

# Electrochemical preparation of composite of poly brilliant cresyl blue (PBCB)–poly 5-amino-2-naphthalenesulfonic acid electrode and electrocatalytic application

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**Abstract** Poly brilliant cresyl blue (PBCB) and poly 5-amino-2-naphthalenesulfonic (PANS) polymer composite modified electrode was fabricated by the electrochemical polymerization of brilliant cresyl blue and 5-amino-2-naphthalenesulfonic acid. When compared polymer composite electrodes with PBCB and PANS electrode, it showed enhanced electrochemical property. The morphology of the resulting composite electrode was characterized by AFM, and the electrochemical properties of the modified electrode were characterized by cyclic voltammetry and amperometry. The composite electrode showed surface-confined and pH-dependent electrochemical property. The composite electrode exhibited high catalytic behavior toward the reduction of hydrogen peroxide at low overpotential. The detection limit and sensitivity of the electrode toward  $\text{H}_2\text{O}_2$  detection was  $5 \mu\text{M}$  and  $1 \mu\text{A}/\text{mM}$ , respectively, and response time was less than 10 s for hydrogen peroxide.

**Keywords** Brilliant cresyl blue · Composite electrode · Electrocatalysis · Hydrogen peroxide

## Introduction

Chemically modified electrodes (CME) are very useful in a series of situations where the analyte requires high over-

potential [1]. This undesirable experimental condition enables other species to interfere in electroanalysis and the electrocatalytic determinations. The CME technique is applied in order to lower the overpotential and can result in an improvement of sensitivity and selectivity. Basically, these kinds of electrodes can be synthesized by anchoring the desired chemical species to direct the process to a very specific application [2]. Many immobilized species are able to mediate the electron transfer processes and, consequently, can be used to fabricate electrochemical sensors [3–5]. However, for various reasons concerning the stability of the irreversibly adsorbed mediator layers, fast heterogeneous electron transfer between electrode and adlayer of the mediator, fast chemical redox reaction between adsorbed mediators and catalytic substrate solution, etc., phenoxazine and phenothiazine mediators are often considered as being most suitable in obtaining CMEs for the electrocatalytic application.

Phenoxazine and phenothiazine mediators are incorporated on the surfaces nafion [6] and DNA film [7, 8] in order to improve electron transfer rate, electrochemical property, selectivity, and sensitivity. Malinauskas et al. [9] and Kubota et al. [10] incorporated dyes into metal phosphate of zirconia and titanium, respectively, and Zaitseva et al. [11] immobilized dyes on porous silica–zirconia–antimonia mixed oxide and have shown good electrochemical properties. Recently, some work has been published using silica gel or zeolite as inert support matrix of the electron mediator species in carbon paste electrodes [12–14]. The use of these materials may be important for catalytic purposes due to such characteristics as high surface area and porosity, facility of mediator immobilization and mechanical resistance [15], and different azines [16] like methylene blue [17], thionine [18, 19], and azine derivatives [20–22] have been electropolymerized at elec-

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trodes, and some of them have been used as amperometric sensors for NADH. As compared to adsorption, electropolymerization presents more versatile technique for immobilization of azine type redox mediators since wide variety of electrode materials could be involved in this process [23–26]. Also, it is expected that electropolymerized layers should be more stable as compared to adsorbed ones. Subsequently, polymer-modified electrodes have received great attention in recent years, as polymer films have good stability, reproducibility, more active sites, homogeneity in electrochemical deposition, and strong adherence to electrode surface [27–31]. Electropolymerization is a good approach to immobilize polymers to prepare modified electrode as adjusting the electrochemical parameters can control film thickness, permeation, and charge transport characteristics. Recently, polyaniline nanonetworks on *p*-aminobenzene sulfonic acid functionalized glassy carbon electrode [32], and DNA/poly(*p*-aminobenzene sulfonic acid) composite electrode [33] has been reported for simultaneous determination of ascorbic acid and uric acid and shows good stability. Similarly, positively charged dye, brilliant cresyl blue (BCB), and negatively charged ANS were electropolymerized and obtained the composite of the PBCB–PANS electrode since the composite electrode has shown superior electrochemical property than polymerized dye or PANS alone. Indeed, Hatchett et al. have reviewed composites of intrinsically conducting polymers as sensing nanomaterials and pointed out that composites of CPs properties have superior over CPs alone [34]. We [35] have also observed enhanced electrochemical properties in the composite electrode of bromo thymol blue–PEDOT.

