



## Theoretical and experimental study of $M^{2+}$ adsorption on biopolymers. III. Comparative kinetic pattern of Pb, Hg and Cd

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### Abstract

Chitosan, pectin and chitosan–pectin pellets were studied as adsorbents for  $Cd^{2+}$  from acidic aqueous solutions at selected conditions. Several adsorption sites were considered and adsorption mechanisms for the biopolymers–metal interactions are proposed taking into account theoretical and experimental results. Langmuir adsorption isotherms for Hg, Pb and Cd on pellets gave  $K_L$  values of 796, 1551 and 7265 L mequiv.<sup>-1</sup>; their  $Me_{max}$  (mequiv./g) being 0.027 (2.71 mg/g), 0.1075 (11.2 mg/g) and 0.022 (1.23 mg/g), respectively. Chitosan adsorption of Hg achieved 85–100% and 70–75% for Pb and Cd, respectively.  $C_i$  (initial concentration) was 47,045.4  $\mu\text{g/g}$  chitosan for Pb, 6863.6  $\mu\text{g/g}$  chitosan for Hg and 5.4436  $\mu\text{g/g}$  chitosan for Cd. When pectin was the adsorbent 15% of Hg and not more than 4% Pb and Cd were adsorbed,  $C_i$  Pb being 9409.1  $\mu\text{g/g}$  pectin  $C_i$  Hg = 1372.7  $\mu\text{g/g}$  pectin and  $C_i$  Cd = 1064.5  $\mu\text{g/g}$  pectin. In the case of pellets, 100% initial Hg, Cd and Pb present initially in solution were adsorbed,  $C_i$  Pb being = 103.5  $\mu\text{g/g}$ ,  $C_i$  Hg = 15  $\mu\text{g/g}$  and  $C_i$  Cd = 12  $\mu\text{g/g}$ .

Molecular mechanics (MM2) and PM3 calculations were done, trying to identify evidence of the proposed kinetic model and an explanation of the equilibrium uptake of the metal adsorption on chitosan–pectin pellets.

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### 1. Introduction

Biosorbents are a very interesting alternative to remove transition and heavy metals from wastewater (Dronnet, Renard, Axelos, & Thibault, 1996; Dronnet, Renard, Axelos, & Thibault, 1997; Hattis & Murray, 1976; Joensen & Villadsen, 1994a,b; Periasamy & Namasivayam, 1994; Roberts, 1992a,b). Chitin, chitosan and pectin will bind several metal ions (Eiden, Jewell, & Wightman, 1980; Maruca, Suder, & Wightman, 1982). Chitosan is more effective than chitin as a scavenger in several cases (Periasamy & Namasivayam, 1994). Many authors believe the presence of  $NH_2$  groups (and  $NH_3^+$ ) is the key to understand both biopolymers adsorption capabilities. Chitosan has a higher adsorption rate of different metals than chitin at acidic pH in aqueous solutions (Dambies, Guimon, Yiacoumi, & Guibal, 2000; Filipovicgrcic, Maysinger, Zor,

& Jalsenjak, 1995; Guibal, Dambies, Milot, & Roussy, 1999a; Guibal, Larkin, Vincent, & Tobin, 1999b; Guibal, Saucedo, Jansson-Charrier, Delanghe, & Le Cloirec; Huang, Chung, & Liou, 1996).

Hg, Pb and Cd are the three of the most contaminating heavy metals. Chitosan with different acetylation and substitution grades has been studied as a heavy metals remover from waste waters (Arguelles-Monal & Peniche-Covas, 1993; Guibal, Milot, & Tobin, 1998; Juang & Ju, 1997; Kawamura, Yoshida, Asai, & Tanibe, 1998; McKay, Blair, & Findon, 1989; Onsoyen & Skaugrud, 1990; Peniche, Alvarez, & Arguelles-Monal, 1992; Saucedo, Guibal, Roussy, & Le Cloriec, 1993; Thomas, Todhar, & Phillip, 1997). There are several reports in recent open literature on pectin as a sorbent for these heavy metals (Dronnet et al., 1996, 1997; Walter, 1991). Available data have proved chitosan is better for  $Hg^{2+}$ , and pectin for  $Pb^{2+}$  adsorption (Dronnet, Renard, Axelos, & Thibault, 1998b; Kertesz, 1951; Paskins-Hurlbut, 1977; Stanstchev, Kratschanov, Popova, Kirstchev, & Mareschev, 1979).

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Additives are required for activated carbon to remove inorganic or high molecular weight organic matter from water. Ion-exchange-resins are effective, but very expensive. Using a natural product obtained from crab shells waste seems a very interesting option. Also, pectin is a low cost adsorbent, especially when obtained 'crude'. Metals removal at the level here presented has been very difficult to achieve by other methods.

Recent excellent review from Guibal (Guibal, 2004) summarizes the literature on chitosan and environmental applications, especially on metal ions. The author concluded that the sorption capacities of chitosan can be of great use for the recovery of valuable metals or the treatment of contaminated effluents. The loading of the polymer matrix with metal can give the support interesting complementary properties for the sorption of other organic and inorganic materials for catalytic applications and electronic and optical devices. Other review with careful analysis of experimental uptake of metal ions by chitosan and derivatives has been published by Kennedy et al. (Varma, Deshpande, & Kennedy, 2004). One of the best-characterized chitosan derivatives are the alginate-chitosan beads for adsorption of metal divalent ions (Gotoh, Matsushima, & Kikuchi, 2004b). Besides, cellulose/chitosan blend beads have been used for sorption of metal ions (Twu, Huan, Chang, & Wang, 2003). Selectivity issues have been addressed by Smidsrod et al. (Vold, Varum, & Guibal, 2003). In the case of  $\text{Hg}^{2+}$ , chemical modification of chitosan has demonstrated to have positive effects on uptake (Jeon & Holl, 2003). Low cost adsorbents for heavy metals uptake are highly desired to optimize decontamination processes (Babel & Kurniawan, 2003). Sorption onto chitosan has been studied with detail recently, especially for  $\text{Cu}^{2+}$  (Ng, Cheung, & McKay, 2002) and  $\text{Cr}^{6+}$  (Sag & Aktay, 2002). Diffusion of metal ions through chitosan gel membranes has been addressed recently (Krajewska, 2001).  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  adsorption have been studied carefully on chitosan derivatives by Strasdeit et al. (Becker, Schlaak, & Strasdeit, 2000). Mn has also been recently analyzed (Gotoh, Matsushima, & Kikuchi, 2004a).

