INFLUENCE OF LIPOPHILIC AND HYDROPHILIC CO-MONOMERS ON THE HYDRODYNAMIC DIAMETER OF THERMOSENSITIVE NIPA DERIVATIVES FOR THERMALLY CONTROLLED DRUG DELIVERY

WITOLD MUSIAŁ1*, MONIKA GASZTYCH1, VANJA KOVOL1, IGOR Mucha1, ALEKSANDRA MALAMIS2, WOJCIECH KOŁODZIEJCZYK1 and AGNIESZKA GOLA3

Wroclaw Medical University, Pharmaceutical Faculty, 1Department of Physical Chemistry, 2Department of Pharmaceutical Technology, Borowska 211, 50-556 Wroclaw, Poland
1University of Maribor, Institute of Engineering Materials and Design, 17, Smetanova St. 2000 Maribor, Slovenia
2Wroclaw Medical University, Pharmaceutical Faculty, Department of Analytical Chemistry, Borowska 211 A, 50-566 Wroclaw, Poland

Abstract: For modern drug delivery, new drug carriers sensitive to various factors and with size in the range of micro- and nanometers are required. The aim of this work was to evaluate the influence of hydrophilic and hydrophobic co-monomers on the hydrodynamic diameter of three co-polymers of N-isopropylacrylamide (NIPA) nanogels synthesized at 70°C in the presence of potassium persulfate (KPS) as the initiator and N,N'-methylene bis-acrylamide (MBA) as the cross-linker. The first batch of nanoparticles was synthesized without co-monomer, whereas poly(ethylene glycol) methyl ether acrylate (PEG-MEA), and N-tert-butylicrylamlide (NTB), were implemented as co-monomers for the second and third batch. Hydrodynamic diameter of nanoparticles was in the range 550-800 nm. The compositions of the synthesized co-polymer nanoparticles were confirmed via IR and NMR analyses. The SFPP conditions resulted in hydrodynamic diameters ranging from approximately 550 to 800 nm at temperatures lower than the volume phase transition temperature (VPTT) and diameters ranging from 250 to 600 nm at temperatures above the VPTT, where the VPTT was between 26 and 41°C. The polydispersity index (PDI) showed a maximum or a minimum value at the VPTT, which was an important indicator of the volume phase transition. According to the PDI observation during thermal cycling, the addition of NTB into the polymeric chain resulted in maximal values of the PDI at the VPTT, similar to the case of nanoparticles without any additional co-monomers. In contrast, in the case of PEG-MEA, the PDI presented a minimal value. Dynamic light scattering (DLS) volume measurements, performed simultaneously with spectral methods, may lead to a fast evaluation of nanoparticles prepared by SFPP.

Keywords: nanoparticles, N-isopropylacrylamide, volume phase transition temperature, drug delivery

Targeting of drug molecules to respective sites of activity and controlling drug delivery have been developing intensively due to the potential of reducing adverse drug effects, as well as the ability to precisely deliver drug molecules to the proper activity sites. An interesting monomer that has been used in the research on new polymeric drug carriers is N-isopropylacrylamide (NIPA) (1). Poly (N-isopropylacrylamide) (pNIPA) was first synthesized by Pelton and Chibante in 1986 (2) and is one of the best and most extensively studied temperature sensitive polymers due to the numerous potential applications in the biomedical and industrial fields, particularly in the controlled drug deliveries of insulin (3, 4), doxorubicin (5), injectable drugs (6), carbazochrome sodium sulfonate (7), proteins (8), indomethacin (9) and other biologically active substances, which can be attributed to specific properties of the polymer (10, 11). Mucoadhesive properties (12), control of enzymatic activities (13-15), tunable optics (16, 17), and chemical separation (18-20) were also studied in pNIPA. pNIPA particles possess a sharp and reversible volume phase transition at the volume phase transition temperature (VPTT) of 32°C (21-23), which corresponds well with the range of physiological temperatures (24, 25). Below the VPTT, the polymer chains are swollen in water, whereas above the VPTT, the polymer chains are hydrophobized and form a more compact structure due to dehydration (26, 27). Nanogels synthesized using NIPA have

* Corresponding author: e-mail: witold.musial@umed.wroc.pl; phone: 0048 71 7840231

