Research Article

Chitosan-Coated Magnetic Nanoparticles with Low Chitosan Content Prepared in One-Step

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Chitosan-coated magnetic nanoparticles (CMNP) were obtained at 50°C in a one-step method comprising coprecipitation in the presence of low chitosan content. CMNP showed high magnetization and superparamagnetism. They were composed of a core of 9.5 nm in average diameter and a very thin chitosan layer in accordance with electron microscopy measurements. The results from Fourier transform infrared spectrometry demonstrated that CMNP were obtained and those from thermogravimetric analysis allowed to determine that they were composed of 95 wt% of magnetic nanoparticles and 5 wt% of chitosan. 67% efficacy in the Pb⁺² removal test indicated that only 60% of amino groups on CMNP surface bound to Pb, probably due to some degree of nanoparticle flocculation during the redispersion. The very low weight ratio chitosan to magnetic nanoparticles obtained in this study, 0.053, and the high yield of the precipitation reactions ($\approx 97\%$) are noticeable.

1. Introduction

Chitosan-coated magnetic nanoparticles constitute a very interesting material because of the number of actual and potential applications. This type of nanoparticles containing a core of a magnetic material, usually a mixture of magnetite (Fe₃O₄) and maghemite (γ -Fe₂O₃), owes its functionality to the free amino and hydroxyl groups that chitosan contains. Through these groups, CMNP are able to bind to a diversity of another chemical groups and ions which leads to a number of applications as protein and metal adsorption [1–3], guided drug and gene delivery [4, 5], magnetic resonance imaging [6], tissue engineering [7], and enzyme immobilization [8–10].

The most used procedure for CMNP preparing is first to obtain the magnetic nanoparticles, followed by chitosan coating [2, 8, 11–13]. CMNP with average diameters ranging from 14 [13] to 25–30 nm [2, 11, 12] and different chitosan

contents have been prepared using this procedure. However, a few of papers have appeared in the specialized literature focused on preparing CMNP by a more simple one-step method, which basically includes coprecipitation in a solution containing Fe⁺², Fe⁺³, and chitosan [14–16]. Using this method, CMNP with relatively large average particle diameter (67 nm) and 0.39 in theoretical weight ratio chitosan to magnetic nanoparticles were obtained [14]. Wu et al. [15] reported the preparation in one-step of CMNP with 50–100 nm in average diameter. Moreover, the theoretical weight ratio chitosan to Fe₃O₄ was as high as 8.7. The enormous difference between the weight ratios chitosan to magnetic nanoparticles in these reports is noticeable. Recently, our group reported the use of the one-step method to obtain CMNP, where magnetic nanoparticles showed 10–11 nm in average diameter and a weight ratio chitosan to magnetic nanoparticles in the composite between 0.06 and 0.24 [16]. It is noteworthy that these ratio values were significantly lower

than those in the reports of Hong and Rhee [14] and Wu et al. [15]. Surprisingly, CMNP with the lowest chitosan content showed the highest Pb^{+2} ions removal efficacy from an aqueous solution containing a known concentration of Pb^{+2} ions. The proposed explanation for this was that particle aggregation and, as a consequence, a decrease in the total surface area available for Pb^{+2} binding, was favored by the use of higher chitosan contents during the magnetic nanoparticles precipitation. Based on these results, it would seem interesting to investigate on the preparation and characterization of CMNP with low chitosan content.

As a continuation of our previous work [16], we report here the preparation of CMNP in one-step with low content of chitosan. In fact, we used the lowest chitosan concentration of those used in our previous work, which would lead to a theoretical weight ratio chitosan to magnetic nanoparticles close to 0.06. The product of the process was characterized in detail and a discussion on the implications of the preparation of CMNP in one-step with low content of chitosan was included.

2. Materials and Methods

2.1. Materials. Chitosan with low molecular weight and 75% deacetylation degree, ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 99%), ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 98%), aqueous ammonia (NH_4OH , 57.6 wt%) and ninhydrine (97%) from Aldrich were used as received. Lead nitrate, $\text{Pb}(\text{NO}_3)_2$, 99.7%, from J. T. Baker, was also used as received. Deionized and triple-distilled water was drawn from a Millipore system.