Application of pectin or pectin-containing substances as efficient adsorbents of metal ions was suggested (Aizenberg, Trachtenberg, Sedina, et al., 1995; Trachtenberg, 1992). Adsorption of  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  was studied on apple pectin (AP), beet-pectin (BP) and citrus pectin (CP). AP exhibits the highest affinity for  $\text{Co}^{2+}$ , BP for  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$ , whereas CP shows a distinct preference for  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Pb}^{2+}$  (Kartel, Kupchik, & Veisov, 2000). The selectivity sequence for pectins was  $\text{Pb}^{2+} \gg \text{Cu}^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} \gg \text{Zn}^{2+} > \text{Cd}^{2+}$ . Adsorption of  $\text{Cd}^{2+}$  by gel beads of sugar-beet pectin has been recently published (Harel, Mignot, Sauvage, & Junter, 1998). Using a concentration of 2% (w/v) of Ca citrus pectin, the removal achieves 7.53 mg/g dry gel, whereas with Ca sugar-beet pectin and 3% (w/v) only 4.5 mg/g dry gel are removed. The extraction mechanism is mainly based on ion exchange

between the solution  $\text{Cd}^{2+}$  and the polymer (chelated  $\text{Ca}^{2+}$ ). Moreover, some cations are extracted by simple diffusion through the polymer structure.

In previously published papers (parts I and II of the series (Ferreira & Gschaider, 2001; Zalba, Debbaudt, Ferreira, & Gschaider, 2001)), we have performed theoretical and experimental studies of adsorption of Hg and Pb by chitosan, pectin and chitosan-pectin pellets. Using the Extended Hückel Method, PM3 (Parameterized Model 3) and MM2 (Molecular Mechanics, version 2) we studied  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  adsorption by intra- and intermolecular chelation of these metals on pectin and chitosan models (as di- or oligosaccharides). Besides, experimental data (kinetic and equilibrium uptake data) were analyzed and discussed.

This third part presents adsorption results for Cd and compares them with those obtained for Hg and Pb, especially in pellets. Pellets have been the most efficient adsorbent. An X-ray analyzer coupled with a scan electron microscopy analyzer (EDAX-SEM) was used to study chitosan-pectin pellets, their structure and pectin and chitosan distribution. One important issue to address is the way chitosan is distributed in pellets, located inside and at the pellet surface. (Are we in the presence of a separate phase or of a new real mixed compound?) The present work also presents adsorption isotherms at 20 °C for the three metals on pellets and a comparative analysis of Langmuir and Freundlich adjustment.

The present model describes a situation in which metal-adsorbent local interactions are taking place. The way the cation achieves its position near the sites is not modeled. The possibility of a second cation adsorption next to a previously adsorbed one has not been considered. There would be no simple model including all the effects involved in adsorption kinetics and equilibrium (e.g. biopolymer particle size, external and intraparticle diffusion, possible pore-blockage mechanism, cooperative effects and others).

## 2. Theoretical studies

### 2.1. Modified MM2

A modified MM2 (Cambridge Soft-Chem 3D 5.0) was used in this study to compute oligosaccharide conformations steric energies and changes introduced by heavy metal presence. The modifications of the original MM2 include:

- a charge dipole interaction term;
- a quartic stretching term.

The definition of the quartic stretching term is related to Eq. (1)

$$E_{\text{stretch}} = 71.94 \sum K_s [(r - r_0)^2 + CS(r - r_0)^3 + Q_s(r - r_0)^4] \quad (1)$$

where  $K_s$  controls the stiffness of the string's stretching (bond stretching force constants) and  $r_0$  defines the equilibrium length of a specific bond (the standard measurement used in building models). Unique  $K_2$  and  $r_0$  parameters are assigned to each pair of bonded atoms based on their atom type (C–C, C–H, O–C). The constant 71.94 is a conversion factor to obtain final units as kcal/mole. CS is the cubic stretch term allowing for an asymmetric shape of a potential well, thereby allowing long bonds to be handled. However, this cubic stretch term is not enough to handle abnormally long bonds. Thus, a quartic stretch or QS term is used for the very long bond correction. Summing up, this equation takes into account longer bonds, and includes cutoffs for electrostatic and Van der Waals term with fifth order polynomial switching function and torsional and non-bonded constraints.

The results here presented account for chitosan–Cd interaction using MM2 and PM3. Results for pectin–Cd interaction were very similar to those found for pectin–Pb interaction and shown in a previously published paper (part I of the series; Ferreira & Gschaidt, 2001). Pectin–metal binding capacity has been reported to be greatly improved after sugar-beet pectin saponification and/or cross-linking suggesting that ion-exchange is the major mechanism involved (Dronnet et al., 1998a; Muzzarelli, 1973).

## 2.2. Inter and intramolecular chelation

$\alpha$ -D(1  $\rightarrow$  4) polygalacturonic acid (PGA) structure was obtained as described by Pérez et al. (Braccini, Grasso, & Perez, 1999). PGA was modeled with four monomers linked in two different ways. So, as to consider intermolecular chelation in the case of these oligosaccharides, two models of polysaccharide molecules (as disaccharide) were used, each one bonded to the heavy metal as a ligand in different ways. When considering intermolecular interaction, in the case of chitosan, we used 93 atoms when only two  $\text{NH}_2$  groups were involved in the coordination with the metal. One hundred and nineteen atoms were used in the case of pectin. When analyzing intramolecular chelation a similar number of atoms was used (Figs. 1 and 2).

Chitosan structure was modeled as derived from  $\alpha$ -chitin, following a description by Roberts (Roberts, 1992a, b). For chitin, the structural features were those of  $\alpha$ -chitin.

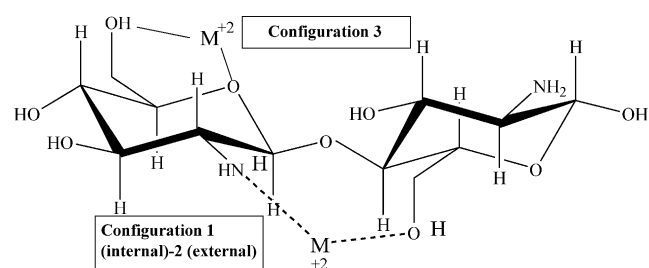


Fig. 1. Intramolecular chelation: configurations 1, 2 and 3.

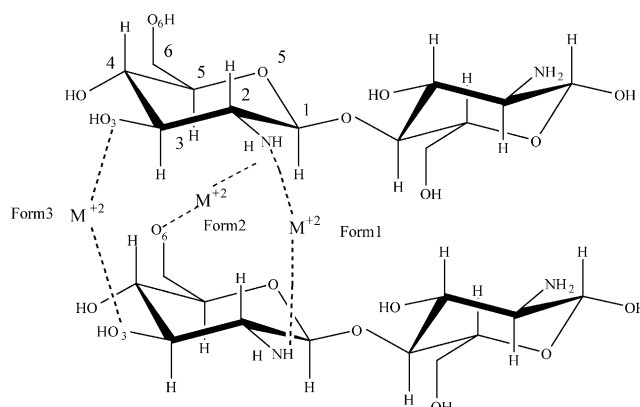


Fig. 2. Intermolecular chelation: forms 1, 2 and 3.

Figs. 1 and 2 show metal cation adsorption positions evaluated as configurations 1, 2 and 3. When the metal is present, the interaction is with  $\text{O}^-$  (no H present). In both cases, four monosaccharides were considered in the models. In the case of intermolecular chelation, biopolymers were modeled as disaccharides in PM3 and MM2 calculations.

