Chemical modification of *in natura* collagen by acidic catalysis – structural characterization and mechanistic features of Cd(II) sorption by solution microcalorimetry

Fernanda Santos Carvalho dos Anjos[1], Esdras Santos Silva[2],
Marcos Antonio Santana Andrade Jr[2], Eunice Fragoso da Silva Vieira[2],
Antonio Reinaldo Cestari[1]

[1] Department of Materials Science, Federal University of Sergipe, CEP 49100-000, São Cristóvão, Sergipe, Brazil.

[2] Laboratory of Materials and Calorimetry, Department of Chemistry/CCET, Federal University of Sergipe, CEP 49100-000, São Cristóvão, Sergipe, Brazil.

e-mail: fernanda.anjos.univasf@gmail.com, arcestari@gmail.com, esdrasquimica@yahoo.com.br, marcos_asaj@hotmail.com, eunice@ufs.br

ABSTRACT

Chemically-modified *in natura* collage was prepared under acidic catalysis for using in sorption of Cd(II) from aqueous solutions. Simultaneous determination of the quantity and energetic parameters of Cd(II) sorption on PGA-scale at pH 8.0 were determined using solution microcalorimetry (SM). The calorimetric enthalpies of Cd(II) sorption on PGA-scale are all exothermic, and Cd(II) adsorption energies decrease as temperature increases. The maximum sorption capacity of PGA-scale for Cd(II) (438 µmol g^{-1}) is superior with many sorption data reported in the literature. Characterization and SM indicate that the main sites for Cd(II) sorption are located in the collagen structure of PGA-scale. It was found that the interactions PGA-scale/Cd(II) are mainly due to chemisorption and diffusion may occur at the PGA-scale/Cd(II) interface. The results of this study underline the good features of PGA-scale as a promising material for sorption of Cd(II) from aqueous media.

Keywords: Cadmium; biosorbents; fish scales; acidic catalysis; solution microcalorimetry; thermodynamics.

1. INTRODUCTION

Cadmium (Cd) is a highly toxic pollutant introduced into natural waters due to the discharge of a variety of industrial wastewaters. In particular, Cd(II) can be released to drinking water from the corrosion of some galvanized plumbing and water main pipe materials. Short-term exposures to Cd(II) cause skin and stomach irritation or ulceration. Long-term exposure can cause damage to liver, nerve tissue, kidney circulation, and death [1].

Several methods have been reported for Cd(II) removal [2]. However, they are not cost-effective, require tight operations and maintenance, and feed concentration must be monitored closely [2]. On the other hand, adsorption offers significant advantages like low cost, availability, profitability, and efficiency, in comparison with conventional methods. In particular, biosorption from aqueous solutions is a relatively new, very promising process in the removal of contaminants from aqueous effluents. The major advantages of biosorption include: low cost, high efficiency, minimization of chemical and/or biological sludge regeneration of biosorbent, no additional nutrient requirement, and the possibility of metal recovery [3]. However, most biosorbents are used in the form of thin particles. The major problems associated with this kind of biosorbents are post-separation of the suspended particles from the treated effluent and regeneration of used biosorbents. In this way, membrane-like materials are considered a good alternative to be used in biosorption processes [4].

In this work, we used chemically crosslinked scales of Brazilian Corvina fish (Micropogonias furnieri), as an adsorbent for Cd(II). Fish scales are composed of an extracellular matrix, mainly type I fibrillar collagen, and hydroxyapatites [$Ca_{10}(PO_4)_6(OH)_2$ and/or $Ca_5(PO_4)_3OH$]. They are a membrane-like sorption material and their adsorption abilities are due to the presence of significant amounts of specific chemical groups

of fish scales collagen, such as hydroxyl, carboxyl, amine and amide that are involved in the biosorption of heavy metals [5-8]. It is well known that fundamental understanding of the chemical processes occurring at adsorbent/adsorbate interfaces is critical when the use of new and economically viable adsorbents moves beyond lab-scale quantities. In this way, the Cd(II) sorption experiments were performed by solution microcalorimetry.

Calorimetry, the measurement of heat, allows simultaneous determination of both the quantity and the energy of sorption. The experimental yield can potentially give information on kinetics and thermodynamics of the processes, energetics and analysis. To date, less attention has been paid to the direct solution calorimetry investigations of heavy metals interaction on biosorbents. However, solution microcalorimetry has been considered invaluable for understanding many mechanistic features occurring at biosorbent/adsorbate interfaces [9].

2. MATERIALS AND METHODS

2.1. Reagents and solvents

Water was used after double-distillation. All the chemicals/reagents used in these studies were of analytical reagent grade. Aqueous solutions of Cd(II) was prepared by dissolving $Cd(NO_3)_2$ (Sigma-Aldrich) in pH 8.0 borax/boric acid buffered solution (ionic strength of 1.0×10^{-4} mol L^{-1}).

2.2. Biosorbent preparation

The raw Corvina fish (Micropogonias furnieri) scales were collected from the Fishermen's Market located in Aracaju, state of Sergipe, Brazil. Mature fish scales were washed repeatedly with water to remove adhering dust and soluble impurities from their surfaces. The scales were dried in direct sun light for 6 h. The dried fish scale (50 g) was then crosslinked under acidic catalysis using a 0.10% (w/v) pH 4.0 buffered solution (sodium acetate/acetic acid) of glutaraldehyde (GA) [7]. The aqueous suspension was mechanically agitated for 3 h at room temperature. The suspension was filtered off and the solid residue washed repeatedly with water and dried at 45 °C for 12h. A pale yellow material was obtained (hereafter described as PGA-scale for simplicity) and cut into small 5 mm x 5 mm square membranes of 0.5 mm of thickness and conditioned in a dark air-free flask.

