Materials Science and Engineering C 65 (2016) 205–214

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Polyaniline–graphene oxide nanocomposite sensor for quantification of calcium channel blocker levamlodipine

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ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form 4 February 2016
Accepted 31 March 2016
Available online 11 April 2016

Keywords:
Nanocomposite
Electrochemical properties
Levamlodipine (LAMP)
Polyaniline–graphene oxide modified glassy carbon electrode (PANI/GO/GCE)

ABSTRACT

A novel polyaniline–graphene oxide nanocomposite (PANI/GO/GCE) sensor has been fabricated for quantification of a calcium channel blocker drug levamlodipine (LAMP). Fabricated sensor has been characterized by electrochemical impedance spectroscopy, square wave and cyclic voltammetry, Raman spectroscopy and Fourier transform infrared (FTIR) spectroscopy. The developed PANI/GO/GCE sensor has excellent analytical performance towards electrocatalytic oxidation as compared to PANI/GCE, GO/GCE and bare GCE. Under optimized experimental conditions, the fabricated sensor exhibits a linear response for LAMP for its oxidation over a concentration range from 1.25 μg mL^{-1} to 13.25 μg mL^{-1} with correlation coefficient of 0.9950 (r^2), detection limit of 1.07 ng mL^{-1} and quantification limit of 3.57 ng mL^{-1}. The sensor shows an excellent performance for detecting LAMP with reproducibility of 2.78% relative standard deviation (RSD). The proposed method has been successfully applied for LAMP determination in pharmaceutical formulation with a recovery from 99.88% to 101.75%.

1. Introduction

Levamlodipine, S-(-)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxy-carbonyl-5-methoxycarbonyl-6-methyl-1, 4-dihydropyridine [A] is basically an S- enantiomer of amlodipine [1,2]. It is a very powerful dihydropyridine calcium channel blocker possessing vasodilation properties and used in the treatment of hypertension and angina [3].

In recent years, polymer nanocomposites have become an interesting area of research due to their large surface to volume ratio, excellent mechanical, optical and electrocatalytic properties and have been extensively employed for various electroanalytical sensing applications [4–17]. Polyaniline (PANI) is one of the highly conducting polymers and has attracted much attention for their use in different types of sensing applications using various electroanalytical techniques [18–30]. It is one of the most promising and unique conducting polymers having good biocompatibility, environmental stability, excellent electronic properties. Carbon based materials exhibit very fascinating and extraordinary electrochemical properties making them an appealing choice as electrode material for various electrochemical studies of different compounds [31–41]. Maleh et al. [42] have proposed a new kind of sensor based on FePt/CNTs nanocomposite/*N*-(4-hydroxyphenyl)-3,5-dinitrobenzamide modified carbon paste electrode for detection of glutathione and piroxicam simultaneously. Also they proposed, a very novel modified carbon paste electrode based on NiO/CNTs nanocomposite and (9, 10-dihydro-9,10-ethanoanthracene-11,12-dicarboximido)-4-ethylbenzene-1,2-diol for simultaneous detection of cysteamine, nicotinamide adenine dinucleotide and folic acid [43]. Mokhtari et al. [44] fabricated multiwall carbon nanotubes paste electrode and characterized by various electrochemical techniques for voltammetric quantification of morphine and diclofenac in biological and pharmaceutical samples. M. Elyasi et al. [45] developed a Pt/CNTs nanocomposite based ionic liquid modified carbon paste electrode (Pt/CNTs/ILCPE) sensor to determine Sudan I using voltammetric techniques. Also Maleh et al. [46] proposed a sensitive biosensor immobilized DNA at pencil graphite electrode modified with polypyrrole/functionalized multiwalled carbon nanotubes to detect 6-mercaptopurine. Due to the high electron transport and electrocatalytic activity, graphene based electrodes exhibit fascinating potential applications as electrochemical sensors and promote quantification of analyte at its surface with enhanced electrocatalytic performance. Nowadays, graphene based electrodes have been extensively reported for various kinds of sensing application for a variety of analytes. Recently, Akhavan et al. [47] have applied reduced graphene nanowalls for ultra sensitive detection of single nucleotide polymorphisms of DNA using differential pulse voltammetry (DPV) and efficiently developed an electrochemical biosensor for the determination of the purine and pyrimidine bases of DNA. They also succeeded in establishing a versatile Mg^{2+}-charged spongy graphene electrodes for sensitive detection of leukemia in blood serum using differential pulse voltammetry and compared with glassy carbon electrode [48]. Akhavan et al. [49] also applied reduced graphene oxide nanowall electrodes for highly sensitive and selective electrochemical detection of leukemia cells using DPV.

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http://dx.doi.org/10.1016/j.msec.2016.03.115
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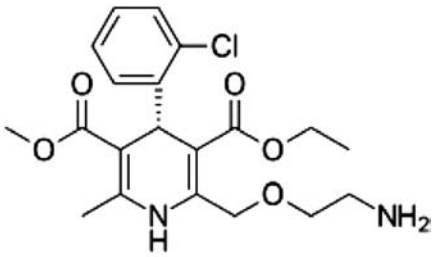


[A]

[A]. : Structure of levamlodipine.

Graphene oxide (GO), an oxidized form of graphene is a two-dimensional nanosheet of covalently bonded carbon atoms. GO has become an interesting area of research as it exhibits very similar properties with graphene. Electrocatalytic studies on various electroactive compounds have been reported using GO as an electrode material [50,51]. The covalent oxygen functional groups in GO induce incredible mechanical strength and can interface with polymers to form GO-intercalated polymer nanocomposites [52].

The electrocatalytic activity of graphene is enhanced by conjugation with other conducting nanomaterials for electrochemical sensing of various compounds. PANI provides a uniform matrix for the immobilization of graphene oxide and hence there is an increase in the effective electrode surface area and electron transfer centers after the immobilization. Thus, conjugation of PANI with graphene oxide significantly enhance the electrocatalytic activity of the resultant electrode and due to their synergistic catalytic effect, there is an enhancement in the voltammetric response of levamlodipine (LAMP). Comparing with CNTs, graphene oxide has shown the advantages of increased conductivity, good biocompatibility, ease of production and function, and low cost source material [53–55]. Moreover, comparing with fullerene, another carbon based electrode material, it is difficult to disperse it in the polymer matrices due to its strong molecular arrangement and crystalline form. Therefore, it is difficult to prepare PANI-fullerene polymer composites [56]. Also, graphene and PANI based electrodes have much more faster electron transfer kinetics and enhanced catalytic response for electro quantification of analytes than metal nanoparticles [57,58]. Therefore, it is highly desirable to explore polyaniline–graphene oxide composite for sensing applications.

