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# Synthesis and Characterization of Thermosensitive Nanoparticles Based on PNIPAAm Core and Chitosan Shell Structure

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**Abstract:** Noble thermosensitive nanoparticles, based on a PNIPAAm-*co*-AA core and a chitosan shell structure, were designed and synthesized for the controlled release of the loaded drug. PNIPAAm nanoparticles containing a carboxylic group on their surface were synthesized using emulsion polymerization. The carboxylic groups were conjugated with the amino group of a low molecular weight, water soluble chitosan. The particle size of the synthesized nanoparticles was decreased from 380 to 25 nm as the temperature of the dispersed medium was increased. Chitosan-conjugated nanoparticles with 2~5 wt% MBA, a crosslinking monomer, induced a stable aqueous dispersion at a concentration of 1 mg/1 mL. The chitosan-conjugated nanoparticles showed thermosensitive behaviors such as LCST and size shrinkage that were affected by the PNIPAAm core and induced some particle aggregation around LCST, which was not shown in the NIPAAm-*co*-AA nanoparticles. These chitosan-conjugated nanoparticles are also expected to be more biocompatible than the PNIPAAm core itself through the chitosan shell structures.

*Keywords*: nanoparticles, chitosan, lower critical solution temperature, thermosensitive.

## Introduction

Poly(*N*-isopropylacrylamide) (PNIPAAm) exhibits a lower critical solution temperature (LCST) in aqueous solutions, and its crosslinked nanogels shows high swelling thermosensitivity in water.[1-3] These nanogels have been investigated for molecular separation, sorption-desorption of solutes, control of enzyme activity, and release of solutes. PNIPPAm are constantly copolymerized with other monomers due to its weak mechanic properties and applications. Ramkisson-Ganorkar, *et al.* developed a modulating insulin system from thermosensitive terpolymers of NIPAAm, butyl acrylate and acrylic acid.[4] They showed that the synthesized terpolymers can be used for controlled release of insulin drug as temperature and pH of the medium varies. Park also reported a pH/temperature hydrogel system composed of NIPAAm and *N,N'*-diethylaminopropyl methacrylamide for controlled release of insulin.[5] Their hydrogel system entrapped insulin resulted in that temperature cycling operations greatly affected the insulin release rate, whereas pH variation did not significantly influence the release profile.

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A new challenge is the development of the thermosensitive nanogels with biological activity, especially for drug delivery applications. In this context, the preparation of thermosensitive gels conjugating with biomaterials is attractive because it allows a drug to be released when it is needed. Several authors have reported the thermosensitive gel systems based on PNIPAAm conjugated with biomaterials. Kono, *et al.* synthsized liposomes coated with a copolymers of NIPAAm and *N,N'*-didodecylacrylamide.[6] The release of calcein from the copolymer-modified liposomes was very slow below LCST, whereas the release was rapid above LCST. Katayama *et al.* reported the thermosensitive PNIPAAm nanogels conjugating with the receptor of protein kinase A.[7] When the molecule contacts with protein kinase A, the LCST of PNIPAAm was changed and the immuno-conjugated polymer was degraded. From this active responding of the immuno-conjugates, the controlled release was achieved.

Chitosan and its derivatives have become useful polysaccharides in the biomedical area because of their numerous and interesting biological properties such as biocompatibility, biodegradability, and nontoxic properties.[8-11] Lee, *et al.* reported the synthesis of PNIPAAm-chitosan complex particles with the method of soap less dispersion polymerization.[12,13] In this reaction system, the chitosan behaved as a

surfactant, preventing polymer particles from coagulation at initial stage, but the reaction mixture soon become heterogeneous, producing PNIPAAm-chitosan complex particles as precipitates. Kim, *et al.* also reported grafted copolymerization or blending of chitosan and PNIPAAm. They found that in the buffer solution with various pHs and temperatures, the chitosan/PNIPAAm blend IPN has higher swelling than that of the chitosan-*g*-PNIPAAm IPN.[14]

Recently our studies are focused on chemical modifications, nanoparticle preparation, and drug-release behaviors of low molecular weight water soluble chitosan (LMWSC).[15-17] In the present study, we have designed novel thermosensitive nanoparticles, based on NIPAAm-*co*-AA core exhibiting LCST behaviors and chitosan shell having biocompatibility, to develop the controlled release of the loaded drug. Chitosan conjugated NIPAAm-*co*-AA nanoparticles with advantages of temperature sensitivity and biodegradability were synthesized. Characterizations and thermosensitive behaviors of the nanoparticles composed of either NIPAAm-*co*-AA or NIPAAm-*co*-AA conjugated with chitosan were also investigated.

