Development of Functional Coating Reagent for Wood Based Materials by using Chitosan

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Abstract

In this report, Chitosan is proposed to be used as a coating reagent for wood based materials based on its characteristics described as follows:

1. Chitosan is not dissolved in organic solvents in general.
2. Chitosan become cationic polymer when dissolved in acidic water.
3. Chitosan has special quality of making good film.
4. Chitosan has quality of moisture-holding.
5. Chitosan forms schiff's base when reacting with aldehyde compounds.

Considering these characteristics, we propose chitosan be applied in the following areas.

1. As a coating reagent to prevent from uneven of coloring for wood based materials(1,3): Chitosan, having quality of making good film and insolved organic solvents, makes uniform coloring possible and inhibits a coloring agent from penetration.
2. As a coating reagent for electrostatic painting(2,4): Chitosan, having quality of moisture-holding and cation, increases the amount of anionic paint sprayed when electrostatic painting.
3. As a remover of formaldehyde released from the plywood (5): Chitosan painted on plywood reacts with formaldehyde released from the glueline and forms schiff's base to remove formaldehyde.

Keywords: Chitin, Chitosan, wood based materials, electrostatic painting, formaldehyde

Materials and methods

Coating reagents of surface of wood based materials for painting [1]

Wood based materials are used widely for furniture or building materials. Wood based materials are often painted for coloring. In

this case, it is often required that finished evenly coloring for fine dress. But the coloring is not evenly finished from porosity of wood based materials. Then in this report, it is examined to utilization of chitosan as surface treatment reagents of wood based materials for even of coloring. The treatments are as follows: The surface of wood based materials are sprayed with 0.5% of chitosan dissolved in 0.5% of aqueous acetic acid solution. After drying, it's sprayed with coloring paints dissolved in organic solvent and after drying it's polished, furthermore sprayed with paints for finished.

Coating reagent for electrostatic painting

Electrostatic painting technique is generally used for painting wood. The specific character of electrostatic painting technique is better than general painting technique in yield of paint. Lacquer paint was used in this experiment. An outline of electrostatic painting is shown in figure 1.

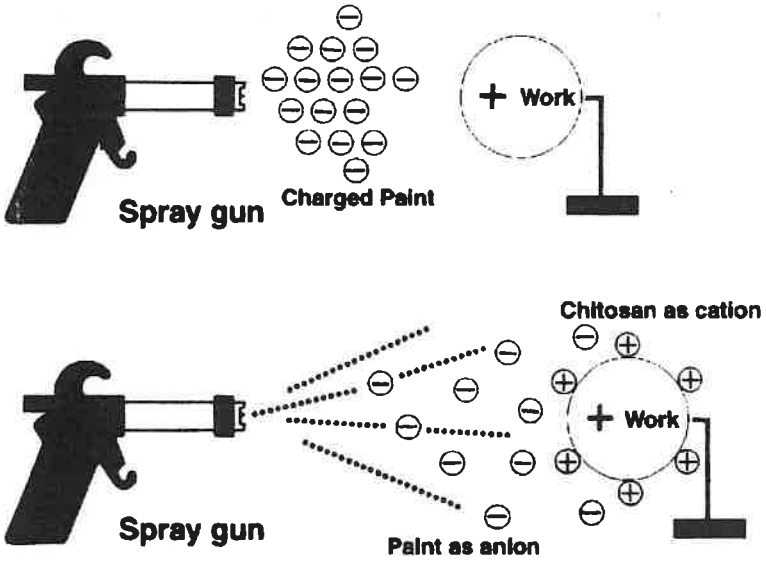

Figure 1 The outline of electrostatic painting

Remover of formaldehyde released from the ply wood

Plywood used for building or furniture is laminated with adhesive involved formaldehyde. The formaldehyde released from glue line of plywood is bad for health. It is one of the most crucial topics that aldehyde released from plywood is removed from environment in house. Therefore, it is examined that chitosan powder is coated on surface of plywood so that formaldehyde is not released from the glue line. Experimental method described as follows:

Ten piecies of plywood(15x5cm) rubbed powdered chitosan to the surface are set in glass made desicater with petridish holded 300 ml

of distilled water. The formaldehyde released is dissolved in 300 ml of distilled water at 20°C for 24 hours. Quantitative analysis of formaldehyde in water is determined by acetylacetone method[2].

Results and discussion

Coating reagents of surface of wood based materials for painting

The effects of chitosan as coating reagents are indicated in figure 2. On the right side of board (Beech wood) is colored evenly with effects of chitosan, on the other hand, on the left side of the surface treated without chitosan is colored unevenly. It is considered that penetration of paints are resisted with chitosan layer but are colored unevenly without treating chitosan.

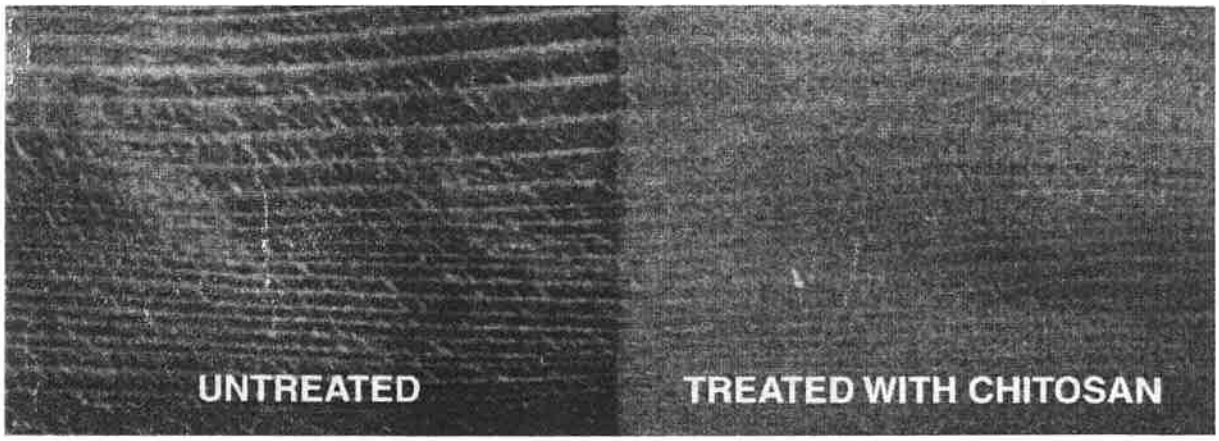

Figure 2. Effects of coated chitosan in coloring

Coating reagent for electrostatic painting

Electrostatic painting method is used widely for metal,plastics and wood based materials. The character are that paint negatively charged with high voltage (70kV) are effectively sprayed to wood based materials grounded, but paint is not stained at times when surface of wood based materials are dryed under the conditions of low humidity. The problems are not happened if chitosan, having quality of moisuture-holding and cation, is coated on surface of wood based materials. As the chitosan is coated, the gloss of film on work is increased and work is painted more dark colored.(Table 1) And the tone of color and the degree of gloss increase with increasing concentration of chitosan. It is obvious that the paint is sprayed abundantly to work as compared to the untreated with chitosan.

Table 1. Effects of coating chitosan in electrostatic painting (Evaluation with naked eyes)

	Tones of color	The degree of gloss
control	++	−
0.5% of chitosan	+++	++
1.0% of chitosan	+++	++
3.0% of chitosan	+++	+++

These results are reconfirmed by gloss meter and color meter.

Remover of formaldehyde released from the plywood

Chitosan is reacted rapidly with aldehyde compound,then formed schiff's base at room temperature[3][4]. In this case, formaldehyde released from glue line of plywood used urea resin is reacted with powdered chitosan rubbed on surface of plywood. Therefore amount of released formaldehyde is decreased with formation of schiff's base. (Table 2, Figure 3) It is obvious that chitosan and urea reduce the formaldehyde released from plywood. But released formaldehyde is not reduce with powdered cellulose.

Therefore, it is obvious that these effects are caused by formation of schiff's base.

Table 2. Reduction of formaldehyde released from plywood with powdered chitosan

Powder	Amount of rubbed Powder (mg)	Amount of released formaldehyde (ppm)	Redution rate of releasedformaldehyde (%)
untreated	-	2.0	-
Powdered cellulose	1206	1.8	10.0
Powdered urea	1123	0.3	85.0
Powdered chitosan (3μm)	1247	0.2	90.0
Powdered chitosan (14μm)	1231	0.3	85.0
Pow dered chitosan (60 mesh pass)	301	1.0	50.0

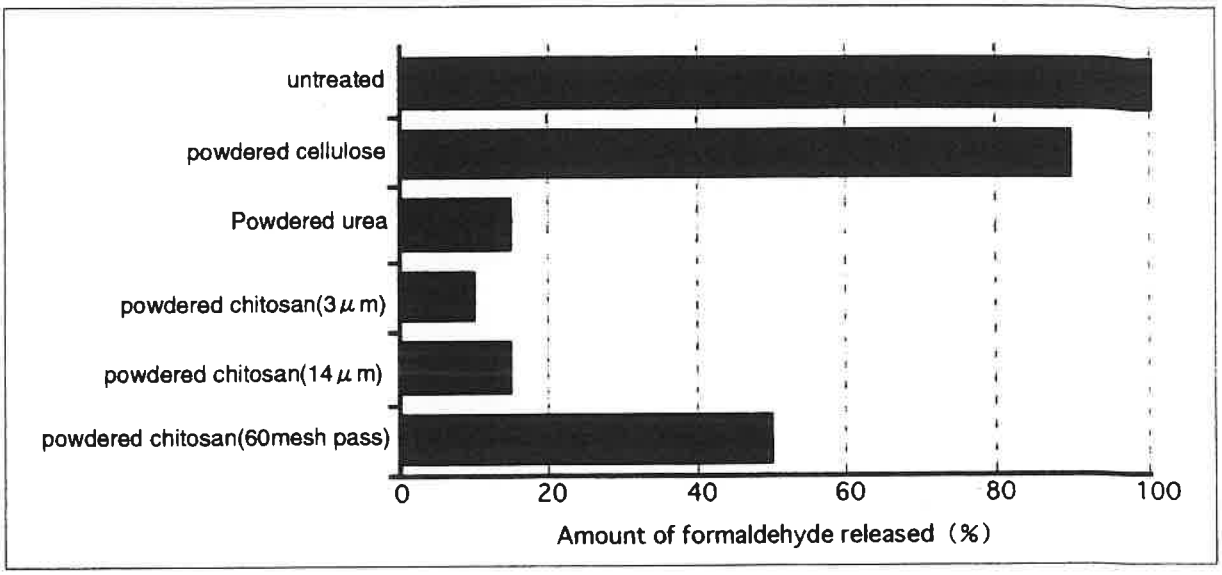

Figure 3. Reduction of formaldehyde released from plywood with powdered chitosan

Conclusion

From this work we conclude that chitosan is good materials as coating reagents for wood based materials, and that the function of chitosan for paint are possible to even of coloring for painting wood, efficient coating in yield for electrostatic painting, reduction of formaldehyde released from plywood by formation of schiff's base with aldehyde.

