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EQUILIBRIUM STUDIES OF CAPTOPRIL AND ITS BIOLOGICAL IRON (II) AND ZINC (II) BINARY COMPLEXES

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ABSTRACT :

Acid-base equilibria of captopril and its complex - formation with iron (II) and zinc (II) have been investigated in aqueous medium and ionic strength (I=0.1 M sodium perchlorate) conditions. Protonation constant of the ligand and the stability constants of 1:2 metal-ligand complexes formed were determined from the obtained potentiometric data. The complexes of Fe (II) and Zn (II) with captopril (cap) have been prepared. They were characterized by elemental analysis, infrared and electronic absorption spectra. Binding sites in the complexes with a special regard to the possible role of -S- and -C=O groups in the coordination have been discussed. The thermal behaviour of the prepered complexes have been studied by thermogravimetric analysis in nitrogen atmosphere up to 750°C.

INTRODUCTION :

Captopril is one of the most important drugs used to control high blood pressure and help in the relief of chronic heart failure. It may have an effect on reduction of left atrial size in mild to moderate hypertension[1,2] and on insulin receptor (tyrosine kinase) activity in essential hypertension[3]. Captopril inhibits tumor growth in a xenograft model of human renal cell carcinona[4] and has a direct protective effect on myocardial cells with anoxic – reperfusion injury[5]. In vitro a combination of captopril with aspirin significantly inhibites the platelet aggregation[6].

A combination of captopril and dopamine may prevent dopamine induced myocardial injury[7]. Co-administration of ferrous sulphate and captopril were shown to decrease its bioavailability[8-10] via forming a stable complex. Captopril bind to zinc[11] in AC- enzyme and increase urinary zinc loss and may deplete zinc stores. The bioavailability of captopril[12] may be reduced in presence of an antiacid. The mechanism of transport of metal ions is poorly understood. Any progress in this area requires equilibrium studies. The objective of this work is to investigate the equilibria of captopril and its binary complexes with iron (II) and zinc (II) in aqueous solution, the composition and stability of the complexes are to be determined. Another goal of this work is to explore the optimal pH conditions of the reaction. In this work spectrophotometric and potentiometric methods were used to study the complex equilibria in solution as a function of pH, concentration of components and ionic strength of solution. To get better information about the equilibria in solution and dissociation constants

of captopril and the stability constants of its complexes a combination of spectrophtometric and potentiometric metheds were used[13-15]. Moreover the present work involves preparation and characterization of solid complexes to elucidate their structure by several techniques viz : microcmical analysis, infrared spectroscopy and thermal analysis.

Most pharmaceuticals contain electron donor groups likely to bind metal ions occurring naturally[16].

EXPERIMENTAL :

1-Apparatus:

A Perkin–Elemer (Lambda 3B) spectro-photometer equipped with 1-cm matched quartz cells and controlled by a Matsube 286/33 DX computer was used for the absorbance measurements.

pH measurements were made with a Cornning 215 pH meter with a combined glass electrode. The pH measurments were done in aqueous solutions. All measurements were carried out at a temperature of ~25ºC.

The infrared spectra were performed by a Jasco FTIR 480 (4000-400 cm^{-1}) computerized spectrophotometer.

Microanalysis were carried out by the unit of microanalysis at Cairo University.

Thermogravimetric analysis (TG) and differential thermal analyis (DTA) were carried out with a Schimadzu Thermal Analyzer 50 H at heating rate of 10ºC/min.

2-Chemicals and solutions:

All chemicals and reagents were of analytical grade. Captopril samples were purchased from Aldrich chemicals. Metal salts: $ZnSO_4.7H_2O$ and $FeSO_4.7H_2O$ of Analar products obtained from Merck (Germany) and were used for preparation of solutions of the corresponding metal ions. Perchloric acid, sodium perchlorate and potassium hydrogen phthalate were all prepared from the Analar grade reagents (Aldrich Chemical Co.). Standard solution of sodium hydroxide was also prepared.

Stock solution ($5X10^{-3}$ M) of the ligand (cap) was prepared by dissolving an accurately weighed amount of the reagent in the appropriate volume of bidistilled water. Stock solutions of iron (II) and zinc (II) were prepared in bidistilled water. The solutions were diluted if necessary to prepare standard working solutions. The metal content of solutions was standardized by the conventional methods[17].

3-Potentiometric study:

In the binary systems studied, the following solutions were titrated potentiometrically with 0.2 mol dm^{-3} standard carbonate – free sodium hydroxide solutions standardized against standard potassium hydrogen phthalate :

a-solution 1 x 10^{-3} mol dm^{-3} perchloric.

b-solution (a) + 1 x 10^{-3} mol dm^{-3} captopril.

c-Solution (b) + 1 x 10^{-3} mol dm^{-3} Fe^{2+} or Zn^{2+}.

