Electrical Responses of Chitosan-based Biosensor Toward Cadmium Ions Adsorption

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ABSTRACT

Chitosan-based biosensors has contributed to the advancement of bio-detection technologies, offering improved sensitivity and selectivity for the detection of various analytes, such as heavy metal ions. The presence of heavy metal ions in the environment poses a significant threat to human health, environmental quality, and ecosystem stability due to their carcinogenic and toxic properties. This study incorporates chitosan film in direct spatial contact with an interdigitated electrode to determine its electrical response. Chitosan films were soaked in cadmium ion solution in various concentrations before measuring the film's resistance, capacitance and impedance. Identification of circuit arrangement was done by employing the Nyquist plot. The results show that ionic concentration is directly proportional to films' adsorption, capacitance and conductivity. However, the impedance and resistance value decreased as the metal ion concentration increased. The findings suggest that chitosan film possesses the capability to adsorb cadmium ions, even at low concentrations. Additionally, alterations in the electrical properties of the film can serve as a reliable indicator of the concentration of ions present in the solution.

1. Introduction

Biosensors are analytical devices that integrate a biological element with a transducer and convert its response into electrical output [1,2]. The electrical response plays a crucial role in detecting and measuring different analytes, making biosensors highly valuable in various applications, including medical diagnostics, environmental monitoring, and food safety. Biosensors offer a more environmentally sustainable option for assessing toxicity than classical approaches. The conversion of biochemical signals into quantifiable electrical signals is greatly influenced by the type of transducer used, such as electrodes or nanomaterial-based structures, consequently affecting the sensitivity, selectivity, and reliability of the electrical response in biosensors.

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The electrical response is essential for detecting and measuring various analytes, making biosensors extremely valuable in a diverse range of applications, such as monitoring heavy metal ions in aqueous environments. Heavy metal ions are present in wastewater and soil, primarily caused by various industries, including mining, paints, batteries, and agriculture. Untreated waste from these industries directly enters rivers and seas, posing a detrimental impact on the drinking water source and the marine ecosystem.

Many heavy metals, including Pb (II), As (V), and Cd (II), exhibit carcinogenic and toxic properties, thereby posing a grave threat to human health. Prolonged consumption of these contaminants can lead to organ damage and adversely affect the nervous system [3]. Hence, the World Health Organization has limited the permissible consumption of heavy metals, especially in drinking water. For example, the maximum permissible limit of cadmium ions in water is 0.005 mg/L, which may cause renal toxicity, hypertension and kidney damage [4]. Thus, monitoring wastewater released to the environment is vital to ensure the discharged water meets the required standards for protecting public health and ecosystems. Heavy metals can cause toxicity in biological systems through the inhibition of membrane transport processes and substitutive ligand binding [5].

Extensive research has been conducted on chitosan due to its cationic nature, which makes it an intriguing option for use in biosensors to detect heavy metal ions. Chitosan is a white, stiff, and inelastic nitrogenous polysaccharide and is produced by the deacetylation of chitin. Chitin is also known as the beta-(1,4)-N-acetyl-D-glucosamine homopolymer. Its structure is very similar to that of cellulose, with the exception that the second carbon atom has a hydroxyl group instead of an acetamido group [6]. It has three reactive functional groups: amine, primary, and secondary hydroxyls, which can be covalently or ionically modified. Moreover, chitosan has excellent film-forming properties [7]. These characteristics make chitosan a promising candidate for biosensor applications. Some examples of chitosan-based biosensors are carbon dots rooted in agarose hydrogel [8], fluorescent sulfur quantum dots embedded in chitosan hydrogels for Cr(VI) ions detection and removal [9], silver nanowires/HPMC/Chitosan/Urease modified electrodes [10], and metal-organic framework-based nanosensors [11]. These biosensors have demonstrated favourable outcomes in the selective detection of heavy metal ions, even at low concentrations.

