APPLICATIONS OF CAPILLARY ELECTROKINETC SEPARATION METHODS

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Capillary electrophoresis (CE) has been used in its various modes, to analyse a wide variety of compounds ranging from inorganic ions and small organic molecules to peptides, proteins, nanoparticles, DNA and RNA.

Electrophoresis in narrow diameter tubes was first reported more than 50 years ago, however, CE in fused silica capillaries under a millimetre internal diameter and high fields, as we know it today, was only mentioned for the first time by Jorgenson and Luckacs in 1981. The next important achievement was the introduction of micellar electrokinetic chromatography (MECK) by Terabe and co-workers in 1984, which enabled the separation of neutral compounds. MECK involves the use of a charged surfactant above its critical micelle concentration, to which the analytes have different affinities, therefore acquiring different mobilities depending on the partition coefficient.

Integrating electrophoresis and chromatography principles has lead to the development of capillary electrochromatography which has also found many applications and has evolved, together with HPLC, to the use of monoliths as stationary phases and lab-on-a-chip technology.

When compared to its closest analytical technique, HPLC, CE is often referred to has having a better separation efficiency; being more environmental friendly; allowing the use of extremes of pH; requiring less sample material and shorter sample preparation; simplifying the interface to MS and enabling chiral separations at lower cost.

CE is particularly simple when applied in free zone to small ionisable compounds. Aqueous or non-aqueous running buffers can be used with several additives that allow tuning selectivity and efficiency. In fact, CE method development for enantiomeric separations is significantly easier and cheaper than HPLC since it can be achieved simply by adding a chiral selector to the running buffer thus avoiding acquisition of expensive chiral columns.

CE has also been frequently used for the determination of dissociation constants based on the different effective mobilities of the analytes depending on the pH of the medium and consequently on the degree of ionization.

It is probably for DNA sequencing, using sieving gels inside the capillary, that CE has been most successful. The technique is termed capillary gel electrophoresis (CGE). By the use of arrays of capillaries and laser induced fluorescence detection (LIF), it was possible to decipher the human genome in much less time than initially predicted. CGE is also frequently used for the analysis of proteins in the form of SDS-protein complexes.

One of the pitfalls of CE, when compared to HPLC, is its lower sensitivity. This is due to the small sample volumes involved and the small optical path provided by the detection window when using spectrophotomectric detectors. The high excitation capability of laser beams can however overcome this drawback whenever fluorescence derivatization is possible and CE-LIF is in fact one of the most sensitive techniques for peptide and protein analysis. Off and on-line derivatization have been used for direct fluorescence labelling but also for immunoreactions with labelled monoclonal and polyclonal antibodies thus allowing detection of compounds inside a single cell.

Other less common applications of CE involve the detection and quantification of nanoparticles, microorganisms and viruses.

This talk illustrates some applications of CE with practical examples from the author and from literature.