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Bioinorganic studies on Fe (II)- zidovudine (azt) complex

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The anti-HIV drug 3'-Azido-3'-deoxythymidine (Zidovudine) AZT has been quantitatively and qualitatively analysed by direct current polarographic (DCP), differential pulse polarographic (DPP) and amperometric techniques.

AZT gives a well-defined polarographic reduction wave/peak with $E_{1/2}$/*E*p = - 1.42/ - 1.44 V versus *SCE* in 0.2 M KCL at *p*H = 6.0 ± 0.1. Fe (II)-AZT complex has been studied both in solid and aqueous phases. The IR spectral data on the drug and its Fe (II)-complex revealed complexation through - N atom of azide group. The data showed a shift in the band due to azide group in the complex from 2170 to 2150 cm^{-1} in case of pure drug zidovudine. Hence, a tentative structure of the complex has been suggested. Antibacterial activity of the complex has been determined using Raper's paper disc method against *Escherichia coli, Salmonella typhi, Vibrio cholerae,* and *Diplococcus pneumoniae* bacteria. Looking at its increased inhibition power against the above test pathogens, it is presumed that the complex may prove to be more potent against HIV, as compared to AZT drug.

Keywords: Bioinorganic, Anti-HIV drug, Polarography, Amperometric

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A survey of relevant literature reveals that Fe(II) is the major constituent of many enzymes involved in the metabolism of DNA and RNA[1]. Recently in our - laboratory some antibiotic, antidiabetic, antiseptic and antihistamic[2] drug metal complexes have been synthesized and their modes for biological effectiveness has been explained. Looking at the importance of role of metal lions in biological system[3-12], attempts have been made to evaluate biological properties of antiAIDS drugs as a result of their interaction with life essential metal ions. The present investigation deals with the bio-inorganic study on Fe (II)-Zidovudine complexation. Change in the biological properties of pure AZT has been evaluated and the possible role of Fe (II) in the anti-AIDS activity has been discussed in the present paper.

Experimental Procedure

All the chemicals used were of analytical grade and were used without further purification. Sigma Laboratory, USA, supplied the anti-AIDS drug, AZT. Standard solution of Fe (II) (0.01 M), AZT (0.01 M) and potassium chloride (2 M) were prepared in conductivity water.

Electrochemical study

All the polarograms were recorded on an Elico (India) DC Polarograph model CL357, comprising of a three-electrode system, a dropping mercury electrode (dme) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a coiled platinum wire as an auxiliary electrode. Amperometric titrations on Fe (II)-AZT system were performed on a manually operated assembly, comprising of an AJCO vernier potentiometer along with a polyflux galvonometer (sensitivity 8.10×10^{-9} amp/div). A dropping mercury electrode and a saturated calomel electrodes, were used as working and reference electrode respectively. *p*H measurements were carried out on an Elico digital *p*H-meter (model Li-120). All measurements were performed at *p*H = 6.0±0.1, adjusted with dil HCI /NaOH solution. The ionic strength of μ = 0.2 M was adjusted with potassium chloride (in polarographic and amperometric studies on AZT system). All the measurements have been carried out at room temperature i.e. 30±0.1°C.

Polarographic study on Fe (II)-AZT complex equilibrium

Sets of solution containing overall concentrations of Fe (II) fixed at 1 mM in 0.2 M KCI with varying concentration of AZT from 0.5 to 15 mM, were prepared. The *p*H of these solutions was adjusted to 6.0±0.1 as discussed earlier. Hydrogen gas was bubbled through the test solution for about 15 min before recording the polarograms.

Amperometric study on Fe(II)-AZT complex equilibrium

For amperometric titrations, sets of solution containing varying concentration of Fe (II) (over all concentration, 0.5 mm, 1.0 mm, 1.5 mm) in 0.2 M KCI as supporting electrolyte were prepared and the *p*H was adjusted to 6.0±0.1, using dil HCl/NaOH. The plateau potential for Fe (II) i.e. -1.0 V versus SEC

was fixed on the potentiometer. The titrations were performed using AZT solution (*p*H= 6.0±0.l) as titrant.

Synthesis of solid complex

A pinkish brown coloured complex was synthesized by refluxing aqueous solution of Fe(II) and AZT in 1:1 molar ratio for about 3 h. The complex was marked by precipitation after reducing the volume of reaction mixture to one fourth of the original volume. The product was filtered, washed, dried over P_4O_{10} and characterised.

Spectrometric measurements

The IR spectrophotometeric analysis was performed in solution phase using Perkin-Elmer 379, IR spectrophotometer.

Antimicrobial screening

Raper's method[13] was followed for the microbial screening of the complex against various pathogenic bacteria *viz. Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, and *Diplococcus pneumoniae*. Number of replicates in each of the cases was three. The percentage inhibition was calculated using the formula, % Inhibition = $a–b/a \times 100$

where a = diameter of inhibition zone for control (AZT) and b = diameter of inhibition zone for complex.

Results and Discussion

Polarographic behaviour of zidovudine (AZT)

In 0.2 M KCI, at *p*H = 6.0±0.l, AZT produced a well-defined polarographic signal in DCP/DPP modes with half wave potential $E_{l/2}$ = -1.42V versus SCE and peak potential E_p = -1.44V versus SCE and i_d/i_p was found to be proportional to the AZT conc. under the above-mentioned conditions. Zidovudine is sold in the market as Retrovir (100 mg caps), which is soluble in distilled water. Method of standard addition[14] was used to determine the AZT content of Retrovir powder. Its manufacturer Burroughs Wellcome, India within 0.01% error limit in agreement with that claimed the observed polarographic results. Thus enabling the quality control of AZT in pharmaceutical formulation.

