Cyclic Voltammetry of Trazodone as [piperazin-1-yl] Antidepressant Drug and Bovine Serum Albumin Binding

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Abstract: The electrochemical behavior of trazodone (TRZ), 2-{3-[4-(3-chlorophenyl)piperazin-1-yl] propyl}-2H,3H-[1,2,4]triazolo[4,3-a]pyridin-3-one has been investigated by cyclic voltammerty measurements at glassy carbon working electrode. The voltammetric method includes selection of supporting electrolyte, pH, scan rate and temperature optimization; and measurements of some other basic voltammetric parameters. The TRZ oxidation peaks are decreased as a result of TRZ-BSA binding. TRZ-BSA binding mechanism is also established.

Keywords: Piperazine -1-yl class, drug-protein binding, electrochemical method development, voltammetric parameter optimization, serotonin antagonist and reuptake inhibitor.

1. INTRODUCTION

Trazodone (2-{3-[4-(3-chlorophenyl)piperazin-1-yl] propyl}-2H,3H-[1,2,4] triazolo [4,3-a]pyridin-3-one) is a well-known chemical compound that is used as second generation antidepressant drug of phenyl piperazine class that belongs to a serotonin antagonist and reuptake inhibitor (SARI) [1]. Trazodone is used as anti-anxiety and also causes sleeping effects [2]. The exact mechanism of trazodone activity is still undiscovered, but it is generally believed that trazodone is used to relieve depression by preventing the uptake of serotonin in the brain [3].

Researchers have found many ways for the determination of trazodone in biological fluids and in pharmaceutical samples including: trazodone selective electrodes [4], capillary electrophoresis [5], high-performance liquid chromatography [6], atomic emission and absorption spectrometries [7], UV-Vis spectrophotometry [8], spectrofluorimetry [9], GC-MS [10], voltammetry [11] etc. Mostly for the analysis of trazodone hydrochloride (TRZ), the chromatographic methods are employed that are time consuming, expensive and utilizes more chemicals comparatively. Voltammetric method is better tool for the quantitative analysis of drug's active components and excipients in different pharmaceutical dosage forms and also in their metabolites of biological samples.

For the past few years, the investigation of the drug-protein binding through cyclic voltammetry (CV) is of keen interest by the scientists. The difference between the pre and post electrochemical signals of the drug indicates interaction between the drug and protein along with their binding mechanism [12].

Human blood plasma contains many proteins, out of these albumin is highly soluble and the most abundant. Serum albumin works as a carrier for binding of biologically active molecules such as fatty acids, metal ions and drugs to their target organs [13, 14]. Therefore, the model of this study is serum albumin as the drug-protein interaction [15]. This drug-protein interaction got attention because of its importance in the field of chemistry, clinical medicine and life sciences. The interaction mechanism helps to understand the change in structural features of protein, the drug toxicity and playing vital role in the field of pharmacodynamics, pharmacology and biochemistry [16].

The aims of this study are to establish the experimental conditions, to investigate the oxidation mechanism of [piperazin-1-yl] class of compounds and to optimize the conditions for quantitative determination of this compound in pharmaceutical formulations using cyclic voltammetry (CV).

2. EXPERIMENTAL

2.1. Materials

Trazodone hydrochloride (CAS # 25332-39-2) (purity ≥ 99%) powder and Bovine serum albumin lyophilized crystalline powder (CAS # 9048-46-8) (purity ≥ 98.0%) are from Sigma-Aldrich™ and both are used without further purification. Deionized water is used throughout the study. Ortho phosphoric acid (85% w/w), Sodium hydroxide and Sodium nitrate are from Merck™. Sodium perchlorate (Analytical reagent grade) is from Fisher scientific ™. Nitrogen gas cylinder

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99.999% (5N) is obtained from Ms. Linde Pakistan Limited, Karachi having O_2< 4.0 ppm, H_2O < 5.0 ppm and THC < 1.0 ppm as impurity assay as mentioned in certificate of analysis. This N_2 gas is used throughout the study for purging in order to remove oxygen and other unwanted dissolved gases from the electrochemical cell.

