

Article

## Metal-Free Polymethyl Methacrylate (PMMA) Nanoparticles by Enamine “Click” Chemistry at Room Temperature

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**Abstract:** “Click” chemistry has become an efficient avenue to unimolecular polymeric nanoparticles through the self-crosslinking of individual polymer chains containing appropriate functional groups. Herein we report the synthesis of ultra-small (7 nm in size) polymethyl methacrylate (PMMA) nanoparticles (NPs) by the “metal-free” cross-linking of PMMA-precursor chains prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization containing  $\beta$ -ketoester functional groups. Intramolecular collapse was performed by the one-pot reaction of  $\beta$ -ketoester moieties with alkyl diamines in tetrahydrofuran at r.t. (*i.e.*, by enamine formation). The collapsing process was followed by size exclusion chromatography and by nuclear magnetic resonance spectroscopy. The size of the resulting PMMA-NPs was determined by dynamic light scattering. Enamine “click” chemistry increases the synthetic toolbox for the efficient synthesis of metal-free, ultra-small polymeric NPs.

**Keywords:** enamine “click” chemistry; intramolecular collapse; unimolecular polymeric nanoparticles

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## 1. Introduction

Unimolecular polymeric nanoparticles (NPs) are emerging soft nano-objects showing dimensions below 10–15 nm displaying promising properties for different end-use applications such as processing additives [1], blend compatibilizers [2], artificial enzymes [3], photostable bio-imaging agents [4] and drug/siRNA-delivery systems [5], among others. Significant efforts have been devoted in recent years to open new routes to unimolecular nanoparticles relying on efficient synthetic methods for the controlled, irreversible intramolecular collapse of individual polymer chains. In this sense, different synthetic approaches have been reported: free-radical cross-linking of vinyl units [6–8], thermal cross-linking of benzocyclobutene and *o*-quinodimethane functional groups by Diels-Alder reactions [9,10], copper-catalyzed azide-alkyne “click” chemistry [11–13] and fast cross-linking of isocyanate groups from appropriate precursor chains by diamines [14].

Pioneering results were obtained by Mecerreyes *et al.* [6] for polycaprolactone (PCL)-, polystyrene (PS)- and polymethyl methacrylate (PMMA)-NPs synthesized by free-radical cross-linking of vinyl units by using ultra-diluted conditions. Following a similar route, Park *et al.* [7] reported the preparation of nanoparticles with sizes ranging from 10 to 30 nm by self-crosslinking of polymetacrylate terpolymers in ultra-dilute solutions. Additionally, amine-functionalized PS-nanoparticles were prepared by Jiang *et al.* [8] from PS precursors with a very high amount of vinyl cross-linking units (33 and 50 mol%). One of the main shortcomings of the ultra-dilute technique was the relatively high dispersity in size of the resulting polymeric NPs.

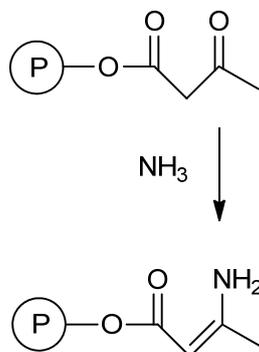
Harth *et al.* [9] developed a continuous addition technique in which intramolecular cross-linking was performed in benzyl ether (BE) at 250 °C from PS-, PMMA- and poly(*n*-butyl acrylate) (PnBA)-precursors containing benzocyclobutene (BCB) cross-linking units. PS-nanoparticles were also synthesized by Croce *et al.* [10] following this route from PS-precursors containing *o*-quinodimethane cross-linking units instead of BCB moieties. A clear limitation of the technique is that reaction conditions are harsh, precluding the use of thermally labile polymers and/or functional groups.

