Electronic Supplementary Material (ESI) for Lab on a Chip.
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Supporting Information

Continuous micellar electrokinetic focusing of neutral species driven by ion concentration polarization

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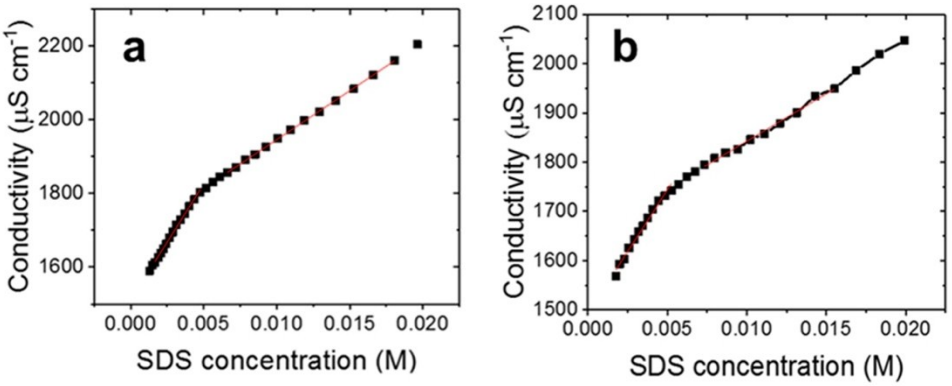
This Supporting Information (SI) includes details of the experimental procedures for 1) conductivity measurements employed to determine the critical micelle concentration (CMC) of sodium dodecyl sulfate (SDS) solutions in 10.0 mM sodium phosphate buffer with and without the addition of BODIPY dye, 2) microfluidic device design and fabrication, and 3) ICP-based extraction experiments that evaluate the impact of concentration (of surfactant and neutral species), flow rate, voltage, and channel geometry on extraction efficiency. Experimental results presented in the SI include fluorescence micrographs that show the extraction process as a function of applied voltage (**Figure S4**), time dependence of extraction efficiency after initiation of driving voltage (**Figure S5**), and extraction efficiency in the presence of surfactant one order of magnitude below the CMC (**Figure S6, S7, S8**) in a device with a 1:9 channel width ratio between upper and lower branches (**Figure S2c**).

Determination of critical micelle concentration. Conductivity measurements were performed to verify the CMC of SDS in 10.0 mM sodium phosphate buffer (pH 7.4, 22 ºC) with and without added BODIPY. The conductivity was measured using an Orion Star A215 pH/Conductivity meter (Thermo Scientific, Waltham, MA).

A 25.00 mL solution of SDS (20.0 mM) in sodium phosphate buffer (10.0 mM, pH 7.4, 22 ºC) was serially diluted under vigorous stirring (1200 rpm). Measurements were taken every minute after each change in concentration. The CMC obtained for SDS in sodium phosphate buffer is 4.53 ± 0.03 mM. These values are in good agreement with those previously published.[1] The CMC of SDS in 10.0 mM sodium phosphate buffer remained in the previously published range even when



BODIPY was added ($CMC_{SDS+BODIPY}$ = 4.53 mM, for both 0.05 mM and 0.10 mM BODIPY).

Figure S1. Solution conductivity as a function of SDS concentration in the absence (a) and presence (b) of 50 μM BODIPY dye. Both (a and b) in 10.0 mM sodium phosphate buffer (pH 7.4).

Device fabrication. The microfluidic devices were fabricated using standard photolithographic processes.[2] Channel molds were patterned using negative photoresist (SU-8 2050, Microchem Corp., Westborough, MD) on Si substrate. Poly(dimethylsiloxane) (PDMS) (Sylgard 184 elastomer kit, Dow Corning Corp., Midland, MI) was used for device fabrication. All microchannels were 46.0 μm tall with a distance of 10.0 mm between the inlet and each outlet and having 500 μm-wide main channel that branched into two channels (each 250 μm wide for 1:1 channel width ratio between upper and lower branches) (**Figure S2a**).

