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Detection of Zn Water Pollution by a Biosensor Based on Alkaloids Derived from Iraqi Catharanthus Roseus

Marwah A. Al-Azzawi[*1,2], Wasan R. Saleh[2]
[1]*Department of Physics, College of Science, Mustansiryah University, Baghdad, Iraq*
[2]*Department of Physics, College of Science, University of Baghdad, Baghdad, Iraq*

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Abstract

In this work, the detection of zinc (Zn) ions that cause water pollution is studied using the CSNPs- Linker-alkaloids compound that was prepared by linking extracted alkaloids from Iraqi Catharanthus roseus plant with Chitosan nanoparticles (CSNPs) using maleic anhydride. This compound is characterized by an X-ray diffractometer (XRD) which shows that it has an orthorhombic structure with crystallite size in the nano dimension. Zeta Potential results show that the CSNPs-Linker-alkaloids carried a positive charge of 54.4 mV, which means it possesses high stability. The Fourier transform infrared spectroscopy (FTIR) shows a new distinct band at 1708.93 cm^{-1} due to C=O esterification. Scanning electron microscope (SEM) images show that the CSNPs- Linker- alkaloids compound have two shapes in the nano dimension: spherical particles and nanotubes, which may be due to nuclei and growth processes, respectively. The energy gap calculated from the photoluminescence spectrum is equal to 2.5 eV. The Hall effect measurements prove that the synthesized CSNPs-Linker-alkaloids compound is a p-type semiconductor. The cycle voltammetry technique was used to detect the Zn ions in different concentrations in the water by modifying the electrochemical system's glassy carbon electrode (GCE) with a CSNPs-Linker-alkaloids compound. The modified electrode was used to detect Zn ions in the range of (1-8) ppm, which causes water pollution. The best sensor sensitivity R^2 equals 0.997 for oxidation and 0.993 for reduction. This modified electrode (GCE /CSNPs- Linker-alkaloids) acts as a good biosensor for heavy metals detection in water as well as for biophysics applications.

Keywords: *Catharanthus roseus, biosensor, CSNPs-Linker-alkaloids compound, cyclic voltammetry, Zinc ions*

الكشف عن تلوث المياه بالزنك بواسطة جهاز الاستشعار الحيوي المستند على قلويدات مشتقة من عين البزون العراقي

مروه عبدالرحمن داود[*1،2] ، وسن رشيد صالح[1]
قسم الفيزياء ، كلية العلوم ، جامعة بغداد ، بغداد ، العراق[1]
قسم الفيزياء ، كلية العلوم ، جامعة المستنصرية ، بغداد ، العراق[2]

*Email: marwa.dawood1104@sc.uobaghdad.edu.iq

الخلاصة:

في هذا العمل ، تمت دراسة الكشف عن أيونات الزنك (Zn) المسببة لتلوث المياه باستخدام مركب CSNPs Linker–alkaloids الناتج عن ربط القلويات المستخرجة من نبات عين البزون العراقي مع جزيئات Chitosan النانوية CSNPs بواسطة أنهيدريد المالئيك للحصول على المركب (CSNPs– Linker– alkaloids تبين ان المركب يمتلك هيكل معيني و بحجم بلوري في البعد النانوي من خلال التشخيص بحيود الاشعة السينية. أظهرت نتائج Zeta أن CSNPs– Linker–alkaloids تحمل شحنة موجبة 54.4 mV ، مما يعني أنها تتمتع باستقرارية عاليه. مطيافية فوريه لتحويل الأشعة تحت الحمراء (FTIR) ، تظهر حزمة مميزة جديدة عند 1708.93 سم$^{-1}$ بسبب الاصره الأسترية C = O. تظهر صور المجهر الإلكتروني الماسح (SEM) أن مركب CSNPs– Linker– alkaloids له شكلين بأبعاد نانوية ؛ الجسيمات الكروية والأنابيب النانوية تكونت بسبب النوى وعمليات النمو على التوالي. فجوة الطاقة المحسوبة من طيف التلألؤ الضوئي تساوي 2.5 فولت. فحص تاثير هول اثبت ان مركب CSNPs– Linker– alkaloids المحضر هو شبه موصل من نوع p–type . للكشف عن أيونات الزنك بتراكيز مختلفة في الماء تم باستخدام قطب كربون زجاجي معدل (GCE) باستخدام تقنية قياس الجهد الدوراني مع مركبCSNPs– Linker– alkaloids . تم استخدام القطب المعدل لاكتشاف أيونات الزنك Zn الملوثه للماء في حدود (1–8) جزء في المليون. أفضل حساسية R^2 تساوي 0.997 للأكسدة و 0.993 للاختزال. يعمل هذا القطب المعدل (GCE / CSNPs– Linker–alkaloids) كمستشعر حيوي جيد للكشف عن المعادن الثقيلة في المياه وكذلك لتطبيقات الفيزياء الاحيائية.