### 2.2.1. Chitosan–chitin model

We considered several situations for intermolecular chelation:

*Form 1.* Metal bonded to two different  $\text{NH}_2$  groups of chitosan (or  $\text{NH}$  groups in the case of chitin).

*Form 2.* Metal bonded to one  $\text{NH}_2$  and one  $\text{O}_6$  from the second disaccharide.

*Form 3.* Metal bonded to two  $\text{O}_3$  from the disaccharide.

*Form 4.* Metal bonded to two O ( $\text{O}_6$  and  $\text{O}_3$ ) from different disaccharides.

### 2.2.2. Digalacturonic acid

Several situations were evaluated for the intermolecular chelation:

*Form 1.* Metal bonded to two different  $\text{COO}$  groups.

*Form 2.* Metal bonded to one  $\text{COO}$  and one  $\text{O}_2$  from the second disaccharide.

*Form 3.* Metal bonded to one  $\text{COO}$  and one  $\text{O}_3$  from the second disaccharide.

*Form 4.* Metal bonded to one  $\text{COO}$  and one  $\text{O}_4$  from the second disaccharide.

*Form 5.* Metal bonded to one  $\text{COO}$  and one O bridge (O) from the second disaccharide.

## 2.3. PM3

We used the parameterized Model version 3 (PM3) method from Chem3D 5.0 (Cambridge Soft)—including their parameters—to compute  $\Delta H_f$  (Standard Formation Enthalpy). A previous MM2 (Molecular Modeling version 2) calculation was performed to obtain a minimum in conformational potential energy space. This software

uses a Molecular Modeling Package menu (MOPAC), which includes a minimization procedure that is very useful when comparing the conformers stability for the same model. This  $\Delta H_f$  in MOPAC is the gas-phase heat of formation at 298 K of 1 mole of a compound from the elements in their standard state

$$\Delta H_f = E_{\text{elect}} + E_{\text{nucl}} + E_{\text{isol}} + E_{\text{atom}} \quad (2)$$

where  $E_{\text{elect}}$  is calculated from the self-consistent field calculation,  $E_{\text{nucl}}$  is the core–core repulsion based on the nuclei in the molecule and  $E_{\text{isol}}$  and  $E_{\text{atoms}}$  are parameters supplied by the potential function for elements in the molecule.

### 3. Experimental

#### 3.1. Chitosan and pectin

Chitin was isolated from crab shells and chitosan was prepared by deacetylating chitin with NaOH 70% (w/v) at 136 °C. The product was washed several times with double-distilled water until a pH of 7 was detected. Later the product was cleaned with ethanol, acetone and finally dried at 105 °C for 1 h. Flakes were then crushed and sieved through a 140–200 mesh. Powder characteristics were: 86% deacetylation degree, 1.18% ash and 3.51% moisture. The deacetylation percentage was determined by conductimetric titration and FTIR. FTIR and conductimetric titration gave comparable and almost identical results.

Pectin used for this study was obtained from citric fruits (SIGMA), with a low degree of methoxylation (DM), 8% and a 79% content of anhydro galacturonic acid. As for pectin, there was a certain concentration of methoxyl group attached to carbonyl not considered in our modeling (see part I: Ferreira & Gschaidner, 2001). Pectin was tested in powder or pellets, obtained by gelation with  $\text{CaCl}_2$  (Zalba *et al.*, 2001).

#### 3.2. Pellet formation

Pellets were formed by mixing chitosan powder with a Na-pectinate suspension, not beads at this stage. This suspension was prepared by pectin reaction with NaOH 2 M. Homogeneous suspensions were immobilized by dropwise addition of a  $\text{CaCl}_2$  solution (4%, w/v). The spheres obtained were left in this medium for 12–24 h (for stabilization) at room temperature. They were washed 4–6 times with double distilled water and then stored at 4–7 °C.

Several unsuccessful trials to obtain pellets were done. Pectin to chitosan weight ratios of 0.45, 1.80, 7.20 and 10 were inadequate. Only at a pectin to chitosan weight ratio of 5.00, the required spheres characteristics were achieved; the best Na-pectinate concentration in the suspension being 2% (w/v). At a Na-pectinate concentration of 1% (w/w) pellets were not uniform. The criterium to select the best

Na-pectinate concentration was the obtention of spherical, well-produced and stable pellets.

Pellets had a 4.3 mm ( $\pm 0.2$  mm) average diameter, measured with a confidence interval of 95%.  $\text{Ca}^{2+}$  is known to be fundamental for the pectin gelation process. However, different salts were used. From all chlorides evaluated (Ca, Mg, Ni, Cr, Co, Fe, and Ba) the best results were achieved with Ca. Pellets FTIR showed the typical chitosan deformation bands at 3350  $\text{cm}^{-1}$ , a band at 1650  $\text{cm}^{-1}$  corresponding to C=O of both pectin and chitosan and also a deformation band between 1300 and 1100  $\text{cm}^{-1}$  for COOR.

#### 3.3. $\text{Cd}^{2+}$ adsorption conditions

The compound used was  $\text{Cd}(\text{NO}_3)_2$  in 0.01 M  $\text{NaClO}_4$ . The initial  $\text{Cd}^{2+}$  solution concentration was  $10^{-5}$  M. Chitosan of 0.0044 g (0.147 g/l) or 0.0225 g for pectin (0.75 g/l) was the sorbent mass used. These weights remained the same as those used for pellet formation. The pellet weight relation was 1/5 (chitosan/pectin) with the same weight used to compare biopolymers in their ‘as obtained forms’ in the solutions used. pH in the  $\text{NaClO}_4$  solution (0.01 M) was 5 and not controlled afterwards. The Cd solution initial pH with the adsorbent present was 6.

Pectin and chitosan adsorption kinetics experiments for Cd were performed in a batch mode. Heavy metals initial concentrations were selected considering the maximum contaminant level allowed in wastewater according to Argentine Legislation: 1 ppm for Hg and 6.5 ppm for Pb. The maximum acceptable Hg level in drinking water is 0.001 mg/l (<1 ng/ml; Thomé, Hugla, & Weltroski, 1992). In the case of Cd, the selected concentration is related to limit levels for this metal in drinking water. One gram of pellets was put in contact with Cd solutions. Noticeably, the major pellets fraction is water and the amount of hydrated pellets used corresponds to 1 g, but the dry matter is 0.0269 g (0.0225 g of pectin plus 0.0044 g of chitosan). Initial conditions were: 1064  $\mu\text{g}$  Cd/g pectin, about 5443  $\mu\text{g}$  Cd/g chitosan and 13  $\mu\text{g}$  Cd/g pellet, the Cd concentration being the same. Metal cation concentrations seemed huge because of the way the results are presented: per gram of adsorbent (1 g in case of pellets, 0.0225 g of pectin and 0.0044 g of chitosan).

#### 3.4. $\text{Cd}^{2+}$ concentration determination

Solutions were put in contact with adsorbents using a magnetic horizontal stirrer. Work temperature was kept at  $20 \pm 1$  °C. Solutions were filtered through a glass-sintered filter. Metal concentrations were determined with a plasma induced emission spectrophotometer SHIMADZU ICPS 1000 III equipped with an ultrasonic aerosol generator UAG 1.

