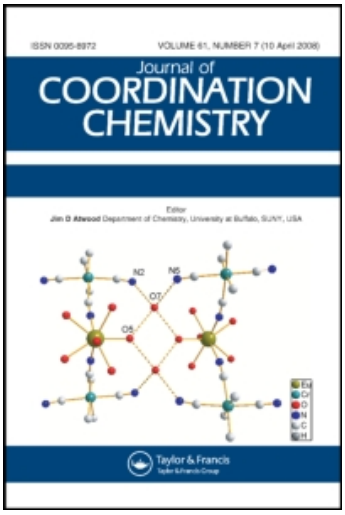


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# The role of Fe(II) in the increased medicinal potency of curcumin analyzed by electrochemical methods

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

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The formation of complexes of curcumin and Fe(II) was studied in aqueous media at pH $5.7 \pm 0.1$ by polarography, amperometry and spectrophotometry. The polarogram indicated formation of complexes between curcumin and Fe(II). Curcumin produces a well-defined direct current polarogram and differential pulse polarogram in 0.1 M ammonium tartrate (supporting electrolyte) at pH $5.7 \pm 0.1$. The stoichiometry of the Fe(II)-curcumin complex is 1 : 1. Anticancer studies on the drug and its metal complex have been performed against sarcoma cells (*in-vitro*), revealing the complex to be more potent in anticancer activity compared to the parent drug.

*Keywords*: Polarography; Amperometry; Curcumin; Iron complex; DCP; DPP



## 1. Introduction

Curcumin, a yellow spice and pigment from curcuma long L. (*Zingiberaceae*) is known for its antioxidant [1–4], anti-inflammatory [5] and anticancer [6, 7] activities. Curcumin and its derivatives are free-radical scavengers, interacting with the oxidative cascade by 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 the diketonic structure [10]. The strong chelating ability of diketones has been investigated towards a number of metal ions and could be of importance in chelating treatment of metal intoxication and overload.

Iron plays an important role in human consumption, such that the deferoxiamine is the only chelating agent used for clinical purposes because of its low gastrointestinal absorption.

In this study we elucidate the active chelating site of curcumin ligands and their complexing ability towards iron(II) by polarography, amperometry, spectrophotometry and IR, continuing our work on the electrochemical, bioinorganic, antibacterial and cytotoxic behavior of some metal-drug complexes [11, 12].

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## 2. Experimental

### 2.1. *Instrumentation*

(1) **Polarography:** All 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 a three-electrode assembly with a saturated calomel electrode (reference electrode) and platinum electrode (auxiliary electrode) and DME (dropping mercury electrode) as working electrode.
(2) **Amperometry:** The amperometry titration was performed manually on a polyflex galvanometer (sensitivity $8.1 \times 10^{-9}$) and an Ajco varnier potentiometer. DME was used as an indicator electrode and a calomel electrode served as reference. 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 a mercury column.
(3) **Spectrophotometry and spectroscopy:** A Systronic digital spectrophotometer 166 was used for complex study. The IR spectrum of solid complex was recorded using KBr pellets on a model 470 IR-spectrophotometer Shimadzu, Japan.



### 2.2. *Chemicals*

The chemicals used were of Analar/BDH grade. The curcumin was from Sigma Chemical Company (St. Louis, MO). Doubly-distilled water and absolute ethanol were solvents; pH adjustment was made using dilute solutions of HCl or NaOH whenever necessary.

### 2.3. *Preparation of complex*

Qualitative and quantitative studies on curcumin were carried out using direct current polarography (DCP) and differential pulse polarography (DPP). The pH of the test solution was adjusted to $5.7 \pm 0.1$ to avoid a matrix effect for electrochemical behavior of curcumin.

Curcumin (0.368 g) was dissolved in 100 mL ethanol and a set of solutions containing varying concentration of curcumin were prepared in 1 M overall concentration of ammonium tartrate at pH $5.7 \pm 0.1$.

