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Polymeric Nanocapsules Templated on Liquid Cores as Efficient Photoreactors

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Polymeric nanocapsules templated on liquid cores were obtained without stabilization by low molecular weight surfactants and applied as photochemical reactors. Toluene cores of the capsules were stabilized by novel graft amphiphilic polyelectrolyte poly(allylamine hydrochloride)-*graft*-poly(vinylnaphthalene) (PAH-*graft*-PVN) that was synthesized via nitroxide-mediated radical polymerization. Self-aggregation of the photoactive copolymer consisting of solvophilic backbone and solvophobic side chains in water as a selective solvent leads to formation of intermolecular aggregates in which hydrophobic perylene fluorophore can be solubilized. However, at the water/toluene interface the macromolecules partially uncoil and polymeric chains are able to stabilize emulsion droplets by anchoring the hydrophobic arms in them. Formation of such capsules was followed using dynamic light scattering and electron microscopy. The obtained capsules showed long term stability. Importantly, energy transfer between the polymeric naphthalene moieties and incorporated perylene chromophores was realized much more efficiently in the core-shell capsules than in the copolymer aggregates. It can be explained in terms of less packed but still confined environment of the nanocapsules. Such an assembly favors migration and transfer of excitation energy, which is otherwise dissipated in naphthalene excimers and aggregates that dominate in the copolymer aggregates formed in an aqueous environment. The obtained nanocapsules were shown to serve as both stable nanocontainers and efficient nanoreactors for photooxidation of perylene as an example reaction. The reaction photosensitized by the polymeric naphthalene chromophores proceeded at least 3 times faster in the capsules than in the aggregates, disregarding pH of the medium. Such properties make those novel capsules promising for numerous applications concerning photosensitized reactions that may be carried out in an aqueous medium and benefit from the confinement effect.

Introduction

The assembly phenomenon of antenna amphiphilic polyelectrolytes has attracted considerable attention in recent years.[1-5] Double chemical nature enables their good solubility in water, while the presence of chromophores makes them photoactive. Their self-assembly in selective solvents depends on topology of macromolecules as well as the balance between hydrophobic and hydrophilic components. Thus, they may be designed to aggregate inter- or intramolecularly leading to desired structures and properties.[6-9] Moreover, during aggregation process they form hydrophobic domains in which sparingly water soluble compounds (e.g. aromatic hydrocarbons) may be solubilized.[10-12] Importantly, the range of possible applications may be easily adjusted by introducing appropriate chromophores into such macromolecules. Antenna polyelectrolytes have been intensively studied as they may mimic the ability of natural photosynthetic systems to provide

sequential energy transfer.[13] They absorb photons which then migrate between chromophores covalently bound to the polymeric chain and can be transferred to suitable energy traps in which photochemical reactions take place.[14-16] Such traps are commonly chemically attached to the excitation energy donor, however amphiphilic antenna polyelectrolytes provide another way to transport the energy. Their aggregation leads to formation of micelle-like structures of high concentration of chromophores in the cores that implies efficient light-harvesting.[17-19] Such structures ensure close proximity between the polymeric chromophores (energy donors) and solubilized molecules (energy acceptors) providing efficient energy migration path.[20] Relatively short donor-acceptor distance as well as partial overlapping of the emission spectrum of donor and the absorption spectrum of acceptor molecules are necessary for the excitation energy transfer to occur.[21]

Such photoactive amphiphilic copolymers can act as photosensitizers in many chemical reactions.[22,23] The systems described so far have been mostly based on statistical and block copolymers. Graft copolymers with solvophobic side chains and solvophilic backbone were studied rarely because the structures they form in water are believed to be less precisely defined.[24,25] Nevertheless, they have been only recently reported as stabilizers

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of oil in water emulsion which leads to the formation of nanocapsules templated on liquid cores.[26,27] The aforementioned technique does not require the use of low molecular weight surfactants and thus opening new possibilities for designing of carriers of drugs or other active agents and nanoreactors useful in e.g. optoelectronic devices, agriculture, environmental protection, wastewater treatment, as well as self-healing and anticorrosion coatings.[28]

A concept of enhancement of chemical and biological processes by utilizing confined environment - on a surface, in zeolite pores, micelles, core-shell formulations, protein cages and in bulk phase - has been widely explored.[29-31] Such approach is of great importance in catalysis, molecular electronics, photonics and molecular biology[32] due to enhancement of kinetics of chemical reactions and their selectivity[33] leading to efficient realization of sequential processes with many synergistic effects.

Ultra-small reactors have been applied so far in numerous reactions including benzene hydroxylation[34] or asymmetric reactions on chiral metal complexes, enhanced dehydrogenation in carbon nanotubes,[35,36] direct synthesis of middle isoparaffins or dimethyl ether from syngas,[37-39] isomerization of organometallic compounds,[40] nitroxide-mediated radical polymerization[41] and photooxidation of perylene solubilized in polymer structures (photozymes).[42] In the latter example, photoactive amphiphilic copolymers forming superstructures enhance the photooxidation via confinement effect but also by photoinduced energy transfer.[42]

Such approach is also important in biomedical field as molecules in living cells commonly experience very confined environment. Formation of artificial biomimetic compartments have great potential for use in biomineralization, bioanalytical devices, sensors and photocatalytic nanoreactors.[43-46] Self-assembled structures, e.g. protein cages as well as naturally occurring systems like virus capsids are capable of encapsulation of enzymes, proteins and metal-ions complexes and are excellent candidates for preparation of bioreactors in which multiple enzymatic reactions take place under kinetic control and product selectivity.[47]

In the work reported herein, we present synthesis of amphiphilic polycation poly(allylamine hydrochloride)-*graft*-poly(vinylnaphthalene) (PAH-*graft*-PVN) and preparation of nanocapsules that act as very effective nanoreactors for perylene photooxidation. Self-assembly of the copolymer in water leads to the formation of molecular aggregates that strongly affect photosensitizing activity. Moreover, its photophysical properties depend on pH that indicates pH-tunable characteristic of used material. At water-oil interface polymeric chains uncoil and less packed chromophores not only stabilize the liquid core of the capsules but also act as energy donor to encapsulated probe. The process occurs in confined environment where both types of molecules are close enough to ensure high efficiency of energy transfer and formation of energy-traps is less probable than in molecular aggregates. Hence, high applicability of nanocapsules stabilized by amphiphilic graft polyelectrolytes is postulated in order to prepare pH-independent nanoreactors for processes requiring substrates operating in environments of different polarity.

Experimental section

Materials. 2-vinylnaphthalene (VN, 95%, Aldrich) was purified by column chromatography using silica gel as the stationary phase (pore size 60 Å, Fluka) and cyclohexane as the eluent (p.a., Avantor Performance Material Poland S.A.). Poly(allylamine hydrochloride) (PAH, M_w=58000 g/mol, Aldrich), 4-Hydroxy-2,2,6,6-

tetramethylpiperidinyl-1-oxyl (HTEMPO, free radical, 98%, Aldrich), 10-camphorsulfonic acid (CSA, anhydrous, 98%, Aldrich), iron(II) sulfate heptahydrate (FeSO$_4$·7H$_2$O, ≥99.0%, Aldrich), sodium hydroxide (NaOH, p.a., Avantor Performance Material Poland S.A.), sodium chloride (NaCl, p.a., Lachner), perylene (Pe, gold label, 99.9%, Aldrich) n-octadecane (C18, p.a., Polyscience Corp.), ferrocene (98%, Aldrich), hydrogen peroxide (H$_2$O$_2$, 30% solution, p.a., Avantor Performance Material Poland S.A., Poland), sulfuric acid (H$_2$SO$_4$, 96%, Chempur), dimethyl sulfoxide (DMSO, p.a., Aldrich), methanol (p.a., Chempur), toluene (p.a., Chempur) were used as received. Deionized water was used to prepare all the solutions.

