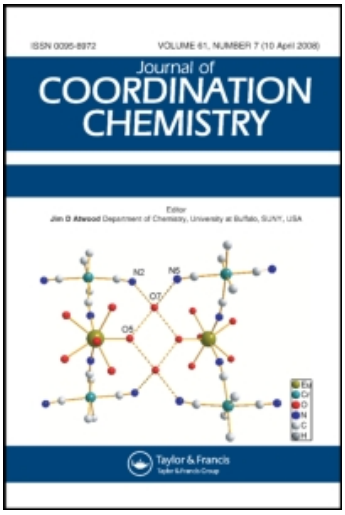

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Voltammetric study of the interaction of pentoxifylline (PTX) with Zn(II) in the presence and absence of cysteine

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Voltammetric study of the interaction of pentoxifylline (PTX) with Zn(II) in the presence and absence of cysteine

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The interaction of pentoxifylline (PTX) with Zn(II) in the presence and absence of cysteine at physiological pH (7.40) was investigated for the first time by square-wave and cyclic voltammetry techniques. The Zn(II)–PTX complex was found to be an electroinactive inert complex, the composition of the formed complex is 1 : 1 (metal : ligand), and the logarithm of its stability constant (log $\beta_{1:1}$) was determined as 3.46 by direct monitoring of the current of free zinc(II). The logarithm of the stability constant (log $\beta_{1:2}$) and stoichiometry of the complexation of Zn(II) with cysteine were determined to be 9.94 and 1 : 2, respectively. The stability constants were in agreement with those calculated from electronic spectral data. In the presence of cysteine, Zn(II)–PTX dissociated and an irreversible peak for Zn(II)–cysteine appeared at −1.342 V. Cysteine prevents complex formation of Zn(II) with PTX.

Keywords: Pentoxifylline; Zinc; Cysteine; Voltammetry

1. Introduction

Pentoxifylline (PTX), a tri-substituted purine and xanthine derivative (scheme 1), is a hemorheologic agent used for the treatment of peripheral arterial disease [1] and intermittent claudication [2]. It improves blood flow through the peripheral circulation by decreasing blood viscosity, inhibiting platelet aggregation, enhancing erythrocyte flexibility and diminishing fibrinogen concentration [3]. Besides these well-known hemorheological properties, PTX has also been found to exert a wide range of immunological activities. It has been reported that pentoxifylline disturbs polarization and migration of human leukocytes [4]. Oxypurines xanthine, hypoxanthine and uric acid are products of metabolism of nucleotides [5].

In humans Zn^{2+} occurs in over 20 metalloenzymes, including several involved in nucleic acid metabolism. Most of the Zn^{2+} in blood is found in the red cells as an

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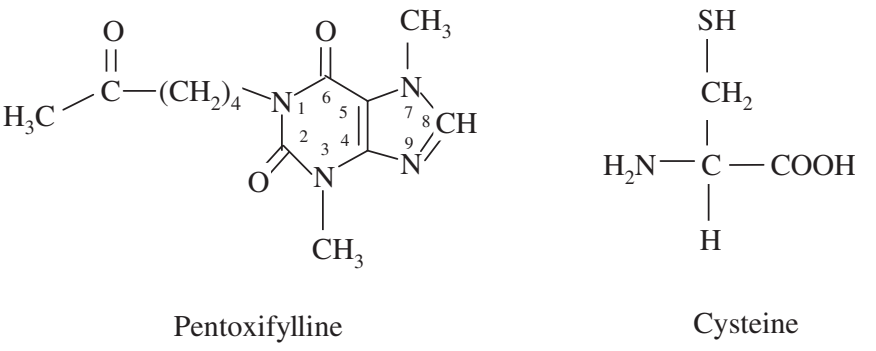


Scheme 1. The molecular structures of pentoxifylline and cysteine.

essential cofactor in the enzyme carbonic anhydrase. The role of Zn^{2+} in enzymes involves either its direct binding to, and polarization of, a substrate or an indirect interaction with water and hydroxide as general acid–base catalysts and nucleophiles [6].

