HYBRID HYROGEL BASED ON POLY (N-ISOPROPYLACRYLAMIDE) COPOLYMER AND SILANOL GROUPS FOR CONTROLLED DELIVERY OF DRUGS

SANDA BUCATARIU[1], MARIETA CONSTANTIN[1], DANIELA RUSU[1], IRINA PRISACARU[2] and GHEORGHE FUNDUEANU[1]

[1]*"Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania ghefun@icmpp.ro*
[2]*"Fiterman Pharma SRL", Iasi, Romania*

By the approached topic, the paper is a homage to Academician Cristofor I. Simionescu, founder of the Romanian school of cellulose, paper and fibers

Hydrogels which adjust their properties in response to environmental factors such as temperature, pH and ionic strength are rapidly evolving, being known as smart materials. A functional monomer, 3-(trimethoxysilyl) propylmethacrylate (TSPM) with methoxysilyl group ($-SiOCH_3$) was copolymerized with N-isopropylacrylamide (NIPAAm), acrylamide (AAm) and N,N'-methylene-bis-acrylamide (Bis), to yield a thermo-sensitive hydrogel [P(NIPAAm-*co*-AAm-*co*-TSPM)]. After treatment with NaOH (0.1 M), these hybrid hydrogels became anionically charged, due to ionisation of silanol and carboxylic groups from hydrolysed TSPM and AAm, respectively. Hydrogel morphology, before and after hydrolysis, as well as the physico-chemical properties, before and after Methylene Blue (MB) loading, were investigated by Scanning Electron Microscopy (SEM), Fourier-transform infrared (FTIR) and Ultraviolet-visible (UV) spectroscopy. The ionized hybrid hydrogels showed delayed release profiles compared to conventional hydrogels, which makes them suitable for controlled drug delivery.

Keywords: hybrid hydrogels, thermo-sensitive hydrogels, drug delivery

1. INTRODUCTION

In the broadest sense, hydrogels are three-dimensional hydrophilic networks that can absorb and retain large amounts of water [1–3]. The special importance of the hydrogels obtained from stimuli-sensitive polymers in both scientific and industrial fields is unanimously acknowledged. Among these polymers, poly(N-isopropylacrylamide) (PNIPAAm) and its copolymers transformed into hydrogels are the most widely used biomaterials for biomedical applications. PNIPAAm presents a lower critical solution temperature (LCST), close to 32 °C [4–14]. In order

to increase the LCST near to human body temperature (37 ^{o}C), NIPAAm needs to be copolymerized with hydrophilic monomers [15]. Accordingly, the hydrogels obtained from these copolymers present a volume phase transition temperature (VPTT); below this temperature, the hydrogels are in swollen state, while above VPTT they undergo volume transition and collapse [16,17]. PNIPAAm-based hydrogels are usually prepared *via* free radical polymerization using acrylamide (AAm), acrylic acid (AAc), hydroxyethylacrylamide (HEAAm), etc. as co-monomers, in the presence of chemical cross-linkers, such as N,N′-methylene-bisacrylamide (Bis) [1,18,19]. For example, copolymerization of NIPAAm with AAm ensures preservation of the acrylamide sequence, maintaining the stimuli-sensitivity of the polymeric network. Consequently, these types of hydrogels are largely used in bio-sensing [20–22], drug delivery [22,23], etc., being the most promising responsive polymers for biomedical applications [20].

Functional polymers based on alkoxysilane have proven to be the most versatile materials used to either modify or introduce functional groups into polymers.

Osváth *et al*. studied the thermo-sensitive behavior of copolymers based on NIPAAm and 3-(trimethoxysilyl)propyl methacrylate (TSPM), which can be widely utilized as a variety of new materials with remarkable thermal stability [4]. TSPM is a reactive monomer with methoxysilyl groups (—SiOCH$_3$) which, at relatively high temperatures, can hydrolyze into silanol [—Si(OH)$_3$] groups and consequently condense into cross-linked polysilsesquioxane [1].

Cao and co-workers have developed poly(NIPAAm-*co*-TSPM)-based microgels with more homogeneous microstructures by simple heating of the copolymer aqueous solution above its LCST [1]. Cross-linked poly(NIPAM-*co*-TSPM) thin films have been also obtained by the same procedure [24].

In our study, hybrid hydrogels were prepared by free radical copolymerization of NIPAAm with AAm, in the presence of variable amounts of TSPM (from 1 to 3% moles *versus* monomers) and *N,N'*-methylene-bis-acrylamide (Bis) used as cross-linker. In order to show that TSPM can also act as an additional cross-linker, hybrid hydrogels without Bis were also obtained. Further, the obtained hydrogels were treated with NaOH (0.1 M) and, as a result, the hybrid hydrogels were anionically charged. The thus prepared hydrogels were characterized in terms of swelling degree and swelling-deswelling kinetics. *In vitro* release studies of methylene blue (MB) were performed in simulated physiological conditions above the VPTT, a prolonged time of drug release being obtained.

