Electrochemcial sensing of anti-diabetic drug using hierarchically deposited PB nanocubes on carbon nanospheres layered on transparent glass electrode

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Abstract

Prussian Blue nanocubes / carbon nanospheres (PB-CNS) heterostructure composed of perfectly cube and spherical composite on indium tin oxide (ITO) surface were developed for in vitro sensing of anti-diabetic drug i.e. Sitagliptin (STA). Detailed morphological, electrochemical, structural and optical characterization of PB-CNS/ITO electrode was done using DLS, SEM, EIS, CV. The sensor showed rapid response time (within 5 s) and the linear range from 0.001 to 10 mM with a shelf life of about 10 weeks under refrigerated conditions. We have also attempted to employ this electrode for assessment of STA in urine samples. The developed sensor exhibited high reproducibility and good storage stability.

Keywords: Sitagliptin, prussian blue nanocubes, carbon nanospheres, sensor, ITO electrode.

Introduction

Sitagliptin ([(R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [**1,2,4**] triazolo [4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine] phosphate monohydrate tablets; STA) is an oral anti-hyperglycemic drug that is used for treatment of type II diabetes [**1-3**]. The analysis of STA in the body is necessary in order to understand the complete mechanism of the drug metabolism like absorption, distribution, metabolism and elimination. Therefore, analysis of STA is of critical importance [**4-5**]. Various methods are available for therapeutic drug monitoring of STA in pharmaceutical dosage forms and in biological fluids including high pressure liquid chromatography (HPLC) [**6**], gas liquid chromatography (GLC) [**7**], capillary electrophoresis [**8**], near infrared spectroscopy [**9**], UV spectrophotometry [**10,11**], conductometry [**12**], or by visual titration [**13**]. Electrochemical techniques are best alternative for sensing STA, because it is easy to operate, can be made available at on-site for monitoring, require minimal sample preparation and sensitive [**14**]. Different nanomaterials are used in electrochemical sensing, because of extraordinary properties like high surface area, inert properties and helps in increasing electron transfer at the interface [**15-17**]. Among them nanocubes and nanospheres are attracting scientist for their exploitation due to their excellent properties as size and shapes are two determining factor for the performance of nanomaterials. In the present work we have used Prussian blue nanocubes/carbon nanospheres (PB-CNS) heterostructure composite for sensing of STA.

PB (ferric hexacyanoferrate) has found a wide use in biosensor field due to its exceptional electron mediating properties. Moreover, its properties such as non-toxicity, high electro catalytic activity, and low over-potential detection [**18**]. Low over-potentials eliminate interferences from ascorbic acid (AA), uric acid and glucose which makes it an important component of sensor. The wide use of PB in electrochemistry is also credited to the fact that PB and its analogues are the model of many polynuclear transition-metal hexacyanometalates having extremely well electrochromic, electrochemical, photo-physical, and magnetic properties and potential analytical applications [**19, 20**].

Carbon nanotubes (CNTs) and nanostructures were one of the flourishing fields of research which have been broadly researched in the past few years [21-22]. The colossal research attention in this field is principally motivated by the distinctive physical and chemical properties of carbon nanomaterials. However, the problem of high production cost of CNT restricts its use in research and hence it is highly desirable to synthesize carbon nanomaterials at relatively simple, low cost and

scalable technique. In the present study, the soot obtained by the pyrrolysis of commercially available kerosene has been used as sensing interface for the fabrication of sensor. Here in this report, we have used PB-CNS heterostructure composite on ITO surface for sensing Sitagliptin. The importance of the unique properties of the combination of PB and CNS has received research interest. CNS are considered to be good mediator for PB-modified electrodes due to their good electric conductivity and the property of being particle carriers and thus we have exploited this combination for sensing STA. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used for rapid and susceptible recognition of STA. To the best of our knowledge, till now there has been no such heterostructure composite formed for ultrasensitive detection of STA. Our research group has also exploited the same heterostructure composite on Fluorine doped tin oxide electrode for sensing metformin via electrochemical impedance analysis only. This heterostructure composite also proved to be amplified sensing interface for sensing STA, as is evident from the results of the CV and EIS.