The biological significance of sulfydryl thiols is well established but it is only recently that their ability to operate as biomarkers has been exploited [7]. The –SH groups have been implicated in enzyme catalysis in general [8]. Cysteine (scheme 1) is an amino acid that contains a thiol (–SH) group. The cysteine thiol group is often a binding ligand for metal centers in enzymes [9]. Cysteine plays a role in many functions of mitochondrial membranes [10], in membrane transport [11] and especially in enzyme catalysis [12].

The binding of metal ions by biologically active ligands (peptides, proteins and drugs) is important in the body. Among electrochemical techniques [13], voltammetry has been widely used to study the interactions between metal ions and ligands [14], including either simple monomeric ligands (such as amino acids [15]) or macromolecular ligands [16].

No voltammetric references on the interaction of pentoxifylline (PTX) with Zn(II) in the presence and absence of cysteine could be found in the literature. In this work, an attempt was made for the first time to determine and analyze the complex formation of PTX with Zn(II) ions by using square-wave voltammetry and cyclic voltammetry. This study focused on obtaining information concerning the structure and properties of the purine metal complexes. Particularly, our interest is focused on the affinity of PTX, an oxypurine derivative, to zinc(II) in the presence of cysteine. This study will also serve in understanding the effect of thiol-amino acids on the interaction of metal(II) ions with purine-based drugs.

2. Experimental

2.1. *Reagents*

L-Cysteine and $ZnCl_2$ were purchased from Merck; PTX was supplied by Sigma. The stock solutions of cysteine, PTX and Zn(II) were prepared by directly dissolving them in deionized and triply-distilled water.

2.2. *Apparatus*

Voltammetric measurements were carried out on a EG&G PAR model 384B polarographic analyzer connected to a EG&G PAR model 303A SMDE electrochemical cell with a hanging mercury drop electrode (working electrode), an $Ag|AgCl|KCl_{sat}$ electrode (reference electrode) and a platinum electrode (auxiliary electrode). Voltammograms were recorded with a DMP-40 XY plotter (Houston Instruments Inc.).

Electronic spectra were recorded on Unicam V2-100 UV-Vis spectrophotometer in the 400–200 nm range at 1 cm cell length.

2.3. *Procedure*

The voltammogram of Zn(II) was recorded to obtain the peak current value (I_0) and the peak potential (E_p^0) in the absence of the ligands. After that, successive additions were made of solutions containing either cysteine, PTX or mixtures of cysteine and PTX. After each addition, voltammograms were recorded to obtain the peak current value and the peak potential of free or bound Zn(II) in the presence of ligand. Solutions and samples were deaerated for about 5 min with pure nitrogen gas before starting the electrochemical experiments. Nitrogen gas was passed over the solutions during the experiments. Each measurement was performed with a fresh mercury drop at room temperature.

The spectra of mixtures with continuous-variation molar concentrations of zinc chloride and PTX or cysteine aqueous solutions at physiological pH (7.4) were recorded. The changes in absorbance at the wavelength of maximum absorptions were followed.

3. Results and discussion

3.1. *Pentoxifylline in the presence of zinc(II) ions*

The voltammogram of PTX in phosphate buffer (pH 7.40) gives two peaks at −0.110 V and −1.524 V, respectively. These peaks have previously been attributed to the reduction of the Hg(I)–PTX complex and carbonyl group on the extended aliphatic chain of the molecule, respectively [17].

