Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

An Experimental Device to Visualize a Protein Stream during Continuous Flow Electrophoresis

H. de Balmann[a]; V. Sanchez[a]
[a] LGC CNRS URA 192 LABORATOIRE DE CHIMIE PHYSIQUE ET ELECTROCHIMIE UNIVERSITE PAUL SABATIER, TOULOUSE CEDEX, FRANCE

To cite this Article de Balmann, H. and Sanchez, V.(1991) 'An Experimental Device to Visualize a Protein Stream during Continuous Flow Electrophoresis', Separation Science and Technology, 26: 1, 127 — 132
To link to this Article: DOI: 10.1080/01496399108050461
URL: http://dx.doi.org/10.1080/01496399108050461

SEPARATION SCIENCE AND TECHNOLOGY, 26(1), pp. 127–132, 1991

NOTE

An Experimental Device to Visualize a Protein Stream during Continuous Flow Electrophoresis

H. de BALMANN and V. SANCHEZ

LGC CNRS URA 192
LABORATOIRE DE CHIMIE PHYSIQUE ET ELECTROCHIMIE
UNIVERSITE PAUL SABATIER
31062 TOULOUSE CEDEX, FRANCE

INTRODUCTION

The quality of separation obtained by continuous flow electrophoresis depends on a number of transport phenomena. Each one of them can modify the originally cylindrical form of the injected stream that contains the species to be separated. Because any spreading out of this stream will cause a loss of resolution, it is necessary to appreciate the influence of these phenomena on the distribution of the components after they flow through the separation chamber.

Among these phenomena, electroosmosis is the most frequently considered to be responsible for spreading the sample stream until it looks like a "crescent" (*1*). Based on the concentration and the residence time of the products in the separation chamber, diffusion and free convection may take place and influence the shape of the sample stream. Some recent experiments dealing with the electrophoretic separation of latex particles (*2*) demonstrated the great influence of another kind of phenomena, called electrohydrodynamics, which is due to the difference in conductivity and dielectric constant between the sample and its surrounding fluid.

Most of the experimental results dealing with continuous flow electrophoresis concern the concentrations profiles at the outlet of the separation chamber, measured in each fraction of the collection port. When comparing these results with any theoretical calculation, it may be difficult to draw any conclusion because of the lack of information about what really takes place inside the separation chamber before the flow is divided into several fractions. Indeed, the measured concentration profiles correspond to the

integral of the sample distribution through the thickness of the separation chamber.

As a result, people interested in the study of continuous flow electrophoresis have looked for an experimental device that could provide information about the shape of the sample stream inside the separation chamber without disturbing the flow. Strickler and Sacks (*1*) were the first to report results obtained with a visualization system that worked with latex particles. By using this system, which involves lighting the outlet of the cell, they visualized the so-called "crescent phenomenon." More recently, Miller et al. (*3*) and Rhodes and Snyder (*2*) used the same kind of device to follow the spreading out of three colored latex fractions during their electrophoretic separation. These studies have demonstrated the usefulness of any visualization device that provides information about the sample stream inside the cell in order to improve understanding of the transport phenomena involved in continuous flow electrophoresis separations. Nevertheless, some questions remain when these observations are translated to the case of electrophoretic processes applied to protein purification because of the great differences in physicochemical and electrical properties existing between these two kinds of products.

In this paper we present a new experimental device that enables a protein stream inside the separation chamber to be visualized during continuous flow electrophoresis, without creating any disturbance on the flow. To illustrate the usefulness of the equipment, some photographs are presented that show the observed protein stream for various operating conditions while working with hemoglobin sample.

EXPERIMENTAL SETUP

The experimental setup consists of three main parts: the electrophoresis cell, the hydraulic system, and the visualization device.

The electrophoresis cell itself is a simple one, having no cooling compartments and no fractions collector at the outlet. The separation chamber is comprised between two quartz walls, 200 mm high, 60 mm wide, and 13 mm thick. The spacing between these walls, which represents the thickness of the chamber, is 3 mm. The carrier buffer is introduced into the separation chamber in such a way that a regular flat flow is established. After flowing through the cell, it is gathered in a single tube without disturbing the flow. The two electrode compartments, containing Pt electrodes and located at both sides of the chamber, are separated from it by ionic exchange membranes. The electrode solution, the composition of which is the same as that of the carrier fluid, is continuously recirculated in order to eliminate the gas bubbles that form when an electrical field is applied. The protein sample is injected at the entrance of the chamber

