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The study of physical properties and formulation of self-assembling nanogel from grafted hydrophilic polymer backbone as stabilization system for curcumin

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ABSTRACT

Introduction: The aim of this work is to formulate and study the thermoresponsive system with hyaluronic acid (HA) as a polysaccharide backbone grafted with poly-(N-isopropylacrylamide) (pNIPAM) for stabilization of highly sensitive molecule. **Methods:** The nanogel forming polymer was synthesized through EDC/NHS coupling reaction. The HA grafted pNIPAM (HA-g-pNIPAM) can be self-assembled to form gel particle. The synthesized HA-g-pNIPAM polymers were characterized by ^{1}H NMR. Size, influence of media to forming nanogel, thermoresponsive behavior and influence of HA-g-pNIPAM nanogel network on curcumin loading capacity and stability were investigated by dynamic light scattering (DLS) and UV-VIS spectrophotometry. **Result and Discussion:** The results showed that the nanogel prepared in ultrapure water is appropriate to form nanogel with submicron-size range. The lower critical solution temperature (LCST) of HA-g-pNIPAM nanogels appeared at 33°C. This study also indicated the importance of polymer network as solubility and stability enhancer of curcumin which could be a model for other labile molecules or insoluble drugs.

Keywords: Curcumin, grafted biopolymer, nanogel, self-assembly, thermoresponsive

INTRODUCTION

Curcumin is a hydrophobic polyphenol derived from the rhizome of *Curcuma longa*. It has been well-established for a wide range of pharmacological activities.[1] Curcumin exhibits antioxidant, anti-inflammatory, and anti-tumor. Apart from its high potential in bioactivity, curcumin possesses strong fluorescence that can be of benefit as biocompatible probe for bio-imaging and theranostics applications.[1-4] However, major problems with curcumin are low water solubility, chemical instability, and poor absorption. These are critical limiting factors against its bioavailability.[1] To overcome the limitations of curcumin, we focused on the development of nanocarriers for incorporation of curcumin. Nanogels are nano-sized hydrogel particles with three-dimensional structure formed by physically or chemically crosslinked polymer networks.[5,6] They have a high-water content and biocompatibility. Natural polysaccharides have been used to form nanogels for biomedical applications because of their hydrophilicity, biocompatibility, and biodegradation properties.[7-9] Hyaluronic acid (HA) is a natural polysaccharide abundant in the body as one of the major components in extracellular matrices and tissue structure.[10] Recent biomedical applications of HA involve, for example, wound healing and scaffolds for tissue engineering.[11,12] HA

is a linear polysaccharide containing repeating disaccharides, glucuronic acid and N-acetylglucosamine.[13] HA can be functionalized to provide a stimuli-responsive function by conjugating stimuli-responsive moieties to their functional groups, for example, -COOH and -NH_2. Stimuli-responsive nanogels have attracted attention over the past few years as system of interest in materials science and nanomedicine, which are advantages as drug delivery carriers.[14,15] These nanogels are capable of responding to external stimuli such as pH and temperature.[16,17] Poly-(N-isopropylacrylamide) (pNIPAM) is a thermoresponsive polymer which contains hydrophobic and hydrophilic moieties. pNIPAM has a lower critical solution temperature (LCST) of 32°C, which can be useful for biomedical applications, since it is close to body temperature.[18] In this study, we have developed a thermoresponsive HA-g-pNIPAM nanogel for incorporation of curcumin. We used HA as a biopolymeric backbone to be grafted with low molecular weight pNIPAM. An influence of media to form self-assembly nanogel and influence of HA-g-pNIPAM nanogel network on curcumin loading capacity and stability were investigated.

MATERIALS AND METHODS

Materials

Curcumin (Purity: ≥98%) was obtained from the Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University. Sodium hyaluronate (M.W. of 45-65 kDa) was purchased from Liuzhou Shengqiang Biotech Co., Ltd., China. pNIPAM (pNIPAM; M.W. 5500 Da) was purchased from Sigma, USA. 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from CreoSalus Inc., USA, Deuterium oxide was purchased from Cambridge Isotope Laboratories, Inc., USA. N-hydroxysuccinimide (NHS) was purchased from Sigma, USA. Ethanol was purchased from RCI Labscan.

Synthesis of HA-grafted pNIPAM Polymer

HA-grafted pNIPAM polymer was synthesized as reported previously[19] HA-g-pNIPAM with 5% degree of grafting were synthesized through EDC/NHS coupling reaction. Sodium hyaluronate (M.W. of 45-65 kDa, 1%w/v*)* was dissolved in ultrapure water followed by adding pNIPAM-NH_2 (M.W. 5500 Da*)* at 1:0.05 of HA*:*pNIPAM molar ratio. A four-fold molar excess of EDC and NHS (1*:*4*:*4 of HA*:*EDC:NHS molar ratio) was added in powder, and the pH set to 5.5 ± 0.3 with NaOH or HCl. After 1 h, pH was adjusted to 7.5 ± 0.3 with NaOH. The reaction was run at room temperature for 48 h and then the products were purified through dialysis for 3 days and freeze-dried. The obtained products were confirmed by ^{1}H NMR. The polymers were named HA-g-pNIPAM x, where x is degree of grafting.

