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# Polypyrrole-Cerium Oxide Nano Composite Glassy Carbon Platform for the Quantification of Torseamide

Archana Pawaiya<sup>1</sup>,  
Nimisha Jadon<sup>2</sup> and  
Rajeev Jain<sup>1\*</sup>

- 1 School of Studies in Chemistry, Jiwaji University, Gwalior, India
- 2 School of Studies in Environmental Chemistry, Jiwaji University, Gwalior, India

## Abstract

Present communication reports, fabrication of a novel polymer film of polypyrrole (PPy)-cerium oxide (CeO<sub>2</sub>) nano composite by chemical polymerization on a glassy carbon electrode (GCE), and thus developed sensor was applied for the quantification of Torseamide drug. The fabricated nano composite was characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). Under optimized conditions, the anodic peak currents of Torseamide drug increased linearly with increase in concentration in the range of 100 ng/mL-6400 ng/mL. The prepared sensor showed good detection limit (33.85 ng/mL) and limit of quantification (102.58 ng/mL) sensitivity and repeatability. The proposed voltammetric sensor was also applied for the quantification of Torseamide in pharmaceutical formulation.

**Keywords:** Torseamide; Electrochemical sensor; Polypyrrole-cerium oxide nano composite; Square wave voltammetry; Differential pulse voltammetry

**Corresponding author:** Rajeev Jain

✉ rajeevjain54@yahoo.co.in

School of Studies in Environmental Chemistry, Jiwaji University, Gwalior-474 011, Madhya Pradesh, India.

**Tel:** +917512442766

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## Highlights

1. Torseamide is used to treat high blood pressure and fluid retention (edema) caused by heart or liver or kidney disease.
2. Sensitive methods are needed to detect Torseamide at low concentrations.
3. First voltammetric study on Torseamide.
4. A polypyrrole-cerium oxide nano composite based electrochemical sensor is developed.
5. The modified electrode detects Torseamide at ng level.

## Introduction

Torseamide (A) is a pyridine-sulfonyl urea type loop diuretic mostly used in the management of edema associated with congestive heart disease [1,2]. In the present studies polypyrrole/cerium oxide composite film modified glassy carbon sensor has been developed for the detection and quantification of Torseamide. On comparing the sensitivity of developed sensor with other electrodes i.e., bare glassy carbon electrode (GCE), polypyrrole modified glassy carbon electrode (PPy/GCE), cerium oxide

modified glassy carbon electrode (CeO<sub>2</sub>/GCE), it was observed that hybrid film of polypyrrole/cerium oxide exhibited increased electro catalytic activity and sensitivity for quantification of Torseamide as compared to bare GCE, PPy/GCE, CeO<sub>2</sub>/GCE. The practical use of the method is demonstrated by determining the concentration of Torseamide in the pharmaceutical sample.

## Experimental

### Reagents and chemicals

Pyrrole <99% (Himedia Laboratories Pvt. Ltd. Mumbai) was distilled under reduced pressure prior to use. Torseamide drug was obtained from commercial sources. Cerium (IV) oxide nano powder, <50 nm particle size (BET), >99.95% was obtained from Sigma Aldrich. Ammonium persulphate (APS) <98% was purchased from Thermo Fisher Scientific India Pvt. Ltd. Mumbai and Sodium p-toluene sulphonamide <98.5% from Himedia Laboratories Pvt. Ltd. Mumbai. All reagents were of analytical grade and used as received without further purification. Stock volume of torseamide (1 mgmL<sup>-1</sup>) for voltammetric measurements was prepared in ethanol 99.9%.

## Instrumentation

Electrochemical measurements were performed using a  $\mu$ Autolab Type III (Eco-Chemie B.V., Utrecht, Netherlands) potentiostat-galvanostat with 757VA Computrace software. The utilized electrodes were PPy-CeO<sub>2</sub> material modified glassy carbon electrode as working electrode, Ag/AgCl (3 M KCl) as reference electrode and platinum wire as auxiliary electrode. All solutions examined by electrochemical techniques were purged for 5 min with purified nitrogen gas. All pH measurements were made on a systronics digital pH model-361 meter fitted with a glass electrode and a saturated calomel electrode as reference, which was previously standardized with buffer solutions of known pH. Fourier transform infra-red (FTIR) spectroscopy (Parkinmer Spectrum Two) of PPy-CeO<sub>2</sub> nano composite sensor was studied in the frequency range 4000-400 cm<sup>-1</sup>. SEM, UV and XRD spectra of the nano composite films were recorded using a Zeiss SEM, DSC-60 plus (Shimadzu Corp. 00244), UV-1280 (Shimadzu Corp. 02059) and powder XRD (Ringku, Miniflex 600) respectively.

## Preparation of PPy-CeO<sub>2</sub> nano composite

The PPy-CeO<sub>2</sub> nano composite was prepared by the chemical oxidative polymerization method using ammonium persulphate (APS) as an oxidizing agent. Certain amount of the cerium (IV) oxide nanoparticles were added in 100 mL of ultra-pure water containing sodium p-toluene sulphonamide (1 M). Then the above mixture was ultrasonically dispersed and freshly distilled pyrrole (1.0 mL) was injected into the mixture with vigorous stirring at 0°C under nitrogen atmosphere. After 30 min, 20 mL of aqueous solution containing APS (0.90 g), as the oxidant, was added drop by drop to the above mixture to initiate the polymerization of pyrrole, stirring continued for 10-12 h, under ice-cold condition. The precipitated powder was washed with ultra-pure water and ethanol several times. Finally, the product was dried in vacuum (60°C for 24 h). For comparison pure PPy sample without CeO<sub>2</sub> nano composite was also prepared by the similar procedure.

## Fabrication of PPy-CeO<sub>2</sub> modified glassy carbon electrode

First of all bare GCE was washed with the help of ultra-pure water and polished with alumina powder of particle size (0.05  $\mu$ m to 0.2  $\mu$ m) on micro cloth pads successively followed by rinsing with ultrapure water till a clean mirror like finish is obtained. PPy-CeO<sub>2</sub> nano composite was dispersed in N, N-dimethyl formamide (DMF), and ultra sonicated for 6 hours to get a homogeneous suspension. The surface of GCE was casted with the help of 10  $\mu$ L of this suspension.

## Optimization of PPy-CeO<sub>2</sub> nano composite on GC surface

The responsive peak current for Torsemide oxidation increases significantly with an increment in the loading amount of PPy-CeO<sub>2</sub> suspension prepared in DMF up to 10  $\mu$ L, after that peak current starts decreasing. The 10  $\mu$ L loading amount of PPy-CeO<sub>2</sub> suspension shown highest conductivity and hence peak current. It may be due to the limited mass transport of Torsemide across a thicker film; therefore 10  $\mu$ L is taken as the loading amount of PPy-CeO<sub>2</sub> for electrode modification (**Figures 1 and 2**).

