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Complex Formation of Copper(II) and Cadmium(II) with Pectin and Polygalacturonic Acid in Aqueous Solution. An ISE-H^+ and ISE-Me^{2+} Electrochemical Study

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The acid-base properties of pectin and its ability to complex with copper(II) or cadmium(II) were quantitatively determined in order to define the ability of this natural polyelectrolyte to sequester metal ions. Potentiometric measurements carried out at different ionic strengths (0.1 - 0.7 mol L^{-1}, in Na^+ ionic medium) allowed us to define the acid-base properties of pectin together with the dependence of the protonation constants on ionic strength and to calculate the protonation constant at infinite dilution. The formation of complex species between pectin and copper(II) or cadmium(II) was investigated potentiometrically (using H^+, Cu^{2+} and Cd^{2+} Ion Selective Electrodes, ISEs) in $NaNO_3$ at 0.1 mol L^{-1} at T = 25°C. The results thus obtained showed the formation of ML and MLH species for both the pectin-Cu^{2+} / Cd^{2+} systems. The acid-base properties and the complexation capacity of a commercial polygalacturonic acid with copper(II) and cadmium(II) ions were also investigated, polygalacturonic acid being the most abundant component of pectin. On the basis of the proposed complexation model, the abilities of pectin and polygalacturonic acid to sequester cadmium(II) and copper(II) ions were evaluated at different pH values. The data obtained on the sequestration capacity of the two ligands used here were compared with previous data for other polycarboxylic polymer systems, including alginate, polyacrylate and polymethacrylates, under the same experimental conditions.

Keywords: polysaccharides as chelating agents; metal sequestering ability; complex formation; speciation studies.

1. INTRODUCTION

Pectin is a polysaccharide that is largely present in the cell wall of plants [1-4]**.** Approximately 70 % of pectin consists of galacturonic acid monomer units, which are linked at the O-1 and the O-4 positions [5] and have varying degrees of methyl-esterification of the C-6 carboxyl groups [6-8] (see structure in Figure 1).

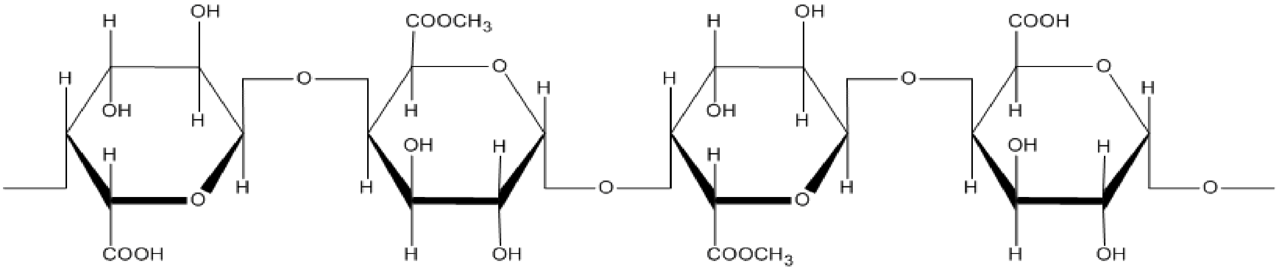

Figure 1. Structure of a generic partially acetylated pectin.

Moreover, the C-2 or C-3 positions of the monomer units can be acetylated to varying degrees. The remaining 30 % of pectin consists of side chains that bear different oligosaccharide residues. Pectin is present in significant amounts in the flesh and peels of many fruits (e.g., in terms of wet weight, pectin comprises up to 2.5 % of apple peel, 5.5 % of orange peel, 4.0 % of lemon flesh, etc.). Pectins are widely used in the alimentary industry [9,10], mostly as gelling agents for food and as stabilizers for pulp drinks, in the production of biodegradable films for foods [11] and in the pharmaceutical industry, where they are primarily used as a carrier for drugs [12-14]. From a chemical point of view, it must be noted that pectin can act as a sequestering agent for metal ions, due to the presence of multiple carboxylate-binding sites in its structure. Metal complexation by naturally occurring polycarboxylic ligands has been widely investigated, with the aim of use in different technical applications such as water and wastewater treatment, heavy metal removal in contaminated sites, metal recovery, etc. Despite the great interest in these processes, few quantitative data on the stability of the complex species formed in these metal-natural polyelectrolyte systems are available in the literature. To confirm the possibility of using naturally occurring polycarboxylates as sequestering agents, the strength of the interactions between fulvic and alginic acids with certain metal ions and organometallic cations in aqueous solution has been investigated in our laboratories in recent years [15-18]. In this work, we have extended our studies to pectin, investigating its acid-base properties and the stability of the complex species that it forms with copper(II) or cadmium(II) ions. Moreover, to confirm the stability of the complex species that are formed in the pectin-metal ion systems, experiments were also carried out to determine the acid-base properties and the complexation capability of a commercial poly-galacturonic acid towards the same metal ions, this poly-electrolyte being the primary component of pectin.

Potentiometric titrations were performed over the pH range from 2 to 7 to determine the protonation constants (using ISE-H^+) of pectin and polygalacturonic acid at different ionic strengths ($0.1 < I$ (Na^+-medium) < 0.7 mol L^{-1}). Potentiometric titrations (using ISE-H^+ and ISE-M^{2+}) were also used to determine the formation constants of the complex species formed between the two polyelectrolytes and copper(II) or cadmium(II) ions in Na^+ ionic medium, at $I = 0.1$ mol L^{-1} and at T =

25°C. The “diprotic-like model” [19,20], already employed successfully to explain the acid-base properties of various polyelectrolytes [20,21], was used to define the protonation constants of the ligands investigated here (more details are reported in the *Results and Discussion* section). A Debye-Hückel type equation was used to evaluate the dependence of the protonation constants on ionic strength.