In an attempt to solve the limitations of the previous techniques, we introduced the macromolecular “click” cycloaddition route [11–13] which relies on the use of alkyne and azide functional groups allowing the synthesis of unimolecular NPs by copper-catalyzed azide-alkyne “click” chemistry. Currently, a great interest exists for developing “click” chemistry procedures beyond the copper-catalyzed azide-alkyne cycloaddition [15], allowing the synthesis of “metal-free” unimolecular polymeric nanoparticles at r.t. In addition to cross-linking of isocyanate by diamines leading to PMMA-NPs of relatively high polydispersity [14], several alternative (metal-free) “click” chemistry reactions are promising candidates, including: (i) enamine formation [16]; (ii) thiol-ene [17] /thiol-yne coupling [18]; (iii) thiol-isocyanate addition [19]; (iv) nitrile oxide-alkyne cycloaddition [20]; and (v) hydroxylamine-carbonyl reaction [21].

Here we disclose intramolecular enamine cross-linking as an alternative “click” technique for the metal-free, room temperature synthesis of unimolecular polymeric nanoparticles. The direct reaction of  $\beta$ -ketoester groups with ammonia to give enamine groups is well-known in polymer chemistry [22] as a useful reaction to protect latexes containing  $\beta$ -ketoester moieties from hydrolysis (see Figure 1). Enamine formation follows the “click” chemistry concept [23,24] since: (i) it can be performed under equimolar conditions and proceeds in appropriate time-scales even at room temperature, with high

yields; (ii) the reaction is insensitive to oxygen and can be performed in solvents that can be easily removed; (iii) products can be easily isolated by simple methods; (iv) tedious fine-tuning of reaction conditions is not required; and (v) there are no side-reactions involved.

**Figure 1.** Schematic illustration of enamine formation upon reaction of a  $\beta$ -ketoester functional group with ammonia.

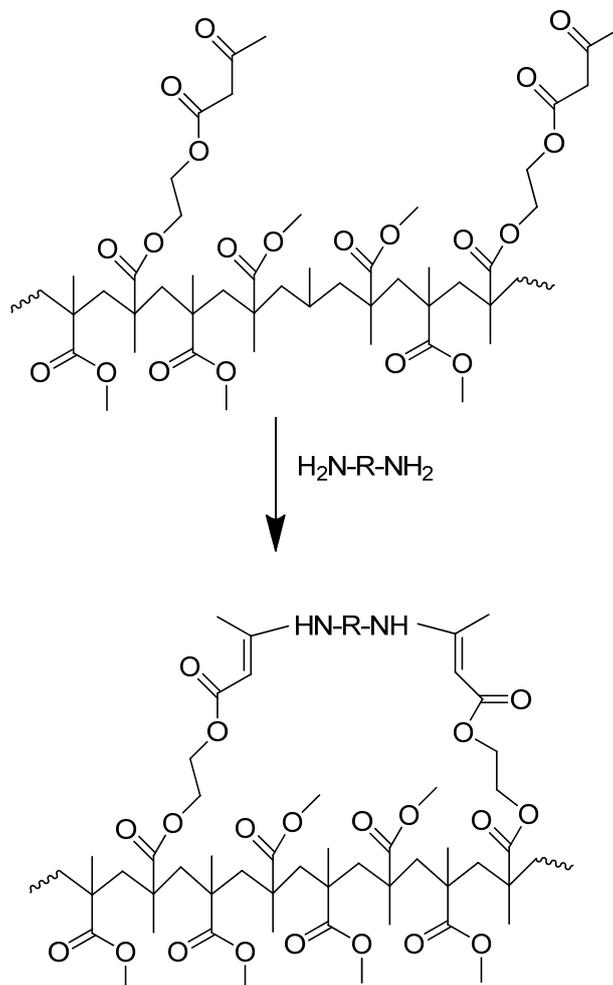


In this communication, we report the synthesis of ultra-small PMMA-NPs synthesized by metal-free enamine cross-linking from PMMA precursors containing  $\beta$ -ketoester units by using alkyl diamines. The collapsing process was followed by size exclusion chromatography and nuclear magnetic resonance spectroscopy. The size of the resulting PMMA-NPs was determined by dynamic light scattering. Similar attempts via reductive amination did not lead to defined nanoparticles and the enamine formation might be the only way to form ultra-small nanoparticles from ketones and amines. Subtle hydrogen bonding effects, affecting the solubility of the resulting NPs upon isolation and drying, are also indicated.

