공학석사학위논문

Hybrid Hydrogels of Laponite and Chitosan made by Electrostatic Interaction

클레이와 키토산의 정전기적 인력을 이용한 하이드로젤 제작 및 점탄성에 관한 연구

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재료공학부

오희선

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이 논문을 공학석사학위논문으로 제출함.

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Abstract

Hydrogels are wet and soft matters consist of three dimensional network structure and high water content. Hydrogels have been applied to various fields such as household items, biomaterials, drug delivery and tissue engineering and so on. But polymer hydrogels have limitations with respect to mechanical and stimuli-responsive properties. Thus, in order to overcome the shortcomings, inorganic materials are introduced to polymer hydrogels and the hybrid hydrogels are prepared.

In this work, the hybrid hydrogel is fabricated by mixing clay and chitosan and improved to mechanical property. Clay, synthetic hectorite, has negative charges on its surface and positive charges on its edge when dispersed in deionized water. And the other component, chitosan, is dissolved in acid aqueous media under pH 6, and amine groups in chitosan chain protonated. Formation of clay-chitosan hybrid hydrogel is initiated through electrostatic interaction between the surface of clay and the amine groups of chitosan. To identify the role of chitosan in the gel formation, systematically we varied the concentration and observe the gel forming capability. And to examine improved mechanical property of clay-chitosan hydrogels, the shear modulus values G′ and G″ are measured clay-chitosan hydrogels which contained all three components or except for one component and also measured depending on chain length and type of chitosan. According to the experiments, we discovered that charge balance is the most important factor to obtain the robust hybrid gels with high modulus. Furthermore, the resulting gel can be moldable and the other materials such as nanoparticles or cell can be easily incorporated inside gel.

Keywords: Hydrogels, hybrid materials, chitosan, electrostatic interaction, self-assembly.

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PAAS/Chitosan 60k = 1/0.08/0.08, (c) Clay/PAAS/Chitosan 330k = 1/0.03/0.08, (d) Clay/PAAS/Chitosan 330k = 1/0.08/0.18.

Chapter 1. Introduction

1.1. What is a gel?

Gels consist of cross-linked three-dimensional networks and swollen liquid.[1] The wet and soft materials are in the states between solid and liquid. The three dimensional structures of gels are made up of hydrophilic polymers, macromolecules, inorganic materials. Gels are classified based on origin, medium, size, cross-linking system and so on. **Table 1-1.** shows various classifications of gels. In many categories, gels can be divided into two types based on the nature of cross-linking, chemical and physical gels. The chemical gels are used covalent bonding for formation of its network structure with or without cross-linker, the physical gels, however, are used non-covalent bonding such as molecular entanglement, electrostatic interaction, hydrogen bonding and hydrophobic interaction. While the chemical gels have excellent mechanical properties, the physical gels are good at response depending on environments. Gels have been exploited a variety of field, especially, hydrogels whose swollen liquid is water, have been applied biomaterial, tissue engineering, drug delivery and so on.[2-6] Some gels which response to external stimuli such as pH, temperature, light, electric and magnetic fields have been also used actuator and micro-valves and so on as well as biotechnology. Also, thin and flexible transistors can be fabricated by ion gels swollen ionic liquid into polymeric 3D structures with polymeric semiconductor or graphene. [7-14]

Table 1-1. Classification of gels.

Origin	Natural gel	
	Synthetic gel	
Medium	Hydrogel	Water
	Organogel	Organic solvent
	Aerogel, Xerogel	Air
Size	Macrogel	
	Microgel	
	Nanogel	
Type of Cross-linking	Chemical gel	Covalent bonding
	Physical gel	Non-covalent interaction (Entanglement, electrostatic interaction, hydrogen bonding and hydrophobic interaction etc.)

1.2. Chitosan hydrogels prepared by physical cross-linking

In diverse forms of chitosan, hydrogel type of chitosan is attractive with respect to applications, mainly biomedical and biotechnology fields.[4, 5, 15] Chitosan hydrogels can be prepared by chemical and physical cross-linking, but our discussion will focus on physically cross-linked approaches. As mentioned above, physical cross-linking is the way that 3D-network structures of a gel are produced without cross-linker, namely used non-covalent bonding such as electrostatic interaction, hydrogen bonding, and hydrophobic interaction. Due to the positive charges of protonated chitosan in acidic media, amine groups of chitosan can interact with either anions or anionic molecules by electrostatic interaction.[16, 17] Sulfate, citrate, tripolyphosphate are commonly used.[18] Although β-glycerol phosphate is also used an anionic molecule for gelation, it does not seem to induce electrostatic interaction. Even though it is not clear what is the role of β-glycerol phosphate in chitosan dispersion, β-glycerol phosphate able chitosan to be dissolved near neutral pH and can be triggered physical gelation upon heating.[19, 20] Chitosan has been known for its chelating properties, so chitosan can be also formed complex or cross-linking with metallic ions by coordinate interactions. Mo(Ⅵ) or Pt(Ⅱ) are commonly used as ionic cross-linkers.[21, 22]

And Domard and co-workers have obtained chitosan physical hydrogels without any cross-linkers.[23] At first an alcohol gel is formed from mixture of chitosan and alcohol by an evaporation of water. And while the alcohol gels immerse in sodium hydroxide and charges inside the alcohol gels are disappeared. Finally, chitosan physical hydrogels that contains water only are obtained. The chitosan hydrogels can be made an onion-like multi-membrane structure by interruption of the neutralization.[24]

1.3. Hybrid hydrogels

Hybrid hydrogel consisting of inorganic and soft materials has many potential applications in stimuli-responsive sensors and actuators as well as biomaterials and biotechnology.[25, 26] Hybrid hydrogels are divided into two groups depending on their purpose. The purpose of one group is the improvement of mechanical performance of hydrogels, and that of the other group is the addition to stimuli-response properties such as temperature, pH, light and electric and magnetic fields.[26] In general, the introduction of clay nanoparticles to polymer system improves mechanical property of polymer hydrogels. In other words, the role of clay in hybrid hydrogels is cross-linking agents and enhancement of network strength.

Clay and poly(ethylene oxide) mixture has one of the forms such as solution, flowing gels, shake gels, or permanent hydrogels depending on the clay and PEO compositions. One of the various types of clay-PEO gels, shake gel, gelation induced by shear forces, is reversible and the gelation is dependent on the polymer concentration, time, temperature, and molecular weight. The shear deforms the large clay-PEO aggregates and exposes fresh surface area for the formation of new polymer bridges, which rapidly form a network that spans the entire solution and forms a gel. When the shaking stops, thermal fluctuations are sufficient to desorb the polymer from the nanoparticle, and the hydrogel relaxes back to a fluid after some time.[27, 28]

Aida and co-workers have fabricated hybrid hydrogels by mixing clay and dendritic molecules linked by PEG. Because guanidinium groups in dendritic molecules have positive charges and the surface of clay has negative charges, the two components would interact with each other, which enable to form 3D-network structures in the hydrogels. Although the physical gels cross-linked by non-covalent interactions are very weak and can be easily disassembled into their components, the hybrid hydrogels have a high mechanical strength. The storage modulus value of the hydrogels at 5.0% clay concentration is 0.5MPa. Interestingly, these hydrogels also have the ability to heal themselves without any reagents or any stimuli. Once the surfaces freshly cut of hydrogel blocks just pushed together, the connection of the blocks takes place.[29]

Haraguchi and co-workers have obtained hydrogels with clay cross-linked PNIPAM by *in situ* free radical polymerization. In this system polymerization of monomer is initiated from the surface of clay, the polymers on the clay grow longer, and then the polymer link between clay particles and form a 3D-network structures. In the view of polymer chains, the clay nanoparticles have a role that is a cross-linker between the chains. The mechanical strength of clay-PNIPAM hydrogels is superior to polymer hydrogels and the hybrid hydrogels also have the ability to self-healing.[30, 31]

The ferrogel, introduced magnetic nanoparticles into polymeric matrix, is an example of stimuli-responsive hydrogel driven by the external fields. Generally, magnetic nanoparticles are synthesized in polymer matrix cross-linked by covalent bonding, but hybrid micelles formed with magnetic nanoparticles and triblock copolymer can be prepared by non-covalent interaction between the micelles.[32] The ferrogel can react different ways depending on uniformity of magnetic field.

1.4. Biopolymer: chitin and chitosan

Chitosan is not wide spread in nature, but that can be obtained from chitin which is the most abundant biopolymer after cellulose and is one of the components in the exoskeleton of crustaceans and insects. Chitosan belongs to the family of linear polysaccharide composed of randomly distributed D-glucosamine and N-acetyl-D-glucosamine unites. It produced by the deacetylation of chitin, and the degree of acetylation of chitosan is below 50%. **Figure 1-1**(a) is chemical structure of chitin and chitosan and (b) is the most popular sources of chitin: crab and shrimp. Although chitin is widespread in nature, up to now the main commercial sources of chitin have been crab and shrimp shells. Chitin is extracted from crab and shrimp shell by acid treatment to dissolve calcium carbonate followed by alkaline extraction to remove proteins.

