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SALT EFFECTS ON THE PROTONATION OF L-HISTIDINE AND L-ASPARTIC ACID: A COMPLEX FORMATION MODEL

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ABSTRACT

Protonation constants of L-histidine (histidinate: his^-) and L-aspartic acid (aspartate: asp^{2-}) were determined potentiometrically, using the (H^+) glass electrode, in aqueous tetraethylammonium iodide (Et_4NI), calcium chloride and sodium chloride solutions, at $0 < I \leq 1$ mol dm^{-3} and $10 \leq T \leq 45$ °C. Differences in protonation constants determined in different salt media were explained by a complex formation model and, according to this model, the presence of the following species was hypothesized: $Ca(his)^+$, $CaH(his)^{2+}$, $CaH_2(his)^{3+}$, $Na(his)^0$, $H_3(his)X^+$, $H_2(his)X^0$, $Et_4N(his)^0$, $Et_4NH(his)^+$, $Ca(asp)^0$, $CaH(asp)^+$, $CaH_2(asp)^{2+}$, $Na(asp)^-$, $NaH(asp)^0$, $H_3(asp)X^0$ and $Et_4N(asp)^-$ ($X^- = Cl^-$ or I^-). Parameters for the dependence on temperature and on ionic strength were determined for all the species. Comparison with similar systems and statistical analysis confirmed the consistency of the model used in explaining the experimental results from this study.

INTRODUCTION

Low molecular weight *O*-ligands and *N*-ligands show some interesting regularities in the stability of their weak complexes, [1–19] which can be summarized as follows.

(a) Monocarboxylic and dicarboxylic ligands form complexes with alkali and alkaline earth metals with a stability fairly independent of the substrate [1–4,9] and do not form any detectable complex species with tetraalkylammonium cations [10].

(b) Amines form very weak complexes with alkaline earth metals and tetraalkylammonium cations, and do not form any detectable complex species with alkali metals [5,7,8,11–13]. Also in this case the complex stability is fairly independent of the substrate.

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(c) Protonated amines form complexes with singlycharged inorganic anions, with a stability fairly independent of both substrate and type of anion [5,7,8,13].

(d) Simple aminoacids show an expected intermediate behavior [5,6].

(e) The ionic strength dependence of formation constants is independent of the ligand and of the metal, and depends on the stoichiometry of the

TABLE 1

Thermodynamic parameters ($T = 25\,°C$, $I = 0$ mol dm^{-3}) for the formation of some weak complexes

Reaction [a]	log K	K	Ref.
1 $Na^+ + mca^- = Na(mca)^0$	−0.17	0.7	1
2 $Na^+ + ac^- = Na(ac)^0$	−0.11	0.8	2, 3
3 $Na^+ + dca^{2-} = Na(dca)^-$	0.70	5.0	1
4 $Na^+ + H(dca)^- = NaH(dca)^0$	−0.17	0.7	1, 2
5 $Na^+ + succ^{2-} = Na(succ)^-$	0.85	7.1	2, 4
6 $Na^+ + H(succ)^- = NaH(succ)^0$	0.15	1.4	2, 4
7 $Na^+ + ama^- = Na(ama)^0$	0.28	1.9	5
8 $Na^+ + H(ama)^0 = NaH(ama)^+$	−0.4	0.4	5
9 $Na^+ + ala^- = Na(ala)^0$	0.2	1.6	6
10 $Na^+ + H(ala)^0 = NaH(ala)^+$	−0.25	0.6	6
11 $Ca^{2+} + ac^- = Ca(ac)^+$	0.93	8.5	2, 3
12 $Ca^{2+} + succ^{2-} = Ca(succ)^0$	2.24	174	2, 4
13 $Ca^{2+} + H(succ)^- = CaH(succ)^+$	1.04	11	2, 4
14 $Ca^{2+} + ala^- = Ca(ala)^+$	1.36	23	6
15 $Ca^{2+} + H(ala)^0 = CaH(ala)^{2+}$	0.35	2.2	6
16 $Ca^{2+} + im^0 = Ca(im)^{2+}$	−0.09	0.8	7
17 $Ca^{2+} + py^0 = Ca(py)^{2+}$	−0.48	0.3	8
18 $H(am)^+ + X^- = H(am)X^0$	−0.34	0.5	5
19 $H_2(am)^{2+} + X^- = H_2(am)X^+$	0.59	3.9	5
20 $H(im)^+ + X^- = H(im)X^0$	−0.27	0.5	7
21 $H(ala)^0 + X^- = H(ala)X^-$	−0.11	0.8	6
22 $H_2(ala)^+ + X^- = H_2(ala)X^0$	0.4	2.5	6
23 $H(ama)^0 + X^- = H(ama)X^-$	−0.54	0.3	5
24 $H_2(ama)^+ + X^- = H_2(ama)X^0$	−0.47	0.3	5
25 $H(py)^+ + X^- = H(py)X^0$	0.0	1.0	8
26 $Et_4N^+ + im^0 = Et_4N(im)^+$	−0.07	0.9	7
27 $Et_4N^+ + ala^- = Et_4N(ala)^0$	0.15	1.4	6
28 $Et_4N^+ + en^0 = Et_4N(en)^+$	0.20	1.6	13
29 $Et_4N^+ + H(en)^+ = Et_4NH(en)^+$	−0.3	0.5	13
30 $Et_4N^+ + py^0 = Et_4N(py)^+$	0.07	1.2	8

[a] Abbreviations: mca^- = monocarboxylate; dca^{2-} = dicarboxylate; ac^- = acetic acid; $succ^{2-}$ = succinic acid; ama^- = aminoacid (general); ala^- = α-alanine; im^0 = imidazole; py^0 = pyridine; am^0 = amine (general); en^0 = ethylenediamine; Et_4N^+ = tetraethylammonium cation.

reaction and on the charge of reactants only [1,2,14–19] if all interactions occurring in solution are taken into consideration.

Histidine and aspartic acid represent two interesting examples of polyfunctional aminoacids. For these ligands the complexing characteristics must lie [according to (a)–(e)] between those of succinic acid [1,2,4] and of α-alanine [5,6] (for aspartic acid) and between those of α-alanine and imidazole [7] (for histidine). Moreover, for protonated histidine a behavior similar to that of diamines in the complexation with X^- (Cl^- or I^-) should be found [5,13].

