Microfluidic Temperature Gradient Focusing

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A new technique is described for the concentration and separation of ionic species in solution within microchannels or capillaries. Concentration is achieved by balancing the electrophoretic velocity of an analyte against the bulk flow of solution in the presence of a temperature gradient. With an appropriate buffer, the temperature gradient can generate a corresponding gradient in the electrophoretic velocity, so that the electrophoretic and bulk velocities sum to zero at a unique point, and the analyte will be focused at that point. The technique is demonstrated for a variety of analytes, including fluorescent dyes, amino acids, DNA, proteins, and particles, and is shown to be capable of greater than 10 000-fold concentration of a dilute analyte.

In the past 10 years, microfluidics¹–⁴ has progressed rapidly from a simple concept to the basis of a new technology that promises tremendous advantages for the field of biotechnology. One challenge posed by miniaturization lies in the detection of very dilute solutions of analytes in ultrasmall volumes, nanoliters or less. In addition, there is frequently a mismatch between the extremely small quantities of sample used for analysis and the much larger quantities needed for loading the sample into the microfluidic device and transporting it to the point of analysis. In a typical example, an electrophoretic separation on a microchip requires the injection of less than 1 nL of sample into the separation channel. However, the channels used to transport the sample to the injection point are also filled with sample, increasing the required volume by a factor of 100 or more. Additionally, the sample is generally loaded into the microchip by pipet, so that 1 μL or more of sample is used, 99.9% of which is discarded as waste. Because sensitivity is challenged in these volume-limited microsystems, it would be advantageous to make use of the entire sample by preconcentrating analytes into a smaller volume suitable for analysis and detection.

Not surprisingly, a great deal of effort has been devoted to addressing this problem⁵–⁹ (and related problems in traditional capillary electrophoresis—for reviews, see refs 10–13), and a number of different preconcentration methods have been investigated. Chemical affinity methods, which concentrate samples using affinity ligands bound to membranes or beads,⁷,¹⁰,¹²,¹³ or directly to the channel or capillary walls,⁵,¹⁰,¹²,¹³ can achieve high degrees of concentration (up to 7000-fold)¹⁰. However, these methods can be difficult to implement, as they require reproducible chemical modification of surfaces and the use of multiple buffers, one to deliver the sample to the preconcentrator and a second to release the sample for analysis. Sample stacking and field-amplified sample injection,¹⁰,¹¹,¹³ in which sample is concentrated as it crosses a boundary between low- and high-conductivity buffers, are some of the simplest and most commonly used concentration techniques for small analytes and achieve fairly high preconcentration factors (100–1000-fold), though they also require multiple buffers. Sweeping⁴,¹⁵ is capable of a very high degree of sample concentration (up to 5000-fold) but is useful only for small hydrophobic analytes with a high affinity for a mobile micellar phase.

Isoelectric focusing (IEF)¹⁶ is a technique that is most commonly used for the concentration and separation of proteins and involves the focusing of analytes at their respective isoelectric points (pIs) along a pH gradient. Successful implementation of this method has been the subject of 40 years of research, most of it pertaining to the generation of stable pH gradients. IEF is limited in application because it is restricted to use with analytes with an accessible pl. Additionally, the concentration to which a protein can be focused with IEF is severely limited by the low solubility of most proteins at their pIs.

Field gradient focusing methods¹⁷–¹⁹ can be relatively simple to implement, offer a number of advantages including the use of

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a single continuous buffer, and, in contrast to IEF, are not limited to analytes with an accessible pI. In previously described field gradient focusing methods, electric field gradients are produced using metal electrodes,18-20 which can introduce unwanted electrochemical products at the electrode—buffer interface. Manipulation of the electric field through membranes18,20 or porous salt bridge-like structures5 eliminates that problem; however, only large molecules or particles that cannot pass through the membrane or salt bridge can be concentrated.

Here we demonstrate a new type of field gradient focusing that can be implemented in a simple straight channel or capillary without the need for embedded electrodes, membranes, or salt bridges. This new technique, which we call temperature gradient focusing (TGF), provides simultaneous concentration and separation in a manner similar to IEF but is applicable to any charged analyte and is not limited to molecules with a specific pI. In addition, we demonstrate that TGF can be used to achieve higher degrees of sample concentration (more than 10 000-fold) than any other single sample preconcentration method.

TGF is similar to electric field gradient focusing (EFGF)18-20 in that both focus analytes by balancing the electrophoretic velocity of an analyte against the bulk velocity of the buffer containing the analyte. If there is an appropriate gradient in the electric field, the total velocity of the analyte (the sum of the bulk and electrophoretic velocities) can be set to zero at a unique point along a channel, and all of the analyte in the system will move toward that point. In contrast to EFGF, where the electric field gradient is applied using a combination of electrodes and membranes, in TGF the necessary electric field gradient is produced simply by the application of a temperature gradient.