The factors such as molecular weight, DA, viscosity are very important to define the quality and properties of chitosan. The molecular weight of chitosan is determined by chromatography, light scattering and viscometry.

Among the methods for the determination of molecular weight, viscometry is an easy and rapid method to be obtained molecular weight of chitosan. The intrinsic viscosity [η] that relate experimental values of constants K and α is expressed by well-known Mark-Houwink equation, $[\eta] = KM^{\alpha}$. Also molecular weight is obtained by light scattering using the dn/dc values. And there are various methods for the determination of its DA, titration, IR, UV, NMR etc. The viscosity of chitosan is influenced by many factors such as molecular weight, DA, ionic strength, pH and temperature. The viscosity of solution decreases as the temperature rises, and the larger molecular weight, the higher viscosity of chitosan solution.

Chitin is insoluble in water or usual organic solvents, but chitosan is soluble in aqueous acidic media, under pH 6. Acetic acid and dilute hydrochloric acid are common solvents for dissolution of chitosan. The solubility of chitosan is also influenced by DA, solvent, chemical modification. Unlike chitin, chitosan is dissolved in acidic media because the amino groups are protonated by protons in acidic solution. Therefore, as the amino groups in the polymer chain increase, the solubility of the polymer solution also increases.

Unlike chitin, chitosan has the amine functions in its chain which are involved in interactions with metal ions, surfactants and oppositely charged polymers. It is possible chitosan to form complex due to these interactions. This chelation of chitosan depends on the state of that and the greater degrees of deacetylation, the better chelation of chitosan because chelation is related to the amino functions

Depending on sources, chitin is divided into three forms, α-, β-, γ-chitin (**Table 1-2**). And chitosan also has three forms, like chitin, but structure and properties of the three forms are similar to those of chitin except for solubility. α-chitin is the most abundant and the most stable thermodynamically in all polymorphs of chitin and is found in lobster, crab tendons, shrimp shells and insects cuticles. β-chitin is rarer than the α form and a common source of β-chitin is squid pens. γ-chitin, the third form of chitin, has a structure of a chain, which comes in mixed parallel and antiparallel arrangements.[33]

Chain structures of α- and β-chitin reveal that α-chitin consists of an antiparallel arrangement of molecules, while β-chitin has a parallel arrangement. Both chitin chains make sheets by intra-sheet hydrogen bonding, CO···NH. In α-chitin sheets, there is another interaction, inter-sheet hydrogen bonding, involving the hydroxymethyl groups, whereas β-chitin does not have this (**Figure 1-2**). Therefore, α-chitin has stronger interaction between chains than the other form, β-chitin. This feature, due to which the sheets of β-chitin are tightly bound themselves but there are no inter-sheet hydrogen bonds, can allow polar molecules such as water or alcohol penetrate easily the lattice of β-chitin. α-chitin, on the other hand, does not penetrate into the lattice by water or alcohol. Instead of the polar molecules in α-chitin, aliphatic diamines permeate the lattice of α-chitin.[34]

Chitin and chitosan are versatile biopolymers in many areas. But chitosan is more useful than chitin due to its solubility in acidic aqueous solution and ability to be used to prepare various forms such as hydrogels, membranes, films, fibers and three dimensional structures. It can be readily converted into those structures due to the solubility of chitosan depending on pH values. Due to non-toxicity, biodegradability, bioactivity and biocompatibility, chitosan has various applications, especially biomedical and biotechnology fields.

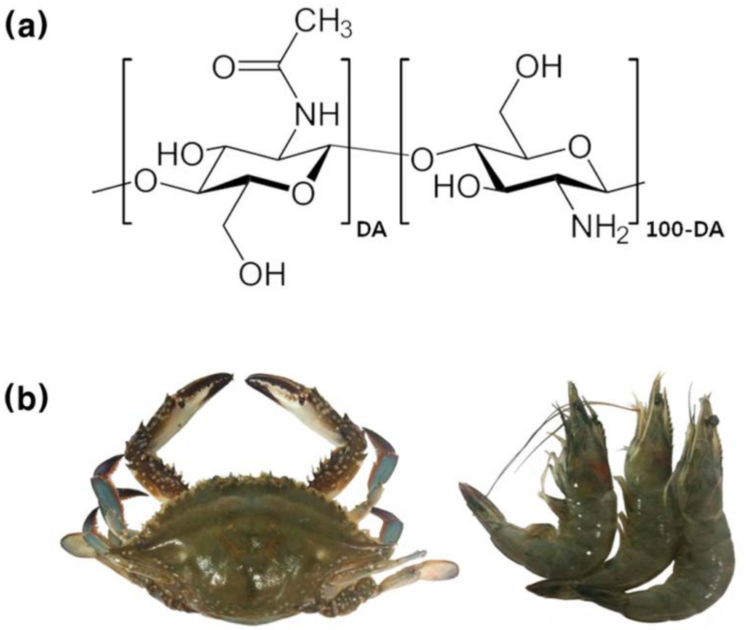

Figure 1-1. Chemical structure and commercial representative sources of chitin and chitosan. (a) Chemical structure of chitin and chitosan, when the value of DA (Degree of Acetylation, %) is more than 50%, it is called chitin. (b) Representative sources of chitin and chitosan: crab and shrimp.

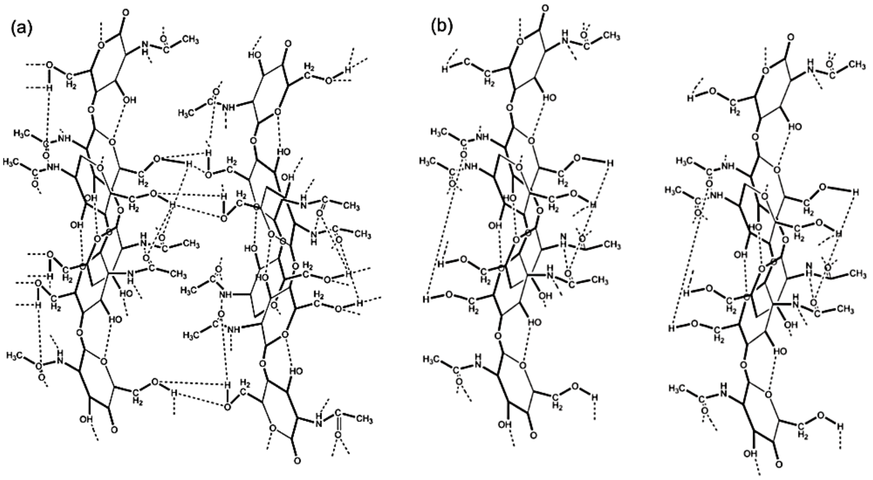

Fig 1-2. Molecular structure of chitin (a) alpha form, antiparallel arrangement, (b) beta form, parallel arrangement, there are no hydrogen bonding between chains.

Table 1-2. Type of chitin based on sources.

Type of chitin	Source	
alpha	Crustaceans	Crab, Lobster Shrimp, Prawn
	Squid (mollusk)	Loligo beak and radula
	Insects	Cuticle Locust apodemes
	Fungi	
beta	Squid (mollusk)	Loligo pen
	Centric diatom	
	Protozoa	
gamma	Squid (mollusk)	Loligo stomach
	Insects	Beetle cocoon

1.5. Clay mineral: Laponite

Clay minerals are a part of phyllosilicates, which are two-dimensional array of silicon-oxygen tetrahedral and aluminum or magnesium oxygen-hydroxyl octahedral. Depending on the number of each layer and structure, clay minerals are classified various categories.[35] Among these clay minerals, laponite, synthetic clay mineral, consist of disc-shaped platelet particles with a thickness of 1nm and an average diameter of 25~30nm. It belongs to the family of (2:1) phyllosilicates and resembles the natural clay mineral hectorite in structure and composition. Laponite are built up of sheets of octahedral coordinated magnesium oxide sandwiched between two parallel sheets of tetrahedral coordinated silica, as shown in **Figure 1-2**. Because some magnesium atoms in octahedral are substituted by lithium, charge of laponite is balanced by interlayer cation, mainly sodium ions.[36] The surface of laponite has a constant negative charge while the charge of edge is positive about 10% of the negative charges. The edge of montmorillonite, family of smectite, has positive or negative charges depending on the pH value,[37] but in the case of laponite, the positive charge is slightly decreasing with increasing pH values up to 11 or neutralized over pH value of 11.[38, 39]

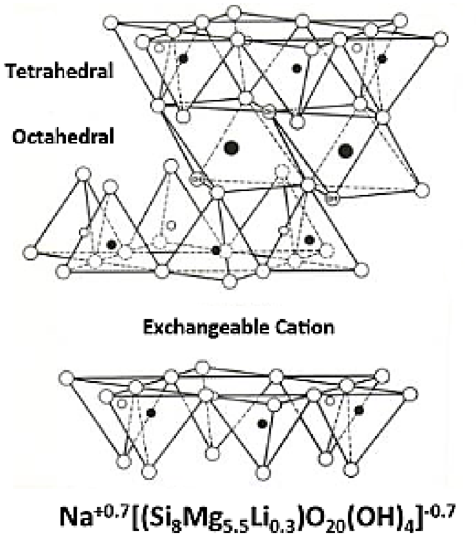

Figure 1-3. Empirical formula and structure of Laponite.

