# Biopolymer–Clay Nanocomposites Based on Chitosan Intercalated in Montmorillonite

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The objective of this work is the intercalation of the cationic biopolymer chitosan in Na⁺– montmorillonite, providing compact and robust three-dimensional nanocomposites with interesting functional properties. CHN chemical analysis, X-ray diffraction, Fourier transform infrared spectroscopy, scanning transmission electron microscopy, energy-dispersion X-ray analysis, and thermal analysis have been employed in the characterization of the nanocomposites, confirming the adsorption in mono- or bilayers of chitosan chains depending on the relative amount of chitosan with respect to the cationic exchange capacity of the clay. The first chitosan layer is adsorbed through a cationic exchange procedure, while the second layer is adsorbed in the acetate salt form. Because the deintercalation of the biopolymer is very difficult, the $-NH_3^+Ac^-$ species belonging to the chitosan second layer act as anionic exchange sites and, in this way, such nanocomposites become suitable systems for the detection of anions. These materials have been successfully used in the development of bulk-modified electrodes exhibiting numerous advantages as easy surface renewal, ruggedness, and long-time stability. The resulting sensors are applied in the potentiometric determination of several anions, showing a higher selectivity toward monovalent anions. This selectivity behavior could be explained by the special arrangement of the polymer as a nanostructured bidimensional system.

## Introduction

As is well-known, polymer–clay nanocomposites are materials of increasing interest because of their structural or functional behavior.[1] For instance, interesting electrical and electrochemical properties of poly(ethylene oxide)–smectites have been extensively reported.[2] The opportunity to combine at the nanometric level clays and a natural polymer (biopolymer) such as chitosan also appears as an attractive way to develop new organic–inorganic hybrid materials provided with properties that are inherent to both types of components.

Chitosan (Figure 1), poly-$\beta$(1,4)-2-amino-2-deoxy-D-glucose, is the deacetylated product of chitin, poly(*N*-acetyl-D-glucosamine), a natural polymer found in the exoskeletons of crustaceans and insects and in the cell wall of fungi and microorganisms.

Chitin with a deacetylation degree (DD) of 75% or above is generally known as chitosan, which can be considered a copolymer composed of glucosamine and *N*-acetylglucosamine units and dissolves readily in dilute organic acids, providing clear, homogeneous, and viscous solutions. Thus, the chemically active groups in the chitosan structure are the free amine groups, located
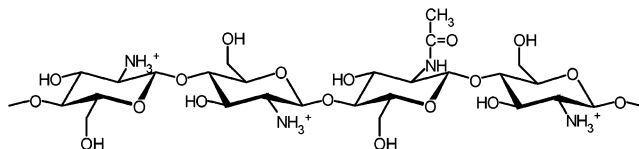


**Figure 1.** Chitosan structure.

in the C2 position of the glucose residue in the polysaccharide chain, and the hydroxyl groups, with both being susceptible to modification. As a primary aliphatic polyamine, chitosan is involved in all of the reactions typical of amines. Most of the applications of chitosan are based on the polyelectrolytic nature and chelating ability of the amine group of the macromolecules, and such properties are mainly governed by the acidity of the $-NH_3^+$ group ($pK_a$ = 6.3).[3] The weak-base anion-exchange ability of pure chitosan has been applied in the development of surface-modified sensors for anion

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detection, based on the casting of chitosan films onto the surface of glassy carbon electrodes.[4,5] Nevertheless, these devices lack of long-term stability probably because of alteration of the characteristics of chitosan films. An appreciable increase in the stability of chitosan films has been obtained by coverage with organosilicic layers generated by sol−gel procedures.[6] In this work, the intercalation of chitosan in layered silicates giving biopolymer−clay nanocomposites appears as an improving way to develop robust and stable sensors useful for anionic detection in aqueous media.

Nanocomposites obtained from different chitosan−clay ratios have been characterized by means of chemical and thermal analysis, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning transmission electron microscopy (STEM), and energy-dispersion X-ray analysis (EDX) techniques. The most remarkable feature of these new nanocomposites is related to their electrochemical response to anions due to the presence of suitable functional groups in the chitosan structure acting as anionic exchange sites. Thus, such nanocomposites are easily processed to construct bulk-modified electrodes for the potentiometric determination of anions, showing a higher selectivity toward monovalent species as a result of the biopolymer bidimensional confinement in the nanostructured solid.

## Experimental Section

**Starting Materials.** Montmorillonite from Crook County, WY (SWy-1), with a cationic exchange capacity (CEC) of 76.4 mequiv/100 g (as supplied) was received from Source Clay Minerals Repository of the Clay Minerals Society, Columbia, MO. A homoionic $Na^+$−montmorillonite sample was prepared by treatment of the <2-$\mu$m-diameter fraction with a 1.0 N NaCl solution. After complete exchange, the sample was washed with bidistilled water until there was an absence of chloride, confirmed by the $AgNO_3$ test.