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Application of Chitosan for Catalyzation Method in Electroless Plating

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Abstract

We developed a novel catalyzation method in electroless plating by a coated film containing chitosan.

Non-conductive substance such as plastics, ceramics, paper, glass, fibers can be metallized by electroless plating. In order to initiate oxidation of a reducing agent in a plating solution, however, the surface of such a non-conductive substance must be subjected to a catalyzation treatment.

It was found that when the surface of the non-conductive substance was coated with a solution containing chitosan before the steps of catalyzation and electroless plating, the obtained film could trap and fix thereon a catalyst metal such as palladium specifically by the activity of chitosan. The amount of the catalyst (palladium) bound to a coated film containing chitosan was enough to be secured in the step of catalyzation and to enable the electroless plating (Cu, Ni) to be uniformly and efficiently obtained on the surface of the substrate.

Keywords: chitosan, non-conductive, palladium, nickel, copper, electroless plating

Materials and methods

Materials

Chitosan (SKD) was purchased from San-Ei Kogyo Co., Ltd. Other reagents were purchased from Kishida Chemical Co., Ltd.

Japanese paper (300 mm square) was obtained from Dai-Inshu Seishi Kyogyo Kumiai.

Plastic test plates (PS: polystyrene, ABS: acrylonitrile-butadien-styrene, 75 mm×125 mm×2 mm t) and paints for plastics were purchased from Ohashi Chemical Industries Ltd.

Preparation of chitosan solution

Chitosan was dissolved in a 1% solution of acetic acid to prepare a 1 W/V% solution of chitosan, which was then diluted with methanol to prepare 0.5% chitosan solution.

Method of electroless plating

A conventional electroless plating operation for a non-conductive substrate was performed by the following steps. After degreasing and cleaning, a non-conductive substrate was treated with the mixture of chromic acid and sulfuric acid at 65℃ for 15 minutes, and then neutralized with 10% NaOH solution at room temperature for 1 minute and cleaned by water ultrasonically. The part was then contacted with a solution of stannous chloride ($SnCl_2 \cdot 2H_2O$: 10 g/l, HCl : 5 ml/l) at 30℃ for 3 minutes and then with a solution of palladium chloride ($PdCl_2 \cdot 2H_2O$: 0.25 g/l, HCl : 5 ml/l) at 30℃ for 3 minutes. Subsequently the part was immersed in a standard electroless nickel plating solution (Table 1) for 7 minutes or a electroless copper plating solution (Table 2) for 15 minutes.

Table 1 Electroless Ni bath composition and operating condition

Component	Concentration
$NiSO_4$	20 g/l
NaH_2PO_4	15 g/l
Citric acid	5 g/l
CH_3COONa	3 g/l
Glycine	2 g/l
Lactic acid	3 g/l
Thiourea	5 ppm
$Pb(NO_3)_2$	3 ppm
Bath pH 6.0	
Bath temperature : 55~60℃	

Our new electroless plating operation for a non-conductive substrate was as follows. After degreasing and cleaning, a chitosan solution was applied to a non-conductive substrate by spraying or dipping. When the chitosan solution did not appear to adhere suitably to this substrate, a paint which was selected for its indication of good adherence to the part was pre-coated. The chitosan solution was allowed to dry at 60℃ for 1 hour. The part was

Table 2 Electroless Cu bath composition and operating condition

Component	Concentration
$CuSO_4$	0.06 mol/l
EDTA	0.12 mol/l
2,2'-Dipyridyl	10 mg/l
Potassium ferrocyanide	10~20 mg/l
Formalin	0.5 mol/l
Bath pH 12.5	
Bath temperature : 60℃	
Agitation with air	

then contacted with a solution of stannous chloride ($SnCl_2 \cdot 2H_2O$: 10 g/l, HCl : 5 ml/l) at 30℃ for 3 minutes and then with a solution of palladium chloride ($PdCl_2 \cdot 2H_2O$: 0.25 g/l, HCl : 5 ml/l) at 30℃ for 3 minutes. Subsequently the part was immersed in a standard electroless nickel plating solution (Table 1) for 7 minutes or a electroless copper plating solution (Table 2) for 15 minutes.

As for the adhesion of a paint or a metal plating layer to a substrate, a 10 mm × 10 mm area of its surface was cross-cut into 100 small squares each having a longitudinal length of 1 mm and a lateral length of 1 mm, and a cellophane adhesive tape was adhered to these area of the coating or the plating and then peeled to evaluate the adhesion in terms of [number of remaining squares of coating or plating / number of all squares].

The thickness of metal layer was measured by a micro XRF element monitor (SEA-5120, Seiko Instruments Inc.).

The surface of a substrate after each treatment was observed by a scanning electron microanalyzer (X-560, HITACHI).

Measurement of the amount of the adsorbed palladium

100 ml of double fold diluted nitric acid was added to the test piece. After 5 minutes 10 ml of the solution was diluted to be 100 ml by distilled water. The amount of released palladium was measured by a multi sequential ICP emission spectrometer (SPS-7700, Seiko Instruments Inc.) .

Results and discussion

Electroless plating on Japanese paper

The chitosan solution was applied to typical Japanese paper by

dipping and then contacted with the palladium chloride solution directly. Subsequently, the paper was immersed in the electroless nickel or copper plating solution. As a result, a uniform nickel or copper deposit could be obtained on the whole surface of the paper. It is considered that in such a case of the paper which consists of cellulose the electroless plating can be performed with facility because of its affinity for chitosan.

Electroless plating on plastics

A PS resin piece was prepared as a substrate. The chitosan solution did not appear to adhere suitably to this substrate, and in fact failed the tape test described above. Consequently, an epoxy-curing type acrylic paint which had good adhesion for PS was precoated by spraying and dried, then the chitosan solution was applied on the cured paint layer to be formed the second layer as an adsorbent of palladium. These double coated layers passed adhesion test completely (100/100). The coated piece was then processed as described above to leave an electroless metal plating uniformly on the surface. The thickness of nickel and copper deposit was 0.38 ㎛ and 0.86 ㎛, respectively. And the adhesion test of these plating were excellent (100/100) .

Repetition of the method using the ABS piece as a substrate and the double coating process also yielded a uniform plating which had a good appearance and an excellent adhesion (100/100) to the surface. The thickness of nickel and copper deposit was 0.41 ㎛ and 1.23 ㎛, respectively. Fig. 1 shows the SEM observation of the surface of nickel and copper plating.

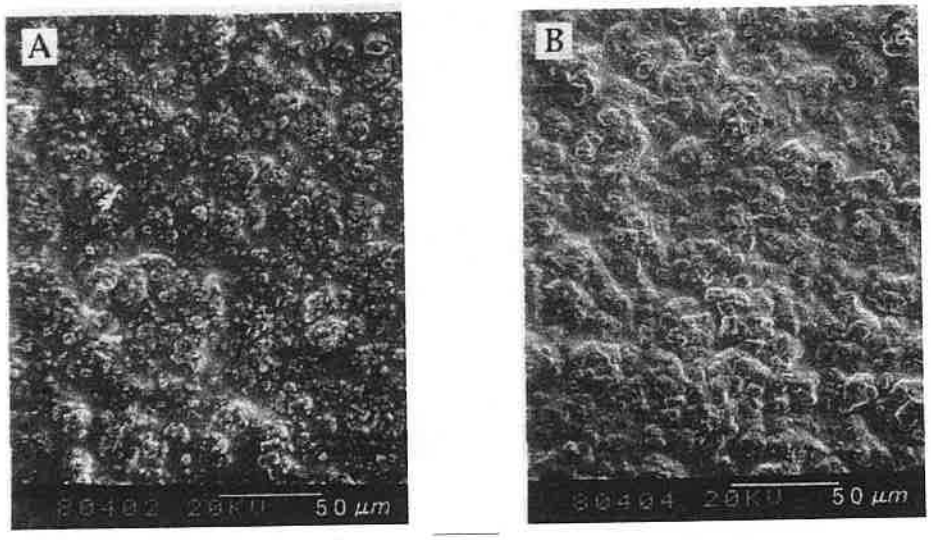

Fig. 1 SEM photograph of electroless nickel and copper plating on ABS
A: Nickel plating B: Copper plating

Measurement of the amount of adsorbed palladium and released palladium into the plating bath

The conventional electroless plating operation for a ABS resin piece (etching method) was performed just before the step of the electroless plating. Subsequently, the piece was immersed in the electroless copper plating solution with the exception of $CuSO_4$ at 60℃ for 15 minutes. The initial amount of adsorbed palladium and the residue of palladium after treatment with the solution were compared with in the case of our new catalyzing method using chitosan under the same condition. As shown in Fig. 2, the initial

