CHEMICAL SPECIATION & BIOAVAILABILITY

Sequestration of organometallic compounds by synthetic and naturally occurring polycarboxylate ligands. Binding of monomethylmercury(II) by polyacrylic and alginic acids

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ABSTRACT

The sequestering capacity of synthetic and naturally occurring polycarboxylate ligands towards monomethylmercury(II) was evaluated by stability quantitative data on the interaction of CH_3Hg^+ with different molecular weight synthetic polyacrylates (2 and 20 kDa average M.wt) and alginate (70–100 kDa) extracted from brown algae *Macrocystis pyrifera*. The influence of ionic medium was evaluated by measurements on the CH_3Hg^+-polyacrylate systems in $NaNO_3$ medium at different ionic strengths (0.10, 0.25, 0.50 and 0.75 mol L^{-1}), and a Debye–Hückel type equation was used for the dependence of complex formation constants on ionic strength. Measurements on the CH_3Hg^+-alginate system were carried out at $I = 0.10$ mol L^{-1} in $NaNO_3$ medium. By using the stability data, the sequestering capacity of both ligands towards monomethylmercury(II) was determined at different pH values. Results obtained show that the binding ability of polyacrylic ligands (PAA) is stronger than the alginate (AA), following the trend PAA (20 kDa) > PAA (2 kDa) > AA.

Keywords: monomethylmercury, sequestration by organic matter, polyacrylic and alginic acids, speciation, equilibrium analysis, complex species formation

INTRODUCTION

The chemistry of mercury in aquatic systems is very complex owing to the variety of valence states and species such as elemental mercury (Hg^0), dissolved inorganic mercury (Hg^{2+}) and organomercury (RHg^+ and R_2Hg^0). Each of these forms shows different toxicity trends and environmental impact. Among the mercury organic derivatives, mono-organomercury is by far the most toxic form of mercury because of its high capacity to enter biological membranes and accumulate in living organisms (Sigel and Sigel, 1997). Along the different steps

of food chain, beginning from phytoplankton (Ullrich *et al.*, 2001), very high biomagnification factors of monomethylmercury are reported: $\sim 10^4$ from water to edible shell-fish (mussels), and 10^6-10^8 from water to big fishes (*e.g.* tuna fish) at the top of food chain (Kwokal and Branica, 2000). Accumulation of methylmercury in living organisms is generally attributed to the well known interaction with thiol groups of cellular constituents, leading to stable complex species, and this may seriously damage the primary functions and structure of sulfur containing proteins. For this reason, the coordination chemistry of inorganic and organic

species of mercury is focused mostly on the sulfide complex species formation (Martell *et al*., 2004). Interactions with thiol groups have been assumed to be the main responsible also for the association of the inorganic (Hg^{2+}) and organic (CH_3Hg^+) mercury with sulfur containing organic matter in soils and in aquatic environments (Amirabaham *et al*., 2002; Benoit *et al*., 2001; Haitzer *et al*., 2003; Hintelmann *et al*. 1995, 1997; Ravichandran, 2004; Qian *et al*., 2002; Schlüter, 1997; Schlüter and Gäth, 1997; Yin *et al*., 1997). On the other hand, it must be noted that other $-O$ and $-N$ binding sites also play an important role in the speciation of natural aquatic systems. The carboxylate groups are by far the most common binding sites in natural organic matter: they are present not only in humic and fulvic substances, where the acidic groups content is mainly due to carboxylic and phenolic groups and to a less extent to thiol and amino groups (Masini *et al*., 1998; Schulten, 1996; Thurmann and Malcom, 1983; Tipping, 2002), but also in many other classes of compounds deriving from both organic production and degradation, such as acidic polysaccharides, hydrolysable amino acids and peptides, fat acids, *etc*. (Buffle, 1988). The interactions of mercury system with carboxylate ligands have been in general little investigated. Some studies were carried out to evaluate the fulvic acid–sulfide ion competition in the mercury ion binding (Loux, 1998; Reddy and Aiken, 2001; Xu and Allard, 1991). Yoon *et al*. (2005) report an X-ray study on the competitive absorption of monomethylmercury by different binding sites in humic substances, including thiol, carboxylate and amino groups. Alderighi *et al*. (2003) report results on the coordination chemistry of CH_3Hg^+ with different low molecular weight inorganic and organic $-O$, $-N$ and $-S$ ligands including carboxylic and amino acids. No more investigations are reported on the stability of monomethylmercury carboxylate systems. For a better understanding of environmental speciation of monomethylmercury, the interactions with natural organic matter containing carboxylate binding sites can not be neglected. To this end we carried out a study on the interaction of monomethylmercury with synthetic (polyacrylate) and natural occurring (alginate) poly-carboxylate ligands. Polyacrylic acid (PAA, Figure 1), as well as polymetacrylic acid, is often used as component of carboxylate based chelating resins for metal ions removal. It is also considered as a good model molecule for soluble fulvic fraction of humic substances (Gamble and
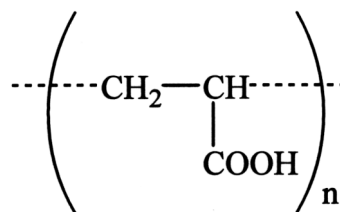

Figure 1 Structure of polyacrylic acid (PAA).

Schnitzer, 1973; Stevenson, 1994). For this reason, results obtained in the investigations on the metal-polyacrylate systems can be compared with those obtained in the coordination studies of metal-fulvate and metal-humate systems, as shown by Clark and Choppin (1996) in studying rare elements complexes with humic acids and, more recently, by Giacalone *et al*. (2006) in a speciation study of organotin compounds with polyacrylate and fulvate ligands. In the present study we used two different polyacrylic acids with molecular weight 2 and 20 kDa in order to evaluate also the dependence of the stability of $CH_3Hg-PAA$ species on the ligand molecular weight (*i.e.* on the number of carboxylic groups).

Alginic acid (AA) is a co-polymer of 1,4 linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues, each containing one carboxylate group per monomeric unit (Figure 2). It is one of the main component of seaweed. According to the algal species, the content of alginic acid may vary from 20 to 40% of algae dry weight (Kelco Inc., 1987; Seki and Suzuki, 1998; Volesky, 2003).

