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# Effects of Chain Conformation and Entanglement on the Electrospinning of Pure Alginate

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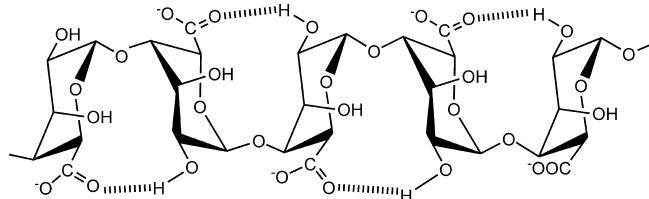
As a natural biopolymer, sodium alginate (SA) has been widely used in the biomedical field in the form of powder, liquid, gel, and compact solid, but not in the form of nanofiber. Electrospinning is an effective method to fabricate nanofibers. However, electrospinning of SA from its aqueous solution is still a challenge. In this study, an effort has been made to solve this problem and find the key reasons that hinder the electrospinning of alginate aqueous solution. Through this research, it was found that pure SA nanofibers could be fabricated successfully by introducing a strong polar cosolvent, glycerol, into the SA aqueous solutions. The study on the properties of the modified SA solution showed that increasing glycerol content increased the viscosity of the SA solution greatly and, meanwhile, decreased the surface tension and the conductivity of the SA solution. The rheological results indicated that the increase in glycerol content could result in the enhanced entanglements of SA chains. Two schematic molecular models were proposed to depict the change of SA chain conformation in aqueous solution with and without glycerol. The main contribution of glycerol to the electrospinning process is to improve the flexibility and entanglement of SA chains by disrupting the strong inter- and intramolecular hydrogen bondings among SA chains, then forming new hydrogen bondings with SA chains.

## Introduction

The linear anionic polysaccharide alginate consists of (1→4) linked  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) units in various composition and sequence and exists widely in many species of brown seaweeds. Due to its nontoxicity, unique tissue compatibility, and biodegradability, alginate has been studied extensively in tissue engineering, including the regeneration of skin,<sup>1</sup> cartilage,<sup>2,3</sup> bone,<sup>4</sup> liver,<sup>5</sup> and cardiac tissue.<sup>6</sup>

From a biological viewpoint, almost all of the human tissues and organs, such as bone, dentin, cartilage, and skin, are deposited in nanofibrous forms or structures.<sup>7</sup> Electrospinning, as a proven method of generating ultrafine fibers on a nanoscopic scale, has attracted a lot of attention in recent years. Compared with the synthesized biopolymers, natural biopolymers normally possess many superior features, such as excellent biocompatibility and biodegradability, good hydrophilicity, nontoxicity, less-immune reaction, enhanced cell adhesion, and proliferation. Therefore, numerous studies on the fabrication of natural biopolymer-based nanofibers and their biomedical applications are being undertaken.<sup>8–17</sup> Up until now, many common natural biopolymers, such as cellulose,<sup>8–10</sup> collagen,<sup>11</sup> gelatin,<sup>12,13</sup> hyaluronic acid,<sup>14,15</sup> chitin,<sup>16</sup> and chitosan,<sup>17</sup> have been successfully electrospun into nanofibers. However, electrospinning of alginate is still a challenge. To our knowledge, pure alginate has not yet been successfully electrospun into nanofibers. Although SA nanofibers have been obtained by electrospinning before,<sup>18–21</sup> they have all been based on blend solutions of SA

**Scheme 1.** Chemical Structure of G Blocks in Alginate



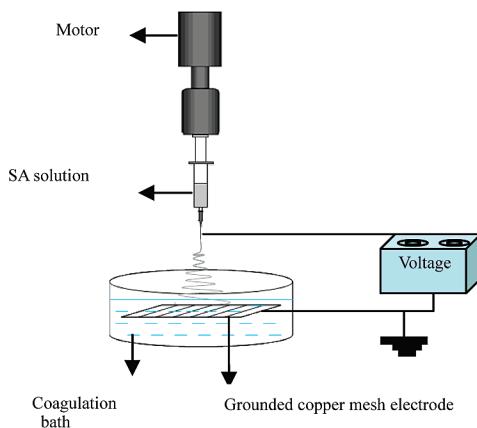
and poly(ethylene oxide) (PEO) or SA and poly(vinyl alcohol) (PVA). Electrospinning of pure SA from its aqueous solution remained a problem, and the basic reason for this problem was not clear. We did not think the difficulty in the SA electrospinning could be simply ascribed to the gelation of SA solution at low concentration (2 wt % in deionized water) or the polyelectrolyte characteristics of alginate. This study was conducted with the goal of exploring the electrospinnability of pure SA and finding the main physical reasons that hinder the electrospinning of SA aqueous solution by investigating the physical properties of the polymer solution.