At physiological pH (7.40), the cyclic voltammogram of 9.80×10^{-6} M Zn(II) ions exhibited a quasi-reversible peak ($\Delta E_p = (E_p)_a - (E_p)_c = 47$ mV; $(I_p)_a/(I_p)_c = 1.113$) at −1.182 V (figure 1). This peak can be inferred from the reduction of free Zn(II) ions to the amalgam ($Zn(II) + 2e^- \rightleftharpoons Zn(Hg)$). After adding PTX into the cell containing 9.80×10^{-6} M Zn(II), no new peak was observed, only free Zn(II) and PTX. At increasing PTX concentration, the peak current of Zn(II) decreases (figure 1) but its peak potential remains unchanged. A metal, which can be reversibly reduced, can form electroinactive inert complexes. Then, the signal of the free metal always appears at a fixed half-wave or peak potential with a peak or limiting current directly proportional to the concentration of the free metal ion [18].

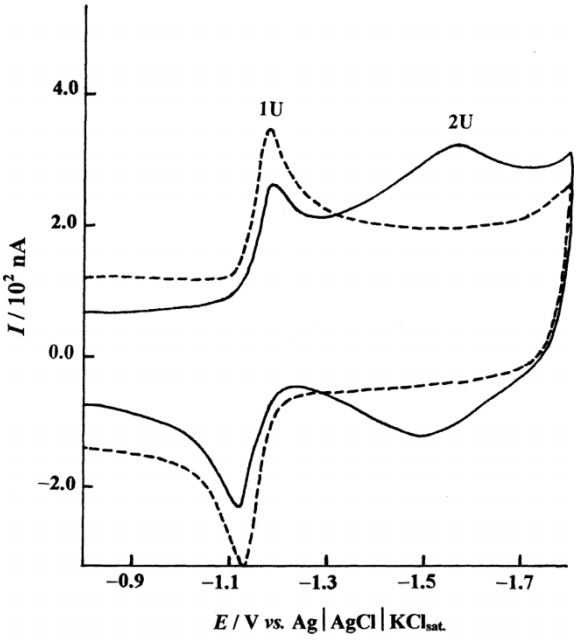


Figure 1. Cyclic voltammograms of 9.80×10^{-6} M Zn(II) solution in the presence (——) and absence (------) of 0.90×10^{-4} M PTX with 0.1 M phosphate buffer (pH 7.40). 1U, the reduction of free Zn(II); 2U, the reduction of carbonyl group of PTX. *Experimental conditions*: scan rate, 500 mV s^{-1}; scan increment, 2 mV; equilibrium time, 5 s; drop size, medium.

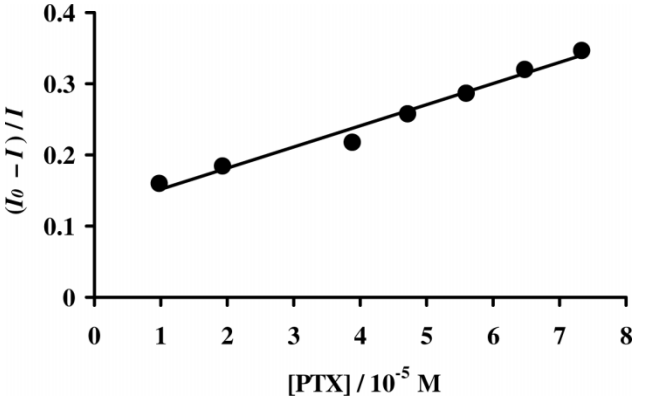


Figure 2. The plot of $(I_0 - I)/I$ *vs* [PTX] with 0.1 M phosphate buffer (pH 7.40).

For an electroinactive inert complex of the electroactive substance Zn(II) with PTX, the electrochemical process can be divided in two steps:

$$\text{Zn–PTX} \rightleftarrows \text{Zn(II)} + \text{PTX} \tag{1}$$

$$\text{Zn(II)} + 2e^- \rightleftarrows \text{Zn(Hg)} \tag{2}$$

In a similar manner to the complexation of Zn(II) with nitrilotriacetic acid (NTA) [19], the experimental data can be fitted to the following equation for inert complexation;

$$\frac{I_0 - I}{I} = \frac{[\text{Zn–PTX}]}{[\text{Zn}]} = \beta_{1:1}[\text{PTX}] \tag{3}$$

the peak current is proportional to the free metal-ion concentration, $\beta_{1:1}$ stands for the conditional stability constant under the experimental conditions of this work, [PTX] is the conditional concentration of the ligand, I and I_0 are the peak currents of Zn(II) in the presence and absence of PTX, respectively.

