Single-electrolyte isotachophoresis : on-chip analyte focusing and separation

Quist, J.W.

4 Tunable Ionic Mobility Filter for Depletion Zone Isotachophoresis

Published in Analytical Chemistry, 2012, 84 (21), pp 9065–9071

We present a novel concept of filtering based on depletion zone isotachophoresis (dzITP). In the micro/nanofluidic filter, compounds are separated according to isotachophoretic principles and simultaneously released selectively along a nanochannel-induced depletion zone. Thus, a tunable low-pass ionic mobility filter is realized. We demonstrate quantitative control of the release of fluorescent compounds through the filter using current and voltage actuation. Two modes of operation are presented. In continuous mode, supply, focusing and separation are synchronized with continuous compound release, resulting in trapping of specific compounds. In pulsed mode, voltage pulses result in release of discrete zones. The dzITP filter was used to enhance detection of 6-carboxyfluorescein 4-fold over fluorescein, even though it had 250x lower starting concentration. Moreover, specific high-mobility analytes were extracted and enriched from diluted raw urine, using fluorescein as an ionic mobility cut-off marker and as a tracer for indirect detection. Tunable ionic filtering is a simple but essential addition to the capabilities of dzITP as a versatile toolkit for biochemical assays.

Filtration is popular amongst sample pretreatment methods as it is fast and simple to implement. Filtration works by placing a barrier in a flow that

allows some compounds to pass while other compounds are trapped behind it. Although most filtration methods are primarily based on size exclusion, several electrokinetic techniques also can be used. For example, in isotachophoresis (ITP) ionic compounds can be trapped very selectively in an ionic mobility window defined by a terminating and a leading electrolyte.1 Compounds outside the ionic mobility window will not be trapped, making filtration possible2. Moreover, impressive concentration factors of trapped compounds can be achieved, up to a million.3 ITP is therefore highly amenable to extract and enrich components from complex biological samples. Notwithstanding its many strengths, the fact that a conventional ITP separation is defined by multiple electrolytes makes it a complex method, especially if fraction recovery or ionic mobility window tuning is desired during experiments.

Counter flow gradient focusing methods are another group of electrokinetic technologies capable of filtration. They rely on a force balance of fluid flow and electrophoresis, which is obtained by inducing an electric field gradient in the fluid flow. Analytes become immobilized at the point where their electrophoretic velocity is equal to the flow velocity.[4] Filter action can be established when some compounds have either too high or too low ionic mobility to be immobilized on the electric field gradient. The electric field gradient can be established in many different ways. For example, in electric field gradient focusing (EFGF) a converging channel is used.[5] With EFGF, undesired low mobility compounds could be removed while desired protein was concentrated.[6] In temperature gradient focusing (TGF), the sensitivity of electrolyte conductivity towards temperature is used to establish an electric field gradient.[7] TGF has been used to focus small molecules at specific

positions, while excluding proteins from the channel using a counterflow.[8] A similar exclusion effect was used by Meighan et al, who concentrated and differentiated proteins in bulk solution near the entrance of a channel.[9]

The integration of nanofluidic components into microfluidic networks has opened up many interesting new perspectives. In some cases, electrostatic or size-based exclusion has been used as a filtering principle[10]. An approach which is less prone to clogging and more interesting from an electrokinetic perspective is the use of a nanochannel-induced ion-depleted zone. Such a depletion zone can be formed by concentration polarization, a process that occurs upon application of an electric field over perm-selective conducts (that is, inside a nanochannel the surface charge of the walls permits counterions, but excludes co-ions).[11, 12] The depletion zone can be maintained at a stable position adjacent to the nanochannel entrance, even if placed in a fluid flow. The high electric field in the depletion zone blocks charged compounds from passing, making such devices suited for highly efficient analyte trapping[13] and water purification[14] applications. Experiments with electrocapture devices, which use nanoporous membranes for depletion zone formation, have indicated that they can be used as a filter that separates between ionic mobilities[15]. Apart from nanoporous channels and membranes, depletion zones also can be formed by several other means, bipolar electrodes being most notable[16].