1. Introduction

Plants are integral to ecosystems. They inhabit and contribute to enriching their environment. Plants improve their habitat by constantly filtering the air, water, and soil they reside in. Phytoremediation removes pollutants by either containing, degrading, or eliminating contaminates such as solvents, pesticides, metals, crude oil, and their derivatives [1].

Catharanthus roseus plant of the Apocynaceae family is one of the most widely explored plants because of its ornamental values and medical properties. The plant has been popularly exploited because of its reservoir for more than 200 alkaloids [2,3].

Nanotechnology has opened up new horizons for the application of nanoparticles and nanostructure in biosensors and bioassays. Nanoparticles with different compositions and dimensions have been widely used in recent years as versatile and sensitive tracers for the electronic, optical, and microgravimetric transduction of different biomolecular recognition events [4].

Water pollution is a problem that threatens the lives of humans and animals alike. The pollution of the ecosystem by toxic heavy metals and organic pollutants is constantly increasing because of human activities [8]. In recent years, the potentially harmful contaminants in the environment have increased tremendously. So, there was a need for more rapid and more efficient methods to monitor the environment continuously, hence the need for monitoring systems such as biosensors. Biosensors can be useful for determining the type and concentration of contaminants in an environment. The primary measurement media for environmental monitoring is water, soil، and air, but there are a variety of other target analytes. An electrochemical biosensor is one type of biosensor that can analyze a biological sample's content by converting the biological event into an electrical signal. They are classified into amperometric, potentiometric, conductometric, and impedimetric [5]. In the amperometric method, the current is measured at a constant potential which shows a logarithmic response. The Cyclic Voltammetry (CV) method is a subclass of the

amperometric method, where the applied potential of the electrode changes when the current is measured. In a suitable potential, the analyte is either oxidized or reduced [6]. Cyclic voltammetry is an extremely sensitive electrochemical technique used to measure the amount of pharmaceutically active compounds in biological samples using glassy carbon or other modified electrodes. Electrochemistry has always provided analytical techniques with instrumental simplicity, moderate cost, and portability.

Heavy metals indicate any metallic element with a high density and is toxic even at low concentrations. Heavy metals are elements that have a density higher than 5 g/cm^3 and are found in very low concentrations in living systems and are highly stable [9]. Metals in the aquatic ecosystem can stay in solution or suspension and deposit on the bottom or be taken by organisms. Zinc (Zn) is one of the main heavy elements in the world; it naturally occurs in the earth's crust and is found, in air, soil, water, and is present in most foods. It is the most important pollutant in surface and groundwater. Because of its acute toxicity and non-biodegradability, zinc-containing liquid and solid wastes are considered hazardous. Ingesting elevated levels of Zn may cause anaemia, damage to the pancreas, and decreased levels of high-density lipoprotein cholesterol [10].

In this work, cycle voltammetry was used to detect Zn ions of different concentrations in water using modified glassy carbon electrodes (GCE) with CSNPs-Linker-alkaloids compound. This compound was prepared by linking extracted alkaloids from Iraqi Catharanthus roseus plant with Chitosan nanoparticles CSNPs by maleic anhydride as a linker. The modified GCE was used as a biosensor for detecting traces of heavy metals such as Zn ions, which cause environmental pollution and affect human health.

2. Experimental work

The chemical materials used in this work were: Chitosan powder (CS) from (Weifang Dongxing Shell Products Factory, Korea); sodium triPolyPhosphate (STPP) with chemical formula ($Na_5P_3O_{10}$) from Andhra Pradesh, India; tetrahydrofuran (THF) of purity 99%; potassium chloride (KCl) of purity 99%; a buffer of sodium acetate (CH_3COONa) of purity 99%; dibasic sodium phosphate (dihydrate) ($Na_2HPO_4 \cdot 2H_2O$) of purity 99%. All these materials supplied from Sigma- Aldrich. Zinc (Zn) stock solution with a concentration of (1000 ppm) supplied from Horiba, France.

Chitosan powder (CS) was used to synthesize Chitosan nanoparticles (CSNPs) in the presence of sodium tripolyphosphate (STPP) compound as a correlation factor. The technique used to produce the nanoparticles is the ionotropic gelation method. A weight of 3 gm of the prepared Chitosan nanoparticles (CSNPs) was dissolved in 10 ml of tetrahydrofuran (THF), and subjected to ultrasonic in a cold-water bath for 5 min. using the sonicated probe. The required concentration of the alkaloid extract from the Catharanthus roseus plant was prepared by melting 1g of the extract in a volume of (THF), then sonicated by a sonicated probe for 5 min. to reach the alkaloid extract to nanoscale size. The nano-sized alkaloid extract was added by distillation to the Chitosan nanoparticles solution with the presence of maleic anhydride, as a connector between them, with magnetic stirring for two hours at a temperature of 45 ºC to obtain the (CSNPs-Linker- alkaloids) compound [11].