Experimental

Materials

Potassium ferrocynide (K_3 [Fe $(CN)_6$]. $3H_2O$), hydrochloric acid (HCl), were procured from Sigma. All other chemicals were of analytic reagent grade. Double distilled water (DW) was used throughout the experiments. Stock standard solutions of STA were prepared by dissolving the appropriate amount in water.

Preparation of carbon nanospheres (CNS) on ITO transparent glass electrodes

For the preparation of the CNS, a laboratory lamp filled with kerosene oil was lighted and allowed to burn in open air. Indium tin oxide-coated glass (ITO) electrode was placed over the flame of the lamp in order to collect the soot emitted and was then allowed to dry. Electrode with carbon nanospheres deposited was obtained (CNS/ITO).

Hierarchical deposition of Prussian blue nanocubes (PB) on carbon nanospheres (CNS) /ITO (PB-CNS heterostructures)

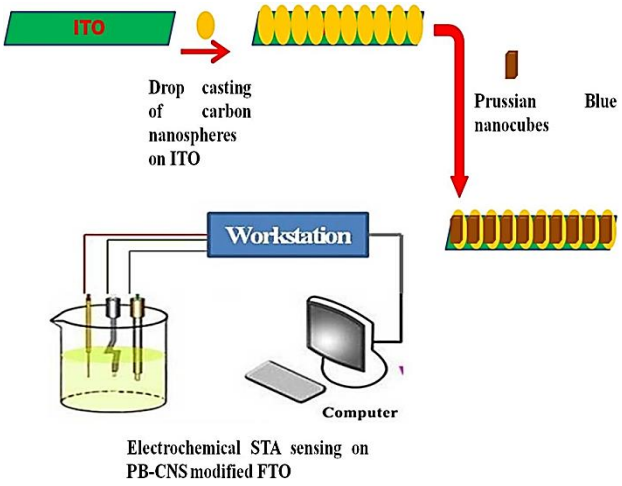
CNS/ITO glass electrodes were placed in a solution with K_3 [Fe $(CN)_6$]. $3H_2O$ in HCL (0.01 M) and kept for continuous stirring of 1 h. Then CNS/ITO glass electrodes containing beaker was kept in an oven for dry coating. Thus obtained PB-CNS heterostructure on ITO glass was washed with ethanol and water thoroughly to remove the unadsorbed nanostructures on the ITO surface. SEM and CV were used for morphological and sensing analysis of PB-CNS heterostructures/ITO working electrode **(Scheme 1).**

Pharmaceutical preparations

Sitagliptin sample was procured from Piramal Healthcare pharmaceutical company (Name: Glucophage). The stock solution of STA (10^{-1} mol L^{-1}) was prepared by dissolving the suitable quantity of STA in DW and kept at 4°C.

Characterization of modified sensing electrode (PB-CNS heterostructures/ITO)

Scanning electron microscopy (SEM) was used to determine the morphology of the modified sensing electrode PB -CNS heterostructures/ITO. DLS was also done in order to confirm the size of the nanospheres and the nano-composites. For DLS, the as prepared CNS and the PB-CNS composites were scraped out from the surface of the electrode and dissolved in distilled water. The prepared solutions were then sonicated and the samples were ready for DLS. Electrochemical measurements were done using EIS and CV. The electrodes composed of PB -CNS heterostructures/ITO as sensing electrode, Ag/AgCl as reference electrode and Pt wire as auxiliary electrode. The measurements were done in PBS (50 mM, pH 6.5, 0.9% NaCl) containing 5 mM $[Fe(CN)_6]^{3-/4}$. For assessment of the performance of sensor, parameters like precision and accuracy were also calculated. Different concentrations of STA were spiked into urine samples obtained from volunteers and the measurements were performed using CV by varying the potential range from -1.0 and +1.0 V at 100 mV s^{-1}.



Scheme 1. Schematic representation of the fabrication of the PB-CNS heterostructures/ITO modified sensor.

