The Binding of Metal Ions by Enalapril Maleate and Lisinopril

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Abstract

A number of side-effects have been observed in patients receiving the antihypertensive agents enalapril maleate and lisinopril. Many of the symptoms could be indicative of trace metal imbalances. Formation constants for the zinc(II) and copper(II) complexes of maleate, enalapril maleate and lisinopril have been measured potentiometrically at 37 °C and $I = 150$ mmol dm^{-3} chloride. The constants have been used in computer simulation models to assess the relative efficacy of the agents in mobilizing zinc and copper from the labile protein complexes in normal plasma. The study indicates that neither maleate, enalapril nor lisinopril mobilize zinc and copper at normal pharmacological levels of the drug.

Introduction

In the western world hypertension is generally considered to be endemic. If untreated, death due to heart disease, stroke or kidney failure may result [1]. Fortunately, hypertension can now be managed by a number of therapeutic agents termed antihypertensives, one of the newest groups of these being the angiotensin converting enzyme (ACE) inhibitors.

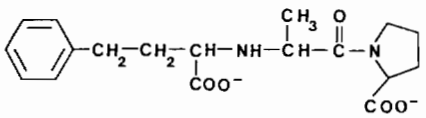

Fig. 1. Chemical structure of enalaprilat.

To date, a number of ACE inhibitors have been developed, one such drug being enalaprilat (1-[*N*-[1(*S*)-carboxy-3-phenylpropyl]-L-alanyl]-L-proline produced by Merck, Sharp & Dohme, Fig. 1). Unfortunately, clinical testing of this drug has shown it to be poorly absorbed when administered orally

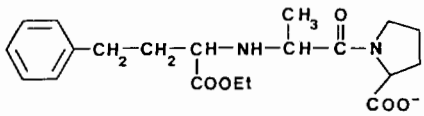

Fig. 2. Chemical structure of enalapril.

[2, 3]. Absorption has, however, been improved upon in two ways:

(i) The chain carboxylate group was esterified to yield the monoethyl ester, designated enalapril (1-[*N*-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, Fig. 2) and produced commercially as the maleate salt.

(ii) The L-alanine fragment of enalaprilat was replaced with L-lysine, the resulting compound being designated lisinopril ([N^2-[(*S*)-1-carboxy-3-phenylpropyl]-L-lysyl]-L-proline, Fig. 3).

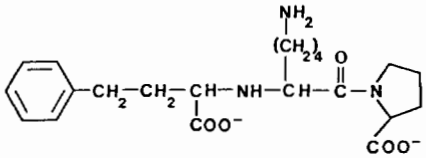

Fig. 3. Chemical structure of lisinopril.

In vitro studies have shown enalapril to lack the activity of enalaprilat; although *in vivo* enalapril undergoes bioactivation to give enalaprilat, so that enalapril acts as a prodrug [4]. Lisinopril, however, requires no bioactivation [4]. During the last five years, both lisinopril and enalapril have been employed in controlling mild to moderate hypertension and in the treatment of congestive heart failure [5, 6].

In general, therapy using both drugs has been well tolerated, with the occurrence of serious adverse reactions being rare [7]. The most frequently reported side-effects have included headaches, dizziness and fatigue, with occasional cases of taste dysfunction, rash and neutropenia [8–10]. The documented cases of these latter three reactions, which are mainly observed with enalapril therapy, and the link of such side-effects to zinc and copper deficiency has already prompted investigations to assess whether the active metabolite of enalapril, *i.e.*

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enalaprilat, could possibly cause depletion of these essential trace metals *in vivo* [11]. This original study indicated that the mobilization of zinc and copper from normal blood plasma would be negligible. In view of this result, a similar study was performed employing the prodrug enalapril to evaluate whether this agent could cause trace metal imbalances. Lisinopril was also studied for comparison.

The studies reported here involved a potentiometric investigation of the interactions of enalapril maleate and lisinopril with zinc and copper, to determine the formation constants for these metal–ligand interactions. The formation constants were then used in the ECCLES (Evaluation of Constituent Concentrations in Large Equilibrium Systems) computer model of blood plasma, so that the effects of enalapril maleate and lisinopril on these trace metals *in vivo* could be predicted [12].

Experimental and Computational Procedures

Materials

Enalapril as the maleate salt and lisinopril were provided by Merck, Sharp & Dohme Ltd. and used without further purification. *Anal.* For enalapril maleate. Found: C, 58.5; H, 6.5; N, 5.7. Calc. for $C_{24}H_{32}O_9N_2$: C, 58.2; H, 6.5; N, 5.7%. For lisinopril. Found: C, 56.8; H, 7.7; N, 9.6. Calc. for $C_{21}H_{31}O_5N_3 \cdot 2H_2O$: C, 57.1; H, 8.0; N, 9.5%. Solutions of the agents were freshly prepared each day by direct weighing.

