MECHANISTIC CHARACTERIZATION OF ALTERNATING CURRENT CLOUD POINT EXTRACTION IN A MICROCHANNEL: EXTRACTION UNDER PHYSIOLOGICAL TEMPERATURE

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ABSTRACT

We present mechanistic characterization of alternating current cloud point extraction (ACPE) in a microchannel. ACPE is our original technique for on-chip extraction and preconcentration of membrane-associated biomolecules [1]. Joule heating of nonionic surfactant micellar solutions plays an important role in ACPE, but temperature of solutions during the extraction has not been investigated. In this study, we estimated the temperature under various experimental conditions using a temperature-dependent fluorescent dye. The ACPE was achieved around 36°C, which is physiological temperature, under optimized conditions. These results provide guidelines for use of the ACPE technique in microfluidic chemical and biochemical analyses.

KEYWORDS

AC electrokinetics, Dielectrophoresis, Phase separation, Surfactant, Micelle

INTRODUCTION

Sample preconcentration before analysis is advantageous to detect low-abundance species, which are often important in bioanalysis. In a previous paper [1], we reported a novel extraction technique termed alternating current cloud point extraction (ACPE) for preconcentration of membrane-associated biomolecules (MAB) including lipids and membrane proteins. To characterize and optimize ACPE, we carried out ACPE experiments under various experimental conditions including amplitude and frequency of applied voltages, flow velocity, and concentration of surfactant, analyte and salt [2]. As described in the following section, Joule heating plays an important role in ACPE to induce aggregation of micelles, but overheating could lead to denaturation of proteins in the solution, which may limit the applicability of the technique in real analyses. Therefore, we investigated solution temperature in ACPE using a temperature-dependent fluorescent dye. The results were compared with those of ACPE experiments to optimize experimental conditions and achieve the extraction under physiological temperature.

PRINCIPLE

The principle of cloud point extraction (CPE), which is widely utilized to extract MAB in a macroscale, is shown in Fig. 1(a). In nonionic surfactant micellar solutions, micelles are aggregated as the temperature of the solution rises above its cloud point (CP). Hydrophobic MAB solubilized by the surfactant are extracted and concentrated into a small volume of the surfactant-rich phase, while hydrophilic molecules are retained in the surfactant-poor phase. The CPE requires two operations: heating and centrifugation. The centrifugation is, however, difficult to miniaturize because the device should be connected to tubes and/or electrical cables to integrate CPE with other analytical steps.

In ACPE (Fig. 1(b)), the micellar 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 CP by Joule heating, which leads to the generation of microdroplets of the surfactant-rich phase by micellar aggregation. At the same time, the microdroplets are trapped just above the central electrode by negative dielectrophoresis under the nonuniform electric fields. As a result, the surfactant-solubilized MAB enriched in the microdroplets are continuously trapped and concentrated above the central electrode.

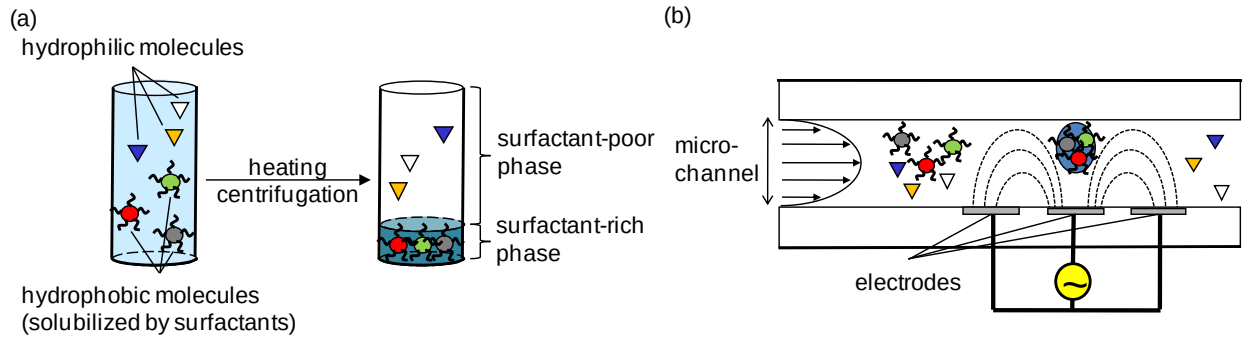

Figure 1: Schematic illustration of (a) CPE in a macroscale and (b) ACPE in a microchannel which has three microband electrodes. The dotted lines indicate electrical flux.

