Efficient Fabrication of Polymer Nanoparticles via Sonogashira Cross-Linking of Linear Polymers in Dilute Solution

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ABSTRACT: The synthesis of single-chain nanoparticles by palladium-catalyzed Sonogashira coupling between a terminal alkyne and a di-halo aryl cross-linker is reported. Statistical copolymers with trimethylsilyl protected alkyne groups pendent to the linear methacrylate back bones were synthesized using reversible addition-fragmentation chain transfer polymerization post polymerization de-protection providing terminal alkyne functionalized linear polymer chains. These linear polymer chains were intramolecularly cross-linked via bifunctional cross-linkers. The resulting well-defined covalently bonded nanoparticles were characterized via triple-detection size exclusion chromatography where MALS detector provided molecular weight information and viscometric detection characterizes particle size and conformations. The particle size could be readily tuned through polymer molecular weight and by degree of cross-linking. © 2015 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2016**, *54*, 209–217

KEYWORDS: nanoparticles; sonogashira coupling; size exclusion chromatography; transmission electron microscopy

INTRODUCTION Advances in polymer science have recently led to novel synthetic methodologies to complex macromolecular structures. The synthesis of structurally well-defined polymeric functional nanoparticles in particular has attracted attention over the past several years. These functionalized nanomaterials have myriad potential applications in the fields of drug delivery,[1,2] biomemtic system,[3,4] chemosensors,[5] and molecular imaging.[6] One route for the synthesis of structurally well-defined polymer nanoparticles is via self assembly and cross-linking of amphiphilic block copolymers.[7] This method, while ubiquitous has a lower size limit of about 20–30 nm. An alternate approach to particles in the 5–20 nm size range involves intramolecular cross-linking of linear polymer chains into single-chain nanoparticles (SCNP); nano-sized cross-linked gels with dimensions smaller than the original solvated polymer coil.[8–10] A range of different cross-linking chemistries comprising intramolecular covalent bonds, noncovalent interactions and dynamic covalent bonds have been investigated in dilute conditions for the synthesis of SCNP.[9,11–13] High-temperature intramolecular dimerization of the benzocylobutene group,[14,15] click reactions,[16–19] photochemically triggered Diels–Alder reaction,[20] photodimerization of anthracene,[21] and Coumarin,[22] Bergman cyclization,[23,24] sulfonyl nitrene insertion,[25] Menschutkin reaction,[26] and olefin metathesis[27] represents a few examples of covalent cross-linking chemistry.

Recent development in fields of various controlled polymerization techniques have enabled the synthesis of polymers with well-defined architecture and reduced heterogeneity across a particular sample.[28] The intrachain covalent linkages result in structures more robust than proteins due to thermal and chemical stability. Albeit rudimentary in structure, an organic chemist's toolbox is enriched with efficient synthetic transformations capable of incorporating reactive handles to the polymer chains that could facilitate intrachain folding.

Requirements for an effective intrachain collapse of single polymer chain include: facile and controllable incorporation of an appropriate reactive precursor to the polymer chain, a highly selective and quantitative post polymerization modification reaction with negligible side products, and mild reaction conditions to favor intrachain cross-linking as compared with interchain cross-linking. One example of such effective synthetic reactions is the Sonogashira coupling. The

Additional Supporting Information may be found in the online version of this article.

Sonogashira coupling, a palladium catalyzed cross-coupling reaction to form carbon–carbon bonds, was first reported by Kenkichi Sonogashira, Yosuo Tohda, and Nobue Hagihara.[29] The coupling has received considerable attention in the field of organic synthesis because of its great potential to carry out various organic transformations with a high degree of regio-selectivity. The high efficacy, selectivity, high yield, negligible byproducts, and mild reaction conditions make the Sonogashira coupling an efficient candidate for a macromolecular reaction. The Sonogashira coupling polymerization has been used to synthesize various conjugated polymers and supramolecular polymers.[30–33] In the present work, we introduce the Sonogashira coupling as a method for the synthesis of SCNPs. We present here the synthesis of polymeric nanoparticles via addition of an external cross-linker. This route involves: (1) Synthesis of random copolymers containing trimethylsilyl protected alkyne groups distributed along the polymer chain. (2) Removal of the trimethylsilyl group to yield the corresponding terminal alkyne functionalized random copolymers. (3) Use of an appropriate bifuctional dihalide cross-linker and application of Sonogashira coupling reaction conditions[34–36] for an efficient intrachain cross-coupling.