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gained interests in the field of pharmaceutical technology and bioengineering. Programmed variations of the composition and the structure of NIPA derivatives allow feasible productions of numerous macromolecules with a preset VPTT in aqueous systems (28-30). The homogenous nucleation of NIPA with co-monomers is performed at high temperatures (31), and the polymer is formed into a microgel structure when the resulting oligomer is insoluble in the solvent (32). The process uses an initiator, yielding initiator radicals, which impart surface-active properties to particles composed of increasing polymer molecules. Particles prepared by this technique are stabilized by sulfate groups derived from the persulfate ion of the attached initiator molecule (33). During the course of pNIPA growth, various structures may be formed from different conditions of the polymerization procedure (34). Moreover, micellization influences the formation of nano- and microparticles (35). Extremely diverse compositions have been used for the synthesis of NIPA derivatives, e.g., acrylic acid (36), methacrylic acid and fumaric acid (37), acrylamide (38), maleic acid (39), and N-tert-butylacrylamide (40, 41). The NIPA derivatives are often studied to obtain specific therapeutic effects, using respective biologically active components, e.g., antibacterial agents, cardiovascular agents, anticancer agents, local anesthetics and non-steroidal anti-inflammatory drugs. The hydrodynamic diameter of the resulting nanogels influences both the possibility of practical applications of the particles to selected human tissues, as well as the loading capacity of the respective biologically active molecules into the pNIPA macromolecule. Implementation of a lipophilic co-monomer should lead to an enhanced binding of lipophilic biologically active substances (42), whereas the implementation of a hydrophilic co-monomer may influence the capability of binding lipophobic substances (43). Previous studies have evaluated some aspects of lipophilized or hydrophilized NIPA derivatives; however, the impact of PDI on the VPTT has not yet been studied.

The aim of the work was to evaluate the influence of hydrophilic and hydrophobic co-monomers on the hydrodynamic radius and the polydispersity index of co-polymers of NIPA nanogels synthesized at 70°C in the presence of a KPS initiator and a MBA cross-linker.

EXPERIMENTAL

Materials
N-isopropylacrylamide (NIPA, 97%, Sigma-Aldrich, Sternheim, Germany), N-tert-butylacrylamide (NTB, 99%, Acros Organics, Geel, Belgium), N,N'-methylene bisacrylamide (MBA, 99%, Sigma-Aldrich, Sternheim, Germany), poly(ethylene glycol) methyl ether acrylate with nine oxyethylene groups (PEG-MEA, 99%, Mₗ = 480 Da, Sigma-Aldrich, Sternheim, Germany), and potassium persulfate (KPS, 98%, Sigma-Aldrich, Sternheim, Germany) were obtained from commercial and industrial suppliers and used without further purification. Dialysis bags with a molecular mass cut-off (MWCO) of 12000–14000 Da were obtained from Visking Medicell International Ltd., London, UK. Deionized water was obtained from an ionic column (System TKA DI 6000, Boutersem, Belgium) and was applied in all procedures.

Methods
Preparation of polymeric nanoparticles
Polymeric nanoparticles and NIPA derivatives were produced by precipitation polymerization without an emulsifier (surfactant free precipitation polymerization, SFPP). A four-necked round bottom flask equipped with a reflux condenser was filled with 600 mL of deionized water and heated up

<table>
<thead>
<tr>
<th>Type of particle</th>
<th>Main monomer</th>
<th>Cross-linker</th>
<th>Co-monomer</th>
<th>Anionic initiator</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>5.00</td>
<td>0.50</td>
<td>-</td>
<td>0.50</td>
</tr>
<tr>
<td>S2</td>
<td>5.00</td>
<td>0.50</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>S3</td>
<td>5.00</td>
<td>0.50</td>
<td>-</td>
<td>0.50</td>
</tr>
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to 70°C, followed by the addition of 0.5 g of KPS as the free radical initiator, which was previously dissolved in 200 mL of deionized water. After stabilizing the temperature, an appropriate mixture of the selected reagents, i.e., NIPA, MBA, and a co-monomer, dissolved in 200 mL of water were added. The substrate compositions are shown in Table 1. The polymerization was carried out at 70°C for 6 h under a nitrogen atmosphere and constant mixing with a magnetic stirrer.

Further purification was performed in that the particles were dialyzed against deionized water until the conductivity was less than 1 µS cm⁻¹. After 21 days, when the purification procedure was completed, the samples were frozen and freeze-dried by Steris LYOVAC GT2 with vacuum pressures in the range of 10 Pa to 100 Pa for 32 h. The yields of the obtained nanoparticles were evaluated via a gravimetric method and found to be 86, 73 and 81% for S1, S2, and S3, respectively.

