MECHANISTIC INVESTIGATION OF ALTERNATING CURRENT CLOUD POINT EXTRACTION IN A MICROCHANNEL

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ABSTRACT

We present mechanistic investigation of alternating current cloud point extraction (ACPE) in a microchannel. We carried out ACPE experiments under various experimental conditions to characterize and optimize the ACPE. The results indicated that ACPE depend on flow velocity and surfactant concentration, whereas not on lipid concentration. Optimum conditions for efficient extraction were also clarified.

KEYWORDS: AC electrokinetics, Dielectrophoresis, Phase separation, Surfactant, Lipids

INTRODUCTION

Sample preconcentration before analysis is important to detect low-abundance biomolecules. Various on-chip preconcentration techniques for DNA [1], mRNA [2] and water-soluble proteins [3] have been reported, but not for membrane-associated biomolecules (MAB). ACPE is our original and unique technique for on-chip preconcentration of MAB [4]. Selective and quantitative extraction of phospholipid as a model of MAB was reported, but the detailed mechanism of the extraction has not been investigated. In this study, we carried out ACPE experiments under various experimental conditions to characterize and optimize the ACPE.

PRINCIPLE

The principle of the ACPE is shown in Figure 1. A nonionic surfactant solution is continuously introduced into a microchannel which has microband electrodes. Application of AC voltages to the electrodes raises the temperature of the solution above its cloud point by Joule heating, which leads to the generation of microdroplets of the surfactant-rich (SR) phase by micellar aggregation. At the same time, the SR microdroplets receive negative dielectrophoretic forces directed toward the lower electric field, and are trapped if the field has a local minimum. The electrode configuration and operation shown in Fig. 1 makes a local minimum in the electric field just above the central electrode. As a result, the surfactant-solubilized target molecules enriched in the SR phase are continuously trapped above the central electrode, as shown in Fig. 2.

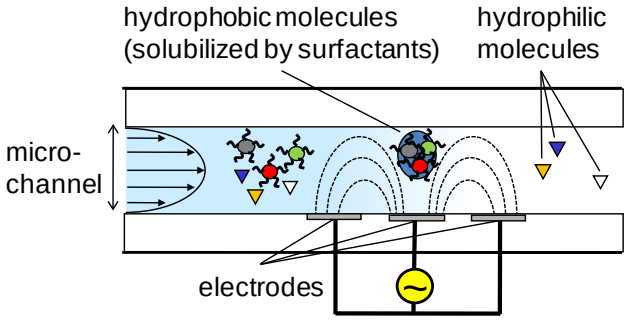

Figure 1: Schematic illustration of the ACPE in a microchannel which has three microband electrodes. The dotted lines indicate electrical flux.

EXPERIMENTAL

A PDMS-glass microchip with Au microelectrodes was fabricated by the standard microfabrication techniques. A test solution containing BODIPY FL-labeled 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (DHPE), Triton X-114 (TX) and 0.15 mol L^{-1} KCl was introduced into the microchannel by a syringe pump. AC voltages (20 $V_{p\text{-}p}$, 5 MHz) were applied to the electrodes by a function generator for 60 s. Extraction processes were visualized by a fluorescence microscope equipped with a CCD camera. The temperature of the microscope stage was kept at 3°C by a temperature controller. For evaluation of the extraction, concentration index (*CI*) was defined as $CI = I_t / I_0$, where I_t and I_0 are mean fluorescence intensities in the measurement area (see Fig. 2(a)) at ACPE time *t* and at the beginning of the experiment, respectively.

RESULTS AND DISCUSSION

Figure 2 shows the results of the ACPE. Bright fluorescence was observed above the central electrode by the application of the AC voltages (Fig. 2(b)-(e)). *CI* reached almost constant value at t = 60 s, so we used data obtained at t = 60 s in the following experiments to study the effects of flow velocity (u), [TX], and [DHPE] on the ACPE.

