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The Binding of Metal Ions by Captopril (SQ 14225). Part II. Complexation of Copper(II)

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Abstract

Formation constants for the interaction of copper(II) with captopril have been measured using (i) histidine and (ii) 2,3-diaminopropionic acid monohydrochloride as competing ligands to prevent redoxing. The main species present in both the binary and ternary experimental systems are reported. Blood plasma models based upon these new formation constants indicate that captopril at high doses may mobilise copper(II) from blood plasma.

Introduction

Captopril, 1-[2(S)-3-mercapto-2-methylpropionyl]-L-proline, SQ14225, the first orally active Angiotensin Converting Enzyme (ACE) inhibitor, is now in widespread use in the treatment of congestive heart failure and hypertension [1]. However, clinical trials have shown that side effects are sometimes associated with the drug [2, 3], especially in patients receiving high doses (>450 mg day^{-1}). These side effects include skin rashes, dysguesia and neutropenia-symptoms often associated with zinc and/or copper depletion.

Previous investigations into the interaction of Zn(II) ions with captopril indicated that it is unlikely that captopril mobilises zinc from the plasma proteins *in vivo* [4]. This communication addresses the complexation of Cu(II) ions by captopril *in vitro* and assesses the ability of this drug to mobilise copper *in vivo*.

Copper–Captopril Interactions

The addition of captopril to an acidic copper solution resulted in the formation of a pale yellow gelatinous precipitate which redissolved at pH = 4. In order to circumvent these solubility problems, a solution of captopril was adjusted to pH = 5 and titrated against an acidic solution of copper. No visible precipitation occurred within the pH range 5.0–3.8. However, the results were not reproducible, indicating that redoxing might well be taking place.

The reduction of copper(II) to copper(I) in the presence of thiol ligands is well known [5, 6], the thiol ligand being oxidised to the dithiol:

$$RSH + Cu(II) = \tfrac{1}{2}RSSR + Cu(I) + H^+$$

In addition, mixed valence complexes may form.

Hallman [7] suggested that, under the conditions pertaining to blood plasma (*i.e.* a free copper(II) concentration of $\approx 10^{-11}$ mol dm^{-3}), cysteine might coordinate to copper(II) rather than reduce it. On this basis, she studied the copper(II)–cysteinate system using histidine as a competing ligand to minimise the free copper concentration. Histidinate is considered to inhibit the oxidation of cysteine due to the formation of very stable histidinate–copper(II) complexes which lower the redox potential of the copper(II)/copper(I) couple such that cysteine is not oxidised, nor the copper(II) reduced [8]. Hallman found that by preparing the copper(II)–histidinate chelate at pH 9, then adding the cysteine and titrating down to pH = 5, redoxing and precipitation could be avoided. Thus, it was decided to adopt an approach similar to that of the cysteine system for copper(II)–captopril interactions.

Two series of investigations were carried out using histidine and 2,3-diaminopropionic acid monohydrochloride (DAP) as competing ligands in order to 'hold' the copper in the +2 oxidation state. These two ligands were chosen since they have similar avidities for Cu(II) ions to that expected for captopril. In order to characterise copper–captopril interactions in the presence of these competing ligands, it was necessary to characterise the protonation and binary copper interactions for both histidinate and DAP.

Experimental

Materials

Analytical grade reagents were used throughout. All solutions were prepared using distilled, doubly deionised, degassed water. Solutions of captopril and DAP were freshly prepared each day. Captopril (E. R.

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TABLE I. Summary of the Titration Data used for Calculating Stability Constants

System	Titration	Number of points	Component concentrations (mmol dm^{-3})			$-\lg[H^+]$ range
			[L]	[L']	[M]	
Histidinate protonation	1	67	13.330			2.8–10.8
	2	66	6.674			1.7–10.7
	3	56	10.000			2.0–9.4
	4	59	4.000			1.7–10.3
	5	56	8.001			1.8–9.3
Cu(II)–histidinate interaction	1	45	10.001		3.292	2.0–8.0
	2	53	10.001		6.583	2.0–7.1
	3	37	10.101		4.937	2.0–6.9
	4	63	11.530		2.821	2.2–7.4
	5	46	10.001		8.977	2.1–5.6
	6	59	5.556		3.292	1.9–5.9
	7	45	6.088		5.102	1.7–5.2
	8	68	15.126		14.750	1.9–5.5
	9	38	5.564		3.350	2.6–5.4
Captopril protonation	1	70	10.052			2.0–10.9
	2	49	5.080			2.3–10.3
	3	75	12.056			1.9–10.3
	4	54	8.058			2.1–10.9
	5	35	3.114			2.5–10.3
Cu(II)–captopril histidinate interaction	1	60	4.537	18.223	4.456	3.6–5.1
	2	72	4.218	16.946	4.143	4.9–6.8
	3	58	4.114	16.523	4.041	5.3–7.8
	4	52	4.026	20.200	3.951	3.6–5.5
	5	51	4.849	14.590	4.758	4.8–7.8
	6	48	6.400	14.613	6.350	4.8–6.1
	7	49	6.542	14.940	6.492	4.4–7.9
DAP protonation	1	47	5.010			2.3–9.9
	2	88	15.336			1.8–10.4
	3	41	5.007			2.3–11.0
	4	61	10.376			2.0–9.8
	5	54	4.074			2.4–10.7
Cu(II)–DAP interaction	1	45	10.283		10.084	2.0–6.0
	2	93	13.710		6.723	2.1–11.0
	3	50	10.119		10.804	2.0–6.0
	4	48	12.023		8.067	2.1–6.7
	5	65	7.049		2.372	2.5–11.0
	6	56	10.656		6.723	2.1–6.6
	7	52	12.105		8.067	2.1–6.6
	8	62	14.410		5.762	2.2–9.2
	9	53	6.783		4.033	2.3–10.5
Cu(II)–captopril–DAP interaction	1	47	20.153	11.619	3.752	9.3–3.9
	2	53	20.102	11.299	3.780	9.3–4.2
	3	49	20.102	11.092	3.711	10.0–4.9
	4	56	20.222	12.085	3.752	9.1–4.3
	5	51	20.222	12.085	3.752	9.0–4.8
	6	58	20.047	11.543	3.752	9.3–4.2
	7	30	20.047	11.643	3.752	6.1–4.6
	8	65	20.549	12.330	3.752	8.8–4.1
	9	62	20.549	12.330	3.752	8.9–4.4
	10	26	29.850	11.801	3.704	6.4–4.8

