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ELECTROCHEMICAL STUDY OF INTERACTION OF THE HEAVY METAL IONS IN HYDROXYLATION REACTION OF SALICYLIC ACID ON GLASSY CARBON ELECTRODE

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Keywords:
Cyclic voltammetry, Hydroxylation reaction, complexation effect, multisweep cycle experiment, interference of heavy metal ions.

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ABSTRACT: The formation of free hydroxyl radical ($^{\cdot}OH$) in oxygen transfers at GCE and the reaction between the electrochemically produced $^{\cdot}OH$ and salicylic acid (SA) is studied by cyclic voltammetry method. Experiments showed that $^{\cdot}OH$ is formed in the potential range of 1.0-1.4V corresponding to the anodic discharge of H_2O at the GCE. The interaction of Cu^{2+}, Hg^{2+}, Cd^{2+}, Mn^{2+} ions on the hydroxylation reaction is studied. The interaction of these metal ions is explained by using derived parameters such as surface coverage, formal potential, transfer co-efficient number and rate constant. The diffusion coefficient values for electroactive species on GCE is determined by using chronoamperomety and chronocoulometry techniques. It shows that Mn-SA has higher diffusion coefficient value as compared to the other systems. Multisweep cycle experiment on GCE surface provided mechanism for the reaction of hydroxylation on salicylic acid. This experiment suggests that metal ions first undergo Fenton-type hydroxylation reaction on salicylic acid and then undergo complexation reaction with 2, 5-dihydroxybenzoic acid.

INTRODUCTION: The complexation of organic compounds with selected metal ions has a wide variety of applications in medicinal chemistry, surface chemistry and analytical chemistry. Complexation of medicinal substances with metal ions influence the bioavailability of drugs in the body and the biological action affects the stability of medicinal compounds since a large number of metals are taken into the body system either with drugs or in the form of diet [1]. The complex formation has been suggested as one of the important mechanisms for certain drug action [2].

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The metal chelating phenomena are used to reduce the toxic effect of drugs in human physiology [3]. The study of redox behavior of biologically and biochemically important compounds are close to natural processes occurring in human and other living organisms.

Salicylic (or) 2-hydroxybenzoic acid (SA) is a compound, which has been shown to play an important signaling role in the activation of various plant defense responses following pathogen attack. These responses include the induction of local and systemic disease resistance, the potentiation of host cell death and the limitation of pathogen spread. SA is also an endogenous growth regulator fulfilling numerous functions in plants under normal conditions.

The presence of heavy metals in the environment and their potentially toxic effects on the health living species is a cause for concern.

This is because heavy metals tend to bioaccumulate in higher trophic levels within the food chain and can be toxic even in trace quantities [4-6]. In the present study, the redox behaviour of salicylic acid (SA) at glassy carbon electrode and its interaction with heavy metals have been investigated in aqueous alcoholic potassium chloride medium by using electrochemical methods.

Reagents: Salicylic acid (SA), ethanol, potassium chloride, copper sulphate, mecurous chloride, cadmium chloride, manganeous sulphate.

MATERIALS AND METHODS:

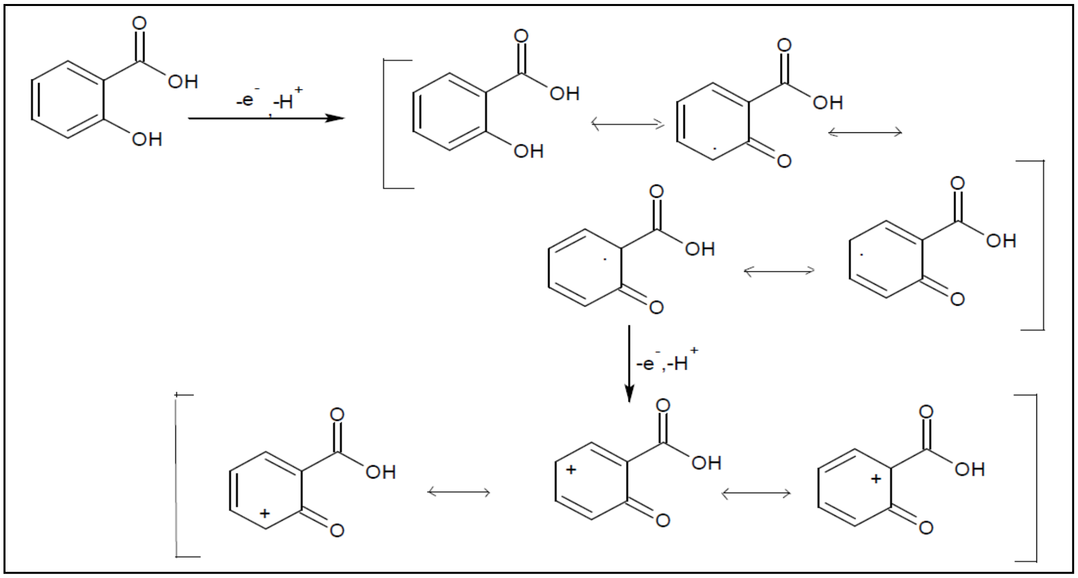
Electrochemical workstations of CHI. USA: Model 600D with potentiostate driven by electroanalytical measuring softwares is connected to PC computer to perform cyclic voltammetry (CV). An Ag/AgCl (3M KCl) and platinum wire are used as a reference and counter electrode respectively.

