Homogeneous Etherification Modification of Chitosan and Preparation of High-Strength Hydrogel

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Abstract. At present, the ocean as a treasure house of human development resources has attracted widespread attention. Chitin is the most abundant biomass resource in the ocean, and it is also a natural macromolecule second only to cellulose in nature. Its deacetylated product, chitosan, is not only easy to modify physically and chemically, but can be processed into various forms and has excellent properties. Biocompatibility, biodegradability, antibacterial, tissue adhesion and other biological activities. Derivatization reactions are often used to improve the properties of chitosan and give it new structures and functions.
In this paper, a series of chitosan ether derivatives were synthesized using alkali/urea aqueous solution as the solvent and homogeneous etherification reaction medium of chitosan, and their structures and properties were characterized. Then, chitosan and quaternized chitosan are dissolved in the acrylic acid monomer aqueous solution, and the chitosan/polyacrylic acid and quaternized high-strength polyelectrolyte composite hydrogel (PEC) are obtained by in-situ polymerization. Finally, the above-mentioned composite hydrogel is treated with Ag+ to obtain the ultra-high strength of the dual interaction of static electricity and coordination bonds, and at the same time a physical cross-linked hydrogel (DPC) with anti-fatigue. The chitosan-based hydrogel with excellent mechanical properties has good application prospects in articular cartilage, wound dressings, bionic stents and the like.

1. Introduction
Chitosan is a renewable natural alkaline polysaccharide with no toxic side effects. Chitosan is a deacetylation product of chitin, an extract from the shell skeleton of shrimp, crab and insects, and is the only alkaline polysaccharide in nature. It has rich sources and is a renewable natural polymer material. Chitosan has the characteristics of good biocompatibility, easy biodegradation, non-toxicity, and environmental friendliness. Chitosan and its derivatives have a wide range of uses, especially in the field of biomedicine, and can be used as drug carriers and artificial soft tissue materials such as Artificial leather, etc.[1,2].

1.1. Main modification pathways of chitin and chitosan
As a product of deacetylation of chitin, it contains both amino and hydroxyl groups. It can form derivatives through acylation, esterification, etherification, alkylation, graft copolymerization, crosslinking and other reactions to improve its physical and chemical properties. It can also improve its selectivity, and has good biocompatibility, antibacterial activity and biodegradability. Chitosan has
a wide range of uses in the fields of food, medicine, tissue engineering, etc.\cite{3, 4}. Chemical modification can give chitosan new functions and properties, and expand its applications.

Chitin and its chitosan are multifunctional group polymers that can undergo a variety of chemical reactions \cite{5}. Especially because of the existence of free amino in the chitosan its reactivity was stronger than chitin, which is received the wide application of chitosan, the main reasons for the different chemical reactions of chitosan modified by, the introduction of the performance of different functional groups in the molecule, can not only improve their solubility, can also be obtained with different performance and effect of derivatives, Thus, its application field is broadened and its application value is improved. The modified chitosan will be widely used in the fields of flocculant, medicine, enzyme carrier, food and so on \cite{6}.

1.1.1. Esterification reaction. Chitin and chitosan have hydroxyl groups on their residues, which can be acylated with a variety of organic acid derivatives such as anhydride and acyl halide. That is, esterification reactions introduce aliphatic or aromatic acyl groups of different molecular weights to form organic acid esters, which is the most studied type of reaction in the chemical reactions of chitosan and chitin \cite{7}.

1.1.2. Etherification reaction. The hydroxyl of chitin and chitosan can react with hydroxylation reagents to generate ethers \cite{8}, such as methyl ether, ethyl ether, benzyl ether, hydroxyethyl ether, cyanoethyl ether, carboxymethyl ether, etc., so as to improve the solubility or give additional properties, and develop some new materials.

1.1.3. Alkylation reaction. Chitosan with halogenated reaction with long carbon chain of chitosan derivatives \cite{9}, such as different carbon chain length of halogenated chemical modification of chitosan and the preparation of ethyl chitosan, butyl of chitosan, octyl and hexadecyl chitosan, the introduction of alkyl substituents weaken the hydrogen bonding interaction of chitosan, can improve its performance. Among them, C2, C4 and C8 alkyl substituted derivatives have good water solubility, while the anticoagulant activity is improved with the increase of alkyl substituted group.

