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Nanoparticle-based Biosensors for Detection of Heavy Metal Ions

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ABSTRACT

Heavy metal pollution is one of the most serious environmental problems in the world. Many efforts have been made to develop biosensors for monitoring heavy metals in the environment. Development of nanoparticle-based biosensors is the most effective way to solve this problem. This review presents the latest technology of nanoparticle-based biosensors for environment monitoring to detect heavy metal ions, which are magnetic chitosan biosensor, colorimetric biosensor, and electrochemical biosensor. Magnetic chitosan biosensor acts as a nano-absorbent, which can easily detect and extract poisonous heavy metal ions such as lead ions and copper ions. There are several methods to prepare the chitosan based on the nanoparticle, which are cross-linking, co-precipitation, multi-cyanoguanidine, and covalent binding method. In colorimetric biosensor, gold and silver nanoparticles are commonly used to detect the lead and mercury ions. In addition, this biosensor is very sensitive, fast and selective to detect metal ions based on the color change of the solution mixture. Meanwhile, electrochemical biosensor is widely used to detect heavy metal ions due to a simple and rapid process, easy, convenient and inexpensive. This biosensor is focused on the surface area, which leads to significant improvement in the performance of devices in terms of sensitivity. The wide surface area can affect the performance of the biosensor due to a limited space for operation of electrode. Therefore, reduced graphene oxide is a suitable material for making the electrochemical biosensor due to a wide surface area, good conductivity and high mechanical strength. In conclusion, these three technologies have their own advantages in making a very useful biosensor in the detection of heavy metal ions.

Keywords: Nanoparticle biosensor; Heavy metal ions, Colorimetric biosensor; Magnetic chitosan biosensor; Electrochemical biosensor

1. INTRODUCTION

Environmental pollutions are increasing day by day and become more serious in line with the rapid urbanization and industrialization. Untreated industrial and agricultural wastes such as heavy metals are highly polluting the water and soil. There are some examples of heavy metals that are high in toxicity and commonly present in our daily life such as lead, mercury, cadmium and arsenic. In recent years, biosensors have established a great potential to solve environmental pollutions. Nanoparticle-based biosensor is an analytical device that combines a biological component with a physicochemical detector in very small size and used for detection of an analyte [1]. There are some reasons to make biosensors in nano-scale such as improving sensitivity and specificity of biomolecule detection, efficient bimolecular recognition, pathogenic diagnosis and environmental

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monitoring. Before the latest technology of nanoparticle-based biosensors are introduced, there are some traditional quantitative methods have been used over a period of time such as atomic absorption or emission spectroscopy [2], inductively coupled plasma/atomic emission spectrometry [3], and cold vapour-atomic fluorescence spectrometry (CVAFS) [4].

The advantages of these traditional analytical methods are extremely sensitive and selective. However, they are very expensive, complicated and require a lot of laborious pre-treatment process. Therefore, nanoparticle-based biosensors have been created and used to replace these traditional methods. The purpose of this paper is to describe some latest examples of nanoparticle-based biosensors for environmental monitoring especially focusing on heavy metal pollutions. The first subtopic that will be discussed in this is paper is about Magnetic Chitosan Biosensor. As the growth of civilizations and industrialization, lead becomes one of the most noxious heavy metals that is released into the environment. Lead ions that accumulate in food chain can cause hazardous effect to human. There are some uncured diseases that can be caused by lead ions such as mental retardation, encephalopathy, seizures and reduction in hemoglobin productions [5]. Poisonous lead ion in water can be adsorbed and removed by magnetic chitosan biosensor [6-7].

Besides, the removal of other heavy metals such as mercury also will be discussed in this topic by using the electrochemical biosensor. Electrochemical techniques and devices are the challenges in the area of heavy metal trace detection [8]. Mercury is a very toxic heavy metal that causes bad impact to both human health and environment [9]. The properties and effect of mercury is almost the same as lead since they can be accumulated through the food chain and cause dangerous diseases. Mercury may cause serious damage from the aspect of cells, then causes cardiovascular, hematological and pulmonary effects to human body [10]. Mercury is also harmful to most of the human body system such as digestive and renal system, immune system, nervous system and others [11]. Thus, research has been done about the detection of mercury ions (II) based on non-cross-linking aggregation of double-stranded DNA modified gold nanoparticles to solve or reduce this problem.

Next, the colorimetric biosensor is also one of the latest technologies used for the detection of heavy metal ions. It is quite commonly used for laboratory tests and industrial applications because of its convenience and easier to be used [12]. Colorimetric biosensors provide semi-quantitative data with the assistance of a calibration chart. Colorimetric biosensor usually classified into gold nanoparticles and silver nanoparticles and they attracted an excellent deal of interest [13]. This is because of their size and shape dependent optical properties and large absorption coefficients [14]. Lastly, nanoparticles nowadays are giving many advantages to human especially from the aspect of its application to detect cancer cells and improve the environmental qualities by reducing the heavy metals. With the aid of these biosensors, the environmental monitoring will be easier to be controlled.

