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Analysis of electrophoretic mobility data for human erythrocytes according to sublayer models

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Abstract

An attempt was made to analyze the electrophoretic mobility data of human erythrocytes in media of different pH values and ionic strengths through cell surface models in which the surface charge layer consists of several ion-penetrable sublayers with a uniform charge distribution in each sublayer. As a result, the three-sublayer model was found to explain the mobility data much better than the two-sublayer model in a wide range of ionic strength at all pH values studied.

Key words: Electrophoretic mobility; Human erythrocytes; Surface charge distribution

1. Introduction

Electrophoresis can provide us with information about the surface structure of human red cells, especially about the distribution of ionogenic groups in the surface region, without producing appreciable alteration or destruction of the cellular organization. For this reason, a large number of studies have been made on the electrophoretic behavior of human red cells [1,2]. Nevertheless, our present knowledge of the distribution of ionogenic groups in the surface region is still limited, due mainly to the lack of proper model for the cell surface. In fact, we cannot use the Smoluchowski formula [3] and the Gouy–Chapman equation [4] to convert the experimentally determined electrophoretic mobility for red cells to the cell surface potential and then to calculate the surface charge density on the cell surface because these equations were derived for solid colloidal particles, the surface charges of which are located only on the ion-impenetrable particle surface of zero thickness while the surface charges of red cells are distributed through an ion-penetrable layer of finite thickness.

In recent years, theoretical studies have been performed on electrophoresis of colloidal particles with a structured surface [5–12]. In particular, Ohshima and Kondo [12] have derived an approximate formula for the electrophoretic mobility of particles with a surface charge layer, which depends on a weighed average of the Donnan potential and the potential at the boundary between the surface charge layer and the sur-

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rounding solution. This formula assumes a uniform charge distribution in an ion-penetrable surface layer of finite thickness and contains two undetermined parameters, charge density and resistance to liquid flow in the surface layer. Since the Donnan potential and the surface potential in the formula are an ionic strength-dependent function of the charge density in the surface layer, curve fitting is necessary to estimate the charge density from the mobility data as a function of the ionic strength of the medium at a fixed pH value using various values of charge density and resistance to liquid flow.

Kawahata et al. [13] have analyzed the electrophoretic mobility data for human red cells at different pH values and ionic strengths with the Ohshima–Kondo formula and found that the formula gives a good agreement between the observed and calculated mobility values as a function of ionic strength in neutral and alkaline media, where the negative charges arising from the ionized acidic groups dominantly contribute to the mobility, but it fails to hold in acidic media, suggesting the presence of the protonated basic groups in the inner part of the surface layer to produce a nonuniform charge distribution.

A similar situation has been observed with other mammalian blood cells such as rat lymphocytes [14] and guinea-pig polymorphonuclear leucocytes [15]. In view of this, it is realistic and reasonable to adopt a surface layer model in which the fixed charges are distributed nonuniformly through the layer of finite thickness to analyze the electrophoretic mobility data for mammalian blood cells.

In the present paper we report the results of an analysis of the electrophoretic mobility data for human erythrocytes with mobility formulas derived on the basis of models in which the surface charge layer consists of several charged sublayers [16,17].

2. Experimental

2.1. Preparation of erythrocyte suspension

All solutions were made up in purified water which was prepared by passing tap water through an ion-exchange resin column and a membrane filter (Ultrapure Water System Milli-Q Plus, Nihon Millipore Kogyo Co., Japan). The chemicals used were of reagent grade unless otherwise noted. Human erythrocytes were obtained from fresh citrated blood supplied by young healthy donors. The cells were separated from the other blood components by centrifugation and washed three times with 0.9% (w/v) NaCl solution (standard saline) on the centrifuge. The washed cells were dispersed in buffer solutions.

2.2. Cell electrophoresis

The electrophoretic mobility of human erythrocytes was measured with a Pen Kem System 3000, an automated electrokinetics analyzer (Pen Kem Inc., USA), in veronal buffer solutions of different pH values and ionic strengths at 310 K. The buffer solutions used had the pH values of 4.0, 4.5, 5.0, 6.0, and 7.4, the ionic strength of which was adjusted to the desired values (0.005–0.154) by the addition of appropriate amounts of NaCl. The solutions were also made isotonic by adding proper amounts of sucrose. Minute precautions were taken to make the measurement more accurate and more reliable than in the earlier work [13]. For example, the electrophoresis cell was thoroughly washed each time when the sample was changed. Mobility readings were repeated 20 times for each sample and the readings were averaged.

