Video Article

Magnetic and Thermal-sensitive Poly(*N*-isopropylacrylamide)-based Microgels for Magnetically Triggered Controlled Release

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Abstract

Magnetically and thermally sensitive poly(*N*-isopropylacrylamide) (PNIPAAm)/Fe₃O₄-NH₂ microgels with the encapsulated anti-cancer drug curcumin (Cur) were designed and fabricated for magnetically triggered release. PNIPAAm-based magnetic microgels with a spherical structure were produced via a temperature-induced emulsion followed with physical-crosslinking by mixing PNIPAAm, polyethylenimine (PEI), and Fe₃O₄-NH₂ magnetic nanoparticles. Because of their dispersity, the Fe₃O₄-NH₂ nanoparticles were embedded inside the polymer matrix. The amine groups exposed on the Fe₃O₄-NH₂ and PEI surface supported the spherical structure by physically crosslinking with the amide groups of the PNIPAAm. The hydrophobic anti-cancer drug curcumin can be dispersed in water after encapsulation into the microgels. The microgels were characterized by transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), and UV-Vis spectral analysis. Furthermore, magnetically triggered release was studied under an external high frequency magnetic field (HFMF). A significant "burst release" of curcumin was observed after applying the HFMF to the microgels due to the magnetic inductive heating (hyperthermia) effect. This manuscript describes the magnetically triggered controlled release of Cur-PNIPAAm/Fe₃O₄-NH₂ encapsulated curcumin, which can be potentially applied for tumor therapy.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55648/>

Introduction

Hydrogels are three-dimensionally (3D) polymeric networks which cannot dissolve but can swell in aqueous solutions¹. The polymeric networks have hydrophilic domains (which can be hydrated to provide the hydrogel structure), and a cross-linked conformation (which can prevent the collapse of the network). Various methods have been investigated for preparation of hydrogels, such as emulsion polymerization, anionic copolymerization, crosslinking of neighboring polymer chains, and inverse micro-emulsion polymerization². Physical and chemical crosslinking are introduced through these methods to obtain structurally stable hydrogels^{1,3}. Chemical crosslinking normally requires the participation of the crosslinking agent, which connects the backbone or the side-chain of the polymers. Compared to chemical crosslinking, physical crosslinking is a better choice to fabricate hydrogels due to the avoidance of a crosslinking agent, since these agents are often toxic for practical applications⁴. Several approaches have been investigated for synthesizing physically cross-linked hydrogels, like crosslinking with ionic interaction, crystallization, bonding between amphiphilic blocks or grafting on the polymer chains, and hydrogen bonding^{4,5,6,7}.

Stimuli-sensitive polymers, which can undergo conformational, chemical or physical property changes in response to different environmental conditions (*i.e.*, temperature, pH, light, ionic strength, and magnetic field), have recently attracted attention as a potential platform for controlled release systems, drug delivery, and anti-cancer therapy^{8,9,10,11,12}. Researchers are focusing on thermo-sensitive polymers where intrinsic temperature can be easily controlled. PNIPAAm is a thermally sensitive polymer, which contains both hydrophilic amide groups and hydrophobic isopropyl groups, and has a lower critical solution temperature (LCST)¹³. Hydrogen bonding between amide groups and water molecules provides the dispersity of PNIPAAm in aqueous solution at low temperatures (below the LCST), while the hydrogen-bonding between polymer chains occurs at high temperatures (above the LCST) and excludes water molecules so that the polymer network collapses. Regarding this unique property, many reports have been published for preparing temperature-triggered, self-assembled hydrogels by adjusting the hydrophobic and hydrophilic ratio of the polymer chain length, such as copolymerization, grafting, or side-chain modification for pharmaceutical platforms^{14,15,16,17}.