Combining the high conducting [59] and electrical properties [60,61] of these polymer nanocomposites with different types of electroanalytical techniques like square wave voltammetry, cyclic and differential pulse voltammetry, a promising sensing platform has now been developed in the area of electrochemical sensing and biosensing [57,58,62–68]. The developed method of determination of levamlodipine using voltammetry acts as an alternative to other techniques of its detection like capillary electrophoresis [69], coupled isotachophoresis-capillary zone electrophoresis with diode array detection [70], spectrophotometric and fluorescence quenching measurements [71], chemiluminescence methods [72] which require complicated extraction, sample preparation, time consuming derivatization and purification processes with expensive and sophisticated instrumentation. In the present paper, using voltammetric technique, a novel method for detection of levamlodipine at PANI/GO/GCE sensor has been developed which offers high sensitivity, accuracy, precision, fast response with relatively low cost simple instrumentation and does not require the tedius procedures like extraction and purification prior to monitoring of analytes. However, the stability of the proposed PANI/GO/GCE sensor has been studied only for 15 days as the anodic current decreases for LAMP oxidation due to deterioration of PANI/GO nanocomposite modification at glassy carbon electrode surface.

To the best of our knowledge, this is the first voltammetric study of S enantiomer levamlodipine at polyaniline/graphene oxide nanocomposite modified glassy carbon electrode. The electrochemical performance of the developed PANI/GO/GCE sensor towards levamlodipine has been compared with the results obtained at individual PANI/GCE, GO/GCE and at bare GC electrode. The fabricated sensor shows reproducible results towards levamlodipine oxidation using voltammetric techniques.

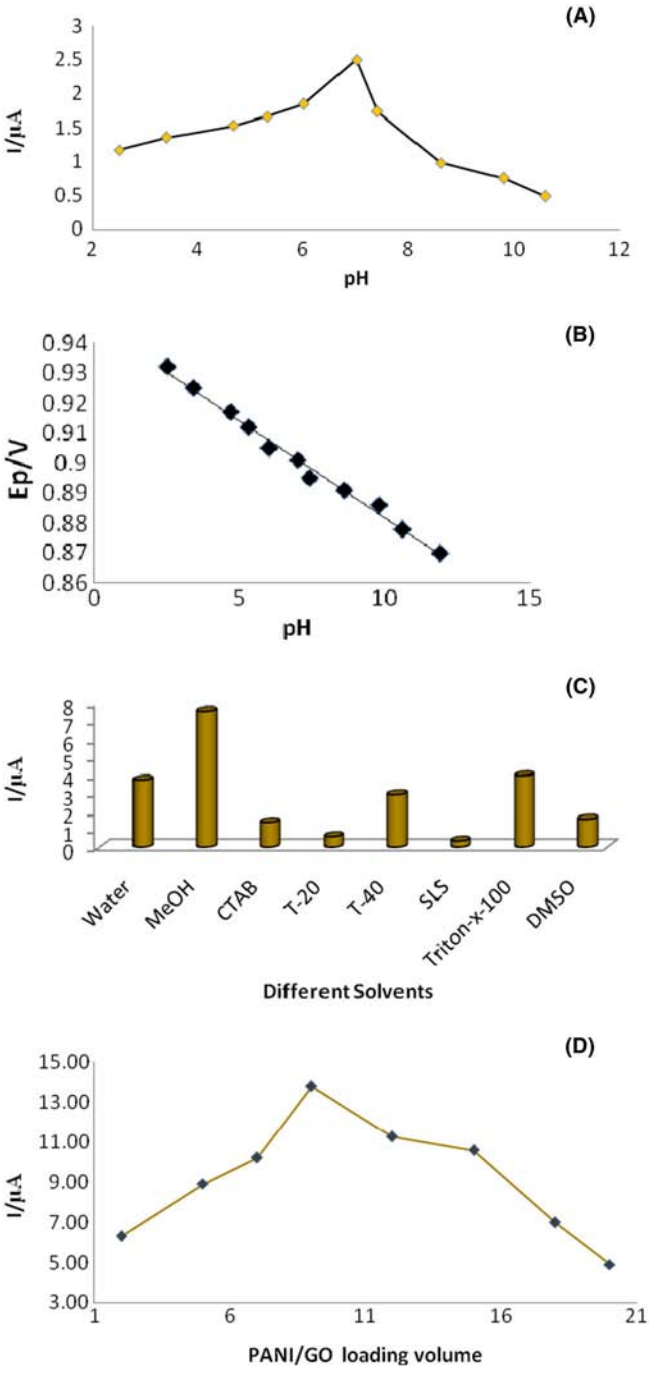


Fig. 1. Optimization parameters for 10 μg mL^{-1} LAMP oxidation. (A) Effect of pH (pH 2.5–10.5). (B) Plot of Ep vs pH. (C) Effect of different solvents. (D) Effect of varying PANI-GO dosage.