1.6. Polymer induced flocculation

Colloidal dispersion is that particles whose dimension is roughly between 1nm and 1μm are dispersed in continuous phases. When colloidal particles meet polymer, there are three ways to interact between colloidal particles and polymers. First, viscosity of the mixture with colloid and polymer is increased only. Second, in the case of block copolymer, some part of segment of the polymer is adsorbed on colloidal particle and the other segment is not. Thus, the polymer prevents from aggregation between colloidal particles and stabilizes the particles in the mixture. Lastly, the addition of polymer in colloidal dispersion affects the stability of that through the changing interfacial energy of colloidal particles. When a polymer is introduced into a colloidal dispersion, flocculation between colloid particles can be observed. And the flocculation is divided into two mechanisms: bridging and depletion flocculation.

When colloid particles approach closely enough, polymer is excluded from the volume between them, leading to a depletion layer. The depletion layer will act to generate an osmotic force, and then aggregation is induced between the particles. That has been termed depletion flocculation. In contrast, when the absorption of polymer onto the colloidal particle surface is favorable at low polymer concentration, a single polymer chain can absorb onto two or more colloid particles simultaneously. That can be induced flocculation between the particles, which has called bridging flocculation. [40, 41]

Chapter 2. Methods

2.1. Purification of chitosan

Chitosan (alpha chitosan was purchased from Sigma-Aldrich and beta chitosan was obtained from Ara Bio, Korea) was dissolved at 0.5% (w/v) in an aqueous acetic acid solution. After complete dissolution, the solution is filtered through 0.45μm pore size membrane (Corning). Then the solution is precipitated with dilute aqueous solution of ammonia and centrifuged. The precipitate is washed with deionized water and centrifuged repeatedly until pH 7 is reached, then purified chitosan is freeze-dried.

2.2. Hydrolysis of beta chitosan[42]

Molecular weight of beta chitosan is much higher than alpha form, so it is difficult to handle in preparation of hydrogels due to high viscosity of beta form. Therefore, beta chitosan chain has to be broken into smaller units, and that depolymerized chain result in reduction of its viscosity. Methods for depolymerized chitosan is refer to [26]. High molecular weight beta chitosan is dispersed at 0.5% (w/v) in 0.1M HCl, and then the chitosan solution is heated at 80℃ for 60h. The hydrolyzed chitosan solution is filtered through 0.45μm pore size membrane (Corning). The chitosan is precipitate with aqueous ammonia and the precipitate is washed with deionized water until pH 7, and then the chitosan is lyophilized.

2.3. Preparation of hydrogels

In laponite 2.5%-PAAS 0.08-Chitosan 0.18 of hydrogelation, clay in 125mg is dispersed in 3.55ml of deionized water. The clay suspension turned to a clear solution after few minutes. And then 1.0ml of 1.0% (w/v) PAAS solution is added to the clay solution for exfoliation of the clay. Purified chitosan is dispersed in deionized water, and then acetic acid was added to the polymer solution for protonation of the $–NH_2$ site (chitosan concentration: 10mg/ml). For adjusting pH values of chitosan dispersion, 0.01M NaOH is added to the chitosan dispersion in 0.05M acetic acid. The 0.45ml of protonated chitosan solution is added to the laponite dipsersion with PAAS dropwise while stirring. The mixture of these components becomes hydrogels immediately or within few minutes.

2.4. Hydrogels incorporated nanoparticles and bacteria

The 125mg of laponite is dispersed in 2.55ml of deionized water and 1ml of 1.0% (w/v) PAAS solution is added to the laponite dispersion. And then 1ml of Au nanoparticle solution which is capped with CTAB is added the above. After a few minutes, 0.45ml of 5.0% (w/v) chitosan dispersion is added to laponite-Au particle dispersion while stirring.

For cyanobacteria incorporated into the hydrogels, the 125mg of laponite is dispersed in 3.55ml of deionized water and 1ml of 1.0% (w/v) PAAS solution is added. The 0.45ml of 5.0% (w/v) chitosan dispersion is added to laponite dispersion while stirring and then 100µl of concentrated bacteria is added incorporation bacteria before finishing the gelation.

2.5. Laponite-chitosan droplets

Chitosan is dispersed in deionized water added acetic acid at 5.0% (w/v) concentration. Laponite is dispersed in deionized water at 2.5% (w/v) concentration and 1.0% (w/v) PAAS solution is added to laponite dispersion. By syringe, needle size 26G, chitosan dispersion is dropped onto the laponite dispersion or injected into the laponite dispersion without stirring. Then, chitosan droplets are obtained.

2.6. Fabrication of micro-sized laponite-chitosan sphere

For micro-sized laponite-chitosan sphere, chitosan is dispersed in deionized water and then acetic acid is added due to being soluble (chitosan concentration: 20mg/ml). And laponite is dispersed in deionized water followed by adding PAAS solution. The weight ratio, laponite/PAAS, is 1/0.08. Chitosan droplets are dispersed in hexane with emulsifier, polyglycerol ester (concentration: 0.5% (v/v)), through SPG membrane (pore size 1.1µm). While the chitosan emulsion in hexane is dispersed in 2.5% (w/v) laponite dispersion with PAAS through SPG membrane (pore size 5.0μm), we can get laponite-chitosan micro-sized sphere. After a few days, hexane in the emulsion is evaporated completely and then the particles are collected onto the solution.

2.7. Characterization: FT-IR, ARES

IR spectra of clay and chitosan are measured with KBr pellets on Nicolet 6700 Fourier transform infrared spectrophotometer (Thermo Scientific, USA) to confirm interaction between amine groups of chitosan chain and laponite. The hydrogels are prepared for measurements by freeze-dried and the measurement of wavenumber range is the 4000~400cm^{-1}. For measurement of rheological property of hydrogels, dynamic frequency sweep (strain 1.0%), TA Instruments ARES is used with a 25mm diameter parallel plate attached to a transducer at 25℃. The gap of the parallel plate is set to be 1.0mm.

Chapter 3. Results and discussion

3.1. Preparation of laponite-chitosan hybrid hydrogels

We present simple and fast method to make hybrid hydrogel of inorganic 2D clay and biopolymer. We have chosen laponite as a two dimensional inorganic clay and chitosan as a biopolymer. Formation of 3D-network structure in hydrogel was initiated through electrostatic interaction between negatively charged 2D planes of laponite and positively charged amine groups of chitosan.

Laponite, synthetic clay mineral, consist of disc-shaped platelet particles with a thickness of 1nm and an average diameter of 25~30nm. It belongs to the family of (2:1) phyllosilicates and resembles the natural clay mineral hectorite in structure and composition. The surface of laponite has a constant negative charge while the charge of edge is positive about 10% of the negative charges when dispersed in deionized water. The positive charge of edge of laponite is slightly decreasing with increasing pH values up to 11 or neutralized over pH value of 11. In our work, because the pH values of hydrogels are always under 10, the surface and the edge of laponite have always negative and positive charges respectively. The chitosan, the other main component, is soluble under pH value of 6, which has positive charge due to protonation of amine group in the chain. The degree of protonation of amine group is various depending on the pH values of solution.

For gelation of laponite-chitosan hydrogels, three components are used: laponite, chitosan and PAAS (poly acrylic acid sodium salt). Summarizing the gelation method, laponite is dispersed in deionized water, and then PAAS solution added to laponite dispersion (**Figure 3-1(a)**). The adding of PAAS can make laponite to have almost negatively charged surface by shield the positive charge of the edge. After 10 minutes, the chitosan dispersion is added to pretreated laponite with PAAS dropwise while stirring (**Figure 3-1(b)**). The hydrogels can be obtained immediately or within a few minutes after mixing three components (**Figure 3-1(c)**). It is surprising to form a hybrid hydrogel just within a few minutes. Without adding any extra chemical, simple mixing of inorganic clays and chitosan dispersion resulted in mechanically strong gel. The gels contained only laponite can be made by dispersed in deionized water at greater than 3.0% (w/v), but it takes 1~2 hours to be a gel. In contrast, the laponite-chitosan hydrogels can be a gelation promptly after adding chitosan to the pretreated laponite dispersion. In colloidal system, increasing the amount of salt, flocculation or sedimentation of large aggregates is occurred due to decrease double layer of colloid particles. Because the pH value of the hydrogels is in the range of 6 to 7 at 2.5% (w/v) of laponite concentration, pH value of laponite dispersion is adjusted by adding

HCl until the same pH value of the hybrid hydrogel and then the gelation of adjusted laponite dispersion is observed. In the adjusted laponite dispersion, gelation is not occurred immediately. Instead of prompt gelation of clay, merely, gelation time is shorter than the initial dispersion. And the hybrid hydrogels introduced chitosan are increasing mechanical strength, but that will be discussed next section. Also, the resulting hydrogel can be moldable and the other materials such as nanoparticles or cell can be easily incorporated inside gel (**Figure 3-2 and Figure 3-3**).