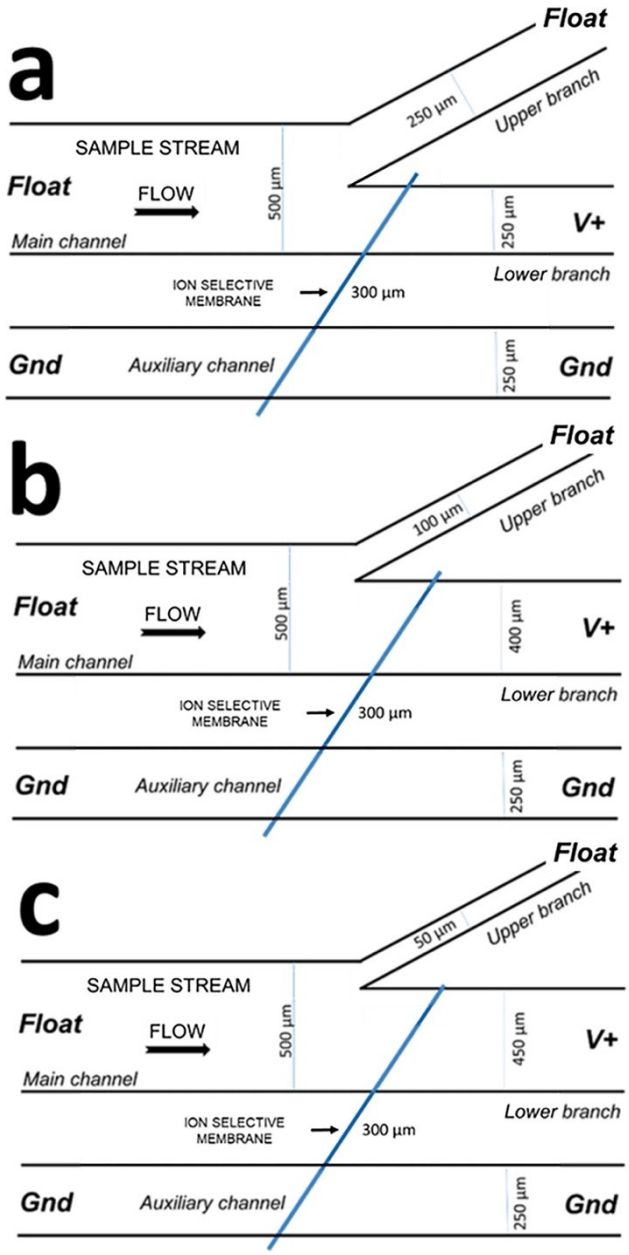


Figure S2. Microfluidic device design schematics with dimensions and experimental setup for continuous ICP separation experiments. Devices with 1:1 (a), 1:4 (b), and 1:9 (c) channel width ratio between the upper and lower branches, respectively.

A second channel (auxiliary channel) 10.0 mm long and 500 µm wide was located parallel to the separation channel at a distance of 300 µm. A 4.0 mm-diameter biopsy punch was used to create the inlet and outlet reservoirs, unless noted otherwise. A mechanical incision was made using a scalpel blade across the lower branch and auxiliary channel, and subsequently filled with 10.0 µL

of *Nafion*®. The membrane was then cured at 95ºC for 10 min. Excess *Nafion*® was removed by applying and peeling away low residue tape. The PDMS layer and glass slide were treated with air plasma (PDC-001, Harrick Plasma, Ithaca, NY) for 60 s (medium RF power) and then bonded together. All microfluidic devices were rinsed with double deionized water and coated with Pluronic (3.0 μM in 10.0 mM phosphate buffer) for at least 18 h. The Pluronic solution was used to suppress the electroosmotic flow. The microfluidic devices were then rinsed with 20.0 mM surfactant solution for 1 h before use to ensure uniform wall charge regardless of the surfactant concentration employed in the experiment. Then just before use, the device was rinsed with 10.0 mM sodium phosphate buffer (pH 7.4) for 15 min to remove the 20 mM surfactant solution. In each experiment, the driving voltage was applied between the outlet of the lower branch (V+, **Figure S2**) and both ends of the auxiliary channel (Gnd, **Figure S2**). Microfluidic devices with high channel width ratio between the upper and lower branches (**Figure S2b,c**) were fabricated and operated using the same procedure.

Calibration of neutral dye (BODIPY) fluorescence intensities in microfluidic device. The device used for calibration was fabricated with the same dimensions and procedure as the devices employed to obtain the data in the main text. The device was rinsed with double deionized water and coated with Pluronic (3.0 μM in 10.0 mM phosphate buffer) for 18 h. The microfluidic device was then rinsed with 20.0 mM SDS solution for 1 h, and then just before use, rinsed with 10.0 mM sodium phosphate buffer (pH 7.4) for 15 min to remove the surfactant solution. The rinsing solution was then replaced with 0.01 mM BODIPY solution in 10.0 mM sodium phosphate buffer (pH 7.4), and fluorescence micrographs were taken to measure mean fluorescence intensity across the lower branch of the microfluidic device 300 μm downstream from the ion selective membrane. Subsequently, the device was rinsed thoroughly with 10.0 mM phosphate buffer for 15 min and

imaged to ascertain the background fluorescence intensity of the channel walls and to account for residual BODIPY adsorption. This procedure was repeated to obtain background subtracted fluorescence intensities for BODIPY concentrations of 0.025, 0.05, 0.075 mM. **Figure S3** demonstrates the linear relationship between background subtracted fluorescence intensity and various BODIPY concentrations.

