공학석사 학위논문

# 이온 복합체 형성을 통해 물리적 성질 조절이 가능한 수화젤 마이크로 수송체 구조에 관한 연구

# Hydrogel Microcontainers with Tunable Physical Properties Prepared by Polyionic Complexation

2013 년 2 월

서울대학교 대학원

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# Abstract

# Hydrogel Microcontainers with Tunable Physical Properties Prepared by Polyionic Complexation

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Synthesis of hydrogel hollow capsules provides the opportunity to extend the applications of gel materials as carriers. We report on biocompatible poly(vinylamine) (PVAm) hydrogel hollow microcontainers derived from well-defined precursor polymer, poly(N-vinylformamide) (PNVF). Herein, PNVF particles were synthesized via dispersion polymerization, varying either of crosslinking agent concentrations or steric stabilizer concentrations with the aim of probing the key factors that facilitate hollow structure. Uniform PVAm hydrogel capsules were consecutively prepared from PNVF, provided PNVF were attained in a sufficiently cross-linked structure.

Subsequently, taking advantage of protonated amine in PVAm at alkaline condition, we constructed PVAm-hyaluronic acid (HA) complexes with two descriptions of HA, possessing different molecular weight. We thus confirmed complex structures with NMR cryoporometry analysis that PVAm-short HA chain complexes (PVAm-SHA) contains denser shell structure than that of PVAm-long HA chain complex (PVAm-LHA). This, in turn, leads to the PVAm-SHA with higher stability against external stimuli, near-infrared (NIR) laser irradiation. For imparting light absorption in NIR region, we introduced gold nanorod (AuNR) to the PVAm-HA complexes. Consequently, PVAm-SHA exhibited improved durability and light stability when compared with PVAm-LHA during the local heating of AuNRs by NIR irradiation.

In this study, we thus practically synthesized hydrogel hollow capsules in different size and also verified the structural change of polyion shell complexes by varying the chain length of HA, leading to enhancement of mechanical strength and light durability. As a consequence, the hollow PVAm-HA capsules developed in the present study can serve as promising microcontainers for therapeutic delivery systems.

**Keywords:** Poly(vinylamine), Hollow Capsule, Dispersion Polymerization, Hyaluronic Acid, Polyionic Complexation, Near-IR irradiation, Pore Size, Light Durability, Mechanical Strength

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# Chapter 1. Introduction

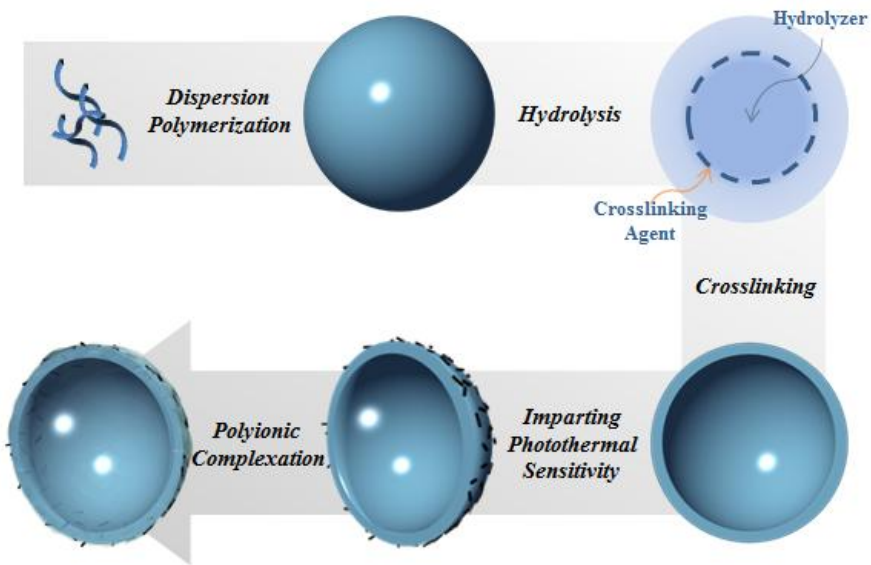
Hydrogels are hydrophilic polymer chains that possess the ability to swell in water and retain a significant amount of water within the confines. The biocompatibility of hydrogels, their high water content and special surface properties, enables mutual function with the natural tissue. Needs in carrier vessels for drugs, therapeutic delivery,[1-4] microreactors,[5,6] and artificial cells[7] urge a development of hydrogel polymeric capsules. The use of polymer capsules as carrier in internal systems defines the requirement for their construction and also demands serious consideration for the composing materials. Among the successful candidates, hydrogel capsules comprised of poly(vinylamine)(PVAm), which is biocompatible polymer with a high density of reactive primary amine group, is a prospective biomaterial carrying high modification feasibility. Since PVAm cannot be synthesized directly from vinylamine monomer, hydrogel capsules have been prepared via precursor polymers that are readily available, herein poly(N-vinylformamide) (PNVF), followed by hydrolysis to PVAm.[8]

There has been a novel method developed by K. Suh et al, alternative to the typical method using template which needs harsh core removal procedure. In template-free method, it is essential to use polymer chains that are both hydrolyzable and disconnectable to covalently crosslink the hydrolyzed polymer chains to each other. Selectively removing the core part in the hydrogel particles enables to generate uniform hydrogel shells without using template.[9] Though, it is worth that this method can conserve biocompatible characteristics of carriers, it is hard to control the capsule size and shell thickness as intended, compared to the

typical procedure taking advantage of the Layer-by-Layer technique to control the thickness of the capsule membrane via the number of deposited polymer layers.[10] Thus, in the aforementioned strategy, attempts to the understanding of the underlying factors about well-controlled size and shape, and finely tuned capsule wall structure, would be meaningful.

Another critical issue is the mechanical stability of microcapsules. Under practical consideration, understanding and controlling the mechanical stability of PVAm capsules is important in that it ensures retention of cargo molecules during delivery. The present study employs one of the promising pathways, physical crosslinking of the capsule walls, wherein two biopolymers are reacted in their completely ionized states to increase the counterion charge density of the polymers, which enhances the overall interaction and hence the mechanical strength of the membrane.[11] Taken together, herein, polyion complexation of PVAm capsules with hyaluronic acid (HA) is carried with the aim to structurally modifying the capsule wall.

This study describes the underlying phenomena about controlling size and shell structure of PVAm hydrogel hollow capsules prepared by PNVF precursor, considering the effect of steric stabilizer concentration and crosslinking agent concentration. Furthermore, we suggest a strategy to manipulate the mechanical stability of prepared PVAm capsules, imparting mechanical strength by incorporation of the HA varied with molecular weight. Several characterizations are then performed to define the structurally tuned carriers.




**Scheme 1.** Schematic illustration of preparing PVAm hydrogel hollow capsules with tunable mechanical stability.

# Chapter 2. Experimental

## 2.1 Materials

N-Vinylformamide (NVF), N,N'-methylenebis(acrylamide) (MBA), poly(2-ethyl-2-oxazoline) (PEtOZO) (Mw ~50K, Mw ~200K), glutaraldehyde (GA) solution (50 wt% in $H_2O$) and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich-Fluka. Free-radical initiator, α,α'-Azobis(isobutyronitrile) (AIBN) was purchased from Junsei Chemical Co., Ltd., and recrystalized in ethanol. Sodium hydroxide (NaOH) and methanol were purchased from Samchun Pure Chemical Co., Ltd. Carboxyl group conjugated gold nanorod (AuNR) was purchased from Nanopartz. Hyaluronate-Rhodamine B (Mw ~20K, Mw ~1600K) was purchased from Creative PEGWorks. Hyaluronic acid sodium salts (Mw ~10K, Mw ~1630K) were purchased from Bioland Co. Ltd. All of the chemicals were used as received, except α,α'-Azobis(isobutyronitrile) (AIBN).

