Journal of Chromatography, 347 (1985) 191–198
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 005

HIGH-PERFORMANCE ELECTROPHORESIS

ELIMINATION OF ELECTROENDOSMOSIS AND SOLUTE ADSORPTION

STELLAN HJERTÉN
Institute of Biochemistry, University of Uppsala, Biomedical Center, P.O. Box 576, S-751 23 Uppsala (Sweden)
(Received July 9th, 1985)

SUMMARY

In ideal electrophoresis in free solution neither electroendosmosis nor adsorption of solutes onto the inside of the electrophoresis chamber should occur. In this paper we show that these two disturbing phenomena are negligible in free high-performance electrophoresis when the narrow-bore electrophoresis tube is coated with a mono-molecular layer of non-cross-linked polyacrylamide. The coating procedure is described in detail.

INTRODUCTION

Zone deformation caused by the Joule heat is often negligible in high-performance electrophoresis, since the separations take place in thin-walled, narrow-bore (0.05–0.3 mm I.D.) tubes. However, charges on the inner surface of the tube may give rise to zone distortion due to adsorption of the substances to be separated. (Solute interactions with the tube wall of other than an electrostatic nature are also possible.) These charges also lead to electroendosmosis. In this paper a new method aimed at the elimination of both electroendosmosis and adsorption is described.

MATERIALS AND METHODS

γ-Methacryloxypropyltrimethoxysilane (Pharmacia, Sweden) was covalently bound to the inside of the glass wall of an electrophoresis tube according to the instructions of the supplier. The carrier ampholytes for isoelectric focusing (Pharmalyte®) were also from Pharmacia. Acrylamide, potassium persulphate and N,N,N′,N′-tetramethylethylenediamine (TEMED) were of electrophoresis grade (Bio-Rad Labs., Richmond, CA, U.S.A.). Terephthalic acid, 4-hydroxybenzoic acid and β-naphthylacetic acid were kindly supplied by Dr. K. G. Wahlund, Department of Analytical Pharmaceutical Chemistry, Biomedical Center, Uppsala, Sweden. Human transferrin was a gift from KABI/VITRUM (Stockholm, Sweden). Human

0021-9673/85/$03.30

haemoglobin was prepared from outdated blood (Academic Hospital, Uppsala, Sweden).

EXPERIMENTAL AND RESULTS

Coating of the inner walls of glass or quartz electrophoresis tubes with a monomolecular polymer layer to eliminate electroendosmosis and adsorption of solutes

The method is based on the use of a bifunctional compound in which one group reacts specifically with the glass wall and the other with a monomer taking part in a polymerization process. Examples of such bifunctional compounds are γ-methacryloxypropyltrimethoxysilane, vinyltriacetoxysilane, vinyltri(β-methoxyethoxy)silane, vinyltrichlorosilane and methylvinyldichlorosilane, where one or two of the methoxy, acetoxy, methoxyethoxy or chloro groups react with the silanol groups in the glass wall, whereas the acryl or vinyl groups with acryl or vinyl monomers to form a polymer, *e.g.*, non-cross-linked polyacrylamide, poly(vinylpyrrolidone), poly(vinyl alcohol). Non-covalently attached polymer is then removed simply by rinsing with water. This procedure gives a thin, well defined monomolecular layer of a polymer covalently bound to the glass wall and thus differs from a previously described method[1] which gives a much thicker layer of the polymer (often methylcellulose).

A detailed description of the new procedure used to coat a capillary tube is given below. Although it applies specifically to polyacrylamide coatings, it can with obvious modifications be used for coatings of other polymers, for instance poly(vinyl alcohol) and poly(vinylpyrrolidone).