EXPERIMENTAL

A PDMS-glass microchip with microelectrodes was fabricated as described previously [1]. In ACPE experiments, a test solution containing fluorescently-labeled lipids (20 mM), Triton X-114 (0.5%), and KCl (15 ~ 150 mM) was continuously introduced into a microchannel (150 μm width, 25 μm depth, 20 mm length) by a syringe pump at 0.33 mm s^{-1}. AC voltages (20 $V_{p\text{-}p}$, 5 MHz) were applied to the electrodes (40 μm width, 20 μm gap) by a function

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generator. The microchannel was visualized by a fluorescence microscope equipped with a CCD camera. For evaluation of the extraction, concentration index (*CI*) was defined as the ratio of mean fluorescence intensities at the ending and the beginning of the experiment. Temperature of the solution was estimated in a separate measurement. Rhodamine B was utilized as a temperature-dependent fluorescent dye [3]. A test solution containing rhodamine B (10 μM), Tween 20 (0.5%), and KCl was continuously introduced into the microchannel, and fluorescence images were obtained in a similar way as the ACPE experiments. Fluorescence intensities in each pixel of the images were converted to the temperature of the solution by a calibration curve obtained in advance.

RESULTS AND DISCUSSION

Figure 2 shows typical fluorescence micrographs of the ACPE experiments with different stage temperatures. When the stage temperature was 3°C, fluorescence was hardly observed from the channel (Fig. 2(a)), which means that the concentration of DHPE was too low to be observed. However, by increasing the temperature above 10°C, bright fluorescence was observed above the central electrode (Figs. 2(b-d)).

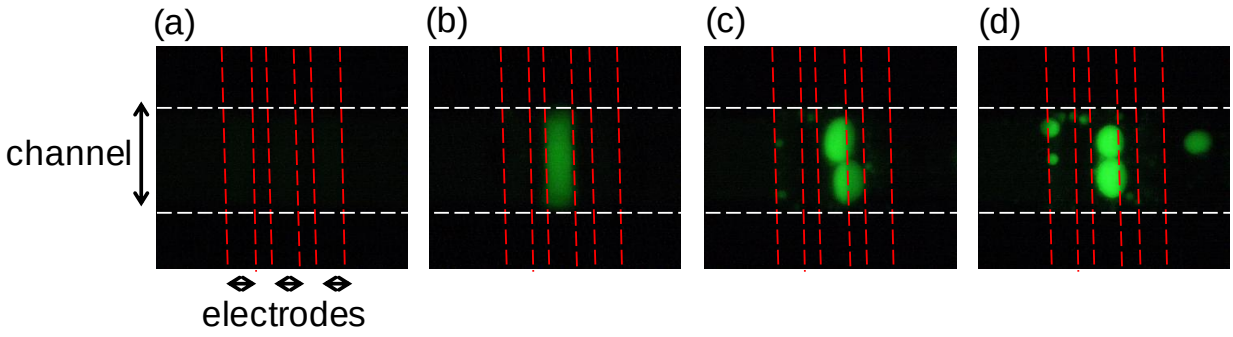

Figure 2. Typical fluorescence micrographs of the ACPE experiments. [KCl] = 50 mM. ACPE time was 60 s. Stage temperature was (a) 3, (b) 10, (c) 15 and (d) 20°C.

Figure 3(a) shows a typical fluorescence image of the microchannel filled with a rhodamine B solution. In the image, the fluorescence intensity from part of the electrodes was enhanced due to reflection effects on the electrode surface. The reflection effects were corrected by normalizing the fluorescence intensity under voltage application by that without applied voltages to allow quantitative discussion. Figure 3(b) shows a false-color image of two-dimensional temperature distribution on the microchip. The temperature increased in the microchannel, particularly at gaps between the electrodes. This result is consistent with numerical calculation of electric field strength *E* in the microchannel (Fig. 3(c)), which exhibits large *E* at interelectrode gaps. In general, thermal energy by Joule heating is proportional to the square of *E*, so the results clearly indicate that the temperature increase is due to Joule heating of the solutions.

