



# Thermoresponsive magnetic composite nanomaterials for multimodal cancer therapy

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## ABSTRACT

The synthesis, characterization and property evaluation of drug-loaded polymer-coated magnetic nanoparticles (MNPs) relevant to multimodal cancer therapy has been studied. The hyperthermia and controlled drug release characteristics of these particles was examined. Magnetite ( $\text{Fe}_3\text{O}_4$ )–poly-*n*-(isopropylacrylamide) (PNIPAM) composite MNPs were synthesized in a core-shell morphology by dispersion polymerization of *n*-(isopropylacrylamide) chains in the presence of a magnetite ferrofluid. These core-shell composite particles, with a core diameter of  $\sim 13$  nm, were loaded with the anti-cancer drug doxorubicin (dox), and the resulting composite nanoparticles (CNPs) exhibit thermoresponsive properties. The magnetic properties of the composite particles are close to those of the uncoated magnetic particles. In an alternating magnetic field (AMF), composite particles loaded with 4.15 wt.% dox exhibit excellent heating properties as well as simultaneous drug release. Drug release testing confirmed that release was much higher above the lower critical solution temperature (LCST) of the CNP, with a release of up to 78.1% of bound dox in 29 h. Controlled drug release testing of the particles reveals that the thermoresponsive property can act as an on/off switch by blocking drug release below the LCST. Our work suggests that these dox-loaded polymer-coated MNPs show excellent *in vitro* hyperthermia and drug release behavior, with the ability to release drugs in the presence of AMF, and the potential to act as agents for combined targeting, hyperthermia and controlled drug release treatment of cancer.

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## 1. Introduction

There has been enormous interest in the exciting area of magnetic nanoparticle (MNP)-based systems for cancer treatment [1–26] as conventional cancer therapies such as radiotherapy, hyperthermia and chemotherapy have serious drawbacks. The limitations of chemotherapy techniques include adverse effects on healthy tissue due to indiscriminate distribution of cytotoxic drugs in the body, insufficient local drug concentration in the tumor and poor control over drug release. In hyperthermia therapy, the tumor is heated in the temperature range of 41–47 °C, causing death of the cancerous cells, but sparing healthy cells [20,27,28]. In conventional hyperthermia techniques—e.g. radiofrequency-based hyperthermia, isolated hepatic perfusion, water baths and heating rods—temperature control is poor, heat distribution is not optimum and there is risk of organ damage due to overheating [20]. Drug-loaded composite MNPs offer a method of simultaneously achieving drug targeting, controlled drug release and hyperthermia treatment for cancer, thus overcoming several limitations of conventional cancer therapy.

The synergistic therapeutic effects of simultaneous chemotherapy and hyperthermia can exceed the individual or sequential application of these techniques [28–31]. Therefore, drug-loaded composite MNPs with a magnetic core and a polymer shell capable of acting as multifunctional agents for combined drug targeting, controlled release and hyperthermia therapy are highly desirable. Such particles can be injected into the appropriate blood vessels and targeted to the tumor by means of a suitable external magnetic field gradient [1,9,24,32,33]. Chemotherapy occurs by drug release from the composite particles which have been targeted to the tumor region. An external alternating magnetic field (AMF) can be applied to generate heat in the targeted particles [13,14,20,34], and the resulting temperature rise can be used for hyperthermia treatment of cancer. If the polymer shell of the nanoparticle is responsive to stimuli such as pH and temperature, drug release can also be controlled.

This paper reports a study of novel iron oxide–poly-*n*-isopropylacrylamide (PNIPAM) composite nanoparticles (CNPs) suitable for magnetic targeting followed by simultaneous magnetic hyperthermia and chemotherapeutic drug release. The core of the CNP consists of iron oxide; PNIPAM is chosen as the thermosensitive polymer shell. PNIPAM is an inverse thermosensitive polymer that has been studied for several biomedical applications [35–41]; it undergoes reversible volume change in water at a lower critical solution temperature (LCST) in the range 30–35 °C. The proximity

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of the LCST to the human body temperature (37 °C) and the ability to tune the LCST by addition of appropriate copolymers makes PNIPAM hydrogels useful for controlled drug delivery. Below the LCST, PNIPAM chains are soluble in water and the polymer is in a swollen state, inhibiting the transport of drug molecules through the matrix, resulting in a slow rate of release. Heat is generated in the iron oxide core upon exposure to an AMF, and this heat is conducted to the polymer shell, increasing the temperature of the PNIPAM. At and above the LCST, the shell collapses, squeezing out drug molecules, resulting in rapid drug release (Fig. 1).

Both Fe<sub>3</sub>O<sub>4</sub> (magnetite) and  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> (maghemite) are known to be biocompatible and non-toxic in concentrations relevant to clinical applications. In vivo experiments on animal models have shown that iron oxides are suitable for drug delivery and hyperthermia [1,17,20,22,42,43]. Human clinical trials for drug delivery [33] and hyperthermia [14,15,25] have been conducted with iron oxide-based ferrofluids, the injected dosage being well tolerated by patients. Superparamagnetic iron oxides (FDA approved) are also used as contrast-enhancing agents for clinical magnetic resonance imaging (MRI) [44–46]. In vitro drug release during AMF-induced heating has been previously studied using drug-loaded thermosensitive carriers [4,6,47] and dye-loaded PNIPAM–magnet systems [3,48]. Drug release from dox-loaded magnetic particles encapsulated by a thermosensitive polymer without an applied magnetic field has recently been studied [26].

The novelty of this work is in the synthesis, characterization and property evaluation of thermosensitive polymer-encapsulated magnetic particles relevant to combined magnetic targeting, hyperthermia and drug release applications. Previously, we have shown that drug-loaded CNPs can be targeted to hepatocellular carcinoma (HCC) in a buffalo rat model, and localization in the tumor was confirmed by MRI and histology [9].