Table 1 reports the thermodynamic parameters for the formation of Na^+, Ca^{2+}, Et_4N^+ and X^- complexes of some low molecular weight ligands, in order to illustrate the complexing features related to those of aspartic acid and histidine.

In this work we report a potentiometric study ((H^+)-glass electrode) on the protonation of L-histidine and L-aspartic acid at different ionic strengths ($0 < I \leq 1$ mol dm^{-3}) and temperatures ($10 \leq T \leq 45\,°C$) in aqueous NaCl, $CaCl_2$ and Et_4NI solutions, with the aim of explaining differences in protonation constants by a complex formation model.

EXPERIMENTAL

Materials

L-Histidine and L-aspartic acid (Fluka puriss. p.a.) were used without further purification; their purity, checked by alkalimetric titration, was always $\geq 99.5\%$. Hydrochloric acid and sodium hydroxide standard solutions were prepared by diluting concentrates from ampoules (C. Erba or Merck) and standardized against sodium carbonate and potassium biphthalate, respectively. Tetraethylammonium iodide was recrystallized twice from methanol [20]. Sodium chloride solution was prepared from Fluka puriss. p.a. product dried in an oven at 140 °C; calcium chloride solution was prepared from Fluka purum p.a. product and standardized against EDTA [21]. Twice-distilled water and grade A glassware were employed.

Apparatus

The hydrogen-ion concentration was measured with a Metrohm E605 potentiometer equipped with a Metrohm glass electrode and an Ingold calomel reference electrode. The titrant was delivered by an Amel dispenser or a Metrohm motorized burette, either having a minimum reading of 0.001 ml. The calibration of the electrode couple, in $-\log c_H$ units (c_H = free proton concentration), was achieved by titrating hydrochloric acid (10–20

mmol dm^{-3}) with standard carbonate-free sodium hydroxide under the same conditions as for the solution being considered (i.e. the same temperature, ionic strength and background salt). The reliability of the calibration in the alkaline range was checked by calculating pK_w values. The measurement cell was thermostatted at $T \pm 0.2$ °C. Magnetic stirring was employed. A stream of purified nitrogen was bubbled through the solution in order to exclude CO_2 and O_2.

Procedure

The titrations were carried out on 25 ml of solution containing 10 mmol dm^{-3} of ligand, variable amounts of Et_4NI, NaCl and $CaCl_2$ as background salts, and an excess of HCl. This last was added in order to complete the protonation of the aminoacids and to calculate the internal $E°$ ($E°_{int}$); by separate calibration we calculated $E°_{ext}$. If $|E°_{int} - E°_{ext}| > 1.5$ mV, the titration was rejected. The junction potential ($E_j = J_a c_H$) was always taken into account at pH < 2. For each ligand, about 20 titrations curves were obtained at each temperature.

Calculations

Potentiometric data were first analyzed by the least squares program ESAB2M [22], which refines the values of conditional protonation constants, along with $E°$, log K_w, junction potential and analytical concentrations (when necessary). Formation constants of weak complexes, together with the parameters for the dependence on temperature and on ionic strength, were refined by the least squares computer program ES2WC [23] (which minimizes the error squares sum of $\bar{p}$, the average number of protons bound to the ligand). The distribution of the species in the various systems investigated was calculated by the computer program ES4EC [24]. This program can also calculate the errors in formation percentages arising from errors in formation constants. Concentrations and thermodynamic quantities are always expressed on the molar scale.

The dependence of formation constants on ionic strength can be taken into account by the Debye–Hückel type equation [25]

$$\log K = \log {}^{T}K - z^* \sqrt{I}/(2 + B\sqrt{I}) + CI + DI^{3/2}$$
$$= \log {}^{T}K - z^* G(B) + L(I) \quad (1)$$

${}^{T}K$ is the formation constant at infinite dilution, $z^* = \Sigma(\text{charge})^2_{\text{reactants}} - \Sigma(\text{charge})^2_{\text{products}}$, $G(B) = \sqrt{I}/(2 + B\sqrt{I})$ and $L(I) = CI + DI^{3/2}$. In general $B = 3$, but in some cases (see below) it is convenient to set $B = 2$ (when weak interactions are not taken into account). C and D are empirical parameters.

Since Cl^- associates weakly with Na^+ and K^+, we considered in the calculations the effective ionic strength, $I_{(e)}$, by using the following degree of dissociation (C = salt concentration)

$$\alpha = 1 - C_{NaCl}^{1/2}\left[0.033 - 9 \times 10^{-4}(T-25)\right] - C_{NaCl}\left[0.219 - 4 \times 10^{-4}(T-25)\right] + 0.079C_{NaCl}^{3/2}$$

$$\alpha = 1 - C_{KCl}^{1/2}\left[0.027 - 5 \times 10^{-4}(T-25)\right] - C_{KCl}\left[0.246 - 1.5 \times 10^{-4}(T-25)\right] + 0.059C_{KCl}^{3/2}$$

These degrees of dissociation were obtained in previous work [25] from a careful analysis of literature data; the error arising from the use of α in calculating the real ionic strength is about $0.05C$.

RESULTS

Protonation constants determined in the various salt media are quite different for both histidine and aspartic acid. At $I \leq 0.5$ mol dm^{-3}, protonation constants can be expressed by an equation similar to eqn. (1), with a one parameter linear term. For aspartic acid ($T = 25\,°C$, $I \leq 0.5$ mol dm^{-3})

$$\log K_1^H = 10.01 - 4G(B) + CI$$

with $B = 2$ and $C = 0.67$ (NaCl), -0.44 ($CaCl_2$) or 1.04 (Et_4NI).

$$\log K_2^H = 3.88 - 2G(B) + CI$$

with $B = 2$ and $C = 0.345$ (NaCl), -0.11 ($CaCl_2$) or 0.61 (Et_4NI).

$$\log K_3^H = 1.92 + CI$$

with $C = 0.13$ (NaCl), -0.095 ($CaCl_2$) or 0.37 (Et_4NI). For histidine [$T = 25\,°C$, $I \leq 0.5$ mol dm^{-3} (NaCl, Et_4NI); $I \leq 0.2$ mol dm^{-3} ($CaCl_2$)]

$$\log K_1^H = 9.30 - 2G(B) + CI$$

with $B = 3$ and $C = 0.44$ (NaCl), -0.18 ($CaCl_2$) or 0.22 (Et_4NI).

$$\log K_2^H = 5.99 + CI$$

with $C = 0.35$ (NaCl), -0.16 ($CaCl_2$) or 0.09 (Et_4NI).

$$\log K_3^H = 1.58 + 2G(B) + CI$$

with $B = 3$ and $C = 0.26$ (NaCl), -0.15 ($CaCl_2$) or -0.05 (Et_4NI).

