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Tandem Isotachophoresis-Zone Electrophoresis via Base-Mediated Destacking for Increased Detection Sensitivity in Microfluidic Systems

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Electrophoresis in microfluidic devices is becoming a useful analytical platform for a variety of biological assays. In this report, we present a method that allows for an increased sensitivity of detection of fluorescent molecules in microfluidic electrophoresis devices. This capability is provided by the implementation of a particular buffer system that is designed to initially function in an isotachophoretic (ITP) mode and, then after a controlled amount of electric current has been applied to the system, to transition to a zone electrophoretic mode. In the initial ITP mode, analytes dissolved in a large volume of injected sample are concentrated into a single narrow zone. After application of a sufficient and adjustable amount of electric current, the system switches into a zone electrophoretic mode, where the concentrated analytes are separated according to their electrophoretic mobilities. Application of this tandem ITP-zone electrophoretic strategy to the concentration, separation, and detection of fluorescent reporter molecules in a standard microfluidic device results in an ∼50-fold increase in detection sensitivity relative to equivalent separations that are obtained with zone electrophoresis alone. Even with very long initial sample plugs (up to 3000 μm), this strategy produces electrophoretic separations with high resolutions and peak efficiencies. This strategy can be implemented to increase detection sensitivity in any standard microfluidic electrophoresis platform and does not require any specialized hardware or microchannel configurations.

Microfluidic-based electrophoresis systems (a type of micro-total analytical system, μTAS) promise to revolutionize the speed and economy with which researchers can obtain genetic, proteomic, chemical, and biochemical information. These advantages stem from the low cost of analysis, high speed, and parallelization attainable in these systems. The ability to control accurately the width of the injected sample plug with cross-channel injectors and the short separation distances afforded by microfabricated devices enable them to perform extremely rapid separations, often in 10% of the time required in more traditional systems such as capillary electrophoresis. The ease of automation for these systems allows the possibility for marked reduction in the cost of operation. Significant effort has gone into the development of fabrication technologies for these systems as well as their detection systems;[1−3] however, until recently, the exploration of novel electrophoretic separation strategies for microfluidic systems has received less attention.[4,5] Techniques that allow for facile, high-throughput, highly sensitive, and selective electrophoretic separation of samples of experimental relevance must be developed in order for μTAS to realize their full potential in economy, throughput, and automation.

Often, a limiting characteristic of μTAS is their ability (or lack thereof) to detect extremely low-concentration species. Because only a small volume of sample is injected into electrophoresis-based μTAS, the sample needs to be of a sufficiently high concentration to allow for detection. Paradoxically, often the most interesting biomolecules are those present at the lowest concentration, making optical detection after separation challenging. In addition, the concentration of a species injected electrokinetically in μTAS depends on the difference in conductivity between the sample and the buffer in the microfluidic channels. To inject a high concentration of sample, it is advantageous if the sample has a lower conductivity than the buffer. Unfortunately, for many biological samples, the opposite is true, and the sample has a higher conductivity than the buffer in the microchannels due to higher salt concentrations. The mismatch in conductivity cannot be compensated by increasing the conductivity of the buffer because the use of higher ionic strength buffers in the microchannels lead to excessive Joule-induced heating in the microfluidic channels. Excessive Joule heating results in poor separation efficiency due to radial thermal gradients in the microchannel. Techniques exist for the removal of excess salt from the sample, but these techniques require additional manipulation of the sample and are often difficult to automate, making them less desirable in a high-throughput, low-cost, automated setting.

With these facts in mind, an electrophoresis protocol that allows for the injection and detection of low-concentration samples,

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samples in high-conductivity buffers, or both would be highly desirable. Here, we present a tandem, transient isotachophoresis-zone electrophoresis (tITP-ZE) method that allows one to introduce a dilute sample in a wide injection zone, concentrate that sample into a narrow, highly concentrated zone, and subsequently separate that sample zone into easily detected bands. This technique relies on a buffer system that initially functions in an ITP mode, where the dilute injected sample is concentrated; after passage of a controlled amount of electrical current, the system transitions to a zone electrophoresis mode, where individual species in this narrow, concentrated zone are separated and detected. When properly employed, this technique allows detection of analytes 2 orders of magnitude lower in concentration than is detectable with traditional, nonstacking, cross-channel injection techniques. This allows one to detect low-concentration samples in microfluidic electrophoresis systems with an increased sensitivity and without the need for off-line salt removal from the sample to enhance electrokinetic injection. This technique requires only that the electrolytes be chosen properly and arranged in the appropriate microfluidic channels at the beginning of the analysis.

