New Development Quantification Methods for Salt Iodine and Urinary Iodine Using Microfluidics Based Nanotechnology

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Abstract. In Malaysia, the first Iodine Deficiency Disorders (IDD) survey was conducted in 1996 and it was discovered that Peninsular Malaysia did not have IDD problem until latter studies showed goitre occurrence of 34.7% in Hulu Langat district and urinary iodine lower than the adequate level of (100-199 ug/L) in Perak and Pahang states (Selamat et al., 2010). Baseline and periodical sampling of children and pregnant woman urine and imported salt commodities for the consumption of the population is mandatory for iodine measurement. Thus, development of quantitative methods of measurement of salt and food iodine is crucial for implementation of the USI program nationwide. In this study, interdigitated electrode (IDE) biosensor, a rapid, sensitive and selective method has been developed to determine the iodine content in both urine and salt. This method includes functionalization and silanization step using 3-aminopropyl triethoxysilane (APTES). The I-V characterization of IDE biosensor was performed using (Keithley 2450), Kickstart software and Probestation. It measures the amount of current flow through IDE which is directly proportional to the concentration of iodine in both urine and salt. Hence, IDE biosensor is proven to be a rapid, selective, sensitive method and can be developed as a new nanotechnology for the elimination of Iodine Deficiency Disorders (IDD) among children and pregnant woman.

1. Introduction
Iodine is an essential nutrient involved in the production of thyroid hormones and is required for the development of fetal nervous system during pregnancy [1]. Pregnant women are mostly susceptible to iodine deficiency because of its higher demand during pregnancy. Iodine deficiency can lead to major health problems, including endemic goiter, cretinism, and growth retardation. Besides that, it can also cause perinatal problem such as congenital anomalies, miscarriage and stillbirth [2]. Iodine deficiency is a serious and preventable health problem affecting more than 2 billion people worldwide [3].

In order to reduce the impact of iodine deficiency, it is essential to determine the population iodine nutrition status and promote adequate iodine intake during pregnancy. Children with adequate iodine nutrition were also shown to have higher intelligent quotient (IQ) compared to those with low iodine nutrition [4].
Measurement of urine iodine need to be carried out since low level of it has been reported to be correlated with the occurrence of Iodine Deficiency Disorders (IDD). The population median urinary iodine values have been classified accordingly by the World Health Organization (WHO) and urinary iodine concentration (UIC) in 2007 (Table 1) to severe, moderate and mild iodine deficiency, adequate iodine nutrition, slight and excessive risk for iodine-induced hyperthyroidism and autoimmune thyroid diseases.

**Table 1.** The classifications of median urinary iodine values related to iodine nutrition status (Source: WHO, 2007).

| Median urinary iodine (µg/L) | Iodine intake | Iodine nutrition status |
|-----------------------------|--------------|------------------------|
| School-age children (6 years or older) |  |  |
| <20 | Insufficient | Severe iodine deficiency |
| 20–49 | Insufficient | Moderate iodine deficiency |
| 50–99 | Insufficient | Mild iodine deficiency |
| 100–199 | Adequate | Adequate iodine nutrition |
| 200–299 | Above requirement | Risk of iodine-induced hyperthyroidism in susceptible groups |
| ≥300 | Excessive | Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease) |

| Pregnant women |  |  |
|----------------|--------------|------------------------|
| <150 | Insufficient |  |
| 150–249 | Adequate |  |
| 250–499 | Above requirements |  |
| ≥500 | Excessive |  |

| Lactating women and children aged less than 2 years |  |  |
|----------------|--------------|------------------------|
| <100 | Insufficient |  |
| ≥100 | Adequate |  |

* The term “excessive” means in excess of the amount required to prevent and control iodine deficiency

One of the most implemented interventions in combating the IDD is through Universal Salt Iodization (USI). Thus, the quantitation of the iodine in salt consignments is crucial upon enactment of USI (Hetzel, 1983). Quantitation of food iodine is also important for food science research including for compilation of food composition database for public use. The International Council for control of iodine Deficiency Disorders (ICCIDD) had proposed the standard method of titration for determining iodine content in salt. Commercially, iodine concentration is exclusively measured calorimetrically by the Sandell-Kolthoff method in which yellow cerium (IV) is reduced to cerium (III) in the presence of iodide ions. This method requires complicated sample preparation, involves a slow response time, and utilizes a toxic reagent that must be properly stored and disposed [5]. Other methods including the Inductively-Coupled Plasma-Mass Spectrometry (ICP-MS) is more sensitive and precise but is very expensive and may not be affordable by small/medium-sized laboratories. However, all these methods are laboratory-based methods and salt sample have to be sampled and taken to the laboratory for analysis.

Recently, researchers use an integrated approach by combining nanoscience technology, electronics, computers and biology to create portable biosensors with extraordinary sensing capabilities.
Thus, in this study nanotechnology biosensor will be develop for a rapid determination of iodine in both urine and salt. This kind of nanotechnology has been widely used in other medical field [12-14], agriculture [14-18] food industry [15-16], and Environment [22].