About 80 μl of γ-methacryloxypropyltrimethoxysilane were mixed with 20 ml of water, which had been adjusted to pH 3.5 by acetic acid. This silane solution was sucked up into the glass capillaries. After reaction at room temperature for 1 h the silane solution was withdrawn. The tubes were washed with water and then filled with a deaerated 3 or 4% (w/v) acrylamide solution containing 1 μl TEMED and 1 mg potassium persulphate per ml solution. After 30 min the excess of (not attached) polyacrylamide was sucked away and the tubes were rinsed with water. Most of the water in the tubes was removed by aspiration and the remainder by drying in an oven at 35°C.

Zone electrophoresis in coated and non-coated tubes

The first experiments were performed with the free zone electrophoresis apparatus where convective disturbances are eliminated by rotating the horizontal quartz electrophoresis tube (380 × 3 mm I.D.)[1]. The sample consisted of an artificial mixture of some aromatic carboxylic acids: terephthalic acid (I), 4-hydroxybenzoic acid (II) and β-naphthylacetic acid (III). The experiments were carried out in a 0.1 *M* Tris–acetic acid buffer (pH 8.6) at 1.840 V (2.6 mA). The rotating tube was scanned with light of wavelengths 280 and 310 nm at the start and after electrophoresis for 5 and 15 min. The ratio between the transmissions at these wavelengths was automatically recorded in order to suppress noise and irregular variations in the baseline of the electropherogram[1]. The experiments were performed in non-coated (Fig. 1a), polyacrylamide-coated (Fig. 1b) and methylcellulose-coated (Fig. 1c) tubes (the methylcellulose treatment is described in ref. 1).

