ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

Graduate Project (Chem.774)

Voltammetric study of paracetamole using glassy carbon electrode

A Graduate project presented to the School of Graduate Studies Addis Ababa University in partial fulfillment of the requirement for the degree of Master of Science in Chemistry.

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July, 2007

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Acknowledgement

I would like to express my deepest gratitude to my advisor Professor Theodros Solomon for his valuable guidance, consistence encouragement and constructive criticism throughout my project work. I feel privileged to be a student of Professor Theodros Solomon because of his hospitability who made me feel free to ask any help from him with out limitation of time.

My heart- felt word of thanks goes to my mother Liemet Wokele, my brothers and my sisters for their material and moral support starting from my elementary school up to this level.

Finally I would to thank Tigray Education Beurou for sponsoring my education, the Ethiopian Pharmaceutical Manufacturing Share Company (EPHARM.SC) for the support of the sample for this project work and AAU department of chemistry for providing me with laboratory and other facilities for this work to accomplish.

Abstract.

This paper describes the use of electro analytical techniques, cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPV) in the study of the electrochemical behavior of paracetamol in aqueous media and at the glassy carbon electrode. The electrochemical reaction was found to be irreversible, involving the transfer of two electrons. Aqueous phosphate buffer pH 7 was used as solvent and supporting electrolyte. An optimization of instrumental parameters was performed for the differential pulse and square wave voltammetric techniques. Differential pulse amplitude, pulse repeat time, and scan rate chosen for differential pulse voltammetry were 30 mV, 0.5 sec, and 5 mv/s respectively. Similarly for square wave voltammetry, the amplitude, frequency, and step potential chosen were 25 mV, 25 Hz, and 5 mV respectively. Finally the interference study of tablet was studied at aqueous phosphate buffer of pH 7.0.

1. INTRODUCTION

1.1. PARACETAMOL.

Paracetamole (N-acetyl-P-amino phenol) is an acetylated aromatic amide (Fig.1), introduced into medicine as an analgesic /antipyretic compound having actions similar to these of aspirin and suitable alternative for patients who are sensitive to aspirin. Paracetamole is a major ingredient in numerous cold, flu medications and in many prescription analgesics. It is remarkably safe in standard doses, but because of its wide availability, deliberate or accidental overdoses are not uncommon [1,2].

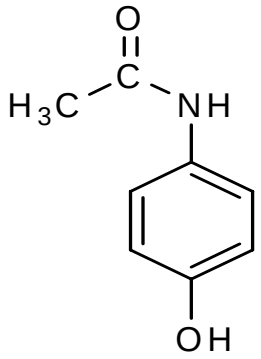


Fig 1-Structure of paracetamole (N-acetyl-p-amino phenol)

Paracetamole, known as acetaminophen, is a painkiller that is popular throughout the world because it is remarkably safe to the stomach. Paracetamole was first discovered in the late nineteenth century. Prior to this cinchona bark, which was also used to make the anti- malaria drug quinine, had been used to treat fevers. As cinchona became scarcer people began to look for cheaper synthetic alternatives. Two of the alternative compounds were acetanilide and phenacetin, developed in 1886 and 1887 respectively. By this time Harmon Northrop Morse had

already synthesized acetaminophen in 1878 through the reduction of p-nitro phenol with tin in glacial acetic acid [3].

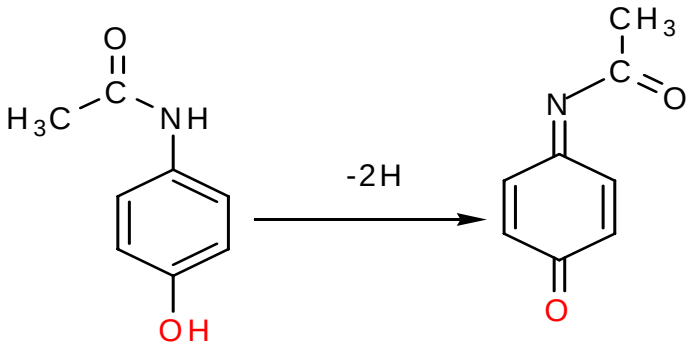
Von Mering in 1893 discovered paracetamole in the urine of individuals who had taken phenacetin. In addition to this the pain killing properties of paracetamol were discovered when similar molecules such as acetanilide were added to patients' prescriptions. But because acetanilide is toxic in moderate doses, chemists modified the structure to try and find a compound that was less harmful but still retained the analgesic properties [4].

Paracetamol attracted little clinical attention in the nineteenth century. However, after it was recognized as the chief metabolite of acetanilide and phenacetin, paracetamol experienced a resurgence of interest. As a derivative of p-amino phenol, paracetamol corresponds to the active principle metabolite of phenacetine. It was commonly assumed at the time that their rapid conversion by the body into paracetamol was actually responsible for the therapeutic effect of both medicines .It was eventually ascertained that phenacetine had its own pharmacological action. A high proportion of phenacetin is converted into paracetamol in the liver [5].

Paracetamol works as a weak prostaglandin inhibitor. It achieves this by blocking the production of prostaglandins, which are involved in the transmission of the pain message to the brain. In this regard paracetamol blocks the pain message at the brain and not at the source of the pains as the others do [5].

Today, paracetamol intoxification represents one of the most commonly used overdose agents in suicide attempts, and in this respect it is potentially more dangerous over the other counter

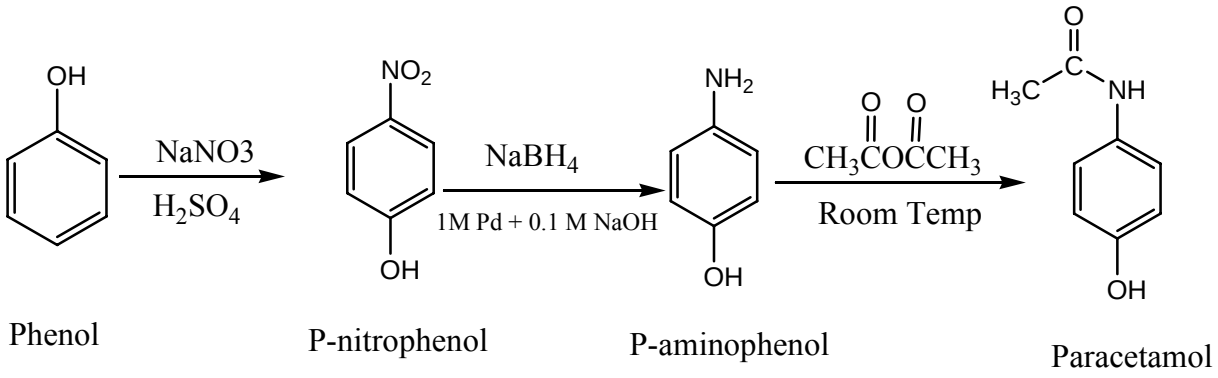
drugs such as aspirin. This is because paracetamol overdoses often cause liver failure, and there have been many attempted suicide cases .The reasons for this poisoning are to do with the responses by which paracetamol is eliminated from the body .It is first metabolized to quinone imine [6].



Quinone imine is extremely toxic, and like other such compounds is eliminated in the liver by reaction with a tripeptide, and glutathione. If insufficient amount of glutathione is present, the toxic quinone will not be eliminated and begins to react with cellular proteins and nucleic acids in the liver, eventually causing irreparable damage [6, 7].

1.2. SYNTHESIS OF PARACETAMOL

Paracetamol is one of the most common drugs in the world, and is manufactured in huge quantities. The starting material for the commercial manufacture of paracetamol is phenol, which is nitrated to give a mixture of the ortho- and para- nitro phenol. The ortho isomer is removed by steam distillation, and the para nitro phenol group is reduced into para aminophenol group. Then this compound is acetylated to give paracetamol [7].



Schem.1.Synthesis of paracetamol from phenol.

1.3. PHYSICAL PROPERTIES OF PARACETAMOL.

Paracetamol is a white, odorless crystalline powder with a bitter taste .It is soluble in water, alcohol, acetone, chloroform and methyl alcohol. It is also soluble in alkali hydroxide, but insoluble in benzene, and ether. The melting point of this compound is between 169-170^{o}c. [7].

1.4. CHEMICAL PROPERTIES OF PARACETAMOL

Paracetamol is most stable at saturated aqueous solutions. Its pKa value is 9.5 in aqueous media. The stability decreases in acid or alkaline conditions. It is slowly broken down into acetic acid and p- aminophenol [7].

1.5. ELECTROCHEMISTRY OF PARACETAMOL

Some reviewed literature reveal that paracetamol was studied with many methods at different solvents. These include titrimetry, chromatography, spectroscopy, high liquid chromatography, and electrochemistry [7, 8, 9, 10, 11]. In most of the methods considerable time is required for purification and extraction steps. But the electroanalytical methods do not require purification and extraction process. Some of the literature describe the electrochemical oxidation of paracetamol both in aqueous and non-aqueous media.

Paracetamol was studied by cyclic voltammetry in 0.1M phosphates and sulfuric acid both as solvent and supporting electrolyte. For this reason in 0.1 M phosphate buffer the maximum peak current is at pH 7 and anodic peak potential appeared at 450 mV using glassy carbon electrode versus the saturated calomel electrode. In addition to this, in sulfuric acid the anodic peak potential appeared at 618 mV in glassy carbon electrode versus Ag/AgCl [10,11,12].

By using amperometric methods, paracetamol has been studied in 0.05 M of phosphate buffer using the cellulose acetate modified glassy carbon electrode. Then the peak potential appeared at 390 mV versus SCE, and the maximum peak current was observed at pH 7 [10].

Electrochemical oxidation of paracetamol was described using differential pulse voltammetry in (1:1) of perchloric acid and methanol mixture, and sulfuric acid. Hence for the former solvent the anodic peak potential appeared at 620 mV versus the SCE electrode. Similarly in

sulfuric acid as a solvent the peak potential observed at 640 mV in pumice mixed acid carbon paste electrode versus SCE [13,14].

Lastly the voltammetric study of paracetamol was described by square wave voltammetry in nanogold modified indium tin oxide (ITO) electrode of 0.1 M phosphate buffer at pH 7.2.The electrode exhibited effective peak potential at 110 mV versus the Ag/AgCl [15].

In this project work the electrochemical oxidation of paracetamol was described using the electro analytical methods (CV, SWV, and DPV) in 0.1 M phosphate buffer using a glassy carbon electrode.

2. ELECTROCHEMICAL TECHNIQUES.

The voltammetric study of pharmaceutical drugs is by far the most common application of electrochemistry in pharmaceutical analysis. As a rule many of the pharmaceutically active constituents are readily oxidized or reduce. The functional groups show excellent voltammetric properties in the redox behavior at the working electrode [16].

Historically, the branch of electrochemistry we now call voltammetry developed from the discovery of polarography in 1922 by the Czech chemist Arousal Heyrovsky, for which he received the 1959 Nobel Prize in chemistry. The early voltammetric methods experienced a number of difficulties, making them less than ideal for routine analytical use. However, in the 1960s and 1970s significant advances were made in all areas of voltammetry (theory,

methodology, and instrumentation), which enhanced the sensitivity and expanded the repertoire of analytical methods.

The coincidence of these advances with the advent of low-cost operational amplifiers also facilitated the rapid commercial development of relatively inexpensive instrumentation.