## 2.2 Synthesis of Poly(N-vinylformamide) Spherical Particles

The standard method and the fixed or varied parameters in the dispersion polymerization are listed in Table 1.

### 2.2.1 Dispersion Polymerization of Poly(N-vinylformamide) Spherical Particles; Control of N,N'-methylenebisacrylamide

In all experiments, 10 mL of methanol dissolving 100 mg of PEtOZO (10 wt% to monomer) was used as a medium. MBA, of which concentration was increased from 0 to 10 wt% relative to monomer, was entirely dissolved in 1 g of NVF, and then the mixture was added in the medium. 5 mg of AIBN (0.5 wt% to monomer) was introduced into the reactor followed by purging nitrogen gas through the solution for deoxygenation. The reactor was put in an oil bath having constant temperature of 70 ℃, stirred well with a magnetic bar at 150 rpm. The reaction mixture was allowed to polymerize for 24 h, then all unreacted monomers and additives were removed by repeated centrifugation with methanol.

### 2.2.2 Dispersion Polymerization of Poly(N-vinylformamide) Spherical Particles; Control of Poly(2-ethyl-2-oxazoline)

1 g of NVF dissolving 30 mg of MBA (3 wt% to monomer) was added in 10 mL of methanol containing PEtOZO (Mw ~50K), of which concentration was increased from 10 to 50 wt% relative to monomer. PEtOZO (Mw ~200K) was used with the concentration of 50 wt% relative to monomer. The following polymerization steps were same above.

## 2.3 Fabrication of Poly(vinylamine) Hydrogel Hollow Capsules

The modified method excerpted from previous works was used.[9] Preparation of mixture was carried out in a 70 mL vial with continuous stirring. An amount of 1 mL PNVF was dispersed in 14 mL methanol and uniformly mixed. 2 g of GA and 5 g of 2 N NaOH aqueous solution were added in sequence. After all the reagents are mixed the reactor was immersed in an oil bath at 70 ℃ and stirred with a magnetic stirrer (500 rpm) for 12 h under nitrogen atmosphere yielding a PVAm hydrogel capsules in methanol. The product was washed in methanol/water (volume %) mixture (70/30, 50/50, 30/70, 0/100 in sequence), leading replacement of dispersion medium from methanol to water. The average size and zeta-potential of capsules were determined by Dynamic Light Scattering Spectrophotometer (DLS-7000)

## 2.4 Preparation of Poly(vinylamine)-Hyaluronic Acid Polyion Complexes

To modify the mechanical properties of the hollow hydrogel capsules, they were treated with counterionic biopolymer, HA solution (Mw ~10K, and 1630K, 1 mg/mL in 0.15 N NaCl), for 4 h and then washed by repeated centrifugation with water. Scheme 2 shows overall reaction preparing PVAm-HA complexes. Prior to carrying HA incorporation, in order to induce photothermal decomposition, a means of demonstrating the light responsiveness of PVAm capsules due to polyionic complexation with HA, using photothermal agent was

found to be effective. Carboxyl polymer-conjugated gold nanorods (AuNR) were selected for agent, taking advantage of wavelength-selective absorption depending on the aspect ratio of AuNR.[12, 13] Out of the wide range of AuNR concentration, 15 nM of AuNR was added to 1 mL of PVAm solution under vigorous stirring. After 4 h, the solution was washed repeatedly to ensure removal of any excess AuNR. Subsequently, HA complexation described above was followed.

## 2.5 Characterization

### 2.5.1 Confocal Laser Scanning Microscopy (CLSM)

The solution (2 mg/mL in water) of FITC-dextran was mixed with the hollow hydrogels. After 4 h, mixture was washed by repeated centrifugation with water. Then, continuously, the FITC-labeled hydrogel capsules were treated by rhodamine B-conjugated hyaluronic acid with two different molecular weights (Mw ~10 K, and 1630K) for 4h followed by washing step as above. The structures of the PVAm-HA complexes were confirmed by direct observation with a Confocal Laser Scanning Microscope (Carl Zeiss LSM710, Germany).

### 2.5.2 Nuclear Magnetic Resonance (NMR) Cryoporometry

To carry quantitative analysis of micropores and mesopores, the NMR cryoporometry measurement was conducted using Nuclear Magnetic Resonance Spectroscopy 500MHz (Bruker Avance 500). A small amount of dry samples (PVAm-SHA, PVAm-LHA, and PVAm capsules) was placed in NMR tube,

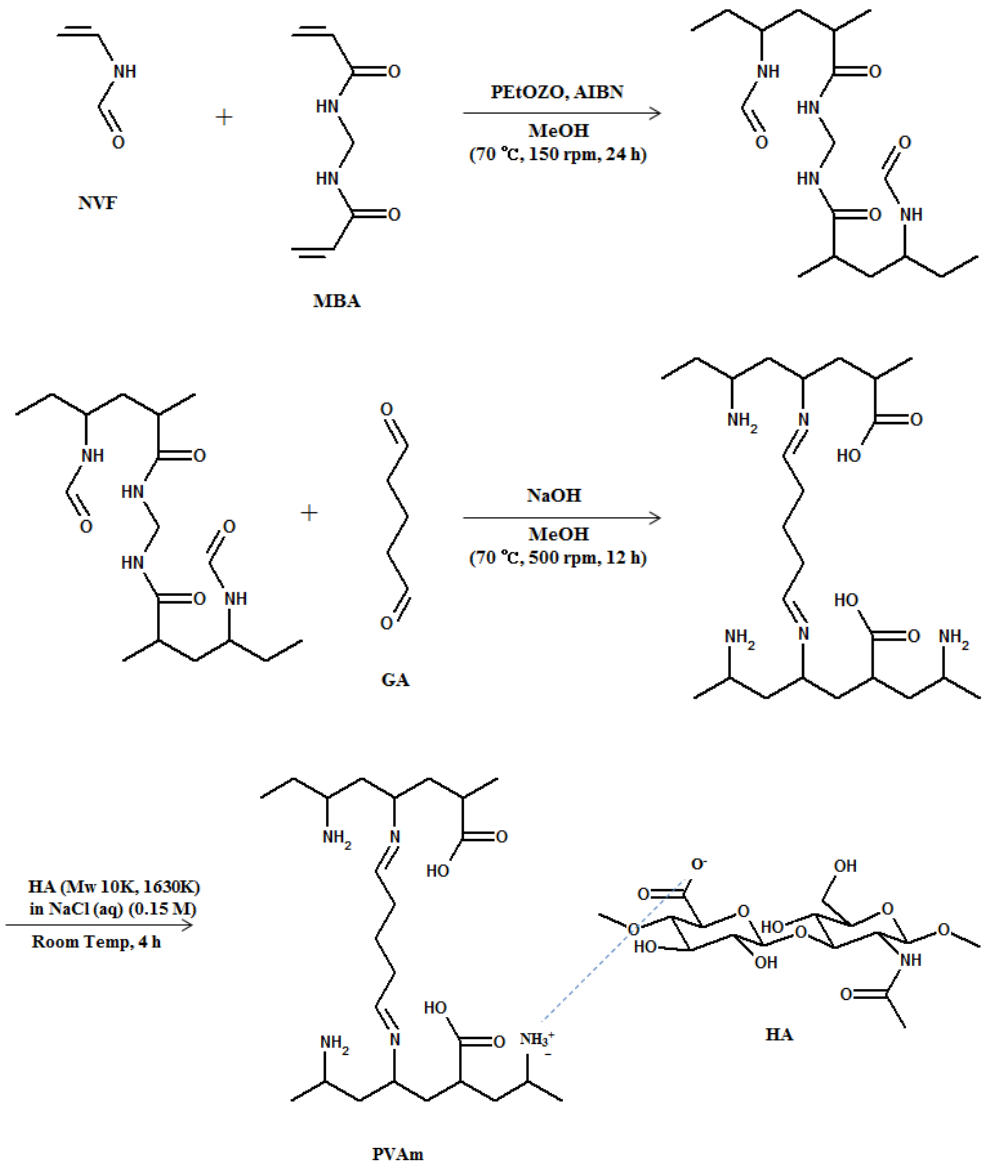
deuterium oxide added. Three samples, to be measured, were prepared in total amount of 600 μl, respectively. To confirm their structure, the temperature of the samples in the NMR probe was gradually decreased by a precision of 1 K within 289 K-275 K, while decreased by 0.5 K within 275 K-267 K. A fraction of deuterium oxide confined in the porous structure was estimated based on the melting temperature depression expressed as a signal of integral intensity relative to temperature.[14, 15]