Chitosan of high molecular weight (MW average = 342 500 g mol$^{-1}$) containing an average number of glucosamine units of 2130 (glucosamine MW = 161 g mol$^{-1}$) was supplied by Aldrich. We have applied a method based on the IR spectra of a chitosan sample to estimate the chitosan DD[7] and confirmed a DD of ca. 75%. Chitosan solutions were prepared by the addition of corresponding amounts of polysaccharide to 1% (v/v) acetic acid (Merck), and after the resulting solution was stirred for about 4 h, the pH of the polysaccharide solution was adjusted to 4.9 with NaOH before being mixed with the clay suspension. Chitosan solutions containing 20.1, 40.2, 80.5, and 161.0 mg of biopolymer in 25 mL of solution were slowly added to a 2% clay suspension (0.5 g of $Na^+$−montmorillonite in 25 mL of bidistilled water), at 323 K, to obtain nanocomposites with initial chitosan−clay ratios of 0.25:1, 0.5:1, 1:1, and 2:1, respectively. For the preparation of nanocomposites with chitosan−clay ratios of 5:1 and 10:1, chitosan solutions with 402.5 mg of biopolymer in 125 mL and 805.0 mg in 250 mL, respectively, were mixed with the clay suspension. In all of the cases, the resulting mixture was stirred for 2 days and finally washed with purified water until free from acetate. For spectroscopic and thermal analysis, the nanocomposites were air-dried at 50 °C and ground to powder.

**Characterization.** The resulting nanocomposites were characterized by chemical analysis (Perkin-Elmer 2400 CHN

analyzer), XRD (Siemens D-500 instrument with a Cu anode and Ni filter), thermal analysis [thermogravimetric analysis (TG) and differential thermal analysis (DTA); SSC/5200 Seiko analyzer], IR spectroscopy (Nicolet 20SXC spectrophotometer), and STEM (LEO-910 microscope, operating at an accelerating voltage of 80 kV, with an EDX analyzer system from Oxford, INCA 200). Potentiometric measurements were performed with a PAR 273A potentiostat from EG&G controlled with M352 software. A conventional two-electrode configuration was employed. The homemade sensor acted as the working electrode, and the potential was measured (at room temperature) against a Ag/AgCl reference electrode.

**Reagents.** Aqueous solutions were prepared from chemicals of analytical reagent grade: NaCl (99.5%, Merck), $NaNO_3$ (99%, Fluka), sodium tetraphenylborate (NaTPB, 99%, Aldrich), sodium acetate (NaAc, 99.5%, Carlo Erba), $Na_2SO_4$ (99%, Aldrich), $K_2Cr_2O_7$ (99%, Carlo Erba), and $K_3Fe(CN)_6$ (99%, Carlo Erba). Deionized water (resistance of 18.2 MΩ cm) was obtained with a Maxima Ultrapure Water system from Elga.

**Potentiometric Sensors.** For the development of potentiometric electrodes, graphite powder (1−2 $\mu$m, synthetic, from Aldrich) was added to the nanocomposite up to a final clay−graphite ratio of 1:0.2 in weight in order to provide the system with electronic conductivity. Such an optimal amount of graphite was determined from the potentiometric determination of nitrate with sensors prepared with different clay−graphite ratios. The graphite−nanocomposite mixture was dried at 50 °C in appropriate moulds, and the resulting disks (diameter 3 mm and height 2 mm) were inserted in polypropylene tubes, sealed with epoxy resin, and finally polished consecutively using 600- and 1200-grit silicon carbide disks (Electron Microscopy Science). The electrical contact was established with a copper wire. The sensors were conditioned for 2 h in a 0.01 M solution of the appropriate salt and then kept in the same solution when not in use.

## Results and Discussion

**(1) Chitosan−Clay Nanocomposites.** Although scarcely applied, positively charged polymers, such as, for instance, $\beta$-(dimethylamino)ethyl methacrylate hydroacetate (DMAEM) and other polycations, can be intercalated in smectite clays following cationic exchange mechanisms.[8,9] Because of the polycationic nature of chitosan in acidic media, this biopolymer also appears as an excellent candidate for intercalation in $Na^+$−montmorillonite by means of cationic exchange processes. For this purpose, $Na^+$−montmorillonite suspensions were treated with solutions of increasing concentration of polysaccharide. The pH value of each chitosan solution was adjusted to 4.9 before being added to the clay suspension in order to avoid any structural alteration of the phyllosilicate. On the other hand, an acidic pH value is necessary to provide −$NH_3^+$ groups in the chitosan structure. Given that the p$K_a$ of the primary amine groups in the chitosan structure is 6.3, a 95% of the amine groups will be protonated at the final pH value (pH 5) of the chitosan−clay mixture. In such conditions, the adsorption process is mainly controlled by a cationic exchange mechanism due to the Coulombic interactions between the positive −$NH_3^+$ groups of the biopolymer and the negative sites in the clay structure. It is important to mention that the nanocomposites are denoted with the initial chitosan−clay ratio employed in their preparation; nevertheless, such a ratio does not indicate the final biopolymer amount adsorbed on the clay.

