Detection of copper ions using microcantilever immunosensors and enzyme-linked immunosorbent assay

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\textbf{Article info}

\textbf{Abstract}

A sensitive and specific monoclonal antibody (designated as mAb6A9) recognizing a Cu(II)–ethylenediamine–N,N,N,N′–tetraacetic acid (EDTA) complex but not metal-free EDTA was obtained by using an 1-(4-aminobenzyl)–EDTA–Cu(II) complex covalently coupled to a carrier protein as an immunogen to immunize the Balb/c mice. A mAb6A9-modified microcantilever sensor (MCS) was developed. A bending response was found to occur at or below 1 ng mL\textsuperscript{−1} of Cu(II)–EDTA complex. An indirect competitive enzyme-linked immunosorbent assay (icELISA) was developed with mAb6A9. The icELISA had a half maximum inhibition concentration and working range of approximately 1.8 and 0.2–17 ng mL\textsuperscript{−1}, respectively. The icELISA showed cross-reactivity of 18.8%, 1.1% and less than 1% with bivalent cobalt, mercury and other metals, respectively. The icELISA and functionalized MCSs were utilized to analyze the content of copper in spiked tap water samples. The assay conditions were optimized. The results of icELISA and MCS correlated well with those obtained by graphite furnace atomic absorption spectrometry.

1. Introduction

Copper, one of very important transition metals, is widely used in industrial and agricultural processes. It has resulted in serious environmental pollution due to the excessive exploitation and utilization. High concentrations of copper are frequently added to pig and poultry feeds to improve their growth [1]. Copper could accumulate in the livers of human body through the food chain [2]. Cu(II) is a potential mutagenic agent [3]. External applications of higher doses of Cu(II) could result in both clastogenic effects and spindle disturbances in mammalian cells [3]. Menkes’ and Wilson’s diseases are two kinds of human genetic maladies that closely relate with the disorder of copper metabolism [4]. China, U.S. Environmental Protection Agency (EPA) and World Health Organization (WHO) have set the copper limits of 1000, 1300 and 2000 ng mL\textsuperscript{−1}, respectively, in drinking water [5]. To monitor the copper content in the environment and study its toxicity, sensitive and specific analytical methods for copper are desirable.

Current conventional methods for copper determination include atomic absorption spectroscopy (AAS) [6,7], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [8], inductively coupled plasma-mass spectrometry (ICP-MS) [9,10], and stripping potentiometry [11]. Although these methods are well used to determine the trace metals, they are expensive, time-consuming and/or inconvenient for in situ monitoring. Other methods have also been developed for copper determination. For example, Gly-Gly-His tripeptide is highly sensitive and selective to Cu(II) and has been used to develop an electrochemical and a microcantilever biosensor for copper ions [12,13]. Chen et al. [14] developed a fluorescence sensor for the highly selective detection of Cu(II) with a limit of detection (LOD) of 3.6 nM based on the aggregation-induced fluorescence quenching of the highly fluorescent glutathione-capped gold nanoparticles. Summer et al. [15] described a wild type form of red fluorescent protein as a highly sensitive, selective, and reversible fluorescence-biosensor for both Cu(I) and Cu(II) and the LOD for Cu(II) was below 1 ppb. Zhou et al. [16] reported a colorimetric method for the detection of Cu(II) based on the color change of the colloid gold in aqueous solutions by using azide and terminal alkyne-functionalized gold nanoparticles, which the minimum concentration of Cu(II) detectable by naked eye was approximately 50 mM. These Cu(II) sensors are based on different principles and have provided alternative methods for the analysis of copper ions.

Enzyme-linked immunosorbent assay (ELISA) is rapid, sensitive, selective and cost-effective. It has been one of the principal detection methods for environmental contaminants [17–19]. Since
plexes and various immunoassays have been developed, such as Cd(II) [21], Co(II) [22], Pb(II) [23,24], Cr(III) [25], UO₂(II) [26,27], Al(III) [28] and Hg(II) [29]. To our knowledge, antibody against Cu(II) has not been reported yet.