Polarographic behaviour of Fe(II)-AZT complex

In 0.2 M KCI at *p*H 6.0±0.l, Fe(II) and its complex with AZT, produced a well defined reversible and diffusion controlled polarographic wave which was revealed by the log plot slope and the plots of i_d versus $\sqrt{h}$ (effective height of mercury column) respectively. On gradual addition of the ligand, the $E_{l/2}$ of the metal shifted to more electronegative value, indicating the formation of Fe(II)-AZT complex. Lingane's, treatment of the observed polarographic data revealed 1:1 [Fe(II)-AZT] complex formation in solution with log $ß_1 = 4.48$.

Amperometric titration of AZT with Fe (II) ion

On gradual addition of Fe(II) ion to the AZT analyte solution, the current goes on decreasing to minimum and then attends a constant value. The plot of i_d $(V + v/V)$ versus volume of titrant added for the said titrant, revealed a L-shaped curve. The end-point was indicated by the intersection of the two lines, which confirmed 1:1, M:L complex formation.

Characterisation of [Fe (II)-AZT] solid complex

Colour: Pinkish brown

Solubility: Highly soluble in water

Elemental analysis

Percentage of C, H, N, O and Fe, found and calculated in complex and AZT drug are summarized in Table 1.

Spectrophotometric analysis

A critical comparision of the IR data of AZT and its complex with Fe(II) shows that the complexation takes place through the 3` position of the drug AZT molecule. IR studies on Fe(II)-Zidovudine complex show that the band due to azide group is shifted to 2150 cm^{-1} from 2170 cm^{-1}, which reveals that coordination is through -N atom of the azide group (Table 2).

On the basis of analytical data, polarographic, amperometric studies and IR spectra, a tentative structure to the Fe(II)-AZT complex may be assigned as shown in Fig. 1.

Table 1 — Elemental analysis (%)

	Complex calculated/(found)	Drug
M [Fe (II)]	9.17 (9.01)	-
C	40.78 (40.09)	44.90
H	4.41 (5.01)	4.86
N	23.79 (23.60)	26.19
O	21.85 (22.29)	24.03

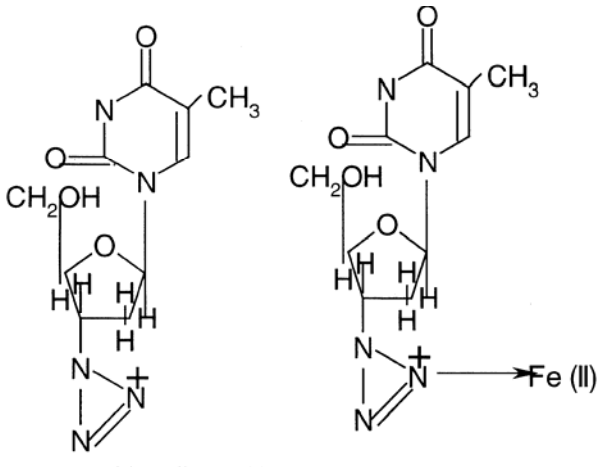

Fig. 1— Structure of zidovudine and its complex with Fe (II)

Antimicrobial activity

Antimicrobial behaviour of the Fe(II)-AZT complex against various pathogenic bacteria has been reported in the Table 3. A perusal of the table reveals that complex shows increased toxic effects against all the pathogenic bacterias under study, as compared to the parent drug AZT.

Possible *in-vivo* mechanism[15]

Fe(II)-Zidovudine complex is phosphorylated *in-vivo* by cellular enzymes to the corresponding deoxyneucleoside triphosphate derivative. In this form the drug inhibits viral RNA-dependent DNA polymerase (reverse transcriptase). Its anti viral selectivity is due to its greater affinity for reverse transcriptase than for human DNA polymerase. As an important part of its mechanism of action, Fe(II)-Zidovudine complex also causes chain termination during DNA synthesis. Thus, if azidothymidine triphosphate is incorporated into a growing strand of DNA, additional nucleoside cannot be added because of the modification in the 3' position of the complex. The antiviral activity of Fe(II)-zidovudine complex is enhanced by acyclovir, interferon, dideoxyadenosine granulocyte-macroppase colonystimulating factor16 and neutralizing antibody, but it is antagonized by thymidine and ribavirine. Antagonism probably results from competition, for phosphorylation, reducing intracellular concentration of zidovudine triphosphate.

AZT, which is known to possess activity against Epstein-Barr virus *in vitro* and also possess activity against gram -ve bacteria, has proved to be a potential anti-HIV drug. On the parallel lines, the Fe(II)-zidovudine complex which has also shown increased antibacterial activity as compared to the AZT may prove to be a comparatively better drug in lieu of AZT. It is, therefore, recommended to the therapeutic experts to decide over the utility of the prepared formulation as an Anti-HIV drug.

Table 2 — IR frequencies (cm^{-1}) and their assignments for zidovudine and its Fe (II) complex

Assignment	Zidovudine	Fe (II) complex
$-N_3$ stretching vibrations	2170	2150
>N-H stretching vibrations	1680	1680
Aromatic ring vibrations	1600, 700	1600, 700
>C=O (5 mem) stretching vibrations	1750	1750
>C=O (6 mem) stretching vibrations	1725	1725
$-CH_2OH$ stretching vibrations	1100	1100

Table 3 —Antimicrobial study on Fe (II) – Zidovudine complex

Test organism	Inhibition zone (mm)		% inhibition
	Complex	Control	
E. coli	35	33	-6.0%
S. typhi	42	31	-35.4
V. cholerae	32	30	-6.6%
D. pneumoniae	36	29	-24.1

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