3. Results and discussion

3.1. Electrophoretic mobility

Fig. 1 shows the experimentally determined electrophoretic mobility of human red cells as a function of the ionic strength of the medium at different pH values. The red cells exhibited negative mobility values in all media used in the present work, showing that the cell surface has a net negative charge or, more exactly, the number of acidic groups far exceeds that of basic groups in the cell surface layer. When the ionic strength of the medium was lower than 0.05 the mobility

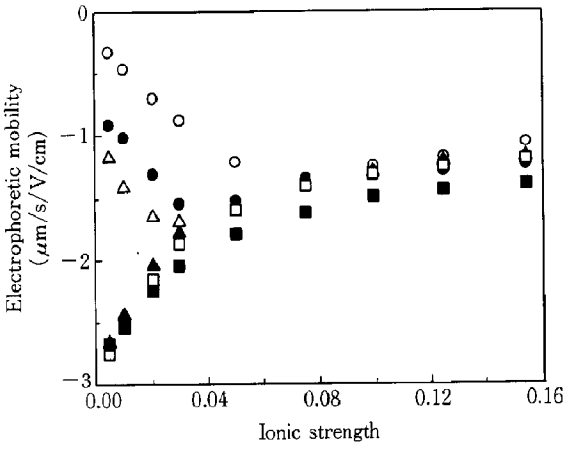

Fig. 1. Electrophoretic mobility of human erythrocytes as a function of medium ionic strength at pH 4.0 (○), 4.5 (●), 5.0 (△), 6.0 (▲), 7.4 (□), and 9.0 (■).

varied in a different way at different pH values with decreasing ionic strength. Thus, the mobility became more negative as the ionic strength of the medium decreased at pH values higher than 6.0. This reflects an increased contribution from the negative charges on the ionized acidic groups located deep inside the surface layer caused by a decreased shielding effect of electrolyte ions. On the contrary, the mobility showed a minimum when plotted as a function of medium ionic strength at pH values between 4.0 and 5.0, where dissociation of the acidic groups is suppressed to some extent, indicating that there are a number of protonated basic groups in the inner part of the surface layer [13], which contribute to make the mobility remarkably less negative at low ionic strengths. This necessitates a proper surface layer model that takes account of the nonuniform charge distribution in the cell surface to analyze the electrophoretic mobility data of human red cells.

3.2. Two-sublayer model

We first try to analyze the mobility data with a two-sublayer model. According to this model, the surface charge layer consists of two oppositely charged sublayers, the outer one (sublayer 1) of which carries negatively charged groups of valence z_1 and number density N_1 and the inner one (sublayer 2) of which carries positively charged groups of valence z_2 and number density N_2. The observed mobility, μ, is directly related

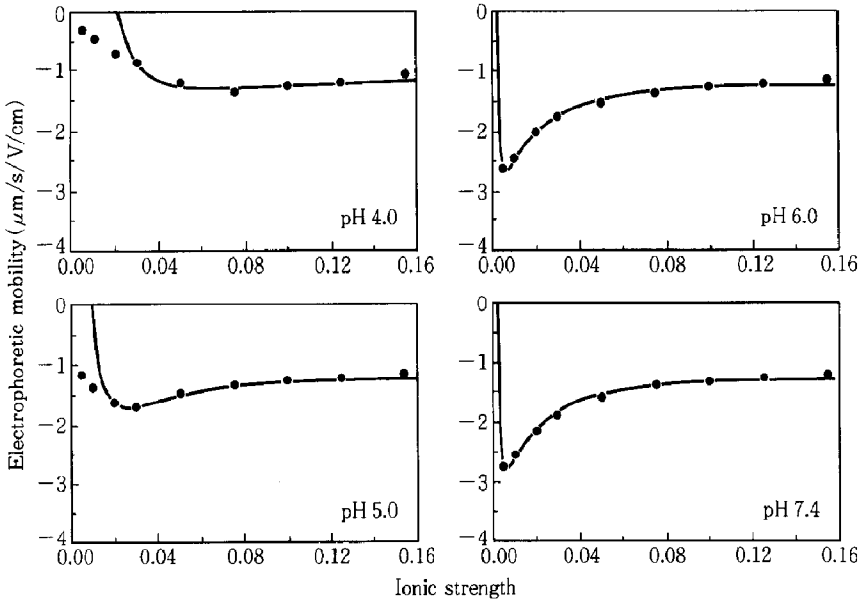

Fig. 2. Electrophoretic mobility–ionic strength curves for human erythrocytes at different pH values. Solid lines are the best fitted curves obtained with the two-sublayer model and closed circles are experimentally observed mobility values.

