New Route to Polymeric Nanoparticles by Click Chemistry Using Bifunctional Cross-Linkers

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Summary: A new route to functional polymeric nanoparticles (PNPs) of different chemical nature in the 3 to 20 nm size range is reported by combining both radical addition fragmentation chain transfer (RAFT) polymerization and "click" chemistry (CC) techniques. RAFT polymerization was employed for the synthesis of well-defined statistical copolymers with pending –Cl groups along the macromolecular chain. After transformation of the –Cl groups to $-N_3$ groups by treatment with sodium azide, an appropriate bifunctional cross-linker is employed to obtain PNPs under CC conditions promoting *intra*molecular cycloaddition (cross-linking). Following this new route, polystyrene, poly(alkyl (meth)acrylate), polymethacrylic acid, poly(sodium styrenesulfonate) and poly(*N*-isopropyl) NPs have been synthesized and in-deep characterized.

Keywords: "click" chemistry; nanoparticles; nanotechnology; reversible addition fragmentation chain transfer (RAFT); synthesis

Introduction

Nanoparticles (NPs) are currently ubiquitous along many research fields, including physics, chemistry, biology and medicine due to new and promising properties of nanoobjects compared to bulk materials.[1] Not surprisingly, the synthesis and characterization of polymeric nanoparticles (PNPs) is becoming a great challenge among polymer and materials scientists, due to the versatility and ease of production of these materials. However, synthetic routes to single-molecule cross-linked PNPs in the 3 - 20 nm size range are certainly scarce. The main approaches employed so far are: (1) collapse and intramolecular coupling of single polymer-chains at ultra-diluted (ca. 10^{-5} - 10^{-6} M) reaction conditions),[2] (2) thermally activated benzocyclobutene-coupling of individual chains by means of a continuous addition technique,[3] (3) microwave-assisted surfactant-free emulsion polymerization (SFEP) in the presence of bifunctional cross-linkers with enhanced reactivity[4] and (4) intramolecular "click" cycloaddition of random terpolymers involving azide and alkyne functionalities along the main chain.[5] Often, poorly defined materials (and in some cases even gels) are obtained using the first strategy. The second route requires high temperature conditions (250 °C) leading to undesirable side-reactions (i.e. oligomerization). Moreover, it cannot be employed with polymers that are not stable at high temperatures, such as acrylic or methacrylic (bio)polymers. The third route implies the use of specific equipment to guarantee a high stability of the microwave power in the reactor and hence a narrow NP size distribution. Finally, random terpolymerization is very difficult to perform in a controlled manner

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for monomers having very different reactivity ratios.

Here, we report a new route for the fabrication of PNPs that involves: (1) synthesis by RAFT polymerization of well-defined statistical copolymers containing –Cl groups pending along the macromolecular chain, (2) replacement of the –Cl groups by $–N_3$ groups by treatment of the copolymer with sodium azide in solution and (3) use of appropriate bifunctional cross-linkers and ‘‘click’’ chemistry[6–8] reaction conditions promoting efficient intramolecular cycloaddition. Following this new route, polystyrene, poly(alkyl (meth)acrylate), polymethacrylic acid, sulfonated polystyrene and poly(*N*-isopropyl acrylamide) NPs have been synthesized and in-deep characterized.

Experimental Part

Materials

Styrene (S, 98%), chloromethylstyrene (ClMS, 97%), *t*-butyl methacrylate (*t*BMA, 98%), 2-chloroethyl methacrylate (ClEMA, 97%), *n*-butyl acrylate (*n*BA, 98%), methyl methacrylate (MMA, 99%), sodium styrenesulfonate (NaSS, > 90%), *N*-isopropylacrylamide (NIPAM, 97%), 4,4′-azobis(4-cyanovaleric acid) (ACV, 98%), copper(I) bromide (CuBr, 98%), 2,2′-bipyridyl (bipy, 99%), sodium azide (NaN_3, 99.5%), trifluoroacetic acid (TFA, 99%) and 1,7-octadiyne (98%) were purchased from Sigma-Aldrich and used as received. 2.2′-Azo(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%) was recrystallized from methanol. Cumyl dithiobenzoate (CDB),[5] 4-cyanopentanoic acid dithiobenzoate (CPADB)[9,10] and S-1-dodecyl-S′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (DDMAT)[11] were synthesized and purified as reported elsewhere.