The total volume was adjusted to 50 ml by adding doubly distilled water and the titrations were performed at ~25 ºC.

4-Preparation of Solid Complexes:

The binary iron–cap and zinc–cap complexes were prepared in stoichiometric ratio 1:2. A solution of $FeSO_4.7H_2O$ or $ZnSO_4.7H_2O$ (0.01 mol) in deionised water was added to an aqueous solution of cap prepared in the minimum amount of water with constant stirring. The pH of the solution was adjusted to 7.7 by adding dilute base. The solution mixture of iron (II) complex turned a dark black product, while a white precipitate of Zinc (II)

complex was formed. The resulting solution mixture was kept at room temperature for one day. The binary complex was filtered and washed rapidly with a few amount of bidistilled water and dried over P_4O_{10}.

RESULTS AND DISCUSSION:

1-Proton–Ligand Dissociation Constants:

The titration curves obtained for cap are shown in Fig. (1). The values of $\bar{n}_H$ (the ligand proton association) as determined according to Irving and Rossotti[18] were complied from the titration data at pH difference equals 0.1. Calculation of proton ligand dissociation constants were carried out by plotting $\bar{n}_H$ against pH values, the values of log K_1H and log K_2H (the first carboxylic and second mercapto proton dissociation constants of the studied captopril) are the pH values corresponding to $\bar{n}_H = 0.5$ and 1.5, respectively. The pK_1 and pK_2 values obtained by treatment of several sets of potentiometric data were found to be 5.45 and 8.45, respectively. Dissociation of carboxyl proton begins at pH ~ 4.4, over pH 6.5 the anion $(cap\text{-}H)^-$ is prevalent. Dissociation of the second mercapto proton originates from pH 7 and the monoanionic species of $(cap\text{-}H)^-$ undergoes ionization on increasing the pH of solution according to the following protonation equilibria :

$$(cap\text{-}H_2) \rightleftharpoons (cap\text{-}H)^- + H^+ \quad (pH\ 4.5 - 6.6) \quad \text{..... 1a}$$

$$K^{H}_{(cap\text{-}H_2)} = [(cap\text{-}H)^-][H^+] / [(cap\text{-}H_2)] \quad \text{..... 1b}$$

$$(cap\text{-}H)^- \rightleftharpoons (cap)^{2-} + H^+ \quad (pH6.7 - 9.5) \quad \text{..... 2a}$$

$$K^{H}_{(cap\text{-}H^-)} = [(cap)^{2-}][H^+] / [(cap\text{-}H)^-] \quad \text{..... 2b}$$

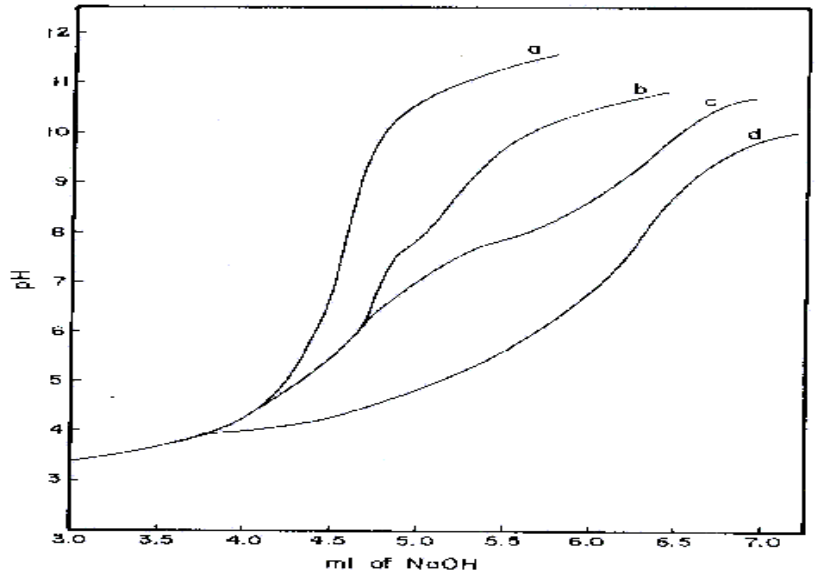
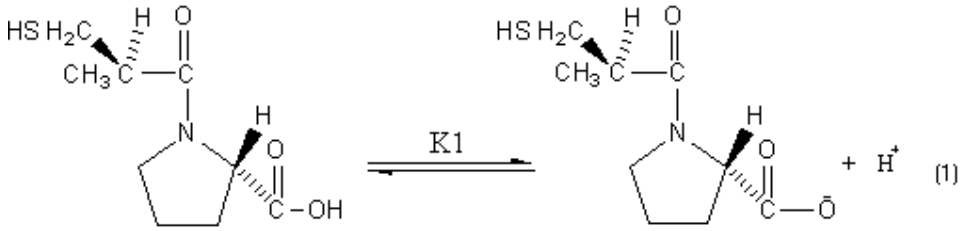
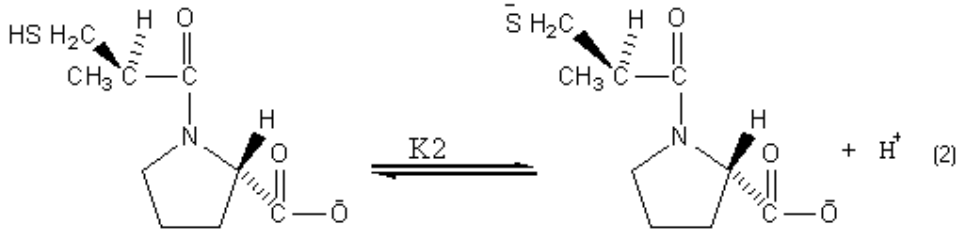


