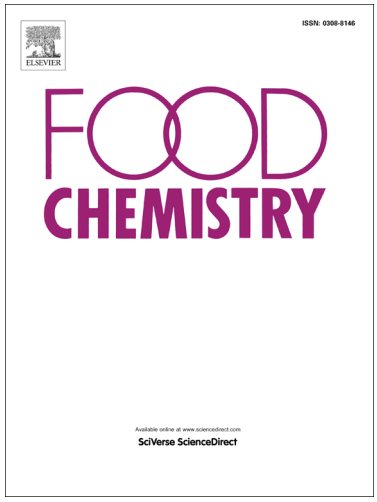


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# Electrochemical DNA Biosensor for Potential Carcinogen Detection in Food Sample

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**Highlights**

A novel electrochemical DNA biosensor was constructed for detecting the presence of carcinogens in food samples:

- A comparison study using three different DNA sequences was performed to prove the effectiveness of guanine rich DNA sequence for optimal binding with carcinogens.
- The DNA was immobilized onto silica nanospheres/gold nanoparticles modified screen-printed electrode.
- The biosensor was used to detect two carcinogens, namely formaldehyde and acrylamide.
- The applicability of the biosensor was evaluated on real food samples and compared using standard Ames test.

**Chemical compounds studied in this article**

23-Aminopropyl-triethoxysilane (PubChem CID: 110154); Glutaraldehyde (PubChem CID: 3485); potassium chloride (PubChem CID: 4873); sulfuric acid (PubChem CID: 1118); Ammonia (PubChem CID: 222); ethanol (PubChem CID: 702); mercaptoethanol (PubChem CID: 1567); tetraethoxysilane (PubChem CID: 6517); potassium dihydrogen phosphate (PubChem CID: 516951); potassium ferricyanide (PubChem CID: 26250); di-potassium hydrogen phosphate (PubChem CID: 24450); Ammonium Acetate (PubChem CID: 517165); Acetic acid (PubChem CID: 176); Acetyl acetone (PubChem CID: 31261)

**Abstract**

The presence of carcinogens in food is a major food safety concern. A nanocomposite-based electrochemical DNA biosensor was constructed for potential carcinogen detection in food samples by immobilizing amine terminated single stranded DNA onto silica nanospheres deposited onto a screen-printed electrode modified using gold nanoparticles. The effect of three different DNA sequences: 15-base guanine, 24-base guanine and 24-base adenine-thymine rich DNA on carcinogen (formaldehyde and acrylamide) detection was evaluated. The competitive binding of the DNA with the carcinogen and electroactive indicator, Methylene blue (MB) was measured using differential pulse voltammetry. Optimization studies were conducted for MB concentration and accumulation time, DNA concentration, buffer concentration, pH and ionic strength. Overall, the 24-base guanine rich DNA yielded the best results with a detection limit of 0.0001 ppm, linear range between 0.0001 ppm to 0.1 ppm and reproducibility below 5% R.S.D. Finally, the results obtained using the biosensor were validated using Ames test.

**Keywords:** Toxicity; Electrochemical biosensor; guanine rich DNA, carcinogens, silica nanospheres, gold nanoparticles

## 1. Introduction

Based on the estimates from the International Agency for Research on Cancer (IARC), in the year 2012 alone, there were 14.1 million of new cancer cases and 8.2 million cancer deaths reported worldwide. The global future burden is also expected to rise at an alarming rate, thus leading towards increased research on cancer prevention and treatment. In general, the substances and exposures that cause cancer are known as carcinogens. Although people can avoid exposure to some cancer-causing environmental exposures such as tobacco smoke and sun rays, it is challenging to avoid toxic pollutants that exist almost everywhere. One of the major sources of toxic pollutant is the substances that are formed during certain food processing operations, which may be carcinogenic in nature and present ill effects on human health. For example, during high temperature cooking such as frying or baking potato-based foods such as French fries or potato chips, a group 2 carcinogen known as acrylamide is released into air (Muttucumaru et al., 2011; Hsu, Chen, Tseng, Cheng, Huang & Yeh, 2016), and the amount of acrylamide formed is highly dependent on the different frying techniques (Danialia, Jinap, Hajeb, Sanny & Tan, 2016). Besides carbohydrate-based foods, a study conducted by Zhu, Ji, Li, Dong & Li (2013) discovered that heating squid extract at high temperature can result in the decomposition of trimethylamine oxide into carcinogens such as formaldehyde and dimethylamine.

A few studies have also reported the formation of formaldehyde in the tissues of stored fish due to enzymatic degradation process (Bianchi, Careri, Musci & Mangi, 2007; Sibirny, Demkiv, Klepach, Honchar & Gonchar, 2011; Wahed, Razzaq, Dharmapuri & Corrales, 2016). Since these carcinogens can easily enter into the human body via inhalation, ingestion, adsorption and direct exposure, it is important to monitor them from entering into the food chain. However, the existing conventional analytical methods used to detect carcinogens in food products lack on-site applicability, and involve expensive and

complicated laborious process that is time consuming and can be handled by trained personnel only. Therefore, the use of DNA biosensors has been recently introduced for on-site determination of potential carcinogens with high selectivity and specificity.

