Surface Modification of Poly(divinylbenzene) Microspheres via Thiol−Ene Chemistry and Alkyne−Azide Click Reactions

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ABSTRACT: We report the functionalization of cross-linked poly(divinylbenzene) (pDVB) microspheres using both thiol−ene chemistry and azide−alkyne click reactions. The RAFT technique was carried out to synthesize SH-functionalized poly(*N*-isopropylacrylamide) (pNIPAAm) and utilized to generate pNIPAAm surface-modified microspheres via thiol−ene modification. The accessible double bonds on the surface of the microspheres allow the direct coupling with thiol-end functionalized pNIPAAm. In a second approach, pDVB microspheres were grafted with poly(2-hydroxyethyl methacrylate) (pHEMA). For this purpose, the residual double bonds on the microspheres surface were used to attach azide groups via the thiol−ene approach of 1-azido-undecane-11-thiol. In a second step, alkyne endfunctionalized pHEMA was used to graft pHEMA to the azide-modified surface via click-chemistry (Huisgen 1,3-dipolar cycloaddition). The surface-sensitive characterization methods X-ray photoelectron spectroscopy, scanning-electron microscopy and FT-IR transmission spectroscopy were employed to characterize the successful surface modification of the microspheres. In addition, fluorescence microscopy confirms the presence of grafted pHEMA chains after labeling with Rhodamine B.

Introduction

In recent years, grafting techniques have been employed to affect the attachment of polymers onto surfaces of nano- and microparticles.[1,2] Surface modification of microspheres to obtain shell-functionalized microspheres is an interesting tool for modifying their properties.[3] Various approaches toward the surface-modification of poly(divinylbenzene) microspheres (pDVB) have been published over the past years. In general, two different approaches can be categorized, the "*grafting to*" and the "*grafting from*" approach. Several groups chose the "*grafting from*" technique because it allows growing polymer chains from the initiators on the substrate, leading to high grafting densities because the monomer units can easily diffuse to the propagating sites. Various living/controlled free polymerizations techniques can be employed for this purpose, e.g. the reversible addition−fragmentation chain transfer (RAFT) process or atom transfer radical polymerization (ATRP). In the "*grafting to*" technique, the polymer chains carry an active terminal group and are coupled to the active surface. Such an approach allows the characterization of the polymer chains before coupling but tends to suffer both from low grafting rates[4] and from low final grafting densities.

The immense amount of scientific interest in "click"-chemsitry in the past years—in particular for the Huisgen cycloaddition—shows the efficiency and the versatile applicability of this reaction.[5,6] The ease of synthesis of the alkyne and azide functionalities, coupled with tolerance to a wide variety of functional groups, stability and reaction conditions, make this coupling process highly attractive for the modification of polymeric materials. Concomitantly, the thiol−ene reaction may be—under certain conditions—an efficient way to couple polymer strands. Therefore, the thiol−ene reaction has started to attract researchers in various areas of material synthesis.[7−13] In our laboratories, the copper-catalyzed Huisgen 1,3-dipolar azide/alkyne cycloaddition process[14−18] as well as the equally effective hetero Diels−Alder conjugation chemistries[19−21] have been used successfully for a number of efficient coupling reactions.

In addition, several groups have applied the "*grafting from*" approach for the modification of microspheres. Zheng and Stöver reported the ring-opening polymerization (ROP) of ε-caprolactone catalyzed by $Al(Et)_3$ and $Al[OCH(CH_3)_2]_3$ from lightly cross-linked poly(DVB80-*co*-HEMA) microspheres[22] as well as the grafting of polystyrene from narrow disperse polymer particles by surface-initiated atom transfer radical polymerization.[23] Barner and co-workers employed RAFT polymerization to exert additional control over the design of core−shell pDVB microspheres and functional particles.[19,24,25] Furthermore, Barner and co-workers applied

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anionic ring-opening polymerization of ethylene oxide to synthesize hydroxyl-functionalized core/shell microspheres.[26]

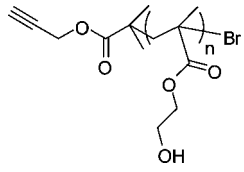
Even though "*grafting to*" techniques can suffer from lower grafting-densities, we demonstrate in here the versatility and success of these two click-techniques via the efficient surface-modification of pDVB microspheres in combination with controlled radical polymerization techniques (ATRP and RAFT).

Experimental Section

Materials. 11-Bromo-1-undecanol (98%, Aldrich), methanol (Merck), tetrahydrofuran (Merck), acetonitrile (Sigma-Aldich), 1,4-dioxane (Fisher Scientific), anisole (99%, Sigma Aldrich), dimethylformamide (BDH, Prolabo), CuBr (99,999%, Aldrich), 2-Bromo-2-isobutyrate,*N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA, Aldrich), 2-(Trimethylsilyloxy)ethyl methacrylate (TMS-HEMA, 96%, Aldrich), sodium azide (Sigma-Aldrich), sodium ascorbate (Sigma), *N*,*N*′-Dicyclohexylcarbodiimide (99%, Sigma-Aldrich), 4-(Dimethylamino)pyridine (99%, Aldrich), Rhodamin B base (97%, Aldrich), phosphorus oxychloride (98%, Fluka) and copper sulfate (Sigma), tris(2-carboxyethyl)phosphine (TCEP, powder, Aldrich), *N*-(1-pyrenyl)maleimide (PM, Sigma) were purchased and used as received. 2,2′-Azoisobutyronitrile (AIBN) was recrystallized from methanol. NIPAAm (*N*-Isopropylacrylamide) was recrystallized from a mixture of benzene and hexane (2:1). The synthesis of the RAFT agent 3-benzylsulfanylthiocarbonylsulfanyl propionic acid (BPATT) has been described elsewhere.[27]

