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

SUPPLEMENTARY INFORMATION

Non-Aqueous Continuous-Flow Electrophoresis (NACFE): Potential Separation Complement for Continuous-Flow Organic Synthesis

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Table of Contents

Additional supplementary files

These files can be found on ChemRxiv (https://doi.org/10.26434/chemrxiv.7840937):

File name	Description/Experiment
Angulagrams.zip	Extracted angulagrams and evaluation parameters for Figure 2, 3, and S4
Angulagrams10h.zip	Extracted angulagrams for the 10-h separation shown in Figure 4
Geometry.zip	Solid Edge files of chip geometry
Plots.zip	Origin files of all plots
Programs.zip	Python programs used to evaluate the data
Rawfiles.zip	Raw image files for Figure 2, 3, and S4
Rawfiles10h.zip	Raw image files for the 10-h separation shown in Figure 4
RawfilesCurrent.zip	Raw data of the current measurements show in Figure S2
Video10h.avi	Video of the 10-h separation
Videocurrent.avi	Video of the current measurements

Properties of tested plastic materials

Table S1. Evaluation parameters of tested plastic materials. PMMA is our reference material and choice for aqueous-based continuous-flow electrophoresis. FEP, PSu, and PVC Type I are suitable candidates for non-aqueous electrophoresis, in principle. However, PVC Type I is optically clear, has the best cost-efficiency, and, thus, was chosen in the present studies.

Name[a]	OC[b]	Millability[c]	PC[d]	Costs[e] (\$/dm^3)	Costs[f] (\$/chip)	Relative costs[g]
ABS	not clear	✔	✘	20	3	1.0
CLPS	not clear	✔	✔	100	13	4.3
CPVC	not clear	✔	✔	55	7	2.3
FEP	**semi-clear**	✔	✔	**535**	**70**	**23.3**
HDPE	not clear	✔	✔	10	2	0.7
PCa	clear	✔	✘	20	3	1.0
PE	not clear	✘	✔	70	9	3.0
PEEK	not clear	✔	✔	655	86	28.7
PETG	clear	✔	✘	15	2	0.7
PMMA	**clear**	✔	✘	**25**	**3**	**1.0**
PP	semi-clear	✘	✔	15	2	0.7
PPS	not clear	✔	✔	755	99	33.0
PS	not clear	✔	✘	15	2	0.7
PSu	**semi-clear**	✔	✔	**185**	**24**	**8.0**
PTFE	not clear	✔	✔	170	22	7.3
PVC Type I	**clear**	✔	✔	**30**	**4**	**1.3**
PVDF	not clear	✔	✔	200	26	8.7
UHMW	not clear	✔	✔	15	2	0.7

[a] ABS = Acrylontrile butadiene styrene, CLPS = Cross-linked polystyrene (Rexolite), CPVC = Chemical-resistant polyvinyl chloride, FEP = Fluorinated ethylene propylene, HDPE = High-density polyethylene, PCa = Polycarbonate, PE = Polyester, PEEK = Polyether ether ketone, PETG = Polyethylene terephthalate glycol, PMMA = Poly(methyl methacrylate), PP = Polypropylene, PPS = Polyphenylene sulphide, PS = Polystyrene, PSu = Polysulfone, PTFE = Polytetrafluoroethylene (Teflon), PVC Type I = Polyvinyl chloride Type I, PVDF = Polyvinylidene fluoride, UHMW = Ultra-high-molecular-weight polyethylene.

[b] Optical clarity in visible spectrum (non-transparent).

[c] Ability to mill plastics using our established protocols for PMMA.**[11]** (✘ = material tends to melt and/or fringe more easily than PMMA).

[d] Compatibility with propylene carbonate (PC): plastic can withstand continuous exposure to PC for at least 48 h without any obvious swelling or disintegration.

[e] Cost is given in Canadian Dollars and based on McMaster-Carr's price list of February 2019. They are listed here for pure illustration and a rough comparison.