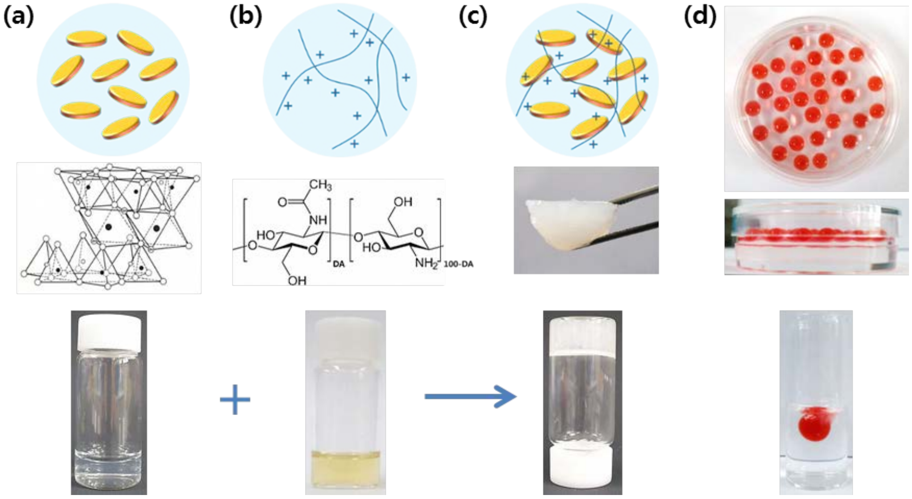

Figure 3-1. Schematic procedure of preparation of hybrid hydrogels by mixing clay, PAAS and chitosan. (a) Chemical structure and image of dispersion of clay, (b) Chemical structure and image of dispersion of chitosan, (c) Images of hybrid hydrogel which is free-standing, (d) Chitosan droplet onto clay dispersion.

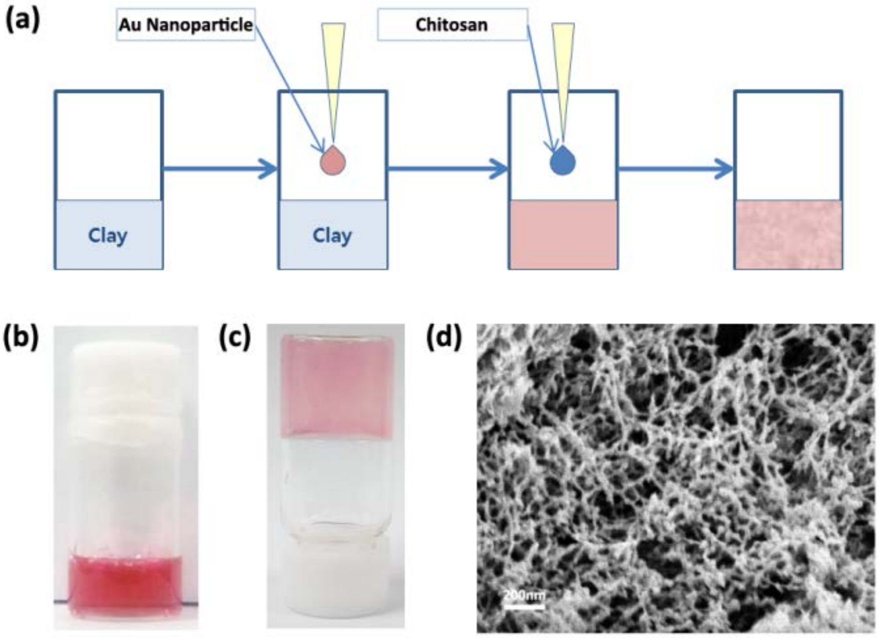

Figure 3-2. The clay-chitosan hydrogel contained Au nanoparticles. (a) Schematic procedures of preparation of clay-chitosan hydrogel contained Au nanoparticles, (b) image of Au nanoparticle solution, (c) image of clay-chitosan hydrogel contained Au nanoparticles, (d) SEM image of clay-chitosan hydrogels contained Au nanoparticles. The hydrogel is dried by using critical point dryer.

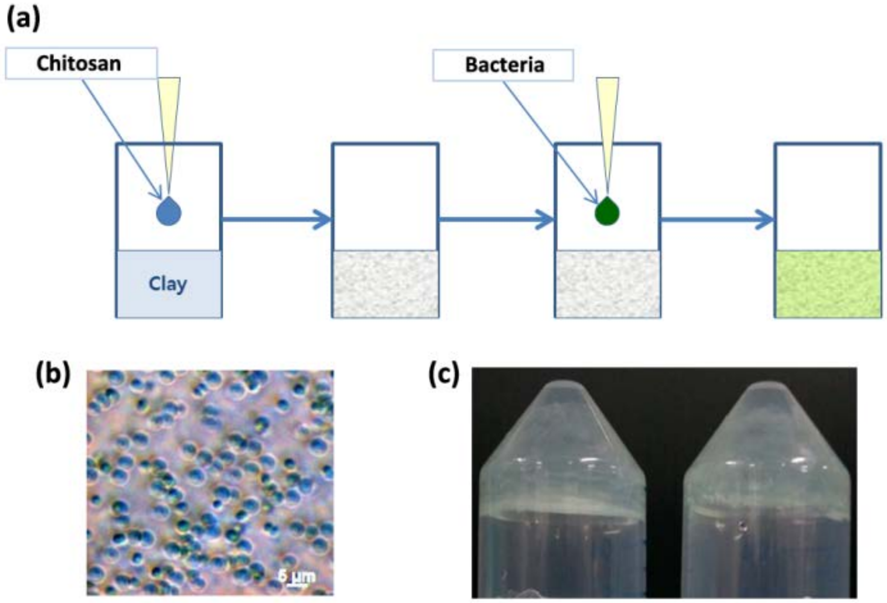

Figure 3-3. The clay-chitosan hydrogel contained cyanobacteria. (a) Schematic procedures of preparation of clay-chitosan hydrogel contained cyanobacteria, (b) optical microscopy image of cyanobacteria, (c) image of clay-chitosan hydrogel contained cyanobacteria.

3.2. Gelation depending on the amount of each component

We confirm that there is the optimum weight ratio of three components for gelation of hybrid hydrogels. Firstly, at greater than 2.5% (w/v) of laponite concentration, the hybrid hydrogels are obtained, however, at 2.0% (w/v) of laponite concentration, gelation is not occurred.

Secondly, the gelation results of hybrid hydrogels (**Fig. 3-4**) are shown that hydrogelation is occurred from 0.08 to 0.20 of the weight ratio range of chitosan to laponite immediately or within a few minutes depending on the amount of chitosan. To identify the role of chitosan in the gel formation, systematically we varied the concentration and observe the gel forming capability. In the lower range of 0.02 to 0.06, gelation is not occurred due to insufficient amount to be formed 3D-network structures in the mixture. On the other hand, over 0.22 of the weight ratio of chitosan to laponite, the hybrid hydrogels are not formed, and the mixture aggregate and sediment at excessive amount of chitosan. In the range of gelation, comparing CEC (cation exchange capacity, meq/100g) of clay and protonated amine group of chitosan (DA(degree of acetylation) = 85%), amine group of chitosan to CEC of clay value is between 0.70 and 1.85.

The addition of polymer in colloidal dispersion affects the stability of that through the changing interfacial energy of colloidal particles. When a polymer is introduced into a colloidal dispersion, flocculation between colloid particles can be observed. There are two mechanisms to be caused flocculation of colloidal system: depletion and bridging flocculation. When colloid particles approach closely enough, polymer is excluded from the volume between them, leading to a depletion layer. The depletion layer will act to generate an osmotic force, and then aggregation is induced between the particles. That has been termed depletion flocculation. In contrast, when the absorption of polymer onto the colloidal particle surface is favorable at low polymer concentration, a single polymer chain can absorb onto two or more colloid particles simultaneously. That can be induced flocculation between the particles, which has called bridging flocculation.

In our system, because clay and chitosan have the opposite charges between them and amine group of chitosan chain strongly attached to the clay surfaces, the absorption of chitosan chain on to clay will be favorable. The radius of gyration of chitosan varied from 60nm to 200nm depending on molecular weight [43-45] and solvent is much large than the diameter of clay primary particle, 25~30nm, [46] so many of the clay particles would be linked by a single chitosan chain. Therefore, the role of chitosan in this system is connection between the clay particles to form the 3D-network structures.

