# Synthesis and characterization of a naphthalimide–dye end-labeled copolymer by reversible addition–fragmentation chain transfer (RAFT) polymerization

**Binxin Li, Daniel Majonis, Peng Liu, and Mitchell A. Winnik**



**Abstract:** We describe the synthesis of an end-functionalized copolymer of *N*-(2-hydroxypropyl)methacrylamide (HPMA) and *N*-hydroxysuccinimide methacrylate (NMS) by reversible addition–fragmentation chain transfer (RAFT) polymerization. To control the polymer composition, the faster reacting monomer (NMS) was added slowly to the reaction mixture beginning 30 min after initating the polymerization (ca. 16% HPMA conversion). One RAFT agent, based on azocyanopentanoic acid, introduced a –COOH group to the chain at one end. Use of a different RAFT agent containing a 4-amino-1,8-naphthalimide dye introduced a UV–vis absorbing and fluorescent group at this chain end. The polymers obtained had molecular weights of 30 000 and 20 000, respectively, and contained about 30 mol% NMS active ester groups.

*Key words:* RAFT, semi-batch polymerization, dye-labeled polymer, HPMA, *N*-hydroxysuccinimide methacrylate.

**Résumé :** On décrit la synthèse d'un copolymère fonctionnalisé aux extrémités du *N*-(2-hydroxypropyl)méthacrylamide (HPMA) et de méthacrylate de *N*-hydroxysuccinimide (NMS) par polymérisation impliquant une addition et une fragmentation radicalaire avec transfert de chaîne (AFRT). Dans le but de contrôler la composition du polymère, le monomère qui réagit le plus rapidement (NMS) est additionné lentement au mélange réactionnel 30 min après que la polymérisation a été initiée (conversion approximative de 16 % du HPMA). Un agent AFRT à base d'acide azocyanopentanoïque permet d'introduire un groupe –COOH à une extrémité de la chaîne. L'utilisation d'un agent AFRT différent, contenant un colorant 4-amino-1,8-naphtalimide, introduit un groupe fluorescent et absorbant dans l'UV–vis à l'extrémité de la chaîne. Les polymères obtenus possèdent respectivement des poids moléculaires de 30 000 et 20 000 et ils contiennent approximativement 30 mol% de groupes esters du NMS.

*Mots-clés :* polymérisation impliquant une addition et une fragmentation radicalaire avec transfert de chaîne (AFRT), polymérisation semi-discontinue, polymère marqué avec un colorant, *N*-(2-hydroxypropyl)méthacrylamide (HPMA), méthacrylate de *N*-hydroxysuccinimide (NMS).

[Traduit par la Rédaction]

## Introduction

Poly-*N*-(2-hydroxypropyl)methacrylate (PHPMA) is a nontoxic, nonimmunogenic, and biocompatible polymer that offers great promise as the host carrier of polymer–drug conjugates. These conjugates (polymer therapeutics) offer some important advantages over conventional chemotherapeutic agents, such as increased solubility, longer circulation times, and lower nonspecific cytotoxicity.[1,2] Early examples of polymer–drug conjugates based on PHPMA involved polymers synthesized by traditional free radical polymerization.[3,4] This led to polymers with rather broad molecular weight distributions. More recently, there has been a strong interest in the synthesis of PHPMA and its copolymers by controlled radical polymerization as a means of obtaining material with a narrower molecular weight distribution. This is important in therapeutic applications because molecular weight can influence the phamacokinetics, toxicity, and inhibitory potency of the conjugate.[5,6]

Several groups have reported an indirect synthesis of PHPMA by atom transfer radical polymerization (ATRP). In this approach, one begins with the polymerization of *N*-methacryloxysuccinimide (NMS) followed by treatment of the PNMS obtained with excess 1-amino-2-propanol.[7] This approach takes advantage of the fact that the succinimide ester group of NMS can react with amines to form amides. For therapeutic applications, complete removal of the Cu ion catalyst can be problematic. This is one reason that a greater effort has been devoted to the development of the synthesis of PHPMA and its copolymers by reversible addition–

Received 26 April 2010. Accepted 29 July 2010. Published on the NRC Research Press Web site at canjchem.nrc.ca on 22 February 2011.

*This article is part of a Special Issue dedicated to Professor J. C. Scaiano.*

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fragmentation chain transfer (RAFT) polymerization. McCormick and co-workers[8] prepared hydrophilic–cationic block copolymers of HPMA and *N*-[3-(dimethylamino)propyl]methacrylamide (DMAPMA) by RAFT polymerization in water. These polymers lack pendant functionality for attachment of drugs or dyes. To address this issue, they developed a synthesis of copolymers of HPMA with *N*-(3-aminopropyl)methacrylamide) (APMA). Here, APMA provided amine functional groups for bioconjugation. They described modification of these copolymers with folic acid[9] or with *N*-succinimidyl 3-(2-pyridyldithio)-propionate to attach 5′-thiolated RNAs through a thiol–disulfide exchange reaction.[10] Hong and Pan[11] reported the synthesis of P(HPMA-*b*-NIPAM) (NIPAM = *N*-isopropylacrylamide) block copolymers by RAFT polymerization. These polymers were obtained with molecular weights in the range of 7 800–26 300 g/mol with narrow polydispersites (PDI = 1.15–1.29). Zentel and co-workers[12] synthesized functional amphiphilic poly(*N*-(2-hydroxypropyl) methacrylamide)-*b*-lauryl methacrylate) by RAFT polymerization. They investigated the influence of nanoaggregates formed by this block polymer on cell viability and on the motility of adherent cells.

A more general strategy for the preparation of functional HPMA copolymers involves the synthesis of an activated polymer with pendant active ester groups along the backbone. A random copolymer of HPMA and NMS would be an effective scaffold for the attachment of amine derivatives of dyes or drugs. The challenge in this synthesis is that the reactivity ratios ($r_{HPMA}$ = 0.12 and $r_{NMS}$ = 3.46)[13] favor rapid consumption of the NMS comonomer. Kane and co-workers[13,14] addressed this problem by using a "semi-batch" approach to the synthesis,[15] by adding the more reactive monomer slowly as the RAFT polymerization proceeded. They monitored the kinetics of the reaction and the composition of the polymer as the reaction proceeded, obtaining polymer that contained ~20 mol% NMS groups throughout the reaction. These polymers could be functionalized with a peptide known to disrupt anthratoxin assembly. The reaction in DMSO involved substitution of the active ester with an amino group from the peptide.