Recently we introduced depletion zone isotachophoresis (dzITP), using a nanochannel-induced depletion zone at the border of which analytes were focused and separated into adjacent zones[17] (chapter 3). As the depletion zone replaces the terminating electrolyte that is used in conventional ITP, dzITP is a single-electrolyte isotachophoretic method, which is an important

simplification. Moreover, dzITP exhibits great versatility, because analyte zones can be precisely positioned by tuning the balance between fluid flow through the separation channel and depletion zone growth. dzITP has been used to study complexation of aptamers and proteins[18]. Here, we greatly leverage the versatility of dzITP by introducing a novel filtering principle that selects compounds based on ionic mobility. The dzITP filter does not depend on physical or chemical properties of the filter substrate, like pore size, nor is it based on a force balance of fluid flow and analyte (electro)migration, in contrast to the electrokinetic technologies mentioned above. Instead, it primarily depends on a balance of fluxes. A limited, controlled stream of compounds is released along the depletion zone. The depletion zone can thereby be imagined as a tunable valve that can be opened or closed in order to control the release of focused compounds. Selectivity is obtained as dzITP orders compounds into distinct zones before they can pass the depletion zone. Since low-mobility compounds are focused closest to the depletion zone, the dzITP filter is a low-pass filter. Ionic mobility cut-offs are tunable by synchronization of isotachophoretic focusing, separation and release of one or multiple zones. Two modes of operation for dzITP filtering are demonstrated. In continuous mode, supply and release of compounds are balanced in order to establish an ionic mobility cut-off, preferably aided by a partially released marker compound. Compounds with lower mobilities than the marker compound are co-released with the marker compound; compounds with higher mobilities are trapped in isotachophoretic zones behind the marker compound zone. In pulsed mode, the flow is temporarily increased to allow one or more individual zones to be released aided by visual feedback control. The device was used for specific enrichment of low-

abundant 6-carboxyfluorescein over highly abundant fluorescein, using continuous release and a spacer compound. Moreover, dzITP filtering was employed for trapping and indirect detection of specific metabolites from urine using fluorescein as a mobility cut-off marker. These experiments demonstrate the applicability of dzITP filtering for real-time monitored pretreatment and analysis of complex biological samples.

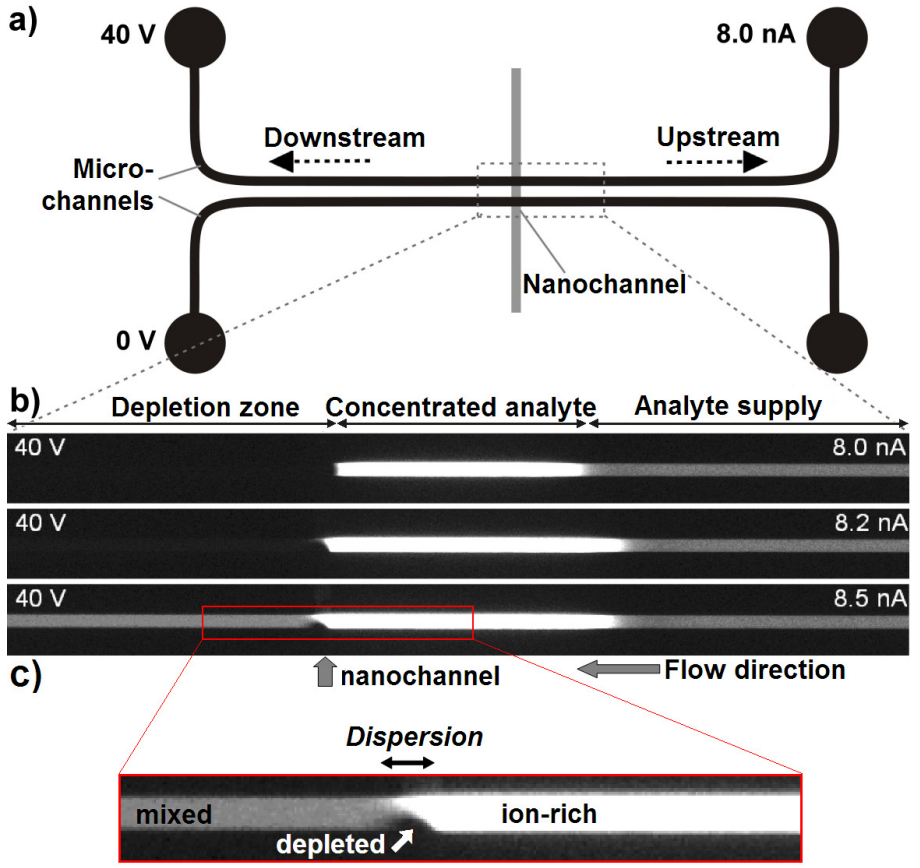

Figure 1. a) Device layout with an example of applied voltages and currents. b) CCD images showing the part of the separation channel near the nanochannel junction. With increasing currents, a focused zone of fluorescein becomes increasingly released. c) Inset showing two laminar streams: depleted fluid from the nanochannel and ion-rich fluid from the dzITP-separation. The two fluid streams are mixed rapidly downstream from the nanochannel.

Experimental Section

Chemicals

Lithium carbonate was purchased from Acros Organics (Geel, Belgium), disodium fluorescein was purchased from Riedel-de Haën (Seelze, Germany), 6-carboxyfluorescein was purchased from Sigma-Aldrich (Steinheim, Germany). FITC-leucine and FITC-glutamate were synthesized as described in chapter 1. 2.0 mmol/L lithium carbonate, pH 10.5, was used as the background electrolyte, unless indicated otherwise.