2.3. Characterization of the materials

The thermogravimetric analyses (TG/DTG, DTA) were made using about 10 mg of material, under nitrogen atmosphere from 25 to 800 °C, at the heating rate of 10 °C min^{-1} in a SDT 2960 thermoanalyzer, from TA Instruments. The morphological characterization of the materials was carried out with a scanning electron microscope (SEM, JEOL-JSM 6360-LV). The samples were previously coated with gold (thickness of about 10 nm). The Raman spectra were acquired with a Bruker Senterra Raman System equipped with an Olympus microscope with a 50x objective to focus a Melles Griot laser beam on the sample. The spectra were excited by the 785 nm line from an air-cooled He–Ne laser. The laser power measured after the microscope objective was ca. 50 mW. The solid-state diffuse reflectance spectra of the samples were recorded on an Ocean Optics UV-Vis spectrophotometer from 400 to 700 cm^{-1} at a resolution of 4.0 cm^{-1}.

2.4. Determination of pH of the point of zero chare (pH$_{pzc}$) of PGA-scale

The determination of the pH$_{pzc}$ of the samples was carried out using a procedure similar as described earlier [10]. Briefly, 50 ml of 0.01 mol L^{-1} NaCl solutions were placed in closed Erlenmeyer flasks. The pH of each solution in each flask was adjusted to specific values by adding HCl 0.1 mol L^{-1} or NaOH 0.1 mol L^{-1} solutions. Then, 0.10 g of PGA-scale was added and the final pH measured after 24 h under agitation at 25 °C. The values of pH$_{pzc}$ of the materials are determined from the points where the initial pH equals the final pH.

2.5. Solution calorimetry measurements of Cd(II) sorption on PGA-scale

Microcalorimetric determinations were performed in a C80 microcalorimeter (SETARAM, France). In this work, it was found that the Cd(II) is maximized using buffered solutions at pH 8.0. Experimental determinations were performed using the membrane breaking (thin Teflon®) technique, as described elsewhere [11]. Briefly, 100 mg of PGA-scale and 0.5 mL of borax/ boric acid buffer solution at pH 8.0 were put into the lower of the calorimetric vessel. Additionally, 2.5 mL of a Cd(II) aqueous pH 8.0 buffer solution were put in

the lower part of the calorimetric vessel. Calorimetric output is of thermal power (dq/dt; mW, power, precision of $\pm$ 0.12 µW) as a function of time (t; s). The thermal effect of thin Teflon® membrane breaking for the empty cell was found to be negligible. In order to evaluate temperature effect, the calorimetric experiments were carried out at 30 °C and 500 °C. Each experiment was performed in triplicate runs. After total stabilization of the calorimeter base line, the residual Cd(II) concentration in solution was determined by the 4-(2-Pyridylazo) resorcinol (PAR) spectrophotometric method [12] at 540 nm wavelength (detection level less than 1.0 mg L^{-1}). The amount of Cd(II) sorbed was calculated by using the following Eq.(1) [13]:

$$n_{int} = \frac{(C_i - C_f).V}{m}$$

(1)

where n_{int} is the fixed quantity of Cd(II) per gram of PGA-scale after a given contact time t, in mol g^{-1}, C_i is the initial concentration of Cd(II) in mol L^{-1}, C_f is the concentration of Cd(II) present in equilibrium, in mol L^{-1}, V is the volume of the solution in L, and m is the mass of PGA-scale in g.

3. RESULTS AND DISCUSSION

3.1 Preliminary information

Fish scales are composed of an extracellular matrix, mainly type I fibrillar collagen, and hydroxyapatites [$Ca_{10}(PO_4)_6(OH)_2$ and/or $Ca_5(PO_4)_3OH$]. The polymeric structure of fibrillar collagen is composed of polypeptide chains with a triple-helical structure, and they are aggregated through hydrogen bonds to form collagen fibers [14]. However, collagens are soluble in both saline and acidic solutions. In order to overcome this undesirable characteristic, chemical crosslinking reactions have been used for efficient insolubilization of collagen structure [6]. In this way, GA is the most successful chemical agent, because GA gives rise to Michael addition or Schiff´s base reactions, which are stable and irreversible [6]. GA can react with several functional groups of collagen, such as amine, thiol, phenol, and imidazole because the most reactive amino acid side-chains are nucleophiles [6,7].

Commercial solutions of GA are usually polymeric glutaraldehyde (PGA) and contain significant amounts of α,β-unsaturated aldehydes that are able to form rings by loss of water molecules by aldol condensation [15]. On the other hand, collagens crosslinked under acidic catalysis are reported to have both low amounts of pendant PGA molecules and high amounts of free organic groups [7]. This is expected to be favorable because the free groups of the crosslinked collagen structure are reported to play an important role in biosorption systems [8].