## Pharmaceutical preparation

Torsemide (0.1  $\mu$ g/mL) was dissolved in different solvents viz. water, acetonitrile (ACN), N, N-dimethyl formamide (DMF), acetone, methanol, ethanol, surfactants such as sodium lauryl sulfate (0.1% SLS), cetyl tri methyl ammonium bromide (0.1% CTAB), and 0.1% Tween-20. Solutions were sonicated for 10-20 min and centrifuged. Clear supernatant liquid was withdrawn. The solutions for recording voltammograms were prepared by mixing an appropriate volume of phosphate-buffered, stock solution and KCl (1.0 M).

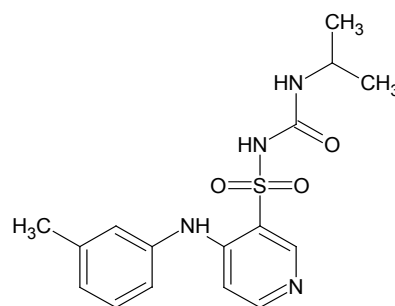
## Results and Discussion

### Scanning electron microscopy (SEM)

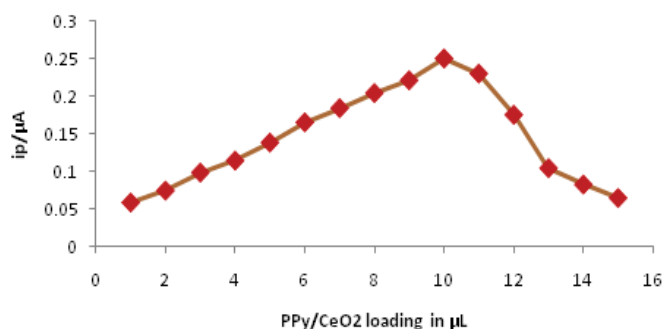
The morphology of PPy (**Figure 3(A)**), CeO<sub>2</sub> nanoparticles (**Figure 3(B)**) and PPy-CeO<sub>2</sub> nano composite (**Figure 3(C)**) were studied by SEM. **Figure 3(A)** shows that PPy has a uniform spherical flower like shape with some of these uniform spherical flowers less than 10  $\mu$ m. whereas **Figure 3(B)** exhibits irregular surface. The SEM image of PPy-CeO<sub>2</sub> shows that incorporation of PPy-CeO<sub>2</sub> results in a moderately increased electro active surface area. Moreover, on the surface of PPy-CeO<sub>2</sub>/GCE many micro-pores can be seen.

### Fourier transform infrared spectroscopy studies (FTIR)

The FT-IR transmission spectrum of PPy-CeO<sub>2</sub> is shown in **Figure 4**. In the spectrum, the specific absorption bands appear at 3507.68 cm<sup>-1</sup> of N-H stretching. The C-O stretching bending of cerium



**Figure 1 (A)** Chemical Structure of Torsemide.



**Figure 2** Effect of varying PPy/CeO<sub>2</sub> loading.

oxide can be observed at  $1124.34\text{ cm}^{-1}$  and  $937.62\text{ cm}^{-1}$ . The spectrum of PPy-CeO<sub>2</sub>, shows out-of-plane bending of the N-H bond located in pyrrole ring appearing at  $3507.68\text{ cm}^{-1}$ , while the sharp band at  $1124.34\text{ cm}^{-1}$  is assigned to the C=O bond stretching vibration present in the dendrimer moiety.

### Effective surface area

The effective surface area of the electrode was calculated in the presence of  $1.0\text{ mM K}_3[\text{Fe}(\text{CN})_6]$  by cyclic voltammetric technique using Randles-Sevcik equation [3].

$$i_p = (2.69 \times 10^5) ACD^{1/2} n^{3/2} \nu^{1/2}$$

Where, A is the effective surface area of the working electrode, C is the concentration of substrate, D is the diffusion coefficient, n is the number of electrons. For  $\text{K}_3[\text{Fe}(\text{CN})_6]$ ,

$$n=1, D=7.6 \times 10^{-6}\text{ cm}^2\text{s}^{-1}.$$

The effective surface areas of PPy-CeO<sub>2</sub>/GC, PPy/GC, CeO<sub>2</sub>/GC and bare GC electrodes were found to be  $1.90\text{ cm}^2$ ,  $1.21\text{ cm}^2$ ,  $0.99\text{ cm}^2$  and  $0.73\text{ cm}^2$  respectively. The electro catalytic effect of PPy/GC was greatly improved after incorporating CeO<sub>2</sub> and the fabricated PPy-CeO<sub>2</sub>/GC exhibited better electro catalytic activity in comparison to PPy/GC, and bare GC electrodes (Figure 5).

### X-ray diffraction analysis

The XRD patterns of the PPy-CeO<sub>2</sub> nano composite showed an intermediary characteristic between the cerium oxide and PPy for both the diffraction angle and peak intensity. The PPy showed a broad and relatively weak diffraction peak at  $2\theta=22^\circ$  from the intermolecular spacing of pyrrole which may be overlapped with the cerium oxide. Cerium oxide was scanned from  $10\text{-}80$  degrees with the scan rate  $2\theta\text{ min}^{-1}$  (Figure 6).

