

# Thermoresponsive Magnetic Micelles for Simultaneous Magnetic Hyperthermia and Drug Delivery

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## ABSTRACT

Multifunctional magnetic nanoparticles encapsulated into a thermoresponsive polymer shell allowed the simultaneous cancer therapy due to the thermo-triggered drug release and a heating for hyperthermia can be performed simultaneously under AC magnetic field.

In our study, micelles made of amphiphilic block copolymer of poly(N-isopropylacrylamide-co-acrylamide)-block-poly( $\epsilon$ -caprolactone), P(NIPAAm-co-AAm)-b-PCL, were combined with iron oxide nanoparticles and doxorubicin anti-cancer drug which are self-assembled at the micelles hydrophobic interior. The feasibility of the synthesized magnetic micelles for the simultaneous therapy was demonstrated through drug release test at the hyperthermic temperature.

**Keywords:** thermoresponsive materials, magnetic nanoparticles, hyperthermia, drug delivery

## 1 INTRODUCTION

Magnetic nanoparticles (MNPs) have extensively studied for therapeutic and diagnostic tools in biomedical applications, ranging from targeted drug delivery and magnetic-hyperthermia to gene transfection and enhancement of medical images [1-4]. This current development of the MNPs provides the promise of improved treatment for many forms of cancer. Polymeric micelles can be employed as a stable drug carrier with MNPs for multifunctional MNPs, because micelles provide important advantages such as significantly enhancement of the water solubility and bioavailability of hydrophobic drugs and minimized aggregation and improved stability in aqueous environments due to the hydrophilic shells application. Polymeric micelles of poly(ethylene glycol) (PEG)-phospholipid, PEG-b-poly(caprolactone) (PCL) loaded with MNP (MNP-micelles) have been applied for MRI contrast and magnetic-field guided drug delivery [5,6]. In our study, we focused on development of thermoresponsive MNP-micelles for simultaneous magnetic hyperthermia and drug delivery to achieve combined chemotherapy and hyperthermia synergic anti-cancer effect. Feasibility of simultaneous hyperthermia and drug delivery using a low critical solution temperature (LCST) of poly(N-isopropyl acrylamide) (PNIPAAm) has been proposed with

a drug release data from the only thermoresponsive polymeric micelles without MNPs [7,8]. A loading of MNPs in polymeric micelles makes a difference in the stability and the thermo-sensitivity. It is expected that the presence of MNPs, especially at high concentrations, will appreciably affect phase diagrams for block copolymers [9]. Therefore, understanding MNP-loading in polymeric micelles are necessary to characterize and optimize MNP-micelles formation for the purpose of the combined therapy. In our study, the micelles made of amphiphilic block copolymer of poly(N-isopropylacrylamide-co-acrylamide)-block-poly( $\epsilon$ -caprolactone), P(NIPAAm-co-AAm)-b-PCL, were combined with magnetite and doxorubicin (DOX) anti-cancer drug which are position at the hydrophobic core. The synthesized thermoresponsive MNP-micelles were characterized and evaluated for the combined therapy of simultaneous magnetic hyperthermia and drug delivery.

## 2 MATERIALS AND METHOD

### 2.1 Materials

Fe(acac)<sub>3</sub>, 1,2-hexadecanediol, oleic acid, oleylamine, benzyl ether, N,N'-dimethylformamide (DMF), tetrahydrofuran (THF), chloroform, hexane, N-isopropylacrylamide (NIPAAm), Acrylamide (AAm), 2-mercaptoethanol (ME), 4,4'-azobis(4-cyanopentanoic acid) (ACPA),  $\epsilon$ -caprolactone (CL), Tin (II) 2-ethylhexanoate (Sn(Oct)<sub>2</sub>), pyrene, DOX·HCl (DOX), triethylamine (TEA) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co. (Saint Louis, MO).