EXPERIMENTAL

Chlorotrimethylsilane, silver chloride, 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU), propargyl methacrylate, tetrabutylammoniumfloride (TBAF), 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid, triethylamine, Copper(I) iodide (CuI), tetrakis(triphenylphosphine)palladium (0) were purchased from Sigma Aldrich and were used as received. Methyl methacrylate (Sigma Aldrich) was passed over a column of basic alumina (Sigma Aldrich) prior to usage. Chloroform-d_1 (Cambridge Isotope Laboratories, Inc.) Hydrochloric acid (EMD), Glacial acetic acid (EMD), anhydrous magnesium sulfate (EMD), Sodium Bicarbonate (Fischer Science Education) were used as received. *n*-Hexane, diethyl ether dichloromethane (DCM), tetrahydrofuran (THF), N,N-dimethylformamide (DMF) were purchased as analytical grade solvents (Sigma Aldrich) and were used as received. ^{1}H NMR (400 MHz) spectra were recorded on a Varian Associates Mercury 400 spectrometer. Solvents ($CDCl_3$) contained 0.03% v/v TMS as an internal reference, chemical shifts (δ) are reported in ppm relative to TMS. Following abbreviations are used for peaks: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Transmission Electron Microscopy (TEM) images were recorded using a Zeiss LEO 922 Ω operating at 120 kV with a Gatan Multi-scan bottom mount digital camera. Samples were prepared by drop casting 2 μL of a nano particle solution (0.002 mg/mL) on to Formvar carbon film coated 400 square mesh copper grids and dried overnight in air while protected from dust. Size exclusion chromatography (SEC) was performed on a Tosoh EcoSEC dual detection (RI and UV) SEC system coupled to an external Wyatt Technologies miniDAWN Treos multiangle light scattering (MALS) detector and a Wyatt Technologies ViscoStarII differential viscometer. The column set was two Tosoh TSKgel SuperMultipore HZ-M columns (4.6 × 150 mm), one Tosoh TSKgel SuperH3000 column (6 × 150 mm) and one Tosoh TSKgel SuperH4000 column (6 × 150 mm). Increment refractive index values (dn/dc) were calculated online assuming 100% mass recovery (RI as the concentration detector) using the Astra 6 software package (Wyatt Technologies) by selecting the entire trace from analyte peak onset to the onset of the solvent peak or flow marker. This method gave the expected values for polystyrene (dn/dc = 0.185, M_n = 30k) when applied to a narrow PDI PS standard supplied by Wyatt. Absolute molecular weights and molecular weight distributions were calculated using the Astra 6 software package. Intrinsic viscosity (η) and viscometric radii (R_η) were calculated from the differential viscometer detector trace and processed using the Astra 6 software.

Synthesis of Monomer Trimethylsilyl Propargyl Methacrylate

To a suspension of silver chloride (0.30 g, 2.1 mmol) in 50 mL of dry dichloromethane was added propargylmethacrylate (2.8 g, 0.02 mol) and 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU), (0.44 g, 2.94 mmol).[37] An appearance of dark color was observed. After stirring at room temperature for about 15 minutes, chloromethylsilane (3.15 g, 0.03 mol) was added dropwise and stirred for 24 hours at 45 °C. The reaction mixture was then diluted with 100 mL of n-hexane and the organic phase was washed with saturated aqueous sodium bicarbonate, 1% hydrochloric acid and water, respectively. The extract was dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a yellow liquid. The crude product was further purified by column chromatography eluting with a solvent mixture of 25:1 *n*-Hexane and diethyl ether to obtain a colorless liquid (75% yield). **^{1}H NMR (400 MHz, $CDCl_3$, δ)**: 6.18 (s, 1H, CH), 5.62 (s, 1H, CH), 4.76 (s, 2H, CH2), 1.97 (s, 3H, CH_3), 0.19 (s, 9H, $Si(CH_3)_3$) (Fig. S1, Supporting Information). **^{13}C NMR (400 MHz, $CDCl_3$, δ)**: 166.96, 136.14, 126.97, 99.55, 92.34, 53.38, 18.10, −0.12 (Fig. S2, Supporting Information).

Control Experiments

Test Reaction between Propargyl Methacrylate and Bromobenzene

Bromobenzene (1 g, 6.3 mmol), $Pd(PPh_3)_4$ (69 mg, 0.06 mmol) and copper iodide (11 mg, 0.06 mmol) were dissolved in 10 mL (6:4, THF/Et_3N) in a schlenk flask and were degassed with argon for 30 minutes.[38] The reaction mixture was magnetically stirred at 70 °C for a period of 30 minutes. Propargyl methacrylate (0.78 g, 6.3 mmol) was added to the mixture via a syringe. The reaction mixture after addition was heated at a temperature of 70 °C over night. Excess of solvent was evaporated in vaccum. The crude product was dissolved in 50 mL of hexanes and passed through a plug of silica to remove any unreacted palladium and copper catalyst. The crude mixture was further analyzed via ^{1}H NMR without further purification (Fig. S5, Supporting Information).