2. MAGNETIC CHITOSAN BIOSENSOR

Chitosan is a type of polyaminosaccharide, which is derived from chitin. It has several interesting features such as hydrophilicity, biocompatibility, biodegradability, non-toxicity and adsorption properties [15]. Recent research found out that the composite product of chitosan and magnetic nanoparticles as nano-adsorbent was able to detect and extract poisonous heavy metal ions such as lead, nickel, copper, and cobalt ions from aqueous solution [16-18]. There were various preparation methods of the chitosan-based nanoparticles such as cross-linking method [19], co-precipitation

method [15], multi-cyanoguanidine method [20], and covalent binding method [21]. Among all the heavy metal ions mentioned above, mercury ions, Hg^{2+} is one of the most harmful ions since it can accumulate in human body and causes variety of symptoms. Hence, researchers are focusing more on the isolation of Hg^{2+} from aqueous by using co-precipitation method. The preparation of chitosan magnetic nanoparticles starts with co-precipitation of Fe^{3+} and Fe^{2+} ions by ammonia solution and treatment under hydrothermal condition [22]. The chemical precipitation can be achieved at 25°C under vigorous stirring by adding ammonia hydroxide, NH_4OH solution. The precipitates can be heated at 80°C for 30 minutes, washed several times with water and ethanol, and then finally dried in a vacuum oven at 70°C [23] and therefore the magnetic iron oxide nanoparticles, Fe_3O_4 were obtained. The chitosan was first carboxymethylated before it could covalently-bound to Fe_3O_4 because chitosan has no suitable functional group to bind directly with Fe_3O_4 [24]. FIGURE 1 illustrated the synthesis route of chitosan-bound Fe_3O_4 nanoparticles and their use as an efficient tool for Hg^{2+} removal by applying an external magnetic field [23]. Meanwhile, FIGURE 2 shows the effect of magnetic Fe_3O_4 nanoparticles react to strong magnet. In order to confirm the presence of chitosan bounded on the Fe_3O_4 surface, FT-IR spectroscopy was used to characterize pure Fe_3O_4 nanoparticles, pure chitosan, and chitosan-bound Fe_3O_4 nanoparticles. The FTIR spectrograph result is as shown in FIGURE 3. In (a), the peak at 585 cm^{-1} is the shifted Fe-O bond of chitosan-bound Fe_3O_4 nanoparticles at about 590 cm^{-1} after shifting. In (b) the peak at 3406 cm^{-1} corresponds to O-H stretching vibrations. The peak at 2860 cm^{-1} is attributed to the C-H stretching vibration of polymer. Where the peak at 1078 cm^{-1} and 1034 cm^{-1} are both attributed to the stretching vibration of C-O. The deformation vibration of N-H in primary amine is observed at the peak of 1611 cm^{-1} and 3406 cm^{-1}. With the presence of all of the special peaks in (c), it can be concluded that Fe_3O_4 nanoparticles is coated by chitosan perfectly.

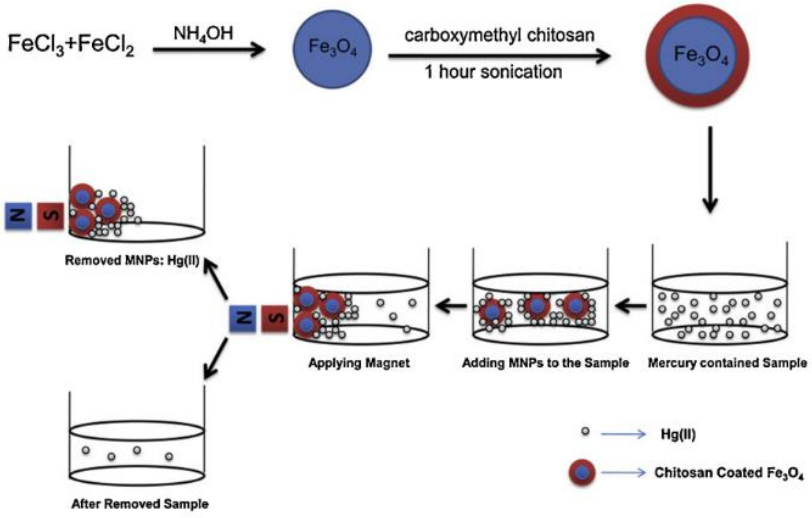

FIGURE 1. Synthesis route of chitosan-bound Fe_3O_4 nanoparticles and their use as an efficient tool for Hg^{2+} removal by applying an external magnetic field [23].