## Experimental

ANS and BCB were purchased from Sigma Aldrich. Stock solutions H<sub>2</sub>O<sub>2</sub> were prepared each time freshly from 34% solution (purchased from Wako). All reagents were of analytical grade and used without any further purification. Solutions were prepared with double distilled water. High purity nitrogen was used for deaeration. The buffer and sample solutions were purged with highly purified nitrogen for at least 10 min prior to the experiments. Nitrogen atmosphere was maintained over the solutions during experiments.

Electrochemical experiments were performed with CH Instruments (Model CHI-400) using CHI-750 potentiostat. Glassy carbon electrode (geometric area=0.07 cm<sup>2</sup>) obtained from BAS served as a working electrode. Pt wire act as counter electrode and Ag/AgCl with the saturated KCl solution used as reference electrode. All the potentials given in this paper were referred with respect to Ag/AgCl (saturated KCl solution) reference electrode.

Prior to modification, glassy carbon electrode was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and used. After being cleaned, the electrode was immersed into 0.1 M PBS solution, pH 7 containing 1 mM ANS and 0.5 mM of BCB, and the potential of working electrode was cycled between –0.75 and 1.0 V at the 100 mV/s for 525 s to prepare PBCB–PANS electrode.

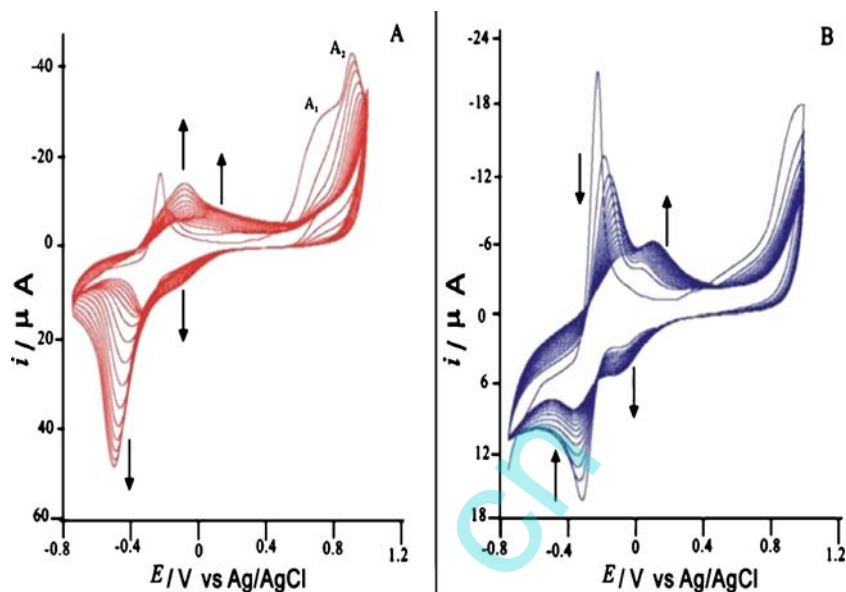
## Results and discussion

### Electrochemical preparation of composite of PBCB–PANS electrode

Preparation of composites of PBCB–PANS electrode was carried out as described in the “Experimental” section. Cyclic voltammograms (CVs) of Fig. 1a indicate formation of composite of PBCB–PANS when the glassy carbon electrode was scanned in the potential range of –0.75 to 1.0 V at the scan rate of 100 mV/s. The potential sweep range, especially the upper limit, is one of the most important factors for the preparation of PBCB–PANS composite electrode. If the potential sweep is confined within the potential region of 0.3 to –0.75 V, a simple CV is observed with the formal potential  $E^{O'}$ , –0.31 V. This value corresponds to redox reaction of BCB monomer (figure not shown). This steady-state cyclic voltammogram persists indefinitely provided that the potential is not made too positive. The sweep in this potential range results in no substantial PBCB film formation. However, electropolymerization of BCB (Fig. 1b) was observed, when the electrode is scanned in the higher anodic potential region at around 0.9 to 1.0 V (vs Ag/AgCl). The gradual decrease of the main cathodic and anodic peak currents of the BCB redox couple at around –0.31 V was well seen after repeated potential scans (Fig. 1). Simultaneous with the decrease of the characteristic redox peaks of the adsorbed species, a new pair of flattened redox couple with formal potential of  $E^{O'} = -0.02$  V in the region of higher