Chip Preparation

Chips were fabricated in Pyrex wafers using standard lithography techniques and dry etching with an SF6/Ar plasma. Details of the chip fabrication procedure can be found in chapter 3. The chips had microchannels with 1.7 µm depth and 20 µm width. The length of the microchannels was 0.9 cm, as measured between the nanochannel junction and the fluid reservoirs. The nanochannels were 60 nm deep, 25 µm wide, and 50 µm long. Before experiments, chip filling was done with ethanol to prevent bubble formation, after which the chips were flushed with electrolyte. Fluid replacement and flushing of channels was done as described in chapter 3. For continuous injections, channels and reservoirs were filled with the same solution containing both electrolyte and sample. For discrete injections, the separation channel was filled with solution containing electrolyte and sample, while the upstream reservoir contained electrolyte only.

Setup and Microscopy

Fluidic reservoirs (40 µL) were created by attaching a custom-build interface to the access holes of the chip using a vacuum. Gold electrodes were used for electrical connections. Voltages were controlled by one or two power supplies (ES 0300 045, Delta Elektronika BV, Zierikzee, The Netherlands), which in turn were controlled by an NI USB 6221 data acquisition system using LabVIEW 8.2 software (National Instruments, Austin, TX). Current actuation was performed with a Keithley 2410 source meter unit (Keithley, Cleveland, OH). In most experiments, current actuation was performed via the electrode connected to the upstream (sample) reservoir, while the downstream reservoir was connected to a constant voltage. In these experiments, only one of the reservoirs of the bottom channel was connected to ground (see figure 1a). However, in the pulsed mode experiments (figure 4), both the upstream and downstream reservoir were connected to voltage sources, while both reservoirs of the bottom channel were connected to ground. For imaging, we used a fluorescence microscope (Olympus IX71, Olympus, Zoeterwoude, The Netherlands) to which a Hamamatsu Orca-ER digital camera was mounted, which was controlled by Hokawo version 2.1 imaging software (Hamamatsu Photonics, Nueremberg, Germany). The integration time for an image was 0.5 s (1 s in figure 3), and the magnification was 40x. Raw CCD images were used in the figures. The false-color image in figure 6 was processed in Hokawo 2.1, to obtain this image, an EXFO X-Cite 120 Fluorescence Illumination System (Lumen Dynamics Group, Missisauga, Canada) was used to increase sensitivity. Fluorescence intensity values were corrected for background signal.

Urine Analysis

Fresh urine sample was obtained from an adult, healthy volunteer and immediately processed for analysis. The sample was 100x diluted in 2.0 mmol/L lithium carbonate containing 100 µmol/L of fluorescein and subsequently injected into the device, without any further sample preparation.

Results and Discussion

Analyte Release

The filtering principle is based on the fact that analytes in dzITP zones can be released along the depletion zone into the downstream part of the separation channel. This is shown in figure 1. A depletion zone is formed in the separation channel upon voltage and current actuation. The analyte, 150 µmol/L of fluorescein, is focused at the upstream border of the depletion zone and forms an isotachophoretic zone. 2.5 mM lithium carbonate was used as the electrolyte. As described in chapter 3, the analyte zone could be positioned at varying distances from the nanochannel by changing the ratio between the voltages and/or currents at the upstream and downstream fluid reservoirs. This positioning is a result of a shifting balance between nanochannel ion pumping capacity and electro-osmotic flow (EOF). Increasing the currents at the upstream reservoir results in increased EOF; therefore the fluorescein zone is positioned closer to the nanochannel. Upon sufficient increase of the EOF, the depletion zone cannot be maintained any longer in the upstream channel[20]. Therefore, compounds are released along the depletion zone into the downstream channel, which enables filter operation. In figure 1b, three situations are shown. When 8.0 nA is applied,

the depletion zone border, as indicated by the concentrated fluorescein zone, is positioned at a small distance from the nanochannel junction. Upon a small relative increase of the EOF (by increasing the current to 8.2 nA) a small stream of non-depleted liquid from the upstream channel, which carries some of the concentrated fluorescein, starts to flow into the downstream channel. If the current is further increased to 8.5 nA, the contribution of the fluorescein-rich fluid stream becomes much larger (figure 1c). In fact, at the nanochannel junction two laminar streams can be discerned, one containing ion-rich fluid from the upstream channel and one containing ion-depleted liquid resulting from the concentration polarization process over the nanochannel (figure 1c). Downstream from the nanochannel, the two streams rapidly mix through dispersion, forming a homogeneous dilution of the released fluorescein.