Measurements

Atomic force microscopy (AFM) images were obtained using a scanning probe microscope (PicoScan from Molecular Imaging) operating in a tapping mode at room temperature. Samples were prepared by casting a drop of diluted sample in water or THF (0.3 mg/20 mL) on the top of a freshly cut mica disk. Proton nuclear magnetic resonance (^1H NMR) experiments were performed on a Bruker AVANCE III spectrometer at 500 MHz. All samples were measured at room temperature in $CDCl_3$ unless otherwise stated. Size exclusion chromatography (SEC) experiments were performed on a Shimadzu SCL 10 AVP machine, connected to three 5 mm-PLgel columns with pore sizes of 10^2, 10^3 and 10^4 Å, using a RID-10A detector and a LC-10ADVP pump. The analysis was performed using either *N,N*-dimethylformamide (DMF) at 60 °C, or using tetrahydrofuran (THF) at 40 °C as eluent. The molecular weights (M_w) and polydispersity indexes (PI) of the polymers were calculated relative to linear polystyrene (PS) standards. Fourier Transformed Infra-Red Spectroscopy (FT-IR) spectra were taken from a Nicolet Avatar 360 apparatus. All samples were measured in KBr disks. Dynamic Light Scattering (DLS) measurements were carried out on a Beckam Coulter N5 apparatus.

Preparation of Poly(styrene–*co*-chloromethylstyrene), P(S-*co*-ClMS)

In a typical procedure, a mixture of styrene (4 g, 38.46 mmol), ClMS (1 g, 6.55 mmol) and CDB (0.006 g, 0.022 mmol) was placed in a *Schlenk* flask and degassed by means of 5 freeze/pump/thaw cycles. The reaction mixture was then placed in a preheated (110 °C) oil bath and stirred for 18 hours at the same temperature. The reaction was then allowed to cool to room temperature and the resulting mixture was precipitated into methanol. The product was filtered and dried under vacuum, to give a slightly pink solid (3.2 g). The composition of the copolymer was determined by ^1H nuclear magnetic resonance (NMR) spectroscopy. The mole fraction of ClMS in the copolymer was found to be 18%. ^1H NMR (*500 MHz, $CDCl_3$*): (δ,ppm) 7.24-6.57 (m, ArH); 4.5 (b, 2H, CH_2Cl); 2-1.25 (m, CH_2, CH). FT-IR: 3081, 3056, 3017, 2926, 2844, 1676, 1611, 1490, 1452, 1339 and 1245 cm^{-1}.

Preparation of Poly(*n*-butyl acrylate-*co*-chloroethyl methacrylate), P(*n*BA-*co*-ClEMA)

In a typical procedure, a solution of *n*BA (2.8 g, 21.84 mmol), ClEMA (0.89 g 5.98 mmol), CDB (0.012 g, 0.044 mmol) and AIBN (0.0015 g, 0.009 mmol) in acetone (2 mL) was placed in a *Schlenk* flask and degassed by means of 5 freeze/pump/thaw cycles. The reaction mixture was then placed in a preheated (65 °C) oil bath and stirred for 18 hours at the same temperature. After this time, the solvent and unreacted monomers were eliminated in a rotary evaporator and the product was dried under vacuum, obtaining the product as a pale orange solid (2.9 g). The composition of the resulting copolymer was determined by ^{1}H NMR spectroscopy. The mole fraction of ClEMA in the copolymer was found to be 19%. ^{1}H NMR (500 MHz, *CDCl$_3$*): (*δ,* ppm) 4.12 (b, 2H, OCH_2), 4.02 (b, 2H, OCH_2), 3.69 (b, 2H, CH_2Cl), 0.9 (b, 3H, CH_3). FT-IR: 2965, 2930, 2874, 1732, 1460, 1387, 1253 and 1163 cm^{-1}.