The glassy carbon electrode (GCE) was polished with 0.05 μm alumina powder using emery papers, rinsed with deionized water، and then cleaned with acetone using an ultrasonic probe for 5 min. 50 μl of the CSNPs- Linker- alkaloids compound was drop cast on the GCE surface and dried at an ambient temperature before being used for measurements. CSNPs-Linker- alkaloids biosensor was investigated by examining their CV peaks current using KCl

of 0.1M and pH=5 as an electrolyte solution, Citrate-Phosphate of volume 1000µl as a buffer, and scan rate equal to 0.5 Vs^{-1}.

X-ray diffractometer (ADX2700 Angstrom model XRD-6000, USA) 40 kV, 30 mA, a wavelength of 1.54 Å was used to study the structural properties. Zeta potential Horiba scientific SZ-100 (Japanese) was used to determine the materials stability. Fourier transform infrared spectrum (using Shimadzu 21 FT-IR spectrometer), and the photoluminescence (PL) spectrum (SL174- spectrophotometer) were used to characterize the morphological and optical properties, respectively. A scanning electron microscope (SEM) (Jsm-7610 F) was used to study the morphological properties. Effect Hall measurements were done with the Van der Pauw Ectopia HMS-3000 Hall measurement system.

3. Results and Discussion

Physical characterization

The structural properties of the CSNPs-Linker-alkaloids compound were studied using an X-ray diffractometer. The XRD pattern of the sample is shown in Figure 1. The characteristic peak of the compound was seen at a diffraction angle 2θ =22.3° corresponding to the (101) plane, which agrees with the results of Antonino et al. [12]; it has an orthorhombic structure which agrees with Okuyama et al. [13]. Thus, the CSNPs-Linker-alkaloids compound chains are arranged in the form of parallel planes giving the orthorhombic form [14].

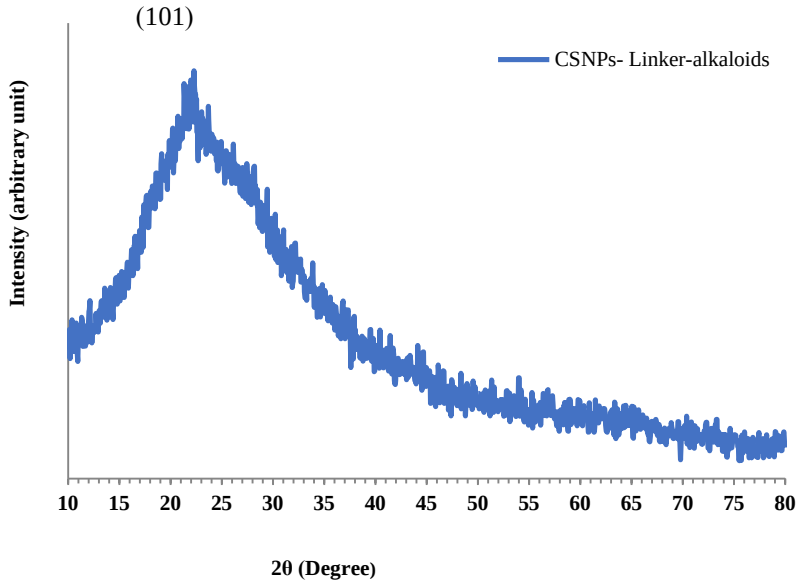

Figure 1: XRD pattern of the CSNPs- Linker- alkaloids compound.

Scherrer's relation [15, 16] was used to calculate the crystallite size (CS) of the synthesized CSNPs- Linker- alkaloids compound:

$$C.S = \frac{K\prime\,\lambda}{\beta\prime\,COS\theta} \quad (1)$$

Where: β′ is the Full Width at Half Maximum, K' is a constant with a value of 0.9, λ is the wavelength which is equal to 1.54 Å and θ is the Bragg's angle. The crystal size calculated using Scherrer's relation was about 1.2 nm.

The Zeta potential of the CSNPs-linker-alkaloids compound was measured at room temperature. Figure 2 represents the zeta potential of CSNPs-Linker- alkaloids compound. From this, it was deduced that the CSNPs- Linker-alkaloids carry a positive charge of 54.4

mV, which means it has high stability. This generated surface charge is essential in maintaining colloidal stability in its natural form without changing. This colloidal is considered more stable with a high value of zeta potential, where with a high positive or negative charge value, the possibility of aggregation is reduced [8].