potential values (Fig. 1b). The changes observed could be interpreted in the usual way. At a high anodic potential exceeding ca. 0.9–1.0 V, the electrooxidation of the adsorbed form of BCB proceeds, yielding some highly reactive species like cation radicals, which initiate the electropolymerization of the adsorbed dye. A gradual decrease of both characteristic redox peaks, as well as the anodic wave in the high potential region, indicates a gradual conversion of BCB to its polymeric derivative.

Electropolymerization of BCB in the presence of ANS is addressed to prepare composite of PBCB–PANS electrode, as shown in Fig. 1a. During anodic scanning of electrode,

**Fig. 1** **a** CVs of PBCB–PANS electrode from solutions containing 0.5 mM ANS and 0.5 mM BCB in 0.1 M PBS solution, pH 6.7, PBS for modification. Scan rate, 0.1 V/s. **b** CVs of PBCB electrode from solutions containing 0.5 mM BCB in 0.1 M PBS solution, pH 6.7, for modification. Scan rate, 0.1 V/s



two irreversible oxidation peak  $A_1$  and  $A_2$  were obtained at the potential of 0.7 and 0.9 V corresponding to oxidation of  $-\text{NH}_2$  group of ANS and BCB oxidation, respectively, and composite of PBCB–PANS electrode was obtained. Moreover, during this process, two reversible redox couple was obtained at the  $E^{O'}$  of  $-0.31$  and  $-0.02$  V corresponding to monomer of BCB and polymer of BCB and or redox peak of ANS, respectively. The redox peak at  $-0.02$  V might be due to overlapping of polymer of BCB and ANS. Importance of this work and close observation is that the monomer peak of BCB at  $E^{O'}$  of  $-0.31$  increased when electrode was scanned in the solution containing BCB and ANS. The increase in the monomer peak might be due to two possible reasons: there are (a) well-known electrostatic interaction between positively charged BCB and negatively charged sulfonate group of ANS and (b) sulfonate group of ANS may have the catalytic effect to augment the monomer growth of BCB dye during electropolymerization. Barsan et al. [36] has studied the effect of sulfonate anion as an electrolyte on the electropolymerization for the preparation of poly(phenazine) electrode and found that monomer peak increased on the successive cycles and concluded that sulfonate group, which is present in the electrolyte, has catalytic effect.

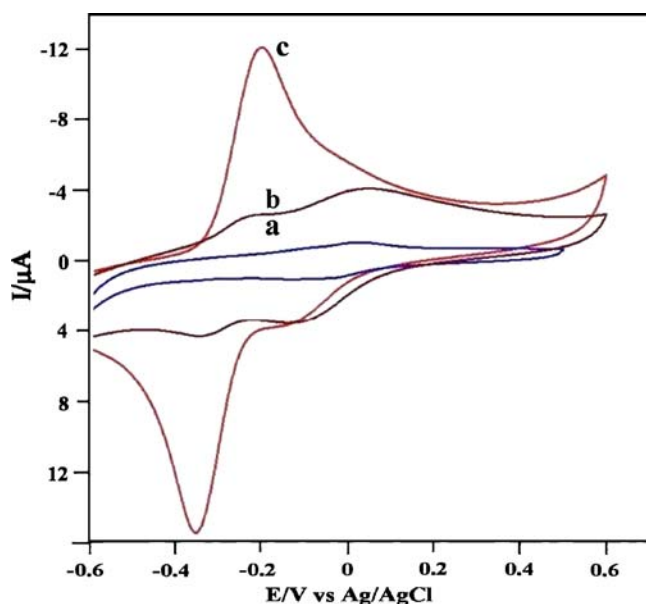
One more interesting result of this work, if one looks at Fig. 1a, is the monomer peak current growing steadily up to the fifth cycle at  $E^{O'}$  of  $-0.31$  while at the same time anodic peak current of  $A_2$  decreased. Interestingly, we could clearly see the sudden growth of peak current after the fifth cycle by the same time anodic peak of  $A_2$  also increased for 5 to 7 cycles and then decreased. We are not able to understand the exact reason for this observation. Meanwhile, Ivanova and Karyakin [37] have observed a

