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Physicochemical Biological and Medicinal Investigation of Co(II)-Decarbazine complex

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ABSTRACT

Drugs are natural or synthetic organic compounds which affects and improving functioning of living body system. They are used in the diagnosis, mitigation, treatment, diseases prevention and relief of discomfort. Decarbazine drug is a chemotherapy medication used in the treatment of melanoma liver cancer, carcinoma of pancreas etc. The discovery and development of the anti-tumor compound cis-platin and its derivatives play an important role in field of medicinal coordination chemistry. Therefore, the biochemical, pharmacological and medicinal studies of drug-metal complexes are very important.

Looking at the wide applicability of metal-drug complexes the present work has been designed to thorough study of drug complexes of life essential metals with an anti-cancer drug Decarbazine.

The electro analysis of the complex has been done using Polarographic and Amperometric methods and IR spectroscopy for the determination of physicochemical parameters. The probable formula for the complex has been worked out to be 1: 1(metal: drug).

Microbial study has been done for knowing it's toxicity against various bacteria and fungi i.e. *Staphylococcus aureus*, *Bacillus pumilus, Proteus mirabillis* and *Escherichia coli*. Raper"s paper disk method has been used for determination of antimicrobial activities. Pharmacological activities i.e. in-vitro and in-vivo studies have been done for its possible therapeutic use. Mouse sarcoma cell line 180 and Balb/C mice were used for the anticancer screening of solid complex in-vitro and in-vivo respectively.

Key words : Anticancer drug, Co- decarbazine Complex

INTRODUCTION

With the application of new and sophisticated machines to study biological and biochemical systems the true role of inorganic salts in living systems can be revealed (1). Inorganic chemistry is not the "Dead Chemistry" that some people may think. Today, it is known that metals are important ingredients in life, just as the organic molecules. For instance, the divalent magnesium and calcium ions play important regulatory roles in cells. Metallothionins are proteins rich in metal ions found in living systems. The divalent cations Zn^{2+}, Ca^{2+} and Mg^{2+} prevent cytotoxicity and *in-vivo* antagonize Cd-induced carcinogenesis.

Cobalt must be added in the diet. It is physiologically active in form of vitamin B_{12}. It is essential metal as it is unusual. It is a part of vitamin B_{12} it contains 0.043 µg. Vitamin B_{12} is first metallo complex in living system. Cobalt metal is used in the treatment of cancer in radio therapy, gamma rays emitted by Co_{60} are in use all over the world of destroying the cancer cell (2). Toxic levels of Co is occurred when addition of it bear causes cardiomyopathy in heavy beer drinkers.

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EXPERIMENTAL

Chemicals and Reagents

All chemicals used were of analytical grade. The drug sample was procured by Sigma Chemical company, USA. Double distilled water and absolute ethanol were used as solvents. Decarbazine was prepared by dissolving the requisite amount in distilled water. Stock solution of 1M potassium chloride was prepared by dissolving a requisite quantity of compound in distilled water. pH adjustment were made using dilute solutions of HCl, NaOH whenever necessary. The test solutions were deaerated by bubbling nitrogen gas for 10 min. before recording the polarogram /Voltammogram.

Apparatus

All the polarographic experiments were performed of the DCP and DPP studies were carried out in exploratory mode and peak analysis in determination mode on software connected Ω metrotism 757VA computrace (ion analyzer). The polarographic cell consisted of a three-electrode assembly and a stirrer having a dropping mercury electrode (DME) as a working electrode a platinum wire as an auxiliary electrode and Ag/AgCl electrode as a reference electrode. The nitrogen gas was bubbled for 15 minutes. A systronics digital pH meter model- 361 was used for pH measurements The amperometric titrations were performed on a manually operated set up, equipped with polyflex galvanometer (sensitivity 8.1 x 10^{-9} amp. per div) and an AJCO vernier potentiometer. The capillary characteristics of the DME had a $m^{2/3}$ $t^{1/6}$ value of 2.5 $mg^{2/3}$ $S^{-1/2}$ at 60 cm effective height of mercury column. The IR spectrum of solid complex was recorded using KBr pellet on an shimadzer, Japan model 470 IR spectrophotometer.

