

Electrochemical behaviour of pyridostigmine bromide-an anticholinergic drug at polyaniline/polypyrrole composite polymer electrode

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A composite polymer surface coated on a tin oxide offers dramatic improvement in the stability and sensitivity of voltammetric measurement of pyridostigmine bromide as compared to individual tin oxide, polyaniline or polypyrrole coated electrode. A linear current response was achieved with a detection limit of 1×10^{-6} M. The stability and morphology of the polymer surfaces were determined by thermogravimetry and scanning electron microscopy respectively. The results suggest that composite polymer electrode is the best detector for pyridostigmine bromide (PB) at lower concentration.

Keywords: Composite polymer, Pyridostigmine bromide, Polyaniline, Polypyrrole, Cyclic voltammetry, Scanning Electron Microscopy, Thermogravimetry

Pyridostigmine bromide (PB) is one of the main drugs currently used to treat myasthenia gravis¹ and also for the treatment of prophylaxis. It is a quaternary ammonium compound, which belongs to a class of neuroactive compound called carbamets². Pyridostigmine is used as a powerful and reversible acetylcholinesterase (AChE) inhibitor, which effectively increases the concentration of acetylcholine at the sites of cholinergic transmission³. PB undergoes hydrolysis by cholinesterase. It is also metabolized by microsomal enzymes in the liver. The main metabolite of PB is 3-hydroxy-*N*-methylpyridinium (3-OH NMP) to which few references of biological action have been made⁴. For understanding the full consequence of the biological activity of PB and its metabolites, various analytical techniques, mainly HPLC and gas chromatography, have been utilized for their measurements in biological samples⁵⁻⁷. No data were available for the voltammetric determination of pyridostigmine at polymer electrode. Electroanalytical methods are highly sensitive, simple in operation, and require low-cost apparatus. Electroanalytical techniques are widely used for the study of different pharmaceutical products⁸⁻¹⁰ because they have proven themselves as fast and sensitive techniques for the determination of compounds. Various chemically modified electrodes have been developed for promoting oxidation-reduction process and detection of compounds of pharmaceutical interest¹¹⁻¹⁶. The modified electrodes

offer a possibility of lowering the over potential and increasing sensitivity and selectivity of some electro active species¹⁷. The use of conducting electroactive polymers (CEPs) to monitor and manipulate biomolecular interactions was reported by Wallace and Kane-Maguire¹⁸. The ability of polymers to interact at molecular level increases their use for the development of new biosensing and bioseparation technologies as well as providing a new means of cellular communications. Several investigations on drug delivery system on modified electrode are also available¹⁹⁻²¹.

Good electrode material must also be associated with good mechanical properties, chemical inertness and stability to ambient condition. Among conducting polymers thin films of polypyrrole and polyaniline are potentially attractive materials²² due to their moderately high conductivity, low cost, relatively high stability and facile production by electrodeposition²³. Composites of PPy with polyethylene glycole, nafion, polyurethane, poly-vinyl chloride and with CMC has been reported extensively²⁴⁻²⁷. The polyaniline-polypyrrole composites²⁸ have conductivity in the range of 10^{-2} to 10^{-3} S/cm. Karakisla and Sacak²⁹ prepared the freestanding polymer film of polyaniline and polypyrrole on insulating polycarbonate electrode.

In this communication, development of polyaniline/polypyrrole composite polymer on tin oxide coated glass electrode for the cyclic

voltammetric response of pyridostigmine bromide in pharmaceutical formulation is reported. Polyaniline shows high conductivity upon doping with simple bronstant acids³⁰ and polypyrrole films doped with *p*-toluene sulphonate gives good mechanical properties and chemical durability against aerial oxidation³¹. It was considered worthwhile to combine the properties of both polymers by the preparation of polyaniline film doped with H₂SO₄ + NaClO₄ by electrochemical deposition on tin oxide coated glass as working electrode. On this polyaniline film polypyrrole film doped with *p*-toluene sulphonate was deposited by electrodeposition at constant potential 0.8 V. The presence of perchlorate with sulphuric acid improves the electropolymerization efficiency³².