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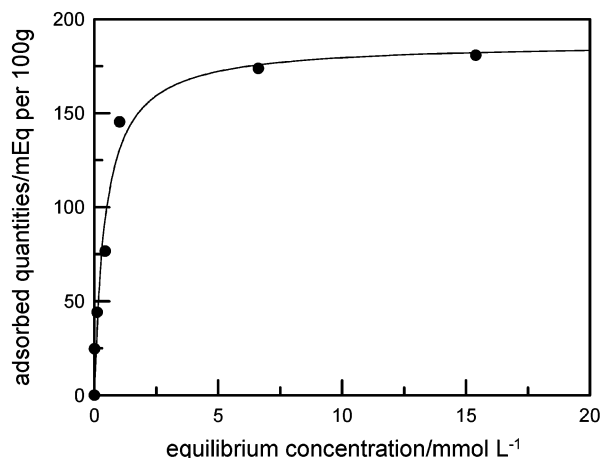(9) Breen, C. *Appl. Clay Sci.* **1999**, *15*, 187−219.

**Figure 2.** Adsorption isotherm of chitosan on $Na^+$−montmorillonite, in an aqueous dispersion at 323 K. Adsorption amounts deduced from CHN chemical analyses.

*Adsorption Isotherm.* Figure 2 shows the adsorption isotherm at 323 K from chitosan solutions on $Na^+$−montmorillonite. The adsorbed amount of chitosan was deduced from the CHN chemical analyses. These results fit well to the L type (Langmuir) of the Giles classification of adsorption isotherms:[10]

$$\Gamma = \frac{bx_m C_s}{1 + bC_s} \qquad (1)$$

where $\Gamma$ is the adsorbed amount of polymer, $C_s$ the equilibrium polymer concentration, $x_m$ the maximum adsorbed amount, and $b$ the affinity constant between polymer interaction sites and clay interaction sites. The good fitting of data to a Langmuir isotherm and the sharp dropping of the slope to zero at full surface coverage are indicative of the high affinity between the chitosan and the clay surface. The $b$ value obtained from the fit of the experimental data to the L-type isotherm was 2.26 $mmol^{-1}$ L, and it is related to the free energy of the adsorption process through the following equation:

$$\Delta G_{ads} = -RT \ln K \qquad (2)$$

where $R$ is the gas constant, $T$ is the adsorption temperature, and $K = (b\rho/4)^2$ for ionic species,[11] with $\rho$ being the ratio of the solvent (water) density to its molecular weight, $\rho \approx 55.6$ mol $L^{-1}$. The Gibbs energy of adsorption was −55.6 kJ $mol^{-1}$, indicating a strong adsorption of the biopolymer on the clay surface.

It is assumed that the cationic exchange sites in the clay, 76.4 mequiv/100 g, impose the adsorption of the same number of $-NH_3^+$ equivalents of the biopolymer. Given that the chitosan is composed of 75% glucosamine units and 25% N-acetylglucosamine units and also that 5% of amine groups in the glucosamine units remains nonprotonated at pH 5, we can estimate the maximum amount of biopolymer adsorbed by Coulombic interactions, 107.2 mequiv/100 g. Such a value agrees well with that calculated considering the average area of each unit

in the chitosan chain ($55 \times 10^{-20}$ $m^2$)[12] and the surface area of the clay interlayer space (700 $m^2$/g),[13] which is 105.7 mequiv/100 g. However, the maximum adsorbed amount of polysaccharide obtained from the fit of the data to the L-type isotherm was 187.4 mequiv/100 g. The excess of biopolymer, 80.2 mequiv/100 g, will not interact electrostatically with the clay surface because the clay CEC has already been balanced. Such an amount of biopolymer may be adsorbed by interactions through the amine and the hydroxyl groups in the chitosan chain. Therefore, the protonated amine groups in the glucosamine units, 57.1 mequiv/100 g, will be balanced with the acetate anions of the starting solution because they do not compensate the electrical charge in the clay mineral. This suggests that the excess of biopolymer should be intercalated as a bilayer with one of the chitosan chains as an acetate salt (Scheme 1), in agreement with the XRD data (see below). The presence of $-NH_3^+Ac^-$ species turns this material, the chitosan−clay nanocomposite, into a potential anion exchanger (57.1 mequiv/100 g of capacity), which is employed in the development of potentiometric sensors for the determination of anions (see below).

The EDX results indicate that the initial $Na^+$ content in the starting montmorillonite is strongly reduced after treatment with the chitosan solutions (chitosan−clay ratios $\geq$ 1). In this way, the predominance of ion-exchange mechanisms driving the intercalation of the biopolymer into the phyllosilicate substrate for the intercalation of the first layer of biopolymer is experimentally confirmed.