(continued)

TABLE I. *(continued)*

System	Titration	Number of points	Component concentrations (mmol dm^{-3})			$-\lg[H^+]$ range
			[L]	[L']	[M]	
	11	59	24.907	11.516	3.704	9.4–3.8
	12	50	19.918	11.176	3.334	10.7–4.8
	13	36	19.918	11.176	3.334	6.6–4.1

TABLE II. Thermodynamic Formation Constants for Histidinate at 37 °C; $I = 150$ mmol dm^{-3} Cl^- (data analysed using MINIQUAD). $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$

Interaction	Species			$\lg \beta_{pqr}$ (σ)[a]	Sum of squared residuals	R_{Factor}	Number of points	Number of titrations
	p	*q*	*r*					
Histidinate	0	1	1	8.712 (0.002)	5.38×10^{-7}	0.002	304	5
protonation	0	1	2	14.508 (0.003)				
	0	1	2	16.209 (0.006)				
Copper(II)–histidinate	1	1	0	9.70 (0.002)	3.22×10^{-7}	0.001	454	9
	1	2	0	17.17 (0.007)				
	1	1	1	13.62 (0.003)				
	1	2	1	22.75 (0.005)				
	1	2	2	25.98 (0.034)				
	2	3	0	29.26 (0.021)				
	1	1	−1	2.43 (0.014)				

[a]Standard deviation.

Squibb & Sons) (*Anal.* Found: C, 50.0; H, 7.0; N, 6.5. Calc. for $C_9H_{15}O_3NS$: C, 49.8; H, 7.0; N, 6.5%); histidine (BDH Biochemicals) (*Anal.* Found: C, 46.1; H, 5.8; N, 27.1. Calc. for $C_6H_9O_2N$: C, 46.4; H, 5.8; N, 27.1%); and 2,3-diaminopropionic acid (Aldrich Chemical Co.) (*Anal.* Found: C, 25.7; H, 6.4; N, 19.8. Calc. for $C_3H_8O_2HCl$: C, 25.6; H, 6.5; N, 19.9%) were used without further purification. Solutions were prepared by direct weighing. A standard copper stock solution (20 mmol dm^{-3}) was prepared from the chloride salt (BDH Analar) and analysed by EDTA complexometric titration [9]. The mineral acid content was determined by titration with standard alkali, the data being analysed by the ESTA suite of programs [10]. Sodium hydroxide (100 mmol dm^{-3}) and hydrochloric acid solutions were prepared from ampoules of concentrated volumetric solutions (BDH Ltd). All solutions were prepared such that the background electrolyte concentration was 150 mmol dm^{-3} chloride.

A summary of the titration data used for calculating the stability constants is given in Table I.

Results

Histidine Protonation

Histidinate has three protonation sites – the carboxylate, imidazole and amino groups. The protonation constants obtained from MAGEC and MINIQUAD cycling of the data [11, 12] are given in Table II.

Copper(II)–Histidinate Interactions

The formation curves obtained for the interaction of Cu(II) ions with histidinate are shown in Fig. 1. Optimisation of the titration data using the OBJE task of ESTA indicated the presence of ML, MLH, MLOH, ML_2, ML_2H and ML_2H_2 species together with an M_2L_3 species (Table II). Although this species never accounted for more than 26% of the copper present its inclusion did significantly decrease the sum of squared residuals. The simulated formation curves calculated on the basis of the model given in Table II (Fig. 2) closely resemble the experimental curves. The experimental and simulated deprotonation curves are shown in Figs. 3 and 4, respectively.

2,3-Diaminopropionate Protonation

The titration data for the protonation of DAP were optimised using the OBJE task of ESTA. The resulting protonation constants corresponding to the two amino groups are given in Table III. The protonation constant for the carboxylate group could not be determined since this group deprotonates at very low pH.

