AC ELECTROKINETIC PHASE SEPARATION, FOCUSING AND CONCENTRATION IN MICRO-CHANNEL

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ABSTRACT
We present a novel technique to concentrate water-insoluble biomolecules in a microchannel utilizing phase separation and focusing under nonuniform electric field. Applying an AC voltage to microelectrodes embedded in a microchannel increased the fluorescence from surfactant-solubilized lipid by 40-fold within 1 min. Electrophoretic transport of the concentrated lipid is also demonstrated.

KEYWORDS: Phase separation, Concentration, AC electrokinetics, Microelectrode

INTRODUCTION
AC electrokinetics [1] has been utilized to pump [2,3] or mix [4,5] fluids in microchannel because of its easy operation and excellent performance. In this study, we developed a novel technique utilizing AC electrokinetics termed AC electrokinetic phase separation and focusing (ACEPF) and applied it to concentration of water-insoluble biomolecules.

PRINCIPLE
A nonionic surfactant solution is known to separate into a small amount of surfactant-rich (SR) phase and a large amount of surfactant-poor (SP) phase upon heating (Figure 1(a)). Therefore, surfactant-solubilized biomolecules are concentrated into the SR phase. In ACEPF, this process is continuously achieved in a microchannel which has three microelectrodes (Figure 1(b)). Application of an AC voltage to them causes the phase separation of fluids by Joule heating. At the same time, the SR phase is focused around the electrodes by negative dielectrophoresis. As a result, efficient concentration of the surfactant-solubilized biomolecules is achieved.

(a)

![water-soluble biomolecules](image)

![heating](image)

![surfactant-solubilized biomolecules](image)

(b)

![electrical flux line](image)

Figure 1. Schematic illustration of concentration by phase separation of a nonionic surfactant solution (a) in a bulk scale and (b) in a microchannel with ACEPF.
EXPERIMENTAL

A PDMS-glass microchip which has Au microelectrodes was fabricated by standard microfabrication techniques. Test solution containing BODIPY FL-labeled 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (DHPE) or Texas Red-labeled bovine serum albumin (BSA), 0.5 % Triton X-114 and 0.15 M KCl was introduced into the microchannel by power-free pumping [6], and an AC voltage (20 V<sub>p-p</sub>, 5 MHz) was applied to the electrodes by a function generator. Concentration processes were visualized by a fluorescence microscope equipped with a CCD camera. The temperature of the microscope stage was kept at 3 °C throughout the study. Mean fluorescence intensity (MFI) on the electrodes was normalized by initial MFI value and used for the evaluation of the concentration.

RESULTS AND DISCUSSION

Figure 2 shows the typical images of concentration by ACEPF. Efficient concentration was observed in Figure 2(d) with the Triton X-114 solubilized DHPE, while concentration was hardly observed with BSA in Figure 2(b). The time course of MFI is shown in Figure 3, which shows the ability of ACEPF to increase the signal of DHPE by 40-fold in 1 min. Such concentration could not be obtained with simple Joule heating or AC actuation on a pair of electrodes, which implies the importance of the three-electrode configuration on ACEPF.

Combination of ACEPF with electrophoresis is demonstrated by electrophoretic transport of concentrated DHPE (Figure 4). The concentrated DHPE were migrated into neighbor channel filled with polymer solution. Application to the preconcentration and electrophoretic separation of lipids is now under investigation. Such operations will enable sensitive and high-throughput analysis of water-insoluble biomolecules which cannot be handled by conventional on-chip concentration and separation methods.
CONCLUSIONS

We have presented the first example of AC electrokinetic liquid-liquid phase separation and its application to the concentration of water-insoluble biomolecules such as lipids. The present ACEPF will be a powerful technique for on-chip analysis of lipids and membrane proteins, which are important for function analysis of biological materials, disease diagnostics and so on.

REFERENCES