The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i, and t. They are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces a change in the concentration of an electro active species at the electrode surface by electrochemically reducing or oxidizing it. The analytical advantages of the various voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1} M), a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times (seconds), simultaneous determination of several analytes, the ability to determine kinetic and mechanistic parameters, a well-developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential waveforms can be generated and small currents measured [16,17].

The electrochemical cell, where the voltammetric experiment is carried out, consists of a working (indicator) electrode, a reference electrode, and usually a counter (auxiliary) electrode. In general, an electrode provides the interface across which a charge can be transferred or its effects felt. Because the working electrode is where the reaction or transfer of interest is taking

place, whenever we refer to the electrode, we always mean the working electrode. The reduction or oxidation of a substance at the surface of a working electrode, at the appropriate applied potential, results in the mass transport of new material to the electrode surface and the generation of a current. Even though the various types of voltammetric techniques may appear to be very different at first glance, their fundamental principles and applications derive from the same electrochemical theory [17,18].

2.1. CYCLIC VOLTAMMERTY (CV)

Cyclic voltammetry is the most versatile electroanalytical techniques for the study of electroactives species. Its versatility combined with ease of measurement has resulted in extensive use of cyclic voltammetry in the field of electrochemistry, in organic chemistry, and biochemistry. The voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions, and on coupled chemical or adsorption processes [18].

It offers a rapid location of redox potentials of the electroactive species and convenient evaluation of the effect of solvent upon the redox process. The potential of the working electrode is controlled versus a reference electrode such as SCE or Ag/AgCl. The resulting triangular waveform is shown in figure (2) a. When the electrode reaction is reversible, the cyclic voltammorgram exhibits peaks on the forward and reverse scans. The parameters of importance are the anodic peak current (ipa), cathodic peak current (ipc), anodic peak potential (Epa) and cathodic peak potentials (Epc).

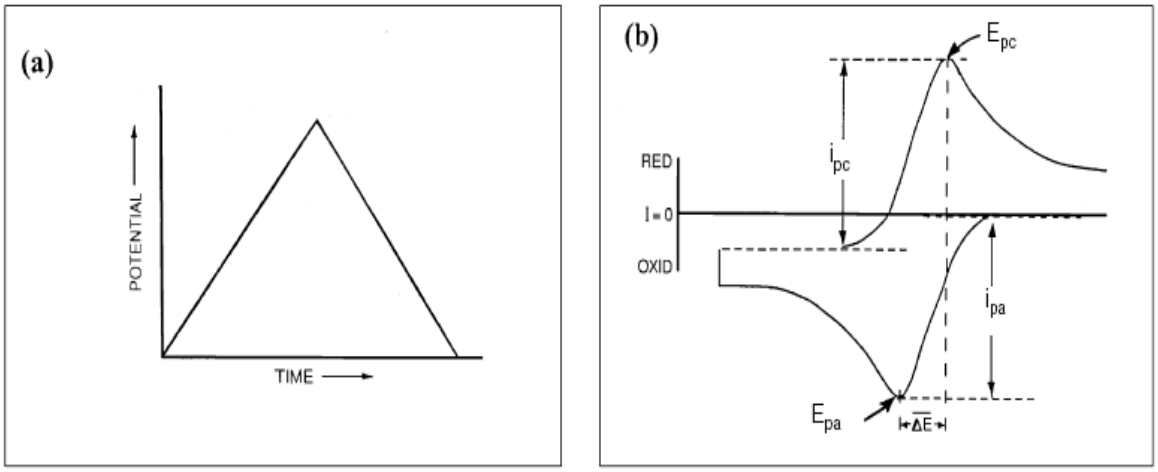


Fig (2) a. Excitation waveform and b) response obtained for the reversible by cyclic voltammetry.

A cyclic voltammogram can quickly show the presence of all species that undergo oxidation-reduction reactions at the working electrode within the limits set by the solvent electrolysis. A redox couple in which both species rapidly exchange electrons with working electrode is termed an electrochemically reversible couple. The formal reduction potential E^0 for a reversible is centered between E_{pa} and E_{pc}:

$$E^0 = (E_{pa} + E_{pc}) / 2 \quad \ldots\ldots (1)$$

The number of electrons (n) transferred in the electrode reaction for the reversible couple can be determined from the separation between the peak potentials:

$$\Delta E = E_{pa} - E_{pc} = 0.059/n \quad \ldots\ldots (2)$$

The peak current for a reversible system is described by the Randles-Sevcik equation for the forward sweep of the first cycle.

$I_p=2.69x10^5\ n^{3/2}\ ACD^{1/2}\ \nu^{1/2}$……(3)

where i_p is the peak current (A), n is the electron stoichiometry, A is the electrode area (cm^2), D is the diffusion coefficient (cm^2/s), C is the concentration (mol/ cm^2), and ν is the scan rate (V/S). Accordingly, for reversible electrode reaction the ratio of i_{pa} to that of i_{pc} is one. The difference in peak potential for two-electron transfer process is 29 mV. i_{pa} increases with $\nu^{1/2}$ and is directly proportional to concentration. For an irreversible process, that is slow (sluggish) electron transfer at the electrode surface, the ratio of i_{pa} to that of i_{pc} is greater than one. The peak current is given by:

$Ip=2.69x10^5n(na\alpha)^{1/2}\ ACD^{1/2}\ \nu^{1/2}$ …….. (4)

where n_a is the number of electrons in the rate determining step and α the transfer coefficient. All other quantities have the same meaning as the equation (3). In the case of a totally irreversible reaction only the peaks on the forward scan is observed. Irreversibility is due to slow electron transfer rate and results in ΔEp greater than 70 mV for one -electron reaction.

2.2. Square-Wave Voltammetry (SWV)

Square wave voltammetry is a type of pulse voltammetry that offers the advantage of speed and high sensitivity. An entire voltammogram is obtained in a few seconds or less. The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude E_{sw} superimposed on a staircase waveform of step height ΔE_s, where the forward pulse of the square

wave coincides with the staircase step (See Fig.3). The net current, i_{ne}, is obtained by taking the difference between the forward and reverse currents ($i_2 - i_1$) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electroactive species. Excellent sensitivity is achieved from the fact that the net current is larger than either the forward or the reverse components, since it is the difference between them and detection limits as low as 1 x 10^{-8} M are possible [19].

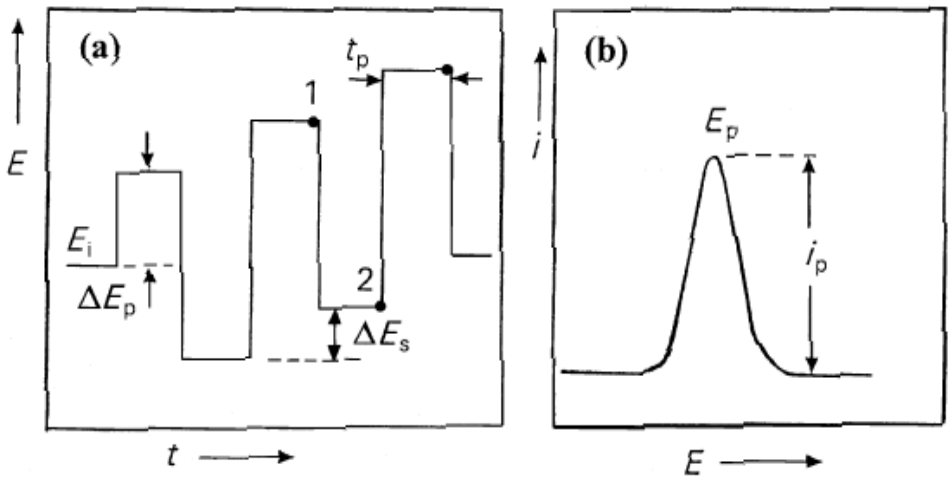


Fig (3). a. Excitation waveform of square wave voltammetry. b. Response obtained by square wave voltammetry.

Square-wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background current. This speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal-to- noise ratio. The net peak current for the irreversible system is given by [19].

I_p=constant.α n^2 ΔE E_{SW} $(f D)^{1/2}$C……(5)

where ΔE is the step potential, f is square wave frequency, E_{SW} is the square wave amplitude, α is the transfer coefficient, n is the over all electron transfer, C is the bulk concentration, and D is the diffusion coefficient of the electroactive species. The effective scan rate is given by fΔE. Kinetic parameters can also benefit from the rapid scanning and the reversal nature of square wave voltammetry. This method possesses both pulse and cyclic voltammetry; hence it is one of the most advanced methods in the family of pulse technique.

Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, determination of some species at trace levels, and its use with electrochemical detection in HPLC [20].

2.3. DIFFERENTIAL PULSE VOLTAMMETRY (DPV).

Differential pulse voltammetry can be considered as a series of regular voltage pulses superimposed on a linearly changing voltage, in which the resulting current is measured between the ramped baseline voltage and the pulse voltage. A digital staircase voltage is commonly used as the ramped baseline (See Fig.4). This technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to100 mV), and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point (1) just before the application of the pulse and the second (2) at the end of the pulse. These sampling points are selected to allow for the decay of the non-faradic

(charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential. [20]. For reversible electrode reaction the peak current is given by:

$$I_p = \frac{nFAD\ ½\ C}{(\pi\ t_m)^{1/2}}\ (1-\sigma/\ 1+\sigma) \quad \text{.....6}$$

where $\sigma = \exp [(nF/\ RT)(\Delta E/2)]$. ΔE is the pulse amplitude.

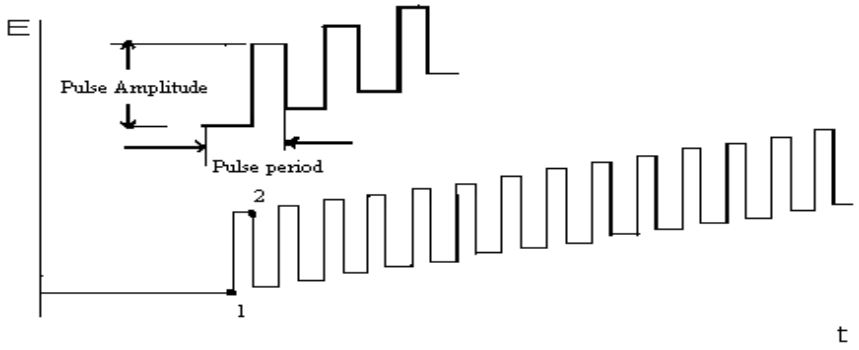


Fig .4, Excitation waveform of the differential pulse voltammetry.

3. OBJECTIVE OF THE STUDY.

The general objective of this study is to develop a less expensive validate voltammetric methods for the oxidation study of paracetamole manufactured in EPHARM.

The specific,

- Development of suitable methods such as CV, SQW, and DPV for paracetamole study using glassy carbon electrode.
- The electrochemistry study of paracetamole at 0.1 M Phosphate buffer solution in different pH values and scan rates.

4. EXPERIMENTAL PART.

4.1. REAGENTS AND CHEMICALS.

The reagents and chemicals used were paracetamole (EPHARM), disodium hydrogen phosphate (Wagtech international Ltd, UK), sodium dihydrogen phosphate (Riedel-deHaen, Germany), HCl (Riedel-deHaen, Germany), NaOH (BDH Poole, England), and sodium bicarbonate (BDH Poole, England). The working electrode was pretreated by polishing it with an alumina and rinsing in distilled water. Doubly distilled water was used through out the work.