When the system includes a reversibly reduced metal ion and a set of successive labile complexes, a single reduction signal is observed, shifted to more negative potentials as the ligand concentration increases, and whose limiting or peak current can decrease if the diffusion coefficients of the complexes are considerably lower than that of the free metal ion [18]. In the presence of a sufficient excess of the ligand, the method of De Ford and Hume [20] allows the calculation of the corresponding stability constants from the ratio between the limiting and peak currents obtained for the metal in the presence (I) and in the absence of the ligand (I_0) and the shift in the half-wave or peak potential (ΔE) caused by the addition of the ligand:

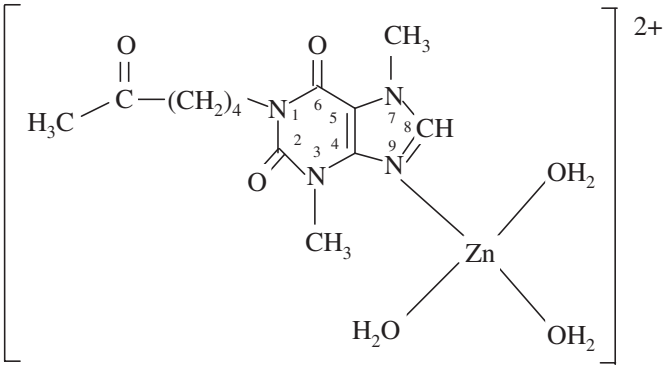
$$F_0 = \exp\left[\frac{-nF\Delta E}{RT} - \ln\frac{I}{I_0}\right] = 1 + \Sigma\beta_i(C_L)^i \tag{4}$$

where F_0 is the zero-order Leden function, $nF/RT = 77.88\ \text{V}^{-1}$ for $n = 2$ and at 25°C, C_L is the bulk concentration of the ligand, and β_i is the overall stability constant of the ML_i complex. Although the method was initially developed for small ligands, de Jong *et al.* [21–23] showed the validity of equation (4) for systems including labile macromolecular complexes, excess ligand, and absence of adsorption phenomena. Equation (3) is equivalent to the zero-order Leden function which is simplified to $F_0 = I_0/I = 1 + \beta_{1:1}$ [PTX] due to ΔE_p being almost zero.

According to equation (3), I_0 and different values of I are determined by holding the concentration of Zn(II) constant and varying the concentration of PTX. The curve of $(I_0 - I)/I$ *versus* [PTX] (figure 2) for the Zn–PTX complex was plotted and a straight line was obtained. From the slope, the logarithm of the conditional stability constant was determined as 3.46. It was only possible to observe the 1 : 1 complex at physiological pH (7.40). Similar to the binding of caffeine (1,3,7-trimethylxanthine) to metal(II) [24, 25] and mercury(I) [26], the coordination of PTX to Zn(II) occurs mainly via the N(9) atom of its heterocyclic ring. Zinc(II) ions in aqueous solution are actually hydrated species, which are better represented by $Zn(H_2O)_4^{2+}$ [27]. Treatment of $Zn(H_2O)_4^{2+}$ species with PTX may cause displacement of one water molecule by PTX (scheme 2).

3.2. *Cysteine in the presence of zinc(II) ions*

Cysteine exhibits two peaks at −0.124 V and −0.596 V in phosphate buffer (pH 7.40). These peaks are attributed to the reduction of mercuric cyteine thiolate ($Hg(RS)_2$)



Scheme 2. The possible structure of Zn(II)–PTX complex.

