UTICAJ OBLIKA NANOČESTICA SREBRA NA FIZIČKOHEMIJSKA SVOJSTVA POLI(*N*-IZOPROPILAKRILAMID) HIDROGEL NANOKOMPOZITA

THE EFFECT OF SILVER NANOPARTICLE SHAPE ON THE PHYSICOCHEMICAL PROPERTIES OF A POLY (*N*-ISOPROPYLACRYLAMIDE) HYDROGEL NANOCOMPOSITES

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Hidrogelovi su materijali koji poseduju specifičnu poroznost, stabilnost, biokompatibilnost i sposobnost apsorpcije okolne tečnosti, kao i svojstva koja su promenljiva i lako podesiva širokom spektru primena, naročito u oblasti medicine i biotehnologije. Posebno je interesantna oblast "inteligentnih" hidrogelova, odnosno materijala koji reaguju na spoljašnje stimulanse i pokazuju značajanu fizičku ili hemijsku promenu pri malim promenama u okruženju. Poli(N-izopropilakrilamid) (PNiPAAm) je najpoznatiji termosenzitivni polimer sa jasno definisanom temperaturom faznog prelaza (VPTT) od oko 32 °C. S druge strane, nanočestice srebra (AgNPs) poseduju optička, katalitička i električna svojstva koja se mogu modifikovati promenom njihove veličine i oblika a imaju i izražen antibakterijski potencijal, pa su stoga, poslednjih decenija intenzivno proučavane. Pokazano je da se anizotropne nanočestice srebra mogu kontrolisano sintetisati sa fizičkohemijskim svojstvima različitim od sfernih analoga, pri čemu su značajna istraživanja usmerena na njihovu praktičnu primenu. Zbog svega navedenog, u okviru ovog istraživanja biće izvedena jednostavna dvostepena sinteza AgNPs/PNiPAAm hidrogel nanokompozita koja uključuje hemijsko formiranje sfernih i trouglastih AgNPs u prvom koraku, a zatim gama-indukovano umrežavanje NiPAAm-a u prisustvu ovih AgNPs. Tehnika gama zračenja objedinjuje sterilizaciju i sintezu u jednom tehnološkom koraku čime se proces optimizuje i otvara širok spektar inovativnih biomedicinskih primena. Formiranje stabilnih i uniformno distribuiranih AgNPs unutar matrice hidrogela potvrđeno je UV-Vis spektroskopijom, a porozna sunđerasta struktura polimerne mreže SEM analizom. Fizičkohemijska karakterizacija izvršena je i ispitivanjem procesa bubrenja i kontrahovanja u vodi na 25°C i 48°C, redom. Pored toga, pokazano je da prisustvo AgNPs utiče na vrednosti temperature faznog prelaza polimera. Osnovni cilj ovog istraživanja je ispitivanje uticaja različite morfologije AgNPs na fizičkohemijska svojstva sintetisanih nanokompozita, imajući u vidu njihovu potencijalnu primenu i stalnu potrebu za širokom upotrebom biokompatibilnih materijala.

Ključne reči: *Hidrogel nanokompoziti; nanočestice srebra; fazni prelaz; svojstva bubrenja*

Hydrogels have unique properties and many potential applications, particularly in medicine and biotechnology. Gel porosity and swelling properties, stability, and biocompatibility are characteristics that are widely variable and easily adjusted. Stimuli-responsive or intelligent hydrogels are a class of these materials that shows a significant response to small changes in the surrounding environment. Poly(N-isopropylacrylamide) (PNiPAAm) is the best-known thermosensitive polymer with a well-defined volume phase transition temperature (VPTT) around 32 °C. On the other hand, silver nanoparticles (AgNPs) have been the subject of intense interest due to their size-dependent optical, catalytic, and electronic properties as well as remarkable antimicrobial potential. Within the last decade, scientists have demonstrated that anisotropic AgNPs can be synthesized in a con-

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trolled manner and that these materials exhibit distinctively different physicochemical properties from their spherical counterparts. A significant challenge that scientists face is establishing how these nanoparticles can be used in a wider spectrum of practical applications. Therefore, we present a simple, straightforward two-step synthesis of AgNPs/PNiPAAm hydrogel nanocomposites that includes the chemical formation of both spherical and triangular AgNPs, followed by gamma irradiation-induced PNiPAAm crosslinking in the presence of nanoparticles. The gamma irradiation technique merges sterilization and synthesis in a single technological step, optimizing the process and opening up a wide range of innovative biomedical applications. The formation of stable and uniformly distributed AgNPs in the polymer was confirmed by UV-VIS spectroscopy, while the network porous sponge-like structure was observed by SEM analysis. Physicochemical characterization was performed by examining the swelling and deswelling processes in water at 25℃ and 48℃, respectively. In addition, AgNPs were shown to have an effect on VPTT values. Our main goal is to investigate how different morphologies of AgNPs affect the physicochemical properties of nanocomposite samples, considering the potential applications and the ongoing need for the widespread use of biocompatible materials.

