CHAPTER 4:

Optimisation of low potential workability of the biosensor

4.1 Introduction

Efficient functioning of the AChE based pesticide biosensors rely on the efficient determination of thiocholine concentration. Thiocholine determination is normally carried out by colorimetric, spectrophotometric, fluorescent measurement and electrochemical detection techniques. Colorimetric method has been successfully used for the estimation of cholinesterase in human serum[1-3] using thiocholine ester such as acetate, propionate, and n-butyrate as the substrate during the assay preparation following Ellman reaction.[4,5] The Ellman reaction involves the reaction of 5, 5-dithio-bis (2-Nitrobenzoic acid) (DTNB) with thiocholine liberated from its esters by enzymatic hydrolysis. The yellow 5-thio-2-nitro-benzoate (TNB) thus formed is measured spectrophotometrically at 412 nm. In Fluorescence measurement method thiocholine is allowed to react with 7-diethylamino-3-(4-maleimidylphenyl)-4-methylcoumarine (CPM) to form a highly fluorescent product enabling picomolar level thiocholine detection.[6]Thiocholine is electrochemically active, thus rendering electrochemical monitoring of residual AChE activity and hence the amount of pesticide possible.

Relative to fluorescence and spectrophotometric determination, electro analysis presents several advantages such as high sensitivity, simplicity, real time detection, and potential to miniaturize the analytical system. Several AChE inhibition based biosensor systems have been proposed for screening of OP and OC neurotoxins by monitoring the electrochemical oxidation of enzymatically generated thiocholine.[7-15]However oxidation of thiocholine on solid electrodes requires a high over potential (~700 mv) that causes interferences from other electro active species and also a high background current. Different strategies have been applied to overcome these drawbacks and to enable an amperometric detection of thiocholine at low applied potential without electrode passivation. These includes use of different mediators such as cobalt(II)pthalocyanine[16-18] Prussian blue[19] and tetracyanoquinodimethane[20,21]; use of pulsed electrochemical detection[22]; use of mercury electrodes; derivatization, and others.[23] Use of composite electrodes such as MWCNT-polymer composite membrane[24,25] and CNT- Ionic liquid gel electrodes[26] were also reported. Polypyrrole (PPy) is a conducting polymer with attractive properties for biosensor application. The porous nature of the electro generated film permits good shelter for the enzyme as well as permits substrate diffusion inside the matrix. PPy film can be generated easily through electrochemical methods; it permits facile elctronic charge flow through the

polymer back bond thus making it highly conducting. Efficient use of PPy as the enzyme-hosting matrix for biosensor fabrication has been demonstrated by several workers.[27-30]In the previous chapter we have demonstrated that PPy in combination with porcine skin gelatin can be used to fabricate highly sensitive and highly stable AChE biosensor for OPs and OCs. We have also demonstrated through CV under sufficient diffusion condition (low potential scan rate) thiocholine oxidation can occur at low over potential of 0.1 V in PPy matrix. In present work we have demonstrated the CA proof of low potential oxidation of thiocholine, optimized the CA parameters for low potential operation of PPy supported AChE biosensor and also had a thorough comparison of the merits and demerits of low potential operation of the biosensor over its conventional high potential operation. A correlation procedure for the two methods has also been worked out.

4.2 Objectives

1. To establish CA evidence of low potential thiocholine oxidation.
2. To optimize the parameters for low potential application.
3. Application of low potential thiocholine oxidation to pesticide analysis taking typical organothiophosphate pesticide ethion.
4. To find out a correlation between the results of low potential analysis to the conventional high potential (0.7V) analysis.

4.3 Experimental

4.3.1 Preparation of the sensor

Enzyme loaded, gelatin-gluteraldehyde-polypyrrole coated platinum electrode (Pt/PPy/AChE-Glut-Geltn electrode) was prepared according to the procedure described in chapter 3.[31] In brief AChE was electro entrapped in PPy at 0.7 V from a 0.5 M solution of the pyrrole in phosphate buffer (PB) pH 7.2 containing 0.02 M KCl and 5 μL(100 U mL^{-1}) of the enzyme. Subsequently gluteraldehyde and gelatin were added in steps and kept the electrode for an aging period of 5 days in -20^0C before use. A three electrodes cell set up comprised of platinum working electrode (diameter 3mm), platinum coil auxiliary electrode and Ag/AgCl saturated with 3 M NaCl as the reference electrode was used for the experiment.

4.3.2 Analysis procedure

Conventional CV was used for qualitative study of the redox behavior of thiocholine at the prepared sensor surface at high and low scan rates throughout the potential range from -0.1 to +0.1 V. CA was used for quantitative evaluation of all parameters mentioned in the article that are linked to the current output. CA experiments were performed with initial potential E_1 = - 0.8 V applied for 180 s or 60 s and final potential E_2= 0.2, 0.4 V or the conventional 0.7 V applied for 300 s. The height of the current transient at the instantaneous step of potential change, after subtraction of the same during PBS addition, was taken as the magnitude of amperometric current due to thiocholine oxidation. The substrate solution was added initially in PB electrolyte and mixed properly before starting any experiment. The experiments were performed at 32^0C in static solution condition under nitrogen atmosphere. Analyses were performed using PBS electrolyte of pH 7.2.

