

## Nerve Agents Detection Using a Cu<sup>2+</sup>/L-Cysteine Bilayer-Coated Microcantilever

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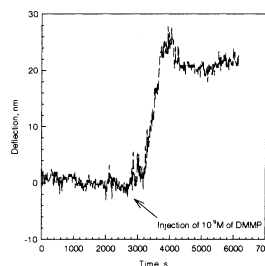
Rapid and sensitive detection of chemical and biological warfare agents, including nerve gases, is of great interest especially after the tragic events of September 11, 2001. All nerve agents belong chemically to the organophosphorus compound group, which is among the most toxic of known substances.<sup>1</sup> The nerve agent, either as a gas, as an aerosol, or as a liquid, enters the body through inhalation or through the skin. Poisoning may also occur through consumption of liquids or foods contaminated with nerve agents. If a person is exposed to a high concentration of nerve agent, for example, 200 mg sarin/m<sup>3</sup>, death may occur within a couple of minutes when the agent is absorbed through the respiratory system. Poisoning takes longer when the nerve agent enters the body through the skin or through consumption.

In recent years, microcantilevers have been used as a platform for the development of many novel physical, chemical, and biological sensors.<sup>2–4</sup> One unique microcantilever feature is that when molecular adsorption is confined to one surface of the microcantilever, it undergoes bending due to adsorption-induced stress.<sup>5–9</sup> Using this concept, we have demonstrated the feasibility of highly sensitive chemical detection of several vapor phase analytes, as well as cations and anions in solution.<sup>2,3</sup>

For chemical sensing, a specific chemical recognition agent can be coated on one surface of a microcantilever to make it molecular specific. Self-assembled monolayers and multilayers are of particular interest<sup>10,11</sup> because of their advantages over other materials for microcantilever surface modification, including good reproducibility, no calibration needed for each microcantilever, and ease of operation. A self-assembled bilayer of Cu<sup>2+</sup>/L-cysteine on a gold surface has recently been characterized.<sup>12,13</sup> Such a Cu<sup>2+</sup>/L-cysteine bilayer could be used to recognize phosphonyl groups due to the formation of strong P=O–Cu<sup>2+</sup> bonds. We report herein a microcantilever sensor for the detection of nerve agents in aqueous solution based on this Cu<sup>2+</sup>/L-cysteine bilayer-modified cantilever. Dimethyl methyl phosphonate (DMMP) was used as a Sarin nerve gas simulant. The cantilevers and the experimental setup were the same as those we have reported previously.<sup>10</sup>

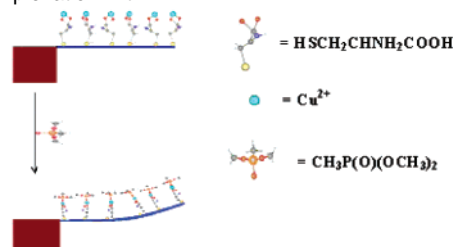
In these experiments, a Cu<sup>2+</sup>/L-cysteine bilayer on a gold-coated microcantilever was formed by immersing the cantilever into a 10<sup>–3</sup> M solution of L-cysteine in tris buffer solution (pH = 5) for 24 h. The microcantilever was then rinsed with a tris buffer solution three times and immersed in a 1 × 10<sup>–3</sup> M CuSO<sub>4</sub> tris buffer solution for 24 h.

The self-assembled Cu<sup>2+</sup>/L-cysteine bilayer-coated microcantilever was initially exposed to a constant flow (4 mL/L) of tris buffer (pH = 5.0), and the cantilever was equilibrated until a stable baseline was obtained (i.e., 0 nm deflection). When a 10<sup>–9</sup> M solution of DMMP in tris buffer (pH = 5.0) is introduced into the fluid cell to replace the buffer solution at the same 4 mL/L flow



**Figure 1.** Bending response as a function of time,  $t$ , for a silicon microcantilever coated with a self-assembled bilayer of Cu<sup>2+</sup>/L-cysteine on the gold surface after injection of a 10<sup>–9</sup> M solution of DMMP in 0.01 M tris buffer at pH = 5.0. The microcantilever was preequilibrated in the 0.01 M tris buffer solution before injection of the DMMP solution.

### Scheme 1. Schematic Representation of the Bending of a Self-Assembled Cu<sup>2+</sup>/L-Cysteine Bilayer-Coated Microcantilever upon Complexation with DMMP

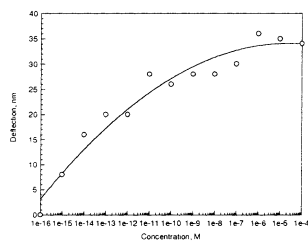


rate, the microcantilever bends up and reaches a maximum amplitude in 15 min. The maximum amplitude of the microcantilever deflection is approximately 25 nm. When a tris buffer solution flows into the fluid cell to replace the DMMP solution, the microcantilever slightly bends down, but does not return to its original position (e.g., 0 nm). The deflection of the microcantilever remains at an amplitude of approximately 22 nm as shown in Figure 1.