HA-g-pNIPAM Nanogel Preparation

HA-g-pNIPAM nanogels were prepared by a simple sonication method. HA-g-pNIPAM polymer was dissolved in ultrapure water, citrate buffer (pH 4.01), and phosphate buffer saline (PBS) solution *(*pH 7.6*)* at 0.5%w/v, under sonication for 30 min. After sonication, nanogels were settled overnight at 4°C.

Curcumin Loading and Stability

10 mM curcumin in ethanol solution was added dropwise into the nanogel formulation where formed with 0.5%w/v polymer in ultrapure water under stirring condition and stirred in 4°C and 25°C for 24 h. The drug-loaded nanogel was sampling at different time point to determine the optimum drug loading time. After incubation, an excess amount of curcumin in drug-loaded nanogel was discarded by centrifugation under 3000 g, 25°C for 5 min. The supernatant was kept, and curcumin amount was determined using UV-visible spectrophotometry with wavelength 420 nm. To investigate an ability of nanogel network as a drug loading enhancer system, the property of each component of the polymer was studied. Sodium hyaluronate (M.W. of 45-65kDa), pNIPAM amine terminated, and HA/pNIPAM (non-modified) were utilized using method mentioned earlier. The polymer's components were utilized in an equal amount as in the polymer structure.

Characterization of Nanogels

Particle size was measured by DLS (Zetasizer Nano ZS, Malvern Instruments, UK). To examine the thermo-responsiveness of the nanogels, average sizes were measured using a controlled temperature program, increasing from 25 to 40°C at 1°C min.

Statistical Analysis

All experiments were performed in triplicate. The results are expressed as mean± standard deviation.

RESULTS AND DISCUSSION

Synthesis of HA-grafted pNIPAM Polymer

HA-grafted pNIPAM polymer was synthesized through EDC/NHS coupling reaction as reported previously.[19,20] The structure of grafting reaction product in polymer synthesis was characterized by ^{1}H NMR. As shown in Figure 1, HA-g-pNIPAM polymer was synthesized with 5% degree of grafting (designated as HA-g-pNIPAM 5). The spectra revealed that modification was successful.

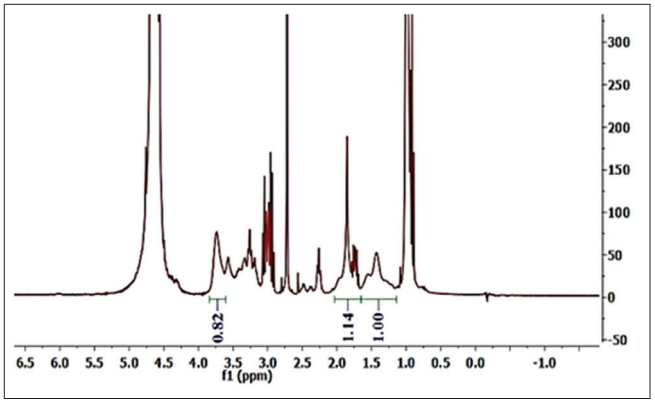

Figure 1: ^{1}H NMR spectra of pNIPAM grafted hyaluronic acid with 5% (HA-g-pNIPAM 5) degree of grafting

An Influence of Media to Form Self- assembly Nanogel Studies

The particle sizes of the nanogels with 5% degree of grafting of HA-g-pNIPAM polymers (HA-g-pNIPAM 5) were investigated by DLS. Nanogel formulations were formed with 0.5%w/v polymer in ultrapure water, citrate buffer (pH 4.01), and PBS (pH 7.6). We have optimized the media that used in forming nanogel with low polydispersity index (PdI), as descripted in Table 1. The results showed that the particle size of all nanogel formulations fell in submicron-size range. However, the PdI of the nanogel prepared in ultrapure water were obviously lower than that in citrate buffer and PBS. Hence, the nanogel prepared in ultrapure water was collected for the next experiment.

Table 1: The size and size distribution (mean±standard deviation) of nanogel formulations resulted from 0.5%w/v of HA-g-pNIPAM 5 with comparing between ultrapure water, citrate buffer (pH 4.01), and PBS (pH 7.6) determined by dynamic light scattering with 3 times at 25°C

Media	z-avg (nm)	PdI
Ultrapure water	547.4±67.05	0.48±0.093
Citrate buffer	428.8±19.49	0.82±0.066
PBS	234.2±25.02	1

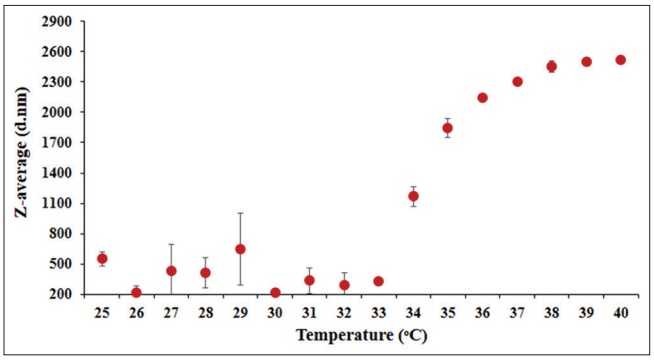

Figure 2: Thermoresponsiveness profile of nanogels measured by dynamic light scattering

