly stable suspension. The experimental cell was fixed in a vertical position. The suspension was introduced into the experimental cell through K by opening the stopcock S, till half of the tube was filled. The stopcock S was then closed and the pure solvent/solution was gently filled in the remainder of the tube PQ so that a distinctly sharp boundary B, was formed. The diameter of the tube PQ was 0.35

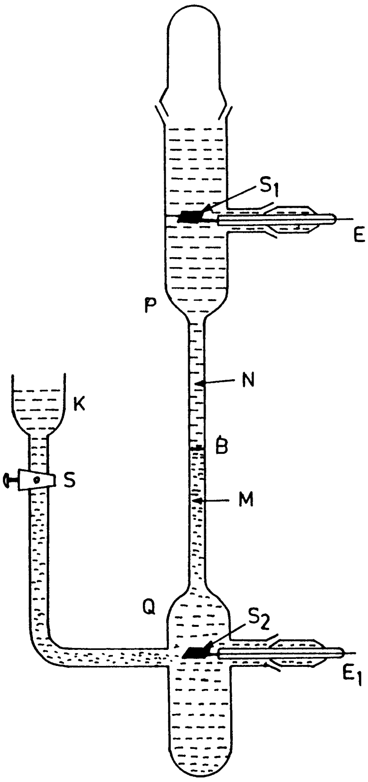


Fig. 1. Apparatus for measurement of electrophoretic velocity, S_1 and S_2 are silver plates coated with silver chloride; E_1 and E_2 are the silver wires attached with S_1 and S_2. S is the stopcock, B; boundary, K; wide mouth side tube for introducing the suspension. M; suspension, N; solvent/solution.

cm. Potential differences up to 50 volts were applied through silver-silver chloride electrodes E_1 and E_2, 15.1 cm apart, with an electronically operated power supply (HIL, India) in such a way that the movement of boundary occurred in the downward direction. The downward displacement of boundary was observed with a cathetometer reading up to ±0.001 cm. The position of boundary at various time intervals was noted and the rate of movement of boundary/electrophoretic velocity was thus determined. Corrections were made for downward movement due to the action of gravitational force. This was done by plotting the observed electrophoretic velocity against applied potential difference. The intercept on the ordinate gives the rate of suspended progesterone particles due to gravity alone. The movement of the boundary was found to occur from anode to cathode. All measurements were made in an air thermostat maintained at 25±0.5 °C.

Results and Discussion

The data on electrophoretic velocities, presented in Figs. 2 and 3, have been corrected by taking sedimentation velocities into account.