Synthesis. *Synthesis of 1-Azido-undecan-11-thiol.* This compound was synthesized by adapting the method by Oyelere et al. ^{1}H NMR ($CDCl_3$, 300 MHz): 3.24 (t, 2H); 2.51 (q, 2H); 1.59 (m, 4H); 1.22−1.41 (m, 14H).[28]

Synthesis of Azido-Functionalized pDVB80 Microspheres (pDVB-N_3). Poly(divinylbenzene) microspheres (pDVB80) were prepared as described by Bai et al.[29] (DVB80, which is composed of isomers of DVB (meta and para), 80%, and 3- and 4-(ethylvinyl)styrene 20%, is used for the synthesis of the microspheres). A 1 g sample of pDVB80 microspheres were mixed with 1-Azidoundecan-11-thiol (1×10^{-5} mol) and AIBN (1×10^{-4} mol) in acetonitrile (10 mL) as solvent. The reaction mixture was stirred for 72 h under reflux. The functionalized microspheres were then isolated by filtration through a 0.45 μm membrane and washed thoroughly with tetrahydrofuran, ethanol, and acetone. Soxhlet extraction has been carried out in acetonitrile for 5 d to remove any unreacted compounds. The microspheres were dried under vacuum before characterization.



Synthesis of ω-Alkyne Poly(HEMA) with ATRP (Alkyne−pHEMA). The pHEMA polymer ($M_n = 21\,000$ g·mol^{-1}, $M_w/M_n = 1.77$) was prepared via the ATRP of TMS-HEMA followed by a deprotection of the TMS groups. The ATRP of TMS-HEMA in anisole runs as follows: after filtration through a silica column, 53.34 g of TMS-HEMA (0.26 mol) monomer was placed in a flask equipped with 164.4 g of anisole, 53.6 mg of CuBr (0.37 mmol), 54.0 mg of 2-propynyl 2-bromo-2-methyl propanoate (0.26 mmol) and a magnetic stirrer bar. The flask was then sealed with a septum and bubbled with nitrogen for 30 min. Then it was heated to 80 °C, and 66 mg of PMDETA (0.38 mmol) was injected under argon to start the polymerization. After 48 h, the reaction was stopped at a conversion of 47.5%. The reaction mixture was purified by filtration over a silica column and dialyzed against THF for 2 weeks.

The cleavage of the TMS protecting groups was carried out by precipitating the p(TMS-HEMA) solution from THF into water in the presence of several drops of concentrated HCl aqueous solution. The white precipitate was freeze-dried from dioxane.

Synthesis of Poly(HEMA) Functionalized pDVB80 (pDVB-g-pHEMA). pDVB-N_3 (0.02 g) was mixed with alkyne-pHEMA (0.2 g, 9.5×10^{-6} mol) in dimethylformamide in a Schlenk flask. Sodium ascorbate (0.19 g, 9.5×10^{-5} mol) dissolved in 1 mL of distilled water was added immediately to the solution. The solution is degassed with nitrogen for 20 min. A degassed flask containing copper sulfate (0.51 mg, 3.2×10^{-6} mol) in distilled water was transferred via a cannula to the Schlenck flask. The solution was stirred for 24 h at 70 °C. Any unreacted compounds were removed by Soxhlet extraction in THF and water.

Synthesis of Rhodamine B Chloride. A solution of Rhodamine B base (2.5 g, 5.6 mmol) in 1,2 dichloromethane (20 mL)—dried over molecular sieve (3 Å) overnight—was stirred under nitrogen, and phosphorus oxychloride (0.98 mL, 10.6 mmol) was slowly added dropwise over 5 min. The solution was refluxed for 5 h (90 °C). The color turned from dark red to dark purple. Thin layer chromatography (MeOH 100%) indicated full conversion after 4 h. After the dark purple solution was filtered by the use of syringe filters and evaporation of the solvent, the dark purple oily product was dried under vacuum (4.5 mbar) at 45 °C overnight, resulting in a dark-bronze colored solid as a crude product that was not purified further.

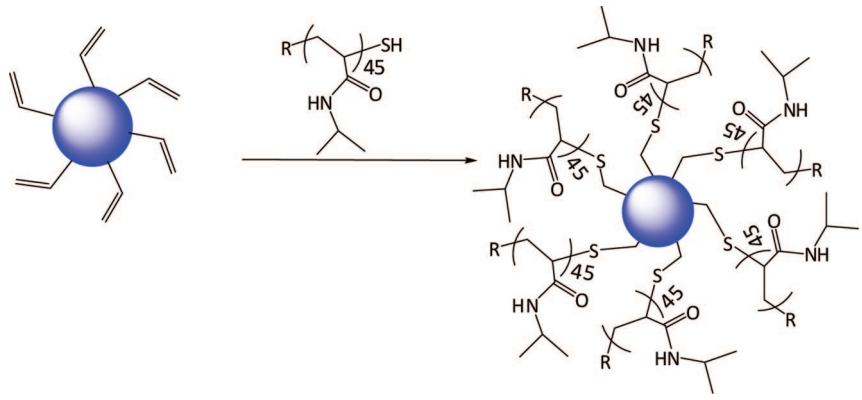
Rhodamine B Chloride-Labeling of pHEMA-Functionalized Microspheres. To fluorescence label the pDVB-*g*-pHEMA, 1 mg of pDVB-*g*-pHEMA grafted microspheres were added to a solution of *N*,*N*′-dicyclohexylcarbodiimide as a dehydrating agent (DCC) (5.0 mg, 5.8×10^{-6} mol), 4-(dimethylamino)pyridine (1.0 mg, 8.2×10^{-6} mol) and Rhodamine B acid chloride (5.2 mg, 1.1×10^{-5} mol) in 2 mL THF. The degassed mixture was stirred for 24 h at room temperature. Particles were washed thoroughly with THF, water and ethanol. As a control experiment, pDVB80 microspheres were submitted to the same reaction conditions.

