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Self-assembled polymer particles based on thermoresponsive biodegradable copolymers of amino acids

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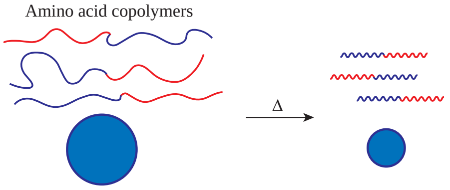
Thermoresponsive amphiphilic PGlu$_{30}$-*b*-PSer$_t$-PSer(OCONHPh)$_p$ copolymers with different hydrophobic unit content were synthesized, characterized and applied for preparation of biodegradable soft particles.



For recent decades, stimuli-responsive (so called ‘smart’) polymers have been widely investigated with respect to the construction of modern drug delivery systems.[1–3] Such polymers are capable of undergoing a reversible phase transition in response to external stimulus, *e.g.*, parameters of media (pH, temperature, ionic strength, *etc*.).[4–6] Amphiphilic block copolymers attract a particular interest due to their ability of self-assembling at supramolecular scale, hydrophilic-hydrophobic interactions, as well as the phase transitions of the blocks in different media, which provide stimuli-responsive properties.[7–10] The use of poly(amino acids) as building blocks for such macromolecular compounds allows one to construct biocompatible and biodegradable drug delivery systems along with their own biological activity, additional functionalities on the surface and controllable phase transition ability due to α-helix–random coil transition and *vice versa*.[11–13] The most common way to obtain poly(amino acid) block copolymers is a stepwise polymerization of *N*-carboxy anhydrides of appropriate amino acids.[11,14–16] The corresponding macromolecular product is acquired by removing the protective groups from a hydrophilic block.

In this research we synthesized the biodegradable thermoresponsive amphiphilic block copolymers containing hydrophilic poly(L-glutamic acid) and poly(L-serine) blocks further modified with hydrophobic fragments, and investigated the possibility of preparation of polymer-based particles.

The synthesis of block copolymer included three steps: (i) the synthesis of homopolymer of γ-benzyl-L-glutamate [PGlu(OBzl)] from its *N*-carboxy anhydride using hexylamine as initiator, (ii) the polymerization of L-Ser(Bzl) from corresponding *N*-carboxy anhydride using PGlu(OBzl) homopolymer as macroinitiator, (iii) the removal of side protective groups with CF_3COOH/CF_3SO_3H mixture (in a ratio of 9:1) (Scheme 1). Molecular mass characteristics of homo- and copolymers were determined using gel permeation chromatography (GPC).[†] The M_n and M_w values for PGlu were 4100 and 4900, respectively. The same characteristics for PGlu-*b*-PSer copolymer obtained were established as 11500 and 15000, respectively. Thus, the synthesized copolymer was characterized with enough narrow molecular weight distribution (M_w/M_n = 1.3) and calculated polymer composition as PGlu$_{30}$-*b*-PSer$_{80}$.

To prepare amphiphilic copolymer based on PGlu$_{30}$-*b*-PSer$_{80}$, PSer block was modified with phenyl isocyanate (see Scheme 1). The modification of the block copolymer was carried out at 50 °C in DMF in the presence of triethylamine using phenyl isocyanate as modifier. The structure of modified [PGlu-*b*-PSer(OCONHPh)] was confirmed by FTIR spectroscopy.[‡] Varying the copolymer/modifier functional group ratio (OH/NCO) as 1:1, 1:2 and 1:4 allowed us to prepare PGlu$_{30}$-*b*-PSer$_{66}$-PSer(OCONHPh)$_{14}$, PGlu$_{30}$-*b*-PSer$_{53}$-PSer(OCONHPh)$_{27}$ and PGlu$_{30}$-*b*-PSer$_{27}$-PSer(OCONHPh)$_{53}$ copolymers (GS1, GS2 and GS3, respectively).

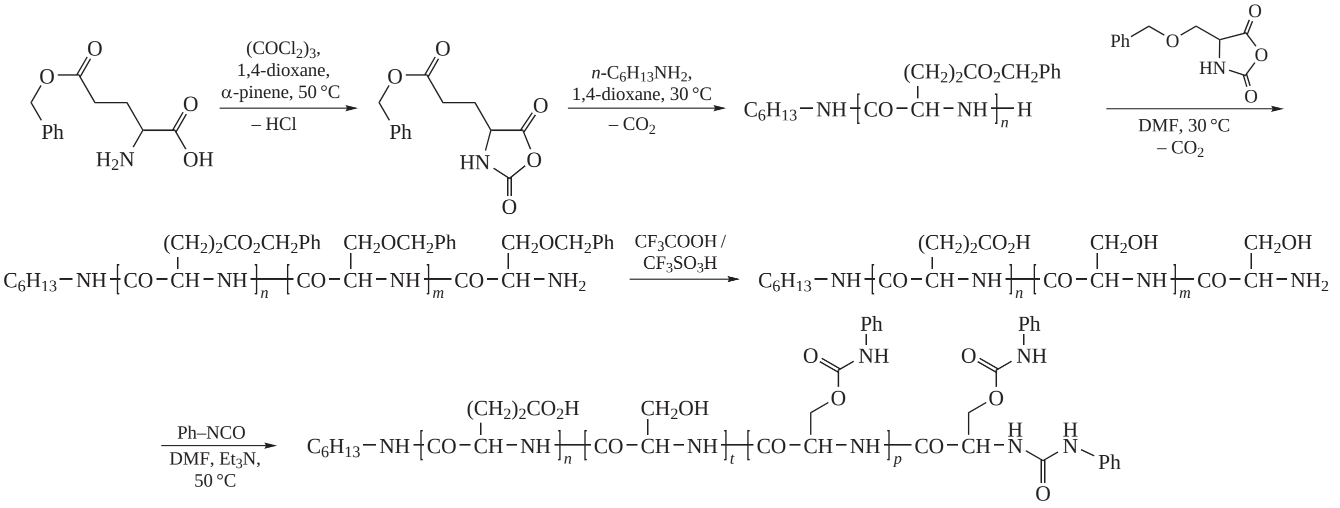
The particles were obtained using the solvent inversion method *via* dialysis from DMSO against 0.1 M phosphate buffer solution (PBS) at pH 7.4 followed by lyophilization. The dispersions of appropriate concentration were prepared by sonication of the copolymer sample in buffer solution.[§]

