Chitosan and Chitosan Modified with Glutaraldehyde Microparticles for Pb(II) Biosorption

I. Microparticles preparation and characterization

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This paper presents the physical and chemical changes in chitosan flakes, having as purpose the improving of mechanical strength, and the enhancing of the resistance of chitosan against acid, alkali and other chemicals. These changes led to obtaining chitosan and cross-linked with glutaraldehyde (GLA) chitosan microparticles, by means of a simple and versatile method, for use in the retention of Pb(II) ions from aqueous solutions. Chitosan and chitosan-GLA particles obtained were analyzed by FT-IR spectroscopy, Scanning Electron Microscopy (SEM) and TG/DSC. Stability of chitosan, and chitosan–GLA particles in media with different pH values was tested. Porous microparticles with high stability in acid solutions were obtained by cross-linking of chitosan with GLA.

Keywords: chitosan microparticles, chitosan-GLA microparticles, physical and chemical

At a global level there is a significant concern regarding the synthesis of the materials with controlled properties, with apllications in environmental remediation processes. Environments contaminated with metals have been extensively studied in recent years, due to the negative effects of the heavy metals on living organisms (plants, animals, humans). The major concerns at global level are environment quality, especially water quality and accessibility of all planet people to safe drinking water. The presence of heavy metals in low concentratios in the ecosystems is a serious problem for society due to their negative effects on human bodies, flora and fauna from environment.

Conventional treatment methods of metal-bearing effluents include ion exchange, chemical precipitation, membrane separation, electrochemical treatment and adsorption. Adsorption-based technology has considered being of high efficiency, cost-effectiveness, simple operation, and environmental friendly [1, 2]. The most commonly used adsorbents are zeolites, activated carbon, clays, inorganic materials, biomass and polymeric materials [3-9]. Some of these adsorbents have low sorption capacity, as well as the inconvenience regarding their separation.

Therefore, there is a need to develop new adsorbents with improved surface characteristics and performance in terms of selectivity and sorption capacity.

Lately, there has been an important concern regarding the synthesis of materials with controlled properties and applications for remediation of contaminated environments.

Chitosan – a linear polysaccharide based on a glucosamine unit derived from chitin is one of the materials extensively studied recently due to its adsorbent properties. The amine and hydroxyl groups of chitosan chains are responsible for the sorbent properties of chitosan. The complexing capacity of chitosan, low toxicity, biocompatibility, and biodegradability are the main characteristics which make it suitable for removal and recovery of metal ions from wastewater [10, 11]. A high capacity for chelating metal ions has obtained for highly deacetylation of chitin. As a result, chelation has related to the content of the amino groups in the polymer chain, and the degree of polymerisation of oligochitosan.

Due to its poor mechanical properties, and low stability in acidic medium, physical and chemical changes have been performed on chitosan. Chitosan based materials have been used in various fields as powders, flakes, particles, membranes, and fibers. It is not recommended to use chitosan flakes as adsorbent because of their low surface area and low porosity. In order to be easily recovered, chitosan was prepared as microspheres with stability to chemicals (acids, bases), and high retention capacity compared to the chitosan flake or powder. In addition, it is well known that the microspheres have different physical and optical properties that can be advantages in areas such as separation, adsorption and biosensors [11, 12]. Chemical modification of chitosan is interesting because major changes do not occur along with its basic network. Materials with new and improved properties can be obtained. Chemical modification of chitosan may include cross-linking, grafting of a new functional group and acetylation. Cross-linking agents such as epichlorohydrin (ECH) [11], ethylene glycol diglycidyl ether (EGDE) [13], glyoxal [14], benzoquinone [15], and cyclodextrin (CD) [16] can be used for improving chitosan stability in acidic media (pH< 2), for enhancing adsorption capacity, or both.

The present study explores the process of obtaining of microparticles of chitosan and chitosan modified using glutaraldehyde (GLA) as crosslinking agent to increase stability in acidic medium. It will achieve complex studies to establish the influence of chitosan-glutaraldehyde ratio, and the washing agent on the particles size, their sphericity, porosity and crystallinity - characteristics that have influenced the sorption capacity.

