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Der Chemica Sinica, 2011, 2 (1): 91-99

Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Increased anticancer activity of curcumin-Zn (II) complex by species sensitive method

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ABSTRACT

The complexing ability of anticancer drug curcumin with Zn(II) was studied in aqueous media with in the pH 6.1±0.1 by means of polarography, amperometry and spectrophotometry. The polarogram of acidic media indicated that a chemical reaction has taken place between curcumin and Zn (II) shows that formation of complexes. The complexes behavior of curcumin with Zn (II), both in solid and liquid phase has been studied by polarography, amperometry and spectrophotometry. Curcumin produce a well defines direct current polarogram and differential pulse polarogram in 0.1 M ammonium tartrate (supporting electrolyte) at pH 6.1±0.1. The stoichiometry of Zn(II) - curcumin complex is 1: 1. Anticancer studies on the drug and its metal complex have been performed against sarcoma cells (In Vitro). The observed results revealed the complex to be more potent in anticancer activity as compared to the parent drug.

Key words- Polarography -Amperometry- Curcumin- DCP- DPP.

INTRODUCTION

Curcumin, a yellow spice and pigment from *curcuma long L.* (Zingiberaceae), is by for known for its antioxidant [1-4], anti-inflammatory [5] and anticancer [6,7] activities. Curcumin and its derivatives have shown the ability of being free-radical scavenger, interacting with oxidative cascade, quenching oxygen and chelating and disarming oxidative properties of metal ions [8,9]. Biological activity of curcumin has been attributed to the benzene rings and also to the diketonic structure [10]. The β diketo moiety of curcumin undergoes a keto-enol tautomerism. The strong chelating ability of diketones has been widely investigated towards a great number of metal ions; therefore, curcumin could be of great importance in the chelating treatment of metal intoxication and overload.

Zinc is the most ubiquitous of all trace elements involved in human metabolism. It is an essential element, necessary for sustaining of life. The WHO expert consultation derived upper limits for Zinc intakes [11]. These are based on the observation that 60 mg of supplemental Zinc/day resulted in adverse interactions with other nutrients and considered that intake should not exceed this amount. Zinc is absorbed mainly in the duodenum and proximal small intestine, about 30% of ingested Zinc is absorbed. Then it is distributed to various organs, the highest turnover rates (retentions) according to radio isotopic studies occurring in the liver, kidneys and spleen.

In this study we have elucidated the active chelating site of these ligand and their complexing ability towards Zinc (II) by electrochemical method polarography, amperometry and spectrophotometry [12-13].

MATERIALS AND METHODS

Instrumentation condition

1. **Polarography**- All the polarograms were recorded on Micro-processor (μp) polarographic analyzer model CL-362. An Elico digital pH meter model 335 was used for pH measurement. The polarographic cell consisted of three-electrode assembly with a saturated calomel electrode (reference electrode) and platinum electrode (auxiliary electrode).
2. **Amperometry**- The amperometry titration was performed on a manually operated set- up equipped with a polyflex galvanometer (sensitivity $8.1* 10^{-9}$) and an Ajco varnier potentiometer. DME was used as an indicator electrode and a calomel electrode served as reference electrode. The capillary characteristic of the DME had an $m^{2/3}$, $t^{1/6}$ value of 2.13 $mg^{2/3}$ $sec^{-1/2}$ at 60 cm effective height of mercury column.
3. **Spectrophotometry**- Systronic digital spectrophotometer 166 was used for complex study by spectrophotometric method.

Chemical and Reagents

The chemicals used were of Analar/ BDH grade. The curcumin was of sigma chemical company (St, Louis, Mo). Double distilled water and absolute ethanol was used as solvent pH adjustment were made using dilute solutions of HCl, NaOH whenever necessary.

Preparation of complex

Qualitative and quantitative studies on curcumin were carried on Direct Current Polarography (DCP) and Differential Pulse Polarography (DPP). The pH of the test solution was adjusted to 6.1±0.1.

0.368 g of authentic curcumin was dissolved in 100ml ethanol, set of solutions containing varying concentration of curcumin were prepared in 1 M overall concentration of ammonium tartrate at pH 6.1±0.1 and following the earlier discussed polarographic and amperometric procedures.