**THEORY**

Before beginning, it is important to clarify the differences between the current focusing method and methods such as sample stacking and isotachophoresis that may be more familiar to some readers. In both cases, samples are focused or concentrated as a result of gradients in their electrophoretic velocities. In sample stacking and isotachophoresis, 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. In contrast, the velocity gradients that produce analyte focusing in TGF result from applied temperature gradients. Consequently, the point at which the focusing occurs is stationary. TGF, then, is analogous to IEF in a gel with immobilized ampholytes.

Consider a long, straight, buffer-filled microchannel with electrode connections at each end as shown in Figure 1a. The velocity of an analyte in the channel is given by the sum of its electrophoretic velocity and the bulk velocity of the buffer:

\[
u_t = \nu_{EP} + \nu_B\] (1)

If there is a gradient in the electrophoretic velocity, the bulk velocity can be adjusted so that the total velocity is equal to zero at a single point along the channel, and the analyte will be focused at that point.

The electrophoretic velocity of an analyte in the microchannel is given by the product of the electric field and the electrophoretic mobility of the analyte: \(u_{EP} = E_{EP}\). In EFGF, the velocity gradient that is used for focusing results from a gradient in the electric field with constant mobility. In TGF, a temperature gradient is applied along the length of the channel as shown in Figure 1b; this results in corresponding gradients in both \(E\) and \(u_{EP}\).

The electric field in the channel is given by

\[E = I/A\sigma\] (2)

where \(I\) is the electric current running through the channel, \(A\) is the channel cross-sectional area, and \(\sigma\) is the conductivity of the buffer. Since the conductivity is temperature-dependent, the electric field is also temperature-dependent. Here, we assume constant current because the current running through any given section of the microchannel will be the same for all parts of the channel, whereas the voltage drop across a portion of the channel and the electric field in it will depend on the temperature of that portion. Most often, the primary temperature dependence of the conductivity is due to the variation of the buffer viscosity with temperature (the viscosity of water is reduced by more than a factor of 3 on going from room temperature to boiling), so it can be written as

\[\sigma = \eta(20)\alpha(20)\eta(T)f(T)\] (3)

![Figure 1. Schematic illustration of temperature gradient focusing in a microchannel. (a) The microchannel is heated on one end and cooled on the other, producing a temperature gradient in the middle. (b) Temperature distribution along the microchannel. (c) The function \(f(T) = \alpha(20)\eta(20)\eta(T)\) (see eq 3) plotted as a function of the distance along the microchannel. (d) Velocity as a function of the distance along the microchannel. The electrophoretic velocity \((u_{EP})\), dashed line, is proportional to \(f(T)\). The average bulk fluid velocity \((u_B\), dotted line) is constant. The total analyte velocity \((u_T\), solid line) is the sum of the electrophoretic and bulk velocities. If \(f(T)\) is nonconstant, then the applied temperature gradient will result in a gradient in \(u_{EP}\). By adjusting \(u_B\), the total velocity can be made to equal zero at a point near the middle of the channel, and all of the analyte in the channel will be focused at that point.](image)

where $T$ is the temperature, $\eta_0$ is the buffer conductivity at $T = 20 \degree C$, $\eta(T)$ is the (temperature-dependent) viscosity, and $f(T)$ is a function that accounts for any other temperature dependence—such as that resulting from a temperature-dependent ionic strength—and is normalized so that $f(20) = 1$. Using eq 2 and eq 3, the temperature-dependent electric field is then given by

$$E = \frac{\eta(20)f(T)}{\eta(20)} \equiv \frac{\eta(T)f(T)}{\eta(20)}$$

(4)

The electrophoretic mobility is also primarily dependent on temperature through the viscosity so that it can be written in a form analogous to that used for the conductivity:

$$\mu_{EP} = \eta(20)\mu_{EP}^0/\eta(T)f_{EP}(T)$$

(5)

where $\mu_{EP}^0$ is the electrophoretic mobility at $T = 20 \degree C$, and $f_{EP}(T)$ accounts for any temperature dependence of the electrophoretic mobility not due to changes in viscosity ($f_{EP}(20) = 1$). The electrophoretic velocity is then given by (assuming that the concentration of analyte is low compared to that of the buffer)

$$u_{EP} = E\mu_{EP}^0/f_{EP}(T)$$

(6)

If $f(T)$ and $f_{EP}(T)$ have the same temperature dependence (if they are both constant, for example, as is the case for most buffers and most analytes), then $u_{EP}$ will not be temperature-dependent, and although a temperature gradient will result in an electric field gradient, it will not result in a velocity gradient. If, on the other hand, $f(T)$ and $f_{EP}(T)$ do not have the same temperature dependence, then temperature gradients will result in gradients in the electrophoretic velocity, which can be used to focus and concentrate analyte.

Most commonly—as is the case for the examples presented here—TGF would be implemented with a buffer characterized by a strongly temperature dependent $f(T)$ and analytes characterized by a constant or nearly constant $f_{EP}(T)$—as shown in Figure 1c. Though it could also be implemented in a system in which $f(T)$ is constant and $f_{EP}(T)$ is not, or in which both $f(T)$ and $f_{EP}(T)$ are nonconstant, but differ in their temperature dependence.