Supporting electrolyte of phosphate buffers (NaH_2PO_4 –Na_2HPO_4) in the pH range 2-12 were prepared from 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 in distilled water. The pH of the solutions were adjusted by adding drops of concentrated HCl and NaOH .The required concentration of paracetamole in phosphate buffer solution was prepared from the EPHARM sample obtained at different pH media.

4.2. APPARATUS.

The voltammetric experiments were performed using the BAS 100A, electrochemical analyzer [Bioanalytical systems (BAS), USA], which was connected to an Acer Pentium computer .A magnetic stirrer with a hot plate from Cole Palmer instrument company was also used for stirring in pH adjustments. The pH of the buffer solution was measured with a Hanna digital pH 301 meter with combination glass electrode. All the potentials are determined with respect to a saturated calomel electrode as a reference electrode.

4.3. ELECTROLYTIC CELLS AND ELECTRODES.

This work was performed using a three-electrode system with a one-compartment glass voltammetric cell, a saturated calomel electrode (SCE) as a reference electrode and platinum wire as an auxiliary electrode. The working electrode was the glassy carbon electrode (GCE) with a diameter of 3mm.

4.4. PROCEDURE.

Cyclic voltammetric measurements were run from –300 to 1500 mV. Square wave voltammetric measurements were run from -200 to 800 mV, and finally the differential pulse voltammetric measurements were run from 0 to 1500 mV. The net current response of the different voltammetric measurements were recorded. The parameters for square wave voltammetric measurements were: a potential step of 5 mV, a square wave amplitude of 25 mV and a peak frequency of 25 Hz. For the differential pulse voltammetric measurements, a pulse amplitude of 30 mV, a scan rate of 5 mV/S, and a pulse period of 0.5 sec was used. All measurements were performed at room temperature (23^{O}_{C}).

5. RESULTS AND DISCUSSION.

In this paper the electrochemical oxidation of paracetamole has been studied using cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry. The optimum pH needed to study the electrochemical behavior of this pharmaceutical compound using the above mentioned electroanalytical techniques was pH 7.

.

5.1. CYCLIC VOLTAMMETRIC INVESTIGATION.

Fig.5 shows the cyclic voltammogram of $7.5x10^{-5}$ M paracetamole at the glassy carbon electrode at pH 7. The cyclic voltammogram of paracetamole shows irreversible oxidation and reduction peaks .The electrochemical reaction of paracetamole at the glassy carbon electrode at pH 7 showed an oxidation peak at 408 mV and a reduction peak at 327 mV .The separation of peak potentials was about 81 mV. The irreversible electrochemical oxidation of paracetamole shows slow electron transfer rates. This is because the separation in peak potentials is greater than for two electrons transfer reversible reaction.

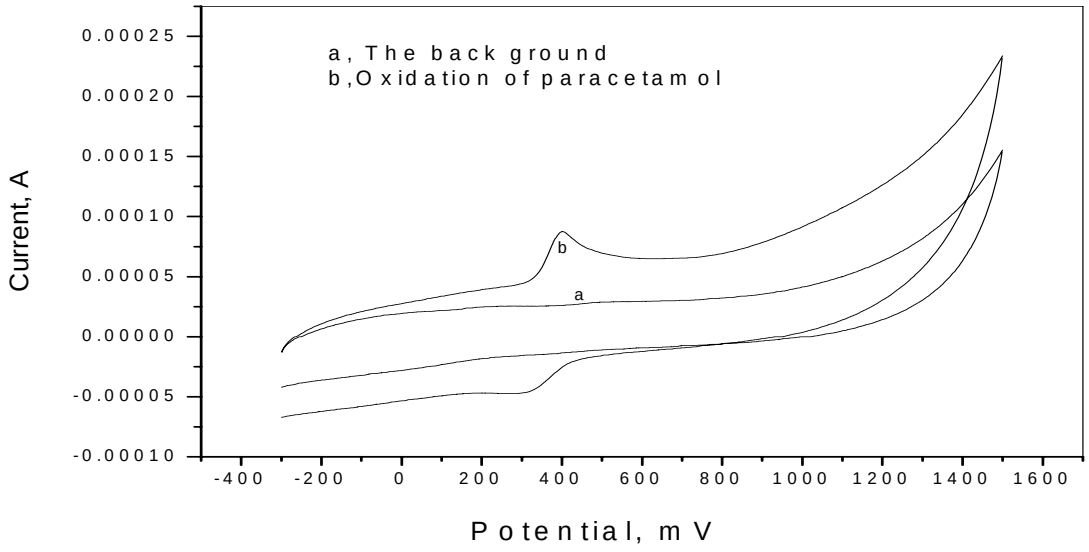


Fig (5). Cyclic voltammogram of 7.5x10-5 M paracetamole in 0.1 M NaH2PO4–Na2HPO4 Buffer (pH = 7.) GCE with scan rate of 100 mV / S

The electrode reaction of paracetamole is similar to non-ionized phenols, and this involves the electrophilic attack on aromatic nucleus with the irreversible removal of two electrons- two protons process to give phenoxonium ion (N-acetyl-P-quinone imine). The proposed electrochemical oxidation of paracetamole at the glassy carbon electrode is given by: [10,12]

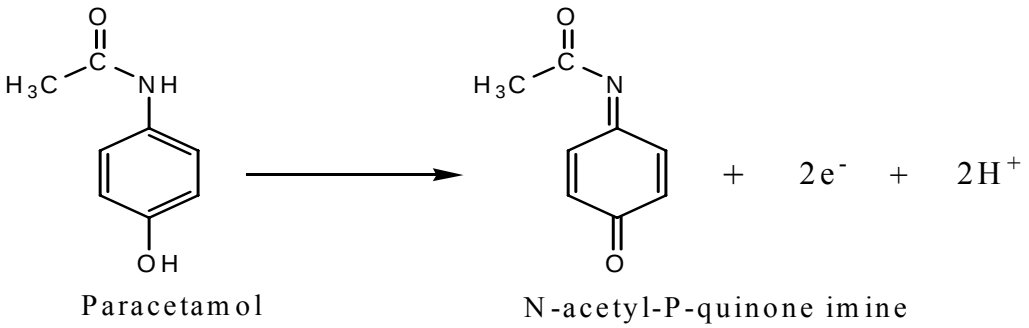


Fig (6). Electrochemical oxidation of paracetamole.

Fig.7 shows the cyclic voltammogram of $7.5x10^{-5}$ M paracetamole for four repetitive cycles. As the number of cycles increased, the peak height (peak current) of the irreversible redox behavior of paracetamole decreased.

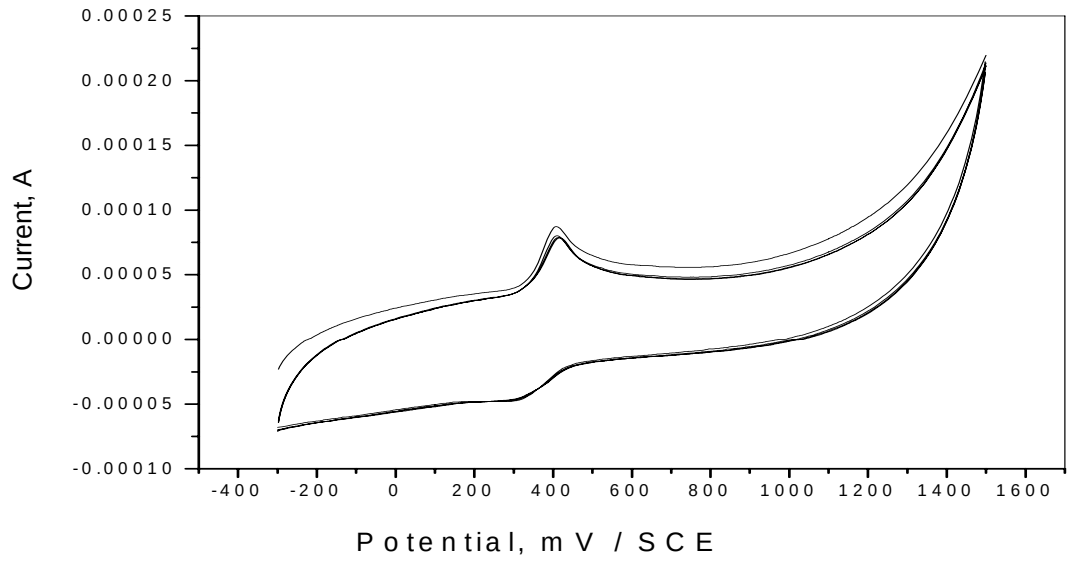


Fig (7). Cyclic voltammogram of $7.5x10^{-5}$ M paracetamole for four repetitive cycles in 0.1 M NaH_2PO_4 –Na_2HPO_4Buffer (pH = 7.0) at a glassy carbon electrode with scan rate of 100 mV/ S.

5.1.1. Effect of concentration.

The effect of concentration was studied using cyclic voltammetry. As can be seen from Fig.5, paracetamole is an electroactive compound showing an oxidation peak. When the concentration of paracetamole was varied the peak current increased successively.

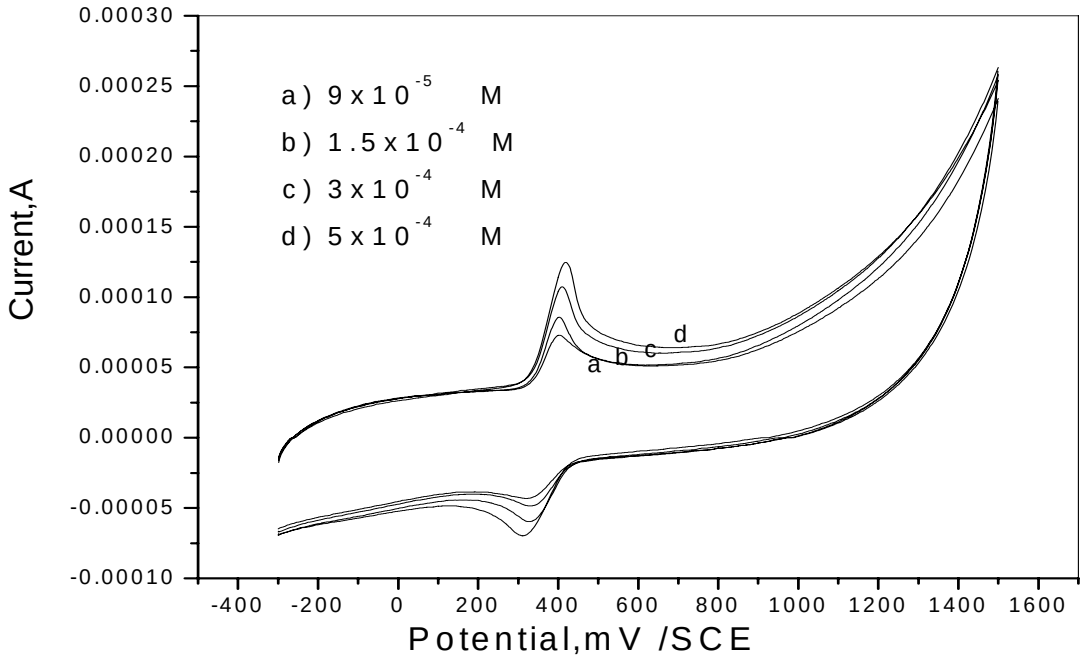


Fig (8). Cyclic voltammogram of paracetamole at different concentrations in 0.1 M NaH_2PO_4 –Na_2HPO_4 buffer (pH = 7.0) at a glassy carbon electrode with scan rate of 100 mV / S.

