Bio-Nanocomposites Based on Layered Double Hydroxides

Margarita Darder,[†] Mar López-Blanco,[†] Pilar Aranda,[†] Fabrice Leroux,[‡] and
Eduardo Ruiz-Hitzky*[,†]

Instituto de Ciencia de Materiales de Madrid, CSIC, Cantoblanco, 28049 Madrid, Spain, and Laboratoire des Matériaux Inorganiques, Université Blaise Pascal, UMR 6002, Aubière Cédex 63177, France

Received September 24, 2004. Revised Manuscript Received February 9, 2005

A new family of functional hybrid nanocomposites based on the intercalation of naturally occurring anionic biopolymers including alginic acid, pectin, κ-carrageenan, ι-carrageenan, and xanthan gum in $[Zn_2Al(OH)_6]Cl\cdot nH_2O$ layered double hydroxide (LDH), have been synthesized. The "coprecipitation" or "co-organized assembly" method has been successfully employed for the intercalation of such polysaccharides within the $[Zn_2Al]$ LDH. However, the "reconstruction" procedure from the calcined LDH in the presence of the anionic polysaccharides only resulted in a partial intercalation of the organic guest. Particular effort was devoted to the study of ι-carrageenan−$[Zn_2Al]$ systems. An essential feature of the prepared biopolymer−$[Zn_2Al]$ nanocomposites is that the anionic exchange capacity of the pristine LDH is turned into a cationic exchange capacity due to negatively charged groups in the polysaccharide structure that do not interact with the positively charged LDH layers. In agreement with the fact that most of the studied biopolymers interact strongly with calcium ions producing homogeneous gels, the prepared biopolymer−$[Zn_2Al]$ nanocomposites were operative as active phases of sensors for the recognition of calcium ions. Hence, the biopolymer−$[Zn_2Al]$ nanocomposites have been incorporated in carbon paste or PVC matrixes for the development of potentiometric sensors. These devices were applied to calcium determination by direct potentiometry and the best responses were obtained for the sensors based on alginate−LDH and ι-carrageenan−LDH nanocomposites.

Introduction

Layered double hydroxides (LDHs) can act as host matrixes for the intercalation of organic polymers in order to synthesize hybrid organic−inorganic nanocomposites.[1] The high charge density on the LDH layers (ca. 300 mEq/100 g LDH) prevents exfoliation of the sheets and, therefore, the intercalation of large anionic species by a delamination and entrapping-restacking process, commonly employed to prepare nanocomposites based on layered clays,[2] is not feasible in many cases. Alternative methods of synthesis, including direct ion-exchange, coprecipitation, and reconstruction of calcined LDH in the presence of the polymer, are usually employed in the intercalation in the layered hydroxides.[3,4] The intercalation of anionic polymers of intermediate molecular weight has been reported for poly(ethylene oxide) derivatives by ion-exchange and coprecipitation[5] and for poly(α,β-aspartate) by coprecipitation method.[6] However, the direct ion-exchange method is generally difficult for the intercalation of larger anionic polymers within LDHs, since this process will be kinetically limited by the slow diffusion of the polymer. To overcome this problem, an alternative method is in situ polymerization after intercalation of the monomers by ion exchange.[7−9] The coprecipitation or "co-organized assembly" method gives rise to monophasic nanocomposites resulting in the intercalation of polymers with high molecular weights (as high as $M_w = 150\,000$ g·mol^{-1}) such as poly(acrylic acid), poly(vinylsulfonate), poly(styrenesulfonate,)[10] and poly(vinyl alcohol)[11] within the LDH sheets.

A special feature of LDHs is that they can be calcined at moderated temperatures (300−500 °C) to yield the corresponding mixed oxides and then recover their original layer structure after treatment with aqueous solutions containing anionic species. This property is useful to incorporate organic species in the LDH structure.[12−16]

---

* To whom correspondence should be addressed. Fax: +34-91-3720623. E-mail: eduardo@icmm.csic.es.

[†] Instituto de Ciencia de Materiales de Madrid.

[‡] Université Blaise Pascal.

(1) Leroux, F.; Besse, J.-P. *Chem. Mater*. **2001**, *13*, 3507−3515 and references therein.

(2) Ruiz-Hitzky, E.; Aranda, P.; Serratosa, J. M. Clay Organic Interactions: Organoclay Complexes and Polymer-Clay Nanocomposites. In *Handbook of Layered Materials*; Auerbach, S. M., Carrado, K. A., Dutta, P. K., Eds.; Marcel Dekker: New York, 2004; Ch. 3.

(3) Carlino, S. *Solid State Ionics* **1997**, *98*, 73−84.

(4) Costantino, U.; Marmottini, F.; Nochetti, M.; Vivani, R. *Eur. J. Inorg. Chem*. **1998**, *10*, 1439−1446.

(5) Leroux, F.; Aranda, P.; Besse, J.-P.; Ruiz-Hitzky, E. *Eur. J. Inorg. Chem.* **2003**, *6*, 1242−1251.

(6) Whilton, N. T.; Vickers, P. J.; Mann, S. *J. Mater. Chem.* **1997**, *7*, 1623−1629.

(7) Sugahara, Y.; Yokoyama, N.; Kurado, K.; Kato, C. *Ceram. Int.* **1988**, *14*, 163−167.

(8) Challier, T.; Slade, R. C. T. *J. Mater. Chem.* **1994**, *4*, 367−371.

(9) Moujahid, E. M.; Besse, J.-P.; Leroux, F. *J. Mater. Chem.* **2002**, *12*, 3324−3330.

(10) Oriakhi, C. O.; Farr, I. V.; Lerner, M. M. *J. Mater. Chem.* **1996**, *6*, 103−107.

(11) Messersmith, P. B.; Stupp, S. I. *Chem. Mater.* **1995**, *7*, 454−460.

(12) Narita, E.; Kaviratna, P.; Pinnavaia, T. J. *Chem. Lett.* **1991**, 805−808.

(13) Kooli, F.; Depege, C.; Ennaqadi, A.; De Roy, A.; Besse, J.-P. *Clays Clay Miner.* **1997**, *45*, 92−98.

(14) Rocha, J.; del Arco, M.; Rives, V.; Ulibarri, M. A. *J. Mater. Chem.* **1999**, *9*, 2499−2503.

(15) Rives, V. *Mater. Chem. Phys.* **2002**, *75*, 19−25.

