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Polyaniline-zeolite nanocomposite material based acetylcholinestrase biosensor for the sensitive detection of acetylcholine and organophosphates

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Abstract

In this work, nanoporous polyaniline-nanocrystalline zeolite organic-inorganic hybrid material was synthesized. Acetylcholinesterase electrochemical biosensor based on polyaniline-zeolite nanocomposite material was fabricated for the sensitive detection of neurotransmitter acetylcholine and the resultant biosensor was further employed for the detection of toxic organophosphate pesticides. The results demonstrate that polyaniline-nanocrystalline zeolite based acetylcholinesterase biosensor exhibited much higher activity when compared to conventional polyaniline. Nanoporous polyaniline-nanocrystalline zeolite based biosensor exhibited good stability, sensitivity, linear range, and limit of detection. The analytical performance of the developed biosensor was demonstrated in the determination of these pesticides in different real samples (apple, cabbage, tap water, and river water) with satisfactory recovery.

Keywords: Nanocrystalline zeolite; polyaniline; enzymatic biosensor; acetylcholine; organophosphate pesticides.

1. Introduction

Acetylcholinesterase (AChE) is a key hydrolase enzyme that promotes the hydrolysis of the neurotransmitter acetylcholine (ACh).[1] ACh is the first discovered neurotransmitter that is found in the brain, neuromuscular junctions, spinal cord, and in both postganglionic terminal buttons of the parasympathetic division of the autonomic nervous system.[2] ACh plays an important role in transmitting messages from motor nerves to muscles, especially to the heart, bladder and stomach.[2] Abnormal ACh level may lead to several neural disorders such as Alzheimer's disease, progressive dementia and schizophrenia.[3-5] Therefore, the development of analytical methods for the determination of ACh is very important. In the recent years, AChE enzyme based biosensors have attracted much attention due to their application for the detection of AChE compounds.[2, 6] Acetylcholinesterase immobilized on conducting polymers, carbon nanotubes, graphene, and carbon paste based materials have been explored for the detection of ACh.[7-12] Several reports are available in literature for the electrochemical detection of ACh based on poly(m-(1,3)-phenylenediamine (detection limit 0.66 μM), ceramic (detection limit 0.2 μM), copper nanoparticles (detection limit 39 μM), and poly(pyrrole)/poly(2-naphthol) (detection limit 100 nM).[2, 13-15] However, the development of a sensitive and selective method for the ACh determination with an improved detection limit is still desirable for analytical application and diagnostic research.

AChE based biosensor can be further used for the detection of toxic organophosphorous pesticides (OP), which is based on the enzyme inhibition property.[16] The irreversible inhibition of AChE by OP pesticides leads to an accumulation of acetylcholine which may cause muscular paralysis, convulsions, bronchial constriction, and even death by asphyxiation in the living organisms.[2] The OP compounds are widely used as pesticides due to their high efficiency in controlling insects. However, the overuse of these pesticides results in pesticide residues in environment. This pesticide contamination is a severe threat to human health due to their long term persistence in the environment and high toxicity to AChE even at very low levels of exposure.[17]

Our research is focused on the synthesis of nanostructured metal oxides, polyaniline, zeolites and their nanocomposites and finds their applications in electrocatalysis.[18-20] In the recent years, zeolites and conducting polymers based electrodes have attracted an increasing interest in electrocatalysis.[21-27] Very recently, our research group have shown the selective

detection of neurotransmitters dopamine and epinephrine using porous polyaniline (PANI) and polyaniline-zeolite composite modified electrodes with high activity and sensitivity compared to conventional polyaniline modified electrode.[20, 28] The objective of this study was to develop a highly sensitive biosensor that can be used for the detection of neurotransmitter ACh and the toxic OP pesticides. PANI can perform dual task, a physicochemical transducer and an immobilization matrix for bio-molecules. Moreover, the biocompatible nature of PANI can help to preserve the activity of enzymes while allowing the permeation of analytes to catalytic sites of the enzymes.[29] Nanoporous polyaniline could provide suitable textural properties for the efficient immobilization of enzyme and analyte interaction, resulting in the improved sensitivity of the biosensor. Therefore in this study, high surface area Nano-ZSM-5 zeolite matrix was used to support porous polyaniline on its surface.

In this work, we present nanoporous polyaniline-zeolite hybrid nanocomposite material (PANI-Nano-ZSM-5) based AChE biosensor for the sensitive detection of neurotransmitter ACh and toxic OP pesticides. PANI-Nano-ZSM-5 was first functionalized with 3-aminopropyltrimethoxysilane to ensure that the polymerization of aniline takes place on zeolite surface only. Then enzyme AChE was loaded on PANI-Nano-ZSM-5 matrix using physical entrapment method. To the best of our knowledge, this is the first report, which deals with the detection of ACh and OP pesticides using nanoporous polyaniline-zeolite based AChE biosensor.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent grade and used as received without further purification. Tetraethylorthosilicate (TEOS, 98%), tetrapropylammonium hydroxide (TPAOH), 3-aminopropyl trimethoxysilane, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) ($EO_{20}PO_{70}EO_{20}$, MW 5800) (hereafter designated as P123), propyltriethoxy silane (PrTES, 97%), acetylcholinesterase (AChE from electric eel, 500 U/mg), acetylcholine chloride, dichlorovos, and monocrotopos were purchased from Sigma Aldrich, India. Aniline, ammonium peroxydisulphate (APS), bovine serum albumin and pralidoxime chloride were obtained from Spectrochem Pvt. Ltd., India. Sodium dodecyl sulfate was obtained from S D Fine Chemical Ltd., India. Deionized water from Millipore Milli-Q system (Resistivity

18 MΩcm) was used in the electrochemical studies. Electrochemical measurements were performed in Phosphate Buffer (***Sorenson's buffer***) solution, which was prepared by mixing NaH_2PO_4 and Na_2HPO_4. All electrochemical experiments were performed in 0.002 M phosphate buffer solution (PBS) containing 0.1 M NaCl at pH 7.4 as supporting electrolyte.