4.3.3 Sensor probe pretreatment and cleaning procedure

After taking out from -20^0C freezer, prior to its application, the sensor was immersed in phosphate buffer saline (PBS) for 1 hr. and then performed few CV and CA runs in PBS for stabilization of the sensor response. Response stabilization can be confirmed from the overlapping of the CA and CV curves toward the end runs (4 to 5 runs). The CV runs were performed in the potential range from 0 V to -0.8 V. The CAs were performed with E_1= -0.8 V, t_1=60 s, E_2= x V (x = 0.2 or 0.4 or 0.7 V), t_2=10s in PBS. The last curve of the overlapping CA serves as the marker for the completeness of the cleaning process applied after each analysis. During inhibition study with pesticides, the CA calibration process was repeated after each analysis because irreversible inhibition changes the base line as well as the marker to a new lower value. Above mentioned CV and CA operations also serve as steps for cleaning the electrode after each analysis. However if low positive potentials, particularly 0.2 V is used for oxidation during analysis, then the working electrode must be potentiostated at 0.7 V for sufficiently long duration, say 300 s, after the analysis and before applying CV/CA cleaning steps, to oxidize the residual thiocholine that remains un oxidized at the electrode surface during the potential step.

4.4 Results and Discussion

4.4.1 Cyclic voltammetric behavior

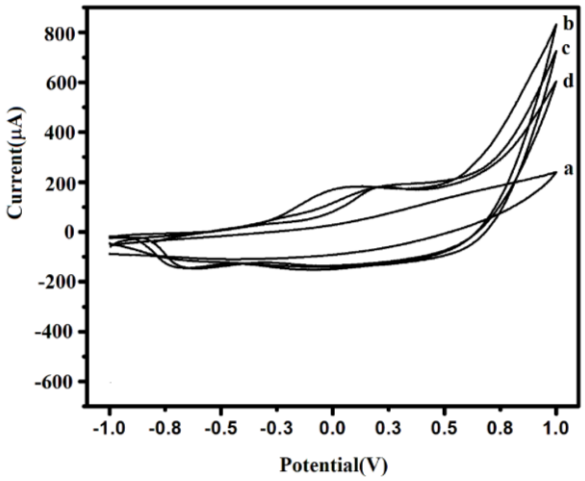

Fig.4.1 Curves b through d, continuous cyclic voltammetric runs in (2 mM) ATChCl – PB mixture at scan rate 1 mV /s using Pt-PPy-AChE-Glut-Geltn electrode. Curve a, the same in PBS.

Figure 4.1 shows the CV at a scan rate of 1 mV/s in ATCh-PB mixture using the enzyme electrode. As seen in the figure thiocholine produces two types of oxidation peaks one low intensity broad peak in the region from -0.3 V to 0.3 V and another sharp peak from 0.5 V onwards.It has been attributed that peak at -0.3 V to 0.3 V region appears due to oxidation of thiocholine ions those diffuse into the PPy matrix, whereas sharp peak at 0.5 V onwards is due to oxidation of thiocholine ions at the film-solution interface. The peak at -0.3 V to 0.3 V region appears in the very first run (b) and get shifted to 0.2 V in the successive runs (c,d). Shifting of peak maxima from 0.0 Vat the initial run to 0.2 V at the final run atributed to readjustment of capacitive behavior upon potential scaning. It is worth mensioning that on bare PPy film the same oxidation peak appears at 0.1 V, as mentioned in the previous chapter. The other sharp peak appearing above 0.5 V onwards, showed decrease in peak current with successive runs and get stablized at the third run (d). The shifting and diminishing intensity is due to change in morphology of PPy film as a result of oxidation at higher potential.[32]

4.4.2 CA measurement

4.4.2.1 CA proof of low potential oxidation

During the cyclic voltammetric study it was observed that the thiocholine oxidation peak at low potential (-0.3 V to 0.3 V range) appears on PPy surface only when a slow scan rate of 1 mV/s is applied. Since the slow scan rate facilitates an infinite diffusion, so one can speculate that at least one condition for low potential oxidation to achieve must be sufficient diffusion of thiocholine ion into the matrix. As a proof of this concept we have examined the feasibility of thiocholine oxidation at a fixed low potential of 0.2 V in CA experiment by applying a forced diffusion condition in which the thiocholine ions were polarized towards the working electrode by applying a sufficiently high negative potential for certain time before subjecting them to oxidation at the second step potential of 0.2 V. The next paragraph to follow is the result of the experiment that clearly proves the validity of the speculation.

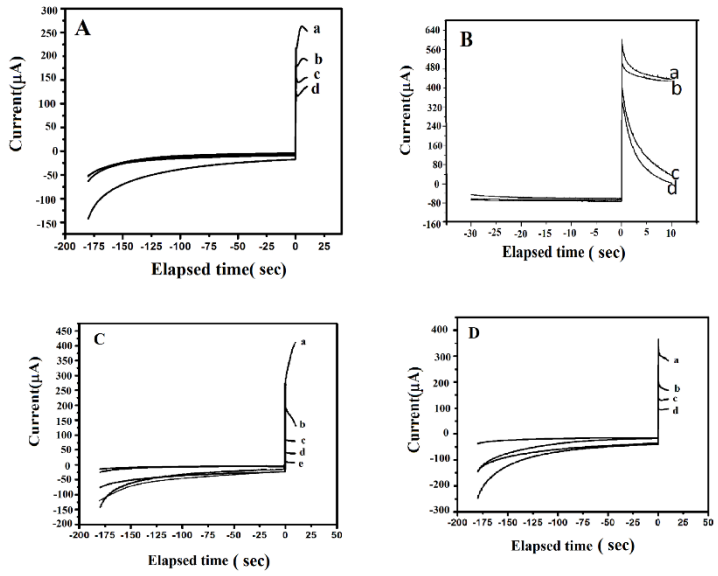

Fig.4.2 CA current transient due to thiocholine (TCh) and acetylthiocholine (ATCh) at 0.7 V and 0.2V under various conditions. A. under pre concentration condition in which a. TCh oxidation at 0.7 V, b. ATCh-PPy charge transfer current at 0.7 V, c. TCh oxidation at 0.2 V d. ATCh-PPy charge transfer current at 0.2 V. B. same as above under normal (non-preconcentrated) condition. C and D for repetition of above experiment A with non-enzyme electrode and deactivated enzyme electrode, respectively.