10.1021/cm0483240 CCC: $30.25

The behavior of LDHs as anionic exchangers[17] is the basis of their application in the electroanalytical field as ionophores in ion-selective electrodes for the determination by direct potentiometry of anions that could be incorporated into the interlayer space, i.e., chloride, nitrate, or sulfate.[18,19] The efficiency of such electrodes depends on the anionic exchange capacity of the LDH, which depends on the nature of both the di- and trivalent metals and the interlayer anion.[20] Other electroanalytical applications of LDHs include the development of amperometric sensors suitable for the determination of oxidizable species[21] as well as the construction of a biosensor for cyanide detection where the interlayer anion acts as a mediator between the redox center of the enzyme and the electrode.[22,23]

The development of bioinspired systems based on nanostructured hybrid organic−inorganic materials is a topic of increasing interest.[24] In this context, the intercalation of biopolymers in layered materials has been previously reported in smectite clays[25,26] as well as in LDHs. In this last case, several biopolymers including poly(α,β-aspartate)[6] and DNA[27,28] have been incorporated in the structure of LDHs. Leroux and co-workers have shown the ability of certain polysaccharides, such as alginate, to intercalate LDHs.[29] The present work tries to extend this behavior to other polysaccharides searching applications based on their complexing capacity. In this way, the objective of this work is the preparation of functional hybrid nanocomposites based on the intercalation of anionic biopolymers, whose structures are shown in Figure 1, in the LDH $[Zn_2Al(OH)_6]Cl\cdot nH_2O$, denoted as $[Zn_2Al]Cl$. Alginic acid, pectin, κ-carrageenan, and ι-carrageenan are natural anionic polysaccharides provided with gelling, thickening, and stabilizing properties. Pectin is extracted from citrus peel and apple pomace, while the natural sources of alginic acid and carrageenans are brown and red seaweeds, respectively. Xanthan gum, prepared from aerobic fermentation of *Xanthomonas campestris*, is also an anionic polyelectrolyte employed as nongelling agent for the control of the viscosity. An interesting feature of the prepared biopolymer−$[Zn_2Al]$ nanocomposites is the possibility to reverse the anionic exchange capacity of the pristine LDH to a cationic exchange capacity due to the negative groups in the polysaccharide structure noninteracting with the positively charged LDH layers. This behavior is opposite to that previously observed when the cationic biopolymer chitosan is intercalated as a bilayer in smectite clays.[26] The best results were obtained in the ι-carrageenan−$[Zn_2Al]$ system, and therefore these materials have been studied more deeply. The resulting biopolymer−$[Zn_2Al]$ nanocomposites will be incorporated in carbon paste or PVC matrixes and will act as active phases of the developed sensors applied to the potentiometric determination of calcium, an important analyte in areas of high relevance such as clinical diagnosis, food analysis, and water quality control.

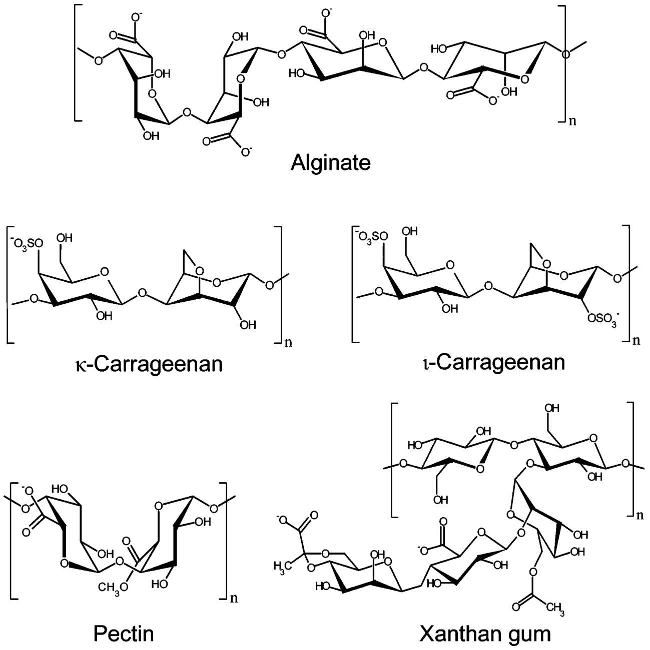

Figure 1. Structures of the studied anionic biopolymers.

Experimental Section

Starting Materials and Reagents. Alginic acid (from Brown Algae), pectin (from Citrus Fruits, galacturonic acid content 87.6%), κ-carrageenan (type I, from Irish Moss), and ι-carrageenan (type II) were obtained from Sigma. Xanthan gum was provided by the ICIDCA (Cuba).

Aqueous solutions were prepared from chemicals of analytical-reagent grade: $AlCl_3\cdot 6H_2O$ (>99%, Fluka), $ZnCl_2$ (98%, Merck), NaOH (97%, Panreac), NaCl (ACS reagent, Riedel-de Haën), $NaNO_3$ (ACS reagent, Fluka), and $CaCl_2\cdot 2H_2O$ (>99.5%, Fluka). Deionized water (resistivity of 18.2 M$\Omega\cdot$cm) was obtained with a Maxima Ultrapure Water system from Elga.

Synthesis Procedure by Coprecipitation Method. Biopolymer (0.91 g) was dissolved in 300 mL of bidistilled water. Alginate (pK_a mannuronic acid units = 3.38, pK_a guluronic acid units = 3.65) and pectin ($pK_a = 2.9$)[30] solutions were prepared by addition of NaOH to the biopolymer suspension to reach a pH value of 8 and ensure complete deprotonation of carboxylic groups. κ-Carrageenan and ι-carrageenan solutions were prepared by slow addition of the biopolymer to hot water (ca. 70 °C) under vigorous magnetic stirring.

---

(16) Latterini, L.; Elisei, F.; Aloisi, G. G.; Costantino, U.; Nochetti, M. *Phys. Chem. Chem. Phys.* **2002**, *4*, 2792−2798.
(17) Meyn, M.; Beneke, K.; Lagaly, G. *Inorg. Chem*. **1990**, *29*, 5201−5207.
(18) Ballarin, B.; Morigi, M.; Scavetta, E.; Seeber, R.; Tonelli, D. *J. Electroanal. Chem.* **2000**, *492*, 7−14.
(19) Morigi, M.; Scavetta, E.; Berrettoni, M.; Giorgetti, M.; Tonelli, D. *Anal. Chim. Acta* **2001**, *439*, 265−272.
(20) Ballarin, B.; Gazzano, M.; Seeber, R.; Tonelli, D.; Vaccari, A. *J. Electroanal. Chem.* **1998**, *445*, 27−37.
(21) Scavetta, E.; Berrettoni, M.; Seeber, R.; Tonelli, D. *Electrochim. Acta* **2001**, *46*, 2681−2692.
(22) Therias, S.; Mousty, C.; Forano, C.; Besse, J.-P. *Langmuir* **1996**, *12*, 4914−4920.
(23) Shan, D.; Cosnier, S.; Mousty, C. *Biosens. Bioelectron*. **2004**, *20*, 390−396.
(24) Ruiz-Hitzky, E. *Chem. Rec.* **2003**, *3*, 88−100.
(25) Krikorian, V.; Kurian, M.; Galvin, M. E.; Nowak, A. P.; Deming, T. J.; Pochan, D. J. *J. Polym. Sci. B: Polym. Phys.* **2002**, *40*, 2579−2586.
(26) (a) Darder, M.; Colilla, M.; Ruiz-Hitzky, E. *Chem. Mater.* **2003**, *15*, 3774−3780. (b) Darder, M.; Colilla, M.; Ruiz-Hitzky, E. *Appl. Clay Sci.* **2005**, *28*, 199−208.
(27) Choy, J. H.; Kwak, S.-Y.; Jeong, Y.-J.; Park, J.-S. *Angew. Chem., Int. Ed.* **2000**, *39*, 4042−4045.
(28) Choy, J. H.; Kwak, S.-Y.; Park, J.-S.; Jeong, Y.-J.; Portier, J. *J. Am. Chem. Soc.* **1999**, *121*, 1399−1400.
(29) Leroux, F.; Gachon, J.; Besse, J.-P. *J. Solid State Chem*. **2004**, *177*, 245−250.