2.2. Preparation of Coated Magnetic Nanoparticles. CMNP were prepared in duplicate (runs C1 and C2) in a 150 mL jacketed glass reactor equipped with a reflux condenser, an inlet for aqueous ammonia feed and a mechanical agitator operated at 400 rpm. To carry out the reaction, 50 mL of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.32 M) and 50 mL of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.2 M) were mixed in the reactor. Then, 0.125 g of chitosan was added to the mixture and the temperature was raised to 50°C. Coprecipitation reaction without chitosan was also carried out in duplicate (runs M1 and M2) as a control. Once Fe^{+2} , Fe^{+3} and chitosan solution reached the desired temperature, the dosing of 20 mL of the aqueous ammonia at 0.67 mL/min was started. After dosing was over, the completion reaction was allowed to proceed for 20 min. At the end of the reaction, the particles were recovered by using a permanent magnet, washed 25 times with deionized water, and lyophilized to obtain the final product.

2.3. Nanocomposites Characterization. X-ray analysis of the products was carried out with a Siemens D-5000 diffractometer using $\text{Cu-K}\alpha$ ($\lambda = 1.5418 \text{ \AA}$) as incident radiation. The size and morphology of the particles were determined in a JEOL JSM-7401F scanning-transmission electron microscope (STEM) and a high-resolution transmission electron microscopy (HRTEM) Titan-300 kV, for which samples were prepared by dispersing the resulting powders in water with ultrasonication and then depositing the dispersion on a copper grid. The magnetic properties of the nanoparticles

were determined using a Physical Properties Measurement System from Quantum Design, model 6000 in mode vibrating sample magnetometer (VSM), with an applied field between -20.0 and 20.0 kOe at room temperature. Fourier transform infrared spectrometry (FTIR) was carried out in a Magna IR 550 from Nicolet with germanium crystal. Thermogravimetric analysis (TGA) of nanoparticles and chitosan was performed in a TGA Q500 from TA Instruments. Analyzed samples were heated from 30 to 800°C at a heating rate of 10°C/min under a nitrogen flow of 50 mL/min. The amino groups on the surface of CMNP were determined by the ninhydrin method, using glycine to construct the calibration curve [17]. To make the measurements, 0.1 g of CMNP were dispersed in 1 mL of water and then 0.6 mL of ninhydrin reagent were added. After that, the dispersion temperature was boiled for 30 min. The amino groups concentration in the dispersion was determined by readings of absorbance at 570 nm.

2.4. Pb^{+2} Removal Test. Typically, 60 mg of dried CMNP were added to 50 mL of 10 ppm $\text{Pb}(\text{NO}_3)_2$ aqueous solution. Then, this mixture was ultrasonicated for 50 min at room temperature taking samples during the process each 10 min. After CMNP were removed, the concentration of Pb^{+2} in the samples was measured by atomic absorption spectroscopy in a Varian SpectrAA 220 equipment.

3. Results and Discussion

There was no visual difference detected between the appearance of the product of the reactions carried out with and without chitosan. In both cases, a black powder was obtained. The X-ray diffraction pattern (XRDP) of the products obtained with chitosan (run C1) and without chitosan (run M1) are shown in Figure 1. XRDPs from the replicates (runs C2 and M2) were not included; however, they show the same signals pattern. Figure 1 also includes the standard patterns of magnetite and maghemite, which were taken from the library of our X-ray equipment. This obeys to the fact that precipitation reactions from $\text{Fe}^{+2} + \text{Fe}^{+3}$ aqueous solutions using aqueous ammonia usually render a mixture of magnetite and maghemite [18, 19]. As it can be seen in Figure 1, XRDPs of magnetite and maghemite display the same signals pattern in the range 30 to 75 $2\theta^\circ$. However, the maghemite pattern shows a signal at 18.22 $2\theta^\circ$, which is not displayed by the magnetite pattern. XRDPs from the products of runs C1 and M1 display the characteristic signals of magnetite and maghemite patterns in the range 30 to 75 $2\theta^\circ$. However, they also show the 18.22 $2\theta^\circ$ signal. From these data, it might be thought that the magnetic nanoparticles are composed only of maghemite. However, the product is not brown, the characteristic color of maghemite, but black, the color of magnetite [20]. Thus, based on the XRDPs of the precipitation products along the product color, it is concluded that the magnetic nanoparticles are composed of a mixture of magnetite and maghemite.

An estimation of the average grain sizes of the magnetic nanoparticles obtained in the precipitation reactions can be

FIGURE 1: X-ray patterns of magnetic nanoparticles prepared by coprecipitation method: (a) without chitosan and (b) with 0.125 (w/v%) of chitosan. It also included (c) magnetite standard pattern and (d) maghemite standard pattern.

obtained by using data from their XRDPs and the well-known Scherrer equation, which is represented as:

$$d = \frac{K\lambda}{\beta \cos \theta}, \quad (1)$$

where d is the average diameter of grain in nm; K is the dimensional factor (0.9); λ is the X-ray wavelength (0.154 nm); β is the line broadening at half the maximum intensity in radians, and θ is the Bragg's angle. d values obtained were 13.05 ± 0.31 for nanoparticles prepared with chitosan and 12.60 ± 0.00 nm for naked magnetic nanoparticles. From these results, it is evident that the presence of chitosan during precipitation reaction does not affect the grain size of magnetic nanoparticles.