Stock solution of TRZ and stock BSA solutions are prepared in deionized water. All solutions under (voltammetric investigations) are prepared in 0.2M phosphate buffer of pH 7.0.

2.2. Equipment

For cyclic voltammetry electrochemical workstation CHI660 (CH Instrument company, USA) is used for electrochemical evaluation. Assembly of three electrode system is employed that is consisting of glassy carbon electrode (GCE), AgIAgCl (3M KCl) and Pt wire as working electrode (WE), reference electrode (RE) and counter electrodes, respectively.

3. RESULTS AND DISCUSSION

Cyclic voltammetry (CV) of trazodone shows two oxidation peaks and no TRZ reduction peak is noticed at glassy carbon electrode (GCE) this means no peaks in reversed scan. The response in forward scan of TRZ is in two oxidation steps at positive potentials i.e. (anodic peaks) and these are well reported peaks [17] (Figure **1**). The peak of potential 0.8 V has higher current that is termed as peak A and other one as B. The current values for peak A are used for further analysis because of its high current intensity and thus response of peak A is more sensitive than B. The potential window of 0 to 1.4 V is selected (Figure **1**).

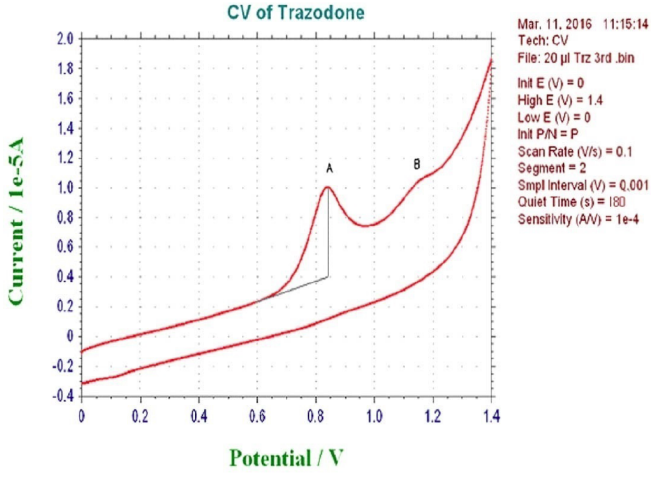


Figure 1: Cyclic Voltammogram of Trazodone.

3.1. Electrode Selection

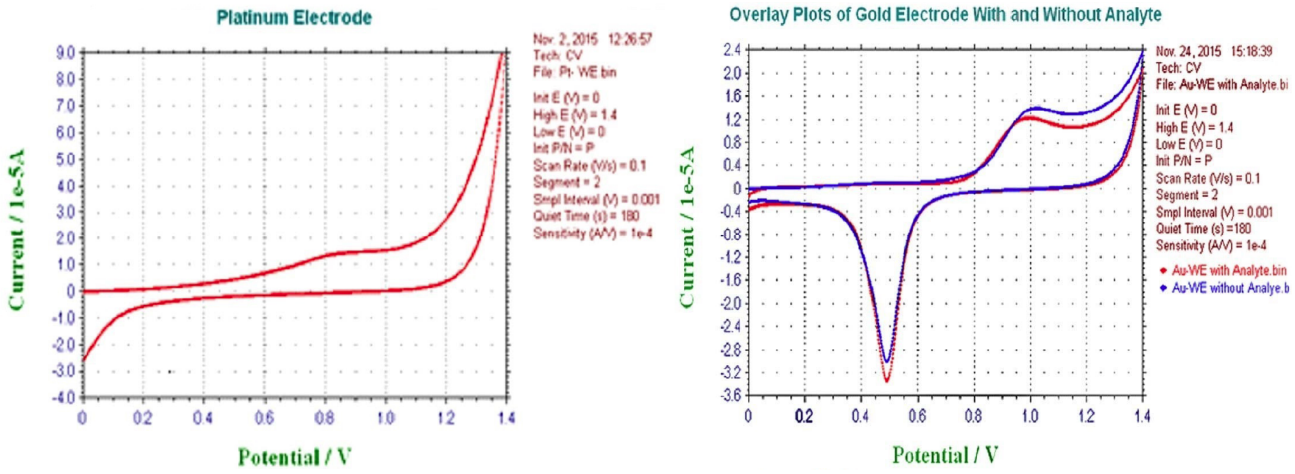
Electrodics is vital for any electrochemical analysis and to achieve improved and optimized outcomes the first factor is the selection of proper electrodes. Different types of electrodes were tested for TRZ analysis to get the better results. Firstly, Pt as working electrodes is compared and found that the TRZ oxidation peak is quite unobvious. The peak at Pt-WE is showing a broader peak that is indicating single oxidation step (Figure **2a**). At GCE peaks are with better intensities and also showing two oxidation steps of TRZ (Figure **1**). Au-WE is also examined but the peaks of TRZ are completely unapparent. At Au-WE the reduction peak is intense but this peak is not of TRZ, since blank solution is also showing the same reducing peak having similar intensity (Figure **2b**). Therefore there will be no or very little TRZ electrochemistry at Au-WE. The conclusion is that GCE is more suitable electrode for the analysis.