Experimental sets were prepared by keeping overall iron (metal ion) ammonium tartrate concentration fixed at 1mM and 0.1M respectively. The ligand concentration was varied from 0 to 15 mM. The pH of the test solution was adjusted to 6.1±0.1.

For amperometric titration experimental sets were prepared by taking different amount of Zn(II) in the cell to which an appropriate amount of ammonium tartrate (supporting electrolyte) was added to make it overall concentration 0.1 M, the pH was adjusted to 6.1±0.1it was then titrated against the standard solution of curcumin at –1.6V Vs saturated calomel electrode (the plateau potential of Zn(II). After each addition of the titrant the current was read on the galvanometer and the current versus volume of titrant added was plotted.

Ethanolic: water (1: 1) solution of curcumin gives an absorbance at 420nm. For spectrophotometeric study of M: L complexation equilibrium. Job's method of continious variation was performed.

Synthesis of solid complex

A brisk red colored solid complex was synthesized by refluxing the 1: 1 aqueous solution of ferrous ammonium sulfate and curcumin solution in water and ethanol (55: 45 v/v) for about 5 hrs. The complexation 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 stored.

Biological study (*In vitro*) of Zn(II) –curcumin complex

Biochemical application is in demand now days. Anticancer activity of metal drug performed against sarcoma 180 cells (13). In vitro cell viability is measured by trypan blue exclusion test that is based on the ability of trypane blue to stain dead cells. A drop of culture is added on aemocytometer and the number of stained, no stained and total number of cells were counted and the percentage inhibition is calculated using following formula

$$\% \text{ Inhibition} = \frac{a - b}{a} \times 100$$

Where 'a' represents the diameter of zone inhibition for control and 'b' for the complex.

Sarcoma 180 cells were purchased from the National Center for Cell Science (NCCS) Pune maintained in DMEM medium (Dulbeco's modified Eagle's medium) supplemented with 10% v/v foetal calf serum, penicillin 100 IU/ ml and streptomycin 100 mg/ml. Cells were obtained as monolayer culture in plastic Roux bottles (corning plastics), cells were harvested using Trypsin version glucose in the exponential growth phase from the Dulbeco's modified eagle medium pre incubated at 37^oC for 24 hrs. The cells were centrifuged to adjust starting cells concentration to 2 X 10^5 cell / ml. A 0.5 ml of DMEM was added to each well and incubated with metal-drug complex containing varying concentrations. It was compared with cells without complex containing similar supplements.

RESULTS AND DISCUSSION

The curcumin sample in 0.1 M ammonium tartrate at pH 6.1±0.1, produced a well defined DC Polarographic curve (Fig 1) with $E_{1/2}$ = -1275 mV Vs SCE, where as the DPP response of the solution resulted in two well defined peaks at (Fig 2) with Ep =-1125 mV and –1275 mV SCE. The peak height of both the peaks in case of DPP of the polarograms was found to be

proportional the curcumin concentration. It was also note that there was no change in E ½ (DCP) and Ep (DPP) values of the resulting polarograms with increasing curcumin electrochemical procedure for the analysis of curcumin.