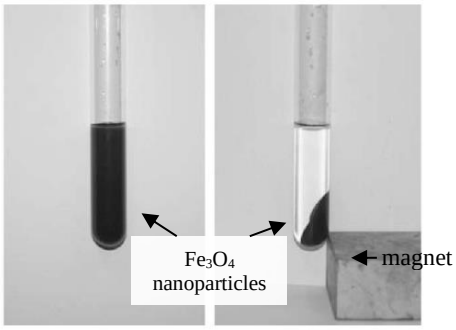

FIGURE 2. The effect of magnetic Fe_3O_4 nanoparticles react to strong magnet [23].

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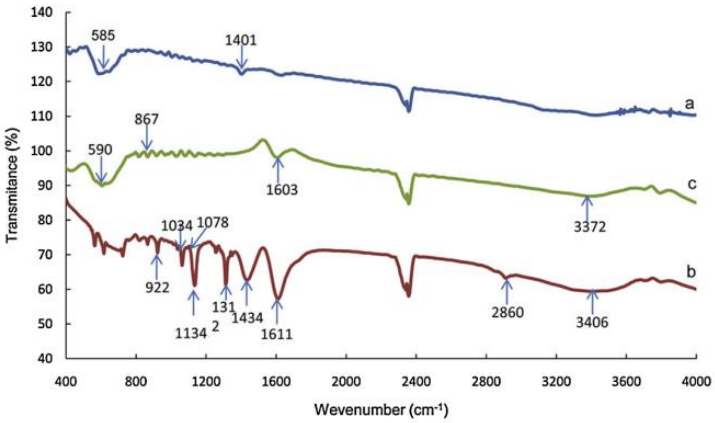

FIGURE 3. FTIR result of (a) naked Fe_3O_4 (b) pure chitosan (c) chitosan-bound Fe_3O_4 [23].

The magnetic properties of chitosan-bound Fe_3O_4 nanoparticles were measured by using vibration sample magnetometer (VSM) at room temperature [23]. A typical magnetization curve is as shown in FIGURE 4. The hysteresis loop shows super paramagnetic property, proved that there is single-domain magnetic nanoparticles coated of the adsorbents. As illustrated in FIGURE 4, the magnetization value of chitosan-bound Fe_3O_4 nanoparticles is slightly lower than that the magnetization value of naked Fe_3O_4 nanoparticles. This is due to the additional of chitosan in Fe_3O_4 nanoparticles that decreases the uniformity of the magnetic moment due to quenching on the surface of naked Fe_3O_4 [25]. Therefore, the chitosan-bound Fe_3O_4 magnetic nanoparticles can be separated by applying external magnetic field easier than naked Fe_3O_4 nanoparticles.

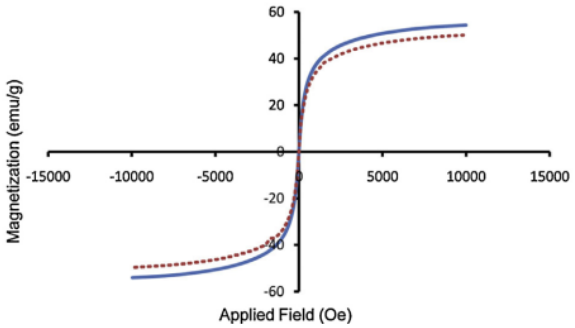

FIGURE 4. Magnetic hysteresis curve of (solid line) naked Fe_3O_4 and (dotted line) chitosan-bound Fe_3O_4 nanoparticles [25].

Adsorption of Hg^{2+} can be categorized into two different adsoptions; chemical and physical adsorptions. Where chemical adsorption is the adhesion of the Hg^{2+} ions on the surface of adsorbent through chemical bonding. Physical adsorption is where the adsorbate adheres to the adsorbent surface through weak Van der Waals force. The research from Nasirimoghaddam [23] found out that physical adsorption has the higher efficiency of about 90% amount of mercury removal. In real time samples, the percentage of removal of Hg^{2+} ions is as shown in FIGURE 5. The removal process was carried out by mixing the chitosan-bound magnetic nanoparticles into the samples by shaking for 4 minutes. Then the magnetic nanoparticles adsorbent was removed from the solution by using strong magnet. From the result in FIGURE 5, it can be concluded that this method has succeeded to remove Hg^{2+} ions. The result shows that most of the samples have an amazing removal percentage of Hg^{2+} ions of about 90%. The chitosan-bound magnetic nanoparticles are able to remove other heavy metal ions if it is more than just Hg^{2+} ions presence in the particular solution. The removal efficiencies for the adsorption of other heavy metal ions using chitosan-bound Fe_3O_4 nanoparticles are as shown in FIGURE 6. The usage of chitosan-bound Fe_3O_4 nanoparticles can also be used as a biosensor for detecting the existence of other heavy metal ions in aqueous solution.

Removal of Hg^{2+} from different waste samples using chitosan-bound Fe_3O_4 magnetic nanoparticles.