[f] About 0.132 dm^3 are required for the assembly of one chip. Electrodes and flow connectors not included in price.

[g] Relative to PMMA.

NACFE chip

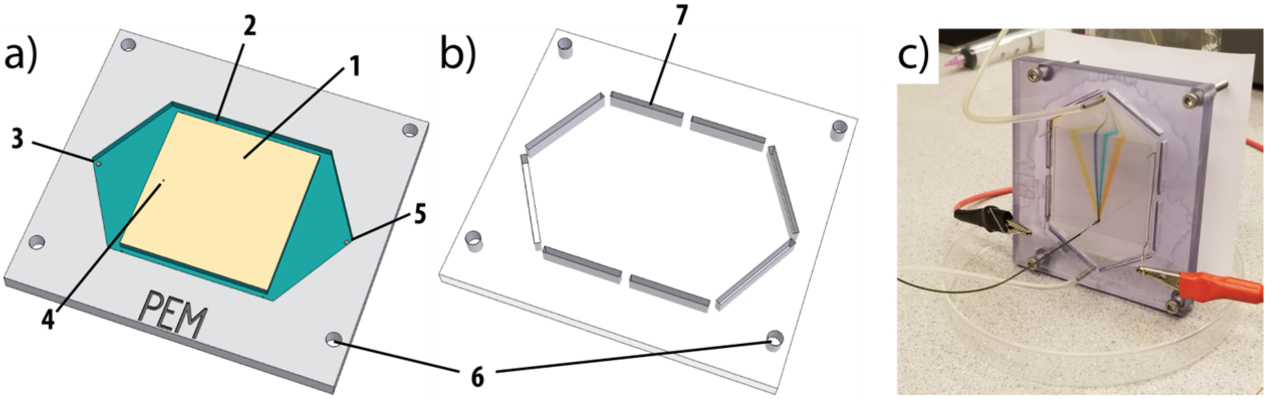


Figure S1. NACFE chip used in this study (named PEM after the city of Pembroke): **a)** bottom plate, **b)** top plate, and **c)** photo of assembled chip during operation. The numbers indicate: the separation zone (1), electrode channels (2), electrolyte inlet (3), sample inlet (4), outlet (5), mounting holes (6), and gluing channels (7). The chip dimensions are 110 mm × 100 mm × 12 mm, and the dimensions of the separation zone are 50 mm × 52 mm × 0.25 mm.

Electric current in NACFE with an electrolyte being TBAA solution in PC

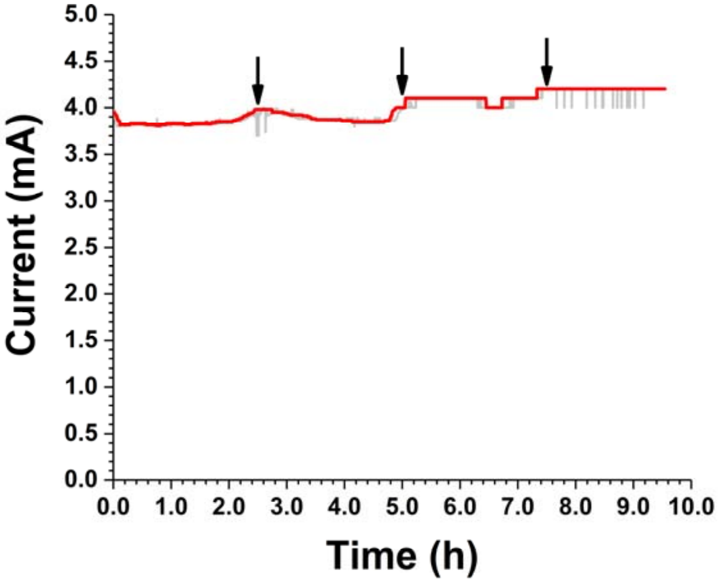


Figure S2. Long-term stability of NACFE using a solution of 30 mM TBAA in PC as an electrolyte. The arrows indicate the times at which the electrolyte was recycled. The grey curve is the signal as measured; the signal was smoothen for clarity by a percentile filter (100-point window; 80%); the red curve is the result of smoothening. The current was stable at 4.00 ± 0.15 mA (*E* = 18.2 V/cm) with only a slight drift towards higher currents due to electrochemical reactions and/or buffer depletion.

