Sonoochemically Prepared GdWNFs/CNFs Nanocomposite as an Electrode Material for the Electrochemical Detection of Antibiotic Drug in Water Bodies

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Abstract

The work demonstrates the development of an electrochemical sensor for quantification of Chloramphenicol (CA) using pencil graphite electrode (PGE) modified with Gadolinium tungstate nano flakes and carbon nano fibers composite (PGE/GWNfs/CNFs). The composite was further characterized and confirmed by X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy, transmission electron microscopy analysis. The prepared GWNfs/CNFs nano composite was fabricated by drop casting method to get PGE/GWNfs/CNFs working electrode. The modified electrode is then analyzed by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) methods for its electrochemical and electrocatalytic property. The electrochemical investigation of developed sensor shows enhanced activity towards electro-oxidation of CA. The DPV studies revealed high efficacy characteristics such as sensitivity in the range 0.03984 µA µM⁻¹ cm⁻², selectivity, good linear range (5–50 µM), and low detection limit (0.4 µM). The study benchmarks the use of GWNfs/CNFs as an excellent transducer material in electrochemical sensing of CA in standard samples thus, it finds an efficient potential application in the analysis of CA in environment sample analysis.

Keywords Chloramphenicol · Gadolinium tungstate nano flakes · Carbon nanofiber · Pencil graphite electrode · Electrochemical sensor

1 Introduction

CA [D (-)-threo-2-dichloroacetamido-1-pnitro-phenyl-1, 3-propanediol is a miasmic active potent broad-spectrum antibiotic which responds gram-positive and gram-negative bacteria [1]. In recent reports, CA is under a high contradiction and are prohibited in many countries like in Europe, the United States, and Canada due to its extremely harmful side effects [2, 3]. At the early discovery of this antibiotic drug was appreciated and used as a remedy for infectious diseases like plague, cholera, typhoid fever, and meningitis [4]. On the post medication survey and research efforts the reliability of the antibiotic was questioned due to its carcinogenicity and aftereffects on human health causing reversible bone marrow suppression, aplastic anemia, enterocolitis and gray baby syndrome [5]. However, the advantages like low economic value and easy accessibility have made the drug more live-in production and consumption [6]. Apart from the synthetic preparation of the CA and dosing in treatment, the CA was also present in the animal feed stock and soil
naturally. An appreciable amount of CA production in soil was initiated by soil bacteria (*Streptomyces venezuelae*) and the cultivated feedstock on this soil have reported with the CA augmentation [7].

Recent advancements in quantification of such analytes with a high sensitivity and selectivity were achieved by the conventional analytical techniques like liquid chromatography (HPLC) [8], gas chromatography-mass spectrometry (GC-MS) [9], capillary electrophoresis [10], antibody-based techniques [11].

The above technique is complex mode of analysis which requires a new method development, time consuming and are incompetent for real time processing. Also, their low limit of detection range is very less, all this snag hinders the operational efficiency in CA sensing. Therefore, owing to the less economic and eco-friendly approach will be highly expected in the current research, this led to the development of electrochemical sensor and its examination. Electrochemical techniques, on the other hand, are the most advantageous because of their simplicity, economy, high sensitivity, easy operation, limited pretreatment, and low solvent need. In other words, electrochemical sensors produce accurate results that are equivalent to those produced by traditional sensors [12].

The low toxicity and excellent ferroelectric properties of the GWNfs used were also highly desired precedence in the current research [13]. Furthermore, the combination of Gd with tetrahedral geometry bearing WO_4 have been widely investigated for excellent photochemical properties and stability. Moreover, their low photon energy, and high quantum yield makes it a potent candidate for diverse applications [14]. The two-dimensional (2D) flake-like nanostructured substrate incorporated have provided acceptable electrical conductivities, inflated active surface areas, and excellent charge transportation [15].

The CNFs have provided a base strength and flexibility and they form the outline layer for the composite. Their unique stacking and active binding sites have provided a stacked carbon nanofibers which forms an outline and helps in rigidity. This sheet gives more active binding sites when compared to other carbon-based materials [16].