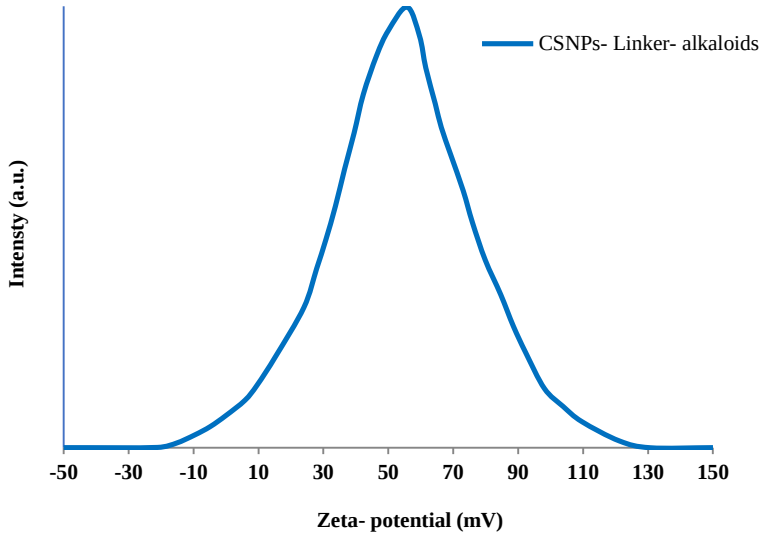

Figure 2: Zeta Potential of CSNPs- Linker- alkaloids compound.

One of the very useful tools for analyzing the optical and structural properties of the CSNPs-Linker-alkaloids compound is the Fourier transform infrared spectrum, which was used to confirm and assess the CSNPs- Linker- alkaloids compound.

Figure 3 shows the spectrum of the CSNPs-Linker-alkaloids compound. The spectrum shows a wide band at 3344.56 cm^{-1}, which is attributed to Chitosan O-H [19], as well as an unreacted or unreactive O-H in Vintafolide which is one of the alkaloid compounds added as it contains more than one O-H group, so the band appeared somewhat wide as this compound contains more than five non-reactive OH compounds. The spectrum has a C-H (aromatic) bond at 3150 cm^{-1}; a band at 2904.79 cm^{-1} and 2855.04 cm^{-1} corresponding to C-H symmetric and asymmetric stretching vibration stretch (C-H), respectively. Also, the spectrum shows a new distinct band at 1708.9cm^{-1}, which may be due to C=O esterification. The bands at 1627.92 cm^{-1} and 1527.26 cm^{-1} are attributed to the NH group, while the band at 1459.04 cm^{-1} is attributed to CH (wagging for the CH = CH group). The band at 1384.89 cm^{-1} is attributed to the P=O overlap with the C-O vibration stretch. The bands at 1087.85 cm^{-1}, 1018.41 cm^{-1}, and 875.68 cm^{-1} are due to the presence of C-O stretching, P-O, or PO_4, and C-H bending (wagging), respectively.

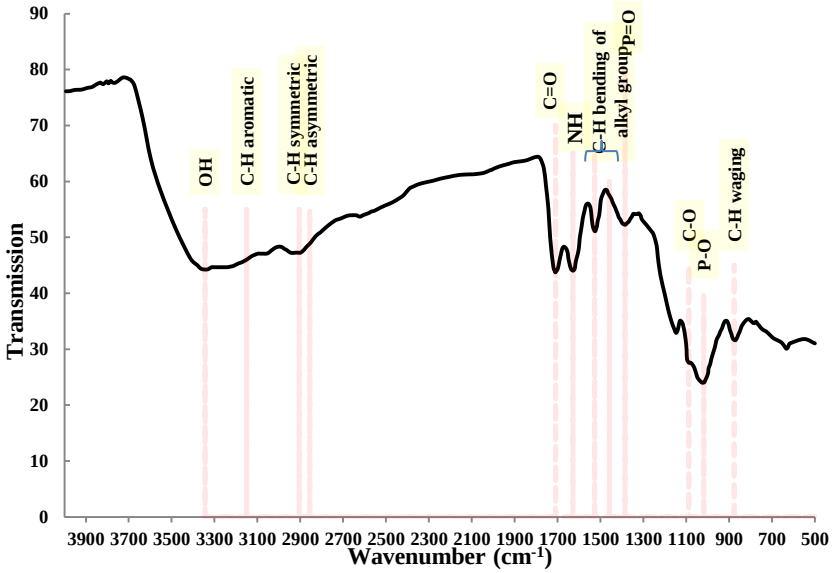

Figure 3: FTIR spectrum of the CSNPs-Linker-alkaloids compound.

Scanning electron microscope (SEM) was used to study the morphology of the CSNPs-Linker-alkaloids compound. Typical example of SEM image is shown in Figure 4.

