ION-ALTERED-FLUORESCENCE IMAGING (IAFI): A NEW, NON-INVASIVE, VISUALIZATION METHOD WHICH SIMULTANEOUSLY IMAGES SCALAR FIELDS AND QUANTIFIES LOCAL ION CONCENTRATION

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ABSTRACT

We present a novel method for full-field scalar visualization and quantification of ion concentration fields: ion-altered-fluorescence imaging (IAFI). The method employs electrically neutral fluorescent dyes whose quantum yields are selectively quenched or enhanced by anionic and/or cationic species. IAFI enables simultaneous imaging of material interfaces and provides non-invasive, scalar-field quantitation of two-dimensional ion density fields. We here demonstrate this method in the full-field visualization of several challenging electrokinetic flows: isotachophoresis (ITP) in both anionic and cationic modes and in a convective electrokinetic instability (EKI) flow.

KEY WORDS

ion visualization, fluorescence quenching, electrokinetic visualization, full-field imaging

INTRODUCTION

Estimation of ion concentration profiles is needed for qualitative detection and quantification of analytes in many microfluidic experiments, such as liquid chromatographic and electrokinetic assays. Traditionally, temperature, conductivity, electrochemical, and UV absorbance detectors have been used to indirectly estimate ion concentration profiles in electrokinetic microfluidic or capillary systems [1, 2]. However, these typically consist of point detectors and thus do not permit dynamic, full-field visualization of unsteady scalar fields. Standard full-field fluorescence visualization offers high sensitivity and is easy to implement in microfluidic devices [3, 4]. However, such visualization provides information associated with the concentration and quantum yield of the fluorescent tracer itself, and little information concerning the concentration of other, non-fluorescent ions endogenous to the flow and its application. To address this, we propose a novel method we term ion-altered-fluorescence imaging (IAFI) which leverages fluorescence quenching or enhancement intrinsic to ions of interest to provide information concerning the concentration and identity of these ions.

IAFI employs electrically neutral fluorescent dyes whose quantum yield is selectively quenched or enhanced by anionic and/or cationic species. IAFI enables simultaneous imaging of material interfaces (between dyed and un-dyed regions) and provides a non-invasive scalar field quantitation of two-dimensional ion density fields. We here demonstrate this method in the full-field visualization several challenging electrokinetic flows: isotachophoresis (ITP) in both anionic and cationic modes and in a convective electrokinetic instability (EKI) flow. We know of no other method which can non-invasively visualize and quantify ion concentration profiles in such a wide range of flow and electrokinetic phenomena. We provide guidelines for selecting and employing these specialized dyes.

CONCEPT AND THEORY

IAFI employs net electroneutral dyes whose fluorescence is strongly quenched or enhanced by ions and/or neutral species. For electrokinetic experiments we select IAFI dyes according to the following criteria: (a) dye is net neutral at and near the pH of interest; (b) dye does not react, complex, or interfere with species of interest or the surfaces of the chamber; (c) dye has a high initial quantum yield and high solubility in the solvent and at pH of interest; (d) dye's fluorescence is strongly quenched or enhanced by ions of interest. To quantify the ion concentration, we also chose dyes whose fluorescence quenching for analytes of interest follows the Stern-Volmer equation

$$\frac{F_0}{F_i} = 1 + K_{Q,i} c_i, \tag{1}$$

where F_0 is the fluorescence intensity in the absence of quencher, F_i is the fluorescence intensity in the presence of the quenching electrolyte i, $K_{Q,i}$ is the quenching constant of the electrolyte i, and c_i is the concentration of ion of interest in the electrolyte i for which the $K_{Q,i}$ is measured.

EXPERIMENTS

To demonstrate IAFI we performed full-field visualization and quantitation of isotachophoresis (ITP) in both anionic and cationic modes and a convective electrokinetic instability (EKI) flow. To demonstrate quantitation using IAFI, we first calibrated fluorescence quenching of dye 6-methoxy-N-(3-sulfopropyl) quinolinium (SPQ) dye in five buffer systems using the Stern-Volmer equation, as shown in Figure 1. We here show (selective) quenching characteristics specific to five buffers: pyradine-HEPES (pH 6.2), imidazole -HEPES (pH 7.1), tris-HEPES (pH 7.3), amediol-HEPES (pH 7.4), and sodium-HEPES (pH 7.4). The experimental data show an excellent fit to the Stern-Volmer equation from 5 to 80 mM analyte (cationic buffer component) concentrations, indicating that SPQ may be used to provide quantitative, calibrated, ion-specific information in electrokinetic flows.

