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Metal complexes and voltammetric determination of enalapril in tablet and urine samples

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ABSTRACT

Twelve metal ions viz; Fe(III), Al(III), Cr(III), La(III), Y(III), Bi(III), Cu(II), Co(II), Ni(II), Mn(II), Pd(II) and Hg(II) were selected to elucidate their interaction with enalapril maleate using potentiometric method. The protonation constant of the ligand and stability constants of complexes formed have been tabulated using three ionic strengths, I = 0.05, 0.1and 0.15 M $NaNO_3$ in aqueous solutions at 25 ± 0.1^0C. Complexes of 1: 1, 1: 2 and/or 1: 3 metal to ligand ratios are formed depending on the nature of the ligand or metal ions. The order of stability constants of the binary complexes was examined. The voltammetric behavior of enalapril was studied using square wave cathodic stripping voltammetric method at carbon paste electrode (CPE) in pharmaceutical dosage forms (tablet) and in biological fluids (spiked and real urine sample) has been developed and evaluated. Different parameters such as medium, pH, accumulation potential, scan rate, accumulation time and ionic strength were tested to optimum condition to optimize the conditions for the determination of Enalapril. The adsorbed form is reduced irreversible at optimal conditions viz; 0.04 M Britton-Robinson buffers (pH~9.00). Linear concentration ranges 0.985-24.62 ng/mL at 15s, 0.985-14.77 at 30 s ng/mL and from 0.985 - 4.925 at 60 s ng/mL, can be determined successfully. The standard addition method was used to determine Lisinopril in pure solutions, tablets and in biological fluids with satisfactory results. The data obtained are compared with standard official method.

KEYWORDS

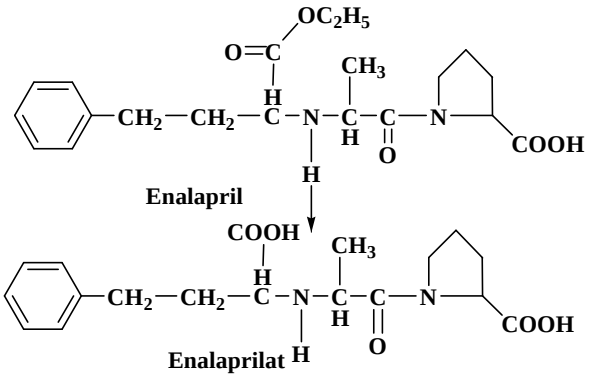
Enalapril;
Stability constants;
Square wave cathodic voltammetry;
Dosage forms;
Urine.

INTRODUCTION

Enalapril maleate (ENP)(N-{N-[(S)-1-ethoxycarbonyl-3-phennylpropyl] L-alanyl)-proline hydrogen maleate)

Enalapril is the ethyl ester of a long - acting angiotensin converting enzyme inhibitor, enalaprilat. Enalapril maleate is indicated for the treatment of essential and renovascular hypertension. Enalaprilat is a pro-drug; following oral administration, it is bioactivated by hy-

drolysis of the ethyl ester to enalaprilat, which is the active angiotensin converting inhibitor[1].



Enalapril has been assayed spectrophotometrically by ion pair-extraction technique[2,3], Potentiometric[4,5] and HPLC[6-8] procedures. In biological fluids, enalapril has been quantitated by radio-enzymic assay[9], GC-MS and GC-negative ion CIMS[10]. Also, different types of analytical procedures have been proposed for the analysis of enalapril in pharmaceuticals formulations. These procedures include capillary electrophoresis[11], polarography[12], voltammetry[13], coulometry[14], amperometry[15], conductometry[16], fluorimetry[17], colorimetry[18-23] and flow injection methods[24]. Most of these methods are sophisticated.

Stripping voltammetry is a very sensitive method for the determination of many traces of organic compounds and metal ions achieving it is low level of detection by combining accumulation process with a voltage scanning measurements[25,26]. Carbon paste electrodes are convenient and often used as working electrodes for the voltammetric measurements because of their attractive properties. From analytical point of view, these electrodes exhibit rather low background currents over a wide range of potentials when compared with other solid electrodes, and after are new ability of their surface as well as a high versatility and simplicity of modification[27,28]. The present work is a continuation of our studies in field of drug analysis using mercury and modified carbon paste electrodes[29-32].