The graph in figure 2 shows how the intensity of released fluorescein in the downstream channel depends on the applied current. A zone of concentrated fluorescein was established by conventional dzITP and subsequently released using different currents on the sample reservoir, while maintaining a constant voltage of 40V at the downstream reservoir. The results of three experimental series using the same conditions are shown, to indicate reproducibility. An important value which can be derived from the graph is the threshold current. Above this threshold, analyte starts to be released.

In the graph, threshold currents can be estimated for each experimental series by interpolation of the point where fluorescence intensity becomes zero. These estimated values vary in the order of 2.5%. Even though between the experimental series the variation in threshold currents is quite small, the corresponding variations in fluorescence intensity are significant. The reason

is that in the filter regime the amount of analyte that is released is quite sensitive to small changes in electric fields. There are several potential causes for small variations in threshold currents, most importantly a shift in electrolyte distribution within the nano/microchannel network. Additional effects include conductivity and pH changes due to CO_2 dissolution from the atmosphere and variations in the zeta potential of the micro- and nanochannel walls. To obtain better reproducibility, applied current settings should therefore be corrected for the threshold current.

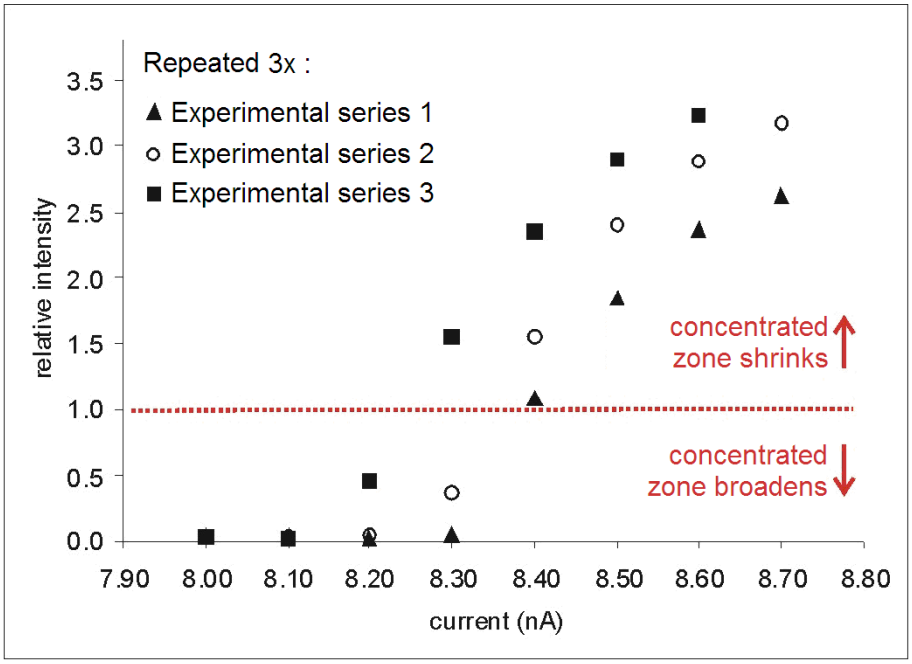

Figure 2. Graph of fluorescence intensity in the downstream channel relative to the intensity of non-concentrated analyte upstream in the channel as a function of the applied current. The red dashed line indicates equal concentration zone growth and release. Below this line the zone is broadening, above it is shrinking. The results of three experimental series with identical experimental conditions show that results are reproducible within 200pA.

The values for the fluorescence intensities in the downstream channel in figure 2 are calculated relative to the intensity in the channel region upstream from the concentrated zone. Therefore, if the relative intensity is equal to one, supply and release of analytes are equal. This particular regime is indicated by the red dashed line in figure 2. At higher values, more analyte is released than supplied and concentrated analyte zones will shrink and ultimately disappear. At lower values, release is smaller than supply and concentrated zones continue to grow. Thus, the growth rate of the concentrated fluorescein zone is indicative for the balance of the supply and release fluxes. This is used for continuous filtering as described in the following sections.

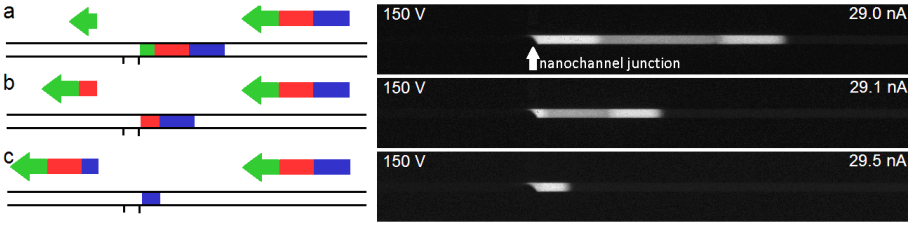

Figure 3. Schematic representation (left) and experimental results (right) showing continuous operation of the dzITP filter. The filter is tuned by balancing release (left arrows) and supply (right arrows) of analytes, determining which analytes pass through the filter and which are trapped in isotachophoretic zones. Analytes are fluorescein, FITC-leucine and 6-carboxyfluorescein. a) Fluorescein is partly released, the other analytes are completely retained. b) Fluorescein is completely released, FITC-leucine partly. c) Fluorescein and FITC-leucine are completely released, 6-carboxyfluorescein partly.

