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Preparation of dual pH- and temperature-sensitive nanogels for curcumin delivery †

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Abstract: Curcumin, an active ingredient in turmeric, has various biological activities, but its low solubility and limited bioavailability hinder its therapeutic use. To address this, we created dual pH- and thermo-sensitive nanogels (NGs) from poly-N-isopropylacrylamide (PNIPAm) and polyacrylamide (PAAm) [P(NIPAAm-co-AAm) NGs] for delivering Curcumin (Cur). We characterized the NGs using various techniques and found them to be biocompatible and low in toxicity. We conducted *in vitro* experiments to demonstrate the pH and temperature-sensitive loading and release of Cur by controlling the swelling and deswelling of the NGs. The PNIPAm-co-PAAm copolymer we synthesized showed ~65% Cur loading. The NGs' zeta potential decreased with increasing pH, and they underwent a phase transition at 40°C with concentration-dependent properties. Almost 100% of Cur was released from the NGs after four hours at pH 5.5 and 40°C. Therefore, these newly synthesized NGs have the potential for solid tumor-targeted therapy by releasing the drug based on physical stimuli such as pH and temperature.

Keywords: Curcumin; P(NIPAAm-co-AAm) nanogels; pH; temperature; drug delivery

1. Introduction

Cur is a powerful active ingredient from turmeric with numerous proven biological activities, such as anti-inflammatory, anti-diabetic, and anti-cancer properties [1, 2]. However, its clinical applications have been limited due to low bioavailability and rapid metabolism [3]. To overcome these limitations, nanocarriers have been developed to enhance the solubility, stability, and targeted delivery of Cur to cancerous tissue. Various nanocarriers, such as bio-polymeric particles and nanoparticles, have been developed. Polymers, in particular, are popular nanocarriers due to their biocompatibility, ease of design, and interesting bio-mimetic characteristics [4]. PNIPAAm nanogels have been developed for drug delivery due to their reversible phase transition property [5]. In this work, a pH- and thermo-sensitive nanocarrier was developed using the polymer P(NIPAAm-co-AAm) NGs to deliver Cur. The NGs were found to have low cytotoxicity and high biocompatibility. The NGs could release Cur according to physical stimuli (pH and temperature) and have potential application value in solid tumor-targeted therapy.

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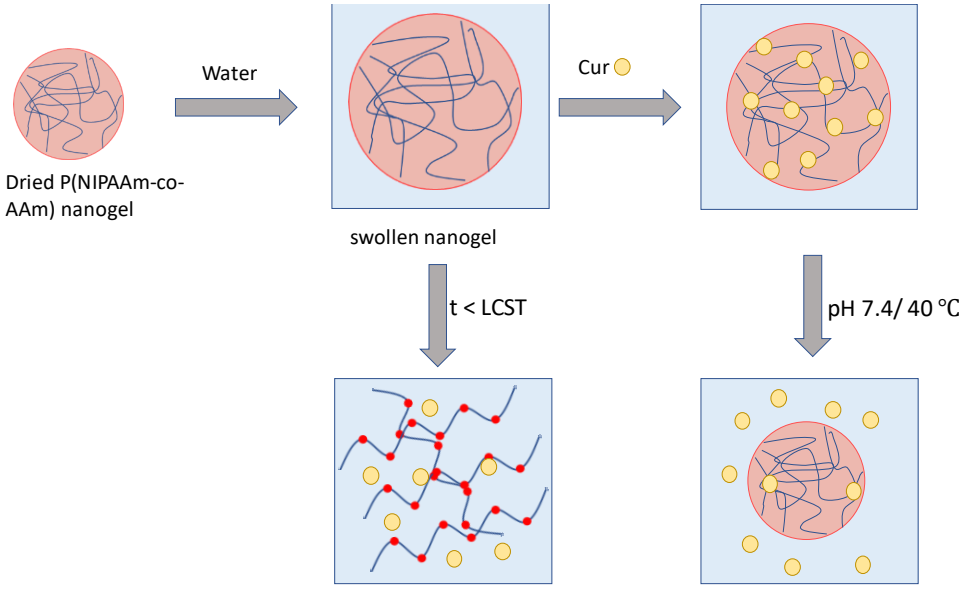

Scheme 1. Illustrating the loading/releasing mechanism of Cur into/from nanogels.

2. Methods

2.1. Nanogel synthesis

The copolymer known as PNIPAm-co-PAAm was produced via free radical polymerization by employing AIBN as an initiator. To commence the synthesis, NIPAm (4.00 g, 35.3 mmol) and AAm (2.75 g, 35.5 mmol) were dissolved in 50 mL of THF within a two-necked round bottom flask. Prior to the addition of AIBN (0.1 g in 1 mL THF), the reaction mixture was purged with N_2 gas for half an hour. The mixture was stirred continuously at a temperature of 70°C for 24 hours. After the reaction was completed, the thick substance was precipitated in 10 mL of diethyl ether. The precipitation process was conducted five times to remove any unreacted monomers. The resulting precipitate was then dried at room temperature in a vacuum oven overnight. The final product was named PNIPAm-co-PAAm copolymer.