Preparation of complex

For the study of metal : ligand (M:L) complexation equilibrium experimental sets were prepared by keeping overall Co (II) and potassium chloride (supporting electrolyte) concentration fixed at 1.0 mM and 0.1M, respectively. The concentration was varied, from 0.0 to 25mM. The volume was made up to 100ml with

distilled water and the pH of each set was adjusted to 6.0 ±0.1 using HCl/NaOH solution. The test solution was deaerated for 5minute before recording the polarogram/voltammogram.

Amperometric titration

Experimental sets, each having different but known amount of Co(II) were prepared in appropriate quantity of supporting electrolyte and pH was adjusted to 6.0 and titrated separately against the standard solution of the titled Decarbazine whose pH was also adjusted to that of the titrate (6.0 using HCl/NaOH) at -0.40 V vs SCE (the plateau potential of Co)(3). The current after each addition of the titrant was read and a curve was plotted between current against volume of titrant added.

Synthesis procedure of solid complex

The complexes were prepared by mixing the decarbazine drug and Co(II) metal ion in the stoichiometric ratio 1:1 (metal : drug). The solution was transferred in a beaker and kept over water bath for an appropriate time so that the volume was reduced to one fourth or less of the initial volume (4). The mixture was kept standing over night. Precipitate so obtain was filtered washed and dried over P_4O_{10}. The refluxed metal drug complex was characterized by Elemental analysis and spectrometric measurements.

Antimicrobial screening

Reper's method (5) was followed for the microbial screening of the complex against various bacteria : *staphylococus aureris, psedomonas mangiferae, salmonella typhi and fungi. A. fumigatus and crysosporium sp.* The number of replicates in each case was calculated using the following formula.

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

Where "A" represents the diameter of the zone of inhibition for control and "B" represents Zone of inhibition for complex.

Pharmacological screening

In-vitro and In-vivo study of anticancer activity of the drug metal complex have been done by following procedure:

Cell Culture Condition

The B16-F10 melanoma tumor cell line was obtained from Jawaharlal Nehru Cancer Hospital & Research Centre(JNCHRC), Idgah Hills, Bhopal, India. and was tested to be pathogen by JNCHRC. The cells were grown in dulbecco's modified Eagles medium (DMEM) supplemented with 10% heated- inactive fetal bivine serum and 1% antibiotic solution (penicillin/streptomycin) at 37°C in 5% CO_2 and 95% humidified atmosphere as a monolayer culture in Roux bottles(6) (Corning Plastic, USA).

Cell Culture and Viability Assay

The cells obtained were cultured in 5ml 24 well culture plates (Corning Plastic, USA) The cells were seeded in dulbecco's modified Eagles medium (DMEM) and media was replaced after every 2 days until the outgrowth had spread to cover at least 50% of the growth surface. Further, the cells were sub cultured by enzymatic method using trypsin. The B16-F10 melanoma tumor cell line was maintained in DMEM media in different culture plates (Corning Plastic, USA). The cultured cell line was treated with 1µM/ml and 10 µM/ml

dose of different pure drugs and metal complexes, than plates were kept in CO_2 incubator. After the treatment, the toxicity of the drug was examined on melanoma tumor cell. The total viable cells in each well were counted daily by trypan-blue exclusion method[19].

Cell Viability count

The dye exclusion test is used to determine the number of viable cells present in cell suspension. It is based on the principal that live cells possess intact cell membrane that exclude certain dyes such as trypan blue, eosin or propidium whereas dead cells do not. Cells viability counts were made by trypan blue dye exclusion method [21]. Two drops of trypan blue were added to each cell culture wall and kept for 15 minutes. Now, a drop of culture was added to hemocytometer (Neubaurs chamber) and the number of stained, non-stained and total number of cells were counted, then, the % inhibition was calculated using the formula:-

$$\text{Inhibition\%} = \frac{\text{No. of viable cells} - \text{No. of viable cells after treatment}}{\text{No. of viable cells without treatment}} \times 100$$