## Experimental

**Materials.** Low molecular weight water soluble chitosan (LMWSC) was obtained from KITOLIFE Co. Korea. LMWSC was modified to enhance water-solubility as described previously as a water-soluble chitosan (M.W. 10,000, deacetylation degree=87%).[15] *N*-Isopropylacrylamide (NIPAAm) was obtained from Fisher Scientific Inc. (Fair Lawn, NJ, USA) and re-crystallized from hexane. *N,N'*-Methylenebis(acrylamide)(MBA), acrylic acid (AA), potassium persulfate (KPS) and sodium dodecyl sulfate (SDS) were purchased from Aldrich (Milwaukee, WI, USA) and used without further purification. Dialysis tubing was commercially obtained from Spectrum.

**Preparation of NIPAAm-*co*-AA Nanoparticles.** NIPAAm-*co*-AA nanoparticle was based on the similar procedure described by Kratz, *et al.* and Pelton.[1,18] Briefly, 6.80 g of NIPAAm (60 mmol), 0.5 g of SDS (1.7 mmol) and 0.14 g of MBA (0.91 mmol) were dissolved in 470 g of double distilled water, degassed with argon. The synthesis was carried out under argon atmosphere to exclude oxygen. After heating the solution to 75 °C, 0.5 g of KPS (1.8 mmol) and 0.3 g of AA (4.2 mmol) in 30 g of water were added under rigorous stirring. The reaction became turbid and the reaction proceeded for 4 h at constant temperature. Diameter of resulting nanoparticle solution was ca. 303.9 nm, measured by the dynamic light scattering at room temperature. The obtained NIPAAm-*co*-AA nanoparticles in aqueous solution were dialyzed with distilled water by using a dialysis membrane (celluSep®, molecular weight cut-off =5,000) for 24 h in order to purify the product.

**Synthesis of Thermosensitive Nanoparticles Based on NIPAAm-*co*-AA Core and Chitosan Shell Structure.** The conjugation of NIPAAm-*co*-AA particle with LMWSC was carried out by the carboxyl-amine reaction. Briefly, 2 mL of NIPAAm-*co*-AA solution (1.2 wt%) was uniformly dispersed in 20 mL of de-mineralized water by ultrasonic for 10 min. The prepared NIPAAm-*co*-AA solution was slowly added into the stirred LMWSC aqueous solution (10 mg in 10 mL de-mineralized water) and preadsorped for 2 h at room temperature. After the preadsorb procedure, 0.5 mg of 1-ethyl-3-(3-dimethyl; aminopropyl) carbodiimide (EDAC, Aldrich), the catalyst of carboxyl-amine conjugation reaction, was added to the solution described above. The nanoparticle-LMWSC conjugation reaction was carried out for 4 h with stirring at 25 °C. The resulting LMWSC-conjugated nanoparticle aqueous solution was dialyzed with distilled water by using a dialysis membrane (celluSep®, molecular weight cut-off =12,000) for 24 h in order to purify the product.

**Characterization of the Thermosensitive Nanoparticles.** NMR measurement was performed with a 300 MHz spectrometer (Bruker, 300 MHz) using $D_2O$ as a solvent at room temperature. The temperature dependent particle size of NIPAAm-*co*-AA and LMWSC conjugate nanoparticles were measured by the dynamic light scattering (LLS, Malvern, Zetasizer, 3000 HS). The particle size was measured in 0.1 wt% of nanoparticles dispersed in doubled distilled water. At each temperature concerned, the solution was stand for 5 min to equilibrium. The gel-collapse temperature of nanoparticle solution, which is analogue to the LCST of the linear temperature, was determined by using a UV/visible spectrophotometer (Shimadzu, UV-1601). The nanoparticles morphology was examined with a JEOL JEM-2000 FX II transmission electron microscope (TEM) and Hitachi S-4800 scanning electron microscope (FESEM).

