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One of the most toxic forms of Hg known is monomethylmercury (CH_3Hg^+ or MM).[1] MM is soluble in water and is incorporated into sludges and sediments. Plant and sedimentary materials are consumed by small fish, which are consumed by increasingly larger fish. MM, being highly lipid soluble, is readily taken up into the food chain but not easily cleared.[2] MM is readily absorbed by humans.[3] Once in the blood, MM is distributed to all tissues, including transport to the nursing infant. MM accumulates in the nervous system, where it leads to neuronal damage.[3]

The development of a scientifically-sound, low-cost, and effective means of detecting MM would significantly reduce the cost of clean-up of Hg-contamination of the environment in the longer term. An extensive survey of the literature, however, suggests that no such sensors have been developed for MM detection. Traditional GC, GC-MS and HPLC are typically used in sample analysis for MM identification and quantification. These methods require instrumentation that is emphatically non-portable, expensive, complex, and unsuited to the analysis of samples in the field.

In recent years, microcantilevers have shown their potential as a platform for the development of many novel physical, chemical, and biological sensors.[4–6] The principle of microcantilever operation in a liquid environment is that when molecular adsorption is confined to one surface of a microcantilever, cantilever bending occurs due to adsorption-induced stress. Using this concept, the feasibility of high-sensitivity chemical detection of a number of vapor phase analytes, and cations and anions in solution has been demonstrated.[4,7,8]

Selective chemical sensors can be prepared by coating or covalently linking a molecular recognition agent (receptor)[9,10] to the microcantilever surface. However, no molecular recognition agents for MM have been developed so far. It is known that monosubstituted organomercury readily forms a complex with a thiol compound. Such complexes are usually very strong, with equilibrium constants of 10^{10}–10^{20} M^{-1}.[11] Thiol compounds also form complexes with many other heavy metal ions, such as Pb^{2+},

Cu^{2+}, Zn^{2+}, *etc.* The complexation is typically used for nonspecific removal and cleanup of heavy metal ions. In this paper we report that 1,6-hexanedithiol monolayer acts as an unusually specific recognition agent for CH_3Hg^+ when a microcantilever is used as the transducer. The mechanism of the sensor is discussed.

A 1,6-hexanedithiol self-assembled monolayer (SAM) on a gold-coated cantilever was formed by immersing the cantilever in a 10^{-2} M solution of 1,6-hexanedithiol in EtOH for 12 h, and rinsing with EtOH three times. The deflection measurements were carried out with an AFM apparatus (Veeco Instruments, USA) with an unmodified cantilever as reference. A schematic diagram of the instrument used in this study has been illustrated previously.[7] In our experiments, we used commercially available silicon microcantilevers (Veeco Instruments). The dimensions of the V-shaped silicon microcantilevers were 180 μm in length, 25 μm in leg width, and 1 μm in thickness. The spring constant was 0.26 N m^{-1}. One side of these cantilevers was covered with a thin film of chromium (3 nm), followed by a 20 nm layer of gold, both deposited by E-beam evaporation. On the uncoated side of the commercial microcantilever was silicon with a 12–19 Å thick, naturally grown SiO_2 layer, which is called "native oxide". Methylmercury chloride was obtained from Aldrich.

1,6-hexanedithiol can form two monolayer structures on a gold(111) surface: upright monosulfide, or the looped disulfide. The experimental conditions are critical in preparing the required monolayer. It is reported that dithiols adopt an upright configuration[12,13] when the gold surface is treated by immersing into a 1×10^{-2} M solution of 1,6-hexanedithiol solution in alcohol (adopted in this work). An upright 1,6-hexanedithiol monolayer film allows binding of CH_3Hg^+ to the microcantilever surface *via* the formation of a S–Hg bond between $HgMe^+$ and a free sulfhydryl (–SH) group on the outer surface of the monolayer. We anticipate that when CH_3Hg^+ is adsorbed on the monolayer surface, the repulsion of CH_3Hg^+ groups will alter the surface stress and consequently bend the cantilever.

