

**A NOVEL PROTOCOL TO ANALYZE SHORT- AND LONG-CHAIN FATTY ACIDS USING NONAQUEOUS MICROCHIP CAPILLARY ELECTROPHORESIS.** M. L. Cable<sup>1</sup>, A. M. Stockton<sup>1</sup>, Maria F. Mora<sup>1</sup> and P. A. Willis<sup>1</sup>, <sup>1</sup>Instrument Electronics and Sensors Section, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109.

**Introduction:** Fatty acids, amphiphilic molecules consisting of an aliphatic chain terminated by a carboxylic acid (-COOH) group, are found in the cell membranes of all three kingdoms of life on Earth [1]. Due to the fact that fatty acids have a polar head group and a nonpolar tail, they are capable of self-assembly into higher-level structures including micelles, monolayers and bilayers.

Most naturally occurring fatty acids have an even number of carbon atoms and range in size from acetic acid (C2) to octacosanoic acid (C28). Because fatty acids are ubiquitous to life as we know it and can survive for extended periods in the environment, they can be used as biomarkers and are often employed in measuring biomass [2]. For example, a sample containing 15-methylpalmitic acid and 14-methylpalmitic acid is likely to have significant populations of gram positive bacteria, while one high in hexadecenoic acid and linoleic acid is indicative of fungi [3].

Fatty acids have been found in cretaceous sedimentary rocks and meteorites, including the Orgueil meteorite [4] and the Murchison meteorite [5]. A sensitive method to detect both short- and long-chain fatty acids could ascertain whether these organics are abiotic or the remnants of ancient cell membranes. Such a method could also be utilized to search for evidence of life on Mars, Enceladus or Europa.

Microchip capillary electrophoresis ( $\mu$ CE) is a liquid-based separation technique that operates with high performance, reagent economy and speed [6]. When coupled to laser-induced fluorescence (LIF) detection, separated species can be identified and quantified with incredible sensitivity (nM to pM) on a platform that is easily automated for remote in situ analysis [7].

Short-chain (C1-C8) fatty acids have been detected with  $\mu$ CE-LIF using the Mars Organic Analyzer Microchip Capillary Electrophoresis System in aqueous conditions [8]. However, compounds with chain lengths longer than C12 are not soluble in aqueous solution [9]. As a result, nonaqueous solvents have been explored to detect long-chain fatty acids (up to C31) in several nonaqueous capillary electrophoresis (NACE) systems, including methanol [10, 11], ethanol, DMSO, DMF, THF, acetone [12], and mixtures such as methanol/water [13], acetonitrile/isopropanol [14], acetonitrile/water [15] and N-methylformamide/dioxane [16-18]. Though most of these reported NACE techniques describe separations with excellent resolution,

the use of a capillary instead of a microfluidic chip, in addition to the complex buffer and solvent ratios, makes adaptation of these techniques for automated in situ analysis challenging.

We propose a new protocol to identify and quantify both short- and long-chain saturated fatty acids in samples of astrobiological interest using nonaqueous microchip capillary electrophoresis ( $\mu$ NACE) with laser-induced fluorescence (LIF).

**Labeling Reaction:** We will use a newly derivatized version of 3-carboxy-6,8-difluoro-7-hydroxycoumarin (Pacific Blue™) specifically synthesized for this work (Figure 1A). The fatty acid is activated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in acidic conditions to form an ester (Figure 1B); the active ester then reacts with Pacific Blue hydrazide in a one-pot synthesis to form the hydrazide amide (PB-labeled acid).

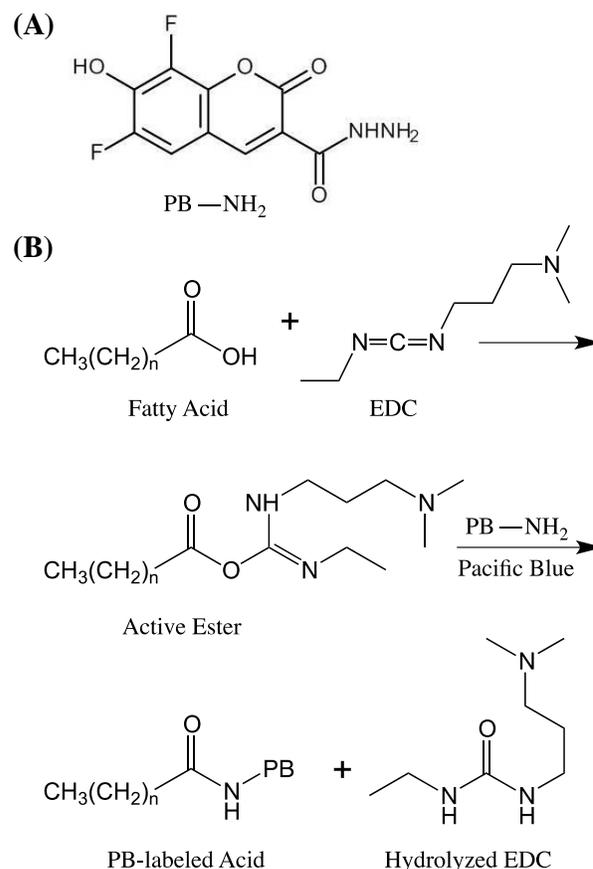


Figure 1. (A) Pacific Blue hydrazide. (B) Reaction to generate PB-labeled fatty acid.

**Proof of Concept:** We have recently reported quantitative compositional analysis of short- and long-chain primary amines using  $\mu$ NACE in ethanol [19]. Amines of length C1 to C18 were identified and quantified, with limits of detection ranging from 1.0-2.6 nm (Figure 2). Labeling was effected with Pacific Blue succinimidyl ester, and resulted in PB-labeled amines that are nearly identical in structure to the PB-labeled fatty acids. As a result, the same solvent and buffer conditions (50 mM ammonium acetate, 1.05 M acetic acid) can be employed in this work.

