STEADY-STATE CONTINUOUS PURIFICATION BY FREE FLOW ELECTROPHORESIS

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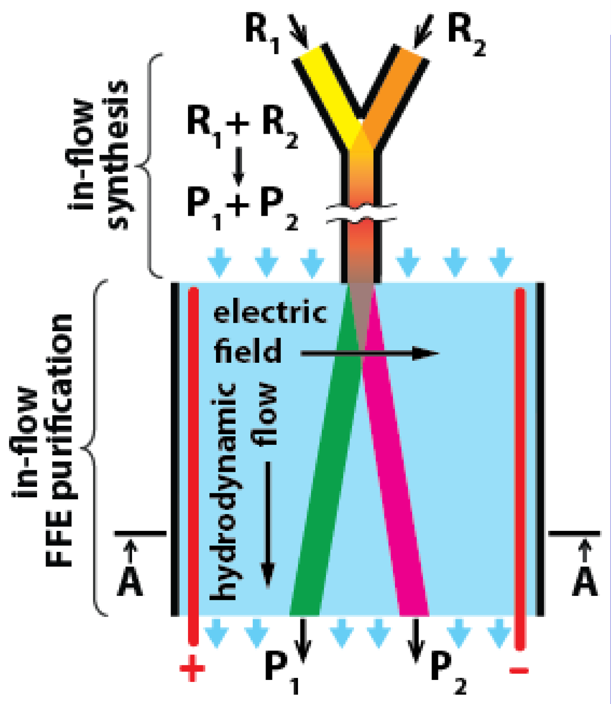
I. Introduction

Continuous microsynthesis has a number of important advantages over batch synthesis. To fully exploit these advantages, continuous-flow microsynthesis should be followed by continuous-flow purification in a compatible scale. Such a combination has not yet been practically realized due to a lack of a suitable purification technique. Our recent research efforts have been motivated by understanding that an existing continuous-flow purification technique, free flow electrophoresis (FFE), is naturally suited for combination with continuous-flow microsynthesis in aqueous solution.

FFE facilitates continuous separation of molecules in a wide separation channel with a uniform hydrodynamic flow of an electrolyte solution and an electrical field non-parallel (typically orthogonal) to this flow. The electric field causes the components of a sample to resolve based on their size to charge ratios. Advantageously, FFE devices can be made in a small scale to suit small flow rates used in continuous-flow microsynthesis.

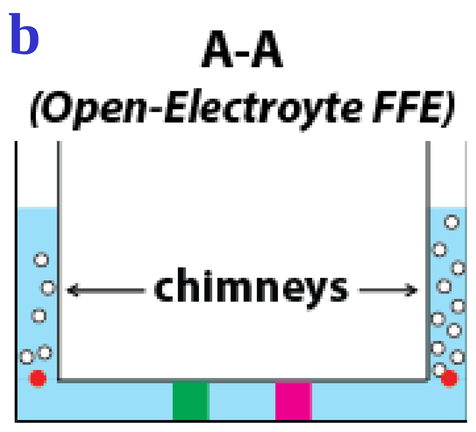


Unfortunately, small-scale FFE cannot be used for steady-state purification. Electrolysis of water leads to the formation of O_2 and H_2 bubbles on the surface of the electrodes. Bubble accumulation on the electrodes and subsequently in other parts of the device leads to progressing electric field distortion and diminishing quality of purification within the first several minutes of operation. The goal of this work was to find an ultimate solution for the problem of FFE instability caused by bubble accumulation, thereby permitting reliable steady-state operation without the distortion of electric field or separation quality. Solving the bubble-accumulation problem is pivotal to FFE integration with other micro-systems.