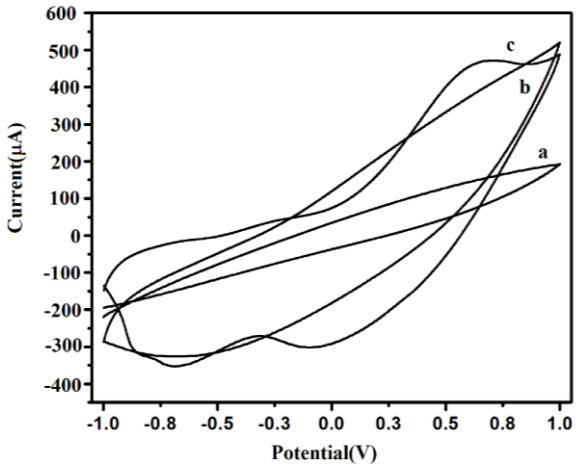

Fig.4.3 Cyclic voltammograms of TCh and ATCh solution using Pt-PPy-Glut-Geltn electrode scan rate of 10 mV/s. Curve b is the CV of (2mM) ATCh, curve c is the CV of (2 mM) TCh and curve a is the same in PBS.

Figure 4.2A,B showing the chronoamperograms of thiocholine (TCh) and acetyl thiocholine (ATCh) on Pt-PPy-Geltn-Glut electrode, respectively under forced (preconcentrated) and normal (non-preconcentrated) conditions. Preconcentration was achieved by subjecting a negative potential of -0.8 V for 180 s. In the figure the current transients at 0 s (at E_2=0.2 V or 0.7 V) get different height when TCh and ATCh are present in the solution, indicating that, it is not merely due to the inherent property of the electrode material or due to the characteristic behavior of the instrument; electrochemical oxidation of either thiocholine only (if hydrolysis is assumed to be fast enough) or both thiocholine and acetylthiocholine must be involved at the two potentials.

That thiocholine oxidation is involved at this current transient is obvious from the difference in peak height between curve a and b. However, ATCh oxidation is not conclusive at this stage because, since the electrode contains enzyme, so it could be possible that ATCh gets hydrolyzed quickly to produce TCh which in turn gets oxidized. To get this point clarified, we have repeated above experiment in two different ways; one with a non-enzyme electrode(Pt-PPy-Geltn-Glut) and the other with same electrode but with a deactivated enzyme(Pt-PPy-AChE*-Geltn-Glut). Deactivation was done through warming the enzyme at70^0C for 30 minutes before immobilization. Deactivation was confirmed through the Ellman assay procedure.[5] Deactivated enzyme was immobilized so as to maintain almost the same morphology inside PPy, that is, to produce the same amount of diffusion channels for electrons and ions like the active enzyme electrode. Figure 4.2C and D are the CAs of TCh

and ATCh, respectively with a non-enzyme and deactivated enzyme electrodes. It has been observed that in both the cases (C and D) the current ratios I_{TCh}:I_{ATCh} becomes much higher as compared to the same in case of active enzyme electrode (1.7 in Fig 4.2C, 1.75 in 4.2D and 1.18 in 4.2A). It indicates that the current transient 'b' obtained in 4.2A is not purely due to ATCh but some contribution of TCh formed from the instant hydrolysis of ATCh also involved. The current ratio in 4.2D is higher than that in 4.2C indicating that the diffusion channels created due to the presence of enzyme inside the PPy matrix can significantly influence the amperometric signal. These observations confirm that ATCh involves in an electron transfer process in the applied experimental condition and is different from thiocholine oxidation. This electron transfer interaction increases with increasing potential and can't be termed as electrochemical oxidation because it doesn't appear as a sharp peak at a well-defined potential range in the CV experiment (Fig.4.3). Figure 4.2 reveals that the TCh oxidation current is more prominent than the electron transfer current, keeping a difference of more than 50 μA in the current transient. This electron transfer process is attributed to the intermolecular electron transfer between ATCh and PPy.

The difference between the intermolecular electron transfer current and TCh oxidation current seems to be affected by two things (i) the applied potential for oxidation, (ii) the preconcentration time. In absence of sufficient preconcentration time, the two oxidations do not get differentiated at low potential 0.2 V, but difference is prominent at 0.7 V. When applied a preconcentration of 180 s the two oxidations get sufficient differentiated at both the potential 0.2 V and 0.7 V. It indicates that for low potential detection of thiocholine a sufficient preconcentration time must be allowed to avoid any erroneous results due to the intermolecular electron transfer between ATCh and PPy.

The difference between the current levels of curves a and b as well as of c and d of Figure 4.2(A,B,C,D) must be the oxidative current of thiocholine oxidation, thus indicating thatthiocholine oxidation on the sensor surface occurs from 0.2 V onwards. This is also substantiated by the observation that the CV peak current shifts from 0.2 V to 0.7 V with increasing scan rate.[31,33]