Synthesis of pNIPAAm$_{45}$. In a round-bottom flask, 4.53 g of NIPAAm (40 mmol), 242.1 mg of 3-benzylsulfanylthiocarbonylsulfany propionic acid (BPATT, 8.9×10^{-4} mol), and 72.99 mg (4.5×10^{-4} mol) of AIBN were dissolved in 27 mL of dioxane. The flask was sealed with a rubber septum and the solution was degassed by nitrogen bubbling for 20 min. Then the flask was put in an oil bath at 60 °C for 24 h. The polymerization was stopped by cooling the reaction to room temperature under air exposure. The solution was concentrated under vacuum and precipitated in diethyl ether. After filtration the yellow powder was dried overnight under vacuum. A conversion of 82% was determined by gravimetric measurement. By analysis of the obtained polymer with a NMP SEC, a molecular weight of 5 300 g·mol^{-1} and a PDI of 1.14 were determined based on a polystyrene calibration.

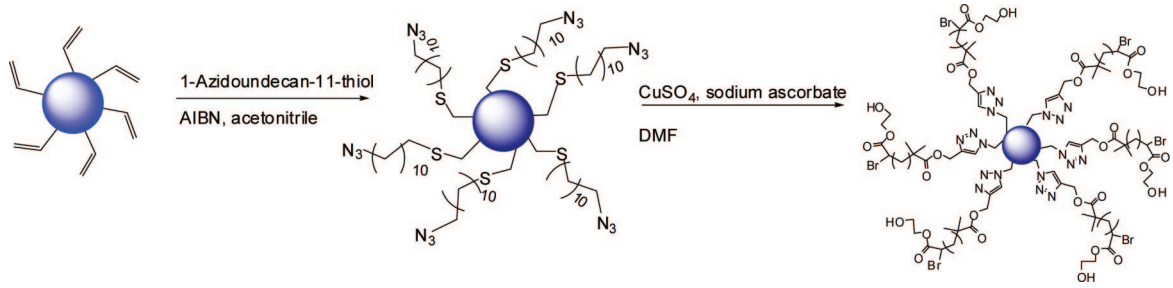
SH End Group Modification of pNIPAAm$_{45}$ (pNIPAAm$_{45}$−SH). Thiol-modification was followed by the procedure published by McCormick and co-workers.[30] To a 50 mL round-bottom flask were added pNIPAAm homopolymer ($M_n = 5300$ g·mol^{-1}, $M_w/M_n = 1.14$) and 15 mL of deionized water. The resulting solution was further diluted with an additional 15 mL solution of 1 M $NaBH_4$, and the mixture was allowed to react for 2 h. Following reduction, the homopolymer solution was dialyzed against water for 3 d and subsequently lyophilized. The resulting dried polymer was then dissolved in DMF, and a solution of tris(2-carboxyethyl phosphine) (TCEP) in DMF was added to yield a 150:1 mol ratio of TCEP to polymer. This solution was allowed to react for 24 h, after which it was charged with a solution of *N*-(1-pyrenyl)maleimide (PM) in DMF to yield a 150:1 mol ratio of PM to polymeric thiol (pNIPAAm-SH).

Thiol−Ene Reaction between pNIPAAm-SH and pDVB80 (pDVB-g-pNIPAAm). pDVB80 (0.05 g) was mixed with pNIPAAm-SH (0.25 g, 4.9×10^{-5} mol) in 10 mL acetonitrile in a Schlenck flask. AIBN (0.025 g, 1.5×10^{-4} mol) was added immediately to the solution. The solution was degassed with nitrogen for 20 min. Subsequently, the solution was stirred for 48 h at 70 °C to ensure complete conversion. Particles were washed thoroughly with acetonitrile and water by Millipore filtration.

Scheme 1. Thiol−Ene Modification of pDVB80 Microspheres with pNIPAAm$_{45}$ in a One-Step Approach (Approach 1)



Scheme 2. PHEMA Grafted Microspheres via Huisgen 1,3-Dipolar Cycloaddition (Approach 2)



Characterization. *NMR Spectroscopy.* ^{1}H NMR spectra were recorded on a Bruker ACF300 300-MHz spectrometer.

SEC. These measurements were performed at room temperature on an apparatus equipped with PSS GRAM columns (30 × 8 mm, 7 μm particle size) with 100 Å and 1 000 Å pore sizes and a precolumn using RI (Bischoff) and UV (270 nm, Waters) detection. NMP with 0.05 M LiBr was used as an eluent in the case of pNIPAAm and DMAC in the case of pHEMA. The flow rate was 1.0 mL·min^{-1} and the WinGPC software was used for evaluation of the obtained data.

X-ray Photoemission Spectroscopy. The samples were introduced through a load look system into an SSX-100 (Surface Science Instruments) photoemission spectrometer with a monochromatic Al Kα X-ray source ($h\nu$ = 1486.6 eV). The base pressure in the spectrometer during the measurements was 10^{-10} mbar. The photoelectron takeoff angle was 37°. The energy resolution was set to 1.3 eV to minimize measuring time. Sample charging was compensated for by directing an electron flood gun onto the sample. Spectral analysis included a Shirley background subtraction and a peak deconvolution that employed Gaussian and Lorentzian functions in a least-squares curve-fitting program (WinSpec) developed at the LISE, University of Namur, Belgium.

Fourier Transform Infrared (FT-IR) Transmission Spectra. These spectra were recorded using a Bruker IFS 66v/s spectrometer under vacuum at a resolution of 4 cm^{-1} using the KBr pellet technique. Spectra were recorded and evaluated with the software OPUS version 4.0 (Bruker).

Scanning Electron Microscopy (SEM). SEM images were recorded on a LEO 1530 (Zeiss) instrument, applying the InLens detector with a slow acceleration voltage of 2 kV and sputtering the microspheres with lead to a sufficient material contrast.