Keywords: *Hydrogel nanocomposites; silver nanoparticles; VPTT; swelling properties*

1 Introduction

In recent years, the technological need for new materials has led to creative approaches to the synthesis and generation of structures, properties, and functions in the field of nanocomposite systems based on polymeric hydrogels and metal nanoparticles. Considering their unique properties and synergistic effect, these materials have become a very attractive class of new materials in various fields of science and technology. Thanks to their crosslinked structure, porous morphology, ability to absorb a large amount of water or biological fluid, and similarity to living tissue, hydrogels have been studied extensively as vehicles for the controlled release of nanoparticles, drugs, therapeutic proteins, enzymes, etc. [1-3]. In addition, for particular applications or delivery active substances on specific sites, it is preferable that hydrogels are sensitive to external stimuli. The most commonly used are pH- and thermosensitive hydrogels because the pH value and temperature are the two parameters that are most frequently changed in physiological systems. Poly(*N*-isopropylacrilamide) (PNiPAAm) hydrogel is suitable for use in biological systems because it is not toxic to tissues and it's thermosensitive in the range of physiologically relevant temperatures (around 32ºC) [4, 5].

On the other hand, due to their unique physical, chemical, and biological properties, silver nanoparticles (AgNPs) have attracted substantial research over the past few decades. In terms of size, shape, crystallinity, and structure, AgNPs outperform their bulk forms. Since they have distinct physical and optical properties as well as biochemical functionality that is tailored by diverse size and shapes, efforts have been made to investigate AgNPs wide range of biomedical applications, including their role as antimicrobial agents, biomedical device coatings, drug-delivery carriers, and medical devices [6-9]. In general, changing the synthesis procedures, reducing agents, and stabilizers is frequently accomplished to obtain precise control of form, size, and size distribution. What caught our attention is to give insights into how different morphologies of AgNPs, nanospheres and nanoprisms used as building blocks of PNIPAAm-based nanocomposites influence their physicochemical properties, that is, to create a material with the best possible characteristics, keeping in mind the intended application. Considering the current trend for broader use of biocompatible materials as antimicrobial agents we predominately focused on evidence that bactericidal activity is shape-dependant and triangular silver nanoprisms exhibit exceptional properties such as increased catalytic activity due to morphologies with highly active (111) facets exceeding spherical and cubical nanoparticles of comparable size [10, 11].

Bearing in mind that the principles of the radiation-chemical method of synthesis (gamma irradiation) are based on biologically harmless and biocompatible radiolytic products of water, this method combines the advantages of environmental reagents with simultaneous stabilization of particles in the polymer matrix. In addition, it is an easy and fast way of synthesis with easy process control, but one of the most important benefits is the possibility of synthesis and sterilization of products in one technological step, which is extremely important for biomedical applications [3, 6, 12]. The aim of this study is to examine how different morphologies of AgNPs affect the physico-chemical properties of nanocomposite samples, taking into account possible applications and the continued demand for biocompatible materials to be widely used.

2 Experimental

2.1 Materials

Silver nitrate ($AgNO_3$), sodium citrate ($Na_3C_6H_5O_7$), polyvinylpyrrolidone (PVP, M_w=29.000 g/mol), sodium borohydride ($NaBH_4$), and *N*-isopropylacrylamide (NiPAAm) were purchased from Sigma Aldrich, while hydrogen peroxide (H_2O_2, 30 wt%) was obtained from J.T. Baker. Solutions were prepared with water obtained from Milli-Q water system (Millipore Corporation).

