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NANOCOMPOSITE HYDROGELS BASED ON POLY(METHACRYLIC ACID) AND LAPONITE XLG

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The present work deals with the synthesis and characterization of some nanocomposite hydrogels based on poly(methacrylic acid) reinforced with different concentrations of Laponite XLG. The hydrogels were synthesized by the radical copolymerization of methacrylic acid and N,N'-methylenebisacrylamide in the presence of the filler, by using ammonium persulfate as the initiator. The resulted materials were characterized by FT-IR, XRD, electron microscopy (SEM, TEM), rheological measurements on the hydrogels in the initial state, swelling studies at different pH values. The release properties were tested by using caffeine as a model drug. The results obtained showed that the introduction of Laponite into the poly(methacrylic acid) matrix led to more rigid hydrogels, characterized by higher storage (G') and loss (G'') modules. In the presence of Laponite the swelling properties also changed, but Laponite itself had no effect on the release properties of the nanocomposite hydrogels.

Keywords: hydrogel, pH-sensitivity, viscoelastic properties

1. Introduction

Hydrogels are three-dimensional polymeric networks with a crosslinked structure capable to absorb a large amount of water or other aqueous solutions without dissolving. In addition to their high water content, they display a variety of useful properties such as biocompatibility and low toxicity, some hydrogels being also biodegradable [1]. Another important property of hydrogels is the ability to respond to different stimuli, like pH, temperature, light irradiation, magnetic/electric field, etc. Due to these properties they have found applications in various fields, such as medicine, pharmacy, agriculture, etc [2,3]. A major disadvantage of hydrogels is their low mechanical strength, especially in the swollen state, which led to the development of a new class of hydrogels, namely (nano)composite hydrogels [4-7]. The pH-sensitive hydrogels represent an important category of hydrogels [8,9]. Their sensitivity to pH change is related to

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the ionizable groups in the hydrogel network which can accept or donate protons depending on the pH value of the environment in which they are immersed. The pH-sensitive hydrogels can be anionic, containing acidic groups like carboxyl or sulphonic groups, or cationic, containing basic groups like amino. A type of well studied hydrogels is the one based on poly(methacrylic acid) (PMAA), which belong to the category pH sensitive anionic hydrogels due to the acidic groups contained. In the case of pH sensitive hydrogels, the swelling degree is the parameter that suffers the most drastic changes as a function of the environmental pH. When the pH of the medium is higher than pKa, the ionization of the hydrogel acidic groups takes place, thus leading to the increase of the swelling degree [10].

Many works investigating the interactions of poly(methacrylic acid) hydrogels with various types of reinforcing agents: gold nanoparticles, carbon nanotubes, clays, have been reported up to now [11-14]. From this point of view, clays have attracted more and more attention as fillers due to their properties, such as large specific surface area and adsorption capacity, optimal rheological properties, chemical inertness and low toxicity. Clays are usually micron- or nanometer-size particles, made of 2D layered silicate sheets. Due to their large surface area, the clay particles display a distinct adsorbent behavior and thus they could be used as delivery systems or as physical crosslinking points of polymeric materials, leading to hydrogels with extraordinary gelling behavior and mechanical strength [15]. Laponite [16,17] and Montmorillonite (MMT) [10,18] are among the most used clays as reinforcing agents for hydrogels. Laponite is a synthetic clay structurally similar with MMT, which is naturally-occurring clay. Both types of clay belong to the smectite group.

The biggest advantage of Laponite is its higher purity and thus the side effects caused by possible impurities of natural clays can be avoided. Laponite crystals are disc-shaped (approximately 25 nm in diameter and 0,92 nm height) and have the empirical formula $Na^{+0.7}[(Si_8Mg_{5.5}Li_{0.3})O_{20}(OH)_4]^{-0.7}$ [19]. Although there are several types of Laponite that have been used to reinforce hydrogels, no studies that compare their properties are reported in literature. The most used sorts of Laponite are RD [20], RDS [21], XLS [22] and XLG [23]. The major advantages of XLG and XLS over RD and RDS are their higher purity and the lower content of heavy metals. Laponite XLG is also non-toxic to human-type cells (hMSCs) [24]. An important difference is that the RD and XLG sorts form gels [25]. The reason for this behavior is that the positively charged edges of Laponite RD/XLG crystals can interact directly with the negatively charged faces of other crystals by electrostatic interaction, thus giving rise to the so-called “House of Cards’’ structure especially at concentrations higher than 2 %. This structure is responsible for increasing the viscosity of Laponite solutions in water. Laponite XLS is actually Laponite XLG modified with pyrophosphate ions that ionically stabilize the structure and prevent the formation of bonds between the edges and the faces of Laponite crystals, and thus the "House of Cards" structure can no longer be formed. As a consequence, Laponite XLS dispersions have a low viscosity even at high concentrations [26,27].