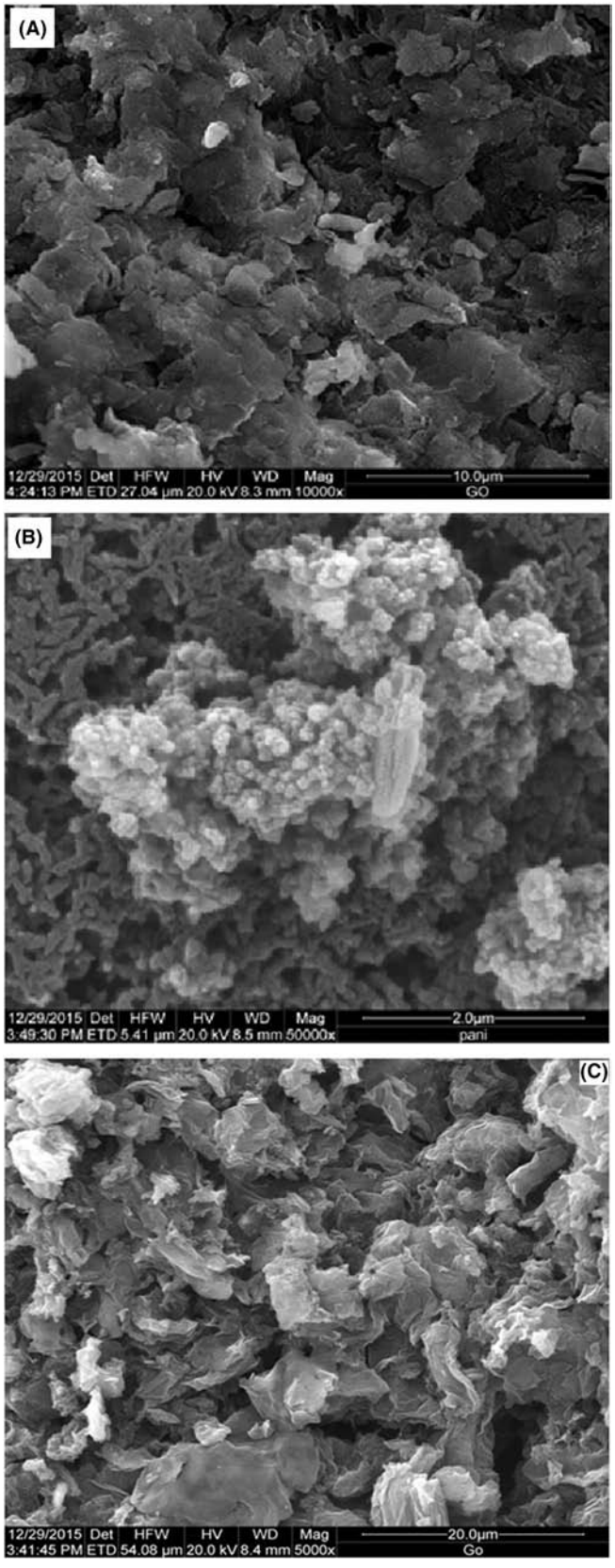


Fig. 2. Scanning electron micrograph of (A) PANI/GCE, (B) GO/GCE, (C) PANI/GO/GCE.

2. Experimental

2.1. Reagents and chemicals

Graphite powder (97% purity) was procured from Loba Chemie, India. Levamlodipine reference standard (98.7% purity) was obtained from Torrent Pharmaceuticals Ltd. Mumbai, India and was used as received. Tablets of levamlodipine labeled 5 mg manufactured by Torrent Pharmaceuticals Ltd, India were obtained from commercial sources. All other chemicals used were of analytical grade and used without further purification. All chemicals were procured at Jiwaji University, Gwalior, India.

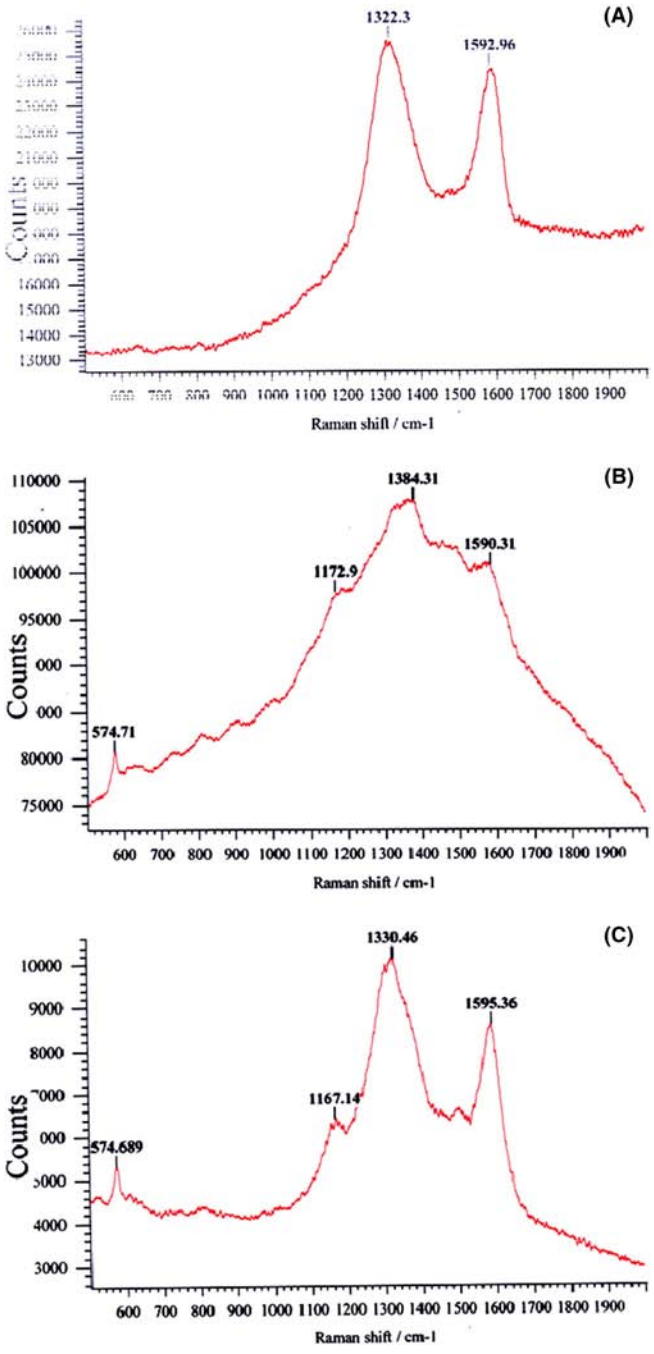


Fig. 3. Raman spectra of (A) GO (B) PANIGCE, (C) PANI/GO at 532 nm.