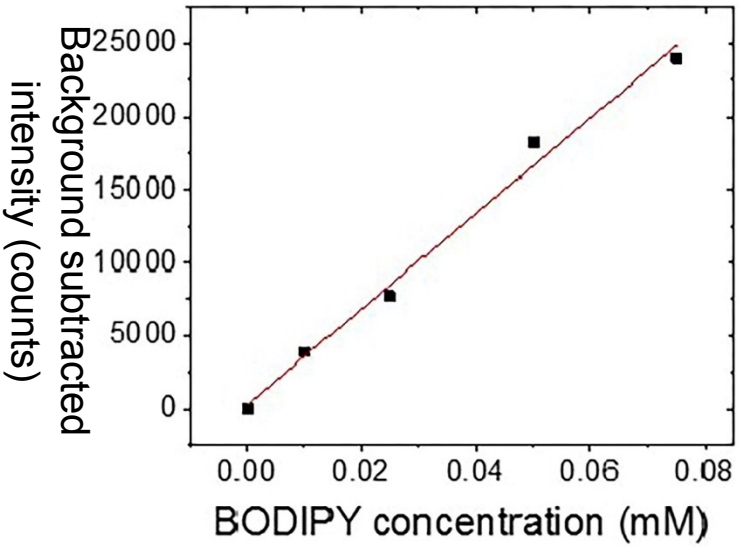


Figure S3. Calibration curve of background subtracted fluorescence intensities for various BODPIY dye concentrations. $y = 327384x + 294.12$; $R^2 = 0.990$.

ICP based extraction of neutral species in the absence of surfactants. The device was rinsed with 10.0 mM sodium phosphate buffer (pH 7.4) for 15 min. The buffer in the inlet reservoir was then replaced with 35.0 µL (resulting height difference ~2.8 mm, concave meniscus) of 0.05 mM BODIPY and 1.0 µM Texas Red solution in 10.0 mM sodium phosphate buffer. Then, the volume in the outlet reservoirs was adjusted to 20.0 µL (resulting height difference ~ 1.6 mm, concave meniscus) to generate pressure driven flow of the solution in the main channel. Finally, a driving voltage of 60.0 V was applied. Fluorescence micrographs (**Figure 1a-b**, main text) were taken 5 min after the driving voltage was applied.

Micellar electrokinetic focusing of a neutral tracer dye by ICP. After the channels were rinsed, the buffer in the inlet reservoir was replaced with 35.0 μL (resulting height difference ~2.8 mm, concave meniscus) of SDS solution (0.0-20.0 mM) and 50 μM BODIPY in sodium phosphate buffer (10.0 mM, pH 7.4). Then, the volume in the outlet reservoirs was adjusted to 20.0 μL (resulting height difference ~ 1.6 mm, concave meniscus) to generate pressure driven flow of the solution in the main channel. Finally, a driving voltage of 60.0 V was applied. Fluorescence micrographs were taken 5 min after the driving voltage was applied (**Figure 2a**, main text). The device was rinsed thoroughly with 10.0 mM phosphate buffer, re-coated for 15 min using 20.0 mM SDS solution in 10.0 mM sodium phosphate buffer, re-rinsed with phosphate buffer (10.0 mM, pH 7.4) in between trials and imaged to ascertain the background fluorescence intensity of the channel walls and to account for residual BODIPY adsorption. Mean fluorescence intensity across the lower branch of the microfluidic device was measured 300 μm downstream from the ion selective membrane and used for quantitative analysis. All fluorescence intensities were background subtracted. Extraction efficiency (EE) was calculated by comparing the intensity (I) at this location to that obtained prior to initiation of ICP (I_0) such that EE = $100\%(1 - I/I_0)$.

Evaluation of the impact of flow rate and voltage on extraction efficiency. For these sets of experiments, a 1.0 mm-diameter biopsy punch was used to create the device inlet reservoir (4.0 mm-diameter outlet). A 10.0 mM SDS solution in sodium phosphate buffer (10.0 mM, pH 7.4) spiked with 0.050 mM BODIPY was continuously flowed into the device using a 0.50 mL Hamilton syringe through a 1.0 mm outer diameter PTF tubing. The 11 Pico Plus Elite Programmable Syringe Pump (Harvard Apparatus, Holliston, MA) was used to control the flow rate. After establishing a flow rate of 50 nL min^{-1} a driving voltage of 100.0 V was applied. After 1 min at this voltage, a fluorescence image was taken. The voltage was then turned off for 1 min.

