Nanogels of Poly(N-Vinylcaprolactam) Core and Polyethyleneglycol Shell by Surfactant Free Emulsion Polymerization

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Polymeric nanogels containing N-vinylcaprolactam (NVCL) and polyethyleneglycol methylether methacrylate (PEGMA) have been prepared by surfactant free emulsion polymerization. The influence of NVCL to PEGMA concentration ratio on the particle size and particle size distribution was studied. PNVCL:PEGMA nanogel particles exhibit thermal responsive properties in water and their transition temperature increases with higher content on PEGMA. It was found that the particle diameter decreased when higher amounts of PEGMA were used in the monomer mixture. The influence of the concentration and type of cross-linker: ethylene glycol dimethacrylate (EGDMA) or 3,9-divinyl-2,4,8,10-tetra-oxaspiro [5.5] undecane (DVA), on polymerization and colloidal characteristics of such temperature sensitive particles was studied. Nanogels, characterized by Fourier Transform Infrared Spectroscopy (FTIR) Differential Scanning Calorimetry (DSC), dynamic and static light scattering (DLS and SLS) and nuclear magnetic resonance spectroscopy (1H-NMR), showed higher incorporation of PEGMA than in the recipe, higher contraction by heating for bigger nanogels and spherical morphology.

Keywords: Surfactant free emulsion polymerization, Poly(N-vinylcaprolactam), Poly(ethylene glycol), Nano/microgels, Thermoresponsive polymers

Introduction

The most popular temperature sensitive polymer is poly(N-isopropylacrylamide) (PNIPAAm) (1–4), but recently another thermoresponsive polymer, poly(N-vinylcaprolactam) (PNVCL), which shows higher bicompatibility compared to PNIPAAm, is being extensively studied (5–12). PNVCL is a polymer with amphiphilic and responsive character. It dissolves in cold water but undergoes a sudden conformational change, collapses and precipitates, upon heating. PNVCL shows LCST in water at temperatures between 30°C and 34°C (near physiological temperatures) depending on molecular weight, end-functionalization, and polymer concentration (13, 14). PNVCL is a suitable polymer for in vivo biomedical applications because it does not suffer hydrolysis in pure water (15). In fact, it has been tested in in vitro cytotoxicity tests showing no obvious cytotoxicity (8, 16).

Emulsion polymerization is the most efficient way to prepare nanogels. This method yields nanogel particles with diameters less than 150 nm. One disadvantage is the relatively large amount of surfactants required and the complete removal of residual surfactant is not always possible. This adds to the cost of raw materials, may result in additional waste-water treatment, and, in some instances, it gives rise to undesired contaminants in the end product. This problem may be overcome by surfactant free emulsion polymerization (SFEP) method. The surfactant-free synthesis of aqueous temperature-sensitive nanogels was pioneered by Pelton’s group (17). Monodisperse PNIPAAm nanogels were obtained by using initiator and cross-linker. In the case of temperature-sensitive microgels based on PNIPAAm or PNVCL, the surfactant-free method is limited in the sense of particle size variation. Using SFEP method, it is difficult to prepare nanogel particles because the initiator residues incorporated into polymer chains are not sufficient to stabilize the extremely large surface area of precursor nanoparticles. For those cases, a surfactant may be added to the reaction mixture to stabilize precursor particles and minimize their growth by aggregation; but the advantages of SFEP method are lost (7, 18). A good alternative to the use of stabilizers during preparation of aqueous nanogels in SFEP method is the use of reactive functional co-monomers into PNVCL-based nanogels, to improve colloidal stability, to help regulate microgel size, and for tuning their properties such as lower critical solution temperature (LCST). The reactive co-monomers participate in the polymerization process and become covalently attached to the polymer chains of nanogel’s network (19–21).
Up to now, only a few studies on the preparation of PNVCL nanogels have been reported (7, 9, 21–24); this may be a result of its polymerization being more complicated and its LCST being not as sharp as for the PNIPAm nanogels. NIPAAm is an acrylamide and NVCL a vinyllactam; on the one side, their rate of polymerization is expected to be very different; NVCL shall polymerize much faster. On the other side, their copolymerization behavior with the most common cross-linkers: bisacrylamides and dimethacrylates is expected to match better to the rate of polymerization of NIPAAm than to the rate of NVCL. These differences give rise to the inequality between work performed on both types of nano-microgels, and the necessity of deeper investigations on the synthesis, functionalization, and characterizations of PNVCL-based nanogels.  

In the present work, we studied the copolymerization of poly(ethylene glycol) methyl ether methacrylate (PEGMA) with N-vinylcaprolactam in water to prepare well-defined temperature-sensitive PNVCL-PEGMA nanogels without the use of surfactant via free radical emulsion polymerization. The reactions were carried out by using different concentrations of initiator and cross-linkers. Nanogels cross-linked with various concentrations of ethylene glycol dimethacrylate (EGDMA) and 3,9-divinyl-2,4,8,10-tetra-oxaspiro [5.5] undecane (DVA) were prepared.

Colloidal characteristics, such as the temperature sensitivity of PNVCL nanogels in water, changes in the volume phase transition temperature (Tc) as a function of the cross-linker, initiator, and NVCL concentrations, were analyzed by DLS. The structure, size, and shape of PNVCL-PEGMA nanogels were characterized by means of FTIR, AFM, DLS, SLS, and NMR.

