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Electrochemical behaviour and biological screening of 4-(substituted) acetophenone salicylyl hydrazones

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ABSTRACT

Electrochemical reduction of 4-(substituted) acetophenone salicylyl hydrazones has been carried out in Britton-Robinson buffers in the pH range of 1.1 to 10.1 containing 50% V/V dimethylformamide. The compounds exhibit a well-defined wave at DME and well-defined cathode peak at HMDE. A plausible mechanism has been suggested on the basis of number of protons and electrons involved in the reduction process.

Keywords: Electrochemical behaviour, hydrazones, microbial activity, reduction**.**

INTRODUCTION

Some hydrazones have been found to play an important role in the medicinal chemistry. Because of their potentially high physiological activities[1], halogen containing organic compounds are associated with antimicrobial[2], antitumor[3], antibacterial[4,5], and anticancer[6] activities. The activity of all hydrazones depends on the toxicity. The toxicity is related to substituents like halogen, sulfur and phosphorus. Nowadays number of herbicides and insecticides [7-10] are c**o**mmercially available that proves the above fact.

In view of this, the electrochemical behavior of acetophenone salicylyl hydrazones and its methyl, methoxy, chloro and bromo substituted derivatives were studied and the results are presented in this paper.

EXPERIMENTAL SECTION

Acetophenone salicylyl hydrazone (A) and its methyl (B), methoxy (C), chloro (D) and bromo substituted derivatives (E) have been synthesized by the literature method, purified by repeated recrystallisation from dimethlformamide-water mixture (1:1) and characterized by spectral studies. Stock solutions (1×10^{-2} M) of the 4-(substituted) acetophenone salicylyl hydrazones are prepared in dimethylformamide (AnalaR grade). Details of experimental setup for polorographic, cyclic voltammetric and coulometric measurements have been described elsewhere [11,12] . All peak potentials and $E_{1/2}$ values are referred with respect to SCE. The number of electrons involved in the reduction process was calculated by millicoulometry [13] using $CdSO_4$ as the reference solution.

RESULTS AND DISCUSSION

(A) Polarography:

All compounds exhibit a single well-defined four electron reduction wave in the pH range 1.1-7.1. The polarographic data are given in Table 1. The observed linear dependence of the limiting current on $h^{1/2}$ (square root of mercury column height) and the concentration of the depolarizer suggests that the wave is diffusion controlled. It is further supported by the low percentage of temperature coefficient [14] (0.85-1.45%K^{-1} between 303 and 333K). The wave is irreversible in the pH range of study. This may be due to the bulky ring attached at the end of the >C=N linkage [15]. The irreversible nature of the wave established by semi log plots of $-E_{dme}$ Vs log $[i/(i_d-i)]$ and the shift of $E_{1/2}$ towards more negative potentials [16] indicated the participation of protons in the reduction process. The fractional value of P (number of protons) observed in the present studies show that heterogeneous proton transfer is taking place [17]. At pH 8.1-10.1, ill-defined waves are observed. The position of the wave on the potential axis together with the reports made in the literature for the reduction of azo [18] , hydrazono linkage [19] indicate that the wave is attributed to the reduction of azo group in hydrazone form [20]. The results are presented in Tables 1 and 2.

Table-1: Effect of pH on Half-wave potential and wave height

Concentration : 1×10^{-3} M
Medium : Aqueous dimethlformamide (50%V/V)
Temperature : Room Temperature (RT)

pH	Half-wave Potential V vs SCE					Wave height (µA)				
	A	B	C	D	E	A	B	C	D	E
1.1	0.54	0.57	0.62	0.47	0.49	7.5	7.9	8.5	6.7	6.9
2.1	0.64	0.68	0.73	0.59	0.6	7.1	7.6	8	6.1	6.5
3.1	0.79	0.84	0.9	0.71	0.72	6.8	7	7.2	5.3	6
4.1	0.85	0.89	0.95	0.79	0.8	6	6.2	6.7	4.2	4.8
5.1	0.94	0.98	1.04	0.87	0.88	4.9	5	5.2	3.4	3.9
6.1	1.06	1.1	1.1	0.99	1	2.9	3.2	3.4	2.1	2.5
7.1	1.15	1.2	1.27	1.09	1.08	1.4	1.6	2.2	1.1	1.7

Table-2: Polarographic characteristics and kinetic parameters of A-E at pH 4.1

Concentration : 1×10^{-3} M
Medium : Aqueous dimethlformamide (50% V/V)
Temperature : Room Temperature (RT)