The present work demonstrates a CA sensor based on GWNfs/CNFs nanocomposite modified PGE. The objective of the work is to make use of abundantly and easily accessible pencil graphite as a working electrode. Further, incorporation of the GWNfs/CNFs proved to amplify catalytic activity towards CA sensing. The proposed sensor demonstrated wide linear detection range and low limit of detection. The sensitivity and the selectivity of the sensor was proved to be efficient. It also asserted the high accuracy, good shelf-life, and reproducibility of results which shows the efficient potency of the nanocomposite (GWNfs/CNFs) material. The practical applicability of the developed sensor is also established through the analysis of CA in different water samples with acceptable recovery results. Finally, critical comparison with earlier sensors, highlighted the superior performance of PGE/GWNfs/CNFs.

2 Experimental

2.1 Chemicals and Apparatus

The CA, Gadolinium (III) nitrate [$\text{Gd} (\text{NO}_3)_3$] and CNFs was acquired from Sigma-Aldrich, Sodium tungstate dihydrate (Na_2WO_4·H_2O), urea, disodium hydrogen phosphate (Na_2HPO_4), monosodium dihydrogen phosphate (NaH_2PO_4), and Potassium chloride (KCl) were acquired from S D fine chemicals India.

Biologic Science potentiostat (model SP-150) with the three-electrode configuration instrument was for the voltmetric studies (CV, EIS, and DPV). The morphological and surface ratio aspects of the nanocomposite are investigated using Talos F200 S transmission electron microscope. The synthesized GWNfs is characterized by a powder X-ray diffractometer (BRUKER D8) to analyze the parameters such as crystallinity, lattice planes etc.

2.2 Synthesis of GWNfs and Preparation of GWNfs/CNFs Composite

GWNfs were achieved by simple sonication method. The starting materials namely, Gd (NO_3)_3 (0.1 M) and Na_2WO_4 (0.2 M) were dissolved in 200 mL of DD water with the constant and uniform agitation. Further 0.5 M (0.6 g in 20 mL) urea was added for possible morphological development. The above is stirred for 1 h at room temperature. The white precipitate obtained was washed with DD water and ethanol to remove impurities and other unreacted materials. The obtained precipitate is dried at 50 °C overnight. Finally, calcinated at a temperature of 800 °C for 3 h in a muffle furnace [17]. Later, GWNfs/CNF composite is prepared. In brief, 5 mg of prepared GWNfs and CNF are added to deionized water. The mixture is then sonication for 20 min to obtain a GWNfs/CNF nano composite.

2.3 Fabrication of Working Electrode

For the proposal of the working electrode, we have selected an efficient and economical PGE as a host along with synthesized GWNfs/CNFs composite. The cylindrical shaped PG rod has a 3 mm diameter. To begin, a tiny section of the rod is cut and polished on one side in preparation for future modification. PGE is polished using PK-3 electrode polishing kit to get a glassy surface. The electrode is sonicated to remove any unconfined particles and further it is rinsed.
dried and the bare PGE is prepared for further modification. The bare PGE is modified by drop-casting 6 µL of GWNfs/CNFs and completely dried at room temperature to get PGE/GWNfs/CNFs working electrode.

2.4 Electrochemical Studies

The electrochemical experiments are performed using a three-electrode configuration system where the modified PGE is the working electrode (active surface area: 7.068 mm²), a saturated calomel as reference electrode, and platinum wire as counter electrode. All the electrochemical properties of bare and modified PGE are characterized using phosphate buffer solution (PBS) of pH 7.0. The active charge transfer at the electrode/electrolyte interface of new modified electrode is studied using the EIS technique in the frequency range of 100 kHz–0.1 Hz with 5 mM [Fe (CN)₆]³⁻/⁴⁻ as the electrochemical probe. All measurements were done at room temperature.

3 Results and Discussion

3.1 SEM, TEM, EDX and XRD Analysis

The surface morphology of prepared GWNfs and GWNfs/CNFs composite was analyzed by SEM and TEM analysis. The microscopic image of the as prepared nanocomposite is documented in Fig. 1A–C. In Fig. 1A it is observed that the synthesized GW was found to be flake like structure. Similarly, in Fig. 1B and C the flakes are overlapped on the fiber like structure, and this can be assigned to carbon nano fibers. Furthermore, the distribution of the elements in GWNfs/CNFs were investigated by EDS and the same is depicted in Fig. 1D. The details of SEM along with the elemental mapping has been given in supplementary data.