3.2. Selection of Supporting Electrolyte

Different supporting electrolytes (i.e. phosphate buffer, sodium nitrate and sodium perchlorate) are examined and the current intensity (for TRZ peak) is higher for phosphate buffer comparatively (Figure **3**). The concentration of phosphate (as SE) is scanned for optimum results and for this four different concentrations of phosphate buffer are run (i.e. 0.1, 0.2, 2.0 and 3.0 M) (Figure **4**). Although peak currents for buffer concentration of 2.0M Phosphate is high but repeatability is poor since repeated scans are not close to each other therefore 2.0M is quite unfeasible buffer concentration. The closeness of repeated scans for 0.2 M (Figure **5**) means good repeatability and thus 0.2 M phosphate buffer is chosen.

3.3. Customization of pH

The phosphate buffer of 0.2M concentration has been chosen. The pH also regulates electrochemical reaction behaviors of the molecules. Effect of the pH on peak current and potentials is therefore studied between pH 2.2 to pH 12.0. By increasing the pH of phosphate buffer the potential of the oxidation peak of trazodone is shifted to less positive values for both oxidation peaks. It is noted that by continuous increase in pH, the potential of peak A is shifted towards less positive value. After pH 7.3 it becomes pH independent and also after pH 9.0 the peak B gradually disappears (Figure **6**) [17]. The pH 7.0 is supposed to be optimum pH for voltammetric determinations. The selection of pH 7.0 instead of pH 7.3 is because of two reasons:



(**a**) (**b**)

Figure 2: Overlaid voltammograms of (**a**) Platinum WE and (**b**) Gold WE.

Firstly, there is no noticeable difference in the current intensities at pH 7.0 and 7.3 Secondly, commercially prepared pH 7.0 buffer is used that has been supplied with certificate of analysis. Meanwhile, the scan rate and other voltammetric parameters are kept fixed.

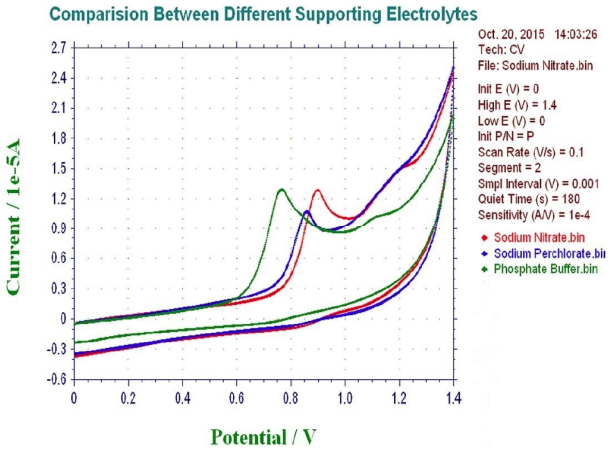


Figure 3: Overlaid voltammograms of 0.2 M Sodium nitrate, 0.2 M Sodium perchlorate and 0.2 M Phosphate buffer pH 7.0.