Isotachophoresis. Isotachophoresis (ITP) has been employed widely for the analysis of inorganic ions and other analytes.[6−9] Sample stacking (a transient form of ITP) is often used to increase the amount of sample introduced in capillary-based electrophoresis systems.[10,11] ITP is implemented with an axially discontinuous electrolyte system. The discontinuous electrolyte system creates step changes in the magnitude of the effective electric field along the separation axis. In its most typical embodiment, ITP has two different buffers present in the separation channel—a leading electrolyte (LE) and a trailing electrolyte (TE). The LE is composed of high-mobility ions and is of a high conductivity relative to the sample ions and TE. Conversely, the TE is composed of low-mobility ions and has a low conductivity compared to the sample ions and LE. As their names suggest, the LE is introduced, with respect to the direction of migration, in front of the sample electrolytes, and the TE behind the sample. With this arrangement, there is a local discontinuity in the electric field at the physical boundary between zones of different conductivity. After application of an electric field for a sufficient period of time, the ions in the separation channel will distribute themselves according to the Kohlrausch regulating function (KRF)[12] given by eq 1 (assuming all ions present are weak

$$\omega(x) = \sum_i \frac{\bar{c}_i(x) z_i}{\mu_i} = \text{constant}(x) \qquad (1)$$

monovalent ions or strong mono- or multivalent ions), where ω is the regulating function value, $\bar{c}_i(x)$ is the concentration of species i at point x along the separation channel, z_i is the number of elementary charges carried by an ion of species i, and μ_i is the electrophoretic mobility of species i. The Kohlrausch function describes the local concentration of each charged species at any position, x, based on the concentrations at the beginning of the electrophoresis. As each electrolyte species migrates along the separation channel, the concentration of each ion modulates so that the value of the regulating function is constant at any spatial point, x, with respect to time. Thus, to obey the constraints of the KRF, dilute ions are concentrated into narrow bands. When a voltage is applied, the ions migrate according to their electrophoretic mobility, and as a result, there is a distribution in the concentration of the various species along the channel. If the initial concentration of analytes that form abutting ITP zones is very low, as a result of attempts to maintain the KRF, the zones are too narrow to support defined, detectable boundaries. However, this ITP-concentrated zone can be subsequently separated by zone electrophoresis (ZE) into facilely detected, distinct sample peaks.

Transition from ITP to Zone Electrophoresis. Many groups have reported methods of coupling ITP and ZE separations, and usually these involve coupling an ITP capillary to a ZE capillary.[13−19] Other researchers have reported methods of inducing controlled transient ITP conditions, after which ZE separation occurs.[20−26]

A technique reported by Hjertén and applied in capillary-based systems places the sample in a buffer with a pH near the isoelectric point of the sample ions; the buffers both aft and in front of the sample are of different and increased pH's. Thus, when electric current is applied, the sample analytes are swept toward the interface between the sample zone and the proximal buffer zone leading to a stacking mechanism. After application of a sufficient electric potential, the boundaries between the zones diffuse and ZE migration occurs, leading to separation.[27]

Karger et al. reported another capillary-based tandem ITP-ZE method where the separation initially is operated in a traditional ITP mode. After application of the electric field for a period sufficient to completely focus the protein analytes, the aft buffer reservoir containing the TE is replaced with LE. This causes migration of high-mobility ions through the sample plug and the ITP separation to transition to a ZE mode. Using this technique, the authors could achieve an increase in UV detection sensitivity of up to 2 orders of magnitude. This technique, while very

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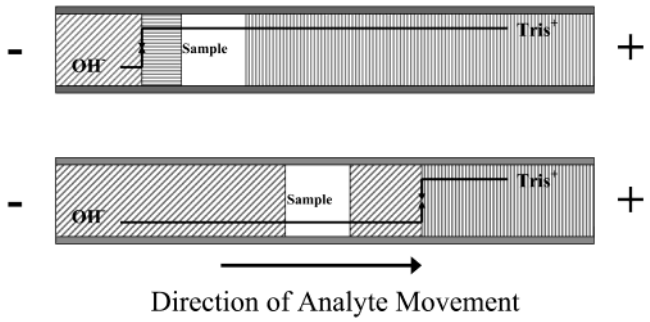