There are several reports in literature on the use of Laponite as a reinforcing agent in various hydrogels obtained from (poly(N-isopropylacrylamide) [28], polyacrylamide [29], poly(vinyl alcohol) [20], alginate [30], etc, but until recently no papers describing nanocomposite hydrogels based on PMAA and Laponite existed. That is why we decided to investigate this topic [31]. However, while this work was in progress, Junior et el. have published a paper describing the synthesis of some hydrogels from PMAA and Laponite RDS [32]. Their work was focused mainly on the study of the swelling properties of those hydrogels at variable pH, in the presence of various concentrations of Laponite RDS. In contrast, the main novelty of our paper consists in the investigation for the first time of the viscoelastic properties of PMAA - Laponite XLG nanocomposite hydrogels as a function of clay concentration at practically the same swelling degree. In addition, we demonstrate unequivocally by TEM analysis, that the nanocomposites formed are of exfoliated type due to the interaction between the PMAA matrix and Laponite. Also, controlled release studies were performed by using caffeine as model drug to investigate the influence of Laponite concentration on the release time. The hydrogels described here may find applications as components of drug delivery systems.

2. Materials and Methods

2.1 Materials

Laponite XLG (BYK Additives & Instruments) was a gift from Cosichem&Analitical, Romania. Methacrylic acid (MAA, 99% Janssen Chimica), N,N'-methylenebisacrylamide (MBA, 99%, Sigma Aldrich) and ammonium persulfate (APS, 98%, Sigma Aldrich) were used as received. Deionized water (DW) was used as the solvent in all cases.

2.2 Synthesis of hydrogels

The hydrogels were obtained by the radical copolymerization of MAA and MBA (2 mol% based on MAA) in aqueous solution, in the presence of APS as the initiator (1 mol% based on MAA) and 0.5-2 wt.% Laponite XLG based on the whole hydrogel composition as the reinforcing agent. The mass composition of the initial mixtures employed for the preparation of hydrogels is shown in Table 1. To prepare the nanocomposite hydrogels, the calculated amount of Laponite XLG was dispersed in DW under magnetic stirring for 2 h, followed by the addition of MAA and MBA. After the complete dissolution of monomers, the initiator was added and the mixture was injected into a mold made up of two glass plates separated by a 1 mm-thick Teflon gasket. The mold was then placed in a water bath at 60°C for 5 h in order the polymerization to occur. At the end of the curing time, the mold was removed from the water bath, allowed to cool to room temperature and opened. Discs of both 8 mm- and 20 mm-diameter were cut from the material. The 20 mm-diameter disks were used immediately for the rheological analysis, while the 8 mm-diameter ones were swelled for 7 days in DW with daily water replacement, in order to remove the unreacted monomers and initiator.

Table 1

The composition of the hydrogel precursor mixture1)

Sample Cod	H_2O (g)	MAA (g)	MBA (g)	APS (g)	Laponite XLG (g)	Laponite XLG %2)	Monomer conversion %
H0%Lap	89.4	10	0.36	0.26	0	0	96
H0.5%Lap	88.9	10	0.36	0.26	0.5	4.8	94
H1%Lap	88.4	10	0.36	0.26	1	9.6	95
H1.5%Lap	87.9	10	0.36	0.26	1.5	14.5	94
H2%Lap	87.4	10	0.36	0.26	2	19.3	92
H2.5%Lap	86.9	10	0.36	0.26	2.5	24.1	91

1) For 100 g amount reaction mixture
2) Based on the total amount of monomers

To determine monomer conversion, part of the hydrogel was dried to constant weight first in air and then over anhydrous $CaCl_2$ and weighed (W_o), and then immersed for purification in DW which was changed daily for 7 days. The final weight of the xerogel after drying was also recorded (W_{ext}).