Polishing of Glassy Carbon Electrode: The glassy carbon electrode is polished with fine alumina powder (0.3micron) on a wet polishing cloth. To do so a part of the cloth is mode wet with deionized water and alumina powder is sprinkled on it. The glassy carbon electrode is then polished on this surface by pressing softly the electrode against the polishing surface in the end for 3-5 minutes. The electrode is then thoroughly washed with deionized water. At this point the electrode surface would look like a shiny black mirror.

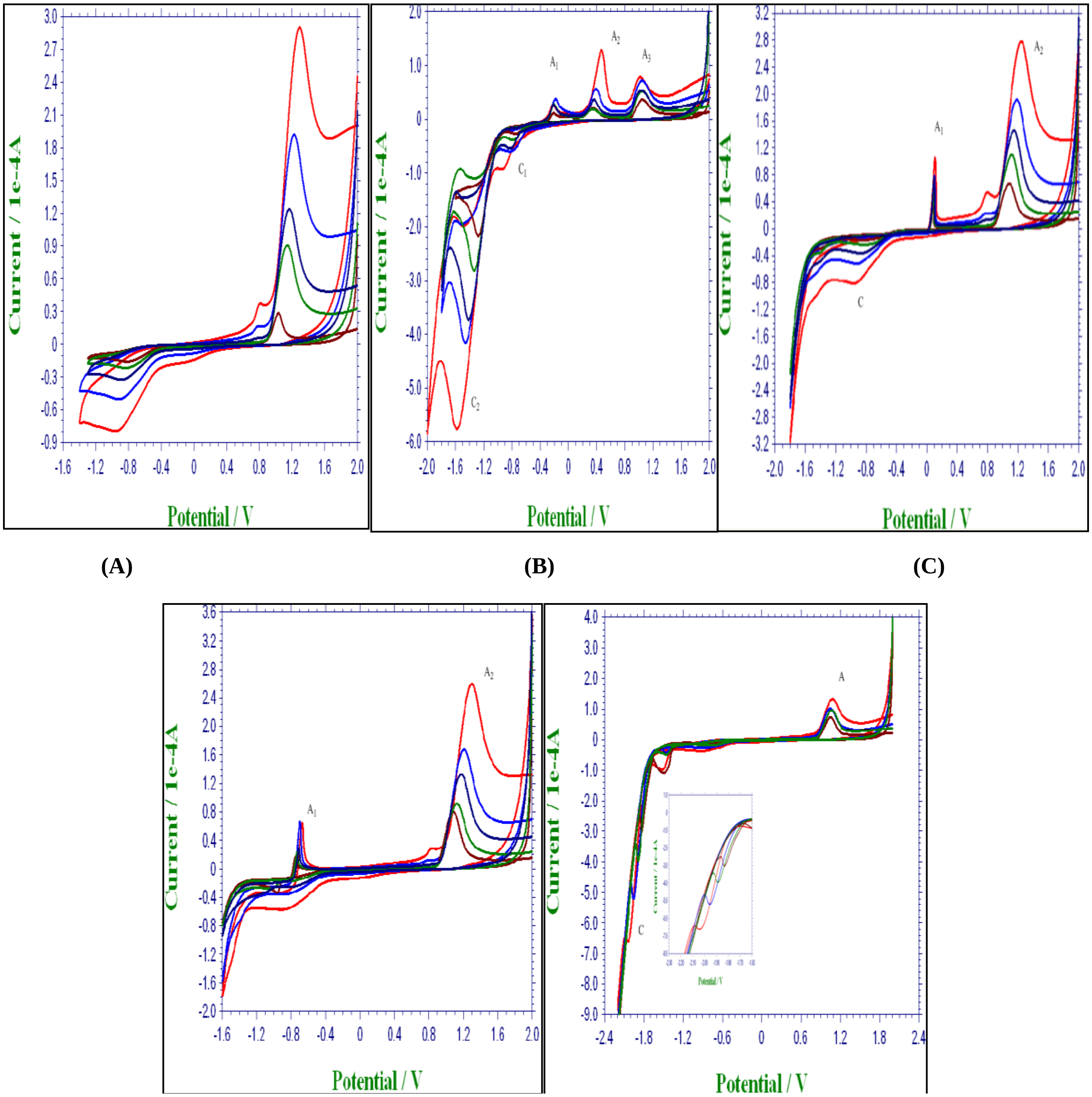
RESULTS AND DISCUSSION:

Scan rate effect: Cyclic Voltammogram for (A) 0.072M SA; (B) 0.072M SA+0.001M Cu^{2+}; (C) 0.072M SA+0.001M Hg^{2+}; (D) 0.072M SA+0.001M Cd^{2+}; (E) 0.072M SA+0.001M Mn^{2+} at different sweep rates in aqueous alcoholic 0.1M KCl solution on glassy carbon electrode are shown in Fig.1. It clearly follows from the results obtained that SA gives the signal at a potential range of 1.0-1.4V which increased with scan rate. Cyclic voltammogram of salicylic acid (Fig.1A) has one cathodic peak at -0.8V and one well defined anodic peak (A) at 1.2V. The anodic peak current increased with increasing scan rate which suggest that diffusion controlled one.

