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Surface plasmon resonance based competitive immunoassay for Cd²⁺†‡

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In this study, a label-free, specific and sensitive surface plasmon resonance (SPR) based competitive immunoassay was used for detecting Cd²⁺ in water samples. The standard curve of the method for Cd²⁺ was constructed in the concentration range of 0–1000 ng mL⁻¹. The values of IC₅₀ and the LOD of the assay were found to be 23.58 and 1.25 ng mL⁻¹. No cross-reactivity was found with other metal ions except Hg²⁺. The proposed method provides an alternative method for sensitive, specific and real-time determination of Cd²⁺ in real aqueous samples.

1. Introduction

Cadmium is one of the most toxic heavy metals for human beings: it has great environmental and health impact and all cadmium compounds have been classified as human carcinogens.^{1,2} Dietary intake of cadmium from water and crops in polluted environments results in various human diseases.³ Therefore, monitoring cadmium in water samples is urgently needed to protect human health and safety.

Up to now, cadmium can be determined by some classical and modern analytical methods, such as atomic absorption spectroscopy (AAS),⁴ inductively coupled plasma mass spectrometry (ICP-MS),⁵ Nonthermal Optical Emission Spectrometry,⁶ graphite furnace atomic absorption spectrometry (GF-AAS),⁷ and miniature dielectric barrier discharge optical emission spectrometric (DBD-OES).⁸ These methods are sensitive and accurate, but there are some disadvantages for these approaches to detect the cadmium, such as longer consumption times, increased technical expertise and tedious sample pretreatments, and some labeling methods are not suitable in some cases, because labeling materials may occupy important binding sites or cause steric hindrance, resulting in false information.

Surface plasmon resonance (SPR) is an optical phenomenon occurred in total internal reflection of light at a metal film–liquid interface,⁹ which is one of the powerful analytical

techniques for direct monitoring of molecular interactions. SPR is based on measuring binding-induced changes in refractive index and this platform thus allows for label-free, real-time analysis of the target molecules. In recent years, SPR has been applied in drug discovery,¹⁰ biomarkers screening,¹¹ food safety^{12,13} and environmental monitoring.^{14,15}

Besides, the immunological detection method based on antigen–antibody has provided a new strategy for the detection of cadmium pollution, and there have been many reports about the immunoassay of cadmium.^{16–18} The members of our research group have also established many methods for detecting metal ions.^{19–24}

Herein, we report a specific and sensitive competitive immunoassay combined SPR for the detection of cadmium (Cd²⁺) in aqueous solution for the first time. At present, all antibodies specific for cadmium recognize a chelated form of Cd²⁺, that is, Cd-EDTA.^{25–29} In our previous study, cadmium ion was respectively coupled to carrier protein (bovine serum albumin, BSA; ovalbumin, OVA) *via* a phenyl isothiocyanate-EDTA (ITCBE) to form the immunogen (Cd-ITCBE-BSA) and coating antigen (Cd-ITCBE-OVA). Using the hybridoma technique we successfully produced monoclonal antibody (mAb) against Cd²⁺, which were then characterized by an indirect competitive enzyme linked immunosorbent assay (ELISA).³⁰ Combining the specific mAb with the sensitive and label-free analytical SPR method, we expect that the SPR based immunoassay will be able to rapid detect Cd²⁺ with high sensitivity and specificity.

2. Experimental

2.1. Chemicals and reagents

N-Hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), bovine serum

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albumin (BSA), chicken egg albumin (OVA), ethanolamine ($\geq 98\%$), glycine (VetecTM reagent grade, 98%) were purchased from Aladdin (Shanghai, China). All other chemical reagents were of analytical grade and used without further purification. The antigen and antibody were self-made in this experiment. Detection experiment of Cd^{2+} in real aqueous samples, lake water sample was collected from Wenyang Lake in Datong city and tap water was obtained from our laboratory. In this work, 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) surfactant P20 was employed as running buffer and 10 mM acetate buffer solution was used as coupling buffer. Ultrapure water was used for the preparation of aqueous solutions.