Voltammetric response of the PB to polyaniline/polypyrrole electrode was examined and electrode sensitivity was found to be greater than that for the film coated with polyaniline or polypyrrole or tin oxide films alone.

Experimental Procedure

Apparatus

Cyclic voltammetric experiments were performed with EG & G Princeton applied research model 273; the potentiostat was controlled by the electrochem software 4.30A. A conventional electrochemical cell of 5 mL capacity, with the three-electrode system equipped with a polyaniline/polypyrrole working electrode, an Ag/AgCl reference electrode, and a platinum-wire counter-electrode was used for voltammetric experiments. The *pH* of the solutions was measured by Systronic *pH* meter.

Scanning electron microscopy (SEM) images of the modified electrodes were obtained with a FEIXL 60 EFSEM electron microscope. Thermal stability of modified electrodes was studied by thermogravimetric method using Dupont thermal analyzer from 25 to 800°C having a heating rate of 10°C per min under the presence of air.

Reagents and standards

Both pyrrole (99%) and aniline (99.8%) were obtained from Aldrich Chemicals and redistilled before use. All solutions and supporting electrolyte were of analytical grade and prepared using ultra pure water. Tin oxide was deposited on glass slide using chemical vaporization technique. Drug solutions in pharmaceutical formulations were prepared immediately before use in acetonitrile and were purified by passing nitrogen gas. The *pH* was adjusted

with Britton Robinson buffers. The ionic strength was maintained with aqueous 1 M KCl.

Electrode preparation

Polyaniline films were deposited on glass slide, which is previously coated with tin oxide (deposited by chemical vapor deposition technique), using cyclic voltammetric technique in H type cell. For electrochemical deposition, tin oxide coated glass slide was used as working electrode, Ag/AgCl was used as reference electrode and graphite rod was used as counter electrode. Sulphuric acid (H₂SO₄, 10⁻¹ M) and sodium perchlorate (NaClO₄, 10⁻² M) were used as doping agents for polyaniline formation. The voltage applied was -0.3 and +1.0 V and the concentration of aniline was taken 0.2 M. On this pre-existing polyaniline film, polypyrrole film was deposited electrochemically by constant potential coulometry method at 0.8 V and the film was allowed to grow till 15 coulomb charge was accumulated. The concentration of pyrrole was 0.2 M and the amount of supporting electrolyte *p*-toluene sulphonate was 0.1 M. All the films were prepared in aqueous media and stored in air at room temperature. To obtain a more sensitive and stable voltammetric response the working electrodes were cyclically scanned 20 times in the potential range -1.4 to + 0.8 V to give a stable background current.

Electroanalysis of pyridostigmine

Ten tablets of pyridostigmine bromide (Bhagat Pharmaceutical, India), each containing 60 mg of PB were powdered in a mortar and the amount corresponding to 1x10⁻³ M of PB was weighed and dissolved in acetonitrile. The contents of the flask were thoroughly agitated for 15 min to affect complete dissolution. After the excipients were settled down, a measured aliquot of the clear supernatant was transferred into a calibrated flask and diluted to a final volume of 10 mL with acetonitrile. Pyridostigmine in working solution was determined by cyclic voltammetric technique. All measurements were performed at room temperature.

