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ANALYSIS METHOD OF ANTI-CANCER DRUG SEMUSTINE FOR CHEMOTHERAPY BY CYCLIC VOLTAMMETRY

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ABSTRACT

Alkylating compounds (melfalan, chlorambucyl, lomustine, and semustine) are chemical compounds commonly used for the treatment of chemotherapy and used as anti-cancer drugs. This is due to its reactivity in stopping the growth of cancer cells by directly reacting to the nucleophile centers of the DNA base. The alkylating compound attacks the target non-selectively, therefore it can attack healthy cells so that it can cause secondary cancer. Because of the hazardous nature of the alkylating compounds which can form secondary cancers, an analytical method for the anti-cancer drug semustine that has been circulating on the market by cyclic voltammetry has been developed. The objectives of this study are (1) to obtain the working stress area for the semustine compound in a water solvent, (2) to determine the effect of using variations of supporting electrolytes and working electrodes on the semustine voltammogram profile. Before the analysis of semustine is made, Ag/AgCl comparison electrodes are made by electrolysis, then the results are characterized using $K_3Fe(CN)_6$ in various supporting electrolytes including KCl, KNO_3, and K_2SO_4. Subsequent research studied the effect of using variations of supporting electrolytes and working electrodes to measure the peak current of the anode and the cathode semustin and the voltammogram profile. The results provide information that KCl was used Pt as a working electrode, gives the highest peak current to the measurements of $K_3Fe(CN)_6$ were compared to K_2SO_4 and KNO_3 on 9.091 mg/L semustine.

Keywords: Semustine, Anti-cancer Drug, Cyclic Voltammetry.

INTRODUCTION

Cancer is a type of disease that causes many deaths and can occur in humans of all age groups and races. Cancer arises due to mutations in normal cells by the influence of radiation, hormones, viruses and carcinogenic chemicals.[1] The nature of cancer cells differs from normal body cells because mitosis of cancer cells is faster, abnormal and uncontrolled.[2] The types of cancer treatments used until now are surgery, radiation, cancer cell killing drugs (chemotherapy) and medicinal plants.[3-5]

Semustine is a methylated derivative of carmustine with antineoplastic activity[6]. As an alkylating agent, semustine forms covalent linkages with necleophlic centers in DNA, causing depurination, base-pair miscoding, strand scission and DNA-DNA cross-linking, which may result in cytotoxicity[7]. Semustine is an organochlorine compound that is urea in which the two hydrogens on one of the amino groups are replaced by nitroso and 2-chloroetyl groups and one hydrogen from the other amino group is replaced by a 4-methylcyxlohexcyl group[8], structural formula of semustine can be seen Fig.-1.

Many methods have been used to detect various anti-cancer drug compounds, including polymer/gold nanoparticles/graphene oxide for determination of an anti-cancer drug imiquimod[9], the determination of pemetrexed using Pd/carbon nanofibers/methyl (trioctyl) ammonium bis (trifluoromethylsulfonyl) imide ($[M3OA]^+[NTF2]^-$) ionic liquid modified carbon paste electrode[10], HPLC-FLD assisted by

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microextraction and solid-phase extraction, for the determination of doxorubicin, daunorubicin, daunorubicin, daunorubicin, daunorubicin epirubicin and irinotecan in hospital effluent[11-12], Determination of the glucuronide metabolite of on 013100, a benzyl styryl sulfone antineoplastic drug, in colon cancer cells using LC/MS/MS[13], liquid chromatography-tandem mass spectrometry (LC-MS/MS) for determination of anticancer drugs, enzalutamide's, in rat plasma,[14] reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of palbociclib.[15] But there have been no reports of research methods for determining the anti-cancer drug semustine.