The stability of ENP in aqueous buffer solutions has been studied as a function of pH. The rate of ENP; loss and the mode of degradation are dependent upon the pH of solution. Little information is available on complexes containing ENP drug. However, aqueous acidic/basic potentiometric titration pk_a values at 25±0.1^{0}C for enalapril were investigated[33-35].

In this manuscript, we report the metal complexes of ENP of the type M^{+n}-ENP such as Fe(III), Al(III), Cr(III), La(III), Y(III), Bi(III), Cu(II), Co(II), Ni(II), Mn(II), Pd(II) and Hg(II), nitrates ions. The dissociation constants of the drug and stability constants of their complexes will be determined.

Also, the voltammetric determination of ENP by square wave adsorptive stripping voltammetry (SWCdSV) at a paraffin oil bare carbon paste electrode has not been studied yet. Thus, the aim was to investigate the square wave cathodic stripping voltammetric determination of ENP in dosage forms (tablets) and in biological fluids (spiked and real urine sample) at a paraffin oil bare carbon paste electrode (CPE).

EXPERIMENTAL

Apparatus

All pH measurements were made with VWR scientific products Model 2000.

All voltammetric experiments were performed with EG, G Princeton Applied Research (PAR Princeton, NJ, USA) Model 273 A potentiostat, controlled by the model 270\250 electrochemical software version 4.30. A three-electrode cell was employed incorporating a hand - make working carbon paste electrode that prepared as previously mentioned[36], an Ag\AgCl (saturated KCl) reference electrode and platinum wire was achieved with a Teflon-Coated bar at approximately 400 rpm using a magnetic stirrer (kikA Labortechinik, Germany).

Ragents and materials

The solutions of Fe(III), Al(III), Cr(III), La(III), Y(III), Bi(III), Cu(II), Co(II), Ni(II), Mn(II), Pd(II) and Hg(II) (Merck, BDH) as nitrates were prepared and titrated complexometrically by EDTA[37]. Sodium hydroxide and enalapril solutions (Merck) were prepared in bidstilled water 50 % ethanol as fresh solution. In voltammetric method; enalapril (Merck) stock standard bidistilled water was prepared at 25 ± 0.1^{0}C and kept in brown volumetric flask. ENP working standard solutions were prepared daily by serial dilution of stock standard solution.

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Pharmaceutical formulations: Ezapril® protect tablet (Kahria Pharm. and Chem. Ind. Co. For Multipharma company. Egypt). to contains 10 mg enalapril Maleate per tablet, real urine sample was taken from heathly voltantear previously taken the drug.

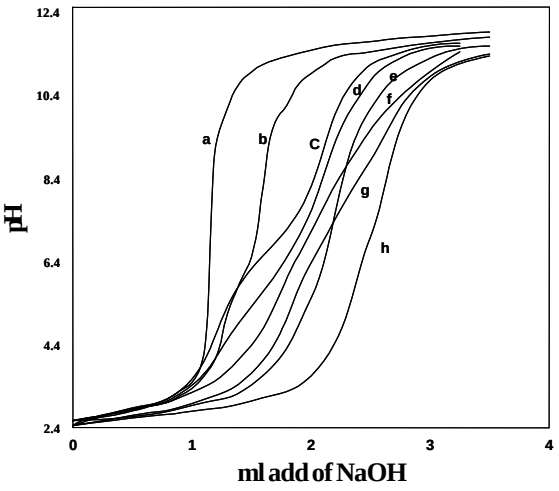

Figure 1 : Titration curves of enalapril with different metal ions at I = 0.05 M $NaNO_3$ (in aqueous - ethanolic solution) a = acid solution, b = solution (a) + ligand (ENP), c = solution (b) + Cu(II) ion, d = b + Cr(III) ion, e = b + Fe (III)ion, f = b + Hg (II) ion, g = b + Y(III) ion, h = b + Pd (II) ion

General analytical procedure

Calvin-Bjerrum, technique as adopted by Irving and Rossoti[38] or Kather and Munshi[39] were used to determine the dissociation constants of the ligand enalapril and the formation constants of their metal complexes with ENP at 25 ± 0.1^{0}C in aqueous solutions.The solutions were titrated potentiometrically with 0.2M standard free sodium hydroxide solution standardized against standard potassium hydrogen phthalate a = 0.001M HNO_3, b = a + 0.001 M. enalapril meleate and c = b + 0.001 M metal nitrate solution. The total volume was adjusted to50 cm^3 by adding doubly-distilled water in each case. The titration's were performed at 25 ± 0.1^{0}C and different ionic strengths of I = 0.1, 0.15 and 0.05 M $NaNO_3$. The preconcentration step was performed by immersing the carbon paste electrode in stirring 15 ml sample solution for a given period of time at potential range from (-0.5-(-1.4V)). was then stopped and a

