Increased Anticancer Potancy of Indole 3 Carbinol by Some Life Essential Metals.

Modi G. [1*] **And Pitre K.S.** [2*]
[1] MODI BHAVAN CHAKRAGHAT [2] Ojaswini Women Engineering College, - Sagar (M.P.) , India * garimanmr@yahoo.com

INTRODUCTION

Nature was not providing antibiotic for cancer. Naturally occurring compounds from Vegetables & herbs exert Chemo preventive properties against carcinogenesis (Ala YI 2006). Indole-3-carbinol is the one of the natural drugs obtained many natural products are available as chemo protective agents against commonly occurring cancers occurring from (Cruciferous vegetable broccoli, cauliflower, cabbage) (McDanell R 1988), that is used in the treatment of breast cancer and lung cancer.

Iron is an essential element for most lives on earth including human beings and most bacterial species, since plants and animals. It is an essential element, necessary for sustaining of life. It is estimated that of the hundreds of 3000 proteins in the human body contain zinc prosthetic groups, one type of which is the so-called zinc finger (World Health Organization 2000). Organic electrochemistry has been increasing interest for industrial applications because these are highly Economic, Less intrusive than other chemical process. It is used as analytical tools to observe, prove and predict biological phenomena (Nilsson E 2000, Brett amo 1997). The earliest copper balance study, reported by Tompsett in 1936(Tomsett SL 1934) showed that human excrete about 2.0 - 2.5 mg copper daily in faces, urine and skin. These values are somewhat higher than current estimates (about 1.6 mg copper per day) (Reck SJ 1980) of the minimal copper intake required to maintain balance, but curiously they are inline with the currently accepted recommended dietary allowances of the World Health Organization and the National Research Council (USA) (World Health Organization 1973) (2.0 - 3.0 mg Cu per day).These three metals are require for human body. Electro analytical techniques mainly polarography, cyclic, square wave (Voltametry) etc. Polarography is the one of the oldest tool for the study of stoichiometry and formation constant of Metal-ligand complexes.

MATERIAL AND METHOD

Chemicals and Reagents- Indole-3-carbinol: [C9H9NO, off white crystals, molecular weight-147.18].0.01M stock solution of Indole-3-carbinol was prepared by dissolving the weight amount of the drug in ethanol.

INSTRUMENTATION

(1) Polarography – DCP and DPP, An Elico Micro-processor polarographic analyzer model CL-362, An Elico Digital pH meter-model 335 was used for pH measurement. Polarography cell consists of 3 electrode assembly – Calomel electrode (reference electrode) and Platinum electrode (Auxiliary electrode) Dropping Mercury electrode (working electrode)
Amperometry – Manually operated set-up equipped with a Polyflex Galvanometer (Sensitivity 8 .1 X10 -9) and Ajco Varner potentiometer

Polarographic study of metal-drug complexation equilibria:
Experimental sets were prepared by keeping overall metal ion concentration fixed at 1.0 mM with varying concentration of each drug (0.5, 1.0, 1.5, 2.0, 2.5 10 mM) in 1M Ammonium Tartrate as

supporting electrolyte. The pH of the experimental sets was adjusted as required, by the addition of necessary amount of dilute HCl/NaOH solution. The ionic strength of the test solution was adjusted to m = 1.0. Then polarogram were recorded. Lingane's method was applied for stock metric study. The dissociation constant can be determined by the following equation.

$$(E1/2)\ c - (E1/2)\ s = 0.0591/n \log Kc - 0.0591/n\ P \log Cx$$

Where, (E1/2) c = half wave potential of complexed species at ligand concentration Cx, (E1/2) s = half wave potential of the simple metal ion, Kc = Dissociation constant of the complex species, Cx = concentration of ligand (in moles), n = Number of electrons involve in the electrode process, P = Number of ligand molecules that are coordinated to one metal ion.

Amperometric study of metal-drug complexation equilibria:
For amperometric titration of metal ions with anticancer drugs experimental sets containing varying concentrations of each of the metal ions coverall (0.5, 1.0, 1.5 and 2.0 mm) in 1M Ammonium Tartrate as supporting electrolyte, were prepared pH of the test solution was adjusted to the desired value. The test analyte was deairated using purified hydrogen gas and its pH was rechecked before performing amperometric titrations. The plateau potential of each metal ion was fixed on the potentiometer and current was read on the polyflex galvanometer.

RESULT AND DISCUSTION

The authentic Indole-3-carbinol sample in 1M ammonium tartrate produced a well defined Direct current polarogram DC curve with half wave potential E1/2 = -1324 mV vs SCE, where as the differential pulse polarogram (DPP) of the solution resulted in two well defined peak at) with peak potential Ep = -1210mV and –1324 mV Vs SCE at pH 8.1±0.1.Thous DPP (differential pulse polarography) is more sensitive than DCP (Direct current polarography).

