Aqueous Mercuric Ion Detection with Microsphere Optical Resonator Sensors

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ABSTRACT

We have developed a novel optical sensor based on fused silica microsphere resonators for mercuric ion Hg(II) detection in an aqueous environment. The whispering gallery mode (WGM) of the sphere is the surface mode. Therefore, its spectral position shifts in response to the binding of Hg(II) to the sphere surface. In addition, the WGM features very high Q-factor (>10^5). This light circulation effect significantly increases the light interaction with the analytes on the sphere surface, resulting in an enhanced sensor sensitivity.

In our experiment, we add Hg(II) to water in which the sensor is immersed. The sensorgram is obtained by monitoring the WGM position in real time. Our experimental results show that a detection limit of 50 ppb is achievable when Hg(II) ions bind to the thiol group on the sphere surface with nearly 1:3 stoichiometry. Controlled experiments with Zn(II) are also performed, and virtually no spectral shift in the WGM is observed.

INTRODUCTION

Optical microsphere resonators [1-14] have been demonstrated as an excellent technical solution for bio/chemical sensors. Several label-free applications have been demonstrated, including the detection of protein and DNA molecules [9,12-13], as well as refractometric measurements of solutions [10-11]. Microspheres are attractive as sensors because of their small size, low optical power requirement, and high light-matter interaction. Light of resonant wavelengths propagates around the surface of the microsphere cavity via total internal reflection in the form of whispering gallery modes (WGM). Microspheres with a Q-factor of greater than 10^9 have been demonstrated [14]. This high degree of optical confinement results in excellent light matter interaction, as photons may circulate the sphere hundreds to thousands of times on average. Thus, a microsphere with sub-millimeter dimensions can have an interaction length on the order of tens to hundreds of centimeters, while only consuming a sample volume on the order of microliters.

The light-matter interaction at the surface of the sphere is a result of the evanescent field of the WGM. As the light propagates around the surface of the sphere, an evanescent field protrudes into the medium beyond the sphere surface, as illustrated in Figure 1. As a result, the spectral position of the WGM is sensitive to the refractive index beyond the surface of the sphere. A change in refractive index of the surrounding solution will cause a shift in the spectral position of the WGM, as will the binding or removal of molecules at the sphere surface. Therefore, quantitative bio/chemical sensing can be performed by measuring the spectral shift of a WGM.

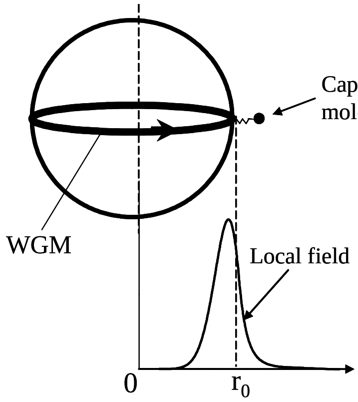

Figure 1: The evanescent field of the WGM extends beyond sphere surface and interacts with the surrounding solution and nearby molecules.

We utilize these capabilities of optical microsphere resonators to demonstrate the detection of the presence of mercuric ions (Hg(II)) in water. This design provides the advantages of simplicity, lower cost, faster measurement time, and compactness, as compared to the standard method of cold vapor atomic fluorescence detection [15]. Other simple optical techniques, including surface plasmon resonance (SPR) [16-19], have already been demonstrated for Hg(II) detection. Detection limits of 0.1 ppm and nanomolar have been achieved respectively by a commercialized SPReeta^{TM} system and by a lab SPR system with the assistance of split-field photodiode detection technology [18-19]. However, due to its small size, low sample consumption, and high light-matter interaction, the microsphere resonator may ultimately prove to be a better concept for heavy metal detection.

According to our experimental results, the label-free optical microsphere sensor system is capable of detecting aqueous Hg(II) on the order of 10-100 ppb (w/w). This is on the same order as previously demonstrated optical sensing systems, despite the fact that microsphere resonator sensors are early experimental prototypes. Also, the specificity of the sensor system is verified in this work by performing control experiments with Zn(II).

EXPERIMENTAL SETUP

The experimental prototype sensor consists of a silica microsphere and a fiber prism, polished into the sidewall of a fluidic cell, as shown in Figure 2. The microspheres are created by melting the tip of a standard single mode fiber optic cable with a CO_2 laser. The surface tension of the melted silica forms a sphere at the end of the cable. In these experiments, the diameters of the microspheres are in the range of 200 to 300 microns.

