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Research Article

Development of Electrochemical Impedance Biosensor using Organic Nanotubes Deposited on Screen Printed Electrodes

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ABSTRACT

Present study reported the development of electrochemical impedance biosensor based on organic nanotubes for nitrate detection. The electrochemical impedance spectroscopy (EIS) technique was used for the fabrication and development of the biosensor. Material impedance to the flow of electric current through was studied in depth. Various performance parameters such as electron diffusion coefficients, charge transfer rates, and charge transfer resistance were determined using EIS with insight into the processes occurring in the solution. Heterogeneous electron transfers rate constant for SPE, MWCNT/SPE and NiR/MWCNT/SPE electrodes were found to be 1.03×10^{-6} cm/s, 2.21×10^{-6} cm/s and 2.44×10^{-6} cm/s, respectively. Using the developed biosensor, sensitivity of $9.01 \times 10^{-4}\ \Omega^{-1}$ $(mg/L)^{-1}$ is obtained for nitrate detection. The lower detection limit was also calculated and found to be 0.02 mg/L. In the methodology of the EIS measuring system, an AC signal was applied to the electrodes in the solution. The collected data was plotted in the complex plane. This is an extremely useful non-destructive technique of examining the complexities of agricultural samples under testing by passage of AC current.

Key words: Electrochemical impedance, organic nanotubes, biosensor, charge transfer resistance, sensitivity

Introduction

Due to increasing use of nitrogen fertilizers in agriculture, the ground water is getting polluted by leaching water and surface runoff water (Ahada and Suthar, 2018). This is further causing severe environmental toxicity. On the other hand, industrial effluents and sewage also contribute significantly to the accumulation of nitrate in soil and hence in food leading to nitrate toxicity (Ziarati, 2018; Zhang *et al.,* 2019). Thus, the detection of nitrate is a growing concern for preventing water pollution, plant nutrient stress management and precision agriculture. Nitrate has now been listed as potential toxicant due to its presence in high levels in fruits, vegetables, water, packaged and processed food products (Ahmed *et al.,* 2017). In this regard, globally, various international agencies have established limiting values for the nitrate intake. As per world health organization, the nitrate concentrations in drinking water should not exceed 10 mg/L (Nicole *et al.,* 2019). The European Union has laid acceptable daily intake limit of NO_3^- to be 3.7 mg/kg body weight per day (European, 2011). In order to prevent nitrate toxicity in humans, it is important to monitor and implement necessary practices in agricultural and horticultural production systems to control excessive food nitrate concentrations. During pandemic (Covid-19), immunity plays an important role in escaping the lethal effects. It is now evident that our food should be healthy and nutritious. Thus, the quality and safety assessment of food is the prime concern today. For this, there is immediate need of smart sensors to detect various toxic chemicals in food. In this regard, upcoming nanotechnology interventions such as biosensors for detection and

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quantification of quality aspects of water, food and environment are gaining popularity (Mutlu, 2016). In present work we have developed electrochemical impedance biosensor for the detection of nitrate in samples. In this technique, the sample is kept under the application of an AC voltage of varying frequencies to record the resulting response (Alahi *et al.,* 2018). The Nyquist plots of the electrochemical impedance data were obtained to study the charge transfer dynamics. A Nyquist plot represents complex plane which depicts the imaginary impedance, consisting of capacitive and resistive character of the cell, versus the real impedance of the cell. In electrochemical impedance spectroscopy (EIS) the heterogeneous electron transfer rate constant (K_e) provided the measurements of resistance to the transfer of electron. The value of K_e obtained for fabricated electrode was found to be depending inversely on the electron transfer resistance (R_{ct}). Thus, immobilization of enzyme (NiR) on MWCNT/SPE electrode caused low R_{ct} values as compared to MWCNT/SPE electrode. Besides various spectroscopic and microscopic characterization studies other characterization parameters such as zeta potential and contact angles for the nanomaterial were also studied. The zeta potential provided the measurement of the magnitude of the electrostatic force of repulsion/attraction among the charged particles dispersed in a liquid medium and guide for the stability of the dispersion. Zeta potential measurements have immense applications in various fields such as ceramics, pharmaceuticals, agriculture and water treatment(Ahmad *et al.,* 2017). When studying the particle size, it was found that when the particle size reduced, the surface properties increasingly become significant for studying the dispersion characteristics. Among the significant surface properties, the surface charge plays determining role in affecting the nature of particles interactions, and hence dispersion properties including stability of the dispersion, viscosity, film formation etc. Another significant parameter related to surface properties of nanofilms i.e., contact angle was also studied. It is the angle formed at the interface of the liquid/solid surface and the liquid/air surface. A high magnitude of contact angle reflects a low solid surface energy and is thus indication of low chemical affinity. Whereas a low contact angle reflects that the solid possess high surface energy and thus high chemical affinity for the compatible bio molecules. There are various laboratory based conventional methods for the nitrate detection, but in view of their limitations such as requirement of lots of chemicals, skills and time, the electrochemical impedance biosensing technique allows fast, accurate and more sensitive detection of the target analyte.