### 2.2 Synthesis of MNPs

Magnetite nanoparticles were synthesized by the reported procedures by Sun et al [10]. Briefly, Fe(acac)<sub>3</sub> (2 mmol) was mixed in benzyl ether (20 ml) with 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), and oleylamine (6 mmol) under nitrogen for the 6 nm magnetite nanoparticles. The mixture was heated at 200 °C for 2 hours under nitrogen and then refluxed at 300 °C for 1 hour. After cooling at room temperature, the solution was precipitated with ethanol and resuspended in hexane. We used the synthesized 6 nm MNPs as seeds to synthesize 8 nm MNPs.

### 2.3 Synthesis of P(NIPAAm-co-AAm)-b-PCL

The synthesis of thermo-responsive amphiphilic polymer is derived from a previously reported method for micelle drug carrier [7,11]. Briefly, P(NIPAAm-co-AAm) was prepared by radical polymerization. NIPAAm (3 g), AAam (0.3063 g), ME (60  $\mu$ l) as a chain transfer agent and ACPA (9 mg) as an initiator were mixed and dissolved in 10 ml of DMF. The solution was bubbled with nitrogen for 30 min and then reacted at 70  $^{\circ}$ C for 24 hours under nitrogen. The reacted solution was precipitated out by addition of 20 ml diethyl ether. The precipitated copolymer was purified by repeated precipitations in diethyl ether and then dried in vacuum. The P(NIPAAm-co-AAm)-b-PCL were synthesized with P(NIPAAm-co-AAm) and CL by ring-opening polymerization. P(NIPAAm-co-AAm) (0.73 g), CL (146  $\mu$ l) and Sn(Oct)<sub>2</sub> (1 mg) as a catalyst were dissolved in 20 ml of toluene. The solution was bubbled with nitrogen for 30 min and reacted at 115  $^{\circ}$ C under nitrogen for 24 hours. The reacted solution was precipitated with diethyl ether and purified by repeated precipitations. The precipitated polymer was dried in vacuum.

### 2.4 Formation of magnetic micelles

MNP-micelles were formed by solvent-evaporation method [12]. P(NIPAAm-co-AAm)-b-PCL (5 mg) and MNPs (5 mg) were dissolved in 10 ml chloroform. After complete evaporation of chloroform with a gentle flow of nitrogen, the dried film was dissolved in THF (1 ml) in a glass vial. The solution was added dropwise to Milli-Q water (10 ml) under vigorous ultrasonication (40 kHz, 130 W, Branson 2510, Branson, Danbury, USA) for 5 min. The solution was open to air, allowing slow evaporation of organic solvent and formation of micelles. The final volume was adjusted to 10 ml with Milli-Q water. The micelles solution was filtered through a nylon syringe filter (pore size 0.2  $\mu$ m, Whatman, Clifton, NJ, USA)

### 2.5 Characterization

The crystal structure, size and magnetic properties of the synthesized samples were characterized by X-ray diffractometer (XRD; Philips X'Pert Pro diffractometer), vibrating sample magnetometer (VSM; Model 7400, Lake Shore, Westerville, OH, USA) and transmission electronic microscopy (TEM; CM 30, Philips, Mahwah, NJ, USA) operating at 120 kV with a LaB 6 cathode. The molecular weight of the copolymers was measured by Voyager DE-PRO MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA). The <sup>1</sup>H-NMR spectra were obtained on Bruker DMX-500 spectrometers in CHCl<sub>3</sub> solution to determine the chemical structure of the obtained copolymer. IR spectra of the samples were recorded with a Bruker FTIR spectrophotometer Model Vertex 70, using ATR technique. For determination of critical micelle concentration, pyrene was used as a hydrophobic

fluorescent probe [13]. The morphologies of the self assembled magnetic micelles were investigated with AFM (Nano V, Veeco, Santa Barbara, CA, USA). The LCST of micelles were measured with a UV-Vis spectrometer (Lambda 950, Perkin Elmer, Waltham, MA, USA). The mean particle size distribution of micelles were investigated by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern, Herrenberg, Germany) equipped with a 4 mW HeNe laser. The samples were equilibrated at each temperature of 25  $^{\circ}$ C and 50  $^{\circ}$ C for 10 min.