Alginate is widely used in different industrial applications, such as food industry, cosmetics, textiles and paper manufacture, *etc*. Due to its chemical structure, alginate may also act as sequestering agent for metal ions removal. The binding
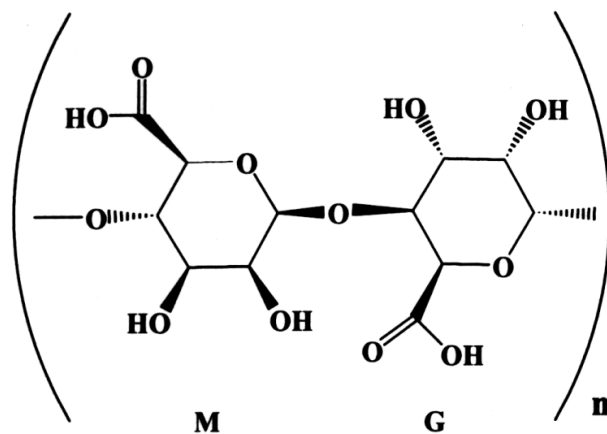

Figure 2 Structure of alginic acid (AA) (G = guluronic acid; M = mannuronic acid).

capacity of metal ions by algal biomass is well known since many years and it has been used as an alternative process, in comparison with the classical treatment, for heavy metal ions removal and recovery (Crist *et al.*, 2000; Darnall *et al.*, 1986; Greene *et al.*, 1987; Hamdy, 2000; Kuyucak and Volesky, 1988; Zimnik and Sneddon, 1988). It is established that metals uptake mainly occurs by carboxylic binding sites of polysaccharides components of marine algae (Crist *et al.*, 1981; Davis *et al.*, 2003a, 2003b; Gardea-Torresdey *et al.*, 1990). The interest in metals biosorption process is increased during last years to provide a low cost technology for soils remediation and wastewaters treatment (Crini, 2005; Veglio and Beolchini, 1997; Vieira and Volesky, 2000; Volesky, 2003; Wase and Forster, 1997). The sequestering ability of alginate has also been recently evaluated towards triorganotin cations (Giacalone *et al.*, 2006) whose high toxicity is well recognised. In this work, we used alginic acid extracted from *Macrocystis pyrifera*. Stability data of species formed in both $CH_3Hg-PAA$ and CH_3Hg-AA systems were used to compare the different sequestration capacity of natural and synthetic polycarboxylate ligands towards monomethylmercury cation. The investigations were performed by potentiometry ($[H^+]-$glass electrode) at $t = 25°C$, in a wide pH range, from about 3 to about 10. The interactions in the $CH_3Hg-PAA$ system were studied using two polyacrylic acids having different molecular weight (2 and 20 kDa) in $NaNO_3$ ionic medium at different ionic strength values (0.10, 0.25, 0.50, 0.75 mol L^{-1}) and measurements in the CH_3Hg-AA system were carried out in $NaNO_3$ medium at $I = 0.10$ mol L^{-1}.

EXPERIMENTAL

Materials

Monomethylmercury was used as chloride salt (Strem Chemicals) without further purification. Commercial alginic acid (AA), extracted from *Macrocystis pyrifera*, was supplied by Sigma (lot 60K1443) with an average content of mannuronic (M) and guluronic (G) acids of 61 and 39%, respectively, *i.e.* an M/G ratio of 1.56. According to technical information by Sigma Company, molecular weight of alginic acid is in the range 70–100 kDa. Polyacrylic acids (2 and 20 kDa) were supplied from Fluka with 26% average water content. Their purity, checked by potentiometric titrations, was always

$> 99.5\%$. Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated Fluka ampoules and standardised against sodium carbonate and potassium hydrogen phthalate, respectively. $NaNO_3$ salt was used as purchased from Fluka, without further purification. All the solutions were prepared using freshly prepared CO_2-free ultra-pure water ($R = 18$ MΩ), and grade A glassware was always employed.

Procedure

Potentiometric titrations were carried out (at $t = 25.0 \pm 0.1°C$) using an apparatus consisting of a Model 713 Metrohm potentiometer, equipped with a combined glass electrode (Ross type 8102, from Orion) and a Model 765 Metrohm motorised burette. The estimated accuracy was ± 0.15 mV and ± 0.003 mL for e.m.f. and titrant volume readings, respectively. The apparatus was connected to a PC, and automatic titrations were performed using a home made computer program to control titrant delivery, data acquisition and to check for e.m.f. stability. All titrations were carried out under magnetic stirring and presaturated N_2 was bubbled through the purified solution in order to exclude O_2 and CO_2 inside. The solutions under investigation contained known amounts of polyacrylic or alginic acid and monomethylmercury(II) chloride, as reported in Table 1. Different amounts of sodium nitrate were added to obtain the pre-established value of ionic strength ($I = 0.10$, 0.25, 0.50, 0.75 mol L^{-1}) in the $CH_3Hg-PAA$ systems. Potentiometric measurements were carried out by titrating 25 mL of the titrand solution with standard NaOH solutions. Alginic acid was used as sodium salt obtained by adding NaOH to the acidic solution up to pH ~ 9. Titrations in the CH_3Hg-AA system were carried out from alkaline to acidic pH values, by means of HCl standard solutions, in $NaNO_3$ medium at 0.10 mol L^{-1} ionic strength. Metal to ligand ratios in the titrand solutions were always $1:1 \leq CH_3Hg:L \leq 1:2$ (L = AA, PAA). Details on the experimental conditions used for both $CH_3Hg-PAA$ and CH_3Hg-AA systems are given in Table 1.

To determine the formal electrode potential E^0_{ext}, independent titrations of HCl solutions were performed for each experiment with standard sodium hydroxide solutions in the same experimental conditions of ionic strength and temperature as the systems under investigation.

Table 1 Experimental conditions for $CH_3Hg-PAA$ and CH_3Hg-AA systems in $NaNO_3$ ionic medium, at $t = 25°C$

I (mol L^{-1})[a]	C_{CH_3Hg}(mmol L^{-1})	C_L /(mmol L^{-1})	pH range	Runs	No. of points
		CH_3Hg-AA			
0.100	0.53−0.59	0.94−1.33	3.10−8.13	5	614
		$CH_3Hg-PAA$ (2 kDa)			
0.097	2.00	4.98−5.02	3.30−10.40	5	587
0.233	2.00	4.95−5.18	3.34−10.41	4	547
0.454	2.00	5.06−5.13	3.23−10.50	6	692
0.672	1.99	5.00	3.17−10.51	5	599
		$CH_3Hg-PAA$ (20 kDa)			
0.106	1.97	4.94	2.89−9.99	3	399
0.233	1.99−4.09	4.94−5.14	3.02−9.97	5	919
0.458	1.97	4.96	2.87−9.96	3	402
0.663	1.99−4.09	4.94−5.00	2.89−10.02	4	601

[a]Effective ionic strength.

Calculations

The following computer programs (De Stefano *et al.*, 1997) were used in the calculations: (i) BSTAC and STACO for the refinement of all the parameters of an acid-base titration (E^0, pK_w, coefficient of junction potential j_a, analytical concentration of reagents) and for the calculation of complex formation constants; (ii) ES4ECI to draw speciation diagrams and to compute species formation percentages; and (iii) LIANA to test the dependence of $\log\beta$ on ionic strength. Formation constants, concentrations and ionic strengths are expressed in the molar (mol L^{-1}) concentration scale. The dependence of formation constants on ionic strength was taken into account using a Debye−Hückel type equation already proposed by our research group (Daniele *et al.*, 1991, 1997),

$$\log\beta = \log^T\beta - z*0.51\ I^{1/2}(1 + 1.5\ I^{1/2})^{-1} + C\ I \tag{1}$$

with

$$z* = \sum(\text{charges})^2_{\text{reactants}} - \sum(\text{charges})^2_{\text{products}} \tag{1a}$$

where β is the conditional formation constant and $^T\beta$ is the formation constant at infinite dilution. C is a parameter dependent on I. All the formation constants refer to the general equilibrium:

$$pCH_3Hg^+ + qL^{z-} + rH^+ = (CH_3Hg)_pL_q(H)_r^{(p-zq+r)}$$
$$\beta_{pqr} \tag{2}$$
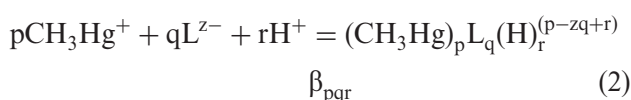
where L = PAA or AA ligand. In all the calculations, ligands protonation and hydrolysis of CH_3Hg^+ and chloride species formation were always taken into account.