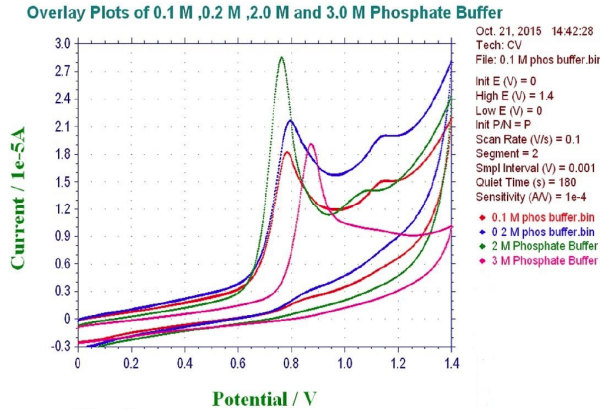


Figure 4: Overlaid voltammograms of 0.1, 0.2, 2.0 and 3.0 M phosphate buffer.

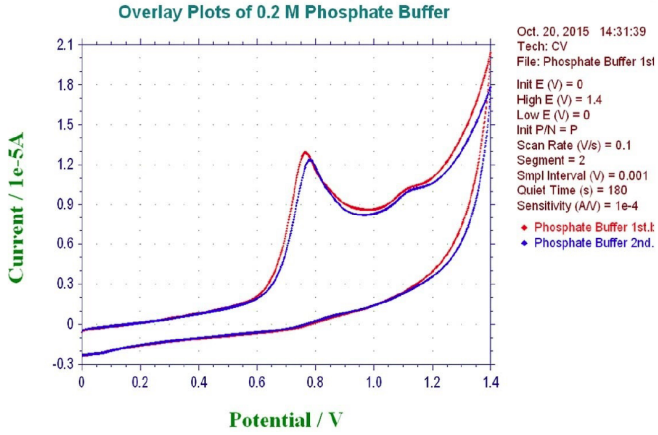


Figure 5: Overlaid voltammograms showing repeatability of runs having 0.2 M Phosphate buffer.

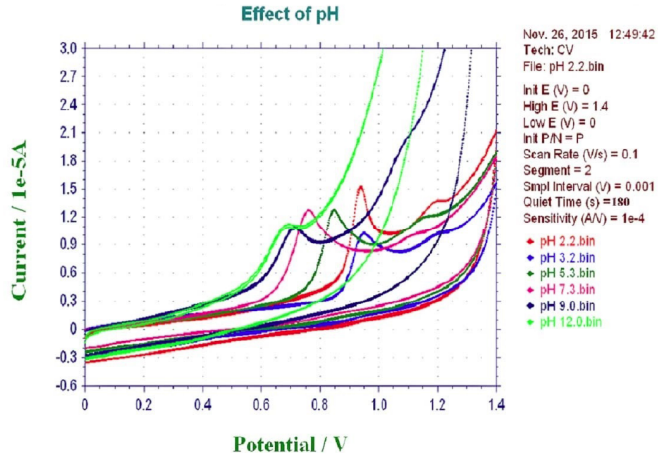


Figure 6: Overlaid voltammograms at different pH.

3.4. Effect of Nitrogen Purge

The cell is also assessed for inertness; nitrogen gas is purged in the electrochemical cell before run to make

inert system. The results are showing prominent effect of N_2 purging since peak intensity is increased after N_2 purging. Since dissolved oxygen in the electrochemical cell interferes the TRZ oxidation and consequently the current intensity of TRZ oxidation peak decreases. Further to this the purging time is optimized and five minutes purging is found sufficient. Since of increasing purging time make no noticeable increase in efficiency but longer purge time increases run time and make analysis quite tedious.

3.5. Effect of Scan Rate

Cell's electrochemistry strongly depends upon scan rate of varying potential in voltammetric runs. Scan rate profile is taken by running voltammograms under identical cell conditions. Scan rates are varied from 0.05-0.5V.s^{-1} (Figure **7**). Peak currents are increasing with scan rates. Usually the statistical correlation coefficient between square root of scan rate and peak currents is evaluated to ensure the mode of mass action for TRZ in cell. The value of such coefficient is found 0.9059.That is showing linear dependency of peak currents on square root of scan rate. Also according to Randles-Savick relationship the cell possess diffusion controlled system. The diffusion layer of uniform thickness is possible.