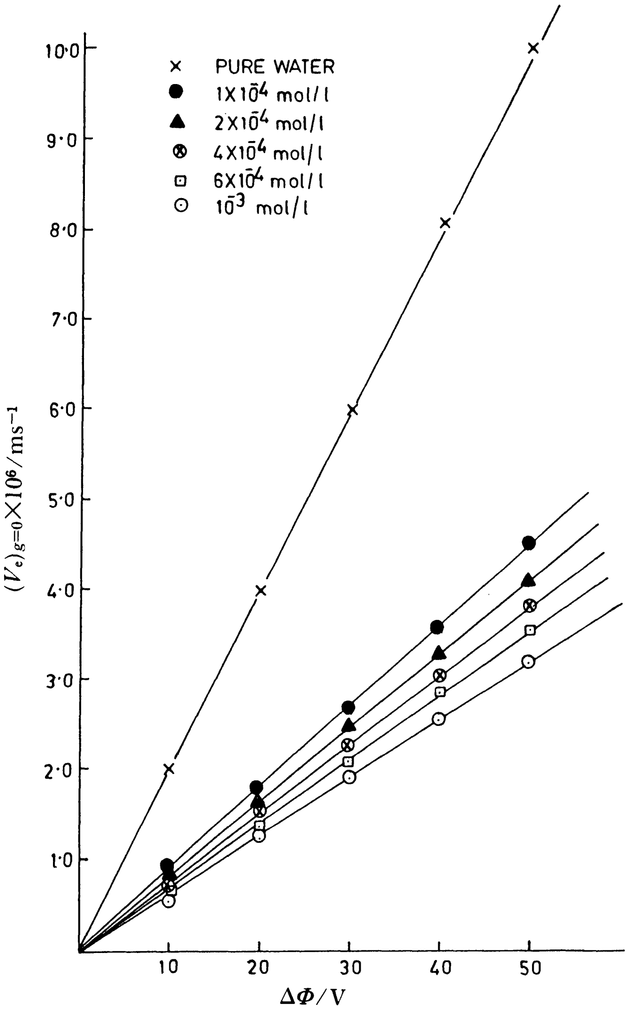


Fig. 2. Dependence of electrophoretic velocity $(V_e)_{g=0}$ on the applied potential difference, $\Delta\Phi$; for progesterone particles dispersed in aqueous solutions of D-glucose of different concentrations. (—X—) Pure water; (—●—) 1X10^{-4}, (—▲—) 2X10^{-4}, (—⊗—) 4X10^{-4}, (—□—) 6X10^{-4}, (—⊙—) 10^{-3} mol l^{-1}.

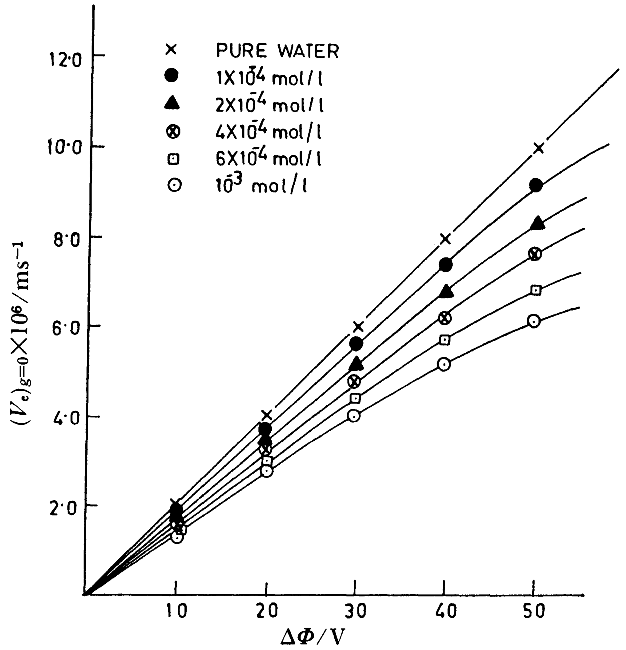


Fig. 3. Dependence of electrophoretic velocity $(V_e)_{g=0}$ on the applied potential difference, $\Delta\Phi$; for progesterone particles dispersed in aqueous solutions of urea at of different concentrations. (—X—) Pure water; (—●—) 1X10^{-4}, (—▲—) 2X10^{-4}, (—⊗—) 4X10^{-4}, (—□—) 6X10^{-4}, (—⊙—) 10^{-3} mol l^{-1}.

The following nonlinear second-order phenomenological equation has been found to satisfy the experimental data in case of progesterone-urea systems

$$(V_e)_{g=0}=L^*_{12}\left(\frac{\Delta\Phi}{T}\right)+\frac{1}{2}L^*_{122}\left(\frac{\Delta\Phi}{T}\right)^2, \tag{1}$$

However, in the case of progesterone-D-glucose systems, $(V_e)_{g=0}$ is found to be linearlity dependent on $\Delta\Phi$ (cf. Fig. 2) satisfying the euqation

$$(V_e)_{g=0}=L^*_{12} \tag{2}$$

with

$$\frac{L_{12}}{T}=\frac{\varepsilon\zeta_{e.p.}}{4\pi\eta l'} . \tag{3}$$

Here L^*_{12} and L^*_{122} are the first and second order electrophoretic transport coefficients respectively, ε is the dielectric constant of the medium, $\zeta_{e.p.}$ is the electrokinetic potential (or zeta potential), η is the coefficient of the viscosity of medium, and l' is the distance between the two electrodes in the electrophoretic cell.

