

MICELLAR GRADIENT FOCUSING

FIELD OF THE INVENTION

[0001] The present invention relates to a method and device for separating and or concentrating one or more analytes in a solution, and in particular, to a method and device which focuses and separates analytes using affinity gradient focusing.

BACKGROUND OF THE INVENTION

[0002] Over the past decade a great deal of research has been focused on the development of micro-total-analytical systems. This technology is based on the concept of integration of a series of microfluidic channels for the movement, separation, reaction, and/or detection of various chemicals, e.g., proteins, DNA, chemical compounds, etc.

[0003] Prior methods for concentrating analytes include stacking and focusing. In the context of this disclosure, focusing refers to methods for manipulating the velocity of an analyte and thereby causing the analyte to move towards a point at which its velocity is zero and where the analyte will therefore accumulate and increase in concentration. In addition, the location of the zero velocity point is often dependent upon some characteristic of the analyte molecule being focused, so that different analyte molecules are focused at different points, and thereby separated.

[0004] In this context, focusing is to be distinguished from stacking, which is a related class of methods in which analytes are moved through a velocity gradient (which is often transient) and the analyte peaks become narrower and more concentrated, but there is no point of zero analyte velocity. In stacking methods, the maximum degree to which analyte concentration can be increased is theoretically limited to the ratio of the velocities on the fast and slow sides of the velocity gradient. In contrast, for focusing at a zero velocity point, there is no theoretical limit to the concentration factor.

[0005] Previously known focusing methods include isoelectric focusing (hereinafter "IEF"), electric field gradient focusing (hereinafter "EMF"), counteracting chromatographic electrophoresis, and temperature gradient focusing (hereinafter "TGF"). In general, all of these previously known methods work by creating a gradient in the electrophoretic velocity of the analyte. Therefore, they only work with analytes that have non-zero electrophoretic mobility. For example, in IEF, the analyte has zero electrophoretic mobility only at the zero velocity point.

[0006] Micellar electrokinetic chromatography and related methods (hereinafter "EKC") take advantage of an analyte's affinity for a pseudostationary phase to facilitate separations using capillary electrophoresis. Traditional EKC separations differ from traditional focusing techniques in that analyte molecules move along a separation channel at an essentially constant velocity, whereas in focusing techniques, analytes migrate through the channel to a point where they have a zero velocity. Separation in EKC is achieved because different analytes migrate with different velocities dependent on their affinity for the pseudostationary phase. Often EKC separations are implemented in conjunction with stacking procedures to preconcentrate analytes and facilitate lower detection limits.

[0007] In EKC (and in chromatography in general), the buffer is referred to as the mobile phase, and a second phase such as a micellar phase is referred to as the pseudostationary phase. The pseudostationary phase serves the same function—to provide selectivity—as a stationary phase in chromatography, but is not actually stationary, and can move with the buffer and/or with its own electrophoretic mobility.

SUMMARY OF THE INVENTION

[0008] The invention provides a method for the preconcentration and or separation of analytes in solution. In particular, it provides a focusing method for analytes that cannot be separated or focused based purely upon their electrophoretic mobilities (neutral species, or chiral species, for example). The separation can be used as a part of a chemical or biochemical analysis in a microfluidic chip or capillary system.

[0009] The new method, described here, combines the two concepts of focusing and EKC to achieve something that could not be done with either of them separately: the focusing of analytes based upon their affinity for a pseudostationary chromatographic phase. The new method combines the characteristics and utility of EKC with those of focusing wherein the pseudostationary phase provides a medium for analytes to move at differing velocities along the separation channel, and hence facilitate a focusing separation modality.

[0010] In one form of the invention, a method is provided for directing at least one analyte in a solution containing a pseudostationary phase. The method includes establishing a steady-state gradient in the retention factor of the pseudostationary phase for the at least one analyte. In one further embodiment the method further includes moving the at least one analyte in the solution, so that the concentration of the at least one analyte is caused to change at one or more positions along the gradient.

[0011] The present invention in another form thereof concerns a device for equilibrium gradient focusing which includes a separation channel and a solution containing a pseudostationary phase located in the separation channel. A first means produces a steady-state gradient in the retention factor of the pseudostationary phase for at least one analyte. In a further embodiment, the device includes a second means which provides movement of at least one analyte within the separation channel so that concentration of the at least one analyte is caused to increase at one or more positions along the separation channel.

[0012] In various alternate further embodiments, the first means provides for a temperature gradient along the length of the separation channel or creates a steady-state gradient in the composition of the solution. The temperature gradient can be produced using a heat sink or a heat source thermally coupled to the separation conduit. In an alternative form, the second means comprises a power supply for applying an electric field along the separator channel and means for applying a bulk flow of the solution through the separation channel, where the means may include a pump, or the means may include the same power supply which provides for moving the analyte, whereby the bulk flow is driven by electroosmosis. In yet another alternative form, the first means includes a fluid chamber containing a second solution to which is largely disconnected from the separation channel