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Fig. 1. Chitosan (a) and chitosan-GLA microparticles (b, c)

Experimental part

Samples of chitosan flakes highly viscous (85% deacetylation, a viscosity > 200cps and the total impurities ≤ 1%) purchased from Sigma Aldrich Chemie GmbH were used for physical and chemical changes. Glutaraldehyde solution 50% was purchased from Fluka.

Chemical precipitation of NaOH 0.5M on a Heidolph stirrer MR Hei-Standard at 250 rpm was applied.

Preparation of chitosan microparticles

Chitosan microparticles were prepared by chemical precipitation using the procedure described in [17]. The chitosan solution was prepared by dissolving 3.5 g of chitosan flakes in 250mL of 5% (v/v) acetic acid. The viscous solution was left overnight. Drops of chitosan solution were added under a continuous stirring into a 500mL of 0.50M NaOH. This, in order to ensure that the acetic acid has neutralized and to coagulate the chitosan gel into spherical uniform, gel beads. After 24 h, the microparticles were separated from the liquid phase and washed out with distilled water. The microparticles obtained were kept for 24 h in an aqueous solution of ethyl alcohol in a continuous stirring in order to maintain their shape and to increase mechanical strength. If they have not been kept in an aqueous solution of ethyl alcohol, they stick to each other, and a compact mass and not beads will be obtained. Finally they were air-dried, and white microparticles were obtained (fig. 1a).

Preparation of chitosan-GLA microparticles

The chitosan microparticles previously prepared were used for the preparation of chitosan–GLA microparticles. By suspending the extensively rinsed chitosan beads in a 0.05M or 0.1M glutaraldehyde solution, ratio of 1:1 and 1:2 with chitosan (mole CHO from GLA:mole NH_2 from chitosan) were obtained. The chitosan beads were kept under a continuous stirring for 24 h in glutaraldehyde solution at room temperature. New resulted chitosan–GLA microparticles were filtered, rinsed out with hot distilled water and then with cold distilled water. These new chitosan–GLA microparticles were maintained for 24 h in an aqueous solution of ethyl alcohol under continuous stirring. They were air dried, and particles from light to dark redbrick were obtained. Particles' colour intensity depends on the cross-linking ratio. The light redbrick particles were obtained for 1:1 glutaraldehyde:chitosan ratio (fig. 1b), and the dark redbrick particles were obtained for 2:1 glutaraldehyde:chitosan ratio (fig. 1c).

Characterization of chitosan, and chitosan-GLA microparticles

Physical characteristics of chitosan particles, chitosan–GLA 1:1, 1:2 ratio particles were determined in order to investigate their adsorption behaviour. Thus, the FT-IR spectra were recorded on a FT-IR 620 (Jasco, Japan) Spectrometer in the 400-4000 cm^{-1} range using potassium bromide pellets. Scanning electron microscopy (SEM) was used to determine morphology, shape and size of particles obtained. CarlsZeiss Auriga scanning electron microscope was used. Thermal analysis was made with a Netzsch STA 449C Jupiter equipment, using Al_2O_3 crucible in air atmosphere (20mL min^{-1}) to 900 °C, with a heating rate of 10K min^{-1}.

Stability of chitosan particles, chitosan–GLA 1:1, 1:2 ratio particles in media with different *p*H values was tested. 5% (v/v) acetic acid, distilled water and 0.10M NaOH solution were used for this purpose.

Results and discussions

Chitosan and chitosan-GLA particles were analyzed by FT-IR spectroscopy, SEM and TG/DSC before the use of Pb(II) ions removal from aqueous solutions.

FT-IR spectra were recorded on chitosan and chitosan - GLA microparticles. The main peaks and their assignments are presented in table 1.

