Synthesis and characterization of poly(N-isopropylmethacrylamide-co-N-isopropylacrylamide) copolymers

Maja Z. Urošević[1], Ljubiša B. Nikolić[1], Snežana Ilić-Stojanović[1], Aleksandar Zdravković[2] and Vesna D. Nikolić[1]

[1]Faculty of Technology, University of Niš, Bulevar Oslobođenja 124, 16000 Leskovac, Serbia
[2]Vocational High School for Technology and Art, Vilema Pušmana 17, 16000 Leskovac, Serbia

Abstract

Copolymeric hydrogels of poly(N-isopropylmethacrylamide-co-N-isopropylacrylamide), p(NIPMAM/NIPAM), are synthesized by radical polymerization of N-isopropylmethacrylamide (NIPMAM) and N-isopropylacrylamide (NIPAM) monomers by using the cross-linker ethylen glycol dimethacrylate (EGDM). The synthesized copolymeric p(NIPMAM/NIPAM) hydrogels, starting monomers and the cross-linker were structurally characterized by using Fourier transform infrared spectroscopy (FTIR). The amounts of residual reactants in the synthesized hydrogels were determined by high-pressure liquid chromatography (HPLC). Swelling of p(NIPMAM/NIPAM) hydrogels was investigated in relation to the temperature and pH value of the solution. The obtained values of residual monomer quantities are within acceptable limits and in the range from 2.69 to 5.25 mg g^{-1} for NIPMAM and 14.55 to 30.80 mg g^{-1} for NIPAM. The synthesized p(NIPMAM/NIPAM) hydrogels are negatively thermosensitive. The most common mechanisms of transport of a swelling solution in p(NIPMAM/NIPAM) hydrogels are polymer chain relaxation, (Case III), and the anomalous type of diffusion (non-Fickian diffusion). The maximal equilibrium swelling degree of 51.19 was reached by the p(NIPMAM/NIPAM) hydrogel with 1.5 mol% of EGDM at the temperature of 25 °C and pH 4, whereas the lowest one of 0.98 was exhibited by the hydrogel with 3 mol% of EGDM at the temperature of 80 °C and pH 7. Due to their low content of residual reactants and a satisfactory degree of swelling at various pH values, synthesized p(NIPMAM/NIPAM) hydrogels can be applied as carriers for the controlled release of pharmaceutically active substances.

Keywords: hydrogel; swelling; Fourier transform infrared spectroscopy FTIR; high-pressure liquid chromatography HPLC

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1. INTRODUCTION

Hydrogels are chemically or physically cross-linked hydrophilic polymer networks capable of absorbing large amounts of water or biological fluids [1-3]. The polymers which constitute three-dimensional hydrogel networks undergo significant physical and chemical changes with slight oscillations in the external environment (temperature, pH value, light, magnetic field *etc.)* [4-8]. The resulting changes are reversible, i.e. hydrogels return to their original state after removing the environmental stimuli [7-9]. Due to this feature, certain hydrogels are known as 'smart' materials [7,10,11]. By selecting appropriate monomers and through adequate synthesis, the hydrogel structure can be designed for a specific purpose [11,12]. One of the most important characteristics of hydrogels is swelling [13]. The process of swelling consists of the diffusion of water molecules or some other solvent through the polymer matrix, followed by polymer chain relaxation due to hydration and polymer matrix expansion after relaxation [14,15]. Swelling of hydrogels is influenced by the hydrogel composition, polymer and solvent compatibility, a degree of cross-linking, temperature

Corresponding author: Maja Z. Urošević, Faculty of Technology, University of Niš, Bulevar Oslobođenja 124, 16000 Leskovac, Serbia
E-mail: maja@tf.ni.ac.rs
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and ionization of the pendant groups [12,16-18]. Two mechanisms of swelling occur during the initial phase of swelling: Fickian diffusion and non-Fickian diffusion [19]. Equation 1 is applied to analyze the nature of the diffusion process for the solvent within hydrogels [20-22].

$$F = \frac{M_t}{M_e} = kt^n \quad (1)$$

For thin plate geometry, the Eq. (1) applies for only up to 60% of the hydrogel swelling process ($0<M_t/M_e<0.6$), whereas the logarithmic form of this equation is:

$$\ln F = \ln (M_t / M_e) = \ln k + n \ln t \quad (2)$$

where F is the fractional sorption, M_t is the mass of the absorbed solvent at the time t, M_e is the mass of the absorbed solvent at the equilibrium state, k is the constant characteristic for the specific type of the polymer network and n is the diffusion exponent.

The most commonly used method for determining the solvent molecule diffusion coefficient in a hydrogel (D) assumes thin plate geometry, taking into account only the initial phase of swelling (up to 60 % swelling) during which the thickness of the sample basically remains constant [19]. For the Fickian diffusion, the constant k is then related to the diffusion coefficient so that Eq. (1) becomes:

$$\frac{M_t}{M_e} = 4\left(\frac{Dt}{\pi l^2}\right)^{1/2} \quad (3)$$

where l is the thickness of the sample (*i.e.* dry hydrogel). By plotting (M_t/M_e) *vs*. $t^{0.5}$, D can be calculated from the slope of the best linear fit. Table 1 shows the values of diffusion exponent n, which determine the mechanism of water or solvent diffusion [23-26].

Table 1. Mechanism of solvent diffusion into the polymer matrix in relation to the value of the diffusion exponent, n

Value of the diffusion exponent	Type of the solvent transport into the polymer matrix
$n < 0.5$	Solvent penetration is considerably slower than the relaxation of polymer chains. The mechanism of the solvent transport belongs to Fickian diffusion and is called “Less Fickian” diffusion.
$n = 0.5$	The solvent transport corresponds to the Fickian diffusion mechanism (Case I). Diffusion rate is much lower than the polymer chain relaxation rate.
$0.5 < n < 1$	The anomalous diffusion mechanism (non-Fickian diffusion) occurs when hydrogel swelling is controlled both by diffusion of solvent into the matrix and the polymer chain relaxation.
$n = 1$	The solvent diffusion process is much faster than the relaxation of polymer chains (Type II, Case II).
$n > 1$	The polymer chain relaxation controls swelling, Type III (Case III, Super Case II).

