

# Recent advances on the electrochemical transduction techniques for the biosensing of pharmaceuticals in aquatic environments

## Abstract

Due to the potential risk to the environment and humans' health, there has been an increasing concern on the development of new analytical methods for monitoring the presence of pharmaceuticals in various aquatic ecosystems, such as in freshwaters (rivers and lakes), sewage and sea water. The conventional techniques, such as liquid chromatography and mass spectrometry are not only laborious but possess other important limitations, such as the high cost and/or the low sensitivity for this application, which needs to be rapidly overwhelmed. In light of this context, the electrochemical biosensors combine the attractive features of biological components (mainly the high selectivity of enzymes, antibodies and nucleic acids) used as units of recognition to the robustness of the electrochemical techniques, which provides stable output signals even at low concentrations of the target analytes. In this mini review, we reported the recent researches in the field of biosensors for the detection of important pharmaceuticals, whose transduction mechanisms are based on amperometric, potentiometric, conductometric and impedimetric methods.

**Keywords:** biosensor, pharmaceutical, electrochemical techniques

Volume 5 Issue 4 - 2019

Wagner Augusto Fiel, Pedro Augusto de Freitas Borges, Vanessa de Freitas Cunha Lins, Ricardo Adriano Dorledo de Faria

Department of Chemical Engineering, Universidade Federal de Minas Gerais, Brazil

**Correspondence:** Ricardo Adriano Dorledo de Faria, Department of Chemical Engineering, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais 30270-901, Brazil. Email ricardo.adriano08@hotmail.com

**Received:** August 18, 2019 | **Published:** September 02, 2019

**Abbreviations:** SELEX, systematic evolution of ligands by exponential enrichment; WE, working electrode; CE, counter-electrode; RE, reference electrode; SPEs, screen-printed electrodes; LOD, limit of detection; EIS, electrochemical impedance spectroscopy; Rct, charge transfer resistance

## Introduction

The presence of pharmaceuticals in the aquatic environment (e.g. rivers, lakes, sewage, estuarine and marine water) merits special attention due to its significant impact as potentially harmful pollutants.<sup>1</sup> The worldwide concern on this issue is due to the concentration in which these substances have been found in water bodies (from nanogram per liter to microgram per liter),<sup>2</sup> overcoming the acceptable levels to ensure the safety of aquatic organisms.<sup>3</sup> The source of these pollutants comprises the human and animal healthcare, which tends to continuously be rising as a function of the advances in the medical treatments, the larger amount of medicines available to combat well known and recently discovered diseases, affordability, the growing number of people in the world and their respective larger life expectancy.<sup>4</sup> Pharmaceuticals and personal care products are considered emerging contaminants, which means that just in the past two decades they have been studied and deeply investigated. Moreover, the fragility of the current analytical tools to detect these molecules at low concentrations hinders the regulation and monitoring of drinking water supplies.<sup>3</sup> Puckowski et al.,<sup>2</sup> complement that few information concerning the presence of pharmaceuticals in the biota are available possibly because of the complexity of the experimental setup to analyze the contaminated samples and due to the possibility of bioaccumulation in the surrounding areas.

Therefore, the development of new technologies and techniques that are more efficient in the detection and analyses of pharmaceuticals is required. Recently, the biosensor technology has been an important method-of-choice for this purpose due to its particularly interesting characteristics, such as its portability, capability of on-site detection, the low time of response and the advantageous lower price in comparison to traditional laboratory techniques.<sup>5</sup> Within this scenario, this mini review presents the types of biosensors currently available, the newest researches performed in the area, the challenges still faced in the monitoring of pharmaceuticals in the aquatic ecosystems, promising futures perspectives as well as the open fields for new researches in this field.

## Most common pharmaceuticals and their effects

To better understand the sensor function in the drugs detection, it is essential an initial analysis of the most common medicines found in river systems. For that, it will be showed the most frequent contaminants in rivers and lakes and their impact in the environment and human life. An important group of pharmaceuticals in the aquatic environment is the one consisting of the acidic drugs like the lipid regulators bezafibrate, gemfibrozil, the antiphlogistics, diclofenac, ibuprofen, indometacine, naproxen, phenazone and the metabolites clofibrac acid, fenofibrac acid and salicylic acid.<sup>6</sup> Amongst them, the clofibrac acid attracts special attention once it has a persistence in the environment of 21 years and has been found in river water in the whole Europe and even in the United States.<sup>7</sup> Another common drug that needs special attention is the antiphlogistic ibuprofen. Therefore, due to the low concentration at which is present in surface water, and

to the appearance of this drug in a large range of rivers worldwide, being considered the third-most popular drug in the world,<sup>8</sup> it raises particular concern. The real toxicology impact of these drugs is still uncertain. A limited amount of studies has been carried out in this field and most of them was performed using acute tests. Based on the available literature, it is possible to affirm the unleash harmful effects to the health of living beings,<sup>9</sup> prompting many groups of research to develop and optimize new technologies for the regulation and monitoring of pharmaceuticals in the aquatic medium.

