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Development of an Electrochemical Microsystin-LR Aptasensor Using Iron Nanoparticles Synthesized from Ripe Banana Peels Extract

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Abstract:
In this work, an electrochemical aptasensor was developed for the detection of Microcystin-leucine arginine (MC-LR) by immobilizing MC-LR targeting aptamers onto electro-deposited polyaniline (PANI) doped with synthesised iron nanoparticles (FeNPs). Aptamer microcystin binding event was monitored and recorded by cyclic voltammetry. The linear range (LR) and the limit of detection (LOD) of the aptasensor were from 0.0 – 1.0 µg $L^{-1}$and 0.06 µgL^{-1} respectively. The World Health Organization (WHO) has set a concentration limit of 1µg$L^{-1}$for MC-LR in drinking water, indicating that the developed aptasensor was enough for practical application. Therefore, this electrochemical aptasensor can be applied for detection of microcystin-LR in drinking water samples.

Keywords: *Polyaniline, iron nanoparticles, aptamers, microcystin-LR, aptasensor*

1. Introduction

Surface waters are currently the main source of drinking water for a large population of the world but due to increased nutrient pollution resulting from eutrophication, most of these surface waters contain toxic cyanobactaria. These cyanobactaria upon ingestion by fish, mammals and birds affects the liver, resulting into death or extensive liver damage [1].

Cyanobacteria, sometimes known as blue-green algae, consist of a family of one celled algae that grow in lakes, slow-moving streams, ponds and other water reservoirs. They thrive in warm and nutritious waters producing a cluster of toxins known as microcystin. Microcystins are cyclic peptides having approximately eighty structural variants and commonly found in water bodies throughout the world. The most common of these variants is Microcystin-LR, Microcystin-YR, Microcystin-RR, and Microcystin-LW. Water-skiing, swimming or boating in Microcystins contaminated water can lead to the exposure to the toxins. These toxins may also be found in fish that are taken from such contaminated water. Reports by World Health Organisation (WHO) have indicated that livestock and pets have died after consuming Microcystins contaminated water [1, 2].

Among the various microcystin, the Microcystin-leucine arginine (MC-LR) is the most common and toxic secondary metabolite produced by freshwater cyanobacteria [2]. It is released in considerable amounts through the bacterial cell lysis. It is chemically stable, highly soluble in water and can survive in the surface water bodies for a long time. The Microcystin poisons can chemically decompose at high temperatures (40°C) or at either very high pH (>9) or low (<1) [3]. The half-life at pH 1 and 40^{o}C is approximately three weeks while at ambient conditions the half-life is ten weeks. The toxins can stay for months or years when released into dark, cooler and natural water bodies. The toxins have been shown to persist even after boiling, implying that cooking does not destroy the toxins [4].

Current technologies used for the detection of Microcystins depends mainly on two methods: analytical methods such as liquid chromatography (LC) or biochemical screening methods like enzyme-linked Immunosorbent assays (ELISA) [5, 6], protein phosphatase inhibition assays (PPIA) [7] or competitive enzyme immunoassays [8]. Analysis of microcystin-LR using HPLC on C-18 following extraction of cells with 75% methanol has achieved a detection limit of 0.1–1mg/liter [9]. A detection limit of 0.1–0.5mg/liter has been achieved using immunoassay kits (ELISA) for Microcystins dissolved in water [10]. Protein phosphatase assay have detected 0.5–1.5mg/liter Microcystins dissolved in water [4, 11].

However, all these methods mentioned above have their inherent disadvantages. For example, the LC methods usually require longer time, consuming sample preparations that involve the pre-concentration of substantial amount of water volumes prior to analysis. The PPIA and ELISA on the other hand might not be able to distinguish between the various Microcystins as well as unrelated protein phosphatase inhibitors [11]. Recently reported are nuclear magnetic resonance and optical measurements [6, 8]. Although offering a high sensitivity, these methods are rather complicated, normally require a lot of time during sample pre-treatment procedures, highly skilled operators and expensive instrumentation, making them

inappropriate for routine monitoring of the microcystin. Also, different biological and chemical sensors have been reconnoitered for detection of these toxins.