### 2.6 Drug loading and in vitro drug release

Doxorubicin (DOX) in chloroform containing triethylamine (TEA) (molar ratio 1:1, 1 mg/ml) was prepared for loading of drug in MNP-micelles. DOX was loaded and placed into core region through adding in micelle formation with the MNPs and polymer. The DOX loaded MNP-micelles (DOX-MNP-micelles) solution was divided into two groups. One group were frozen at -80  $^{\circ}$ C. Then the frozen samples were lyophilized using a freeze dry system (FreeZone 1 L, Labconco, Kansas, MO, USA) for 36 h at a temperature of -40  $^{\circ}$ C. The freeze dried micelles were dissolved in DMSO for determining a drug loading efficiency (efficiency (%))=(weight of incorporated drug in micelles/weight of initial fed drug)\*100). The only DOX-MNP-micelles from the other group solution were separated with a magnet for drug release test. The separated micelles were dispersed in PBS and dialyzed in Milli-Q water for 24 hours. The dialyzed DOX-MNP-micelles solution (4 ml) was placed into a membrane bag (Spectra/Por MWCO 3,500, Spectrum, Los Angeles, CA, USA) and then immersed into 40 ml of PBS. The temperature of medium was controlled to 37  $^{\circ}$ C or 45  $^{\circ}$ C in water bath. At specific time intervals, PBS medium was taken and replaced with fresh medium. The concentration of released DOX was determined by UV-Vis spectrometer (Lambda 950, Perkin Elmer, Waltham, MA, USA) at 480 nm.

## 3 RESULTS AND DISCUSSION

The P(NIPAAm-co-AAm) as a thermoresponsive part in MNP-micelles was synthesized by radical polymerization with NIPAAm and AAam monomers. The LCST of P(NIPAAm-co-AAm) was adjusted to hyperthermia temperature (42  $^{\circ}$ C-45  $^{\circ}$ C) by controlling a molar ratio (86:14) of NIPAAm:AAam. The P(NIPAAm-co-AAm)-b-PCL was synthesized using the synthesized P(NIPAAm-co-AAm) by ring-opening polymerization with CL. The <sup>1</sup>H-NMR spectrum of the P(NIPAAm-co-AAm)-b-PCL in CDCl<sub>3</sub> exhibited a signal at 4.02 ppm, which is assigned to the hydrogen of N-CH in N-isopropylacrylamide repeating units, and a triplet at 2.64 ppm, which is assigned to the hydrogen of -CH<sub>2</sub>CH<sub>2</sub>CO- in CL, respectively. The molecular weight of P(NIPAAm-co-AAm) (Mn=11,545) and P(NIPAAm-co-AAm)-b-PCL (Mn=13,828) was determined by MALDI-TOF mass spectroscopy,

respectively. The synthesized polymers also were also characterized by FT-IR (Fig. 1). An Absorbance band at  $1645\text{ cm}^{-1}$  contributed by the amide groups of P(NIPAAm-co-AAm) and the bending frequency of amide N-H appeared at  $1550\text{ cm}^{-1}$ . After copolymerization with PCL, the  $1720\text{ cm}^{-1}$  caused by the stretch vibration of C=O in PCL pronounced the synthesis of the P(NIPAAm-co-AAm)-b-PCL [11].

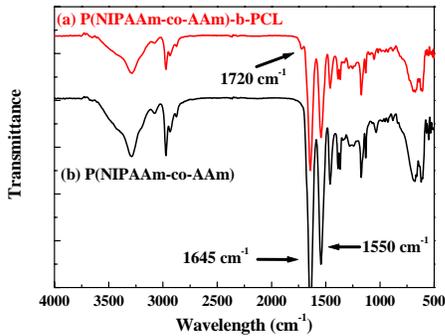


Figure 1 : FT-IR spectra of (a) P(NIPAAm-co-AAm) and (b) P(NIPAAm-co-AAm)-b-PCL polymer.