The crystalline nature of prepared GWNfs, CNF and GWNfs/CNFs is analyzed using XRD. The XRD pattern is shown in Fig. 2A. As seen in Fig. 2A all the diffraction peaks can be assigned to the pure monoclinic crystal

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**Fig. 1** A TEM analysis of GWNfs, B and C TEM analysis of GWNfs/CNFs composite, and D Shows the elemental composition results obtained using EDX
structure of GW. All the diffraction peaks are in very well consistent with the crystal structure from the JCPDS No-23-1076 [18]. The average crystalline size (D) was found to be 16.46 nm. The CNF exhibits distinct peak at 26.5° is credited to the (002) plane and the results are in correlation with the reported papers in the literature. Similarly, the XRD patterns of GWNfs/CNFs confirms the formation of composite.

### 3.2 Electrochemical Characterization of the Modified PGE by EIS

The EIS study is carried out in 0.1 M PBS (pH 7.0) containing 5 mM $\text{[Fe (CN)}_6\text{]}^{4-}/3-$ at an amplitude of 5 mV and between 100 kHz and 0.1 Hz range. EIS spectrum of PGE and PGE/GWNfs/CNFs is represented in Fig. 2B as a Nyquist plot. EIS data of the modified electrodes are used for the calculation of the charge transfer resistance ($R_{\text{ct}}$) from the best-fit Randle’s electrical equivalent circuit. In addition, $R_{\text{ct}}$ for bare PGE is 1870 kΩ. Interestingly, for GWNfs/CNFs, $R_{\text{ct}}$ decreased to 892 kΩ owing to the high rate of electron transfer. This indicates the promising sensing ability of the as-proposed PGE/GWNfs/CNFs for CA analysis.

To verify the enhanced catalytic response of modified PGE in terms of increased active electrode surface area, Randle’s–Sevcik equation (Eq. 1) is applied [19].

$$I_p = (2.69 \times 10^5)n^{3/2}A_{\text{el}}D_R^{1/2}v^{1/2}C_0$$  \hspace{1cm} (1)

where, $I_p$ is the anodic peak current of PGE/GWNfs/CNFs ($I_p=0.0011$ A), ‘n’ is the number of electrons transferred in the redox reaction of CA (n = 2), ‘$A_{\text{el}}$’ (cm²) is the electroactive surface area to be determined, ‘$D_R$’ (cm²/s) is the solution diffusion coefficient ($6 \times 10^{-6}$ for $\text{[Fe (CN)}_6\text{]}^{4-}/3-$), ‘$C_0$’ (mole/cm³) is the concentration of the reaction species in electrolyte ($10^{-6}$ for $\text{[Fe(CN)}_6\text{]}^{4-}$), and, ‘$v$’ (V/s) is the scan rate [20]. Therefore, the PGE/GWNfs/CNFs possessed 3.60 cm² of active electrode surface area, which shows the increased surface area of the electrode.

### 3.3 Effect of Scan Rate and pH

The modified PGE/GWNfs/CNFs and its effective relationship with scan rate is analyzed using CV. The responsive relationship of achieved PGE/GWNfs/CNFs with different scan rates ranging from 25, 50, 75, 100, 125, up to 250 mV s⁻¹ in the presence of 10 μM CA were analyzed and reported in Fig. 3A. The CV showed a good amplification in peak currents with the gradual increase in scan rate. Furthermore, the peak currents show a good linear relationship with the scan rate and square root of scan rate shown in Fig. 3B and C. The calculated regression coefficients ($R^2$) for peak current responses show a good activation of the surface of the PGE/GWNfs/CNFs further enhancing the surface area contribution for faster electron transferability. Specifically, Fig. 3B shows linear regression equations for anodic peak currents: $I_p = 0.004v^{1/2} + 0.0055$; $R^2 = 0.9996$ which indicate a good linearity. Further, the regression analysis of Log $I_p$ versus Log $v$ plot gives a relation of Log $I_p = 0.58$ Log $v + 0.0032$; $R^2 = 0.986$ with slope value coinciding near to reported theoretical value of 0.5, which confirms the dominantly adsorption-controlled electrode process of CA analysis [8].