2.2. Synthesis of PANI-Nano-ZSM-5 nanocomposite

Nanocrystalline ZSM-5 zeolite (Nano-ZSM-5) was prepared using molar composition TEOS/10 PrTES/2.5 Al_2O_3/3.3 Na_2O/25 TPAOH/2500 H_2O by following the reported procedure.[18] Propylamine was then covalently anchored on Nano-ZSM-5. Nano-ZSM-5 (2 g) and (3-aminopropyl)trimethoxysilane (1.43 g) were taken in 50 mL toluene and the reaction mixture was refluxed for 12 h. After the reaction, the solid was filtered and washed with toluene followed by acetone and dried at 323 K for 24 h in oven to obtain amine-functionalized Nano-ZSM-5 (hereafter represented as Nano-ZSM-5-Pr-NH_2).

In a typical synthesis of PANI-Nano-ZSM-5 nanocomposite, first P123 (0.150 g) was dispersed in 600 mL deionized water. Then 0.5 g of Nano-ZSM-5-Pr-NH_2 was added to this mixture followed by the addition of sodium dodecyl sulfate (0.312 g). The mixture was then ultrasonically dispersed for 15 min. A solution of aniline (0.5 g) and HCl (10 mL, 1 M) was added drop wise to the above mixture with vigorous stirring. The mixture was then kept in cold water bath (~ 290 K) and mechanically stirred for 30 minutes. Then an ice cold aqueous solution of APS (2.45 g of APS in 10 mL of deionized water) was added into the above mixture instantly to start the oxidative polymerization and the reaction was allowed to proceed for 5 h under mechanical stirring. After the reaction, the resulting precipitate was washed with 0.1 M ammonium acetate followed by deionized water and ethanol several times. The final product was dried in vacuum at 333 K for 12 h to obtain the PANI-Nano-ZSM-5 nanocomposite. For comparison, conventional PANI was prepared using the reported procedure.[30]

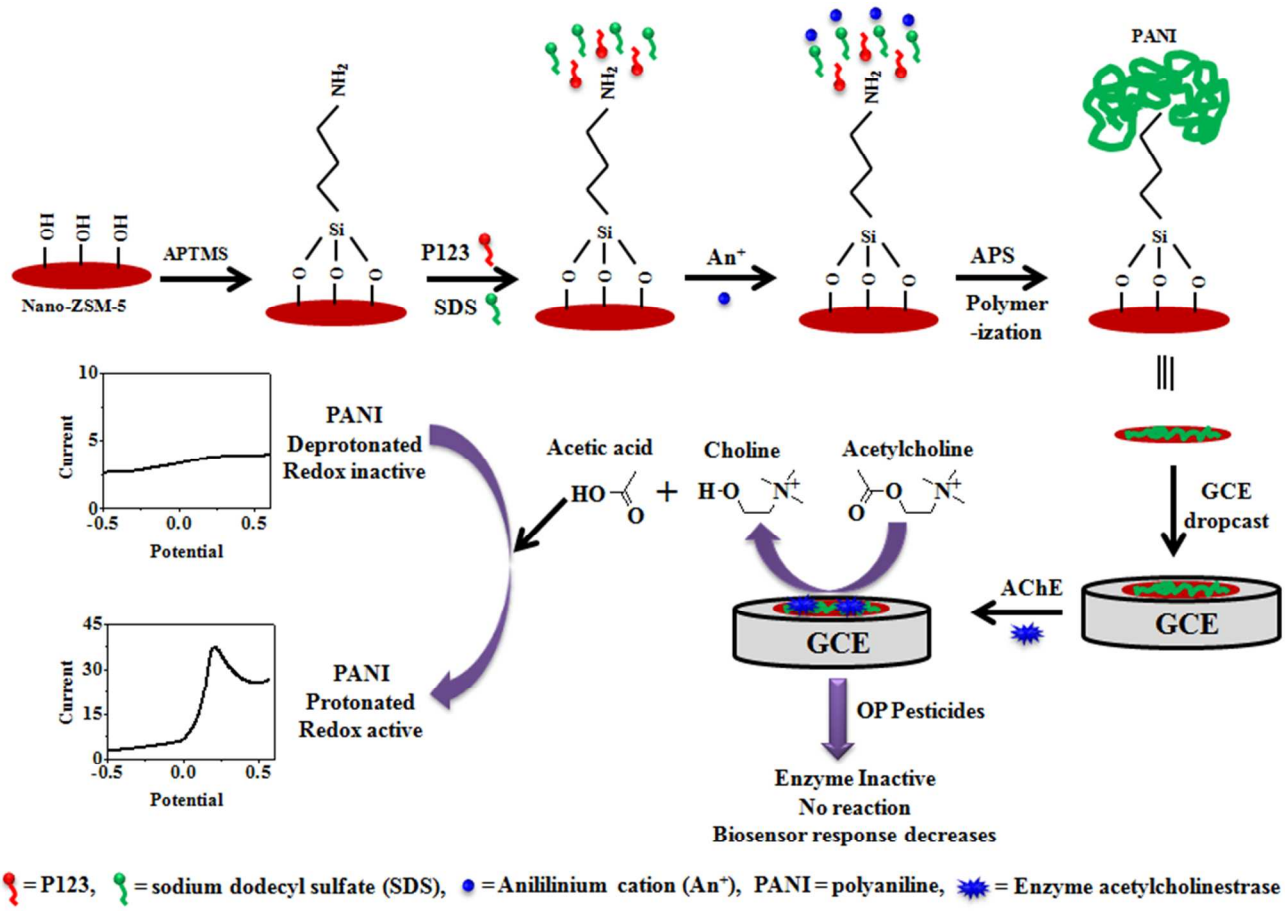
2.3. Instrumentation

X-ray diffraction (XRD) patterns were recorded in the 2θ range of 5-50° with a scan speed of 2°/min on a PANalytical X'PERT PRO diffractometer, using Cu Kα radiation (λ=0.1542 nm, 40 kV, 40 mA) and a proportional counter detector. Nitrogen adsorption measurements were performed at 77 K by Quantachrome Instruments, Autosorb-IQ volumetric