## Results and Discussion

**Preparation of NIPAAm-*co*-Acrylic Acid Nanoparticles.** The NIPAAm-*co*-AA particles were synthesized by emulsion polymerization of NIPAAm monomer and acrylic acid in the presence of MBA as a cross-linker, KPS, as an initiator and SDS as a surfactant. The sample number and detail ingredient of the NIPAAm-*co*-AA nanoparticles were shown in Table I. As the polymerization proceed, the polymerization process started with a transparent solution and the reaction mixture became turbid due to the insolubility of the prepared nanoparticles at 75 °C.

The surfactant, SDS, was necessary because the NIPAAm at higher temperature than LCST would have no capability to stabilize the nanoparticles. From Table I, high conversions (>95%) of all nanoparticles were measured. SEM and TEM images of PNIAA-4 nanoparticle are shown in Figure 1. The electron micrographs show the synthesized nanoparticles are spheres. Although the degree of crosslinking was not high, a ball-shaped morphology was still observed. In

**Table I. Ingredients and Conversions for the Synthesis of NIPAAm-*co*-AA Nanoparticles**

| Sample No. | PNIAA-4(2) | PNIAA-4(5) | PNIAA-8(5) | PNIAA-12(5) |
|---|---|---|---|---|
| Water(g) | 500 | 500 | 500 | 500 |
| NIPAAm(g) | 6.80(95%) | 6.85(91%) | 6.75(87%) | 6.70(83%) |
| AA(g) | 0.3 (4%) | 0.3(4%) | 0.623(8%) | 0.961(12%) |
| MBA(g) | 0.14 (2%) | 0.41(5%) | 0.41(5%) | 0.40(5%) |
| SDS(g) | 0.5 | 0.5 | 0.5 | 0.5 |
| KPS(g) | 0.5 | 0.5 | 0.5 | 0.5 |
| Conversion (%)[a] | 98.1 | 97.8 | 98.6 | 98.3 |

[a]Conversion was measured by gravimetric method (120 °C × 30 min) after dialysis of the resulting nanoparticle solution.