Although it is generally believed that the initial stage in oxidation of phenols leads to formation of a phenooxonium ion **(Scheme.1),** there are reports that the principal product also may be a phenoxy radical. These products may undergo further chemical reactions such as coupling, proton loss or nucleophilic attack [7]. Though a number of possible phenoxonium ion intermediates may be formed, the structure in which a positive charge is on a carbon atom that already suffers an electron deficiency due to the electron-withdrawing effect of a COOH group is unlikely. Therefore salicylic acid peaks are shifted toward positive potential. In the case of o-hydroxybenzoic acid steric effects may also contribute to the shift of potential.



SCHEME 1: ELECTROCHEMICAL OXIDATION OF SALICYLIC ACID



(A) (B) (C)

(D) (E)

FIG.1. CYCLIC VOLTAMMOGRAM FOR (A) 0.072M SA; (B) 0.072M SA+0.001M CU^{2+}; (C) 0.072M SA+0.001M HG^{2+}; (D) 0.072M SA+0.001M CD^{2+}; (E) 0.072M SA+0.001M MN^{2+} AT DIFFERENT SWEEP RATES IN AQUEOUS ALCOHOLIC 0.1M KCL SOLUTION ON GLASSY CARBON ELECTRODE

One reduction peak at -0.8V is observed for the reduction of oxygen to give hydrogen peroxide. The decomposition of hydrogen peroxide can occur rapidly when it has been exposed to current. The reactions are following

$$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$$

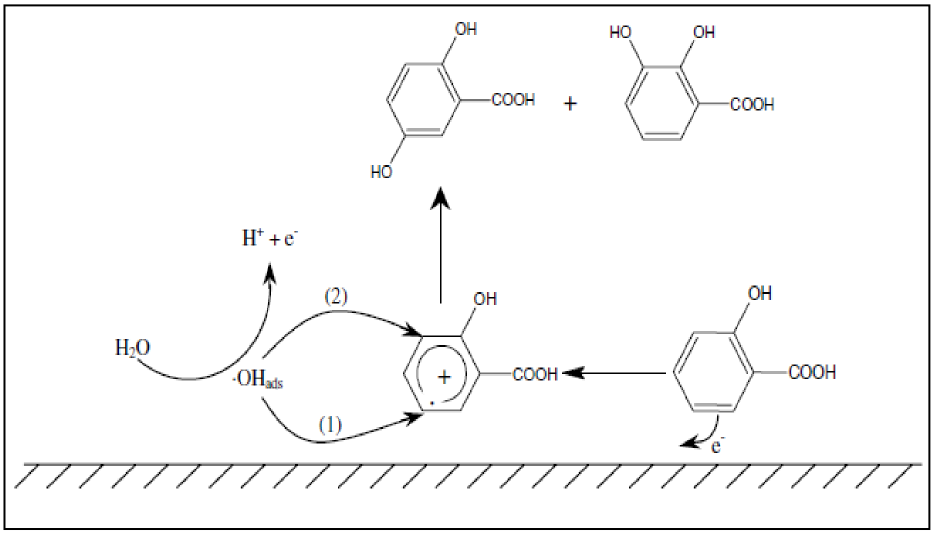
$$2H_2O_2 \longrightarrow 2H_2O(l) + O_2(g)$$

The $^{\cdot}$OH is formed in the potential range of 1.0-1.4V corresponding to the anodic discharge of H_2O at the GCE.

$$H_2O \longrightarrow {}^{\cdot}OH_{(ads)} + H^+ + e^-$$

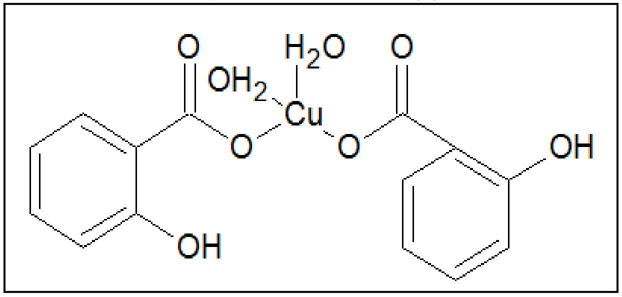
It is also found that when SA is used to trap $^{\cdot}$OH produced on the GCE surface, the amount of 2,5dihydroxybenzoic acid (2,5DHBA) produced is much more than that of 2,3dihydroxybenzoic acid (2,3DHBA). The scheme.2 explains SA first forms an active aromatic radical on the GCE surface before trapping $^{\cdot}$OH and $^{\cdot}$OH performs a stronger preference in attacking at the 5-position than that at the 3-position of the aromatic radical.

