

Synthesis, characterization and study of covalently modified triazole

LAPONITE® edges

Carmelo Giuseppe Colletti,^a Marina Massaro,^a Giuseppe Lazzara,^{b,*} Giuseppe Cavallaro,^b Stefana Milioto,^b Ivana Pibiri,^a Renato Noto^a and Serena Riela^{a,*}.

^a Dipartimento STEBICEF, Sez. Chimica, Università degli Studi di Palermo, Viale delle Scienze, Ed. 17, 90128 Palermo, Italy.

^b Dipartimento di Fisica e Chimica, Università degli Studi di Palermo, Viale delle Scienze, Ed. 17, 90128 Palermo, Italy.

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Abstract

LAPONITE® (Lap) clay mineral was successful functionalized by triazole groups in a two steps procedure. First, the Lap edges were modified with 3-azidopropyltrimethoxysilane by traditional heating and microwave irradiation. MW irradiation allowed us to obtain high loading onto the Lap surface in lower times compared to those obtained through conventional method. Afterwards, the triazole moieties were obtained by reaction between azido functionalized Lap and propargyl alcohol. The successful functionalization of Lap was proved by thermogravimetric analysis, FT-IR spectroscopy, dynamic light scattering and ζ -potential measurements. Finally, the effects of the surface modification on the gel formation ability of Lap were studied by gelation tests and the morphology of the gel phases was investigated by polarized optical microscopy measurements and diffusion experiments.

Introduction

In the last years clay minerals have attracted considerable attention due to their appealing properties.

Hectorite is a natural layered magnesium-lithium silicate belonging to the smectite group with an ideal chemical formula of $\text{Na}_{0.3}\text{Mg}_{2.7}\text{Li}_{0.3}\text{Si}_4\text{O}_{10}(\text{OH})_4$ (Zhang et al., 2019). Due to its interesting features, such as environmental friendliness, swelling properties, tunable composition, etc.,

hectorite finds applications in several fields. However, hectorite deposits in nature are rare. Furthermore, due to the geological environments and conditions, natural hectorite at different deposits has various impurities and variable crystallinity, which limit the clay uses.

LAPONITE® (Lap), a synthetic analogous of hectorite, produced by BYK Additives and Instruments, is a layered hydrous magnesium silicate belonging to the family of (2:1) phyllosilicates built up of sheets of octahedrally coordinated magnesium oxide sandwiched between two parallel sheets of tetrahedrally coordinated silica ($\text{Na}_{0.7}^+[(\text{Si}_8\text{Mg}_{5.5}\text{Li}_{0.3})\text{O}_{20}(\text{OH})_4]_{0.7}^-$) (Becher et al., 2019; Ruzicka and Zaccarelli, 2011).

In the years, as others clay minerals (Massaro et al., 2019a; Massaro et al., 2018a; Massaro et al., 2018b), Lap was widely used in several fields: i) as filler for enhancing polymer properties (Boyaci and Orakdogan, 2016) and for environmental remediation (Charaabi et al., 2019); ii) as scaffolds for tissue engineering (Atrian et al., 2019; Boyer et al., 2018; Xavier et al., 2015), osteoinductive agents (Gaharwar et al., 2013), drug carrier (Li et al., 2014; Wu et al., 2014) and blood coagulators (Gaharwar et al., 2014; Li et al., 2015), due its ability to form stable gels in aqueous medium (Agoda-Tandjawa et al., 2016).

