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The effect of metal ions on the electrochemistry of the furazolidone

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Abstract

A study of the complexing properties of the furazolidone (Fu) with cadmium, copper, nickel, zinc and cobalt was performed in dimethylformamide (DMF) using cyclic voltammetry (CV) and differential pulse (DP) methods with glassy carbon (GC) and dropping mercury electrode (DME). The applied methods allow one to distinguish between the free drugs, free metal ions and their complexes as well as provide evidence that different forms of complexation exist. In aprotic DMF medium, the four electrons reductive peak of the nitro group (NO_2) to hydroxylamine (RNHOH) was changed to a one electron reversible peak corresponding to $RNO_2^{\bullet -}$ and to a subsequent more negative three electrons irreversible peak due to RNHOH.

The one electron reduction of Fu to the nitro radical anion $RNO_2^{\bullet -}$ is slightly shifted to positive potentials upon addition of Cd(II), Ni(II), Zn(II), Cu(II) and Co(II) and is consistent with the formation of complexes involving the nitro group and the oxygen of the adjacent furan of Fu. The redox potential of the hydroxylamine in Fu was also shifted to a more positive potential in the presence of metal ions, but their currents decreased. The complexation at different mole ratios was also investigated. The results suggest that in vivo, metal ions, such as Cd(II), Ni(II), Zn(II), Cu(II) and Co(II) facilitate the initial reduction of Fu by capturing the $RNO_2^{\bullet -}$ after Fu is reduced by biological reductants.

Keywords: Furazolidone; Cyclic voltammetry; Metal ions; Complex; Nitro radical anion

1. Introduction

Furazolidone and other nitrofuran derivatives have been used for more than 30 years in medicine for the treatment of gastrointestinal infections in animals and humans. The main pharmaceutical uses of nitro aromatic compounds (RNO_2) are as antibacterial and anticancer agents [1]. It has been reported that nitro compounds generate a reversible one electron process due to the formation of the nitro radical anion ($RNO_2^{\bullet -}$) and an irreversible three electrons process corresponding to the formation of the hydroxylamine (RNHOH) in aprotic media [2,3]. Redox properties control most biological responses of nitro compounds, and the formation of the couple $RNO_2/RNO_2^{\bullet -}$, seems to be an obligatory intermediate for therapeutic selectivity towards anaerobes [4].

Free radicals are in general reactive species that can be of benefit to an organism, e.g., the radicals produced during phagocytosis, or it can be a liability and produce DNA damage or lipid peroxidation [5,6]. Furthermore, it can be concluded that Fu can potentially behave as a cytotoxic drug, which produces the $RNO_2^{\bullet -}$ and affects the oxygen tension present in cells. The relevance of the latter factor lies in the fact that it permits the rapid reoxidation of the radical. The risk of cytotoxity is minimized in aerobic mammalian cells [6,7]. Studies on the action of nitroaromatic drugs suggest that the reduction of the nitro group produces a transient species that interacts with DNA resulting in damage characterized by the helix destabilization and strand breakage [5]. In common with other DNA-cleavaging drugs containing the nitro group, in vivo experiments have provided clear evidence for free radical-mediated DNA strand cleavage by Fu in a process

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involving metal ions and oxygen [6]. The redox potential of the nitro group is an essential feature in causing DNA damage, and a number of species including $RNO_2^{\bullet -}$ radicals have been postulated to cause DNA damage [8–14].

Of the vast array of therapeutic agents, majority of them are known to enhance their biological properties upon complex formation [15]. Metal complexes are well known for their biological activity [16]. Several studies have been reported in the literature on the polarographic determination of Fu [17,18]. Furthermore, electrochemical and antimicrobial studies on Cu(II), Zn(II) and Co(II) chelates of histamine and chloroamphincal have been performed by a number of workers [19].

In the best of our knowledge, there is no report on the complexation of furazolidone except one in which the bacterial effect of furazolidone and some of its synthesized metal complexes were investigated [20]. They investigated the remarkable efficacy of some chemically synthesized metal furazolidone complexes against the various strains of bacteria and fungi, viz., pseudomonas, mangiferae, *Bacillus pumillus* and *Salmonella typhi* and suggested their application for the treatment of fungal skin infections.