Magnetic materials such as iron, cobalt, and nickel have also received increased attention during the past decades for biochemical applications¹⁸. Among those candidates, iron oxide is the most widely used because of its stability and low toxicity. Nano-sized iron oxides respond instantly to the magnetic field and behave as superparamagnetic atoms. However, such small particles easily aggregate; this reduces the surface energy, and therefore they lose their dispersity. In order to improve the water-dispersity, grafting or coating to protect the layer are commonly applied not only to separate each individual particle for stability but also to further functionalize the reaction site¹⁹.

Here, we fabricated magnetic PNIPAAm-based microgels to serve as drug carriers for controlled release systems. The synthesis process is described and shown in **Figure 1**. Instead of complicated copolymerization and chemical crosslinking, the novel temperature-induced emulsion of PNIPAAm followed by physical crosslinking was employed for obtaining the microgels without additional surfactant or crosslinking agents. This simplified the synthesis and prevented undesired toxicity. Within such a simple preparation protocol, the as-synthesized microgels offered water-dispersity for both the magnetic iron oxide nanoparticles and the hydrophobic, anti-cancer drug, curcumin. FT-IR, TEM, and imaging provided evidence of dispersion and encapsulation. Due to the embedded $\text{Fe}_3\text{O}_4\text{-NH}_2$, the magnetic microgels showed potential for serving as micro-devices for controlled release under HFMF.

Protocol

1. Synthesis of Surface-modified, Water-dispersible, Magnetic Nanoparticles, Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{-NH}_2$

1. Add 14.02 g of FeCl_3 , 8.6 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 250 mL water to a 500-mL beaker.
2. Connect the rotor and controller to set up mechanic stirring. Mix the solution at 300 rpm for 30 min at room temperature (RT).
3. Add 25 mL of ammonium hydroxide (33%) into the solution at RT and keep stirring (300 rpm) for 30 min. Keep the beaker open.
CAUTION: Ammonium hydroxide may cause nose irritation if inhaled. This step must be performed inside an appropriate fume hood.
4. To collect the magnetic iron oxides (Fe_3O_4), remove the mechanic stirring. Put a magnet under the beaker to collect the black particles.
 1. After the Fe_3O_4 nanoparticles are entirely precipitated, carefully remove the supernatant. Do not shake the beaker while pouring the supernatant to avoid loss of Fe_3O_4 .
 2. Remove the magnet and add 50 mL of fresh water to the beaker.
 3. Shake the beaker to re-disperse the Fe_3O_4 . Repeat steps 1.4 to 1.4.2 three times to purify the Fe_3O_4 .
5. After the last wash, transfer all the Fe_3O_4 (10 g) into a 100-mL glass bottle. Add water until the total solution volume is 100 mL. Shake the glass bottle vigorously until no lumps are visible.
NOTE: The protocol can be paused here. The Fe_3O_4 nanoparticles are prepared.
6. Modify the Fe_3O_4 with aminosilane ($\text{Fe}_3\text{O}_4\text{-NH}_2$).
 1. Take the 100-mL solution from step 1.5 and transfer into a 1,000-mL beaker. Add 10 mL of ammonia solution, 90 mL of water, and 900 mL of ethanol to the beaker.
 2. Use a magnetic stir bar to mix the solution at 300 rpm. Add 500 μL of (3-aminopropyl)triethoxysilane (APTES) dropwise to the beaker at RT and stir for another 12 h.
7. Purify and collect the $\text{Fe}_3\text{O}_4\text{-NH}_2$ as described in section 1.4.
8. Re-disperse 1 g of $\text{Fe}_3\text{O}_4\text{-NH}_2$ (from step 1.7) in a 20-mL glass bottle with 20 mL water.
NOTE: The protocol can be paused here. The $\text{Fe}_3\text{O}_4\text{-NH}_2$ nanoparticles are prepared.