adsorption analyzer. Sample was out-gassed at 393 K for 3 h in the degas port of the adsorption apparatus. The specific surface area was calculated from the adsorption data points obtained at P/P_0 between 0.05-0.3 using the Brunauer-Emmett-Teller (BET) equation. The pore diameter was estimated using the Barret–Joyner–Halenda (BJH) method. Scanning electron microscopy (SEM) measurements were carried out on a JEOL JSM-6610LV, to investigate the morphology of the materials. Thermogravimetric analysis (TGA) was performed on a TGA/DSC 1 STAR^e SYSTEM from Mettler Toledo instrument with temperature increments of 10 K/min in air stream. Cyclic voltammetry (CV), and square wave voltammetry (SWV) studies were performed using Autolab PGSTAT302N. A three-electrode electrochemical cell was employed with Ag/AgCl as the reference electrode (3M KCl), AChE/PANI-Nano-ZSM-5 mounted glassy carbon (2 mm diameter) as the working electrode and Pt foil as the counter electrode.

2.4. Fabrication of acetylcholinesterase biosensor

The glassy carbon electrode (GCE) was first polished to a mirror like surface with alumina slurry and then ultrasonicated in ethanol and deionized water for 5 min, respectively. 2 mg of PANI-Nano-ZSM-5 and 10 µL of Nafion were added to 1 mL of deionized water in order to prepare the suspension and this mixture was further sonicated for 30 minutes to obtain a uniform suspension. 10 µL aliquot of obtained PANI-Nano-ZSM-5 suspension was then placed onto the GCE surface. The electrode was dried in air leaving the material mounted onto the GCE surface. The obtained electrode was then coated with 5 µL AChE solution (1 mL PBS (pH 7.4) containing 100 mU AChE and 1 mg bovine serum albumin to maintain the stability of AChE) and allowed to incubate for one hour at 277 K. The resulting electrode was then washed thoroughly with PBS (pH 7.4) to remove the unbound AChE and stored at 277 K. The fabricated AChE biosensor is designated as AChE/PANI-Nano-ZSM-5/GCE. For comparison, AChE/PANI/GCE biosensor was also fabricated in the similar way. All the experiments were carried out at ambient temperature. When not in use, the biosensor electrode was stored in an airtight container at 277 K. The various steps involved in the fabrication and working of acetylcholinesterase biosensor are shown in Scheme 1.



Scheme 1. Schematic representation for the fabrication of AChE/PANI-Nano-ZSM-5/GCE biosensor.

2.5. Real sample preparation

For the analysis of monocrotopos in apple and cabbage, samples were washed with water and chopped. Then, 2 g of each sample was sprayed with known concentration of monocrotopos and stored at 277 K for 24 h. These samples were then mixed with 10 mL acetone - PBS (pH 7.4) (1:9, v:v). After that, the mixture was sonicated for 30 min and centrifuged. Required amount of supernatants liquids were directly taken for the SWV analysis.

2.6. Electrochemical measurements

Enzyme substrate kinetics for the biosensor was calculated from the current-time plot for the biosensor at 0.2 V after the successive addition of ACh to PBS (pH 7.4) under stirring. The

apparent Michaelis-Menten constant (K_m) of the biosensor was then calculated following the Lineweaver-Burk equation:

$$\frac{1}{I_s} = \frac{K_m}{I_{max}} \cdot \frac{1}{C} + \frac{1}{I_{max}}$$

Where, I_s is the steady-state current after the addition of substrate ACh, I_{max} is the maximum current obtained under saturated substrate condition, and C is the bulk concentration of the substrate. Value of K_m was determined by the analysis of the slope and intercept for the plot of $1/I_s$ versus 1/C (where C = ACh concentration).

The AChE/PANI-Nano-ZSM-5/GCE biosensor was employed for the detection of OP pesticides using square wave voltammetry (SWV) method with the potential range from -0.5 to 0.6 V at 10 mV/s scan rate. The performance of the biosensor was tested by measuring its SWV response in PBS (pH 7.4) containing 1 mM ACh. Then the electrode was rinsed with water and incubated in PBS (pH 7.4) containing desired concentration of OP pesticides solution for 5 min and finally transferred into the electrochemical cell containing 10 mL PBS (pH 7.4) and 1 mM ACh to record SWV response. The inhibition rate of pesticide was calculated as follows:

$$Inhibition\ (\%) = \frac{I_{max} - I_{in}}{I_{max}} \text{x } 100$$

Where, I_{max} is the peak current of ACh at AChE/PANI-Nano-ZSM-5/GCE and I_{in} is the corresponding peak current of ACh with pesticide inhibition.

After inhibition by pesticide, AChE/ PANI-Nano-ZSM-5/GCE was washed with PBS and reactivated by immersing into 4.0 mM pralidoxime chloride for 7 min, then transferred into electrochemical cell containing 10 mL, PBS (pH 7.4) and 1 mM ACh to record SWV response. The reactivation efficiency was estimated as follows:

$$Reactivation\ (\%) = \frac{I_r - I_{in}}{I_{max} - I_{in}} \text{x } 100$$

Where, I_r is the peak current of ACh at AChE/PANI-Nano-ZSM-5/GCE after pralidoxime chloride reactivation.

