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The Study of Electrochemical Reduction of 4-Amino-1-[(2R, 5S)-2-(Hydroxymethyl)-1,3-Oxathiolan-5-yl] Pyrimidin-2-one (an Anti -Retro Viral Drug-Lamivudine) at Dropping Mercury Electrode

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Abstract: The electrochemical behavior of Lamivudine (an anti-retro viral drug) has been investigated at dropping mercury electrode. The compound exhibit one well defined irreversible wave. The reduction was diffusion controlled as evidenced from linear plots of i_d against $h^{1/2}$. The mechanism of reduction and effect of concentration, pH, different buffers and temperature has been studied. Slope values indicate irreversible nature of wave so the kinetic parameters (K^0_{fh}, αn) has been evaluated using Meites-Israel and Gaur-Bhargava's method. Thermodynamic parameters (ΔH_p, ΔH_v, ΔG, ΔS) have been determined between 15^0C and 40^0C.

Key words- Lamivudine, Antiretroviral (ARV), D.C. Polarography, Kinetic parameters.

Introduction

Lamivudine is a synthetic nucleoside analogue with activity against HIV-1 and HBV[1-4]. Lamivudine is the negative enatiomer of a dideoxy analogue of cytidine[5]. Lamivudine has been used for the treatment of chronic hepatitis-B at a lower dose than for treatment of HIV. Lamivudine was invented by Bernard Belleau while at work at McGill University and Nghe Nguyen-Ga at the Montreal-based IAF BioChem International, Inc. laboratories in 1989. Initially designed as an antiviral agent, the drug's effectiveness for treating HIV in combination with AZT was discovered by Yung-Chi (Tommy) Cheng at Yale University.

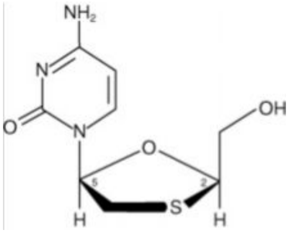
Lamivudine was approved by the Food and Drug Administration (FDA) on November 17, 1995 for use with zidovudine (AZT) and again in 2002 as a once-a-day dosed medication. The fifth antiretroviral drug on the market, it was the last NRTI for three years while the approval process switched to protease inhibitors.

Mechanism of Action: Intracellularly, lamivudine is phosphorylated to its active 5′-triphosphate metabolite, lamivudine triphosphate (3TC-TP). The principal mode of action of 3TC-TP is the inhibition of HIV-1 reverse transcriptase (RT) via DNA chain termination after incorporation of the nucleotide analogue into viral DNA. 3TC-TP is a weak inhibitor of mammalian DNA polymerases α, β, and γ[6]. Lamivudine is more effective against disease when given in combination with zidovudine[7].Lamivudine has very low cellular cytotoxicity. It is rapidly absorbed with bioavailability of 80%[8,9].

The drug is officially listed in Martindale, the extra pharmacopoeia[10].

IUPAC Name: 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2- one
Molecular formula: $C_8H_{11}N_3O_3S$
Molecular mass: 229.26 g/mol.
Appearance: White to off white crystalline solid.
Solubility in water: 70mg/mL at 20°C.
CAS Number: 134678-17-4

Structure:



Lamivudine

Till date several analytical methods that have been reported for the estimation of Lamivudine, such as high performance liquid chromatography[11-14], spectrophotometry[15-18], attenuated total reflectance-IR spectroscopy (ATR-IR)[19], high performance thin layer chromatography(HPTLC) [20-22], LC-MS-MS[23,24], micellar electrokinetic chromatography method (MEKC) [25], titrimetric[26], capillary zone electrophoresis[27]. Voltammetric study of Lamivudine carried out by many workers[28,29]. The objective of this study was to evaluate Lamivudine by polarography.

Apparatus

A model CL 357 (Elico Ltd.) polarograph and the cell described by Meites[30] were used for the polarographic measurement. The three electrode system was completed using a working electrode (dropping mercury electrode, D.M.E.), reference electrode (saturated calomel electrode) and counter electrode (platinum electrode). A polarographic capillary 120 mm long and 0.05 mm in diameter was used.

