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RESEARCH ARTICLE

ANALYTICAL CHEMISTRY

THE POLAROGRAPHIC REDUCTION AND ELECTRODE KINETICS OF ANTIRETROVIRAL DRUG ZIDOVUDINE

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ABSTRACT

The polarographic reduction of Zidovudine has been done in various conditions, at different temperatures, at different pH and at different concentrations by D. C. Polarography. The system is irreversible and exhibit diffusion controlled reduction. Single well defined irreversible wave obtained. So the kinetic parameters (K^0_{fh}, αn) has been evaluated using Meites-Israel and Gaur-Bhargava's method.

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KEY WORD

Zidovudine, Antiretroviral (ARV), D.C. Polarography, Kinetic parameters,

INTRODUCTION

Zidovudine (ZDV) or Azidothymidine (AZT) is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug. Zidovudine was the first approved treatment for HIV (Human Immuno Deficiency Virus) disease. It is an analog of thymidine.

Zidovudine was a major breakthrough in AIDS (Acquired Immuno Deficiency Syndrome) therapy in the 1990s that significantly altered the course of the illness and human being as in early 90s that AIDS was an instant death sentence.

Zidovudine was the first drug approved for the treatment of AIDS and HIV infection. Zerome Horwitz of Barbara Ann Karmanos Cancer Institute and J. Chua and M. Noel of Wayne State University School of Medicine first synthesized AZT in 1964[1]. AZT was originally intended as an anticancer drug, but was shelved after it proved insufficiently effective against tumors in mice.

In 1974 W. Ostertag from the Max Plank Institute, Germany proved that AZT was active in a mouse retrovirus culture system[2].

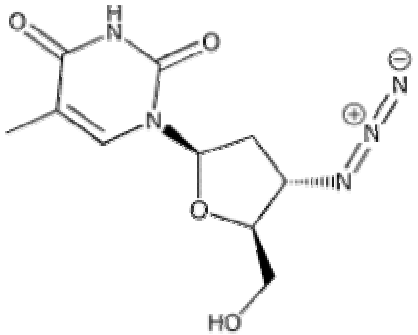
Burroughs Wellcome Co. filed for a patent on AZT in 1985. The Food and Drug Administration (FDA) approved the drug (via the then-new FDA accelerated approval system) for use against

AZT is combined with other drugs in order to prevent mutation of HIV into an AZT-resistant form[3-4].

AZT works by inhibiting the action of reverse transcriptase, the enzyme that HIV uses to make a DNA copy of its RNA. Reverse transcription is necessary for production of the viral double-stranded DNA, which is subsequently integrated into the genetic material of the infected cell[5-6].

IUPAC Name: 1-[(2R,4S,5S)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione.

Molecular formula : $C_{10}H_{13}N_5O_4$
Molecular mass : 267.242 g/mol.
CAS Number : 30516- 87-1
Structure :



Zidovudine

Experimental

Apparatus

The D.C. recording polarograph (CL 357) of Elico Ltd. was used for study. The three electrode system was completed using a working 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

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

Zidovudine was obtained from Rajasthan Drugs Pharmaceuticals Ltd.

Zidovudine solution was prepared freshly every 7 days.

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 nitrogen for 15 min. The cell was placed in the thermostat and the capillary was inserted in solution. The current were measured at various applied voltage.

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 Zidovudine has been studies in different supporting electrolytes in aqueous medium. Reduction of azide group in these media gave one well defined wave.

The reduction was found to be diffusion controlled [the low value of temperature coefficient (below2%K^{-1}) and the direct proportionality observed for i_d versus concentration and i_d versus $h^{1/2}$.]

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. 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 agreement with each other.

The value of diffusion coefficient ($D^{1/2}$) has been determined by Ilkovic equation[7] $I_d = 607\ n\ D^{1/2}\ M^{2/3}T^{1/6}\ C$

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[8]:

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

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[9]:

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

Polarographic reduction of Zidovudine in different supporting electrolytes:

1.0 Reduction of Zidovudine at DME in Ammonium Chloride buffer at pH 9.4:

A well defined irreversible and diffusion controlled wave for Zidovudine was observed in 0.01M ammonium chloride buffer at pH 9.4. The value of $E_{1/2}^{irreversible}$ for Zidovudine was found to be -1.0050 to -1.1068 volts (at various concentrations) vs. SCE.

With increase in concentration of Zidovudine, $E_{1/2}$ shifted to more negative potential confirmed the irreversible nature of wave[10]. The value of I_d is linear with concentration from 0.0001M to 0.0009M. Value of R^2 for linearity is 0.9976.