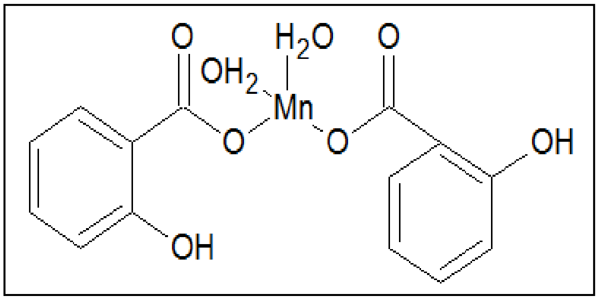
This mechanism is very useful in biological research, such as the unclear mechanism that only 2,5DHBA, not 2,3DHBA is produced by an enzymatic hydroxylation pathway through the cytochrome p-450 microsomal system [8].



SCHEME 2: REACTION OF SA WITH ·OH AT THE GCE SURFACE

From **Fig. 1 (B & E),** Cu (II) and Mn (II) forms complex with salicylic acid which is observed from the characteristic peak at -1.8V. In the **Fig. 1(C & D)** such kind of peak is not observed in case of interaction of Hg (II) & Cd (II) with salicylic acid. In all metal ion-SA cases have the characteristic oxidation peak for the metal ions and there is absence of reduction peaks which suggest that the metal ions involved in the electrocatalytic effect in the hydroxylation of SA via fenton type mechanism. But in cases of Cu-SA and Mn-SA have the characteristic reduction peaks in the potential range of -1.8V. This suggest that the Cu (II) and Mn(II) ions are involved in the formation of complex reaction with SA. The possible structure of the complex is follow [9];





Figs. 1(B-E) show the characteristic peak for hydroxylation reaction. In the case of heavy metal ion interaction with SA, hydroxylation reaction is provided by the fenton type mechanism **(Scheme.3)**

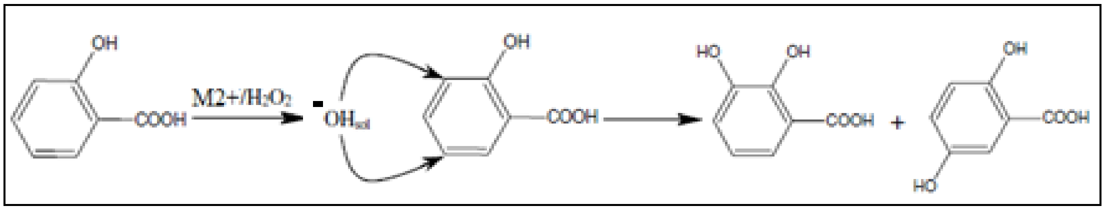
$$M^{2+} + H_2O_2 \longrightarrow M^{+} + {}^{\cdot}OOH + H^{+}$$

$$M^{+} + H_2O_2 \longrightarrow {}^{\cdot}OH + HO^{-} + M^{2+}$$

M = Cu, Hg, Cd, Mn

SCHEME 3: GENERATION OF ·OH RADICAL VIA FENTON TYPE MECHANISM

The overall hydroxylation reaction on the salicylic acid via fenton type mechanism is given in the **Scheme 4.**



SCHEME 4: HYDROXYLATION REACTION ON THE SALICYLIC ACID VIA FENTON TYPE MECHANISM

Cyclic voltammetric parameters for (A) 0.072M SA; (B) 0.072M SA+0.001M Cu^{2+}; (C) 0.072M SA+0.001M Hg^{2+}; (D) 0.072M SA+0.001M Cd^{2+}; (E) 0.072M SA+0.001M Mn^{2+} at different sweep rates in aqueous alcoholic 0.1M KCl solution on glassy carbon electrode are tabulated in **Table 1.** The anodic peak potential is in the order of SA > Hg-SA = Cd-SA > Mn-SA > Cu-Hg. Thus the hydroxylation reaction is highly facilitated by copper ions. The anodic and cathodic peak currents are increased with increasing scan rate which suggest that the diffusion controlled process.

TABLE 1: CYCLIC VOLTAMMETRIC PARAMETERS FOR (A) 0.072M SA; (B) 0.072M SA+0.001M CU^{2+}; (C) 0.072M SA+0.001M HG^{2+}; (D) 0.072M SA+0.001M CD^{2+}; (E) 0.072M SA+0.001M MN^{2+} AT DIFFERENT SWEEP RATES IN AQUEOUS ALCOHOLIC 0.1M KCL SOLUTION ON GLASSY CARBON ELECTRODE

(A)

Scan rate (mV/s)	Ipc x 10^5 (A)	Epc (V)	Ipa x 10^4 (A)	Epa (V)
10	-1.626	-0.7955	0.2950	1.0342
20	-2.234	-0.8426	0.9167	1.1398
40	-3.376	-0.8896	1.250	1.175
80	-5.158	-0.9131	1.937	1.2224
160	-8.106	-0.9424	2.927	1.2930

(B)

Scan rate (mV/s)	Ipc^1 x 10^5 (A)	Epc^1 (V)	Ipc^2 x 10^4 (A)	Epc^2 (V)	Ipa^1 x 10^5 (A)	Epa^1 (V)	Ipa^2 x 10^4 (A)	Epa^2 (V)	Ipa^3 x 10^5 (A)	Epa^3 (V)
10	-2.748	-0.7422	-2.188	-1.2774	1.312	-0.2013	0.2064	0.3517	3.752	1.0520
20	-4.128	-0.7856	-2.838	-1.3280	3.248	-0.2037	0.2128	0.3515	5.504	1.0512
40	-5.728	-0.8181	-3.752	-1.4197	3.167	-0.2037	0.3895	0.3712	5.649	1.0379
80	-6.155	-0.8117	-4.179	-1.4587	4.155	-0.1845	0.5879	0.3909	7.367	1.0443
160	-9.333	-0.9296	-5.783	-1.5765	2.997	-0.2171	1.300	0.4626	8.171	1.0181