Industrial samples	Spiked Hg^{2+} content	% Removal Hg^{2+}
Sample 1*	2 ppm	89.5
Sample 2**	2 ppm	88.4
Sample 3***	2 ppm	88
Sample 4****	2 ppm	86.2

* Urban wastewater.
** Wastewater of arak petroleum industry.
*** Kur river.
**** Wastewater of asaluiye petroleum industry.

FIGURE 5. Removal of Hg^{2+} ions using chitosan-bound Fe_3O_4 nanoparticles [25].

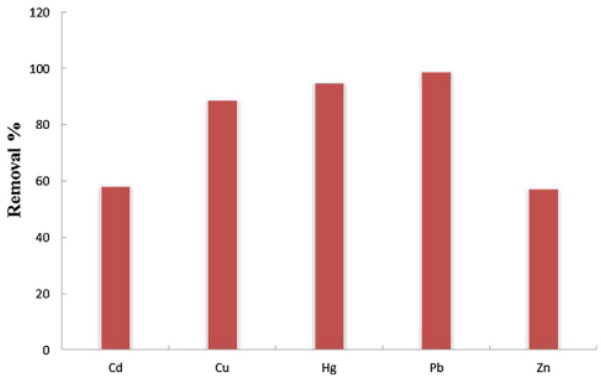

FIGURE 6. Removal efficiencies of chitosan-bound Fe_3O_4 for other heavy metal ions adsorption [23].

3. COLORIMETRIC BIOSENSOR

Colorimetric biosensor is a simple biosensor that can minimize the cost of metal ion biosensor production and make the real-time detection much easier [26]. Colorimetric biosensor is fast, sensitive, and selective for various of metal ions detection such as lead (Pb), mercury (Hg) [27], magnesium (Mn), arsenic (As), chromium (Cr), copper (Cu), cadmium (Cd), and other alkaline earth metals calcium (Ca), barium (Ba), and strontium (Sr)) reported by Priyadarshini and Pradhan [28]. Besides, colorimetric biosensor has a tunable dynamic range and wide, different concentration ranges metal ion detection [29–33]. Colorimetric biosensor is very convenient where the presence of analyte and ions can be seen through the color change of the solution mixture. Colorimetric biosensor will make a change in color and SPR absorbance peak through mechanism of metal aggregation and disassembly of aggregated particle as in FIGURE 7. Different aggregation states of nanoparticles can make a distinctive change in color.

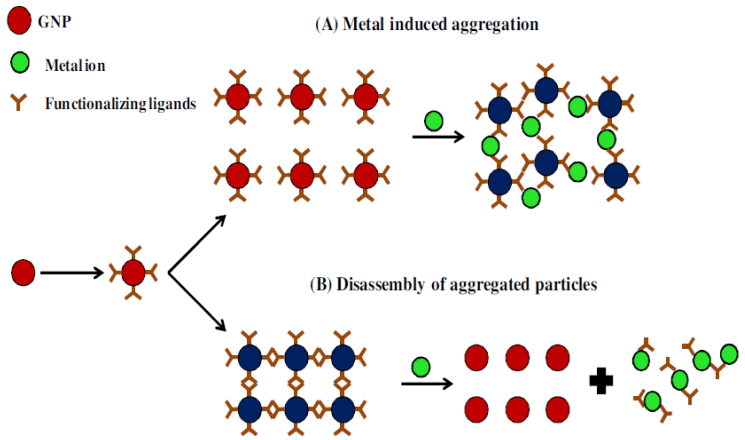

FIGURE 7. Colorimetric biosensor mechanisms [28].

Most of the researchers such as Liu and Lu [34], Xue et al. [33], Lee et al. [29], Zhang et al. [35], Song et al. [36] and Wang et al. [37] use gold nanoparticle (AuNP) for the colorimetric biosensors. AuNP is commonly used for the sense of lead ions (Pb^{2+}) and mercury ions (Hg^{2+}) in the environment, especially heavy metal ions in river water and leaded paint [36]. Liu and Lu [34] reported that, the mixture of AuNP and Pb^{2+} will cause the change of colorimetric biosensor from blue to purple to red color with a change in concentration of Pb^{2+} as shown in FIGURE 8. Results show that colorimetric biosensor is also selective in the detection of the presence of Pb^{2+}. Aggregation of AuNP will result in blue color solution with the absence of Pb^{2+}. However, with the presence of Pb^{2+}, the AuNP will catalyze with the cleavage of gold substrate strand, which will prevent the aggregation of AuNP effectively.