Instruments and procedures. Steady-state fluorescence spectra were measured at room temperature using SLM Aminco 8100 spectrofluorimeter equipped with a 450 W xenon lamp as a source of light. In fluorescence polarization measurements calcite polarizers (Glan-Thompson) were used. ATR-FTIR spectra were collected on diamond using ALPHA FT-IR Spectrometer (Bruker). Malvern Zetasizer Nano ZS instrument working at 173° detection angle was used for dynamic light scattering (DLS) measurements. All the measurements were performed at 22 °C and the reported data represents the averages from three series of measurements (10-100 runs each) and their standard deviations. General purpose mode was used as distribution analysis algorithm. Ultrasonic bath was used for preparation of the capsules (540 W, Sonic-6, Polsonic). Field emission scanning electron microscopy (FE-SEM, Hitachi S-4700) and transmission electron microscopy (TEM, Tecnai F20 Twin) was used for imaging the nanocapsules. Elemental analysis measurements were performed using Vario Microf Cube CHNS Analizer (Elementar). The samples were freeze-dried using Christ Alpha 1-2 LD plus freeze dryer. Rayonet photochemical reactor (model RPR-100) equipped with 2 lamps, 300 nm each, was used to irradiate samples for the photooxidation studies. Total intensity of light measured at 312 nm by radiometer VLX 3W was 4.8 mW/cm^2.

Polymer synthesis. Nitroxide-mediated free radical polymerization was used to synthesize poly(allylamine hydrochloride)-*graft*-poly(vinylnaphthalene) (PAH-*graft*-PVN) according to general procedure described earlier for a polyanion.[48] Briefly, the graft copolymer was obtained in a two-step process. At first, PAH-*graft*-HTEMPO macroinitiator was synthesized in the reaction between HTEMPO and radicals generated on PAH chains using Fenton's reagent (a mixture of H$_2$O$_2$ and FeSO$_4$, 1:1 molar ratio). The molar ratio of HTEMPO : FeSO$_4$: H$_2$O$_2$: PAH (with respect to PAH mers) was 0.1:0.3:0.3:1. That reaction was carried out in water at room temperature for three hours and after that the mixture was heated to 40°C to remove the excess of H$_2$O$_2$. Ferric cations were precipitated with NaOH solution and filtered with syringe filters of pore size of 0.22 μm. The resulted PAH-*graft*-HTEMPO was dialyzed against water for 5 days using cellulose tubing (12000-14000 g/mol cut-off, Aldrich). The graft copolymer was then obtained by mixing 106 mg of previously synthesized macroinitiator PAH-*graft*-HTEMPO and 107 mg of VN. The polymerization was carried out in 15 ml of DMSO in a presence of 10-camphorsulfonic acid (CSA, 0.01 M) as a rate-accelerating additive for 3 hours at 130°C under continuous bubbling with nitrogen. The resulted PAH-*graft*-PVN copolymer (Scheme 1) was purified by dialysis against DMSO and water (8 days in each solvent) followed by extraction with cyclohexane in order to remove non-reacted VN and subsequently freeze-dried.
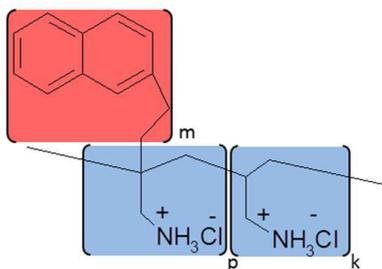
Scheme 1 Structure of the amphiphilic PAH-*graft*-PVN copolymer.

Preparation of capsules on liquid cores. Polymeric capsules were obtained according to previously described procedure[27] with some modifications (the volume ratio between reagents was changed). Briefly, PAH-*graft*-PVN (1 g/L in 0.1 M NaCl) was mixed with toluene in 100:1 volume ratio and the mixture was sonicated for 30 minutes at room temperature in ultrasonic bath. For encapsulation studies solution of Pe in toluene (ca. 10^{-5} M) was used instead of pure toluene.

Preparation of samples for SEM imaging. PAH-*graft*-PVN copolymer and capsules were deposited on silicon surface for SEM imaging. A silicon wafer was previously cleaned with "piranha" solution (mixture of H_2SO_4 (96%) and H_2O_2 (30%), 1:1 v/v, caution! It is a highly corrosive and oxidative mixture), rinsed with deionized water and dried in a stream of argon. The capsules for SEM measurements were prepared in ultrasonic bath at 32°C by direct emulsification (30 min) of PAH-*graft*-PVN (1 g/L in 0.1M NaCl) with C18 (100:1 volume ratio) that was previously warmed up to c.a. 35°C. Such obtained capsules were then cooled down to room temperature which caused solidification of C18 cores and deposited on the silicon wafer by placing a droplet of the emulsion on the cleaned silicon surface. After that, the surface was carefully rinsed with water and dried in a stream of argon.
PAH-*graft*-PVN (1 g/L in 0.1 M NaCl) was deposited on the silicon surface in the same way as the capsules. Droplets of the solution placed on silicon substrate were carefully dried in a stream of argon, rinsed with water to remove salt and non-adsorbed polymer and dried in a stream of argon.

Preparation of samples for TEM imaging. PAH-*graft*-PVN capsules were prepared according to the procedure described for SEM experiment. In order to increase the contrast in TEM images ferrocene was dissolved in C18 (3 g/L) and such a mixture was used as an oil phase.

Solubilization procedure. Pe was solubilized in aqueous solutions of the copolymer at pH =3 and pH =7 by slow injection of microliter quantities of the probe solution (c≈10^{-4} M in methanol) into milliliter quantities of the polymer solution (2 g/L). The solutions were vigorously stirred, purged with argon in order to remove the residues of methanol and equilibrated in darkness for at least 2 hours before measurements.

Photooxidation studies. The samples with solubilized or encapsulated Pe (as described in previously mentioned procedures) were saturated with oxygen, placed in quartz cuvettes and irradiated in Rayonet photoreactor for predetermined periods of time. After each irradiation cycle emission spectra were recorded.

