Biosensing with thermosensitive fluorescent quantum dot-containing polymer particles

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ABSTRACT

In the past decades, increasing attention has been paid to the preparation of “smart” functionalized polymer particles reversibly responding to slight environmental changes, such as variations in temperature, pH, and ionic strength. The composite polymer particles consisting of a solid poly(acrolein-co-styrene) core and a poly(N-vinylcaprolactam) (PVCL) polymer shell doped with CdSe/ZnS semiconductor quantum dots (QDs) were prepared. The thermosensitive response of the composite particles was observed as a decrease in their hydrodynamic diameter upon heating above the lower critical solution temperature of the thermosensitive PVCL polymer used as a shell. Embedding QDs in the PVCL shell makes it possible to obtain particles whose fluorescence is sensitive to temperature changes. The temperature-dependent fluorescence of particles was determined by reversible variation of the distances between QDs in the PVCL shell as a result of temperature-driven conformational changes in this polymer. In addition, these particles can be used as carriers of biomolecule (e.g., bovine serum albumin, BSA) characterized by reversibly temperature-dependent fluorescence, which can serve as the basis for optical detection methods in bioassays, such as the measurement of local temperature in nanovolumes, biosensing, etc.

Keywords: colloidal nanocrystals, thermosensitive polymer, biosensing, quantum dots, local temperature

1. INTRODUCTION

Dispersions of polymer particles have implications in various fields of analytical chemistry, biosensing, and clinical diagnosis, since they are characterized by a large surface area of particles, ease of fabrication, functionality, and portability. Recently, increasing attention has been paid to the preparation of functionalized “smart” polymer particles that can reversibly respond to very slight changes in their environment, such as temperature, pH, and ionic strength variations. In particular, unique properties of thermosensitive polymer particles (TPPs) determine their diversified applications, especially in biology, such as the measurement of local temperature in a single cell and in volumes less than 10^{-18} m^3.

At present optical detection methods are becoming the technological frontier. In this connection, the design of thermosensitive polymer particles containing an optical label with temperature-dependent properties attracts much attention. For example, photoluminescent (PL) nanocrystals, such as CdSe/ZnS semiconductor nanocrystals known as quantum dots (QDs), a considered promising as labels for optical detection methods that are based on changes in the fluorescence intensity and/or peak position of QDs. Along with high quantum yields and an exceptional resistance to
both chemical degradation and photodegradation, QDs of different sizes can be excited efficiently at a single wavelength while emitting at distinctly different wavelengths in the visible and near-IR regions with characteristic narrow, strictly symmetrical spectra, thus allowing multicolor detection.

The QD fluorescence intensity exhibits a linear temperature response, which is sensitive to QD local environment. Interestingly, QD-capping agents are known to make QD fluorescence temperature-insensitive. For example, QD fluorescence intensity becomes independent of temperature both when denatured ovalbumin is used as a capping agent and when QDs are embedded in polymer particles.

The excellent fluorescent properties of QDs for temperature detection can be used, as reported by Lee and Kotov, in superstructures based on assemblies of QDs connected with molecular springs. Assemblies of QDs can be obtained by surface modification followed by linking of surface functional groups with polymers or suitable biomaterials. An example of such a superstructure whose PL intensity exhibits reversible changes in response to the temperature variation has been described. These authors have developed a reversible nanothermometer based on a dynamic superstructure of two types of nanoparticles; it consists of a central gold core and a corona-like structure of CdTe QDs. These are linked via a polyethylene glycol spacer acting as a temperature-dependent molecular spring in the aqueous medium.

Here, we describe a superstructure that makes it possible to use only one type of nanoparticles, namely QDs, and replace the expensive gold core whose diameter is practically invariable with a synthetic polymer core of a required functionality whose diameter is easily controlled by the synthetic procedure. The superstructure is based on advanced architecture consisting of a colloidal solid polymer core and a shell containing QDs. In order to make the distance between QDs, which determines the fluorescence intensity, changeable, we covered the core with a shell of a temperature-sensitive “smart” polymer. Such polymers exhibit critical phenomena, including phase transitions, in response to external stimuli, e.g., changes in temperature. They undergo reversible conformational or phase-separation changes at temperatures above the so-called lower critical solution temperature (LCST).

We chose poly(acrolein-co-styrene) as the material for this core, because its outer layer bears double bonds, which make it easy to form a shell over it. The shell consisted of the temperature-sensitive polymer poly(N-vinylcaprolactam) (PVCL). Fluorescent TPP can be obtained by embedding QDs in the PVCL shell. Temperature variation about LCST causes conformational changes in PVCL and, hence, changes in the distances between QDs, which, in turn, results in fluorescence changes (Fig. 1).