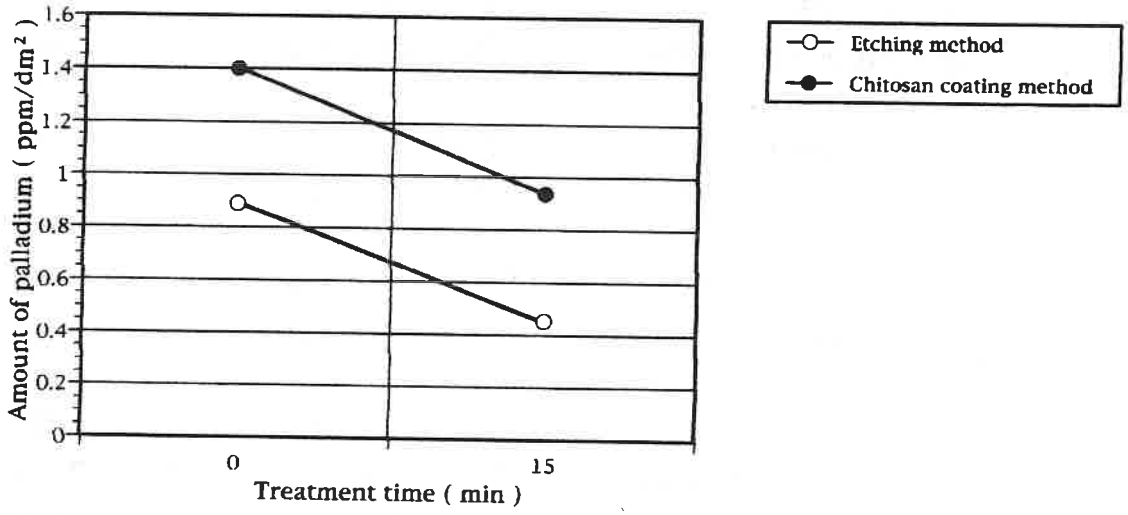

Fig. 2 Amount of palladium after treatment with electroless plating solution

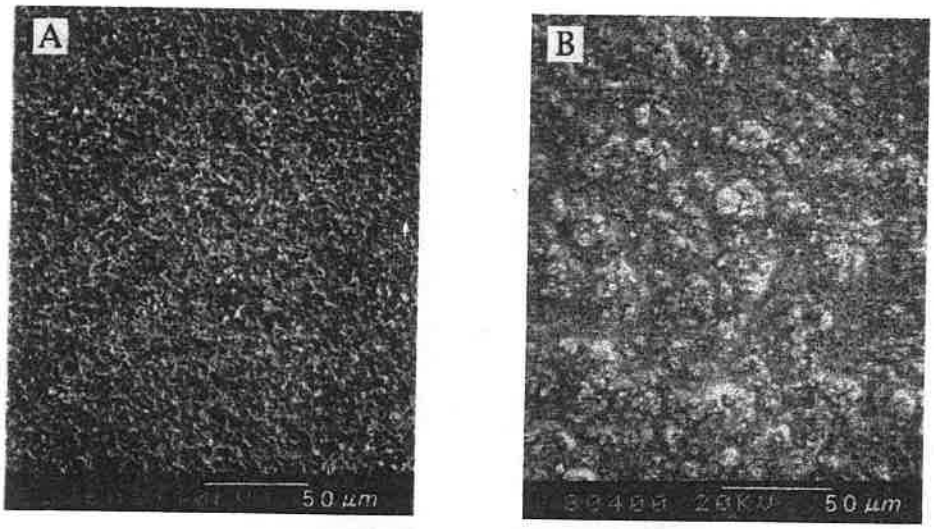

Fig. 3 SEM photograph of treated surface
A: Etching method B: Chitosan coating method

adsorbed amount of palladium in the case of our new chitosan

coating method was higher than that of the conventional method, and even after the treating under such a high alkaline condition, the residue of palladium was still high level. Fig. 3 shows the SEM observation of the surface of chemical etching and the coated film containing chitosan on the ABS piece. The surface treated with chemical etching is very porous and roughened.

Conclusion

According to the sensitizing-activating method [1], the catalyst-accelerator method [2], etc. as the catalyzation methods of the conventional erectroless plating processes, a catalyst metal (palladium) is borne on the surface of a substrate microscopically roughened by chemical etching through multiple stages of reactions effected on the surface of the substrate and by means of physical adsorption of the reaction product on the surface of the substrate, with the result that the residue of palladium is so unstable as to bring about adverse effects such as a failure in adhesion during the course of formation of the plating in many cases. On the contrary, according to our new catalyzation method, a treatment solution containing chitosan is applied on the surface of a substrate to form a kind of catalyst metal-fixing carrier, on which a catalyst metal (palladium) is strongly borne by chemisorption thereof, thus enabling a uniform metallic deposit good in adhesion to the substrate to be formed by electroless plating. Further, the catalyst is borne only on the pretreated portions of the surface of the substrate whereon the treatment solution is applied, thus the partial electroless plating may be obtained. Furthermore, chemical etching by chromic acid etc. can be dispensed with to decrease the number of steps and to simplify the waste water treatment, greatly contributing to an improvement in environmental problems.

Acknowledgment

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The Benefits of Chitosan to Postharvest Storage and the Quality of Fresh Strawberries

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Abstract

Chitosan shows antimicrobial property, and it can form semipermiable membrane with its ability to modify internal atmosphere in the tissue. The addition of Ca-ion may change the rate of gas permeation (CO_2/O_2) in the chitosan film. In this study, different degrees of deacetylation (DD) and concentrations of Ca-ion in chitosan solution were used to control postharvest decay of strawberries.

The results showed the best MA (Modified Atmosphere) effects on storability of fresh strawberries occurred when they were treated with 80% (DD) chitosan solution containing 0.1% Ca-ion concentration. The Ca-ion added in chitosan solution significantly reduced the decay of berries caused by *Botrytis cinerea* and *Rhizopus stolonifer* as compared to pure chitosan solution. Chitosan-coated berries were firmer, higher in citric acid and synthesized anthocyanin at a slower rate than nontreated berries. In the organoleptic test, astringency or bitter taste were not found for chitosan-coated strawberries. However, the flavor was lost to some extent when the berries treated with 80% (DD) chitosan solution contained 0.1% Ca-ions concentration. These results indicated that preservative coating with chitosan containing Ca-ions has a potential to prolong storage life.

Keywords: Chitin, chitosan, strawberry, modified atmosphere, storage

Materials and methods

Fruits

Strawberries from Taiwan were selected on the basis of uniform 50% red color, moderate size, touch-firmness and lack of physical damage. Selected berries were weighed into samples of 15 g each. The berries were randomly distributed into groups of 70 fruits. Each group represented one replicate, and for each treatment three replicates were used.

Chitosan coating solutions

Chitosan was prepared by deacetylating chitin with NaOH (45%, w/w) at 100 °C. The 60% and 80% deacetylation chitin were obtained, respectively. Two g (dry wt) of chitosan powder was dissolved in 200 ml of acetic acid (1 %) solution to yield a 1 % stock solution. Into the stock solution was added 0.1 g $CaCl_2$ and the chitosan solution containing Ca^{2+} was formed. Four kinds of chitosan solution including de-80 Ca, de-80, de-60 Ca, and de-60 were used.

Inoculum preparation

Fungal cultures of *Botrytis cinerea* and *Rhizopus stolonifer* used in this study were obtained from Food Industry Research and Development Institute in Taiwan. Spores of these molds were recovered by filtering the mycelial suspension of 2-wk old culture and the

concentration of the spore suspension was 2×10^5 spore/mL.

Decay control

Strawberries were immersed in spore suspensions of *Botrytis cinerea* and *Rhizopus stolonifer* respectively and allowed to air dry at room temperature for three hours. Inoculated berries were dipped in chitosan solutions or in water for control. After drying, the berries were stored at 13 °C. Berries were examined for mold infection every 5 days.

Quality evaluation

In these experiments, the berries were stored at 2 °C and the quality of the berries was evaluated every 5 days. A sample of 5-7 berries was randomly removed from each treatment and analyzed for firmness, citric acid and anthocyanin content. The firmness of the berries was measured with Rheometer using an adapter (NO.6). Citric acid was determined using 5 g aliquot of berry juice in 10 mL deionized water and measured by HPLC. The separating column was a nucleosil $5C_{18}$ column of Vercopak. The mobile phase was 1 % phosphate buffer solution. Anthocyanin content was extracted with acidified ethanol according to the method of Fuleki and Francis (1968).

Sensory evaluation

Twelve judges, aged 22-40 years, evaluated color, flavor, taste, and acceptability. The judges scored the treated-berries for each sensory characteristic on a 1 to 9 rating scale. Sensory evaluation data were computered and analysis of variance was applied to all quantitative analyses to test the significance of differences.

Results and discussion

Decay control

Berries were considered infected when a visible lesion was observed. The results were expressed as percentage of berries infected. Figure 1 indicates that the decay of berries was reduced significantly ($P<0.05$) when inoculated berries were treated with chitosan.

There was added benefit to decay control by increasing the degree of deacetylation especially at the end of storage. The addition of Ca-ion to chitosan solution increased the antifungal capacity in comparison with pure chitosan . At the end of storage (30 days), decayed berries in control were about 95% for *Botrytis cinerea* and 100% for *Rhizopus stolonifer* (data not shown). In contrast, the level of decay in de-80-Ca and de-60-Ca coated berries was 25% and 38%, respectively. Chitosan can induce chitinase and elicit phytoalexins to inhibit growth of fungi (Mauch *et al.*, 1984). Semi-permeable coatings formed from chitosan solution containing Ca-ion advanced to improve storability of berries by elevating CO_2/O_2 ratio in the coatings.

Quality evaluation

Chitosan coating had a beneficial effect on flesh firmness, and retarded both the conversion of citric acid and the synthesis of anthocyanin in strawberries (Fig. 2 and 3). Those coated with "Ca-chitosan" were firmer and higher in citric acid than pure chitosan-treated berries (Fig. 2). High citric acid and low anthocyanin contents indicated that the ripening of strawberries was inhibited. From figure 2B, we know that the concentrations of critic acid in berries were decreased overtime; however, an unexpected breakpoint was found in control. Fleix *et al.* (1982) regarded the phenomenon as an over-ripening indicator

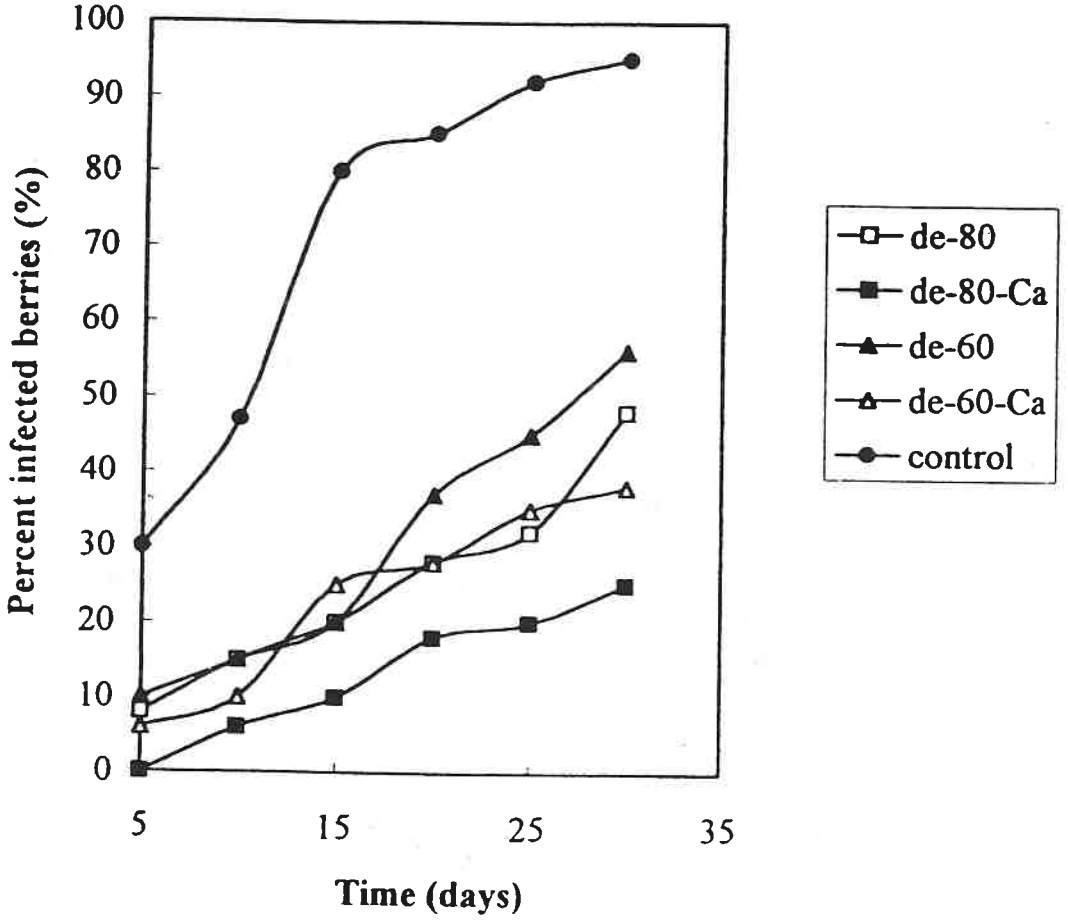

Figure 1. Effect of different treatments on the decay control of strawberries for *Botrytis cinerea*.