Figure 2 shows STEM micrographs of nanoparticles prepared without and with chitosan (Figures 2(a) and 2(b), resp.). Measurements of more than 1000 nanoparticles from different micrographs of each of the samples were carried out by using an image analysis program (ImageJ 1.37c). These results were used to construct the corresponding histograms of particle diameters, also included in Figure 2. In addition, using the data from nanoparticle size measurements, the number-average diameter (D_n) was calculated with the following equation [21]:

$$D_n = \frac{\sum n_i D_i}{\sum n_i}, \quad (2)$$

where n_i is the number of particles of diameter d_i .

STEM micrographs in Figure 2 show nanoparticles with spheroidal morphology. A difference between naked nanoparticles and those prepared with chitosan was not visually detected in this figure, however, a HRTEM micrograph of a single nanoparticle prepared with chitosan (Figure 3), shows that a chitosan layer on magnetic nanoparticles was formed. In this figure, the crystal planes of the nanoparticle can be identified as parallel lines crossing the particle image. However, these lines do not extend to the edges of the nanoparticle. Instead, there is a central area (bounded by

a red line in the image), which includes the crystal planes, that is, those of the magnetic nanoparticle. The remaining area near the edges of the nanoparticle, that does not include the crystal planes, would correspond to an amorphous substance, in this case, chitosan. It is remarkable the very thin layer of chitosan on the magnetic nanoparticle, which is in accordance with that expected because of the chitosan low content in the recipe.

D_n values of naked nanoparticles and those prepared with chitosan were 9.5 ± 0.1 and 9.9 ± 0.2 nm, respectively. In spite that coprecipitation is a widely used method for preparing magnetic nanoparticles, it is not common obtaining nanoparticles with average diameters smaller than 10 nm, as those reported in this work. In fact, the average diameters of magnetic nanoparticles prepared by coprecipitation method range from 14 [13] to 25–30 nm [2, 11, 12]. Although, it cannot be excluded that other authors using this method have obtained magnetic nanoparticles with similar sizes to those obtained in this work, they were not identified in our search in the specialized literature.

A comparison of the magnetic nanoparticles size determined from STEM with the average grain size estimated by using (1) indicates that the latter is slightly higher than the former. However, taking into account that Scherrer equation gives a rough estimation of the average grain size of the material, it can be said that the size of magnetic nanoparticles and that of the grains are similar. In turn, this would lead to conclude that the magnetic nanoparticles are composed of only one grain, even when chitosan was present during the precipitation reaction.

Figure 4 shows the magnetization curves at room temperature of the nanoparticles obtained with and without chitosan. These curves were prepared using the averages of the magnetization values from the products obtained in the reactions and their replicates. The magnetization of naked nanoparticles attained a value of 73.2 ± 1.7 emu/g at the maximum magnetic field applied (20 kOe), while that of nanoparticles obtained in the presence of chitosan was 65.6 ± 0.1 emu/g. These values are similar to those

FIGURE 2: SEM micrographs of particles prepared by coprecipitation method (a) without chitosan and (b) with 0.125 (w/v%) of chitosan. Corresponding nanoparticle diameter histograms are also included.

FIGURE 3: HRTEM micrograph of a nanoparticle prepared by coprecipitation method with 0.125 (w/v%) of chitosan. The red line delimits the extension of the crystalline region of the nanoparticle.

reported for the naked magnetic nanoparticles and CMNP with 0.125% (w/v) of chitosan in the formulation in our previous work [16]. Statistically, it can be said that naked nanoparticles magnetized more than those prepared with chitosan, which is ascribed to the fraction of nonmagnetic

material (chitosan) that the nanoparticles would contain. It is noteworthy that the curves in Figure 4 do not attain magnetic saturation values. This is explained as a consequence of the difficulty for aligning the magnetic moments in the direction of applied magnetic field experimented by the surface atoms, which constitute a significant fraction of the total atoms in very small particles [22]. It is also noticeable the very low values of remnant magnetization and coercivity field of prepared nanoparticles. Naked nanoparticles attained 1.6 ± 0.3 emu/g and 17.8 ± 2.7 Oe in remnant magnetization and coercivity field, respectively, while the corresponding values for the magnetic nanoparticles prepared with chitosan were 1.5 ± 0.9 emu/g and 22.7 ± 3.8 Oe. These values are very small and indicate a superparamagnetic behavior, which is characteristic of magnetite and maghemite nanoparticles with diameters smaller than 10–15 nm [23]. The most attractive feature of superparamagnetic nanoparticles is their demagnetization once the applied magnetic field is removed, which allows their reuse.