In-vivo

Animals and Tumor Treatment

Experiments were performed on Male C57BL/6J black mice, 6-8 weeks old, weighing 20-25 gm purchased from laboratory animals center Jawaharlal Nehru Cancer Hospital & Research Centre (JNCHRC), Idgah Hills, Bhopal, India. Mice were kept in cages with sawdust bedding and given food and water ad libitum air conditioned animal house. Environmentally controlled at a temperature of 25±2˚C and with a 12:12 light-dark cycle. All animals experiments were carried out with approval of institutional animal care and use committee as per norms CPCSEA registration No-500/01/a/CPCSEA/2001B16-F10 cells cultured and maintained at ≤ 90% confluence, were suspended by trypsinization and washes complete media and allow to recover at 37˚C for 4h, followed by three washes with PBS, pH 7.4. The cells were then counted using a hemocytometer, and diluted to given a final concentration of 5.0×10^6 cells/ml in PBS, pH 7.4. The mice were bilaterally injected subcutaneously in the dosal rear hindquarter skin with 100µL (5.0×10^6 cells) of B16-F10 viable tumor cell per site. Once the tumor became palpable, diameter in three per-pendicular planes (D_1, D_2, D_3) were measure on alternated days using a vernier caliper as described earlier[22]. The tumor volume (V) was calculate from the formula

$$V = (\pi/6)\ (D_1, D_2, D_3)$$

Tumor were measuring 100±10 mm^2 were taken for the experiments.

After the treatment, the tumor response was assessed by parameters volume doubling time and growth delay. Volume doubling time (V.D.T.) is the time required to double the tumor volume from 100 mm^3 to 200mm^3 while growth delay is the difference in time, in days, between the treatment and untreated tumor to reach 300 mm^3 from 100mm^3 . Then after find out inhibition rate was calculate from the formula(7).

$$\text{Inhibition Rate (IR)} = \frac{\text{Untreated (V.D.T.)} - \text{Treated(V.D.T.)}}{\text{(Untreated VDT)}} \times 100$$

RESULT AND DISCUSSION

Polarographic Analysis of Bio-metal Co((II) – Decarbazine Co(II) give a well-defined polarographic waves and peaks with Half wave potential $\mathbf{E_{1/2}}$ -0.56 V in 0.1M potassium Chloride as supporting electrolyte and 0.001% gelatin as maximum suppressor at pH 4.0 ± 0.1 respectively. The shift in half wave potential and peak potential towards a more negative value with increasing of ligand (Decarbazine) and decease in diffusion current indicated complex formation between above mentioned metal ions and drug decarbazine. The results of which are depicted in Figure 1.

The metals and its complexes with the ligand understudy were found to be reversibly reduced involving two and three electrons. Which was evidenced by the plots of log I / (id-i) versus Potential (E). The shift in half wave potentials towards a more negative value a with increasing concentration of ligand and decrease in diffusion current indicated complex formation between bio metal ions to be used i.e Co(II) with drug Decarbazine.

Graph between $\Delta E_{1/2}$ = Shift in the $E_{1/2} = (E_{1/2})C - (E_{1/2})S$ and log Cx (logarithms of concentration of the ligand) is plotted to study the concentration and the formation constant of the complex. The plot was linear indicating the formation of single complex species in solution. Lingan's treatment **(8)** of the observed polarographic data revealed 1:1 (Metal:Decarbazine) ratio complex formation with formation constant log log $\beta 1(Co) = 5.67$.

Amperometric determination of decarbazine with Co (II)

Co (II) gives a well-defined polarographic wave in 0.1 M KCl at pH 6.0. The diffusion current was found to be proportional to its concentration. Decarbazine does not produce any wave under the said experimental conditions. The platue potential for the polarographic wave of Co (II) i.e. -0.6 V vs SCE, was applied on the potentiometer for carrying out amperometric titration. Co(II) was taken as titrate and the drug was taken as titrant. The current volume plots resulted in L shaped curve. The end point as located by graphical method revealed metal to drug ratio of 1:1 which is in agreement with author's observation on the metal ligand complexation equilibrium using polarographic method. The standardised method was found be accurate for the analysis of complexes.