TABLE 1: Protonation constants of ENP (ligand) and stability constants of metal ion complexes with three ionic strengths at 25^{0}C ± 0.1

Ionic strenght/Metal ion	I= 0.05 M			I= 0.1 M			I= 0.15 M		
	Log K_1 (M:L)*	Log K_2 (M:L)*	Log K_3 (M:L)*	Log K_1 (M:L)*	Log K_2 (M:L)*	Log K_3 (M:L)*	Log K_1 (M:L)*	Log K_2 (M:L)*	Log K_3 (M:L)*
H^+	7.2	5.2	-	6.7	4.7	-	5.1	4.0	-
Fe(III)	7.422 1:1	4.092 1:2	2.421 1:3	5.45 1:1	3.22 1:2	2.216 1:3	-	3.691 1:2	1.474 1:3
Al(III)	6.669 1:1	5.668 1:2	4.62 1:3	4.875 1:1	3.957 1:2	3.282 1:3	4.708 1:1	3.25 1:2	2.359 1:3
Cr(III)	5.698 1:1	3.851 1:2	1.986 1:3	5.698 1:1	3.971 1:2	-	5.403 1:1	2.704 1:2	1.571 1:3
La(III)	6.277 1:1	3.00 1:2	1.217 1:3	2.558 1:1	3.778 1:2	4.58 1:3	3.717 1:1	2.636 1:2	-
Y(III)	7.009 1:1	5.371 1:2	2.082 1:3	3.407 1:1	2.531 1:2	-	4.34 1:1	-	-
Bi(III)	-	-	-	5.453 1:1	3.391 1:2	-	-	-	-
Cu(II)	4.80 1:1	-	-	4.28 1:1	2.37 1:2	-	2.781 1:1	1.397 1:2	-
Co(II)	-	-	-	3.407 1:1	-	-	-	-	-
Ni(II)	2. 1:1	-	-	-	-	-	2.779 1:1	-	-
Mn(II)	5.62 1:1	-	-	4.58 1:1	-	-	-	-	-
Pd(II)	5.85 1:1	3.3 1:2	-	-	-	-	3.34 1:1	-	-
Hg(II)	6.50 1:1	3.89 1:2	-	4.35 1:1	2.07 1:2	-	4.263 1:1	1.998 1:2	-

***These ratios are form potentiometric and conductometric methods**

delay period of 10s to settle the solution and decrease the background current, square wave voltammogram was recorded in the negative potential direction. A renewed carbon paste electrode surface was used for each measurement.

For determination of enalapril in biological fluids (spiked and real urine samples), 1mL aliquot of urine (blank or containing drug) was transferred to 250 mL separating funnel containing 5ml of diethyl ether (Merck). The mixture was thoroughly shaken for 15 min, then, the organic layer was transferred to a glass tube, and the solvent was evaporated in water bath to dryness. The residue was reconstituted in methanol. Then, 30µL urine sample (containing 0.985 ng/mL of drug in case of spiked urine and unknown amount of excreted drug in real urine samples), was added to 15 ml 0.04 M Britton - Robinson buffer pH ~ 9.0.The solution was stirred at 400 rmp at open circuit conditions and the square wave voltammogram was recorded. Also, in case of dosage forms, 10 tablets of the drug were weighed into a small dish, powdered and mixed well. A knowledge portion was weighed and dissolved in 100 mL of methanol, shaken well and filtered was then transferred into a calibrated flask and it was completed to volume with bidistteled water. 30µL of each solution was then added to the measurement cell. In all measurements the square wave voltammogram was recorded in negative potential direction. The optimum operational parameters selected for the determination of ENP by (SWCSV) using CPE was tabulated in TABLE 2.