**Evaluation of the obtained polymeric nanoparticles**

**Compositions of the resulting macromolecules by IR and NMR**

Dry mass analysis of the nanogels showed a dispersion concentration on the order of 0.5 mass %. IR assessments were performed using an infrared spectrophotometer (M80, Specord, Carl Zeiss, Jena, Germany) on KBr pellets. In order to prepare the KBr pellets, approximately 0.5-0.7 mg of the sample was mixed with 180-200 mg of potassium bromide powder and compressed into a disc using a hydraulic press. Spectra were recorded in the range of 4000-400 cm⁻¹. NMR spectra were recorded on a Bruker 300 MHz spectrometer. Ten milligrams of each assessed polymer was dissolved in 0.6 mL of DMSO-d₆. The temperature of the analysis was 26°C.

**Hydrodynamic diameter and VPTT by DLS**

Hydrodynamic diameter was measured in aqueous dispersions of the synthesized nanoparticles in a DLS Zetasizer Nano device (Malvern Instruments) at a wavelength of 678 nm. The hydrodynamic diameter was assessed in aqueous dispersions by dynamic light scattering (DLS). From approximately 50 mL of the sample, 50 µL of the filtered liquid was placed into a polystyrene single-use fluorimeter cuvette. Then, 1 mL of deionized water, filtered through a 0.2 µm PVDF Whatman nanofilter, was added to the cuvette, and the sample was inserted into the device using the 173° backscatter measurement condition and the Mark-Houwink parameter setting. The duration of the measurements was extended for the case of large particles, with a relaxation time multiplier of 100000, according to the instrument preset. Using the automatic option in the measurement duration setting, the software automatically determined the most appropriate time for the measurements in order to increase the reproducibility and quality of the data. The worst data were rejected, and acceptable data were used to calculate the final results. The default number of repetitions was set automatically and depended on the stability of the sample and the size of the particles. The measurements settings were automated to seek an optimum position; the attenuator selection was also automated. Every measurement was conducted five times. Two sets of data were obtained from each

![Figure 1. Comparison of the IR spectra of NIPA (A) and synthesized NIPA derivatives: S1 (B), S2 (C), and S3 (D)](image_url)
Figure 2. $^1$H NMR spectra of monomers, cross-linkers, co-monomers, and the resulting polymers S1 (A), S2 (B), S3 (C), where NIPA is $N$-isopropylacrylamide, MBA is $N,N'$-methylene bisacrylamide, PEG is methyl ether acrylate with nine oxyethylene groups ($M_w = 480$ Da), and NTB is $N$-tert-butylacrylamide. Details are presented in the text.
Influence of lipophilic and hydrophilic co-monomers on the hydrodynamic... measurement, i.e., the average diameter of the nanogels and the polydispersity index (PDI) of the assessed dispersions, from the Zetasizer Nano Version 5.03 Software.

RESULTS AND DISCUSSION

Composition of the synthesized nanoparticles via IR

The IR evaluations of the samples revealed the characteristic groups of the NIPA monomer. The maximum at 1620 cm\(^{-1}\) was assigned to the absorption of the stretching vibrations of the C=C bond (44), and this peak is often observed without conjugated groups at approximately 1650 cm\(^{-1}\). This peak was not present after the synthesis of the polymer due to the saturation of the vinyl group. The peaks at 808 cm\(^{-1}\) and 664 cm\(^{-1}\) disappeared after the saturation, which are assigned to the =CH out-of-plane deformation vibration in the C=C bond conjugated to C=O and to the C-H wagging in the vinyl group, respectively (45). In the spectra of the S1, S2, and S3 polymers and accounting for the fact that the vinyl bond was saturated, we observed the respective frequencies of the saturated hydrocarbon chain groups. During the course of the precipitation polymerization, unsaturated compounds become saturated, and bands higher than 3000 cm\(^{-1}\) should disappear (46). The peaks observed in the monomer at 3104 cm\(^{-1}\) and 3030 cm\(^{-1}\), which can be assigned to the stretching vibrations of the unsaturated C=C bond, vanished from the spectrum after the reaction. The spectra of NIPA and S1 – S3 are presented in Figure 1.