The sphere is placed in contact with a fiber optic prism, as shown in Figure 2. Laser light is coupled into the sphere through the prism via frustrated total internal reflection, as was first demonstrated in [20]. In order to couple the light efficiently into the WGM of a sphere, the phase-matching condition between the fiber prism and the WGM of the microsphere should be met. This can be achieved by polishing the fiber at an angle θ such that $n_{fiber}\sin\theta = n_{WGM}$, where n_{fiber} and n_{WGM} are the effective refractive index of the fiber mode and the WGM, respectively. In the experiments presented here, the prism is polished at an angle of 74°.

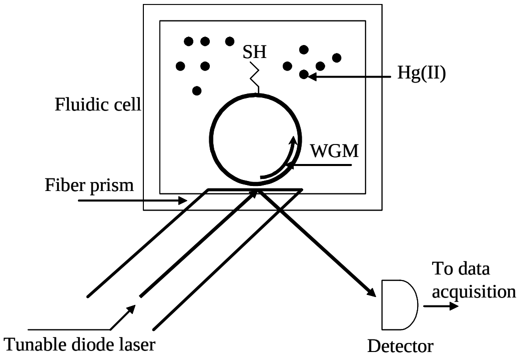

Figure 2: Experimental setup.

To identify and monitor the WGMs of the microsphere, excitation light is coupled into the sphere via the prism from a tunable laser diode at approximately 980 nm (300 kHz linewidth). The laser scans across a spectral range of approximately 100 pm at a frequency of 3 Hz. Light reflected by the prism is collected by a photodetector and monitored with a data acquisition card and computer. When the wavelength of the tunable laser is on resonance with a WGM of the sphere, the power of the light reflected at the prism decreases, and a spectral dip is recorded. As changes occur near the surface of the microsphere, the spectral position of this dip changes. An example of the spectral shift of a WGM is shown in Figure 3.

Before the microsphere is ready for Hg(II) sensing, it must first undergo preparation steps. The spheres are first placed in boiling de-ionized (DI) water for 30 minutes after fabrication, followed by a 30-minute incubation at room temperature in 1% (3-mercaptopropyl)trimethoxysilane (3-MTS) prepared in a 90/10 (v/v) ethanol/water mixture. In separate experiments, the adsorption of 3-MTS to the sphere surface was monitored using the spectral shift in the WGMs. It was verified that Langmuir saturation was reached within five minutes, and thus a 30-minute incubation time is sufficient in these experiments.

Following silanization, the spheres are rinsed with anhydrous ethanol and DI water five times each and baked in an oven at 100^{o}C for 30 minutes. One sphere is then submerged in DI water inside the fluidic cell, as shown in Figure 2. Varying concentrations of $HgCl_2$ are then added to the fluidic cell to achieve the desired concentrations of Hg(II). The concentrations in this work range from 140 nM to 11 mM. According to previous studies, the maximum density of binding sites on the sphere for silane agents is approximately 5×10^{14} cm^{-2} [21]. Therefore, even at the lowest concentration, the number of Hg(II) ions is still 100 times more than the maximum binding sites on the sphere surface. As a result, concentration depletion is ignored in our experiments and the concentration of Hg(II) in the cell after each injection is regarded as constant during the binding process.

Immediately after each incremental addition of $HgCl_2$, the spectral shift of a WGM is monitored and recorded by a computer. This data is then used to quantify the amount of Hg(II) that was captured by the functionalized microsphere surface.

RESULTS AND DISCUSSION

Upon the injection of $HgCl_2$, the WGM spectral position shifts to a higher wavelength in response to the binding of Hg(II) to the functionalized sphere surface, as shown in Figure 4. Sufficient time is given after each injection to ensure that equilibrium is reached. Then, a higher concentration of Hg(II) is injected, resulting in a new equilibrium that further shifts the WGM to

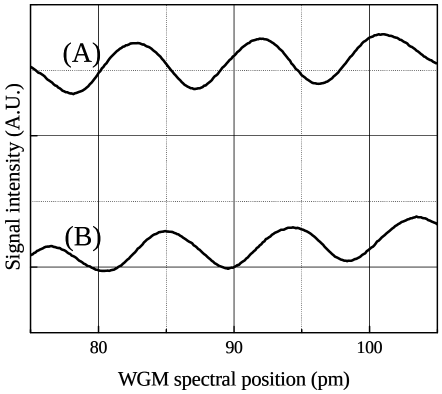

Figure 3: The spectral dip indicates the location of the WGM. The spectral shift from (A) to (B) is due to the binding of Hg(II) at the surface. The data for each trace is adjusted vertically for clarity.

a longer wavelength. The WGM spectra are monitored and recorded throughout the experiment at a rate of 3 Hz. Following the experiment, one of the spectral dips is analyzed and the recorded traces are used to plot the ladder-like sensorgram, a part of which is shown in Figure 4.