DNA biosensors have been used widely for other purposes as well like the detection of genetically modified organisms (Ulianas et al., 2012), DNA sequence of dangerous bacteria and pathogens (Rodriguex-Mozaz et al., 2004) or for the determination of certain species and its sex. For example, Tombelli et al. (2000) constructed a DNA piezoelectric biosensor to detect the presence of bacterial toxicity in environmental samples particularly from the *Aeromonas* strain. Hybridisation of its 23-mer probes with complementary sequences proved the presence of different *Aeromonas* bacterial strain in water, vegetable and human specimens. This biosensor was proven to be sensitive towards this particular strain only when interaction with non-specific samples yielded negative results. Abdalhai et al. (2005) built a DNA biosensor to detect *Escherichia coli*. The probes were modified with thiol (SH) and amine (NH2) to be bonded with cadmium sulphide particles (CdSNPs) immobilized onto a gold electrode surface. This biosensor was tested on contaminated meat and yielded a detection limit of 1.97 × 10–14 M with a correlation coefficient of 0.989. Marazza et al. (1999) described a DNA biosensor based on calf thymus DNA adsorbed onto graphite screen-printed electrode at a controlled potential which was able to detect intercalating and groove binding environmental pollutant compounds in river water samples like daunomycin, polychlorinated biphenyls (PCBs) and aflatoxin B1with detection limits as low as 0.2 mg/L.

In 2012, Ensafi, Razaei, Aminia and Heydari-Bafrooei constructed a simple DNA biosensor using salmon sperm ds-DNA-modified pencil graphite electrode (PGE) to detect the presence of Sudan II, using differential pulse voltammetry. This biosensor could detect the presence this carcinogenic food dye as low as 0.00007 $\mu g\, mL^{-1.}$ . Stobiecka, Radecka, and Radeck (2006), constructed a voltammetric biosensor to detect acrylamide in food using

a carbon-paste electrode modified with hemoglobin (Hb), which displays a decreasing peak current of Hb-Fe(III) reduction with a very low detection limit of $1.2 \times 10^{-10}$ M.

An electrochemical immunosensor was developed by Paniel, Radoi and Marty in 2010 to detect trace amounts of aflatoxin M1 in food products using a competitive immunoassay using horseradish peroxidase (HRP) as a tag and magnetic nanoparticles coated with antibody (anti-$AFM_1$) which were deposited on the surface of screen-printed carbon electrodes. The enzymatic response was measured amperometrically with a detection limit of 0.01 ppb. Generally, when humans are exposed to potential carcinogens, they will bind to human DNA, creating DNA adducts that leads to the damage of the DNA. If the damaged DNA is not repaired, the mutated DNA will then be replicated and transcribed, leading towards the pathogenesis of cancer (Flora, Izzoti, Randerath, Rederath, & Lewtas, 1996). Therefore, since DNA is the main target for potential carcinogens, it is logical to use DNA in the construction of electrochemical biosensors to detect potential carcinogens (Bohunicky & Mousa 2011).

In literature, many studies have proven that carcinogens seem to have preference towards certain sites within a DNA sequence, preferably guanine base sites (Lewis & Parry 2004). This indicates that mutations does not occur randomly, but occur within certain DNA sequences known as mutational hotspots (Ziegler, Leffell, Kunala, Sharma & Brash 1993; Denssenko, Kaoudriakova, Smith, O'Connor, Riggs & Pfeifer 1998) . When a carcinogen binds to a certain base within the DNA sequence, it will change the structure of the base. Besides DNA sequence, the immobilization technique of DNA onto the electrode surface of electrochemical biosensors will also affect the biosensors' response and performance. This is because the immobilization of the DNA will determine the conformation of the DNA on the surface of the electrode, thus dictating the accessibility of the carcinogens to the DNA binding sites (Diculescu, Paquim & Brett, 2005).

Therefore, in this study, the DNA probe was immobilized onto SiNSp to ensure that it was positioned in an ideal conformation for optimum competitive binding with carcinogen and MB. The nanocomposite of gold nanoparticles (AuNPs) mixed with SiNSp functions to improve the electron transfer rate, thus enhancing the overall electrochemical response of the biosensor. To the best of our knowledge, this is the first reported SiNSp/AuNPs nanocomposite-based DNA electrochemical biosensor, which was fabricated for the detection of potential carcinogens for food safety application.

## 2. Experimental Section

### *2.1 Instrumentation*

All electrochemical measurements were conducted using an AUTO-LAB PGSTAT potentiostat and GPES software package. The electrochemical system consists of a carbon working electrode on screen printed electrode (SPE), carbon pencil counter electrode and Ag/AgCl reference electrode. The differential pulse voltammetry (DPV) was scanned from -0.4 V to 0 V with a step potential of 0.01 V and scan rate of 0.005 V/s. For validation, the real samples were measured using UV-Visible Spectrophotometer Cary 50 and High Performance Liquid Chromatography PU-980 Intelligent HPLC pump. The Ames test was conducted in a Safemate 2.0 biosafety cabinet. The samples were kept in a Memmert Waterbath, while Memmert incubator was used for incubation purpose.