**Experimental**

**Reagents**

N-Vinylcaprolactam (NVCL) from Sigma-Aldrich, México was purified by recrystallization in hexane and dried under vacuum prior to use. Ethylene glycol dimethacrylate (EGDMA, 98%), from Sigma-Aldrich, México, was purified by passing through an inhibitor remover column (Sigma-Aldrich, Mexico). Poly(ethylene glycol) methyl ether methacrylate (PEGMA) (Mn = 1100 g/mol), ammonium persulfate (APS, 98%), 3,9-divinyl-2,4,8,10-tetra-oxaspiro [5.5] undecane (DVA, 98%), Sodium chloride (99.4%), disodium phosphate (98%), and potassium phosphate (99%) were supplied by Sigma-Aldrich México and used as received. Phosphate buffer solutions (pH 7.4 and pH 5) were prepared at 0.05 M total concentration with adjusted ionic strength using NaCl at 0.1 M. Water was deionized by a Barnstead apparatus. Deionized water and buffers were filtered through 0.2 μm Nylon filters to eliminate any particulate matter, prior to use.

**Synthesis of Poly(NVCL-co-PEGMA) Nanogels**

Poly(NVCL-co-PEGMA) nanogels were prepared by a surfactant-free emulsion polymerization (SFEP) method with different ratios of NVCL:PEGMA monomers using EGDMA or DVA as cross-linkers and the initiator APS (Scheme 1). In a typical synthesis, NVCL was mixed with the proper amounts of PEGMA, cross-linker (EDGMA or DVA), and dissolved in 48 mL of deionized water at room temperature. The mixture was bubbled with nitrogen for 30 min for the removal of any dissolved oxygen. The monomer mixture was then heated to 85°C in a preheated oil bath and vigorously stirred. Ammonium persulfate was dissolved in 2 mL of deionized water and it was added to the system to act as a thermal initiator. The polymerization process was allowed to continue for different reaction times and was stopped by cooling. The resulting dispersions were purified via dialysis (Spectra/Por dialysis membrane, MWCO: 12000-14000, VWR) against deionized water. During dialysis, water was changed frequently for 5 days at room temperature to remove any unreacted monomer and other impurities. The nanogels were recovered by freeze drying: 5 mL of purified dispersion was filled in 10 mL glass vials. The samples were frozen at -4°C in a conventional freezer for 12 h and then placed into the drying chamber of a Labconco Freeze Dry System Freezone 4.5 (Kansas City, MI, USA), precooled to -50°C. Drying was performed at a pressure of 0.05 mbar for 48 h. After drying the samples, these can be used for later re-dispersion and/or characterization.

**Nanogels Characterization**

The chemical structure of the poly(NVCL-co-PEGMA) nanogels was analyzed by Fourier transform Infrared Spectroscopy (FTIR) using a Spectrum 400, FT-IR/FT-NIR Spectrometer (Perkin Elmer) with a diamond attenuated total reflection (ATR) accessory in the spectral range from 4000 cm⁻¹ to 650 cm⁻¹. The samples were used directly as dried powders and 16 scans were collected.

The content of NVCL in the nanogels was evaluated by proton nuclear magnetic resonance spectroscopy (¹H-NMR) by using a Varian Mercury, 200 MHz equipment. Dried nanogels of known weight (0.1 g) were dispersed in 20 mL of deuterated chloroform (CDCl₃) by using an ultrasonic bath for 60 min keeping the dispersion cold.

The size distribution of the nanogel products was evaluated by dynamic light scattering (DLS) using a Zetasizer Nano ZS (ZEN3690; Malvern Instruments, Miami, FL) equipped with a red laser of 630 nm. The angle of measurement is 90°. Purified and reconstituted samples were analyzed. The freeze-dried samples were re-suspended to form a 1 wt% dispersion in deionized water under magnetic stirring for 60 min before measurement. The size distribution reported is the distribution by volume using CONTIN analysis. The reported hydrodynamic diameters (Dh) were calculated using the Stokes–Einstein equation for spheres, as usual (25). The effect of the temperature on the particle size of the nanogel products was studied by DLS using the same Zetasizer Nano equipment by a temperature trend method edited to go from 5°C to 50°C, equilibrating 4 min once the measurement temperature was achieved.

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¹A quick search on WEB of Knowledge demonstrated that 141 papers on NIPAAm nano- and microgels versus 15 papers on NVCL nano- and 625 microgels were published up to June 20th 2013; among those, NVCL nanogels count for 3 papers.
The refractive index increment (dn/dc) for selected nanogels in deionized water was measured at 25°C and λ = 633 nm in a differential refractometer model DAWN DSP, Wyatt Technology Corporation, Sta. Barbara, CA. A base dispersion of nanogels in deionized water of concentration 1 mg/mL and dilutions of this were used for the measurements.

Values of the weight-average molecular weight (Mw), radius of gyration (Rg), and second virial coefficient (A2) were measured by static light scattering (SLS) on a multi-angle equipment of Wyatt Technology Corporation, Sta. Barbara, CA (model: Dawn-F), equipped with a laser of λ = 633 nm. An initial dispersion of nanogels with a concentration of 0.25 mg/mL was prepared by stirring overnight in deionized water. Just before measurements, dispersed samples were filtered off using filters of 0.45 μm pore size (Nylon). Intensity of scattered light was measured for at least 5 dilutions of each sample in the angular range from 30° to 135°. Berry-plot algorithm was applied for the evaluation of results (26).

Differential scanning calorimetry (DSC) was used to determine melting point (Tm) and glass transition temperature (Tg) events in the nanogels. The equipment used was a Modulated DSC, TA Instruments model 2920. Samples were cooled to −10°C, maintained isothermal for 5 min; afterward the temperature was modulated to +/− 0.5°C every 60 s; and then heated with modulation at a rate of 5°C/min to 200°C in nitrogen atmosphere. Two cycles were run and the results reported corresponded to the second cycle.

Atomic force microscopy (AFM) images were obtained by using Agilent SPM 5100 (Molecular Imaging) equipment. For these measurements nanogel dispersions containing 0.1 mg/mL in water were dropped on fresh cleaved mica surfaces and air dried at a controlled temperature of 25°C for 48 h. Images were acquired in intermittent contact form by using silicon cantilevers (Budget Sensors). Topography, phase, and amplitude images were evaluated by using the WSxM software (27).