In our previous work, we successfully synthesized Iron nanoparticles using eco-friendly, rapid, simple and low-cost approach through reduction of ferric chloride solution in a green method using ripe banana peels extract as the reducing agent [12]. This work focuses on developing an electrochemical apt sensor utilizing the ripe banana peels extract (RBPE) synthesized iron nanoparticles for determination of microcystin-LR (MC-LR). The aptamer based nanobiosensor was prepared with aptamer deposited on glassy carbon electrode (GCE) electrocatalytically activated with metalized polymeric nanocomposite system. The polymeric nanocomposites system was synthesized with polyaniline (PANI) imprignated with iron nanoparticles (FeNPs). The aptasensor response toward microcystin-LR was monitored using cyclic voltammetry.

2. Experimental

2.1. Chemicals and Reagents

Iron (III) chloride was purchased from Merck and was used without further purification. The distilled de-ionized water (DDW) was obtained from the Biochemistry laboratory at the University of Nairobi. Microcystin target aptamer were isolated by SELEX process from a random ss-DNA library by Kim et al. (2007) [32]. Microcystin-LR was bought from Enzo Life Sciences, USA and was dissolved into 50 mM Tris, pH 7.5, (150mM NaCl, 2mM $MgCl_2$) binding buffer. This stock was further diluted to the required concentrations during the experiments.

2.2. Electro-Immobilization of the Nanocomposite Film

Electro-polymerization of polyaniline (PANI) onto the bare glassy carbon electrode (GCE) was achieved by the use of aniline and ammonium hydrate salt in 97% hydrochloric acid. Platinum wire and Ag/AgCl in saturated potassium chloride (KCl) were employed as the counter and reference electrodes respectively. The potential was swept between -200mV and 900mV. The resulting electrode was labeled PANI/GCE. Electrodeposition of the iron nanoparticles onto the PANI/GCE was carried out for one hour using 10μL of the iron nanoparticles dispersed in water. Ag/AgCl in saturated KCl and Platinum wire were used as the references and counter electrodes respectively. The potential window was set between -200mV and 900mV. The electrode was then rinsed with ultra-purified water and dried in open air. The resulting electrode was referred to as the FeNPs/PANI/GCE.

2.3 Immobilization of the Aptamer onto GCE Modified Surface (FeNPs/PANI/GCE)

The self-assembly of microcystin targeting aptamer onto the PANI/FeNPs modified GCE was prepared as follows: the aptamer was denatured through heating at 90^{o}C for 15 minutes, quickly cooled to 5^{o}C for 10 minutes then incubated at 4^{o}C for 4 min so as to allow re-naturation and attainment of the most stable conformation. For the aptamer immobilization, PANI/FeNPs modified GCE was kept in a solution containing the microcystin targeting aptamer for 16 hours at 5 ^{o}C. Finally, the PANI/FeNPs/aptamer was rinsed with ultra-purified water to remove unbound aptamers, followed by air drying. The resulting electrode was then employed as the aptasensor for the detection of microcystin-LR.

2.4. Voltammetry

Basi Epsilon Ec-ver. 2.00.71_XP work station equipped with three-electrode cell system from BioAnalytical systems, BAS, US, was used to carry out the voltammetric experiments. A glassy carbon electrode of 3mm diameter and area of 0.071 cm^2 was used as the working electrode. A platinum (Pt) wire from Sigma Aldrich was used as the auxiliary electrode while the reference electrodes were silver/silver chloride (Ag/AgCl) electrodes. Alumina slurries of 1.0, 0.3 and 0.05 μm and micro polishing pads were obtained from Buehler, IL, USA and employed in the cleaning and polishing of the GCE. All the cell solutions used were bubbled prior to each experiment with analytical grade argon to degas them. All the experiments were performed at room temperature and pressure and the resulting voltammograms recorded in a computer interfaced to the Basi Epsilon workstation. All the solutions used for the cyclic voltammetry were kept in a refrigerator at -20 ^{o}C when not in use.

3. Results and Discussions

Atypical cyclic voltammogram of PANI being grown on a glassy carbon electrode through oxidative polymerization of 0.01 M aniline in 0.5 M HCl is shown in *figure 1*.

