Full Paper

Determination of the Reactivity of the Anti-cancer Nitrogen Mustard-Mechlorethamine: A Cyclic Voltammetric Investigation

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Abstract- We have investigated the electrochemical interaction between mechlorethamine and organic solvents i.e. acetone and acetonitrile, in the presence and absence of 4-chloro butyronitrile (CBN) using cyclic voltammetric technique. We found the reactivity (chemical reactivity) of mechlorethamine through the value of the forward reaction rate constants (K_f) in acetone and acetonitrile, in the presence and absence of CBN were 0.1601 s^{-1}, 0.4828 s^{-1}, 0.1365 s^{-1}, 0.2724 s^{-1}, respectively. These values of K_f were determined from the curves of K_ft versus t with correlation coefficient values are higher than 0.9500. The values of K_f affected by the donor number (DN) of solvents and also the structure of the nucleophilic compound were added. This is due to acetone has a larger DN than acetonitrile therefore K_f in acetone is higher than that in acetonitrile. CBN reduces the value of the chemical reactivities of mechlorethamine in both solvents. This can occur because CBN is a simple structure that is why it can interact directly with mechlorethamine.

Keywords- Reactivity, Mechlorethamine, Cyclic Voltammetry, Organic Solvent

1. INTRODUCTION

Voltammetry is a sensitive enough technique, effective and convenient for the analysis of important molecules including drugs in pharmaceutical formulations and in human body fluids. This technique is very popular and widely used due to low cost, simplicity, and relatively short analysis time as compared with other analytical techniques [1-11]. One of the several techniques for voltammetry analysis is the cyclic voltammetry, which is very useful in answering the problems of oxidation-reduction reaction, kinetics, and reaction at the electrode surface, such as determining the reaction rate constant of anticancer drugs [12,13]. The reaction rate constants of the anticancer nitrogen mustards mechlorethamine has not been previously reported using this technique.

The anticancer nitrogen mustards have been reported to be potentially carcinogenic, mutagenic and promote secondary cancer [14-17]. These potential were highly correlated to their chemical reactivities or their reaction rate constants, as we have previously reported on chlorambucil [12,13]. Several important compounds in the class of nitrogen mustards i.e. mechlorethamine, chlorambucil, melphalan, cyclophosphamide and ifosfamide, have different chemical reactivities. Mechlorethamine has the highest chemical reactivity, followed by melphalan and chlorambucil, respectively. The chemical reactivities of cyclophosphamide and ifosfamide depend on their parent molecule binder, which is evidenced through the quantum mechanical calculations of isophosphoramide and phosphoramide which have different chemical reactivities [18].

The goal of our present work was to show the chemical reactivity value of mechlorethamine, to show that the value is higher than chlorambucil and study the electrochemical behavior of these compounds by cyclic voltammetric technique.

2. EXPERIMENTAL

2.1. Apparatus and Procedures

Cyclic voltammetry (CV) measurements were conducted using BASi Epsilon Electrochemical analyzer. A standard three-electrode electrochemical cell was used for all electrochemical measurements with a platinum electrode (1 mm diameter) as working electrode, a platinum (Pt) wire as an auxiliary electrode and an Ag/AgCl (with 3 M KCl) as a reference electrode. Cyclic voltammetry measurements were conducted in a homemade 10 mL glass cell. Working electrode was polished with an alumina suspension.

Measured solution was made by dissolving a certain amount of mechlorethamine in solvents containing 0.2 M sodium perchlorate. Potassium dihydrogen phosphate buffer solution was added to the solution before measurements were conducted.

2.2. Chemicals

All Chemicals used in this study were p.a grade. Mechlorethamine was obtained from SIGMA while acetone, acetonitrile, sodium perchlorate, potassium dihydrogen phosphate, 4-chloro butyronitrile (CBN) and sodium hydroxide were obtained from MERCK.