similar property in FAD during electropolymerization, which is having azine unit in the backbone of its structure, i.e., the monomer irreversible oxidation peak current is gradually increased in the course of continuous cycling. Such significant improvement of monomer oxidation in the course of film growth is the property of highly conductive polymers like polypyrrole, polyaniline, etc. From the above point, we concluded that a similar type of property is expected in the case of composite film of PBCB–PANS. We did not observe such irreversible anodic peak current growth when we electropolymerized BCB and ANS individually. So, we suggest that formed film might be the result of the formation of composite of PBCB–PANS.

In addition, we compared currents of PBCB, PANS, and PBCB–PANS electrode. After modification, electrode was scanned in PBS solution (base electrolyte only), and results of voltammogram of PBCB, PANS, and PBCB–PANS electrode are shown in Fig. 2. The PBCB–PANS (Fig. 2c) electrode current was ten times higher than PBCB electrode (Fig. 2b); such significant enhancement in the current might be raised due to the presence of PANS in the composite of PBCB–PANS electrode and electrostatic interaction between BCB and ANS.

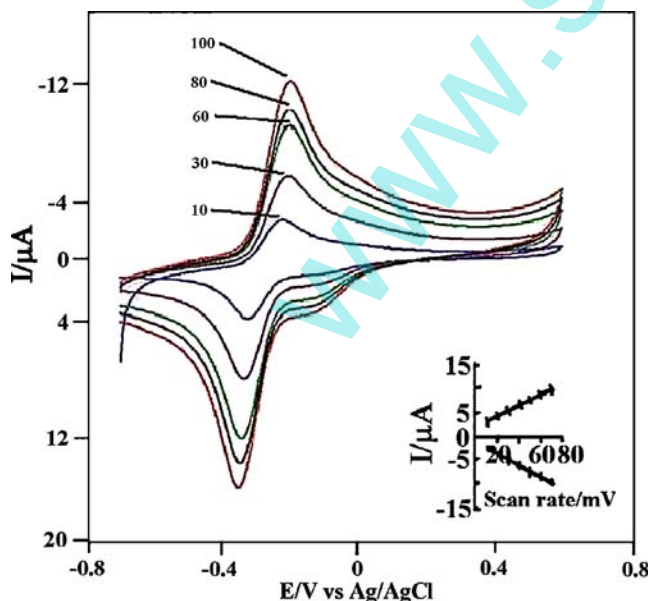
#### Electrochemical properties of PBCB–PANS film electrode

Figure 3 shows the cyclic voltammograms of PBCB–PANS film modified electrodes in a deaerated PBS solution at different scan rates. A redox couple with well-defined peaks appeared. Both anodic and cathodic currents (at  $-0.31$  V) increase linearly with scan rates up to 0.07 V/s as shown in the inset of Fig. 3. Also, the ratio of anodic to cathodic peak currents is nearly unity for all scan rates studied.