2. Synthesis of Organic-inorganic Hybrid Microgels by Thermo-induced Emulsion

1. Preparation of **Solution 1-1 and 1-2**.
 1. For solution 1-1, add 0.25 g of PNIPAAm, 5 mL of Fe_3O_4 solution (from step 1.5), and 0.2 g of PEI to a 50-mL glass bottle. Add 20 mL of water and use a magnetic stir bar to stir at 300 rpm for 30 min.
 2. For solution 1-2, repeat step 2.1.1, but replace Fe_3O_4 as $\text{Fe}_3\text{O}_4\text{-NH}_2$ solution (from step 1.8).
2. To prepare **Solution 2**, add 0.8 g of PEI and 18.2 mL of water to a 50-mL glass bottle. Use a water bath to heat up the solution to 70 °C for 30 min. Prepare a second bottle of **Solution 2**.
3. Preparation of **PNIPAAm/ Fe_3O_4** .
 1. Use an ultrasonic cell disruptor to sonicate (50 w), a magnetic stir bar to stir (300 rpm), and a water bath to heat **Solution 2** (70 °C).
 2. Add **Solution 1-1** to the heated **Solution 2** dropwise using a 3-mL syringe at a rate of 1 mL/min.
 3. Continue sonication, stirring and heating at 70 °C for 30 min.
 4. Cool the solution to RT. Remove the solution from the cell disruptor and water bath.
 5. Collect the microgels by placing the magnet close to the glass bottle.
 6. Remove the supernatant after the microgels have precipitated to the bottom of the glass bottle.
 7. Add another 25 mL of water to the glass bottle and re-disperse the microgels by vortexing. This solution is PNIPAAm/ Fe_3O_4 .
NOTE: The protocol can be paused here.
4. Preparation of **PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$** .
 1. Use an ultrasonic cell disruptor to sonicate (50 w), a magnetic stir bar to stir (300 rpm), and a water bath to heat **Solution 2** (70 °C).
 2. Add **Solution 1-2** to the heated **Solution 2** dropwise using a 3-mL syringe at a rate of 1 mL/min.
 3. Continue sonication, stirring and heating at 70 °C for 30 min.
 4. Cool the solution to RT. Remove the solution from the cell disruptor and water bath.
 5. Collect the microgels by placing the magnet close to the glass bottle.
 6. Once the microgels precipitate, remove the supernatant.

7. Add another 25 mL of water to the glass bottle and re-disperse the microgels by vortexing. This solution is PNIPAAm/Fe₃O₄-NH₂.
NOTE: The protocol can be paused here.

3. Preparation of Curcumin-loaded Microgels (Cur-PNIPAAm/Fe₃O₄-NH₂)

NOTE: These steps must be performed in the dark.

1. Add 100 mg of Cur and 20 mL of ethanol to a 20-mL of glass bottle.
2. Take 2 mL of the Cur solution and transfer to the PNIPAAm/Fe₃O₄-NH₂ solution (step 2.4.7). Stir at 400 rpm and RT overnight.
3. After stirring at 400 rpm and RT overnight, use the magnet to collect PNIPAAm/Fe₃O₄-NH₂ as described in steps 2.4.5 and 2.4.6.
4. Add another 25 mL of water to the glass bottle and re-disperse the microgels by vortexing. This solution is Cur-PNIPAAm/Fe₃O₄-NH₂.

4. Magnetically Triggered Drug Release

1. Transfer 10 mL of the Cur-PNIPAAm/Fe₃O₄-NH₂ solution and add 2 mL of water to a 15-mL centrifugation tube.
2. Place the centrifugation tube in the center of the coil for applying the HFMF²⁰. Apply HFMF at 15 KHz for 20 min.
3. Withdraw 0.5 mL of the HFMF solution and replace with fresh 0.5 mL of water at every 2 min interval while applying the HFMF.
4. Transfer the withdrawn solution to the 1-mL cuvette.
5. Measure the absorption of the withdrawn solution by UV/Vis at 482 nm²¹.
6. Determine the concentration of the released drugs by using the relationship of absorption and concentration from a standard calibration curve²².