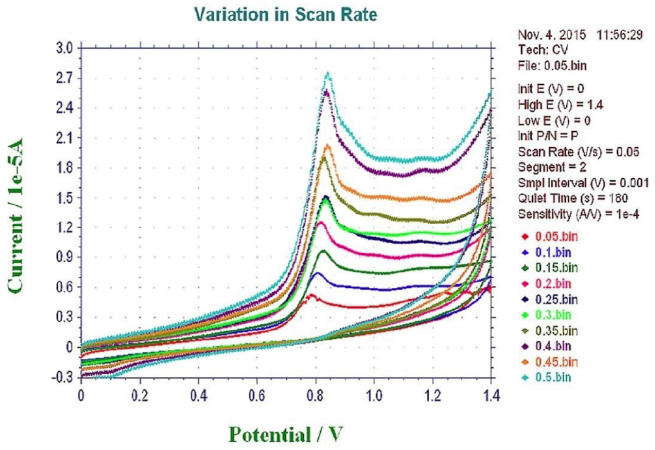


Figure 7: Overlaid voltammograms for different scan rates.

The correlation coefficient obtained from the plot of square root of scan rate and current is 0.905. As shown in Figure **8** that by increase in scan rate the current is also increasing and there is very clear periodic trend is obtained i.e. a well definite zigzag pattern is observed in higher scan rate from 0.35 to 0.5 V.s^{-1}. But this amplitude of this periodicity is very low in lower scan rate and high for higher scan rates. The reason behind this could be at high scan rates e.g. 0.3V.s^{-1} the possibility of electron loss by TRZ at 1st oxidation site is got difficult so current is reduced but as scan rates reached to above 0.3V.s^{-1} the current increases that ability of electron loss is easy by TRZ and similar pattern of up and down results are obtained up till 0.5 V.s^{-1} scan rate. The scan rate selected throughout for our study is 0.1V.s^{-1}. The linear scan rate range (0.1 to 0.25 V.s^{-1}) is selected. Although maximum current intensity is obtained at 0.5 V.s^{-1} but because of oscillation after 0.25 to 0.5 V.s^{-1} these scan rates are neglected for the minimization of uncertainty in analysis.

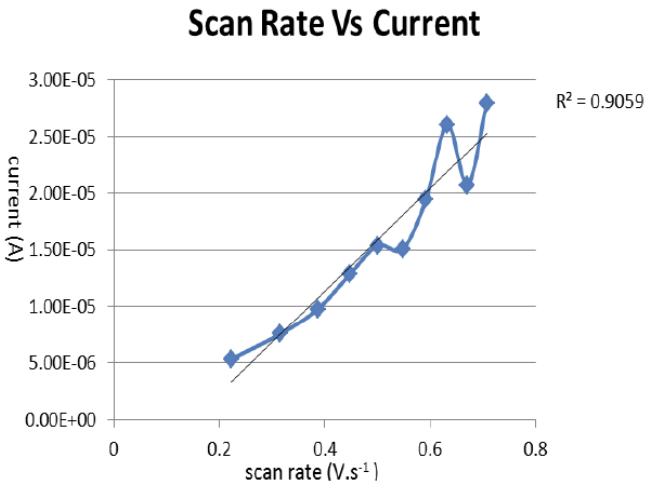


Figure 8: Plot of Square root of scan rate versus current.