## 2. Results and Discussion

The “metal-free” synthetic pathway to PMMA-NPs is summarized in Figure 2. As PMMA-nanoparticle precursors we targeted random copolymers of methyl methacrylate (MMA) and (2-acetoacetoxy)ethyl methacrylate (AEMA) containing different molar fractions of  $\beta$ -ketoester units and/or different molecular weight (denoted as **1a**, **1b** and **1c** in Table 1). The precursors were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization using 2-cyanoprop-2-yl-dithiobenzoate (CDB) as chain transfer agent and 2,2'-azobis(2-methylpropionitrile) (AIBN) as free-radical initiator. RAFT is a very convenient polymerization technique for obtaining nanoparticle precursors with low molecular-weight dispersity [11-13] and hence nanoparticles with uniform size. The main characteristics of the random P(MMA-*co*-AEMA) copolymers investigated in this work are summarized in Table 1, as determined by size exclusion chromatography with absolute molar mass characterization and proton nuclear magnetic resonance spectroscopy. Well-defined copolymers showing low molecular-weight dispersity ( $\mathcal{D} = M_w/M_n$ ) values were obtained. The copolymer composition was found to be very close to the feed monomer composition, as expected for a statistical copolymerization process (reactivity ratios:  $r_{\text{MMA}} = 0.90$  and  $r_{\text{AEMA}} = 0.95$ ) allowing the random placement of the  $\beta$ -ketoester moieties along the copolymer chains.

**Figure 2.** Enamine “click” chemistry route for the synthesis of ultra-small polymethyl methacrylate nanoparticles (PMMA-NPs).

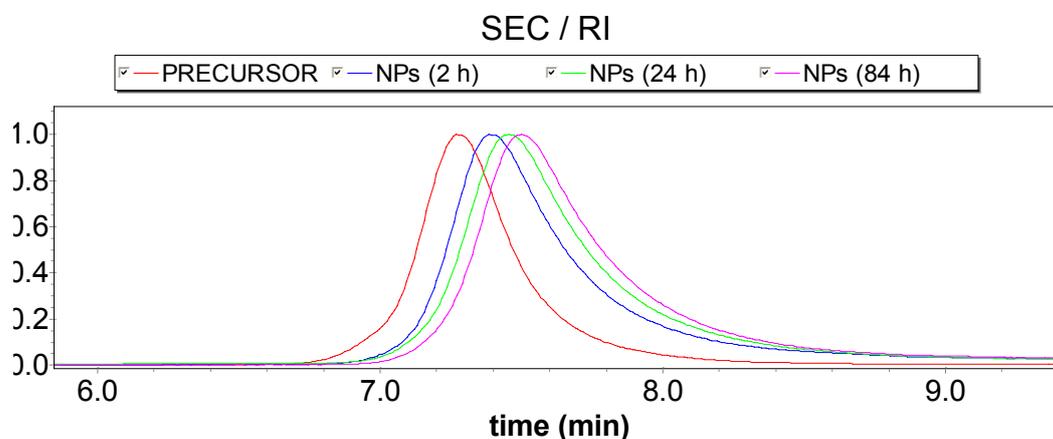


**Table 1.** Characteristics of different precursors of ultra-small PMMA-NPs synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization.

Precursor	$M_w$ (g/mol)	Dispersity	AEMA (mol%)
<b>1a</b>	57,100	1.2	35
<b>1b</b>	35,000	1.1	34
<b>1c</b>	43,000	1.1	25

The collapsing process upon enamine formation was followed by size-exclusion chromatography (SEC) which is a technique very valuable to discriminate macromolecules according to their hydrodynamic radius ( $R_H$ ). In this sense, a significant reduction in  $R_H$  is expected by SEC/refractive index (RI) upon collapse of individual polymer chains to ultra-small nanoparticles. Conversely, for totally collapsed single-chain NPs only a slight increase of the *actual* molecular weight due to enamine cross-linking (*i.e.*, less than 10%) is expected by SEC/multi-angle laser light scattering (MALLS) when compared to the molecular weight of the starting linear precursor chains. A significant increase in retention time (corresponding to a clear reduction in  $R_H$ ) is observed in Figure 3 accompanying NP formation by enamine cross-linking.