The formation of complex species in the pectin-Cu^{2+}/Cd^{2+} and the polygalacturonate-Cu^{2+}/Cd^{2+} systems was also investigated using an ISE-M^{2+} technique over a wide range of ligand-to-metal concentration ratios, using I = 0.1 mol L^{-1} and pH ~ 5.5 in order to avoid hydrolysis of the cadmium ion and to minimize that of the copper(II) ion (see details in the *Results and Discussion* section). Analysis of the experimental data provided evidence for the formation of ML and MLH species in all of the systems investigated. The data obtained on stability were used to quantitatively define the sequestering capacity of pectin and polygalacturonate for Cd^{2+} and Cu^{2+} ions. To this end, we used a parameter designated $pL_{0.5}$, whose significance and calculation is reported below. The $pL_{0.5}$ values calculated for these systems were compared with results obtained for other metal-polycarboxylate systems, with particular reference to metal-alginate systems. The pH-dependence of the $pL_{0.5}$ values of pectin, polygalacturonate, and alginic acid was also evaluated, and an empirical relationship was reported.

2. EXPERIMENTAL PART

2.1 Chemicals and materials

Cadmium(II) nitrate tetrahydrate and copper(II) nitrate hydrate were analytical grade, high purity (> 99.5 %) products from Aldrich. Commercial pectin potassium salt, extracted from citrus peel (PECT, lot. 077K1583), and polygalacturonic acid (PGA, lot 066K3784) were supplied by Aldrich. The purity of the ligands was confirmed by potentiometric titrations, and water content (14 and 17 %, for PECT and PGA, respectively) was estimated by Thermo Gravimetric Analysis (TGA, TA INSTRUMENTS, model Q5000 IR); these values were taken into account in all calculations. Sodium chloride and nitrate solutions were prepared by weighing pure salts (Fluka) after drying at 110°C. Hydrochloric or nitric acid and sodium hydroxide solutions were prepared by diluting concentrated Fluka ampoules and were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. All solutions were prepared using water obtained from reverse osmosis with a specific resistivity ≥ 18 MΩ cm.

2.2 Experimental equipment and procedure for potentiometric measurements.

ISE-H^+ measurements were carried out at T = 25.0 ± 0.1°C using a 809 Metrohm *Titrando* apparatus equipped with a combined Orion Ross glass electrode (type 8102). The estimated accuracy was ± 0.20 mV and ± 0.02 mL for the e.m.f. and titrant volume readings, respectively. The apparatus was connected to a PC, and automatic titrations were performed using the Metrohm *TiAMO* 1.0

software to control titrant delivery and data acquisition and to check for e.m.f. stability. ISE-M^{2+} (M^{2+} = Cu^{2+} or Cd^{2+}) measurements were carried out using an apparatus that consisted of a Model 654 Metrohm potentiometer connected to a Model 765 Metrohm motorized burette. The following ISE-M^{2+} electrodes were used: a cadmium membrane electrode (type 9448SC) and a cupric membrane electrode (type 9429SC) coupled with a double junction reference electrode (Ross, type 900200), for ISE-Cd^{2+} and ISE-Cu^{2+} measurements, respectively. The estimated accuracy was ± 0.15 mV and ± 0.003 mL for the e.m.f. and titrant volume readings, respectively. The apparatus was connected to a PC, and automatic titrations were performed using a homemade computer program to control titrant delivery and data acquisition and to check for e.m.f. stability. Several series of potentiometric titrations were performed to investigate the behavior of PGA and pectin in aqueous solution. The first set of experiments included ISE-H^+ measurements, which were performed to determine both the acid-base properties of the biopolymers and the stability constants of the complex species formed between the two polyelectrolytes and copper(II) or cadmium(II) ions. To determine the ligand protonation constants, 25 - 40 mL of a solution containing the ligand under study ($0.5 \leq C_L \leq 4$ mmol L^{-1}) and the amount of NaCl needed to reach the desired ionic strength ($0 < I \leq 0.7$ mol L^{-1}) were titrated with a sodium hydroxide solution from pH 2 to 7. To study metal-ligand complex species formation, solutions were prepared by dissolving different amounts of ligands and cadmium(II) or copper(II) salts to obtain concentration ratios (L to M) that ranged from 1:1 to 3:1. To have the ligands in their fully protonated form, hydrochloric or nitric acid was added to the solutions.

A second set of potentiometric titrations was carried out with the ISE-M^{2+}, according to two different procedures. In the first procedure, 25 mL of a solution containing a known amount of Cu^{2+}or Cd^{2+} at 0.4 - 1.1 mmol L^{-1} were titrated with a sodium salt solution of the polyelectrolyte (PGA or PECT) at 2 - 6 mmol L^{-1} (*procedure 1*). In the second procedure, 25 mL of a solution containing known amounts of the ligand L (L = PGA or PECT) at 0.5 - 2 mmol L^{-1} were titrated with standard metal solutions (*procedure 2*). In both of these procedures, sodium nitrate was added to maintain the ionic strength at $I = 0.10$ mol L^{-1} and, at the same time, the pH of solution under investigation was monitored by using a combined ISE-H^+ glass electrode. By using procedures 1 and 2, we obtained experimental results for a wide range of M/PGA or M/PECT concentration ratios at pH ~ 5.

For each experiment, independent titrations of nitric or hydrochloric acid with NaOH standard solutions were performed using the same experimental conditions of temperature and ionic strength as for the systems under study, in order to determine the formal electrode potential E° of the combined ISE-H^+ glass electrode. For the experiments with the ISE-M^{2+}, an electrode calibration was performed by titrating a solution containing sodium nitrate at $I = 0.1$ mol L^{-1} with a standard cupric or cadmium nitrate solution. Pure nitrogen was bubbled through the solutions in the titration cells in order to displace O_2 and CO_2, and the solutions were stirred magnetically.

2.3 Calculations

The nonlinear least squares computer program ESAB2M [22] was used for refinement of all of the parameters of the acid-base titration [E_0, K_w, liquid junction potential coefficient (j_a) and analytical concentration of reagents]. The BSTAC and STACO computer programs [23] were used in the calculation of the protonation and complex formation constants. Both programs can accommodate measurements at different ionic strengths. The ES4ECI [24] program was used to draw speciation diagrams and to calculate the percentages of species formation. The LIANA [25] program was used to fit the different linear and nonlinear functions.

