

CHARACTERISTICS OF SOLUTION FLOW IN SUPPORTING MEDIA
IN THIN-LAYER ELECTROPHORESIS IN MOIST CHAMBERS

JERZY KOWALCZYK

*Department of Technical Analytical Chemistry and Testing of Materials,
Faculty of Chemistry, Technical University,
Gdańsk (Poland)*

(First received June 14th, 1963)

(Modified October 15th, 1963)

As is generally known the process of electrophoresis (ionophoresis) in supporting media is always associated with electro-osmotic movement. The solution movement is complicated only in moist chambers, as it is a resultant of two components, the electro-osmotic flow and the flow caused by water evaporation from the surface of the supporting media. MACHEBOEUF *et al.*^{1,2} were the first to investigate this problem. Their experiments concerned the conditions of separation of the fractions of serum protein. Later investigations on solution flow were also carried out by DE WAEL³, PEETERS AND VUYLSTEKE⁴, AUDUBERT AND DE MENDE⁵, SCHILLING AND WALDMANN-MAYER^{6,7} and others. All the results obtained dealt with electrophoresis on filter paper. In the work described below experiments involving systems which do not contain filter paper were performed in moist chambers. We believe that the results of these investigations may be of interest as moist chambers are much used. In addition there has lately been considerable development of thin-layer electrophoresis and ionophoresis involving a variety of supporting media.

EXPERIMENTAL

Apparatus

A rectangular PCV wall chamber (25 × 10 × 10 cm), containing two 350 ml electrolyte vessels. Compact layers of cotton wool, rendering the flow of the products of electrolysis more difficult, were placed between the electrodes and the points of immersion of the electrophoretic media. The electrodes were made of platinum wire. The rectangular lid of the moist chamber was made of plexiglass.

Sources of voltage

(a) A d.c. valve rectifier of a stabilized output voltage range of 50–600 V and (b) an a.c. autotransformer of a stabilized output voltage range 0–250 V, 50 c/s.

Materials

(1) *Solutions*: 0.05 N hydrochloric acid; veronal-acetate buffer solution, pH = 8.6, prepared according to MICHAELIS⁸.

(2) *Substances for supporting media*: Whatman No. 1 filter paper; glass fiber filter paper (60 × 80 threads of 0.3 mm gauge per cm²); silica gel precipitated by the

method of HARNIS AND WICK⁹; alumina prepared from a commercial product according to ref. 10; titanium dioxide (E. Merck, Darmstadt); kaolin (Carter Products, Inc., New York, No. 606); starch (E. Merck, Darmstadt).

(3) *Solution flow indicator*: 1.5% glucose solution in 0.05 N hydrochloric acid or veronal-acetate buffer, pH = 8.6.

(4) *Reagent for development of the glucose spots*: 0.93 g aniline + 1.66 g phthalic acid and 100 ml of butyl alcohol¹¹.

Methods

(1) *Preparation of the layers of supporting media*: except for filter paper and glass fiber paper all the above substances were sieved and grains of maximum diameter of 0.06 mm were used. A glass plate measuring 15 × 5 cm and having a cavity of 2 mm depth was used. The cavity was filled with even wet layers of the substances. Pieces of Whatman No. 1 paper were attached to both ends of the layers. The plate with the layer thus prepared was immersed in the electrolyte solution and the chamber was closed hermetically and left overnight. The next day the excess of moist medium was removed from the plate surface leaving an evenly wet layer which measured exactly 15 × 5 × 0.2 cm³.

(2) *Measurement of the solution flow velocities in the supporting media*: (a) Evaporation flow (Joule effect flow): by applying an alternating current, the effects of electro-osmosis were avoided and the flow obtained was due solely to evaporation of water from the medium surface. (b) Electro-osmotic flow: electro-osmotic flow velocities were calculated from the difference between the resultant flow velocity and the velocity of the flow occurring under the influence of water evaporation. (c) Resultant flow: on applying a direct current the solution flow obtained was that occurring normally in moist chambers as the result of electro-osmosis and evaporation.

Measurement of the fluid movement was carried out according to BERMES AND McDONALD¹², using glucose as a neutral indicator independent of electric field influences. From the distance moved by the glucose it was possible to calculate the liquid velocities at various points along the supporting medium layers.

