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Structural, Antimicrobial and Pharmacological Studies of Fe (II)-5-Fluorouracil Complex

Shuchita Agrawal

B. T. Institute of Research & Technology, Sironja Sagar MP, India

Abstract: ***The coordinating character of 5-Fluorouracil (5-FU) with N and O sites towards metal ion Fe(II) have been synthesized and characterized by elemental, spectral (UV and IR spectroscopy) data as well as by magnetic moment values and conductivity measurements. The amperometric & elemental analysis suggests that the stoichiometry of the Metal: 5-FU 1:1. The low molar conductance values reveal the non-electrolytic nature of the complex. Magnetic moment values in concert with the electronic spectra indicate that 5-FU coordinates with the metal ion in a bidentate manner through the C = O and N atoms. The antimicrobial activity of the synthesized complex was screened against bacteria viz. Bacillus subtilis, Staphylococcus saphyphiticus, Staphylococcus aureus & Escherichia coli. In-Vitro Cytotoxicity of 5-Fluorouracil -Fe complex is screened against Sarcoma-180 tumor Cells. Fe (II): 5-FU ligand complex shows an increased activity in comparison to the control.***

Keywords: Metal liand complex, 5-Fluorouracil, Spectral studies and Antimicrobial activity

1. Introduction

Structural change of 5-Fluorouracil (5-FU), a mono fluorinated result of uracil, has utterly different biological properties than uracil which has substantial biological applications when it forms complexes with metal ions [1].

As the time of its synthesis, 5-FU has been ever more in work alone or in combination with other cytotoxic drugs and hormones in the medical treatment of solid tumors. It has also been used in the healing of different carcinomas [2]. This is owing to the presence of fluorine atom at the C-5 position which can considerably modify the electronic properties as indicated experimentally by changes in the electronic spectra [3].Complexes of amino acids are involved in the exchange and transport mechanism of trace metal ions in the human body. Synthesis and characterization of transition metal complexes of Cu (II), Ni (II) and Zn (II) with 5-Fluorouracil and some amino acids has been reported [4-7]. The literature reveals that number of drugs have been used to synthesize the complexes by means of many metals with an observation to improve their therapeutic action [8 -11].

In this paper, we report the synthesis, spectral and biological activities of ligand Fe (II) complex involving 5-Fluorouracil (5-FU).This study helped in understanding the coordination environment of the ligands around the metal ion and to investigate the antimicrobial activities.

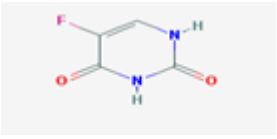

Figure 1: Structure of 5-Fluorouracil (5-FU).
Molecular formula- $C_4H_3FN_2O_2$

2. Experimental

All the ligands are extra pure Sigma Aldrich, Fluka (Puriss) products and they are used without further purification. C, H and N analytical data estimated with the help of elemental analyser at CDRI Lucknow (U.P.).Metal content of the mixed ligand complexes were estimated gravimetrically by the standard procedure. Molar conductance (1×10^{-3} M) was measured using an Elico CM 180 conductivity bridge by using 0.01 M KCl solution as calibrant. Magnetic susceptibility measurements were carried out on a Gouy balance at room temperature using mercuric tetra (thio-cyanato) cobaltate (II) as the calibrant. Diamagnetic corrections were applied in compliance with Pascal's constant [12]. Electronic absorption spectra were recorded with a double beam spectrophotometer. Vibrational spectra were recorded on a FTIR (Simadzu, model 8400) spectrometer, in the 400–4000 cm–1 range. In vitro antimicrobial activities of 5-Fluorouracil and its Fe (II)–5–FU(A) complex in DMSO medium were tested against three Gram–positive pathogenic bacterial strains: Bacillus subtilis, Staphylococcus saphyphiticus and Staphylococcus aureus, two Gram–negative bacterial strains: Escherichia coli and Pseudomonas aeruginosa using Muller Hinton agar nutrient by well diffusion technique [13].

3. Synthesis of Complex

5–Fluorouracil (0.013 g, 10 m mol) was dissolved in aqueous (10 ml) solution containing a few drops of concentrated ammonia and stirred. When 5-fluorouracil was completely dissolved, an aqueous (10 ml) solution of metal salt (10 m mol, 0.025 g Fe $(CH_3COO)_2$) was added slowly and stirred at room temperature .The pH (6.3) of the reaction mixture, adjusted by adding few drops of aqueous Na_2CO_3 solution (0.0105 g, 10 m mol). The resulting solution were reduced to 1/2 of its original volume by water bath and kept overnight. On standing, the mixed ligand complexes were obtained and collected by *vacuum* filtration, washed several times with cold water, ethanol and anhydrous ether. The mixed ligand complexes were dried in air and stored over anhydrous $CaCl_2$ at room temperature. The yield of the isolated complexes was found to be 63% (app) [14].

