

DYNAMIC LIGHT SCATTERING INVESTIGATION OF PNIPAM-co-MAA MICROGEL SOLUTION

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Received February 27, 2014; accepted March 20, 2014; published online March 25, 2014

The paper presents results of a study of the effect of the environment temperature and pH on the size of particles based on poly N (isopropylacrylamide) chain. The tested substance was the copolymer PNIPAM-co-MAA. The particle size measurements were performed by dynamic light scattering. It was found that the copolymer tested reacts specifically to temperature increase by shrinking more than two times. Important for stabilization of the structure are the chemical groups -COOH present in methacrylic acid that undergoes dissociation. Gradual increase in temperature results in a decrease in the dissociation constant, in binding of protons and thus causes shrinkage of the entire particle. It was also shown that PNIPAM-co-MAA in high concentrations undergoes crystallization.

INTRODUCTION

Nanotechnology is one of the fastest developing branches of science. Nanoparticles are defined as structures whose diameter ranges between 1 and 100nm (Ravichandran, 2009). In practice, molecules with diameters close to 1000nm are also treated as nanoparticles. One of the most interesting present or future applications of nanoparticles is in medicine as drug carriers for targeted drug delivery or as contrast agents (Sattler 2010).

Many classifications of nanoparticles for medical applications have been proposed. Faraji and Wipf have divided nanostructures for drug delivery into: inorganic particles, polymers, solid lipids, liposomes, nanocrystals and nanotubes (Faraji & Wipf, 2009). Polymers belong to the most popular materials, whose properties, such as particle size or addition of external or internal of reactive centers can be easily controlled.

Multi-stimuli responsive polymer nanoparticles have been recently widely studied as a promising material in medical application. The unique properties of poly(N-isopropylacrylamide) (PNIPAM) based polymers and copolymers predestined them as innovative drug carriers for drug delivery systems (Chen et al., 2012) diagnostic substance carriers and or as biosensors (i.e. nanothermometer) (Chen & Chen, 2011).

PNIPAM-based polymers undergo changes in conformation in response to external stimuli such changes in pH, temperature, ionic strength, salt concentration, electric and magnetic field, light or

ultrasounds (Meid et al 2012, Schmaljohann 2006). The materials which react specifically to changes in their environment are also called “smart materials” (Karg, Hellweg 2009). PNIPAM-base polymers exhibit the ability to change their properties from the hydrophilic, in low temperatures, to hydrophobic, in high temperatures. The critical temperature of the phase transition (LCST) is in the range of 32 – 34°C (Chen, Jiang, & Sun, 2013). The phenomenon of particle shrinkage is based on the dipole interaction between polymer chains, which can be used in specific immobilization of some antibodies (Zhou, Veron, Elaissari, Delair & Pichot, 2004). Also the hydrogen bonds play an important role in the shrinkage behavior (Kratz, Hellweg & Eimer, 2000), that may be caused by interaction of -COOH groups coming from the copolymeric part of the chain. The consequence of changes in these interactions is the binding of water by a hydrophilic molecule that causes its swelling below LCST and above LCST a sharp contraction of the molecule takes place and the water, previously bounded inside, is released to the environment. This process may be corrupted by addition of ethanol to the solution, because ethanol particles adhere to the polymer chain and substitute the water bonded inside the particle (Biswas et al, 2012). The effect of change in the size and release of the inner substance can be potentially used in controlled drug delivery systems in which PNIPAM would act as a carrier of an active substance or drug would release it in a targeted place (Eeckman et al, 2004, Margaret A. Phillips et al, 2010). The particles

may also take the complex form like core-shell type with a magnetic core and PNIPAM chain shell (Zhang & Wang, 2009). This composition allows transportation of the particle by magnetic field to the place of drug release and heats it up for a more effective PNIPAM collapse.

MATERIALS AND METHODS

1. Polymer synthesis

Polymer synthesis was made following the procedure described in (Ito et al, 1999). In brief P(NiPAM-co-MAA) was synthesized from NiPAM and methacrylic acid (MAA). The cross-linker was BIS (N,N-methylenebisacrylamide). The polymerization process was initiated by KPS (potassium persulfate). For the fluorescence tests the sample was dyed by rhodamine B. PNiPAM microgel solution was centrifuged, then freeze-dried.

2. Hydrodynamic radius

In this study, we measured the hydrodynamic radius of the particles by dynamic light scattering (DLS). The measuring system was based on a goniometer (ALV, Germany) with a correlator ALV/5000E and the avalanche photodiode (SPCM-AQR). The laser system used in this experiment was a HeNe 633 nm.