SA is a water-soluble polymer and can not be dissolved in most organic solvents. To better understand what difficulties there may be in the electrospinning of SA solution, we looked into the chain conformations of alginate in water. According to Smidsrød's report,<sup>22</sup> the molecular chains of alginate are extended in water, even under an infinitely high salt concentration. Mackie reported<sup>23</sup> that the rigidity of the alginate chain is mainly caused by the G units, which are linked by diaxial linkages and stabilized by hydrogen bondings, as shown in Scheme 1. This makes the chain rigidity of alginate have a similarity to the cellulosic chain. It was speculated that the rigid and extended worm-like molecular chains of alginate could not

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**Figure 1.** Schematic diagram of the electrospinning setup.

form effective chain entanglements in its aqueous solution. This may be the reason that electrospinning of pure alginate from its aqueous solution is so difficult or impossible to be achieved. In this investigation, a strong polar cosolvent, glycerol, was used to solve the aforementioned problem. For the first time, pure alginate nanofibers were successfully fabricated by the electrospinning process.

## Experimental Section

**Materials.** Sodium alginate (SA, 3500 cps for a 2% solution at 25 °C) was purchased from Sigma. Glycerol, ethanol, and calcium chloride ( $\text{CaCl}_2$ ) were obtained from Beijing Chem. Co. (Beijing, China). All the reagents were used as received.

**Electrospinning.** SA solutions were prepared by dissolving a precise amount of SA in solvents through gentle stirring. The mixture of glycerol and double distilled water was used as the solvent, and the volume ratio of glycerol to double distilled water was varied from 0/1 to 2/1. The concentration unit used was in w/v% (w in g and v in mL). A high voltage power supply (The Beijing Machinery & Electricity Institute, China) was employed to generate the high voltage (0–50 kV). The applied voltage was fixed at 28 kV in this study, and the tip-to-collector distance was 12 cm. A copper mesh used as the collector was immersed in a coagulating bath containing ethanol and 10 wt %  $\text{CaCl}_2$  aqueous solution (the volume ratio of ethanol to 10 wt %  $\text{CaCl}_2$  aqueous solution was 5/1). The copper mesh was placed 1 cm beneath the surface of the liquid, as shown in Figure 1. The SA solution was loaded into a 5 mL syringe with a capillary tip with an inner diameter of 0.3 mm. A syringe pump was then used to feed the SA solution into the needle tip at a rate of 105  $\mu\text{L}/\text{min}$ . After electrospinning, the SA fibers were immersed in ethanol for 30 min and then rinsed with ethanol to remove the glycerol. The SA fibrous membranes were dried in a vacuum oven at a temperature of 50 °C overnight to dry off any remaining solvent.

**Characterization.** The morphologies of the electrospun fibers were observed using scanning electron microscope (SEM, JEOL JSM-6700F, Japan) at an accelerating voltage of 5 kV. Each sample was sputter-coated with gold for analysis. The surface tensions of SA solutions were measured by a surface tension meter (Dataphysics) at 25 °C. Their conductivities were measured by a conductivity meter (DDS-307A, Rex Shanghai). All rheological measurements were performed on a strain-controlled rheometer ARES with couette geometry (TA Instruments, U.S.A.) at 25 °C. Frequency sweeps were carried out for angular frequencies  $\omega = 0.1\text{--}100 \text{ rad/s}$  at a strain amplitude of 1%. Shear measurements were performed in a range of shear rates from 0.05 to 90  $\text{s}^{-1}$ .