The method was used in determining chemical reactivity by cyclic voltammetry. This method was chosen because it is: simple and has good precision and accuracy,[16-19] so that it is able to provide useful information related to the chemical reactivity of certain compounds,[20] for example: the rate and rate of reaction constants, reaction mechanisms and studies of the oxidation and reduction processes of a compound that is not obtained with other analytical methods.[21-22]

In this study, a method of analyzing the anti-cancer drug semustine for chemotherapy was developed on the market by cyclic voltammetry, by measuring the chemical reactivity of semustine as an anti-cancer drug. Before the analysis of semustine is made, Ag/AgCl reference electrodes are made by electrolysis, then the results are characterized using $K_3Fe(CN)_6$ in various supporting electrolytes including KCl, KNO_3, and K_2SO_4. Subsequent research studied the effect of using variations of supporting electrolytes and work electrodes to measure the peak current of the anode and the cathode semustine and the voltammogram profile. The objectives of this study obtain the working stress area for the semustine compound in water solvent and determine the effect of using variations of supporting electrolytes and working electrodes on the semustine voltammogram profile.

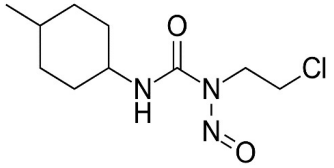


Fig.-1: Structural Formula of Semustine

EXPERIMENTAL

Instruments and Materials

The instruments were used in this study are Mettler AE-200 analytical balance, magnetic stirrer, BAS Epsilon potentiostat version 1.60.70, Orion pH/ISE 710A pH meter, 10 mL sample cell size, Ag/AgCl references electrode, working electrode platinum and platinum wire auxiliary electrodes.

The materials were used in the study had a purity of pro analysis without further purification. These include semustine (Sigma-Aldrich), sodium perchlorate monohydrate (Merck), potassium nitrate (Merck) and potassium chloride (Merck), potassium sulfate, double distilled water, silver chloride, potassium ferricyanide, and sodium chloride, for the manufacture and characterization of comparative electrodes, nitrogen gas (BOC gases).

Preparation and Determination of the Performance of the Ag/AgCl References Electrode

At the stage of making Ag/AgCl reference electrodes, the electrodes are made by electrolysis of silver wire (Ag) in 3M NaCl solution. The wire which has been coated with AgCl is then inserted into the glass tube electrode body which has a border at the end and has been filled with 3M KCl solution. The electrodes are then closed and the connectors mounted. These comparative electrodes are then compared with commercial electrodes (BAS). The electrodes were then qualified for performance using a solution of $K_3Fe(CN)_6$ in 0.1 M potassium chloride (KCl), potassium nitrate (KNO_3) and potassium sulfate solutions (K_2SO_4) supporting electrolytes solution.

Measuring the Range of Potential Work forBlanks and Analytes (Semustine) Analyzed

Determination of the range of work stresses is carried out with the aim to obtain the area of stress used in analytical research (semustine). In this study, the working voltage area will be tried from -1.0 V to 1.3V. The area of work stress obtained will be used to identify the peak current from the aqueous solution of

semustine in water solvents. Prior to analyzing the analyte, blank measurements are taken, i.e. solvents used water, both solvents with supporting electrolytes only (KCl, KNO_3, and K_2SO_4 respectively 0.1M) and solvents with supporting electrolytes.

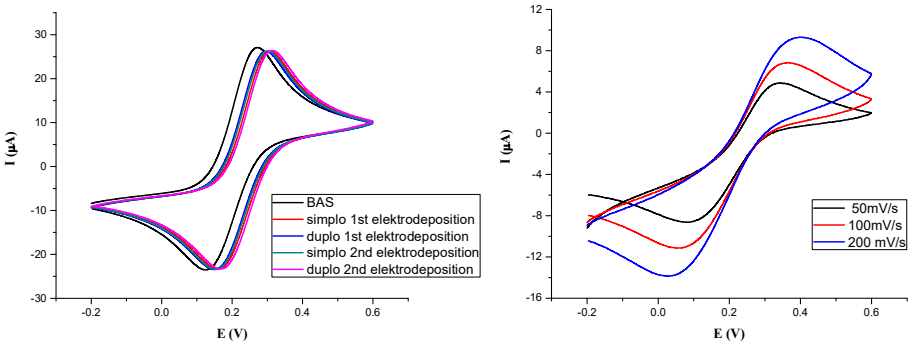
Measurement of Semustine in a 0.1 M KCl Supporting Electrolyte Solution (Water)