The IR spectral data of dacarbazine drug gives IR bands at frequency 880 cm^{-1} and 1601 cm^{-1}, whereas the IR bands in drug complex are shifted to 850 cm^{-1} and 1565 cm^{-1}, respectively due to the involvement of the two nitrogen(10), one each of primary amide and triazo (attached to dimethyl group) groups of the drug in complex(11). The results of IR spectrometry of pure drug and its Co (II)-dacarbazine complex are reported in Table-1.

Antimicrobial activity of Co (II) Decarbazine complex- Antimicrobial activity of the complex is presented in Table-2 of the various human and plant pathogens studied. This complex was found to be most toxic against psedomonas magniferae bacteria.

Decarbazine and its metal complex with Bio metals Co(II) against Mouse Sarcoma cell line 180

The results of in-vitro pharmacology are suggested that Decarbazine-Co(II) complex was found to be more effective as compared to other complexes. The drug and its complex under study are shows increased inhibition against the Mouse**.** The percentage inhibition activity at 1µM/ml concentration of Decarbazine and its metal complexes of Co(II) 28.91 ± 1.08, and another set of experiment at 10µM/ml concentration of Decarbazine and its metal Co(II) shows the increase inhibition activity 88.88 ± 1.70. The data was statistically significant as at P< 0.04.

Decarbazine and its Bio metal complex of Co(II) against B16F10 melanoma tumor

The results of the anticancer activity of mice tumor size against Decarbazine drug and its Co(II) complex understudy were calculated using the parameters of inhibition rate (IR), Growth delay (GD), and Volume of tumor doubling time (VDT). The inhibition rate was 77.81 for Co-Decarbazine . The growth delay was also increased in different groups i.e. 7.32 ± 0.89, 6.92 ± 0.52 for decarbazine drug and its Co(II) complex. The volume of tumor doubling time of Untreated (10) group were 2.15 ± 0.09 (days) for pure drug, for Co-decarbazine. Volume of tumor doubling time (VDT) was recorded 12.80 ± 0.57 (days) respectively. The differences in the values of the results of experimental groups were statistically analyzed and found to be significant as compared to the control group (p< 0.05).

REFERENCES

1. Greenwell, M., and Rahman P.K.R.M. "Medicinal plants: their use in anticancer treatment." *International journal of pharmaceutical sciences and research* 6.10 (2015): 4103.
2. Anand, S. P., and Sati N. "Artificial preservatives and their harmful effects: looking toward nature for safer alternatives." *Int. J. Pharm. Sci. Res* 4.7 (2013): 2496-2501.
3. Basset J, Denny R.C. and Jeffery G.H. "Textbook of Quantitative Analysis" VII Edition *ELBS Pub* (2004): 440.
4. Chérifa Boulechfar et. all "Synthesis, electrochemical, and quantum chemical studies of some metal complexes: Mn (II), Co(II), and Zn(II) with 2-furaldehyde semicarbazone" *J Molecular Structure 1271*.(2023): 134007.
5. Ghosh M.N. "Fundamentals of experimental pharmacology" *Scientific book agency, Calcutta*. (1984): 61.
6. Chouhan Priyanka and Mishra Jyotsna. "Role of Zinc (II) Bio metal in complexation of Aspirin as Anti Stroke Drug. Research Journal of Pharmacy and Technology. 15. 10 (2022} 4416. doi: 10.52711/0974-360X.2022.00740
7. Shukla Savinash Mani and Mishra Jyotsna "Herbal Mouthwash For Enhanced Oral Health" International J Creative Research Thought. 11.11 (2023) 2320.
8. Trivedi A.V. Shukla J and Pitre K.S. "Bioinorganic study on [Fe(promethazine)2H2O Cl]2+ complex" Indian J Exp Biol. 36. 11 (1998) 1125-9. PMID: 10085782

9. K. Nakamoto, IR and Raman Spectra of Inorganic and Co-ordination compounds, **Vol-III, Inter science**, New York, 1977.
10. R. Dayer, Application of Absorption spectroscopy of Organic Compounds, Printice Hall of India Pvt. Ltd., New Delhi, 1974.