Results and Discussion

SEM study

The SEM micrographs of different polymer electrodes are shown in Fig. 1 (a-d). The micrograph of polyaniline film surface in Fig. 1a shows the morphology and needle shaped structure of polyaniline³⁹. Figure 1b shows the micrograph of polypyrrole alone revealing average particle size of

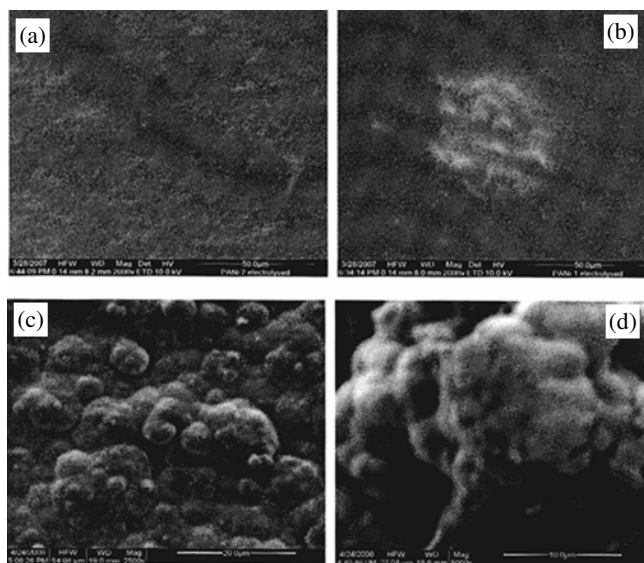


Fig. 1—SEM Micrograph of: (a) polyaniline doped with $\text{H}_2\text{SO}_4 + \text{NaClO}_4$ (2000X magnification), (b) polypyrrole doped with *p*-toluenesulphonate (2000X magnification), (c) polyaniline/polypyrrole composite (2500X magnification), and (d) single particle of PANI/PPY composite (5000X magnification).

approximately 3-5 μm . Figures 1c and 1d show the SEMs of polyaniline/polypyrrole composite film in which the particle size increases (approx 6-10 μm) because of the composite formation, which makes an effective change in molecular structure. The size of the grain varied due to the variation in polyaniline and polypyrrole chain. SEM studies show a rough surface, which may be responsible for good current response at this electrode. It is true that smaller particle size of polypyrrole (3-5 μm) offers more surface area compared to large particle size of composite (6-7 μm), even than the current response will be poor, because the conductivity of polypyrrole is less, this is also evident from Fig. 2b where the current is low as compared to composite electrode in cyclic voltammetric experiment.

Electrochemical behaviour of PB

Figure 2 exhibits cyclic voltammograms (CV) at (a) tin oxide coated glass modified electrode, (b) polypyrrole- modified electrode and (c) polyaniline/polypyrrole modified tin oxide coated glass electrode in 1×10^{-3} M solution. Among the four modified electrode surfaces only one modified surface of polyaniline/polypyrrole composite film deposited on tin oxide coated glass surface display a well defined reduction peak (Fig. 2c). In case of tin oxide coated electrode the reduction peak is absent for PB