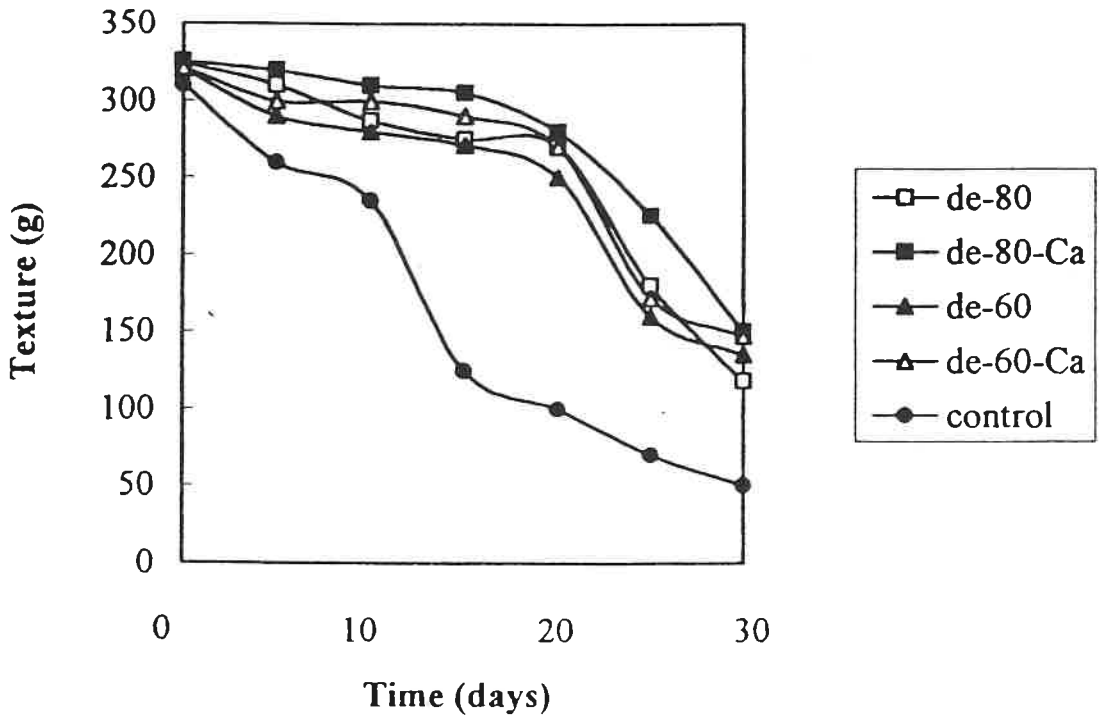

Figure 2. (A)

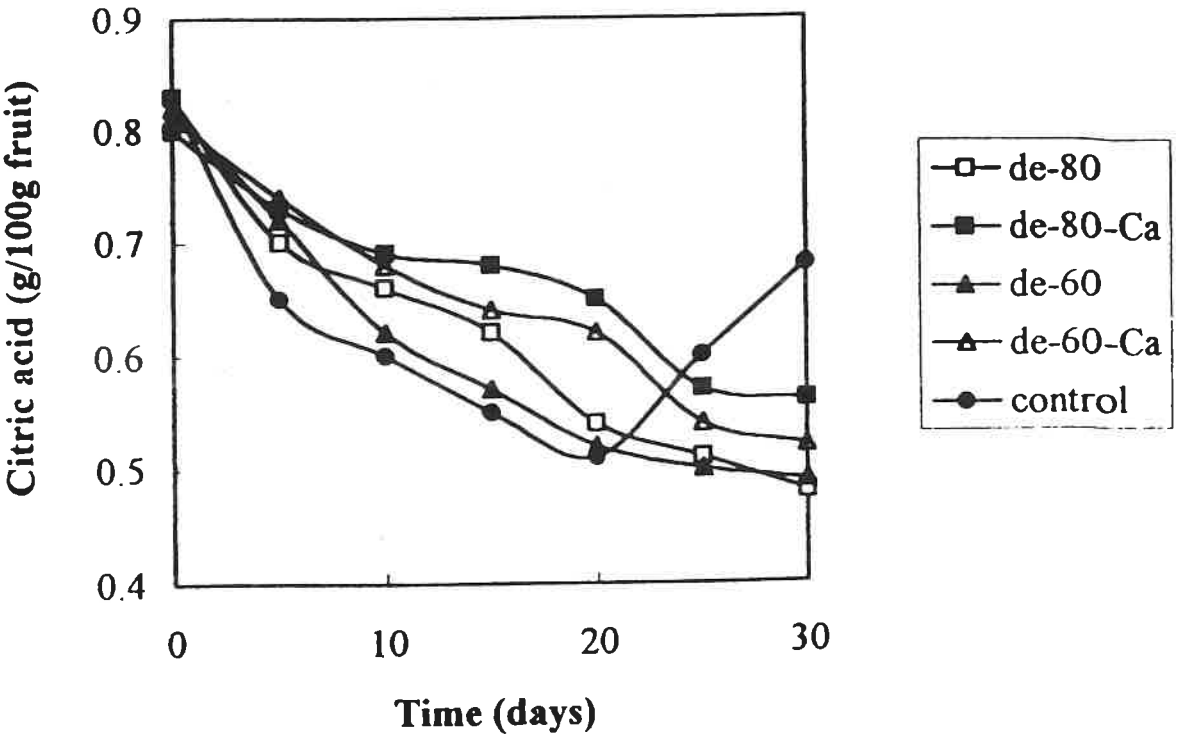

Figure 2. (B)

Figure 2. Change in (A) Texture and (B) Citric acid of the different chitosan-coated strawberries during storage (n=3).

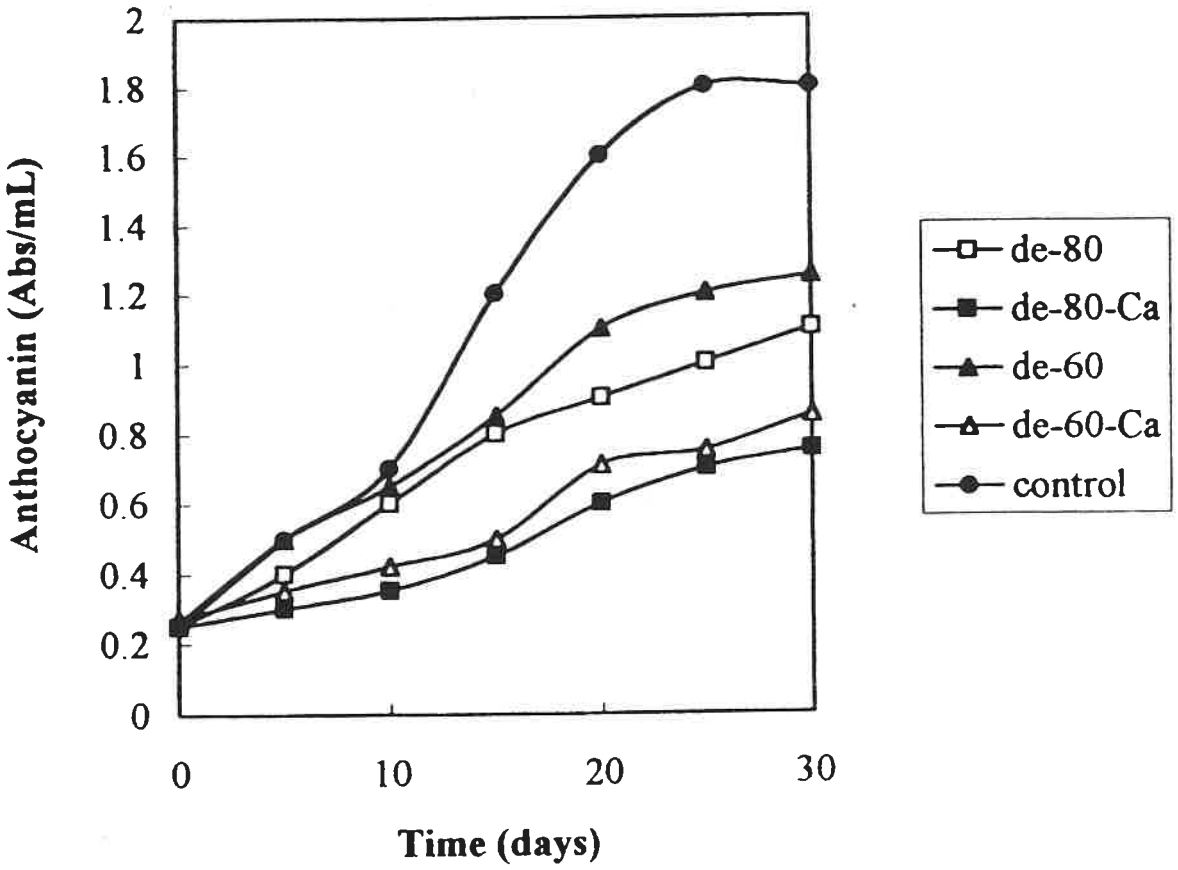

Figure 3. Anthocyanin content of the different chitosan-coated strawberries during storage.

in nontreated-berries. The retention of firmness, lower citric acid and slower rates of anthocyanin production in "Ca-chitosan" coated berries hinted that Ca-chitosan film was more selectively permeable to O_2 than to CO_2.