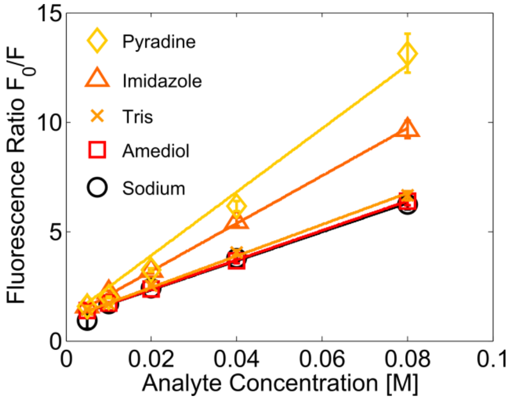

Figure 1. Stern-Volmer plot for quenching of 6-methoxy-N-(3-sulfopropyl) quinolinium (SPQ) by buffers pyradine-HEPES (◊, pH 6.2), imidazole-HEPES(△, pH 7.1), tris-HEPES (x, *pH 7.3), amediol-HEPES (□, pH 7.4), and sodium-HEPES (○, pH 7.4). The regression coefficients* R^2 *are greater than 0.99 for analyte (cationic buffer component) concentrations from 5 to 80 mM, demonstrating the potential for highly accurate and selective quantitation of specific ions using IAFI.*

Next we demonstrate visualization and simultaneous quantiation of ion concentrations in an electrokinetic experiment, cationic isotachophoresis. In Figure 2 we show cationic ITP experiments where SPQ and 10-(3-sulfopropyl)acridinium betaine (SAB) were used to visualize and quantify spatiotemporal development of distinctly five cation zones in ITP. Importantly, SPQ and SAB are each electrically net neutral between pH 0 - 12 and therefore do not interfere with buffer conductivity, buffer pH, or the electrokinetic process (including ITP dynamics). We estimate the cationic component concentration of trailing electrolyte zone using Stern-Volmer quenching constants (Figure 1). The measured concentrations via IAFI agrees to within 10% or better versus predicted concentrations.

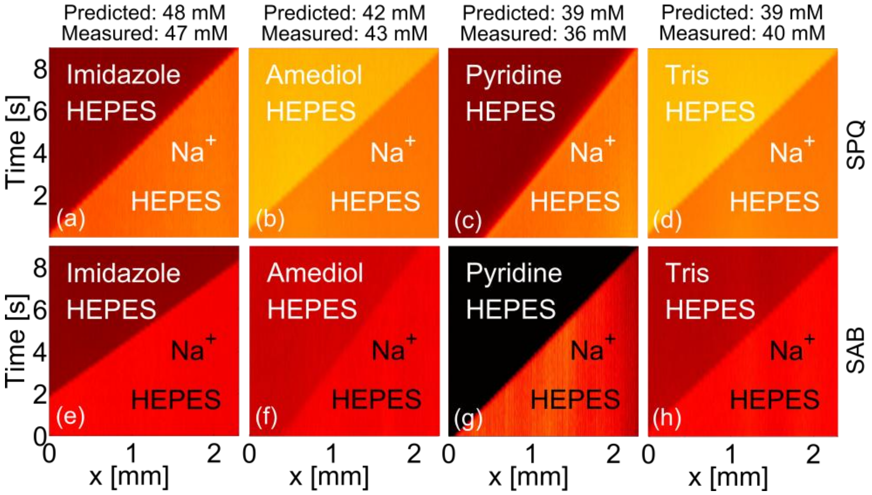

Figure 2. Spatiotemporal plots of eight cationic isotachophoresis (ITP) experiments visualized and quantified with SPQ (a-d) and visualized with 10-(3-sulfopropyl)acridinium betaine (SAB) (e-h) (both 2.5 mM). Each box shows cross-sectional area-averaged intensity of dye fluorescence vs. distance along the channel (horizontal axis, x = 0 to 2.3 mm) and time (vertical axis, 0 to 9 s). The top left region of each plot quantifies the trailing electrolyte (TE) development and the bottom right region the leading electrolyte (LE). Each dye is able to clearly visualize the interface between LE and TE and quantify each of the five analyte concentrations of interest (imidazole, amediol, pyradine, or tris). The measured concentrations of TE ions agree to within 10% or better of predictions for each case.