Microcantilever sensor (MCS) is a technique utilizing the bending response of microcantilever, which is driven by the formation of surface stress induced from the adsorption of molecules onto the functional layer of a bi-material microcantilever, and recording of either optical or electronic signals [30]. Since the 1990s, MCSs have been studied for the detection of various biomacromolecules [31–34], however, only a few reports were found for the detection of small molecules [35–38]. Xi et al. [39] reported an unmodified gold-coated microcantilever having a LOD of 10⁻¹¹ M Hg(II). Ji and Thundat [40] developed a microcantilever modified with 11-mercaptoundecanol or 2-(4-mercaptophenonyl)-N,N-diethylacetamide to form ion-selective self-assembled monolayer, which could be used to detect a concentration of 10⁻⁸ M Ca(II). Cherian et al. [41] reported the detection of heavy metal ions using protein (AgNt84-6)-functionalized microcantilever sensors. Microcantilevers were also developed for the detection of CrO₄²⁻ based on either hydrogel swelling MCS [42] or a self-assembled monolayer MCS modified with triethyl-12-mercaptododecylammonium bromide [43]. However, these MCSs developed for the detection of heavy metals are often not highly selective [40–43]. Microcantilever immunosensors utilize highly selective antibodies and combine the advantages of both ELISA and MCSs. As compared with ELISA, microcantilever immunosensors do not need enzyme-tracers and can detect multiple analytes in a single step in real-time, in situ monitoring, and consequently lower the analytical cost.

In the present study, a microcantilever immunosensor and an icELISA, which a highly sensitive and specific monoclonal antibody against copper–chelate complex is used, have been developed. According to the copper limit criteria for drinking water set by China, EPA and WHO, the two methods were used to detect the content of copper in tap water and were validated by comparison with graphite furnace atomic absorption spectrometry (GFAAS).

2. Experimental

2.1. Chemicals and apparatus

1-(4-Aminobenzy)-ethylenediamine-N,N,N',N”-tetraacetic acid (A–Bz–EDTA) was purchased from Dojindo Laboratories (kumamoto, Japan) and was used, without further purification, to form a Cu–EDTA complex as the hapten (Fig. 1). Bovine serum albumin (BSA), ovalbumin (OVA), ethylenediamine–N,N,N',N”–tetraacetic acid (EDTA), iminodiacetic acid (IDA), 2-iminohiothiolane hydrochloride, 3,3,5,5’-tetramethylbenzidine (TMB), copper chloride (99.999%) and other atomic absorption grade metal ions were purchased from Sigma–Aldrich (St. Louis, MO). Goat anti-mouse IgG conjugated with horseradish peroxidase (IgG-HRP) was purchased from Jackson Immunoresearch Laboratories (West Grove, PA). All other reagents were obtained from Beijing Chemical Reagents Company (Beijing, China).

Cell culture plates and 96-well polystyrene microtiter plates were obtained from Costar (Corning, NY). Silicon nitride microcantilevers (Veeco Instruments, Plainview, NY) were used. The dimensions of the V-shaped microcantilevers were 0.6 µm × 40 µm × 200 µm. One side of the microcantilever had a thin film of chromium (15 nm) covered with a 60-nm layer of gold deposited by e-beam evaporation.

2.2. Buffers and solutions

Buffers and solutions used include coating buffer (0.05 M carbonate buffer, pH 9.6), phosphate-buffered saline (PBS) (0.1 M phosphate buffer containing 0.5% sodium chloride, pH 7.5), PBS with 0.1% (v/v) Tween-20 (PBST), citrate-phosphate buffer (0.01 M citric acid and 0.03 M monosodium phosphate, pH 5.5), substrate solution (0.4 mg urea peroxide added to 10.0 mL of citrate-phosphate buffer containing 0.1 mg mL⁻¹ TMB), and a stop solution (2.0 M sulfuric acid). Deionized water used for making buffers and solutions was collected from a Millipore Water Purification System (Millipore Co., Billerica, MA).

2.3. Preparation of immunogen and coating antigen

To a solution of 4 mg A–Bz–EDTA dissolved in 2 mL of 1 M hydrochloric acid was added dropwise 60 µL of fresh 0.2 M NaNO₂ solution at 0 °C. After being stirred for 15 min at 0 °C, the solution was adjusted to pH 7.5, PBS with 0.1% (v/v) Tween-20 (PBST), citrate-phosphate buffer (0.01 M citric acid and 0.03 M monosodium phosphate, pH 5.5), substrate solution (0.4 mg urea peroxide added to 10.0 mL of citrate-phosphate buffer containing 0.1 mg mL⁻¹ TMB), and a stop solution (2.0 M sulfuric acid). Deionized water used for making buffers and solutions was collected from a Millipore Water Purification System (Millipore Co., Billerica, MA).

