

## APPARATUS FOR CAPILLARY ELECTROPHORESIS AND ASSOCIATED METHOD

This application is a continuation-in-part of Provisional Application No. 60/108,528, which was filed on Nov. 16, 1998, a date to which priority is claimed under 35 U.S.C. §120 and 37 CFR §1.78, and which Provisional Application is incorporated by reference herein in its entirety.

The claimed invention was made with financial support from the United States Government and the inventors hereby acknowledge that the government may have certain rights in the invention, as specified by law.

### FIELD OF THE INVENTION

The present invention relates to the field of molecular separations and, more particularly, to molecular separation by electrophoresis with a polyelectrolyte multilayer positioned within a very small passage such as in a capillary tube.

### BACKGROUND OF THE INVENTION

Electrophoresis is a method for separation of individual molecular species from a mixture by the application of an electric field. The technique relies on the migration of charged molecules through a solution in the electric field. Separation of the molecules occurs due to their different rates of movement through the solution, the rate being influenced by factors such as the pH of the solution, the mass and charge of the molecule, and the strength and duration of the electric field.

The electrophoretic separation may be carried out in a support medium wherein the molecules to be separated are loaded. Common support media for electrophoretic molecular separation include gels of various chemical formulations and physical configurations. Support gels, however, may be difficult to prepare, handle, and process, thereby resulting in reproducibility problems.

One approach for increasing reproducibility has been the use of capillary tubes, but without a support medium for the separation, other than the electrophoresis buffer itself. A capillary tube for use in this technique is substantially a small tube having a void space in the form of a very narrow passage therein. The electrophoretic separation is carried out within the narrow passage. For example, in the late fifties Hjerten reported success in electrophoretic molecular separations using a quartz capillary tube having an internal diameter of about 1–3 mm and using only a suspending solution as the separation medium. Hjerten, S., *Arkivkem.* 1958, 13, 151. Hjerten's system was never commercialized due to problems related to complex design and insufficient heat dissipation during the process. Over the succeeding years other authors reported improved separations and increased heat dissipation using thinner capillaries. In addition, what may be considered the first apparatus for capillary zone electrophoresis was described by Jorgenson and Lukacs. See Jorgenson, J, and K. D. Lukacs, *Anal. Chem.*, 1981, 53, 1298; and *Science*, 1983, 222, 266.

As known in the prior art, capillary zone electrophoresis (CZE) is generally performed as follows. An apparatus for CZE preferably includes a power supply which may provide for reversing polarity, the power supply being connected by each of two electrodes to each of two buffer reservoirs. A fused silica capillary is positioned so as to form a connecting bridge between the two reservoirs. The capillary is generally from about 20 cm to 1 m long, and includes a passage of

from about 25 to 100  $\mu\text{m}$  internal diameter. The capillary generally has an outer layer of polyimide to provide added flexibility, as well as durability. Detection of molecular species is performed in an area, or window, of the capillary where the polyimide coating has been stripped away. Suitable detection methods include absorbance, laser-induced fluorescence, refractive index conductivity, electrochemical detection, and even mass spectrometry, although this last approach requires an interface other than the capillary tube.

A sample containing the molecular species to be separated may generally be introduced in the capillary either hydrodynamically or electrokinetically. Those skilled in the art will know that hydrodynamic injection of the sample may be variously accomplished. The capillary may be elevated at one end to inject the sample by substantially syphoning it into the passage. A sample vial may be positioned in fluid connection with the passage, and fluid pressure may be applied to the capillary or to the sample vial to thereby move the sample into the passage. Conversely, suction may be applied at a second end of the passage to draw sample from a sample vial connected to a first end of the capillary. Injection may also be accomplished by means of a syringe, and may preferably include a sample splitter. Electrokinetic injection relies on the application of an initial voltage through the passage to initiate sufficient fluid flow to bring the sample into the passage, thereafter initiating predetermined electrophoretic separation conditions.

Commercially available systems for CZE also include features for rinsing, and for added heat dissipation. Rinsing is accomplished by flushing a rinse fluid through the passage, the rinse fluid usually being water, a buffer, or another predetermined solution. Rinse cycles may be effected by applying pressure to the system to thereby flush the rinse fluid through the microchannel. For added heat dissipation, commercial systems include a coolant feature. For example a fluorocarbon fluid may be used to bathe the capillary so as to prevent uneven heat dissipation during the electrophoresis.

Molecular separation by electrophoresis relies on the electrical interactions affecting the molecular species being separated. The passage walls defining the passage have naturally occurring electrical charges on their surfaces. In a fused silica capillary, for example, surface silanol groups (Si—OH) are substantially deprotonated at a pH above 2, the wall thereby having negative charges on its surface. A tightly adsorbed, substantially stagnant layer of cations from a fluid contained in the passage will localize adjacent the negatively charged wall so as to partially neutralize the negative charge on the wall. The remaining negative charge on the wall is neutralized by excess cations, which remain in the fluid in a more diffuse layer of mobile, solvated cations. The electrical potential across the double layer comprising the wall and the cations is known in the art as "zeta potential". In an electric field, cations are attracted to the cathode, and anions are attracted to the anode. In CZE, the cations in the diffuse layer migrate toward the cathode and, since they are solvated, pull solvent molecules along in their migration, creating a flow of solvent. This solvent flow induced by the electric field, is known as electroosmotic flow (EOF). The velocity of the EOF may be calculated according to equations well known in the art. During electrophoresis, molecules are separated by the EOF in relation to their charge and size. Because fluid flow is generally toward the cathode, molecules tend to elute (be released) from the capillary cations first, followed by neutral molecules having substantially no net charge, followed by anions. Neutral molecules tend not be separated from each