Maleic acid (supplied by BDH Chemicals Ltd.) was prepared as a single stock solution and stored at room temperature. *Anal.* Found: C, 41.2; H, 3.5. Calc. for $C_4H_4O_4$: C, 41.4; H, 3.5%.

Acidified solutions of zinc and copper were prepared from their oxide and chloride salts, respectively (BDH AnalaR). The solutions were analysed by titration with EDTA for metal ion concentration and with sodium hydroxide for hydrogen ion content.

Sodium chloride (BDH AnalaR) was employed to maintain a background electrolyte concentration of 150 mmol dm^{-3} chloride in all solutions.

Method

All potentiometric titrations were performed at 37 °C and I = 150 mmol dm^{-3} chloride, following our usual procedure [13]. Ligand–proton and metal–ligand ratios were varied over the set of experiments performed for each system, as shown in the summary (Table I).

Data Treatment

The potentiometric titration data was analysed using the ESTA (Equilibrium Simulation for Titration Analysis) library of computer programs [14]. Initial

TABLE I. Summary of Titration Data Used in Formation Constant Calculations[a]

System	C_M	C_L	C_X	C_H	pH range
Proton–maleate		15.03		14.83	1.68–10.87
		12.01		11.92	1.75–10.81
		9.06		8.82	1.87–10.96
		6.03		5.85	2.02–10.89
		9.01		8.85	1.86–11.04
		11.95		11.88	1.76–10.90
Zn–maleate	3.65	11.36		3.76	1.94–6.79
	3.65	11.36		3.76	1.94–6.82
	3.75	11.36		3.77	1.94–6.73
	4.86	10.10		5.01	1.93–6.82
	6.64	8.26		6.84	1.90–6.73
	2.92	12.12		3.01	1.95–6.74
Cu–maleate	3.03	12.63		2.99	1.94–6.4
	3.79	11.36		3.84	1.95–6.29
	6.89	8.26		6.98	1.91–5.4
	5.05	10.10		5.05	1.92–5.99
	2.53	12.63		2.53	1.97–6.46
	3.79	11.36		3.79	1.96–6.23
	3.79	11.36		3.79	1.96–6.25
Proton–enalapril maleate		14.83	14.83	15.03	1.99–10.81
		11.71	11.71	19.95	1.85–10.88
		9.02	9.02	16.00	1.92–10.93
		5.99	5.99	15.99	1.88–10.95
		12.04	12.04	15.82	1.94–10.99
Zn–enalapril maleate	6.64	8.33	8.33	6.84	2.21–6.81
	3.65	6.00	6.00	3.76	2.37–6.50
	3.65	11.15	11.15	3.76	2.30–6.79
	2.66	8.14	8.14	2.74	2.45–6.92
	6.35	4.23	4.23	6.55	2.20–6.81
Cu–enalapril maleate	3.79	11.24	11.27	11.28	2.04–4.69
	2.53	12.60	12.60	10.85	2.08–4.49
	5.05	9.87	9.87	11.71	2.02–4.34
	6.59	8.03	8.03	12.24	1.98–4.46
	3.03	11.81	11.81	9.42	2.13–4.61
	3.79	11.08	11.08	9.78	2.11–4.55
	3.79	11.42	11.42	3.79	2.38–5.07
Proton–lisinopril		4.78			4.97–11.06
		4.78			1.82–4.95
		9.77			4.93–10.95
		9.77			1.73–4.91
		13.96			4.90–10.93
		13.96			1.75–4.91
		4.84			4.94–10.92
		4.84			1.79–4.99
		14.88			4.88–10.93
		14.88			1.71–4.91
		10.02			4.92–10.95
		10.02			1.71–4.93
Zn–lisinopril	10.06	10.92		10.05	2.70–7.38
	10.06	10.92		10.05	2.72–7.19
	5.03	14.12		5.02	3.42–8.07
	5.03	14.12		5.02	3.43–8.01
	6.70	12.55		6.70	3.17–7.76

(continued)

TABLE I. *(continued)*

System	C_M	C_L	C_X	C_H	pH range
Cu–lisinopril	5.02	14.95		4.99	2.95–9.41
	6.69	13.31		6.66	2.75–8.93
	8.02	11.38		7.97	2.60–8.03
	3.34	11.73		3.33	3.10–9.49
	8.02	8.45		7.97	2.51–6.91

[a]Initial total concentrations of metal (C_M), ligand (C_L), second ligand (C_X), mineral acid (C_H), and pH range investigated. All concentrations are expressed in mmol dm^{-3}.

estimates of the stoichiometries of possible complexes and their formation constants were obtained using the ZBAR and QBAR tasks for the binary systems and QBAR alone for the ternary system. The OBJE option was employed to refine the formation constants for the various systems to produce a number of possible models. The final model selection was based on graphical comparisons between the calculated and the experimental formation (ZBAR) and deprotonation (QBAR) curves as well as the various statistical criteria. The ERR% task was used to assess the extent of formation of each postulated complex in each experimental titration, allowing identification of significant and minor complexes. Species distribution profiles as a function of pH ($-\log[H^+]$) were computed using the SPEC task. This affords easy identification of the predominant species over a range of pH values.