Coefficient C for the linear term is very different for the three salts and, in turn, is different from C_{model}, which represents the value calculated for several systems by taking into account the majority of weak interactions occurring in solution, in addition to the main complexing reaction [19]: $C_{model} = (0.1 + 0.145z^*)I$ ($I \leq 0.5$ mol dm^{-3}, $T = 25\,°C$). Thus, the dif-

ferences among protonation constants in NaCl, $CaCl_2$ and Et_4NI may be explained according to factors (a)–(e) (see introduction) by the formation of the following species (in addition to simple protonation species).

For aspartic acid

$Et_4N(asp)^-$

$H_i(asp)X^{(3-i)-}$ $[i = 1(*), 2(*), 3]$

$CaH_i(asp)^{i+}$ $[i = 0, 1, 2]$

$NaH_i(asp)^{(1-i)+}$ $[i = 0, 1, 2(*)]$

For histidine

$Et_4NH_i(his)^{i+}$ $[i = 0, 1]$

$H_i(his)X^{(2-i)-}$ $[i = 1(*), 2, 3]$

$CaH_i(his)^{(i+1)+}$ $[i = 0, 1, 2]$

$NaH_i(his)^{i+}$ $[i = 0, 1(*), 2(*)]$

ES2WC calculations showed that most of the above species form in significant percentages. Species marked by (*) were not found because either they do not form or they form in too small an amount. In Table 2 we report the thermodynamic parameters for the species formed in the two systems.

In Figs. 1–4, the distributions vs. pH of the species in the systems H^+–Na^+–his^-–X^-, H^+–Et_4N^+–Ca^{2+}–his^-–X^-, H^+–Na^+–asp^{2-}–X^- and H^+–Et_4N^+–Ca^{2+}–asp^{2-}–X^- are reported. All the systems show high formation percentages of weak species hypothesized in this work, in particular if one considers that only Ca^{2+} complexes were taken into account in the literature up to now. To show the relevance of the various species, together with their statistical significance, we report in Table 3 some formation percentages with their confidence intervals (± 3 std. dev.). In Table 4, the values of thermodynamic parameters at different temperatures and ionic strengths are shown for all the species found in this work.

DISCUSSION

Calcium complexes of aspartic acid and histidine show high stability in comparison with that of amines and of other aminoacids (e.g. α-alanine). In particular, aspartate complexes are stronger than those of both α-alanine

TABLE 2

Thermodynamic parameters for the formation of aspartate and histidinate species in aqueous solution at infinite dilution and $T = 25\,^{\circ}C$

Species	$\log \beta$ [a]	$-\Delta G^{\circ}$ [b]	ΔH° [b]	ΔS° [b]
$H(asp)^-$	10.01 ± 0.02 [c]	57.13 ± 0.11 [c]	-40 ± 4 [c]	59 ± 13 [c]
$H_2(asp)^0$	13.89 ± 0.02	79.27 ± 0.11	-43 ± 4	121 ± 14
$H_3(asp)^+$	15.81 ± 0.04	90.2 ± 0.2	-45 ± 7	152 ± 20
$Et_4N(asp)^-$	-0.27 ± 0.2	-1.5 ± 1	60 ± 20	200 ± 70
$H_3(asp)X^0$	15.34 ± 0.25	88.0 ± 2	-4 ± 20	280 ± 70
$Ca(asp)^0$	2.52 ± 0.04	14.4 ± 0.2	1 ± 6	53 ± 20
$CaH(asp)^+$	11.44 ± 0.08	65.3 ± 0.5	-44 ± 8	71 ± 30
$CaH_2(asp)^{2+}$	14.33 ± 0.15	-81.8 ± 0.9	-54 ± 15	92 ± 50
$Na(asp)^-$	0.42 ± 0.10	-2.4 ± 0.6	14 ± 9	54 ± 35
$NaH(asp)^0$	9.73 ± 0.15	-56.0 ± 1	-17 ± 10	130 ± 40
$H(his)^0$	9.30 ± 0.02	53.8 ± 0.11	-46 ± 4	24 ± 14
$H_2(his)^+$	15.29 ± 0.02	87.26 ± 0.11	-78 ± 5	30 ± 14
$H_3(his)^{2+}$	16.87 ± 0.05	96.3 ± 0.3	-73 ± 9	78 ± 20
$Et_4N(his)^0$	0.23 ± 0.2	13.0 ± 1	-26 ± 10	-81 ± 30
$Et_4NH(his)^+$	9.06 ± 0.2	52.0 ± 1	-58 ± 8	-20 ± 30
$H_2(his)X^0$	15.22 ± 0.15	87.0 ± 1	-65 ± 13	75 ± 40
$H_3(his)X^+$	17.28 ± 0.15	99.0 ± 1	-61 ± 15	125 ± 50
$Ca(his)^+$	1.55 ± 0.06	8.9 ± 0.3	-3 ± 9	18 ± 30
$CaH(his)^{2+}$	9.95 ± 0.10	56.8 ± 0.6	-39 ± 20	59 ± 70
$CaH_2(his)^{3+}$	15.43 ± 0.15	-88.0 ± 1	-46 ± 20	141 ± 70
$Na(his)^0$	-0.5 ± 0.3	-3.0 ± 2	–	–

[a] β = Overall formation constant;
[b] ΔG° and ΔH° in kJ mol^{-1}; ΔS° in J K^{-1} mol^{-1};
[c] ± 3 std. dev.

