

Surface Modification of Synthetic Clay Aimed at Biomolecule Adsorption: Synthesis and Characterization

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This work describes the process for functionalization of laponite through the grafting of 3-mercaptopropyltrimethoxysilane (MPTS). Laponite is synthetic smectite clay with surface area of 350 m²/g. The samples, prior to and after functionalization, were characterized by chemical analyses, thermogravimetric analysis (TGA), x ray diffraction (XRD), diffuse reflectance infrared Fourier transform spectroscopy (DRIFT), scanning electron microscopy and energy dispersive spectrometry (MEV/EDS). Infrared spectroscopy and elemental analyses confirmed the presence of organic chains and thiol groups in the modified clay. The immobilized and available thiol group, measured according to the Volhard method, totaled 1.4 meq/g of clay, with approximately 90% accessible for Ag⁺ trapping. These results represent an improvement as compared to other works concerning the functionalization of smectite-type clays in which the effect produced by functional group blockage limits the access of species to less than 10% of the complexing sites.

Keywords: laponite, functionalization, biomolecule adsorption

1. Introduction

Mineral clays of the smectite group present a set of structural characteristics that make them attractive for the development of catalysts, sorbents, supports for drug or enzymes, and intercalation of organic molecules¹⁻⁵. Organosilanes are widely used for the modification of silica surfaces. The silylation mechanism for the attachment of organosilane to amorphous silica and alumina surfaces has been commonly reported⁶⁻⁸. Recently, organosilanes have been employed for the modification of layered silicates, with smectites and vermiculites being the most commonly clays used to investigate the functionalization processes⁹⁻¹². Among the smectites, montmorillonite has been the most common choice, mostly due to its availability and adequate characteristics of expandability¹². More recently, the functionalization of other clays, such as the synthetic laponite, has been also studied for various applications. These include i) the preparation of organosilanes-quinoxane-laponite clay films on solid electrodes and their subsequent use as electrochemical (bio) sensors^{2,13,14}; ii) the immobilization of several enzymes within clay matrices¹⁵; iii) the immobilization of catalysts⁵ and iv) the removal of undesirable biomolecules from extractors and slurry¹⁶.

Laponite is a layered hydrous magnesium silicate, hydrothermally synthesized from mixing silicate, lithium, and magnesium salts in the presence of mineralizing agents. This clay is normally used as a rheology modifier for a wide range of industrial and consumer products and to produce electrically conductive, antistatic, and barrier thin coatings. Laponite is an entirely synthetic smectite clay, which closely resembles the natural clay mineral hectorite in both structure and composition, and has a specific surface area of 350 m²/g. Laponite can be described by the chemical formula of Na_{0.7} [(Si₈ Mg_{5.5} Li_{0.3}) O₂₀(OH)₄] and is made up of small disc-like particles. Each single-particle is a crystalline disc with an average radius of 20 nm and a thickness of 1.0 nm. Only in dilute suspension, the charged platelets

become dispersed as individual units. In a dry form, these units are usually agglomerated into larger clusters. The silanol groups located at the edges of the laponite sheets may be used to covalently attach themselves to organic compounds. The lamellar spacing of laponite may be expanded to incorporate intercalants¹⁷.

Surface modification is a key step concerning the application of clays in biotechnology. One of the main obstacles hindering these applications is the interaction of organic molecules with the acid and hydrophilic clay surface, which in turn may cause the denaturation of enzymes¹⁵. This limitation can be overcome by passivating the acid surface sites and creating a more organophilic clay matrix with different functionalities. The clay surface may be modified through reactions with various functional groups, such as thiol, amine, or long carbon chains.