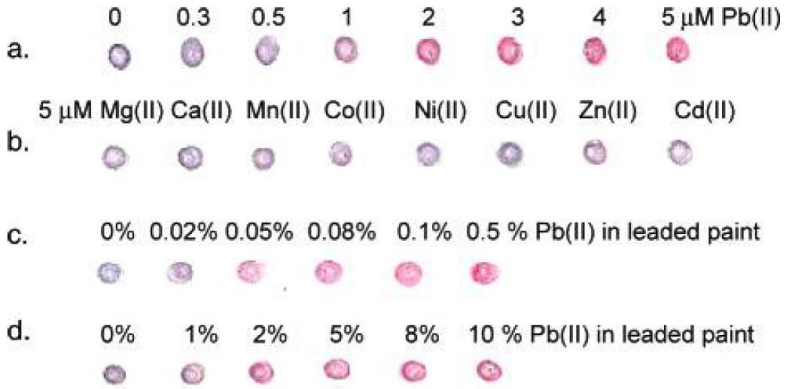

FIGURE 8. Colorimetric selectivity and sensitivity detection of different concentration of Pb^{2+} [34].

Global mercury contamination alerts the importance and awareness on the mercury exposure. AuNP biosensors also enable the colorimetric detection of Hg^{2+} in aqueous media. AuNP colorimetric biosensor is simple, practical, economical and can operate with better sensitivity and reliability [38]. Different mechanism can occur in the detection of Hg^{2+}, in which a clear red-to-pinkish colorimetric response can be detected, as shown in FIGURE 9, where the Hg^{2+} will selectively bind and stabilize the T-T to T-Hg^{2+}-T bonding. This will lead to AuNP aggregation. The enzyme free DNA-AuNP can be used to detect Hg^{2+} in aqueous solution since it has very high selectivity and sensitivity and do not require specialized equipment, except that it needs a temperature control unit. Lee et al. [29] reported that an increasing in the concentration of Hg^{2+} metal ions will increase the need in the contribution of required melting point.

FIGURE 9. Colorimetric results of different heavy metal ions obtained at 47°C [29].

The invention of novel colorimetric biosensor for Uranyl (UO_2^{2+}) using label-free DNAzyme AuNP by Lee et al, where AuNP are highly negative charge due to phosphate backbone of DNA [39]. Label-free colorimetric biosensor need no of DNA attaches on the AuNP. AuNP substrate strand will form a cleavage and shorten the length of the weakest complementary part in aggregates with the presence of UO_2^{2+}. In consequence, it will reduce the melting point of AuNP aggregates with the substrate cleavage. No interaction between the complex and AuNP when UO_2^{2+} is absent, and hence will induce the aggregation of AuNP due to screening effect of NaCl and color change from red back to blue.

Chen et al. has invented a colorimetric assay for Pb^{2+} based leaching of AuNP [31]. Shi et al. also has improved and introduced a better version of label-free colorimetric biosensor for the detection of Pb^{2+} based on the acceleration of gold leaching by graphene oxide (GO) [32]. GO concentration of 10 µg/mL is selected due to the best performance of solution at pH 8.0. GO modified AuNP can be dissolved in Pb^{2+}-$S_2O_3^{2-}$ for 2 hours to test the presence of Pb^{2+} ions which is shown in FIGURE 10. The presence of Pb^{2+} ions and the increase in the concentration of Pb^{2+} ions lead to colorless solution as shown in FIGURE 11. Detection of Pb^{2+} in real life such as in drinking water and river water has been observed and analyzed using colorimetric biosensor shown in FIGURE 12.

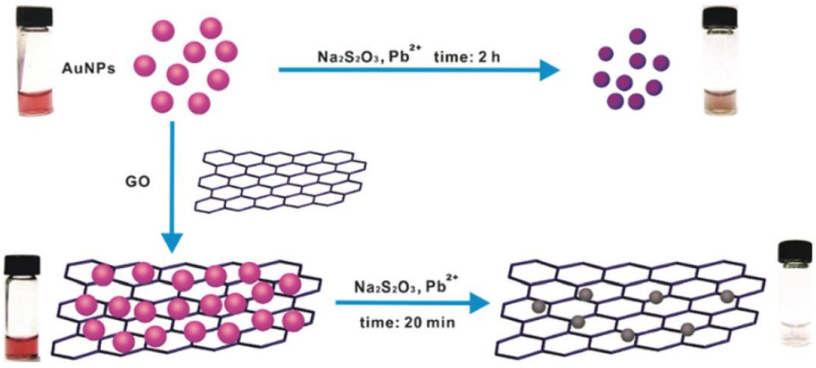

FIGURE 10. Colorimetric sensing of Pb^{2+} using GO enhanced AuNP [32].