Results and Discussion

Effect of PEGMA Concentration

It is assumed that the nanogels prepared in this work may possess core-shell morphology. This morphology, in principle, is supported by the miscibility of the co-monomers and their corresponding polymers due to the migration by diffusion of the more hydrophilic PEG to the outer surface leaving the less hydrophilic PNVCL toward the core. This assumption is also supported by the fact that at the polymerization temperature used in this work, PNVCL phase separates from water as this temperature is much higher than the LCST of PNVCL.

The co-monomers can improve colloidal stability of the nanogels and help to regulate nanogel size (28). We studied the influence of the PNVCL:PEGMA weight ratio on particle size and particle size distribution. PEGMA concentrations (30, 35, 37.5, 40, 42.5, and 45 wt%) were investigated maintaining NVCL as main component.

Experimental data summarized in Fig. 1 indicate that when the PEGMA content in monomer mixture is lower than 40 wt% of the particle size distribution becomes bimodal with an overall increase in particle size. The colloidal stability at the synthesis temperature is for reactions using less than 40 wt% of PEGMA not granted so that the system tends to aggregate. The formation of smaller particles at high NVCL content can be explained by increasing amount of NVCL dissolved in water and formation of higher number of nucleation sites in reaction medium in the very beginning of the polymerization process. It is clear from Fig. 1 that incorporation of large amounts of PEGMA into microgel structure reduces the particle size. This behavior is not surprising as effective steric stabilization at room temperature arises from PEG segments in PEGMA, which probably form a hairy layer around NVCL particles. Without PEGMA co-monomer, the microgel size is very large and its dispersion is unstable, which confirms that such NVCL-based latex particles are mainly sterically stabilized and electrostatic stabilization, originating from charged group introduced by initiator, is much less significant.

Effect of Initiator Concentration

The effect of initiator concentration on the size of the nanogels was studied based on PNVCL:PEGMA 60:40 weight ratio. Table 1 shows the evolution of the average diameters measured in reactions where the amount of initiator was varied from 1 to 3 mol% with respect to NVCL. An increase of APS concentration produced smaller particles with narrow particle size distribution.

The reduction of particle size might be explained by the fact that increasing the APS concentration resulted in the formation
Fig. 1. Size distribution of PNVCL nanogels synthesized with different proportions of PEGMA (cross-linked with EDGMA 2 mol%).

Table 1. Results for reactions varying the initiator concentration.

| Nanogel | NVCL (w%)a | PEGMA (w%)a | APS (mol%)b | \( D_h \) (nm) | PDI  |
|---------|------------|------------|------------|-------------|------|
| NG1     | 60         | 40         | 1          | 520, 5100   | 0.444|
| NG2     | 60         | 40         | 2          | 330         | 0.265|
| NG3     | 60         | 40         | 4          | 78          | 0.204|

Note: Weight ratio NVCL: PEGMA (60:40); 2 mol% EGDMA; \( T = 85^\circ C; t = 30 \text{ min.} \)

a the total weight of co-monomers is 0.5g.
b mol% with respect to NVCL.

of large number of nuclei at the initial stage of polymerization, so that the final particle size becomes smaller for a given amount of monomer used (29). A similar behavior was reported in the synthesis of PNIPAAm nano/microgels (30). As can be seen, when the initiator is used in 1 mol% concentration, large particles and bimodal distributions were found (Table 1 and Fig. 2).

**Effect of Cross-Linker Type and Concentration**

The effect of the cross-linker type in the formation of nano/microparticles has not been studied very well. A recent publication has shown that this effect can be very important (31). The variation of the cross-linker type used for the synthesis of temperature-sensitive nanogels provides an additional possibility for influencing their size and swelling properties. EGDMA and DVA were used and were successfully employed as cross-linkers for NVCL. The effect of cross-linker concentration on the formation of PNVCL-PEGMA nanogels was studied through variation of EGDMA amounts (see Table 2). The average hydrodynamic particle diameters in the swollen state at room temperature were determined by DLS measurements. As can be seen in Table 2, there are significant differences between the diameters obtained. The cross-linker concentration dramatically affects the particle diameters. It is noteworthy that an increase in cross-linker concentration results in increased average diameter, showing that EGDMA influences particle nucleation during the synthesis of the nanogel particles. The optimal amount of EGDMA for this case is also dependent on the NVCL/PEGMA composition ratio in the feed. For NVCL/PEGMA 70:30 wt% ratio, nanogels were only obtained when EGDMA concentration was 1 mol%.

Lower concentration on EGDMA (0.5 mol% and 0.75 mol%) were not sufficient to cross-link the growing polymer chains, yielding most likely branched molecules; whereas a concentration of 2 mol% cross-linker yielded two distributions of sizes (see Fig. S1 in the Supplementary material file). Likewise, it is possible to observe that for a composition NVCL/PEGMA...
55:45 wt% and EGDMA concentrations ranging from 2 to 3.5 mol%, nanogels of different diameter with unimodal distributions of sizes were obtained. For some reactions where a higher amount of cross-linker was added, two distributions of sizes with formation of microlayer particles were observed.