Test Reaction between Polymer Poly(propargyl methacrylate-co-methyl methacrylate) (PgMA-co-MMA) and Bromobenzene

The procedure for this test reaction was adapted from the test reaction done on the model monomer. Poly(propargyl methacrylate-co-methyl methacrylate) (PgMA-co-MMA) 100 mg, $M_w = 16$ kg/mol, 0.006 mmol), containing 35% of alkyne (0.0021 mmol) $Pd(PPh_3)_4$ (0.5 mg, 0.0004 mmol) and copper iodide (0.076 mg, 0.0004 mmol) were dissolved in a 10 mL solution of (6:4, THF/Et_3N) in a schlenk and was degassed with argon for 30 minutes. Bromobenzene (0.32 mg, 0.0021 mmol) was added to the mixture via a syringe. The reaction mixture after addition was heated over-night at a temperature of 70 °C. The resulting brown solution was passed through a silica column flushed with THF to remove any unreacted palladium and copper catalyst. The solution was evaporated under reduced pressure to a volume of approximately 2 mL and was precipitated in a minimum amount of cold water to obtain a yellowish powder (^{1}H NMR, Fig. S6, Supporting Information).

General Procedure for Synthesis of Trimethylsilyl Protected Copolymer

Synthesis of Poly(trimethylsilyl propargyl methacrylate-co-methyl methacrylate) (PTMSPMA-co-MMA) P1-TMS

An oven dried 10 mL schlenk flask was charged with a mixture of MMA (0.33 g, 3.3 mmol) and TMSPgMA (0.65 g, 3.3 mmol). 4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid (12 mg, 0.04 mmol) and AIBN (0.65 mg, 0.004 mmol) were then added, in stock solutions in dimethylformamide with a final volume of 2 mL. The polymerization mixture was degassed by three freeze-pump-thaw cycles. The schlenk flask was then heated in an oil bath at 80 °C for 24 hours. After the polymerization was complete the schlenk flask was opened to air. The resulting viscous polymer was diluted with 3 mL of THF and precipitated in 10 mL of ice-cold water. **1H NMR (400 MHz, CDCl3, δ)**: 4.50–4.70 (s, CH2—C, TMSPgMA), 3.54–3.67 (s, CH3—O— (O)C), MMA,), 1.60–1.41(br, (CH_2—C(CH_3), MMA, TMSPgMA), 0.84–1.02 (br, (CH_3(C), MMA, TMSPgMA), 0.16 (s, (CH_3)$_3$Si—) TMSPgMA) (Fig. S7, Supporting Information).

General Procedure for Deprotection

Synthesis of Poly(propargyl methacrylate-co-methyl methacryate) (PgPMA-co-MMA) P1

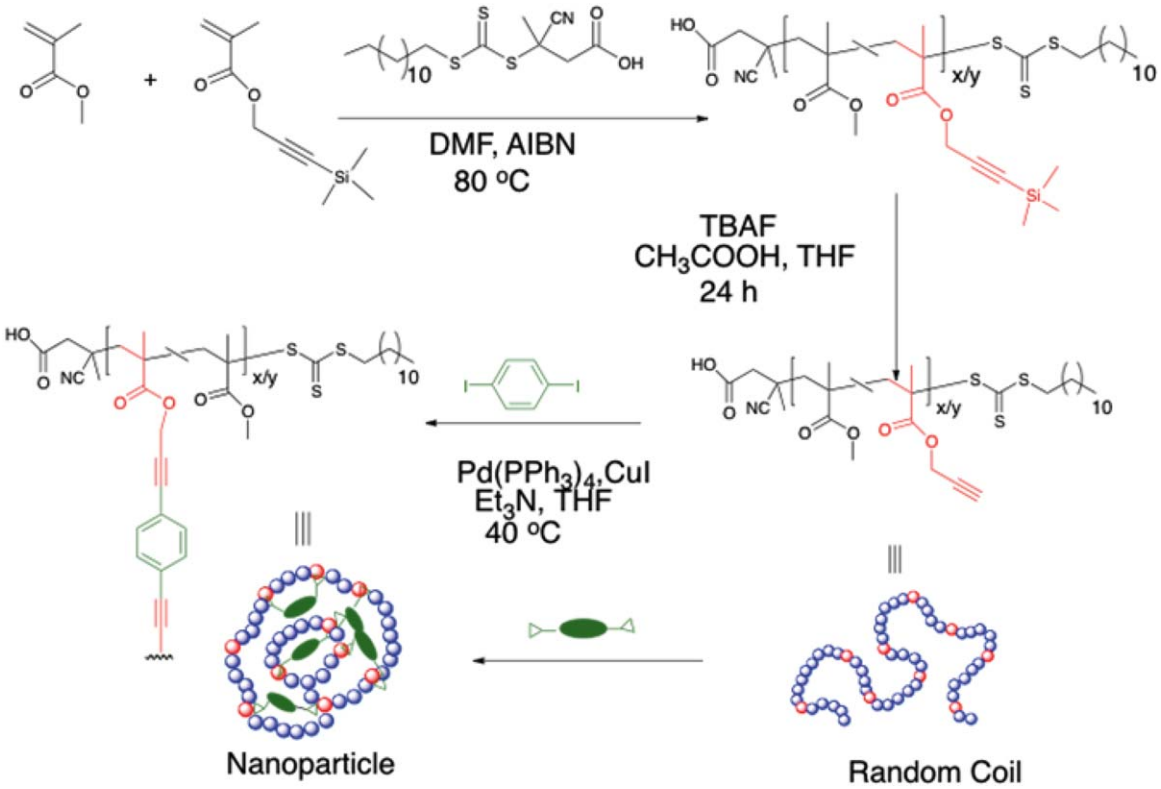
The copolymer **P1** (865 mg, 0.049 mmol) containing 45% (0.022 mmol) of the trimethylsilyl protected alkyne was dissolved in argon purged 100 mL THF. Acetic acid (0.002 mL, 0.033 mmol) was added to the solution. Argon was bubbled to the solution (∼30 min) and the solution was cooled to −5 °C in an ice-salt–water bath. A 1 M solution of tetrabutylammoniumfloride (TBAF) (0.033 mL, 0.033 mmol) was added slowly via a syringe (∼5 min). The resulting mixture was stirred at this temperature for 30 minutes and then warmed to ambient temperature followed by stirring for 24 hours. The solution was passed through a short silica column to remove the excess of TBAF, followed by washing with additional THF. The resulting solution was then concentrated under a reduced pressure to a small volume, which was then precipitated in to cold water. The precipitate was then filtered and dried under vacuum to give a **P1** as a white powder. **^{1}H NMR (400 MHz, $CDCl_3$, δ)**: 4.50–4.70 (s, CH_2—C, PgMA), 3.54–3.67 (s, CH_3—O— (O)C), MMA,), 2.47 (S, (CH, PgMA), 1.60–1.41(br, (CH_2—C(CH_3), MMA, PgMA), 0.84–1.02 (br, (CH_3(C), MMA, PgMA) (Fig. S8, Supporting Information) ($M_n = 17.5$ kg/mol, $M_w = 21.1$ kg/mol, PDI = 1.2).