2.4. Immunization protocol, monoclonal antibody production, purification and characterization

Six female Balb/c mice, 7 weeks old, were immunized with the immunogen (Cu(II)–EDTA–Bz–OVA). The protocols of immunization, fusion, antibody production and purification were the same as described previously [44]. The cross-reactivity with other metals was detected with icELISA, which the procedure is described in details in Supplementary content.

2.5. Monoclonal antibody sulfhydrylation

The sulfhydrylation protocol was the same as previously described [38]. The dialyzed sulfhydrated mAbs were diluted to 1.0 mg mL⁻¹ with PBS containing 0.1 mM EDTA and stored at −40 °C. The binding activities of the antibodies before and after sulfhydrylation were tested by non-competitive ELISA, which the procedure is detailed in Supplementary content.

Fig. 1. A general synthesis schematic and chemical structure of the immunogen and coating antigen.
2.6. Functionalization of microcantilever sensor with 2-iminothiolane sulfhydrylated mAbs

The functionalization was performed in a microplate well that was replaced to a new one after each step. The microcantilever was immersed in washing solution (3 mL sulfuric acid and 1 mL 30% H₂O₂) for 10 min before use, and then was washed with deionized water. The microcantilever was placed into a microplate well and blocked with 200 µL of 3% non-fat milk in PBS for 30 min at 37 °C, and then was washed with PBST 4 times. The microcantilever was immersed in 200 µL of 5.0 µg mL⁻¹ sulfhydrylated mAbs solution in PBS for 30 min at 37 °C. The functionalized microcantilever was washed with PBST 4 times and dried under a gentle stream of nitrogen gas for use.

2.7. Characterization of activity of antibody immobilized on microcantilever using ELISA

Non-competitive ELISA was used to confirm the antibodies immobilized on the microcantilever. The decorated microcantilever and naked microcantilever were separately put into microplate wells. To the wells 200 µL of goat anti-mouse IgG-HRP diluted in PBS was added. After incubated at 37 °C for 30 min, the microcantilevers were washed 4 times with PBST. After the two microcantilevers were transferred to new wells, 200 µL per well of substrate solution was added and incubated at 37 °C for 15 min. The reaction was stopped by adding 50 µL of the stop solution. The microcantilevers were removed from the wells and the absorbance was read at 450 nm on a microplate reader.

2.8. Microcantilever immunosensor detection

The functionalized microcantilever was mounted onto the fluid cell filled with PBS (Fig. 2). The PBS was circulated through the cell by a peristaltic pump. The flow rate was controlled at 4 mL h⁻¹ and kept constant during each experiment. The temperature of fluid cell was maintained at 310.00 ± 0.01 K while the room temperature was maintained at 301 ± 0.5 K. After the deflection baseline was stabilized, the analytes dissolved in 2 mL PBS containing 2000 ng mL⁻¹ EDTA were injected into the fluid cell. The detection data of each analyte was calculated by using the OriginPro 7.5 software. A linear calibration curve was made between the deflection responses and the varying concentrations of Cu(II) dissolved in PBS containing 2000 ng mL⁻¹ EDTA and the concentrations of Cu(II) in samples were calculated using the calibration curve.

2.9. Preparation and analysis of tap water samples

All glassware was washed with a washing solution of 60% sulfuric acid, 30% nitric acid and 10% deionized water and then rinsed with deionized water. All plastic ware was soaked overnight in 3 M HCl and rinsed with deionized water prior to use [45]. Tap water samples 1–6 were collected from the taps in the laboratory (i.e., Beijing municipal water supply) in 5-day intervals. The water samples were acidified by adding 10 µL concentrated HCl in 1 mL water sample, then filtered through a 0.45 µm syringe filter and stored in pre-cleaned 50-mL centrifuge tubes. A series of copper-spiked water samples were prepared at concentrations ranging from 100 to 2000 ng mL⁻¹ by adding a copper standard solution (0.1 mg mL⁻¹ Cu(II) in the deionized water). The sample pH was adjusted to 7.5 prior to the assays. The copper content in all test samples were also detected by GFAAS.