1.1.4. Acylation reaction. Chitosan can react with a variety of organic acid derivatives, such as anhydride and acyl halide (mainly acyl chloride), and introduce aliphatic or aromatic acyl groups with different molecular weights. The solubility of the resulting products in solvents can be greatly improved, which is the most studied reaction in the chemical reactions of chitosan \cite{10}. There are both light group and amino group on the sugar residue of chitosan molecular chain. The acylation reaction on oxygen is called esterification reaction. The sugar residue of chitosan has two hydroxyl groups, the primary hydroxyl group at C6 position and the secondary hydroxyl group at C3 position. It can also occur on amino groups to form amides. The order of acylation activity was C2 amino larger than C6 hydroxyl group and C3 hydroxyl group. Chitosan can also be esterified with inorganic oxygen-containing acids. Generate sulfuric acid ester, nitrate ester, phosphate ester and other compounds. A great influence on the degree of acylation reaction medium, chitosan in the condition of high swelling jelly, heterogeneous high-speed acylation reaction, can be in aprotic solvent such as methanol/ethanol or methanol/formamide) completely in the acylation, and only get a part in methanol/acetic acid acylating product, it can be in trichloroacetic acid/ethylene dichloride or acetic acid solvent such as homogeneous reaction. For example, Jiang Juzhen \cite{11} et al used formamide as solvent; The ratio of concentrated sulfuric acid and chlorosulfonic acid is 1:2 mixture, the reaction temperature is -5°C-0°C, the reaction time is 4 hours, the preparation of sulfur content of more than 15% of the main product is C6 hydroxyl chitosan sulfate. Wang Zhouyu et al. \cite{12} used acetic acid and acetone as reaction media and acylated chitosan with maleic anhydride under homogeneous reaction conditions to obtain chitosan derivatives acylated on water-soluble N.

1.1.5. Quaternary ammonium reaction. There is a free amino group on each sugar residue of chitosan molecule, and quaternary ammonium salt can be easily obtained by alkylation on N. For example, n-trimethyl iodide chitosan can be obtained by nitrogen protection, n-methyl-pyrrolidone solvent,
mixture of NaOH and CH3I, and reaction at 36°C for 15 hours [13]. By exchange with chloride anion exchange resin, n-trimethyl chloride chitosan is converted to a more stable.

1.1.6. Graft copolymerization of chitosan. The graft copolymerization of chitosan mainly includes chemical method and radiation grafting. In terms of reaction mechanism, it can be divided into ion initiation and radical initiation grafting. Chitosan can improve its properties and expand its application by grafting.

1.1.6.1 Chemical grafting copolymerization.

The REDOX system initiated graft copolymerization in which the alkenes were grafted onto chitosan in heterogeneous aqueous solution using ammonium cerium nitrate or ammonium cerium sulfate. Peng Xianghong et al. [14] used Ce4+ ion as initiator to initiate graft of chitosan and alkenes monomers such as methacrylic acid, acrylamide and styrene onto sugar residues. In the free radical initiator system, initiators include persulfate, diphenylphthalide peroxide and azodiisoo-butanol to initiate graft copolymerization. For example, Liu Zheng and Jiang Xianmin [15] graft copolymerization of acrylonitrile to chitosan with potassium persulfate and sodium bisulfite as the initiator system. Jian-hua cheng [16] through free radical grafting reaction, such as in the ammonium persulfate and sodium sulfite oxidation reduction system of grafted acrylic acid ethyl ester under N2 protection dimethyl amine quaternary ammonium salt, synthesised a grafted polymer quaternary ammonium salt of chitosan polymer, and used as a conditioner in shampoo, can significantly improve polymer quaternary ammonium salt type adsorption accumulated and make the hair smooth and soft Disadvantages, has a great market application prospect [17].

1.1.6.2 Radiation grafting copolymerization

γ -ray irradiation, electron beam irradiation and ultraviolet radiation produced by low pressure mercury lamp can be used for graft copolymerization of chitosan. The grafting rate of acrylamide, methyl methacrylate and hydroxyethyl propylene ester on chitosan with 60Co γ ray was over 90% in water and methanol. Irradiation grafting can be carried out at room temperature without initiator and is suitable for biomedical field [18-22].

