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Study of Electrohydrodynamic Phenomena during Purification of Proteins by Continuous Flow Electrophoresis

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Abstract

Continuous flow electrophoresis is a process that enables one to purify or to separate proteins on a preparative scale. Its performance depends on a number of transport phenomena that act together to spread the protein stream, which may cause a loss of resolution. This paper is devoted to the study of one of them, called electrohydrodynamics, which is due to the difference existing between the electrical properties of the protein sample and those of its surrounding fluid. An experimental study was performed by using a device that provides visualization of the sample stream inside the separation chamber by working with an alternating current which enables only the influence of electrohydrodynamic effects to be studied without any interference from the other transport phenomena, such as electroosmosis and electrophoretic migration. The experimental results are found to be in very good agreement with the theoretical predictions, which give the sample elongation under different operating conditions, such as residence time, conductivities, and electric field strength.

INTRODUCTION

The quality of any purification or separation performed by continuous flow electrophoresis processes depends on a number of transport phenomena. Each one of these phenomena can modify the originally cylindrical form of the injected stream that contains the species to be separated. Because any spreading of that stream may cause a loss of resolution, it is important to appreciate the influence of these transport phenomena on the distribution of the components after they flow through the electrophoretic chamber.

Upon applying an electric field, in addition to the electrophoretic migration and to the carrier buffer flow, the sample components may be

submitted to an electroosmotic velocity due to the charges on the cell walls. That velocity is directed parallel to the electrical field. That electroosmotic velocity, due to the fixed charges existing on the walls of the separation chamber, depends on both the material of these walls and on the composition of the fluid used as the carrier buffer as well as on the electric field strength. Combined with electrophoretic migration and carrier buffer flow, this electroosmotic effect causes a deformation of the sample stream which is expected to look like a crescent at the outlet of the separation chamber (*1*).

According to the concentration and to the residence time of the components in the electrophoretic apparatus, diffusion phenomena can become significant and contribute to spreading of the sample stream. Moreover, during ground-based experiments, free convection may occur due to concentration or temperature gradients, and it can disturb the flow pattern of the fluids inside the chamber (*2*). All these phenomena are well known and have been studied, either theoretically or experimentally, by many authors working with electrophoretic separations.

Some recent experiments dealing with the electrophoretic separation of different colored latex fractions (*3*) have demonstrated the influence of the conductivity or dielectric constant of the sample compared to those of the carrier fluid. More precisely, increasing the conductivity of the injected latex sample was found to widen the corresponding peaks observed by measuring the concentration in each fraction of the collection port. That kind of observation can be explained, as proposed by Rhodes et al. (*4*), by taking account of another kind of transport phenomena called the "electrohydrodynamic phenomenon," previously studied as part of the physics of dielectric fluids. Indeed, Taylor (*5*) demonstrated that upon applying an electric field, the difference in conductivity between a sample and its surrounding fluid induces local distortions of the stream lines that result in an heterogeneity of the stresses acting on the surface of the sample. As a result, the sample stream elongates and its final form results from the combination of the different stresses that act on its external boundary.

During any separation in continuous flow electrophoresis, these transport phenomena—electroosmosis, diffusion, free convection, and electrohydrodynamics—take place simultaneously and determine the resolution of the process.

Most of the experimental results dealing with electrophoretic separation are reported as concentration profiles measured in each fraction of the collection port and located at the outlet of the electrophoretic chamber. On the other hand, some mathematical models have been developed to calculate these concentration profiles according to some of the transport phenomena involved in the process. Most of them take account of the carrier buffer Poiseuille flow, electroosmosis, and sometimes diffusion (*6*–

8). When comparing the experimental observations with the theoretical predictions, some difficulties may arise about the validity of the calculation. These difficulties are due to the lack of information concerning what really happens inside the chamber, from the injection point to the outlet, before the flow is collected as several fractions.

