MICRO KINETIC EXCLUSION ASSAY FOR CADMIUM ANALYSIS

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ABSTRACT

Pollution of the environment by heavy metal species is a worldwide problem on account of their toxicity. Instrumental analysis, which is time-consuming and expensive, is generally used for the heavy metal analysis. Simple and rapid analysis method is useful for screening the pollution. We developed highly sensitive cadmium immunoassay based on kinetic exclusion assay in a microchip. The microfluidic immunoassay was completed within 7 min. The lower detection limit for cadmium was 1.85×10^2 ng/L, which satisfied much stricter regulation in Japan.

KEYWORDS

Immunoassay, Biosensor, Heavy Metal, Kinetic Exclusion Assay, Absorbance.

INTRODUCTION

Monitoring of heavy metal species is important from the view point of human health because of their toxicity. The conventional analysis methods of the heavy metal species are AAS, ICP-AES and ICP-MS. Although their sensitivity and accuracy are attractive, they are also time-consuming and expensive. Immunoassays have a potential for facile and cost-effective analysis methods because pretreatment can be simplified by utilizing antibody specific binding to the analytes. We have developed antibodies specific for heavy metal species, such as cadmium-EDTA, and chromium-EDTA, etc.[1,2] Microfluidic immunoassays have attractive features, such as high throughput, short analysis time, small amount of samples, and high sensitivity. Although they are promising candidates for rapid and low-cost analysis methods, applications to small analytes are difficult. Conventionally, sandwich and competitive ELISA are used for microfluidic immunoassays. Although the sandwich ELISA can achieve high sensitivity by using two kinds of antibodies captured by at least two epitopes, small analytes lack two epitopes that can be recognized simultaneously. Although the competitive ELISA can detect small analytes, the sensitivity is low. As one of approaches, Ueda's group has developed a non-competitive open-sandwich ELISA (OS-ELISA) on a microfluidic device.[3] However, the preparation and stabilization of antibody variable domains have many problems. We have reported kinetic exclusion assay for small analytes.[4] The kinetic exclusion assay can apply to the small analytes with high sensitivity and without complex preparations. Simple and low-cost immunoassay for small analytes is expected by applying the kinetic exclusion assay to microfluidics. Here, we report a micro kinetic exclusion assay for small analytes, cadmium.

THEORY

Figure 1 shows a schematic of the kinetic exclusion assay, which can reach the detection limit on the basis of the antibody dissociation constant K_d.[4] Usually, once free antibody binds the solid phase, antigen-antibody complex are dissociated to replace the bound free antibody. When interaction time is less than the dissociation time under high flow rate condition, a change in the sample concentration is kinetically excluded. Reaction rate is a few percent. Since the free antibody is accumulated on the beads under the initial equilibrium condition, small analytes can be measured with high sensitivity.

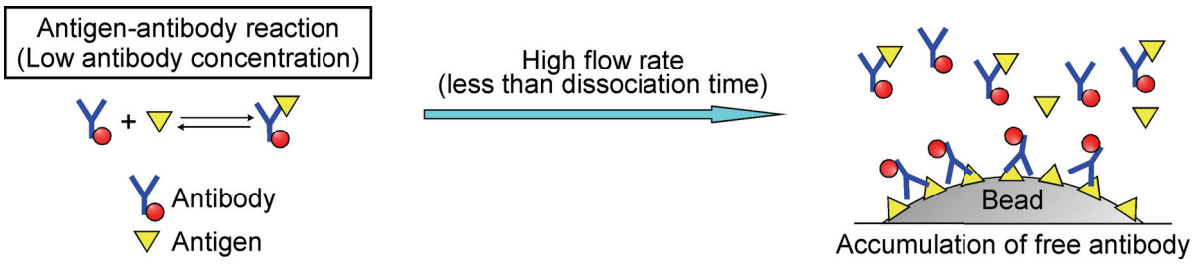

Figure 1. Schematic of the principle of the kinetic exclusion assay.

EXPERIMENT

The microfluidic device was fabricated using photolithography and soft lithography in PDMS. A main microchannel and a sub microchannel were designed for the flow of the sample solution and washing buffer, and solid phase packing, respectively as shown in Figure 2. Microchannel width and depth were 2 mm and 125 μm, respectively. Pillars having a width of 100 μm were fabricated at intervals of 50 μm. The solid phase packing area had 2 mm square. Microchannel surfaces were blocked by bovine serum albumin (BSA). Microflow was controlled by electro-osmotic pump (EO pump). Antigens were immobilized on PMMA beads having 100-μm

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diameter by reacting 1-(4-isotihocyanobenzyl)EDTA with BSA physically adsorbed on beads. Antigen immobilized beads were packed as a monolayer because of 100 μm-dimater, while double layer in our previous paper.[5] In this work, we evaluated the micro kinetic exclusion assay using cadmium-EDTA antibody (NX2C3) labeling gold nanoparticles. K_d and the cross reactivities were shown in Table 1. We evaluated the absorbance dependence of gold nanoparticles on the cadmium concentration by a green LED and photodiode powered by AAA butteries (Figure 3). The incident light irradiated the solid phase packing area through a 0.7-mm pinhole for preventing scattering light from non-intensive area.