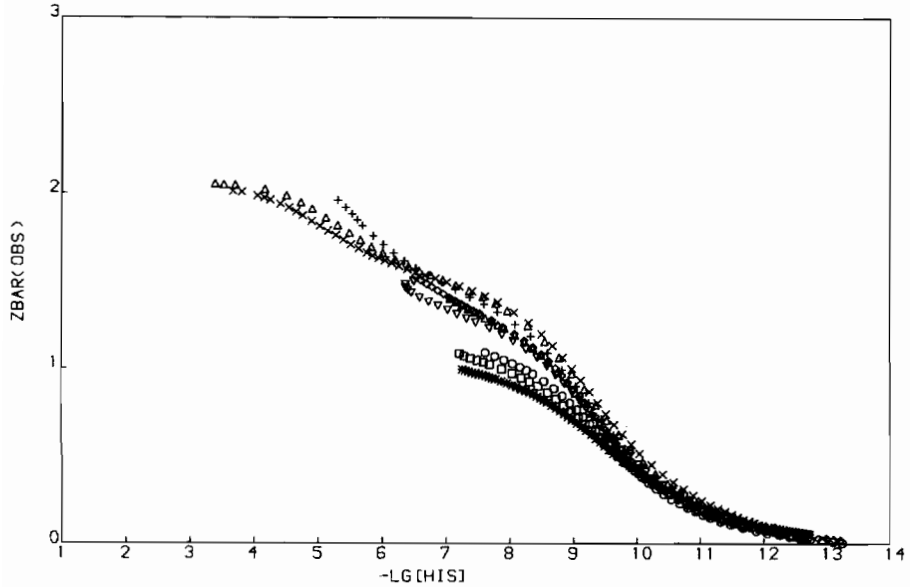

Fig. 1. Experimental copper(II)–histidinate formation curve.

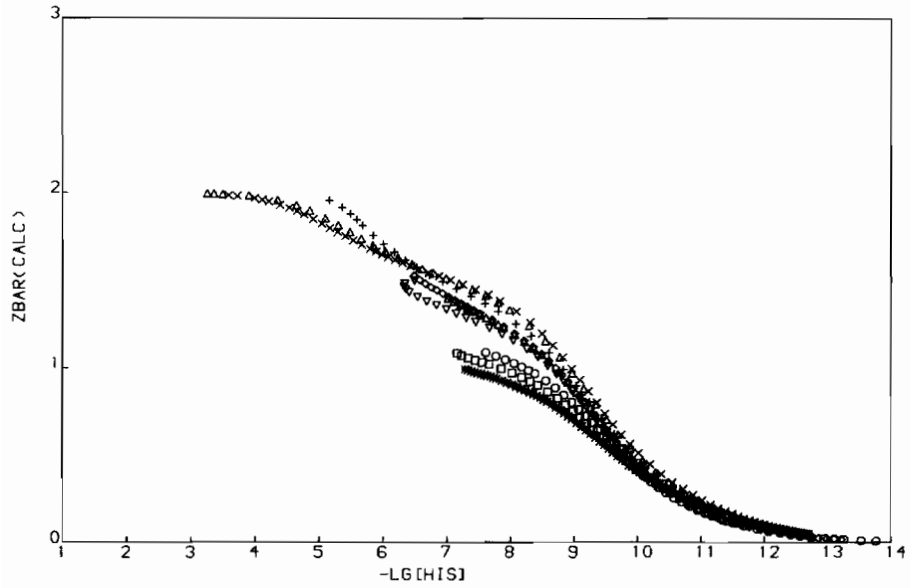

Fig. 2. Simulated copper(II)–histidinate formation curve.

TABLE III. Thermodynamic Formation Constants for 2,3-Diaminopropionate at 37 °C; I = 150 mmol dm^{-3} Cl^- (data analysed using ESTA). $\beta_{pqr} = [M_qL_qH_r]/[M]^p[L]^q[H]^r$

Interaction	Species			lg β_{pqr} (σ)	Objective function	R_{Factor}	Number of points	Number of titrations
	p	q	r					
DAP–protonation	0	1	1	9.048 (0.002)	62	0.003	291	5
	0	1	2	15.400 (0.003)				
Copper(II)–DAP	1	1	0	9.87 (0.01)	1539	0.011	524	9
	1	2	0	18.67 (0.01)				
	1	1	1	14.72 (0.01)				
	1	2	1	24.06 (0.01)				
	1	2	2	28.72 (0.01)				
	1	1	−1	2.15 (0.05)				
	1	2	−2	−4.19 (0.06)				

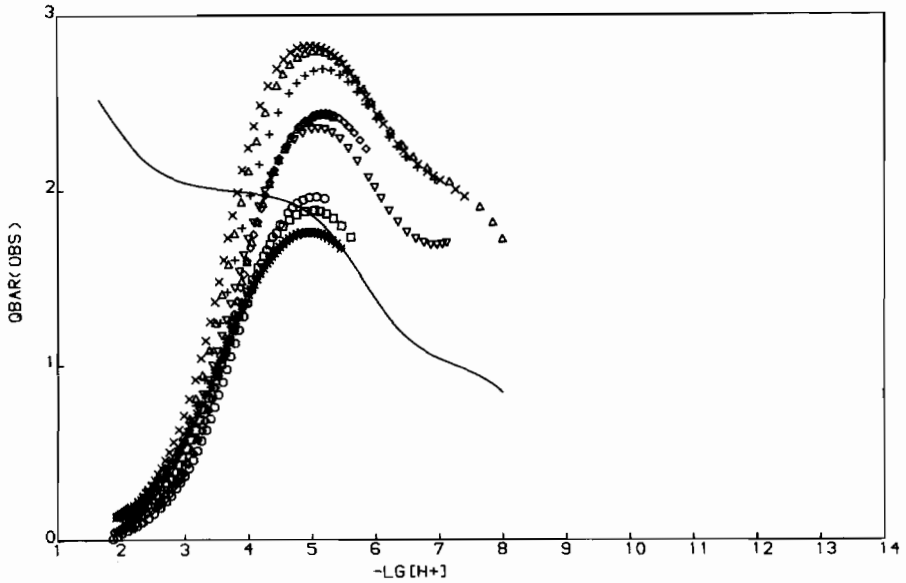

Fig. 3. Experimental copper(II)–histidinate deprotonation curve.