The protonation constants of the polyelectrolytes, the hydrolysis constants of the metal ions, and the complex formation constants for the M^{2+}-L species ($M^{2+} = Cu^{2+}$ or Cd^{2+} ions and L = PGA or PECT) are given according to the equilibria of eqs. (1), (2) and (3), respectively:

$$H_{i-1}L^{z+i-1} + H^+ = H_iL^{z+i} \qquad \beta_i^H \qquad (1)$$

$$pM^{2+} + qH_2O = M_p(OH)_q^{(2p-q)} + qH^+ \qquad \beta_{pq}^{\ *} \qquad (2)$$

$$iM^{2+} + L^{z-} + jH^+ = M_iLH_j^{(2i-z+j)} \qquad \beta_{i1j} \qquad (3)$$

with z = effective charge of the polyelectrolyte.

The dependence of the protonation constants on ionic strength was taken into account by the following Debye–Hückel type equation:

$$\log K = \log {}^TK - z^* \, 0.51\, I^{1/2}(1 + 1.5\, I^{1/2})^{-1} + C\, I \qquad (4)$$

where K is the formation constant and ^TK is the relative value at infinite dilution. *C* is an empirical parameter for the dependence on *I* and z* is given by

$$z^* = \Sigma\, (\text{charges})^2_{\text{reactants}} - \Sigma\, (\text{charges})^2_{\text{products}} \qquad (4a)$$

The protonation and formation constants, reagent concentrations, and ionic strengths are expressed in terms of molar (mol L^{-1}) concentrations.

3. RESULTS AND DISCUSSION

3.1 Protonation of polyelectrolytes and hydrolysis of metal ions

To define the speciation model for the metal-ligand systems considered here, the protonation constants of ligands and the hydrolysis of metal ions must be determined. The protonation constants of polygalacturonic acid and pectin were calculated from the potentiometric data by using the "Diprotic-Like Model" (DLM) [19,20], which was previously proposed to explain the acid-base properties of a variety of synthetic and natural polyelectrolytes [20,21]. According to this model, only two protonation constants (log K_1^H and log K_2^H) are necessary to define the protonation of a polycarboxylate polyelectrolyte, which reduces the difficulty in data processing with a negligible loss of accuracy compared with both the classical Henderson Hasselbalch equation, based on the Katchalsky theory [26, 27], and with the three parameter equation proposed by Högfeldt et al. [28].

In Table 1, the protonation constants of PGA and PECT are reported in NaCl medium at different ionic strengths.

Table 1. Protonation constants, calculated according to the "Diprotic-Like Model", of PGA and PECT, in NaCl ionic medium at different ionic strengths and at T = 25°C.

I / mol L^{-1}	log K_1^{H}	log β_2^{H}
	PGA	
0.100	3.975 ± 0.009 a)	6.78 ± 0.02 a)
0.238	3.643 ± 0.003	6.561 ± 0.005
0.475	3.472 ± 0.002	6.205 ± 0.005
0.709	3.520 ± 0.003	6.377 ± 0.005
	PECT	
0.104	3.774 ± 0.003 a)	6.94 ± 0.01 a)
0.236	3.54 ± 0.03	6.45 ± 0.02
0.480	3.279 ± 0.006	5.927 ± 0.007
0.713	3.117 ± 0.006	5.319 ± 0.007

a) ± std. dev.

As expected, the data reported in Table 1 show a very similar proton exchange capacity of PECT and PGA, as seen by a comparison of the values of $\log K_1^{H}$, and $\log \beta_2^{H}$ of the two polyelectrolytes. The dependence of the protonation constants on the ionic strength was evaluated by using the extended Debye-Hückel type equation [eq. (4)]. Using the same equation, the value of the effective charge of the polyelectrolytes was calculated. The values of thermodynamic constants, of the parameter *C* from eq. (4), and of the effective charge *z* of the polyelectrolytes are reported in Table 2.

Table 2. Protonation constants of PGA and PECT at infinite dilution, together with the parameter *C* from eq. (4) for the dependence on ionic strength, in NaCl at T = 25°C.

Polyelectrolyte	-z a)	log ${}^{T}K_1^{H}$	C	σ b)	log ${}^{T}\beta_2^{H}$	C	σ b)
PGA	3.5	4.64	0.23	0.06	8.01	0.87	0.08
Pectin	3.5	4.58	-0.18	0.006	8.41	-1.06	0.08

a) effective charge of the polyelectrolyte; b) std dev. of the fit.

The hydrolysis of cadmium(II), which according to Baes and Mesmer [29] occurs at pH > 7, can be neglected in the pH range investigated in our measurements. On the contrary, the hydrolysis of copper(II), which starts at pH ≈ 5.5 [29], was considered in the speciation models. For the copper(II) cation, the formation of the hydrolytic species $Cu(OH)^{+}$ and $Cu_2(OH)_2^{2+}$ was accounted for by using the following hydrolysis constants: log β^*_{11} = -7.7 and log β^*_{22} = -10.77, at I = 0.1 mol L^{-1} ($NaClO_4$) and T = 25 °C [30].

3.2. Copper(II) and cadmium(II)-biopolymer complex species

Table 3 reports the formation constants for the complex species that are formed by PGA and PECT with copper(II) or cadmium(II) at I = 0.1 mol L^{-1} and T = 25°C, as obtained by ISE-H^+ and ISE-M^{2+} potentiometric measurements.

The experimental data obtained from the ISE-H^+ measurements, carried out over the pH range from 2 to 7, gave evidence for the formation of ML and MLH species in all of the systems that were investigated (M = Cu^{2+}, Cd^{2+}; L = PECT, PGA). The formation of MLH species is in accordance with the diprotic-like model that was used here to describe the acid-base properties of pectin and polygalacturonic acid. The formation constants of the ML species are in excellent agreement with those obtained from ISE-M^{2+} experiments at pH ≈ 5 according to the two different procedures, which were discussed in the *Experimental equipment and procedure* section (paragraph 2) .