This pattern was subsequently repeated such that the voltage was decreased by 10.0 V increments to 10.0 V and images were acquired 1 min after each voltage step (**Figure S4**). These experiments were repeated three times each at flow rates of , 50, 60 and 70 nL min^{-1}. In each case, the mean fluorescence intensity across the lower branch of the microfluidic device was measured 300 μm downstream from the ion selective membrane and used for quantitative analysis. After the full voltage sequence, the device was thoroughly rinsed for 15 min using phosphate buffer (10.0 mM, pH 7.4), and imaged to obtain background fluorescence intensity of the channel walls. Background subtracted fluorescence intensities were used to quantify extraction efficiency at each voltage. **Figure 2c** in the main text shows the average extraction efficiency obtained over a range of applied voltages over this series of flow rates.

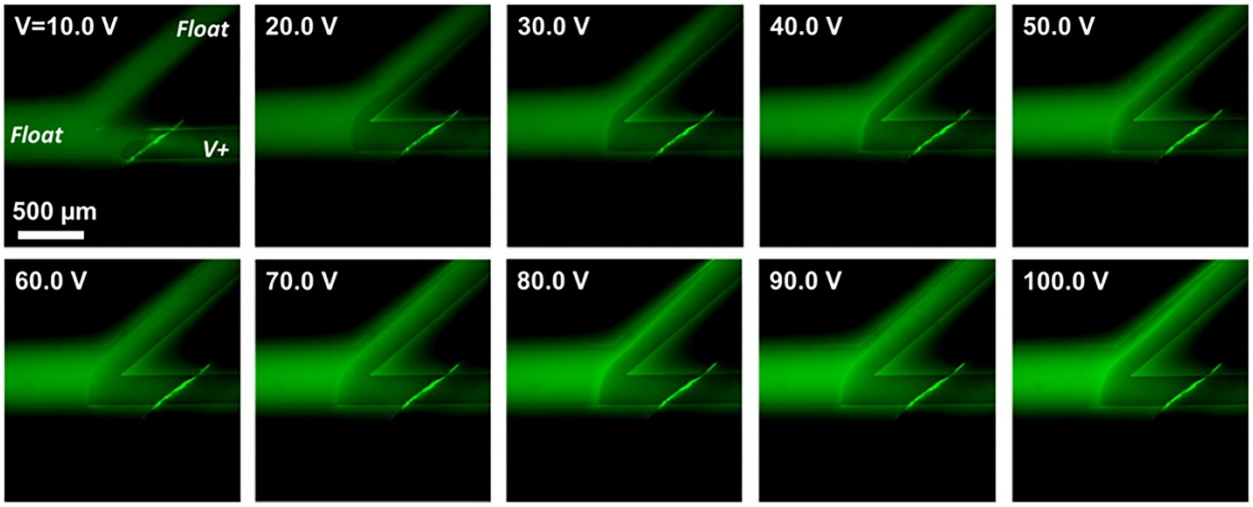


Figure S4. Fluorescence micrographs showing the location of neutral analyte in the presence of 10.0 mM SDS and flow rate of 50 nL/min for voltage sequence 10.0-100.0 V.

Evaluation of the impact of flow rate and voltage on extraction efficiency at longer periods of time. For these sets of experiments, a 1.0 mm-diameter biopsy punch was used to create the device inlet (4.0 mm outlet). A solution of 0.050 mM BODIPY and 10.0 mM SDS in sodium phosphate buffer (10.0 mM, pH 7.4) was continuously flowed into the device using a 0.50 mL Hamilton syringe through 1.0 mm O.D. PTF tubing. The syringe pump was used to control the flow rate. After establishing a flow rate of 60 nL min^{-1} a driving voltage of 60.0 V was applied. A fluorescence image was taken every 1 min after ICP initiation for a total of 10 min (**Figure S4**). After the full voltage sequence, the device was thoroughly rinsed for 15 min using phosphate buffer (10.0 mM, pH 7.4), and imaged to obtain the background fluorescence intensity of the channel walls. These experiments were repeated three times. **Figure S5** shows the average extraction efficiency obtained over a time period of 10 min. The mean fluorescence intensity across the lower branch of microfluidic device was measured 300 μm downstream from the ion selective membrane and used for quantitative analysis. All fluorescence intensities were background subtracted.