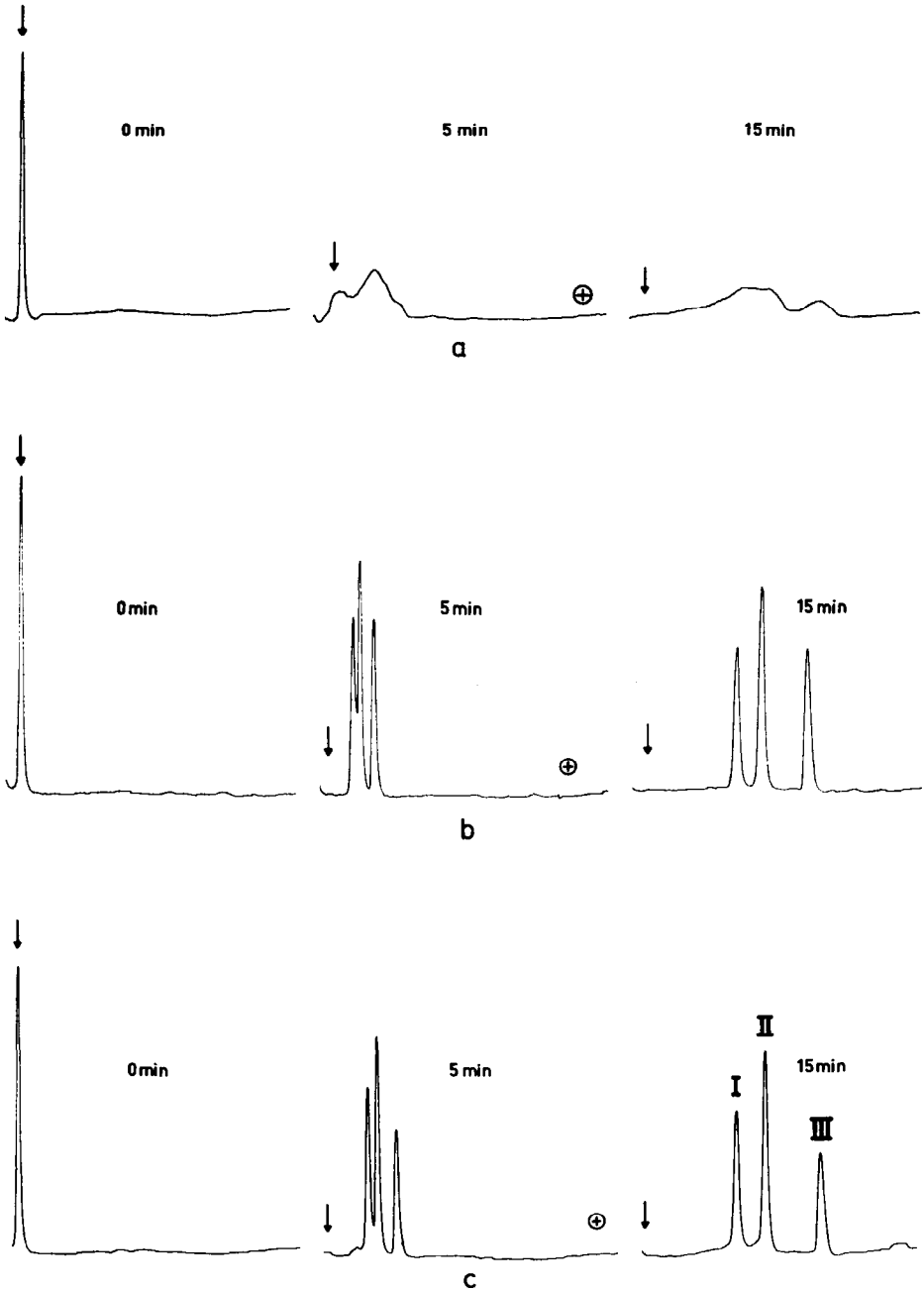

Fig. 1. Zone electrophoresis of aromatic carboxylic acids in a tube of inner diameter 3 mm. The experiments were carried out in the free zone electrophoresis apparatus with a rotating quartz electrophoresis tube of length 380 mm. The experiments were conducted in a non-coated tube (a), in a tube coated with non-cross-linked polyacrylamide (b), as described herein, and in a tube coated with methylcellulose (c), as described in ref. 1. The very marked zone broadening in Fig. 1a is chiefly caused by the hydrodynamic reflow attendent upon the electroendosmosis. No indications of electroendosmosis (or reflow) or adsorption are seen in Fig. 1b and c.

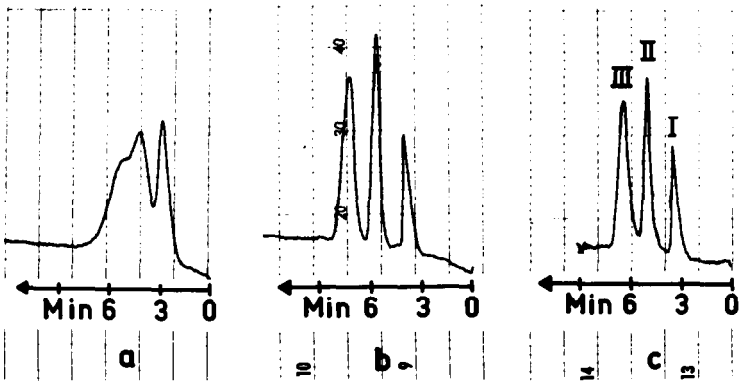

Fig. 2. Zone electrophoresis of aromatic carboxylic acids in a tube of inner diameter 0.2 mm. The experiments were conducted in the high-performance electrophoresis apparatus equipped with a glass tube of length 160 mm, in a non-coated tube (a), in a tube coated with non-cross-linked polyacrylamide (b), as described herein, and in a tube coated with methylcellulose (c), as described in ref. 1.

The above experiments were then repeated in the high-performance electrophoresis apparatus[2–6]. The electrophoresis tube had dimensions 160 × 0.2 I.D. × 0.4 mm O.D. The voltage applied was 2000 V (50 μA). The recording was done by on-tube absorption measurements[2,3] at 280 nm (Fig. 2).