**Fig. 2** Cyclic voltammogram of (a) PBCB–PANS, (b) PANS, and (c) PBCB electrode in 0.1 M PBS solution, pH 6.7. Scan rate, 0.1 V/s

These observations indicate that the electron transfer process involves a surface-confined species, and the charge transfer is fast in the coating [38] and the kinetics of heterogeneous electron transfer rate constant ( $k_s$ ) can be calculated by using Laviron's equation [39]. The  $\Delta E_p$  values obtained using various scan rates for less than 200 mV and the calculated  $k_s$  value was found to be  $3.2 \text{ s}^{-1}$ . The obtained rate constant was comparable with methylene blue doped silica nanoparticle modified electrode [40]. We



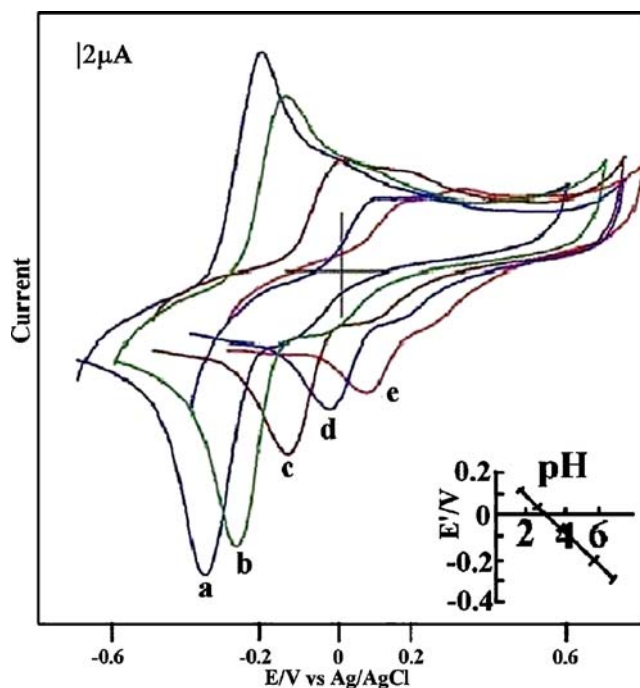
**Fig. 3** Cyclic voltammogram of PBCB–PANS in 0.1 M PBS, pH 6.7 at different scan rates; inset scan rate vs current

have estimated the apparent surface coverage  $\Gamma$  by using the following equation

$$\Gamma = Q_t/nFA_e$$

where  $Q_t$  is charge from the area under the PBCB–PANS composite electrode oxidation peak corrected for the base line (at the low scan rate of 10 mV/s) and the other symbols have their usual meanings. In the present case, the calculated surface coverage ( $\Gamma$ ) is  $1.05 \times 10^{-9} \text{ mol cm}^{-2}$  for  $n=2$ .

One of the major drawbacks of the polyaromatic quinoid-type mediators, being redox species of two electron–proton acceptors/donors, is the dependence of their midpoint potential  $E_m$  on the solution pH. In contrast, the mediators that have a metal ion as redox center are only electron acceptors/donors, in this way having a midpoint potential, which has no pH dependence [41]. It has been found that when phenazine dyes are in solution or chemically adsorbed at electrode surfaces, they possess a midpoint potential, which is highly pH dependent [42]. However, recent findings have shown that when mediators like phenoxazine and phenothiazine are immobilized into metal phosphate like zirconium phosphate [43] and/or zeolite [44], their formal potential values remain invariant with pH. It was therefore necessary to investigate the variation of midpoint potential with pH for the electropolymerized dyes since this influences the applied potential at which the redox-mediated biosensors can be used.



**Fig. 4** Cyclic voltammograms of PBCB–PANS at different pH: (a) 6.7, (b) 5.8, (c) 3.8, (d) 2.6, (e) 1.5 solution. Scan rate, 100 mV/s

