The role of biometal Fe (II) in the increased medicinal potency of curcumin analysed by electrochemical methods

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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.

Iron is one of the ions studied with the curcumin, which has gained quite a relevant position, given its great importance in biological processes such as in oxygen transfer and the DNA synthesis, to mention but a couple of the most important one. The aforementioned underlines the fact that iron plays an important role in human consumption, such that the deferoxiamine turns out to be the only chelating agent used for clinical purpose, because of its low gastrointestinal absorption.

Materials and methods

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 was 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 5.7±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 5.7±0.1 and following the earlier discussed polarographic 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 5.7±0.1.

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 Fe (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 haemocytometer and the number of stained, nonstained and total number of cells were counted and the percentage inhibition is calculated using following formula

$$(\%\ \text{Inhibition}\ ) = \frac{a - b}{a}\ X\ 100$$

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 versin 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 5.7±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.

Polarographic study of M: L complexation equilibrium-

Both Fe (II) and its complex with curcumin ligand produce a reversible two-electrone reduction wave in 0.1 M ammonium tartrate at pH 5.7±0.1. The complex formation between Fe (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 Fe (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 C_x (logarithim 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 Fe (II) - curcumin complex formation with formation constant log β=4.98.

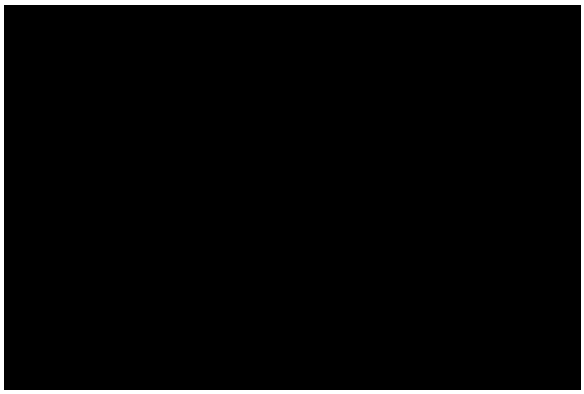

Fig 1 : DCP of 0.0012 M Curcumin in 0.1 M Ammonium tartrate, pH 5.7 ± 0.1

Fig 2: DPP of 0.0012 M Curcumin in 0.1 M Ammonium tartrate, pH 5.7 ± 0.1

3a 3b 3c

Fig-3: Direct current polarogram of Fe (II) in 0.1 M Ammonium tartrate, pH 5.7 ± 0.1 presence of curcumin in fig. 3(a) 0.0M, 3(b) 0.0012M, 3(c) 0.005M.

4a 4b 4c

Fig-4: Differential pulse polarogram of Fe (II) in 0.1 M Ammonium tartrate, pH 5.7 ± 0.1 presence of curcumin in fig. 4(a) 0.0M, 4(b) 0.0012M, 4(c) 0.005M.

Biological study (*In vitro)*

Table1 represent the antitumor behavior of curcumin and its Fe (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 Fe (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 Fe (II)-curcumin complex is more effective against the sarcoma-180 cell line as compared to the parent drug.

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
Fe (II)-curcumin complex	10	11.7	23.5
	50	35.5	54.5
	100	57.8	78.5

Table1- Antitumor behaviour of curcumin and its Fe (II) complex

Conclusion-

The polarographic 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 Fe complex shows increased therapeutic experts for its possible use as a more potent anticancer drug.

References-

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