Sensory evaluation

The intensity scores of most sensory attributes were different among the different treatments and storage time (Table 1). The highest ratings for strawberry color, flavor, taste and acceptability for berries were found at day 15 for control. However, the score ratings for total evaluation of strawberries increased with storage time for coated-berries. Compared to strawberries coated in pure chitosan, those coated in Ca-chitosan had slightly lower attribute ratings after 25 days in storage, particularly in flavor. The significant change in flavor attributes of strawberries coated by "de-80-Ca" chitosan may be related to changes in O_2 and CO_2 levels in the film. With storage time, levels of O_2 decreased and CO_2 increased in the chitosan-film, which caused anaerobic respiration and development of undesirable attributes (Carlin *et al.*, 1990). In fact, the slight ethanol-flavor was emitted from strawberries treated by de-80-Ca chitosan solution.

Table 1. Effect of different chitosan-coated strawberries on sensory characteristics

Treatment	Days in Storage	Evaluation criteria scored			
		Color	Flavor	Taste	Acceptability
control	5	6.2	5.6	5.1	5.8^{d}
	15	7.4	7.6	7.3	7.0^{d}
	25	6.5	4.5	5.1	4.3^{d}
de-60	5	5.0	5.1	4.1	4.3^{c}
	15	5.4	5.8	5.5	5.6^{c}
	25	6.5	7.2	7.1	7.2^{b}
de-60-Ca	5	4.5	4.8	3.1	4.2^{c}
	15	5.6	5.3	4.5	5.1^{a}
	25	6.2	6.2	7.1	6.2^{c}
de-80	5	4.3	5.1	3.9	4.6^{b}
	15	5.9	4.5	3.5	4.2^{b}
	25	6.5	6.7	6.5	6.4^{c}
de-80-Ca	5	4.3	5.5	2.7	3.8^{a}
	15	5.1	6.0	3.5	5.0^{a}
	25	6.8	2.1	6.8	3.8^{a}

Conclusion

Ca-chitosan coating was more effective than chitosan treatment in controlling postharvest decay of strawberries. During quality evaluation, it was found that berries coated with Ca-chitosan were firmer and lower in the concentration of citric acid than chitosan-treated or control berries. Our study indicates high deacetyling degree of chitosan and the addition of Ca ion to chitosan would increase the permeability of CO_2/O_2 and has a potential to prolong storage life. According to the sensory evaluation, the berries coated with de-80-Ca chitosan lost their fresh flavor after 25 days in storage. For this reason, it is

recommended that strawberries be coated with de-60-Ca chitosan.

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COMPARATIVE ESTIMATION OF BACTERICIDAL AND SORPTION PROPERTIES OF CHITIN AND ITS DERIVATIVES BEING OBTAINED BY ELECTROCHEMICAL AND TRADITIONAL METHODS

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Introduction

Practical application of sorbing materials in ecology technologies, drinking water purification technologies, medicine, agriculture and other fields dictates the necessity of these polymers having not only high speed and depth of sorption, selectivity of sorption towards various admixtures, ecological harmlessness, biological compatibility, etc. which are characteristic of chitin and its derivatives, but also bacteriostatic and bactericide qualities.

Most of the known sorbents not only have no bactericide properties but also are a favourable medium for sorption and multiplication of patogenes.

The purpose of the work was to carry out comparative estimation of bacteriostatic, bactericide and fungicide properties of chitin and chitosan obtained by traditional method and activated chitin produced by electrochemical method in combination with treatment with surfactants.

Materials and methods

The subject of this research was represented by chitin and chitosan produced from freshwater shrimp *Gammarus* by traditional acid-alkaline method and activated chitin (ACh) produced by original patented production method [2] with additional activation by treatment of intermediate product with anionic surface-active substance. Deproteinization and demineralization of raw material was carried out during single technological process on exposure to products of electrochemical decomposition of water, redox-potential, electric field in weakly salty electrolyzers of original construction [2] with specified parameters (current, voltage, time, temperature, concentration).

Activation of chitin was carried out after deproteinization stage with the aim of saponification of lipides hydroplyzation products, formed, probably, in the process of raw material deproteinization, and deeper degreasing of raw material.

Chitin modification with the aim of giving it bactericide and fungicide properties was also carried out by electrochemical treatment in electrolyzers on exposure to products of water electrolysis and weak salt solutions. Modification was assisted by activation of reaction centres of chitin connected with its treatment with surfactants and suppression of hydration effects in electric field [3].

Assessment of chitin and its derivatives' physical and chemical properties has been carried out according traditional parameters and methods: amount of mineral residue after ashing, total and amine nitrogen, rate of solubility in acetic acid in the presence of soduim chloride. Chitosan molecular mass calculation was based on the value of characteristic viscosity of these solutions measured by the Ostwald viscosimeter, rate of deacetilation was defined by the back conductometric titration with OK-102 conductometer (Hungary made). Bacteriostatic and bactericide properties were assessed according unique method based on microbiological analysis of water, contaminated with *Esherichia coli* cells from their suspension in 0.5% sterile solution of NaCl, preset amount of which (1000 ml) was filtered with specified speed (40 ml/min) through sterile cartridge

with sorbent (100 x 100 mm). The cartridge was later thermostatted at the temperature of 37°C during 48 hours in order to provide favourable conditions for growth and multiplication of the bacteria. Then the cartridge was repeatedly washed with 1000 ml of water and microbiological analysis of the washing water was carried out.

Cells of *E.coli* content in initial suspension and filtrates received was estimated by limiting tenfold dilution method [4] with inoculation to liquid elective Kod's medium. The inoculations were thermostatted at the temperature of 37°C during 24 hours. Counting of the cells was carried out using McCready tables by method of most probable numbers.

Study of effect of chitin and its derivatives to dynamics of *E.coli* growth was carried out by method of moving culture of this bacteria to liquid glucose-saline medium [6] and incubating with intensive stirring at the temperature of 37°C during 60 min. Then studied samples were introduced to the medium with concentration of 100 mcg/ml and incubated again.

For live cells calculation samples were taken in 0, 45, 90 and 180 minutes. The time of sample introduction was taken as beginning of time counting. Colonies calculations were done by dispersion of a number of successive tenfold dilutions to ENDO medium in Petri dishes, which were thermostatted at the temperature of 37°C during 24 hours. Before the inoculation the medium was 50 timed diluted.

Assessment of fungicide activity of the tested materials according to the modified method of agar blocks which was modified by us [7] . The preparations were introduced into flasks with melted nutritional agar (potato-sugar agar) until final concentration of the preparation in the medium was 0.25-0.5%. After that melted agar was poured in sterile Petri dishes. After agar had solidified disks cut from 3-5 days old mycelium of phitopathogenic fungi (4 disks for 1 disk) were placed on the surface of the medium. For control of each fungus Petri dishes with disks introduced in the nutritional medium without adding the tested material were taken. Petri dishes with disks of fungi were incubated in dark at the temperature of 25°C. Diameters of the fungi colonies were measured on the 3rd and 6th days of cultivation. Inhibitor activity of the preparations was assessed on the basis of suppressing the growth of mycelium of each fungus using Abbot formula [7]:

$$I = \frac{Dc - De}{Dc} \cdot 100\%$$

where D_c - arithmetic mean of diameters of fungi colonies taken for control, mm

D_e - arithmetic mean of diameters of fungi colonies taken for experiment,mm

I - suppressing of growth of fungi mycelium, %

Results and discussion

Major physical and chemical properties of studied specimens are shown in table 1. Viscosity of chitin was determined in its solution in dymethilacetamide in presence of LiCl, viscosity of chitosan - in solution of acetic acid in presence of NaCl (in order to suppress polyelectrolyte effects).

Antimicrobial properties of traditionally obtained chitin and chitosan compared to those of activated chitin (ACh) in respect to pipe water contaminated with *E.coli* cells studied by unique method are given in Tables 2 and 3, in Fig.1, 2.

As test organizm imitating microbiological contamination of water *E.coli* culture of line 2339 was taken. The culture was made available by the specialists

of Institute for Vaccines and Serum (St.Petersburg, Russia) and which is a widely accepted indicator of water contamination.

Table 1

Physical and chemical properties of the studied specimens

Properties / Specimens	Ash, %	Nitrogen total, %	Nitrogen amine, %	Deacetylation deg.	Characteristic viscosity, dl/g	Molecular mass, c.u.
Activated chitin (ACh)	0.01	7.06	2.61	0.35	7.12	113,000
Chitin	0.13	6.10	2.19	0.10	7.98	143,000
Chitosan	0.00	8.00	7.17	0.95	4.97	95,000

Advantage of the chosen method of study of sorbents' antimicrobial properties is that the method allows not only to assess their bactericide effectiveness as matter able to sorb bacteria in dynamic regime of water flowing through the filter cartridge, but also to study their bacteriostatic properties during thermostatting of inoculated filter cartridge.

Number of *E.coli* cells contained in initial contaminated water and in the flushing water before and after filtering and thermostatting of the filter cartridge is given in Table 2.

Table 2

Contents of *E.coli* cells in water suspension on different stages of filter purification

Specimen	Content of cells in 1 ml of		
	initial suspension	filtrate	filtrate after thermostatting of the filter
ACh	50	0.2	0.0
Chitosan	130	80	250
Chitin	50	5.0	0.0

Percent ratio of number of *E.coli* cells in the filtrate before and after thermostatting of the filter cartridge in relation to initial suspension is illustrated by diagrams in Fig.1.

When analysing the received results it is possible to note that specimen ACh has the highest antimicrobial activity which is due, probably, to its containing low molecular products of water and electrolytes electrolysis which were physically and chemically bound during the process of treatment in electrolyser.

Bactericide properties of the specimen are characterised with decreasing of *E.coli* cells number in the filtrate in comparison with initial suspension by 99.6%.

ACh is not a substrate for *E.coli* culture growth and can not be a source of secondary inoculation of water as after thermostatting of the filter cartridge filled with this material after having filtered through it a suspension of bacterial cells (at the temperature of 37± 1°C during 48 hours) no *E.coli* cells were found in the flushing water.

The data received demonstrated that though specimen of chitin has bacteriostatic properties (in flushing water from the filter cartridge filled with this material after its thermostatting growth of *E.coli* cells was not noticed)its bactericide qualities are worse than those of ACh as decrease of cells number during filtration of bacterial suspension amounted to 90% in comparison with initial concentration.

