ROLE OF BIO-METAL Co(II) IN ANTI-AIDS EFFECT OF ZIDOVUDINE (AZT)

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ABSTRACT

Physicochemical and microbial studies on Co(II)-Zidovudine complex have been done in solid and aqueous phase. On the basis of elemental analysis, polarographic studies and amperometric titrations, the metal:drug ratio has been worked out to be 1: 1. The metal ligand interaction has been studied using polarographic method at 25±1°C and at ionic strength of μ = 1.0 (KCl). IR spectral studies have been used to ascertain metal:ligand binding site which speaks of the complex formation between the metal and the zidovudine ligand through the nitrogen of the azide group. Microbial studies on the complex was done against various pathogenic bacteria viz. *Eschirichia coli, Salmonella typhi, Vibrio cholerae* and *Diplococcus pneumoniae.* The results of the microbial studies with the metal:drug complex revealed that the drug: metal complex is more potent against the pathogenic bacteria as compared to the pure drug, Zidovudine. As such Co(II)-zidovudine complex may be recommended to the therapeutic experts for its possible use as more potent anti-AIDS drugs.

Key Words: Bio metal, Anti-AIDS, Co(II)-Zidovudine complex

INTRODUCTION

Metal constitutes about 3% of human body weight but the intricate biological systems depend very much upon the metals. Most of the drugs are organic compounds and they have a great tendency to form complexes with these metal ions. Cobalt is a life essential element; it is a part of vitamin B_{12}.

Vitamin B_{12} is the first metallocomplex in living system to be studied in great depth /1-3/. Complexation behaviour of Co(II) for medicinal purposes has been widely published /4/. In our laboratory, enhanced activity of Co(II)-complexes with some anti histaminic drugs /5/, antiseptic and antidiabetic drugs /6/ has been studied. The biochemical, pharmacological and medicinal importance of metal drug complexes is very well established /7,8/. In continuation of the work done in our laboratory, the present paper deals with the said studies on the Co(II)-Zidovudine (3' -Azido-3' deoxythimidine) (Anti-AIDS drug) complex.

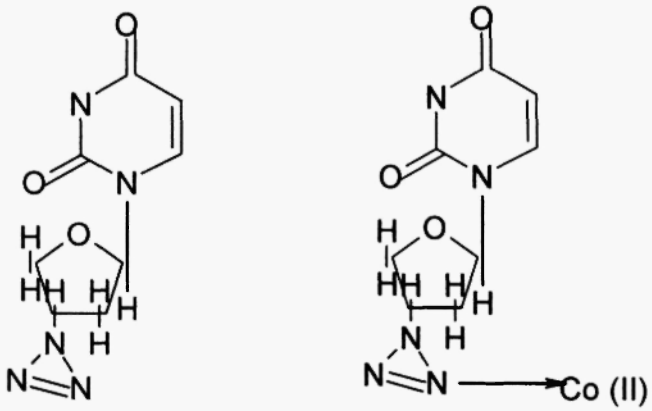

Fig. 1: Zidovudine and its complex with Co(II)

EXPERIMENTAL

Reagents

All the chemical used were of Anala - R / BDH grade. The drug Zidovudine (Zidovir) was procured from Sigma Chemical Company, USA. Double distilled water was used as solvent.

Apparatus

Polarographic measurements were made on an Elico (India) DC Polarograph model no. 357. The electrode system consisted of a Dropping Mercury Electrode (DME) as a working electrode, a coiled platinum wire as an auxillary electrode and saturated calomel electrode (SCE) as reference electrode. The amperometric titration was performed on a manually operated

setup comprising of a polyflex galvanometer (sensitivity 8.1 x 10^{-9}amp/div.) and an AJCO Varnier Potentiometer. A DME was used as an indicator electrode and a calomel electrode served as reference electrode. The capillary characteristics of the DME had a $m^{2/3}$, $t^{1/6}$ = 2.13 $mg^{2/3}$ $sec^{-1/2}$ at 50cm. effective height of the mercury column. The pH of the test solutions was measured on an Elico digital pH meter Model LI-108. The elemental i.e. C, H, and N analysis of the complex was done on a Heraeus Varlo Erba elemental analyser model-1108. The IR spectrum of the solid complex was recorded using KBr pallets on a Perkin-Elmer-IR Spectrophotometer, model-379.

General Procedure

Polarographic Measurements

Experimental sets were prepared by keeping overall cobalt (metal ion) and potassium chloride (supporting electrolyte) concentration fixed at 1.0mM and 1.0M respectively. The ligand concentration was varied from 0.0mM to 15mM. The pH of the test solution was adjusted to 6.0±0.1 using, HCl/NaOH solution.

Amperometric Titrations

Experimental sets, each having different but known amounts of the drug under study, were prepared in appropriate quantity of supporting electrolyte (potassium chloride) at pH = 6.0± 0.1 and titrated separately against the standard solution of the titled Co(II) ions whose pH has been adjusted to that of the titrate (6.0±0.1).

Synthesis Procedure of the Solid Complex

Cobalt nitrate and Zidovudine (drug) solution were separately prepared in distilled water and were mixed in 1: 1 molar ratio the mixture was then refluxed in a round bottom flask for 1-2 hrs. The residue (complex) was filtered and washed thoroughly to remove any unreacted materials. The complex was dried at 40°C and stored over P_4H_{10}.