Formation of brown precipitate at the cathode for the imidazolium-based electrolyte

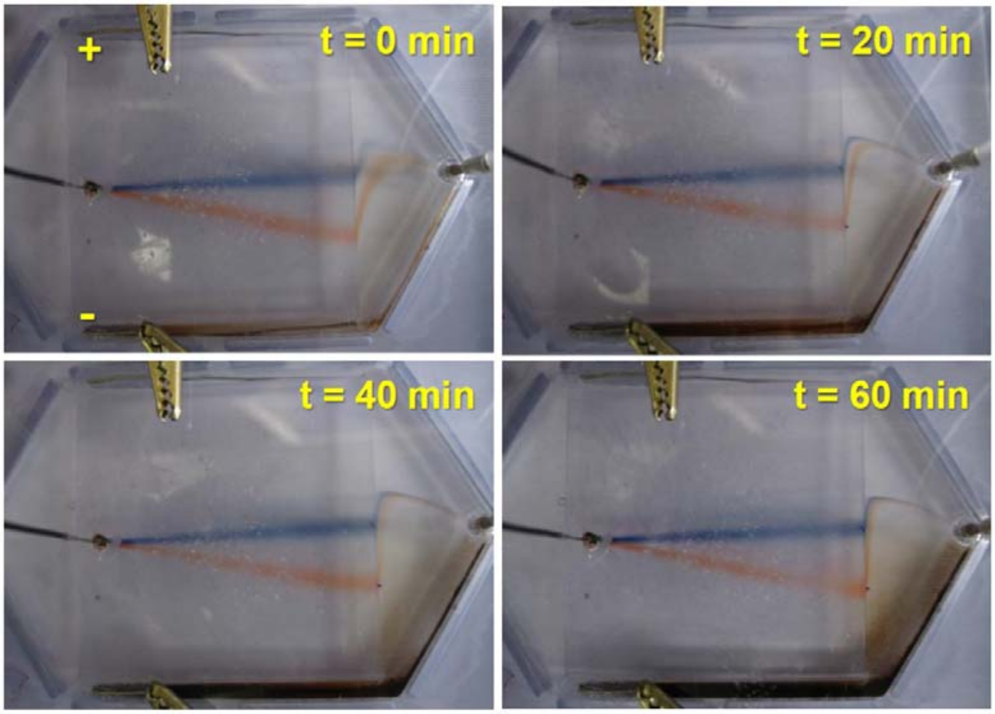


Figure S3. Separation of Sudan black B and Rhodamine 6G (3 mM each) in 21 mM imidazolium ethyl sulfate in PC. The excessive precipitation of a brown product at the cathode (bottom) affects optical clarity of the chip.

