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Review

Electrophoresis: The march of pennies, the march of dimes

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Abstract

The present review encompasses ca. 65 years of history of developments in electrokinetic separations, taking as a starting point the year 1937, i.e. the official launching of Tiselius' moving boundary electrophoresis (MBE). The 1950s have been particularly rich in introducing novel methodologies in zone electrophoresis (ZE), thus bringing about the decline of MBE. Among them of extraordinary importance was the development of electrophoresis on agar gels coupled to immuno-diffusion at right angles, which brought a big revolution not only in biochemistry but also in clinical chemistry. Also the by now forgotten paper electrophoresis was a landmark in separation science, in that it implemented, in its "fingerprinting" version, the first genuine two-dimensional (2D) map, coupling orthogonally a charge to a hydrophobic scale separation, while permitting for the first time the detection of spot mutations, i.e. single amino acid replacements in a polypeptide chain, that paved the way to modern genetic analysis. Equally important was the introduction of starch-block electrophoresis, that brought about the notion of sieving and the first discontinuous buffers, refined, in the 1960s, by Ornstein and Davies with their classical papers combining multiphasic buffer systems to polyacrylamide gels, that went down to history as disc-electrophoresis. The 1960s also contributed with two fundamental techniques, isoelectric focusing (IEF) and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) that permitted to discriminate proteins solely on the basis of surface charge and molecular mass, respectively. The 1970s gave other fundamental contributions, such as isotachophoresis, the first example of a fully instrumental approach to electrophoresis, both in its analytical and preparative version (Tachophor and Tachofrac), 2D maps combining IEF to SDS–PAGE at right angles and silver staining techniques, that incremented sensitivity by 3 orders of magnitude. The 1980s generated immobilized pH gradients and capillary zone electrophoresis (CZE), two big players that dominated the electrokinetic horizon for all the 1990s and still in vigorous use in present days. The review terminates with a glimpse, in the third millennium, onto microchip technology and hyphenated techniques, notably direct interfacing of various electrophoretic separation methods with mass spectrometry (MS).

Keywords: Electrophoretic methodologies; Isoelectric focusing; SDS-electrophoresis; Isotachophoresis; Immuno-electrophoresis; Disc-electrophoresis; Capillary zone electrophoresis; Two-dimensional maps; Microchip electrophoresis; Hyphenated techniques; Silver stains

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1. Introduction

Eheu fugaces, Postume, Postume,
Labuntur anni, nec pietas moram
Rugis et instanti senectae
Adferet indomitaeque mortis . . .
(Quintus Horatius Flaccus, Carmina 2, 14).

It is a privilege to have been asked to write this review in honour of Professor Csaba Horvath, not only a very good friend of mine, but also one of the most brilliant scientists and most charming human being I have ever met in my long scientific career. I only hope he will not revolt in his tomb for this lousy treatise and bear with me with the same patience and tolerance he always exhibited any time I approached him to illuminate me in the obscure paths of science. Although he went down to history as one of the most sagacious inventors in chromatography (his first prototype of an HPLC instrument was still standing in his office at Yale last time I visited him), he has also made some outstanding contributions in the field of electrophoresis, when he moved into it through the rising star of the 1990s, capillary zone electrophoresis (CZE), bringing to this field his unique knowledge in chromatography. It was the "Marriage of Figaro", although the effervescent music of Mozart was missing. Albeit the leitmotif of this special issue is "separation science: Past, Present and Future" I have decided to concentrate this historical survey especially on the past (though not disdaining the present) for an educational purpose: since journal issues have been placed in the Internet only starting from ca. 1997, it turns out that the vast and incredibly rich body of science of pre-Internet origin is rapidly falling into oblivion. Nobody spends time in libraries any longer (a most unfortunate situation), so all past history is rapidly forgotten and the new generations tend to re-invent the wheel over and over again. If we do not learn from our past we will never be able to build a radiant future. This chronicle will start from an incipit, i.e. the first genuine example of a most powerful analytical technique, the moving boundary electrophoresis (MBE) a la Tiselius and inch its way through modern times. A march that encompasses some 65 years in electrokinetic methodologies.

2. 1937: crossing the columns of Hercules

Was MBE the start of modern separation science? Surely it was. Some people like to quote, as the point of origin, the work of Ferdinand Frédéric Reuss (an officer in the Czar's army in Moscow, although he was born in Tuebingen), who, while moonlighting on the banks of the Moskwa River, instead of fighting Napoleon's army, discovered the phenomenon of electroendoosmosis (EOF, to be rediscovered almost two centuries later in CZE; surely enough, he had filled his U-tube apparatus with sand collected on the river shore, i.e. silica powder!) [1]. His was a rich man experiment: his power supply was a voltaic pile composed of 92 silver rubles and an equal number of zinc plates, something that not even count Volta could afford (more humbly, his pile was a stack of alternate zinc and copper disks, separated by cotton sponges imbued with dilute sulphuric acid). But this would be like claiming that Homer, in his poem the Iliad, laid the foundations of modern war, with its super-extermination weapon power! Tiselius "Moving Boundary Electrophoresis" [2], just like the ultracentrifuge, was a highly sophisticated instrument, equipped with in situ observations devices, able to monitor the movement of proteins molecules in the electric field (perhaps scientists could be the ancestors of present day "pippin toms"). The detection principle was very much the same as that of the ultracentrifuge [3,4]: light shed along the migration path detected the "moving boundaries", i.e. the regions of strong variation of refractive index upon the passage from one macromolecular ion to the next one (in fact to the mixture of all ions having lower mobilities!) (Fig. 1, left side). Since these boundaries would be displayed as sigmoidal transitions, a complex schlieren optics device would transform them into peaks, easily amenable to computational treatment (Fig. 1, right side). The Tiselius instrument made thus possible, for the first time, the accurate measurement of absolute mobilities of proteins [5], since the movement of the macroions was not hindered by the viscous drag of density gradients or by the tortuous channels of support media, as later on occurring in "zone electrophoresis". Moreover, since both, the ascending and descending boundaries in the two limbs of the cell U-tube could be monitored, it was soon realized that departure from enantiography afforded clues to interactions among the species under investigation. Thus, the