Data presented in table 1 indicate that chitosan-GLA microparticles have a new peak at ~ 1660 cm^{-1} specific to imine bond (C=N) [18]. This peak is not presented in chitosan FT-IR spectrum and can be due to the reaction between amino groups of chitosan and aldehyde groups of GLA. The absence of peak specific to carbonyl bond (C=O) that can be found in FT-IR spectrum at ~ 1710 cm^{-1} confirms involving of the carbonyl group in cross-linking. Furthermore, the peak at 1102 cm^{-1} was shifted to peaks at 1116 cm^{-1} and 1120 cm^{-1}, respectively. This means that nitrogen atom of amino group of chitosan is involved in cross-linking with aldehyde group of GLA.

All three FT-IR spectra present a peak in the wavenumbers range 3400-3500 cm^{-1}, which is specific to

Table 1

FT-IR PEAK ASSIGNMENTS OF CHITOSAN, CHITOSAN-GLA 1:1 AND CHITOSAN-GLA 1:2 MICROPARTICLES

Materials	FT-IR peaks					
	ν(O-H) (cm^{-1})	ν(C-H) (cm^{-1})	ν(C=N) (cm^{-1})	ν(C=C) (cm^{-1})	ν(C-O) (cm^{-1})	ν(C-N) (cm^{-1})
Chitosan microparticles	3400	2875	-	1584	1370	1102
Chitosan-GLA 1:1 microparticles	3455	2890	1658	1570	1375	1116
Chitosan-GLA 1:2 microparticles	3430	2890	1660	1563	1378	1120