The relation between paracetamole concentration and cyclic voltammetric anodic peak current (I_{pa}) is linear as given in equation 4. The linear dependence of peak current on paracetamole concentration is depicted from the calibration curve shown Fig.9, with correlation coefficient almost unity (r=0.9973) and slope –0.1184. Hence linear fit of I_{pa} versus concentration is given by:

$$I_{pa} = -6.6026 \times 10^{-5} - 0.1184\ [APAP]$$

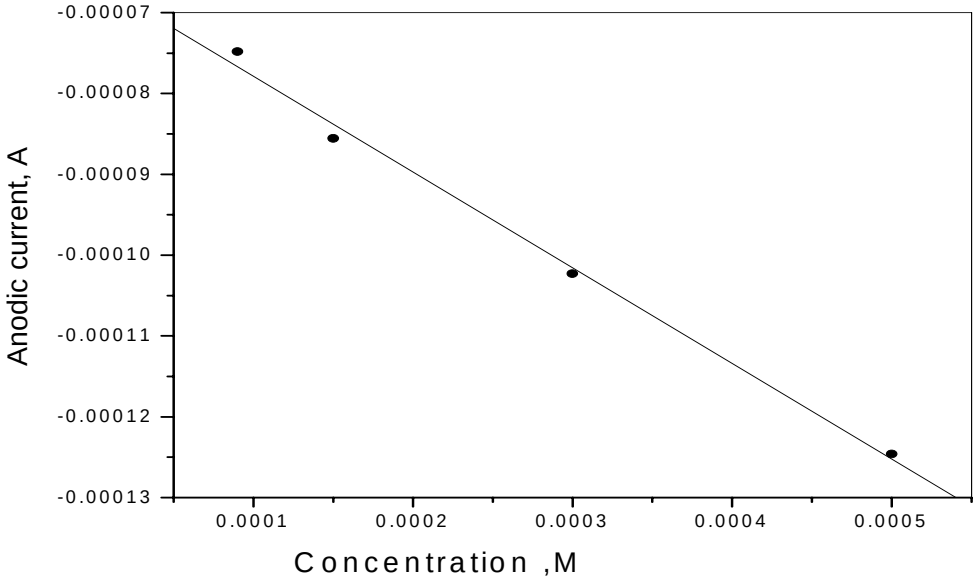


Fig .9 Plot of CV anodic peak current as a function of concentrations in 0.1 M NaH_2PO_4 –Na_2HPO_4 buffer (pH= 7) at a glassy carbon electrode with scan rate of 100 mV/ S

5.1.2. Effect of pH

The optimum buffer in this study was the phosphate buffer (NaH_2PO_4 –Na_2HPO_4) .The influence of pH on peak current of paracetamole has been studied in the range of 2-12. Fig.10 shows the dependence of peak current for the cyclic voltammetry. Peak current decreases below pH 6.0 and beyond pH 8.0. The maximum peak current was observed at pH 7.

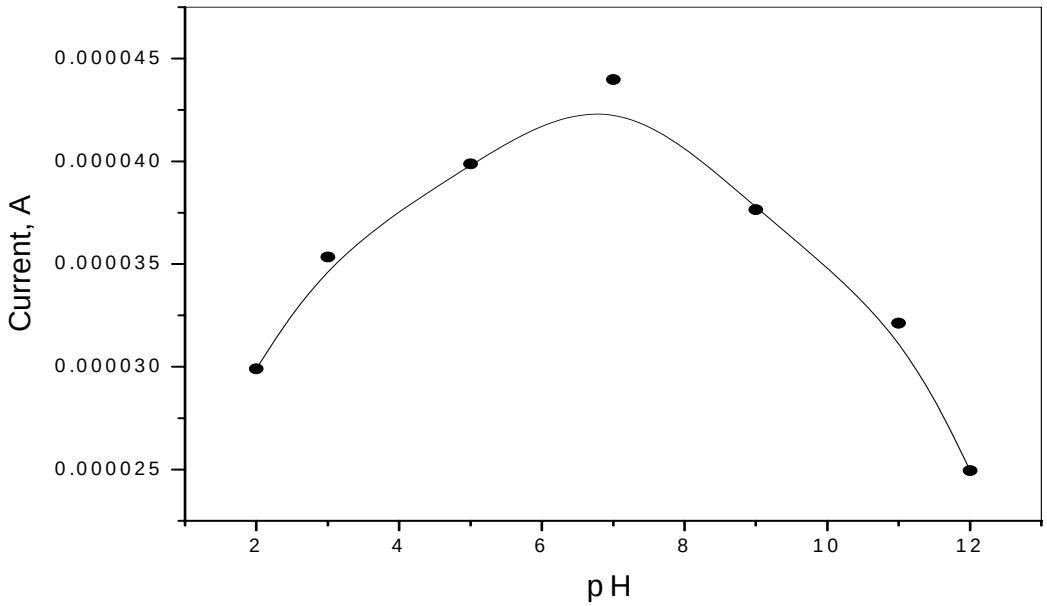


Fig.10. Plot of CV anodic peak current as a function of pH of $5x10^{-5}$ M, paracetamole.

5.1.3. Effect of scan rate.

The cyclic voltammogram of paracetamole solution was run at different scan rates. As the scan rate changes from 50 to 500 mV /S, there is a shift in the anodic peak potentials, and anodic peak currents. The electrode reaction of paracetamole at surface of the electrode is dependent on the scan rates.

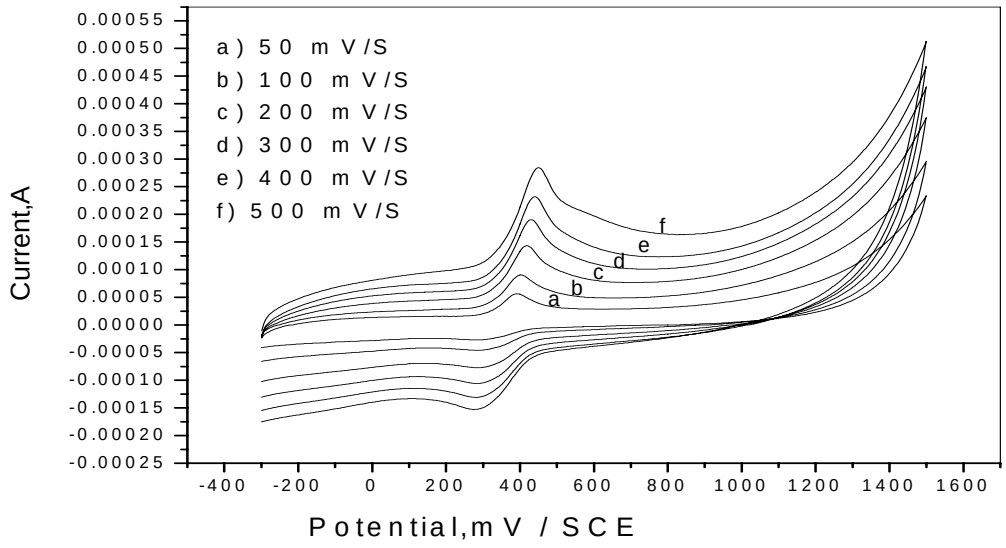


Fig .11 Cyclic voltammogram of 7.5 x 10^{-5} M paracetamole at different scan rates.

The dependence of peak current on the square root of scan rates is shown in Fig.12.

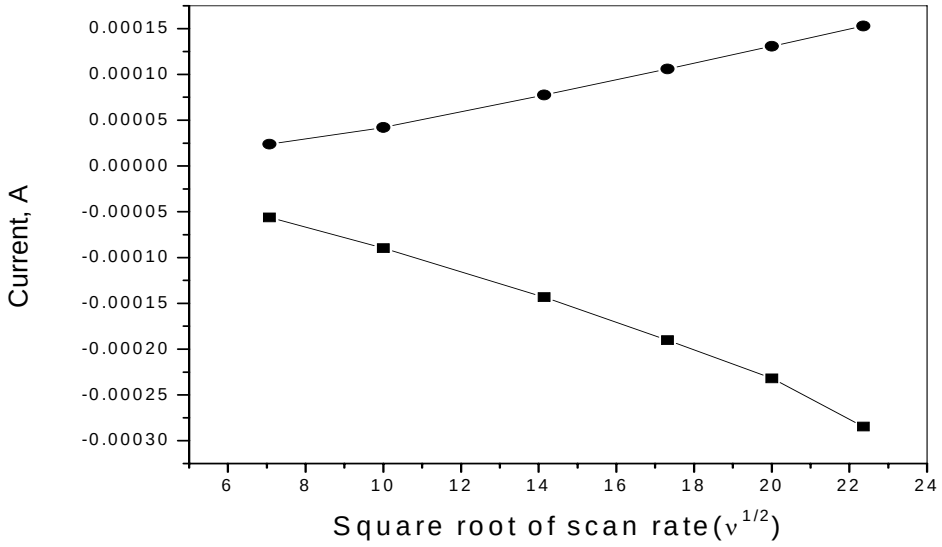


Fig.12 Plot of cathodic peak current as a function of $\nu^{1/2}$(●), and anodic peak current as a function of $\nu^{1/2}$ (■)

Table (1). Shows the influence of scan rate on peak current and peak potential for the oxidation of paracetamole, with cyclic voltammogram of 7.5 x 10^{-5} M paracetamole in 0.1 M NaH_2PO_4 –Na_2HPO_4 buffer (pH=7).

Scan rate mV/s	I_{pa} /10^{-5} A	I_{pc} /10^{-5} A	I_{pa} / I_{pc}	E_{pa} /mV	E_{PC} /mV	ΔE_P/mV
50	-5.617	2.388	-2.35	391	330	61
100	-8.982	4.203	-2.14	408	327	81
150	-11.24	6.132	-1.83	410	293	117
200	-14.32	7.748	-1.82	421	290	128
250	-16.16	8.961	-1.81	424	290	134
300	-19.03	10.59	-1.80	432	290	139
350	-20.85	11.64	-1.79	435	286	149
400	-23.19	13.09	-1.77	440	286	154
450	-25.42	14.43	-1.75	443	286	157
500	-28.45	16.53	-1.72	450	283	167

From table (1) it is easy to conclude that, as the scan rate increases the peak-to-peak separation (ΔEp) also changes. The ratio of the anodic peak current to the cathodic peak current is also different from unity. Hence the electrochemical reaction of paracetamole is irreversible.

5.2. SQUARE WAVE VOLTAMMETRIC INVESTIGATION.

Fig.13 shows the square wave voltammogram of 3 x 10^{-4} M paracetamole. Only one definite peak is observed at 410 mV, which indicates that paracetamole undergoes only one step electrochemical reaction at the glassy carbon electrode when the potential was run in the positive direction.