to the charge densities, N_1, and N_2, through the following formula [17]:

$$\mu = \frac{e}{\eta\lambda^2}\left\{ z_1 N_1 \left[1 + \left(\frac{\lambda}{\kappa}\right)^2 \frac{1+\lambda/2\kappa}{1+\lambda/\kappa} \right] + (z_1 N_1 - z_2 N_2)\left[\left(\frac{\lambda}{\kappa}\right)^2 \frac{\lambda}{2(\kappa-\lambda)} e^{-\kappa d} - \frac{\kappa^2}{\kappa^2-\lambda^2} e^{-\lambda d} \right] \right\}, \quad (1)$$

where e is the elementary electric charge, η the viscosity of the medium, d the thickness of the outer sublayer (the total thickness of the surface layer is d_c), κ the Debye–Hückel parameter of the medium, and λ a parameter characterizing the resistance to liquid flow in the surface layer.

In order to check the suitability of this formula and to determine the values of z_1N_1, z_2N_2, d, and λ, curve fitting was performed for the mobility data shown in Fig. 1 using various values for these parameters. The results obtained that gave the best fitting are shown in Fig. 2 and Table 1. Although the theoretical curve at each pH value was found to fit in well with the experimental data points over a wide range of medium ionic strength when the values given in Table 1 for the parameters were employed, the pH dependence of the charge density, z_1N_1, is not reasonable at all. It increases instead of decreasing as the pH of the medium decreases, whereas dissociation of the acidic groups should decrease with decreasing pH of the medium. Hence, there must be something wrong with this surface layer model. In fact, the model assumes the presence of a sudden jump in the charge distribution somewhere in the surface layer while the real charge distribution would be such that the outer part is rich in negative charges, the inner part is abundant in positive charges, and the transitional zone between the two parts has no or little charge as the result of the compensation of opposite charges.

Table 1
Negative and positive charge densities, d and $1/\lambda$ for human erythrocytes as a function of pH

pH	Charge density z_1N_1(M)	Charge density z_2N_2(M)	d (nm)	$1/\lambda$ (nm)
4.0	−0.110	0.850	4.5	0.6
4.5	−0.072	0.720	6.5	0.9
5.0	−0.072	0.520	6.5	0.9
6.0	−0.062	0.066	5.0	1.0
7.4	−0.064	0.066	5.0	1.0

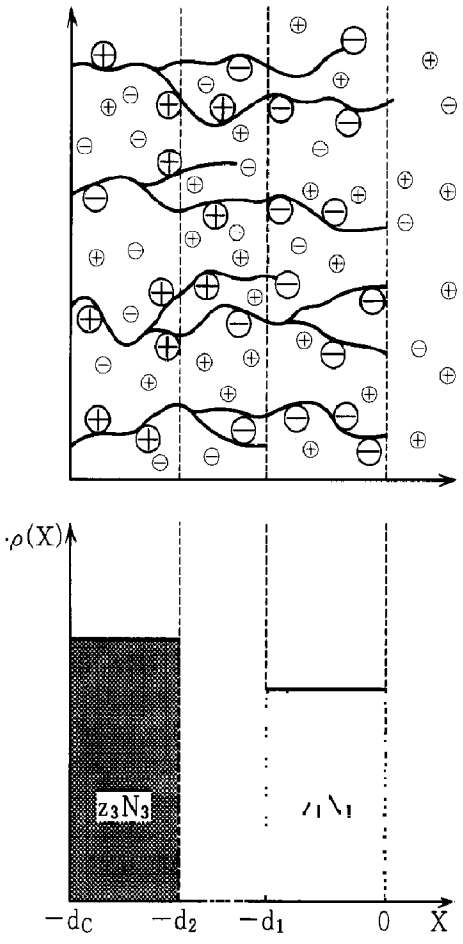

Fig. 3. Schematic representation of fixed-charge distribution in the surface layer of red blood cells in the three-sublayer model.

3.3. Three-sublayer model

In an attempt to improve the two-sublayer model discussed in the previous section, we set up a three-sublayer model in which the cell surface charge layer consists of three sublayers, the outer sublayer (sublayer 1) bearing negatively charged groups of valence z_1 and number density N_1, the middle sublayer (sublayer 2) bearing no

charge, where negative and positive charges are compensated with each other, and the inner sublayer (sublayer 3) bearing positively charged groups of valence z_3 and number density N_3 (Fig. 3).

