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Chitosan-Coating of Cellulosic Materials Using an Aqueous Chitosan-CO_2 Solution

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ABSTRACT: Chitosan was dissolved in water using carbonic acid gas (CO_2) *via* preparation of the gel. The suspension of the gel became transparent and the viscosity increased by the bubbling of CO_2 gas, showing that chitosan was dissolved by CO_2. Cellulosic materials were easily coated with chitosan using this solution (chitosan-CO_2 solution), because it is unnecessary to remove the acid usually required in the case of the coating using chitosan-acid solutions. Cotton fibers were dipped into chitosan-CO_2 solution, and then dried. The degree of the chitosan coating was examined by adsorbing acidic fluorescent probe on the chitosan-coated cotton and observing it by fluorescence microscope. Chitosan-coating was also performed on paper and the degree of the coating was examined by Fourier transform infrared-attenuated total reflection (FT-IR-ATR) spectroscopy. Antimicrobiabil activity of the chitosan coated on cotton gauze to *Staphylococcus aureus* was examined, and effective activity was ascertained. These results showed that the chitosan-coating using chitosan-CO_2 solution was hopeful to coat cellulosic materials with chitosan and render pharmacological activities to them.

KEY WORDS Chitosan / Carbonic Acid Gas / Viscosity / Fluorescent Microscope / Fourier Transform Infrared-Attenuated Total Reflection (FT-IR-ATR) / Antimicrobial Activity /

Chitosan, a hydrolyzed product of chitin, is a naturally-occurring, biodegradable polymer[1] and show various pharmacological activities such as anti-fungal,[2,3] anti-allergic,[4] anti-tumor,[5] immune-activating[6,7] effects and so on. Recently, these properties of chitosan have been focused on from the viewpoint of industrial usage. Anti-allergic textiles were developed by mixing powdery chitosan into the substrate polymer and some of them are on the market. However, mixing of powdery chitosan seems not to be effective for fully revelation of the property of chitosan. Chitosan buried in the substrate polymer does not work; The material becomes heterogeneous, which usually lowers the flexibility and the stability of the materials. For economically effective use, chitosan had better be coated on the substrate polymer.

Chitosan-coating is a very promising technique to render the various pharmacological properties of chitosan on the substrates. For this purpose, chitosan must be dissolved in a solvent and the solution painted on the material. After drying the solvent, chitosan must be left as a film without the solvent. Enough adhesion between chitosan film and the substrate material is required. However, chitosan is not dissolved in water and in usual organic solvents except for some fluorine-containing solvents which are expensive and toxic, so it is difficult to find an adequate solvent for chitosan-coating.

Chitosan is an only naturally occurring basic polymer possessing amino groups and, therefore, is possible to be dissolved in various aqueous solutions containing organic and inorganic acids. Therefore, it might be expected to use these solutions for chitosan-coating. However, acid remains in the film after drying the solution, and, as a result, resulting film is water-soluble. This is not desirable, so the treatment with alkali solution is necessary to eliminate the remaining acid. This is troublesome and requires time and money. In addition, such treatment might damage the chitosan film and the substrate material. For ideal chitosan-coating, a substance, which acts as an acid for dissolving chitosan in water and is removed naturally in the process of drying, must be very useful from an industrial point of view. We found that carbonic acid gas (CO_2 gas) was useful as such material.[8]

EXPERIMENTAL

Materials

Chitosan (PSH-80) was supplied from Yaizu Suisan Co. Ltd., the molecular weight being 8×10^5. Other chemicals purchased from Wako Co. Ltd., were of

guaranteed grade.

Preparation of Chitosan-CO_2 Solution

One gram of powdery chitosan was dispersed in 100 mL of water, and 10 mL of 1 N HCl solution was added to dissolve chitosan. The solution was neutralized by adding a small excess equivalent of an aqueous 0.5 N NaOH solution and agitating the mixture. A translucent soft gel was precipitated and was collected, and then washed with distilled water repeatedly until the washed water show neutral pH. The gel was dispersed in water and was broken into small pieces with a mixer. CO_2 gas was bubbled in the suspension using a capillary tube. The concentration of chitosan was determined by weighing the chitosan in the gel after drying, before dispersing in water.

Measurement of the Viscosity of Chitosan Solution

To measure the change of the viscosity during the process of dissolving chitosan with CO_2, the concentration of chitosan was set as 1%, and a part of the suspension was taken out for viscosity measurement. As the gel was a disturber for exact measurement, it was separated by quick centrifugation before measurement. Viscosity was measured using a B type rotating viscometer (RE80 Viscometer, Toki Sangyo Co. Ltd.,).