4.4.3 Optimization of preconcentration parameters:

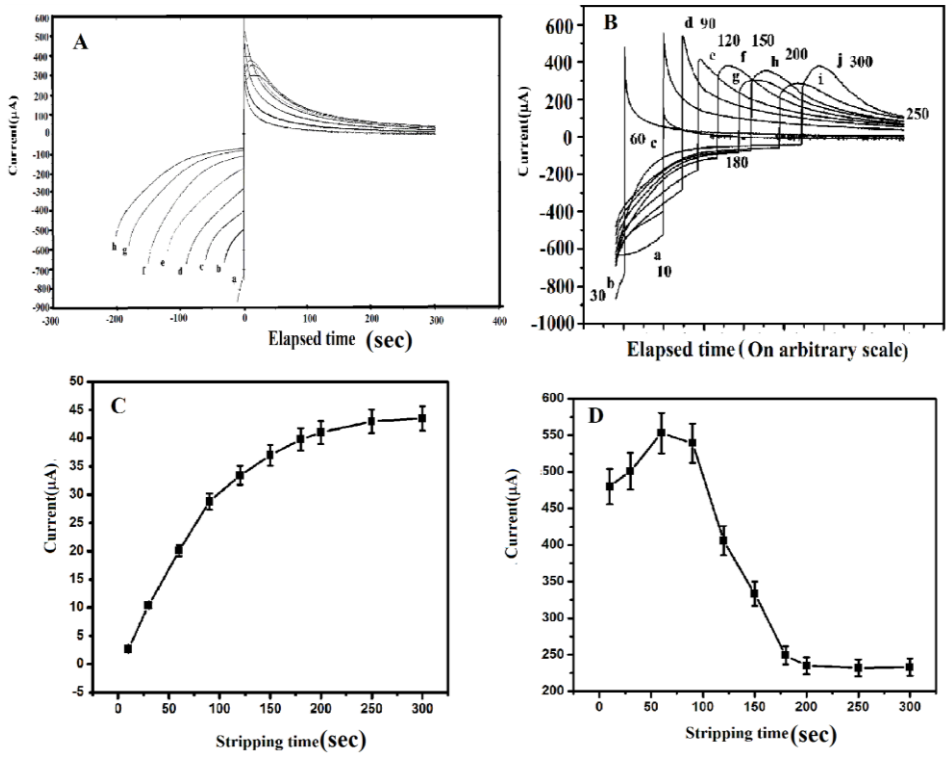

Fig.4.4 Effect of preconcentration time on the CA current. A. Curves a-h are the CAs of (2mM) ATChCl solution using enzyme electrode for different preconcentration time. B. expanded CA plots to show the effect of preconcentration time on the peak shape C. The plot of preconcentration time vs. current while recording the later at the end of the CAs, and D. the same when recorded at the current transient.CA parameters used were E_0=-0.8 V, E_1=0.7 V, $t_0$variable, t_1=300 s.

Figure 4.4A,B showing the successive CA runs obtained by applying different preconcentration (polarization) time at -0.8 V while subjecting the oxidation at 0.7 V. Fig.4.4C is the plot of preconcentration time vs. current while recording the later at the end (300 s) of the CAs and 4.4D the same when recorded at the peak. It is seen that the current at the end increases gradually with preconcentration time and get saturated at about 180s. The peak current on the other hand increases initially up to 75 s and then starts decrease until a steady value was obtained at about 180 s. This decrease is due to hindrance in electron movement caused by excess accumulation of dithiobischoline at the electrode surface as a result of continuous polarized oxidation. This hindered electron transfer is obvious from the lowering of the electron diffusion coefficient in the polarized case as compared to the unpolarized case obtained through impedance calculation.[34]This indicates that a proper cross

checking of the electrode cleaning step before each new experiment must be done for repeated use of the electrode when the amperometric signal is measured at the peak and when a longer preconcentration time is used. With longer preconcentration time the current transient take different shape. It shows upward rise for certain time, reaches a maxima and then decreases slowly (Fig.4.4B). This feature is characteristic of oxidation at 0.7 V only. It indicates that maximum oxidation of thiocholine occurs at 0.7 V. At lower potential, incomplete oxidation takes place. The results of this experiment together with that of sec.4.4.2.1 led to the conclusion that better estimate of Faradaic current of thiocholine oxidation can be made from the CA current transient after applying proper preconcentration procedure and applying electrode cleaning step before each new experiment.

Effect of applied negative preconcentration potential on the signal intensity was studied through CA experiment to find out appropriate preconcentration potential. Figure 4.5 shows the plot of preconcentration potential versus current signal and it is seen that the maximum signal obtained when the applied potential is -0.8 V.

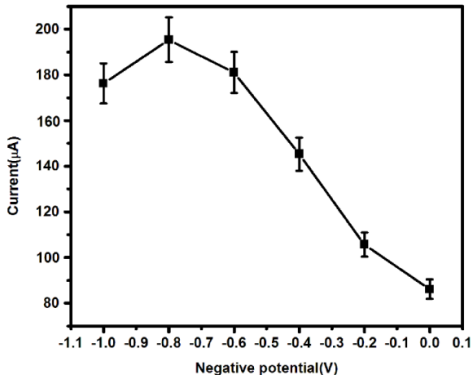

Fig.4.5 Plot of preconcentration potentials vs. peak currents when a constant preconcentration time of 180 s was applied during CA experiment. CA parameters used were E_0 variable, E_1= 0.7 V, t_0=180 s, t_1=300 s.