NOTE: The standard calibration relation is:

$$\text{Concentration} = 17.23 \times \text{Absorption} - 0.05$$

where the correlation coefficient is 0.9993.

5. Characterization of the Magnetic Microgels

1. Thermogravimetric analyzer (TGA)²³.
 1. Measure the weight loss of PNIPAAm/Fe₃O₄ and PNIPAAm/Fe₃O₄-NH₂ vs. temperature under air atmosphere by TGA.
 1. Heat the sample from RT to 100 °C and keep at this temperature for 10 min to eliminate humidity. Heat the sample from 100 °C to 800 °C at a rate of 10 °C/min. Weigh the samples.
 2. Plot the weight loss vs. temperature of both PNIPAAm/Fe₃O₄ and PNIPAAm/Fe₃O₄-NH₂.
NOTE: The residue weight is either Fe₃O₄ or Fe₃O₄-NH₂, while the lost weight is PNIPAAm.
2. FT-IR²⁴.
 1. Dry 10 mg of sample with 1 g of KBr at 100 °C overnight.
 2. Press the mixture from step 5.2.1 into pellets as described in the following steps (5.2.2.1 - 5.2.2.5):
 1. Grind the materials from step 5.2.1 into a fine powder by using a mortar and pestle.
 2. Place the assembled apparatus (mortar and pestle) into the pellet press. Align the apparatus in the exact middle of the press.
 3. Pump the press until a pressure of 20,000 psi is reached. Let the pellet sit at that pressure for 5 min.
CAUTION: Align the apparatus in the exact middle of the press otherwise sample will disperse out of the mortar and cause injury from exposure.
 4. Remove the die containing the pellet and the piston from the press.
 5. Turn it upside down and pump the piston to force the pellet out.
 3. Record the FT-IR absorption spectra of samples by FT-IR at frequencies ranging from 400 to 4,000 cm⁻¹ with 4 cm⁻¹ resolution²⁴.
3. Morphology observations by TEM²⁵.
 1. Drop the sample solution onto a copper-grid coated with a collodion and then dry at RT or in a 70 °C oven overnight.
 2. Take TEM images.
NOTE: Strong electron beams can damage the samples. Therefore, TEM images should be taken as quickly as possible.
4. Aqueous-dispersion abilities of polymers and microgels.
 1. To prepare PNIPAAm solution, add 7 mg of PNIPAAm and 7 mL of water to a 7-mL glass bottle. Use a vortex to mix the solution until there are no aggregates.
 2. To prepare PNIPAAm/Fe₃O₄-NH₂ solution, transfer 0.7 mL of PNIPAAm/Fe₃O₄-NH₂ solution (step 2.4.7) to a 7-mL glass bottle and add 6.3 mL of water. Use a vortex to mix the solution until there is no precipitation.
 3. To prepare Cur-PNIPAAm/Fe₃O₄-NH₂ solution, transfer 0.7 mL of Cur-PNIPAAm/Fe₃O₄-NH₂ solution (step 3.4) to a 7-mL glass bottle and add 6.3 mL of water. Use a vortex to mix the solution until there is no precipitation.
 4. Take a picture of the solutions (steps 5.4.1 - 5.4.3) using a digital camera.
 5. Place the solutions into an oven and set the temperature to 70 °C. Wait 2 h until equilibrium.
 6. Take another picture of the solutions. To maintain the temperature, take the picture within 1 min. Avoid shaking the glass bottle as this can re-disperse the precipitations.
5. For magnetic collection of microgels, place the strong magnet close to the Cur-PNIPAAm/Fe₃O₄-NH₂ solution (step 5.4.3). Wait until the microgels are fully collected, then take a picture.