We are interested in this approach to the synthesis of functional HPMA copolymers, but with a higher NMS content. Here, we report a modified synthesis that follows the Kane and co-workers[13] strategy, but introduces functional and dye-containing chain-transfer agents (CTAs). As is well-established for RAFT polymerization, these transfer agents allow one to introduce functional groups or dyes at the initiating end of the polymer chains.[16–18] We are particularly interested in dyes based on the naphthalimide ring structure. The 1,8-naphthalimide chromophore and its derivatives have been studied for many years.[19–22] Many of these derivatives are easily synthesized from the corresponding naphthalic anhydrides. They have relatively high fluorescent quantum yields, high photostability, and tunable spectroscopic properties. Many 1,8-naphthalimide derivatives have been incorporated covalently or non-covalently into various polymeric materials to study the properties and behavior of the polymers. Our group has examined a family of 1,8-naphthalimide derivatives as donor–acceptor pairs to monitor polymer morphology or the diffusion of polymers by Förster resonance energy transfer (FRET) measurements.[23] Polymers containing the 1,8-naphthalimide group have also been studied for other applications such as sensors, probes, OLEDs, and solar cells, as well as photochemotherapy.[24]

Here, we describe the synthesis of a copolymer of HPMA and NMS with a naphthalimide-labeled end through RAFT polymerization. This dye-containing polymeric precursor can be further modified and potentially used in bioconjugation, drug delivery, and gene delivery applications.

## Experimental

### General characterization

$^1$H NMR (400 MHz) spectra were recorded on a Varian Mercury 400 spectrometer. $CDCl_3$ and $d_6$-DMSO were purchased from Cambridge Isotope Laboratories, Inc. 3-(Trimethylsilyl)propionic acid-$d_4$ sodium salt (TSP) was purchased from Sigma-Aldrich. For gel permeation chromatography (GPC) measurements, the eluant was 1-methyl-2-pyrrolidinone (NMP) containing 0.2 wt% LiCl, and the column was calibrated with poly(methyl methacrylate) (PMMA) standards. The analysis was carried out at 80 °C at a flow rate of 0.6 mL/min using a Viscotek VE1121 GPC solvent pump, with a Viscotek VE 3580 refractive index detector and a Viscotek VE3210 UV–vis absorbance detector. UV–vis measurements were carried out with a PerkinElmer Lambda 6 spectrometer.

### Materials

4-Bromo-1,8-naphthalimide anhydride (BNA) (95%, Sigma-Aldrich), isobutylamine (99%, Sigma-Aldrich), ethylenediamine (99%, Sigma-Aldrich), oxalyl chloride (98%, Sigma-Aldrich), 2,2′-azobis(2-methylpropionitrile) (AIBN) (98%, Sigma-Aldrich), 1,3,5-trioxane (≥99%, Sigma-Aldrich), *tert*-butyl alcohol (*t*-BuOH) (≥99.5%, Sigma-Aldrich), *N*,*N*-dimethylformamide (DMF) (99.8%, Sigma-Aldrich), 1-methyl-2-pyrrolidinone (NMP) (≥99%, Sigma-Aldrich), and other chemicals were used as received. *N*-(2-Hydroxypropyl)methacrylamide (HPMA) was prepared according the literature procedure reported by Ulbrich et al.[25] *N*-Methacryloxysuccinimide (NMS) was synthesized according to the literature procedure reported by Shunmugam and Tew.[26] 4-Cyanopentanoic acid dithiobenzoate (CIDB) was synthesized as described previously.[27]

### Synthesis of 9-isobutyl-4-ethylenediamino-1,8-naphthalimide

9-Isobutyl-4-ethylenediamino-1,8-naphthalimide was synthesized in two steps. 4-Bromo-1,8-naphthalic anhydride (1.4 g, 5.1 mmol) and isobutylamine (2.0 mL, 21 mmol) were added to 1,4-dioxane (40 mL) at room temperature. The solution was stirred for 4.5 h at 100 °C. The solvent was removed by rotary evaporation. The yellow solid was purified by silica column chromatography using heptanes–acetone (12:1, v:v) as the eluent. Finally, the yellow solid product (9-isobutyl-4-bromo-1,8-naphthalimide) was dried under vacuum at 50 °C overnight. Yield: 1.14 g (72%); mp 130.0–131.5 °C. $^1$H NMR ($CDCl_3$, ppm) δ: 0.98 (d, 6H), 2.24 (m, 1H), 4.04 (d, 2H), 7.85 (dd, 1H), 8.04 (d, 1H), 8.42 (d, 1H), 8.58 (d, 1H), 8.66 (d, 1H). Elemental anal. calcd: C 57.85, H 4.25, N 4.22; found: C 57.98, H 4.32, N 4.50.

**Table 1.** Characteristics of the naphthalimide–dye end-labeled poly(HPMA-co-NMS).

| NPMA mol%[a] | NMS mol%[a] | $DP_{HPMA}$[a] | $DP_{NMS}$[a] | $M_n$ (NMR)[a] | $M_n$ (NMR)[b] | $M_n$ (UV)[c] |
|---|---|---|---|---|---|---|
| 69 | 31 | 54 | 24 | 16 000 | 18 000 | 18 000 |

**Note:** HPMA, *N*-(2-hydroxypropyl)methacrylamide; NMS, *N*-methacryloxysuccinimide; $DP_{HPMA}$, mean number of HPMA units per polymer; and $DP_{NMS}$, mean number of NMS units per polymer.

[a]Calculated with eqs. [1] and [2].

[b]From the determination of the moles of the dye end group using TPS as an internal standard, calculated as $M_n$ = [mass of poly(HPMA-co-NMS)]/[moles of naphthalimide–dye].