The present study was aimed at developing clay-based materials that can be used as sorbents for biomolecules. This work focused on the functionalization of a synthetic clay with thiol groups through the grafting of 3-mercaptopropyltrimethoxysilane (MPTS). A detailed material characterization was provided by chemical analysis, diffuse reflectance infrared Fourier transform spectroscopy-DRIFT, x ray diffraction-XRD, scanning electron microscopy and energy dispersive spectrometry – MEV/EDS. The sorption capacity of the mercaptanyl functional groups in the clay was evaluated using the Volhard silver nitrate method.

2. Experimental Procedures

2.1. Materials

The laponite sample used in the experiments was supplied by Rockwood Specialties Inc. and is identified by the chemical formula Na_{0.7} [(Si₈ Mg_{5.5} Li_{0.3}) O₂₀(OH)₄]. According to the supplier, the

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material has a specific density of 2.53 and cation exchange capacity (CEC) of 50 to 60 meq/100 g of clay. When fully dispersed in water, the particles display disc-shaped, nano-sized crystals of approximately 20 nm in diameter and 1 nm in thickness. The organosilane-grafting agent, the 3-mercaptopropyltrimethoxysilane (MPTMS) ($\text{Si}(\text{OCH}_3)_3\text{C}_3\text{H}_6\text{SH}$), 98% pure, was purchased from Sigma-Aldrich and used without further purification. Toluene (99.8%) was also provided by Sigma-Aldrich. The solutions of AgNO_3 , KSCN, and $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2$ used in the adsorption experiments were prepared with reagent grade chemicals (Merck). All solutions were prepared with high-purity water ($18 \text{ M}\Omega\cdot\text{cm}^{-1}$) obtained from a MilliQ water purification system.

2.2. Modification process

Prior to the grafting process, 1 g of the sodic laponite (LNa) was acid activated with 100 mL of 0.1 mol/L HCl solution. This suspension was maintained under agitation for 6 hours; the resulting sample was denominated LH. The acid activation was used to remove the sodium ions from the interlamellar region. In a next step, 1 g of acid activated laponite, previously dried at 150°C for 4 hours, was dispersed in 50 cm^3 of 0.1 mol/L MPTMS in dry toluene. The resulting mixture was refluxed and agitated for 24 hours within an inert atmosphere of N_2 to avoid the oxidation of the group SH. The obtained solid was sequentially washed with toluene, absolute ethyl alcohol, and MilliQ water, filtered and dried for 2 hours at 120°C in an inert nitrogen atmosphere. The functionalized sample was denominated LSH. This method is similar to that described by Walcarius et al.⁶ for the functionalization of silica gel.

2.3. Characterization methods

The x ray diffraction patterns of modified and unmodified clay samples were obtained with a Philips model PW1710 diffractometer, fitted with a Cu tube ($\lambda = 1.5418 \text{ \AA}$, 40 kV and 20 mA, step size $0.06^\circ 2\theta$, 5 s/step). DRIFT analyses were performed using a FT/IR- spectrophotometer, model SPECTRUM-1000, Perkin Elmer (32 scans of accumulation; resolution of 4 cm^{-1}). The IR measurements were taken at room temperature, in the spectral range of $400\text{--}4000 \text{ cm}^{-1}$. The samples were mixed with KBr powder in 5 wt. (%) and a pure KBr spectrum was used as reference. Thermogravimetric curves were obtained in a TGA model TGA50 Shimadzu under in N_2 environment, (20 mL/min), with temperature ramp of $10^\circ\text{C}/\text{min}$ between 30°C - 1000°C . The morphological and semi-quantitative analyses of the samples was carried out in a Scanning Electron Microscopy (Jeol instrument model JSM-6360LV, operating at 25 kV and with a Noran energy-dispersive spectrometer (ZAF corrections coupled).