Figure 1. Engineering of thermosensitive polymer particles

These fluorescence changes may serve as a basis for optical detection methods in bioassays. Thus, the purpose of this study was to design polymer particles with thermosensitive fluorescence and estimate their properties, in particular, as carriers of biologically active compounds (exemplified by bovine serum albumin, BSA) and as nanothermometers for measuring local temperature changes (exemplified by monitoring the temperature in the course of chemical reactions).

2. MATERIALS AND METHODS

2.1 Materials

Acrolein was purchased from Fluka, Germany. It was distilled three times at the atmospheric pressure, and the fraction with a boiling point of 56°C, ρ = 0.806 g/cm³, and ng = 1.40 was used. Styrene was also purchased from Fluka, Germany, purified with a 5% sodium hydroxide aqueous solution to remove the stabilizer, rinsed with water until pH
became neutral, dried over calcium chloride, and distilled twice in vacuum. The fraction with a boiling point of 51°C (2.1 kPa), ρ_420 = 0.906 g/cm³, and nD_20 = 1.54 was used.

The following materials were purchased from Sigma–Aldrich and used without further purification: N-vinylcaprolactam (VCL), potassium persulfate (PP), α,α'-azo-isobutyronitrile (AIBN), sodium chloride, sodium borate buffer, bovine serum albumin (BSA), and sodium azide. Ethanol, methanol, propanol-2, and chloroform (Aldrich) were of analytical grade.

Semiconductor CdSe/ZnS core–shell nanocrystals were synthesized as described earlier 19. In the present study, hydrophobic nanocrystals with diameters of 3.5 nm (with a PL emission peak at 554 nm), and 6 nm (with a PL emission peak at 610 nm) were used. Their PL was excited at λ_ex = 480 nm in all experiments.

2.2 Methods

Optical and fluorescent characteristics were measured using an UV/vis Beckman DU-700 spectrophotometer, a Shimadzu RF-551 spectrofluorimeter, and a BioDoc-IT System UV-Transilluminator. The FT–IR spectra were recorded using a Varian 3100 FT–IR spectrophotometer.

2.2.1 Synthesis of poly(acrolein-co-styrene) core particles

Emulsifier-free radical copolymerization was carried out in distilled water at a comonomer-to-water ratio of 1:9 and an acrolein-to-styrene molar ratio of 10:1. A homogeneous styrene–water mixture was prepared, and acrolein was added into the reactor. The reaction mixture was deoxygenated by purging with N₂ for 30 min, and PP (0.5 wt.% relative to the monomer mixture) was added as an initiator. The temperature of the polymerizing mixture was adjusted to 65°C. Polymerization was carried out under nitrogen for 12 h while stirring.

2.2.2 Radical seed polymerization of N-vinylcaprolactam

VCL (0.5 ml) at a seed particle to VCL ratio of 1:0.5 w/w in a 20:1 water–propanol-2 mixture was added to 1 ml of 1-wt.% dispersion of seed particles and left for swelling at 4°C for 12 h. Then, 0.15 ml of AIBN (0.2 wt.% relative to VCL) in a 20:1 water–propanol-2 mixture was added, and the temperature of the polymerizing mixture was adjusted to 70°C. Polymerization was carried out under nitrogen for 3 h while stirring.

2.2.3. Measurement of the hydrodynamic radius of polymer particles

The hydrodynamic radius (R) of the polymer particles was measured using the dynamic light scattering technique. The dispersion was diluted with water to obtain the concentration required for the light scattering experiments according to the manufacturer's recommendations and then poured into a cuvette 20. The cuvette holder was kept at the desired temperature between 20 and 45°C. The particle size was measured using a Coulter N4-MD sub-micron particle analyzer.

2.2.4 Measurement of the acrolein oligomer concentration

The acrolein oligomer concentration in the supernatant obtained after centrifugation of the polymer suspension was measured against water at λ_max = 273 nm using a Beckman DU-70 spectrophotometer 21. The results obtained (in absorbance units) were represented as the oligomer mass using a calibration graph of the optical absorption of known quantities of the oligomer dissolved in water.