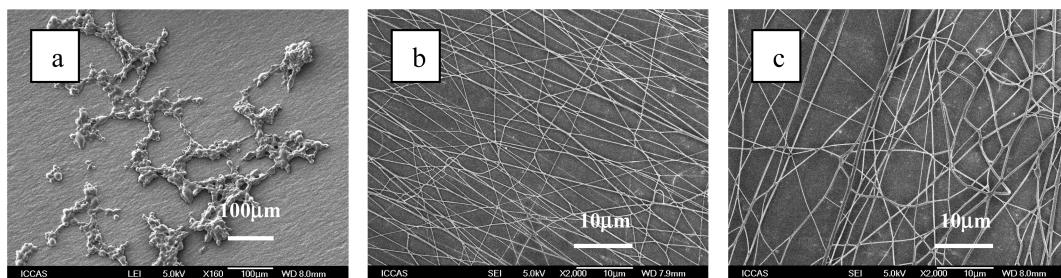
## Results and Discussion

SA, as a natural polyelectrolyte, can dissolve very well in water in a wide range of concentrations. In this study, electro-

spinning of various SA aqueous solutions with concentrations ranging from 1.5 to 4.5 w/v % was tried. It was observed that droplets fell down from the spinneret and no continuous jets formed even under a high applied voltage (30 kV). It seems that the alginate aqueous solutions were not gelled nor had a very high viscoelasticity to form a continuous stream in the spinning process. In addition, we do not think the reason that the SA solutions cannot be electrospun can be simply blamed on their polyelectrolyte characteristics.

In view of the rigid and extended chain characteristics of alginate molecules in aqueous solution, increasing the flexibility and entanglements of SA chains may be essential in resolving the aforementioned problem. It has been reported that the inclusion of polyols such as glycerol or poly(ethylene glycol) can improve the flexibility of SA chains.<sup>24</sup> Glycerol is a low-cost polar bioreagent and can mix with water in any ratio without any SA precipitate forming. In this study, glycerol was used as a cosolvent. Then the electrospinnability and the physical properties of the SA/water/glycerol system were studied for the first time. The spinning solutions were fixed at a specific concentration of 2 w/v %, and the volume ratio of glycerol to water was varied from 0.5 to 2. The SEM images of the electrospun SA fibers are shown in Figure 2. When the volume ratio of glycerol to water was 0.5, electrospinning could not be carried out and only irregular droplets were collected (Figure 2a). When the volume ratio of glycerol to water was 1, ultrathin fibers could be obtained (Figure 2b), although the electrospinning process was not continuous. When the volume ratio of glycerol to water was increased to 2, a continuous and stable electrospinning jet was observed. As seen in Figure 2c, uniform fibers without beads could be produced, with an average fiber diameter of about 200 nm. It can be concluded that the increase in glycerol content improves the electrospinnability of the SA solution. Following this discovery, the physical properties of SA solutions with and without glycerol were studied. As shown in Table 1, the properties of the solution changed greatly when glycerol was introduced into the SA solution. In our study, the viscosity of 2 w/v % SA aqueous solution obtained from ARES Rheometer at a shear rate of 0.5  $\text{s}^{-1}$  was 22.2 Pa s. However, the viscosity of 2 w/v % SA solution increased significantly with the increase in volume ratio of glycerol to water. When the volume ratio of glycerol to water was 2, the viscosity of the SA solution at a shear rate of 0.5  $\text{s}^{-1}$  increased to 701.3 Pa s. We know that the viscosity of glycerol itself (1.5 Pa s) cannot affect the viscosity of SA/water/glycerol system very much. Therefore, it can be deduced that the glycerol must have changed the SA chain conformation in the solution, and the SA chain entanglements may have been greatly enhanced, which led to the increase in viscosity of the SA solution. In addition, a significant decrease in both the surface tension and the conductivity of SA solution was observed with the increase in glycerol content. It was indicated that all the changes of the modified SA solution, including the decreased surface tension and conductivity and increased viscosity jointly contributed to the improved electrospinnability of the SA solution. We conclude that glycerol as the cosolvent plays an important role in modifying the properties of the SA solution.

The effect of SA concentration in the range of 1.6–2.4 w/v % on the electrospinning process was studied at a fixed volume ratio of glycerol to water of 2. It was found that the average fiber diameter increased from 120 to 300 nm with the increase in polymer concentration (Figure 3). However, when the SA concentration was increased to 2.4 w/v %, the SA solution was too viscous to be electrospun smoothly. In cases where fibers



**Figure 2.** SEM images of SA fibers electrospun from SA solutions with different volume ratio of glycerol to water (a) 0.5, (b) 1, and (c) 2. SA concentration was fixed at 2 w/v %.