A major advantage of this method over other methods of EFGF is that the concentration of the buffer salts is unaffected by the focusing. Because the buffer conductivity is proportional to the mobility of the salts in it, if the buffer salt is considered as an analyte, then, $f_{EP}(T) = f(T)$ so that the electrophoretic velocity of the buffer salts is temperature-independent, and they are not focused. In other words, the temperature dependence of the electrophoretic mobility of the buffer salts is what gives rise to the temperature dependence of the buffer conductivity and hence the electric field (eqs 3–4). Consequently, the product of the electric field and the electrophoretic mobility of the buffer salts is constant, and the velocity of the buffer salts is constant.

A given analyte will be focused at the point along the temperature gradient where $u_T = u_{EP} + u_B = 0$, so that (using eq 6)

$$u_B = -E\mu_{EP}^0/f_{EP}(T)$$

or

$$\mu_{EP} = -\frac{u_B f_{EP}(T)}{E f(T)}$$

(7)

Assuming that $f_{EP}(T) = \text{const} = 1$ and $\partial f(T)/\partial T < 0$ (as is the case for the experiments described below), multiple analytes (with different mobilities) can be simultaneously focused (on the same temperature gradient, and with the same $u_B$) provided that they have mobilities that fall within a certain range:

$$\mu_{min} < |\mu_{EP}| < \mu_{max}$$

(8)

The extent of that range is dependent on the properties of the buffer ($f(T)$) and the high- and low-temperature extremes of the applied temperature gradient. If $T_H$ and $T_C$ are the high- and low-temperature extremes of the applied temperature gradient, then

$$\mu_{max}/\mu_{min} = f(T_C)/f(T_H)$$

(9)

Therefore, the range of analyte mobilities that can be simultaneously focused is given by the range of $f(T)$. This does not mean that TGF is restricted to use with analytes having a mobility in any particular range. It is only the range of analytes that can be simultaneously focused that is limited. By adjusting $u_B$, the span of the temperature gradient, or both, TGF can be used to focus any analyte with nonzero electrophoretic mobility.

The average bulk fluid velocity, $u_B$, can be driven using either an applied pressure gradient, electroosmosis, or a combination of the two. Because of the low dispersion inherent to electroosmotic flow (EOF), TGF will work best if most or all of the bulk velocity results from EOF.

In a channel of nonuniform temperature, the electric field and electroosmotic mobility will also be nonuniform, and the average electroosmotic velocity will be given by

$$\langle u_{EO} \rangle = \frac{1}{l} \int_0^l E(T(z))\mu_{EO}(T(z)) \, dz$$

(10)

where $z$ is the spatial coordinate along the length of the channel and $l$ is the channel length. The electroosmotic mobility, $\mu_{EO}$, is typically written as

$$\mu_{EO}(T) = \frac{\epsilon z}{4\pi \eta(T)} \equiv \mu_{EO}^0 \eta(20)/\eta(T)$$

(11)

where $\epsilon$ is the dielectric constant of the buffer and $z$ is the zeta potential of the wall–buffer interface. Assuming constant $\epsilon$ and $z$ (and $\mu_{EO}^0$), the average electroosmotic mobility becomes

$$\langle u_{EO} \rangle = \frac{E \mu_{EO}^0}{l} \int_0^l f(T(z)) \, dz$$

(12)

If the temperature gradient is sharp compared to the length of the channel, $T(z)$ can be approximated by a step function:

where \( x \) is the fraction of the channel that is at \( T_H \).
The integral in (12) then becomes

\[
\frac{1}{f(T_H)} \int_0^\infty f(T(z)) \, dz = \frac{x}{f(T_H)} + (1-x)f(T_C)
\]  

(14)

indicating that the \( u_{EO} \) can be tuned by varying \( x \), the fraction of the channel that is at \( T_H \).

If the bulk flow is driven entirely by EOF, \( u_B = \langle u_{EO} \rangle \), the condition for focusing can be written as

\[
f(T_H) + (1-x) < \frac{-\mu_{EP}^0}{\mu_{EO}^0} < x + (1-x)\frac{f(T_C)}{f(T_H)} - \text{substitute in from (14)}
\]  

(15)

The width of a focused band of analyte can be calculated using the steady-state diffusion equation:

\[
D_{\text{eff}} \frac{dC(z)}{dz} = u_T C(z) = [u_B + E_{\theta\,EP}^0 f(T(z))] C(z)
\]  

(16)

where \( C(z) \) is the analyte concentration and \( D_{\text{eff}} \) is an effective dispersion coefficient. If \( f(T(z)) \) is assumed to be a linear function of position, \( f(T(z)) = f_0 + f_2z \), the solution to (16) is a Gaussian:

\[
C(z) = C_0 \exp[-(z-z_0)^2/2\alpha^2]
\]  