3. Results and discussion

In our previous study, a different synthesis strategy was used to prepare PANI-zeolite nanocomposite material.[20] With this approach, both, bulk PANI and PANI coated zeolite phases were obtained. To inhibit the growth of bulk PANI, in this study, two steps synthesis strategy was adopted for the preparation of PANI-zeolite nanocomposite material. The details of the characterization of PANI and Nano-ZSM-5 are provided in our previous report.[20] The details of textural characterization (XRD, N_2-adsorption, SEM, and TGA analysis) for PANI-Nano-ZSM-5 nanocomposite synthesized in this study are provided in supporting information section (Fig. S1-S3). In the present case, PANI formed a thin layer on the surface of Nano-ZSM-5 selectively and no bulk PANI phase was observed in SEM image (Fig. S2). Detail of mechanism for the formation of PANI-Nano-ZSM-5 nanocomposite material is provided in supporting information section. PANI-Nano-ZSM-5 nanocompoposite material based AChE biosensor was developed for the sensitive electrochemical detection of neurotransmitter acetylcholine and the resultant biosensor was further applied to detect toxic organophosphate pesticides by the enzyme inhibition protocol.

3.1. Electrochemical behavior of ACh at AChE/PANI-Nano-ZSM-5/GCE

Prior to employ the AChE/PANI-Nano-ZSM-5/GCE for acetylcholine study, the electrochemical behavior of PANI-Nano-ZSM-5, PANI, and Nano-ZSM-5 was first investigated using CV in PBS (pH 5) (Fig. 1). The CV of PANI-Nano-ZSM-5/GCE exhibited four redox peaks (i)-(iv) at pH 1 corresponding to the electron transitions between the redox states leucoemeraldine/emeraldine and emeraldine/pernigraniline of PANI (Fig. 1, inset).[31] However, due to the pH dependence of the emeraldine/pernigraniline redox reaction, the two sets of peaks overlapped at pH 5 and a common set of peaks corresponding to leucoemeraldine/pernigraniline redox reaction was observed.[31] PANI/GCE also exhibited a pair of redox peaks at pH 5 whereas no redox peak was observed at Nano-ZSM-5/GCE (Fig. 1).

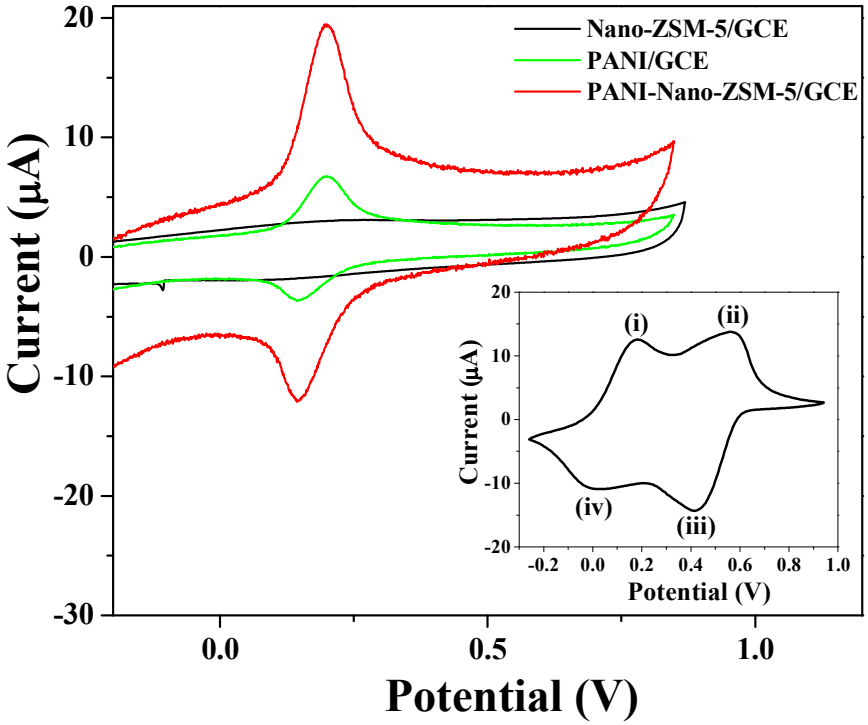


Fig. 1. CV for Nano-ZSM-5/GCE, PANI/GCE, and PANI-Nano-ZSM-5/GCE in PBS (pH 5) at a scan rate 50 mV/s. Inset shows CV for PANI-Nano-ZSM-5/GCE in PBS (pH 1) at a scan rate 50 mV/s.

Fig. 2 shows the SWV at AChE/PANI-Nano-ZSM-5/GCE in the absence and in the presence of 1 mM ACh in PBS (pH 7.4) at a scan rate 10 mV/s. The results show that no peak was observed in the absence of ACh (Fig. 2). Furthermore, no peak was observed at PANI-Nano-ZSM-5/GCE in the presence of ACh (Fig. S4). However, AChE/PANI-Nano-ZSM-5/GCE and AChE/PANI/GCE exhibited an oxidation peak at 0.2 V in the presence of 1.0 mM ACh (Fig. 2 and Fig. S4). These observations confirm that the oxidation peak was obtained due to the hydrolysis of ACh by enzyme AChE. Enzyme AChE is characterized by a catalytic triad which is a coordinated structure consisting of three essential amino acids histidine, serine, and aspartic acid. The AChE catalysis start when the serine hydroxyl group in AChE attacks and cleaves the ester group of ACh followed by its deprotonation by a neighbouring histidine group in the triad (Scheme S1a). Hence, AChE catalyzes the hydrolysis of ACh to produce choline and acetic acid as the final products (Scheme S1a). The acetic acid molecules could diffuse into the electrode

surface resulting in the protonation of PANI film.[12] Therefore, the key step of this biosensor is AChE-acetylcholine enzymatic reaction which causes the small changes of local pH in the vicinity of electrode surface. It is well documented in literature that PANI redox behavior depends on the pH.[12] The changes in the redox activity of PANI are directly related to the pH in its vicinity. It may be noted that the deprotonated form of PANI is redox inactive and the protonated form of PANI is redox active (Scheme 1). Furthermore, it may be noted that the oxidation peak current for AChE/PANI/GCE is very low when compared to AChE/PANI-Nano-ZSM-5/GCE (Fig. S4). The high activity of PANI-Nano-ZSM-5 can be attributed to the nanoporous film formed at the high surface area Nano-ZSM-5 surface which is favorable for the fast diffusion of acetic acid molecules onto the electrode surface. Therefore, PANI film formed at Nano-ZSM-5 exhibited dual functionality, first as an efficient pH sensitive layer and second as a support matrix for AChE immobilization.