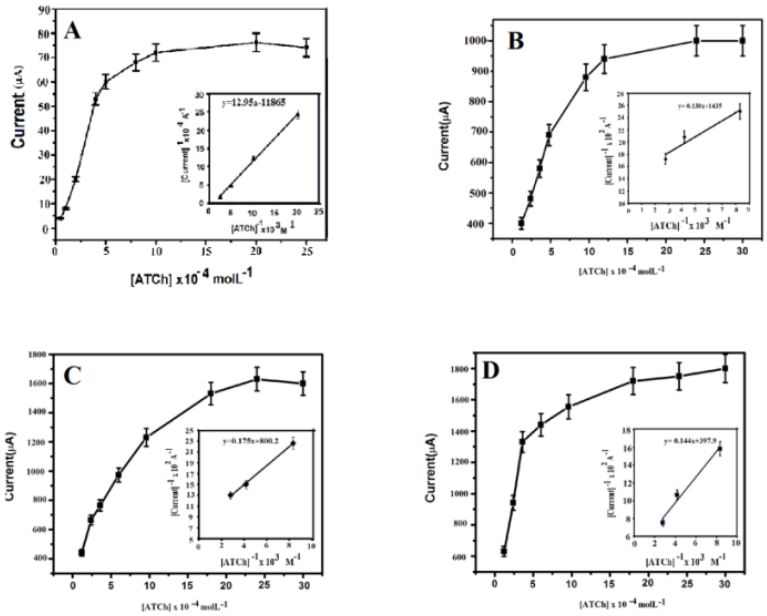

Fig.4.6 Michealis-Menteen plots under normal and preconcentration conditions with a fixed amount (10µL) ofenzyme loading. Parameters are (A) E_0= 0.0 V: t_0=60 s: E_1= 0.7 V : t_1=10 s (B-D) t_0=180 s, E_1= - 0.8V, t_1=10 s and E_1=0.2, 0.4 and 0.7 V, respectively.

4.4.4 Effect of polarized oxidation on kinetic parameter

Saturated substrate concentration (S), apparent Michealis-Menteen constant (K_m^{app}) and the sensitivity of the sensor probe were determined through Michealis-Menteen plot under the pre-polarized oxidation at that three potentials. Michealis-Menteen plots are shown in Figure 4.6. Parameters obtained from the calculations have been summarized in table 4.1.

Table 4.1 Effect of potential on kinetic parameters and on LODs

Evaluated parameters	CA parameters			
	E_0= -0.8V t_0=180s E_1= 0.2V	E_0= -0.8V t_0=180s E_1= 0.4 V	E_0= -0.8V t_0=180s E_1= 0.7 V	E_0=0.0 V t_0=60 s E_1=0.7 V
K_m^{app} (mmol L $^{-1}$)	0.09	0.22	0.36	1.09
S(mmol L^{-1})	2.40	2.40	2.40	2.00
Sensitivity(mA/M)	750	1350	2916	143
LOD(TCh) M	6.4 x 10^{-7}	3.5 x 10^{-7}	1.6 x 10^{-7}	2.1 x 10^{-6}
LOD (ethion) ppb	2	0.5	0.3	0.3

Table 4.2 Effect of preconcentration on kinetic parameters and LODs

Evaluated Parameters	CA parameters					
	E_0= -0.8V E_1= 0.2 V		E_0= -0.8V E_1= 0.4V		E_0= -0.8V E_1= 0.7 V	
	t_0=60s	t_0=180s	t_0=60s	t_0=180s	t_0=60s,	t_0=180s
K_m^{app} (mmol L^{-1})	0.11	0.09	0.38	0.22	0.65	0.36
S(mmol L^{-1})	2.4	2.4	2.4	2.4	2.4	2.4
Sensitivity(mA/M)	170	750	698	1350	1660	2916
LOD(TCh) M	2.8×10^{-6}	6.4×10^{-7}	7×10^{-7}	3.5×10^{-7}	2.9×10^{-7}	1.6×10^{-7}
LOD (ethion) ppb	2	2	0.5	0.5	0.3	0.3

As seen in table 4.1, thiocholine sensitivity without application of preconcentration is found to be143 mA/M. It is clear from the table that thiocholine sensitivity increases sharply when preconcentration is applied. Increase is proportional to the applied oxidation potential. As expected, a reverse trend is seen in the LOD values of thiocholine; LOD is higher when lower oxidation potential and lower preconcentration are applied as is evident from table 4.2.

It appears that the apparent Michealis-Menten constant was affected by three things. It decreases when a negative preconcentration potential was applied. Further decrement was seen when the negative potential was applied for higher (180s) preconcentration time. This is attributed to the increase of K_a through enhanced enzyme substrate contact via polarization.

$$E + S \rightleftharpoons ES \qquad k_a, k_{a'}$$

$$ES \longrightarrow P \qquad k_b$$

$$\boldsymbol{K_m = (k_a' + k_b)/k_a}$$

Scheme 4.1 Michalies-Menten mechanism

Lowering of K_m^{app} value was also observed when the oxidation potential was lowered. This is attributed to the insufficient oxidation at lower potentials that gives a lower estimate of K_b (Scheme 4.1). Relatively higher value of K_m (1.09 mmol L^{-1}) was obtained during normal, non-polarized oxidation at 0.7 V. [31]

4.4.5 Effect low potential oxidation on precision and stability

The inter assay precision for the prepared sensor found to be 6.52%. Intra assay precision determined under polarized or preconcentrated condition were found to be 1.32%, 1.15%, and 3.10%, respectively, at 0.7 V, 0.4 V, and 0.2 V as final potentials (calculations in appendix).The lowering of precision at 0.2 V is probably due to incomplete oxidation of TCh at this marginal potential.

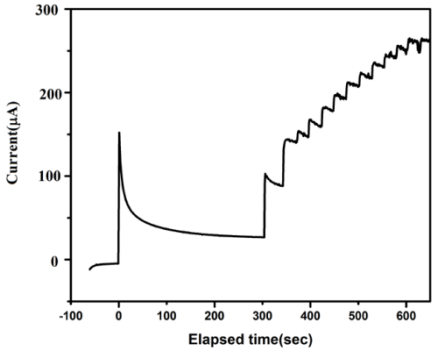

Fig.4.7 Sensor response to successive addition of acetylthiocholine (50 µL, 2 mM) solution.

In absence of proper electrode cleaning procedure, which is inevitable when thiocholine is added to the electrolytic cell during CA method keeping the electrode potentiostated at a fixed potential, huge deviation in signal intensity is observed. The current intensities were higher for the first two runs followed by 8-10 low intensity signal leading to low precision (RSD 22% for all runs and 10.01% for all but the first two runs, Fig.4.7). This is attributed to the electrode fouling through oxidized thiocholine ions getting trapped in the film material.

