FIREFLIES-ON-A-CHIP
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ABSTRACT

We present microfluidic droplet-based ‘fireflies’ for non-invasive chemical and biological sensing applications. These ‘fireflies’ are bi-compartmental aqueous-ionic liquid droplets in which metal (gold) ions from the aqueous compartment partition into the adjacent ionic liquid compartment and catalyze a fluorescence producing reaction. We report detailed measurements of the kinetics of fluorescence emission as a function of metal concentration and droplet flow speed. Finally, we demonstrate complete, passive detachment of the two compartments at a hydrodynamic obstruction.

KEYWORDS: Multiphase microfluidics, Compound droplet, Ionic liquid, Chemical sensing.

INTRODUCTION

A majority of droplet-based microfluidics applications focus on complete chemical isolation of the droplets from the surroundings [1]; however, there is another regime where a droplet is deliberately made to interact with continuous phase or neighboring droplets [2]. We have recently demonstrated a three-phase droplet system in which a chemically active third fluid, an ionic liquid, is introduced along with aqueous and fluorinated oil (FO) phases to generate microfluidic ‘compound’ droplets [3]. The ionic liquid segment can initiate and control chemical communication with the aqueous compartment. Ionic liquids (ILs) are room-temperature liquid salts of organic cations and organic/inorganic anions and have attracted immense attention due to their unique physical and chemical characteristics [4]. Fluid properties such as viscosity, density, hydrophobicity and chemical reactivity can be predictably engineered and tuned by appropriate selection of the cations and anions.

The enchanting glow of fireflies is produced by a magnesium-aided enzymatic bioluminescence reaction [5]. Inspired by this natural ingenuity, we demonstrate here microfluidic droplet-based ‘fireflies’ for chemical and biological sensing applications. We create aqueous-ionic liquid compound droplets in which metal (gold) ions from the aqueous compartment partition into the adjacent ionic liquid compartment and catalyze a chemical reaction accompanied by fluorescence, thus forming a bright fluid ‘firefly’ structure (Figure 1(c)). The catalytic reaction and fluorescence emission is entirely localized within the ionic liquid, a segment of the overall structure, analogous to localized light emission within specific abdominal segments in fireflies. We present detailed measurements of the kinetics of fluorescence emission as a function of aqueous metal concentration and droplet flow speed. Since both the substrate and fluorescent product remain in the IL compartment due to their preferential solubility in IL, the detection of metal ion occurs in non-invasive fashion, thus preventing sample contamination. Also, the ability to detach the “detector” IL compartment from the aqueous analyte segment would enable further downstream chemical processing, such as multi-step detection. Therefore, we demonstrate complete, passive detachment of the two compartments at a hydrodynamic obstruction.

CONCEPT

A conceptual scheme of the presented microfluidic fireflies is provided in Figure 1(a). Gold ions, initially present only in the aqueous phase, partition into the ionic liquid compartment and catalyze the conversion of a weakly fluorescing aryl alkyne substrate (I) into (II), a highly fluorescent coumarin-derivative that causes the bright glow in the ionic liquid part [6]. Although gold ions are highly soluble in water, they can partition into the polar IL (1-methyl-3-ethylimidazolium bis(trifluoromethanesulfonyl)imide, [EMIm][NTf₂]) segment which is pre-doped with a very weakly fluorescing aryl alkyne substrate (I). We have selected Au(III) ion (gold salt, HAuCl₄·3H₂O) as a water-soluble model analyte that has attracted much interest as a powerful catalyst in organic synthesis and as a common precursor in nanomaterials synthesis.

EXPERIMENTAL

Individual syringe pumps were used to deliver carrier fluid, IL ([EMIm][NTf₂]) and aqueous solutions (HAuCl₄) to the microfluidic device. A 10:1 (v/v) mixture of perfluorodecalin (PFD) and perfluorooctanol (PFO) was used as the carrier fluid (FO). A microfluidic device scheme shown in Figure 1(d) was used to generate the aqueous-ionic liquid compound droplets flowing in an immiscible carrier phase of fluorinated oil. The devices were fabricated in poly(dimethyl siloxane) (PDMS) using standard soft lithographic techniques. Aqueous HAuCl₄ solutions of varying concentrations (5 mM, 10 mM, and 40 mM) were used while the substrate (I) solution was 0.4 M. The individual flow rates of each stream, Qfo, QIL and QAq were 6 μL/min⁻¹, 1 μL/min⁻¹ and 3 μL/min⁻¹, respectively. To evaluate the effect of flow speed on the time-development of fluorescence, we measured fluorescence intensity within the IL compartment for three different total flow speeds (2.4, 4.5
and 6.7 mm/sec) within the operational range of the current microfluidic device, while maintaining constant H\text{AuCl}_4 aqueous concentration. Once steady state operation was achieved images of the flow were recorded at 21 fps by a CCD camera mounted on a stereomicroscope. These images were subsequently analyzed using image-processing algorithms implemented in MATLAB.