The binding process between Hg(II) and thiol groups on the sphere surface can be described by:

$$mHg(II) + nS \Leftrightarrow Hg_m S_n \, . \qquad (1)$$

The dissociation constant K_d is therefore given by

$$K_d = \frac{[Hg(II)]^m [S]^n}{[Hg_m S_n]} \, . \qquad (2)$$

Only one layer of Hg(II) will form on the sphere surface due to the binding of Hg(II) with the thiol groups, and thus the wavelength shift can be related to the binding kinetics. We define the normalized WGM shift, δ, as the ratio of occupied thiol sites to the total binding sites $[S]_0$:

$$\delta = n[Hg_m S_n]/[S]_0 \, . \qquad (3)$$

The inset in Figure 4 shows the detail of the binding process for [Hg(II)] = 2.67 μM. The binding rate of 0.012 s^{-1} obtained from the simulated curve indicates that the detection can be completed in a few minutes. This detection time, even at a much lower Hg(II) concentration, is still at least five times shorter than that in SPR-based mercury sensors [19].

The normalized WGM spectral shift at equilibrium, δ_{eq}, is derived from Eqs. (1) through (3) and expressed as follows:

$$\frac{\delta_{eq}}{(1-\delta_{eq})^n} = \frac{n}{K_d}[S]_0^{\;n-1}[Hg(II)]^m = \gamma [Hg(II)]^m \, . \qquad (4)$$

Experimental results for the normalized and absolute WGM shift as a function of Hg(II) concentration are obtained from the data in Figure 4 plotted in Figure 5.

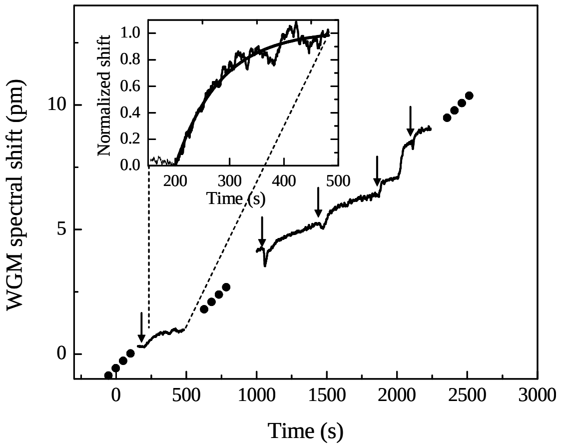

Figure 4: Sensorgram showing the binding of Hg(II) of various concentrations to thiol groups on the sphere surface. Arrows indicate the time when $HgCl_2$ was added. Inset: normalized sensorgram for [Hg(II)] = 2.67 μM. The simulated curve is obtained by the first-order Langmuir model with $k = 0.012$ s^{-1}.

In order to determine the binding stoichiometry, we fit the experimental results with different integer combinations of m and n and plot them against the experimental data in Figure 5. As can be seen in the plot, the best fit occurs when m:n = 1:3 and when $\gamma = 2.5\text{x}10^4$. This 1:3 stoichiometry binding was also observed previously with SPR, and was attributed to the formation of bulky mercury chloride-hydroxyl complexes $HgCl_4(OH)^{3-}$ [19]. According to the inset in Figure 5, the microsphere sensor begins to show saturation at approximately 80 μM Hg(II) and the linear log-log response range covers from $2\text{x}10^{-7}$ to $6\text{x}10^{-5}$ M.

The detection limit can be deduced by considering the spectral resolution along with the experimental results. The linewidth of the WGM is 4 pm, which corresponds to a Q-factor of approximately $2.5\text{x}10^5$. Assuming that one-fiftieth of the WGM linewidth can be resolved, as in [12], the resolution of the system is 0.08 pm. As shown in Figure 5, the saturation level for the sphere under study is 13.5 pm. Using m, n, and γ provided above, the detection limit of our sensor is estimated to be $[\text{Hg(II)}]_{\text{min}}$ = 240 nM, or 50 ppb (w/w). This detection limit is close to what was observed experimentally. In fact, the two lowest detectable concentrations used in our experiment are 140 nM and 380 nM, corresponding to 28 ppb and 76 ppb, respectively.