2.4. Binding capacity of the mercaptyl functional groups

In the present investigation, the amount of the immobilized and accessible thiol groups was successfully determined according to a protocol based on the Volhard silver nitrate method¹⁸ developed by the authors. The determination is based on the chemical affinity of silver by the thiol groups. Following the protocol, 25 mL of 0.01 mol/L silver nitrate solution were added to flasks containing 100 mg of dry modified clay. The flasks were covered to avoid exposure to light and stirred in a shaker for 3 h prior to testing. The remaining silver nitrate was titrated with standardized 0.01 mol/L potassium thiocyanate (KSCN) using iron ammonium sulfate hexahydrate as an indicator. The endpoint was detected when an excess of thiocyanate anion formed a red colored complex with the iron compound. The binding capacity of the mercaptyl functional group was expressed in meq/g of clay. In all cases the tests were carried out in triplicate and the results

compared with those obtained using a sample of unmodified clay as a reference. Another flask containing pure solution was submitted to the same process in order to evaluate eventual precipitation. This methodology was used as protocol to quantify the accessible SH groups. The sequence of reactions is described below:

Adsorption step:

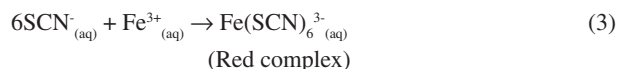


(modified clay)

Titration step:



End point:



3. Results and Discussion

3.1. Functionalization process

The silylation of silicates involves a direct condensation reaction between the terminal (OH) groups from the silicate surface and the species derived from the hydrolysis of the alkoxy silanes. It is believed that the silylation of silicate is favored over the polysiloxane formation if the reaction is conducted in a dry environment. Figure 1 depicts a schematic illustration of the functionalization process followed by biomolecule adsorption. The silane coupling agents are a family of organosilicon monomers with the general structure of R-SiX_3 . X is the hydrolyzable alkoxy group, typically methoxy ($-\text{OCH}_3$) or ethoxy ($-\text{OC}_2\text{H}_5$), which releases methanol and ethanol during the coupling reactions. R is an organo-functional group attached to silicon. Some recent works have shown that it is possible to create specific chemical activity through the selection of an adequate functional group, in a way that the functionalized clay will provide specific sites for the adsorption of specific enzymes⁴. In the present work, the mercapto group was selected as functional group due to its great affinity to many biomolecules.

Following functionalization, the concentration of the immobilized thiol groups in the clay was measured according to the Volhard

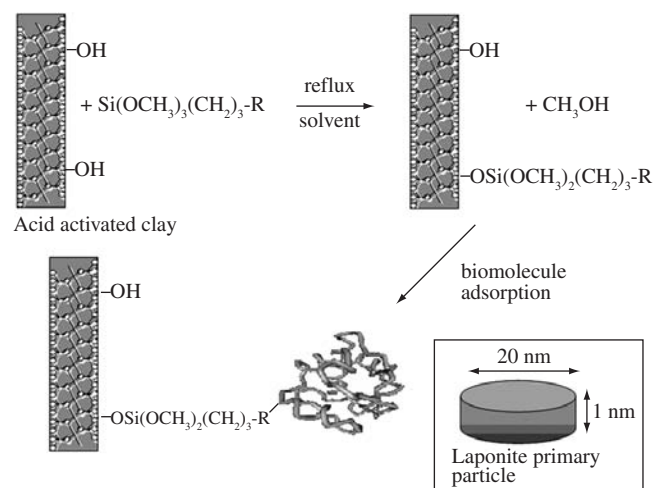


Figure 1. Schematic illustration of the direct hydrolysis and condensation reaction followed by biomolecule adsorption. The inset shows a schematic representation of the size and morphology of the individual clay platelets (the “primary particles”).

method. The total binding capacity was determined as 1.4 meq/g and 0.15 meq/g for the functionalized and the original clay (LNa), respectively. It indicates that the mechanism of adsorption involves primarily silver ion complexation by the thiol groups instead of cation exchange. The unmodified sample exhibits some ion immobilization capacity that may be related to a cation exchange mechanism, even though the original sample shows a very low affinity for silver ions. The average CEC indicated by the supplier is of 0.55 meq/g.