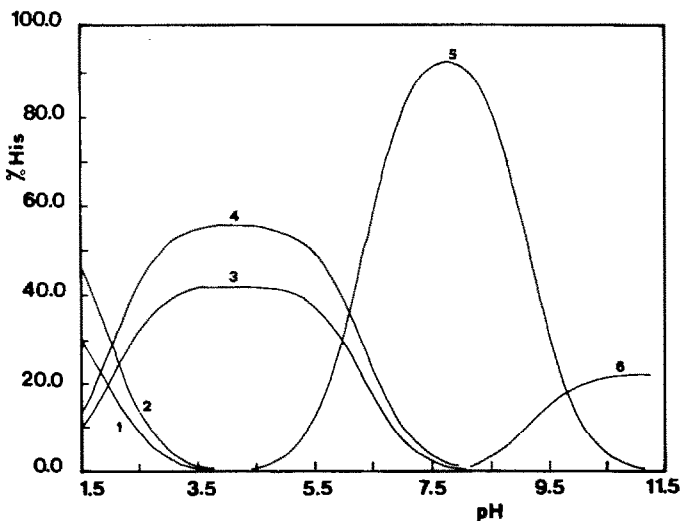

Fig. 1. Distribution of species vs. pH in the system H^+–Na^+–his^-–X^- ($X^- = Cl^-$ or I^-) at $T = 25\,^{\circ}C$; $C_{Na} = C_X = 1$ mol dm^{-3}; $C_{his} = 1$ mmol dm^{-3}; (1) $H_3(his)^{2+}$; (2) $H_3(his)X^+$; (3) $H_2(his)X^0$; (4) $H_2(his)^+$; (5) $H(his)^0$; (6) $Na(his)^0$.

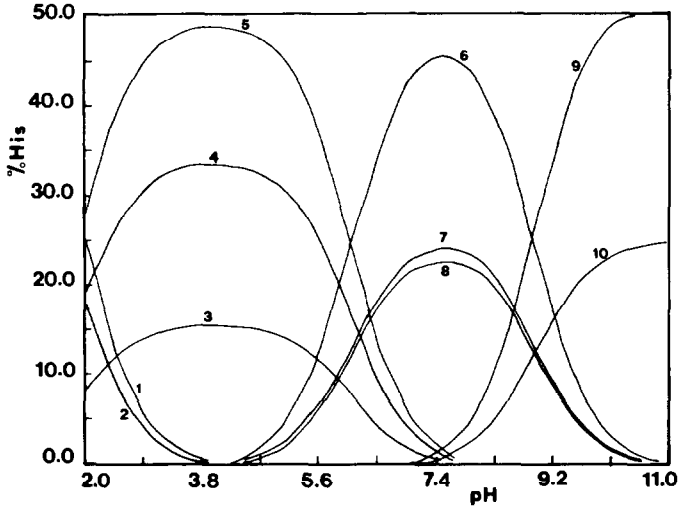

Fig. 2. Distribution of species vs. pH in the system H^+–Et_4N^+–Ca^{2+}–his^-–X^- ($X^- = Cl^-$ or I^-) at $T = 25\,°C$; $C_{Et_4N} = 0.7$, $C_{Ca} = 0.1$, $C_X = 0.9$ mol dm^{-3}; $C_{his} = 1$ mmol dm^{-3}; (1) $H_3(his)X^+$; (2) $H_3(his)^{2+}$; (3) $CaH_2(his)^{3+}$; (4) $H_2(his)X^0$; (5) $H_2(his)^+$; (6) $H(his)^0$; (7) $CaH(his)^{2+}$; (8) $Et_4NH(his)^+$; (9) $Ca(his)^+$; (10) $Et_4N(his)^0$.

and succinic acid. This suggests that all potentially coordinating groups (three in both histidine and aspartic acid) are involved in the formation of Ca^{2+} complexes. The sodium complexes are very weak by comparison with those of carboxylic acids, similarly to aminoacids [1,2,6]. Tetraethylam-

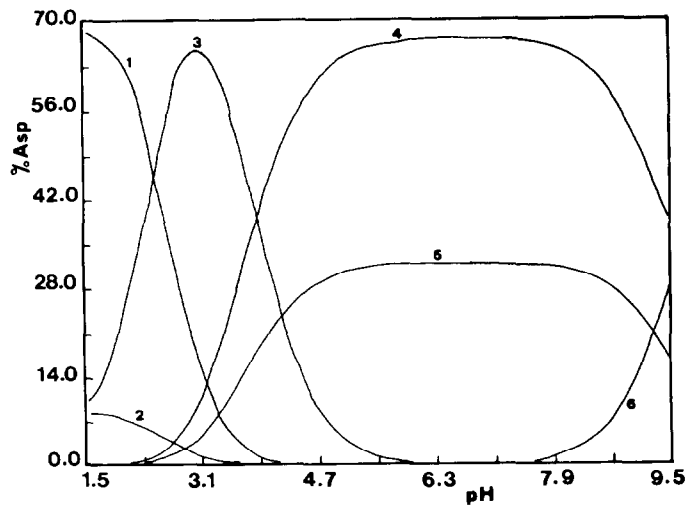

Fig. 3. Distribution of species vs. pH in the system H^+–Na^+–asp^{2-}–X^- ($X^- = Cl^-$ or I^-) at $T = 25\,°C$; $C_{Na} = C_X = 1$ mol dm^{-3}; $C_{asp} = 1$ mmol dm^{-3}; (1) $H_3(asp)^+$; (2) $H_3(asp)X^0$; (3) $H_2(asp)^0$; (4) $H(asp)^-$; (5) $NaH(asp)^0$; (6) $Na(asp)^-$.

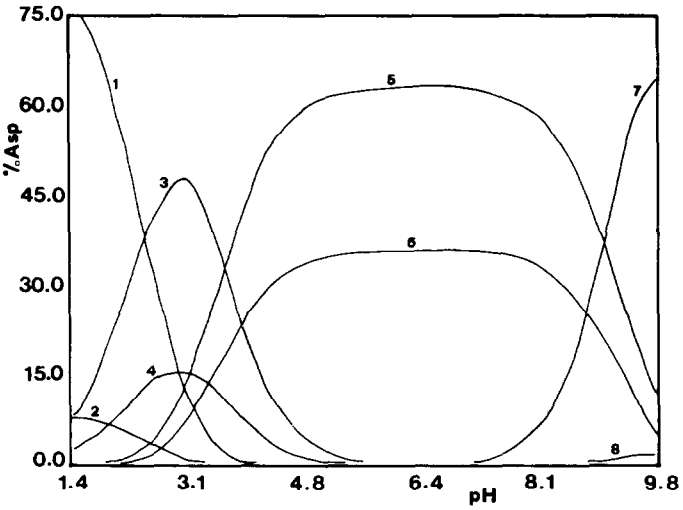

Fig. 4. Distribution of species vs. pH in the system $H^+-Et_4N^+-Ca^{2+}-asp^{2-}-X^-$ ($X^- = Cl^-$ or I^-) at $T = 25\,°C$; $C_{Et_4N} = 0.7$, $C_{Ca} = 0.1$, $C_X = 0.9$ mol dm^{-3}, $C_{asp} = 1$ mmol dm^{-3}; (1) $H_3(asp)^+$; (2) $H_3(asp)X^0$; (3) $H_2(asp)^0$; (4) $CaH_2(asp)^{2+}$; (5) $CaH(asp)^+$; (6) $H(asp)^-$; (7) $Ca(asp)^0$; (8) $Et_4N(asp)^-$ (negligible, $\approx 2\%$ at pH = 10).

monium complexes and X^- complexes show essentially the same stability as that found for similar systems [6,7,8].