### 2.5.3 Near-Infrared (NIR) Laser Irradiation

100 ul of PVAm-HA complexes was deposited onto the silicon wafer confined by a unit well. A Near-IR femto-second laser beam (Oscillator, repetition rate (80 MHz), 2 mW @800 nm) with varying duration time (1 min, 2 min, 4 min, and 7 min) was employed as the laser source, reason of relatively not injurious stimuli to cellular system.[16] After irradiation, investigation on the disrupted structure of PVAm-HA complexes was executed with a scanning electron microscope (JEOL JSM-6701F)

| Parameter | Standard Method | Variation Level |
|---|---|---|
| MBA concentration (wt % to monomer) | 3 | 0,1,3,5,10 |
| PEtOZO concentration (wt % to monomer) | 10 | 10,20,30,40,50 |
| AIBN concentration (wt % to monomer) | 0.5 | Fixed |
| NVF concentration (wt % to solvent) | 10 | Fixed |
| Polymerization media | Methyl alcohol | Fixed |
| Steric stabilizer type (g $mol^{-1}$) | PEtOZO (Mw ~50K) | PEtOZO (Mw ~200K) |
| Initiator type | AIBN | Fixed |
| Polymerization temperature(℃) | 70 | Fixed |
| Polymerization time (h) | 24 | Fixed |
| Agitation rate (rpm) | 150 | Fixed |

**Table 1.** Standard method and fixed/varied parameters in dispersion polymerization.




**Scheme 2.** Scheme of preparing PVAm-HA polyion complexes. (1) Preparation of PNVF through dispersion polymerization. (2) Amide bond hydrolysis followed by crosslinking of amine group with GA. (3) Polyionic complexation of PVAm with HA to obtain PVAm-HA complexes.

# Chapter 3. Result and Discussion

## 3.1 Dispersion Polymerization of Poly(N-vinylformamide-co-N,N'-methylenebisacrylamide) by Adjusting Synthetic Parameters

PNVF particles can be prepared by dispersion polymerization in a wide variation of parameters. The reaction requires that the monomer, initiator, and steric stabilizer are all soluble in the starting medium, and then the product polymer precipitates during the reaction.[17, 18] This method puts certain constraints on the types of solvents that can be used with starting materials. Temperature, of course, influences both the thermodynamic factors, especially solubility, as well as the kinetics of initiator decomposition, which in turn determines the rate of polymerization and particle nucleation. These factors can affect the structure of polymer product. In order to investigate on the effect of parameters chosen on particle formation in the dispersion polymerization of NVF, we set some criteria of medium, reaction temperature, and agitation rate.

### 3.1.1 Control of Crosslinking Agent Concentration

The dispersion polymerization of NVF in the presence of crosslinking agent was performed to produce hydrogel microspheres with novel crosslinking network. The crosslinking agent used was divinyl compound; MBA. Figure 1 displays TEM

micrographs of the particles prepared by dispersion polymerization with varying amounts of the crosslinking agent MBA. In this study the noncrosslinked PNVF particles were obtained in methyl alcohol medium with AIBN as the initiator and PEtOZO as the steric stabilizer (P1). Crosslinked (1 wt % (P2), 3 wt % (P3), 5 wt % (P4), and 10 wt % (P5) on the basis of NVF monomer) particles were also obtained in the same condition. In all cases examined, the copolymerization of NVF with variation of MBA concentration afforded relatively monodisperse particles. Furthermore, using the intersection method[19], originated by Mayo and Lewis[20], copolymerization reactivity ratio of NVF and MBA were calculated. Mayo-Lewis equation [eq. (1) below] can be represented by eq. (2), where $M_1$ and $M_2$ are the initial composition of NVF and MBA, respectively.

$$dM_1/dM_2 = [M_1(r_1M_1 + M_2)]/[M_2(r_2M_2 + M_1) \quad (1)$$

$$r_1 = r_2(m_1M_2^2/m_2M_1^2) + (M_2/M_1)[(m_1/m_2) - 1] \quad (2)$$

Treating $m_1M_2^2/ m_2M_1^2$ and $(M_2/M_1)[(m_1/m_2)-1]$ as the slope and intercept, each experiment represents a straight line, where exists intersect at a point in the $r_1$, $r_2$ plane.[21] From the values of $M_1/M_2$ and $m_1/m_2$ calculated from the ratios of the areas under peaks assigned in NMR spectra, as measured by the integral curves, reactivity ratios were obtained as shown in Table 3. The reactivity ratio indicates that MBA has tendency to be constructed into blocky part in the NVF/MBA copolymer.

### 3.1.2 Control of Steric Stabilizer Concentration

PEtOZO was reported to be an effective steric stabilizer for the preparation of micron-size monodisperse particles by dispersion polymerization.[22-24] Extensive literature demonstrating that NVF polymer particles ranging from 0.5 to 2 μm can be synthesized by varying the steric stabilizer concentration. Kobayashi group showed that where the PEtOZO concentration was higher than 10 wt% for NVF, relatively stabilized particles in micron range were obtained. Therefore, in this study, the dispersion polymerization of NVF with MBA was performed by using PEtOZO as a disepersant and 3 wt% of MBA.

The effect of PEtOZO concentration on the particle size is shown in Figure 2 and Table 4. The particle size decreased with increasing the steric stabilizer concentration, indicating that the steric stabilizer of higher concentration stabilized the particles more efficiently at early stage of growth to produce smaller polymer particles. Additionally, Change in molecular weight of steric stabilizer exerted a straightforward result that the particle size was found to decrease with increasing molecular weight of the PEtOZO, as illustrated in Figure 2, readily explained as the higher molecular weight PEtOZO results in a higher viscosity of the medium, leading to a higher equilibrium amount of adsorbed steric stabilizer and thus a thicker stabilizing layer.