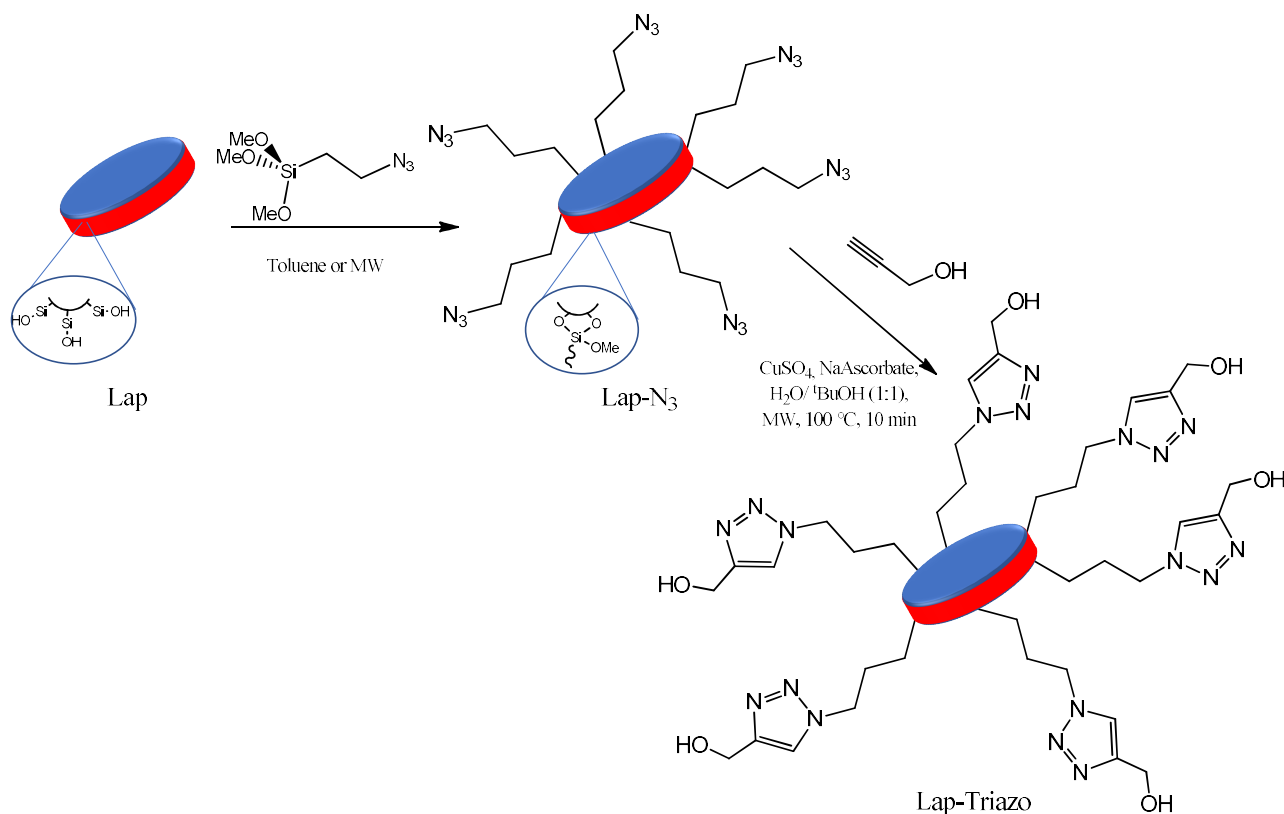
Surface functionalization of LAPONITE® can improve its dispersion in a polymeric matrix, enhance exfoliation and expand the application fields (Hegyesei et al., 2019; Herrera et al., 2005).

Herein, we report the covalent modification of Lap edges by means of grafting of organosilane bearing azido groups which were further functionalized to obtain Lap nanocomposite with some triazole functionalities. The experimental conditions for the organosilane grafting were optimized and the effects of the surface modification on the gel formation ability of Lap were studied by gelation tests, dynamic light scattering and ζ -potential measurements. The morphology of the gel phases was investigated by POM measurements and diffusion experiments.

Results and Discussion

LAPONITE® was functionalized with triazole units in a two steps procedure (Scheme 1). First, the silanol groups present at the edges of the clay were reacted with an excess of 3-azido

propyltrimethoxysilane to obtain an azido functionalized laponite (Lap-N₃) which was further modified by a copper catalyzed click reaction among the azido groups at laponite edges and an alkyne to give the final compound Lap-Triazo.



Scheme 1. Schematic representation of the synthetic pathway used for the synthesis of Lap-Triazo.

The experimental conditions adopted for the synthesis of Lap-N₃ were investigated in terms of heating source, solvent, temperature and time (Table 1).

