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Research Article

A method to determine quasi-steady state in constant voltage mode isotachophoresis

Identification of the steady state is very challenging in isotachophoresis (ITP); especially in complex microgeometries, such as dog-leg channels or cross-channel junctions. In this work, an elastic matching method is applied to determine the quasi-steady state in microscale ITP. In the elastic matching method, the similarity between two profiles is calculated by comparing intensity distribution of two images or profiles. To demonstrate this similarity-based analysis technique for ITP, a constant voltage mode ITP model is developed and applied to a five-component ITP system. Hydrochloric acid and caproic acid are used as the leader and terminator, respectively, while histidine is used as the counter-ion. Two sample components, acetic acid and benzoic acid, are separated under the action of an applied electric field in both straight and dog-leg microchannels. This analysis shows that conductivity profiles provide a better measure to determine the quasi-steady state in an ITP process. For a straight microchannel, the quasi-steady state is achieved in less than a minute with a total potential drop of 100 V in a 2 cm long channel. In a straight channel, a true steady state can be achieved for ITP with appropriate countercurrent flow where stationary zones are formed, but the time it takes to reach the steady state is much longer than the without counter flow case. The numerical results indicate that a steady state cannot be reached in a dog-leg microchannel because of sample dispersion and refocusing at and near the intersections and at the branch channels. However, the elastic matching method can be used to determine the quasi-steady state in a dog-leg microchannel.

Keywords:
Elastic matching method / ITP / Microfluidic / Quasi-steady time
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1 Introduction

In recent years, microscale ITP has become very popular as a preconcentration technique for multi-dimensional separation platforms [1–3]. In ITP, a mixture of separands is generally introduced between a leading and a terminating electrolyte, and an electric current/field is applied for stacking/separation of sample components [4]. The electrophoretic mobility of a sample component should be more than that of a terminating electrolyte and less than that of a leading electrolyte. Hence, in the presence of an electric field, all analytes are arranged according to their mobilities and migrate toward cathode or anode depending on their charges. For instance, in anionic ITP components move toward the anode, while in cationic ITP the migration is toward the cathode. During transient ITP, the zones of sample analytes develop slowly and migrate at different speeds depending on their mobilities. The concentration within each analyte zone changes until it is proportional to the concentration of the leading electrolyte, which allows for simultaneous fractionation and concentration of dilute sample species [5, 6]. When the sample components reach a fully stacked state, all zones, including the leader and terminator, move with an equal velocity, and this condition is called quasi or (pseudo)-steady state.

At quasi-steady state, the target component(s) can be transferred to another high-resolution separation column for further separation, manipulation, and/or sensing [7, 8]. Hence, the identification of quasi-steady time and location is very important in ITP. Harrison and Ivory [9] developed an analytical model to predict the position of stationary steady-state zones in a vortex-stabilized ITP column. Their model requires a countercurrent flow and that model can only be applied in a straight channel ITP.

In experimental studies, quasi-steady or steady states are generally determined from the concentration profiles of each component [10]. Through a comprehensive experimental study, Baumann and Chrambach [11] have shown that the

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time taken to reach quasi-steady state increases with an increase in the sample loading in a system. However, the visual technique cannot be applied to a system where concentration of one or more component cannot be determined from optical images. Furthermore, even if the concentration distributions are known, it is quite difficult to quantify the steady-state time when ITP trains move. Beckers [12] proposed a simplified mathematical model to calculate the steady-state ITP behavior using electrolyte pH, electric field, and the total concentration of co-ions and counter-ions. But this steady-state algebraic model cannot quantify the time required to reach quasi-steady state. Determination of quasi-steady time is very important for injection of the stacked zone(s) to the next separation dimension such as in capillary zone electrophoresis (CZE). If the injection takes place too early, only a fraction of the sample will be transferred to the CZE column. Until now, there is no analysis method to quantify the quasi-steady state in ITP.

In this study, the elastic matching method is used to determine the quasi-steady state in microchip ITP. The elastic matching method is a well-known technique in computer science which measures the similarity among a series of patterns or images. In the last decade, numerous studies were reported on elastic matching for handwriting and facial identification [13–16]. Details of the elastic matching method are presented in Section 3. To demonstrate the usefulness of the elastic matching method, numerical simulation of ITP is carried out in straight and dog-leg microchannels, and steady-state times are investigated in both channels based on the conductivity profiles as conductivity carries information about all individual components.