3. RESULTS AND DISCUSSION

3.1. Effect of Scan Rate

The effect of varying the potential scan rate on the oxidation-reduction peak currents of mechlorethamine were studied. The cyclic voltammograms were recorded in 0.2 M potassium dihydrogen phosphate buffers of pH 7.2 in acetone and pH 7.0 in acetonitrile. The oxidation and reduction peak currents in acetone increased linearly with the square root of the scan rate over the range 0.15 to 1.00 Vs^{-1} with a correlation coefficient (R^2) from 0.9791 and 0.9475, respectively. The process of diffusion controlled is in this region as shown in the Fig. 1b. From Fig.1a, the observed peak's shift in peak potential towards more positive values with an increase in scan rate is a typical behavior of an irreversible electron transfer process [19,20].
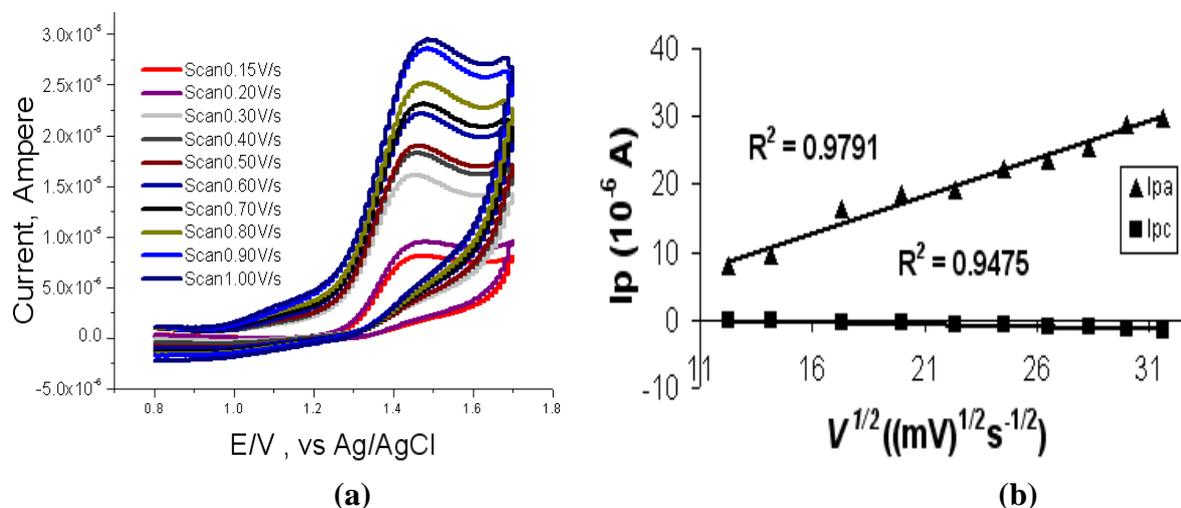
Fig. 1. Effect of scan rate variation of 2.0×10^{-3} M mechlorethamine in acetone at Pt electrode with 0.2 M potassium dihydrogen phosphate buffer, pH 7.2 (a) current *vs* potential, (b) current *vs* the square root of the scan rate

Similar behavior also occurred in voltammogram of mechlorethamine in acetonitrile. In the concentration of 2.0×10^{-3} M mechlorethamine, the oxidation-reduction peak currents increased linearly against the square root of the scan rate with correlation coefficient values of 0.9488 and 0.9429, respectively (Fig. 2b). Based on this data, it can be proposed that the processes occurring at the electrode surface are diffusion controlled.