First of all, the reaction was carried out following the experimental conditions usually adopted in literature for similar compounds (Daniel et al., 2008). In details, the grafting of the silane at the clay edge was performed in toluene, at room temperature and different times (Entries 1-4). All samples were sonicated for 30 min to force exfoliation of the clay exposing more Si-OH regions (Daniel et al., 2008) in the presence of the organosilane and left to stir (Entries 2-5). After sonication, the degree of functionalization of laponite, estimated by TGA, was 0.25 mmol g⁻¹ (Entry 1).

As it is possible to note from Table 1, the amount of silane grafted increases with increasing of the aging time. The best result, 9 wt% of loading, was obtained by aging the mixture for 168 h (Entry 4). A slight improvement in the silane loading was achieved by increasing temperature (from r.t. to

110 °C) (Entry 5) for 24 h. With the idea in mind of decrease the reaction time and increase the greenness of the reaction, we performed further experiments by using microwave irradiation with or without solvent at 100 °C for 1 h (Entries 6-7) (Massaro et al., 2019a). It is noteworthy that just 1 h of MW irradiation is enough to obtain a loading of the silane that approaches to that obtained under traditional conditions (Entries 5 and 6).

Finally, we evaluated the influence of the initial amount of organosilane used for the reaction, by further experiments performed at different organosilane/laponite ratio. Figure 1 reports the amount of silane grafted as a function of initial organosilane concentration. Surprisingly, by increasing the amount of organosilane added in the reaction mixture, a decrease in the degree of functionalization was observed. This could be due to the presence of side reactions of the organosilane, such as its hydrolysis and/or condensation, which could occur at higher concentration, affecting the grafting reaction (Brochier Salon et al., 2008).

Table 1. Experimental conditions for the synthesis of Lap-N₃.

Entry	Solvent	Time (h)	Temperature (°C)	Degree of functionalization (mmol g ⁻¹)	Functionalization (wt%)
1 ^a	Toluene	0	r.t.	0.25	2.1
2	Toluene	24	r.t.	0.76	6.4
3	Toluene	48	r.t.	1.00	8.7
4	Toluene	168	r.t.	1.10	9.0
5	Toluene	24	110	1.15	9.7
6	Toluene	1	100	1.15	9.6
7	/	1	100	1.10	9.0

^aThe dispersion was sonicated for 30 min, the powder was filtered, washed and analyzed by TGA.

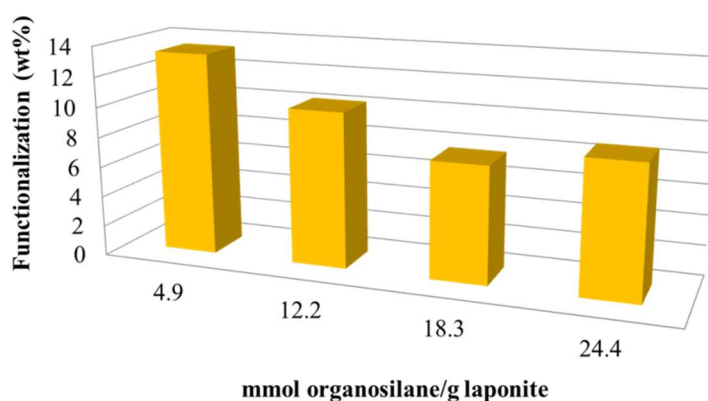


Figure 1. Degree of Lap functionalization as a function of organosilane/laponite ratio. Relative errors are $\pm 0.2\%$.

Lap-N₃ was subjected to the copper catalyzed Huisgen 1,3 dipolar cycloaddition in the presence of propargyl alcohol (Scheme 1). The reaction was carried out by microwave irradiation for 10 min at 100 °C. After work-up, Lap-Triazo, a white powder, was obtained with a loading of 3 wt% with respect to Lap-N₃.

Characterization of Lap-N₃ and Lap-Triazo

As above mentioned, the amount of organic moiety linked to the Lap edges was determined by TG. It should be noted that, in agreement with the literature (Cavallaro et al., 2011), Lap does not show any relevant mass loss in the temperature range, with the exception of the water loss up to 120 °C (see Figure S1). The TG data normalized by the water content are provided in Figure 2a. The Lap-N₃ sample shows the mass loss at ca. 250 °C indicating the volatilization of the functionalization moiety. The further organic modification generated a clear degradation step at ca. 390 °C that is in agreement with literature findings for triazole bonded to clay minerals (Riela et al., 2014).