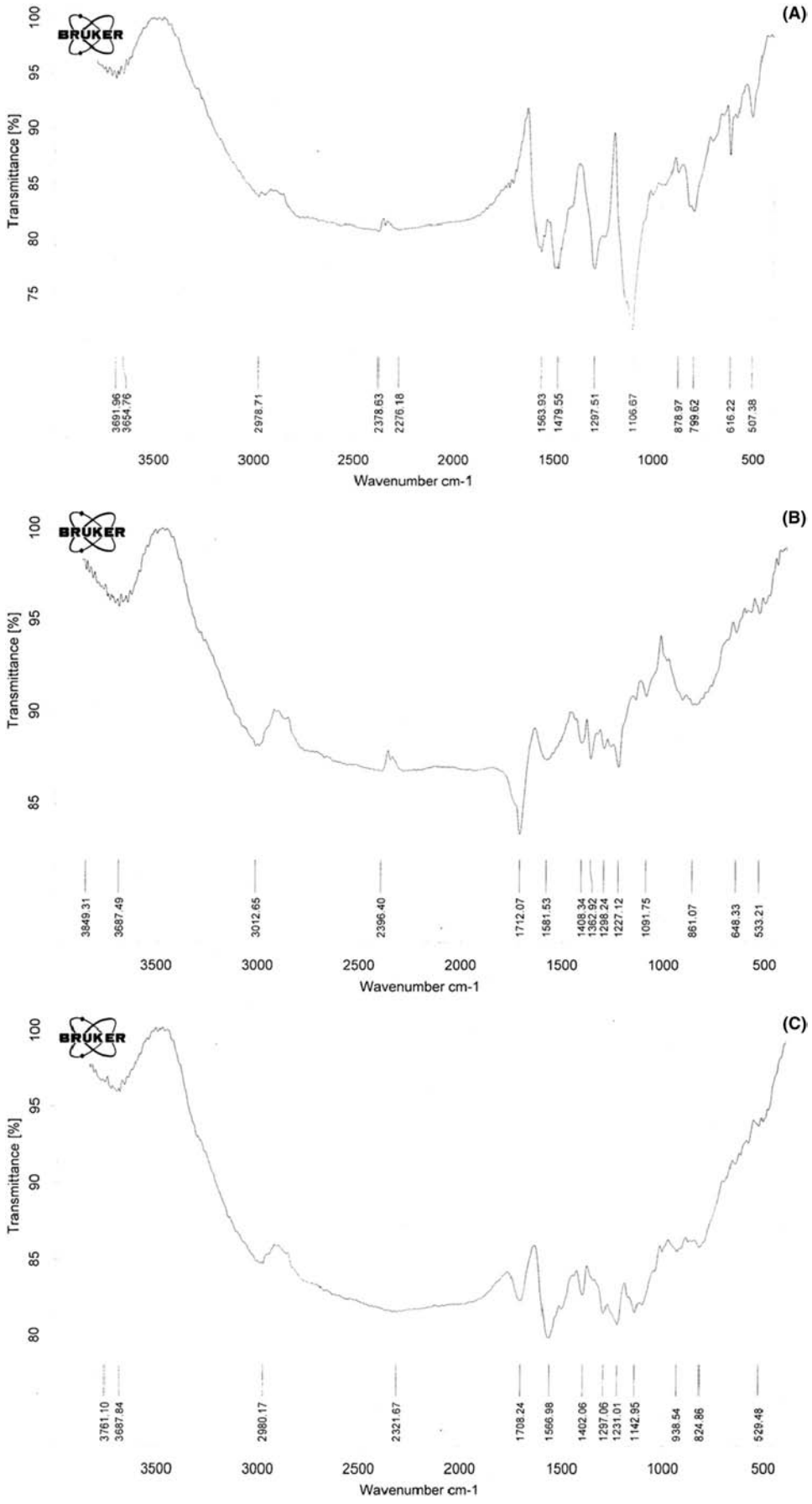


Fig. 4. FTIR spectra of (A) PANI (B) GO, (C) PANI/GO.

2.2. Apparatus

All electrochemical measurements were carried out at μ-AUTOLAB potentiostat-galvanostat with NOVA 1.10 software. PANI/GO nanocomposite film modified glassy carbon electrode was employed as working, Ag/AgCl (3 M KCl) as reference and platinum wire as an auxiliary electrode. Before carrying out the electrochemical investigations all the solutions used were purged for 10 min with purified nitrogen gas. Decible DB-1011 digital pH meter fixed with a glass electrode was used for carrying out pH-metric measurements. FTIR spectra of samples were recorded using Bruker Equinox spectrometer. Renishaw inVia Raman microscope was used for recording Raman spectra of samples.

2.3. Pharmaceutical preparation

The average weight of 20 tablets was determined and grinded finely in a motor pestle. The powdered material equivalent to 10.0 mg of levamlodipine was transferred to calibrated flask containing 10.0 mL of methanol. The contents of the flask were sonicated for 30 min to mix it properly. After proper mixing, the suspension was subjected to centrifugation at 500 rpm for 15 min. An aliquot of the sample was then analysed according to the proposed voltammetric procedure.

2.4. Preparation of graphene oxide (GO)

GO was prepared according to modified Hummers method [28]. 0.5 g of graphite powder and 0.5 g of sodium nitrate ($NaNO_3$) were mixed with 23.0 mL conc. H_2SO_4 (98%) and swirled for 30 min in an ice bath. 4.0 g of potassium permanganate ($KMnO_4$) was added to the suspension maintaining the stirring vigorously. The rate of addition of $KMnO_4$ was adjusted in order to keep the temperature lower than 35 °C. After completing the addition of $KMnO_4$, ice bath was removed and the mixture was continuously stirred at 35 °C in a water bath for further 2 h. On completion of the reaction, 40.0 mL of ultrapure water was added gradually to the reaction mixture and was kept stirring at 90 °C for 1 h. Thereafter, the mixture was treated with further 100 mL of ultrapure water followed by the addition of 3.0 mL H_2O_2 (30%) changing the colour of reaction mixture from dark brown to yellow. GO was extracted from the reaction mixture by centrifugation followed by washing with 5% HCl and then with ultrapure water. After drying under vacuum, GO was obtained.

2.5. Preparation of polyaniline/graphene oxide (PANI/GO) nanocomposite

PANI was synthesized by interfacial polymerization [73], which was prepared with the reactant ratio of aniline:ammonium persulphate (APS):Camphor sulphonic acid (CSA) = 2:1:1. First aniline (0.1 M) was dissolved in carbon tetrachloride (100.0 mL) as organic phase and aqueous phase containing APS (0.1 M) was mixed with CSA (0.1 M) each dissolved in 25 mL of double distilled water (50.0 mL). Then, the water phase was transferred to organic phase for interfacial polymerization at 5–7 °C for overnight. GO/PANI nanocomposite was prepared following similar procedures but replacing water with known concentration of GO aqueous solutions, the ratio of aniline, APS and CSA were maintained as above. Specifically, 0.03 g of GO was dispersed in 100.0 mL water under sonication for 3 h to obtain a homogeneous brown solution. After that 0.57 g APS (0.1 M) and 0.581 g CSA (0.1 M) were added to the solution and sonicated for an additional hour. Resulting mixture was transferred into organic phase for interfacial polymerization at 5–7 °C for overnight.

2.6. Fabrication of polyaniline–graphene oxide modified GCE sensor electrode

Before electrode modification, bare GC electrode was rinsed ultrasonically with ethanol followed by ultrapure water. GCE surface was then polished with alumina powder of particle size ranging from 0.05 μm to 0.1 μm on microcloth pads followed by constant washing with ultrapure water till a clear and clean surface was obtained and dried in vacuum dessicator for 15 min. The PANI/GO nanocomposite, weighed 2.0 mg was dispersed in 2.0 mL of N, N-dimethylformamide (DMF) to give a solution of 1.0 mg mL^{-1} with ultrasonication for 1–2 h to get homogenous suspension. The surface of GCE was modified with PANI/GO suspension by casting the known volume (9 μL) using a micro syringe and used for the proposed voltammetric analysis.