**Figure 3.** Evolution of the size-exclusion chromatography (SEC) traces with reaction time during the synthesis of PMMA-NPs by enamine “click” chemistry from precursor **1a**. All curves from the SEC/refractive index (RI) detector are normalized to the same ordinate scale for clarity of presentation.



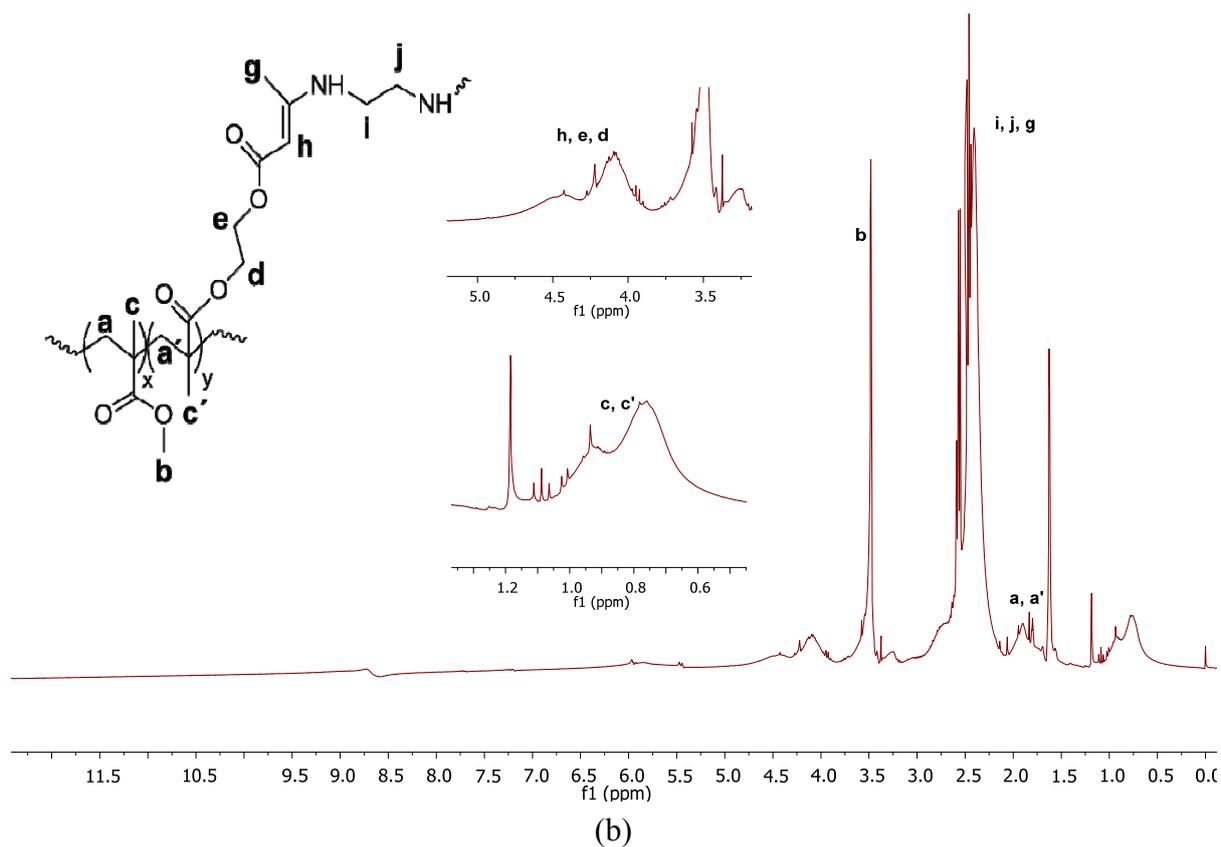
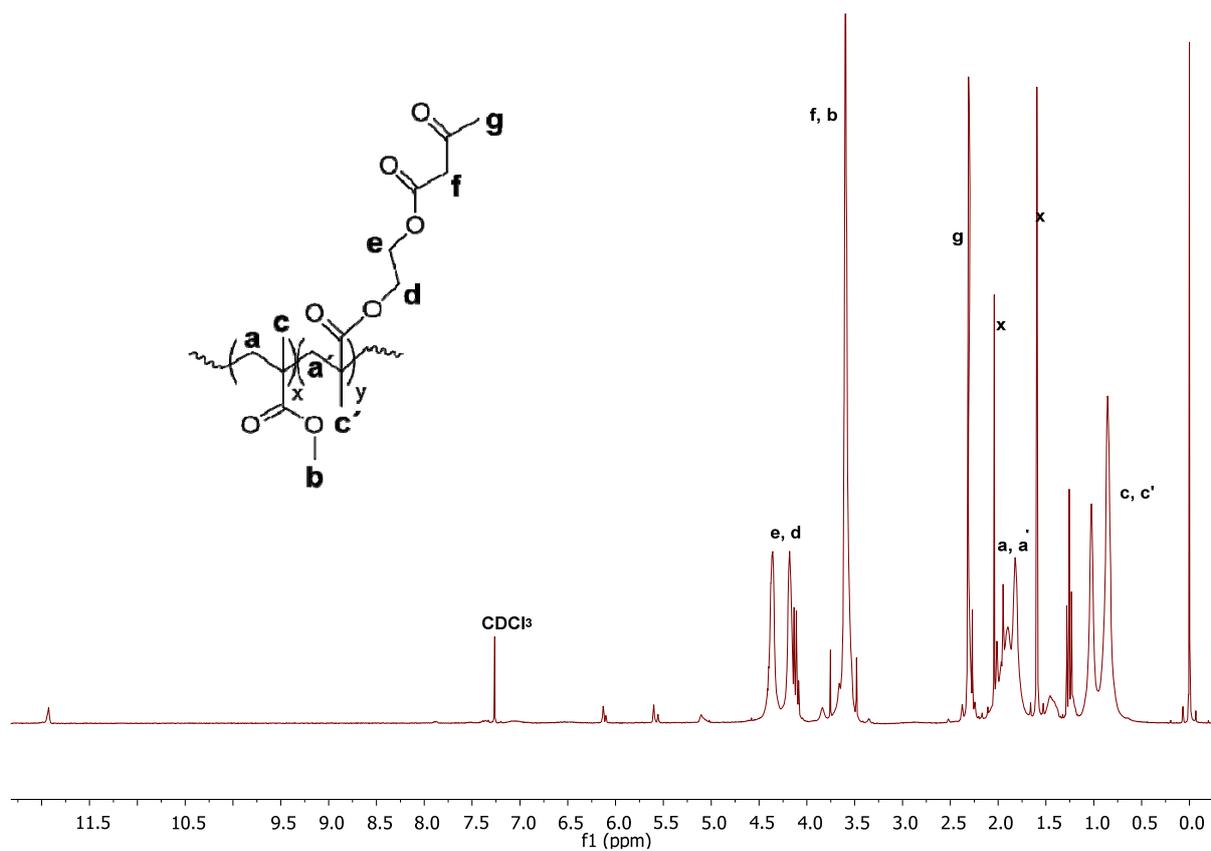
It is noteworthy that since intermolecular reactions give rise inevitably to a large increase in the molecular weight (*i.e.*,  $\geq 100\%$ ), an “apparent” molecular weight reduction, as deduced from Figure 3 due to the increase in retention time upon enamine cross-linking, is a clear signature of chain collapse. A slight increase in “absolute” mass-average molecular weight,  $M_w$ , upon reaction was found by SEC/MALLS ( $M_w = 89,000$  g/mol) when compared to the expected value ( $M_w = 61,900$  g/mol) suggesting that some degree of inter-particle coupling could be also present in addition to the principal single-chain intramolecular cross-linking process. On average, the number of polymer chains per nanoparticle is estimated to be around  $89,000/61,900 = 1.4$ .