The aqueous micelle solution was prepared using the characterized copolymers by the solvent-evaporation method. The critical micellar concentration (CMC) of the synthesized P(NIPAAm-co-AAm)-b-PCL was determined to  $17.8\text{ mg/L}$  by measuring fluorescence of pyrene which is placed as a probe at the hydrophobic core during micelle formation. This low CMC could offer a stable formation of core-shell micelle structure even in diluted solution.

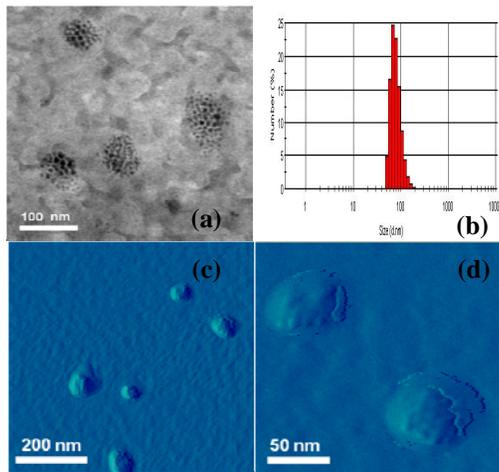


Figure 2 : (a) TEM, (b) size distribution by DLS and (c, d) AFM images of MNP-micelles

When relatively higher concentration of MNPs or MNP-micelles was added in an aqueous solution during micelles formation, the MNP-micelles were aggregated and precipitated. A 1:1 weight ratio of amphiphilic P(NIPAAm-co-AAm)-b-PCL and MNPs with a  $1\text{ mg/ml}$  of MNP-micelle concentration allowed stable dispersion and core-

shell structure of MNP-micelles in Milli-Q water or PBS (Fig 2). TEM and AFM images of the dried MNP-micelles showed a diameter of  $80\text{-}90\text{ nm}$  with self-assembled MNPs at the core region in the MNP-micelles (Fig. 2). The hydrodynamic micelle size by DLS shows the size of MNP-micelles in hydrated state. The size of hydrated MNP-micelles was  $112\pm 10\text{ nm}$  (Fig. 2(b)).

Hyperthermia and drug delivery both benefit from high magnetic moment. However, one of the limitations in MNP-micelles is a small magnetic moment not enough to perform the combined therapy due to small MNP-loading amount [4,9].

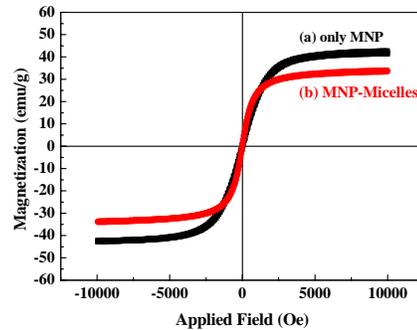


Figure 3 : Magnetization curves of (a) MNPs and (b) MNP-micelles at room temperature.

The magnetization curve of the MNP-micelles was compared with only MNPs (Fig. 3). The decrease rate of the magnetization after MNP-micelle formation was only 20.4 % from the only MNPs. Although the weight ratio of MNPs and polymer was 1:1, the saturation magnetization (emu/g) of the MNP-micelles was higher than half of the only MNPs magnetization by the interaction between the assembled MNPs at the core of MNP-micelles [14].

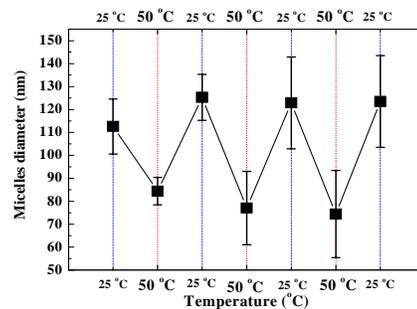


Figure 4 : Thermoresponsive size changes of MNP-micelles at  $25\text{ }^{\circ}\text{C}$  and  $50\text{ }^{\circ}\text{C}$  by DLS.