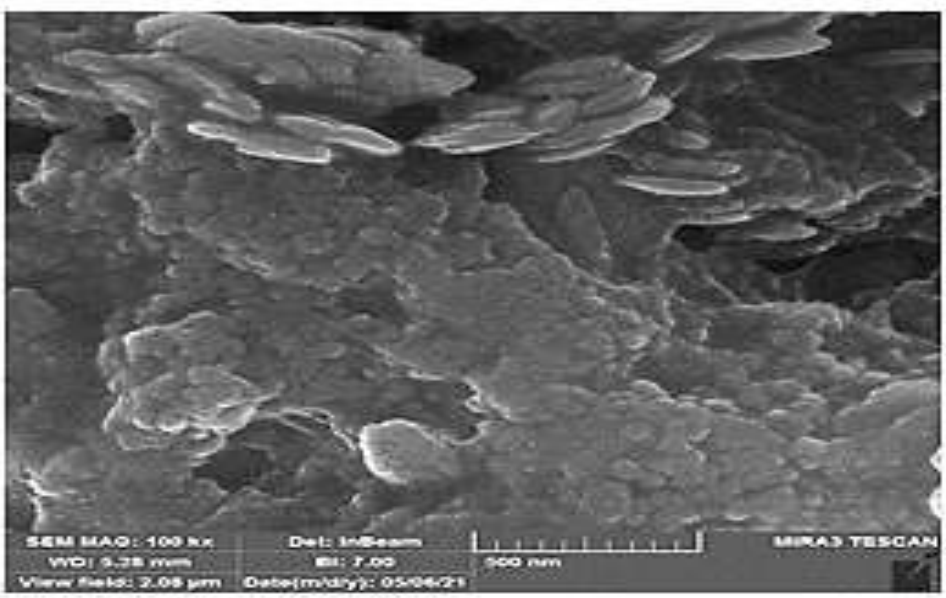

Figure 4: SEM image of CSNPs-Linker-alkaloids compound at magnifications of 100kx.

The image shows that the compound has two shapes of nanostructures: spherical and nanorods due to nuclei and growth processes. The spherical nanoparticles are gathered in the shape of cauliflower as a result of the surface energy, which led to an increase in the surface adsorption of metal ions in water, while the nanorods were formed as a result of surface tension. These nanostructures are regular and have dimensions with a diameter range of (13-44) nm.

The photoluminescence (PL) spectra of the CSNPs- Linker- alkaloids compound was examined at room temperature using a PL spectrofluorometer covering a range within (200-800) nm. Photoluminescence (PL) studies provide information on different energy states between valence and conduction bands responsible for irradiative recombination. Figure 5

shows the PL spectrum for the CSNPs-Linker-alkaloids compounds, which has a peak at 496 nm at excitation wavelengths of 400 nm according to the absorption peak reported by Al-Azzawi et al. [11].

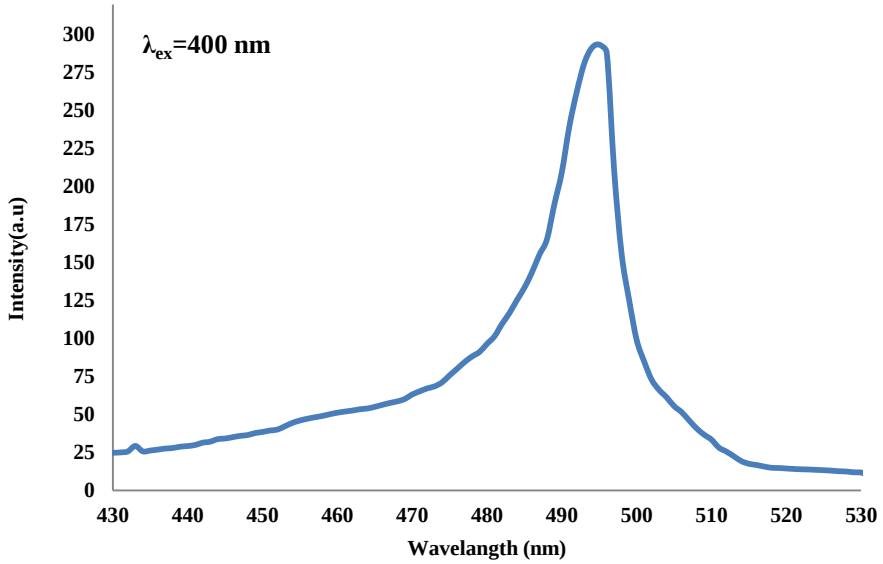

Figure 5: Photoluminescence spectra CSNPs- Linker- alkaloids compound.

The optical energy gap (Eg) value was calculated using Equation (2) [20], where λ_{peak} represents the wavelength peak of the compound.

$$Eg = \frac{1240}{\lambda\ peak\ (nm)} \quad (2)$$

It is necessary to determine whether the CSNPs- Linker- alkaloids compound is an n-type or p-type semiconductor. Table 1 shows the parameters deduced from Hall Effect measurements for CSNPs- Linker- alkaloids compound.