In Table 5, some comparison between the stability of complexes here reported and that for other ligands with similar coordination groups is

TABLE 3

Species percentages (calculated with respect to the ligand), together with errors expressed as three times the standard deviation

Species	Percentage $\pm 3s$	pH	Analytical concentration
$H_3(his)X^+$	38± 8	1.75	as in Fig. 1
$H_2(his)X^0$	43± 8	4.00	
$Na(his)^0$	20±11	10.00	
$CaH_2(his)^{3+}$	15± 5	3.50	as in Fig. 2
$CaH(his)^{2+}$	24± 5	7.50	
$Ca(his)^+$	42± 5	9.50	
$Et_4NH(his)^+$	23± 8	7.50	
$Et_4N(his)^0$	23± 8	10.00	
$H_3(asp)X^0$	10± 5	1.40	as in Fig. 3
$NaH(asp)^0$	32± 8	7.40	
$Na(asp)^-$	40± 6	9.80	
$CaH_2(asp)^{2+}$	14± 4	3.40	as in Fig. 4
$CaH(asp)^+$	63± 5	7.40	
$Ca(asp)^0$	57± 3	9.40	
$Et_4N(asp)^-$	2± 1	9.80	

TABLE 4a

Thermodynamic parameters for the formation of aspartate species in aqueous solution at different temperatures and ionic strengths

Species	I [a]	T [b]	$\log \beta$ [c]	$\Delta G°$ [d]	$\Delta H°$ [d]	$\Delta S°$ [d]
$H(asp)^-$	0.1	10	10.13	−54.9	−54	4
	0.1	25	9.67	−55.2	−44	36
	0.1	37	9.40	−55.8	−35	68
	0.1	45	9.27	−56.5	−27	92
	0.5	10	10.12	−54.8	−50	16
	0.5	25	9.69	−55.3	−40	50
	0.5	37	9.45	−56.1	−30	83
	0.5	45	9.34	−56.9	−23	107
	1.0	10	9.96	−54.0	−20	119
	1.0	25	9.83	−56.1	−7	164
	1.0	37	9.82	−58.3	6	206
	1.0	45	9.87	−60.1	15	236
$H_2(asp)^0$	0.1	10	13.91	−75.4	−61	50
	0.1	25	13.39	−76.4	−51	85
	0.1	37	13.07	−77.6	−41	119
	0.1	45	12.92	−78.7	−33	145
	0.5	10	13.94	−75.5	−59	59
	0.5	25	13.44	−76.7	−48	95
	0.5	37	13.14	−78.0	−38	130
	0.5	45	13.00	−79.2	−30	156
	1.0	10	13.77	−74.6	−18	201
	1.0	25	13.67	−78.0	−3	252
	1.0	37	13.70	−81.3	12	300
	1.0	45	13.77	−83.9	22	334
$H_3(asp)^+$	0.1	10	16.35	−88.6	−59	106
	0.1	25	15.84	−90.4	−50	136
	0.1	37	15.53	−92.2	−41	167
	0.1	45	15.38	−93.6	−33	189
	0.5	10	16.64	−90.2	−77	48
	0.5	25	15.96	−91.1	−70	72
	0.5	37	15.51	−92.1	−62	97
	0.5	45	15.26	−93.0	−56	116
	1.0	10	17.01	−92.2	−99	−23
	1.0	25	16.11	−91.9	−94	−8
	1.0	37	15.49	−92.0	−89	10
	1.0	45	15.12	−92.1	−84	25
$Et_4N(asp)^-$	0.1	10	−1.12	6.1	53	164
	0.1	25	−0.61	3.5	58	184
	0.1	37	−0.20	1.2	63	200
	0.1	45	0.08	−0.5	66	210
	0.5	10	−1.14	6.2	56	177
	0.5	25	−0.59	3.4	62	198
	0.5	37	−0.15	0.9	67	214
	0.5	45	0.14	−0.9	71	226

TABLE 4a (continued)

Species	I [a]	T [b]	log β [c]	$\Delta G°$ [d]	$\Delta H°$ [d]	$\Delta S°$ [d]
	1.0	10	−1.29	7.0	86	279
	1.0	25	−0.45	2.6	95	312
	1.0	37	0.22	−1.3	103	337
	1.0	45	0.67	−4.1	109	354
$H_3(asp)X^0$	0.1	10	14.82	−80.3	−14	233
	0.1	25	14.68	−83.8	−16	227
	0.1	37	14.57	−86.5	−17	223
	0.1	45	14.49	−88.3	−18	220
	0.5	10	14.99	−81.3	−19	219
	0.5	25	14.80	−84.5	−21	212
	0.5	37	14.65	−87.0	−23	206
	0.5	45	14.55	−88.6	−24	202
	1.0	10	14.93	−80.9	26	377
	1.0	25	15.18	−86.6	29	386
	1.0	37	15.38	−91.3	31	394
	1.0	45	15.52	−94.5	33	399
$Ca(asp)^0$	0.1	10	1.89	−10.2	−6	16
	0.1	25	1.83	−10.4	−6	14
	0.1	37	1.79	−10.6	−7	12
	0.1	45	1.76	−10.7	−7	11
	0.5	10	1.76	−9.6	7	60
	0.5	25	1.83	−10.5	8	63
	0.5	37	1.89	−11.2	9	65
	0.5	45	1.93	−11.8	9	66
	1.0	10	1.33	−7.2	75	289
	1.0	25	2.06	−11.8	83	317
	1.0	37	2.64	−15.7	90	340
	1.0	45	3.03	−18.5	94	355
$CaH(asp)^+$	0.1	10	11.23	−60.9	−48	44
	0.1	25	10.76	−61.4	−54	26
	0.1	37	10.38	−61.6	−58	12
	0.1	45	10.13	−61.7	−61	2
	0.5	10	11.21	−60.7	−41	69
	0.5	25	10.80	−61.7	−46	54
	0.5	37	10.48	−62.2	−49	41
	0.5	45	10.27	−62.5	−52	33
	1.0	10	10.90	−59.1	19	275
	1.0	25	11.08	−63.2	21	282
	1.0	37	11.23	−66.7	22	287
	1.0	45	11.32	−69.0	24	291
$CaH_2(asp)^{2+}$	0.1	10	14.40	−78.0	−58	72
	0.1	25	13.84	−79.0	−64	50
	0.1	37	13.38	−79.5	−69	33
	0.1	45	13.08	−79.7	−73	22
	0.5	10	14.53	−78.7	−61	62
	0.5	25	13.93	−79.5	−68	39
	0.5	37	13.45	−79.8	−73	21
	0.5	45	13.13	−80.0	−77	8