Comparing NACFE with weak- and strong-basicity anions in the electrolyte

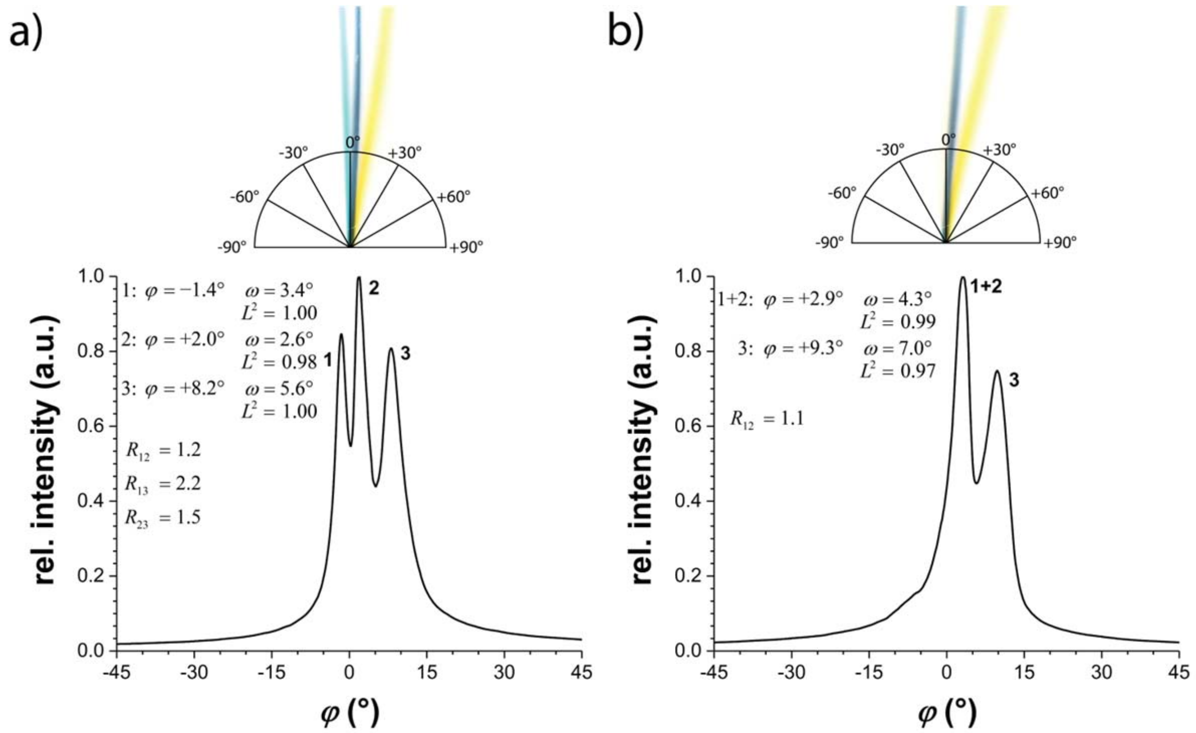


Figure S4. Separations with **a)** TBAA (strong base anion) and **b)** tetrabutylammonium hydrogen sulfate (weak base anion) in PC as electrolyte. Analytes were α-naphtholbenzein (1), Sudan black B (2), and DMAS (3). Only the strong-base anion was able to separate all three analytes. The conditions were: electrolyte flow rate = 3 mL/min, sample flow rate = 2 µL/min, and E = 27.3 V/cm ($I \approx 8.8$ mA). The anode and cathode are towards negative and positive angles, respectively.

Acetonitrile as solvent in non-aqueous electrolyte

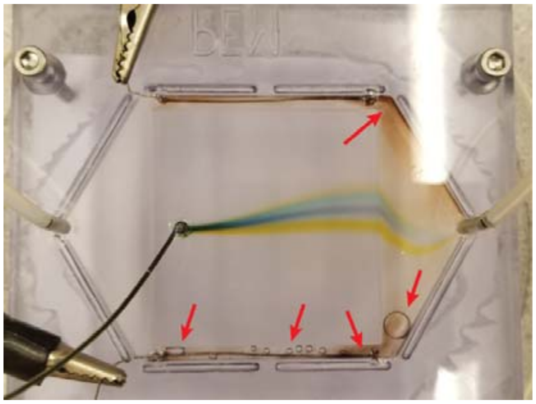


Figure S5. NACFE of four analytes (fluorescein, α-naphtholbenzein, Sudan black B, and DMAS; 1.25 mM each) in an electrolyte containing 30 mM TBAA in acetonitrile. Bubble formation (red arrows) and lack of bubble dislodging from the electrodes are evident. Bands are broader and higher electric fields are needed to achieve resolution similar to that in the PC-based electrolyte. Conditions were: electrolyte flow rate = 4 mL/min, sample flow rate = 2 µL/min, and *E* = 5.3 V/cm (*I* ≈ 8.0 mA).