1. Remove the magnet and vortex the microgel solution until fully dispersed. Take another picture.

Representative Results

The schematic for synthesis of PNIPAAm/PEI/Fe₃O₄-NH₂ microgels is shown in **Figure 1**. TGA was applied to estimate the relative composition of the organic compound against the whole microgel. Since only the organic compound PNIPAAm could be burned, the relative composition of PNIPAAm and Fe₃O₄ (or Fe₃O₄-NH₂) was determined and is shown in **Table 1**. Why do PNIPAAm/Fe₃O₄-NH₂ microgels display the better dispersity but hold lower contents of iron oxides? Owing to the stronger interaction and better dispersion in PNIPAAm/Fe₃O₄-NH₂ than that in PNIPAAm/Fe₃O₄, Fe₃O₄-NH₂ is easier to cross-link PNIPAAm than Fe₃O₄. As a result, the yields of PNIPAAm/Fe₃O₄-NH₂ microgels are much higher than those of PNIPAAm/Fe₃O₄. Due to the collection processes (steps 2.3.3 - 2.3.5 and 2.4.3 - 2.4.5), un-crosslinked PNIPAAm was removed with the supernatant since only the magnetic iron oxide with microgels can be magnetically absorbed. As a consequence, the weight percentages of PNIPAAm in the microgels are 32.37% (PNIPAAm/Fe₃O₄) and 68.56% (PNIPAAm/Fe₃O₄-NH₂). The Fe₃O₄-NH₂ nanoparticles can physically crosslink much more PNIPAAm compared to Fe₃O₄ nanoparticles.

The TEM images of PNIPAAm solutions and magnetic microgels were taken by digital camera at room temperature. As shown in **Figure 2a**, there are no specific structures in a pure PNIPAAm solution at room temperature. However, regular spherical iron oxide particles (**Figure 2b**) were observed in both PNIPAAm/Fe₃O₄ (**Figure 2c**) and PNIPAAm/Fe₃O₄-NH₂ (**Figure 2d**) microgels, which provided evidence of physical crosslinking resulting from the hydrogen bonding between PNIPAAm and PEI. Most of the Fe₃O₄ nanoparticles can only be adsorbed on the surface of the PNIPAAm-based matrix and produced aggregation clusters (**Figure 2c**). However, APTES-modified iron oxide nanoparticles, Fe₃O₄-NH₂ can be embedded into the particles, due to the greater water dispersity and smaller size of the magnetic nanoparticles (**Figure 3d**), compared to bare Fe₃O₄ nanoparticles. After loaded with curcumin, the morphology of Cur-PNIPAAm/Fe₃O₄-NH₂ (**Figure 2e**) was much more isolated than that of magnetic microgels owing to the hydrophobic characteristic of Cur. The results also suggest that Cur was not only encapsulated inside but also absorbed on the surface of the microgels.

Microgel preparation and therapeutic-molecule encapsulation were identified by FT-IR analysis as shown in **Figure 3**. Compared to Fe₃O₄ from the literature^{19,26}, the newly-appeared absorption peaks at 2927, 1203, 987, and 472 cm⁻¹ were attributed to the vibration of C-H stretching, Si-O-Si stretching, Si-O stretching, and Si-O bending, respectively, which suggested the successful modification of APTES to cover the surface of the Fe₃O₄ nanoparticles. Fe-O vibration peaks (584 cm⁻¹) also were observed in both PNIPAAm/Fe₃O₄ and PNIPAAm/Fe₃O₄-NH₂. However, the relative intensity of Fe-O vibration was higher in PNIPAAm/Fe₃O₄ than that in PNIPAAm/Fe₃O₄-NH₂, which also supported our description of the composition, that a better water-dispersity led to a better structural distribution. After the loading process, characteristic absorption peaks of curcumin at 1509 and 3511 cm⁻¹ referring to aromatic C=C bending and O-H stretching, respectively, appeared in FT-IR spectra of Cur-PNIPAAm/Fe₃O₄-NH₂, which indicated the successful encapsulation of curcumin.