As a result, people interested in the study of electrophoretic processes have looked for an experimental device capable of giving some information about the sample stream shape inside the separation chamber without creating any significant disturbance. Strickler and Sachs (*1*) were the first to propose a visualization system for work with latex particle samples. They used it to demonstrate the existence of a "crescent" deformation due to the combination of the carrier buffer parabolic flow and electroosmosis. Rhodes and Snyder (*3*) and Miller et al. (*9*) also used the same kind of device to study the deformation due to conductivity gradients during electrophoretic separation of latex. These studies dealing with latex particles demonstrated the usefulness of any visualization system providing an image of the sample stream during flow through the electrophoretic chamber in order to improve understanding of the various transport phenomena involved in electrophoresis processes. However, it remains difficult to translate the results that come from these studies directly to the case of electrophoretic purification of proteins because of the great differences existing between physicochemical and electrical properties of latex and proteins. For example, in working with protein solutions, any variation in the concentration of the sample can change the conductivity and thus modify the influence of any conductivity-dependent phenomena.

This paper deals with a study of the relevance of electrohydrodynamic phenomena during electrophoretic purification of protein solutions. The experimental study was performed by using an original device which was specially designed in our laboratory (*10*). It enables the sample stream in any cross section inside the separation chamber to be visualized. From these observations one can measure the sample elongation from the inlet to the outlet of the apparatus. The experimental results obtained with different proteins in different buffers are compared with theoretical predictions, and they deal with the influence of such operating parameters as the electric field strength and the conductivity on sample distortion due to electrohydrodynamic effects.

II. MATERIAL AND METHODS

1. Experimental Set-up

The equipment used for the experimental study allows observation of the protein stream in a cross section inside the electrophoretic chamber.

To do this, a simplified electrophoretic chamber having no cooling compartments and no collection port is employed. That chamber, located between two quartz plates, is 150 mm long, 60 mm wide, and 3 mm thick. The sample is injected at the top of the chamber through a needle, the internal diameter of which is about 0.8 mm. Two compartments containing Pt electrodes are located on both sides of the chamber and separated from it by ionic exchange membranes. The visualization system, represented in Fig. 1, has been described into detail elsewhere (*10*). It consists of a laser light, at the outlet of which is placed a cylindrical mirror in order to provide a light sheet, the thickness of which does not exceed 1 mm. That sheet lights the electrophoretic chamber in a plane perpendicular to the main fluid flow. A CCD camera allows visualization in real time of the protein stream in a cross section of the chamber. The position for observation can be varied from the injection plane to the outlet of the chamber, which allows the change in the protein stream section during flow through the cell to be followed.

2. Fluids

Two carrier fluids, both at pH 6.9, were used for the experiments: a Tris-borate buffer (electrical conductivity $\approx$ 140 μS/cm) and an ampholyte solution at 1%, purchased from LKB (electrical conductivity $\approx$ 54 μS/cm). The samples injected into the electrophoretic chamber were protein solutions, obtained by dissolving lyophilized products and purchased from

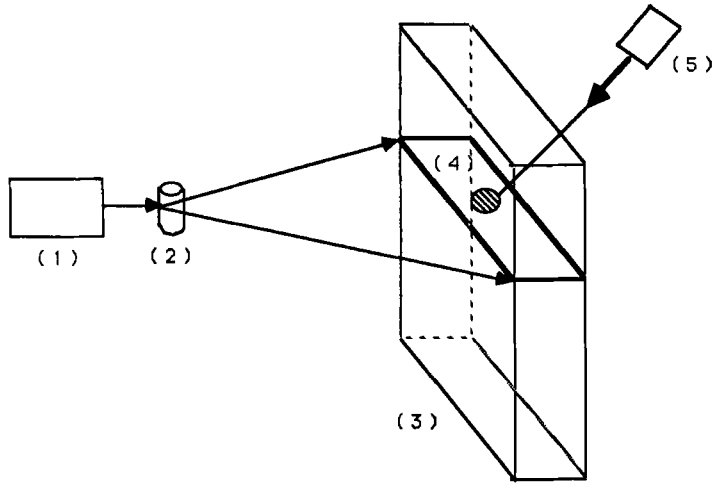

FIG. 1. System for protein stream visualization: (1) 5 mW He-Ne laser; (2) cylindrical mirror; (3) electrophoresis cell; (4) illuminated cross section of the cell; (5) CCD camera.