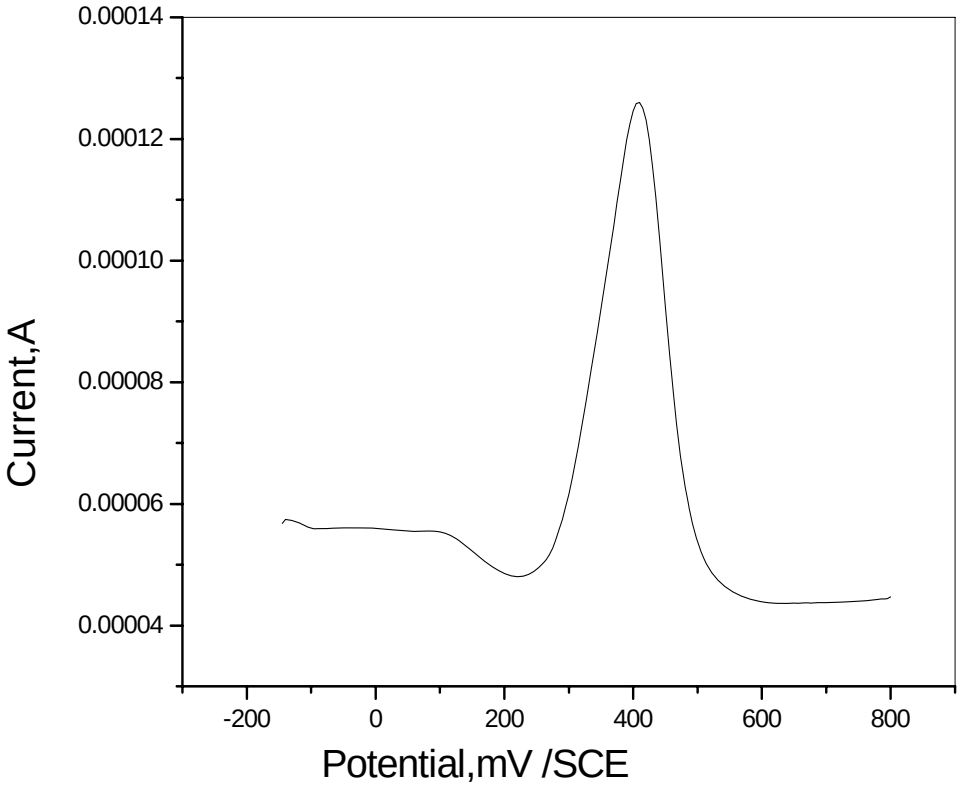


Fig.13 Square wave voltammogram of 3 x 10^{-4} M paracetamole in 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH = 7) at a glassy carbon electrode with scan rate of 100 mV / S.

5.2.1. Effect of concentration.

Similarly the effects of concentrations have been studied using square wave voltammetry. The peak current was found to be proportional to the paracetamole concentration.

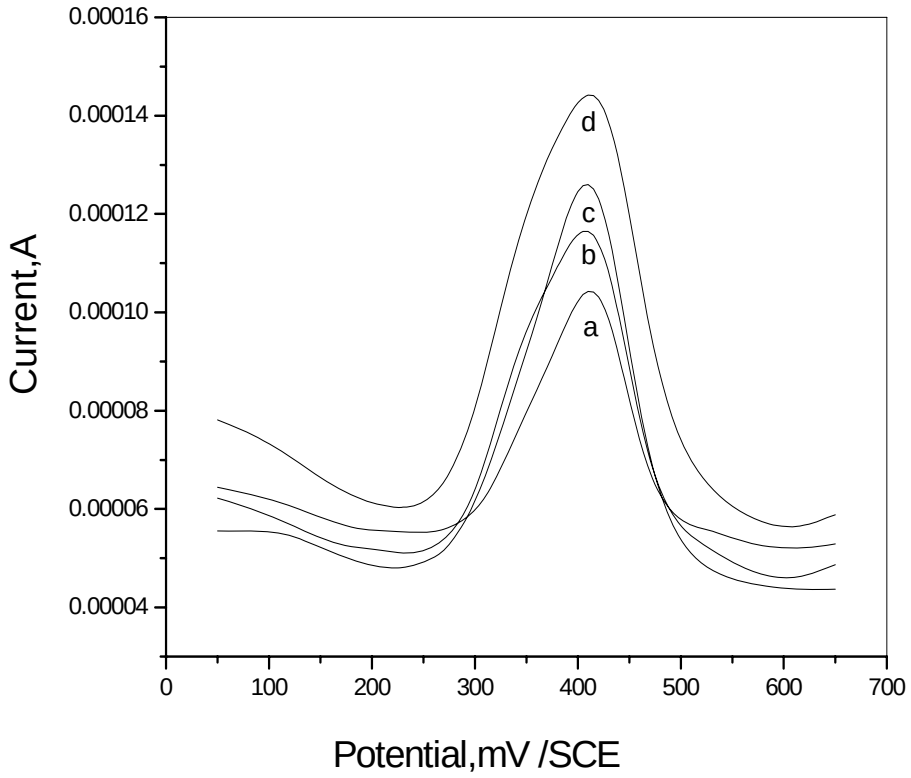


Fig.14 Square wave voltammogram of paracetamole in 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH=7) at a glassy carbon electrode with scan rate of 100 mV /S. a) $1.5x10^{-4}$ M, b) 2.5 x 10^{-4} M, c) $3x10^{-4}$ M, d) $6x10^{-4}$ M.

The dependence of the SWV peak current on paracetamole concentration is shown Fig15. A linear plot was obtained with a correlation coefficient of 0.9881,and slope of –0.08524.

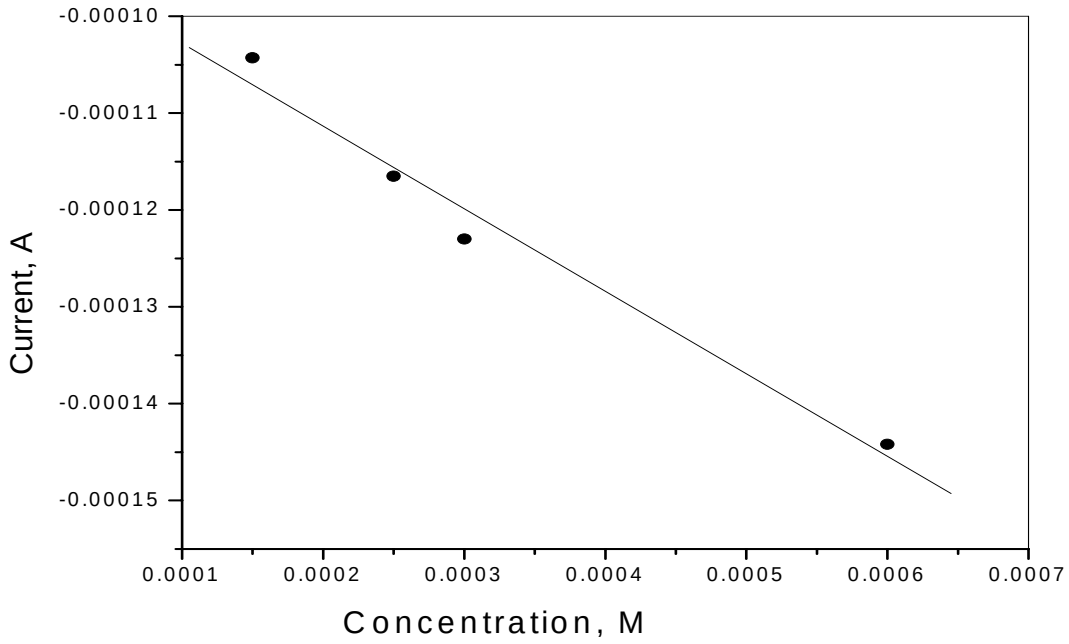


Fig.15 Shows plot of SQW anodic peak current as a function of concentrations in 0.1 M NaH_2PO_4 –Na_2HPO_4 buffer (pH =7) at a glassy carbon electrode with scan rate of 100 mV / S.

5.2.2. Effect of pH

The influence of pH on peak current of paracetamole has been studied using square wave voltammetry. Fig.16, (a) and (b) show the dependence of peak current for the square wave voltammetry, and maximum peak current was observed at pH 7.

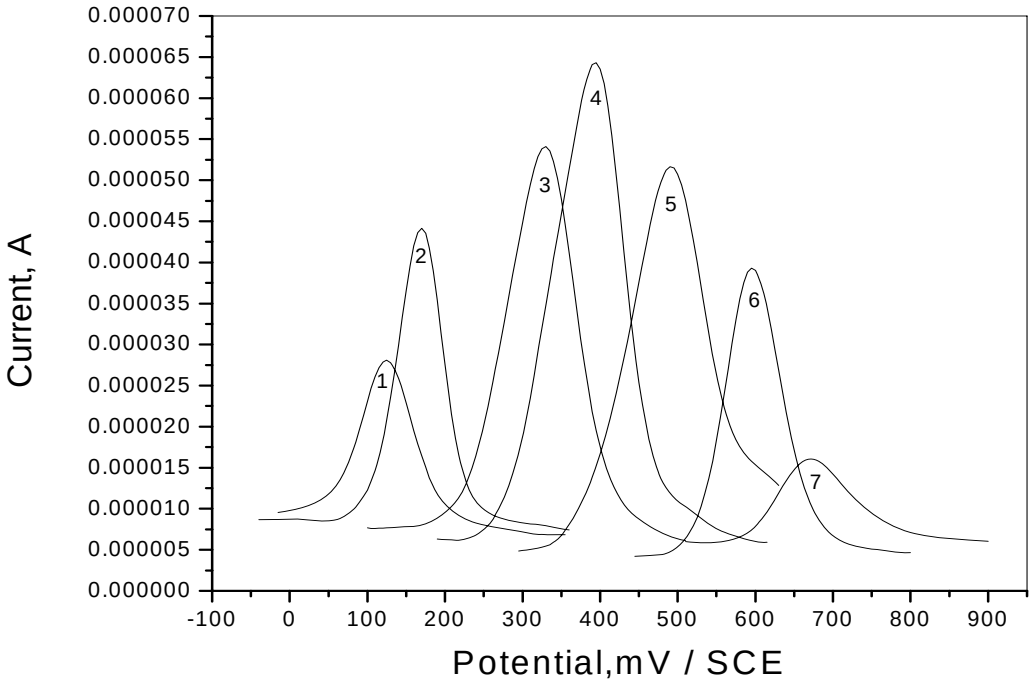


Fig.16 (a). Square wave voltammogram of $9x10^{-5}$ M, paracetamole. 1) pH=12, 2) pH=11 3) pH=9, 4) pH=7, 5) pH=5, 6) pH=3, 7) pH=2.

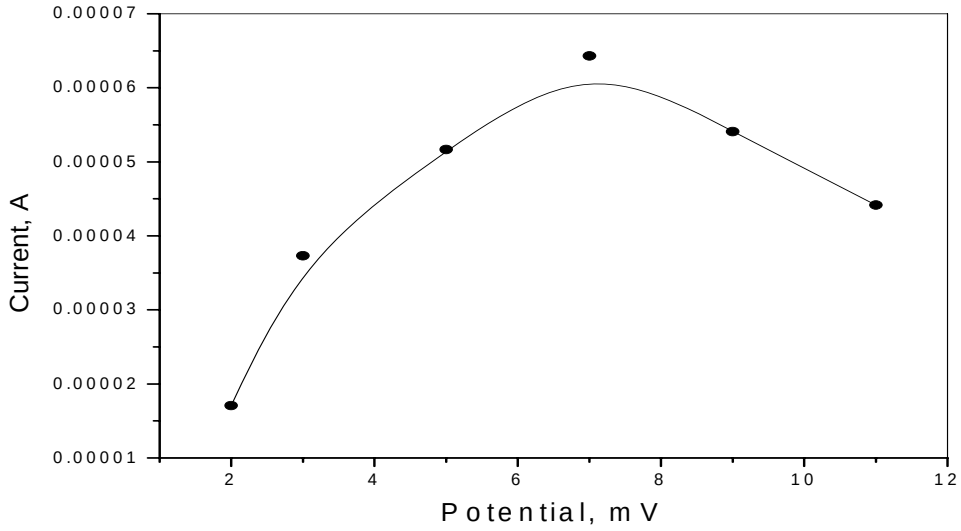


Fig.16 (b). Plot of SQW anodic peak current as a function of pH of $9x10^{-5}$ M, paracetamole.