*XRD.* Figure 3 shows the XRD patterns of clay and chitosan samples and chitosan−clay nanocomposites. The intercalation of the biopolymer in the clay interlayer is confirmed by the decrease of $2\theta$ values while the chitosan−clay ratio increases. The $d_{001}$ spacings are obtained using the first rational orders corresponding to the 00*l* reflections. Taking into account the thickness of the silicate layer (about 0.96 nm), the increase of the interlayer distance ($\Delta d_L$) in each nanocomposite is calculated from the corresponding $d_{001}$ spacing.

In acidic solutions, chitosan shows an extended structure that may facilitate the biopolymer intercalation in the clay interlayer space[14] in opposition to analogous polysaccharides with coiled or helicoidal structures that are only adsorbed in the external surface of clays.[15] We have found from the XRD pattern of a chitosan film a $d_{001}$ spacing of 0.38 nm, indicating that chitosan film consists of arrays of parallel sheets of chains in a way similar to that of chitin.[12,16] Therefore, the interlayer space in the nanocomposites prepared from chitosan−clay ratios of 0.25:1 and 0.5:1 can be related to the thickness of one chitosan sheet and, thus,

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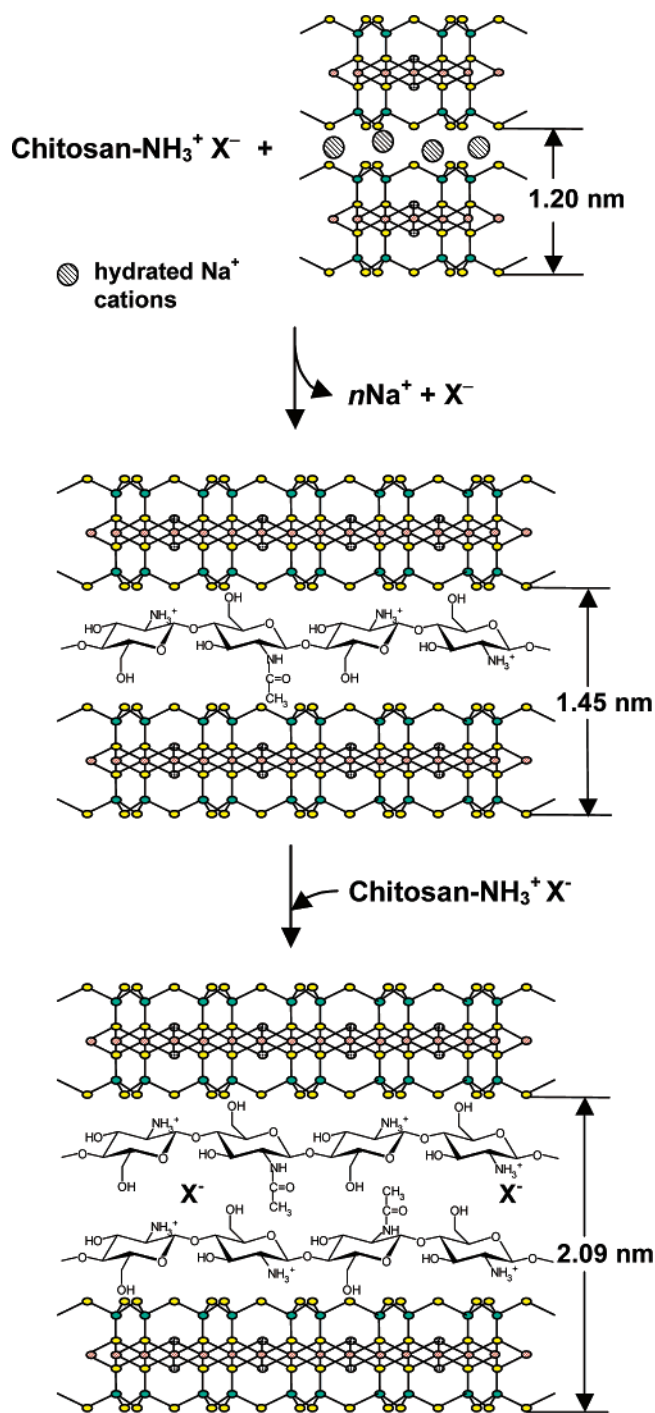(14) Pan, J. R.; Huang, C.; Chen, S.; Chung, Y.-C. *Colloids Surf. A* **1999**, *147*, 359−364.
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**Scheme 1. Intercalation of Chitosan into Na⁺−Montmorillonite**





**Figure 3.** XRD patterns of (a) Na⁺−montmorillonite, (b) chitosan film, and nanocomposites prepared from chitosan−clay ratios of (c) 0.25:1, (d) 0.5:1, (e) 1:1, (f) 2:1, (g) 5:1, and (h) 10:1.