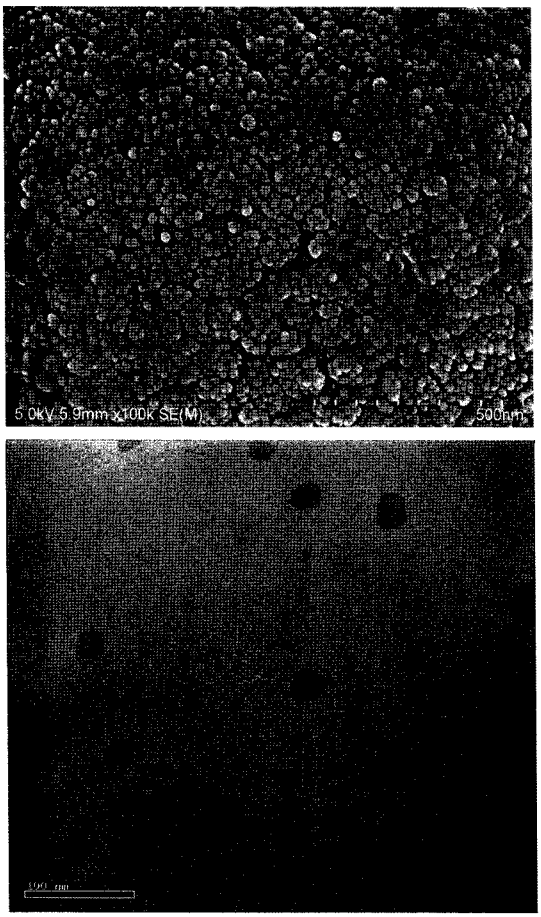


**Figure 1.** SEM and TEM images of the PNIAA-4(5) (Table I) nanoparticles.

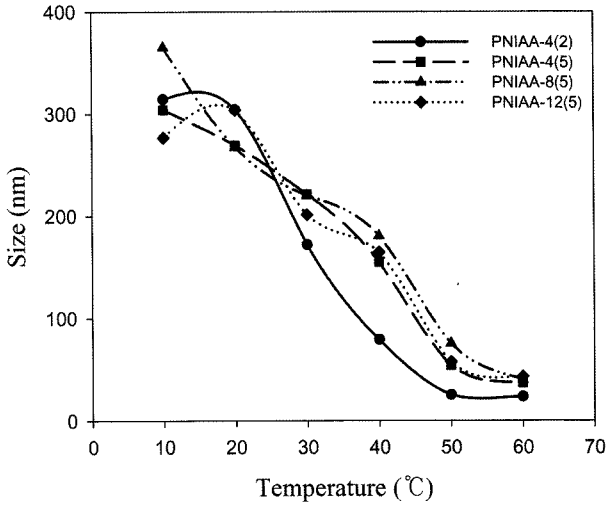


**Figure 2.** Thermosensitive behaviors of NIPAAm-*co*-AA nanoparticles as a function of temperature.

aqueous solutions, such as water or buffers, PNIAAm-*co*-AA particles were uniformly dispersed and stabilized due to the repulsive forces between each particle originated from the charge groups, including carboxylic groups, surfactant and initiators located at the surface of the nanoparticles.

**Particle Size and the Gel-collapse Temperature of NIPAAm-*co*-AA Particles.** The NIPAAm-*co*-AA nanoparticles exhibit different thermosensitive behaviors with particle concentration in water. At highly dilute concentration of NIPAAm-*co*-AA particles (1 mg of particles/1 mL of water), the particles shows shrinkage in size without particles aggregation. Figure 2 summarized the results of NIPAAm-*co*-AA particles obtained from dynamic light scattering at various temperatures. The effects of MBA and acid content on particle size were investigated, and the particle size of NIPAAm-*co*-AA particles is decreased as a function of temperature, due to the PNIPAAm polymer chain's aggregation. Figure 2 also showed the significant decrease of particle size as the temperature increased from 10 to 50 °C. "Normally, above the LCST the shrinkage of nanoparticles aggregation caused by PNIPAAm polymer chain occurs, which results in the increase of the particle size. However, our experiment showed that the effect of temperature on particle size was influenced by the nanoparticle concentration. As the temperature increases, the highly dilute concentration of the nanoparticles may inhibit the aggregation between nanoparticles, but leads the PNIPAAm polymer chain's aggregation of the internal nanoparticles. This

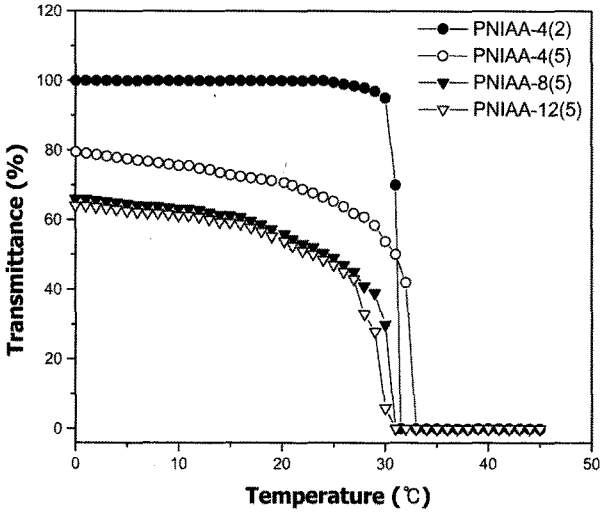


**Figure 3.** Effect of AA content on the gel-collapse temperature of NIPAAm-*co*-AA nanoparticles in doubles distilled water.

explains that the particle size decreases at below and above the LCST in the case of the highly dilute NIPAAm-*co*-AA nanoparticles." Results of transmittance measurement of NIPAAm-*co*-AA particles at higher concentration (15 mg of particles/1 mL of water) as a function of temperature are shown in Figure 3. The transmittance can be related to the particle size and the precipitation of polymer or lattices. In Figure 3, all of the NIPAAm-*co*-AA nanoparticles showed similar trends of transmittance; that is, the transmittance of the particle with different AA content dropped abruptly around 32 °C, which may be related a lower critical solution temperature (LCST) in aqueous solutions. When the volume phase transition of a particle occurred, the light transmittance of the particle solution was lowered (clouded), which implies the gel-collapse temperature of the solution. With the transmittance results of Figure 3, gel-collapse temperature seemed to be decreased with increasing AA content. "The increased content of the hydrophilic AA may promotes the hydration of the nanoparticles with water to decrease the gel-collapse temperature of the NIPAAm-*co*-AA nanoparicles." The slightly crosslinked sample (PNIAA4(2)) also showed higher transmittance than those of highly crosslinked samples (PNIAA4(5), PNIPAA8(5) and PNIPAA12(5) at the same temperature. Low transmittance of the highly crosslinked samples may be due to the localized gel networks in the swollen nanoparticle.