The successful functionalization was further verified by FT-IR spectroscopy (Figure 2b). Compared to pristine Lap (Yu et al., 2019), Lap-N₃ exhibits, in the FT-IR spectrum, the vibration band for C-H stretching of methylene groups around 2980 cm⁻¹ and a strong band around 2100 cm⁻¹ due to the –N₃ groups of the grafted azido silane. The latter band disappears in the FT-IR spectrum of Lap-Triazo as a consequence of the formation of triazole units at the laponite edges.

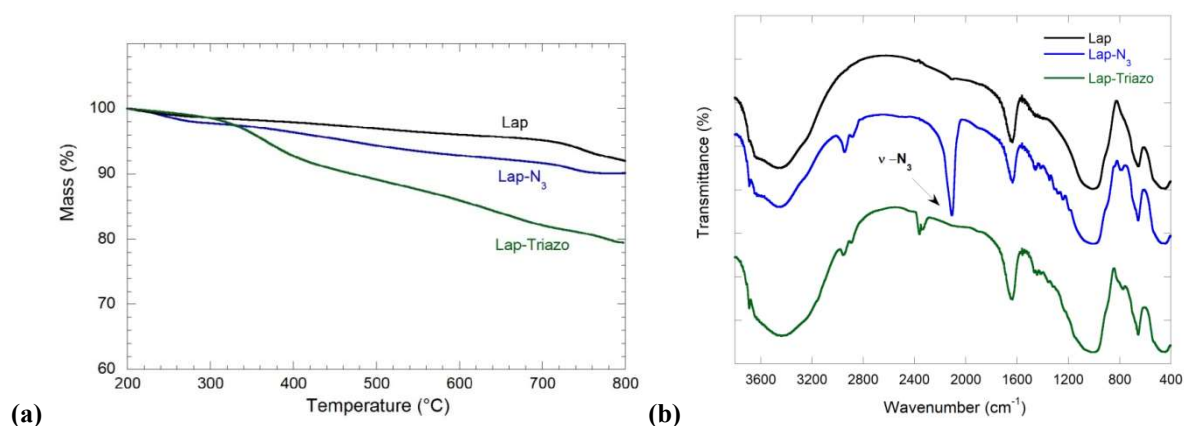


Figure 2. (a) Thermograms of Lap, Lap-N₃ and Lap-Triazo; (b) FT-IR spectra of Lap, Lap-N₃ and Lap-Triazo.

Lap-N₃ and Lap-Triazo properties

As reported in literature (Ruzicka and Zaccarelli, 2011), the grafting of silane at the laponite edges could modify the clay properties in aqueous solution, preventing the formation of hydrogel network. Indeed, in the modified clays, the house of card structure could be not developed as a consequence of the lacking of the electrostatic interactions, responsible of the silicate network formation (Figure 3a).

To confirm the above hypothesis, we performed some gelation tests in different solvents, namely water, PBS buffer and water/ethanol (0.5 v/v%) and PBS/ethanol (0.5 v/v%). The obtained results, reported in Table S1, showed that, conversely to Lap, in all cases investigated the formation of the gel phases did not occur. This could be due to a combination of weakly screened Coulomb repulsions, between the platelet faces that stabilized the clusters, and attractive interactions (for example π - π interaction among the different triazole units in aqueous medium) that acts as the driving force for clustering (Figure 3b).