The value is comparable to the values obtained by Dominguez & Yue in a synthesis of mercaptal fibers (1.7 meq/g)¹⁸. It is important to note that the method of silver titration may provide non-differentiating results for both thiols and disulfide groups, since the latter also form very stable complexes with silver ions¹⁸.

Elemental semi-quantitative analyses provided by EDS are summarized in Table 1. The atomic % ratio, Si/Mg, increases from 1.9 to 2.4 after acid treatment, thus indicating that clay dissolution has occurred to some extent with a consequent release of Mg²⁺ from the clay lattice. We believe that this reaction may increase the density of Mg-OH and Si-OH groups on clay surface and thereby improve the organosilane immobilization, since this immobilization involves a reaction with the silicate's OH groups. The increase of the atomic % ratio, Si/Mg, in the LSHH-Ag sample is related to the incorporation of the organosilane molecule. The atomic % ratio, Ag/S, in this sample suggests that almost all of the mercaptal groups (~90%) were accessible to silver binding. This presents a positive result, once Mercier & Detellier who reported that the intercalation of 3-(mercaptopropyl)trimethoxysilane in the galleries of smectite-type clay led to loadings corresponding to less than 10% of the total number of thiol groups in the adsorbent¹⁰. An improvement of the previous result was later reported by Mercier & Pinnavaia who prepared an MPTS-fluorohectorite and found that 67% of the thiol groups were accessible for Hg(II) ions⁹. This result is similar to those reported by Celis et al. for MPTS-sepiolite¹. These authors attributed the relatively low loading to the blockade of pore space by intercalated molecules.

3.2. Sample characterization

The DRIFT spectra of modified and unmodified clay are presented in Figure 2. Changes following the modification process can be observed in all frequency range of the FTIR spectra. The spectrum of the sodium laponite (LNa) exhibits a broad shoulder at around 3700-3400 cm⁻¹, in a range of frequencies usually assigned to surface hydroxyl groups and sorbed water. This poorly resolved shoulder consists of the overlapping of two components: Si-OH (at 3628 cm⁻¹) and Mg-OH (at 3686 cm⁻¹) stretching vibrations⁵ and the ν (O-H) stretching frequency at 3450 cm⁻¹ due to physisorbed water. The δ (O-H) deformation band at 1640 cm⁻¹ is yet another indication of the presence of water¹⁹. In the low energy region, the spectrum shows one broad band with a maximum peak at 1010 cm⁻¹ assigned to Si-O and Si-O-Si stretching vibrations, one band around 660 cm⁻¹ due to O-H bending vibration from adsorbed water, and one band at 520 cm⁻¹ assigned to Mg-O vibration¹⁵. After acid activation, some changes in the FTIR spectra can be observed. In the high-energy range, the band

Table 1. EDS Semi-quantitative analyses for the clays samples.

Samples	Atomic % ratio		
	Si/Mg	S/Mg	Ag/S
LNa	1.9	-	-
LH	2.4	-	-
LSHH + Ag	3.5	0.8	0.9

LSHH + Ag indicates the functionalized Laponite after silver adsorption.

intensity at 3800-2600 cm⁻¹ and at 1640 cm⁻¹ increase, thus suggesting that the LH sample is more hydrophilic than the LNa. It is possible to observe a broadening of the band assigned to the Si-O and Si-O-Si stretching vibrations, an indication that acid activation may have promoted some minor structural modification. After functionalization, the LSHH sample shows the characteristic SH stretching vibration at 2562 cm⁻¹ and CH stretching vibration at 2800-3000 cm⁻¹, thus indicating that the organic molecules were effectively grafted to the surface silanol groups²⁰. The relatively lower intensity of the band centered at 3450 cm⁻¹ and the δ (O-H) deformation band at 1640 cm⁻¹ indicates a reduction of the adsorbed water content, with a consequent increase of the hydrophobicity of the LSHH sample.