As expected, nanoparticle collapse by enamine formation introduces significant changes in the  $^1\text{H-NMR}$  spectrum of the material. Figure 4(a) illustrates the  $^1\text{H-NMR}$  spectrum of the starting precursor **1a**, whereas Figure 4(b) gives the spectrum of the resulting nanoparticles. The corresponding peak assignment is also indicated.

The peak broadening observed in Figure 4(b) is a clear signature of NP formation and is well-documented in the literature [9]. This band broadening cannot be attributed to an increase of the “absolute” molecular weight upon nanoparticle formation but to its own nanoparticle structure upon changing from a solvated random coil to a compact, globular object. As an example, insets in Figure 4 show band broadening in signals from  $-\text{CH}=\text{}$  protons of enamine groups (4.5 ppm, broad) and  $-\text{CH}_3$  protons of main-chain methyl groups (0.5–1.1 ppm, very broad).

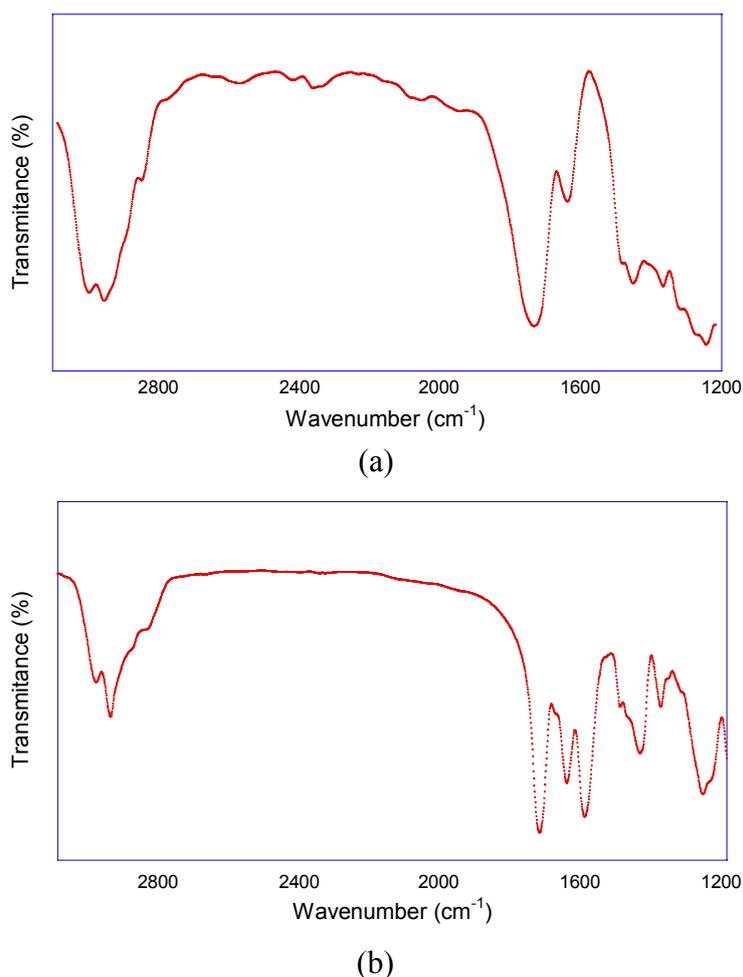
The signal from the  $=\text{N-H}$  protons of enamine groups is visible in the  $^1\text{H-NMR}$  spectrum at 8.7 ppm. It is worth noting that the weak signal from the proton enol-form which can be seen at around 12 ppm in the precursor (Figure 4(a)) disappears upon nanoparticle formation.

**Figure 4.** (a)  $^1\text{H-NMR}$  spectrum of the starting P(MMA-*co*-AEMA) precursor **1a**. (b)  $^1\text{H-NMR}$  spectrum in tetrahydrofuran (THF)- $\text{d}_8$  of the PMMA-NPs, **2a**, synthesized by enamine “click” chemistry. The sharp, intense band around 1.7 arises from solvent (THF- $\text{d}_8$ ).