In the case of using DVA as the cross-linker for the preparation of NVCL nanogels, it is important to note that DVA is an acid-degradable cross-linker. It is stable in base, hydrolyzes at very slow rates at the physiological pH of 7.4, and become progressively more labile as the pH is lowered (32). Here, it should be noted that the polymerization reactions using DVA were carried out in pH 7.4 phosphate buffered saline (PBS) in order to control the pH. Table 3 shows the diameters measured for the nanogels in the swollen state cross-linked with various concentrations of DVA. One general observation is that the particle diameters are larger when using DVA as compared to the use of EGDM as a cross-linker. A possible explanation for this behavior depends on the solubility of DVA in water, which is lower than that of EGDM. Consequently, in the reaction carried out by using DVA, fewer growing radicals containing cross-linkers are formed and higher diameters are obtained than in the case of using EGDM as the cross-linker. When the concentration of DVA is 0.5 mol%, no formation of nanogel particles was observed, while 1 mol% DVA produced microgels with 679 nm and 2 mol% of DVA leads to bimodal particle distributions, in majority as large aggregates. The concentration window by usage of DVA as the cross-linker is very narrow for preparation of NVCL nanogels. As a result, experiments under the same conditions described in Table 3 second row, but stopping the polymerization in 10 min and 20 min, yielded the results reported in the fourth and fifth row of Table 3. As can be seen, the nanogel diameter decreased with decreasing polymerization time.

55:45 wt% and EGDM as a cross-linker.

### Characterization by Nuclear Magnetic Resonance Spectroscopy

As FT-IR is not a straightforward quantitative method for determination of copolymer composition, the molar ratios of PNVCL-PEGMA in the resulting nanogel particles were determined by quantitative proton nuclear magnetic resonance spectroscopy (1H-NMR). Fig. 4 shows the 1H NMR spectrum of nanogel (NG3) as an example. The signal at 1.6 ppm is assigned to the CH2-CH2-CH2- groups of the lactam ring and polymer backbone (a); the signal at 2.7 ppm corresponds to the two hydrogens of the methylene group (-C(O)-CH2-) next to carbonyl (d) of lactam; the signal at 4.36 ppm corresponds to -CH- of the backbone of polymer (b); and the signal at 3.1 ppm corresponds to the methylene group of –CH2- (c). PEGMA has one main chemical shift at approximately 3.6 ppm, which corresponds to the methylene group of –NCH2- (d).

### Characterization of NVCL-PEGMA Nano/Microgels

Identification of the main components of dried nanogels was pursued by FT-IR spectroscopy. For this, the spectrum of purified nanogels was compared with PNVCL lineal homopolymer (60,000 g/mol) and pure PEGMA monomer as illustrated in Fig. 3. The linear polymer PNVCL shows absorption signals at 1622 cm\(^{-1}\), assigned to C=O stretching vibration in lactam ring; at 1480 cm\(^{-1}\) assigned to C-N stretching of lactam ring and at 2874 cm\(^{-1}\) assigned to aliphatic C-H stretching vibration. Pure PEGMA shows an absorption signal at 1720 cm\(^{-1}\), assigned to C=O stretching vibration of ester group; whereas the aliphatic C-H stretching peaks were located at 2876 cm\(^{-1}\). As expected, FT-IR spectrum of PNVCL-PEGMA nanogels show absorption signals at 1720 and 1630 cm\(^{-1}\) corresponding to C=O stretching vibrations of PEGMA and NVCL, respectively; moreover, the aliphatic C-H stretching peaks were located at 2880 cm\(^{-1}\) and a new broad absorption band was observed for the nanogels at 3507 cm\(^{-1}\), which corresponds to O-H stretching.

Nanogels absorb moisture from the air easily; therefore, this broad O-H stretching signal might be a result of the absorption of water from the air. It is evident that the intensity of absorption bands corresponding to carbonyl stretching vibrations of NVCL and PEGMA units are similar, a not expected result as NG3 contains more NVCL in the recipe (60 wt%).

### Table 2. Results by DLS of nanogels synthesized at different concentrations of EGDMA (APS = 4 mol%; T = 85°C; t = 30 min)

| Nanogel | NVCL (wt%) | PEGMA (wt%) | EGDMA (mol%) | D\(h\) (nm) | PDI |
|---------|------------|-------------|--------------|-------------|-----|
| NG6     | 70         | 30          | 2.00         | 1705\(\pm5\), 43 | 0.722 |
| NG7     | 70         | 30          | 1.00         | 56          | 0.251 |
| NG8     | 70         | 30          | 0.75         | 5           | 0.459 |
| NG9     | 70         | 30          | 0.50         | 5           | 0.451 |
| NG5     | 60         | 40          | 3.00         | 1603\(\pm2,5\), 52 | 0.523 |
| NG3     | 60         | 40          | 2.00         | 78          | 0.204 |
| NG4     | 60         | 40          | 1.00         | 6           | 0.441 |
| NG35    | 55         | 45          | 3.50         | 133         | 0.222 |
| NG10    | 55         | 45          | 3.00         | 82          | 0.173 |
| NG11    | 55         | 45          | 2.50         | 49          | 0.245 |
| NG12    | 55         | 45          | 2.00         | 7           | 0.267 |

### Table 3. Nanogel formation varying DVA cross-linker concentration and polymerization time

| Nanogel | NVCL (wt%) | PEGMA (wt%) | DVA (mol%) | Time (min) | D\(h\) (nm) | PDI |
|---------|------------|-------------|------------|------------|-------------|-----|
| NG20    | 60         | 40          | 2.00       | 30         | 3832\(\pm5\), 416 | 0.521 |
| NG21    | 60         | 40          | 1.00       | 30         | 679         | 0.312 |
| NG22    | 60         | 40          | 0.50       | 30         | 6           | 0.203 |
| NG25    | 60         | 40          | 1.00       | 20         | 413         | 0.205 |
| NG26    | 60         | 40          | 1.00       | 10         | 303         | 0.223 |

Note: Weight ratio PNVCL:PEGMA (60:40), APS = 4 mol%; in Buffer pH = 7.4; T = 85°C.

*a*total weight of co-monomers is 0.5 g.

*b*mol% with respect to NVCL.
Fig. 3. FT-IR spectrum for a linear polymer of PNVCL, PEGMA, and nanogel NG3.

