Research Article

Initial Investigation of Using Planar Interdigital Sensors for Assessment of Quality in Seafood

A. R. Mohd Syaifudin,¹ K. P. Jayasundera,² and S. C. Mukhopadhyay¹

¹ School of Engineering and Advanced Technology, College of Sciences, Massey University, Palmerston North 4442, New Zealand

² Institute of Fundamental Sciences, College of Sciences, Massey University, Palmerston North 4442, New Zealand

Correspondence should be addressed to S. C. Mukhopadhyay, s.c.mukhopadhyay@massey.ac.nz

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A planar interdigital sensor-based sensing system has been fabricated for assessment of seafood quality. Our main objective is to sense the molecule of domoic acid presence in mussels. Three peptide derivatives namely sarcosine, proline, and hydroxyproline were used for the initial studies which are structurally closely related to our target molecule. The proline molecule is arguably the most important amino acid in peptide conformation, containing the basic structural similarity to the domoic acid. Three novel interdigital sensors have been designed and fabricated. The initial result shows that sensors respond very well to the chemicals and it is possible to discriminate the different chemicals from the output of the sensor. Results from the analysis have shown that Sensor_1 has better sensitivity compared to other sensors. Sensor_1 was chosen for further analysis with real mussels. The changes in sensor impedance were then analysed with real mussels before and after adding the proline. The presence of proline on the mussel samples was detected by the sensor. Results also showed a good correlation of $R^2 = 0.717$ between sensitivity and sample thickness.

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1. INTRODUCTION

In late 19th and early 20th century, a large number of illnesses were linked with the consumption of raw oysters, clams, and mussels. The problem still exists today and time to time it appears in the society. There were some incident of deaths in recent times which has made this problem is worthy of research. It was found that these illnesses were related to the ingestion of domoic acid-contaminated mussels which led to amnesic shellfish poisoning (ASP) [1–4]. ASP is characterized according to both gastrointestinal and neurological symptoms, including severe headache, confusion, and either temporary or permanent memory loss.

Domoic acid (DA) is a naturally occurring toxin produced by microscopic algae, specifically the diatom species *Pseudo-nitzschia*. DA is a chemical that is produced by algae or plankton when it blooms. Shellfish and filter-feeding fish (anchovies, sardines, krill, etc.) ingest these algae, where the toxin concentrates. Both can accumulate this toxin without apparent ill effects. However, in marine mammals and humans, DA is tetracarboxylic acid that acts as a neurotoxin.

The toxin is not destroyed by cooking or freezing. Figure 1 shows the chemical structure of domoic acid.

The presence of DA in shellfish has been reported in various regions of the world [1]. There have been numerous reports of toxicity in a variety of wildlife species indicating that DA moves up the food chain in marine ecosystems [1–4].

Studies have proven that certain amount of DA can cause health problems to animals and humans [5–10]. In 1987, DA was identified as the toxin responsible for an outbreak of illness in Prince Edward Island, Canada [11]. It was caused by eating blue mussels. Effects on both the gastrointestinal tract and the nervous system were observed. It was reported that 107 patients (all adults) met the case definition [2]. Dose-related symptoms included nausea, vomiting, abdominal cramps, diarrhoea, headache, memory loss, and convulsions, and several deaths were attributed to the toxin. As a result of the episode of human illness in Canada, a regulatory level of 20 $\mu\text{g/g}$ of DA in shellfish meat was established.

In order to protect consumers from shellfish poisoning, most countries have a set of regulatory guidelines. All the

FIGURE 1: Chemical structure of domoic acid.

FIGURE 2: Representation of planar interdigital sensors. (a) Conventional interdigital sensor. (b) Novel interdigital sensor (Sensor_1).

products need to be tested for the toxin, which places a heavy workload on the laboratories and is extremely expensive to the industry. This has motivated us toward the development of a sensing system which can detect the presence of DA and their related analogs without much difficulty and at a low cost.

2. EXISTING METHOD

The existing method to detect DA is either based on using HPLC or SPR.