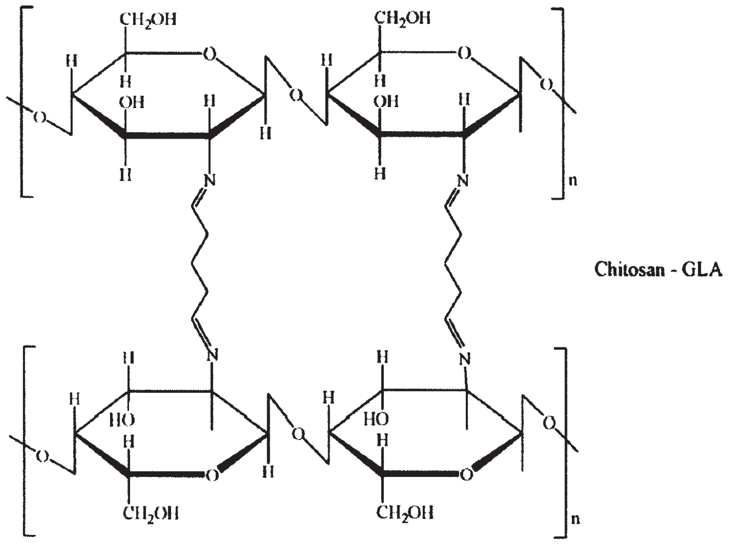

Fig. 2. Chemical structure of chitosan-GLA particles

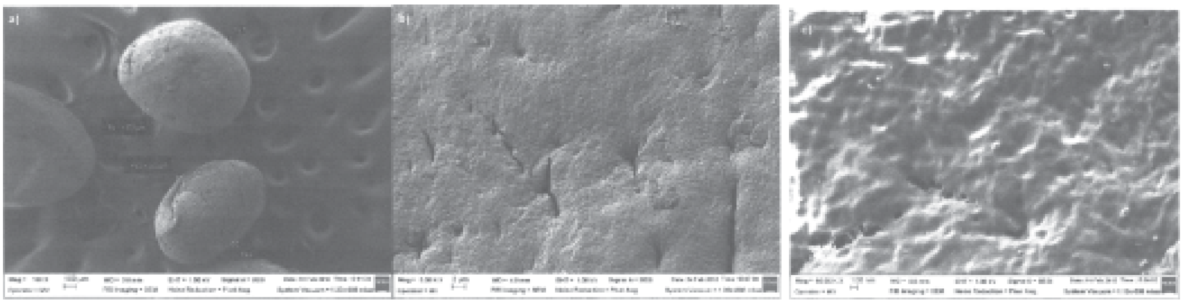

Fig. 3. SEM images of chitosan particles obtained at different magnifications

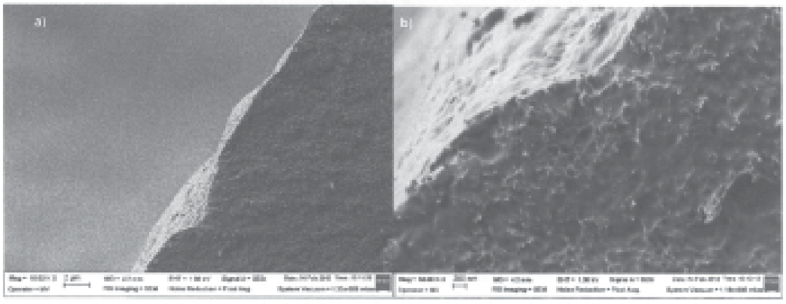

Fig. 4. SEM images of the chitosan particle's surface obtained by cutting the particles

–O-H stretching vibration [19, 20], and a peak at ~1380 cm^{-1} assigned to –C-O- stretching of primary alcoholic group [21]. The peak at 3400-3500 cm^{-1} overlaps with peak assigned to -N-H stretching vibration [22-24]. Other important aspect regarding FT-IR spectra of chitosan-GLA microparticles is that, the intensity of peaks increases with the increasing of chitosan:GLA ratio. These results of FT-IR analysis indicated that chitosan reacts with GLA by amino groups of chitosan and aldehyde groups of glutaraldehyde according with a Schiff base reaction (fig. 2).

Scanning electron microscopy was used to determine shape, morphology and size of the particles obtained. SEM images obtained are presented in the figures 3-6.

The particles resulted by physical modification of chitosan are of hemispherical origin (some look like frozen drops), slightly stretched with an average diameter between 600 and 900μm (fig. 3a). The average diameter value of chitosan particles makes them be part of microparticles category. By analysing the surface morphology, it was found that this material presents several types of agglomeration of material (fig. 3b and c). The surface presents smooth areas (one polymer layer) that cause a rheological flow aspect (fig. 3b and c). Cutting microparticles, the section presented in figures 4a and b was obtained.

These figures show a closed porosity (not a connected porosity) of chitosan microparticles. The shape of the synthesised chitosan-GLA particles is not very different from that of chitosan microparticles obtained. From the morphological point of view, it can be also seen a different surface organization (fig. 5). Another organization of surface from morphological point of view can also be observed.

Figure 5a shows that the chitosan-GLA (1:1) particles are spherical slightly stretched. The average diameter of these particles is between 1 and 1.29 mm. Consequently, they can be included in the microparticles category. It can be observed (fig. 5b and c) that the cross-linked polymer has a more porous structure (numerous interconnected pores) than that of chitosan microparticles.

The shape of chitosan-GLA (1:2) particles is the same with that of the chitosan-GLA (1:1) particles (fig. 6a), but they are different from the morphological point of view (fig. 6b, c).

Differences in height from topographic point of view (fig. 6b and c) can be observed for chitosan-GLA (1:2) particles. Porosity is increased, and a fibrillar structure is obtained. The size of particles is in the range from 1 to 1.33 mm (fig. 6a). Surface morphology is completely different (fig. 6b, c, and d). Furthermore, a large amount of cross-