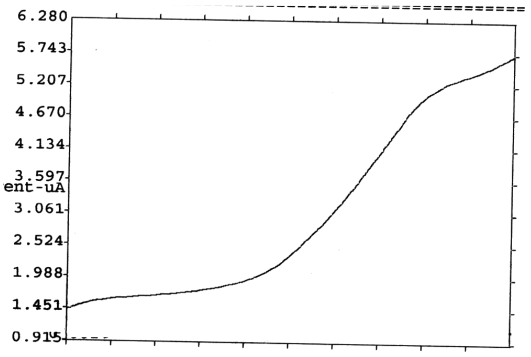

Fig 1 : DCP of 0.0012 M Curcumin in 0.1 M Ammonium tartrate, pH 5.7 ± 0.1

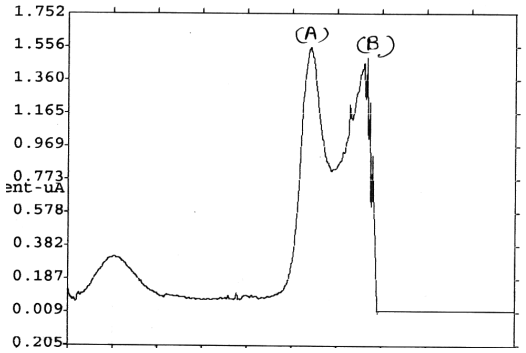

Fig 2: DPP of 0.0012 M Curcumin in 0.1 M Ammonium tartrate, pH 6.1 ± 0.1

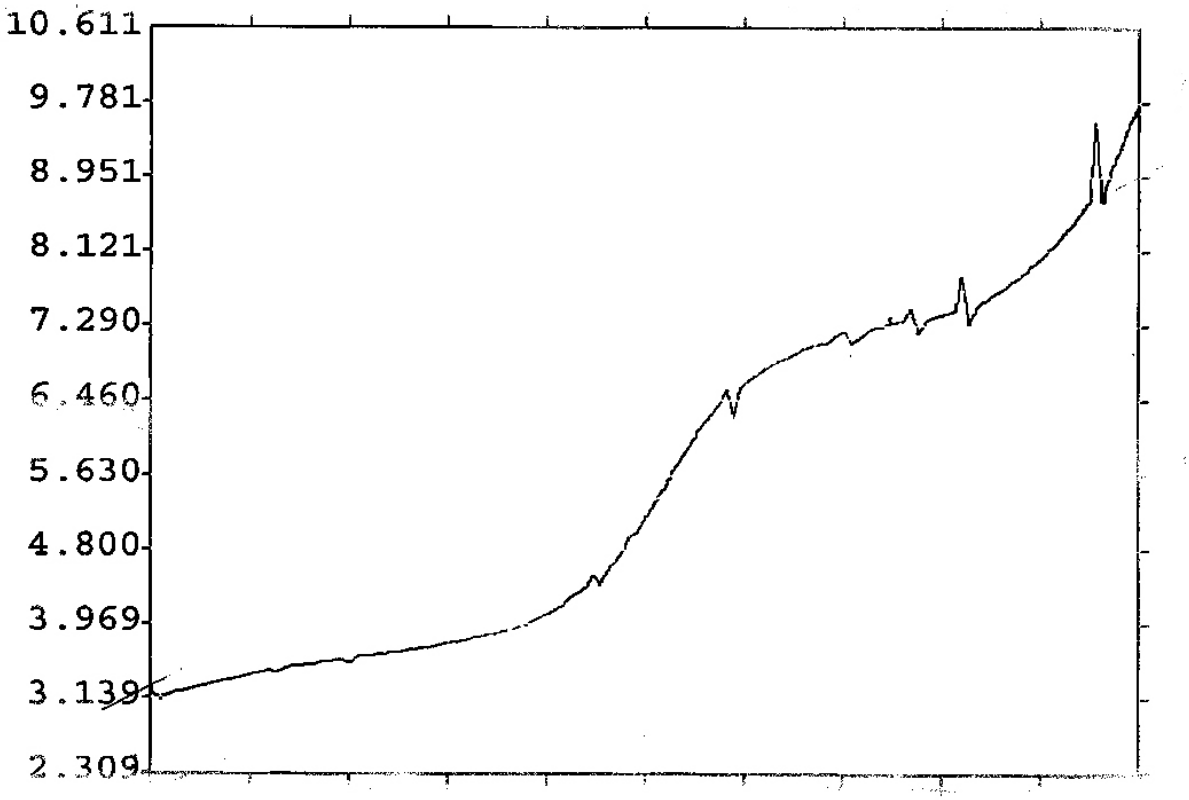

Fig. 3 (a)