Fu at protic media shows two reductive peaks due to the 4e-reduction of the nitro group to form the corresponding hydroxylamine (RNHOH) and 2e-reduction of the corresponding hydroxylamine to form the amine (RNH_2), respectively as is usual for nitroaromatic compounds [17,21]. However, at aprotic media (in DMF or high pH) the peak due to a 4e-transfer was changed to a 1e-reversible peak corresponding to $RNO_2/RNO_2^{\bullet -}$ couple with a subsequent more negative 3e-irreversible peak due to RNHOH according to the following equations:

$$RNO_2 + 1e \rightleftharpoons RNO_2^{\bullet -}$$

$$RNO_2^{\bullet -} + 3e + 4H^+ \rightarrow RNHOH + H_2O$$

In the previous works [22,23], we reported on the stabilization of $RNO_2^{\bullet -}$ at aprotic and obtained some of its kinetic parameters. In continuation of our reports on the redox chemistry of Fu in aprotic DMF solution and the effect of metal ions on the redox potential of Fu, we investigated whether it is feasible for metal ions to be agents for the activation of biological processes. Finally, these data are essential for the design of stable metal complexes of Fu as second generation Fu derivatives with potential clinical application.

Various metal ions have been added to the Fu solution and changes in the composition of the solution have been monitored using cyclic voltammetry and differential pulse methods with the aim of investigating the binding and metal exchange properties.

2. Experimental

2.1. Apparatus

Electrochemical experiments were performed using a Metrohm model 746 VA trace analyzer connected a 747 VA stand. A glassy carbon electrode (0.2 mm diameter) was used as the working electrode which was polished sequentially with alumina powder. A multimode mercury electrode (Metrohm) was also used as a working electrode. A platinum wire and a commercial KCl saturated Ag/AgCl electrode from Metrohm were used as the auxiliary and reference electrodes, respectively. The potential of the peak was measured to within ±0.01 V. The solutions were purged with 99.999% argon for 10 min before the start of the experiments. All of the studies were carried out in an inert atmosphere at room temperature.

2.2. Reagents

Fu was purchased from Sigma for basic studies. All the other reagents employed were of analytical grade without further purification. Dimethylformamide (DMF) and tetrabutylammonium perchlorate (TBAP) from Merck were used as solvent and supporting electrolyte, respectively. The nitrate salts of all metal ions were used.

3. Results and discussion

3.1. Cyclic voltammetry of Fu

The cyclic voltammogram of Fu in a wide potential range shows two cathodic peaks at −0.89 and −1.48 V. The linear dependence of the current on concentration shows a diffusion controlled process. In this work, we focus on the effect of various metal ions on the reduction mechanism of Fu. The possible complex formation reaction between either the toxic, non-essential metal ions, e.g., Cd(II), Co(II), Ni(II) or the essential but toxic metal ions, e.g., Cu(II) and Zn(II) with Fu is investigated. Their complexation behavior was verified by two cyclic voltammetry and differential pulse methods on GC and DME electrodes.

3.2. The effect of various metal ions on the reduction of Fu

Fig. 1 shows comparative voltammograms for: (a) blank (0.1 TBAP in DMF); (b) furazolidone alone; (c) the furazolidone and the various metal ions (1:1). As indicated from the figure, there is an evidence of a complex formation reaction between furazolidone and all metal ions. This is obvious from the positive shift in reduction potentials and decreasing diffusion currents. Upon the addition of one equivalent of a metal ion, the potential of first cathodic peak, E_{c1}, ($RNO_2/RNO_2^{\bullet -}$ couple) of Fu slightly shifted to positive potential (about 10–80 mV). The small positive shifts in the first reduction potential is consistent with the complexation of the $Fu^{\bullet -}$ to the metal ions, which is due to stabilization of nitro radical anion ($RNO_2^{\bullet -}$) with respect to the Fu by the positive charge of the metal ions. Thus, it can be suggested that Fu binds via the NO_2 group and the adjacent oxygen of the furan ring.