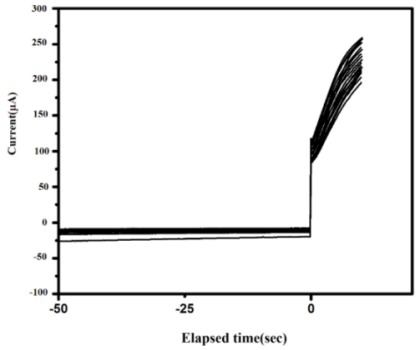

Fig.4.8 Operational stability of the sensor at 0.2 V. Figure contains fifty continuous CA runs in ATChCl with R.S.D 3.28**%**.

4.4.6 Effect of low potential operation on sensor operational stability

Operational stability determined for repeated measurement of thiocholine oxidation under preconcentration condition in absence of pesticides found to be different at low and high oxidation potentials. It was found to be 40 at 0.7 V, and over 50 at low potentials (0.4 V and 0.2 V) within 3.10% RSD (Fig.4.8).Getting of relatively lower operational stability at 0.7 V as compared to low potentials of 0.4 V and 0.2 V is attributed to the change in PPy film morphology and porosity, in addition to enzyme deactivation, upon prolonged oxidation at 0.7 V.

4.4.7 Application to Organophosphate analysis

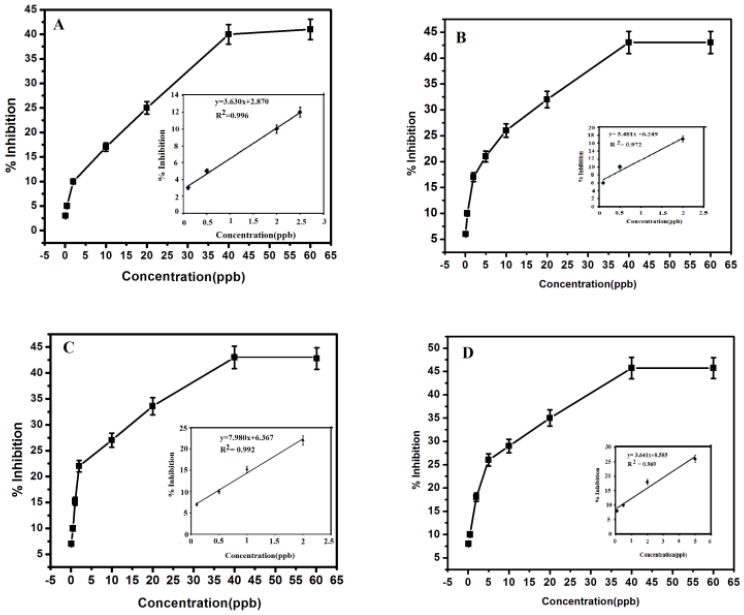

Fig.4.9 Calibration curves for ethion at different CA conditions. Parameters are (A-C):E_0=-0.8V t_0=180 s, t_1=10 s and E_1=0.2, 0.4 and 0.7 V, respectively. D.E_0= 0.0 V t_0= 60 s, t_1=10 s.

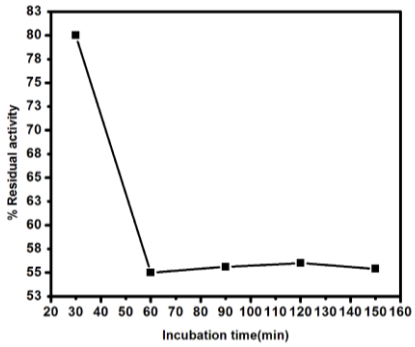

Fig.4.10 Incubation time vs. % residual activity plots with 20 ppb ethion solution.

Low potential workability of the sensor was tested taking ethion as a sample OP pesticide, preparing the solutions in 5% acetonitrile. Incubation time for analysis of the pesticide was at first determined by taking a 20 ppb ethion solution and then determining the variation in residual activity (Ar % Eqn.2.1) with time. Maximum decrease in Ar% found to occur after 60 minutes (Fig.4.10). Reactivation of the sensor was done by keeping immersed in 0.05 M NaF solution for half an hour. Calibration curves were then obtained at different sets of conditions taking 1 hr. as the inhibition time and using Eqn.2.2 (Fig.4.9).The limit of detection (LOD) of ethion was calculated for each curve by noting the concentration of pesticide corresponding to 10% inhibition. Table 4.1 and 4.2 show the LODs of ethion under different CA conditions. It is seen that the LOD is not affected by preconcentration technique but to some extent by the operating potential; LOD increases when the oxidation potential is lowered.

The reason why the LOD of the pesticide is not affected by the polarization or preconcentration technique is obvious. The LOD of the pesticide, as per the adopted definition, is dependent on the percent inhibition that does not depend on the preconcentration. Moreover, thiocholine preconcentration on the electrode surface becomes limited once it is covered by the pesticide. Thiocholine LOD was determined using the equation (Eqn.4.1)

$$LOD = 3 \times \frac{S_b}{m} \qquad (4.1)$$

Where s_b is the standard deviation of the blank (phosphate buffer), m is the slope of the calibration curve. Table 4.3 shows a comparison of LODs of thiocholine obtained at low potentials by different mediator based methods and the present method.

