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Physiochemical, Microbial and Pharmacological studies of Fe (II) - Chlorambucil complex

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Abstract

The anticancer drug **Chlorambucil** and its complex with Fe (II) have been qualitative and quantitative analyzed spectrometrically and electrochemically.

All the studies have been done in both phases i.e. solid and liquid. On the basis of elemental analysis, IR spectrometry, Polarography and Amperometric methods the probable formula of the complex has been worked out to be 1:2 (M: L).

Antimicrobial activity of complex has been determined using Disc diffusion method against various pathogenic bacteria and fungi. Obtained results is increased inhibition efficiency against the prepared complex, it is presumed that the complex may prove to be more potent as compared to **Chlorambucil** drug.

Pharmacological studies (In-Vitro and In-vivo) of prepared drug complex were done on **sarcoma-180 tumor** cell. The observed results revealed that the complex is more potent as compared to the pure drug in all above mentioned activities. As such Fe (II) **Chlorambucil** drug complex may be recommended to the therapeutic expert for its possible use as more potent anticancer drug.

Key words : Bioinorganic, anticancer drug, Iron complex, polarography, pharmacology.

Introduction

Chlorambucil is an anti neo plastic drug. It is also used as immunosuppressive agent[1]. Chemically it is a ([4-(bis-2Chloroethyle) amino] L-phenyl amine) and its biological activity is related to the ability to function as an alkylating agent in physiological conditions. The designs of a drug for treatment of any particular disease depend on the development of suitable chemical criteria for in-vitro and in-vivo reactions.

Fe (II) is recognized as an essential metal for normal functioning of our biological system[2]. Fe deficiency is associated with impaired growth and large number of diseases[3].

It has been observed in favorable cases that

metal drug complexes show increase potency then the parent drug[4,5]. Keeping this view in mind the present investigation deals with the bioinorganic studies of the interacting such biologically essential metal Fe (II) and an anticancer drug **Chlorambucil.**

Change in the biological properties of the **Chlorambucil** have been evaluated and underlying role of Fe (II) in the anticancer activity of pure drug has been discussed. The study on Iron complex of anticancer drug **Chlorambucil** have carried out by physico- chemically, microbially and pharmacologically. The metal ligand complexation equilibrium has been studied using elemental analysis, amperometric titration and voltametric / polarographic studies. Besides, IR spectral analysis has been worked out which gives probable formula for the complex is to be1:2.

Various pathogenic bacteria like **Klebsiella Pneumonia, Pseudomonas, Staphylococcus aureus and fungi i.e. Asperginus Niger, Nigrosporan sp.** have been used to microbial study using disc diffusion method.

Screening of the prepared metallo-drugs will be done on **sarcoma-180 tumor** cell using respective parent drug as control. Viability of tumor cells will be measured by trypane blue exclusion test[6-7]. The result of physicochemical method, microbial and pharmacological studies with the Fe- **Chlorambucil** complex suggested that the recommendation of prepared complex to the therapeutic experts for its possible use as more potent anticancer drug.

Experimental

All the chemicals used were of analytical grade, sigma laboratory, USA supplied the **Chlorambucil** drug. Standard solution of Fe (II) 0.5 mM, **Chlorambucil** 1mM and potassium chloride 0.1 M were 5% of 95% ethyl alcohol prepared was kept.

Electrochemical studies

The DCP and DPP studies were carried out in Exploratory mode and peak analysis in determination mode on a software connected Ω Metrohm 757 VA 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 reference electrodes. The nitrogen gas was bubbled for 5 minutes. A systronics digital pH meter model-361 was used for pH measurements.

Different sets of solution containing over all concentration of Fe (II) 0.5 mM in 0.1M KCl with varying concentration of **Chlorambucil** from 0.5 to 15mM, were prepared. The pH of these solutions was adjusted to 6.0±0. Nitrogen gas was bubbled through the test solution for about 15 minutes before recording the polarograms.

The AJCO potentiometer apparatus was used for amperometric titration consisted of a DME as working electrode, a calomel electrode as reference electrode and attached to an AJCO Vernier Potentiometer. Characteristics of the DME had $m^{2/3}t^{1/6} = 2.5mg^{2/3}sec^{-1/2}$ at 60cm effective height of mercury column.