2 Theory

2.1 Mathematical model for ITP

In the literature, there exist numerous works on numerical modeling of ITP [17–20]. In 1983, Bier et al. [21] proposed a full-scale, unified mathematical model for transient electrophoretic separation processes and presented simulation results for different modes of electrophoresis. Mosher et al. [22] described a computer model that takes care of diffusive effects for the accurate prediction of the boundary shape for weak electrolytes. Recently, we presented a mathematical model for microchannel ITP [23]. This mathematical model is a minor departure from the generalized vector treatment of [22, 24] in which we treat each component i, as being composed of a set of species j, which are summed over j to recover each of the i components in our system, i.e.

$$C_i = \sum_{j=1}^{J_i+1} S_{ij} \tag{1}$$

where C and S are the concentration of component and species, respectively. This allows us to treat all components as being mathematically equivalent. If we assume the diffusion coefficient (D) and absolute mobility (ω) of each species is same (i.e. $D_{ij} = D_i$ and $\omega_{ij} = \omega_i$) in a component, then the mass conservation equation for each component is obtained as

$$\frac{\partial}{\partial t} C_i + \nabla \cdot \left[(\vec{U} + \langle z_i \rangle \omega_i \vec{E}) C_i - D_i \nabla C_i \right] = 0 \tag{2}$$

where $\vec{E}$ is the electric field ($-\nabla\phi$) and $\vec{U}$ is the bulk flow velocity. The effective valence of a component can be defined as

$$\langle z_i \rangle = \sum_{j=1}^{J_i+1} \frac{z_{ij} S_{ij}}{C_i} \tag{3}$$

where z_{ij} is the valence of jth species that makes up each ith component in the system. If the dissociation reactions are fast, we also have J_i algebraic relations for equilibrium constants (K_{ij}) among the component i species, $K_{ij} = (C_{H^+} S_{ij})/S_{ij+1}$, which must be solved together with mass conservation equations for each component. For a system with M components, the charge conservation equation is expressed as [24]

$$\begin{aligned} \nabla \cdot (\sigma \vec{E}) = F \Bigg[& \sum_{i=1}^{M} \sum_{j=1}^{J_i+1} z_{ij} D_{ij} \nabla^2 S_{ij} \\ & + \left(D_{H^+} \nabla^2 C_{H^+} - D_{OH^-} \nabla^2 C_{OH^-} \right) \\ & + \vec{U} \left(\sum_{i=1}^{M} \sum_{j=1}^{J_i+1} z_{ij} S_{ij} + C_{H^+} - C_{OH^-} \right) \Bigg] \end{aligned} \tag{4}$$

where F is the Faraday constant and σ is the conductivity. The electrical conductivity is given as

$$\sigma = F \left[\sum_{i=1}^{M} \sum_{j=1}^{J_i+1} z_{ij} \mu_{ij} S_{ij} + (\mu_{H^+} C_{H^+} - \mu_{OH^-} C_{OH^-}) \right] \tag{5}$$

Here H^+ and OH^+ represent hydronium and hydroxyl ions, respectively, and μ is the effective mobility. For microscale ITP, the electroneutrality constraint must be satisfied over the channel, which can be expressed as [24]

$$C_{H^+} - \frac{K_W}{C_{H^+}} = -\sum_{i=1}^{M} \langle z_i \rangle C_i \tag{6}$$

where K_W is the equilibrium constant for water.

2.2 Assumptions

This model neglects Joule heating since relatively small electric fields are used for separation. Also, electric field-induced electroosmotic flow is not considered here because in most experimental work the channel surfaces are coated with methylcellulose or other chemicals to suppress electroosmosis. Ionic strength-dependent mobility corrections are not applied in this study, as their contribution is negligible [25].

2.3 Boundary conditions

ITP simulation is strongly dependent on the initial distribution of all charged species. In this study, it is assumed that leader, terminator, and two samples are

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initially distributed along the channel in an arbitrary fashion as presented in Table 1. In solving the mass conservation equations, the net flux through the channel walls and end wells (reservoirs) is set to zero. The electric potential is subjected to insulating boundary conditions ($\nabla\phi \cdot \vec{n} = 0$) at the walls, and constant electric potentials are maintained at the electrode (end) wells.

3 Elastic matching method

The similarity measure between patterns is a basis of many pattern matching and recognition techniques in computer vision. The resemblance between two images is generally carried out by a geometry-based (shape) method or an intensity-based (color and texture) method [13]. A "similarity parameter" is a function defined on a pair of patterns/images to indicate the degree of resemblance between two patterns/images. One of the widely used similarity measure techniques is elastic matching method [14]. Here, the match is called "elastic" since the preservation can be approximate rather than rigid. This technique can even be applied to two images that are not of the same size or shape [16].