Preparation of Poly(methyl methacrylate-*co*-chloromethylstyrene), P(MMA-*co*-ClMS)

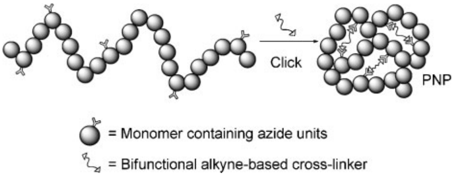
In a typical procedure, a mixture of MMA (2.9 g, 28.9 mmol), ClMS (1.1 g 7.2 mmol) and CDB (0.014 g, 0.05 mmol) was placed in a *Schlenk* flask and degassed by means of 5 freeze/pump/thaw cycles. The reaction mixture was placed in a preheated (110 °C) oil bath and stirred for 20 hours at the same temperature. The reaction was then allowed to cool to room temperature and the resulting mixture was precipitated into methanol. The product was filtered and dried under vacuum, to give a slightly pink solid (2.6 g). The composition of the copolymer was determined by ^{1}H NMR spectroscopy. The mole fraction of ClMS in the copolymer was found to be 34%. ^{1}H NMR (*500 MHz, CDCl$_3$*): (*δ, ppm)* 7.24-6.75 (m, ArH); 4.5 (b, 2H, CH_2-Cl), 3.6 (s, 3H OCH_3), 2-0.5 (m, CH_3, CH_2, CH). FT-IR: 3006, 2943, 1728, 1676, 1602, 1450, 1383, 1236, 1197 and 1145 cm^{-1}.

Preparation of Poly(*t*-butyl methacrylate-*co*-chloroethyl methacrylate), P(*t*BMA-*co*-ClEMA)

In a typical procedure, a solution of containing *t*BMA (3.17 g, 22.3 mmol), ClEMA (0.9 g, 6.05 mmol), CDB (0.014 g, 0.05 mmol) and AIBN (0.0011 g, 0.015 mmol) in acetone (2 mL) was placed in a *Schlenk* flask and degassed by means of 5 freeze/pump/thaw cycles. The reaction mixture was placed in a preheated (65 °C) oil bath and stirred for 18 hours at the same temperature. After this time, the reaction was allowed to cool to room temperature and the crude was precipitated into a methanol/water (50/50) mixture. The product was filtered and dried under vacuum, to give a slightly pink solid (3.1 g). The composition of the copolymer was determined by ^{1}H NMR spectroscopy. The mole fraction of ClEMA in the copolymer was found to be 17%. ^{1}H NMR (*500 MHz, DMSO-d$_6$*): (*δ*, ppm) 4.12 (b, 2H, OCH_2), 3.69 (b, 2H, CH_2Cl), 1.42 (b, 9H, *t*-Bu). FT-IR: 2979, 2936, 1724, 1480, 1459, 1394, 1368, 1252, 1139, 849 and 750 cm^{-1}.

Preparation of Poly(sodium styrenesulfonate-*co*-chloromethylstyrene), P(NaSS-*co*-ClMS)

In a typical procedure, NaSS (1 g, 4.85 mmol), ClMS (0.3 g 1.9 mmol), CPADB (0.007 g, 0.025 mmol) and ACV (0.002 g, 0.007 mmol) were dissolved in 10 mL of a mixture of water/DMF (70/30) in a *Schlenk* flask. After degassing by means of 5 freeze/pump/thaw cycles, the reaction mixture was placed in a preheated (75 °C) oil bath and stirred for 24 hours at the same temperature. After this time the solvent and unreacted monomer were removed under vacuum. The crude copolymer was purified by dialysis against water and then freeze-dried to give a white solid (0.7 g). The composition of the copolymer was determined by ^{1}H NMR spectroscopy. The mole fraction of ClMS in the copolymer was found to be 30%. ^{1}H NMR (*500 MHz, DMSO-d$_6$*): (*δ*, ppm) 7,46-6.5 (m, ArH), 4.5 (b, 2H, CH_2Cl); 2-1.25 (m, CH_2, CH). FT-IR: 3340, 3056, 2927, 2854, 1650, 1490, 1435,1409 and 1383 cm^{-1}.