Chitosan-based biosensors offer several advantages for heavy metal ion detection. Chitosan is characterised by its high sorption capacity for metal ions, facilitating targeted and efficient detection [12]. Upon exposure to a solution containing heavy metal ions, chitosan undergoes a process whereby the amino and hydroxyl groups on its surface interact with the metal ions, forming stable chelates [13]. As such, it has potential for real-time detection of heavy metal ions [14].

Chitosan is the only alkaline polysaccharide in nature, compared with other types of polysaccharides like agar, cellulose, pectin, or starch, which are either acidic or neutral [15]. Chitosan and its derivative ability to absorb heavy metals and dyes even at low concentrations is a fundamental characteristic that endows it with efficacy in identifying heavy metal ions. The study by Hermanto *et al.,* [16] demonstrates the potential of chitosan as a biosensor for quantifying the concentration of Hg(II) concentration in aqueous samples. Another study shows that the amalgamation of chitosan with magnetic materials enables it to effectively adsorb heavy metals and antibiotics across a broad spectrum of pH levels [17]. Several factors, including pH, temperature, contact time, initial metal ion concentration, chitosan particle size, and the presence of other substances in the water, can influence the adsorption capacity and efficiency of chitosan for heavy metal ions.

Although extensive studies on chitosan have been successfully carried out to test its applications in various fields, only a few studies employed pure chitosan and discussed its electrical properties. Therefore, in the present work, the electrical characteristics of chitosan film when soaked in Cd^{2+} ions at various concentrations were measured.

2. Materials and Method

Medium molecular weight chitosan powder was purchased from Sigma Aldrich. Cadmium solution in nitric acid (1000 ppm) were brought from Fisher Scientific UK. All reagents used were analytical grade chemical.

Chitosan film was prepared by dissolving 1 g of chitosan powder in 100 mL of 1% (v/v) acetic acid at 55 °C and stirred for 24 hours until a uniform solution was obtained. Then, the solution was spread onto a plastic substrate and allowed to dry at room temperature. The dried chitosan film had an approximate thickness of 0.132 mm. The film on the substrate was then sectioned into small square pieces with dimensions 8 mm × 8 mm and inspected for any defect, such as delamination or dirt, that could affect the absorption ability.

The metal ion used in this study is Cd^{2+} with concentrations 1 mg/L, 2 mg/L, 10 mg/L, 30 mg/L and 50 mg/L. The prepared chitosan films were immersed in the metal ions solutions for 20 minutes. The films were then removed from the solution and dapped gently with tissue paper to blot the excess solution. The remaining solution was sent for Atomic Absorption Spectrometry (AAS) testing. AAS can be used to determine of elemental composition or trace elements present in solutions owing to its ability to analyse low-concentration samples.

The film was attached to an Interdigitated Electrode (IDE) and connected to an LCR meter to characterise its electrical properties. IDEs are widely used as sensing platforms for detecting various analytes in chemical and biological systems. It can specifically attach to target molecules, causing detectable alterations in electrical characteristics like impedance, capacitance, or conductivity. Other advantages of IDEs include not requiring extra electrodes, straightforward and inexpensive mass production, and the capability to easily customise them for various analytes. In this study, IDE employed was made from gold electrodes over a ceramic substrate, with bands/gaps of 200 μm, and 1 mm thickness. The schematic diagram of IDE and its properties is shown in Figure 1. The gold electrode was used due to its ability to measure small changes in the sample precisely.
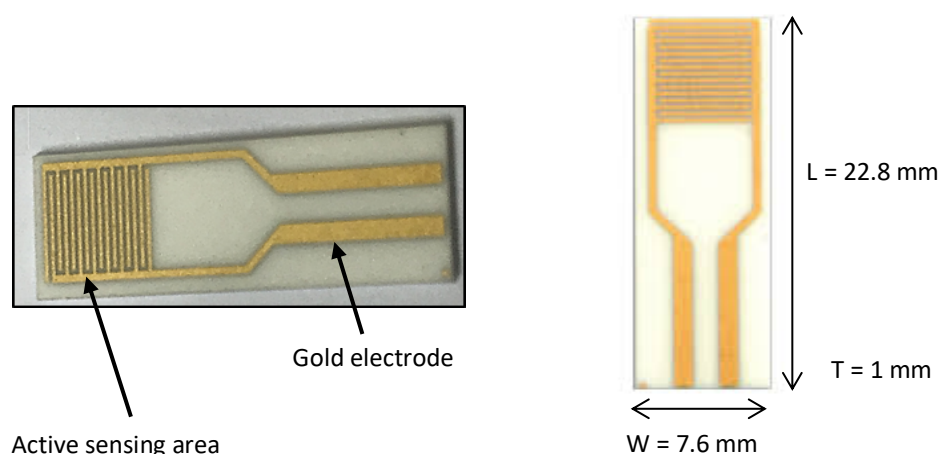
Fig. 1. Gold Interdigitated Electrode (IDE) on ceramic substrate with dimension for bands/gaps of 200μm