Observation by a Fluorescent Microscope

A commercial sanitary cotton (Ohsaki Cutter Cotton) was dipped into chitosan-CO_2 solution for a few minute, and taken out and dried at 60°C. This chitosan-treated cotton (content of chitosan, 1%) was dipped into ethanol solution containing fluorescein (25%) as a fluorescent probe for 1 h, and then washed with water. Chitosan-nontreated cotton was also treated with the fluorescein solution. These cottons were observed by a fluorescent microscope (Olympus IX70).

Measurement of the FT-IR-ATR Spectra

A commercial filter paper (Advantec Toyo, No. 2) was dipped into chitosan-CO_2 solution for a few minute, taken out and dried at 60°C. The content of chitosan (2%) coated on the paper was calculated by comparing the weight of the sample before and after treatment. IR spectra of the chitosan-treated and nontreated filter papers were measured with the accumulation time of 50 using a JASCO FT/IR-670 plus spectrometer equipped with an ATR accessory fitted up a ZnSe crystal (MIRacle, Pike Co.).

Measurement of Antimicrobial Activity of Chitosan-Coated Gauze

A commercial gauze (Pipfujimoto Co. Ltd.), produced according to Japanese Pharmacopeia was dipped in chitosan-CO_2 solution for 5 min, and dried at 60°C. The content of chitosan was 5%. Antimicrobial activity of this chitosan-coated gauze to *Staphylococcus aureus* was measured by the method (Quantitative test of JIS L 1902 (1998)) of Japanese Industrial Standard (JIS).[9] The *Staphylococcus aureus* (IFO 12732) was supplied from Institute for Fermentation, Osaka.

RESULTS AND DISCUSSION

CO_2 gas dissolves in water (0.82 mL mL^{-1} H_2O, 25°C, 1 atm) and the dissolved CO_2 molecule reacts with H_2O molecule forming H_2CO_3. This H_2CO_3 molecule is an acid and dissociates into H^+ and HCO_3^- ions. H_2CO_3 is known as a very weak acid (pK_a = 6.4)[10] but this is the value calculated assuming that all the CO_2 molecules dissolved in water changes into H_2CO_3. However, the reaction, $CO_2 + H_2O = H_2CO_3$, inclines extremely to the left, so the exact acidity of H_2CO_3 must be re-calculated considering the equilibrium constant of the above equilibrium reaction. Garg and Maren obtained the equilibrium constant, $K = [CO_2]/[H_2CO_3] = 4.6 \times 10^2$, in water using a stopped flow method.[11] The real value of pK_a was 3.8 using the above equilibrium constant, which means that H_2CO_3 is an acid stronger than acetic acid (pK_a = 4.6) and near lactic acid (pK_a = 3.7). As acetic and lactic acids have the ability to dissolve chitosan, CO_2 should dissolve chitosan. Chitosan gel and deacetylated chitin (DAC 50) dispersed in water were easily dissolved by bubbling CO_2 gas into the emulsion.[8] We tried to use this aqueous chitosan solution dissolved by the bubbling of CO_2 gas (abbreviated as chitosan-CO_2 solution) for chitosan coating. When usual acid solutions of chitosan were used for coating, the acid or anion combined with amino group is not eliminated in the process of drying. In the case of chitosan-CO_2 solution, however, dissolved CO_2 is easily transferred to the air as gaseous CO_2 during evaporation of water. Accompanying it, H_2CO_3 molecule decomposes into CO_2, and this change also induces the decrease of HCO_3^- ion which is responsible for ionizing and dissolving chitosan. As a result, ionized amino groups of dissolved chitosan loses the charge and precipitates forming a film without acid.[8] Nevertheless of this speculation, there is a possibility that HCO_3^- ion is left in the film after drying and combines with cationized amino group as a salt. This ionic pair is a kind of ammonium hydrogen carbonate, and it is known that ammonium hydrogen carbonate is unstable and easily decomposes into ammonia, water and CO_2. Therefore, we thought that CO_2 is perfectly eliminable during the process of drying. The postula-

Table I. Viscosities and the pH values of chitosan solutions (1%) dissolved in various acids

	Hydrochloric acid	Acetic acid	Butyric acid	Carbonic acid gas
Viscosity/mPa s	166.7	92.8	415.6	1065.7
pH	0.41	3.38	2.49	6.61

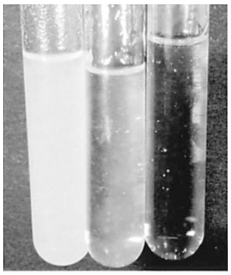

Figure 1. Photographs of chitosan suspension 0 (A), 1 (B), and 3 (C) min after beginning of the CO_2 bubbling.