In the series of copolymers GS1, GS2 and GS3 at 25 °C, the hydrodynamic diameters of the particles in 0.1 M PBS (pH 7.4) were 1296±88, 1131±111 and 960±95 nm, respectively. PDI for all samples was lower than 0.3. These data show only slight influence of hydrophobic part content (13, 25 or 48%) on the particle size. The ζ-potential value of all samples at 25 °C was close to –40 mV. The preparation of particles under more alkaline conditions (pH 8.7) at the same temperature led to reducing of the particle size to 599±83 nm. The decrease in size of nanoparticles is caused by different behavior of self-assemble process at different pH. The higher pH provides the higher level of carboxylic group ionization. In turn, it favors the repulsion between negatively charged polymeric chains that prevents the self-assembly into large nanoparticles.

[†] GPC was performed on a Shimadzu LC-20 Prominence instrument equipped with refractometric detector RID 10-A (Japan) and Styragel, 7.8×300 mm column, HMW 6E, 15–20 μm (USA) at 60 °C with 0.1 M LiBr in DMF as a mobile phase (Figure S1, see Online Supplementary Materials). The flow rate was 0.3 ml min^{-1}. Poly(methyl methacrylate) standards with M_w in the range 17000–250000 and M_w/M_n ratio no more than 1.14 were applied for calibration.

[‡] FTIR spectroscopy was carried out on Shimadzu IR Prestige-21 in KBr in 4000–400 cm^{-1} region (Figure S2).

[§] Dynamic light scattering measurements for determination of hydrodynamic parameters and ζ-potentials of the particles were made on Zetasizer ZS (Malvern, Great Britain) at angle 113° and using colloids containing 0.5 mg ml^{-1} of copolymer; the temperature range was 25–38 °C.



Scheme 1

The influence of the temperature on the particle size determined in the range of 25–38 °C is presented in Figure 1. When the temperature arose up to 27 °C and higher, the hydrodynamic particle size decreased approximately by a factor of 3. This effect can be caused by the change of poly(L-glutamic acid) block conformation from extended random coil to the compact ordered structures. Similarly to the proteins, the folding of random coil structures of synthetic polypeptides into α-helixes or β-sheets leads to macromolecule compaction.[13] The presence of so-called lower critical solution temperature above which the polypeptide changes shape from random coil to ordered conformation was previously shown[17] for elastin-mimetic di- and tri-block peptide polymers. Particularly, it was established by differential scanning calorimetry (DSC) that the transition temperature for [(Val-Pro-Gly-Val-Gly)$_4$Val-Pro-Gly-Ile-Gly]$_{16}$ polypeptide was 28 °C.[17]

In our case, DSC experiment was also applied to determine the temperature at which the conformational transition as well as the change of polymer particle size occurred (see Figure 1).¶ The conformation transition followed by a decrease in hydrodynamic diameter of particles was detected at 26.7 °C.

The ζ-potential value remains the same with the temperature increase, which reveals no sufficient influence of temperature on the colloid stability. The GS1 and GS3 samples demonstrated a similar trend.

In conclusion, the herein suggested method for the preparation of amphiphilic block copolymers makes it possible to regulate hydrophobic/hydrophilic balance in the polymer along with the amount of the neutral/charged functionalities on the particle surface. The obtained PGlu$_{30}$-*b*-PSer$_t$-PSer(OCONHPh)$_p$ block copolymers are thermoresponsive due to conformation change of polypeptide. Such kind of block-copolymers can be of interest with regard to the modern drug delivery system creation.

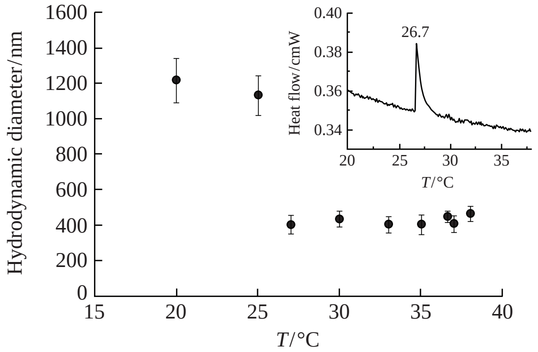


Figure 1 Influence of the temperature on hydrodynamic diameter of GS2 particles. Conditions: 0.1 M PBS, pH 7.4, concentration 0.5 mg ml^{-1}. Insert: DSC analysis.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2017.03.015.

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¶ The DSC experiment was carried out using Setaram μDSC 3 EVO (Setaram Instrumentation, France) at temperature span of 10–45 °C.