Figure 1. Schematic representation of tITP-ZE in an arbitrary microchannel. Vertical lines represent areas with leading electrolyte, horizontal lines represent areas with trailing electrolyte, and diagonal lines represent areas with unbuffered hydroxyl ions.

successful in capillary-based systems would be difficult to implement in a microfluidic system where the buffers in reservoirs are not as easy to replace.[26]

Another clever technique, developed by Swerdlow and co-workers,[28] uses an injection of hydroxyl ions to neutralize cationic Tris buffer ions. This results in a section of the separation channel that exists transiently as a low-conductivity, high-electric field zone, causing the sample ions to concentrate next to the boundary between the neutralized Tris zone and the high-conductivity, low-electric field "normal" buffer. These techniques can lead to dramatic increases in the amount of sample introduced into a separation channel without any increase in the initial sample zone width. Thus far, these systems have been demonstrated primarily in capillaries and in most cases require physically separate ITP and ZE separation channels. In this report, we demonstrate the coupling of ITP to ZE in a single channel with control of the relative duration of ITP and ZE within the same channel. Furthermore, this technique can be implemented in standard microfluidic electrophoresis systems with cross-channel injector geometries and does not require specific channel designs or additional hardware to control the ITP to ZE transition.

During electrophoresis in aqueous systems, H^+ and OH^- ions are produced continuously at the electrodes by electrolysis of water. When anions are analyzed by ITP, OH^- production at the cathode (negative potential) must be sufficiently buffered by a cationic agent. If the hydroxyl ions are not sufficiently neutralized by a cationic buffering ion, then these very high-mobility OH^- ions migrate into the separation channel, passing through the sample zone to become the predominant electric current-carrying ions proximal to the sample ions. This system is schematically illustrated in Figure 1.

It is common practice to avoid this situation, and the ensuing pH changes, as it can affect the charge state of the analytes and the efficiency of the separation. However, if the pH changes during analysis do not significantly affect the charge on the sample ions (e.g., if the pH of the separation buffer is always greater than the pK_a of the sample ions), then the presence of OH^- ions proximal to the sample zone can be used to "destack" a sample plug. After hydroxyl migration through the sample zone, the conductivity of the zones in front of and behind the sample zone are essentially the same. This eliminates the step changes in the effective electric field necessary for ITP and ZE ensues.

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Table 1. Chemical Data of the Ions Used in This Study[a]

ion	pK_a	electrophoretic mobility ($\times 10^{-9}$) ($m^2/V\cdot s$)
chloride		−79.1
Tris	8.1	29.5
TAPS	8.4	−8.3
eTag reporter 1	6.4	−1.05
eTag reporter 2	6.4	−0.813

[a] Mobilities refer to the fully ionized species. The values for the eTag reporters refer to the highest pK_a of the molecule.

The buffering capacity in the separation channel determines the number of OH^- ions that migrate through the separation channel and hence how long the sample remains under ITP conditions. Thus, the concentration of buffering cation determines how much of the separation channel (or alternatively how much of the separation time) is used for ITP versus ZE. By adjusting the concentration of the cationic buffering agent properly, controlled amounts of the separation distance can be allocated for ITP concentration of sample ions and the remainder of the separation distance is used for ZE separation. This technique differs from that of Swerdlow and co-workers in that the presence of OH^- ions is used to destack the sample ions, whereas with the former technique, OH^- are used to stack the sample ions.

To properly implement tandem ITP-ZE, it is helpful to know beforehand the chemical and physical properties of the sample to be analyzed such as electrophoretic mobility, pK_a, and number of charges. ACLARA's eTag reporter molecules are amenable to this requirement (Table 1). These proprietary, fluorescent molecules have defined and unique electrophoretic mobilities and have been demonstrated to be applicable for use in highly multiplexed genotyping, gene expression profiling, and protein profiling. Free eTag reporter molecules are generated in biochemical reactions as a result of a cleavage following a specific binding event, e.g., the specific binding of a nucleic acid probe to a target or the binding of an antibody to an antigen. This binding event is detected and quantified by electrophoretic analysis of the released eTag reporters, yielding information about the original biological sample.