Materials and Methods

DropSens (DS 110) screen-printed electrodes (3.4 × 1.0 × 0.05 cm) procured from Metrohm, India were used for the fabrication of biosensor. Screen-printed electrode (SPE) with integrated system having reference electrode of Ag/AgCl, the graphite working electrode was (4mm) and graphite counter electrode. The absorption characteristics of the nanomaterial were conducted using UV–visible spectrophotometer in the wavelength range 200–800nm. The organic nanotubes of carbon, multi walled carbon nanotubes (MWCNT) as synthesized in previously reported studies were used for the fabrication of MWCNT/SPE and NiR/MWCNT/SPE sensors using the drop casting method. For the further characterization during the development stages of the biosensor, zeta potential measurements were recorded as in the previous work to get insight into the electrostatic repulsion forces between similarly charged particles in dispersion medium (Kundu *et al.,* 2021).The nanoparticles were dispersed in medium possessing higher zeta potentials to understand the dispersion stability and nanoparticles tendency to agglomerate. The set-up of the contact angle measurement system consisted of goniometer, microscope and sample holder. First of all, a drop was placed on the sample holder very slowly from the graduated injecting syringe using precise and fixed volume of the liquid using the software. The drop on the solid surface was then photographed and the image captured was further used to calculate the contact angle. The recorded image was then processed using digital technique and the software (Image J) assisted in analysing the image obtained. The contact angle measurements of MWCNT films were measured by sessile drop method (Kundu *et al.* 2019). The drop volume was optimized (2 µl) to obtain the symmetric drop and the flow rate was kept at medium. The drop was viewed through a CCD camera attached to a microscope. The image was grabbed and further digitized for each measurement. For each of the measurements the contact angle values were measured thrice to obtain an average value. All the measurements of contact angles were conducted at room temperature. Impedance spectroscopy of SPE, MWMWCNT/SPE, and NiR/MWMWCNT/SPE biosensors have been studied in a PBS, pH 7.4, with 5mM $[Fe(CN)_6]^{3''/4''}$ redox couple at a fixed biasing voltage of 0.30 V (frequency 0.01-10^4 Hz). Nyquist plots for SPE, MWMWCNT/SPE, and NiR/MWMWCNT/SPE biosensors have been plotted. Heterogeneous electron transfer rate constant (K_e) were calculated for SPE, MWCNT/SPE, and NiR/MWCNT /SP electrodes by using following equation is

$$K_e = \frac{RT}{n^2F^2AR_{CT}S} \quad (1)$$

Where R stands for gas constant (8.314 /mol/K), T represents absolute temperature (K), F is the Faraday's constant (C/mol), A stands for electrode surface area (cm^2), S represents the bulk solution concentration (mol/cm^3), n stands for constant denoting number of electron transferred in electrochemical reaction.

The relation among frequency for maximum impedance –Z”, R_{ct} and C_{dl} can be expressed as (Massah and Vakilian 2019),

$$\boldsymbol{R_{ct}C_{dl} = \frac{1}{2\pi f_m} = \tau} \quad (2)$$

Here f_m stands for the maximum frequency and τ is the time constant. In our study, electrochemical impedance study was conducted for different electrodes and the characteristic parameters measured.