[c]From the absorbance at 430 nm using a value of $\varepsilon = 1.3 \times 10^4$ $(mol/L)^{-1}$ $cm^{-1}$.



9-Isobutyl-4-bromo-1,8-naphthalimide (3.020 g, 9.123 mmol) was added to ethylenediamine (50 mL, 0.75 mol). The solution was stirred at 50 °C for 2.5 h. The reaction mixture was treated with toluene (200 mL), and then the volatile liquids were removed by rotary evaporation. The crude product was dissolved in 1 mol/L aq HCl (70 mL). The product was precipitated by adding 1 mol/L aq NaOH until the solution became weakly basic (pH ~8). The mixture was then filtered. The yellow solid product (9-isobutyl-4-ethylenediamino-1,8-naphthalimide) was dried under vacuum at 50 °C overnight. Yield: 2.66 g (94%); mp 162–164 °C. $^1$H NMR ($CDCl_3$, ppm) δ: 0.98 (d, 6H), 1.24 (broad, 2H), 2.25 (m, 1H), 3.18 (t, 2H), 3.42 (m, 2H), 4.03 (d, 2H), 6.14 (broad, 1H), 6.72 (d, 1H), 7.62 (t, 1H), 8.16 (d, 1H), 8.45 (d, 1H), 8.58 (d, 1H). Elemental anal. calcd.: C 69.43, H 6.80, N 13.49; found: C 68.52, H 6.66, N 13.66.

### Synthesis of bis(benzenethiocarbonyl) disulfide

Magnesium turnings (3.00 g, 0.125 mol) were placed into a round-bottom flask with a catalytic amount of iodine. Bromobenzene (18.84 g, 0.1200 mol) was mixed with dry THF (90 mL). Then a 10 mL mixture of bromobenzene and THF was added to the flask and heated slightly. The remaining mixture was added slowly, while the temperature of reaction remained below 40 °C. The reaction was then stirred at room temperature for 1 h, after which the flask was cooled to 0 °C. Carbon disulfide (9.15 g, 0.120 mol) was added to the Grignard mixture at 0 °C. When the reaction finished after 2 h, deionized water (350 mL) was added, and the salts were removed by filtration. Concentrated HCl (~10 mL) was added to the filtrate and the mixture was extracted with diethyl ether. After evaporating the solvent with a rotary evaporator, absolute ethanol (100 mL) was added into the dithiobenzoic acid along with DMSO (18.75 g, 0.24 mol) and a catalytic amount of iodine. The reaction proceeded at room temperature for 2 h, and then the mixture was filtered. The purple solid (bis(thiocarbonyl) disulfide) was dried under vacuum at room temperature overnight. Yield: 11.04 g (60%); mp 96–98 °C. $^1$H NMR ($CDCl_3$, ppm) δ: 7.45 (t, 4H), 7.61 (t, 2H), 8.07 (t, 4H).

### Synthesis of the naphthalimide–dye-labeled CTA (NapDB)

Oxalyl chloride (5.3 mL, 62 mmol) was added to a stirred suspension of 4,4′-azobis(4-cyanovaleric acid) (1.708 g, 6.775 mmol) in anhyd $CH_2Cl_2$ with a catalytic amount of *N*,*N*-dimethylformamide at room temperature. After 3 h, the reaction mixture turned clear and was evaporated with a rotary evaporator to leave a yellow solid of 4,4′-(diazene-1,2-diyl)bis(4-cyanopentanoyl chloride). Yield: 1.820 g (95.6%). $^1$H NMR ($CDCl_3$, ppm) δ: 1.72 (d, 6H), 2.62 (m, 4H), 3.14 (m, 4H).

9-Isobutyl-4-ethylenediamino-1,8-naphthalimide (1.557 g, 4.788 mmol) was dissolved in 100 mL anhyd $CH_2Cl_2$. The solution was cooled to 0 °C. A solution of 4,4′-(diazene-1,2-diyl)bis(4-cyanopentanoyl chloride) (0.632 g, 2.19 mmol) and *N*,*N*-diisopropylethylamine (2.29 mL, 13.1 mmol) in 20 mL anhyd $CH_2Cl_2$ was added dropwise to the dye solution via an addition funnel under $N_2$ protection. The reaction was stirred for 12 h at room temperature and then concentrated with a rotary evaporator. The mixture was washed with saturated sodium bicarbonate solution, 1% aq HCl, and finally 2 mol/L aq NaCl. The organic layer was dried over magnesium sulfate, filtered, and concentrated. The naphthalimide–dye-labeled CTA precursor was precipitated in diethyl ether and lyophilized overnight. Yield: 1.36 g (79%). $^1$H NMR ($CDCl_3$, ppm) δ: 0.98 (d, 12H, $CH_3$), 1.69 (d, 6H, $CH_3$), 2.22–2.52 (m, 8H, $CH_2CH_2$), 3.44 (m, 4H, $CH_2$), 3.68 (t, 4H, $CH_2$), 4.01 (d, 4H, $CH_2$), 6.57 (d, 2H), 7.60 (t, 2H), 8.12 (d, 2H), 8.35 (d, 2H), 8.51 (d, 2H).

A solution of bis(thiocarbonyl) disulfide (0.156 g, 0.510 mmol) and the naphthalimide–dye-labeled CTA precursor (0.213 g, 0.246 mmol) in ethyl acetate was degassed with nitrogen and heated at 80 °C for 20 h. The solvent was removed with a rotary evaporator, and the residue was purified by silica column chromatography using ethyl acetate – hexane as the eluent. Yield: 0.049 g (34%); mp 90–93 °C. $^1$H NMR ($CDCl_3$, ppm) δ: 0.99 (d, 6H, $CH_3$), 1.93 (s, 3H, $CH_3$), 2.44–2.71 (m, 4H, $CH_2CH_2$), 3.54 (t, 2H, $CH_2$), 3.77 (t, 2H, $CH_2$), 4.04 (d, 2H, $CH_2$), 6.17 (broad, 1H, NH), 6.60 (d, 1H, naphthalic-H), 6.91 (broad, 1H, NH), 7.66 (t, 1H), 7.35 (m, 2H, m-ArH), 7.38 (t, 1H, p-ArH), 7.84 (dd, 2H, o-ArH), 8.24 (d, 1H), 8.45 (d, 1H), 8.57 (d, 1H). Elemental anal. calcd.: C 65.01, H 5.63, N 9.78; found: C 64.83, H 5.77, N 9.23.