3.2. Characterization of the materials

Some SEM micrographs of the materials are shown in Figure 1. The internal sections of fish scales are rich in inorganic material containing high proportions of calcium and phosphorus, whereas their surfaces are composed by superimposed vertically oriented collagen fibers. However, Needle-like or flaky crystals of apatites were also detected by SEM/EDX analysis (details not shown), in the outer layer of the scales.
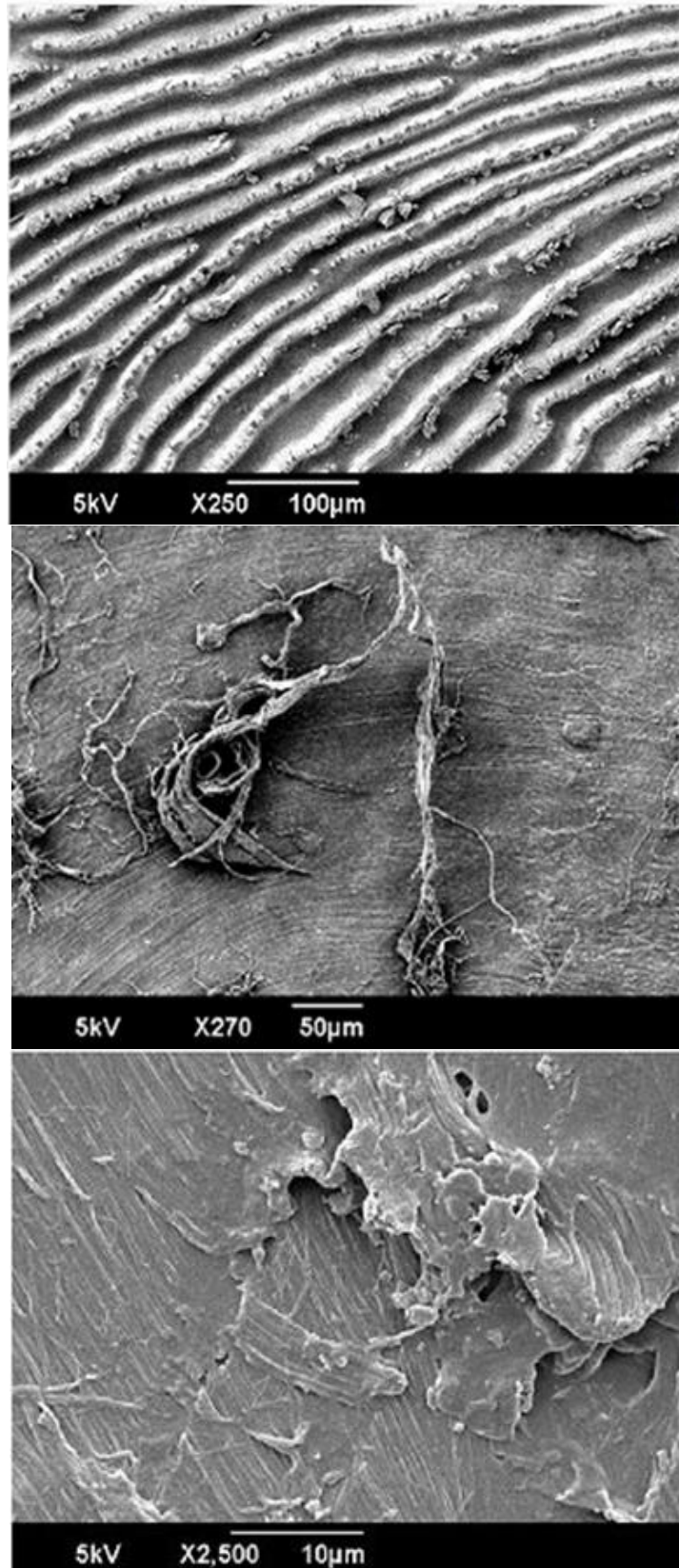
Figure 1: SEM images of the raw Corvina scale (upper part), PGA-scale (middle part) and PGA-scale/Cd(II) (lower part), with magnifications of 250X, 270X and 2,500X, respectively.

After crosslinking, the fish scale collagen fibers seem to be closer one another in relation to the raw

scale and micrometer-sized aggregates can also be observed. It has been attributed to the partial distortion (or disruption) of the fibrillar structure of the fish scale collagen after PGA reaction, in an attempt to accommodate molecules of different lengths on fish scale collagen structure [15]. The PGA crosslinking reaction can induce the collagen fibers to be combined to form plywood-type patterns and the fusion of some smaller pores to generate larger ones [16, 17]. The modification of color and rigidity of the scales are also indicative of chemical and/or structural modifications. It is observed that PGA-scale did not change its main surface morphological features after Cd(II) sorption.

The TG-DTG plots of the materials are shown in Figure 2. The identification of components of biocomposites, such as fish scales using TG/DTG is not easy due to overlapping processes [18,19]. Upon continuous heating, fish scales have a sequence of irreversible thermal decomposition reactions. For the TG/DTG plots of the raw scale, it has shown four main mass loss regions: 25–215, 215-410, 410-500, 500-750 °C. The first event has been related to the superficial water releasing and the denaturation of fish scale collagen. The others correspond to the thermal degradation of the polymeric chains of collagen, possible dehydroxylation of hydroxyapatite and carbon material elimination [19]. A slightly change of slope of the TG curve at about 100 °C is due to the water removal of the first hydration shell of the collagen structure [20]. At low temperatures, collagen-water interactions are stabilized by hydrogen bonding between water molecules and terminal -OH groups, both of the primary and the secondary hydration layers. As the temperature increases, the loosely bound water molecules become free resulting in a lower residence time of the water molecules in the hydration layer of collagen [21].
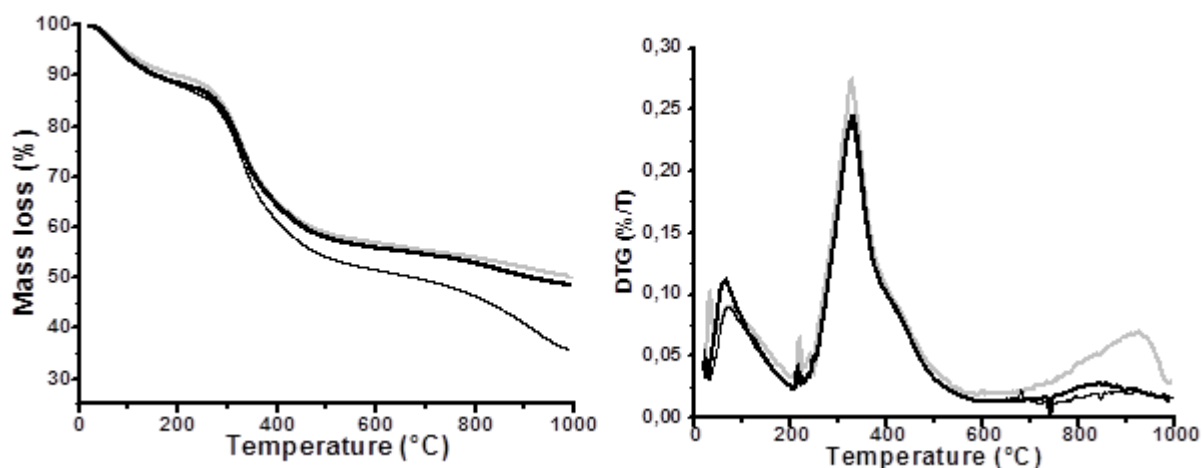

Figure 2. TG (left) and DTG (right) curves of the raw Corvina scale (thin lines), PGA-scale (thick lines) and PGA-scale/Cd(II) (grey lines).