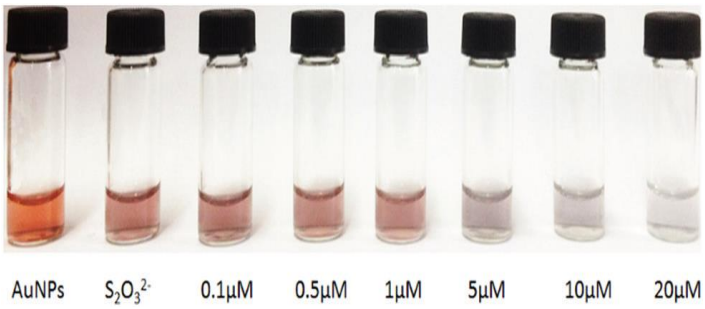

FIGURE 11. Colorimetric response for pure AuNP, addition of $S_2O_3^{2-}$ and different concentration Pb^{2+} [32].

Samples	Pb^{2+} ($\mu g\ L^{-1}$)	Addition (µM)	Recovery (%)	RSD (%) ($n = 3$)
Pure drinking water	0	0.2	93	1.54
	0	5	102.4	7.23
	0	10	105.3	2.12
River water	474.5	—	—	3.73

FIGURE 12. Sample test in drinking water and river water using GO modified colorimetric biosensor [32].

FIGURE 13 shows the comparison between labeled and label-free colorimetric UO_2^{2+} sensors. Label-free colorimetric biosensors have more advantages compared to labeled colorimetric biosensor in terms of detection range, detection limit and working time.

sensors	labeled sensor	label-free sensor
Detection range	50 nM-2 μM	1 nM-700 nM
Detection limit	50 nM	1 nM
Linear range	50−500 nM	1−100 nM
Saturation point	2 μM	700 nM
Working time	30 min	6 min
Working temperature	Room temperature	Room temperature
Operation step	1 step	3 steps
Quenching	No	Possible (by shifting pH)
Std. Dev.	~10% of the signal change when saturated	~10% of the signal change when saturated
Color Change	Purple to red (in the presence of analyte)	Red to blue (in the absence of analyte)
Type	Turn on	Turn off
Stability of AuNPs	Stable	Less stable

FIGURE 13. Comparison between labeled and label-free colorimetric UO_2^{2+} sensor [39].

For much greener, and environmental-friendly colorimetric biosensor, Annadhasan et al. studied on green synthesized silver nanoparticle (AgNP) for colorimetric detection of Hg^{2+}, Pb^{2+} and Mn^{2+} in aqueous medium [40]. SPR intensity and color change based on Hg^{2+} and Mn^{2+} ions, where color of Hg^{2+} solution change from yellow to colorless caused by the oxidation of Ag0 to Ag+ during the reduction process of Hg^{2+} ions. The color of Mn^{2+} solution change to brownish yellow when the ions are added into AgNP due to rapid aggregation of AgNP. Figure 14 shows the AgNP and AuNP interaction with different metal ions.

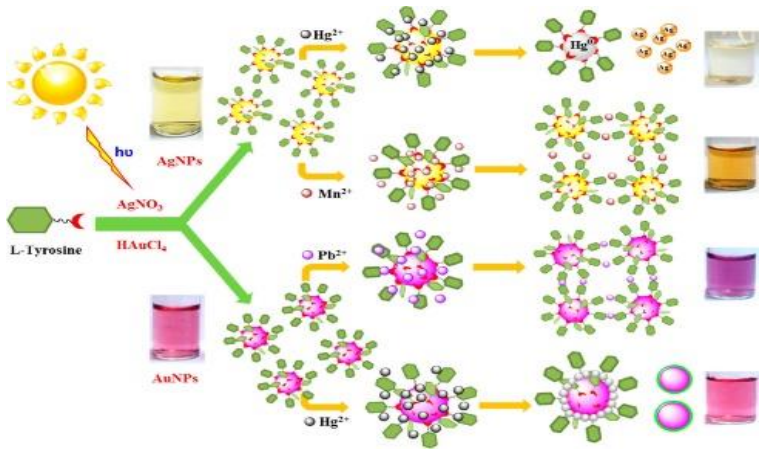

FIGURE 14. AgNP and AuNP interaction with different metal ions (Hg^{2+}, Mn^{2+} and Pb^{2+})[40].

4. ELECTROCHEMICAL BIOSENSOR

Electrochemical sensor is widely used because it is simple, rapid, easy, convenient and inexpensive for detection of different type of analytes in different applications, such as in food testing, clinical diagnosis and environmental monitoring [41- 43]. However, the electrochemical sensors have one main problem, which is a limited surface area of the electrode. This is due to electrode coverage that is designed in a small area to provide an easy and convenient sensor for on-site monitoring and portable for simple tracing process [44]. Therefore, the sensitivity of this electrochemical biosensor is lower compared to other types of biosensors discussed previously.

Graphene is a material that can be obtained through simple chemical processing of graphite [45], and it is a very suitable candidate for a biosensor electrode due to a very good electrical conductivity, possible of wide surface area and high mechanical strength. Besides that, an option of using a three-dimensional reduced graphene oxide (3D-rGO) can be an ideal material for an electrochemical biosensor due to its good in physical and chemical characteristic, like large surface area, very good conductivity and excellent mechanical performances.