Table 1: Hall effect parameters for CSNPs- Linker- alkaloids compound.

n $(cm)^{-3}$	R_H $(cm^{-3}\ C^{-1})$	σ $(\Omega.cm)^{-1}$	μ $(cm^2V^{-1}\ s^{-1})$
$6.44x10^{14}$	$9.69x10^{3}$	$1.88x10^{-1}$	$1.82x10^{3}$

where n is the bulk concentration, R_H is the Hall coefficient, σ is the conductivity, and μ is the mobility.

According to Equation 2 and Hall Effect measurements, the optical energy gap was equal to 2.5 eV, so that the synthesized compound is a p- type semiconductor.

Detection of Heavy Metals (Zn) by electrochemical characterization

One of the most severe environmental issues is heavy metals contamination. Industrial waste can damage the nervous system of human beings because it contains Zinc ions (Zn).

To detect water heavy metals, such as Zn ions, pollution, Zn ions were prepared from the maximum concentration of the stock solution (1000 ppm). The necessary dilution was carried out with distilled water based on the chemical formula $C_1V_1=C_2V_2$ where C_1 and C_2 represent the concentration of a stock solution (ppm) and final concentration (ppm), respectively, while

V_1 and V_2 represent the volume obtained from the stock solution (ml) and the final volume (ml), respectively [21]. Concentrations of (1-8) ppm were obtained by diluting the KCl electrolyte solution of pH=5. An electrochemical system (IRASOl PGS-10) with potentiostats driven by electroanalytical measuring software was connected to a computer to characterize the cyclic voltammogram (CV) of the CSNPs- Linker- alkaloids compound. All measurements were carried out in a three-cell containing a platinum electrode as the electrode (CE), Ag/AgCl reference electrode (RE), and Glassy Carbon Electrode (GCE) as the working electrode (WE) [22]. The electrochemical analysis method was used for the detection of Zn ions at low concentrations (1-4) ppm and high concentrations (6-8) ppm as well as 5 ppm, for both types of modified electrode extract plate and CSNPs- Linker- alkaloids compound.
Figure 6 shows the cyclic voltammogram (CV) of the extracted plant and CSNPs-Linker-alkaloids compound using the modified glassy carbon electrodes, which were used to detect Zn ions at a concentration of 5 ppm, (which the maximum allowed concentration of heavy metals in rivers and water and is the recommended value for the system of maintaining rivers from pollution according to Iraqi specifications no. (25 for 1967)).

The CV curves show an increase in the oxidation-reduction current peaks for the modified electrode (GCE/CSNPs-Linker-alkaloid).

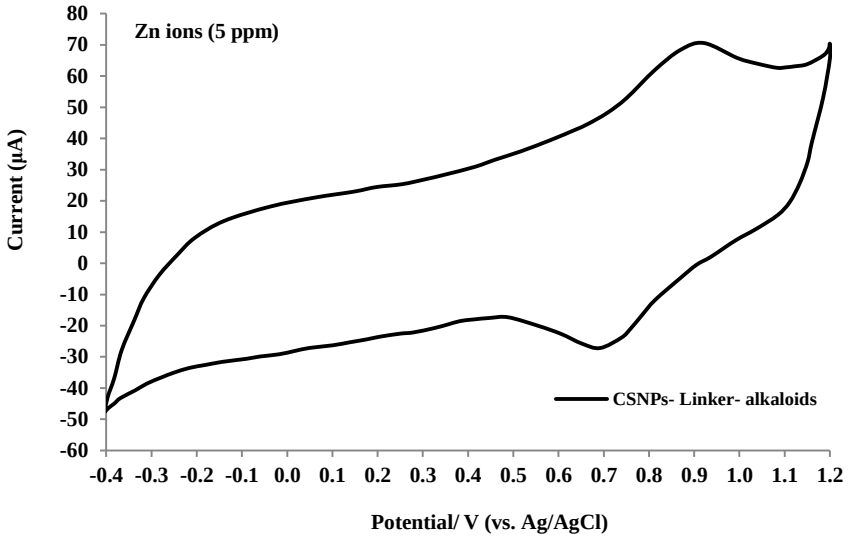

Figure 6: Cyclic voltammogram of the max. allowed concentrations of Zn ions (5 ppm) for modified electrodes GCE/CSNPs- Linker- alkaloids against reference electrode (Ag/AgCl) with KCl electrolytes of pH=5 and scan rates 0.5 Vs^{-1}.

Figures 7 and 8 show the cyclic voltammograms of the GCE /CSNPs-Linker-alkaloids modified electrodes of low and high Zn ions concentrations. These figures revealed that at a concentration of 1 ppm, there is no current (the sensor does not respond). In comparison, at the range of (2-8) ppm, the oxidation and reduction current peaks increase with the increase of the Zn ions concentration.