(C)

Scan rate (mV/s)	Ipc x 10^5 (A)	Epc (V)	Ipa^1 x 10^4 (A)	Epa^1 (V)	Ipa^2 x 10^4 (A)	Epa^2 (V)
10	-1.749	-0.7187	0.5083	0.0810	0.7003	1.0870
20	-2.496	-0.7269	0.6248	0.0901	1.138	1.1227
40	-3.872	-0.8443	0.8000	0.0965	1.475	1.1621
80	-5.496	-0.9163	0.8128	0.0965	1.950	1.1883
160	-8.397	-0.9488	1.093	0.1035	2.800	1.2469

(D)

Scan rate (mV/s)	Ipa^1 x 10^5 (A)	Epa^1 (V)	Ipa^2 x 10^4 (A)	Epa^2 (V)
10	1.980	-0.7542	0.8127	1.0870
20	2.636	-0.7307	0.9324	1.1283
40	3.333	-0.7072	1.344	1.1754
80	6.800	-0.6952	1.700	1.2104
160	6.835	-0.6717	2.620	1.3045

(E)

Scan rate (mV/s)	Ipc x 10^4 (A)	Epc (V)	Ipa x 10^4 (A)	Epa (V)
10	-3.058	-1.8291	0.7912	1.0573
20	-4.000	-1.8918	1.000	1.0726
40	-5.303	-1.9546	1.112	1.0496
80	-6.670	-2.0486	1.372	1.0886
160	-8.296	-2.1427	1.833	1.1360

Cyclic voltammetric derived parameters for (A) 0.072M SA; (B) 0.072M SA+0.001M Cu^{2+}; (C) 0.072M SA+0.001M Hg^{2+}; (D) 0.072M SA+0.001M Cd^{2+}; (E) 0.072M SA+0.001M Mn^{2+} at different sweep rates in aqueous alcoholic 0.1M KCl solution on glassy carbon electrode is tabulated in **Table 2.** The surface concentration of the electroactive species (Γ) can be estimated according to the following equation [10];

$$Ip = n^2F^2A.\Gamma\ v/\ 4RT$$

Laviron's equation [11] was used to estimate αn and ks values, as follows:

$$Ep = E^0 + RT/\alpha nF\ [\ln(RTks/\alpha nF)\text{- } \ln v]$$

Where α is the electron transfer coefficient, ks is the standard rate constant of the surface reaction, v is the scan rate, n is the electron transfer number, and E^0 is the formal potential. Rate constant (ks) and electron transfer coefficient number(αn) values can be calculated from the intercept and slope of the linear plot of Ep with respect to Ln v, if the value is E^0 is known (plots are not shown).

The E^0 value can be deduced from the intercept of Ep vs v plot on the ordinate by extrapolating the line to v = 0. Knowing E^0 and from the graphical representations of Ep vs Ln v, the values of αn and ks are obtained from the slope and intercept, respectively.

TABLE 2: CYCLIC VOLTAMMETRIC DERIVED PARAMETERS FOR(A) 0.072M SA; (B) 0.072M SA+0.001M CU^{2+}; (C) 0.072M SA+0.001M HG^{2+}; (D) 0.072M SA+0.001M CD^{2+}; (E) 0.072M SA+0.001M MN^{2+} AT DIFFERENT SWEEP RATES IN AQUEOUS ALCOHOLIC 0.1M KCL SOLUTION ON GLASSY CARBON ELECTRODE

Compound	Γ_c x 10^{10} (mol/cm)	E^0_c (V)	αn_c	k_s^c (s^{-1})	Γ_a x 10^{10} (mol/cm^2)	E^0_a (V)	αn_a	k_s^a (s^{-1})
(A) SA	-	-	-	-	4.865	1.084	0.298	0.7907
(B) Cu-SA	1.596	-1.298	0.2445	1.294 x 10^2	0.760	1.053	2.567	1.487 x 10^3
(C) Hg-SA	-	-	-	-	3.953	1.101	0.467	1.299
(D) Cd-SA	-	-	-	-	3.345	1.098	0.347	0.992
(E) Mn-SA	2.432	-1.851	0.227	1.199 x 10^2	1.824	1.048	1.0269	3.589

The derived parameters for hydroxylation reaction with and without interaction of heavy metal ions are compared as follows.

The surface concentration of electroactive species (Γ) is given the order of

SA > Hg-SA > Cd-SA > Mn-SA > Cu-SA

The transport co-efficient number (αn) is in the order of

Cu-SA > Mn-SA > Hg-SA > Cd-SA > SA

The rate constant (ks) is in the order of

Cu-SA > Mn-SA > Hg-SA > Cd-SA > SA

The order of electrocatalytic activity of heavy metal ions in hydroxylation of SA is Cu > Mn > Hg > Cd. This is also supported by the appearance of the special characteristic reduction peaks in the potential range of -1.8V for Cu-SA and Mn-SA.