(30) http://www.lsbu.ac.uk/water/hydro.html and references therein.

Table 1. Composition Obtained from Chemical Analysis (Al and Zn by ICP/AES; Biopolymer Content from CHNS Elemental Analysis; and Water Content from TG Analysis) and Proposed Formulas of the Biopolymer−[Zn_2Al] Nanocomposites

bio-nanocomposite	Zn %	Al %	biopolymer %	H_2O %	formula
ι-carrageenan−LDH	27.15	7.35	43.1	11.4	$[Zn_2Al_{1.3}(OH)_6]$ (ι-carrageenan)$_{1.1}$·3.7H_2O
κ-carrageenan−LDH	34.06	8.44	33.1	10.0	$[Zn_2Al_{1.2}(OH)_6]$ (κ-carrageenan)$_{0.8}$·2.6H_2O
alginate−LDH[a]	21.71	4.25	40.5	13.2	$[Zn_2Al_{0.95}(OH)_6]$ (alginate)$_{1.4}$·4.4H_2O
pectin−LDH	24.93	6.27	44.5	9.9	$[Zn_2Al_{1.2}(OH)_6]$ (pectin)$_{1.4}$·3.1H_2O

[a] Data from ref 29.

$AlCl_3 \cdot 6H_2O$ (0.68 g) and 0.603 g of $ZnCl_2$ were dissolved in 500 mL of decarbonated bidistilled water. This solution was added dropwise with a peristaltic pump to the biopolymer solution, while kept under nitrogen atmosphere. Simultaneously, a solution of NaOH 1 M was also added dropwise to the biopolymer solution through an automatic dispenser (Dosimat 765 with an 806 Exchange Unit, from Metrohm) controlled by a 781 pH/Ion Meter (Metrohm) to keep a constant pH of 9 during the synthesis. The precipitate was aged in the mother liquid for 24 h, centrifuged, and then washed three times with bidistilled water and finally dried at 40 °C. The $[Zn_2Al(OH)_6]Cl \cdot nH_2O$ LDH without intercalated biopolymer, denoted as $[Zn_2Al]Cl$, was synthesized following the same procedure but replacing the biopolymer solution with bidistilled water.

Characterization. The resulting $[Zn_2Al]Cl$ LDH and biopolymer−[Zn_2Al] nanocomposites were characterized by chemical analysis (Perkin-Elmer 2400 CHNS/O Series II analyzer; Thermo Jarrel Ash "IRIS Advantage" ICP/AES for Al and Zn determination; Cl^- ion-selective electrode DX235-Cl Mettler-Toledo; samples were dissolved by 0.1 M nitric acid treatment at moderate temperature), XRD (Siemens D-500 instrument with a Cu anode and Ni filter), thermal analysis (TG and DTA) (SSC/5200 Seiko analyzer), IR spectroscopy (Nicolet 20SXC spectrophotometer), and SEM (ZEISS DSM-960 microscope working at 15.0 kV).

^{13}C CP MAS NMR spectra were obtained in a Bruker Avance 400 spectrometer, using a standard cross-polarization pulse sequence. Samples were spun at 10 kHz. Spectrometer frequencies were set to 100.62 and 400.13 MHz for ^{13}C and ^1H, respectively. A contact time of 2 ms and a period between successive accumulations of 5 s were used. The number of scans was of 800. Chemical shift values were referenced to tetramethylsilane (TMS). ^{27}Al MAS NMR spectra were also registered with a Bruker Avance 400 spectrometer at 104.26 MHz, using in this case a standard single pulse sequence, with 2 μs pulse width and a period between successive accumulations of 5 s. Samples were spun at 10 kHz and the number of scans was 400. Chemical shift values were given with respect to an external aqueous $AlCl_3$ solution.

Potentiometric measurements were performed with an Orion EA920 Ion Selective Meter. The homemade sensors acted as the working electrode in a conventional two-electrode configuration and the potential was measured (at room temperature) against a Ag/AgCl reference electrode.

Potentiometric Sensors. Modified carbon paste electrodes (CPEs) were prepared by the following procedure. Pristine $[Zn_2Al]Cl$ LDH or biopolymer−[Zn_2Al] nanocomposite (20 mg) was ground in a mortar to obtain a very fine powder. Then, the powder was mixed into 40 mg of graphite powder (1−2 μm, synthetic, from Aldrich) and 40 mg of paraffin oil as liquid binder and thoroughly mixed in an agate mortar. The modified carbon paste was packed firmly into the cavity of an electrode body (internal diameter 3 mm), and the electrical contact was assured by a copper wire. The electrode surface was then polished on paper until a smooth surface was obtained.

Modified PVC-based membranes were prepared by incorporating 50 mg of pristine $[Zn_2Al]Cl$ LDH or biopolymer−[Zn_2Al] nanocomposite (particle size lower than 74 μm) to a mixture containing 50 mg of poly(vinyl chloride) (PVC, high molecular weight from Fluka) and 100 mg of dibutyl phthalate (DBP, from M&B) in 750 μL of tetrahydrofuran (THF, from Merck). The mixture was dispersed in an ultrasonic bath for 10 min. A 40-μL portion of the mixture were suspended at the end of a polypropylene tube (diameter 2.5 mm) and the solvent was allowed to evaporate for 3 h. The potentiometric membrane electrode was constructed by filling the tube with $CaCl_2$ 10^{-3} M as internal reference solution and introducing an internal Ag/AgCl reference electrode. The modified PVC based membrane was conditioned in $NaNO_3$ 10^{-3} M for 24 h prior to use.

Results and Discussion

Intercalation of Anionic Biopolymers in Layered Double Hydroxides by Coprecipitation Method. As indicated by Oriakhi et al.,[10] coprecipitation method is a useful way to intercalate anionic polymers of high molecular weight within layered double hydroxides. This method consists of the "co-organized assembly" synthesis of the LDH in the presence of the polymer, which will be incorporated between the LDH sheets. Recently, it has been successfully applied to intercalate an anionic biopolymer, alginate, within a LDH.[29] In the present work, the incorporation of analogous naturally occurring anionic polysaccharides including pectin, xanthan gum, ι-carrageenan, and κ-carrageenan in the [Zn_2Al] structure, will be studied by the coprecipitation method and compared to the alginate−[Zn_2Al] nanocomposite.