2.2. Instrumentation

The Biacore XTM (Uppsala, Sweden) and CM5 sensor chip were used for all *in situ* surface bioaffinity detection. All solutions for experiments were filtered by 0.22 μm microporous membrane and degassed before use. Running buffer was HBS-EP buffer solution (pH 7.4) throughout the instrument at 10 $\mu\text{L min}^{-1}$ flow rate. CM5 sensor chips and all solutions were kept at room temperature before use.

2.3. Immobilization of antigen

Many methods of antibody immobilization used in SPR have been developed,^{31,32} however, the antigen was immobilized to determine Cd^{2+} in this experiment. Under optimal assay conditions, the self-made coating antigen (Cd-ITCBE-OVA) immobilization experiment was carried out at first, the whole process are shown in Fig. 1. A sensor chip CM5 was docked into the Biacore instrument firstly and primed with running buffer (HBS-EP, pH 7.4) at 10 $\mu\text{L min}^{-1}$ flow rate. The EDC/NHS mixed solution was injected (70 μL , 10 $\mu\text{L min}^{-1}$) to activate the surface of the sensor chip CM5. This was immediately followed by immobilization antigen (Cd-ITCBE-OVA) (70 μL , 10 $\mu\text{L min}^{-1}$) prepared in 10 mM sodium acetate buffer (pH 3.75). Then the remaining activate sites of the sensor chip CM5 surface was blocked with 70 μL ethanolamine (pH 8.5). In this way, the immobilization of antigen was completed.

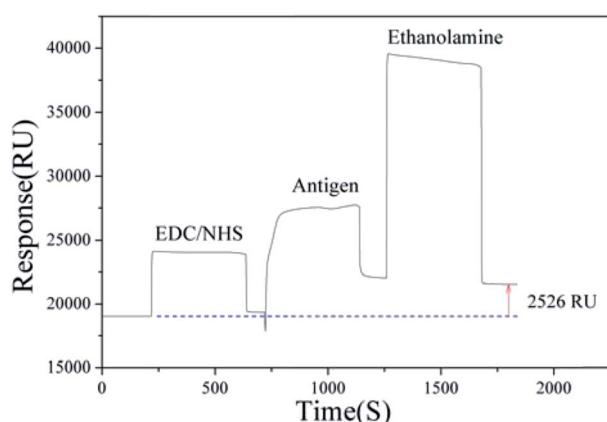
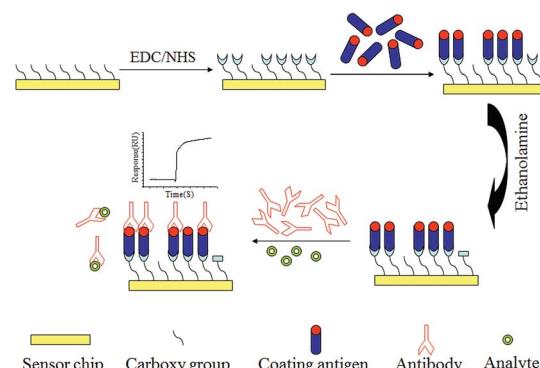


Fig. 1 Immobilization of the antigen.



Scheme 1 The principle and process of the SPR based competitive immunoassay for Cd^{2+} .

Eventually, it was found that the corresponding signal value of the combinative amount of antigen was 2526 RU.

2.4. The whole process of Cd^{2+} detection assay

The principle and process of the method are illustrated in Scheme 1. The CM5 dextran chip surface was firstly activated by EDC/NHS solution, after the coating antigen was immobilized, then the surface was deactivated with ethanolamine. The coating antigen competed for the mAbs with Cd^{2+} in the solution. The SPR response change decreased due to the less amount of the mAbs bound on the chip surface, which was caused by the competition of the high concentration of Cd^{2+} in the solution.

