Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting Information for

On-line Electrophoretic Sample Clean-up for Sensitive and Reproducible μCE Immunoanalysis

Qiong Pan,[a] Soongweon Hong,[b] Xiaocui Zhu,[a] Meiping Zhao*[a] and Luke P. Lee*[b]

[a] Beijing National Laboratory for Molecular Sciences, MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.

[b] Berkeley Sensor and Actuator Center, Department of Bioengineering, University of California, Berkeley, USA.

Corresponding authors: mpzhao@pku.edu.cn; lplee@berkeley.edu

Calculation Process for μCE Step 2 Selective Field-amplified Concentrating

At the end of the loading step, L_2 is almost filled up with sample zone, other parts of the channel are filled with running buffer (Figure 1a, I). At time t_x, the sample zone is pushed back toward *SR*, and the length of the sample zone is l_x (see Figure 1a, II). The electric field strength across L_2 is described by eq 1.

$$E_{2x} = \frac{\gamma U_2}{[(l - l_x) + \gamma l_x]} \qquad \text{eq 1a}$$

$$E_{x0} = \frac{U_2}{[(l - l_x) + \gamma l_x]} \qquad \text{eq 1b}$$

where U_2 is the potential on L_2, E_{2x} is the electric field strength across sample zone, which has a length of l_x; and E_{x0} is the electric field strength across (l_2-l_x), γ is the conductivity ratio of the running buffer and the sample buffer.

Accordingly, bulk flow velocity (V_b) in channel L_2 is the vector sum of the local electroosmotic velocity (V_{eo}) and the pressure-driven velocity (V_h) in each channel. Because of the uneven liquid level between SR and other reservoirs, there exists a hydrodynamic pressure from SR to the intersection. The bulk flow velocity is expressed in eq2.

$$V_b = V_{eo} - V_h \qquad \text{eq 2}$$

V_{eo} is the weighted average of local velocity in sample zone and running buffer zone, as described by eq 3.

$$V_{eo} = (1 - \frac{l_x}{l})\mu_{eox0} E_{x0} + (\frac{l_x}{l})\mu_{eo2x} E_{2x} \qquad \text{eq 3}$$

where μ_{eox0} and μ_{eo2x} are the electroosmotic mobility for the running buffer and the sample buffer. If we define $x = \frac{l_x}{l}$, as the ratio of sample zone length in L_2, from eq 1, 2 and 3, the bulk flow in L_2 (V_b) in terms of x can be obtained as in eq 4.

$$V_b = \frac{(1 - x)\mu_{eox0} + \gamma x \mu_{eo2x}}{1 + (\gamma - 1)x} \bullet \frac{U_2}{l} - V_h \qquad \text{eq 4}$$

Because

$$U_i \propto I_i l_i,\ U_2 + U_3 = V_3 - V_2,\ U_4 + U_3 = V_3 - V_4$$

where U_i and I_i represent the potential and current on each channel, l_i stands for the length of each channel, and V_i stands for the voltage applied to the reservoir. U_2 can be calculated with voltages on each reservoir and the current on each channel. U_2 under different voltage applied to the reservoir is shown in Table S1.

Under the applied voltage, the negatively charged analyte in the sample buffer has an electrophoretic velocity ($V_{ep}=\mu_{ep}E_{2x}$) and in the direction opposite to EOF. The electrophoretic velocity, V_{ep} of the analyte anions in sample buffer can be expressed as in eq 5.

$$V_{ep} = \mu_{ep}E_{2x} = \frac{\mu_{ep}\gamma}{[(1-x)+\gamma x]} \bullet \frac{U_2}{l} \quad \text{eq 5}$$

Accordingly, the electrophoretic velocity of analytes in running buffer is:

$$V_{ep} = \mu_{ep}E_{x0} = \frac{\mu_{ep}}{[(1-x)+\gamma x]} \bullet \frac{U_2}{l} \quad \text{eq 6}$$

In the two buffer system, anion analytes have much higher electrophoretic velocity in sample buffer due to its high electric field. The analytes reduced its velocity when they enter running buffer zone, so that they are stacked at the running buffer side of the interface. So the analyte velocities in running buffer zone determined their movement after being stacked.

