

Preparation of Polyion Complex Capsule and Fiber of Chitosan and Gellan-Sulfate at Aqueous Interface

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The electrostatic interaction between polysaccharides has been studied. Different characteristic surface structures, such as true spheres and fibrous forms, are made from gellan-sulfate (GS) and chitosan via polyion complex (PIC) formation. When a chitosan solution is added dropwise into a GS solution, spherical droplets form in the GS solution. The procedure makes true spherical capsules with various diameters, which are stable enough for finger pinching or magnetic stirring in distilled water. The soft droplet capsule is acid resistant, alkali resistant, and stable in boiling water. When an aqueous GS solution is added into an aqueous chitosan solution without mixing, a film of PIC is formed at the interface. When this PIC film is withdrawn from the interface, a fiber line forms in the wet states. After the intact wet fiber is dried in air, a strong fiber forms. The tensile strength of the strongest fiber created is 196 MPa. The swelling degree of the capsules and the strength of the fibers depend on the degree of sulfation of gellan.

A new polysaccharide material, gellan, which is composed of tetrasaccharide repeat units comprised of [→3)-β-D-glucose-(1→4)-β-D-glucuronic acid-(1→4)-β-D-glucose-(1→4)-α-L-rhamnose-(1→],[1] was first found in freshwater weeds in a Pennsylvania pond in 1970'. Later, gellan has been used as a promising biomaterial in food additives, culture media, and so on. In parallel, based on the biomedical interests, the weak acidity of gellan was changed to a stronger acidity by introducing sulfo moieties. As a biomedical selective artificial ligand, gellan-sulfate (GS) was successfully synthesized for use in removing a complex of extra domain A containing fibronectin from plasma in rheumatoid arthritis patients.[2–4]

Polyion complexes (PICs) are formed by the reaction of a polyelectrolyte with an oppositely charged polyelectrolyte in aqueous solution. PICs have numerous applications such as membranes, antistatic coatings, and microcapsules, all of which have been widely studied.[5,6]

In the course of our continuing work on the creation of biomimetic hybrid materials,[7–16] we found that the interaction between anionic and cationic polyelectrolytes gives characteristic structures at the interface between aqueous solutions. In this article we report different characteristic surface structures, such as capsules and fibers, between cationic chitosan,[17,18] which is composed of [(1→4)-2-amino-2-deoxy-β-D-glucan] with amino functional groups, and anionic GS with carboxyl and sulfo functional groups via PIC formation at aqueous interface.

Experimental

Materials and Methods. Chitosan (chitosan 10, 100, and 1000), *N*,*N*-dimethylformamide dehydrated (DMF), and chlorosulfonic acid ($ClSO_3H$) were purchased from Wako Pure Chemical Ind., Japan. The viscosity average molecular weights of chitosan are in the 210000–1800000 range from the viscosity equation.[19] Gellan gum was kindly given to us by San-Ei Gen F.F.I., Inc., Japan.

Preparation of GS. GS was prepared by mixing gellan and $ClSO_3H$ in DMF, as reported in the earlier articles.[2–4] After neutralization, the reaction mixture was treated with acetone for precipitation. The precipitate was dissolved in water, dialyzed, and freeze-dried. The degree of substitution for sulfation (DS) was measured by titration with barium chloride using an indicator, dimethylsulfonazo Ⅲ, in water.[20]

Preparation of PIC Hybrid Materials. A 1.5% chitosan solution in 0.15 M (1 M = 1 mol dm^{-3}) acetic acid was prepared. 0.75% aqueous solutions of GSs with different DSs were prepared. When a chitosan solution is added dropwise into a GS solution, spherical droplets form in the GS solution spontaneously. When a GS solution is added onto a chitosan solution and the interface is withdrawn, a long fiber line forms from the interface in the wet states. Wet reactive spinning in water can be done using a roll up apparatus after dehydration in alcohol.