For study of stoichiometry and formation of the complex, Lingane's polarographic method was used, a simple method for study of metal ligand equilibria in cases where only one complex is formed over the entire range of ligand concentration.

Experimental solutions were prepared by keeping overall iron (metal ion) and ammonium tartrate concentration fixed at 1 mM and 0.1 M, respectively, while varying the ligand concentration from 0 to 15 mM. The pH was adjusted to $5.7 \pm 0.1$, and the solution was deaerated with purified $H_2$ gas. Polaragram was recorded keeping the initial potential set to $-1100$ mV.

For amperometric titration solutions were prepared by taking different amounts of Fe(II) in the cell to which an appropriate amount of ammonium tartrate (supporting electrolyte) was added to make 0.1 M, the pH was adjusted to $5.7 \pm 0.1$, then titrated against a standard solution of curcumin at $-1.6$ V vs. saturated calomel electrode (the plateau potential of Fe(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 420 nm. For spectrophotometric study of M : L equilibrium, Job's method of continuous variation was performed.

### 2.4. *Synthesis of solid complex*

A brick red solid was synthesized by refluxing 1 : 1 aqueous solution of ferrous ammonium sulfate and curcumin in water and ethanol (55 : 45 v/v) for about 5 h. The complexation was marked by precipitation after reducing the volume to one fourth of the original volume. The product was filtered, washed, dried over $P_4O_{10}$ and stored.

### 2.5. *Biological study of Fe(II) curcumin complex*

The activity of bacteria and some fungi on compounds gives information about the complex, prompting us to screen the complex and precursors to determine which part of the molecule is responsible for its physicochemical activity.



### 2.6. *Antimicrobial study*

Various methods are available for the evaluation of the antibacterial and antifungal activity [13, 14]. In the present study, antimicrobial activity of the complex was evaluated by the well diffusion method [15]. Antibacterial activity was done by using *Bacillus pumilus* and *Salmonella typhi*. Antifungal activity was performed by using *Aspergillus niger* and *Fusarium oxysporum*.

### 2.7. In vitro *cytotoxic study on Fe(II)–curcumin complex*

Cytotoxic activity of metal drug was performed against sarcoma 180 cells [16]. *In vitro* cell viability is measured by trypan blue exclusion test 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 the following formula

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

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 fetal calf serum, penicillin 100 IU $mL^{-1}$ and streptomycin 100 mg $mL^{-1}$. Cells were obtained as monolayer culture in plastic Roux bottles (Corning plastics) and were harvested using trypsin versin glucose in the exponential growth phase from the Dulbeco's modified eagle medium pre-incubated at 37°C for 24 h. The cells were centrifuged to adjust starting cell concentration to $2 \times 10^5$ cell $mL^{-1}$.

DMEM (0.5 mL) was added to each well and incubated with metal-drug complex containing varying concentrations. The results were compared with cells without complex, but containing similar supplements.

## 3. Results and discussion

Curcumin in 0.1 M ammonium tartrate at pH 5.7 ± 0.1 produced a well-defined DC polarographic curve (figure 1) with $E_{1/2} = -1275$ mV vs. SCE, whereas the DPP response of the solution resulted in two well-defined peaks (figure 2) with $E\mathrm{p} = -1125$ mV and −1275 mV SCE. Both peak heights for DPP were proportional to curcumin concentration. Curcumin is polarographically active in both acidic and basic enviorments.

### 3.1. *Polarographic study of M : L complexation equilibrium*

Both Fe(II) and its complex with curcumin produce a reversible two-electron reduction wave in 0.1 M ammonium tartrate at pH 5.7 ± 0.1. Complex formation between Fe(II) and curcumin (Supplementary Material) was revealed by the shift in half-wave potential and peak potential to a more negative 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 $\log C_x$ (logarithm of the concentration of ligand) resulted in a linear plot (figure 3), showing formation of a single complex in solution. Lingane treatment of the observed polarographic data revealed 1 : 1 Fe(II)-curcumin complex with formation constant $\log \beta = 4.98$.