U_2 can be calculated by detecting the current at SR end from the electrode. It should be noted that U_2 is a variable depending on x. In this experiment, we monitored the electric current through L_2. It increased initially within the first few seconds and then remained virtually a constant later on, indicating a relatively stable potential along L_2. In order to simplify the calculation, U_2 is assumed to be a constant. The calculated U_2 values are listed in Table S1.

Table S1. U_2 under Different Voltage Applied to Each Reservoir

V_1 (V)	V_2 (V)	V_3 (V)	V_4 (V)	I_1 (mA)	I_2 (mA)	I_3 (mA)	I_4 (mA)	U_2 (V)
1680	1680	2000	0	4.5	4.5	20.5	11.5	76
1620	1620	2000	0	5.0	5.0	21.0	11.0	86
1600	1600	2000	0	5.5	5.5	22.0	11.0	94
1550	1550	2000	0	6.0	6.0	23.0	11.0	100
1510	1510	2000	0	6.5	6.5	24.0	11.0	106

For a given μCE, the voltages applied to all reservoirs and the buffer conductivity ratio was kept constant. In our case, U_2=100 V and γ=40; l=0.50 cm. The electroosmotic mobility for sample solution (2.25 mM Tris and 2.25 mM tetraboric acid, 0.5% BSA, pH=8.5, 50% ethanol) and running buffer solution (44.5 mM Tris base, 44.5 mM tetraborate acid, 2% BSA, pH8.5) are 6.07×10^{-4} and 3.14×10^{-4} cm^2/Vs, respectively. The electrophoretic mobilities of GFP-IGF-I and anti-IGF-I are 1.10×10^{-4} cm^2/Vs and 0.31×10^{-4} cm^2/Vs. The bulk velocity driven by the static pressure is 0.09 cm/s. Substituting these values into equations 1a and 1b, V_{2b} and V_{ep} can be expressed as a function of x, which is shown in Fig. 2a.