EXPERIMENTAL SECTION

Electrophoresis Conditions. All electrophoretic separations were conducted in microchannel electrophoresis devices fabricated from poly(methyl methacrylate) (ACLARA BioSciences Inc., Mountain View, CA), filled with 1% poly(ethylene oxide) ($M_W \sim$ 500 000) (Aldrich Chemical, Santa Clara, CA) dissolved in the specific electrolyte solution described in each experiment. Electrolyte/polymer solutions were loaded sequentially into each arm of the electrophoresis chip manually by application of a vacuum, with the order of addition chosen to give the desired arrangement of electrolytes in the microfluidic system, with the terminating electrolyte in section A, the sample in section B, and the leading electrolyte in section C as depicted in Figure 2. Electrophoresis chips had a cross-channel injector design, with either a 250-μm injector offset and a 4.5-cm separation length or a 3000-μm injector offset and a 5.5-cm separation length. Electrophoretic separations were performed with a breadboard electrophoresis

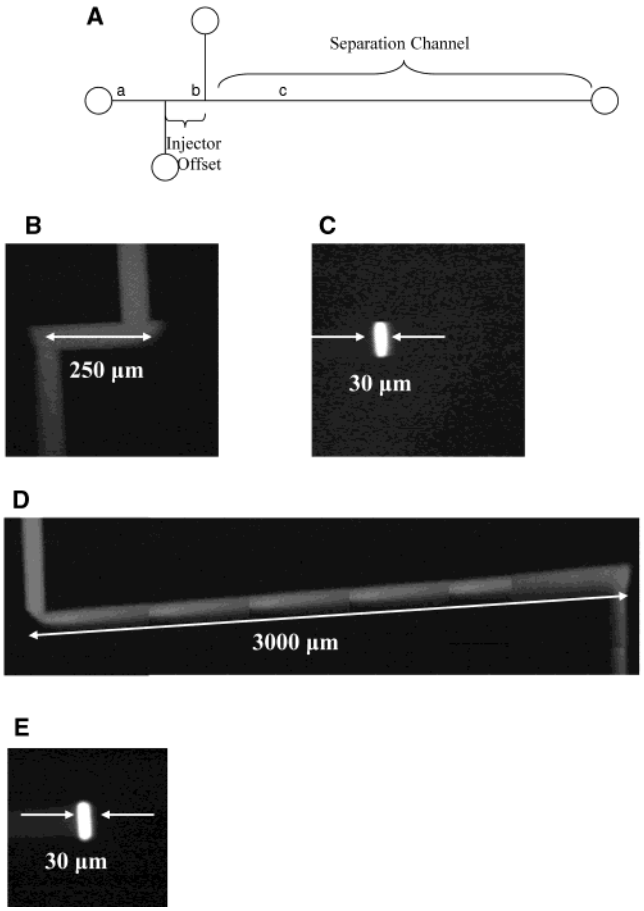

Figure 2. Fluorescence CCD images of tITP-ZE separation during injection (panels B and D) and after ITP concentration (panels C and E). Panel a shows the general microfluidic channel configuration. Panels B and C show results obtained with a 250-μm injector and panels D and E with a 3000-μm injector. Conditions: Sample was fluorescein at 1 mM. Leading electrolyte: 25 mM $Tris^+$, 25 mM Cl^-. Trailing electrolyte: 25 mM $Tris^+$, 25 mM $TAPS^-$. Injection field: 300 V/cm, current ~ 18 μA. Separation field: 200 V/cm, current ~10 μA.

system designed and built in-house at ACLARA.[29] The confocal laser-induced fluorescence detection system consists of a 488-nm Ar+ excitation laser, a 10× microscope objective (Nikon, Melville, NY) and a photomultiplier tube (Hamamatsu, Bridgewater, NJ) and recorded fluorescence emission at 520 nm at a fixed point near the end of the separation channel. Fluorescent imaging was carried out with an identical system, except that the laser was replaced with a high-intensity mercury arc lamp (Nikon), the PMT was replaced with a charge-coupled device (CCD) camera (Hamamatsu), and a lower-magnification, 4× objective was used (Nikon).

Sample Preparation. Fluorescent eTag reporter molecules, eTag reporter 1 and eTag reporter 2, were synthesized at ACLARA, diluted into the desired electrolyte at the desired concentration, and used directly.

Buffer Preparation. Buffers were prepared from 18 MΩ·cm deionized water (Millipore, Bedford, MA). Stock solutions were made of the free acid and free base of each ion. Leading and trailing electrolytes were then prepared by mixing of stock solutions to give the desired ionic concentrations.