2.2.5 Incorporation of quantum dots into TPPs

Solvents for QD incorporation into TPPs were selected among water, methanol, ethanol, propanol, propanol-2, butanol, hexane, chloroform, and their mixtures at ratios of 1:1, 5:1, and 10:1. It was required that the solvent do not affect the size of TPPs, their aggregation, or colloid formation during incubation. QDs (0.2 mg) were purified from TOP/TOPO by dispersing in chloroform and precipitating with methanol (at a chloroform-to-methanol ratio of 1:3). The purified QDs were dispersed in 1 ml of propanol-2 and added to 0.5 ml of a 1-wt.% TPP dispersion in a 20:1 water–propanol-2
mixture. The mixture was stirred vigorously, sonicated for 2 min, incubated for 20 min while stirring, shaken for 1 h at room temperature, and centrifuged at 7000 rpm for 10 min with addition of water. The pellet was then dispersed in 0.5 ml of water. To remove propanol-2, the obtained TPPs embedded with QDs were dialyzed against the water–propanol-2 mixture.

2.2.6 Bovine serum albumin immobilization on TPPs

An aliquot (0.125 ml) of a 1-wt.% dispersion of TPPs containing QDs was incubated with BSA (1.6–15 mg/g polymer solids) in a 0.1 M sodium borate buffer solution at 20°C for 2 h and in a water bath at 40°C for 0.5, 1, or 2 h. To block the groups that had not reacted, 0.5 ml of a glycine solution buffered with 0.1 M borate buffer (10 mg/ml) was added. Then, the excess protein was removed by three centrifugation–dispersion cycles, and the pellet was dispersed in 1 ml of a glycine solution buffered with 0.1 M borate buffer (10 mg/ml). The concentration of unbound BSA was determined by Bradford’s method at $\lambda = 595$ nm, with allowance for dilution during the adsorption procedure.

2.2.7 Exothermic reaction in the presence of TPPs

Trypsin digestion of bovine serum albumin. The reaction was carried out in 2 ml of Tris buffer solution (pH 7.8) containing 0.5 mg/ml BSA and a 0.005-wt.% TPP dispersion in a quartz cuvette. Trypsin was added to a concentration of 0.01 mg/ml. The temperature of the mixture was monitored in the course of the reaction by means of fluorimetry at an excitation wavelength of 488 nm.

Cross-linking of chitosan plasticized with PEG using butanediol diglycidyl polyether. The reaction was carried out in 2 ml of PBS (pH 7.2) containing 0.05% of chitosan, 0.005 wt.% of PEG (MM = 4000 Da), and a 0.005-wt.% TPP dispersion in a quartz cuvette. Butanediol diglycidyl ether was added to a concentration of 0.05 wt.%. The temperature of the mixture was monitored in the course of the reaction by means of fluorimetry at an excitation wavelength of 488 nm.

3. RESULTS AND DISCUSSIONS

3.1 Preparation of TPPs

Thermosensitive composite particles were obtained using a two-stage reaction: first, core particles were synthesized via emulsifier-free radical copolymerization; then, the particles were modified with the thermosensitive polymer (Fig. 1).

The first step was the synthesis of core particles based on the copolymer of styrene and acrolein. This type of cores possessed the properties of polystyrene particles 22; in addition, the polyacrolein component provided hydrophilicity of the surface and contained double bonds due to the specific characteristics of acrolein polymerization 23. The particle size can be easily varied by changing the ratio of the polymerized monomers 24. We used emulsifier-free radical copolymerization of acrolein and styrene in water with an acrolein-to-styrene monomer ratio of 10:1 in the presence of K$_2$S$_2$O$_8$ to prepare polymer particles with a hydrodynamic diameter of 185 ± 15 nm. This diameter was sufficiently small to preclude spontaneous sedimentation during measurements.

The second step was the formation of the PVCL thermosensitive shell by radical seed polymerization of VCL. This polymerization was carried out after swelling of seed particles with VCL in the presence of an initiator. Under these conditions, the grafting of the forming PVCL molecules mainly occurred via double bonds of polyacrolein. This type of polymerization allowed the formation of composite particles practically without generation of new particles 25. The thermosensitive properties of the obtained particles were evaluated by measuring the dynamic light scattering. It is noteworthy that the hydrodynamic particle sizes of the TPPs decreased with increasing temperature above the LCST due to conformational changes of PVCL from a hydrated coil to a collapsed hydrophobic globule 26. TPPs collapsed dramatically at 32°C, which is the LCST of PVCL.
3.2 Characterization of TPPs

The FT–IR technique was used to control the desired surface modification of TPPs (Fig.2). In these spectra, one can see an adsorption peak at 1650 cm\(^{-1}\), which is characteristic of C=O vibration of amide groups\(^\text{27}\). These results provide evidence for the grafting of PVCL onto poly(acrolein-co-styrene) cores.