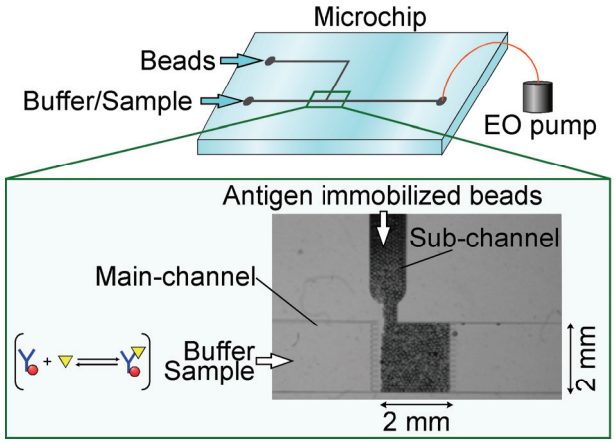

Figure 2. Illustration of the microchip with pillars.

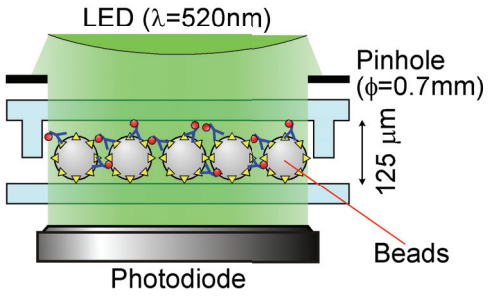

Figure 3. Schematic of the absorbance measurement.

Table 1. Antibody K_d and the cross reactivities to the metals.

Antibody	K_d (nM)	Cross Reactivity (%)							
		Cd(II)	Cu(II)	Mn(II)	Zn(II)	Fe(II)	Mg(II)	Cr(III)	Pb(II)
NX2C3	60.0	100	1.35	0.722	0.565	0.031	0.005	0.001	0.001

RESULTS AND DISCUSSIONS

After beads packing, the flow from the sub microchannel to the packing area was not observed because of the high pressure loss in the sub microchannel.[5] Valves are usually used for these flow controls, they are not necessary for our system. To achieve the theoretical level of detection, three measurement conditions, antibody concentration, flow volume, and flow rate, had to be optimized.[4] First, beads were packed in the pillar area. Second, the sample solution was flowed through the beads. Third, unbound free antibody was washed with 1 mL of phosphate buffered saline with 1 mg/mL BSA. Finally, absorbance was measured. The microfluidic device was repeatedly used in the experiment by removing the solid phases. Figure 4 shows the absorbance dependences on antibody concentration, flow volume, and flow rate.[6] The linear relation between the resulting absorbance signals, and the antibody concentration and the flow colume was observed under the experimental conditions. We selected 200 pM of the antibody concentration and 2.0 mL of the flow volume. The relative absorbance became constant value over 400 μL/min. However, bubbles were frequently contaminated into the beads area over 600 μL/min. The bubbles disturb flow conditions for binding the antibody to the antigen on the solid phase and optical path for correct absorbance detection. Therefore, we selected 400 μL/min of the flow rate.

