Amperometric Sensors Based on Mercaptopyridine−Montmorillonite Intercalation Compounds

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The objective of this work is the study of the intercalation of 2- and 4- mercapto-substituted pyridines (2Mpy and 4Mpy, respectively) in Na^+−montmorillonite, providing intercalation compounds with interesting functional properties derived from the presence of the mercapto group. CHN chemical analysis, X-ray diffraction, Fourier transform infrared spectroscopy, and ^{13}C NMR spectroscopy have been employed for the characterization of the intercalation compounds. The molecular arrangement of such species in the interlayer space depends on the intercalation medium (neutral or acidic) and on the position of the mercapto group in the starting mercaptopyridine. In methanol medium, only 4Mpy can be intercalated, the pyridine ring interacting with the interlayer cations through water bridges. 2Mpy and 4Mpy can be intercalated by cation exchange from the corresponding protonated (pyridinium) derivatives, formed by addition of 1 M HCl to the intercalation medium. Tautomeric equilibrium between thiol and thione species of such mercaptopyridines must be taken into account to explain the arrangement of molecular aggregates and their particular orientation in the interlayer space. We have tried to correlate the structural arrangements of the intercalated species with the complexing ability of mercapto groups toward heavy metal ions. In this way, these materials have been tested as the active phase of carbon paste electrodes (CPEs) for the amperometric determination of Cd(II), Pb(II), Cu(II), and Hg(II) ions in aqueous solutions. CPEs based on pure 2- or 4-mercaptopyridines alone do not give an electroanalytical response toward heavy metal ions, whereas CPEs of the nanostructured materials show good electroanalytical behavior due to the particular arrangement of the guest species in the interlayer space of the clay.

Introduction

Research on hybrid organic−inorganic materials is one of the most attractive topics currently being investigated for the development of tunable systems in which the synergy between its components may allow the finding of properties that are unattainable by only one of either the organic or inorganic components.[1] In this context, our research group has experience in the preparation and characterization of different types of hybrid materials including intercalation compounds based on clays.[2] Among them are those showing interesting electroactive properties such as (i) those derived from the intercalative complexation of interlayer cations by crown ethers and cryptands in smectites,[3] (ii) the first described polymer−clay nanocomposite with properties as solid electrolytes based on the intercalation of poly(ethylene oxide) in montmorillonite,[4] and (iii) biopolymer−clay nanocomposites able to act as the active phase of ion-selective sensors; for example, the intercalation of chitosan transforms the clay into an anion exchanger with sensitivity toward anions.[5] Following this direction, the present paper concerns the adsorption of two isomers of mercaptopyridine molecules into montmorillonite and their application as active phases of electroactive sensors based on carbon paste electrodes (CPEs). CPEs have been demonstrated as operative in the determination of heavy metal ions, for instance Hg(II) in aqueous solution, and several other electroanalytical applications.[6] Intercalation of pyridine and related derivatives in clays has been extensively studied for several decades.[7,8] Their intercalation can be achieved from the neutral molecules, by interaction of the pyridine ring with the interlayer cations through water bridges, or by cation exchange, from the corresponding protonated (pyridinium) derivatives. Especially interesting is the fact that intercalated pyridines can

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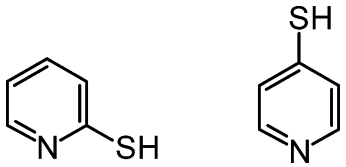

Figure 1. Molecular structures of 2-mercaptopyridine (2Mpy) and 4-mercaptopyridine (4Mpy) used in this work.

adopt different orientation in the interlayer space depending on both the nature of such molecules and the electrical charge of the clay, as first evidenced by Serratosa by means of dichroism studies of certain IR bands.[7] We report here the intercalation of 2- and 4-mercaptopyridines (Figure 1) into Na^+−montmorillonite by treatment of the clay with a solution of the pyridine derivative (in methanol or methanol/HCl), which takes place spontaneously at room temperature. The properties of the resulting materials and the mechanisms governing the interaction of each pyridine derivative with the clay have been studied from the analysis of the adsorption isotherms and the corresponding X-ray diffraction (XRD) patterns, IR spectroscopic data, ^{13}C NMR spectroscopic data, and chemical analyses. The final aim of this work is to take advantage of the affinity of the mercapto group toward transition metals[9] to develop modified electrodes[10] for the amperometric determination of heavy metal ions in aqueous solution. The potential application of these materials, which can be predicted on the basis of the different behavior of the electrodes, will be discussed, taking into account the different molecular arrangement of each isomer in the interlayer space.

Experimental Section

Starting Materials. Montmorillonite from Crook County, Wyoming (SWy-1) with cationic exchange capacity (CEC) of 76.4 mequiv/100 g was received from the source clay minerals repository of the Clay Minerals Society, Columbia, MO. A natural montmorillonite free of paramagnetic impurities, Gel White (GW) from Georgia Kaolin Company, whose characteristics have been published by Tilak et al.,[11] was used for solid state NMR characterization of montmorillonite.

Mercaptopyridine derivatives *o*-(2-mercaptopyridine, 2Mpy) and *p*-(4-mercaptopyridine, 4Mpy) were supplied by Aldrich (99% and 95% purity, respectively). Methanol (99.9%) and HCl (32%) were purchased from Fluka, acetic acid (100%) was purchased from Merck, and HNO_3 (60%) was purchased from Panreac. Aqueous solutions of heavy metal nitrates were prepared from chemicals of analytical-reagent grade: $Pb(NO_3)_2 \cdot H_2O$ (≥99%, Riedel-de Haën), $Cu(NO_3)_2 \cdot (1/2)H_2O$ (≥99%, Riedel-de Haën), $Cd(NO_3)_2 \cdot 4H_2O$ (99%, Riedel-de Haën), and $Hg(NO_3)_2 \cdot H_2O$ (≥99.0%, Fluka). Deionized water (resistivity of 18.2 MΩ·cm) was obtained with a Maxima Ultrapure Water system from Elga.