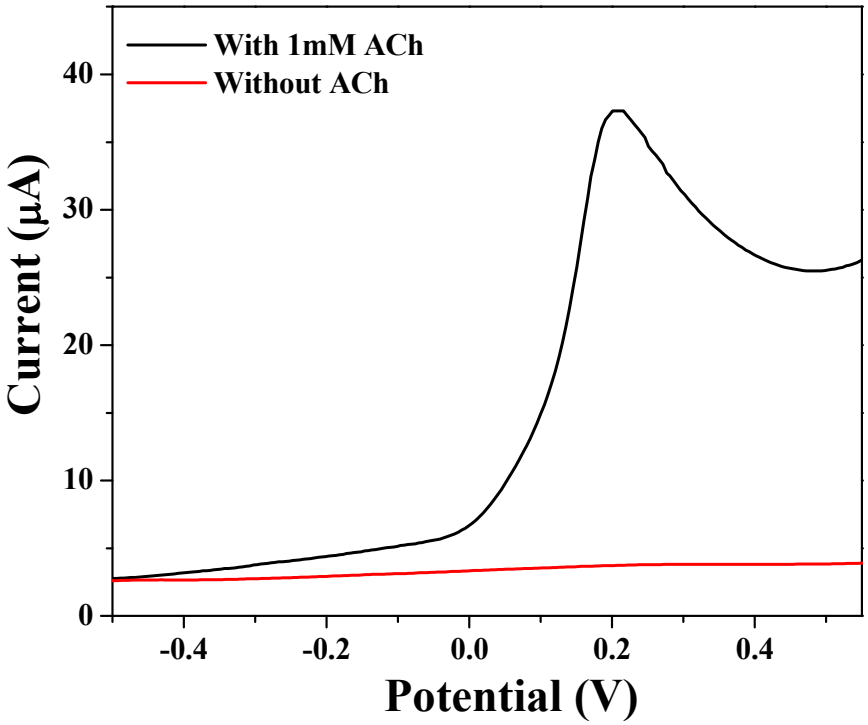


Fig. 2. SWV response at AChE/PANI-Nano-ZSM-5/GCE in 0.002 M PBS containing 0.1 M NaCl (pH 7.4) in the absence and in the presence of 1 mM ACh at a scan rate of 10 mV/s.

3.2. Optimization of experimental parameters

In order to get the maximum sensitivity of the biosensor AChE/PANI-Nano-ZSM-5/GCE, various important parameters such as influence of buffer solution, pH of buffer solution, and enzyme loading were optimized in PBS containing 1 mM ACh using SWV.

The biosensor response depends on the buffer capacity of the working solution. Strong buffer concentration neutralizes the acetic acid produced. Therefore, 0.002 M PBS containing 0.1 M NaCl was used to study the biosensor response. Furthermore, the bioactivity of the immobilized AChE depends on the pH of the supporting electrolyte. Therefore, the influence of pH on the enzyme activity was also investigated. The maximum enzyme activity was observed in the pH range 7 to 8. Therefore, in order to mimic the physiological environment, pH 7.4 was chosen as the optimum pH value for all the experiments.

Amount of enzyme AChE is an important parameter for the preparation of biosensor. In order to optimize the enzyme loading, amount of AChE was varied from 1 µL to 7 µL during the fabrication of biosensor. Fig. S5 shows the influence of AChE amount on the biosensor response. With increase in AChE amount, the current response was found to increase and reached a maximum value at 5 µL. With further increase in AChE amount, current response was found to decrease. This decrease in current response can be attributed to the increase in AChE film thickness at electrode surface, which causes higher resistance for the electrochemical processes. Therefore, 5 µL was taken as optimum enzyme amount in the fabrication of biosensor.

3.3. Electrochemical detection of ACh at AChE/PANI-Nano-ZSM-5/GCE

AChE/PANI-Nano-ZSM-5/GCE was employed for the electrochemical detection of neurotransmitter ACh because of its higher electro-catalytic activity. The amperometric response of AChE/PANI-Nano-ZSM-5/GCE biosensor was investigated at an applied potential of 0.2 V with the successive additions of different concentrations of ACh in the electrochemical cell containing PBS (pH 7.4). Fig. 3 shows a current-time curve for the electrochemical detection of ACh at AChE/PANI-Nano-ZSM-5/GCE. The amperometric current obtained was proportional to the concentration of ACh in the electrochemical cell. The current response obtained was found to be linearly dependent on the concentration of ACh in the range of 1 µM to 1 mM with the regression equation I (µA) = 10.447 + 0.024 C (µM) with a correlation coefficient of 0.9945 (Fig. 3, inset). The sensitivity and limit of detection (S/N = 3) of the biosensor were found to be

0.76 μA/μM cm^2 and 30 nM, respectively. The response curve follows a Michaelis-Menten behavior showing linearity in initial stages and approaching saturation at higher substrate concentration. It may be noted that the K_m value for the free enzyme is 100 μM.[32] The apparent value of Michaelis-Menten constant (K_m) was found to be 232 μM according to Lineweaver-Burk equation (Fig. S6). The K_m value obtained in this study is close to the K_m value (220 μM) obtained at Acetylcholinesterase-TiO_2-decorated graphene nanohybrid.[33] The K_m value obtained at AChE/PANI-Nano-ZSM-5/GCE is lower than many literature reports for AChE biosensors (Table S1), indicating a higher affinity of the AChE bound to PANI-Nano-ZSM-5 surface. A comparison of the electro-catalytic activity of AChE/PANI-Nano-ZSM-5/GCE, and AChE/PANI/GCE towards ACh detection is provided in Fig. 4. The results confirmed that AChE/PANI-Nano-ZSM-5/GCE exhibited much higher sensitivity toward ACh when compared to AChE/PANI/GCE biosensor. The high activity of the developed biosensor can be correlated to the large surface area of the nanocomposite material and nanoporous PANI film formed on the high surface area Nano-ZSM-5, which are favorable for the fast diffusion of analyte molecules onto the electrode surface.