2. EXPERIMENTAL

2.1. CHEMICALS AND MATERIALS

N-isopropylacrylamide (NIPAAm) from Sigma-Aldrich Chemie GmbH (Germany) was recrystallized from hexane. Acrylamide (AAm) from Aldrich (Germany), 3-(trimethoxysilyl) propylmethacrylate (TSPM), potassium persulfate (KPS), N,N'-methylenebisacrylamide (Bis), *N,N,N',N'*-tetramethylethylenediamine

(TEMED) from Fluka (Germany), have been used as received. Methylene blue (MB) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Standard phosphate buffer solution (PBS) at pH=7.4 (50 mM Na_2HPO_4 + NaOH) was prepared in our laboratory.

2.2. HYBRID HYDROGELS PREPARATION

The hybrid hydrogels were prepared by free radical copolymerization of NIPAAm and AAm in aqueous solution, in the presence of different TSPM molar ratios, previously dissolved in methanol. *N,N'*-methylenebisacrylamide (Bis) was used as cross-linker (1% mole *versus* monomers) and a redox system based on potassium persulfate (KPS) and *N,N,N',N'*-tetramethylethylenediamine (TEMED) was used as initiator. Dry nitrogen was bubbled through the solution for 45 min before polymerization. Next, the initiator (2.5% moles KPS *vs* monomers) was added and kept for 5 min under nitrogen atmosphere, to generate free radicals. Finally, the polymerization accelerator (100 μL of TEMED) was added, the mixture was homogenized, and then quickly transferred into a 10 mL syringe. The polymerization reaction took place for 24 hours inside the syringe. Then, the sample was extracted from the syringe and cut into small cylindrical pieces 10 mm in diameter and 7 mm height. Similar preparation conditions were used to obtain a series of samples without Bis, to verify the cross-linking ability of TSPM and also for the synthesis of conventional hydrogel without TSPM, used as reference.

For the synthesis of ionized hydrogels, the samples were kept in 0.1 N NaOH for 24 h at ambient temperature, then purified by extensive washing at room temperature.

2.3. HYBRID HYDROGELS CHARACTERIZATION

2.3.1. Morphological and elemental composition analysis

The morphological and elemental composition of conventional, un-ionized and ionized hydrogels, was examined on an Environmental Scanning Electron Microscope (ESEM), type Quanta 200, operating with secondary electrons in Low Vacuum, at 20 kV, coupled to an energy-dispersive X-ray (EDX) analyzer. For this analysis, the samples were quickly frozen in liquid nitrogen and then dried in vacuum (-57 ^{o}C, 5.5×10^{-4} mbar) for 48 h, after which the dried hydrogels were carefully fractured for observing their inner morphology.

2.3.2. ATR-FTIR analysis

The chemical structure of the hydrogels has been confirmed by Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR) using a FT-IR Vertex 70 spectrophotometer (Bruker, Austria). The lyophilized hydrogels were placed on a KRS-5 support; the frequency ranged between 4000 and 600

cm^{-1}; 128 scans were recorded; the resolution was 4 cm^{-1}. Data processing was performed with the OPUS 6.5 software (Bruker Optics, Austria).

2.4. SWELLING STUDIES

2.4.1. Equilibrium swelling ratios

The swelling ratio of hybrid hydrogels was measured at different temperatures ranging from 20 to 65°C, controlled up to 0.2°C, using a thermostated water bath. Pre-weighed samples were equilibrated in simulated physiological fluids (standard phosphate buffer (pH 7.4, 50 mM Na_2HPO_4 + NaOH)) for 12 h at each predetermined temperature. Then, the samples were taken out from the water bath, the excess liquid was wiped off from the surface with moistened filter paper, and weighed. The swelling ratio was determined according to Equation (1):

$$\text{Swelling ratio (SR)} = \frac{W_s - W_d}{W_d} \tag{1}$$

where W_s is the weight of the swollen hydrogel at equilibrium at each temperature and W_d is the weight of the dry sample.

The VPTT of the hydrogel was determined as the inflexion point of the curve swelling ratio as a function of temperature, by Boltzmann fitting of the experimental data.

2.4.2. Swelling kinetics

The dry hydrogel sample was weighed and then immersed in an excess of standard phosphate buffer (pH 7.4), at room temperature (25 °C). Then, the hydrogel was removed from medium at various time intervals and weighed after the excessive solution on the surface was blotted with moistened filter paper. The swelling ratio was calculated for three sample of each hydrogel.

2.4.3. Deswelling kinetics

The samples were swollen in PBS (pH 7.4), at room temperature until equilibrium was reached. The equilibrated samples were quickly transferred to hot solutions at 50°C. At a predetermined time interval, the samples were removed and weighed after wiping off the excess solution on the surface with a filter paper. The water retention capacity (WR) as a function of time was determined using Equation (2):

$$WR_t = (W_t - W_d) / (W_s - W_d) \times 100 \tag{2}$$

where W_t is the weight of the hydrogel at various exposure times, W_d is the weight of the dried hydrogel, and W_s is the weight of the swollen hydrogel at equilibrium (25 °C).

2.5. DRUG LOADING AND RELEASE STUDIES

For MB loading, each dried hydrogel sample (~0.100 g) was soaked in 10 mL of MB aqueous solution (C_{MB}=1 mg/mL), and maintained immersed for seven days at room temperatures (20°C). Then, the MB-loaded hydrogel was removed, washed twice with distilled water and then dried in a vacuum oven. The amount of MB loaded in each sample was determined according to Equation (3):

$$MB_{loaded} = [MB]_i - [MB]_f \qquad (3)$$

where $[MB]_i$ and $[MB]_f$ represent the MB concentration in solution before and after the loading process, respectively.