Results and discussions

SEM, DLS, CV and EIS assay of PB-CNS heterostructures modified sensor

The general morphologies of the modified electrode (PB-CNS heterostructures/ITO) were characterized by SEM and shown in **Fig. 1**. SEM imaging technique confirmed that the decorated structures are spherical shape carbon nanospheres with uniform morphologies **(Fig. 1 (A)).** It was observed from the SEM images that the diameters of the perfectly spherical shape carbon nanospheres showed homogenous structures. However, it can be seen that nanocubes of prussian blue are joined together through their bases with carbon nanospheres and formed heterostructure (PB-CNS) morphologies **(Fig. 1 (B)).**

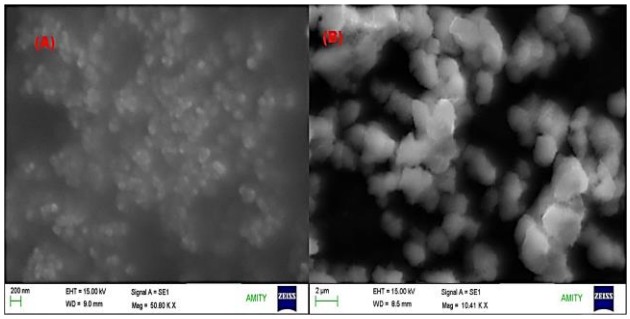


Fig. 1. SEM image of (A) CNS/ITO (B) PB-CNS heterostructures/ITO.

Further size characterization of the as-grown spherical shaped carbon nanospheres and PB-CNS heterostructure was done by the differential light scattering (DLS) and represented in **Fig. 2 (A)** and **(B). Fig. 2** exhibits the DLS spectrum of spherical shaped CNS (A) and PB-CNS heterostructure (B). A well defined peak was observed in the spectrum at 65 nm, which is for the pure CNS and 85 nm for PB-CNS heterostructure. The peaks observed that the sensing material formed is of nanomaterial range.

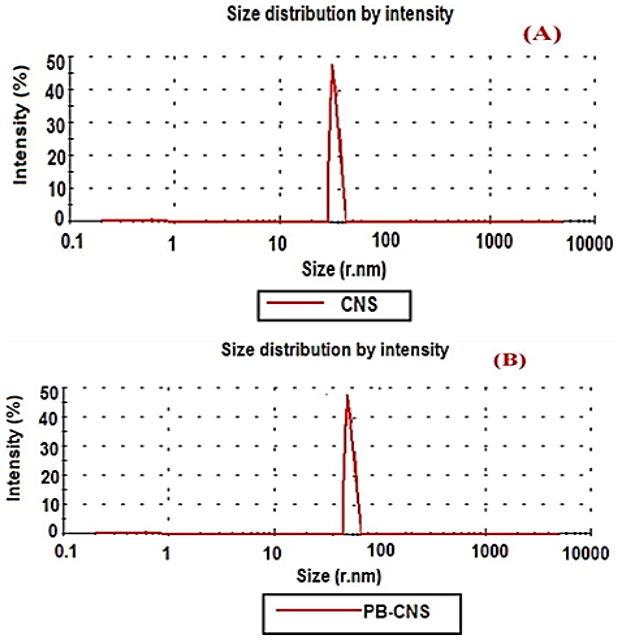


Fig. 2. (A) DLS spectra of carbon nanospheres (CNS) and (B) DLS spectra of prussian blue nanocubes-Carbon nanospheres (PB-CNS).