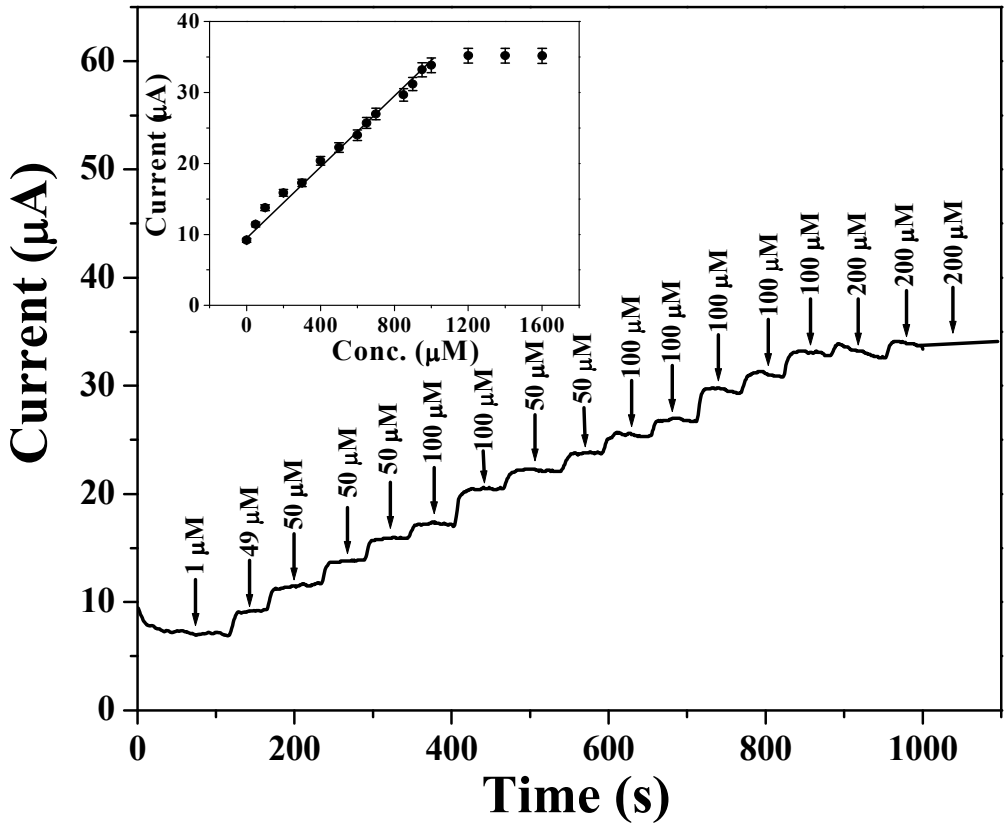


Fig. 3. Amperometric response of AChE/PANI-Nano-ZSM-5/GCE in 0.002 M PBS containing 0.1 M NaCl (pH 7.4) at an applied potential of 0.2 V with successive additions of ACh. Inset shows the calibration plot.

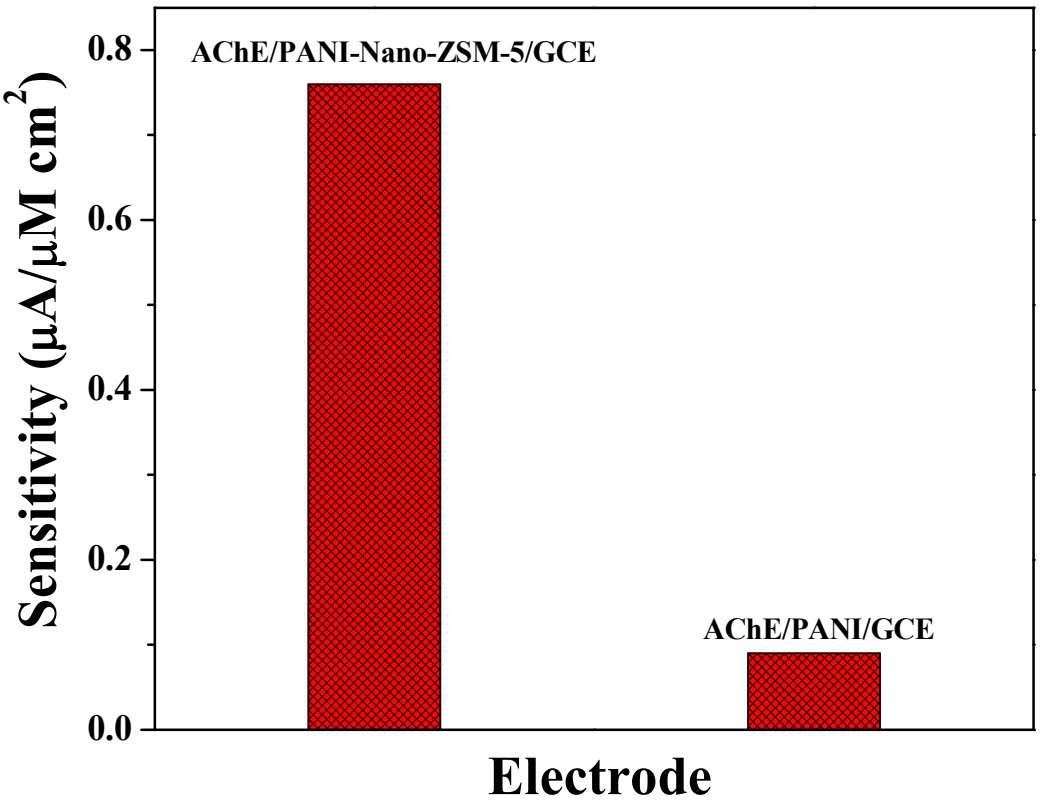


Fig. 4. Comparison of the sensitivity towards ACh at AChE/PANI-Nano-ZSM-5/GCE, and AChE/PANI/GCE biosensors investigated in this study.