Scheme 1.
Illustration of the new route to polymeric nanoparticles (PNPs) from azide-containing random copolymers and bifunctional cross-linkers under conditions promoting intramolecular "click" cycloaddition.

Preparation of Poly(*N*-isopropylacrylamide-*co*-chloroethyl methacrylate), P(NIPAM-*co*-ClEMA)

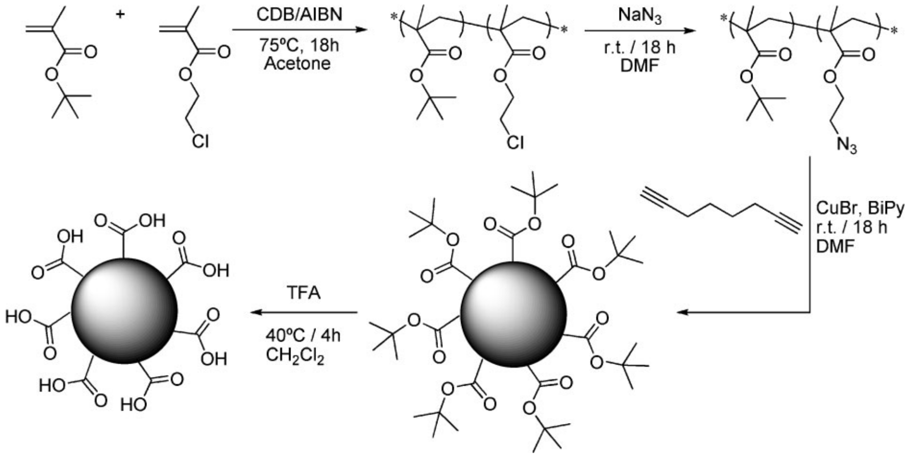
In a typical procedure, a solution of NIPAM (2 g, 17.67 mmol), ClEMA (0.29 g 1.95 mmol), DDMAT (0.017 g, 0.046 mmol) and AIBN (0.0016 g, 0.009 mmol) in DMF (3 mL) was placed in a *Schlenk* flask and degassed by means of 5 freeze/pump/thaw cycles. The reaction mixture was then placed in a preheated (70 °C) oil bath and stirred for 18 hours at the same temperature. After this time, the reaction was allowed to cool to room temperature and the resulting mixture was precipitated into diethyl ether. The product was filtered and dried under vacuum, to give a white solid (1.5 g). The composition of the copolymer was determined by ^{1}H NMR spectroscopy. The mole fraction of ClEMA in the copolymer was found to be 11%. ^{1}H NMR (*500 MHz, CDCl$_3$*): (δ, ppm) 4.12 (b, 2H, OCH_2), 3.98 (b, 1H NHCH) 3.69 (b, 2H, CH_2Cl), 0.98 (b, 6H, CH(CH_3)$_2$). FT-IR: 3295, 3071, 2969, 2866, 1725, 1636, 1540, 1168 and 1034 cm^{-1}.

General Procedure for the Preparation of Azide-Functionalized Copolymers

In a typical procedure, a Cl-containing copolymer (500 mg) was dissolved in DMF (20 mL) and treated with sodium azide (2 equiv.). The reaction was stirred at room temperature for 24 h. After this time, the crude was poured into a methanol/water mixture, and the product recovered by filtration. The reaction was followed by ^{1}H NMR, observing the disappearance of the CH_2-Cl signal (at 4.5 ppm for ClMS and at 3.6 ppm for ClEMA), and the appearance of the new CH_2-N$_3$ signal at 4.25 and 3.4 ppm, respectively. Alternatively, the reaction can be followed by FT-IR spectra observing the formation of a new azide stretching band at 2102 cm^{-1}.