Protonation of polyelectrolytes

The protonation constants of polycarboxylates considered in this work can be calculated in different ways, namely by Högfeldt three parameter equation (Högfeldt *et al.*, 1989), by the modified Hasselbalch−Henderson equation as proposed by Katchalsky (Katchalsky, 1954; Katchalsky and Spitnik, 1947) or by using a low molecular weight model which considers a polycarboxylic monomeric unit. Several trials carried out on different natural and synthetic polycarboxylate systems (Crea *et al.*, work in progress) showed that for both polyacrylates and alginate it is sufficient to consider a dicarboxylic unit. The present calculations were performed by using this model. The effective charges for the polyelectrolyte units are -3.0, -4.6 and -3.5, for PAA 2 kDa, PAA 20 kDa and alginate, respectively. The values of $\log K^H$ of polyacrylic acids (2 and 20 kDa) and alginic acid used in this work (Crea *et al.*, work in progress) are reported in Table 2. In the same table we report the protonation constants of the polyacrilyc acids under investigation for $I \to 0$ mol L^{-1}, calculated by using Eqn (1) for the dependence on ionic strength.

Protonation constants reported in Table 2 were used for the calculations of formation constants of complex species in the $CH_3Hg-PAA$ and CH_3Hg-AA systems.

Table 2 Protonation constants of polyacrylic and alginic acids in NaNO$_{3aq}$ at different ionic strengths, together with the protonation constants at $I \to 0$ mol L^{-1}(for PAA systems) calculated with a Debye−Hückel type equation (1), $t = 25°$C

I (mol L^{-1})	$\log K^H_I$	$\log \beta^H_2$
	PAA 2 kDa	
0.00	6.46 $\pm$ 0.06[a]	11.30 $\pm$ 0.10[a]
0.10	5.79 $\pm$ 0.02	10.17 $\pm$ 0.04
0.25	5.58 $\pm$ 0.02	9.82 $\pm$ 0.02
0.50	5.41 $\pm$ 0.01	9.56 $\pm$ 0.02
0.75	5.32 $\pm$ 0.01	9.42 $\pm$ 0.02
	PAA 20 kDa	
0.00	7.21 $\pm$ 0.04	12.56 $\pm$ 0.05
0.10	6.23 $\pm$ 0.02	10.82 $\pm$ 0.01
0.25	5.93 $\pm$ 0.02	10.30 $\pm$ 0.02
0.50	5.71 $\pm$ 0.03	9.94 $\pm$ 0.03
0.75	5.61 $\pm$ 0.04	9.78 $\pm$ 0.05
	AA	
0.10	3.98 $\pm$ 0.01	6.21 $\pm$ 0.01

[a]Standard deviation.

## *Hydrolysis and chloride species formation for monomethylmercury*

Monomethylmercury, as well as inorganic mercury(II) ion, undergoes hydrolysis leading to a quite stable hydroxo species in aqueous solution (Alderighi *et al.*, 2003; De Robertis *et al.*, 1998; Sanz *et al.*, 2002). Therefore, hydrolysis of CH$_3$Hg$^+$ can not be neglected in the studies of organomercury(II)-ligand interaction in aqueous solution. In this work we used the values of hydrolysis constants of CH$_3$Hg$^+$ reported by De Robertis *et al.* (1998) at different ionic strengths in NaNO$_3$ medium. Moreover, also CH$_3$Hg − chloride interaction was considered using values of formation constants reported by the same authors. Both values of hydrolysis and chloride species formation, reported in Table 3, were considered in the calculations to define the speciation model of both CH$_3$Hg−PAA and CH$_3$Hg−AA systems.

Table 3 Hydrolysis and chloride formation constants[a] of CH$_3$Hg$^+$ at different ionic strengths (NaNO$_3$ medium) and at $t = 25°$C

I (mol L^{-1})	$-\log\beta_{1-1}$[b]	I (mol L^{-1})	$\log K_{Cl}$
0.01	4.529	0.00	5.45
0.09	4.538	0.10	5.25
0.25	4.556	0.25	5.19
0.49	4.582	0.50	5.16
0.81	4.618	1.00	5.13

[a]Values from De Robertis *et al.*, 1998.
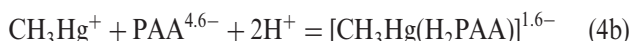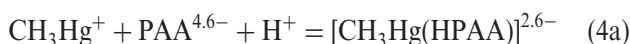[b]log β values refer to equilibrium
  CH$_3$Hg$^+$ + H$_2$O = CH$_3$Hg(OH)^0 + H$^+$.
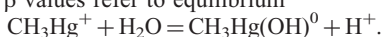
RESULTS AND DISCUSSION

The complex formation model obtained from the best fit of experimental measurements shows, for both CH$_3$Hg−L (L = PAA, AA) systems, the formation of the 110 [CH$_3$Hg(L)], 111 [CH$_3$Hg(HL)] and 112 [CH$_3$Hg(H$_2$L)] species according to the following equilibria (Equations 3−5).

CH$_3$Hg−PAA (2 kDa) system

$$CH_3Hg^+ + PAA^{3-} = [CH_3Hg(PAA)]^{2-} \quad (3)$$

$$CH_3Hg^+ + PAA^{3-} + H^+ = CH_3Hg(HPAA)]^- \quad (3a)$$

$$CH_3Hg^+ + PAA^{3-} + 2H^+ = [CH_3Hg(H_2PAA)]^0 \quad (3b)$$
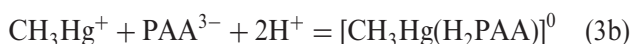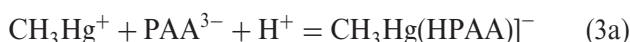
CH$_3$Hg−PAA (20 kDa) system

$$CH_3Hg^+ + PAA^{4.6-} = [CH_3Hg(PAA)]^{3.6-} \quad (4)$$

$$CH_3Hg^+ + PAA^{4.6-} + H^+ = [CH_3Hg(HPAA)]^{2.6-} \quad (4a)$$

$$CH_3Hg^+ + PAA^{4.6-} + 2H^+ = [CH_3Hg(H_2PAA)]^{1.6-} \quad (4b)$$

CH$_3$Hg−AA system

$$CH_3Hg^+ + AA^{3.5-} = [CH_3Hg(AA)]^{2.5-} \quad (5)$$

$$CH_3Hg^+ + AA^{3.5-} + H^+ = [CH_3Hg(HAA)]^{1.5-} \quad (5a)$$

$$CH_3Hg^+ + AA^{3.5-} + 2H^+ = [CH_3Hg(H_2AA)]^{0.5-} \quad (5b)$$
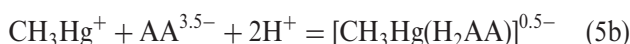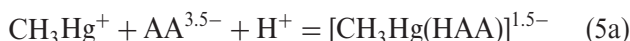
In Table 4, the values of formation constants of CH$_3$Hg$^+$ complex species with polyacrylates and alginate are reported at different ionic strengths (0.10 mol L^{-1} for CH$_3$Hg−AA system) in NaNO$_3$ medium.