Table 3. Formation constants of Cu^{2+} and Cd^{2+}-PGA and -PECT complex species, in $NaNO_3$ ionic medium at I = 0.1 mol L^{-1} and at T = 25°C

Ligand	ISE-M^{2+} measurements	ISE-H^+ measurements	
	log β_{110} [a)b)]	log β_{110} [a)b)]	log β_{111} [a)b)]
Cu^{2+} complexes			
PGA	4.863 ± 0.001 [c)]	4.72 ± 0.05	7.63 ± 0.04
	4.78 ± 0.01[d)]		
PECT	5.240 ± 0.001 [c)]	5.31 ± 0.02	8.66 ± 0.01
	5.278 ± 0.003 [d)]		
Cd^{2+} complexes			
PGA	3.660 ± 0.001 [c)]	3.68 ± 0.01	7.77 ± 0.01
	3.668 ± 0.001 [d)]		
PECT	3.636 ± 0.001 [c)]	3.35 ± 0.02	7.547 ± 0.003
	3.382 ± 0.001 [d)]		

[a)] log β_{i1j} refers to the reaction of eq. (3); [b)] ± std. dev; [c)] values obtained using the polyelectrolyte solution as a titrant for the metal ion solution; [d)] values obtained using the metal ion solution as a titrant for the polyelectrolyte solution.

As seen from the data reported in Table 3, the log β_{110} values that were calculated with ISE-H^+ and ISE-M^{2+} measurements are very similar and confirm the validity of the methodology used and the accuracy of the results obtained using each technique. As expected, the stability of the copper complex species is higher than that of the corresponding cadmium species, nearly one or two log units in terms of log K. A similar difference was noted in a previous investigation of the formation of complex species between copper(II) or cadmium(II) and polyacrylates or polymethacrylates of different molecular weight [18], and an analogous trend in stability was also shown for complex species of Cu^{2+}

and Cd^{2+} with low molecular weight carboxylic ligands [31,32]. The formation constants of the complex species that are reported in Table 3 were used to draw the speciation profiles (Figures 2-5) for all the systems investigated in the $2 \leq pH \leq 7$ range. In particular, the values of the formation constants of the ML species that were used in the calculations are the mean values obtained from the ISE-H^+ and ISE-M^{2+} measurements.

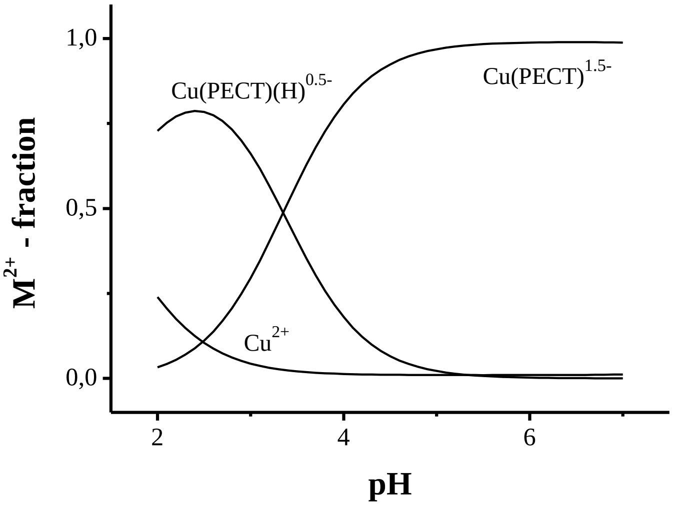

Figure 2. Speciation diagram for the Cu^{2+}-$PECT^{3.5-}$ system at I = 0.1 mol L^{-1} and T = 25°C. Experimental conditions: C_{Cu} = 0.5 mmol L^{-1}; C_{PECT} = 1 mmol L^{-1}.

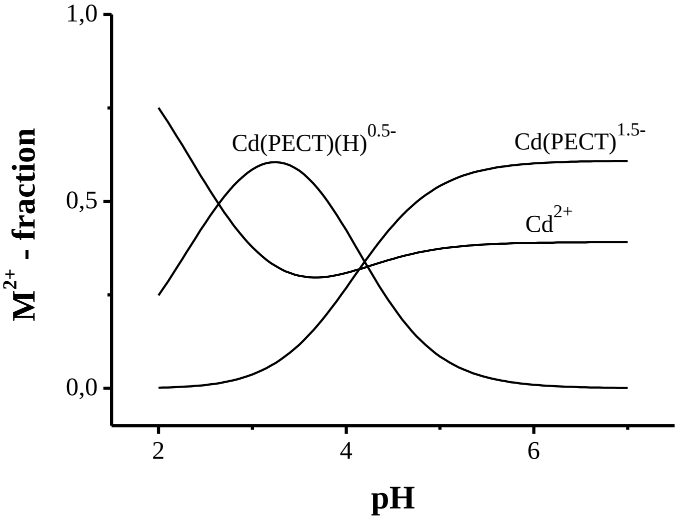

Figure 3. Speciation diagram for the Cd^{2+} - $PECT^{3.5-}$ system at I = 0.1 mol L^{-1} and T = 25°C. Experimental conditions: C_{Cd} = 0.5 mmol L^{-1}; C_{PECT} = 1 mmol L^{-1}.

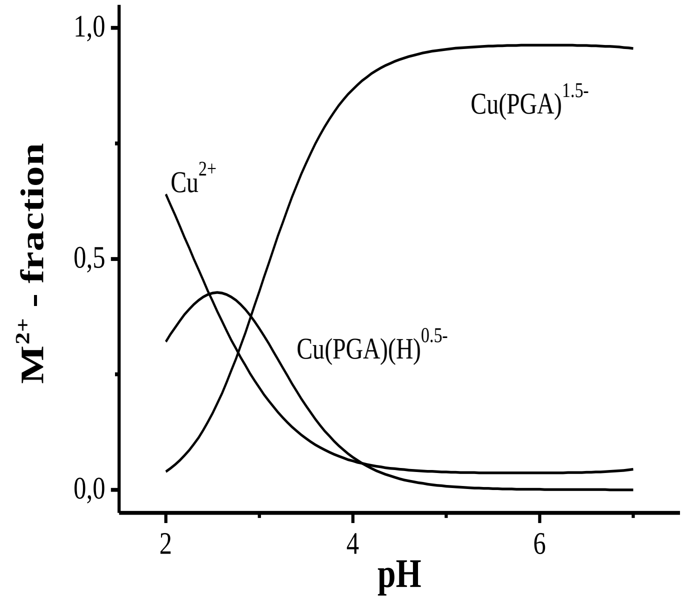

Figure 4. Speciation diagram for the Cu^{2+} - $PGA^{3.5-}$ system at I = 0.1 mol L^{-1} and T = 25°C. Experimental conditions: C_{Cu} = 0.5 mmol L^{-1}; C_{PGA} = 1 mmol L^{-1}.