The LCST of MNP-micelles by outer P(NIPAAm-co-AAm) was found to be approximately  $43\text{ }^{\circ}\text{C}$  in a turbidity test by measuring absorbance at  $500\text{ nm}$  using UV-vis spectrometer. The temperature dependent size change of MNP-micelles was measured at  $25\text{ }^{\circ}\text{C}$  and  $50\text{ }^{\circ}\text{C}$ . The size was reversibly changed from approximately  $121\text{ nm}$  at  $25\text{ }^{\circ}\text{C}$  to  $78.6\text{ nm}$  at  $50\text{ }^{\circ}\text{C}$ , respectively (Fig. 4). Calculated

reversible volume change of MNP-micelle was approximately 74.6 % between 25 °C and 50 °C.

The controlled drug release at the hyperthermia temperature for the combined therapy by the volume changes of the MNP-micelles were measured upon temperature alteration. The drug loading efficiency of MNP-micelles was 65.7 % by measuring the loaded and unloaded DOX amount. The controlled drug release by heat was performed with 2 cycle temperature rising from 37 to 45 °C (Fig. 5). The cumulative DOX drug-release percent was sharply increased to 6.58 % (1<sup>st</sup> cycle) and 11.81% (2<sup>nd</sup> cycle) at the hyperthermia temperature (45 °C) for 30 min and this burst effect was not observed at the returning to 37 °C after 45 °C.

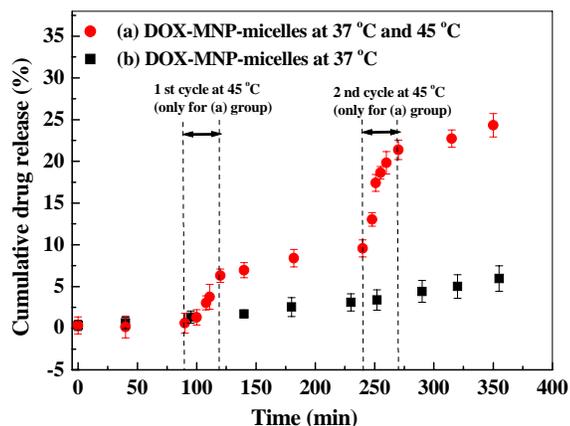


Figure 5 : Cumulative *in vitro* drug release of DOX-MNP-micelles at 37 °C and 45 °C.

## 4 CONCLUSION

P(NIPAAm-co-AAm)-b-PCL block copolymer (LCST= 43 °C (hyperthermia temperature)) was synthesized to encapsulate MNPs and a drug. Thermo-responsive MNP-micelles were successfully fabricated as a core-shell structure by controlling concentration of MNPs and P(NIPAAm-co-AAm)-b-PCL block copolymer. A reversible volume change of MNP-micelle was approximately 74.6 % between 25 °C and 50 °C. The huge volume change of the DOX-MNP-micelles could enhance the rate of DOX drug release at the hyperthermia temperature. Therefore, the synthesized MNP-micelles can give impact to the new development of cancer therapy tools with synergic effect of magnetic hyperthermia and chemotherapy.

## ACKNOWLEDGEMENT

This work as well as the use of the Center for Nanoscale Materials at Argonne National Laboratory and the Electron Microscopy Center was supported by UChicago Argonne, LLC, Operator of Argonne National Laboratory ("Argonne"). Argonne, a U.S. Department of Energy Office

of Science laboratory, is operated under Contract No. DE-AC02-06CH11357. Technical support from the Nanofabrication and NanoBio Interfaces group at the CNM, under User Proposal No. 704, is gratefully acknowledged.

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