Chronoamperometry and Chronocoulometry study

Fig. 2 represents the chronoamperometry (A) and chronocoulometry (B) plot for SA and heavy metal ions-SA compounds in 0.1M aqueous alcoholic potassium chloride medium. The diffusion coefficient values for different electroactive species on glassy carbon electrode is tabulated in **Table 3.**

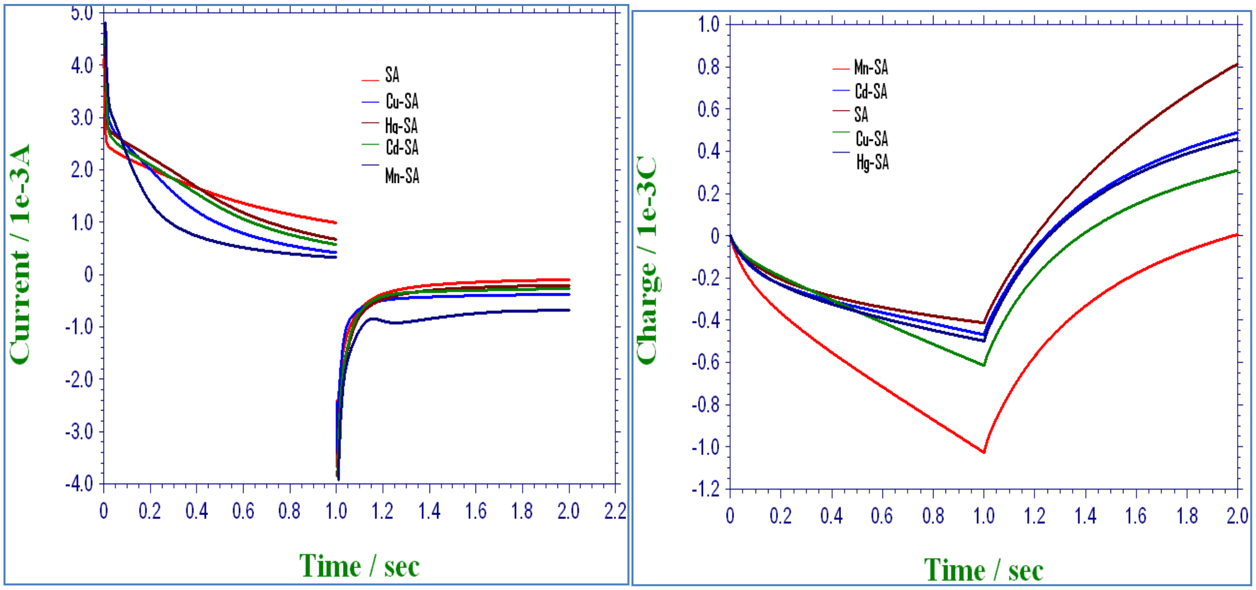
Diffusion coefficient values is in the order of;

For forward scan (f) (CA): Hg-SA > Cd-SA > Cu-SA > SA > Mn-SA

(CC): Mn-SA > Cu-SA> Hg-SA > Cd-SA > SA

For reverse scan(r) (CA): Mn-SA > SA >Hg-SA > Cd-SA > Cu-SA

(CC): SA > Mn-SA > Cd-SA > Cu-SA > Hg-SA



(A) **(B)**

FIG. 2: CHRONOAMPEROMETRY (A) AND CHRONOCOULOMETRY (B) PLOT FOR SA AND HEAVY METAL IONS-SA COMPOUNDS IN 0.1M AQUEOUS ALCOHOLIC KCL SOLUTION

TABLE 3: DIFFUSION COEFFICIENT VALUES FOR SA AND HEAVY METAL IONS-SA ON GLASSY CARBON ELECTRODE IN 0.1 M AQUEOUS ALCOHOLIC KCL SOLUTIONS

Compound	Chronoamperometry method(CA)		Chronocoulometry method (CC)	
	D_f x 10^8 (cm^2/s)	D_r x 10^{10} (cm^2/s)	D_f x 10^9 (cm^2/s)	D_r x 10^8 (cm^2/s)
SA	0.451	3.60	0.1764	1.721
Cu-SA	1.005	0.6161	0.7993	0.6291
Hg-SA	1.110	3.314	0.3005	0.00708
Cd-SA	1.056	0.8352	0.2330	0.7513
Mn-SA	0.343	4.987	1.915	0.8283

The result shows that more number of Mn and salicylic acid molecules is adsorbed on the GCE surface. This is because Mn^{2+} ion is undergo formation of $Mn(OH)_2$ with $^{\cdot}OH$ radical. Nucleation growth and a characteristic peak at -1.8V is observed in the Mn-SA case study. Thus hydroxylation reaction in SA with presence of heavy metal is time and charge dependent.

