Journal of Chromatography, 270 (1983) 1–6
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,099

HIGH-PERFORMANCE ELECTROPHORESIS: THE ELECTROPHORETIC COUNTERPART OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

STELLAN HJERTÉN
Institute of Biochemistry, University of Uppsala, Biomedical Center, P.O. Box 576, S-751 23 Uppsala (Sweden)
(Received June 24th, 1983)

SUMMARY

High-performance electrophoresis is the electrophoretic equivalent of high-performance liquid chromatography. Very efficient cooling is obtained because the experiments are performed in glass tubes of small diameter (0.05–0.3 mm) with a wall thickness of only 0.1–0.2 mm. Under these conditions the thermal deformation of a zone is small even at high field strengths, which allows high resolution and short run times. The solutes are recorded directly in the glass tube as they migrate electrophoretically through the stationary UV detector of a free-zone electrophoresis or gel-scanning apparatus. Accordingly there is no delay in the detection caused by time-consuming derivatization (staining).

The electrophoresis can be performed both in sieving and non-sieving gels and also in carrier-free solution. An analysis, which may comprise low-molecular-weight compounds, proteins, nucleic acids, viruses, etc., takes at present 10–60 min. This time can be considerably reduced by using a liquid-cooled electrophoresis tube. About 0.01–1 μg of material is suitable in the case of proteins.

INTRODUCTION

Electrophoresis, centrifugation and chromatography are the most effective and widely used methods for the separation of biopolymers. All three methods are based on the differential transport velocities of the solutes in the separation chamber, *i.e.* in the electrophoresis cell, the centrifuge tube or the chromatography column. In electrophoresis these velocities are determined by the charges of the solutes, in centrifugation by their sizes (densities) and in chromatography by their affinities for the bed material. The significant principal difference between electrophoresis, centrifugation, and chromatography is that the transport of the solutes is evoked differently: namely by an electrical field, a centrifugal field and a liquid flow, respectively.

These qualitative considerations indicate considerable analogies among electrophoresis, centrifugation and chromatography as regards the separation mechanisms. It is therefore natural that for example a new chromatographic method will

0021-9673/83/$03.00

be followed shortly by its counterpart in electrophoresis or centrifugation*. We shall now describe a method which is the electrophoretic counterpart of high-performance liquid chromatography (HPLC) and which therefore may be termed high-performance electrophoresis (HPE).

CHARACTERISTIC FEATURES OF HIGH-PERFORMANCE ELECTROPHORESIS

HPE is defined as the electrophoretic counterpart of HPLC and should therefore have the same attractive characteristic features as HPLC, *i.e.* high resolution, short run times, high sensitivity, and on-line monitoring of the solute, *i.e.* no time-consuming derivatization (staining).

High resolution

High chromatographic resolution can be obtained by minimizing eddy diffusion and improving the rate of mass exchange so that quasi-equilibrium is achieved in a very short time. To accomplish this the beads forming the bed should be small, uniform and very regularly packed. Also in electrophoresis, high resolution requires a homogeneous medium. Radial temperature gradients in the separation chamber are equally detrimental in all methods, but are a practical problem only in the case of electrophoresis, where significant heat evolution occurs in the separation chamber itself. High-resolution electrophoresis therefore requires very efficient dissipation of the Joule heat to minimize parabolic distortion of the zone[1] (see below).

Short run times

In chromatography the duration of a run can be shortened by the use of short columns and/or high flow-rates. The same is true in the case of electrophoresis by the use of short columns and/or high field strengths. However, the nature of the separation mechanism requires a separation chamber of reasonable length to allow any separation at all. Rapid separations therefore require high field strengths. The large amount of heat evolved must then be rapidly transported to the coolant in order to minimize thermal deformation of the electrophoretic zones. This can be accomplished by making the electrophoresis column from a material with a high thermal conductivity and very thin walls. In addition, the diameter or the thickness of the electrophoresis chamber should be kept at a minimum. All of these requirements are fulfilled if glass capillaries of an inner diameter of 0.05 0.3 mm and a wall thickness of 0.1 0.2 mm are used as electrophoresis tubes.

High sensitivity

In electrophoresis the sensitivity depends (as in chromatography) on the quality of the detection system.