### *2.2 Chemicals*

The reagents 23-Aminopropyl-triethoxysilane (APTS, 99 %), DNA (15-base guanine rich, 24-base guanine rich and 24-base AT rich DNA), glutaraldehyde (25 %), gold colloid (20 nm), potassium chloride (KCl) and sulfuric acid ($H_2SO_4$) were purchased from Sigma Aldrich. The potential carcinogens, formaldehyde and acrylamide, and MB solution were

purchased from Sigma Aldrich and Systerm, respectively. Ammonia (25%) and ethanol (95%) were obtained from Systerm, whereas mercaptoethanol (MCE) and tetraethoxysilane (TEOS) were bought from Fluka. Both, potassium dihydrogen phosphate ($KH_2PO_4$) and potassium ferricyanide ($K_3[Fe(CN)_6]$) were obtained from Merck, while dipotassium hydrogen phosphate ($K_2HPO_4$) was purchased from BDH. For extraction of formaldehyde from fish sample, trichloroacetic acid was purchased from Sigma Aldrich, ammonium acetate from Merck, and both, acetic acid and acetyl acetone from Systerm. All chemicals were of analytical grade and used without further purification. All standard buffer and chemical solutions were prepared freshly with Milli-Q deionized water.

*2.3 Preparation of solutions*

MB solution (1 mM) was prepared by dissolving powdered MB in deionized water. To prepare DNA solutions of various concentrations, DNA stock solution (100 μM) was diluted with potassium phosphate buffer (pH 7) and stored at -5 $^{o}$C when not in use. All DNA sequences used in this study are as below:

15 bases guanine rich DNA : CGG-CGG-CGG-CGG-CGG

24 bases guanine rich DNA : CGG-CGG-CGG-CGG-CGG-CGG-CGG-CGG

24 base adenine-thymine DNA : AT-AT-AT-AT-AT-AT-AT-AT-AT-AT-AT-AT

Nash Reagent solution for the analysis of formaldehyde content in fish extract sample was prepared by mixing 75 g of ammonium acetate with 1.5 mL acetic acid500 mL of deionized water was added in the solution.

*2.4 Synthesis of silica nanospheres (SiNSp)*

2 mL of deionised water and 5 mL of ammonia solution were added into 20 mL of ethanol. The mixture was sonicated for 10 min, followed by an addition of 2 ml of TEOS and 4 mL of ethanol. The mixture was then sonicated for another 30 to 40 min at 55 $^{o}$C. Subsequently, the mixture was treated with 2 mL of APTS (99%) and stirred overnight. The following day, the APTS-silica solution was centrifuged at 4000 rpm for 20 min, and washed thoroughly with deionised water and ethanol for at least four times. The collected silica slurry was then air dried overnight before being used for the biosensor construction.

*2.5 Fabrication of electrochemical DNA biosensor*

The electrochemical DNA biosensor was fabricated as illustrated in Figure 1. First, 15 µL of gold colloid was dropped onto the screen printed electrode (SPE) and air dried overnight. This was followed by 10 µL of SiNSp (1.5 mg of SiNSp dispersed in 1 mL of ethanol) onto the SPE. The modified SPE was then immersed in 10% glutaraldehyde solution for an hour, before being immersed in 10 µM of DNA overnight. The next day, the SPEs were washed, immersed in potential carcinogens and MB before being scanned using DPV.

**Figure 1**. Diagram of the construction of the electrochemical DNA biosensor based on DNA immobilized onto SiNSp/AuNPs nanocomposite-modified SPE. The amine terminated single stranded DNA were immobilized onto the SiNSpvia glutaraldehyde linkers.

*2.6 Real sample preparation*

Two food samples were prepared for the purpose of comparing the performance of the developed biosensor to Ames test. The first sample was Mackerel fish purchased from a local supermarket, which was reported to contain high levels of formaldehyde, while the second

sample was cassava chips, which was reported to have high levels of acrylamide due to its preparation at high temperature.

The fish extract sample was prepared by homogenizing 30 g of Mackerel fish tissuein 60 mL of 5 % trichloroacetic acid (95%) for 30 min. The resulting liquid was then filtered using Whatman number 2 filter paper in order to separate the digested tissues.

On the other hand, the cassava chips were extracted by grinding 2 g of the sample, adding 20 mL of deionized water, and shaking it using an orbital shaker paper in a water bath for 30 min at 50 $^{o}$C. The mixture was then centrifuged at 12000 rpm for 20 min at 4 $^{o}$C. Finally, the resulting supernatant was filtered using an injection filter through a filter membrane measuring 13 mm in diameter with pore size of 0.22 µm.

*2.7 Real sample (Mackerel fish extract) analysis using UV-Visible spectrophotometer*

First, the calibration curve of formaldehyde standards was constructed between 0.01 ppm to 10 ppm formaldehyde, where 5 mL of the standard solution was mixed with 2 mL of Nash Reagent and heated in a water bath for 10 min at 60 $^{o}$C . The absorption of each standard solution was then measured at 415 nm wavelength. Finally, the fish extract samples with spiked concentrations of 0. 8, 10 and 15 ppm formaldehyde were tested likewise.

*2.8 Real sample (cassava chips extract) analysis using HPLC*

HPLC with reverse phase separation mode was used to determine the content of acrylamide in cassava chips extract sample. The HPLC system consists of one pump and connected to an injector. The HPLC column used was $C_{18}$ with a UV-Visible detector set at 210 nm wavelength. The analysis used methanol to air at ratio of 10: 90 for mobile phase and a flow rate of 1 mL/min. Before the analysis of the cassava chips extract sample, the calibration curve of acrylamide standards was constructed from 0.05 ppm to 1 ppm acrylamide.