The principles of the DLS method are given in detail in (Berne & Pecora, 1976). In general the scattered light intensity fluctuates because of the Brownian motion of the particles. The intensity autocorrelation function is given by Eq. (1):

$$g^2(\tau) = B \left[1 + \beta |g^1(\tau)|^2 \right], \quad (1)$$

where B is the baseline, β is the coherence factor. The $g^1(\tau)$ stands for the 1-st order (field) autocorrelation function and for monodisperse solution is given by Eq. (2):

$$g^1(\tau) = \exp(\Gamma \tau), \quad (2)$$

Where Γ is proportional to the square of scattering angle q , Eq. (3):

$$\Gamma = Dq^2, \quad (3)$$

where D is the diffusion coefficient obtained from the experiment. For a mixture of a few components in solution the cumulant expansion of autocorrelation function might be calculated according to Eq. (4):

$$\ln g^1(\tau) = \ln A + \Gamma_1 \tau + \frac{1}{2!} \Gamma_2 \tau^2 + \frac{1}{3!} \Gamma_3 \tau^3, \quad (4)$$

This equation is used for the fitting procedure. On the basis of the constants from the cumulant expression, the polydispersity index (pdi) can be calculated from Eq. (5):

$$pdi = \frac{\Gamma_2}{\Gamma_1^2}, \quad (5)$$

Hydrodynamic radius is obtained indirectly from the diffusion coefficient given by the Stokes-Einstein Eq. (6):

$$D = \frac{k_B T}{6\pi\eta R_h}, \quad (6)$$

where k_B is the Boltzmann constant and η - the solvent viscosity, T - temperature, R_h - hydrodynamic radius.

For the tests PNIPAM-co-MAA was diluted with deionized water to a concentration of 0.02 mg / ml samples. The solutions were stabilized by buffers (Chempur) at pH 2-10. All tests, DLS and particle tracking were made in the temperature range 20-50°C at every 2 degree.

3. Temperature and pH dependence

The tests were made using a standard pH electrode and a resistive heater. The PNIPAM-co-MAA solution was prepared by dissolving dry polymer in Millipore water without buffer. The final concentration was 0.5mg/mL. The investigation was carried in a closed test-tube with an embedded pH electrode. The solution was heated and measured at every 0.2°C. The pH measurements were corrected for the effect of temperature changes.

4. Structural investigations

The structure of fluorescent PNIPAM particles was studied using confocal microscopy by a Zeiss Laser Scanning Microscope LSM780. The images were taken with a microscope water immersion objective ZEISS C-Apochromat 40x/1.2 W Corr M27. For the measurements, the chamber coverglass (8 WELL from LAB-TEK) and filled up slide channels (1 μ -Slide from Ibidi) were used.

RESULTS AND DISCUSSION

PNIPAM-co-MAA copolymer was selected for the test because it contains additional -COOH groups with respect to those in the native PNIPAM, and these groups

play an important role in the contraction of the molecule caused by pH and temperature changes. The choice of pH values was dictated by the pK_a of the methacrylic acid that is 4.25 (Tiwari *et al* 2012). Below the pK_a the polymer chains are neutral and carboxylic groups are not dissociated. Above the pK_a they became negatively charged, dissociated to COO^- and protons and highly hydrated because of the increase in osmotic pressure inside the particle (Himstedt, Marshall & Wickramasinghe, 2011) that may inhibit the particle collapse.

The DLS results presented in Figs 1-4 illustrate the apparent changes in particles size above the critical temperature. For pK_a below the critical value the initial particle size at 20°C is around 450 nm from DLS and it slightly decreases until reaching LCST. At the critical temperature a rapid particle contraction is observed. This behavior might be employed in systems of controlled drug delivery.

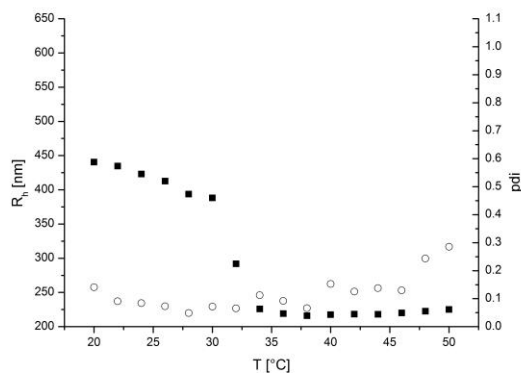


Fig. 1. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 2.