The conversion was calculated using equation 1:

$$C\ \% = \frac{Wext - WLap}{Wo - WLap} * 100 \qquad (1)$$

where W_{Lap} represents the amount of Laponite contained by the hydrogel part used to determine the conversion.

2.3 Drug release

For loading with the model drug, dry xerogel disks weighing approximately 6-7 mg were placed in 2 ml of a caffeine solution of 9.15 mg/ml concentration and kept to swell at room temperature for 48 h. The hydrogels were then removed from solution and dried to constant mass. The release experiments were performed in two 50-ml centrifuge tubes containing solutions of different pH, acidic (pH 1.2, HCl solution) and basic (pH 7.4, PBS solution). The xerogels were immersed in 30 ml solution at 37°C and kept under continuous orbital stirring in a heating - cooling dry plate (Torrey Pines Inc.). At predetermined time intervals, the centrifuge tube was additionally stirred manually and 1-ml samples were taken, diluted to a volume of 7 ml, and the caffeine concentration was determined (λ = 273 nm) by UV-VIS spectrometry (UV-Vis Thermo instrument). To keep the solution volume constant, 1 ml of fresh solution was introduced at 37°C after each sampling.

2.4 Characterization

Fourier Transform Infrared (FT-IR) spectra were recorded in the 400-4000 cm^{-1} range on a Tensor 37 Bruker spectrophotometer (Woodstock, NY, USA), equipped with a Golden Gate ATR unit, by using ground xerogels.

X-ray diffraction (XRD) spectra were obtained by using an X-ray diffractometer (Rigaku Ultima IV, Tokyo, Japan) with CuK radiation (= 1.5406 Å), operated at 40 kV and 30 mA. Scanning Electron Microscopy (SEM) analyses were performed on previously freeze dried swollen hydrogel samples (ALPHA 1-2 LDplus, Martin Christ) by means of an ambient scanning electron microscope (ESEM-FEI Quanta 200, Eindhoven, The Netherlands). The morphology of the nanocomposite xerogels was obtained by transmission electron microscopy (TEM) carried out on a Tecnai G2 F20 TWIN instrument (FEI Company, Eindhoven, The Netherlands). The viscoelastic properties of the hydrogels were tested immediately after synthesis by using a Kinexus Pro rheometer (Malvern Instruments, UK, software 1.60). Amplitude and frequency sweep measurements were performed at 25°C by applying a normal force of 0.5 N and employing parallel plates with roughened finish to prevent slippage. In the case of amplitude measurements, the measurements were carried out at 1 Hz constant frequency, while in the case of frequency measurements; deformation was kept constant at a value of $5 \cdot 10^{-3}$ within the linear viscoelasticity range.The swelling degree of the hydrogels after 24 h was determined at 37°C in 3 different-pH aqueous solutions (HCl solution - pH 1.2; deionized water- pH 5.4; PBS - pH 7.4). Weighed dry xerogel disks (mX) were immersed in 30 ml of solution, and then the swollen disks were removed from solution after 24 h, wiped superficially with filter paper and weighed (mH) and reintroduced into solution. The degree of swelling (G) was calculated as the ratio between the amount of water absorbed and the mass of polymer in xerogel, according to equation 2:

$$G\ g/g = (mH - mX)/(mX\ x\frac{(100 - \%\ Lap)}{100}) \qquad (2)$$

where % Lap stands for the Laponite percentage of the xerogel calculated on the basis of the amount of monomers and Laponite introduced in the polymerization and the total conversion of the monomers (Table 1).

3. Results and Discussion

Nanocomposite hydrogels were synthesized by the free-radical copolymerization of MAA and MBA in the presence of Laponite XLG. High conversions were obtained in all cases, with values close to 100% (Table 1). The resulted hydrogels were characterized structurally by FT-IR and XRD analyses, morphologically by SEM and TEM, while the viscoelastic properties were determined by rheological measurements. Swelling studies at different pH values were performed as well, and the ability of hydrogels to control the release of caffeine, used as a model drug, was investigated.