For amperometric titration, set of solution containing varying concentration of Fe(II) (over all concentration, 0.5mM, 1mM, 1.5mM) in 0.1M KCl as supporting electrolyte were prepared and pH was adjusted to 6.0±0.1, using dil HCl/ NaOH. The plateau potential for Fe (II) is -1.1V versus SCE was fixed on the potentiometer. The titrations were performed using **Chlorambucil** solution as titrant.

Synthesis of Solid Complex :

A brown colored complex was synthesized by refluxing aqueous solution of M:L Fe (II) and **Chlorambucil** (1:2) molar ratio for about 3 hours. 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 characterized. The elemental C,H,N,O, analysis of complex was done on a hearaus varlo Erba Elemental analyzer Model- 1108, at CDRI Lucknow, where as volumetric method was used for the estimation of Iron in Fe- **Chlorambucil** complex[8].

Spectrometric measurement :

The IR spectrophotometric analysis was performed in solution phase using Shimadzu Corporation FTIR-spectrometer, model-8400S

Antimicrobial Screening :

Disc diffusion method [9-10] was followed for the microbial screening of Fe (II)- **Chlorambucil** complex against bacteria Viz Klebsiella pneumonia, Pseudomonas aeruginasa, Straphylococus aureus and fungi i.e. Asperginus Niger Nigrosporas SP.. Number of replicates in each of the case was three; percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{a-b}{a} \times 100$$

Where a= diameter of inhibition zone for control (**Chlorambucil)**

b=diameter of inhibition zone for complex

Pharmacological studies :

In-vitro study of anticancer activity of prepared metal drug complex have been done by following procedure[11-13]

In-vitro: - **sarcoma-180 tumor cell** were obtained from national center of cell science, Pune India, as a monolayer culture in roux bottles (corning plastics, USA).

The cells obtained were cultured in 5ml 24 well culture plate (corning plastics, USA). The cells were seeded in $2x10^5$ cells per well and 1.0 ml of Dulbecco's modified Eagles medium (DMEM) contain 10% V/V foetalcalf serum, penicillin 100 µg/ml and streptomycin 100 µg/ml was added to each well. The cells were kept in incubator at 37°C of 4h in 5% CO_2 atmosphere and 95% humidity. The cell counter was made on neubaurs chamber (fine optic, Germany). Three dilution Viz 1µm, 10 µm, and 100µm of pure drug and its Fe Complex were made and then the cells were treated as follows

Column	Free drug	column	Metal complex
A	1µm (1ML)	A	1µm (1ML)
B	10µm (1ML)	B	10µm (1ML)
C	100µm (1ML)	C	100µm (1ML)

After addition of respective solutions, the culture plate was incubated at 37°C for four hour's finally the cell counts were made as under. There are compared with the cell cultured in DMEM with treatment.

$$\% \text{ of inhibition} = \frac{\text{Total no. of viable cells} - \text{total no. Of Viable cells after experiment}}{\text{Total no. of viable cells.}} \times 100$$

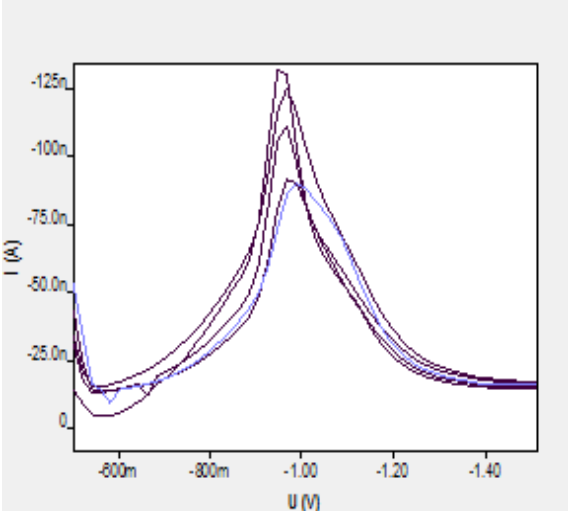

Fig-1 polarogram of Fe(II) (0.5mM) in 0.1M KCL supporting electrolyte at pH 6.0±0.01 And 1.0mM Chlorambucil.