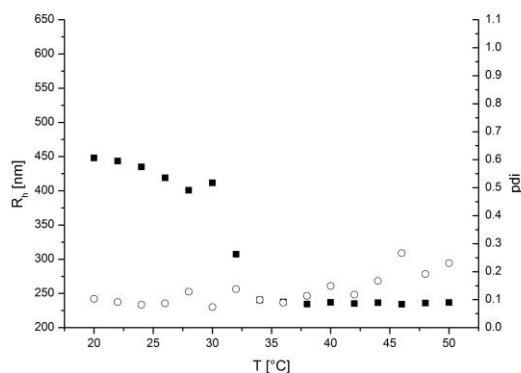


Fig. 2. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 3.

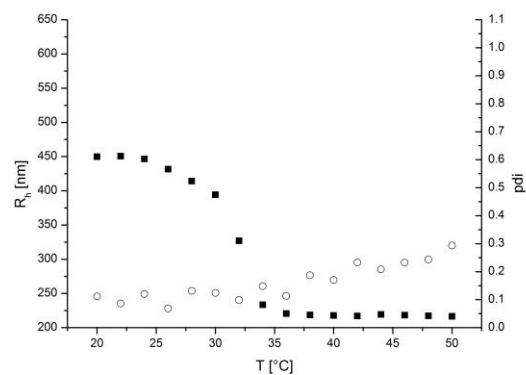


Fig. 3. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 4.

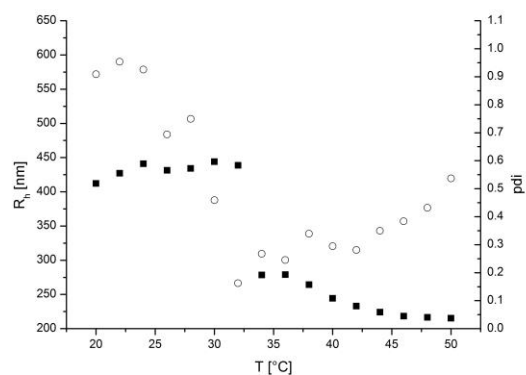


Fig. 4. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 5.

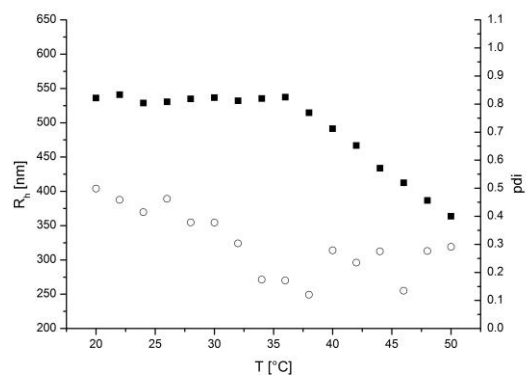


Fig. 5. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 6.

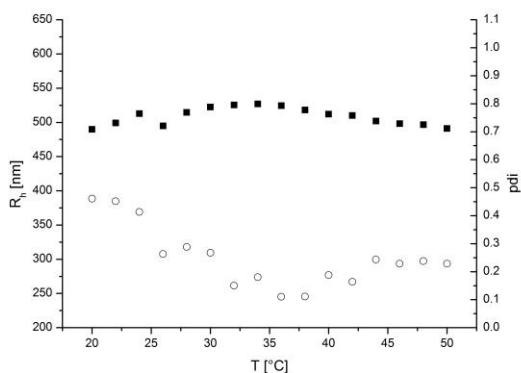


Fig. 6. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 7.

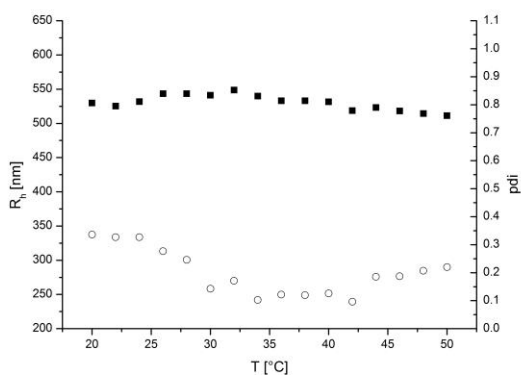


Fig. 7. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 8.

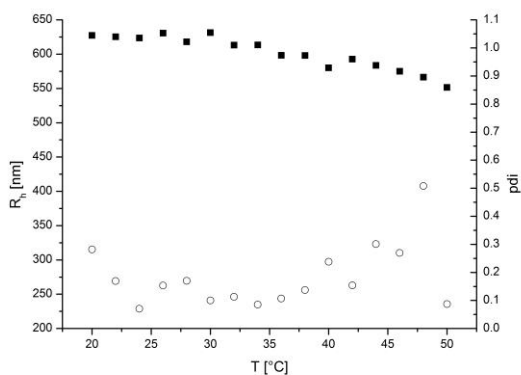


Fig. 8. Hydrodynamic radius (black squares) and polydispersity (white dots) index versus temperature changes at pH 9.