Fluorescence Microscopy. The fluorescence microscope (Leica DMRX) was operated with a HBO lamp as an excitation light source and a filter cube consisting of an excitation bandpass-filter (BP 450−490 nm), a dichroic beamsplitter with a cut off wavelength of 510 nm and a detection filter (LP 515 nm). With this combination we could observe the emission of the microspheres. We used objectives with several magnifications (20 ×, C Plan; 63 ×, HCX PL Fluotar; 100 ×, PL Fluotar; Leica). For each CCD-recorded frame (ColorView III, Soft imaging system) we chose an integration time of 50 s for all measured samples.

Confocal Fluorescence Microscopy. These images were captured using a Zeiss LSM 510 confocal laser scanning microscope. All images were captured using an oil immersion lens NA 1.3 (Objective Plan-Neofluar 40 × /1.3 oil). Rhodamine B was excited by a 488 nm Argon laser. A main beam splitter (MFT) was used with a long pass filter (488 nm/543 nm). Emission was captured by a spectral detection unit set 560 nm (LP).

Turbidity Study. A titration device, Metrohm automatic 809 Titrando system, was used and a temperature ramp from 20 to 70 °C was applied with a temperature increase of 1 °C·min^{-1}.

Elemental Analysis. This was performed using a Thermo Flash Elemental Analyzer (1112 Series), and D,L-methionin was used for calibration.

Results and Discussion

In the following section, we describe in detail the characterization of the core−shell microspheres which were synthesized via two approaches. For both approaches, poly(divinylbenzene) (pDVB80) particles were prepared by precipitation polymerization, having diameters of 1.3 μm. These microspheres have a thin surface layer consisting of lightly cross-linked and swellable poly (divinyl benzene)[31] and contain vinyl groups on their surfaces which are accessible for modification, i.e. direct surface modification via "*grafting to*" techniques. The RAFT technique was used to synthesize SH-functionalized poly(*N*-isopropylacrylamide) (pNIPAAm-SH) polymers to generate surface-modified microspheres via thiol−ene reaction (Scheme 1). In a second approach, pDVB80 microspheres were grafted with alkyne-functionalized pHEMA (Scheme 2). For this purpose, the residual double bonds on the microsphere surface were converted into azide groups via a thio-click approach using a thiol-azide compound (1-azido-undecane-11-thiol). In a second step, the alkyne end-functionalized pHEMA was grafted to the azide-modified surface via click-chemistry.

Approach 1. This method results in pDVB-*g*-pNIPAAm$_{45}$ microspheres. This approach is a simple way to modify pDVB80

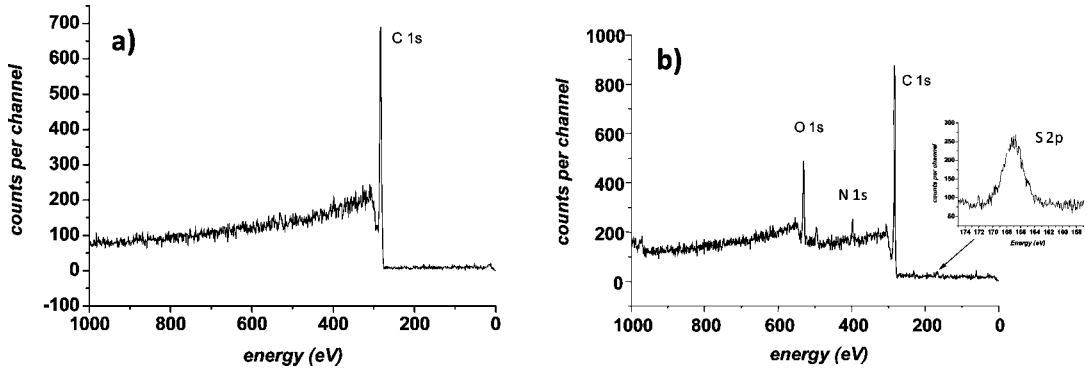


Figure 1. XPS spectrum of (a) poly(divinylbenzene) microspheres (pDVB80) and (b) pDVB80-*g*-pNIPAAm$_{45}$ microspheres. The inset shows the S2p XPS spectrum.

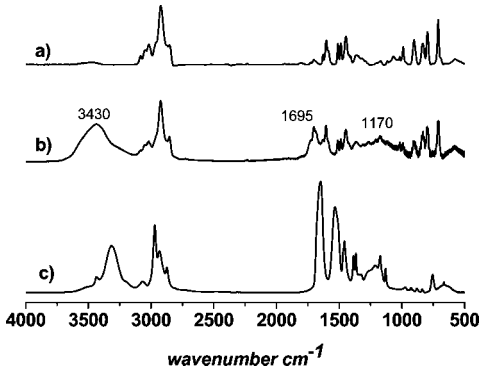


Figure 2. FT-IR transmissions spectra of (a) pDVB80 microspheres, (b) pDVB80-*g*-pNIPAAm$_{45}$ microspheres and (c) pNIPAAm$_{45}$ as reference.

microspheres due to the direct coupling of thiol-modified polymer to the residual free and accessible double bonds on the surface. The surface-modified microspheres were characterized with elemental analysis, SEM, FT-IR transmission spectra, and XPS. PNIPAAm is a stimuli-responsive polymer which shows response to change in temperature resulting in an LCST (lower critical solution temperature) around 32 °C. On the one hand, it has an expanded conformation due to hydration below 32 °C. On the other hand, it contracts in aqueous solution above the LCST. Among such diverse stimuli as temperature, pH, solvent composition, and electric fields, temperature is one of the most broadly used stimuli in environment-responsive polymer systems because it is easy to control.

XPS was used to identify the chemical composition at the surface of the modified microspheres. Figure 1a shows the XPS spectra of the pDVB80 microspheres and Figure 1b the spectra of the pDVB80-*g*-pNIPAAm$_{45}$ microspheres. Inspection of the Figures clearly shows that the poly(divinylbenzene) microspheres only display a signal for carbon while the grafted microspheres display additional signals for nitrogen, sulfur and oxygen atoms as expected for a pNPIAAm-containing surface. Thus the XPS data clearly confirm the attachment of pNIPAAM onto the surface of the microspheres.