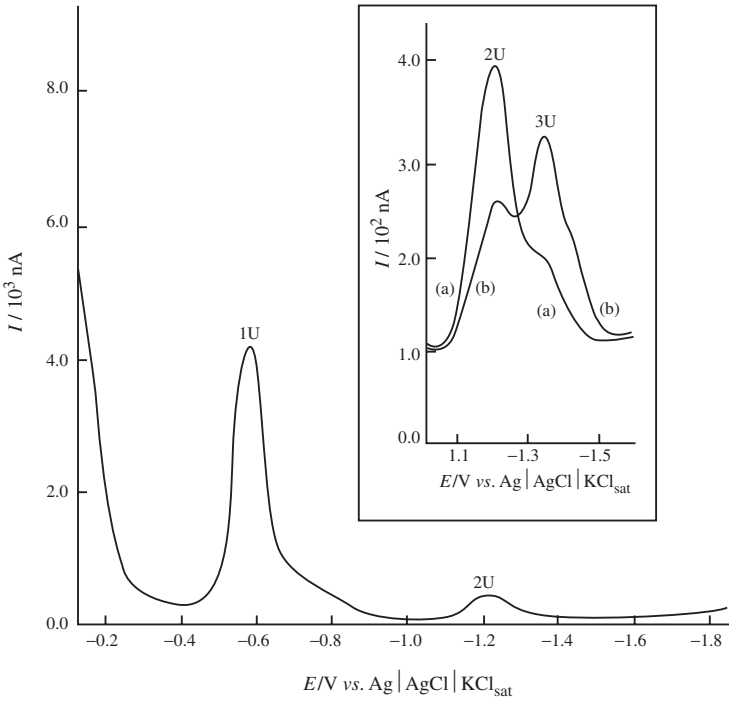


Figure 3. Square-wave voltammogram of 9.80×10^{-6} M Zn(II) solution containing 9.80×10^{-6} M cysteine at 0.1 M phosphate buffer (pH 7.40). Inset: Square-wave voltammograms of 9.80×10^{-6} M Zn(II) solution containing (a) 1.94×10^{-5} M; (b) 0.90×10^{-4} M cysteine with 0.1 M phosphate buffer (pH 7.40). 1U, the reduction of $Hg_2(RS)_2$ to metallic mercury and free thiolate (RS^-) ions; 2U, the reduction of free Zn(II); 3U, the reduction of the Zn(II)–cysteine complex. *Experimental conditions*: scan rate, 200 mV s^{-1}; scan increment, 2 mV; equilibrium time, 5 s; drop size, medium.

to mercurous cysteine thiolate ($Hg_2(RS)_2$) and the reduction of $Hg_2(RS)_2$ to metallic mercury and free thiolate (RS^-) ions [28–30]. However, the reduction of $Hg(RS)_2$ to $Hg_2(RS)_2$ was observed at high cysteine concentrations. The peak currents of these peaks also depend on the experimental conditions.