2.2 Synthesis of anisotropic silver nanoparticles

A seedless approach was used to create triangular Ag nanoprisms. Aqueous solutions of $AgNO_3$ (0.1 mM, 25 mL), $Na_3C_6H_5O_7$ (30 mM, 1.5 mL), H_2O_2 (30 wt%, 60 µL), and PVP (0.7 mM, 1.5 mL) were vigorously mixed at room temperature (25°C). The solution changed color after the addition of $NaBH_4$ (0.1 M, 150 µL). After adding $NaBH_4$, the solution instantly turned pale yellow, indicating a nucleation process and the creation of small AgNPs. During 30 minutes of stirring, the colloidal solution changed color from dark yellow to orange, then pink, and ultimately blue. The blue color denotes the end of the reaction, i.e., the completion of the growing process of triangular Ag nanoprisms. For a few months, the colloidal solution remains stable at room temperature with no color change. Spherical AgNPs were prepared when 250 mL of the aqueous solution containing $AgNO_3$ (1 mM) was heated with a heating mantle in a two-neck round-bottomed flask under vigorous stirring. A condenser was used to prevent the evaporation of the solvent. After boiling had commenced, 5 mL of $Na_3C_6H_5O_7$ (1 wt.%) was injected into this solution. The solution became yellow immediately, indicating the presence of spherical AgNPs, and was left under stirring without heating for 30 min. The concentration of silver in the colloidal solution was determined by Philips PYU UNICAM SP9 atomic absorption spectrometry (AAS). Before the AAS measurements, 0.1 ml of concentrated Ag colloid sample (centrifugated at 12000 rpm) was dissolved in 0.9 mL of concentrated hydrochloric acid. The obtained concentrations are 11 mg l^{-1} and 27 mg l^{-1} for colloidal solutions of nanoprisms and nanospheres, respectively.

2.3 Synthesis of AgNPs/PNiPAAm nanocomposite hydrogels

The solution of NiPAAm monomer (10 wt.%) was prepared in distilled water and bubbled with argon in order to remove oxygen. A colloidal dispersion of spherical and triangular AgNPs was added, with the final concentration of nanoparticles in the monomer solution being 2.5 x 10^{-4} mol L^{-1}. The prepared AgNPs/NiPAAm solutions were poured into specially designed molds (consisting of glass plates separated by rubber spacer). The samples were irradiated by γ-rays, at a dose rate of 0.22 kGy/h up to an absorbed dose of 25 kGy, to obtain crosslinked polymer networks, i.e., nanocomposite hydrogel samples.

2.4 Methods of Characterization

Gel content. After the completion of the crosslinking process, obtained hydrogels were cut into discs (dimension 10 x 4 mm), dried to the constant mass, and then extracted in distilled water (obtained from Milli-Q water system) for a week to remove unreacted reactants. Extracted hydrogels were dried again to the constant weight in order to determine the gel fraction. The gel fraction (W_g) was calculated from the following equation:

$$W_g(\%) = \frac{m_{ae}}{m_{be}} \cdot 100 \qquad (1)$$

where m_{ae} and m_{be} were the weights of dry samples after and before extraction, respectively [3].

Optical properties. The absorption spectra of the colloidal dispersion of anisotropic AgNPs, and AgNPs/PNiPAAm hydrogel nanocomposites were recorded on a Thermo Fisher Scientific Evolution 600 UV-Vis spectrophotometer in the wavelength of 300–850 nm.

Transmision electron microscopy (TEM). The size and shape of AgNPs collected on carbon-coated copper grids were observed with a PHILIPS EM-400 transmission electron microscopy (TEM) operated at 100 kV.

Scanning electron microscopy (SEM). The internal morphology of the hydrogel nanocomposite samples was performed by JEOL JSM-6610LV scanning electron microscope, operated at 20 kV. Before the observation, samples were swollen in water, frozen at −20°C, and then lyophilized using a Martin Christ Freezedryer Alpha 1-2 Ldplus (T = − 32°C at 0.310 mbar).

Swelling degree studies. Dynamic swelling properties of hydrogels were examined in distilled water at room temperature. The swelling process was monitored gravimetrically by measuring the mass of the swollen gel at predetermined time intervals until the equilibrium swelling degree (SD_{eq}) was reached [3]. All swelling experiments were performed in triplicate.

Deswelling studies. The equilibrium swollen hydrogels in water at 25°C (below the VPTT) were transferred into the water at 48°C (above the VPTT), for the deswelling kinetics studies. The weight of hydrogels was measured at predetermined time intervals during deswelling until a constant weight was reached [3]. The deswelling kinetics of each sample was monitored in triplicate.