A digital pH meter model 111 E was used for measuring the pH of the analytes.

Reagents

Solution of Lamivudine drug: A stock solution of 0.1M Lamivudine was prepared in double distilled water and stored in a dark glass bottle at 4^0C. Solution prepared freshly after 7 days.

Lamivudine was obtained from Rajasthan Drugs Pharmaceuticals Ltd.

Supporting electrolyte: Supporting electrolytes were prepared in double distilled water as and when required.

All the solutions were prepared from double distilled water and analytical reagent grade chemicals (MERCK).

Procedure

The working procedure for D.C. polarography was as follows:

A 10 ml of experimental solution was placed in a polarographic cell and deoxygenated with ultra pure nitrogen which had been passed through acidified vanadous chloride solution for 5 minutes. The cell was placed in the thermostat and the capillary was inserted in solution. The current values were measured at various applied voltages.

The potential was applied to the working electrode with 150 mV/min span rate and 100 nA/div. sensitivity of current measurement.

Result and Discussion

Electrochemical reduction of Lamivudine has been studies in different supporting electrolytes in aqueous medium. Reduction of ketonic group in these media gave one well defined wave.

As buffer medium is essential for reduction process of organic compounds because most of the reductions of organic compounds are dependent on the pH of the solution[31], so in present study supporting electrolytes used are buffers.

The reduction was found to be diffusion controlled [the low value of temperature coefficient (below 2%K^{-1}) and the direct proportionality observed for i_d versus concentration and i_d versus $h^{1/2}$.] [32]

Linear plots were obtained for log i/i_d-I versus E_{de} with various slope values. Slope values are indicated irreversible nature of wave (plot of E_{de} versus log i/id-i is linear and slope have a value more than 30 mV for 2 electron reduction system[33]). Reduction process of most carbonyl compounds is irreversible[34]. Hence for irreversible wave kinetic parameters were calculated from Meites-Israil method as well as by Gaur-Bhargava's method. Results by both methods are in close agreement with each other.

The value of diffusion coefficient (D_o) has been determined by Ilkovic equation[35]:

$$I_d = 607\, n\, D^{1/2}\, m^{2/3} t^{1/6}\, c$$

Where n = number of electrons transferred in the process,

m = rate of mercury flow in mg/sec,

D = diffusion constant of depolarizer in cm^2/s,

t = drop time in s,

c = depolarizer concentration in millimoles/litre,

I_d = diffusion current in micro amperes.

The value of heterogeneous rate constant ($k^o_{f,h}$) has been evaluated by Meites-Israel equation[36]:

$$-E_{1/2} = \frac{0.05915}{\alpha n} \log \frac{1.349\, k^o_{f,h}\, t^{1/2}}{D^{1/2}}$$

Where αn = product of transfer coefficient (α) and number of electrons transferred in the rate determining step.

Meites Israel has extended the Koutecky's graphical method into comparatively more precise mathematical form. Further, Gaur-Bhargava has also extended the Koutecky's treatment for irreversible wave, since according to them the diffusion to the electrode surface (mercury drop) is spherical and not a linear one as assumed by Meites and Israel.

Gaur Bhargava's modification[37]:

$$-E_{1/2} = \frac{0.05915}{\alpha n} \log \frac{1.349\, k^{o}_{f,h}\, t^{1/2}}{(\text{antilog } c)D^{1/2}}$$

Electrochemical reduction of Lamivudine:

Reduction of Lamivudine at DME in Acetate buffer at pH 3.5 and 4.5:

Electrochemical reduction of Lamivudine was carried out in Acetate buffer (0.01M) at pH 3.5 and 4.5. The concentration of drug varies from 2mM to 4mM as this concentration range is most suitable for polarographic analysis[38]. The single irreversible wave [value of slope of E_{de} v/s log(i/id-i) varies from 87-109 mV for various concentration] was obtained. The value of $E_{1/2}$ irreversible varies from 1.47 V to 1.44 V. This reduction potential value is in close agreement with previous workers findings[39,40]. Shifting of the values of $E_{1/2}$ to less negative potential with increase in concentration confirmed the irreversible nature of wave[41]. The value of K^{o}_{fh} decreases with increase in concentration. A well defined irreversible and diffusion controlled wave for Lamivudine was observed in 0.01M Acetate buffer at pH 3.5 & 4.5. The value of diffusion current (I_d) is linear with concentration from 0.2mM to0.8mM. Value of regression coefficient (R^2) for linearity is 0.9902. The forward rate constant (K^{o}_{fh}) values for the compound studied was found to decrease with increase in pH. This trend shows that the electrode process becomes more and more irreversible with increase in pH of the solution; rate constant values are observed to be high in acidic media in general in Lamivudine indicating that the rate of the reaction was fast in this media because of the protonation[42].

Table: 1 Electrochemical reduction of Lamivudine in Acetate buffer pH 3.5 & 4.5

S.No.	pH	Drug Concentration (mM)	$E_{1/2}$ (V)	I_d (μA)	D_o (cm^2/s)	$K^{o}_{f,h}$ (ME Method) (cm/s)	$K^{o}_{f,h}$ (GB Method) (cm/s)
1.	3.5	0.2	-1.4723	3.6	91.33	1.06398^{-13}	1.43597^{-13}
2.		0.4	-1.4506	4.3	32.58	5.65665^{-14}	$7.63786E^{-14}$
3.		0.6	-1.4472	5.2	21.17	3.52219^{-15}	4.758^{-15}
4.		0.8	-1.4424	6.3	17.48	4.11282^{-17}	5.55842^{-17}
5.	4.5	0.2	-1.4882	3.3	76.75	1.17862^{-16}	1.59069^{-16}
6.		0.4	-1.4882	4.5	35.68	2.39247^{-17}	3.23041^{-17}
7.		0.6	-1.4837	6.0	28.19	3.42977^{-19}	4.63316^{-19}
8.		0.8	-1.4794	7.1	22.20	6.9348^{-19}	9.37229^{-19}

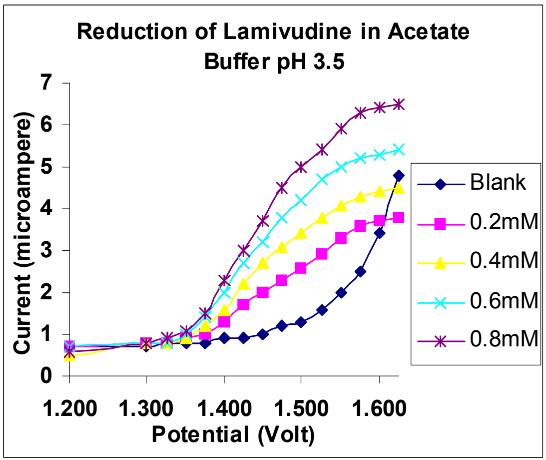


Fig 2: Reduction of Lamivudine in Acetate Buffer pH 3.5

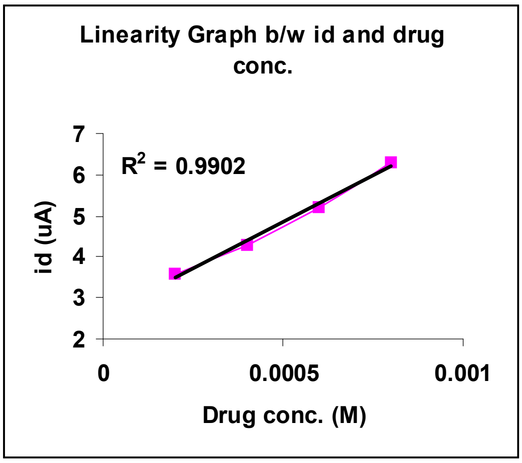


Fig 3: Linearity graph between reduction current and drug concentration

Reduction of Lamivudine at DME in Ammonium Acetate buffer at pH 8.0 and 10.0:

Electrochemical reduction of Lamivudine was carried out in Ammonium acetate buffer (0.01M) at pH 8.0 and 10.0. All of observations (effect and cause) are same as above.