Investigation of the Thermoresponsive Properties

Properties of the control nanogel and the stable dispersion in water at 0.5% w/v are shown in Figure 2. The DLS measurements were conducted from 25 to 40°C. The LCST was measured as the temperature at the size of the nanogels changed abruptly. The LCST of HA-g-pNIPAM 5 nanogel prepared in ultrapure water was investigated. The results showed that the LCST of HA-g-pNIPAM nanogels at the polymer concentration of 0.5%w/v was 33°C, where their sizes dramatically changed from 547.4 nm to 2.5 μm. For HA-g-pNIPAM 5 an average sizes of about 547.4 nm were observed at 25°C, which is the temperature below the LCST. At 40°C, which is the highest temperature tested above the LCST, the particles became as large as 2.5 μm. This finding indicated that pNIPAM chains on the HA resulted in the increased hydrophobicity at temperature above the LCST. The aggregation occurred in nanogel formulation leading to the larger sizes observed.[21]

An Influence of HA-g-pNIPAM Nanogel Network on Curcumin Loading Capacity and Stability Studies

After the determination of curcumin in HA-g-pNIPAM polymer, unmodified HA and pNIPAM native polymer, HA-g-pNIPAM nanogel showed the most drug loading capacity comparing to the each polymer component alone or non-modified HA physically mixed with native pNIPAM at both 4 and 25°C [Figure 3]. The results indicated that network of HA-g-pNIPAM in nanogel formulation provided the better system for curcumin solubility. This possibly is a result of hydrophobic part of pNIPAM, which might contribute an appropriate condition for curcumin to fit in, and linked with the hydrated structure of HA assembled into nanogel particle. In this study, we also investigated an optimum curcumin-loading time for HA-g-pNIPAM polymer. Interestingly, the maximum drug load could be observed initially with 6-h drug-nanogel incubation time. However, the curcumin was previously loaded in this formulation precipitated out after 24-h storage. Therefore, we selected 24 h as the optimum incubation time which the formulation did not shown

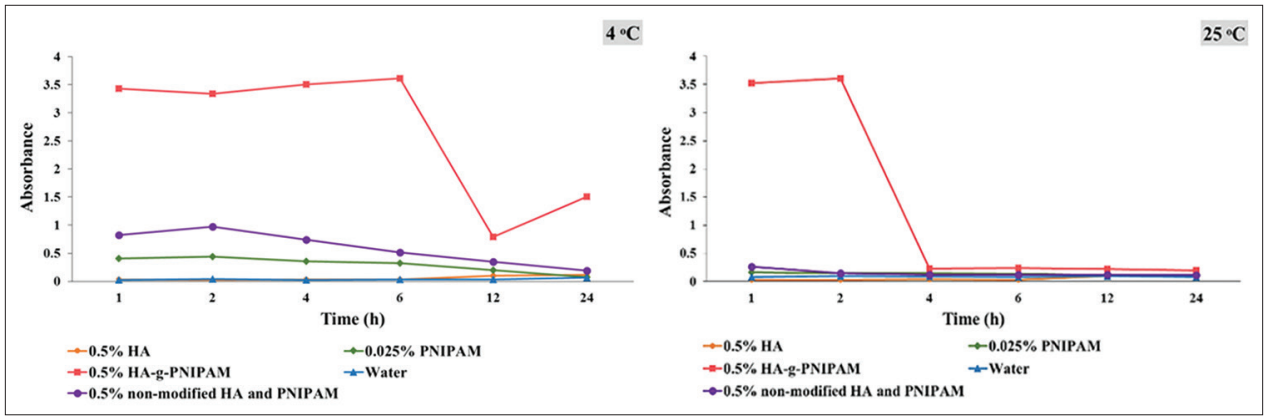

Figure 3: The curcumin absorbance of HA-g-pNIPAM nanogel and polymer components at each time point

curcumin precipitated during 3 days of storage. Moreover, we also found that curcumin incubation should be performed under 4°C condition, according to the better curcumin absorbance from HA-g-pNIPAM nanogel which was incubated the drug under 4°C.

CONCLUSION

This study formulated thermoresponsive nanogel particles from HA that modified with a pNIPAM to enhance the aqueous solubility of curcumin. We studied the effect pNIPAM grafting on the nanogel assembly of HA. Then, the nanogel formulations with different media containing 0.5% w/v HA-g-pNIPAM polymers were investigated for an appropriate of media to form self-assembly nanogel and it was found that ultrapure water is appropriate to form nanogel. In the optimization of drug loading process, we found that curcumin stock solution should be loaded dropwise into the stirring nanogel under 4°C and kept stirring for 24 h due to the drug loading content and stability. Interestingly, the network of polymer in nanogel formulation seemed to have an influence on curcumin solubility and stability according to the absorbance data compared with curcumin in polymer's components separately. The colloidal stability provided by the polymer network should be further investigated to confirm the impact of nanogel on drug loading efficiency.

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