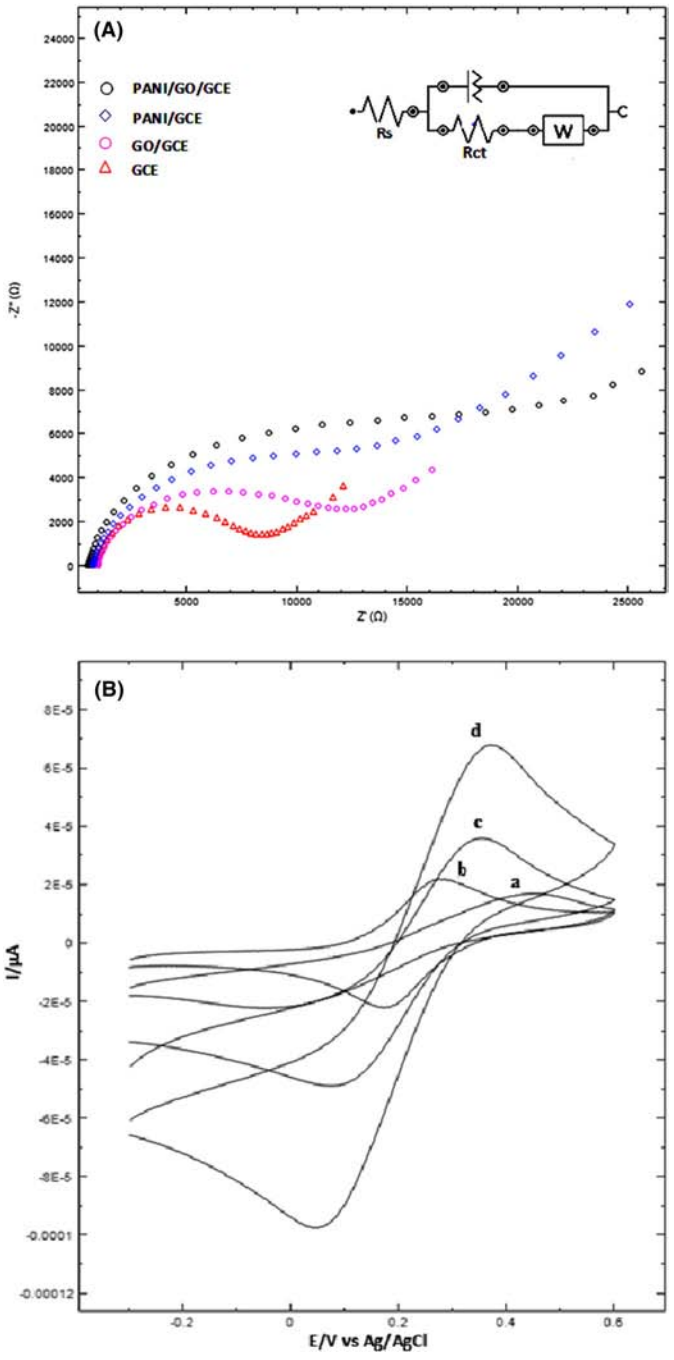
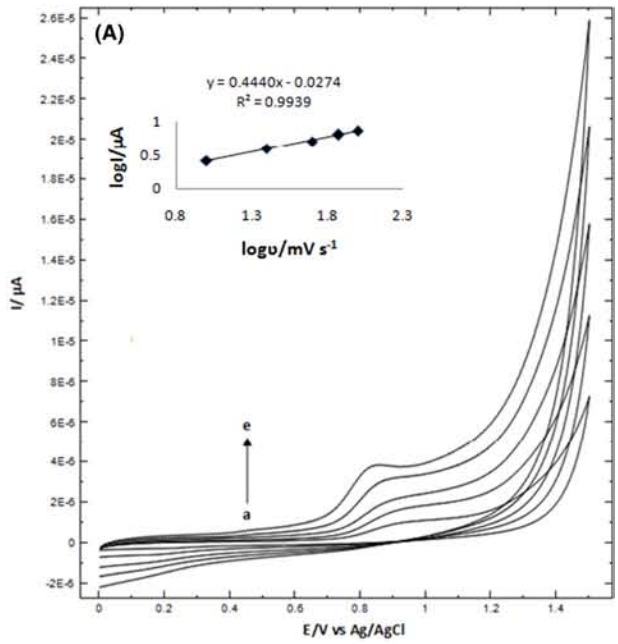
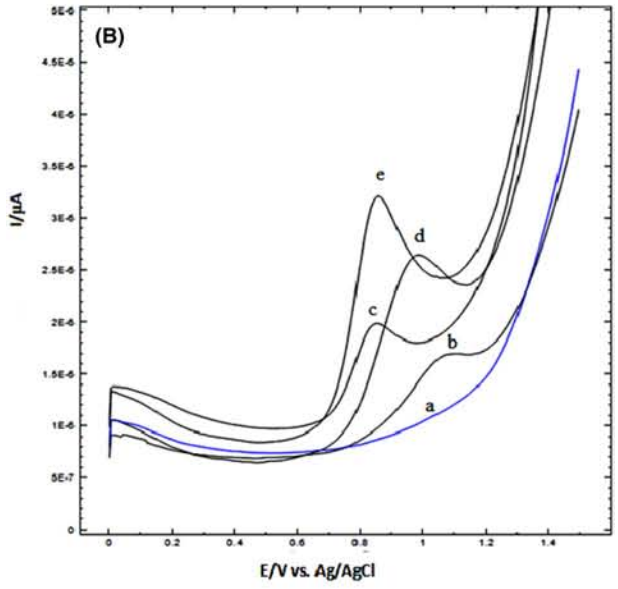
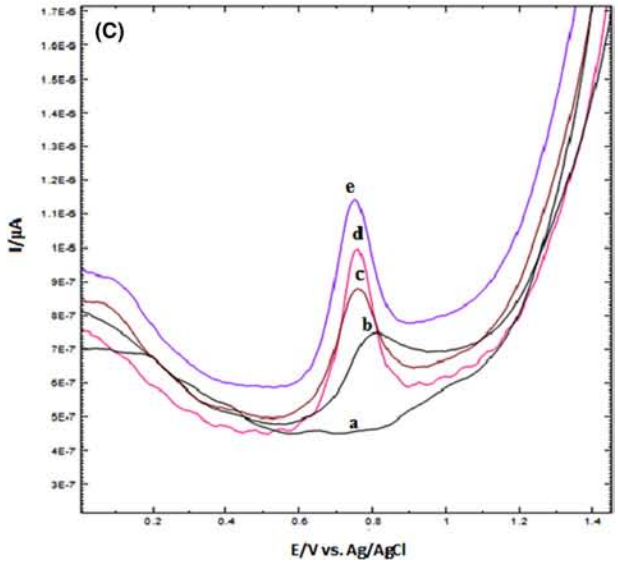


Fig. 5. (A) Nyquist Plot of GCE (curve a), GO/GCE (curve b), PANI/GCE (curve c) and PANI/GO/GCE (curve d) in 5.0 mM $K_3Fe(CN)_6$, Inset: Randles circuit used to fit the impedance data. (B) Cyclic voltammetric behaviour of 1.0 mM $K_3Fe(CN)_6$ at bare GCE (curve a), at GO/GCE (curve b), PANI/GCE (curve c), and PANI/GO/GCE (curve d).