Thermosensitive hydrogels contain polymers whose interactions with water molecules can be controlled by the change in temperature [27-29]. In negatively thermosensitive hydrogels, the polymer swells at the temperatures lower than the phase transition temperature, while it contracts with the rise in temperature above the phase transition temperature [27,30]. Poly(*N*-isopropylmethacrylamide), p(NIPMAM), and poly(*N*-isopropylacrylamide), p(NIPAM), are representatives of negatively thermosensitive hydrogels [31]. The aqueous solution of p(NIPAM) exhibits the lower critical solution temperature (LCST) of about 32 °C [32], whereas this value for the aqueous solution of p(NIPMAM) is in the range of 38-42 °C [33]. Properties of thermosensitive hydrogels can be modified by incorporation of hydrophilic or hydrophobic comonomers [34]. In the available literature, the information about a variety of synthesized copolymers, microgels and nanogels based on *N*-isopropylmethacrylamide (NIPMAM) and *N*-isopropylacrylamide (NIPAM) can be found [35-38]. The p(NIPAM/NIPMAM) copolymer in the monomer molar ratio of 51:49 exhibits a LCST close to the human body temperature (36.8 °C), whereas microgels of this copolymer in the same monomer ratio exhibit a volume phase transition temperature (VPTT) slightly lower than the LCST [35]. p(NIPMAM/NIPAM) microgels were synthesized

by radical polymerization of NIPAM and NIPMAM monomers in the presence of the *N*,*N*'-methylenebisacrylamide cross-linker in the mixture of methanol/water, 1/1 v/v, at room temperature. The volume swelling degree of the microgels was ≈28 [35]. P(NIPAM/NIPMAM) nano-sized gels were also synthesized by precipitation polymerization at the elevated temperature (70 °C) and using the *N*,*N*'-methylenebisacrylamide cross-linker and the initiator ammonium persulfate [36]. Copolymer hydrogels of p(NIPAM/NIPMAM) are used for the controlled release of a drug [35] and the immobilization of an enzyme [39]. In the paper by Naseem *et al*, poly(*N*-isopropylmethacrylamide-*co*-acrylic acid), p(NIPMAM/AA) microgels were used as adsorbents for the removal of cationic and anionic dyes from aqueous media [40]. p(NIPMAM/AA) microgels were shown to be efficient adsorbents of cationic dyes (methylene blue and Rhodamine B) owing to the presence of negatively charged carboxylic groups at high pH and a low temperature. However, they have also exhibited a large capacity for removing anionic Congo red dye due to high hydrophobicity of microgel particles at low pH values and high temperatures [40].

The aim of this paper is the synthesis and characterization of p(NIPMAM/NIPAM) hydrogels as potential drug carriers and the investigation of the influence of the environmental pH value and the temperature on hydrogel swelling properties.

2. EXPERIMENTAL

2. 1. Reagents

The *N*-isopropylmethacrylamide monomer (NIPMAM, 97 % purity degree, Acros Organics, New Jersey, USA), *N*-isopropylacrylamide comonomer (NIPAM, 99 % purity degree, Acros Organics, New Jersey, USA) and ethylene glycol dimethacrylate cross-linker (EGDM, 97 % purity degree, Fluka Chemical Corp, CH) were used for the synthesis of hydrogels. The initiation of the polymerization reaction was conducted with 2,2'-Azobis(2-methylpropionitrile), AIBN (98% purity degree, Acros Organics, New Jersey, USA). Ethanol (99.5 % purity degree, Merck KGaA, Darmstadt, DE) and methanol (99.9 % HPLC purity, Merck KGaA, Darmstadt, DE) were used as solvents. Monomers, cross-linker and initiator were used without previous purification.

2. 2. Synthesis of hydrogels

Copolymeric hydrogels poly(*N*-isopropylmethacrylamide-*co*-*N*-isopropylacrylamide), p(NIPMAM/NIPAM) were synthesized by radical polymerization of NIPMAM and NIPAM monomers in the molar ratio 40/60 with 1.5, 2 and 3 mol% EGDM cross-linker. Sample labels and hydrogel compositions are shown in Table 2. The initiator AIBN was used in the concentration of 2.8 mol% for initiating the polymerization reaction.

Table 2. Sample labels and hydrogel compositions

	Concentration, mol%*		
Sample label	NIPMAM*	NIPAM*	EGDM**
p(NIPMAM/NIPAM) 40/60/1.5	40	60	1.5
(NIPMAM/NIPAM) 40/60/2	40	60	2
(NIPMAM/NIPAM) 40/60/3	40	60	3

*molar percent in relation to the NIPMAM-NIPAM mixture;
**molar percent of the cross-linker in relation to the overall monomer quantity

Measured amounts of reactants were dissolved in ethanol in order to obtain homogenous mixtures. The concentrations of reactants used in the reaction of polymerization are listed in Table 3.

Glass tubes sealed after injecting homogenous mixtures of the samples were used for performing the polymerization reaction. Thermal initiation of the polymerization reaction for all three samples was conducted at 70 °C for 30 min, at 80 °C for 120 min and at 85 °C for 30 min. Schematic representation of the reaction mixture and a possible structure of the obtained p(NIPMAM/NIPAM) hydrogels is shown in Figure 1.

Table 3. Concentrations of reactants used in the synthesis of p(NIPMAM/NIPAM) hydrogels

	Concentration, mol dm^{-3}		
	p(NIPMAM/NIPAM) 40/60/1.5	(NIPMAM/NIPAM) 40/60/2	(NIPMAM/NIPAM) 40/60/3
NIPMAM	1.30	1.30	1.30
NIPAM	1.95	1.95	1.95
AIBN	0.09	0.09	0.09
EGDM	0.048	0.065	0.097