Continuous Filtering

Figure 3 shows how the dzITP filter is operated in order to collect compounds above a certain mobility cut-off, while continuously releasing other compounds. In this experiment, fluorescein, 6-carboxyfluorescein and FITC-leucine, each 50 µmol/L, were injected continuously by placing the sample solution in the upstream reservoir. A depletion zone was established and

subsequently the current that was applied through the upstream reservoir was tuned just above the threshold current (figure 3a). This resulted in continuous release of analyte along the depletion zone. The flux of released analyte was smaller than the supply flux of fluorescein. Therefore, fluorescein was only partly released and the concentrated zone of fluorescein continued to broaden. The other two analytes focused in zones behind the fluorescein zone and therefore were not released at all. This condition can be written as

$$0 < J_{release} < J_{fluorescein} \quad (1)$$

where $J_{release}$ and $J_{fluorescein}$ are the fluxes (in mol/s) of released analyte and supplied fluorescein, respectively. When increasing the current, more fluorescein was released than supplied; therefore no fluorescein zone was formed. Additionally, part of the FITC-leucine was released. Since release of FITC-leucine was only partial, a FITC-leucine zone was still formed, behind which all 6-carboxyfluorescein was collected in a second zone (figure 3b). This filtering condition can be written as

$$J_{fluorescein} < J_{release} < (J_{fluorescein} + J_{FITC\text{-}leucine}) \quad (2)$$

In this filtering regime still a narrow bright band can be observed at the depletion zone border, presumably this is due to limited stacking of fluorescein. However, full release of fluorescein is evidenced by the fact that this band does not broaden over time. Moreover, the FITC-leucine zone is growing less rapidly, which is evidence for partial release of FITC-leucine. A third regime is shown in figure 3c, in which all FITC-leucine (together with all fluorescein) and part of the 6-carboxyfluorescein is released. Here, the filtering condition is

$$(J_{fluorescein} + J_{FITC\text{-}leucine}) < J_{release} < (J_{fluorescein} + J_{FITC\text{-}leucine} + J_{6\text{-}carboxyfluorescein}) \quad (3)$$

Finally, at sufficiently high current, no filtering and no analyte zone formation is observed yielding the condition

$$J_{release} > (J_{fluorescein} + J_{FITC\text{-}leucine} + J_{6\text{-}carboxyfluorescein}) \quad (4)$$

In this regime, the electrolyte is co-released with all the analytes. The continuous filtering mode can be automated by setting the zone width of a specific, continuously injected marker compound to a predefined value, using a real-time image analysis algorithm as a feedback for applied voltages and currents. The marker compound zone can maintain a stable width only if continuous marker compound supply is balanced by partial release of the marker compound. The ionic mobility of the marker compound then precisely defines the ionic mobility cut-off of the dzITP filter: isotachophoretic principles guarantee that all ions with lower mobilities than the marker compound will be co-released while all higher mobility compounds will be focused and separated adjacent to the marker zone.

dzITP filtering is based on release along the depletion zone, yielding a temporary two-stream profile of analyte-rich and ion-depleted fluid (see also figures 1 b and c), and resulting in a balance of fluxes. The depletion zone may therefore be compared to a valve that allows to tune the quantity of compound that is to be released. This mechanism is in contrast with release through the depletion zone (or any other region with increasing electric field), in which case filtering can be achieved by tuning the force balance between fluid flow and opposite electrophoretic drift. The latter mechanism is quite common for gradient focusing methods, including EFGF and TGF. It has also been exploited in electrocapture devices, in which peptides and other compounds could be released sequentially across enrichment and depletion zones formed by concentration polarization[15, 19].

Pulsed Filtering

Figure 4 shows dzITP filtering in pulsed mode. A discrete amount of sample containing fluorescein (60 µmol/L), 6-carboxyfluorescein, FITC-leucine, and FITC-glutamate (each 30 µmol/L), was injected by filling the separation channel with electrolyte plus sample solution, while the upstream "sample" reservoir contained electrolyte without sample.