Table 4.3 LODs of thiocholine obtained during low potential oxidation under different methods. Response characteristics of thiocholine on different electrochemical transducers

Electrode	Working potential[a](V)	Detection limit	Reference
Graphite/epoxy resin	0.7	5x 10^{-6} M	33
Cobalt phthalocyanine/screen printed electrode	0.1	5.0x10^{-7}M	17
Prussian blue/screen printed electrode	0.2	5x10^{-6} M	19
Tetracyanoquinodimethane/epoxy resin/graphite	0.3	2.5x 10^{-6} M	20
PPy-AChE-Geltn-Glut/platinum electrode	0.2	6.4x 10^{-7}M	present work

[a]All potential via Ag/AgCl

4.4.8 Correlation between low and conventional high potential analysis

Correlation procedure for mapping the low potential (0.4 V) data onto conventional high potential (0.7 V) data has been worked out as shown below

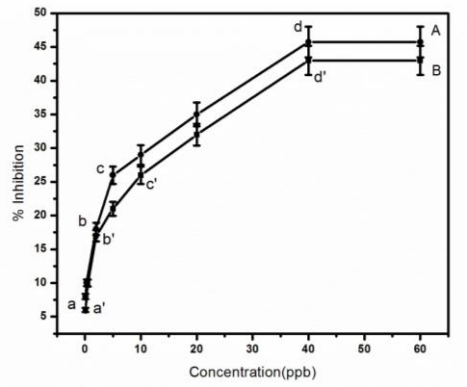

Fig.4.11Calibration curves for ethion at two different CA conditions. Parameters are A: E_0= 0.0 V t_0= 60 s, t_1=10 s, E_1= 0.7 V and B: E_0= -0.8 V, t_0= 180 s, t_1=10 s, E_1= 0.4 V

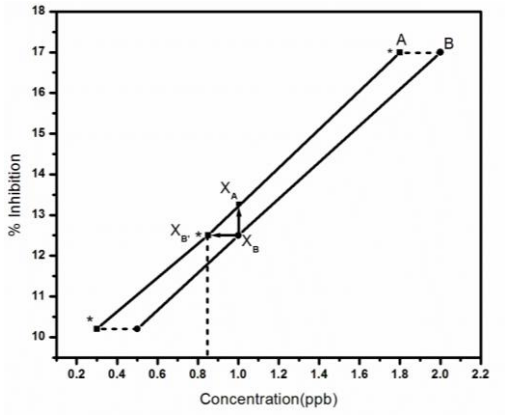

Fig.4.12 Segment 1of Fig.4.11before regression : a a´b b´ A- 0.3 to 1.8 ppb, B- 0.5 to 2 ppb, I%= 10.2 to 17.0

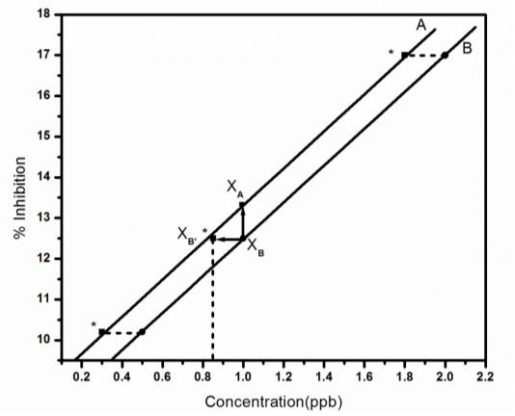

Fig.4.13 Segment 1of Fig.4.11 after regression.

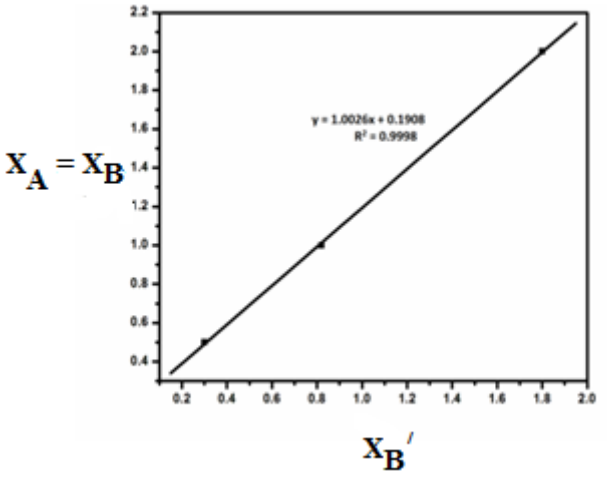

Fig.4.14 plot of Common Concentration vs. Shifted Concentration of B on curve A for segment 1

Table 4.4 Correlation of concentration in segment 1

Common Concentration $X_B = X_A$ (ppb)	Shifted Concentration of B on curve A, $X_{B'}$ (ppb)	% Inhibition Common to both A and B
0.50	0.30	10.2
1.00	0.82	12.5
2.00	1.80	17.0

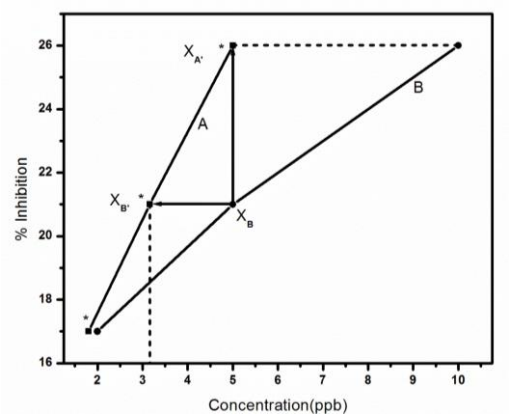

Fig.4.15 Segment 2 of Fig. 4.11 before regression: b b′cc′, A= 1.8 to 5ppb, B- 2 to 10 ppb, I%= 17 to 26