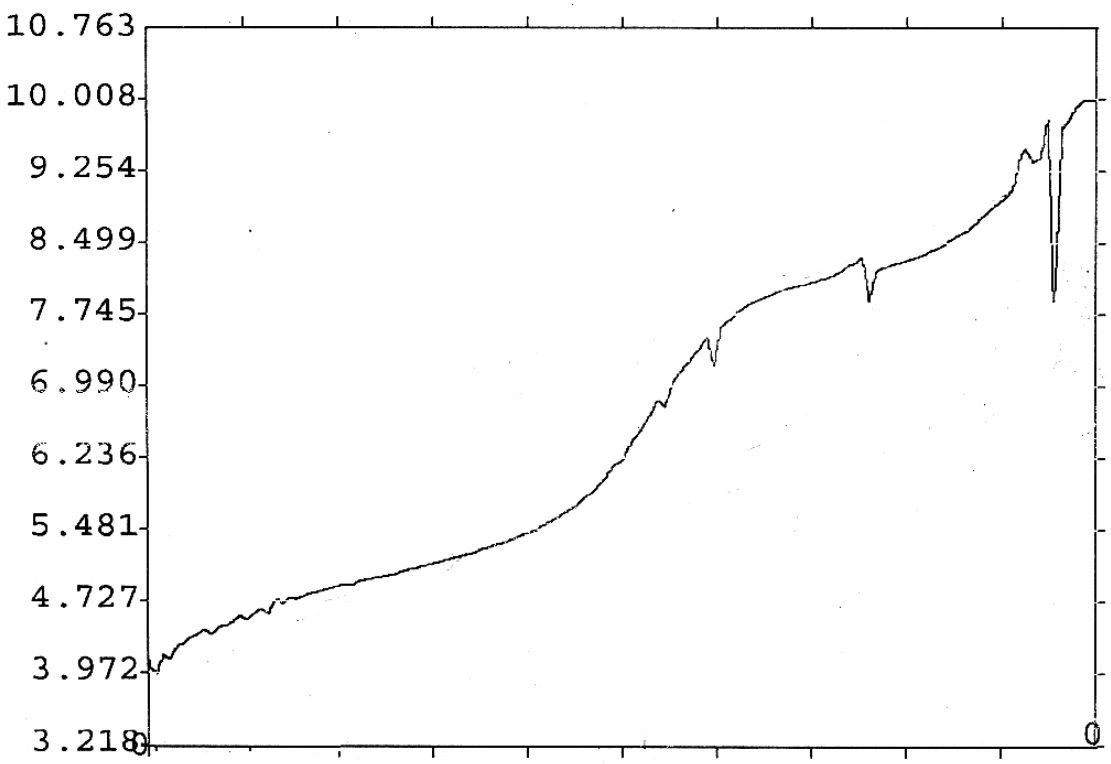

Fig. 3 (b)