The plasma mobilization of metal ions by the antihypertensive agents was simulated using the ECCLES program.

Results and Discussion

Interactions of Enalapril Maleate and Lisinopril with Zinc and Copper

Enalapril is commercially produced as the 1:1 maleate salt [15]. In view of this, the potentiometric studies involving this compound were treated as ternary systems. Thus, in order to evaluate the interactions of enalapril with protons, Zn(II) and Cu(II) ions, the maleate binary system was first characterized.

The formation constants for maleate, enalapril maleate and lisinopril obtained from this present study are given in Tables II–IV.

Experimental formation curves for the binary systems and deprotonation curves for the ternary systems are shown in Figs. 4–9, whilst Fig. 10 serves as an example of the model selection procedure. An example of a species distribution profile is given in Fig. 11, with Table V listing the significant species postulated as being formed at pH 7.4, as determined from the speciation profiles of all the systems examined. The computation of speciation profiles employed ligand-to-metal ratios of 3:1 throughout.

Metal–Ligand Complexation by Maleate

Maleate has two carboxylate groups representing two potential binding sites. The protonation con-

TABLE II. Formation Constants for Proton–, Zn(II)– and Cu(II)–Maleate Interaction at 37 °C[a]

$\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$

Interaction	Species			log β_{pqr}	Standard deviation	Objective function	*R* factor	No. of points	No. of titrations
	p	*q*	*r*						
Maleate protonation	1	0	1	5.819	0.001	27	0.001	559	6
	1	0	2	7.589	0.003				
Zn(II)–maleate	1	1	0	2.19	0.014				
Model A	1	2	2	15.72	0.011				
	1	2	1	10.52	0.012	54	0.0025	458	7
	1	2	0	4.67	0.013				
	1	1	−1	−5.54	0.027				
Model B	1	1	0	2.21	0.014				
	1	2	2	15.72	0.011				
	1	2	1	10.51	0.012	54	0.0025	458	7
	1	2	0	4.65	0.013				
	1	2	−1	−3.22	0.027				
Cu(II)–maleate	1	1	0	3.57	0.003				
	1	1	−1	−3.51	0.012	61	0.003	440	7
	1	2	2	15.35	0.012				
	1	2	0	5.50	0.008				

[a]I = 150 mmol dm^{-3} chloride.

TABLE III. Formation Constants for Proton–, Zn(II)– and Cu(II)–Enalapril Maleate Interaction at 37 °C[a]

$\beta_{pqq'r} = [M_pL_qL'_{q'}H_r]/[M]^p[L]^q[L']^{q'}[H]^r$

Interaction	Species				log $\beta_{pqq'r}$	Standard deviation	Objective function	R factor	No. of points	No. of titrations
	p	q	q'	r						
Enalapril maleate protonation	0	0	1	1	5.819					
	0	0	1	2	7.589					
	0	1	0	1	5.292	0.002	220	0.005	488	5
	0	1	0	2	8.317	0.004				
Zn(II)–enalapril maleate	1	0	1	0	2.19					
	1	0	1	−1	−5.54					
Model A	1	0	2	2	15.72					
	1	0	2	1	10.52					
	1	0	2	0	4.67		148	0.005	358	6
	1	1	0	1	7.32	0.009				
	1	1	0	0	2.33	0.010				
	1	1	0	−1	−4.96	0.014				
Model B	1	0	1	0	2.21					
	1	0	2	2	15.72					
	1	0	2	1	10.51					
	1	0	2	0	4.65					
	1	0	2	−1	−3.22		151	0.005	358	6
	1	1	0	1	7.32	0.009				
	1	1	0	0	2.33	0.010				
	1	1	0	−1	−4.94	0.014				
Cu(II)–enalapril maleate	1	0	1	0	3.57					
	1	0	1	−1	−3.51					
	1	0	2	2	15.35					
	1	0	2	0	5.50		90	0.002	343	7
	1	1	0	0	3.12	0.006				
	1	2	0	2	14.18	0.016				
	1	1	1	0	6.47	0.007				

[a] I = 150 mmol dm^{-3} chloride; L = enalapril; L′ = maleate.