(a)   **(b)**
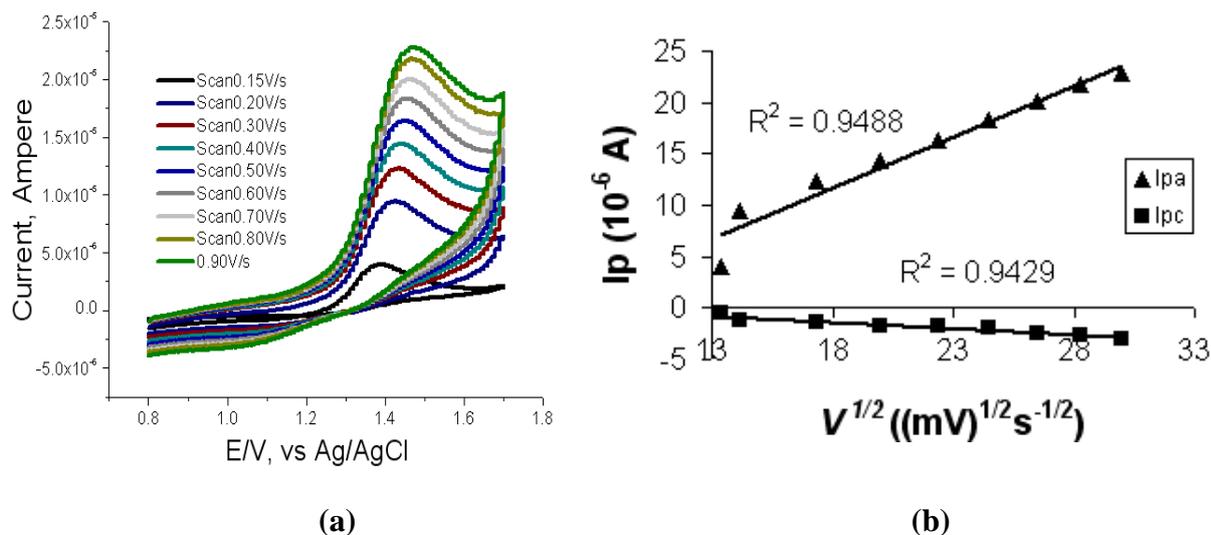
Fig. 2. Effect of scan rate variation of 2.0×10^{-3} M mechlorethamine in acetonitrile at Pt electrode with 0.2 M potassium dihydrogen phosphate buffer, pH 7.0 (a) current *vs.* potential, (b) current *vs.* the square root of the scan rate

3.2. Effect of Solvent Used

Mechlorethamine has a reversible electrochemical reaction which is followed by an irreversible chemical reaction mechanism (E_rC_i). Tests on the reversible electrochemical reaction mechanism have been done through fulfillment of Randles-Sevcik equation. This is demonstrated by the curve of Ip values against $V^{1/2}$ for each voltammogram and the R^2 constant values close to 1 (Fig. 1(b) and Fig. 2(b)). Table 1 showed that test on the irreversible chemical reaction which is also evident from the values of Ip_c/Ip_a less than 1.

Table 1. The values of Ip_c/Ip_a on the applied scan rate of mechlorethamine in acetone and acetonitrile

V (V/s)	Ip_c/Ip_a (acetone)	Ip_c/Ip_a (acetonitrile)
0.15	0.552	0.478
0.20	0.555	0.537
0.30	0.565	0.553
0.40	0.572	0.558
0.50	0.572	0.559
0.60	0.581	0.561
0.70	0.583	0.578
0.80	0.587	0.589
0.90	0.603	0.615

Both cyclic voltammograms of mechlorethamine in acetone and acetonitrile gave almost the same shape of the curve. In Fig. 3, voltammogram of mechlorethamine in acetone formed a good peak on the banisters forward direction, but rather ramps on the reverse direction, with the difference in the values of the Ip_c and Ip_a relatively long distances. In contrast, the voltammogram of mechlorethamine in acetonitrile gave the difference in the values of the Ip_c and Ip_a with the values of Ip_c/Ip_a almost all smaller than that in acetone. The differences are caused by the physical properties of the solvents used i.e the DN, which had a role in the alkylation reaction of mechlorethamine. Acetone has a larger DN than acetonitrile. In such cases mechlorethamine is more prone to solvation by acetone than acetonitrile. As a result, the amount of mechlorethamine reacted in acetone will be more than in acetonitrile. This will have a direct impact on the values of Ip_a, Ip_c and also Ip_c/Ip_a. Therefore, the values of the Ip_c/Ip_a of this compound in acetone are relatively higher than in acetonitrile.