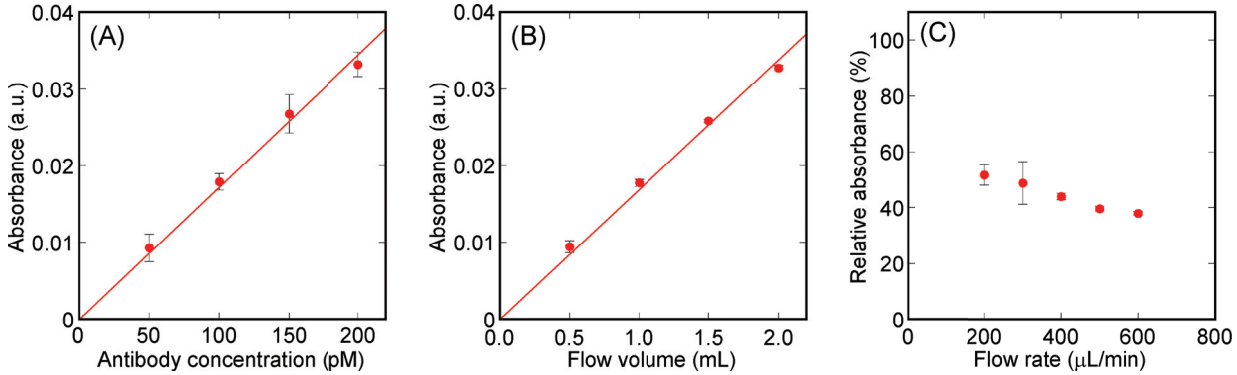

Figure 4. Absorbance dependence on (A) antibody concentration and (B) flow volume. (C) Relative absorbance dependence on flow rate.

Assay samples consisted of 200 pM antibody mixed with cadmium ion and 10 μM EDTA in 50 mM Tris buffer (pH 7.5). Under the optimized conditions, the relative absorbance dependence on cadmium concentration was examined (Figure 5).[6] The results were fitted with four-parameter logistic equation. The detection limit and dynamic range defined as the concentration with 30% relative standard deviation (RSD) and the region with <10% RSD [7] were evaluated to be 1.85×10^2 ng/L and 0.64−57.0 μg/L (Figure6), which satisfied much stricter regulation of 10 μg/L in Japan. The microfluidic immunoassay was completed within 7 min.

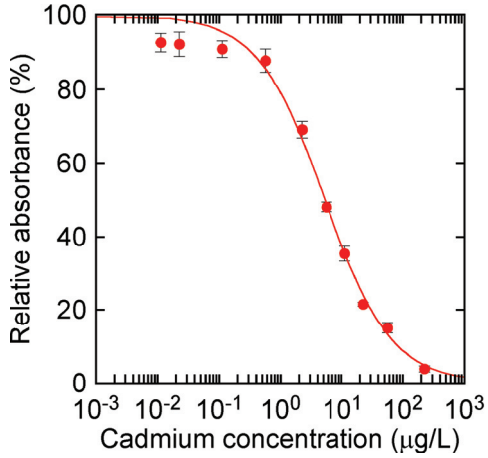

Figure 5. Standard curves for cadmium measured by the micro kinetic exclusion assay.

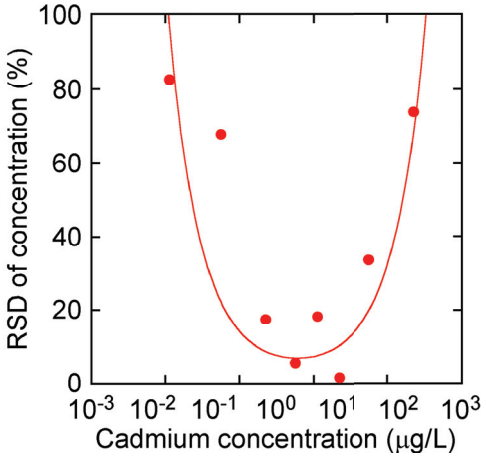

Figure 6. Precision profile of the standard curve in Figure 5.

In order to verify the K_d-limited detection of the micro kinetic exclusion assay, K_d was compared with the half-maximal inhibitory concentration. The half-maximal inhibitory concentration was estimated to be 5.3 μg/L, which is nearly the same as the K_d of NX2C3 of 60 nM (6.7 μg/L). Therefore, theoretical detection was achieved using the micro kinetic exclusion assay.

Our developed method can generally apply to immunoassays of other small analytes, such as heavy metal complex, dioxin, and polyaromatic hydrocarbons. The absorbance measurement system is simple, especially the LED and the photodiode powered by AAA butteries. Therefore, our system has a potential for on-site analysis system. In real environmental samples, some metal ions, such as Mg(II) and Fe(II), are contained at relatively high concentrations. Although the antibody has a high selectivity, those metal species cannot be ignored. Therefore, it can be useful by combining with adequate pretreatment, for example, the solid phase extraction for cadmium purification in our previous paper.[1]

CONCLUTIONS

We developed a micro kinetic exclusion assay for cadmium analysis with high sensitivity. Simple absorbance measurement system powered by AAA butteries was developed. Microflow control without valves was demonstrated. Antibody concentration, flow volume, and flow rate were optimized to achieve the theoretical level of detection for the micro kinetic exclusion assay. The detection limit and dynamic range were carefully considered and evaluated to be 1.85×10^2 ng/L and 0.64−57.0 μg/L, respectively. The micro kinetic exclusion assay can be a powerful tool for on-site analysis of heavy metal species.

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