Table: 1

Electrochemical reduction of Zidovudine in Ammonium acetate buffer

S.No.	Drug Concentration (mM)	E ½ (V)	I_d (µA)	$D_o^{1/2}$ (cm^2/s)	$K^o_{f,h}$ (MI Method) (cm/s)	$K^o_{f,h}$ (GB Method) (cm/s)
1.	0.1	-1.0050	3.4	20.7870	$3.4424x10^{-7}$	$4.6449x10^{-7}$
2.	0.2	-1.0380	3.9	11.9220	$9.3188x10^{-9}$	$1.2576x10^{-9}$
3.	0.3	-1.0481	4.4	8.9669	$1.4652x10^{-10}$	$1.9780x10^{-10}$
4.	0.4	-1.0689	5.4	8.2537	$6.7479x10^{-9}$	$9.1113x10^{-9}$
5.	0.5	-1.0765	6.0	7.3366	$1.0192x10^{-9}$	$1.3766x10^{-9}$
6.	0.7	-1.0777	7.3	6.3758	$1.7453x10^{-10}$	$1.3582x10^{-10}$
7.	0.9	-1.1068	8.8	5.9780	$3.7851x10^{-11}$	$5.1167x10^{-11}$

2.0 Reduction of Zidovudine at DME in Acetate buffer at pH 4.5:

A well defined irreversible and diffusion controlled wave for Zidovudine was observed in 0.01M Acetate buffer at pH 4.5. The value of $E_{1/2}^{irreversible}$ for Zidovudine was found to be -0.9461 to -1.0204 volts (at various concentrations) vs. SCE.

With increase in concentration $E_{1/2}$ shifted to more negative potential. The value of I_d is linear with concentration from 0.0002M to 0.0008M. Value of R^2 for linearity is 1.0.

Table: 2

Electrochemical reduction of Zidovudine in Acetate buffer

S.No.	Drug Concentration (mM)	E ½ (V)	I_d (µA)	$D_o^{1/2}$ (cm^2/s)	$K^o_{f,h}$ (MI Method) (cm/s)	$K^o_{f,h}$ (GB Method) (cm/s)
1.	0.2	-0.9461	3.8	11.6163	$1.3721x10^{-11}$	$1.8518x10^{-11}$
2.	0.4	-0.9532	5.2	7.9480	$1.4626x10^{-13}$	$1.9748x10^{-13}$
3.	0.6	-0.9850	6.8	6.9290	$1.4940x10^{-15}$	$1.0182x10^{-15}$
4.	0.8	-1.0204	8.3	6.3431	$1.0282x10^{-17}$	$1.3896x10^{-17}$

3.0 Reduction of Zidovudine at DME in B.R. Buffer at various pH :

A well defined irreversible and diffusion controlled wave for Zidovudine was observed in 0.01M B.R. buffer at pH 1.4, 3, 5 and 7. The shift in $E_{1/2}^{irreversible}$ towards more negative potential with increase in pH clearly shows the participation of protons in the reduction process. The value of I_d is linear with concentration from 0.0002M to 0.0008M.