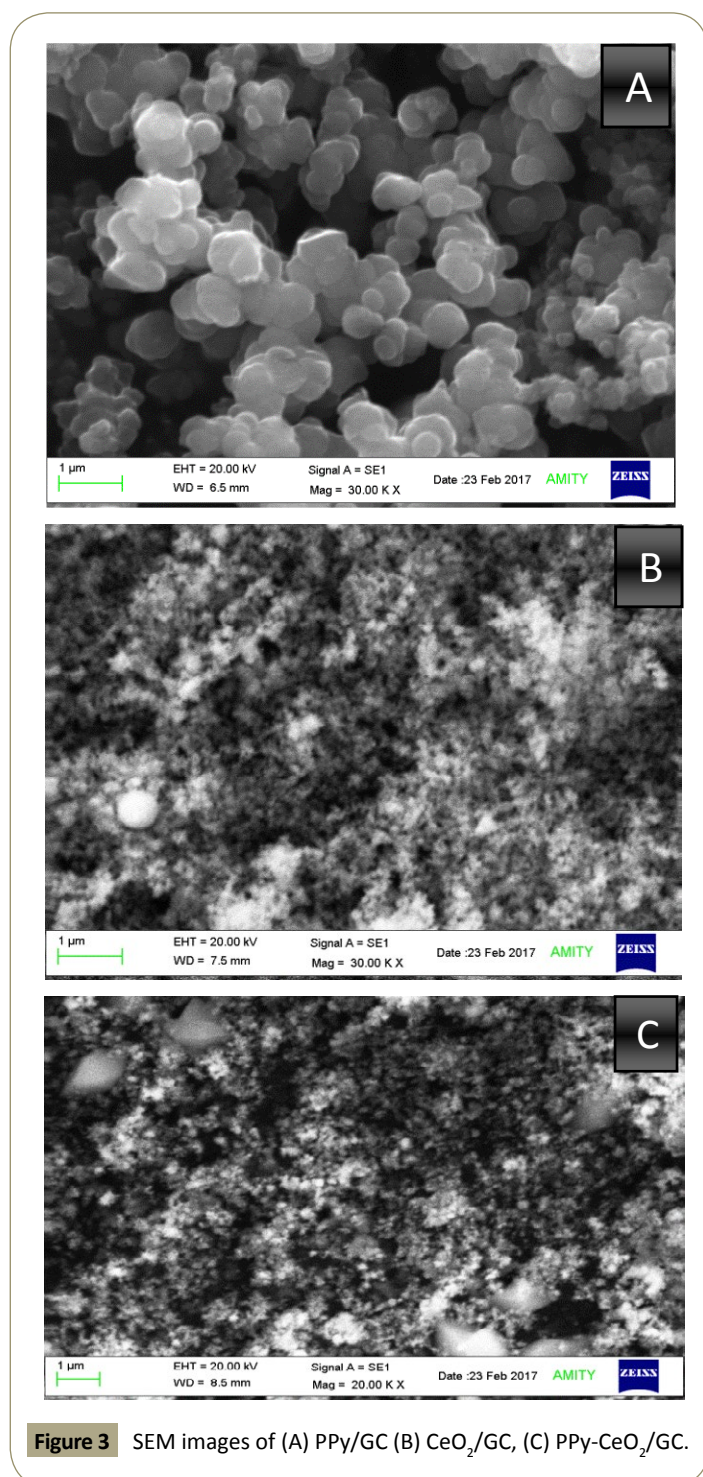
### Electrochemical impedance spectroscopy

The behavior of GCE before and after modification by PPy, CeO<sub>2</sub> and PPy-CeO<sub>2</sub> nano composite was also investigated by applying the EIS at room temperature. Figure 7 shows Nyquist plot of the unmodified GCE and PPy/GC, CeO<sub>2</sub>/GC and PPy-CeO<sub>2</sub>/GC in  $5.0\text{ mM} [\text{Fe}(\text{CN})_6]^{4-/3-}$  solution containing PBS (0.1 M, pH 7.0) at  $0.2\text{ V}$ . The EIS includes a semicircular and a linear part. The semicircular part at higher frequencies corresponds to the electron transfer limited process, and diameter is equivalent to charge transfer resistance (R<sub>ct</sub>). The linear part at lower frequencies corresponds to the diffusion process [4]. As shown in Figure 8, after modifying GC with PPy-CeO<sub>2</sub> nano composite the diameter of the semicircle decreases, which indicate that, the impedance of the electrode decreases in the presence of PPy-CeO<sub>2</sub> nano composite. The R<sub>ct</sub> values for unmodified GC and after modification with PPy/GC, CeO<sub>2</sub>/GC and PPy-CeO<sub>2</sub>/GC are  $410\text{ k}\Omega$ ,  $66.4\text{ k}\Omega$ ,  $361\text{ k}\Omega$  and  $4.70\text{ k}\Omega$ , respectively. These results demonstrate that PPy-CeO<sub>2</sub> nano composite have an excellent potential to be an electric conductive material which are able to accelerate the electron transfer [5].

### Electrochemical behavior of torsemide at PPy-CeO<sub>2</sub>/GC polymer nano composite sensor

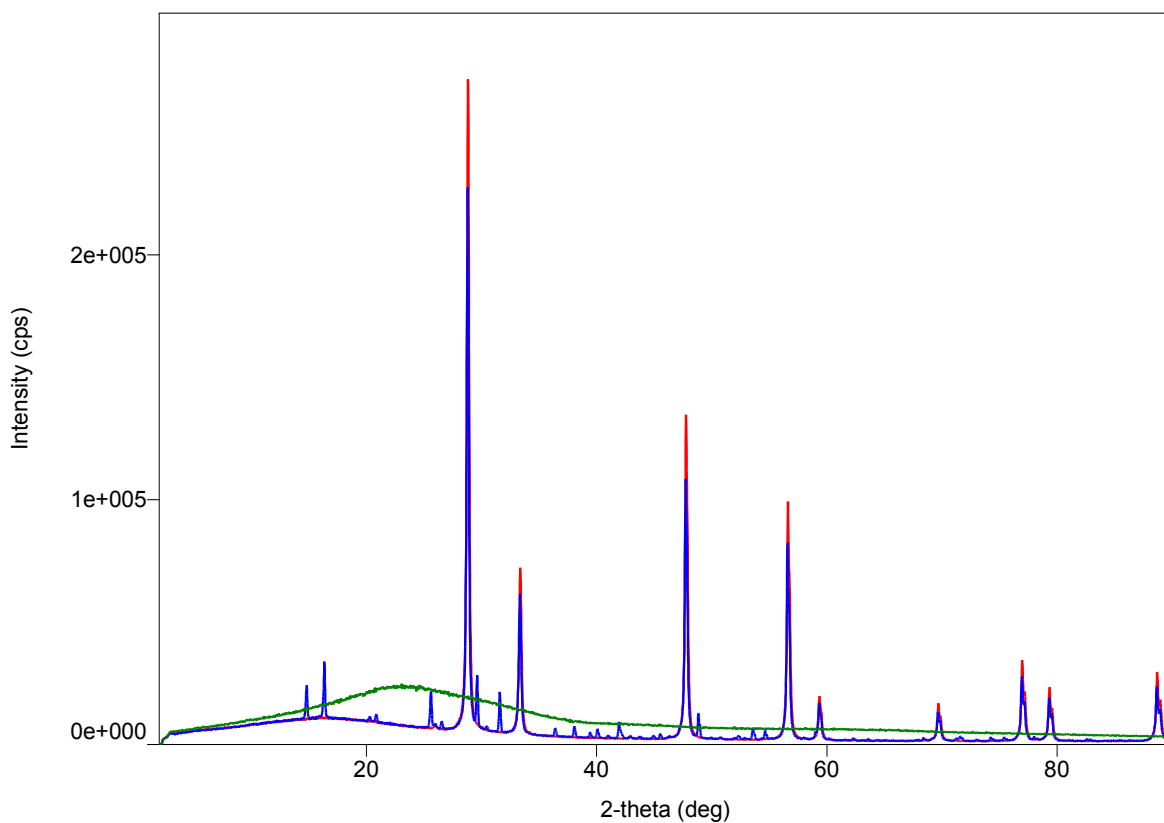
The electrochemical performance of Torsemide at PPy-CeO<sub>2</sub>/GC sensor was studied using square wave (SWV) and differential pulse voltammetry (DPV).

