Generation of Streaming Potential by Liposomes in Cylindrical Capillary. A Physical Model

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Abstract. The streaming potential, U, of liposome solution during flow through a capillary was determined and the physical model of this effect is presented. Equations describing correlations between streaming potential data and the electrokinetic parameters of liposomes (mean surface charge density σ, liposome radius, r_0), and the parameters of the capillary and the solvent are presented.

Key words: Streaming potential — Capillary flow — Liposome

Introduction

It has been found that electrokinetic properties of vesicles can be correlated with their efficiency in carrying drugs. Measurements of changes in electrokinetic potential allow to identify the bond constants for the various substances making up the liposomal membrane (Mikes and Kovar 1981; Nałęcz and Wojtczak 1982). Such measurements usually involve probing with fluorescent, cation and anion or spin markers (Fernandez 1981; Nakagaki et al. 1981; Lukac 1983) the properties of which differ in polar and apolar membrane phase.

Some measurements of electrokinetic properties are based on the determination of electrophoretic mobility and zeta-potential (Haydon 1961; Hattenbach et al. 1985; Zschöring et al. 1985; Woodle and Collins 1992).

It can be proven that zeta-potential is closely related to electrokinetic potential, ψ_0.

The effect of flow in vesicles coated with an electric diffuse layer of ions (EDL) is recorded on the ends of a cylindrical capillary as the streaming potential, U or current, I.

This effect has not been analysed yet. Recently, it has been shown experimentally that added streaming potential can be registered during a flow of liposomes across a cylindrical capillary. The potential depends upon the type of liposomes and the conditions of their flow (Zawada 1990, 1991). An exact analysis of the streaming potential generated by the flow of ion solution through a rectangular

capillary has been performed by Babchin et al. (1976), Lyklema (1977), Gur and Ravina (1979). It has been found that electrolyte solutions put near to phase are characterised by special properties, and the structure of EDL has been described (Gur 1979; Tang et al. 1992). Similarly the generation of streaming potential by colloidal particles in a cylindrical capillary can be explained.

The streaming potential of liposome solution

Spherical liposomes with radius r_0 and surface charge density σ are coated with electric double layer (EDL) with electrokinetic potential, ψ_0. The liposome membrane is unpenetrable to ions. Liposome solution is defined by a certain dielectric constant, ε and viscosity η, and these values hold for all bulk solutions. During the flow through a capillary, the components of the velocity of liquid v_l, according to the capillary axis, in the surroundings of the liposome differ from the components v_{l0} of the liposome surface point. If the mean difference of velocities $v_l - v_{l0}$, is different from zero, electric charge flow is to be observed (EDL), i.e. the generating of a definite streaming potential, U. The following expression defining the streaming potential, U may be written for N liposomes flowing across a capillary per one second:

$$U = N \cdot \Omega \cdot \iint_S (v_0 - v_{l0}) \cdot \rho \cdot \mathrm{d}S \tag{1}$$

where Ω is the resistance of solution, ρ is the macroscopic density of electric charges in EDL, and $\mathrm{d}S$ is the integral area of the capillary slide.

The value of U should be defined as a mean value since it depends on the velocity of each point in the ion atmosphere of each liposome in the capillary and their transitory position. The determination of the EDL structure and velocity distributions around the liposomes in the capillary makes it necessary to find an analytic solution to equation (1).

Electric double layer of spherical particles

The techniques testing liposome EDL are based upon the Gouy and Chapman model (G-C) (Scheludko 1969). The conditions of this model often meet the requirements, but small liposomes are better described by the Debye- Hückel model of ion atmosphere (Libuś and Libuś 1987). We obtain the following expressions for charge density, ρ and electrokinetic potential, ψ_0 for $z_+ \cdot z_-$ electrolyte, using the D-Hü theory to describe the EDL the of small liposome keeping the formalism of the G-C theory:

$$\rho = -\frac{a^2 r_0^2 \sigma}{1 + a r_0} \cdot \mathrm{e}^{a r_0} \cdot \frac{1}{r} \cdot \mathrm{e}^{-a r} \tag{2}$$

$$\Psi_r = \frac{8\pi r_0^2 \sigma}{\varepsilon\varepsilon_0(1+ar_0)} \cdot e^{ar_0} \cdot \frac{1}{r} \tag{3}$$

$$a^2 = \frac{8\pi e^2}{\varepsilon\varepsilon_0 kT} \sum_i c_i z_i^2 \tag{4}$$

where r_0 is liposome radius, r is the distance from the centre of the liposome to the EDL point, c_i is the i-ion concentration in the solution, σ is the surface charge density, ε is the dielectric constant, ε_0 is the permittivity of free space, and e, k, T have the usual meanings.