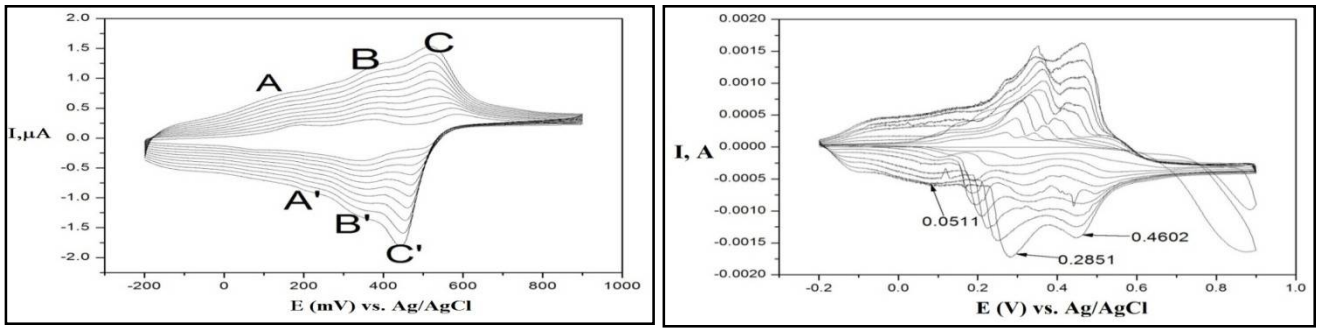

Figure 1: Electrodeposition of PANI onto the GCE at a scan rate of 50mV/s. A/A', B/B' and C/C' are redox couples for polyaniline

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Figure 2: Electrodeposition of Fe nanoparticles onto PANI/GCE at a scan rate of 50mV/s. 0.4602V, 0.2851V and 0.0511V are reduction potentials for FeNPs/PANI/GCE

The three peaks of reductive reaction and three peaks of oxidative reaction that were observed were consistent with the results obtained by Mathebe *et al*. [13] and [14] (*figure 1*). In the electrodeposition of the iron nanoparticles onto the PANI/GCE, the voltammograms still had three sets of redox couples (A/A', B/B' and C/C') though the peaks were not well defined (*figure 2*). The three sets of redox couples were as a result of characteristic redox processes of PANI as observed there earlier [13, 14]. The electron diffusion took place along the chain of an adsorbed electroactive polymer [15, 16]. The role of the PANI film in the aptasensor construction was thus to serve as a point of attachment for the nanoparticles and to enhance the catalytic activity of the aptamers for the detection of microcystin-LR while iron nanoparticles provides a large specific surface area for increased immobilization of the aptamer [33]. As shown in figure 2, the peaks were well defined and remain unaltered during the cycles, reflecting the stability of the iron nanoparticles coating onto the PANI/GCE and the strong interaction between PANI and iron nanoparticles [17].

An aptasensor (Aptamer/FeNPs/PANI/GCE) was developed by keeping FeNPs/PANI/GCE fabricated electrode surface in a solution containing microcystin targeting aptamer. Sensing of microcystin-LR was anchored on the relative change in current response. Figure 3 shows cyclic voltammetric responses of the aptasensor before and after the addition of microcystin-LR.

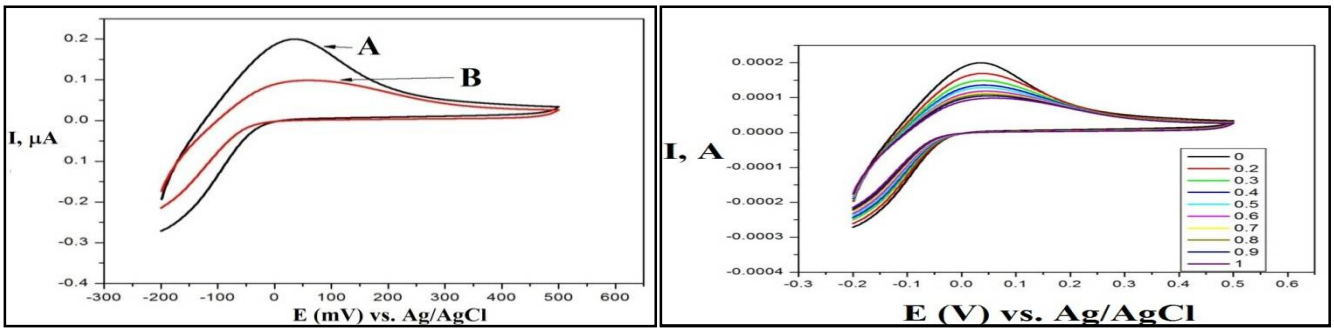

Figure 3: Cyclic voltammetric responses of the aptasensor before (A) and after (B) aggregation following addition of 0.1µM of microcystin-LR at 37°C.

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Figure 4: Cyclic voltammetry of aptasensor with different MC-LR concentration (0 – 1µM) at a scan rate of 100 mV s^{-1} at 37 °C.