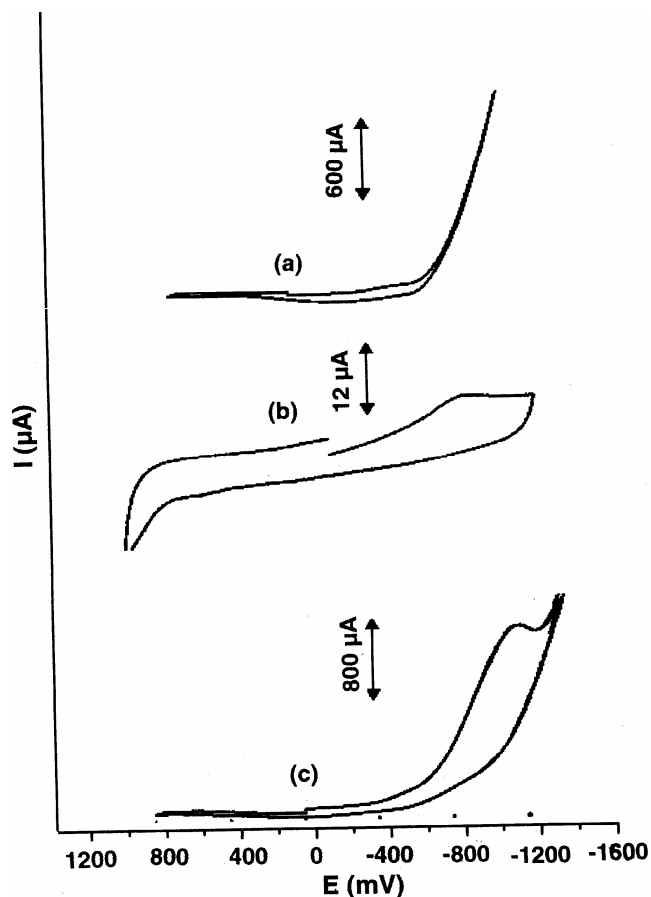


Fig. 2—Cyclic voltammograms of (a) tin oxide coated glass slide, (b) polypyrrole film coated on tin oxide coated glass slide electrode and (c) polyaniline/polypyrrole composite film coated on tin oxide glass slide electrode, recorded in 1×10^{-3} M pyridostigmine bromide solution.

although the current reaches up to 600 μA , which indicates poor response of the tin oxide coated glass slide. Polyaniline films doped with $\text{H}_2\text{SO}_4 + \text{NaClO}_4$ was not found to be stable against acetonitrile solution which was used as solvent for pyridostigmine drug. Modified electrode prepared by electrodeposition of polypyrrole film doped with *p*-toluene sulphonate electrode, exhibited a well-defined peak of 12 μA at -0.87 V for PB. This reduction in current is due to the poor conductivity of the polypyrrole film. The polypyrrole coated tin oxide glass slide electrode shows substantially higher potential and poor current response (12 μA) compared to the PANI/PPY composite modified electrode which shows more negative potential (-1.05 V) and better current (800 μA) in cyclic voltammetric response of PB.

The pH dependence of PB was studied in pH range between 2.0 to 12.0. The voltammetric response was

markedly dependent on pH . PB gave a well-defined peak up to pH 6.8 and it did not give well-defined peaks beyond this value of pH (Table 1). This behaviour is supported by the fact that PB is stable in acidic medium and at higher pH PB is extremely unstable^{24,25} (Table 1). Cyclic voltammogram of 1 mM solution of PB, at a scan rate of 100 mV/s, exhibited a single well-defined irreversible reduction peak up to pH 6.8 (Fig. 3). No peak was observed in the reversal scan corresponding to cathodic peak. Hence, this observation confirmed the irreversibility of the reduction process. The reduction peak potential of the PB observed at the composite electrode with pH is plotted in Fig. 4. Peak potential is parabolic with pH and a break is observed at pH 6.8 which can be associated with the pK_a value of PB. This break could be due to protonation-deprotonation process of the electroactive molecule.

The effect of different scan rate on the electrode process was studied by recording cyclic voltammograms at various sweep rates from 50 to 700 mV/s. The reduction peak shifted to more negative potential with increase in scan rate as shown in Fig. 5a. The peak current was found to increase proportionally with square root of scan rate (Fig. 5b), which confirmed the diffusion controlled nature of the electrode process (Table 2). The shift in peak potential also indicated the irreversible nature of the electrode process. The cyclic voltammograms were