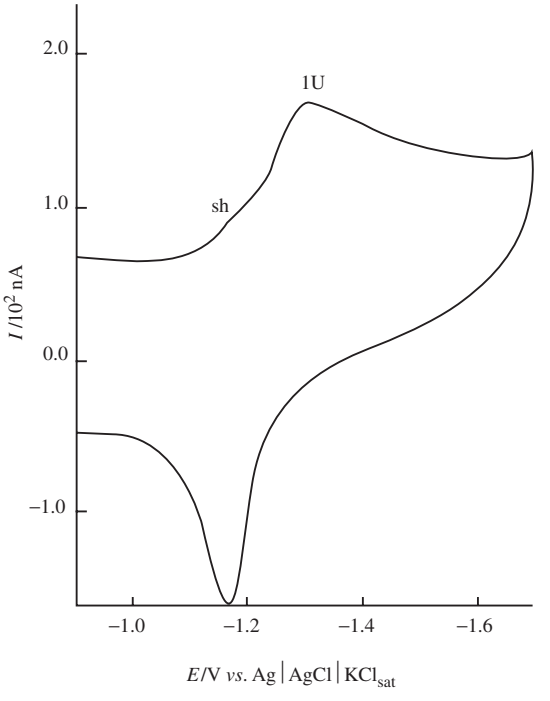


Figure 4. Cyclic voltammogram of 9.80×10^{-6} M Zn(II) solution containing 0.90×10^{-4} M cysteine with 0.1 M phosphate buffer (pH 7.40). sh (shoulder), the reduction of free Zn(II); 1U, the reduction of the Zn(II)–cysteine complex. *Experimental conditions*: as described in figure 1.

Gradually increasing cysteine concentration in the cell containing 9.80×10^{-6} M zinc(II) results in both a sharp decrease in the peak corresponding to the reduction of free zinc(II) and the appearance of a new irreversible peak at more negative potential (−1.342 V) (figures 3 and 4). The potential of this new peak is different than that of free Zn(II) ($E_{Zn(II)} = -1.182$ V). The peak at −1.342 V may be due to the reduction of Zn(II) ions complexed with cysteine according to following equilibrium:

$$Zn(II)(RS)_2 + 2e^- \rightarrow Zn(Hg) + 2RS^- \tag{5}$$

This is expected thermodynamically. The reducible ion is stabilized by complex formation and more difficult to reduce. A similar reduction potential for $Zn(II)(RS)_2$ was obtained from the polarogram of cystine and zinc(II) in 0.1 M NH_3–NH_4Cl solution [31].

As shown in figure 4, the full width at half height of the reduction peak current of $Zn(II)(RS)_2$ at −1.342 V indicates a possible role of adsorption. Since the adsorption is weak, the additional peak does not appear. In order to obtain the coordination number (m) and the stability constant (β) of the complex, the following equation [32] can be used:

$$\frac{1}{I_p} = \frac{1}{I_{p,\max}} + \frac{1}{\beta I_{p,\max}[RSH]^m} \tag{6}$$

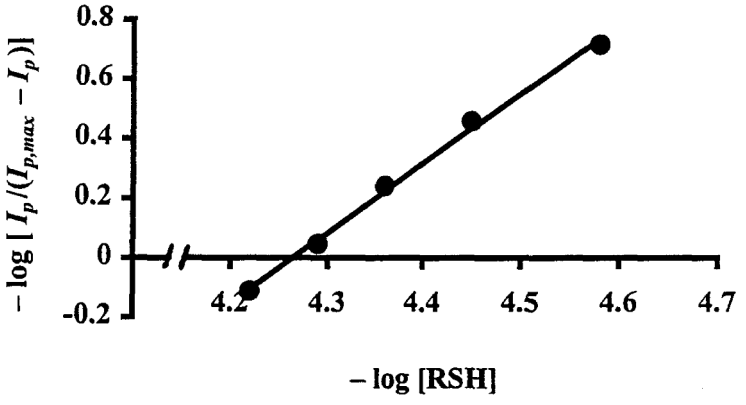
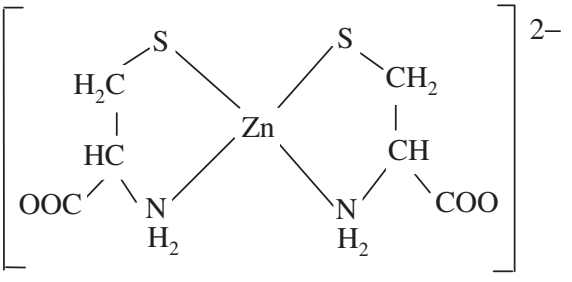


Figure 5. The plot of $-\log [I_p/(I_{p,\max} - I_p)]$ *vs* $-\log$[RSH] with 0.1 M phosphate buffer (pH 7.40).