Intercalation Compounds. Homo-ionic Na^+−montmorillonite samples were prepared by treatment of the <2-μm-diameter fraction with 1.0 M NaCl solution. After complete exchange, the samples were washed and dialyzed with bidistilled water until the absence of chloride ions, confirmed by the $AgNO_3$ test. Montmorillonite films were prepared from a 1% clay suspension (200 mg of Na^+−montmorillonite in 20 mL of bidistilled water) slowly filtered through a Millipore microporous membrane (0.22 μm) and left to dry at room temperature.

For intercalation studies in acidic medium the clay films were immersed in mercaptopyridine solutions of different concentrations in methanol:1 M HCl (1:1 v/v) overnight. Some intercalation studies were also performed in neutral medium, with the clay films immersed in 0.3 M mercaptopyridine methanol solutions overnight. The resulting films were washed with methanol and left to dry at room temperature. These films consist of oriented aggregates and are mechanically adequate to be comfortably handled for characterization by different techniques.

To evaluate the effect of acidic medium in the starting mercaptopyridine, 0.3 M solutions of pure 2Mpy and pure 4Mpy were treated with methanol:HCl (1:1), with the mixture maintained under continuous magnetic stirring during several hours at room temperature. After that, the solvent was eliminated in a rotary evaporator to recover the resulting dried solids.

Characterization. The resulting intercalation materials were characterized by chemical analysis (Perkin-Elmer 2400 CHN analyzer), XRD (Siemens D-500 instrument with a Cu anode and Ni filter), and IR spectroscopy (Nicolet 20SXC spectrophotometer). ^{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 ^{1}H, respectively. A contact time of 1 ms and a period between successive accumulations of 5 s were used. The number of scans was 400. Chemical shift values were referenced to tetramethylsilane (TMS).

Carbon paste electrodes (CPEs)[12] were prepared by mixing 40 mg of the prepared clay−mercaptopyridines and 80 mg of graphite powder (1−2 μm, synthetic, from Aldrich) in an agate mortar. Then 96 μL of paraffin oil (Aldrich) was added to a final intercalation compound percentage of 20% and the mixture was mixed until an uniform paste was obtained. The carbon paste was then packed at the end (ca. 1 mm deep) of a glass tube (3 mm internal diameter), and provided with an unmodified carbon paste−copper contact. Appropriate packing of the carbon paste was achieved by pressing the surface electrode against a filter paper. An unmodified CPE (tested as a blank for comparative purpose) was prepared in the same way but without incorporation of the intercalation compound. Anodic stripping cyclic voltammetry (ASCV) measurements were performed with a EG&G PAR 273A galvanostat/potentiostat controlled with PARC M270 software, version 3.0, using a conventional three-electrode configuration. The homemade sensor acted as the working electrode; a coiled platinum wire served as the counter electrode, always measuring against an Ag/AgCl reference electrode. The experimental parameters used in ASCV were the following: (i) For the preconcentration step, the electrode was immersed in an accumulation cell for 20 min at open circuit, containing a stirred solution of 5 mg·L^{-1} Cd(II), Pb(II), Cu(II), and Hg(II) in 10 mM acetic/acetate buffer (HAc/NaAc) pH 5.0. (ii) For the measurement step, the electrode was removed, rinsed carefully with water, and placed in the measurement cell containing 0.1 M HCl as supporting electrolyte; a constant potential of −1.0 V was applied during 20 s for metal reduction, and a cyclic potential

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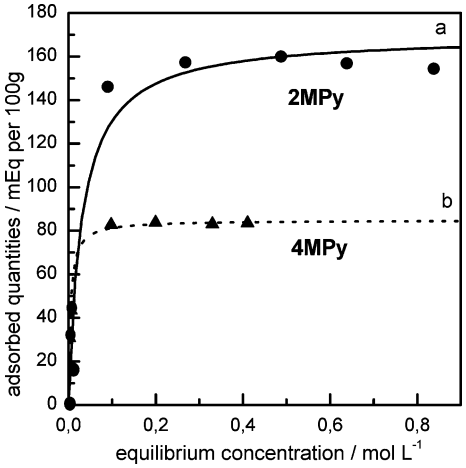

Figure 2. Adsorption isotherm at 298 K of 2Mpy (a) and 4Mpy (b) on Na^+−montmorillonite from methanol:1 M HCl (1:1) dispersions. Adsorption amounts deduced from CHN chemical analyses.

sweep between −1.0 and +1.0 V, at a scan rate of 500 mV·s^{-1}, was then performed, causing the metal reoxidation and leading to voltammetric signals. Prior to the amperometric determination, the electrode surface was activated by successive potential sweeps in the measurement electrolyte, followed by a few preconcentration and measurement cycles. Regeneration of the electrode after measurement was done by applying several cyclic sweeps until no metal response was observed. The electrode was stored in air when not in use, and under these conditions it can be used over several weeks without further surface renewal.

Results and Discussion

Mercaptopyridine−Montmorillonite Intercalation Compounds. *Adsorption Isotherms.* Figure 2 shows the adsorption isotherms at 298 K from 2Mpy (a) and 4Mpy (b) solutions on Na^+−montmorillonite working in acidic medium (methanol/HCl) as described in the Experimental Section. The adsorbed amount of mercaptopyridine was deduced from CHN elemental analysis. These results fit well to the L-type (Langmuir) of the Giles classification of adsorption isotherms:[13]

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

where Γ is the adsorbed amount of the organic species, C_s is the equilibrium molecular concentration, x_m is the maximum adsorbed amount, and b is the affinity constant between the organic adsorbate and clay adsorbent. In both cases 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 mercaptopyridines 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:

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

R is the gas constant, T is the adsorption temperature, and K = $(b\rho/4)^2$ for ionic species,[14] where ρ is the ratio of the solvent density to its molecular weight. The Gibbs energy of adsorption of 2Mpy and 4Mpy in montmorillonite was $\Delta G_{ads} = -30.3$ kJ mol^{-1} and $\Delta G_{ads} = -39.9$ kJ mol^{-1}, respectively, indicating a strong tendency to adsorption of the mercaptopyridine species on the clay surface, being stronger in the case of 4Mpy.