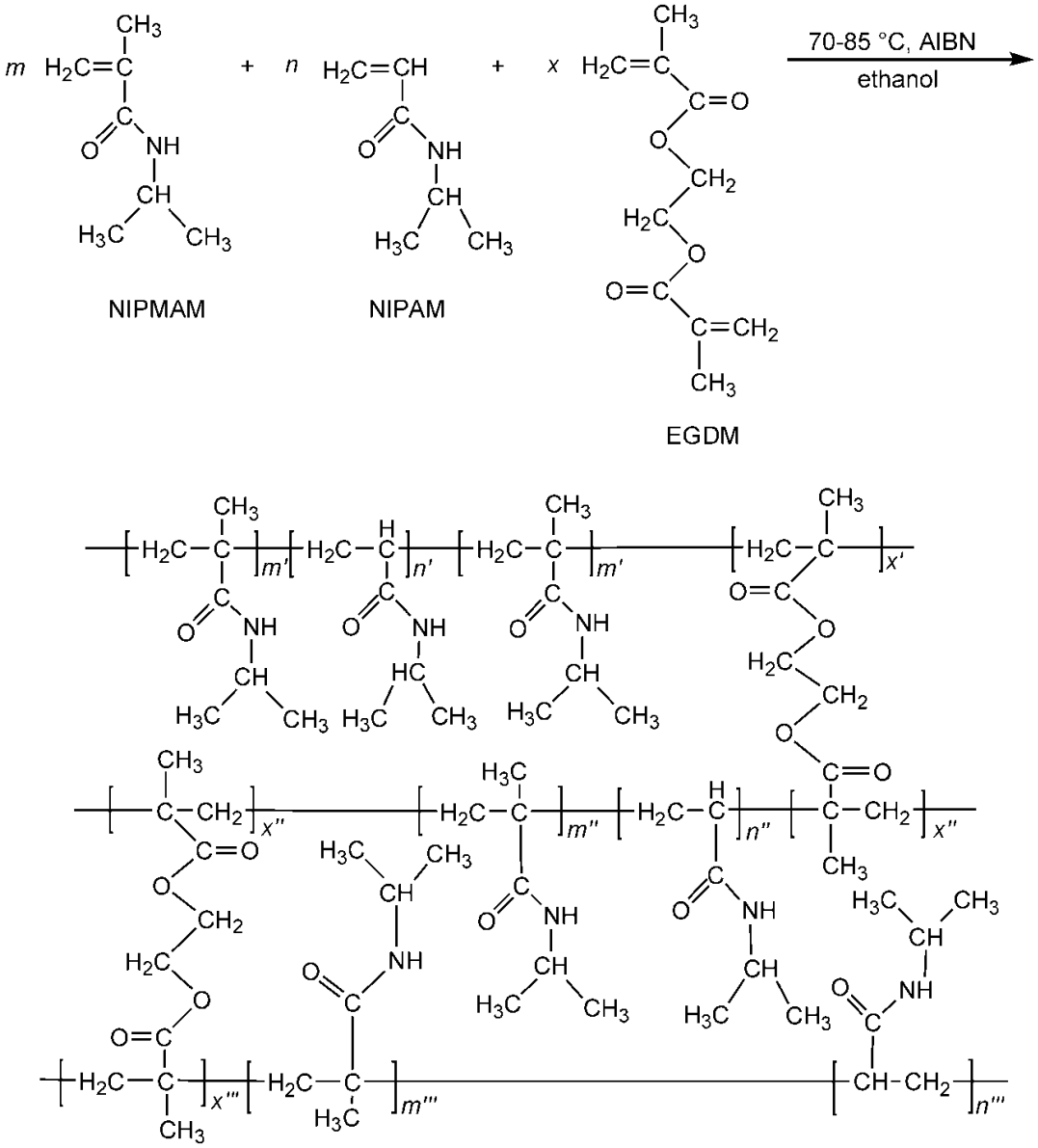

Figure 1. Schematic representation of the synthesis and a possible structure of p(NIPMAM/NIPAM) hydrogels

The p(NIPMAM/NIPAM) hydrogels were cooled after the synthesis, and the obtained cylinders were taken out from the glass tubes. In order to remove residual reactants, hydrogels were treated with methanol for 168 h. Upon the extraction with methanol, hydrogels were dried in a drying oven at the temperature of 45 °C to the constant mass.

2. 3. Analysis of residual reactants

The contents of residual reactants in the synthesized samples of p(NIPMAM/NIPAM) hydrogels were determined by applying the high-pressure liquid chromatography (HPLC) method. The methanol extracts were filtered through a

cellulous membrane filter (0.45 μm pore diameter) and analyzed on the apparatus HPLC Agilent 1100 Series with a diode-array detector, DAD 1200 Series (Agilent Technologies, Santa Clara, USA). The column ZORBAX Eclipse XDB-C18 (4.6×250 mm, 5 μm, Agilent Technologies, Santa Clara, USA) was set at the temperature of 25 °C. The injected volume of the sample for the analysis was 10 μl. The detection was conducted at the wavelength of 210 nm. The mixture of methanol/redistilled water in the ratio 70/30 v/v was used as a mobile phase, at the flow rate of 0.5 cm^3 min^{-1}. Calibration curves for determining the contents of monomers and the cross-linker were constructed by preparing a series of solutions with known concentrations.

2. 4. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) spectra of synthesized p(NIPMAM/NIPAM) hydrogel samples and starting monomers were recorded by using thin transparent tablets with potassium bromide of spectroscopic purity, which were vacuumed and pressed under the pressure of about 200 MPa. In order to make tablets, 150 mg of KBr and 0.7 mg of the samples were measured, then ground to powder in an amalgamator (WIG-L-BVG, 31210-3A, USA). The cross-linker was recorded in the shape of a thin film between two plates of zinc selenide (ZnSe). The measurements were conducted within the wavenumber range from 4000 to 400 cm^{-1} on the FTIR Bomem Hartmann & Braun MB-series spectrophotometer (Hartmann & Braun, Baptiste, Canada). The spectra were processed by using Win-Bomem Easy software.

2.5. Swelling behavior

Swelling of synthesized p(NIPMAM/NIPAM) xerogels (5 mm in diameter and 2 mm thick discs) was monitored gravimetrically. A specific amount of a p(NIPMAM/NIPAM) xerogel was immersed in a solution of a specific pH value, and the sample mass was measured at specific time intervals until the equilibrium was reached, i.e. constant mass of the hydrogel. The swelling degree, α, was calculated by the following equation:

$$\alpha = \frac{m - m_0}{m_0} \tag{4}$$

where m_0 is the xerogel mass, and m is the mass of the swollen hydrogel at the time t.

The swelling studies were performed in solutions of pH values of 4, 7 and 8 and at the temperatures of 25, 37, 60 and 80 °C. The aqueous media for swelling were prepared by adjusting the pH value by adding 0.1 M solution of sodium hydroxide (Centrohem, Belgrade, Serbia) or 0.1 M solution of hydrochloric acid (Zorka, Šabac, Serbia). pH values were measured by a pH meter (HI9318-HI9219, Hanna, Portugal). Thermosensitivity of hydrogels was tested within the temperature range from 25 to 80 °C in a water bath (Sutjeska, Belgrade, Serbia). For the determination of swelling degrees, three measurements per sample were performed.

3. RESULTS AND DISCUSSION

Figure 2 shows FTIR spectra of NIPMAM and NIPAM monomers, EGDM and a p(NIPMAM/NIPAM) copolymer sample 40/60/3.

In the FTIR spectrum of the NIPMAM monomer (Fig. 2a), an absorption band appears at 3291 cm^{-1} as a result of valence N-H vibrations, ν(N-H). The asymmetric valence vibrations of C-H bond from the vinyl group, ν_{as}(=C-H), produce a band with the maximum at 3061 cm^{-1}, whereas deformation vibrations in the plane give an absorption band with the maximum at 1296 cm^{-1}, δ(=C-H). Absorption bands with the maxima at 2973 and 2878 cm^{-1} originate from asymmetric and symmetric valence vibrations of methyl groups within the monomer structure, respectively. Evidence of the presence of an amide group within the monomer structure is provided by the absorption bands with the maxima at 1653 cm^{-1} (amide band I) and 1539 cm^{-1} (amide band II). The amide band I is assigned to valence vibrations of the keto group, whereas the amide band II is formed by coupling N-H deformation vibrations and valence C-N vibrations. Valence vibrations of the C=C bond in the FTIR spectrum of the NIPMAM monomer (Fig. 2a) produce an absorption band with the maximum at 1606 cm^{-1}. The absorption band of asymmetric deformation C-H vibrations in the plane of CH_3-C group

appears at 1459 cm^{-1}. Consequence of C-H bond vibrations from the isopropyl group is an absorption band with the maximum at 1363 cm^{-1}. The existence of the isopropyl group is indicated by the bands at 1157 and 1011 cm^{-1}.