Fig. 3 (A) shows CVs obtained for ITO, CNS/ITO and PB-CNS heterostructures/ITO electrodes in between the potential range of -1.2 to + 1.6 V at a sweep rate of 100 mVs^{-1}. The increase in the anodic current obtained for the PB-CNS heterostructures/ITO [0.330 A, curve (iii)] compared to that of CNS/ITO [0.250 A, curve (ii)] indicates PB-CNS heterostructures forms conducting layer on ITO surface in comparison to the bare ITO [0.150 A, curve (i)]. EIS provides an efficient method to examine the electronic features of surface-modified electrodes. Nyquist plots are obtained for ITO, CNS/ITO and PB-CNS heterostructures/ITO electrodes; respectively in frequency range of 0.01–10 KHz. The value of charge transfer resistance (Rct) is obtained from the Nyquist diameter (real axis value at lower frequency intercept). It can be defined as hindrance between electrolyte and sensing interface which can be controlled by modifying the surface of sensing electrode with nanomaterials. It can be seen that Rct value for the ITO electrode (> 500 Ω) obtained is higher (i) than that of CNS/ITO (ii) electrode (350 Ω) and can be attributed to the conducting nature of carbon nanospheres that promotes permeability of $[Fe(CN)_6]^{3-/4-}$ to electrode surface. After decoration of prussian blue nanocubes (iii), RCT value further decreases to 290 Ω indicating formation of sensing electrode **(Fig. 3 (B)).**

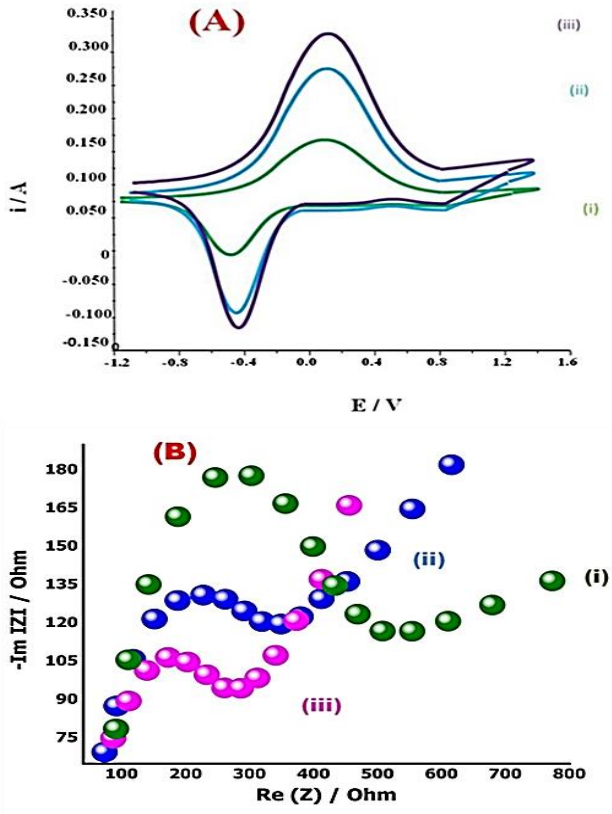


Fig. 3. (A) CV pattern of bare ITO (i), CNS/ITO (ii) and PB-CNS heterostructures/ITO (iii) in the potential range of -1.2 to +1.6 V s^{-1} at 100 mVs^{-1} in 50 mM phosphate buffer (pH 7). (B) EIS of (i) bare ITO, (ii) CNS/ITO and (iii) PB-CNS heterostructures/ITO containing 1 mM $Fe(CN)_6^{3-/4-}$ with 0.1 M KCl at 0.20 mV s^{-1} (frequency range of 0.01 Hz –10 kHz).

Electrochemical response of STA with PB-CNS heterostructures modified sensor

Scheme 1 shows the modification of ITO with PB-CNS heterostructure composed of spherical and cube shaped PB-CNS. The sensing electrode in the presence of STA showed redox process. The triazole/triazolium redox couple in the STA shows quasi-reversible oxidation in the potential scan from -0.1 to +0.1. The oxidation process is related to oxidation of the triazole group to triazolium group, and then this oxidized form obtained is reduced back at negative potential. These processes are quasi-reversible [27].