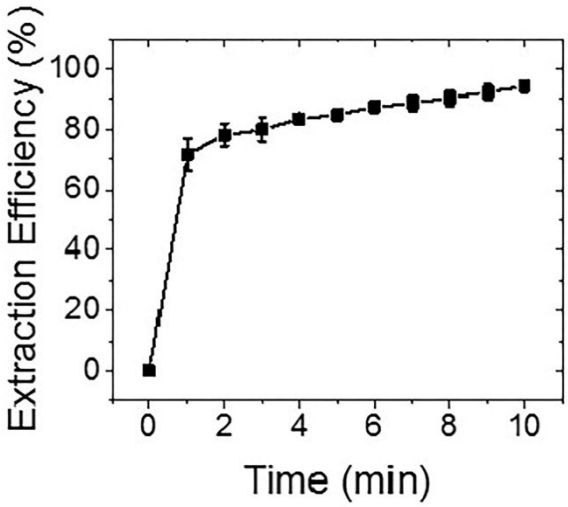


Figure S5. Neutral species extraction efficiency at different time points after initiation of ICP. Results demonstrated using 10.0 mM SDS in 10.0 mM phosphate buffer at flow rate of 60 nL min^{-1}, and under applied voltage of 60.0 V. Error bars represent the standard deviation for three replicates.

Influence of the concentration of the neutral species on the efficiency of CMEKS. After treatment with SDS and rinsing with phosphate buffer, the buffer in the inlet reservoir was replaced with 35.0 μL (resulting height difference ~2.8 mm, concave meniscus) of 10.0 mM SDS in sodium phosphate buffer (10.0 mM, pH 7.4) with BODIPY at 0.05, 0.10, 0.50, 1.0 or 5.0 mM. Then, the volume in the outlet reservoirs was adjusted to 20.0 μL (resulting height difference ~ 1.6 mm, concave meniscus) to generate pressure driven flow of the solution in the main channel. Finally, a driving voltage of 60.0 V was applied. Fluorescence micrographs were taken 5 min after the driving voltage was applied (**Figure 3**, main text). Then, the voltage was turned off, and the device was rinsed 3 times with 10.0 mM phosphate buffer, re-coated for 15 min using 20.0 mM SDS in 10.0 mM sodium phosphate buffer, and imaged to obtain background fluorescence intensity of the channel walls in between the trials. Extraction efficiency was calculated as described in the preceding paragraphs.

ICP enrichment and extraction of neutral species at surfactant concentrations below the CMC (1.0 and 0.5 mM SDS). For these experiments, a 1.0 mm-diameter biopsy punch was used to create the device inlet. A 1.0 mM SDS solution in sodium phosphate buffer (10.0 mM, pH 7.4) spiked with 0.050 mM BODIPY was continuously flowed into the device using a 0.50 mL Hamilton syringe and 1.0 mm O.D. PTF tubing. The syringe pump was used to control the flow rate. After establishing a flow rate of 60.0 nL min^{-1} a driving voltage of 60.0 V was applied. A series of fluorescence micrographs was acquired over a period of 25 min (images taken at 1, 3, 5, 10, 15, 20, and 25 min) after the start of the experiment. The device was thoroughly rinsed with 10.0 mM phosphate buffer for 15 min, and imaged to obtain the background fluorescence intensity of the channel walls. Separation efficiency was calculated as described in the preceding paragraphs. **Figure S6** shows the resulting extraction efficiency obtained for 0.050 mM BODIPY

dye in the presence of 0.50 mM SDS over a period of 25 min. **Figure S7** shows the increase of background subtracted intensity across IDZ boundary obtained for 0.050 mM BODIPY dye in the presence of 1.0 mM SDS over a period of 25 min.

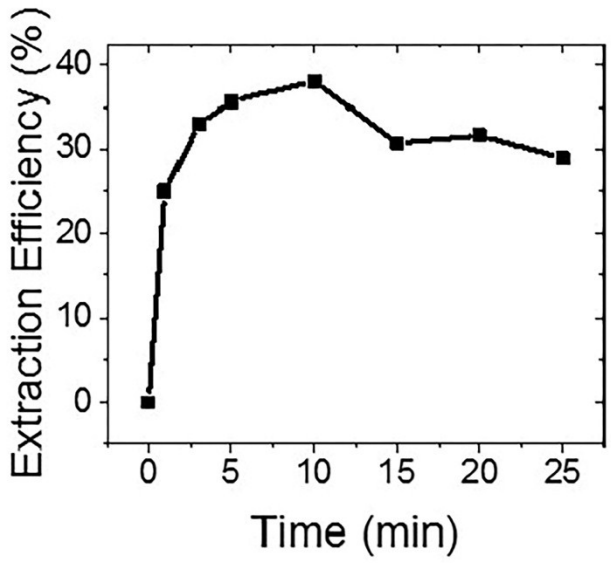


Figure S6. Neutral species extraction efficiency at different time points after initiation of ICP in a device with a branching ratio of 1:9. Results demonstrated using 0.50 mM SDS in 10.0 mM phosphate buffer at flow rate of 60 nL min^{-1}, and under applied voltage of 60.0 V.