Results and discussion

General characterization of polymers. The formation of PAH-*graft*-HTEMPO was confirmed by elemental analysis and IR spectroscopy. The degree of substitution (DS) of HTEMPO in the macroinitiator was calculated based on the content of C and N in the sample to be 5.2±0.1%. The FT-IR spectrum of PAH-*graft*-HTEMPO shows the band with the maximum at 1375 cm^{-1} which may be assigned to N-O stretching vibration confirming formation of the macroinitiator.[49]
The process of formation of PVN grafts on PAH chains was confirmed by FT-IR spectroscopy (Figure S1 in SI). The bands characteristic for aromatic groups could be clearly assigned in the spectrum of PAH-*graft*-PVN. Moreover, IR spectroscopy was also used for determination of the composition of the graft copolymer using appropriate calibration curve (Figure S2 in SI). Unfortunately, neither elemental analysis (underestimated content of nitrogen due to formation of solid residues) nor ^{1}H NMR spectroscopy (restricted mobility of aromatic rings in the confined environment)[50] could be used for determination of the composition of PAH-*graft*-PVN. Taking into account molecular weight of PAH equal to 58000 g/mol and calculated grafting density the content of VN in macromolecule was found to be equal to 12.7±0.03 wt. % which indicates that there were c.a. 48 VN units per each macromolecule and on average ca. 1.5 per graft (based on the composition of PAH-*graft*-HTEMPO).

Conformation of PAH-*graft*-PVN macromolecules. Conformation of the copolymer macromolecules in an aqueous solution was investigated using dynamic light scattering (DLS) measurements. As the charge of PAH depends on the ionic strength of the solution all the measurements were carried out in solutions of pH=3, where PAH is completely protonated and pH=7, where it is partially deionized.[51] The obtained results showed that in both examined conditions the polymeric chains form structures of average hydrodynamic diameter equal to 75 and 102 nm for lower and higher pH, respectively (Figure 1, Table S1 in SI). Taking into account average diameters of PAH homopolymer macromolecules at given pH (9.8±0.3 nm at pH=3 and 10.8±0.7 at pH=7, as determined using DLS) formation of intermolecular aggregates was postulated.
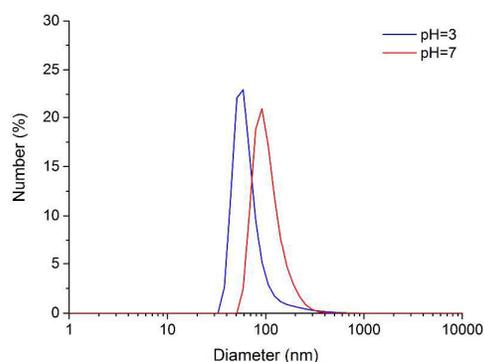

Figure 1 Number-weighted hydrodynamic diameter distribution of PAH-*graft*-PVN dissolved in 0.1 M NaCl (c=1 g/L) at pH = 3 and pH = 7.

About 40% increase of the aggregate size at higher pH is quite surprising as usually lower charge of polyelectrolyte macromolecules favors their more compact conformation resulting from smaller electrostatic repulsion. The observed phenomenon might be a consequence of reorganization of naphthalene chromophores within the photoactive aggregates. Such an

explanation is supported by fluorescence studies as the emission spectra of the copolymer measured at both pH conditions differ significantly (see further). Conformational changes within the hydrophobic domains at higher pH are probably induced by swelling of the aggregates containing less charged and thus more flexible chains than at lower pH. Charged macromolecules (at lower pH) also stronger repeal each other that may destabilize the aggregates leading to the reduction of their sizes. Moreover, diameters of PAH homopolymer vary only a little within the studied pH values suggesting that the observed changes are indeed related to the presence of hydrophobic groups grafted onto PAH chains.[52]

Formation of spherical objects of diameter between 40-100 nm was also followed using SEM. It confirmed formation of intermolecular aggregates (Figure S3 and S4 in SI). Similar aggregates of average hydrodynamic diameter equal to 255 nm were previously observed for uncharged poly(vinyl alcohol)-*graft*-poly(vinylnaphthalene) (PVA-*graft*-PVN),[53] however, they existed only in an aqueous solution, while PAH-*graft*-PVN intermolecular associates are stable enough to be observed upon deposition on silicon surface and SEM imaging.

Fluorescence studies. Conformation of the copolymer chains was also investigated spectroscopically as varying interactions between the polymeric naphathalene chromophores imply changes of their photophysical properties.[54] Steady-state emission spectra of PAH-

graft-PVN show bands of high intensity upon excitation of the isolated chromophores at 280 nm, however quite intensive emission is also observed upon excitation at wavelengths longer than 400 nm that cannot be absorbed by isolated naphthalene units (Figure 2). It indicates strong interactions between the chromophores within the formed aggregates absorbing at lower energies which results in red-shifted fluorescence.

Formation of the aggregates was also verified by fluorescence excitation spectra based on bands with maxima up to c.a. 360-385 nm. Importantly, emission with maxima at c.a. 400 nm indicates that even if the chromophores are quite packed in the aggregates they have enough rotational freedom to form fully overlapped ("normal") excimers.[55]

For previously reported poly(sodium styrenesulfonate-*block*-vinylnaphthalene) (PSSS-*block*-VN) copolymer with VN units the emission spectrum (λ_{ex}=280 nm) was dominated by the "second" excimer what indicated formation of microenvironment in which the chromophores interact even stronger than here.[56] It may be rationalized by strong repulsive interaction between the highly charged sulfonate groups that tend to squeeze the hydrophobic parts of the macromolecules. Here we can observe reduction of the excimer intensity with respect to the monomeric emission while
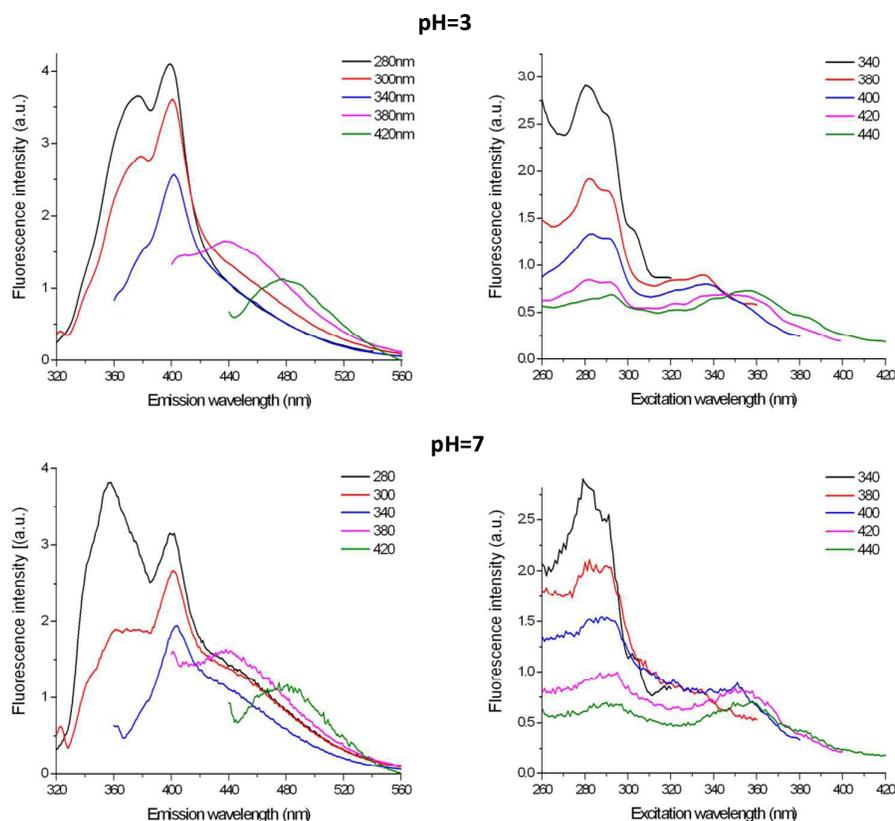

pH=3

pH=7

Figure 2 Steady-state emission (left panel, for different excitation wavelength given in the legends) and excitation (right panel, for different emission wavelength given in the legends) spectra of solutions of PAH-*graft*-PVN (0.1 g/L in 0.1 M NaCl) at pH= 3 and pH=

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increasing pH of the polymer solution (Figure S5 in SI). It suggests relaxation of the aggregates being a result of partial deionization of PAH chains. It is in agreement with the sizes of aggregates obtained from DLS and SEM measurements (see further).