Blanks in the form of 0.1 M KCl solution (water) as much as 10 mL were measured first. After that, some of the semustine are injected, stirred with a magnetic stirrer for 1 minute and then measured. The electrodes used were gold discs as work electrodes, Pt wire as comparative electrodes, Ag/AgCl electrodes as a referenc eleectrodes. The scan rate varies by 50 mV/s; 100 mV/s ; 200 mV/s ; 400 mV/s. The same thing was also carried out measurement of semustine with 0.1 M KNO_3 and K_2SO_4. Measurements were made using a cyclic voltammetry technique with platinum working electrodes, Ag/AgCl KCl 3M reference electrodes and platinum wire supporting electrodes.

RESULTS AND DISCUSSION

Determination of Ag/AgCl Reference Electrode Performance

Electrodes are made by electrolysis of silver wire (Ag) in 3 M NaCl solution. A silver wire coated with Ag/AgCl is inserted into the body (place) of electrodes that have been filled with KCl 3M, can be seen in Fig.-2.



(a) (b)

Fig.-2: Cyclic Voltammogram of BAS and Ag/AgCl (a), Solution $K_3Fe(CN)_6$ 0.01M (b)

Figure-2a can be seen the comparison of Ip_a and Ip_c between BAS and Ag/AgCl electrodes was 1.18 with 1.13, then these reference electrodes are compared with electrodes that are has been commercial (BAS). Figure 2a, it can be seen that the average Ip_a and Ip_c of BAS are 26.29 and -22.29 mA while for Ag/AgCl are 25.30 and -22.66 mA. Ipa/Ipc ratio is 1.18 and 1.12 for BAS and Ag/AgCl. The t-test results it can be concluded that the electrodes made (Ag/AgCl) results were not significantly different from fabricant electrodes (BAS) can be seen the following equation: $t_{\exp} = \frac{|\bar{d}|\sqrt{n}}{s} = \frac{10{,}11\times\sqrt{5}}{15{,}086} = 1.4985$ dan $t_{crit} = 2.78$. Because texp<tcrit there is no significant difference between the BAS electrode and the artificial electrode-1 and electrode-2. $t_{\exp} = \frac{|\bar{d}|\sqrt{n}}{s} = \frac{17{,}23\times\sqrt{5}}{19{,}469} = 1.979$ dan $t_{crit} = 2.78$, Because $t_{exp} < t_{crit}$ there is no significant difference between the BAS electrode and the artificial electrode-2.

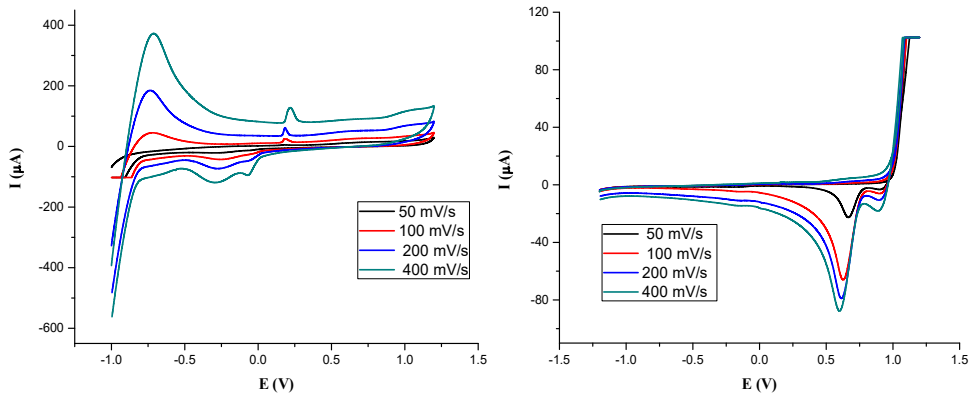
Then the performance qualification is tested by measuring the $K_3Fe(CN)_6$ 0.01 M solution in KCl, KNO_3 and K_2SO_4 0.1 M supporting electrolyte solutions using cyclic voltammetry techniques. In testing the performance of this comparative electrode used platinum working electrodes and platinum wire supporting electrodes. The results of performance measurements of $K_3Fe(CN)_6$ 0.01 M in a solution of several supporting electrolytes can be seen in Fig.-2b.