2.1. Based on HPLC

High-pressure liquid chromatography (HPLC) has been used to determine DA [2, 11–13]. Ten samples of commercial blue mussels (*Mytilus edulis*) from Japan were analyzed for DA. DA was found in all samples at low concentrations (0.11–1.81 ng/g mussel tissue) [14]. A new sensitive determination

FIGURE 3: Sensor_2 and Sensor_3 of different configurations.

method of DA using high-performance liquid chromatography with electrogenerated tris has been reported [15].

2.2. Based on SPR

A portable surface plasmon resonance (SPR) biosensor system has been developed for the detection of domoic acid [16]. A rapid and sensitive immuno-based screening method was reported to detect DA present in extracts of shellfish species using a surface plasmon resonance-based optical biosensor [17].

The existing methods required expensive equipments and also trained personnel. The procedure to prepare the sample and to conduct the experiment is tedious and time consuming. Looking at the complexity of the existing methods, we have designed and fabricated novel planar interdigital sensor-based sensing system with the purpose of an easy detection of molecules for DA. The developed sensing system should be reliable and cost effective.

3. DEVELOPMENT OF PLANAR INTERDIGITAL SENSORS FOR ASSESSMENT OF DOMOIC ACID

Planar interdigital sensor has been used for estimation of properties of dielectric material such as milk, saxophone reeds, meat, and leather [18–22]. The electric field lines generated by the sensor penetrate into the material under test (MUT) and will change the impedance of the sensor. The sensor behaves as a capacitor in which the capacitive reactance becomes a function of system properties. Therefore by measuring the capacitive reactance the system properties can be evaluated. The capacitance of parallel plate capacitor can be calculated by

$$C = \frac{\epsilon_0 \epsilon_r A}{d}, \quad (1)$$

where C is the capacitance (in farads), ϵ_0 is the absolute permittivity (8.854×10^{-12} F/m), ϵ_r is the relative permittivity of material under test, A is the area (in square meters), and d is the spacing (in meters).

(a)

(b)

(c)

FIGURE 4: The equivalent circuit of novel sensors. (a) Sensor_1 with configuration 1-11-1. (b) Sensor_2 with configuration 1-5-1-5-1. (c) Sensor_3 with configuration 1-3-1-3-1-3-1.

TABLE 1: Relationship between excitation voltage, sensing voltage, current, and impedance.

Sensor type	Excitation		Sensing			Impedance (MΩ)
	Vin (V)	Freq (kHz)	Vout (mV)	Phase	I (mA)	
Sensor_1	10.0	10.0	156.3	83.0	1.303	7.678
Sensor_2	10.0	10.0	187.5	83.0	1.563	6.400
Sensor_3	10.0	10.0	192.1	83.0	1.601	6.247

FIGURE 5: The estimation of capacitance of each sensor geometry.

FIGURE 6: The fabricated interdigital sensors.

3.1. Sensor design

A novel planar interdigital sensor, different from conventional interdigital sensor has been used. Three types of novel interdigital sensors have been designed and fabricated. The initial goal is to evaluate which of these three sensors give a good response and could discriminate between three different chemical samples. Each sensor has the same effective area of 4.75 mm by 5.00 mm and having pitches (the distance between adjacent positive and negative electrode) of 0.25 mm. The positive and negative electrodes have the same length and width of 4.75 mm and 0.125 mm, respectively. All sensors were designed to have the same effective number of electrodes (thirteen). Sensor_1 was designed to have 2

FIGURE 7: The signal waveform of Sensor_1.

TABLE 2: Prices of different chemicals in the market.

No.	Chemical name	Quantity	Price (USD)
1	Sarcosine	100 g	\$53.30
2	L-Proline	100 g	\$100.00
3	Hydroxy proline	25 g	\$102.80
4	Domoic acid	1 mg	\$508.00
5	Kaimic acid	10 mg	\$54.80

positive electrodes at each end separated by 11 negative electrodes. Sensor_2 and Sensor_3 were designed with the same dimensions but with different configurations. Sensor_2 has 3 positive electrodes with 10 negative electrodes while Sensor_3 has 4 positive electrodes with 9 negative electrodes. Figures 2(a) and 2(b) show the representation of conventional and novel interdigital sensors. Figure 3 shows Sensor_2 and Sensor_3 with different configurations.