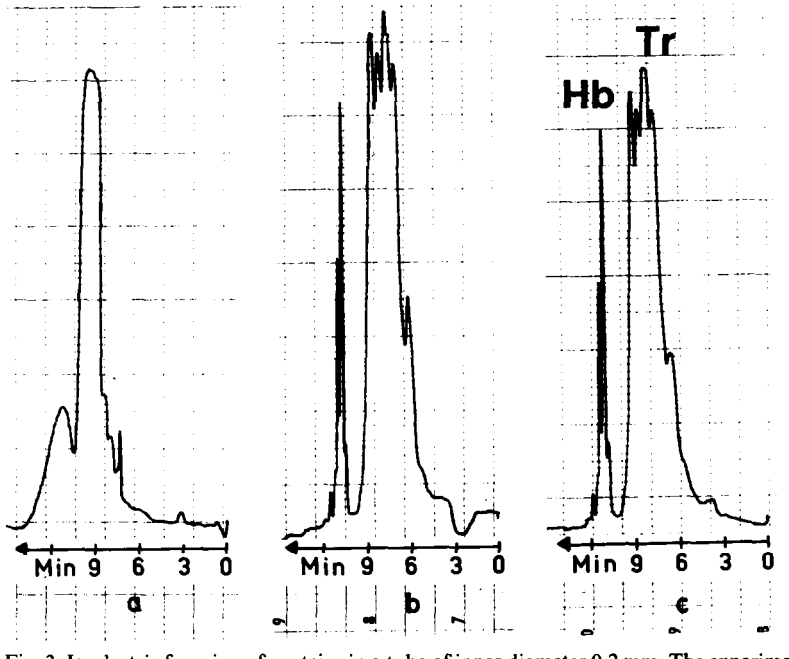

Fig. 3. Isoelectric focusing of proteins in a tube of inner diameter 0.2 mm. The experiments were conducted in the high-performance electrophoresis apparatus in a glass tube of length 120 mm. The sample consisted of transferrin (Tr) and haemoglobin (Hb). Details of tubes a–c as in Fig. 2.

Isoelectric focusing in coated and non-coated tubes

The experiments were performed in glass tubes, 120 × 0.2 I.D. × 0.4 mm O.D., filled with a mixture of human haemoglobin (final concentration: 3 μg/μl), human transferrin (final concentration: 5 μg/μl) and Pharmalyte®, pH 3–10 (final concentration: 1%, v/v). The tube was coated with non-cross-linked polyacrylamide as described above. Focusing was carried out in the high-performance electrophoresis apparatus with on-tube detection at 280 nm at 2000 V for about 15 min with 0.02 *M* phosphoric acid as anolyte and 0.02 *M* sodium hydroxide as catholyte. Elution of the focused protein zones was achieved by replacing the anolyte with 0.02 *M* sodium hydroxide (for details of the elution procedure, see ref. 6). The protein pattern is shown in Fig. 3a. The experiment was repeated in glass tubes coated with non-cross-linked polyacrylamide (Fig. 3b) and methylcellulose (Fig. 3c).

DISCUSSION

Electroendosmosis, which is caused by the presence of fixed charges in the electrophoresis tube (chamber), causes primarily only a displacement of the electrophoretically migrating zones. Although this displacement does not itself cause distortion of the zones, it precludes any possibility of determining accurately the absolute values of the electrophoretic mobilities. Electroendosmosis may, however, be attended by a hydrodynamic reflow, which causes the zones to become strongly parabolically distorted (see Fig. 16 and eqn. 56 in ref. 1). A reflow occurs when the resistance to hydrodynamic flow in the electrophoresis tube is relatively low. For instance, the electroendosmotic reflow is very pronounced in free electrophoresis in a non-coated tube of length 380 mm when the inner diameter is 3 mm (Fig. 1a), but is strongly suppressed when the diameter is reduced to 0.2 mm (Figs. 2a and 3a). Supporting media, such as cellulose powder and gels of agarose or cross-linked polyacrylamide, give too high a flow resistance to allow any reflow.