II. Open Electrolyte FFE

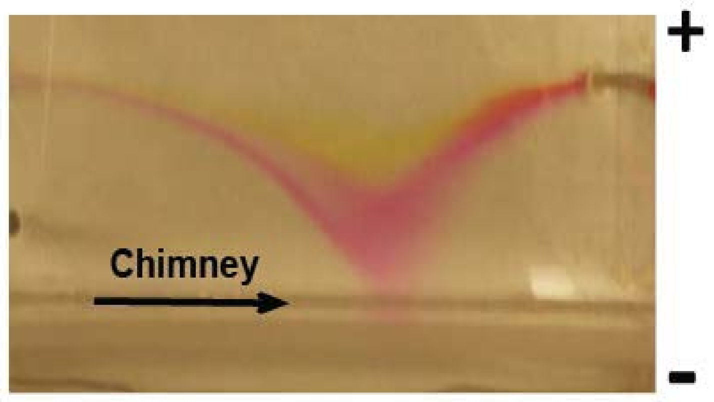
a

b



An ultimate solution to the bubble-accumulation problem could be achieved by breaking a paradigm of a closed FFE device. Bubble removal into the atmosphere could be easy and natural if the electrolyte above the electrodes was open to the atmosphere. Further, engineering the "open-concept" FFE device requires vertical chimneys to hold a column of electrolyte that hydrostatically balances the pressure inside the device. We term this approach Open-Electrolyte FFE (OEFFE). The left figure schematically illustrates the differences between the classical planar FFE device (a) and an OEFFE device with chimneys (b).

III. Initial Problem

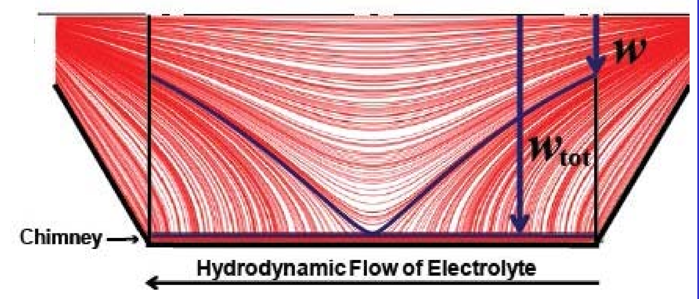


Despite the apparent simplicity of OEFFE, all of our initial attempts to construct a functional OEFFE device had failed. The flow through the separation channel was not uniform and diverted from the separation channel into the chimneys.

IV. *In-Silico* Optimizing

1.

COMSOL Multi-Physics Simulation: 3-D modeling of flow trajectories; shown on right is a simulation of the initial problem (above).

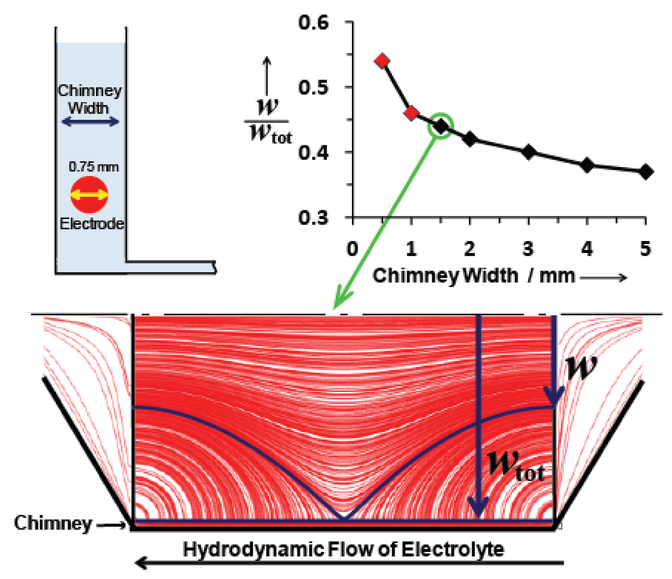


2.

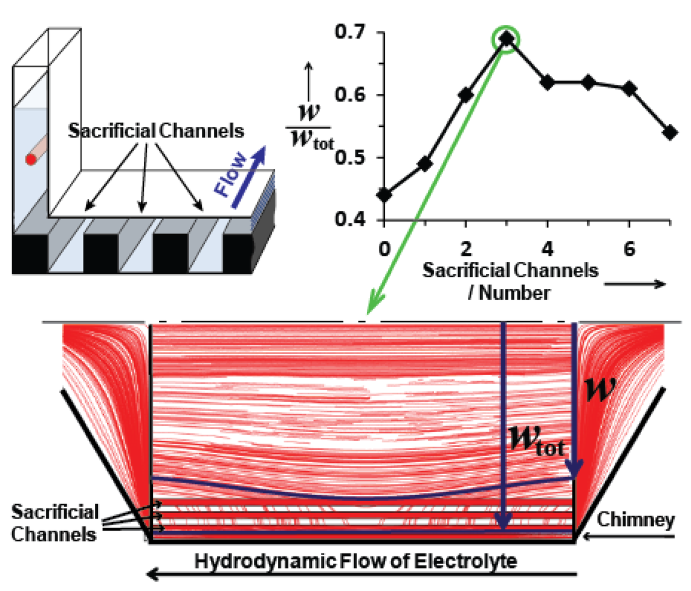
Designing and Optimization Parameter w/w_{tot}: w is the width of the part of the separation channel entry gate which incorporates only streamlines that do not leave the main part of the separation channel and w_{tot} is the total width of the entry gate that does not include chimneys. The value of w/w_{tot} in the above example is 0.26.