Chemical Analysis. CHNS chemical analyses of the biopolymer−[Zn_2Al] nanocomposites confirm the incorporation of the biopolymers in the LDH. From such results, the amount of biopolymer retained by the LDH 2D-network, expressed in mEq of biopolymer per 100 g of LDH, can be calculated taking into account the content of negatively charged groups in the biopolymer structure (Figure 1). The values obtained for each nanocomposite are 316, 401, 326, and 128 mEq/100 g for alginate, pectin, ι-carrageenan, and κ-carrageenan, respectively. Taking into account other analytical results for Zn, Al, and H_2O content the formulas proposed for the nanocomposites are collected in Table 1. It is important to remark the absence of chloride in all the samples as deduced from both EDX and potentiometric analyses. This last technique confirms the presence of Cl^- in the LDH of reference, obtained in absence of biopolymers, which has $[Zn_2Al_{1.2}(OH)_6]Cl_{1.6} \cdot 1.1H_2O$ as formula. The same technique applied to analyze the chloride content in the nanocomposites gives negative results, indicating a Cl^- concentration below the detection limit ($< 4 \times 10^{-5}$ M). The Al excess from the theoretical Zn/Al ratio (2:1) is tentatively attributed to the formation of aluminate and/or oxyhydroxide species that could remain associated to nonintercalated biopolymers, in agreement with the phase diagrams described for Al(III) species vs pH. The presence of aluminate species could be explained when the biopolymer content in the

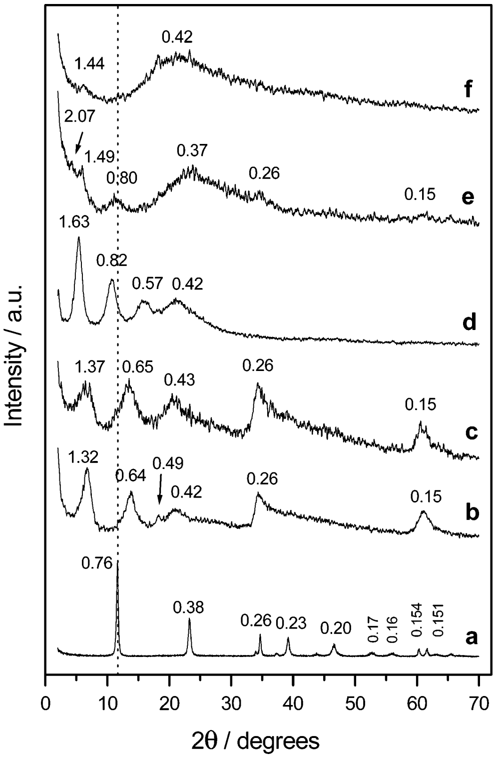

Figure 2. X-ray diffraction patterns of (a) starting LDH ([Zn_2Al]Cl) and biopolymer−[Zn_2Al] nanocomposites prepared with (b) alginate, (c) pectin, (d) ι-carrageenan, (e) κ-carrageenan, and (f) xanthan (d_{00l} values in nm).

nanocomposite is below the expected stoichiometry (see for instance the κ-carrageenan−LDH), whereas charged oxy-hydroxide species could be compensating the electrical charge in the case of nanocomposites containing an excess of biopolymer (see for instance the ι-carrageenan−LDH).

The anionic exchange capacity (AEC) of LDH with a Zn/Al ratio of 2 is approximately 340 mEq/100 g LDH. The amounts of alginate and ι-carrageenan intercalated in the LDH, provided by the chemical analysis, indicate that the positive charges in the LDH structure may be compensated at the theoretical extent by the negative charges present in the biopolymers. In the case of pectin, an excess of polysaccharide seems to be incorporated in the LDH suggesting that the positive charge of LDH is reversed to negative by the excess of pectin. In contrast, only a small amount of κ-carrageenan is intercalated in the LDH and it is expected that a great part of the LDH positive charges remain compensated by Cl^- anions. The former case suggests the presence of adsorbed biopolymer which would give rise to anionic affinity as observed by measurements of the electrophoretic mobility on other negatively charged polymers.[31]

X-ray Diffraction. The XRD patterns of the pristine [Zn_2Al]Cl as well as the anionic biopolymer intercalated LDHs are shown in Figure 2. The increase in the basal spacing confirms the intercalation of alginate, pectin, and ι-carrageenan within the LDH. The d_{00l} spacings are obtained using the first rational orders corresponding to the 00*l* reflections, with values of 1.32, 1.37, and 1.63 nm for alginate, pectin, and ι-carrageenan (Figure 2b−d), respectively. The increase of the interlayer distance (Δd_L) in the nanocomposites is calculated from the d_{00l} spacing and the thickness of the inorganic layer, estimated to 0.48 nm in comparison to the brucite structure. For the mentioned biopolymer−LDH nanocomposites the Δd_L values are 0.84 and 0.89 nm for alginate and pectin, respectively, which indicate their intercalation as a monolayer, in good agreement with the previous work by Leroux et al.[29] In the case of ι-carrageenan, the Δd_L value of 1.15 nm indicates that the polymer could be intercalated as a double helix. It has been found that the ι-carrageenan molecule forms a half-staggered, parallel, 3-fold, right-handed double helix stabilized by interchain hydrogen bonds from 2- and 6-hydroxyl groups in the galactosyl units.[32] The thickness of the ι-carrageenan double helix, 1.09 nm, is consistent with the measured value of Δd_L in the ι-carrageenan−[Zn_2Al] nanocomposite.

In the case of κ-carrageenan (Figure 2e), the XRD pattern shows two peaks of very low intensity that correspond to d_{00l} spacings of 2.07 and 1.49 nm. In Figure 2f, the XRD pattern obtained for xanthan shows a similar peak of very low intensity corresponding to a d_{00l} spacing of 1.44 nm. In both cases, these ill-defined diffraction peaks of low intensity indicate that κ-carrageenan and xanthan give poor crystalline materials, indicating a low ordering (low stacking arrangement) in the structural arrangement of the resulting compounds, contrary to that observed in the intercalations of alginate, pectin, or ι-carrageenan. In the case of κ-carrageenan this fact may be due to the low content of negatively charged groups in this polymer, i.e., 1 charge/dimer, in comparison to the other polysaccharides. The anionic charges in the xanthan structure are located at the end of the side chains, while the other anionic polyelectrolytes are linear polymers. The presence of side chains in the xanthan structure is found to render difficult the incorporation of this nonlinear biopolymer within the LDH.

Concerning the (110) reflection typical of the LDH structures, it should be noted that it is clearly observed in Figure 2b, c, and e, but it is not observed in the diffractograms of ι-carrageenan−LDH and xanthan−LDH (Figure 2d and f). In the case of ι-carrageenan (Figure 2d) the EDX analysis indicates a Zn/Al ratio close to 2 (excess of Al is discussed below) indicating the formation of LDH which is corroborated by the IR spectra (vide infra). It is assumed that highly pronounced platelets stacking usually give rise to a (110) diffraction peak of very small intensity that sometimes is not observed in the XRD pattern as occurs in the intercalation of large molecules such as DNA.[27,28] It underlines merely that the so-called co-organized assembly is difficult to tackle when the organic macromolecules are strongly orientating the LDH platelets. In a different way, in the case of xanthan (Figure 2f) according to the EDX analysis indicating a very high content in Al vs. Zn, the XRD indicates that the LDH, and therefore the related nanocomposite, is not formed.

For comparison with the coprecipitation method, alternative methods including direct ion-exchange, reconstruction

---

(31) Wilson, O. C., Jr.; Olorunyolemi, T.; Jaworski, A.; Borum, L.; Young, D.; Siriwat, A.; Dickens, E.; Oriakhi, C.; Lerner, M. *Appl. Clay Sci.* **1999**, *15*, 265−279.

(32) Janaswamy, S.; Chandrasekaran, R. *Carbohydr. Res.* **2002**, *337*, 523−535.