TABLE IV. Formation Constants for Proton–, Zn(II)– and Cu(II)–Lisinopril Interaction at 37 °C[a]

$\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$

Interaction	Species			log β_{pqr}	Standard deviation	Objective function	R factor	No. of points	No. of titrations
	p	q	r						
Lisinopril protonation	0	1	1	10.401	0.002				
	0	1	2	17.364	0.003	21	0.001	464	12
	0	1	2	20.468	0.004				
	0	1	4	21.942	0.010				
Zn(II)–lisinopril	1	1	1	13.96	0.002				
	1	1	0	5.65	0.007	152	0.007	327	6
	1	2	2	26.81	0.004				
Cu(II)–lisinopril	1	1	1	16.90	0.004				
	1	1	0	9.21	0.006	375	0.007	303	6
	1	1	−1	−0.51	0.014				
	1	2	2	30.99	0.005				

[a] I = 150 mmol dm^{-3} chloride.

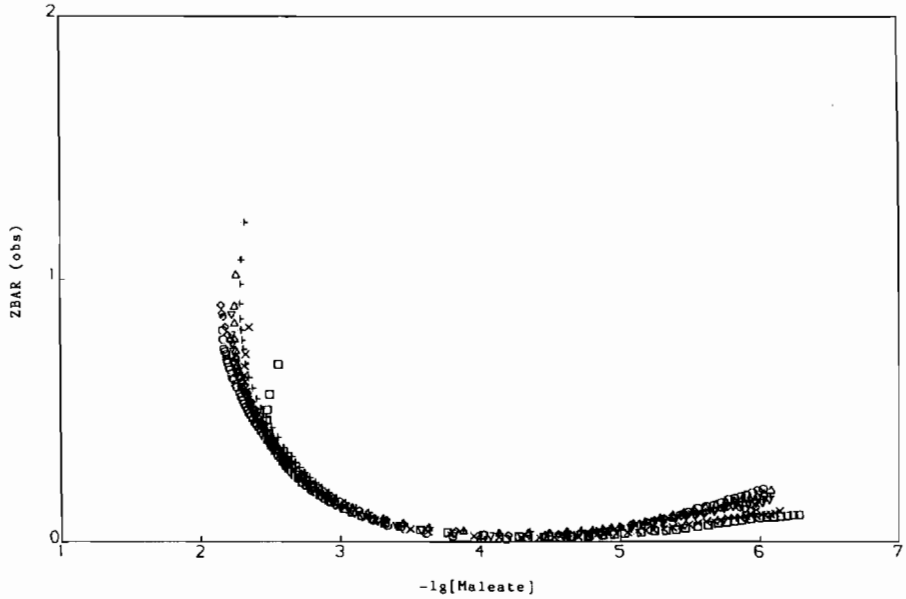

Fig. 4. Experimental formation curve for the Zn(II)–maleate system.

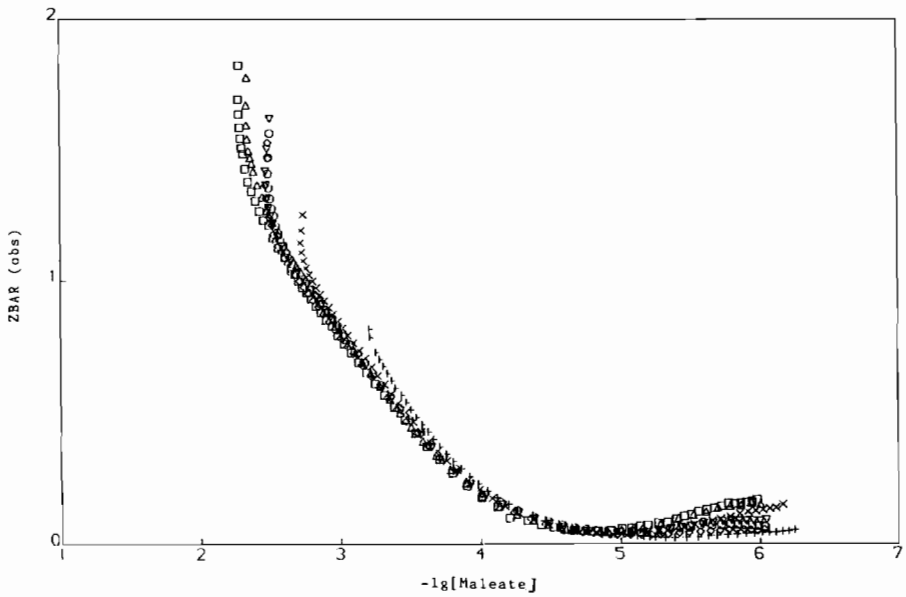

Fig. 5. Experimental formation curve for the Cu(II)–maleate system.

stants derived from the optimization of the experimental data are given in Table II. The constants obtained from this study compare favourably with those of previous investigations [16–18].