Determination of volume phase transition temperatures. In order to determine the VPTT of hydrogels and hydrogel nanocomposites, samples were equilibrated at the predetermined temperature, in the temperature range from 14°C up to 48°C. The samples were allowed to swell to equilibrium at each defined temperature, and their corresponding equilibrium swelling degree was determined using Equation (2).

3 Results and discussion

3.1 Synthesis of AgNPs/PNiPAAm hydrogel nanocomposites

In this study, a two-step synthesis of AgNPs/PNiPAAm hydrogel nanocomposites was performed. The first step includes the chemical formation of both spherical and triangular AgNPs, followed by gamma irradiation-induced PNiPAAm crosslinking in the presence of nanoparticles. The radiolytic method (“green method”) is a highly suitable tool for the crosslinking of polymers, and it does not require harmful initiators or crosslinkers, eliminating the problem of removing them at the end of the process. To evaluate the efficiency of the chosen crosslinking method, the percentage of gelation was calculated by using Equation (1). The obtained results for the gel fraction (Table 1) show that the chosen synthesis method is appropriate because it gives high yields of polymer networks without unreacted residues.

3.2 Optical properties of anisotropic AgNPs and AgNPs/PNiPAAm hydrogel nanocomposites

The formation of a colloidal dispersion of spherical as well as triangular AgNPs, and the presence of incorporated AgNPs within the PNiPAAm hydrogel network is confirmed by UV-Vis spectral studies.

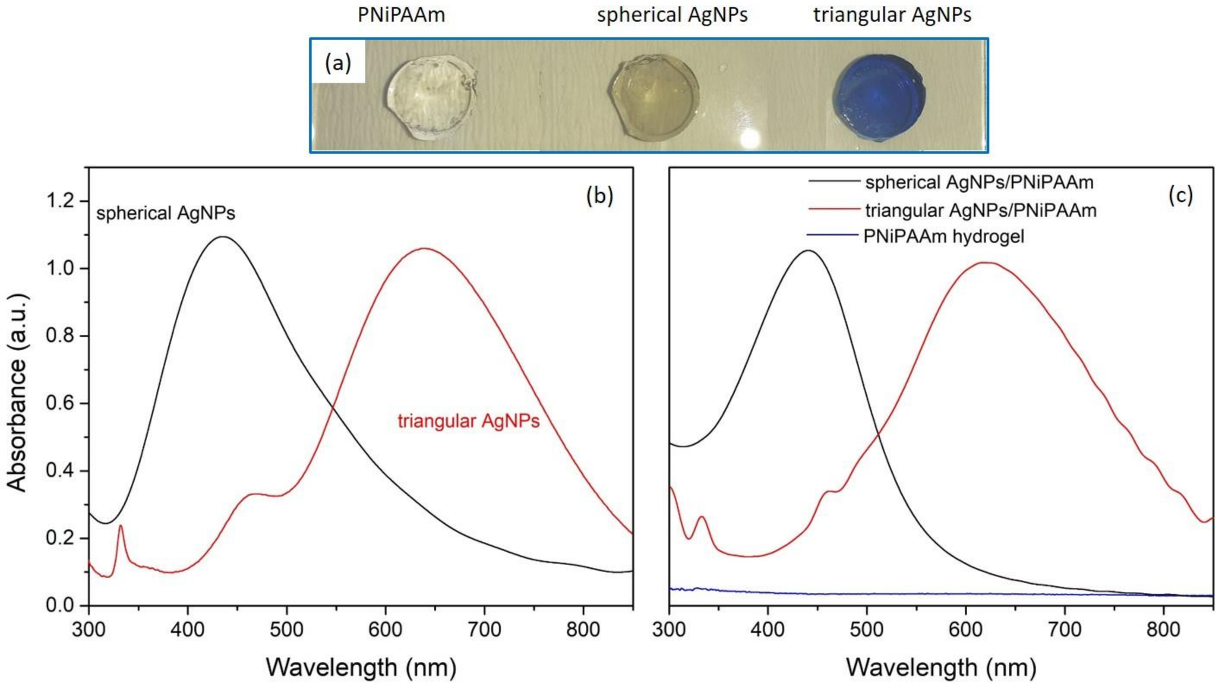

Figure 1. Photography of prepared hydrogels (a), UV-Vis absorption spectra of anisotropic AgNPs (b), and AgNPs/PNiPAAm hydrogel nanocomposites (c).