However, (1) can be used to calculate the capacitance of a planar type capacitor by introducing two factors, one for change of distance, “ d ” and another for change of area, “ A ”. Only “ d ” was changed in the design. It is important during the sensor design stage, to optimise the number of negative electrodes and to estimate the equivalent capacitance within sensor geometry. The equivalent circuit to estimate the equivalent capacitance within sensor geometry is shown in Figure 4. The equivalent capacitance can be calculated by

$$\begin{aligned} C_{12} &= \frac{1}{2} C_{11}; \dots; C_{1n} = \frac{1}{n} C_{11}, \\ C_{21} &= \frac{1}{2} C_{21}; \dots; C_{2n} = \frac{1}{n} C_{21}, \end{aligned} \quad (2)$$

where $C_{11} = C_{21}$.

FIGURE 8: Circuit diagram for full-wave signal rectification.

FIGURE 9: Sarcosine, proline, and hydroxyproline molecules used for initial studies.

FIGURE 10: The sample on the sensor of measurement.

FIGURE 11: The experimental setup.

Figure 5 shows the estimation of capacitance within sensor geometry. Results from circuit analysis have shown that Sensor_1 has better uniformity compare to Sensor_2 and Sensor_3. This uniformity is very important for achieving largest sensitivity of measurement. Figure 6 shows the picture of all fabricated sensors.

4. RESULTS AND DISCUSSIONS

The sensor is connected with a series surface mount resistor of 120 k Ω to measure the current through the sensor. A

frequency of 10 kHz with 10 V_{pp} (voltage peak to peak) is applied to the exciting coil using agilent function waveform generator. The electric field will be created by the exciting coil of the sensor in the system under test. The output signal from the interdigital sensor is small and alternating in nature. Figure 7 shows the signal waveform from Sensor_1 and Table 1 shows the relationship between excitation voltage, sensing voltage, current, and impedance for all sensors.

A signal rectification circuit shown in Figure 8 is required to rectify and amplify the alternating signal. Two LM324 low power quad operational amplifiers were used in the

FIGURE 12: Comparison of sensors sensitivity with different sensor configurations.

FIGURE 13: Comparison of sensors sensitivity with different chemical samples.

circuit. A full-wave rectifier will convert the signal waveform to one constant polarity (+ve and -ve) at its output. The amplify signal will pass through a low-pass filter with cut off frequency of 13 kHz. The output signal from the circuit is fed to the analog input of microcontroller SiLab C8051F020 to obtain digital value.

4.1. Laboratory experiments

Before experimenting with the actual samples from mussels, we have explored the response of the sensors with three peptide derivatives namely sarcosine, proline, and hydroxyproline for the initial studies, which are structurally and closely related to our target molecule. N-methyl glycine represents the simplest structure. The proline molecule is arguably the most important amino acid in peptide

FIGURE 14: The samples were cut from each mussel.

FIGURE 15: The sample (mussel) on the sensor of measurement.

conformation, containing the basic structural similarity to the domoic acid. The hydroxyproline containing hydroxyl group at position 4 represents the substituent at C4, which is particularly crucial for the binding. Figure 9 shows the chemical structure of the three samples used for the initial studies. These three samples were chosen because their prices were cheaper compared to the domoic acid. Table 2 shows the prices of each chemical in the market.

A small amount of samples of sarcosine, proline, and hydroxyproline (1.4 mg) were used for the experiment. Each chemical was placed on the effective area of the sensor as shown in Figure 10. The electrodes are separated from the sample with the help of glad wrap. The experimental setup is shown in Figure 11. Measurement data of air (V_{air}) for calibration was first collected before measuring the chemical samples. The output voltage (V_{out}) from the rectification circuit and digital value from microcontroller were collected. Data of sensor sensitivity was analyzed. The sensitivity can be calculated by

$$\text{Sensitivity} = \frac{(V_{\text{out}} - V_{\text{Air}})}{V_{\text{Air}}} \times 100, \quad (3)$$

where V_{Air} is voltage air or voltage without sample and V_{out} is voltage output with sample.