The performance of the $K_3Fe(CN)_6$ 0.01 M solution in a 0.1 M KCl supporting electrolyte solution, were obtained anodic peak current (Ip_a) +22.98 µA and the cathodic peak current (Ip_c) is -22.98 µA. Based on the analysis of the measurement potential value, the number of electrons involved (n) can be calculated

based on the ratio of anodic peak currents and cathodic peak currents obtained, then the comparison value of $Ip_a/Ip_c$for $K_3Fe(CN)_6$ 0.01 M in KCl 0.1 M is: 22.98/22.98 = 1.00.
The data generated above, the voltammogram profile is shown in Fig.-2b for the system $Fe(CN)_6^{3-}$ /$Fe(CN)_6^{4-}$ in KCl 0.1M have referred to cyclic voltammograms as well as systems $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ in $KNO_3$and K_2SO_4 0.1 Mwhich were calculated some researchers had previously[3].The difference in potential values at the anodic peak and the cathodic peak is more due to the difference and concentration of the supporting electrolyte solution used. The number of electrons involved (n) equal to 1 and ΔEp of 238 mV indicates that the Ag/AgCl comparison electrodes made have quite good criteria and can be used for further experiments.

Measurement Blanks

The blanks made with cyclic voltammograms are water solvents, each of which contains a KCl-supporting electrolyte solution. KNO_3 and K_2SO_4 with variations of the working electrodes used namely, Pt wire and gold discs. Blank cyclic voltammogram measured as many as 4 cycles of the variation of scan rate: 50mV/s; 100V/s; 200V/s and 400 mV/s.
The blank voltammogram measured in potential areas of measurement of -1.0 to 1.25 V, it can be seen that a water solvent containing a 0.1 M KCl supporting electrolyte solution using a Pt working electrode, provides a cyclic voltammogram which shows the peak oxidation peaks and reduction peaks. The oxidation peak gives the anode potential at 0.222 V with the anode peak current of 49.4 µA, while the reduction peak gives the cathode potential at -0.274 V with the cathode top current of 64.8 µA. The same was done by measuring the water solvent with a 0.1 M KCl supporting electrolyte solution using a gold disc working electrode. In the resulting voltammogram, there were no oxidation peaks while the reduction peaks gave a cathode potential of 0.598 V and the peak current of the cathode -74.92 µA can be seen Fig.-3a and 3b.



(a) (b)

Fig.-3: Cyclic Voltammogram of Water Solvent in KCl 0.1 M with Pt (A) and Au (B) as a Working Electrodes