Table 1
Properties of the Interdigitated Electrode (IDE) used

IDE Sensor	Frequency (Hz)	Conductivity range	K_{cell} (cm^{-1})	Descriptions
DRP-IDE AU200 (gold)	28 kHz	7.39-1478μS/cm (135.3 to 0.68 kΩ x cm)	0.0166 ±0.0003	- 200 μm bands/gaps widths - Ceramic substrate

The IDE was connected to Rohde & Schwarz LCR Meter to measure the electrical properties of chitosan film such as resistance, capacitance, impedance and phase angle (Table 1). The LCR meter, which stands for Inductance (L), Capacitance (C), and Resistance (R) meter, is commonly used to measure the electrical properties of passive electronic components. The voltage provided by the LCR meter is 1 V within the frequency range of 10 Hz to 200 kHz. The results from the LCR meter were then analysed to obtain the Nyquist plot for identifying circuit components.

3. Result and Discussion

Cadmium ion solution was prepared in five different concentrations: 1 mg/L, 2 mg/L, 10 mg/L, 30 mg/L, and 50 mg/L. A piece of chitosan film was immersed in each solution and removed after 20 minutes. Then, the remaining cadmium solution was analysed using AAS to check for the amount of metal ions left in the solution, and the quantity of ions absorbed by the chitosan film was calculated. AAS works based on the principle of the absorption of particular wavelengths of light by atoms in their ground state in the gas phase that is directly related to the concentration of metal ions in the sample, enabling precise quantitative analysis. The results were tabulated in Table 2, and the trend shows adsorption escalates as the cadmium concentration increases. This finding is comparable with Abdel-Mohdy *et al.*, [18], who state that chitosan's metal cation uptake was measured as a function of metal concentration and metal cation type in the solution. The adsorption was due to the existence of amino and hydroxyl groups in chitosan molecules, which contribute to various possible adsorption interactions between chitosan and pollutants such as metals, dyes and phenols [19].

The electrical responses of biosensors are influenced by the materials used in their construction. This study incorporated gold IDE in the sensing heavy metal ions, converting electrochemical reactions in chitosan film into measurable electrical signals. These signals were then interpreted by LCR meter in digital form. LCR meter calculates impedance, Z, and phase angle, θ, by measuring the current flow and voltage across the measurement target terminals.