Fig.(1) : Potentiometric titration curves of captopril :
a) 0.0037 M $HClO_4$, b) a + 0.001 M captopril , c) b+ 0.001 M Zn(II), d) b+ 0.001 M Fe(II)

Thus, the acid–base equilibria to be considered in the pH range 4.5-9.5 can be shown in the following scheme :





Scheme (1) : the dissociation constants of captopril

The two dissociation constants of carboxyl and mercapto proton were calculated and given in Table (1).

Table (1): Formation constants captopril and its metal complexes

Central ion	Log K_1	Log k_2
Cap	5.45	8.45
Fe (II)	6.84	7.46
Zn (II)	4.8	5.34

2-Binary metal – ligand systems :

Potentiometric equilibrium titration curves of Iron(II) – cap is taken as being representative example (Fig. 1). In the titration curve, the inflection is significantly lower with respect to that of the free captopril, indicating the formation of iron-complex by release of protons from SH groups.

The potentiometric data could be fitted by assuming the formation of 1:2 complex with the formation of two six member rings via mercapto and oxopropyl groups.

The titration curves of zinc(II)–cap solutions [Fig.1(c)] differs well the curve separated at pH 6.70.

Captopril [Fig. 1(b)], demonstrating replacement of two SH protons due to complexation. This shows that captopril binds to zinc through a thiol group[19] and gives 2:1 ligand – metal complex.

The initial volume of titration solution in each case was V°. Values V^I, V^{II} and V^{III} of alkali were consumed in titrations (a), (b) and (c) 0respectively to give identical values of pH. A ligand–proton formation curve was obtained by plotting the degree of formation ($\bar{n}_H$) the ligand–proton association against pH (Fig. 2),

using the relationship derived by Irving and Rossotti [17].

$$\bar{n}_H = Y + \frac{(V^{I} - V^{II})}{(V^{\circ} + V^{I})} \frac{(N^{\circ} + E^{\circ})}{T_{C_L^{\circ}}} \quad -----(3)$$