For type I collagen, complete denaturation is observed at about 215 °C, due to the breaking of the hydrogen bonds between α-chains of collagen [18]. When collagen is subjected to high temperatures, its triple helix unfolds to produce random chains of denaturated collagen that can remain covalently linked to each other or not depending on the degree of heating. The denaturation phenomenon -distinct from degradation- implies that the rupture of interchain hydrogen bonds leads to the formation of an amorphous polymer, typically called as gelatin [18].

For the TG/DTG plots of PGA-scale, the peak due to the complete fish scale collagen denaturation is narrower in relation to the raw scale. In addition, the denaturation temperature of PGA-scale collagen is shifted toward a higher temperature (about 220 °C). It allows concluding that the thermal stability of fish scale collagen is increased due to the presence of PGA. Typically, PGA cross-linking in collagen restricts water intrusion into the collagen structure due to the decrease of the hydrophilic groups in the cross linked material [17]. Figure 3 displays the DTA curves of the scales, before and after glutaraldehyde crosslinking.

The curve of the uncrosslinked scale exhibits an initial peak centered at about 32 °C, associated to the denaturation temperature (Td) of the fish scale collagen. However, it is observed the shift of T_d to a higher value (about 55 °C) after PGA crosslinking. The increased thermal stability exhibited by fish scale collagen has been ascribed to the presence of covalent bonds formed by the crosslinking reaction [17].
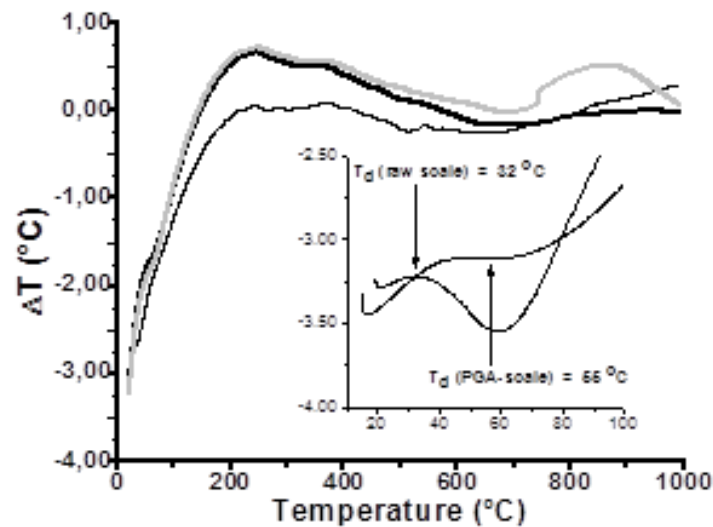
Figure 3: DTA curves of raw Corvina scale (thin line), PGA-scale (thick line) and PGA-scale/Cd(II) (grey line). The inset figure exhibits the denaturation temperatures (T_d) of raw Corvina scale and the PGA-scale.

The Raman spectra of the materials are shown in Figure 4. Raman spectra are typically obtained for surface samples just a few microns thick and with minimal interferences from surface water. However, analysis of Raman spectra of naturally-occurring materials is difficult due to the presence of broad bands and band overlappings [21]. The most prominent peaks were found in the range of Raman shift from 400 to 1800 cm^{-1} and only the strongest absorbing modes were assigned. For the raw fish scale, the strong Raman bands centered at 1676, 1450 and 1250 cm^{-1} have been assigned, respectively, to amides I, II and III vibrational modes, which involves C=O and C-N stretching, C-C-N bending, and N-H in-plane bending groups or vibration of thioester forms due to the presence of R-O-S-C- groups, all of the peptide groups of the fish scale collagen [21]. A band centered around 1070 cm^{-1} is due to vibrations of C-C, which is characteristic of α-helices of collagen. Bands for proline residues appear around 870 cm^{-1}. A sharp band centered at 645 cm^{-1} has been assigned to C=O out-of plane bending vibrations. Bands that appeared centered at 585 cm^{-1} are attributed to the symmetric O–P–O bending mode in the apatite lattice and the band centered at 870 cm^{-1} corresponds to carbonate anions substituted for phosphate ions in the apatite lattice [22]. It suggests that the fish scale is a composite consisting of type I collagen and calcium-deficient apatite containing carbonate ions.
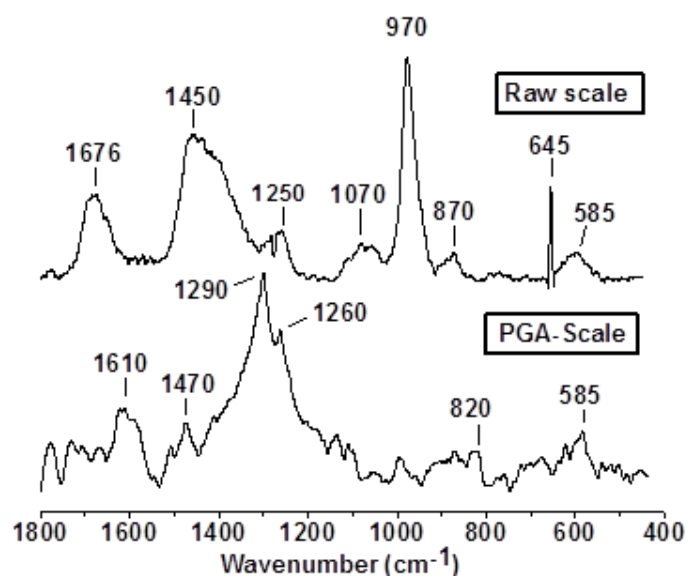

Figure 4: Raman spectra of raw Corvina scale and PGA-scale.