Hydrodynamics of liposome solution in a cylindrical capillary

The description of spherical particle motion in laminar stream is based upon the Nevier-Stokes equation:

$$\eta \Delta V = \text{grad } P \tag{5}$$

$$\text{div } V = 0 \tag{6}$$

where η is the solution viscosity, V is the liquid velocity, P is the hydrostatic pressure, and Δ is the Laplace's operator.

For stationary flow, when $\Delta P = 0$, the following condition is fulfilled (Einstein 1906):

$$\frac{P}{\eta} = \Delta W \tag{7}$$

where W is the liquid velocity potential.

The rigid, spherical particle of radius r_0 is at distance b from the capillary inlet, at distance h from the capillary axis, of radius R and length L. The beginning of the Cartesian coordinate system $(d+b+x,\ y,\ z+h)$ lies at $Q(0,0,0)$. The beginning of the x, y, z, coordinate system lies at $Q'(d+b,0,h)$, and the centre of the particle lies at this point (Fig. 1):

$d+b$ is the translation vector of coordinates system x, y, z;

x axis has the same direction as the capillary axis;

z axis passes through the particle centre and the capillary axis.

The distance to any point of the capillary is:

$$g^2 = (x+d+b)^2 + y^2 + (z+h)^2.$$

The distance to a point on the inner wall of the capillary is:

$$g_1^2 = (x+d+b)^2 + R^2.$$

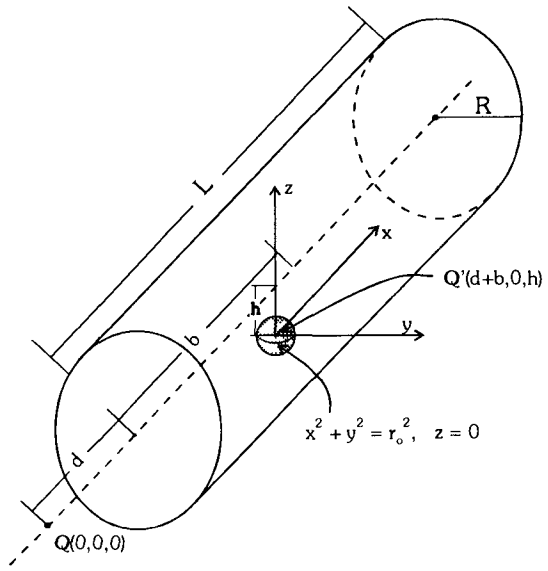

Figure 1. Schematic representation of a spherical particle in the capillary (for explanation see the text).

The distance to a point on the surface plane of liposome is:

$$g_2^2 = (x + d + b)^2 + y^2 + (z + h)^2$$

where: $x^2 + y^2 + z^2 = r_0^2$. The liquid has a constant viscosity, η and a dielectric permittivity $\varepsilon \cdot \varepsilon_0$ in all its volume.

The solutions to equation (7) can be fulfilled in many individual hydrodynamic states, while the equation form depends upon the number of the boundary conditions and the distribution of the assumed or known hydrodynamic pressure. In order to make equation (1) solvable, despite the almost polar symmetry of flow in the capillary, equation (7) has to be presented in coordinates meeting the condition of spherical symmetry of distribution of EDL ions around the liposome. The form of function W that describes the liquid velocity potential in a capillary is in accord with that used by Einstein (1906) with the exception of the term characterising the pressure influence. It was assumed that the pressure vector takes a linear form and follows the capillary axis.