SIGMA Ltd., in the appropriate buffer. Two kind of proteins were used: γ-globulin (Gg) and hemoglobin (Hb). Various quantities of protein were dissolved in the buffers to alter the resulting conductivity of the samples.

3. Experimental Data

The visualization device enabled images showing the sample stream in any cross section of the electrophoretic chamber to be recorded. It was possible to measure the characteristic dimensions of the sample stream from photographs.

4. Procedure

Experiments were carried out by applying an alternating current (ac), whose frequency was 50 Hz, between the two electrodes.

Under these conditions it is assumed that electroosmosis and electrophoretic migration are negligible (*11*), so that only the influence of the electrohydrodynamic phenomena on sample distortion can be seen.

5. Temperature

All experiments were performed at room temperature (25°C). According to chamber geometry, the residence times, and the current intensities investigated, the temperature increase between the inlet and the outlet of the electrophoretic chamber is always lower than 1°C, so one can assume that the working temperature remains constant.

III. ELECTROHYDRODYNAMIC PHENOMENA

Electrohydrodynamic phenomena were first discussed by Taylor (*5*) when he was working on the physics of dielectric fluids. He showed that when a drop of any dielectric fluid is placed in another dielectric fluid whose electrical properties (electrical conductivity or dielectric constant) are different and an electric field is applied, this drop can be distorted. Distortion results from the heterogeneity of the stresses that act on the external surface of the drop due to local variations of the electrical field lines. According to the relative properties of the two dielectric fluids, the direction of drop elongation can be either parallel or perpendicular to that of the electric field. The rate of elongation u depends on the electrical properties of the fluids, on the size of the drop, as well as on the electric field strength. It can be expressed by using the following relationships:

$$u = FD \cos 2\theta \tag{1}$$

where

$$F = [rE^2\epsilon_e]/[12\pi(\mu_e + \mu_i)(R + 1)^2]$$

and

$$D = R^2 + R + 1 - 3S$$

As a result, the maximum elongation, obtained for $\theta = 0$, can be expressed as a function of the residence time t_s:

$$(Y - r_0) = FDt_s \tag{2}$$

assuming that the value of R remains constant as the sample elongates.

In these relationships, the subscripts i and e indicate the drop and its surrounding fluid, respectively. ϵ, σ, and μ are the dielectric constants, the conductivities, and the viscosities of the fluids, respectively, while r is the radius of the circular drop in the absence of any electric field. R and S represent, respectively, the ratios of the dielectric constants and the conductivities between the two fluids, i.e., $R = \sigma_i/\sigma_e$ and $S = \epsilon_i/\epsilon_e$. The direction of elongation is determined by the sign of D. When it is positive, the drop elongates in a direction parallel to that of the electrical field lines at infinity, while when it is negative, the drop elongates in a direction perpendicular to the electric field.

Recently Rhodes et al. (*4*) demonstrated the relevance of that phenomenon in the case of the electrophoretic processes in order to explain their experimental observations concerning the electrophoretic separation of latex particles under microgravity.

One can assume that the dielectric constant ratio S is close to unity when working with protein solutions. As a result, the main parameter is the conductivity ratio R between the protein sample and its surrounding fluid. The direction of elongation is then parallel or perpendicular to the field lines at infinity as the sample conductivity is respectively higher or lower than that of the external fluid. Furthermore, sample elongation is expected to increase as its conductivity differs more and more from that of the surrounding fluid. This means that for protein solutions, increasing the concentration of the sample, which generally leads to an increase in its electrical conductivity, should result in a more elongated sample.

IV. RESULTS AND DISCUSSION

Figure 2 gives some examples of photographs from which the sample elongations were determined. Note that at zero electrical field, which rep-