Stability of the formed aggregates at different pH conditions was examined by heating aqueous solutions of PAH-*graft*-PVN to 50°C and maintaining this temperature for 10 minutes, after which the samples were cooled down to the room temperature. The samples were measured using DLS apparatus and spectrofluorimeter before and after heating. No significant changes, neither in size of the formed structures (Table S1 in SI) nor in the fluorescence spectra were observed after heating. It indicates that the observed structures are not just kinetically frozen but the aggregates also seem to be stable in polar media at the examined conditions.

Capsules templated on liquid cores. Formation of aqueous suspensions of nanocapsules composed of PAH-*graft*-PVN shells and liquid hydrophobic cores was followed by DLS measurements (Figure 3) as well as by SEM (Figure 4 and 5) and TEM (Figure S7 in SI). They were formed by simple mixing the aqueous solution of PAH-*graft*-PVN with a hydrophobic solvent (toluene or C18) at appropriate ratios, followed by sonication. The graft copolymer sufficiently stabilizes the nanocapsules, so no addition of low molecular weight surfactants was necessary and the capsules were not sensitive to dilution. DLS studies showed that the capsules were stable even upon 50 times dilution of the originally obtained dispersion. Lower concentration could not be measured because of the detection limit of DLS apparatus. The capsules for SEM imaging were fabricated on liquid C18 at 32°C and cooled down to the room temperature which caused solidification of C18 preserving the shape of the formed capsules under vacuum during SEM experiment. Importantly, no significant differences between parameters describing the capsules formed on toluene and C18 cores were observed. Both diameters obtained from DLS and SEM images show that regardless of the pH of the solution in which the graft copolymer was dissolved, the capsules of diameter c.a. 50-100 nm and quite narrow size distribution (PDI=0.3) are formed. Moreover, the obtained capsules do not tend to aggregate or sediment at both studied pH conditions what is of particular importance for further applications (Figures 4, 5 and S6 in SI).
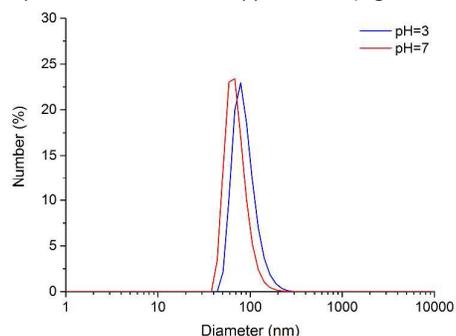

Figure 3 Number-weighted hydrodynamic diameter distribution of the capsules stabilized by PAH-*graft*-PVN in an aqueous dispersion (0.1 M NaCl) at pH=3 and pH=7.

Judging from similar diameters of the aggregates and capsules one can doubt whether the capsules were indeed obtained in the applied procedure. In order to verify the type of the obtained structure fluorescent probe studies were carried out. Pe was used as the probe because of its low solubility in water (1.2·10^{-9} M) and an efficient fluorescence emission appearing only in non-polar environment.[57] Comparison of the polarization values (p) obtained for Pe (λ_{ex} = 410 nm, λ_{em} = 450 nm) solubilized in the aggregates formed in an aqueous solution of the graft copolymer and encapsulated in toluene cores of the capsules indicates formation of objects having core-shell structure. In the packed hydrophobic domains of molecular aggregates the values of polarization were found to be high (p=0.18-0.30) indicating that the probe experiences highly viscous environment.[56] Meanwhile, polarization value measured for the capsules' cores was close to p=0.1 that indicates significantly higher rotational freedom of Pe. Thus, microviscosity of the hydrophobic domains experienced by the Pe probe estimated using Perrin equation[58] is 2-2.5 times larger for the capsules than for bulk toluene, while for the aggregates it is 2-3 times larger than for the capsules and significantly varies with pH (Table 1). It suggests that microviscosity of the aggregates strongly depends on pH, while structure of toluene cores of the capsules is pH-independent.

Table 1 Microviscosity of the polymeric (PAH-*graft*-PVN) systems determined with Pe fluorescent probe (λ_{ex} = 410 nm, λ_{em} = 450 nm) and application of Perrin equation .

	Copolymer solution		Capsules		Toluene (c$_{Pe}$=2.5 mg/L)
	pH=3	pH=7	pH=3	pH=7	
Microviscosity (cP)	33.7	61.8	16.6	20.7	8.4

Different photophysical properties were observed for Pe in each type of systems (see further) that also indicates different morphologies of the obtained structures. Furthermore, milky emulsion was obtained in sonication and no coalescence of droplets was observed for two weeks. That confirms that oil droplets were well protected by the amphiphilic polyelectrolyte.

High values of zeta potential of the capsules measured immediately after sonication (Figure 6 and Table 2) suggest good electrostatic stabilization of the emulsion droplets. As expected, better stability expressed by higher value of the zeta potential was provided by the copolymer dissolved in acidic solution because of its higher electrostatic charge (Table 2). However, even at pH=7 the zeta potential values were reasonably high.

Table 2 Zeta potential values of PAH homopolymer, graft copolymer PAH-*graft*-PVN and the capsules at pH=3 and pH=7.

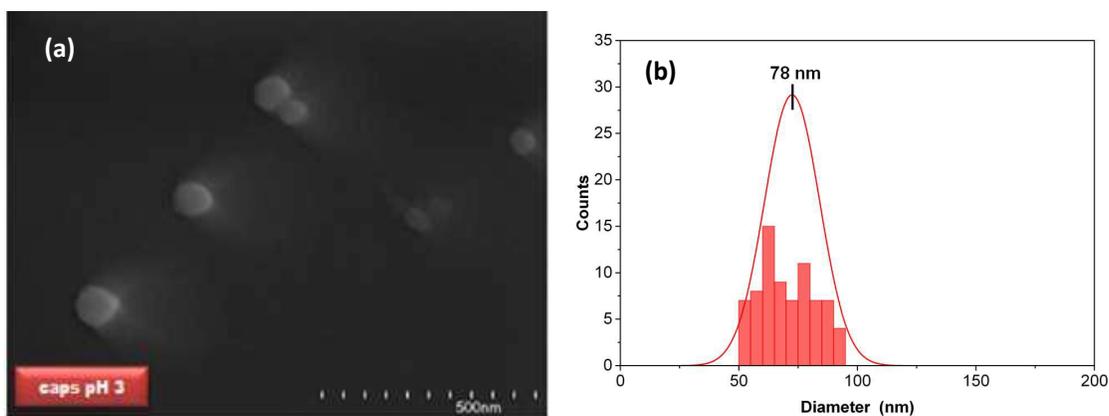|	PAH	PAH-*graft*-PVN	Capsules
	Zeta potential (mV)		
pH=3	+26.4±5.0	+44.1±1.1	+41.7±0.9
pH=7	+24.5±2.1	+23.9±1.9	+26.9±2.4