## Biosensors

Biosensors are devices that include a biological recognition element, called bioreceptor, which is responsible for binding the target analyte from a certain medium, providing a specific response, such as light, heat, liberation of protons and/or electrons, etc.<sup>10</sup> The generated output signal from the biorecognition is received by a transducer element and converted into a measurable signal, which is usually proportional to the concentration of bond analyte. A key point in the biosensor technology is the search for improving its sensitivity and selectivity. To achieve these features, several researches have focused on studying different pairs of bioreceptors and transducers.

### Bioreceptors

Among the plural possibilities of bioreceptors (enzymes, whole cells, organelles, microorganisms and tissues for instance),<sup>8</sup> many studies have presented particular interest on immunosensors, subset of biosensors that uses antibodies (or antigens) to interact with the target analyte with high affinity.<sup>5,11</sup> As disadvantages, immunosensors traditionally require laborious conditions to obtain specific and pure antibodies/antigens as bioreceptors. In cases in which the bioreceptor is not highly specific towards the molecule of interest, other analyte-like molecules may interact with the antibodies at the surface of the sensor in a process called cross-reactivity, which hinders the detection of target analyte at low concentrations.<sup>11,12</sup> Other researches move towards improving aptamer-based bioreceptors. Aptamers are molecules made up of DNA or RNA that conform to 3D structures due to chemical bonds between their nucleotides.<sup>13</sup> These structures are produced by Systematic Evolution of Ligands by Exponential Enrichment (SELEX), which consists of an iterative *in vitro* selection process that allows selection and augmentation of the nucleotide sequence.<sup>13,14</sup> In this procedure, highly selective aptamers are produced to interact with the target molecules in the transducer matrix.<sup>13</sup> Thus, aptamers with characteristics superior to antibodies can be produced, such as: high affinity with specific analyte, good stability, easy reproduction and chemical modification.<sup>13</sup>

### Transducers

There are four main types of transducers, depending on the stimulus they pick up from the biorecognition: optical, thermal, piezoelectric, and electrochemical.<sup>14</sup> Optical sensors emit output signals in the presence of light promoted by chemical or biological reactions between the bioreceptor and the target molecule. In this case, optical fibers are used to guide the emitted light to suitable sensors. Thermal sensors detect the heat emitted in the biochemical reaction. However, it is an inaccurate method, as most of the energy released is lost to the environment without being detected by the sensor, which impairs the reading. Piezoelectric sensors detect changes in mass caused by the formation of chemical bonds between bioreceptors and analyte molecules. This causes a change in mechanical stress

and, consequently, in the oscillation frequency of a quartz crystal.<sup>16</sup> A special subset of transducers relies on the electrochemical sensors. The electrochemical transducers are frequently employed in analytical contexts where high accuracy, reliability, robustness, ease application, high sensitivity and selectivity and relative low cost are required in the sensing of a target molecule.<sup>13,17,18</sup> The main types of electrochemical biosensors comprises the amperometric, potentiometric, conductometry and impedimetric devices.<sup>16</sup>

## Electrochemical biosensing of pharmaceuticals

The experimental electrochemical setup traditionally relies on a three-electrode cell consisting of an electrolyte, which is a conductive solution that allows the charge transport, a working electrode (WE) that is the biosensor in which the electrochemical transduction takes place; a counter-electrode (CE) controlled by the potentiostat to set a certain potential to the WE and to provide a pathway to the current flow, and a reference electrode (RE) kept close to the WE and at a known distance from the reactive region to provide a stable information regarding the WE potential to the equipment.<sup>19</sup> An important concern on the electrochemical biosensors for pharmaceutical analysis is the adsorption of molecules on the electrode surface (fouling) and the passivation of the surface, which alters the physicochemical characteristics of the transducer and, consequently, the sensitivity and performance of the biosensor. To overcome this problem, Couto et al.,<sup>20</sup> suggested the use of screen-printed electrodes (SPEs) as a promising candidate as an electrochemical transducer, since its low cost allows one to discard the electrode after each analysis. Faria et al.,<sup>21</sup> reported that the SPEs are amenable for *in situ* analysis because of the possibility of using in miniaturized systems, which is particularly interesting for monitoring pharmaceuticals in aquatic environments.