3. Results and discussion

3.1. Optimization of experimental conditions

3.1.1. Effect of pH

The electrocatalytic oxidation studies of levamlodipine at PANI/GO sensor were performed using square wave voltammetry in phosphate buffer at different pH ranging from 2.5 to 11.9. It is evident from Fig. 1 (A) that the oxidative current gradually increases with increase in pH. It attains maxima at pH 7.0 and then starts to decrease with further increase in pH. Therefore, pH 7.0 was chosen as an optimum pH to carry out the electrochemical studies of levamlodipine. A linear relation was observed between peak potential (E_p) and pH, which can be expressed by equation (Fig. 1B) (i),

$$\text{SWV: } E_p/V\ (Ag/AgCl) = 0.0065pH + 0.8557, r^2 = 0.9966 \quad \text{(i)}$$

3.1.2. Effect of ionic strength of supporting electrolyte

The dependence of peak current on ionic strength of supporting electrolyte KCl was studied and varied in the range from 0.5 M to 2.0 M. Results indicated that current I increases by increasing KCl concentration significantly upto 1 M and no significant increase in current was observed and no interferences observed on the peak current for the oxidation studies of levamlodipine at PANI/GO/GCE.

3.1.3. Effect of different solvents

The effect of different solvent systems on levamlodipine oxidation has been studied by square wave voltammetry. LAMP exhibits maximum peak current in methanol as compared to other solvents and surfactants and therefore methanol was selected as the solvent medium for the electroanalytical studies of levamlodipine (Fig. 1C).

3.1.4. Effect of loading volume of PANI/GO film at GCE surface

The effect of loading volume of PANI/GO/DMF suspension for casting GCE surface on anodic current has been observed from 2 μL to 20 μL. The peak current increases significantly up to 9 μL. However, the current starts decreasing after 9 μL (Fig. 1D). Therefore, 9 μL was selected as an optimum loading volume for modification of GCE surface.

3.2. Characterization of PANI/GO nanocomposite sensor film on GCE surface

The surface characteristics of the fabricated PANI/GO/GCE sensor were studied by scanning electron microscopy (SEM). Fig. 2 clearly exhibits the scanning electron micrographs of PANI/GCE (2A), GO/GCE (2B) and PANI/GO/GCE (2C) suggesting that PANI and GO make a hybrid film on the surface of GCE retaining original morphology.

Raman spectroscopic analysis was studied at 532 nm for GO (Fig. 3A), PANI (Fig. 3B) and PANI/GO (Fig. 3C) nanocomposite. Results for GO clearly show two sharp peaks at 1322.3 cm^{-1} and 1592.9 cm^{-1} which correspond to D and G band respectively confirming its graphitized structure along with its monolayer property which is in good agreement with the literature [74–80]. Intense and sharp spectral peaks obtained for of PANI (Fig. 3B) and GO can be clearly seen in the spectra obtained for PANI/GO nanocomposite (Fig. 3 C) indicating the formation of nanocomposite.

Fourier transform infrared spectra (FTIR) of PANI (Fig. 4 A), GO (Fig. 4 B) were recorded. For GO, the FTIR peaks obtained between

Fig. 6. (A) Cyclic voltammetric behaviour of LAMP at PANI/GO/GCE in phosphate buffer 7 at different scan rates (a–e): 10, 25, 50, 70 and 100 mVs^{-1}, Inset: plot of log Ip vs. log ν. (B) Square wave voltammograms of 10 μg mL^{-1} LAMP: Blank (curve a), at GCE (curve b), at GO/GCE (curve c), PANI/GCE (curve d) and at PANI/GO/GCE (curve e). (C) Differential Pulse voltammograms of 10 μg mL^{-1} LAMP: Blank (curve a), at GCE (curve b), at GO/GCE (curve c), PANI/GCE (curve d) and at PANI/GO/GCE (curve e).

900 and 1200 cm^{-1} correspond to C—O stretching. Again the peak between 1227 and 1298 cm^{-1} and peak around 1408 cm^{-1} may be assigned to C—O—C and C—OH bond respectively. Peak near 1620 cm^{-1} shows the similar peak as obtained for graphite. The two clear peaks in the FTIR spectrum of GO between 1500 and 2000 cm^{-1} clearly corresponds to CO and CC which finds good accordance with the other reported data [81–83]. Again the sharp FTIR peaks obtained for PANI at 1563 cm^{-1} and 1479 cm^{-1} assigned to the nonsymmetric vibration mode of quinoid and benzenoid ring system of polyaniline [84]. The FTIR data obtained for PANI/GO (Fig. 4 C) have been compared with the spectra obtained for PANI and GO which clearly suggest the formation of nanocomposite.

The electron transfer characteristics of the fabricated PANI/GO/GCE sensor during the fabrication of the GCE surface were studied using electrochemical impedance spectroscopy. 5.0 mM of $K_3Fe(CN)_6$ in 0.1 M KCl was used as redox probe. The obtained charge transfer resistance (R_{ct}) values for the bare GCE, PANI/GCE, GO/GCE and PANI/GO/GCE were found to be 9.98 KΩ, 8.02 KΩ, 7.23 KΩ and 6.03 KΩ respectively. The results clearly indicate that the modification of the electrode surface by PANI/GO/GCE results in the enhanced electrical conductivity and electrochemical performance of the nanocomposite sensor towards LAMP oxidation as compared to PANI/GCE, GO/GCE and bare GC electrode (Fig. 5A).

3.3. Effective surface area of PANI/GO nanocomposite sensor

The effective surface area studies of bare GCE, PANI/GCE, GO/GCE and PANI/GO/GCE sensor were performed using cyclic voltammetry taking 1.0 mM solution of $K_3Fe(CN)_6$ in 0.1 M KCl using Randles–Sevcik equation. It was calculated from slope of the plot between $\nu^{1/2}$ and I based on the Randles–Sevcik Eq. (ii) [85],

$$I = \left(2.69 \times 10^5\right) ACD^{1/2}\, n^{3/2}\, \nu^{1/2} \tag{ii}$$

Where, A represents the effective surface area of the electrodes in cm^2, n is the number of electrons taking part in charge transfer process, D is the diffusion coefficient of the analyte in the solution, C is the concentration of $K_3Fe(CN)_6$ solution. The values of n and D for $K_3Fe(CN)_6$ are 1 and 7.6×10^{-6} $cm^2\ s^{-1}$ respectively [86]. The results obtained reveal that the PANI/GO/GCE nanocomposite sensor possesses greater surface area (0.2134 cm^2) than PANI/GCE (0.1558 cm^2), GO/GCE (0.1033 cm^2) and bare GCE (0.0261 cm^2) suggesting an enhanced electrocatalytic performance of the fabricated PANI/GO/GCE towards LAMP oxidation (Fig. 5B).