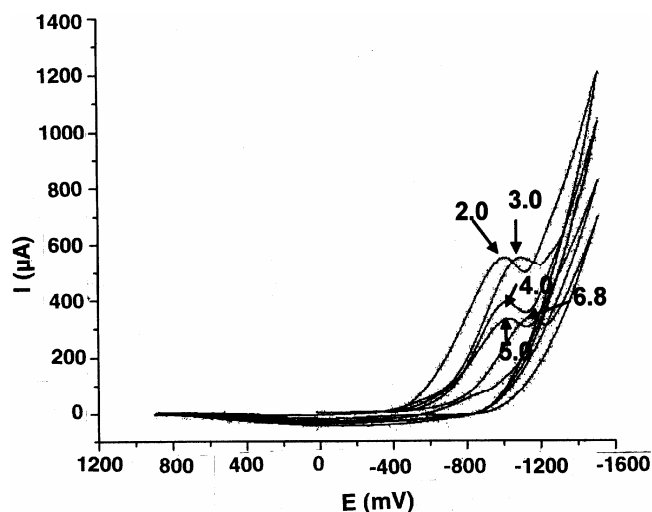


Fig. 3—Dependence of cathodic peak potential on pH in the supporting electrolyte of 1.0 mM solution of PB at polyaniline/polypyrrole composite electrode in phosphate buffer at scan rate 100 mV/s. (a) $pH=2$, (b) $pH=3$, (c) $pH=4$, (d) $pH=5$ and (e) $pH=6.8$.

also recorded in the concentration range 1-0.01 mM of PB (Fig. 6a). The plot of peak current (i_p) versus concentration (Fig. 6b) is linear in lower concentration range which reflects the diffusion control nature of the reduction process but at higher concentration the graph shows a tendency to limit the diffusion process which is due to the adsorption of PB at the polymer surface that restricts the flow due to diffusion.

On the basis of this electrochemical study of PB at polyaniline/polypyrrole composite film modified

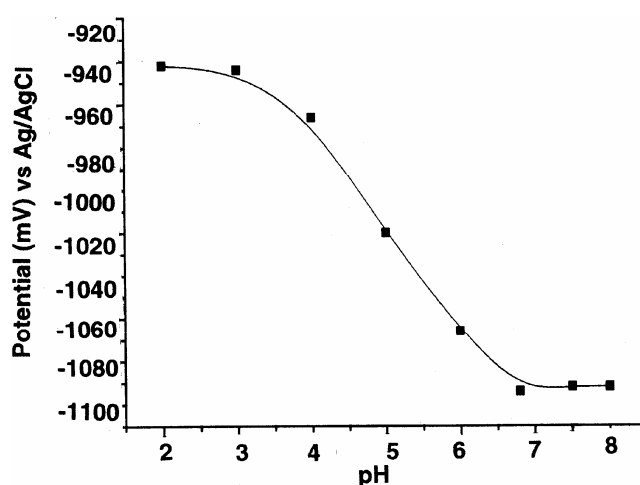


Fig. 4—Plot of peak current versus pH of the solution of PB.

Table 1—Effect of pH on reduction peak of PB at polyaniline/polypyrrole composite electrode.

S. No.	pH	Potential (E_p) (mV)
1	2	-942
2	3	-944
3	4	-966
4	5	-1020
5	6	-1066
6	6.8	-1092
7	8	-1092

Table 2—Effect of scan rate on peak currents of 1.0 mM solution of PB at polyaniline/polypyrrole composite polymer electrode.

S. No	Scan rate (v) (mV/S)	(v) ^{1/2} (mV/S)	Peak current (i_p) (mA)
1	50	7.07	310
2	100	10.0	421
3	250	15.81	673.23
4	400	20	846.44
5	550	23.45	990.93
6	700	26.45	1338