3.6. Influence of Quiet Time

Bimolecular reaction usually forms equilibrium. There is always need to ensure time dependency for such analysis. The quiet time is adjusted and profile of quiet time is experimentally done. The diffusion current is increasing with quiet time and the optimum current is obtained at 180 seconds. After each successive run the

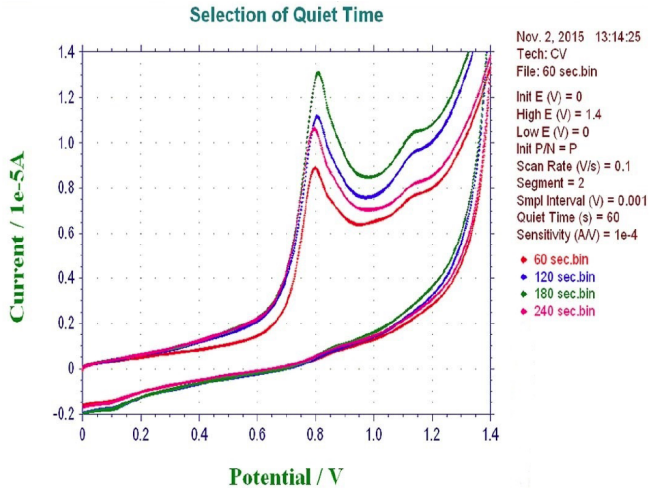


Figure 9: Overlaid voltammograms for different quiet time.

peak intensity of TRZ decreases gradually indicating the adsorption of drug on the surface of electrode. This behavior is reported [17] that such decrease in analyte's signal is due to adsorption controlled system. Therefore the GCE is polished prior to every run throughout the study (Figure **9**).

3.7. Temperature Dependency

Effect of temperature (on the reaction) is noticed by obtaining the voltammograms at different temperatures. As shown in Figure **10** that by increasing the temperature, the signal intensity also increases linearly and at lower temperatures, the signal intensity is reduced. This is due to high collision possibility, since at high temperature, molecular collision is increased. Therefore current is high and vice versa. Temperature also influences the diffusion coefficient. Since current is depending upon temperature, therefore to avoid uncertainties the temperature is maintained, the voltammetric experiments are usually done at ambient conditions. Although the laboratory temperature is always maintained and monitored at 25^{o}C and the temperature is altered by employing, circulatory water bath coupled with jacketed electrochemical cell.

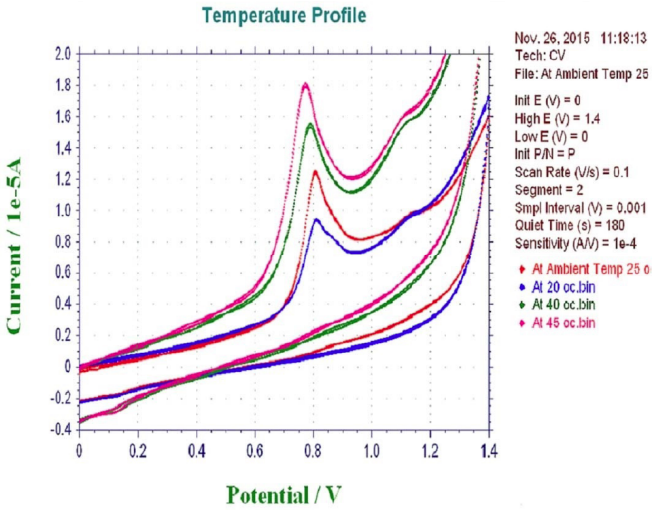
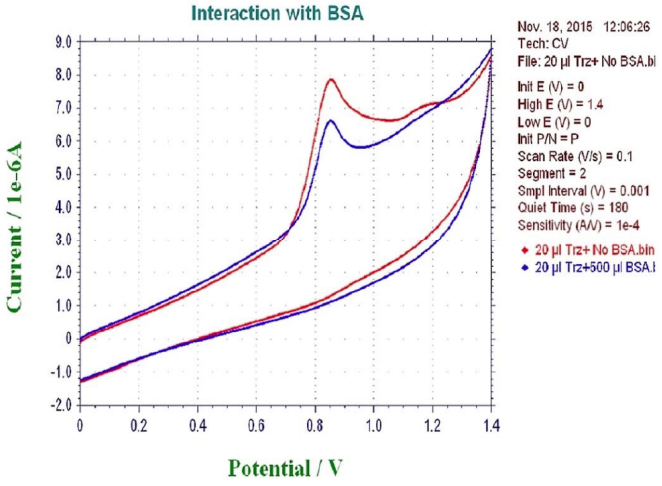