Chemical modification with PGA might cause effects on some amino acids of fish scale collagen. It is suggested by the observation of absence of the peak at 1070 cm^{-1}, due to reactions of PGA with amide III of α-helices of collagen [23]. The band at 1676 cm^{-1} decreased its intensity and was shifted to about 1610 cm^{-1}, due probably to reactions of PGA with amide I groups. The broad bands centered at 1470, 1290 and 1260 cm^{-1} have been attributed to the –CH$_2$ bending mode of PGA [22]. The Raman spectrum of PGA-scale with Cd(II) sorbed (not shown) has shown almost total absence of the bands observed in the PGA-Scale spectrum. However, the mineral phosphate spectral region (900-1200 cm^{-1}) was not affected. So, it is more likely that the main Cd(II) sorption sites are located in the collagen structure of the PGA-Scale.

The pH of a solution can greatly affect surface charge because functional groups present on the surface of particles can often contain oxygen or nitrogen, two atoms which can be protonated or deprotonated to become charged. Collagens contain ionizable groups, which serve to provide a surface charge when submerged in an aqueous solution [24]. Thus, as the pH changes, so does the surface charge of the material. The pH where the net total particle charge is zero is called the point of zero charge (pHpzc). If the pH is above its pzc the adsorbent surface will have a net negative charge and predominantly exhibit an ability to exchange, while the adsorbent will mainly retain anions (electrostatically) if its pH is below its pzc. Typically, the collagen net charge depends on the number and location of amino acids and the solvent pH [24].

Most collagens contain many lysine residues, usually located on the protein surface (i.e., exposed to the aqueous medium) because of the polarity of amine groups [6]. The unprotonated amino groups are very reactive as nucleophilic agents. It should be noted that lysine ε-amino groups have pK$_a$ > 9.5, but it is presumed that the small percentage of amines present in their unprotonated form at lower pH is sufficient to react with GA, which then drives the acid-base equilibrium to deprotonation of these groups for further reaction. The α-carboxyl group of proteins has a pK$_a$ of about 2 and the α-amino group has a pK$_a$ of around 9.5. Asp and glu have pK$_a$ around 4.0, cys, thr and lys have around 10, arg has around 12.5, and his at around 6.0. At neutral pH, aspartic and glutamic acids carry a negative charge and arg and lys carry negative charges.

When the pH becomes more alkaline, lys and arg residues lose their positive charge and become neutral at pH 12. If the pH is made more acidic, aspartic and glutamic acids shed their negative charge and become neutral. Collagen also possesses amino acids having pK$_a$ ~ 11, which become more positively charged at low pH values and the majority of these residues should remain positive even at high pH. Aldehydes are expected to form Schiff bases upon nucleophilic attack by lysine residues in collagens. However, some Schiff bases are unstable in solution and tend to break down to regenerate the aldehyde in solution and collagen-linked amines [6, 7]. These amine groups are expected to be strongly basic, which can increase the value of the pH$_{pzc}$ of PGA-scale (7.60), compared to the pH$_{pzc}$ of the raw scale (7.00) [13].

In this work, solid-state diffuse reflectance (DR) is used to complement the morphological studies of the fish scales. However, band overlapping and poor spectral resolutions preclude details of the chemical environment of the materials by DR. The DR spectra of the materials are shown in Figure 5. For the raw scale, it is observed a broad and small peak centered at about 415 nm, due to the presence of chromophores of collagen [25]. For PGA-scale, there are the presence of two broad peak centered at 400 and 440 nm, due to PGA itself. After interaction with Cd(II), a new broad peak centered at 465 nm is also observed, due probably to the presence of Cd(II)-collagen complexes [25]. However, we are unable to discuss additional structural features of the fish scale collagen-sorbed Cd(II) compounds, using the experimental results of this work. In addition, this specific aspect has assumed to be beyond the scope of this work.
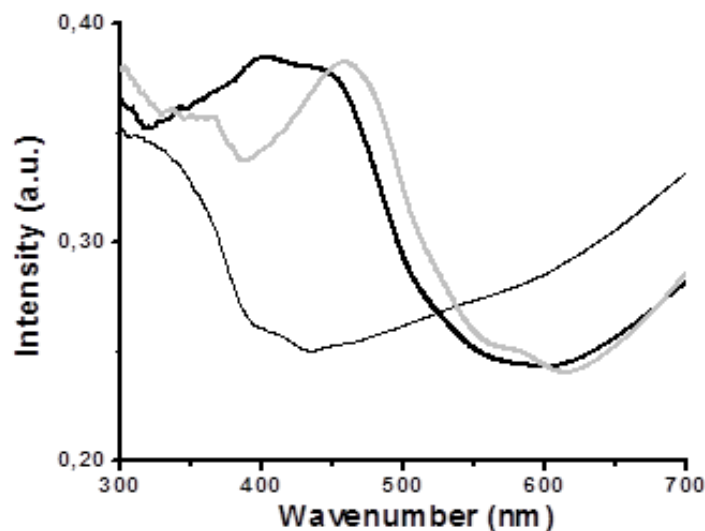
Figure 5: Solid-state diffuse reflectance spectra of the raw Corvina scale (thin line), PGA-scale (thick line) and PGA-scale/Cd(II) (grey line).

3.3. Analysis of Cd(II) sorption on PGA-scale by solution calorimetry

Mechanisms of interaction at solid/solution interfaces, whether surface adsorption, surface precipitation, co-precipitation, and pure solid formation are often difficult to distinguish experimentally. Typically, interaction involves a progression of these processes. The term sorption is used when the actual interaction mechanism is not known [1]. The highest adsorbed amounts of Cd(II) on PGA-scale were found using Cd(II) solutions at pH of 8.0, above the pH_{pzc} of PGA-scale (7.60). In this work, no precipitation of cadmium hydroxides in solution was detected.