(17)

with

\[
z_0 = -\frac{f_0}{f_1} - \frac{u_B}{E_{\theta\,EP}^0 f_1}
\]  

(18)

and

\[
\alpha = \sqrt{D_{\text{eff}} E_{\theta\,EP}^0 f_1}
\]  

(19)

Two closely spaced analyte bands can be resolved if the distance between them, \( \Delta z_0 > 2\alpha \), or (using eq 7, eq 18, and eq 19)

\[
\frac{\Delta \mu_{EP}^0}{\mu_{EP}^0} > 2 \sqrt{\frac{D_{\text{eff}} f_1}{E_{\theta\,EP}^0 f(T)^2}}
\]  

(20)

where \( \Delta \mu_{EP}^0 \) is the difference in mobilities of two similar analytes. So, as with IEF and EFGF, analyte bands in TGF are more tightly focused using sharper temperature gradients, whereas the ability to resolve analytes with similar mobilities is improved by spreading out the temperature gradient.

The amplitude of the Gaussian (eq 17) is

\[
C_0 = \frac{N_{\text{tot}}}{\sqrt{2\pi\alpha_A}}
\]  

(21)

where \( N_{\text{tot}} \) is the total amount of analyte that has been focused. If analyte is continuously fed into the microchannel from a sample reservoir, as is the case in all of the examples presented below, the total amount of analyte and therefore the peak amplitude, \( C_0 \), will increase linearly with time. If the input concentration is much less than peak concentration, \( C_0 \ll C_0 \), the system can be considered as quasi-steady state, and the shape of the analyte band will still be well described by the Gaussian form (eq 17). In that case,

\[
N_{\text{tot}} = C_{in} A u_T (T_{in}) t
\]  

(22)

and

\[
C_0 = C_{in} E_{\theta\,EP}^0 f(T_{in}) - f(T_0) - \frac{1}{\sqrt{2\pi\alpha_A}}
\]  

(23)

where \( T_{in} \) is the temperature of the sample input reservoir, \( T_0 \) is the temperature at the point where the analyte is focused (\( z = z_0 \)), and \( t \) is the focusing time. It should be noted that when the concentration of the focused analyte becomes comparable to the concentration of the buffer, it will begin to have a nonnegligible effect on the solution conductivity, and the shape of the analyte band will no longer be well described by eq 17 and eq 23.

**EXPERIMENTAL SECTION**

**Chemicals and Materials.** All aqueous solutions were prepared using water from a Millipore Milli-Q \(^{22} \) system (Bedford, MA). DMSO was obtained from Matheson, Coleman and Bell Co. \(^{22} \) (Norwood, OH). Methanol and amino acids were obtained from Aldrich \(^{22} \) (Milwaukee, WI). Tris(hydroxymethyl)aminomethane base (Tris) was obtained from Sigma \(^{22} \) (St. Louis, MO). Boric acid was obtained from Mallinckrodt \(^{22} \) (Phillipsburg, NJ). Amino acids were obtained from Aldrich. \(^{22} \) Potassium cyanide, 3(4-carboxybenzoyl)quinoline-2-carboxaldehyde (CBQCA), 3(2-furoyl)quinoline-2-carboxaldehyde (FQ), Oregon Green 488 \(^{22} \) carboxylic acid and Cascade Blue \(^{22} \) hydrazide were obtained from Molecular Probes \(^{22} \) (Eugene, OR). Recombinant green fluorescence protein was obtained from Clontech \(^{22} \) (Palo Alto, CA). Fluorescein-labeled DNA was obtained from Mosaic Technologies \(^{22} \) (Waltham, MA). TAMRA-labeled DNA was obtained from Operon Technologies \(^{22} \) (Huntsville, AL). Fluorescently labeled polystyrene microspheres (6\( \mu \)m diameter) were obtained from Polysciences \(^{22} \) (Warrington, PA). All reagents were used as received.

\(^{22} \) Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.
Amino Acid Labeling. Serine and tyrosine were labeled with CBQCA according to the following procedure. Serine was dissolved at a concentration of 50 mM in 200 mM carbonate buffer, pH 9.4. Tyrosine was dissolved at a concentration approaching its solubility limit (<25 mM) in 200 mM carbonate buffer, pH 9.4. Each amino acid was (separately) labeled by combining 10 µL of 10 mM KCN (in water), 10 µL of 10 mM CBQCA (in DMSO), and 10 µL of amino acid solution. The mixtures were then protected from light exposure and vortexed at room temperature for 4-10 h. The labeled serine solution was diluted 200× into concentrated Tris/boric acid buffer (900 mM Tris, 900 mM boric acid). The labeled tyrosine solution was diluted 50× into Tris/boric acid buffer. The labeled amino acid solutions were used immediately after dilution. The labeled amino acids were individually focused using the techniques described below to verify that only one fluorescent product resulted from the labeling of each amino acid. The solution used for the separation/focusing described below was a mixture of the two amino acid solutions in Tris/boric acid buffer with a ratio of 2:1 tyrosine solution/serine solution.