### The homopolymerization of HPMA

The polymerizations were carried out under an Argon (Ar) atmosphere using the Shlenck technique. A typical polymerization procedure is described in the following. A stock solution was prepared comprised of AIBN (140.08 mg, 0.851 mmol), CIDB (41.08 mg, 0.155 mmol), and 1,3,5-trioxane (internal standard, 449 mg, 4.98 mmol) in degassed DMF (5 mL). HPMA (0.458 g, 3.55 mmol) was evacuated and back-filled with Ar three times. The degassed *t*-BuOH (3.2 mL) was injected into a round-bottom flask containing HPMA to form a 1 mol/L solution, and a solution of AIBN, CIDB, and 1,3,5-trioxane in DMF (200 μL) was transferred

into the same flask. The reaction was carried out at 80 °C immediately and finally quenched in an acetone – dry ice bath. Aliquots (0.1 mL) were taken out for NMR analysis throughout the reaction. The final polymer was precipitated using a mixture of anhydrous diethyl ether and anhydrous acetone (1:1 v/v), recovered by centrifugation, and then lyophilized overnight for further analysis by NMR and GPC.

### The copolymerization of HPMA and NMS

The copolymerization reactions were carried out under an Argon (Ar) atmosphere on a Shlenck line. A typical copolymerization procedure (Table 1) is described in the following. A stock solution was prepared consisting of AIBN (6.6 mg, 0.040 mmol), NCTA (45.7 mg, 0.0799 mmol), and 1,3,5-trioxane (internal standard, 77.6 mg. 0.861 mmol) in degassed DMF (1 mL). The monomers were evacuated and backfilled with Ar three times. The solvents were degassed by bubbling with Ar gas. *t*-BuOH (1.9 mL) was added to a round-bottom flask containing HPMA (0.275 g, 2.13 mmol) to form a 1 mol/L solution, and then the solution of AIBN, NCTA, and 1,3,5-trioxane in DMF (200 μL) was transferred into the flask. The mixture was heated at 80 °C for 30 min, and then a solution of NMS in DMF (0.5 mol/L) was added continuously into the reaction mixture at 0.43 mL/h through an airtight syringe by a syringe pump (KD Scientific, model 780100). After the addition of the NMS solution, the reaction was kept at 80 °C for an additional 30 min and was then quenched in an acetone – dry ice bath. Aliquots (0.1 mL) were taken out for NMR analysis throughout the reaction. The final polymer was precipitated using a mixture of anhydrous diethyl ether and anhydrous acetone (1:1 v/v), recovered by centrifugation, and then lyophilized overnight. A sample for end-group analysis was subjected to a second precipitation from DMF into ether–acetone. The polymer product (26 mg) was stored at 4 °C.

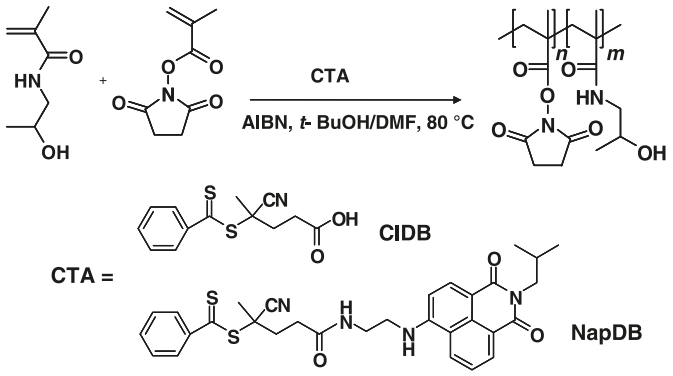
### Molecular weight determination

A sample of the dye-labeled polymer was examined by UV–vis spectroscopy. A sample of the dye-labeled poly(HPMA-co-NMS) (1.134 mg) was dissolved in 1.0 mL DMF to form a 1.13 g/L solution. The calibration curve of the UV absorbance of the naphthalimide–dye was built using 9-isobutyl-4-ethylenediamino-1,8-naphthalimide (BEAN) as a model compound. A series of solutions of BEAN in DMF was prepared with accurately known concentrations. The absorbance of these solutions at 440 nm was measured by UV–vis spectrometry. For the $^1$H NMR experiments, dye-labeled copolymers were dissolved into DMSO-$d_6$ to form solutions at 10 mg/mL. In the GPC experiments, dye-labeled polymers were dissolved in NMP containing 0.2 wt% LiCl to form 1 mg/mL solutions.

## Results and discussion

In a RAFT polymerization employing a thiocarbonylthio compound ZC(=S)SR as a chain transfer agent, the R group will become attached to the initiating end of the chain. The –SC(=S)Z at the other end can act as a protected thiol for further transformation of the polymer. In the initial polymerization reactions described below, CIDB (see below, Scheme 1) was used as the chain-transfer agent (CTA). It

**Scheme 1.** Reversible addition–fragmentation chain transfer (RAFT) copolymerization of *N*-(2-hydroxypropyl)methacrylamide (HPMA) and *N*-methacryloxysuccinimide (NMS).




was synthesized as described in ref. 27. This CTA introduces a carboxylic acid into the R-group. If the R-group bears important functionality (such as a fluorescent dye) for applications in aqueous media, it is important that the attachment of the dye not involve ester groups which can slowly hydrolyze in water. In the design of the naphthalimide chain transfer agent used in these experiments, it is incorporated as an amide. The synthesis of this CTA is shown in Scheme 2. It was synthesized from bis(benzenethiocarbonyl) disulfide and the dye-containing azo-initiator in a single step. The yield was only 34% after column chromatography, but $^1$H NMR and elemental analysis indicated that the product was pure. It showed only one spot by TLC.