In spite of the fact that antimicrobial properties of chitosan are often mentioned in literature, in our experiment this specimen did not demonstrate much of bactericide properties. During filtration of bacterial suspension *E.coli* cells content decreased by 38.5%. Chitosan did not show also bacteriostatic properties. On the contrary, in the flushing water from the filter cartridge a large number of cells was found (192,3% in comparison to initial concentration) i.e. in 2 days number of bacteria increased 5 times. This means that chitosan can be a substrate for *E.coli* growth by itself, without additional factors of the growth. This phenomenon is, probably, explained by *E.coli* cells having chitosanese activity.

When studying effect of activated chitin (ACh), chitosan and chitin on *E.coli* culture growth dynamic information on growth of pure culture on glucose-salt medium at the temperature of 37± 1°C with constant stirring during 180 min and culture growth in presence of specimens in the same conditions was acquired. The information is presented in table 3 and on Fig.2.

Table 3

Effect of specimens on *E.coli* culture growth dynamics

Specimen	Number of cells in 1 ml of sample after incubation with the specimen after			
	0 min	45 min	90 min	180 min
Pure culture	$1.73 \cdot 10^5$	$1.2 \cdot 10^6$	$3.5 \cdot 10^6$	$6 \cdot 10^6$
ACh	$1.27 \cdot 10^6$	$1 \cdot 10^3$	0	0
Chitosan	$9 \cdot 10^4$	$6.7 \cdot 10^4$	$7 \cdot 10^4$	$8.7 \cdot 10^4$
Chitin	$2.33 \cdot 10^6$	$3 \cdot 10^6$	$5 \cdot 10^6$	$6 \cdot 10^5$

Analysis of the received results demonstrated that disappearing of *E.coli* culture in presence of ACh was noticed 90 min after the specimen introduction.

After introduction of chitin in cell substance certain inhibiting of cells growth was noticed during last 90 minutes.

During incubation of the culture with chitosan static effect of the specimen on the cells' growth was noticed.

Suppressing effect of activated chitin ACh on *E.coli* culture is explained by its containing bound products of water and electrolytes electrolysis.

Certain inhibiting effect of chitin is, probably, connected with its interaction with components of the nutritional medium which prevents from the substrate utilization by the cells.

Effect of chitosan on *E.coli* cells coincides with the phenomenon described in literature [8] and correlates with the results of study of its bacteriostatic and bactericide properties as a filter agent.

In order to study fungistatic activity ACh and chitosan produced from ACh by traditional method were chosen. Fungistatic activity of the studied polymers was tested in relation to 8 test objects - phythopathogenic fungi *Fusarium oxysporum, Fuzarium culmorum, Fuzarium solani, Alternaria solani, Botrytis solani, Selerotinia batoticola, Helminthosporum teres, Bipolaris sorokiniana.*

Received results showed that at the chosen concentration of ACh and chitosan equal to 0.25-0.5% they either had no fungistatic activity or this activity was expressed weakly, which corresponds to the known information [9] on these polymers having no direct inhibitor effect on phythopathogens.

Growth of fungi mycelium was limited only at ACh and chitosan concentrations equal to 0.5% only by 5-20%.

But in relation to two phythopathogens *(B.cinereae* and *H.teres)* in presence of ACh only (0.5% concentration) considerable effect on inhibiting of their

growth by 47.4-39.5 and 42.8-50.2% accordingly was noted. Using of chitosan in relation to these pathgogenes is also not promising (see Table 4).
The received results also show absence of chitinase activity of phythopathogens while chitosanase activity is present.

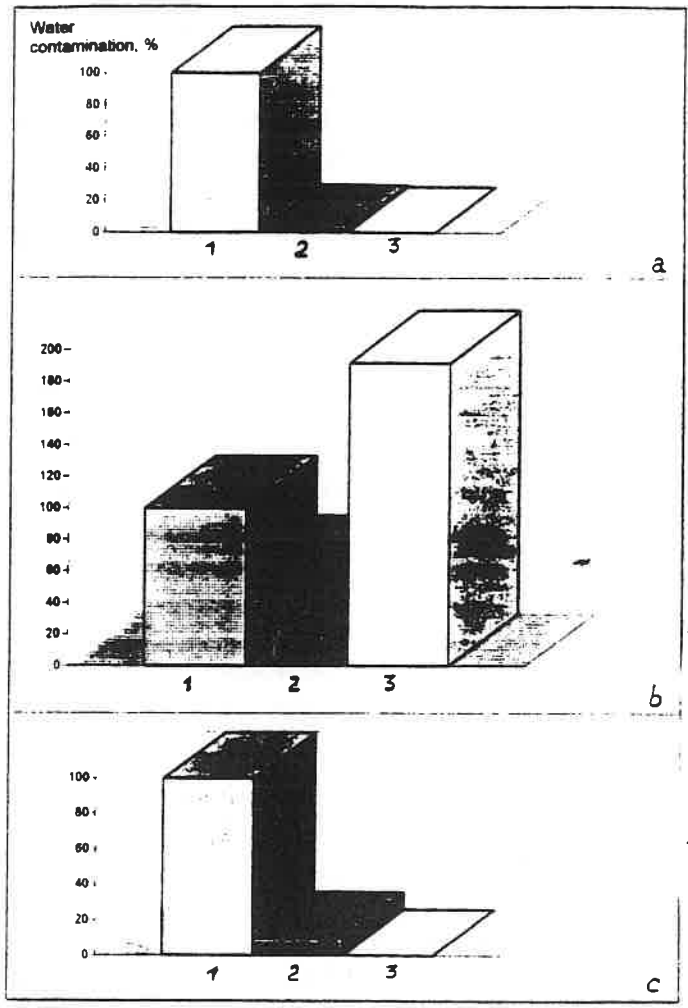

Fig. 1 Water contamination with *E.coli* cells in relation to contamination of initial suspension - 1
in filtrate - 2
in filtrate after thermostatting of the filter cartridge -3
after filter purification with specimens of
a) ACh
b) chitosan
c) chitin

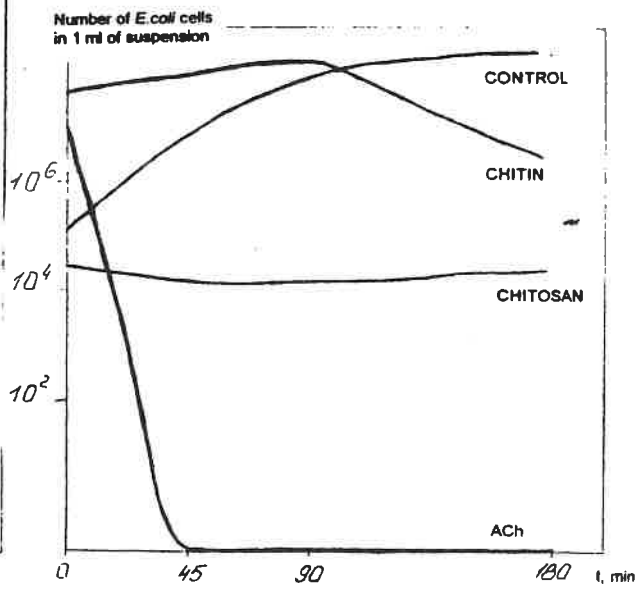

Fig.2 Effect of the specimens: Ach, chitin and chitosan on *E.coli* culture growth dynamic in glucose-salt medium in comparison with control (pure) culture

Conclusion

Activated chitin (ACh) having enforced antimicrobial properties was produced from shell-containing raw material according to unique electrochemical method combined with treatment with surfactants.

According to the unique method imitating work of the sorbent in filter cartridge bactericide and bacteriostatic properties of the produced polymer were studied in relation to test bacteria *E.coli*.

Fungistatic activity of ACh in experiments *in vitro* was studied in relation to 8 test phythopathogens.

Considerable advantage of activated chitin as antimicrobial substance in comparison with chitin produced by traditional method in relation to *E.coli* bacteria and *B.cinereae* and *H.teres* fungi is demonstrated. Absence of antimicrobial properties of chitosan in relation to the objects of the study is shown.

Table 4

Fungistatic activity of activated chitin and chitosan

Phythopathogen type	Polymer concentration in the medium		Mean diameter of fungi colonies , mm		Inhibiting of fungi colonies growth, % to control	
			Day of Cultivation			
			3rd	6th	3rd	6th
B.cinereae	control	0.0	24.7±0.8	40.0±3.0	-	-
	chitosan	0.5	20.5±1.2	27.3±1.2	17.0	31.7
		0.25	21.7±1.1	35.5±1.6	12.0	11.2
	ACh	0.5	13.0±0.4	24.2±0.4	47.4	39.5
		0.25	15.8±0.5	30.0±1.3	36.0	25.0
H.teres	control	0.0	14.0±0.5	37.8±1.2	-	-
	chitosan	0.5	3.0±0.5	36.3±1.2	7.0	3.9
	ACh	0.5	8.0±0.3	18.8±0.6	42.8	50.2
		0.25	11.5±0.5	29.0±0.5	18.0	23.3

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CHITOSAN-COATED SAND: PREPARATION AND DYE-ADSORPTION BEHAVIOUR

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Abstract
Chitosan may be applied as a thin coating on sand particles and subsequently cross-linked using glutaraldehyde. The coated sand is an efficient adsorbent for a representative anionic dye and can be regenerated by treatment with NaOH solution without any appreciable loss of dye adsorption capacity. The cross-linked coating shows good stability in acid media. The results demonstrate the product's suitability for treating dyehouse effluent to remove colour.

Keywords: Chitosan, coatings, sand, dye adsorption, regeneration

Introduction
One major problem with dyehouse effluent is that, even when present at concentrations which are relatively low in terms of COD and/or BOD, dyes can produce unacceptable colour in the effluent and hence must be removed. Chitosan, poly[β-(1→4)-2-amino-2-deoxy-**D**-glucopyranose], is a very efficient adsorbent of anionic dyes from acidified solution[1-3] but, because of its relatively high cost, it is important that the chitosan is available in the correct state to ensure efficient usage. Chitosan is normally supplied in the form of flakes of irregular size and shape and in this form it is unsuitable for use as packing in conventional columns. Attempts have been made to overcome this problem and chitosan-based adsorbents in the form of ion-exchange beads[4] or filters[5,6] have been reported. However, because the contact time available in exchange columns is short, much of the chitosan in such adsorbents would not be involved in dye adsorption and the current work is an attempt to produce chitosan in the form of a thin coating, on a suitable particulate substrate, and evaluate its effectiveness as a dye adsorbent.