Lastly, anionic polymer, PAAS, helps the interactions between laponite and chitosan for gelation. PAAS has negative charge dissolved in deionized water. PAAS added to laponite dispersion plays a role in completely exfoliation of laponite particles through passivation of positive charge in laponite, so amine groups in chitosan chain would interact with surface of laponite. In case of gelation without PAAS, we obtain only a few gelation samples at 3.0% (w/v) of laponite concentration. Additionally, at 2.5% (w/v) of laponite concentration, the hydrogels are formed, but the hydrogel do not maintain its shape and finally collapse. However, over 0.10 of weight ratio of PAAS to laponite, chitosan droplet in pretreated laponite is obtained instead of laponite-chitosan hydrogel. In existence of anionic polymer, PAAS, generally, the clay-chitosan hydrogels are obtained by adding chitosan dispersion in the weight ratio range of chitosan 0.08 to 0.20. But, over 0.10 of PAAS weight ratio, chitosan droplets are formed in laponite dispersion (**Figure 3-1(d) and Figure 3-4**). It seems that the rest of PAAS after wrapped the edge of laponite disturbs interactions for gelation between surface of laponite and amine group of chitosan.

We find that the result of gelation is different depending on pH values of chitosan dispersion. Chitosan is soluble under pH 6, but degree of protonated amine groups is different as pH values of chitosan dispersion. Thus, to identify the effect on gelation with respect to pH value of chitosan dispersion, chitosan dispersion adjusted pH by base is added to laponite dispersion whether gelation is occurred or not. One of chitosan dispersion is adjusted to 4.67, and the other is 5.99 by adding 0.01M NaOH solution. In case of the addition of chitosan dispersion pH value of 4.67 to clay dispersion, the gelation is occurred in the chitosan weight ratio range of 0.08 to 0.18. On the other hand, in case of the addition of chitosan dispersion pH value of 5.99 to clay dispersion, the mixture is flowed instead of gelation, and laponite and chitosan dispersion are not mixed well. Therefore, in order to obtain laponite-chitosan hydrogel, the amount of positive charges in chitosan chain is important. Also, the electrostatic interaction between surface of clay and amine group of chitosan is essential to be hydrogels. After the gelation is occurred, the hydrogels are immersed in various buffers in the pH range of 4 to 10: citric, sodium acetate, phosphate and sodium carbonate. After a week, the hydrogels maintain their shape without collapse (**Figure 3-5**). Therefore, it is thought that pH values are important at initial step of the gelation, but the fabricated hydrogels are not affected by the pH value. The mechanical strength depending on the pH value of chitosan dispersion will be discussed next section.

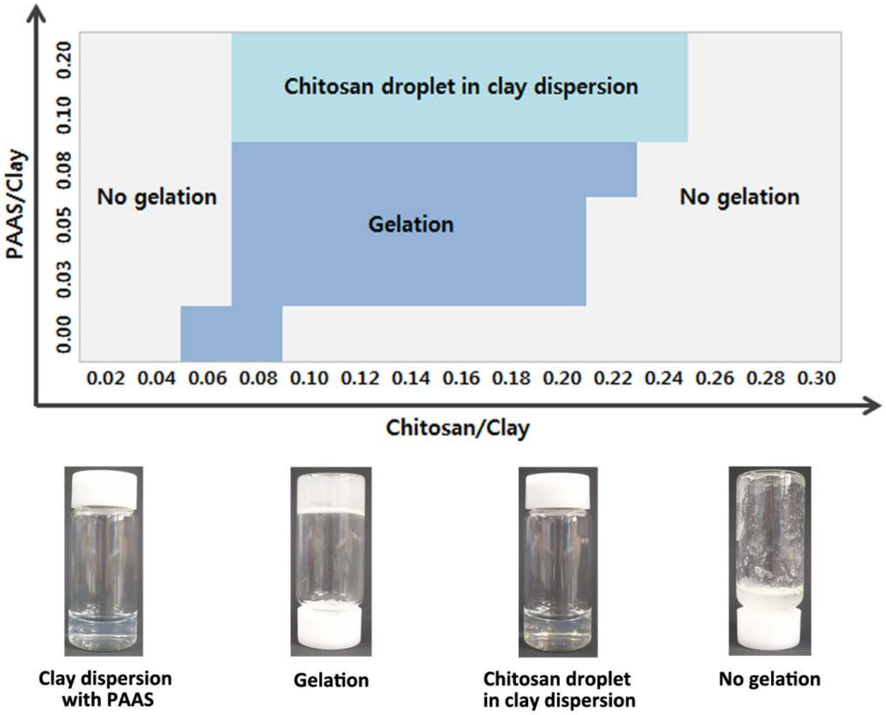

Figure 3-4. Phase diagram for clay-chitosan hydrogels. The gelation result such as the phase diagram is at 3.0% (w/v) clay concentration. The identical gelation results are observed at greater than 2.5% (w/v) clay concentration.

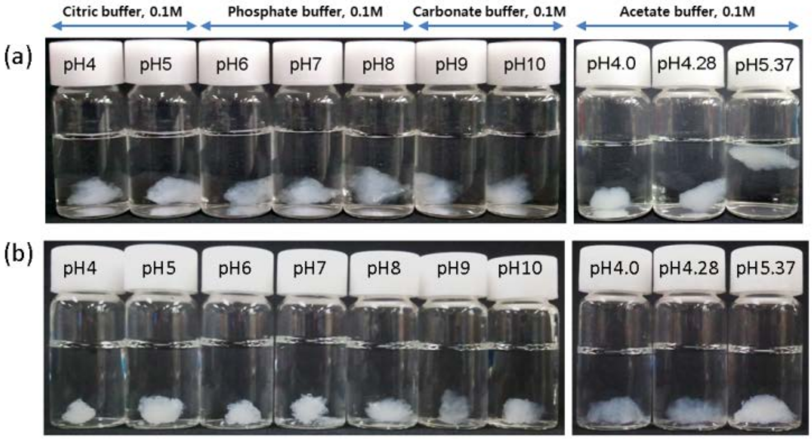

Figure 3-5. The laponite-chitosan hydrogels are immersed in various buffers. (a) Immediately after immersion the hydrogels in various buffers, (b) after a week, the hydrogels maintain their shape.

3.3. Fabrication of micro-sized laponite-chitosan sphere

In previous section, chitosan droplets in laponite dispersion with PAAS are observed over 0.10 of PAAS weight ratio and maintain their shape in laponite dispersion after a few days. When chitosan dispersion is injected into laponite dispersion by syringe slowly instead of dropping the chitosan dispersion, the drop of chitosan also maintains its shape rather than burst into laponite dispersion. However, when chitosan dispersion is injected into basic buffer which is equivalent to laponite pH value by syringe, chitosan drop is not observed (**Figure 3-6**). According to that, it is thought to be interaction with laponite onto the surface of chitosan drop.

Above the method, dropping or injection chitosan dispersion by syringe, there is limitation in controlling size of droplet. For fabrication micro sized laponite-chitosan droplets, W/O/W emulsion is made from laponite, chitosan, and hexane using SPG membrane because components, laponite and chitosan, dispersed in deionized water respectively are to be a gel by mixing immediately.

Summarizing method (**Figure 3-7(a)**), first of all, W/O emulsion is prepared that chitosan in deionized water added acetic acid is dispersed into oil phase with emulsifier and its size is about 4~5μm. The emulsion at the first step is dispersed into laponite solution with PAAS, and then W/O/W emulsion is produced and its size is about 20μm. At the second step, laponite particles play a role in emulsifier or stabilizer, which is stabilized by solid particles, SiO_2 or TiO_2, adsorbing onto the interface between the two phases, called Pickering emulsion.[47-49]

The solution dispersed chitosan droplets is divided into three layers hexane, emulsion and water. After a day, the emulsion layer decrease, and the solution is divided into two layers, hexane at the top and water at the bottom, and the particles are between two phases.

After a few days, hexane in the W/O/W emulsion is evaporated completely, and then the particles are collected. The collected particles are observed by SEM and EDS and the results are shown in **Figure 3-7(b), (c)**. According to the results, we can observe micro sized spheres and their size is 5~10μm, which is thought to have laponite and chitosan from EDS peaks.

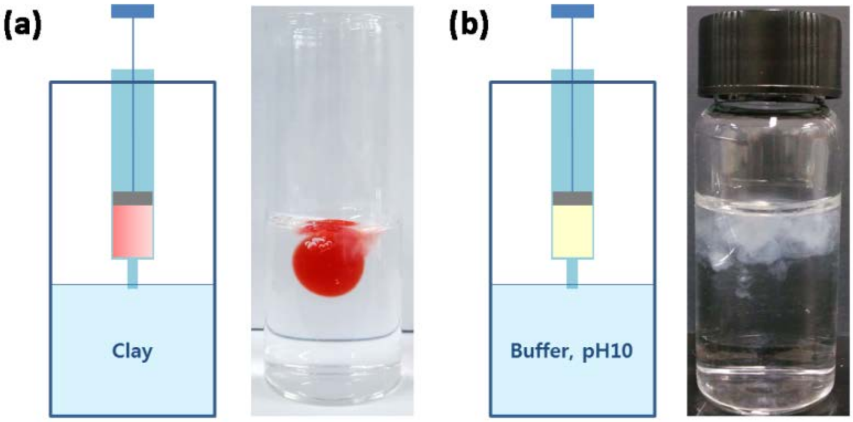

Figure 3-6. The images of chitosan drop in (a) laponite dispersion with PAAS, (b) basic buffer injected by syringe.