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RESULTS AND DISCUSSION

Sample Concentration in Base-Mediated tITP-ZE. Since microfluidic-based electrophoretic separations are extremely short in duration (usually on the order of seconds), diffusional peak broadening is usually minimal and initial injection width commonly limits separation efficiency. The tITP portion of a tITP-ZE analysis concentrates dilute sample ions into narrow, highly concentrated zones. This allows large sample volumes to be introduced via an injector with a long offset without loss of separation efficiency (i.e., with narrow peaks). The sample ions in the offset, which can account for more than 5% of the total separation distance, are then concentrated into a narrow zone via ITP. Under the parameters investigated here, tITP-ZE produces effective initial sample zone widths that are independent of the length of the injector offset, with samples zones being compressed into 30-μm plugs during the tITP portion of the separation.

This effect is seen in Figure 2, which presents CCD images of a tITP-ZE analysis of 1 mM fluorescein. The initial panel (Figure 2A) shows the layout for an arbitrary cross-channel injector microfluidic electrophoresis device for clarity in interpreting subsequent panels in Figure 2. In Figure 2B, the fluorescein sample has been introduced into a 250-μm offset injector by the application of an electrical potential across the channel arms perpendicular to the separation channel. After introduction of the sample into the offset, the separation field was applied in the following manner: the application of an electrical potential between the ends of the separation channel serves to migrate ions through the main separation channel, while an additional, small electrical potential is applied between the perpendicularly opposed sample introduction channels and the separation cathode to prevent the diffusive "leakage" of sample ions into the separation channel during the separation (a so-called "pull-back" voltage). Upon application of the separation voltage, the fluorescein was concentrated rapidly into a narrow, ~30-μm zone after it had migrated less than 50 μm down the separation channel, as shown in Figure 2C. When the identical analysis is done using a 3000-μm injector assembly, the fluorescein is again concentrated in a narrow, ~ 30-μm zone, as is seen in Figure 2D and E. In both Figure 2C and 2E, the CCD signal is saturating. This illustration shows that tITP-ZE allows one to uncouple the concentration of the original sample from the effective concentration of the injected sample, facilitating easier detection. Most importantly, under conditions studied here, the effective minimum sample zone width is independent of the initial width (the length of the offset of the cross-channel injector).

Control of the Relative Durations of ITP and ZE. The rate at which the boundary between the electrolytically produced unbuffered hydroxyl ions and buffering counterions moves down the separation channel is determined both by the rate of hydroxyl ion production and by the buffering capacity of the counterion. The latter of these is easily modulated to control the rate at which the destacking boundary moves down the separation channel by adjusting the concentration of the buffering counterion. In tITP-ZE, the concentration of the buffering counterion ($Tris^+$ in this work) controls the effective lengths of the separation channel used for ITP concentration and ZE separations of sample ions. If the $Tris^+$ concentration is high enough, the migration of the hydroxyl-Tris boundary is slow, and so it does not overtake the

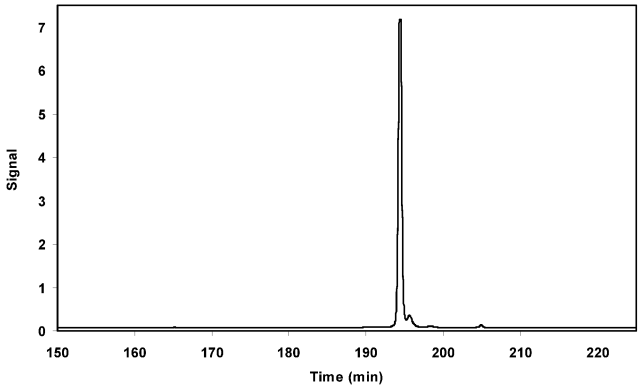

Figure 3. ITP analysis of two eTag reporter molecules. Conditions: sample was eTag reporter 1 and eTag reporter 2 at 250 pM each. Leading electrolyte: 17 mM Amediol$^+$, 10 mM Cl$^-$. Trailing electrolyte: 17 mM Amediol$^+$, 20 mM Ala$^-$. Separation field: 200 V/cm, current ~10 μA. Separation length: 6.0 cm. Injector offset: 3000 μm.