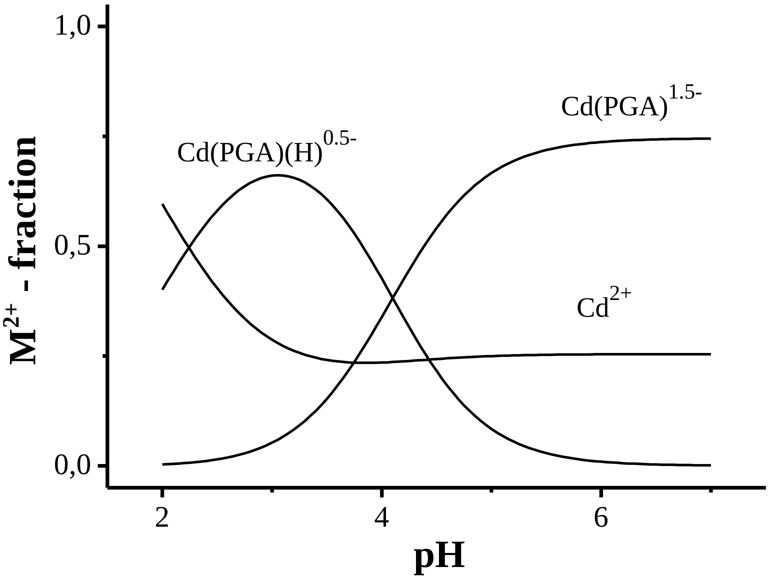

Figure 5. Speciation diagram for the Cd^{2+} - $PGA^{3.5-}$ system at I = 0.1 mol L^{-1} and T = 25°C. Experimental conditions: C_{Cd} = 0.5 mmol L^{-1}; C_{PGA} = 1 mmol L^{-1}.

The speciation diagrams reported in Figures 2-5 show the coexistence of mono-protonated MLH and un-protonated ML species at pH < 4 in all the systems. At 4 < pH < 7, the un-protonated species predominate.

The fraction of metal containing complex species formed in the systems investigated here is, in general, quite high. In particular the fractions of the MLH and ML species range from 0.5 to 0.75 and from 0.5 to 1, respectively.

In accordance with the stability data that were obtained for the complex species formed in the PECT-Cu/Cd and PGA-Cu/Cd systems, a negligible fraction of free copper(II) ion is present, while a considerable fraction of free cadmium(II) ion can be observed at all pH values investigated. As expected, no hydrolytic species of cadmium(II) or copper(II) ions are formed in this pH range.

3.3. Comparison with analogous systems

Very few papers in the literature provide quantitative stability data for the formation of complex species of copper(II) and cadmium(II) with the polymer ligands considered here. Using several polarographic techniques, Garrigosa et al. [33] obtained the following Cd-PGA complex formation constants in 0.01 mol L^{-1} KNO_3 at pH = 6: log K = 4.38 (by Reverse Pulse Polarography, RPP), log K = 4.35 (by Alternating Current Polarography, ACP), and log K = 4.18 (by Differential Pulse Polarography, DPP), yielding an average value of 4.30.

Using ISE-Cu^{2+} potentiometric and voltammetric (differential pulse polarography and square-wave voltammetry) measurements, Vilhena C. et al. [34] calculated a value of log $\beta_{CuL} = 3.5$ for the formation of Cu-PECT complex species. The authors also report the formation of an ML_2 species with log $\beta_{CuL_2} = 8.0$, which was obtained at high L:M concentration ratio. Despite the use of different chemical models, these formation constants were calculated to be at the same order of magnitude in the literature and in this study, which used the diprotic-like model.

Data comparisons can also be made by considering the interaction of Cd^{2+} and Cu^{2+} ions with alginic acid (AA), which, like pectin, is an acidic polysaccharide. Alginic acid consists of monomer units of mannuronic and guluronic acids, which are very similar in structure to galacturonic acid, the main component of natural pectins. The only quantitative data on the protonation of alginate and the stability of species in the Cu/Cd-alginate system were reported previously by our laboratories [35,18] and are shown in Table 4. It can be seen that the protonation constants of AA are comparable to those of PGA and PECT, which were reported in Table 1. For example, at $I = 0.1$ mol L^{-1}, log $\beta_2^H = 6.78$, 6.94 and 6.42 for PGA, PECT and AA, respectively. This observation confirms the similar acid-base behavior of the three polymers.

A comparison of the formation constants of the ML species in the previously reported alginate-Cu/Cd system [18] (see Table 4) and the PECT-Cu/Cd and PGA-Cu/Cd systems shows a slightly lower stability for the species that were formed in the alginate system, according to the trend PECT > PGA > AA, for both copper(II) and cadmium(II) complexes. Similarly, a decrease in the stability of metal-alginate species compared to complexes with other synthetic and naturally occurring polycarboxylate ligands has been registered in previous studies [15-18], which investigated the interactions of metal and organometallic cations with fulvic, polyacrylic and alginic acids.

Table 4. Literature data for the alginic acid protonation [20] and Cu^{2+} and Cd^{2+} alginate complex species [18], at I = 0.1 mol L^{-1} and at T = 25°C.