Characterization of Fibers. Scanning electron microscopy (SEM) was performed using a Hitachi S-5000 microscope to observe the surfaces of the fibers (accelerating voltage, 3 kV). The diameters of the fibers were measured both by an optical microscope and SEM. The stress/strain curves of a single thread of the fibers were measured using a Tensilon (STA-1150, Orientec Co.). The initial gauge length was 20 mm, and the drawing speed was 20 mm/min. The average stress/strain curves were determined from 10 independent measurements.

Results and Discussion

Sulfated gellan was prepared according to earlier articles.[2–4] After some modifications of the reaction conditions were done, the DSs were analyzed to be in the 6–44% range (Fig. 1). The GSs were then used in the PIC creation reactions.

When a 1.5% chitosan (M_w = 1800000) solution in 0.15 M acetic acid at pH 5 and 20–60 °C is added dropwise into a

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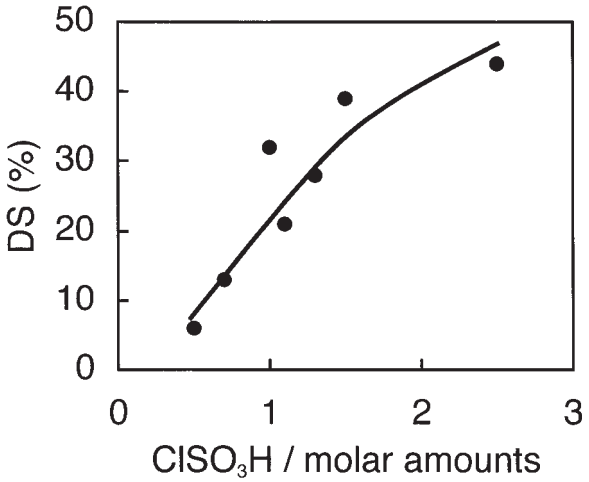

Fig. 1. Relationship between the degree of sulfation (DS) of gellan and the added sulfating agent, chlorosulfonic acid ($ClSO_3H$). Molar amounts; $ClSO_3H$ amounts to one hydroxy group of gellan.

0.75% GS solution in water at pH 4–6 and 20–60 °C, spherical droplets form in the GS solution (Fig. 2(a)). This procedure makes true spherical droplet structures with various diameters. When lower M_w chitosan samples ($M_w = 210000$ and 1310000) are used, the solutions keep floating on the GS solution and the spherical droplets are not formed. The spherical droplets, the inside of which is chitosan and the outside surface GS, are stable enough for finger pinching or magnetic stirring in distilled water to rinse extra GS off the droplets' outside surfaces. The soft droplet capsule is acid resistant, alkali resistant, and largely stable in boiling water. The swelling degree of the droplet capsule depends on the DS of gellan. For example, when a chitosan solution and a GS-6 (DS = 6%) solution reacted for 30 min at 60 °C, a droplet with a diameter (d_0) = 4.9 mm was obtained and the droplet swelled to a diameter (d) = 6.1 mm after being immersed for 24 h in distilled water. When a chitosan solution and a GS-39 (DS = 39%) solution were allowed to react, a droplet with a $d_0 = 5.8$ mm was obtained and remained unchanged at $d = 5.8$ mm after being immersed for 24 h in distilled water. In 80–100% ethanol the droplets shrink to white turbid solid particles.

Next, when an aqueous GS solution is carefully added into an aqueous chitosan ($M_w = 210000$ and 1310000) solution at pH 5 and room temperature without mixing, a film of PIC is formed at the interface. When this interface film is removed without stirring, it is instantly and continuously replaced. When this PIC film is withdrawn from the interface and is hung up over glass rod, a fiber line forms in a wet state (Fig. 2(b)). After the intact wet fiber is dehydrated in alcohol and dried in air after, a strong fiber forms (Fig. 2(c)). This fiber has a double counter ion structure; that is, the outside layer