Elemental Analysis

The elemental i.e. C, H, and N analysis of the complex was done at CDRI, Lucknow, whereas the gravimetric method was used for the

estimation of cobalt in the complex /9/.

IR Spectra Analysis

The IR spectrum of the solid complex was recorded using KBr pallets. Biological Study on the Co(II)-Zidovudine Complex

Microbial study

Raper's /10/ paper disc method was followed for the microbial screening of Co(II)-Zidovudine complex against various bacteria viz. *Eschirichia coli, Salmonella typhi, Vibrio cholarae* and *Diplococcus pneumoniae.* Sterilized filter paper discs (6mm diameter) were dipped into the complex solutions of O.O1 M concentration. Prior to this, the bacteria were separately homogenised with nutrient agar and potato dextrose media (at 27 30°C) plated on to the sterilised petri-dishes. Dipped filter paper discs were placed on seeded medium. After 24hrs. of incubation, antimicrobial activities were recorded by measuring the inhibition zone against complex under study. Similar experiment was repeated with the control drug (Zidovudine).

The number of replicates in each case was three, percentage inhibition was calculated using the following formula

$$\% \text{ inhibition} = A\text{-}B / A \times 100$$

where A represents the diameter of the inhibition zone for control (Zidovudine) and B represents the diameter of the inhibition zone for sample (Co(II)-Zidovudine complex)

RESULTS AND DISCUSSION

Polarographic Measurements

Polarographic behaviour of zidovudine with Co(II) and its complex with the ligand under study were found to be reversibly reduced, involving two electrons, which was evidenced from the plots of log plot slopes. The reduction was found to be diffusion controlled, which was evidenced by the plot of id vs.$\sqrt{h}$ corr.

On gradual increase of the Zidovudine concentrations, the half wave

potential of Co(II) shifted to more negative value and the diffusion current shortened thereby showing complex formation between Co(II) with Zidovudine.

To study the composition and formation constant of the complex, plot of $\Delta E_{1/2}$ (shift in $E_{1/2}$) i.e. $(E_{1/2})c - (E_{1/2})s$ against log Cx (logarithm of the concentration of the ligand) was drawn. The plot is a linear line showing the formation of single complex species in solution. Lingane's treatment /11/ of the observed polarographic data reveals 1: 1 metal:zidovudine complex formation with log $\beta_1 = 5.29$.

Amperometric Determination of Zidovudine with Co(II)

Co(II) gives a well defined polarographic wave in 1.0M KCl at pH = 6.0± 0.1. The diffusion current was found to be proportional to the concentraton of Co(II). The Zidovudine drug does not produce a wave under the said experimental conditions. The plateau potential for the polarographic wave of Co(II) (-1.2V) vs. Hg pool was applied for carrying out amperometric titrations. On performing the amperometric titration of drug solution with standard solution of Co(II), the current volume plots resulted in L-shaped curves. The end point as located by graphical method revealed metal to the drug ratio of 1: 1, which is in agreement with the authors observations on the metal:ligand equilibria using polarographic method.

Characterization of Co(II)-Zidovudine Complex

Elemental Analysis

The results of elemental analysis (Table 1) of the drug and its complex with Co(II) revealed 1: 1, metal : drug ratio in the complex, which supports authors' findings using polarographic and amperometric methods.

IR Spectra

The structurally important frequencies of IR bands for Zidovudine and its complex with Co(II) metal ions have been tabulated in Table 2. A comparision of the IR data for the drug and its Co(II) complex reveals that the band at 2170cm-l in the spectrum of the drug is shifted to 2150cm-l in the spectrum of the complex, indicating the involvement of -N of the Azide group of the drug in the complex formation. On the basis of above data a

tentative structure to the Co(II)-Zidovudine complex /12/ may be given as in Figure-1.

Table 1

Elemental Analysis percentage calculated / [found]

Complex		Drug
M%[Co(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%

Table 2

Principle IR Signals (cm^{-1}) and their assignments for Zidovudine and its Co(II) complex

Assignment	Zidovudine	Co(II) Complex
$-N_3$ stretching vibrations	2170	2150
> N-H stretching vibrations	1680	1680
Aromatic ring vibration	1600,700	1600, 700
>C=0 (5 mem) stretching vibrations	1750	1750
>C=0 (6 mem) stretching vibrations	1725	1725
$-CH_2OH$ stretching vibrations	1100	1100

Microbial Study

Results of antimicrobial activities of the Co(II)-Zidovudine complex are shown in Table 3. A perusal of the data in the table clearly shows that Co(II) Zidovudine complex is found to be more toxic as compared to the control drug against test bacteria.

Table 3
Antimicrobial study on Co(II) – Zidovudine complex

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

Possible In-Vivo Mechanism /13/

Co(II)-Zidovudine complex is phosphorylated *in-vivo* by cellular enzymes to the corresponding deoxynucleoside triphosphate derivative. In this form the drug inhibits viral RNA-dependent DNA polymerase (reverse transcriptase). Its antiviral selectivity is due to its greater affinity for reverse transcriptase than for human DNA polymerase. As an important part of its mechanism of action, Co(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.

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 parallel lines the Co(II)-Zidovudine complex which has also shown increased antibacterial activity as compared to the Zidovudine may prove to be a comparatively better drug in lieu of Zidovudine drug. It is therefore recommended to the therapeutic experts to decide over the utility of the prepared formulation as an anti-HIV drug.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Chemistry, Dr. H.S. Gour University, Sagar, for providing necessary laboratory facilities.

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