Polyaniline (PANI) is a versatile material in electrochemical application due to its cheaper, good environmental stability, interesting electro-activity and irregular doping or de-doping [46]. The combination of graphene and PANI can be obtained through the situ electrochemical synthesis and situ polymerization. This composite is proven to have high electrochemical stability and good electrical conductivity. As discussed previously, mercury, Hg^{2+} detection is based on the special bonding of T-Hg^{2+}-T coordination [47], therefore the composite can be a functional layer that has high affinity towards DNA immobilization. DNA is always anchors onto PANI surface due to its high intensity of amino group. There are two advantages of electrochemical biosensor based on 3D-rGO@PANI nano-composite suitability for Hg^{2+} detection; which are to improve the limited surface area problem and electrochemical performance, and to ensure the DNA immobilized anchor on PANI surface due to high intensity of amino groups. Therefore, it has high sensitivity for Hg^{2+} detection [44]. FIGURE 15 shows that process of the DNA electrochemical biosensor-based 3D-rGO@PANI nano-composites to detect the Hg^{2+} ions and electrochemical signal is formed. Three-electrode cells were used to measure impedance spectra from -0.2V to -0.8V and 100mV pulse amplitude. There are three electrodes, which are reference electrode (Ag@AgCl electrode), counter electrode (platinum slides) and working electrode (gold electrode).

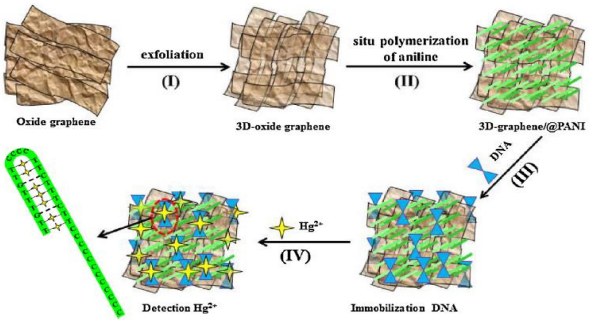

FIGURE 15. The detection of Hg^{2+} ions by using the electrochemical DNA biosensor based on 3D-rGO@PANI nano-composite [44].

FIGURE 16 demonstrates a modified Randle's equivalent circuit and it represented an interfacial phenomena model. This circuit has four elements, which is the ohmic resistance of the electrolyte, R_s, the Warbury impedance (WD), the constant phase element (CPE) between solution and

electrode and the electron transfer resistance, R_{ct}. The bare gold electrode has low charge-transfer resistance (0.18 kΩ) as in FIGURE 16 (a). After 3D-rGO@PANI is placed on the surface of a bare gold electrode, the value of R_{ct} became smaller (0.65 kΩ). At the same time, DNA also increases the electron transfer in the blocking layer and increases the R_{ct}. When Hg^{2+} is appeared, the T-Hg^{2+}-T bond is formed and the thickness of film increased, therefore the R_{ct} also increased [48], [49]. The CV results are shown in FIGURE 16 (b). During the concentration of Hg^{2+} testing, the values of R_{ct} are directly proportional to Hg^{2+} concentration, which mean, the device has high sensitivity to Hg^{2+} ions as shown in FIGURE 17(a)). In FIGURE17 (b), the change in R_{ct}, that is given by ΔR_{ct} represented a relative amount and quantitative behavior of this detection [50]. The graph in FIGURE 17(b) demonstrates an equation ΔR_{ct}= 574.7 + 391.9 log CHg^{2+} and linear correlation coefficient, R = 0.9872. This detection of Hg^{2+} biosensor has higher sensitivity than conventional method because of the limitation of detection has been improved and a lower limit of detection (LOD) is obtained.

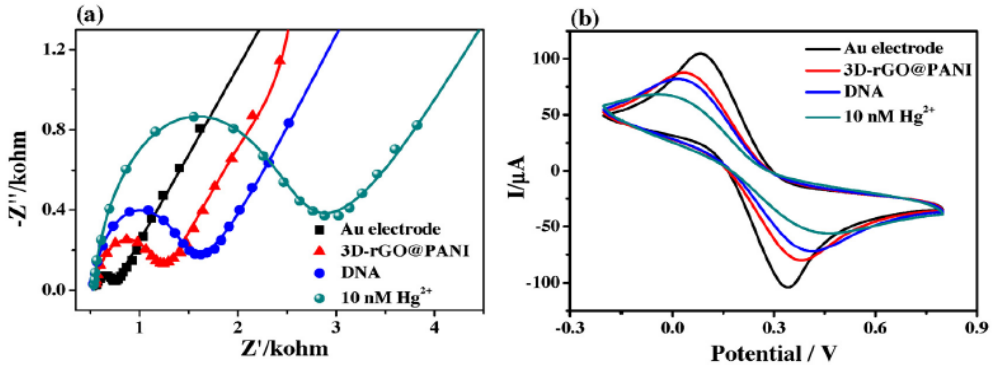

FIGURE 16. (a) EIS Nyquist plots and (b) CV curves of different modified electrode types [44].