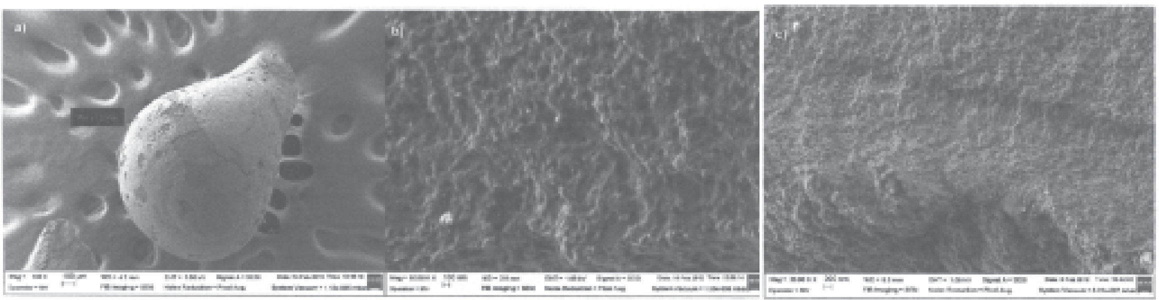

Fig. 5. SEM images of chitosan-GLA (1:1) particles and chitosan-GLA particle's surface obtained at different magnifications

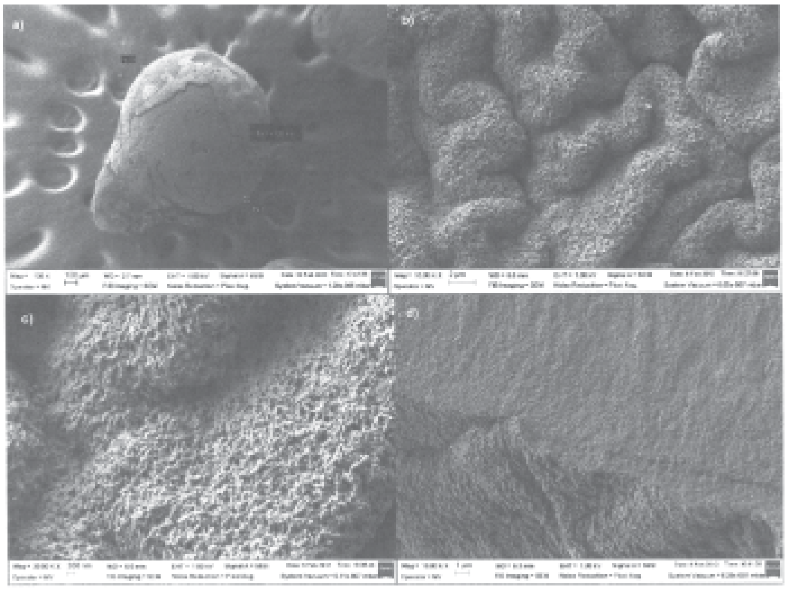

Fig. 6. SEM images of chitosan-GLA (1:2) particles and chitosan-GLA particle's surface obtained at different magnifications

linking agents leads to the obtaining of a higher porosity material. For chitosan-GLA (1:2) microparticles, it can be observed a more porous, expanded structure (intrinsic porosity) (figs. 6b, c and d). Conclusion is that cross-linking has as result the increasing of the porosity, and consequently the increasing of the adsorption capacity.

Thermal analysis of the microparticles obtained was performed in order to determine the influence of cross-linking to the thermal stability of the chitosan microparticles. Thus, TG/DSC curves of chitosan flakes, chitosan microparticles, and chitosan-GLA (1:2) microparticles are presented on the figure 7.

One initial weight loss up to 160°C accompanied by an endothermic effect on DSC curve of 7.83% (fig. 7a) was observed for chitosan flakes. This loss of weight can be attributed to remove water that is embedded on chitosan flakes. This step is followed by a stability phase up to 235°C when the second step of decomposion starts. Because this step is followed by an exothermic effect (with a peak at 296°C) it can be regarded as partial oxidation of chitosan molecules. Complet combustion of chitosan takes place slowly between 310°C and 620°C. This step is accompanied by the releasing of a large amount of energy which can be seen on DSC curve as a broad and intense peak. The combustion of carbon traces remained after the previous decompositions takes place at around 700°C.

The loss of weight due to the water molecules takes place up to 160°C (6% weight loss) for chitosan microparticles. The second step at 160-310°C takes place with a loss of weight at about 43.01%. At temperatures higher than 310°C chitosan microparticles are slowly and continously decomposed, much faster after 500°C. This is probbaly due to the oxidation reaction of carbon mass which remains after the previous decomposition process. The oxidation process is supported by a strong exothermic effect accompanying the weight loss.