During the reduction process, the formation of spherical AgNPs is visually indicated by the change of the reaction solution from colorless to yellow-brownish. Metal nanoparticles have free electrons, which create a surface plasmon resonance (SPR) absorption band as a result of the electrons mutual vibration in resonance with the light wave. The appearance of the single peak at 452 nm (Figure 1b) reveals the surface plasmon resonance assigned to the presence of spherical AgNPs. This absorption is highly dependent on particle size and shape, dielectric medium, and particle environment. On the other side, the absorption spectrum of triangular Ag nanoprisms has three distinct peaks (Figure 1b). According to the Mie theory, the blue colloid dispersion of the anisotropic AgNPs exhibits two more surface plasmon resonance bands that reflect the geometry of the particles. According to the theoretical discrete dipole approximation method for the triangular nanoprisms, the first peak at 332 nm corresponds to the quadrupole transversal out-of-plane resonance, the second band wavelength at 494 nm to the in-plane quadrupole resonance, and the third at 638 nm to the in-plane dipole plasmon resonance [13, 14]. These results are in agreement with the TEM analysis showing spherical and triangular AgNPs (Figure 2a and 2b). After the gamma irradiation-induced PNiPAAm crosslinking in the presence of both spherical and triangular AgNPs, it can be seen that there is no evidence of changed morphological characteristics of the AgNPs (Figure 1c). The slight shift in peak position is most likely due to the increase in the refractive index of the particle environment from 1.333 (water) to 1.503 (PNiPAAm) [10, 11].

3.3 Morphological characterization of AgNPs and polymer network

Morphological properties of the AgNPs. The TEM analysis of prepared colloidal anisotropic AgNPs confirmed the formation of spherical and triangular nanoparticles. Figure 2a shows spherical AgNPs with an average size of around 40 nm, while triangular Ag nanoprisms with size ranging from 40 nm to 50 nm are shown in Figure 2b. The mixture of different sizes is obtained as a consequence of the presence of statistical thermodynamics of the free energies and kinetic effects during the growth process [10, 11].

Morphological properties of the polymer network. The internal morphology of the investigated hydrogel nanocomposites was examined using SEM, and obtained micrographs are shown in Figure 2 (c and d). According to the SEM micrographs, the crosslinking process occurred uniformly throughout the hydrogel samples, and the expected porous sponge-like structure was confirmed.

Compared to PNiPAAm hydrogel, it was observed that the incorporation of AgNPs into the polymer network has no significant influence on internal morphology.