Electroendosmosis without reflow does not cause significant distortion of a zone, as stated above. A prerequisite is, however, that the zeta potential of the glass wall is the same throughout the length of the tube. This is not always the case. For instance, a non-uniform adsorption of solutes to the charged glass wall may lead to a non-uniform charge distribution on it, which will, in turn, give locally different electroendosmotic migration velocities, resulting in asymmetric zones (peaks).

The surface charges causing electroendosmosis may also give rise to adsorption of the sample solutes onto the glass tube wall. If the wall is coated with a neutral polymer to eliminate electroendosmosis the solutes may be sterically prevented from coming into contact with the wall and therefore from being adsorbed. In this way interactions other than the electrostatic ones are also suppressed. From a comparison of Fig. 1a with b and c, Fig. 2a with b and c and Fig. 3a with b and c, it is obvious that a monomolecular layer of non-cross-linked polyacrylamide (applied by the method described herein) is as efficient as a thicker layer of methylcellulose (applied as previously descriubed[1]) in suppressing zone broadening caused by adsorption or/and electroendosmosis. The absence of adsorption onto the coated glass walls is evident from the fact that the peaks are symmetrical.

Macromolecular substances often show greater tendencies to adsorb to any surface than do low-molecular-weight compounds. The reason is that macromole-

cules have many more binding sites and accordingly can be adsorbed by multipoint attachment[7]. Therefore, it is particularly important to coat the tube when the sample consists of biopolymers, such as proteins, and when the electrophoresis is performed in dilute buffers, since such buffers often do not efficiently suppress the electrostatic interactions between the solute and the glass wall. Although analysis times can be shortened by the use of low buffer concentrations, excessively low concentrations should not be used because they will give rise to relatively large differences in conductivity between a zone and the surrounding buffer, resulting in skewed peaks even in the absence of adsorption; see eqn. 34b and Fig. 12 in ref. 8. In this paper, with the aid of isoelectric focusing experiments, the significance of treating the tube wall with polymers to avoid distortion of protein zones has been demonstrated (Fig. 3). However, the distortions are often more marked in zone electrophoresis, since in this technique the zones are not automatically sharpened as in isoelectric focusing.

Jorgensen and DeArman Lukacs[9] modified the surfaces of silica tubes with glycol groups to decrease the adsorption of proteins. However, if the largest peak in the serum electropherogram in Fig. 9 of ref. 9 corresponds to albumin, electroendosmosis is still very pronounced.

The treatment of the glass tubes with methylcellulose involves baking at elevated temperature and low pH in the presence of formaldehyde[1]. These conditions make the coating more stable because the methylcellulose molecules become cross-linked and perhaps covalently attached to the silanols of the glass wall. In a study of various coating agents for suppression of electroendosmosis, Vanderhoff *et al.*[10] confirmed the high efficiency of methylcellulose. They used γ-glycidoxypropyltrimethoxysilane in an attempt to link the methylcellulose covalently to the tube wall via the epoxide group of the silane molecule. It is, however, very questionable whether this reaction took place under the conditions used (opening of the epoxide ring requires an alkaline pH). The method is very time-consuming, since the authors state that "extensive rinsing of the coated columns for a period of at least 3 days is required to remove the "physically adsorbed" methylcellulose from the surface". In addition, the "physically adsorbed" methylcellulose "may desorb from the cell wall and readsorb onto the colloidal particles" which "would obivate the electrophoretic separation". The coating procedure described herein is rapid and creates a well defined monomolecular polymer layer with so little leakage of polymer material that possible adsorption of released polymer to the substances to be separated will not change their mobilities.

Radola[11] has described isoelectric focusing in gels of cross-linked polyacrylamide covalently bound to glass plates or polyester films pretreated with methacryloxypropyltrimethoxysilane.