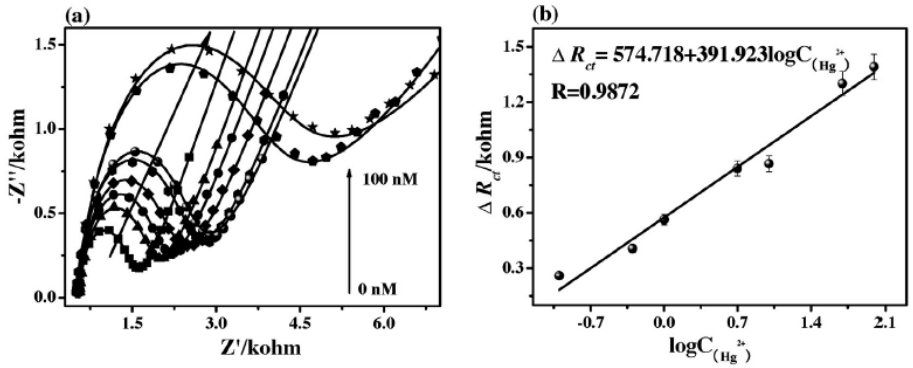

FIGURE 17. (a) Nyquist plots of EIS for mercury ion detection and (b) linear relationship between difference in the R_{ct} and the log C (Hg^{2+}) [44].

To test the device's sensitivity, the solution was initially contained metal ions, which are Pb^{2+}, Ni^{2+}, Ca^{2+}, Ag^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, and Fe^{3+}. The results as recorded in FIGURE 18(a) shows positive response to mercury, Hg2+ ions and negative response to other metal ions. This condition demonstrated that the sensor has a good sensitivity response to Hg^{2+} compared to other metal ions. This DNA electrochemical biosensor-based 3D-rGO@PANI nano-composites can be cleaned up easily and requires less time-consuming in its reproduction of new electrodes because it involve immersion of the electrode in a stirred solution containing 1.0M HNO_3, 1.0 KCl and 1.0mM EDTA in

only one minute. FIGURE18 (b) shows that the sensor has high reproducibility in mercury ion detection after 10 repetitive measurements. The results show that the DNA electrochemical biosensor-based 3D-rGO@PANI nano-composites is very suitable for mercury detection because it has good sensitivity, excellent selectivity, lower LOD and repeatability of the proposed production method.

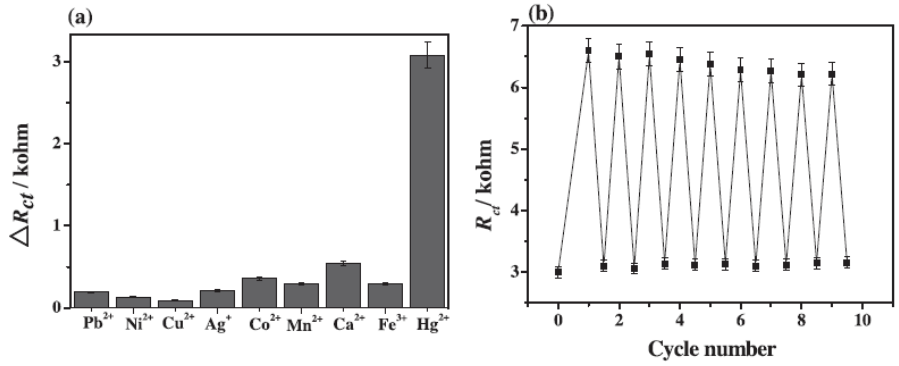

FIGURE 18. (a) R_{ct} for different metal ions (b) reusability of sensor [44].

5. CONCLUSION

In conclusion, several techniques have been discussed in the detection of heavy metal ions, which are magnetic chitosan biosensor, electrochemical biosensor, and colorimetric biosensor. Recent researches have shown that magnetic chitosan biosensor can easily detect and extract poisonous heavy metal ions. On the other hand, the colorimetric biosensor can detect the heavy metal ions easily in real-time that are common in laboratory tests and industrial applications. Its detection is also fast, sensitive, and selective for various types of metal ions. However, the electrochemical biosensor has one main problem related to its limited surface area of the electrode, which decreases its sensitivity, even though it is a simple, rapid, and inexpensive technique for determining heavy metals at different areas and different types of analyses. Therefore, the colorimetric biosensor is the most suitable biosensor to detect the heavy metal ions due to its latest technology, save in its production cost, convenient, and easy usage especially due to the inclusion of gold nanoparticles. It can improve the environmental quality and provide many advantages to human lives, especially in the detection of cancer cells.

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