3. Results and discussion

3.1. Characteristics of monoclonal antibody against Cu(II)–EDTA–Bz–ova conjugate

A monoclonal antibody, designated as mAb6A9, was generated with Cu(II)–EDTA–Bz–ova as the immunogen. The dissociation constant (Kd) of the antibody was determined with the method of Beatty [46]. The Kd value was 2.3 × 10⁻⁹ M. The mAb6A9 is an IgG1 isotype that has x light chains. In this study, a cost-effective, commercially available chelate A–Bz–EDTA was used to synthesize the immunogen of copper ion. Several bifunctional chelate reagents were reported for the synthesis of metal immunogens via coupling with carrier proteins and formation of metal–chelate complexes. Those reagents include isothiocyanato-benzyl-ethylendiamine–N,N,N,N–tetraacetic acid (ITCBE) [21], isothiocyanato-benzyl-diethylenetriamine pentacetic acid (ITCBDETA) [22], N-(2-amino-3-p-isothiocyanatophenyl-propyl)-trans-cyclohexane-1,2-diamine-N,N,N,N′,N′-diethylenetriamine pentacetic acid (CHX-A) [23] and 5-isothiocyanato-1,10-phenanthroline-2,9-dicarboxylic acid (ITCCDCP) [26]. The conjugation of those chelate reagents with carrier proteins is very simple and readily occurs in a weakly basic condition. However, those reagents are more expensive than A–Bz–EDTA. It is noteworthy that the chelating reactions between metal ions and chelating reagents must be carried out after the formation of chelate–protein conjugate when using the A–Bz–EDTA as the bifunctional reagent, because the diazotization of A–Bz–EDTA needs a strong acid condition, which could dissociate metal–chelate complex. A–Bz–EDTA has been successfully used for production of antibodies recognizing other heavy metals such as Cd(II), Pb(II) and Hg(II) in our laboratory (the detailed data will be published in other manuscripts).

The cross-reactivity with the other metals was investigated with icELISA. Table 1 shows that the cross-reactivity of mAb6A9 with Co(II), Hg(II), Ag(I), Cd(II), Pb(II), Al(III) and Fe(III) is 18.8%, 1.1%, 0.9%, 0.8%, 0.3%, 0.2% and 0.1%, respectively, and below 0.1% with other metals.

3.2. Optimization of icELISA

The optimal concentrations of coating antigen, mAb and goat anti-mouse IgG-HRP were screened by checkerboard titration. Concentrations of 0.25 µg mL⁻¹ of Cu(II)–EDTA–Bz–BSA, 0.1 µg mL⁻¹ of mAb and 0.1 µg mL⁻¹ of goat anti-mouse IgG-HRP were selected and used throughout this work. The protocol of icELISA was described in Supplementary content. Fig. 3 shows a representative inhibition curve for Cu(II) by icELISA. The IC₅₀ values of the icELISA
were approximately 1.8 ng mL\(^{-1}\), and the calibration range, based on 10–90% of inhibition of binding of mAb6A9 to Cu(II)–EDTA, was 0.2–17 ng mL\(^{-1}\).

3.3. Activities of antibodies functionalized on microcantilever

The 2-iminothiolane hydrochloride, a thiolating reagent for primary amines, was used to introduce free sulfhydryl groups by amide bonds on the antibodies for microcantilever functionalization in a single step [47]. The effect of sulfhydrylation on antibody activity was investigated by non-competitive ELISA. The results showed that sulfhydrylation of the mAb by using 2-iminothiolane caused loss of approximately 18% of the binding activities (sulfhydrylation decreased OD from 1.256 to 1.026, the data was obtained by triplicate determinations in the same ELISA plate), which are similar with the result of our previous report [38]. The activity decrease may be attributed to disulfide bond breakage and denaturation of the antibody during the sulfhydrylation process.

Non-competitive ELISA was used to confirm the antibody activities immobilized on the microcantilever. The OD values of the decorated and naked microcantilevers by the non-competitive ELISA were 0.638 and 0.059, respectively, which indirectly verified the successful functionalization of the microcantilevers.