If the liquid velocity potential amounts to:

$$W = -c\frac{(x + b + d)^3}{3} + B \cdot \frac{\partial^2}{\partial x^2}\frac{1}{g} + \frac{A}{2} \cdot \left[(x + b + d)^2 - \frac{y^2 + (z + h)^2}{2}\right] \qquad (8)$$

the component liquid velocities V_x, V_y, V_z are given by:

$$V_x = \frac{\partial W}{\partial x} + u' \qquad V_y = \frac{\partial W}{\partial y} + v' \qquad V_z = \frac{\partial W}{\partial z} + w' \qquad (9a, b, c)$$

and

$$u' = c\left[\frac{R^2}{2} + (x+b+d)^2 - \frac{y^2+(z+h)^2}{2}\right] \quad v' = 0 \quad w' = 0 \qquad (10\text{a, b, c})$$

where A, B, c are constants determined from boundary conditions.

The pressure distribution in the capillary can be written as:

$$\frac{P}{\eta} = -2c\,(x+b+d). \qquad (11)$$

If the hydrostatic pressure at the inlet to the capillary is P_1 and that at the outlet is P_2, the experimental hydrostatic pressure is $P_0 = P_1 - P_2$, and:

$$\frac{P_0}{\eta} = 2cL. \qquad (12)$$

Knowing the distribution of the hydrostatic pressure in the capillary, we can solve equation (9 a,b,c) and the following expressions defining component velocities V_x, V_y, V_z can be obtained:

$$V_t = \frac{c}{2}\left[R^2 - y^2 - (z+h)^2\right] + B\frac{\partial^3}{\partial x^3}\frac{1}{g} + A(x+b+d) \qquad (13)$$

$$V_y = B\frac{\partial}{\partial y}\frac{\partial^2}{\partial x^2}\frac{1}{g} - \frac{A}{2}y \qquad (14)$$

$$V_z = B\frac{\partial}{\partial z}\frac{\partial^2}{\partial x^2}\frac{1}{g} - \frac{A}{2}(z+h). \qquad (15)$$

The velocity of the liquid in a laminar flow takes a value of 0(*) at the capillary wall, while a rigid particle, if positioned outside of the capillary axis, is rotating.

The velocity of the points indicated on the particle surface within the equatorial plane takes a constant value. (**)

In this case the boundary conditions assume the following form:

* – for points on the inner face of the capillary $g = g_1$ and V_x, V_y, $V_z = 0$

** – for points on a surface of the particle: $z = 0$ and $x^2 + y^2 = r_0^2$,

the V_x = constant and then $\dfrac{\partial V_x}{\partial x} = 0$

Following the conditions mentioned above we obtain:

$$A = -6B \cdot R^{-5} \cdot \left(\frac{2}{3}\right)^{\frac{7}{2}}$$

$$3B = c \cdot r_0^2 \left[\frac{2(d+b+r_0)^2 - h^2}{[(d+b+r_0)^2+h^2]^{\frac{5}{2}}} - \frac{2(d+b)^2 - h^2}{[(d+b)^2+h^2]^{\frac{5}{2}}}\right]^{-1}$$

and taking into account lack of radial symmetry, the condition that normalises the value of parameter d is given in the form of:

$$2(d+b+x)^2 = R^2$$

The component of velocity V_x takes the following form:

$$V_x = c \cdot \left[\frac{R^2 - y^2 - (z+h)^2}{2}\right] + B\frac{\partial^3}{\partial x^3}\frac{1}{g} + A(x+b+d) \tag{16}$$

The streaming potential of liposome solutions in a single cylindrical capillary

In order to introduce an expression defining the velocity in expression (1), rearrangement is needed into a form that is characteristics for the spherical coordinate system:

$$x = r \cdot \sin\nu \cdot \cos\varphi \qquad y = r \cdot \sin\nu \cdot \sin\varphi \qquad x = z \cdot \cos\nu$$

$$\mathrm{d}S = \mathrm{d}y \cdot \mathrm{d}z = r \cdot \sin\varphi \cdot \mathrm{d}r \cdot \mathrm{d}\nu$$