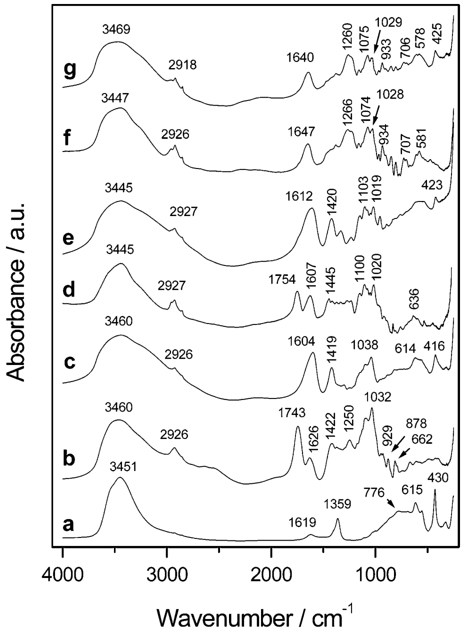

Figure 3. IR spectra (4000−350 cm^{-1} region) of (a) [Zn_2Al]Cl LDH, (b) alginate, (c) alginate−LDH, (d) pectin, (e) pectin−LDH, (f) ι-carrageenan, and (g) ι-carrageenan−LDH.

in aqueous medium,[13] reconstruction in H_2O/glycerol medium,[33] and microwave-assisted rehydration[34] were followed to intercalate the ι-carrageenan in the LDH. The aim is to check the efficiency of the reconstruction method and its suitability in order to incorporate κ-carrageenan and xanthan. ι-Carrageenan was chosen as a model due to the good results obtained with the previously assayed method.

Rehydration in the presence of ι-carrageenan in H_2O/glycerol medium failed to incorporate the biopolymer in the LDH structure, with the inorganic host showing its unmodified characteristic XRD peaks (data not shown). The other methods yielded a partial intercalation of ι-carrageenan, and the XRD pattern of the samples showed the characteristic diffraction peaks of the original LDH together with a peak of very low intensity corresponding to a d_{00l} spacing of 1.73 nm ($\Delta d_L = 1.25$ nm), which is attributed to the intercalated biopolymer. This fact indicates that only a small amount of biopolymer is intercalated in the LDH, being most likely present at the border of the LDH microcrystals,[16] and points out that the coprecipitation method is here the best way to intercalate the studied polysaccharides into the LDH structure.

IR Spectroscopy. FTIR spectra, in the 4000−350 cm^{-1} wavenumber range, of the pristine [Zn_2Al]Cl LDH, the pure biopolymers, and the corresponding biopolymer−[Zn_2Al] nanocomposites are shown in Figure 3. A strong vibration band around 3451 cm^{-1} is observed in all the spectra and is attributed to the stretching vibration ν_{OH} of water molecules and hydroxyl groups belonging to brucite layers. The spectrum of [Zn_2Al]Cl LDH (Figure 3a) shows vibration bands at 1619 cm^{-1} (δ_{HOH}), 1359 cm^{-1} ($\nu_{3\ \mathrm{asym}\ C-O}$ stretching mode of the carbonate anion), 776 and 625 cm^{-1} (ν_{M-O}), and 430 cm^{-1} (δ_{O-M-O}). In the spectra of alginate and pectin biopolymers (Figure 3b and d), a vibration band related to the stretching vibration ($\nu_{C=O}$) of carboxylate groups appears at 1743 and 1754 cm^{-1}, respectively. When the biopolymers are incorporated within the LDH lamellae (Figure 3c and e), the band is shifted toward a lower wavenumber, 1604 and 1612 cm^{-1} in each case, indicating a strong interaction of these negative groups with the positively charged LDH sheets. These bands cannot be attributed to water molecules due to their low intensity, but they could involve the band related to the bending vibration of water. Peaks at 1422 and 1445 cm^{-1} for alginate and pectin biopolymers, respectively, which are also related to the stretching vibration of carboxylate groups, are shifted to a lower frequency (1419 and 1420 cm^{-1}) in the corresponding nanocomposites spectra.

The spectrum of ι-carrageenan (Figure 3f) shows the characteristic vibration bands of the biopolymer: 1237 cm^{-1} ($\nu_{S=O}$ of SO_4), 930 cm^{-1} (3,6-anhydro-L-galactose), 849 cm^{-1} (C−O−S of SO_4-4, secondary axial C-4), 805 cm^{-1} (C−O−S of 3,6-anhydro-L-galactose), and 902 cm^{-1} attributed to the glycosidic bond in the polysaccharide structure.[35] These vibration bands are also observed in the spectrum of the corresponding nanocomposite (Figure 3g), but, in contrast to the alginate− and pectin−LDHs, the bands related to the sulfate group are not shifted toward a lower wavenumber, indicating a weak interaction between the ι-carrageenan and the LDH sheets.

The IR spectra of the nanocomposites exhibit in all the cases a peak around 420 cm^{-1} which is attributed to the δ_{O-M-O} deformation vibrations in the LDH sheets,[13] indicating that the LDH structure is truly formed when biopolymers are incorporated between the layers, as confirmed previously by XRD.

^{13}C NMR Spectroscopy. Solid-state high-resolution NMR spectroscopy was applied to study the incorporation of the biopolymer ι-carrageenan into the LDH structure. The ^{13}C NMR spectrum of the biopolymer (Figure 4) exhibits signals at 102.4, 74.4, and 62.4 ppm that can be assigned to G1, G4-G5, and G6, respectively, in the galactose residues, while the signals at 90.9 and 81.0 ppm are attributed to A1 and A3-A4 in the anhydrogalactose residues (carbon atoms are labeled as G and A according to the residue, as shown in Figure 4). The signal at 77.3 ppm may be assigned to several carbon nuclei in both residues, G3 and A5, whereas the signal at 69.2 ppm corresponds to G2, A2, and A6. Signals at 104.9 and 98.8 ppm may be due to small fractions of ν-carrageenan, the precursor of ι-carrageenan, present in the commercial preparation of the biopolymer.[35] The ^{13}C NMR spectrum of the intercalated biopolymer (Figure 4b) shows a slight shift of the signals attributed to A2 and G4 (at 69.2 and 74.4 ppm), which correspond to the carbon atoms linked to the sulfate groups. The minor shift of both signals may indicate a weak interaction between the negatively charged sulfate groups in the biopolymer and the positively charged LDH layers, which is in good agreement with the results obtained from FTIR. The current results are similar to what is usually

---

(33) Dimotakis, E. D.; Pinnavaia, T. J. *Inorg. Chem.* **1990**, *29*, 2393−2394.

(34) Hussein, M. Z.; Zainal, Z.; Yahaya, A. H.; Foo, D. W. V. *J. Mater. Synth. Process.* **2002**, *10*, 89−95.

(35) (a) Bellion, C.; Brigand, G.; Prome, J.-C.; Welti, D.; Bociek, S. *Carbohydr. Res.* **1983**, *119*, 31−48. (b) Greer, C. W.; Shomer, I.; Goldstein, M. E.; Yaphe, W. *Carbohydr. Res.* **1984**, *129*, 189−196.