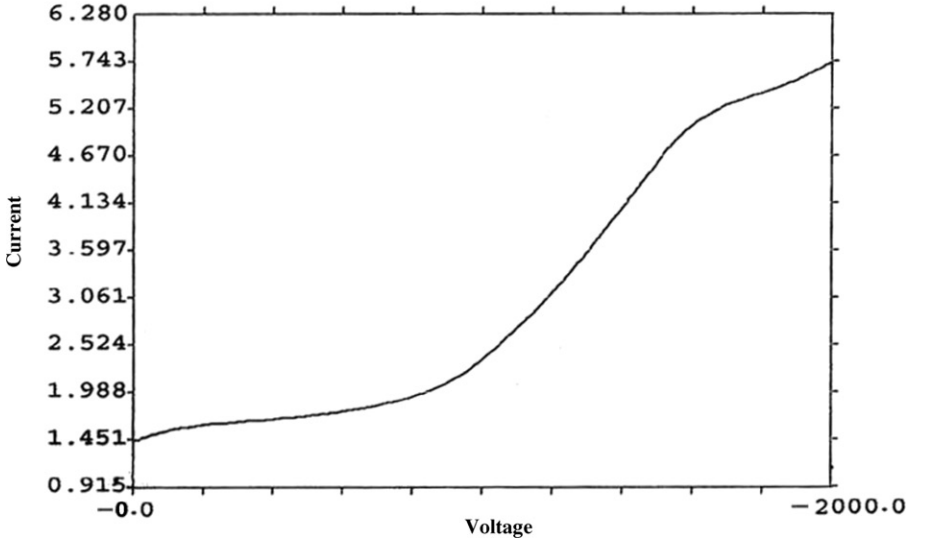



Figure 1. DCP of 0.0012 M curcumin in 0.1 M ammonium tartrate, pH 5.7 ± 0.1.

### 3.2. *Amperometric determination of curcumin with Fe(II)*

Fe(II) gives a well-defined polarographic wave in 0.1 M ammonium tartrate at pH $5.7 \pm 0.1$ with diffusion current proportional to Fe(II) concentration. The plateau potential for the polarographic wave of Fe(II), $-1.6$ V vs. SCE, was applied for amperometric titration, Fe(II) was taken as titrate and curcumin as titrant. The current volume plots resulted in an L-shaped curve (figure 4). The end point located by graphic method revealed a metal to drug ratio of 1 : 1, in agreement with the metal : ligand complexation equilibrium using polarographic method.

### 3.3. *IR spectral analysis of Fe(II)-curcumin complex*

Comparing IR spectra of curcumin and its Fe(II) complex (table 1), show bands at 1640 to 1586 $cm^{-1}$ due to C=O $\beta$-diketone (enolic) [9] form for curcumin, which disappear in