TABLE 2: The optimum operational parameters selected for the determination of LSP by SWCSV at CPE

Parameter	Selected value
Accumulation potential	-0.5 V
Final potential	-1.4 V
Modulation time	10S.
Frequency	50 HZ
Scan increment	2 mV
Accumulation time	Various
PH	9.00
Buffer type	0.04M Britton- Robinson universal buffer

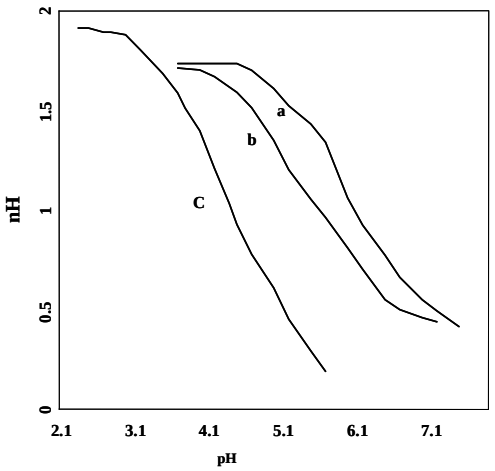

Figure 2 : protonation constantes curves of enalapril maleate at a = 0.05 M, b = 0.1 M, C = 0.15 M

RESULTS AND DISSCUTION

Metal complexes of enalapril

The titration curves are shown in figure 1 the average number of proton attached per ligand, $\bar{n}H$, was calculated[38].

$$\bar{n}H = Y + \frac{(V_1 - V_2)(N^0 + E^0)}{(V_0 - V_1)(Tcl^0)} \quad (1)$$

Where Y = 2 (number of aissociable protons in the ligand), V° is the initial volume, V_1 and V_2 are the volume of alkali required to reach the same pH in mineral acid (HNO_3) and (HNO_3+ENP) respectively. $T_CL°$ is the total concentration of ligand, N° is the normality of the alkali and E° is the initial concentration of free acid.

Calculation of proton ligand association constants were carried out by plotting $\bar{n}_H$ against pH at the three ionic strengths are shown in figure 2. The values of $\log K_1^H$ and $\log K_2^H$ (the first and second proton association constants of the enalapril) are the pH values corresponding to = 0.5 and 1.5, respectively. The SUPERQUAD computer program[40,41] was used to refine the overall protonation or formation constants by a least squares fit. The value of $\log K_1^H$ TABLE 1 agrees quite well with those previously reported[38], in aqueous solution medium and at 25 ± 0.1°C.

It is worth mentioning that the ligand does not hydrolysis under the experimental conditions. This is indicated by the rapid attainment of equilibrium during the titration time. The titration curves of the metal - ligand solutions (c) are well separated from the ligand solution (b) in figure 1.Thus replacement of H^+ ion is due to complexation. From these titration curves, $\bar{n}$ (average number of ligand molecules attached per metal ion) and pL (free ligand exponent) values were calculated using Irving and Rossoti equation[38].

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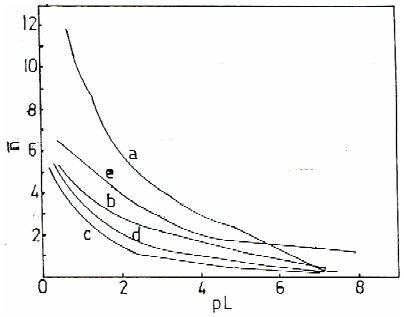


Figure 3 : Formation curves of binary metal ion complexes with ENP (ligand) at I = 0.05M

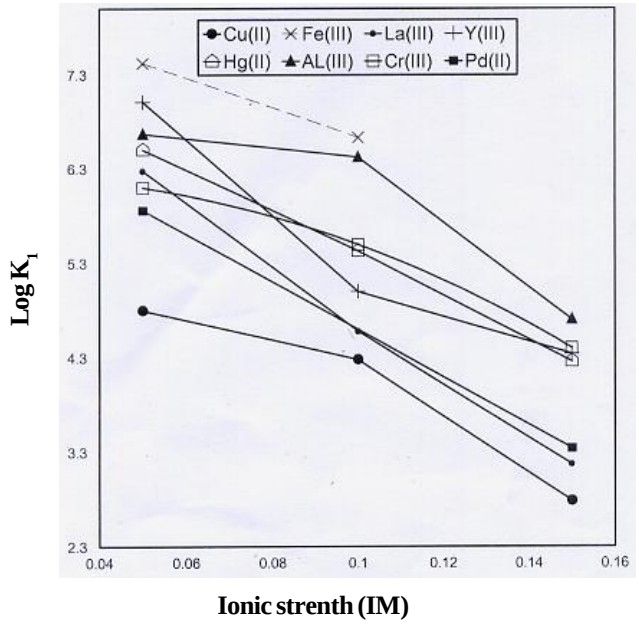


Figure 4 : The relationship between the first stability constants (logK_1) of metal ion complexes and ionic strength