The term defining the mean value $\overline{U}$ (1)

$$\iint_{r,\nu} (V_{r,\nu} - V_{r_0,\nu}) \cdot \rho \cdot r \cdot \sin\varphi \mathrm{d}r\mathrm{d}\nu$$

considering parameter φ, has the following solution:

$$\frac{\overline{U}}{\Omega \cdot N} = c \cdot \frac{4}{3} \cdot \frac{\pi r_0^2 \sigma}{a} - 3B \cdot \frac{4h}{5\pi(d+b)} \cdot \frac{a^2 r_0^2 \sigma}{1+ar_0} \cdot \mathrm{e}^{ar_0} \cdot \sum(-1)^n \frac{\pi^{2n-1}}{(2n-1)!} \cdot$$

$$\cdot \int_{r_0}^{\infty} \left[\frac{r}{[(d+b)^2 + (h-r)^2]^{\frac{5}{2}}} + \frac{r}{[(d+b)^2 + (h+r)^2]^{\frac{5}{2}}} - \right. \tag{17}$$

$$\left. - \frac{r_0}{[(d+b)^2 + (h-r_0)^2]^{\frac{5}{2}}} - \frac{r_0}{[(d+b)^2 + (h+r_0)^2]^{\frac{5}{2}}}\right] \cdot \mathrm{e}^{-ar}\,\mathrm{d}r + \cdots$$

Both terms (Eq 16):

$$\frac{c}{2} \cdot \left[R - y^2 - (z+h)^2\right] \quad \text{and} \quad B \cdot \frac{\partial^3}{\partial x^3}\frac{1}{g}$$

give non-zero solutions for the streaming potential (Eq 17).

The first term of equation (17) describes the linear delay of the particle in relation to the mean liquid velocity; the second one portrays the influence of the

particle rotation. The analysis of the equation indicates that liposome rotation depends linearly on the liposome distance from the capillary axis, i.e. the value of h. This effect can be observed only for very small ionic forces of the order of 10^{-5}, when Debay's radius $1/a$ takes high values. Even in such conditions the contribution of the rotation term to a whole effect is very small of the order of a 10^{-10} fraction of the first term. The conditions are fulfilled for liposomes of diameters of $10^{-5} - 10^{-7}$ m, when the capillary diameter R is $2.4 \cdot 10^{-4}$ m and length $L = 0.25$ m.

Taking into account the latter, the flow potential of the solution of liposomes or other rigid, spherical particles of the colloidal dimensions has the following value:

$$U = \frac{2 \cdot \pi}{3} \cdot \frac{P_0}{\eta L} \cdot \frac{r_0^2 \sigma}{a} \cdot N \cdot \Omega \tag{18}$$

The model presented does not take into account the effects coming from the inlet and the outlet of the liposomes in to and out of the capillary, i.e., those accompanying the changes of the system geometry. The Ohm resistance of a capillary filled with an electrolyte solution is proportional to $L/\kappa \cdot R^2$, where κ means the specific conductivity of the solution. At the same time, the number of particles flowing through the capillary within a time unit is proportional to R^4; therefore, the flow potential U should be proportional to R^2. Concentration together with the type and the valence of the electrolyte ions have influence on the Debay's radius $1/a$ and the conductivity of the electrolyte, thus strongly affecting the flow potential U.

A flow of the bare electrolyte results in a flow potential that can be connected with the electrolyte movement in relation to the capillary wall; therefore, the effect can be a source of artifacts coming from the colloid adsorption on the glass surface of the capillary taking place during the flow of liposome solution. It has been found (Zawada 1991), that the adsorption effect can be eliminated by covering the capillary inner surface with a phospholipid film composed so as to give the capillary surface potential $\zeta = 0$. This cover is durable enough to take a series of measurements, and can be easily removed by washing the capillary with organic solvents.

With the known the geometry of the capillary given by N and r_0 and using Eq 18, the electrokinetic potential ψ_0 or surface charge density σ can be calculated for liposomes and other colloidal particles. This potential was determined by the hydrodynamic method; therefore it has the physical meaning of the ζ potential. Depending on the size of the liposomes, we determine their mean radius by light or electron microscopy, gel chromatography (Dusset at al. 1982), light scattering or other methods.

The presented model of the generation of streaming potential can be used to describe measurements of electrokinetic properties of colloidal particles. The measurements of the electric properties of the particles surface allow to set the

absolute sign of EDL on the basis of the sign of potential U, which is probably the most important benefit of the method.

The measurements of the surface potential are performed to characterise the adsorption of some drugs on the outer surface of liposomes.

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Final version accepted March 28, 1996