MB release studies were performed by immersing the MB loaded hydrogels (about 0.100 g) in PBS (pH 7.4) at 37°C. At predetermined time intervals, 3.0 mL of the release solution was taken out and MB concentration was determined spectrophotometrically at 288 nm, using a previously plotted calibration curve. The same volume of fresh release medium was added, to maintain the volume of the release system unchanged.

3. RESULTS AND DISCUSSION

3.1. HYBRID HYDROGELS PREPARATION

A new hybrid hydrogel based on NIPAAm, AAm, and TSPM was obtained. TSPM was incorporated because it is a very reactive compound, due to the methoxysilyl groups transformed, after hydrolysis, into silanol groups (Fig. 1).
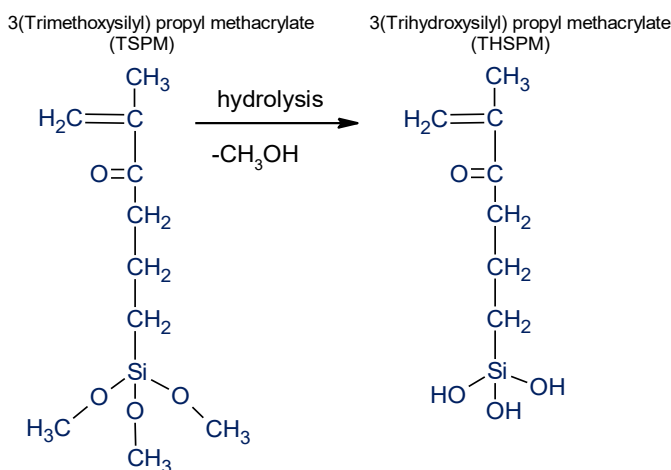
Fig. 1. Hydrolysis of TSPM to THSPM.

In this study, TSPM was used as both monomer and cross-linker. After previous solubilization in methanol, TSPM was mixed with the monomer solution at different molar percentages related to NIPAAm and AAm. Then, the hybrid hydrogels were prepared by free radical copolymerization of monomers, at a molar ratio NIPAAm: AAm of 4:1, in the presence/ absence of Bis. This molar ratio was previously established to ensure a poly(NIPAAm-*co*-AAm) copolymer with a VPTT value in PBS (pH 7.4) close to that of the human body. A redox system based on KPS and *N,N,N',N'*- TEMED was used to initiate polymerization (Fig. 2).
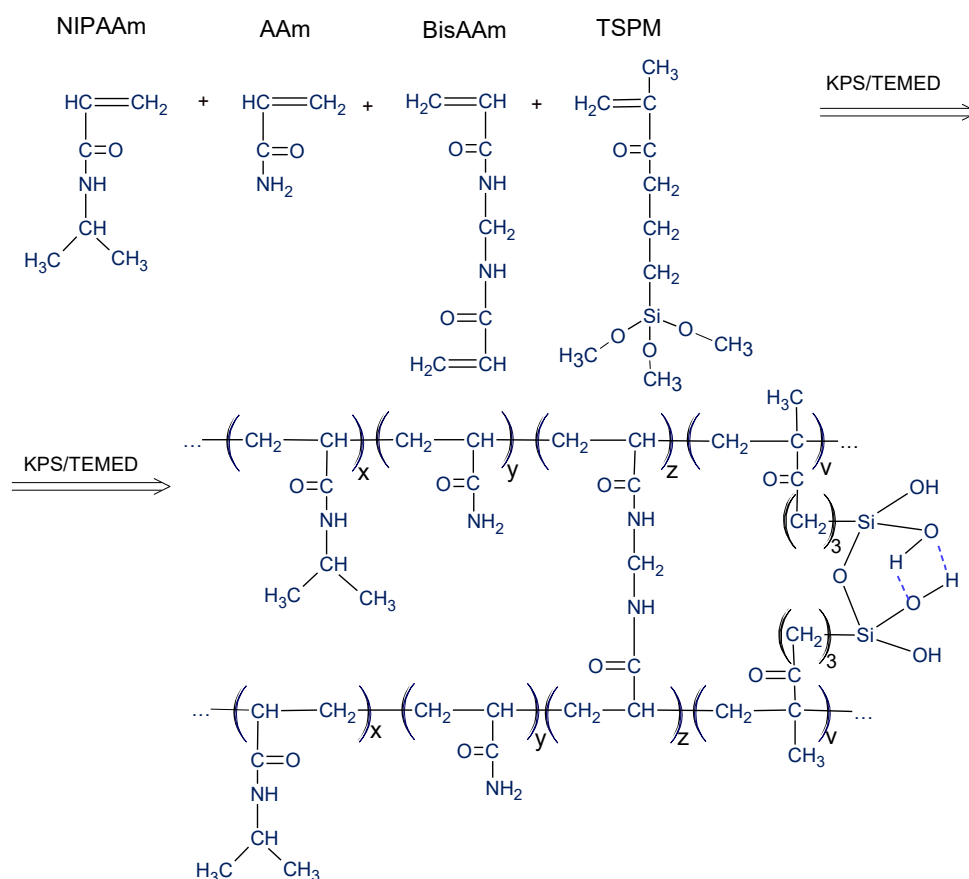
Fig. 2. Schematic representation of the synthesis of hybrid hydrogels.