General Procedure for the Preparation of PNPs from Azide-Functionalized Copolymers

In a typical procedure, CuBr (0,2 g, 1.39 mmol), bipy (0.2 g, 1.28 mmol) and the



Scheme 2.
Synthesis of poly(*t*-butyl methacrylate) (P*t*BMA) NPs and water-soluble poly(methacrylic acid) (PMAAc) NPs thereof.

corresponding azide-containing copolymer (200 mg) were dissolved in previously degassed DMF (200 mL) under N_2 atmosphere. While this solution was vigorously stirred, a solution containing an alkyne bifunctional cross-linker, typically 1,7-octadiine, (equimolar amounts of alkyne and azide groups) in DMF (10 mL) was added dropwise via a syringe pump (LHS 300/600 Brand GmbH) at a rate of 2 mL/h under N_2. After the addition was complete, the solution was stirred for further 2 h at room temperature. Then, CH_2Cl_2 was added and the organic layer was washed multiple times with saturated aqueous NH_4Cl, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by precipitation into a methanol/water mixture. Almost quantitative intramolecular cycloaddition (*c.a.* $> 95\%$) leading to new 1,2,3-triazole units was confirmed by FT-IR, in which the almost complete disappearance of the azide band is observed ($2102\,cm^{-1}$). This was further corroborated by ^1H NMR spectra in which the corresponding disappearance of the signal of the azide methylene at 4.25ppm (N_3-MS) and 3.4 ppm (N_3-EMA) is observed. In addition, the PNPs were further characterized by AFM, SEC and DLS measurements.

Synthesis of Water-Soluble PMAAc Nanoparticles

*t*BMA PNPs (300 mg) were dissolved in CH_2Cl_2 (20 mL) at room temperature. TFA (2 mL) was then added in small portions. The reaction flask was stopped with a glass stopper and the reaction mixture was stirred at 40 °C for 4 hours. After removing the solvent and unreacted TFA by rotary evaporation, the crude product was dissolved in a small amount of methanol and precipitated into diethyl ether. The product was filtrated and dried under vacuum to give a white solid (150 mg).

Results and Discussion

The new route towards the synthesis of PNPs proposed in this work is illustrated in Scheme 1.

Single-molecule polymeric nanoparticles were obtained by intramolecular "click" cycloaddition from a random copolymer containing azide functional groups and a bifunctional cross-linker under dilute reaction conditions. Several copolymers of very different chemical nature and low polydispersity index (PDI) have been synthesized by RAFT to demonstrate the versatility of this approach (Table 1). The RAFT polymerization technique permits to obtain a series of copolymers with pending −Cl groups along the macromolecular chains of very narrow PDI in a well-

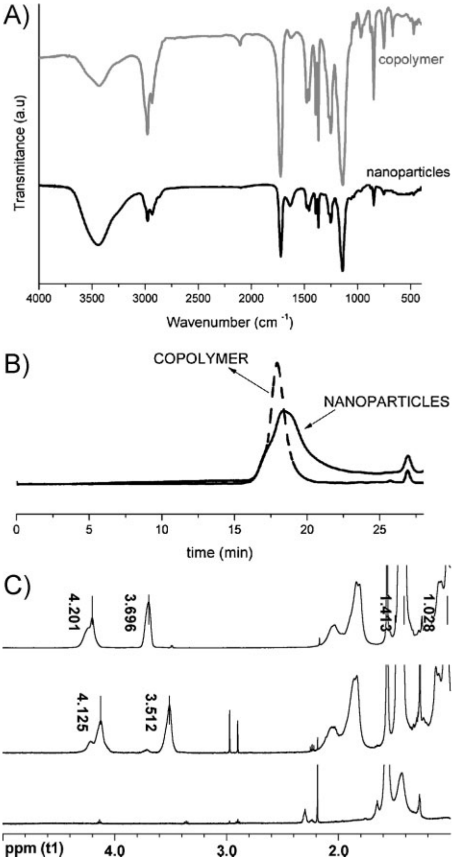


Figure 1.
Comparison of FT-IR (A) and SEC (B) data of the azide-containing PtBMA copolymer and the resulting NPs upon "click" cycloaddition with a dialkyne cross-linker. (C) ^1H NMR spectra corresponding (from top to bottom) to the Cl-containing PtBMA copolymer, the azide-containing copolymer and the resulting NPs.