The photos of various microgels at 25 °C or 70 °C are shown in **Figure 4**, in which the milky and brown solutions represent the aggregation of PNIPAAm and iron oxides, respectively. Compared to **Figures 4a-c**, there were no obviously visible aggregations in PNIPAAm, PNIPAAm/Fe₃O₄-NH₂, and Cur-PNIPAAm/Fe₃O₄-NH₂ solutions at room temperature (25 °C). PNIPAAm solution and magnetic microgels then became opaque when the solutions were heated higher than the LCST of PNIPAAm as shown in **Figure 4d-f**. Both magnetic microgels were milky but dispersed without any precipitation, and this indicated the great dispersity and strong, physical bonding between PNIPAAm, Fe₃O₄-NH₂, and curcumin. As shown in **Figure 5**, the magnetic microgels could be easily collected with the magnet and re-dispersed into the aqueous solution without any aggregation after removing the magnet. The results indicated that these magnetic microgels could potentially be applied to an aqueous delivery system like the human body for clinical applications.

In vitro release behaviors of magnetic microgels were monitored via HFMF. The experimental apparatus set up is shown in **Figure 6**, where the centrifugation tube should be in the center of the coil bearing the magnetic field. The brown precipitation located in the tube center was the magnetic microgels, which were separated from the solutions under HFMF treatments.

Magnetic release percentage with and without HFMF was monitored and is shown in **Figure 7**. Compared to the percentage of release without HFMF within identical periods (20 min), the release percentage increased 2.5 times under HFMF treatment, and the temperature of bulk solution could be raised to over 50 °C simultaneously. Owing to the containment of thermo-sensitive polymers, PNIPAAm, magnetic microgels could squeeze out the encapsulated drug (Cur), resulting from the PNIPAAm polymer matrix becoming hydrophobic and then conjugated under high temperature (50 °C). Meanwhile, curcumin could be released to accomplish the anti-cancer therapy by applying the HFMF. Local heating from magnetic induction upon HFMF can destroy the binding between Cur and PNIPAAm, even though the hydrophobic Cur is expected to bind to the hydrophobic PNIPAAm at high temperatures. Furthermore, the volume change (from hydrophilic to hydrophobic and lower to higher temperature) of the magnetic microgels would also squeeze out the Cur.

The temperature increase of the bulk solution was recorded and shown in **Figure 7** as the red curve with the diamond symbol. As shown, the temperature first increased with heating time and plateaued after 14 min. The plateau should be the saturation of the magnetic inductive heating (hyperthermia) in the bulk water. However, the localized temperature should be high enough to squeeze out the Cur.

Figure 1. Schematic Synthesis Process for PNIPAAm/PEI/Fe₃O₄-NH₂ Microgels.

Mix the PNIPAAm, $\text{Fe}_3\text{O}_4\text{-NH}_2$, and PEI together and heat up the mixture to 70 °C so as to introduce H-bonding for the microgel preparation. [Please click here to view a larger version of this figure.](#)

Figure 2. TEM images of PNIPAAm Solutions and Magnetic Microgels. a) PNIPAAm, b) Fe_3O_4 , c) PNIPAAm/ Fe_3O_4 , d) PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$, and e) Cur-PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$. The TEM images were taken to monitor the dispersity and morphology of the samples. The TEM samples were prepared at RT. [Please click here to view a larger version of this figure.](#)

Figure 3. FT-IR Spectra of PNIPAAm, $\text{Fe}_3\text{O}_4\text{-NH}_2$, PNIPAAm/ Fe_3O_4 , PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$, and Cur-PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$. The as-synthesized microgels were blended with KBr and pressed into pellets. FRIR was then applied to clarify the interactions of PNIPAAm, $\text{Fe}_3\text{O}_4\text{-NH}_2$, PEI, and curcumin by monitoring the absorption changes of function groups. [Please click here to view a larger version of this figure.](#)