3.4. Detection of Cu(II) by microcantilever immunosensors

In the functionalized microcantilever immunosensors, Cu(II)–EDTA standard sample was added into the fluid cells at a concentration range from 0.1 to 100 ng mL\(^{-1}\). Fig. 4 shows the deflections of microcantilever in response to varying concentrations of Cu(II). When LOD is defined as a signal of 3-fold of the background noises, the results showed that the LOD of MCS for Cu(II) is approximately 1 ng mL\(^{-1}\).

Velanki et al. [48] reported the detection of Cd(II) using microcantilever sensors functionalized with antibodies via a layer-by-layer multilayer self-assembly technique. This multilayer approach was very complicated. Furthermore, the content of Cd(II) was detected by injecting the Cd(II)–EDTA–Bz–BSA conjugate but not Cd(II)–EDTA to a flowing fluid stream, which heavy metals could not be specifically adsorbed onto the BSA and thus cause the result unrepeatable [28,49].

3.5. Regeneration and reproducibility of microcantilever immunosensor

A high degree of uniformity of physical features is required for the microcantilevers. In the present study, the microcantilevers were not regenerated for reuse. Five microcantilevers were used to estimate the degree of uniformity in the same functionalized method and assay condition. The results showed that at 10 ng mL\(^{-1}\) of Cu(II), the bending amplitude of the micro-beams was 59 nm with the relative standard deviation (RSD) of 5.7%, indicating acceptable reproducibility.

3.6. Microcantilever immunosensor, ELISA and GFAAS analyses of copper ions in spiked tap water

Minimizing matrix interferences is important for successful applications of metal ions immunoassays. A strategy is to improve the specificity and sensitivity of the antibody against Cu(II)–EDTA through molecular biotechnology, such as phage display technique [19,50]. This approach can be adopted to solve the problem of cross-reactivity. It is, however, time-consuming and technically complex. China, EPA and WHO have recommended that the copper limits in drinking water be 1000, 1300 and 2000 ng mL\(^{-1}\), respectively. The highly sensitive MCS and icELISA established allow more than 1000-fold dilution of the samples to reduce the matrix interference and thus increase the accuracy of the measurements. To determine the matrix interference at 100, 500, 1000-fold dilutions, samples 1–5 were prepared and fortified at 100, 500, 1000 ng mL\(^{-1}\) of Cu(II). The content of copper ions in the unfortified samples ranged from 6 to 8 ng mL\(^{-1}\) detected by GFAAS. PBS spiked with the corresponding levels of Cu(II) was the control. All spiked samples were diluted to 1 ng mL\(^{-1}\). Each sample was analyzed for three times (3 microplate wells per sample each time) by icELISA. When the fortified samples were diluted for 500- or 1000-fold, the recoveries were between 94% and 112%. The recoveries of 100-fold diluted samples ranged from 109% to 127%, suggesting some matrix interference (Table S1).

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Table 1

<table>
<thead>
<tr>
<th>Ion</th>
<th>IC(_{50}) (ng mL(^{-1}))</th>
<th>Cross-reactivity (%)</th>
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<tbody>
<tr>
<td>Cu(II)</td>
<td>1.8 ± 0.2*</td>
<td>100 ± 6.7</td>
</tr>
<tr>
<td>Co(II)</td>
<td>9.6 ± 0.9</td>
<td>18.8 ± 1.8</td>
</tr>
<tr>
<td>Hg(II)</td>
<td>168.8 ± 15.9</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Ag(I)</td>
<td>195.8 ± 16.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>227.5 ± 19.5</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>240.2 ± 0.2</td>
<td>0.3 ± 0.03</td>
</tr>
<tr>
<td>Al(III)</td>
<td>1195.2 ± 153.4</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>1523.4 ± 127.4</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>&gt;2000</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>In(III)</td>
<td>&gt;2000</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>&gt;2000</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Mg(II)</td>
<td>&gt;2000</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>&gt;2000</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

*Values are mean of triplicate determinations ± SD (standard deviation).
The average recovery and coefficient of variation after 1000-fold dilution was 101% and 7.7%, respectively, and those after 100, 500-fold dilution were 118% and 13.3%, 106% and 8.9%, respectively. Fig. 5 shows that no noticeable matrix effect was observed when the tap water was detected by MCS after 1000-fold dilution, but a little matrix interference after only 100-fold dilution.