Figure 4 SEM image of the capsules with C18 core deposited from aqueous dispersion at pH=3 (a) and histogram of their size distribution (b).
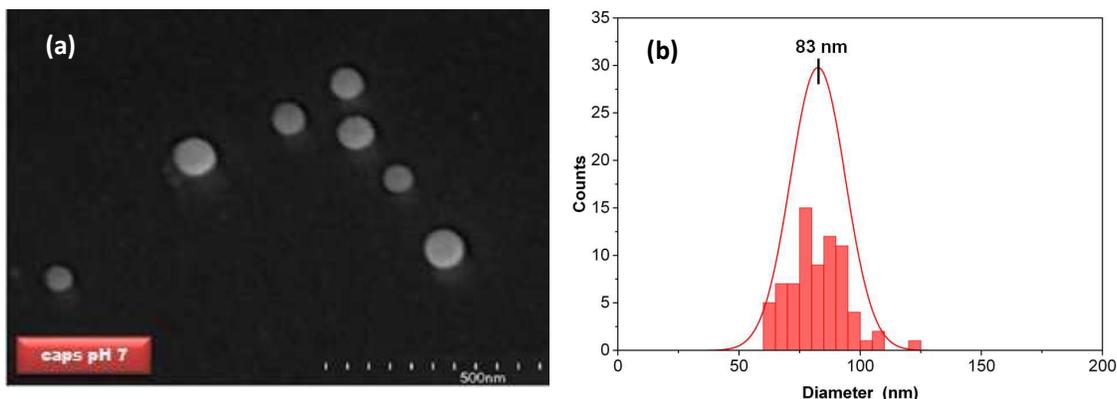

Figure 5 SEM image of the capsules with C18 core deposited from aqueous dispersion at pH=7 (a) and histogram of their size distribution (b).

During two weeks storage at room temperature only slight variations in zeta potential values were observed indicating good long-term stability of the nanocapsules based on PAH-*graft*-PVN. It confirms high potential of graft amphiphilic polyelectrolytes for formation of stable nanocapsules templated on liquid cores. What needs to be emphasized is that the comb-like copolymer architecture is crucial for the efficient stabilization of emulsion droplets as homopolymer macromolecules cannot stabilize such nanocapsules. After emulsification of PAH and toluene the objects of diameter in the range 13-15.5 nm were measured by DLS but phase separation occurred very quickly after sonication.

Steady-state emission spectra of the capsules were measured in order to investigate if the formation of capsules is influenced by the pH changes. However, the obtained spectra (data not shown) did not differ very significantly from the respective spectra of the aggregates measured at given pH (Figure 2). It was surprising as one can expect the decrease in the intensity of excimer band while macromolecules uncoil being transferred from an aqueous solution to the water-oil interface. Partial contribution of the residual copolymer aggregates in these observations cannot be excluded as the systems were not centrifuged for purification in order to avoid possible damages of soft capsules. Nevertheless, photochemical studies (see further) indicate significant structural differences between aggregates and capsules.

Photophysical properties of Pe in PAH-graft-PVN aggregates and capsules. Molecular aggregates formed by PAH-*graft*-PVN amphiphilic polyelectrolyte dissolved in polar medium enable

solubilization of large aromatic hydrocarbons like Pe and support efficient transport of excitation energy absorbed by the naphthalene units to the probe molecules. Nevertheless, the interactions between the chromophores in such confined environment affect photophysical properties of molecules they interact with, especially with regard to the efficiency of energy transfer. To compare different behavior of the hydrophobic probe interacting with VN units, three systems were studied. In the first one VN and Pe were dissolved in toluene ($c_{VN}=10^{-3}$ M, $c_{Pe}=10^{-5}$ M; concentrations comparable to overall concentrations of VN and Pe in the capsules' dispersion) and steady-state emission spectra were recorded (Figure S9 in SI). One can notice that while Pe and VN are dissolved in non-polar solvent they do not tend to interact in a manner observed for molecular aggregates trapping Pe.
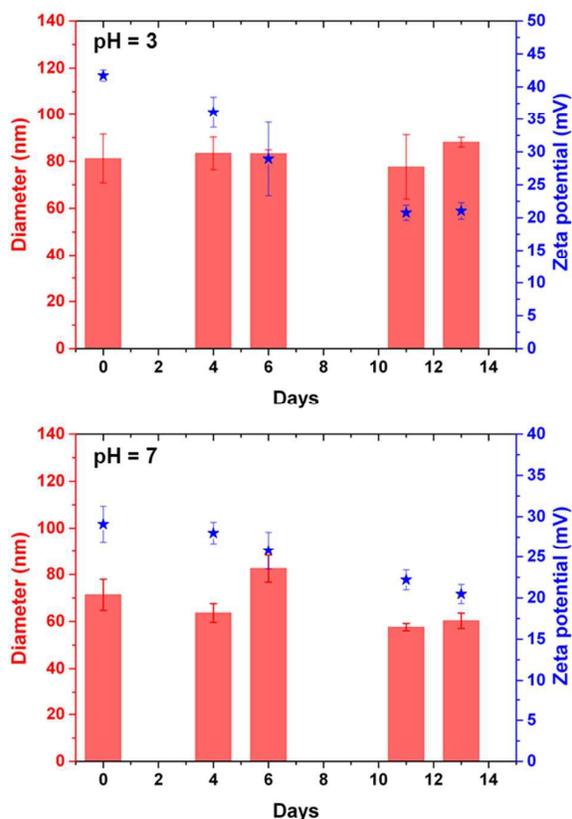

Figure 6 Number-weighted hydrodynamic diameters and zeta potential of the capsules stabilized by PAH-*graft*-PVN at various periods after preparation.

The average distance between the molecules of both types is not short enough to ensure efficient singlet energy migration from VN excited at 280 nm to Pe as no emission of Pe serving as energy acceptor is observed upon excitation of VN. Note, that the low-intensity emission observed between 450-500 nm is not the effect of energy transfer but rather direct excitation of Pe which slightly absorbs also at 280 nm (see Figure S10 in SI). Interestingly, for Pe solubilized in the hydrophobic pockets of PAH-*graft*-PVN, relatively high efficiency of energy transfer was observed (Figure 7). The

relative energy transfer efficiency was calculated using ratiometric equation (for details see SI) as the donor/acceptor intensity ratio to be 41% and 35% for solution at pH=3 and pH=7, respectively. Similar results were obtained for the system consisted of perylene trapped in domains formed by poly(sodium 4-styrenesulfonate)-*block*-vinylnaphthalene.[59] It confirms that perylene molecules are in close proximity to VN units and accept the energy absorbed by the chromophores most likely following Förster resonance energy transfer (FRET) mechanism. Transfer of an excitation energy to Pe is parallel with the decrease in the intensity of fluorescence of VN comparing to the spectra recorded for PAH-*graft*-PVN copolymer without the solubilized energy acceptor.