In Fig. 3, the oxidation peak potentials of mechlorethamine in acetone and acetonitrile are 1.48 V and 1.46 V, respectively. This implies that mechlorethamine is more easily oxidized in acetonitrile. In terms of response, this compound in acetone provides the oxidation peak current better than that in acetonitrile. The use of different solvents will give different responses and also different oxidation-reduction potential peaks, but does not cause a shift of current at the point of initial-final potential.
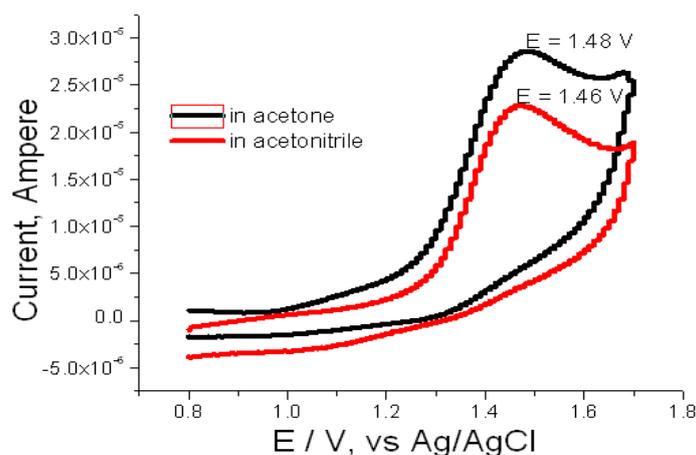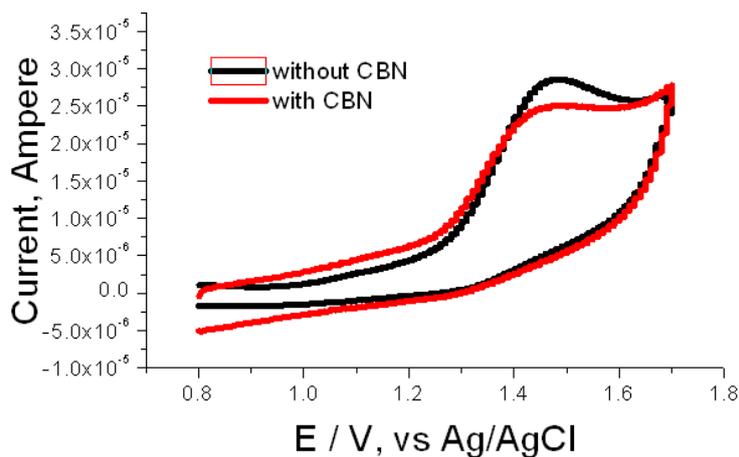

Fig. 3. Cyclic voltammogram of 2.0×10^{-3} M mechlorethamine in acetone (black line) and in acetonitrile (red line) with 0.2 M potassium dihydrogen phosphate buffer at 0.9 V/s of scan rate
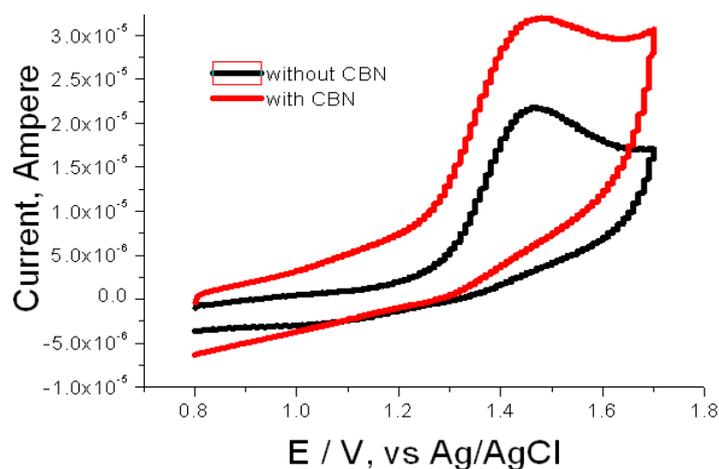
3.3. Cyclic Voltammetry of Mechlorethamine in the Presence of 4-Chloro Butyronitrile Nucleophile

Addition effect of nucleophilic compounds, i.e. CBN on mechlorethamine in acetone and acetonitrile is shown in Figs. 4 and 5 respectively. The effect of the nucleophile added to

mechlorethamine in solvents was tested through characterization from their voltammogram. After the addition of CBN, the voltammogram produced a change of Ip_a and Ip_c values were proportional to the square root of the scan rate with average correlation coefficient, R^2 in acetone and acetonitrile are 0.9964; 0.9965 and 0.9986; 0.9870, respectively. This implies that the condition has fulfilled Randles-Sevcik equation. The comparison of the values of Ip_c and Ip_a obtained was below 1, which means that the E_rC_i mechanism was unchanged.