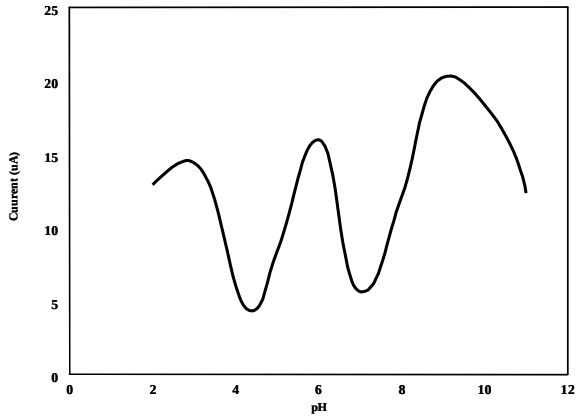


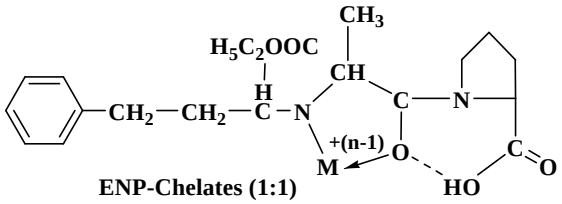
Figure 5 : Plot of Ip versus different Britton -Robinson universal buffer solutions pH values at 492.52 ng\mL of enalapril

$$\bar{n} = \frac{(V_3 - V_2)(N^0 + E^0)}{(V_0 + V_2)\,n\,HTcM^o} \quad (2)$$

$$pL = Log\left[\frac{(1 + K_1^H[H^+] + K_2^H[H^+]^2 + K_3^H[H^+]^3 + ---)}{(Tcl^0 - \bar{n}\,TcM^0)} \times \frac{V_0 + V_3}{V_0}\right] \quad (3)$$

Where V_1, V_2, V_3 are the amounts of the alkali reach the same pH in the free acid, free acid + ligand and free acid + ligand + metal, respectively. $T_cM°$denotes the total concentration of metal present in the solution. The $\bar{n}$ values were plotted against the corresponding pL values to get the formation curves of the metal complexation equilibria. The formation curves are shown in figure 3. From these formation curves, the values of stability constants at diffrerent ionic strengths I = 0.1, 0.05 and 0.15 M are listed in TABLE 1 were determined using the half - integral method[38]. Also, from the relation between logK_1 and the ionic strengths figure 4, we can conclude that the stability constants of metal - ligand complexes (1:1) were increased as the ionic strength decreased.

Enalapril maleate has two sites, the first site is protonation of imino group (NH) and the other site is the dissociation of proton in the carboxylic group (COOH), these sites are shown as follow:



ENP-Chelates (1:1)

The order of stability constants of the different binary complexes formed between enalapril and transition metal ions investigated in this study is in the expected Irving - Williams order[42] for (1:1)metal to ligand at different ionic strengths $NaNO_3$: Fe (III)>Y (III)> Al (III)>Hg(II)>La (III)>Pd (II)>Cr (III)>Mn (II)>Cu (II)>Ni (II).

Square wave voltammetry of enalapril

Effect of buffer type, pH, ionic strength

The effect of type of buffer used as electrolyte (Acetate buffer, citrate buffer, phosphate buffer, HCl - sodium acetate buffer and Britton - Robinson universal buffer) on the analytical signal was tested. Both the peak height and peak shape were taking into consideration

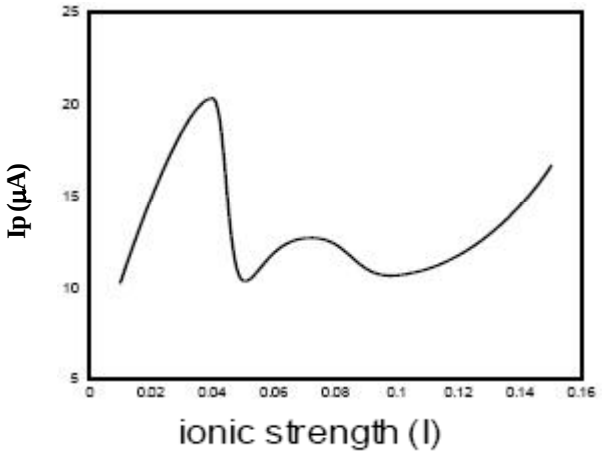


Figure 6 : Plot of Ip versus different ionic strengths of pH ~ (9.0) at ng\mL of ENP