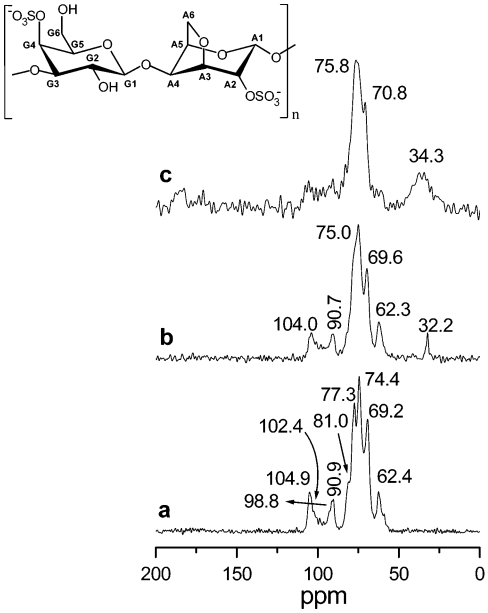

Figure 4. ^{13}C NMR spectra of (a) the biopolymer *ι*-carrageenan, (b) the *ι*-carrageenan−LDH nanocomposite and (c) the biopolymer *ι*-carrageenan after alkaline treatment.

observed in the case of the incorporation of large guest species such as polymers;[9−11] more precisely, the case of carrageenan is close to alginate[29] where the cumbersome biopolymer was found to strongly orient the morphology of the hybrid phase (next section). In Figure 4b, can be also observed a signal at 32.2 ppm that does not appear in the spectrum of the pristine biopolymer. This signal is ascribed to some changes in the biopolymer structure after the alkaline treatment applied during the "coprecipitation" procedure rather than to the intercalation. Figure 4c shows the spectrum of the biopolymer after treatment in 1 M NaOH overnight. Since the 6-sulfated galactose units in the fraction of the precursor, *ν*-carrageenan, are vulnerable to alkali, they may undergo a cyclization to 3,6-anhydrogalactose under such alkaline treatment.[36]

SEM-EDX Study. The SEM image of the [Zn_2Al]Cl material (Figure 5a) shows the usual "sand-rose" morphology of the LDHs, which is quite different from that observed in the SEM image of the *ι*-carrageenan−[Zn_2Al] nanocomposite (Figure 5b). The intercalated biopolymer seems to facilitate the aggregation of the [Zn_2Al] particles, providing compactness to the resulting material. The morphology offering such orientation of the LDH platelets by the incorporation of polymers has been evidenced for other relative nanocomposite systems.[9,11]

In agreement with ICP/AES results, the EDX data indicate that the Zn/Al = 2 theoretical ratio in the nanocomposites diminishes, showing an increase in the Al content that may rise till 30% in weight. As above-discussed, extra-Al content incorporated during the coprecipitation synthesis could be attributed to the presence of oxyhydroxide polymeric Al species, including Al(13) Keggin cages, and probably aluminate species according to the high pH of the medium.[37] The extent of such species in the complex system must be related to the nature and the amount of the biopolymer. The image of that complex system includes the presence of the LDH-nanocomposite associated to a biopolymer excess whose charge is equilibrated by positive Al-polycations. According to the scarce content in chloride ions it could be also proposed that AlO_2^- species act as counterions compensating the positive LDH charge which is not balanced by the biopolymer due to steric hindrance. We have unsuccessfully tried to confirm the nature of the components related to Al species from ^{27}Al NMR spectra but only a single signal around 13.7 ppm, which corresponds to Al(III) in octahedral environments, appears in all the studied samples. The same type of signal is also observed in the LDH without polymer (i.e., [$Zn_2Al_{1.2}(OH)_6$]$Cl_{1.6}$·$1.1H_2O$).

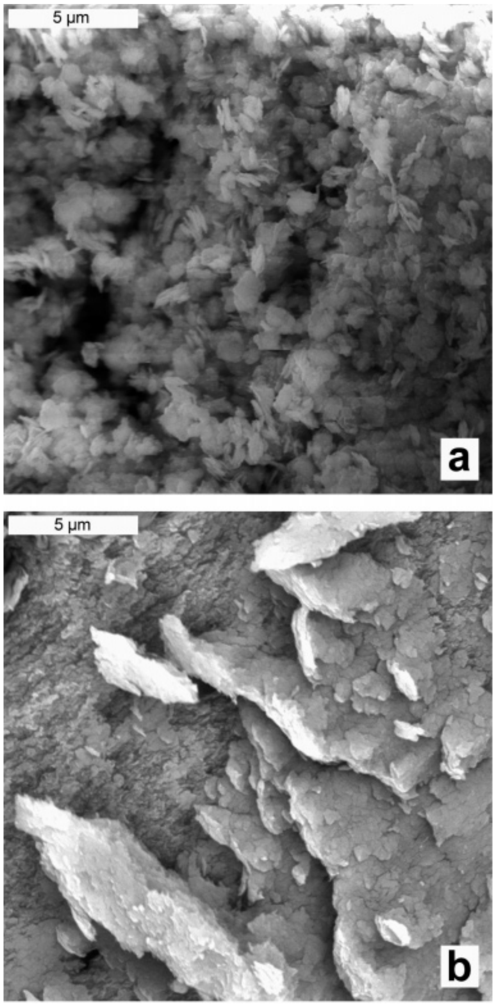

Figure 5. SEM images of the [Zn_2Al]Cl material (a) and the *ι*-carrageenan−[Zn_2Al] nanocomposite (b).

Thermal Stability. A TG curve corresponding to the [Zn_2Al]Cl starting LDH (Figure 6a) shows a mass loss with an endothermic peak at 136 °C, attributed to the loss of physically adsorbed water molecules located at the exterior of the LDH particles and in the interlayer space (16% between 25 and 200 °C). A second mass loss with also an endothermic effect centered at 248 °C is related to a partial dehydroxylation of the brucite-type layers (10% between 200

---

(36) (a) Rees, D. A. *J. Chem. Soc.* **1961**, 5168−5171. (b) Navarro, D. A.; Stortz, C. A. *Carbohydr. Res.* **2003**, *338*, 2111−2118.

(37) Bottero, J. I.; Axelos, M.; Tchoubar, D.; Cases, J. M.; Fripiat, J. J.; Fiessinger, F. *J. Colloid Interface Sci.* **1987**, *117*, 47−57.