The effect of various metal ions on the second cathodic peak (formation of hydroxylamine) showed similar behavior and suggests that a number of different processes

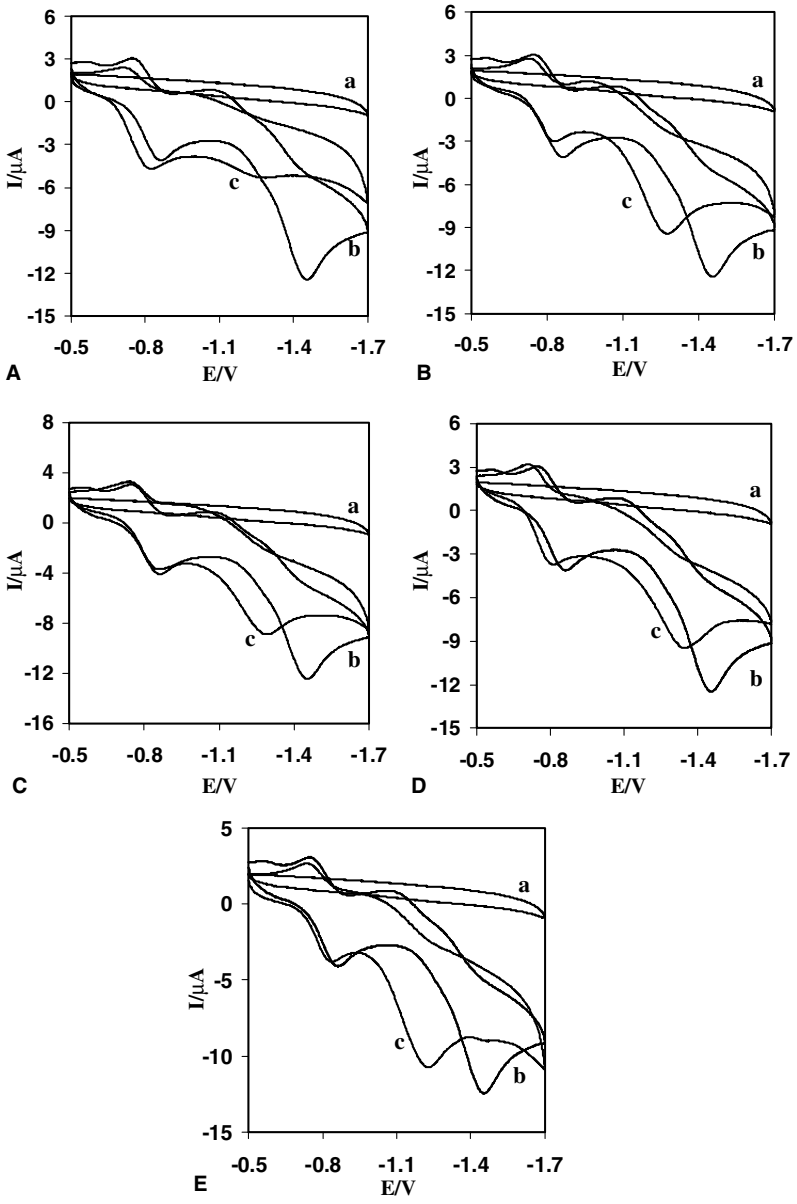


Fig. 1. CVs of Fu(1 mM) in the presence of: (A) Co(II), (B) Ni(II), (C) Zn(II), (D) Cd(II), and (E) Cu(II). (a) Blank, (b) Fu alone, (c) 1:1 complex of metal-Fu, in DMF, TBAP 0.1 M, on GC, scan rate 100 mV s^{-1}.

involving metal complexes are taking place. In all cases, the second peak (E_{c2}) showed a large positive shift (110–230 mV) with further reducing of its current. The change in E_{c2} value of Fu upon the addition of one equivalent of a metal ion followed the order Cu(II) > Co(II) > Ni(II) ≈ Zn(II) > Cd(II). In the case of Co(II), the current approximately disappeared. The effect of metal ions on the potential and the current of Fu are summarized in Table 1. It should be mentioned that in agreement with the forgoing results for first cathodic peak, the values of potential obtained for second peak confirm the complex formation. On the other hand, the positive shift in the reduction potential of the complexed Fu corresponds to the reduction of the negative charge of $RNO_2^{\bullet -}$ due to its complexation with the metal ion which increases its tendency to reduce.