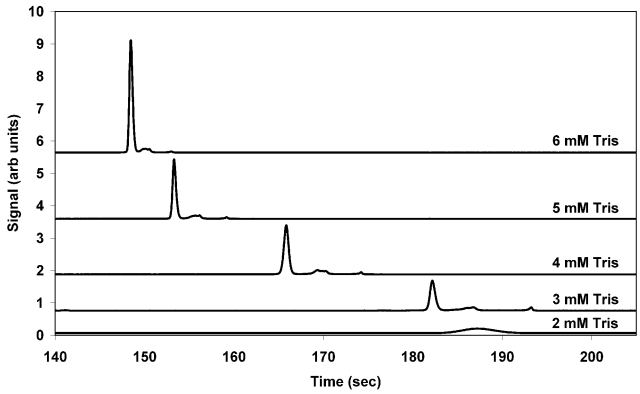

Figure 4. tITP-ZE analysis of a single eTag reporter molecule. Conditions: sample eTag reporter 1 at 250 pM. Leading electrolyte: 2 mM Cl$^-$, Tris$^+$ as specified in each trace. Trailing electrolyte: 2 mM TAPS$^-$, Tris$^+$ as specified in each trace. Each buffer was adjusted to pH ~8.4 with NaOH. Separation field: 200 V/cm, current ~2 μA. Separation length: 5.5 cm. Injector offset: 3000 μm.

sample ions before they reach the detector, resulting in a purely ITP-driven process. This is seen in Figure 3, where a mixture of two different eTag reporter molecules with substantially different electrophoretic mobilities is detected as a single peak. As will be seen later (in Figure 5), when the same mixture of eTag reporter molecules is separated in buffers of lower Tris$^+$ concentration, the reporters are well separated. This single peak for the two reporters is observed because the low sample concentration causes the boundary between abutting analyte zones to be too narrow to be detected via optical fluorescence.

Figure 4 and Table 2 present data that demonstrate the influence of Tris$^+$ concentration on the duration of ITP versus ZE. In all analyses, the sample was a single eTag reporter species at 250 pM in the corresponding leading electrolyte. The concentration of Tris$^+$ ion in both the leading and trailing electrolytes was varied according to Figure 4. Analyses were performed in a chip with a 3000-μm offset injector and a 5.5-cm separation channel. When the analysis is conducted with high concentration of counterion, the reaction boundary between unbuffered hydroxyl ions and the buffering Tris$^+$ counterions moves slowly toward the anode and does not overtake the sample until it has migrated a substantial distance down the separation channel. In other words,

Table 2. Peak Data for TITP-ZE Separation at Varying Concentrations of the Buffering Counterion

[Tris] (mM)	peak amplitude (arb units)	fwhm (s)	fwhm (mm)
2	0.137	4.07	1.20
3	0.892	0.678	0.205
4	1.48	0.606	0.201
5	1.78	0.437	0.157
6	3.43	0.415	0.154

ITP of the sample ions has persisted for a relatively longer portion of the separation time and ZE for a relatively shorter period in comparison to analyses using lower Tris$^+$ concentrations. Experimentally, this condition manifests itself as a tall narrow peak in the electropherogram because ITP conditions suppress diffusional peak broadening for a significant portion of the migration time.

In contrast, when the separation is conducted in buffer systems with low concentrations of buffering counterion, ITP persists for a shorter period of time and the sample spends more time in the ZE mode where diffusional peak broadening occurs to a greater extent.. This can be seen in the electropherograms of Figure 4, where the sample peak widens and becomes less intense as Tris$^+$ concentration decreases, although the sample was introduced under the same conditions for each analysis. At 2 mM Tris$^+$, the lowest concentration of buffering counterions investigated in this study, the hydroxyl-Tris$^+$ boundary moves so quickly through the sample zone that ITP does not persist long enough to concentrate the sample to any appreciable extent. This can be seen in Figure 4, in the 2 mM Tris$^+$ trace, which shows a wide, low-amplitude peak approximately equivalent to that which would be obtained in purely ZE separation in a homogeneous buffer. At the highest Tris concentration (6 mM), the peak has a fwhm of 0.415 s (corresponding to a half width of 0.154 mm), while at the lowest Tris concentration the fwhm is 4.07 s or 1.20 mm. Thus, there is a nearly 8-fold reduction in peak width between analyses with 2 and 6 mM Tris$^+$. This decrease is caused merely by changing the concentration of Tris in the buffer, therefore causing ITP to persist for a longer portion of the migration time.