As can be seen the stability of the species formed in CH$_3$Hg−PAA and CH$_3$Hg−AA systems follows the trend: PAA(20 kDa) > PAA(2 kDa) > AA. By using Equation (1) for the dependence on ionic strength, formation constants at infinite dilution (for $I \to 0$ mol L^{-1}) of complex species formed in the CH$_3$Hg−PAA systems were calculated together with the empirical parameter C. Results are reported in Table 5.

Figure 3 shows the dependence on ionic strength of formation constants for the species CH$_3$Hg−L (L = PAA 2 kDa and 20 kDa) obtained by fitting the experimental values to the Debye−Hückel type Equation (1).

Table 4 Formation constants of CH$_3$Hg−PAA and CH$_3$Hg−AA complex species in NaNO$_{3aq}$, at different ionic strengths and at $t = 25°$C

I (mol L^{-1})	log β_{112}	log β_{111}	log β_{110}
	CH$_3$Hg−PAA 2 kDa		
0.097	15.20 ± 0.01[a]	11.457 ± 0.005[a]	5.730 ± 0.004[a]
0.233	14.72 ± 0.01	11.050 ± 0.006	5.495 ± 0.004
0.454	14.21 ± 0.02	10.881 ± 0.009	5.428 ± 0.006
0.672	13.77 ± 0.04	10.502 ± 0.009	5.154 ± 0.006
	CH$_3$Hg−PAA 20 kDa		
0.106	16.326 ± 0.009[a]	12.086 ± 0.006[a]	6.042 ± 0.004[a]
0.233	15.771 ± 0.008	11.697 ± 0.005	5.878 ± 0.003
0.458	15.426 ± 0.007	11.398 ± 0.006	5.625 ± 0.005
0.663	15.38 ± 0.01	11.322 ± 0.008	5.668 ± 0.005
	CH$_3$Hg−AA		
0.100	12.61 ± 0.06	9.34 ± 0.07	4.85 ± 0.02

[a]Standard deviation.

The dependence of formation constants on ionic strength allows us to know the stability of the systems under investigation at each effective ionic strength value in the range 0−0.70 mol L^{-1}, without carry out further experimental measurements. This is of fundamental importance when speciation studies are performed in natural fluids where ionic strength values range from about 0.08 for fresh waters to about 0.70 mol L^{-1} for saline and marine waters, while biological fluids show ionic strength values ~ 0.15 mol L^{-1}. In Table 6 we report the smoothed formation constant values for the systems CH$_3$Hg− PAA 2 and −PAA 20 kDa. As one can see, the accordance between the experimental values and the calculated ones is quite satisfactory and indicates that the proposed model for the dependence on ionic strength is correct.

Speciation profiles and sequestering capacity of polyacrylic and alginic ligands

In Figure 4 we report the speciation diagrams for CH$_3$Hg−AA and CH$_3$Hg−PAA (2 and 20 kDa)

systems in the pH range 3 to 9, at two different ligand concentrations, *i.e.*, 10^{-2} and 10^{-3} mol L^{-1} (Figures 4a,c,e and 4b,d,f, respectively), with a concentration of CH$_3$Hg$^+$ = 10^{-8} mol L^{-1}. Protonated (112) and (111) species are present in all the acidic pH range, being the formation of the 111 species quite consistent even above pH 5, especially in the CH$_3$Hg−PAA systems. In the pH range 6 to 9, monomethylmercury(II) is mainly present as complex species 110 [CH$_3$Hg(L)], with little contribution of the monoprotonated [CH$_3$Hg(HL)] species, in both polyacrylate and alginate systems. In this pH range, the hydrolytic species [CH$_3$Hg(OH)] is also present in both systems, with a noticeable formation percentage especially for a metal to ligand ratio of 10^{-8}/10^{-3}.

The formation of the simple hydrolytic species [CH$_3$Hg(OH)] is more evident in the CH$_3$Hg−AA system owing to the weaker stability of the complex species in this system in comparison to CH$_3$Hg− PAA systems (see Table 4). In the CH$_3$Hg−PAA (2 and 20 kDa) systems, the more stable CH$_3$Hg(PAA)

Table 5 Formation constants of CH$_3$Hg−PAA complexes at $I \rightarrow 0$ mol L^{-1} and $t = 25°$C, together with empirical parameter C for the dependence on ionic strength [Eqn (1)]

pqr	log$^T \beta_{pqr}$[a]	C	σ[b]
	CH$_3$Hg−PAA 2 kDa		
112	16.61 ± 0.04[c]	−0.8 ± 0.1[c]	0.03
111	12.56 ± 0.03	− 0.2 ± 0.1	0.07
110	6.39 ± 0.04	− 0.1 ± 0.1	0.06
	CH$_3$Hg−PAA 20 kDa		
112	18.52 ± 0.03	1.16 ± 0.07	0.02
111	13.79 ± 0.01	0.79 ± 0.03	0.007
110	7.00 ± 0.03	0.5 ± 0.08	0.04

[a]Complex formation constants refer to equilibrium of Eqn (2).
Standard deviation: [b]on the fit; [c]on the parameters.
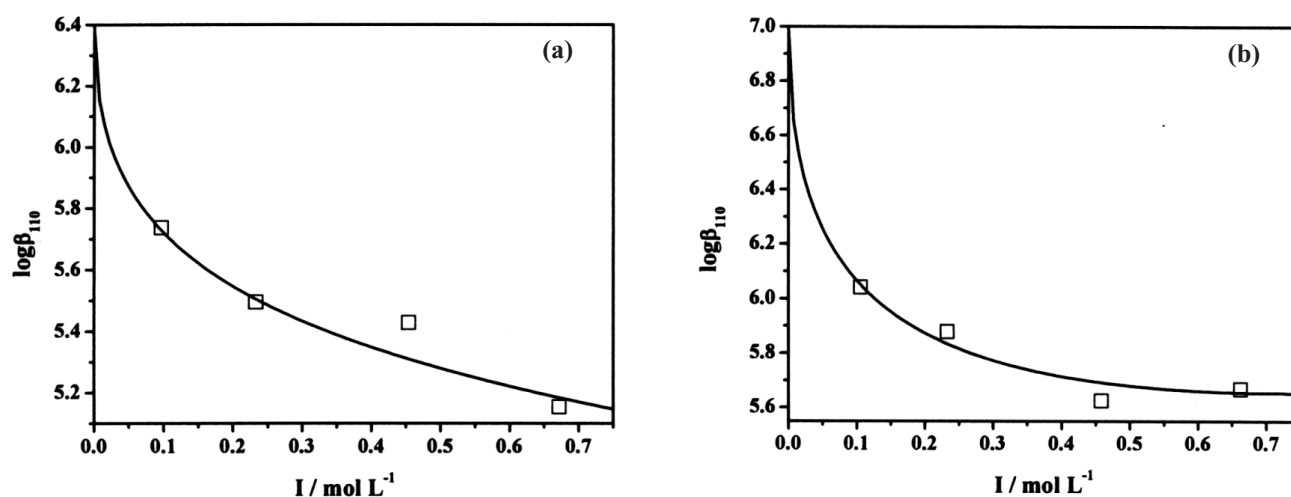
Figure 3 Dependence of formation constants on ionic strength, according to the Eqn (1), for (a) $CH_3Hg–PAA$ (2 kDa) and (b) $CH_3Hg–PAA$ (20 kDa) complex species at $t = 25°C$.