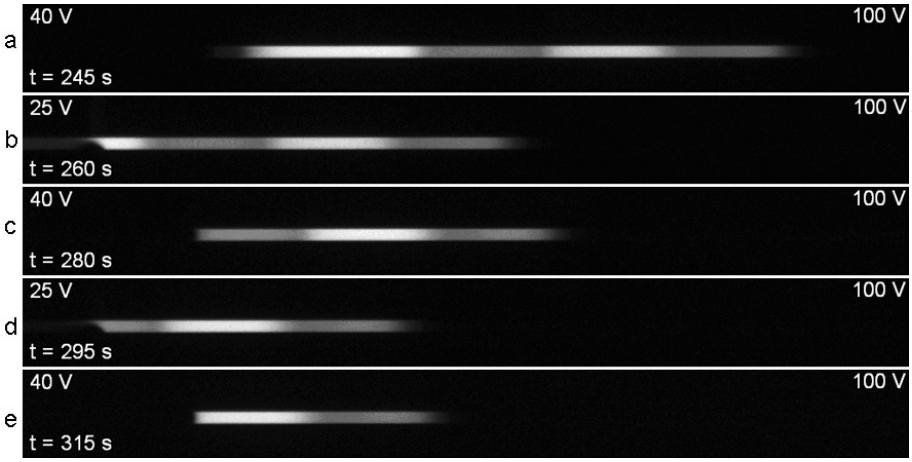

Figure 4. Pulsed operation of the dzITP filter. a) Completed dzITP-separation of a discrete injection of fluorescein, FITC-leucine, 6-carboxyfluorescein and FITC-glutamate. b) Release of the fluorescein zone. c) Retained zones after fluorescein release. c) Release of the FITC-leucine zone. d) Retained zones after fluorescein and FITC-leucine release.

First, a depletion zone was established in the upstream part of the channel, at the border of which the analytes were focused. Fluorescein focused closest to the depletion zone border, followed by FITC-leucine, 6-carboxyfluorescein and finally FITC-glutamate. During this stage, applied voltages were 120 V (upstream) and 40 V (downstream). After 3 minutes of voltage actuation, the dzITP separation was completed, resulting in the starting situation for the second stage, in which pulsed release of compounds was performed (figure 4a). The downstream voltage was temporarily lowered to 25 V. The analyte

zones were transported into the downstream direction until the nanochannel junction was reached, after which the first analyte zone (fluorescein) started to be released (figure 4b). All analyte zones would have been released if the downstream voltage were maintained at the lowered value. To prevent this, the downstream voltage was increased again to 40 V after the fluorescein zone was released. The depletion zone was re-established rapidly (sub-second scale) and the upstream depletion zone border slowly returned to its original position (~30 seconds). Behind the depletion zone border, the remaining analyte zones were retained (figure 4c). This procedure was subsequently repeated twice to release the FITC-leucine (figure 4d,e) and 6-carboxyfluorescein zones respectively.

In this experiment, the voltage actuation steps that ended the release of an individual zone was done manually, based on visual clues. Voltage actuation based on feedback from a sensor or from automatic image analysis might yield a more reliable procedure. It should be taken into account that in isotachophoretic separations there is always overlap between neighboring zones due to diffusion. Several approaches to this problem can be envisioned. First, if one desires “pure” analyte (for example, for identification during downstream analysis), only the heart of the corresponding zone can be selected, excluding other dzITP-separated compounds. Second, if one wants to ensure to select all of a certain zone, the “cuts” can be made in the centers of the neighboring zones. Finally, analytes can be released with regular time intervals to fractionate the dzITP-separation while accepting that a single compound might appear in multiple fractions, or that a single fraction contains multiple compounds.

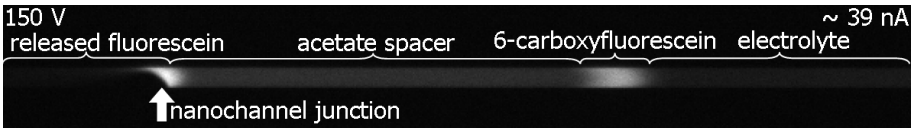

Figure 5. Selective enrichment of low-abundant 6-carboxyfluorescein over high-abundant fluorescein using acetate as a spacer compound. The image was obtained after 10 minutes of actuation