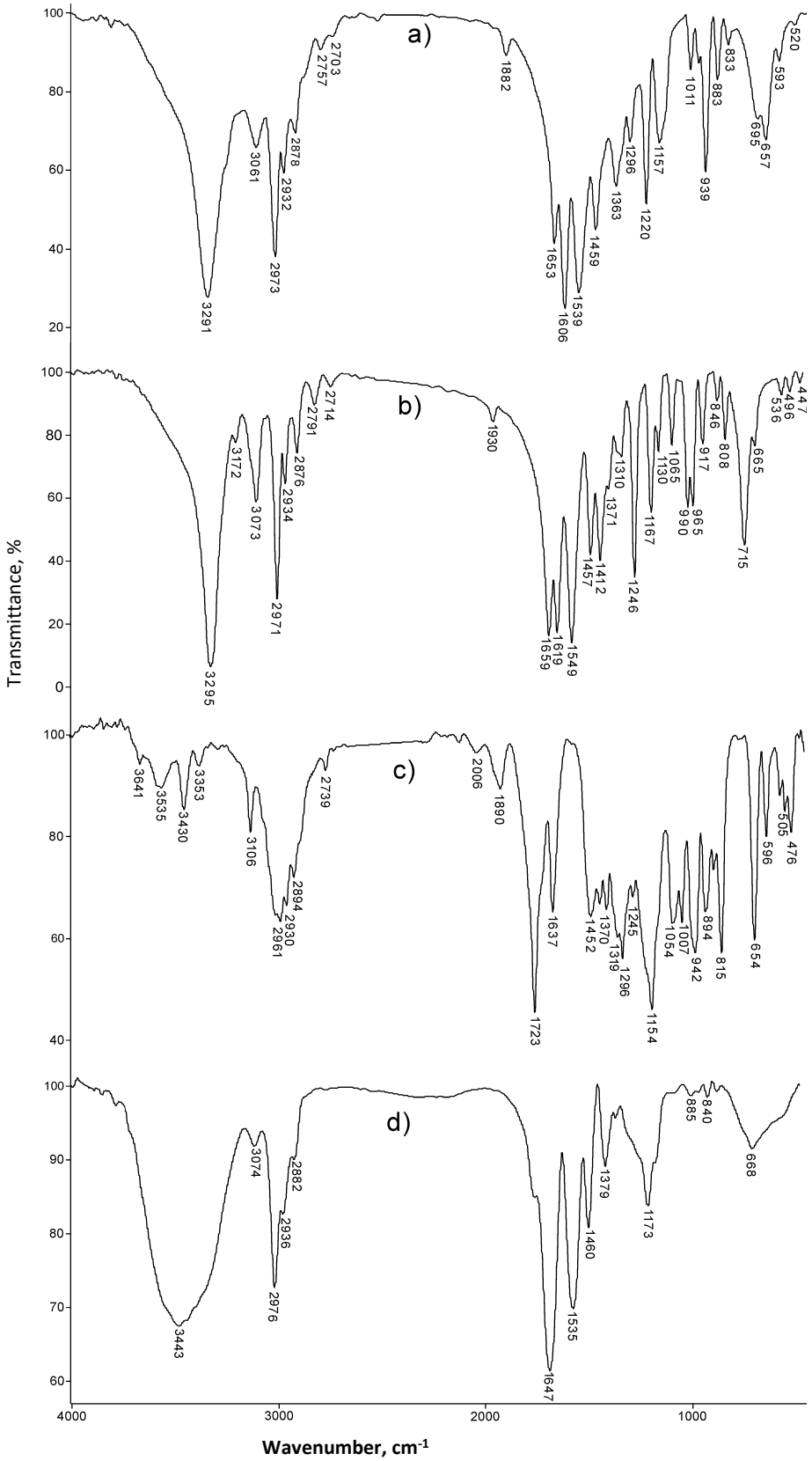

Figure 2. FTIR spectra of NIPMAM (a), NIPAM (b), EGDM (c) and p(NIPMAM/NIPAM) sample 40/60/3 (d)

In the range of wavenumbers over 3000 cm^{-1} in the FTIR spectrum of the NIPAM monomer (Fig. 2b), the characteristic absorption band of strong intensity is at the maximum of 3295 cm^{-1}, which is assigned to valence vibrations of the secondary amino-group, ν(N-H) [41] and the absorption band at 3073 cm^{-1}, which is a result of asymmetric vibrations of the vinyl group, ν_{as}(=C-H). Absorption bands with the maxima at 2971 cm^{-1} and 2876 cm^{-1} in the NIPAM FTIR spectrum are the result of asymmetric and symmetric valence vibrations of the C-H bond in the methyl group, respectively [42]. A medium intensity absorption band with the maximum at 2934 cm^{-1} originates from asymmetric valence vibrations of the C-H bond in the NIPAM isopropyl group, ν_{as}(C-H). The amide bands I, II and III with the maxima at 1659, 1549 cm^{-1} [43,44], and 1310 cm^{-1}, respectively, confirm the presence of the amide group in the NIPAM molecule. The result of C=C bond vibrations is a strong intensity absorption band with the maximum at 1619 cm^{-1} [45]. A medium intensity absorption band at 1371 cm^{-1} corresponds to deformation vibrations in the plane of C-H bond from the NIPAM isopropyl group, δ(C-H) [42]. Evidence of the existence of an isopropyl group in the structure of NIPAM is a strong intensity absorption band with two maxima at 1167 and 1130 cm^{-1}. Deformation vibrations in the plane of the vinyl group, δ(=C-H), produce a band at 1412 cm^{-1} [46], whereas deformations out of the plane, γ(=C-H), produce bands at 990 and 917 cm^{-1} [44].

In the EGDM FTIR spectrum (Fig. 2c), there are bands characteristic for ester and vinyl functional groups. The characteristic absorption band with the maximum at 1723 cm^{-1} in the EGDM FTIR spectrum is assigned to C=O valence vibrations, ν(C=O). Valence vibrations of C-O bond produce a band with the absorption maximum at 1154 cm^{-1}. The absorption band with the maximum at 1636 cm^{-1} originates from the absorption of the C=C bond. In the EGDM FTIR spectrum (Fig. 2c), there are also bands with the maxima at 2894 cm^{-1} originating from $\nu_s(CH_3)$, at 2960 cm^{-1} from $\nu_{as}(CH_3)$, at 2930 cm^{-1} from $\nu_{as}(CH_2)$ and at 3105 cm^{-1} originating from the vinyl group ν_{as}(=CH).

In the FTIR spectrum of the p(NIPMAM/NIPAM) copolymer sample (Fig. 2d), certain characteristic absorption bands which are present in the spectra of NIPMAM and NIPAM monomers are absent, thus indicating the formation of a new structure. Bonding of monomers in the polymerization reaction was made by breaking C=C bonds, which is indicated by the absence of bands originating from valence vibrations of C=C bonds, ν(C=C) which appear in the range of wavenumbers from 1600-1640 cm^{-1}, as well as deformation vibrations in the plane of the vinyl group δ(=C-H) in the range of wavenumbers 1290-1300 cm^{-1} and 1410-1420 cm^{-1}. A low intensity absorption band with the maximum at 3074 cm^{-1} is probably the result of C-H valence vibrations of the EGDM vinyl group, which can indicate the existence of dangling chains in the structure of the synthesized p(NIPMAM/NIPAM) copolymer. A wide band within the range from 3100 to 3700 cm^{-1}, with the maximum at 3443 cm^{-1} originates from valence vibrations of N-H groups in both monomers, ν(N-H). The centroid of this band is shifted to higher wavenumbers in relation to the position of the same band in the monomer FTIR spectra (Figs 2a and b). The width of this absorption band in the copolymer FTIR spectrum indicates the formation of intramolecular hydrogen bonds between chains in the hydrogel via -NH group as the proton donor. A strong intensity amide band I appears at 1647 cm^{-1} and is shifted by 6 and 12 units towards lower wavenumbers in relation to the position of the same band in the NIPMAM and NIPAM FTIR spectra, respectively. The amide band II appears at 1535 cm^{-1} (Fig. 2d), with the maximum shifted by 4 and 14 units towards lower wavenumbers in relation to the position of the same band in the NIPMAM (Fig. 2a) and NIPAM FTIR spectra, respectively (Figs 2a and b). Shifting of these maxima towards lower wavenumbers indicates that N-H and C=O groups of NIPMAM and NIPAM monomers participate in formation of the hydrogen bond.