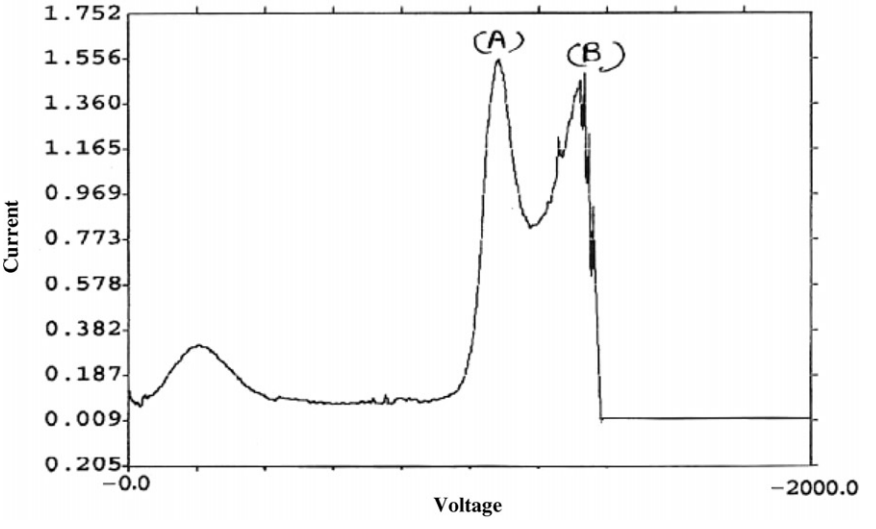



Figure 2. DPP of 0.0012 M curcumin in 0.1 M ammonium tartrate, pH $5.7 \pm 0.1$.

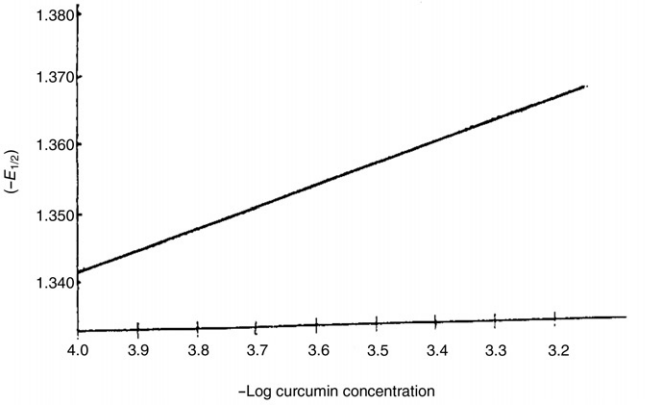



Figure 3. Plot of $-E_{1/2}$ *vs.* $\log C_x$ for Fe(II)-curcumin complex.

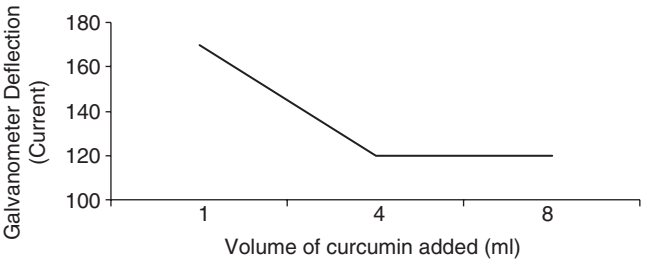


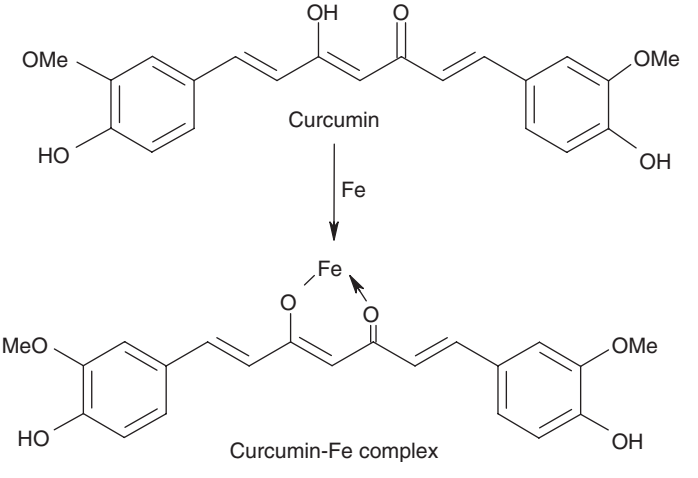

Figure 4. Amperometric titration of 10 mM/50 mL analyte Fe(II) with 2.5 mM curcumin in 0.1 M ammonium tartrate, pH 5.7 ± 0.1.