Figure 2 shows the FT-IR transmission spectra of (a) pDVB80 microspheres, (b) pDVB80-*g*-pNIPAAm$_{45}$ microspheres and (c) pNIPAAm$_{45}$. Clearly, characteristic peaks of pNIPAAm can be detected in the spectrum of the surface modified microspheres, indicating the successful grafting (3 435 cm^{-1} (ν(N−H)$_{free}$, (ν(N−H)$_{bonded}$, amide), 1 705 cm^{-1} (amide stretch), 1 169 cm^{-1} (CH_3 and CH_2 skeletal).[22]

Suspension studies of pDVB80-*g*-pNIPAAm$_{45}$ microspheres demonstrate an appealing gain of hydrophilicity when grafted with pNIPAAm$_{45}$. PDVB80 and pDVB80-*g*-pNIPAAm$_{45}$ microspheres were stirred vigorously in deionized water. Prior to their functionalization, the particles show hydrophobicity, accumulate on the water surface, and adhere to the wall of the glass vial. However, pNIPAAm-grafted particles can easily be suspended in water due to their hydrophilic outer pNIPAAm-layer. This clearly indicates the disparate behavior of modified and unmodified microspheres. As mentioned above, pNIPAAm exhibits a lower critical solution temperature (LCST) in aqueous solution and a sharp reversible phase transition is observed at 32 °C in water.[24] Above the LCST of PNIPAAm (32 °C) the hydrophobic pDVB80-*g*-pNIPAAm$_{45}$ microspheres are sticking to the glass vial due to increasing hydrophobicity. The hydrophobicity of the microspheres leads to the continuous adsorption of the particles to the glass vial (see arrows in Figure 3).

This observation was supported by a turbidimetric study from 20 to 70 °C. Up to approximately 40 °C a slight increase in transparency is detected. Above 40 °C, which is slightly higher than the LCST of pNIPAAm (32 °C), a sharp increase of the transmission is observed (Figure 3). Near or above the LCST the pNIPAAm chains collapse and induce a more hydrophobic environment and therefore decrease the dispersibility of the microspheres. At this transition point, the microspheres aggregate and move to the water surface leading to a more transparent solution. As can be seen in Figure 3, the microspheres adhere to the glass vial above the LCST as a result of the increasing hydrophobicity. Although the "grafting to" approach has a general tendency toward lower grafting densities, the grafting density of pDVB80-*g*-pNIPAAm$_{45}$ is obviously sufficient to promote the stimuli-responsive behavior.

In addition, the microspheres were visualized by scanning electron microscopy (SEM). Figure 4a shows an image of a single pDVB80 microsphere. pNIPAAm-grafted particles (Figure 4b) clearly show a significantly more coarse and rough surface which is due to the attached polymer on the surface.

Approach 2. In a second study, we describe the synthesis and characterization of polyHEMA grafted pDVB80 microspheres (Scheme 2). Here, the Huisgen 1,3-dipolar cycloaddition is used to attach azido-functionalized pHEMA to the surface.

This approach is a very versatile and orthogonal method to attach any compound, polymer, or biomacromolecule carrying an alkyne-group to a surface. Therefore, multifunctional azido-microspheres (pDVB-N_3) were synthesized via the thiol−ene reaction. The surface-modified microspheres were characterized by elemental analysis, SEM, FT-IR, fluorescence spectroscopy and XPS. XPS analysis of the pDVB80-*g*-pHEMA microspheres (Figure 5c) exhibits the characteristic signals for bromine, nitrogen, sulfur and oxygen atoms.

The N 1s spectra of pDVB-N_3 shows two peaks at 402 and 399 eV (see Figure 5d). The ratio of the areas of these two peaks is approximately 2:1. The peak at 402 eV corresponds to

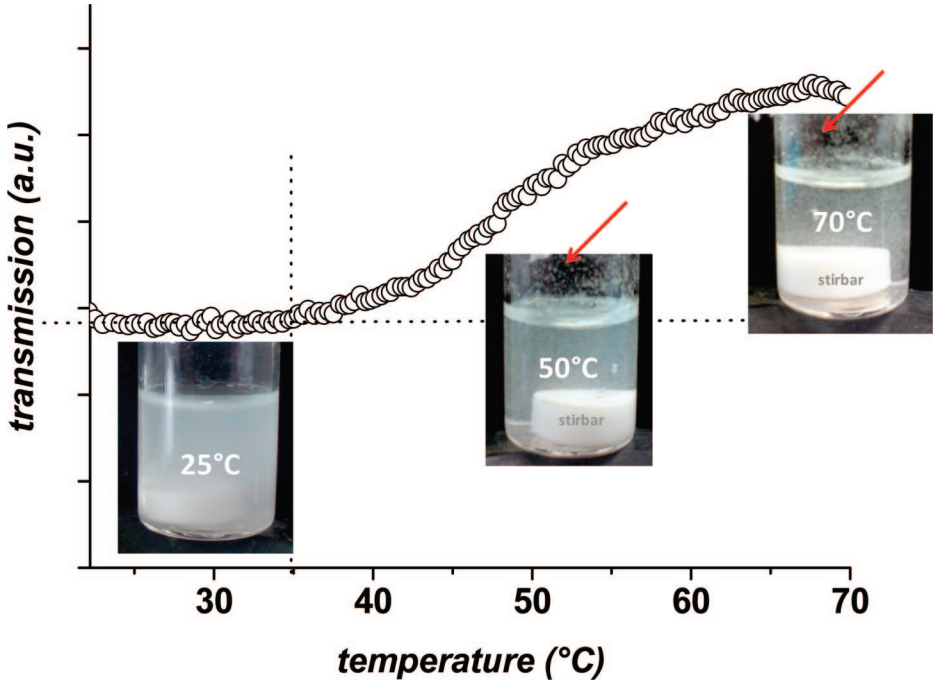


Figure 3. Temperature-dependent turbidity measurement of pDVB80-*g*-pNIPAAm$_{45}$ microspheres (20−70 °C). Suspension study in water for pDVB80-*g*-pNIPAAm$_{45}$ microspheres clearly showing the dispersibility of pDVB80-*g*-pNIPAAm$_{45}$ microspheres and increasing transmission with increasing temperature.