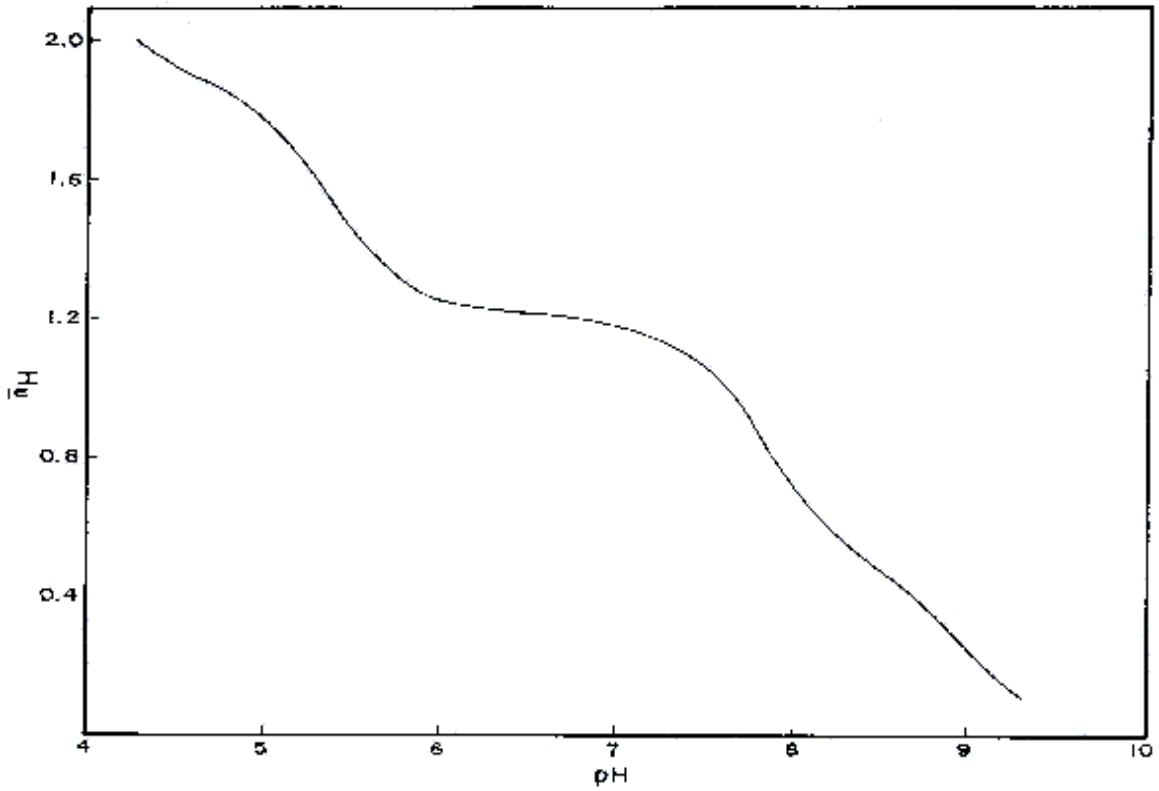


Fig. (2): Proton - ligand formation curve of captopril

Where Y is the total number of dissocitiable protons per ligand molecule added at the beginning of the titration. From which the acid dissociation constants of the ligand were obtained. The $pK_1$and pK_2 values corresponding to $\bar{n}_H$ =1.5 and $\bar{n}_H$ = 0.5 respectively were obtained from the curve.

The formation curves of the complexation equilibria (Fig. 3) obtained by plotting the degree of formation of the complex ($\bar{n}$) against the negative logarithm of the concentration of non – protonated ligand (pL) using the following two relationships.

$$\bar{n} = \frac{(V^{III} - V^{II})(N^{\circ} + E^{\circ}) + T_{C_L^{\circ}}(Y - \bar{n}_H)}{(V^{\circ} + V^{I})\bar{n}_H T_{C_M^{\circ}}} \quad ------(4)$$

$$pL = \log \frac{\sum_{n=0}^{n=i} \beta_n^H [H^+]^n}{T_{C_L^{\circ}} - \bar{n}\, T_{C_M^{\circ}}} \cdot \frac{V^{\circ} + V^{III}}{V^{\circ}} \quad ---(5)$$

Where β_n^H is the reciprocal acid dissociation constant of the ligand (proton-ligand stability constant).

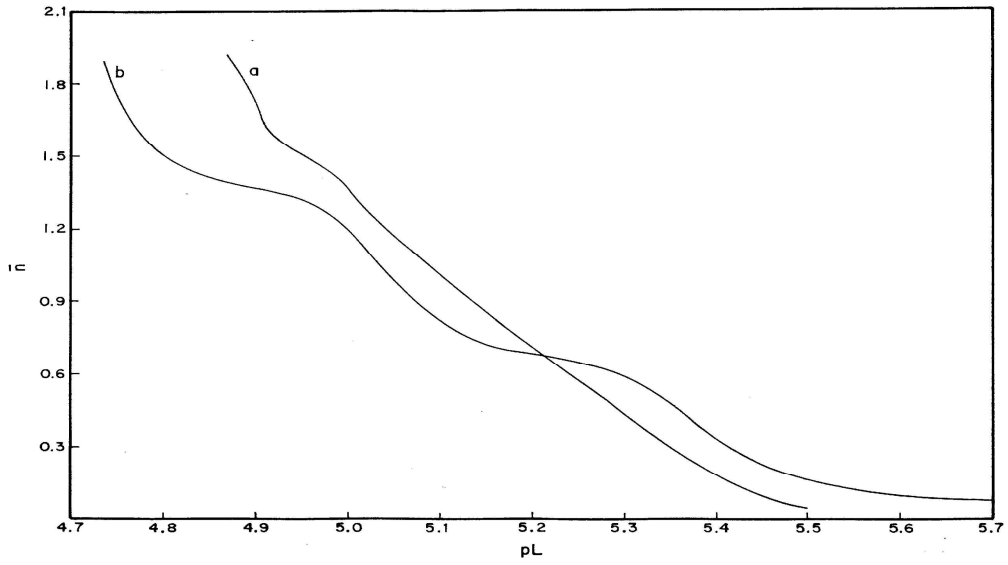


Fig. (3) : Metal ion captopril formation curves ; a) Zn(II), b) Fe(II)

The stability constants for the complexation equilibria corresponding to the 1 : 1 (eq. 6) and 1:2 (eq. 7) metal–ligand complexes were calculated from potentiometric titration curves with 1:2 metal: ligand ratio. The stability constants of the complexes formed with captopril listed in Table (1) are in the order Fe (II) > Zn (II).