RESULTS AND DISCUSSION

Fig. 1 illustrates the effect of water evaporation on the solution flow. Results are shown for processes occurring under identical conditions (all supporting media had the same dimensions with the exception of filter paper layers and glass fiber paper). Considerable differences in flow velocities were evident although in these cases the current intensities were similar (*cf.* the current intensities for silica gel, alumina and titanium dioxide in Fig. 1). In addition, Fig. 1 shows that in each case described the solution flow conformed to the description of paper electrophoresis given by MACHEBOEUF^{1,2}:

- (a) the fluid movement was symmetrical to the centre of the layers,
- (b) the straight line on the diagram characterized the velocities as a function of the distance from the beginning of the layers and
- (c) the velocity was zero at the centre of the layers.

A subsequent determination of electro-osmotic flow velocities under the same conditions was attempted.

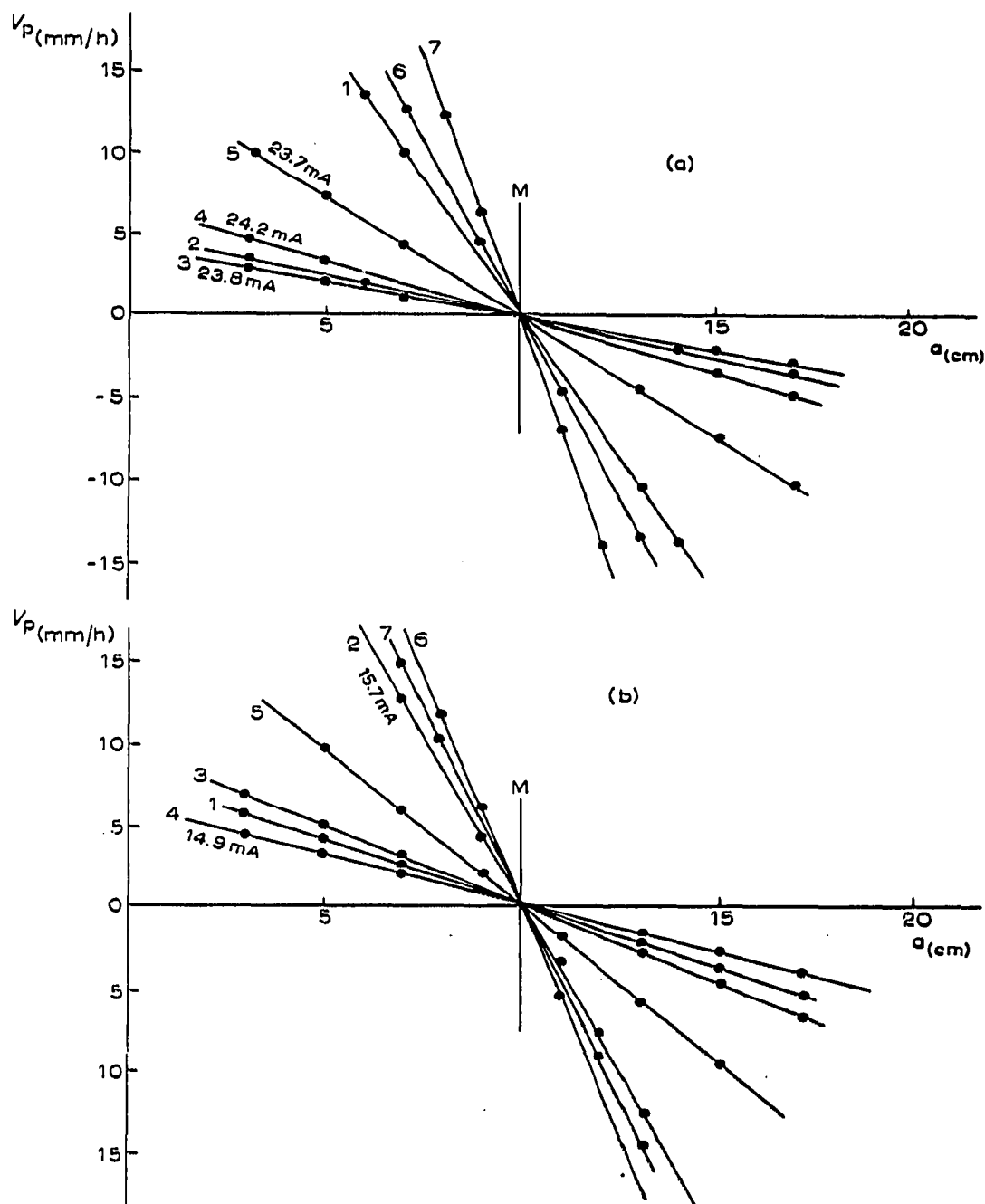


Fig. 1. Diagram of the solution flow, due to water evaporation. Curves of flow velocities, plotted as functions of the position on the medium layers. Solutions: Fig. a = 0.05 *N* hydrochloric acid; Fig. b = veronal-acetate buffer pH = 8.6. Supporting media: 1 = starch; 2 = titanium dioxide; 3 = alumina; 4 = silica gel; 5 = kaolin; 6 = glass fiber filter paper; 7 = Whatman No. 1 filter paper. Voltage 7.5 V/cm, a.c. 50 c/s. Time = 60 min.