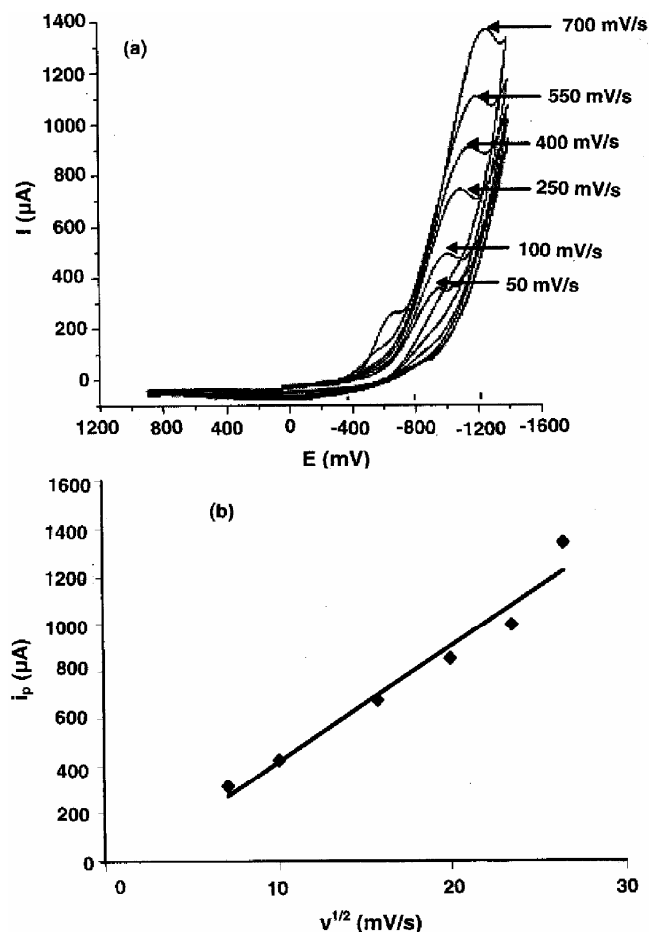


Fig. 5—Dependence of peak current on different scan rates (a) Cyclic voltammograms of 1.0 mM solution of PB solution at different scan rates at pH 3.0. Scan rates (I) 50, (II) 100, (III) 250, (IV) 400, (V) 550, and (VI) 700 mV/s-300 mV/s; (b) Dependence of peak current (i_p) on square root of scan rates of 1.0 mM solution of pyridostigmine bromide

electrode, a reduction mechanism may be postulated as depicted in Fig.7.

Analytical application

The electrochemical behaviour of pyridostigmine on polyaniline/polypyrrole modified electrode was established and studied for the first time. PB is irreversibly reduced at negative potential. It shows that the PB concentration in pharmaceutical formulations can be determined by using voltammetric technique on the basis of reduction process. This behaviour provides a useful tool for the detection and quantification of such drugs at low levels of concentration. The procedure could be adopted for pharmacokinetic studies as well as for quality control laboratory studies.