In this study, the elastic matching method is used to compare the conductivity profiles obtained from ITP simulation to determine the steady state. The conductivity profile is chosen instead of individual component distributions because its distribution is unique and it has a contribution from all the components including the leader, terminator, and counter-ions. At steady state, all components reach a stable value and the similarity parameter will approach zero. However, in ITP a true steady state cannot be obtained without a counter flow as preconcentrated sample zones continue to move toward the anode or cathode for anionic or cationic ITP. Therefore, we shift our focus to quasi-steady state at which the conductivity profile stops evolving but migrates toward electrode with ITP velocity. Here, our objective is to find the quasi-steady time by comparing conductivity profiles from two successive time steps using the elastic matching method.

Let $X = \{X^k | k = 1, 2, \ldots, L\}$ which denotes a profile set that contains all ITP steps, where L is the total number of time steps. A profile can represent a two-dimensional image or a one-dimensional intensity value. We used the latter and express the kth profile like $X^k = [x_1^k, x_2^k, \ldots x_l^k, \ldots x_n^k]$, where n is the number of grid points and x_l^k means the intensity of the lth grid points at the kth time step. Then the similarity distance (parameter) between a profile X^k and its subsequent profile X^{k+1} is obtained by solving a point-to-point correspondence optimization problem using the elastic matching method [14].

An example of the elastic matching method is illustrated in Fig. 1A for two different profiles, where eight and nine points are extracted from the X^k and X^{k+1} profile, respectively to check the similarity between them. We calculate the similarity distance between two profiles by finding the optimum point-to-point correspondence. The similarity distance from the elastic matching can be computed using the following dynamic programming function [14]:

$$D(i,j) = \min\left\{\begin{array}{c} D(i-1,j) \\ D(i-1,j-1) \\ D(i,j-1) \end{array}\right\} + d(i,j), i = 2, \ldots, m, \ \ j = 2, \ldots, n \qquad (7)$$

Table 1. Physicochemical properties and initial concentration distributions of different components used in the numerical simulation of ITP. The maximum charge state of leader, terminator, and samples is −1, but that of counter ion is 2

Type	Name	ω 10E−9 (m^2/V s)	pK_1	pK_2	pK_3	Initial distribution (mM) where x is in m
Leader	Hydrochloric acid	79.1	−2	–	–	$0\ (x<0.0015),\ 10-10\ e^{-3125(x-0.0004)^2}(x\geq 0.0015)$
Terminator	Caproic acid	30.2	4.857	–	–	$10(x<0.0004),\ 10e^{-3125(x-0.004)^2}(x\geq 0.0004)$
Sample A	Acetic acid	42.4	4.756	–	–	$5\,e^{-3125(x-0.0015)^2}$
Sample B	Benzoic acid	33.6	4.203	–	–	$5\,e^{-3125(x-0.0015)^2}$
Counter ion	Histidine	28.8	2	6.04	9.33	20

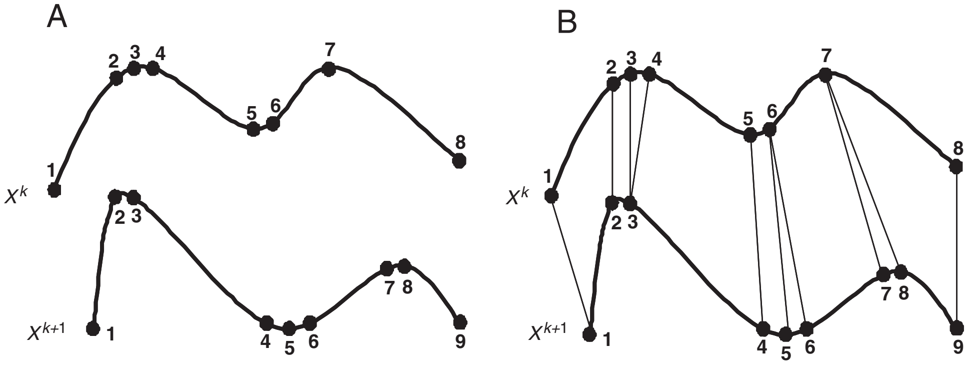

Figure 1. Illustration of elastic matching method. (A) Feature points extracted from two profiles X^k and X^{k+1}, where $X^k = [x_1^k, x_2^k, \ldots, x_8^k]$ and $X^{k+1} = [x_1^{k+1}, x_2^{k+1}, \ldots, x_9^{k+1}]$. (B) Correspondence by elastic matching based on similarity distance.