**Torsemide behavior in solubilized system:** Different organic solvent viz methanol, ethanol, acetone, water, acetonitrile, N, N-dimethyl formamide (DMF) and in different surfactants such as cetyl trimethyl ammonium bromide (0.1%CTAB), sodium lauryl sulfate (0.1%SLS) and 0.1%Tween-20 were used to study voltammetric behaviour of Torsemide. In square wave voltammogram maximum peak current was obtained in methanol (Figure 8).

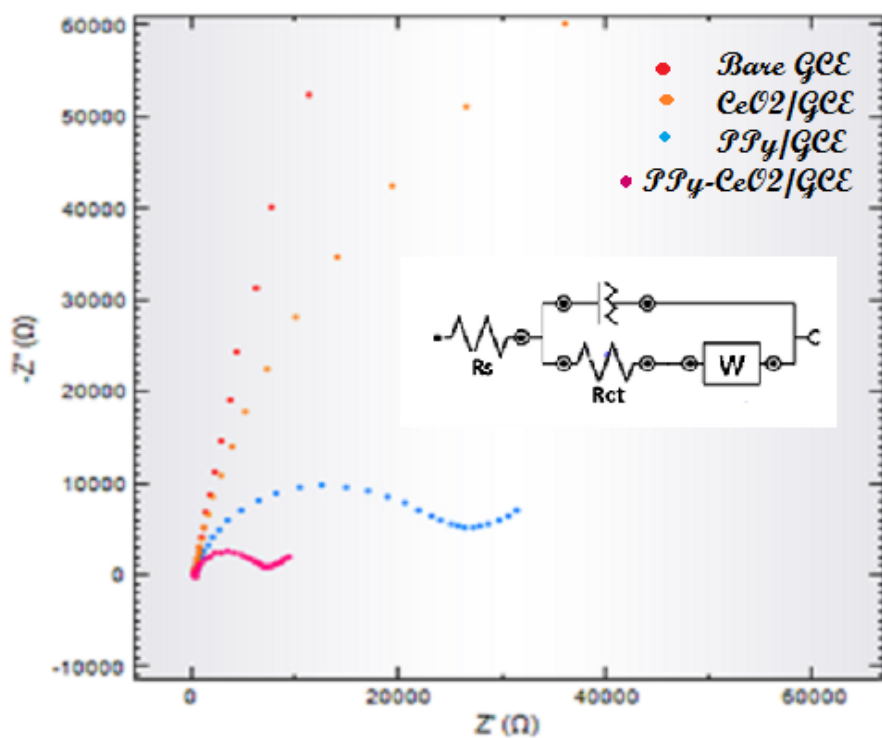


**Figure 3** SEM images of (A) PPy/GC (B) CeO<sub>2</sub>/GC, (C) PPy-CeO<sub>2</sub>/GC.

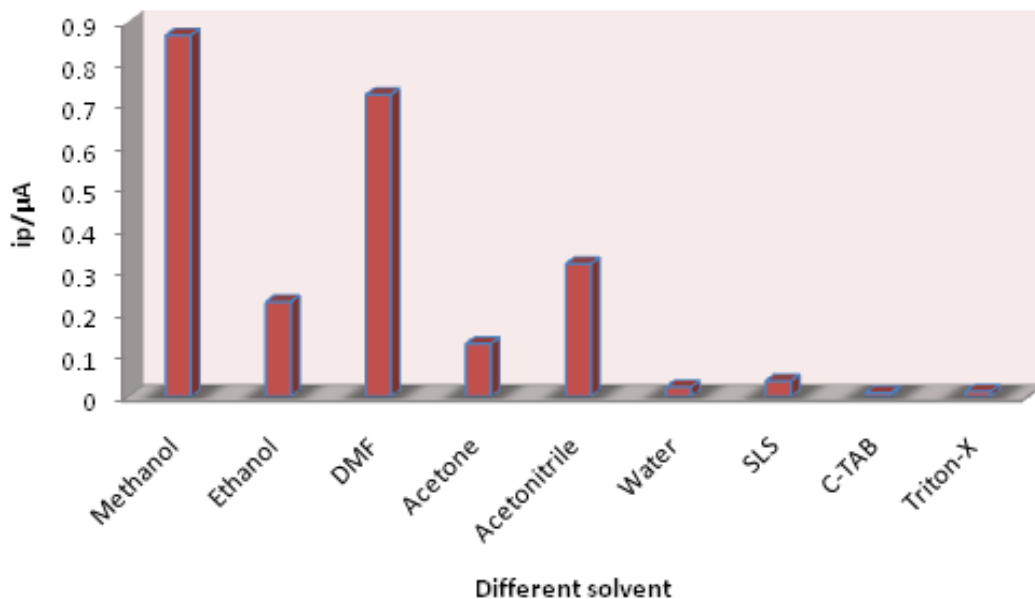




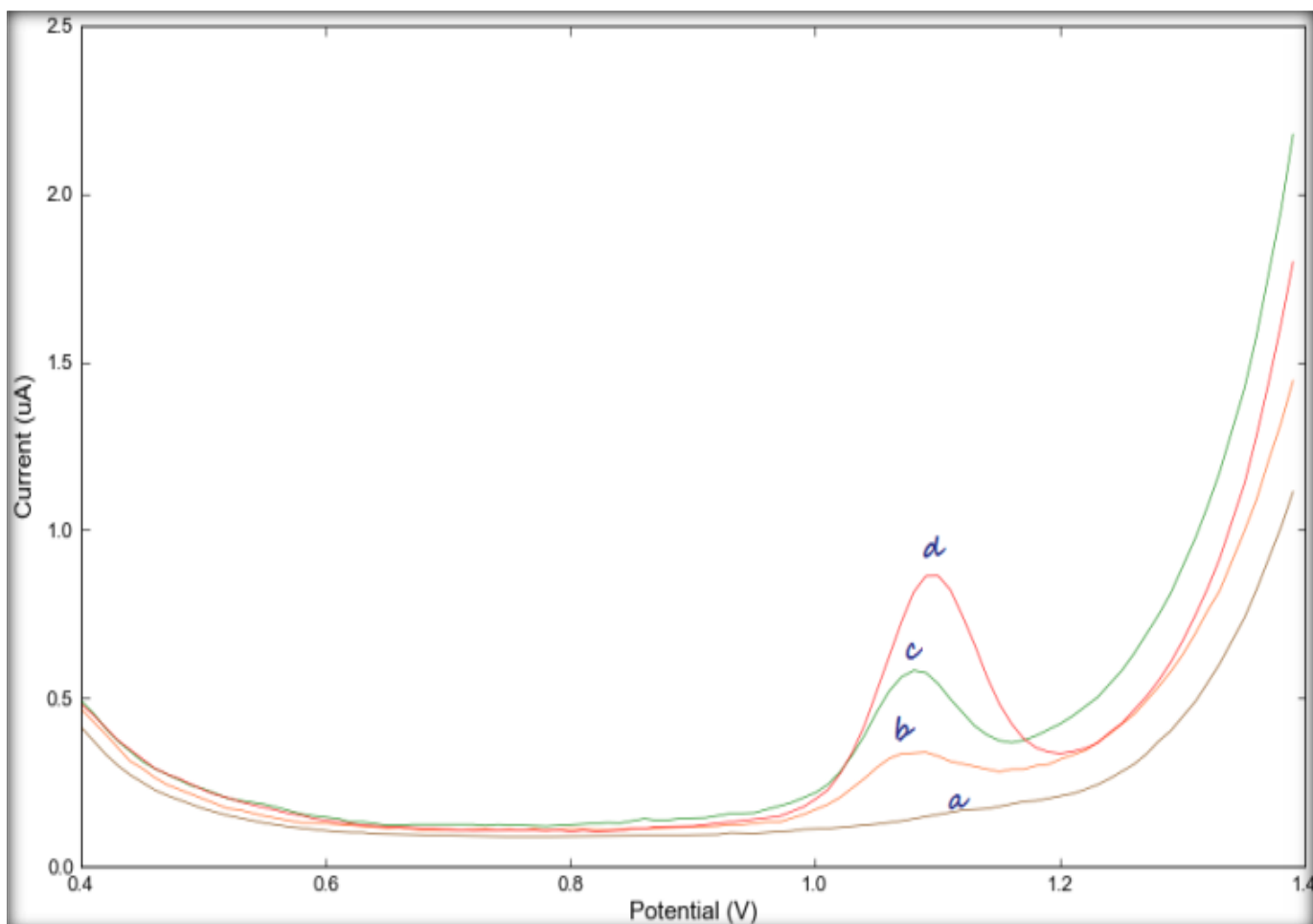
**Figure 6** XRD pattern of PPy and CeO<sub>2</sub>, PPy-CeO<sub>2</sub> nanocomposite.



**Figure 7** (A) Nyquist plot of GC (curve a), CeO<sub>2</sub>/GC (curve b), PPy/GC (curve c) and PPy-CeO<sub>2</sub>/GC in 5.0 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>], Inset: Equivalent circuit used to fit the impedance data.



**Figure 8** Comparison of peak currents of Torsamide in different systems.



**Figure 9** Square wave voltammetric behaviour of Torsamide (100 ng/mL); in methanol (pH 3.05) (a) GC (b)  $\text{CeO}_2/\text{GC}$  (c)  $\text{PPy}/\text{GC}$ , and (d)  $\text{PPy-CeO}_2/\text{GC}$ .

**Square wave voltammetric behaviour of torsemide:** Anodic peaks of Torsemide at bare GC (curve a), CeO<sub>2</sub>/GC (curve b), PPy/GC (curve c) and PPy-CeO<sub>2</sub>/GC (curve d) electrodes were observed at 0.040 μA/1.090 V, 0.110 μA/1.040 V, 0.288 μA/1.050 V and 0.962 μA/1.070 V. The anodic peak current obtained at PPy-CeO<sub>2</sub>/GC at least 25 times higher than that observed at GC, which is a clear evidence of good electro catalytic activity of PPy-CeO<sub>2</sub> toward Torsemide.