Interestingly, the efficiency of energy transfer increases significantly while moving from the copolymer solution to the core-shell capsules stabilized by PAH-*graft*-PVN. The observed intensity of Pe emission upon excitation at 280 nm was much higher than in solution which suggest that the distance between Pe and VN is relatively small and comparable to the the Förster radius for Pe-naphthalene pair that is equal to 31.2±0.4 Å.[60] Excitation energy is transferred mainly from monomeric VN that was manifested by more significant decrease of intensity of the short wavelength emission of the chromophores in the systems with encapsulated Pe (Figure 7). The relative FRET efficiency was calculated to be 63% and 68% for the capsules obtained in solution of pH=3 and pH=7, respectively, that are exceptionally high values.

It suggests that the efficiency of energy migration between VN chromophores is higher in the capsules than in PAH-*graft*-PVN domains formed in an aqueous solution. Moreover, in comparison to aggregates significant decrease in emission intensity of monomeric naphthalene in the capsules at the expense of perylene emission was observed. Slightly higher efficiency obtained for the capsules at pH=7 as compare to pH=3 may be due to the more relaxed conformation of the macromolecules that leads to lower probability of excimer formation which is noticeable in the emission spectra (Figure S8 in SI). Importantly, no structural changes in energy acceptor molecule were observed after indirect excitation as its emission spectrum was essentially unchanged.

The efficiency of energy transfer in the presented systems may be affected by two phenomena. On one hand, the confinement of the space that surrounds Pe molecules gives rise to the decrease in the distance between donor and acceptor molecules which may increase the FRET efficiency. On the other hand, aggregation of naphthalene chromophores observed in an aqueous solution promotes formation of excimers or aggregates that act as energy traps and decrease the FRET efficiency. Polymer chains in the capsules are located at the interface and are more uncoiled due to the presence of hydrophobic molecules solvating the chromophores. It reduces probability of formation of excimers and aggregates in the capsules. At the same time, the hydrophobic phase is restricted to the size of the nanocapsules which makes the distance between VN and Pe short enough to ensure effective energy transfer. Hydrophobic domains in the aggregates ensure short donor-acceptor distance, however the environment is very crowded that limits the rotational freedom of Pe leading to the entrapment of the excitation energy. Differences in the structures of the aggregates and capsules that lead to significant differences in excitation energy transfer efficiency are depicted in Scheme 2.
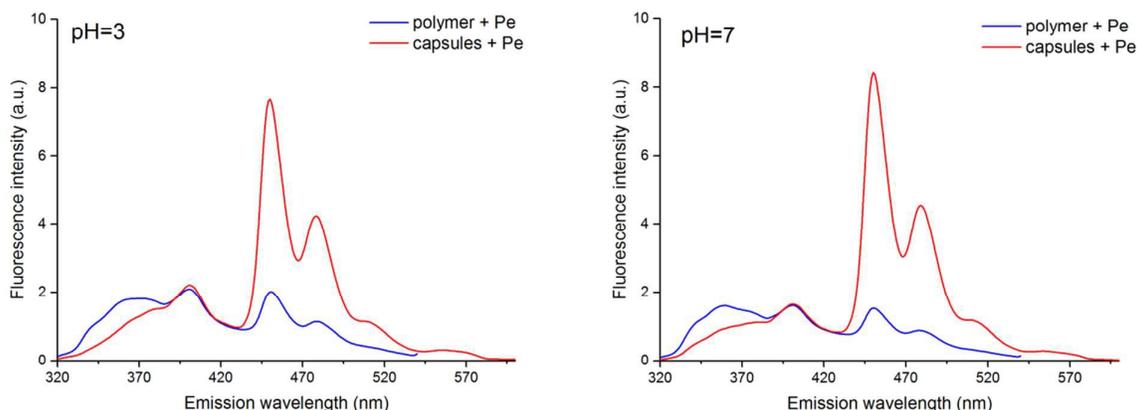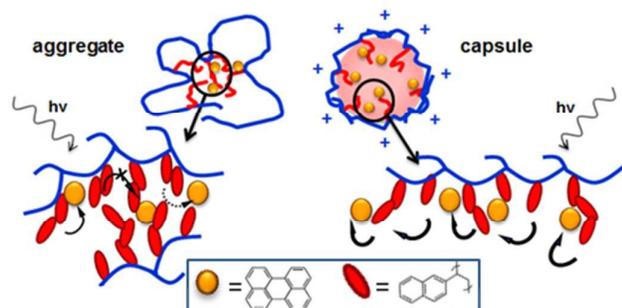
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Figure 7 Steady-state emission spectra (λ_{ex} = 280 nm) of aqueous solution of PAH-*graft*-PVN with solubilized Pe and dispersions of the capsules with encapsulated Pe.

Scheme 2 Model structures of the aggregates and capsules made of PAH-*graft*-PVN copolymer containing solubilized Pe. The curved arrows indicate excitation energy transfer occurring in the systems – the thicker the line, the more efficient energy transfer.

Photosensitized oxidation of solubilized and encapsulated Pe. The ability of the capsules to transfer excitation energy from the polymeric chromophores to the probe molecules can be very useful for designing effective nanoreactors, e.g. for removal of hydrophobic environmental pollutants. Photooxidation of Pe was used to check the possibility of the capsules to act as photosensitizers. Photooxidation of Pe leads to non-flourescent perylenequinones[61] so the extent of the reaction may be easily followed by measuring fluorescence of Pe during irradiation. Thus, the sensitized reaction carried out via irradiation at the wavelength absorbed almost exclusively by naphthalene chromophores (280 nm) is manifested by the decrease in the intensity of Pe emission (Figure S11 and S13 in SI).

The actual extent of the reaction was measured by following Pe fluorescence intensity (I) at λ_{em}= 450 nm (Figure S12 and S14).

Based on these data the photooxidation rate constants were determined as the slopes of the fitted lines in Figure 8. One can notice some pH dependency of the measured rate constants for the copolymer aggregates and insignificant differences for the capsules. Importantly, while comparing the rate constants for the capsules and copolymers at the same pH it is clear that the photooxidation of Pe is at least 3 times faster in the core-shell formulation. It may be a consequence of rearrangement of the chromophores while transferred from the solution to water/oil interface. In polar medium excimers acting as energy traps are formed within polymeric aggregates that lowers the energy transfer efficiency. At water/oil interface chromophores are less packed and able to provide more efficient energy transfer to Pe (see Figure 7 and Scheme 2). It suggests that polymeric capsules stabilized by graft copolymers with photoactive side chains serve as more efficient and insensitive to pH changes reactors for photooxidation than commonly used photozymes. For the capsules serving as nanophotoreactors Pe emission decreased almost completely after 10 min while for the aggregates 35 min was required to reach similar conversion (Figure S12 and S14).

