Stimuli-responsive nanogels by e-beam irradiation of dilute aqueous micellar solutions: Nanogels with pH controlled LCST

L. A. Picos-Corrales[*], A. Licea-Claverie[*] and K. F. Arndt[**]

[*] Instituto Tecnológico de Tijuana, Centro de Graduados e Investigación en Química, A.P. 1166, C.P. 22000 Tijuana, B.C., Mexico, aliceac@tectijuana.mx

[**] Dresden University of Technology, Department of Chemistry, Physical Chemistry of Polymers, D-01062 Dresden, Germany, karl-friedrich.arndt@chemie.tu-dresden.de

ABSTRACT

Stimuli-responsive nanogels were prepared starting with amphiphilic block copolymers that were crosslinked in the micellar state by means of electron beam irradiation. The materials were thoroughly characterized by dynamic and static light scattering before and after the irradiation. The effect of the irradiation dose and the polymer concentration were tested. The size of the nanogels decreased slightly with increasing irradiation dose. The resulting nanogels have a T/pH-sensitive shell and a hydrophobic core that could be used as a reservoir for drugs. Nanogels were also prepared starting from a mixture of different block copolymers showing a temperature and pH sensitive behavior with characteristics of an ideal drug carrier.

Keywords: nanogels, e-beam irradiation, crosslinked micelles, light scattering, temperature sensitive polymers.

1 INTRODUCTION

Nanogels are nanometric sized swollen polymer networks with particular physicochemical properties such as good solubility in both organic and aqueous solvents, stability in a broad range of pH and temperatures, good degree of flexibility and high surface/volume ratio [1]. These properties makes them candidates for applications in controlled drug delivery, regenerative medicine, bioimaging and sensors [2,3]. Sensitive nanogels as carriers for the transport of drugs could improve the efficacy of therapies [4].

The potential applications of nanogels have increased the interest in developing synthetic strategies that allow for a successful preparation of nanogels with defined morphology, controlled size and functionality. The synthesis of chemically crosslinked micro- and nanogels from monomers and/or preformed polymers, have been carried out by means of radical crosslinking copolymerization techniques and irradiation methods. For example, heterogeneous free radical polymerization, reversible addition-fragmentation chain transfer (RAFT), atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP) [5,6], electron beam irradiation [7,8], irradiation with gamma rays [9,10], and irradiation with ultraviolet light [11,12] have been used. With those synthetic strategies, homogeneous [7] and core-shell [8,9] nanogels have been prepared. Electron beam irradiation has become a powerful tool for the preparation of nanogels. The procedure applied is usually based on the irradiation of dilute polymer solutions with a dose sufficient to obtain nanogels. When dilute aqueous polymer solutions are subjected to ionizing radiation, polymer radicals are formed, which may decay by disproportion and recombination (crosslinking). Depending on the polymer concentration and on the dose rate, two different crosslinking reactions can take place: inter- and intramolecular crosslinking. If the irradiation dose is high and the distance between single polymer chains is long, the recombination of the formed radicals results mainly in intramolecular crosslinking [7]. Electron beam irradiation can be used as a standard tool for the formation of nanogels starting with aggregates of amphiphilic copolymers locking herewith the aggregate structure [8].

2 EXPERIMENTAL PART

2.1 Materials

Amphiphilic block copolymers were prepared by RAFT polymerization following a synthetic strategy described previously [13]. PNIPAAm-*b*-PSt (22,120 g/mol; 84:16 mol%), PNIPAAm-*b*-PHA (23,440 g/mol; 81:19 mol%), poly(NIPAAm-co-5MPA)-*b*-PSt (24,030 g/mol; 79:11:10 mol%) and poly(NIPAAm-co-5MPA)-*b*-PHA (26,640 g/mol; 85:7:8 mol%) were used (Figure 1); where HA is *n*-hexylacrylate, NIPAAm is *N*-isopropylacrylamide, St is styrene and 5MPA is 5-methacryloyl-oxy-pentanoic acid.

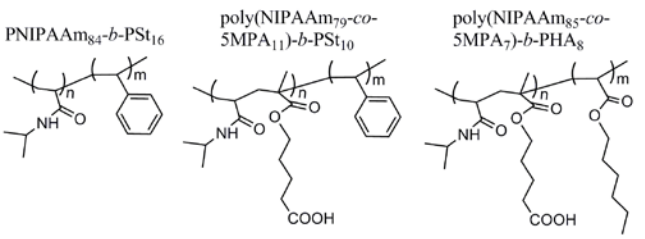


Figure 1. Amphiphilic block copolymers used.