**Differential pulse voltammetric behaviour of torsemide:** Differential pulse voltammograms (DPV) were recorded at bare and modified electrodes. Anodic peak currents of Torsemide at 1.184 μA/1.040 V clearly indicate the electro catalytic activity of PPy-CeO<sub>2</sub>/GC sensor.

**Effect of deposition potential and time:** Different potential range, time and frequency have been analyzed for proper electro deposition of Torsemide particles into pre-synthesized PPy-CeO<sub>2</sub> nano composite film. The oxidation potential of Torsemide in electrolyte is found to be approximately 0.1 V, while the anodic peak current of Torsemide appears at more positive potential around 1.080 V. This shows a kinetic hindrance of the Torsemide in PPy-CeO<sub>2</sub> nano composite film. In this study, as the deposition

potential and time increased, it shows a decrease in peak current. Equation shows current time behaviour for the electrode position of Torsemide.

$$I(\mu\text{A})=0.012 (\text{mV s}^{-1})+0.124; r^2=0.997$$

### Validation of the proposed method

**Scan rate study:** Cyclic voltammetric behaviour of 100.0 μg mL<sup>-1</sup> Torsemide at PPy-CeO<sub>2</sub>/GC sensor has been studied at different scan rates ranging from 25 mV s<sup>-1</sup> to 125 mV s<sup>-1</sup> and shown in **Figures 9-11**. A linear relation of square root of scan rate (v<sup>1/2</sup>) and current (I) can be expressed by Equation:

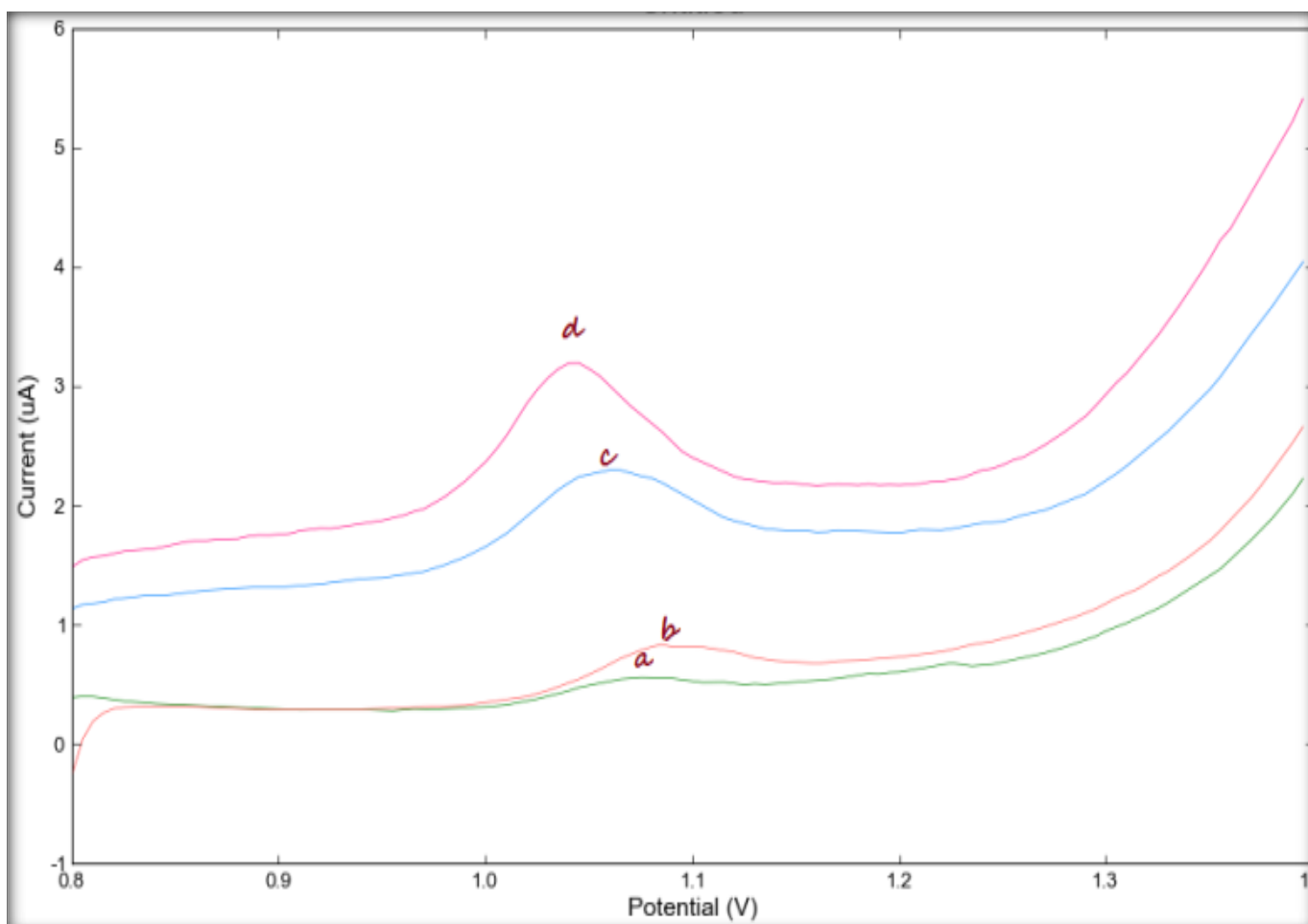
$$I (\mu\text{A})=0.018 (\text{mV s}^{-1})-2.532; r^2=0.981$$

Diffusion controlled process of the electrode reaction has been confirmed by the linear plot of log v vs log I expressed by the following Equation:

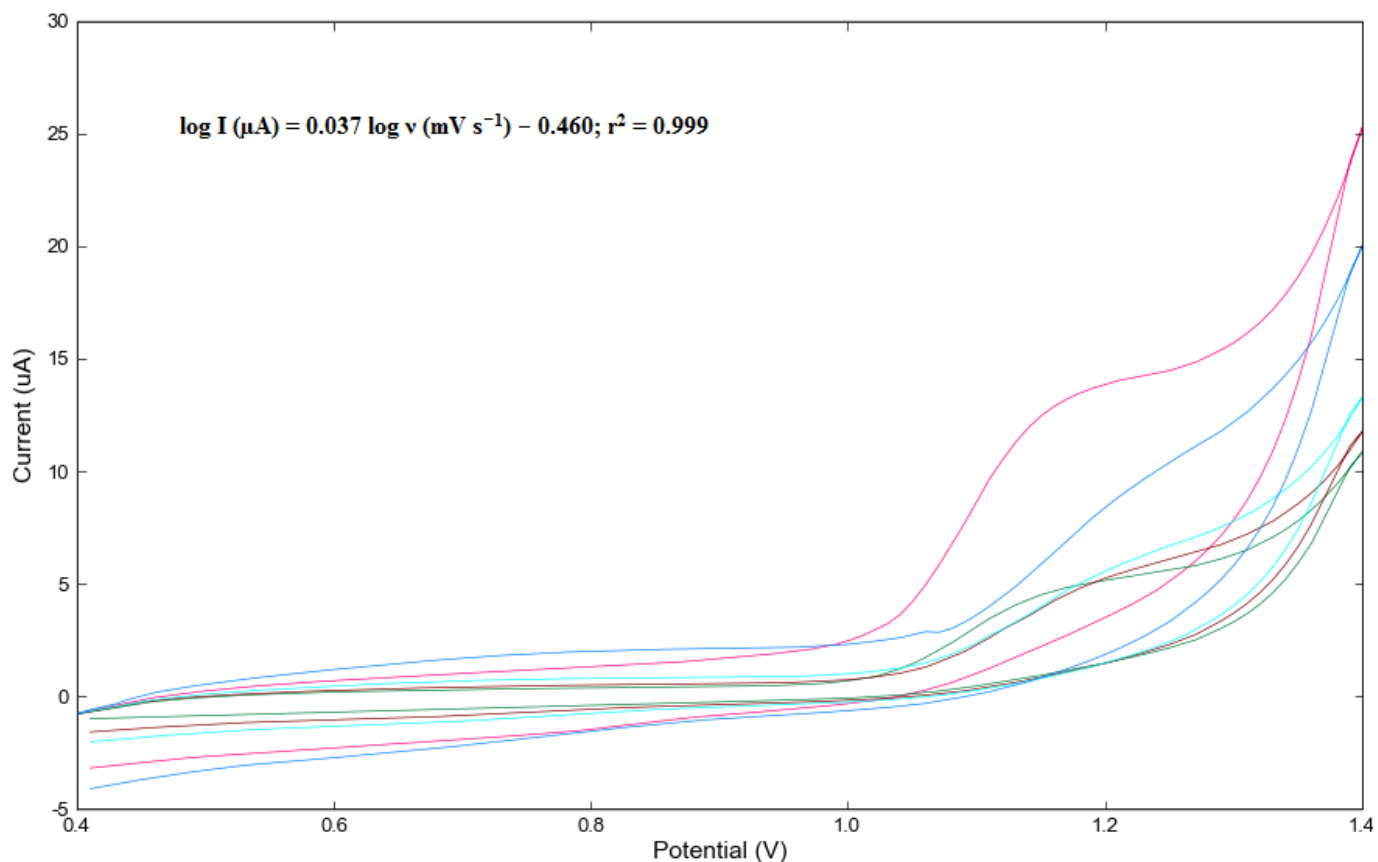
$$\log I (\mu\text{A})=0.037 \log v (\text{mV s}^{-1})-0.460; r^2=0.999$$

Obtained slope 0.037 confirms the diffusion controlled nature of the electrode process [6,7].