We initially examined the kinetics of the polymerization of HPMA at 80 °C in the presence of CIDB as the chain-transfer agent and AIBN as the initiator. The ratio of [HPMA]–[CIDB]–[AIBN] was 320:2:1. As shown in the Supplementary data, $\ln([M]_0/[M]_t)$ increased linearly with reaction time over the first 4 h of the polymerization. Later, the rate of the polymerization became slower. The molecular weight increased monotonically but not quite linearly throughout the polymerization (see Fig. S2 in the Supplementary data), and maintained a narrow polydispersity. At 70% conversion, the sample was characterized by GPC (PMMA standards), yielding $M_n$ = 32 000 with PDI = 1.3. These results establish that CIDB is an effective CTA for this RAFT polymerization. These results also provide information about the monomer conversion kinetics that we used to design the addition rate of NMS for the copolymerization of HPMA and NMS.

### Semi-batch copolymerization of HPMA and NMS with CIDB as the CTA

Initial studies of the copolymerization of HPMA and NMS were carried out using CIDB as the RAFT agent. This reaction is shown in Scheme 1. In this copolymerization, all of the reactants except NMS (that is, AIBN, the CTA, 1,3,5-trioxane as an internal standard, and HPMA monomer, in a mixture of DMF and *tert*-butyl alcohol as the solvent) were introduced into a flask on a Schlenk line equipped with a septum and thoroughly outgassed as described in the Experimental section. The reaction was initiated by heating the solution to 80 °C and, after 30 min, a solution of NMS

**Scheme 2.** The synthesis of the naphthalimide–dye-labeled chain-transfer agent (CTA).

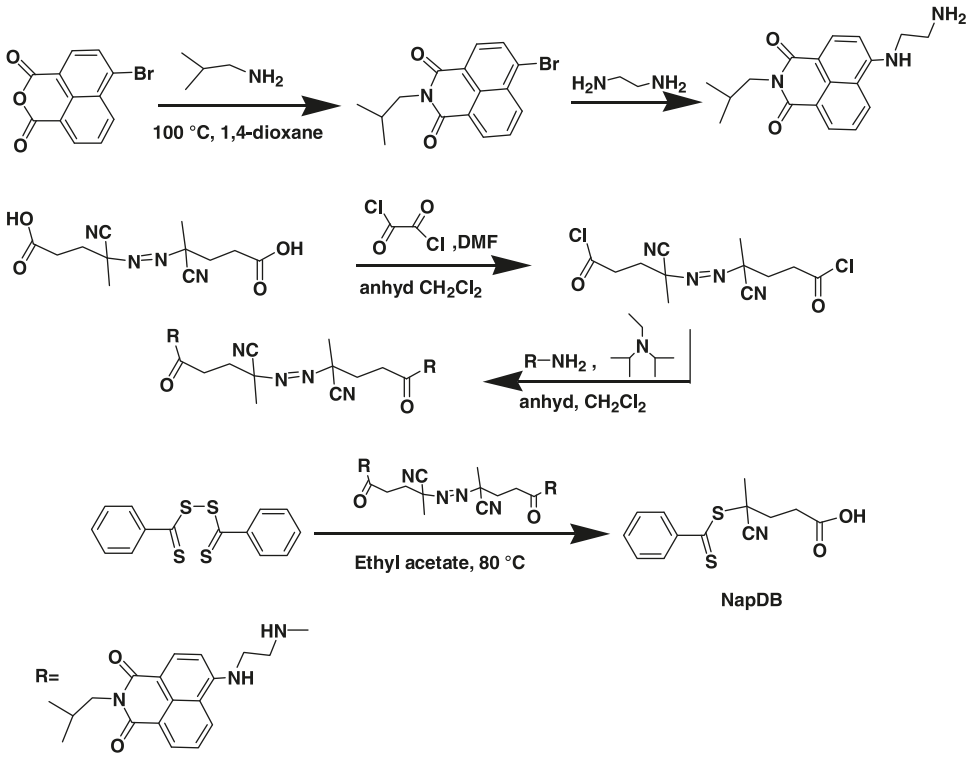
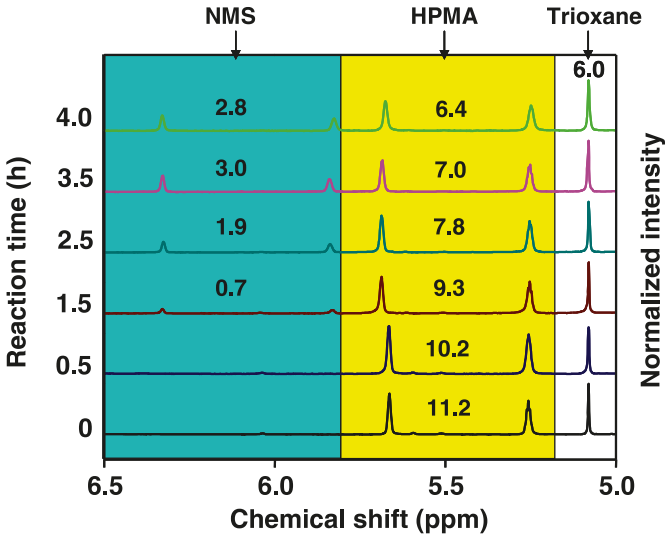



**Fig. 1.** $^1$H NMR spectra for the conversion of *N*-(2-hydroxypropyl)methacrylamide (HPMA) and *N*-methacryloxysuccinimide (NMS) in reversible addition–fragmentation chain transfer (RAFT) copolymerization. The numbers in different colour zones indicate the average value of the vinyl protons of each corresponding monomer compared with the peak of the internal standard.