**Amperometric method:** The amperometry technique is based on the application of a constant potential to the WE and the measurement of the resultant electrical current over the time due to the faradaic reactions involving the electroactive analyte.<sup>22</sup> In the context of the detection of organic pollutants in wastewater, Ejeian et al.,<sup>23</sup> affirmed that the amperometry is the most successful transduction mechanism of biosensors, which is possibly credited to the fact that in this technique the use of a specific potential is associated to a given analyte, minimizing the interference of other electroactive species on the electrochemical response of the biosensor and increasing its selectivity and sensitivity.<sup>24</sup> Gil and Melo<sup>25</sup> pointed out that the amperometric technique combined to the use of enzymes is the best option to the analysis of oxidizable drugs in the context of pharmaceutical analysis. According to the authors, there are three main mechanisms involving the charge transfer from the enzymatic reaction to the transducer substrate: the first generation biosensors, which are based on the reductive/oxidative features of cofactors  $\beta$ -NAD(P)<sup>+</sup>; the employment of electron mediators aiming to increase the sensitivity of device by means of the improvement of the electron transfer (second generation) and the direct mechanism of electron transfer from the enzyme to the electrode, as called third generation. Del Torno-de Román et al.,<sup>26</sup> researched the use of tyrosinase in disposable screen-printed carbon electrodes for the recognition of sulfamethoxazole. The biosensor presented good reproducibility, repeatability and a limit of detection (LOD) equal to  $22.6 \pm 2.1 \mu\text{M}$ . Moreover, satisfactory recovery was observed at a 95% confidence level when the analyte was diluted in spiked water. In their work, Apetrei et al.,<sup>27</sup> reported the development of an amperometric biosensor based on carbon nanofibers as an electrochemical transducer matrix and the enzyme diamine oxidase

as a bioreceptor for the detection of norepinephrine, a vasopressor drug. In this case, the cathodic current developed at -0.6 V with respect to a Ag pseudo-reference electrode. At optimal conditions, a LOD of 0.23 mM was achieved and the enzymatic biosensor showed high selectivity to norepinephrine due to a Hill coefficient,  $h=1.02$ . Enzymatic biosensors were also developed by Tomassetti et al.,<sup>28</sup> and by the Beitollahi<sup>29</sup> group. The first reported the detection of chloramphenicol by means of a catalytic direct methanol fuel cell containing immobilized dehydrogenase enzyme. The LOD was equal to  $8.0 \times 10^{-7}$  M within a linear concentration range from 1.0 to 6.0 mM. On the other hand, Beitollahi et al.,<sup>29</sup> functionalized a carbon paste electrode with anti-prolactin antibodies to linearly detect prolactin at concentrations ranging from 25.0 to 2000.0 mIU.L<sup>-1</sup> yielding a LOD of 12.5 mIU.L<sup>-1</sup>.

**Potentiometric method:** The potentiometry relies on the potential difference between the WE and the RE in the presence of a negligible current flow. The measured potential is proportional to the ion activity and can be calculated from the Nernst equation.<sup>30</sup> Atif et al.,<sup>31</sup> assessed the effect of urea on the morphology of Fe<sub>3</sub>O<sub>4</sub> nanoparticles used as electrochemical transducers in a potentiometric biosensor containing urease as a bioreceptor. With a rapid response time approximately equal to 12 s, the proposed enzymatic biosensor responded to a logarithmic concentration of 0.1 mM to 80 mM urea with sensitivity equal to 42 mV.decade<sup>-1</sup>. The paper authored by Ismail & Adeloju<sup>32</sup> dealt with the detection of penicillin by means of a penicillinase-based potentiometric biosensor. Their device exhibited a LOD of 1.7  $\mu$ M and a linear concentration range from 7.5  $\mu$ M to 283.0  $\mu$ M. Despite the low concentrations detectable by the sensor, the authors concluded that their device was more amenable for the detection of the analyte at higher concentrations (from 10 ppm to 20 ppm). One year later, the same authors published another paper<sup>33</sup> reporting the detection of penicillin and other antibiotics in the context of a potentiometric sensor. In this new report, the research compared a single layer and a bilayer biosensor, both containing penicillinase enzyme as a bioreceptor. The results shown that the single layer device presented a lower LOD (3  $\mu$ M) if compared to the bilayer sensor (LOD = 3.3  $\mu$ M). Furthermore, according to the authors, the percentage recovery ( $103 \pm 5\%$ ) was in good agreement with the standard titrimetric method ( $105 \pm 5\%$ ).