Table 2
Adsorption of Cd^{2+} by Chitosan Film

Film	Initial concentration of Cd^{2+} Ions (mg/L)	Amount of adsorption (mg/L)
A	1	0.3918
B	2	0.2895
C	10	0.585
D	30	3.24
E	50	5.53

Figure 2 (a) – (c) displays the electrical resistance, capacitance and impedance of soaked chitosan films at various Cd^{2+} concentrations against the LCR frequency. The resistance versus frequency graph was plotted in Figure 2 (a). The lowest cadmium concentration reflects the lowest cadmium adsorbance by chitosan film, which possesses the highest resistance value. On the other hand, the film that adsorbed Cd^{2+} the most experienced the least resistance. Another trend that can be observed is the value of resistance drop as frequency increases owing to the internal measurement system of the LCR meter that employs the Electrical Impedance Spectroscopy (EIS) technique. This spectroscopy method exploits Faraday's Law to investigate the chemical reaction of a material in terms of electrical measurement. It utilises alternating current (AC) to excite the device under test (DUT) and measure the response. Accordingly, the voltage applied across the DUT was in the sinusoidal wave, and the range of frequency used in this measurement was 10 Hz to 200 kHz. As the

frequency increases, the mobility of the charge carrier becomes higher, causing the film's resistance to decrease.

The amount of ions adsorbed is directly proportional to the film's ability to store charges; the more ions adsorbed by the film, the higher its capacitance value as featured in Figure 2 (b). The graph displays an identical pattern as in the resistance plot, where the frequency increment causes the capacitance value to drop. Impedance is the complex value containing both resistance and reactance, where reactance accounts for the effects of capacitance and inductance in the circuit.
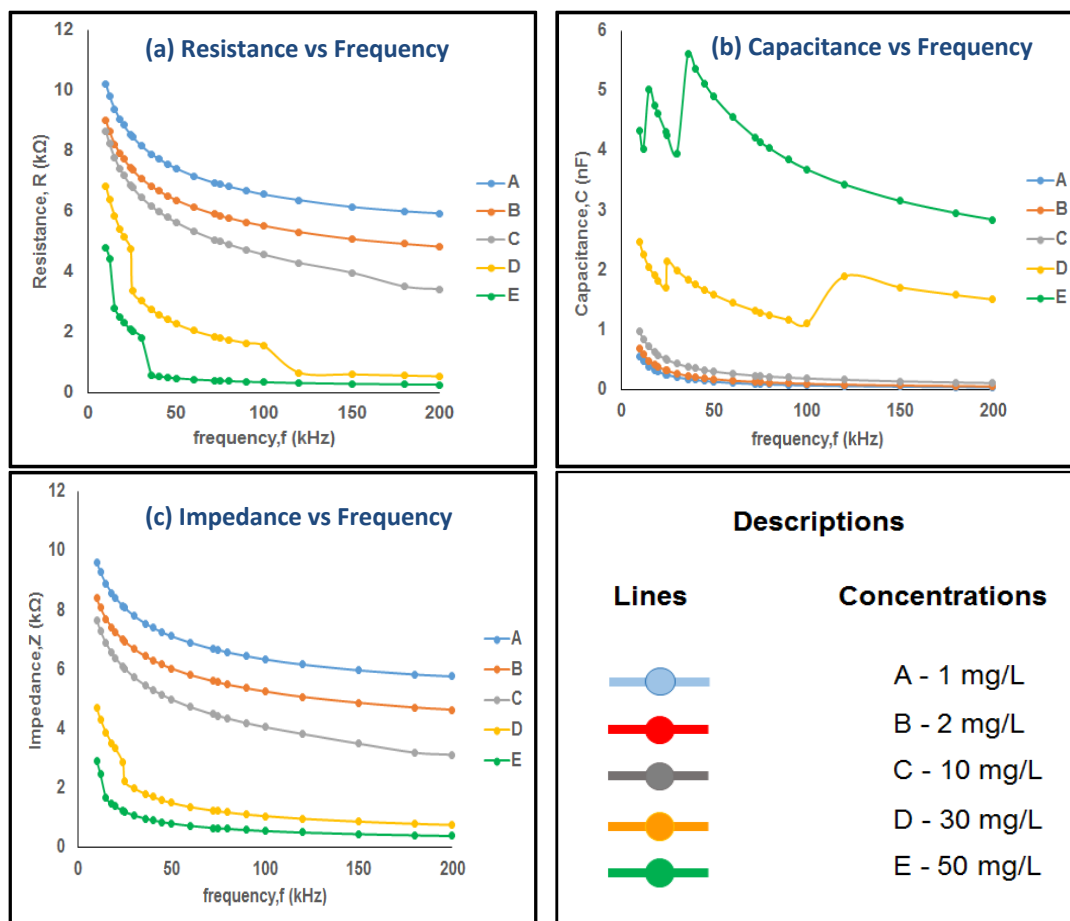
Fig. 2. (a) Resistance; (b) Capacitance; and (c) Impedance of chitosan film soaked in cadmium ions solution against the LCR frequency