The mobility formula corresponding to this model is given as below,

$$\mu = \frac{e}{\eta\lambda^2}\left\{ z_1 N_1 \left[1 + \left(\frac{\lambda}{\kappa}\right)^2 \frac{1+\lambda/2\kappa}{1+\lambda/\kappa}\right] + \left(z_1 N_1 e^{-\kappa d_1} - z_3 N_3 e^{-\kappa d_2}\right)\left(\frac{\lambda}{\kappa}\right)^2 \frac{\lambda}{2(\kappa-\lambda)} - \left(z_1 N_1 e^{-\lambda d_1} - z_3 N_3 e^{-\lambda d_2}\right)\left(\frac{\lambda}{\kappa}\right)^2 \frac{\lambda^2}{\kappa^2-\lambda^2}\right\}, \quad (2)$$

where $d_2 - d_1$ is the thickness of sublayer 2 and $d_c - d_2$ the thickness of sublayer 3. This three-sublayer model is expected to yield a more reasonable fitting since it provides a less abrupt change in the charge distribution than that given by the two-sublayer model. The results of curve fitting are shown in Fig. 4 and Table 2, in which the values of the parameters that produced the best fitting are listed. The agreement between the observed and calculated mobility values is as good as that attained with the two-sublayer model (Fig. 2) in a wide range of medium ionic strength while the values of d_1, d_2, and λ are fixed. In addition, the value of z_1N_1 becomes more negative as the pH of the medium increases, in accordance with increasing dissociation of the acidic groups. In view of this, the three-sublayer model is superior to the two-sublayer model. However, the model still gives mobility values much higher than those experimentally measured at very low ionic strengths. This suggests that the charge density is not uniform in the deep inside of the surface layer (sublayer 3) since the mobility values at very low ionic strengths reflect an increased contribu-

Table 2
Negative and positive charge densities, d_1, d_2 and $1/\lambda$ for human erythrocytes as a function of pH

pH	Charge density z_1N_1(M)	Charge density z_3N_3(M)	d_1 (nm)	d_2 (nm)	$1/\lambda$ (nm)
4.0	−0.070	0.900	4.0	6.0	0.9
4.5	−0.072	0.420	4.0	6.0	0.9
5.0	−0.072	0.280	4.0	6.0	0.9
6.0	−0.072	0.088	4.0	6.0	0.9
7.4	−0.074	0.088	4.0	6.0	0.9

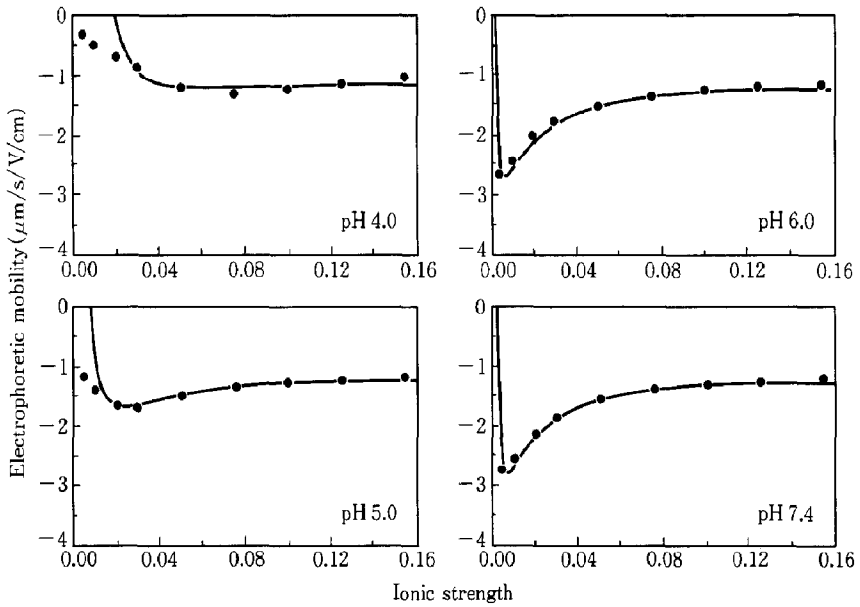

Fig. 4. Electrophoretic mobility–ionic strength curves for human erythrocytes at different pH values. Solid lines are the best fitted curves obtained with the three-sublayer model and closed circles are experimentally observed mobility values.

tion from the charges located in this part of the surface layer. The downward deviation of the experimentally determined mobility values from the theoretical curve would indicate that the density of the basic groups decreases toward the deep inside of sublayer 3.

In conclusion, the present work shows that the electrophoretic behavior of human erythrocytes can be explained by the three-sublayer model much better than by the two-sublayer model in a wide range of medium ionic strength at any pH.

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