Zinc interacts with maleate to form both protonated and hydroxy species, indicated by the non-superimposability of the formation curves in both the acidic and alkaline regions (Fig. 4). OBJE analysis of the titration data resulted in two equivalent models (Table II), both of which could be considered to equally well describe the experimental system. Species distribution analyses show the hydroxy complexes of both models to be minor species, accounting for less than 5% of the total zinc in each titration. However, their inclusion provides a better overall analysis of the system which is reflected by the improvement in statistics. The zinc maleate models differ by only the hydroxy species; in view of this, the possible coexistence of these species was investigated. The inclusion of both hydroxy complexes resulted in a statistically inferior model which, however, suggests the MLOH and ML_2OH complexes to be mutually exclusive species in that they form in identical pH regions within the titrations.

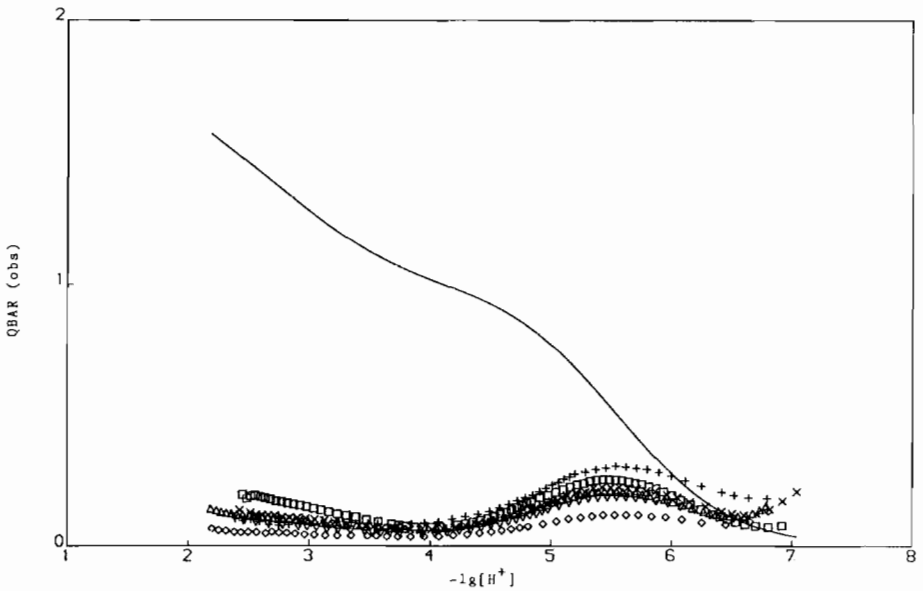

Fig. 6. Experimental deprotonation curve for the Zn(II)–enalapril maleate system. Different symbols refer to different titrations having different ligand:metal ratios and different total ligand and total metal concentrations. On each deprotonation curve a plot of *n versus* $-\log[H^+]$ appears as a solid black line.

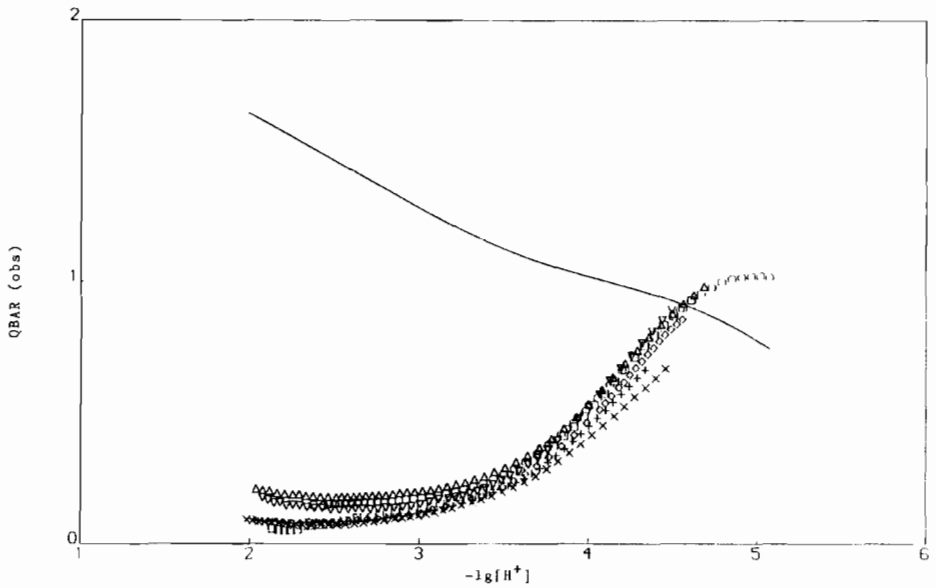

Fig. 7. Experimental deprotonation curve for the Cu(II)–enalapril maleate system.

Since the zinc maleate models cannot be differentiated either statistically or graphically, and because of the importance of correctly defining the binary system, both models have, therefore, been used in the analysis of the zinc enalapril maleate ternary data.