For comparison purposes, it is mentioned that the final magnetization value of the naked nanoparticles prepared in this study is higher than those reported by Ge et al. [13] and

FIGURE 4: Magnetization curves determined at room temperature for nanoparticles prepared by coprecipitation method: (●) without chitosan and (■) with 0.125 (w/v%) of chitosan.

Wu et al. [15], similar to that obtained by Pan et al. [12] but lower than those reported by Liu et al. [2] and Kuo et al. [8]. The latter authors obtained values of magnetic saturation around 80 emu/g, however, due to the relatively large size of the nanoparticles (≈ 25 nm in average diameter) [2], they showed a relatively high values of remnant magnetization. This characteristic would lead to nanoparticles agglomeration after the magnetic field removal, which is not attractive for some applications where the re-use of the nanoparticles is required. On the other hand, CMNP obtained in this study show final magnetization values similar to those reported by other authors [8, 12, 13] and higher than that reported by Wu et al. [15], who obtained a value of 35.5 emu/g.

The direct dependence between magnetization of small particles (diameters smaller than ca. 15 nm) and particle size [24, 25] is well known. Because of this, it is noticeable the relatively high values of final magnetization obtained in this study, taking into account that the sizes of the corresponding magnetic nanoparticles are in the bottom of the sizes range reported in the literature on preparation of magnetic nanoparticles by coprecipitation method [2, 8, 11–15]. Considering that magnetization in magnetic nanoparticles directly depends on crystallinity [26], the higher magnetization values obtained in this study could be ascribed to a possible improvement in the magnetic nanoparticles crystallinity.

Figure 5 shows the FTIR spectra of pure chitosan and magnetic nanoparticles prepared with chitosan (run C1). The spectrum of the product from run C2 (not shown) displays a similar signals pattern to that from run C1. In accordance with that reported in the literature [27, 28], the characteristic absorption bands for chitosan in Figure 5 appear at 3457 (O-H and N-H stretching vibrations), 2875 (C-H stretching vibrations), 1658 (N-H bending vibrations), 1425 (C-N stretching vibrations) and 1076 cm^{-1} (C-O-C stretching vibrations). The spectrum of the product obtained

FIGURE 5: FTIR spectra of nanoparticles prepared by (a) coprecipitation method with 0.125 (w/v%) of chitosan and (b) that of pure chitosan.

using chitosan show four of the five characteristic absorption bands for this polymer (signal at 1425 cm^{-1} disappeared) and one additional at 570 cm^{-1} , which corresponds to Fe-O stretching vibrations in magnetite and maghemite [8]. Considering that the products of the precipitation reactions in presence of chitosan were exhaustively washed and magnetically recovered, free chitosan, if there was, was removed. Thus, it was concluded that all the chitosan in the final product is chemically bound to the magnetic nanoparticles. FTIR spectra results demonstrated that the precipitation reactions carried out with chitosan rendered magnetic nanoparticles coated by this polymer.

The results of TGA characterization of CMNP (run C1), naked nanoparticles (run M1), and pure chitosan are shown in Figure 6. From these measurements and those of the replicates, an average weight loss of $91.52 \pm 0.03\%$ was calculated for CMNP, while naked nanoparticles and pure chitosan showed weight losses of 3.98 ± 0.13 and $96.39 \pm 0.40\%$, respectively. From these results and the weight of the CMNP samples before and after TGA characterization, a content of $5.0 \pm 0.3\text{ wt}\%$ of chitosan in CMNP was estimated, which equals to 52.6 mg of chitosan immobilized on one gram of magnetic nanoparticles or 0.053 in weight ratio chitosan to magnetic nanoparticles. The theoretical value of immobilized chitosan calculated from the recipe of precipitation reactions, assuming that the reaction proceeded to 100% and only magnetite was obtained, was 64.4 mg/g or 0.065 in weight ratio chitosan to magnetic nanoparticles. Furthermore, it is interesting to mention that the weight of the final product recovered from runs C1 and C2 was 1.9800 and 1.9880 g, respectively. Considering the chitosan and magnetic nanoparticles content in CMNP as determined from the results by TGA and assuming that the precipitation reactions render only magnetite, it was estimated a yield close to 96.7% for both of precipitation reactions. Also, it was estimated that only around 76% of the chitosan in the recipe

FIGURE 6: Thermal degradation curves of nanoparticles prepared by coprecipitation method: (a) without chitosan and (b) with 0.125 (w/v) of chitosan. It is also included that of (c) pure chitosan.