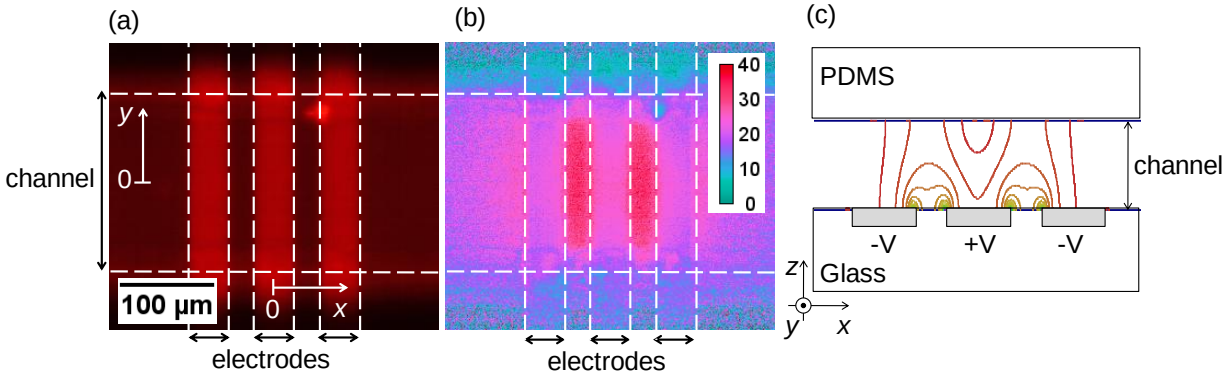

Figure 3. (a) Typical fluorescence image of the microchannel filled with a rhodamine B solution. (b) False-color image of two-dimensional temperature distribution on the microchip. (c) Contour plot of numerically calculated electric field strength E in the microchannel.

Figure 4(a) shows temperature profile along the microchannel at $y = 0$ and at different stage temperatures. When the stage temperature was 3°C, only a small part of the solution was heated above its CP. However, by increasing the temperature to 10°C, most part of the solution near the electrodes was heated above its CP. Further increase in the stage temperature (15 and 20°C) resulted in increased solution temperature. The results of ACPE experiments under these conditions are shown in Fig. 4(b). The increase in *CI* was observed when the stage temperature was 10°C, which is consistent with the results in Fig. 4(a). However, further increase in the stage temperature resulted in almost the same *CI*. These results suggest that the ACPE can be achieved if the solution temperature is above its CP, in a similar way as CPE in a macroscale, and a much higher solution temperature than its CP does not increase *CI*. In addition, when the stage temperature was 10°C, the maximum solution temperature was 36°C. Therefore, the ACPE can be conducted under physiological temperature.

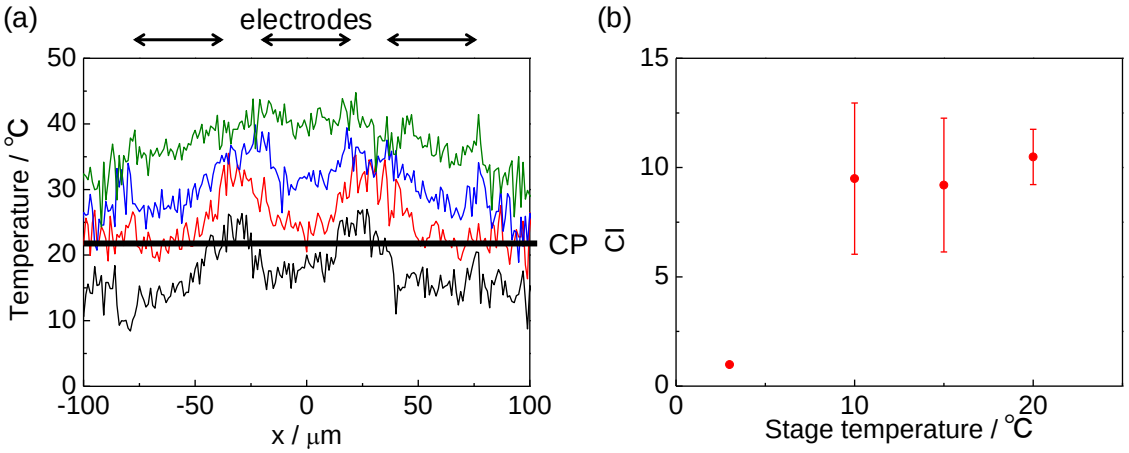

Figure 4. (a) Temperature profile along the microchannel at y = 0. Stage temperature was 3 (black), 10 (red), 15 (blue) and 20°C (green). [KCl] = 50 mM. See Fig. 3(a) for coordinates. (b) Dependence of CI on the stage temperature.

CONCLUSION

We have estimated the solution temperature in the ACPE. ACPE was successfully conducted under physiological temperature, so the technique can be applied not only to analyze the amount of target molecules but also to analyze their function. Since conventional CPE in a macroscale can also be applied to metal and organic compounds, ACPE is also expected to be applied to analyze them and broaden the applicability of chemical analyses on microfluidic devices.

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