Microchip Nonaqueous Capillary Electrophoresis of Saturated Fatty Acids Using a New Fluorescent Dye

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Supporting Information

Materials

Methanoic (formic, C1) acid, ethanoic (acetic, C2) acid, butanoic (butyric, C4) acid, hexanoic (caproic, C6) acid, octanoic (caprylic, C8) acid, decanoic (capric, C10) acid, dodecanoic (lauric, C12) acid, tetradecanoic (myristic, C14) acid, hexadecanoic (palmitic, C16) acid, octadecanoic (stearic, C18) acid, eicosanoic (arachidic, C20) acid, docosanoic (behenic, C22) acid, tetracosanoic (lignoceric, C24) acid, hexacosanoic (cerotic, C26) acid, octacosanoic (montanic, C28) acid and triacontanoic (melissic, C30) acid were purchased from Sigma Aldrich. All acids were 98% purity or greater, with the exceptions of methanoic acid (≥96%) and hexacosanoic acid (≥95%). Ethyl alcohol (99.5%, ACS reagent, absolute) was purchased from Acros Organics. Ammonium acetate (99.99%), N,N-diisopropylethylamine (DIEA, 99.5%) and hydrochloric acid (ACS standard, 2.0 N in water) were purchased from Sigma-Aldrich. Acetic acid (glacial, 17.4 N, 99.9%), dimethylsulfoxide (DMSO, ACS reagent grade) and N,N-dimethylformamide (DMF, ACS reagent grade) were purchased from Fisher Scientific. Hexanes (ACS Reagent Grade) were purchased from J.T. Baker. Butyl alcohol (n-butanol, ≥99.7%) was purchased from EMD Millipore Chemicals. Potassium hydroxide was purchased from J. T. Baker. O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) was purchased from EMD Millipore Chemicals and stored at 4 °C. 7-Diethylaminocoumarin-3-carboxylic acid hydrazide (DCCH) was purchased from Invitrogen (Life Technologies Corp.) and stored at -20 °C as a 20 mM solution in DMF. All reagents were used without further purification. All solutions were filtered prior to use with disposable 0.22-µm nylon membrane syringe filters (Fisherbrand).

Pacific Blue hydrazide (PB), a novel dye, was custom synthesized by Invitrogen and stored at -20 °C as a 20 mM solution in DMF. To our knowledge, this is the first use of the Pacific Blue dye with hydrazide functionalization to target carboxylic acids.

µNACE protocol optimization

Initially, a commercially available dye was investigated for this work. DCCH has similar excitation and emission properties to Pacific Blue, a dye used previously in our laboratory. Though labeling was performed successfully using DCCH in basic conditions in DMF, separations were poor (Fig. S1), even when the field strength was increased to the maximum possible with our system, 1.7 $kV \cdot cm^{-1}$. We believe this is due to the ternary amine functionality on the DCCH dye, which is positively charged in conditions of low effective pH. This attempt highlights the importance of pH and charge in obtaining effective electrophoretic separations on microfluidic devices.