In the polymerization process, complete conversion of reactants into the polymer is not achieved and hence there are some amounts of unreacted reactants in the polymer product. The presence of residual reactants in the polymer may affect the properties of the related polymer. However, a much more serious hazard comes from the fact that residual monomers are toxic for the operators in the manufacturing process and for the consumers of polymer products. The liquid and gas chromatography (HPLC and GC) methods are used to analyze residual reactants in specific extracts. The aim of the polymer industry is to reduce the content of residual reactants in the process of the polymer production to the minimum [47- 52].

The HPLC method was used to analyze methanol extracts of the obtained copolymer in order to determine the amounts of residual unreacted monomers and the cross-linker. To conduct the investigation, a detection wavelength of

210 nm was used. Under the selected chromatographic conditions, retention times of 6.693, 6.176 and 12.812 min correspond to the NIPMAM, NIPAM and EGDM, respectively.

Figure 3 shows the HPLC chromatograms of NIPMAM and NIPAM monomers and EGDM cross-linker.

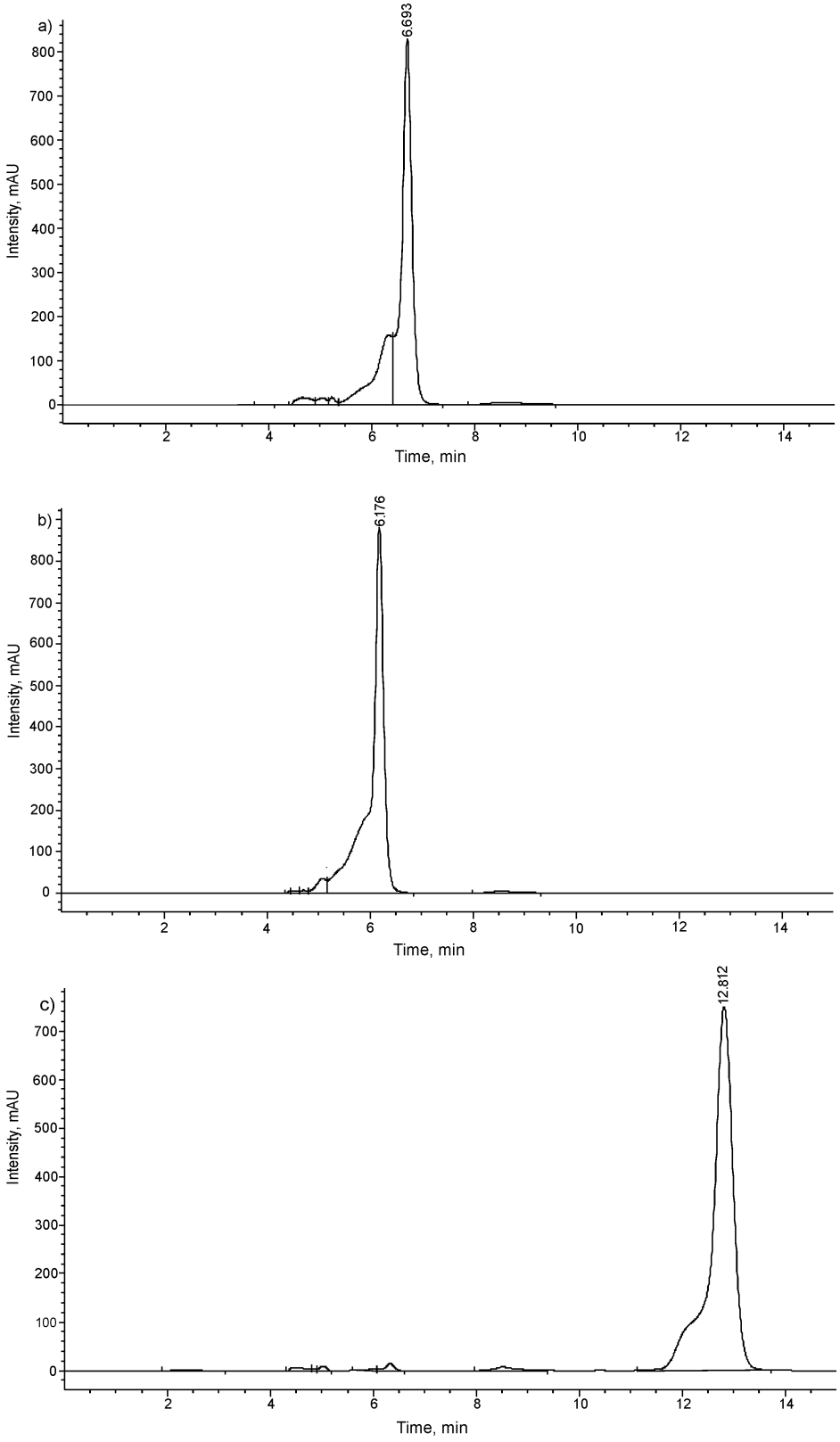

Figure 3. HPLC chromatograms of NIPMAM (a), NIPAM (b) and EGDM (c)

The unreacted amounts of NIPMAM and NIPAM in the course of polymerization in relation to the total mass of synthesized p(NIPMAM/NIPAM) xerogels are presented in Table 4.

Table 4. Masses of unreacted NIPMAM and NIPAM monomers in relation to the total weight of p(NIPMAM/NIPAM) xerogels

Xerogel sample	Concentration of monomer, mg g^{-1}	
p(NIPMAM/NIPAM)	NIPMAM	NIPAM
40/60/1.5	5.50	30.80
40/60/2	5.25	22.58
40/60/3	2.69	14.55

The EGDM cross-linker was not detected in the methanol extracts, thus indicating that the total added EGDM amount reacted in the process of p(NIPMAM/NIPAM) hydrogel synthesis. The obtained values of the amount of residual monomers range from 2.69 to 5.25 mg g^{-1} for NIPMAM and 14.55 to 30.80 mg g^{-1} for NIPAM, calculated per the mass of p(NIPMAM/NIPAM) xerogels. These amounts of residual monomers are within acceptable limits, because monomers are toxic in much higher amounts [53]. Residual reactants may be removed from the hydrogel by evaporation or extraction with suitable solvents (water, methanol and acetone). The change of the solvent in which hydrogels were washed was reported to contributes to the decrease in the amounts of reactants in a hydrogel [51].