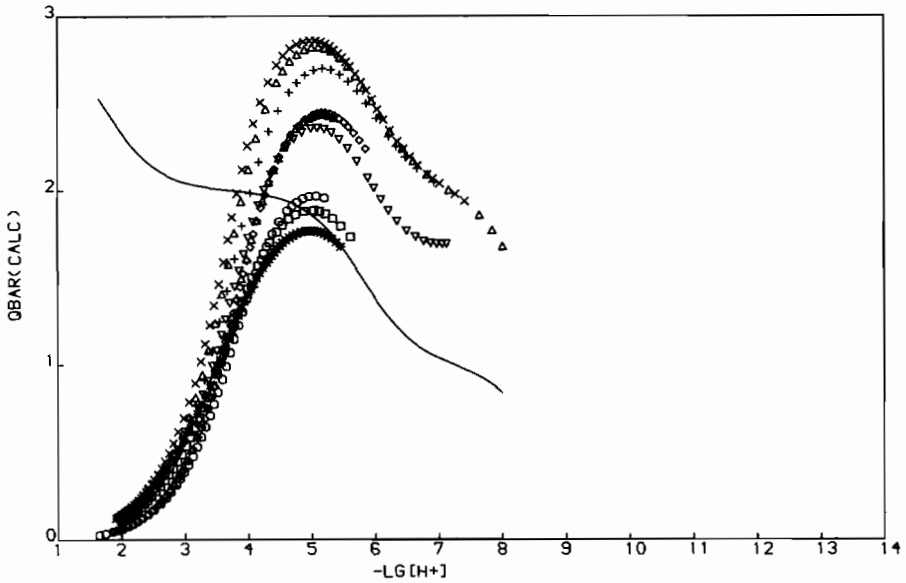

Fig. 4. Simulated copper(II)–histidinate deprotonation curve.

Copper(II)–2,3-Diaminopropionate Interactions

In contrast to histidine, DAP forms only mononuclear complexes with copper. The formation constants for the interaction of DAP with copper(II) ions are given in Table III. Analysis of the data showed the best model contained the species ML, ML_2, MLH, ML_2H, ML_2H_2, MLOH and $ML_2(OH)_2$. At a ligand to metal ratio of 7.9:4.0 mmol dm^{-3}, the ML_2 species predominates from pH 6–10, binding 98% of the total metal concentration by pH 8. At pH > 11, the $ML_2(OH)_2$ accounts for up to 90% of the metal complexed. Previous studies have not indicated the presence of the hydroxy species [13, 14], but inclusion of the species improved the statistical output and gave a close fit between the experimental and calculated data. The experimental and simulated formation and deprotonation curves are shown in Figs. 5–8.

Captopril Protonation

The experimental protonation curves for captopril show that there are two well separated protonation sites. These correspond to the carboxylate and sulphydryl donor groups. The protonation constants resulting from ESTA analysis of the data are given

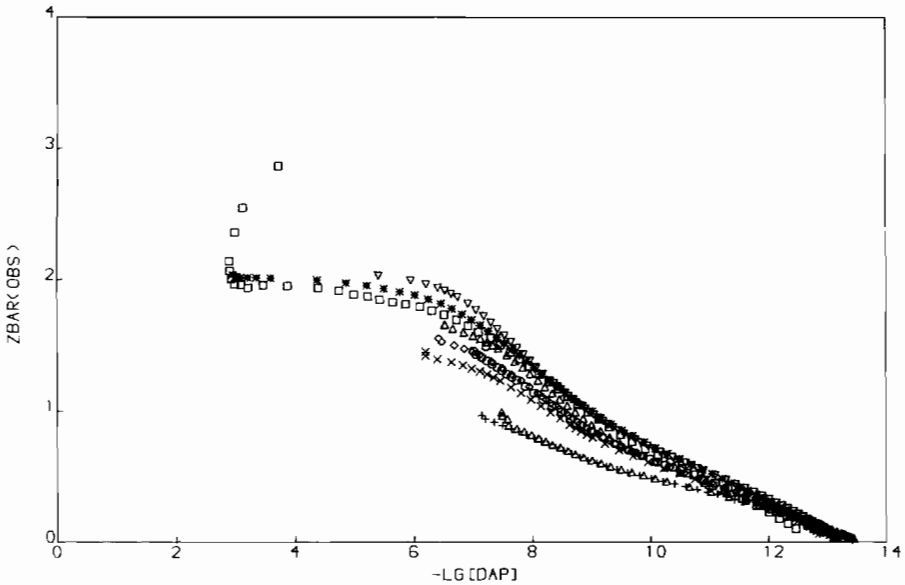

Fig. 5. Experimental copper(II)–DAP formation curve.