Generally, the sorption of cations is favored at $pH>pH_{pzc}$. As the pH increases, the sorption sites of the adsorbent are progressively deprotonated, leading to an in increase of sorption of cationic species. The sorption of metals on solid adsorbents is also affected by the electrostatic attraction/repulsion between the surface charges and the dissolved ions in solution, as well as the protonation of the sorption sites on the adsorbent [26]. The sorption behavior reflects both the speciation of Cd(II) in solution as well as the surface charge characteristics of the solid. The adsorbent surface charge will be positive below PZC and negative above this pH. Dominative cadmium species in solution are "free" Cd^{2+} (at pH between 2 − 8) and $[CdOH]^{+}$ (at pH between 8.5 − 9.5) [27]. Indeed, at $pH>pH_{pzc}$, an appreciable Cd(II) sorption capacity was observed, suggesting that in addition to the electrostatic interaction, other mechanism may also coexist during the sorption processes.

The initial concentration provides an important driving force to overcome all mass transfer resistances of the adsorbate between the aqueous and solid phases [1]. In this work, a higher initial concentration of Cd(II) has enhanced the sorption amounts, as demonstrated in Table 1. These results indicate that the observed affinity of Cd(II) for PGA-scale is concentration dependent. The maximum sorption capacity observed of 438 $\mu mol\ g^{-1}$ (about 49.5 $mg\ g^{-1}$) for Cd(II) on PGA-scale is superior with many reported data in the literature [28].

Table 1: Solution calorimetry parameters of Cd(II) sorption on PGA-scale.

TEMP. °C	$C_I / 10^{-3}/$ MOL L^{-1}	$-Q_{INT}/$ J G^{-1}	$N_{INT}/$ MMOL G^{-1}	$-\Delta_{INT}H/$ KJ MOL^{-1}
30	4.50	0.65	52.4	12.5
50	4.50	45.72	71.2	642.2
30	45.0	0.83	375.0	2.2
50	45.0	51.86	438.0	118.4

The average SD of the results was less than 6.7 %

As most chemical and physical processes are accompanied by heat effects, isothermal solution calorimetry represents a unique technique to obtain direct information of thermodynamics and kinetics. A typical profile of the calorimetric interaction processes is illustrated in Figure 6. It should be known that the heat measured from solution calorimetry is an integral heat since there are different sites for Cd(II) interaction in PGA-scale [29]. The base line used for the integrations was selected as linear from first to last point, joining the two extreme points selected on the curves. The calorimetric parameters of the Cd(II) sorption on PGA-scale are presented in Table 1. The non-symmetric shapes of the calorimetric plots indicate that the Cd(II) sorption sites of PGA-scale are not energetically uniform. For low crystalline materials, such as PGA-scale the sorption sites are typically heterogeneous, and the number of these sites depends on their distribution and the structural defects of sorption sites on the surface [30]. In general, a sharp increase in heat flow occurred in the first 5 min of interaction followed by a sharply decreasing with coverage. The sorption is very fast between t=0 and 25 min, and only a few calorimetric points could be measured in this period with the calorimeter set up. At sorption time > 30 min the Cd(II) sorption takes place at slower rates, suggesting that available sites on the adsorbent are the limiting factor for the sorption. Although it seems to reach completion at around 50-60 min, some long-term calorimetric experiments showed that sorption can continue for longer times, but very slowly. Indeed, after 60 min, the evolved heat becomes too low to be accurately separated from the instrumental noise.

The interaction enthalpy ($\Delta_{int}H$) can be calculated directly by the following Eq. (2) [29]:

$$\Delta_{int} H = \frac{\int_{t_i}^{t_f} dQ_{int}}{n_{int}} \tag{2}$$

where Q_{int} the integral interaction energy (J g^{-1}) and n_{int} is the number of moles of Cd(II) adsorbed (mol g^{-1}).

The enthalpies of Cd(II) sorption on PGA-scale are all exothermic. The magnitude of $\Delta_{int}H$ increased as the Cd(II) sorption is increased. Indeed, the increasing of $\Delta_{int}H$ with adsorbent coverage is the most frequent situation. At higher loading, repulsive lateral interactions between the sorbed species, which are endothermic, might increase, decreasing the exothermic net $\Delta_{int}H$ [31]. The $\Delta_{int}H$ value (calorimetrically measured) can also be used as a measure of the interaction forces between adsorbate and adsorbent, giving indications of the bonding strengths. Exothermic $\Delta_{int}H$ may suggest the dominance of attractive forces between the sorption sites and the adsorbing species or attractive forces between sorbed molecules on the sorption sites [30].
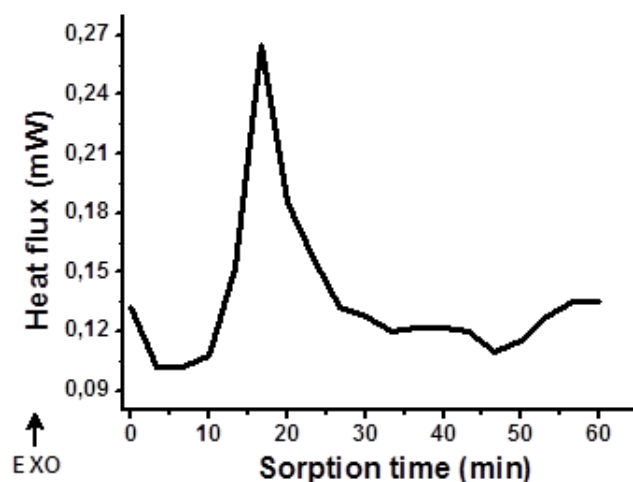
Figure 6: Typical solution microcalorimetry plot of Cd(II) sorption on PGA-scale. Experimental conditions: Initial Cd(II) concentration in solution = 4.50×10^{-4} mol L^{-1}, mass of adsorbent: 100 mg, temperature: 30 °C.