Figure 4 shows the dependence of the swelling degree of a p(NIPMAM/NIPAM) hydrogel on time in solutions with different pH values (4, 7 and 8) at 25 °C.

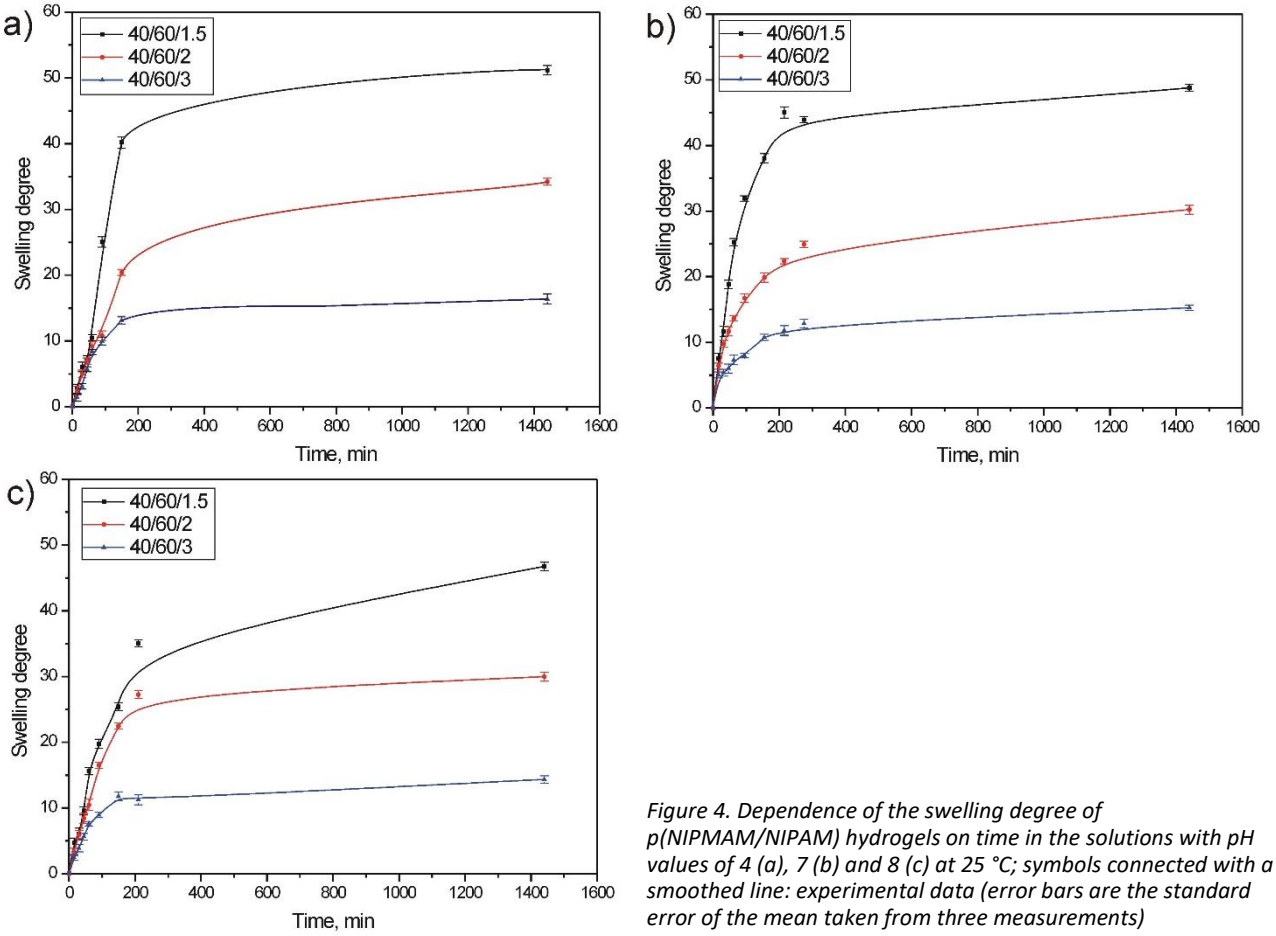

Figure 4. Dependence of the swelling degree of p(NIPMAM/NIPAM) hydrogels on time in the solutions with pH values of 4 (a), 7 (b) and 8 (c) at 25 °C; symbols connected with a smoothed line: experimental data (error bars are the standard error of the mean taken from three measurements)

In mildly acidic environment (pH 4), hydrogels exhibited slightly higher swelling degrees as compared to the neutral or alkaline environment. Secondary amino groups of NIPMAM and NIPAM in the acidic environment can exist in the protonated state (NH_2^+), which leads to the electrostatic repulsion of polymer chains and a higher degree of hydrogel

swelling. A pH increase in the solution from 7 to 8 did not significantly affect the swelling degree of p(NIPMAM/NIPAM) hydrogels. Synthesized p(NIPMAM/NIPAM) hydrogels did not react significantly to the changes in the environmental pH value, i.e. they exhibited weak pH sensitivity.

The swelling degree of p(NIPMAM/NIPAM) hydrogels depended on the concentration of the EGDM cross-linker (Fig. 4). The swelling equilibrium degree of hydrogels decreaseds with the increase in the cross-linker concentration, which can be explained by the formation of a denser and a more compact polymer network. At low concentrations of the EGDM cross-linker, the distance between nodes in the polymer network is larger and hence it enables the penetration of larger quantities of the solution, that is, a higher degree of hydrogel swelling.

The p(NIPMAM/NIPAM) hydrogel with 1.5 mol% of EGDM absorbed the largest amount of the solution at the pH value of 4, resulting in the equilibrium swelling degree of α_e = 51.19. It can be noticed that p(NIPMAM/NIPAM) hydrogels reached swelling degrees close to the equilibrium ones within the first 200 min. Copolymeric p(NIPMAM/NIPAM) hydrogels exhibited significantly higher equilibrium swelling degrees (15.25-48.77) at the pH 7 and at 25 °C compared to the swelling degree of the a p(NIPAM) hydrogel ($\alpha_e \approx 3.50$) synthesized in the study of Shekhar and associates [54]. This result is probably due to the presence of the thermosensitive component of NIPMAM in the copolymer structure. The p(NIPAM/NIPMAM) microgels synthesized in the work of Fundenau et al. reached the volume swelling degree of about 23, under simulated physiological conditions (phosphate buffer solution pH 7.4) at 25 °C [35]. The p(NIPMAM/NIPAM) hydrogels synthesized in this work exhibited higher swelling degrees than the p(NIPAM/NIPMAM) microgels and the homopolymer p(NIPAM).

Table 5 presents the values of kinetic parameters for swelling of hydrogel series at various pH values (4, 7 and 8) and at the temperature of 25 °C.