General Procedure for Synthesis of Nanoparticle NP1

About 100 mg of polymer **P1** (0.005 mmol) containing 45% of the alkyne functionality (0.002 mmol), and copper iodide (CuI) (0.002 mmol, 0.49 mg) were dissolved in a solution of dry and already degassed 10 mL solution of (5:5, THF/Et_3N) under argon in an oven dried three neck round bottom flask. The solution was heated at 40 °C for approximately 30 minutes. To the resulting solution 1,4-diiodobenzene (0.32 mg, 0.001 mmol) and tetrakis(triphenylphosphine)palladium (0) ($Pd(PPh_3)_4$) (2.3 mg, 0.002 mmol) with respect to the alkyne functionality in a 10 mL solution of (5:5, THF/Et_3N) was added drop wise over a period of 1 hour. The reaction mixture was heated at 40 °C overnight and turned brown in color. The resulting brown solution was passed through a silica column flushed with THF to remove any unreacted palladium and copper catalyst. The solution was evaporated under reduced pressure to a volume of approximately 2 mL and was precipitated in a minimum amount of cold water to obtain a yellowish powder**. ^{1}H NMR (400 MHz, $CDCl_3$, δ)**: 7.8–7.9 (Ar), 4.50–4.70 (s, CH_2—C, PgMA), 3.54–3.67 (s, CH_3—O— (O)C), MMA,), 1.60–1.41(br, (CH_2—C(CH_3), MMA, PgMA), 0.84–1.02 (br, (CH_3(C), MMA, PgMA) ($M_n = 19.4$ kg/mol, $M_w = 27.2$ kg/mol, PDI = 1.4).

RESULTS AND DISCUSSION

The alkyne functional group is a versatile functional group, which has been used extensively in organic chemistry for a variety of reactions. In polymer chemistry, alkyne functionality has been exploited as a coupling technique via the copper-catalyzed azide click cycloaddition. Ruiz de Luzuriaga and coworkers used the copper catalyzed C—N click reaction to synthesize SCNPs for the first time.[17] SCNPs of various chemical nature were synthesized using an intrachain cross-coupling click cycloaddition with a di-alkyne cross-linker.[39] In any macromolecular synthesis, protection of the alkyne bond has been mandated to avoid any chain transfer reactions and branching events during radical polymerization.[40,41] However, in an interesting example SCNPs has been synthesized by using Glaser–Hay coupling using an unprotected alkyne functionality.[42] In a recent work photoactivated thio-yne coupling have been explored as an efficient synthetic approach to synthesize SCNPs.[43] To our knowledge synthesis of SCNP via the palladium catalyzed Sonogashira coupling has not been demonstrated yet. The monomer trimethylsily propargylmethacrylate (TMSPgMA) was synthesized according to a literature procedure.[37] Copolymers of methylmethacrylate (MMA) and TMSPgMA with different chain lengths and varying amount of TMSPgMA were synthesized using reversible addition-fragmentation chain transfer



SCHEME 1 Schematic illustration of the synthesis of SCNPs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(RAFT) polymerization (Scheme 1). RAFT polymerization was selected due to its excellent control over molecular weight and a narrow molecular weight distribution.[44] ^{1}H NMR spectroscopy was used to determine the composition of various linear copolymers. The mole fraction of TMSPgMA and MMA were calculated from the ratio of the peak area around 0.19 ppm, corresponding to nine methyl protons in trimethylsilyl propargyl group, to the total area of peak at 3.6 ppm, which is attributed to methyl protons of MMA (Fig. S3, Supporting Information). Deprotection of these linear copolymers using TBAF/CH_3COOH yielded the corresponding alkyne functionalized copolymers.[40] The mole fraction of PgMA and MMA were calculated from the ratio of the peak area at 2.5 ppm, corresponding to alkyne protons of propargyl methacrylate, to the area of peak methyl protons of MMA at 3.6 ppm (Fig. S4, Supporting Information).