General handling procedures and experiences with NACFE

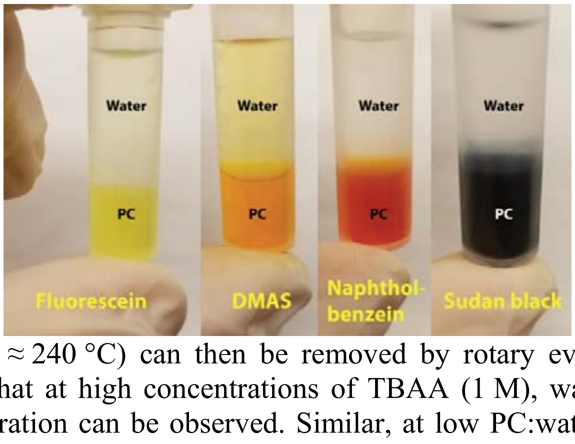
Materials and chemicals. All solutions were prepared using analytical grade reagents. Acetonitrile, α-naphtholbenzein, 2-[4-(dimethylamino)styryl]-1-methylpyridinium iodide, fluorescein sodium salt, imidazolium ethyl sulfate, PC, rhodamine 6G, Sudan black B, and TBAA were purchased from Sigma Aldrich (Oakville, ON, Canada). Electrolytes were solutions of imidazolium or tetrabutylammonium salts in PC or acetonitrile. Stock solutions of analytes were prepared in respective electrolytes as solvents. All chip materials listed in **Table S1** were purchased from McMaster Carr (Elmhurst, IL, USA).

Instrumentation. The electrolyte was delivered to the NAFCE chip with an NE-9000G peristaltic pump from New Era Pump Systems, Inc. (Farmingdale, NY, USA). The pump was equipped with a Masterflex pulse dampener from Cole Palmer (Vernon Hills, IL, USA) to suppress flow pulsation. Analyte solutions were delivered to the NACFE chip with a Model 11 syringe pump from Harvard Apparatus (Holliston, MA, USA). Separation voltage was applied to the platinum electrodes inside the separation zone from an EPS 3501 XL power supply from GE Healthcare (Chicago, IL, USA). NACFE chips were fabricated using a MODELA MDX-540 Benchtop Milling Machine from Roland DGA (Irvine, CA, USA).

Chip Fabrication. NACFE chips were designed in Solid Edge (see model files in **geometry.zip**) and fabricated of PVC Type I according to our previously developed fabrication procedure for PMMA chips (*J. Sep. Sci.* **2011**, *34*, 556−564, DOI: **10.1002/jssc.201000758**; *Lab Chip* **2017**, *17*, 256-266, DOI: **10.1039/C6LC01381C**). Details on chip fabrication and chip components used (apart from the chip material) may be found in these two previous works.

General experimental details. Electrolyte and analyte flow rates were in the ranges of 2−4 mL/min and 1−2 µL/min, respectively. 30−150 V were applied as separation voltages resulting in field strengths of 5−28 V/cm (distance between electrodes = 5.5 cm), respectively. Recycling for the 10-h separation was done in discrete steps transferring the electrolyte from the outlet container to the source container (feeding the peristaltic pump) roughly every 2.5 h.

Mixing behaviour of PC. PC and water are not miscible at similar ratios (*e.g.* 50:50). Therefore, water can be used to extract TBAA or other salts from the PC phase; aqueous workup of TBA-salts is a common technique (see *e.g.* DOI: 10.1021/ol063113h). The organic compounds will remain mainly in the PC phase (extraction coefficients can be further tuned by adding acid or base to water or by washing with diethyl ether):



PC (boiling point at 1 atm ≈ 240 °C) can then be removed by rotary evaporation to yield the pure organic compounds. Note that at high concentrations of TBAA (1 M), water and PC phases become miscible and no phase separation can be observed. Similar, at low PC:water ratios (lower than 20:80, see DOI: 10.1021/je00028a012), PC and water mix and form a one-phase system. However, any organic compound previously dissolved in PC will precipitate; the precipitate can be further washed with cold water to remove remaining TBAA (and other salts). PC is also not miscible with hexane; therefore, hexane can be used to directly extract the organic compounds from PC if applicable (see *e.g.* DOI: 10.1021/cr900393d).

