ABSTRACT

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The primary aim of this thesis was to develop analytical tools and methods to facilitate the study of biological processes down to single cell and sub-cellular levels.

Using capillary electroporesis with confocal fluorescence detection, analysis of glutamate, labeled with fluorescein isothiocyanate (FITC), was performed with a concentration detection limit at about 300 femtomolar (femto = 10^{-15}). A constricted detection window, size-matched with the probe volume, was used to force the molecules into the probe volume and also to minimize the contribution from spherical aberration.

For the fabrication of submicrometer fused-silica tips and detection windows a pulling device based on tantalum heating filament was developed. The device use the self-tension of a bent capillary as pulling force and reproducible tips and constricted regions were produced from commercial fused silica and borosilicate capillaries.

Single secretory vesicles from the atrial gland of *Aplysia californica* were analyzed, regarding the contents of amino acids and peptides. By using optical trapping, a single vesicle that had a volume of attoliters (10^{-18} liters) was introduced into the tapered inlet of a separation capillary. Prior to electrophoretic separation the vesicle was lyzed and its components were labeled on-column with naphtalene-2,3-dicarboxaldehyde/cyanide (NDA/CN^-). Detection of the derivatives was performed with laser-induced fluorescence. The resulting electropherograms indicated distinct variations in the contents of single vesicles.

A selective chiral determination of aspartic (asp) and glutamic acid (glu) by micellar electrokinetic chromatography (MEKC) with laser-induced fluorescence or UV-absorbance detection was developed. The amino acids were derivatized using the fluorogenic chiral reagent *o*-phtaldialdehyde/2,3,4,6-tetra-O-acetyl-1-thio-beta-D-glucopyranose (OPA/TATG) prior to separation. Using the neutral surfactant octyglucoside (OG), full resolution of the enantiomers was achieved even in samples with complex matrices, such as human serum and urine.

The study of enzyme-substrate reaction dynamics in confined biomimetic containers was performed using liposomes of femto- to attoliter volumes and computer simulations of the collision frequency. The liposomes were optically trapped and the hydrolysis of fluorescein diphosphate catalyzed by alkaline phosphatase was followed by confocal microscopy. The study showed that wall interactions are a central part for estimations of the reaction dynamics.

A method for miniaturized electroporation of single cells and organelles is also described. This method use carbon-fiber microelectrodes and a highly spatially focused electrical field to introduce polar cell-impermeant solutes, such as fluorescent dyes, fluorogenic reagents and DNA, into single cells.

Key Words: capillary electrophoresis, confocal fluorescence detection, constricted detection window, confocal microscopy, single vesicle, electroporation, amino acid, single molecule detection, liposome, dye, biomimetic.
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