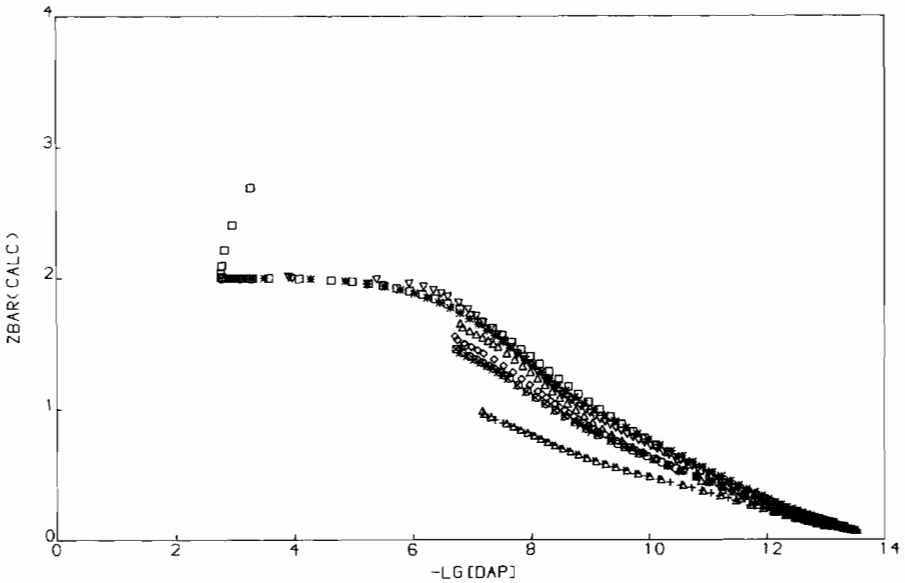

Fig. 6. Simulated copper(II)–DAP formation curve.

TABLE IV. Protonation Constants for Captopril at 37 °C, 150 mmol dm^{-3} Cl^- (data analysed using ESTA). $\beta_{qr} = [L_qH_r]/[L]^q[H]^r$

Species		lg β_{qr} (σ)	Objective function	R_{Factor}	Number of points	Number of titrations
q	r					
1	1	9.686 (0.002)	27	0.001	283	5
1	2	13.145 (0.002)				

in Table IV. Thus, the pK_a for the proline carboxylate function is 3.46 and that for the thiol group 9.69. There is no indication that the tertiary nitrogen of the proline ring protonates. This is consistent with the findings of other workers studying ligands with proline as the carboxylate terminal moiety [15].

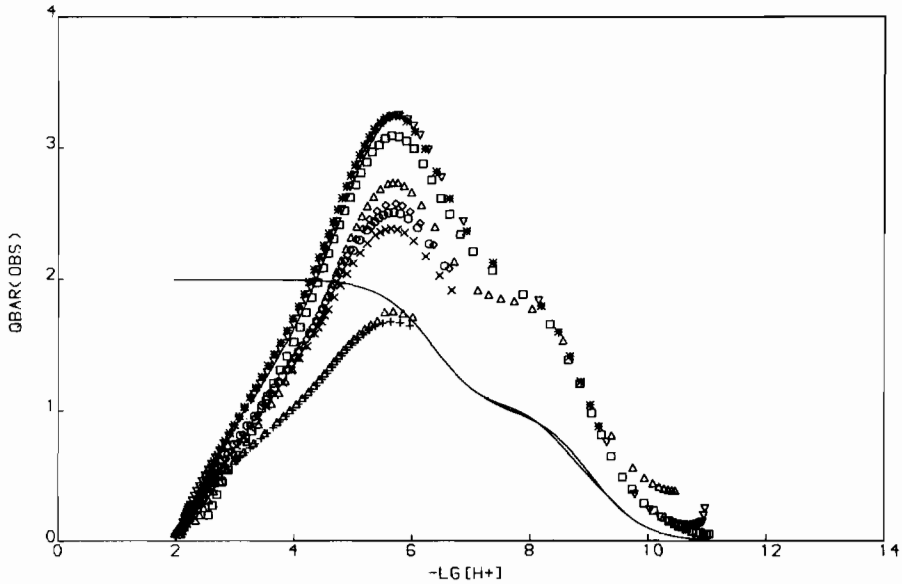

Fig. 7. Experimental copper(II)–DAP deprotonation curve. Different symbols refer to different titrations having different ligand: metal ratios and different total ligand and total metal concentrations. On each deprotonation curve, a plot of $\bar{n}$ *vs.* $-\lg[H^+]$ appears as a solid line.