**Reproducibility and stability of sensor:** The stability of the



**Figure 10** Differential pulse voltammogram of 100 ng/mL, Torsemide in methanol (pH 3.05) at (a) GC (b) CeO<sub>2</sub>/GC (c) PPy/GC, and (d) PPy-CeO<sub>2</sub>/GC.



**Figure 11** (A) Cyclic voltammograms of Torsemide at PPy-CeO<sub>2</sub>/GC at pH 3.05 at different scan rates (a-e): 25, 50, 75, 100 and 125 mVs<sup>-1</sup> and (B) plot of sweep rate versus current.

PPy-CeO<sub>2</sub>/GC sensor for the oxidation of Torsemide was studied by recording the current response at a fixed Torsemide concentration of 100 ng/mL over a period of 1 week using the same PPy-CeO<sub>2</sub>/GC sensor stored in a dry place. This sensor maintained about 89% of the initial current response. It indicates that the PPy-CeO<sub>2</sub>/GC sensor has good long term stability for the determination of Torsemide. The repeatability of the modified sensor was investigated by replicate recordings of voltammograms at a fixed Torsemide concentration of 100 ng/mL. The coefficient of variance (%RSD) for the peak currents in SWV based on five replicates is 1.81%, indicating an excellent repeatability of the response at PPy-CeO<sub>2</sub>/GC sensor.

**Limit of detection and limit of quantification:** The sensitivity was investigated in terms of LOD (Limit of detection) and LOQ (Limit of quantification) by applying formula  $3S/b$  and  $10S/b$ , where  $S$  denoted as standard deviation of response i.e., current value and  $b$  is slope of calibration curve which is shown in **Figure 12**. In square-wave voltammetry (SWV) Torsemide drug current response is directly proportional to its concentration in the range of 10 ng/mL-6400 ng/mL (**Figure 12**) with the following linear regression equation (4):

$$I_p (\mu A) = 0.0318(\text{ng/mL}) - 0.1439, r^2 = 0.992$$

Limit of detection (LOD) and limit of quantitation (LOQ) are 33.85 ng/mL and 102.58 ng/mL respectively.

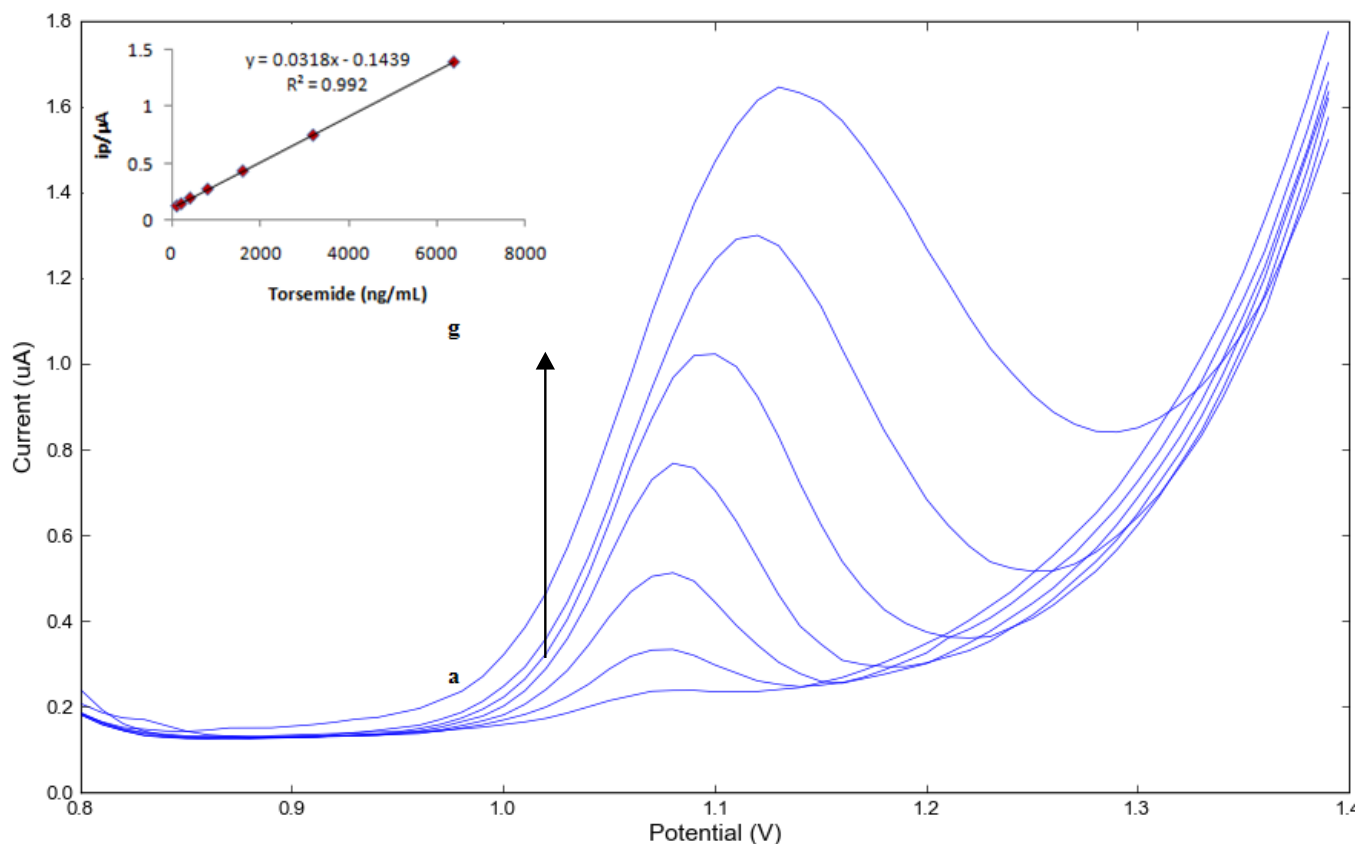
**Precision:** Intraday and interday precision study of Torsemide was carried out by estimating different concentrations of Torsemide. The results are reported in terms of %RSD. Inter-day variation of the same concentration of Torsemide was analyzed for three consecutive days by performing five measurements on each day. The average %RSD value is 0.88%, which demonstrates good repeatability of the method at the developed sensor (**Table 1**).

