

## MIXING REACTIONS BY TEMPERATURE GRADIENT FOCUSING

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 10/197,331 filed on Jul. 18, 2002 now U.S. Pat. No. 7,029,561 B2; which claims benefit of the filing date of both Provisional Patent Application Nos. 60/307,691, filed on Jul. 25, 2001, and 60/323,404, filed on Sep. 19, 2001 and this application claims benefit of the filing date of Provisional Patent Application No. 60/588,237 filed on Jul. 15, 2004.

### STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made by employees of the United States Government and may be manufactured and used by or for the Government for governmental purposes without the payment of any royalties.

### FIELD OF THE INVENTION

The present invention relates to a method for electrokinetic focusing of materials in a fluid, and in particular, methods for electro-focusing materials in fluidic devices using electric field gradients.

### BACKGROUND OF THE INVENTION

Over the past decade a great deal of research has been focused on the development of technology related to micro-total-analytical systems. This technology is based on the concept of a series of microfluidic channels also known as micro-channels for the movement, separation, reaction, and/or detection of various materials such as chemicals; biological compounds such as amino acids, proteins, and DNA; or cells or colloidal particles.

One disadvantage with prior microfluidic devices and methods is that there is frequently a mismatch between the extremely small quantities of sample material used for analysis and the often much larger quantities needed for loading the sample into the microfluidic device and transporting the sample to the point of analysis. For example, a typical analysis sample may be around one nanoliter or less of a liquid containing one or materials that is injected into a separation channel and then separated electrokinetically as it moves down the channel to a detection region. However, the channels used to transport the sample materials to the injection point are typically also filled with the sample, thus increasing the required amount of the sample by a factor of 100 or more. In addition, the sample is typically loaded onto the microfluidic device into a reservoir from a pipette so that in all, approximately 99.9% of the sample is discarded as waste.

One general way of addressing the problem of requiring a large sample for analysis is to use any one of a number of focusing techniques. In the context of this disclosure, "focusing" refers to methods for manipulating the velocity of a material and thereby causing it to move towards a position at which the velocity is zero. At the zero velocity position the material will accumulate and increase in concentration, i.e., it will become focused. In addition, the location of the zero velocity position is often dependent upon some characteristic of the material or molecule being focused, so that different materials are focused at different positions, and can thereby be separated.

In this context, focusing is to be distinguished from stacking, which is a related class of methods. Stacking involves moving materials through a velocity gradient (which is often transient) so that the materials accumulate along the velocity gradient. However with stacking, there is no point where the material velocity is zero. Hence, in stacking methods the maximum degree to which material 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 position, there is no theoretical limit to the concentration factor.

Prior focusing methods include isoelectric focusing; electromobility focusing; counteracting chromatographic electrophoresis; temperature gradient focusing, disclosed in U.S. Patent Application Publication No. 2003/0019752, herein incorporated by reference; and most recently, chiral temperature gradient focusing, disclosed in co-pending U.S. patent application Ser. No. 11/039,955, herein incorporated by reference; and micellar affinity gradient focusing, disclosed in U.S. Patent Application Publication No. 2004/0206626, herein incorporated by reference. With the exception of the recently described micellar affinity gradient focusing method, the focusing methods all separate different materials based upon their electrophoretic properties, e.g., mobility in the case of electromobility focusing and temperature gradient focusing, and the isoelectric point in the case of isoelectric focusing.

Electric field gradient or electromobility focusing is one technique which can be used to concentrate samples at a given position within a microfluidic device before the analysis step. Further, the electric field gradient can be used to concentrate the entire sample at the beginning of the separation channel so that very little of the sample would be wasted.

Electric field gradient focusing is accomplished by the application of an electric field gradient within a microchannel. In response to the electric field gradient, there is a corresponding gradient in the electrophoretic velocity of any ionic material within the microchannel. The total velocity of the material is the sum of its electrophoretic velocity and the bulk fluid velocity. If these two components of the velocity are in opposite directions, they can be balanced so that the material will have zero total velocity.

When there is a gradient in the electrophoretic velocity, the balance between bulk and electrokinetic velocities can occur at a single position along the microchannel and therefore can result in focusing of the material at that position. Typically, the electric field gradient used in focusing is generated by the external manipulation of the electric field in the middle of the microchannel through the use of conducting wires, salt bridges, porous membranes, or other structures that will pass electric current but will restrict the flow of bulk fluid and the materials that are to be focused.

Several recent developments with regard to focusing methods in microfluidics, and in particular, the use of electric field gradients, have been made. A description of related methods of focusing can be found in C. F. Ivory, W. S. Koegler, R. L. Greenlee, and V. Surdugio, Abstracts of Papers of the American Chemical Society 207, 177-BTEC (1994); C. F. Ivory, Separation Science and Technology 35, 1777 (2000); Z. Huang and C. F. Ivory, Analytical Chemistry 71, 1628 (1999); W. S. Koegler and C. F. Ivory, Journal of Chromatography a 726, 229 (1996); and P. H. Ofarrell, Science 227, 1586 (1985), all of which are hereby incorporated by reference.

To illustrate the basic principles disclosed in these publications, reference is made to FIG. 1(a) which depicts a length of fluid-filled microchannel of constant cross-sectional area with an electrode, denoted 4, in the middle, and two further