3.1 Hydrogel structure

FT-IR spectroscopy was employed to demonstrate the presence of clay in the hydrogel matrix and to observe if interactions between components occurred (Fig.1a).

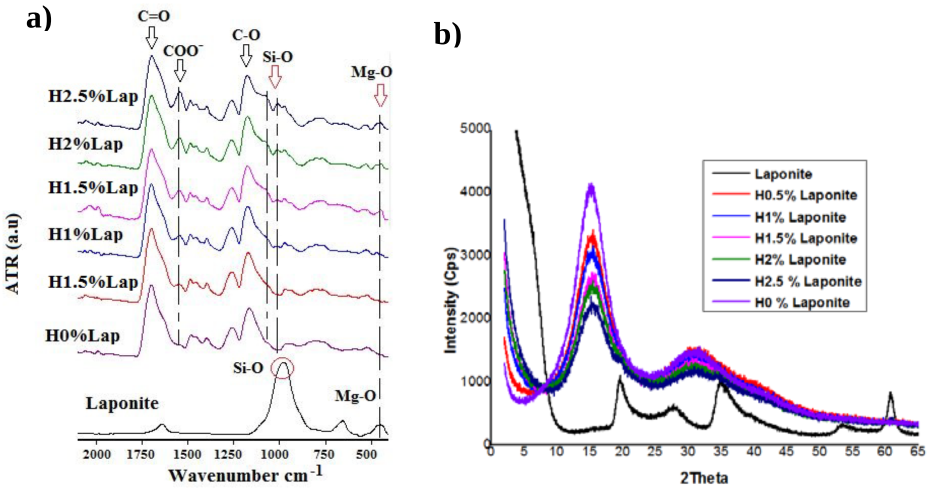

Fig. 1.FT-IR (a) and XRD (b) spectra of hydrogels reinforced with different concentrations of Laponite XLG

All xerogel spectra displayed the characteristic peaks of the carboxylic group (1692 cm^{-1} C=O, 1160 cm^{-1} C-O, Fig.1a) of the PMAA matrix [17,33]. In the case of the nanocomposite hydrogels, the characteristic peaks of Laponite were also identified at 970 cm^{-1} (stretching vibrations of the Si-O and Si-O-Si bonds) and 442 cm^{-1} (Mg-O bond stretching vibration) [24,25], their intensity increasing with the amount of Laponite in the hydrogel. It was also observed that the peak corresponding to the Si-O bond stretching vibration shifted as a function of Laponite concentration from 970 cm^{-1} in neat Laponite to 961 cm^{-1} in H0.5%Lap, 964 cm^{-1} in H1%Lap, 965 cm^{-1} in H1.5%Lap, 1004 cm^{-1} in H2%Lap, 1003 cm^{-1} in H2.5%Lap, thus proving that a stronger interaction occurred when using a larger amount of Laponite. One could be noticed as well the presence of a new peak at 1538 cm^{-1}, indicative for the existence of COO^{-} groups formed as a result of the reaction of MAA units with the basic groups of Laponite [34]. The intensity of this new peak increased with Laponite concentration. The XRD analysis was performed to monitor the position (2θ), shape and intensity of the diffraction line characteristic to the clay in the composite structure, in order to determine the existence of the phenomenon of intercalation / exfoliation of clay layers (Fig.1b).

In the case of the unreinforced xerogel, a peak characteristic for PMAA ($2\theta = 15^0$) was observed, which suggests the crystallinity of the polymer used in the synthesis of the hydrogel [35]. Once the Laponite content of the xerogel increased, the intensity of this peak decreased, indicating the decrease of the crystallinity degree. The same effect was observed by Yu et al., but for a hydrogel based on PVA, starch and Laponite [36]. Also, in the composite xerogels the peaks characteristic for Laponite diminished or completely disappeared, suggesting that predominantly exfoliated nanocomposite hydrogels were obtained. This assumption was supported by the TEM analysis which showed that Laponite was substantially exfoliated and uniformly dispersed in the polymer matrix