Figure 3. Visualization of the SFPP product via SEM: A – S1, B – S2, C – S3, and the obtained size distributions from DLS measurements at temperatures over the VPTT (42°C, left peak, a) and below the VPTT (18°C, right peak, b) for S1 (D) and for S2 (E). The graph F represents the decrease in diameter with the increase of temperature from 18°C to 42°C (from right to left). The numbers on the x-axis represent the diameter on a logarithmic scale. The bar represents 5 µm (A, B) and 1 µm (C)
Composition of nanoparticles via NMR

In order to evaluate the chemical structures of the obtained polymers, 1H NMR spectra of the basic monomer NIPA, the cross-linking agent MBA, the co-monomers, i.e., PEG-MEA and NTB, and the synthesized polymers, i.e., S1, S2 and S3, were obtained. As a result of the comparative analysis of these spectra, it was found that in the spectra of the synthesized polymers S1-S3, the signals from protons associated with the –C=CH group, which were present in the spectra of the monomers disappeared: 5.6-6.2 ppm for the cross linker MBA, 5.9-6.4 ppm for the co-monomer PEG-MEA, and 5.4-6.3 ppm for the co-monomer NTB. This indicates that in the resulting polymers, the double bonds were saturated during the polymerization. Furthermore, the PEG-MEA co-monomer was successfully implemented into the polymer, according to the band shift at 3.20-3.50 ppm in the spectrum of the S2 polymer. This shift is characteristic of the protons of the ethoxyl groups present in the PEG-MEA monomer. The incorporation of NTB into the polymer led to a band shift of 1.5 ppm in the spectrum of the S3 polymer, which originated from the characteristic protons of the tert-butyl group of the NTB monomer. We also observed a peak at 4.5 ppm in all of the S1-S3 spectra, which is characteristic of the protons associated with the -HCNN group in the MBA cross linker. The spectra are shown in Fig. 2A-B.

Hydrodynamic diameter and electrokinetic potential

From DLS measurements, the synthesis of polymer nanoparticles using an anionic initiator generated S1, S2, and S3 with hydrodynamic diameters of approximately 600, 800, and 550 nm, respectively. A visualization of the SFPP product showed that no observable nanoparticles were embedded in the hydrogel matrix (Fig. 3A-C). The figures show a xerogel, which was produced via freeze-drying of the product dispersions obtained from the SFPP procedure. The cavities in the xerogel may reflect places where particles were embedded, where some of them were extracted from the surface due to the high vacuum conditions in the SEM device. The sizes of the particles observed in SEM were different compared to those obtained from DLS measurements, as shown in a previous paper (40). The size distributions of nanoparticles assessed in the DLS measurements are presented in Figure 3D-F.

Changes in the hydrodynamic diameters of the nanoparticles induced by heating aqueous suspensions of the polymers are summarized in Figure 4A-C. As shown in the graph in Figure 4A, with increasing temperature, the hydrodynamic diameter of the S1 nanoparticles decreased from approximately 600 to 250 nm. In the temperature range of 34-36°C, a clear decrease was observed in the hydrodynamic diameter, reflecting the volume phase transition. In the temperature range of 18-32°C, the hydrodynamic diameter declined with fluctuations in the range of 450-620 nm. However, above the temperature of 38°C, the hydrodynamic diameter remained constant, and the nanoparticles maintained a stable size. The hydrodynamic diameter of the S2 polymer varied in the range of 580-960 nm during the temperature interval of 18-28°C (Fig. 4B). However, above 28°C, S2 demonstrated a consistent decrease in the hydrodynamic diameter, which proceeded in a wider temperature range compared with the decrease in the hydrodynamic diameter of S1. The hydrodynamic diameter of S2 clearly decreased from approximately 800 to 300 nm while heating the suspension from 28 to 40°C. Above this temperature, a slight increase of 50 nm in the hydrodynamic diameter was observed. This was not further studied because the temperature exceeded the physiological temperature of the living body. Changes in the hydrodynamic diameter of the S3 nanoparticles are similar to the changes of the hydrodynamic diameter of the S1 nanoparticles, where the hydrodynamic diameter in the temperature range of 18-27°C did not change significantly (Fig. 4C). At approximately 27°C, there was a dramatic increase of the hydrodynamic diameter up to 800 nm. Further heating of the dispersion led to a drop of the hydrodynamic diameter. The hydrodynamic diameter stabilized to approximately 270 nm at a temperature above approximately 36°C. Electrokinetic potential allows for the assessment of the stability of colloidal suspensions. The electrokinetic potentials of the evaluated nanoparticles ranged from -21.40 mV to -19.27 mV. The detailed values are summarized in Table 2.