The reduction of its current is due to the bulky structure of one mole of metal ion complexed by a ligand. This causes reduction of its diffusion current. Compare curves in Fig. 1.

The redox potential of the Fu was shifted positive in the presence of the metal ions (Table 1), but both the magnitude of the increase and the relative influence of the metals were different. As it can be seen in Fig. 1, Cu(II) complex shows the most positive shift in second cathodic peak, while the less positive shift is observed for Cd(II) complex. This means that copper complex could be reduced more easily than the other metal complexes. It was previously reported that most of the copper complexes with antihistaminic and antiseptic drugs are more potent than as compared to free drugs [24].

Table 1
The cathodic potentials and currents of Fu in the presence of 1:1 various metal (1:1) in DMF on glassy carbon electrode

Metal	$-E_{c1}$ (V)	$-E_{a1}$ (V)	$-E_{c2}$ (V)	$-I_{c2}$ (μA)
Fu	0.89	0.77	1.48	9.0
Cd(II)	0.82	0.73	1.37	5.5
Zn(II)	0.88	0.75	1.31	5.0
Ni(II)	0.82	0.74	1.32	5.6
Cu(II)	0.82	0.75	1.25	6.1
Co(II)	0.82	0.73	1.23	1.0

In comparing the diffusion currents during complex formation, it is noticed that copper retains its well-defined peak and the decrease in the diffusion current is less than in the other metal ions. On the other hand, Co(II)'s second reductive peak virtually disappeared. It appears that Co(II) in its complex with Fu forms a solid complex, and no reductive peak was observed.

3.3. Complex formation at different mole ratios

In order to understand the stoichiometries of the complexes of Fu with various metal ions, voltammetric measurements were carried out in two different ways, either metal solution was added to the Fu solution or a Fu solution was added to the metal solution. In both cases, differential pulse polarograms were recorded at different ligand-to-metal ratios (L/M).

Fig. 2 shows the DPs of Fu in the presence of different L/M ratios of typically Zn(II) ion on DME. DPs of Fu show two 1e- and 3e-peaks corresponding to $RNO_2/RNO_2^{\bullet -}$ ($E_{c1} = -0.80$ V) and hydroxylamine ($E_{c2} = -1.29$ V), respectively at approximately same potential in cyclic voltammetry (Table 1). A new complex peak at the potential about −0.64 V appeared which is near the first cathodic peak of Fu and results in the broadening of this peak. Since the current of both peaks decreased it can be concluded that this metal ion complexes with the NO_2 group when the concentration of Fu is high. The plot of the current of the second cathodic peak (I_{c2}) vs. L/M ratios is shown in Fig. 2, inset. It is observed from the plot that by adding a metal (thus decreasing the L/M ratio) the peak height of I_{c2} is decreased. This plot shows an inflection point at 1:1 ratio, which confirms the formation of the ML complex.

The DPs of the Zn(II) ion in the different L/M ratios of Fu are shown in Fig. 3. Note that the Zn(II) ion shows a reductive peak at $E = -0.96$ V on DME in DMF in which the addition of the ligand-to-metal solution results in the reduction of the metal peak current (I_{c2}) and an increase in the current of the new complex peak at −0.60 V (I_{c1}) up to L/M ratio of 0.5 (Fig. 3A). As it is observed in Fig. 3B, by continuing the Fu addition (mole ratios higher than 0.5) two reductive peaks of Fu were appeared at $E_{c3} = -0.80$ V and $E_{c4} = -1.29$ V and their currents were increased by increasing concentration. In this manner, the plot of current of the second peak (I_{c2}) vs. L/M ratios shows an inflection at 1:2 ligand:metal ratio and L/M = 0.5 (Fig. 3A, inset). Thus, as the concentration of Fu increases, the reduction of the peak current of Zn(II) is due to the binding of two Zn(II) ions with two complexing sites in a molecule of Fu and the formation of M_2L.