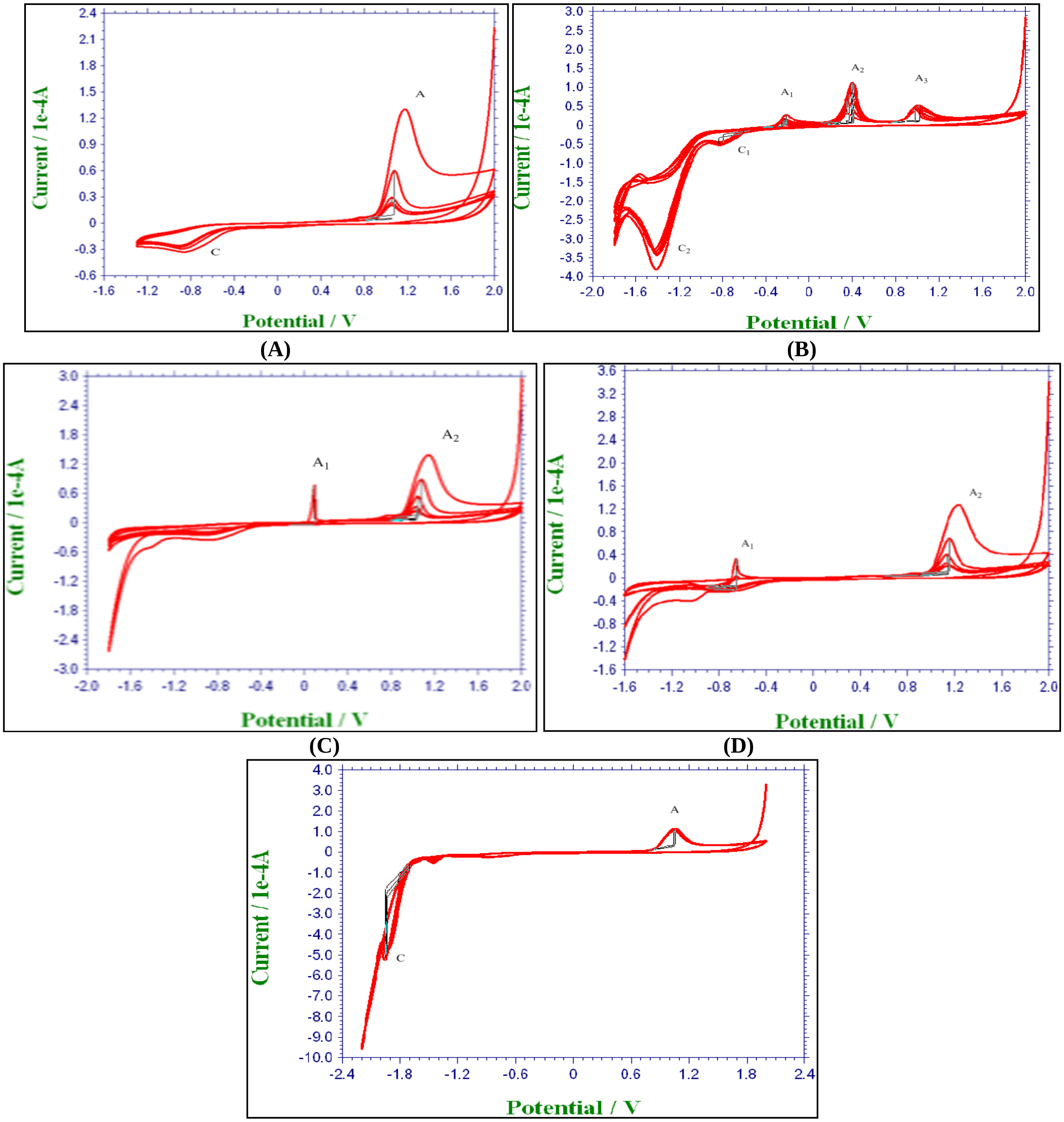
Multisweep cycle experiment: Fig. 3 represents the multi sweep cyclic voltammogram for (A) 0.072M SA; (B) 0.072M SA+0.001M Cu^{2+}; (C) 0.072M SA+0.001M Hg^{2+}; (D) 0.072M SA+0.001M Cd^{2+}; (E) 0.072M SA+0.001M Mn^{2+} at 40mV/s in aqueous alcoholic 0.1M KCl solution on glassy carbon electrode. In all cases anodic peak current for the hydroxylation reaction is decreased with increasing scan number. This is due to the occupying of the electroactive surface by 2,5-dihydroxybenzoic acid (2,5-DHBA).

After the first cycle Hg-SA and Cd-SA exhibits no characteristic peak for the metal ions. But the anodic peak current for the hydroxylation is decreased with increasing scan number. This suggest that the Hg^{2+} and Cd^{2+} ions facilitates the deposition of the 2, 5-DHBA. But in case of Mn-SA there is no change in the anodic peak current for hydroxylation reaction.

Cyclic voltammogram for Mn-SA exhibits nucleation growth above the potential of -1.8V. This suggests that adsorption of stable Mn-SA complex on the glassy carbon electrode surface. The structure of the Mn-SA complex is already given. In case of Cu-SA, anodic peak current (A_1 and A_2) for the copper metal ion is increased with increasing concentration. But the anodic peak for hydroxylation reaction (A_3) is closely appeared.

In the **scheme 5 & 6**, the initial step is the oxidation of 2, 3-DHBA by Cu^{2+} involves the formation of a Cu (II)-DHBA complex. The next step may involve intramolecular electron transfer within the Cu (II)-DHBA complex to produce Cu (I) and the DHBA radical.