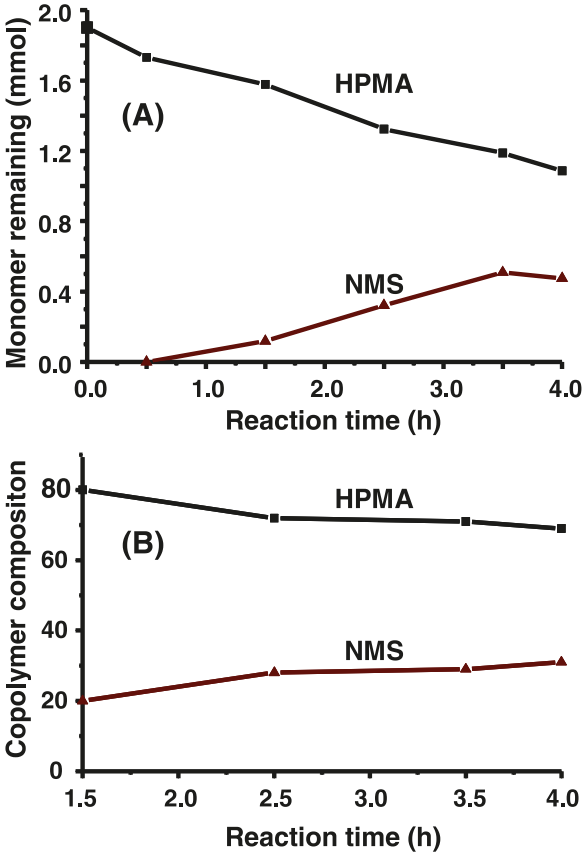

(0.5 mol/L) in DMF was added continuously into the reaction mixture at 0.43 mL/h via a syringe pump.

The conversion of HPMA and NMS was determined by $^1$H NMR (in $CDCl_3$) (Fig. 1). The peaks at 5.2 and 5.7 ppm are ascribed to the vinyl protons of HPMA, and the peaks at 5.8 and 6.3 ppm are ascribed to the vinyl protons of NMS. The peak of 1,3,5-trioxane at 5.1 ppm was used as an internal standard to determine the amounts of HPMA and NMS remaining in the reaction mixture. In Fig. 1, the integral of this peak is arbitrarily assigned an integration value of 6.0. The numbers in the yellow zone of the spectra represent the average of the integrals of the two vinyl protons of HPMA, whereas the numbers in the blue zones of the spectra are the corresponding values of the two vinyl protons of NMS. These integral values indicate the amount of HPMA and NMS remaining at each point in the reaction. Over the first 30 min of the reaction, only HPMA was present. Then the addition of NMS began. Over the next 3.5 h, one can see a decrease in the concentration of HPMA in the reaction, accompanied by a slow steady build-up of NMS monomer in the reaction mixture. Even though NMS is more reactive in this copolymerization than HPMA, the consumption rate of NMS in the reaction was slower than its feed rate.

In Fig. 2A, we plot these data in terms of the time evolution of the amount of the monomers present in the system. The amount of HPMA in the reaction decreased essentially linearly with reaction time, and the amount of free NMS in the reaction mixture increased over time. After 4 h, 42% of the HPMA in the reaction and 24% of the NMS added were consumed. The composition of the poly(HPMA-co-NMS) obtained at different intervals of time was determined by $^1$H NMR (in $d_6$-DMSO). Figure 2B shows that the percentage of HPMA in poly(HPMA-co-NMS) decreased slightly from 80% to 69% as the incorporation of NMS increased from 20% to 31%. The relatively high percentage of HPMA in the copolymer at 1.5 h is a consequence of the poly(HPMA) segment formed during the initial 30 min of the polymerization.

GPC measurements showed that the molecular weight of the copolymer increased over the first 3.5 h of the reaction. Individual traces were symmetrical (shown in Fig. S3B in

**Fig. 2.** (A) Amounts of monomer remaining at different reaction times in the semi-batch reversible addition–fragmentation chain transfer (RAFT) copolymerization of *N*-(2-hydroxypropyl)methacrylamide (HPMA) and *N*-methacryloxysuccinimide (NMS) with 4-cyanopentanoic acid dithiobenzoate (CIDB) as the chain-trnasfer agent (CTA). (B) The composition of the copolymer at these reaction times determined by $^1$H NMR.

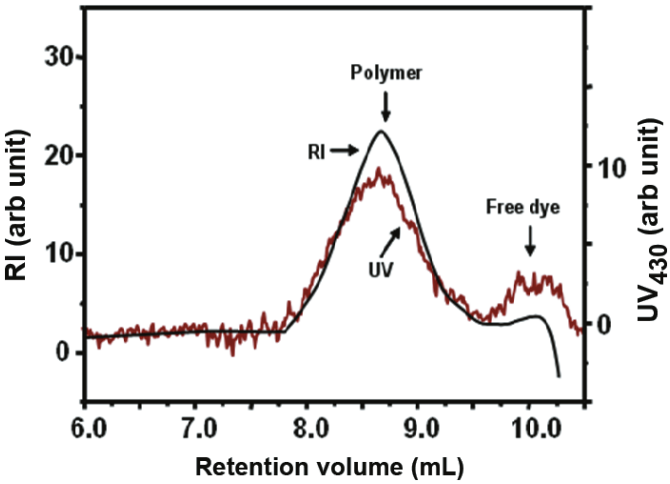



the Supplementary data), and the polydispersities were relatively narrow (PDI = 1.3–1.4). Based upon PMMA standards, $M_n$ values increased from 10 000 g/mol after 1.5 h to 18 000 g/mol after 3.5 h, with little additional change found after 4 h, when the reaction was terminated. The poly(HPMA-co-NMS) obtained had $M_n$= 18 000 g/mol and PDI = 1.4. Analysis of the polymer by $^1$H NMR revealed that the incorporation of NMS in the copolymer increased gradually as the copolymerization proceeded. We infer that the semi-batch method of synthesis enabled the formation of a polymer with a quasi-random distribution of NMS units, except for the segment of poly(HPMA) formed at the beginning of the polymerization.