**Figure 4.** IR spectra (4000−350 cm⁻¹ region) of the starting Na⁺−montmorillonite (a), the chitosan film (b), and two nanocomposites with starting chitosan−clay ratios of 1:1 (c) and 5:1 (d).

to its intercalation as a monolayer covering the interlayer surface of the clay, as shown in Scheme 1. Above such chitosan−clay ratios, the increase of the basal spacing can be explained as the uptake of two chitosan layers by the clay. As mentioned above, the second polymer layer should correspond to the adsorption of chitosan in its acetate salt form, with the $\Delta d_L$ values obtained for the nanocomposites prepared from the highest chitosan−clay ratios corresponding to the thickness of two chitosan layers together with the thickness of the acetate anion.

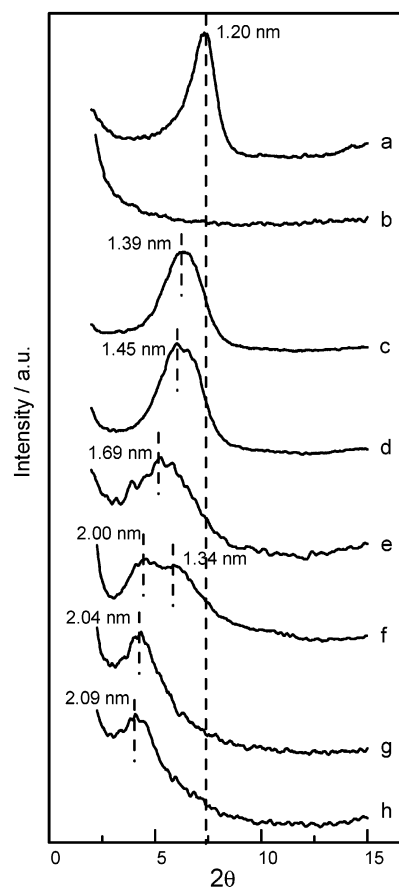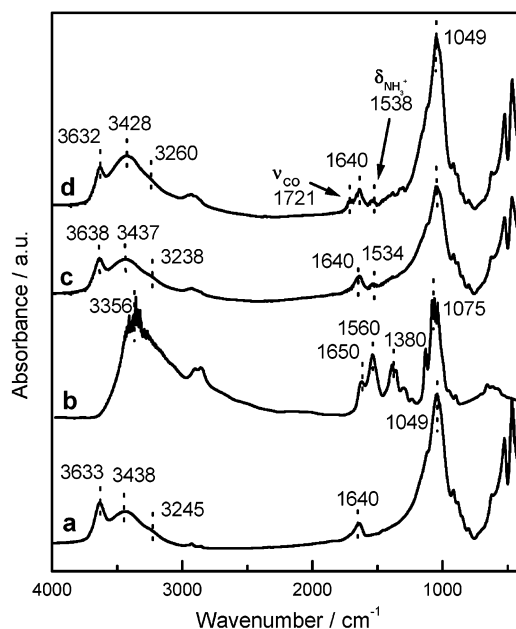*IR Spectroscopy.* Figure 4 shows the IR spectra of Na⁺−montmorillonite, chitosan, and two chitosan−clay nanocomposites prepared from 1:1 and 5:1 biopolymer−clay ratios, respectively, in the 4000−350 cm⁻¹ wavenumber range. Besides the vibrational band characteristics of the silicate ($\nu_{OH}$ of Al,Mg(OH) $\sim$ 3635 cm⁻¹; $\nu_{OH}$

**Table 1. Frequency Values of Vibrational Bands Corresponding to the Water Molecules Associated with the Silicate and with the Protonated Amine Group in the Chitosan Chain**

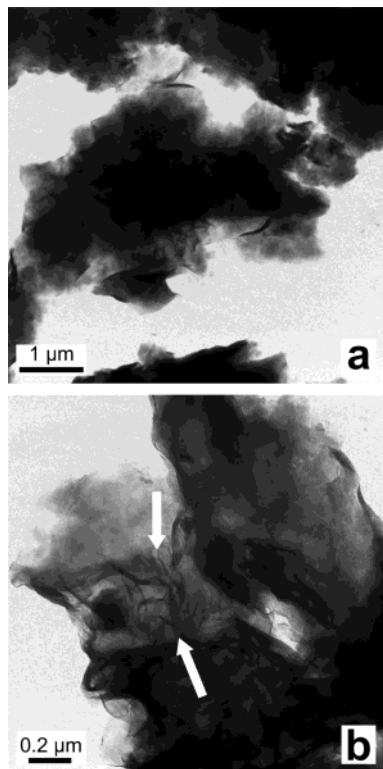| | clay | chitosan film | chitosan−clay nanocomposites | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0.5:1 | 1:1 | 2:1 | 5:1 | 10:1 |
| $\delta_{HOH}$ (cm$^{-1}$) | 1640 | 1650[a] | 1640 | 1640 | 1640 | 1640 | 1640 |
| $\delta_{NH_3}$ (cm$^{-1}$) | | 1560 | 1531 | 1534 | 1534 | 1538 | 1541 |

[a] Stretching band of amide I (C=O).