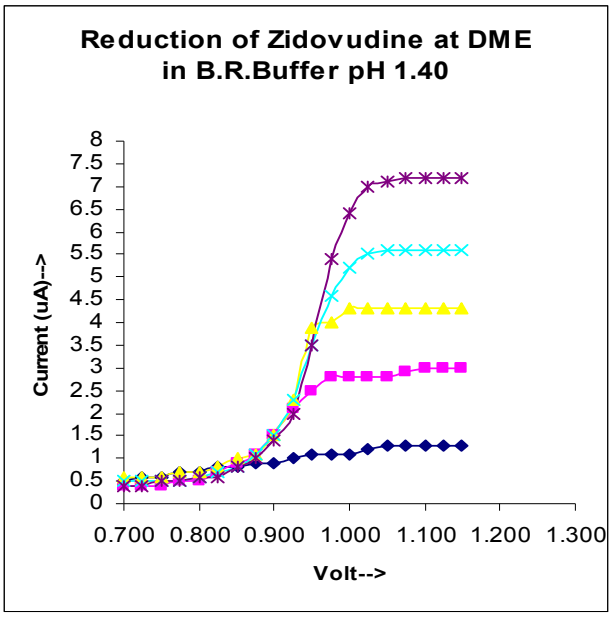
Table: 3

Electrochemical reduction of Zidovudine in BR buffer at various pH

S.No.	pH	Drug Concentration (mM)	$E_{½}$ (V)	I_d (µA)	$D_o^{1/2}$ (cm^2/s)	$K^o_{f,h}$ (MI Method) (cm/s)	$K^o_{f,h}$ (GB Method) (cm/s)
1.	1.40	0.2	-0.8937	2.8	7.4331	$3.3389x10^{-11}$	$4.5063x10^{-11}$
2.		0.4	-0.9077	4.3	5.7076	$3.0956x10^{-13}$	$4.1798x10^{-13}$
3.		0.6	-0.9266	5.2	4.6015	$1.9710x10^{-13}$	$2.6626x10^{-13}$
4.		0.8	-0.9403	7.2	4.7784	$9.5553x10^{-15}$	$1.2914x10^{-14}$
5.	3.0	0.2	-0.9125	2.7	7.1677	$2.6898x10^{-14}$	$3.6312x10^{-14}$
6.		0.4	-0.9292	4.2	5.5748	$1.7908x10^{-15}$	$2.4180x10^{-15}$
7.		0.6	-0.9381	5.6	4.9554	$4.7570x10^{-16}$	$6.4260x10^{-16}$
8.		0.8	-0.9483	7.5	4.9775	$1.1226x10^{-16}$	$1.5172x10^{-16}$
9.	5.0	0.2	-0.9343	3.3	8.7605	$9.8361x10^{-13}$	$1.3275x10^{-12}$
10.		0.4	-0.9426	4.8	6.3712	$1.2402x10^{-15}$	$1.6746x10^{-15}$
11.		0.6	-0.9561	6.5	5.7518	$6.6168x10^{-15}$	$8.9383x10^{-15}$
12.		0.8	-0.9714	8.3	5.5085	$9.1716x10^{-15}$	$1.2395x10^{-14}$
13.	7.0	0.2	-1.0132	3.0	7.9641	$7.4188x10^{-10}$	$1.0013x10^{-9}$
14.		0.4	-1.0391	4.3	5.7076	$3.7370x10^{-10}$	$5.0458x10^{-10}$
15.		0.6	-1.0639	5.6	4.9554	$3.4966x10^{-10}$	$4.7234x10^{-10}$
16.		0.8	-1.0798	7.9	5.2430	$4.8633x10^{-10}$	$6.5726x10^{-10}$

Fig.1

Reduction of Zidovudine (various concentration) at DME in B.R. Buffer pH 1.40



4.0 Reduction of Zidovudine at DME in B.R. Buffer (pH-5.0) 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 50^0C. (Table-4)

Table: 4

Electrochemical Reduction of Zidovudine at DME in B.R. Buffer (pH-5.0) at various Temperatures

S.No.	Temp (K)	Drug Concentration (mM)	$E_{½}$ (V)	αn	I_d (µA)	$D_o^{1/2}$ (cm^2/s)	$K^o_{f,h}$ (MI Method) (cm/s)	$K^o_{f,h}$ (GB Method) (cm/s)
1.	288	0.8	-1.0106	0.9992	5.6	3.5189	$1.16599x10^{-17}$	$1.57582x10^{-17}$
2.	292	0.8	-1.0128	0.8244	6.6	4.1473	$9.32783x10^{-14}$	$1.26064x10^{-14}$
3.	297	0.8	-1.0060	0.7732	7.1	4.4615	$1.41604x10^{-14}$	$1.91376x10^{-14}$
4.	302	0.8	-1.0082	0.7422	7.6	4.7756	$6.65813x10^{-13}$	$8.99838x10^{-13}$
5.	307	0.8	-1.0066	0.7302	8.0	5.0270	$4.59918x10^{-13}$	$6.21573x10^{-13}$
6.	312	0.8	-1.0090	0.7196	8.4	5.2783	$1.09343x10^{-12}$	$1.47776x10^{-12}$
7.	317	0.8	-1.0049	0.6951	8.6	5.4040	$3.2771x10^{-12}$	$4.42896x10^{-12}$
8.	322	0.8	-1.0082	0.6706	8.8	5.5279	$8.00011x10^{-12}$	$1.08121x10^{-11}$

The value of K^0_{fh} at various experimental conditions comes out to be of the order of $10^{-13\pm4}$which indicates irreversible nature of reaction. 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[11]. 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 higher temperature.

CONCLUSIONS

The value of K^0_{fh} at various experimental conditions comes out to be of the order of $10^{-13\pm4}$which indicates irreversible nature of reaction. The values of K^0_{fh} increases with increase in pH which suggests that irrereversibility decrease with increase in pH, this implies that reduction products of drug are stable in acidic medium. 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[11]. 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 higher temperature.

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