The same thing has studied the effect of KNO_3 and K_2SO_4 solutions as supporting electrolytes and Pt and Au discs as working electrodes on blank cyclic voltammograms. The voltammogram produced is measured in potential areas of measurement of -1.0 to 1.25 V with a scan rate variation: 50 mV/s; 100; 200 and 400 mV/s, the results can be seen Fig.- 4.
Figure-4a, can be seen a cyclic voltammogram of water with $KNO_3$0.1 M using Pt as a working electrode, can be measured anode and cathode potentials of 0.612 V and -0.298 V, anode peak current 3.14 µA and cathode peak current 5.251 µA. When Au is used as a working electrode (Fig.-4b), the anode and cathode potentials are 0.476 V and -0.126 V respectively, while the anode peak current is 0.768 µA and the cathode is -0.798 µA. On a cyclic voltammogram of water with 0.1 M K_2SO_4 solution using Pt (Fig.-4c); anode potential of 0.452 V and cathode potential of -0.064 V, anode peak current of 5.7 µA and -6.7 µA while on a cyclic voltammogram of water with K_2SO_4 0.1 M solution using Au (Fig.-4d), anode and cathode potential were obtained respectively 0.044 and 0.22 Volt, anode peak current 1.277 µA and cathode peak current -1.867 µA. From the results of variations of the supporting electrolytes and working

electrodes, it was found that the optimum conditions were, the supporting electrolyte of KCl 0.1 M solution and the working electrode was Pt.

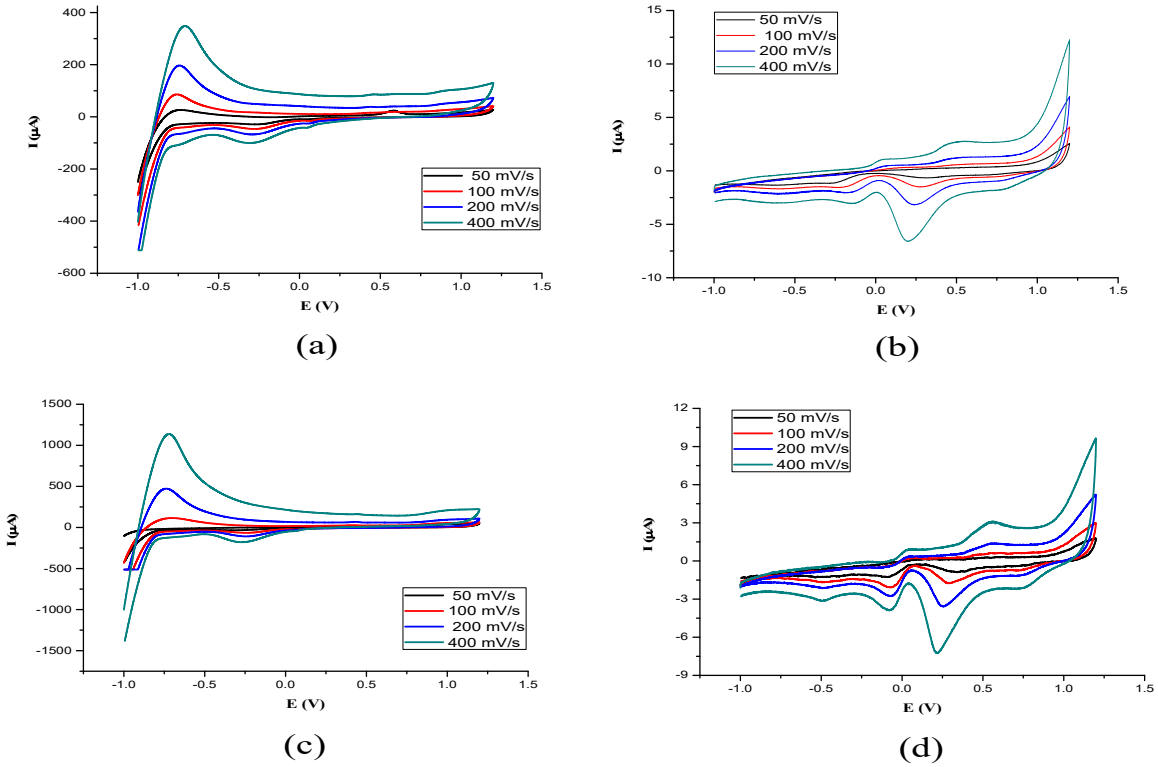


Fig.-4: Cyclic Voltammogram of Water Solvent in KNO_3 0.1M with Pt (a) and Au (b); K_2SO_4 0.1M with Pt (c) and Au (d) as a Working Electrodes