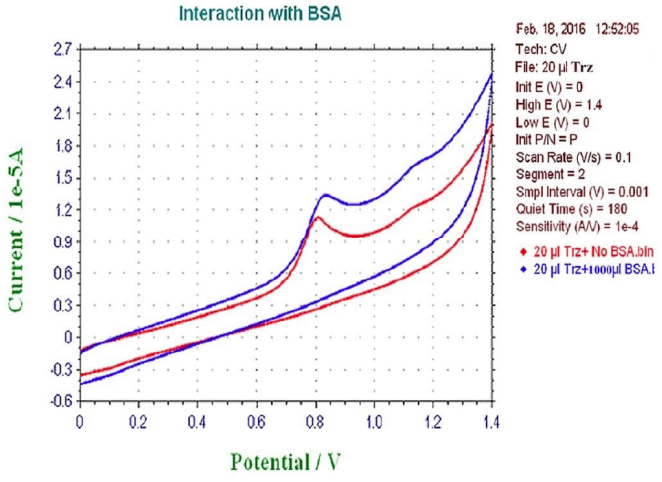
Figure 10: Overlaid voltammograms at different temperatures.

3.8. Interferences Effect

After optimization and selection of fundamental voltammetric parameters for TRZ analysis the interaction of the TRZ with the protein BSA is studied. The similar experiments are conducted for the characterization of binding mechanism of TRZ with BSA. The current intensity of TRZ is significantly decreased by the addition of BSA in the electrochemical cell, suggesting the exist stance of interaction between TRZ and BSA interaction (Figure **11a & b**). Scope of the study is to developed and optimize analytical method, therefore the study is concerned is to analyze *in vitro* interaction of drug with BSA along with interfering species (excipients) of drug, inspite of TRZ-BSA interaction in living bodies.



(**a**)



(**b**)

Figure 11: Overlaid voltammograms showing interaction of TRZ with BSA.

TRZ has two oxidation sites (Figure **12**) I and II. The interaction is engaging these electron donating sites with BSA. Since BSA is a macromolecule and a variety of bindings are probable, this depends upon stereochemistry of both TRZ and BSA. Firstly the interaction at lower BSA concentrations is showing that the blocking of second oxidation is lesser than first oxidation but at higher BSA concentrations the decrease ratio is quite identical for both oxidation peaks (Figure **11b**). The decrease in oxidation signal could be due to two reasons; one is binding of the TRZ

electrons that are supposed to undergo oxidation in voltammetric runs. Second reason is the physical coverage of BSA as a result of variety of docking and or binding with TRZ makes the TRZ-BSA composite not able to allow efficient diffusion and inhibit interaction of TRZ oxidation sites with WE. There may be any one or both mechanisms for inhibition in voltammetric oxidation of TRZ.

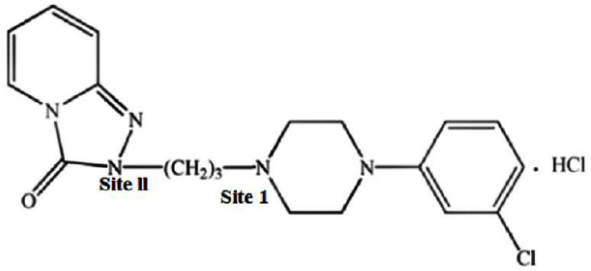


Figure 12: Structure showing oxidation sites for TRZ.

3.9. Voltammetric Current as a Function of TRZ Concentration

The calibration curve is plot i.e. current as a function of concentration. The sensitivity obtained from this plot is 0.677 nA.M^{-1}. The correlation coefficient is 0.9879 (Figure **13**). The range of TRZ concentration is from 1x10^{-5} to 1x10^{-9} M but the results are showing non-linear response. At nano level concentration i.e. 2.00 x10^{-9} M the curve is showing non-linearity but at higher concentrations, a good linear relationship is established between the concentration and current. Therefore the range is divided in to two categories i.e. low and high concentrations of TRZ (Figure **14a & b**).