**Synthesis of Nanoparticles Based on PNIPAAm Core and Chitosan Shell Structure.** The carboxylic groups of NIPAAm-*co*-AA nanoparticles were applied to conjugate with the amino group of the low molecular weight water soluble chitosan (LMWSC). As the NIPAAm-*co*-AA nanoparticles slowly added into a highly diluted LMWSC solution, LMWSC might be adsorbed at the surface of the nanoparticles by carboxyl-amine interaction, and the conjugation reaction of NIPAAm-*co*-AA particle with LMWSC was carried out by using 3-(3-dimethyl aminopropyl) carbodiimide (EDAC). The resulting LMWSC-conjugated nanoparticle aqueous solution was dialyzed to cut off the un-reacted LMWSC by using a dialysis membrane. Figure 4 shows $^1$H NMR spectra of NIPAAm-AA-LMWSC nanoparticles (A),

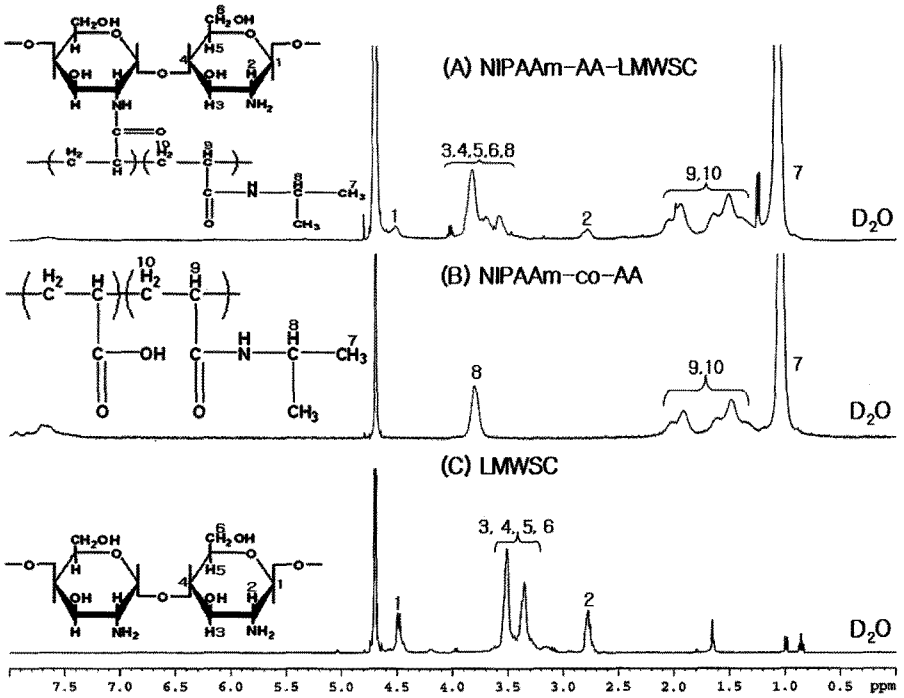


**Figure 4.** $^1$H NMR spectra of NIPAAm-AA-LMWSC nanoparticles (A), NIPAAm-*co*-AA nanoparticles (B), and LMWSC (C).

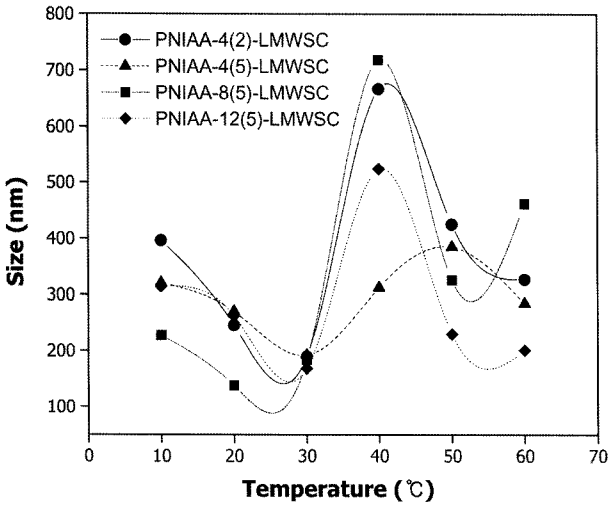


**Figure 5.** Thermosensitive behaviors of chitosan conjugated PNIPAAm nanoparticles as a function of temperature.

NIPAAm-*co*-AA nanoparticles (B), and LMWSC (C). As shown in Figure 4, NIPAAm-*co*-AA nanoparticles and LMWSC itself showed their specific peak characteristics in $^1$H NMR spectra of NIPAAm-AA-LMWSC nanoparticles. In our experiment, LMWSC may be conjugated on the surface of cross-linked NIPAAm-nanoparticles, resulting nanoparticles with NIPAAm-AA core and crosslinked chitosan shell structures.

**Thermosensitive Behaviors of Nanoparticles Based on PNIPAAm Core and Chitosan Shell Structure.** Thermosensitive behaviors of the LMWSC conjugated NIPAAm-AA particles were investigated by measuring the average particle size and gel-collapse temperature as the temperature increases. Figure 5 shows the size data of PNIAA-LMWSC nanoparticles. The particles size was decreased at below LCST, increased