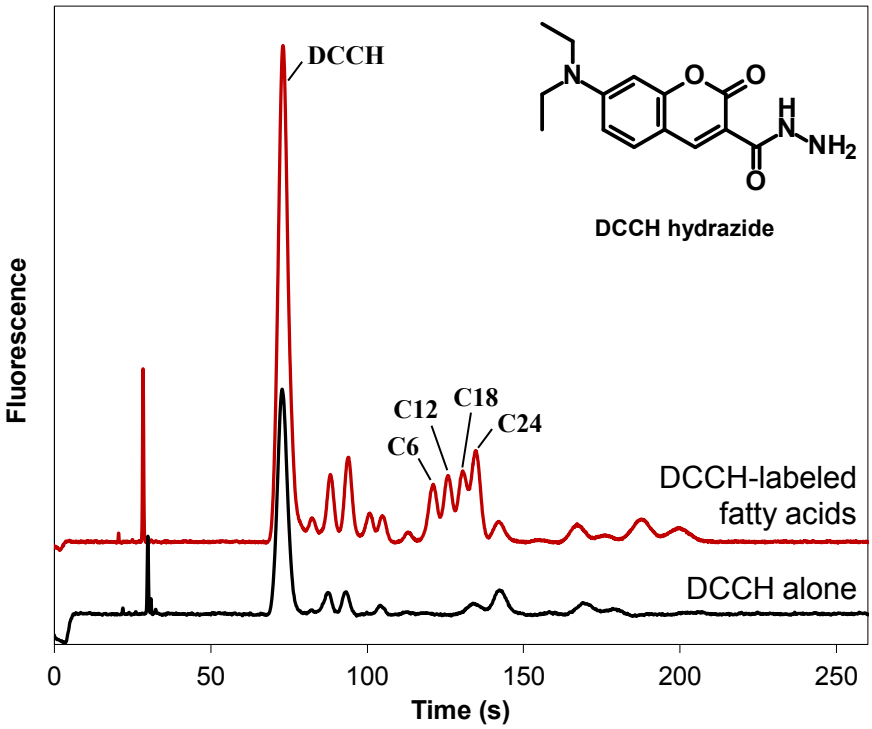

Fig. S1. Separation of four fatty acids labeled with DCCH (see structure, upper right). Though labeling was successful, resolution of fatty acids was poor due to the ternary amine on the DCCH which is positively charged in conditions of low effective pH. Separations performed in 400 mM ammonium acetate with 700 mM acetic acid in ethanol, using shorter chips (Micronit X3550, 35 mm separation channel) with reversed potentials (sample well positive, waste well negative).

We then turned to the synthetic route, and worked with Life Technologies to synthesize a Pacific Blue dye with a hydrazide functional group to couple to carboxylic acids. Previous work in our laboratory has resulted in effective separations of primary amines up to C18, and the labeled product of the Pacific Blue hydrazide should be similar enough to the labeled primary amine that separations can be performed in the same conditions.

Labeling was performed in DMF, as ethanol shuts down the HCTU coupling agent (data not shown). Further, labeling was found to be effective only at concentrations over 1 mM for the Pacific Blue hydrazide dye, and 2 mM for HCTU (Fig. S2).

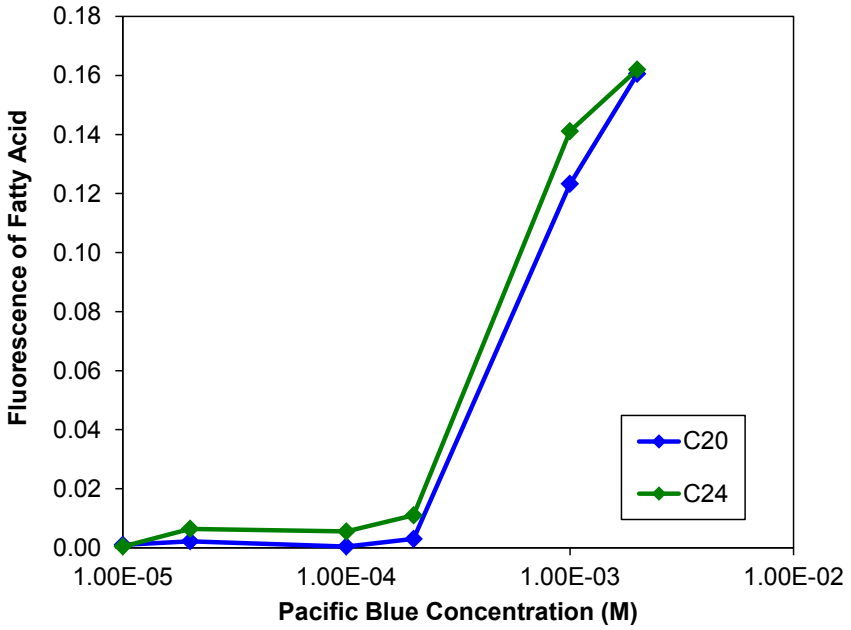

Fig. S2. Labeling optimization in DMF. Variation of PB dye concentration in labeling of C20 and C24 acids. Below 1 mM, the acids are no longer labeled. Concentration of acids to dye was maintained at 1:2, with 2 mM HCTU and 50 mM DIEA. Separations in 100 mM ammonium acetate and 1 M acetic acid in ethanol.

Following labeling optimization, separations were optimized by changing buffer concentrations (ammonium acetate and acetic acid) to maximize both resolution between fatty acids and between fatty acids and Pacific Blue contamination peaks (Fig. S3). Ionic strength was maintained at 1.1 M.