Fig. 4. Analysis by $^1$H-RMN of nanogel PNVCL:PEGMA (60:40) cross-linked with EGDMA 2 mol% (NG3).

corresponding to methylenes of ethyleneglycol (g). The composition was calculated by integration of signals at chemical shifts of 2.7 ppm (NVCL) and at chemical shifts of 3.6 ppm (PEGMA). Results described in Table 4 demonstrate that in all cases NVCL is incorporated into the nanogel structure in a lesser amount than used in the recipe. This may result from the fact that polymerization of NVCL yields a non-stabilized polymer radical, while PEGMA yields a tertiary stabilized radical. This may lead to the preferred incorporation of PEGMA units rather than NVCL. This would be a result of copolymerization parameters that are unknown for the polymerization system used.
Table 4. Composition of nanogels calculated from NMR results

| Nanogel          | Dₜ (nm) | NVCL (mass)   | PEGMA (mass) |
|------------------|---------|---------------|--------------|
| NG3  | 78      | 81.95 mol% (36.4) | 18.05 mol% (63.6) |
| NG17 | 220     | 84.53 mol% (40.8) | 15.47 mol% (59.2) |
| NG35 | 128     | 86.57 mol% (44.9) | 13.43 mol% (55.1) |
| NG25 | 413     | 87.21 mol% (46.3) | 12.79 mol% (53.7) |
| NG26 | 303     | 83.45 mol% (38.9) | 16.55 mol% (61.1) |

However, the values reported for the copolymerization of NVCL with glycidylmethacrylate (GMA) in Dioxane can serve as an orientation as they are 0.039 and 6.75, respectively (33); values that anticipate the copolymerization of NVCL with PEGMA being not effective in incorporating NVCL. Therefore, the results are not surprising.

**Characterization by Differential Scanning Calorimetry**

The influence of co-monomer ratio, the cross-linker concentration (EGDMA or DVA) on the thermal behavior of PNVCL:PEGMA lyophilized nanogel particles, was investigated by means of DSC. Fig. 5 shows an example of thermogram for nanogel NG25. Two thermal transitions were observed: a sharp crystalline melting transition peak corresponding to PEG units (Tₚ) and a glass transition corresponding to the cross-linked PNVCL (Tₜ). It is noteworthy that an increase in cross-linker concentration results in an increase in Tₚ and Tₜ (see Table 5).

Table 5 shows the Tₚ and Tₜ for PNVCL:PEGMA nanogels prepared using 60:40 and 55:45 weight ratios. The sharp crystalline melting transition peak (PEG) increases from 25.9°C to 38.3°C and to 37.6°C as the cross-linker concentration increases from 2.5 mol% to 3 mol% and to 3.5 mol%, respectively. With regard to the glass transition temperature for PNVCL in this same nanogels, Tₜ is increased from 99.8°C, to 116.3°C, and to 136.7°C as the cross-linker concentration is increased from 2.5 mol% to 3 mol%, and to 3.5 mol%, respectively. These Tₜ values are much lower than those of dry and pure linear PNVCL (145°C) determined experimentally by Kirsh et al. (34).

Incorporation of PEGMA units in nanogel lead to a decrease in the glass transition temperature of the network, probably because it traps water that acts as a plasticizer lowering the Tₜ values more than for pure PNVCL. With respect to nanogels prepared using DVA as the cross-linker, thermal transition values were higher than those obtained by similar nanogels prepared using EGDMA as the cross-linker. Compare, for example, the values measured for sample NG25 (PNVCL:PEGMA 60:40 weight ratio, 1% DVA), Tₚ = 35.0°C and Tₜ = 135.1°C with those reported for NG3 (Table 5).

**Characterization by Static Light Scattering**

Microgel/nanogel PNVCL/PEGMA particles were studied by static light scattering at 25°C. These measurements enabled...
Table 5. T<sub>M</sub> and T<sub>g</sub> results by DSC of nanogels PNVCL:PEGMA prepared at different reaction conditions, specially concentration of cross-linker

| Nanogel | D<sub>h</sub> (nm) | T<sub>M</sub> (°C) | T<sub>g</sub> (°C) |
|---------|-------------------|-------------------|-------------------|
| NG3     | NVCL:PEGMA(60:40)/EGDMA<sub>2 mol%</sub>(85 °C) | 78                | 24.8              | 126.1            |
| NG17    | NVCL:PEGMA(60:40)/EGDMA<sub>2 mol%</sub>(90 °C) | 220               | 32.2              | 128.8            |
| NG11    | NVCL:PEGMA(55:45)/EGDMA<sub>2.5 mol%</sub>(85 °C) | 49                | 25.9              | 99.8             |
| NG10    | NVCL:PEGMA(55:45)/EGDMA<sub>3 mol%</sub>(85 °C) | 82                | 28.3              | 116.3            |
| NG35    | NVCL:PEGMA(55:45)/EGDMA<sub>3.5 mol%</sub>(85 °C) | 128               | 37.6              | 136.7            |
| NG25    | NVCL:PEGMA(60:40)/DVA<sub>1 mol%</sub>(85 °C) | 413               | 35.0              | 135.1            |