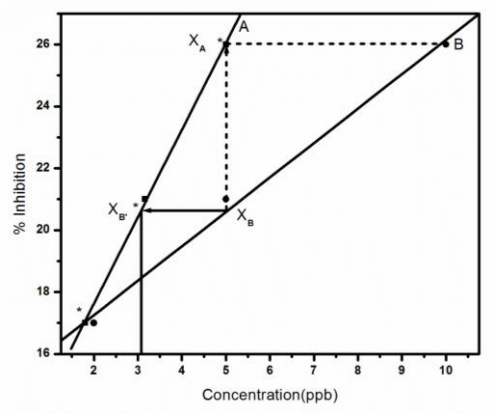

Fig.4.16 Segment 2of Fig.4.11 after regression

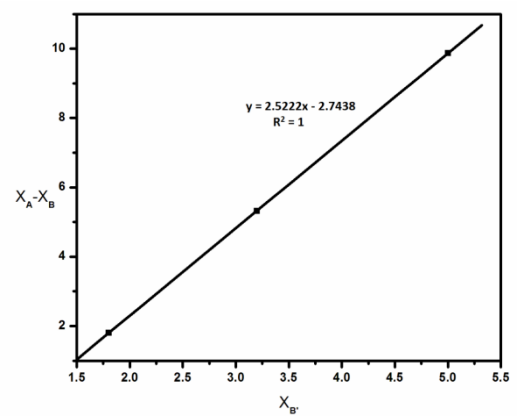

Fig.4.17 plot of Common Concentration vs. Shifted Concentration of B on curve A for segment 2

Table 4.5 Correlation of concentration in segment 2

Common Concentration $X_B = X_A$ (ppb)	Shifted Concentration of B on curve A, $X_{B'}$ (ppb)	%Inhibition Common to both A and B
1.80	1.80	17.0
5.32	3.20	21.0
9.87	5.00	26.0

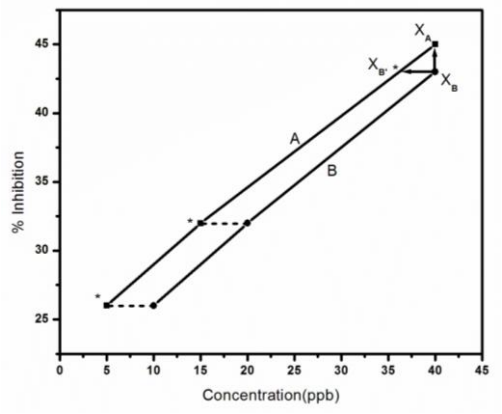

Fig.4.18 Segment 3 of Fig. 4.11 before regression cc´dd´: A= 5 to 40ppb, B- 10 to 40 ppb, I%= 26 to 43

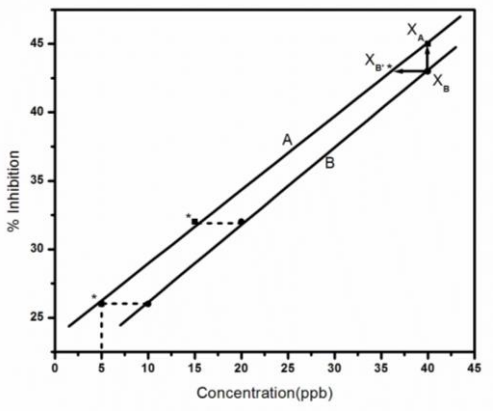

Fig.4.19 Segment 3of Fig.4.11 after regression

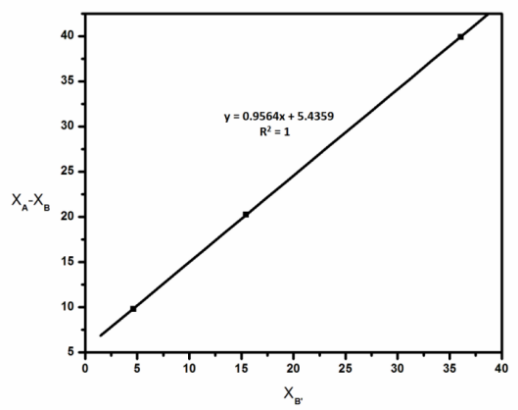

Fig.4.20 plot of Common Concentration vs. Shifted Concentration of B on curve A for segment 3

Table 4.6 Correlation of concentration in segment 3

Common Concentration $X_B = X_A$ (ppb)	Shifted Concentration of B on curve A, $X_{B'}$ (ppb)	% Inhibition Common to both A and B
9.82	4.61	26.0
20.24	15.44	32.0
39.90	36.05	43.0

Correlation equations for the three segmentsare y= 1.0026x +0.1908 for segment aa′bb′with I% 10to 17, y= 2.522x-2.7438 for segment bb′cc′with I % 17 to 26, y=0.9564x+5.4359 for segment cc′dd′with I % 26 to 43. The % deviations of the low potential method in the three regions found to be 16.20, 37.80 and 24.17% respectively for segments 1, 2 and 3.

4.5 Conclusion

We have shown throughCA method that PPy can lower the oxidation potential of thiocholine close to 0.2 V from its conventional value of 0.7 V. The low potential oxidation of thiocholine can be utilized through amperometric method for quantification of thiocholine as well as organophosphate pesticide. Low potential analysis enhances the operational stability of the PPy entrapped AChE biosensor but gives lower estimate (higher LODs) of both thiocholine and pesticides. An optimized set of operating condition for low potential detection of thiocholine from amperometric current transient has been worked out that involves using of a preconcentration potential of -0.8 V and apreconcentration time of 180 s prior to oxidation. Though the LOD of thiocholineis significantly lowered by the preconcentration method, the same of pesticide is not significantly affected.The work has also demonstrated that unhydrolyzedacetylthiocholine also contribute a significant amount of charge transfer current to the amperometric transient with PPy coated Pt electrode. This current is lower in magnitude than the thiocholine oxidation current and attributed to the intermolecular electron transfer between PPy and acetylthiocholine catalyzed by PPy.

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