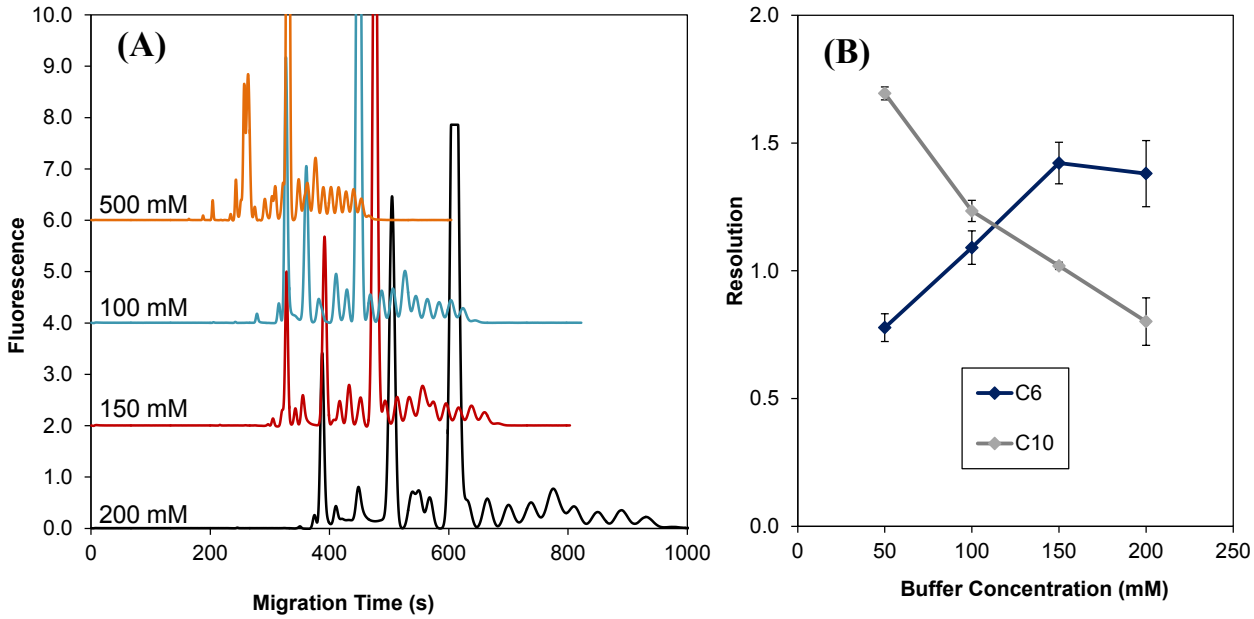

Fig. S3. Separation optimization. (A) Electropherograms of 2.5 µM C2-C30 fatty acids. (B) At 100 mM buffer (ammonium acetate) concentration, the resolution of hexanoic (C6) and decanoic (C10) acids with the Pacific Blue hydrolysis peak (largest peak in the electropherogram) is maximized.

Fatty acid extraction from Snake Pit hydrothermal vent

A 2.0112 g aliquot of sediment was extracted with 2.00 mL of 1:2.5 hexane/ethanol (vol/vol) using sonication (10 min). Saponification was effected with 0.1 g of potassium hydroxide and heat (60 °C) for 1 hr. A 700 µL aliquot of 2.0 N HCl was added to acidify the fatty acid salts and liberate the free fatty acids, which were dried and extracted into 1.00 mL of n- butanol. The solution was then decanted and filtered using a 0.2 µm nylon syringe filter. The filtrate was evaporated to dryness (150 °C for 1 hr, 70 °C for 1 hr) and resolubilized in 100 µL of DMF containing 1 mM PB-NH2 and 2 mM HCTU with 50 mM diisopropylethylamine (DIEA). After 2 hrs, the solution was diluted 1:4 into separation buffer (100 mM ammonium acetate and 1.0 M acetic acid in ethanol) and analyzed using µNACE-LIF.

Resolution and theoretical plates

Data fitting was performed with PeakFit (Seasolve Software, Inc., v4.12). Peak efficiencies were calculated using Nmoment and a separation distance of 75 mm (Table S1).

Table S1. Peak efficiencies of fatty acids (2.5 µM C2-C26 acids, 5 µM C28, C30 acids) separated in 100 mM ammonium acetate with 1.0 M acetic acid in ethanol.

Fatty Acid	Plates/m
C2	178,643
C4	211,039
C6	218,335
C10	246,578
C12	232,156
C14	177,886
C16	152,235
C18	248,531
C20	278,081
C22	244,206
C24	206,753
C26	218,036
C28	233,643
C30	349,664

Table S2. Resolution of fatty acid peaks (2.5 μM C10-C26 acids, 5 μM C28, C30 acids) separated in 100 mM ammonium acetate with 1.0 M acetic acid in ethanol. With the exception of C14 and C16, the resolution of all fatty acids differing by 2 methyl units is above 1.

Fatty Acids	Resolution
C10-C12	1.14 ± 0.03
C12-C14	0.84 ± 0.01
C14-C16	0.67 ± 0.03
C16-C18	1.00 ± 0.03
C18-C20	1.20 ± 0.08
C20-C22	1.18 ± 0.13
C22-C24	1.08 ± 0.11
C24-C26	1.03 ± 0.09
C26-C28	1.04 ± 0.08
C28-C30	1.11 ± 0.08