Table 6. Results by SLS at 25°C of PNVCL:PEGMA nanogels cross-linked with EGDMA and DVA

| Nanogel | M<sub>w</sub> (g/mol) | A<sub>2</sub> (mol/L m) | R<sub>g</sub> (nm) | R<sub>h</sub> <sup>a</sup> (nm) | Yield <sup>b</sup> (%) |
|---------|----------------------|------------------------|------------------|----------------------------|----------------------|
| NG7     | NVCL:PEGMA(70:30)/EGDMA<sub>1 mol%</sub> | 1.278 x 10<sup>6</sup> | 1.138 x 10<sup>-3</sup> | 35.9±8.4 | 28.0 | 1.28 | 49.1 |
| NG3     | NVCL:PEGMA(60:40)/EGDMA<sub>2 mol%</sub>(85 °C) | 2.898 x 10<sup>6</sup> | 1.292 x 10<sup>-4</sup> | 50.7±7.5 | 39.0 | 1.30 | 43.2 |
| NG19    | NVCL:PEGMA(60:40)/EGDMA<sub>2 mol%</sub>(90 °C) | 3.956 x 10<sup>6</sup> | 6.218 x 10<sup>-4</sup> | 63.3±10.4 | 110.0 | 0.50 | 42.2 |
| NG10    | NVCL:PEGMA(55:45)/EGDMA<sub>3 mol%</sub> | 2.550 x 10<sup>6</sup> | 1.145 x 10<sup>-4</sup> | 40.3±4.8 | 41.0 | 0.98 | 47.1 |
| NG35    | NVCL:PEGMA(55:45)/EGDMA<sub>3.5 mol%</sub> | 1.281 x 10<sup>7</sup> | 2.192 x 10<sup>-4</sup> | 73.8±13.4 | 64.0 | 1.15 | 43.8 |
| NG26    | NVCL:PEGMA(60:40)/DVA<sub>1 mol%</sub>(303 nm) | 4.107 x 10<sup>7</sup> | 1.073 x 10<sup>-3</sup> | 127±9.0 | 151.5 | 0.80 | 51.1 |

<sup>a</sup>By DLS R<sub>h</sub> = D<sub>h</sub>/2).
<sup>b</sup>Calculated using ρ = R<sub>g</sub>/R<sub>h</sub>.

Combining the experimental data measured by static and dynamic light scattering we calculated the parameter ρ (R<sub>g</sub>/R<sub>h</sub>), which is structure-sensitive and is frequently used to obtain structural information regarding the nanogel/microgel particles. The experimental data show a number of features that are not observed with common flexible linear chains and not with hard spheres: high molecular weights accompanied with ρ-parameters ranging from typical micelle-like aggregates to microgel particles (25). For example, sample NG19 shows ρ = 0.5, a value that falls within the reported range of 0.35–0.6 for microgel particles (25); and NG26 is close to the value of hard spheres (0.8) whereas other nanogels show values that resemble more spherical micelles (ρ around 1). Some authors suggest that values below 1.0 are due to a cross-linking density higher in the core than in the outer region. If the surface of the nano/microgel is covered by dangling chains, this would increase the hydrodynamic radius but have little influence on the radius of gyration and the ρ-parameter is expected to be smaller than one.

Spherical morphology was confirmed for some nanogels by atomic force microscopy (AFM) studies. Diluted dispersions were deposited onto freshly cleaved mica sheets and dried in air. Fig. 6 show images of two nanogels NVCL:PEGMA 55:45 cross-linked with different amounts on EGDMA that resulted in different size nanogels. Both show values of ρ parameter close to

![AFM images of nano/microgels over mica surface: (a) NG35 NVCL:PEGMA (55:45), EGDMA (3.5%) D<sub>h</sub> = 133 nm; (b) NG10 NVCL:PEGMA (55:45), EGDMA (3%) D<sub>h</sub> = 82 nm.](image-url)
1.0 (soft spheres); and the images show a spherical morphology with different diameters. The diameters do not exactly fit the values from dynamic light scattering (DLS); however, consideration should be made as dry diameters cannot be equal to average diameters swollen in water (DLS). Nevertheless, the trend in sizes is maintained: NG35 is bigger than NG10.

**Temperature Sensitivity of PNVCL/PEGMA Nanogels**

The effect of the temperature of water on the average diameters of the prepared nano/microgels, as a function of co-monomer ratio, initiator concentration and cross-linker was analyzed by DLS. The results show that all nano/micorgel particles synthesized swell by decreasing temperature and shrink at temperatures above the transition temperature ($T_\text{tr}$), which was as expected. Volume transition temperature ($T_\text{v}$) values for nano/microgels were calculated from the first derivative of the $D_h$-$T$ trend curves. Prior to transition temperature analysis of the nanogel particles, nanogels were cleaned by means of dialysis to remove non-reacted reagents and impurities.

In Table 7 the temperature transitions for nanogels prepared with different compositions of PNVCL-PEGMA in water are presented.

Sample NG3 (PNVCL-PEGMA 60/40 wt%) shows a $T_\text{tr}$ around 36.3°C, it has a diameter of 78 nm and was obtained after 30 min polymerization time. Nanogel NG19, polymerized under the same conditions as NG3 but at longer polymerization time (50 min) increased its size to 330 nm, and showed a transition temperature of only 32°C (see Fig. 7). Most likely, as the reaction progresses, the NVCL content increases in the nanogel, decreasing in proportion the overall percentage of the hydrophilic co-monomer PEGMA; consequently, a decrease in the transition temperature is observed. As can be seen in Fig. 7, the nanogels show a higher contrast if the size of nanogel is higher. For example sample NG3 shrinks about 10 nm (12.8%) compared with sample NG19 which shrinks 110 nm (33.3%).

For PNVCL/PEGMA nanogels prepared with a composition 55/45 wt% and 2.5% EGDMA (sample NG11), transition temperature was 35.8°C, and nanogel with 3.5% EGDMA (sample NG35), $T_\text{tr}$ decreased to 30°C.

Samples NG11 and NG35 show a similar behavior as discussed for samples NG3 and NG19. Sample NG11, which belongs to a nanogel that has a diameter of 49 nm, exhibited a smaller contraction (6 nm, 12.2%) than sample NG35, which belongs to a nanogel with diameter of 128 nm (contraction 30 nm, 22.6%); see Fig. 7. In the case of NG19, Fig. 7(b) shows apparently a two-step transition, but considering the non-equilibrium measurement (constant heating rate) and the experimental error for determination of $D_h$, this trend can be misleading. In any case, the $T_\text{tr}$ (where the biggest change in size occurs) is 31.7°C.