complex species formed in the pH range 5 to 9 is able to lower the formation of $CH_3Hg(OH)$ species. For a metal to ligand concentration ratio of 10^{-8} to 10^{-3} mol L^{-1} more than 75% of $CH_3Hg(AA)$ species is formed at pH about 5.5 (Figure 4b), and more than 70% of $CH_3Hg(PAA)$ species is achieved at pH 6.5 and 7 for PAA 2 and PAA 20 kDa, respectively (Figures 4d,f). For a 10 times higher concentration of ligands (10^{-2} mol L^{-1}) the formation percentage of $CH_3Hg(L)$ ($L = AA$ and PAA) species increases up to about 90% (Figures 4a,c,e) and a consistent lowering of $CH_3Hg(OH)$ species formation occurs: for example, the formation percentage of $CH_3Hg(OH)$ in the $CH_3Hg–AA$ system decreases from about 70% to about 25% at pH 7 for an alginate ligand concentration increasing from 10^{-3} mol L^{-1} to 10^{-2} mol L^{-1}.

The sum of the formation percentage of the complex species obtained in each system at different pH values gives quantitative information on the sequestration of monomethylmercury(II) by the ligands under investigation. To this end we used the following Boltzmann type equation (6)

$$P = P_\infty + \frac{P_0 - P_\infty}{1 + \exp[(pL - pL_{50})/S]} \qquad (6)$$

where P is the total percentage of monomethyl mercury(II) complex species [*i.e.* $CH_3Hg(L) + CH_3Hg(HL)$ with $L =$ alginate or polyacrylate ligand] calculated with respect to metal concentration; $pL = -\log[L]_T$; P_0 and P_∞ are the percentages for $pL \rightarrow 0$ and $pL \rightarrow \infty$, respectively; $pL_{50} = pL$ (for $P = 50\%$); S is an adjustable parameter which accounts for the slope in the flex of sigmoid curve.

Table 6 Smoothed values of formation constants of $CH_3Hg–PAA$ (2 and 20 kDa) complex species in $NaNO_{3aq}$, at different ionic strengths and at $t = 25°C$

I (mol L^{-1})	$\log\beta_{112}$	$\|\Delta \log \beta_{calc-exp}\|$	$\log\beta_{111}$[a]	$\|\Delta \log \beta_{calc-exp}\|$	$\log\beta_{110}$[a]	$\|\Delta \log \beta_{calc-exp}\|$
			$CH_3Hg–PAA$ 2 kDa			
0.097	15.23 ± 0.03[b]	0.03	11.46 ± 0.02[b]	0.00	5.73 ± 0.03[b]	0.00
0.233	14.70 ± 0.03	0.02	11.08 ± 0.02	0.03	5.51 ± 0.02	0.01
0.454	14.18 ± 0.02	0.03	10.75 ± 0.04	0.13	5.31 ± 0.04	0.11
0.672	13.79 ± 0.04	0.02	10.53 ± 0.06	0.03	5.19 ± 0.06	0.03
			$CH_3Hg–PAA$ 20 kDa			
0.106	16.30 ± 0.02	0.03	12.09 ± 0.01	0.00	6.05 ± 0.02	0.00
0.233	15.79 ± 0.02	0.02	11.69 ± 0.01	0.00	5.83 ± 0.02	0.05
0.458	15.45 ± 0.02	0.03	11.41 ± 0.01	0.01	5.69 ± 0.02	0.06
0.663	15.36 ± 0.03	0.02	11.32 ± 0.01	0.00	5.66 ± 0.03	0.01

[a]Complex formation constants refer to equilibrium of Eqn (2); [b]Standard deviation.
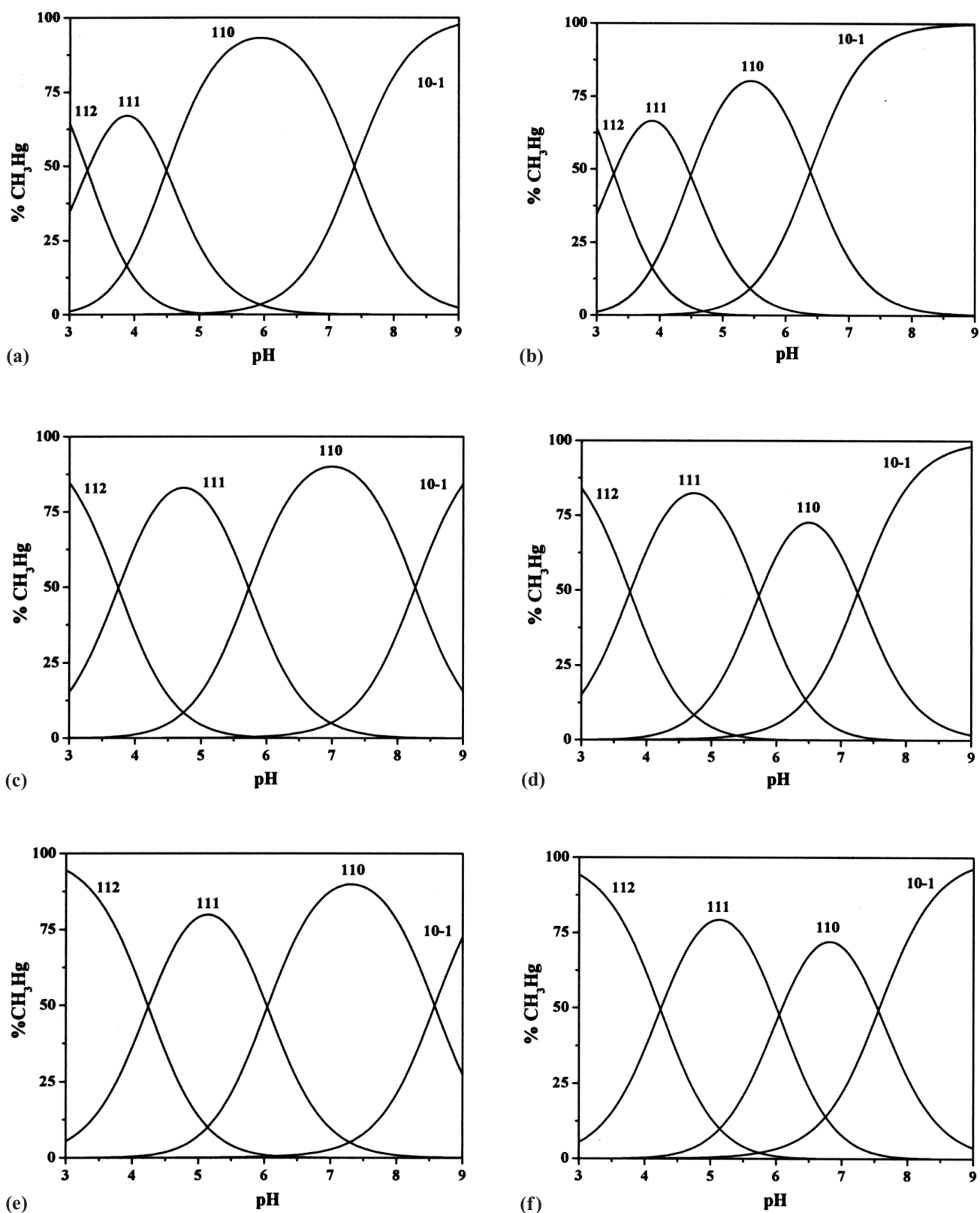
Figure 4 Speciation diagrams for the CH_3Hg-AA (**a,b**) and $CH_3Hg-PAA$ [(2 kDa (**c,d**) and 20 kDa (**e,f**)] systems at $I = 0.10$ mol L^{-1} (NaNO$_3$) and $t = 25°C$.