For comparative purposes, a conventional hydrogel cross-linked with Bis, without TSPM, was synthesized under similar preparation conditions. The conventional, as well as the double cross-linked hydrogels were successfully prepared at an around 88% yield, whereas hybrid hydrogels, cross-linked only with TSPM, were obtained at a less than 65% yield (Table 1). The hybrid hydrogel samples were obtained at a NIPAAm:AAm molar ratio of 4:1 and coded as

"NAmT$_{xBis}$" or "NAmT$_x$" where "x" is the molar percent of TSPM *vs* monomers. Abbreviation "Bis" in the sample code indicated that Bis was used as a cross-linker at a concentration of 1% (mol) *vs* monomers. The composition of the reaction mixture is given in Table 1.

Table 1

Feed compositions used in the synthesis of conventional and hybrid hydrogels

Sample code	Comonomer composition in feed x 10^{-3} M (% moles)		Bis (% to monomers)	TSPM (% to monomers)	Yield (%)[d]
	NIPAAm	AAm			
CONVENTIONAL HYDROGELS – cross-linked with Bis					
NAm	4.8 (80)	1.2 (20)	1	–	88.78
HYBRID HYDROGELS – double cross-linked with Bis and TSPM					
NAmT$_{1Bis}$[b]	4.8 (80)	1.2 (20)	1	1	88.50
NAmT$_{2Bis}$				2	88.70
NAmT$_{3Bis}$				3	88.85
HYBRID HYDROGELS – cross-linked with TSPM					
NAmT$_1$[c]	4.8 (80)	1.2 (20)	–	1	54.16
NAmT$_2$				2	60.12
NAmT$_3$				3	62.48

NIPAAm+AAm, 1.2M; KPS, 30 mM; Bis, 12 mM (1% vs comonomers)

[a]*NAm – notation for conventional hydrogel, where the molar ratio of co-monomers NIPAAm:AAm in 5 mL reaction mixture is 4:1.* [b]*NAmT$_{xBis}$ – notation for hybrid hydrogels double cross-linked, where "Bis" stands for N,N'-methylenebisacrylamide; "x" is the molar percentage of TSPM vs monomers .*[c]*NAmT$_x$ – notation for hybrid hydrogels, where "x" has the same meaning as for double cross-linked hybrid hydrogels.* [d]*The yield was determined as the ratio between dry weight of the obtained hydrogel and total amount of monomer in the feed.*

3.2. MORPHOLOGICAL ANALYSIS

In order to study the morphology of hydrogels in their natural state, the samples were first swollen in distilled water, then frozen and lyophilized. The morphology of NAmT$_{xBis}$ and NAmT$_x$ is shown in Figure 3. It can be seen that the size of pores slightly decreases with increasing the content of TSPM in the cross-linked hybrid hydrogels (NAmT$_{xBis}$). Therefore, the network is denser, due to the cross-linkable silanol groups of hydrolyzed TSPM (THSPM), which are able to form either very strong hydrogen bounds or siloxane bridges. The capacity of THSPM to form a dense cross-linked network is also showed in the cross-section representation of the hydrogel obtained without BisAAm (NAmT$_x$).