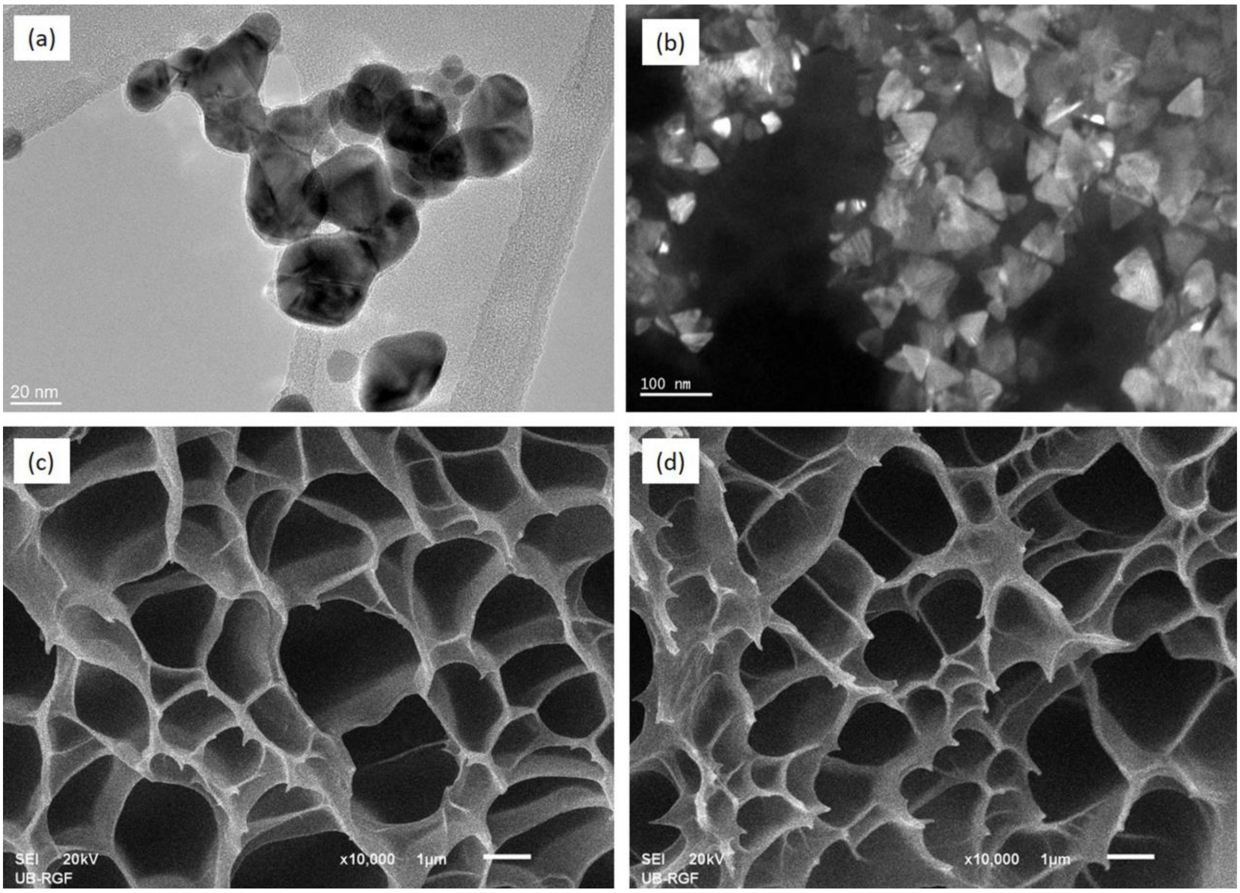

Figure 2. TEM micrographs of colloidal spherical (a) and triangular (b) AgNPs, and SEM micrographs of PNiPAAm hydrogel (c) and triangular AgNPs/PNiPAAm hydrogel nanocomposites (d).

3.4 Physicochemical characterization

Swelling and fluid transport properties. Given the possible biomedical use of the synthesized system, swelling capacity and swelling kinetic parameters are important for physicochemical characterization. The swelling capacity and diffusion properties of hydrogels were investigated in a neutral medium (distilled water) at room temperature. The swelling degree was calculated by the equation [3].

$$SD = \frac{m_t - m_o}{m_0} \tag{2}$$

where m_t is the weight of the swollen hydrogel at time t, and m_0 is the weight of xerogel. In addition, swelling kinetics and the liquid transport mechanism were investigated by applying the equation that follows the change in the hydrogel mass during the swelling process [3, 15]:

$$\frac{SD}{SD_{eq}} = k \cdot t^n \tag{3}$$

where k is the kinetic constant related to the structure of the polymer network and n is the diffusion exponent. From the logarithmic form of the given equation for the initial period of swelling (first 60% of SD_{eq}), values for n and k can be determined from the slope and intercept, respectively (Figure 3b).

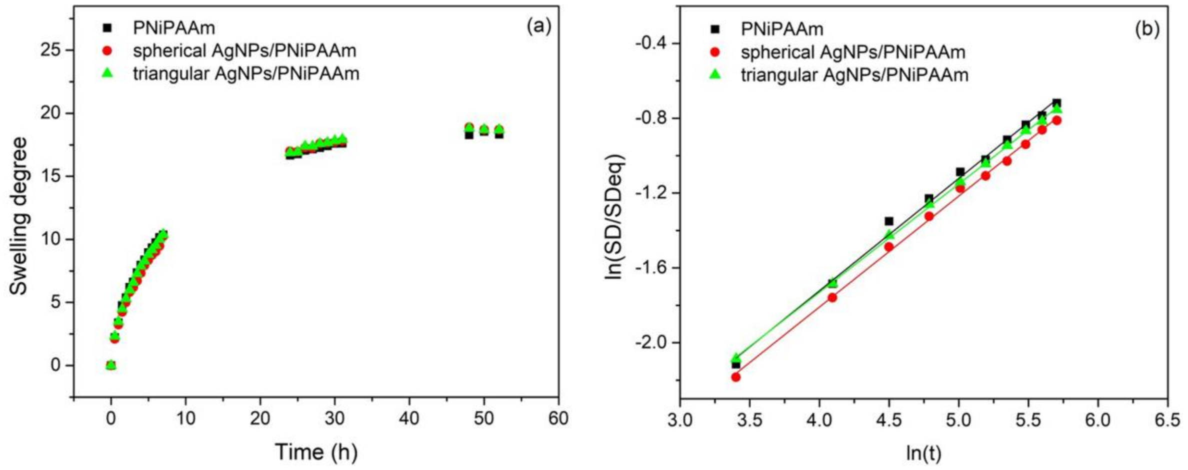

Figure 3. Swelling curves of PNiPAAm hydrogel and AgNPs/PNiPAAm hydrogel nanocomposites(a), and corresponding plots of swelling kinetic curves (b).