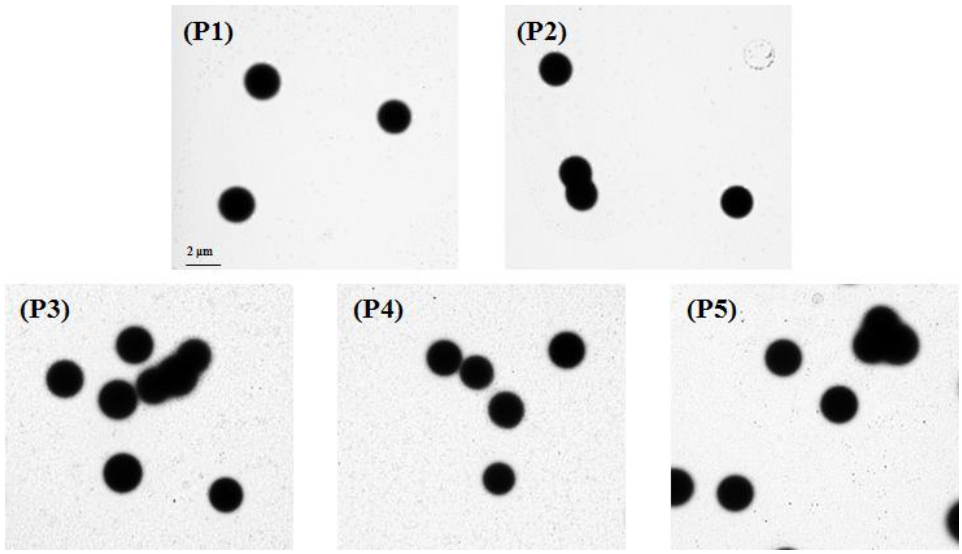



**Figure 1.** TEM micrographs of poly(N-vinylformamide) particles crosslinked by (a) 0 wt%, (b) 1 wt%, (c) 3 wt%, (d) 5 wt%, and (e) 10 wt%(P5) of N,N'-methylene bisacrylamide.

| Parameter | P1 | P2 | P3 | P4 | P5 |
|---|---|---|---|---|---|
| **MBA concentration (wt %)** | 0 | 1 | 3 | 5 | 10 |
| **Average size (µm)** | 2.00 | 1.72 | 1.94 | 2.10 | 2.11 |

**Table 2.** Variations and sizes of PNVF synthesized by adjusting MBA concentration.

| Comonomer pair | | Molar ratio in monomer mixture ($M_1/M_2$) | Molar ratio in copolymer ($m_1/m_2$) | Reactivity ratio |
|---|---|---|---|---|
| $M_1$ | $M_2$ | | | |
| **NVF** | **MBA** | 0.0055 | 174.56 | $r_1$=0.97 |
| | | 0.0400 | 22.11 | $r_2$=3.83 |

**Table 3.** Reactivity ratios calculated by NMR spectral analysis.

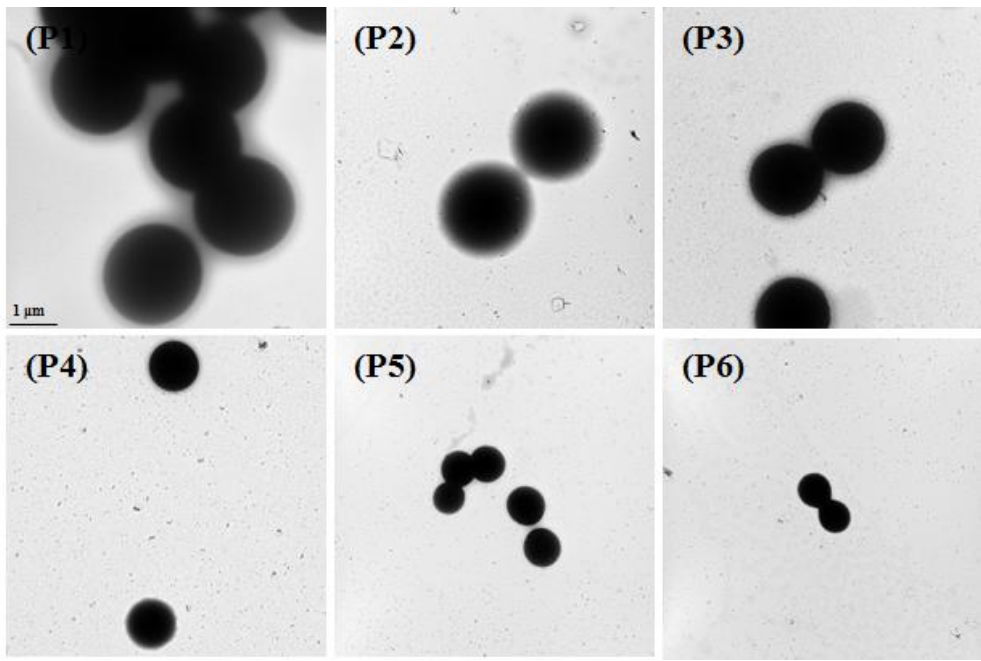



**Figure 2.** TEM micrographs of poly(N-vinylformamide) particles crosslinked by 3wt% of N,N'-methylenebisacrylamide prepared in different PEtOZO concentrations and molecular weights.

| **Parameter** | **P1** | **P2** | **P3** | **P4** | **P5** | **P6** |
|---|---|---|---|---|---|---|
| **PEtOZO (wt %)** | 10 | 20 | 30 | 40 | 50 | 50 |
| **PEtOZO type (g mol$^{-1}$)** | | | Mw ~50K | | | Mw ~200K |
| **Average size ( $\mu$ m)** | 2.16 | 1.81 | 1.54 | 1.03 | 0.77 | 0.56 |

**Table 4.** Variations and sizes of PNVF synthesized by adjusting PEtOZO concentration.

## 3.2 Poly(vinylamine) Hydrogel Hollow Capsules; Effect of Crosslinking Agent

Poly(vinylamine) (PVAm) is a polyamine possessing primary amino group directly linked to the backbone. The synthesis of PVAm was achieved by polymerization of PNVF followed by basic hydrolysis. In this study, the base concentration, reaction temperature, and reaction time were set at level of 2 N, 70 ℃, and 12 h, respectively. In given conditions, the hydrolysis of amide group in NVF proceeded to a complete conversion.[8] Both the hydrolysis of vinylformamide to vinylamine and the some breakage of amide bonds between NVF and MBA, which then allows the cleaved PVAm chains to diffuse out of the particles, seem to be essential for the generation of hollow capsule structure without using templates.[25] On preparing PVAm hollow capsules, crosslinking reaction of GA occurred consistent with basic hydrolysis reaction for additional crosslinking.

### 3.2.1 Control of N,N'-methylenebisacrylamide Concentration

Depending on the MBA concentration, PNVF in Figure 1 (PNVF with various MBA concentrations) resulted in dissimilar form of PVAm. Figure 3 is the TEM image of PVAm constructed with GA as a crosslinking agent. As shown in Figure 3, MBA concentration up to 1 wt% (The percentage of MBA herein referred to relative weight to monomer) failed to produce PVAm hollow capsules, merely formed agglomerated polymer chain. Primary amine group generated by hydrolyzing of amide bond in PNVF enable to form a new crosslinking, represented as C=N imine bond, with the portion of aldehyde group in GA.