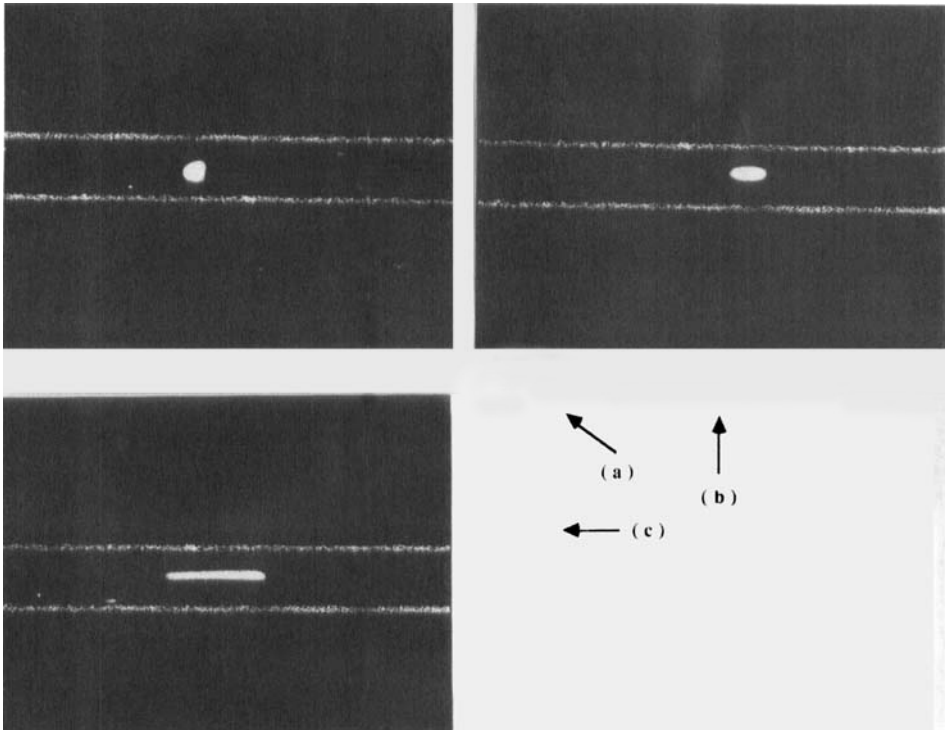

FIG. 2. Sample stream distortion for a hemoglobin solution as observed with the visualization system upon applying an AC current. *Operating conditions:* sample injection rate = 6.4 mL/h, buffer flow rate = 27 mL/min. *Carrier buffer:* Tris-borate, pH = 6.9, conductivity = 140 μS/cm. *Sample:* hemoglobin solution at 1.2% in Tris-borate buffer, pH = 6.9, conductivity = 206 μS/cm. (a) E = 0 V/cm; (b) E = 15 V/cm; (c) E = 30 V/cm. Sample residence time = 25 s.

resents the reference state for our experiments, the sample is located in the center of the chamber. More precisely, under normal operating conditions the value of r/d, which is the ratio of the sample radius over the half chamber thickness, remains lower than 0.4, so that the residence time can be considered to be almost constant over the sample cross-section.

Different sets of experiments were performed corresponding to conductivity ratios, i.e., sample conductivity over buffer conductivity, either lower or higher than unity.

For a sample whose conductivity is lower than that of the carrier fluid, it was found that deformation takes place in a direction perpendicular to the electric field, as expected from theory, and that it increases as the electric field strength increases. However, because the thickness of the

chamber was only 3 mm, it was found that the elongation is such that the sample reaches the walls even under smooth operating conditions such as, for instance, for $E = 15$ V/cm and $R = 0.8$. Consequently, the results reported and discussed later on concern only samples with conductivities higher than that of the carrier fluid in order to allow greater variations of the operating parameters.

The observation device enables us to visualize the sample stream in any cross section of the separation chamber. We first studied the influence of the residence time on sample elongation. Elongation is defined as the sample width at a given value of the electric field minus that at zero electric field. The experimental results obtained for various electric field strengths are reported on Fig. 3, and they show the variations of sample elongation versus residence time. For these experiments the residence time was varied by changing the observation position from the inlet to the outlet of the electrophoretic chamber.

One can observe that the sample elongation which equals zero in the injection plane increases continuously as the sample flows through the chamber. Furthermore, these variations are found to be linear. This means that in this range of residence time, the assumption that the acting force, which is the difference in conductivity between the sample and its surrounding fluid, remains constant is correct, so that the rate of elongation also remains constant.