The typical swelling curves of hydrogels (Figure 3a) show that the incorporation of AgNPs into the polymer network has no significant influence on swelling capacity. Only a slight increase of SD_{eq} (3.9% for spherical and 4.4% for triangular AgNPs) was observed for AgNPs/PNiPAAm hydrogel nanocomposites, compared to PNiPAAm hydrogel (Table 1). This probably occurs due to a partially restricted crosslinking process, caused by the presence of nanoparticles, which results in a decrease in crosslinking density, followed by a slight decrease in gel content as shown in Table 1. The shape of the particles has no effect on the swelling capacity because the values obtained for spherical and triangular AgNPs are approximately the same. Moreover, all investigated samples showed non-Fickian diffusion ($0.5 < n < 1$), indicating that both diffusion and polymer relaxation processes controlled the fluid transport. By monitoring the swelling kinetics, it is possible to determine the diffusion coefficient (D) by using the following equation [3, 16]:

$$D = \left(\frac{k \cdot \pi \cdot r^2}{4}\right)^{1/n} \tag{4}$$

where r is the radius of the xerogels. According to the results presented in Table 1, diffusion coefficients are higher for nanocomposite samples, indicating a slightly higher porosity of the polymer network and easier transport of water into it. Such behavior of hydrogel nanocomposites is expected, considering the values of SD_{eq} and k.

Table 1. Parameters obtained from physicochemical characterization.

	Wg, %	SD_{eq}	k, min^{-1}	n	$D\times10^{6}$, $cm^2\ min^{-1}$	K_d, min^{-1}	$VPTT$
PNiPAAm	99.0	18.0	0.0015	0.60	2.9	0.096	28.1
Spherical AgNPs/PNiPAAm	98.9	18.7	0.0016	0.59	3.5	0.076	28.6
Triangular AgNPs/PNiPAAm	98.8	18.8	0.0017	0.58	3.7	0.079	28.7

Deswelling studies. Bearing in mind that PNiPAAm is a thermosensitive polymer, the response of all samples at temperatures above the VPTT was examined (Figure 4a). Water retention (WR) was measured in order to evaluate their deswelling properties, according to the following equation [3]:

$$WR = \frac{m_t - m_{col}}{m_{eq} - m_{col}} \tag{5}$$

where m_t is the weight of deswollen hydrogel at predetermined time intervals, m_{eq} is the weight of equilibrium swollen hydrogel, and m_{col} is the weight of deswelled i.e. collapsed hydrogel. To investigate the deswelling kinetics quantitatively, a semi-logarithmic plot of first-order rate analysis was used to fit the time dependence of the deswelling, where values of deswelling rate constant (K_d) were obtained from the slope of the plot given by the equation [17]:

$$ln\left[\frac{m_t - m_{col}}{m_{eq} - m_{col}}\right] = -K_d \cdot t \qquad (6)$$

Although the swelling rate (k) is approximately the same for all samples, the deswelling rate (K_d) is lower for the hydrogel nanocomposites. It is well known that PNiPAAm-based hydrogels can form a dense surface skin layer, which acted as a diffusion barrier. Namely, when the equilibrium swollen hydrogel is transferred into the water at a higher temperature, the surface PNiPAAm molecules shrink first, resulting in the formation of a dense skin layer on the hydrogel surface, which can hinder the diffusion of water molecules from the hydrogel interior [3, 18]. Moreover, the hydrogel nanocomposites absorbed more water molecules (higher SD_{eq}) which are bind to AgNPs and form a solvation shell around them [19], resulting in decreased deswelling rate.