Simultaneously, amide bond in MBA became hydrolyzed, leading to determination of laterally crosslinked-structure. Herein, surface stiffening by crosslinking occurs consecutively with the evolution of residues diffusing out, as described in Figure 3, the result that maintenance of shell structure over MBA 3 wt%, indicates crosslinking networks formed by new bonding of GA can be only possible when the concentration of MBA is above a significant level. Additionally, the shell structure failed to be retained in basic hydrolysis, where reaction time, 12 h, was sufficient for cleavage of amide bond in NVF, without insertion of GA (not shown in this paper). Even though MBA hardly maintained the shell structure of PVAm unaided, it can functioned as holding material of whole structure until imine bonds are formed by GA diffused toward the interior.

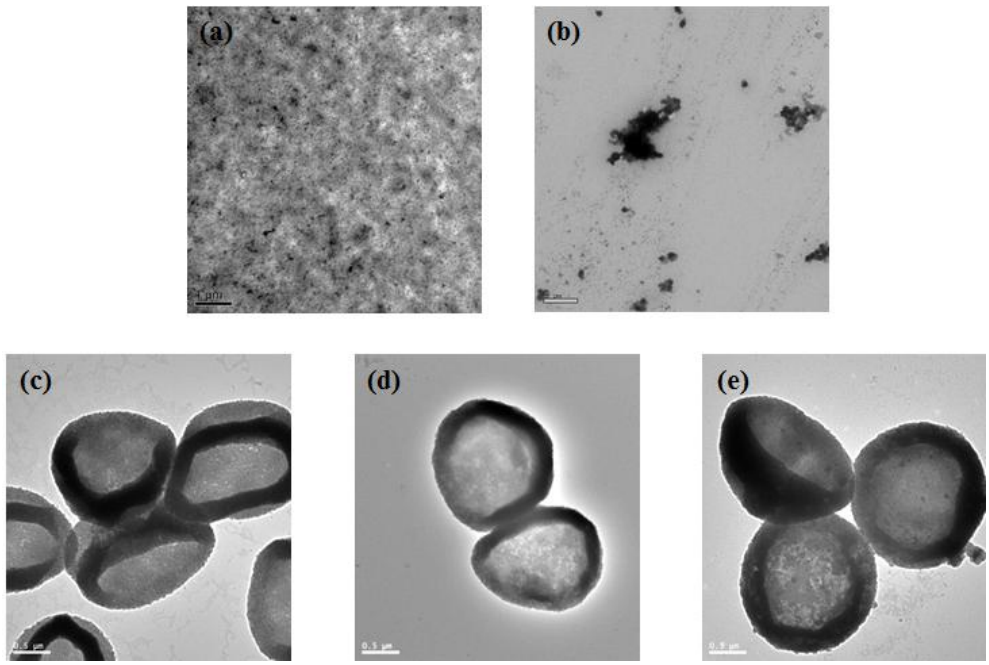



**Figure 3.** TEM images to show the PVAm capsules formed in a concentration series of PNVF particles varied with MBA concentration. (a), (b), (c), (d), and (e) are corresponding to 0 wt%, 1 wt%, 3 wt%, 5 wt%, and 10 wt% of MBA, respectively.

## 3.3 Poly(vinylamine) Hydrogel Hollow Capsules; Effect of Particle Size

As mentioned above, when hollow capsules are formed, both hydrolyzing of amide bond and crosslinking by GA exhibit significant consequences. To analyze the tendency of getting hollow enables to compare the relative reaction rate of hydrolysis and GA crosslinking. To examine this, varying the size of precursor, PNVF particle, was conducted at the given conditions displayed in Figure 2. It is worthwhile noting that the images presented in the Figure 4 give indication of reaction rate of NaOH as hydrolyzer and GA as crosslinking agent. Irrespective of the precursor size variation, to the limit of 700 nm, hollow capsules were realized. It can be stated that NaOH can penetrate entirely close to the core of PNVF, leading to complete elimination of inner part whereas there exists the limiting penetration depth of GA. In addition, though it is not possible to practically measure the shell thickness of PVAm capsules, they were almost same 150 nm in size, which means, definitely, diffusion layer of GA attained to a definite range. Note that this could be explicated that the shell thickness is determined dominantly by the diffusion layer limitation of GA.

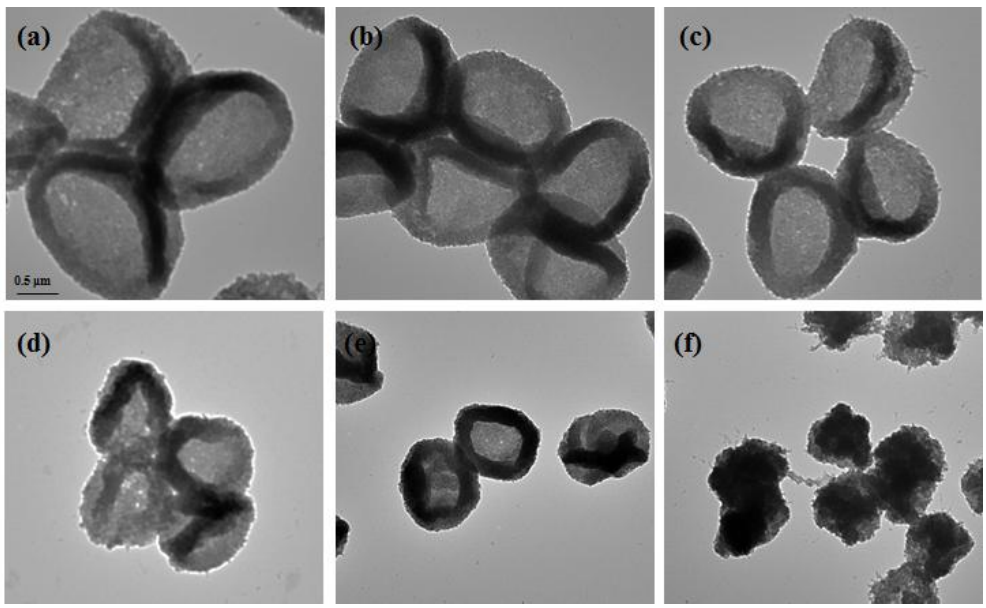



**Figure 4.** TEM images to show the PVAm capsules formed in a concentration series of PNVF particles varied with PEtOZO concentration and molecular weight. (a), (b), (c), (d), and (e) are corresponding to 10 wt%, 20 wt%, 30 wt%, 40 wt%, and 50 wt% of PEtOZO (Mw ~50K), respectively. (f) corresponds to 50 wt% of PEtOZO (Mw ~200K).

## 3.4 Poly(vinylamine)-Hyaluronic Acid Complexation; Modification of Network System

Surface modification of poly(vinylamine) (PVAm) capsules to polyion complex composed of hyaluronic acid, a sort of biopolymer, was conducted in order to control the mechanical property of capsules. The formation of polyanion–polycation (polyelectrolyte) complexes for stability improvement is mainly driven by an electrostatic mechanism where charge neutralization and possible local overcompensation or bridging (such as hydrogen bonding, Coulomb forces, van der Waals forces, and transfer forces) mediated by a multivalent counterion induces attraction between segments of the polyelectrolytes.[11] Reaction in their highly ionized states to increase the counterion charge density of the polymers, which increases the overall interaction and hence the mechanical strength of the PVAm-HA complexes, could be achieved by maintaining the pH values of the PVAm and HA solutions at neutral pH. In given conditions, polyionic complexation between positive amine group and negative carboxylate group resulted in enhancement of structural stability.