In order to better understand Sonogashira coupling as an effective candidate for this macromolecular conjugation reaction, the standard coupling was performed on monomeric propargyl methacrylate and bromobenzene as a model aryl halide counterpart.[38] ^{1}H NMR spectroscopy was used to monitor the reaction and showed appearance of peaks in the aromatic region as well as decrease in the intensity of the alkyne peak in the crude mixture after 24 hours reflux (Fig. S5, Supporting Information). Having shown that the reaction gave a quantitative conversion on a model monomer, a test reaction was carried on a model linear alkyne functionalized random copolymer with a mono functionalized halogen counterpart. The coupling reaction was applied to a model linear copolymer PgMA-co-MMA with bromobenzene as a model mono functionalized aryl halide. ^{1}H NMR again showed a quantitative conversion with disappearance of the alkyne peak and appearance of additional peaks in the aromatic region (Fig. S6, Supporting Information). Having shown that the model mono-functionalized aryl halide readily undergoes a quantitative macromolecular reaction, copolymers were submitted to Sonogashira coupling conditions to induce collapse by using a difunctionalized cross-linker (Scheme 1) In order to adapt Sonogashira coupling conditions that are successful for small molecules, any SCNP synthesis reaction requires consideration of some important issues. Since Sonogashira coupling is sensitive to reaction conditions and substrates, changes in reaction components often necessitates changes in other reaction conditions (i.e., solvent, reaction temperature, catalyst, base, copper salt). We have investigated various reaction conditions for an efficient nanoparticle synthesis, and the ideal conditions are given in the "Experimental" section. 1,4-Diiodobenzene is an efficient and appropriate choice for the external cross-linker due to its high reactivity as well as its commercial availability.

The nomenclature used in this work is as follows: Each linear random copolymer is given a prefix (**P**) and is assigned with a number. The corresponding nanoparticles obtained by the cross-coupling reaction is given a prefix NP with a corresponding number. Initial attempts of the cross-coupling reaction were performed on a parent polymer scaffold **P1,**

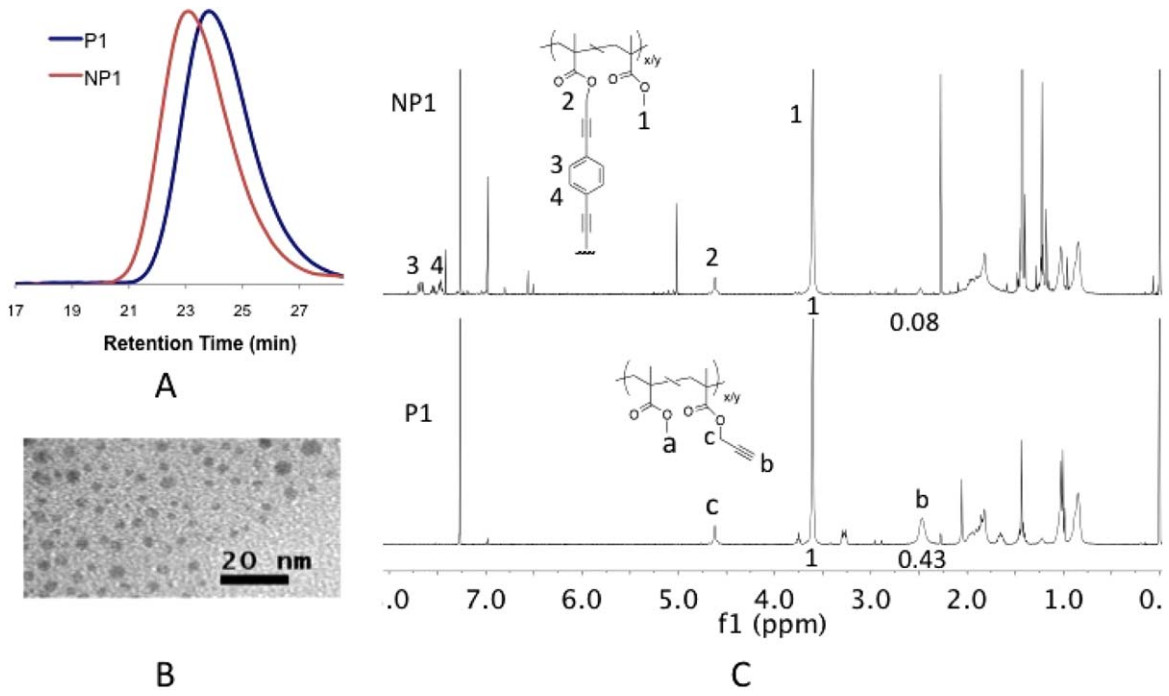


FIGURE 1 (A) Normalized SEC traces (MALS, THF) before cross-linking of linear polymer precursors and after cross-linking of the corresponding nanoparticles (**P1, NP1**). (**B**) TEM image of the nanoparticle (**NP1**). (**C**) ^{1}H NMR overlay spectrum of polymer **P1** and nanoparticle **NP1**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