The impedance magnitude represents the overall resistance to current flow, while the impedance phase indicates the relationship between voltage and current in an AC circuit. At lower frequencies, the impedance may be dominated by the resistive component. On the other hand, the reactive components such as inductance and capacitance may have a more significant impact on the overall impedance at higher frequencies. The decrement in impedance signifies an increment in the material's conductivity due to more ions available and being able to move more freely through the material [20]. Impedance value at low frequency is related to the space charge polarisation, whereas the higher frequency is due to the hoping electron between the localised ions [21].

The Nyquist plot provides a graphical representation of the frequency response function of a linear system, depicting the impedance of the system in the complex plane and, accordingly, facilitates the assessment of the electrical behaviour of various materials and systems. It typically consists of a plot of the real impedance (Z') and the imaginary impedance (Z''), providing valuable information about the electrochemical processes occurring within a system.

Figure 3 shows the Nyquist plot as well the circuit arrangement between chitosan film, interdigitated electrode, and bulk resistance, R_b. As can be observed from the graph, a semicircle is absent at the high-frequency region, leaving only a spike at the lower frequency. These observations suggest a high level of ionic conduction on the surface of the chitosan film which provides more mobile ion and hence causes an increase in conductivity. The diameter of the semicircle in the Nyquist plot is indicative of the charge transfer resistance [22] where a smaller diameter of the semicircle implies a significant charge transfer efficiency [23] and increment of the bias voltage [24]. The parting of the tilted spike below 90º referred to constant phase element (CPE) which is imperfect capacitor and bulk conduction process. CPE is the circuit element that exist to describe the capacitance that occur in the electrochemical system due to the surface roughness or distribution of reaction rates. In low frequency region, the electrode-electrolyte undergo polarisation effect as a result of diffusion-controlled Warburg impedance. This leads to resistive component of the polymer electrolytes occur and prevail ionic conduction directly.

The same trend was obtained by Mattos *et al*., [25] in the studies of the ionic conductivity of polymer blends. They also reported that pure chitosan exhibited a lack of semicircle at high frequency and a residual tail at low frequency. On the other hand, Yusof *et al*., [26] proposed that the absence of a semicircle at higher concentrations of metal ions can be attributed to the presence of only the resistive component of the polymer electrolytes within a specific frequency range.
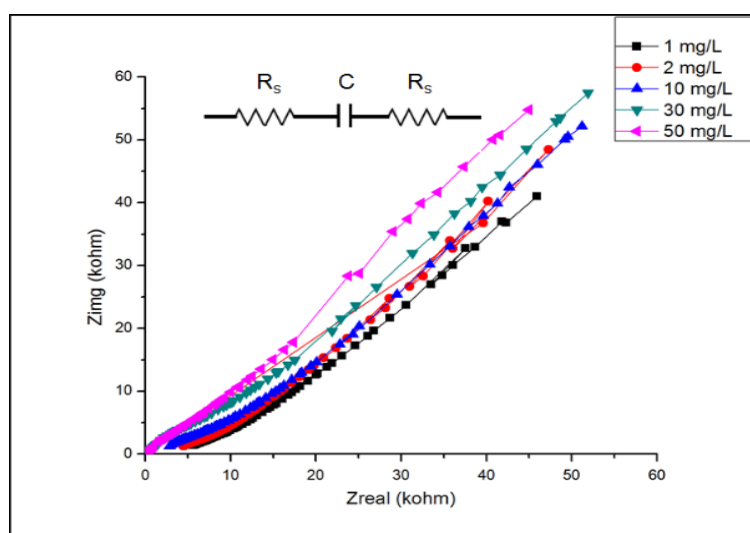