Captopril behaviour may be based on the bidentate nature which coordinates through thiol group and oxygen of carbonyl group forming stable six – membered chelate rings.

The corresponding equilibria may be represented as follows :

$$Fe^{2+} + (cap\text{-}H_2) \rightleftharpoons Fe(cap) + 2H^+, \quad K_{Fe\text{-}cap}^{Fe} \quad \ldots\ldots (6)$$

$$Fe(cap) + (cap\text{-}H_2) \rightleftharpoons Fe(cap)_2^{--} + 2H^+, \quad K_{Fe(cap)2}^{Fe(cap)} \quad \ldots\ldots (7)$$

$$Zn^{2+} + (cap\text{-}H)^- \rightleftharpoons Zn(cap) + H^+, \quad K_{Zn(cap)}^{Zn} \quad \ldots\ldots (8)$$

$$Zn(cap) + (cap\text{-}H)^- \rightleftharpoons Zn(cap)_2^{--} + H^+, \quad K_{Zn(cap)_2}^{Zn(cap)} \quad \ldots\ldots (9)$$

Fig. (1) displays the reaction of zinc and captopril started when the carboxylic proton has been dissociated.

The use of large single charged anions such as perchlorate (radius ~ 0.235 nm) minimize the electrostatic interaction that exist between anions and metal ions. The absence of polynuclear species was confirmed by repeating the experiments using several concentrations of the reactants, where the results obtained were identical. The released two protons in Zn – cap complex consumes one mole of alkali and second mole of alkali is required for deprotonation of two mercapto group of two ligand molecules. The potentiometric titration curve of binary system containing Zn-cap in a 1:1 molar ratio exhibit a distinct inflection at m= 1 in addition to other inflection at m =2, (m= number of moles of alkali added for mole of metal ion), which calculated from the Fig. (1), reverses to the distance between c and b curves corresponding to stepwise formation of ML and ML_2 complex species. The results obtained for the formation of the binary complexes investigated are shown in Table (1).

3-Electronic Absorption Spectra :

The absorption spectra of captopril in bidistilled water was studied in UV region (200–330 nm) at different values of pH. The spectra obtained display an absorption band with maximum wavelength λ at 210 nm at pH 7.7 and in 0.1 M ionic strength ($NaClO_4$).

The complex formation of Fe^{2+} with captopril was examined at different pH's in equimolar solutions ($1.0x10^{-4}$M). The absorption spectra of the binary system reflect the formation of a complex with λ_{max} = 280 nm at pH 7.7. The formation of binary complex is accompanied by a bathochromic shift of the band displayed in the absorption spectrum. In alkaline media, pH>7.7 the band is shifted to longer wavelengths, a beahviour which refers to probable formation of another type of complex species which may be bonded to the S atom at λ= 340 nm.

The absorbance of the band increases with rise of pH attaining a maximum value (pH 8.0), then decreases again. The absorption curve of equimolar solutions (C_M=$C_{L=}$1X10^{-4} M) of Zn-cap. The absorption spectra of the system were recorded in the pH range 6.0-9.6 using a blank containing the same concentration of cap.

On comparison of the electronic absorption spectra of the free ligand cap with that of the chelated Fe(II) or Zn(II), it reveals that the addition of metal ion to cap ligand solution make the spectrum band of the ligand shifted to

longer wavelength and this is evidence for the formation of the coordination compound.

4-Stoichiometry of the complexes :

The stoichiometric ratio of Fe^{2+} or Zn^{2+}-cap complexes was determined by using the usual two spectrophotometric methods of namely Job's continuous variation method[20] and The molar ratio method[21].

The two methods confirmed the formation of 1:2 (M:L) complex.

5-Infrared Spectra:

The IR spectrum of captopril ligand show a weak band near 2700-2500 cm^{-1} (Figure 4) which may be assigned to υ_{OH} stretching mode of carboxylic group. This band disappeared in the spectrum of the Fe^{2+} and Zn^{2+} complexes (Figs 4 and 5). This is true and in accordance with our potentiometric data, we prepared the solid complexes at pH 7.7 which is beyond the ionization range of carboxylic proton in pH 4.5–6.6 as describe previously. The bands due to υ_{as} (COO^-) and υ_s (COO^-) of carboxylic group in the spectrum of Fe^{2+} or Z^{2+}-cap complex are observed in the regions of 1650 – 1600 and 1450–1400 cm^{-1}. This proves that the oxygen atom of carboxylic group did not participate. Vibrational evidence for S coordination of captopril[18], the peek at 2550 cm^{-1} in the spectrum of captopril which may be assigned to υ_{SH} stretching mode this peak disappeared in the spectrum of the two iron and zinc binary complexes. The absence of υ_{SH} confirms the deprotonation of SH group and participation of sulfur in the coordination to metal ions[22]. The strongest absorption peak at 1745 cm^{-1} is due to C=O stretching vibration. This peak became weak and shifted to 1610 cm^{-1} show a bathochromic shift in the spectrum of complexes which suggests bonding of captopril through C=O group. However, the IR spectra of Fe^{2+} and Zn^{2+} binary complexes displayed new bands in the range 3450–3300 cm^{-1} which can be assigned to coordination of water molecules[23].