Experimental conditions:

$C_{CH_3Hg} = 10^{-8}$ mol L^{-1}; $C_{AA/PAA\ 2\,kDa/PAA\ 20\,kDa} = 10^{-2}$ mol L^{-1} (**a,c,e**)

$C_{CH_3Hg} = 10^{-8}$ mol L^{-1}; $C_{AA/PAA\ 2\,kDa/PAA\ 20\,kDa} = 10^{-3}$ mol L^{-1} (**b,d,f**)

Table 7 Parameters of the Boltzman equation (6a) for the solutions containing $CH_3Hg_{(tot)} = 10^{-8}$ mol L^{-1} at $I = 0.10$ mol L^{-1}(NaNO$_{3aq}$), at $t = 25°C$ and different pH values

pH	CH$_3$Hg−PAA (2 kDa)		CH$_3$Hg−PAA (20 kDa)		CH$_3$Hg−AA	
	pL$_{50}$	S	pL$_{50}$	S	pL$_{50}$	S
6	4.227	0.434	4.451	0.434	3.382	0.434
7	3.263	0.434	3.555	0.434	2.387	0.434
8	2.267	0.434	2.577	0.434	1.388	0.434

Being in our case $P_\infty = 0$ and $P_0 = 100$, Equation (6) becomes

$$P\% = \frac{100}{1 + \exp[(pL - pL_{50})/S]} \qquad (6a)$$

where P% is the percentage of CH$_3$Hg−PAA or CH$_3$Hg−AA complex species , pL$_{50}$ is the ligand concentration necessary to sequestrate 50% of the CH$_3$Hg$^+$. This equation allows to calculate the CH$_3$Hg$^+$ sequestration percentage for each pH and ligand concentration value, when the formation constants for the systems under investigation and the species formation percentages *vs*. pH have been defined. In Table 7 we report the values of pL$_{50}$ and S of Boltzman equation calculated for the systems CH$_3$Hg−AA and −PAA at different pH and for a CH$_3$Hg$^+$ concentration 10^{-8} mol L^{-1}.
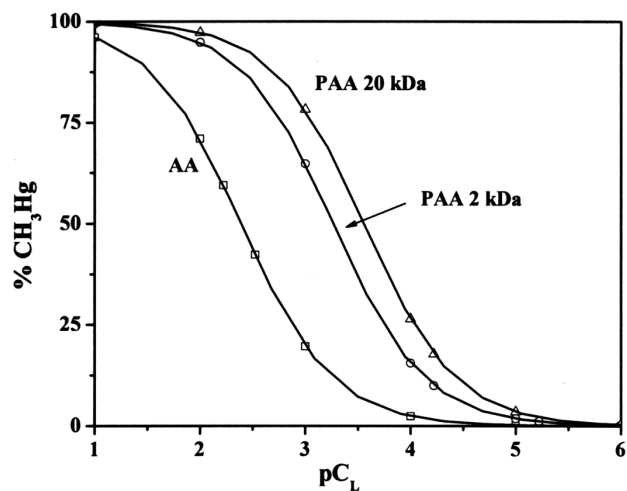
Figure 5 shows the sequestration percentage of CH$_3$Hg$^+$ cation by alginate as function of ligand concentration, at different pH values. Curves refer to the 111 [CH$_3$Hg(HL)] and 110 [CH$_3$Hg(L)] complex

species formation without considering the formation of CH$_3$Hg$^+$ hydrolytic species [CH$_3$Hg(OH)].

As expected (see speciation diagrams, Figure 4), binding of monomethylmercury by alginate is strongly dependent on pH conditions. The most favourable condition is at pH = 6. At this pH value, the amount of ligand needed to bind 50% of organo-metal ion (0.5 10^{-8} mol L^{-1}, *i.e.* 5 nmol L^{-1}) is about $10^{-3.4}$ mol L^{-1} (*i.e.*, less than 400 µmol L^{-1}). To bind the same amount of CH$_3$Hg$^+$ cation at pH 7 and 8, about $10^{-2.4}$ mol L^{-1} and $10^{-1.4}$ mol L^{-1} of ligand are necessary, respectively. Such a great difference, depends on a consistent formation of the very stable hydrolytic species which, at pH > 6, lowers strongly the formation of CH$_3$Hg(PAA) and CH$_3$Hg(AA) complex species. This is more evident in the CH$_3$Hg−AA system where the complex species formed are slightly weaker than the corresponding ones in the CH$_3$Hg−PAA systems (see Table 4). In Figure 6, a comparison between sequestering capacity of alginate and polyacrylate ligands (2 and 20 kDa) is reported at pH = 7.
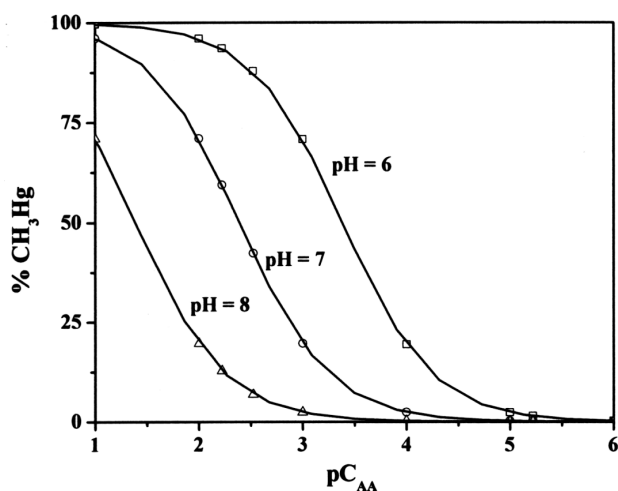
Figure 5 Percentage of methylmercury(II) as complex species *vs* pC$_{AA}$ at different pH values. Experimental conditions: $t = 25°C$, $I(NaNO_3) = 0.10$ mol L^{-1}, CH$_3$Hg$_{tot}$ = 10^{-8} mol L^{-1}.

Figure 6 Percentage of methylmercury(II) as complex species *vs*. pC$_L$ (L = AA, PAA 2 kDa and PAA 20 kDa) at pH = 7. Experimental conditions: $t = 25°C$, $I(NaNO_3) = 0.10$ mol L^{-1}, CH$_3$Hg$_{tot}$ = 10^{-8} mol L^{-1}.