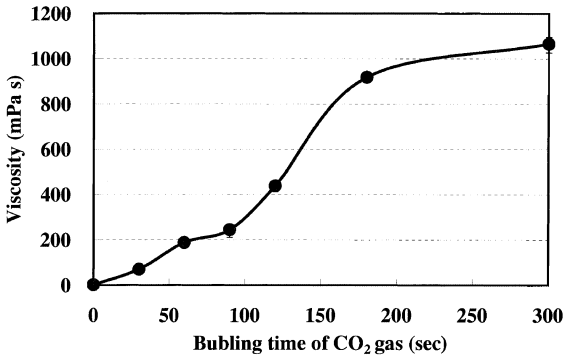

Figure 2. Viscosity change of the chitosan suspension during the process of CO_2 bubbling.

tion that the CO_2 is easily eliminable from chitosan-CO_2 solution was also proved by an experiment of the bubbling of N_2 gas. Powdery chitosan was precipitated when N_2 gas was bubbled into chitosan-CO_2 solution, showing that CO_2 was easily expelled by this operation.

The appearance of the gel changed depending on the concentration of NaOH used for pH neutralization. When NaOH concentration was high, powdery chitosan was precipitated and it was very hard to dissolve it with CO_2. Reversely, the gel was soft and translucent when the concentration was low. In this case, the gel was easily dissolved by bubbling with CO_2. These results means that the pH value around chitosan molecule becomes locally large when high concentration of NaOH was used, and dissolved chitosan loses most of charges and forms crystal structure and precipitates. However, chitosan can still hold the charges when low concentration of NaOH is used and only small parts of chitosan lose charges and coagulates, resulting in the bridging between the molecules, namely the formation of the gel. Although the gel was easily dissolved, dissolving of powdery chitosan took considerable long time. This is because CO_2 molecule is able to enter into the gel and ionize the non-charged part of the gel, but is not able to enter into solid chitosan and, as a result, only slow dissolving from the surface occurs. Figure 1 shows the photographs of the chitosan gel emulsion before and after bubbling CO_2 gas. Here, chitosan gel was prepared by the method described in the Experimental section. The emulsion was opaque due to the dispersed gel but changed transparent for a few minutes during the bubbling of CO_2 gas, showing that the gel was dissolved.

Figure 2 shows the change of the viscosity of the emulsion after the start of bubbling CO_2 gas. Viscosity increased in parallel with the increase of the transparency of the solution, which means that chitosan was dissolved molecularly with dissolved CO_2.

Table I shows the viscosities of chitosan solutions (1%) dissolved in various acids. The concentration of acid was 1 N, but that of CO_2 was not determined. In this experiment, chitosan-CO_2 solution was stood for a day after the preparation. Within the time, viscosity increased accompanying the change of pH, 5.96 (just after the bubbling), 6.08 (1 h), 6.24 (3 h) and 6.61 (24 h), showing that dissolved CO_2 was evaporated. As a result, pH increased, which suppressed the ionization of chitosan, resulting in the increase in the viscosity. Chitosan-CO_2 solution was more viscous than other solutions. The concentrated chitosan-CO_2 solutions (3–4%) changed slowly into transparent soft gel when it was stood for days.

The chitosan-CO_2 solution was spread on a glass surface and then dried at room temperature. A transparent thin film of chitosan was left on the glass surface and the film strongly adhered to the glass and was difficult to strip with a spatula. This film was not dissolved in water even when immersed. This means that CO_2 was eliminated during the evaporation of water and water-insoluble chitosan film was formed, as expected.

Chitosan is a polysaccharide and has a very similar structure with cellulose, so it was thought that coating of cellulosic materials is easier than that of other substances. We, therefore, tried the coating of the cotton fibers. Cotton was dipped in a chitosan-CO_2 solution and then dried. To examine whether the cotton was chitosan-coated, fluorescent probe was used. Cotton treated with chitosan-CO_2 solution was again dipped in the solution containing the acidic fluorescent probe, fluorescein, then washed repeatedly with water. Non-treated cotton was also treated similarly with the flu-

Table II. Antibacterial activity of chitosan-treated gauze

Concentration of inoculm (*Staphylococcus aureus*)	Growth rate (F)	Value of bacteriostasis activity (S)
1×10^5 cfu mL^{-1}	3.1	2.2

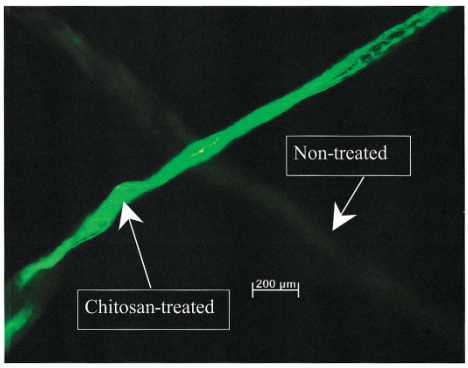

Figure 3. Fluorescent microscope photographs of the chitosan-treated and the non-treated cotton fibers.

