SH-SAW Sensor Based Microfluidic System for the Detection of Heavy Metal Compounds in Liquid Environments

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Abstract

In this work, a guided shear horizontal mode surface acoustic wave (SH-SAW) sensor, fabricated by patterning gold (Au) interdigitated electrodes (IDE) on a 64° YX-LiNbO₃ based piezoelectric substrate, was used for the detection of heavy metal compounds. A flow cell, with a reservoir volume of 3 µl, which employs inlet and outlet valves for the microfluidic chamber and polydimethylsiloxane (PDMS) based microfluidic channels, was also designed and fabricated using an acrylic material. The frequency based response of the SAW sensor towards varying concentrations of heavy metal compounds such as lead nitrate (PbNO₃) and cadmium nitrate (CdNO₃) were investigated. As the surface acoustic wave propagates on the substrate, between input and output IDEs, a shift in the resonant frequency of the SAW device was observed due to the change in velocity of the wave caused by the varying concentrations of the test analytes. The results obtained demonstrated the capability of the system to detect picomolar level concentrations. The response of the SAW sensor is analyzed and presented in this paper.

Keywords: Heavy metal compounds; Microfluidic; Shear horizontal SAW sensor; Surface acoustic wave device;

1. Introduction

The toxic effects of heavy metals have been a growing concern in the biomedical and environmental industries, and hence a major focus of research in the development of different sensing systems. Although, heavy metals are typically formulated as non-toxic components, it has been shown that they can cause serious health issues even at micro molar concentration levels [1]. Some of the most common heavy metal pollutants are lead (Pb) and cadmium (Cd) [2]. Pb has been categorized as a neurotoxic element due to its severe impact on the nervous system causing neurotoxicity and nephrotoxicity in the gastrointestinal and renal systems [3-6]. Cd, which has been increasingly used in industrial product lines, is known to cause kidney failure, hypercalciuria and osteoporosis [7-9]. The detection of these heavy metal compounds is thus of utmost importance.

The rising demand for heavy metals in applications such as plating, mining, solar cells, pharmaceutical and chemical industries exacerbate their role in environmental pollution factors [10, 11]. Heavy metal detection methods including spectrometric techniques such as cold vapor atomic absorption spectroscopy, inductively coupled plasma mass spectroscopy, electrochemical impedance spectroscopy, UV-visible absorbance spectroscopy, x-ray, laser and colorimetric analysis as well as biosensing detection techniques based on enzyme activation or bacteria have been reported [12-19]. However, these techniques are often expensive, time consuming, laborious, hazardous and require complex labeling methods. Therefore, it is necessary to develop sensing systems with easy-to-use, cost effective, highly sensitive and rapid detection techniques.

Surface acoustic wave (SAW) devices have been used in various sensing applications for measuring the changes in SAW device characteristics such as magnitude or phase shift in frequency response [20]. Several advantageous features such as their smaller size, higher

resonant frequency, lower power consumption and better compatibility with CMOS technology makes them an appropriate choice when compared to micro-cantilever and natural clay-modified electrode based sensing systems [21-24]. Even though different SAW device configurations have been used, since its discovery by Lord Rayleigh in 1885 [25], the use of interdigital electrodes (IDEs) and reflectors by White and Voltmer in 1965 [26] and Staples et al. in 1974 [27], respectively has proven to minimize the power dissipation and hence obtain optimized SAW generation and detection.

SAW devices have been typically used in the Rayleigh and shear horizontal (SH) SAW propagation modes [28]. In the Rayleigh propagation mode, particle displacement has two components: one is parallel to the SAW propagation direction and the other is normal to the substrate, thus forming an elliptical trajectory for the particle. Most of the SAW energy is confined to the surface of the substrate and is often diminished to zero at a substrate depth of 4 to 5 times the wavelength [29]. Due to the normal component of the particle displacement and interference with other media, above the substrate surface, this mode is not suitable for the liquid environment. On the other hand, in the SH-SAW propagation mode, the particle displacement happens only in parallel with the substrate surface which prevents the vibration vector from transferring into any secondary media and hence prohibits SAW energy attenuation when the substrate comes into contact with a liquid [30]. The use of the SH-SAW device is thus a promising solution for heavy metal compound detection in liquid environments.

In this study, an efficient SH-SAW sensor, consisting of gold (Au) IDEs on a 64° YX-LiNbO₃ based piezoelectric substrate, was fabricated for the detection of heavy metal compounds. A flow cell, which consists of inlet and outlet ports for the microfluidic chamber as well as polydimethylsiloxane (PDMS) based microfluidic channels, was also fabricated using

acrylic material. A chemical sensing layer, naphtho[2,3-a]dipyrido[3,2-h:2',3'-f]phenazine-5,18-dione (QDPPZ) was used to selectively bind to the heavy metal ions. The capability of the system for detecting different concentrations of heavy metal compounds such as lead nitrate (PbNO_3) and cadmium nitrate (CdNO_3) in liquid environments, through phase shifts in the frequency based response of the SH-SAW sensor was investigated.