($M_n = 17.5$ kg/mol) containing 45% of alkyne functionality (Fig. S8, Supporting Information) with a total polymer concentration of approximately 5 mg/mL. The resulting nanoparticles were characterized by triple detection SEC. A decrease in retention time was observed in all SEC detector traces [MALS, Fig. 1(A)] (RI, Fig. S10, Supporting Information) indicating that the nanoparticles obtained are bigger in size than the parent polymer. Formation of nanoparticle is confirmed by a decrease in the intrinsic viscosity (η), indicating a more compact globule. An increase in viscometric radius (R_η), provided by SEC detection with a differential viscometer, with a concomitant increase in the value of molecular weight confirms the addition of an external cross-linker in to the parent polymer. The viscometer data, molecular weight data, and peak retention time for the polymer as well as for the nanoparticles are given in Table 1. The observed phenomenon could be explained due to a relative high polymer concentration in the reaction mixture as well as of high cross-linking density. The visual evidence for nanoparticle formation was provided by transmission electron microscopy (TEM) [Fig. 1(B)], which agrees with the size provided by the viscometric detector. The cross-linked nanoparticles were further characterized by ^{1}H NMR spectroscopy, for the transformation of **P1** in to **NP1** [Fig. 1(C)]. The decrease in

TABLE 1 SEC Data for Polymers P1, P2, P3, P4, and Corresponding Nanoparticles NP1, NP2, NP3, NP4

Sample	M_n (kg/mol[a])	M_w (kg/mol[b])	PDI	% Alkyne	Retention Time (min[c])	η (mL/g[d])	R_η (nm[e])
P1	17.5	21.1	1.2	45	23.8	51.3	5.6
NP1	19.4	27.2	1.4	13	23.0	47.9	6.3
P2	11.3	13.4	1.2	50	24.9	30.0	4.2
NP2	21.1	24.1	1.2	na	25.6	11.3	3.5
P3	34.5	55.4	1.6	35	22.0	57.2	7.1
NP3	39.4	43.4	1.1	na	23.9	21.3	5.0
P4	16.4	28.1	1.7	23	22.7	36.2	4.6
NP4	29.4	32.1	1.2	na	24.3	21.9	4.1

All SEC data collected at 40 °C in THF.
[a] Calculated from RI data.
[b] Absolute M_w calculated from MALS data.
[c] Calculated from MALS detector trace.
[d] Calculated from viscometric trace.
[e] Calculated from viscometric trace.

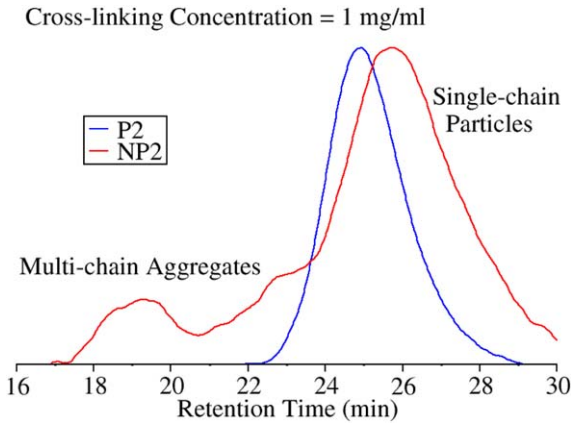


FIGURE 2 Normalized SEC trace (THF, MALS) of linear copolymer **P2** and corresponding nanoparticle **NP2**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

intensity of the characteristic alkyne resonance at $\delta = 2.5$ ppm confirmed incorporation of the external cross-linker in to the parent polymer.