Figure 3 presents the weight loss and the peak of the first derivative in order to illustrate the most significant events regarding weight loss events. In contrast to the original clay, which that does not show any important weight loss in the temperature range of 200 °C to 600 °C, the functionalized sample exhibits a sharp DTG peak at 280-330 °C. This feature was mainly ascribed to the loss of the mercaptopropyl group. A smaller and broader peak is also observed at temperatures of 700 °C. This typical behavior of organoclays can be used as an indication of how much organic has been loaded onto the clay particles¹⁰. At higher temperature (~780 °C), the dehydroxylation of the clay sheets takes place. The increase of hydrophobicity following functionalization is indicated by the decrease of adsorbed water from 14.5 to 5.1% for the LH and LSHH samples, respectively, at 30-200 °C. Taking into account the weight loss in the range 200 to 700 °C, one may calculate 1.7 mmol of mercaptopropyl groups per gram of grafted clay. Comparing this value to the binding capacity

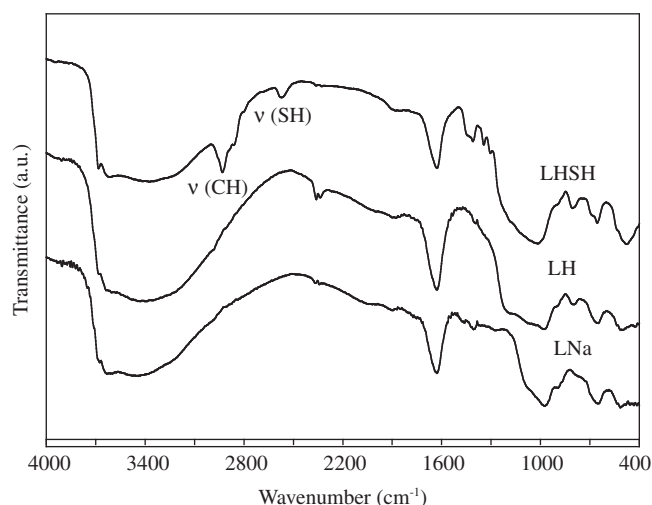


Figure 2. FTIR spectra of sodium (LNa), acid activated (LH) and functionalized (LSHH) laponite samples.

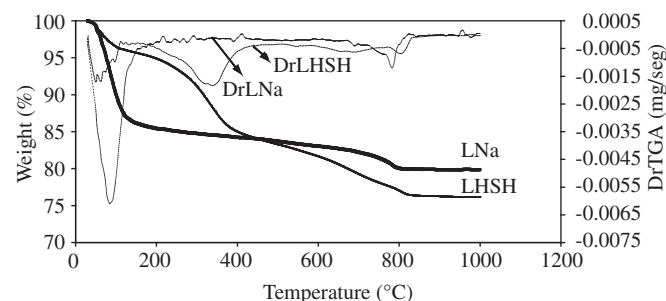
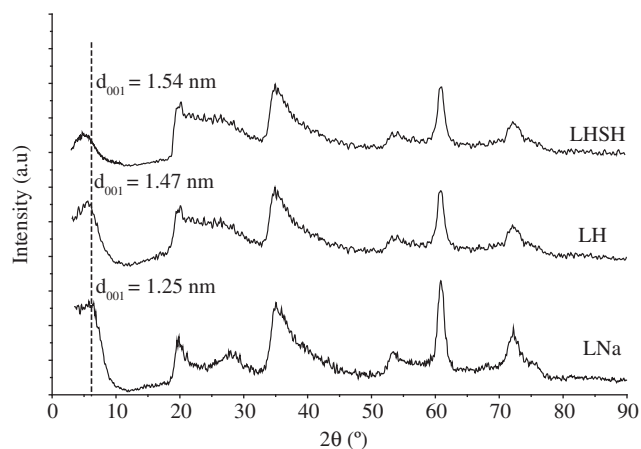


Figure 3. TG and DrTG curves of laponite samples.