4. Results and Discussion

Polarographic Behavior of 5-Fluorouracil with Fe (II)

In 0.1 M KCl at pH 10.4±0.2 the Fe (II) and its complex with ligand under study were found to be reversible and diffusion controlled Polarographic wave which revealed by the log plot slop id versus √h respectively on gradual addition of ligand the E1/2 of metal shifted towards more electronegative value indicating the formation of complex (Figure **2**). Lingane's treatment [15] of observed Polarographic data revealed 1:1 [M: L] Complex formation in solution.

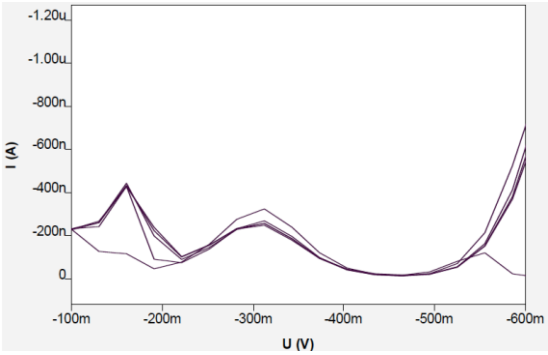

Figure 2: Polarogram of Fe (II) (2.0 mM) in 0.1M Ammonium buffer Solution at pH 7.0±0.1 and 2.0 mM **5-Fluorouracil**

Amperometric Determination of 5-Fluorouracil with Fe (II)

5-Fluorouracil with Fe (II) gives a well defined polarographic waves / peak in 0.1 M KCl at 10.4±0.2 pH the diffusion current was found proportional to the concentration of Fe(II). The platue potential for the polarographic wave of Fe (II) (-0.40V) 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 versus volume (V+vV) of titrant added, revealed L shaped curve (Figure **3**). The end point was indicated by the intersection of the two lines, which confirmed 1:1 [M: L] complex formation.

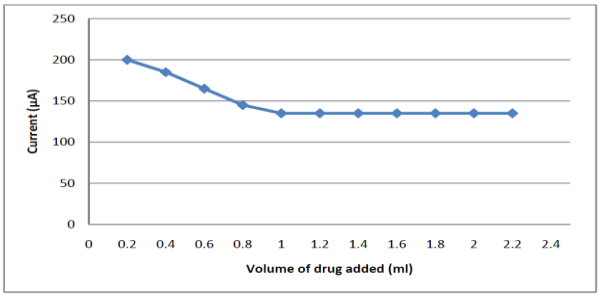

Figure 3: Amperometry titration of (2mM/10ml) **5-Fluorouracil** (2mM/ml) Fe (II) solution in 0.1 M Ammonium buffer Solution.

The elemental analysis and Molar conductance

The analytical data shows, the stoichiometry of Fe (II): 5-Fluorouracil is to be 1:1. The observed low molar conductance values (1×10^{-3} M, DMSO solution) at room temperature are reliable with the non electrolytic nature due to the absence of counter ions in the proposed structure [16].

Empirical Formula of complex- Fe $[C_4H_2FO_2N_2]$
Color of complex- Brown
Yield (%)-63%
Molar conductance- 11.1 (Ω^{-1} cm^2 mol^{-1})

Table 1: *Physico-chemical properties*
Elemental analysis, found (calculated) %

	Fe	C	H	F	O	N
found	30.56	26.39	1.067	9.57	17.29	14.99
calculated	30.43	26.08	1.086	9.78	17.39	15.21

Vibrational spectra

The IR spectra provide valuable information on the subject of the nature of functional group attached to the metal ion. The characteristic IR spectral data (KBr pellet, cm-1) with the principal IR frequencies of 5-FU and its complex are given in (Table 2). The presence of coordinated water molecules ν (OH) is confirmed by the rocking, twisting and wagging vibrational modes at 3101 – 2931 cm-1, 954 – 949 cm-1 and 747 – 739 cm-1respectively [17] .ν (M–N) and ν (M–O) bands are tentatively assigned in the region 439–432 cm-1 and 551–542 cm-1indicating the complexation of the ligands with transition metal ions [17] respectively.

Table 2: *IR spectral data (in cm-1)*

	υ (C=O)	δ (N-H)	υ(C-F)	υ(M-N)	υ(M-O)
5-Fluorouracil	1704, 1685	1513, 1430	1470	-	-
Fe(II)-5-FU	1691, 1652	1517,1408	1473	432	542

From the data, the ligand 5–FU acts as bidentate which form metal chelate all the way through the deprotonated N3 and C4 = O of carbonyl oxygen atoms. The magnitude of Δυ value falls in the range 237–252 cm-1 suggesting the bidentate coordination in complexation.