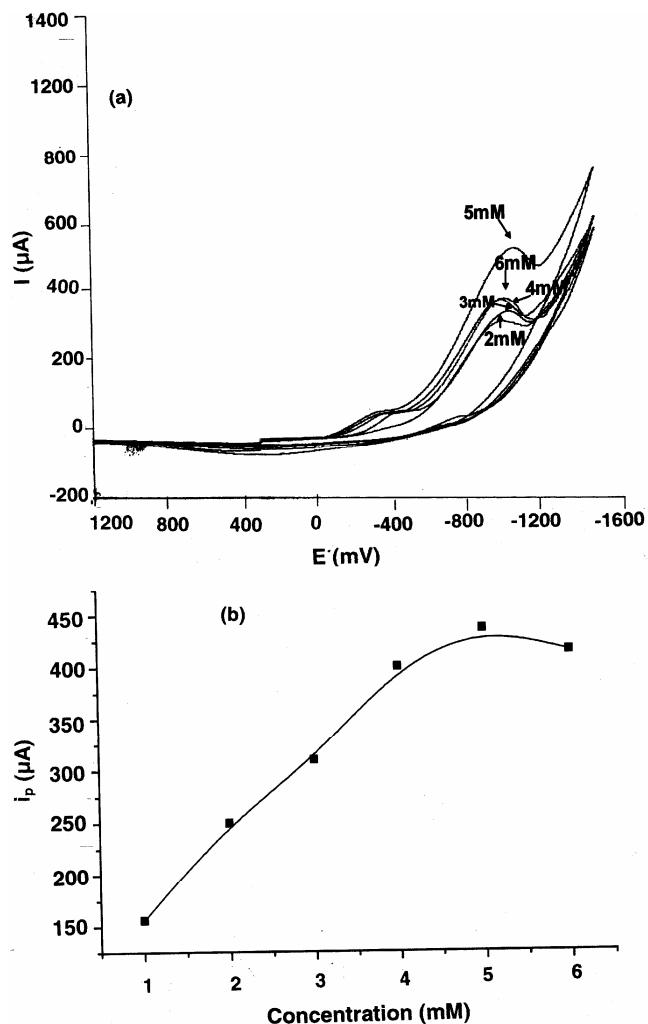


Fig. 6—Dependence of peak current on different concentrations of PB: (a) Cyclic voltammogram of PB at different concentrations at pH 3.0 and scan rate 100 mV/s at 1-0.01 mM concentrations; (b) plot of peak current (i_p) versus concentration of PB at pH 3.0 and scan rate 100 mV/s at 10 -0.01 mM concentrations.

Conclusion

In the present studies it has been found that polyaniline/polypyrrole modified tin oxide coated glass electrode exhibited greater electrochemical activity towards the reduction of PB as compared to its tin oxide, polyaniline and polypyrrole counterparts. The improvements observed are attributed to enhanced activity due to the formation of composite. The SEM images show assembly of polypyrrole particles along the polyaniline chain. This composite polymer modified electrode also shows good adherence to the substrate. Polyaniline/polypyrrole composite polymer modified electrode provide a sensitive and selective method of pyridostigmine

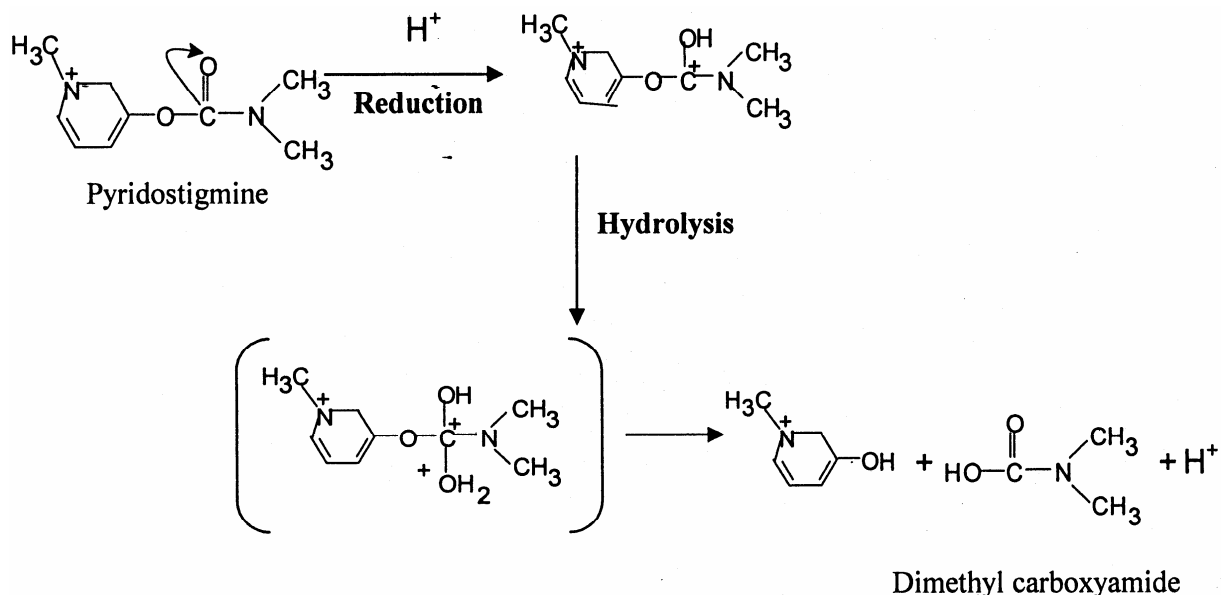


Fig. 7—Proposed mechanism for the reduction of PB.

bromide analysis. The improvement in PB detection indicates good promise for qualitative and quantitative analysis.

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