**Figure 5.** STEM images of nanocomposites prepared from chitosan−clay ratios of (a) 1:1 and (b) 5:1.

of H$_2$O $\sim$ 3430 and 3250 cm$^{-1}$; $\delta_{HOH} \sim$ 1640 cm$^{-1}$; and $\nu_{SiO}$ of Si−O−Si $\sim$ 1050 cm$^{-1}$), the bands attributed to the intercalated chitosan are also observed in the spectra of both nanocomposites (Figure 4c,d). The frequency of vibrational bands at 1560 cm$^{-1}$ in the starting chitosan, which corresponds to the deformation vibration ($\delta_{NH_3}$) of the protonated amine group, is shifted toward lower frequency values depending on the amount of the intercalated biopolymer (Table 1). This fact can be related to the electrostatic interaction between such groups and the negatively charged sites in the clay structure. The vibrational bands in the starting chitosan ($\nu_{CO}$ of C−O−C $\sim$ 1075 cm$^{-1}$, characteristic of the piranose ring; and $\nu_{OH} \sim$ 3356 cm$^{-1}$) overlap with the bands of the silicate. In the case of the nanocomposite prepared from the highest chitosan−clay ratio (Figure 4d), the band at 1721 cm$^{-1}$ may be attributed to the $\nu_{CO}$ stretching band of acetate ions associated with the second layer of chitosan, as described in Scheme 1.

*STEM.* The STEM images (Figure 5) for the different chitosan−clay ratio materials show the characteristic platelets of the montmorillonite tactoids. Excess of polymer is not observed at least for chitosan−clay ratios of 5:1 or lower, confirming that the nanocomposites consist essentially of well-intercalated phases. Corruga-
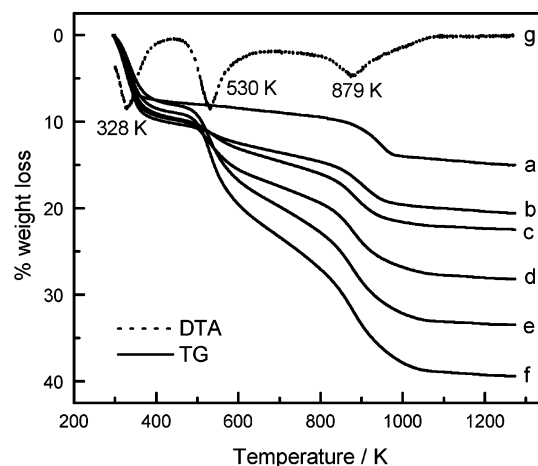


**Figure 6.** TG curves in the 300−1270 K temperature range (obtained under an air atmosphere) of (a) Na$^+$−montmorillonite and nanocomposites prepared from chitosan−clay ratios of (b) 0.25:1, (c) 0.5:1, (d) 1:1, (e) 2:1, and (f) 5:1. The DTA curve corresponds to the 5:1 nanocomposite (g).

tions in the clay layers (see the arrows in Figure 5b) are indicative of the constrained action of the polymer interacting with the clay substrate.

*Stability.* Previous studies have demonstrated that polycation adsorption processes in clay minerals are essentially irreversible because desorption requires the simultaneous desorption of all of the ionic sites in a polymer chain and the diffusion away from the clay surface.[9,17] In our case, the chitosan−clay nanocomposites treated with concentrated NaCl aqueous solutions (1.0 N) for long periods of time (>3 weeks) preserve their structural arrangement in agreement with the XRD results.

Thermal stability was investigated from DTA and TG curves recorded in the 300−1200 K range, under air flow conditions (Figure 6). The weight loss between room temperature and about 500 K is related to the adsorbed water molecules. Such a weight loss is about 7.9% in the starting silicate, while the biopolymer−clay nanocomposites show losses slightly higher, ranging from 8.4 to 10.5%. This fact indicates the high water-retention capacity of chitosan. The high thermal stability of these materials is evidenced by the elevated temperature required to eliminate the organic matter associated with the clay. This fact occurs between 500 and 800 K, corresponding to the combustion of the intercalated chitosan.