The shift in SWV peak potential as a function of pH was studied and a linear dependence was observed. Fig17 shows that the peak potential of paracetamole becomes more negative potential as the pH increases. This indicates that for pH greater than 9, paracetamole is converted to the phenoxide ion. The oxidation of this anionic species involves one less proton, which is in close agreement with the literature [10].

The dependence of peak potential on pH had slopes of 50 mV and 63 mV per pH unit for the square wave voltammetry .The number of protons involved in the oxidation changes from two to one as the pH increases for (n=2). Electrode reactions involving a weak acid or base have potential - pH variations that show a change in slope at pH = pKa. From the intersection of lines of the plots, the pKa value of paracetamole was estimated to be 9.4. This is a good agreement with literature value [7]. As can be seen from Fig.17, two linear ranges were obtained for SQW.

E_{pa} (V versus SCE) = 0.762-0.050{pH}, r=0.9986 (pH 2- 7)

E_{pa} (V versus SCE) = 0.82-0.063{pH}, r=0.9965 (pH 7-12)

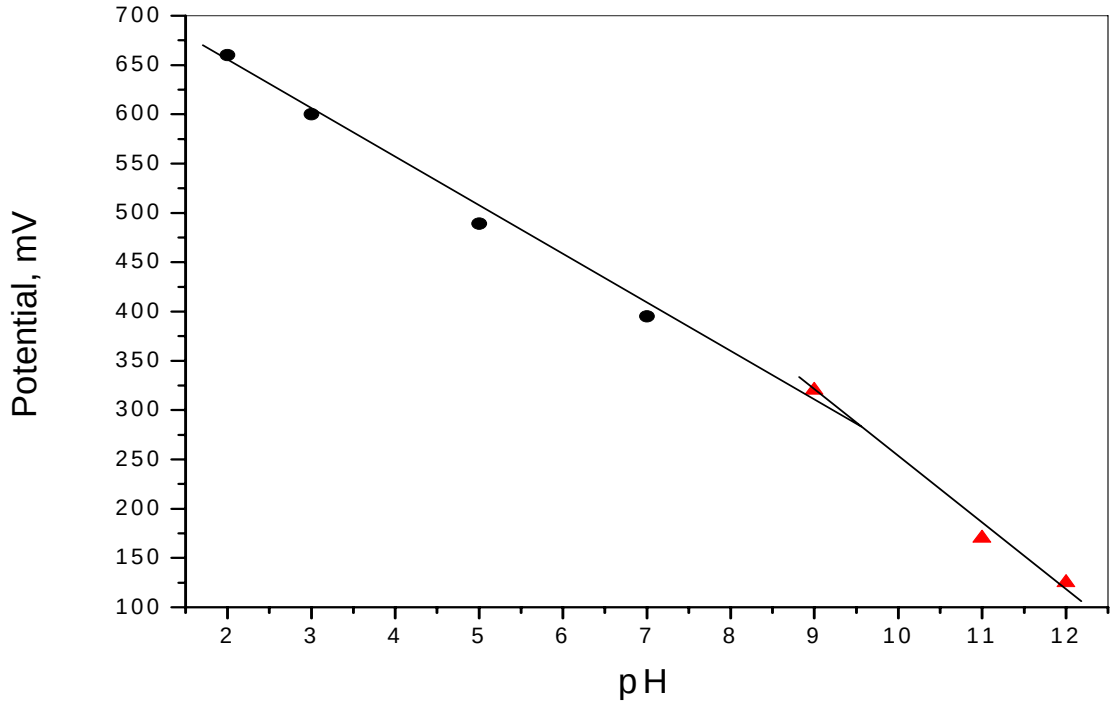


Fig.17 Plot of SQV anodic peak potential as a function of pH for 9 x 10^{-5} M Paracetamole.

In Square wave voltammetry, the analyte signals can be dependent on instrumental parameters such as square wave amplitude, frequency, and step potentials. These parameters have an effect on peak current and peak potential.

5.2.3. Effect of square wave frequency

It was found that an increase in square wave frequency results in an increase in the effective scan rate, which inturn increases, the current. However at very high frequency, the peak current is unstable and obscured by large residual current. On the other hand very low frequency gives a low but narrow signal in the total analyte time. Hence the selection of the frequency usually

requires a compromise among sensitivity, resolution, and speed. Therefore the optimum frequency chosen for this work was 25 HZ.

5.2.4. Effect of square wave amplitude

When the square wave amplitude varies from 10-60 mV the peak current increases with the increase of the square wave amplitude. Unfortunately, a broaden peak shape was obtained. The optimum amplitude must be found to maximize the peak current, and with resolution of small peak width. Hence 25 mV was chosen for this work. The effect of square wave amplitude on peak current is shown in Fig 18.

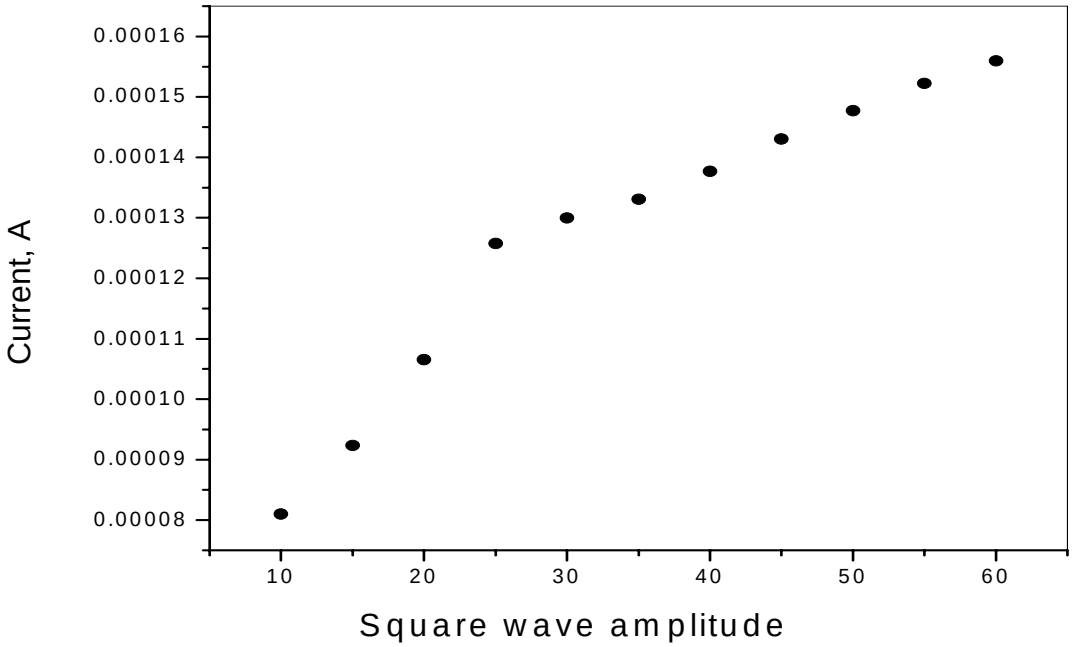


Fig.18 Plot of anodic peak current as a function of square wave amplitude.

5.2.5. Effect of step potential

The net current increases as the step potential increases. However it is accompanied by peak broadening, and loss of peak symmetry. Therefore, the optimum step potential chosen to maximize sensitivity and to obtain better peak symmetry was 5 mV.

5.3. DIFFERENTIAL PULSE VOLTAMMETRIC INVESTIGATION.

The differential pulse voltammogram of $7.5x10^{-5}$ M paracetamole shows an anodic peak at 400 mV, similar to the one step reaction in square wave voltammetry.

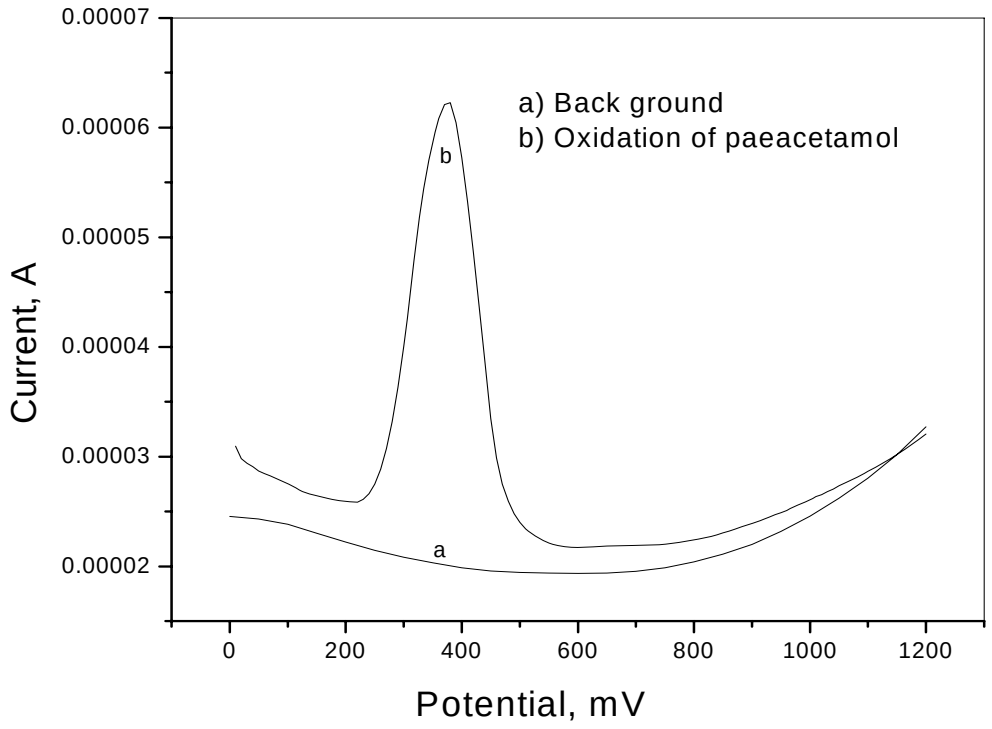


Fig.19 Differential pulse voltammogram of 7.5 x 10^{-5} M paracetamole in 0.1 M NaH_2PO_4 – Na_2HPO_4 buffer (pH = 7.0) at a glassy carbon electrode with scan rate of 100 mV / S.

5.3.1. Effect of concentration

The effect of concentration can be shown by recording the DPV at each concentration (2-7.5 x 10^{-5} M). The resulting differential pulse voltammogram consists of current peaks, the height of which is directly proportional to the paracetamole concentration.