Upon NP formation, a shift of a significant part of the original infra-red C=O band of AEMA from  $1,732\text{ cm}^{-1}$  to  $1,656\text{ cm}^{-1}$  is observed as well as the presence of a new band at  $1,605\text{ cm}^{-1}$  which can be assigned to stretching vibrations of enamine double bonds (Figure 5). Based on previous works on model compounds [25,26], the large shifts observed can be attributed to the presence of significant NH/C=O hydrogen bonding interactions upon enamine formation.

**Figure 5.** (a) FTIR spectrum of precursor **1a** in the solid state. (b) FTIR spectrum of the isolated PMMA-NPs **2a** synthesized by enamine “click” chemistry.

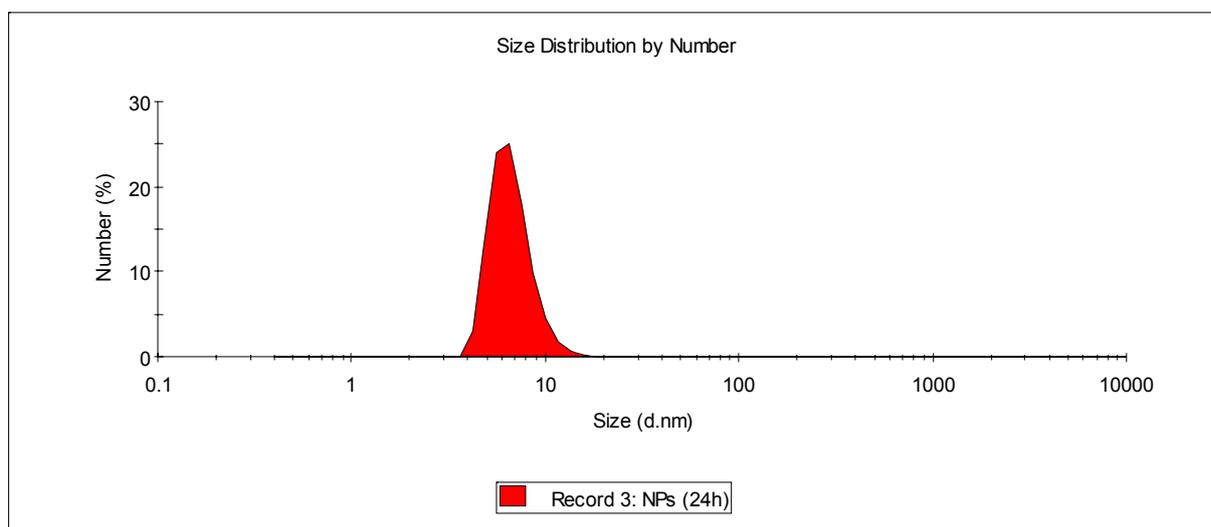


As depicted in Figure 6, nanoparticle size measurements by DLS supported the SEC and  $^1\text{H-NMR}$  results. Ultra-small PMMA-NPs with 7 nm in size were routinely obtained at r.t. upon reaction completion. Interestingly, the size of the PMMA-NPs remained unaltered while stored in dilute THF solutions ( $c < 1\text{ g/L}$ ).

However, when isolated by precipitation and further drying the resulting PMMA-NPs became insoluble in THF probably as a consequence of strong inter-nanoparticle hydrogen bonding (HB) interactions [14]. Attempts to solubilize the nanoparticles by adding lithium chloride, methanol, or trifluoroacetic acid failed. Similar attempts to change from THF solvent to dimethyl formamide before precipitation do not avoid nanoparticle aggregation presumably by cooperative HB interactions upon isolation.

Work is in progress to investigate the influence of the diamine structure on this striking behavior by using diamines containing bulky *tert*-butyl groups in the structure, as well as to determine the precise NP morphology by transmission electron microscopy and atomic force microscopy. Such research, which is outside the scope of the present paper, will be published in the near future.