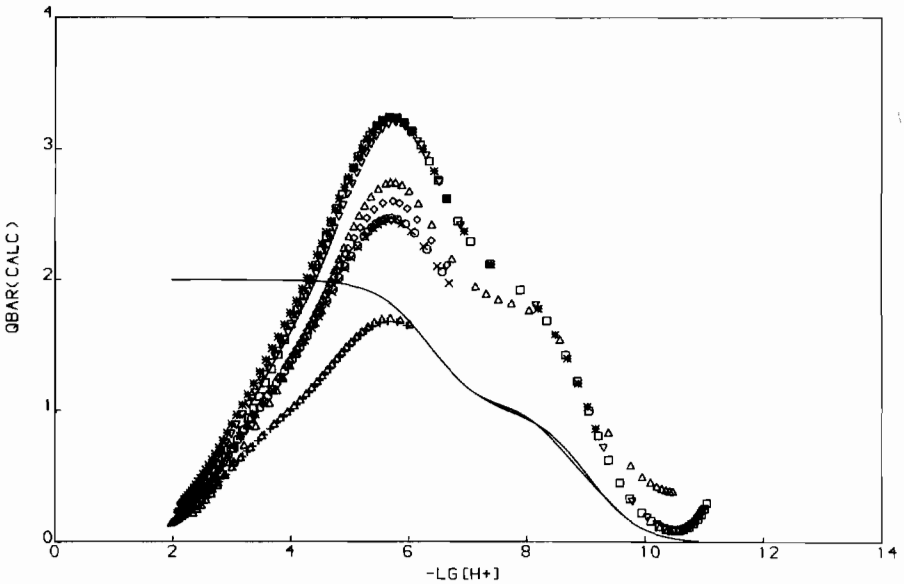

Fig. 8. Simulated copper(II)–DAP deprotonation curve.

The results of this study are in close agreement with a previous determination of the protonation constants of captopril in which MINIQUAD was used to analyse the data [4].

Copper(II)–Captopril Interactions

The interaction of copper(II) ions with captopril was investigated in two separate studies using histidine and DAP as competing ligands.

In the copper–captopril–histidinate studies, the following approach was adopted. A solution of copper–histidine was prepared at the pH at which the free copper(II) concentration was minimised (as determined by the ESTA 'SPEC' program) [10]. Captopril was then added to this copper–histidine chelate and the resulting solution titrated with alkali. In order to minimise any possible redoxing, readings were taken 45 s after addition of alkali (e.m.f. readings were stable after 45 s and remained constant for 30 s, but after this drifting began to occur) and titrations were limited to 50 points such that the duration of any one titration did not exceed 40 min.

TABLE V. Thermodynamic Formation Constants for Copper(II)–Captopril Interactions at 37 °C, I = 150 mmol dm^{-3} Cl$^-$, using Histidinate as a Competing Ligands (data analysed using MINIQUAD). $\beta_{pqq'r} = [M_pL_qL'_{q'}H_r]/[M]^p[L]^q[L']^{q'}[H]^r$; L represents histidinate; L' represents captopril

Species				lg β	(σ)	Sum of squared residuals	R_{Factor}	Number of points	Number of titrations
p	q	q'	r						
1	0	1	0	11.52	(0.02)	3.31×10^{-6}	0.003	390	7
1	0	1	−1	7.62	(0.02)				

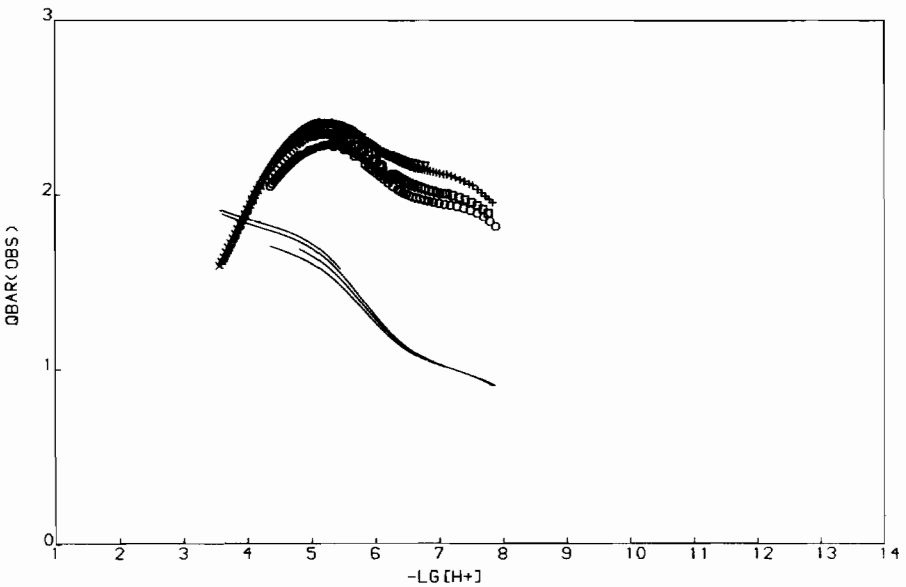

Fig. 9. Experimental copper(II)–captopril–histidinate deprotonation curve.

A slightly different approach was adopted for the copper–captopril–DAP system. Commencing the ternary titrations at the pH of maximum copper–DAP complexation (pH 9.0) meant that only a very narrow pH range could be covered during the course of the titration. Moreover, the copper–DAP binary systems were ill-characterised at this high pH. To avoid such problems, an alkaline solution of copper–DAP was titrated against an acidic solution of captopril. Placing the captopril in the burette ensured that the concentrations of this ligand were low throughout the course of the titration and that a much wider pH range could be covered.