The Nyquist plots of NiR/MWCNT/SPE biosensor were obtained for the detection of nitrate in the range 0.05 mg/L to 0.5 mg/L (Fig. 4). From these plots linearity curve was plotted between measured R_{ct} and concentration. From the slope of the curve the regression equation was obtained for calculating the unknown concentrations in the analyte (R_{ct}^{-1} (Ω^{-1}) =9.01 concentration (mg/L) +0.0022) (Fig. 5). Then to calculate the time constant, the relation among the frequency for maximum impedance (f_m), time constant (τ), electron transfer resistance (R_{ct}) and capacitance (C_{dl}) of the interfacial double layer as used is given by equation (Charbaji *et al.,* 2021),

$$R_{ct}C_{dl} = \frac{1}{2\pi f_m} = \tau \quad (3)$$

Limit of detection (LOD) depending upon standard deviation of calibration curve (σ) and sensitivity of biosensor (S) was calculated using formula(Ahmad *et al.* 2017),

$$Detection\ limit = \frac{3\sigma}{S} \quad (4)$$

Results and Discussion

Multiwalled carbon nanotube (MWCNT) shows a zeta potential of -28.4±4.5 mV due to presence of abundant number of functional groups such as OH, C=O, and COOH (Fig. 1). The zeta potential values indicate the high stability of the dispersions. Dispersions with a low zeta potential get agglomerate under the action of Van der Waals forces (Asiri *et al.* 2020). The contact angle values were found to be decreasing as surface roughness increased. The corresponding values for immobilized biosensor were found to be lower than material electrodes (Fig. 2). The low values of contact angle for the NiR/MWCNT/SPE biosensor were accredited to enhanced surface energy of the films due to increased surface area induced by roughness (Alahi *et al.* 2018).The charge transfer resistance (R_{ct}) value obtained for SPE, MWMWCNT/SPE and NiR/MWMWCNT/SPE electrodes were 356.2 Ω, 320.9 Ω and 235.8 Ω, respectively. The decrease in charge transfer resistance after immobilization of enzyme onto MWCNT/SPE to 235.8 Ω highlighted successful modification of MWCNT/SPE surface

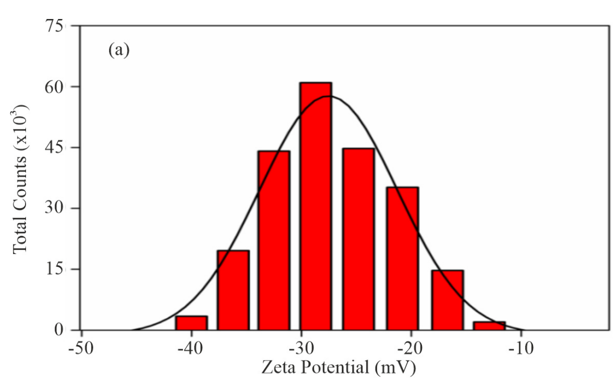

Fig. 1. Surface charge variation on nanotubes

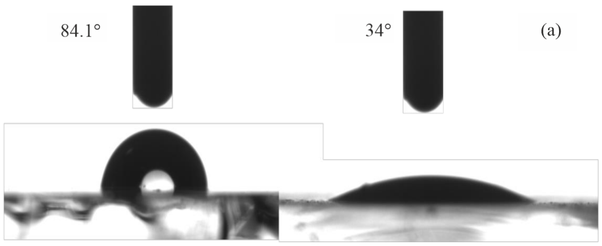

Fig. 2. Contact angle measurements for (a) MWCNT/SPE and NiR/MWCNT/SPE

with nitrate reductase enzyme. The decrease in the value of electrochemical impedance may be possibly due to orientation of NiR enzyme with the MWMWCNT structure resulting in enhanced electron conduction towards the sensing electrode. After the incorporation of BSA molecules, the R_{ct} value increased to 308.2 Ω (Fig. 3). The possible cause of this falling impedance might be perhaps the steric hindrance of bulk generated in redox reaction (Kundu *et al.,* 2017; Kundu *et al.,* 2019). Heterogeneous electron transfer rate constant (K_e) for SPE, MWCNT/SPE, NiR/MWCNT/SPE electrodes were 1.03×10^{-6} cm/s, 2.21×10^{-6} cm/s, 2.44×10^{-6} cm/s, respectively. Highest electron transfer rate constant for NiR immobilized electrode indicates that the complex structure formation through binding of enzyme onto nanocomposite coated SPE promotes faster electron transport via reduction of resistive hindrance (Kundu *et al.,* 2019).The time constant for SPE, MWCNT/SPE, and NiR/MWCNT/SPE electrodes has been found to be 0.001 s, 0.016 s, 0.007 respectively. The time constant