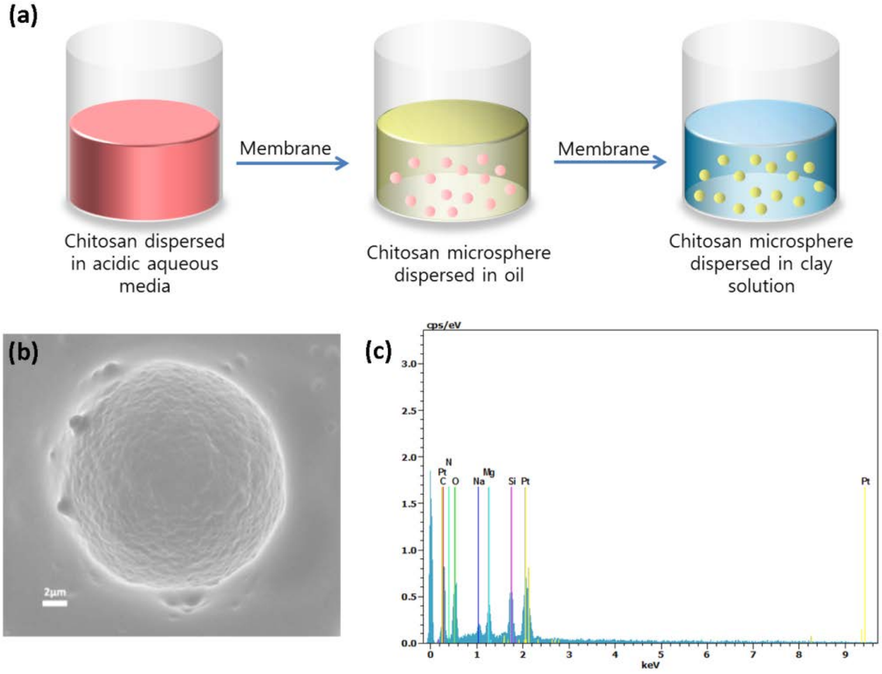

Figure 3-7. The laponite-chitosan microspheres using Pickering emusion though SPG membrane. (a) The schematic procedure of preparation the laponite-chitosan microspheres, (b) the SEM image of the laponite-chitosan microspheres, (c) the EDS result of the laponite-chitosan microsphere.

3.4. Rheological measurements

Shear modulus values of the clay-chitosan gels, G′ and G″, are measured by ARES for characterization of mechanical property, and frequency sweep test are conducted. All the clay-chitosan hydrogels have a single plateau region in dynamic modulus, and the storage modulus G′ values are always greater than the loss modulus G″ values over the entire range of frequencies in dynamic frequency sweep test. The maximum G′ value of laponite-chitosan hydrogels at 3.0% (w/v) of laponite concentration (50kPa) is slightly lower than that of Aida and co-workers' hydrogels (90kPa).

In order to confirm the effect of chitosan in mechanical property, the storage modulus G′ values are compared clay gels without PAAS and chitosan at 2.5% (w/v) concentration of clay and clay-chitosan hydrogels contained 2.5% clay, 0.2% PAAS and 0.45% chitosan. In **Figure 3-8(a)**, the storage modulus G′ values of clay-chitosan hydrogels as function of angular frequency at a fixed strain, $\gamma = 1.0\%$, are always greater than that of the hydrogels contained clay only. The G′ values of clay-chitosan hydrogels is about 20kPa, which is increased in 400 times than that of the other sample. The hydrogel contained clay only is formed by electrostatic interaction between the surface of clay and the edge of clay, but it is very weak hydrogel and flow easily under small deformation. In contrast, the hydrogel contained clay and chitosan is harder than the hydrogel contained clay only due to interaction between negatively charged surface of clay and positively charged amine group of chitosan. The FT-IR results are shown that the peak of amine group of clay-chitosan hydrogel is shifted toward the smaller wavenumber, and it means that there is an interaction between clay and the amine group of chitosan. In **Figure 3-8(b)**, it is shown that the G′ values of clay-chitosan hydrogels made of pretreated clay with PAAS have three times higher than that of clay-chitosan hydrogels without PAAS. For exfoliation and homogenous dispersion of clay in deionized water, the anionic polymer, PAAS, is added to the clay dispersion. The interruption of interaction between the surface and the edge of clay is result in the increase in G′ values of clay-chitosan hydrogels as well as help the interaction between clay and chitosan for gelation in **Figure 3-5** and **Figure 3-8(b)**. The G′ values in **Figure 3-8(c)** are as function of the clay concentration, the more increase clay concentration, the greater G′ values are obtained. While each hydrogel contained clay only or chitosan only is very weak, the mechanical strength of clay-chitosan hybrid hydrogels is superior to them. The anionic polymer, PAAS, not only helps for formation of 3D-network structures but also improves the mechanical strength of clay-chitosan hydrogels.

Depending on the chain length (or molecular weight) of added chitosan and form of chitosan, alpha or beta chitosan, the storage modulus G′ values are measured, and the results are in **Figure 3-9(a), (b)**. While the effect of the chain length of chitosan on the G′ values is existing, there is no difference depending on the form of chitosan. In the weight ratio of chitosan to clay between 0.08 and 0.12, the G′ values of clay-chitosan hydrogels with chitosan-330k ($\overline{M_v} = 330{,}000$) is greater than that of clay-chitosan hydrogels with chitosan-60k ($\overline{M_v} = 60{,}000$), but in the range of 0.12 to 0.18 of chitosan to clay, while the increase of the G′ values of chitosan-330k is reduced, the G′ values of chitosan-60k is increased continuously. Thus, the G′ values of chitosan-60k are higher than that of chitosan-330k over 0.12 weight ratio. Owing to that the positive charges of amine groups in chitosan chain is affected by pH value of acidic media, the strength of clay-chitosan hydrogel is expected to decline with increase pH value of chitosan dispersion.

In **Figure 3-9(c)**, the results of the storage modulus values are shown depending on the pH value of chitosan as function of the amount of chitosan. The hybrid hydrogels for rheological measurements are prepared using pH values of chitosan dispersion: 2.20, 4.67 and 5.99, respectively. Whereas the hydrogelation is occurred at 2.20 and 4.67 of pH values, aggregation is observed in the mixture with 5.99 pH value of chitosan dispersion. So, the storage modulus is measured the hydrogel samples using 2.20 and 4.67 of pH value and compared them. According to the measurements, as increased the pH values of chitosan dispersion, the storage modulus values are decreased. To obtain clay-chitosan hydrogels with high mechanical strength, the initial pH value of chitosan dispersion is important. We also confirm that amount of charges of components is critical factor for robust laponote- chitosan hydrogels through the experiment.

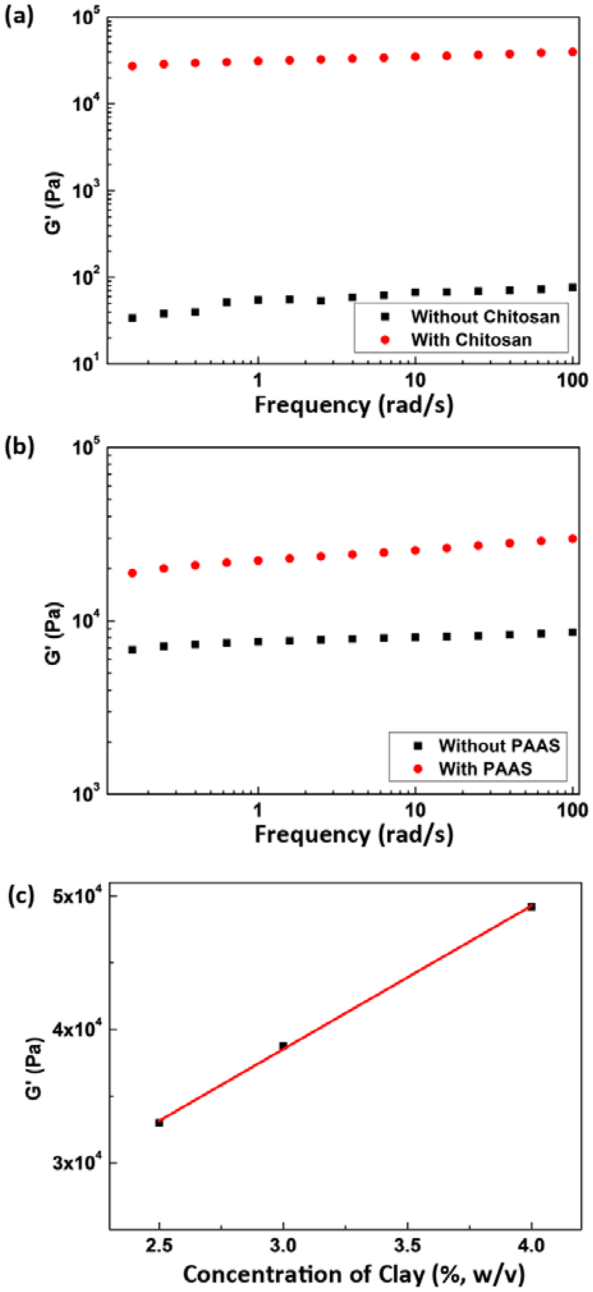

Figure 3-8. Rheological properties of clay-chitosan hydrogels with 2.5% (w/v) clay concentration depending on with or without one component and clay concentration. (a) Storage modulus G′ values with or without