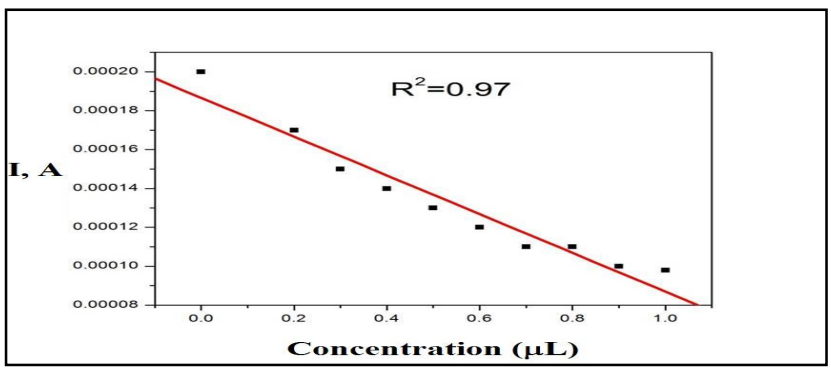

Figure 5: A calibration plot showing the relationship between peak current and concentration of microcystin-LR

The aggregation following the introduction of microcystin-LR caused a sharp decrease in the peak current (*Figure 3B*). This was as result of the binding of the microcystin-LR to the aptamer thereby reducing conductance of the aptamer immobilized on the FeNPs/PANI/GCE. Figure 4 shows cyclic voltammograms taken from a series of varied microcystin-LR concentrations.

No.	Method	LR (µg/L)	LOD (µg/L)	Ref.
1.	HPLC	-	0.017µg/g	19
2.	ELISA	0.022 – 0.8	0.022	20
3.	Mass spectrometry	-	0.5	21
4.	Protein phosphatise inhibition	0.93 – 40.32	0.93	22
5.	Colorimetric inhibition assay	0.098 – 1.56	0.05	23
6.	Colorimetric sensor	0.5 – 7.5x10^3	0.37	24
7.	Fluorescent aptasensor	0.4 – 1.2x10^3	0.137	25
8.	Chemiluminescence enzyme immunosensor	0.062 – 0.65	0.032	26
9.	Electrochemical impedence aptasensor	0.0498 – 100	0.0179	27
10.	Electrochemical immunosensor	0.01 - 50	0.007	28
11.	Label-free voltammetryc aptasensor	-	0.0019	29
12.	Amperommetric immunosensor	0.01 - 100	0.01	30
13.	Label-free electrochemical immunosensor	0.05ng/mL– 25µg/mL	0.017	31
14.	Electrochemical aptasensor	0.00 – 1.00	0.06	Current

Table 1: Comparison of different reported methods of Microcystin-LR detection
Note: LR = Linear range; LOD = Limit of detection; Ref = reference

The interaction of the aptamer with the microcystin-LR was confirmed by the drop in the peak current as the concentration of microcystin-LR increases with a correlation coefficient (R^2) equals to 0.97 (*figure 5*). After optimization of the dose response curve (*figure 5*) and based on the average standard deviation of the measurements (σ) and the slope (S) of the fitting curve as $3\sigma/S$ [18], the limit of detection (LOD) for the aptasensor was found to be 0.06 µg/L. This sensitivity was obtained using cyclic voltammetry. Further improvement in sensitivity can be obtained if a more sensitive technique like square wave or differential pulse voltammetry is used.

The performance of the current electrochemical aptasensor was compared with the reported techniques of microcystin-LR detection. As shown in Table 1, its detection limit and linear range compares favorably well with earlier work. It also met with the need of WHO guideline limit of 1 µg/L in drinking water, indicating that it was enough for practical application. Therefore, this electrochemical aptasensor can be applied for detection of microcystin-LR in drinking water samples.

4. Conclusion

A simple and highly sensitive GCE/PANI/FeNPs/aptamer based electrochemical biosensor has been developed for the detection of Microcystin-LR. This GCE/PANI/FeNPs/aptamer biosensor was able to detect Microcystin-LR up to 0.06 µg/L. The high sensitivity of this aptasensor for the target was attributed to high surface area exhibited by the PANI/FeNPs nanocomposite which significantly enhanced the aptamer loading.