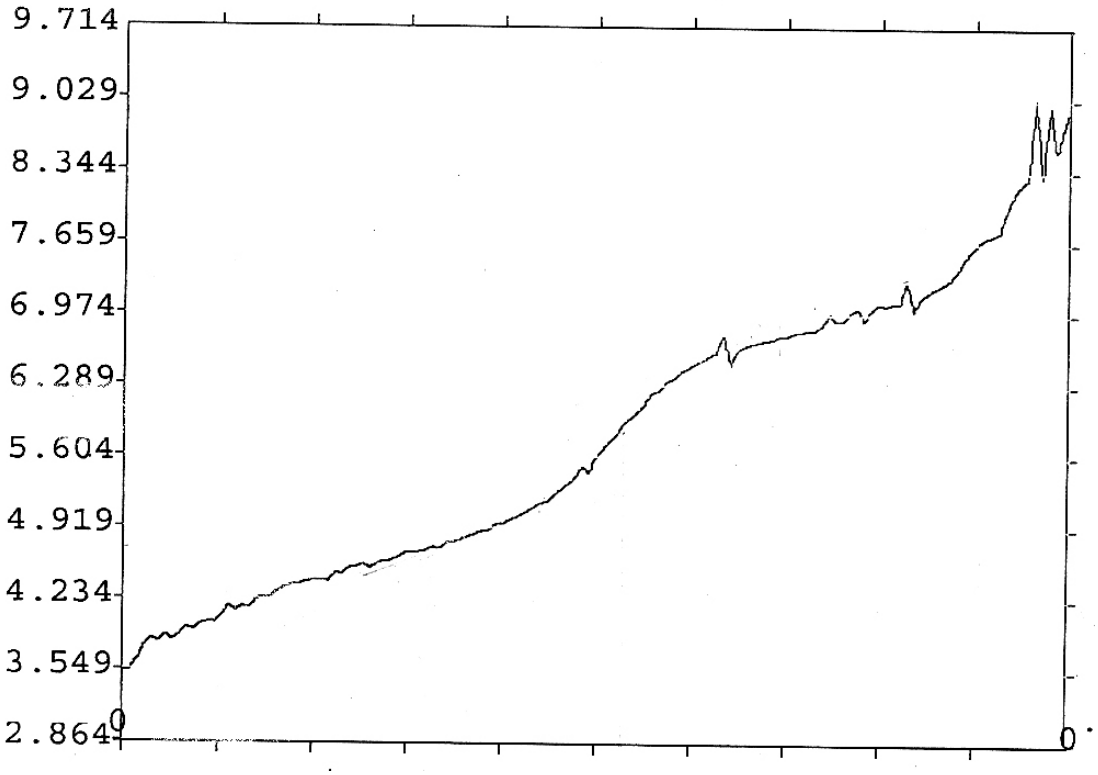

Fig 3 (c)

Fig-3: Direct current polarogram of Zn(II) in 0.1 M Ammonium tartrate, pH 6.1 ± 0.1 presence of curcumin in fig. 3(a) 0.0M, 3(b) 0.0012M, 3(c) 0.005M

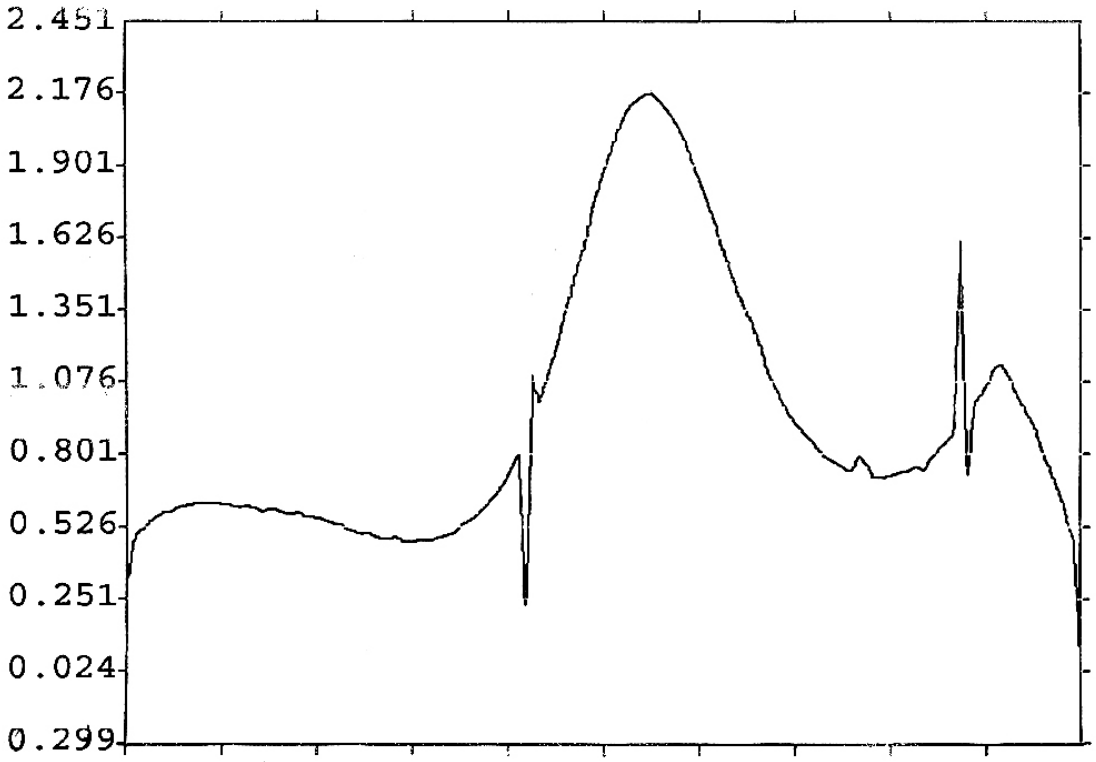
2.451
2.176
1.901
1.626
1.351
1.076
0.801
0.526
0.251
0.024
0.299

Fig 4 (a)