The obtained particles remained stable for a long time and were unaffected by electrolyte (0.15 M NaCl, physiological saline). This stability was also preserved at high temperatures, when the PVCL particles were shrunken. The modification of copolymer particles with PVCL made it possible to decrease the amount of low-molecular-weight products in the dispersion media. These products are formed because of partial degradation of polyacrolein during storage, with oligomers released into the dispersion medium\(^\text{28}\). The amount of low-molecular-weight products (0.11 mg/ml) was found to be one-quarter as much as that in the case of unmodified copolymer particles (0.46 mg/ml).

Figure 3 shows the temperature dependence of the hydrodynamic radius of TPPs. With increasing temperature, the radii of TPPs gradually decreased as the dispersion was heated to 29\(^\circ\)C and then drastically decreased as the temperature further increased to 32\(^\circ\)C. This sharp decrease was due to hydrophobic aggregation of PVCL chains. The results indicated that the particles drastically collapsed at 32\(^\circ\)C, which is the LCST for PVCL. Thus, colloidally and chemically stable TPPs can be obtained by two-stage synthesis of core-shell particles. The following part of the study was aimed at obtaining fluorescent TPPs and studying their properties.
3.3. TPPs embedded with QDs

Semiconductor QDs emitting light at about 555 nm were incorporated into the PVCL shells of TPPs from a 1:20 chloroform–propanol-2 mixture after removal of TOPO as described earlier. The resultant TPPs doped with QDs displayed intense green fluorescence. Efficient incorporation of QDs into TPPs was proved by the absence of free QDs in the dispersion medium after centrifugation of the TPP suspension: the fluorescence intensity of the supernatant fraction after centrifugation was found to be negligible.

It is worth noting that QDs had almost no effect on the thermosensitive properties of TPPs. Figure 3B shows that TPPs containing QDs responded to heating in almost the same way as TPPs without QDs. However, the embedding of QDs resulted in a slight LCST shift towards lower temperatures. This phenomenon can be explained in terms of the effect of QDs, as hydrophobic components of the composite particle, on the conformation of the aqueous associations of PVCL, which results in breakage of cross-linking hydrogen bonds.

In Fig. 4A, the fluorescence spectra of the obtained TPPs at various temperatures are compared with the fluorescence spectra of seed poly(acrolein-co-styrene) particles doped with the same amount of QDs by means of the swelling procedure described earlier. It should be noted that heating of TPPs reduced their fluorescence intensity, but the fluorescence intensity of seed copolymer particles was almost unchanged at higher temperatures (Fig. 4A). Moreover, the intensity of TPP fluorescence recorded at 20°C was almost fourfold higher compared to that of seed particles doped with QDs by swelling. Thus, the inclusion of QDs into TPPs seems to be preferable over their inclusion into seed copolymer particles. As noted by Nida et al., the ZnS shell may be damaged by a solvent (e.g., chloroform), which results in coordinative unsaturation (the surface emitting state) of QDs and, consequently, fluorescence quenching. PVCL around each QD is likely to occupy the vacant coordinate sites on the QD surface and efficiently passivate the surface emitting state (as compared to copolymer chains of seed particles), which results in an increase in the TPP fluorescence.

![Figure 4A](image1.png)

Figure 4. (A) The fluorescence spectra of poly(acrolein-co-styrene) particles at (1) 20°C and (2) 40°C and TPPs at (3) 20°C and (4) 40°C. (B) Temperature dependence of the fluorescence spectra of a mixture containing TPPs embedded with QDs ($\lambda_{em} = 550$ nm) and unmodified seed copolymer particles embedded with QDs ($\lambda_{em} = 610$ nm) using the swelling procedure.

The temperature effect on the fluorescence intensity of TPPs can be also confirmed by the fact that only the peak corresponding to TPPs was decreased (Fig. 4B) upon heating the mixture containing QD-embedded TPPs ($\lambda_{em} = 550$ nm) and QD-embedded unmodified seed copolymer particles ($\lambda_{em} = 610$ nm).