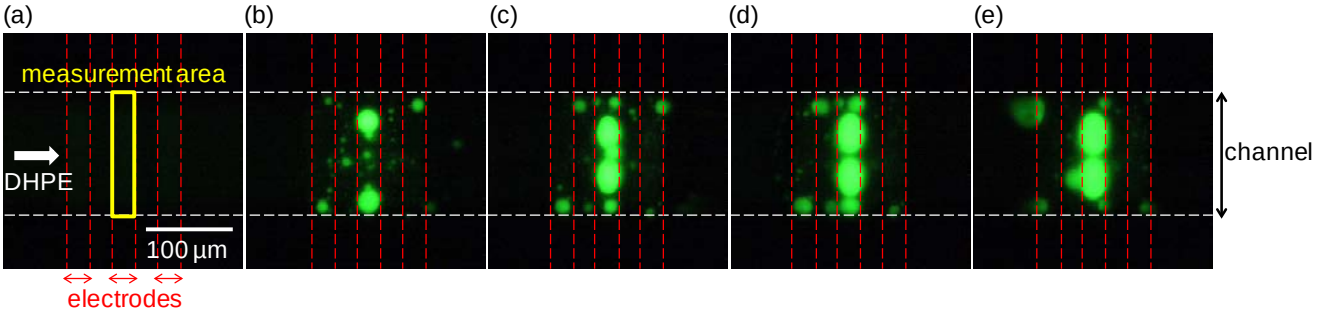

Figure 2: Typical fluorescence micrographs of the ACPE experiments. [DHPE] = 20 mM, [TX] = 0.5%, and u = 0.33 mm s^{-1}. (a) t = 0, (b) t = 15, (c) t = 30, (d) t = 45, and (e) t = 60 s.

Figure 3(a) shows dependence of the *CI* on u. *CI* was ~1 (no concentration) for u = 0, suggesting that the number of the micelles near the electrodes is too small to achieve efficient concentration enrichment (Fig. 3(b)). *CI* took maximum values for u = 0.10~0.67 mm s^{-1}. Under these conditions, supply of the micelles from upstream by fluid flow is sufficient to achieve efficient concentration enrichment (Fig. 3(c)). For u > 1.3 mm s^{-1}, *CI* decreased since the viscous drag force by fluid flow exceed the dielectrophoretic force which trap the SR microdroplets above the central electrode (Fig. 3(d)).

