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J. A. Loo
H. R. Udseth
R. D. Smith

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Pacific Northwest Laboratory
Richland, Washington 99352

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CAPILLARY ZONE ELECTROPHORESIS-MASS SPECTROMETRY OF PEPTIDES AND PROTEINS

Joseph A. Loo , Harold R. Udseth, and Richard D. Smith
Chemical Methods and Separations Group
Chemical Sciences Department
Pacific Northwest Laboratory*
Richland, WA 99352

Capillary zone electrophoresis (CZE) is attracting extensive attention as a fast, high resolution analytical and micro-preparative separations technique for systems of biological interest. In zone electrophoresis, a column is filled with a single electrolyte having a specific conductivity. The mixture of substances to be separated is applied as a narrow band to the head of a buffer filled column in a band whose width is much less than the length of the column and at a concentration too low to affect the buffer conductivity. An electric field is then applied across the length of the column and the individual substances migrate and separate according to their net electrophoretic velocities. Zone electrophoresis carried out in small diameter (< 100 μm) fused silica capillaries is a relatively new approach to the high resolution separation of aqueous samples. Very small volume samples (picoliter range) with separation efficiencies on the order of 10^6 theoretical plates for amino acids have been achieved (1). The method can be further enhanced by the dynamic combination of detection sensitivity and selectivity offered by mass spectrometry (MS). The on-line marriage of mass spectrometry to CZE is accomplished by an atmospheric pressure electrospray ionization source interface. Electrospray ionization (ESI) is rapidly growing as an ionization method of choice for nonvolatile biomolecules since the pioneering experiments of Malcolm Dole (2) and later by John Fenn (3). Fenn's group first demonstrated that ESI is a "soft" ionization technique, yielding little or no fragmentation, capable of producing intact multiply charged molecular ions for proteins with molecular weights (MW) exceeding 30 kDa from highly charge liquid droplets at atmospheric pressure through a field-assisted ion desorption process (4). Our research efforts have demonstrated that proteins with MW's greater than 100 kDa can be analyzed using a conventional quadrupole mass spectrometer with an upper m/z limit of only 1700.

Previous CZE-MS experiments have demonstrated the feasibility to analyze amino acids and small peptides. However, the CZE separation of larger protein systems in untreated fused silica capillaries has often resulted in broad, tailing peaks due to Coulombic attraction and adsorption of analyte on active sites to the negatively charged silica wall. Separations performed in buffered systems with the pH above the isoelectric point (pI) of the proteins allow both the proteins and the capillary wall to be negatively charged and mutually repulsive to greatly improve the resolution (5), as demonstrated in Figure 1. Subpicomole CZE-MS separations of myoglobins and smaller peptides can be easily performed in this manner, with over 125,000 theoretical plates obtained. CZE protein separations can also be achieved through the use of very low pH aqueous phosphate buffers, lower than the pI of the analytes (6). Preliminary CZE-MS results have been obtained for a mixture of horse and whale myoglobins and a variety of cytochrome c proteins at pH 2.4.

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