The fluorescence intensities of TPPs were also measured in heating–cooling cycles with the temperature varying between 20 and 45°C (Fig. 5A). Note that the process was totally reversible, with photodegradation being negligible in each temperature cycle (20 min). The maximum variation of the fluorescence intensity corresponded to the largest changes in the TPP radius at temperatures between 27 and 31°C. The sensitivity of temperature measurement within this range was about 0.1°C (Fig. 5B). The region of the maximum sensitivity of temperature measurement may be varied by changing the type of the thermosensitive polymer used. For example, the use of the copolymer of PVCL and poly-N-vinylpyrrolidone allows shifting the range of the maximum sensitivity toward higher temperatures, whereas the use of the copolymer of PVCL and vinyl alcohol results in a downshift of the region of the maximum sensitivity.
The fluorescence of TPP supernatants after centrifugation was vanishingly low, which confirmed that the QDs were not lost from the TPPs during heating–cooling cycles. The sensors developed were very stable, with no more than 10% of the fluorescence lost during 10 heating–cooling cycles, 20 min per cycle. The shelf life of the TPPs was found to be more than 2 years without any change in their fluorescence properties.

The phenomenon of reversible temperature-dependent fluorescence of TPPs can be explained as follows. The increase in temperature altered the conformation of PVCL on the surface of seed copolymer particles, resulting in the formation of hydrophobic globules \(^{30}\). As mentioned above, this may be observed as shrinkage of the PVCL layer and, hence, a decrease in the particle size. The shrinkage of the PVCL layer seems to decrease the distances between the embedded QDs, which is the crucial factor in quenching QD fluorescence \(^{31}\). In addition, the peaks were slightly red-shifted at temperatures above the LCST, which also indicated that QDs were located close to one another.

Our calculations of the distance between QDs before and after heating confirmed this suggestion. Since our previous data showed that QDs could not be incorporated into seed copolymer particles without a preliminary swelling for at least 1 h, the distance was calculated on the assumption on predominant QD penetration into the PVCL shell \(^{24}\). UV–vis measurements show that about \(10^4\) QDs could be incorporated into each TPP, which gives a mean distance between QD centers of about 9.5 nm at 20°C. According to \(^{32}\), this distance is typical of films with a relatively low QD density. Heating to 40°C induced shrinkage of the PVCL layer, and the mean distance between QDs was decreased to 4.2 nm. This agrees with published data on films of densely packed QDs (4.0–4.1 nm) \(^{32}\). Thus, the PVCL shells on copolymer particles analyzed at different temperatures may be regarded as films with different QD densities. The films containing QDs at a low density were characterized by narrow fluorescence spectral bands and a relatively intense fluorescence that could be quenched by increasing the density of QDs accompanied by a red shift of the emission peak \(^{33,34}\). This quenching resulted from nonradiative excitation transfer between QDs \(^{32}\) and interaction of the dipole moments related to the QD asymmetry \(^{35}\). To summarize, we may conclude that the above analogy between PVCL shells containing QDs and QD films supports the effectiveness of our approach to the formation of TPPs with temperature-dependent fluorescence, where temperature-dependent conformational changes of PVCL cause changes in the distances between QDs.

The cooling procedure resulted in expansion of PVCL chains, and the distance between QDs probably returned to the initial value, which resulted in fluorescence recovery. This behavior ensured reversibility of the fluorescence intensity changes. Thus, incorporation of fluorescence labels, including QDs, into TPPs is a promising approach to the production of optically sensitive polymer particles with temperature-dependent fluorescence. In addition, the fluorescence intensity of these particles is reversible during a heating–cooling cycle, which is an advantage in terms of the development of optical detection methods for bioassays.

**3.4. Bovine serum albumin immobilization on TPPs**

For all bioanalytical applications, particles should be conjugated with a specific bioligand, preferably protein or peptide. It is known that PVCL is capable of complexing with various compounds. In the case of its interaction with proteins, PVCL amide groups form hydrogen bonds with carboxyl or amino groups of proteins. The conditions and efficiency of this complexing were estimated using a model of immobilization of BSA at concentrations from 1.6 to 15 mg/g polymer.
It can be seen in Fig. 6A that the amount of the adsorbed protein increased with increasing protein concentration in the solution until signal saturation was reached at a concentration of 10 mg/g polymer. Apparently, the plateau corresponded to the situation where the surfaces of the polymer particles were completely covered with the attached protein macromolecules, and there was no free space left for more protein. An increase in the amount of immobilized BSA to 10 mg/g caused a decrease in the TPP fluorescence intensity (Fig. 6A), which remained practically unchanged as the BSA concentration further increased. BSA at the saturating surface concentration seemed to form a complex with PVCL, which gave rise to conformational changes and decreased the PVCL capacity for passivating the surface emitting states of QDs. After saturation of the surface with BSA (at a concentration of 10 mg/g), the PVCL shell probably underwent no further conformational changes, and the fluorescence remained practically unvaried. In addition, the BSA adsorption had almost no effect on the thermosensitive properties of TPPs embedded with QDs: in the vicinity of 32°C, the hydrodynamic radii of TPPs decreased by about 48 nm. Therefore, we estimated the amount of added BSA that corresponded to saturation.