Materials and methods
Materials - The chitosan used in this work was supplied by Aber Technologies (France). The mole fraction of *N*-acetylated residues (F_A) was found to be 0.02 by dye adsorption[3]. The dye used was C.I. Acid Orange 7 which was purified by recrystallising twice from aqueous ethanol and drying under vacuum at 45-50°C. The glutaraldehyde was obtained as an aqueous solution (approximately 50 wt.%) and was used as supplied.
Preparation of low molecular weight chitosan - To a chitosan solution (10 g/L) in 1% (v/v) aqueous acetic acid was added sodium nitrite (mole ratio $NaNO_2$: $-NH_2$ of 1:100). The solution was stirred, portions removed at intervals and the chitosan precipitated by the addition of ammonia, washed with distilled water and dried at 60°C under vacuum. The Limiting Viscosity Number was measured by the dilution method using a Ubbelohde

viscometer with internal sintered glass filter. The solvent was 0.2 M CH_3COOH/0.1 M CH_3COONa.

Preparation of chitosan-coated sand - A portion of sand was steeped in a solution of chitosan in 1% acetic acid, filtered and cross-linked by one of two methods.

Method 1 - Completely dry (air dried) chitosan-coated sand (25 g) was stirred for 1 hour at room temperature in 100 mL of 0.1 M NaOH containing 0.2 mL of glutaraldehyde.

Method 2 - Wet or semi-dry chitosan-coated sand was added directly to methanol and stirred for 30 minutes after addition of the calculated amount of glutaraldehyde.

Measurement of dye adsorption - A solution, approximately 5 x 10^{-3} M, of C.I. Acid Orange 7 in 0.1 M aqueous acetic acid was prepared. Approximately 5 g (accurately weighed) of chitosan-coated sand was heated at 40°C in 200 mL of this dye solution for 16 hours. The amount of dye adsorbed by the chitosan-coated sand was determined from the initial and final concentrations of the solution measured at 484 nm.

Stability of chitosan-coated sand - A portion of chitosan-coated sand (approximately 10 g) was steeped in 100 mL 1% aqueous acetic acid. Aliquots (20 mL) were removed after various intervals and the flow time measured in an Ubbelohde viscometer at 30°C.

Results and discussion

Preparation of chitosan-coated sand filters

These were prepared by first coating the sand with chitosan using different concentrations of chitosan solution followed by cross-linking with glutaraldehyde. The known stoichiometry of the interaction of C. I. Acid Orange 7 with protonated amine groups on chitosan[3] was used to determine the amount of chitosan coating on the sand prior to, or after, cross-linking.

In the first series cross-linking was carried out in 0.1 M NaOH solution following Method 1 above. Table 1 gives the percentage weight of coating and maximum dye adsorption before and after cross-linking.

Table 1.

$C_{chitosan}$ (w/v)	1	2	3.5	5
Weight of coating (wt %)	0.17	0.32	0.59	0.78
Dye adsorption after coating (g/kg)	3.6	6.9	12.8	16.9
Dye adsorption after cross-linking (g/kg)	-	6.3	11.6	15.8

The results demonstrate that both the weight of coating and the dye adsorption capacity, both before and after cross-linking, increase in an approximately linear manner with increase in the concentration of the chitosan solution used. In all cases there is a small decrease in dye adsorption capacity on cross-linking; this may be due to reduced accessibility of the amine groups. Attempts to further increase the weight of coating by increasing the concentration of chitosan in the treating solution failed for two reasons: formation of gels in solution at these higher concentrations and aggregation of the dried coated sand particles, making it very difficult to separate them for subsequent cross-linking. The tendency to aggregate was found to increase with increase in the concentration of chitosan in the solution.

It was found that aggregation could be prevented by carrying out the cross-linking on wet or incompletely dried (80-90% dry) material using Method 2, which involves slurrying in methanol containing glutaraldehyde. Although some of the chitosan is lost in this process it is possible to build up the weight of the chitosan coating by multiple treatments involving coating and cross-linking (Table 2).

Table 2. Dye adsorption capacity following multiple treatments with a 5% chitosan solution using Method 2 for cross-linking wet or semi-dry products.

Number of treatments	1	2	3
Wet: dye adsorption (g/kg)	5.8	10.9	20.1
Semi-dry: dye adsorption (g/kg)	13.1	23.7	32.0

Chitosan solutions having higher solids contents were prepared using lower molecular weight chitosan produced by nitrous acid degradation. The effect of increasing chitosan concentration in solution on the weight of coating obtained and the subsequent dye adsorption capacity is shown in Table 3.

Table 3. Dye adsorption capacity following the use of lower molecular weight chitosan in a single application process and Method 2 for cross-linking the semi-dry product.

Sample Number	1	2	3
Degradation time (h)	0	1	24
[η] (mL/g)	750	569	216
$M_v \times 10^{-5}$	5.50	3.91	0.41
$C_{chitosan}$ (w/v)	5	6	8
Weight of coating (wt %)	0.61	0.66	0.74
Dye adsorption (g/kg)	13.1	14.3	15.5

The molecular weights of the chitosan samples were calculated using the Mark-Houwink constants of Wang *et al.*[7].

The 8% chitosan solution was then used in a multiple application process to increase the dye capacity of the chitosan-coated sand particles. The results are given in Table 4.

Table 4. Dye adsorption using lower molecular weight chitosan in a multiple application process and Method 2 for cross-linking the semi-dry product.

Number of treatments	1	2	3
Sample designation	SF31	SF32	SF33
Weight of coating (wt %)	0.74	1.40	2.01
Dye adsorption (g/kg)	15.5	30.1	41.2

Stability of the cross-linked chitosan coating on sand particles

The stability of the chitosan coatings to acid media were tested as described in Materials and methods section using two solvents: a) 0.2 M CH_3COOH/0.1 M CH_3COONa (pH 4.45); b) 0.1 M CH_3COOH (pH 2.91). After more than three months the solvents were still clear and the flow time as measured in a Ubbelohde viscometer had not changed indicating that the cross-linked coatings were stable under these conditions.

Regeneration of dye adsorption capability

Regeneration of the coating was carried out using 0.1 M NaOH for the desorption step. The results show that the chitosan-coated sand particles can be used for a number of adsorption/desorption cycles without any appreciable loss in capacity (Table 5).

Table 5. Dye adsorption capacity following repeated adsorption/desorption cycles.

Regeneration cycles	0	1	2	3	4
Sample SF32	30.1	28.9	29.4	27.6	27.1
Sample SF33	41.2	38.6	37.3	39.5	-

Conclusions

Chitosan, in the form of a glutaraldehyde cross-linked coating on sand particles, is an effective column packing material for decolourising dyehouse effluent. Coating weights of up to 2% of the total weight were obtained and at this level the dye adsorption capacity was in excess of 40 g of C.I. Acid Orange 7 per kilogram of product. The adsorbent could be regenerated by treatment with an alkaline solution and a minimum of 90% of the dye adsorption capacity was retained over a number of adsorption/desorption cycles. The cross-linked coating was found to be stable in acid media for at least three months.

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Effect of Chitosan from Shrimp, Squid and Crab on the State of Water and Denaturation of Myofibriliar Protein during Drying Process

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Abstract

Drying has been one of the most ancient process to preserve food. As because of these, many studies have been published specially in the drying behavior of proteins, with special emphasis in denaturation. The difficulty in finding water activities (Aw) lowering agents that are safe, economical, effective, tasteless and colorless make us considering chitosan as a very good alternative as an Aw lowering agent in fish products.

Chitosans were extracted from crab, squid, and shrimp. Changes in the state of water and progress of denaturation of fish myofibrils in presence of 2.5-12.5% of chitosan during drying process were studied. The addition of chitosan resulted in a decrease in water activity (Aw), indicating the change in the state of water in myofibrils. Furthermore, it was found that an ideal concentration was at 7.5% of chitosan on myofibrils. Chitosan from crab had higher effect than those from squid and shrimp. Chitosan has a suppressive effects, resulting from drying, and there is a correlation between the suppressive effects and the state of water. These results suggest that the suppressive effects on the denaturation are likely to be attributed to the stabilization of the hydrated water surrounding myofibrils by the addition of chitosan.

Keywords: Chitosan, drying, water activity, myofibrils,

One of man's earliest discoveries for food preservation must have been the fact that fresh foods become much less perishable when their water content is drastically reduced. Apart from physical changes, all types of deterioration, including microbial growth, are based on chemical and biochemical reactions. Formally it can be stated that except for a diffusion limitations the speed of any reaction is determined by its substrate concentration. Drying has been one of the most ancient process to preserve food. As

because of these, many studies have been published specially in the drying behavior of proteins, with special emphasis in denaturation but there is not information available regarding effect of additives on the state of water and denaturation of fish protein by dehydration process. The difficulty in finding water activities (Aw) lowering agents that are safe, economical, effective, tasteless and colorless make us considering chitosan as a very good alternative to be used as an Aw lowering agent in fish products.

As traditional foods, we have been eating mushrooms, soft shrimps and baker yeast, all of which contain chitin and chitosan as constituents. Chitin and chitosan as been approved officially as food additives in Japan.

Chitosan is a very useful derivative form of Chitin. Chitin and chitosan are natural resources waiting for a market and currently the subject of a big number of applied research projects, directed towards the commercial exploitation. Is comun to find information about chitin and chitosan in interchangeably way. Many different areas have been investigated to find possible uses for these polymers over the years. Among these potential areas we can find: paper production, heavy metals chelating agents, cements, photogragraphic products, food technology, etc., In this research we have used chitosan from three different sources of crustaceans and it was tested as a water activity (Aw) lowering agent and the effect on the state of water in fish myofibrills was observed.

MATERIALS AND METHODS

The samples of Japanese fan lobster (*Ibacus ciliatus*), spear squid (*Doriteuthis blekeri*) and Japanese swimming crab (*Portinus trituberculatus*), were obtained from a food processing factory in Nagasaki city. Chitin was isolated from this three species using the method of Hackman[1)] and it was pulverized and pass through a 40 mesh sieve.

Preparation of chitosan

The samples of spear squid, Japanese swimming crab and Japanese fan lobster were crushed and then inmerse in 20 volumes of 2N HCl solution for 48 hours (the solution was changed every 12 hrs). The precipitated was washed with distilled water and adjust to pH 7.0. Then 20 volumes of 1N NaOH were added (this solution was changed every 6 hours) during heating at 98°C for 36 hours. The samples were washed with distilled water and the pH was adjusted at

7.0 and finally lyophilized to obtain chitin. Then, 20 volumes of 60% NaOH are added to the chitin powder (150 g) while heating at 130°C for 3 hours. The sample is washed until get a pH 7.0. acetic acid solution (10%) is added and the sample is stirring for 12 hours. Centrifuged at 10,000 x g, for 30 minutes and the precipitate is washed with distilled water until reach pH 7.0. Finally the precipitate is lyophilized and a white chitosan powder is produced.