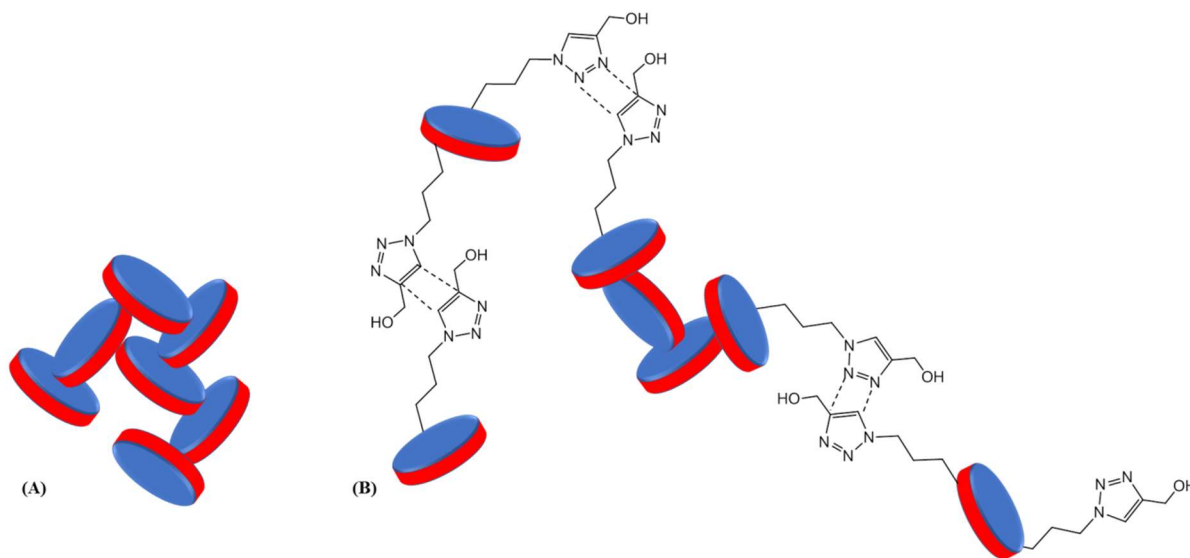


Figure 3. Cartoon representation of (a) organization of Lap and (b) possible arrangement of Lap-Triazo in aqueous medium.

Different results were obtained by using Lap-N₃ and Lap-Triazo as filler (5 wt%) for Lap hydrogels. As reported in Table S1, in each case investigated, by adding, to Lap dispersion, the filler (Lap-N₃ or Lap-Triazo), we obtained opaque and thixotropic hydrogels.

The morphology of the different Lap hydrogels obtained, both in water and in PBS buffer solution, was observed by POM measurements (Figures 4 and S2). Analysis of POM images evidences a homogeneous gel matrix for Lap hydrogels in water (Figure 4a) which turn into less homogeneous with the presence of some clusters in PBS buffer solution (Figure 4b).

To evaluate any effects of the degree of functionalization on the abilities of Lap-N₃ to act as filler, we also analyzed the of Lap hydrogels obtained with the Lap-N₃ with the lowest (2 wt%) and highest (9 wt%) degree of functionalization.

The effect of the functionalization of Lap-N₃ is clearly detected by POM images (Figure 4c-d). As it is possible to observe from Figure 4c, lower degree of functionalization offers a better organization in the gel network in water.

The introduction of Lap-Triazo as filler, causes the formation of less homogeneous hydrogels compared to pristine Lap (Figure 4e) in water, with the presence of some black lumps probably due to clustering effects deriving from π - π interactions among the aromatic groups in aqueous medium. Similar conclusions can be done for the hydrogel obtained in PBS buffer solution (Figure S2).

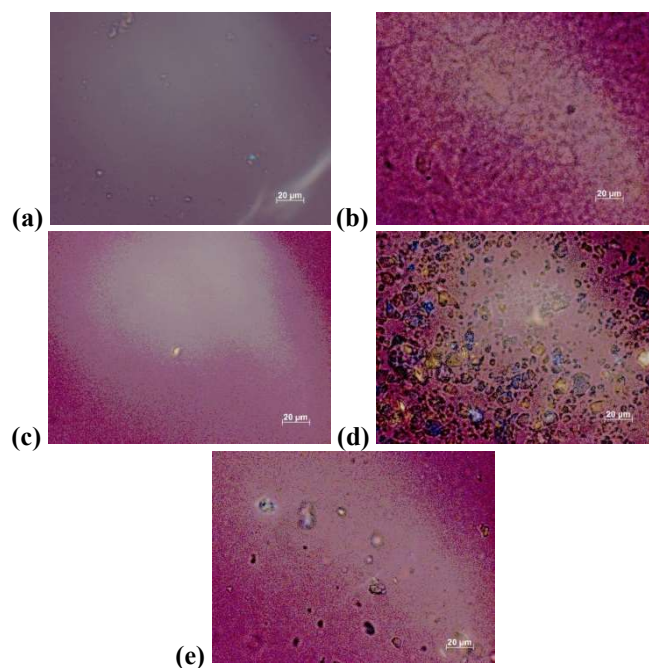


Figure 4. POM images of the hydrogel obtained with (a) Lap in water; (b) Lap in PBS (1:10) buffer solution; (c) Lap with 5 wt% of Lap-N₃ (2 wt% loading) in water; (d) Lap with 5 wt% of Lap-N₃ (9 wt% loading) in water; (e) Lap with 5 wt% of Lap-Triazo in water.