Control experiments are performed with $ZnCl_2$ to demonstrate the sensor's strong binding affinity for Hg(II). It is known that Zn(II) has a lower binding affinity than Hg(II) [19]. Again the sphere is prepared as previously. $ZnCl_2$ solution is first injected into the fluidic cell to make the Zn(II) concentration 120 μM. Only a small subsequent WGM spectral shift is observed, as shown in Figure 6. In contrast, a much larger shift is induced when Hg(II) is added, even when the concentration of Hg(II) is only 16 μM. With this experiment, the possibility that Cl^{2-} is responsible for the WGM shift can also be eliminated.

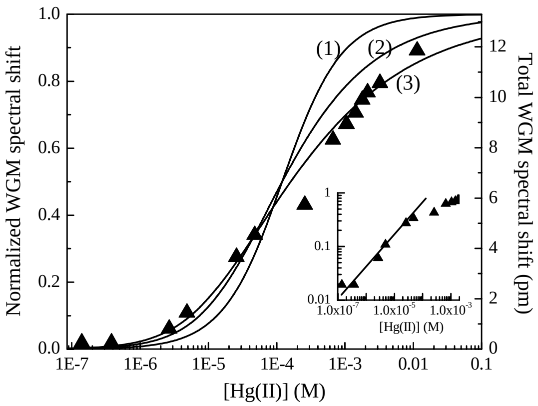

Figure 5: Triangles are the experimental results for normalized (left axis) and absolute (right axis) sensor response as a function of the Hg(II) concentration. The sensor saturation level is approximately 13.5 pm. Solid curves are the fit using Eq. (4): (1) m:n = 1:1, γ = 8.33x10^3; (2) *m*:*n* = 1:2, γ = 1.67x10^4; (3) *m*:*n* = 1:3, γ = 2.5x10^4. For clarity, the same results are also plotted in the log-log scale in the inset.

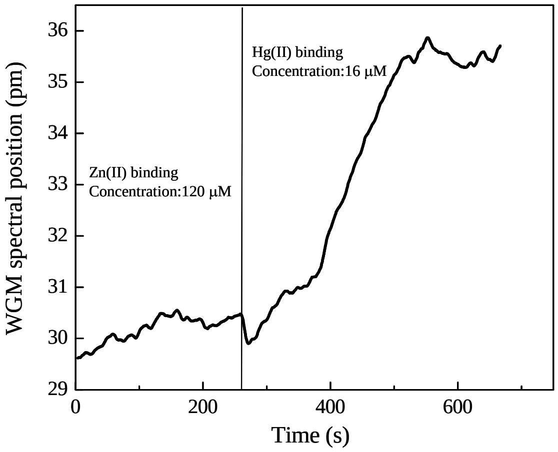

Figure 6: Control experiment with Zn(II) (concentration 120 μM) and Hg(II) (16 μM) showed that thiol groups bound selectively to Hg(II).

SUMMARY AND DISCUSSION

We have demonstrated a novel label-free optical sensor for mercury detection in water using an optical microsphere resonator. The detection limit is estimated to be 50 ppb, comparable to results obtained using SPR. Our experiments have also shown that mercuric ions bind to thiol groups on the sphere surface in 1:3 stoichiometry. Furthermore, the detection time for our sensors is only a few minutes, faster than typical SPR-based sensors. Various control experiments were also carried out to verify the specific binding of Hg(II).

Improvements can be made to the detection limit in future experiments. The Q-factor, which determines the sensor's spectral resolution and hence the detection limit, can be enhanced by shifting the operating wavelength to the region near 500 nm where the absorption of the light by water surrounding the sphere can be significantly reduced [22]. In addition, using a smaller sphere will further increase the detection limit, as the sensor sensitivity is inversely proportional to the sphere size [11-12]. Moreover, the transduction signal can be amplified by using a sandwich-type detection in which an agent that has strong binding affinity for Hg(II) is introduced to the sensor following the Hg(II). 2-mercaptobenzothiazole (2-MBT) is currently under investigation for this function, and appears to significantly increase the sensitivity [18]. Given the high sensitivity and quick sensing response demonstrated in this work, along with the potential for improvement, this research will open a door to a series of applications in chemical sensing, especially heavy metal detection using microsphere resonator sensors.

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