A Joint Conference
57th ICASS
57th International Conference on Analytical Sciences and Spectroscopy
August 29-31, 2011 Toronto, Ontario

Milli-free flow electrophoresis: I. Fast prototyping of mFFE devices

CCACC 2011
3rd Canada-China Analytical Chemistry Conference
第三届中-加分析化学会议
August 29-31, 2011
Toronto, Ontario

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redefine THE POSSIBLE.

I. Abstract

We coin a term of **milli-free flow electrophoresis (mFFE)** to describe mid-scale FFE with flow rates intermediate to macro-FFE and micro-FFE (μFFE). We view mFFE as a viable purification complement to continuous synthesis in capillary reactors with product flow rates of 5 to 2000 μL/min, too small for macro-FFE but too large for μFFE. The development of the tandem of continuous synthesis/purification will require the production and evaluation of a large number of prototypes of mFFE devices. As the first step, we developed a fast (~24 h) and economical ($10) method for prototyping mFFE devices using a robotic milling machine. mFFE prototypes are constructed from two machined matching poly(methyl methacrylate) (PMMA) substrates, which are bonded in 10 min using dichloromethane to provide a strong and irreversible seal. Using the developed prototyping technology, we designed and evaluated 25 prototypes of mFFE devices. By optimizing the feed rates and rotational speeds of the drills, the depth of the electrode channels, and the sample flow rate, we were able to achieve indefinitely long operation of the device with cycles of alternating 15 min electrophoresis and 0.5 min regeneration (bubble removal). The test analytes, rhodamine B and fluorescein, were baseline resolved by mFFE for flow rates ranging from 10 to 600 μL/min. These results prove that our prototyping approach is suitable for the challenging task of multi-parameter optimization of mFFE devices.

II. Experimental

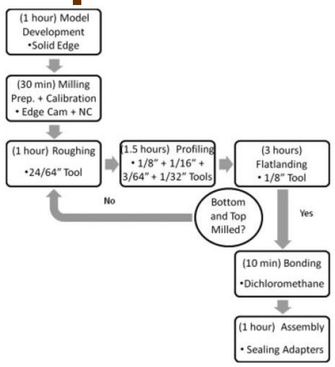
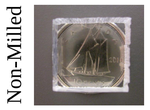


Figure1: General milling procedure for **mFFE devices**

III. Results

A. Optimization of PMMA Transparency and Clarity



LEGEND	Tool Diameter (mm)	Feedrate (mm/min)	RPM
A	0.8	800	10^4
B	0.8	150	6×10^3
C	3.2	800	10^4
D	3.2	200	3×10^3

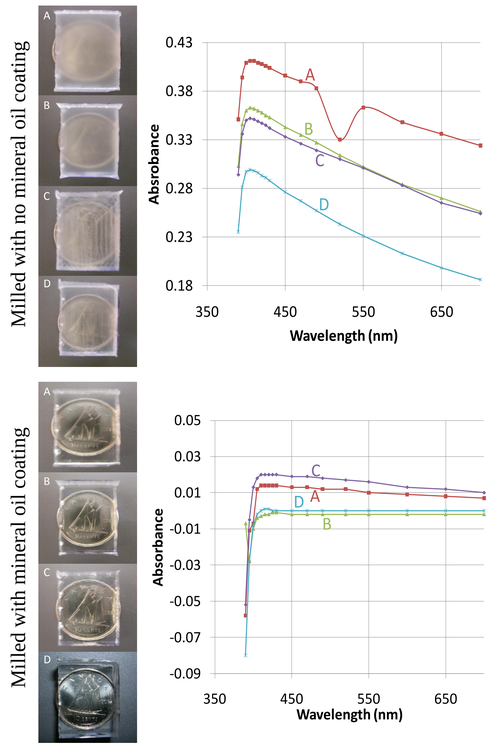


Figure 2: Absorbance spectra of milled PMMA relative to a non-milled piece

B. Optimization of Bubble Removal

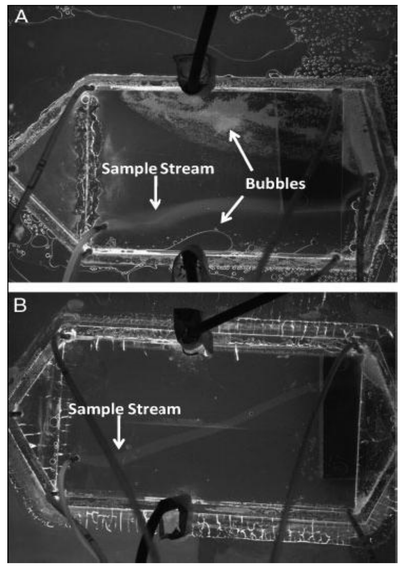


Figure 3: Optimization of bubble removal. Electrode channels that are deeper than the separation channel (200 μm) permit higher flow rates which limit their interference with the electric field. The electrode channels are 1.00 mm and 1.15 mm in **A** and **B** respectively. The electric field strength here was 800 V/cm.

C. Resolution of Fluorescein and Rhodamine B

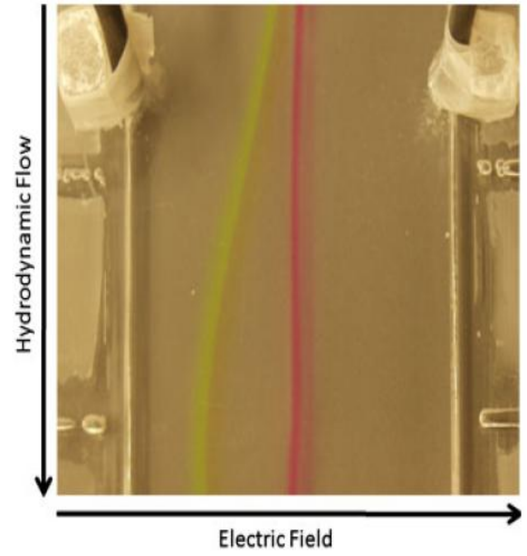


Figure 4: Continuous separation of 500 μM rhodamine B from 500 μM fluorescein. A resolution of 9.5 was achieved by applying a separation voltage of 400 V (E = 67 V/cm). The hydrodynamic flow rate was 0.66 ± 0.02 mL/min, and the sample flow rate was 10 μL/min. The buffer composition was 25 mM HEPES and 300 mM Triton X-100 at pH 7.00.

IV. Conclusions

Our work will reinforce the area with a tool for fast prototyping of mFFE devices. The fabrication and optimization of a fully functional ‘‘milli’’-free-flow electrophoresis device have been demonstrated. Fluorescein was resolved from rhodamine B at 400 V (67 V/cm), R_s = 9.5. A milling machine was used to manufacture the device in less than 24 h. The sample flow rates administered in the ‘‘milli’’-chip can be compatible with most continuous flow microreactors. mFFE would serve as a complementary means of continuous purification. As a method of prototyping, milling offers an inexpensive and time-efficient alternative to modern microfabrication techniques based on photolithography, hot embossing or laser ablation, whenever larger devices are involved. By following the protocols described in this article, practitioners will be able to design, manufacture and test devices in a much shorter time frame than previously experienced. In the near future we plan on introducing new ways of eliminating the current bubble problem associated with microfluidic systems that require the integration of an electric field. Doing this will indefinitely increase the operational longevity, and open up the potential for various mFFE applications.

V. Reference

Agostino, Fletcher J., Evenhuis, Christopher J, Krylov, Sergey, N. *J. Sep. Sci.* 2011, *34*, 556-564