In most cases reported in literature, SCNP synthesis involves a post-polymerization transformation in dilute solution of a concentration typically 1 mg/mL. So we decided to perform the cross-linking reaction with concentration of 1 mg/mL on a polymer (**P2**) containing 50% alkyne (Fig. S12, Supporting Information). A greater shift in retention time in all SEC traces confirmed formation of SCNPs and interestingly the MALS trace showed a shoulder indicating a small amount of interchain aggregates (MALS, Fig. 2) (RI, Fig. S14, Supporting Information). Another cross-linking reaction was performed on a polymer scaffold with a relative high molecular weight (Table 1) and 35% alkyne (Fig. S16, Supporting Information). As expected intrachain cross-linking was observed in all SEC traces with a small interchain aggregate (MALS, Fig. 3) (RI, Fig. S17, Supporting Information). With an increase in the

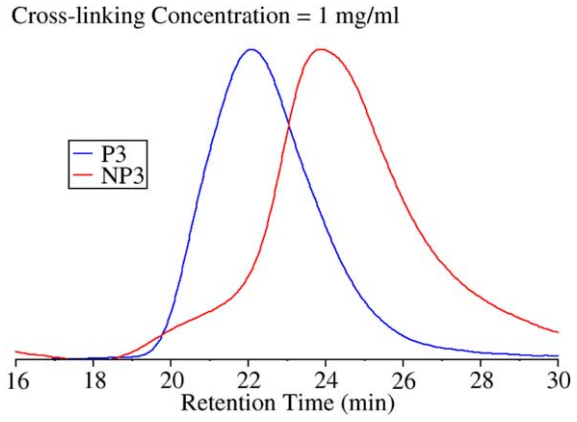


FIGURE 3 Normalized SEC trace (THF, MALS) of linear copolymer **P3** and corresponding nanoparticle **NP3**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

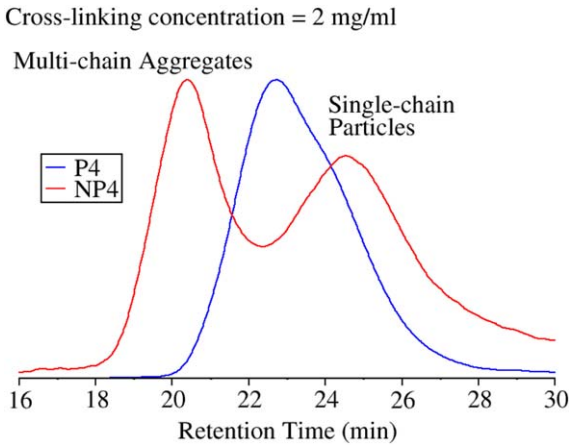


FIGURE 4 Normalized SEC trace (THF, MALS) of linear copolymer **P4** and corresponding nanoparticle **NP4**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

amount of reactive alkyne moiety in the parent polymer, an increase in the retention time was observed in all the SEC traces of the corresponding nanoparticles. This observation correlates with the fact that increase in intrachain cross-linking is driven by the amount of reactive alkyne functionality present.

Further experiments were carried out to study the effect of polymer concentration in the reaction mixture on the cross-linking phenomenon. The cross-linking reaction was carried out on another parent polymer P4 with 23% alkyne, (Fig. S20, Supporting Information) with a final polymer concentration of 2 mg/mL. The SEC traces showed both interchain as well as intrachain cross-linking (MALS, Fig. 4) (RI, Fig. S22, Supporting Information). The presence of a significant amount of interchain aggregates was evident by a big peak in the MALS trace, while a small shoulder was also observed in the RI detector. Decrease in the value of the intrinsic viscosity (η) as well as the viscometric radius (R_η) corroborates the formation of nanoparticle (Table 1). The observed phenomenon confirms that changing the concentration of the reaction mixture can modulate the cross-linking. In summary, this study demonstrated that interchain cross-linking occurred at a higher concentration (5 mg/mL). At intermediate concentration (2 mg/mL) both inter and intrachain cross-linked particles were observed. At a low concentration (1 mg/mL), intrachain cross-linked nanoparticles were obtained with a shift to greater retention time in the SEC traces.

Alternatively, the cross-linking phenomenon was investigated for polymers (**P5–P6**) with the same amount of alkyne functionality and different molecular weight (Table 2). The cross-linking coupling reaction was performed on a relatively high molecular weight **P5** with 30% alkyne (Fig. S24, Supporting Information). All SEC traces showed an increase in retention time with the MALS trace showing some amount of interchain aggregates (MALS, Fig. 5) (RI, Fig. S2, Supporting

TABLE 2 SEC Data for Polymer P5, P6 and Corresponding Nanoparticles NP5 and NP6

Sample	M_n (kg/mol[a])	M_w (kg/mol[b])	PDI	% Alkyne	Retention Time (min[c])	η (mL/g[d])	R_η (nm[e])
P5	78.2	91.8	1.2	30	21.12	38.00	8.1
NP5	61.3	72.3	1.1	na	23.11	30.80	6.9
P6	30.1	34.8	1.1	30	24.24	15.12	4.3
NP6	21.1	24.1	1.1	na	25.74	6.84	3.9
P7	23.3	28.8	1.2	50	29.23	50.88	5.8
NP7	25.4	30.5	1.2	na	29.29	53.72	5.3

All SEC data collected at 40 °C in THF.
[a] Calculated from RI data.
[b] Absolute M_w calculated from MALS data.
[c] Calculated from MALS detector trace.
[d] Calculated from viscometric trace.
[e] Calculated from viscometric trace.