## Synthesis and characterization of the naphthalimide–dye end-labeled copolymer

To obtain a corresponding copolymer of HPMA and NMS with a strongly fluorescent dye at one end, the polymerization reaction was repeated using NapDB as the RAFT agent. This reaction is shown in Scheme 1. The copolymerization was carried out in the semi-batch mode at 80 °C over 4 h, with the addition of NMS (0.43 mL/h) initiated at the 30 min point of the reaction. The ratio of monomers

**Fig. 3.** Gel permeation chromatography (GPC) traces (UV and refractive index (RI) detectors) of the naphthalimide–dye end-labeled poly(HPMA-co-NMS) synthesized using a mol ratio ([HPMA + NMS]/[CTA]/[AIBN] = 320:2:1). The UV signal was monitored at 430 nm. HPMA, *N*-(2-hydroxypropyl)methacrylamide; NMS, *N*-methacryloxysuccinimide; CTA, chain-transfer agent; and AIBN, 2,2′-azobis(2-methylpropionitrile).




(HPMA + NMS) to NapDB to AIBN was maintained at 320:2:1. The ratio of HPMA to NMS remained 3:1. The conversion of HPMA and NMS was determined by $^1$H NMR, using 1,3,5-trioxane as an internal standard. From GPC measurements on the dye-labeled poly(HPMA-co-NMS), using the refractive index (RI) detector in conjunction with PMMA standards, we obtained a nominal molecular weight of $M_n$ = 21 000, $M_w$ = 31 000, PDI = 1.5 (Fig. 3).

This sample also shows two peaks in the UV trace ($\lambda$ = 430 nm) at retention times of 14.5 and 16.7 min. The former corresponds to the polymer and demonstrates that the dye is part of the polymer structure. The peak at longer retention time is due to residual CTA in the sample. The amount present is too small to give an appreciable signal in the RI detector.

The $^1$H NMR spectrum of the purified naphthalimide–dye end-labeled poly(HPMA-co-NMS) in $d_6$-DMSO is presented in Fig. 4 along with the corresponding peak assignments. The characteristic resonances for the poly(HPMA-co-NMS) backbone appear at 0.4–1.4 ppm, along with the methacrylamide methyl protons and the methyl protons (k) of the HPMA unit. The peaks at 2.8 and 3.0 ppm are ascribed to the protons of the succinimide (o) and to the protons of the amide methylene (i), respectively. The signal of the alcohol methine (j) appears at 3.7 ppm. The signals of hydroxyl proton and amine proton appear at 4.7 and 7.3 ppm, respectively. The signals of the naphthalimide are also very clear and easily identified, such as the proton a at 8.6 ppm, the proton e at 8.5 ppm, and the proton c at 8.3 ppm. The peak at 3.3 ppm is due to traces of water present in the deuterated DMSO used as the NMR solvent.

For quantitative interpretation of the $^1$H NMR signals, a spectrum was acquired with 1280 scans and a relaxation delay time of 25 s. The mean number of HPMA units per polymer ($DP_{HPMA}$) and of NMS units per polymer ($DP_{NMS}$) were

**Fig. 4.** [1]H NMR spectrum of the naphthalimide–dye end-labeled poly(HPMA-co-NMS) in DMSO-$d_6$. The inset is an enlargement of the region from 6.8 to 8.7 ppm.

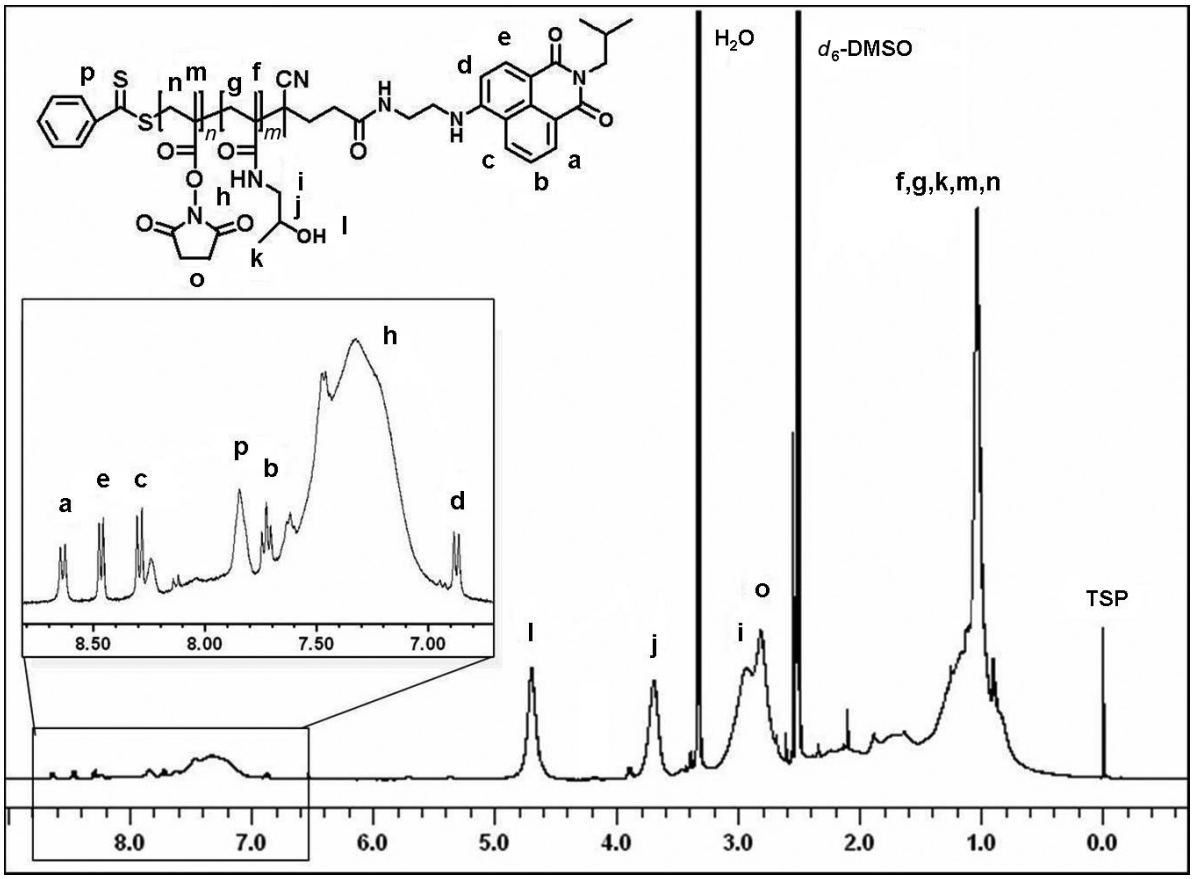
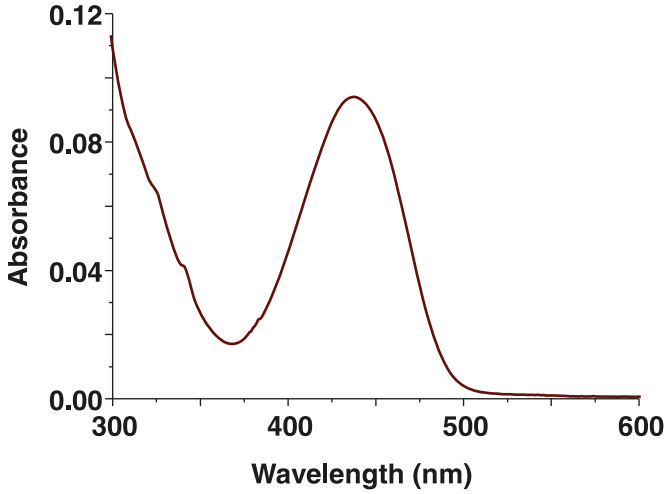