Figure 4. Aqueous-dispersion Abilities of Microgels Under and Above the LCST: a) PNIPAAm, b) PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$, and c) Cur-PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$ at 25 °C. d) PNIPAAm, e) PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$, and f) Cur-PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$ at 70 °C. The sample solutions were prepared at RT and heated up to 70 °C. The photographs were taken under RT and 70 °C in order to observe the water-dispersivity of synthesized microgels. [Please click here to view a larger version of this figure.](#)

Figure 5. Collection of Curcumin-loaded Magnetic Microgels by a Magnet. Cur-PNIPAAm/ $\text{Fe}_3\text{O}_4\text{-NH}_2$ were dispersed in aqueous solution (left) and collected by a magnet (right). [Please click here to view a larger version of this figure.](#)

Figure 6. Experimental Apparatus for Magnetic-triggered Release with HFMF. The white ring is the copper coil. The centrifugation tube containing the magnetic microgels is shown. [Please click here to view a larger version of this figure.](#)

Figure 7. Controlled Release of Cur-PNIPAAm/Fe₃O₄-NH₂ Microgels at pH 7.4 with (Square Symbol) and without (Circle Symbol) HFMF. The curcumin release percentage of magnetic microgels with (black; squares) and without (black; circles) applying the HFMF is shown. The increase in temperature of Cur-PNIPAAm/Fe₃O₄-NH₂ microgels with HFMF is displayed in red (diamond). Error bars represent SD. [Please click here to view a larger version of this figure.](#)

Samples	PNIPAAm (%)	Fe ₃ O ₄ (%)
PNIPAAm/Fe ₃ O ₄	32.37	68.63 (Fe ₃ O ₄)
PNIPAAm/Fe ₃ O ₄ -NH ₂	68.56	31.44 (Fe ₃ O ₄ -NH ₂)

Table 1. Relative Composition (% weight) of the Magnetic Nanoparticles and PNIPAAm in the Microgels. The relative composition of the magnetic microgels was calculated using TGA analysis.

Discussion

The most important steps of the preparation are in protocol section 2, for the synthesis of the magnetic microgels by thermo-induced emulsion. As shown in **Figure 2** (TEM images), the spherical structure of microgels could be maintained at RT (lower than the LCST) due to the physical crosslinking resulting from the strong H-bonding between PNIPAAm (amide groups), PEI (amine groups) and Fe₃O₄-NH₂ (amine groups). Based on the comparison in **Figure 4**, the magnetic microgels are well-dispersed at low (25 °C) or high (70 °C) temperature. The microgels can also be collected by a magnet and re-dispersed into homogenous solution as shown in **Figure 5**.

Traditional preparation of hydrogels synthesized with both hydrophobic and hydrophilic monomers normally requires the introduction of crosslinking agents to obtain 3D networks^{4,5,6,7}. However, crosslinking agents are difficult to remove and often cause side-effects in their application.

PNIPAAm can aggregate or self-assemble into particles under high temperature and also re-disperse into homogenous solution when the temperature is lower than its LCST. Crosslinking and chemical modification are often employed for hydrogel preparation to prevent the collapse of 3D networks. Thermo-induced crosslinking via hydrogen bonding is applied here to replace chemical reactions, thus simplifying the synthesis and preparation process.

Critical to the success of hydrogel fabrication are the polymerization- and crosslinking- free process and the encapsulation of hydrophobic drugs. Without polymerization, the hydrogel could remove the unreacted initiators and monomers that often lead to strong toxicity. Here, we successfully accomplished the dispersion and encapsulation of inorganic compounds (iron oxide) and hydrophobic molecules (curcumin) via surface-modification and solvent-introduction.

Through *in vitro* release tests (**Figure 7**), we found that the magnetic microgels had an efficient increase of both temperature and release percentage in the external magnetic field (HFMF) by the magnetic inductive heating (hyperthermia) effect. With the aforementioned properties, these PNIPAAm-based magnetic microgels are potential candidates for magnetically and thermally triggered, targeted delivery of tumor therapy.

Disclosures

The authors have nothing to disclosure.

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