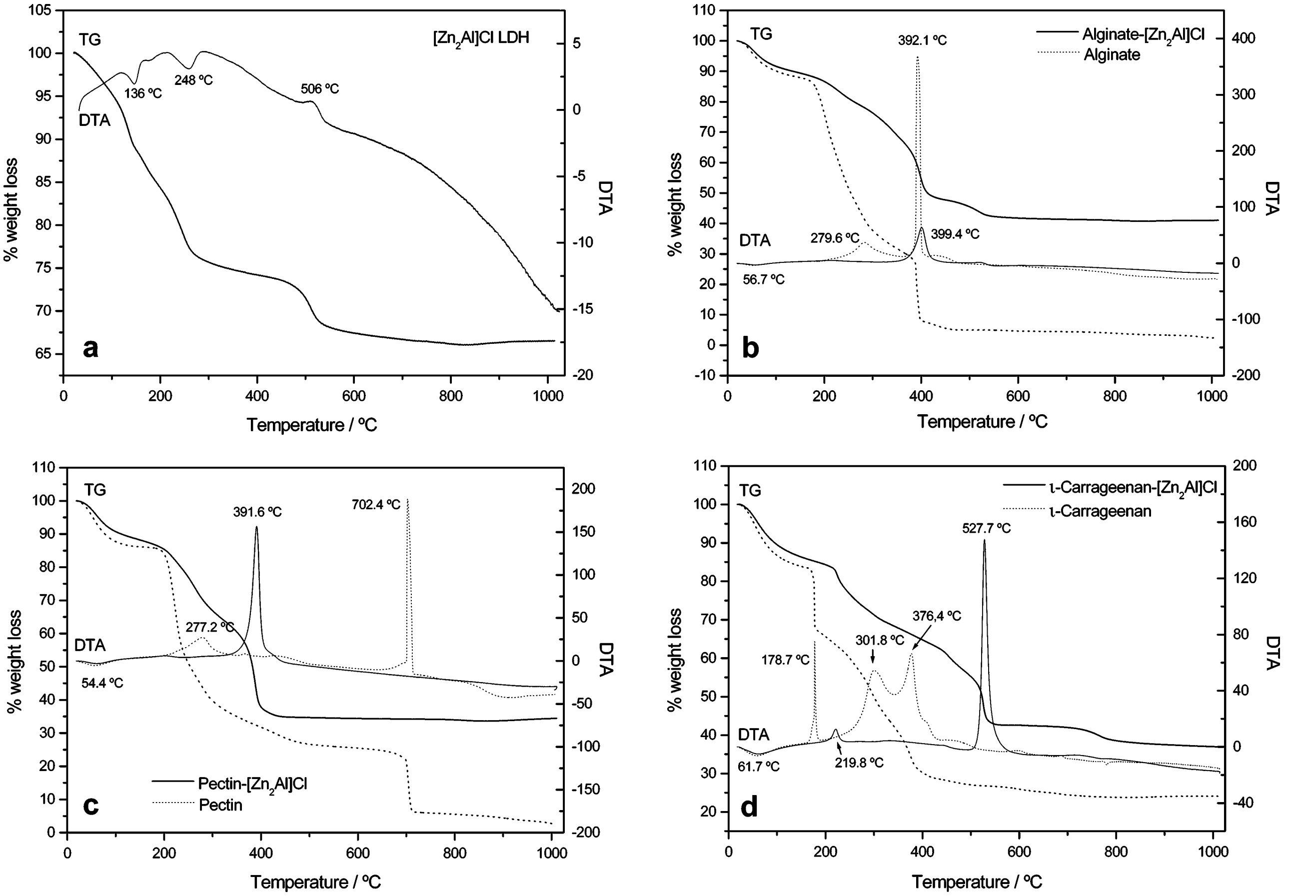

Figure 6. TG and DTA curves obtained for the [Zn$_2$Al]Cl material (a) and the biopolymer−[Zn$_2$Al] nanocomposites with alginate (b), pectin (c), and *ι*-carrageenan (d). In all graphs, dashed lines correspond to both the TG and the DTA curves of the pure biopolymers and solid lines correspond to TG and DTA curves of the biopolymer−[Zn$_2$Al] nanocomposites.

and 450 °C). A third weight loss of 8% associated with an exothermic peak at 506 °C could be attributed to the carbonate decomposition and to the elimination of chloride species.[38]

Figure 6b, c, and d show the TG and DTA curves of the biopolymer−LDH nanocomposites containing alginate, pectin, and *ι*-carrageenan, respectively. In all cases, a weight loss is observed when heating between 25 and 200 °C (12% for alginate and pectin, and 15% for *ι*-carrageenan), and it is related to water molecules physically adsorbed. Heating at temperatures higher than 200 °C produces two mass loss events for all the biopolymers. The weight loss between 200 and 350 °C is attributed to the dehydroxylation of the LDH layers and also to a partial decomposition of the biopolymer (19% for alginate, and 26% for pectin). In the *ι*-carrageenan-LDH nanocomposite a weight loss of 20% is observed between 200 and 400 °C. The second weight loss at heating temperatures higher than 350−400 °C is related to combustion of the intercalated biopolymer (28% for alginate, 27% for pectin, and 28% for *ι*-carrageenan).

The biopolymer-LDH intercalation materials based on alginate and *ι*-carrageenan show decomposition temperatures of the organic matter at almost the same temperature (in the case of alginate), or even at higher temperature (in the case of *ι*-carrageenan), than the pristine biopolymers. This behavior is consistent with a protecting effect increasing the thermal stability, which is particularly enhanced in the case of the *ι*-carrageenan. A different behavior is observed for the pectin−LDH nanocomposite (Figure 6c), exhibiting a decrease of 310 °C in the decomposition temperature of the intercalated biopolymer with respect to the nonintercalated pectin. Previous studies have revealed a high-temperature oxidation reaction of pectins and related carbohydrates above 600 °C.[39] In this case, the higher thermal stability of the pristine polysaccharide could be attributed to its complex structure consisting of blocks of “smooth” and “hairy” regions, with helical conformations that may contribute to the enhanced thermal stability of the native biopolymer.[40] In contrast, the conformation of the intercalated pectin must be quite similar to that of the intercalated alginate, both exhibiting comparable decomposition temperatures close to 400 °C.

Potentiometric Sensors Based on the Biopolymer−[Zn$_2$Al] Nanocomposites. LDHs have been applied as ionophores in potentiometric sensors for the determination of anionic species due to their behavior as anionic exchangers.[18−20] In this work, a CPE based on the [Zn$_2$Al]Cl material has been tested by direct potentiometry for the determination of CaCl$_2$ in aqueous solution. The resulting curve (Figure 7) shows the negative slope expected for a

---

[38] Leroux, F.; Adachi-Pagano, M.; Intissar, M.; Chauvière, S.; Forano, C.; Besse, J.-P. *J. Mater. Chem.* **2001**, *11*, 105−112.

[39] Waymack, B. E.; Belote, J. L.; Baliga, V. L.; Hajaligol, M. R. *Fuel* **2004**, *83*, 1505−1518.

[40] Pérez, S.; Mazeau, K.; Hervé du Penhoat, C. *Plant Physiol. Biochem.* **2000**, *38*, 37−55.

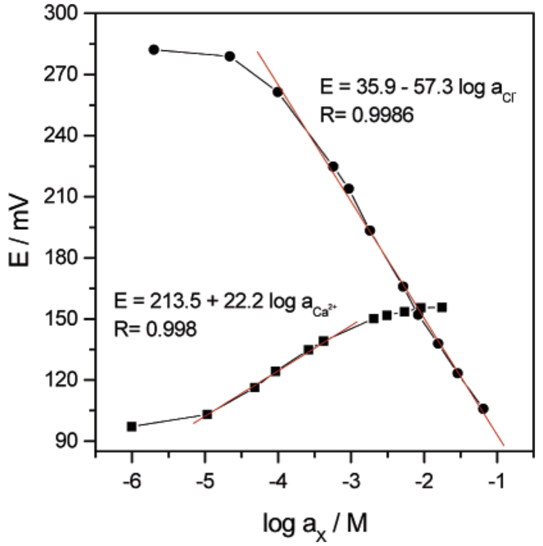

Figure 7. Potentiometric responses of the CPEs prepared with the $[Zn_2Al]Cl$ material (●) and the ι-carrageenan-LDH nanocomposite (■) in $CaCl_2$ solutions of increasing activity.

sensor that responds to the Cl^- anions. In contrast, when the studied biopolymers are intercalated in the LDH and the resulting biopolymer−LDH nanocomposite-based CPEs are employed in the potentiometric measurements of the same $CaCl_2$ solutions, a positive slope is obtained indicating the sensor response toward Ca^{2+} ions. Figure 7 shows the potentiometric response for the ι-carrageenan−LDH nanocomposite. Similar behaviors have been found for all the tested polysaccharides, pointing out the resulting behavior of the biopolymer−$[Zn_2Al]$ nanocomposites as cationic exchangers.