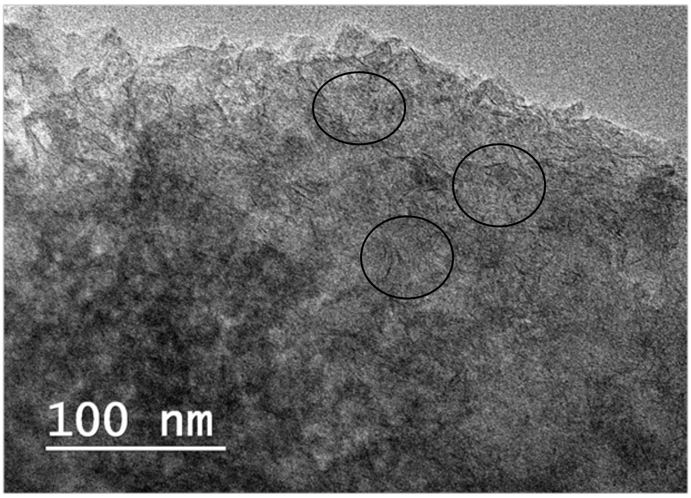

Fig. 2. TEM image of the H2%Lap xerogel

The SEM analyses of water-swollen and freeze-dried hydrogels revealed a structure with large interconnected pores, in the case of both unreinforced and reinforced hydrogels (Fig.3)

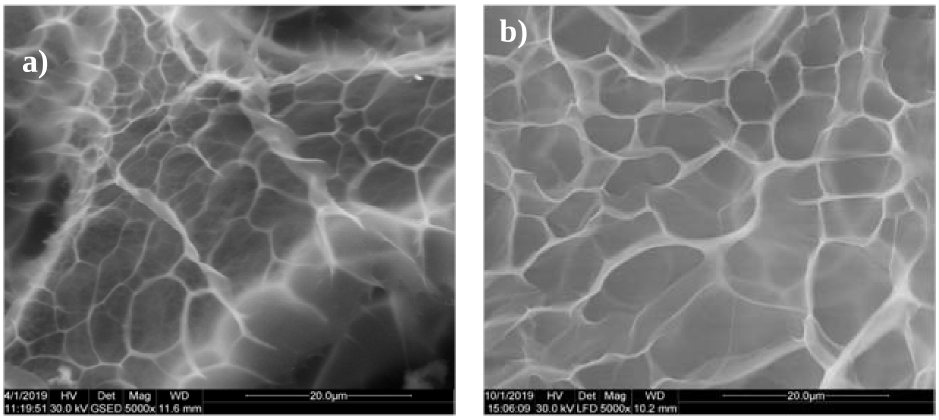

Fig. 3. SEM images the freeze dried a) H0 and b) H2%Lap hydrogels

3.2 Viscoelastic properties

The viscoelastic properties of hydrogels were investigated immediately after synthesis, when the swelling degree of hydrogels was practically identical in all cases, in order to be able to make a more accurate assessment of the influence of the Laponite amount. The amplitude sweep measurements showed that the viscoelastic properties remained constant over a wide range of deformation in all cases (Fig.4a), indicating a high elasticity of hydrogels. The frequency sweep measurements conducted at constant deformation revealed that G’ was higher than G’’ over the whole frequency range investigated (Fig.4b), which is characteristic for crosslinked materials. The increasing dependence of G' and G" on frequency indicated a network with large meshes for all hydrogels [37], in agreement with the relatively low concentration of the crosslinking agent in comparison with MAA. G' increased with increasing Laponite concentration (Fig.4b, 5a), while, despite G" increase (Fig.5a), the loss angle decreased (Fig.5b), indicating that the reinforcing agent determined an improvement of the hydrogel elastic character [38].

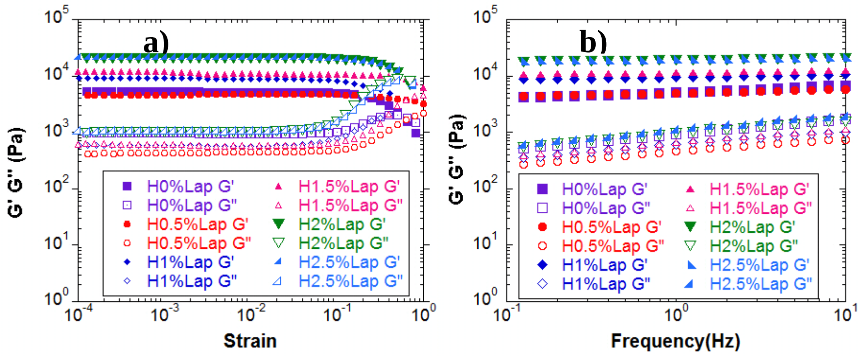

Fig. 4. Amplitude (a) and frequency (b) sweep rheological measurements upon the hydrogels synthesized