**Table 1.** Physical Properties of SA Solutions

$V_{\text{glycerol}}/V_{\text{water}}$ (SA concentration, 2 w/v %)	conductivity ( $\mu\text{S}/\text{cm}$ )	surface tension (mN/m)	$\eta^a$ (Pa s)
0	4180	61.4	22.2
0.5	1310	45.0	76.9
1	597	42.0	196.3
2	193	37.5	701.3

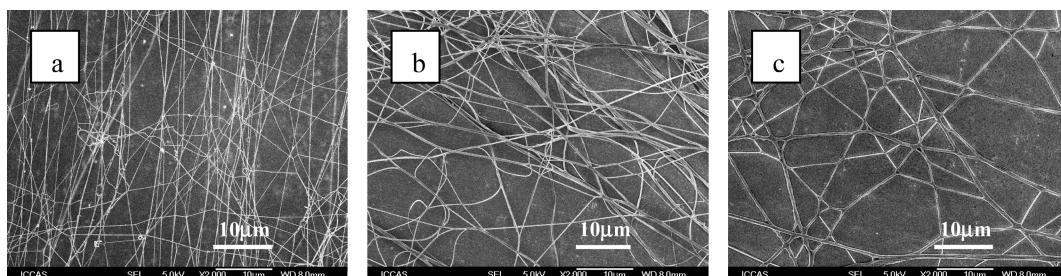
<sup>a</sup> Viscosity was obtained by ARES Rheometer at a shear rate of 0.5  $\text{s}^{-1}$ .

can be formed, most of the glycerol could be removed and still obtain cross-linked SA nanofibers through a coagulating bath containing ethanol and  $\text{CaCl}_2$  solution.

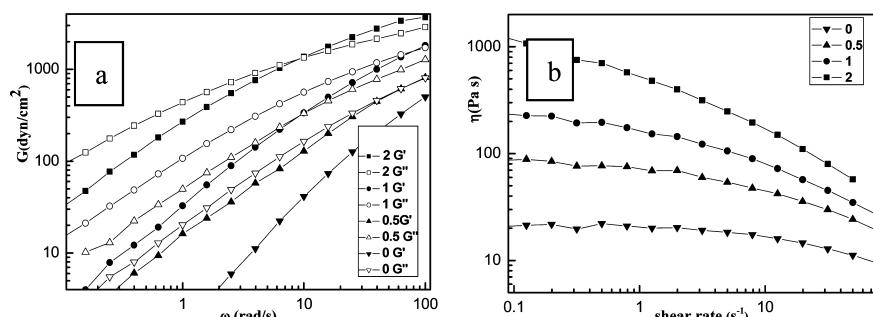
Rheological measurements were also carried out to understand the effect of glycerol on the chain conformation and hydrodynamic behavior of the SA solution. It can be seen from Figure 4 that both  $G'$  and  $G''$  increased with the increase in glycerol content, which indicates that both the viscosity and elasticity of the system increase with the increase in glycerol content. When the volume ratio of glycerol to water was less than 1, no crossover point ( $G' = G''$ ) could be observed within the referred frequency. However, when the volume ratio of glycerol to water was increased to 1, the crossover point was observed and moved to lower frequency with the additional increase in glycerol content (Figure 4a). A high correlation was found in our study

between a lower crossover frequency and the higher quality of electrospinning. It has been accepted that the shift of the crossover point could reflect the change of physical crosslinking degree of the studied system.<sup>25,26</sup> In other words, the shift of the dynamic crossover point to a lower frequency is an indication that the networks caused by either chain entanglement or crosslinking are enhanced and tighter. Glycerol, as a very strong polar solvent with three hydroxyl groups per molecule, can interact with SA by disrupting a large number of inter- and intramolecular hydrogen bondings of SA chains and then forming solvent–polymer hydrogen bondings. As a result, the apparent flexibility of SA chains may have increased and the formation of the entanglements of SA chains becomes possible. In Figure 4b, a typical Newtonian liquid behavior is observed within a wide range of shear rates in the SA/water system. However, when the glycerol content is increased in SA solution, shear-thinning behavior becomes increasingly apparent. This effect can also be explained in terms of the enhanced entanglement networks, which will be disturbed at lower and lower shear rates in a shear rate-dependent measurement.