2. Theory

The piezoelectric based SH-SAW devices, generally consists of two sets of metalized input and output IDEs mounted on a piezoelectric substrate. The surface acoustic wave is generated by an input electrical signal applied at the input IDEs. It then propagates on the piezoelectric substrate through the delay line which is the distance between the input and output IDEs and is converted back to an electrical signal. The SH-SAW device output signal can finally be obtained from the output IDEs. The SAW resonant frequency can be mathematically calculated by [28]

$$f_0 = \frac{v_s}{\lambda} \quad (1)$$

$$\lambda = 4 \times w \quad (2)$$

where, f_0 is the resonant frequency of the SAW, v_s is the saw propagation velocity on piezoelectric substrate, λ is the SAW wavelength and w is the width of the IDEs. The resonant frequency of the SH-SAW sensor is dependent on the velocity of the acoustic wave passing through the piezoelectric substrate. The velocity of the wave is known to vary due to mass, stiffness, conductivity, dielectric coefficient, temperature and pressure changes [31]. The varying concentrations of the heavy metal compounds cause a change in the SAW propagation velocity between input and output IDEs, due to the chemical adsorbent (QDPPZ) that was deposited as a

sensitive layer, and this resulted in a change in the resonant frequency, which can be used to quantify the concentration levels [32].

3. Experimental

3.1 Chemicals and Sample Preparation

PbNO₃ and CdNO₃, in crystalline form, were purchased from the Sigma Aldrich Chemical Company. PbNO₃ in concentrations of 1 pM, 100 pM, 1 nM, 100 nM, 1 μM, 50 μM, 100 μM and 750 μM and 1 pM, 100 pM, 1 nM, 100 nM, 1 μM, 50 μM, 100 μM, 250 μM and CdNO₃ in concentrations of 750 μM were prepared by mixing in deionized (DI) water. PbNO₃ and CdNO₃ are soluble in the DI water and the process will not be affected by NO₃⁻ anions [33]. All test analytes were stored at -20 °C in 10 ml aliquots before use. Tubing (Inner diameter - 0.01"; Outer diameter - 0.0625") and tube connection accessories for sample transfer was purchased from Upchurch Scientific.

3.2 SH-SAW Sensor Fabrication

Figure 1(a) illustrates the schematic of the SH-SAW device. The SH-SAW sensor was fabricated on a 64° YX-LiNbO₃ piezoelectric substrate using photolithography techniques. Eight pairs of input and output IDEs, 0.1 μm thick Au, were patterned by metal sputtering technique on the piezoelectric substrate. The electrode aperture was 760 μm, while electrode width and gap were 10 μm thereby resulting in a 40 μm acoustic wave length (λ). The SH-SAW sensor also consisted of 120 reflectors fabricated on outer side of input and output IDEs with similar dimensions and 20 reflectors in between the IDEs to reduce the scattering losses of surface acoustic waves [34]. Since the SAW propagation velocity on the 64° YX-LiNbO₃ piezoelectric substrate is 4474 m/s and the designed wavelength is 40 μm, the resultant resonant frequency will be 111.8 MHz [35]. The measured resonant frequency of the fabricated sensor was

112 MHz. The photograph of the fabricated SH-SAW sensor, with overall device dimensions of $11 \times 12 \text{ mm}^2$ is shown in Fig. 1(b).

3.3 Synthesis of the Chemical Sensing Layer

The chemical sensing layer QDPPZ was synthesized using a two-step process. In the first step, concentrated sulfuric acid (H_2SO_4 , 20 mL) and concentrated nitric acid (HNO_3 , 10 mL) were added dropwise to a mixture of 1,10-phenanthroline (1.00 g, 5.56 mmol) in the presence of potassium bromide (KBr) (5.95 g, 50 mmol) at 0°C . The solution was refluxed for 2 hours then cooled to room temperature, yielding a black, oily product. The contents of the flask were diluted with 400 mL deionized water and neutralized with sodium bicarbonate (NaHCO_3), yielding a clear yellow solution. The product was extracted with methylene chloride and dried over anhydrous magnesium sulfate (MgSO_4). The solvents were removed using a rotary evaporator, resulting in a yellow solid. The product was purified by recrystallization from methanol. The average yield of the product, 1,10-phenanthroline-5,6-dione was (1.11 g, 5.31 mmol) which was calculated to be 95%. ^1H NMR (400 MHz, CDCl_3 , 25°C) δ : 9.12-9.10 (t, 2H, $J = 2.95 \text{ Hz}$), 8.51-8.48 (d, 2H, $J = 1.83 \text{ Hz}$), 7.60-7.55 (m, 2H, $J = 4.71 \text{ Hz}$).