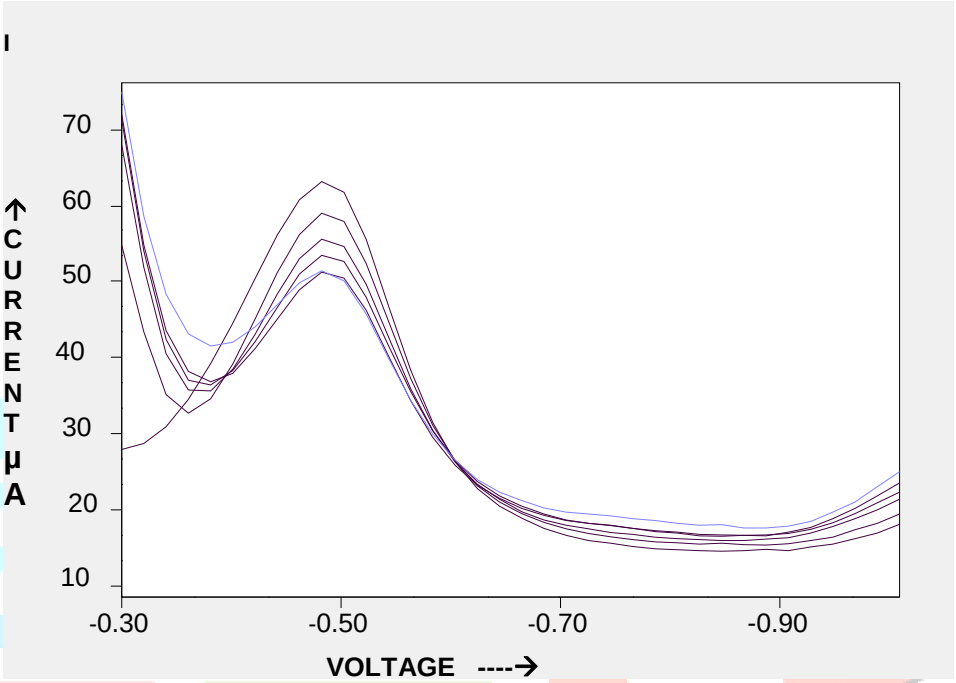

Figure 1- Differential Pulse Polarogram of Co(II) in 0.1M 0.1M Britton Robinson Buffer + 0.001% Gelatin at pH 5.0±0.1 I.without and with ii. 0.1mM iii. 0.2mM iv. 0.4 mM Metotraxate