The modification of carbon paste is a simple and fast procedure for the development of ion-selective electrodes (ISEs), but such sensors usually lack good mechanical properties.[41] Due to the low reproducibility and the instability of the biopolymer−LDH modified CPEs, a different design of sensor with internal reference electrolyte and based on modified PVC membranes was usually preferred. The resulting sensors were tested in solutions of $CaCl_2$ of increasing activity and in all cases the expected positive slope was obtained (Figure 8). The best potentiometric responses were observed for the nanocomposites based on alginate and ι-carrageenan biopolymers. Both sensors show also a good reproducibility and stability, providing average slopes of 21.6 ± 2.3 mV decade^{-1} ($n = 5$) and 23.1 ± 1.5 mV decade^{-1} ($n = 4$) for alginate and ι-carrageenan, respectively. A linear response range between 10^{-5} and 10^{-3} M and a detection limit of 10^{-5} M were obtained for the alginate−LDH-based sensor, while these parameters were 10^{-4} to 10^{-2} M and 3.5×10^{-5} M, respectively, for the ι-carrageenan−LDH-based sensor. Even if chemical analysis confirmed the intercalation of alginate and ι-carrageenan in an amount equivalent to the anion exchange capacity (AEC) of the LDH, the positive site charges in the LDH layers are not totally compensated by the biopolymer negative charges due to the relatively high molecular size and thus the stiffness of the monosaccharide units. Since the charge density of the biopolymer structure can be supposed to be much lower than the charge density present in the LDH layers, it is assumed that a part of the positive charge of the inorganic host is compensated by counteranions. Among these anions, apart from hydroxyls and a low amount of chloride ions, can also be considered the presence of AlO_2^- species as above-discussed. At the same time, an important number of negative groups (carboxylate or sulfate) in the biopolymer structure may remain available to interact with cationic species in solution. The potentiometric responses obtained for sensors based on alginate−LDH and ι-carrageenan−LDH nanocomposites point out the predominant cationic exchange behavior of both nanocomposites, but they also indicate a small contribution of the LDH anionic exchange sites, giving rise to slopes slightly lower than the nernstian value expected for divalent cations.

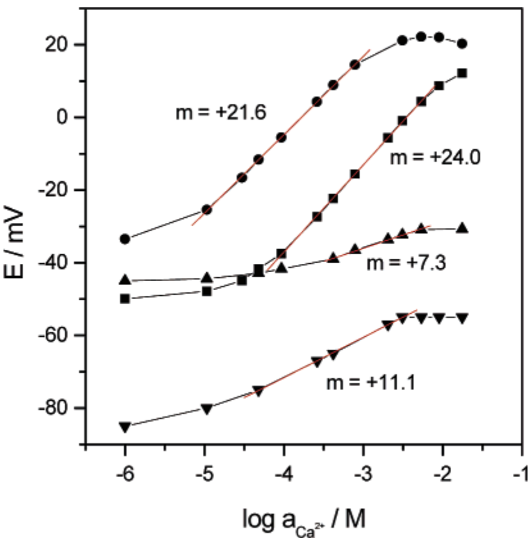

Figure 8. Potentiometric responses of the PVC membrane-based electrodes prepared with the alginate−LDH (●), ι-carrageenan−LDH (■), pectin−LDH (▼), and κ-carrageenan−LDH (▲) nanocomposites in $CaCl_2$ solutions of increasing activity.

In contrast, sensors based on the pectin−LDH and κ-carrageenan−LDH nanocomposites present a low sensitivity as well as a small reproducibility toward Ca^{2+} solutions. In the case of κ-carrageenan, this fact agrees with previous results indicating that the biopolymer is not well intercalated between the inorganic layers. The low amount of adsorbed κ-carrageenan may leave available positively charged sites in the LDH structure, compensated with OH^- ions, and probably AlO_2^- that are acting as anionic exchangers. Thus, the global response of the sensor to $CaCl_2$ solutions may be an intermediate response to both Ca^{2+} and Cl^- ions.

In the case of the pectin-based nanocomposite, it is difficult to explain the achieved subnernstian response, since chemical analysis confirms the adsorption of a high amount of biopolymer that could turn the anionic exchange capacity of the LDH into a cationic exchange capacity. This behavior could be attributed to the complex structure of pectin,[40] and probably also to its interaction with the LDH layers, varying the balance between the available anionic and cationic exchange sites and giving an intermediate response similar to that obtained for the κ-carrageenan−LDH nanocomposite.

---

(41) Kalcher, K.; Kauffmann, J.-M.; Wang, J.; Svancara, I.; Vytras, K.; Neuhold, C.; Yang, Z. *Electroanalysis* **1995**, *7*, 5−22.

Conclusions

The chemistry of functional hybrid nanostructured materials is now enriched with new biopolymer nanocomposites, in this case deriving from a Zn_2Al LDH. These hydroxides acquire the behaviors characteristic of the guest molecule, that is to say of the biopolymers, such as the ability to specifically complex Ca^{2+} ions, that could be applied for the development of electrochemical sensors useful for determining such species.

The "coprecipitation" vs the "reconstruction" method, is confirmed as the best method for the intercalation of anionic biopolysaccharides of high molecular weight, such as alginic acid, pectin, κ-carrageenan, ι-carrageenan, and xanthan gum, within the LDH. XRD clearly shows the intercalation of alginate, pectin, and ι-carrageenan between the layers of Zn_2Al LDH. In agreement with these results, alginate and pectin are intercalated as a monolayer of the biopolymer, whereas ι-carrageenan showing a higher Δd_L is intercalated as a bilayer or most likely as a double helix. It is difficult at this stage of the investigation to confirm this last conformation. IR, EDX, and ^{13}C NMR techniques corroborate that the intercalation is always driven by electrostatic interactions between the negatively charged groups of the biopolymer and the positively charged LDH layers.

As above-indicated, an important feature of the prepared biopolymer−[Zn_2Al] nanocomposites is that the anionic exchange behavior of the pristine LDH is turned into a cationic exchange capacity due to the presence of free negatively charged groups in the polysaccharide structure that do not interact strongly with the positively charged LDH layers. The biopolymer−[Zn_2Al] nanocomposites have been incorporated in carbon paste or PVC matrixes for the development of potentiometric sensors, being tested as active phases of such devices for the recognition of calcium ions. The determination of Ca^{2+} was carried out by direct potentiometry and the best responses were obtained for the sensors based on alginate−LDH and ι-carrageenan−LDH nanocomposites.

Acknowledgment. This work was supported by the CICYT (Spain; projects MAT2003-06003-C02-01 and BTE2003-05757-C02-02), by the Comunidad Autónoma de Madrid (Spain; project 07N/0070/2002), and by the Junta de Andalucia (Spain; IFAPA-2002.000890). We thank Dr. A. Alvarez (ICIDCA, Cuba) for supplying the xanthan gum sample. M. D. acknowledges support by a fellowship from the Comunidad Autónoma de Madrid. We also thank Dr. I. Sobrados for her assistance in NMR studies and F. Pinto for technical assistance in SEM and EDX studies.

CM0483240