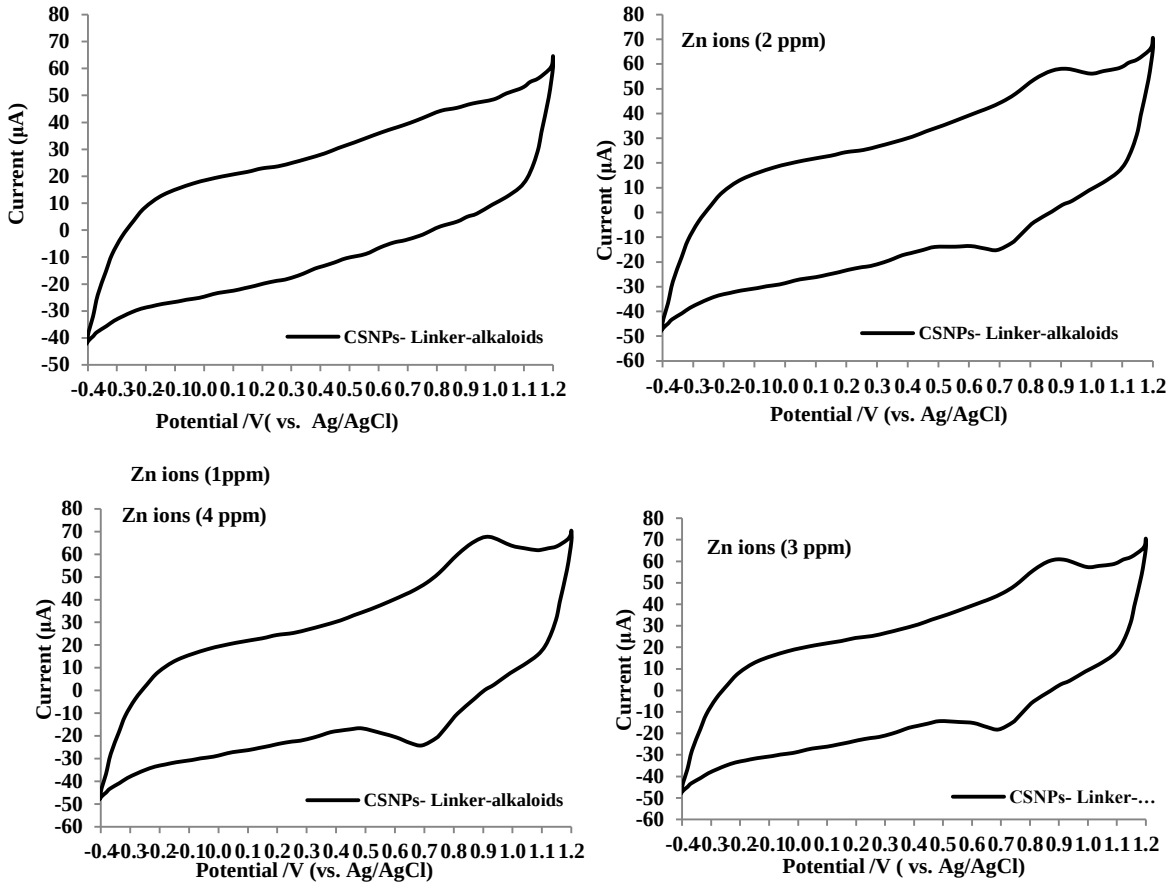

Figure 7: Cyclic voltammogram of modified electrodes GCE/ CSNPs- Linker- alkaloids at low concentrations of Zn ions (1-4) ppm against to reference electrode (Ag/AgCl) with KCl electrolytes of pH=5 and scan rates 0.5 Vs^{-1}.