1.1.7 Cross-linking modification of chitosan

The purpose of cross-linking is to make the product insoluble and form a stable gel that can be used as a carrier for embedding drugs or immobilized enzymes. Crosslinking agents commonly used in chitosan include aldehydes, polyanions and cycloalkanes. The three types of crosslinking agents have their own characteristics: the aldehyde group first generates schiff base structure with the amino group of chitosan, and then reacts with the hydroxyl group, and the obtained Schiff base structure can also be reduced to the amino group; Polyanion and cation shell are crosslinked by electrostatic action. Epichlorohydrin or cyclothiohydrin can react directly with the amino group, directly cross-linking, but will reduce the amino content. Under the action of strong base, it can also react with the primary hydroxyl group of chitosan to produce the products of transaction linking [23].

On the other hand, hydrogels are an important class of functional polymer materials. Using chitosan as the matrix and adding other active components can form a hydrogel system [24,25]. Hydrogel is a kind of hydrophilic three-dimensional network polymer polymer. Its water absorption weight can reach dozens to hundreds of times its own weight. It is insoluble in water and can maintain its morphology and characteristics in water. Due to its good hydrophilicity and biocompatibility, hydrogels have been widely used in the fields of heat preservation, sanitary products, drug loading, burn dressings, postoperative anti-adhesion, etc., and will have a wider application prospect [26-28].

Most of these hydrogels are prepared by chemical cross-linking between a cross-linking agent and chitosan molecules, which are called chemical hydrogels. Compared with chemical hydrogels, intermolecular low-energy bonds (such as hydrogen bonds, coordination bonds, hydrophobic interactions, etc.) are cross-linked to form hydrogels with a network structure, which are called physical hydrogels. In order to improve the strength of the hydrogel, Gong et al. proposed a method for preparing amphoteric polyelectrolyte hydrogel [29]. The high-density ionic bond can be used as a strong interaction to improve the mechanical properties of the hydrogel, and can also be used as a recoverable sacrificial bond to give it anti-fatigue and self-healing properties. This work intends to
perform homogeneous etherification modification on chitosan in a low-temperature freezing-thawing-dissolving chitosan system by combining urea aqueous solution, and then construct a high-strength physical hydrocoagulation through in-situ polymerization, water balance, and Ag+ crosslink glue.

2. Experiment
Disperse chitosan in a KOH/LiOH/urea aqueous solution, freeze-thaw at low temperature to obtain a transparent chitosan solution. The solution is neutralized to slightly acidic with hydrochloric acid, and then protonated chitosan is obtained after dialysis and lyophilization. At the same time, different etherifying agents are added to the chitosan solution to prepare a series of chitosan etherified derivatives. Dissolve the protonated chitosan and the quaternized chitosan in the aqueous solution of acrylic monomer, add the initiator—ammonium persulfate, and pour the solution into the mold after the solution is evenly mixed, and remove it by repeated vacuum-filling with nitrogen. The dissolved oxygen in the solution was polymerized at 70°C for 18 hours to obtain a hydrogel.

It was placed in deionized water and dialyzed for a week to obtain a PEC hydrogel. The PEC hydrogel was soaked in Ag+ solution for 12 hours, and then washed repeatedly with deionized water to obtain a high-strength physically crosslinked hydrogel (DPC).

3. Results and Discussion
At low temperatures, chitosan and OH- and urea molecules in the solvent form a hydrogen bond network to dissolve. At the same time, since the KOH/LiOH/urea aqueous solution has strong alkalinity, it is also a very suitable medium for homogeneous etherification reaction. By changing the type and amount of etherifying agent, the author synthesized a series of methylated chitosan, carboxymethyl chitosan, quaternized chitosan, hydroxyethyl chitosan, hydroxypropyl with different degrees of substitution. For chitosan, studies have shown that chitosan ethers with high degree of substitution can be synthesized through a one-step reaction in a homogeneous system, and amino substitution and hydroxyl substitution are carried out simultaneously. In addition, protonated chitosan has good water solubility, which provides the possibility to construct high-strength polyelectrolyte hydrogels.