The variation in redox potential for Fu metal complexes in comparison to the Fu free drug is most likely related to the different coordination geometries and donor atoms in Fu metal complex. When the Fu concentration is high at the first step of metal ion addition to Fu solution, each

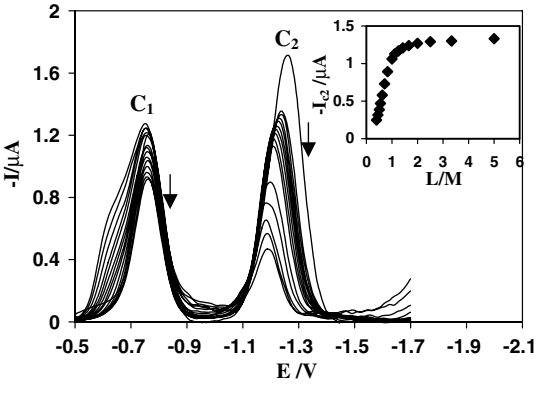


Fig. 2. DPs of Fu (1 mM) in the presence of different ligand-to-metal ratios; Fu/Zn: 0.40, 0.45, 0.50, 0.55, 0.62, 0.71, 0.85, 1.00, 1.11, 1.25, 1.42, 1.66, 2.00, 2.50, 3.33 and 5.00 in DMF on DME. Inset: the plot of current of second peak vs. L/M ratios.

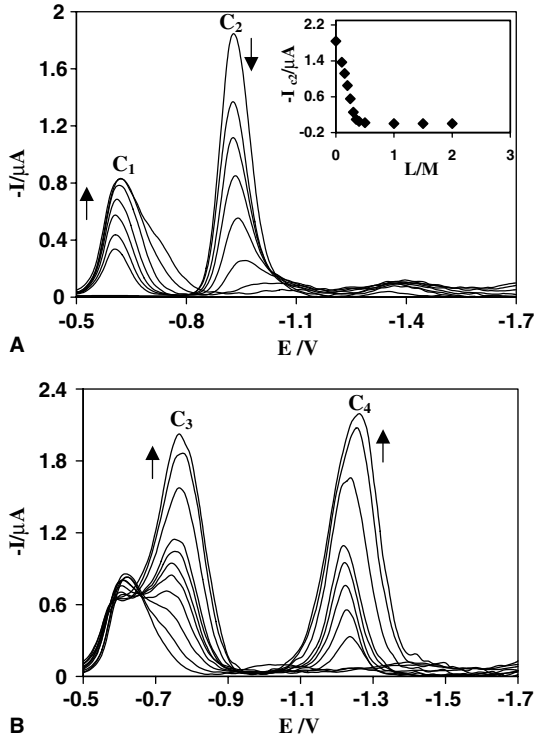
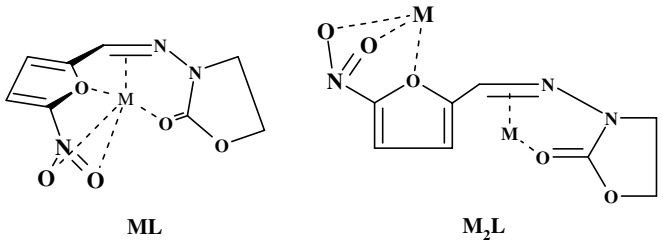
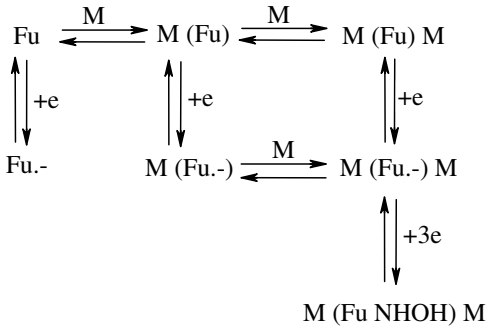


Fig. 3. (A) DPs of Zn(II) (1 mM) in the presence of different ligand-to-metal ratios; Fu/Zn: 0.00, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 in DMF on DME. Inset: the plot of current of second peak vs. L/M ratios. (B) Same as (A), continuing mole ratios, Fu/Zn: 0.50, 1.00, 1.50, 2.00, 2.10, 2.30, 2.50, 2.80, 3.00, 3.50, 4.00.