6-Microanalysis:

The elemental analysis data (Table 2) of the isolated complexes of iron (II) and zinc (II) with captopril are in good agreement with the stoichiometry of the metal complexes were ascertained by using the continuous variation and molar ratio methods[20, 21] and also agree with the results of potentiometric and thermal analysis studies. The results reveale that the stoichiometry is 1:2 (metal : ligand) ratio for each complex.

7-Thermal Analysis:

The thermogravimetric studies of iron and zinc-cap complexes were carried out. The metal complexes were stable at room temperature but started to show a significant mass loss at 120-180ºC. Such a thermogravimetric behavior of the complex indicates the presence of coordinated water molecules in the complex. Three decomposition steps are observed also in the TGA curve for every complex at 185, 350 and 465ºC. The step at 185-350ºC range was recorded corresponds to the loss of two aliphatic methyl groups and two molecules of CO_2. The second decomposition step at 350–465ºC can be attributed to the removal of two hetero saturated five member rings. The complex shows another decomposition step occurs in the temperature range 465–680ºC. The mean loss for both the two complexes about 26 and 29% respectively. A constant weight is attained at 690ºC corresponding to the metal oxide as a final product.

Table (2): Microanalytical data of captopril complexes

Compound	Melting point	Elemental analysis (found)			
		%C	%H	%N	%S
Fe $(cap)_2.2H_2O$	342	41.38 (40.9)	6.11 (6.50)	5.34 (5.31)	12.21 (12.90)
Zn $(cap)_2.2H_2O$	328	41.35 (40.1)	6.11 (6.35)	5.34 (5.25)	12.21 (11.81)

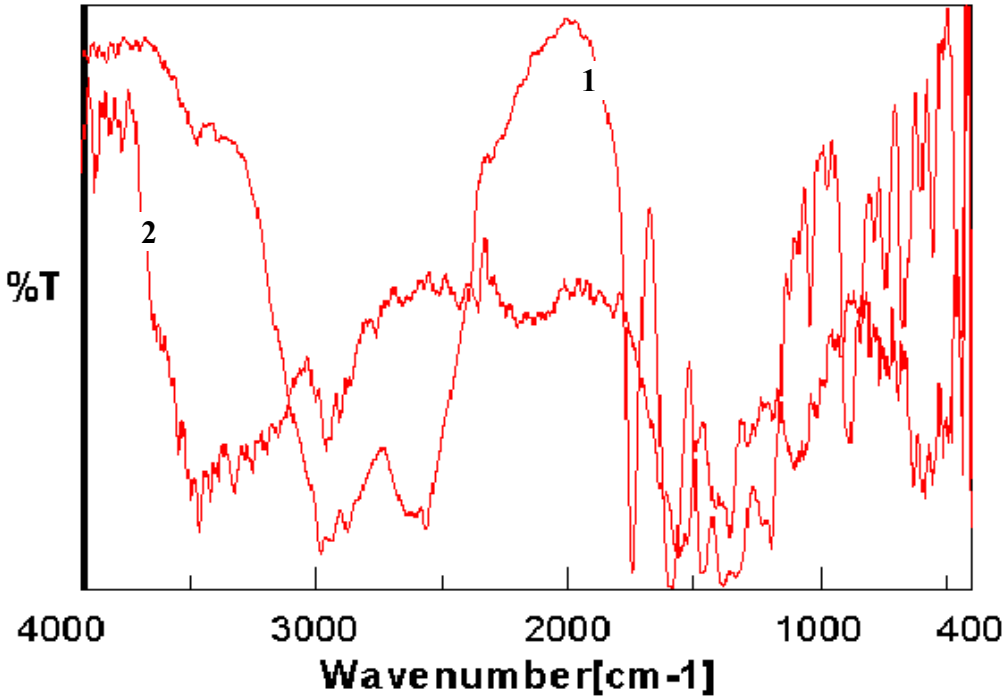
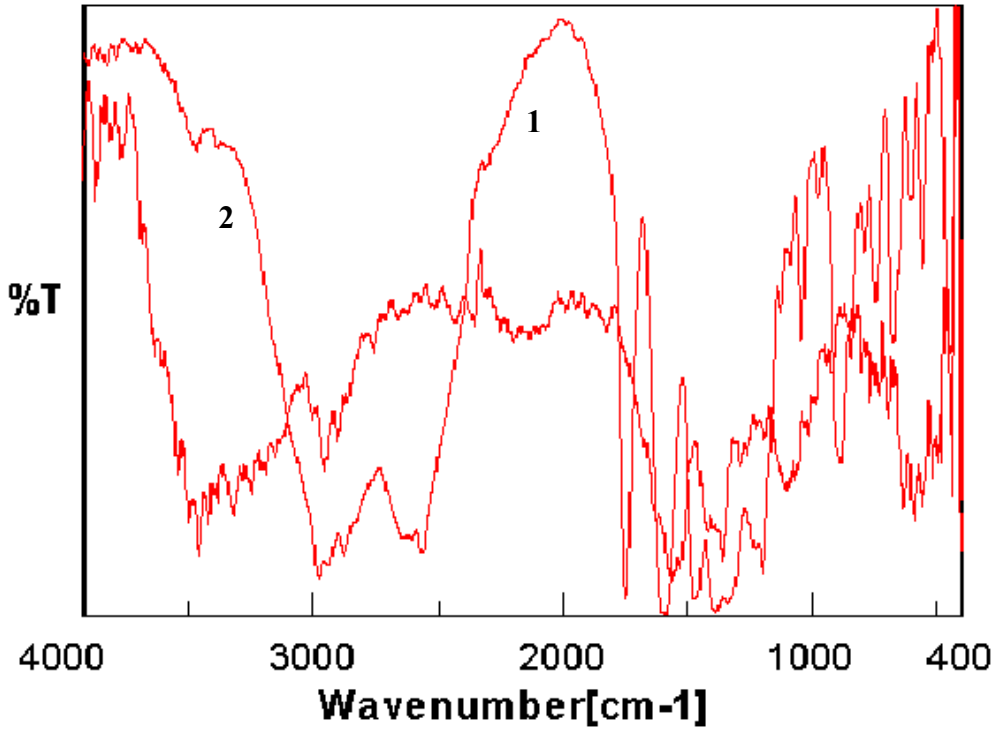