The sequestration curves show that polyacrylic ligand 20 kDa is the best sequestering agent for CH_3Hg^+ cation: a quite low concentration of this ligand ($\sim 10^{-3.5}$ mol L^{-1}) is sufficient to bind 50% of methylmercury here considered (0.5×10^{-8} mol L^{-1}). The binding capacity of alginic ligand is lower than both 2 and 20 kDa polyacrylic ligands: at the pH value considered, an alginate concentration of about $10^{-2.4}$ mol L^{-1} is needed to remove 50% of organomercury(II) cation. In spite of its weaker binding capacity, alginate ligand can also be considered a good metal ion removal agent due to its high concentration in brown algae [20 to 40% of dry weight, according to Volesky (2003)].

CONCLUSIONS

Interaction of monomethylmercury(II) with carboxylic ligands has been very little investigated. Libich and Rabenstein (1973) determined formation constants of methylmercury(II) with some mono carboxylic ligands by NMR studies. More recently, Alderighi *et al.* (2003) report a thermodynamic study on the binding of methylmercury with different −O, −N and −S donor ligands, including carboxylic and amino-carboxylic ligands. No investigation was carried out till now on the monomethylmercury − polycarboxylate systems. Therefore, results obtained in this work are the first ones obtained in the study of these systems and no comparison can be made with other literature data. The stability data obtained by the equilibrium analysis on the CH_3Hg−PAA and CH_3Hg−AA systems in aqueous solution allowed us to quantitatively define the binding capacity of the ligands under investigation towards mono methylmercury(II). Polyacrylate ligands show a higher sequestering capacity than the alginate one, following the trend: PAA $(20 > 2) > AA$. The same trend $(PAA > AA)$ was found in a recent work (Giacalone *et al.*, 2006) where the sequestering capacity of polyacrylate, alginate and fulvate ligands was investigated towards $(CH_3)_3Sn^+$ cation, having the same charge as methylmercury(II) cation. Results obtained show that also in the presence of polycarboxylic ligands, hydrolysis of monomethylmercury can not be neglected, especially above pH 6.

REFERENCES

Alderighi, L., Gans, P., Midollini, S. and Vacca, A. 2003. Co-ordination chemistry of the methylmercury(II) ion in aqueous solution: a thermodynamic investigation. *Inorg. Chim. Acta*, **356**, 8−18.

Amirabaham, A., Reid, A.L., Haines, T.A., Kahl, J.S. and Arnold, C. 2002. Association of methylmercury with dissolved humic acids. *Environ. Sci. Technol.*, **36**, 690−695.

Benoit, J.M., Mason, R.P., Gilmour, C.C. and Aiken, G.R. 2001. Constants for mercury binding by dissolved organic matter isolates from the Florida Everglades. *Geochim. Cosmochim. Acta*, **65**, 4445−4451.

Buffle, J. 1988. *Complexation Reactions in Aquatic Systems: An Analytical Approach*, Chap. 4, p. 146. Ellis Horwood, Chichester.

Clark, S.B. and Choppin, G.R. 1996. A comparison of the dissociation kinetics of rare earth element complexes with synthetic polyelectrolytes and humic acid. In: Gaffney, J.S., Marley, N.A. and Clark, S.B. (eds.), *Humic and fulvic acids. Isolation, Structure and Environmental Role*, ACS Symposium Series no. 651, pp. 207−219.

Crea, F., De Stefano, C., Gianguzza, A., Pettignano, A., Piazzese, D. and Sammartano, S. Acid-base properties of synthetic and natural polyelectrolytes: experimental results and models for the dependence on different aqueous media. (work in progress).

Crea, F., Giacalone, A., Gianguzza, A., Piazzese, D. and Sammartano, S. 2006. Modelling of natural and synthetic polyelectrolyte interactions in natural waters. *Mar. Chem.*, **99**, 93−105.

Crini, G. 2005. Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. *Progr. Polym. Sci.*, **30**, 38−70.

Crist, R.H., Oberholser, K., Shank, N. and Nguyen, M. 1981. Nature of bonding between metallic ions and algal cell walls. *Environ. Sci. Technol.*, **15**, 1212−1217.

Crist, R.H., Martin, J.R., Guptili, P.W. and Eslinger, J.M. 2000. Interaction of metals and protons with algae. 2. Ion exchange in adsorption and metal displacement by protons. *Environ. Sci. Technol.*, **24**, 337−342.

Daniele, P.G., De Robertis, A., De Stefano, C. and Sammartano, S. 1991. Ionic Strength Dependence of Formation Constants. XIII. A Critical Examination of Preceding Results. In: Alegret, S., Arias, J.J., Barceló, D., Casal, J. and Router, G. (eds.), *Miscellany of scientific papers offered to Enric Casassas*, pp. 121−126. Barcelona, Spain.

Daniele, P.G., De Stefano, C., Foti, C. and Sammartano, S. 1997. The effect of ionic strength and ionic medium on thermodynamic parameters of protonation and complex formation. *Curr. Topics Sol. Chem.*, **2**, 253−274.

Darnall, D.W., Greene, B., Henzel, M.T., Hosea, J.M., McPherson, R.A., Sneddon, J. and Alexander, M.D. 1986. Selective recovery of gold and other metal ions from an algal biomass. *Environ. Sci. Technol.*, **20**, 206−208.

Davis, T.A., Volesky, B. and Mucci, A. 2003a. A review of the biochemistry of heavy metal biosorption by brown algae. *Water. Res.*, **37**, 4311−4330.

Davis, T.A., Llanes, F., Volesky, B. and Mucci, A. 2003b. Metal selectivity of Sargassum spp. And their alginates in relation to their α-L-guluronic acid content and conformation. *Environ. Sci. Technol.*, **37**, 261−267.

De Robertis, A., Foti, C., Patanè, G. and Sammartano, S. 1998. Hydrolysis of $(CH_3)Hg^+$ in different ionic media: salt effects and complex formation. *J. Chem. Eng. Data*, **43**, 957−960.

De Stefano, C., Mineo, P., Rigano, C. and Sammartano, S. 1997. Computer tools for the speciation of natural fluids. In: Gianguzza, A., Pelizzetti, E. and Sammartano, S. (eds.), *Marine Chemistry − An Environmental Analytical Chemistry Approach*, pp. 71−83. Kluwer, Amsterdam.

Gamble, D.S. and Schnitzer, M. 1973. The chemistry of fulvic acid and its reactions with metal ions. In: Singer, P.C. (ed.), *Trace Metals and Metal-Organic Interactions in Natural Waters*, pp. 265−302. Ann Arbor Science.

Gardea-Torresdey, J.L., Becker-Hapak, M.K., Hosea, J.M. and Darnall, D.W. 1990. Effect of chemical modification of algal carboxyl groups on metal ion binding. *Environ. Sci. Technol.*, **24**, 1372−1378.

Giacalone, A., Gianguzza, A., Pettignano, A. and Sammartano, S. 2006. Sequestration of organometallic compounds by natural organic matter. Binding of trimethyltin(iv) by fulvic and alginic acids. *Appl. Organomet. Chem.*, **20**, 706−717.