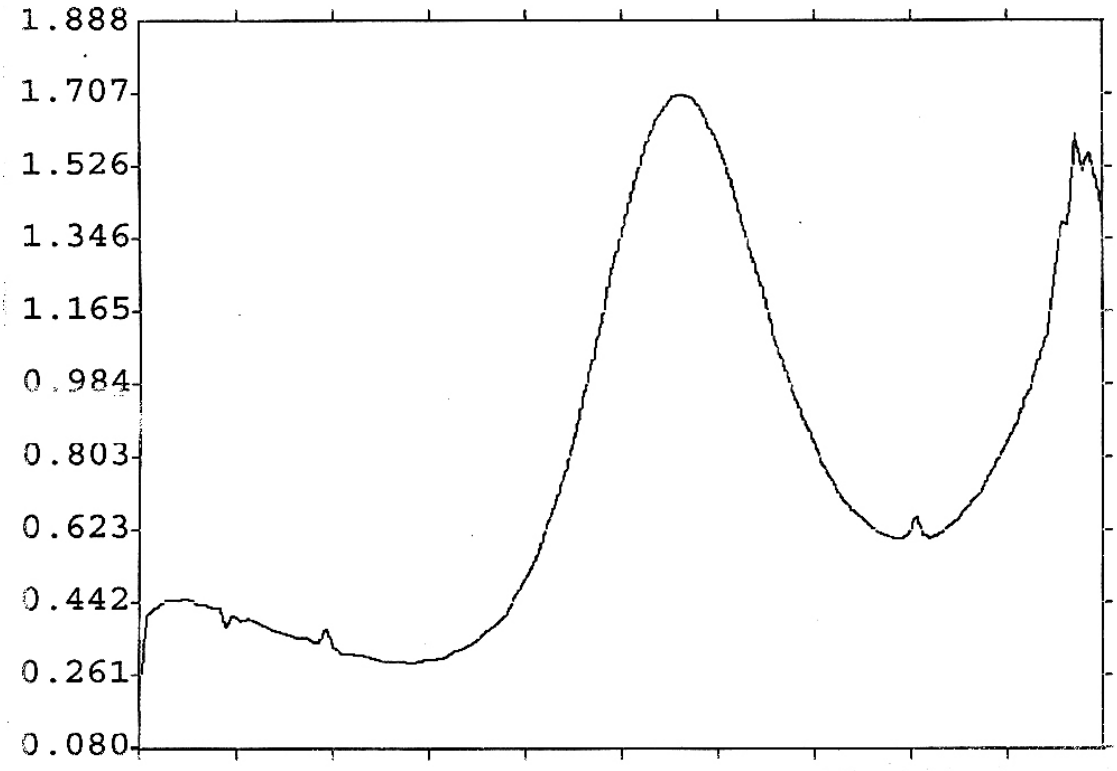
1.888
1.707
1.526
1.346
1.165
0.984
0.803
0.623
0.442
0.261
0.080

Fig 4 (b)

