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# This Week's Citation Classic™

**Poulik M D.** Starch gel electrophoresis in a discontinuous system of buffers.
*Nature* **180**:1477-9, 1957.
[Dept. Public Health, Univ. Toronto, Canada]

**The introduction of starch gel electrophoresis in a discontinuous system of buffers was instrumental in the discovery of many polymorphic systems especially of serum proteins and enzymes. The underlying principle (Kohlrausch regulating function) accounts for the superior resolving power of similar electrophoretic methods, e.g., disc polyacrylamide electrophoresis. [The *SCI*® indicates that this paper has been cited in over 1,560 publications since 1957. ]**

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"In 1948, I escaped from my native country, Czechoslovakia, and in 1953, I entered the University of Toronto Medical School to complete my medical education. To support myself, I worked in the department of hygiene and preventive medicine. My work was directed by F.T. Frazer, a prominent immunologist at that time. My project was to enhance the purity of diphtheria toxin that was treated with formalin to produce the diphtheria toxoid for mass immunization.

"I had previously developed a prototypic immunolectrophoresis method[1] that I now applied to the problem of identifying the multitude of chromogens and proteins present in the crude diphtheria toxin. However, this original method was not suited to produce a toxin more pure than that already available. Fortunately, O. Smithies was working in a nearby laboratory and he was developing the methodology for starch gel electrophoresis: the most powerful electrophoretic method known at the time. I adapted Smithies's techniques in my project. However, each experimental separation of the impurities in the toxin required eight hours of electrophoresis. This consumption of time was not conducive to a smooth course in either my medical school work or my marriage, even though my wife, Emily, was working with me as a research technician. Consequently, we both tried to devise a less time-consuming procedure.

"By chance, I had noticed an article describing the use of tris (hydroxymethyl) aminomethane for acidimetric work.[2] I thought it would be interesting to try this compound in electrophoresis, and suggested that Emily prepare starch gel in 0.076 molar tris and adjust the pH to 8.65 with 0.005 molar citric acid. She did so and applied our standard 'dirty' toxin to the slab for separation at about 9 a.m. one day. When I arrived at the laboratory after my morning classes to have lunch with Emily, to my amazement the 'run' was nearly completed. All of the chromogenic bands known to be in the toxin were visibly separated and a strange 'brown-line' was present at the position of the fastest migrating protein. This proved later to be impurities of the buffer compounds stacked at the highest voltage gradient along the gel. The experiment proved to be reproducible and separation markedly improved.

"Further experimentation showed that the reason for the superior resolving power of this new system was the serendipitous omission by Emily of not placing the tris-citrate buffer in the electrode vessels as well as in the gel slab. The new 'discontinuous' system (tris-citrate buffer in the gel and borate buffer in the electrode vessels) worked in accordance with the Kohlrausch regulating function described in 1897 for separation of ionic species.[3] A thorough study of the phenomenon by L. Ornstein[4] explained the 'steady-state stackup' and led to the development of the disc polyacrylamide electrophoresis technique that eventually replaced starch gel electrophoresis in many laboratories. In the years from 1957 to 1964, starch gel electrophoresis in a discontinuous system of buffers became a method of choice, and was used by a great number of investigators and thus led to the discovery of a multitude of polymorphic systems of proteins. See reference 5 for a report of my most recent work."

1. **Poulik M D.** Filter paper electrophoresis of purified diphtheria toxoid. *Can. J. Med Sci.* **30**:417-19, 1952.
2. **Fossum J H, Markunas P C & Riddick J A.** Tris (hydroxymethyl) aminomethane as an acidimetric standard. *Anal. Chem.* **23**:491-3, 1951. (Cited 55 times.)
3. **Kohlrausch F.** Über Konzentrations Verschiebungen durch Electrolyse im Innern von Lösungen und Losungsgemischen. *Ann. Phys.* **62**:209-11, 1897. (Cited 110 times since 1955.)
4. **Ornstenin L.** Disc electrophoresis. I. Background and theory. *Ann. NY Acad. Sci.* **121**:321-49, 1964. (Cited 3.520 times.)
5. **Lillehoj E & Poulik M D.** $\beta_2$-microglobulin and membrane proteins. (Ioachim H L. ed.) *Pathobiolngy annual 1979.* New York: Raven Press. 1979. Vol. 9. p. 49-80.