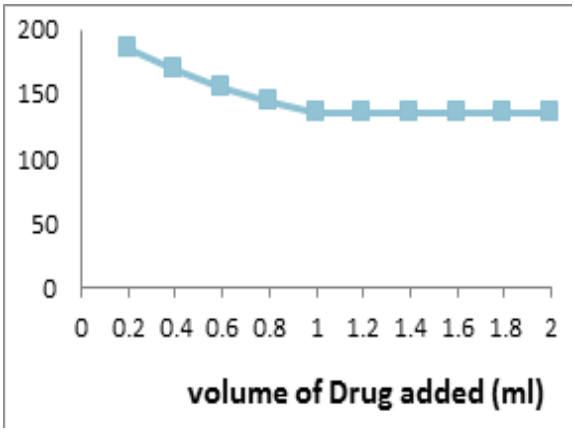

Fig-2 Amperometric titration of (1mM/10ml) Chlorambucil. (0.5mM/ml) & Fe (II) solution in 0.1 M KCl Solution

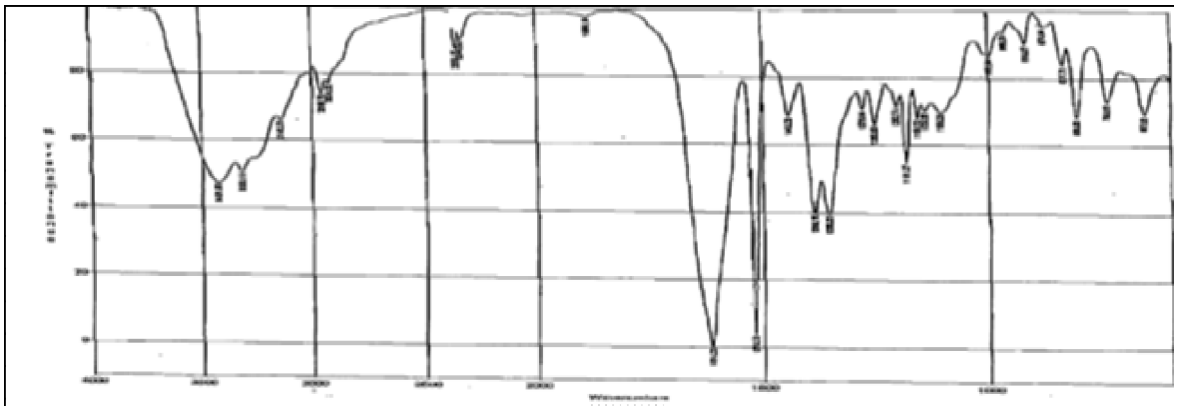

Fig-3 IR Spectrogram of Fe (II) Chlorambucil complex

Table 1. Result of Elemental Analysis (%)

Element	Chlorambucil	Fe (II)-Chlorambucil
Fe	-	9.22 (10.43)
C	51.12 (51.1)	43.98 (44.01)
H	5.97 (6.0)	5.36 (5.62)
O	14.48 (14.51)	13.52 (13.30)
N	9.08 (8.9)	7.98 (7.72)
Cl	23.32 (23.3)	19.98 (19.91)

Table-2. Result of Antibacterial Activity of Chlorambucil and It's Fe (II) - Chlorambucil complex

Test Organism (A-*Bacteria)*	Zone of inhibition		% Inhibition
	Control (mm)	Complex (mm)	
Staphylococcus aureus	7	9	-28.57
Klebsiella pneumonia	-	3	-
Prosteus	8	10	-25.0
*(*B-*Fungi)*	6	9	-33.3
Asperginus Niger			
Nigrosporan SP	05	07	-40

Table-3. Results of In vitro cytotoxicity of Chlorambucil and It's Fe (II)- Chlorambucil complex against sarcoma-180 tumor cell Line