Also, the increase of the complex modulus G* (Fig.5b) and simultaneously of G'(Fig.5a), about 4 times with the increase of the reinforcing agent concentration from about 5% to 24% (relative to the polymer) indicated a clear stiffening / improving effect of the mechanical properties of the hydrogel under the action of the clay [37,40].

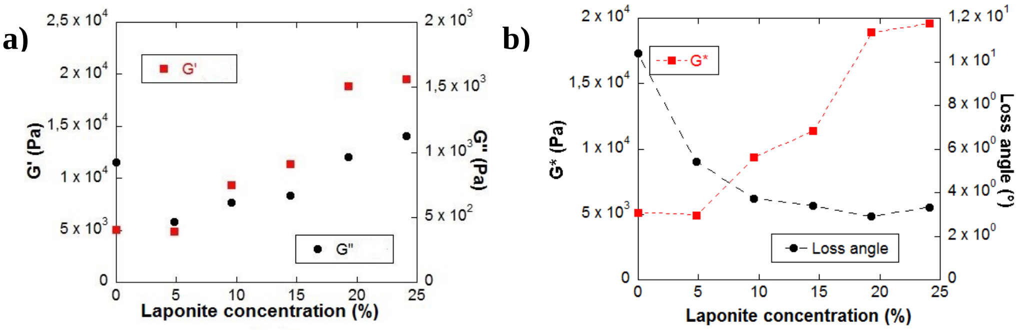

Fig. 5. The dependence of a) G’ and G’’ and b) G* and loss angle at 1 Hz on Laponite concentration (Frequency sweep measurements)

3.3 Swelling studies

The ability of hydrogels to absorb water is decisive for their use as biomaterials, including as supports for controlled drug release. In this study, the influence of Laponite concentration on the swelling degree of hydrogels at three different pH values (1.2;5.4;7.4) was investigated. It is worth mentioning that the proportion of Laponite within the xerogel varied between 0 wt.% in the case of H0%Lap and approximately 24 wt.% based on polymer in the case of H2.5%Lap. The swelling degree for all hydrogels synthesized varied with the pH value, in agreement with the pH-responsive character of the PMAA hydrogels. The swelling degree increased with the pH value of the medium because at a pH larger than 5 the carboxyl groups in the hydrogel ionized and the polymer chains became negatively charged. The higher pH, the larger the concentration of the negative charges within the polymer was. This negative charge was responsible for a higher swelling degree of the hydrogel at a more basic pH (Fig.6) [10], the additional hydrogel network expansion occurring because of both the electrostatic repulsion between the carboxylate ions and the additional osmotic pressure due to the formation of negative charges inside the hydrogel [38]. Both phenomena are more pronounced when the concentration of the negative charges is higher.

At pH 1.2, where the strong acid environment did not allow the formation of additional carboxylate groups, which could have facilitated the swelling of the hydrogel [39], the increase of Laponite concentration affected very little the swelling degree, which varied between 4.6 in the absence of Laponite and 4.1 in the case of H2.5%Lap, the effect being similar to that of Laponite RDS [32]. A possible explanation for this slight decrease may consist in the existence of a weak physical interaction between Laponite, whose basic groups are neutralized under these conditions, and the PMAA chains, which behave as a slight additional crosslinking.

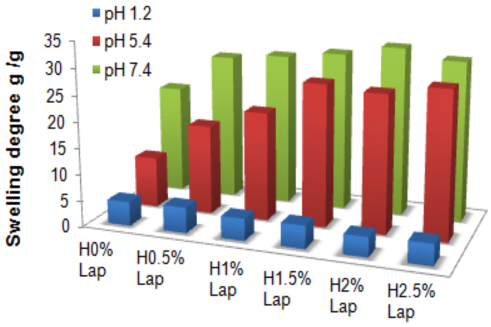

Fig. 6. Dependence of the swelling degree of the nanocomposite hydrogels on Laponite concentration and pH of the medium. Swelling time 24 h; temperature 37 °C.