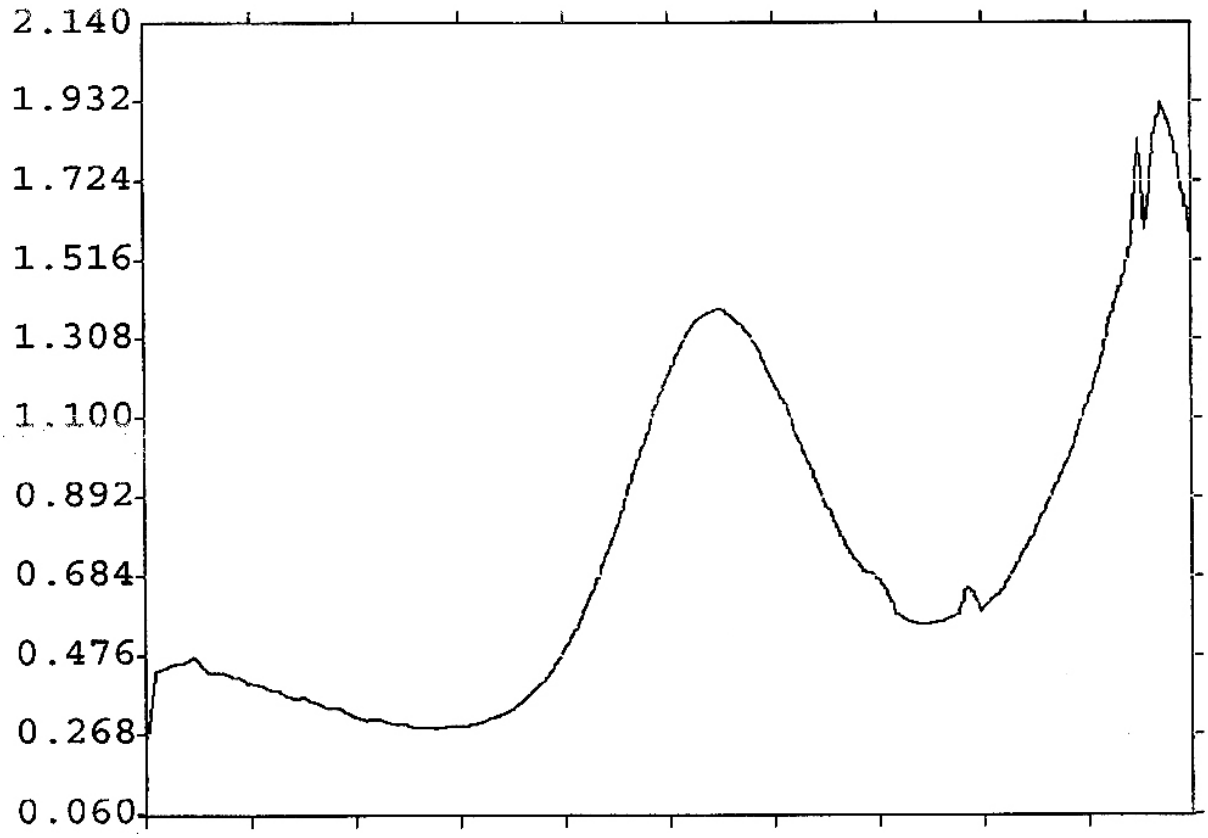

Fig 4 (c)

Fig-3: Diferential pulse polarogram of Zn(II) in 0.1 M Ammonium tartrate, pH 6.1 ± 0.1 presence of curcumin in fig. 4(a) 0.0M, 4(b) 0.0012M, 4(c) 0.005M

Polarographic study of M: L complexation equilibrium

Both Zn(II) and its complex with curcumin ligand produce a reversible two-electrone reduction wave in 0.1 M ammonium tartrate at pH 6.1±0.1. The complex formation between Zn(II) and curcumin (Fig 3 a,b,c) (Fig 4 a,b,c) was revealed by the shift in half-wave potential and peak potential of Zn(II) metal ion to a more electronegative value and decrease in the height of the diffusion current with gradual increase of the curcumin concentration. Plot of $\Delta E_{½}$ [shift in the $E_{½} = (E_{½})_c - (E_{½})_s$] against log Cx (logarithm of the concentration of ligand) resulted in a linear plot. Thus showed the formation of single complex species in solution.

Lingane treatment of the observed polarographic data revealed 1: 1 Zn(II)- curcumin complex formation with formation constant log β=4.10.

Amperometric determination of Curcumin with Zn(II)

As has been mention above that Zn(II) gives a well-defined polarographic wave in 0.1 M ammonium tartrate at pH 6.1±0.1. The diffusion current was found to be proportional to Zn(II) concentration. The plateau potential for the polarographic wave of Zn(II), -1.0V Vs SCE anode was applied for carrying out amperometric titration, Zn(II) was taken as titrate and the drug solution was taken as titrant. The current volume plots resulted in an L Shaped curve (Fig. 5). The end point as located by a graphic method revealed a metal to drug ratio of 1: 1 which is in

agreement with the author's observation on the metal: ligand complexation equilibrium using a polarographic method.