2.2 Preparation of Nanogels

Aggregates were prepared either via direct dispersion in water or Buffer of pH 7.4 (copolymers containing acid comonomers); or laternatively by an emulsion method consisting in disolving the block-copolymers in dichloromethane followed by drop by drop addition of the solution to water/Buffer of pH 7.4, under vigorous stirring until dichloromethane was evaporated. In all cases a concentration of 1 mg/mL of amphiphilic block copolymer in aqueous media was prepared. Dispersions were degassed by means of argon flux. The samples were irradiated by e-beam (Figure 2) at a dose of 30, 60 or 120 kGy at room temperature in oxygen-free closed PE-bags. The electron beam irradiation was performed with an accelerator ELV-2 (Budker Institute of Nuclear Physics, Novosibirsk, Russia). The accelerator was operated with an energy of 1.0 MeV.

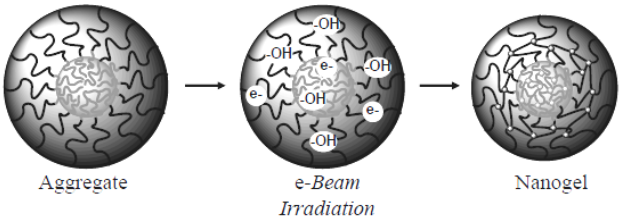


Figure 2. Nanogels prepared by e-beam irradiation

2.3 Characterization

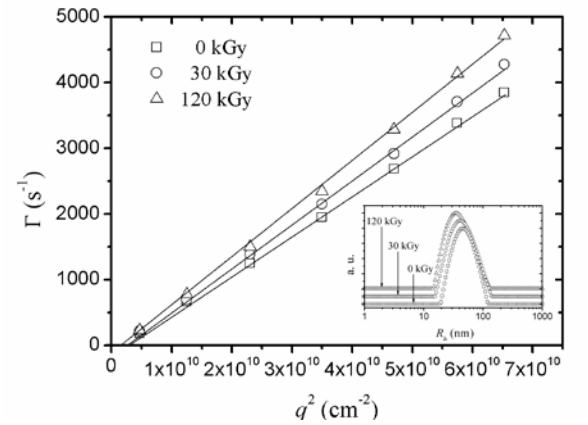
Weight-average molecular weights (M_w) and radius of gyration (R_g) were determined by static light scattering (SLS) using a modified FICA 50 (SLS Systemtechnik G. Baur, Denzlingen, Germany) apparatus, with a laser of λ=632.8 nm at scattering angles between 50 and 140° in steps of 5°. Dynamic light scattering (DLS) was used to determine the hydrodynamic radius (R_h) and polydispersity index (PDI=μ_2/Γ'^2, from the cumulant method). Measurements were carried out at 25 °C using a laser light scattering spectrometer (ALV/DLS/SLS-5000) equipped with an ALV-5000/EPP multiple digital time correlator, laser goniometer system ALV/CGS-8F S/N 025 and a helium-neon laser (Uniphase 1145P, output power of 22 mW, wavelength 632.8 nm). Measurements were performed at scattering angles between 50 and 130° in steps of 20°. In all cases, polymer aqueous solutions/dispersions of 1 mg/mL were prepared, then filtered off using a 0.45 microns syringe filter for eliminating dust, and measured.

3 RESULTS AND DISCUSSION

3.1 Preparation of nanogels starting with poly(NIPAAm-*co*-5MPA$_{5\%}$)-*b*-PHA

Amphiphilic block copolymers self-assemble in aqueous environments to micelle like aggregates. Using this behavior, the title amphiphilic block copolymer was irradiated in the micellar state by electron beam at high irradiation dose to lock the nanostructure obtaining core-shell nanogels. In this case, inter and intramolecular crosslinking may take place. The SLS and DLS analysis of *Net*-[poly(NIPAAm-co-5MPA$_{5\%}$)-*b*-PHA] prepared at different doses (30, 60 and 120 kGy) in buffer of pH 7.4 are shown in Figure 3. In DLS, the linear relationship indicates that the samples have a single diffusivity and thus have a narrow size distribution (Figure 3A) [14]. In SLS, the linear dependence on the scattering angle is also observed (Figure 3B). The radius of gyration (R_g) and hydrodynamic radius (R_h) decrease slightly with increasing irradiation dose. The size of the nanogels decreases due to the increased crosslinking density. The values of A_2 decrease with increasing irradiation dose, this is an indication that the interactions within the polymer segments are preferred to the interaction between the polymer segments and the solvent molecules (Figure 4).