Fig. 2 shows the results of the calculations.

The magnitude and direction of the electro-osmotic movement show considerable differences on the above mentioned media. As was expected in these cases, the liquid flowed with a constant velocity along the layers. The resultant solution flow is shown in Fig. 3.

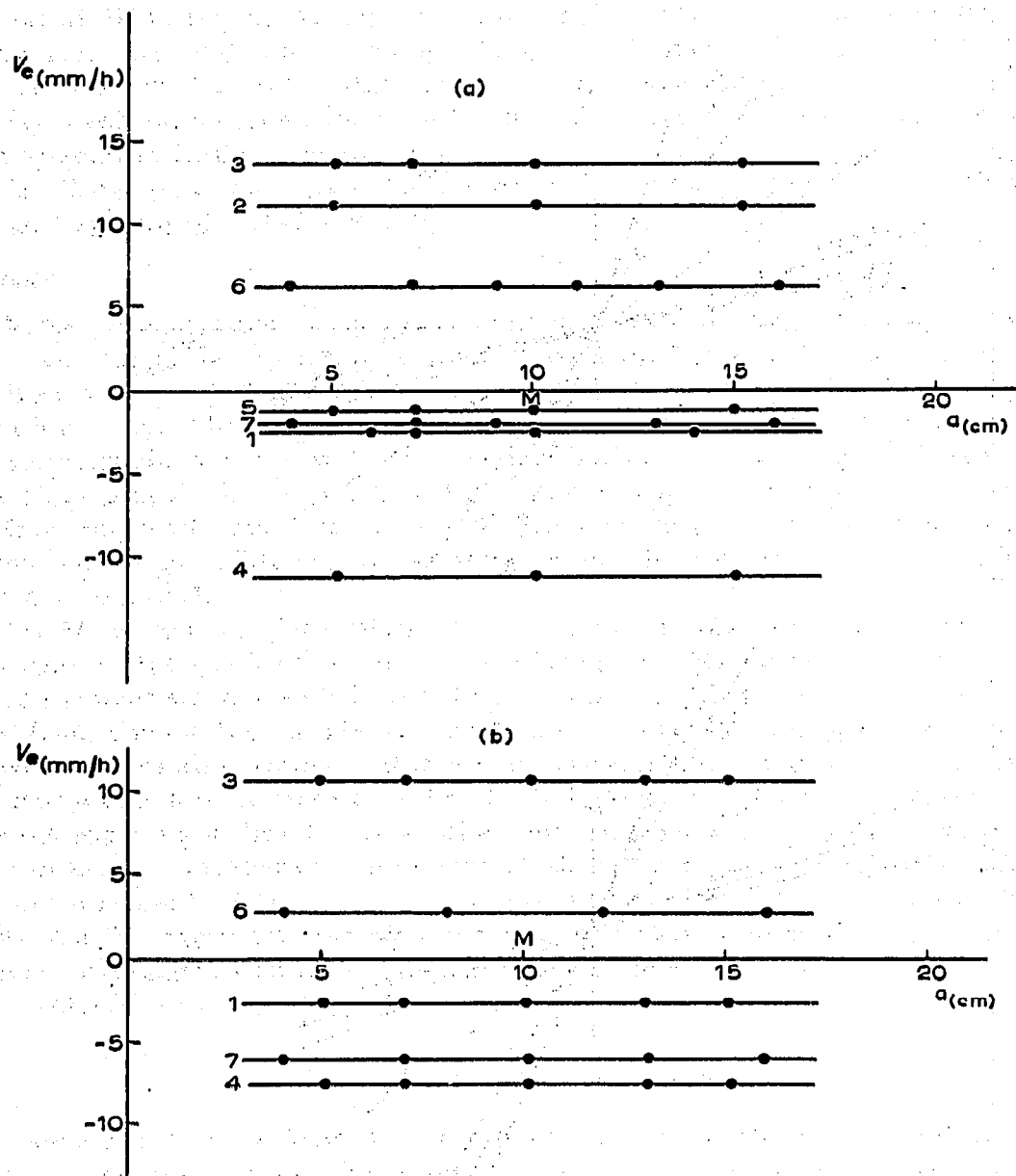


Fig. 2. Diagram of electroosmotic flow. Curves of solution flow velocities plotted as functions of position on the medium layers. Solutions: Fig. a = 0.05 *N* hydrochloric acid; Fig. b = veronal-acetate buffer pH = 8.6. Supporting media: 1 = starch; 2 = titanium dioxide; 3 = alumina; 4 = silica gel; 5 = kaolin; 6 = glass fiber filter paper; 7 = Whatman No. 1 filter paper. Voltage = 7.5 V/cm, d.c. Time = 60 min.