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The initial conditions of $D(i, j)$ become:

$$
\begin{aligned}
D(1,1) &= 0\\
D(i,1) &= D(i-1,1)+d(i,1), \quad 2 \le i \le m \qquad (8)\\
D(1,j) &= D(1,j-1)+d(1,j), \quad 2 \le j \le n
\end{aligned}
$$

Therefore, the similarity index, the cumulative distance of all correspondence between a profile X^k (from point 1 to m) and a subsequent profile X^{k+1} (from point 1 to n) can be defined as $D^k = D(m, n)$. The local distance function $d(i, j)$ between the ith point of X^k and the j^{th} point of X^{k+1} varies with the application. For our application we used a conductivity profile that has an intensity value, so the local distance function is defined as

$$d(i,j) = \sqrt{(I(i) - I(j))^2} \qquad (9)$$

where $I(i)$ means an intensity value of the i^{th} point of X^k and $I(j)$ means an intensity value of the jth point of X^{k+1}. Figure. 1B shows corresponding points by elastic matching where the path from (1, 1) to (m, n) indicates the best correspondence between feature points of two profiles. In this numerical study, the value of intensity (conductivity) is calculated at each grid point for the similarity index (D^k) calculation.

4 Results and discussion

Two-dimensional numerical simulation of transient ITP is performed for straight and dog-leg microchannels using the finite volume method [23]. For both channel configurations, the channel width is 250 μm and the channel length is 2 cm. In a dog-leg microchannel, the width and length of the branch channels are 250 μm and 5 mm, respectively, and the branch channels are located 5 mm away from the anodic and ground reservoirs. Table 1 shows the physiochemical properties of different components used for numerical simulation of ITP. The simulation results obtained from the straight microchannel are compared with the published results [19] and an excellent agreement is obtained between them.

4.1 ITP in a straight microchannel

The transient developments of sample components in a straight microchannel are presented in Fig. 2 for a total potential drop of 100 V across a 2 cm long channel. The anode reservoir is located at the right end of the channel ($x = 2$ cm), while the ground reservoir is at the left end ($x = 0$ cm) for the anionic ITP presented here. At the

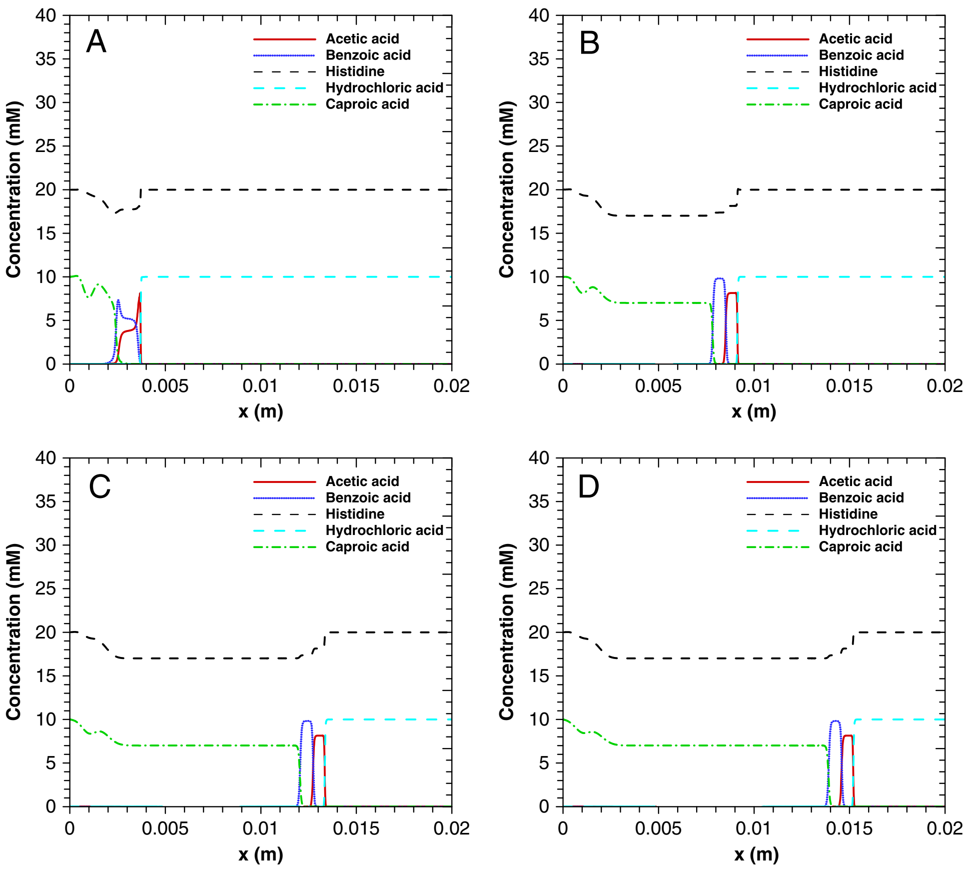

Figure 2. Concentration distributions of different components along a straight microchannel at (A) 5 s, (B) 25 s, (C) 45 s and (D) 55 s. The anode potential is 100 V and the ground potential is 0 V. All simulation conditions are shown in Table 1.