Aspartic acid was labeled with FQ according to the following procedure. Aspartic acid was dissolved at a concentration of 50 mM in 200 mM carbonate buffer, pH 9.4. It was then labeled by combining 10 µL of 10 mM KCN (in water), 10 µL of 10 mM FQ (in methanol), and 10 µL of amino acid solution. The mixture was then protected from light exposure and vortexed at room temperature for 4 h. The labeled aspartic acid solution was diluted 15× into Tris/boric acid buffer. The solution was then immediately used for focusing.

Safety Precautions. Potassium cyanide is highly poisonous and reacts readily with acids to form lethal HCN gas. Handle with extreme care.

Microchannel Fabrication. Polymer microchannels were formed by imprinting with a micromachined silicon template and then sealing with similar polymeric material as has been previously described.23,24 Thin polycarbonate microchannel chips were made using 125-μm-thick sheets of polycarbonate ( McMaster-Carr,23 Atlanta, GA). Microchannels were first imprinted into one sheet of polycarbonate (25 mm × 38 mm) at 155 °C under an applied force of 13000 N. The polycarbonate was then cooled to 120 °C and released from the silicon master. A second 125-μm-thick sheet of polycarbonate—with holes for fluid access to the channels—was pressed against the first and sealed to it at a temperature of 135 °C for 1 h. The resulting thin polycarbonate chips were cut into strips (6–7 mm wide), each containing one microchannel. Typical microchannels were trapezoidal in cross section and were 75 μm wide at the base, 25 μm wide at the top, and 30 μm deep.

For the focusing and separation of green fluorescence protein, a 30-μm-i.d. silica capillary (Polymicro Technologies Inc.,22 Phoenix, AR) was embedded between two 125-μm-thick polycarbonate sheets. The two polycarbonate sheets were pressed together around the silica capillary and bonded at 13000 N, 150 °C for 10 min.

For the focusing on a temperature gradient produced by Joule heating, a junction of narrow and wide microchannels was imprinted into 2-mm-thick acrylic sheet (Acrylite OP-4,22 Cyro Industries, Mt. Arlington, NJ) using a micromachined silicon template. A second sheet of acrylic, with holes drilled through it for fluid access to the microchannels, was sealed to the first at 103 °C for 8 min. The microchannels were trapezoidal in cross section. The narrow portion of the microchannel was 65 μm wide at the base, 20 μm wide at the top, and 30 μm deep. The wide portion of the microchannel was 325 μm wide at the base, 280 μm wide at the top, and 30 μm deep.

Copper Heating/ Cooling Blocks. For most of the examples of TGF described below, zones of different temperatures and the temperature gradients between them were produced by thermally anchoring the thin polycarbonate microchannel chips to alternately heated or cooled copper blocks. Three temperature zones were used: two cold zones covering much of the ends of the channels and the buffer reservoirs, and a narrow hot zone near the middle of the channel. The middle, heated copper block was heated using a small high-power resistor embedded into the copper, and its temperature was regulated using a PID temperature controller (Omega Engineering Inc.,22 Stamford, CT). To regulate the temperature of the cold zones, 1/8-in.-diameter holes were drilled through the cooled copper blocks, and cold water from a thermostated bath (Neslab,22 Portsmouth, NH) was passed through them. The heated and cooled copper blocks were arranged so that there was a 1-mm gap between the hot and cold blocks on one side and a 2-mm gap on the other.

Thin polycarbonate microchannel chips (see above) were attached to the copper blocks using thermally conductive adhesive transfer tape (3M22). The chips were pressed against the copper blocks from above with 3-mm-thick PDMS (Sylgard 184,22 Dow Corning, M idland, MI) gaskets and a 2-mm-thick acrylic (Acrylite OP-4,22 Cyro Industries) top plate, which was secured to the outer copper blocks using nylon screws.

Two-Color Thermometry. The temperature gradient produced in a microchannel using the copper heating/cooling blocks was characterized using a two-color fluorescence thermometry technique.25 Briefly, a buffer containing a mixture of two different fluorophores was introduced into the microchannel. One fluorophore, rhodamine B, has been shown to be useful for fluorescence-based thermometry because it has a quantum efficiency that is strongly temperature-dependent.26,27 The second fluorophore, carboxyfluorescein, is only weakly temperature-dependent and so can be used as an internal reference. Emission from the two fluorophores was simultaneously imaged using a dual band fluorescein/ rhodamine filter set (Chroma,22 Brattleboro, VT) and a 3-CCD color camera (Dage-MTI,22 Michigan City, IN). The ratio of the signals from the red and green channels of the camera output was used to calculate the temperature at each point along the channel using a calibration curve generated from images of the same dye in a thermostated silica capillary.24