2.2. Loading and release of Cur

The hydrophobic anticancer drug, Cur, was loaded into PNIPAm-co-PAAm copolymer through swelling diffusion at a weight to weight ratio of 10:1. Firstly, 0.1 g of dried PNIPAm-co-PAAm copolymer powder was mixed with 10 mg of Cur drug in 3 mL of deionized water, and the resultant solution was mixed for 24 hours at 25°C. The drug-loaded polymer sample was centrifuged at 40°C, and the supernatant was analyzed using a UV-Vis spectrophotometer at 427 nm to determine the amount of drugs loaded into the PNIPAm-co-PAAm sample. The Cur loaded sample was named PNIPAm-co-PAAm@Cur, and it was estimated that the percentage of Cur loading was approximately 65%.

To investigate the release of Cur from PNIPAm-co-PAAm@Cur, various experiments were conducted under different conditions such as pH 7.4 and pH 5.5, 25°C and 40°C, and pH 7.4/40°C and pH 5.5/40°C. The sample was placed inside a dialysis bag with a molecular weight cut-off of 3500 kDa and immersed in 25 mL of PBS solution. At different intervals, samples were withdrawn and Cur was quantified at 427 nm. The cumulative release of Cur was determined using the following formula: Cur release (%) = (Amount of Cur released at time t / Total amount of Cur in the sample) × 100.

2.3. In vitro cytotoxicity of nanogels

In order to evaluate the biocompatibility, an MTT assay was conducted on HepG2 cells that were grown in a 96-well plate with different concentrations of free Cur, PNIPAm-co-PAAm, and Cur loaded PNIPAm-co-PAAm@Cur samples. After 4 hours, MTT solution was added and then incubated for another 4 hours. The formazan crystals were dissolved with DMSO and the absorbance was measured at 595 nm using an ELISA microplate reader.

3. Results and discussions

3.1. Characterization of nanogels

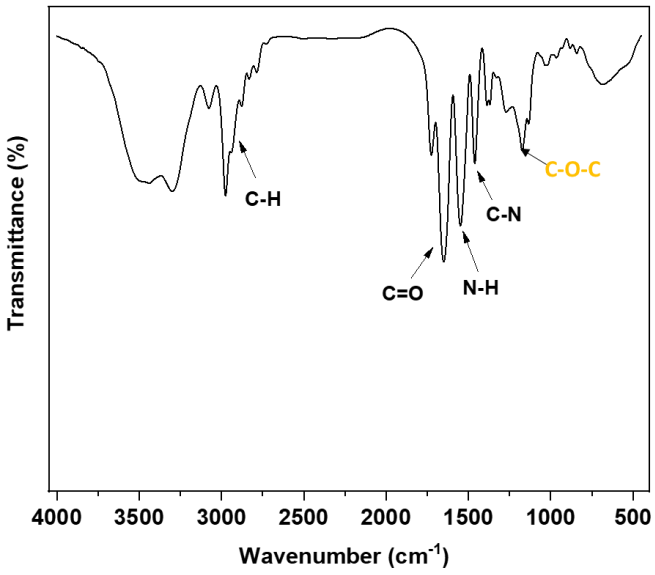

Figure 1. FTIR spectrum of PNIPAm-co-PAAm@Cur copolymer.

In Figure 1, the FT-IR spectrum of PNIPAm-co-PAAm@Cur copolymer is shown. The vibrational band ranging from 2793 to 2834 cm^{-1} indicates the alkyl C-H stretch of the NIPAm groups. The band observed at 1679 cm^{-1} can be attributed to the C=O group of two monomers. The N-H groups of the AAm groups are responsible for the stretching vibrations observed at 1518 cm^{-1}. The intense band detected at 1376 cm^{-1} corresponds to the C-N stretching of the PNIPAm segments present in the PNIPAm-co-PAAm HG NGs. Additionally, the appearance of the C-O-C peak at 1250 cm^{-1} indicates the presence of aromatic Cur molecules.

Below the low critical solution temperature (LCST=40°C), the copolymer dispersion was homogeneous and transparent, indicating a linear chain structure with high water absorption (Figure 1). However, at 40°C, there was a decrease in transmittance, indicating a globule structure.