Protonation equilibria			
Medium	I/mol L^{-1}	Log K_1^H	Log β_2^H
NaCl	0.10	3.499	6.421
	0.25	3.247	6.008
	0.50	3.135	5.896
	0.75	3.060	5.743
Complexation equilibria			
		Log β_{110}	
$NaNO_3$	0.10	ISE-M^{2+} measurements	ISE-H^+ measurements
		Cu^{2+}-complexes	
		3.626	3.586
		Cd^{2+}-complexes	
		3.072	3.027

[a)] log β_i^H refers to the reaction of eq. (1); [b)] log β_{i1j} refers to the reaction of eq. (3)

3.4. Ability of biopolymers to sequester cadmium(II) and copper(II) ions

The stability constants of the complex species that were formed in the systems investigated here allow us to quantitatively define the ability of pectin and polygalacturonic acid to sequester cadmium(II) and copper(II) ions. This information is useful when planning the removal of toxic metal ions from contaminated waters, either by using these biopolymers directly in solution or in a gel phase.

The sequestering power of a ligand towards a metal ion can be evaluated by determining the total fraction of metal that is complexed (x) as a function of total ligand concentration, expressed as pL (pL = -log $[L]_{tot}$). Because this function is typically a sigmoidal curve, which rapidly increases over a relatively small change in concentration, we can use a Boltzmann-type equation with asymptotes of 0 for pL→ ∞ and 1 for pL→ 0:

$$x = \frac{1}{1+10^{(pL-pL_{0.5})}} \tag{6}$$

where $pL_{0.5}$ is an empirical parameter numerically corresponding to the ligand concentration that is required to sequester one-half of the metal ions, *i.e.* to reach x =0.5 [36]. This parameter and its analogous pL_{50}, (i.e., the ligand concentration needed for the sequestration of 50% of the metal ions) previously have been tested successfully to define the sequestering capacity of different ligands towards metal ions and organometallic cations [15-18,36-39]. By using this equation, the sequestering capacities of poly-galacturonic acid and pectin were calculated for both of the metal ions over the pH range investigated here, in $NaNO_3$ ionic medium at I = 0.1 mol L^{-1} and at T = 25°C. In all of the calculations, we assumed that the concentration of metal ions was equal to 10^{-12} mol L^{-1}. The parameters of Boltzmann equation are reported in Table 5. In the same table we also report, for comparison, the sequestering parameters that were previously obtained for alginate-Cu/Cd systems [18] under the same experimental conditions.

Table 5. Parameters of the Boltzmann equation for solutions containing trace metal (10^{-12} mol L^{-1}) concentrations of Cd^{2+} or Cu^{2+} ions, at different pH values, in $NaNO_3$ ionic medium at *I* = 0.1 mol L^{-1} and at T = 25°C

pH	Metal ion	$pL_{0.5}$		
	Copper(II)	PECT	PGA	AA
3		4.63	3.76	2.79
4		5.17	4.45	3.50
5		5.29	4.68	3.61
6		5.30	4.71	3.62
7		5.23	4.64	3.54
	Cadmium(II)			
3		3.38	3.59	2.24
4		3.53	3.73	2.94
5		3.39	3.69	3.06
6		3.35	3.68	3.07
7		3.35	3.68	3.07

The data reported in Table 5 show that the ability of alginate to sequester Cu^{2+} or Cd^{2+} ions is always lower than that of PGA and pectin, over the entire pH range investigated; this is a result of the differences in the stability of the metal complex species formed with the three poly-electrolytes. Figures 6 and 7 show, as examples, the sequestration curves of PECT, PGA and AA towards the two divalent metal ions at pH = 6. At this pH, the sequestering capacity of the three polyelectrolytes for Cu^{2+} ions follows the trend AA < PGA < PECT, whilst for Cd^{2+} the trend is AA < PECT < PGA.

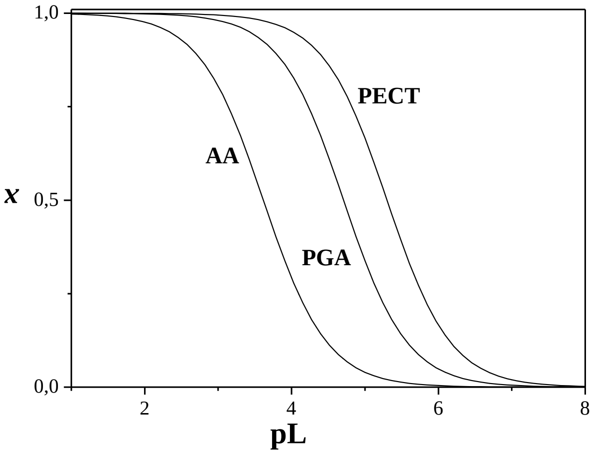

Figure 6. Total fraction of Cu^{2+} ions complexed (*x*) by L *vs.* pL [L = AA, PGA, PECT]. Experimental conditions: $C_{Cu^{2+}} = 10^{-12}$ mol·L^{-1}, pH = 6, *I* ($NaNO_3$) = 0.1 mol L^{-1} and T = 25°C.

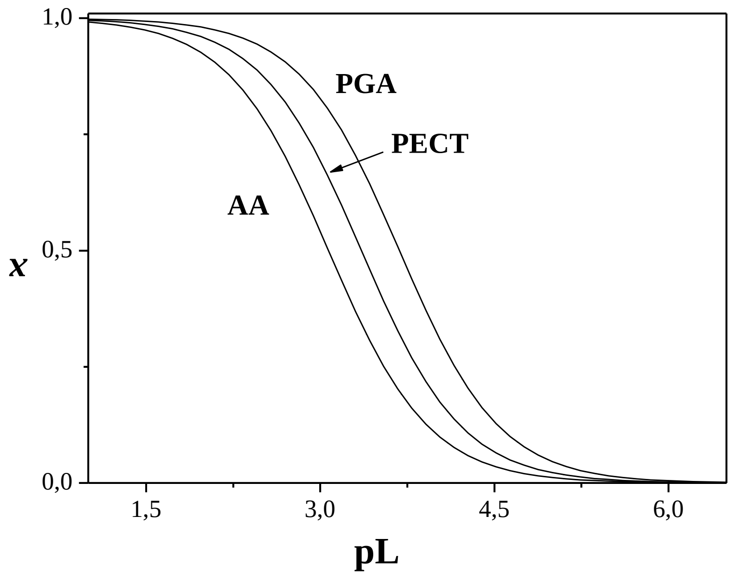

Figure 7. Total fraction of Cd^{2+} ions complexed (x) by L *vs.* pL [L = AA, PGA, PECT]. Experimental conditions: $C_{Cd^{2+}} = 10^{-12}$ mol L^{-1}, pH = 6, I ($NaNO_3$) = 0.1 mol L^{-1} and T = 25°C.