Table 1.
Characteristics of the copolymers synthesized by RAFT polymerization.

Copolymer	Mn [a)]	PDI [a)]
P(S-*co*-ClMS)	99000	1.2
P(*n*BA-*co*-ClEMA)	42300	1.3
P(MMA-*co*-ClMS)	48000	1.3
P(*t*BMA-*co*-ClEMA)	29000	1.17
P(NaSS-*co*-ClMS)	[b)]	[b)]
P(NIPAM-*co*-ClEMA)	39000 [c)]	1.3 [c)]

[a)] Measured by SEC in THF.
[b)] Insoluble in THF.
[c)] Measured by SEC in DMF.
P(S-*co*-ClMS) = poly(styrene-*co*-chloromethylstyrene); P(*n*BA-*co*-ClEMA) = poly(*n*-butyl acrylate-*co*-chloroethyl methacrylate); P(MMA-*co*-ClMS) = poly(methyl methacrylate-*co*-chloromethylstyrene); P(*t*BMA-*co*-ClEMA) = poly(*t*-butyl methacrylate-*co*-chloroethyl methacrylate); P(NaSS-*co*-ClMS) = poly(sodium 4-styrenesulfonate–*co*-chloromethylstyrene); P(NIPAM-*co*-ClEMA) = Poly(*N*-isopropyl acrylamide-*co*-chloroethyl methacrylate).

controlled manner. Additionally, these –Cl groups are easily (and nearly quantitatively) replaced by $-N_3$ groups upon treatment with sodium azide at room temperature.

As a typical example, Scheme 2 shows the synthetic route to poly(*t*-butyl methacrylate) (P*t*BMA) NPs, and subsequent deprotection to give the corresponding water-soluble poly(methacrylic acid) (PMAAc) NPs.

The disappearance of the azide groups upon reaction with the alkyne-containing bifunctional cross-linker leading to the formation of triazole groups was monitored by FT-IR (Figure 1A). A very small percentage of azide (< 5%) is still noticeable in the FT-IR spectrum of the P*t*BMA PNPs. In this sense, it is worth noticing that a very small percentage of azide could be very useful for further functionalization of the PNPs with targeting moieties.[5] Collapse of the macromolecular chains upon crosslinking was clearly observed by SEC (Figure 1B). Additional evidence of the collapse was obtained by AFM (Figure 2) and DLS (see Table 2) results. Figure 1 C shows the evolution (from top to bottom) of the H^1 NMR spectra corresponding to the Cl-containing P*t*BMA copolymer, the cor-

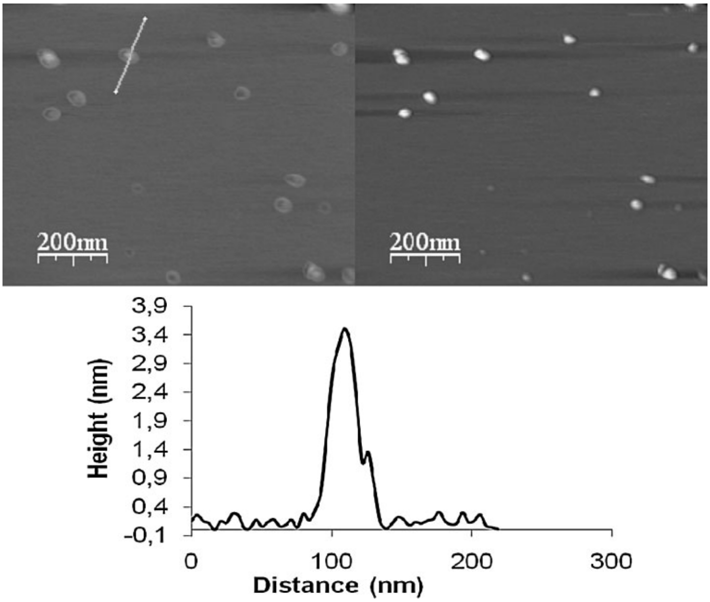


Figure 2.
Topographic AFM images and corresponding profiles of PtBMA PNPs (in the dry state) showing a mean particle diameter of 3.5 nm.