*2.9 Comparison with Ames test*

The agar medium used was composed of two layers. The top layer (2 mL) consisted of 6% of agar and 0.6 % of NaCl, where the agar layer contained trace amounts of histidine and biotine with concentrations around 0.05 mM. The bottom layer was known as minimal glucose agar, which was composed of Vogel-Bonner E medium with 2% w/v glucose and 1.5% w/v agar. 20 mL of the agar medium was poured into the plate and kept in the refrigerator after being wrapped in plastic.

The fish and cassava chip extract samples were first filtered using sterile filter paper. The samples were then diluted using deionized water into 100%, 50%, 25%, 12.5% and 6.25% before being used for the Ames test. Next, negative control samples were prepared by using deionized water only, while positive control samples utilised mutagenic chemicals 4-nitro-*o*-phenylenediamine for the TA 98 bacterial strain and sodium azide for the TA 100 bacterial strain.

The bacterium that was used in this test was *Salmonella typhimurium* of the TA 98 and TA 100 strains. Before conducting the test, the bacterial culture was inoculated for 18 hours. The test tubes and agar plates were ensured to be sterile and labelled. The S9 metabolic mix for the purpose of activating any metabolites in the sample was prior prepared and kept in a cool place. The top agar must be in liquid form and kept at 43 $^{o}$C to 48 $^{o}$C before usage.

The sterile test tubes were added with 2mL of the liquid top layer agar, 0.5 mL of S9 mix or buffer solution, 0.05 mL of extract sample and 0.1 mL bacterial culture. The mixture was then poured on top of the minimal glucose agar plate. When the top layer agar hardened, the plate was inverted and kept in the incubator at 37 $^{o}$C for 48 hours. After this period, the amount of colony was counted and recorded. A positive result indicated a mutagenic sample,

which has a colony count that was double in amount to the negative control colony count. The results were then compared to the biosensor results.

## 3. Results and discussion

### *3.1 Confirmation of the DNA immobilization onto the modified SPE*

**Figure 2** shows the various DPV peaks at -0.25 V upon binding of MB to the guanine-rich DNA immobilized on the modified SPE. It can be observed that there is no peak present in the absence of MB (Figure 2(D), while a slight increase in the peak occurs upon the addition of MB (Figure 2(C)). The highest peak (Figure 2 (A)) was produced in the presence of both, DNA and MB, as the MB molecules bind to the bases of the DNA. However, a decrease in the peak (Figure 2 (B)) was observed in the presence of DNA, MB and carcinogen (formaldehyde), as the carcinogen molecules occupied the binding spaces of the DNA and decreased the amount of MB molecules that can bind to the bases of the DNA. The peaks that were observed in Figure 2 (A) and Figure 2 (B) proved that the DNA was successfully bonded covalently to the SiNSp via the linkers on the SPE.

MB molecules that are positively charged may bind electrostatically to the phosphate group of the DNA that is negatively charged, as well as bind to the bases of the DNA (Ulianas, Lee, Sharina and Tan, 2006). A recent study conducted by Ortiz, Fragosoa, Ortiz, O'Sullivan (2011) on the binding properties of MB molecules to single stranded DNA (ssDNA) reported that MB binds through electrostatic binding to the guanine bases of the DNA. This was observed using UV-visible spectroscopy, where quenching of MB fluorescence was favoured towards the guanine base, and produced lesser signal when MB and ssDNA with the base sequence of GGG-GGG-GGG were interacted in comparison to repetitive adenine, cytosine and thymine DNA base sequences (Lucarelli, Marraza, Turner, & Mascini 2004).

**Figure 2**: The DPV peak under the condition: a) AuNPs, SiNSp, DNA and MB immobilized onto the SPE; (b) AuNPs, SiNSp, DNA, 0.2 ppm formaldehyde and MB immobilized onto the SPE; (c) AuNPs, SiNSp and MB immobilized onto the SPE; and (d) AuNPs and SiNSp with DNA only immobilized onto the SPE.

*3.2 Optimization of DNA biosensor response*

All the optimized parameters for the DNA electrochemical biosensor are shown in TABLE 1. It is noteworthy that SiNSp are non-conductive in nature, thus no electron transfer takes place via this layer from the redox indicator to the electrode. Therefore, it is important to optimize the amount of AuNPs that acts as an excellent electron transfer material for optimum biosensor performance. From the experiments conducted, it can be observed that an increase in AuNPs, results in an increase in the response of the biosensor. This is because the AuNPs function to amplify the conduction pathways, thus enabling more electrons to be transferred.

**Table 1**: The optimum parameters for the DNA electrochemical biosensor.

| **PARAMETERS** | **OPTIMUM AMOUNT** |
|---|---|
| DNA concentration | 5 µM |
| Amount of gold colloid | 15µL |
| Amount of silica nanosphere | 0.012 mg |
| % of glutaraldehyde | 10 |
| Concentration of Methylene blue | 40 µM |
| Carcinogen accumulation time | 60 s |
| Methylene blue accumulation time | 90 s |
| Potassium phosphate buffer concentration | 0.15 M |

| | |
|---|---|
| Potassium phosphate buffer pH | pH 7 |
| Ionic strength | 0.15 M |

An increase in biosensor response was found with increasing SiNSp loading from 0.4 mg to 0.6 mg due to the increased binding of MB molecules to the DNA immobilized on the modified SPE. However, a decrease in the peak current was observed at above 0.6 mg of SiNSp loading because of the presence of additional non-conductive microspheres that were blocking the surface of the SPE, which indirectly reduced the electron transfer rate. This indicates the importance of the immobilization matrix, as well as the amount of immobilized biomolecules in order to ensure that the molecules are in a suitable orientation for optimal biosensor response and performance. This is in agreement with the study by Arotiba et al. (2008) that stated that immobilization matrix functions as a large electrode surface that will orientate the biomolecules for optimum immobilization.