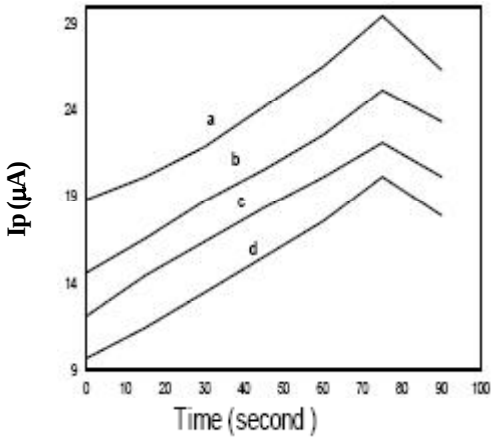


Figure 7 : Plot Ip -current time of ENP at (a): 492.52 ng/ mL^{-1} (b): 49.25 ng/mL^{-1}(c): 4.925ng/mL^{-1} (d): 0.492ng/ mL^{-1}

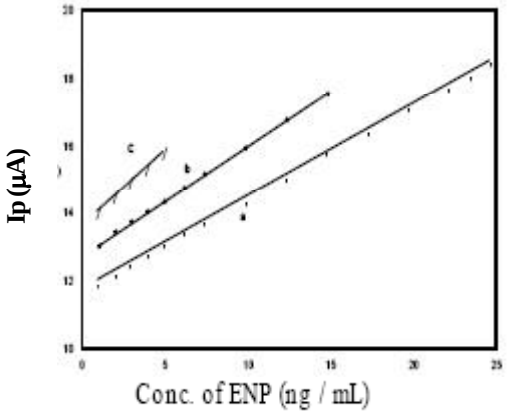


Figure 8 : Plot of Ip versus concentrations of enalapril using 0.04 M of Britton - Robinson buffer (pH~9.0) at different accumulation times: (a): 15 s (b): 30 s (c): 60 s

when choosing type of buffer. A study of the influence of the ionic strength of the medium on definition of the voltammeteric peak revealed that minimal background current. The best curve and the highest peak were ob-

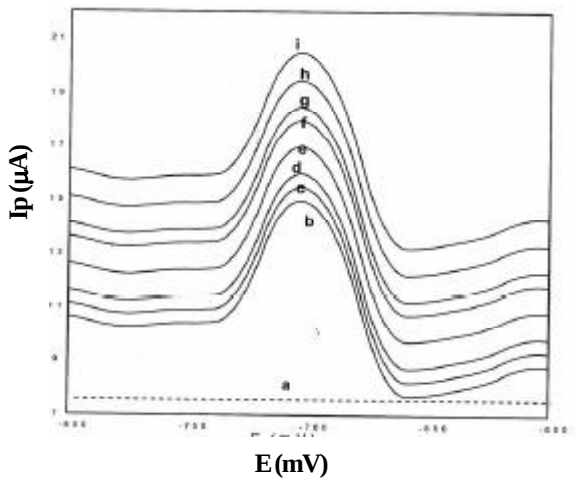


Figure 9 : Typical SWCS voltammograms of Enalapril different concentrations in presence of 0.04 M Britton - Robinson buffer (pH ~9.0) at 60 s

tained in 0.04 M of Britton -Robinson buffer. The effect of pH - on the reduction of ENP at CPE s was studied over the pH range 2.8-12 at concentration 942 ng/ mL ENP by square wave voltammetry as shown in figure 5. A small current was observed at pH ~2.8, which increased gradually up to pH~9.0 which used in all measurements.The potential cathodic of ENP is shifted linearly towards less negative values with increasing the pH over than 9.0. The influence of ionic strength on the efficiency of accumulation was studied for 492 ng/mL ENP per concentration time. The buffer concentration range from 0.02-0.15 M in the chosen buffer type varied the ionic strength. The result showed that increasing ionic strengths were found to be of great significance on the degree of accumulation. This indicated that the process responsible for accumulation of the drug at the electrode surface was mainly electrostatic in nature.

Effect of accumulation potential

The effect of accumulation potential on peak current was also investigated in potential range from 0.0 to -5.0 V at 30s perconcentration time for 985 ng/mL of ENP solution (pH~9.0). Experiments proved that the peak current of ENP increases with negative shifting of starting potential in the range from -0.3 to -0.5 V and the decease with negative shifting from -0.5 to - 1.4V. The peak current has its maximum value at initial potential -0.5 V, which was used in the subsequent examination of other decencies.