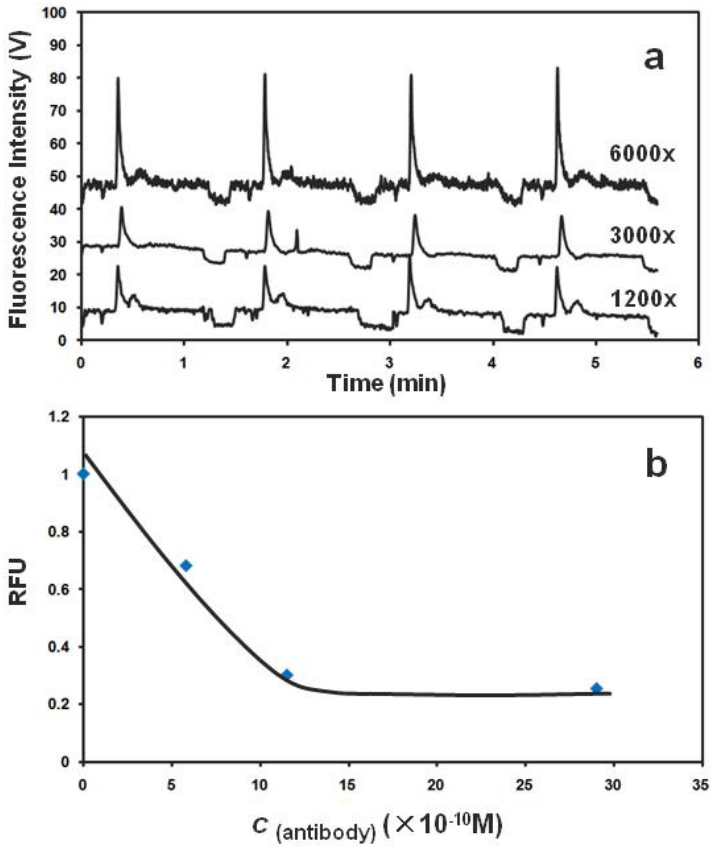

Figure S1. Optimization of the amount of antibody in the competitive immunoassay. a) Electropherograms of the three immunoreaction mixtures with different antibody concentrations (6000, 3000 and 1200 times dilution of the original antibody solution). The GFP-IGF-I concentration was fixed at 7.6×10^{-10} M. b) Relative peak height ratio between the immunoreaction mixture solution with different amount of antibodies and the GFP-IGF-I solution without antibody. All reactions were carried out in the electrophoretic sample buffer and 50% ethanol was added right before loading the sample on the chip.

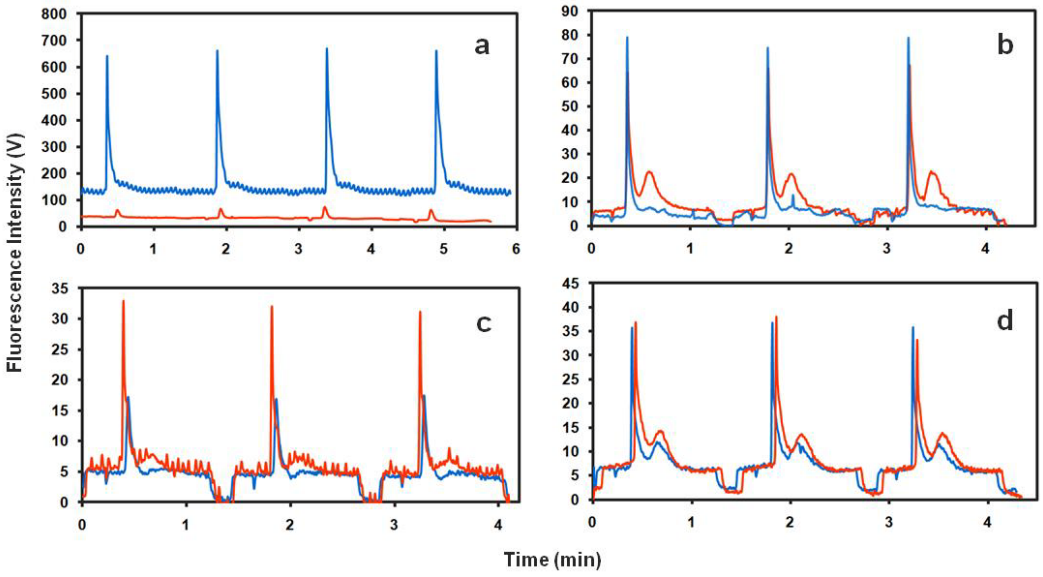

Figure S2. Effects of addition of 50% ethanol in the sample buffer on the stability of GFP-IGF-I and the μCE performance. a) Ethanol was added to the tested GFP-IGF-I solution right before performing μCE. The peak height was increased up to 10 times (blue curve) in comparison with that detected without addition of ethanol (red curve). b) Ethanol was added to the tested GFP-IGF-I solution and incubated for four hours before performing μCE (red curve). The extra peaks in comparison with that detected right after addition of ethanol (blue curve) indicate partial denature of GFP-IGF-I. c) Ethanol was added to the immunoreaction solution right before performing μCE. The immunoreaction solution contains 3.3×10^{-10} M (2.5 μg/mL) IGF-I, 8.3×10^{-10} M antibody and 7.6×10^{-10} M GFP-IGF-I for the red curve and only same concentration of GFP-IGF-I and antibody for the blue curve. d) Ethanol was added to the immunoreaction solution and incubated for 1 h before performing μCE. The immunoreaction condition were the same as in c).

Table S2. Recovery Test Results of the Two Human Serum Samples (n=3)

Sample Number	IGF-I Spiked (ng/mL)	IGF-I Found (ng/mL)	Recovery (%)
1#	0	2.56	91.1
	2.49	4.83	
2#	0	4.67	106
	2.49	7.32	