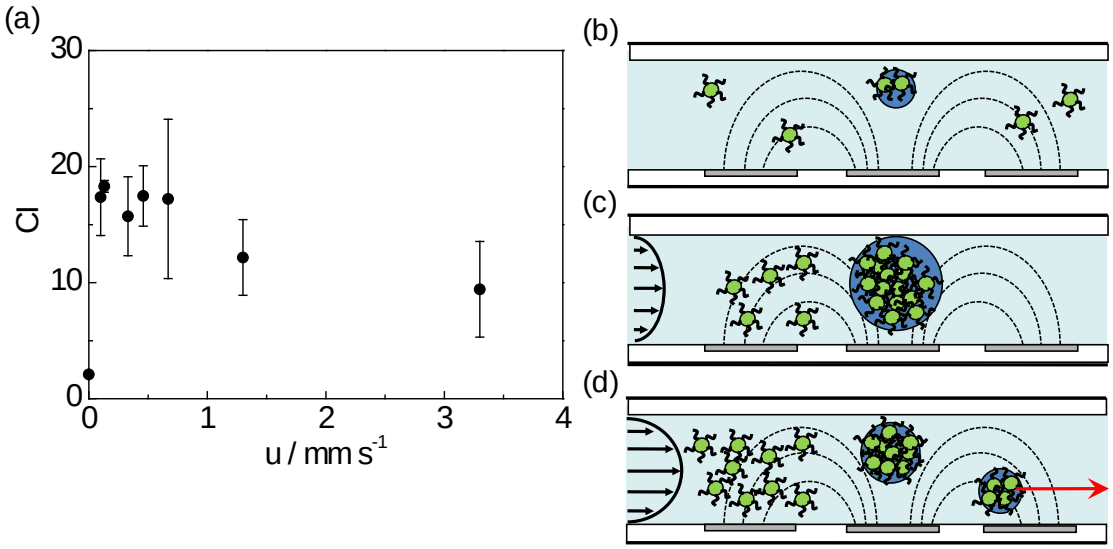

Figure 3: (a) Dependence of the CI on flow velocity. [DHPE] = 20 mM, [TX] = 0.5%. The error bars indicate ±1SD of at least three experiments. (b-d) Schematic illustrations of ACPE at each condition. (b) u = 0 mm s^{-1}. (c) u = 0.10~0.67 mm s^{-1}. (d) u > 1.3 mm s^{-1}.

Dependence of *CI* on [TX] is shown in Fig. 4. *CI* decreased at low and high [TX], and took maximum values for [TX] = 0.1~1%. To clarify the dependence, we estimated the concentration of the micelles ([Micelle]) for each condition using the reported values of critical micelle concentration (0.015%) and aggregation number (50) of [TX] [5]. Then we calculated the ratio of [Micelle] to [DHPE]. These values are also indicated in Fig. 4. At low [TX], *CI* decreased since the number of the micelles in the solution was small and the SR microdroplets cannot become large enough to be trapped by the dielectrophoretic force. *CI* also decreased at high [TX] since the number of the micelles was too large and micelles which did not contain lipids were also trapped. In contrast, *CI* did not depend on [DHPE] as shown Fig. 5. Therefore, optimum conditions of ACPE were u = 0.10~0.67 mm s^{-1} and [TX] = 0.1~1%, regardless of the concentration of analytes.

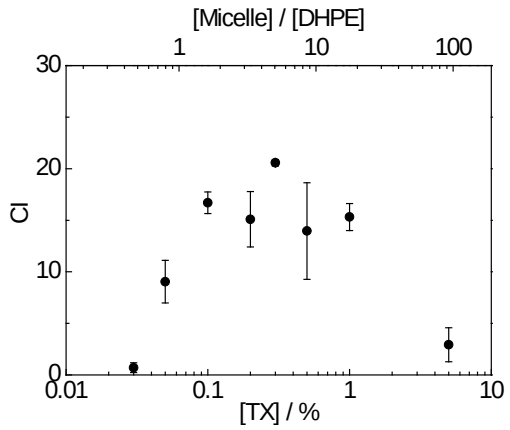

Figure 4. Dependence of the CI on surfactant concentration. Ratio of the number of micelles to the number of lipids is also indicated on top X-axis. [DHPE] = 20 mM, u = 0.33 mm s^{-1}.

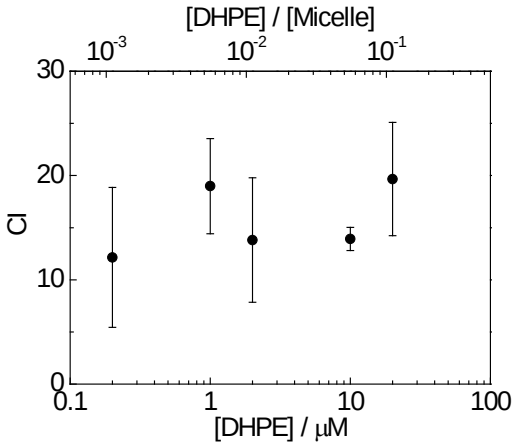

Figure 5. Dependence of the CI on lipid concentration. Ratio of the number of lipids to the number of micelles is also indicated on top X-axis. [TX] = 0.5%, u = 0.33 mm s^{-1}.

CONCLUSION

We have carried out ACPE experiments under various experimental conditions to characterize and optimize the ACPE. The results indicated that ACPE depend on flow velocity and surfactant concentration, whereas not on lipid concentration. Optimum conditions for efficient extraction were also clarified. These results offer the guidelines to utilize ACPE as a component of integrated on-chip bioanalysis.

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