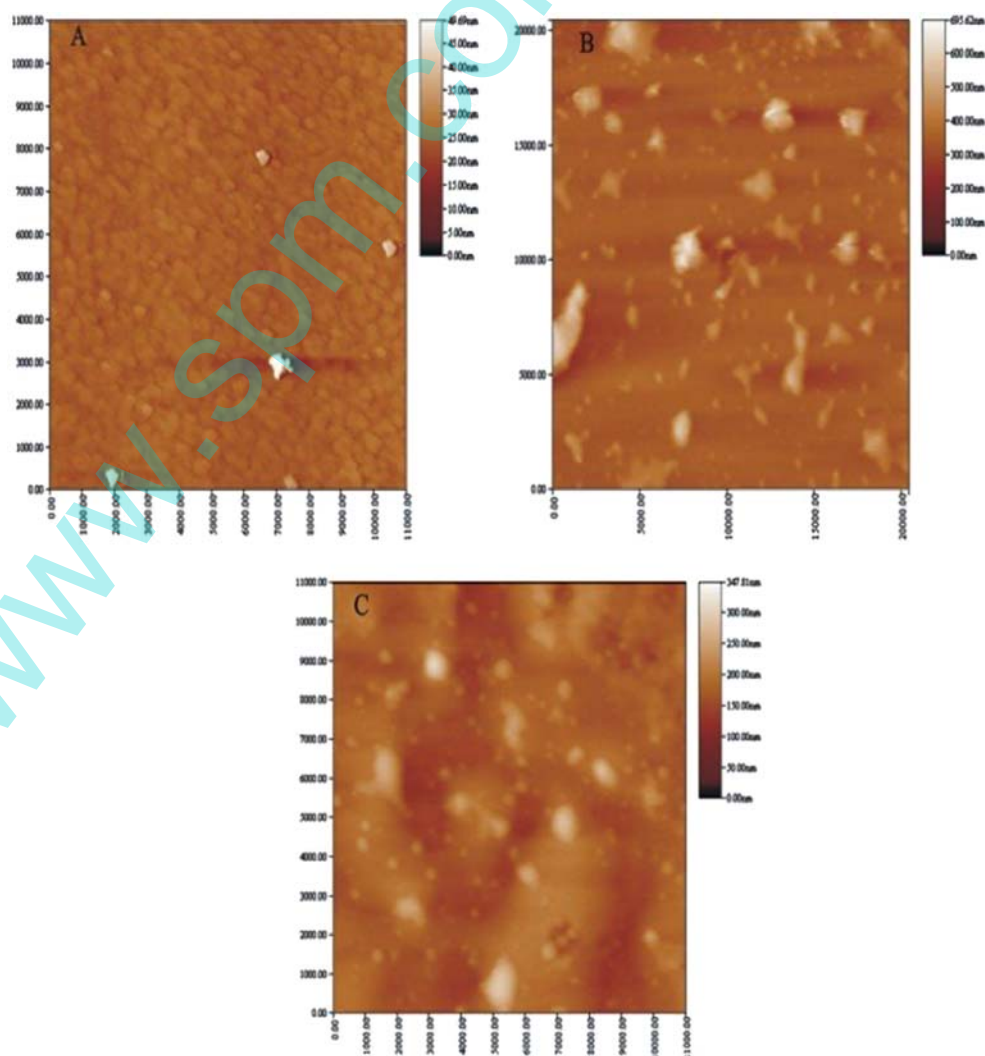


So, it is necessary to study the response of composite of PBCB–PANS film electrode toward different pH solutions, shown in Fig. 4. As shown in Fig. 4, our results exhibited the pH dependence of the voltammetric peak potentials, i.e., the anodic and cathodic peak potentials of the modified electrode were shifted to a less positive value with increasing pH of the contacting solution. The half-wave potential ( $E_{1/2}$ ) of the PBCB–PANS film electrode was evaluated as the mean of the anodic and cathodic peak potentials of the cyclic voltammograms recorded at various pH values. The  $E_{1/2}$  vs pH plot yields straight line with a slope of 65 mV per unit change in solution pH as shown in the inset of Fig. 4. It suggests that the overall redox reaction of the film comprises equal number of electron and proton involved.