Selective enrichment

dzITP filtering can be used to concentrate specific low-abundant analytes and to enrich them selectively over compounds of which the initial concentration is much higher. To demonstrate this, we continuously injected a sample mixture containing 100 µmol/L fluorescein and 400 nmol/L 6-carboxyfluorescein. The mixture contained also 100 µmol/L sodium acetate; the acetate ion has intermediate mobility between fluorescein and 6-carboxyfluorescein and therefore acted as a non-fluorescent spacer in isotachophoretic separations. Currents and voltages were tuned such that the acetate spacer zone was released partly, establishing the cut-off of the filter. Fluorescein, having a lower mobility, was co-released with acetate, while 6-carboxyfluorescein was trapped and concentrated at the upstream border of the acetate spacer zone (figure 5). The fluorescein traveling through the acetate zone is increased in concentration ~2.2 times compared to its background concentration in the electrolyte, allowing indirect detection of the upstream acetate zone border where 6-carboxyfluorescein is to be focused. Already after 1 minute, the 6-carboxyfluorescein started to become visible as a peak. In 10 minutes, the intensity of the 6-carboxyfluorescein peak was increased 4-fold over the intensity of the fluorescein in the acetate zone and almost 10-fold over the fluorescence intensity in the background

electrolyte. Recalling that the starting concentration of 6-carboxyfluorescein was 250x lower than the fluorescein starting concentration, it is clear that dzITP filtering can be used for rapid enrichment of specific-low abundant compounds even in the presence of similar high-abundant compounds. This can be highly advantageous for biological samples, where the concentrations of analytes of interest can differ many orders of magnitude.

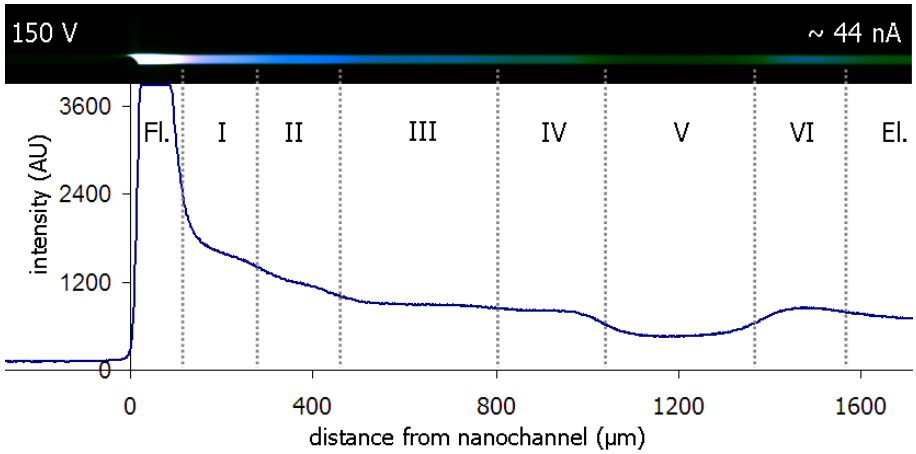

Figure 6. False-color CCD image showing dzITP filtration of urine after 5 minutes of actuation, using partly released fluorescein as a marker for ionic mobility cut-off and for indirect detection. The fluorescence profile of the CCD image shows six putative analyte zones, indicated by the Roman numerals I-VI. Fl. = fluorescein marker zone; El. = background electrolyte zone. The image was obtained after 6 minutes of actuation

Urine Sample

Figure 6 shows a proof of principle for the application of the dzITP filter to a complex biological sample, using fluorescein as a marker compound. A continuous dzITP injection was performed. Without low-pass dzITP filtering, fluorescein and undesired low-mobility compounds would accumulate,

rapidly covering the complete read-out window. Therefore, the upstream current was tuned such that a fluorescein zone was established with an approximately stable width. Behind this fluorescein zone, compounds from the urine sample formed several other zones. The current had to be modulated slightly (45-43 nA) to keep release of fluorescein approximately constant, possibly due to changes in overall conductivity caused by broadening isotachophoretic zones. The zones are visualized by indirect detection. In isotachophoretic separations, each zone has its specific conductivity. While the continuously injected fluorescein migrates through these zones, its concentration is being adjusted to the local conductivity. The fluorescein thus is not only used as a marker, but also acts as tracer; it is a so-called "underspeeder"[21]. In the fluorescence profile in figure 6, six putative analyte zones are indicated. As in each isotachophoretic separation, the zones have overlap at the borders. The putative zone borders are therefore indicated at the inflection points between the zones in the fluorescence profile.

Theoretically, with this mode of indirect detection a stair-like fluorescence profile is expected, with increasing fluorescence intensities for each zone that is closer to the fluorescein zone. However, zone V in figure 6 has significantly lower fluorescence intensity. Possibly, this zone contains an analyte which quenches fluorescence.

The identity of the detected analytes is not known. However, the fluorescein marker and the carbonate electrolyte define a window of ionic mobilities, which contains only a limited number of metabolites out of the thousands of compounds in urine. These probably include only very small molecules or molecules that have, like fluorescein, at least a double negative charge. Notable metabolites that fulfill these conditions include acetate, aspartate,

glutamate and several citric acid cycle products. The carbonate electrolyte defines the upper limit of the mobility window and excludes some small ions, particularly chloride. Low-mobility compounds are co-released with the fluorescein.