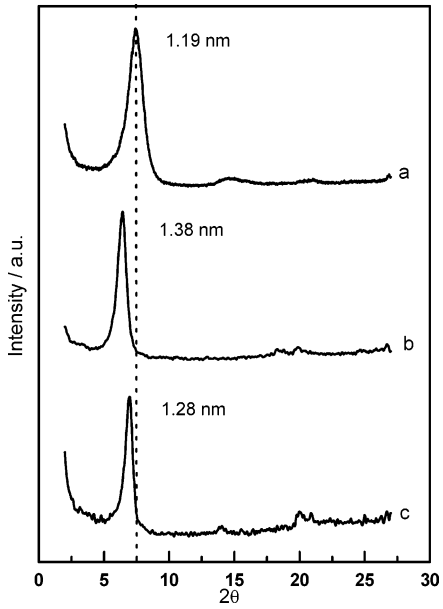

Figure 3. X-ray diffraction patterns of Na^+−montmorillonite untreated (a) and treated with 2Mpy (b) and 4Mpy (c) in methanol:1 M HCl (1:1).

Considering the average area (molecular modeling) of mercaptopyridine molecules (42×10^{-20} m^2) and the available surface area of the clay interlayer space (ca. 700 m^2/g),[15] the theoretical maximum amount of adsorbed molecules is 138.0 mequiv/100 g if they are lying flat along the plane. For 4Mpy, the maximum adsorbed amount of molecules obtained from the fit of the data to the L-type isotherm was 84.8 mequiv/100 g, coinciding with the CEC of the clay. In the case of 2Mpy, the maximum adsorbed amount of molecules was 169.9 mequiv/100 g, corresponding to approximately double the CEC of the clay.

X-ray Diffraction. Figure 3 shows the XRD patterns of the starting homo-ionic clay untreated and after treatments with 2Mpy and 4Mpy in methanol:1 M HCl (1:1). The intercalation of both mercaptopyridine molecules in the clay interlayer is confirmed by the decrease of 2θ values. The d_{00l} spaces are calculated using the first rational orders corresponding to the 00l reflections. Taking into account the thickness of the silicate layer (about 0.96 nm), the increase of the interlayer distance (Δd_L) for each intercalation compound is calculated from the corresponding d_{00l} space. We have found, from the XRD pattern of clay film treated with 4Mpy, a Δd_L value of 0.32 nm corresponding to the thickness of mercaptopyridine molecules disposed with the ring parallel to the plane defined by the silicate layers. The larger increase of the basal space in the case of 2Mpy (Δd_L

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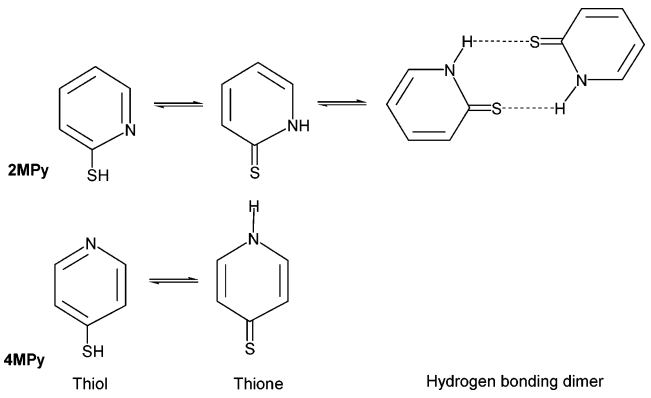
Scheme 1. Chemical Structures of the Molecules Studied in This Work and Their Possible Thione−Thiol Tautomerism



$= 0.42$ nm) compared to 4Mpy ($\Delta d_L = 0.32$ nm) is consistent with the greater amount of these molecules in the interlayer space deduced from the elemental analysis. This suggests that the 2Mpy molecules adopt an inclined disposition related to the plane defined by the silicate layers.

IR Spectroscopy. Attempts to intercalate 2Mpy in a neutral medium (methanol) in montmorillonite appeared from XRD, FTIR spectroscopy and chemical analysis to fail. Upon addition of HCl to protonate this mercaptopyridine isomer, intercalation proceeded. It is well-known that in solid state 2Mpy predominates in its thione tautomer conformation, forming molecular aggregates that are organized as dimers through hydrogen bonding interactions (Scheme 1).[16] With the aim to determine the configuration adopted by the 2Mpy molecules in the interlayer space of montmorillonite, after intercalation in acidic medium, i.e., as mercaptopyridinium cations, we have studied the resulting hybrid materials by IR spectroscopy.

Figure 4 shows the IR spectra of the starting mercaptopyridine (2Mpy) and the untreated clay (Na^+−montmorillonite) as well as the montmorillonite treated with 2Mpy in methanol/1 M HCl (1:1). The vibration bands characteristics of the silicate (ν_{OH} of Al, Mg(OH) ∼ 3635 cm^{-1}; ν_{OH} of H_2O ∼ 3430 and 3250 cm^{-1}; δ_{HOH} ∼ 1640 cm^{-1}; ν_{SiO} of Si−O−Si ∼ 1050 cm^{-1}) are clearly identified in the clay spectrum (Figure 4a).