5. **Acknowledgement**

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6. References

i. Niazi, J. H., Lee, S. J., Kim, Y. S., and Gu, M. B. (2008). ssDNA aptamers that selectively bind oxytetracycline. *Bioorganic & medicinal chemistry*,16, 1254-1261.

ii. Lobón, G. S., Yepez, A., Garcia, L. F., Morais, R. L., Vaz, B. G., Carvalho, V. V., de Oliveira, G. A. R., Luque, R. & Gil, E. de-S. (2017) Efficient electrochemical remediation of microcystin-LR in tap water using designer TiO2@ carbon electrodes. *Scientific Reports,* 7, 41326. doi: 10.1038/srep41326.

iii. Lahti, K., Rapala, J., Färdig, M., Niemelä, M., & Sivonen, K. (1997). Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. *Water Research*, 31(5), 1005-1012.

iv. Mountfort, D. O., Holland, P. & Sprosen, J. (2005). Method for detecting classes of microcystins by combination of protein phosphatase inhibition assay and ELISA: comparison with LC-MS. *Toxicon,* 45, 199-206.

v. Pyo, D., Choi, J., Hong, J. & Hlaing O. H. (2006). Rapid analytical detection of microcystins using gold colloidal immunochromatographic strip. *Journal of Immunoassay and Immunochemistry*, 27(4), 291-302.

vi. Lindner, P., Molz, R., Yacoub-George, E., Dürkop, A. & Wolf, H. (2004). Development of a highly sensitive inhibition immunoassay for microcystin-LR. *Analytica chimica acta,* 521(1), 37- 44.

vii. Cai, H., Lee, T. M. H., & Hsing, I. M. (2006). Label-free protein recognition using an aptamer-based sensor. *Sensors and Actuators B-Chemical*, 114(1), 433 – 437.

viii. Long, F., He, M., Zhu, A. N., & Shi, H. C. (2009). Portable optical immunosensor for highly sensitive detection of microcystin-LR in water samples. *Biosensors and Bioelectronics,* 24(8), 2346 - 2351.

ix. Song, S., Wang, L., Li, J., Fan, C., & Zhao, J. (2008). Aptamer-based biosensors. *Trends in Analytical Chemistry*, 27, 108-117.

x. Moreira, C., Ramos, V., Azevedo, J., & Vasconcelos, V. (2014). Methods to detect cyanobacteria and their toxins in the environment. *Applied microbiology and biotechnology*, 98(19), 8073 - 8082.

xi. Sangolkar, L. N., Maske, S. S., & Chakrabarti, T. (2006). Methods for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria. *Water research*, 40(19), 3485 - 3496.

xii. Ondiek J. K. O. & Guto, P. M. (2018), Green Synthesis and Characterization of Ripe Banana Peels Stabilized Iron Nanoparticles. International *Journal of Science and Technology. Submitted.*

xiii. Mathebe, N. G., Morrin, A. & Iwuoha, E. I. (2004). Electrochemistry and scanning electron microscopy of polyaniline/peroxidase-based biosensor. *Talanta*, 64, 115 - 120.

xiv. Iwuoha, E. I., Saenz de Villaverde, D., Garcia, N. P., Smyth, M. R. & Pingarron, J. M. (1997). Reactivities of organic phase biosensors. 2. The amperometric behaviour of horseradish peroxidase immobilised on a platinum electrode modified with an electrosynthetic polyaniline film. *Biosensors and Bioelectronics*, 12, 749 - 761.

xv. Kanungo, M., Kumar, A. & Contractor, A. Q. (2002). Studies on electropolymerization of aniline in the presence of sodium dodecyl sulfate and its application in sensing urea. *Journal of Electroanalytical Chemistry*, 528, 46 - 56.

xvi. Bistolas, N., Wollenberger, Jung, U. C. & Scheller, F. W. (2005). Cytochrome P450 biosensors—a review. *Biosensors and Bioelectronics*, 20, 2408 - 242.

xvii. Peulon, S. & Lincot, D. (1998). Mechanistic study of cathodic electrodeposition of zinc oxide and zinc hydroxychloride films from oxygenated aqueous zinc chloride solutions. *Journal of the Electrochemical Society*, 145(3), 864 - 874.

xviii. Oyagi, M. O., Onyatta, J. O., Kamau, G. N. & Guto, P. M. (2016). Validation of the Polyacrylic acid/Glassy Carbon Differential Pulse Anodic Stripping Voltammetric Sensor for Simultaneous Analysis of Lead(II), Cadmium(II) and Cobalt(II) Ions. *International Journal of Electrochemical Science (IJES)*, 11, 3852 - 3861.

xix. Aguete, E. C., Gago-Mart´ınez, A., Le˜ao, Rodr´ıguez-V´azquez, J. M., Men`ard, J. A., C. & Lawrence, J. F. (2003). HPLC and HPCE analysis of Microcystins RR, LR and YR present in Cyanobactaria and Water by using Immunoaffinity extraction. *Talanta*, 59, 697–705.