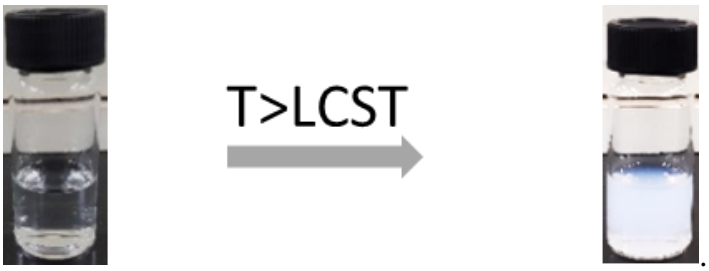

Figure 2. The photographs of nanogels at below and above the low critical solution temperature.

To analyze the swelling-deswelling behavior of PNIPAm-co-PAAm copolymer in response to pH changes, the zeta potential was measured to determine its electrical charge. The zeta potential of this copolymer is sensitive to pH because of the carbonyl and amide groups present in it. As the pH increased from 3 to 9, the zeta potential decreased from +12 to +2 mV. At pH levels lower than 5.5, the hydrophobic part of the PNIPAm segments aggregates into micelle cores, whereas the hydrophilic PAAm segments form a globule-like structure around them. At higher pH levels, the copolymer becomes less protonated and more hydrophilic, allowing it to transition into a sol phase with a linear copolymer, as illustrated in Figure 2.

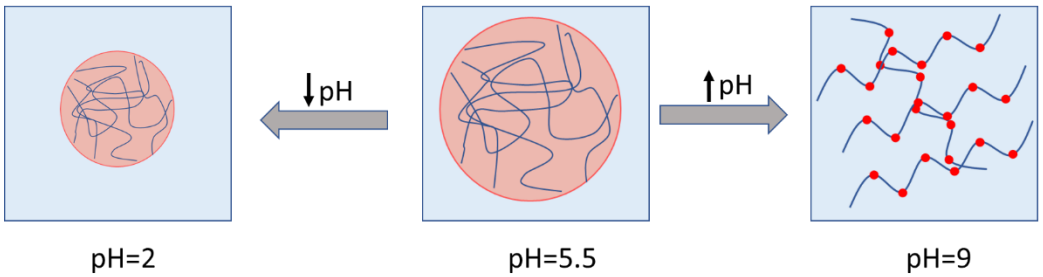

Figure 3. Illustrate the phase transition of P(NIPAAm-co-AAm) NGs under the pH stimulus. Highlighting the hydrophobic domain (red) and hydrophilic polymer segments (blue) in an aqueous environment (light blue).

3.2. Release of Cur

Curcumin release from nanogels system was recorded under different conditions (pH 7.4/5.5, 25℃/40℃). Cur release was enhanced at pH 5.5 and 40℃, reaching nearly 100% after 2 hours. The optimal performance for releasing Cur was achieved by subjecting it to a combination of thermal and pH stimuli.

3.3. Cytotoxicity of Cur

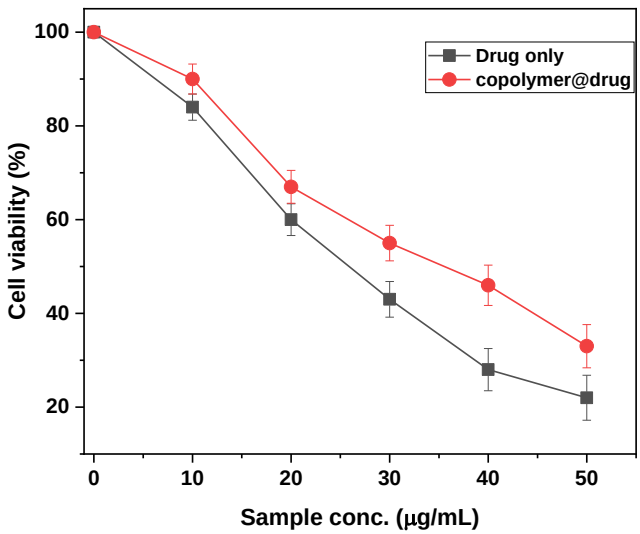

Figure 4. The percentage of cell viability was measured for PNIPAm-co-PAAm@Cur nanogels' in vitro cytotoxicity at 37 °C.

HepG2 cells were used to evaluate the biocompatibility of the PNIPAm-co-PAAm copolymer both with and without Cur loading at 37°C (Figure 4). The copolymer exhibited around 90% viability, whereas the Cur-loaded copolymer exhibited toxicity that var-

ied with concentration. Nonetheless, the Cur-loaded copolymer exhibited higher cell viability compared to pure Cur at the same concentrations due to lower Cur release. These findings suggest that the copolymer is safe for biological use and can be employed for loading and releasing anticancer agents in tumor microenvironments.

3. Conclusion

In conclusion, a pH and thermos-responsive copolymer system (PNIPAm-co-PAAm) was prepared by in-situ copolymerization of NIPAm and AAm monomers for drug delivery. Characterization was conducted using various instruments and showed high drug loading and complete release under pH and temperature stimuli. Biocompatibility was also confirmed, indicating potential use in cancer therapy.

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