It is probable that more than six urine metabolites are retained in the dzITP zones, however they might have insufficient starting concentrations and therefore are not forming individual zones, but rather are present as non-detectable peaks between the zones. For sample analysis it is therefore desirable to continue this continuous filtration step with a number of pulsed releases as described above. This way, fractions of enriched analytes can be sent to a detector located along the downstream channel for identification and quantification.

For our metabolomics research, the dzITP filter will be enabling, because it can be used to concentrate and fractionate many metabolites in the presence of at least two undesired classes of compounds in ultrasmall complex samples: proteins, which have lower mobility, and salts, which have higher mobility than most metabolites.

Conclusions

We have developed a dynamic low-pass filter that separates compounds based on ionic mobility. The dzITP filter works by voltage or current-controlled release of compounds along a nanochannel-induced depletion zone. Isotachophoretic separation of compounds before the filter results in selectivity. In pulsed mode, dzITP-separated compounds are fractionated in plugs that are released sequentially along the depletion zone. In continuous mode, a certain (marker) compound is partially released, whereby the ionic

mobility of this compound defines the cut-off of the filter. Compounds with lower mobility are co-released, compounds with higher mobility are trapped in isotachophoretic zones. Importantly, this cut-off can be simply and rapidly tuned by voltage or current actuation. The dzITP filter has been demonstrated for the selective enrichment of low-abundant 6-carboxyfluorescein over highly abundant fluorescein. dzITP filtering was also applied to diluted raw urine sample, using fluorescein as a marker. Out of the thousands of compounds in urine, a small ionic mobility window was selected wherein five analyte zones were indirectly detected. The dzITP filter can thus enrich specific compounds from complex biological samples and enable real-time monitoring and detection.

References

1. G. Garcia-Schwarz, A. Rogacs, S. S. Bahga and J. G. Santiago, J. Visualized Exp (61), e3890 (2012).
2. A. Persat and J. G. Santiago, Analytical Chemistry **83** (6), 2310-2316 (2011).
3. B. Jung, R. Bharadwaj and J. G. Santiago, Analytical Chemistry **78** (7), 2319-2327 (2006).
4. J. G. Shackman and D. Ross, Electrophoresis **28** (4), 556-571 (2007).
5. W. S. Koegler and C. F. Ivory, Biotechnology Progress **12** (6), 822-836 (1996).
6. S.-L. Lin, Y. Li, H. D. Tolley, P. H. Humble and M. L. Lee, Journal of Chromatography A **1125** (2), 254-262 (2006).
7. D. Ross and L. E. Locascio, Analytical Chemistry **74** (11), 2556-2564 (2002).
8. M. S. Munson, J. M. Meacham, L. E. Locascio and D. Ross, Analytical Chemistry **80** (1), 172-178 (2007).
9. M. M. Meighan, J. Vasquez, L. Dziubcynski, S. Hews and M. A. Hayes, Analytical Chemistry **83** (1), 368-373 (2010).
10. J. Han, J. Fu and R. B. Schoch, Lab on a Chip **8** (1), 23-33 (2008).
11. Q. Pu, J. Yun, H. Temkin and S. Liu, Nano Letters **4** (6), 1099-1103 (2004).
12. T. A. Zangle, A. Mani and J. G. Santiago, Chemical Society Reviews **39** (3), 1014-1035 (2010).

13. Y.-C. Wang, A. L. Stevens and J. Han, Analytical Chemistry **77** (14), 4293-4299 (2005).
14. S. J. Kim, S. H. Ko, K. H. Kang and J. Han, Nat Nano **5** (4), 297-301 (2010).
15. J. Astorga-Wells, S. Vollmer, T. Bergman and H. Jörnvall, Analytical Chemistry **79** (3), 1057-1063 (2007).
16. F. o. Mavré, R. K. Anand, D. R. Laws, K.-F. Chow, B.-Y. Chang, J. A. Crooks and R. M. Crooks, Analytical Chemistry **82** (21), 8766-8774 (2010).
17. J. Quist, K. G. H. Janssen, P. Vulto, T. Hankemeier and H. J. van der Linden, Analytical Chemistry **83** (20), 7910-7915 (2011).
18. L. F. Cheow and J. Han, Analytical Chemistry **83** (18), 7086-7093 (2011).
19. J. Astorga-Wells, S. Vollmer, S. Tryggvason, T. Bergman and H. Jörnvall, Analytical Chemistry **77** (22), 7131-7136 (2005).
20. S. J. Kim, L. D. Li and J. Han, Langmuir **25** (13), 7759-7765 (2009).
21. R. D. Chambers and J. G. Santiago, Analytical Chemistry **81** (8), 3022-3028 (2009).