## 2. Materials and methods

### 2.1. Synthesis of MNPs

Fe<sub>3</sub>O<sub>4</sub> MNPs were synthesized by a reverse co-precipitation method adapted from Aono et al. [49] to obtain particles with an

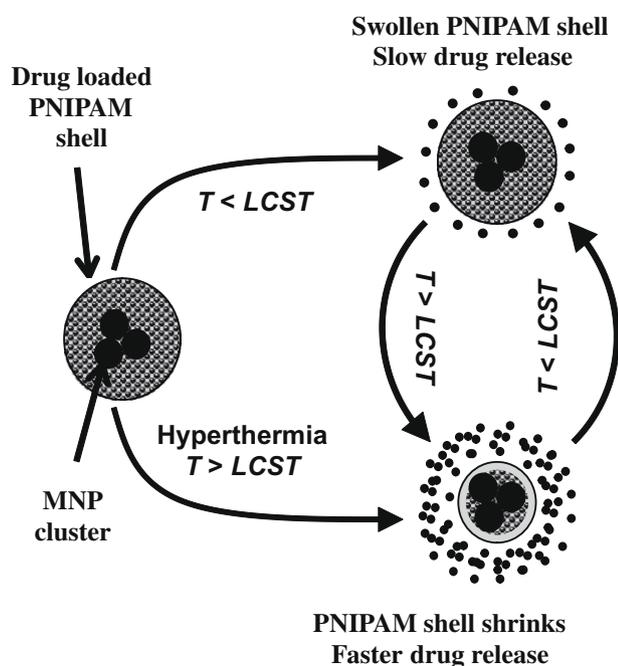


Fig. 1. Schematic diagram showing drug release from PNIPAM-coated MNPs below and above the polymer LCST.

average size of 10–14 nm. In a typical experiment, FeCl<sub>3</sub>·6H<sub>2</sub>O (0.1 M, 27 g) (Merck) and FeCl<sub>2</sub>·4H<sub>2</sub>O (0.05 M, 10 g) (Merck) were dissolved in 200 ml of ultrapure MilliQ water and heated to 80 °C. The synthesis was performed inside a glovebox in an inert atmosphere, and nitrogen was bubbled through all solutions for 30 min before use. Under vigorous stirring, 140 ml of NaOH solution (0.14 M, 5.6 g in 140 ml) (Merck) was dripped into this solution at a rate of 6 ml min<sup>-1</sup>, upon which the solution turned black, indicating the precipitation of Fe<sub>3</sub>O<sub>4</sub> (magnetite). The mixture was maintained at 80 °C for 30 min, cooled to room temperature and centrifuged at 8000 rpm for 15 min. The supernatant was discarded and the particles were extracted. After washing several times with MilliQ water and ethanol to remove residual reactants, the particles were dried in vacuum for 24 h.

### 2.2. Size selection by magnetic separation

The dried MNPs were ultrasonically redispersed in 500 ml of ethanol until a dark suspension was obtained. A 0.8 T block permanent magnet was placed on the outer wall of the beaker to attract the most magnetically responsive particles in the suspension. The supernatant was extracted with a peristaltic pump (Simon, Manostat). The responsive particles were redispersed in ethanol and the process was repeated three times. The magnetically separated particles were dried in vacuum and stored in a vacuum oven at room temperature.

### 2.3. Preparation of iron oxide–polymer CNPs

CNPs were prepared, in the presence of MNPs, by free radical dispersion polymerization of NIPAM monomer in water with *N,N*-methylene(bis)acrylamide (BAAm) as the cross-linker, *N,N,N',N'*-tetramethylene diamine (TEMED) as the accelerator and ammonium persulfate (APS) as the initiator [3,50]. Our procedure does not involve surfactants which can alter the LCST [40,51]. MilliQ water was used, through which nitrogen was bubbled for 15 min prior to use. The NIPAM monomer was purified by recrystallization from hexane and dried in vacuum for 24 h before use. In a typical experiment 35 ml of ferrofluid containing 850 mg MNP was added to a solution of 960 mg NIPAM (Aldrich, 97%) in 30 ml water and ultrasonicated for 10 min. The mixture was transferred to a beaker and stirred at 24,000 rpm by an IKA-Werke T25 Ultra Turrax high-speed dispersion unit while 2.5 ml of BAAm (Fluka, 98%) solution (8 mg in 5 ml) and 0.5 ml of APS (J.T. Baker, 98.5%) solution (180 mg in 5 ml) were added to the mixture. Finally 1 ml of TEMED (Lancaster, 99%) was added dropwise with continuous stirring. After 20 min of stirring, the mixture was left undisturbed for 2 h, after which the CNPs were magnetically separated and washed several times with MilliQ water. Some of the CNPs were dried in vacuum for 24 h, resulting in dehydration of the particles: the remainder were used as-synthesized in the swollen state for dynamic light scattering (DLS) and microcalorimetry experiments.

### 2.4. Drug loading

The water-soluble anti-cancer drug doxorubicin (dox) was chosen as a model drug. Typically, 26.5 mg (loading target ~10 wt.% of CNP) of doxorubicin hydrochloride was dissolved in 20 ml of MilliQ by ultrasonication for 10 min. Two hundred and forty-five milligrams of dehydrated CNPs was added to the dox solution and ultrasonicated for an additional 10 min. The bottle was sealed and gently shaken in a rotary shaker in the dark for 20 h at 24 °C to facilitate both reswelling of the PNIPAM shell in the aqueous dox solution as well as to facilitate dox uptake. After loading, the dox-loaded CNPs (Dox-CNPs) were magnetically separated. To determine residual dox content, the supernatant was analyzed in

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