(1.4 meq/g) determined by the Volhard Method we assume that 82% of SH group are actually accessible to complexation of Ag^+ ions. This finding is in good agreement with accessibility of 90% inferred by the atomic Ag/S ratio determined by EDS analysis (Table 1). For comparative purposes, a sample of laponite-Na was submitted to the same modification process but without acid activation. A value of 0.83 mmol of mercaptopropyl groups per gram of grafted clay was determined by TG analysis, 104% less than the previous of 1.7 mmol/g. Therefore, the acid pretreatment showed a significant influence on improving the grafting process without collapse of the structure. It is interesting to notice that the differences in the temperature of dehydroxylation (at 780 °C in LNa sample and at 808 °C in LSH) suggests changes in the structure after the grafting process.

As illustrated in Figure 4, the XRD patterns for all the laponite samples are quite similar, with only the reflection at $2\theta \sim 28^\circ$ showing some loss of definition. A comparison of the overall patterns indicates that the original structure was preserved after modification. The main d-spacing parameters of all samples are indicated. An approximate value of 1.25 nm was estimated for the interlayer distance in the LNa sample, despite the broadness of the d_{001} peak. Other authors report similar findings, which were attributed to the very low dimensions and low crystallinity of the laponite crystals^{5,19}. Acid activation led to an increase in the interlayer spacing (1.25 to 1.47 nm), most likely due to water adsorption. The increased hydrophilic feature of the LH sample with respect to the starting laponite was previously indicated by the DRIFT spectra. No significant differences were observed in the basal spacing of the LH and LSH samples (from 1.47 to 1.54 nm). Normally, the intercalation of molecules in interlayer region of clay mineral cause significant changes in the interlayer distance¹⁹. In spite of this, it was observed by TG analysis an important decreasing in amount of adsorbed water. Usually the decrease of water content causes a significant decreasing in d_{001} spacing¹⁰. Taken into account that such reduction has not happened, our hypothesis is that there was replacement of some water molecule to organosilane in interlayer region of the clay. It indicates that silylation may take place at the surface of the particles and also within the interlayer galleries. Prado et al. and Park et al. report similar results^{17,19}. It was possible to observe that the peak d_{001} becomes broader and less intense following

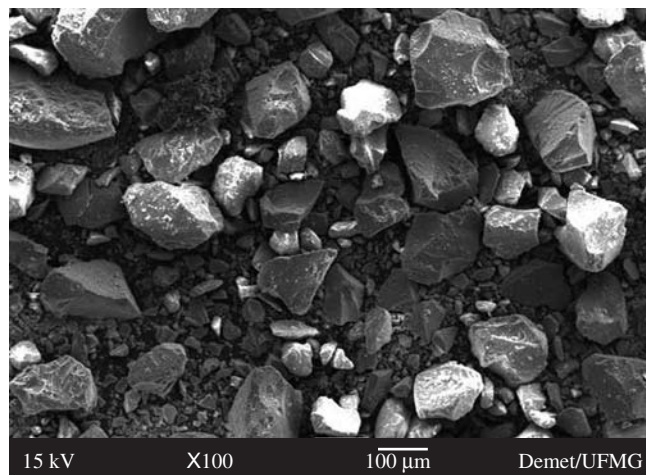


Sample	d-spacing/nm (Bragg angle/degree)
LNa	1.25 (7.0); 0.45 (19.6); 0.32 (28.1); 0.25 (35.1); 0.15 (60.8);
LH	1.47 (6.1); 0.45 (19.6); broad 0.26 (34.1); 0.15 (60.8)
LSH	1.54 (5.6); 0.45 (19.6); broad 0.26 (34.1); 0.15 (60.8)