Higher cross-linker content results in bigger nanogels and higher contraction; this means that additional cross-linker bridges structures of smaller nanogels into a composite nanogel with higher NVCL content. It looks like the cross-linking density is not increased. The high content on NVCL is confirmed by NMR (Table 4, compare NG17 with NG35). Focusing now on the temperature-sensitivity for nanogels prepared with DVA as the cross-linker (PNVCL-PEGMA 60/40 wt% and 1% DVA as the cross-linker), the $T_\text{tr}$ of samples NG25 and NG26 are 28.7°C and 33.3°C, respectively (see Supplementary material). As explained earlier, the diameters measured for the nanogels in the swollen state were 413 and 303 nm for samples NG25 and NG26, respectively. The diameters of nanogel particles when using DVA as the cross-linker, are larger than when using EGDMA as the cross-linker as previously discussed. This may arise from the solubility differences in water of both cross-linkers: DVA is less water soluble than EGDMA.

To verify that the amount of hydrophilic co-monomer PEGMA is related to transition temperature, molar ratios of PEGMA and PNVCL content were determined by NMR in some samples (see Table 4).

For example, sample NG3 contained 63.6 mass% of PEGMA and showed $T_\text{tr}$ 36.3°C, whereas samples NG26 and NG25 contained 61.1 mass% and 53.7 mass% of PEGMA, respectively. They show transition temperatures of 33.3°C and 28.4°C, respectively, confirming that nanogels with a higher hydrophilic co-monomer PEGMA content show higher transition temperatures.

**Conclusions**

The synthesis of a series of thermosensitive, spherical, core-shell nanogels with poly(N-vinylcaprolactam) core and poly(ethylene glycol) methyl ether methacrylate shell, was accomplished by surfactant free emulsion polymerization. The influence of polymerization parameters, such as temperature, time, and composition in the recipe, into the nanogel properties was assessed. The cross-linker type has a big impact on properties: usage of DVA results in bigger nanogels (microgels with diameter >300 nm) with higher content on NVCL and lower transition temperature. When using EGDMA, the size can be controlled by changing polymerization temperature and cross-linker content, such that nanogels from 49 nm to 330 nm can be prepared. The increase of PEGMA content in the nanogel structure leads to smaller nanogels and, in general terms, to higher transition temperature. The thermosensitive properties of the nanogels depend significantly on their diameters, their composition, and polymerization parameters. Core-shell nanogels containing 12–18 mol% PEGMA are good candidates for biomedical applications.
Fig. 7. Mean average size of PNvCL:PEGMA nanogels in water as a function of temperature: (A) 60:40 wt%, 30 min of reaction time, 2 mol% EGDMA (NG3); (B) 60:40 wt%, 50 min of reaction time, 2 mol% EGDMA (NG19); (C) 55:45 wt%, 30 min time, 2.5 mol% (NG11); (D) 55:45 wt%, 30 min time, 3.5 mol% (NG35).

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Supplemental Material

Supplemental data for this article can be accessed on the publisher’s website.

References

1. Wu, X., Pelton, R.H., Hamielec, A.E., Woods, D.R., and McPhee, W. (1994) The kinetics of poly(N-isopropylacrylamide) microgel latex formation. Colloid. Polym. Sci., 272:467–477.
2. Schild, H.G. (1992) Poly(N-isopropylacrylamide): experiment, theory and application. Prog. Polym. Sci., 17:163–249.
3. Hirokawa, Y., and Tanaka, T. (1984) Volume phase transition in a nonionic gel. J. Chem. Phys., 81:6379–6380.
4. Pelton, R.H., Pelton, H.M., Morfesis, A., and Rowell, R.L. (1989) Particle sizes and electrophoretic mobilities of poly(N-isopropylacrylamide) latex. Langmuir, 5:816–818.
5. Solomon, O.E., Corciovei, M., Ciuta, I., and Boghina, C. (1968) Properties of solutions of Poly(N-vinylcaprolactam). J. Appl. Polym. Sci., 12:1835–1842.
6. Lozinsky, V.I., Simenen, I.A., Kurskaya, E.A., Kulakova, V.K., Galaev, I.Y., Mattiasson, B., Grinberg, V.Y., Grinberg, N.V., and Khokhlov, A.R. (2000) Synthesis of N-vinylcaprolactam polymers in water containing media. Polymer., 41:6507–6518.
7. Pich, A., Tessier, A., Boyko, V., Lu, Y., Arndt, K.F., and Adler, H.J.P. (2006) Synthesis and characterization of poly(N-vinylcaprolactam)-based microgels exhibiting temperature and pH-sensitive properties. Macromolecules, 39:7701–7707.
8. Viñols, H., Laukkanen, A., Hirvonen, J., and Tenhu, H. (2002) Binding and release of drugs into and from thermosensitive poly(N-vinyl caprolactam) nanoparticles. Eur. J. Pharm. Sci., 16:69–74.
9. Peng, S.F., and Wu, C. (2001) Surfactant Effect on pH and Temperature Sensitivities of Poly(N-vinylcaprolactam-co-sodium acrylate) Microparticles. Macromolecules, 34:568–571.
10. Ivanov, A.E., Kazakov, S.V., Iyu, G., and Mattiasson, B. (2001) Thermosensitive copolymer of N-vinylcaprolactam and 1-vinylimidazole: Molecular characterization and separation by immobilized metal affinity chromatography. Polymer., 42:3373–3381.
11. Maksaeva, E.E., Tenhu, H., and Khokhlov, A.R. (2002) Behavior of poly(N-vinylcaprolactam-co-methacrylic acid) macromolecules in aqueous solution: Interplay between Coulombic and hydrophobic interaction. Macromolecules, 35:1870–1876.
12. Laukkanen, A., Hietala, S., Maunu, S.L., and Tenhu, H. (2000) Poly(N-vinylcaprolactam) Microgel Particles Grafted with Amphiphilic Chains. Macromolecules, 33:8703–8708.
13. Shostakovsky, M.F., Sidolakovsky, F.P., and Zelenskaya, M.G. (1952) Synthesis and transformations of Vinylcaprolactam Part 1. Polymerization in presence of hydrogen peroxide. Bull. Acad. Sci. U.S.S.R., Div. Chem. Sci., 4:633–636.
14. Ponce-Vargas, S.M., Cortez-Lemus, N.A., and Licea-Claverie, A. (2012) Preparation of poly(N-vinylcaprolactam) (NVCL) and statistical copolymers of NVCL with variable cloud point temperature.
by using a tritiocarbonate RAFT agent. *Macromol. Symp.*, 325–326:56–70.