The hybrid hydrogels were treated with 0.1 N NaOH, to obtain ionized carboxyl and silanol groups (Fig. 4). The resulted samples were coded as NAcT$_{xBis}$, considering that poly(NIPAAm-*co*-AAm) became poly(N-isopropylacrylamide-*co*-acrylic acid) (poly(NIPAAm-*co*-Ac)). Also observed was that the hydrogels cross-linked only with TSPM (NAmT$_X$), without Bis, solubilized after the treatment with NaOH; this demonstrates that the stability of the hydrogel is based on the hydrogen bonds between the silanol groups, rather than on the siloxanes bridges.
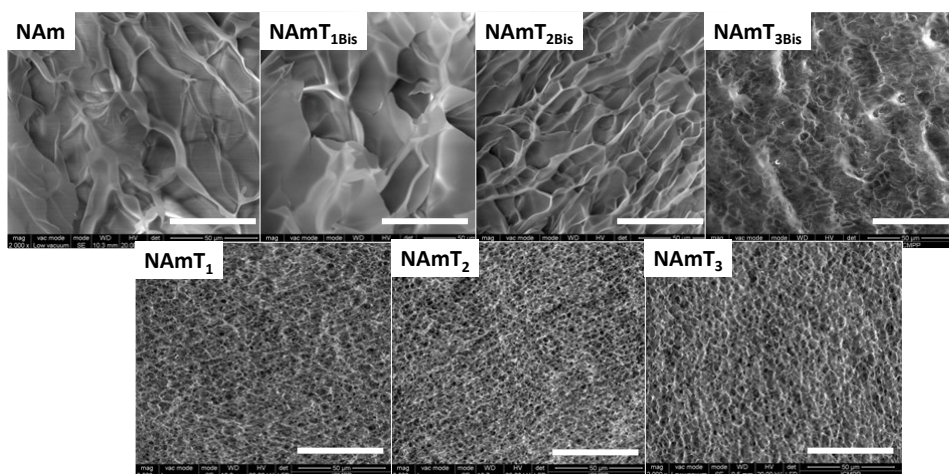
Fig. 3. Scanning electron micrographs of conventional hydrogel (NAm), hybrid hydrogels double cross-linked with Bis and TSPM (NAmT$_{xBis}$), and hybrid hydrogels cross-linked only with TSPM (NAmT$_x$). The bar corresponds to 50 µm
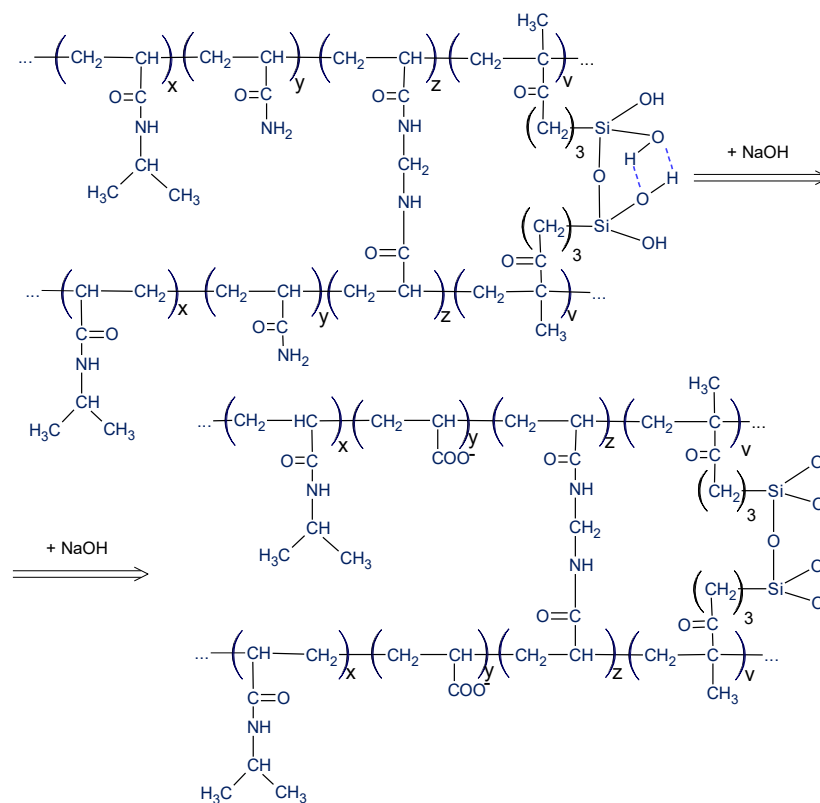

Fig. 4. Schematic representation of the ionization of hybrid hydrogels.

The morphology of the hydrogel before and after ionization is illustrated in Figure 5. The images do not show evident differences in the morphology of hydrogels before and after ionization. However, in the case of the hydrogel with 3% TSPM (sample $NAmT_{3Bis}$ vs $NAcT_{3Bis}$ in Fig. 5), a slight difference in the aspect of pores can be observed. They seem better defined after ionization, while the polymer network looks less dense. This difference can be explained by solubilization of inorganic silica-based micro-domains in the presence of NaOH.
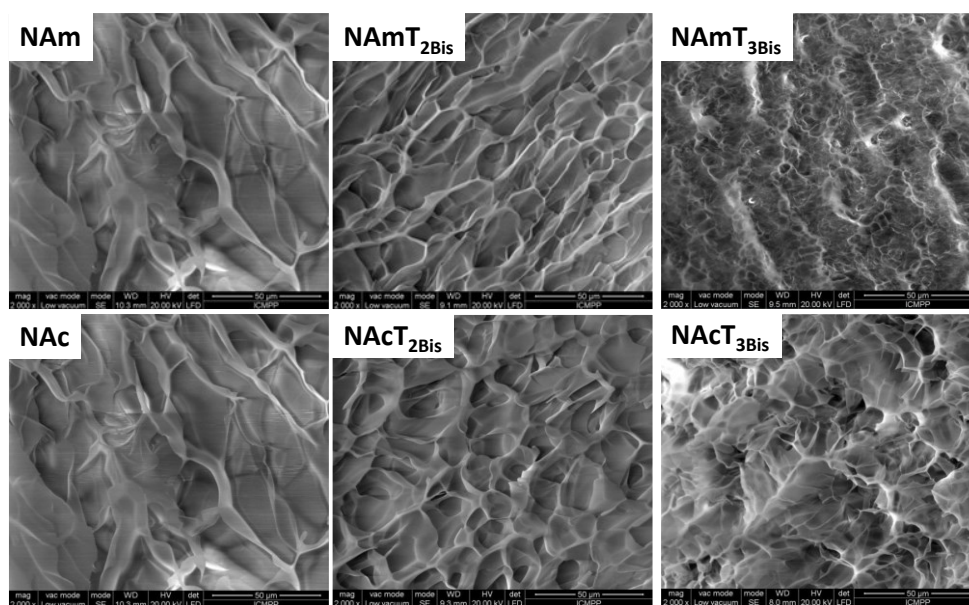
Fig. 5. Scanning electron micrographs of conventional hydrogel (NAm and NAc) and double cross-linked hybrid hydrogels before ($NAmT_{2Bis}$, $NAmT_{3Bis}$) and after ionization ($NAcT_{2Bis}$, $NAcT_{3Bis}$).
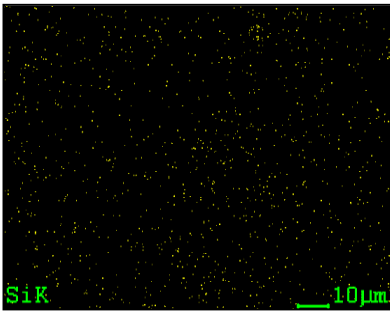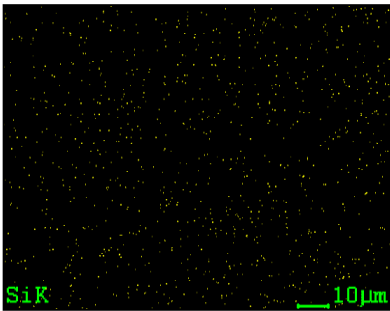
Moreover, EDX analysis (Table 2) shows that the amount of silica in $NAmT_{xBis}$ is reduced to less than half after ionization of hydrogels, due to the dissolution of inorganic micro-domains. It is only the silanol groups attached to THSPM that remained in the hydrogel.