According to Eq. 1 a plot of $(V_e/\Delta\Phi)$ against $\Delta\Phi$ yields a straight line as shown in Fig. 4.

The slope gives L^*_{122} and the intercept L^*_{12} for progesterone-urea systems. The value of L^*_{12} for progesterone-D-glucose system was obtained from the slope of linear plots of Eq. 2. The values of L^*_{12} and L^*_{122} evaluated in this way are recorded in Table 1.

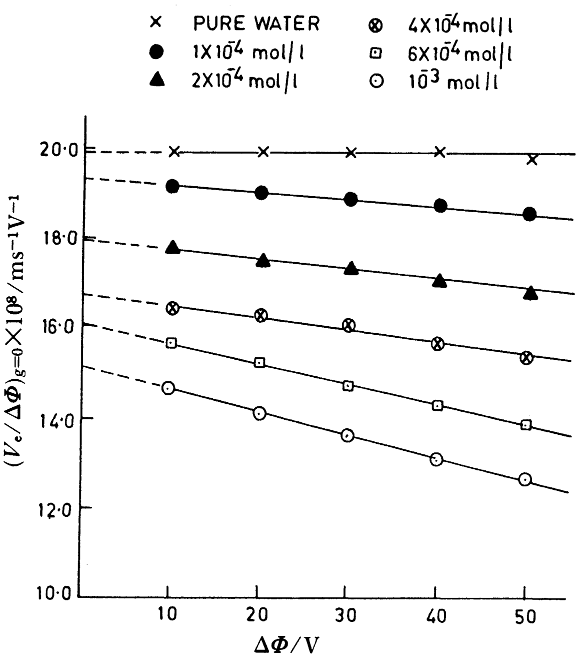


Fig. 4. Test of the applicability of the second order phenomenological equation (Eq. 1) for progesterone/aqueous urea solution systems.
(—×—) Pure water; (—●—) 1×10^{-4}, (—▲—) 2×10^{-4}, (—⊗—) 4×10^{-4}, (—□—) 6×10^{-4}, (—○—) 10^{-3} mol l^{-1}.

Evaluation of Zeta Potential. Electrophoresis, an electrokinetic phenomenon, is directly related to the nature of the mobile part of the electrical double layer, and may, therefore, only be interpreted in terms of zeta potential or the charge density at the surface of shear. Since no direct information is given about the charge density at the surface of the particles, only the zeta potential can give better information regarding the electrical nature of progesterone particles. Hence zeta potential was evaluated from the electrophoretic flux data by using the following equation.[5]

$$\zeta_{\text{e.p.}} = \frac{4\pi\eta l'}{\varepsilon} \times \left(\frac{L^*_{12}}{T}\right) \times 9 \times 10^4 \quad \text{volts} \qquad (4)$$

The occurrence of electrophoretic movement towards the cathode is an indication that an electrical double layer is formed at the surface of progesterone particles dispersed in these solutions due to adsorption of ions particularly cations (protonated urea and glucose) as shown in Fig. 5. From the schematic representation of the electrical double layer it is evident that all the progesterone particles bear a net positive charge facilitating their movement towards cathode when potential differences, $\Delta\Phi$, are imposed.