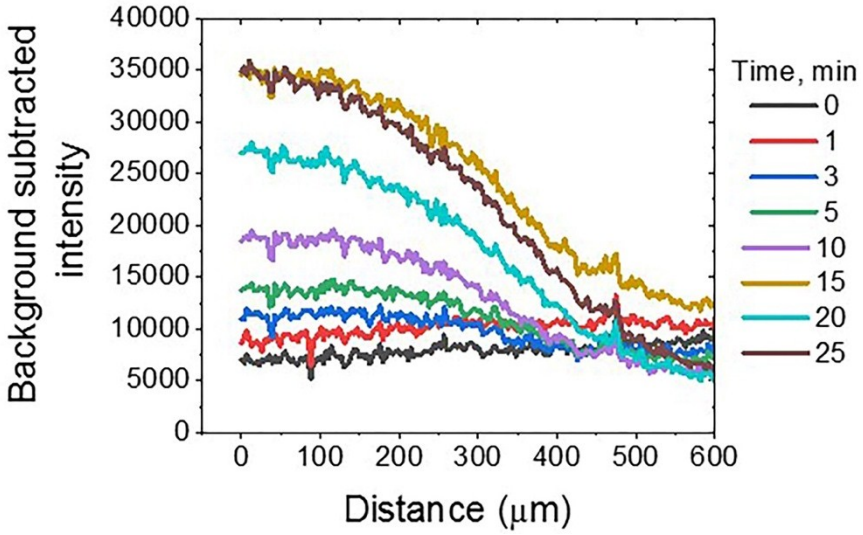
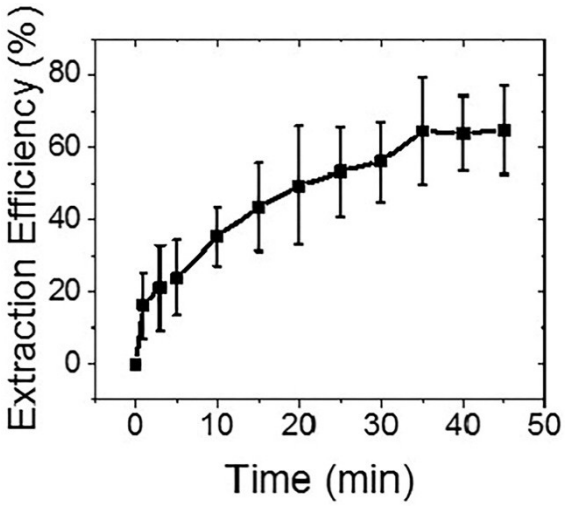


Figure S7. Increase of background subtracted intensity across IDZ boundary of neutral species at different time points after initiation of ICP in a device with a branching channel width ratio of 1:9. Results demonstrated using 1.0 mM SDS in 10.0 mM phosphate buffer at flow rate of 60 nL min^{-1}, and under applied voltage of 60.0 V

ICP enrichment and extraction of neutral species at sodium cholate concentrations below the CMC. For these experiments, a 1.0 mm-diameter biopsy punch was used to create the device inlet. A 3.0 mM SC solution in sodium phosphate buffer (10.0 mM, pH 7.4) spiked with 0.050 mM BODIPY was continuously flowed into the device using a 0.50 mL Hamilton syringe and 1.0 mm O.D. PTF tubing. A syringe pump was used to control the flow rate. After establishing a flow rate of 60.0 nL min^{-1} a driving voltage of 80.0 V was applied. A series of fluorescence micrographs was acquired over a period of 45 min (images taken at 1, 3, 5, 10, 15, 20, 25, 30, 35, 40 and 45 min) after the start of the experiment. The device was thoroughly rinsed with 10.0 mM phosphate buffer for 15 min and imaged to obtain background fluorescence intensity of the channel walls. Extraction efficiency was calculated as described in the preceding paragraphs. **Figure S8** shows the resulting separation efficiency obtained for 0.050 mM BODIPY dye in the presence of 3.0 mM sodium cholate over a period of 45 min. **Figure S9** shows the fluorescence micrographs of neutral analyte (0.050 mM BODIPY) at different time points in the presence of 3.0 mM sodium cholate



over a period of 45 min.

Figure S8. Neutral species extraction efficiency at different time points after initiation of ICP in a device with a branching channel width ratio of 1:9. Results demonstrated using 3.0 mM sodium cholate in 10.0 mM phosphate buffer at flow rate of 60 nL min^{-1}, and under applied voltage of 80.0 V.