3.4. Scan rate studies and estimation of charge transfer coefficient

Cyclic voltammetric behaviour of 15 ng mL^{-1} LAMP at PANI/GO/GC electrode was studied at different scan rates (Fig. 6A). It was observed that the oxidation peak current (I) increases linearly with the square root of scan rate ($\nu^{1/2}$) in the range of 10–100 mV s^{-1}, and can be expressed by the Eq. (iii) [87],

$$I(\mu A) = 0.6933(mV\,s^{-1}) - 0.4061;\ r^2 = 0.9932 \tag{iii}$$

The obtained slope 0.4440 is very near 0.5 which confirms diffusion controlled electrode process and by plotting log I vs. log ν given by the Eq. (iv)) [88],

$$\log I(\mu A) = 0.4440 \log \nu\left(mV\ s^{-1}\right) - 0.0274;\ r^2 = 0.9939 \tag{iv}$$

For a typical diffusion controlled electrode process, the peak potential (E_p) and scan rate (v) are expressed by the Eq. (v),

$$E_p = E^\circ + (RT/\alpha nF)\ \ln\left(RTk^\circ/\alpha nF\right) + (RT/\alpha nF)\ \ln \nu \tag{v}$$

Where k° is the standard rate of reaction, E° is the formal potential, n is the number of electrons involved in the electrode process, α is the transfer coefficient and ν is the scan rate. During the oxidation process of LAMP at PANI/GO/GCE, E_p linearly depends on the logarithm of the scan rate in the range of 10–100 mVs^{-1} which can be expressed by the following Eq. (vi),

$$E_p = 0.0289(\ln \nu) + 0.8583,\ r^2 = 0.9807 \tag{vi}$$

Combining Eqs. (v) and (vi) we obtain,

$$(RT/\alpha nF) = 0.0289 \tag{vii}$$

Where, T = 298 K, R = 8.314 J K^{-1} mol^{-1} and F = 96,500 C. From Eq. (vii) αn can be calculated as 0.8883. Taking 2 as the number of electrons (n) involved in the oxidation of LAMP [3,89], value of charge transfer coefficient α was obtained as 0.4441 which is theoretically close to 0.5 that further confirms the irreversible behaviour of the electrode process [86].

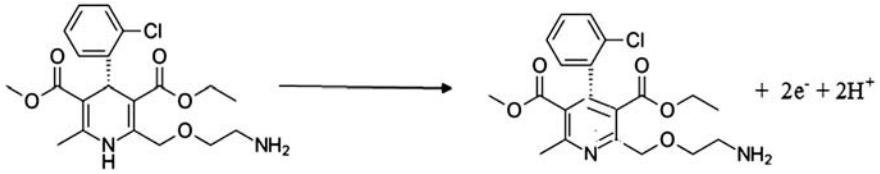
On the basis of square wave and cyclic voltammetric studies, a possible electrode mechanism for the oxidation of levamlodipine at PANI/GO/GC sensor has been postulated (Scheme 1) [3,90].

3.5. PANI/GO/GCE sensor for oxidation of levamlodipine

The electrocatalytic oxidation of levamlodipine was studied by square wave (SWV) and differential pulse voltammetry (DPV). A 10 μg mL^{-1} of LAMP in methanol in phosphate buffer at pH 7.0 exhibits a well defined oxidation peak at PANI/GO/GCE nanocomposite sensor. The obtained square wave voltammograms (Fig.6B) and differential pulse voltammograms (Fig.6C) clearly illustrate that the fabricated PANI/GO/GCE sensor is much more sensitive and shows better electrochemical performance towards LAMP oxidation with respect to other GO/GCE, PANI/GCE and bare GC electrode.

3.6. Calibration curve and limit of detection

Square wave voltammograms (Fig. 7) show the effect of the increasing concentration of levamlodipine on its oxidation which indicates that anodic peak linearly increases with the increase in the concentration of LAMP with a correlation coefficient (r^2) of 0.9950. Under optimized conditions, a calibration curve has been plotted over an increasing



Scheme 1. Proposed electrode mechanism for oxidation of LAMP.

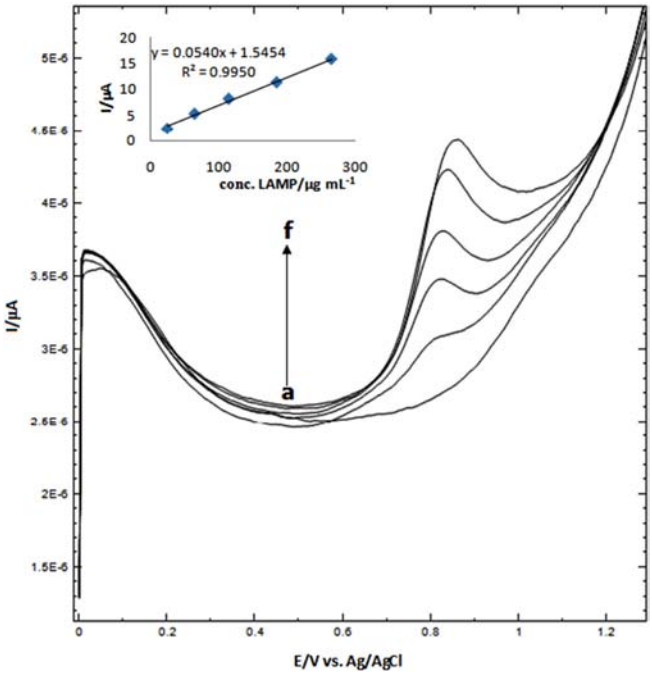


Fig. 7. Square wave voltammograms of Levamlodipine at PANI/GO/GCE sensor at different concentrations (b–f), (a) blank, (b) 1.25 μg mL^{-1}, (c) 3.25 μg mL^{-1} (d) 5.75 μg mL^{-1}, (e) 9.25 μg mL^{-1}, (f) 13.25 μg mL^{-1} Inset: Plot of peak current, I vs. LAMP concentration.

concentration range from 1.25 μg mL^{-1} to 13.25 μg mL^{-1} and peak current can be expressed by the Eq. (viii),

$$I/\mu A = 0.0540\left(\mu g\, mL^{-1}\right) + 1.5454, r^2 = 0.9950 \quad \text{(viii)}$$

Limit of detection (LOD, 1.07 ng mL^{-1}) and limit of quantification (LOQ, 3.57 ng mL^{-1}) were calculated as 3S/m and 10S/m respectively where S represents the standard deviation of the peak current and m is slope of calibration curve.