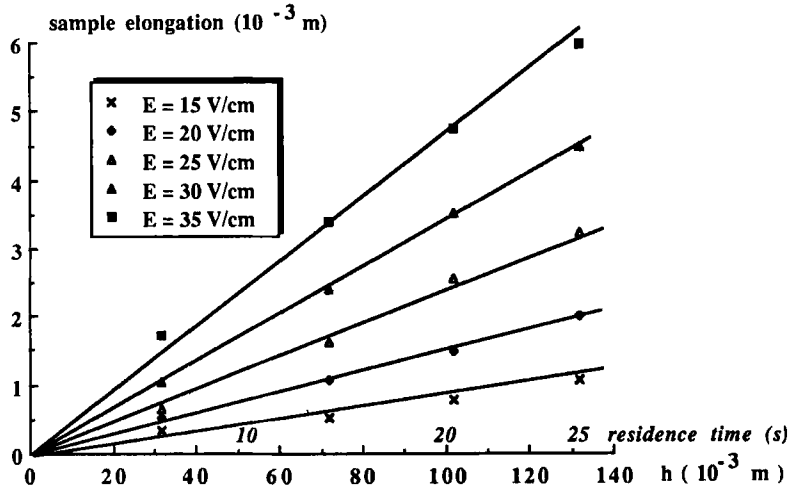

FIG. 3. Variations of the sample elongation as a function of its height in the chamber. Operating conditions and fluids as in Fig. 2.

On the other hand, the results show that for a given residence time the sample elongation becomes more and more important as the electric field increases, as expected from Eq. (2).

Because of the concentration conductivity dependence of protein solutions, we then investigated the influence of sample concentration on the elongation of a hemoglobin stream for various electrical field strengths. The values obtained are reported in Fig. 4 which gives the variations of the elongation versus the square of the electric field for three different concentrations. The first observation is that these variations look like straight lines, the slope of which increases as the protein concentration increases. That linear dependence of elongation versus E^2 is in very good agreement with the electrohydrodynamic theory that leads to Eq. (2). The same kind of behavior was reported by Rhodes et al. (*4*) who worked with latex samples in a phosphate buffer. Furthermore, according to Eq. (2) the sample width is expected to increase with conductivity and then with concentration, as is experimentally observed. For given operating conditions and carrier fluid properties, Eq. (2) can be rewritten as

$$\Delta y = (Y - 2r_0) = Bt_s f(R)E^2 \tag{3}$$

where

$$f(R) = [R^2 + R - 2]/[R + 1]^2$$

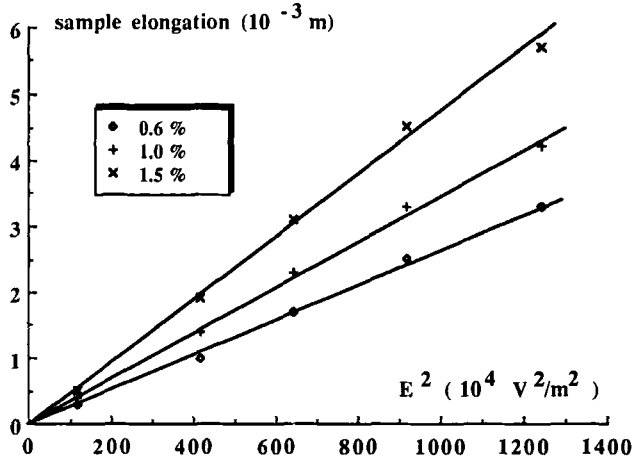

FIG. 4. Influence of the protein concentration on the variations of the sample elongation versus E^2 for an hemoglobin stream. Operating conditions and carrier buffer as in Fig. 2. Sample residence time = 25 s.

TABLE 1
Influence of the Protein Concentration (Fig. 4): Calculation of the Parameters Involved in Eq. (3)

Protein concentration (%)	Sample conductivity (μS/cm)	R	$f(R)$	Residence time (s)
0.6	180	1.286	0.180	25
1.0	198	1.414	0.242	25
1.5	216	1.543	0.297	25

In Table 1 we give the values of the parameters involved in Eq. (3) for the three concentrations investigated. From these values we then calculated those of the term $\Delta y/f(R)$, and they are plotted in Fig. 5 versus the square of the electric field. It is observed that the straight lines obtained for the different concentrations thus become superimposed.