Fig. 4. In the absence (black line) and presence (red line) of CBN nucleophile for cyclic voltammogram of 2.0×10^{-3} M mechlorethamine in acetone with 0.2 M potassium dihydrogen phosphate buffer, pH 7.2 at 0.9 V/s of scan rate

Fig. 5. In the absence (black line) and presence (red line) of CBN nucleophile for cyclic voltammogram of 2.0×10^{-3} M mechlorethamine in acetonitrile with 0.2 M potassium dihydrogen phosphate buffer, pH 7.0 at 0.9 V/s of scan rate

The oxidation peak potentials of mechlorethamine in acetone and acetonitrile both in the absence and presence of CBN were 1.48 V; 1.44 V; 1.46 V and 1.47 V, respectively. In acetone, the addition of CBN enhanced the oxidation of mechlorethamine. Conversely, in acetonitrile, its oxidation was hampered. A Similar phenomenon occurs in the current response. In acetone, the presence of CBN reduced the current response, the opposite occurred in acetonitrile (Fig. 4 and Fig. 5). Both Figs. 4 and 5 show a similar trend where CBN raised current's difference in the initial-final potentials of the cyclic voltammogram both in acetone and acetonitrile. The differences may occur due to changes in the process of diffusion and mass transport on the surface of the working electrode. These changes may affect its chemical reactivity.

3.4. The forward rate constants of mechlorethamine (K_f)

We calculated the parameters of each analyte in the cyclic voltammogram to obtain the values of I_{pc}/I_{pa}, E^0 and t. The determination of $K_f t$ values was based on the working curve plots of Nicholson i.e. I_{pc}/I_{pa} versus log $K_f t$. The value was determined as the value of the slope from the curve of $K_f t$ versus t of each analyte. Both the reduction-oxidation peaks were determined by using I_{pc}/I_{pa} calculation with the correction of Nicholson equation [21-24]. The correlation coefficient of mechlorethamine, obtained from the curves of $K_f t$ versus t, in acetone and acetonitrile, in the presence and absence of CBN was quite good, i.e. 0.9906; 0.9795; 0.9516; 0.9806, respectively. The K_f values of mechlorethamine can be seen in Table 2. This table shows that the K_f value of mechlorethamine in acetone is higher than in acetonitrile. These results are consistent with the previous studies showing that the reactivity of mechlorethamine is higher than chlorambucil [13]. These results also prove that the most dominant property of solvent that affects the value of the chemical reactivities or the forward reaction rate constants is the (DN).

Table 2. The forward reaction rate constants (K_f) of mechlorethamine in different solvents and in the presence of CBN

Solvents	K_f (s^{-1})	K_f relative (s^{-1})
Acetone	0.4828	3.5370
Acetonitrile	0.2724	1.9956
Acetone + CBN	0.1601	1.1729
Acetonitrile + CBN	0.1365	1.0000

In Table 2, it is clear that the presence of CBN reduces the values of the chemical reactivities of mechlorethamine both in acetone and acetonitrile. CBN is not a bulky molecule and has a relatively simple molecular structure. Due to simple structure, CBN can interact directly with mechlorethamine, and thus reduces the chemical reactivities of mechlorethamine in both acetone and acetonitrile as solvents.

4. CONCLUSION

In this investigation, the interaction of mechlorethamine with acetone and acetonitrile both in the absence and presence of CBN was reported. The overall forward reaction rate constants (K_f) of mechlorethamine in four conditions were determined successfully by using cyclic voltammetric technique. The use of different solvents and the addition of a nucleophilic compound, does not alter the mechanisms that occur only reactivity are shifted. Finally, the chemical reactivities of mechlorethamine under these conditions are higher than that of chlorambucil [13].

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