### 3.5. pH variation measurements

pH was measured during Cd adsorption on both biopolymers and pellets. Adsorption experiments were done in triplicate or further repeated until convincing results were obtained. Data presented were from reproducible experiments. pH measurements have an error of only  $\pm 0.05$ . This error was verified by reproducible experiments.

### 3.6. EDAX-SEM studies

A JEOL 35CF, operated between 15 and 21 kV, with an EDAX of Si–Li was used. These studies were performed on chitosan–pectin pellets to analyze the structure and distribution of each component.

### 3.7. X-ray fluorescence

A PHILIPS PW 1400 Spectrophotometer with an Rh anode, working at 60 kV and 40 mA with a LiF200 vacuum atmosphere was used. In order to study metal distribution in pellets, a comparative analysis was performed with used and unused pellets. Pellets were polished to eliminate their external surface. The study was done on blank pellets (unused), whole pellets (used but unpolished pellets) and polished pellets (used pellets lacking their external surface). The amount of Ca was also determined in fresh and used samples.

### 3.8. Adsorption isotherms for Pb, Cd and Hg

Adsorption isotherms were obtained at different metal concentrations at  $20 \pm 1$  °C:  $(1-5) \times 10^{-4}$  M up to  $2 \times 10^{-3}$ , for Pb or  $5 \times 10^{-4}$  in the case of Hg and Cd,

using 15 ml  $\text{NaClO}_4$  0.01 M (pH 5.0). Freundlich and Langmuir adjustments were applied in each case. Table 3 presents the results of these correlations. pH was measured in the filtrate at the equilibrium condition. The Langmuir isotherm approximates to monomolecular adsorption of gases and is widely applied to experimental data in case of adsorption from a solution by a surface active-solid when random adsorption occurs onto independent sites.

## 4. Results

### 4.1. $\text{Cd}^{2+}$ adsorption in biopolymers

$\text{Cd}^{2+}$  adsorption on chitosan showed an oscillating behavior. Successive adsorption–desorption steps were evidenced with time, Cd surface release increased  $\text{Cd}^{2+}$  concentration in the solution until the adsorption rate increased, etc. (Fig. 3). Although this behavior seems physically unreasonable, this result was quite reproducible.

In the case of pectin, although a high uptake was found at 60 min of contact, metal cation release from surface was very important after 60 min (Fig. 4). Both cations initial concentrations were the same. Total Cd adsorption (100%) is achieved on pellets after 150 min (Fig. 5).

### 4.2. pH variations vs uptake using chitosan and pectin

Cd adsorption kinetics showed an atypical behavior. pH remained in the range of 6–6.3 for chitosan and near 4.5–5 for pectin. Maximum adsorption uptakes were 70% on chitosan (Fig. 3) and near 75% on pectin at 60 min (Fig. 4). Pectin showed efficient Cd adsorption at short times (not more than 60 min) under these conditions.

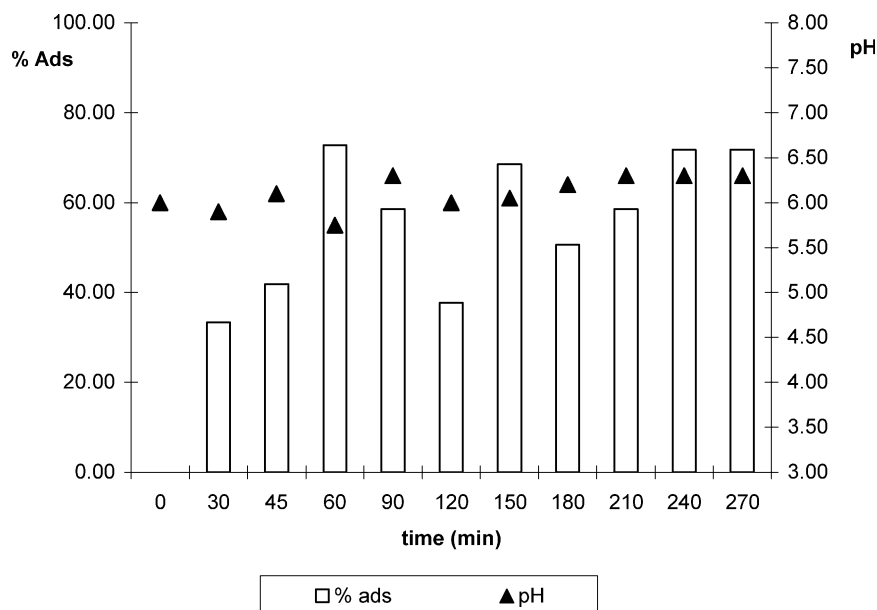


Fig. 3. Kinetics course of Cd adsorption on chitosan.

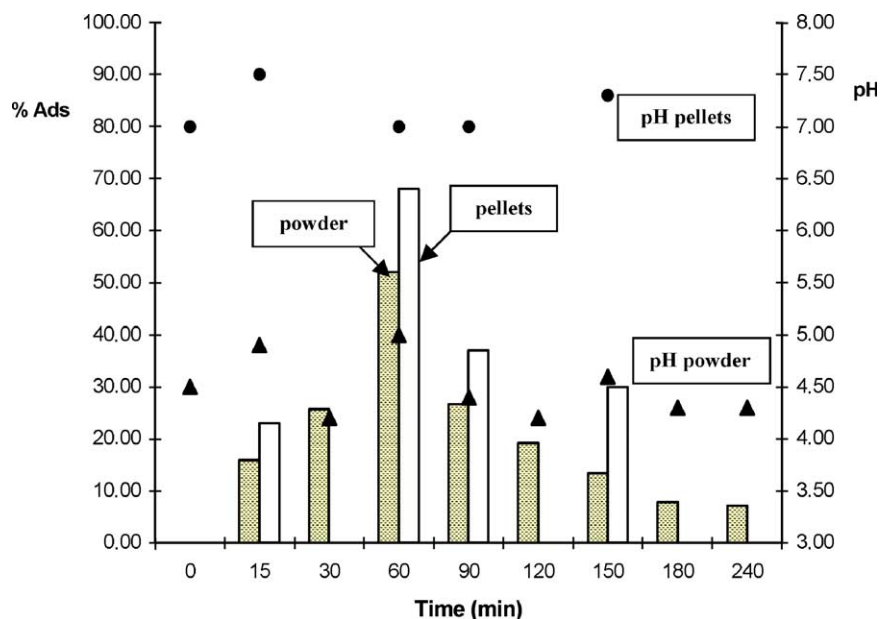


Fig. 4. Kinetics course of Cd adsorption on pectin powder and pectin pellets.

Let us discuss a very important point: pH differences in experiments with pectin, chitosan and pellets. Evidently, pH variation using either pellets or chitosan is almost the same—from 6 to 6.3–7. When pectin is used, the pH is rather low (4.5–5). This difference will be further discussed later in this paper because of its importance in understanding  $M^{2+}$  adsorption on biopolymers and pellets.