The dependence of $pL_{0.5}$ on pH for each of the three ligands is shown in Figures 8 and 9. It can be seen that the trends in the sequestering capacity for both copper(II) and cadmium(II) ions were confirmed over the entire pH range investigated.

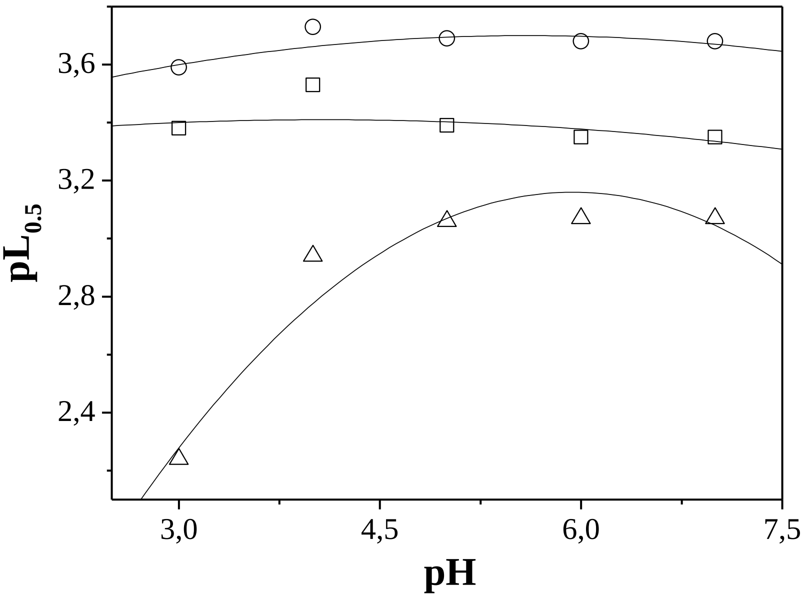

Figure 8. Dependence on pH of $pL_{0.5}$ of PECT (□), PGA (○), and AA (△) toward cadmium(II). Experimental conditions: $NaNO_3$ ionic medium, I = 0.1 mol L^{-1} and T = 25°C.

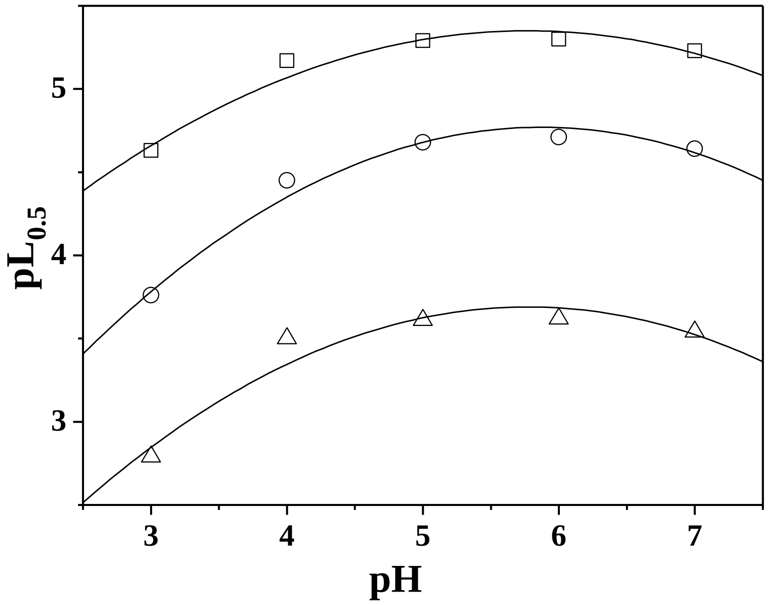

Figure 9. Dependence on pH of $pL_{0.5}$ of PECT (□), PGA (○), and AA (△) toward copper(II). Experimental conditions: $NaNO_3$ ionic medium, *I* = 0.1 mol L^{-1} and T = 25°C.

The empirical relationship described by eq. (7) was used to study the dependence of $pL_{0.5}$ on pH.

$$pL_{0.5} = p_1 + p_2\,(pH - p_3)^2 \tag{7}$$

where p_1, p_2 and p_3 are empirical parameters whose values, together with the standard deviation of the fits, are reported in Table 6.

Table 6. Parameters of eq. (7) for the dependence of $pL_{0.5}$ on pH in $NaNO_3$ ionic medium at *I* = 0.1 mol L^{-1} and at T = 25°C.

Ligand	p_1	p_2	p_3	σ of fit
		Copper(II)		
PECT	5.35	-0.09	5.78	0.05
PGA	4.77	-0.12	5.87	0.05
AA	3.69	-0.11	5.77	0.07
		Cadmium(II)		
PECT	3.41	-0.01	4.07	0.05
PGA	3.71	-0.15	5.59	0.03
AA	3.16	-0.10	5.94	0.08

For the cadmium(II)-L (L = PGA, PECT) systems, the sequestering capacity ($pL_{0.5}$) is almost independent of the pH in the experimental range investigated. For the cadmium(II)-AA system, more marked changes can be observed, particularly an increase in $pL_{0.5}$ from 2.29 (at pH = 3) to 3.06 (at pH = 7). For the copper(II)-L (L = PGA, PECT, AA) systems, the sequestering capacities of the three ligands show a relevant dependence on pH in the pH range 3-5, and, for all the ligands, the $pL_{0.5}$ values decrease slightly at pH > 5. This decrease can be attributed to the formation of non-negligible amounts of metal hydrolytic species at pH > 5.

4. FINAL REMARKS

– The acid-base properties of poly-galacturonic acid and pectin were reported here for the first time. Protonation constants were calculated at different ionic strengths and the values at $I \rightarrow 0$ mol L^{-1} were extrapolated by using a Debye–Hückel type equation.