Comparison of Results from ZE and tITP-ZE Separations. The tITP-ZE separation methodology allows for separations with markedly decreased limits of detection (LOD) while maintaining equivalent separation efficiencies and increased resolutions with increased (but still extremely short) analysis times. The decreased LOD results from the ability to load a large volume of dilute sample and to concentrate that sample into a narrow zone that is more easily detected. To obtain efficient separations in microfluidic-based systems without ITP concentration, one must use narrow injector geometries, which limit the total amount of sample that can be introduced into the analysis system and lead to detection difficulties with dilute samples. Panels a, c, and e of Figure 5 present the results of a ZE separation performed using a 250-μm injector, 4.5-cm separation channel microfluidic device. Panels b, d, and f present the results of a tITP-ZE separation performed using a 3000-μm injector assembly, 5.5-cm separation channel microfluidic device. The ZE experiment used a homogeneous buffer of 6 mM Tris$^+$, 2 mM Cl$^-$. The tITP-ZE system used a discontinuous buffer system with an LE of 6 mM Tris$^+$, 2 mM Cl$^-$ and a TE of 6 mM Tris$^+$, 2 mM TAPS$^-$. In each case, the

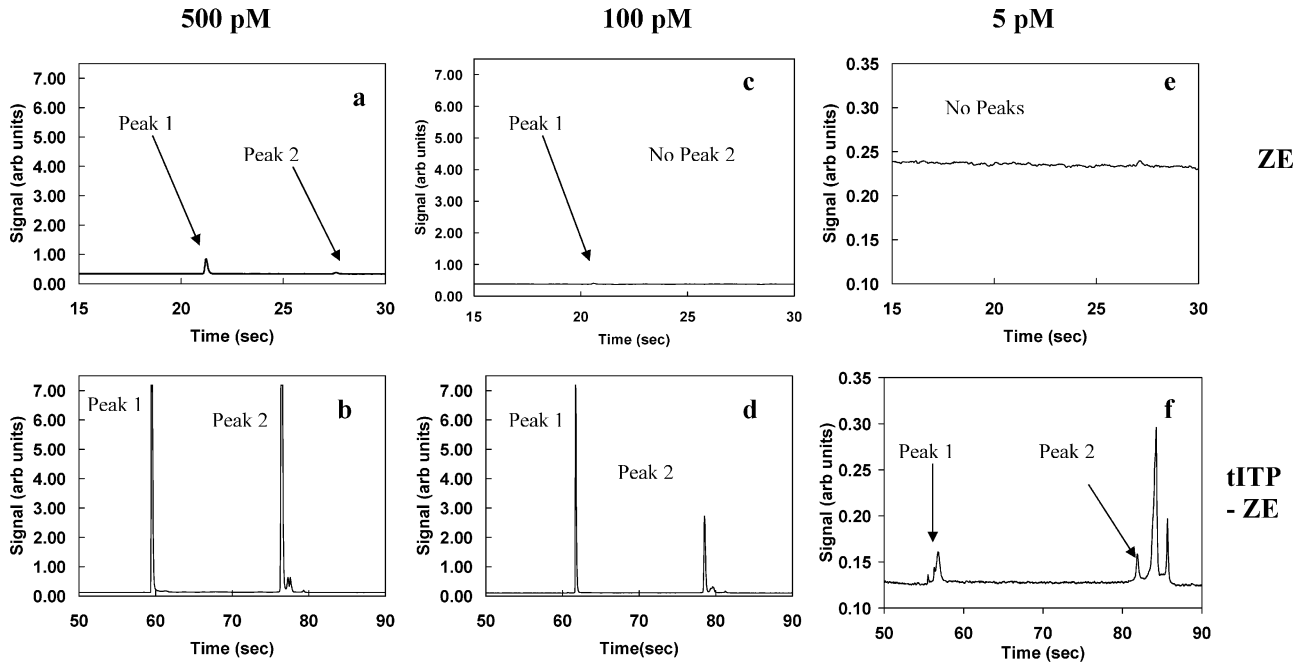

Figure 5. Comparison of tITP-ZE and ZE separations of two eTag reporter molecules. tITP-ZE conditions: sample eTag reporter 1 and eTag reporter 2 at specified concentrations. Leading electrolyte: 6 mM $Tris^+$, 2 mM Cl^-, pH 8.4. Trailing electrolyte: 6 mM $Tris^+$, 2 mM $TAPS^-$, pH 8.3. Separation field: 200 V/cm, current ~2 μA. Separation length: 5.5 cm. Injector offset: 3000 μm. ZE conditions: sample eTag reporter 1 and eTag reporter 2 at specified concentrations. Electrolyte: 6 mM $Tris^+$, 2 mM Cl^-, pH 8.3. Separation field: 200 V/cm, current ~2.5 μA. Separation length: 4.5 cm. Injector offset: 250 μm.