The bands of 2Mpy have been assigned by means of theoretical calculation considering its thiol−thione tautomeric forms.[17] A marked difference in the IR spectrum of 2Mpy before and after intercalation (Figure 4) was found. As expected, the characteristic IR bands observed in the spectrum of pure 2Mpy (Figure 4b) agree well with the theoretical bands predicted for 2Mpy in its thione form (Table 1).

However, after intercalation (Figure 4c), characteristic bands of the thiol form (1612, 1558, and 1450 cm^{-1}) are observed in addition to the thione bands. Moreover, the band at 1582 cm^{-1} that is predicted in the thiol form, corresponding to stretching vibrations of the pyridine ring and to −SH bending, appears in our case as a strong band at 1583 cm^{-1}. It can be concluded that 2Mpy in acidic medium intercalates

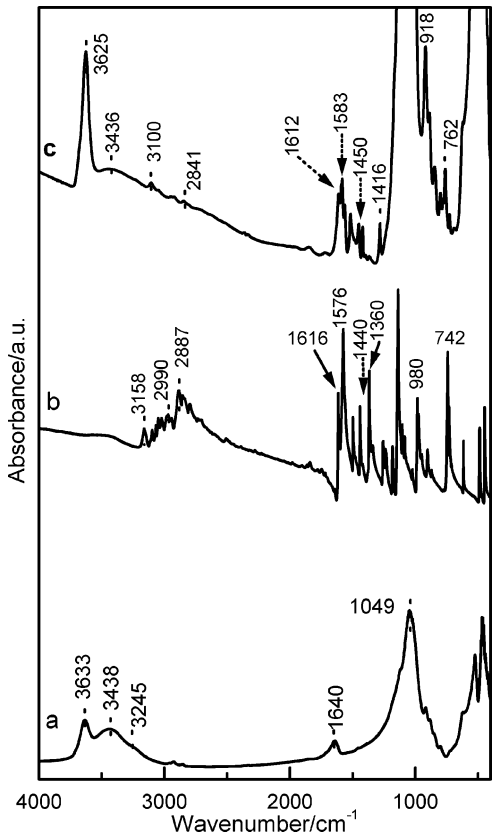


Figure 4. IR spectra (4000−400 cm^{-1} region) of a film of the starting Na^+−montmorillonite (a), 2Mpy in KBr pellets (b), and clay film treated with 2Mpy in methanol:1 M HCl (c).

in montmorillonite as protonated dimers resulting from hydrogen bonding between thiol and thione tautomeric forms, as we propose in Figure 5.

It is important to remark that the characteristic ν_{OH} bands of water molecules (at 3438, 3245 cm^{-1}) and δ_{OH} (at 1640 cm^{-1}) initially present in the interlayer space of the clay are not observed in the spectrum (Figure 4c), indicating that the intercalation of protonated 2Mpy-thiol-thione in its proposed dimer configuration partially replaces these water molecules. Besides, it can be evidenced that the molecular disposition in this case is not entirely planar in agreement with the absence of dichroism (varying the incidence angle of IR beam relative to the sample film between 0° and 45°)[7] of the band at 762 cm^{-1}, assigned to C−H vibrational modes out of plane. This is consistent with the amount of intercalated species deduced from the chemical analysis (169.9 mequiv/100 g) and with the increase of the basal space obtained from the XRD patterns ($\Delta d_L = 0.42$ nm). Thus, a structural arrangement in the interlayer space showing an inclination of the dimers with respect to the plane defined by the silicate layers can be assumed. In addition, the slight inclination between the two rings integrated in the dimer must be taken into account.[19] Figure 6 shows the IR spectra of pure 4Mpy and intercalated under the same experimental conditions that reported for 2Mpy intercalations. It is known that 4Mpy in the solid state exists mainly in the thione form,

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Table 1. Frequencies (cm^{-1}) and Assignment of Vibrational Modes in the IR Spectrum of 2Mpy Molecule in its Thiol−Thione Tautomeric Forms[a]

2Mpy solid[b]	SWy−2Mpy (MeOH/1 M HCl)[c]	2Mpy-thione (calcd)	assignment	2Mpy-thiol (calcd)	assignment
1616	1612	1641	ν_{8b}	1601	ν_{8b}
	1583			1582	ν_{8b}, δ_{CSH}
1576	1558	1569	ν_{8a}, δ_{CNH}		
1440	1450	1467	ν_{19a}, δ_{CNH}	1461	ν_{14}
	1416			1416	ν_{19b}
1360		1345	ν_3		
742	762	733	ν_{11}, $\delta_{CNH(out\ of\ plane)}$	764	ν_{10b}

[a] The assignment of the vibrational modes is taken from the normal mode assignment of benzene.[18] All the frequencies are expressed in wavenumbers. [b] KBr pellets. [c] Film.

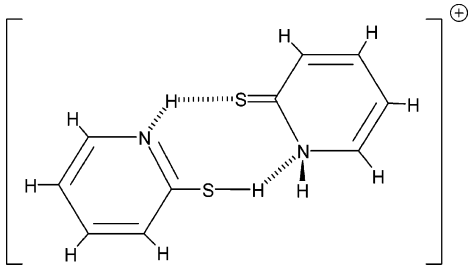

Figure 5. Molecular structure proposed for protonated 2Mpy-thiol-thione dimer.