xx. Lotierzo, M., Abuknesha, R., Davis, F. & Tothill, I. E. (2012). A membrane-Based ELISA Assay and Electrochemical Immunosensor for Microcystin-LR in water samples. *Environmental. Science and Technology,* 46(10), 5504–5510.

xxi. Liu, S., Deng, C. & Zhang, X. (2016). Facile synthesis of Cu^{2+} -modified mesoporous silica-coated magnetic grapheme composite for enrichment of microcystin-LR followed by mass spectrometry analysis. *Talanta*, 154, 183–189.

xxii. Catanante, G., Espin, L. & Marty, J. (2015). Sensitive biosensor based on recombinant PP1α for microcystin detection. *Biosensors and Bioelectron*ics, 67, 700–707. https://doi.org/10.1016/j.bios.2014.10.030

xxiii. Sassolas, A., Catanante, G., Fournier, D. & Marty, J. L. (2011). Development of a Colorimetric inhibition assay for microcystin-LR detection: Comparison of the sensitivity of different protein phosphatases. *Talanta,* 85, 2498–2503.

xxiv. Li, X., Cheng, R., Shi, H., Tang, B., Xiao, H. & Zhao, G. (2016). A simple highly sensitive and selective aptamer-based colorimetric sensor for environmental toxins microcystin-LR in water samples. *Journal of Hazardous Materials,* 304, 474–480. https://doi.org/10.1016/j.jhazmat.2015.11.016

xxv. Taghdisi, S. M., Danesh, N. M., Ramezani, M., Ghows, N., Shaegh, S. A. M. & Abnous, K. (2017). A novel fluorescent aptasensor for ultrasensitive detection of microcystin-LR based on single-walled carbon nanotubes and dapoxyl. *Talanta,* 166, 187–192.

xxvi. Long, F., Shi, H. C., He, M., Sheng, J. W. & Wang, J. F. (2009). Sensitive and rapid chemiluminescence enzyme immunoassay for microcystin-LR in water samples. *Analytica Chimica Acta,* 649(1), 123–127. https://doi.org/10.1016/j.aca.2009.07.026

xxvii. Lin, Z., Huang, H., Xu, Y., Gao, X., Qiu, B., Chen, X. & Chen, G. (2013). Dtermination of microcystin-LR in water by a label-free aptamer based electrochemical impedance biosensor. *Talanta,* 103, 371–374.

xxviii. He, Z., Wei, J., Gan, C., Liu, W. & Liu, Y. (2017). A rolling circle amplification signal-enhanced immunosensor for ultrasensitive microcystin-LR detection based on a magnetic grapheme functionalized electrode. *RSC Advances*, 7, 39906 – 39913. DOI: 10.1039/c7ra07696g

xxix. Shimaa, E., Andy, N., Mohamed, S. & Mohammed Z. (2014). Label-Free VoltammetricAptasensor for the Sensitive Detection of Microcystin-LR Using Graphene-Modified Electrodes. *Analytical Chemistry*, 86 (15), 7551–7557. DOI: 10.1021/ac501335k

xxx. Lesedi, L., Jongjit, J., Martin, H. & Bo, M. (2017). Electrochemical Flow-ELISA for Rapid and Sensitive Determination of Microcystin-LR Using Automated Sequential Injection System. *Sensors,* 17, 1639. doi:10.3390/s17071639

xxxi. Fu, X., Zhao, C., Liu, X., Zhao, J., Niu, X., Zheng, L. & Yang Y. (2016). A layer-by-layer assembly label-free electrochemical immunosensor for the detection of microcystin-LR based on CHIT/PAMAM dendrimer/silver nanocubes. *International Journal of Environmental Analytical Chemistry*, 96(3), 284-297. DOI: 10.1080/03067319.2016.1150460

xxxii. Kim, Y. S., Jung, H. S., Matsura, T., Lee, H. Y., Kawai, T. & Gu, M. B. (2007). Electrochemical detection of 17β-estradiol using a DNA aptamer immobilized gold electrode chip. *Biosensors and Bioelectronics,* 22, 2525–2531.

xxxiii. Michira, I. N., Katithi, D. N., Guto, P. M., Kamau, G. N., Baker, P. & Iwuoha, E. (2014). Synthesis, Characterization of Green Tea Stabilized Iron Nanoparticles and their Synergistic Effect on Polyaniline. *International Journal of Sciences: Basic and Applied Research (IJSBAR)*, 13(2), 63-76.