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## FLUIDIC TEMPERATURE GRADIENT FOCUSING

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of the filing date of both copending Provisional Patent Application Nos. 60/307,691, filed on Jul. 25, 2001, and 60/323,404, filed on Sep. 19, 2001.

### 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 samples, and in particular, methods for electro-focusing samples 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 microchannels for the movement, separation, reaction, and/or detection of various chemicals or biological compounds such as amino acids, proteins, and DNA.

One disadvantage with prior microfluidic devices is that there is frequently a mismatch between the extremely small quantities of sample 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 sample 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 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.

Electric field gradient focusing is one way of addressing the problem of requiring a large sample for analysis due to the inefficiencies of conventional devices which result in wasted sample. Electric field gradient focusing can be used to concentrate samples at a given point within a microfluidic device before the analysis step. Further, the electric field gradient can be used to concentrate all of the 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 ion within the microchannel. The total velocity of the ion is the sum of its electrophoretic velocity and the bulk fluid velocity. If these two components of the velocity are in

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opposite directions, they can be balanced so that the molecule 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 point within the microchannel and therefore in focusing of ions at that point. 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 analytes 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. Surdigio, 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 buffer-filled microchannel of constant cross-sectional area with an electrode, denoted 4, in the middle, and two further electrodes at each end, denoted 3 and 5, so that the voltages  $V_1$ ,  $V_3$  at the ends and the voltage  $V_2$  at the middle of the channel can be controlled. A single species of negatively charged analyte is present in a buffer that is provided to the microchannel. The electrical connection, represented as electrode 4, can be accomplished with a simple metal wire as depicted in FIG. 1(a), or through a more complicated structure consisting of additional fluid channels and porous membrane structures or salt bridges.

The electric field in the section 1, i.e., the channel between electrodes 3 and 4 is  $E_1=(V_2-V_1)/(l/2)$  and the electric field in section 2, i.e., between electrodes 4 and 5, is  $E_2=(V_3-V_2)/(l/2)$ , where  $V_1$ ,  $V_2$ , and  $V_3$  are the voltages applied to the three electrodes 3, 4, and 5, and  $l$  is the length of the microchannel. If,  $E_1$  differs from  $E_2$  as shown in FIG. 1(b), the electrophoretic velocity of the analyte in the channel,  $u_{EP}$ , will be different in section 1 than in section 2. If an overall bulk fluid velocity,  $u_B < 0$ , is applied, e.g., either electro-osmotic or pressure-driven, the bulk fluid velocity must be the same, due to continuity, in all parts of the microchannel. The total velocity of the analyte,  $u_T = u_B + u_{EP}$ , will then be the sum of the electrophoretic and bulk velocities, which can differ in section 1 from section 2.

The use of the microchannel device of FIG. 1(a) for focusing of the ions is illustrated in FIG. 2 where  $u_{T,1} > 0 > u_{T,2}$ , so that the ions flow into the middle from both directions and are thus focused in the middle of the channel near electrode 4.

One major drawback to electric field gradient focusing is that the microchannel device tends to be difficult to construct and that it requires the control of voltage on an additional electrode, e.g. 4 of FIG. 1(a), that is used to apply the electric field gradient. In addition, if electrodes are used to generate electric field gradients, unwanted chemical products will be generated electrochemically at the buffer-electrode interface. If the electric field gradient is produced through the use of a salt bridge or membrane, the electro-