**Figure 6.** Size distribution of PMMA-NPs **2a** in THF by DLS.



### 3. Experimental Section

#### 3.1. Materials

2,2'-Azobis(2-methylpropionitrile) (AIBN), methyl methacrylate (MMA), (2-acetoacetoxy)ethyl methacrylate (AEMA), diethylenetriamine (DETA), deuterated tetrahydrofuran (THF- $d_8$ ) and deuterated chloroform (99.96 atom% D) containing 0.03% (v/v) tetramethylsilane (TMS) were supplied by Aldrich. AIBN was purified by re-crystallization. MMA was distilled under reduced pressure (Buchi Glass Oven B-585) immediately before use. AEMA was purified by passing through a neutral alumina column. 2-Cyanoprop-2-yl-dithiobenzoate (CDB) and HPLC grade tetrahydrofuran (THF) were obtained from Strem Chemicals and Scharlau, respectively, and were used as received.

#### 3.2. Methods

Size-exclusion chromatography (SEC) was performed with an Agilent G-1310A liquid chromatograph equipped with a high performance PLgel Mixed column and on-line differential refractometer (RI) and multi-angle laser light scattering (MALLS) Wyatt detectors (Optilab-Rex and Mini-Dawn Treos, respectively) and THF (flow rate of 1.0 mL/min) was used as eluent at 30 °C.  $^1\text{H-NMR}$  spectra were recorded on a Bruker AVANCE spectrometer (300 MHz). Nanoparticle size was measured by dynamic light scattering (DLS) in a Malvern Zetasizer apparatus at r.t. Infrared (IR) spectra were recorded in a Jasco 3600 FT-IR Spectrometer.

### 3.3. Precursor Synthesis

In a typical procedure, a solution containing AIBN (1 mg, 6  $\mu$ mol), CDB (9 mg, 40  $\mu$ mol), MMA (1 mL, 9.3 mmol) and AEMA (1.2 mL, 6.2 mmol) was degassed and heated to 65 °C under magnetic stirring. After reaction, the resulting copolymer, **1a**, was purified by precipitation in methanol and drying under dynamic vacuum for 24 h. Yield (gravimetric): 1.52 g (67%). Mass-average molecular weight and dispersity (SEC/RI/MALLS): 57,100 g/mol and 1.1, respectively. Molar fraction of MMA ( $^1$ H-NMR): 0.65.

### 3.4. Nanoparticle Synthesis

On a round-bottom flask, the precursor **1a** (150 mg, 0.3 mmol of AEMA) was dissolved in 25 mL of THF. On a separate flask, DETA (equimolar amount of amine groups) was dissolved in 25 mL of THF. Both solutions were mixed at once and allowed to stir at r.t. Samples were removed periodically from the reaction medium for SEC/RI/MALLS and DLS analysis. In separate experiments, nanoparticle formation was monitored by  $^1$ H-NMR by using THF- $d_8$  as solvent. After reaction completion, the transparent solution was concentrated and precipitated onto methanol to give **2a** as a powder which was dried under dynamic vacuum for 24 h. Reaction yield: 100 mg, (67%). Average NP size (by DLS in THF): 7 nm.

## 4. Conclusions

A new, “metal-free” route to PMMA-NPs has been developed based on enamine “click” chemistry. As appropriate precursors, well-defined random copolymers of MMA and AEMA have been synthesized by RAFT polymerization, showing low molecular-weight dispersity and a random placement of  $\beta$ -ketoester moieties along the copolymer chains. Intramolecular collapse of the PMMA-NP precursors was performed by the one-pot reaction of the  $\beta$ -ketoester moieties with alkyl diamines in THF at r.t. (*i.e.*, by enamine formation). Significant changes were observed both in the  $^1$ H-NMR and FTIR spectra upon enamine cross-linking.

Nanoparticle formation was followed by SEC/RI/MALLS and, in separate experiments, by  $^1$ H-NMR spectroscopy in THF- $d_8$ . The size of the resulting PMMA-NPs was <10 nm as determined by DLS and remained unaltered while the nanoparticles were stored in dilute THF solutions ( $c < 1$  g/L). Strong inter-nanoparticle hydrogen bonding upon nanoparticle drying rendered the isolated PMMA-NPs insoluble in THF.

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