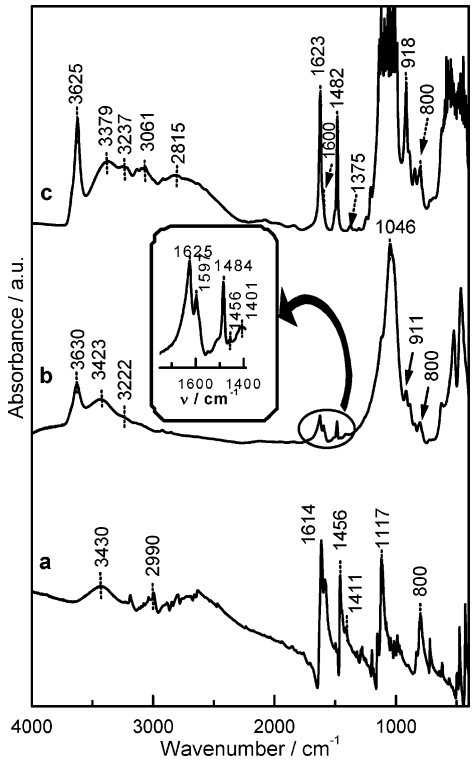

Figure 6. IR spectra (4000−400 cm^{-1} region) of pure 4Mpy (a), 4Mpy intercalated in Na^+−montmorillonite in methanol (b) (KBr pellets), and clay film treated with 4Mpy in methanol:1 M HCl (c). The inset of the (b) spectrum represents a magnification in the 1400−1700 cm^{-1} region.

which is experimentally corroborated by the similitude of observed IR bands and predicted values for 4Mpy-thione (Figure 6a, Table 2). Contrary to 2Mpy, 4Mpy could be intercalated in Na^+−montmorillonite using neutral solutions (methanol). In this case the IR spectrum of 4Mpy/Na^+−montmorillonite shows a shift of the characteristic bands of pure 4Mpy. The bands corresponding to stretching modes of the aromatic ring (skeleton modes) appear at 1597, 1484, and 1401 cm^{-1}, frequencies that are quite similar to those calculated for 4Mpy-thiol. Besides, it can be evidenced that stretching vibration bands of the ring observed at 1625 and 1456 cm^{-1} are close to the theoretical values predicted for 4Mpy-thione. The band at 800 cm^{-1} is assigned to δ_{CH} bending out-of-plane vibrations. The bands appearing at 3423 and 3222 cm^{-1} are assignable to ν_{OH} stretching vibrations of water, and the absorption in the 1600−1650 cm^{-1} region could contain the δ_{HOH} bending vibrations of the H_2O. The ν_{OH} bands characteristic of interlayer water molecules are slightly shifted toward lower wavenumber values, whereas the δ_{HOH} band is shifted toward higher wavenumber values. This indicates that water molecules are not completely replaced after intercalation, being in interaction (water bridges) with the introduced species.[8]

The situation is quite different when the intercalation of 4Mpy in Na^+−montmorillonite takes place in an acidic medium (methanol/HCl) and the spectrum in Figure 6c shows a broad adsorption band between 3300 and 2800 cm^{-1}, which is typical of ν_{NH} stretching vibrations of the $-(NH)^+$ group of pyridinium cations.[8] The band at 1623 cm^{-1} corresponds to ν_{8b} vibrational mode of pyridinium cation ring,[7] confirming the protonation of 4Mpy. The theoretical model for tautomer 4Mpy-thiol predicts bands at 1601, 1482, and 1398 cm^{-1}, which are associated with the ring vibrational modes. Experimentally, these bands appear at 1600, 1482, and 1375 cm^{-1}, in good agreement with the calculated values. In this case, only the thiol form is detected, the typical IR bands of 4Mpy-thione being absent. Finally, the predicted band corresponding to C−H (out-of-plane) bending vibrations, at 810 cm^{-1}, appears at 800 cm^{-1}. The dichroism of this last band, together with the XRD data ($\Delta d_L = 0.32$ nm), indicates that in this case the 4Mpy protonated molecules are intercalated with the ring parallel to the montmorillonite layers. This arrangement in the monolayer is consistent with the molecular content deduced from the chemical analysis (84.8 mequiv/100 g) that is close to the CEC of the clay.

In the O−H stretching region, the 3379 and 3237 cm^{-1} bands could be assigned to ν_{OH} vibrations of water, slightly shifted toward lower wavenumber values. Because the corresponding δ_{HOH} band around 1640 cm^{-1} is not observed, it could be deduced that only a low content in water molecules still remains after the intercalation process. It could be also admitted that protonated 4Mpy (thiol form) interacts with these molecules through water bridges.[8]

^{13}C NMR Spectroscopy. Solid-state high-resolution NMR spectroscopy was applied to study 2- and 4-mercaptopyridine intercalated in acidic medium into Gel White montmorillonite (GW). XRD and CHN chemical analysis of the mercapto-

Table 2. Frequencies (cm^{-1}) and Assignment of Vibrational Modes in IR Spectrum of 4Mpy Molecule in Its Thiol−Thione Tautomeric Forms[a]

4Mpy pure solid[b]	SWy−4Mpy (methanol)[c]	SWy−4Mpy (acidic medium)	4Mpy-thione (calcd)	assignment	4Mpy-thiol (calcd)	assignment
1614	1625		1623	ν_{8b}		
	1597	1600			1601	ν_{8b}
	1484	1482			1482	ν_{14}
1456	1456		1475	ν_{14}		
1411			1433	ν_{19a}		
	1401	1375			1398	ν_{19}
800	800	800	819	$\delta_{\text{ring o.p.}}$	810	$\delta_{\text{ring i.p.}}$

[a] The specification of the vibrational modes is taken from the normal mode assignment of benzene.[18] All the frequencies are in wavenumbers. o.p. denotes "out of plane" and i.p. denotes "in plane". [b] KBr pellets. [c] Film.

pyridine/GW samples yielded values of $d_{001} = 1.43$ nm ($\Delta d_L = 0.47$ nm) and 138.9 mequiv of adsorbed 2Mpy per 100 g of clay, respectively, and values of $d_{001} = 1.26$ nm ($\Delta d_L = 0.30$ nm) and 65.4 mequiv of adsorbed 4Mpy per 100 g of clay, respectively. These results confirmed the intercalation of 2Mpy and 4Mpy in the GW clay interlayer space, resulting in systems analogous to the mercaptopyridine−montmorillonite previously discussed.