This means that term B in Eq. (3) is independent of the concentration, because the residence time was kept constant. Therefore, for a given protein sample, the influence of concentration on the distortion seems to be due only to the resulting change in conductivity.

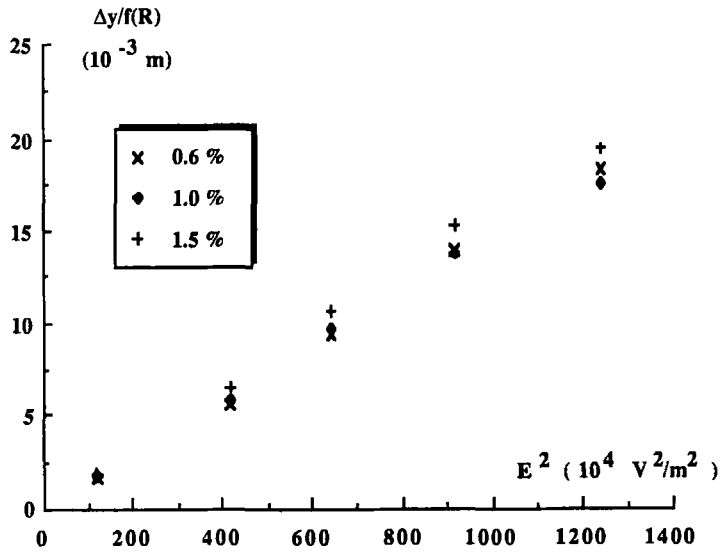

FIG. 5. Influence of the sample concentration on the electrohydrodynamic distortion. Data from Fig. 4 and Table 1.

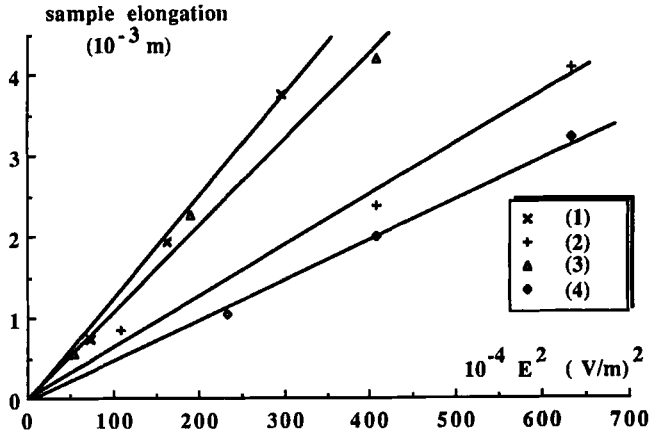

FIG. 6. Variations of the sample elongation versus E^2 for different proteins in different buffers. Data in Table 2.

To go further into an understanding of these phenomena, we also investigated the influence of the protein and of the carrier buffer. Some experimental results are reported in Fig. 6, which gives the variations of sample elongation versus the square of the electric field for two protein samples and two different buffers. Table 2 provides information concerning the fluids as well as the operating conditions used for these experiments. The first observation is that whatever the protein or the buffer, sample elongation varies linearly with E^2, as was already observed in one particular case. However, Fig. 6 shows that the straight lines that correspond to different protein–buffer associations are not superimposed. From these kinds of raw data which give the sample elongation under various operating conditions, it is difficult to draw any conclusion about the influence of

TABLE 2
Influence of the Protein–Buffer Association on the Electrohydrodynamic Distortion: Data Used for Figs. 6 and 7

Sample	Buffer	R (conductivity ratio)	$f(R)$	Residence time (s)
1. γ-Globulin, 0.3%	Tris-borate	1.62	0.33	47
2. γ-Globulin, 0.3%	Ampholytes	2.67	0.58	45
3. Hemoglobin, 0.6%	Ampholytes	1.74	0.37	43
4. Hemoglobin, 1.2%	Tris-borate	1.47	0.27	25