**Recovery:** For recovery studies, voltammetric measurements were made on three different levels of concentrations in six replicates with standard and formulation. Interferences of the pharmaceutical sample were also studied by comparing the obtained peak current values under optimized conditions. It is observed that the recoveries vary in the range of 98.37-99.79% (**Table 2**). These results show an excellent recovery for the Torsemide and also no interference of pharmaceutical sample was observed in the voltammetric quantification of Torsemide using PPy-CeO<sub>2</sub>/GC sensor.

## Analytical Application of Sensor to Pharmaceutical Formulation

The developed procedure could be applied successfully for the quantification of Torsemide in commercial pharmaceutical dosage forms at PPy-CeO<sub>2</sub>/GC sensor. The recovery study indicates that the PPy-CeO<sub>2</sub>/GC sensor can be effectively used for the selective





**Figure 12** (A) Square-wave voltammometric peak current of Torsamide in methanol (ng/mL); (B) Plot of current vs. concentrations of Torsamide in methanol (pH 3.05).

determination of Torsamide in pharmaceutical formulation. The quantitative analysis of the drug was based on the dependence of the anodic peak current on the concentration of Torsamide. The current is proportional to the concentration over a convenient range (100 ng/mL-6400 ng/mL) with good correlation coefficient ( $r^2=0.999$ ). This behavior provides a useful tool for the detection and quantification of the drug at low concentrations. The procedure can also be adopted for pharmacokinetic studies as well as for quality control laboratory studies.

## Conclusion

The PPy-CeO<sub>2</sub> nanocomposite was prepared by chemical polymerization method and used to modify GCE for the investigation of Torsamide by square wave voltammetry (SWV) and differential pulse voltammetry (DPV) in PB (pH 3.05). SEM, XRD, and FTIR studies showed the existence of a certain interaction between PPy-CeO<sub>2</sub>. The square-wave voltammogram of Torsamide at PPy-CeO<sub>2</sub>/GC sensor exhibited around 25 times enhancement in peak current, as compared to bare GC. The advantage of the PPy-CeO<sub>2</sub>/GC sensor is its favorable surface stability which provides great possibilities for successful application. Calibration plot reveals linearity within the range of 100-6400 ng/mL with a correlation coefficient of 0.992. Lower limit of detection (33.85 ng/mL) clearly demonstrates the sensitivity of the proposed method (**Table 3**).

**Table 1** Repeatability experiments at PPy-CeO<sub>2</sub>/GC sensor with 100 ng/mL Torsamide.

Time	Inter day repeatability		Intraday repeatability	
	Average current	RSD%	Average current	RSD%
Day 1	0.114	1.81	0.1144	1.81
Day 2	0.112	1.89		
Day 3	0.113	1.83		
Average current	0.113	0.88		

**Table 2** Recovery studies of Torsamide in the presence of pharmaceutical sample.

Added (ng/mL) <sup>a</sup>	Found (ng/mL)	Recovery (%)	RSD <sup>b</sup> (%)
0.25	0.24	98.37	1.81
0.50	0.49	98.71	1.89
0.75	0.74	99.79	1.83

**Table 3** Comparison of the present work with the other reported methods.

Reference method	Linearity range/Detection limit	References
RP-HPLC	50-100 µg/mL	[8]
Spectrophotometric	5-25 µg/mL	[9]
HPLC-UV	0.05-5 µg/mL	[10]
Spectroscopic	4-24 µg/mL	[11]
HPTLC	80-31 ng/mL	[12]
Spectrophotometric	0.94 µg/mL	[13]
SWV (PPy-CeO <sub>2</sub> /GCE)	33.85 ng/mL	Present work

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## References

- Dunn CJ, Fitton A, Brogden RN (1995) Torasemide. An update of its pharmacological properties and therapeutic efficacy. *Drugs* 49: 121-142.
- Roush GC, Kaur R, Ernst ME (2014) Diuretics: A review and update. *J Cardiovasc Pharmacol Ther* 19: 5-13.
- Jain R, Dhanjai D, Sharma S (2013) *Colloids Surf A* 436: 178-184.
- Suni II (2008) Impedance methods for electrochemical sensors using nanomaterials. *Trends Anal Chem* 27: 604-611.
- Ghoreishi SM, Saeidinejad F, Behpour M, Masoum S (2015) Application of multivariate optimization to electrochemical determination of methyldopa drug in the presence of diclofenac at a nanostructured electrochemical sensor. *Sens Actuators B Chem* 221: 576-585.
- Rather JA, Pilehvar S, Wael KD (2013) A biosensor fabricated by incorporation of a redox mediator into a carbon nanotube/nafion composite for tyrosinase immobilization: detection of matairesinol, an endocrine disruptor. *Analyst* 138: 204-210.
- Rather JA, Wael KD (2012) C60-funtionalized MWCNT based sensor for sensitive detection of endocrine disruptor vinclozolin in solubilized system and wastewater. *Sens Actuators B: Chem* 171-172: 907-915.
- Shukla R, Bhavsar N, Pandey V, Golhani D, Pal Jain A, et al. (2012) RP-HPLC Determination of Torsemide in Pharmaceutical Formulation by Liquid Chromatography. *Asian Journal of Bio and Pharm Sci* 2: 45-48.
- Bagade SB, Patil SK (2010) Spectrophotometric Estimation of Torsemide in Tablet Dosage Form Using Chemical Derivatization Technique. *International Journal of Pharmaceutical Quality Assurance* 2: 52-55.
- Cho HY, Kang HA, Pank CH, Kim SM, Kim DH, et al. (2005) Bioequivalence of Boryung Torsemide tablet to Toren Tablet (Torsemide 10 mg) by High Performance Liquid Chromatography/UV detector. *Kor Pharm Sci* 35: 323-328.
- Bhadja SA, Chhalotiya UK, Shah DA, Mehta FA, Bhatt KK, et al. (2014) Simultaneous Estimation of Torsemide and Amiloride Hydrochloride in their Pharmaceutical Dosage form by dual Wavelength UV Spectroscopic Method. *Adv J Pharm Sci Res* 2: 21-28.
- Kakde R, Chaudhary N, Barsagade A, Kale D (2011) Stability-Indicating HPTLC Method for Analysis of Torsemide in Pharmaceutical Preparations. *Acta Chromatographica* 23: 145-155.
- Krishna MV, Sankar DG (2008) Simple Spectrophotometric Determination of Torasemide in Bulk Drug and in Formulations. *E Journal of Chemistry* 5: 473-478.