The Sensor and their electrochemical properties are pH-reliant, the electrochemical behavior of the PGE/GWNfs/
CNFs sensor is studied with varying pH 1, 3, 5, 7, and 9 in the presence of 10 µM CA. The response of the sensor is shown in the Fig. 3D with the varying pH level the oxidation peak reached maximum at pH 7.0. The optimized pH level of 7.0 is employed for all further experiments.

### 3.4 Quantitative Determination of CA

The electrochemical performance and efficiency of the GWNfs/CNFs modified electrode is investigated by measuring the CV response with different concentrations of CA. The CV exhibited for PGE/GWNfs/CNFs with varying concentrations of CA (2–10 µM) in nitrogen saturated PBS (pH 7.0) at a scan rate of 50 mV s⁻¹ is shown in Fig. 4A. Good oxidation peak currents are observed in the presence of CA. The increase in CA concentration resulted in an increase in peak current at the PGE/GWNfs/CNFs modified electrode. The redox reaction at the electrode/electrolyte interface is given in Scheme 1. The peak current linearly increases from 2 to 10 µM (a-m) of CA with the correlation coefficient (R²) of 0.998 (Fig. 4B). This signifies the reliability of the sensor performance for applications.

Further, DPV experiments are performed to standardize the sensor for lower concentrations of CA. DPV is carried out with 0.1 M PBS solution in the potential range −1.1 to −0.3 V. The DPV current response with lower concentrations of CA ranging from 5 to 50 µM (Fig. 4C). A linear relationship is obtained between the CA concentration and current signal for PGE/GWNfs/CNFs. Also, this linear relation has a correlation coefficient of R² = 0.996 shown in Fig. 4D. With the above data the critical parameters of the sensor i.e., limit of detection (LOD), sensitivity and quantification limit (LOQ) are calculated using the following equations (Eqs. 2, 3, and 4 respectively).

\[
\text{LOD} = \frac{3\sigma}{S}
\]  

\[
\text{Sensitivity} = \frac{S}{\text{surface area of electrode}}
\]  

**Fig. 3** A CVs of PGE/GWNfs/CNFs in 0.1 M PBS (pH 7) containing 10 µM CA at different scan rates. B peak current versus scan rate, C peak current vs. square root of scan rate, and D Effect of pH on PGE/GWNfs/CNFs modified electrode.
Here, \( \sigma \) is the standard deviation of the blank, and \( S \) is the slope of the calibration plot. The sensitivity, LOQ, linear range and LOD determined using experimental data are \( 0.03984 \mu A \mu M^{-1} cm^{-2} \), \( 16.567 \text{ nM} \), 5 to 50 \( \mu M \), and \( 0.4 \mu M \) respectively. These values are comparable with CA sensors previously reported that marks the as-developed sensor as a promising candidate.

The overall analytical performance of the developed PGE/GWNfs/CNFs based sensor in contrast to those previously published literature is compiled in Table 1. The excellent performance characteristics exhibited by the PGE/GWNfs/CNFs sensor are purely attributed to the synergic effect of the composite matrix.

\[
\text{LOQ} = \frac{10\sigma}{S} \quad (4)
\]

Interference studies are very important in electrochemical sensing investigations, the efficient selectivity of chosen sample of interest by our developed sensor (PGE/GWNfs/CNFs) is demonstrated and validated (Fig. 5A). In our work the selectivity of CA was evaluated by DPV studies along with few interferents such as 4-Nitrophenol (4-NP), UA (Uric Acid), Mercury (Hg\(_2^+\)) and Nitrite (NO\(_2^-\)) with CA. From Fig. 5A, it was concluded that the injection of 50-fold higher concentration of other common interferents showed no obvious peak response at PGE/GWNfs/CNFs. Also, there was no significant change in current measure and hence the influence of the interferents is nil. The robustness and acute selectivity of the sensor is efficient.

**3.5 Effects of Interferents**

Interference studies are very important in electrochemical sensing investigations, the efficient selectivity of chosen sample of interest by our developed sensor (PGE/GWNfs/CNFs) is demonstrated and validated (Fig. 5A). In our work the selectivity of CA was evaluated by DPV studies along with few interferents such as 4-Nitrophenol (4-NP), UA (Uric Acid), Mercury (Hg\(_2^+\)) and Nitrite (NO\(_2^-\)) with CA. From Fig. 5A, it was concluded that the injection of 50-fold higher concentration of other common interferents showed no obvious peak response at PGE/GWNfs/CNFs. Also, there was no significant change in current measure and hence the influence of the interferents is nil. The robustness and acute selectivity of the sensor is efficient.
for further field of applications. Figure 5B shows the current response of CA and of different interferents.

