

3

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 negatively charged material to be focused is present in a fluid 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 material 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 material,  $u_T = u_B + u_{EP}$ , 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 material is illustrated in FIG. 2 where  $u_{T,1} > 0 > u_{T,2}$ , so that the material moves into the middle from both directions and is 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), which 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 fluid-electrode interface. If the electric field gradient is produced through the use of a salt bridge or membrane, the electrochemical products can be avoided, however only materials that cannot pass through the membrane or salt bridge can be focused.

Two additional methods for concentrating a sample include sample stacking and field amplified sample injection in which a sample is concentrated as the sample crosses a boundary between low and high conductivity fluids. These methods can achieve preconcentration factors of 100 to 1000-fold although these methods require multiple fluids. Sweeping is yet another concentration method which is capable of a very high degree of sample concentration (e.g., up to 5000-fold), but is useful only for small hydrophobic molecules with a high affinity for a mobile micellar phase.

An additional technique for concentrating an ionic material includes isoelectric focusing. Isoelectric focusing is commonly used for the concentration and separation of proteins and involves the focusing of materials at their respective isoelectric points along a pH gradient.

Two examples of recent isoelectric focusing techniques are provided by U.S. Pat. No. 3,664,939 to Luner et al. and U.S. Pat. No. 5,759,370 to Pawliszyn. Both references relate to isoelectric focusing with pH gradients that are created by the application of a temperature gradient. Isoelectric focusing uses a pH gradient to focus materials and in particular proteins, at positions along the pH gradient where the local pH is equal to the isoelectric points of the materials. The isoelectric point is the pH at which the material has zero electrophoretic mobility, i.e., approximately zero charge. pH gradients for isoelectric focusing are typically generated using ampholyte mixtures or immobilized ampholytes in gels. The two above referenced patents are included here as examples of prior art

4

uses of temperature gradients for focusing. It is, however, very unusual for isoelectric focusing to be done with a pH gradient generated using a temperature gradient.

One disadvantage of isoelectric focusing is that it is limited in application because it can only be used with materials having an accessible isoelectric point such as proteins and peptides. Additionally, the concentration to which a protein can be focused with isoelectric focusing is severely limited due to the low solubility of most proteins at their isoelectric points.

#### BRIEF SUMMARY OF THE INVENTION

Broadly speaking, the present invention involves temperature gradient focusing, in conjunction with the concept that a molecular species or other material in a fluid can be focused and thereby spatially localized, e.g., as a focused band, and then allowed to interact or mix with another molecular species or material that is carried by a bulk fluid flow or electrophoresis through the focused band of the first material. If the interaction, binding, or reaction of the two materials produces a third material, that third material can then also be focused on the temperature gradient. Detection of the focused band of the third material, which is the product of the interaction or reaction of the first two materials, signals that the interaction has occurred. If the product of the interaction is a duplex of the first two materials, further manipulations can then be used to determine the strength of the binding of that duplex by measuring its melting curve and melting temperature.

In the context of this disclosure, "duplex" refers to a pair of two different molecular species or other subject materials that are bound together through specific or non-specific interactions, including biochemical, physiological, and/or pharmaceutical interactions. Examples of types of molecules or materials that would typically be involved in duplex formation include: proteins, nucleic acids, glycoproteins, carbohydrates, hormones, pharmaceuticals, antibodies, and aptamers. Specific examples of duplex forming pairs include: DNA/DNA, DNA/PNA, DNA/RNA, RNA/PNA, antibody/antigen, antibody/hapten, nucleic acid aptamer/protein, drug molecule/drug target molecule, enzyme/substrate, enzyme/inhibitor, enzyme/cofactor, binding protein/substrate, carrier protein/substrate, lectin/carbohydrate, receptor/hormone, receptor/effector, ligand/cell surface receptor, virus/ligand, etc.

Temperature gradient focusing is conducted using a method and device for concentrating and separating materials in a fluid within fluid conduits which include channels, microchannels, and capillary tubes. The concentration is achieved by balancing the electrophoretic velocity of a material against the bulk flow of fluid in the presence of a temperature gradient. Using an appropriate fluid, the temperature gradient can generate a corresponding gradient in the electrophoretic velocity of the material so that the electrophoretic and bulk velocities sum to zero at a given position and the material will be focused at that position.

One aspect of the present invention concerns a method for analyzing the interaction between a first material and a second material in a fluid. The interaction gives rise to a third material. The method includes the steps of introducing a first material into a fluid and applying an electric field to the fluid thereby causing the first material to move electrophoretically with an electrophoretic velocity. A temperature gradient is established in the fluid having a significant component substantially aligned with the electrophoretic motion of the first material, thereby generating a gradient in the electrophoretic velocity of the first material. A flow is produced in the fluid