**Fig. 5.** UV–vis absorption spectrum of the naphthalimide–dye end-labeled poly(HPMA-co-NMS) (1.13 g/L) in *N*,*N*-dimethylformamide (DMF), with an absorbance at $\lambda_{max}$ (440 nm) of 0.094.




evaluated from the ratios of the peak integrals, using the signal a at 8.65 ppm as a measure of the naphthalimide end group. The signal j at 3.7 ppm represents one proton per HPMA unit. The signals i + o at 2.8–3.1 ppm represent contributions from the NMS group (4H) and 2H from the HPMA group. The desired information was calculated using eqs. [1] and [2].

$$[1] \qquad DP_{HPMA} = \frac{I_j n_a}{I_a n_j}$$

$$[2] \qquad DP_{NMS} = \frac{(I_{i+o} - I_j n_i / n_j) n_a}{I_a n_i}$$

Here $I_a$ is the integral of peak a. $I_i$, $I_j$, and $I_o$ are the integrals of peaks i, j, and o, respectively. $n_a$ is the number of peak a protons. $n_i$, $n_j$, and $n_o$ are the numbers of peak i, j, and o protons), respectively. The absolute number – average molecular weight of poly(HPMA-co-NMS) was calculated from the degrees of polymerization of monomers (HPMA and NMS), as well as the mass of the naphthalimide chain end assuming one dye molecule per chain.

As a check on this value of $M_n$, we used TSP as an external standard for the quantitative determination of $M_n$ by NMR. In this procedure, a sample of the dye-labeled poly(HPMA-co-NMS) (6.17 mg) was dissolved in $d_6$-DMSO (0.6913 g), and a known amount (19.8 mg) of a solution of TSP in $d_6$-DMSO (1.0 mg/g) was added. The [1]H NMR spectrum was measured as described previously. The TSP signal can be seen as the peak at 0.0 ppm in Fig. 4. The integral of this signal was compared with the signal of the naphthalimide–dye at 8.65 ppm to obtain the mol ratio of the naphthalimide dye to TSP. The results obtained from [1]H NMR ($d_6$-DMSO) are summarized in Table 1. The $M_n$ values determined with both methods are in good agreement. The mol ratio of

HPMA–NMS determined by NMR (69:31) is somewhat enhanced in its NMS content compared with the 3:1 mol ratio of monomers in the feed (25 mol%) NMS.

Figure 5 presents a UV–vis spectrum of the polymer in DMF solution. The solution shows $\lambda_{max}$ at 440 nm. To estimate $M_n$ based upon this absorption, we determined a value of the molar extinction coefficient of the chromophore in DMF ($\varepsilon = 1.3 \times 10^4$ (mol/L)$^{-1}$ cm$^{-1}$) using 9-isobutyl-4-ethylenediamino-1,8-naphthalimide as a model compound. In this way we calculated $M_n^{UV} = 18\,000$. These $M_n$ values are compared in Table 1 and are seen to be in good agreement with each other. The value of $M_n$ obtained from analysis of the GPC trace with a calibration curve based on PMMA standards was 21 000. The difference between this value and those obtained by NMR and UV–vis is an indication of the different hydrodynamic volumes of poly(HPMA-co-NMS) and PMMA in NMP at 80 °C.

## Summary

Copolymers poly(HPMA-co-NMS) with molecular weight of ca. 20 000 and 30 000 were synthesized by RAFT polymerization, using two different RAFT agents. In one example, we used the carboxylic acid functionalized CIDB, whereas in the second example, we used NapDB containing a naphthalimide–dye derivative with an absorption $\lambda_{max}$ at 430 nm. Both polymers contained ~30 mol% NMS groups. This pair of monomers is characterized by a serious reactivity ratio mismatch ($r_{HPMA} = 0.12$ and $r_{NMS} = 3.46$).[13] To overcome this problem, we followed the example of Kane and co-workers[13,14] and fed the more reactive monomer (NMS) into the reaction slowly as the polymerization proceeded. We began the addition of NMS as a solution in DMF 30 min after initiation of the polymerization of HPMA (~15% conversion). The reaction was not run under monomer-starved conditions. By NMR, we could show that the amount of free NMS monomer in the reaction built up slowly over the 3.5 h that it was fed into the reaction. An aliquot of the polymer taken after 1 h of reaction time contained 20 mol% NMS and, after 4 h, the polymer contained 30 mol% NMS. Values of $M_n$ determined by end-group analysis by $^1$H NMR and by UV–vis spectroscopy were in good agreement with each other.

## Supplementary data

Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca).

## Acknowledgment

The authors thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for their support of this research.

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