Table 1. Principal IR signals ($cm^{-1}$) and their assignments for Fe(II)-curcumin complex with parent drug.

| Assignments | Curcumin ($cm^{-1}$) | Fe(II) complex ($cm^{-1}$) |
|---|---|---|
| Phenolichydroxyl OH | 3508 | 3508 |
| C=O $\beta$ diketone (enolic) | 1640–1586 | Disappear |
| C=C (s) | 1489 | 1483 |
| C–H bending | 1387 | 1387 |
| Aromatic C–H (s) | 963–856 | 856 |
| C–O, C–C–C | 1153–1260 | 1153–1260 |



the spectrum of its Fe(II) complex. The sharp OH signal at 3508 $cm^{-1}$ for curcumin is not shifted in the spectrum of Fe(II)-curcumin complex. Thus, on the basis of polarographic, amperometric data and IR studies a tentative structure to 1 : 1, Fe(II)-curcumin complex may be:




### 3.4. *Spectrophotometric determination*

Methanol : water (1 : 1) of curcumin gives an absorption maximum at 420 nm used to study M : L complexation. Job's method of continuous variation was used and the absorption intensity of each set was recorded at 420 nm. Gradual increase

Table 2. Antimicrobial study of Fe(II)-curcumin complex.

| S. No. | Organism | Inhibition-zone (mm) Concentration of complex 0.05 mM | 0.25 mM | 0.5 mM | 1.0 mM [B] | Control metal [A] 1.0 mM | Percent change over control metal (A−B/A)100 | Control drug (Y) 1.0 mM | Percent change over control drug (Y−B/Y)100 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Bacillus pumilus* | 14 | 20 | 28 | 34 | 57 | 40 | 25 | −36 |
| 2 | *Salmonella typhi* | 10 | 24 | 30 | 37 | 41 | 10 | 23 | −61 |
| 3 | *Aspergillus niger* | – | 14 | 20 | 26 | 53 | 51 | 21 | −24 |
| 4 | *Fusarium oxysporum* | – | – | 10 | 12 | 15 | 20 | 11 | −9 |

Table 3. Percent inhibition of curcumin and complex against sarcoma 180.

| Compound | Concentration ($\mu g\,mL^{-1}$) | Percentage inhibition after 2 h | Percentage inhibition after 4 h |
|---|---|---|---|
| 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 |

of $[Fe^{2+}]$ in the solutions resulted in decrease in the intensity of absorption at 420 nm and 1 : 1 Fe(II)–curcumin complex was confirmed (Supplementary Material).



### 3.5. *Antimicrobial study of Fe(II) curcumin complex*

Curcumin is a well-known antibacterial and anticancer agent. The complex exhibits variable activity against *Bacillus pumillus*. The change over control metal is 40% and over control drug is −36%. For *Salmonella typhi* the change over control metal and drug is 10 and −61%, respectively. The metal drug and their complex show toxicity against *Aspergillus niger* and *Fusarium oxysporum*. The results of antimicrobial study are shown in table 2.

### 3.6. *Biological study* (in vitro)

Table 3 represents the cytotoxic behavior of curcumin and its Fe(II) complex against sarcoma 180 cell line. The table clearly shows that on increasing drug/complex concentration form 10 to 100 $\mu g\,mL^{-1}$, the percentage inhibition continues increasing from 5.6 to 53.4% after 2 h and 15.6 to 73.8% after 4 h of inhibition with pure drug. Fe(II)-curcumin complex shows increased inhibition using similar concentration, i.e. 11.7 to 57.8% after 2 h, 23.5 to 78.5% after 4 h. The Fe(II)-curcumin complex is more effective against the sarcoma-180 cell line than the parent drug.

## 4. Conclusion

The data show stoichiometric ratio of 1 : 1 for the Fe(II) curcumin complex.

Antimicrobial studies on the metal-drug complex show toxicity against bacteria and fungi and the complexes are more potent than curcumin.

The polarographic/amperometric methods are used for qualitative and quantitative analysis of curcumin and are recommended for quality control in the drug industry. Statistical treatment of the observed amperometric data clearly reveals the accuracy and precision of curcumin determination. The increased potency of the complex may allow use as a potent anticancer drug.

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