The 1,6-hexanedithiol SAM modified microcantilever was initially exposed to a constant flow of de-ionized water at 4 mL h^{-1} and the cantilever was equilibrated until a stable baseline was obtained. Since flow rate changes will alter the baseline, a constant flow rate was maintained during each experiment. Defined concentrations of CH_3Hg^+ solution were injected and passed through the fluid cell at the same 4 mL h^{-1} flow rate. A new cantilever was used for each sample injection. When a 10^{-5} M solution of CH_3Hg^+ was switched into the fluid cell, the cantilever immediately bent downward and irreversibly reached its maximum (saturated) deflection of approximately 22 nm in

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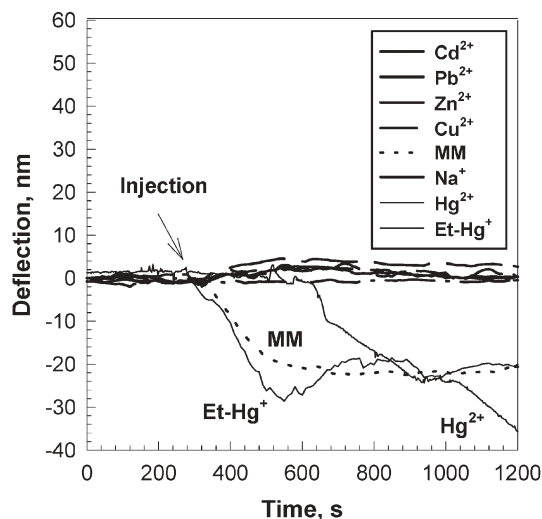
Fig. 1 The bending response of a silicon cantilever coated with a self-assembled monolayer of 1,6-hexanedithiol on the gold surface, to CH_3Hg^+ and different cations at the same concentration (1×10^{-5} M) in water.

Fig. 2 Deflection of silicon cantilevers coated with a self-assembled monolayer of 1,6-hexanedithiol on the gold surfaces as a function of the change in concentration of CH_3Hg^+ in deionized water. A binding constant of 3×10^{-12} M^{-1} was obtained using a Langmuir model based non-linear curve fitting method (solid line), which is in the range of 10^{-12}–10^{-13} at pH = 7.0 reported by Hintelmann et al.[14]

about 3 min (Fig. 1). Blank tests performed on an unmodified bare gold coated silicon cantilever revealed that unmodified cantilevers do not bend in response to CH_3Hg^+ even for concentrations as high as 10^{-3} M. This ruled out a nonspecific salt effect on the unmodified microcantilever surface.

It was recently established that molecular adsorption-induced changes in surface stress result in a microcantilever bending motion.[9,10] For the 22 nm deflection, the surface stress change is calculated to be 0.045 N m^{-1}, i.e. 8.1×10^{-6} N on the cantilever surface, according to eqn (1):[7]

$$\Delta Z = \left(\frac{3(1-v)L^2}{Et^2}\right)\delta s \qquad (1)$$

where ΔZ is the observed deflection at the end of the cantilever, v and E are Poisson's ratio (0.2152) and Young's modulus (155.8 GPa) for the silicon substrate, respectively, t and L are the thickness (1 μm) and length (180 μm) of the cantilever, respectively, and δs is the differential stress on the cantilever.

Fig. 2 shows that the cantilever deflection at equilibrium for different concentrations of CH_3Hg^+ in aqueous solution varies with concentration in the range 10^{-16}–10^{-5} M. Deflection was observed at a CH_3Hg^+ concentration as low as 10^{-14} M. The low detection limit arises from the strong affinity of the CH_3Hg^+ cation for sulfur. The adsorption does not fit the Langmuir model well, indicating that adsorbate–adsorbate repulsion could play a role here. We are investigating different models and AFM images and IR for the surface characteristics of the MM.