Table: 2 Electrochemical reduction of Lamivudine in Ammonium Acetate buffer pH 8.0 & 10.0

S.No.	pH	Drug Concentration (mM)	E ½ (V)	I_d (µA)	D_o (cm^2/s)	$K^{o}_{f,h}$ (ME Method) (cm/s)	$K^{o}_{f,h}$ (GB Method) (cm/s)
1.	8.0	0.2	-1.7185	2.8	55.251	1.55256^{-17}	2.09537^{-17}
2.		0.4	-1.7137	3.8	25.441	4.52852^{-23}	6.1146^{-23}
3.		0.6	-1.7072	5.1	20.367	2.39348^{-26}	3.23327^{-26}
4.		0.8	-1.7082	6.2	16.931	2.67424^{-31}	3.61421^{-31}
5.	10.0	0.2	-1.7979	3.6	91.33	3.35737^{-19}	4.53118^{-19}
6.		0.4	-1.7906	5.8	59.27	3.82024^{-20}	5.15825^{-20}
7.		0.6	-1.7877	8.0	50.11	1.33796^{-20}	1.80741^{-20}
8.		0.8	-1.7821	9.9	43.17	4.86105^{-21}	6.56964^{-21}

Reduction of Lamivudine at DME in Britton Robinson (B.R.) buffer at pH 2.87, 5.0 and 7.2:

Electrochemical reduction of Lamivudine was carried out in B.R. buffer (0.01M) at pH 2.87, 5.0 and 7.2. All of observations (effect and cause) are same as above.

Table: 3 Electrochemical reduction of Lamivudine in B.R. buffer (0.01M) at pH 2.87, 5.0 and 7.2

S.No.	pH	Drug Concentration (mM)	E ½ (V)	I_d (µA)	D_o (cm^2/s)	$K^{o}_{f,h}$ (ME Method) (cm/s)	$K^{o}_{f,h}$ (GB Method) (cm/s)
1.	2.85	0.2	-1.4717	6.0	253.70	5.52527^{-9}	7.45702^{-9}
2.		0.4	-1.4551	6.5	74.44	2.53657^{-9}	3.42499^{-9}
3.		0.6	-1.4440	7.2	40.59	1.7101^{-9}	2.31012^{-9}
4.		0.8	-1.4449	7.6	25.44	1.47422^{-10}	1.99239^{-10}
5.	5.0	0.2	-1.5634	3.8	101.76	2.08939^{-7}	2.81989^{-7}
6.		0.4	-1.5455	4.5	35.68	1.50377^{-8}	2.03045^{-8}
7.		0.6	-1.5418	6.1	29.14	1.25695^{-10}	1.69797^{-10}
8.		0.8	-1.5379	6.2	16.93	3.81277^{-12}	5.15291^{-12}
9.	7.2	0.2	-1.7310	3.5	86.33	9.73778^{-12}	1.31423^{-11}
10.		0.4	-1.7300	5.0	44.05	8.70297^{-14}	1.17511^{-13}
11.		0.6	-1.7292	6.1	29.14	2.51623^{-14}	3.39909^{-14}
12.		0.8	-1.7276	7.3	23.47	5.12743^{-15}	6.92966^{-15}