**Conductometry method:** The conductometric sensing is based on the measurement of system conductance due to variations in the amount of ions that can be generated or consumed due to biochemical reactions in bioreceptors. Soldatkina et al.,<sup>34</sup> developed a conductometry arginine biosensor consisting of arginase and urease as bioreceptors. The analytic response of the sensor towards the target analyte was linear at the concentration range of 2.5 - 500  $\mu$ M with a response time of 20 s, a LOD of 2.5  $\mu$ M and sensitivity of  $13.4 \pm 2.4 \mu$ S.mM<sup>-1</sup>. Kolahchi et al.,<sup>35</sup> studied the detection of phenols, which are frequently found in wastewater due to the discard of pharmaceuticals, by means of a *Pseudomonas sp.* (GSN23) based biosensor. Their conductometric device was sensitive to phenol at the concentration range of 1.0–300 mg.L<sup>-1</sup> yielding a LOD of 0.2 mg.L<sup>-1</sup>. The authors further investigated the performance of the biosensor in real water samples collected from the Rhone River in Lyon (France). After some steps of preparation of the water sample, known amounts of phenol were injected to the water up to concentrations of 80 mg.L<sup>-1</sup>. The results indicated there was no significant influence of the saline and acidic nature of the river water in the sensor's response.

**Impedimetric method:** Based on the Electrochemical Impedance Spectroscopy (EIS) technique, the impedimetric method relies on the changes in the impedance at the biosensor surface due to the binding of the target analyte. Briefly, in this technique a small sinusoidal perturbation is applied to the electrochemical system generating a resultant sinusoidal signal. Typically, an AC potential is applied and the information regarding the consequent frequency-dependent current is collected by a potentiostat. As a result, the impedance ( $Z$ ) is expressed as a function of a real ( $Z'$ ) and an imaginary component ( $Z''$ ) and is interpreted by modelling the data to an appropriate electrical equivalent circuit consisting of resistors, capacitors and inductors elements.<sup>36</sup> Jiménez et al.,<sup>12</sup> highlighted the importance to detect progesterone at the environmental and clinical contexts due to the harmful effect of this hormone at excessive concentrations. Progesterone, which is used in the menopausal therapy to regulate the menstrual cycles and the hormone levels in the body, can provoke plenty of side effects to humans' health from sneezing and vomiting to breast and lung cancer in women as well as the decrease of testosterone secretion in men.<sup>12</sup> The authors attached ssDNA aptamers with affinity towards progesterone (P4) to a gold electrode and evaluated changes in the electron transfer resistance of the as-obtained biosensor. The developed device was capable to detect P4 in an analytical range varying from 10 to 60 ng.mL<sup>-1</sup>, presented a limit of detection of 0.90 ng.mL<sup>-1</sup> with a good recovery percentage in spiked tap water samples. In another work, Shiravand & Azadbakht<sup>37</sup> decorated a glassy carbon electrode with acid-oxidized carbon nanotubes, platinum and silver nanoparticles as transducer elements, and aminated capture probe (ssDNA1) and diclofenac aptamer (ssDNA2) as bioreceptors for diclofenac recognition. The researchers monitored the changes in the charge transfer resistance ( $R_{ct}$ ) at the interface between the biosensor and an electrolyte containing ferri/ferrocyanide ions as redox probe. A dynamic analytical range from 10 pM to 800 nM with a limit of detection of 2.8 pM was obtained and the authors observed that the sensor was highly selective towards the target pharmaceutical. In the presence of ascorbic acid, paracetamol, glucose, bisphenol A, tryptophan and dopamine at a thousand times higher concentration, the proposed sensor exhibited a very negligible change in the  $R_{ct}$ . Khan et al.,<sup>38</sup> bet on developing a molecularly imprinted polymer-based biosensor to detect the digoxin, a drug prescribed to the treatment of some heart diseases (atrial fibrillation and flutter for example). Their results indicated the possibility of recognizing digoxin from  $1.0 \times 10^{-9}$  M to  $5.0 \times 10^{-8}$  M with a sensitivity estimated to be equal to  $33.33 \Omega.nM^{-1}$  and the biosensor presented a limit of detection of  $6.95 \times 10^{-11}$  M.<sup>39</sup> In the same context, Kjelstrup et al.,<sup>39</sup> demonstrated the fabrication of a DNA-based sensor capable to detect this pharmaceutical in plasma at concentrations above 10 nM within a timeframe of 30 min.