Table 5. Kinetic swelling parameters for p(NIPMAM/NIPAM) hydrogel series in the solutions with pH values of 4, 7 and 8 at 25 °C

pH	Sample	n	$K / 10^3$ min$^{1/n}$	R^2
4	40/60/1.5	1.14	2.27	0.970
	40/60/2	0.88	6.90	0.994
	40/60/3	1.29	2.54	0.998
7	40/60/1.5	0.93	10.60	0.990
	40/60/2	0.55	45.47	0.997
	40/60/3	0.29	137.28	0.956
8	40/60/1.5	0.81	10.14	0.977
	40/60/2	0.88	10.06	0.998
	40/60/3	0.80	19.00	0.993

In the solution with the pH value of 4, the process of p(NIPMAM/NIPAM) hydrogel swelling is controlled by relaxation of polymer chains, $n > 1$ (Case III), except for the case of the hydrogel with 2 mol% of EGDM, where swelling is controlled both by diffusion of the solution into the polymer matrix and the relaxation of polymer chains, non-Fickian diffusion. The transport of the solution in the polymer matrix at the solution pH values of 7 and 8 corresponds to the anomalous diffusion type (non-Fickian diffusion), $0.5<n<1$. For the p(NIPMAM/NIPAM) hydrogel sample 40/60/3 the diffusion exponent value at the pH value of 7 is 0.29, which indicates that hydrogel swelling in this environment is determined by diffusion.

Figure 5 shows a change in the swelling degree of p(NIPMAM/NIPAM) hydrogels with time in the solutions with pH values of 4, 7 and 8 at the temperatures of 37, 60 and 80 °C.

By analyzing the graphs in Figure 5, it can be noticed that the highest swelling degree at all tested pH values and temperatures is exhibited by the p(NIPMAM/NIPAM) hydrogel sample with 1.5 mol% of EGDM. As mentioned above, the cross-linking degree affects the swelling degree of hydrogels and, with the increase in the cross-linker concentration there is a decrease in the equilibrium swelling degree of the hydrogel. The p(NIPMAM/NIPAM) hydrogel sample with 1.5 mol% of EGDM (Fig. 5a) at 37 °C and pH 4 had the highest equilibrium swelling degree (α_e = 3.20), whereas the hydrogel sample with 3 mol% of EGDM at 80 °C and pH = 7 exhibited the lowest equilibrium swelling degree (α_e = 0.98). A change in the pH values slightly affected the equilibrium swelling degree of p(NIPMAM/NIPAM) hydrogels at the tested temperatures. The increase in temperature from 37 to 80 °C did not cause a significant decrease in the swelling degree of p(NIPMAM/NIPAM) hydrogels.

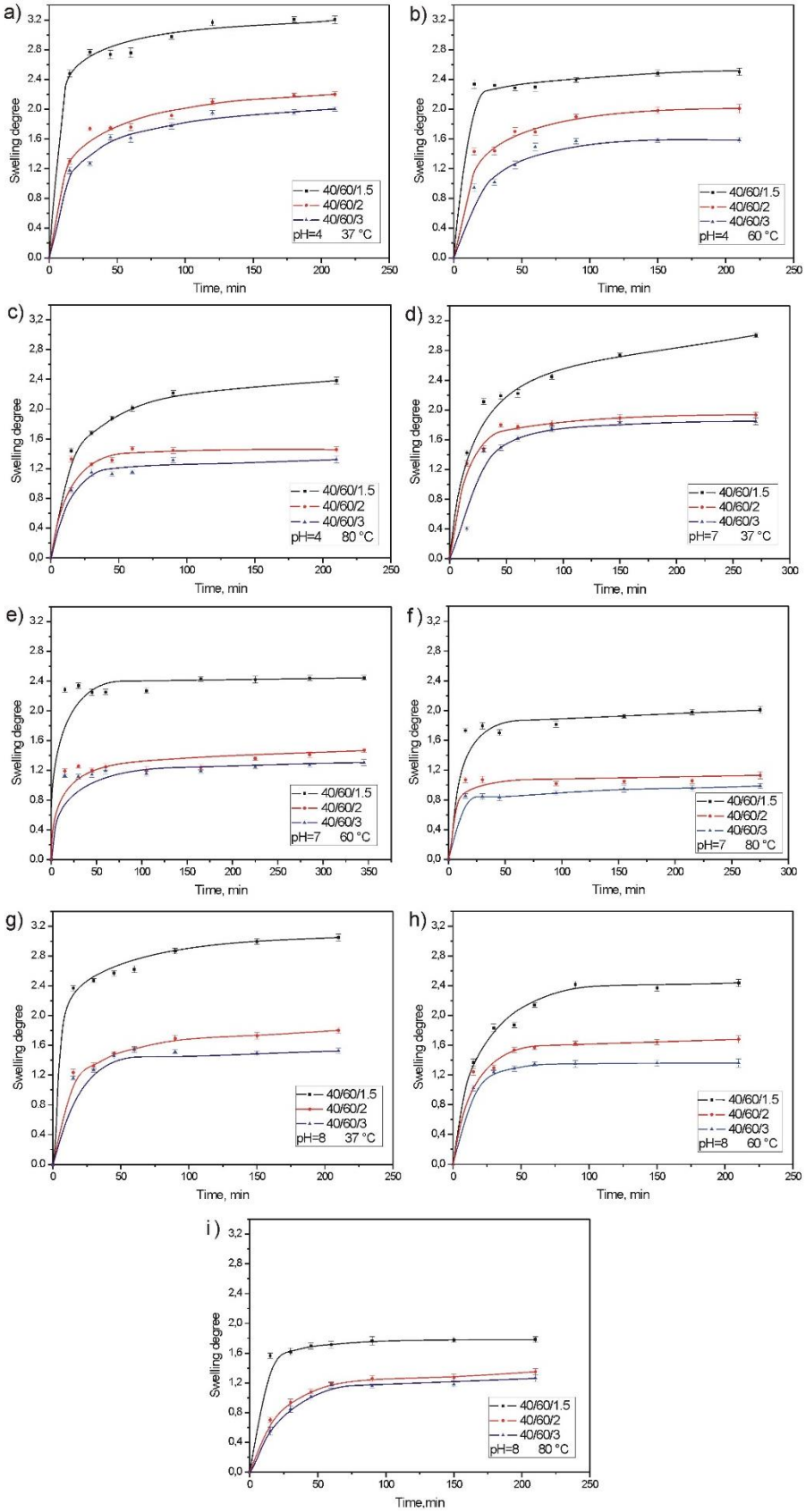

Figure 5. Dependence of the swelling degree of p(NIPMAM/NIPAM) hydrogels on time in the solutions at different pH values and temperatures: pH 4 (37 °C - a, 60 °C - b, 80 °C - c), pH 7 (37 °C - d, 60 °C - e, 80 °C - f) and pH 8 (37 °C - g, 60 °C - h, 80 °C - i); symbols connected with a smoothed line: experimental data (error bars are the standard error of the mean taken from three measurements)

By analyzing the graphs in Figure 5, it can be noticed that the highest swelling degree at all tested pH values and temperatures is exhibited by the p(NIPMAM/NIPAM) hydrogel sample with 1.5 mol% of EGDM. As mentioned above, the cross-linking degree affects the swelling degree of hydrogels and, with the increase in the cross-linker concentration there is a decrease in the equilibrium swelling degree of the hydrogel. The p(NIPMAM/NIPAM) hydrogel sample with 1.5 mol% of EGDM (Fig. 5a) at 37 °C and pH 4 had the highest equilibrium swelling degree (α_e = 3.20), whereas the hydrogel sample with 3 mol% of EGDM at 80 °C and pH 7 exhibited the lowest equilibrium swelling degree (α_e = 0.98). A change in the pH values slightly affected the equilibrium swelling degree of p(NIPMAM/NIPAM) hydrogels at the tested temperatures. The increase in temperature from 37 to 80 °C did not cause a significant decrease in the swelling degree of p(NIPMAM/NIPAM) hydrogels.