3.3. FT-IR ANALYSIS

Figure 6 shows the FTIR spectra of the conventional hydrogel and of hybrid hydrogels with different chemistries. Comparing the FTIR spectra of NAm with those of $NAmT_{xBis}$ and $NAmT_x$, it is clearly evident that the shoulder bands at 1725 cm^{-1} (C=O from TSPM) and at ~1050–1080 cm^{-1} (Si-O-Si groups) appeared only in TSPM-containing hydrogels.

Table 2

Elemental composition obtained by the EDX of conventional hydrogel (sample NAm) and double cross-linked hybrid hydrogel: non-ionized (sample $NAmT_{3Bis}$) and ionized (sampe $NAcT_{3Bis}$)

NAm	**Element**	**Wt%**	**At%**
	CK	69.40	73.89
	NK	14.60	13.32
	OK	16.00	12.77
	Matrix	Correction	ZAF

NAmT$_{3BIS}$	**Element**	**Wt%**	**At%**	
	CK	73.87	78.11	
	NK	11.99	10.87	
	OK	13.55	10.76	
	SiK	00.59	00.27	
	Matrix	Correction	ZAF	

NAcT$_{3BIS}$	**Element**	**Wt%**	**At%**	
	CK	69.99	74.51	
	NK	13.93	12.72	
	OK	15.85	12.67	
	SiK	00.23	00.11	
	Matrix	Correction	ZAF	

3.4. THERMO-SENSITIVE PROPERTIES

The most important characteristic of the PNIPAAm based-hydrogels designed for biomedical applications is to display rapid changes in volume transition at a temperature close to that of the human body [25].

3.4.1. Determination of VPTT at equilibrium

The swelling ratios (SR) of conventional and hybrid hydrogels before and after ionization are shown in Figures 7A and 7B, respectively. The experiments were carried out in PBS, over a temperature range from 25 to 60°C. VPTT represents the

inflection point of the curve, which was obtained from SR variation as a function of temperature. All hydrogels showed a relatively sharp transition from high to low swelling degrees around the human body temperature (37°C). The presence of TSPM in hybrid hydrogels does not affect significantly the value of VPTT, however there is some influence on the degrees of swelling.
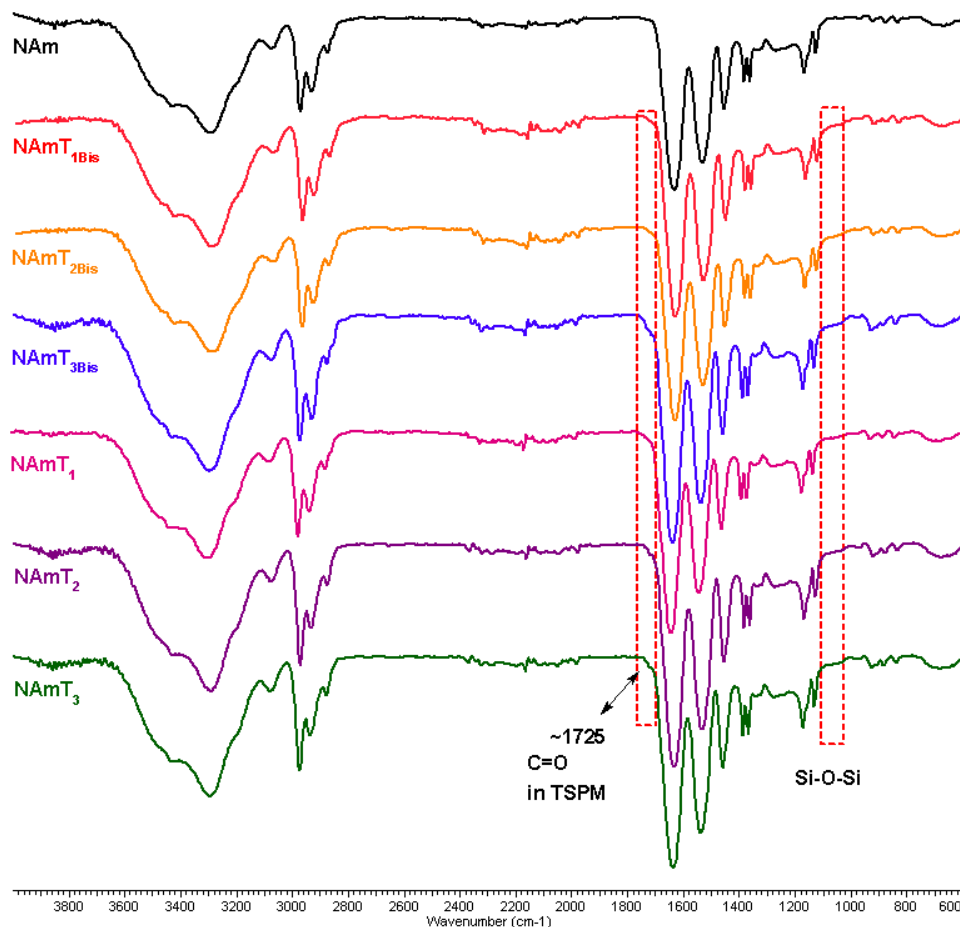