**(2) Potentiometric Sensors Based on Clay−Chitosan Nanocomposites.** One of the most remarkable features of these materials is related to their good mechanical properties. The combination of chitosan and montmorillonite reduces the film-forming capabilities of both separated components, the chitosan solutions and the clay suspensions. We have observed that chitosan−clay mixtures tend to shrink as the chitosan−clay ratio increases, providing robust and handy three-dimensional networks that can be employed to construct bulk-modified electrodes just by the addition of graphite powder necessary to confer the required electronic conductivity to the system. The excellent mechanical

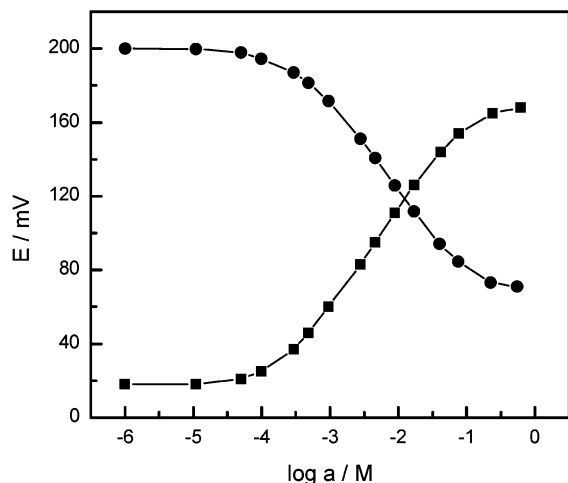(17) Theng, B. K. G. *Formation and Properties of Clay−Polymer Complexes*; Elsevier: New York, 1979.

**Figure 7.** Dependence of the electrode potential on the activity of NaCl solutions for sensors based on nanocomposites resulting from chitosan−clay ratios of (■) 1:1 and (●) 5:1.



**Figure 8.** Dependence of the electrode potential on the anion activity for the sensor based on a 5:1 chitosan−clay nanocomposite when potentiometric measurements are carried out in solutions containing (a) $Cr_2O_7^{2-}$, (b) $Fe(CN)_6^{3-}$, (c) $NO_3^-$, (d) $CH_3COO^-$, (e) $SO_4^{2-}$, and (f) tetraphenylborate.

behavior of the chitosan−clay−graphite system, allowing the construction of robust electrodes without the addition of interparticular cross-linking agents as epoxides or organosilanes, should be mentioned. In addition, given that such electrodes are composed solely of natural materials, i.e., clay, chitosan, and graphite, they are of low environmental impact.

On the other hand, the presence of $-NH_3^+Ac^-$ species acting as anionic exchangers in nanocomposites with the higher amounts of biopolymer, exceeding the clay CEC, allows for these systems to be applied as sensors for anions. This fact has been confirmed by the potentiometric responses of sensors based on the nanocomposites with starting chitosan−clay ratios of 1:1 and 5:1, respectively, which provided different responses when measuring the same NaCl solutions (Figure 7). The positive slope (+52.7 mV/decade) exhibited by the first sensor indicates the cationic exchange behavior of the nanocomposite obtained from a chitosan−clay ratio of 1:1. This is in agreement with the CHN chemical analysis and the EDX results, which revealed the presence of residual $Na^+$ ions because the amount of chitosan adsorbed on the clay was not enough to balance the initial negative charges. In contrast, the sensor constructed with the 5:1 chitosan−clay nanocomposite gave the expected decrease in the electric potential as the anion activity increases (negative slope of −48.2 mV/decade) because of the presence of anionic exchange sites in the second layer of adsorbed chitosan. These results reveal the ability of modifying the initial cationic exchange property of the clay, depending on the intercalated chitosan amount, and turning the nanocomposite into an anionic exchanger when chitosan is intercalated as a bilayer.

The response of the sensor with a chitosan−clay−graphite ratio of 5:1:0.2 was studied in solutions of different anions. As shown in Figure 8, the sensor exhibits a Nernstian response to nitrate (−57.4 mV/decade), sub-Nernstian responses toward acetate and sulfate (−36.4 and −24.9 mV/decade, respectively), and super-Nernstian responses toward dichromate and ferricyanide (−43.8 and −29.1 mV/decade, respectively). It is important to mention that the sensor shows negative slopes for all of the anions measured except for tetraphenylborate. In this case, a positive slope (+25.6
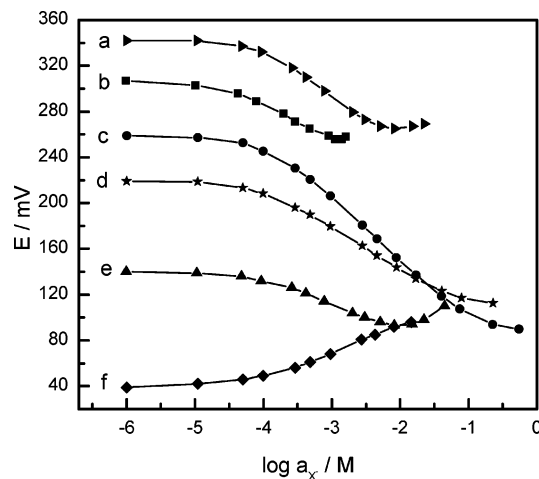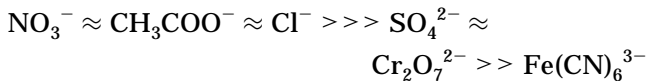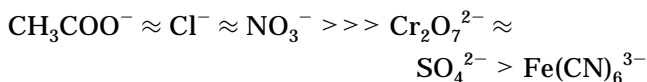
mV/decade) is obtained, indicating the sensor response toward the cation, $Na^+$. Such behavior may be related to the rejection of the anion due to its hydrophobic character and its large size.