We studied different conditions of BSA immobilization on TPPs, including incubation at 20°C for 1 h and incubation at 40°C for 0.5, 1, and 2 h. The maximum amount of adsorbed BSA (~80% of the amount added) on TPPs was found in the case of incubation at 40°C for 1 h. With increasing temperature, the hydrophobic interactions between PVCL and BSA became stronger. It is known that deformation of protein molecules due to their interaction with PVCL, which is facilitated by the rise of temperature, promotes mechanical entrapment of BSA during the shell shrinkage. This effect increased the amount of adsorbed BSA at 40°C as compared to that at 20°C (Fig. 6B).

Although the BSA adsorption at higher temperatures decreased the fluorescence intensity of TPPs, this decrease was smaller than in the case of BSA adsorption at 20°C (Fig. 6B). Figure 5B also shows the reversible fluorescence dependence on temperature during a cooling–heating cycle for TPPs. Thus, QD-containing TPPs can be efficiently assembled with protein molecules (as exemplified by BSA) in such a manner that TPPs retained their thermosensitive properties, including the reversible dependence on temperature, with a relatively small loss of fluorescence intensity under the optimal protein adsorption conditions.

3.5. The use of TPPs for monitoring local temperature

Below are two examples demonstrating the possible practical bioanalytical applications of TPPs. Figure 7 (reaction 1) shows the time course of the exothermic reaction of trypsin digestion of BSA carried out in the presence of TPPs. As one can see from the figure, the TPP fluorescence intensity was decreasing for nearly 5 min; this was accompanied by an increase in temperature by 2°C. After that, the fluorescence intensity increased almost to the initial level. It is known that most enzymatic reactions are exothermal and proceed for 5–10 min. Thus, the observed decrease in TPP fluorescence can be considered to reflect the heat release during the reaction. When the reaction was completed, the fluorescence was almost entirely restored.
The second reaction (Fig. 7, reaction 2) was the reaction of chitosan cross-linking, which has important medical applications because it is involved in the preparation of chitosan films. The quality of the films prepared strongly depends on the possibility to precisely identify the moment when the cross-linking reaction completed and the mixture may be applied onto a substrate. This is why we chose this exothermic reaction. The fluorescence decrease of TPPs during reaction 2 followed the same pattern as that of reaction 1 except that it was longer (as judged by the moment when the TPP fluorescence intensity stopped decreasing), and the temperature effect was about two times stronger. When the cuvette cooled after the reaction had been completed, the time course of the temperature decrease was about the same in both cases. This is evidenced by the same slope of the curves at the stage when the fluorescence intensity was restored (cf. Figs. 7A and 7B).

Thus, it has been unambiguously demonstrated that the TPPs obtained enable the detection of temperature variations as small as 0.1°C in bioanalytical applications.

4. CONCLUSIONS

Novel composite particles consisting of a solid poly(acrolein-co-styrene) core and a thermosensitive PVCL shell containing semiconductor nanocrystals have been obtained. Embedding of QDs in these TPPs makes it possible to obtain particles that are characterized by a high fluorescence intensity reversibly changing during heating–cooling cycles. This property is explained by the variation of the mean distance between QDs in the PVCL shell caused by conformational changes of polymer chains. Heating above the LCST gives rise to the globular conformation of PVCL and, hence, shrinkage of the TPP shell. This leads to a decrease in the distance between QDs, which, in turn, results in fluorescence quenching.

The properties of these particles as carriers of biomolecules have been studied using BSA as a model biomolecule. The influence of operating parameters such as the temperature, time of immobilization, initial BSA concentration for the adsorption of BSA on the TPPs, and fluorescence intensity have been evaluated. Optimal adsorption condition (immobilization at 40°C for 1 h) allowed the fabrication of TPPs tagged with the biomolecule (BSA) that exhibit reversibly temperature-dependent fluorescence.

Finally, bioanalytical applications of TPPs have been illustrated by examples of their use for real-time remote monitoring of the local temperature in a reaction mixture in the course of exothermic chemical reactions. Thus, the TPPs obtained in this study can be regarded as potential bioreagents for measurement of the local temperature and for the use in optical detection methods in bioassays.

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