other. Various factors may affect the velocity of the EOF, and hence also affect molecular separation. Factors affecting EOF velocity and molecular separation include viscosity of the suspending fluid, particularly adjacent the passage wall, a change in the electrical charge of the wall itself, or alterations to the neutralizing charges overlying the wall.

Polyelectrolytes have been previously used for modifying the electrophoretic properties of fused silica capillary passages. Adsorption of a cationic polyelectrolyte to the negatively charged silica surface effectively reverses the surface charge from negative to positive. This charge reversal causes fluid flow to be toward the anode so that anions elute first, followed by neutral molecules, followed lastly by cations. Polyelectrolytes previously used to coat silica surfaces include polyarginine, chitosan, poly (diallyldimethylammonium chloride) (PDADMAC), and polyethylenimine. Prior electrophoretic techniques have employed single layers of polyelectrolyte.

A method for forming multilayers of polyelectrolytes has now been described. Decher, G. and J. Schmitt, J. *Prog. Colloid Polym. Sci.*, 1992, 89, 160; and Decher, G., *Science*, 1997, 277, 1232. However, the advantages of polyelectrolyte multilayers for capillary electrophoresis have not been recognized before the present invention.

SUMMARY OF THE INVENTION

With the foregoing in mind, the present invention advantageously provides a capillary tube having a multilayer comprising a polyelectrolyte and positioned for analytical separations of molecules.

It is an object of the invention to provide increased electrophoretic efficiency, and substantially equal efficiency at pH of about 4 and about 6.

It is a further object of the invention to provide substantially reproducible electroosmotic mobility among capillaries manufactured using the same procedure.

It is yet another object of the invention to provide a capillary coated with a polyelectrolyte multilayer which may be used for many analytical cycles while yielding substantially reproducible results.

It is a further object of the invention to provide a coated capillary which substantially reduces irreversible adsorption of large polyions such as proteins to the passage wall.

It is an additional object of the invention to provide a coated capillary which is easily manufactured.

It is also an object of the invention to provide a coating for electrophoretic separations which also functions as a partition medium allowing separation of neutral and/or hydrophobic analytes.

It is still another object of the invention to provide a capillary zone electrophoresis system which requires no pre-analysis equilibration, so that a relatively stable electroosmotic flow is obtained substantially more rapidly.

Accordingly, the capillary tube comprises a generally cylindrical void space, or passage, having a lengthwise dimension and a cross section dimension of from about five micrometers to about one hundred micrometers. The multilayer comprising a plurality of polyelectrolyte layers is positioned within the cylindrical void adjacent the walls. The capillary tube may preferably comprise a plurality of layers of a cationic polyelectrolyte and an anionic polyelectrolyte.

An embodiment of the invention includes a plate having a multilayer for analytical separation of macromolecules. The plate comprises a passage substantially defined by passage walls, and a multilayer positioned within the passage adjacent the walls, the multilayer comprising a plurality of polyelectrolyte layers. The passage may preferably be positioned within a capillary tube or within a plate. In addition, the plate may comprise a plurality of passages. The passage preferably comprises walls of fused silica.

In yet another embodiment of the invention, the passage coated with the polyelectrolyte multilayer may further comprise particles coated with polyelectrolyte multilayers. The particles may preferably comprise non-porous silica in approximate sizes from about 1–5 μm, but may also comprise other suitable materials. Presence of these multilayer coated particles improves separation of neutral molecules by increasing transport of molecular species from the fluid flow into the multilayer. Multilayer coated particles may be included in any of the other embodiments of the present invention, for example in a capillary, or a plate. In addition, the coated particles may also be included in an apparatus embodiment of the invention.

The present invention also includes an apparatus for electrophoretic separation of macromolecules. The apparatus comprises a power supply having a positive electrode and a negative electrode for generating an electric field; a multilayer positioned substantially in a passage formed by passage walls, the passage having a first end electrically connected to the positive electrode and a second end electrically connected to the negative electrode to thereby generate an electric field through the passage, and wherein the multilayer comprises a plurality of polyelectrolyte layers; and a sensor positioned adjacent the passage for sensing macromolecules.

The invention further includes a method for analytical separation of macromolecules. The method comprises the step of forming a passage defined by passage walls. A second step in the method includes positioning a multilayer substantially within the passage adjoining the walls, wherein the multilayer comprises a plurality of polyelectrolyte layers. A third step includes positioning a sample containing macromolecules substantially within the passage. A fourth step includes generating a flow of a predetermined fluid through the passage to thereby substantially separate macromolecules from the sample responsive to an interaction with the multilayer. The flow of fluid may preferably be generated by passing an electric field through the passage, also known as electrophoresis, or by applying pressure to thereby generate the fluid flow.

BRIEF DESCRIPTION OF THE DRAWINGS

Some of the features, advantages, and benefits of the present invention having been stated, others will become apparent as the description proceeds when taken in conjunction with the accompanying drawings in which:

FIG. **1** is a schematic diagram of a capillary tube having a passage wall coated with a polyelectrolyte multilayer according to an embodiment of the present invention;

FIG. **2** is a schematic diagram of a microchannel plate;

FIG. **3** is a schematic view of the capillary tube passage of FIG. **1** including multilayer coated particles;

FIG. **4** is a schematic diagram of the apparatus of the present invention;

FIG. **5** shows electrophoretic separation of several proteins using a single layer of polyelectrolyte (PDADMAC) at about pH 4, as described in Example 1;

FIG. **6** shows electrophoretic separation of several proteins using a multilayer of polyelectrolytes at about pH 4, as described in Example 1;