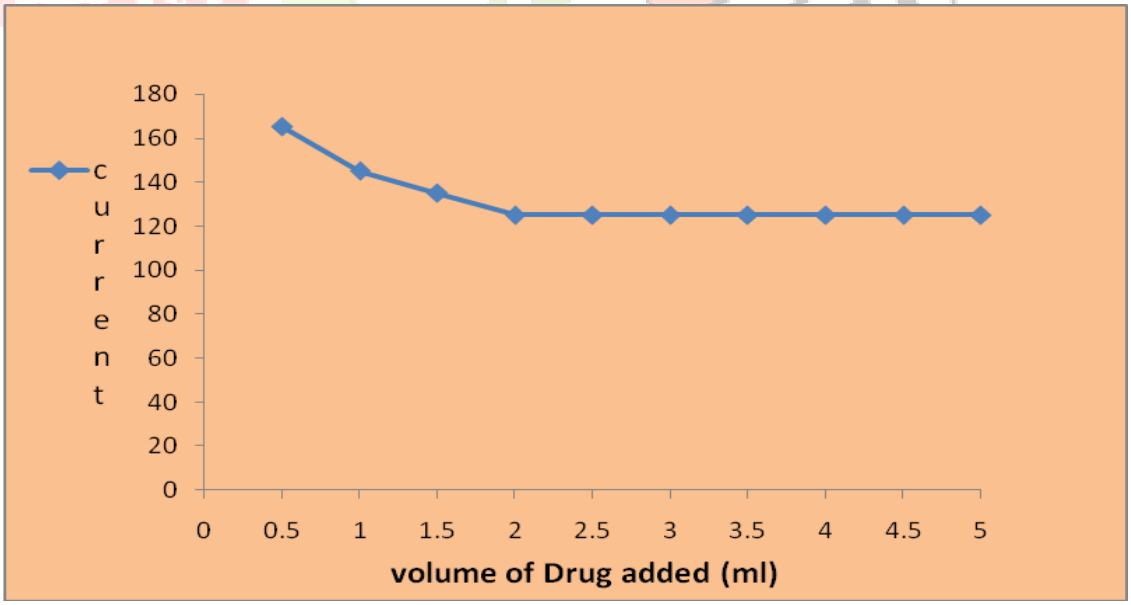

Figure 2 - Amperometric titration of 2mM/10 ml analysis Co (II) with 1mM Decarbazine in 0.1M Potassium Chloride

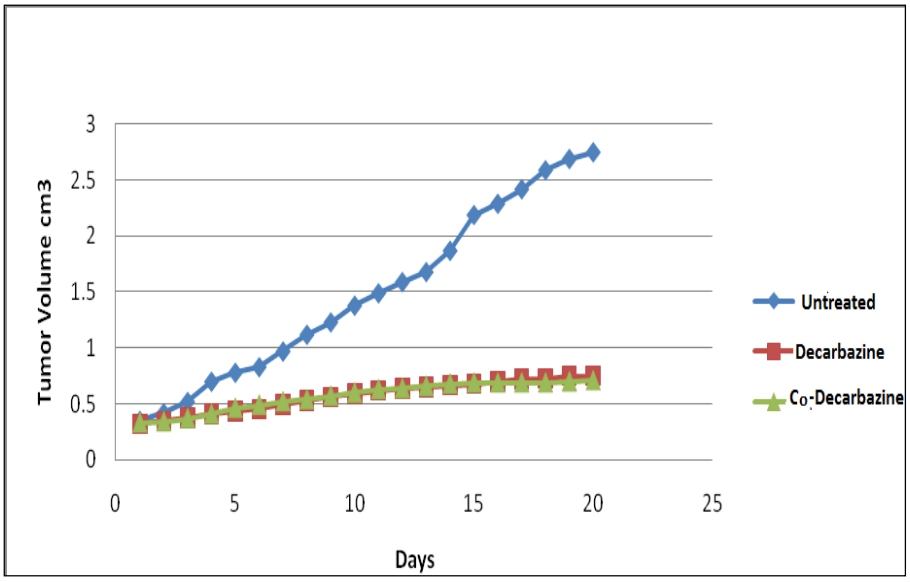

Figure 3 Effect of Co(II)-Decarbazine complex on tumor volume. A-Without drug, B- With Bleomycin, C-With Co(II)- Decarbazine

Table-1

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

Decarbazine	Assignments	Complex
620sharp 650sharp	Imidazole Vibrations	620 sharp 650 sharp
880sharp	$CONH_2$ stretching vibrations	850 sharp
1280weak 1325 weak 1340 weak	-N_2 stretching	1280weak 1325 weak 1340 weak
1430 sharp	-N=N stretching vibrations	1430s
1601broad	C-N aliphatic vibrations	1565broad

Table- 2

Antimicrobial study of Co(II) - Decarbazine Complex

Test Organism	**Inhibition zone (mm) Con. of complex 2mM/10ml (B)**	**Control Cu(II) metal 1.0 mM/10ml (A)**	**% Change (A-B/A) ×100**	**Control Clopidogrel drug 1.0mM/10ml (Y)**	**% Change (Y-B/Y) ×100**
Staphylococcus aureus	15	19	21	21	28.5
Bacillus pumilus	14	17	18	23	39.1
Proteus mirabillis	10	11	10	13	23.1
Escherichia coli	11	13	15	11	--

Including diameter of filter paper disc 6mm.