Fig. 4 (A) displays the cyclic voltammograms for the PB-CNS heterostructure without and with 1.0 mM STA in 50 mM PBS (pH 7, 0.9% NaCl) containing 5mM $[Fe(CN)_6]^{3-/4}$ in the range of -0.1 to +0.125 V at scan rate

of 100 mV/s. Low sensing signal observed from the modified electrode in the absence of STA **(Fig. 4 (A) (i))** however a pair of well-defined redox peaks appear from the modified electrode in presence of 1 mM STA **(Fig. 4 (A) (ii)).** The change in redox peaks can also be observed from the CV curve of PB-CNS heterostructures/ITO electrode with and without STA in PBS. This may be attributed to the fact high surface area of PB-CNS heterostructures facilitates the fast and direct electron transfer.

Electrochemical response of PB-CNS heterostructures modified sensor with different concentrations of STA.

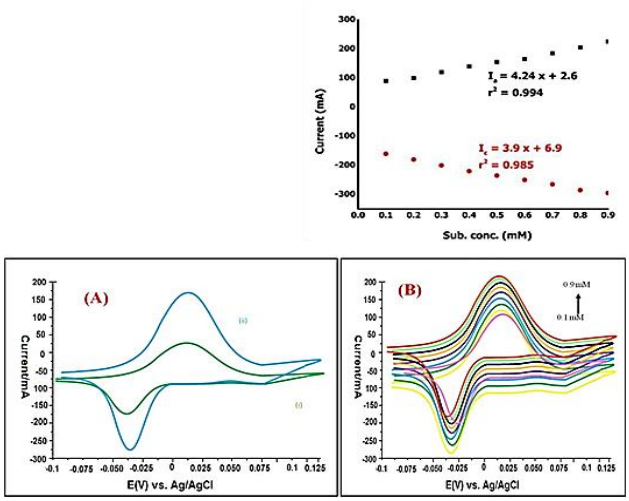


Fig. 4. (A) CV response of PB-CNS heterostructures/ITO modified sensor with STA (ii) and without STA (i) (B) CVs of PB-CNS heterostructures/ITO using 0.1 to 0.9 mM Sitagliptin, [INSET: calibration plot between susbtrate concentration (mM) and current (A)]

In **Fig. 4 (B)** the cyclic voltammograms are recorded at different concentrations of STA ranging from 0.1to 0.9 mM of STA. All these experiments were measured at PB-CNS heterostructures/ITO electrodes in 50 mM PBS buffer (pH 7) 100 mVs^{-1}. The experiments were carried out in duplicate sets, thereby disclosing the reproducibility of the system surrounded by 1%. Limit of detection was found to be 0.001 mM. The extent of the oxidation peak at about +0.02 V (due to the quasi-reversible redox processes) increases owing to the rising concentrations of STA. It is also very obviously visible from the voltammograms that the response current also increases on subsequent additions of STA. The incubation time for the sensing electrode was very fast, i.e. less than 5 s, which display am elevated electron transfer quality of the PB-CNS heterostructures.

Study of electrochemical response of PB-CNS heterostructures modified sensor with varying pH, temperature and time

The effect of pH on the oxidation STA was studied over the pH range of 4.0 – 9.0 at a steady STA concentration of 1.0 mM and potential from − 0.3 to + 0.75 V 100 mV/s as represented in **Fig. 5 (a).** There is dependency of cathodic peak to pH range. Highest response was observed for pH 9. With increase in pH there is a shift in the cathodic peak potential to more negative values while the anodic peak shift is not significant. It means oxidation process involved in the redox process is not affected as compared to reduction process. It can be ascribed to reactions involving oxhydryl groups as reported by Itaya et al. [18]. So, the optimum pH was kept at pH9, and was selected for the subsequent experiments. Thus, the high-sensitivity and limit of detection for the STA sensor can be said due to the heterostructure morphologies of the used PB-CNS. The effect of temperature was also studied by varying it from 15 to 40 ^{0}C. The highest signal was observed at 35 ^{o}C, suggesting the PB-CNS sensor works finest at room temperature **(Fig. 5 (b).** A steady potential was also applied for a span of 5 s to 55 s. **Fig. 5 (c)** shows that the maximum amplification was obtained at 5 s and no considerable differences were observed from 10 s to 55 s. Consequently, pH of 9, temperature of 35 ^{o}C and 5 s response time were found to be the optimum conditions.