Copper(II)–Captopril–Histidinate Interactions

Data for the copper–captopril–histidinate system were analysed using the MINIQUAD program (at the time when these investigations were carried out the ESTA program was not available). In the analysis of the data, the copper(II)–histidinate formation constants were fixed and constants for the copper-(II)–captopril species optimised. Over forty different models were considered. Of these, the model containing an ML and MLOH copper(II)–captopril species was considered to describe most adequately the experimental data (Table V). With the advent of the ESTA program, the copper–captopril–histidinate system was represented graphically in terms of the deprotonation function, Qbar [10]. Comparison of the experimental deprotonation curves (Fig. 9) with the simulated curves (Fig. 10), based on the data in Table V, indicated the formation of ML and MLOH copper(II)–captopril species described reasonably well the experimental data.

Copper(II)–Captopril–DAP Interactions

Titration data for the copper(II)–captopril–DAP system were analysed using the ESTA suite of programs. The same parameters as those for the copper(II)–captopril–histidinate system were optimised. The final selection of species was based on graphical comparison between the experimental and simulated Qbar curves (Figs. 11 and 12), in addition to the various statistical criteria. The model containing the ML and MLOH species (Table VI) gave the best agreement with observed data. The copper–captopril constants measured for this system show a very close agreement with those

TABLE VI. Thermodynamic Formation Constants for Copper(II)–Captopril Interactions at 37 °C, I = 150 mmol dm^{-3} Cl^{-1}, using DAP as the Competing Ligand (data analysed using MINIQUAD). $\beta_{pqq'r} = [M_pL_qL'_{q'}H_r]/[M]^p[L]^q[L']^{q'}[H]^r$; L represents DAP; L' represents captopril

Species				lg β	(σ)	Objective function	R_{Factor}	Number of points	Number of titrations
p	q	q'	r						
1	0	1	0	11.52	(0.02)	3209	0.064	642	13
1	0	1	−1	7.20	(0.02)				

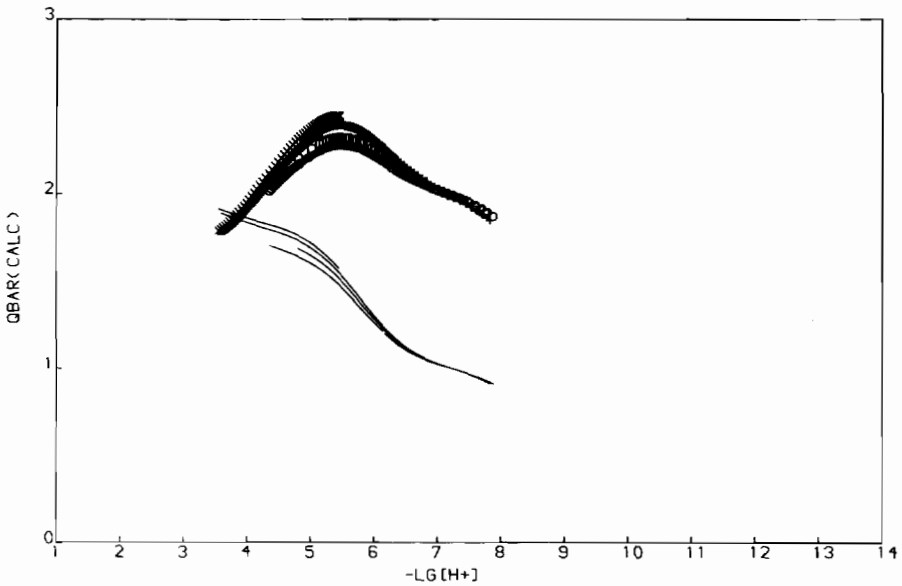

Fig. 10. Simulated copper(II)–captopril–histidinate deprotonation curve.

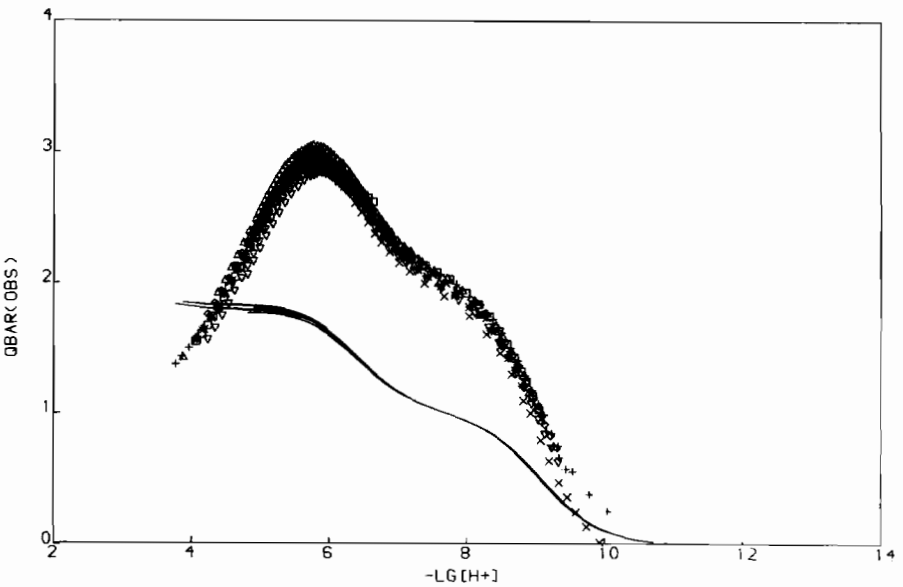

Fig. 11. Experimental copper(II)–captopril–DAP deprotonation curve.