A)



B)

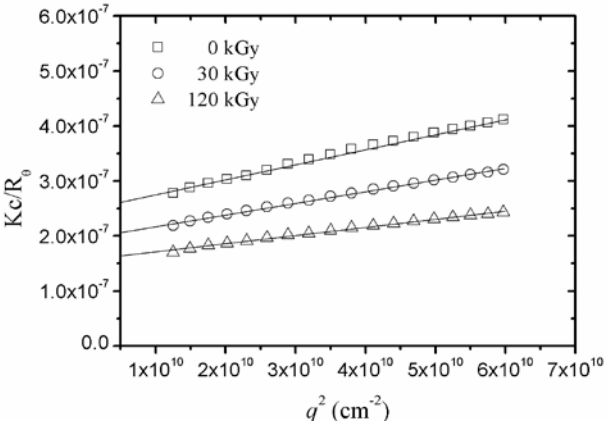


Figure 3. DLS (A) and SLS (B) of nanogels prepared at different irradiation doses.

In Figure 5 the sensitive behavior of these samples can be observed. At pH 7.4, the nanogels showed thermal stability in the range of 24 to 46 °C. However, at pH 5.8 a decrease in radius with increasing temperature is observed for samples irradiated at 30 kGy and 60 kGy. The sample irradiated with less energy (30 kGy) underwent the largest contraction with temperature: R_h=41 nm at 24 °C; R_h=35 nm at 38 °C and R_h=33 nm at 46 °C (at pH 5.8). A transition temperature of 30 °C can be postulated although a sharp transition was not observed. At a lower pH (4.0) the same sample exhibited a lower transition temperature of 18 °C inline with the fact that at a lower pH the acid groups of 5MPA units in the polymer are less ionized yielding the nano polymer network more hydrophobic. The sample irradiated at 60 kGy showed, at pH 5.8, a linear decrease in size with temperature within the range of 24 to 42 °C at pH 5.8. A transition temperature cannot be postulated while temperature sensitivity is observed. The sample irradiated with the highest dose used in this study (120 kGy) showed no temperature sensitivity, suggesting a very rigid polymer network. In all cases, aggregation of the nanogels was not observed in the temperature range studied, indicating no LCST. As comparison, without irradiation the block copolymer used for this experiments, showed at pH 5.8 a transition temperature of 38 °C followed by self-aggregation and precipitation (LCST); while no temperature sensitivity was observed at pH 7.4.

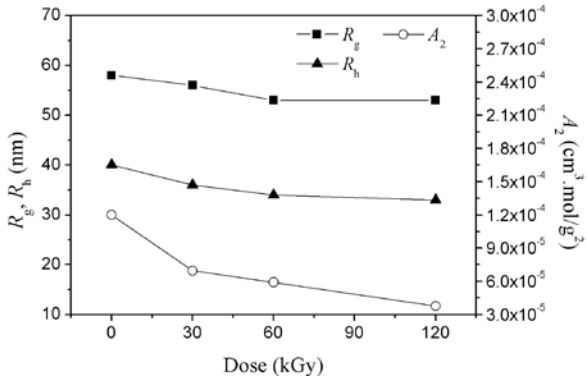


Figure 4. Effect of the irradiation dose on size.

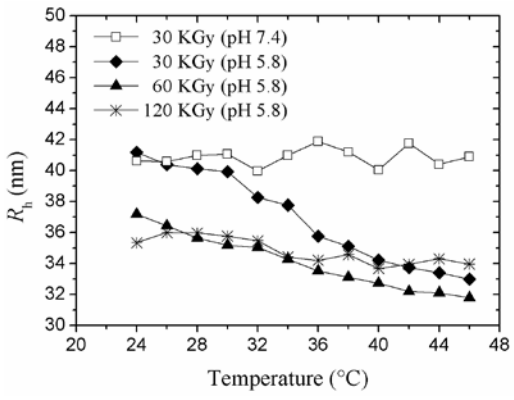


Figure 5. Analysis of thermosensitivity of nanogels.