Scheme 3. The structure of the Zn(II)–cysteine complex.

where I_p stands for the peak current of the Zn(II)–cysteine complex, $I_{p,\max}$ is the peak current when all the metal ion forms the complex and [RSH] is the concentration of cysteine. The plot of $-\log [I_p/(I_{p,\max} - I_p)]$ *versus* $-\log$ [RSH] is linear with slope of m (figure 5). The results of $m = 2$ and $\log \beta_{1:2} = 9.94$ were obtained, which means that only the $Zn(II)(RS)_2$ complex is formed. Cysteine has three coordination sites viz., –SH, $–NH_2$ and –COOH (scheme 1). Previously, the structure of the complex of Zn(II) with cysteine was proposed as shown in scheme 3 [33, 34].

3.3. *Spectroscopic measurements*

To compare the stability of the Zn(II)–PTX and Zn(II)–cysteine complexes, electronic spectral studies were also carried out. The stability constants and stoichiometries of these complexes were determined by Job's method. The absorption maxima of the ligands and their Zn(II) complexes are given in table 1. The UV absorption bands of Zn(II)–PTX and Zn(II)–cysteine complexes may be assigned to the ligand-to-metal charge-transfer bands. The stoichiometries of the complexes determined from electronic spectral data are in agreement with the voltammetric data. In addition, the stability constants of the complexes are close to the values found by voltammetric data (table 1).

Table 1. Logarithms of the conditional stability constants obtained from electronic spectra and voltammetric data for Zn(II)–PTX and Zn(II)–cysteine complexes.

Compound	λ_{max}/nm	Conditional stability constants		Calculation method
		log $\beta_{1:1}$	log $\beta_{1:2}$	
PTX	268	–	–	–
Cysteine	229	–	–	–
Zn(II)–PTX complex	274	3.46	–	Equation (3)
		3.42	–	Job's method
Zn(II)–cysteine complex	225	–	9.94	Equation (6)
	–	–	9.17	Job's method

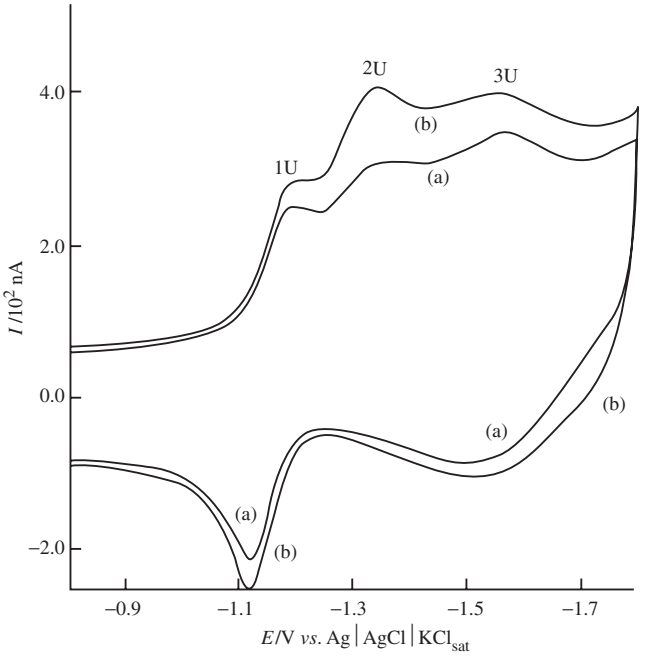


Figure 6. Cyclic voltammograms of 9.80×10^{-6} M Zn(II) and 0.90×10^{-4} M PTX mixture containing (a) 8.93×10^{-6} M; (b) 3.48×10^{-5} M cysteine at 0.1 M phosphate buffer (pH 7.40). 1U, the reduction of free Zn(II); 2U, the reduction of the Zn(II)–cysteine complex; 3U, the reduction of the carbonyl group of PTX. *Experimental conditions*: as described in figure 1.