The formation of the initial Cu-DHAB complex likely alters the electron density distribution increasing the probability that the semiquinone can form by one electron oxidation with Cu (I) [12]. The semiquinone structure is then further oxidized at the surface of GCE to form the quinone species.

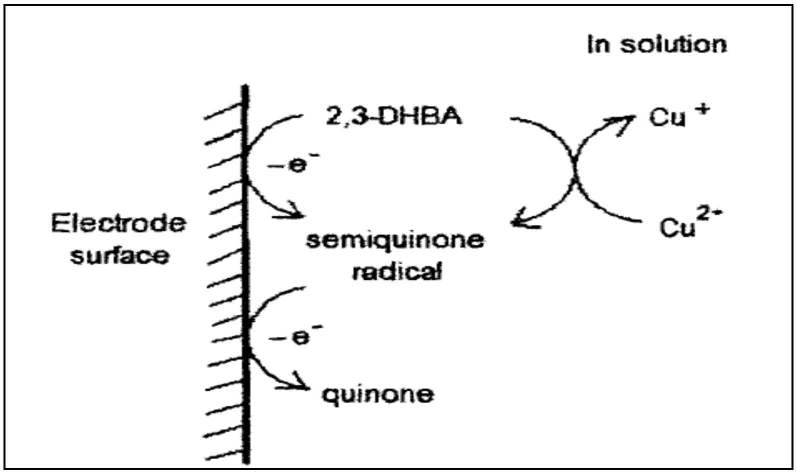


(A) (B)

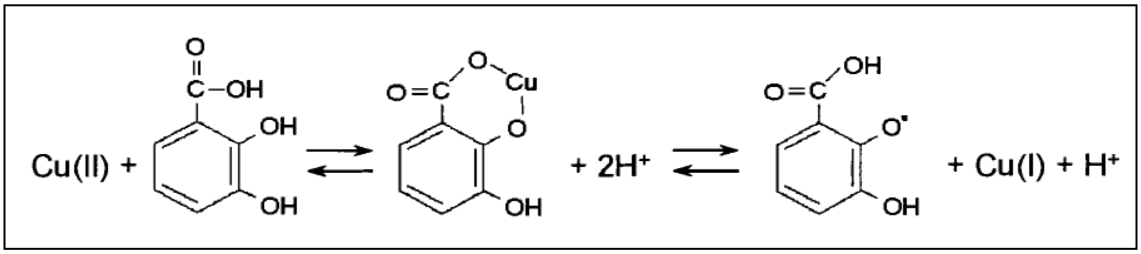
(C) (D)

(E)

FIG. 3: MULTISWEEP CYCLIC VOLTAMMOGRAM FOR (A) 0.072M SA; (B) 0.072M SA+0.001M CU^{2+}; (C) 0.072M SA+0.001M HG^{2+}; (D) 0.072M SA+0.001M CD^{2+}; (E) 0.072M SA+0.001M MN^{2+} AT 40MV/S IN AQUEOUS ALCOHOLIC 0.1M KCL SOLUTION ON GLASSY CARBON ELECTRODE

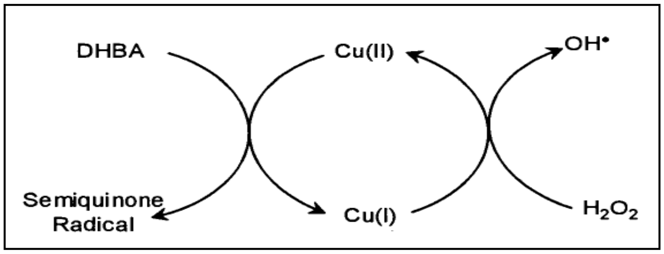


SCHEME 5: REDOX SCHEME OF CU-DHBA COMPLEX



SCHEME 6: INTERACTION BETWEEN 2,3DHBA AND CU

The electrochemically produced H_2O_2 react with Cu-DHBA complex produce ˙OH radical. Thus at the first cycle, ˙OH is produced via fenton type mechanism. After the first cycle, the ˙OH may be produced via reaction between H_2O_2 and Cu-DHBA complex. The production of ˙OH radical is given in **Scheme 7.**



SCHEME 7: THE REDOX CYCLING SCHEME OF CU-2, 3-DHBA-H_2O_2 SYSTEM

CONCLUSION: Measuring of the reducing capacity and electrochemical behavior of phenolic compounds may provide useful information about the free radical scavenging acitivity of natural occurring phenols. Salicylic acid acts as modest suppression agent for hydroxyl radical. Cu^{2+} and Mn^{2+} ions are border line acids. Hg^{2+} and Cd^{2+} ions are soft acids. Hydroxy group is a hard base. According to HSAB theory, salicylic acid is formed complex with Cu^{2+} and Mn^{2+} ions.

In case of Cu-SA, Mn-SA mixtures, metal chelation mechanism is predominant and restricts the accessibility of the Cu^{2+} and Mn^{2+} ions for participation in fenton-type reaction. Hg^{2+} and Cd^{2+} ions have electrocatalytic activity in the hydroxylation reaction of salicylic acid.

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