### 3.4.1 Influence of Hyaluronic Acid Molecular Weight on Complexation

As shown in the Figure 5, the PVAm capsules were prepared in standard method, resulting in the average diameter of 2.48 μm. These samples were modified with varied HAs to demonstrate the effect of complexation on the structure. Degree of polyionic complexation, resulting in change of mechanical

stability can be influenced by the intensity of electrostatic interaction related to the charge density and local accessibility. We suggested accessibility to counterion, the latter factor, would be varied with the molecular weight of hyaluronic acid, consisting of 10K and 1630K. PVAm-HA complexes, different in HA molecular weight, were prepared in following steps. (1) Preparation of PVAm hollow capsules in water medium, (2) polyionic complexation between PVAm and HA. To confirm the presence of the complexed moiety, the labeling of amino groups with fluorescein isothiocyanate(FITC) followed by complexation with Rhodamine B-conjugated HA was conducted (Figure 6). That both PVAm-LHA and PVAm-SHA displayed yellow around the shell of PVAm capsules means that interchain complexation reaction is successfully complicated. And the predicted structure of each complex is described in Scheme 3. In regard of the dependency of long-range self-diffusion coefficient of a polymer chain on the chain length,[26] there exists discrepancy in diffusivity between LHA and SHA, leading to difference in network construction.

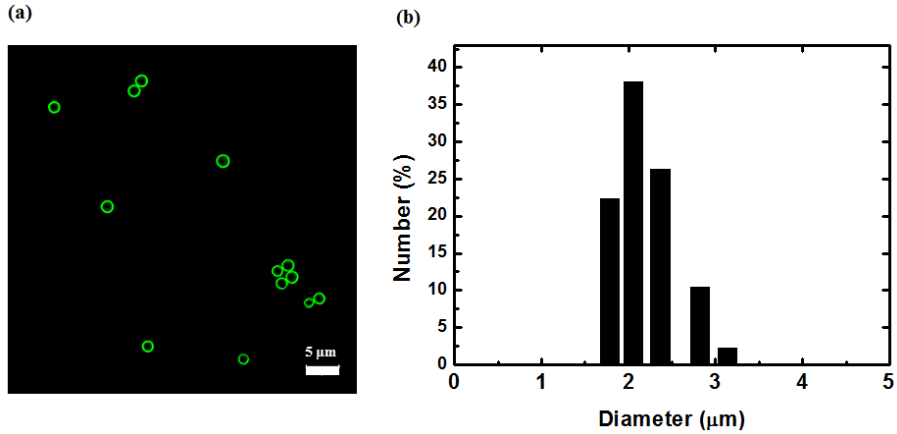



**Figure 5.** PVAm hydrogel hollow capsules. (a) CLSM image and (b) size distributions of PVAm capsules. The average size of PVAm capsules is 2.48 ± 0.2 μm in diameter.

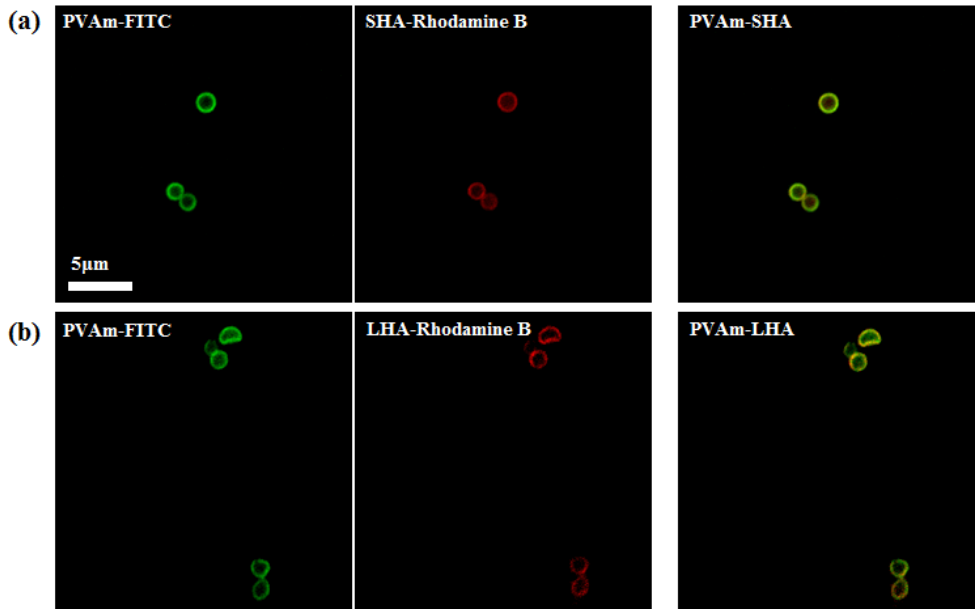
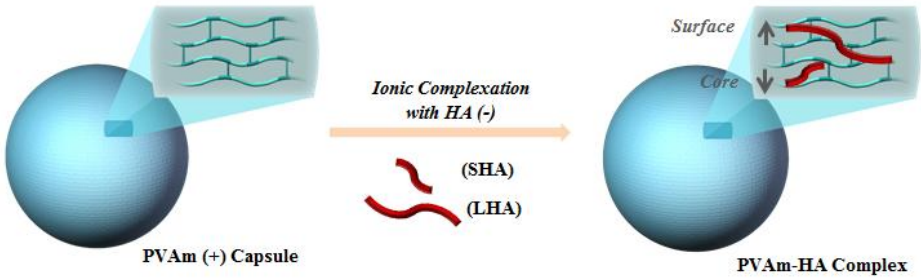



**Figure 6.** CLSM images of positively charged PVAm capsules complexed with negatively charged (a) short HA chains (SHA, 10K-20K) and (b) long HA chains (LHA, 1600K-1630K). FITC-labeled PVAm capsules (green, left) were associated with rhodamine b-conjugated HA (red, middle). Yellow fluorescence (right image) indicates that PVAm capsules were readily formed the ionic complexations with both SHA and LHA.




**Scheme 3.** PVAm-HA polyionic complexation. Schematic illustration on interchain physical crosslinking by two different molecular weights of HA.

### 3.4.2 Pore Size Distribution within Poly(vinylamine)-Hyaluronic Acid Complexes

Analysis on the enhancement in mechanical stability of crosslinked structures is available via assessment of exhibiting pore size after complexation. While traditionally, scanning electron microscopy (SEM) and mercury porosimetry have been used to characterize microparticle porosities and morphology, NMR cryoporometry have become alternative to precisely deal with the complexities of biodegradable microparticles. By determining the amount of liquid at different temperatures, an apparent pore size distribution can be calculated. The pore size distribution, p(d), was calculated from I(T) using the Gibbs-Thompson relation for the melting point depression as (Strange et al., 1993).

$$\mathrm{p(d)} = \frac{K}{d^2}\frac{\partial I}{\partial T} \tag{3}$$

where d is an effective diameter for assumedly cylindrical pores, and the material constant $K=(2\upsilon\gamma_{sl}T^0/\Delta H)$, where $\upsilon$ is the molar volume, $\gamma_{sl}$ is the free energy of the solid-liquid interface, and $\Delta H$ is the latent heat of melting of the pore-filling material of water set to the generally accepted value of 50 K nm.[14] From the I-T curve obtained, (Figure 7) the average pore size can be estimated as exhibiting difference in the extent of tightening. The sizes correspond to 18.9 nm, 14.7 nm, and 10.3 nm for PVAm, PVAm-LHA, and PVAm-SHA, respectively. Namely, the pore size becomes smaller when polyionic complexation was induced, particullary, using the shorter HA chain. In-depth explanation on these results will be discussed in later chaper.