Figs. 3a and 3b show the cases where a point of hydrostatic equilibrium^{1,2}, π , on the supporting medium exists. In this zone π , the resultant fluid motion equalled zero. The curves in Figs. 3c and 3d show on the other hand the solution flow in cases where there was no point π on the supporting media (π being a hypothetical zone lying beyond the medium layers). The results obtained in Fig. 3 are clear, taking into consideration the differences in the values obtained for both flow components illustrated in Figs. 1 and 2.

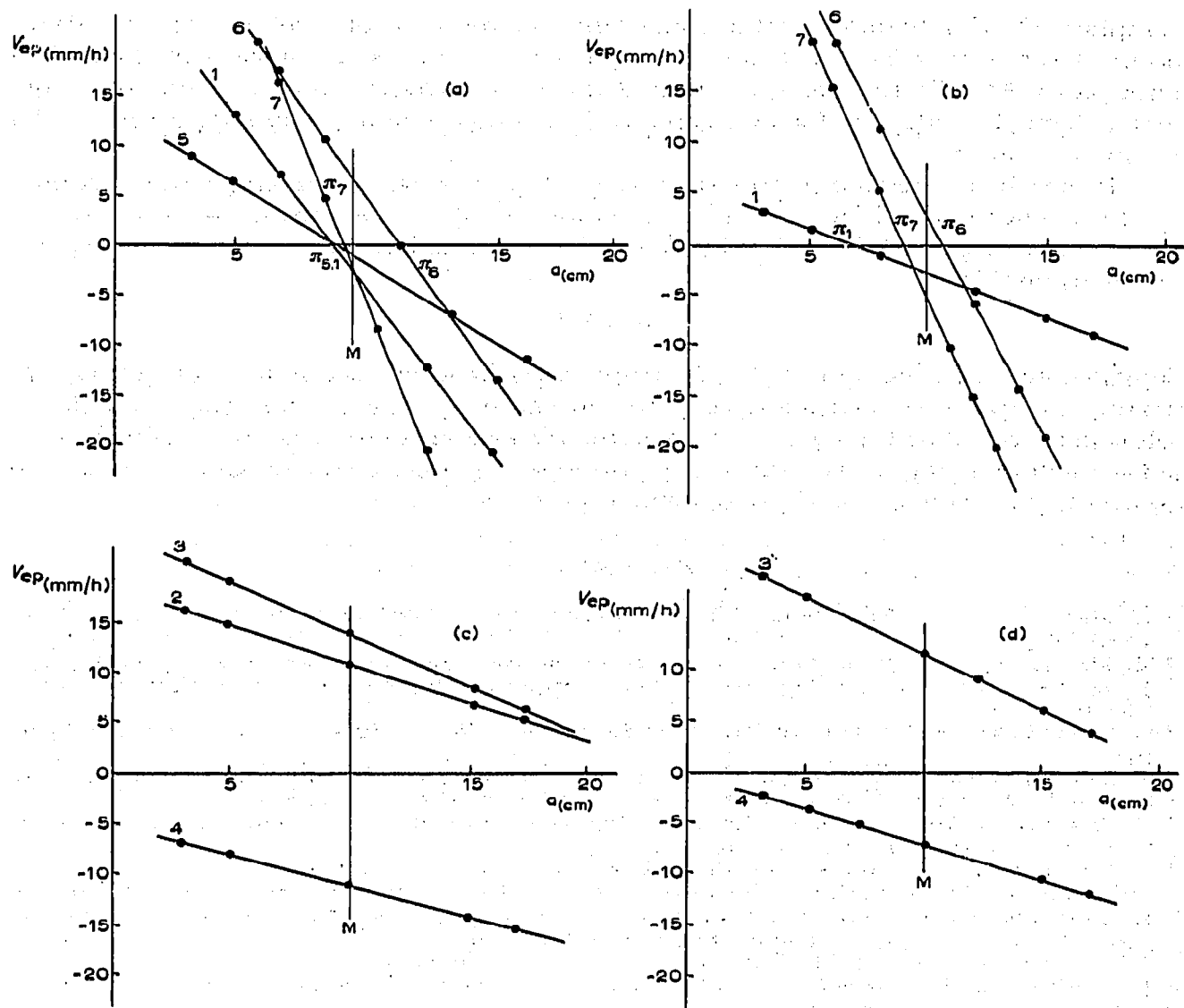


Fig. 3. Diagram of resultant solution flow in supporting media during electrophoresis in moist chambers. Curves describing flow velocities as function of position along the medium layers. Figs. a and b = systems in which there is a zone of hydrostatic equilibrium π , where the liquid velocity equals zero. Figs. c and d = systems in which there is no zone π . Solutions: Figs. a and c = 0.05 N hydrochloric acid, Figs. b and d = veronal acetate buffer pH = 8.6. Supporting media: 1 = starch; 2 = titanium dioxide; 3 = alumina; 4 = silica gel; 5 = kaolin; 6 = glass fiber filter paper; 7 = Whatman No. 1 filter paper. Voltage = 7.5 V/cm, d.c. Time = 60 min.

CONCLUSIONS

Two groups of systems, supporting medium + solution, exist under the conditions usually present in electrophoresis (ionophoresis) in moist chambers. They are: (1) systems in which a zone of hydrostatic equilibrium, π , exists in the medium layer and (2) systems in which there is no zone π .

As can be seen in Fig. 3, in systems of the first kind the solutions flow in a variable and complicated way from both electrolyte vessels to the zone π . In systems without a zone π , the solution flow, being unidirectional and uniformly increasing, is less

complicated. Furthermore, after some time, changes of electrolyte concentrations occur at the zone π . This fact has already been stated by some authors in the case of filter paper^{1,2,6,7}. On this basis, one may deduce the existence of more stable and more easily defined conditions for electrophoresis in moist chambers in supporting media having no zone π .