beginning of the ITP process, sharp boundaries between the terminator (caproic acid) and sample B (Benzoic acid), and between the leader (hydrochloric acid) and sample A (acetic acid) are formed, but the two samples (acetic acid and benzoic acid) are not separated from each other in Fig. 2A. As time progresses, the two samples start forming sharp interfaces as shown in Fig. 2B–D. The fractionation process was completed in more than 25 s, after which the fractionated bands move toward the anode with a constant speed. However, it is really challenging to identify the quasi-steady-state time from the concentration distributions or from the speed of the component boundaries. The process of finding the quasi-steady state gets even trickier as the number of sample components increases in a system.

To find the quasi-steady state in a systematic manner, the change in conductivity profiles is investigated during the ITP process because the conductivity profile is directly related to the mobilities and concentration of each component. In other words, a change in mobility and/or concentration of a component will result in a change in conductivity profile. Figure 3 shows the conductivity distribution along the channel for some selected time steps. Initially, the change in conductivity is very high, but once ITP approaches quasi-steady state the conductivity profiles are very similar. It is noteworthy to mention that the conductivity profile simplifies the information content significantly; however, it is still difficult to identify the time when the profile does not change anymore. In this work, the elastic matching method is employed to find the quasi-steady-state time from the conductivity profiles. The similarity index (D^k) is calculated from the numerical results of the conductivity distribution at each time step by comparing conductivity profiles at the kth and $(k+1)$th time step.

Figure 4 presents how the similarity index changes with time for straight channel ITP. In the similarity index calculation, only the domain with changes in conductivity is taken into consideration. In other words, the regions occupied by the leader and terminator are excluded from the comparison study as these regions remain unchanged in the ITP process. Based on the similarity index plot (Fig. 4), it is clear that the ITP reaches quasi-steady state at 42 s. We also plotted the current flow through the channel with time in Fig. 4 to investigate whether it could also be used as an indicator for steady state. The current is calculated as

$$I=\int_{A_c}\vec{I}\,\mathrm{d}A_c \tag{10}$$

where A_c is the cross-sectional area, and the current density is given as

$$\vec{I}=F\sum_{i=1}^{M+2}\left(-D\nabla(\langle z_i\rangle C_i)+\langle z_i\rangle\vec{U}C_i+\langle z_i^2\rangle\omega_i\vec{E}C_i\right) \tag{11}$$

As seen from Fig. 4, the current continues to decrease with time as the highly conductive leader leaves the channel and the less conductive terminator enters into the channel. Therefore, we can conclude that the similarity index calculated from the elastic matching method is a good quantitative tool for finding the quasi-steady time in ITP process. On the other hand, the temporal distribution of current cannot be used as an indicator for finding steady state in ITP.

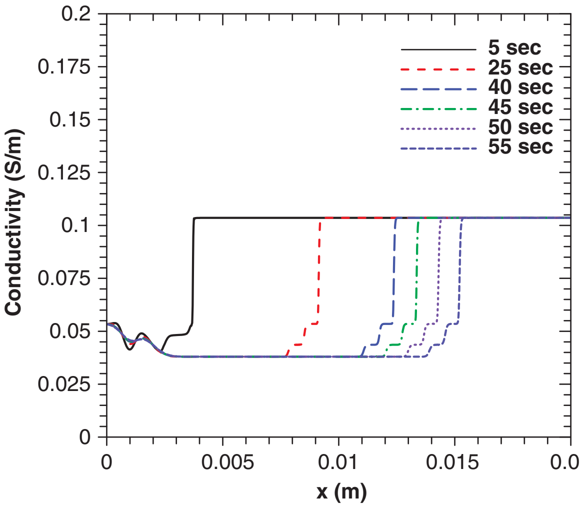

Figure 3. Conductivity distribution along the straight microchannel at different times during ITP separation process. All other conditions remain same as Fig. 2.