Originally, we anticipated that such a cantilever would also respond to other heavy metal ions since those ions also form complexes with thiol groups. However, our study showed that heavy metal ions, such as Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+} as well as alkali and alkaline Na^+, K^+, and Ca^{2+} etc., did not cause bending responses (Fig. 1). Although other organometallic compounds, such as methylzinc and tetraphenylarsonium complex with the 1,6-hexanedithiol monolayer also bend the cantilever in anhydrous ethanol (figure not shown), all of these organometallic compounds decompose in water. This cantilever also deflected when exposed
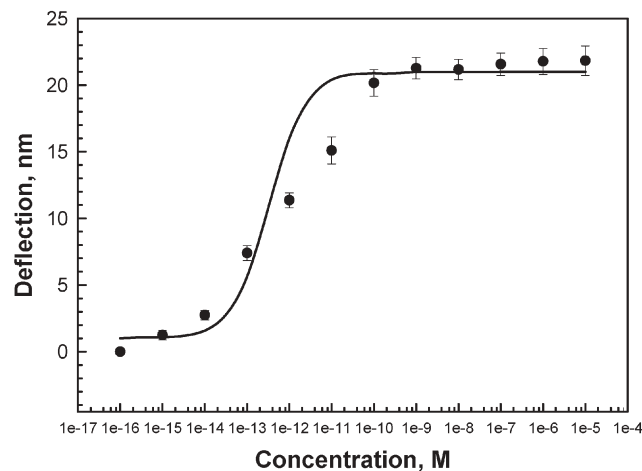
to ethylmercury (Et–Hg$^+$) (Fig. 1). However, the sensor is more valuable for methylmercury detection since most of the organomercuric compounds found in nature and in fish is methylmercury.[15] Beside MM and ethylmercury, other organomercuric species, such as phenylmercury, generally decompose to Hg^{2+} in water. These results indicated that this 1,6-hexanethiol monolayer modified cantilever could be a potential specific CH_3Hg^+ sensor with high sensitivity. This 1,6-hexanedithiol SAM modified cantilever responds more slowly to Hg^{2+} than MM on the time scale of our experiment (Fig. 1). However, Hg^{2+} may interfere with the detection since the Hg^{2+} can gradually penetrate the SAM film and react with Au, as discussed in our previous report.[7] Another control experiment showed that organic compounds in the environment, such as ethanol, and sodium dodecyl sulfate (SDS), don't interfere with the MM detection. Polyelectrolytes, such as poly(styrene sulfonate, sodium salt), on the other hand, did cause some bending, because they stick on almost any surfaces due to hydrophobic effects.

The microcantilever bending is generally caused by molecular adsorption-induced surface stress changes. The selectivity may be explained by the repulsion energy between 1,6-hexanedithiol molecules after their complexation with MM and heavy metal ions. The steric effect of the large methyl groups in MM would force the hexanedithiol units apart, bending the cantilever. On the other hand, heavy metal ions, such as Zn^{2+}, are known to form complexes with two SH groups, which would bring two dithiol units closer and result in a *negative* repulsion energy on the cantilever surface. Slightly upward deflections of the microcantilevers have been observed in such cases.

To confirm these hypotheses, we calculated the repulsion energy between two MM-complexed hexanedithiol groups on the cantilever surface by using Density Functional Theory (DFT).

The distance between two SH groups on the SAM surface is a critical parameter for accurate analysis of repulsion energy on the cantilever surface. However, the molecular structure of the monolayers has not been solved for unknown reasons.[16,17] No

ordered phase was observed for the solution-grown monolayers by a couple of surface microscopic methods. Assuming the monolayer structure is similar to that of closely packed structure of alkanethiol hexanethiol[15] with a 5 Å distance between two S atoms, DFT calculations predict the repulsion force between two MM-complexed hexanedithiols to be 4.7×10^{-10} N. This corresponds to 2.6×10^{-5} N over the whole cantilever surface on multiplying the number of hexanedithiol-pairs on the cantilever by the cantilever length. Given the assumptions and simplifications made, this result is quite close to the measured surface stress change mentioned above eqn (1). The DFT calculations also suggest that the binding of hexanedithiol to heavy metals can cause a negative surface stress change on the cantilever surface: a Cd^{2+} atom brought into the vicinity of two hexanedithiol units preferentially forms the dimer in which the Cd^{2+} is bonded to both units. This brings the formerly free ends of the dithiols closer together, which is *opposite* to that in the case of MM. The calculations thus provide a plausible explanation for the selectivity for MM exhibited by microcantilevers.

In conclusion, these results show that although the sulfhydryl group in a hexanedithiol molecule is not a selective recognition agent, its monolayer could potentially be a unique receptor for alkylmercury when cantilever and/or maybe other surface stress based sensing platforms (such as bridge, belt, *etc.*) are used as the transducer.

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