The preparation of chitosan/polyacrylic acid (quaternized chitosan/polyacrylic acid) PEC hydrogel and DPC hydrogel was carried out according to schematic of Figure 1. Because the newly prepared PEC hydrogel has not reached the electrostatic equilibrium, the ionic bond has not been formed completely, and its mechanical properties are poor. After being fully immersed in deionized water, chitosan (or quaternized chitosan) and chitosan/polyacrylic acid have a strong electrostatic interaction, and the mechanical properties of the hydrogel are significantly improved.

Table 1 shows the influence of acrylic acid concentration on tensile strength. Taking chitosan/polyacrylic acid PEC hydrogel as an example, as the acrylic acid concentration increases from 20wt% to 25wt%, its tensile strength increases from 1.1MPa to 1.27MPa; further increasing the acrylic acid concentration to 40wt%, its tensile strength The strength is reduced to 0.54MPa. Quaternized chitosan has better water solubility and higher charge density than chitosan, and can form stronger electrostatic interaction with polyacrylic acid. The maximum tensile strength of the quaternized PEC hydrogel reached 16.1 MPa, and the elastic modulus reached 63.8 MPa (see Table 2).

By changing the ratio of chitosan or quaternized chitosan to polyacrylic acid, the strength of the hydrogel can be adjusted in a wide range.

After the PEC hydrogel is immersed in Ag+ solution, since chitosan (or quaternized chitosan) contains a large amount of amino groups (-NH2), and polyacrylic acid contains a large amount of carboxyl groups (-COOH), they are both can be complexed with Ag+ to form a DPC hydrogel with dual interaction of ionic bond and coordination bond. The introduction of coordination bonds greatly improves the strength and toughness of the hydrogel. For example, the maximum tensile strength of the chitosan/polyacrylic acid (quaternized chitosan/polyacrylic acid) DPC hydrogel reaches 24.0MPa, the Young's modulus reaches 56.4MPa, and the elongation at break is still as high as 510%.
Figure 1. Schematic diagram of the preparation of PEC and DPC hydrogels.

Table 1. Tests on the mechanical properties of different concentrations of chitosan/acrylic acid PEC hydrogels.

| Samples | Chitosan concentration (wt%) | Acrylic acid concentration (wt%) | Tensile Strength (MPa) | Elastic Modulus (MPa) |
|---------|-----------------------------|----------------------------------|------------------------|-----------------------|
| 1       | 4                           | 12                               | 0.43                   | 1.26                  |
| 2       | 8                           | 18                               | 0.81                   | 1.89                  |
| 3       | 10                          | 20                               | 1.13                   | 3.52                  |
| 4       | 10                          | 25                               | 1.27                   | 4.36                  |
| 5       | 12                          | 30                               | 0.83                   | 0.78                  |
| 6       | 12                          | 40                               | 0.54                   | 0.13                  |

Table 2. Tests of mechanical properties of different concentrations of quaternized chitosan/acrylic acid PEC hydrogels.

| Samples | Concentration of quaternized chitosan (wt%) | Acrylic acid concentration (wt%) | Tensile Strength (MPa) | Elastic Modulus (MPa) |
|---------|---------------------------------------------|----------------------------------|------------------------|-----------------------|
| 1       | 4                                           | 12                               | 1.99                   | 0.98                  |
| 2       | 8                                           | 18                               | 5.96                   | 3.57                  |
| 3       | 10                                          | 20                               | 16.11                  | 63.85                 |
| 4       | 10                                          | 25                               | 14.73                  | 8.20                  |
| 5       | 12                                          | 30                               | 6.77                   | 3.45                  |
| 6       | 12                                          | 40                               | 2.24                   | 0.52                  |
4. Conclusion
For the first time, chitosan ether with high degree of substitution was synthesized homogeneously in an alkali/urea aqueous solution. In a homogeneous system, the amino and hydroxyl groups of chitosan have similar reactivity. Furthermore, chitosan or quaternized chitosan is polymerized in situ in an aqueous acrylic acid solution to obtain a high-strength, anti-fatigue PEC hydrogel with a tensile strength of 16.1 MPa and an elastic modulus of 63.8 MPa. Ag+ is introduced into the PEC hydrogel, and a high-strength and high-toughness DPC hydrogel is constructed through its complexation with amino and carboxyl groups. The tensile strength reaches 24.0 MPa and the elongation at break reaches 510%. As a polysaccharide-based hydrogel, its excellent physical properties and good biocompatibility provide a broader prospect for the biomimetic application of hydrogels.

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