TABLE 4a (continued)

Species	I [a]	T [b]	log β [c]	$\Delta G°$ [d]	$\Delta H°$ [d]	$\Delta S°$ [d]
	1.0	10	14.48	−78.5	−27	180
	1.0	25	14.21	−81.1	−30	170
	1.0	37	13.99	−83.1	−33	162
	1.0	45	13.85	−84.4	−35	156
$Na(asp)^-$	0.1	10	0.00	0.0	8	28
	0.1	25	0.08	−0.5	9	32
	0.1	37	0.14	−0.8	10	34
	0.1	45	0.19	−1.1	10	36
	0.5	10	−0.01	0.1	12	41
	0.5	25	0.10	−0.6	13	45
	0.5	37	0.19	−1.1	14	49
	0.5	45	0.25	−1.6	15	51
	1.0	10	−0.17	0.9	42	144
	1.0	25	0.24	−1.4	46	159
	1.0	37	0.56	−3.4	50	172
	1.0	45	0.78	−4.8	53	180
$NaH(asp)^0$	0.1	10	9.44	−51.2	−22	102
	0.1	25	9.23	−52.6	−25	93
	0.1	37	9.05	−53.7	−27	87
	0.1	45	8.93	−54.4	−28	82
	0.5	10	9.47	−51.4	−20	111
	0.5	25	9.28	−53.0	−22	103
	0.5	37	9.12	−54.2	−24	97
	0.5	45	9.02	−54.9	−25	93
	1.0	10	9.30	−50.4	21	253
	1.0	25	9.51	−54.3	23	261
	1.0	37	9.68	−57.4	25	267
	1.0	45	9.79	−59.6	27	271

[a] I in mol dm^{-3};
[b] T in °C;
[c] β is the overall formation constant;
[d] $\Delta G°$ and $\Delta H°$ in kJ mol^{-1}; $\Delta S°$ in J K^{-1} mol^{-1}.

shown. At present, only qualitative or semiquantitative comparison can be made; nevertheless, it is interesting to note that some formation constants can be guessed with good accuracy, taking into consideration the relatively high errors accompanying thermodynamic formation parameters for these weak and very weak complexes.

The reliability of the model here proposed can be checked in two ways: (i) by comparison with other similar systems, and (ii) by considering the consistency of the experimental data with the model, using statistical parameters. As regards (i), we have seen that the species found for both ligands, and their stability, are comparable with other similar systems. As regards (ii), we report in Table 6 some statistical parameters for the fit obtained by

TABLE 4b

Thermodynamic parameters for the formation of histidinate species in aqueous solution at different temperatures and ionic strengths [a]

Species	I	T	$\log \beta$	$\Delta G°$	$\Delta H°$	$\Delta S°$
$H(his)^0$	0.1	10	9.55	−51.7	−42	34
	0.1	25	9.13	−52.1	−47	19
	0.1	37	8.81	−52.3	−50	6
	0.1	45	8.59	−52.3	−53	−2
	0.5	10	9.59	−52.0	−43	31
	0.5	25	9.17	−52.3	−48	15
	0.5	37	8.83	−52.4	−52	2
	0.5	45	8.60	−52.4	−54	−6
	1.0	10	9.68	−52.5	−43	33
	1.0	25	9.26	−52.9	−48	17
	1.0	37	8.92	−53.0	−52	5
	1.0	45	8.70	−53.0	−54	−4
$H_2(his)^+$	0.1	10	15.84	−85.8	−72	49
	0.1	25	15.13	−86.4	−80	22
	0.1	37	14.57	−86.5	−86	1
	0.1	45	14.20	−86.5	−91	−13
	0.5	10	15.95	−86.4	−76	38
	0.5	25	15.21	−86.8	−84	9
	0.5	37	14.61	−86.8	−91	−13
	0.5	45	14.22	−86.6	−96	−29
	1.0	10	16.12	−87.4	−79	29
	1.0	25	15.35	−87.6	−88	−1
	1.0	37	14.73	−87.5	−95	−24
	1.0	45	14.32	−87.2	−100	−40
$H_3(his)^{2+}$	0.1	10	17.57	−95.2	−68	96
	0.1	25	16.90	−96.5	−76	70
	0.1	37	16.37	−97.2	−82	50
	0.1	45	16.01	−97.5	−86	36
	0.5	10	17.77	−96.3	−77	70
	0.5	25	17.02	−97.1	−85	41
	0.5	37	16.42	−97.5	−92	18
	0.5	45	16.02	−97.6	−97	3
	1.0	10	18.02	−97.7	−87	37
	1.0	25	17.17	−98.0	−97	4
	1.0	37	16.49	−97.9	−105	−22
	1.0	45	16.03	−97.7	−110	−39
$Et_4N(his)^0$	0.1	10	0.30	−1.6	−24	−78
	0.1	25	0.07	−0.4	−26	−86
	0.1	37	−0.12	0.7	−28	−94
	0.1	45	−0.24	1.5	−30	−98
	0.5	10	0.34	−1.8	−25	−81
	0.5	25	0.10	−0.6	−27	−90
	0.5	37	−0.10	0.6	−30	−97
	0.5	45	−0.23	1.4	−31	−102

TABLE 4b (continued)