#### Surface morphology of PBCB–PANS electrode

In order to study surface morphology of modified electrode, we have measured AFM by tapping mode, shown in Fig. 5. Figure 5a shows tapping mode AFM images of PANS

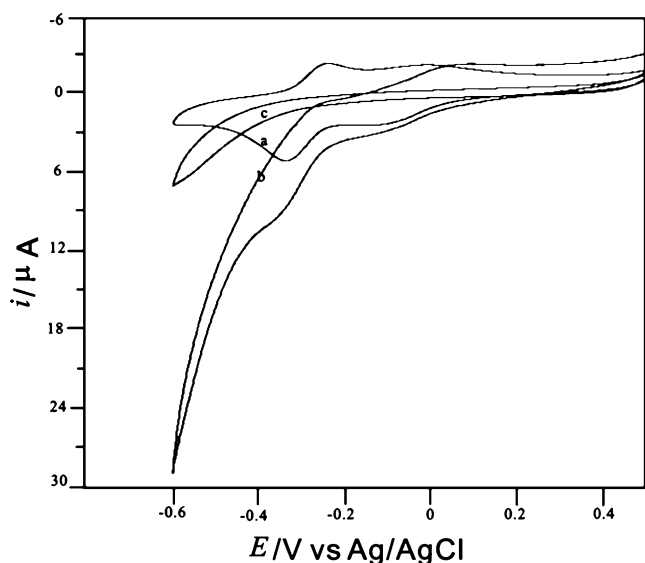
**Fig. 5** Tapping mode AFM image of **a** PANS electrode, **b** PBCB, and **c** PBCB–PANS film coated on ITO glass



electrode. Figure 5b shows AFM images of PBCB, which is randomly oriented on the surface of ITO glass electrode, and the thickness of the film was measured to be ~600 nm. AFM images of composite of PBCB–PANS electrode is shown in Fig. 5c in which dye molecules are uniformly and smoothly oriented on the surface of ITO electrode and the thickness of the film was measured to be ~330 nm. If we compare the morphology of PBCB and composite of PBCB–PANS electrode, the composite electrode exhibits as smooth it might be due to the presence of PANS in the composite.

#### Electrocatalytic reduction of hydrogen peroxide at PBCB–PANS film electrode

In our experiments, we found that the PBCB–PANS film electrode has a catalytic effect on the reduction of  $H_2O_2$ . As is known, the electroreduction of  $H_2O_2$  requires a large overpotential, and no obvious response is observed in the range of 0.5 to –0.6 V on bare electrode in pH 6.7 PBS solution containing 0.2 mM  $H_2O_2$  (Fig. 6). From Fig. 6b, it



**Fig. 6** PBCB–PANS composite electrode response: **a** absence of 0.2 mM  $\text{H}_2\text{O}_2$ , **b** in the presence of 0.2 mM  $\text{H}_2\text{O}_2$ , and **c** bare electrode. Electrolyte: PBS, pH 6.7 solution

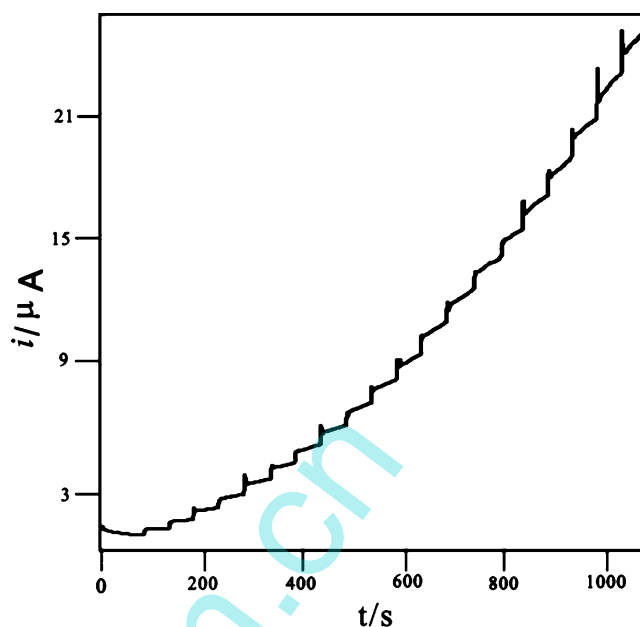
can be seen that upon addition of  $\text{H}_2\text{O}_2$  reduction, peak current of PBCB–PANS film electrode increased, and the corresponding oxidation peak currents decreased, as shown in Fig. 6b. The increase in the cathodic peak current and concomitant decrease in the anodic peak current indicate that PBCB–PANS electrode can catalyze electrocatalytic reduction of  $\text{H}_2\text{O}_2$ .