Figures 12 and 13 show the comparative values of the sensor output for three different sensors for the three samples. It can be said that the sensors respond very well to the chemicals. It is seen that it is possible to discriminate

FIGURE 16: Sensor sensitivity with sample before and after adding proline.

FIGURE 17: Results of mussel samples from different location.

the different chemicals from the output of the sensor. This provides an opportunity to develop a sensing system to detect the presence of DA in raw oysters, claws, and mussels. Result from initial studies concludes that Sensor_1 shows a good response and give better sensitivity to the chemical structure. It can clearly discriminate between three samples compared to Sensor_2 and Sensor_3. Therefore, Sensor_1 was chosen for further analysis with real mussels.

4.2. Experiment with mussels

Assuming all mussels in the market are good, containing DA level less than $20 \mu\text{g/g}$ of mussel meat. A set of 8 mussels were randomly selected and were cut from 5 different positions. Figure 14 shows that how the samples were cut

FIGURE 18: The relationship between sensor sensitivity with sample thickness.

FIGURE 19: Sensor sensitivity with DA.

from each mussel. All samples were placed onto the nonstick paper and left them to be dried at a controlled laboratory temperature of 22.9°C with humidity of 40%. Each sample thickness and surface temperature was measured using digital calliper and temperature tester, respectively. The samples thickness measured were between 1.6 mm to 3.2 mm having the temperature of range between 21.9°C to 22.5°C . The experimental setup shown in Figure 10 was constructed to test the samples. Each sample was placed on the sensor as shown in Figure 15.

The output voltage from the signal rectification circuit and the digital values from microcontroller were taken. Data of each sample was taken for analysis before and after a small amount of proline (0.7 mg) were added to the samples. Proline was used in the experiment because it has a close chemical structure to domoic acid. Result in Figure 16 shows that there was a significant difference of Sensor_1 sensitivity before and after adding the proline. It is shown that Sensor_1 was able to detect the presence of proline on the sample. Graph on Figure 17 shows the average sensor sensitivity is higher at location 5 and lower at location 1. This is because the average thickness at location 5 is 2.7 mm, which is thicker compared to other locations. The sensitivity is lowest at location 1 of having average thickness of 2.3 mm. The graph in Figure 18 shows a good correlation with

$R^2 = 0.717$, between sensor sensitivity with sample thickness. Experiment with DA was also conducted. Small amount of DA of 0.1 mg was diluted in 2.0 mL (2.0 g) of water to give 50 ppm of DA. Results in Figure 19 shows the respond of Sensor_1 with small amount of DA (0.5 μg) injected to the samples.

5. CONCLUSION

Initial studies on three different chemicals proved that the sensors can respond very well to each chemical and can clearly discriminate between them. Sensor_1 was selected for further analysis with real mussels because it has better sensitivity. Analysis of results with real mussels shows that Sensor_1 can detect the presence of proline on the samples. It can be said that Sensor_1 was able to detect the small amount of proline which is structurally close to DA chemical structure. Experiment with DA also shown that Sensor_1 was able to detect the presence of DA in mussel meat. Therefore, novel planar interdigital sensor can be used to assess the chemicals structure in mussels. The sensing system can provide a fast and rapid analysis of DA in shellfish meats. A low cost and reliable sensing system for commercial use will be developed for future work. The proposed system will use the microcontroller-based sensing system where the calibration of sensor and analysis can be done instantaneously. Calibration of air will be taken first and then measurement data of sample. A set of threshold values (sensor sensitivity) at different sample thickness need to be set. This threshold values can be obtained from the experiment with more mussels with injected DA. A result of pass and fail analysis will be programmed into the microcontroller according to this threshold values.

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