Information). Similarly, a cross-linking reaction was carried out on a low molecular weight polymer **P6** with the same amount of alkyne present (Fig. S28, Supporting Information). Intrachain cross-linking was observed leading to an increase in the retention time in all the three SEC traces (MALS, Fig. 6) (RI, Fig. S30, Supporting Information). As expected, there is a greater shift in the retention time of the high molecular weight polymer nanoparticle as compared with the low molecular weight polymer nanoparticle.

A decrease in intrinsic viscosity (η), along with the reduction in viscometric radius R_η confirms the formation of the corresponding nanoparticles (Table 2). The retention time of the nanoparticle formed from the high molecular weight polymer was shorter as compared with the retention time of the nanoparticle formed from a low molecular weight polymer. The above phenomenon can be explained by the fact that the higher molecular weight polymer (**P5**) formed a bigger nanoparticle (**NP5**) with a longer retention time as compared with the lower molecular weight (**P6**) forming a smaller nanoparticle (**NP6**) with a shorter retention time.

The alkyne functional moiety is capable of undergoing a self click homocoupling reaction via the Glaser–Hay reaction mechanism. The homocoupling of acetylenic functionality is often concomitant to the metal catalyzed cross-coupling reactions. In order to ascertain that the cross-linking reaction is taking place via the palladium catalyzed Sonogashira coupling between and alkyne and an aryl halide, rather than by an alkyne homocoupling, a control experiment was performed. Polymer (**P7**) was subjected to Sonogashira coupling conditions without addition of an external dihalo cross-linker. There was not any significant change in the retention time before and after the cross-linking, suggesting that an aryl halide is required under these reaction conditions (Table 2, Fig. S32, Supporting Information). We believe that the selectivity of the Sonogashira coupling over the alkyne homocoupling under the given conditions is dependent on the choice of base as well as the solvent. A detailed study of reaction optimization with different catalysts, bases, as well as external cross-linkers is undergoing in our lab.

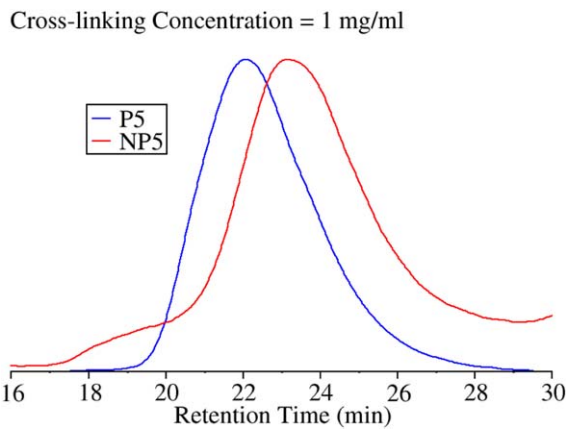


FIGURE 5 Normalized SEC trace (THF, MALS) of linear copolymer **P5** and corresponding nanoparticle **NP5**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

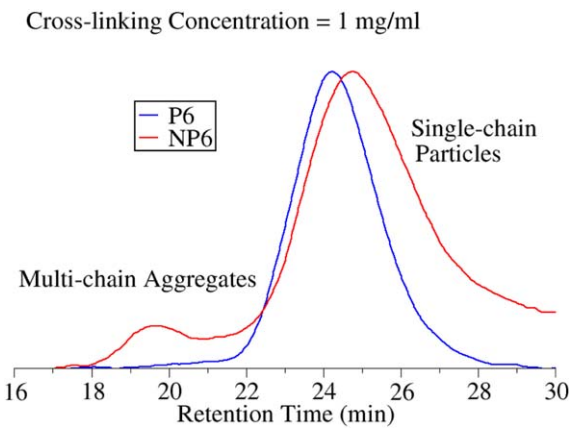


FIGURE 6 Normalized SEC trace (THF, MALS) of linear copolymer **P6** and corresponding nanoparticle **NP6**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSIONS

Random copolymers containing terminal alkyne moieties have been prepared by RAFT polymerization. We have shown that both inter and intrachain cross-linked polymer nanoparticles can be synthesized via palladium catalyzed Sonogashira coupling. At a high polymer concentration in the reaction mixture, interchain cross-linking predominated, while by decreasing the concentration intrachain cross-linking was favored. We also demonstrated that by changing the molar percent of alkyne functionality in the parent polymer, the size of resulting nanoparticle could be controlled. Although current SCNP literature has many examples of nanoparticle synthesis, it still lacks a thorough study on how the nanoparticle formation is affected by the manner in which intrachain cross-linking is performed. In this perspective, we have presented the synthesis of polymer nanoparticles from single polymer chains by the Sonogashira coupling reaction conditions and further, we envision to study the effect of polymer architecture on the single-chain folding behavior. The Sonogashira coupling reaction can be applied in inducing intrachain covalent bond formation either with in-built reactive precursors, or via an external cross-linker. Currently, research efforts are going on in our lab to synthesize single-chain nanoparticles under Sonogashira coupling reaction conditions from in-built reactive precursor functionalized polymers. We are testing similar synthetic conditions with the same reactive partners placed in a variety of positions.

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