Sensitivity of hydrogels to temperature changes from 25 to 80 °C, in the solution of the pH value of 7 is shown in Figure 6.

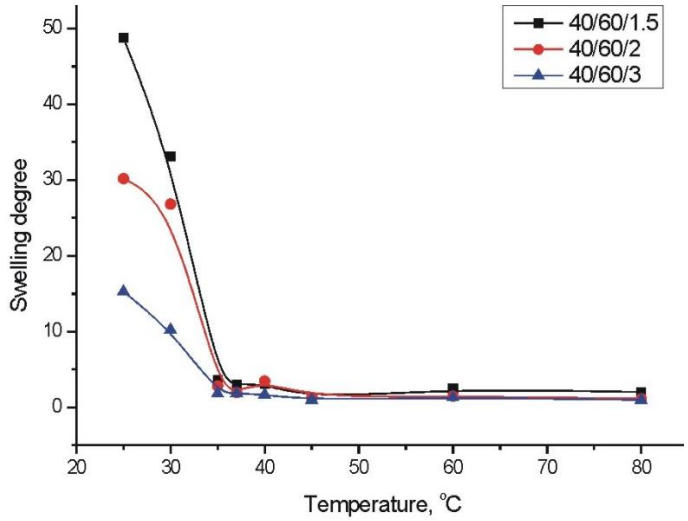

Figure 6. Dependence of the equilibrium swelling degree of p(NIPMAM/NIPAM) hydrogels on temperature in the solution of pH = 7

With the increase in temperature above 25 °C, there is a significant decrease in the equilibrium swelling degree (Fig. 6) and hence we can classify the investigated hydrogels in the negatively temperature-sensitive group. In the range of temperatures from 25 to 35 °C, the effect of cross-linking is more significant, while at higher temperatures this effect is less pronounced. Contraction and elimination of water from the hydrogel structure at temperatures above the phase transition is a consequence of breaking hydrogen bonds with water molecules, whereupon hydrophobic interactions between the groups of polymer networks become more dominant. Temperatures below the VPTT in hydrogels cause hydration of groups, i.e. there are intermolecular hydrogen interactions causing hydrogel swelling [55].

4. CONCLUSION

In this work, p(NIPMAM/NIPAM) hydrogels were synthesized by radical polymerization at the molar ratio of monomers 40/60 with EGDM as a cross-linker in the concentrations of 1.5, 2 and 3 mol%. FTIR analysis has shown the absence of absorption bands corresponding to valence C=C vibrations, as well as deformation vibrations in the plane of vinyl groups in monomers and the cross-linker in the copolymer spectrum indicating completed polymerization by breakage of double bonds. By using the HPLC method, it was determined that the amounts of residual monomers are within the acceptable limits. Swelling of p(NIPMAM/NIPAM) hydrogels depended on temperature and the cross-linker content. The p(NIPMAM/NIPAM) hydrogels are negatively thermosensitive since the hydrogel swelling degree decreased with the increase in temperature. The highest equilibrium swelling degree (α_e = 51.19) was achieved by a p(NIPMAM/NIPAM) hydrogel with 1.5 mol% of EGDM in the solution with a pH value of 4 and at the temperature of 25 °C. The hydrogel swelling degree decreased with the increase in the cross-linker content; hence, p(NIPMAM/NIPAM)

hydrogel with 3 mol% of EGDM at 80 °C in the solution with a pH value of 7 exhibited the lowest equilibrium swelling degree (α_e = 0.98). Due to their low content of residual reactants and a satisfactory degree of swelling at various pH values, synthesized p(NIPMAM/NIPAM) hydrogels can be applied as carriers for the controlled release of pharmaceutically active substances.

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SAŽETAK

Sinteza i karakterizacija kopolimera poli(N-izopropilmetakrilamida-ko-N-izopropilakrilamida)

Maja Z. Urošević[1], Ljubiša B. Nikolić[1], Snežana Ilić-Stojanović[1], Aleksandar Zdravković[2] i Vesna D. Nikolić[1]

[1]Tehnološki fakutet, Univerzitet u Nišu, Bulevar Oslobođenja 124, 16000 Leskovac, Srbija
[2]Visoka tehnološko umetnička strukovna škola, Vilema Pušmana 17, 16000 Leskovac, Srbija

(Naučni rad)

Kopolimerni hidrogelovi poli(N-izopropilmetakrilamida-ko-N-izopropilakrilamida), p(NIPMAM/NIPAM), sintetisani su radikalnom polimerizacijom monomera N-izopropilmetakrilamida (NIPMAM) i N-izopropilakrilamida (NIPAM) primenom umreživača etilenglikoldimetakrilata (EGDM). Sintetisani kopolimerni hidrogelovi p(NIPMAM/NIPAM), polazni monomeri i umreživač strukturno su okarakterisani infracrvenom spektroskopijom sa Furijeovom transformacijom (FTIR). Količine rezidualnih reaktanata u sintetisanim hidrogelovima su određene tečnom hromatografijom pod visokim pritiskom (HPLC). Bubrenje hidrogelova p(NIPMAM/NIPAM) ispitano je u zavisnosti od temperature i pH vrednosti rastvora. Dobijene vrednosti količine rezidualnih monomera nalaze se u prihvatljivim granicama i kreću se od 2,69 do 5,25 mg g^{-1} za NIPMAM i 14,55 do 30,80 mg g^{-1} za NIPAM. Sintetisani hidrogelovi p(NIPMAM/NIPAM) su negativno temperaturno osetljivi. Najzastupljeniji mehanizmi transporta rastvora kod hidrogelova p(NIPMAM/NIPAM) su relaksacija polimernih lanaca, (Slučaj III) i anomalni tip difuzije (ne-Fikova difuzija). Najveći ravnotežni stepen bubrenja dostiže hidrogel p(NIPMAM/NIPAM) sa 1,5 mol% EGDM pri temperaturi 25 °C i pH 4 (α_e = 51,19), a najniži, hidrogel p(NIPMAM/NIPAM) sa 3 mol% EGDM pri temperaturi 80 °C i pH 7 (α_e = 0,98). Sintetisani p(NIPMAM/NIPAM) hidrogelovi zahvaljujući niskom sadržaju rezidualnih reaktanata i zadovoljavajućem stepenu bubrenja u sredini različitih pH vrednosti mogu biti primenjeni kao nosači za kontrolisano otpuštanje farmaceutski aktivnih supstanci.

Ključne reči: hidrogel; bubrenje; infracrvena spektroskopija sa Furijeovom transformacijom FTIR; tečna hromatografija pod visokim pritiskom HPLC