Species	I	T	$\log \beta$	$\Delta G°$	$\Delta H°$	$\Delta S°$
	1.0	10	0.43	−2.3	−25	−78
	1.0	25	0.19	−1.1	−27	−88
	1.0	37	0.00	0.0	−29	−95
	1.0	45	−0.13	0.8	−31	−100
$Et_4NH(his)^+$	0.1	10	9.43	−51.1	−53	−8
	0.1	25	8.90	−50.8	−59	−28
	0.1	37	8.49	−50.4	−64	−44
	0.1	45	8.21	−50.0	−67	−55
	0.5	10	9.54	−51.7	−57	−20
	0.5	25	8.98	−51.2	−64	−42
	0.5	37	8.53	−50.6	−69	−59
	0.5	45	8.23	−50.1	−72	−70
	1.0	10	9.71	−52.6	−61	−29
	1.0	25	9.12	−52.0	−67	−51
	1.0	37	8.64	−51.3	−73	−70
	1.0	45	8.33	−50.7	−77	−82
$H_2(his)X^0$	0.1	10	15.49	−84.0	−60	84
	0.1	25	14.90	−85.0	−67	61
	0.1	37	14.43	−85.7	−72	43
	0.1	45	14.12	−86.0	−76	31
	0.5	10	15.64	−84.8	−65	69
	0.5	25	15.00	−85.6	−72	45
	0.5	37	14.49	−86.0	−78	25
	0.5	45	14.15	−86.2	−82	12
	1.0	10	15.91	−86.2	−68	63
	1.0	25	15.24	−87.0	−76	37
	1.0	37	14.71	−87.3	−82	17
	1.0	45	14.35	−87.4	−86	3
$H_3(his)X^+$	0.1	10	17.54	−95.0	−58	131
	0.1	25	16.97	−96.9	−64	110
	0.1	37	16.52	−98.1	−69	93
	0.1	45	16.22	−98.8	−73	81
	0.5	10	17.75	−96.2	−66	108
	0.5	25	17.11	−97.7	−73	83
	0.5	37	16.60	−98.5	−79	64
	0.5	45	16.26	−99.0	−83	51
	1.0	10	18.11	−98.2	−72	91
	1.0	25	17.40	−99.3	−80	64
	1.0	37	16.83	−99.9	−87	42
	1.0	45	16.46	−100.2	−91	27
$Ca(his)^+$	0.1	10	1.25	−6.7	−4	11
	0.1	25	1.21	−6.9	−4	10
	0.1	37	1.18	−7.0	−4	9
	0.1	45	1.17	−7.1	−4	8
	0.5	10	1.26	−6.8	−3	14
	0.5	25	1.23	−7.0	−3	13

TABLE 4b (continued)

Species	I	T	$\log \beta$	$\Delta G°$	$\Delta H°$	$\Delta S°$
	0.5	37	1.21	−7.2	−3	12
	0.5	45	1.20	−7.3	−4	11
	1.0	10	1.36	−7.4	1	29
	1.0	25	1.37	−7.8	1	30
	1.0	37	1.38	−8.2	1	30
	1.0	45	1.38	−8.4	1	30
$CaH(his)^{2+}$	0.1	10	10.15	−55.0	−37	65
	0.1	25	9.79	−55.9	−41	51
	0.1	37	9.51	−56.5	−44	40
	0.1	45	9.32	−56.7	−46	33
	0.5	10	10.26	−55.6	−40	53
	0.5	25	9.87	−56.3	−45	38
	0.5	37	9.55	−56.7	−49	26
	0.5	45	9.34	−56.9	−51	18
	1.0	10	10.44	−56.6	−44	45
	1.0	25	10.01	−57.1	−49	28
	1.0	37	9.67	−57.4	−53	15
	1.0	45	9.44	−57.5	−55	6
$CaH_2(his)^{3+}$	0.1	10	16.06	−87.1	−44	153
	0.1	25	15.63	−89.2	−48	137
	0.1	37	15.29	−90.8	−52	124
	0.1	45	15.06	−91.7	−55	115
	0.5	10	16.29	−88.3	−54	121
	0.5	25	15.76	−90.0	−60	101
	0.5	37	15.34	−91.1	−65	85
	0.5	45	15.06	−91.7	−68	74
	1.0	10	16.54	−89.6	−68	76
	1.0	25	15.87	−90.6	−76	50
	1.0	37	15.34	−91.1	−82	30
	1.0	45	14.98	−91.2	−86	16
$Na(his)^{0}$	0.1	10	−0.66	3.6		
	0.1	25	−0.67	3.8		
	0.1	37	−0.67	4.0		
	0.1	45	−0.67	4.1		
	0.5	10	−0.62	3.4		
	0.5	25	−0.63	3.6		
	0.5	37	−0.65	3.8		
	0.5	45	−0.66	4.0		
	1.0	10	−0.53	2.9		
	1.0	25	−0.54	3.1		
	1.0	37	−0.55	3.3		
	1.0	45	−0.56	3.4		

[a] Footnotes as for Table 4a.

TABLE 5

Comparison between the stability of aspartate and histidinate complexes and that of similar systems

Reaction	K [a]	Notes [b]
$H_3(asp)^+ + X^- = H_3(asp)X^0$	0.3	(XXIII), [0.3]
$H_2(his)^+ + X^- = H_2(his)X^0$	0.9	(XXII) + (XX), [0.8]
$H_3(his)^{2+} + X^- = H_3(his)X^+$	2.6	(XIX), [3.9] *
$Na^+ + H(asp)^- = NaH(asp)^0$	0.5	(I), (II), (IV), [0.9] (VIII), [0.4]
$Na^+ + (asp)^{2-} = Na(asp)^-$	2.6	[(I) + (III)]/2, [2.8] [(VIII) + (III)]/2, [2.7]
$Na^+ + his^- = Na(his)^0$	0.3	(VIII), [0.4]
$Ca^{2+} + asp^{2-} = Ca(asp)^0$	330	(XII) + (XIV), [200] **
$Ca^{2+} + H(asp)^- = CaH(asp)^+$	27	(XIV), [23]
$Ca^{2+} H_2(asp)^0 = CaH_2(asp)^{2+}$	2.8	(XV), [2.2]
$Ca^{2+} + his^- = Ca(his)^+$	35	(XIV) + (XVI), [28]
$Ca^{2+} + H(his)^0 = CaH(his)^{2+}$	4.5	(XV) + (XVI), [3]
$Ca^{2+} + H_2(his)^+ = CaH_2(his)^{3+}$	1.4	(XI), [3.5] *
$Et_4N^+ + asp^{2-} = Et_4N(asp)^-$	0.5	(XXVII), [1.4]
$Et_4N^+ + his^- = Et_4N(his)^0$	1.7	(XXVIII), [1.6]
$Et_4N^+ + H(his)^0 = Et_4NH(his)^+$	0.4	(XXIX), [0.5]

[a] Formation constant in $mol^{-1}\ dm^3$;

[b] Roman numbers indicate the corresponding equilibrium in Table 1. In square brackets is reported the value of the formation constant for the comparison equilibrium (or the sum of comparison equilibria).