Several possible mechanisms have been proposed for sorption processes at solid/solution interfaces, including surface precipitation, intraparticle diffusion or diffusion into pores, surface binding heterogeneity and others [1]. Some effects may contribute to the sorption enthalpies, rendering it a complex quantity: the energies of the surface bonds, changes in degrees of freedom of the atoms/molecules, the energies of interactions between the adsorbed species, surface relaxations or rearrangements. The values of sorption enthalpies at solid/solution interfaces are average results of exothermic chemical bonding and endothermic diffusional interaction processes [29]. Solvent and solute transport through adsorbent materials is generally described following sorption–diffusion mechanisms, where molecules first diffuse from the bulk phase to the adsorbent surface. Next, they adsorb to the sites on the surface and/or diffuse through the adsorbent structure, driven by the chemical potential gradient within the adsorbent pores [29]. Typically, interactions that occur with adsorbate diffusion present small values of $\Delta_{int}H$ (in this work, -2.2 and -12.5 kJ mol^{-1}).

The forces involved in sorption processes include non specific van der Waals or electrostatic interactions, and specific forces involved in the formation of chemical bonds. Sorption processes that involve only non specific interactions are generally referred to as "physical sorption" while those in which stronger interactions occur are termed "chemisorption". Most covalent bond energies fall in the range of -100 to -1000 kJ mol^{-1} and generally decrease in strength as the bond length increases [32]. In this work, the high exothermic results observed are in agreement with interaction of Cd(II) to sorption sites at the PGA-scale surface and chemisorption may be an important mechanism. However, from data in Table 1 it is clear that the sorption energies and enthalpies vary with the initial Cd(II) concentration in solution and temperature. Thus, a simple sorption mechanism in a single step cannot be postulated and a more complicated sorption mechanism seems to be operating.

4. CONCLUSION

In this work, Brazilian Corvina fish scales were cross linked with polyglutaraldehyde under acidic catalysis and used for Cd(II) sorption from aqueous solutions. Characterization has pointed out a higher denaturation temperature of fish scale collagen after PGA crosslinking. The Raman spectra of the materials have suggested that the main sites for Cd(II) sorption are located in the collagen structure of the PGA-Scale.

The interaction of Cd(II) with PGA-scale from aqueous solutions was studied by isothermal solution calorimetry. The non-symmetric shapes of the calorimetric plots indicate that the sorption sites of the adsorbent are not energetically uniform. The values of $\Delta_{int}H$ of Cd(II) sorption on PGA-scale were all exothermic. The magnitude of $\Delta_{int}H$ increased as the surface coverage is increased, suggesting repulsive lateral interactions between adsorbed species and that chemisorption may occur at the PGA-scale/Cd(II) interfaces. The analysis of the calorimetric results has suggested that affinity of Cd(II) for PGA-scale is both concentration and temperature dependent. The maximum sorption capacity observed for Cd(II) on PGA-scale is superior with many usual biosorbents.

Additional features of the Cd(II)/PGA-scale interactions will be determined in future using sorption isotherms. Anyway, the results of the present work underline the good features of the crosslinked Corvina fish scales as a promising material for sorption of Cd(II) from aqueous media.

5. ACKNOWLEDGEMENTS

6. BIBLIOGRAPHY

[1] RAO, KS, MOHAPATRA, M, ANAND, S, *et al*., "Review on cadmium removal from aqueous solutions", *International journal of engineering science and technology,* v. 2, n. 7, pp. 81-103, Jan. 2010.

[2] FU, F., WANG, Q. "Removal of heavy metals ions from wastewaters: A Review", *Journal of Environmental Management*, v. 92, pp 407-418, Mar. 2011.

[3] LODEIRO, A.P, HERRERO, A.R, VICENTE, M.E.S. "Thermodynamic and Kinetic Aspects on the Biosorption of Cadmium by Low Cost Materials: A Review", *Environmental Chemistry*, v. 3, n. 6, pp. 400-418, Dec. 2006.

[4] STOQUART, C, SERVAIS, P, BÉRUBÉ, PR, *et al*., "Hybrid Membrane Processes using activated carbon treatment for drinking water: A review", *Journal of Membrane Science*, v. 411–412, pp. 1–12, Sep. 2012.

[5] PATI, F., ADHIKARI, B., DHARA S. "Isolation and characterization of fish scale collagen of higher thermal stability", *Bioresource Technology*, v. 101, pp. 3737–4372, May 2010.

[6] MIGNEAULT, I., DARTIGUENAVE, C., BERTRAND, M.J., *et al*., "Glutaraldehyde: behavior in aqueous solution, reaction with proteins, and application to enzyme crosslinking", *Biotechniques,* v. 37, n, 5, pp. 790-802, Nov. 2004.

[7] DAMINK, L.H.H.O, DIJKSTRA, P.J, VAN LUYN, M.J.A, *et al*., "Glutaraldehyde as a crosslinking agent for collagen-based biomaterials", *Journal of Materials Science: Materials in Medicine*, v. 6, pp. 460-472, Aug. 1995.

[8] SRIVIDYA, K., MOHANTY, K. "Biosorption of hexavalent chromium from aqueous solutions by Catla catla scales: Equilibrium and kinetics studies", *Chemical Engineering Journal*, v. 155, pp. 666–673, Dec. 2009.

[9] FERGUSON, H.F, FRURIP, D.J., PASTOR, A.J, *et al*., "A review of analytical applications of calorimetry", *Thermochimica Acta,* v. 363, n. 1, pp. 1-21, Nov. 2000.

[10] PUZIY, A.M., PODDUBNAYA, O.I, MARTÍNEZ-ALONSO, A., *et al*., "Synthetic carbons activated with phosphoric acid: I. Surface chemistry and ion binding properties", *Carbon,* v. 40, pp. 1493-1505, Aug. 2002.