At pH higher than 5 (Figs 5-8), the average initial particle size is bigger than at pH lower than 5 and the LCST contraction is unobserved.

At pH lower than pK_a , the pDI is above 0.3 which

implies that the particles are quite monodisperse. At pH 5, pDI undergoes unexpected changes that may be interpreted as related to transition state of the molecule.

Above the critical temperature in stabilized environment changes in particle size were observed. This observation has prompted us to make an experiment under the change in pH in a solution without a buffer. Because change in particle size caused by heating involves modification in hydrophilic properties as a result of changes in the degree of dissociation of chemical groups in the solution, the change in pH in non-stabilized solution can be expected as a result of temperature changes.

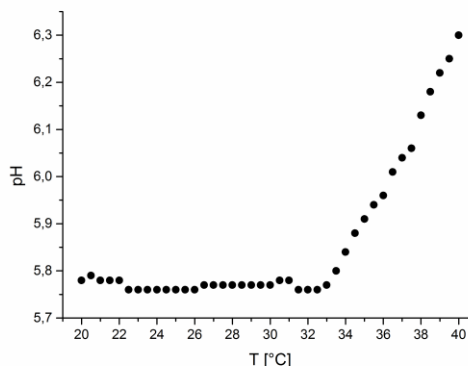


Fig. 9. Temperature dependence of pH

According to the results presented in Fig. 9, below LCST the pH changes are not observed. When temperature exceeds LCST a marked increase in pH is noticed that indicates attachment of protons to the dissociated $-\text{COO}^-$ groups.

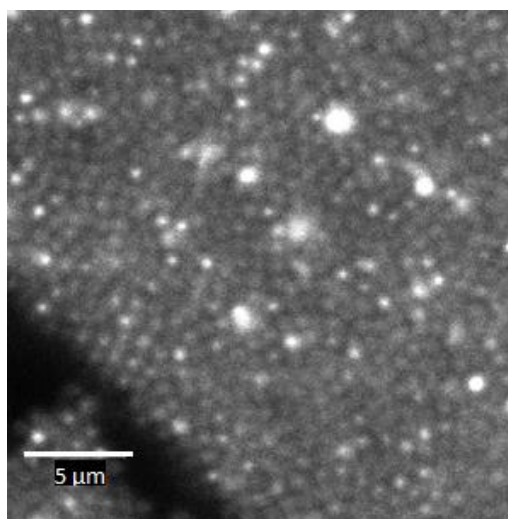


Fig. 10. LSM scan, PNIPAM-co-MAA dyed with rhodamine B.

PNIPAM-co-MAA particles were labeled with rhodamine B dye during the synthesis to make them detectable using confocal scanning fluorescence microscopy. The following pictures show the morphology of concentrated solution in water mixture. It can be seen that the particles are characterized by the ability to aggregate at high concentrations, Fig. 10.

CONCLUSION

The experiments have shown that the copolymer of PNIPAM and methacrylic acid exhibit volume phase transition accompanied with shrinkage in response to temperature change at acidic pH. The observed changes in the particle size are approximately 2-times. Above the pK_a of acrylic acid the size of PNIPAM-co-MAA particle is bigger and almost constant on the sample heating.

ACKNOWLEDGMENTS

Authors would like to thank Dzina Kleshchanok from SABIC Europe, Science and Technology Centre, Geleen for the synthesis of the sample, Adam Patkowski and Jacek Gapinski for help in analysis of the obtained results.

Tomasz Śliwa thanks the NanoBioMedical Centre in Poznań, Adam Mickiewicz University and Faculty of Physics where Ph.D. thesis is carried under the project co-financed by European Union, programme Human Capital National Cohesion Strategy funded by European Social Fund.

Maciej Jarzębski wishes to thank the Foundation for Polish Science and NanoBioMedical Centre in Poznań Adam Mickiewicz University, Faculty of Physics that the work was supported by the International PhD Projects Programme of Foundation for Polish Science operated within the Innovative Economy Operational Programme (IE OP) 2007-2013 within European Regional Development Fund. Financial support from National Centre for Research and Development under research grant "Nanomaterials and their application to biomedicine" contract number PBS1/A9/13/2012, is gratefully acknowledged.

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