Besides that, increasing concentration of DNA also affected the biosensors' response due to the increased availability of binding spaces. Based on the experiments, an increase in biosensor response was observed from 1 µM to 5 µM DNA concentrations, but the response starts to plateau beyond that. This is in correlation with the capacity of DNA immobilized on the SiNSp/AuNPS modified SPE. At the optimum DNA concentration of 5 µM, all the available amine groups and glutaraldehyde linkers were fully occupied. The increase in the amount of analyte detected electrochemically can be carried out by increasing the amount of immobilized biomolecules so that more analyte can be captured efficiently. This is in accordance with the basic principle of electrochemical response which is directly proportional with the amount of analyte species captured.

The percentage of glutaraldehyde was also optimised in this study. An increase in biosensor response was found from 2 % to 10 % glutaraldehyde, and the biosensors' response

plateaus beyond that. Glutaraldehyde act as linkers between the aminated DNA to the amine groups on the SiNSp. The plateau after the 10 % glutaraldehyde is due to the limited amount of DNA available to accommodate the linkers. However, if the amount of DNA is increased, the response of the biosensor will also increase with increasing amount of glutaraldehyde.

MB plays an important role in this biosensor as it is the indicator of the response of the biosensor. Therefore, it is imperative that its concentration, as well as its incubation time with DNA is optimized. It can be observed that there is an increase in biosensor response with MB concentration from 10 µM to 40 µM. This is in accordance with the capacity of MB molecules to bind to the DNA. At the optimum concentration of 40 µM, the DNA is fully bonded to MB molecules, and the biosensors' response stabilizes after this value as the DNA is already saturated with MB molecules. The time needed for MB molecules to fully bind to DNA is optimized at 90 s as the biosensor response increases from 30 s to 90 s. This is succeeded by a plateau of response as the binding spaces on DNA can no longer accommodate more MB molecules. This observation is in agreement with Ozkan et al (2002) and Kerman, Meric, Ozkan, Kara, Erdem, & Ozsoz (2002) that stated that the voltammetric signal of MB will decrease when MB molecules can no longer bind to guanine bases in DNA.

In addition, the response of this biosensor also increased from pH 6 to pH 7, but declines at pH 8. Generally, an acidic or basic medium can causedamage to DNA,thus resulting in the decrease in response at these pH values (Hianik, Ostana, Sonlajnerova & Grman 2007)Hence, the optimum pH for the buffer is at pH 7. The buffer used in this study is potassium phosphate buffer with potassium chloride as its salt. There is an increase in the response of the biosensor as the buffer capacity and ionic strength was increased from 0.01 M to 0.15 M. This is because high salt concentrations can stabilize the configuration of the DNA (Ortiz, Fragosoa, Ortiz, O'Sullivan 2011), thus giving better access for the MB and carcinogen molecules to bind to the DNA.

The performance of this biosensor was then tested on formaldehyde and acrylamide using the optimized conditions.

*3.3 Biosensor performance based on 24-base adenine-thymine (AT) rich DNA sequence*

The biosensor was first constructed using the 24-base AT rich DNA sequence. The performance of the biosensor was tested by assessing its linear range, correlation factor ($R^2$), detection limit, sensitivity and reproducibility on the monitoring of the potential carcinogens, acrylamide and formaldehyde, respectively. Each potential carcinogen gave different responses as seen in Figure 5, but on average the linear range for this type of DNA sequence was between 0.001 ppm to 0.1 ppm. The $R^2$ value on average was around 0.94, while its lower detection limit was 0.001 ppm. The % RSD (relative standard deviation) for this sensor on the detection of formaldehyde was 16.9 %, while for acrylamide was 14.37 %.

*3.4 Biosensor performance based on 15-base guanine rich DNA sequence*

The biosensor was then constructed used 15-base guanine rich DNA sequence. Each of the potential carcinogens gave different response as shown in Figure 6, but on average the linear range for this type of DNA sequence was narrower between 0.0001 ppm to 0.01 ppm. However, the linearity $R^2$ value on average was higher compared to AT-rich DNA around 0.97, while its lower detection limit was 0.0001 ppm. The % RSD for this sensor was also better than the biosensor constructed using 24-AT rich DNA sequence, where the % RSD for the detection of formaldehyde was 5.11 % and acrylamide was 7.46 %.

*3.5 Biosensor based on 24-base guanine rich DNA sequence*

On the other hand, the biosensor constructed using 24-base guanine rich DNA sequence demonstrated the linear range on average between 0.0001 ppm to 0.1 ppm. The average $R^2$ value was around 0.96, while its lower detection limit was 0.0001 ppm. The % RSD for this

sensor was excellent with the value for the detection of formaldehyde at 3.4 % and acrylamide at 5.6 %.

Each potential carcinogen gave different responses because they interact differently with DNA. They also act differently with different types of DNA. This is due to the fact that different carcinogens bind on different sites on the base of the DNA. Huang et al. (2015) reported the presence of a strong interaction between guanine and acrylamide on the N7 position of the base leading to the formation of DNA-acrylamide adduct. The formation of this adduct was detected from the reduction of DPV peak current due to DNA oxidation.