Fig. 3. Nyquist plot

The appearance of semicircle impedance represents the parallel arrangement of equivalent circuit resistance-capacitance. The capacitor refers to the immobile chitosan backboned chain and becomes polarised in the alternating field, whilst the resistor indicates the migration of the proton ions [27]. If the capacitance is ideal, the spike will be perpendicular to the real impedance axis. However, due to the missing semicircle, the capacitance is not in parallel arrangement but in a series equivalent circuit. According to the analysis by Ramesh and Arof [28], the spike is inclined at an angle below 90º due to the electrode's roughness or inhomogeneous distribution of the metal ion onto the chitosan film. The depressed semicircle of the Nyquist plot represents the bulk material.

The bulk resistance, R_b value can be obtain from the fitting Nyquist graph with linear line where the interception indicates the R_b value. Under normal conditions for the impedance plot, bulk resistance, R_b was extrapolated from interception between the semicircle at a high frequency and spike line of low frequency. However, due to the missing semicircle in the measured plot, the bulk resistance was calculated directly from the intercept of the line and the x-axis using linear Randle

models. It can also be calculated by using Eq. (1), where the value of $Z_{imaginary}$ is obtained from *y*-axis interception.

$$Z_{img} = \frac{R_b}{2} \tag{1}$$

Table 3 shows that the smallest R_b could be achieved by soaking the chitosan film in the highest ionic concentration. The results are in parallel with the earlier resistance results. R_b decrement leads to diminishing of amorphousness in polymer electrolyte structure [26].

Table 3
Value of bulk resistance, R_b at different concentrations

Concentration of metal ions (mg/L)	R_b value (Ω)
1	5842.09
2	4850.16
10	3751.63
30	961.96
50	566.53

Ionic conduction dominates the process of electrolytes at higher frequencies. The blocking electrode effect can be observed as a straight line that is parallel to the imaginary axis at a lower frequency range. By virtue of the electrode-electrolyte interface, this blocking electrode spike is reckoned as capacitance, where the ideal spikes should be perpendicular to the real impedance. However, slightly tilted spikes in the Nyquist plot suggested an uneven or non-homogenous electrolyte-electrode interface.

4. Conclusions

The present study employed chitosan as a prospective adsorbent for cadmium ions. The study aimed to investigate the electrical properties of the interaction between chitosan and cadmium ions across a range of concentrations. The initial dry state of chitosan films was found to exhibit inadequate conductivity. Nevertheless, chitosan's conductivity increases when immersed in an aqueous environment. The rise in metal ion concentration resulted in a corresponding increase in the quantity of metal ion adsorbed.

The findings indicate that the adsorption process of metal ions onto the chitosan surface resulted in an augmentation of the capacitance of the chitosan film. This is because a higher concentration contains more free mobile ions. As the concentration of metal ions increased, both impedance and resistance decreased.

The utilisation of Nyquist plot analysis facilitated the determination of the equivalent circuit between the chitosan film and IDE. It was observed that the capacitance is present in a circuit equivalent to a series configuration. An increase in concentration decreased impedance and allowed free electrons to flow from one division to the next. When the number of mobile ions increases, capacitance and conductivity increase while impedance, dielectric constant, and dielectric loss decrease.

The chitosan film had the lowest bulk resistance when it was subjected to the highest ionic concentration available. These findings are in agreement with the resistance findings that were discovered earlier. When the bulk resistance of the polymer electrolyte is decreased, the amorphousness of the structure is also reduced.

Acknowledgement

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