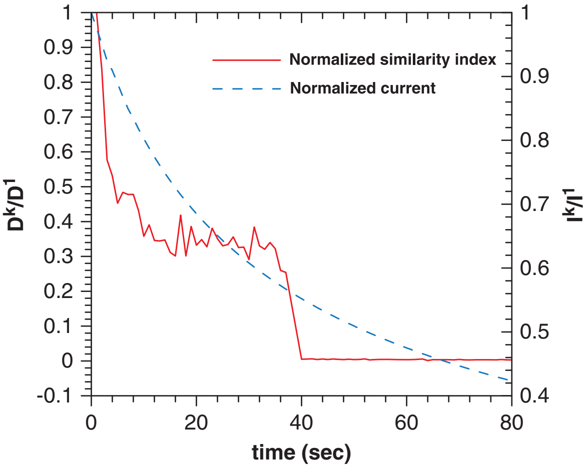

Figure 4. Normalized similarity index and normalized current for ITP in a straight microchannel. Similarity index show that the ITP reaches quasi-steady state in 42 s. The current continues to change throughout the duration of ITP as the total mass of leader and terminator changes during the ITP process.

4.2 ITP in a dog-leg microchannel

Next, the elastic matching method is expanded to 2D complex geometries to find the quasi-steady state time. ITP is simulated in a 2 cm long dog-leg microchannel using five components, as described in the straight microchannel part. For the sake of simplicity, we have used the same concentration distribution as that of a straight channel.

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The anodic reservoir is located at the right end and the ground reservoir is at the left end. A potential difference of 100 V is used across the 2 cm long channel.

The concentration contours for acetic acid and benzoic acid are shown in Fig. 5A and B, respectively. The focused bands of the acetic acid and benzoic acid are straight before passing through the T-junction as shown at 5 and 20 s, but they disperse when they enter the left T-junction at 24 s (Fig. 5). Deformation of the electric field in the T-junction is the primary source of dispersion for samples. Moreover, there is a significant loss of samples through the side channel while they cross the junction at 24 s. At 32 s, the band containing acetic acid leaves the first junction completely, and starts to resharpen to minimize the lateral dispersion. The acetic acid forms a closely tight band at 41 s (Fig. 5A) and continues to move toward the right. The acetic acid band starts deforming again at around 110 s when it enters the right T-channel junction. A similar behavior is observed for the benzoic acid, but the benzoic acid band always trails the acetic acid band since the mobility of benzoic acid is smaller than that of acetic acid. At 134 s, both bands leave the second T-junction and move toward the anode.

Similar to the straight channel case, the conductivity profiles are obtained (Supporting Information Fig. 1) for the dog-leg channel at each time step, and these profiles are used to create the similarity index distribution (Fig. 6) using the elastic matching method. In this case, the whole domain (including branch channels) is used for the similarity index calculation. The formation of peaks in the similarity index is due to band dispersion and distortion at the T-channel junctions. Unlike a straight channel case, the similarity index does not approach zero even when both samples leave from the second (right) branch channel. The minor activity, such as redistribution of components, at the branch channel is primarily responsible for the non-zero value of the similarity index. Nevertheless, the similarity index comes down to a small value after 120 s, and remains stable for the duration of ITP. Therefore, based on the similarity index distribution, it can be concluded that the ITP reaches the quasi-steady state at around 120 s for the dog-leg channel presented here. On the other hand, the current value continues to decrease with time (Fig. 6), and similar to straight channel ITP, current cannot be used to identify the quasi-steady state.