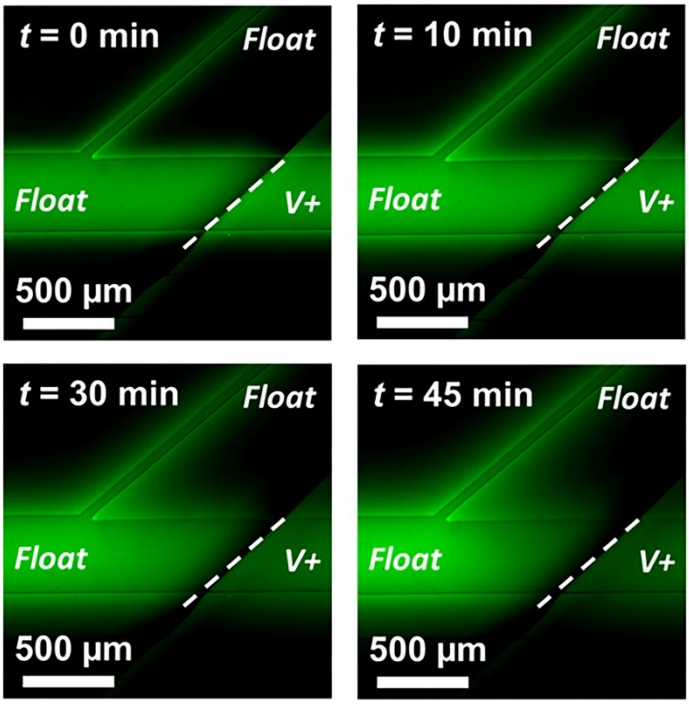


Figure S9. Fluorescence micrographs showing the location of neutral analyte (0.050 mM BODIPY) at different time points in the presence of 3.0 mM SC at a flow rate of 60 nL/min, and under applied voltage of 60.0 V.

Simultaneous solubilization and extraction of two neutral species (BODIPY and pyrene) from solution by continuous micellar electrokinetic focusing. The device was rinsed with 20.0 mM sodium cholate solution in phosphate buffer (10.0 mM) for 1 h. Further, the device was rinsed with 10.0 mM sodium phosphate buffer (pH 7.4) for 15 min to remove excess surfactant. The buffer in the inlet reservoir was then replaced with 35.0 µL (resulting height difference ~2.8 mm, concave meniscus) of the sample solution (0.05 mM BODIPY, 0.15 mM pyrene, and 40.0 mM sodium cholate, in 10.0 mM sodium phosphate buffer). Then, the volume in the outlet reservoirs was adjusted to 20.0 µL (resulting height difference ~ 1.6 mm, concave meniscus) to generate pressure driven flow of the solution in the main channel. Finally, a driving voltage of 60.0 V was

applied. Fluorescence micrographs (**Figures 6a-f**, main text) were taken at the times indicated after the driving voltage was applied. **Figure S10** demonstrates the filling of the main channel with fresh solution from the inlet 1 min after releasing the driving voltage.

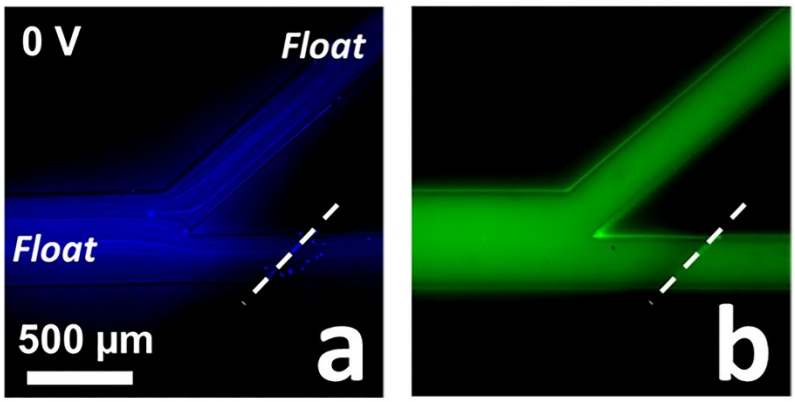
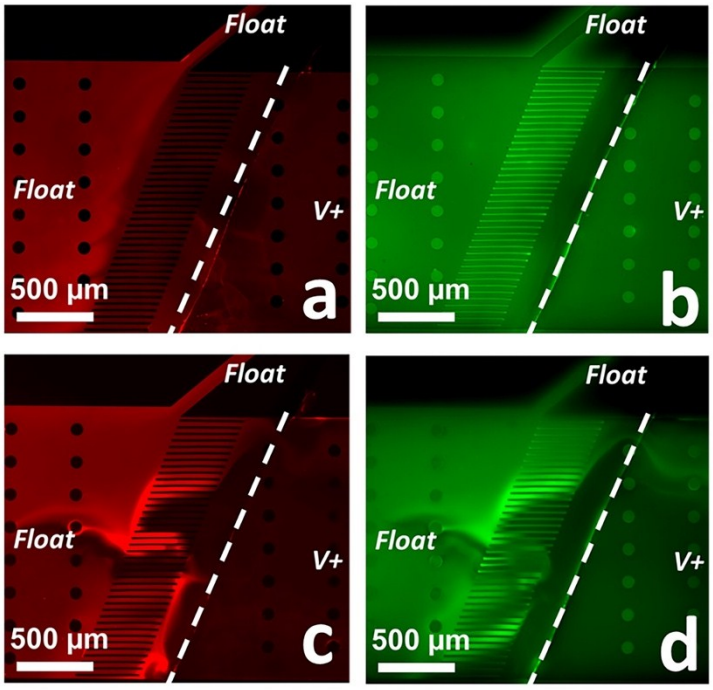