Compound	Concentration µM/ml	% inhibition after 4h
Chlorambucil	1.0	35.4 ± 1.0(a)
	10.0	(b)
Fe(II)-	100.0	53.7 ± 1.6
Chlorambucil	1.0	77.6 ± 1.3
complex	10.0	51.1 ± 1.1
	100.0	64.8 ± 1.4
		90.5 ± 1.5

Result and Discussion

Polarographic behavior of Fe (II)- Chlorambucil complex :

In 0.1 M KCl at 6.0±0.1 pH, Fe (II) and its complexes with Chlorambucil, produced a well defined reversible and diffusion controlled polarographic wave which revealed by the log plot slop id versus √h (effective height of mercury column) respectively. On gradual addition of ligand, the $E_{½}$ of metal shifted to more electronegative value, indicating the formation of complex (Fig.-1). Lingane treatment[14] of observed polarographic data reveled 1:2 [M: L] complexes formation in solution with $\log\beta_1 = 5.675$.

Amperometric titration of Chlorambucil with Fe (II):

Fe (II) with **Chlorambucil** gives a well defined polarographic waves/peak in 0.1M KCl at 6.0±0.1 pH. the diffusion current was found proportional to the concentration of Fe (II). The platue potential for the polarographic wave Fe (II) (-1.1v) vs Hg pool was applied for carrying out amperometric titration. The current goes on decreasing to minimum and then attends a constant value. The plot of id (V+vV) versus volume of titrate added, revealed L shaped curve (Fig-2). The end point was indicated by the intersection of the two lines, which confirmed 1:2, [M: L] complexes formation.

Characterization of
(Fe(II)Chlorambucil) solid complex
Color: - Brown
Solubility: - Water soluble

Elemental analysis :

Percentage of C, N, O, H, and Fe found and calculated in complex and **Chlorambucil** drug are summarized in Table-1

Spectrometric analysis

The structurally important frequencies of IR bands for **Chlorambucil** and its complexes with Fe (II) metal ion have been shows in Fig-3.

A comparison of IR data for the drug and it's complexes reveals that the bands at 1400 and 1590 cm^{-1} in the drug are shifted to 1740cm^{-1} in the spectrum of complex, indicated the involvement of carboxylic group and amino group in complex formation.

On the basis of elemental analysis, polarographic, amperometric studies and IR spectra, a tentative structure to the Fe (II)- **Chlorambucil** complex may be assigned as shown in Fig-4.

Antimicrobial activity :

Antimicrobial behavior of the Fe (II) - **Chlorambucil** complex against various pathogenic bacteria and fungi has been reported in the Table-2. A perusal of the data in Table reveals that complex shown increased toxic effects against all the pathogenic bacteria under study, as compared to the parent drug **Chlorambucil.**

Pharmacological studies :

In Vitro

The result of in vitro experiments of pure drug and its complex are shown in Table-3. A perusal of the results data in Table that Fe (II) - **Chlorambucil** complex was found to be more effective than pure drug (14-17). The complex under study shows in increased inhibition against the **sarcoma-180 tumor** cells at all the test concentration i.e. 110,100 µm /ML. The increased inhibition activity of the complex was 51.1±1.1%, 64.8±1.4% and 90.5±1.5% as against 35.4±1.0%, 53.7±1.6% and 77.6±01.3% shown by the drug, respectively. The statistical treatment of observed inhibition data i.e. standard deviation and coefficient of variance which never exceeded 0.9 and 18% respectively, speaks the reliability of the observed inhibition data[18].

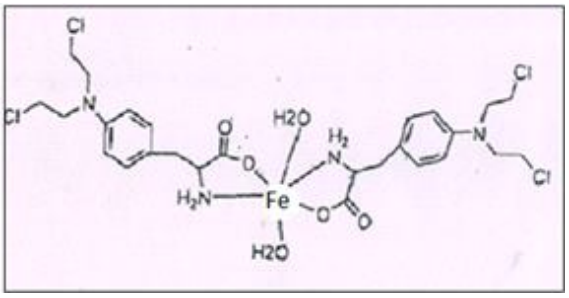

Fig-4 Fe (II) - Chlorambucil complex Structure.

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