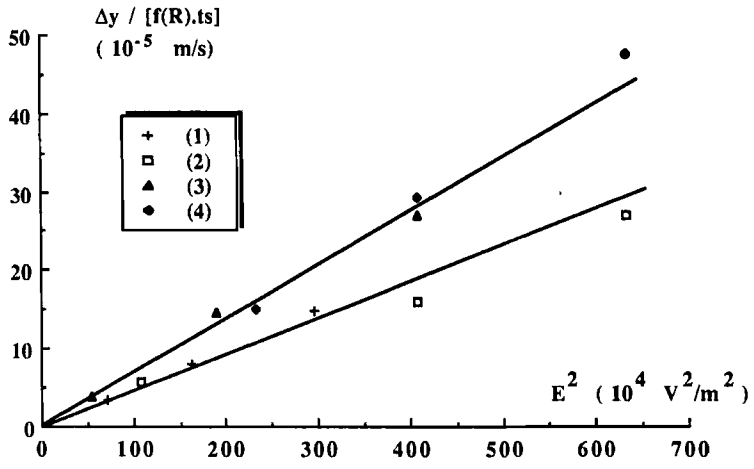

FIG. 7. Influence of protein and buffer properties on the electrohydrodynamic distortion. Data from Fig. 6 and Table 2.

protein or buffer because no general tendency is observed. One reason for this is that changing the buffer leads to a change in the conductivity of the sample, and that was found to modify sample elongation. To deal with this problem, we calculated the values of the parameters involved in Eq. (3). The values obtained are reported in Table 2 for the two proteins and the two buffers. They were used to plot the variations of the term $\Delta y/[f(R)t_s]$ versus the square of the electric field in Figure 7. From that plot it is possible to draw some conclusions about the influence of the fluid's properties. Indeed, Fig. 7 shows that for a given protein, either γ-globulin or hemoglobin, the values of $\Delta y/[f(R)t_s]$ obtained for the two buffer solutions are located on the same straight line. This means that the buffer itself has no influence on electrohydrodynamic distortion. Figure 7 also shows that the lines corresponding to γ-globulin and hemoglobin are not superimposed, and that the slope of the straight line obtained with hemoglobin is higher than that obtained with γ-globulin. This means that the nature of the protein seems to have an influence on electrohydrodynamic distortion, and that for the conditions we used the hemoglobin sample is more sensitive to electrohydrodynamic distortion than is the γ-globulin sample.

V. CONCLUSION

We were interested in the relevance of electrohydrodynamic phenomena due to differences in conductivity or dielectric constant when dealing with the electrophoretic separation of proteins.

An experimental study was carried out by using a visualization system that allowed measurement of the real dimensions of a protein sample inside

an electrophoretic chamber. An alternating current was used because it permitted us to study only the influence of electrohydrodynamics without any interference from other transport phenomena such as electroosmosis or electrophoretic migration.

We first demonstrated that sample elongation due to electrohydrodynamic phenomena varies continuously from the injection point to the outlet of the chamber. More precisely, we found that for given operating conditions the variations of sample elongation versus chamber height is linear in the range of residence time investigated.

We found that the influence of the electrical field is exactly as expected from theoretical predictions, i.e., that sample elongation varies linearly with the square of the electric field.

Because of the concentration conductivity dependence of protein solutions, which is one of the main differences between proteins and other kinds of products such as latex, we then investigated the influence of sample concentration. We found that sample elongation for a given protein becomes more and more important as the concentration increases, but that this can be explained by taking into account the resulting change in conductivity.

Finally, by working with different kinds of products, we have shown that for given operating conditions, sample elongation depends on the protein as well as on the carrier buffer. Through comparison of the experimental results with the theoretical predictions, we have proposed a way to understand the influence of fluids properties. In this way it was found that the nature of the protein has an intrinsic influence on sample elongation due to electrohydrodynamic phenomena. It was also found that the buffer seems to have no intrinsic influence on electrohydrodynamic distortion.

In order to study electrohydrodynamic phenomena, a mathematical model is presently being used to investigate, from a theoretical point of view, the time dependence of stream elongation, as well as its shape, in the separation chamber. These theoretical predictions will be compared with the experimental results provided by the cross-section illuminator.

We are also working both experimentally and theoretically on coupling between electrohydrodynamic phenomena and the other transport phenomena involved in electrophoretic processes applied to protein separations or purifications.

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