Figure S10. Fluorescence micrographs showing the neutral species (green – BODIPY, 50 µM input concentration; blue – pyrene, 150 µM input concentration) in the presence of 40.0 mM sodium cholate. Image taken 1 min after releasing the driving voltage ($V+ = 0$).

ICP based focusing of charged and neutral species in complex matrices (spent dialysate). An ICP-based device yielding increased volumetric throughput was fabricated according to a previously described procedure. The microchannel had a distance of 10.0 mm between the inlet and each outlet and a 2.1 µm-wide main channel that branched into two channels (0.1 mm and 2.0 mm wide). Microfin structures were 0.5 mm long and 0.020 mm wide with a 0.030 mm gap between microslits). A second channel (auxiliary channel) 10.0 mm long and 0.5 µm wide was located parallel to the separation channel at a distance of 300 µm. A 1.0 mm-diameter biopsy punch was used to create the inlet reservoir. A 4.0 mm-diameter biopsy punch was used to create the outlet reservoirs. A mechanical incision was made using a scalpel blade across the lower branch and auxiliary channel, and subsequently filled with 20.0 µL of *Nafion*®. The membrane was then cured at 95ºC for 10 min. Excess *Nafion*® was removed by applying and peeling away low residue tape. The PDMS layer and glass slide were treated with air plasma (PDC-001, Harrick Plasma,

Ithaca, NY) for 60 s (medium RF power) and then bonded to a glass slide to form the channel

floor. The microfluidic device was rinsed with double deionized water and coated with Pluronic (3.0 μM in 10.0 mM phosphate buffer) for at least 18 h. The microfluidic device was then rinsed with 20.0 mM SDS solution for 1 h before use. Then just before use, the device was rinsed with 10.0 mM sodium phosphate buffer (pH 7.4) for 15 min to remove the 20 mM SDS solution. Undiluted spent dialysate was spiked with BODIPY (to 0.050 mM) and dye-linked albumin (0.1 mg mL^{-1}). This solution was continuously flowed into the device using a 0.50 mL Hamilton syringe and 1.0 mm O.D. PTF tubing. A syringe pump was used to control the flow rate. After establishing a flow rate of 0.2 μL min^{-1}, a driving voltage of 120.0 V was applied. Fluorescence micrographs (**Figures S11a,b**) were obtained 5 min after applying the driving voltage. Further, the device was thoroughly rinsed with 10.0 mM phosphate buffer for 15 min and imaged to obtain background



fluorescence intensity of the channel walls. Further, the device was used to repeat the experiment employing spent dialysate spiked with 10.0 mM SDS, 0.050 mM BODIPY and 0.1 mg mL^{-1} dye linked albumin.

Figure S11. Fluorescence micrographs showing neutral species (green – BODIPY, 50 μM input concentration; red – dye-linked albumin, 0.1 mg mL^{-1} input concentration) in the absence (a,b) and presence (c,d) of 10.0 mM SDS. Flow rate, 0.2 μL/min.; $V+$ = 120.0 V. Images taken 5 min after applying the driving voltage.

References

(1) Fuguet, E.; Ràfols, C.; Rosés, M.; Bosch, E. Critical Micelle Concentration of Surfactants in Aqueous Buffered and Unbuffered Systems. *Anal. Chim. Acta* **2005**, *548* (1–2), 95–100.

(2) McDonald, J. C.; Whitesides, G. M. Poly (Dimethylsiloxane) as a Material for Fabricating Microfluidic Devices. *Acc. Chem. Res.* **2002**, *35* (7), 491–499.