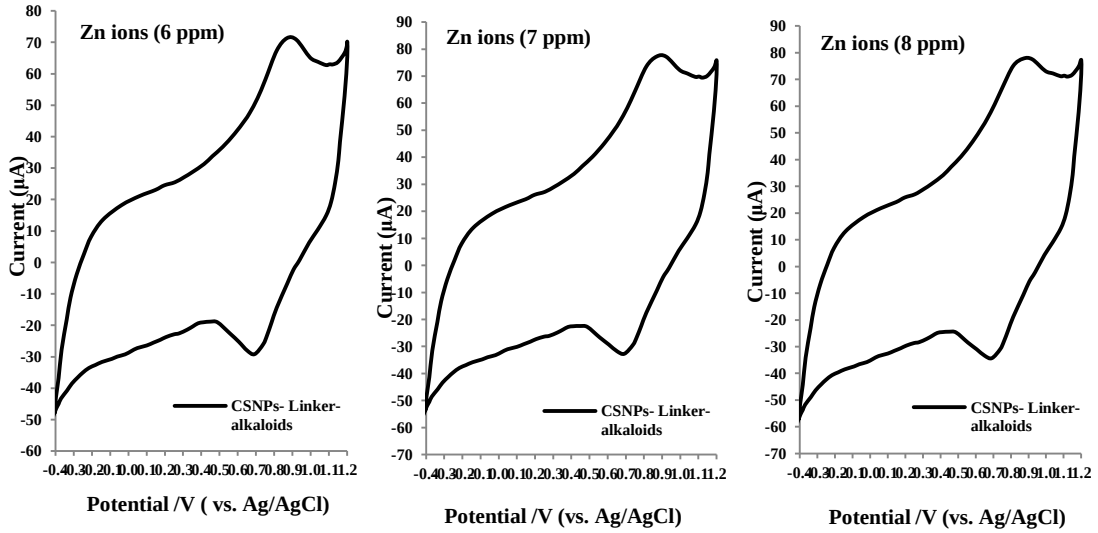

Figure 8: Cyclic voltammogram of modified electrodes GCE/ CSNPs- Linker- alkaloids at high concentrations of Zn ions (6-8) ppm against reference electrode (Ag/AgCl) with KCl electrolytes of pH=5 and scan
rates 0.5 Vs^{-1}.

The calibration graphs shown in Figure 9 display the relation between the oxidation-reducing current peaks resulting from CV curves in Figures (5-7) with the compound concentrations for (1-8) ppm concentration of Zn metal ions.

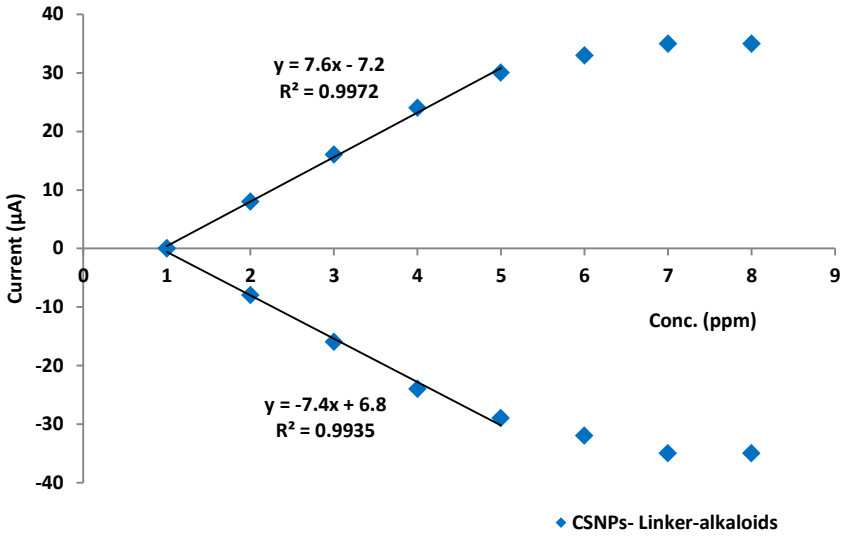

Figure 9: The relationship of steady-state current vs. different concentrations of Zn ions.

The calibration curve in Figure 9 shows that up to 1 ppm, there is no response. The sensitivity R^2 of 0.997 is high for oxidation and 0.993 for reduction. Thus, the sensor is highly sensitive at low and high concentrations of pollutants. At concentrations of (2-5) ppm, the relationship is linear, while at concentrations of (6-8) ppm, the relation is non-linear; this may be because the current has reached its saturation level. Accordingly, the biosensor sensitivity range is (2-5) ppm.

5. Conclusions

Alkaloids were successfully extracted from Iraqi Catharanthus roseus plant and converted into a nanocomposite using nano-chitosan linker as well as maleic anhydride linker to obtain the final product (CSNPs-Linker-alkaloids) compound. An electrochemical biosensor was fabricated by modifying the glassy carbon electrode (GCE) with CSNPs-Linker-alkaloids compound resulting from linking the extracted alkaloids from Iraqi Catharanthus roseus plant for the quantitative determination of water pollution with zinc metal ions. Satisfactory results were obtained. XRD results proved that the synthesized CSNPs-Linker-alkaloids compound has an orthorhombic structure with crystal size in the nano range. The zeta potential of the CSNPs-Linker-alkaloids compound was 54.4 mV which means that the compound has long-term stability. FTIR spectrum for CSNPs- Linker- alkaloids showed a new distinct band at 1708.93 cm^{-1}, which is attributed to C=O esterification, which is an evidence of the success of the synthesis process. The SEM image proves that the CSNPs-Linker- alkaloids compound was formed in two nano dimensions shapes: spherical particles and nanotubes due to nuclei and growth processes. The Hall effect measurements proved that the synthesized CSNPs-Linker-alkaloids compound is a p-type semiconductor. The fabricated biosensors proved to be efficient in the sensitivity range (2-5) ppm for the detection of zinc ions in polluted water.

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