#### 4.3. pH variation using pellets

When pellets were used, no cycles of adsorption–desorption or release from surface were found. Moreover, at the same adsorption time we used for Hg (150 min), but higher than that for Pb (90 min) 100% adsorption uptake is

achieved and maintained with no pH variations. Table 1 shows initial and final pHs found for biopolymers and pellets adsorption. It is clear that pellets behavior implies a pH increase, increasing adsorption time and metal uptake, until 100% uptake is achieved. With  $Pb^{2+}$ , the  $\Delta pH$  (final pH – initial pH) is 1.6, whereas with  $Hg^{2+}$  it is 0.5. In the case of Cd adsorption on pellets,  $\Delta pH$  is 1.0. The pH measured inside the pellets (by means of an electrode used for soft food) was 8.5–9.0.

#### 4.4. MM2–PM3 results Cd-chitosan

Intramolecular co-ordination followed this trend in steric energies:  $Pb > Cd > Hg$ , for conf. 1 and  $Pb > Hg > Cd$  for

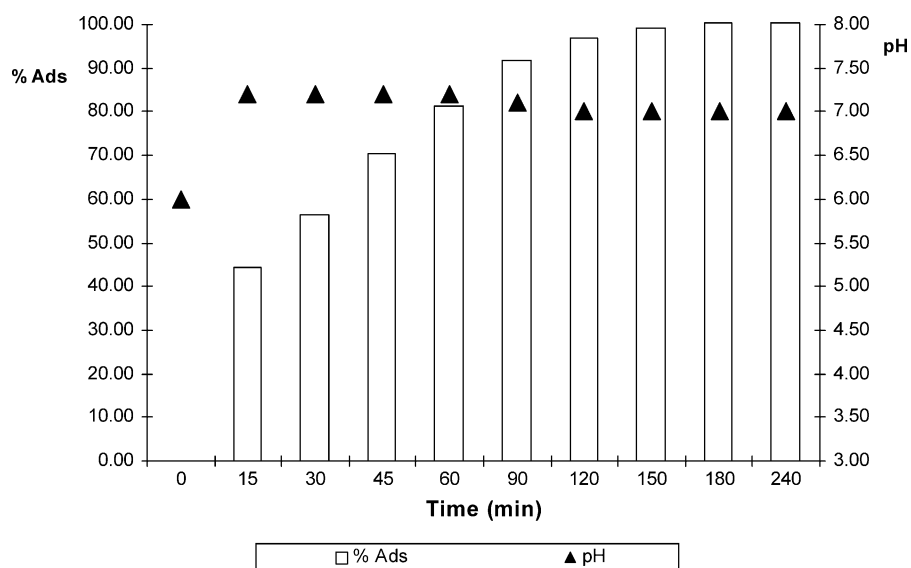


Fig. 5. Kinetics course of Cd adsorption on chitosan–pectin pellets.

Table 1  
Percentage adsorption of Pb, Cd and Hg

Metal	Pectin			Chitosan			Pellets		
	Hg	Pb	Cd	Hg	Pb	Cd	Hg	Pb	Cd
%	15	4	4	85–100	70	75	100	100	100
Final pH	4.2	4.6	4.2	6.5	6	6	7.9	7.5	7

Initial pH 5. Concentration in  $\mu\text{g/ml}$  solution (constant). Pellets  $C_i \text{ Pb} = 103.5 \mu\text{g/g}$ ;  $C_i \text{ Hg} = 15 \mu\text{g/g}$ ;  $C_i \text{ Cd} = 12 \mu\text{g/g}$ . Chitosan  $C_i \text{ Pb} = 47,045.4 \mu\text{g/g}$ ;  $C_i \text{ Hg} = 6863.6 \mu\text{g/g}$ ;  $C_i \text{ Cd} = 5443.6 \mu\text{g/g}$ . Pectin  $C_i \text{ Pb} = 9409.1 \mu\text{g/g}$ ;  $C_i \text{ Hg} = 1372.7 \mu\text{g/g}$ ;  $C_i \text{ Cd} = 1064.5 \mu\text{g/g}$ .

Table 2  
SE and  $-\Delta H_f$  1, 2 and 3 intramolecular and intermolecular chelation for Pb, Hg and Cd (kJ/mol) with chitosan

Metal	Conf. 1			Conf. 2			Conf. 3			Inter		
	Hg	Pb	Cd	Hg	Pb	Cd	Hg	Pb	Cd	Hg	Pb	Cd
SE	295	317	305	348	371	314	294	299	485	107	199	162
$-\Delta H_f$	1611	1561	1713	1548	1544	1614	2330	2313	1640	1476	1159	803

conf. 2, whereas for conf. 3 was  $\text{Cd} > \text{Hg} > \text{Pb}$ . Intermolecular chelation order was  $\text{Pb} > \text{Cd} > \text{Hg}$ . The highest the steric energy (SE), the lowest the total entropy. In terms of enthalpy, Cd was enthalpically favored for intramolecular chelation but not for the intermolecular one. Clearly, Hg was the most favored enthalpically, whereas Pb was entropically disfavored vs Cd. Table 2 shows these results.

#### 4.5. EDAX-SEM studies

Pellet photographs of a dried pellet and its transversal cut show its compact inner structure and irregular heterogeneous surface. Wet pellets showed a uniform surface (results not shown).

#### 4.6. XR fluorescence

Hg was detected in whole pellets and in polished pellets, though in a much lower concentration (Fig. 6). The same results were obtained for Pb (Fig. 7). It was impossible to

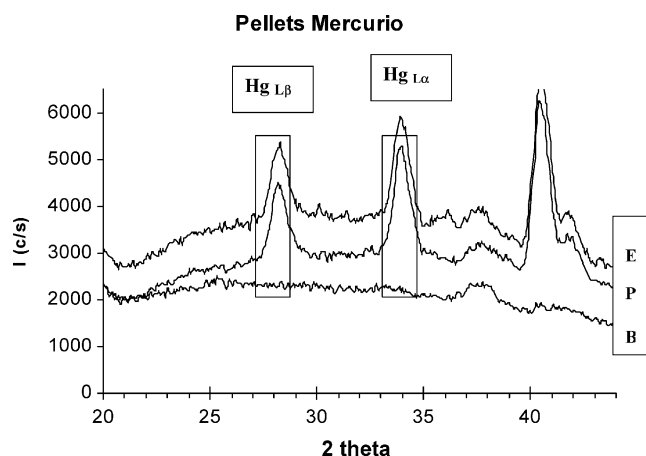


Fig. 6. XRF for Hg detection-E entire B blank P polished.

analyze Cd because its signal superimposes the Rh signal used in fluorescence equipment. The Ca determination demonstrated that in used pellets, a minor amount of Ca is found.

#### 4.7. Adsorption studies on pellets

##### 4.7.1. Adsorption isotherms–Langmuir–Freundlich analysis

Table 3 shows the parameters found for Langmuir–Freundlich isotherms.  $\text{Me}_{\text{max}}$  was similar for Hg and Cd, and lower than that for Pb. In the case of Freundlich adjustment, pellets affinity was almost three times higher for Hg than for Cd. It seems that Langmuir equation fits adsorption data better.