Due to the complexity of ^{13}C NMR spectra when tautomeric species coexist, it has been necessary to use theoretical models based on spectroscopic simulation tools.[20] In the case of 4Mpy we have taken into account the number of equivalent carbons as well as their intensity ratio, although in some cases the proposed assignments do not coincide with the calculated data. To perform the assignments of signals in thione forms, we have additionally considered several studies reported in the literature.[21]

The ^{13}C NMR spectrum of pure 2Mpy (Figure 7a) shows well-defined signals at 140.2, 138.5, 133.1, and 113.6 ppm, which can be attributed to C6*, C4*, C3*, and C5* carbons in 2Mpy-thione tautomer, respectively. A lower intensity signal at 177.4 ppm, assignable to C2* carbon, is also observed. The ^{13}C NMR spectrum of the montmorillonite−2Mpy intercalation compound prepared in acidic medium (Figure 7b) is not well-defined, but several signals (118.7, 122.4, 128.1, 133.5, 138.6, 144.8, 149.9, 153.6, and 177.4 ppm) can be distinguished using deconvolution methods. These signals can be assigned to C5*, C5−C6, C4, C3*, C4*, C3, C6*, C2, and C2*, respectively, evidencing in this case the coexistence of both tautomeric species (thiol−thione), in agreement with the protonated dimer model proposed above and also with the IR spectroscopic results (Figure 5).

The assignment of ^{13}C NMR signals in the case of the 4Mpy−montmorillonite intercalation compound, also prepared in acidic medium, is more complicated. It has been necessary to compare the ^{13}C NMR spectrum of 4Mpy treated with 1 M HCl, to correlate the different signals assigned to the corresponding carbon nuclei. Figure 8a shows the signals observed for pure 4Mpy (117.2, 129.4, 130.0, 134.8, 152.9, and 194.9 ppm), which can be assigned to carbons denoted in the figure as C3*, C2*, C3, C2, C4, and C4*, respectively. The coexistence of both tautomeric forms, thiol and thione,

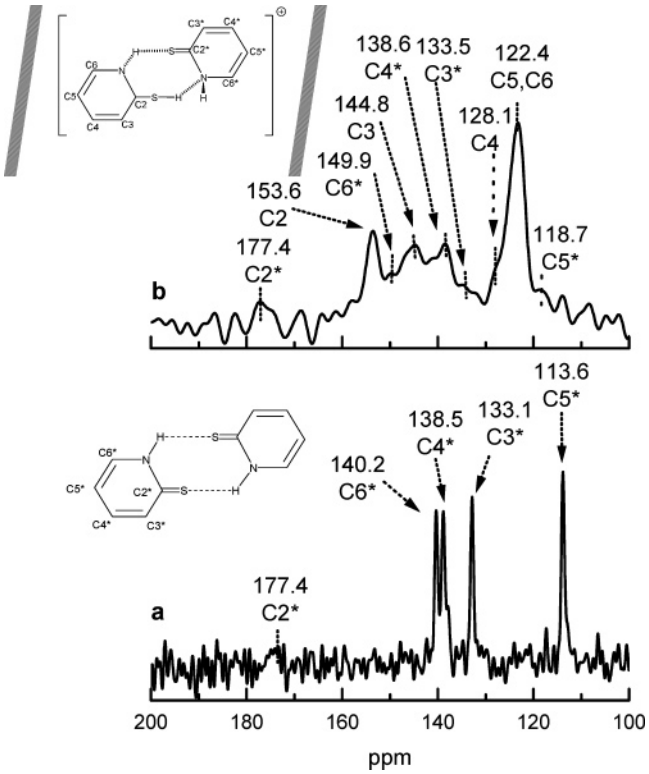

Figure 7. ^{13}C NMR spectra in the 100−200 ppm region of (a) pure 2Mpy and (b) montmorillonite treated with 2Mpy in acidic medium (methanol:1 M HCl (1:1)).

is here evidenced. The ^{13}C NMR spectrum of 4Mpy treated with 1 M HCl (Figure 8b) shows well-defined signals at 127.7, 140.2, and 159.4 ppm, attributable to the C3, C2, and C4 carbons of the 4Mpy in its thiol form. This means that the acidic treatment stabilizes the thiol tautomer. Shifts ($\Delta\delta \approx 6$ ppm) of C2 and C4 signals toward lower field can be ascribed to the protonation effect that takes place preferentially at the N heteroatom of the 4Mpy-thiol, in good agreement with the reported behavior of monoaminopyridines in acidic medium.[22]

The montmorillonite−4Mpy intercalation compound prepared in acidic medium (Figure 8c) shows a poor ^{13}C NMR spectrum with a low signal-to-noise ratio that was not possible to improve experimentally. We propose the existence of three main signals centered at 121.4, 140.3, and 156.9 ppm, attributable to C3, C2, and C4 carbons in the protonated 4Mpy-thiol, which are comparable to the observed pattern of the ^{13}C NMR spectrum of 4Mpy treated with 1 M HCl. In that spectrum it can be observed that the band at 121.4

(20) *JCAMP ^{13}C−Chemical Shift Calculation* (http://www.chem.uni-Potsdam.de/tools/calcnmr.html, Institute for Organic Chemistry and Structure Elucidation, University of Postdam, Germany); *UpSol NMR Prediction* (Scientific Software Engineering).