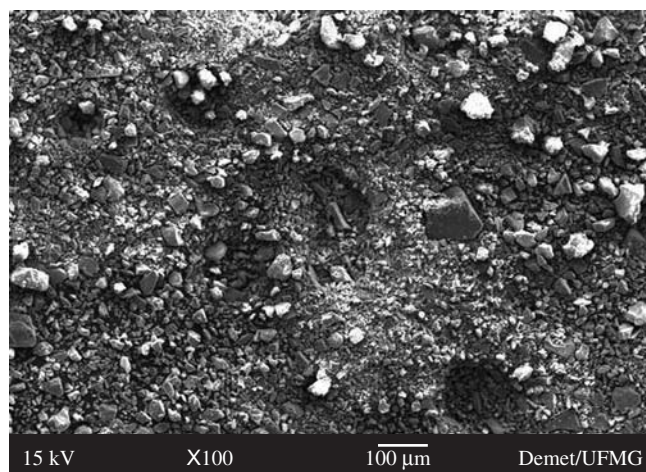
Figure 4. XRD patterns of sodium (LNa), acid activated (LH) and functionalized (LSH) laponite. The inset shows the precise peak indexation.

functionalization, thus indicating that this process may have caused some degree of disorder of the crystallites.

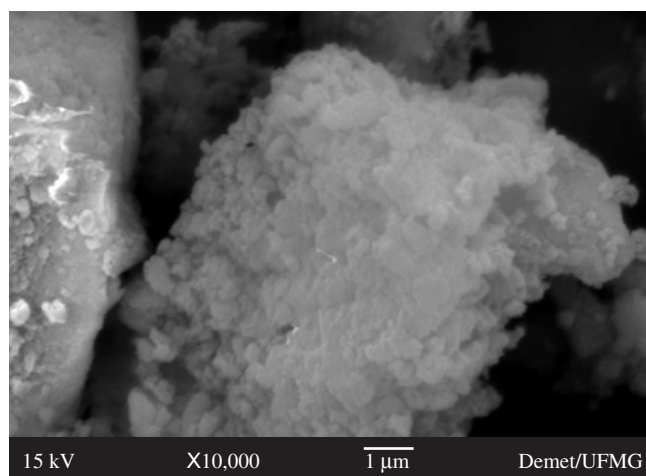
Figure 5a shows a SEM micrograph of the Na-laponite sample. One may observe particles formed by agglomerates of irregular



(a)



(b)



(c)

Figure 5. SEM micrographs of a) sodium laponite (LNa), b) and c) functionalized laponite (LHSH).

shapes and flat surfaces. The strong tendency toward aggregation and the compact aspect of the material can be observed. The images 4b and 4c display the SEM micrographs of the functionalized clay. In this case, the particles are apparently smaller in size than the previous sample and are composed of disordered thin sheet particles aggregates (Figure 5c). One can conclude that functionalization promotes the formation of disordered and less cohesive aggregates, probably due to a reduction of the edge-to-edge and face-to-face interactions. Unlike the hydrophilic laponite (LNa), which formed large size aggregates after drying, the LSH sample was hardly dispersed in water (hydrophobic) and easily kept as a loose powder after drying. This change constitutes an important advantage considering the product application in commercial units.

4. Conclusion

Laponite, a synthetic silicate, was functionalized through a straightforward reflux with organosilanes, in a route that seems attractive for the preparation of a selective sorbent for biomolecules. Analyses by XRD showed that the silylation process did not significantly affect the crystallographic structure of the clay as no important changes were observed in the basal spacing after functionalization. Analyses by XRD and TGA analysis showed that the silylation drastically reduced adsorbed water without causing basal spacing contraction, suggesting that intercalation may have occurred also in the interlayer region. The DRIFT results confirmed the presence of thiol groups and organic compounds on the modified clay. The Volhard method indicated a binding capacity of 1.4 meq/g of clay and this method showed to be a fast and efficient methodology for binding capacity determination to be applied to thiol functionalized substrates.

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