To measure the temperature profile produced with the copper heating/cooling block apparatus, a solution of 100 μM carboxyfluorescein and 100 μM rhodamine B in 60 mM carbonate buffer, pH 9.4, was injected into a thin polycarbonate microchannel that was anchored to the copper heating/cooling blocks as described above. For Te = 10 °C and Tc = 80 °C, the resulting temperature

RESULTS AND DISCUSSION

In order for TGF to work with most analytes, a buffer is required with a nonconstant \( f(T) \). Of the buffers that we had available in our laboratory, the one that was found to work the best was an equimolar mixture of Tris and boric acid at either a low concentration or a high concentration. The measured \( f(T) \) for this buffer at three different concentrations is shown in Figure 3. Both low-concentration (9 mM Tris, 9 mM boric acid) and high-concentration (900 mM Tris, 900 mM boric acid) Tris/boric buffers have nonconstant \( f(T) \)—though in opposite directions—and could therefore be used for TGF. For the intermediate-concentration (90 mM Tris, 90 mM boric acid) Tris/boric buffer, \( f(T) \) is fairly steep at high temperatures, but it is nonmonotonic, making it a less than ideal buffer for TGF.

TGF was found to work with both the low- and high-concentration Tris/boric buffers, but problems were encountered with the low-concentration buffer because of its low electrical conductivity. Therefore, the high-concentration buffer (900 mM Tris, 900 mM boric acid) was used for all of the examples described below. Despite its high concentration, the 900 mM buffer actually had a fairly low conductivity—comparable to 20 mM carbonate buffer. Consequently, moderately high voltages could be applied to the buffer-filled microchannels without significant Joule heating. For example, in the measurements described below and shown in Figures 4 and 5, at a typical applied voltage of 1000 V, the current in the microchannel was \( \approx 20 \mu A \), for a dissipated power of less than 10 mW/cm. Measurements on similar microchannels indicate a temperature rise of only a few degrees under these conditions.

The pH of the buffer was also temperature-dependent. For the 900 mM buffer, the measured pH varied from 8.6 at 20 °C to 7.5 at 70 °C, which is consistent with expectations based on the temperature dependence of the \( pK_a \)'s of boric acid and Tris. If an analyte had a \( pK_a \) in or near this range of pH, then its electro-photophoretic mobility would gain some additional temperature dependence \( (\zeta_p(T)) \) would be nonconstant) that would serve to reinforce the focusing. For the fluorescent dyes and labeled amino acids used here, the \( pK_a \)'s are all well below this pH range, so that for these analytes \( \zeta_p(T) \) can be considered constant.

To demonstrate the wide range of applicability of TGF, a variety of different analytes were focused and separated in microchannels with an applied temperature gradient spanning a 2-mm gap between two temperature-regulated copper blocks as described above. The apparatus is illustrated schematically in Figure 4a. The focusing electric field was applied through platinum wire electrodes placed in the reservoirs at each end of the microchannel. The counterbalancing bulk flow was primarily driven by electroosmosis and was fine-tuned by adjusting the amount of fluid in the reservoirs.

In the examples shown in Figure 4b—d and f—h, imprinted polycarbonate microchannels were used; for (e), a 30-μm-i.d. silica capillary, embedded into the middle of the polycarbonate chip, was used. In all the examples, the total microchannel/capillary length was 2.3 cm.

Figure 4b shows the separation and focusing of a mixture of two fluorescent dyes, Oregon Green 488 carboxylic acid and Cascade Blue hydrazide, at an applied voltage of \(-1500\) V with...
Figure 4. Demonstration of focusing and separation of a variety of different analytes using TGF. (a) Schematic drawing of the apparatus. (b–g) Fluorescence images of focusing zone for various analytes. Magnification is constant for all images. The total length of each image is 1.9 mm. (b) Oregon Green 488 carboxylic acid and Cascade Blue hydrazide. (c) The two products resulting from labeling of aspartic acid with FQ. (d) Mixture of CBQCA-labeled serine and tyrosine. (e) Green fluorescence protein. (f) Fluorescein- and TAMRA-labeled acid with FQ. (g) 6-mm-diameter fluorescently labeled polystyrene particles. (h) Same as (g), but after the channel was rinsed and refilled with the unfocused particle solution.

$T_H = 80 \, ^\circ\text{C}$ and $T_C = 10 \, ^\circ\text{C}$. The image was taken after ~30 min of focusing. At this high driving voltage, the velocities are quite high, and the effects of Taylor dispersion\(^{(28)}\) can clearly be seen on the shapes of the bands.

Figure 4c shows the separation and focusing of two fluorescent products that resulted from the labeling of aspartic acid with FQ. The image was taken after 15 min of focusing at an applied voltage of $-1000 \, \text{V}$ with $T_H = 80 \, ^\circ\text{C}$ and $T_C = 10 \, ^\circ\text{C}$. The fluorescence was imaged using a long-wavelength-pass emission filter so that the red-shifted emission from the more mobile product (the red band to the left of the image) could be observed.

