

a semi-permeable structure connecting the fluid chamber to the separation channel, allowing passage of one or more components of the solution and the second solution.

[0013] In its implementation, the present method functions to focus or separate analytes using either a neutral or charged pseudostationary phase. For example, the pseudostationary phase may be charged micelles such as negatively charged micelles, although the same principles discussed apply for positively charged micelles. A voltage is applied so that the charged micelles move from the region of high retention to the region of low retention. The background buffer is made to flow in the opposite direction, from the region of low retention to the region of high retention. In the region of high retention, the analyte is predominantly located within the micelles and so the analyte moves with the micelles. In the region of low retention, the analyte is predominantly located outside the micelles, and so moves with the buffer, in the opposite direction. Somewhere between the regions of high and low retention, the net velocity of the analyte is zero, and the analyte is focused at that point.

[0014] An advantage of this method over all of the previous focusing techniques is that it can be used with neutral analytes, which, in their native state, have zero electrophoretic mobility. An additional advantage over other focusing techniques is that it provides for focusing based upon properties of the analyte molecules other than the electrophoretic mobility of the molecule.

[0015] An advantage of this method over the previous EKC techniques is that the present method is a focusing technique and as such has no theoretical upper limit to the concentration factor. An additional advantage over EKC stacking techniques is that the position of the focused analyte can be manipulated, e.g., held stationary or moved, as needed to maneuver the sample for detection or further analysis while maintaining the sample in a narrow focused plug.

[0016] An advantage of this method over prior art stacking or sweeping methods is that this method employs a steady-state gradient in the retention factor. This differs from stacking or sweeping methods in which a transient retention factor gradient is established by sequentially injecting two or more solutions with different compositions into a separation channel in order to form a composition (and therefore retention factor) gradient at the interface between two different solutions. These types of gradients used for stacking or sweeping change over time after they have been established either being dissipated by dispersion/diffusion and/or moving along the length of the separation channel. In contrast, the steady state gradients used by this method are established and maintained by the externally-controllable parameters of the method (the applied voltage, bulk flow rate, temperature, etc.), and as long as those parameters are kept constant, the gradient will persist. With this method, it is possible, however, to vary the gradient over time, in a controlled manner, by varying the externally-controllable parameters of the method.

[0017] Further features and advantages of the present invention will be set forth in, or apparent from, the detailed description of preferred embodiments thereof which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1 is a schematic of micellar gradient focusing in a separation channel such as a microchannel or capillary;

[0019] FIG. 2 is a schematic drawing of a fluidic device according to one embodiment of the present invention;

[0020] FIGS. 3a-f depict fluorescence microscopy images of focusing of rhodamine B where the images of FIGS. 3a-f were taken just before initiating the focusing, i.e., 0 seconds, and at 10, 20, 30, 40, and 50 seconds, respectively, after the voltage was turned on;

[0021] FIG. 4 is a plot of peak concentration vs. time for focusing of rhodamine B in a capillary;

[0022] FIG. 5 depicts micellar gradient focusing and separation of two neutral fluorescent dyes, rhodamine B (left) and rhodamine 110 (right); and

[0023] FIG. 6 is a schematic of micellar gradient focusing in a separation conduit according to another embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0024] The present method provides focusing, i.e., concentrations and/or separation, based upon affinity of an analyte for a pseudostationary phase such as a micellar phase. The method works by creating a steady-state gradient in the retention factor of the solute of interest to the pseudostationary phase (i.e., affinity of solute for the micelles) in a separation channel such as in capillary systems or in a microfluidic chip. The solute has an inherent electrophoretic mobility when free in solution. When interacting with the pseudostationary phase, the solute assumes the electrophoretic mobility of the pseudostationary phase. On one side of the gradient, the solutes strongly interact with the pseudostationary phase and have a net mobility dominated by that of the pseudostationary phase. On the other side of the gradient, the retention factor is low and the solute assumes its native electrophoretic mobility. If the pseudostationary phase is charged, a combination of electrokinetic and pressure-driven flow can be applied so that the pseudostationary phase and the mobile phase move in opposite directions. Conversely, focusing can be performed with a neutral pseudostationary phase if the analyte is charged and made to migrate in the opposite direction of the mobile phase. Under these conditions, the analyte can be made to focus at a point along the retention factor gradient. Different analytes with different affinities for the pseudostationary phase (or different electrophoretic mobilities) will focus at different points. The present method thereby provides a focusing equivalent of EKC.

[0025] Referring now to FIG. 1, on the left side of channel 10, the retention factor of an analyte in the micellar phase, or other pseudostationary phase as represented by micelles 12, is high, so that the analyte is predominantly found in the micelles 12. In the context of this patent, a separation channel refers to any microchannel, capillary, or other tube or separation column where the focusing and separation takes place. On the right side of channel 10, the retention factor is low, and the analyte is predominantly found in the mobile phase, i.e., buffer 14. The mobile phase is pumped via pump system 16, either electroosmotically, with pressure