3.2 Preparation of nanogels starting with a mixture of block copolymers

With the goal in mind to fine tune the temperature response of the nanogels, to mimic the behavior of an ideal drug carrier; mixtures of block copolymers were irradiated. For that, the hydrophilicity of the nanogels was decreased starting with a mixture (50:50 wt.%) of block copolymers with the same type of hydrophobic block. The mixtures consisted of PNIPAAm-*b*-PSt and poly(NIPAAm-co-5MPA)-*b*-PSt on the one side; and PNIPAAm-*b*-PHA and poly(NIPAAm-co-5MPA)-*b*-PHA, on the other side. Without irradiation the block copolymers non containing acid comonomer showed a transition temperature in water of 32 °C and no pH effect on that. For the block copolymers containing acid comonomers at pH 5.8 a transition temperature of 38 °C and 40 °C was observed for poly(NIPAAm-co-5MPA)-*b*-PHA and poly(NIPAAm-co-5MPA)-*b*-PSt, respectively; and no transition temperature at pH 7.4. The mixtures of block copolymers were irradiated at a dose of 30 kGy in buffer of pH 7.4. Characteristics of the obtained nanogels are shown in the columns of Tables 1 and 2. The hydrodynamic radii of nanogels are smaller than for aggregates. The nanogels have a high molecular weight. The values of second virial coefficient indicate a good interaction between the nanogels and the solvent. The polydispersity index of these nanogels was close to 0.20. The ρ-ratio of the nanogels is consistent with the experimental values obtained for core-shell morphology of flexible polymers [15].

Table 1. Radii of aggregates and nanogels.

Nanogel	$R_{h(0\ kGy)}$ (nm)	$R_{h(30\ kGy)}$ (nm)	$R_{g(30\ kGy)}$ (nm)
Net-[Blend-PSt]	29	21	28
Net-[Blend-PHA]	39	23	31

Table 2. Molecular weight and ρ-parameter of nanogels.

Nanogel	M_w (g/mol)	A_2 (cm^3.mol/g^2)	ρ ($R_g/R_{h,\ 0\rightarrow 0}$)
Net-[Blend-PSt]	388,000	0.0015	1.22
Net-[Blend-PHA]	239,000	0.0053	1.29

In Figure 6 the sensitive behavior of these nanogels is observed. At pH 7.4, both types of nanogels showed thermal stability between 24 and 46 °C. At pH 5.8, the hydrophilicity of nanogels decreases with decreasing ionization of carboxylic acid. Both samples exhibit a transition temperature followed by self-agregation and precipitation (LCST) at 33 °C (pH 5.8). Therefore, these nanogels are stable at 37 °C and pH 7.4 (normal physiological conditions), but unstable at 37 °C and pH 5.8

(endosomal pH) Therefore this nanogels are candidates for drug delivery into the cell. This study demonstrates that the mixture of block copolymers with different LCST allows for optimizing the T/pH-sensitive behaviour of nanogels. At pH 5.8, the nanogels formed starting with 100 wt.% of block copolymers with acid groups did not showed a clear phase transition, but a more or less continuous shrinkage. However, blend-nanogels exhibit a phase transition because their hydrophobic-hydrophilic balance is more adequate for self aggregation. These results open the door for adjusting the temperature sensitivity by mixing block-copolymers having different sensitivity behavior into "whish sensitive" nanogels.

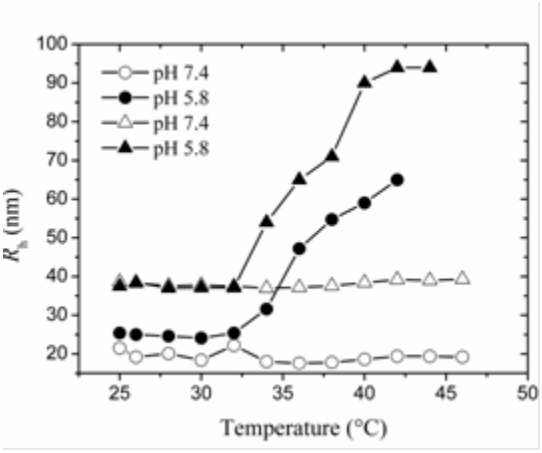


Figure 6. LCST study of *net*-[Blend-PSt] (○, pH 7.4; ●, pH 5.8) and *net*-[Blend-PHA] (Δ, pH 7.4; ▲, pH 5.8).