Measurement of Working Voltage (Potential) Range

Measurement of voltammetry used a three electrodes system, namely platinum working electrodes or gold electrodes, self-made Ag/AgCl comparators and platinum wire supporting electrodes, on a solution of 4.7619 ppm semustine compounds in several different solvents, at a certain range of potential work. The use of a certain range of potential work in this measurement is a preliminary analysis or a qualitative analysis of analytes conducted using cyclic voltammetry techniques. This determination aims to determine the potential area that will be used in the measurement of a compound.

In this study, the range of work potential determined on the measurement using the cyclic voltammetry technique to identify the peak current of the semustine compound is -1.0 to 1.25 V in water. The semustine cyclic voltammogram produced, in some electrolytes is shown in Fig.-5.

Semustine in a 0.1 M KCl solution and Au as a working electrode in water (Fig.-5b) gives a cathodic peak potential (Ep_c) of 0.606 V while the anode potential and the anode peak current are not readable, meaning the semustine compound in the gold working electrode is not electroactive. From the results of variations in the working electrodes that provide peak anode and cathode peak currents for semustine analysis are Pt. The same is also studied semustine analysis using 0.1 M potassium nitrate (KNO_3) solution in water in the potential range of -1.0 to 1.2 V with Pt as the working electrode can be seen Fig.-5c, produces an oxidation peak current at the anodic peak potential (Ep_a) of +0.452 V and a reduction peak current at the cathodic peak potential (Ep_c) of -0.246 V with a standard potential value (E0) calculated using Equation $E^0 = \frac{Ep_a + Ep_c}{2}$ is 0.349 V versus E^0 Ag/AgCl, the anode peak current produced is 4.867 µA and the cathode peak current is -76,635 µA. Semustine in a 0.1 M KNO_3 solution and Au as a working electrode in water (Fig.-5d) gives the anode peak potential (Ep_a) is +0.302 V and the reduction peak current at the cathodic peak potential (Ep_c) is 0.352 V with a standard potential value (E^0) equal to 0.327 V versus E^0

Ag/AgCl, the anode peak current produced is 0.506 µA and the current is the cathode peak is -1.597 µA. The results of the cyclic semustine voltammogram in a 0.1 M KNO_3 solution in water using Pt and Au as a working electrodes are electroactive meaning they provide peak anode and cathode currents.