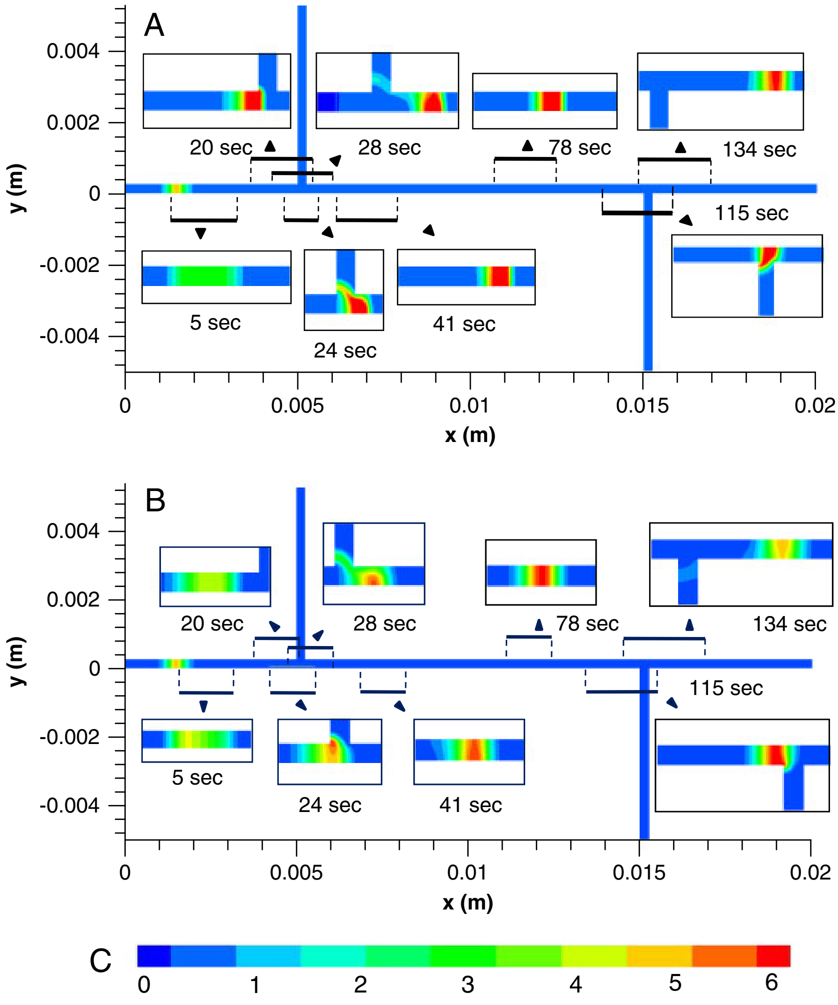

Figure 5. Concentration contours of (A) acetic acid and (B) benzoic acid during ITP in a dog-leg microchannel; (C) Contour label for (A) and (B) where concentrations are presented in millimolar. The anode potential is 100 V and the ground potential is 0 V. All simulation conditions are shown in Table 1.

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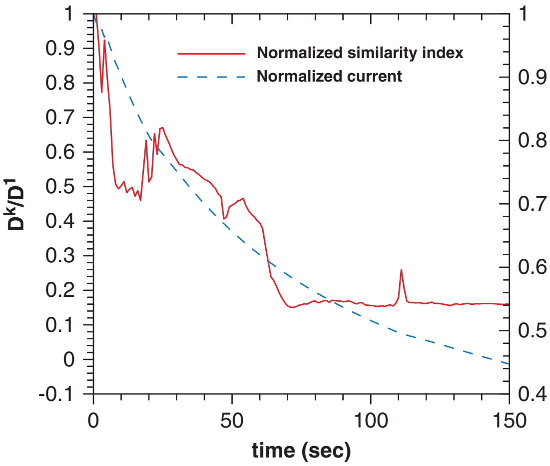

Figure 6. Normalized similarity index and current change for ITP in a dog-leg microchannel. All simulation conditions are same as Fig. 5. The last peak (at 112 s) on the similarity index profile is due to band dispersion at the right T-channel junction. The similarity index value does not reach zero due to minor activities in the two branch channels.

4.3 ITP in a straight microchannel with counterflow

Finally, we applied the elastic matching method to find the quasi-steady state in straight channel ITP with a counterflow. In the ITP process, it is sometimes useful to stop the ITP train at any chosen position within a channel for further processing, separation or sensing. Generally, a counterflow is used to stop the stacked band at a predetermined location, and the average velocity of the countercurrent flow should be in between that of a pure leader zone and of a pure trailer zone. Recently, Harrison and Ivory [9] developed an analytical model to predict the location of stationary zone in a countercurrent flow ITP. They also proposed a relationship between the length of the leader zone (L_L) and the counterflow velocity (v_{cf}) as [9]

$$v_{cf} = \frac{\mu_T \mu_L \Delta\phi}{L_L(\mu_L - \mu_T) - L_C \mu_L} \tag{12}$$

where L_C is the total length of the separation column, $\Delta\phi$ is the total nominal voltage across the separation channel, and μ_L and μ_T are absolute mobilities of leader and terminator, respectively.

In this part, we have reconsidered the straight channel ITP but included a countercurrent flow. Equation (12) is used to find the counterflow velocity needed to stop the leader within 2 mm of the ground reservoir. Based on the model input presented in Table 1, the calculated counterflow velocity is 3.35E−04 m/s in a 2 cm long separation channel for a nominal potential drop of 100 V. The straight channel ITP results are recreated using this counterflow velocity, while keeping all other conditions the same as Fig. 2. The effect of countercurrent flow velocity is obtained by introducing a uniform flow velocity in the model.