The formation curve for the interaction of Cu(II) ions with maleate (Fig. 5) once again indicates the formation of hydroxy and protonated species. Analysis of the titration data using OBJE confirmed the presence of these species and showed the system to be characterized by the formation of the ML_2H_2, ML_2, ML and MLOH complexes in solution. The results of this study differ from those of Bonomo *et al.*, who characterized the system in terms of the stepwise ML and ML_2 complexes [17]. Comparison of the formation constants for the ML and ML_2 species shows good agreement, considering the different experimental conditions and computational techniques employed.

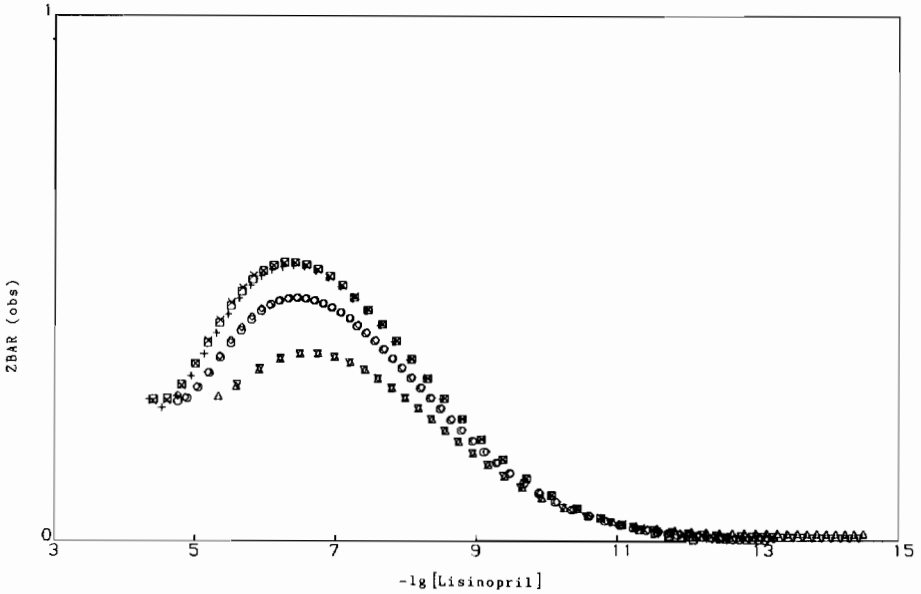

Fig. 8. Experimental formation curve for the Zn(II)–lisinopril system.

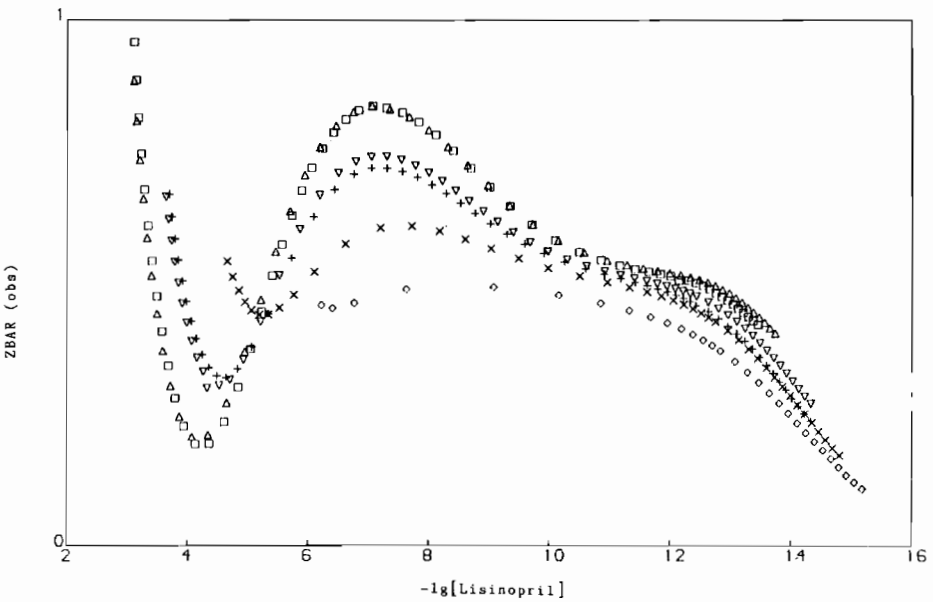

Fig. 9. Experimental formation curve for the Cu(II)–lisinopril system.

Metal–Ligand Complexation by Enalapril Maleate

Enalapril maleate has four possible protonation sites; these correspond to the two carboxylate groups of the maleate anion and the secondary amino and carboxylate group of the enalapril cation. In the analysis of the titration data, the salt was considered to consist of two separate ligands. Therefore, during the refinement procedure only the enalapril protonation constants were optimized, those for the maleate system being held constant. The resultant constants can be found in Table III.

Analysis of the zinc and copper enalapril maleate ternary titration data was carried out using the OBJE task of ESTA and the previously defined zinc and copper maleate constants (Table II). Throughout the optimization procedures, the constants for the maleate species were held constant whilst the enalapril binary and enalapril maleate ternary constants were refined.