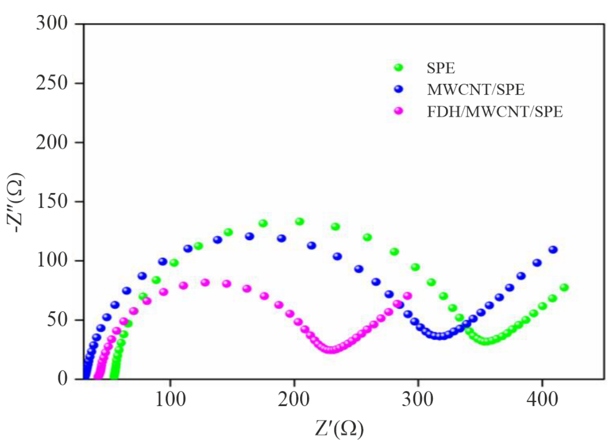

Fig. 3. Nyquist plots for the SPE, MWCNT/SPE and NiR/MWCNT/SPE

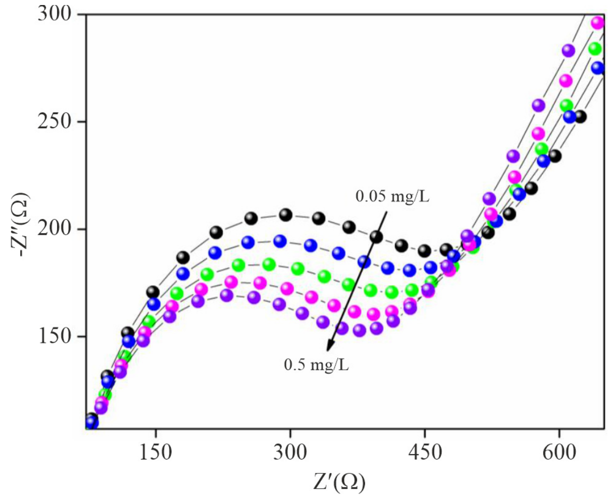

Fig. 4. Nyquist plot for the MWCNT biosensor with varying analyte concentration

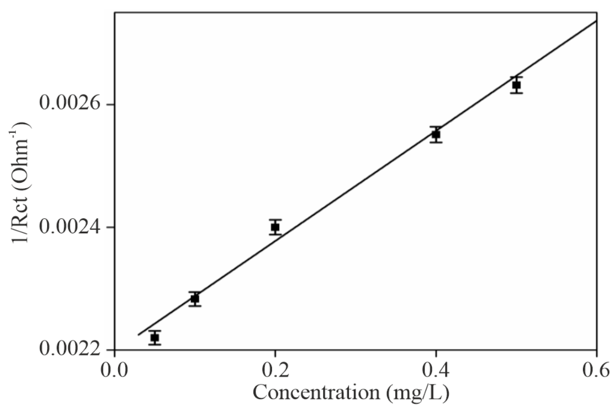

Fig. 5. Calibration plot for the organic nanotubes-based bio sensor for nitrate detection (R_{ct}^{-1} (Ω^{-1}) =9.01 concentration (mg/L) +0.0022)

for MWCNT/SPE was found higher than others owing to sluggish movement of $[Fe(CN)_6]^{3-/4-}$ ions through the interface but again a decrease of time constant was observed when enzyme was immobilized. It was found that NiR/MWCNT/SPE bio sensor exhibited high sensitivity of 9.01×10^{-4} Ω^{-1} $(mg/L)^{-1}$ within detection range of 0.05-0.50 mg/L. The LOD was found to be 0.02 mg/L for detection of nitrate. The obtained high sensitivity and lower detection limit might be probably attributed due to large surface area of MWCNT and presence of large number of active functional groups on electrode surface which consequently leads to high loading of enzyme (Ali *et al.,* 2017).

Conclusion

The organic nanotubes based electrochemical impedance biosensor was successfully developed for the detection of nitrate. The impedance spectroscopy based biosensing techniques allow study of binding events, electro catalytic mechanisms and electrode/ solution interfacial layer. The present study paved way for the exploration of novel nanostructures and immobilization methods for the improvement in the sensitivity, stability and limit of detection of the present biosensors. The impedance biosensor has huge scope for application in the development of portable devices for nitrate detection in water, soil and food. It will be highly popular among the end users (consumers, farmers, researchers, industries etc.) for future applications in agriculture.

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