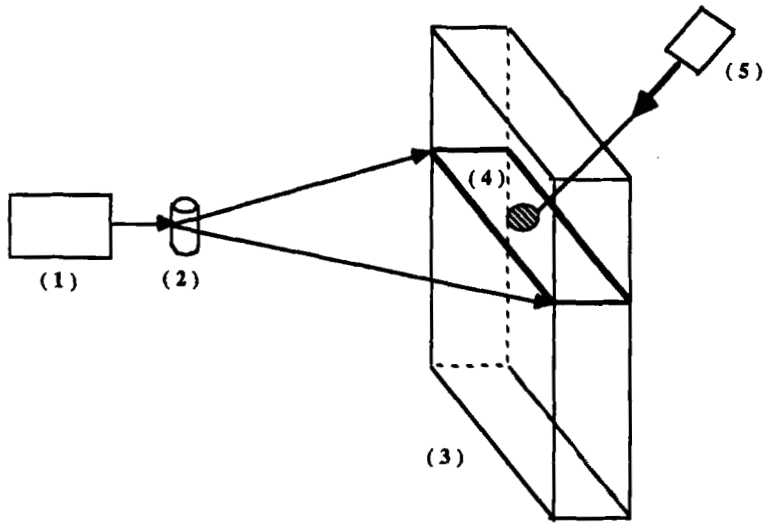

FIG. 1. Schematic of the visualization device: (1) 5 mW He-Ne laser, (2) cylindrical mirror, (3) electrophoresis cell, (4) illuminated cross section of the cell, (5) CCD camera.

through a nonmetallic hollow needle, the diameter of which is less than 1 mm.

The visualization device (Fig. 1) provides a light sheet that lights the cell in a plane perpendicular to the direction of the fluid flow. This sheet is less than 1 mm thick, and its position can be varied from the injection plane to the outlet of the electrophoretic chamber. Another function of the visualization system is to allow observation and recording of the images formed in that plane due to the light scattered by the protein stream. The optical bench used to create the light sheet consists of 5-mW He-Ne laser source, at the outlet of which a cylindrical mirror is placed. On the other side of the cell, with respect to the incident light, a CCD camera connected to a video screen enables the images to be followed in real time. A video recorder is used to store the results.

RESULTS

Figure 2 shows an example of the images that can be obtained by this method.

The carrier fluid used during these experiments was a Tris-borate buffer with a pH equal to 7.0 and an electrical conductivity around 140 μS/cm. The protein sample consisted of a hemoglobin solution prepared by dissolving a given quantity of powder purchased from Sigma Ltd. in a Tris-borate buffer in order to obtain a sample with a given conductivity. The protein concentration equaled 0.6% in weight. The carrier buffer was care-

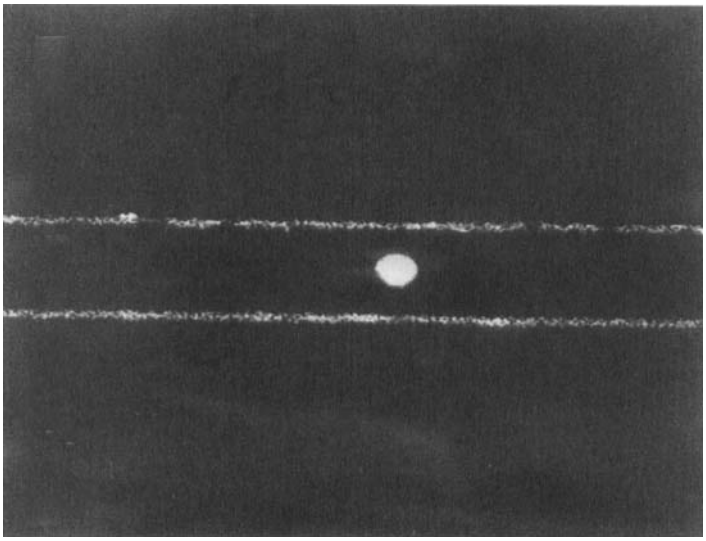

FIG. 2. Visualization of an hemoglobin stream. (a) Before applying the electric field. (b) Example of distortion observed under alternating current. (c) Example of distortion observed under direct current.

fully ultrafiltered and degassed before each run in order to remove any bacterial contaminators or bubbles.

Figure 2(a) shows the result obtained before applying the electric field. In this case the protein stream has a circular shape, located at the center of the separation chamber.