In Figure 3 we show two anionic ITP experiments where SPQ and SAB were used to simultaneously visualize and quantify spatiotemporal development of distinctly five anion zones in ITP. In Figure 3a we employed 5 mM SPQ to observe 20 mM chloride leading electrolyte (LE), 20 mM nitrate, 16 mM carbonic acid, 13 mM 3,5-dinitrobenzoic acid, and 12 mM HEPES trailing electrolyte (TE) zones in a single ITP experiment. Tris served as the counterion in all zones. In Figure 3b we employed 2 mM SAB to observe 50 mM formic acid (LE), 40 mM nicotinic acid, 32 mM HEPES, and 38 mM L-Asparagine (TE). Tris again served as the counterion in all zones. The zone observed between HEPES and L-Asparagine in Figure 3b is likely an impurity. Each dye clearly visualizes interfaces between individual ion zones in these highly unsteady, non-uniform flows with high gradients.

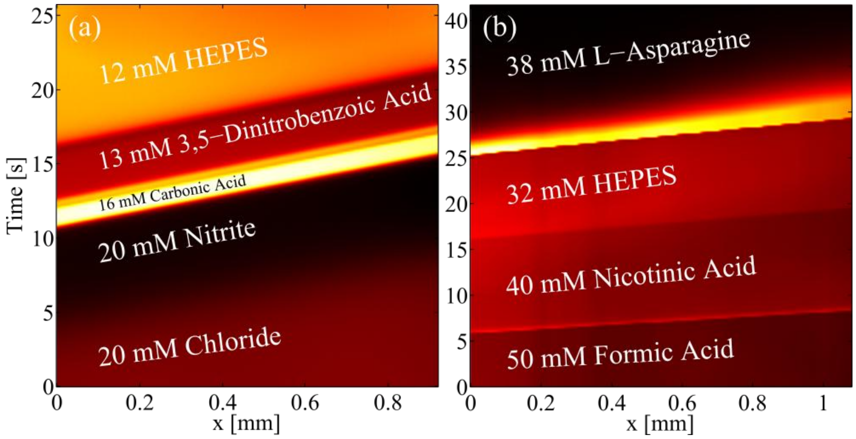

Figure 3. Spatiotemporal plots from IAFI showing development of five ion zones migrating under ITP. Here we use (a) SPQ (5 mM) and (b) SAB (2 mM) IAFI dyes. In (a) IAFI visualizes the spatiotemporal development of five distinct anion zones: chloride (LE), nitrite, carbonic acid, 3,5-dinitrobenzoic acid, and HEPES (TE). The blurred interface between the chloride and nitrite zones likely indicates zone mixing (i.e. a "shared" ITP zone). In (b) IAFI visualizes the spatiotemporal development of formic acid (LE) nicotinic acid, HEPES, and L-asparagine (TE). The zone observed between HEPES and L-Asparagine is likely an impurity.

Lastly, in Figure 5 we show convective EKI experiments [6] visualized and quantified with IAFI. Shown are three images of a highlight unsteady, fully-chaotic flow instability in an electrokinetic flow focusing experiment. IAFI captures both the transverse velocity fluctuations of the secondary flow and quantitatively measures local ion densities as the EKI mixes the center stream with two sheath streams.

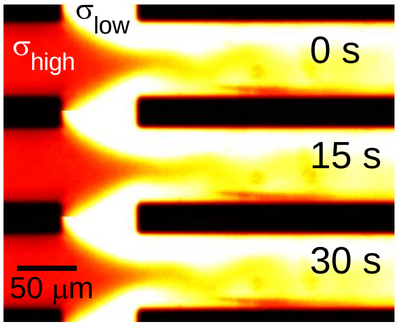

Figure 4. Two dimensional, full-field visualization of a convective electrokinetic instability (EKI) near a cross-type intersection of an electrokinetic chip. Shown are three images taken 15 s apart and visualized via IAFI using SPQ at 4 mM. Low conductivity streams (white, 60 µS/cm, 10 mM HEPES, 300 µM KCl) from top and bottom were mixed with a high conductivity center stream (red (or gray in bw), 4 mS/cm, 10 mM HEPES, 30 mM KCl) and driven by order 400 V/cm electric field. This configuration is well known to result in EKI at high fields [6]. IAFI is able to simultaneously visualize material lines between the two streams and quantify the dynamics of the chloride concentration in the entire field.

ACKNOWLEDGEMENTS

We gratefully acknowledge support from the National Science Foundation for a Graduate Research Fellowship for Viktor Shkolnikov. This work was supported by National Science Foundation grant CBET-0967600-000.

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