##### 4.7.2. Adsorption kinetics of Pb, Cd and Hg on pellets using different metal concentration

Figs. 8–10 show different kinetic pattern graphs at 3–4 Pb, Hg and Cd concentrations using pellets as adsorbents. Evidently, the process is much faster for Pb and Cd than for Hg. Adsorption rate increases in the order:  $\text{Hg} < \text{Cd} < \text{Pb}$ .

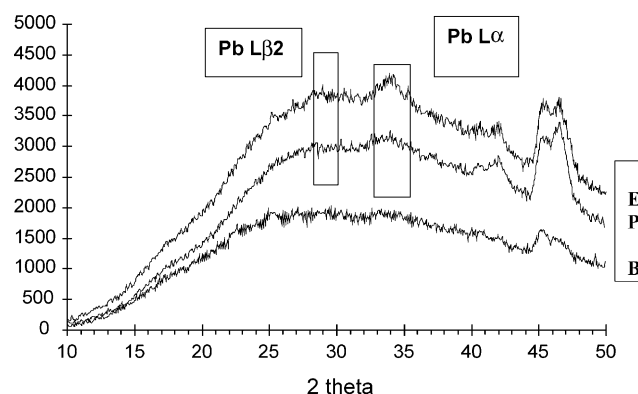


Fig. 7. XRD for Pb detection-E entire B blank P polished.

Table 3  
Langmuir–Freundlich analysis of adsorption isotherms for Hg, Pb and Cd at 20 °C

Cation	Hg	Pb	Cd
Range in pH	7.6–7.7	7.4–7.5	6.9–7.1
$C_p$ ( $\text{g l}^{-1}$ )	67	67	67
$K_L$ ( $\text{l mequiv.}^{-1}$ )	796	1551	7265
$Me_{\max}$ (mequiv./g)	0.027,	0.1075,	0.022,
	2.71 mg/g	11.2 mg/g	1.23 mg/g
$Me_{\max}$ (mequiv./g) g dried pellet	1.0, 101 mg/g	4, 414 mg/g	0.82, 46.1 mg/g
$R_2$	0.9923	0.999	0.999
Ref. chitosan	370 mg/g	130 mg/g	13 mg/g
$K_f$	0.1802		0.0643
$1/n$	0.4149		0.1991
$R_2$	0.9750		0.7749

$K_L$ : adsorption equilibrium constant.  $Me_{\max}$ : monolayer adsorbed quantity.  $K_f$ : sorbent adsorption capacity.

## 5. Discussion

### 5.1. Adsorption sites in chitosan

The chitosan behavior in aqueous media is controlled by deacetylation degree, solubility, molecular mobility, molecular weight, pH and ionic strength. Considering fully deacetylated chitin, we have one cationic charge in  $\text{NH}_3^+$  every 0.514 nm in chitosan. We also must take into account chain accessibility, mobility and charge density. Chitosan exhibited an  $-\text{NH}_3^+$  fraction of 0.9 at pH 4 and of 0.5 at pH 6. At pH 6 the electrostatic interaction would be weakened (Domard, 1997; Domard, Rinaudo, & Terrassin, 1989; Muzarelli, 1977). As adsorption capacity depends on chitosan amine-group concentration, amine group availability is important (Roberts, 1992a).

Although opinions remain divided, the most accepted theory proposes that when chitosan powder is at a pH where chitosan is not soluble, dispersion is minimum and hydration is maximum leading to crystallinity very close

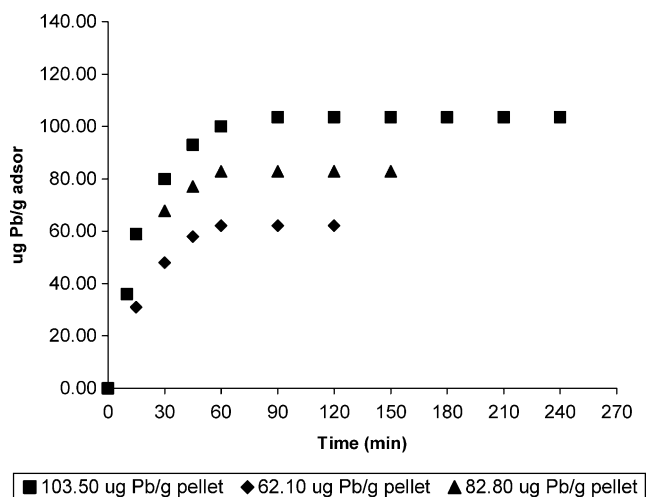


Fig. 8. Adsorption of Pb on chitosan–pectin pellets.

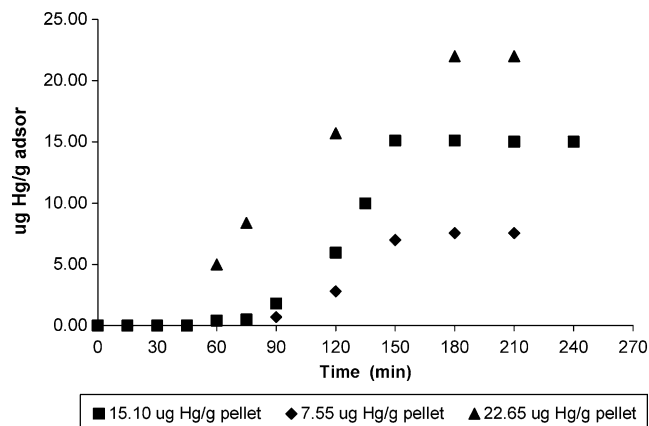


Fig. 9. Adsorption of Hg on chitosan–pectin pellets.

to zero. In the case of pectin, water molecules break intermolecular H bonding, depending on pH (Deuel & Stutz, 1958). Under the conditions here reported diffusion limitations or swelling effects seemed not to be as important as electrostatic interaction and cation exchange, especially for Pb and Cd.

### 5.2. $\text{Cd}^{2+}$ adsorption on chitosan—experimental and theoretical characterization

$\text{Cd}^{2+}$ ,  $\text{Cd}(\text{OH})^+$ ,  $\text{Cd}(\text{OH})_2$  and  $\text{Cd}(\text{OH})_4^{2-}$  can be present depending on the pH level. When  $\text{Cd}^{2+}$  was in a perchlorate solution at the pH range here included, the only species present is  $\text{Cd}(\text{OH})^+$ . With chitosan, Cd uptake was near 75% of the total Cd solution concentration after 60 min of contact. Following this step, a  $\text{Cd}^{2+}$  surface release took place. The experiment at 120 min was repeated several times and it is reproducible. It remains to be established whether  $\text{Cd}^{2+}$  can displace a  $\text{H}^+$  from the  $\text{NH}_3^+$ . This seems unlikely since no pH modification occurs and only minor pH changes are detected (6–6.3) in the case of Cd adsorption.