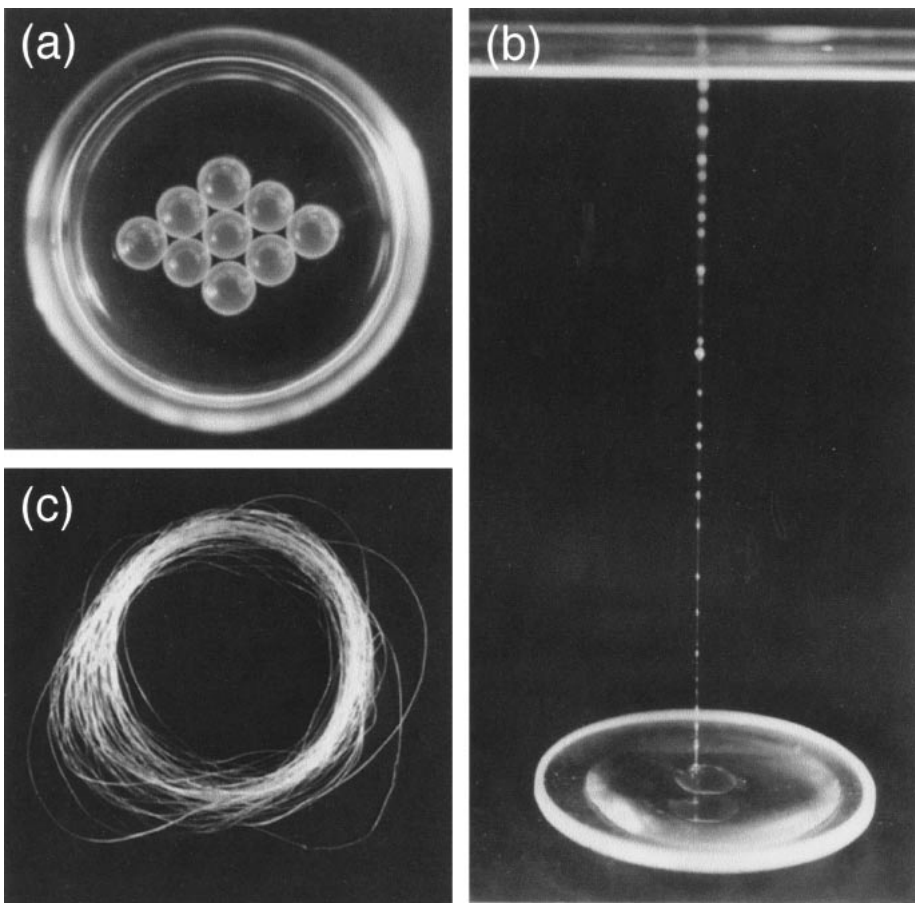

Fig. 2. Characteristic capsule and fiber structures via polyion complex formation. (a) True spheres (top view). (b) Regularly spaced droplets in the wet states. (c) Fiber after drying.