* Probable unfavorable steric structure.

** Probable additional chelate stabilization.

ES2WC. The body of results shows a high degree of self-consistency. On the other hand, as shown in Table 3, the errors ($\pm$std. dev.) relative to the species percentages are quite reasonable and allow the speciation for these systems to be made correctly.

The dependence on ionic strength for the stability constants of all the species is very close to that proposed in several earlier publications. In general, the difference between the experimental log K ($I \neq 0$) and the value calculated by eqn. (1), using C and D taken from Refs. 1, 2, 14 and 19 [according to point (e) of the introduction] is less than 50% for minor species and less than 10% for major species, at $T = 25\,^\circ C$.

The thermodynamic parameters reported in Tables 2 and 4 are affected by large errors (except those referring to protonation) and must be regarded as indicative. Nevertheless, it is interesting to note the great variability of ΔH° and ΔS° as a function of I and T, and this indicates that speciation and structural considerations cannot be made if the function $\Delta Y^\circ = f(I, T)$ ($Y = G, H, S$) is not known.

TABLE 6

Statistical analysis for ES2WC calculations

		$Y = f(Y_{calcd})$		$Y = f(Y_{calcd}$, intercept = 0)		
		Slope	Intercept	Slope	r [a]	% [b]
(A) Residuals *						
his^-	$\log K_1^H$	0.997	0.028	1.00015	0.99999	8
	$\log K_2^H$	0.988	0.075	1.00063	0.99999	61
	$\log K_3^H$	0.912	0.158	0.99870	0.99939	24
asp^{2-}	$\log K_1^H$	1.037	0.345	1.00026	0.99999	85
	$\log K_2^H$	1.033	−0.126	0.99841	0.99998	94
	$\log K_3^H$	0.954	0.104	1.00680	0.99985	98

(B) Statistical parameters for the fit **

	Histidine	*Aspartic acid*
$s(\bar{p})$	0.024	0.020
$\epsilon(\bar{p})$	0.017	0.015
$\epsilon(\log K_1^H)$	0.025	0.027
$\epsilon(\log K_2^H)$	0.019	0.017
$\epsilon(\log K_3^H)$	0.052	0.030

[a] Correlation coefficient.

[b] Level for joint hypothesis 0 intercept/1 slope; the hypothesis is accepted if this parameter is < 99% (99% confidence interval) or 95% (95% confidence interval).

* Residuals $Y - Y_{calcd}$ [in ES2WC $Y = \bar{p}$ (average number of protons bound to the ligand)] must have zero mean, i.e., $Y = f(Y_{calcd})$ must be a straight line with zero intercept and unity slope.

** s = Standard deviation; ϵ = mean deviation; these parameters are calculated by ES2WC.

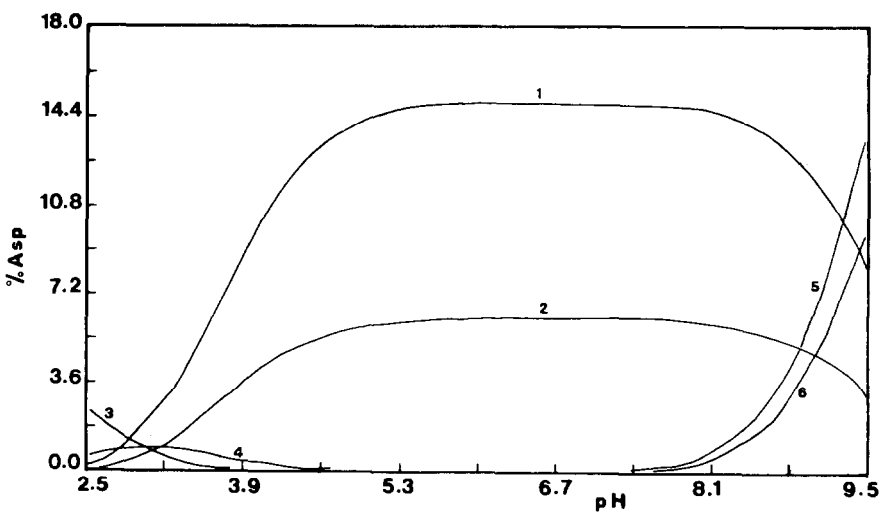

Fig. 5. Distribution of species vs. pH in the system H^+–Na^+–Ca^{2+}–Cl^-–asp^{2-}, at $T = 25\,°C$. $C_{Na} = 0.4$, $C_{Cl} = 0.5$, $C_{Ca} = 0.0045$ mol dm^{-3} (free concentrations in 3.5% salinity marine water); $C_{asp} = 1$ mmol dm^{-3} (trace); $I \approx 0.55$ mol dm^{-3}; (1) $NaH(asp)^0$; (2) $CaH(asp)^+$; (3) $H_3(asp)Cl^0$; (4) $H_2(asp)Cl^-$; (5) $Na(asp)^-$; (6) $Ca(asp)^0$.

One of the most important fields of application of the studies of complexing ability of naturally occurring ligands towards alkali and alkaline earth metals and towards inorganic anions is the speciation of natural fluids, such as marine water, blood plasma, urine, etc. As an example, we report in Fig. 5 the distribution of the species in the system aspartic acid (trace)–marine water (3.5% salinity). In this case the free concentrations of Ref. 26 were used for Ca^{2+}, Na^{+} and Cl^{-}, and the presence of Mg^{2+} was neglected. At the pH values of sea water both Ca^{2+} and Na^{+} complexes are present in significant amounts (note that, if considering Mg^{2+} complexes too, the percentage of aspartate present as complex species should be much higher), and this indicates the importance of these type of complexes in the speciation of natural fluids, also for ligands generally considered as having low complexing ability towards alkali and alkaline earth metals.

As regards literature findings [27], it is impossible in practice to make comparisons, since the complexes found here were not reported on or, for calcium complexes, were studied without considering the weak complexes (Na^{+}, X^{-}) formed by the ligand with the salt used to keep the ionic strength constant. Further studies on other polyfunctional *O*- and *N*-ligands and on Mg^{2+} complexes are in progress.

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