It has been shown that phosphonyl groups strongly bind with Cu<sup>2+</sup> and copper complexes.<sup>14–17</sup> Due, in part, to the affinity of phosphonyl groups for copper complexes, several copper complexes have been used as catalysts for the hydrolysis of the phosphorus ester moiety of organophosphorus compounds.<sup>17</sup> Organophosphorus compounds are fairly unstable at high pH; for example, the half-life of Sarin is 2 min at pH = 10. However, the half-life of Sarin dramatically increases to approximately 8 days at pH = 5. The catalytic effect of copper complexes for hydrolysis at the phosphorus ester site is only enhanced when the pH is above 7. In the present study, fresh DMMP solutions were prepared 1 h before each measurement.<sup>17</sup> DMMP is more stable than Sarin. Thus, under our experimental conditions (pH = 5) and time scale (30 min), the hydrolysis of DMMP can be neglected. Given this, the up-bending of the microcantilever upon exposure to DMMP is most likely caused from the complexation of DMMP with the Cu<sup>2+</sup>/L-cysteine

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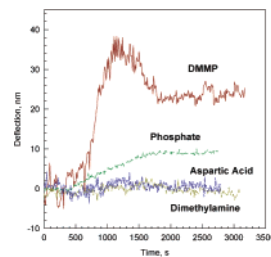
**Figure 2.** Maximum deflection of a silicon cantilever coated with a self-assembled bilayer of  $\text{Cu}^{2+}$ /L-cysteine on the gold surface as a function of the concentration of DMMP in 0.01 M tris buffer at pH = 5.0.

bilayer on the microcantilever surface through  $\text{Cu}^{2+}$ -O=P bonds that alter the surface tension of the microcantilever as shown in Scheme 1. For this 25 nm deflection, the surface stress caused by the binding of DMMP on the  $\text{Cu}^{2+}$ /L-cysteine bilayer surface is  $1.07 \times 10^{-9}$  N/m.<sup>4</sup> Contact angle experiments were conducted to characterize the surface polarity of the  $\text{Cu}^{2+}$ /L-cysteine bilayer surface on the gold before and after exposure to DMMP. A contact angle of  $77.6^\circ$  was observed for the  $\text{Cu}^{2+}$ /L-cysteine bilayer, and a contact angle of  $81.2^\circ$  was noted after exposure of this coating to DMMP. This is consistent with our conclusion that the DMMP is adsorbed on the  $\text{Cu}^{2+}$ /L-cysteine bilayer surface.

The slight back-bending of the microcantilever after the DMMP solution was replaced by tris buffer in the fluid cell might be caused by the physical absorption of DMMP on the surface of the  $\text{Cu}^{2+}$ /L-cysteine bilayer on the microcantilever that could be readily washed off of the surface when the buffer was switched into the fluid cell.

Figure 2 shows the maximum deflection amplitude of a  $\text{Cu}^{2+}$ /L-cysteine bilayer-coated microcantilever versus the concentration of DMMP in the solution. DMMP varies from a low concentration of  $10^{-16}$  M to a high concentration of  $10^{-4}$  M. The plot shows that this microcantilever can be used for the detection of DMMP with a concentration as low as  $10^{-15}$  M in aqueous solution. The high sensitivity for DMMP detection suggests that there is a strong complexation of the  $\text{Cu}^{2+}$ /L-cysteine bilayer with DMMP at pH = 5.

Control experiments were performed on unmodified microcantilevers and on microcantilevers modified with a  $\text{Cu}^{2+}$ -free L-cysteine monolayer. No deflection of the cantilever was observed upon exposure to a  $10^{-5}$  M solution of DMMP. The effects of other compounds that can complex with  $\text{Cu}^{2+}$ , such as DL-aspartic acid, dimethylamine, 1,10-phenanthroline, acetic acid, acetonitrile, and sodium phosphate, on the deflections of this  $\text{Cu}^{2+}$ /L-cysteine bilayer-coated cantilever were investigated. Dimethylamine and DL-aspartic acid caused a slight 1 and 3 nm deflection, respectively, as shown in Figure 3. No microcantilever bending was observed upon exposure to 1,10-phenanthroline, acetonitrile, and acetic acid (lines not shown for clarity). An approximately 10 nm up-bending deflection of the microcantilever was observed when sodium phosphate interacted with the copper coating on the microcantilever. To elucidate whether phosphate ions may interfere with the detection of organophosphorus compounds using this microcantilever, a comparison experiment was performed on the bending response of a  $\text{Cu}^{2+}$ /L-cysteine bilayer-coated microcantilever to DMMP in the presence and absence of phosphate ions. An approximately 27 nm deflection of the cantilever was observed



**Figure 3.** Bending response as a function of time,  $t$ , for a silicon cantilever coated with a self-assembled bilayer of  $\text{Cu}^{2+}$ /L-cysteine on the gold surface of the microcantilever after injection of a  $10^{-6}$  M solution of various electrolytes in 0.01 M tris buffer at pH = 5.0.

when the cantilever was exposed to a  $10^{-9}$  M solution of DMMP in the presence of  $10^{-5}$  M phosphate ions, which is similar to that obtained without phosphate ions in the solution. These results suggest that the  $\text{Cu}^{2+}$ /L-cysteine bilayer-coated microcantilever could be a potential organophosphorus sensor with high selectivity.

In summary, the  $\text{Cu}^{2+}$ /L-cysteine bilayer-coated microcantilever demonstrated high sensitivity and selectivity toward organophosphorus compounds in aqueous solution. The microcantilever undergoes bending upon exposure to DMMP at concentrations as low as  $10^{-15}$  M due to the complexation of the phosphonyl group and the  $\text{Cu}^{2+}$ /L-cysteine bilayer on the microcantilever surface. We are currently investigating the sensitivity of this sensor in air.

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