3.4. Detection of OP pesticides

Due to the higher electrochemical response and sensitivity of AChE/PANI-Nano-ZSM-5/GCE, it was further employed for the electrochemical detection of OP pesticides monocrotopos and dichlorvos. It may be noted that monocrotopos and dichlorvos were chosen as model OP pesticides. The applicability can be extended to other OP pesticides also. After the incubation of AChE/PANI-Nano-ZSM-5/GCE in standard OP pesticide solution, OP pesticides irreversibly bind to the serine of peptide located at the active site of AChE to form phosphorylated adduct resulting in the deactivation of enzyme (Scheme S1b).[34] This leads to decrease in the activity and biological function of enzyme. The inhibition property of AChE was exploited to detect and monitor OP pesticide at AChE/PANI-Nano-ZSM-5/GCE. SWV was employed for the detection of OP pesticides at AChE/PANI-Nano-ZSM-5/GCE in PBS (pH 7.4) containing 1 mM ACh (Fig. 5). Fig. 5 shows the SWV before and after incubation of the biosensor in different concentrations of monocrotopos. The peak current was found to decrease with increase in

concentration of monocrotopos. This decrease in current response was directly proportional to the pesticide concentration. A linear response was obtained from 1 ppb to 1000 ppb with a limit of detection (S/N=3) of 0.1 ppb. In the similar manner, AChE/PANI-Nano-ZSM-5/GCE biosensor was also employed for the detection of dichlorvos and the results are summarized in Table S2. The comparison of the results shown in this paper with the published literature is provided in Table S1. Table S1 shows that AChE/PANI-Nano-ZSM-5/GCE biosensor shows a good linear range and improved detection limit when compared to many literature reports.

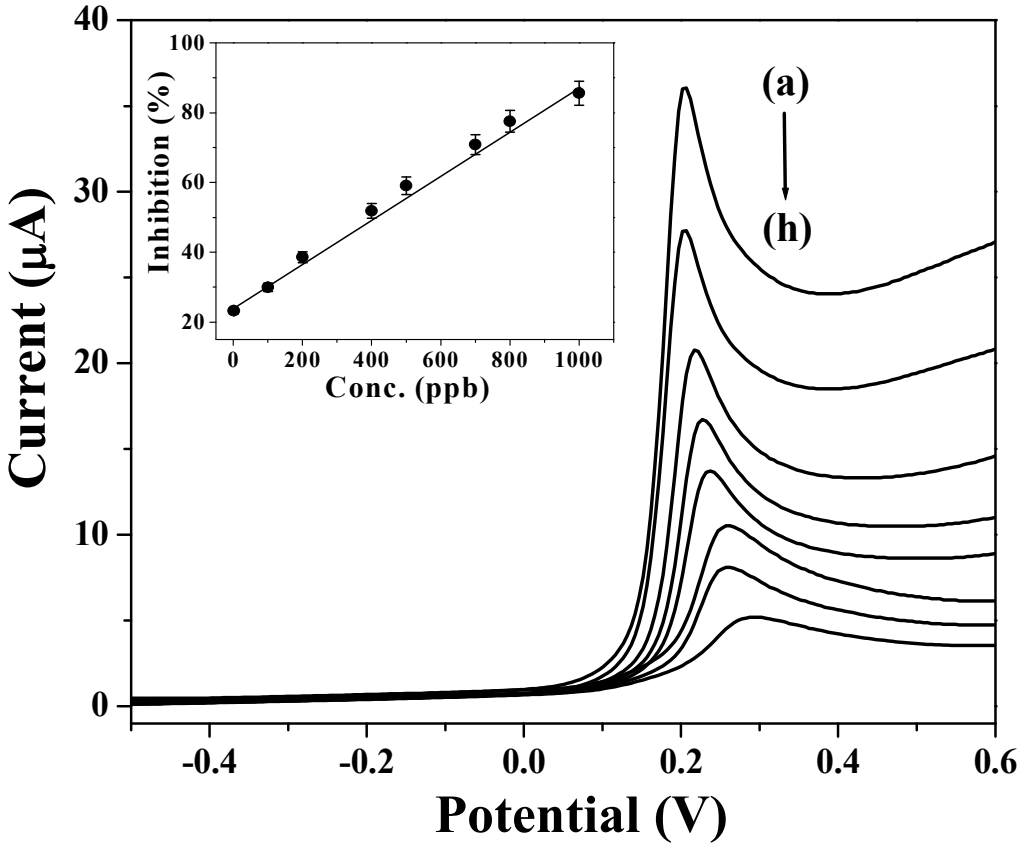


Fig. 5. SWVs at AChE/PANI-Nano-ZSM-5/GCE in 0.002 M PBS containing 0.1 M NaCl (pH 7.4) with 1 mM ACh after inhibition by monocrotopos of different concentrations (a - h): 0, 1, 100, 200, 400, 500, 800, 1000 ppb under the optimum conditions. Inset shows relationship between inhibition rates and monocrotopos concentrations.

The inhibition time is one of the important parameters in pesticide analysis. First, AChE/PANI-Nano-ZSM-5/GCE was immersed in 50 ppb of OP pesticides for a certain period of

time and then transferred into electrochemical cell containing 1 mM ACh in PBS (pH 7.4) to study the electrochemical response. It was observed that that with increase in inhibition time the inhibition percentage increased and then attained a stable value after the inhibition time of 5 min. Therefore, 5 min was chosen as optimum inhibition time for pesticides determination.