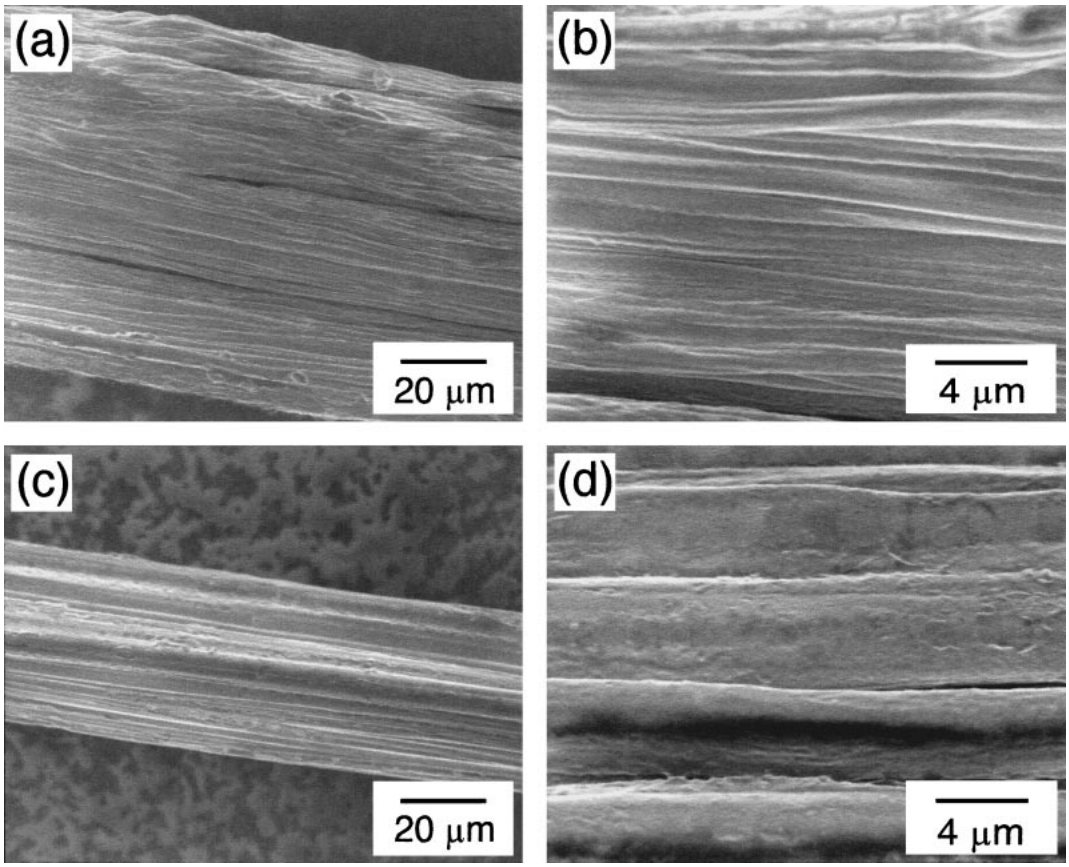

Fig. 3. SEM images of PIC fibers. Chitosan–gellan fiber; (a) (magnification: ×1000) and (b) detailed close-up view (×5000). Chitosan–GS fiber; (c) (×1000) and (d) detailed close-up view (×5000).

is chitosan and the inside layer is GS. Likewise, a strong fiber, which is made in a similar way as described above, but whose outside layer is GS and inside layer is chitosan ($M_w = 210000$ and 1310000), is spinnable. When chitosan ($M_w = 1800000$) is used as the inside or outside material, a droplet is obtained instead of a fiber because the chitosan solution has a high viscosity.

SEM images of a single thread of the PIC fibers are shown in Fig. 3. The diameters of the chitosan–GS fibers were in about the 30–90 micron range (Fig. 3(c)), and much thinner than the chitosan–gellan fibers (Fig. 3(a)) reported in our earlier articles.[7,9] A number of fibrils are assembled into a fiber. The fibril structures of both fibers are aligned (Figs. 3(b) and (d)).

The stress/strain curves of the PIC fibers are shown in Fig. 4. The sulfation procedure led to stronger fibers, while the strains were only changed between 6 (GS-13) and 11% (GS-0). The numbers of sulfate groups in the GS-13, 28, and 44 fibers were measured, and the ratios of GS to chitosan residues were 4:1, 1:3, and 1:9, respectively. The excess un-interacting molecules are assumed to be folded. When there are many folds in a chain molecule, the initial elastic moduli (the initial slopes of stress/strain curves) are decreased.[21] For GS-44, which is highly folded, the initial elastic modulus was the lowest. The relationship between fiber tensile strength and the DS of gellan is shown in Fig. 5(a). The strength of the fibers depends on the DS of gellan. At DSs of 21–28%, the strongest fibers

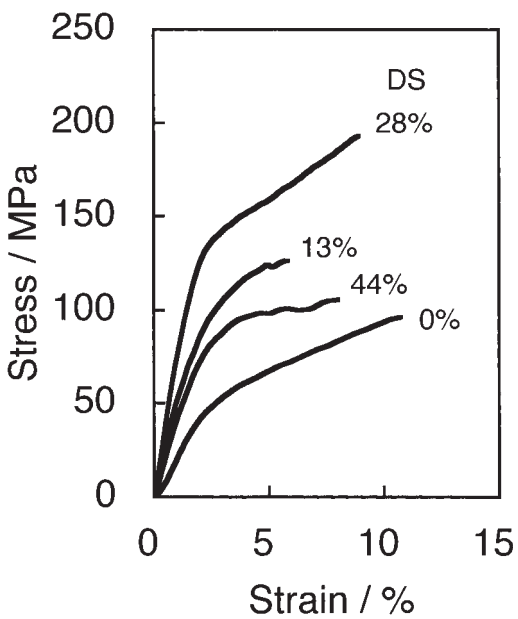

Fig. 4. Stress/strain curves of the chitosan–GS fibers.