The theoretical explanation for the use of a polymer coating to eliminate electroendosmosis is given below. Electrophoresis and electroendosmosis are roughly governed by the classical equations

$$u_{ep} = \varepsilon\zeta_{ep}/4\pi\eta \tag{1}$$

$$u_{eo} = \varepsilon\zeta_{eo}/4\pi\eta \tag{2}$$

where u_{ep} is the electrophoretic mobility, u_{eo} the electroendosmotic mobility, ζ_{ep} the

zeta potential of the solute, ζ_{eo} the zeta potential of the tube wall, ε the dielectric constant and η the bulk viscosity. These equations show that there is no net gain in suppressing electroendosmosis by increasing the viscosity of the buffer, since the electrophoretic mobility will also decrease by the same extent as the electroendosmotic mobility. According to these equations, a feasible way to suppress electroendosmosis, *i.e.*, to get $| u_{eo} | \ll | u_{ep} |$, is to operate under conditions such that $| \zeta_{eo} | \ll | \zeta_{ep} |$. However, in practice, it is very difficult to find materials for the electrophoresis tubes such that this inequality obtains: even the most inert plastic tubes give considerable electroendosmosis[1]. However, the following formula, which I derived several years ago and which is more general than the Helmholz formula (eqn. 2), indicates another way to suppress electroendosmosis[1]

$$u_{eo} = \frac{\varepsilon}{4\pi} \int_0^{\zeta_{eo}} \frac{1}{\eta} \cdot d\psi \tag{3}$$

where ψ is the electric potential. The value of the integral will approach zero when the viscosity, η, in the double layer close to the tube wall approaches infinity. Accordingly, if the inner surface of an electrophoresis tube (chamber) is coated with a polymer solution of high viscosity, electroendosmosis will be virtually eliminated. Any neutral polymer that is soluble or swells in water can be used, for instance methylcellulose or non-cross-linked polyacrylamide. It should be mentioned that these polymers, dissolved in the buffer, will also suppress electroendosmosis[1,2], probably because the polymers tend to adhere to the tube wall and thereby create a thin surface layer of high viscosity. The electrophoretic mobility is, of course, higher in buffer alone than in a polymer-containing (and therefore viscous) buffer (see eqn. 1), which is an obvious advantage when short analysis times are desired.

Since in free solution electroendosmosis causes all electrophoretically migrating zones to be displaced to the same extent, it cannot give rise to any separation of the sample solutes in a carrier-free medium, contrary to what has been stated.

ACKNOWLEDGEMENTS

All experiments have been skilfully carried out by Mrs. Karin Elenbring which is greatfully acknowledged. The work was financially supported by the Swedish Natural Science Research Council and the Wallenberg Foundation.

REFERENCES

1 S. Hjertén, *Chromatogr. Rev.*, 9 (1967) 122–219.
2 S. Hjertén, *J. Chromatogr.*, 270 (1983) 1–6.
3 S. Hjertén, in H. Hirai (Editor), *Electrophoresis '83, Proceedings of the Third Meeting of the International Electrophoresis Society*, Walter de Gruyter, Berlin, 1983, pp. 71–79.
4 S. Hjertén and M.-D. Zhu, *Pure Appl. Chem.*, (1985) in press.
5 S. Hjertén and M.-D. Zhu, *J. Chromatogr.*, 327 (1985) 157–164.
6 S. Hjertén and M.-D. Zhu, *J. Chromatogr.*, 346 (1985) 265.
7 S. Hjertén, J. Rosengren and S. Påhlman, *J. Chromatogr.*, 101 (1974) 281–288.

8 S. Hjertén, in G. Milazzo (Editor), *Topics in Bioelectrochemistry and Bioenergetics*, Vol. 2, Wiley, New York, 1978, pp. 89–128.
9 J. W. Jorgensen and K. DeArman Lukacs, *Science, (Washington, DC)*, 222 (1983) 266–272.
10 J. W. Vanderhoff, F. J. Micale and P. H. Krumrine, *Sep. Purif. Methods*, 6 (1977) 61–87.
11 B. J. Radola, *Electrophoresis*, 1 (1980) 43–56.
12 S. Hjertén, *Ark. Kemi*, 13 (1958) 151–152.