Figure 4d shows the separation and focusing of serine and tyrosine labeled with CBQCA. The image was taken after 10 min of focusing at $-1000 \, \text{V}$, $T_H = 80 \, ^\circ\text{C}$, and $T_C = 10 \, ^\circ\text{C}$. It was verified that CBQCA labeling resulted in only one fluorescent product per amino acid. From the measured temperature gradient (Figure 2), the two peaks are $\sim 20 ^\circ\text{C}$ apart. Using eq 7 and the data of Figure 3, this indicates that the ratio of electrophoretic mobilities of the two labeled amino acids is $\sim 1.1$. This is to be compared to the expected value of 1.17 calculated using the charge-to-mass ratios of the labeled amino acids.

Figure 4e shows the focusing and separation of recombinant green fluorescence protein. In this case, the separation was done in a 30-μm-i.d. silica capillary that was embedded into the middle of a thin polycarbonate chip. The silica capillary was used to reduce the problem of protein adsorption to the walls of the plastic microchannels. The image was taken after 40 min of focusing at $-550 \, \text{V}$, $T_H = 60 \, ^\circ\text{C}$, and $T_C = 10 \, ^\circ\text{C}$. $T_H$ was lowered to 60 $^\circ\text{C}$ to avoid denaturing the protein. Four fluorescent bands (the forth one, in the far left of the image, is very faint) were found, indicating some heterogeneity of the protein. This is to be compared to the three bands found for the same protein using slab gel IEF.\(^{(29)}\)

Figure 4f shows the focusing and separation of a mixture of fluorescein- and TAMRA-labeled DNA fragments. The image was taken after $\sim 30 \, \text{min}$ of focusing at $-1000 \, \text{V}$, $T_H = 38 \, ^\circ\text{C}$, and $T_C = 10 \, ^\circ\text{C}$. For this separation, $T_H$ was lowered to 38 $^\circ\text{C}$ in order to decrease the temperature gradient (decrease $f_i$) to help partially separate the overlapping peaks. Since the electrophoretic mobility of DNA molecules is essentially independent of length and sequence, the mobilities of the two DNA molecules used here differ only by a small amount due to the different fluorescent dyes used to label them. The fluorescein-labeled DNA (green; sequence: CGT GGA ACA GTA CTA GGT AG) is slightly more mobile than and so is shifted to the left of the TAMRA-labeled DNA (red; sequence: TCC GGG CCC TTG CAT AAG TG).

Figure 4g shows the focusing of fluorescently labeled polystyrene microspheres (6 μm diameter). The image was taken after


Demonstration of TGF using temperature gradient generated by internal Joule heating. (a) Schematic showing arrangement of microchannels used. With an applied voltage of −1900 V, Joule heating resulted in higher temperatures in the narrow microchannel, and a sharp temperature gradient at the junction with the wide channel. (b) White light microscopy image of the microchannel junction. (c) Fluorescence microscopy image taken before the application of the high voltage. (d–g) Fluorescence microscopy images taken 40, 90, 140, and 190 s, respectively, after the application of the high voltage. Measurements of the peak fluorescence intensity indicated a greater than 300-fold increase in concentration. For scale, each of the images is 945 μm × 788 μm.

45 min at −1000 V, TH = 80 °C, and TC = 10 °C. The focused band is much more diffuse than that achieved with the smaller molecules because of the polydispersity of the particle mobilities and the extremely small diffusion constant of the particles. Nevertheless, a high degree of focusing is possible. The image shows the “head” of the focused band of particles; the “tail” extended for several millimeters to the left of the image; for comparison, note the green band of Figure 4b. The same section of microchannel is shown in Figure 4h after the focused band was rinsed out and the channel was refilled with the unfocused particle solution.

Because TGF does not rely on the use of membranes or salt bridges, there is no loss of analyte, large or small, from the focusing zone. This allows TGF to be used as an essentially perfect preconcentrator. With this method, it is possible to flow a relatively large amount of fluid containing a dilute analyte through the temperature gradient, collecting all of the analyte at the focusing point for subsequent analysis. In addition, TGF can be very selective, as only those analytes with mobilities that lie within a selected range will be focused. The degree to which TGF can be used to concentrate samples is limited only when the sample becomes so concentrated that it has a significant effect on the electrical conductivity of the solution or when the maximum solubility is approached. Starting with a very dilute analyte, concentration factors are in practice limited only by the time allowed for the concentration to occur.

To demonstrate the high degree to which a dilute analyte can be concentrated using TGF, an 8 nM solution of Oregon Green 488 carboxylic acid was focused in a thin polycarbonate microchannel chip with an applied temperature gradient spanning a 1-mm gap between the two temperature-regulated copper blocks as described above. The procedure was otherwise similar to that used for the examples shown in Figure 4 with an applied voltage of −1250 V, TH = 80 °C, and TC = 10 °C. Figure 5a is a fluorescence micrograph of the focused sample plug at the end of a focusing run (after ~100 min of focusing). The arrows schematically show the relative magnitudes of the electrophoretic (uH) and bulk (uL) velocities and the resulting total velocity (uT) in both the hot and cold portions of the channel.