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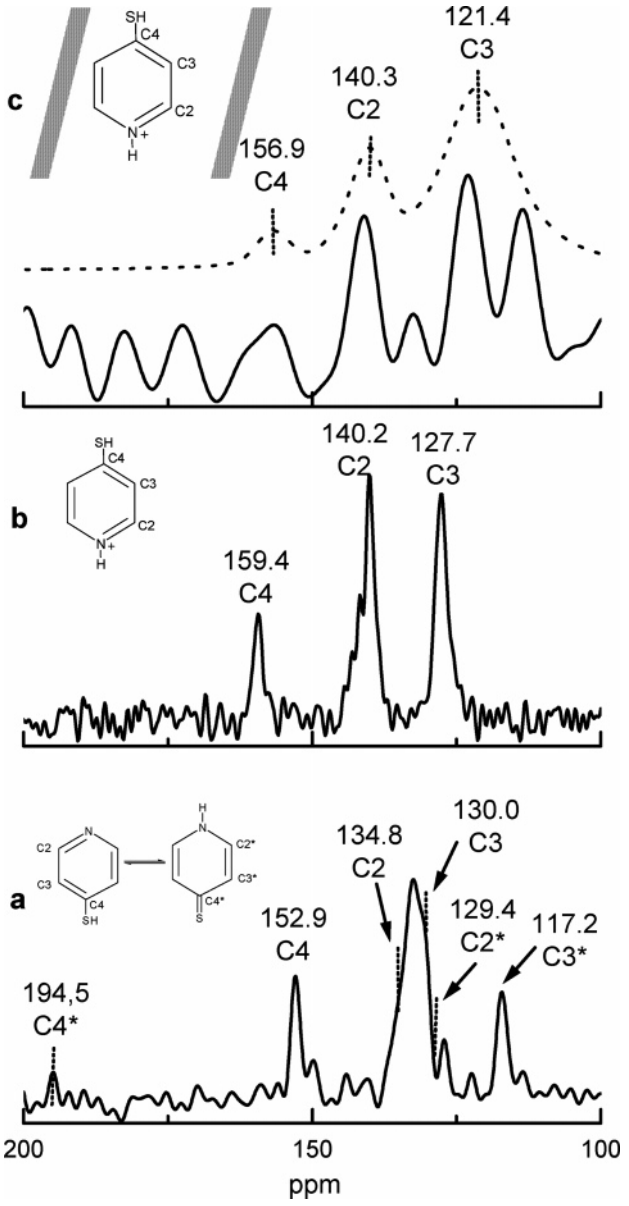

Figure 8. ^{13}C NMR spectra in the 100−200 ppm region of pure 4Mpy (a), 4Mpy treated with 1 M HCl (b), and 4Mpy intercalated in montmorillonite in acidic medium (methanol:1 M HCl (1:1)) (c).

(C3) appears split into two peaks appearing at 113.4 and 122.4 ppm. It is difficult to clearly establish the intercalation mechanisms of these compounds in clays, and it is known that the behavior of organic molecules in the confined interlayer space is different from that in open surfaces or in solution.[2] Thus, it is possible to postulate several phenomena that could produce an splitting of such signal, such as the adduct formation of the type 4Mpy···H···4Mpy in the interlayer space, in a way similar to that observed in the intercalation of other pyridine molecules.[22,23] Besides, the formation of intramolecular hydrogen bonds between NH^+ and SH groups of adjacent molecules could also produce the observed spectral splitting.

It can be assumed that the studies from ^{13}C NMR corroborate the IR spectroscopic results, being able to distinguish between the predominant tautomer species in each case as well as the protonation effects in the acidic medium.

Although it is known that the presence of acids in contact with clay samples produces a slight extraction of metallic elements of the silicate network (mainly from the octahedral layers), in our adopted conditions no appreciable changes in the XRD patterns or in the IR spectrum are observed, which is indicative of a nonsignificant alteration of the phyllosilicate.

Molecular Arrangement Models. From the results discussed above we can postulate various molecular models to explain the interaction of 2Mpy and 4Mpy with Na^+−montmorillonite in acidic and neutral media (Scheme 2).

Amperometric Sensors Based on Mercaptopyridine−Montmorillonite Intercalation Compounds. Carbon paste electrodes containing the previously described materials were employed for the amperometric detection of Cd(II), Pb(II), Cu(II), and Hg(II). The objective was to study the ability of mercaptopyridine−montmorillonite intercalation compounds to give complexes with heavy metal ions in aqueous solutions. It is important to point out that the incorporation of homo-ionic montmorillonite, without intercalated mercaptopyridines, in the CPEs results in an unstable electrochemical signal due to the swelling properties of the clay in aqueous media, and the lack of compactness of the resulting sensors.

The electrochemical behavior of CPEs was studied by anodic stripping cyclic voltammetry (ASCV) following the procedure described in the Experimental Section. Amperometric sensors based on intercalation compounds of 4Mpy in montmorillonite in methanol (Figure 9A, solid line) and acidic medium (Figure 9B, solid line) present a good response toward different heavy metal cations. In the first case, the cation retention phenomenon takes place through the free −SH groups of 4Mpy-thiol in equilibrium with thione species. However, the existence of a higher amount of free −SH in the case of 4Mpy intercalated in acidic medium (as 4Mpy-thiol) generates a better response toward Hg(II) ions. In addition, it is important to mention the intermetallic effect between metals present in solution, mainly mercury and copper, previously reported in the literature.[24] This support effect could cause the peak for Cu(II) to be shifted when the Hg(II) signal increases.

The CPEs modified with the intercalation compound derived from the treatment of montmorillonite with 2Mpy in acidic medium (Figure 9B, dashed line) do not show a significant response toward the tested heavy metal ions. This fact could be explained by the assumption of molecular aggregate formation proposed in Figure 5 (2Mpy-thiol-thione dimer). This configuration determines the absence of free SH groups capable of coordinating heavy metal ions, since these thiol groups are involved in hydrogen bonding.

CPEs containing the starting compounds, i.e., pure 4Mpy and 2Mpy, were also tested, showing that they do not offer response toward the checked heavy metal ions. This is a coherent result taking into account that the predominant species in the crystalline state of these compounds is the

(23) Marincola, F. C.; Cadoni E.; Gelli, G.; Lai, A.; Beltrame, P. *Magn. Reson. Chem.* **1999**, *37*, 600−601.