Even the TG/DSC curve of the chitosan-GLA curve has a similar shape, although there are some significant differences (fig. 7c). The first loss weight step takes place slowly up to 160°C, and then faster up to 200°C (5.84% weight loss) than the first step. This can indicate a small amount of GLA retained in the chitosan-GLA structure that requires a higher energy to be removed (GLA is retained by strong bonds or can be encapsulated on chitosan structure). This step is followed by a weak endothermic effect represented by a flattened peak on DSC curve (fig. 7c).

The first decomposition step takes place at a lower temperature than in case of chitosan flakes, and reaction starts at 200°C being accompanied by two extothermic effects with lower intensity on DSC curve. The fact that two effects can be identified means that the decomposition step consists of at least two separate reactions. Complete combustion takes place then up to 640°C. This value is higher than in case of chitosan flakes (620°C) due to the cross-linking of GLA molecules on chitosan structure. It can be noticed also that combustion reaction is accompanied by a high release of energy. The wide and strong peak that accompanies this process on DSC curve has a maximum at 590°C that is different from 574°C for chitosan flakes, and 546°C for chitosan microparticles.

Differences between thermal decomposition of chitosan flakes and chitosan microparticles might be due to the physical structure of the particles. Thus, water is removed from microparticles (high volume and small surface) more slowly than from flakes with a larger surface. On the other hand, the decomposition temperature is higher with about 20°C in case of flakes rather than microparticles. This trend changes at temperatures higher than 400°C. It can be considered that carbon mass remained after chitosan decomposition is less compact in case of flakes than in case of microparticles, and its decomposition takes place much faster. As a conclusion, the decomposition way is