Compound No.	$-E_{1/2}$ (V)	$\Delta E^{1/2}/\Delta pH$	α_n	Number of Protons	$DX10^6$ cm^2S^{-1}	I^*X10^3	$K^0_{f,h}$ cmS^{-1}	ΔG^0 kcal/mol
A	0.85	0.1	0.57	0.93	1.88	3.33	$1.23X10^{-9}$	8.35
B	0.89	0.104	0.51	0.99	2.01	3.44	$2.24X10^{-9}$	8.4
C	0.95	0.109	0.52	0.99	2.35	3.72	$7.25X10^{-10}$	8.49
D	0.79	0.106	0.57	1.08	0.92	2.33	$3.31X10^{-9}$	8.09
E	0.8	0.097	0.57	0.98	1.2	2.67	$1.05X10^{-8}$	7.79

$I^* = i_d/cm^{2/3}t^{1/6}$

(B) Cyclic Voltammetry:

Cyclic voltammetry at hanging mercury drop electrode (HMDE) shows that the compounds exhibit two cathodic peaks at high scan rates (100-500mVS^{-1}) and one cathodic peak at low scan rates (10-50mVS^{-1}) in the pH range 2.1-6.1. On the other hand, no peak is observed at all scan rates under the experimental conditions in the pH range 8.1-10.1. But in contrast to this only a single reduction wave is observed in the DC polarographic studies in the pH range 1.1-7.1 and this was attributed to the 4-electron reductive cleavage. The two cathodic peaks observed in acidic pHs at high scan rates therefore suggest that the reduction is taking place in two steps. Further, the two peaks observed in cyclic voltammetry at high scan rates only indicate that these steps are quite fast. This is probably the reason for the presence of a single wave instead of two waves in DC Polarography. In alkaline solutions (pH 8.1-10.1) no peak is observed either in DC Polarography or Cyclic voltammetric studies under the experimental conditions. This is attributed to the chemical cleavage of azomethine anionic form(II) [21]. i_{PC} vs. $\nu^{1/2}$ is a linear plot passing through the origin and $i_{PC}/\nu^{1/2}$ values are nearly unaltered. This suggests that the reduction is diffusion controlled one. The diffusion controlled nature of the electrode process is confirmed by the increase of the peak currents with the increase in concentration of the polarizer. The plot of $(i_{PC}/\nu^{1/2})$ vs ν indicated that the reduction process is an irreversible charge transfer process [22]. This is further substantiated by the absence of anodic peak in the reverse scan. The cathodic peak potential shifts to more negative values with the rise in the pH of the solution. The results

are similar to those obtained in DC Polarography. Hence the reduction mechanism at HMDE is assumed to be same as at DME. The cyclic voltammetric data of the compounds (A-E) are presented in Table 3.

Table-3: Cyclic voltammetric data of compounds A-E at pH 4.1

Concentration : 1×10^{-3} M
Medium : Aqueous dimethlformamide (50% V/V)
Temperature : Room Temperature (RT)

Compound No.	Scan rate VS^{-1}	$-E_{PC\,I}$ V	$-E_{PC\,II}$ V	$I_{PC\,I}$	$I_{PC\,II}$
A	0.01	1.05	-	2	-
	0.02	1.08	-	3	-
	0.05	1.11	-	5	-
	0.1	1.14	1.3	7.2	8.1
	0.2	1.18	1.34	10.5	11.7
	0.3	1.22	1.38	13	14.5
	0.5	1.26	1.42	17	18.5
B	0.01	1.01	-	2.8	-
	0.02	1.04	-	4.2	-
	0.05	1.07	-	7	-
	0.1	1.1	1.27	10.5	10.1
	0.2	1.14	1.3	16	16
	0.3	1.18	1.33	18.9	18.4
	0.5	1.22	1.36	24.5	24.4
C	0.01	1.05	-	2.3	-
	0.02	1.08	-	3.5	-
	0.05	1.11	-	5	-
	0.1	1.14	1.32	6.9	6
	0.2	1.18	1.32	9.8	9.2
	0.3	1.22	1.38	12.4	10.9
	0.5	1.26	1.41	16.3	14.3
D	0.01	0.98	-	2.7	-
	0.02	1.01	-	4.1	-
	0.05	1.04	-	6.8	-
	0.1	1.07	1.22	9.8	9.5
	0.2	1.11	1.25	15	14.3
	0.3	1.15	1.28	17.8	17.1
	0.5	1.19	1.31	23.4	22.5
E	0.01	1.01	-	2.5	-
	0.02	1.04	-	3.3	-
	0.05	1.07	-	7.2	-
	0.1	1.1	1.25	8.9	8.4
	0.2	1.14	1.28	15.1	14.2
	0.3	1.18	1.31	17.8	17.4
	0.5	1.22	134	23.8	21.8