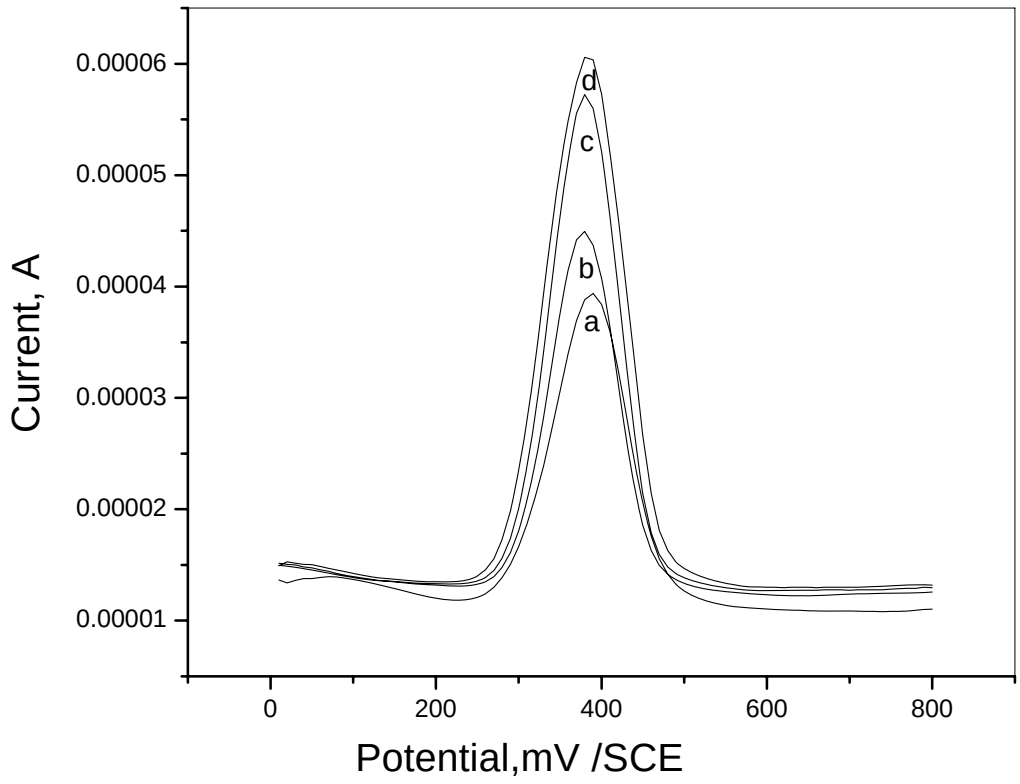


Fig.20 Differential pulse voltammogram of paracetamole in 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH= 7) at a glassy carbon electrode with scan rate of 100 mV / S, a) 2 $x10^{-5}$, b) 3 x 10^{-5}, c) 5 x 10^{-5}, d) 7.5$x10^{-5}$ M.

5.3.2.Effect of pH

The effect of pH on peak current of paracetamole has been studied using differential pulse voltammetry. Fig.21, (a) and (b) show the dependence of peak current on pH for the differential pulse voltammetry, and maximum peak current was observed at pH 7. This pH was chosen as the optimum pH

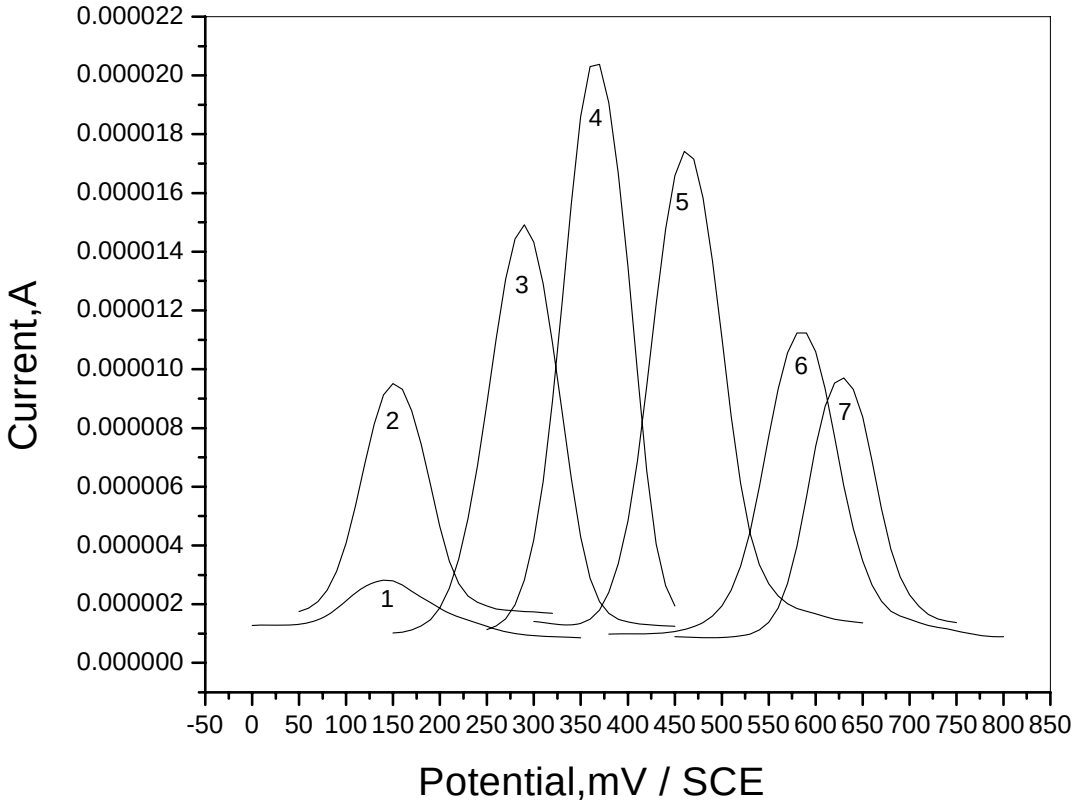


Fig.21 (a). Differential pulse voltammogram of $5x10^{-5}$ M paracetamol.1) pH=12, 2) pH=11,3) pH=9, 4) pH=7, 5) pH=5, 6) pH=3, 7) pH=2.

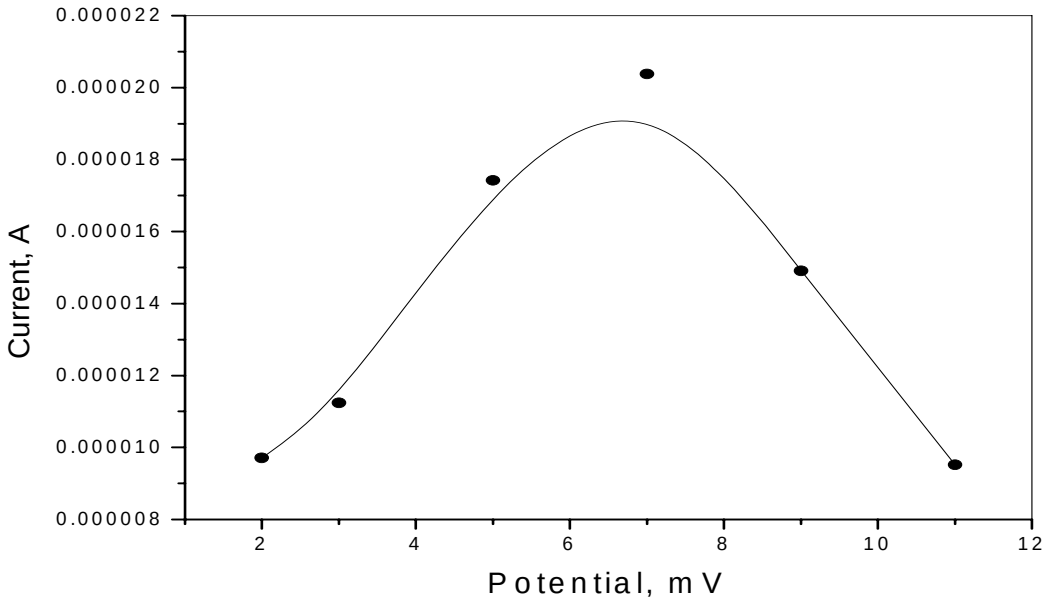


Fig.21 (b). Plot of DPV anodic peak current as a function of pH of 5 x 10^{-5} M, paracetamole.

The shift in DPV peak potential as a function of pH was studied and a linear dependence was observed. Paracetamole is a weak acid and its redox behavior is strongly pH dependent. Fig.22 shows that the peak potential of paracetamole shifted towards more negative potential as the pH increases, which denotes the ease of oxidation of the protonated molecule.

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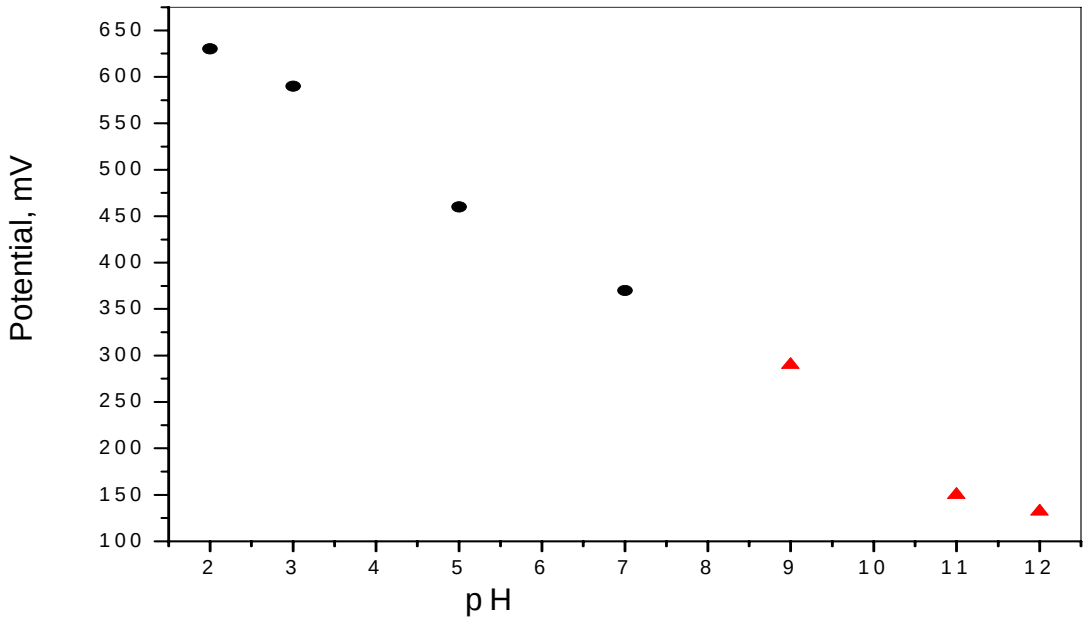

Fig.22 Plot of DPV anodic peak potential as a function of pH for 5 x 10^{-5} M Paracetamole.

5.3.3. Effect of pulse amplitude

The effect of pulse amplitude was varied from 10-50 mV. Peak current increased with increasing of pulse amplitude. There was peak broadening when the amplitude was greater than 30 mV. The effect of differential pulse amplitude on peak current is shown in Fig 23.An amplitude of 30 mV was chosen for these investigations.