3.

Optimizing chimney width to maximize w/w_{tot}.

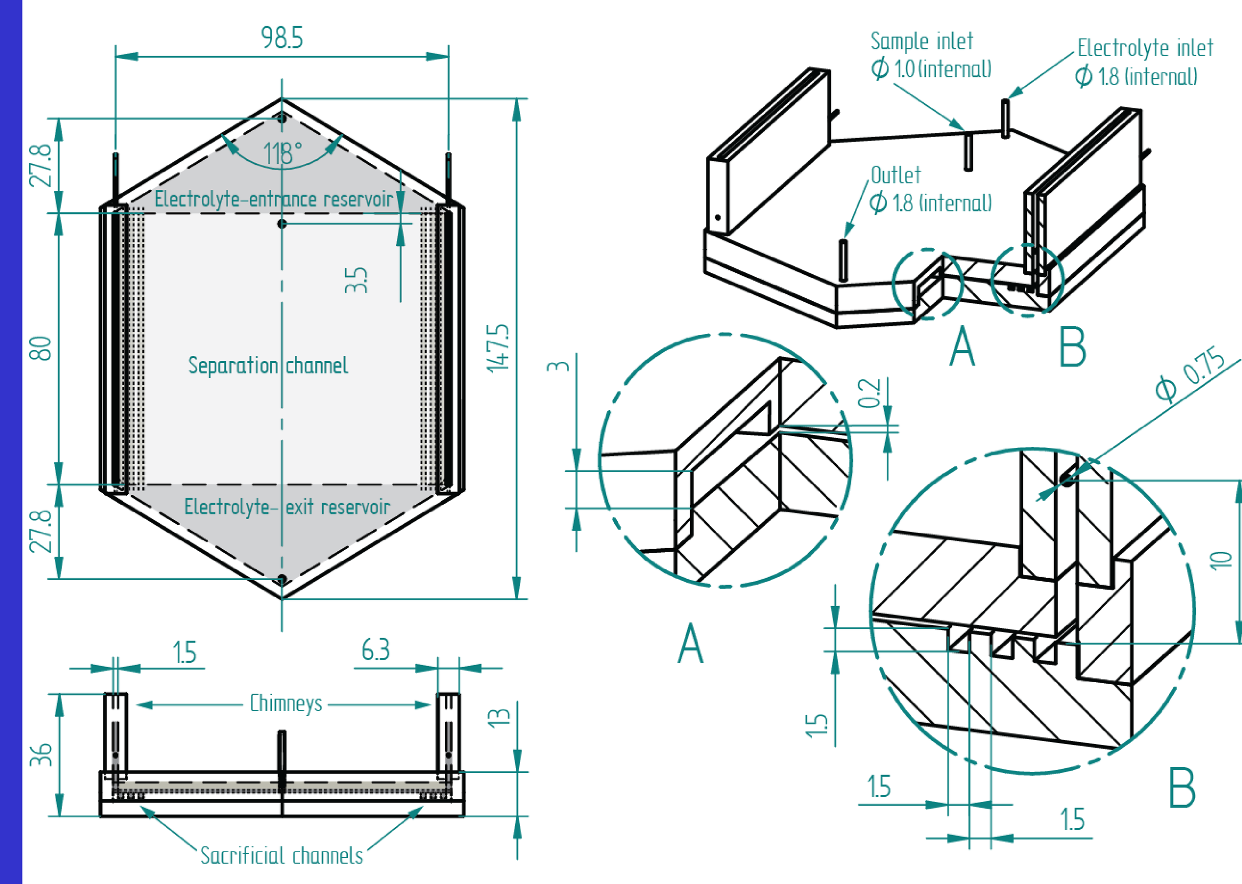


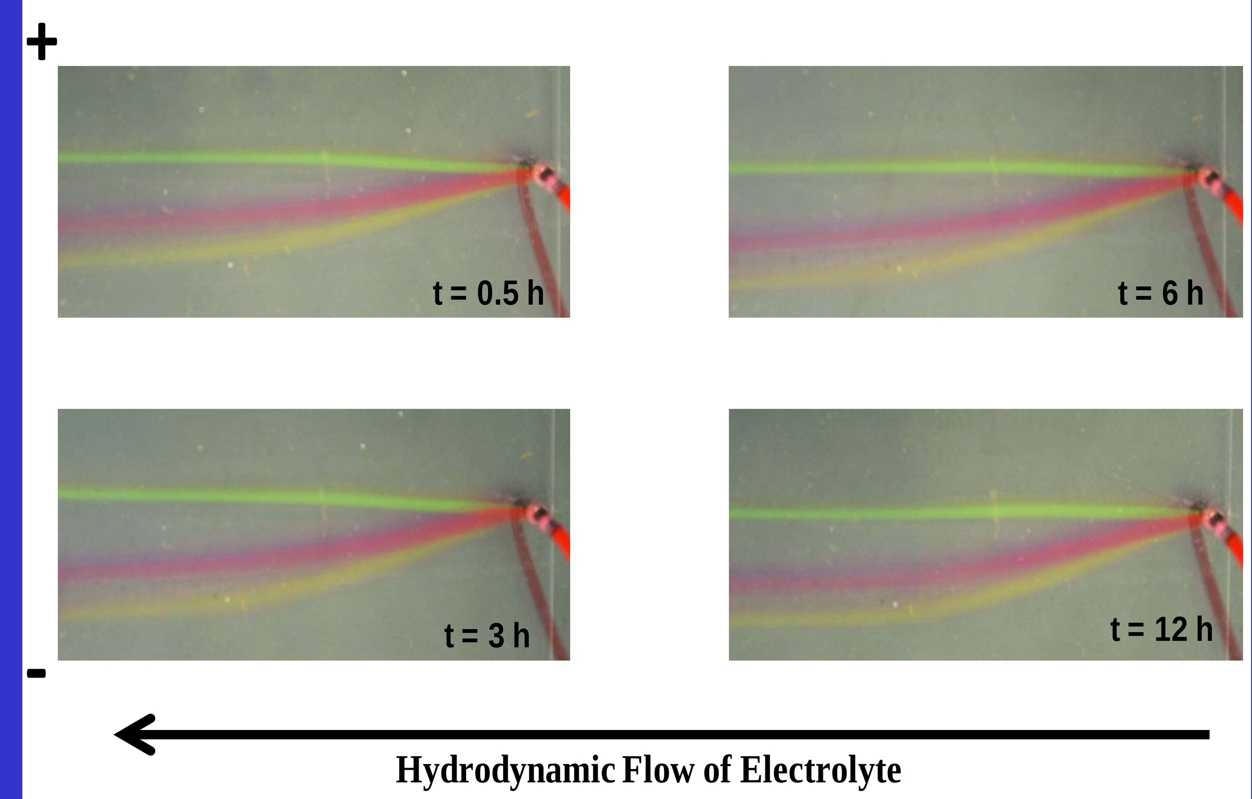
4.



Sacrificial Channels: narrow and deep channels improve flow uniformity. A maximum value of w/w_{tot} = 0.69 was reached when 3 sacrificial channels were introduced.

V. Prototype and Results





Steady-state continuous separation of 250 mM fluorescein (green), 250 mM rhodamine B (pink), and 250 mM rhodamine 6G (yellow) by OEFFE. An electric field of 50.0 V/cm was applied across the chip for a 12-h period. The hydrodynamic flow rate of the electrolyte was 5.00 ± 0.5 mL/min. A mixture of the three dyes was introduced at a flow rate of 4.00 ± 0.01 µL/min. Current reading was stable at 25 ± 1 mA. Removing the bubbles from the OEFFE device prevented electric field distortion and supported its steady-state separation with constant quality of separation.

VI. Conclusion

OEFFE ultimately solves the problem of the deterioration of separation quality over time by preventing the accumulation of bubbles in the device. With the assistance of COMSOL simulations, we found the appropriate geometries of the chimneys and sacrificial channels to achieve acceptable flow uniformity. We have successfully demonstrated steady-state small-scale continuous separation. Solving the bubble-accumulation problem is pivotal to FFE integration with other micro-systems.

VII. Reference

Agostino, F. J., Cherney, L., Galievsky, V., Krylov, S. N. *Submitted to Angew. Chem. Int. Ed.*