On-line monitoring

In chromatography the solutes are often detected spectrophotometrically by

* Examples of such analogous methods are: isopycnic centrifugation, isoelectrioc focusing and chromatofocusing; displacement electrophoresis, displacement chromatography and displacement centrifugation. Unfortunately, many separation methods are given names which do not refer to the analogous methods, which makes it difficult for an uninitiated person to see the similarities between different techniques and therefore learn them rapidly and use them properly.

means of a flow cuvette as they leave the column. In electrophoresis the use of such an external cuvette is seldom practicable. It is more appropriate to use a segment of the electrophoresis tube itself as the "cuvette" for a stationary UV beam. As in chromatography, steps involving derivatization or staining and destaining are then superfluous.

THE EQUIPMENT

The electrophoresis tube

As mentioned above, we use glass tubes with a wall thickness of only 0.1–0.2 mm to minimize thermal deformation of the zones. Another advantage of these thin-walled glass tubes is that they allow transmission of UV light. It is thus unnecessary to use brittle and more expensive quartz tubes. The electrophoresis tube should not be made of plastic owing to the low thermal conductivity of plastics compared with glass. Furthermore, gels such as agarose and polyacrylamide do not adhere firmly to these hydrophobic materials, which is a prerequisite for high resolution runs. Also the quality of the glass is of importance to get proper adhesion. Some glass tubes have very poor adhesion properties. The glass tubes are drawn over a Bunsen burner to the dimensions desired. All experiments reported here were done in columns without active cooling.

The detection system

In the free-zone electrophoresis apparatus the solutes are localized by scanning the rotating electrophoresis tube[1]. This technique is very attractive, since it allows repeated recordings during the course of a run, but is not a suitable detection method for HPE because the optical properties of the thin-walled electrophoresis tube vary considerably along the length of the tube, causing serious irregularities in the baseline. Therefore we let the solutes migrate electrophoretically past a stationary UV detection system (a narrow light beam passes the electrophoresis tube and strikes a photomultiplier connected to a recorder). Owing to the short light path a high-sensitivity detection sytem is required for analyses of the extremely small amounts of material applied. We are constructing such a detector system. However, while awaiting its completion we use the UV detector of the free-zone electrophoresis[1] or that of a home-built equipment[2] for scanning of polyacrylamide gels (without moving the electrophoresis tube).

Sample application

The sample is applied by layering it on top of the gel. The application is facilitated by the addition of sucrose to the sample, which is electrophoresed into the gel at a relatively low voltage (20 V/cm) to avoid thermal convection. A narrow starting zone is obtained if the sample is given a conductivity about five times lower than that of the buffer. In experiments with extremely narrow-bore electrophoresis tubes the layering step has been omitted.

EXPERIMENTAL

All the experiments described were performed with a very provisional equipment without liquid cooling. The duration of a run varies from 10 to 60 min. The

apparatus we plan to build will give an efficient cooling which will permit higher field strengths and consequently much faster runs. Lower buffer concentrations than those used in this investigation will also allow shorter run times.

Fig. 1 refers to an electrophoresis experiment with low-molecular-weight compounds in a glass tube of *ca.* 0.15 mm I.D. and filled with a polyacrylamide gel of the composition $T = 10\%$; $C = 3\%$ (these parameters are defined in ref. 3). The experiment was conducted in a 0.1 *M* Tris–acetic acid buffer, pH 8.6, at a field strength of 100 V/cm and a current of 0.6 mA. The distance between the gel surface and the detector (the migration distance) was 8 cm. The recording, made at 265 nm, showed a baseline separation of the sample components (aromatic carboxylic acids). The analysis time was 10 min, which is comparable with the duration of HPLC runs.

High-molecular-weight substances have also been analyzed by the HPE technique. An example is shown in Fig. 2, which illustrates a run done with normal human serum. The experiment was performed in a 0.4% agarose gel as supporting medium; 0.1 *M* Tris–acetic acid, pH 8.6, was employed as buffer. At a field strength of 100 V/cm the current was 0.18 mA. The protein zones were recorded at 280 nm when they had migrated a distance of 6.5 cm. The pattern obtained is that expected when serum is electrophoretically analyzed in a non-sieving medium, such as an agarose gel.