DISCUSSION

Changes of the hydrodynamic diameter in the initial phase of the process

As clearly seen from a comparison of the changes in the hydrodynamic diameters of S1, S2 and S3, the initial heating of the aqueous nanoparticle dispersions moderately influenced the hydrodynamic diameter of the nanoparticles. The hydrodynamic diameter decreased when the polymer-water system reached the VPTT. However, in the case of S1, the hydrodynamic diameter varied only in the range of 477-616 nm, while S2 presented significantly greater variations in the hydrodynamic diam-
Figure 4. Influence of increasing temperature on the hydrodynamic diameter of synthesized nanoparticles S1 (A), S2 (B), and S3 (C)
Figure 5. Influence of the temperature on the polydispersity index (PDI) of the evaluated NIPA derivatives: S1 (A), S2 (B), S3 (C), in the terms of hydrodynamic diameter from DLS.
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der due to the presence of polyoxyethylene glycol (PEG) chains (with lengths of nine oxyethylene units) implemented into the structure. The resulting fluctuations indicated the possibility of a free movement of the hydrophilic PEG chains anchored to the generated nanoparticles. The maximum value of the hydrodynamic diameter can be attributed to the maximum length of the upright PEG chain over the surface of the nanoparticle, while the lowest value corresponds to an almost parallel position of the PEG chain on the surface of the macromolecule; the valley observed between 23 and 25°C may have resulted from the fluctuations. With the further increase of temperature, the hydrodynamic diameter decreased, similar to the case of a simple NIPA polymer. A similar influence of the PEG chain on the hydrodynamic diameter measured at elevated temperatures was shown by Hiwatari et al. (47). Contrary to S1 and S2, the S3 nanoparticles are characterized by slight variations of the hydrodynamic diameter in the temperature range between 18°C and the VPTT. This may have resulted from the introduction of the lipophilic co-monomer NTB. The covalent lipophilization of the nanoparticles enhanced the stabilization of the macromolecules during the precipitation polymerization process and prevented agglomeration and deagglomeration with increasing temperatures up to the VPTT values. The influence of NTB on the thermal stability of polymeric derivatives was also evaluated by Bharathi and Pazhanisamy (48).

Changes of the hydrodynamic diameter in the vicinity of the phase transition temperature

With increasing temperature, the hydrodynamic diameters of the S1-S3 nanoparticles, after a period of relative stability, exhibited rapid changes, as shown in Figure 4. The hydrodynamic diameter of the S1 nanoparticles decreased immediately once the temperature of the system reached 33°C, falling steadily from approximately 600 nm to a final value of 300 nm at 37°C. The implementation of the lipophilic agent NTB had a positive effect on the ability of the nanoparticles to expel water bound in the polymer network at lower temperatures. The presence of NTB may favor the aggregation of particles at temperatures close to the VPTT, due to the collapse of particles and water expulsion. In the vicinity of the VPTT, the particles may adhere to themselves as they gain lipophilic properties but soon after, they lose interactions between each other due to the high volume of aqueous dispersants. A parallel phenomenon was observed in the case of mesoscopically periodic photonic materials derived from the NIPA monomer (49).

This concept was confirmed by the occurrence of VPTT at a lower temperature in the case of the S3 polymer, which contained a lipophilic agent, compared to S1 without NTB and S2 with a PEG chain. Clearly, by comparing Figure 4A (S1) and Figure 4C (S3), the S3 polymer started to expel water at a lower temperature in the presence of a lipophilic group. However, the process of water expulsion and collapse of the nanoparticles took place at a wider temperature interval of 8°C. The respective data are in Table 3. The aqueous system with S2 passed into the VPTT at lower temperatures, but the range of the VPTT area was very wide, i.e., 12°C. The S2 polymer component, i.e., PEG-MEA, significantly elongated the temperature interval in which there was a systematic decrease in the hydrodynamic diameter of the nanoparticles. Consequently, the system fell into equilibrium at higher temperatures, as shown in Table 3.