chitosan, (red, circle) Clay/PAAS/Chitosan = 1/0.08/0.18, (black, square) Clay/PAAS/Chitosan = 1/0.00/0.00. (b) Storage modulus G′ values with or without PAAS, (red, circle) Clay/PAAS/Chitosan = 1/0.05/0.18, (black, square) Clay/PAAS/Chitosan = 1/0.00/0.08. (c) Storage modulus G′ values depending on clay concentration, Clay/PAAS/Chitosan = 1/0.08/0.18.

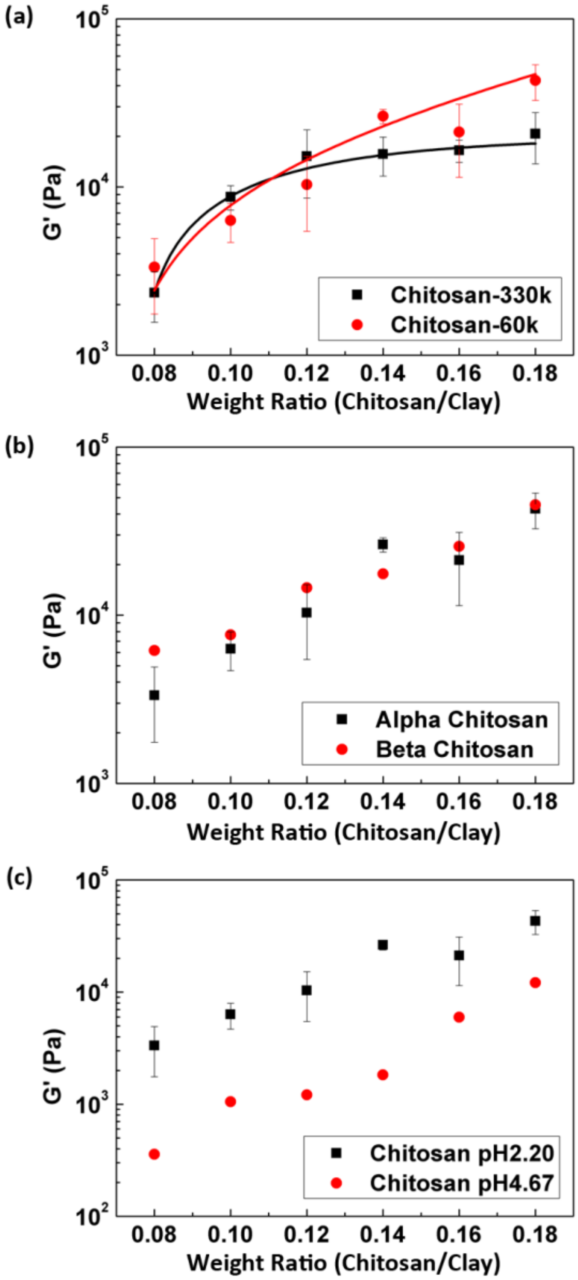

Figure 3-9. Rheological properties of clay-chitosan hydrogels with 2.5% (w/v) clay concentration depending on type of chitosan chain and pH values of chitosan dispersion. (a) Storage modulus G′ values depending

on chain length of chitosan as function of weight ratio chitosan to clay, molecular weight of (red, circle) is 60,000 (chitosan-60k) and molecular weight of (black, square) is 330,000 (chitosan-330k). (b) Storage modulus G′ values of alpha chitosan (black, square) and depolymerized beta chitosan (red, circle). (c) Storage modulus G′ values depending on pH value of chitosan (chitosan-60k) dispersion, pH value of (black, square) is 2.20 and pH value of (red, circle) is 4.67.

3.5. FT-IR measurement

IR spectra of clay and chitosan are measured to confirm interactions between laponite and amine group of chitosan. The laponite-chitosan hydrogels are prepared by freeze-dried and the measurements are conducted with KBr pellets in the 4000~400cm^{-1} wavenumber range. The results spectra of the hydrogels are shown **Figure 3-10**. Two types of vibration occur in primary amine: Stretch and bending vibration. The N-H stretch vibration of primary amine is in the region The N-H bending vibration of primary amine is observed in the region 3300-3000cm^{-1}, and the N-H bending vibration of primary amine is observed in the region 1650-1580cm^{-1}. But the former bands appear in the same region of the O-H stretches and are weaker and sharper than those of the O-H stretches. So, it is difficult to distinguish the stretch vibration of primary amine. In **Figure 3-10(a)**, the bending vibration of primary amine is observed at 1598cm^{-1}, which is shifted toward lower wavenumber in clay-chitosan hydrogel (**Figure 3-10(c)**). It means that there is an interaction between laponite and the amine group of chitosan.

In **Figure 3-10**, FT-IR spectra of hydrogels are compared between different compositions. At the composition that clay/PAAS/chitosan 60k is 1/0.08/0.08, we confirm that the peak correspond to amine group of chitosan chain is shifted toward lower wavenumber, 1571cm^{-1}

(**Figure 3-10(c) and Figure 3-11(b)**). In accordance with the results of **Figure 3-11(a-c)**, there is no difference shifted peak depending on weight ratio of PAAS and molecular weight of chitosan. However, in the case of 0.18 of chitosan weight ratio, the peak correspond to amine group is less shifted toward 1577cm^{-1} and broader (**Figure 3-11(d)**) than that of the others. It seems that PAAS do not affect the interaction between surface of laponite and the amine group of chitosan directly. Instead, PAAS is thought to help interaction between surface of laponite and amine group of chitosan.

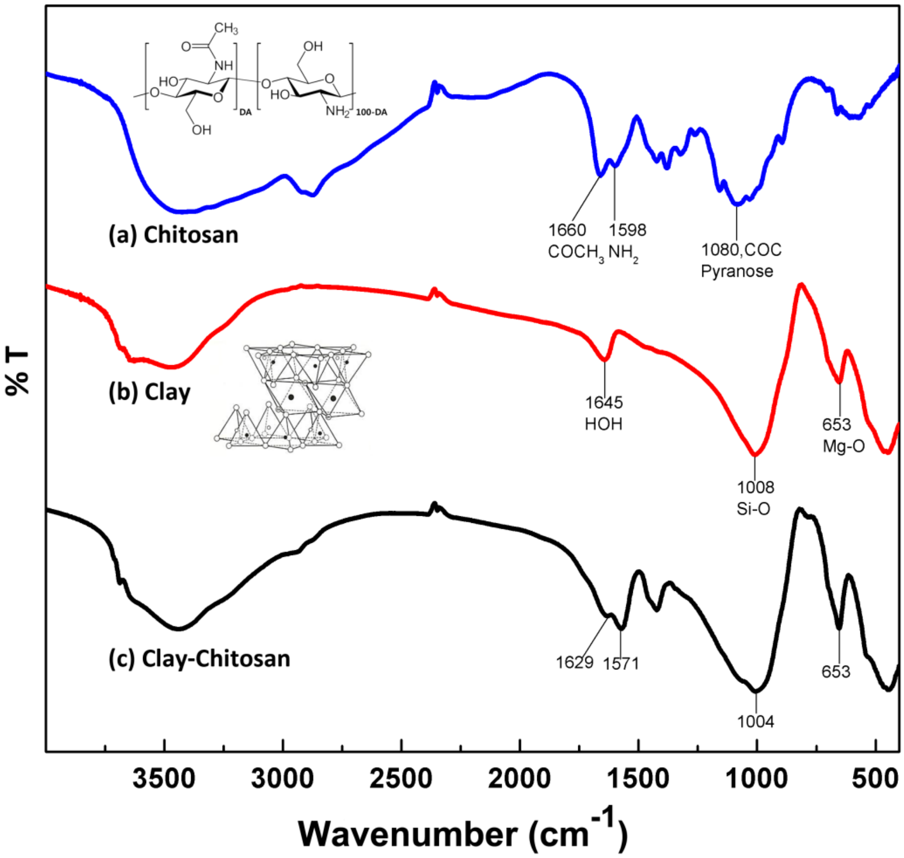

Figure 3-10. FT-IR spectra of clay, chitosan and the hydrogels. (a) chitosan: alpha, $\overline{M_v} = 60{,}000$, (b) clay: laponite RD, (c) clay-chitosan hydrogel (Clay/PAAS/Chitosan 60k = 1/0.08/0.08) prepared by freeze-dried.