3.7. Reproducibility, stability and interference studies of fabricated sensor

The reproducibility of the fabricated sensor was studied taking 10 μg mL^{-1} of levamlodipine at three different electrodes fabricated separately (Table 1). The results reveal acceptable reproducibility of the sensor with relative standard deviation (RSD) of 2.78%. Stability measurements were carried out by measuring the anodic current response of 10 μg mL^{-1} of LAMP for 15 days. In the meantime, the fabricated sensor was stored at 5 °C. After 15 days, the sensor exhibits 89% of its preliminary current response. Determination of levamlodipine in the presence of various interfering organic molecules and common excipients was investigated to study the anti-interference ability of the fabricated sensor. 10 μg mL^{-1} of LAMP in the presence of 50-fold increased concentration of glucose, lactose, magnesium starate and starch with a deviation of 1.6% indicates practical analytical application of the fabricated sensor.

Table 1
Reproducibility data for 10 μg mL^{-1} levamlodipine at PANI/GO/GCE sensor.

Sensor	Sensor reproducibility		Single sensor repeatability	
	Mean current (I/μA)	RSD (%)	Mean current (I/μA)	RSD (%)
Sensor 1	1.71[a]	0.62	1.71[a]	0.62
Sensor 2	1.65[a]	3.91		
Sensor 3	1.62[a]	3.32		
Average	1.66[b]	2.78		

[a] Mean of five replicate readings.
[b] Mean of three sensors.

Table 2
Accuracy and precision for determination of levamlodipine by SWV (n = 3).

Added (μg mL^{-1})	Found (μg mL^{-1})	Precision[a] (μg mL^{-1})	Coefficient of variation (%)	Accuracy[b] (%)
3.00	2.96	2.96 ± 0.07	2.53	−0.03
7.00	6.95	6.95 ± 0.09	1.38	−0.04
10.00	10.12	10.12 ± 0.25	2.51	0.12

Coefficient of variation = S.D./Mean × 100.
[a] Mean ± S.D.
[b] Accuracy = [found –added/added] × 100.

3.8. Accuracy and precision

The accuracy and precision of the proposed method were calculated taking a succession of three different concentrations of levamlodipine standard (3.00, 7.00, 10.00 μg mL^{-1}). The accuracy of the method was determined by the variation in the percentage relative error between the obtained and added concentration of LAMP standard. The precision of the method was measured by calculating % RSD and coefficient of variation. The results obtained (Table 2) clearly show that the fabricated PANI/GO/GCE sensor is sensitive for LAMP oxidation at its surface.

3.9. Analytical utility of the fabricated PANI/GO/GCE sensor to pharmaceutical formulation

To justify the analytical efficacy of the fabricated PANI/GO/GCE sensor, it was applied to commercial tablet dosage form of levamlodipine labeled 5 mg for its detection. After crushing the tablets, sample was weighed equivalent to 10 mg of levamlodipine and dissolved in 10 mL methanol. The mixture was ultrasonicated for 15 min and centrifuged at 500 rpm for 15 min. A series of levamlodipine (3.00, 7.00, 10.00 μg mL^{-1}) was added to measure percentage recovery using square wave voltammetry. The results obtained (Table 3) confirm the sensitivity and selectivity of the fabricated PANI/GO/GCE for analysis of levamlodipine in pharmaceutical formulation with an acceptable recovery from 99.88% to 101.75%.

3.10. Comparison of the proposed method with other reported methods

On the basis of the reported data for the detection limit of R enantiomer amlodipine at various electrodes [3,89–98], the electrochemical quantification of S enantiomer levamlodipine at PANI/GO/GC sensor has been compared in Table 4. The results clearly depicts that the proposed method of determination of LAMP at fabricated PANI/GO/GC electrode is much more sensitive for its oxidation studies at its surface, however this is the first reported method for voltammetric studies of S levamlodipine at PANI/GO/GCE.

Table 3
Recovery study of levamlodipine in pharmaceutical formulation.

Added (μg mL^{-1})	Found[a] (μg mL^{-1})	RSD (%)	Recovery (%)
3.00	2.99	3.02	99.88
7.00	7.02	2.56	100.28
10.00	10.17	0.76	101.75

[a] Mean of three replicates.

Table 4
Comparison of detection limit of proposed method with other electrochemical methods reported for R amlodipine.

Reference method	Sensor	Detection parameter (LOD)	Reference
SWV	SWCNT/EPPGE	1.0×10^{-9} M	[3]
SWV	MWCNT/EPPGE	5.0×10^{-9} M	[3]
AdSWV	CPE	2.0×10^{-10} M	[89]
DPSV	MWCNT/GCE	0.001 μg mL^{-1}	[90]
DPV	GR/CS/GCE	0.6 μM L^{-1}	[91]
DPV	GCE	0.012 mg mL^{-1}	[92]
DPSV	MWCNT/GCE	0.005 μg mL^{-1}	[93]
DPV	MWCNT/Graphite	1.0 μg mL^{-1}	[94]
DPV	GCE	0.31 μM L^{-1}	[95]
SWV	MWCNTsP	0.049 μM L^{-1}	[96]
DPV	BDD	28.6 μg mL^{-1}	[97]
SWAdSV	GCE	1.4×10^{-8}M	[98]
SWV of LAMP (S enantiomer)	PANI/GO/GCE	1.07 ng mL^{-1} (2.61×10^{-12} M)	Present work

Abbreviations: SWV: Square wave voltammetry, SWCNT: Singlewalled carbon nanotubes, MWCNT: Multiwalled carbon nanotubes, EPPGE: Edge plane pyrolytic graphite electrode PANI: Polyaniline, GO: graphene Oxide, GCE: Glassy Carbon Electrode, SWV: Square Wave Voltammetry, DPV: Differential Voltammetry, DPV: Differential Pulse Voltammetry, DPAdSV: Square wave adsorptive voltammetry, DPSV: Differential Pulse Stripping voltammetry, CPE: Carbon paste electrode, CS: Chitosan, GR: Graphene, BDD: Boron doped diamond electrode, MWCNTsP: Multiwalled carbon nanotubes paste electrode, LAMP: Levamlodipine.

4. Conclusion

The present paper reports a sensitive and suitable method for the electrocatalytic determination of levamlodipine at PANI/GO modified glassy carbon electrode in methanol. The synergistic effect of PANI and GO leads to higher voltammetric response with lower background current, overpotential, limits of detection with reproducible results for LAMP oxidation at PANI/GO/GCE. Results clearly show that under optimized conditions, fabricated PANI/GO/GC sensor is much sensitive towards electrocatalytic determination of levamlodipine and proposed method can be successfully applied to the detection of LAMP in pharmaceutical formulations with satisfied recovery limits.

Acknowledgment

The authors are grateful to the Department of Science and Technology, New Delhi, India for providing INSPIRE fellowship to one of them (A S).

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