On the other hand, formaldehyde interacts with DNA to produce N-hydroxylmethyl adducts at the N2 position of guanine, as well as forms cross-linking between one guanine base to another (Lu, Collins, Ru, Bermudez, & Swenberg, 2010). The formation of this cross-linking will affect the wellbeing of cells if they are not removed during the DNA repair process. This explains the reason that these adducts are mostly found in the lungs of heavy smokers (Kawanishi, Matsuda & Yagi, 2014). Table 2 shows a comparison between the performances of the three types of DNA. The 24-base guanine rich DNA sequencegave the widest linear range between 0.0001 ppm to 0.1 ppm and also gave the biggest peak current value. The detection limit for both, the guanine rich DNA sequence is the same at around 0.0001 ppm, while AT rich DNA sequence was 0.001 ppm only. In terms of sensitivity, the 24-base guanine rich DNA and AT rich DNA sequence displayed high sensitivity, but the AT rich DNA sequence biosensor gave low reproducibility. The best reproducibility was produced by the 24 base guanine rich DNA sequence biosensor.

In terms of performance, it can be concluded that the best response was shown by the 24-base guanine DNA sequence biosensor. This may be due to the fact that carcinogens prefer to bind to guanine. This was also evinced when the 24-base guanine rich DNA sequence yielded better response compare to the shorter 15-base guanine rich DNA sequence

biosensor. Therefore, the biosensor using 24-guanine rich DNA sequence was further used for the detection of non-carcinogenic chemicals to determine this biosensor's sensitivity towards potential carcinogens, as well as for real sample testing.

**Table 2**: Comparison of the performance of the three different DNA biosensors for acrylamide determination.

| | **24 base GC** | **15 base GC** | **24 base AT** |
|---|---|---|---|
| **Linear range (ppm)** | 0.0001 to 0.1 ppm | 0.0001 to 0.01 ppm | 0.001 to 0.01 ppm |
| **Regression equation** | y = -1.629ln(x) + 13.801 | y = -0.982ln(x) + 6.3944 | y = -0.808ln(x) + 10.073 |
| **R2** | 0.9123 | 0.9735 | 0.9571 |
| **Lower detection limit (ppm)** | 0.00008 | 0.00009 | 0.0006 |
| **Sensitivity (ΔδCurrent/decade)** | -1.6 | -0.9 | -0.8 |
| **Relative standard Deviation (%)** | 4 | 7 | 11 |

**Table 3**: Comparison of the performance of the three different DNA biosensors for formaldehyde determination.

| | **24 base GC** | **15 base GC** | **24 base AT** |
|---|---|---|---|
| **Linear range (ppm)** | 0.0001 to 0.1 ppm | 0.0001 to 0.01 | 0.001 to 0.01 ppm |

| | | ppm | |
|---|---|---|---|
| **Regression equation** | y = -0.74ln(x) + 28.471 | y = -0.649ln(x) + 13.516 | y = -1.808ln(x) - 3.1212 |
| **R2** | 0.9747 | 0.9683 | 0.9437 |
| **Lower detection limit (ppm)** | 0.00008 | 0.00007 | 0.0006 |
| **Sensitivity (ΔδCurrent/decade)** | -0.7 | -0.6 | -1.8 |
| **Relative standard Deviation (%)** | 3 % | 8 % | 11 % |

*3.7 Comparison of real sample analysis using standard Nash method and biosensor for formaldehyde determination in mackerel fish extract*

A standard calibration curve was constructed based on Nash method and regression equation of y = 0.0141x + 0.0198 ($R^2$=0.9961) was obtained. Four mackerel fish extract samples were then prepared, where one sample was not modified in any way and the remaining three samples were spiked with formaldehyde to obtain concentrations of 8, 10 and 15 ppm, respectively. The unmodified sample was found to contain 7.64 ppm of formaldehyde, while the other three spiked samples contained 9.57, 11.64 and 16.57 ppm of formaldehyde, respectively using the Nash method.

The same analysis was then repeated using the 24-base guanine rich DNA biosensor. The biosensor detected the presence of formaldehyde of 6.74 ppm in the unmodified sample, while the other three spiked samples were found to contain 7.55, 13.12 and 15.78 ppm of formaldehyde, respectively.

Subsequently, a two-way T- test was conducted (p = 0.05 with a degree of freedom of 2 and the t distribution was 4.303) and the t value obtained from the calculation was 2.1057. Therefore, it can be concluded there is no significant difference between both the methods.

*3.8 Comparison of real sample analysis using standard HPLC-UV method and biosensor for acrylamide determination in cassava chips extract*

A standard calibration curve was constructed using HPLC-UV at retention time of 4.2 min and absorbance of 210 nm wavelength, where regression equation of y = 10431x - 148.63 ($R^2$= 0.9953) was obtained. Four cassava chips extract samples were then prepared, where one sample was not modified in any way and the remaining three samples were spiked with acrylamide to obtain concentrations of 1, 2.5 and 5 ppm, respectively. The unmodified sample was found to contain 0.81 ppm of acrylamide, while the other three spiked samples contained 3.07, 3.32 and 5.07 ppm of acrylamide, respectively using the HPLC-UV method.