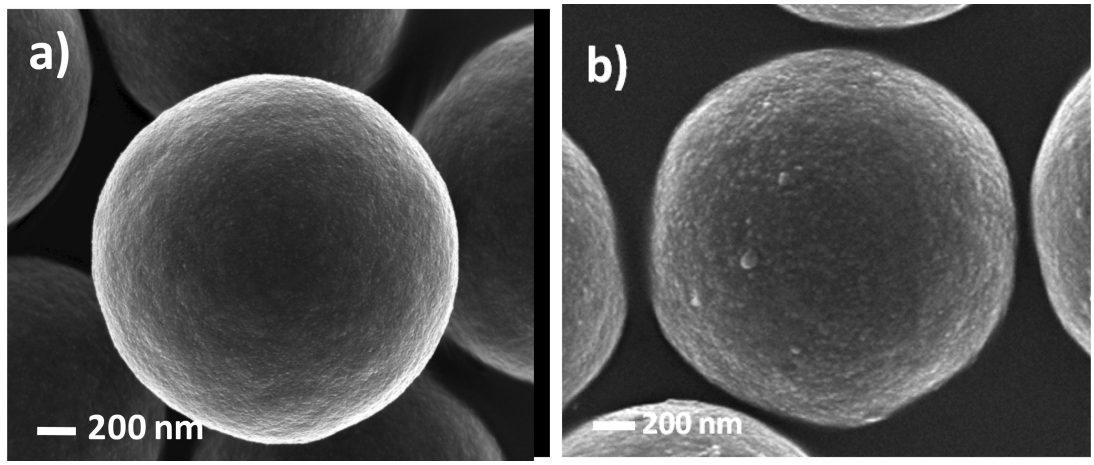


Figure 4. SEM images of (a) poly(divinylbenzene) microspheres (pDVB80) and (b) pDVB80-*g*-pNIPAAm$_{45}$ core−shell microspheres. The surface structure of pNIPAAm grafted microspheres is distinctly coarser compared to the blank microspheres.

the relatively electron poor middle N atom of the azide group[32] (Please note that the peaks are shifted due to charging effects). After reaction of the pHEMA with pDVB-N_3 only one N 1s signal can be observed at 397 eV. This is in accordance with previous results by Collman et al.[33] and London et al.[34] proving that the reaction took place. The N 1s signal at 397 eV observed in the case of *pDVB80-g-pHEMA microspheres* is quite broad which is caused by a high binding energy shoulder due to unreacted azide groups. Fitting the N 1s spectrum (see Figure 5d) shows that 41% of all azide groups have reacted. This is in good accordance with the values found with FT-IR spectroscopy.

Figure 6 shows the FT-IR transmission spectra of (a) pDVB80-*g*-pHEMA, (b) pDVB80-N_3 and (c) pDVB80 microspheres. The spectrum of the pDVB80-N_3 microspheres clearly shows the characteristic N_3-vibration at 2100 cm^{-1}. After reaction with alkyne-modified pHEMA, the peak decreases significantly but not completely. This indicates that not all azide groups have reacted. Comparing the areas under the N_3-vibration peaks at 2100 cm^{-1} before and after reaction shows that about 39% of all azide groups have reacted which is in good accordance with the XPS results. The increase in grafting density hinders further grafting in the vicinity of grafted polymer chains. Characteristic peaks for pHEMA after the click-reaction with N_3-functionalized microspheres can also be detected (3500 cm^{-1} (OH stretching), 1745 cm^{-1} (C═O), 1640 cm^{-1}, 1266 cm^{-1} (CH_2), 1018 cm^{-1} (CO(H) stretching)) proving a successful grafting.

To prove the effective attachment of the pHEMA chains to the surface of the azido-functionalized microspheres, the functional OH-groups of the pHEMA chains were used to label them with a fluorescent dye, e.g., Rhodamine B. This fluorescent tag has a functional carboxyl group which can react with hydroxy-functional end groups.

Figure 8a represents a fluorescence image of the pDVB80-*g*-pHEMA microspheres measured with a Leica DMRX. The homogeneous fluorescence clearly confirms that the microspheres were functionalized with pHEMA. The control experiment with pDVB80 microspheres under identical conditions

