

solution of Oregon Green 488 carboxylic acid in 1.8 M Tris/boric, 100 min of focusing resulted in a focused plug of Oregon Green 488 carboxylic acid with a peak concentration over 80 μM —a greater than 10000-fold increase in concentration.

It will become readily apparent to one of ordinary skill in the art that the present method provides for use in numerous applications. For example, temperature gradient focusing could be used as a preconcentration step before an analysis or separation or as a simultaneous concentration and separation technique.

In addition, temperature gradient focusing may be used with any charged species in solution and not just small molecules. For example, the analytes may include larger molecules such as proteins and DNA, or even particles and cells. Further, the present method can be used with particles to create packed beds of particles or cells for use in other analysis steps. In addition, the present method can be adapted for use to sort particles or cells by electrophoretic mobility.

In one separation mode, the bulk velocity could be ramped over time to scan focused sample peaks past a fixed detector, e.g. the detector shown in FIG. 5. This would produce results similar to capillary electrophoresis but the widths of the sample peaks would be determined by the applied gradients and the peak heights would be determined by how long a given peak was in the focusing “window”. If the ramp speed were halved, the peak heights would all be doubled, so that the ramp rate could be chosen dependent on the concentration limit of detection necessary. Alternatively, the focusing window could remain fixed and a scanning or imaging detector could be used to locate the separate peaks.

In a further embodiment, the method may be adapted for a system where temperature dependence is due to something other than the ionic strength. An example is a system having $f(T)$ constant but $f_{EP}(T)$ not constant, or variable. One way to accomplish this would be to use a buffer with a temperature dependent pH. In such a system, this embodiment of the present invention is similar to isoelectric focusing schemes. However, the present environment differs from isoelectric focusing in that, in the present system, an opposing buffer flow is applied so that analytes are focused at a pH other than their isoelectric points.

When using any of the embodiments of the present method, operating parameters which include voltage, bulk flow rate, and temperature of the different zones may be held constant with time or varied with time to affect the position and width of focused sample peaks. Varying of parameters may be accomplished using any of a number of methods which include the methods previously described above in which the focused sample peaks are scanned past a fixed detector.

Advantageously, in order to achieve the fastest accumulation of analyte in the focused peak, the highest possible voltage should be used. However, a higher applied voltage requires a faster bulk flow which results in greater dispersion, i.e., wider focused peaks, which is disadvantageous for separation and for achieving preconcentration of a sample to a high concentration in a very narrow peak. Therefore, a high voltage and fast bulk flow could be used for the initial accumulation of analyte into a relatively broad peak, and the voltage flow and flow rate could be reduced to the point at which the peak is narrowest. Further, temperature gradients could be turned on and off to first concentrate the sample and then release the focused peak and allow it to flow on down the channel. Further, the temperature gradient can be adjusted to be linear or nonlinear, and the temperature

gradient may be monotonic or non-monotonic. Thus, operating parameters may be adjusted to achieve the desired results.

While the previously disclosed embodiments are directed to a microchannel or microfluid device, the present method may be adapted for incorporation for use with substantially larger channels which may include millimeter and centimeter if not larger in dimension which should now be apparent to one of ordinary skill in the art. Because temperature gradient focusing uses low conductivity buffers, one can adapt the present method for use in much larger scale geometries than the micron-sized channels and capillaries described in detail herein.

Further, the previously described method can be adapted for use in modified capillary fluidic systems known to one of ordinary skill in the art. FIG. 6 depicts a capillary fluidic system having a capillary tube 70 spanning between two buffer reservoirs 77. Two temperature blocks, denoted as heated block 72 and cooling block 73 are located along the length of the capillary tube 70 to provide a desired temperature gradient in the capillary tube 70. Alternatively, temperature blocks being both cooling, both heated, both at ambient temperature, or any combination, thereof, may be substituted to provide the desired temperature gradient.

The buffer reservoirs 77 contain a buffer with temperature dependent ionic strength. Electrodes 74, 75 are connected at one end to a power supply and on the other end, are in contact with the buffer solution in the buffer reservoirs 77. The power supply applies a driving voltage through the capillary tube 70. A source of bulk flow is driven either by electro-osmosis with the applied driving voltage, by a pressure gradient applied, e.g. by a pump, or a combination of the two. Detector 80 is used to detect analytes present in the buffer solution.

One of ordinary skill in the art now will readily appreciate that the present temperature gradient focusing differs from prior art methods such as sample stacking and isotachopheresis. In both cases, samples are focused or concentrated as a result of gradients in their electrophoretic velocities. In sample stacking and isotachopheresis, the velocity gradients are generated at the interfaces between buffers of different composition, and the point at which the concentration or focusing occurs is not stationary, but moves along with the electroosmotic flow in the channel or capillary. In contrast to both sample stacking and isotachopheresis, the velocity gradients that produce analyte focusing in the present temperature gradient focusing result from applied temperature gradients.

Further, one skilled in the art will recognized that the present temperature gradient focusing differs from isoelectric focusing techniques such as those disclosed in U.S. Pat. Nos. 3,664,939 and 5,759,370. Unlike isoelectric focusing techniques in which the pH gradient is established by using a buffer system that has a temperature dependent pH, the present temperature gradient focusing utilizes a buffer that has a temperature dependent ionic strength. When a temperature gradient and a voltage are applied to a microchannel, the ionic strength gradient of the buffer gives rise to a velocity gradient, which is used for focusing. As a result, an analyte present in the buffer is focused at a point where the analyte's total velocity, i.e., the sum of the electrophoretic velocity and the bulk velocity of the buffer is zero. Therefore, in the present temperature gradient focusing, the pH and the isoelectric point of the analyte are not critical.

It will now be apparent to one of ordinary skill in the art that the present microfluidic device and temperature gradient focusing method provide numerous advantages over prior