On the light of the above findings, we thought interesting to evaluate some colloidal properties of the Lap nanodisks before and after modifications in order to highlight the effect of the organic moiety. The electrostatic interactions have been investigated by means of electrophoretic mobility measurements and ζ -potential data. As showed in Table 2, the surface modification did not alter the surface charge on the nanodisks. This result can be interpreted considering that the net charge of Laponite is dominated by the negative face-side surface while the positive edge contributes just a little (Massaro et al., 2019b; Ruzicka and Zaccarelli, 2011). The solvent effect is also negligible on this respect (Table 2).

As concerns the hydrodynamic diameter, first one should keep in mind that the obtained values have to be considered as radius for the equivalent sphere possessing the same translational diffusion coefficient of the anisotropic nanoparticles. Nevertheless, the method can provide a clear evidence on aggregation and stability of the nanodisks induced by external parameters and/or surface modifications (Cavallaro et al., 2012). The pristine Lap showed a hydrodynamic diameter consistent with the literature value in water (Massaro et al., 2019b) and the PBS generate clustering in agreement with the salt screening effect and phase diagram reported for laponite (Ruzicka and Zaccarelli, 2011). Regardless of the modification, the Lap appears instable in PBS solvent where aggregation and clustering are observed with a significant increase of the hydrodynamic diameter. Going deeper, Lap-N₃ appears with a lower aggregation tendency than both Lap and Lap-Triazo. Conversely, in water media, all the surface modifications generated a clustering being more pronounced for Lap-Triazo.

Table 2. ζ -potential and dynamic light scattering for 0.05 wt% suspensions of Lap, Lap-N₃ and Lap-Triazo.

Sample	ζ -potential (mV)	Hydrodynamic diameter (nm)	ζ -potential (mV)	Hydrodynamic diameter (nm)
PBS (1:10) as a solvent		In water		
Lap	-33 ± 4	891 ± 65	-34 ± 4	18.7 ± 1.6
Lap-Triazo	-29 ± 3	909 ± 104	-31 ± 3	825 ± 93
Lap-N ₃ (9 wt%)	-33 ± 4	526 ± 17	-32 ± 3	433 ± 7
Lap-N ₃ (2 wt%)	-36 ± 4	531 ± 31	-35 ± 4	446 ± 10

The dye diffusion experiments were carried out on the laponite gel obtained at 5 wt% of functionalized Lap nanocomposites in water and in PBS. These experiments allowed us to evidence the Brownian diffusion of the dye within the gel matrix providing indirect information on the gel tortuosity and mesoscopic structure (Figure 5 and S3). The dye diffusion coefficients of Lap indicate that the clustering induced by PBS reduces the dye mobility in the medium. As concerns the diffusion in water based gels, all the functionalized Lap nanocomposites generated a decrease of the dye mobility in agreement with the observed clustering effect evidenced by DLS results and POM investigations (Figure 4). In PBS medium, the results are dependent on the surface modifications, in particular the gel based on Lap-Triazo, despite the largest clustering effect is observed by DLS, shows an increase of the dye diffusion coefficient with respect to Lap, according to the morphology observed by POM measurements. Differently Lap-N₃ showed nearly negligible differences compared to bare Lap.

Table 3. Results from dye diffusion experiments.