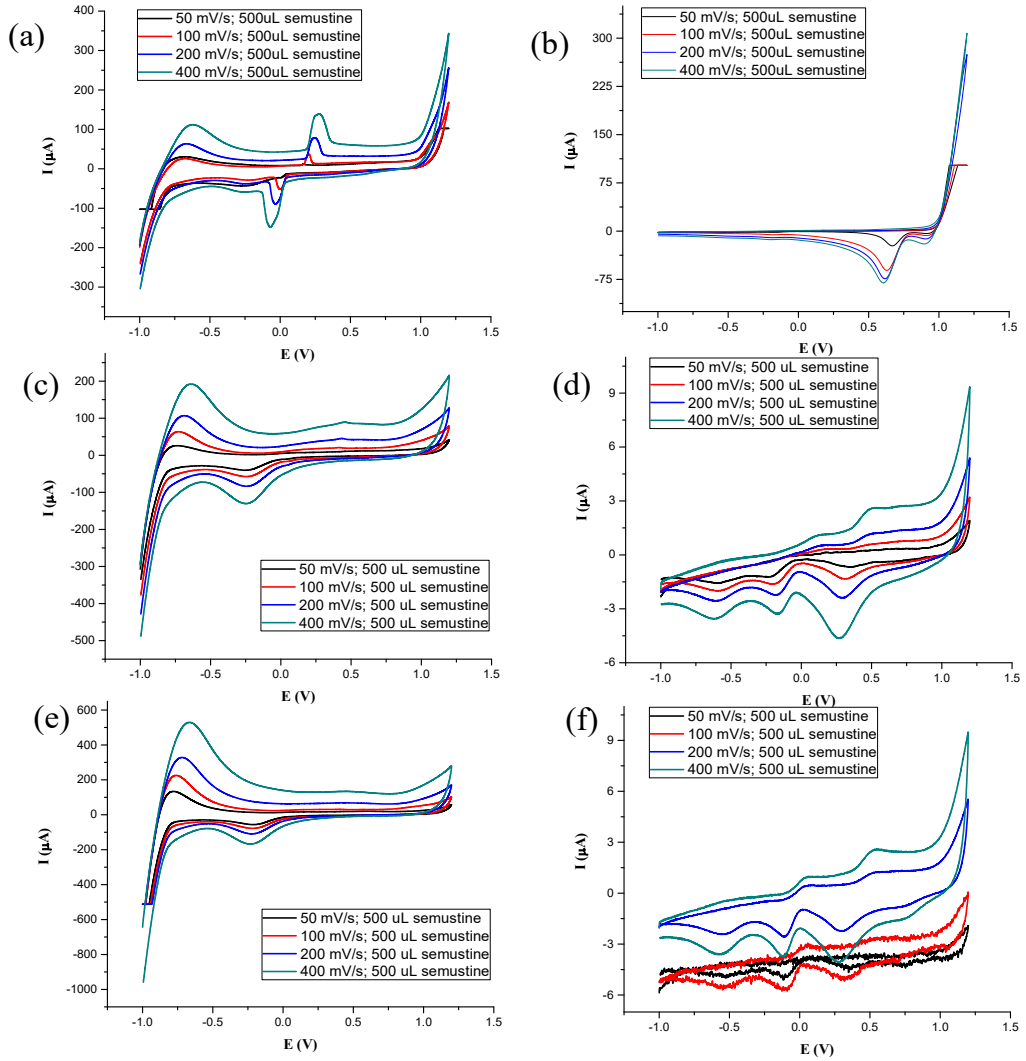


Fig.-5: Cyclic Voltammogram of Semustine in Water with KCl 0.1 M with Pt (a) and Au (b), KNO_3 0.1M with Pt (c) and Au (d), K_2SO_4 0.1 M with Pt (e) and Au (f) as a Working Electrodes

In this research also studied the effect of using 0.1 M K_2SO_4 solution as a supporting electrolyte solution in water for semustine analysis using Pt working electrodes the results can be seen in Fig.-5e. Semustine cyclic voltammogram in water in the potential range of -1.0 V to 1.2 V using a 0.1 M K_2SO_4 solution, giving the cathode peak potential (Ep_c) at -0.228 V and the cathode peak current (Ip_c) -112.3 µA and the anode peak potential and the anode peak current are absent, whereas by using Au as a working electrode an anode peak potential is obtained at 0.281 V, the average cathode peak potential at -0.319 V. with a standard potential value (E^0) equal to 0.323 V versus E^0 Ag/AgCl. The results of a semustine voltammogram in water using a 0.1 M K_2SO_4 solution and Pt as a non-electro selective working electrode means no redox reaction occurs.

CONCLUSION

Based on t-test result, reference electrodes (Ag/AgCl) was made to compare with Fabricant electrode (BAS) not significantly different. Ag/AgCl characterization using $K_3[Fe(CN)_6]$ 0.01 M solution in a 0.1 M KCl supporting electrolyte gives the highest potential and peak current compared to KNO_3 and K_2SO_4.Cyclic voltammetry measurement provide useful information about the current and potential

values in the oxidation-reduction of semustine. The measurement of semustine in a 0.1 M KCl supporting electrolyte solution (water) with Platinum wire (Pt) as a working electrodes, produces anode and cathode peak currents. The higher the concentration of semustine the peak current produced is increasing, while using a gold disc electrode the peak current of the anode does not appear.

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