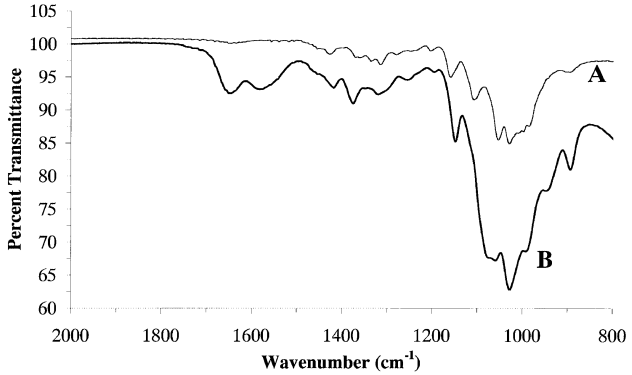

Figure 4. FT-IR-ATR spectra of the non-treated (A) and the chitosan-treated filter papers.

orescent probe solution and was used as a reference. Figure 3 shows a photograph taken by a fluorescent microscope. Chitosan-treated and non-treated cotton fibers were crossed and chitosan-treated fibers had intense brilliancy compared to those of the non-treated. This means that more probe was adsorbed on chitosan-treated cotton. As the fluorescein molecule has a carboxyl group, it interacts with amino groups of the chitosan by electrostatic interaction. Therefore, above result proved that chitosan was really coated on the cotton fibers. Chitosan-treated cotton has the almost same appearance as the non-treated and it was difficult to discriminate them visually, but the feel was different; treated one was more rigid than the non-treated.

Papers are mainly consisted of cellulose, so chitosan-coating on papers was expected to be rather easy. A filter paper was dipped in chitosan-CO_2 solution then dried like the case of cotton fiber. In the case of the paper, however, it was possible to paint a concentrated chitosan-CO_2 solution on a paper using a brush. Transparent, thick chitosan film was formed by this method, and it was possible to control the thickness of the film. Fluorescent technique was difficult to apply for examining the coating in this case, because of the thickness of the paper. Therefore, we used FT-IR-ATR spectroscopy to obtain the IR spectra of the surface region of a few micrometers. Figure 4 shows the FT-IR-ATR spectra of chitosan-treated and non-treated samples. The absorption band due to the bending mode of amino group emerged around 1600 cm^{-1} in the spectrum of the treated sample, showing that chitosan was really coated on the paper.

We examined whether the pharmacological properties of chitosan was rendered to the substrate by this coating. As an example, antimicrobial activity of chitosan-coated gauze to *Staphylococcus aureus* was examined according to the method authorized by JIS. The result is shown in Table II. In this table, concentration of inoculum means the value in the solution ; 0.2 mL of this solution being spread on the treated and non-treated samples (size ; 1.8 cm × 1.8 cm). *Staphylococcus aureus* implanted on the samples in this way was cultured for 18 h at 37°C. After that, the bacteria were washed out by saline water and the number of inoculm was counted.

The growth rate (F) is defined as follows.

$$F = Mb - Ma$$

Ma: Logarithm of the number of inoculm on the non-treated sample just after the implantation.

Mb: Logarithm of the number of inoculm on the non-treated sample after 18 h at 37°C.

The value of F means the growing activity of the bacteria on the non-treated sample.

The value of bacteriostasis activity (S) is defined as follows.

$$S = Mb - Mc$$

Mc: Logarithm of the number of inoculm on the treated sample after 18 h at 37°C.

The value S means the logarithm of the ratio of the numbers of the inoculum on the non-treated and on the treated samples after 18 h cultivation at 37°C.

According to JIS L 1902 (1998),[9] it is all right to declare that this treatment is anti-bacterial when S is larger than 2.0. It is clear that this chitosan-treated gauze has the antimicrobial activity to *Staphylococcus aureus*.

From these results and discussion, it was proved that chitosan-CO_2 solution is very useful for chitosan-coating of cellulosic materials like cottons and papers and is able to render pharmacological properties like antimicrobial activity to the substrate materials. This method seems to open a new area for industrial applications.

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