Sample	D	D/D ₀	D	D/D ₀
	(cm ² s ⁻¹)		(cm ² s ⁻¹)	
	PBS (1:10) as a solvent		In water	
Lap	(5.33 ± 0.08)×10 ⁻⁶		(9.2 ± 0.2)×10 ⁻⁶	
Lap-Triazo	(6.91 ± 0.07)×10 ⁻⁶	1.14±0.03	(6.30 ± 0.07)×10 ⁻⁶	0.83±0.02
Lap-N ₃ (2 wt%)	(4.6 ± 0.1)×10 ⁻⁶	0.93±0.03	(5.13 ± 0.08)×10 ⁻⁶	0.75±0.03
Lap-N ₃ (9 wt%)	(5.2 ± 0.1)×10 ⁻⁶	0.98±0.04	(6.12 ± 0.07)×10 ⁻⁶	0.82±0.02

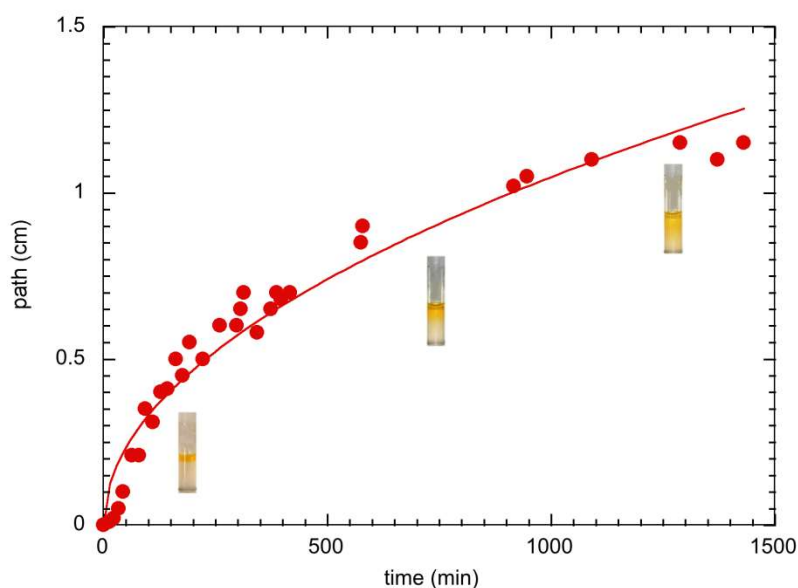


Figure 5. Diffusion of the methylo range through Lap hydrogel in water as a function of the time.

Conclusions

In summary, LAPONITE® nanodisks were functionalized with azido groups which were used as scaffold for further modifications to finally obtain a triazole modified Lap. The successful functionalization was confirmed by FT-IR spectroscopy and the amount of organic portions grafted were estimated by thermogravimetric analysis. Gelation tests showed the existence of strong supramolecular interactions among the different triazole units linked at the Lap edges which prevents the formation of the house of card structure in aqueous medium.

The obtained nanomaterials were, thus, used as filler for Lap hydrogel, and the properties of the resulting hydrogels were investigated. POM images highlighted different morphology of the Lap gels depending on the filler used (Lap-N₃ or Lap-Triazo, respectively) as confirmed by diffusion experiments, DLS and ζ-potential measurements.

Experimental

Materials and Methods

All reagents needed were purchased from Sigma-Aldrich and used without further purification.

Laponite is a BYK Additives and Instruments product.

FT-IR spectra (KBr) were recorded with an Agilent Technologies Cary 630 FT-IR spectrometer.

Specimens for these measurements were prepared by mixing 5 mg of the sample powder with 100 mg of KBr.

Microwave-assisted syntheses were carried out with a CEM DISCOVER monomode system in a closed vessel.

Dynamic Light Scattering (DLS) experiments were carried out by means of a Zetasizer NANO-ZS (Malvern Instruments) at 25.0 ± 0.1 °C. The field-time autocorrelation functions were analyzed by ILT analysis, which provides the decay rate (Γ) profiles of the diffusive modes. For the translational motion, the collective diffusion coefficient is $D_t = \Gamma/q^2$ where q is the scattering vector given by $4\pi n \lambda^{-1} \sin(\theta/2)$ being n the solvent refractive index, λ the laser wavelength (632.8 nm) and θ the

scattering angle (173°). The apparent hydrodynamic diameter for the equivalent sphere was calculated by means of the Stoke-Einstein equation. ζ -potential measurements were carried out by means of a Zetasizer NANO-ZS (Malvern Instruments) at $t = 25.0 \pm 0.1$ °C. The measurements were conducted using folded capillary cell, which is a disposable cell. Measurements were repeated three times and average values with standard deviations are provided.