## Conclusion

This mini review has addressed the importance of the electrochemical techniques in the biosensing of pharmaceuticals. The enhanced focus on the monitoring of these molecules has been done due to the risk they represent to the environment as a function of the extended persistence time they possess in aquatic ecosystems and the significant negative impact on animals' and humans' health. Despite the importance in detecting these pharmaceuticals, the traditional analytical methods currently available are still somehow problematic regarding their cost and the limited sensitivity. Contrary, the technology of electrochemical biosensors comprises a group of



devices with tremendous potential to the detection and quantification of these molecules in aquatic environments since they are usually more cost-effective, because they are more amenable for in situ applications they usually present higher sensitivity and selectivity. Amperometric, potentiometric, conductometric and impedimetric biosensors have been recently reported in the literature as promising candidates to assess the changes in the electrical features of transducer matrix as a response to the binding of pharmaceuticals of environmental interest, providing very low limits of detection and stable response in large linear concentration ranges.

## Funding details

None.

## Acknowledgements

None.

## Conflict of interests

Authors declare that there is no conflict of interest.

## References

1. Almeida HFD, Marrucho IM, Freire MG. Removal of non-steroidal anti-inflammatory drugs from aqueous environments with reusable ionic-liquid-based systems. *ACS Sustain Chem Eng*. 2017;5(3):2428–2436.
2. Puckowski A, Mioduszecka K, Łukaszewicz P, et al. Bioaccumulation and analytics of pharmaceutical residues in the environment: a review. *J Pharm Biomed Anal*. 2016;127:232–255.
3. Ebele AJ, Abdallah MAE, Harrad S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg Contam*. 2017;3:1–16.
4. Comber S, Gardner M, Sörme P, et al. Active pharmaceutical ingredients entering the aquatic environment from wastewater treatments works: a cause for concern? *Sci Total Environ*. 2018;613–614:538–47.
5. Mehrotra P. Biosensors and their applications—a review. *J Oral Biol Craniofac Res*. 2016;6:153–159.
6. Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. *Water Res*. 1998;32(11):3245–3260.
7. Jennifer L Packer, Jeffrey J Werner, Douglas E Latch, et al. Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibrac acid, and ibuprofen. *Aquatic Sci*. 2003;65(4):342–351.
8. Buser HR, Poiger T, Müller MD. Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater. *Environ Sci Technol*. 1999;33(15):2529–2535.
9. Ragugnetti M, Adams M, Guimarães A, et al. Ibuprofen Genotoxicity in Aquatic Environment: An Experimental Model Using *Oreochromis niloticus*. *Water, Air Soil Poll*. 2011;(1):361–364.
10. Thévenot DR, Toth K, Durst RA, et al. Electrochemical biosensors: recommended definitions and classification. *Pure Appl Chem*. 1999;71(12):2333–2348.
11. Fan L, Zhao G, Shi H, et al. A simple and label-free aptasensor based on nickel hexacyanoferrate nanoparticles as signal probe for highly sensitive detection of 17 $\beta$ -estradiol. *Bios Bioelectron*. 2015;68:303–309.
12. Jiménez GC, Eissa S, Ng A, et al. Aptamer-based label-free impedimetric biosensor for detection of progesterone. *Anal Chem*. 2015;87(2):1075–1082.
13. Li F, Yu Z, Han X, et al. Electrochemical aptamer-based sensors for food and water analysis: A review. *Anal Chim Acta*. 2019;1051:1–23.
14. Guo W, Sun N, Pei M, et al. A novel electrochemical aptasensor for ultrasensitive detection of kanamycin based on MWCNTs–HMIMPF6 and nanoporous PtTi alloy. *Bios Bioelectron*. 2015;74:691–697.
15. Alawad A, Istamboulié G, Calas-Blanchard C, et al. A reagentless aptasensor based on intrinsic aptamer redox activity for the detection of tetracycline in water. *Sens Act B: Chem*. 2019;288:141–146.
16. Silva LMC. *Development of electrochemical biosensors for phenol and urea focusing on environmental application*. Universidade Federal Do Rio de Janeiro; 2011.
17. Ensafi AA. An introduction to sensors and biosensors. *Electrochem Bios*. 2019;1:1–10.
18. Faria RAD, Heneine LGD, Matencio T, Messaddeq Y. Recent trends in the electroanalytical detection of food fraud. *Int J Bios Bioelectron*. 2019;5(3):63–67.
19. Rezaei B, Irannejad N. Electrochemical detection techniques in biosensor applications. *Electrochem Bios*. 2019;2:11–43.
20. Couto RAS, Lima JLFC, Quinaz MB. Recent developments, characteristics and potential applications of screen-printed electrodes in pharmaceutical and biological analysis. *Talanta*. 2016;146:801–814.
21. Faria RAD, Messaddeq Y, Heneine LGD, et al. Application of screen-printed carbon electrode as an electrochemical transducer in biosensors. *Int J Bios Bioelectron*. 2019;5(1):1–2.
22. Beitollahi H, Safaei M, Tajik S. Voltammetric and amperometric sensors for determination of epinephrine: a short review (2013–2017). *J Electrochem Sci Eng*. 2019;9(1):27–43.
23. Ejeian F, Etedali P, Mansouri-Tehrani HÁ, et al. Biosensors for wastewater monitoring: a review. *Bios Bioelectron*. 2018;118:66–79.
24. Moon JM, Thapliyal N, Hussain KK, et al. Conducting polymer-based electrochemical biosensors for neurotransmitters: a review. *Bios Bioelectron*. 2018;102:540–552.
25. Gil ES, Melo GR. Electrochemical biosensors in pharmaceutical analysis. *Braz J Pharm Sci*. 2010;46(3):375–391.
26. Del Torno-de Román L, Alonso-Lomillo MA, Dominguez-Renedo O, et al. Tyrosinase based biosensor for the electrochemical determination of sulfamethoxazole. *Sens Act B: Chem*. 201;227:48–53.
27. Apetrei IM, Diaconu C, Apetrei C, et al. Electrochemical biosensor based on carbon nanofibers and diamine oxidase for detection of norepinephrine. *Rom Biotech Letters*. 2016;21(1):11092–11102.
28. Tomassetti M, Angeloni R, Martini E, et al. Enzymatic DMFC device used for direct analysis of chloramphenicol and a comparison with the competitive immunosensor method. *Sens Act B: Chem*. 2018;255(2):1545–1552.
29. Beitollahi H, Nekooei S, Torkzadeh-Mahani M. Amperometric immunosensor for prolactin hormone measurement using antibodies loaded on a nano-Au monolayer modified ionic liquid carbon paste electrode. *Talanta*. 2018;188:701–707.
30. Naveen MH, Gurudatt NG, Shim YB. Applications of conducting polymer composites to electrochemical sensors: a review. *App Mat Today*. 2017;9:419–433.
31. Atif M, Ali A, Al Salhi MS, et al. Effect of urea on the morphology of Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles and their application in potentiometric urea biosensors. *Silicon*. 2019;11:1371–1376.