Greene, B., McPherson, R. and Darnall, D. 1987. Algal sorbents for selective metal ion recovery. In: Patterson, J.W. and Passino, R. (eds.), *Metal speciation separation and recovery*, pp. 315−338. Lewis Chelsea, ML.

Hamdy, A.A. 2000. Biosorption of heavy metals by marine algae. *Curr. Microbiol.*, **41**, 232−238.

Haitzer, M., Aiken, G.R. and Ryan, J.N. 2003. Binding of Mercury(II) to aquatic humic substances: influence of pH and source of humic substances. *Environ. Sci. Technol.*, **37**, 2436−2441.

Hintelmann, H., Welbourn, P.M. and Evans, R.D. 1995. Binding of methylmercury compounds by humic and fulvic acids. *Water Air Soil Pollut.*, **80**, 1031−1034.

Hintelmann, H., Welbourn, P.M. and Evans, R.D. 1997. Measurements of complexation of methylmercury compounds by freshwater humic substances using equilibrium dialysis. *Environ. Sci. Technol.*, **31**, 489−495.

Högfeldt, E., Miyajima, T., Marinsky, J.A. and Muhammed, M. 1989. Application of a simple three parameter mode to titration data for some polyelectrolytes. *Acta Chem. Scand.*, **43**, 496−499.

Katchalsky, A. 1954. Problems in the physical chemistry of polyelectrolytes. *J. Polymer Sci.*, **12**, 159−184.

Katchalsky, A. and Spitnik, P. 1947. Potentiometric titrations of polymethacrylic acid. *J. Polymer Sci.*, **2**, 432−437.

Kelco Inc. 1987. *Alginate products for scientific water control*, 3rd edn. San Diego, CA.

Kuyucak, N. and Volesky, B. 1988. Biosorbent for recovery of metals from industrial solutions. *Biotechnol. Lett.*, **10**, 137−142.

Kwokal, Z. and Branica, M. 2000. Determination of dissolved monomethylmercury in saline, estuarine and fresh waters of Croatia. *Croat. Chem. Acta*, **73**, 97−109.

Libich, S. and Rabenstein, D.L. 1973. Nuclear Magnetic Resonance studies of the solution chemistry of metal complexes. Determination of formation constants of methylmercury complexes of selected carboxylic acids. *Analyt. Chem.*, **45**, 118−124.

Loux, N.T. 1998. An assessment of mercury-species-dependent binding with natural organic carbon. *Chem. Spec. Bioavail.*, **10**, 127−136.

Martell, A.E., Smith, R.M. and Motekaitis, R.J. 2004. NIST Standard Reference Database 46, vers. 8, Gaithersburg.

Masini, J.C., Abate, G., Lima, E.C., Hahn, L.C., Nakamura, J.L. and Nagatomy, H.R. 1998. Comparison of methodologies for determination of carboxylic and phenolic groups in humic acids. *Analyt. Chim. Acta*, **364**, 223−233.

Qian, J., Skyllberg, U., Frech, W., Bleam, W.F., Bloom, P.R. and Petit, P.E. 2002. Bonding of methyl mercury to reduced sulfur groups in soil and stream organic matter as determined by X-ray absorption spectroscopy and binding affinity studies. *Geochim. Cosmochim. Acta*, **66**, 3873−3885.

Ravichandran, M. 2004. Interactions between mercury and dissolved organic matter. A review. *Chemosphere*, **55**, 319−331.

Reddy, M.M. and Aiken, G.R. 2001. Fulvic acid-sulfide ion competition for mercury ion binding in the Florida Everglades. *Water, Air Soil Pollut.*, **132**, 89−104.

Sanz, J., Raposo, J.C., de Diego, A. and Madariaga, J.M. 2002. Complexation of CH_3Hg^+ with chloride, sulfate and carbonate in $NaClO_4$: construction of thermodynamic models. *Appl. Organomet. Chem.*, **16**, 339−346.

Schlüter, K. 1997. Sorption of inorganic mercury and monomethyl mercury in an iron-humus podzol soil of southern Norway studied by batch experiments. *Environ. Geol.*, **30**, 266−279.

Schlüter, K. and Gäth, S. 1997. Modelling leaching of inorganic Hg(II) in a Scandinavian iron-humus Podzol. Validation and long-term leaching under various deposition rates. *Water, Air Soil Pollut.*, **96**, 301−320.

Schulten, H.-R. 1996. A new approach to the structural analysis of humic substances in water and in soils. In: Gaffney, J.S., Marley, N.A. and Clark, S.B. (eds.), *Humic and fulvic acids. Isolation, structure and environmental role*, Chap. 4, pp. 42−56. ACS Symposium series 651. (1996), American Chemical Society, Washington DC.

Seki, H. and Suzuki, S. 1998. Biosorption of heavy metal ions to brown algae Macrocystis pyrifera, Kjellmaniella crassifloria and Undaria pinnatifida. *J. Coll. Interface Sci.*, **206**, 297−301.

Sigel, A. and Sigel, H. (eds.). 1997. Mercury and its effects on environment and biology, In: *Metal ions in biological systems*, Vol. 34. Marcel Dekker, INC. New York.

Stevenson, F.J. 1994. *Humus chemistry. Genesis, composition, reactions*. 2nd edn., John Wiley & Sons Inc., N.Y.

Tipping, E. 2002. *Cation binding by humic substances*, Cambridge Environmental Chemistry series, no. 12, Cambridge University Press (UK).

Thurmann, E.M. and Malcom, R.L. 1983. In: Christman, R.F. and Gjessing, E.T. (eds.), *Aquatic and Terrestrial Humic Materials*, Chap. 1. Ann Arbor Science.

Ullrich, S.M., Tanton, T.W. and Abdrashitova, S.A. 2001. Mercury in the aquatic environment: a review of factors affecting methylation. *Crit. Rev. Environ. Sci. Technol.*, **31**, 241−293.

Veglio, F. and Beolchini, F. 1997. Removal of metals by biosorption: a review. *Hydrometallurgy*, **44**, 301−316.

Vieira, R.H.S.F. and Volesky, B. 2000. Biosorption: a solution to pollution? *Int. Microbiol.*, **3**, 17−24.

Volesky, B. 2003. *Sorption and Biosorption*, BV Sorbex Inc. Montreal.

Wase, J. and Forster, C. 1997. *Biosorbents for metal ions*. Taylor & Francis Ltd. London.

Xu, H. and Allard, B. 1991. Effects of a fulvic acid on the speciation and mobility of mercury in aqueous solutions. *Water, Air Soil Pollut.*, **56**, 709−717.

Yin, Y., Allen, H.E., Huang, C.P. and Sanders, P.F. 1997. Interaction of Hg(II) with soil-derived humic substances. *Analyt. Chim. Acta*, **341**, 73−82.

Yoon, S-J., Diener, L.M., Bloom, P.R., Nater, E.A. and Bleam, W.F. 2005. X-ray absorption studies of CH_3Hg^+ -binding sites in humic substances. *Geochim. Cosmochim. Acta*, **69**, 1111−1121.

Zimnik, P.R. and Sneddon, J. 1988. Binding and removal of aluminium ions in water by an algal biomass. *Analyt. Lett.*, **20**, 755−760.