It may be interesting to note that we used filter paper as our supporting medium. As this is a system with zone π , it is therefore one of the less advantageous systems. Further investigations on this subject will be continued and described in subsequent publications^{13,14}.

SUMMARY

Solution flows during thin-layer electrophoresis in moist chambers, for seven supporting media, were characterized. Two groups of systems, supporting medium + solution, were defined. They are: (1) systems with a zone of hydrostatic equilibrium, π , in the medium layer, (2) systems having no zone π . It may be deduced that the second group of systems is more advantageous in practice.

REFERENCES

- ¹ M. MACHEBOEUF, P. REBEYROTTE, J. M. DUBERT AND M. BRUNERIE, *Bull. Soc. Chim. Biol.*, 35 (1953) 334.
- ² M. MACHEBOEUF, J. M. DUBERT AND P. REBEYROTTE, *Bull. Soc. Chim. Biol.*, 35 (1953) 346.
- ³ J. DE WAEL, *CIBA Foundation Symposium on Paper Electrophoresis*, J. & A. Churchill, Ltd., London, 1956.
- ⁴ H. PEETERS AND P. VUYLSTEKE, *Colloq. St. Jans. Hosp., Brugge, Belg.*, 4me, De Tempel, Brugge, 1956.
- ⁵ R. AUDUBERT AND S. DE MENDE, *The Principles of Electrophoresis*, Hutchinson, London, 1959.
- ⁶ K. SCHILLING AND H. WALDMANN-MEYER, *Acta Chem. Scand.*, 13 (1959) 1.
- ⁷ H. WALDMANN-MEYER, *Chromatographic Reviews* 5, Elsevier, Amsterdam, 1963, pp. 1-45.
- ⁸ L. MICHAELIS, *Biochem. Z.*, 234 (1931) 139.
- ⁹ R. HARMIS AND A. N. WICK, *Ind. Eng. Chem., Anal. Ed.*, 18 (1946) 276.
- ¹⁰ E. LEDERER AND M. LEDERER, *Chromatography*, 2nd Ed., Elsevier, Amsterdam, 1957, p. 24.
- ¹¹ R. J. BLOCK, E. L. DURRUM AND G. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, 2nd Ed., Academic Press, New-York, 1958.
- ¹² E. W. BERMES AND H. J. McDONALD, *J. Chromatog.*, 4 (1960) 34.
- ¹³ J. KOWALCZYK, *Chem. Anal. (Warsaw)*, 8 (1963) 659, 823, 835; 9 (1964) 21, 29.
- ¹⁴ J. KOWALCZYK, in the press.

J. Chromatog., 14 (1964) 411-416