

ing provides the ability to the concentrate targets prior to the assay reaction, with no theoretical limit to the concentration factor. This leads to assays with improved detection limits. This is in contrast to prior methods of concentrating a target which is difficult in affinity based capillary electrophoresis assays and typically involves a pre-concentration step.

Another advantage of the present method is the ability to mix constituents within a microfluidic geometry that does not require complex channel geometries or channel modifications. Because of the low Reynold's number in microfluidic devices, mixing two fluid streams together by diffusion alone can require unacceptably long channel lengths. One way to overcome this is to introduce geometries that facilitate mixing such as serpentine channels or slanted wells ablated into channels. With temperature gradient focusing, the focusing mechanism isolates one of the species to be mixed in a unique spatial location while the second species is allowed to flow through the focused band by bulk flow (or by a combination of bulk flow and electrophoretic motion if the second species is charged). This allows for mixing to occur over small length scales, e.g. 50-200 μm defined by the length of the focused band. In addition, the labeling for detection, e.g. target or probe is simplified because only one needs to be labeled since the product of the target and probe focuses in a unique location.

A further advantage of processing PNA/DNA duplexes through a temperature gradient, e.g., determining melting temperatures of duplexes, is that the present method requires much lower concentration samples. A conventional UV absorbance-based melting temperature measurement typically requires μM levels of DNA samples while temperature gradient focusing only requires nM to pM levels, an improvement of 3-6 orders of magnitude.

In addition, the present temperature gradient focusing-based analysis can also be performed faster than the conventional UV absorbance-based melting temperature measurement, e.g., 5 min compared to 60 min as initially demonstrated. In addition to melting curves of PNA/DNA duplexes, the scanning temperature gradient focusing can be used for any temperature dependent assay, e.g. polymer precipitation reactions.

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 solutions of different composition, and the position 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 material 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 fluid system that has a temperature dependent pH, the present temperature gradient focusing typically utilizes a fluid 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 fluid gives rise to a velocity gradient, which is used for focusing. As a result, a material present in the fluid is focused at a position where the materi-

al's total velocity, i.e., the sum of the electrophoretic velocity of the material and the bulk velocity of the fluid is zero. Therefore, in the present temperature gradient focusing, the pH and the isoelectric point of the material are typically not critical. In the embodiment of the present invention that utilizes fluids with a temperature-dependent pH, and thereby uses a thermally generated pH gradient for focusing, a bulk fluid flow is applied so that rather than focusing at its isoelectric point as in isoelectric focusing, a material will focus at a position along the pH gradient where the local pH is unequal to the isoelectric point of the material.

It will now be apparent to one of ordinary skill in the art that the present temperature gradient focusing method provides numerous advantages over prior methods. The present method is simpler to implement as no imbedded electrodes or salt bridges are necessary. In addition, like isoelectric focusing, temperature gradient focusing can be used to both concentrate and separate materials, but without the disadvantages associated with isoelectric focusing.

A further advantage of the present invention is provided in that only a single, continuous fluid system is required. Solid phase extraction and related preconcentration methods of the prior art require multiple fluids where one fluid is used to carry the material to the preconcentrator and a second fluid is used to release the material from the preconcentrator. Further examples of multiple fluid systems include sample stacking, field amplified injection, isotachopheresis, and sweeping.

Further, the present temperature gradient focusing provides enhanced concentration when compared with the prior art of other single preconcentration methods.

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.

What is claimed is:

1. A method for analyzing the interaction between a first material and a second material in a fluid, said interaction giving rise to a third material, said method comprising the steps of:

- introducing a first material to a fluid;
- applying an electric field to said fluid thereby causing said first material to move electrophoretically with an electrophoretic velocity;
- establishing a temperature gradient in said fluid having a significant component substantially aligned with the electrophoretic motion of said first material, thereby generating a gradient of the electrophoretic velocity of said first material;
- producing a flow of said fluid having a significant component substantially aligned in a direction opposite a direction of the electrophoretic motion of said first material; wherein magnitudes of said electric field, said temperature gradient, and said flow are such that said first material will accumulate or be focused at a first position along said temperature gradient; and
- introducing a second material to said fluid so that said second material moves through said first position, thereby interacting with said first material to form a third material;
- whereby said third material of said interaction is then focused at a second position along said temperature gradient.

2. The method of claim 1, wherein an ionic strength of said fluid is temperature dependent and said temperature gradient establishes a gradient in the ionic strength of said fluid.