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Effect of accumulation time and reproducibility

The dependence of the peak current on accumulation time was studied for four levels of concentration named as (0.492, 4.92, 49.2 and 492 ng/mL) of ENP. The stripping signal increased linearly with increase accumulation time up to 75 s for all concentrations figure 7. Repeating three experiments on 4.92 ng/ mL at 60s accumulation time showed the reproducibility of the adsorption process.

Effect of concentration and detection limit

The square wave cathodic stripping peak for ENP yields a well-defined concentration dependence using SWCdSV method. Calibration plots over the ENP concentration range, following different preconcentration times ware investigated. One peak was observed on addition of pure drug to sample at (-0.71) V as shown in figure 8. On increasing the ENP concentration, however, a well-defined peak was observed over the concentration 0.985-24.62 ng/mL at 15s, 0.985- 14.77 at 30 s ng/mL and from 0.985 - 4.925 at 60 s ng/mL, with the stirring at - 0.5 V, the results are shown in figure 9. However, TABLE 3 illustrates the linearity ranges. The detection limits estimated as $3\sigma/b$ where b is the slope and σ = standard deviation of the intercept[26,29,30], and quantitative limits was also computed as $10\sigma/$ b. The results obtained from the proposed method show that ENP can be detected from 2×10^{-10} M (0.0985 ng/mL); with relative standard deviation ± 0.01%, Correlation coefficient r = 0.9964 (n = 5) at accumulation time 60s.

TABLE 3 : Characteristic of linear regression of calibration curves for enalapril in 0.04 M Britton-Robinson buffer (pH~9.0) using SWCdS at different deposition times

Deposition time (s)	Linearity range (ng/mL)	Correlation coefficient	Slope (μA/ngmL^{-1}) ± SD	Intercept (μA) ± SD
15	0.985-24.62	0.9965	3.57 ± 0.42	4.704± 0.43
30	0.985-14.77	0.9998	3.09 ±0.37	4.967± 0.38
60	0.985-4.92	0.9980	2.22 ± 0.56	5.335± 0.53

Effect of interferences

To investigate the efficiency and selectivity of the proposed analytical method to Pharmaceutical formulation, a synthetic solution containing a fixed amount of enalapril (ENP) (1×10^{-6} M) was spiked excess amount of some common excipients and additives (1:10000) that are used in Pharmaceutical preparation (e.g. Glycine, DL- alanine, DL- Valine (amino acids), Urea, and some metal ions such as Fe (III), Cu (II) and Cd (II)).

These additives and excipients were added to voltammetric cell to study the effect of such additives under the optimum experimental conditions on the efficiency and selectivity of the proposed analytical method to pharmaceutical formation.

However, different concentrations of DL- valine ranged from 1×10^{-6} to 1×10^{-5} M was added, and then the voltammograms were recorded. The results showed that the addition of 1×10^{-6} M of DL-Valine increase the current response of ENP by about 15.78 % and the addition of 1×10^{-5} M increase the current peak by about 21.15 %. On the other hand it was observed the addition of 1×10^{-6} and 1×10^{-5} M of DL-alanine increase the peak response by 9.68 % and 10.69 % respectively. Using the same concentrations, the concentrations 1×10^{-6} and 1×10^{-5} M Glycine was observed no significant interference. Ascorbic acid and

TABEL 4: Analysis of lisinopril in tablet, spiked and real urine samples

Sample	Accumulation time (s)	Detection limits (ng/mL)	Linearity range (ng/mL)	Slope (μA/ ng mL^{-1}) ± SD	Intercept (ng/mL) ± SD	Correlation coefficient
Ezapril® tablet[a]	15	0.86	0.985-14.77	0.84 ± 0.01	5.04 ± 0.24	0.9994
	30	0. 78	0.985-14.77	0.92 ± 0.02	5.59 ± 0.24	0.9992
	60	0.71	0.985-4.92	1.4 ± 0.03	6.53 ± 0.33	0.9980
Spiked urine sample	15	0.04	0.0985-0.49	27.39 ± 0.33	27.39 ± 0.33	0.9972
	30	0.03	0.098-0.39	25.18 ± 0.83	29.69 ± 0.23	0.9984
	60	0.02	0.098-0.39	28.20 ± 1.01	33.13 ± 0.20	0.9937
Real urine sample after 12 h	15	0.84	9.85-49.25	0.43 ± 0.01	31.14 ± 0.12	0.9999
	30	0.74	9.85- 39.40	0.41 ± 0.01	30.84 ± 0.10	0.9990
	60	0.71	9.85-39.40	0.42 ± 0.01	35.30 ± 0.10	0.9962
Real urine sample after 24 h	15	0.65	0.985-20.83	0.69 ± 0.02	32.15 ± 0.15	0.9987
	30	0.47	0.985-12.50	0.77 ± 0.02	33.36 ± 0.12	0.9985
	60	0.43	0.985-4.16	1.74 ± 0.09	33.510 ± 0.25	0.9961