Figure 1: a) A schematic of the general concept: metal catalyzed reaction-induced fluorescence generation within the ionic liquid compartment of a microfluidic ‘firefly’ b) Reaction scheme: gold ions are transferred from the aqueous to ionic liquid compartment and catalyze the conversion of a weak fluorescing substrate (I) into a strongly fluorescing product (II), triggering bright fluorescence (excitation: 365nm, Emission: 488nm). c) Stereomicroscope image of a firefly-on-a-chip. Scale bar=300\mu m d) A schematic illustrating firefly formation in our microfluidic devices.

RESULTS AND DISCUSSION

In order to investigate the physico-chemical aspects of our analytical method, we carried out detailed time-resolved measurements of fluorescence emission from IL compartments, for various metal ion concentrations and at different flow speeds (Figure 2(a-d)). The fluorescence intensity within IL segments gradually increases as the compound droplets translate along the length of microchannel (Figure 2(a)). We examined the effect of Au(III) concentrations on the kinetics of fluorescence emission. The normalized fluorescence intensity curves versus reaction time, which is defined by the distance travelled along the microchannel divided by the velocity of the compound droplets, get steeper with increasing gold concentration in the aqueous compartment (Figure 2(c)). The time required to reach 10% of the maximum fluorescence intensity can be applied as a criterion to detect the concentration of Au(III) in aqueous sample. As can be seen in Figure 2(c), it takes 8, 15 and 21 seconds respectively for the 40, 10 and 5mM H\text{AuCl}_4 samples.

Further, we recorded the fluorescence intensity at various time points corresponding to various locations along the length of the microchannel (relative to the drop dispensing junction) for three different droplet speeds (for all cases [Au(III)] = 10 mM). As shown in Figure 2(d), when the normalized fluorescence intensity for all these cases is plotted against the reaction time, all three curves collapse onto each other. The fluorescence emission kinetics follow a non-linear logistic form, independent of flow speed, a reasonable behavior in light of accelerated mass transfer processes in such ‘compound’ droplets [3]. The fluorescence intensity exhibits an induction period of approximately 13 seconds and approaches a plateau within about 60 seconds, corresponding to depletion of substrate (I) in the IL compartment. This overall behavior is governed by the coupled processes of convection-enhanced mass transfer and chemical reaction.

Finally, we demonstrate passive decoupling of the IL ‘sensor’ from aqueous ‘analyte’ by simple flow through a hydrodynamic obstruction. An asymmetric orifice-like constriction with sharp corners (Figure 2(e)-(h)) inserted in the microchannel modifies the local flows and favors necking at the liquid-liquid interface, leading to complete disengagement.
The ability to controllably fabricate and disengage such chemically functional fluid structures makes this technique compatible with operation in complex microfluidic networks involving additional chemical and physical processing steps on the droplets.

**Figure 2:** Stereomicroscope images showing a) increase in fluorescence intensity within the ionic liquid compartment of a compound droplet with time as it travels along the microchannel and b) still fireflies in microchannel. c) Plots of normalized fluorescence intensity of IL compartments versus time for three different gold concentrations in the aqueous compartment, flowing at 4.5 mm/sec. d) Plots of normalized fluorescence intensity versus time for two different flow speeds at constant aqueous gold concentration; the plots are seen to collapse onto the same curve indicating a reaction controlled regime. e-h) Stereomicroscope images of a compound droplet encountering an obstacle in the microchannel, causing detachment of sensor compartment from the analyte (sample) compartment. All scale bars=300 µm.

**CONCLUSION**

Microfluidic firefly structures are an exciting addition to the rapidly expanding toolkit of digital microfluidic methods, and enable unique non-invasive 'on-drop' measurements for sensing applications.

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