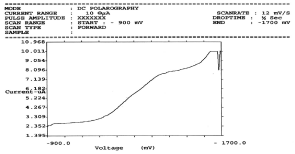

Figure 1: Direct Current Polarogram

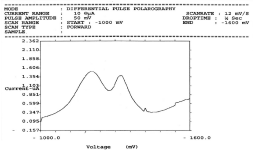

Figure 2: Differential Pulse Polarogram

DCP and DPP of 0.0015 M Indole-3-carbinol in 1 M Ammonium Tartrate at pH 8.1 ± 0.1

Proposed reduction mechanism at DME (Dropping mercury electrode)

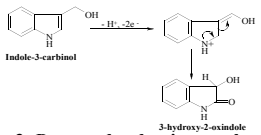

Figure 3: Proposed reduction mechanism at DME

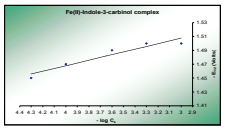

Figure 4: Plots of –E1/2 vs –log Cx for Fe(II)-Indole-3-Carbinol complexes

Results of Lingane's method

On reveling the polarograms of Fe(II),Zn(II),Cu(II) and its complexes shows that on gradual increase of drug concentration the half wave potential of the metal ion shifted to more negative value with the decrease in the diffusion current, thereby revealing the complex formation of Fe(II) Zn(II),Cu(II)- Indole-3-Carbinol.

Complex	Stoichiometry	Formation constant
Cu(II) - Indole-3-Carbinol	1:1	4.82
Fe-(II) - Indole-3-Carbinol	1:1	4.45
Zn-(II) - Indole-3-Carbinol	1:1	5.03

Table 1 : Metal Ligand Complex

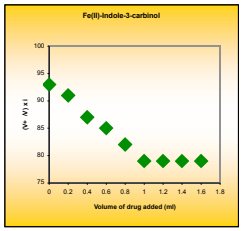

Figure 5 : Plot of L Shape Curve

Fe(II) gives a very well defined two electron cathodic reduction wave in 1.0 M Ammonium Tartrate at pH = 5.5±0.1.Fe(II) solution as titrate and fixing the potential at -1.6 V vs SCE (plateau potential of Fe(II)), the id Vs volume of titrant. Result plot yielded L shaped curve was obtained. The end point as located by graphical method revealed metal to drug ratio of 1:1 which is in agreement with the author's observation on the metal: ligand complexation equilibrium using polarographic method.

Assignments	Indole-3-Carbinol (cm-1)	Fe(II) complex (cm-1)
NH Secondary (s)	3387	Disappear
Aromatic ring joint with 5 membered ring	3059	3059

Table 2: IR spectral analysis of Fe (II), - Indole-3-Carbinol complex

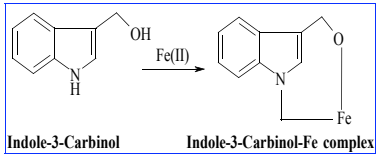

Figure 6: Structure Of Complex with Indole-3-Carbinol

Same result was obtain with Cu-I3C,Zn-I3C complexs

S. No.	Organism	Inhibition-zone (mm)				Control Metal [A]	Percent Change over control metal (A-B/A)100	Control drug (Y)	Percent change over control drug (Y-B/Y)100
		Concentration of complex (mM)				1.0 mM		1.0 mM	
		0.05	0.25	0.5	1.0 [B]				
1	Bacillus pumilus	-	-	18.00	34.00	41.00	12.19	22.00	-63.00
2	Salmonella typhi	-	-	24.00	41.00	53.00	22.64	37.00	-10.81
3	Aspergillus niger	-	-	-	9.00	31.00	70.96	14.00	35.71
4	Fusarium oxysporum	-	-	9.00	15.00	34.00	55.88	17.00	11.76

Table 3: Antimicrobial screening of Zn(II)-Indole-3-carbinol complex

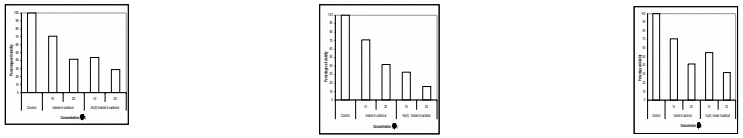

Figure 7: In-vitro Cytotoxic study on Fe(II),Cu(II),Zn(II) - Indole-3-Carbinol complex- by MTT test against HELLA Cell Line

CONCLUSION-

From the data of polarographic, amperometric studies, and IR studies mentioned in different tables and figures in the paper, it is quite clear that Cu (II), Fe (II), Zn (II) complexes of, Indole-3-carbinol, show stoichiometric ratio of 1:1. The complexes of Cu (II) Indole-3-carbinol are not effective against Fusarium oxysporum fungi. On the basis of pharmacological data reveal that complexes are more potent in comparison to parent drugs. At last I would like to say that these complexes are significantly useful in the field of cancer chemotherapy.

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