Next, the same analysis was repeated using the 24-base guanine rich DNA biosensor. The biosensor detected the presence of acrylamide of 1.06 ppm in the unmodified sample, while the other three spiked samples were found to contain 1.96, 2.48 and 4.38 ppm of acrylamide, respectively.

Subsequently, a two-way T- test was conducted (p = 0.05 with a degree of freedom of 2 and the t distribution was 4.303) and the t value obtained from the calculation was 1.0921. Therefore, it can be concluded that there is no significant difference between both the methods.

*3.9 Comparison of real sample analysis using Ames test and biosensor*

Ames test was conducted to compare the biosensors' performance with an internationally recognized standard mutagenic test. The test was conducted on two samples, in which sample A is the cassava chips extract and sample B is the Mackerel fish extract. Each of these sample also underwent two tests each, one with the S9 mix and the other without the mix.

The colony growth on the agar plate indicated that the bacteria has mutated to its original wild type that can survive without histidine. The presence of mutagens in the sample will damage the bacteria's DNA that cannot survive without histidine to its original wild type that can survive without histidine.

The results of Ames test that was conducted on the cassava chips extract sample exhibited almost similar colony count with the negative control in all of the sample plates for both the strains. This means that the Ames test did not detect the presence of mutagens in this sample. On the other hand, the positive control showed 10 fold amounts of colony count compared to the other plates indicating that the bacteria is functional as it is able to cause mutations in the presence of a mutagen.

On the other hand, the results of Ames test that was conducted on the mackerel fish extract sample showed that all the bacteria strain, except TA 98 without the s9 mix showed negative results as the colony count on the sample plates were the same amount as the colony count on the negative control plates. However, the sample plates from the TA 98 strain without s9 displayed an interesting trend. The 100% plate showed almost double in amount of colony count compared to its negative control. This was followed by a gradual decrease in colony count from 100 % sample to 6.25 % sample. This is an indication that the sample is probably mutagenic also.

Based on the results from Ames test, it was evinced that the cassava chips extract sample does not contain any mutagenic chemicals. However, the 24-base guanine rich DNA biosensor detected the presence of low concentration of acrylamide around 0.021 ppm, which is beyond the detection limit of Ames test. Furthermore, IARC has classified acrylamide substance as both, carcinogenic and mutagenic, which may be the reason that this sample did not cause any mutagenic effects to the bacteria.

Next, based on the biosensor response for Mackerel fish extract sample, it can be concluded the sample might be mutagenic for bacterial strain TA 98 without s9 mix. The mutagenic characteristics of this sample was mild because the sample was diluted few times when it was added with the bacterial culture and agar. In addition, according to Kawanishi et al. (2014), the mutagenicity of formaldehyde is not so strong compared to other mutagens like 4-nitroquinolene-1-oxide. Formaldehyde mutates the bacterial DNA by causing base change mutation and frame shift mutations without the presence of a metabolic activator.

*3.10 Calculation of carcinogenicity percentage for the real samples*

Next, the carcinogenicity percentage of the real samples were calculated, in which the calculation valuewas used to determine the carcinogenic strength of the samples. The percentage was also compared with the biosensors' result, as well as the Ames test results. The percentage calculation method that was based on the work by Xu, Ye, Yang, Li, & Yang (2014) used the results obtained from the detection of real samples using the 24-base guanine rich DNA biosensor, as well as the baseline value obtained from the detection of the non-carcinogenic chemicals::

% carcinogenicity, $I_C = [(I_{max\ peak\ height} - I_{biosensor+sample})/I_{max\ peak\ height}] \times 100\%$

Where

$I_{biosensor+sample}$ represents the maximum peak height that was obtained when the samples were tested using the biosensor

$I_{max\ peak\ height}$ represents the maximum peak height that was obtained from the calibration curves of formaldehyde and acrylamide

While the Baseline % was calculated using the formula:

Baseline %= $(I_{biosensor+non\text{-}carcinogenic\ toxicants}/I_{max\ peak\ height}) \times 100$

Where

$I_{biosensor+non\text{-}carcinogenic\ toxicants}$ represent the average peak height that was obtained from the detection of non-carcinogenic toxicants taken from section 3.7 with the value of 5.62525 µA.

If the carcinogenicity % obtained was above the baseline %, it can be interpreted that the sample is carcinogenic and vice versa. Therefore, the cassava chips extract sample is not carcinogenic as the carcinogenicity % was slightly below the baseline %. This result is comparable to the result obtained in the Ames test, where the sample did not exhibit any mutagenicity effect towards the bacteria. However, the mackerel fish extract sample showed a carcinogenicity % above the baseline % by 34.51 % more, which is in agreement with the Ames test results. This confirms that the mackerel fish extract sample was capable of mutating the bacterial strain of TA98.

## 4. CONCLUSION

The performance of three different electrochemical DNA biosensors based on different DNA sequences was successfully evaluated and compared. This study evinced that potential carcinogens prefer to bind to guanine base, thus increasing the amounts of guanine bases within the DNA sequence yields better responses in terms of sensitivity, detection limit, linear range and reproducibility. The use of SiNSp and AuNPs have contributed significantly to produce a highly sensitive and selective DNA biosensor. The constructed biosensor was

also shown to be capable of detecting the presence of potential carcinogens or mutagens in accordance with the widely-employed Ames test.