3.5. Reproducibility, stability, and reactivation of the biosensor

The reproducibility of the fabricated biosensor was evaluated with five different freshly prepared AChE/PANI-Nano-ZSM-5/GCE electrodes in 1 mM ACh after being immersed in 50 ppb monocrotopos for 5 min using SWV. A relative standard deviation (RSD) of 2.3 % was obtained, confirming a good reproducibility of the biosensor electrode (Fig. S7). Similarly, the repeatability of the biosensor was studied by carrying out five different measurements using the same AChE/PANI-Nano-ZSM-5/GCE (Fig. S8). The electrode was regenerated using pralidoxime chloride after each experiment resuming 99.2 % of its original activity for one regeneration measurement. A RSD of 3.8 % was obtained for five different measurements using 50 ppb monocrotopos, indicating an acceptable repeatability of measurements (Fig. S8). The long term stability of the biosensor was evaluated by measuring its sensitivity toward 1 mM concentration of ACh for 15 days. The biosensor was stored in refrigerator at 277 K and its sensitivity was tested at the interval of 3 days. No significant decrease in the response of ACh was observed in the 15 day storage, indicating the stability of developed biosensor. After a 15 day storage period, the sensor retained 92 % of its initial current response, indicating the acceptable stability of biosensor.

The inhibition of AChE by OP pesticide can be reversed by the use of nucleophilic compounds such as pralidoxime chloride. It was observed that AChE on electrode surface inhibited by monocrotopos after complete saturation with monocrotopos (approximately 1000 pbb) can resume 95 % of its original activity after immersion in 4.0 mM pralidoxime chloride for 7 min. Therefore, the proposed biosensor can be used repeatedly based on this reactivation procedure with an acceptable reproducibility.

3.6. Real sample analysis

In order to evaluate the practical applicability and accuracy of the proposed biosensor, experiments were performed to determine the concentration of monocrotopos in different real

samples (tap water, river water, apple, and cabbage). Experiments were performed at AChE/PANI-Nano-ZSM-5/GCE to determine the concentration of monocrotopos under the optimized conditions and the results are listed in Table 1. The values of recovery were in the range from 98 to 102 %, suggesting the high accuracy of AChE/PANI-Nano-ZSM-5/GCE based biosensor.

Table 1. Determination of monocrotopos in different real samples at AChE/PANI-Nano-ZSM-5/GCE biosensor.

S.No.	Sample (ppb)	Taken (ppb)	Found by biosensor (ppb)[a]	Recovery (%)[a]	Detected by GC (ppb) [b]
1.	Apple	100	101.6	101.6	100.4
		200	203.8	101.9	201.3
2.	Cabbage	100	98.2	98.2	100.8
		200	204.3	102.1	201.7
3.	Artificial tap water sample containing known amounts of analytes	100	101.7	101.7	100.6
		200	202.7	101.3	200.8
4.	Sutlej river water (Rupnagar, Punjab, India)	100	101.8	101.8	100.4
		200	204.5	102.3	201.6

[a] AChE/PANI-Nano-ZSM-5/GCE biosensor;
[b] GC: Gas chromatography.

4. Conclusions

In summary, an acetylcholinesterase immobilized polyaniline-zeolite based electrochemical biosensor was developed for the detection of neurotransmitter acetylcholine and the resultant biosensor was further employed for the detection of toxic organophosphate pesticides monocrotopos, and dichlorvos. Results demonstrate that the developed biosensor exhibited the high electro-catalytic activity with good stability, and sensitivity. The high activity

of the developed biosensor can be correlated to the large surface area of the nanocomposite material and nanoporous polyaniline film formed on the high surface area Nano-ZSM-5, which in turn was favorable for the fast diffusion of analyte molecules onto the electrode surface. The apparent value of Michaelis-Menten constant was found to be 232 μM which is lower than many acetylcholinesterase based biosensors reported in the literature. The analytical performance of the developed biosensor was extended in the determination of organophosphate pesticides in different food samples and water bodies with satisfactory results. This methodology can further be extended to different enzymes and antibodies and hence can be considered as promising candidate for the construction of many other important biosensors.

Acknowledgements

Authors thank Department of Science and Technology, New Delhi for financial assistance (DST grant SB/S1/PC-91/2012). BK is grateful to CSIR, New Delhi for SRF fellowship. Authors are grateful to Director IIT Ropar for constant encouragement.

Supporting Information

The supporting information contains additional experimental details and results and discussion as noted in text.

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Figures, Tables and Scheme captions

Figures

Fig. 1 CV for Nano-ZSM-5/GCE, PANI/GCE, and PANI-Nano-ZSM-5/GCE in PBS (pH 5) at a scan rate 50 mV/s. Inset shows CV for PANI-Nano-ZSM-5/GCE in PBS (pH 1) at a scan rate 50 mV/s.

Fig. 2 SWV response at AChE/PANI-Nano-ZSM-5/GCE in 0.002 M PBS containing 0.1 M NaCl (pH 7.4) in the absence and in the presence of 1 mM ACh at a scan rate of 10 mV/s.

Fig. 3 Amperometric response of AChE/PANI-Nano-ZSM-5/GCE in 0.002 M PBS containing 0.1 M NaCl (pH 7.4) at an applied potential of 0.2 V with successive additions of ACh. Inset shows the calibration plot.

Fig. 4 Comparison of the sensitivity towards ACh at AChE/PANI-Nano-ZSM-5/GCE and AChE/PANI/GCE biosensors investigated in this study.

Fig. 5 SWVs at AChE/PANI-Nano-ZSM-5/GCE in 0.002 M PBS containing 0.1 M NaCl (pH 7.4) with 1 mM ACh after inhibition by monocrotopos of different concentrations (a - h): 0, 1, 100, 200, 400, 500, 800, 1000 ppb under the optimum conditions. Inset shows relationship between inhibition rates and monocrotopos concentrations.

Table

Table 1 Determination of monocrotopos in different real samples at AChE/PANI-Nano-ZSM-5/GCE biosensor.

Scheme

Scheme 1 Schematic representation for the fabrication of AChE/PANI-Nano-ZSM-5/GCE biosensor.