

Michl H. Über Papierionophorese bei Spannungsgefällen von 50 Volt/cm.
Monatsh. Chem. 82:489-93, 1951.
[I. Chemischen Laboratorium der Universität Wien, Austria]

Electrophoretic separations of low-molecular substances can be accomplished by use of high voltages within 20-30 min., separations which are impossible at low voltage. Photos show the separation of amino acids, polypeptides, organic acids, and inorganic ions. [The *SCI*[®] indicates that this paper has been cited over 280 times since 1961.]

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"In 1948, after World War II, I finished my thesis at the former Second Chemical Institute of the University of Vienna, Austria, where I got my first job as an assistant. At that time, there was a shortage of nearly everything. There were no grants or special equipment available. We had to synthesize reagents like ninhydrin and, for instance, solder power supplies by ourselves. We did not even have filter paper suitable for paper chromatography, which was one reason to search for other methods of separation of low-molecular substances.

"So in 1949 I constructed a very simplified electrophoresis apparatus of the Tiselius-Svensson type.¹ It worked very well with serum analyses. However, all experiments with low-molecular substances like amino acids were unsatisfactory. The reason was, of course, diffusion, which destroyed the initially sharp boundary between solution and buffer within the time necessary for separation. Principally, it is possible to accelerate electrophoretic separations by raising the applied voltage. On the other hand, it was impossible to avoid buffer convections, even when improved methods of heat exchange were used.

"Paper electrophoresis—at that time a brand new method—provided similar difficulties. Heat exchange by evaporation of salt-containing buffers leads to feedback effects: the salt concentration rises and causes an increase of electrical current, followed by more heating and evaporation until the circuit breaks by burning the paper. Therefore it was necessary to perform heat exchange, for instance by heat conduction and (or) develop volatile buffers, which don't change their pH values by evaporation. In the paper mentioned above, liquid heat exchangers were preferred because the contact between the wet paper and the cooling liquid works without pressure, and the liquid heat exchanger in vertical arrangements cooled itself by thermosiphon effects. Furthermore, the electroosmosis was controlled by buffer flow and buoyancy. A lucky chance helped solve the problem of the volatile buffer. At first, diluted solutions of organic acids or bases were tried. The low electric conductivity and buffer capacity of these electrolytes caused a high sensitivity against overloading.

"One day, I used a particularly bad smelling brand of technically pure toluene as a cooling liquid. Suddenly, the electrophoretic separations were improved markedly: the toluene was contaminated with pyridine, collidins, and related compounds. So the development of a pyridine-acetate buffer became obvious. I have to admit, however, that I never again got such good separations of basic amino acids and peptides as in that special brand of toluene.

"For this paper I received the Fritz Pregel Prize of the Austrian Academy of Sciences.

"I think the method became popular because, first, it was useful in elucidating primary structures of proteins and nucleic acids.^{2,3} Secondly, the method was born of deficiency, therefore it was simple. And thirdly, colleagues—among them later Nobel prizewinners—were fair enough to cite me in spite of the fact that publications in *Monatshfte für Chemie* were reputed to be top secrets."

1. Michl H. Eine vereinfachte Elektrophoreseeinrichtung für runde Küvetten.
Monatsh. Chem. 82:23-31, 1951.

2. Heftmann E, ed. *Chromatography*. New York: Van Nostrand-Reinhold, 1975. p. 282-94.

3. Brownlee G G. *Determinations of sequences in RNA*. Amsterdam: North-Holland, 1972. p. 54-66.