Fig. 6. IR spectra of conventional hydrogel (NAm), double cross-linked (NAmT$_{xBis}$) and single cross-linked hydrogels with TSPM (NAmT$_x$).

As shown in Figure 7A, under the VPTT, all hybrid hydrogels have lower swelling ratios than those of conventional ones. Moreover, swelling ratios decrease with increasing the amount of TSPM in hydrogels, because TSPM act also as a cross-linker. However, the situation changes after ionization (Fig. 7B), when the swelling degree is higher for the hybrid hydrogels, comparatively with the conventional ones. This behavior could be explained by the presence of ionized silanol

groups that can no longer form hydrogen bonds. Moreover, hybrid hydrogels became more hydrophilic after ionization of silanol groups.
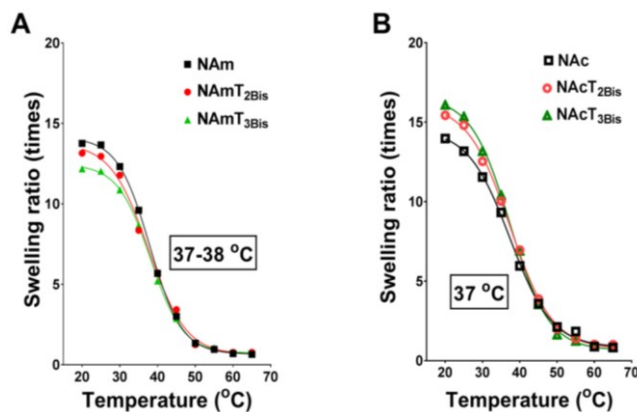

Fig. 7. Swelling ratios of hybrid hydrogels as a function of temperature before (A) and after (B) ionization

3.4.2. Swelling kinetics

Swelling kinetics of hydrogels was investigated in PBS (pH 7.4) in relation with temperature and amount of TSPM in the hydrogel.

Figure 8 illustrates the swelling kinetics of hydrogels before (A) and after (B) ionization. The measurements were performed in PBS at 25°C, under VPTT. Both conventional and hybrid hydrogels displayed low swelling rates; they reach equilibrium after 100h. However, after ionization of the functional groups, the swelling rates increased due to the increased hydrophilicity of hydrogels.
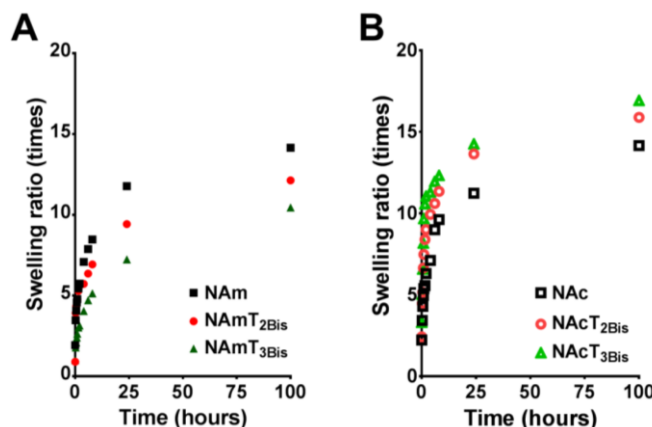

Fig. 8. Swelling kinetic curves of conventional and hybrid hydrogels before (A) and after (B) ionization. The measurements were performed in PBS at pH 7.4 and 25°C.

3.4.3. Deswelling kinetics

The deswelling rate of hydrogels is mainly due to the diffusion of water through the polymer network. Thus, at high hydrogel porosity, water diffusion is rapid and, consequently, the responsivity of hydrogel to temperature is faster.