Some changes in the intensity of VN emission during irradiation were also observed indicating some quenching of its fluorescence by oxygen[56] or partial oxidation. However, some structural changes may also take place what is especially noticeable while comparing the copolymer with capsules at different pH. The VN emission of PAH-*graft*-PVN copolymer at pH=3 decreases by 20-30%, however at pH=7 it changes only slightly after 35 min of irradiation and surprisingly, after 10 min even slightly increases. It suggests that some changes in the polymer conformation may occur that would reduce the number of energy traps (excimers, aggregates). Importantly, for the capsules the decrease in VN emission intensity is almost 20% at pH= 7 and nearly 40% at lower pH that indicates that formation of the capsules causes changes in molecular organization of the chromophores along the polymeric chains.
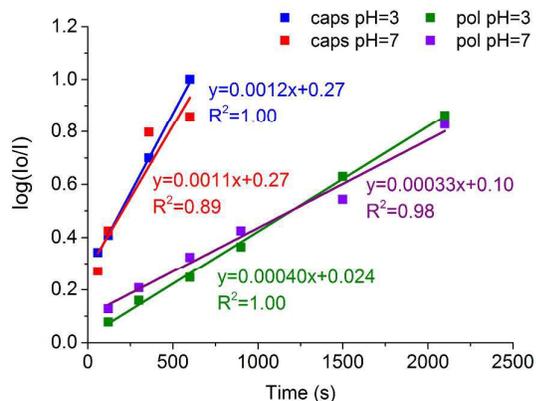
Figure 8 Kinetics of photooxidation of Pe solubilized in copolymer aggregates (a) and encapsulated in oil cores of capsules (b) excited via VN chromophores at 280 nm.

Conclusions

Graft amphiphilic copolymer, PAH-*graft*-PVN, composed of solvophilic backbone and solvophobic side chains was synthesized via nitroxide-mediated radical polymerization and used for stabilization of hydrophobic liquid droplets (toluene, n-octadecane) leading to formation of capsules of the diameter of c.a. 50-100 nm. Self-assembly of the synthesized copolymer in an aqueous solution led to the formation of intermolecular aggregates in which perylene, an exemplary sparingly water-soluble molecule, can be solubilized. Close proximity of perylene and polymeric naphthalene chromophores that are highly packed within the hydrophobic polymeric domains led to the efficient energy transfer which makes such copolymer a promising photosensitizer.

Relaxation of the aggregated PAH-*graft*-PVN structure after addition of an oil phase combined with emulsification led to the deposition of the copolymer at the water/oil interface and enabled stabilization of such nanoemulsion by anchoring hydrophobic polymeric arms in the nanodroplets. Importantly, no low molecular weight surfactant was needed in the proposed strategy as the applied amphiphilic polyelectrolyte acted as an efficient stabilizer of the droplets. The obtained capsules showed good stability during storage that is important for their application as nanocontainers for hydrophobic compounds such as perylene. Moreover, the efficiency of energy transfer between the naphthalene polymeric units and Pe solubilized in the cores of capsules was much higher (reached almost 70%) than for the molecular aggregates formed in an aqueous solution of PAH-*graft*-PVN. These observations could be explained in terms of specific polymer structure (partial uncoiling) adopted at the water/oil interface in the capsules. Such structures enable reduction of the formation of naphthalene excimers/aggregates that may serve as excitation energy traps. Moreover, the rate constant of the photosensitized oxidation of Pe was found to be c.a. 3 times larger for the capsules than for aggregate systems.

The proposed here photoactive nanocapsules are very promising for the development of nanoreactors for numerous photosensitized reactions that can be carried out in an aqueous medium and benefit from the confinement effect. This approach may be easily extended for other chromophores that may utilize also visible light.

Literature

1   M. Nowakowska, S. Zapotoczny and A. Karewicz, *Polymer*, 2001, **42**, 1817-1823.
2   S. Zapotoczny, M. Golonka and M. Nowakowska, *Langmuir*, 2008, **24**, 5868-5876.
3   M. Nowakowska, A. Karewicz, M. Kłos and S. Zapotoczny, *Macromolecules*, 2003, **36**, 4134-4139.
4   A. Bhattacharya and B. N. Misra, *Prog. Polym. Sci*., 2004 **29**, 767-814.
5   J. Bohrisch, C. D. Eisenbach, W. Jaeger, H. Mori, A. H. E. Müller, M. Rehahn, C. Schaller, C. Traser and P. Wittmeyer, *Adv. Polym. Sci*., 2004, **165**, 1-41.
6   S. Förster and M. Antonietti, *Adv. Mater*., 1998, **10**, 195-217.
7   L. Wang, J. Lin and L. Zhang, *Macronmolecules*, 2010, **43**, 1602-1609.
8   J. Wang and L. M. Leong, *Eur. Polym. J*., 2013, **49**, 3722-3733.
9   B. E. McKenzie, H. Friedrich, M. J. M. Wirix, J. F. de Visser, O. R. Monaghan, P. H. H. Bomans, F. Nudelman, S. J. Holder and N. A. J. M. Sommerdijk, *Angew. Chem. Int. Ed*., 2015, **54**, 2457-2461.
10 R. Nagarajan, *Curr. Opin. Colloid Interface Sci.,* 1996, **1**, 391-401.
11 P. J. M. Lebens and J. T. F. Keurentjes*, Ind. Eng. Chem. Res*.,1996, **35**, 3415-3421.
12 Y. Lin and P. Alexandridis*, J. Phys. Chem. B,* 2002, **106**, 10845-10853.
13 K.-Y. Peng, S.-A. Chen and W.-S. Fann, *J. Am. Chem. Soc*., 2001, **123**, 11388-11397.
14 S. Kirstein and S. Dähne, *Int. J. Photoenergy*, 2006, **20363**, 1.
15 S. E. Webber*, Chem. Rev*., 1990, **90**, 1469-1482.
16 J.-F. Baussard, J.-L. Habib-Jiwan and A. Laschewsky, *Langmuir*, 2003, **19**, 7963-7969.
17 I. Capek, *Adv. Colloid Interface Sci*., 2002, **97**, 91-149.
18 V. G. Rao, S. Mandal, S. Ghosh, C. Banerjee and N. Sarkar, *J. Phys. Chem. B*, 2012, **116**, 12021-12029.
19 D. Cringus, A. Bakulin, J. Lindner, P. Vöhringer, M. S. Pshenichnikov and D. A. Wiersma. *Phys. Chem. B,* 2007, **111**, 14193-14207.
20 P. Rajdev, D. Basak and S. Ghosh, *Macromolecules*, 2015, **48**, 3360-3367.
21 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 3rd edn., 2006.
22 M. Nowakowska, M. Kępczyński and K. Szczubiałka, *Pure Appl. Chem*., 2001, **73**, 491-495.
23 K. Y. Cho, J. W. Choi, S.-H. Lee, S. S. Hwang and K.-Y. Baek, *Polym. Chem*., 2013, **4**, 2400-2005.
24 Y. Li, Y. Zhang, D. Yang, C. Feng, S. Zhai, J. Hu, G. Lu and X. Huang, *J. Polym. Sci. A: Polym. Chem*., 2009, **47**, 6032-6043.
25 L. Wang, T. Jiang and J. Lin, *RSC Adv*., 2013, **3**, 19481-19491.