The experiment was run twice—on different days and in different microchannels. The measured peak concentration versus time for the two runs is plotted in Figure 5b. The peak concentration for each of the two runs followed a similar, roughly linear increase as predicted by eq 23. The small variations around the linear trend were due to changes in the amount of fluid in the reservoirs resulting from electroosmotic pumping and evaporation and the periodic manual adjustments that were necessary to offset them. The concentration was deduced from the measured fluorescence intensity using a calibration curve obtained from intensity measurements with the microchannels filled with various solutions of known, uniform concentration. The calibration measurements were made with the same applied temperature gradient as was used for the focusing experiments. The concentrations used for the calibration curve were 8, 25, 50, and 100 μM. The correlation coefficient (R²) of the calibration curve was 0.990.

After 100 min of focusing, the peak concentration was ~90 μM, more than a 10 000-fold increase over the concentration of the initial solution, and still rising. At this point, the integrated amount of sample in the focused peak was 1.6 × 10⁻¹⁴ mol, or the equivalent of ~2 μL of the original 8 nM solution. As far as we are aware, the factor of 10 000 concentration reported here surpasses that reported for any other single focusing or preconcentration method.

Although 100 min may seem like a long time, it is comparable to the times required for similarly high degrees of preconcentration using chemical affinity methods—though TGF is much simpler to implement. In addition, the performance of TGF on shorter time scales is similar to or better than that reported for other methods. For the examples of TGF demonstrated here, the degree of focusing after 1 min ranged from 2-fold to 100-fold, depending on the analyte mobility and the applied voltage and temperature gradient. This is to be compared with the results of other reported methods: with IEF, 100-fold concentration can be achieved.

(30) Although the bulk flow is primarily driven electroosmotically, the temperature and electric field gradients give rise to pressure gradients within the microchannel. The resulting Taylor dispersion has the surprising property that the effective dispersion constant is inversely proportional to the diffusion constant.
achieved in 20 min or longer; with DNA preconcentration on metal electrodes, 5-fold concentration can be achieved between 30 s and 3 min.\textsuperscript{6,9} with the use of a porous membrane structure for DNA preconcentration, up to 100-fold concentration has been achieved in 4 or 5 min;\textsuperscript{8} with EGFG, greater than 100-fold concentration has been demonstrated in 10–30 min;\textsuperscript{10} and with affinity methods, 1000-fold or greater concentration can be achieved in times of the order of 100 min.\textsuperscript{13}

In its simplest form, the temperature gradient required for TGF can be generated by the same applied electric field that drives the focusing. This is illustrated in Figure 6. The focusing occurs at a junction of two microchannels of different cross-sectional area. Because the buffer-filled channels have finite electrical resistivity, the application of a high voltage causes a flow of current through the channels that dissipates energy in the form of heat—a phenomenon referred to as Joule heating. Because the total current passing through the channel is constant, the current density in the narrow channel is higher than in the wide channel. Consequently, more heat is dissipated and the temperature is higher in the narrow channel, and a sharp temperature gradient forms at the junction.

An 8 \( \mu \)M solution of Oregon Green 488 carboxylic acid was used for the results shown in Figure 6. The total length of the microchannel used was 1.7 cm; the narrow portion was 1.3 cm long, and the wide portion was 0.4 cm long. At the applied voltage of \(-1900 \text{ V}\), single-color fluorescence thermometry\textsuperscript{24,31} measurements showed that, at steady state, the temperature gradient spanned the range from \( T_C = 23 \) °C to \( T_H = 39 \) °C, with a maximum gradient of 150 °C/\( \text{mm} \).

Figure 6b shows a white light microscopy image of the area around the junction of the wide and narrow portions of the microchannel. The series of fluorescence images shown in Figure 6c–g were taken just before and 40, 90, 140, and 190 s after the high voltage (\(-1900 \text{ V}\)) was turned on. Measurements of the peak fluorescence intensity indicated a greater than 300-fold increase in concentration during this time. The white light image of Figure 6b was actually taken after \( \sim 6 \text{ min} \) of focusing; the diffuse amber spot at the center of the image is the concentrated sample plug imaged with transmitted white light through the 30-\( \mu \)m depth of the microchannel, indicating that TGF can easily produce concentrations sufficient for the use of absorbance detection, even in microfluidic systems.

CONCLUSIONS

We have described and demonstrated a new technique, temperature gradient focusing, that uses a temperature gradient for the focusing and separation of ionic species in microfluidic channels or capillaries. With TGF, electrophoretic velocity gradients are created without the use of embedded electrodes, membranes, or salt bridges; this potentially simplifies device construction and operation and allows for highly efficient focusing of any charged analyte. The first experiments with TGF, described here, indicate that it can provide performance comparable to and in some ways better than that achieved with established methods such as IEF, EFGF, and other sample concentration methods.

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