Table 2.
Characteristics of the PNPs synthesized from the copolymers in Table 1 by "click" chemistsry using bifunctional cross-linkers.

Nanoparticle	Mn[a)]	PDI [a)]	Size by DLS (nm) [a)]
PS	45000	1.7	10 ± 2
PnBA	15000	1.3	8 ± 2
PMMA	13000	1.3	7 ± 3
PtBMA	18000	1.2	7 ± 2
PNaSS	b)	b)	11 ± 3 c)
PNIPAM	b)	b)	10 ± 2 c)

[a)] Measured in THF unless otherwise stated.
[b)] Insoluble in THF.
[c)] Measured in DMF.
PS = polystyrene; PnBA = poly(n-butyl acrylate); PMMA = poly(methyl methacrylate); PtBMA = poly(t-butyl methacrylate); PNaSS = poly(sodium 4-styrenesulfonate); PNIPAM = Poly(N-isopropyl acrylamide).

responding azide-containing copolymer and the resulting PNPs.

The general validity of the method is illustrated in Table 2 in which physical data from several PNPs of very different chemical nature are reported.

It is worth noticing that PNaSS PNPs can be easily obtained by this method. These particular PNPs display interesting viscosity behavior in salt-free water solutions at r.t. when compared to the corresponding copolymer precursor. Additionally, the resulting P*n*BMA PNPs showing a glass transition temperature below r.t. (− 8 °C) are useful as simultaneous rheology and impact (toughening) modifiers in nanocomposites. PNIPAM NPs are interesting for nanomedicine applications such as drug delivery.[12] Also, water-soluble PMMAc PNPs with appropriate chelating cross-linker molecules are promising agents for nuclear magnetic imaging (MRI) applications.[13] By tailoring the nature of the dialkyne cross-linker, even fluorescent PNaSS PNPs have been prepared.[14]

Conclusion

A new, simple and very effective route to PNPs in the 3-20 nm range has been reported. The generality of the method has been demonstrated by synthesizing PNPs with different chemical structure, solubility, functionality, physico-chemical characteristics and hence potential applications covering from unconventional polyelectrolytes and melt-flow/toughening modifiers for nanocomposites to drug delivery nanocarriers, fluorescent probes[14] or MRI contrast agents for nanomedicine.[13]

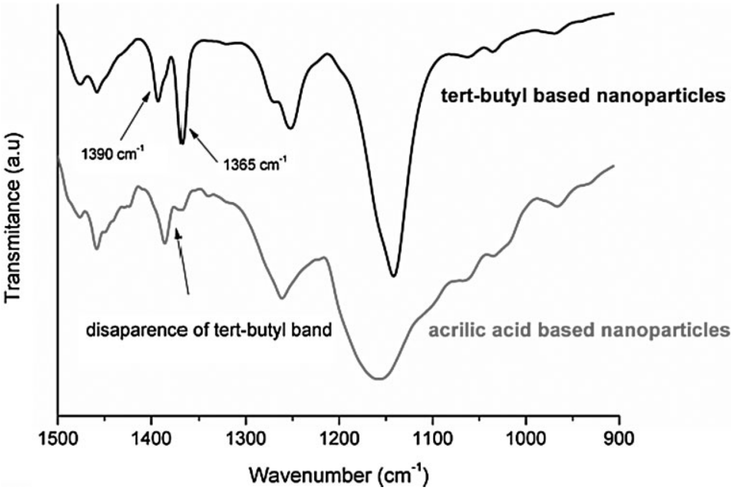


Figure 3.
Comparative FT-IR spectra of PtBMA NPs and PMAAc NPs obtained thereof in the 1500-900 cm^{-1} region.

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