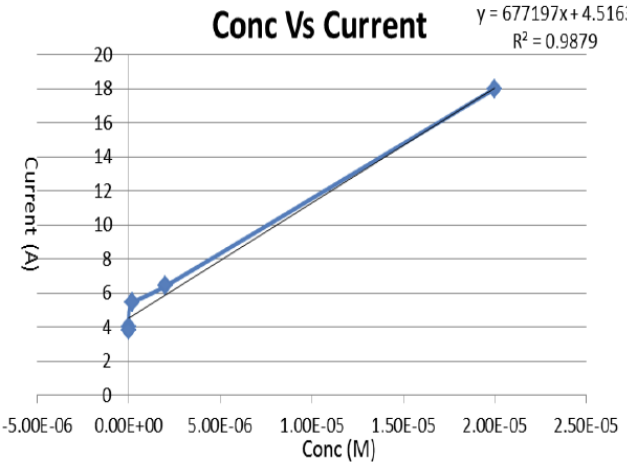
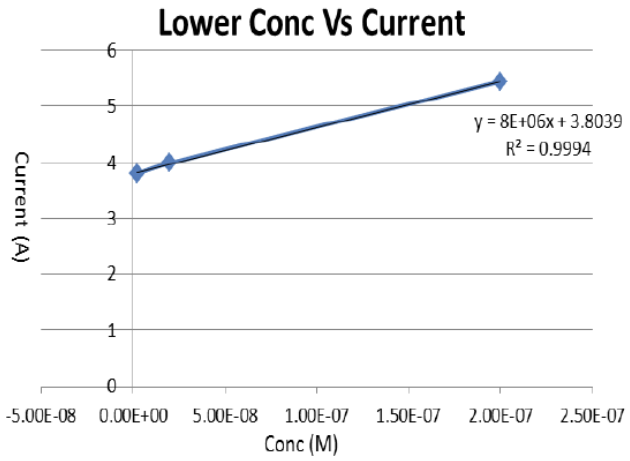
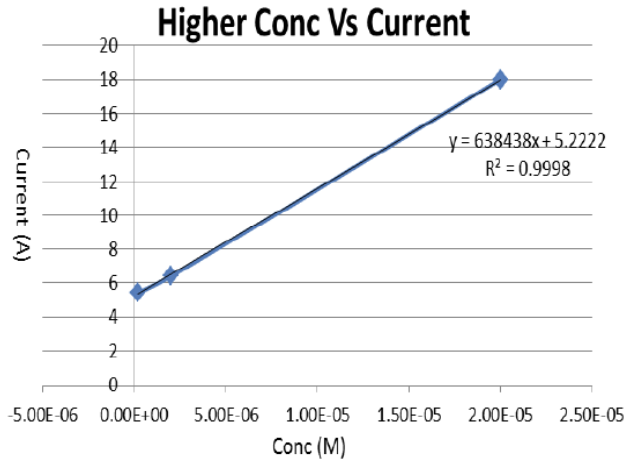


Figure 13: Plot of concentration versus current.

The correlation coefficient obtained for the set of data for lower values is 0.9994 whereas for higher TRZ concentrations it is 0.9998. For the lower values the signal is very low that the signal to noise ratio (S/N) is close to unity. This means there will be uncertainty that either this value is of signal or noise. For example, the signal for the 2.00 x 10^{-9} M is very small and could be considered as noise. Regarding higher concentrations, the (S/N) is higher and there is no possibility of any uncertainty and other factors involved in the analysis therefore this is more feasible range for quantitative determination.



(**a**)



(**b**)

Figure 14: (**a**) plot of lower concentration Vs current (**b**) plot of higher concentration Vs current.

4. CONCLUSION

In this study, the electrochemistry of the antidepressant drug Trazodone hydrochloride is successfully done through cyclic voltammetry. Basic voltammetric parameters are established and oxidation mechanism is studied. Along with this the interaction between drug and protein is also proven through cyclic voltammetry. These results could be useful for determination of TRZ or BSA in biological samples.

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