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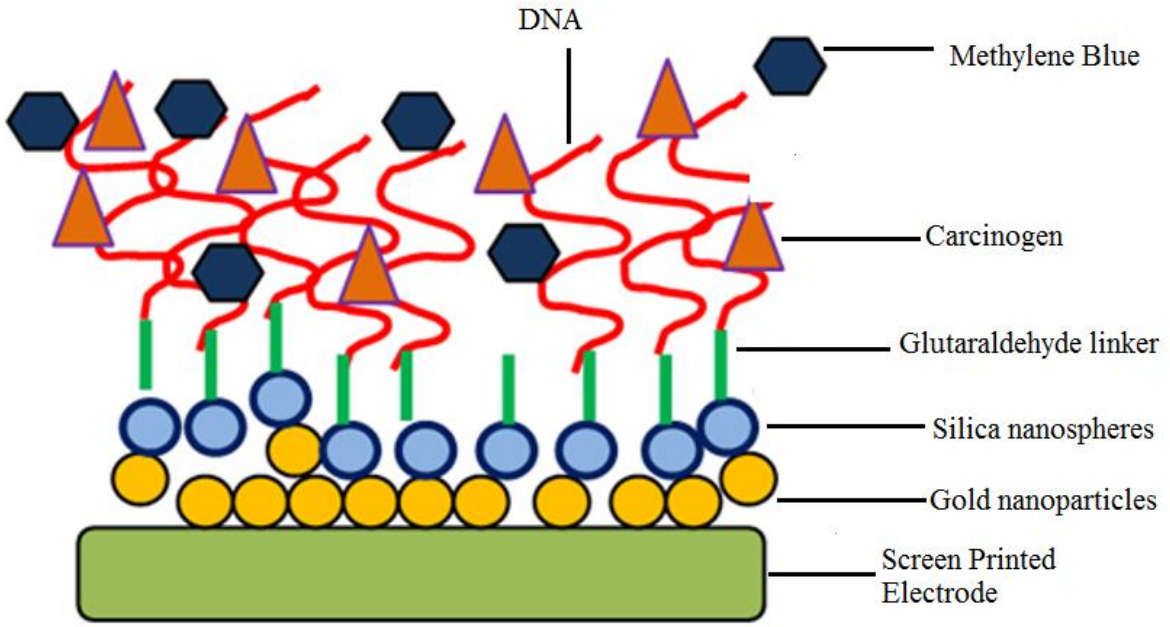


**Figure 1**. Diagram of the construction of DNA toxicity biosensor based on DNA immobilized onto silica and gold nanosphere modified SPE. Amine terminated single stranded DNA will be immobilized onto the silica nanospeheres via glutaraldehyde linkers.

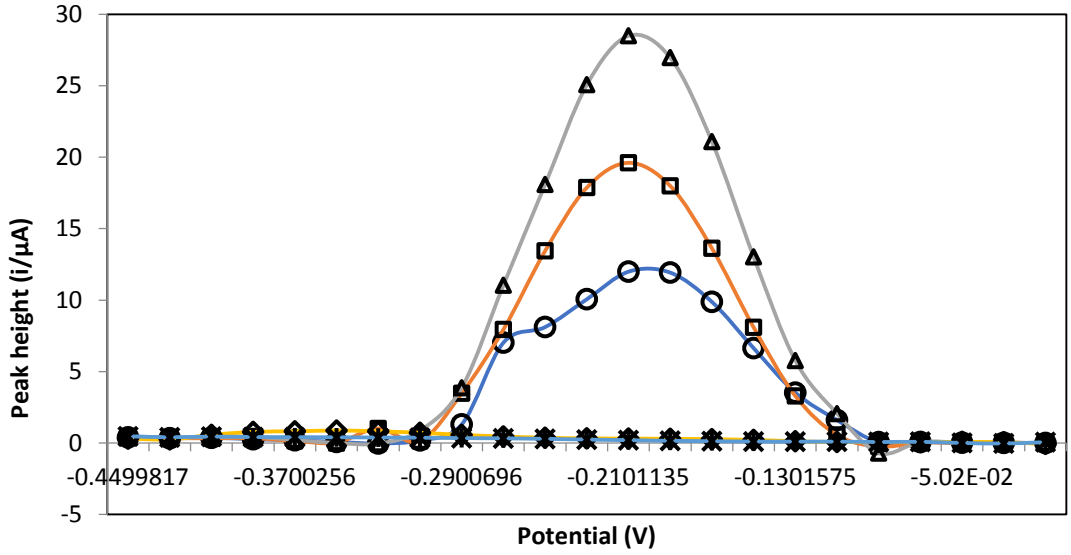


**Figure** 2: a) gold nanoparticles, silica nanopsheres, DNA and MB immobilized onto SPE (b) gold nanoparticles, silica nanopsheres, DNA , 0.2 ppm formaldehyde and MB immobilized onto SPE (c) gold nanoparticles, silica nanopsheres and MB immobilized onto SPE (d) gold nanoparticles and silica nanopsheres with DNA only immobilized onto SPE

**Highlights**

A novel electrochemical DNA biosensor was constructed for detecting the presence of carcinogens in food samples:

- A comparison study using three different DNA sequences was performed to prove the effectiveness of guanine rich DNA sequence for optimal binding with carcinogens.
- The DNA was immobilized onto silica nanospheres/gold nanoparticles modified screen-printed electrode.
- The biosensor was used to detect two carcinogens, namely formaldehyde and acrylamide.
- The applicability of the biosensor was evaluated on real food samples and compared using standard Ames test.