2. Material and method
2.1 Materials
Ethanol, 3-aminopropyl triethoxysilane (APTES), sodium chloride, commercial salt: rock salt and iodised salt. All analytical grade reagents were purchased from Sigma Aldrich.

2.2 Preparation of standard solution of iodine
A series of eight standard solution of iodine solutions were prepared by diluting 1000 µg/L of their stock solution with distilled water until calibration mark. The standard solutions were prepared at eight different concentrations depending on the sensitivity of the interdigitated electrode (IDE). The lowest concentration of iodine at 0.001 µg/L was prepared until 1000 µg/L.

2.3 I-V Characterization for detection of iodine concentration using interdigitated electrode (IDE)
Firstly, the IDE electrode was cleaned with ethanol and distilled water and left to dry for 5 minutes. After that, 2µL of APTES solution was pipetted on the active area of IDE electrode and left to dry in the dry cabinet at room temperature of 27 °C for 30 minutes. It builds an active layer on the IDE. Then, it was washed with distilled water to remove the unbounded APTES. APTES is an aminosilane frequently used in process of salinization and functionalization of the surfaces with alkoxysilane molecules. Lastly, 2µL of standard iodine and was pipetted on the active area of IDE electrode and kept inside the dry cabinet for 30 minutes. This step was repeated with 2µL salt solution. The I-V characterization then was completed using (Keithley 2450), Kickstart software and Probestation.

![Figure 1. I-V characterization of iodine and salt solution.](image)

3. Result and Discussion
The I-V characterization was performed using (Keithley 2450), Kickstart software and Probestation with the IDE biosensor to get the I-V characteristic as shown in Figure 2. The I-V characterization measures the amount of current flow through IDE. During the I-V characterization, picoammeter voltage was supplied from 0 to 1 V only, if the voltage applied more than that suitable range, it will damage the IDE biosensor [20]. Figure 2 shows the I-V characteristic for bare IDE, APTES and different concentration of standard iodine ranged from 0 ppb to 1000 ppb.

From the I-V measurement (Figure 2), the bare IDE gives the current value $2.27 \times 10^{-11}$ A whereas for the functionalize IDE with APTES gives the current value $1.19 \times 10^{-9}$. Then, the current value recorded for 0 ppb, 0.001 ppb, 0.01 ppb, 0.1 ppb, 1 ppb, 10 ppb, 100 ppb and 1000 ppb were $1.01 \times 10^{-9}$ A, $1.51 \times 10^{-9}$ A, $2.94 \times 10^{-9}$ A, $3.34 \times 10^{-9}$ A, $6.92 \times 10^{-9}$ A, $9.01 \times 10^{-9}$ A, $1.01 \times 10^{-8}$ A and $2.39 \times 10^{-8}$ A respectively. It can be seen that, the current values increase as the concentration of standard iodine increase. Hence, IDE biosensor has high sensitivity that can measure until the lowest concentration of iodine. The usage of the IDE sensors in the biosensor field is tremendous in these days because of the large number of comb structured finger electrodes that gain high sensitivity [23].
Figure 2. I-V characteristic of bare IDE, APTES and different concentration of standard iodine.

Figure 3. I-V characteristic of bare IDE, APTES and different concentration of standard iodine.

The I-V measurement of standard iodine then was repeated using 0 ppb, 20 ppb, 40 ppb, 60 ppb, 80 ppb and 100 ppb. The current values were $1.01 \times 10^{-9}$, $8.83 \times 10^{-9}$, $1.47 \times 10^{-8}$, $2.62 \times 10^{-8}$, $2.63 \times 10^{-8}$, $4.76 \times 10^{-8}$ and $6.34 \times 10^{-8}$ respectively. It shows that in Figure 3, the concentration of iodine is directly proportional to the current value, the current will continue to increase as the concentration of iodine increase. Thus, IDE biosensor capable for detection starting from low to high concentration of iodine.

Figure 4 shows the I-V characterization for different brands of salt where sodium chloride, NaCl act as a control. The current value for NaCl, sea salt and rock salt were $2.37 \times 10^{-8}$, $4.76 \times 10^{-8}$ and $2.42 \times 10^{-8}$ respectively. From the graph above, it clearly shown that, sea salt gives the highest current value which is $4.76 \times 10^{-8}$ compare to others. Sea salt is iodized salt which contain the highest amount of iodine among the other salt. IDE biosensor is proven to be a very sensitive and rapid method to detect the present of iodine in the samples. This advance nanotechnology biosensor can be applied in biomedical field for monitoring and intervention programme of Universal Salt Iodization (USI) for elimination of Iodine Deficiency Disorders (IDD) in pregnant woman and schoolchildren.
4. Conclusion
In this research, iodine was successfully detected using IDE biosensor. This nanotechnology biosensor can be developed and applied in biomedical field due to the simple, rapid, selective and sensitive method compared to the conventional method that requires complex laboratory handling methods and instrumentation.

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