Analysis of proteins in a molecular-sieving polyacrylamide gel was also performed. Fig. 3 illustrates a separation of monomers, dimers, trimers, tetramers and pentamers of bovine serum albumin. The experimental conditions were similar to those in the serum run with the difference that the field strength was 150 V/cm and the current 0.66 mA (the gel had the composition $T = 6\%$; $C = 3\%$).

Further applications, including an analysis of a sample of tobacco mosaic virus, are illustrated in ref. 4.

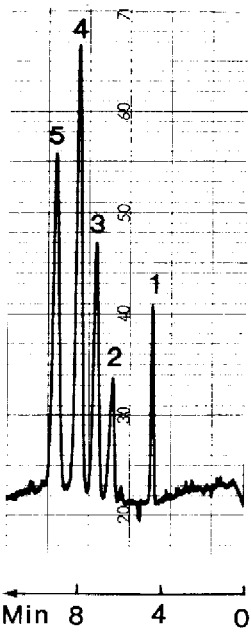
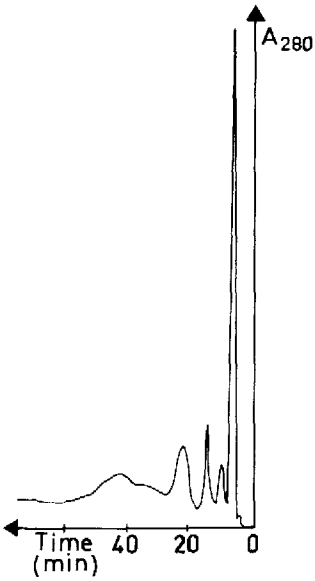


Fig. 1. High-performance electrophoresis of aromatic carboxylic acids. Supporting medium, polyacrylamide gel; sample, a mixture of terephthalic acid (1), benzoic acid (2), 4-hydroxybenzoic acid (3), 4-hydroxy-3-methoxybenzoic acid (4), and β-naphthylacetic acid (5); total amount of sample, 0.01 μg.



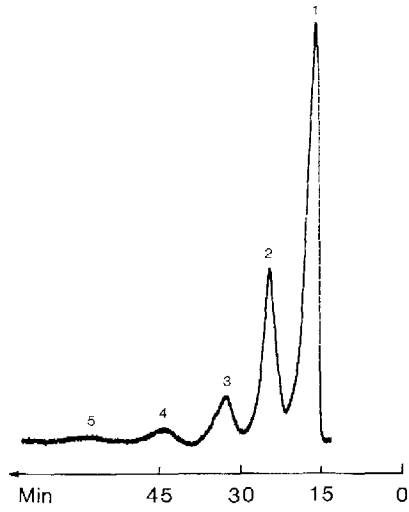


Fig. 2. High-performance electrophoresis of human serum. Supporting medium, agarose gel; total amount of sample, 1 μg.

Fig. 3. High-performance electrophoresis of bovine serum albumin. Supporting medium, polyacrylamide gel; total amount of sample, 0.1 μg. Peaks: 1 = monomer; 2 = dimer; 3 = trimer; 4 = tetramer; 5 = pentamer.

DISCUSSION

The thin-walled narrow-bore electrophoresis tubes used in this study can also be employed for runs in buffer alone without any supporting medium. One should recall, however, that electroendosmosis and convection then can cause zone-broadening, *i.e.* a decrease in resolution. Electroendosmosis can be eliminated by coating the electrophoresis tube with a polymer such as methylcellulose[1] or by performing the run in a buffer containing the polymer[5]. Convection is not so pronounced for low-molecular-weight compounds, as for macromolecules (such as proteins and nucleic acids) and particles (virus, bacteria, etc.). The convection in a carrier-free medium can, however, be almost completely eliminated if the horizontal electrophoresis tube is slowly rotated about its long axis[1]. This free-zone electrophoresis technique has also the advantage that it permits UV detection of solutes not only by the technique described herein (*i.e.* by recording the solutes as they pass electrophoretically a stationary UV beam), but also by automatic scans of the rotating electrophoresis tube at desired time intervals. This tube, made of quartz, has an inner diameter of 1–3 mm, which means that the usable field strengths are lower than with the electrophoresis tubes used in this study (the wall thickness is also much larger).

The UV detection technique described herein has been used also in displacement electrophoresis[6] and is standard in HPLC.

