Isotachophoresis

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Isotachophoresis (ITP) is a nonlinear electrophoretic technique used in the separation of a variety of ionic compounds, ranging from small molecules like metal ions [1], to large molecules like proteins [2]. Unlike "linear" zone electrophoresis in which separating solute bands continually spread by diffusion or dispersion, ITP forms self-sharpening, adjacent zones of substantially pure solute whose concentrations often exceed several mgs/ml. In ITP a multianalyte sample is usually introduced between the leading electrolyte (LE, containing leading ion) and the terminating electrolyte (TE, containing terminating ion) where the leading ion, the terminating ion and the sample components must have the same charge polarity, and the sample ions must have lower electrophoretic mobilities than the leading ion but larger than the terminating ion. After application of a fixed electric current, sample components move forward behind the leading ion and in front of the terminating ion and form discrete, contiguous zones in order of their electrophoretic mobilities. Then, following a brief transient period where the discrete solute zones are formed, this ITP "stack" assumes a fixed concentration profile with a constant velocity moving in the direction of the leader.

Kohlrausch [3] developed the basic theory of ITP 110 years ago, but it hadn’t received much attention until the development of capillary electrophoresis in the 1970’s. Since then ITP, along with zone electrophoresis (ZE) and isoelectric focusing (IEF), became indispensable analytical tools, especially for high resolution and fast analysis of biological samples. This development formed the foundation in the early 90’s of the book “The Dynamics of Electrophoresis” [4], which comprehensively covers electrophoresis theory, modern applications and computer simulations of electrophoresis. During the past decade the rapid development of microfluidic-based electrophoresis makes it one of the most promising candidates to replace gel and capillary electrophoresis. ITP has played an increasingly important role in the application of microchip electrophoresis due to two unique features:

First, ITP is an extremely powerful concentration method. No matter how low the sample concentration is, it can be concentrated to a plateau concentration which, in the ideal case, is described by the following equation:

\[
C_{Sample-plateau} = C_{LE} \left( \frac{|\omega_{LE}| + |\omega_{Counter-ion}|}{|\omega_{Sample}| + |\omega_{Counter-ion}|} \right) C_{Sample} \left( \frac{|\omega_{LE}| + |\omega_{Counter-ion}|}{|\omega_{Sample}| + |\omega_{Counter-ion}|} \right),
\]

where \(C\) is the concentration, \(z\) the charge and \(\omega\), the electrophoretic mobility. This unique characteristic of ITP is very helpful when it comes to microchip electrophoresis where detection is challenging due to the low sample mass loadings and small detection window in a microfluidic chip. The best way to increase the loading capacity of a microfluidic chip is to pre-concentrate the sample; and ITP provides a simple and powerful concentration method which can be easily integrated onto a chip prior to other on-chip operations, especially ZE. It has been reported recently that a two million fold concentration increase of Alexa Fluor 488 was achieved by on-chip ITP [5].
Second, ITP is self-resharpening, i.e., the stacked zones can quickly recover their shape after a dispersive event. This feature makes ITP a very desirable method for microchip applications where T-junctions, intersections and U-turns frequently present as dispersion sources. For example, Cui and Ivory [6] have demonstrated the self-sharpening ability of ITP after dispersion by a T-junction in a networked microfluidic chip both by experiments and 2D simulations, as shown in Figure 1A where the sharp leading boundary of a moving zone of proteins approaches a T-junction. As the stack passes through the junction, the upper part of the leading boundary is stretched and sharply twisted as it was drawn about 150 µm (roughly half the channel width) into the junction channel while the lower part of the stack continued moving left to right, stretching and dispersing as it crossed the T-junction (Figure 1B). The trailing zone boundaries then catch up with the top of the leading boundary and execute the same maneuver. After the protein zones have left the vicinity of the junction, they eventually finished resharpening with slightly tilted boundaries (Figure 1C). This feature was also simulated, as shown in Figure 1D-F by an ITP model developed in COMSOL (Burlington, MA), a finite-element based program that is widely available and easy-to-use.

![Figure 1. The time-series photos A-C shows how an ITP train of three fluorescent proteins first disperses and then resharpenes as it migrates straight through a T-junction. D-F are part of an ITP simulation using three “virtual” proteins to illustrate how the ITP model mimics the essential mechanisms of dispersion and resharpening.](image)

ITP has already shown its superiority to other electrophoretic separation techniques in terms of those two features discussed above. With more features, e.g., prediction of ITP steady-state zone positions [7] and turn-induced isotachophoretic focusing in microfluidic channels [8], being discovered, ITP should find wider use in biological applications such as microscale protein purification and enzymatic assays on compact microfluidic chips.
References


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