At pH 5.4 and 7.4, the swelling degree increased with the concentration of Laponite in the hydrogel, the increase being steeper at pH 5.4. The phenomenon is explained by the presence basic groups in the structure of Laponite XLG, leading to basic character aqueous dispersions [40]. The pH values of the aqueous Laponite XLG dispersions in water with the concentrations displayed in Table 1 (only water and Laponite) increased from 8.7 in the case of H0.5%Lap to 9.6 for H2.5%Lap, as determined by means of a pH-meter. Their reaction with the polymer MAA units led to the formation of additional carboxylate anionic groups, as shown by the FT-IR analysis (Fig. 1a), which increased the swelling degree. The steeper increase of the swelling degree at pH 5.4 may be explained by a higher PMAA carboxylic acid groups/basic Laponite groups ratio than at pH 7,4 so that the proportion of carboxylate groups resulted in this case from the reaction with Laponite out of the total carboxylate groups on the PMAA chain was higher. At pH 7.4, the proportion of acidic carboxyl groups in PMAA was lower, while the number of carboxylate groups was higher due to the basic pH. As a result, the proportion of new carboxylate groups that formed by reaction with the basic Laponite groups was lower and therefore the increase in swelling with the Laponite concentration was slower. However, at pH 7.4, due to the basic environment, the concentration of carboxylate groups in the hydrogel is always higher than at pH 5.4 and therefore the swelling degree was higher at pH 7.4 for all Laponite concentrations.

3.4. Release properties

The release properties of hydrogels were tested by means of a model drug, namely caffeine (Fig.7), which over time has been used against fatigue, headaches, breathing problems, excessive weight, and also as an antioxidant and digestive stimulant[41-43]. The experiments aimed at investigating whether the presence of Laponite in the hydrogel has any effect on the rate of drug release. There are several studies in the literature in which caffeine has been used by other researchers as a model drug to investigate the release properties of several types of hydrogels [44-47] .

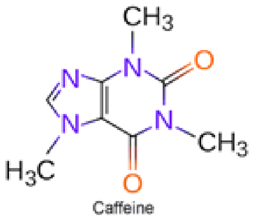

Fig. 7. Caffeine chemical structure

The hydrogels were loaded with the drug by swelling the xerogel in a solution of caffeine in deionized water with a concentration of 9.15 mg / ml. The presence of caffeine in xerogels was demonstrated by FTIR and EDAX analyses.

In the case of loaded hydrogel, the FT-IR spectra highlighted the peak characteristic for the C=O group in caffeine and its shift from 1693 cm^{-1} to 1699 cm^{-1}. Changes due to the interaction between caffeine and Laponite were noticed within the 1100-900 cm $^{-1}$ range, being observed shifts for the characteristic peaks of both Laponite and caffeine (Fig.8).

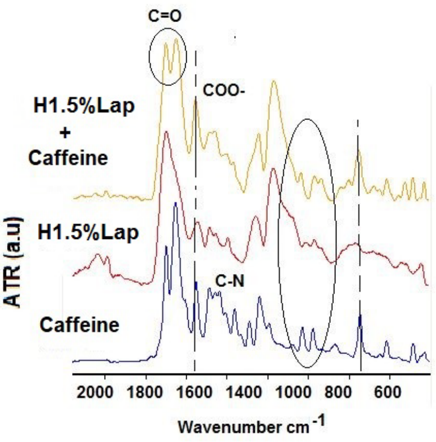

Fig. 8. FT-IR comparative spectra for caffeine, H1.5%Lap and drug-loaded H1.5%Lap.

The EDAX analysis, performed upon an unloaded xerogel (Fig.9a), a loaded xerogel (Fig. 9b), and on a nanocomposite xerogel loaded with drug (Fig.9c) showed the absence of nitrogen in the case of the caffeine-free sample and its presence in the case of the other two samples, demonstrating that caffeine was loaded. Also, in the case of the Laponite-containing sample, the presence of Si and Mg characteristic peaks could be observed (Fig.9 c).