– The abilities of pectin and polygalacturonic acid to sequester cadmium(II) and copper(II) were defined using the stability parameters of the complex species that are formed in these systems.

– The most suitable pH conditions for sequestration of metal ions were identified using speciation studies involving the protonation of the ligands, the hydrolysis of the metal ions, and the metal-ligand interactions**.**

– The observed difference in the stability of the complexes (copper > cadmium) was as expected in accordance with information from low molecular weight carboxylic ligands.

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References

1. D. Mohnen, *Pectins and their Manipulation*, Blackwell Publishing and CRC Press, Oxford (2002)
2. H.V. Scheller, J.K. Jensen, S.O. Sørensen, J. Harholt, and N. Geshi, *Plant Physiol* 129 (2007) 283
3. D. Mohnen, R. Lou Doong, K. Liljebjelke, G. Fralish, and J. Chan, *Progress in Biotechnology*, Elsevier, Amsterdam (1996)
4. C.D May, *Carbohyd. Polym.*, 12 (1990), 79-99.
5. D.A. Rees and A.W. Wright, *J. Chem. Soc.*, Section B 2 (1971) 1366
6. B.L. Ridley, M.A. O'Neill and D. Mohnen, *Phytochem.*, 57 (2001) 929
7. Z.K. Mukhiddinov, D.K. Khalikov, F.T. Abdusamiev and C.C. Avloev *Talanta*, 53 (2000), 171
8. J.A. DeVries, M. Hansen, J. Søderberg, P.E. Glahn and J.K. Pedersen, *Carbohyd. Polym.*, 6 (1986) 165
9. I.L. Novosel'skaya, N. L. Voropaeva, L. N. Semenova and S. Sh. Rashidova, *Chem. Nat. Compd.*, 36 (2000) 1
10. B.R. Thakur, R.K. Singh and A. K. Handa, *Crit. Rev. Food Sci.*, *37* (1997) 47
11. C. Rolin, *Industrial gums*, , Academic Press, New York (1993)
12. P. Sriamornsak, and J. Nunthanid, *Int. J. Pharm.*, 160 (1998) 207
13. A. Ashford, *J. Control. Release*, 26 (1993) 213
14. P. Liu, and T.R. Krishnan, *J Pharm. Pharmacol.*, 51 (1999) 141
15. A. Giacalone, A. Gianguzza, A. Pettignano and S. Sammartano, *Appl. Organomet. Chem.*, 20 (2006) 706

16. C. De Stefano, A. Gianguzza, A. Pettignano, S. Sammartano and S. Sciarrino, *Chem. Spec. Bioavailab.*, 19 (2007) 131
17. C. De Stefano, A. Gianguzza, A. Pettignano, D. Piazzese and S. Sammartano, *J. Radioanal. Nucl. Chem.* 289 (2011) 689
18. C. De Stefano, A. Gianguzza, A. Pettignano, S. Sammartano and S. Sciarrino, *J. Chem. Eng. Data*, 55 (2010) 714
19. R.M. Cigala, F.Crea and S. Sammartano, *J. Mol. Liq.*, 143 (2008) 129
20. F. Crea, C. De Stefano, A. Gianguzza, A. Pettignano, D. Piazzese and S. Sammartano, *J Chem Eng Data*, 54 (2009) 589
21. S. Cataldo, F. Crea, A. Gianguzza, A. Pettignano and D. Piazzese, *J. Mol. Liq.*, 148 (2009) 120
22. C. De Stefano, P. Princi, C. Rigano, and S. Sammartano, *Ann. Chim. (Rome)*, 77 (1987) 643
23. C. De Stefano, P. Mineo, C. Rigano and S. Sammartano, *Ann. Chim. (Rome)*, 83 (1993) 243
24. C. De Stefano, C. Foti, O. Giuffré, P. Mineo, C. Rigano and S. Sammartano, *Ann. Chim. (Rome)* 86 (1996) 257
25. C. De Stefano, S. Sammartano, P. Mineo and C. Rigano, *Marine Chemistry - An Environmental Analytical Chemistry Approach*, Kluwer Academic Publishers: Amsterdam, (1997)
26. A. Katchalsky and P. Spitnik, *J. Polym. Sci.*, 2(1947) 432
27. A. Katchalsky, *J. Polym. Sci.*, 2 (1954) 159
28. E. Högfeldt, T. Miyajima, J.A. Marinsky and M. Muhammed, *Acta Chem. Scand.*, 43 (1989) 496
29. C.F. Baes and R.E. Mesmer, *The Hydrolysis of Cations*; John Wyley & Sons New York, (1976)
30. A. De Robertis, C. De Stefano, C. Foti and G. Signorino, *Talanta*, 44 (1997) 1839
31. A.E. Martell, R.M. Smith and R.J. Motekaitis, *NIST Standard Reference Database 46, vers.8* Gaithersburg, (2004)
32. D. Pettit and K. Powell, *IUPAC Stability Constants Database*; Academic Software: Otley, UK, (1997)
33. A.M. Garrigosa, R. Gusmão, C. Arino, J.M. Diaz-Cruz and M. Esteban, *Talanta* 73 (2007) 776
34. C. Vilhena, M.L. Gonçalves and A.M. Mota, *Electroanal.*, 16, (2004) 2065
35. C. De Stefano, A. Gianguzza, D. Piazzese and S. Sammartano *Anal. Bioanal. Chem.*, 383 (2005) 587
36. A. Gianguzza, O. Giuffré, D. Piazzese and S. Sammartano, *Coord. Chem. Rev.*, 256 (2012) 222
37. C. De Stefano, A. Gianguzza, D. Milea, A. Pettignano, and S. Sammartano, *J. Alloys Compd.*, 424 (2006) 93
38. F. Crea, C. De Stefano, D. Milea and S. Sammartano, *J. Sol. Chem.*, 38 (2009) 115
39. A. Gianguzza, D. Milea, A. Pettignano and S. Sammartano, *Environ. Chem.*, 7 (2010) 259