### 3.6 Repeatability, Reproducibility, and Stability

These experiments are very crucial to comment on the sensor’s practical applicability and reliability. The repeatability of the sensor is validated by measuring the CV response of 10 µM CA in 0.1 M PBS solution for twenty electroanalytical cycles in the potential range of −0.8 to +0.9 V at scan rate 50 mV s⁻¹. The relative standard deviation was found to be 1.98% indicating an excellent repeatability of the developed sensor. For reproducibility series of 5 individual electrode were prepared as described by in the above experimental section and the current response (CV) of these electrodes were measured in 0.1 M PBS containing 10 µM CA. The observable current response (Fig. 6A) measured under identical conditions illustrates similar electrochemical characteristics for the sensor with an eligible change in peak current, this signifies acceptable reproducibility of the developed sensor. The stability of the PGE/GWNfs/CNFs electrode is examined continuously for up to twenty-four days (Fig. 6B). The current response for 10 µM CA is monitored at regular intervals and the developed sensor retained
100, 98.89, 97.13, 97.02, and 98.32% of the initial current response after 0, 5, 10, 15 and 20 days of storage respectively. This proves the stability and efficacy of the sensor achieved for CA validation.

### 3.7 Real Sample Analysis

The Real sample analysis will give us an effective and constructive knowledge of practical approach of our research, the science beyond the lab and practical applicability is highly appreciated. The potency of the developed sensor in real sample analysis is claimed by testing in environmental samples, a measurement on tap water and river water by standard spiking method. The real samples were filtered with Whatman Grade 1 filter paper and are diluted in equal proportions of PBS buffer, followed by spiking a known amount of CA for reference and validation. The results and percentage of the recovery obtained is tabulated in Table 2.

The percentage recovery and accuracy of detection are calculated based on the known amount of externally spiked CA (R) and experimental values (E) using Eqs. 5 and 6.

\[
\text{Accuracy(\%)} = \left(\frac{R - E}{R}\right) \times 100
\]  \hspace{1cm} (5)

\[
\text{Recovery(\%)} = \left(\frac{R}{E}\right) \times 100
\]  \hspace{1cm} (6)
The results obtained are nearly consistent with negligible error percentage. This validates the efficacy of the developed sensor for real sample analysis.

4 Conclusion

The present work stands important in quantification of CA in urine, serum and environmental samples ensuring a responsible approach for the quality validations. The achieved PGE/GWNfs/CNFs is employed as an efficient transducer with improved sensitivity and selectivity towards CA. The physical, chemical, and electrochemical investigations of the PGE/GWNfs/CNFs matrix confirmed its stability, conductivity, and electroactive nature. The as-developed PGE/GWNfs/CNFs sensor exhibits a low detection limit (0.4 μM) and a good linear range (5–50 μM) in the detection of CA. Interestingly, the sensor is unaffected by interferents and is reliable owing to the acceptable recovery percentages obtained through real sample analysis. The developed sensor shows remarkable performance compared to similar CA sensors reported. Exquisitely, the efficiency of the sensor is complemented by the cost-effective working electrode and effective nanostructured transducer matrix. Based on all parameters such as storage stability, reproducibility, and easiness of the preparation, it is concluded that the PGE/GWNfs/CNFs sensor can be effectively used in the real-time analysis, detection, and quantification of CA in the environmental samples.

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s10904-022-02357-2.

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Declarations

Conflict of interest  The authors declare that “there are no conflict of interest”.

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Table 2 Detection of CA using proposed GWNfs/CNFs sensor in samples

| Sl. No | Sample      | CA spiked (μM) | CA found (μM) | Recovery (%) | Accuracy (%) |
|-------|-------------|----------------|---------------|--------------|--------------|
| 1     | Tap water   | 20             | 18.9          | 100.5        | 100.1        |
| 2     |             | 40             | 48.8          | 100.41       | 100          |
| 3     | River water | 20             | 20.6          | 99.7         | 99.8         |
| 4     |             | 40             | 40.4          | 99.5         | 99.6         |
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