3.4. *Pentoxifylline–zinc complex in the presence of cysteine*

When cysteine was added to the cell containing 0.90×10^{-4} M PTX and 9.80×10^{-6} M zinc(II), the irreversible reduction peak of the Zn(II)–cysteine complex at −1.342 V appeared while the peak currents of free Zn(II) (formed from the dissociation of the Zn(II)–PTX complex in the presence of cysteine) and free PTX increased (figures 6, 7). The peak current of the Zn(II)–cysteine complex increased with increasing cysteine concentration. As shown in figure 7, the increase in the peak current of free Zn(II) which formed from the dissociation of the Zn(II)–PTX complex in the

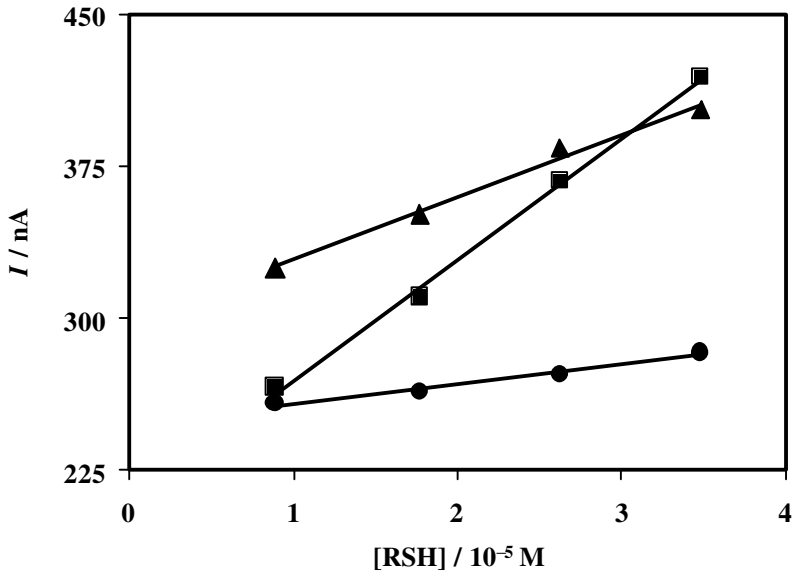
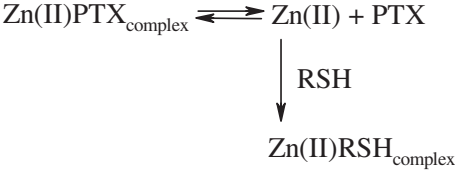


Figure 7. The dependence of the peak currents with added cysteine (RSH) concentration to the 9.80×10^{-6} M Zn(II) and 0.90×10^{-4} M PTX mixture. The peak currents of free Zn(II) (•); Zn(II)–cysteine complex (■); free PTX (▲).



Scheme 4. The interaction of the Zn(II)–PTX complex with cysteine (RSH).

presence of cysteine, is less than those of the Zn(II)–cysteine complex and free PTX, due to complexation of free Zn(II) with cysteine. Evidentally, cysteine prevents complexation of Zn(II) ions with PTX (scheme 4); in the presence of PTX and cysteine, zinc(II) prefers binding with cysteine instead of formation of the mixed-ligand complex.

4. Conclusion

In this article, the interaction of PTX with Zn(II) ions in the presence and absence of cysteine is reported. The results obtained in the study of Zn(II)–PTX by square-wave and cyclic voltammetry techniques offer data for metal–drug interactions. The stability-constant and stoichiometry values and also a possible mechanism of the Zn(II)–PTX interaction have been determined by using a simple and rapid voltammetric procedure.

The prevention of interaction of Zn(II) with PTX in the presence of cysteine is a model for the relationship of a drug with the metallothioneins in the living body.

Quantitative knowledge of the strength of Zn(II) binding to cysteine will help us to understand its interaction with PTX in the presence of cysteine.

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