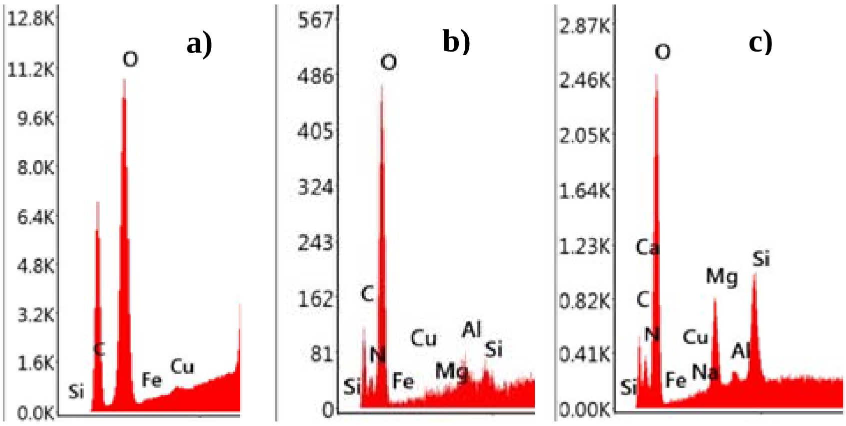

Fig. 9. Comparative EDAX spectra: a) H0%Lap b) H0%Lap loaded with caffeine c) H1,5%Lap loaded with caffeine

The drug release experiment was performed at both pH 1.2 and 7.4 and at a constant temperature of 37°C. Figure 10 displays the cumulative amount of drug released over time. It can be seen that the caffeine release took place quickly in both environments, the equilibrium being reached after about 60 minutes, both in the presence of Laponite and in its absence.

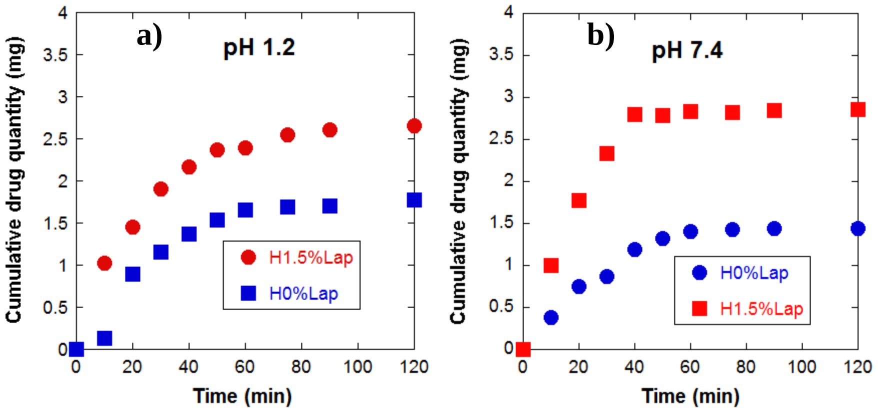

Fig. 10. Caffeine release a) pH 1.2; b) pH 7.4

Therefore, the results showed that Laponite had practically no influence on the release properties of the nanocomposite hydrogels under the experimental conditions used. By contrast, the addition of Laponite influenced the amount of drug absorbed by the hydrogel. The H1.5%Lap nanocomposite hydrogel (containing about 15 wt.% Laponite based on polymer) absorbed about twice the amount of drug as compared with the hydrogel without Laponite, which can be explained by the higher swelling capacity in DW (pH approx. 5.5) which led to the absorption of a larger amount of drug solution.

6. Conclusions

PMAA-based crosslinked hydrogels reinforced with different concentrations of Laponite were synthesized by radical copolymerization. The synthesized hydrogels were characterized by FT-IR, XRD, TEM, SEM analyses that demonstrated the existence of interactions between the organic polymer matrix and the inorganic reinforcing agent, as well as the formation of exfoliated nanocomposite hydrogels with a porous structure. Rheological measurements, performed on hydrogels immediately after their synthesis, showed the improved mechanical behavior brought by Laponite addition.

Swelling studies showed that pH-sensitive nanocomposite hydrogels were obtained, which can change their swelling degree depending on the amount of Laponite in the matrix, but also depending on the pH of the swelling medium. The nanocomposite hydrogels were also tested as a potential vehicle for the controlled release of drugs using caffeine as a model drug, and it turned out that Laponite had no effect on the release properties.

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