3. Results and discussion

In order to obtain much more sensitive results in this study, the SPR relevant experimental conditions were optimized respectively before the antigen binding experiment. To select the optimal pH value of acetate buffer solution (the antigen diluent), nine parallel experiments were measured so that the corresponding signals were obtained, as shown in Fig. S1 (ESI[†]), it was seen that the corresponding signal of pH value 3.75 was the strongest one, so the acetate buffer solution of pH value 3.75 was selected. Then choosing the appropriate antigen concentration, five experiments with different antigen dilution rates were carried out, as exhibited in Fig. S2 (ESI[†]), it was seen that with the dilution rate increasing, the response values were significantly decreased, but due to the high cost of antigen, resulting in 1 : 10 antigen dilution rate was selected. Finally, four parallel experiments with different flow rates were measured respectively, as exhibited in Fig. S3 (ESI[†]), the 10 $\mu\text{L min}^{-1}$ flow rate was chosen after comprehensive consideration. After screening, it was concluded that the results were satisfactory under these optimal conditions.

The resulting chip above was used as a sensing surface for detecting Cd^{2+} . The mixed solution (v/v = 1 : 1) of the cadmium mAb solution and Cd-EDTA standard solution was prepared, and Cd-EDTA standard solution was diluted into different concentration with HBS-EP before mixing. These solutions were then injected at 10 $\mu\text{L min}^{-1}$ flow rate over the coating



antigen (Cd-ITCBA-OVA) modified surface for 3 min to allow binding assays. Following this, the sensor surface was regenerated by injected 10 μ L 10 mM glycine-HCl (pH 1.7). The corresponding signals and the calibration curve of the SPR based immunoassay for Cd^{2+} in different standard concentrations are shown in Fig. 2 and 3. It was found that the SPR signal steadily decreased as the concentration of Cd^{2+} increased. The IC_{50} value was found to be 23.58 ng mL^{-1} , the limit of detection (LOD) was 1.25 ng mL^{-1} and the linear range of concentration was 3.57–758.37 ng mL^{-1} . Compared with other methods for Cd^{2+} detection, our method had a similar or superior linear range and detection limit (Table S1, ESI[†]).

In order to demonstrate the binding of cadmium mAbs to Cd^{2+} was specific, metal ions such as Cu^{2+} , Ni^{2+} , Co^{2+} , Cr^{3+} , Pb^{2+} , Mg^{2+} and Zn^{2+} were selected to test the cross-reactivity. The concentration of the cross-reacting ions were 0–1000 ng mL^{-1} and applied according to the procedure of the assay, as shown in Fig. 4. One could conclude that no cross-reactivity of the mAbs with Cu^{2+} , Ni^{2+} , Co^{2+} , Cr^{3+} , Pb^{2+} , Mg^{2+} and Zn^{2+} respectively, but the cross-reactivity of the mAbs with Hg^{2+} was 73.12%, which was actually rather serious. The results obtained in this approach were similar to the ELISA using the same mAb.³⁰ It could be caused by the spatial configuration of the chelates that is respectively formed by Hg^{2+} and Cd^{2+} with EDTA are quite similar and the interactions of the chelates with the antibody.

The practical application of the proposed biosensor was evaluated in lake water and tap water. Samples of lake water and tap water were collected from the Wenyang Lake and our laboratory, respectively. Prior to testing, the collected water samples were filtered by 0.22 μ m filter membrane to remove insoluble substances. Then, the SPR based competitive immunoassay was applied to detect the filtrates which were spiked with Cd -EDTA at the concentration of 10 ng mL^{-1} , 20 ng mL^{-1} and 50 ng mL^{-1} , respectively. Acceptable recovery rates of 96.80–102.37% were obtained (as shown in Table 1), which suggested that

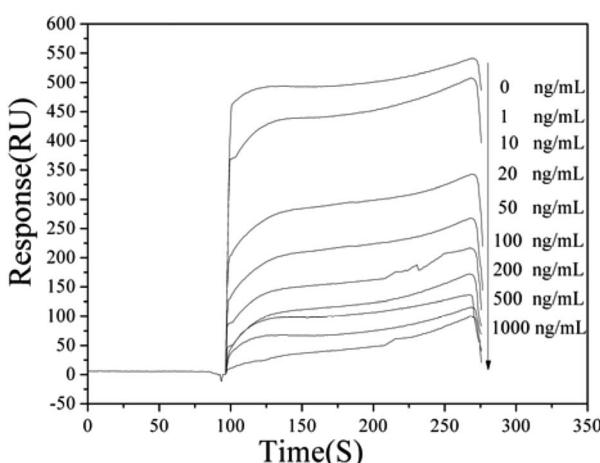


Fig. 2 The SPR signals of the SPR based immunoassay for Cd^{2+} with different standard concentrations. The arrow shows the signal changes with increasing Cd -EDTA concentrations (0, 1, 10, 20, 50, 100, 200, 500, 1000 ng mL^{-1}).