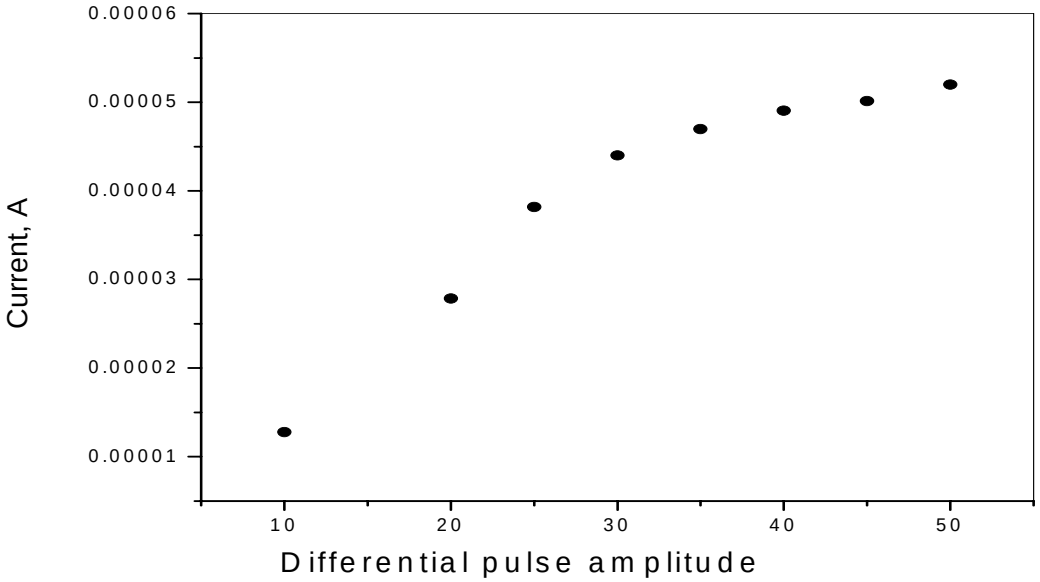

Fig.23 Plot of anodic peak current as a function of differential pulse amplitude.

5.3.4. Effect of pulse repeat time.

As the pulse time increases the peak current decreases. On the other hand at very low pulse repeat time the peak current increases accompanied by peak broadening, when the pulse repeat time is less than 0.5 sec. Hence a pulse repeat time of 0.5 sec was chosen for these investigations

6. INTERFERENCE STUDY.

Excipients are pharmaceutical additives, which are inactive ingredients tested to make up medication, but do not affect on the bioavailability of the drug. They include preservatives, dyes, flavors, lubricants, binders and many classifications. The common excipients present in paracetamole are starch, talc, magnesium stearat, and sodium alginate. The effect of these

ingredients on peak current and peak potential had been studied using cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry.

Cyclic voltammogram of pure paracetamole peak (1), and the tablet formulation peak (2) is shown in Fig.24a. These peaks indicate that anodic peak potential and peak current in the pure form of the sample of paracetamole was appeared at 406 mV, and -8.2x10^{-5}A respectively, and for the tablet formulation is at 414mV,and -6.85x10^{-5}A. Hence the presences of the additives in the tablet preparation have small effect on peak potential, but the peak current decreased.

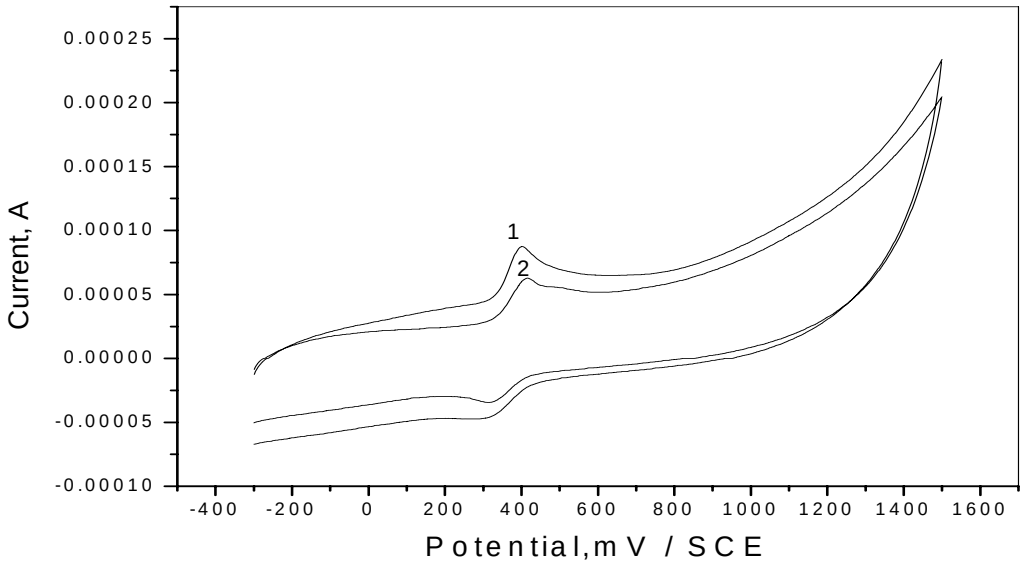

Fig.24 (a) The cyclic voltammogram of 1.5x 10^{-4} M paracetamole in glassy carbon electrode. Peak (1) pure paracetamole**,** Peak (2) the tablet form.

Using the square wave voltammetry, the addition of the ingredients into the pure form of paracetamole causes the peak current to decrease and small shift in peak potential. (Fig.24b) Peak (1) for the pure paracetamole the anodic peak potential and peak current appeared at

405 mV, and –6.95 x 10^{-5}A respectively. Peak (2) for the tablet, peak current and peak potential appeared at –6.19 x 10-5A,and 412 mV respectively.

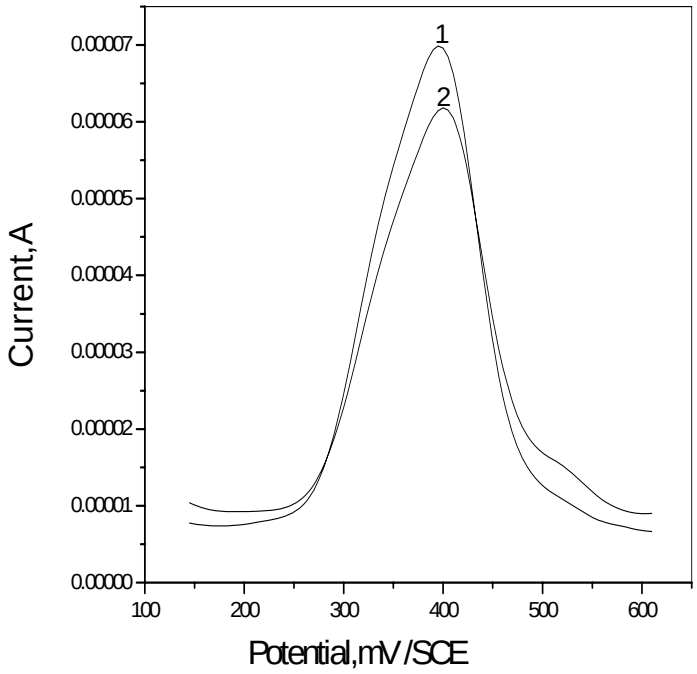

Fig.24 (b) Square wave voltammogram of 7 x 10^{-5} M paracetamole at glassy carbon electrode in phosphate buffer. Peak (1) pure paracetamole, Peak (2) the tablet form.

Lastly the effect of additives in the tablet preparations was studied by differential pulse voltammetry. The peak (1) for the pure paracetamole anodic peak potential and peak current appeared at 398 mV, and -3.34x10^{-5}A respectively; peak (2) for the tablet form it was observed

at 400 mV, and -2.48x10^{-5}A. Therefore the anodic peak current decreased in the presence of additives.

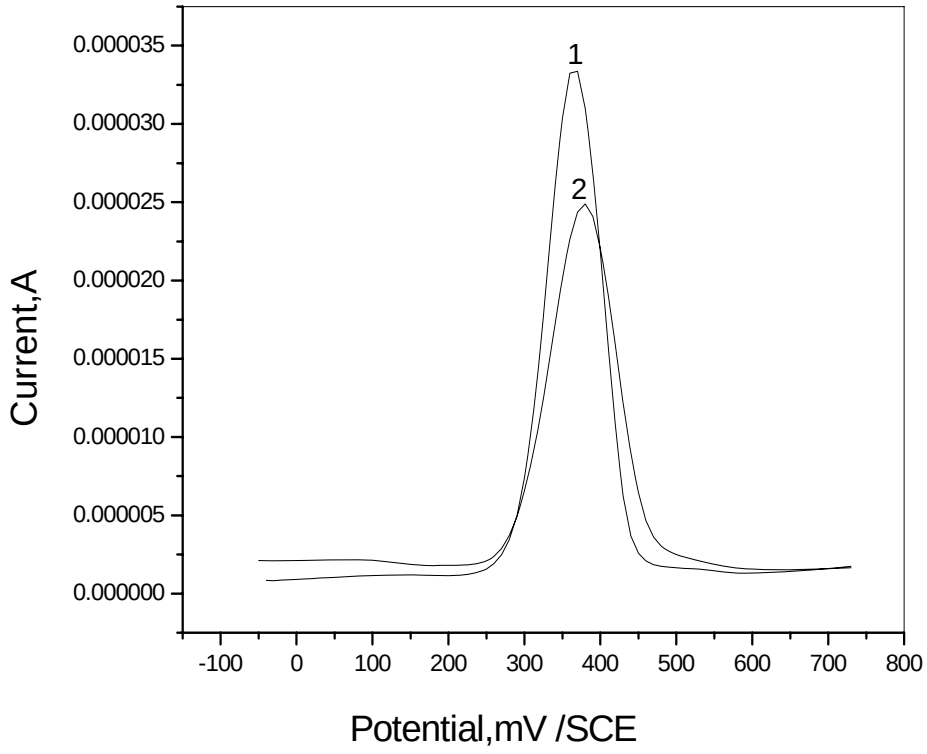

Fig.24(c) Differential pulse voltammogram of 1.5 x 10^{-5}M paracetamole at the glassy carbon electrode in phosphate buffer. Peak (1) pure paracetamole, Peak (2) the tablet form.

Generally the addition of preservatives, sweetening, and flavoring agents used in the tablet preparation were found not to cause any interference in anodic peak potential of the proposed methods. But the presences of the additives in tablet formulation affect the anodic peak current.

7. CONCLUSION.

The electrochemical oxidation of paracetamole in aqueous media was successfully studied by three electroanalytical techniques such as CV, SWV, and DPV with glassy carbon electrode. Several voltammetric parameters have been optimized and their influence in peak current peak potential was studied. An irreversible reaction with the transfer of two electrons per molecule of paracetamole was observed. In addition to this the pH effect of the supporting electrolyte has been studied in CV, SWV, and DPV. As pH increases the anodic peak potential drastically shifts to the negative potential, which indicates that hydrogen ion takes part in the electrochemical oxidation of paracetamole. The presence of excipeints in the tablet shows a current difference from the pure form, but a negligible difference in peak potential. In comparing the results obtained with literature values, the anodic peak potential differs by 40 mV using cyclic voltammetry. This may be due to the sample purity, or shift in the potential of the SCE. As pH increases, peak potential shifts to the negative direction, which is consistence with the literature. While comparing the peak current, the maximum peak current was observed at pH 7,which is in good agreement with the literature. In addition to this the presence of excipients in the tablets do not affect the peak potential, but the additives in the tablet formulation affects the peak current and show close agreement with reference method. To sum up, the voltammetric study of paracetamole requires no purification or extraction steps. Electroanalytical techniques have a major advantage in time and economy in study of the pharmaceutical drugs.

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