at around LCST and re-decreased at the above LCST. The particle size increase at around LCST may be due to some nanoparticles aggregation caused by PNIPAAm polymer chain shrinkage, which was not shown in NIPAAm-*co*-AA nanoparticles. Several research groups also reported similar results of that PNIPAAm gels can be aggregation at around LCST.[19,20] The results of light transmission experiment of chitosan conjugated nanoparticles were shown in Figure 6. From the Figure 6, it is clearly shown that the cloud point of chitosan conjugated PNIPAAm-AA nanoparticles solution was elevated about 2~3 °C compared with pure particles. The explanation is that the LMWSC of chitosan conjugated PNIPAAm-AA nanoparticles is water soluble. The water soluble shell structure may increase the cloud point of PNIAA-LMWSC nanoparticles.

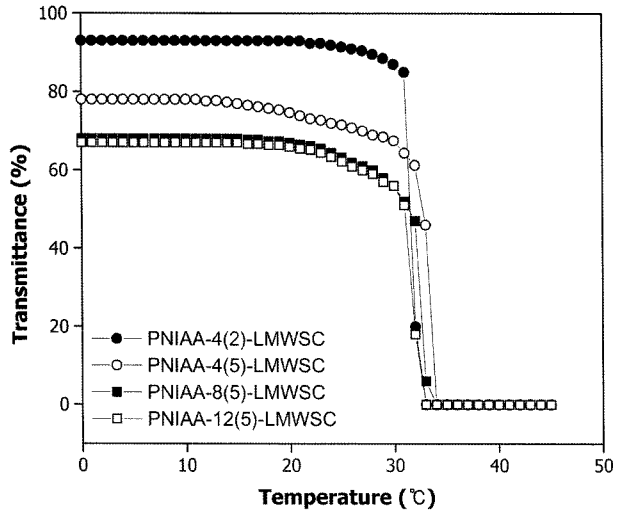


**Figure 6.** UV transmittance of chitosan conjugated PNIPAAm nanoparticles at various temperatures.

## Conclusions

We have synthesized the first example of thermosensitive nanoparticles of PNIPAAm-AA core and chitosan shell structure for the controlled release of the loaded drug. Characterizations and thermosensitive behaviors of nanoparticles composed of either NIPAAm-*co*-AA or NIPAAm-*co*-AA conjugated with chitosan were also investigated. The synthesized nanoparticles showed thermosensitive behaviors such as LCST and particle shrinkage affected by the crosslinked PNIPAAm core, which is more biocompatible than PNIPAAm core itself through the chitosan-conjugated shell structures. Therefore, the thermosensitive characteristics of chitosan conjugated nanoparticles make it potentially useful in the design of a new type of intelligent drug capsules that is compatible with cells to release its pharmacological activity. Those results will be reported elsewhere.

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