Changes of the hydrodynamic diameter after reaching the VPTT

Both minimal diameters of S1 and S3 were stabilized at approximately 270 nm, after overcoming temperatures of 37 and 34°C, respectively. The slightly higher diameter of the S3 nanoparticles could be associated with the lipophilic nature of the co-polymer NTB. In contrast, in the case of S2, we observed a further decrease in the diameter, possibly due to the collapse of the polymer network in which the long chains of PEG-MEA played an important role. This was confirmed by the fact that at 34 and 37°C, aqueous dispersions of the S1 and S3 poly-

Table 2. Electrokinetic potentials of the synthesized nanoparticles at 18°C.

<table>
<thead>
<tr>
<th>Co-polymer type</th>
<th>Electrokinetic potential [mV]</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>-19.27</td>
<td>0.23</td>
</tr>
<tr>
<td>S2</td>
<td>-21.40</td>
<td>0.44</td>
</tr>
<tr>
<td>S3</td>
<td>-20.33</td>
<td>0.47</td>
</tr>
</tbody>
</table>

ΔT - the difference between the final and initial temperatures measured during the evaluation of the VPTT.
mers were in equilibrium, while at the same temperature, the S2 polymer still had the ability to reduce its diameter.

**Evolution of the polydispersity index during the evaluation of the VPTT**

A deeper PDI analysis showed that the values were good indicators of achieving the VPTT by the test systems. Values of the PDI in the case of S1 and S3 transformed in similar manners when the systems were heated. The PDI was initially maintained at a constant level and then steadily increased, reaching a maximum at around the VPTT, as illustrated in Figures 5A and 5C; then, the values decreased to those observed at much lower temperatures. The systematic increase in the vicinity of the VPTT resulted from the coexistence of macromolecules with hydrodynamic diameters reduced to various extents.

More complex changes occurred in the S2 polymer. The increasing temperature resulted in a clear and systematic decrease in the PDI, until it reached a minimum at the point of the VPTT. At this point, a maximum homogeneity of the nanoparticles may be observed, as shown in Figure 5B. This may indicate that as the temperature increased, there was a decrease in the interaction between the nanoparticles. Deglomeration probably took place, according to the collapse of the hydrophilic PEG chains. This phenomenon confirmed the existence of interactions between macromolecules containing PEG groups. At temperatures below the VPTT, these effects were severe and may be associated with the free movements of PEG ends associated with the surfaces of the nanoparticles, where various sizes of agglomerated nanoparticles were observed. At the VPTT, the polymeric nanoparticles finally collapsed. However, at the temperature above VPTT, the S2 sample also exhibited a decrease of its diameter, but this was associated with a systematic decrease due to the temperature of the system and not due to the random deglomeration of the agglomerated nanoparticles. The synthesized particles may be applied in release experiments due to their thermosensitivities, similar to what had been shown for NIPA derivatives with various terminal groups, which was evaluated by our team for the controlled release of a model cationic drug (50).

**CONCLUSIONS**

The evaluation of the hydrodynamic diameter in conjunction with NMR and IR assessments enabled deep insights into the structure of the nanoparticles synthesized in the SFPP process. The compositions of the synthesized co-polymer nanoparticles were confirmed via IR and NMR analyses. Saturation of the vinyl bonds was easily detected due to the disappearance of the characteristic bands from the IR and NMR spectra. Successful implementation of the PEG groups into the pNIPA co-polymer was also shown via NMR. The proposed conditions of SFPP resulted in samples with hydrodynamic diameters in the range of approximately 550 to 800 nm at temperatures below the VPTT and 250 to 600 nm at temperatures above the VPTT, where the VPTT was between 26 and 41°C. The PDI presented either a maximum or a minimum value at the VPTT temperature, which was an important indication of the phase transition. According to the observation of the PDI during the course of thermal cycling, the implementation of the PEG-MEA into the polymeric chain resulted in maximal values of the PDI at the VPTT, similar to the case of nanoparticles without any additional co-monomers. In contrast, in the case of PEG-MEA, the PDI exhibited a minimal value. The DLS measurements, performed simultaneously with spectral methods, may lead to a fast evaluation of nanoparticles prepared by SFPP. The PDI can also be a good parameter, which may be applied for the observation of the VPTT. Both the S1 and S3 structures were characterized by a distinct VPTT, with stable values of the hydrodynamic diameter at temperatures below and above the VPTT, which can be beneficial for controlled drug delivery. In the case of S2, there was a large variation in the hydrodynamic diameter at temperatures below the VPTT; hence, there may be challenges in using S2 for potential applications in drug delivery.

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**REFERENCES**

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