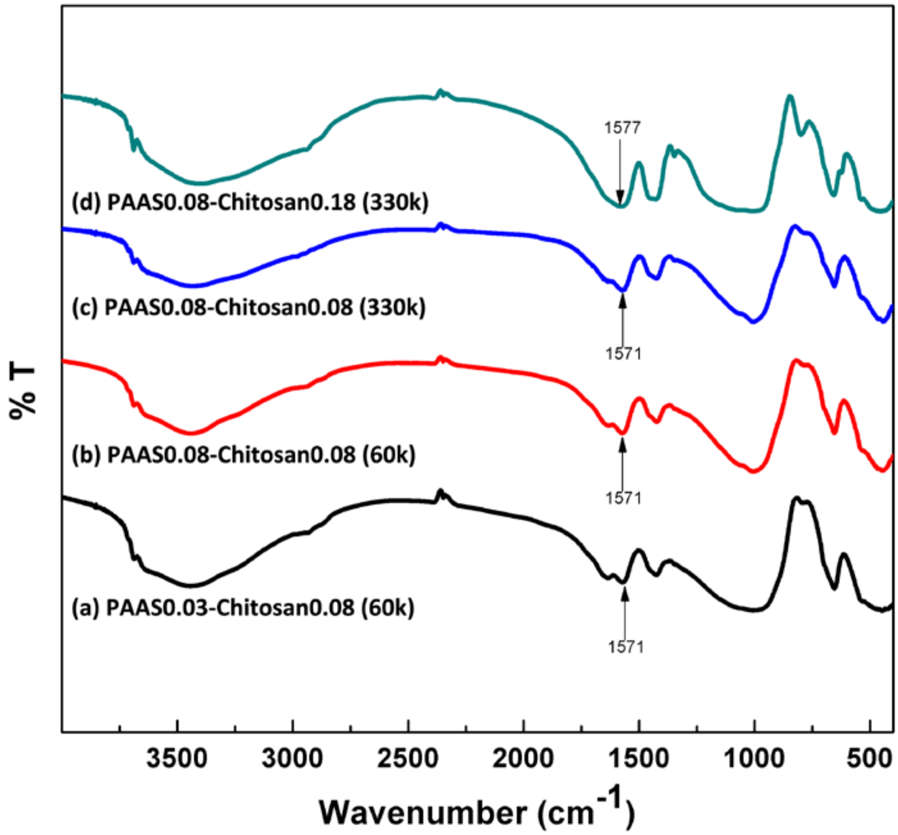

Figure 3-11. FT-IR spectra of laponite-chitosan hydrogels at 3.0% (w/v) of laponite concentration. The laponite-chitosan hydrogels are prepared by freeze-dried. (a) Clay/PAAS/Chitosan 60k = 1/0.03/0.08, (b) Clay/PAAS/Chitosan 60k = 1/0.08/0.08, (c) Clay/PAAS/Chitosan 330k = 1/0.03/0.08, (d) Clay/PAAS/Chitosan 330k = 1/0.08/0.18.

Chapter 4. Conclusion

In conclusion, we fabricate moldable and robust laponite-chitosan hydrogel by mixing non-modified chitosan and clay nanoplatelets. The hydrogels are obtained by mixing pretreated laponite with PAAS and chitosan within a few minutes. For gelation of laponite-chitosan hydrogels, electrostatic interaction is essential between surface of laponite and amine group of chitosan through that gelation of laponite-chitosan hydrogels is occurred or not depending pH values of chitosan dispersion. After the hydrogelation, the hydrogels are not collapsed in a variety of pH values. Thus, it is though that the pH values affect to the initial gelation. Also, we find the optimum ratio of three components for robust hydrogels, therefore, the charge balance between laponite and chitosan is important factor for the high mechanical strength.

In the range of hydrogelation, the more amount of chitosan, the strength of hydrogels are increased. While there is no effect based on form of chitosan, the storage modulus of hydrogels using smaller molecular weight of chitosan is higher than that of larger molecular weight of chitosan. Also, we find that the amount of charge in chitosan chain affects the mechanical strength of the hydrogels through the experiment of gelation using adjusted pH of chitosan dispersion.

According to the results from our work, the laponite-chitosan hybrid hydrogels have not only higher mechanical strength but also the potential to confer functionalities by incorporation of inorganic materials, cell or bacteria inside the hydrogels. Also, by a variety of methods such as dropping, injection or emulsification, we confirm that the laponite-chitosan hydrogels are fabricated from macro to micro size.

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Abstract in Korean

하이드로젤은 친수성 고분자로 이루어진 3차원 구조와 물로 구성되어있으며, 고체와 액체의 특성을 모두 갖는 것으로 알려져 있다. 이러한 젤은 생활용품에서부터 의료용 재료 등 다양한 영역에서 응용 가능하다. 친수성 고분자를 이용하여 만든 하이드로젤은 기계적 강도가 약하거나 외부자극에 반응하기 어렵다. 이러한 점을 극복하기 위해 3차원 구조체에 다른 물질을 도입하여 하이드로젤의 기계적 강도를 높이거나, 외부 자극에 반응할 수 있는 물질을 도입하여 하이드로젤에 기능을 부여하기도 한다.

본 연구에서는 수용액상에서 콜로이드 형태로 존재하는 클레이 입자에 키토산을 도입하여 기계적 성질이 우수한 하이드로젤을 제작하였다. 클레이 입자는 물에 분산되어 음의 전하를 가지며, 키토산은 pH가 6이하인 산성 수용액에서 양의 전하를 갖는다. 따라서 두 전하 사이의 상호작용에 의해 3차원 구조가 형성되면서 하이드로젤이 만들어진다.

클레이 농도가 3.0% (w/v)이상인 경우, 물에 분산시킨 후 1~2시간 이내에 점도가 증가하며 젤이 되는데, 약한 힘을 가하면 그 형태를 유지하지 못하고 용액과 같은 형태로 변하게 된다. 그러나 클레이 농도 2.5% 이상인 경우, 키토산을 넣은 직후 또는 수 분 이내에 젤이 되는 것을 확인하였다.

기계적 성질이 우수한 단단한 클레이-키토산 하이드로젤을 만들기 위한 최적의 비율이 존재함을 확인하였다. 첨가하는 키토산의 양이 적을 경우, 3차원 구조를 형성하지 못하며, 너무

많은 양의 키토산을 첨가하였을 경우 3차원 구조를 유지하지 못하고 클레이와 키토산이 응집 후 침전하게 된다. 또한 클레이 입자를 잘 분산시키기 위해 첨가하는 PAAS의 양이 너무 많을 경우에는 하이드로젤을 형성하지 못하고 클레이 분산액 내부에 키토산 방울을 얻게 된다.

점탄성 측정을 통해 제작한 하이드로젤의 기계적 강도를 측정하였다. 하이드로젤이 형성되는 키토산 범위에서, 첨가하는 키토산의 양이 증가할수록 하이드로젤의 강도가 증가함을 알 수 있었다. 그리고 PAAS가 첨가된 경우 하이드로젤의 강도가 높아졌으나 그 효과가 크지 않고, 클레이의 농도가 증가할 수록 강도가 증가함을 확인하였다. 또한 키토산 사슬 길이가 짧을수록 젤의 강도가 높았으며, 키토산 형태에 따른 강도는 큰 차이가 없음을 확인하였다. 첨가하는 키토산 분산액의 pH를 변화하여 제작 후 측정한 결과 pH값이 높은 키토산 분산액을 이용한 하이드로젤의 강도가 약함을 알 수 있었다.

클레이와 키토산을 가지고 단단한 하이드로젤을 만들었으며, 키토산의 양전하가 많을수록 더 단단한 하이드로젤을 얻을 수 있었고, 키토산 사슬에 전하의 양이 적은 경우 젤이 되지 않음을 확인하였다. 이를 통해 클레이-키토산 하이드로젤은 정전기적 상호작용에 의해 3차원 구조를 형성하여 하이드로젤이 됨을 알 수 있다. 따라서 단단한 하이드로젤을 얻기 위해 최적의 비율이 존재하게 된다. 본 연구에서는 하이드로젤의 기계적 강도를 향상시킴과 동시에 나노입자 또는 박테리아를 쉽게 젤 내부에 도입할 수 있는 것을 통해, 하이드로젤에 부가적인 기능을 부여할 수 있는 가능성을 확인하였다.