#### Amperometric determination of hydrogen peroxide at PBCB–PANS film electrode

Quantitative determination of  $\text{H}_2\text{O}_2$  was also carried out amperometrically in a stirred solution of PBS solution maintained at pH 6.7 solution. Figure 7 depicts the typical amperometric response of the PBCB–PANS film electrode recorded at an applied potential of  $-0.35$  V for successive addition of 1 mM  $\text{H}_2\text{O}_2$  solution. A rapid and well-defined response was observed for each addition of  $\text{H}_2\text{O}_2$ . The sensor responds quickly to the concentration of  $\text{H}_2\text{O}_2$ , a steady-state current being reached within less than 10 s. Under optimum conditions, the modified electrode showed a linear response to  $\text{H}_2\text{O}_2$  in the concentration range of  $5 \mu\text{M}$  to 0.2 mM, and detection limit ( $S/N=3$ ) was found to be  $5 \mu\text{M}$  and sensitivity of  $1 \mu\text{A}/\text{mM}$  for  $\text{H}_2\text{O}_2$  at the composite electrode. The results indicate that the modified electrode maintained a good sensitivity and had a stable amperometric response under hydrodynamic condition.

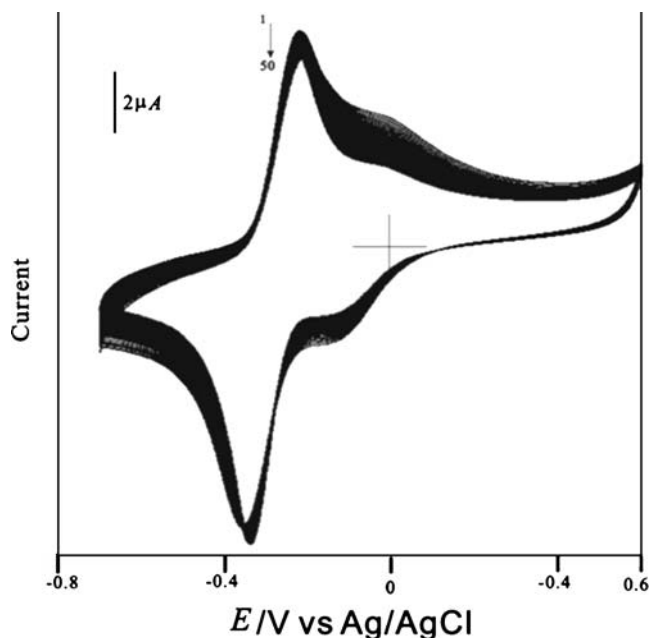
#### Stability of PBCB–PANS film electrode

The stability of the PBCB–PANS film electrode was studied. After electrode preparation, electrode was washed



**Fig. 7** Amperometric response to  $\text{H}_2\text{O}_2$  at PBCB–PANS composite electrode condition. PBS, pH 6.7 under stirred condition. Operating potential,  $-0.35$  V

in double distilled water followed by the electrode that was cycled in PBS, pH 6.7 solution, potential between  $-0.7$  and  $0.6$  V in  $50$  mV/s. The change in current was observed (Fig. 8) to be 7% between the first cycle and 50th cycle. The reason expected for this increased stability is due to composite formation between PBCB and PANS.



**Fig. 8** Cyclic voltammograms of PBCB–PANS composite electrode in background electrolyte of PBS, pH 6.7. Scanned between  $-0.7$  and  $0.6$  V. Scan rate,  $50$  mV/s. Number of cycles, 50

## Conclusion

Polymer composite of PBCB–PANS electrode was prepared by cyclic voltammetry. The composite electrode, PBCB–PANS electrode, was shown to have good electrochemical response over PBCB and PANS electrode. The composite electrode shows surface-confined and pH-dependent electrochemical property. The composite electrode exhibits catalytically active toward the reduction of hydrogen peroxide, and response time for detection of hydrogen peroxide by amperometry is less than 10 s.

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