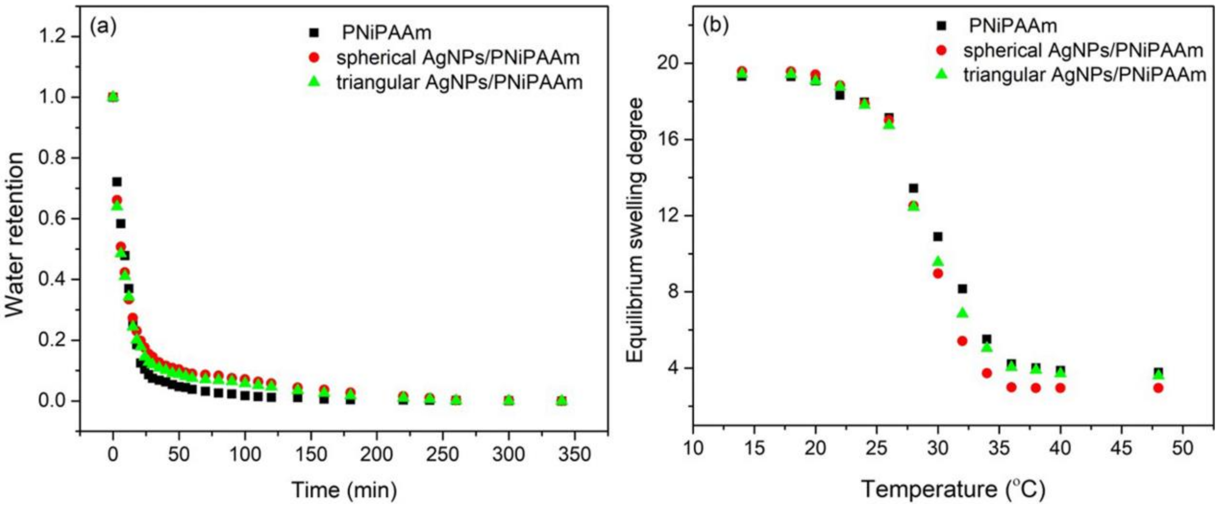

Figure 4. Deswelling curves (a) and temperature dependence of SD_{eq} (b) for PNiPAAm hydrogel and AgNPs/PNiPAAm hydrogel nanocomposite samples.

Determination of volume phase transition temperatures. The VPTT value close to the human body temperature is the most important characteristic of PNiPAAm, which makes it one of the most studied thermosensitive polymers. At low temperatures, under VPTT, the formation of hydrogen bonds between the water molecules and the hydrophilic groups in the PNiPAAm side chains causes water uptake and swelling. As the temperature of the surrounding media increases above VPTT the hydrogen bonds between water and PNiPAAm are disrupted, and PNiPAAm becomes hydrophobic, causing the leaching of water and collapsing of the polymer network.

In this investigation, the influence of the incorporation of AgNPs into the polymer network on VPTT values was examined, and results are presented in Figure 4b, and in Table 1. According to the presented results, the presence of nanoparticles between polymer chains leads to a slight increase in VPTT values. This phenomenon may be due to the fact that a more porous network structure makes it easier for water to diffuse in or out of the matrix, and thus the effect of temperature variation on phase separation is rapidly manifested [2, 3, 16]. As in the case of swelling capacity, the different shape of the nanoparticles has no significant effect on the VPTT value.

4 Conclusion

In summary, PNiPAAm hydrogel and AgNPs/PNiPAAm hydrogel nanocomposites were successfully synthesized by a two-step procedure combining chemical and gamma irradiation methods. UV-Vis and TEM analysis confirmed the formation of both, spherical and triangular AgNPs, with an average size ranging from 40 nm to 50 nm. After incorporation into the polymer matrix, the spherical and triangular AgNPs remain stable which was confirmed by UV-VIS. The SEM analysis showed a stable porous sponge-like structure of the crosslinked polymer network, while the incorporation of nanoparticles has no significant effect on the internal morphology. The AgNPs/PNiPAAm hydrogel nanocomposites, compared to PNiPAAm hydrogel samples, exhibited a slightly higher swelling capacity, diffusion coefficient, as well as VPTT values. Because the geometry of AgNPs has no effect on the basic physical properties of the hydrogel polymer network, various particles can be combined to enhance their biological potential without affecting the properties of the polymer matrix. To conclude, the desired AgNPs/PNiPAAm hydrogel nanocomposites were successfully synthesized. Each component retained its primary physicochemical properties, resulting in a stable material with the desired predominant characteristics. This provides a good platform for further in-depth investigation in order to obtain materials for tailored biomedical applications.

4.1 Acknowledgement

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5 Nomenclature

PNiPAAm – poly(*N*-isopropylacrylamide)
VPTT – volume phase transition temperature
AgNPs - silver nanoparticles
PVP - polyvinylpyrrolidone
AAS - atomic absorption spectrometry
SPR - surface plasmon resonance
SEM - scanning electron microscopy
TEM - transmision electron microscopy

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