4 CONCLUSIONS

Core-shell nanogels based on amphiphilic block copolymers crosslinked in the micellar state were prepared successfully by means of electron beam irradiation. The size of *net*-[poly(NIPAAm$_{85\%}$-*co*-5MPA$_{7\%}$)-*b*-PHA$_{8\%}$] decreased slightly with increasing irradiation dose (R_h=40 nm at 0 kGy; R_h=36 nm at 30 kGy and R_h=33 nm at 120 kGy). In the explored concentration range (0.25-1.0 mg/mL), the size of the nanogels did not showed a strong dependence on the block copolymer concentration. The nanogels have suitable sizes for their application as controlled drugs delivery systems (10-100 nm). The variation of size as a function of temperature decreases with increasing radiation dose, because of the increase in the rigidity of the structure. *Net*-[poly(NIPAAm$_{85\%}$-*co*-5MPA$_{7\%}$)-*b*-PHA$_{8\%}$] prepared at 30 kGy underwent more variation of size depending on the temperature (R_h=41 nm at 24 °C; R_h=35 nm at 38 °C; R_h=33 nm at 46 °C) at pH 5.8. Nanogels based on mixtures of different block copolymers showed a temperature and pH sensitive behavior that mimics an ideal drug carrier; they are stable at physiological conditions (pH 7.4 and 37 °C) but unstable at pH 5.8 and 37 °C (endosomal pH).

ACKNOWLEDGEMENTS

L.A. Picos-Corrales thank DAAD for financing his PhD sandwich program. This investigation was supported by CONACYT (Mexico) SEP2006-60792. Technical support by Franziska Krahl, Stefan Lehmann and David Rupsch (TU- Dresden), I.A. Rivero and A. Ochoa (IT-Tijuana), is gratefully acknowledged.

REFERENCES

[1] M. Resmini, K. Flavin and D. Carboni, "Microgels and nanogels with catalytic activity", Springer, 1-37, 2010.
[2] A. V. Kabanov and S. V. Vinogradov, Angew. Chem. Int. Ed., 48, 5418-5429, 2009.
[3] Y. Sasaki and K. Akiyoshi, The Chemical Record, 10, 366-376, 2010.
[4] J. H. Park, S. Lee, J. H. Kim, K. Park, K. Kim and I. C. Kwon, Prog. Polym. Sci., 33, 113-137, 2008.
[5] N. Sanson and J. Rieger, Polym. Chem., 1, 965-977, 2010.
[6] J. K. Oh, R. Drumright, D. J. Siegwart and K. Matyjaszewski, Prog. Polym. Sci., 33, 448-477, 2008.
[7] T. Schmidt, I. Janik, S. Kadlubowski, P. Ulanski, J. M. Rosiak, R. Reichelt and K. F. Arndt, Polymer, 46, 9908-9918, 2005.
[8] S. Zschoche, J. Rueda, V. Boyko, F. Krahl, K. F. Arndt and B. Voit, Macromol. Chem. Phys., 211, 1035-1042, 2010.
[9] T. Narita, K. Terao, T. Dobashi, N. Nagasawa and F. Yoshii, Colloids. Surf. B, 38, 187-190, 2004.
[10] G. Valdés-Díaz, S. Rodríguez-Calvo, A. Pérez-Gramatges, M. Rapado-Paneque, F. A. Fernandez-Lima, C. R. Ponciano and E. F. da Silveira, J. Colloid Interface Sci., 311, 253–261, 2007.
[11] D. Kuckling, C. D. Vo, S. E. Wohlrab, Langmuir, 18, 4263-4269, 2002.
[12] S. Yusa, M. Sugahara, T. Endo, Y. Morishima, Langmuir, 25, 5258-5265, 2009.
[13] L. A. Picos-Corrales, A. Licea-Claverie, J. M. Cornejo-Bravo, S. Schwarz, K. F. Arndt, Macromol. Chem. Phys., 213, 301-314, 2012.
[14] A. E. Smith, X. Xu, S. E. Kirkland-York, D. A. Savin and C. L. McCormick, Macromolecules, 43, 1210-1217, 2010.
[15] J. Yun, R. Faust, L. S. Szilágyi, S. Kéki and M. Zsuga, Macromolecules, 36, 1717-1723, 2003.