

## CAPILLARY ELECTROPHORESIS ENZYME IMMUNOASSAY

This is a continuation-in-part of U.S. patent application Ser. No. 08/386,224, filed on Feb. 9, 1995, which, in turn, is a continuation of U.S. patent application Ser. No. 07/944,846, filed on Sep. 14, 1992, now abandoned.

### FIELD OF THE INVENTION

The invention relates in general to techniques for analysis of chemical species, and in particular to analyses involving electrokinetic separation.

### BACKGROUND OF THE INVENTION

Capillary electrophoresis is a well-known procedure for separation of chemical components. A sample solution containing molecules to be separated is placed in a length of capillary tubing containing an electrophoretic medium. Upon application of an electric field across the capillary, different components within the sample migrate at distinct rates towards the oppositely charged end of the capillary dependent upon their relative electrophoretic mobilities in the electrophoretic medium. Due to the varying electromigratory rates, the sample components become increasingly separated into distinct zones or groups as they progress along the capillary. At some position along the capillary, the components of the sample are detected.

Electrophoresis has been applied to the separation of charged materials such as proteins, nucleic acids, and cells. These separations depend upon differences in charge density, molecular size, and partitioning or complexation with a mobile phase additive. U.S. Pat. No. 5,061,361 relates to a capillary zone electrophoresis system in which a nanoliter volume of sample is introduced into the capillary tube, and an electric field is imposed on the system to effect separation of the charged components. After migration along the length of the tube, the sample components are detected via ultra-violet absorbance. U.S. Pat. No. 5,084,150 relates to an electrokinetic method of separation in which the surface of moving charged colloidal particles is treated so as to interact selectively with the sample molecules to be separated. An electric field is imposed on a capillary tube containing the colloidal particles and the sample to achieve separation. U.S. Pat. No. 5,045,172 relates to a capillary electrophoresis apparatus in which electrodes are attached at each end of a capillary tube, and a detector is coupled to the tube. U.S. Pat. No. 4,181,589 relates to a method for separating biological cells using an electric field. The above-described U.S. patents are hereby incorporated by reference.

Various types of assays are used as clinical diagnostics. Immunoassays of various formats widely are used in clinical diagnosis to measure analytes in body fluids. Immunoassays also are used for the sensitive and specific measurement of analytes in complex mixtures in research, industrial and environmental applications. For example, an enzyme linked immunosorbent assay ("ELISA") has been used to determine levels of cytokines, Thyroid-Stimulating Hormone ("TSH") and other analytes in serum and other biological samples. Engvall, *Methods in Enzymology*, 70:419 (1980); Schurrs and VanWeeman, *J. Immunoassay*, 1:229 (1980); and Scharpe, et al., *Clin. Chem.*, 22:733 (1976) are general references to enzyme immunoassay techniques and are herein incorporated by reference in their entirety.

Patent Cooperation Treaty ("PCT") publications WO 93/22053 and WO 93/22054, and U.S. Pat. No. 5,304,487, herein incorporated by reference, describe various types of

assays performed in microscale analytical devices. Devices for detecting the presence of a preselected analyte in a fluid sample or for analyzing a fluid cell containing a sample are disclosed. The devices typically are made of a solid substrate that is microfabricated to have a sample inlet port and a mesoscale flow system. Other embodiments have various flow restriction designs. The solid substrate may be a few millimeters thick and about 0.2 to 2.0 cm square. The flow channel typically on the order of 0.1  $\mu\text{m}$  to 500  $\mu\text{m}$ . The devices are used in a wide range of automated, sensitive and rapid tests using various flow inducing means.

Immunoassays using electrophoretic separation of free and bound antigen have been explored. Assays using capillary electrophoresis have provided rapid separation and accurate quantitation. Moreover, the microvolume scale of capillary electrophoresis reduces the amount of sample and reagents required, as well as decreasing the amount of waste generated. However, many assays require analyte quantitation to be at levels of  $10^{-10}$  to  $10^{-12}$  Molar. Given these low concentrations of analyte, even laser induced fluorescence detection coupled with capillary electrophoresis may not be sufficient for the detection of an analyte. Overall, sensitivity, speed and cost are important factors to be considered in designing and conducting an immunoassay.

### SUMMARY OF THE INVENTION

The invention encompasses methods of analysis of an analyte in a sample, and is based on the discovery that analyte determination may be performed rapidly using capillary electrophoresis on exceedingly small amounts of sample by performing enzyme amplification with a compound having a biorecognition moiety in a capillary electrophoresis format.

One object of the invention is to provide methods and apparatus for performing highly sensitive enzyme-amplified assays using capillary electrophoresis of an enzyme having a biorecognition moiety. Combining a binding reaction of an analyte with an enzyme having a biorecognition moiety and an electrophoretic separation which uses enzyme-amplified detection methodology, assay methods and apparatus are provided that offer simplicity, quantitative sensitivity and rapid analysis. Another object of the invention is to provide highly sensitive enzyme-amplified assays using a capillary electrophoresis format in which the binding reaction prior to electrophoresis is heterogeneous or homogeneous. Another object of the invention is to provide enzyme-amplified immunoassays using capillary electrophoresis. Yet another object of the invention is to provide methods and microscale apparatus for conducting sensitive automated enzyme-amplified assays using electrophoresis.

Unlike traditional electrophoresis, electrophoresis utilized according to the invention exploits inherent or induced differences in electrophoretic velocities of a detectable product and/or a competitor (the amount of competitor being proportional to an analyte of interest), and a reactant in a given electrophoretic medium in order to mix and separate these components. The electrophoretic mixing of chemical species confers a special advantage over the traditional methods in the field of chemical analysis; i.e., electrophoretic mixing of competitor and reactant is performed without substantial dilution of the chemical species contained within the zones. Under the influence of an applied potential and a chosen electrophoretic medium, a chemical species may possess a distinct electrophoretic mobility which will allow it to electrophorese essentially independently of the bulk solution. Thus, a zone of competitor and