FIGURE 7: Pb²⁺ removal as a function of time using chitosan-coated magnetic nanoparticles prepared by coprecipitation method with 0.125% (w/v) of chitosan.

was covering the magnetic nanoparticles for CMNP from run C1 and 84% for those from run C2. This is the reason why the experimental value of immobilized chitosan is less than the theoretical one.

To verify the reacting availability of the amino groups on the surface of CMNP, an evaluation of their efficacy for Pb²⁺ removal from a Pb(NO₃)₂ aqueous solution was carried out. This test is based on the ability of chitosan to chelate heavy metal ions through its amino groups. The average results of the behavior of CMNP from runs C1 and C2 are shown in Figure 7. This figure shows that the Pb²⁺ concentration drops from its initial value (10 ppm) to around 4.5 ppm in the first 10 minutes. Then, it slowly decreases to attain a final value of 3.22 ppm at the end of the test, which equals to a removal efficacy of 67.8%. In spite that this value looks interesting, it is only a part of the picture. It still remains

unclear if all or only a fraction of the amino groups on the CMNP surface bound to Pb²⁺ ions. To elucidate this, it was proceeded as follows. TGA results allowed to estimate that CMNP was composed of 95 wt% of magnetic nanoparticles and 5 wt% of chitosan. From TEM measurements, a D_n value of 9.5 nm for magnetic nanoparticles was determined. With these data and density values of 5.2 g/mL for magnetite [29] and 1.342 g/mL for chitosan [30], it was estimated that one gram of CMNP contains 4.07×10^{17} nanoparticles and that the chitosan layer on a magnetic nanoparticle is close to 0.3 nm in thickness. The latter result implies that CMNP have an average diameter around 10.1 nm, which roughly agrees with that determined from TEM measurements (9.9 nm). On the other hand, ninhydrin method allowed to estimate that one CMNP gram contained 0.0911 mmoles of amino groups distributed on their surface. With this amount and considering that one CMNP gram is composed of 4.07×10^{17} nanoparticles, a value of 135 amino groups per each coated-magnetic nanoparticle was obtained. Simple calculations from results obtained in the Pb²⁺ removal test, indicate that 3.39×10^{-4} g of Pb²⁺ ions were chelated by the CMNP in the test. This value along that of nanoparticle number contained in one gram of CMNP lead to an estimation of 40 Pb²⁺ ions chelated by each coated-magnetic nanoparticle and, from here, 3.4 amino groups per each Pb²⁺ ion. Taking into account that two amino groups are required to chelate one Pb²⁺ ion, it is concluded that only around 60% of amino groups were used in the chelation process. Now, the question is why an important fraction of the amino groups, 40%, on the surface of CMNP is not able to chelate Pb²⁺ ions. An explanation to this behavior would arise from an incomplete redispersion of CMNP, previously recovered as a powder, during the Pb²⁺ removal test. If so, a fraction of CMNP would form clusters of flocculated nanoparticles, which would reduce the available CMNP area, and as a consequence, the available amino groups for chelating Pb²⁺ ions.

4. Conclusions

Superparamagnetic nanoparticles composed of a magnetite-maghemite mixture with relatively high magnetization final values were precipitated in the presence of chitosan. This one-step method allowed to obtain CMNP, which consist of a magnetic nanoparticle core of 9.5 nm in average diameter coated by a thin layer of chitosan. From the TGA results it was calculated that CMNP were composed of 5 wt% of chitosan and 95 wt% of magnetic nanoparticles, and that the chitosan layer on a magnetic nanoparticle was close to 0.3 nm in thickness. In the Pb²⁺ removal test, CMNP showed an efficacy close to 68%, which implies that only 60% of the amino groups on the surface of CMNP bound to Pb²⁺ ions. The explanation for this behavior arose from a possible CMNP flocculation, which would lead to a reduction in the available amino groups for chelating Pb²⁺ ions. Finally, the very low weight ratio chitosan to magnetic nanoparticles obtained in this study, 0.053, is remarkable, which is almost the minimum necessary to cover each nanoparticle, considering that the polymer is homogeneously distributed.

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