Scheme 1.



Scheme 2.

metal ion is complexed with one Fu and the resulting complex is ML (Fig. 2). In contrast when Fu concentration is low (the addition of Fu to metal ion solution) M_2L complex formed (Fig. 3). In this manner, each Fu was complexed from two complexing sites by two ions (Scheme 1). Similar observations have been reported previously [20].

The similarity in the behavior of all metal ions and the large effect on the electrochemistry in each case suggest that Fu binds via two different complexing sites. The NO_2 group and adjacent oxygen of furan ring (site 1) is one complexing site and the other one is imine moiety (C=N) and the oxygen of heterocycle (site 2).

In a high concentration of Fu (ML complex), the metal ion binds via the NO_2 group and the adjacent oxygen of the furan ring. It seems that after the formation of ML, the molecule wraps around the metal ion as shown in scheme 1 and decreases the probability of binding of the second metal ion. When the concentration of the metal ion is high, the addition of Fu to the metal solution causes the metal ions to complex with both sites and results in an M_2L complex (Scheme 2). It can be seen that the reduction potentials of two different complexes, ML and M_2L are -0.64 and -0.60 V, respectively, which is a good indication of formation of two different complexes.

4. Conclusions

It is well known that deficiencies of trace elements can occur for general reasons, i.e., vitamin deficiencies [25]. The possible complex formation reaction that may occur between metal ions and Fu may give an image of what happens when administrating a drug. For this purpose, electrochemical properties of Fu with two complexing sites, RNO_2 and C–N groups, in the presence of various metal ions was investigated to elucidate and confirm the possible complexation reaction of Fu with used metal ions.

The voltammetric measurements indicated the existence of 1:1 metal:drug ratio (when the concentration of drug is high) and 2:1 metal:drug ratio (when the concentration of metal is high) which is in a good agreement with the stoichiometric of the isolated solid complexes [20].

It is worthwhile to mention the involvement of the two different sites of drug during the complexation with metal ions, imine moiety (C=N) and the RNO_2 group. The potentials of two cathodic peaks of Fu were shifted positive in the presence of the metal ions, but the magnitude of the positive shift in second peak is more significant. Since the nitro anion radical, $RNO_2^{\bullet -}$, was produced at first cathodic peak and then consumed at second one, complexation of $RNO_2^{\bullet -}$ with metal ions results to more positive shift in second peak. By uptaking one electron by Fu, the electron is localized on the oxygen atoms of RNO_2 group of the furan ring. Complexation of $Fu^{\bullet -}$ with one equivalent of each metal significantly changes the electron distribution in the nitro group, shifting the electron density to the heterocycle ring. The small positive shift in first cathodic peak is corresponds to a stabilization of $Fu^{\bullet -}$ with respect to Fu, consistent with the stabilization of the local negative charge of the drug by the metal ions. On the other hand, the more positive shift in second cathodic peak is due to decreasing the negative charge of $Fu^{\bullet -}$ upon its complexation with metal ions which increases its tendency to reduction in 3e-irreversible peak.

The results of this work suggest that in vivo, the reduction of Fu is a reversible one-electron process, forming $Fu^{\bullet -}$ which is complexed by metal ions which could be delivered to DNA. Cu(II) acetate has been shown to accelerate the rate of streptonigrin-mediated DNA cleavage in the presence of NADH [14]. In contrast, Co(II) has no effect on the streptonigrin-mediated DNA cleavage [14], and Co (II) has been reported to act as radical inhibitor [26]. This study has shown that divalent transition metal increased the accessibility of the nitro redox chemistry to biological reductants. While many nitro radical anions anticancer drugs have been studied in detailed, the relationship between the rate of DNA cleavage and anticancer activity is unknown. The results of this work suggest that metal complexes of Fu will accelerate the rate of DNA cleavage compared to the free drug.

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