Urea were showed no significant interference on peak response of ENP.

Furthermore, the effect of some metal ions such as Fe (III), Cu (II) and Cd (II) on the peak response of 1×10^{-6} M of ENP was studied. Different concentrations of Fe (III), Cu(II) and Cd(II) ranged from 1×10^{-6} to 1×10^{-4} M were added. In the presence of Fe (III) the current decrease by 3.58 % at 1×10^{-6}, 3.86 % at 1×10^{-5} M and 5.00 % at 1×10^{-4} M on peak response of ENP.

But in the case of Cu (II), it is showed the current increase by 8.08 % at 1×10^{-6} M and 10.28% at 1×10^{-5} M in ENP. In the case of Cd (II) was observed no significant interference on peak response of ENP.

Analytical applications

The proposed method was successfully applied to determine enalapril in pharmaceutical preparations, spiked and real urine samples.

Pharmaceutical preparations

The square wave voltammogram of the tablet sample was recorded after preconcentration time for 15, 30 and 60 s in 0.04 M Britton-Robinson buffer (pH~9.0). The content of the tablet in the cell was determined by standard addition method[43]. One peak was observed on addition of pure drug to sample at (-0.58 V) on increasing the ENP concentration, the peak current was increased linearly from 0.985 - 14.77 ng/mL at 15, 30 s and from 0.985 - 4.92 ng/mL at 60s, which fitted equation $Y = 0.84 X + 5.04$ with correlation coefficient 0.9994, $Y = 0.68 X + 5.59$ with correlation coefficient 0.9992, $Y = 0.78 x + 6.53$ with correlation coefficient 0.9980 at 15, 30 and 60 s, respectively.

The obtained values were compared statistically by studied t - test for accuracy and f -test for precision with the official method[44,45].

Real urine samples

The proposed method was also applied to the determination of ENP in human urine samples from healthy voluntress who received as single oral dose of 10 mg of Ezapril® tablet. The samples of individual were collected up to 24 h after a dministration of tablet and urinary volumes were recorded as well. ENP was well separated from organic components and excipients did not interfere[45]. The result obtained summarized in TABLE 4, shown that a small amount of an administered dose are excreted in the urine. The results showed a high correlation coefficient $r > 0.9992$. Also, the obtained result from the proposed method for voltammetric method[44,45], in which about 60 % of an oral dose is excreted in human urine in the first 24 hours.

Accuracy and repeatability

Applying the proposed method for the analysis of ENP in dosage forms, spiked and real urine samples exhibited the correlation coefficient of 0.9980, 0.9937, 0.9962 at 60s respectively, the standard deviation of both slopes ± 0.71, ± 0.28, ± 0.10 % at 60 s respectively, and the intercept of 20.73, 33.13, 35.30 at 60 s respectively, indicating adequate precision and accuracy of the proposed method.

CONCLUSION

Potentiometric method is excellent method for calculation of stability constant of metal ligand complexes. ENA forms 1:1, 1:2 and/or 1:3 metal to ligand complexes. Also, the SWCdSV method with carbon paste electrode for the quantitative determination of ENP was found to be simple and highly sensitive in dosage forms and biological fluids. A detection limit of 2×10^{-10} M (0.0985 ng/mL) at 60 s accumulation time with the standard deviation ± 1.007 was obtained in pure solution. The method can be used successfully to assay the drug in dosage form, as well as in spiked and real urine samples. It has some distinct advantages over existing methods regarding sensitivity; time saving and minimum detect ability. Moreover, it can be directly applied to the determination of ENP in urine without prior extraction, and this is an advantage over HPLC that necessitates a clean-up procedure before application. The method is sensitive enough to monitor the drug level after therapeutic doses.

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