Polyacrylamide gels in the form of narrow columns[7–9] or thin slabs[10] have previously been used as a supporting medium in electrophoresis. When operated at high field strengths these gels give high resolution because the diffusional zone broadening is then small owing to the short run times, and the thermal zone deformation is kept at a minimum owing to the small gel thickness, which allows a rapid dissipation of the Joule heat. Since the glass tubes used in this study have extremely thin walls through which the Joule heat can be transported very efficiently it should be possible to use higher field strengths than in the tubes with thicker walls without

serious thermal zone broadening. One can therefore expect the thin-walled glass tubes to give a higher resolution than that previously obtained in (micro)electrophoresis, particularly when they are surrounded by a rapidly streaming coolant.

In the case of polyacrylamide gel electrophoresis in narrow-bore tubes the proteins have been detected by staining[7–9]. The thin-walled electrophoresis tubes we have employed have the advantage of transmitting UV light, thereby permitting on-line detection, which must be a characteristic feature for a method defined as the equivalence of HPLC. The sensitivity compares favorably with that achieved by staining. When analyzing proteins we have used *ca.* 0.01–1 μg of material in most of our experiments in volumes of 0.01–0.1 μl.

Jorgensen and DeArman Lukacs[11] have recently published a method based on electroendosmosis —or a combination of electroendosmosis and electrophoresis— for the separation of low-molecular-weight substances in a carrier-free medium in 1-m long glass capillaries. The substances, prelabelled with fluorescamine, were detected by on-column measurement of the fluorescence.

NOTE ADDED IN PROOF

I would also like to refer to an interesting article by Lederer[12] ("High-performance" paper electrophoresis) in which some inorganic ions were separated within 5 min.

The experiments presented herein indicate that the run times in HPE experiments are about the same as in HPLC; the resolution might be higher in the former technique, at least for macromolecules when a run is performed in a gel such as polyacrylamide, owing in part to the fact that a chromatographic bed cannot be sufficiently well packed so as to compete in uniformity with a homogeneous, coherent electrophoresis gel and therefore gives a larger eddy diffusion. The high-resolving molecular-sieve electrophoresis experiment in Fig. 3 showing a separation of monomers, dimers, trimers, tetramers and pentamers of albumin, is an example; if this experiment is performed as a molecular-sieve chromatography in the HPLC mode on cross-linked agarose[13] or a TSK SW gel[14], the tetramers and pentamers cannot be resolved.

ACKNOWLEDGEMENTS

I am very grateful to Mrs. Karin Elenbring for the many experiments she has performed in order to overcome all the difficulties we met in trying to make the equipment operate satisfactorily. The work has been financially supported by the Swedish Natural Science Research Council.

REFERENCES

1 S. Hjertén, *Chromatogr. Rev.*, 9 (1967) 122.
2 E. Fries and S. Hjertén, *Anal. Biochem.*, 64 (1975) 466.
3 S. Hjertén, *Arch. Biochem. Biophys.*, Suppl. 1 (1962) 147.
4 S. Hjertén, in H. Hirai (Editor), *Electrophoresis '83, Proceedings of the International Conference on Electrophoresis, Tokyo, May 9–12, 1983*. Walter de Gruyter, in press, 1983.
5 S. Hjertén, *Arkiv Kemi*, 13 (1958) 151.
6 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verhegen, *Isotachophoresis: Theory, Instrumentation and Application*, Elsevier, Amsterdam, 1967, pp. 153–170.
7 U. Grossbach, *Biochim. Biophys. Acta*, 107 (1965) 180.
8 H. Hydén, K. Bjurstam and B. McEwen, *Anal. Biochem.*, 17 (1966) 1.
9 V. Neuhoff, *Arzneim.-Forsch.*, 18 (1968) 35.
10 W. Ansorge and L. de Maeyer, *J. Chromatogr.*, 202 (1980) 45.
11 J. W. Jorgenson and K. DeArman Lukacs, *J. Chromatogr.*, 218 (1981) 209.
12 M. Lederer, *J. Chromatogr.*, 171 (1979) 403.
13 S. Hjertén, *Acta Chem. Scand.*, B36 (1982) 203.
14 M. E. Himmel and P. G. Squire, *Int. J. Peptide Protein Res.*, 17 (1981) 365.