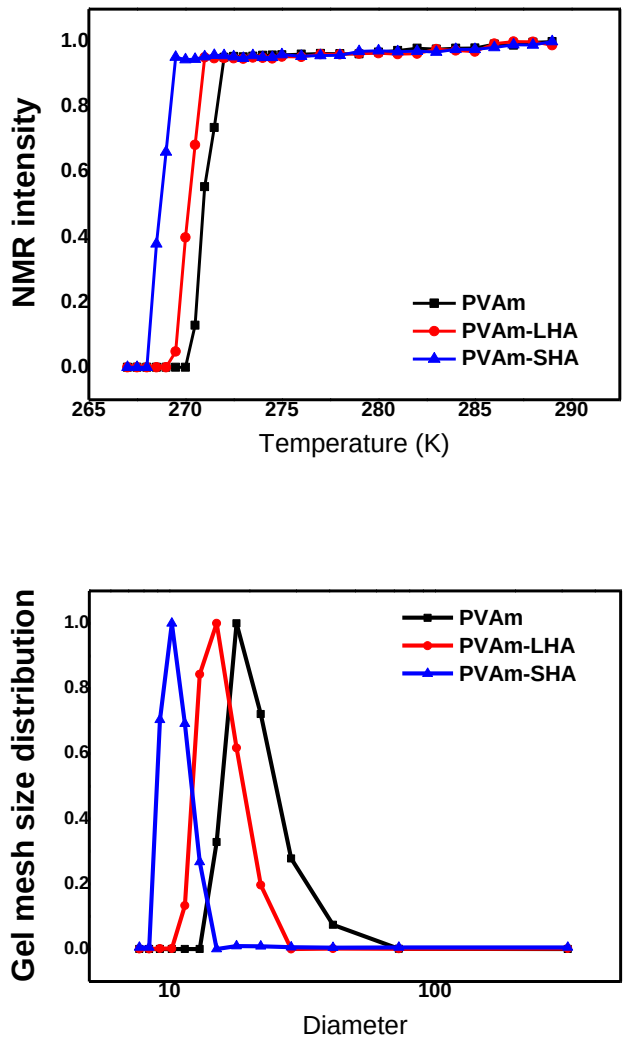



**Figure 7.** Hydrogel mesh size of PVAm-HA complexes characterized by NMR cryoporometry. The pore size of PVAm, PVAm-LHA, and PVAm-SHA was estimated at 18.9, 14.7, and 10.3 nm, respectively.

### 3.4.3 Light Durability of Poly(vinylamine)-Hyaluronic Acid Complexes

To investigate the modified stability against external stimuli as well as to span our research to the field of bioengineering inside living cells, laser irradiation in the near-infrared region, where light absorption by tissue is minimal,[27] was selected as a device of stimulation. To design the complexes with enhanced absorption in the near-infrared region, particularly 800 nm wavelength, AuNR with aspect ratio of 4.0 was introduced.[27-28] With an aim to manipulating for finely dispersed nanorods on the capsule wall through electrostatic interaction with positive amine group, carboxyl polymer-conjugated AuNR were used. Figure 10a, b display the PVAm-AuNR hybrids with a surface plasmon resonance peak around 800 nm. After exposed to 2 mW NIR laser irradiation, the PVAm-AuNR hybrids were entirely burned out (Figure 8c). It is clearly apparent in Figure 9 that there exists distinct discrepancy between the samples. The PVAm capsules containing long chain HA were severely deformed after 7 min, whereas the capsules with short chain HA are seen retained intact. In accordance with those results, the PVAm capsules without complexation were completely burned out as presented in Figure 9. Based on this observation, we speculate that the reinforced mechanical stability might be induced by polyion complexation, herein, which the only variation was the molecular weight of counterpart molecule. As considered above (Scheme 3), with the smaller chain length, it was possible for HA to deeply penetrate into the capsule contrary to the long chain, and thereby promote lateral tightening throughout the shell structure.

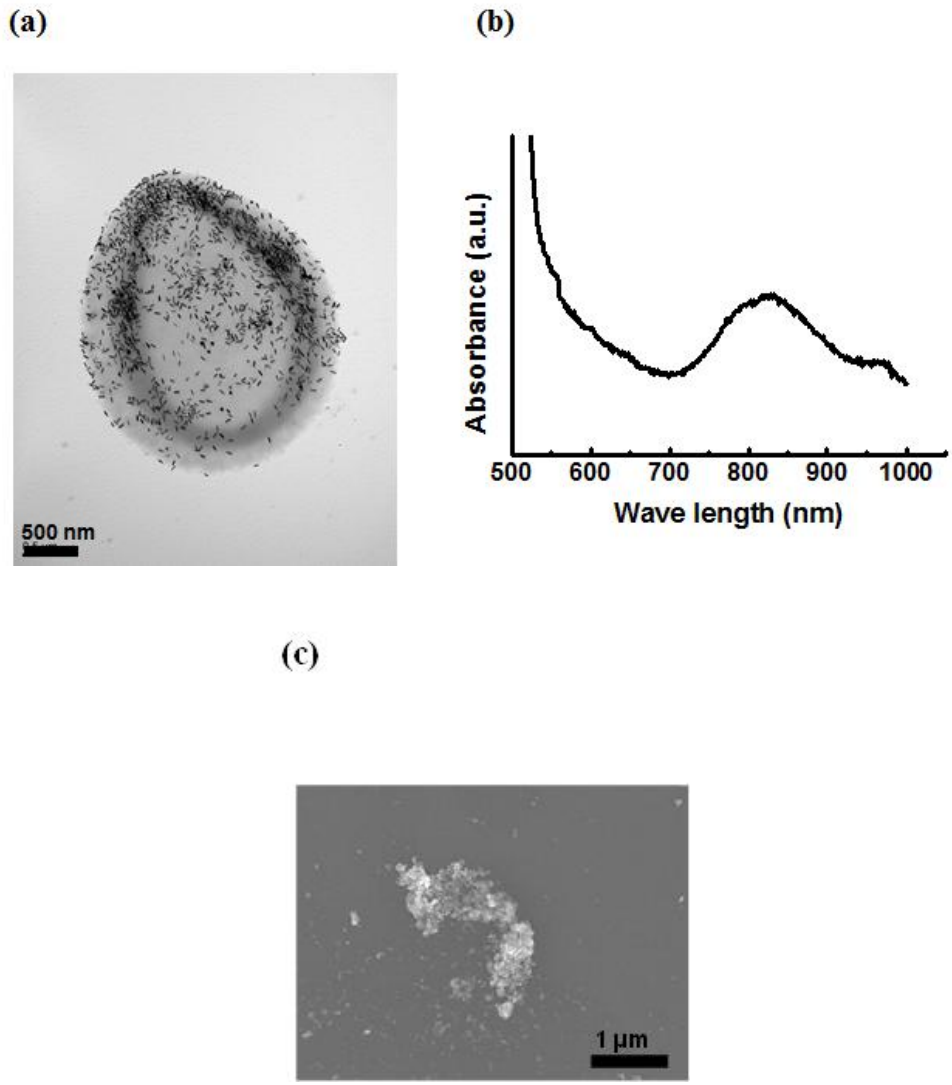



**Figure 8.** Characterization of PVAm-AuNR hybrids. The AuNRs are carboxyl polymer-conjugated type, exhibiting negative zeta potential of -25.7 mV with aspect ratio of 4.0 (a) TEM image and (b) absorption spectrum of PVAm-AuNR hybrids with a surface plasmon resonance peak around 800 nm. (c) SEM image of PVAm-AuNR hybrids after 7 min NIR light irradiation. All capsules were burned out.