[11] VIEIRA, E.F.S, CESTARI, A.R, SILVA, R.G, *et al*., "Use of calorimetry to evaluate cement slurry resistance to the attack of acid solutions", *Thermochimica Acta,* v. 419, pp. 45-49, Sep. 2004.

[12] HASHEM, E.Y, "Spectrophotometric studies on the simultaneous determination of cadmium and mercury with 4-(2-pyridylazo)-resorcinol", *Spectrochimica Acta,* v. 58, pp. 1401-1410, May 2002.

[13] MOURA, K.O, VIEIRA, E.F.S, CESTARI, A.R. "The use of solution microcalorimetry to evaluate chemically modified fish scales as a viable adsorbent for heavy metals", *Journal of Thermal Analysis and Calorimetry*, v. 107, pp. 999-1005, May 2011.

[14] BIGI, A., BURGHAMMER, M., FALCONI, R., *et al*., "Twisted plywood pattern of collagen fibrils in teleost scales: An X-ray diffraction investigation", *Journal of Structural Biology,* v. 136, n. 2, pp. 137-143, Nov. 2001.

[15] TANRISEVEN, A., ZEHRA, O. "A novel method for the immobilization of glucoamylase onto polyglutaraldehyde-activated gelatin", *Biochemical Engineering Journal*, v. 39, pp. 430-434, May 2008.

[16] MA, L., GAO, C., MAO, Z., *et al*., "Collagen/chitosan porous scaffolds with improved biostability for skin tissue Engineering", *Biomaterials,* v. 24, n. 26, pp. 4833–4841, Nov. 2003.

[17] KIKUCHI, M., MATSUMOTO, H.N, YAMADA, T., *et al*., "Glutaraldehyde cross-linked hydroxyapatite/collagen self-organized nanocomposites", *Biomaterials,* v. 25, n. 1, pp. 63–69, Jan. 2004.

[18] SAMOUILLAN, V., DELAUNAY, F., DANDURAND, J., *et al.*, "The use of thermal techniques for the characterization and selection of natural biomaterials", *Journal of Functional Biomaterials*, v. 2, n. 3, pp. 230-248, Sep. 2011.

[19] XIA, Z., CALDERON-COLONA, X., TREXLERA, M., *et al.*, "Thermal denaturation of type I collagen vitrified gels", *Thermochimica Acta,* v. 527, pp. 172–179, Jan. 2012.

[20] HORN, M.M, MARTINS, V.C.A, PLEPIS, A.M.G. "Interaction of anionic collagen with chitosan: Effect on thermal and morphological characteristics", *Carbohydrate Polymers,* v. 77, pp. 239–243, Jun. 2009.

[21] IKOMA, T., KOBAYASHI, H., TANAKA, J., *et al.*,"Microstructure, mechanical, and biomimetic properties of fish scales from Pagrus major", *Journal of Structural Biology,* v. 142, pp. 327–333, Jun. 2003.

[22] KUMAR, R., SRIPRIYA, R., BALAJI, S., *et al.*, "Physical characterization of succinylated type I collagen by Raman spectra and MALDI-TOF/MS and in vitro evaluation for biomedical applications", *Journal of Molecular Structure,* v. 994, pp. 117-124, May 2011.

[23] GU, Y.W, TAY, B.Y., LIM, C.S., *et al.*, "Biomimetic deposition of apatite coating on surface-modified NiTi alloy", *Biomaterials,* v. 26, pp. 6916-6923, Dec. 2005.

[24] PARK, J.J, WEIGER, M.C, LACERDA, S.H.P., *et al.*, "Characterization of Non-Equilibrium Nanoparticle Adsorption on a Model Biological Substrate", *Langmuir,* v. 26, n. 7, pp. 4822–4830, Jan. 2010.

[25] LIU, X., TANG, R., HE, Q., *et al.*, "Fe(III)-Immobilized Collagen Fiber: A Renewable Heterogeneous Catalyst for the Photoassisted Decomposition of Orange II", *Industrial & Engineering Chemistry Research*, v. 48, n. 3, pp. 1458–1463, Jan. 2009.

[26] HU, J-L., HE, X-W, WANG, C-R, *et al.*, "Cadmium adsorption characteristic of alkali modified sewage sludge", *Bioresource Technology,* v. 121, pp. 25-30, Oct. 2012.

[27] FERNÁNDEZ-NAVA, Y., ULMANU, M., ANGER, I., *et al.*,"Use of Granular Bentonite in the Removal of Mercury (II), Cadmium (II) and Lead (II) from Aqueous Solutions", *Water, Air, & Soil Pollution*, v. 215, pp. 239–249, Feb. 2011.

[28] YADANAPARTHI, S.K.R, GRAYBILL, D., VON WANDRUSZKA, R. "Adsorbents for the removal of of arsenic, cadmium and lead from contaminated waters", *Journal of Hazardous Materials*, v. 171, pp. 1-15, Nov. 2009.

[29] VIEIRA, E.F.S., COSTA, L.P, CESTARI, A.R. "Preparation and characterization of polyalginate-glutaraldehyde membranes-swelling analysis by microcalorimetry and adsorption kinetics of cationic dye", *Journal of Applied Polymer Science,* v. 118, pp. 857-865, Oct. 2010.

[30] DRAGO, B., GERVASINI, A., DUMITRIU, E., *et al.*, "Calorimetric determination of the acidic character of amorphous and crystalline aluminosilicates", *Thermochimica Acta,* v. 420, pp. 127-134, 2004.

[31] BOLESLAV, T. "Adsorption heats of phenol on activated carbon using adapted method of immersion calorimetry", *Journal of Thermal Analysis and Calorimetry*, v. 107, pp. 923-926, Mar. 2012.

[32] OSTROVSKII, V. E. "Review of the molar heats of chemisorption and chemabsorption by crystalline oxides and the surface "homogeneity versus heterogeneity" problem", *Journal of Thermal Analysis and Calorimetry,* v. 108, n. 2, pp. 559-581, Jun. 2012.