(C) Structural Effect:

The parameters α_n, $\Delta E^{1/2}/\Delta pH$ and I (diffusion current coefficient) lie practically in the same range for all the members of the reaction series (A-E). Hummet's linear free energy relations area applied to investigate the influence of the substituents on the cathodic peak potentials. Plots are drawn between the cathodic peak potential and Hammett substituent constant[23] and the specific reaction constant (σ) plot is linear. It is observed from the plot that the electron withdrawing substituent (σ is positive), viz., -Cl, -Br shift the $E_{1/2}$ towards more positive values. This suggests that the substituents do not affect the mechanism of the reaction but only make the reduction easy. The value of reaction constant (ρ = 0.3 volts) obtained presently is in good agreement with the values reported earlier in the literature [24].

(D) Millicoulometry:

The number of electrons involved in the reduction of 4-(substituted) acetophenone salicylylhydrazone is found to be four in Britton-Robinson buffers of pH containing 50% V/V. The millicoulometer of Devries and Kroon [13] with mercury pool cathode was employed for determining the value of n. The results are presented in Table 4.

Table-4: Millicoulometric data of acetophenone salicylyl hydrazone (1×10^{-3} M) at pH 4.1
Medium: Aqueous dimethylformamide (50%V/V)

pH	Current (µA)	Time (Sec)	n value (Sec)
4.1	6	0	-
	5.3	7200	3.6
	5	10800	3.7

(E) Effect of temperature:
The polarograms of 4-(substituted) acetophenone salicylyl hydrazone at pH 4.1 are recorded at 303, 313, 323, 333 K to study the effect of temperature on the limiting current and the half-wave potential and the results are presented in Table 5. The compounds exhibit well-defined single cathodic waves at all temperatures in the pH range of study. The linear plots of i_l versus $h^{1/2}$ suggest that the limiting current is diffusion controlled at all temperatures. The diffusion current increases with increase in the temperature and the temperature coefficient of the diffusion current lies between 1.0 to 1.6% per degree. These values are in good agreement with the values reported in the literature for other similar compounds by Meites [25]. Half-wave potential shifts to more negative value with rise in temperature and αn_a value with increase in temperature may be ascribed to the decrease in α value. A decrease in the α value indicates that the transfer of electrons is made increasingly difficult as the temperature is elevated. A decrease in αn_a values [26-29] suggests that the system is tending to become more irreversible. Literature survey reveals [30, 31] that similar observations were made for other organic compounds.

Table-5; Effect of temperature on the polarographic characteristics of A-E at pH 4.1
Concentration : 1×10-3 M
Medium : Aqueous dimethlformamide (50% V/V)

Compound No	Temperature	$-E_{1/2}$ V vs SCE	Limiting current (µA)	Temperature coefficient (% deg^{-1})	α_n	$DX10^6$ cm^2S^{-1}
A	303	0.85	6	-	0.61	1.88
	313	0.91	6.8	1.25	0.57	2.42
	323	0.97	7.8	1.37	0.54	3.17
	333	1.03	9	1.43	0.52	4.24
B	303	0.89	6.2	-	0.49	2.01
	313	0.96	7	1.21	0.47	2.56
	323	1.02	8	1.33	0.45	3.35
	333	1.07	9.2	1.39	0.43	4.43
C	303	0.95	6.7	-	0.47	2.35
	313	1.01	7.3	0.86	0.45	2.79
	323	1.08	8.1	1.04	0.43	3.42
	333	1.13	9.1	1.16	0.41	4.32
D	303	0.8	4.8	-	0.64	1.2
	313	0.85	5.4	1.18	0.61	1.53
	323	0.91	6.1	1.22	0.57	2.02
	333	0.98	7.1	1.36	0.54	2.72
E	303	0.79	4.2	-	0.52	0.92
	313	0.84	4.7	1.12	0.49	1.21
	323	0.9	5.3	1.2	0.47	1.64
	333	0.97	6	1.24	0.45	2.28

Mechanism:
Based on the results obtained, the following mechanism is suggested for the electro-reduction of 4-(substituted) acetophenone salicylyl hydrazone. The mechanism is consistent with the earlier mechanism suggested for the reduction of azomethine group [32].