RSC Advances Accepted Manuscript

26 F. Liu, J. Hu, G. Liu, C. Hou, S. Lin, H. Zou, G. Zhang, J. Sun, H. Luo and Y. Tu, *Macromolecules*, 2013, **46**, 2646-2657.
27 J. Szafraniec, M. Janik, J. Odrobińska and S. Zapotoczny, *Nanoscale*, 2015, **7**, 5525-5536.
28 H. Wei, Y. Wang, J. Guo, N. Z. Shen, D. Jiang, X. Zhang, X. Yan, J. Zhu, Q. Wang, L. Shao, H. Lin, S. Wei and Z. Guo, *J. Mater. Chem. A*, 2015, **3**, 469-480.
29 Y. D. Tretyakov, A. V. Lukashin and A. A. Eliseev, *Russ. Chem. Rev.*, 2004, 73, 899-921.
30 K. Kim, *Chem. Soc. Rev.,* 2002, 31, 96-107.
31 S. A. Bode, I. J. Minten, R. J. M. Nolte and J. J. L. M. Cornelissen, *Nanoscale*, 2011, 3, 2376-2389.
32 P. Anzenbacher Jr and M. A. Palacios, *Nat. Chem.*, 2009, **1**, 80-87.
33 W. A. Solomonsz, G. A. Rance, B. J. Harris and A. N. Khlobystov, *Nanoscale*, 2013, 5, 12200-12205.
34 H. Zhang X., Pan, X. Han, X. Liu, X. Wang, W. Shen and X. Bao, *Chem. Sci.*, 2013, **4**, 1075-1078.
35 Q. Yang, D. Han, H. Yang and C. Li, *Chem. Asian J.,* 2008, **3**, 1214-1229.
36 L. Zhang, G. Xia, Y. Ge, C. Wang, Z. Guo, X. Li and X. Yu*, J. Mater. Chem. A*, 2015, **3**, 20494-20499.
37 J. He, Z. Liu, Y. Yoneyama, N. Nishiyama and N. Tsubaki, *Chem. Eur. J.*, 2006, **12**, 8296-8304.
38 J. He, Y. Yoneyama, B. Xu, N. Nishiyama and N. Tsubaki, *Langmuir*, 2005, **21**, 1699-1702.
39 G. Yang, N. Tsubaki, J. Shamoto, Y. Yoneyama and Y. Zhang, *J. Am. Chem. Soc.*, 2010, **132**, 8129-8136.
40 N. J. Coville and D. C. Levendis*, Eur. J. Inorg. Chem.*, 2002, **2002**, 3067-3078.
41 P. B. Zetterlund, *Polym. Chem.*, 2011, **2**, 534-549.
42 N. A. D. Burke, M. Templin and J. E. Guillet, *J. Photochem. Photobiol. A: Chem.*, 1996, **100**, 93-100.
43 M. Karlsson, M. Davidson, R. Karlsson, A. Karlsson, J. Bergenholtz, Z. Konkoli, A. Jesorka, T. Lobovkina, J. Hurtig, M. Voinova and O. Orwar, *Annu. Rev. Phys. Chem.*, 2004, **55**, 613-649.
44 J. Bain and S. S. Staniland, *Phys. Chem. Chem. Phys.*, 2015, **17**, 15508-15521.
45 N. Nandi, *Annu. Rev. Phys. Chem.*, 2009, **28**, 11-167.
46 H.-J. Kooa, O. D. Velev, *J. Mater. Chem. A*, 2013, **1**, 11106-11110.
47 B. Maity, K. Fujita and T. Ueno, *Curr. Opin. Chem. Biol.*, 2015, **25**, 88-97.
48 M. Rymarczyk-Machał, J. Szafraniec, S. Zapotoczny and M. Nowakowska, *Eur. Polym. J.*, 2014, **55**, 76-85.
49 L. Rintoul, A. S. Micallef and S. E. Bottle, *Spectrochim. Acta Mol. Biomol. Spectrosc.,* 2008, **70**, 713-717.
50 S. Goldstein and D. Meyerstein, *Acc. Chem. Res.*, 1999, **32**, 547-550.
51 S. W. Cranford, C. Ortiz and M. J. Buehler, *Soft Matter*, 2010, **6**, 4175-4188.
52 B. Jachimska, T. Jasiński, P. Warszyński, Z. Adamczyk, Colloids and Surfaces A: Physicochem. Eng. Aspects, 2010, **355**, 7–15.
53 M. Rymarczyk-Machał, S. Zapotoczny and M. Nowakowska, *J. Polym. Sci. A Polym. Chem.*, 2006, **44**, 2675-2683.
54 S. Zapotoczny, M. Rymarczyk-Machał, A. Stradomska, P. Petelenz and M. Nowakowska*, J. Phys. Chem. B.,* 2007, **111**, 10088-10094.
55 Y. Morishima, *Prog. Polym. Sci.*, 1990, **15**, 949-997.
56 M. Nowakowska, S. Zapotoczny and A. Karewicz, *Macromolecules*, 2000, **33**, 7345-7348.
57 M. M. Miller, S. P. Wasik, G.-L. Huang, W.-Y. Shiu and D. Mackay, *Environ. Sci. Technol.*, 1985, **19**, 522-529.
58 C. C. Ruiz, *J. Colloid Interface Sci.*, 2000, **221**, 262-267.
59 S. Zapotoczny, M. Golonka and M. Nowakowska, *Macromol. Rapid Commun.*, 2005, **26**, 1049-1054.
60 M. Nowakowska, B. White and J. E. Guillet, *Macromolecules*, 1989, **22**, 2317-2324.
61 P. Sotero and R. Arce, *J. Photochem. Photobiol. A: Chem.*, 2008, **199**, 14-22.
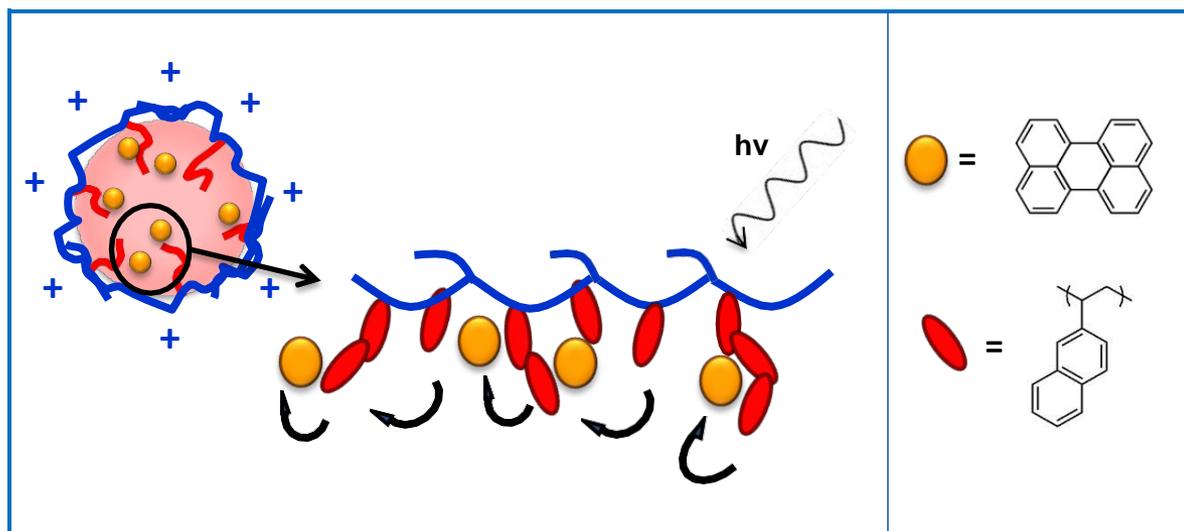
Polymeric nanocapsules templated on liquid core, stabilized by graft amphiphilic polyelectrolytes were fabricated and applied as photochemical reactors