Fig.(4): 1) IR spectra of cap and 2) its binary complex Fe(II)-cap



Fig(5): 1) IR spectra of cap and 2) its binary complex Zn(II)-cap

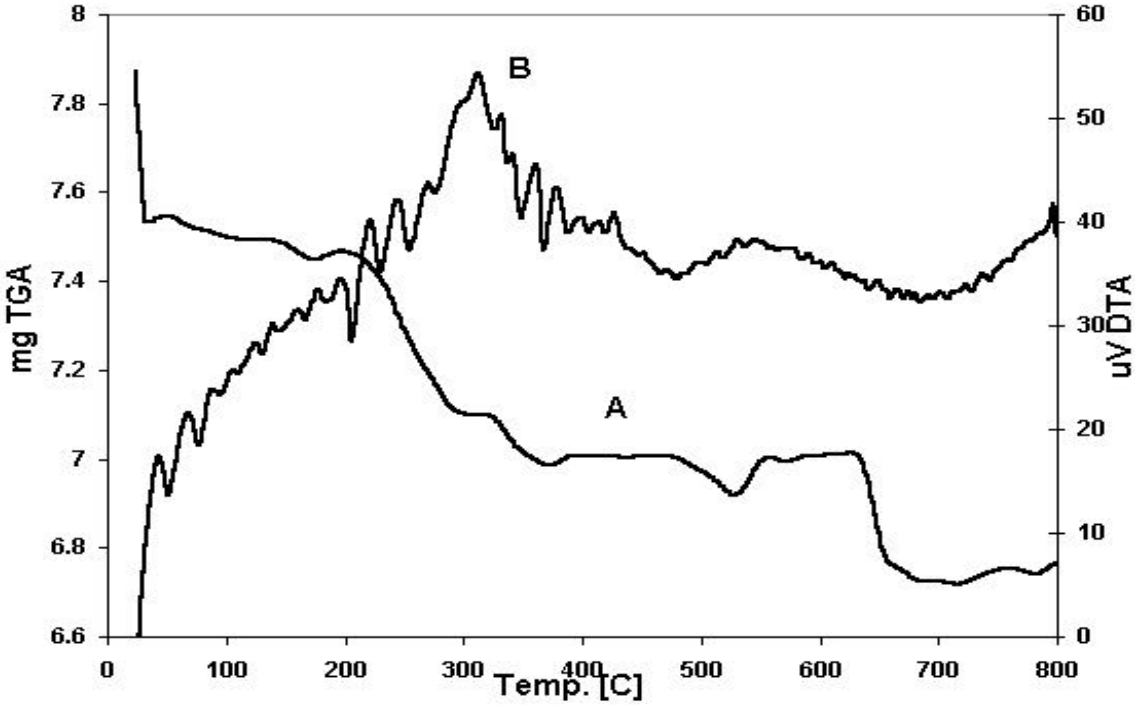


Fig.(6): A) Thermogravemetric analysis of Fe(II)-cap binary complex and B) its differential thermal analysis.