Cd adsorbed neared 0.0045 g/g of chitosan. One explanation for adsorption–desorption cycles would be

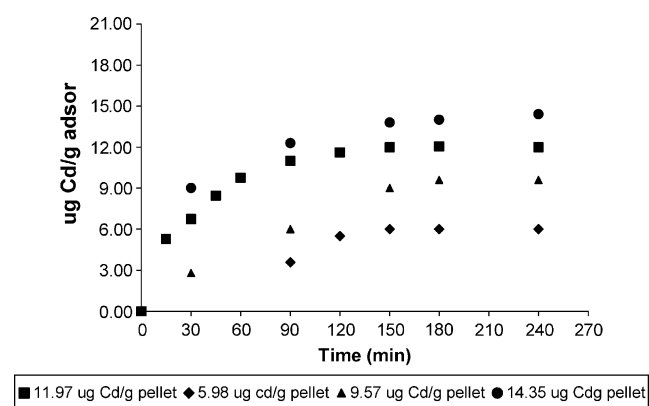


Fig. 10. Adsorption of Cd on chitosan–pectin pellets.



that adsorption and desorption kinetic constants are similar and that some electrostatic interaction takes place between  $\text{Cd}^{2+}$  adsorbed on close sites. A similar two steps mechanism could be acting for Cd, as in case of Pb (see Part II: Zalba et al. (2001)).

MM2 and PM3 calculations showed a tendency, in electronic terms, and with respect to equilibrium uptake, that would be lower for Pb and Cd than for Hg.  $\text{Pb}^{2+}$  produced more perturbation in chitosan chains (intramolecular chelation conf. 1 and 2), although in the most favorable intermolecular chelation conformation (through two  $\text{NH}_2$ ) presented a higher SE than that found for Hg and Pb (Table 2). In this case, Cd presented an intermediate SE. When PM3 results were analyzed the lowest  $-\Delta H_f$  was for Cd. When considering intramolecular chelation, confs. 1 and 2 presented the highest  $-\Delta H_f$ .

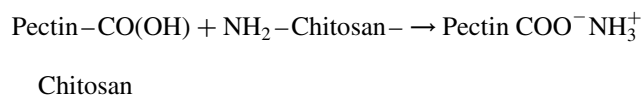
### 5.3. $\text{Cd}^{2+}$ adsorption on pectin

Heavy metal adsorption on pectin with different structure has been widely reported (Franco, Chagas, & Jorge, 2002; Garnier, Axelos, & Thibault, 1994; Jorge & Chagas, 1988; Malikhova & Kohn, 1982, 1983; Turakhozaev, Dzhakhanrigov, Khodzhaeva, Ryabchenko, & Sharikov, 1995; Wellner et al., 1998). Wellner et al. have reported FTIR studies of pectate and petinate gels formed by divalent cations (Wellner et al., 1998), whereas Turakhozaev et al. have presented a preparation method for partially esterified pectin as a sorbent for heavy metals (Turakhozaev et al., 1995). Malikhova and Kohn (1982) reported Cd binding to pectin and divalent cations binding with pectins of different structures have been reported by Franco et al. (2002), Garnier et al. (1994), Jorge and Chagas (1988), and Malikhova and Kohn (1983).

Ca–alginate brought a negative charge on the majority of functional groups ( $\text{RO}^-$ ) at pH 5 (Huang et al., 1996). Besides the  $\text{COO}^-$  group, ionized RO groups can be involved in reactions using pectin. If this biopolymer has a crystalline structure, the rearrangement to obtain the complexation would be difficult. Metal adsorption can induce changes in bioadsorbents. At pH 5.5–6 polyglutamic acid is normally in a completely ionized form, but in the presence of divalent metal ions a significant helix formation can be induced. The pH found with pectin and the three metal cations (4.2–4.6) in solutions before adsorption, was that of a polygalacturonic acid solution. Since pH did not diminish, neither Pb(Cd) nor Hg displaced H from COOH. Adsorption must have been taking place on the  $\text{COO}^-$  groups, perhaps between different pectin molecules, to produce  $(\text{COO})_2 \text{M}$  groups. MM2 calculation predicts that intermolecular chelation is strongly favored.

### 5.4. $\text{Cd}^{2+}$ adsorption on pellets

Chitosan amine groups do not cross-link with remaining COOH pectin groups but they can form salt-type bonds at the pH range used:



This reaction may occur if chitosan is soluble at the pH of pellet preparation. It has been reported that salt-type bonds are the main reaction product when chitosan reacts with carboxylic acids. Nevertheless, as EDAX-SEM and pellet pH studies have demonstrated chitosan does not form a homogeneous mixed compound with pectin inside the pellet. Therefore, pellets are composed of gelled pectin and additionally of a heterogeneous phase of insoluble chitosan inside the pellet. In order to understand the inside/outside pellet structure we propose a pellet composition model, based on experimental characterization. First, the pellet surface in contact with the initial adsorption solution at pH 6.5–7 has a different metal affinity than that of the whole pellet, probably presenting not crystallized chitosan with hydrated exposed pectin. This is a speculation because this fact is very difficult to prove. Pellets are produced by gelation and therefore exposed to moisture; water diffusing into the biopolymer amorphous regions. The biopolymer swelling destroys residual crystallinity, thereby increasing the solute accessibility to adsorption sites (Piron, Accominotti, & Domard, 1997). The high  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  uptake can be explained considering pellets as ion-exchangers ( $\text{Ca}^{2+}$  exchanged by  $\text{M}^{2+}$ ). Thus, gelation and cross-linking generate a structure with a higher exchange-capability than pectin itself. We propose that  $\text{Ca}^{2+}$  exchange with  $\text{Pb}^{2+}/\text{Cd}^{2+}$  take place in pellets. An open, hydrated network is an excellent ion-exchanger. This proposal has support in XRF results. In Ca–alginate/chitosan pellets (Huang et al., 1996), the  $\text{Cu}^{2+}$  binding capacity decrease is related to chitosan immobilization onto alginate beads because of the exposed lower affinity chitosan. Our results seem to indicate that there is an exposed surface probably containing chitosan in its structure. Clearly, pectin pellets themselves have different adsorption properties than chitosan–pectin pellets (compare Figs. 4 and 5). The gelation-induced decrease in COOH group concentration can be related to the pH variation found when comparing pectin pellets and pectin in powder. When chitosan–pectin pellets were analyzed chitosan played an essential role in adsorption. In fact, when chitosan was present, pH remained constant and near 7 after 15 min, whereas with pectin pellets, it reached 7.5 and evidenced an adsorption oscillating behavior.

If pellet structure and internal/external pH differ, the relative amounts of  $\text{NH}_3^+$ ,  $\text{NH}_2$  and  $\text{COO}^-$  also vary, internally and externally. The same happened when metal concentrations were considered. Depending on pH values,

hydrolyzed forms of metal cation concentrations may be higher internally than externally in pellets. Therefore, the metal concentration gradient we found, testing pellets by XRF, could be assigned to both diffusion problems when getting the inside the pellet and chemical changes in pellet-metal interaction. This interaction not only gives rise to a higher internal–external pH in the wet pellet and to more  $\text{NH}_2$  or  $\text{COO}^-$  adsorption sites, but also to an hydrolyzed metal cation and therefore a lower affinity for these adsorption sites.