Because metal ions are ubiquitous in the environment, it was important to investigate the effect of non-target metals on the reliability of the two assays. However, it is difficult to estimate the interference of each non-target metal to the Cu(II) assay. Therefore, some common metals were selected to test. Although the cross-reactivity of mAb6A9 with Co(II), Hg(II), Ag(I), Cd(II), Al(III) and Fe(III) is relatively higher than the other metals (Table 1), the interferences from Co(II), Hg(II), Ag(I) and Cd(II) can be neglected due to their very low concentrations in tap water, particularly after 1000-fold dilution. The metal ions including Ca(II), Mg(II), Al(III) and Fe(III) were relatively high concentrations in the tap water matrix would interfere the assay results. Sasaki et al. [51] reported a simple method to reduce interference from excess Mg(II) in Cd(II) immunoassays by controlling the added EDTA content in samples, and presented a two-step model in which Cd(II) and Mg(II) competed for EDTA due to the different formation constants following the order Mg(II) > Ca(II) > Al(III) > Fe(III). According to the method reported by Sasaki et al. [51], the influence of EDTA on the MCS and icELISA was investigated in a concentration range of 0.2–17 ng mL\(^{-1}\) of EDTA without Mg(II) in Cd(II) immunoassays by controlling the added EDTA content in samples, and presented a two-step model in which Cd(II) and Mg(II) competed for EDTA due to the different formation constants followed by Cd(II)–EDTA and Mg(II)–EDTA competing for antibodies based on the different affinities. This method [51] relies on that the formation constant of non-target metal–EDTA is much lower than that of target metal–EDTA, thus, it is also effective for Ca(II) but not for Al(III) and Fe(III).

4. Conclusions

To our knowledge, it is the first report on monoclonal antibody-based microcantilevers and ELISAs for copper ions. The IC\(_{50}\) values of the icELISA were approximately 1.8 ng mL\(^{-1}\), and the working range, based on 10–90% of inhibition of binding of mAb6A9 to Cu(II)–EDTA, was 0.2–17 ng mL\(^{-1}\). The LOD of MCS for Cu(II) is approximately 1 ng mL\(^{-1}\). The two assays developed in the present study are sensitive enough for monitoring of Cu(II) in drinking water at the levels set by China, EPA and WHO. The samples need only simple dilution in PBS containing 2000 ng mL\(^{-1}\) EDTA without other sample preparation steps. Further applications may include analysis of copper ions in other matrices, such as contaminated soils or foods.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2010.07.041.

References


Table 2

<table>
<thead>
<tr>
<th>Cu(II) fortified (ng mL(^{-1}))</th>
<th>icELISA</th>
<th>MCS</th>
<th>GFAAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND(^a)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>500(^b)</td>
<td>467.5 ± 37.8(^c)</td>
<td>437.6 ± 30.2</td>
<td>556.1 ± 32.7</td>
</tr>
<tr>
<td>1000</td>
<td>1101.3 ± 101.3</td>
<td>1113.8 ± 89.7</td>
<td>989.6 ± 106.9</td>
</tr>
<tr>
<td>1500</td>
<td>1539.6 ± 102.6</td>
<td>1432.2 ± 112.3</td>
<td>1689.0 ± 134.4</td>
</tr>
<tr>
<td>2000</td>
<td>2187.5 ± 205.3</td>
<td>1923.1 ± 152.5</td>
<td>2085.3 ± 252.3</td>
</tr>
</tbody>
</table>

\(^a\) ND means not detected.
\(^b\) Samples spiked 500 ng mL\(^{-1}\) of Cu(II) were diluted 500-fold.
\(^c\) Values are mean of triplicate determinations ± SD (standard deviation).

Fig. 5. Effects of interferences in tap water on microcantilever deflection. (A) The tap water spiked 100 ng mL\(^{-1}\) of Cu(II) was diluted 100-fold for detection. (B) The tap water spiked 1000 ng mL\(^{-1}\) of Cu(II) was diluted 1000-fold for detection. (C) PBS spiked 1 ng mL\(^{-1}\) Cu(II) for detection. (D) The tap water was diluted 100-fold for detection. (E) The tap water was diluted 1000-fold for detection.

Comparison of icELISA, MCS and GFAAS for the analysis of tap water spiked with Cu(II).