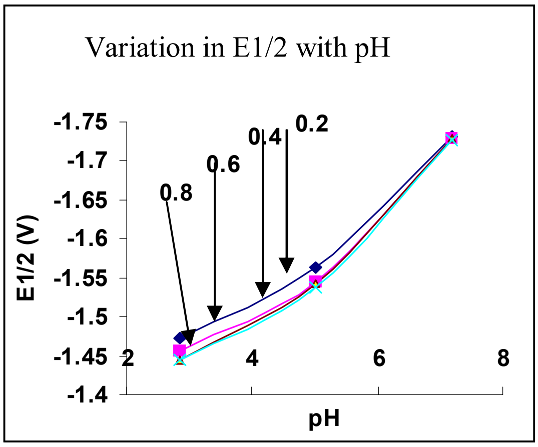


Fig 4: Variation of E1/2 with pH on concentrate on 0.2mM, 0.4mM, 0.6mM and 0.8mM

Reduction of Lamivudine at DME in Acetate Buffer (pH-3.5) at various Temperatures*:*

A gradual change in diffusion current and half wave potential was observed when the solution temperature was increased from 15^0C to 40^0C.

The value of K^0_{fh} at various experimental conditions comes out to be of the order of $10^{-9\pm2}$which indicates irreversible nature of reaction. $E_{1/2}$ shifted towards less negative potential confirms irreversible nature of reduction[43]. The value of αn decreases with increase in temperature (Table- 4). A decrease in value of αn implies that transfer of electrons becomes difficult as temperature was elevated[44]. Further the values of K^0_{fh} increases with increase in temperature which suggests that irrereversibility decrease with increase in temperature, this implies that reduction products of drug are stable at lower temperature. In other words the electrode reaction was rendered more irreversible at lower temperature. By above data thermodynamic parameters were calculated. (Table 5)

The positive values of ΔH indicate endothermic nature of reaction, sufficiently large negative values of ΔG showed that reaction is spontaneous[45]. Reaction is entropically favorable as values of ΔS are positive[46].

Conclusions

The electrochemical behavior of Lamivudine at the mercury electrode was studied and discussed. Drug reduced at electrode due to reduction of C=O group in the structure. Half wave potential (E ½) varies from -1.42V to -1.75V, which is in close agreement with previous workers[39,40].

The value of K^0_{fh} at various experimental conditions comes out to be of the order of 10^{-9} to 10^{-21} which indicates irreversible nature of reaction. The values of K^0_{fh} decreases with increase in pH which suggests that irrereversibility increase with increase in pH, this implies that reduction products of drug are stable in alkaline medium. The value of αn decreases with increase in temperature. A decrease in value of αn implies that transfer of electrons becomes difficult as temperature was elevated. Further the values of K^0_{fh} increases with increase in temperature which suggests that irrereversibility decrease with increase in temperature. In other words the electrode reaction was rendered more irreversible at higher temperature.

Table: 4 Electrochemical Reduction of Lamivudine at DME in Acetate Buffer (pH-3.5) at various Temperatures:

S.No.	Temp (K)	Drug Concentration (mM)	E ½ (V)	αn	I_d (μA)	D_o (cm^2/s)	$K^o_{f,h}$ (ME Method) (cm/s)	$K^o_{f,h}$ (GB Method) (cm/s)
1.	293.15	0.6	-1.4433	0.4388	6.6	34.1092	8.85998E-11	1.19686E-10
2.	298.15	0.6	-1.4441	0.4378	7.1	39.4730	9.91614E-11	1.33954E-10
3.	303.15	0.6	-1.4444	0.4234	7.8	47.6402	2.38369E-10	3.22005E-10
4.	308.15	0.6	-1.4452	0.4068	8.0	50.1146	5.98194E-10	8.0808E-10
5.	313.15	0.6	-1.4461	0.3876	8.5	56.5746	1.79168E-09	2.42031E-09

Table 5: Thermodynamic parameters:

S.No.	Temperature (K)	ΔH_p Kcal/mole	ΔH_V Kcal/mole	ΔS Cal/degree/mole	ΔG Kcal/mole
1.	293.15	148.98	146.54	625.75	-36.9
2.	298.15	148.98	146.50	616.54	-37.4
3.	303.15	148.98	146.46	605.54	-37.1
4.	308.15	148.98	146.42	594.30	-36.7
5.	313.15	148.98	146.37	582.68	-36.1

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