Evaluation procedures for NACFE images

The following procedures are mostly an implementation and extension of the concepts and programs in our previous work: *Anal. Chem.* **2018**, *90*, 9504−9509, DOI: **10.1021/acs.analchem.8b02186**. The basic idea is to represent molecular stream separation by a single plot, called angulagram. In an angulagram, every stream is represented by a single peak. Peak properties, such as position and shape, contain all information required to calculate quantitative stream characteristics: stream deflection, stream width, and stream linearity. The source code of all programs described here can be found in the Supplementary Files on Github (DOI: **10.5281/zenodo.2592588**) and ChemRxiv (DOI: **10.26434/chemrxiv.7840937**).

Constructing angulagrams from reflectometric images. The general procedure of angulagram construction is the same as the one used for fluorescence images in the previous work, namely i) aligning the image (rotation/mirroring), ii) cropping the region of interest (separation zone), iii) transferring the data from Cartesian to polar coordinates, and iv) integrating the signal over the radius. In contrast to fluorescence images, a reflectometric input image naturally includes a high background, which can be filtered out by lowering the color saturation and extracting only the hue levels of interest. The respective parameters were manually determined in Photoshop using the ruler tool (rotation angle, inlet position, and separation zone size) and adjustments tools (Image → Adjustments → Hue/saturation; Image → Adjustments → Levels); these parameters were then fed into a Python program (**angureflexin.py**) that performed the construction (see source code for details). The generated output consisted of an angulagram, a parameter file that listed all used parameters, an image of the separation zone in polar coordinates, and a preview image that was used as thumbnail for the angulagrams in this work.

Extracting stream parameters form angulagrams. Another Python script (**evolutin.py**) was used to find the stream peaks in the angulagrams and determine their parameters (deflection, width, linearity, and resolution) as described in our previous work. See the source code for details.

Evaluation of the 10-h separation. Angulagrams for all 3587 images (taken every 10 s) were created using batch processing by a Python script (**angulagrams10h.py**, processing time: 8.5 h) using the Python script (**angureflexin.py**) and the method described above. Parameters (rotation angle, color saturation, hue levels, etc.) were pre-determined for a set of 38 images across the whole separation (see **sampleparameters.csv**). They were found to be similar for these 38 images; therefore, their averages were used for the whole set of 3587 images. The resulting angulagrams were subsequently evaluated and stream parameters were extracted (**streameval10h.py** and **streampara10h.py**, processing times: about 45 min each) using the Python script (**evolutin.py**) and the method described above. Parameters needed for evaluation (background, window size for extrema finding, etc.) were pre-determined for a sample set. Before extracting stream parameters from the angulagrams, angulagrams were smoothened by a Savitzky-Golay filter (window size = 31, polynomial order = 3) to ensure the stability of the used numerical methods for finding minima/maxima etc. Finally, an integrated image based on all 3587 images was calculated (**integrate10h.py**) and presented in Figure 4d. A video files was generated by reducing the resolution of the images and encoding them with the XVID codec (**makevideo10h.py**).

Electrical current measurements. An EPS 3501 XL Electrophoresis Power Supply was used to set the electrophoresis voltage and measure the current. However, this device has no direct output (RS232 or the like) to sample the current data. Therefore, we used a camera to observe the display and then used an optical character recognition (OCR) approach to extract the current values. This was implemented as another Python script (**extract_currents.py**) using OpenCV (Open Source Computer Vision Library, https://opencv.org/) and Google's Tesseract engine (https://github.com/tesseract-ocr/tesseract). See source code for details. A video files was generated by reducing the resolution of the images and encoding them with the XVID codec (**makevideo10h.py**).