The compounds under study are reduced at dropping mercury electrode (DME) through a mechanism which involves the azomethine group. The azomethine moiety in 4-(substituted) acetophenone salicylyl hydrazone is protonated to yield protonated hydrazone form.

Biological Screening:
Anti microbial activity:
The synthesized compounds were screened for their anti fungal and anti bacterial activities. The *in-vitro* anti microbial activities of the synthesized compounds was assessed against fungi and bacteria. The fungi used were C. albicans and A. fumigates. The bacteria used were S. aureus and E. coli.

Amphotericin B and Vancomycin were used as standards for comparison for anti fungal and anti bacterial activities respectively. The activities were determined by measuring the diameter of the inhibition zone in mm.

None of the compounds is suit able candidate for anti fungal and anti bacterial indication as shown in the Table 6.

Table-6: Anti microbial activity of compounds (A-E) – Zone of inhibition in mm

Compound	Concentration (µg/mL)	Anti fungal Test		Anti bacterial Test		Remark
		C. albicans ATCC 14503	*A. fumigates ATCC 16424*	*S. aureus 209P*	*E. coli ATCC 25922*	
A	100	-	-	-	-	1
	1000	-	14 hr	15 hr	-	2
B	100	-	-	-	-	1
	1000	-	13 hr	17 hr	-	2
C	100	-	-	9 hr	-	1
	1000	-	10 hr	16 hr	-	2
D	100	-	-	51 hr	-	3
	1000	-	12 hr	78 hr	-	3
E	100	-	-	48 hr	-	3
	1000	-	12 hr	16 hr	-	3

1. Inactive
2. Activity in both indication
3. Poor antibacterial spectrum

Anti-viral activity:
The antiviral activity of the compounds (A-E) was determined against Herpes Simplex Virus-2 by CPE inhibition assay, vero cells -African green monkey kidney cell line-ATCC CCL-81) were cultivated as mono layers in 5% carbon dioxide at 37^0C, in Dulbecco's modified Eagle medium (MEM) with 5% fetal bovine serum (FBS).

The diluted extracts (100g/mL) were transferred to the aspirated vero cell mono layers. Cultures were incubated at 37^0C for 60 minutes. 100µL of virus ($100TCID_{50}$) was added to each well. The tray was transferred to an environmental chamber (37^0C).

Cultures were inspected periodically for virus-induced cytopathic effect (viral CPE). Absence of CPE indicated complete inactivation of the virus. Partial inhibition was considered to be a negative result. Some of the synthesized compounds were found to b e active as shown in the Table 7.

Table-7: Antiviral activity of the compounds A-E
Standard Famiclovir (10µg/mL)

Compound	% CPE Inhibition
A	0-25%
B	0-25%
C	0-25%
D	0-25%
E	0-25%

Antioxidant activity:
The antioxidant activity if some of the synthesized compounds were determined by DPPH method using Trolox as a reference standard. Amongst the compounds screened for antioxidant activity, some of the synthesized compounds were found to be active as shown in Tables 8 and 9.

Table-8: Antioxidant activity of the compounds A-E
(Primary screening data of DPPH assay with test concentration 250µg/mL)

Compound	% AO activity
A	93.25
B	92.48
C	93.65
D	92.68
E	91.88

Table-9: Evaluation of primary positives to dose dependent DPPH assay

Compound	Test concentration (µg/mL)				
	125	62.5	31.25	15.625	7.8125
A	92.46	91.28	90.12	65.23	32.88
B	91.02	89.86	88.99	52.33	26.22
C	91.86	90.66	89.00	44.11	28.23
D	90.98	89.88	87.98	62.22	31.88
E	90.88	88.99	85.42	55.82	32.22

CONCLUSION

Electrochemical behavior:
4-(substituted) acetophenone salicylyl hydrazones undergo irreversible, diffusion controlled, substituent position dependent polarographic reduction at the dropping mercury electrode.

The effect of pH, concentration of the depolarizer, height of the mercury column, temperature on the polarographic behavior was presented.

The number of electrons involved in each step of the mechanism was evaluated and presented.

Microbial Screening
Some of the synthesized compounds showed poor antimicrobial activity. In primary screening of antiviral activity, the compounds were found to be active. Hence they were selected for the dose dependent study but they get precipitated at higher concentration. The compounds were also tested for antioxidant activity. In primary screening, the compounds were found to be active and were further tested for dose dependent study and were found active in dose dependent study.

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