In the second step, 1,10-phenanthroline-5,6-dione (0.50 g, 2.38 mmol) was refluxed in ethanol for 15 min. 9,10-diaminoanthroquinone (0.981 g, 2.38 mmol) was then added, resulting in a purple solution, and the solution was refluxed for 4 hours. The dark purple product was collected using vacuum filtration, washed with methanol and concentrated in vacuum. The reaction yield was 80%. ¹H NMR (400 MHz, CDCl₃, 25 °C): δ 9.83 (d, 1H), 9.64 (d, 1H), 9.28 (d, 2H), 8.64 (dd, 2H), 8.27 (q, 2H), 7.82 (m, 4H). The final structure is shown in Fig. 2.

3.4 Microfluidic Flow Cell Fabrication

The flow cell, with overall device dimension of 70 × 50 × 52 mm (w/l/h), was designed in AutoCAD™ and CNC machined using acrylic material (Fig. 3). A microfluidic flow channel, with dimensions of 710 × 6800 × 710 μm (w/l/h) and a total channel volume of approximately 3.4 μl, was also fabricated with PDMS. The flow of the test analyte through the PDMS microfluidic channel was obtained by integrating two sets of inlet and outlet ports in the flow cell. The effective closing of the flow cell, which results in the tight sealing of the PDMS microfluidic flow channel around the sensing area of the SH-SAW sensor, was made possible by the use of an axially magnetized set of neodymium magnets (Diameter - 0.25"; Thickness - 0.375"; Magnetic strength – 13,200 Gauss) purchased from K&J Magnets, Inc.

3.5 Experiment Setup

The experiment setup is shown in Fig. 4. The SH-SAW sensor was placed in the sensor groove of the flow cell. Calibration for the wires and probes was done before the measurements. Before use and at the end of each experiment, the sensor was cleaned with acetone, and then blow dried with pressurized air. The measurements were performed at constant room temperature (25°C), since any changes in the temperature will affect the SAW velocity and attenuation [36]. Initially, a reference signal for deionized (DI) water was obtained. Then,

varying concentrations of PbNO_3 (1 pM, 100 pM, 1 nM, 100 nM, 1 μM , 50 μM , 100 μM and 750 μM), and CdNO_3 (1 pM, 100 pM, 1 nM, 100 nM, 1 μM , 50 μM , 100 μM , 250 μM and 750 μM) were injected into the flow cell using a KD Scientific (KDS210P) programmable syringe pump, at a flow rate of 50 $\mu\text{L}/\text{min}$. An Agilent 4395A network analyzer was used to measure the phase response (S_{21}) of the SH-SAW sensor towards the test analytes. System control, data acquisition and post processing of the network analyzer measurements were performed using a LabView™ based application.

4. Results and Discussion

The development of a selective and sensitive chemical sensing layer relied on the deposition of the organic ligand, QDPPZ, which is a 1,10-phenanthroline derivative that consists of nitrogen and oxygen groups that will coordinate to the heavy metal ions. Initial work has demonstrated that the quinolone unit was essential in inducing selectivity towards the heavy metal ions that was not observed if we used 1,10-phenanthroline alone. The binding of QDPPZ to the heavy metal ions causes an increase in mass between the electrodes, thus affecting the surface acoustic wave function. As the ions flow through the sample channels, QDPPZ molecules coordinate with the metal ions, forming a complex and increase the mass of the material between the electrodes.

Figure 5 shows the phase response (S_{21}) of the SH-SAW towards different concentrations of PbNO_3 . It was observed that the resonant frequency of the reference signal established by DI water shifted from 108.564 MHz to 108.551 MHz, 108.543 MHz, 108.529 MHz, 108.508 MHz, 108.503 MHz, 108.499 MHz, 108.494 MHz and 108.479 MHz for the 1 pM, 100 pM, 1 nM, 100 nM, 1 μM , 50 μM , 100 μM and 750 μM concentrations of PbNO_3 solution, respectively (Fig. 5(a)). This results in a frequency shift of 13 kHz, 21 kHz, 35 kHz, 56 kHz, 61 kHz, 65 kHz,

70 kHz and 85 kHz along with a percentage change of 0.011 %, 0.019 %, 0.032 % , 0.051 %, 0.056 %, 0.059 %, 0.064 % and 0.078 % for the 1 pM, 100 pM, 1 nM, 100 nM, 1 μ M, 50 μ M, 100 μ M and 750 μ M concentrations of PbNO_3 solution, respectively when compared with DI water (Fig. 5(b)). The experiment was repeated seven times using two different fabricated sensors and standard deviation of 3464 Hz, 1732 Hz, 2081 Hz, 6806 Hz, 3464 Hz, 4582 Hz, 2081 Hz and 5567 Hz from the average value of 15 kHz, 22 kHz, 33 kHz, 48 kHz, 57 kHz, 64 kHz, 69 kHz and 80 kHz were achieved for the 1 pM, 100 pM, 1 nM, 100 nM, 1 μ M, 50 μ M, 100 μ M and 750 μ M concentrations of PbNO_3 solution, respectively (Fig. 5(b)).