The interaction of Zn(II) ions with enalapril maleate is characterized by the formation of the MLH, ML and MLOH enalapril complexes, as well as

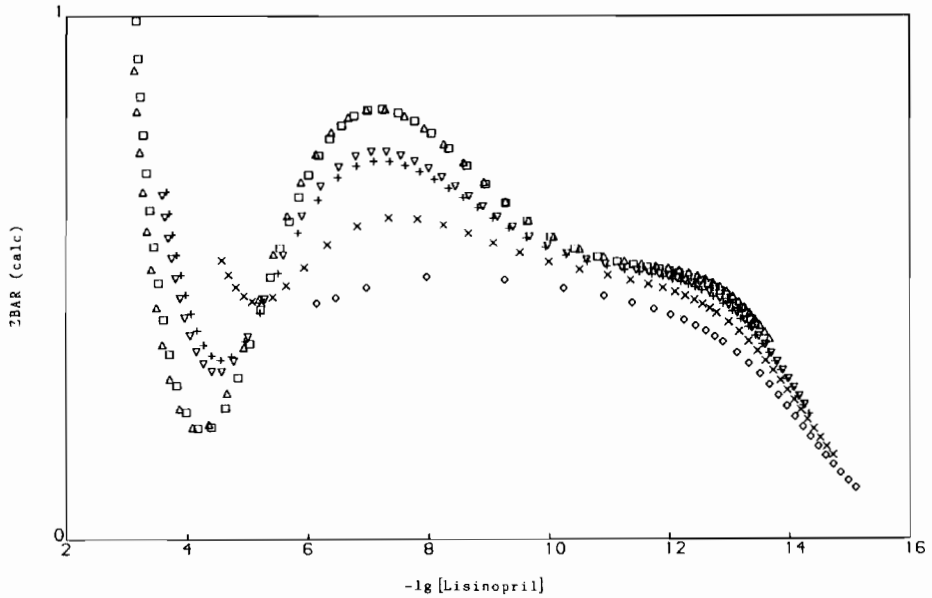

Fig. 10. Calculated formation curve for the Cu(II)–lisinopril system.

TABLE V. Major Metal–Ligand Species Postulated as Being Formed at pH 7.4[a]

Interaction	Species formed	Charge	Total metal present in species (%)
Zinc maleate			
Model A	$Zn(MAL)_2$	−2	50.6
	Zn(MAL)	0	20.9
Model B	$Zn(MAL)_2$	−2	44.4
	Zn(MAL)	0	21.4
	$Zn(MAL)_2OH$	−3	15.3
Copper maleate	Cu(MAL)OH	−1	53.1
	Cu(MAL)	0	25.1
	$Cu(MAL)_2$	−2	19.3
Zinc–enalapril maleate			
Model A	$Zn(MAL)_2$	−2	33.9
	Zn(ENA)OH	0	23.9
	Zn(ENA)	+1	18.4
Model B	$Zn(MAL)_2$	−2	29.6
	Zn(ENA)OH	0	24.2
	Zn(ENA)	+1	17.7
Copper–enalapril maleate	Cu(ENA)(MAL)	−1	67.4
	Cu(MAL)OH	−1	15.7
Zinc–lisinopril	$Zn(LIS)_2H_2$	0	56.4
	Zn(LIS)H	+1	37.0
Copper–lisinopril	$Cu(LIS)H_2$	0	92.7

[a]MAL = maleate; ENA = enalapril; LIS = lisinopril.

the maleate species listed in Table III. Analogous results were obtained utilizing both of the previously defined zinc maleate models. In addition to the binary complexes, mixed ligand species were tested for, but their presence was not detected in the optimization procedure. ERR% analyses showed the enalapril and maleate hydroxy complexes to be minor species; however, their inclusion resulted in a better overall analysis of the system as reflected in the improved statistics and curve fitting. The experimental deprotonation curve for the Zn(II)–enalapril maleate system is given in Fig. 6.

The titrations involving the interaction of Cu(II) ions with enalapril maleate were terminated at a pH of 5 due to precipitation. OBJE analysis of the titration data yielded several possible models. Of these, the model containing the MLL′ mixed ligand complex and the ML_2H_2 and ML enalapril binary species was considered to best describe the experimental data. The Cu(II)–enalapril maleate deprotonation curve is displayed in Fig. 7.

Metal–Ligand Complexation by Lisinopril

Lisinopril has four possible protonation sites; these correspond to the two carboxylate and two amino groups of the ligand. The results of the OBJE analysis of the protonation data are shown in Table IV.