Thermogravimetric analyses were done using the instrument Q5000 IR (TA Instruments) under nitrogen flow ($25 \text{ cm}^3 \text{ min}^{-1}$) by heating the samples from room temperature to 800 °C. Each sample (ca. 5 mg) was placed in a platinum pan and heated at $20 \text{ }^\circ\text{C min}^{-1}$.

POM measurements the samples were casted between two glasses. The samples were heated to their sol phases and cooled. The instrument used was a Zeiss Imager.A2m microscope equipped with crossed polarizers, a Zeiss AX10 heating stage, a Photometrics AXIOCAM ICC1 camera interfaced to a computer, and a Linkam microprocessor thermometer connected to a K thermocouple.

Synthesis of HNT-N₃ *via* traditional heating

In a typical synthetic run, 1 g of 3-azidopropyl trimethoxysilane (Riela et al., 2014) were dissolved in 20 mL of dry toluene and 0.200 g of Lap were added. The mixture was sonicated for 30 min, and then stirred for the appropriate time. Afterwards, the crude solid was filtered, washed with several aliquots of CH₃OH and dried overnight at 80 °C under vacuum.

Synthesis of HNT-N₃ *via* microwave irradiation

0.100 g of Lap were weighed in an MW test tube provided with a cap, and the appropriate amount of 3-azidopropyltrimethoxysilane was added dropwise with or without 2 mL of toluene. The mixture was dispersed ultrasonically for 30 min at room temperature and inserted in an MW apparatus at 100 °C under constant stirring for 1 h. The powder was filtered, rinsed with MeOH and dried at 80 °C under vacuum.

Synthesis of Lap-Triazo

Lap-N₃ (0.050 g), propargyl alcohol (2.5 mg, 6.1×10^{-3} mmol), CuSO₄ × 5H₂O (1 mg) and sodium ascorbate (1.5 mg) were weighed in a microwave test tube provided with a cup. Afterwards, 1 mL

of a mixture $\text{H}_2\text{O}/^t\text{BuOH}$ (1:1) was added and the dispersion was subjected to ultrasound for ca. 1 min. The crude was inserted in the MW apparatus at 100 °C, under constant stirring, for 10 min. Successively, the solid was collected by centrifuge, washed three times with water and three times with CH_2Cl_2 and finally dried at 40 °C under vacuum.

Preparation of gels.

Pure gels were prepared by weighing into a screw-capped sample vial (diameter 1.5 cm) the amount of Lap and solvent (~ 1 g). The mixture was first dispersed for 5 minutes with ultrasound irradiation and left at room temperature until a gel was obtained. Hybrid gels were prepared by weighing into a screw-capped sample vial (diameter 1.5 cm) the amount of Lap, Lap- N_3 and Lap-Triazo and solvent (~ 1 g). The mixture was first dispersed for 5 minutes with ultrasound irradiation and subsequently left at room temperature until a gel was obtained.

Thixotropic and Behaviour.

The mechanical stimulus was involved by stirring the gel phase with a stirring bar of 8 mm of length and 3 mm of height at 1000 rpm for 5 min. Thereafter, the materials were stored at room temperature overnight. When the samples were stable to the tube-inversion test, the gels were defined as thixotropic.

Diffusion analysis

The gels were placed in a glass vials (length of 1 cm and height of 5 cm) and 100 μL of dye solution were added carefully on the top. Two dyes (anionic and cationic) were tested and in particular methyloange and rhodamine B. It should be noted that the cationic dye did not show any diffusion after 1 week while the anionic dye coloured all the gel in ca. 24 hours. The cationic dye immobilization is explained by electrostatic interactions with the negatively charged Lap surface.

The anionic dye diffusion coefficient was determined by fitting the average displacement vs time profiles by assuming a Brownian motion equation. In particular the quadratic mean displacement ($\langle x^2 \rangle$) is proportional to the time (t) as described by

$$\langle x^2 \rangle = 2 D t$$

where D is the dye diffusion coefficient. The dye motion was detected by optical images (at an acquisition rate of 4 images/h) and ImageJ software was used to determine the dye front.

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