32. Ismail F, Adeloju SB. The use of poly (vinyl alcohol) to cross-link penicillinase for the fabrication of a penicillin potentiometric biosensor. *Electroanal.* 2014;26(12):2701–2709.
33. Ismail F, Adeloju SB. Comparison of single layer and bilayer biosensors based on crosslinking of penicillinase for potentiometric detection of penicillin in milk and antibiotics. *Electroanal.* 2015;27(6):1523–1531.
34. Soldatkina OV, Soldatkin OO, Velychko TP, et al. Conductometric biosensor for arginine determination in pharmaceuticals. *Bioelectrochem.* 2018;124:40–46.
35. Kolahchi N, Braiek M, Ebrahimipour G, et al. Direct detection of phenol using a new bacterial strain-based conductometric biosensor. *J Environ Chem Eng.* 2018;6(1):478–484.
36. Faria RAD, Heneine LGD, Matencio T, et al. Faradaic and non-faradaic electrochemical impedance spectroscopy as transduction techniques for sensing applications. *Int J Bios Bioelectron.* 2019;5(1):29–31.
37. Shiravand T, Azadbakht A. Impedimetric biosensor based on bimetallic AgPt nanoparticle-decorated carbon nanotubes as highly conductive film surface. *J Solid State Electrochem.* 2017;21(6):1699–1711.
38. Khan SI, Hillawar RR, Tadi KK, et al. Determination of digoxin in biological and pharmaceutical samples. *Curr Anal Chem.* 2018;14:1–9.
39. Kjelstrup MV, Nielsen LDF, Hansen-Bruhn, et al. A DNA-based assay for digoxin detection. *Bios.* 2018;8(19):1–12.