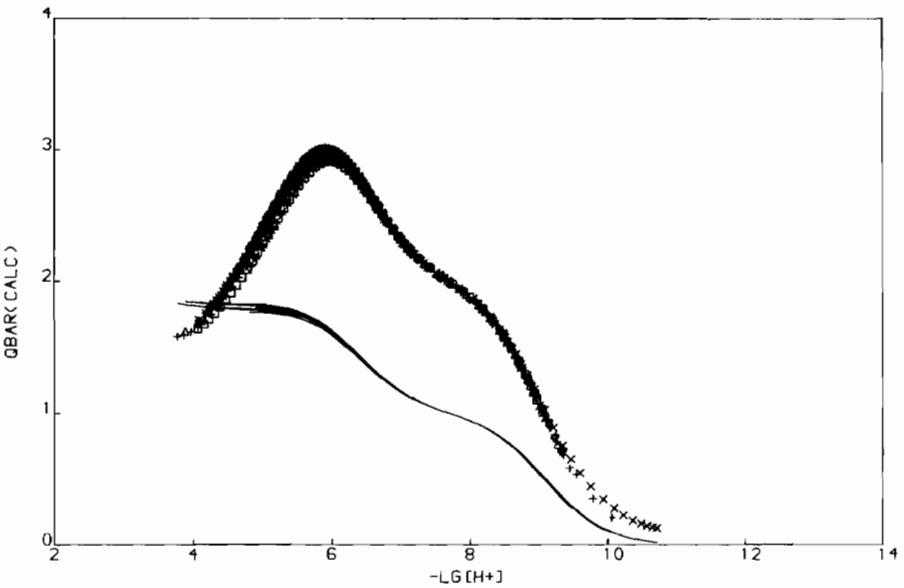

Fig. 12. Simulated copper(II)–captopril–DAP deprotonation curve.

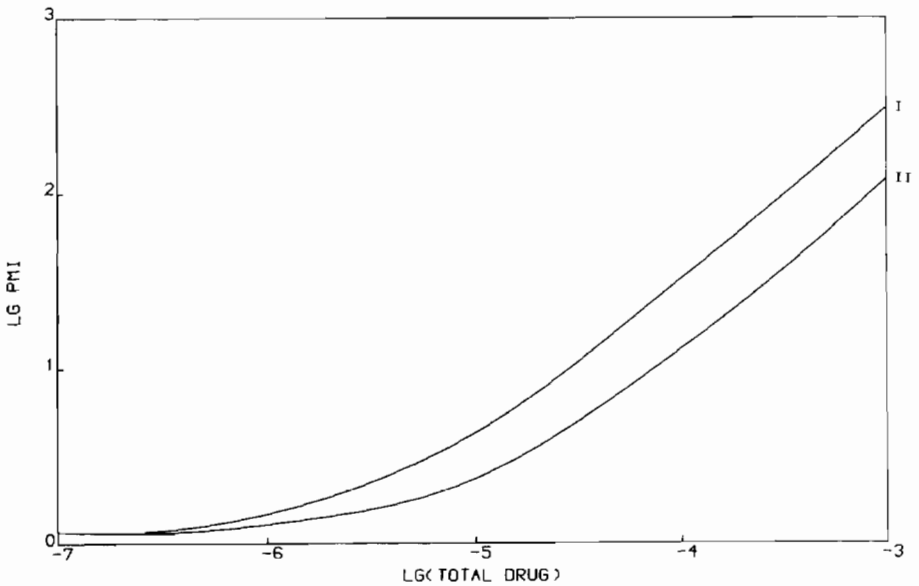

Fig. 13. PMI curves for the mobilisation of copper(II) ions by captopril using (I) histidine and (II) DAP as competing ligands.

determined for the copper(II)–captopril–histidinate system (Table V).

Blood Plasma Model

The ability of captopril to mobilise copper(II) ions from the labile protein complexes in plasma was evaluated using the ECCLES* program [16]. The efficacy of a drug to mobilise the metal from plasma can be quantified in terms of the Plasma Mobilising Index (*PMI*) [17]. Simulations were performed separately using constants from either Table V or Table VI.

At a captopril concentration of 10^{-5} mol dm^{-3}, using the constants in Table V, the ECCLES simulation shows 73% of copper being bound in the complex CuCapOH. This fell to 51% upon substituting the respective constants from Table VI. However, the percentage of copper bound in the CuCapOH complex would rise to 99% (using either set of constants) at a captopril concentration of 10^{-3} mol dm^{-3}, such a concentration of captopril being higher than would normally be found *in vivo*.

*Evaluation of constituent concentrations in large equilibrium systems.

The PMI curves for the mobilisation of copper(II) ions by captopril are shown in Fig. 13. These studies suggest that captopril could mobilise copper(II) in blood plasma and such mobilisation is dependent upon the CuCapOH species being present. However, clinical studies indicate that even at large doses of captopril the plasma concentration does not reach 10^{-5} mol dm^{-3} [18–20]. In addition, our conclusions are complicated by the possibility of redoxing, as mentioned in the introduction, and more experimental studies are desirable to fully elucidate copper(II)–captopril binding.

An ESR study of the copper–captopril system and clinical studies to monitor the urinary excretion of copper and zinc in hypertensive patients undergoing captopril therapy are currently being undertaken and will be reported in a subsequent communication.

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