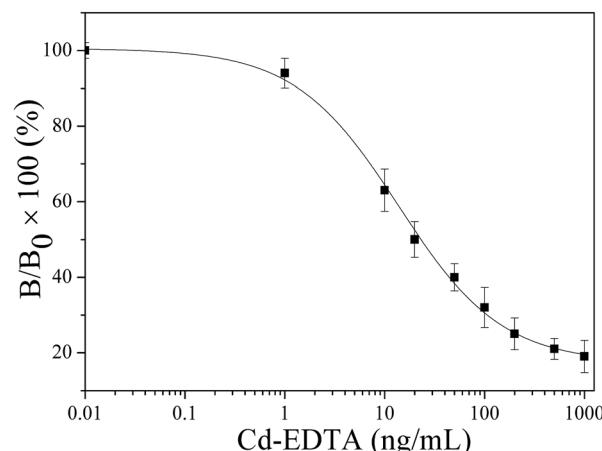


Fig. 3 The calibration curve of the SPR based immunoassay for Cd^{2+} with different standard concentrations, where B and B_0 are the SPR signals of the analyte at the standard point and at the zero concentration of the analyte, respectively.

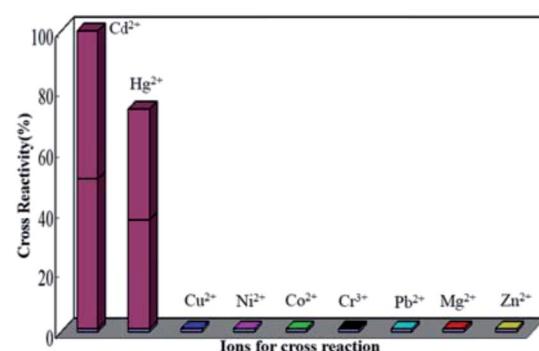


Fig. 4 The cross-reactivity values of the monoclonal antibody with Cd^{2+} and other ions tested by the proposed SPR based competitive immunoassay.

the method could be used for the detection of Cd^{2+} in real aqueous samples.

Note that various cellphone (CP)-based technologies have been developed for multifarious applications.^{33–35} What's more, combining an angle-resolved SPR detection system with cellphone-based devices (CBDs) has been developed and employed for the detection of β_2 microglobulin ($\beta_2\text{M}$),³⁶ and a good analysis result was obtained. Hence, in the near future,

Table 1 Determination of Cd^{2+} in water samples using the proposed method

Samples	Added (ng mL^{-1})	Found, mean \pm SD (ng mL^{-1} , $n = 3$)	Recovery (%)
Lake water	10	9.85 \pm 0.63	98.54
	20	19.36 \pm 1.12	96.80
	50	48.42 \pm 3.07	96.84
Tap water	10	10.24 \pm 0.59	102.37
	20	19.52 \pm 1.23	97.60
	50	49.16 \pm 2.85	98.31



heavy metal ions may also be detected by combining SPR with CBDs technique so that develop a prospective upcoming devices in the environmental and food monitoring.

4. Conclusions

In summary, a sensitive and specific SPR based competitive immunoassay for the detection of Cd^{2+} was developed. It provided a new theoretical and technical support for the detection of heavy metal ions. Based on this novel method, the values of IC_{50} (23.58 ng mL^{-1}) and LOD (1.25 ng mL^{-1}) were obtained. In addition to Hg^{2+} , this method showed good selectivity for Cd^{2+} without interference of some other heavy metal ions. Moreover, the sensing strategy could real-time monitor and shorten the analytical time, which is shorter than previously reported methods.³⁷⁻⁴⁰ This novel biosensor approach not only provides an alternative, sensitive and specific analytical method for the detection of Cd^{2+} , but can also be extended as a useful model for the detection of other small molecular compounds in biological, food and environmental areas.

Conflicts of interest

There are no conflicts of interest to declare.

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