### 5.5. Adsorption isotherms

Adsorption isotherms data using chitosan as adsorbent have been widely reported using Langmuir model (Allen & Brown, 1995; De Rome & Gadd, 1987; Guibal, Saucedo, Roussay, Roulph, & Le Cloirec, 1993; Jansson-Charrier, Guibal, Roussay, Delanghe, & Le Clorie, 1996; Jha & Leels, 1988; Langmuir, 1918; Navarro, Sumi, Fujill, & Malsumura, 1996; Perez, Kouwijzer, Mazeau, & Engelsen). Experiments for the three metals adsorption on pellets were well fitted by Langmuir treatment. It was evident that  $K_L$  (binding equilibrium constant) increased twice from Hg to Pb and 10 times from Hg to Cd. However,  $\text{Me}_{\text{max}}$  was similar for Hg and Cd, whereas it was five times higher for Pb. Surface heterogeneity was not an essential factor. Our data fitted better Langmuir than Freundlich equation.  $K_L$  Langmuir parameter decreased in the order  $\text{Cd} \gg \text{Pb} > \text{Hg}$ . However, the quantity adsorbed by monolayer decreased from Pb to Cd ( $\text{Pb} \gg \text{Hg} > \text{Cd}$ ) almost 10-fold (Table 3). Different adsorption enthalpies can affect these data. In case of Freundlich,  $K_f$  was higher for Hg than for Cd.  $n$  values were both higher than 1. We support electrostatics as being the main mechanism for Hg adsorption, ion-exchange for Pb adsorption and combined mechanisms for Cd adsorption in pellets. Chelation cannot be ruled out as an additional adsorption process. Opposite selectivities have been reported for sugar-beet pectins and polycarboxylate-containing substances (Dronnet et al., 1998b; Gotoh et al., 2004b). If adsorption uptakes are reported by pectin and chitosan masses, they must be adjusted by a factor of 37.2. Data presented per gram of dried pellet can be compared with published results for Cd and Pb adsorption on sugar-beet pulp. Dronnet reported a 0.58 mequiv./g  $\text{Me}_{\text{max}}$  for Pb of and 0.47 mequiv./g for Cd using  $14.55 \text{ g l}^{-1}$  of sugar-beet pectin (Gotoh et al., 2004b). We increased adsorption capacity for Pb 7-fold and 2-fold for Cd in chitosan–pectin pellets. In case of recent literature, the increase achieved 5-fold, comparing pellets with citrus pectin (Harel et al., 1998).

### 5.6. Modeling polysaccharides: validity of the present approach

There are not many experimental means other than nuclear magnetic resonance (NMR) suitable for probing

carbohydrate conformations and evaluating calculated potential energy surfaces. Saccharides optical activity depends on their chemical composition, configuration and conformation. Models have been developed and applied to disaccharides in aqueous solutions. These studies result in the location of preferred regions in the configurational space, rather than in the location of some-well defined points. Primary structures of polysaccharides permit an almost infinite array of chemical structures and conformations. A thorough description of the conformational space available for a disaccharide has been shown (Perez et al.). Perez et al. reported the solution behavior of pectin polysaccharides investigated by small angle neutron scattering, viscosimetric and molecular modeling studies. The persistence lengths ranged from 10 to 17 monomer units. These authors evaluated the accessible conformational space of eight disaccharides that represent the constituent repeating segments of the homogalacturonan and rhamnogalacturonan polysaccharides. Each dimer was analyzed conformationally and the results were used to access the configurational statistics of representative polysaccharide chains. The inclusion of varying amounts of rhamnose units (5–25%) only slightly reduced the persistence length and the extended overall chain conformation remained unchanged (Cros, Garnier, Axelos, Imberty, & Perez, 1996). NMR studies and molecular modeling of disaccharides have been applied to pectin as polysaccharide (Cros, Hervé du Penhoat, Bouchemal, Imberty, & Pérez, 1992). The main aim when pectin/chitosan was modeled was to test LOCAL interactions of pectin/chitosan active sites with Cd. Other results from the same group support the use of reduced models to test local interactions with adsorbates (Imberty & Perez, 2000; Mazeau, Perez, & Rinaudo, 2000; Rogovina, Vikhoreva, Akhopova, Gorbacheva, & Zelenetskii, 1998).

Although MM2 results of SE have no physical meaning, difference between steric energies in conformers is an approach to total energy change. Only an approach because vibrational term is not considered in MM2 and therefore no entropy changes can be calculated. However, comparing the same total atom number conformers, and taking into account the qualitative nature of the approach, the results are in line with a coherent explanation of the experimental results.

Finally, in chitosan N from  $\text{NH}_2$  in its non-protonated form (and covalent bonding) play central role in metal ion adsorption and we demonstrated this in previous work (Ferreira & Gschaidler, 2001; Zalba et al., 2001) and recent published manuscripts support this proposal (Rhazi et al., 2002a,b). In the case of pectin, ion-exchange properties are the main properties for metal adsorption from aqueous solutions.

## 6. Conclusions

- Cd, Pb and Hg can be removed from wastewaters at levels below the *maximum permitted* ones by using

chitosan–pectin pellets without pH control. Pectin or chitosan separately are not so efficient.

- Pectin, chitosan and chitosan–pectin pellets kinetic patterns are totally different for the three metals. The cation solution chemistry as well as the chemistry of the exposed surface of the three adsorbents at the working pH is crucial to understand these behaviors.
- MM2, PM3 are additional tools to understand and propose the kind of interaction taking place: chelation, ion exchange or electrostatic bonding. We suggest that electrostatic interactions are governing the process in the case of Hg, whereas ion-exchanging mechanisms seem to be more suitable for Pb. When analyzing Cd, a combined mechanism seems to be appropriate, electrostatic interactions being also important. Chelation with dissociated/non-dissociated sites at the adsorption surfaces cannot be ruled out in all cases. Langmuir isotherms on pellets showed that  $V_{\max}$  decreases in the order  $\text{Pb} > \text{Hg} > \text{Cd}$ , whereas  $K_L$  follows the trend  $\text{Cd} > \text{Pb} > \text{Hg}$ . It seems more sites are available for Pb than for Cd or Hg adsorption. This can be related to the relative excess of available pectin in pellets structure.
- Langmuir isotherms for the adsorption of the three metals at 20 °C show a higher binding capacity of pectin–chitosan pellets than the one reported for sugar-beet pectins and even that of chitosan alone.
- Pellets seem to have a surface covered with a phase of crosslinked chitosan–pectin with excess pectin and an inside with heterogeneously distributed insoluble chitosan.
- EDAX-fluorescence studies allow us to conclude that a cation diffusion from pellet surface to the inner portion of pellets takes place; the surface remaining the main adsorption place.  $\text{Ca}^{2+}$  exchange takes place. A complex diffusion-reaction model considering internal/external pH differences explains the abrupt change in metal concentration comparing internal/external pellets surfaces.

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