Then, varying concentrations of CdNO_3 was injected onto the SH-SAW sensor. It was observed that the resonant frequency of the reference signal established by DI water shifted from 109.290 MHz to 109.100 MHz, 109.076 MHz, 108.989 MHz, 108.979 MHz, 108.963 MHz, 108.948 MHz, 108.944 MHz, 108.935 MHz and 108.933 MHz for the 1 pM, 100 pM, 1 nM, 100 nM, 1 μ M, 50 μ M 100 μ M, 250 μ M and 750 μ M concentrations of CdNO_3 solution, respectively. The phase response (S_{21}) of the SH-SAW sensor demonstrated a frequency shift of 190 kHz, 214 kHz, 301 kHz, 311 kHz, 327 kHz, 342 kHz, 346 kHz, 355 kHz and 357 kHz along with a percentage change of 0.173 %, 0.195 %, 0.275 %, 0.284 %, 0.299 %, 0.312 %, 0.316 %, 0.324 % and 0.326 %for the 1 pM, 100 pM, 1 nM, 100 nM, 1 μ M, 50 μ M 100 μ M, 250 μ M and 750 μ M concentrations of CdNO_3 solution, respectively when compared with DI water (Fig. 6). The experiment was repeated eight times using two different fabricated sensors and standard deviation of 4242 Hz, 11313 Hz, 11313 Hz, 15556 Hz, 16970 Hz, 24748 Hz, 15556 Hz, 14849 Hz and 12727 Hz were obtained from the average value of 109 kHz, 129 kHz, 175 kHz, 192 kHz, 203 kHz, 217 kHz, 221 kHz, 235 kHz and 271 kHz for the 1 pM, 100 pM, 1 nM,

100 nM, 1 μ M, 50 μ M 100 μ M, 250 μ M and 750 μ M concentrations of CdNO₃ solution, respectively.

The resonant frequency phase shifts can be attributed to the change in SAW propagation velocity, in the delay line, caused by the varying concentrations of the test analytes. These sets of phase responses also displayed detection levels as low as picomolar concentrations and the ability of the SH-SAW sensor to distinguish among a wide range (micro, nano and pico level) of sample concentrations. It is worth noting that the approved toxicity level of Pb and Cd according to the United States Food and Drug Administration (USFDA) is 1.25 mM and 207 μ M, respectively [37].

5. Conclusion

In this work, a SH-SAW sensor was successfully fabricated on a 64° YX-LiNbO₃ piezoelectric substrate. An efficient flow cell was also designed and fabricated using acrylic material. The flow cell consisted of inlet and outlet ports for the microfluidic chamber and PDMS based microfluidic channels. The feasibility of using the SH-Saw sensor for detecting heavy metal compounds was demonstrated through the quantitative detection of PbNO₃ and CdNO₃. The resonant frequency of the SH-SAW sensor increased from 108.551 MHz to 108.479 MHz and 109.100 MHz to 108.933 MHz as the concentration of PbNO₃ and CdNO₃ were increased from 1 pM to 750 μ M, respectively. The measured phase response (S_{21}) of the SH-SAW sensor showed a 13 kHz and 190 kHz frequency shift for the 1 pM concentration of PbNO₃ and CdNO₃, respectively. The results obtained demonstrated the capability of the SH-SAW sensor to detect heavy metal compounds at picomolar concentrations, which are several orders of magnitude lower than the approved USFDA toxicity limits. The ability to integrate the

SH-SAW sensor into a hand-held and portable detection system as well as research to improve the sensitivity and selectivity of the SH-SAW sensor to a wider variety of bio/chemicals is part of our future work.

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Figures Captions

Fig. 1. (a) Schematic of the SH-SAW device and (b) fabricated SH-SAW sensor.

Fig. 2. Flow cell with the SH-SAW sensor in the sensor groove (Inset: PDMS microfluidic flow channel).

Fig. 3. Structure of QDPPZ.

Fig. 4. Experimental setup.

Fig. 5. (a) SH-SAW sensor phase response (S_{21}) towards varying concentrations of PbNO_3 and (b) percentage change in frequency shift of SH-SAW sensor response.

Fig. 6. SH-SAW sensor frequency shift towards varying concentrations CdNO_3 .

(a)

(b)

Figure 1

Figure 2

Figure 3

Figure 5

Figure 6