15. Maeda, Y., Nakamura, T., and Ikeda, I. (2002) Hydration and phase behavior of poly(N-vinylcaprolactam) and poly(N-vinylpyrrolidone) in water. *Macromolecules*, 35:217–222.

16. Vihola, H., Laukkanen, A., Vihola, L., Tenhu, H., and Hirvonen, J. (2005) Cytotoxicity of thermosensitive polymers poly(N-isopropylacrylamide), poly(N-vinylcaprolactam) and amphiphilically modified poly(N-vinylcaprolactam). *Biomaterials*, 26:3055–3064.

17. Pelton, R.H., and Chibante, P. (1986) Preparation of aqueous lattices with N-isopropylacrylamide. *Colloid. Surf.*, 20:247–256.

18. Griffin, J.M., Robb, I., and Bismarck, A. (2007) Preparation and characterization of surfactant-free stimuli-sensitive microgel dispersions. *J. Appl. Polym. Sci.*, 104:1912–1919.

19. Boyko, V., Richter, S., Grillo, I., and Geissler, E. (2005) Structure of thermosensitive poly(N-vinylcaprolactam-co-N-vinylpyrrolidone) microgels. *Macromolecules*, 38:5266–5270.

20. Gao, Y., Au-Yeung, S.C.F., and Wu, C. (1999) Interaction between surfactant and poly(N-vinylcaprolactam) microgels. *Macromolecules*, 32:3674–3677.

21. Pich, A., Berger, S., Ornatsky, O., Baranov, V., and Winnik, M.A. (2009) The influence of PEG macromonomers on the size and properties of thermosensitive aqueous microgels. *Colloid. Polym. Sci.*, 287:269–275.

22. Imaz, A., and Forcada, J. (2008) N-vinylcaprolactam-based microgels: Synthesis and characterization. *J. Polym. Sci. Part A: Polym. Chem.*, 46:2510–2524.

23. Imaz, A., and Forcada, J. (2008) N-vinylcaprolactam-based microgels: Effect of the concentration and type of cross-linker. *J. Polym. Sci. Part A: Polym. Chem.*, 46:2766–2775.

24. Imaz, A., and Forcada, J. (2009) Optimized buffered polymerizations to produce N-vinylcaprolactam-based microgels. *Eur. Polym. J.*, 11:3164–3175.

25. Burchard, W. (1999) Solution properties of branched macromolecules. *Adv. Polym. Sci.*, 143:113–194.

26. He, E., Ravy, P., and Tam, K.C. (2007) Synthesis and self-assembly behavior of four-arm poly(ethylene oxide)-b-(poly(2-diethylamino)ethyl methacrylate) star block copolymer in salt solutions. *Langmuir*, 23:2382–2388.

27. Horcas, I., Fernández, R., Gomez-Rodriguez, J.M., Colchero, J., Gomez-Herrero, J., and Baro, A.M. (2007) WSXM: a software for scanning probe microscopy and a tool for nanotechnology. *Rev. Sci. Instrum.*, 78:013705.

28. Boyko, V., Richter, S., Pich, A., and Arndt, K.F. (2003) Poly(N-vinylcaprolactam) microgels. Polymeric stabilization with poly(vinyl alcohol). *Colloid. Polym. Sci.*, 282:127–132.

29. Pich, A., Boyko, V., Lu, Y., Richter, S., Adler, H.J., and Arndt, K.F. (2003) Preparation of PEGMA-functionalized latex particles. 2. System styrene/N-vinylcaprolactam. *Colloid. Polym. Sci.*, 281:916–920.

30. Serrano-Medina, A., Cornejo-Bravo, J.M., and Licea-Claverie, A. (2012) Synthesis of pH and temperature sensitive, core–shell nano/microgels, by one pot, soap-free emulsion polymerization. *J. Colloid Interface Sci.*, 369:82–90.

31. Obeso-Vera, C., Cornejo-Bravo, J.M., Serrano-Medina, A., and Licea-Claverie, A. (2013) Effect of crosslinkers on size and temperature sensitivity of poly(N-isopropylacrylamide) microgels. *Polym. Bull.*, 70:653–664.

32. Chiriac, A.P., Nita, L.E., and Nistor, M.T. (2011) Nanonetwork with dual temperature and pH responsiveness based on copolymers of 2-hydroxyethyl methacrylate with 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]-undecane. *J. Nanopart. Res.*, 13:6953–6962.

33. Qiu, X., and Sukhishvili, S.A. (2006) Copolymerization of N-vinylcaprolactam and glycidyl methacrylate: Reactivity ratio and composition control. *J. Polym. Sci. Part A: Polym. Chem.*, 44:183–191.

34. Kirsh, Y.E., Yanul, N.A., and Kalninsh, K.K. (1999) Structural transformation of water associate interactions in poly-N-vinylcaprolactam-water system. *Eur. Polym. J.*, 35:305–316.