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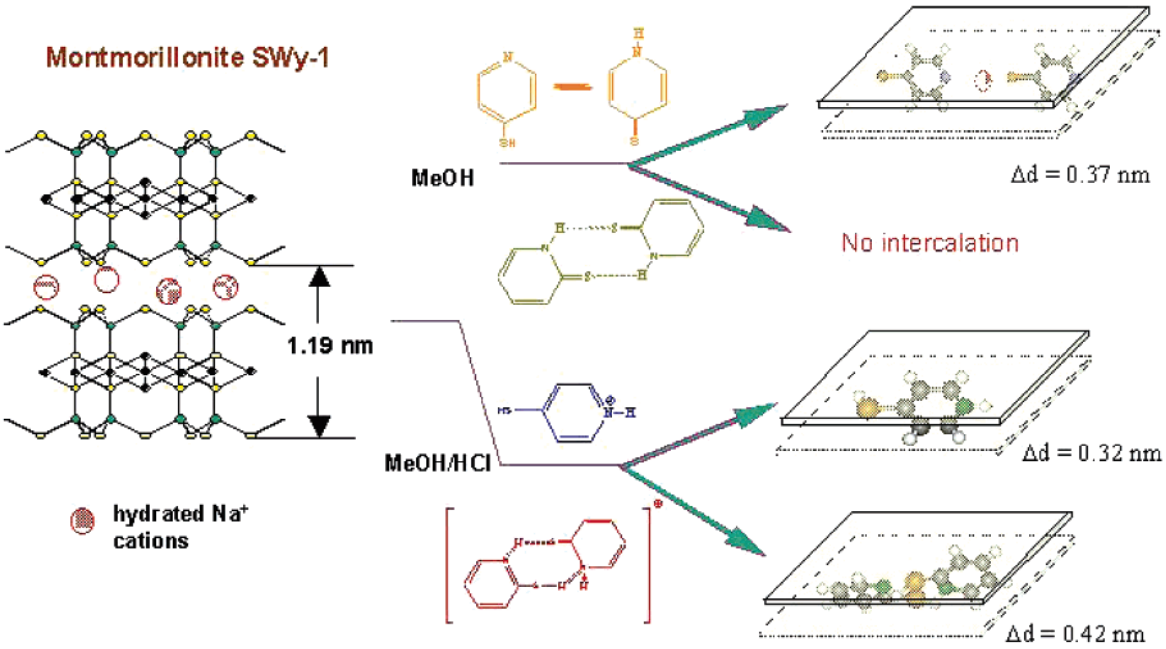
Scheme 2. Schematic Summary of Proposed Molecular Models in the Interaction of 2Mpy and 4Mpy with Na^+−Montmorillonite Intercalated from Neutral (Methanol) or Acidic Medium (Methanol:1 M HCl (1:1))



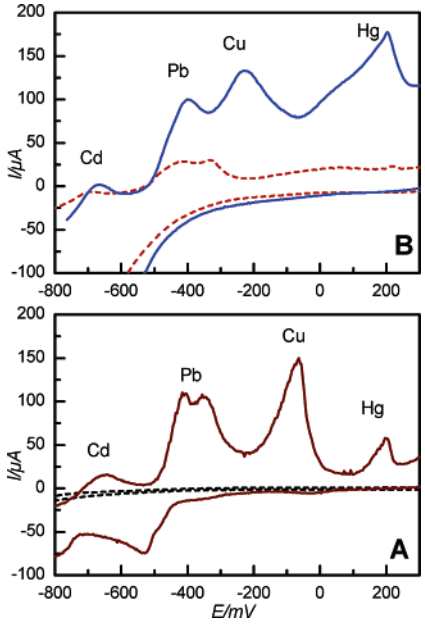


Figure 9. ASCV responses of unmodified CPE (A, dashed line), 4Mpy−montmorillonite (obtained in methanol) modified CPE (A, solid line), 2Mpy−montmorillonite (obtained in acidic medium) modified CPE (B, dashed line), and 4Mpy−montmorillonite (obtained in acidic medium) modified CPE (B, solid line).

corresponding thione form, i.e., without free −SH groups able to interact with such metal ions.

Conclusions

The intercalation of mercaptopyridines into Na^+−montmorillonite through a cation-exchange process provides compounds with interesting structural and functional properties. The techniques employed in the characterization of the nanocomposites indicate which are the appropriate conditions to intercalate the mercaptopyridines as well as their tendency to form molecular aggregates. This last ability depends on the predominant tautomeric species present in the intercalation medium. The 2Mpy molecule cannot be intercalated from methanol solution, but the addition of HCl to the 2Mpy/MeOH solution allows the intercalation by means of an ion-exchange mechanism of protonated dimers, consisting of thiol−thione tautomers arranged as aggregated species. The intercalation of 4Mpy from methanol solution takes place through water-bridge interactions of both thiol and thione species with the hydrated Na^+ interlayer cations. The addition of HCl to the 4Mpy/MeOH solution gives $4MpyH^+$ species in their thiol tautomeric form that intercalate by exchanging the interlayer Na^+ ions. CPEs prepared from the intercalation compounds show different electrochemical responses toward heavy metal ions (Cd(II), Pb(II), Cu(II), and Hg(II)) in aqueous medium depending on the nature of the intercalation compound. The existence of intercalation species provided of free −SH groups, i.e., in the case of 4Mpy intercalated in acidic medium, results in an enhanced response toward Hg(II) ions.

The present results constitute a good example illustrating that the incorporation of molecular species into inorganic solids could provide functional nanostructured materials, useful in this case for application as electrochemical sensors. Such molecular species (mercaptopyridines) alone are unable by themselves to give such behaviors, but their intercalation in layered silicates procures a particular arrangement in the interlayer space that makes possible their use as active phases of modified electrodes.

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