Table 3. Peak Efficiencies and Resolutions for TITP-ZE Separations with Samples at Differing Concentrations

concn (pM) (peak no.)	peak efficiency (plates/m)		resolution	
	ZE	tITP-ZE	ZE	tITP-ZE
500 (1)	136 700	>363 000	18.8	52.6
500 (2)	69 400	484 000	na[a]	na
100 (1)	86 000	663 000	nd[b]	57.0
100 (2)	nd	466 000	na	na
5 (1)	nd	34 800	nd	57.5
5 (2)	nd	82 300	na	na

[a] nd, no data; na, not applicable.

sample contained the same two eTag reporter molecules used in Figure 3 and was dissolved in the leading electrolyte. All other experimental parameters were equivalent. The signal and time scales are the same at each concentration to facilitate easy comparison of peak amplitude. As can be seen in Figure 5, the peaks produced by both the ZE and tITP-ZE separations are well resolved, but the tITP-ZE peaks are of markedly increased amplitude. In fact, at a concentration of 500 pM, presented in Figure 5a, both peaks produced in the tITP-ZE separation saturate the detector, while the ZE separation produces peaks with markedly lower signal.

Also evident in the tITP-ZE electropherograms is the presence of so-called "system peaks", immediately following peak 2. We believe that these peaks are due to low levels of fluorescent contaminants present in the buffer and specifically result from the concentration of buffer impurities to detectable levels by the same mechanism as the analytes. Multiple lots and brands (all of the highest purity available) of buffer salts, and multiple water sources, were tested in tITP-ZE, and all produced system peaks, but of varying amplitude. Further, the system peak amplitude was independent of the eTag reporter molecule concentration, and in fact it was present in the absence of any analytes. These observations are in agreement with postulation that these peaks represent a ubiquitous buffer salt contaminant.

Panels c and d of Figure 5 show ZE and tITP-ZE electropherograms obtained for the separation of eTag reporters at 100 pM nominal concentration. In the ZE electropherogram (panel c), the first eTag reporter is still detectable, but the second peak is absent because the eTag reporter concentration is below the LOD for this system in pure ZE mode. In contrast, the tITP-ZE peaks are both easily detectable, with peak 1 being nearly 200 times the signal intensity of the ZE peak.

Finally, in Figure 5, Panels e and f, neither of the eTag reporters is detectable in the purely ZE separation; however, both reporters are still easily detected in the tITP-ZE separation. The tITP-ZE separations show increased resolution and efficiency over the corresponding pure ZE separations, despite the fact that the initial sample zone in the tITP-ZE separation is 12 times larger than the ZE separation (in a microfluidic chip, due to the speed of separation, the initial sample width usually limits the separation efficiency).

Table 3 presents the peak efficiency and resolution of separations at each concentration. In all cases, tITP-ZE produces higher resolution and higher efficiency separations than pure ZE. On average, the separation efficiency is nearly 6 times greater for tITP-ZE peaks than for ZE peaks. Additionally, the tITP-ZE peaks are on average 50 times more intense. tITP-ZE separations take ~3 times longer to perform under these experimental conditions but still are complete in less than 1.5 min.

CONCLUSIONS

We have presented a technique that allows for the coupling of ITP and ZE electrophoretic separation modalities in a single microfluidic channel. Operating under tITP-ZE conditions produced peaks that are on average 50-fold greater in amplitude when compared to pure ZE separations of the same analytes. This electrophoresis technique produces chip-based separations of greater efficiency and higher resolution than in comparable ZE separations. These benefits come at the expense of an ~3-fold increase in analysis time, but analysis time remains under 1.5 min. Implementation of this technique requires knowledge of the analytes' chemical characteristics beforehand and the matching of the electrolyte system to the pK_a's of the analytes. Although these requirements may limit the broad applicability of this approach, this technique can be implemented for the analysis of dilute sample ions where a priori, their mobilities are known or fall within a well-defined range. In addition to the application presented in this paper, other examples of systems in which this is true include DNA sequencing, double-stranded DNA sizing, and the analysis of single-nucleotide polymorphisms by primer extension via end-labeled free-solution electrophoresis.[30]

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