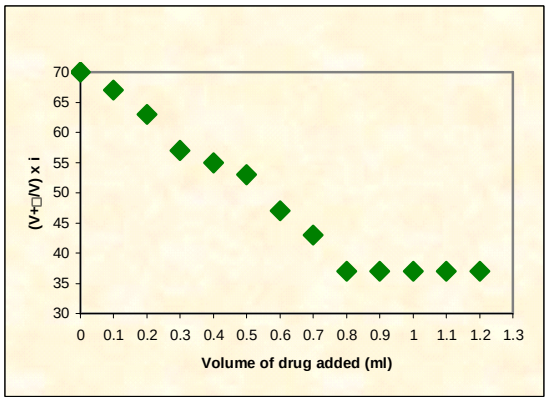

Fig-5: Amperometric titration of 10 mM / 50 ml analyte Zn(II) with 2.5 mM Curcumin in 0.1 M Ammonium tartrate, pH 6.1 ±0.1

Spectrophotometric Determination

Methanolic: water (1:1) solution of curcumin gives a absorption maxima at 420nm for spectrophotometric study of M: L complexation equilibrium, Job's method of continuous variation was used to prepare the test solution (sets) and the absorption intensity of each set was recorded at 420 nm.

The gradual increase of Zn^{2+} concentration in the test solutions resulted in decrease in the intensity of absorption at 420 nm. Thus confirming Zn(II) –curcumin complex formation 1: 1, M: L stoichiometry was observed on plotting

$\frac{[Zn^{2+}]}{[Zn^{2+}]\,[Cur]}$ Vs absorption intensity (Fig 6)

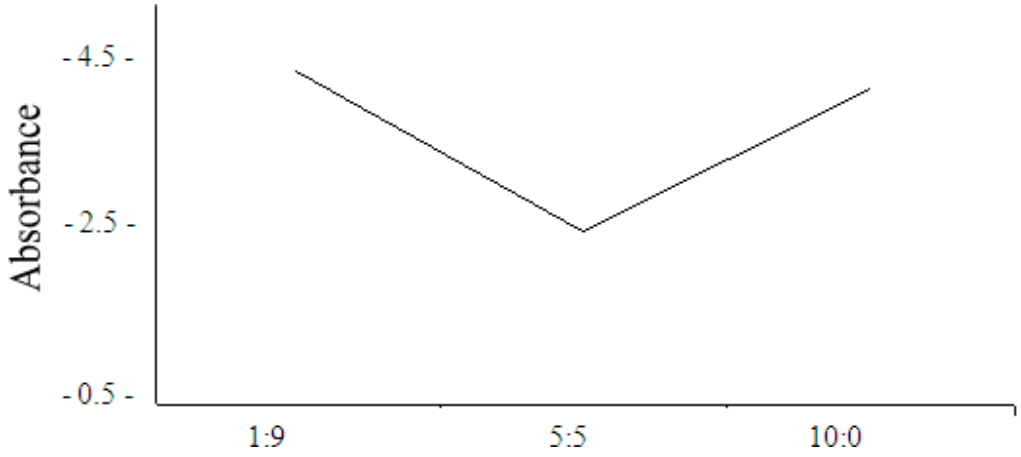

Fig-6: Spectrophotometric determination of Zn(II)- Curcumin complex at pH 6.1 ± 0.1

Biological study (*In vitro)*

Table1 represent the antitumor behavior of curcumin and its Zn(II) complex against sarcoma 180 cells line. The table clearly shows that on increasing drug/ complex concentration form 10 to 100

ng/ml, the percentage inhibition goes on increasing from 5.6% to 53.4% after 2 hrs, 15.6% to 73.8% after 4 hrs of inhibition with pure drug. Whereas Zn(II)- curcumin complex show increased percentage inhibition using similar complex concentration 10 to 100 ng/ ml for the said time intervals i.e. 11.7% to 57.8% after 2 hrs, 23.5% to 78.5% after 4 hrs. It is quite clear that the prepared Zn(II)- curcumin complex is more effective against the sarcoma-180 cell line as compared to the parent drug.

Table 1

Compound	Concentration (ng)	Percentage inhibition after	
		2 hrs	4 hrs
Curcumin	10	5.6	15.6
	50	29.7	49.2
	100	53.4	73.8
Zn(II)-curcumin complex	10	11.7	23.5
	50	35.5	54.5
	100	57.8	78.5

CONCLUSION

The polarographic and amperometric methods could be successfully used for the qualitative and quantitative analysis of curcumin and could be recommended for its use for quality control purpose in the drug industry. In addition in view of the increased to the therapeutic experts for its possible use as a more potent anticancer drug. Statistical treatment of the observed amperometric data clearly reveals the accuracy and precision of curcumin determination.

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