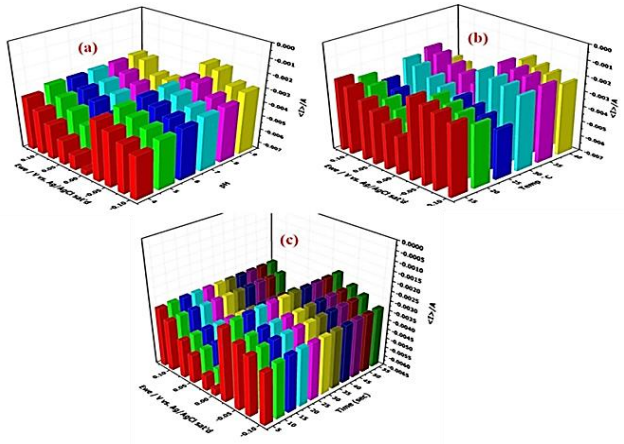


Fig. 5. 3D representation of the cyclic voltammograms at different (a) pH (b) temperature (c) time.

Analytical application and validation of PB-CNS heterostructures modified sensor

In order to evaluate analytic recovery, precision and accuracy of proposed method, known amount of added STA was calculated by PB-CNS heterostructures modified sensor. 97.8 ± 1.2 and 98.1 ± 1.9 were calculated as the mean analytic recoveries of 300 and 400 mM STA respectively.

Table 1. Selectivity analysis of PB-CNS modified ITO electrode for STA analysis (at).

Interferents	Relative response (%)
Glucose	110
Ascorbic acid	130
Urea	100
Uric acid	97
Cholesterol	100
Bilirubin	100
Pyruate	100

According to **Table 1,** the PB-CNS sensor is found to be very specific for the determination of STA. The reproducibility (single day; within batch) and reliability of the PB-CNS modified sensor was assessed on the basis of ten concentrations of STA spiked urine samples and five

times again after storage at −20 ◦C **(Table 2).** The PB-CNS electrode was found to be 99% accurate for measuring different concentrations STA in spiked urine samples. As per the values of Rct, which increased only to 1 % after 1 week and 20 % after 10 weeks, the sensor is said to be stable. The comparison of the various parameters of other sensors developed for detection of STA is presented in **Table 3.**

Table 2. Reproducibility of STA by PB-CNS modified ITO electrode obtained in spiked urine samples.

Spiked Urine Samples (μM)	Present Method (μM)
10	10
20	21
40	42
60	60
80	80
100	102
200	205
300	310
400	400
500	500

Table 3. A comparison of analytical characteristics of various STA sensors.

Matrix/ method	Response time	Limit of Detection (μM)	Linear range (μM)	Stability	References
HPLC–UV/Chromatography	NR	0.1	1–75	NR	23
Linear sweep voltammetry (LSV), differential pulse voltammetry (DPV)	15 s	0.018 and 0.059	0.05 to 4	NR	24
Cyclodextrins (β-CDs) based polyvinylchloride (PVC) electrodes /potentiometric	8 s	7	1 to 10000	NR	25
$BiVO_4$ modified carbon paste electrode	20 s	400	700 to 6000	NR	26
*PB nanocubes/ carbon nanospheres heterostructure/ITO	5 s	1	1 to 10000	1 month	Present

Conclusion

The present work demonstrates the use of PB nanocubes/ carbon nanospheres heterostructure composite on ITO glass as sensing platform for detection of STA. The quasi-reversible processes are restricted by the STA diffusion to the electrode surface. The proposed electrode shows CV graphs in the concentration range of 0.001 -10 mM, analytical recovery from 97.8 ± 1.2 and 98.1 ± 1.9 and 99% accuracy towards the measurement of STA. The present investigation of this PB-CNS nanocomposites lay base for being commercialized as a device for further use in clinical applications.

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Author's contributions

Conceived the plan: J.N., C.S.P; Performed the expeirments: C. S.; Data analysis: V.K., J.N., C.S.P; Wrote the paper: J.N., C.S.P, C. S. Authors have no competing financial interests.

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