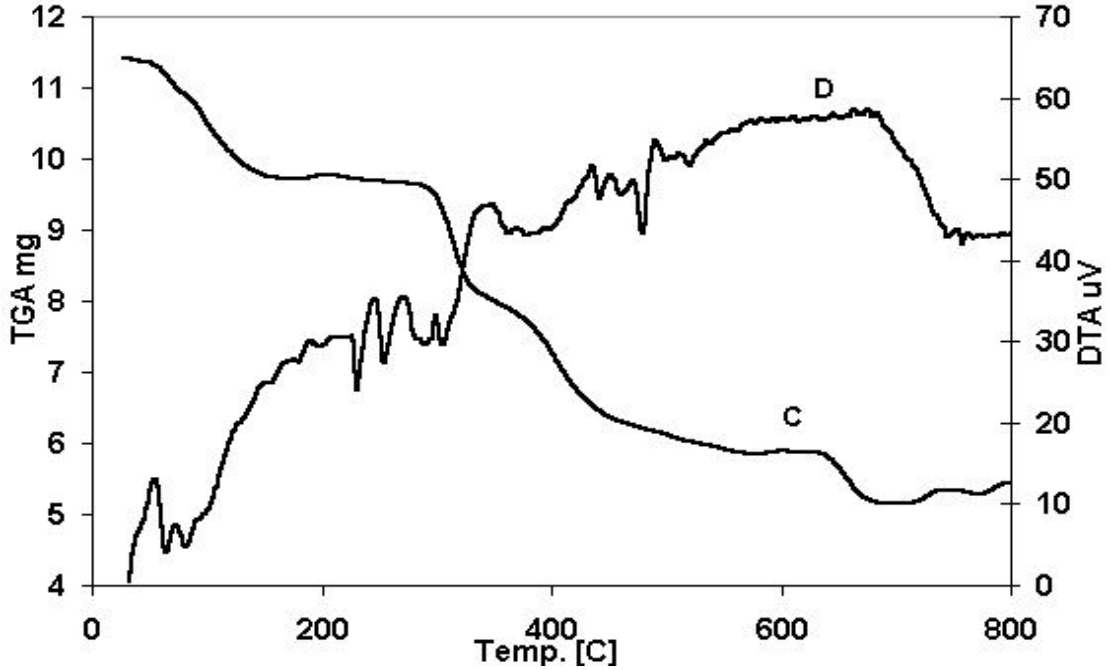


Fig.(7): C) Thermogravemetric analysis of Zn(II)-cap binary complex and D) its differential thermal analysis

CONCLUSIONS:

Captopril is one of ACE inhibitors that can form metal complexes. It can bind to zinc and iron through thiol and carbonyl groups. Captopril react with zinc and iron in pH values which approximately similar to that found in biological fluids. The dissociation constants of captopril and the stability constants of the binary iron and zinc were calculated. It was found that iron (II) or zinc (II) forms 1 : 2 complexes with captopril. The elucidation of the complex structure has been carried out by several techniques such as microchemical analysis, electronic and infrared spectroscopy, thermal analysis and potentiometric titrations.

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دراسة حالات الاتزان لمركب الكابتوبريل ومتراكباته البيولوجية مع أيونات الحديدوز والزنك ثنائى التكافؤ

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اشتمل البحث على دراسة حالة الاتزان فى محلول مائى لمتراكبات الحديدوز والزنك الثنائى التكافؤ مع مركب الكابتوبريل ذات الأهمية الصيدلية باستخدام الطريقة الجهدية للمعايرة فى محاليل ذات قوة أيونية 0.1 مول من بيركلورات الصوديوم وحسبت من طريقة المعايرة الجهدية ثوابت التأين للمركب الكابتوبريل، وكذلك ثوابت التكوين والاستقرار للمتراكبات الناتجة لأيونات الحديدوز والزنك حيث دلت المراجع أن تعاطى عقار الكابتوبريل فى العلاج يسبب نقص معدن الحديد والزنك داخل جسم الإنسان وللتعرف على ميكانيكية التفاعل والظروف المثلى للتفاعل يلزم دراسة حالات الاتزان القائمة فى المحلول عند تغير درجة الأس الهيدروجينى وهذا ما تمت دراسته فى هذا البحث وكذلك تم توصيف المتراكبات الصلبة باستخدام التحليل الكيميائى الدقيق، واستخدام الأشعة تحت الحمراء لمعرفة مراكز الترابط بين المعدن والمركب الصيدلى، وكذلك استخدم التحليل الحرارى فى جو خامل من النيتروجين لمعرفة الصيغة التكوينية لهذه المتراكبات، وكذلك تم معرفة قيم الطول الموجى للامتصاص الإلكترونى للمركب والمتراكبات موضع الدراسة.