The formation curve for the interaction of zinc with lisinopril (Fig. 8) indicates the presence of protonated and/or polynuclear species. Optimization of the titration data confirmed the existence of protonated complexes as opposed to polynuclear species. Table IV shows the final model to contain the ML_2H_2, MLH and ML species. During the optimization procedure, an MLH_2 type species was also detected. However, the extent of formation of this complex was not considered to be significant, thus, it was excluded from the final model. Species

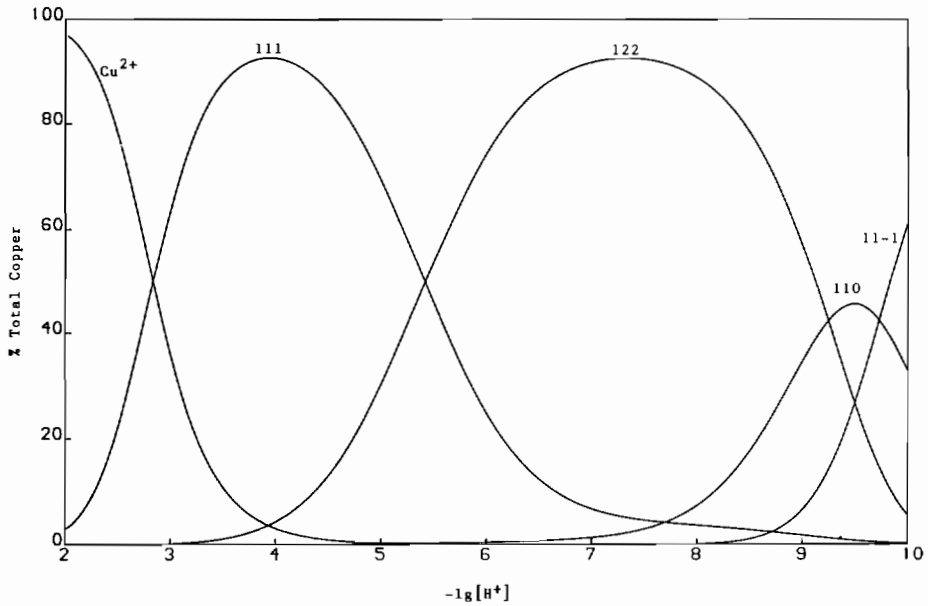

Fig. 11. Cu(II)–lisinopril species distribution ([L]:[M] = 15:5 mmol dm^{-3}).

distribution analyses show the protonated species to predominate in solution. The ML complex forms to a much lesser extent and occurs only in the final stages of the titrations.

Cu(II) ions interact with lisinopril to form a variety of complexes. Optimization of the titration data yielded a number of possible models, many of which contained the polynuclear species, M_2L_3H. For a number of reasons, the existence of this species was considered improbable:

(i) Polynuclear complexes normally start to form in the acid region of the titration, where the concentration of deprotonated ligand is low and the concentration of free metal high. In this case, the formation of the M_2L_3H species is not initiated until a pH of 7–8.

(ii) Failure to detect other polynuclear complexes in the optimization procedure.

(iii) The size of the ligand implies the species would be unlikely due to steric factors.

In view of these factors, the model containing the ML_2H_2. MLH, ML and MLOH species was considered to best represent the experimental data, in preference to the identical model containing the additional M_2L_3H complex. The experimental and simulated formation curves for the interaction of copper with lisinopril are given in Figs. 9 and 10, respectively. The species distribution for this system is displayed in Fig. 11.

Blood Plasma Simulation Studies

The relative abilities of the antihypertensive agents enalapril maleate and lisinopril to mobilize copper and zinc from the labile protein complexes in plasma were evaluated using the ECCLES program. The computer simulation studies were performed over a range of drug concentrations utilizing the formation constants obtained from the potentiometric investigations.

The findings of the simulations indicate negligible mobilization of Cu(II) and Zn(II) ions by maleate and enalapril. Therefore, the side-effects observed with enalapril maleate therapy are probably not directly dependent on mobilization of these metals in plasma. Preliminary investigations have been carried out in healthy adult volunteers to determine the effects of enalapril on the urinary excretion of electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+} and Zn^{2+}) [19]. The results of this study show the amount of zinc excreted in 24 h at a drug concentration of 6.65×10^{-6} mol dm^{-3} was not significantly different from the normal range. This finding, therefore, supports the computer predictions on enalapril and enalaprilat that zinc would not be mobilized into the low-molecular-mass fraction of plasma leading to increased excretion. Unfortunately, the copper excretion levels were not monitored in this experimental programme.

The studies involving lisinopril show that Zn(II) and Cu(II) mobilization is initiated at a very high plasma drug concentration of 10^{-3} mol dm^{-3} (two to three orders of magnitude greater than the concentrations actually attained *in vivo*). This mobilization is insignificant in comparison to that obtained with known chelators of zinc and copper, such as D-penicillamine and triethylenetetramine hydrochloride [20, 21].

The findings of the computer simulation study involving lisinopril are yet to be corroborated. Again,

validation of the results could be achieved through monitoring the levels of zinc and copper in urine.

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