The use of Pacific Blue to detect both primary amines and carboxylic acids has several advantages. First, this enables multiple analyte detection capability without the need to add a second excitation source, keeping the instrument package small. Second, maintaining the same buffer effective pH and concentration for multiple analyte detection protocols allows for higher throughput with minimal microchannel rinsing and conditioning steps.

**Applications:** A nonaqueous microfluidic protocol to analyze fatty acid content in a rapid, automated fashion will enable biomass classification with high sensitivity of environmental samples in the field, in addition to enabling ultrasensitive detection of cell membrane constituents on bodies of astrobiological interest (Mars, Europa, Enceladus). This technique is also capable of handling highly complex organic samples such as Titan tholins (Figure 3), aerosols generated under simulated Titan atmospheric conditions [19]. This new protocol could therefore be used to search for oxygen incorporation in Titan organic material in situ, which could yield evidence of active surface chemistry on this moon [20].

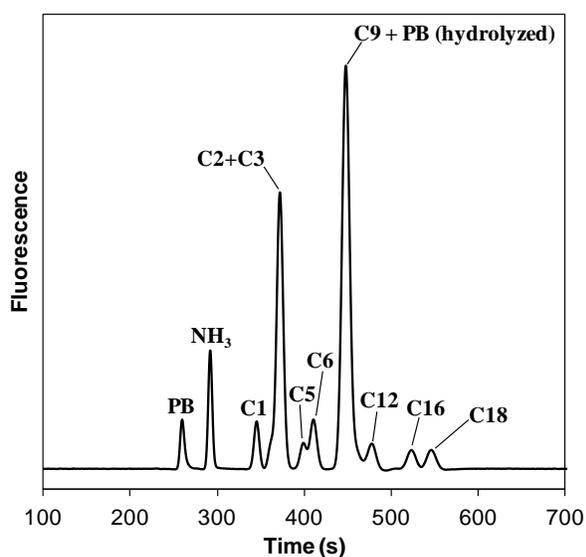


Figure 2. Separation of C1-C18 PB-labeled primary amines using  $\mu$ NACE in ethanol.

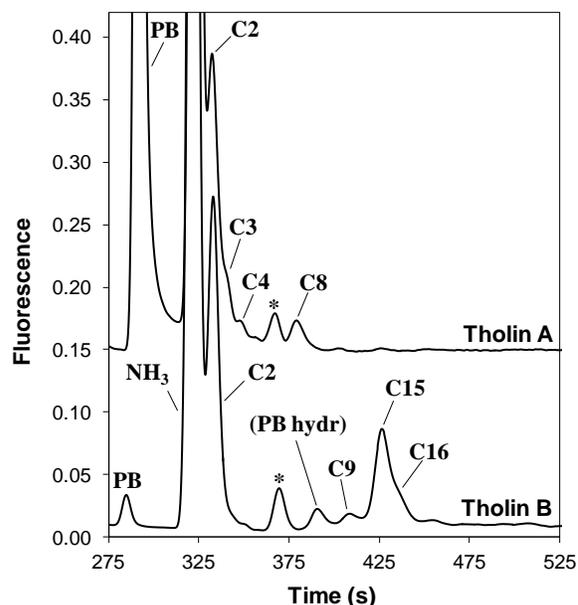


Figure 3.  $\mu$ NACE of two tholin samples, showing different primary amines in each sample.

**References:** [1] Hoover R. B. et al. (2005) in *Perspectives in Astrobiology, Series I: Life and Behavioural Sciences*, eds. Rozanov A. Y. and Paeppe R., IOS Press, 43-65. [2] Buyer J. S. and Sasser M. (2012) *Appl. Soil Ecol.*, *61*, 127-130. [3] Zelles L. et al. (1995) *Biol. Fert. Soils*, *19*, 115-123. [4] Nagy B. and Bitz M. C. (1963) *Arch. Biochem. Biophys.*, *101*, 240-248. [5] Botta O. and Bada J. L. (2002) *Surv. Geophys.*, *23*, 411-467. [6] Landers J. P. (2008) CRC Press, Taylor & Francis Group. [7] Mora M. F. et al. (2012) *Electrophoresis*, *33*, 2624-2638. [8] Stockton A. M. et al. (2011) *Astrobiology*, *11*, 519-528. [9] Otieno A. C. and Mwangela S. M. (2008) *Anal. Chim. Acta*, *624*, 163-174. [10] Gallaher D. L. and Johnson M. E. (2000) *Anal. Chem.*, *72*, 2080-2086. [11] Salimi-Moosavi H. and Cassidy R. M. (1996) *Anal. Chem.*, *68*, 293-299. [12] Breadmore M. C. et al. (2007) *Electrophoresis*, *28*, 1252-1258. [13] Roldan-Assad R. and Gareil P. (1995) *J. Chromatogr. A*, *708*, 339-350. [14] Vergara-Barberán M. et al. (2011) *J. Agr. Food Chem.*, *59*, 10775-10780. [15] Santa T. et al. (2002) *Biomed. Chromatogr.*, *16*, 523-528. [16] Bannore Y. C. et al. (2008) *J. Sep. Sci.*, *31*, 2667-2676. [17] Drange E. and Lundanes E. (1997) *J. Chromatogr. A*, *771*, 301-309. [18] Haddadian F. et al. (1999) *J. Chromatogr. Sci.*, *37*, 103-107. [19] Cable M. L. et al. (2013) *Anal. Chem.*, in press. [20] Cable M. L. et al. (2012) *Chem. Rev.*, *112*, 1882-1909.