were obtained, but at DSs higher than 30%, the strengths of the fibers decreased. The strongest fiber thus created was 196 MPa (20.0 kgf/mm^2). The numbers of anions and cations in the fibers of GS-13, 28, and 44 were calculated. The lesser

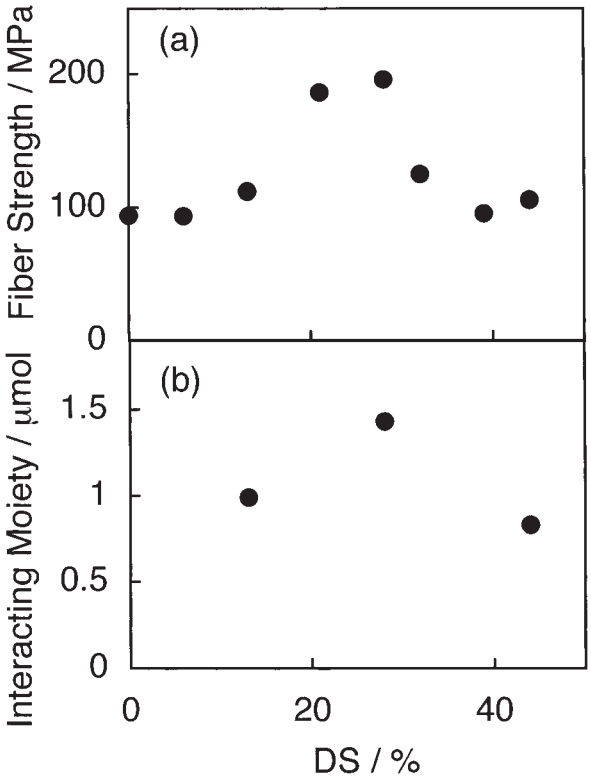

Fig. 5. (a) Relationship between the tensile strength of the fibers and the DS of gellan. (b) Relationship between the number of moles of possible interacting moieties in the fibers and the DS of gellan.

numbers are the numbers of possible interactions. The numbers of the interacting moieties are shown in Fig. 5(b). As the bridge structure becomes denser, the fiber becomes stronger. The results of Fig. 5(a) are in good agreement with those of Fig. 5(b).

Thus, a stable spherical capsule and a strong fiber can be prepared via this PIC between chitosan and GS. They can be biodegraded by microorganisms and enzymes[22–24] involved in keeping both land and sea ecology clean, and may be used for selective adsorption of cell adhesion molecules and for the suppression of cancer cell metastasis.[2–4] The results themselves are scientifically interesting, and might offer additional knowledge toward understanding biological interaction, such as the self-assembly of proteins, antigen–antibody reactions, and the transmission of genetic information.

In our PIC hybrid material studies, we first created fibers and capsules having sulfate functional groups as the polyanionic component. These findings may also have the prospect of developing new biomimetic materials in aqueous circumstances. As we have reported, given the selective adsorption ability of anionic (acetylsalicylic acid and acidic amino acids) and cationic (phenethylamine) molecules to the inside of matrices,[25,26] it can be anticipated that charged molecules will adsorb onto/into the capsules and the fibers by changing the weakly acidic carboxyl to the strongly acidic sulfo groups. When the capsules and the fibers are biodegraded, the digested oligochitosan fragments are expected to exhibit anti-infection actions as reported earlier.[27,28] Thus, PIC capsules and fibers having strongly acidic sulfo moieties may show additional promise in the diverse medical, agricultural, and fiber industrial applications.

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