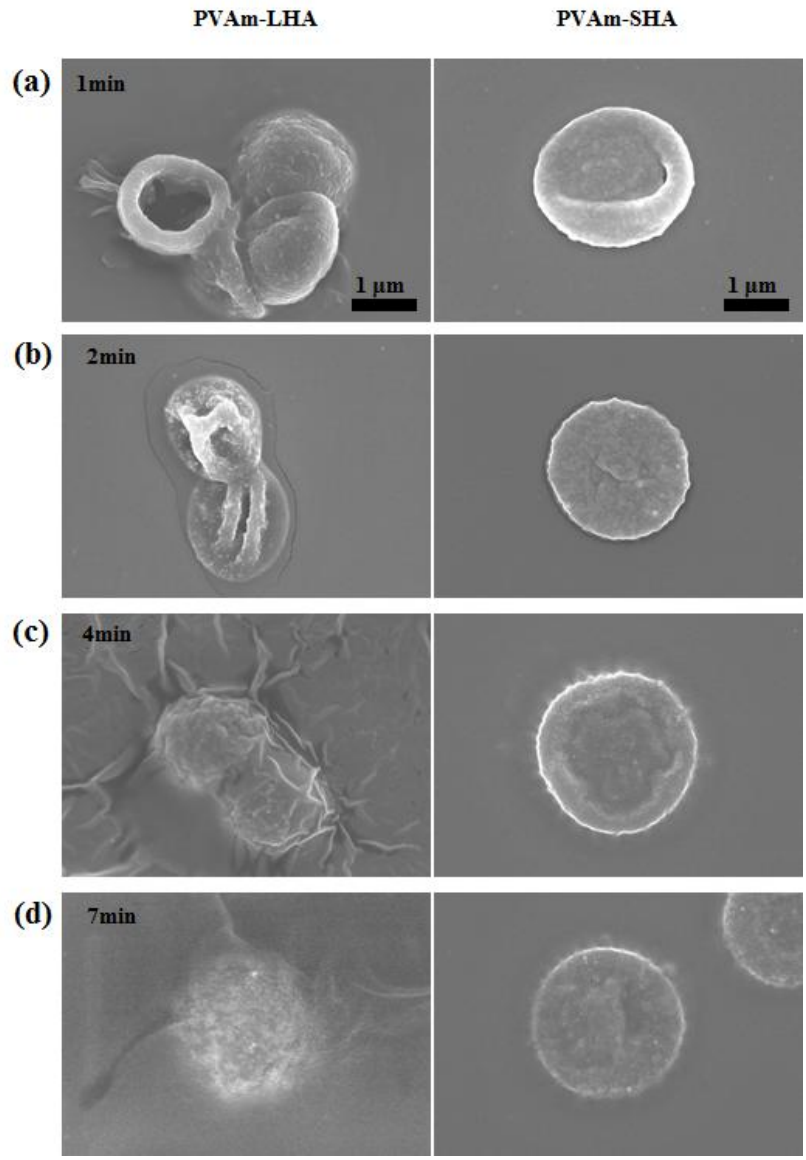



**Figure 9.** Light stability of PVAm-HA capsule complexes. SEM images of PVAm-SHA and PVAm-LHA after (a) 1 min, (b) 2 min, (c) 4 min, and (d) 7 min of NIR light irradiation. PVAm-LHA exhibited destruction after 4 min and consequently burned out at 7 min, while PVAm-SHA remained intact in the given range of duration time.

# Chapter 4. Conclusion

Synthesis of PVAm hydrogel hollow capsules provides the opportunity to extend the applications of gel materials as carriers. In this report, poly(N-vinylformamide) was polymerized by dispersion polymerization and hydrolyzed to PVAm, concurrently there existed newly generated crosslinking bond by glutaraldehyde. We studied several parameters that govern the structure of PVAm, adjusting the crosslinking density and particle size of PNVF. A minimum of 3 wt% of crosslinking agent MBA was necessary to facilitate PVAm hollow capsules with stable shell structure. In addition, increasing the steric stabilizer concentration up to 50 wt% (Mw ~50K), leading to decrease in size below 800 nm, allowed PNVF to be obtained as PVAm hollow capsules, while PNVF of which diameter is close to 500nm, almost double of the shell thickness, failed to realize hollow structure. These imply that the primary factor to control hollow shell thickness might be GA, namely penetration depth of GA, not MBA which can only serves as a structure holding material against amide bond hydrolysis. Continuously, to improve mechanical stability of capsules, a simple and convenient polyionic complexation was suggested here, that is physical crosslinking between two counterions. With two different molecular weights of HA, we successfully demonstrated the feasibly enhanced structure of PVAm. Particularly, using short chain HA lead to decrease in pore size by 45 % compared to non-complexed PVAm capsules, while with the long chain HA, the pore size decreased by 22 %. In addition, the durability test against near-infrared laser irradiation displayed the results in accordance with NMR cryoporometry measurement. Thereupon, the PVAm-HA capsules prepared

by aforementioned method are to be a prospective template wherein mechanical stability can be practically modulated. In advance of presented results, future aims lie in the use of PVAm hydrogel capsules possessing specific structural properties as desired.

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# 초 록

본 연구는 폴리비닐포름아미드 구형 입자로부터 중공형 폴리비닐아민 하이드로젤 캡슐을 주형없이 제조한 후, 히알루론산과 복합체를 형성시킴으로써 물리적 강도를 제어할 수 있는 전달체를 구현하기 위한 연구이며, 형성된 복합체의 구조적 특성을 다각적으로 분석하였다. 일반적으로 주형을 사용하지 않고 합성한 중공형 캡슐의 경우, 크기가 균일하지 않지만 본 연구에서는 분산중합을 통해 합성한 구형 입자로부터 중공형 캡슐을 제조하였기에 균일도가 매우 높은 캡슐을 구현할 수 있었다. 중공형 구조가 형성되는 원리를 규명하기 위해 구형 입자의 가교도 및 크기를 변화시키면서 캡슐을 제조하였으며, 그로부터 가교제인 메틸렌비스아크릴아미드와 글루타알데히드는 각각 중공형 구조의 지지체 및 캡슐 외곽 두께의 결정 요인이라는 것을 확인하였다. 또한 중공형 캡슐의 물리적 특성 제어를 위한 복합체 형성 물질로 고분자 사슬의 길이가 다른 두 가지 (10 K, 1630 K)의 히알루론산을 이용하였으며, 결과적으로 고분자 사슬 길이에 따라 최종 복합체의 기공 크기, 기계적 강도 및 광내열성의 차이를 구현할 수 있었다.

**주 요 어:** 폴리비닐아민, 중공형 하이드로젤 캡슐, 분산 중합, 히알루론산, 근적외선 조사, 기계적 강도

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