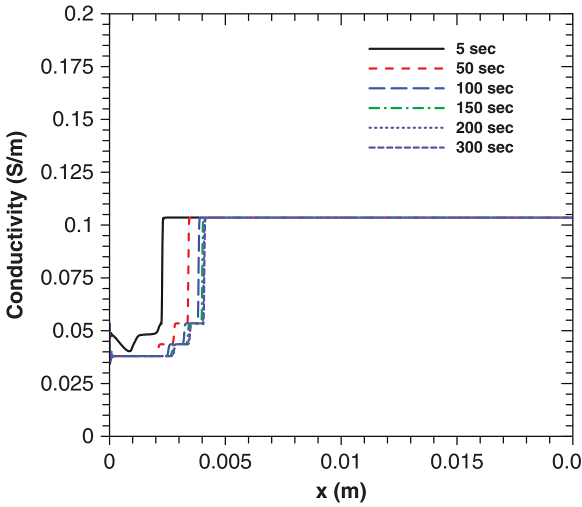

Figure 7. Conductivity distribution along the straight microchannel for ITP with countercurrent flow. The counterflow velocity is calculated based on the analytical model presented in [9]. Here, $L_C = 0.02$ m, $L_L = 0.018$ m, $\phi_C = 100$ V, and the counterflow velocity is 3.35E−04 m/s.

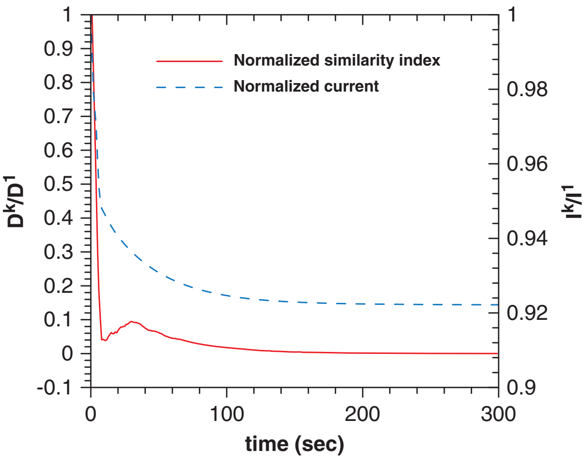

Figure 8. Normalized similarity index and current value for ITP in a straight microchannel with appropriate counterflow. Both the elastic matching method and current based technique predict the true steady state where stacked zones are immobilized.

Figure 7 shows the conductivity distribution along the straight channel at different times when the countercurrent flow is applied in the straight channel. The conductivity profile changes very rapidly in first 25 s, primarily at the locations of acetic and benzoic acid. The conductivity profile takes step-like profile once all components form distinct zones with sharp boundaries, but it takes longer time to form a stable conductivity profile. The delay in reaching steady state is largely due to the inclusion of a constant hydrodynamic component in the system, which interacts with the electromigration and diffusion terms. The countercurrent flow is responsible for the slower response in the analyte boundary formation. Unlike ITP without back flow,

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the normalized similarity index variation (Fig. 8) is quite moderate during transient times due to slower changes in concentration profiles. Here it is noteworthy to mention that both the similarity index and current reading can be used as effective tools to determine a true steady state for ITP with bulk flow. This is because of the fact that no component is leaving the separation channel at steady state.

5 Concluding remarks

The elastic matching method is applied to identify the quasi-equilibrium state during microchannel ITP. In this method, resemblance between two consecutive conductivity profiles is quantified and presented as a similarity index. The similarity parameter/index approaches zero when a process reaches steady or pseudo-steady state. To test the applicability of this pattern recognition technique in ITP, a numerical model is developed to simulate ITP in microchannels. This in-house numerical model is based on the flux conservation at the cell boundary, and it can predict the ITP zone formation at both steady and transient states. The numerical results for the concentration of components, current density and conductivity are obtained in a straight channel (2 cm long and 250 μm wide) for two different scenarios: ITP with and without countercurrent flow. In the case of no bulk counterflow, the stacked zones reach quasi-steady state within 42 s of initiating the electric current in the channel for a nominal electric field of 50 V/cm. In this case, the stacked zones continue to move toward the anode after reaching the quasi-steady state. On the other hand, ITP with counterflow case was able to immobilize the sample zones, but requires a significantly longer time to reach an equilibrium state. Both the current-based and the elastic matching methods were able to predict the true steady state in ITP with a countercurrent bulk flow. But the current-based technique cannot be used for ITP without counterflow as current density continuously decreases with time for this case. The elastic matching method is also used to quantify the quasi-steady state in a dog-leg microchannel from the numerical results of conductivity distributions in the channel. Similar to the straight channel case, the conductivity profiles provide an excellent choice to determine the quasi-steady state of ITP in a dog-leg microchannel.

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The authors have declared no conflict.

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