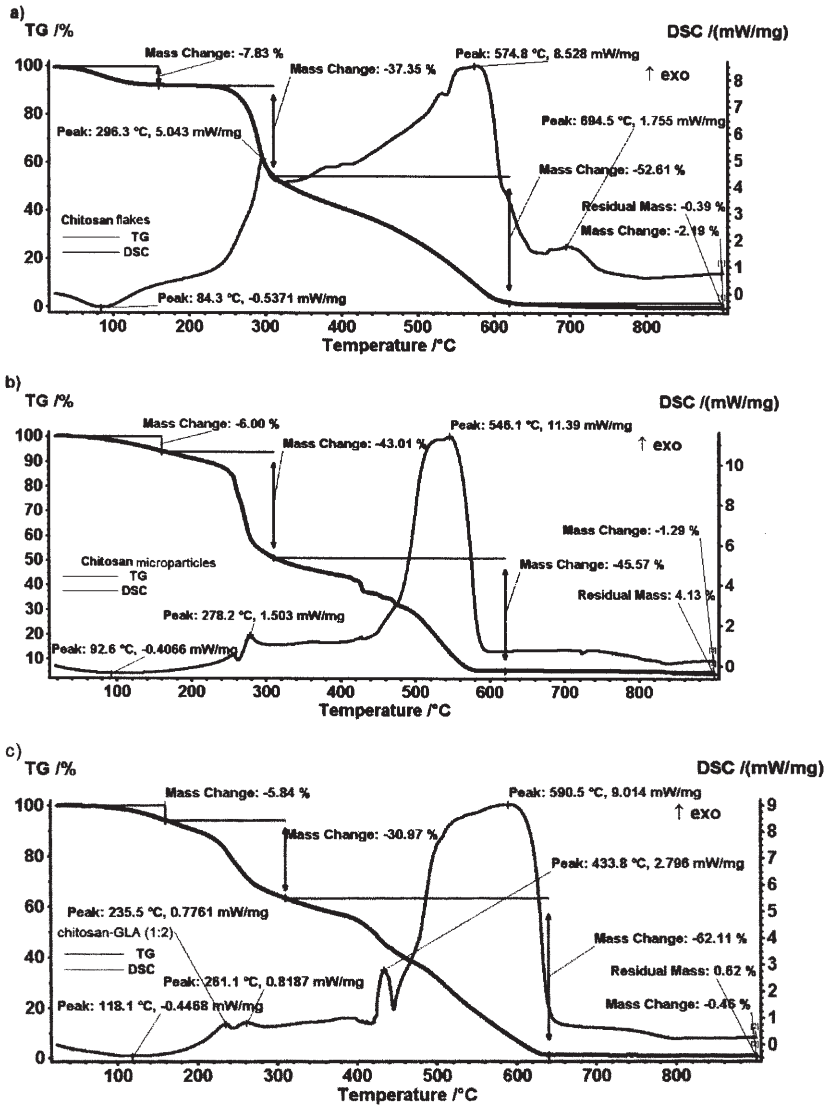

Fig. 7. TG/DSC curves of chitosan flakes (a), chitosan microparticles (b), and chitosan-GLA (1:2)

Table 2
SOLUBILITY EFFECT OF CHITOSAN FLAKES, CHITOSAN MICROPARTICLES, CHITOSAN-GLA 1:1 AND CHITOSAN-GLA 1:2 MICROPARTICLES

Sorbent	Solubility tests		
	5% (v/v) acetic acid	distilled water	0.5M NaOH
Chitosan flakes	soluble	insoluble	insoluble
Chitosan microparticles	soluble	insoluble	insoluble
Chitosan-GLA 1:1 microparticles	insoluble	insoluble	insoluble
Chitosan-GLA 1:2 microparticles	insoluble	insoluble	insoluble

similar, but not the same in case of flakes and microparticles.

The different thermal behaviour of chitosan-GLA (1:2) microparticles indicates changes in the chitosan structure, stronger bonds to be broken, and higher energy to be decomposed. This behaviour confirms the cross-linking of chitosan with GLA.

Thus, physical and chemical modification of chitosan has as results changes in physical and chemical properties of chitosan.

It has been demonstrated that chitosan has a high solubility in acid solutions. This represents one disadvantage in case of use it in wastewater treatment because most industrial effluents have a lower *p*H value. Chitosan flakes, chitosan microparticles, chitosan-GLA 1:1 and chitosan-GLA 1:2 microparticles were tested with regard to their solubility in 5% (v/v) acetic acid, distilled water and 0.5M NaOH solution. The results obtained are presented in table 2.

Table 2 reveals that chitosan flakes, and chitosan microparticles are insoluble in distillated water, and 0.5M NaOH solution, but they were dissolved in the acetic acid solution. This is due to the protonation of amino groups from the chitosan structure at low *p*H values. Physical modification of chitosan does not change this behaviour. Chemical modification on chitosan can overcome this weakness. Table 2 indicates that the cross-linked chitosan microparticles did not dissolve in acidic solution, in distillated water and alkaline solution. Thus, chemical modification on chitosan can improve the swelling effect on the biosorbent due to the fact that the amino groups from the chitosan chains will be involved in cross-linking with aldehyde groups from GLA, and they will not be free to be protonated by H^+ from acidic solution. Chitosan-GLA microparticles will not be dissolved. Consequently, chitosan-GLA microparticles can be easy removed from treated effluents after the remediation.

Physical and chemical modification of chitosan was performed in order to obtain materials with controlled properties that can be used for remediation of wastewater with low heavy metals content.

Conclusions

An experimental study has been performed to obtain chitosan and chitosan-GLA particles with physico-chemical properties improved by a simple and versatile method. FT-IR spectra analysis indicated that cross-linking of chitosan occurs through a chemical reaction between the amino groups of chitosan and aldehyde groups of glutaraldehyde. Microparticles stick to each other, and a compact mass was obtained by washing only with an aqueous solution. Washing with an aqueous solution of ethyl alcohol was performed to overcome this inconvenience, and spherical beads were obtained. Cross-linking was confirmed by thermal analysis. Porous microparticles with high stability in acid solutions (or wastewater with low *p*H) were obtained by cross-linking of chitosan with GLA. These microparticles will be tested for lead removal from aqueous solutions in order to determine their sorption properties.

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