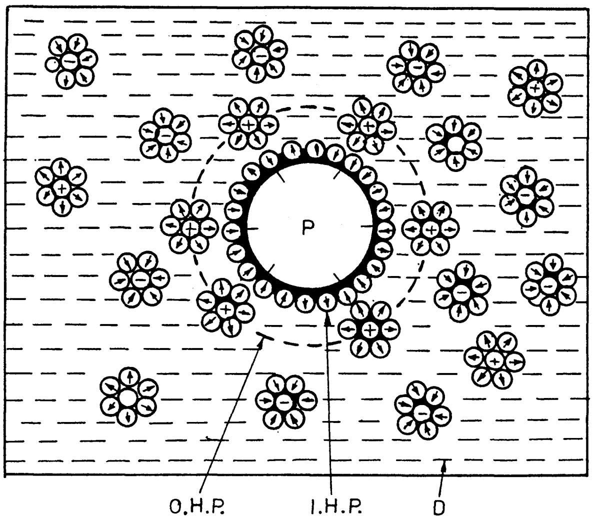
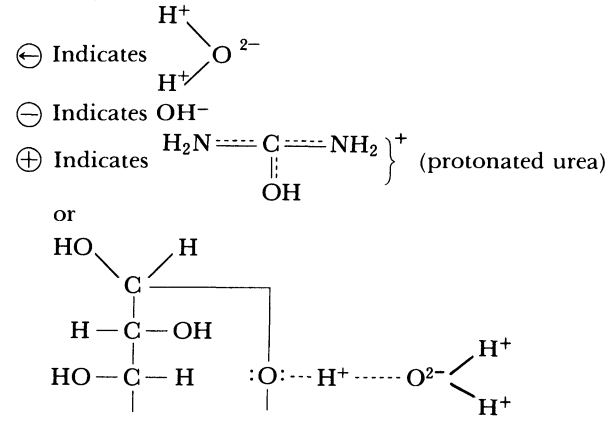


Fig. 5. Postulated picture of the electrical double layer at the progesterone/solution interface:
P=progesterone particle
D=diffused part of the electrical double layer



Glucose molecule surrounded with water molecules.

Origin of Nonlinearity. The nonlinear dependence of electrophoretic velocity, $(V_e)_{g=0}$ on applied electrical potential difference $\Delta\Phi$, for progesterone-urea system indicates that significant polarization of the electrical

Table 1. Phenological Coefficients and Zeta Potentials

$C\times10^4$ / mol l^{-1}	$\frac{L^*_{12}}{T}\times10^8$ / $\text{ms}^{-1}\text{V}^{-1}\text{K}^{-1}$		$\frac{1}{2}\frac{L^*_{122}}{T^2}\times10^{10}$ / $\text{ms}^{-1}\text{V}^{-2}\text{K}^{-2}$	$\zeta\times10^3$ / V	
	D-Glucose	Urea	Urea	D-Glucose	Urea
0.0	19.98	19.92	0.00	39.5	39.4
1.0	8.96	19.28	−1.67	15.98	34.3
2.0	8.31	17.93	−2.12	14.88	32.2
4.0	7.56	16.74	−2.67	13.86	30.0
6.0	7.16	16.10	−4.41	13.27	28.9
10.0	6.44	15.18	−5.00	12.48	27.3

double layer has occurred during electrophoresis, since water and urea both have permanent dipole moments ($\mu_{\text{urea}}=4.56\text{D}$, $\mu_{H_2O}=1.85\text{D}$). The polarizability α, may be represented as Ref. 10a

$$\alpha=\alpha_0+\alpha_\mu, \tag{5}$$

where α_0 is the distortion polarizability and α_μ is the orientation-polarizability. The orientation-polarizability defined by[10b)]

$$\alpha_\mu=\frac{\mu^2}{3kT}, \tag{6}$$

arises from the tendency of the permanent dipole moments to be oriented in the direction of the applied field.

Further, the polarizability α, is also dependent on the dipole moment that water and urea molecules acquire in an electric field $\Delta\Phi$, by the relation.[11)]

$$\alpha=\frac{\mu}{\Delta\Phi} \tag{7}$$

Rastogi and Shabd[12)] used Bockris[13)] model of the electrical double layer for explaining the nonlinearity in electroosmotic measurements. According to them the zeta potential is regarded to be made up of two terms, $\Delta\psi$ the charge contribution potential and $\Delta\chi$, the dipolar contribution potential i.e.

$$\zeta=\Delta\psi+\Delta\chi_{\text{eff}} \tag{8}$$

with

$$\Delta\chi_{\text{eff}}=\Delta\chi_{H_2O}+\Delta\chi_{\text{urea}}. \tag{9}$$

The applied electrical potential may affect either of these contributions.