Figures 2(b) and 2(c) show the images under an applied electric field under various operating conditions. In the first case (b) an alternating current was used to study the influence of the electrohydrodynamic phenomena on the spreading of the sample. There is elongation of the protein stream compared to its shape before a current was applied. In the second case (c) a direct current was used. It is thus possible to visualize the resulting shape of the stream where all the transport phenomena act simultaneously and to observe the "crescent" deformation of the sample.

CONCLUSION

In this paper we briefly present a new equipment to visualize a protein stream inside a separation chamber during continuous flow electrophoresis. That equipment consists of an optical system that lights the cell in a plane perpendicular to the main fluid flow. The position of the light sheet can be varied in order to follow the change in the sample stream shape from the injection to the outlet of the electrophoretic chamber.

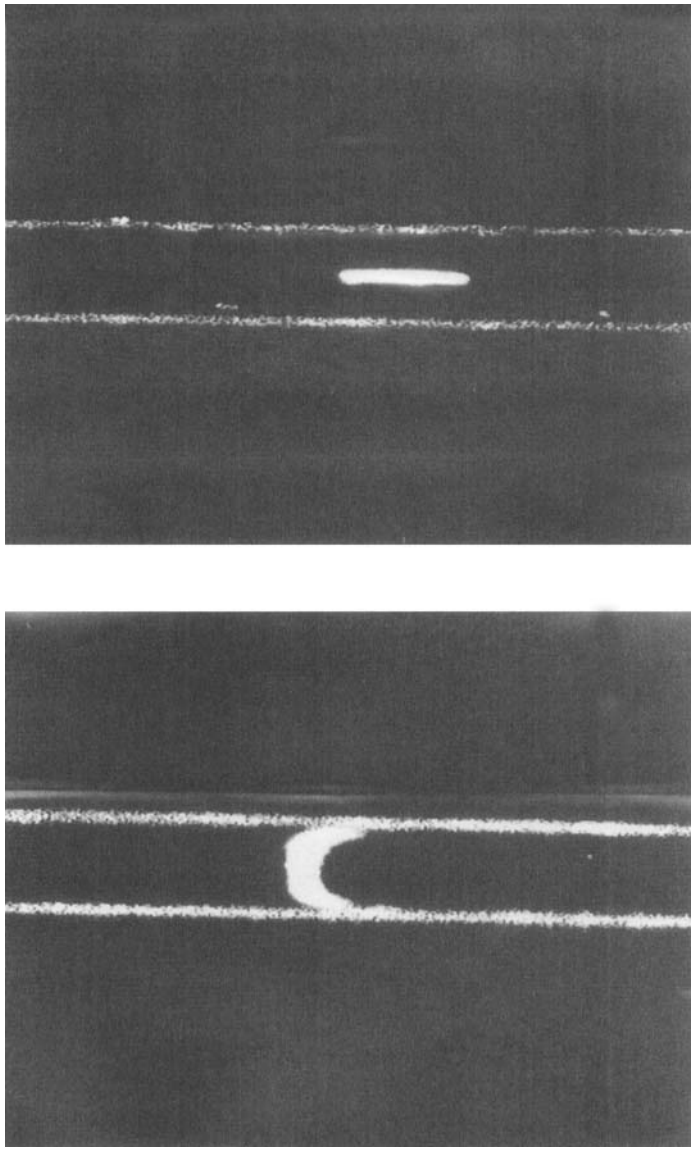

FIG. 2 (*continued*).

Working with a hemoglobin sample, we demonstrated that it is possible to use this device in order to study the influence of the various transport phenomena on the deformation of the stream during its flow through the electrophoresis cell.

This kind of information has now to be used more systematically in order to complete the traditional experimental measurements that give the concentration profiles of the products after they flow through the fractions collector. It will thus become possible to compare in a better way the experimental results with theoretical predictions in order to determine the validity of any assumption made in the calculations or to determine the main phenomena with respect to the spread of the stream according to the operating conditions and the fluid properties.

Finally, the equipment presented here is not specific for studies dealing with electrophoresis. It may be useful for other applications in which the visualization of a protein stream can provide more information compared with traditional measurements.

REFERENCES

1. A. Strickler and T. Sachs, *Ann. N. Y. Acad. Sci., 20,* 497 (1973).
2. P. H. Rhodes and R. S. Snyder, *Electrophoresis, 7,* 113 (1986).
3. T. Y. Miller, G. O. Williams, and R. S. Snyder, *Ibid., 6,* 377, (1985).

Received by editor December 18, 1989