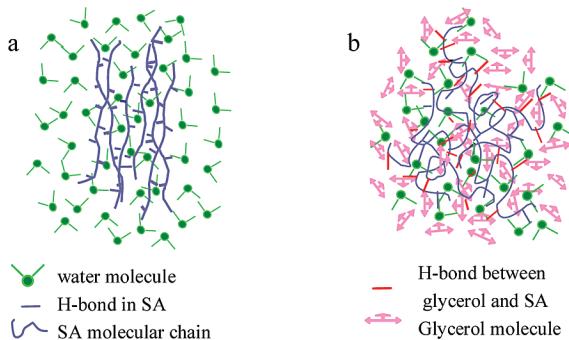
Based on the aforementioned results, we can conclude that glycerol greatly improves the electrospinnability of the SA solutions. The combined contribution of enhanced chain entanglement (or both viscosity and elasticity) and decreased



**Figure 3.** SEM images of SA fibers electrospun from SA solution with different concentration: (a) 1.6, (b) 2.0 and (c) 2.4 w/v %. Volume ratio of glycerol to water was constant (2/1).



**Figure 4.** Effect of glycerol content on (a) storage ( $G'$ ) and loss ( $G''$ ) moduli of the SA solutions as functions of frequency at 25 °C and (b) viscosity of the SA solutions as a function of shear rate. SA concentration was fixed at 2 w/v %. The following, 0, 0.5, 1, and 2, represent the volume ratio of glycerol to water.



**Figure 5.** (a) SA molecular model in aqueous solution and (b) SA molecular model in glycerol–water mixed solution.

surface tension and conductivity may be the key reasons for the improved spinnability. Therefore, the previously mentioned factors should be adjusted to achieve the optimal spinnability. Although the viscosity can be adjusted by changing the concentration of SA, the increase in SA concentration up to 4.5 w/v % has very little positive impact on the spinnability of SA solution. It has been reported that PEO can benefit the SA fiber formation, however, the surface tension increased contrarily with the addition of PEO.<sup>19</sup> Hence, we can further draw the conclusion that the key factor hindering SA electrospinning from its aqueous solution is due to the insufficient chain entanglements. As previously mentioned, glycerol, as a strong polar cosolvent, can improve the SA chain entanglements. The decrease in the surface tension and conductivity may also contribute to the improvement in the electrospinnability of SA, however, its effects may not be as important in the SA/water/glycerol system.

To better illustrate the role of glycerol and the change of the SA chain conformation with and without glycerol, two schematic illustrations are shown in Figure 5. Figure 5a depicts the original SA molecular chain conformation in aqueous solution, which represents the extended and rigid chains stabilized by the hydrogen bondings, and few entanglements can be observed. However, these SA chains become flexible and chain entanglements occur with the introduction of glycerol, which can form hydrogen bonding directly with the SA molecules and results in a more flexible SA chain conformation. This new SA chain conformation and entanglement networks are depicted in Figure 5b. These pictures indicate that the chain conformation of natural biopolymers, which normally have many polar groups and charges, may play an important role in the electrospinnability of their aqueous solution. The enhanced or improved chain entanglements will improve the fabrication of nanofibers. It is expected that the SA nanofibrous materials with improved water-resistance by CaCl<sub>2</sub> crosslinking will have numerous potential applications in biomedical fields such as wound dressing, tissue engineering scaffold, drug delivery, and so on.

## Conclusions

In this study, pure SA was successfully electrospun by using glycerol as a cosolvent. Continuous jets could be observed and smooth uniform SA fibers were fabricated by simply adjusting the volume ratio of glycerol to water in the solvents. The studies on the properties of the SA solutions showed that the increase in glycerol content led to the decrease in the surface tension and conductivity, as well as a noticeable increase in viscosity and elasticity. Rheological measurements indicated that glycerol

could enhance the SA chain entanglements by forming new hydrogen bondings with SA chains after disrupting some or most of the inter- and intramolecular hydrogen bondings of SA chains. Two schematic model pictures were used to depict the SA chain conformations in aqueous solution with and without glycerol. The main factor that hindered the electrospinning of SA may be the lack of chain entanglements due to the rigid and extended SA chain conformations in aqueous solution. This study also provided a fundamental understanding and possible solution to other natural biopolymer solutions with problematic electrospinning processes. It is expected that SA nanofibrous membranes will have potential applications as novel biomedical materials.

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