Similar considerations apply even in the case of electrophoresis. The electrical double layer around the particle is considered to be formed on account of —(i) uneven distribution of ions produced by the partial ionization of the dissociable groups of the particle itself, (ii) self dissociation of the solvent molecules, and (iii) preferential orientation of the adsorbed solvent dipoles at the particle surface.

However, the possibility of dissociation of progesterone particles under the influence of applied electrical potential does not exist.

Bockris, Devanathan, and Muller have taken into account the competition between the molecules of water and an organic substance for a site in the double layer and have emphasized the role of charge dependent orientation of water dipoles at the interface.[14)] The charge on the particle will stimulate the water molecules to orient themselves (charge implies fields and dipoles tend to align with fields), resulting a water layer of oriented water dipoles at the surface. However, it must not be imagined that the water molecules are unaffected by the presence of their neighbors (urea molecule in this case). After all dipoles interact with dipoles; hence, the oriented water molecules experience lateral interaction — a phenomenon which affects the number of water molecules oriented in one direction and therefore the value of dipole potential $\Delta\chi$. Through, $\Delta\chi$, the potential difference across the interface and the structure of the interface will be affected. Thus the observed nonlinear variation of electrophoretic velocity and hence the ζ potential may be attributed to the enhanced dipolar contribution when $\Delta\Phi$ is varied. In addition, pronounced nonlinearity is observed at higher concentrations owing to strong interactions.

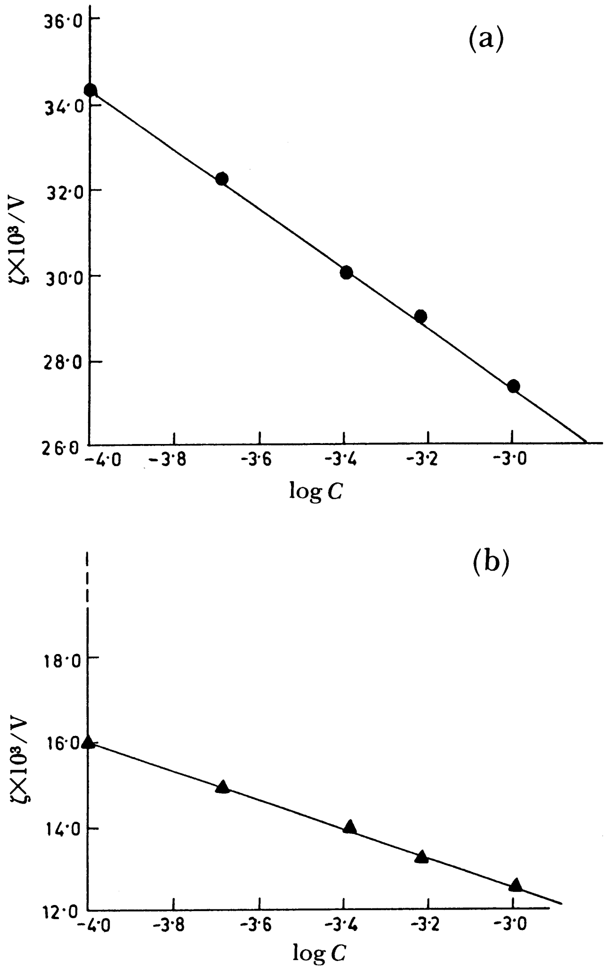


Fig. 6. Dependence of zeta potential (ζ) on log C for progesterone/aqueous solutions of urea (a); D-glucose (b).

Zeta potential varies nonlinearly with concentration. However, the following linear equation[5,6)] satisfies the dependence of zeta potential on log C.

$$\zeta=A-B\log C \tag{10}$$

where A may be taken as approximately constant for the conditions under which the charge on the membrance surface does not change sign and B is another constant which depends on topography of the surface. The results are shown in Fig. 6.

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