

pseudostationary phase, a gradient in the retention factor will still produce a gradient in velocity (if $u_{EP} \neq 0$), and so a neutral micellar or other pseudostationary phase can be used for focusing. If the analyte is neutral, however, the pseudostationary phase must be charged.

[0049] The present micellar gradient focusing can also be implemented through the superposition of multiple separation modalities to yield highly selective chiral separations. Traditionally, microchannel-based separations of chiral analytes are conducted by adding a chiral selector to the medium in which the separation is performed. Commonly these chiral selectors have a chiral handedness associated with their structure and thereby interact more strongly with either the D or L enantiomer of a racemic analyte mixture. This interaction either will lead to a retardation of the electrophoretic velocity of one enantiomer over the other and or differentially effect the partitioning into the pseudostationary phase, and thereby provide their separation.

[0050] Implementation of chiral selectors in the present method can be done by adding the desired chiral selector (in addition to the pseudostationary phase) to the buffer medium. Thus, the addition of the chiral selector would effect the velocities (and therefore the focusing points) of the two enantiomers differently in one or both of two ways: the chiral selector could either differentially modify the electrophoretic mobilities of the two enantiomers and/or it could differentially effect their partitioning into the pseudostationary phase. Thus micellar gradient focusing, where a spatial gradient in pseudostationary phase affinity for the analyte exists, allows for focusing of enantiomers at differing spatial points in the channel when combined with a chiral selector. Note that chiral separations could also be accomplished within this new method by using a chiral pseudostationary phase, such as micelles formed with chiral surfactants, either with or without an additional chiral selector additive.

[0051] It will now be apparent to one skilled in the art that the present method combines focusing with EKC and thereby incorporates the advantages of both. Advantages inherent to focusing include the high degree of analyte concentration that can be achieved which translates to lower concentration limits of detection, simultaneous separation and concentration of one or more analytes of interest, and the ability to manipulate the sample peak while maintaining focusing.

[0052] The combination of focusing with micellar chromatography adds the ability to focus and separate analytes that cannot be focused or separated with any other prior focusing technique. In particular, neutral species now can be focused, and different analytes with identical electrophoretic mobilities, such as stereo-isomers, can be separated.

[0053] Further, the pseudostationary-phase-based focusing of the present invention also allows for the tuning of the property of the analyte molecules that is the basis for the focusing and separation. For example, different pseudostationary phases can be used to separate analytes based upon their hydrophobicity, chirality, or specific affinity for a ligand. Any of the pseudostationary phases that have been developed for EKC can potentially be used in a focusing mode with the present novel method.

[0054] In addition, the present micellar gradient focusing technique, which separates molecules on the basis of prop-

erties other than electrophoretic mobility, can be used in conjunction with one of the previously developed focusing techniques or in combination with a different variation or embodiment of this present micellar gradient focusing technique to implement a 2D separation scheme in which both separation dimensions result from focusing techniques.

[0055] Since the retention factor gradient can be imposed using a temperature gradient, the present method potentially shares the advantage of the simplicity of implementation of temperature gradient focusing.

[0056] Although the invention has been described above in relation to preferred embodiments thereof, it will be understood by those skilled in the art that variations and modifications can be effected in these preferred embodiments without departing from the scope and spirit of the invention.

1. A method for directing at least one analyte in a solution containing a pseudostationary phase, the pseudostationary phase having a retention factor for said at least one analyte, said method comprising the step of:

establishing a steady-state spatial gradient in the retention factor in the pseudostationary phase for the at least one analyte.

2. The method of claim 1 further comprising 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 said gradient.

3. The method of claim 2, wherein the step of moving the at least one analyte is accomplished using a combination of an applied electric field and an applied bulk solution flow.

4. The method of claim 3, wherein:

the pseudostationary phase is an ionic pseudostationary phase;

the analyte is either neutral or charged;

the applied electric field has a significant component substantially aligned with said gradient and in a direction such that the resulting electrophoretic motion of the ionic pseudostationary phase is substantially aligned in a direction from a region of high retention factor to a region of low retention factor; and

the applied bulk solution flow has a significant component substantially aligned in a direction opposite the direction of the electrophoretic motion of the ionic pseudostationary phase.

5. The method of claim 4, wherein said step of establishing a steady-state spatial gradient in the retention factor comprises producing a temperature gradient in the solution.

6. The method of claim 4, wherein said step of establishing a steady-state spatial gradient in the retention factor comprises producing a gradient in the composition of the solution.

7. The method of claim 4, further comprising adding a chiral selector to the solution.

8. The method of claim 4, wherein the pseudostationary phase is selected from the group comprising micelles, microemulsions, lyosomes, and dendrimers.

9. The method of claim 3, wherein:

the pseudostationary phase is a neutral pseudostationary phase;