

Capillary electrophoresis in contemporary analytical chemistry

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The phenomenon of the migration of charged species under the influence of an externally applied electric field is known under the name of *electrophoresis*. Differences in the mobility of the analytes due to their average charge, size, shape, and the properties of the used electrolyte solution form a basis of a valuable separation method in chemistry.

According to [1] the Russian physicist Reuss carried out the first separations based on this principle already in 1809. He studied the migration of colloidal clay particles and discovered that the liquid adjacent to the negatively charged surface of the wall migrated towards the negative electrode under the influence of an externally applied electric field. The theoretical aspects of this electrokinetic phenomenon (*electroosmosis* by Reuss) were formulated in 1897 by Kohlrausch [2]. In the late 1800s and early 1900s, electrophoretic separations were carried out by several researchers in the so-called U-shaped tubes. Tiselius, starting in 1925 with his PhD thesis on the development of free moving boundary electrophoresis, advanced the analytical aspects of electrophoresis. This resulted in the separation of complex protein mixtures based on differences in electrophoretic mobilities [3]. In 1948 Tiselius was awarded the Nobel Prize in Chemistry for his work on electrophoresis.

Possibilities of performing electrophoresis in capillaries were investigated by Hjertén [4], Everaerts [5], and Virtanen [6] but their work did not attract much attention to capillary electrophoresis (CE). The situation changed with the work by Jorgenson & Lukacs [7]. They performed separation of fluorescent dansylated amino acids in a glass capillary with an inner diameter of 75 μm . Applying voltages up to 30 kV, they provided efficiencies more than 400 000 within

25 min [7]. Such efficiency, not seen in the separation science before, was mainly due to the fact that at diameters less than 0.1 μm the capillary wall dissipated all the Joule heating generated in the buffer by the electric current. The analyte band broadening during its movement along the capillary is determined then (in most cases) by its diffusion, which is very low in liquids. After the landmark work by Jorgenson & Lukacs, interest in CE started to grow rapidly. (For example, performing search with SciFinder[®] Scholar[™] program (American Chemical Society) on the keyword “capillary electrophoresis” results in about 30 thousand hits.)

Although CE was initially heralded for its speed and low sample volume, the technique is valuable because it is quantitative, can be automated, and will separate compounds that have been traditionally difficult to handle by high performance liquid chromatography (HPLC). CE played a crucial role in determining the human genome sequence and it is the basis for virtually all micro-fluidics for “lab-on-a-chip” devices. CE can separate polar substances, which are notoriously difficult to analyse with HPLC. Chiral separations are another area in which use of CE has expanded. The small sample volumes required for CE can be an advantage in the case of a limited amount of sample.

Nowadays companies like Agilent sell “lab-on-a-chip” devices that use electrophoretic separations. Beckman Coulter has eight automated, application-based capillary array systems, marketed under the name P/ACE MDQ. The application-based systems – chiral, carbohydrate, molecular characterization, DNA, glycoprotein, education, and quality control – differ in the way they are configured and the types of detectors and chemistries coupled to them. An area closely related to CE that has yet to reach its stride is capillary electrochromatography (CEC), which combines features of CE with LC by using capillaries, packed with chromatographic materials. Although no products have yet hit the CEC market, some of the problems have been solved, so they may start appearing over the next few years.

Will CE ever overtake HPLC? This is not an appropriate question any more. CE comes into its own for large molecules and when the sample size is limited. This tends to give its best applications a biological flavour, and it can powerfully address problems where HPLC has little chance of success – the Human Genome Project being an obvious example. Now that CE has been officially recognized by several regulatory agencies – the Food and Drug Administration and the Center for Drug Evaluation and Research among them – it is finding a niche in quality assurance and quality control labs as well. And with CE’s hugely successful foray into genomics, which is the undisputed reason for the Human Genome Project having been finished well ahead of schedule, the question is whether it can do the same for proteomics, the next phase of genomic research.

In Estonia CE was introduced in the early 1990s at the Department of Analytical Chemistry, Institute of Chemistry (then at the Estonian Academy of Sciences) by efforts of E. Urbas, A. Ebber, and R. Kuldvee. Recognizing the need for responding to the general trend in separation science, reasonable CE systems were built from components available at the laboratory at that time without any capital investment. Ease of composing one’s own, “tailor made”

systems appeared to be one more important advantage of CE (unfortunately nonscientific) for low budget laboratories willing to carry out competitive research (which could not definitely be the case with e.g. mass spectrometry). Automation and computerization of sampling process in CE was found to be the “niche” for research. Since then, the application of computerized sampling in monitoring bioprocesses has been of our interest. Recently nonaqueous buffer systems, especially use of ionic liquids as buffer ingredients, was added to our research topics. This special issue of the *Proceedings of the Estonian Academy of Sciences* and the next issue summarize the results of this research.

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