Electronic absorption spectra coupled with Magnetic moment values

The spectra suggested octahedral Fe (II) ligand complex. The magnetic moment values of these mixed ligand complexes in Bohr Magneton (μ_{eff} (BM) =2.98) supports the proposed geometry [18].

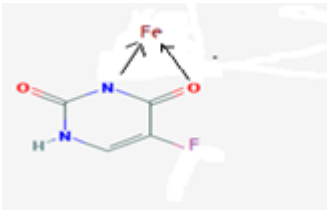

Figure 4: Structure of 5-Fluorouracil (5-FU)-Fe complex.
Molecular formula- Fe $[C_4H_2FO_2N_2]$

Antimicrobial activity

In vitro minimum inhibitory concentration (MIC) values of the ligand complex of Fe (II) 5–FU with free ligand (5–FU) were tested against some microorganisms and are summarized in Table 3. Commercially existing standard drugs Ampicillin (antibacterial control) and Nystain (antifungal control) are used as control. The antimicrobial activities of the prepared complex is normally better than the free ligands and the activities depend upon the Fe (II) ions i.e., size, charge distribution, shape and redox potential of the metal chelat [19]. The mixed ligand complexes explain moderate activity than the corresponding free ligands and

metal salts. From the Table 3, the MIC value of the mixed ligand complex is also confirmed by the liquid dilution route [20] in which the efficacy was also observed at very low concentrations.

Table 3: Antimicrobial activity of the free ligand (A) and its complex with Fe (II)

Complex	Minimum Inhibitory Concentration values (mg / ml)			
	Bacillus subtilis	Staphylococcus saphyphiticus	Staphylococcus aureus	E. coli
Control	5.4	4.8	7.2	6.2
5-FU(A)	12.1	14.6	16.6	21.8
Fe(II)-5-FU	9.2	9.9	8.9	15.7

The measured MIC values (mg / ml) of the control, ligand complex with free ligands against microorganisms, can be explained on the basis of chelation theory [22, 23]. From the MIC values, Fe (II) 5–FU (A) complex show moderate activity against all the bacterial strains even than the standard drug. Also the antibacterial activity of complex is due to the presence of electron withdrawing (C5–F) [24].

Also, it was established that the pathogenic Gram positive bacterial strains like *Bacillus subtilis*, *Escherichia coli* strains shows remarkable activities against the free ligand and their complexes.

Pharmacological Studies

In Vitro

The result of *in-vitro* experiments of pure drugs and its complex are shown in Table **4**. Perusals of the data it is compared shown that Fe (II) 5–FU complex was found to be more effective than pure drug. The complex under study showed an increased inhibition against the **Sarcoma-180 tumor** cell line at all the test concentrations i.e. 1, 10, µm/ML. The increased inhibition activity of complex was 26.68 ± 1.15%, 53.08 ± 1.70% as against 19.98 ± 0.43, 41.97 ± 0.98 shown by the drug, respectively. The data were statistically significant as at P< 0.05.

Table 4: *In-Vitro* Cytotoxicity of 5-Fluorouracil -Fe complex Against Sarcoma-180 tumor Cell

Compound	Concentration µ M/ml	% inhibition after 8h
5-Fluorouracil	1.0 10	19.98 ± 0.43 (a) (b) 41.97 ± 0.98
Fe (II)5-Fluorouracil Complex	1.0 10	26.68 ± 1.15 53.08 ± 1.70

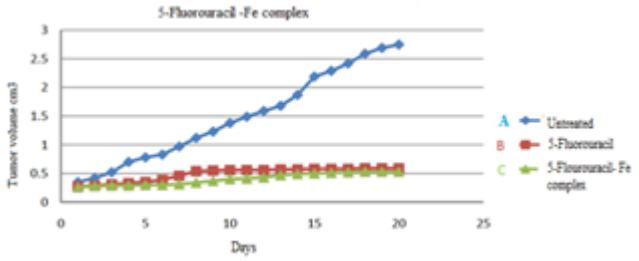

Figure 4: In-Vitro **Cyto-toxic activity of** 5-Fluorouracil (5-FU)-Fe complex

5. Conclusion

The present study explains geometry and nature of the ligand complex which is studied by the electronic absorption spectra. From the spectral studies, the ligand 5–FU (A) and the Fe (II) ion via, de-protonated N3, C_4 = O of carbonyl oxygen atom forming a stable 4 member chelate ring. The Fe (II) ligand complex shows tetrahedral geometry, which is supported by the magnetic moment.

The *in vitro* antimicrobial evaluations show more potent activities for Fe (II) 5 –FU ligand complex [25].

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Author Profile

Dr Shuchita Agrawal has done Ph.D in Chemistry. She has teaching experience of more than 25 years. She has 25 research papers published and presented in International and National Journals.