The potentiometric selectivity coefficients of the sensor for nitrate against different anions ($K_{NO_3^-,X^-}^{POT}$) were determined by the separate solutions method.[18] The potential was measured in separate solutions containing different anions at the same activity as that of the main anion solution ($a_{NO_3^-} = a_{X^-} = 10^{-3}$ M), with the sensor being previously conditioned in a $10^{-2}$ M nitrate solution. The sensor showed the following selectivity sequence:

$$NO_3^- \approx CH_3COO^- \approx Cl^- >>> SO_4^{2-} \approx$$
$$Cr_2O_7^{2-} >> Fe(CN)_6^{3-}$$

The sensor is about 25 times more selective to nitrate than to divalent anions and about 60 times more selective than to trivalent anions, but it shows an important interference due to chloride and acetate. Morigi et al.[19] reported the application of hydrotalcite-like solids (LDHs) as ionophore toward the anion present in their interlayer. According to these results, the increase of the selectivity toward $SO_4^{2-}$ would be expected after conditioning the chitosan-montmorillonite based sensor in a 0.01 M sulfate solution. Nevertheless, the resulting selectivity sequence was quite similar to that obtained after conditioning the sensor in a nitrate solution:

$$CH_3COO^- \approx Cl^- \approx NO_3^- >>> Cr_2O_7^{2-} \approx$$
$$SO_4^{2-} > Fe(CN)_6^{3-}$$

These preliminary results, which will be more extensively studied elsewhere,[20] confirm the remarkable selectivity of the sensor for monovalent anions, which may be related to the special arrangement of the

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(19) Morigi, M.; Scavetta, E.; Berrettoni, M.; Giorgetti, M.; Tonelli, D. *Anal. Chim. Acta* **2001**, *439*, 265−272.

chitosan layers, in an extended conformation, into the montmorillonite interlayer space. Because the adsorbed biopolymer is confined in such a nanostructured region, it may be unable to change its conformation to balance more than one negative charge, giving the best response toward monovalent anions. This fact makes the employment of the sensor as an ion-selective electrode for the determination of a given anion in a complex sample with a mixture of monovalent species difficult. However, such partial selectivity to different anions (cross-sensitivity) becomes an important feature for the development of expert systems based on electrode arrays, with their responses being evaluated by artificial intelligence methods.[21,22] In this sense, we have developed an improved pattern recognition method (case-based reasoning, CBR) for ionic detection in liquid complex samples.[23]

## Conclusions

The intercalation of the cationic biopolymer chitosan into $Na^+$−montmorillonite through a cationic exchange process provides nanocomposites with both interesting structural and functional properties. The clay reduces the film-forming capability of chitosan leading to compact, robust, and handy three-dimensional nanocomposites that can be combined with graphite to construct bulk-modified electrodes. The functional properties are due to the $-NH_3^+$ groups in the chitosan structure that may act as an ionophore in the detection of anions.

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(21) Bos, M.; Bos, A.; Van der Linden, W. E. *Anal. Chim. Acta* **1990**, *233*, 31−39.

(22) Banet, M.; Massart, D. L.; Fabry, P.; Conesa, F.; Eichener, C.; Menardo, C. *Talanta* **2000**, *51*, 863−877.

(23) Colilla, M.; Fernández, C. J.; Ruiz-Hitzky, E. *Analyst* **2002**, *127*, 1580−1582.

The techniques employed in the characterization of the nanocomposites, CHN chemical analysis, XRD, FTIR spectroscopy, STEM, EDX, and thermal analysis, confirm the high affinity between the clay substrate and the biopolymer as well as the special arrangement of chitosan as a bilayer when the biopolymer amount exceeds the CEC of the clay. The intercalation of the first layer of chitosan takes place mainly by electrostatic interactions between the $-NH_3^+$ groups in the chitosan chain and the negative sites in the clay. In contrast, hydrogen bonds between amino and hydroxyl groups of chitosan and the clay substrate are established in the adsorption of the second layer. Thus, the presence of $-NH_3^+Ac^-$ species in the bilayer turns the resulting material into a potential anion exchanger (57.1 mequiv/100 g of capacity), which is employed in the development of bulk-modified electrodes for the potentiometric determination of anions. The high selectivity of such sensors toward monovalent anions may be related to the particularly extended conformation of the biopolymer bilayer in the clay interlayer space. Because of this partial selectivity to different anions (cross-sensitivity), these sensors can be regarded as promising devices for the development of electrode arrays combined with artificial intelligence methods for the characterization of complex liquids containing mixtures of different anionic species.

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