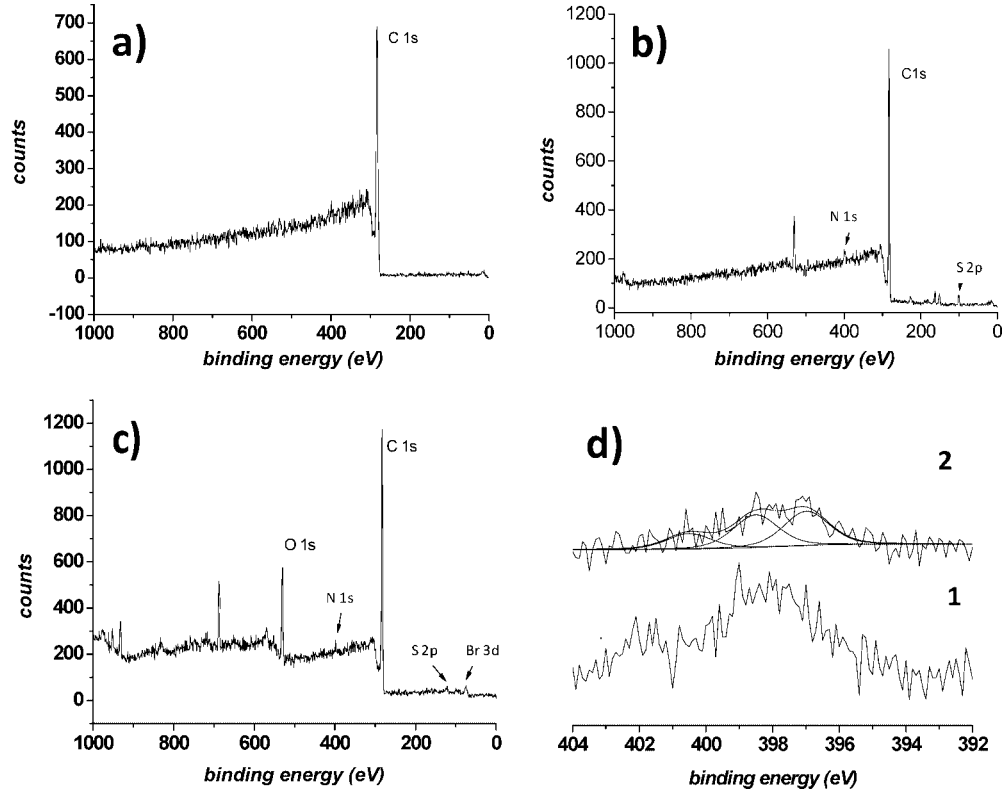


Figure 5. XPS spectra of (a) pDVB80, (b) pDVB-N_3 and (b) pDVB80-*g*-pHEMA microspheres. The inset shows the N 1s XPS spectrum. The peak at 688 eV results from residual $CuSO_4$ from click-reaction (Cu 2p). (d) N 1s XPS spectra of (1) pDVB-N_3 and (2) pDVB80-*g*-pHEMA microspheres.

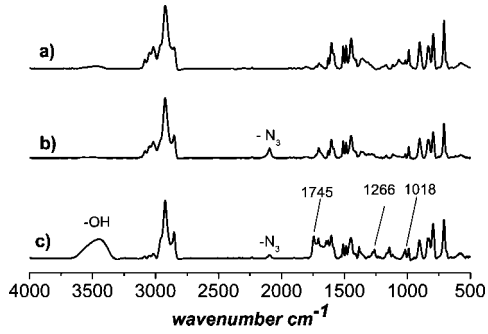


Figure 6. FT-IR transmission spectra of (a) pDVB80 microspheres, (b) pDVB80-N_3 and (c) pDVB80-*g*-pHEMA.

shows no fluorescence. Moreover, the fluorescent derivatization of the particles demonstrated a homogeneous distribution of OH groups on the surface of the particle (Figure 8a). Figure 8b shows the Rhodamine B-labeled microspheres on a filter paper after intensive washing. The particles have a pink color due to the covalently bound Rhodamine B to the pHEMA chains attached to the pDVB80 surface. As expected, the control experiment which was carried out under identical conditions with pDVB80 microspheres, results in nonfluorescent particles. Furthermore, the microspheres keep their white color, which indicates that no Rhodamine B is attached to the surface (Figure 8c).

Additionally, the Rhodamine B tagged microspheres were studied via confocal microscopy. Hence, it is possible to select the Z-dimension (three-dimensional function) which provides image depth and enables the fabrication of cross-sectional slices of the images. The image shown in Figure 9 represents a cross-sectional slice of fluorescence-labeled pHEMA microspheres. It clearly shows the fluorescence in the outer shell (and no fluorescence in the core of the particle) and therefore confirms the exclusive functionalization with pHEMA

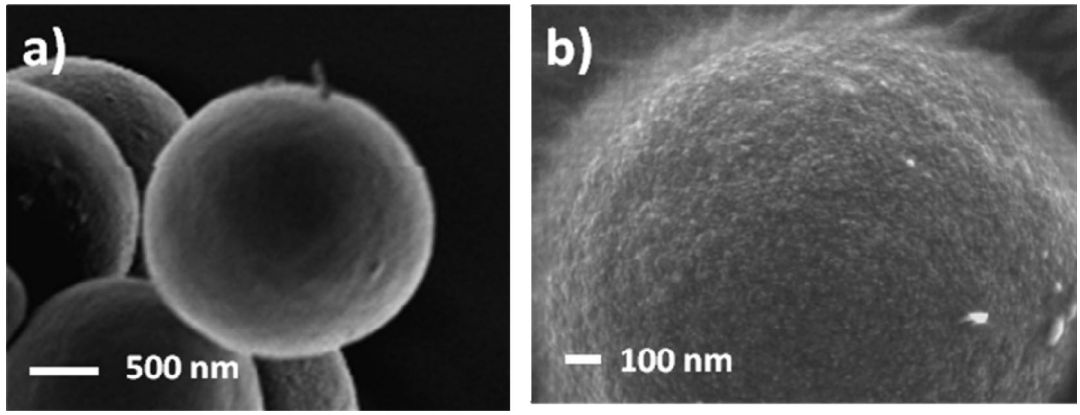


Figure 7. SEM images of (a) pDVB-*g*-pHEMA and (b) the same image magnified.