Figure 9 presents the colapsing kinetics, expressed as a PBS retention function of time, of the conventional and hybrid hydrogels, before (A) and after (B) ionization. One may notice that the collapsing rates are higher in ionized hybrid hydrogels because of the inertial response, when the buffer is ejected during the collapse of hydrogels. This fact could be attributed to the ionization of silanol groups, which makes the hydrogel network more compressible than in unionized hydrogels. In fact, by ionization, the hydrogen bonds are destroyed and the three-dimensional network becomes less rigid. It should be underlined that the deswelling rates are higher than the swelling rates because, during collapsing, water is expelled mechanically through convection, a process much faster than diffusion into the hydrogel.
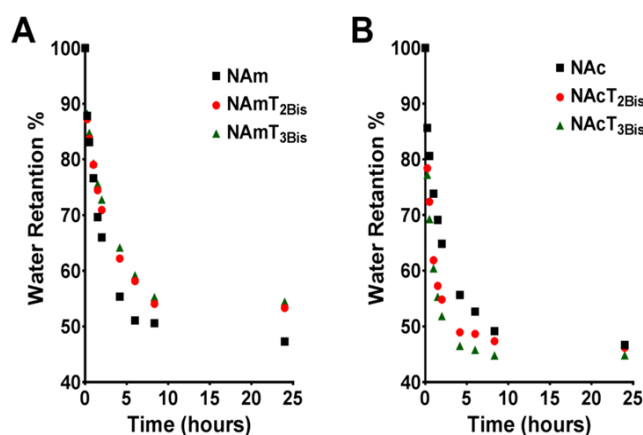

Fig. 9. Deswelling kinetic curves of conventional and hybrid hydrogels before (A) and after (B) ionization. The measurements were performed in PBS at pH 7.4 and 50°C.

3.5. DRUG LOADING AND RELEASE STUDIES

MB was selected as a drug model to evaluate the drug loading/ release properties of hybrid hydrogels. MB is a cationic molecule expected to interact electrostatically with the oppositely charged carboxyl and silanol groups of the ionized hydrogels.

Indeed, as plotted in Figure 10, the amount of MB retained in the hydrogel increased with increasing the amount of TSPM in *hydrogel*. Moreover, after ionization of the functional groups, the amount of retained MB increased significantly.
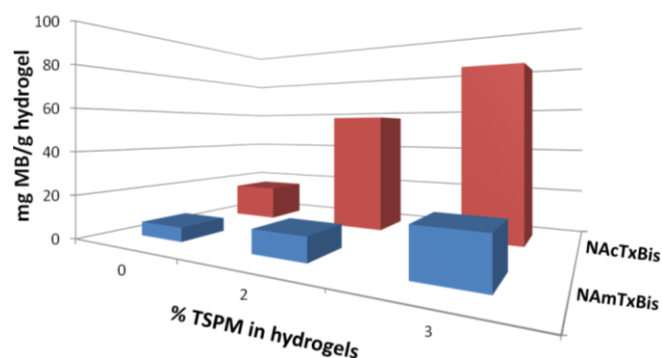
Fig. 10. Graphical representation of MB retention in hybrid hydrogel before ($NAmT_{xBis}$)
and after ($NAcT_{xBis}$) ionization.

The release kinetics of MB from hydrogels was studied under simulated physiological conditions (PBS at pH 7.4 and 37°C) (Fig. 11). It is expected that the release profiles of MB into the release medium should be controlled by the steric interactions between the drug and the polymer network, as well as by the electrostatic interactions between the oppositely charged molecules. The release kinetics was expressed first as the amount of MB (in mg) released from 1 g of hydrogel (Fig.11A) in order to show that the ionized hybrid hydrogel released eight times more drug than the conventional one and four times more than non-ionized hybrid hydrogels. Secondly, the release profile was expressed as percent of MB released from the total amount of entrapped drug (Fig. 11B). It is absolutely clear that ionized hybrid hydrogels exhibit a delayed release pattern, comparatively with the fast release from the conventional hydrogel. This behavior can be attributed both to the electrostatic interactions between the drug and the ionized groups from the polymer network, and to the physical retention of the drug.
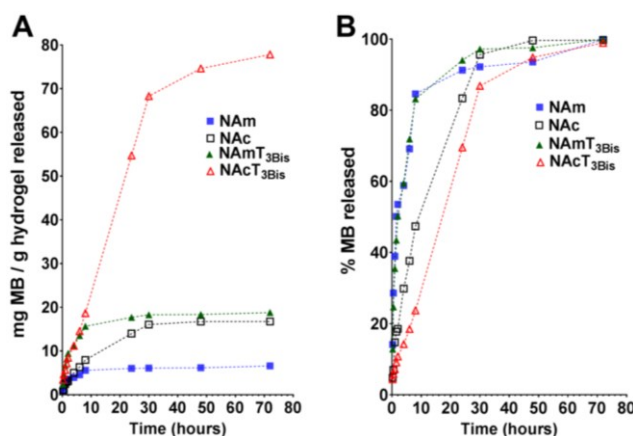

Fig. 11. Release kinetics expressed as the amount (mg) of MB from 1g hydrogels (A)
and percent of MB (B) in PBS (pH = 7.4) at 37°C. For comparative purposes, the release profile
of MB from the conventional hydrogel was also represented before and after ionization.

4. CONCLUSIONS

Thermoresponsive hybrid hydrogels based on poly(NIPAAm-*co*-AAm-*co*-TSPM) were successfully prepared by free-radical polymerization of NIPAAm, AAm, TSPM, and a suitable cross-linker (Bis). After the treatment with NaOH, a ionized hybrid hydrogel was formed, which was able to retain a large amount of MB. SEM micrographs of dried hydrogels showed that the size of pores was controlled by the amount of TSPM. EDX analyses indicated the presence of silica in hybrid hydrogels. Ionized hybrid hydrogels displayed a controllable swelling/deswelling process. The ionized hybrid hydrogels presented delayed release profiles compared with conventional hydrogels, which makes them suitable as support for controlled drug delivery.

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