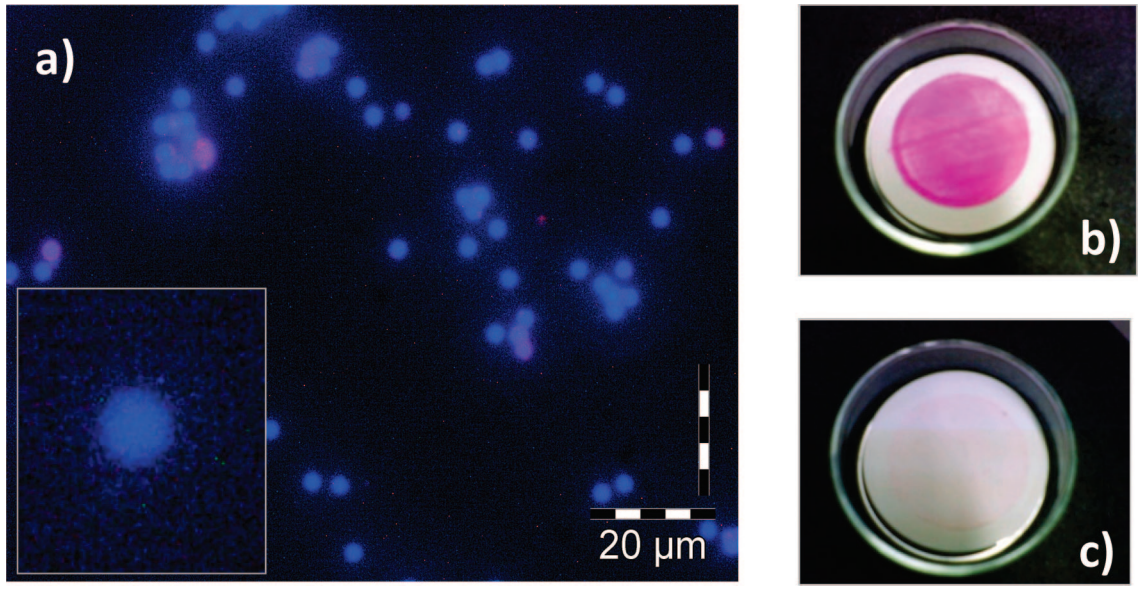


Figure 8. (a) Wide-field fluorescence microscopy images of Rhodamine B-labeled pDVB80-*g*-pHEMA (excitation filter: 450−490 nm), and (b) Rhodamine B-labeled microspheres on a filter paper (pink). The pink color results from the covalently bounding of the Rhodamine B. (c) Control experiment: identical conditions for ungrafted pDVB80 microspheres on a filter paper (white).

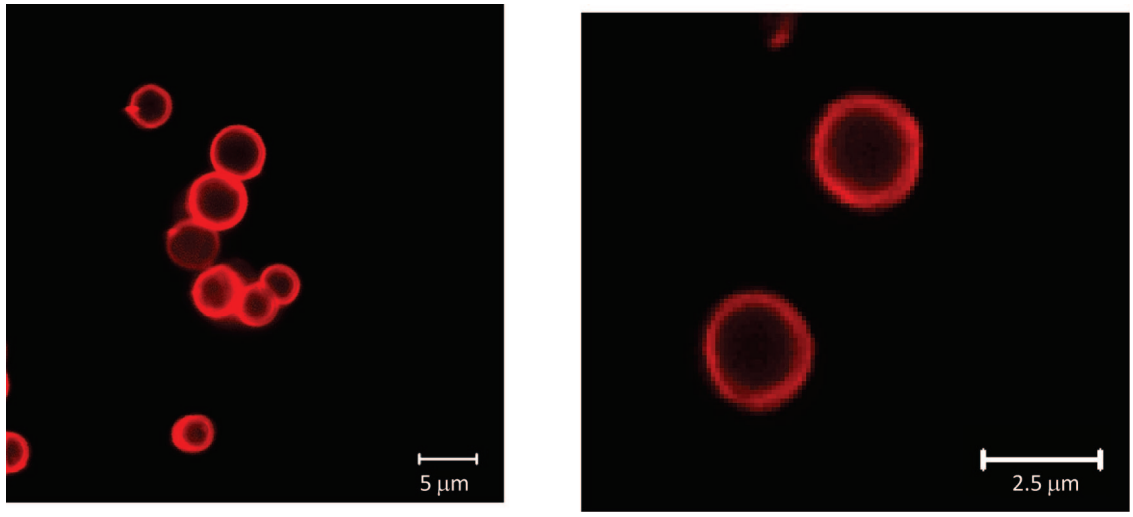


Figure 9. Confocal microscopy image of pDVB80-*g*-pHEMA microspheres functionalized with a Rhodamine B- fluorescent tag.

on the surface of the microspheres. Furthermore, the control experiment with nonfunctionalized pDVB80 microspheres under identical reaction conditions with Rhodamine B shows no fluorescence.

Conclusions

We demonstrate the successful grafting of polymer chains via thiol−ene chemistry and azide−alkyne click-reactions. The thiol−ene approach for grafting ω-thiol-functionalized polymers is a straightforward and effective method to directly graft polymers to the residual accessible double bonds of pDVB80 microspheres in a one-step process. As a model reaction, we chose SH-functionalized pNIPAAm, synthesized via RAFT polymerization. This approach can be extended toward the attachment of any thiol-functionalized compound to the surface (e.g., various thiol-functionalized responsive polymers and proteins). We showed the successful grafting via surface analysis methods (FT-IR transmission spectroscopy and XPS) and temperature dependent turbidity studies. The visualization of the particles was carried out with scanning electron microscopy (SEM).

In an alternative approach, the 1,3 Huisgen dipolar cycloaddition was used to click alkyne-functionalized pHEMA to N_3-functionalized pDVB80. This approach sufficiently extends our *"grafting to"* approach to further agents not carrying a thiol group. For this purpose multifunctional azido-functionalized microspheres were prepared via the thiol−ene reaction of 1-azido-undecan-11-thiol with residual double bonds on the surface. These surface-modified particles are grafted with pHEMA and characterized with FT-IR transmission spectroscopy, XPS, SEM and fluorescence microscopy. The presented grafting techniques therefore provide a facile and near global access to an enormous variety of functional grafted microspheres. Grafting of hydrophilic polymers to hydrophobic particles can truly enhance the suspension properties of the particles in aqueous environment.

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