The degree of Acetylation was determined by spectrophotometry using Muzzarelli's method[2] and also by potentiometric titration according with Broussignac's method[3]. The average molecular weight and the degree of polimerization was determined using Robert's and Domszy method[4].

Preparation of fish myofibrils

Fish myofibrils were prepared from lizardfish (*Saurida wanieso*) meat. After heading and eviscerating, the fish was washed in ice water. The skin and bones were removed with a meat separating machine. Fish meat was washed in 5 volumes of 0.1M KCl adjusting the pH to 7.0 with 20mM tris-maleate buffer. This washed process was repeated three times. After excess water was pressed out, the resulting residue was homogenized with volume of the same buffer as above. The suspension thus obtained was filtered through a nylon net (#16) and 1% triton x-100 was added then was kept standing at 5°C for 30 minutes, then was centrifuged at 1,400 x g for 10 minutes. After being washed again with 5 volumes of the same buffer as stated earlier, the residue was centrifuged at 1,400 for 10 minutes. The washing treatment with the same buffer was repeated until the supernatant became clean. The residue was suspended in 5 volumes of cold distilled water and centrifuged first at 3,800 x g for 10 minutes and then at 9,200 x g for 20 minutes to produce the myofibrils.

Determination of the Water Activity (Aw)

Water activity defined as an index to represent mobility of water molecules in a substance and expressed as a ratio of the partial water vapor pressure at the same temperature. The partial water vapor was determined at 20°C after equilibrium for 1 or 2 hours and using Akiba method[5] (1951) measuring the water vapor pressure with an oil manometer.

Determination of the moisture content.

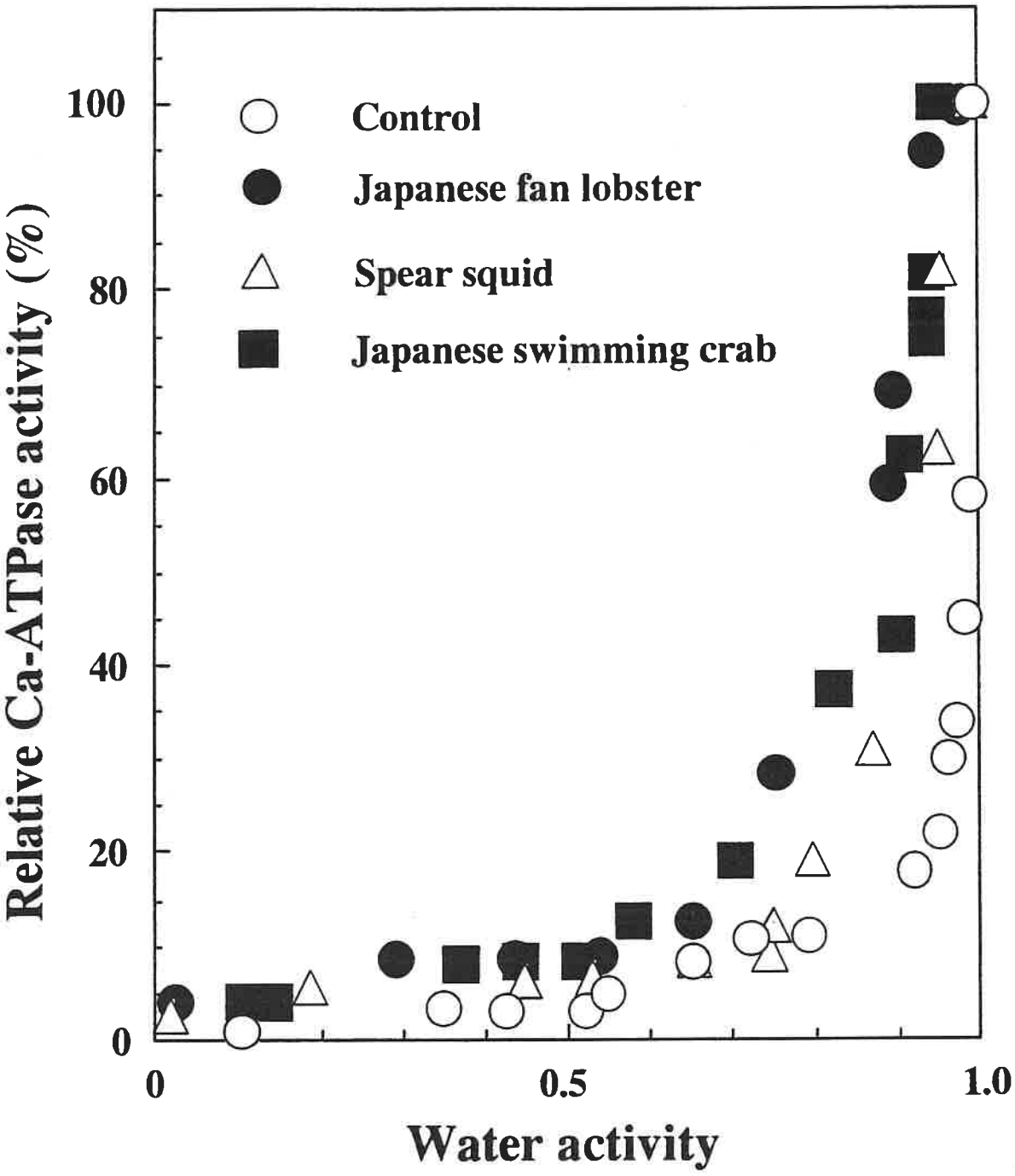
The moisture content was determined as the loss of water in a sample by oven drying at 105°C for 16 to 18 hours.

Results and Discussions

A water sorption isotherm of materials represents relationship between moisture content and water activity of myofibrils at a particular temperature. In accordance with this relation, the water activity of myofibrils without or with 5% chitosan at 20°C during dehydration process was plotted as a function of the moisture content in the fig.

All the desorption isotherms shows a sigmoidal curve which has two bending within the 0.05-0.15. Water activity of myofibrils with all of chitosan decreased remarkably throughout dehydration process and of which were lower than that of chitosan (control) without for the same moisture content.

In order to evaluate the characteristics of state of water in myofibrils from above desorption isotherms, the amount of monolayer water (M1) and the amount of multilayer water (M2) in myofibrils were calculated by the BET method[6] and Bull's method[7] respectively. The M1 and the M2, water activity, Aw1 and Aw2 at the M2 points, remaining relative Ca-ATPase activity among others parameters (table 1).



The relative Ca-ATP activity and water activity (20°C) to show the effect of chitosan 5% from different crustaceans added to lizardfish myofibrils during dehydration process.

Table 1. Amount of monolayer and multilayer water, sorption surface area of lizard fish myofibrils added 5% of various chitosans from desorption isotherm at 20°C and remaining Ca-ATPase activity of the myofibrils corresponding with monolayer or multilayer absorbed water

System	Monolayer water[*1]		Aw_1[*2]	T_{a1}[*3]	Multilayer water[*4]		Aw_2[*5]	T_{a2}[*6]	M_2/M_1	S[*7]
	M_1[*8]	M_{d1}[*9]			M_2[*8]	M_{d2}[*9]				
Control	8.2	0.089	0.110	1.66	15.9	0.189	0.610	4.80	1.93	0.311
Japanese fan lobster	8.6	0.094	0.102	4.61	18.5	0.226	0.655	11.60	2.15	0.328
Spear squid	8.7	0.095	0.055	3.07	18.2	0.222	0.593	7.47	2.09	0.332
Japanese swimming crab	9.4	0.103	0.096	4.46	21.7	0.277	0.425	14.15	2.30	0.360

[*1]Estimated by B.E.T. analysis
[*2]Water activity of the sample at the M1 point
[*3]Remaining myofibril relative Ca-ATPase activity (%) of the sample at the M1 point.
[*4]Estimated by Bull's analysis
[*5]Water activity of the sample at the M2 point.
[*6]Remaining myofibril relative Ca-ATPase activity (%) of the sample at the M2 point.
[*7]Sorption surface area (m^2/mg) of sample.
[*8]Moisture content (g/100 g of sample).
[*9]Moisture content (g/g of dried matter).

CONCLUSIONS

Chitosan has a suppresive effects, resulting from drying, and there is a correlation between the suppresive effects and the state of water. These results suggest that the suppressive effects on the denaturation are likely to be attributed to the stabilization of the hydrated water surrounding myofibrils by the addition of chitosan. Chitosan from crab had higher effect than those from squid and shrimp.

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SEPARATIONS OF ORGANIC LIQUID MIXTURES THROUGH CHITOSAN DERIVATIVE MEMBRANES

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Abstract

Benzoylchitosans with different degree of benzoylation were synthesized as membrane materials having a good durability for the separation of benzene/cyclohexane mixtures. Characteristics of benzoylchitosan membranes such as contact angle, crystallinity and degree of swelling were significantly influenced by degree of benzoylation. When benzoylchitosan membranes were applied to the permeation and separation for the benzene/cyclohexane mixtures in pervaporation, both the permeation rate and benzene concentration in the permeate increased with increasing benzene concentration in the feed, and these membranes showed a benzene-permselectivity and a difference of the benzene-permselectivity for the benzoylchitosan membranes with different degree of benzoylation mainly depended on a difference of physical structure of the membranes based on the characteristics of these membranes. Characteristics of permeation and separation for the benzene/cyclohexane mixtures through the benzoylchitosan membrane were analyzed by the solution-diffusion model. It was found that the benzene-permselectivity was dependent on both the sorption selectivity and diffusion selectivity but was significantly governed by the latter selectivity, also a tentative model for the benzene-permselectivity is discussed.

Keywords: Benzoylchitosan, membrane, pervaporation, benzene/cyclohexane, permselectivity, separation mechanism

Introduction

The separation of the benzene/cyclohexane mixtures is very important in the petrochemical industry. However, it is very difficult to remove directly by distillation one component from benzene/cyclohexane mixtures, which are close-boiling point chemicals. Recently, much attention has been paid to membrane separation tequniques for the separation and concentration of liquid and gas mixtures. In particular, pervaporation (PV) is very useful for the separation of organic liquid mixtures. A swelling of polymer membranes with the feed mixtures in the membrane separation gives high permeability but low selectivity for the separation. In PV, the characteristics of permeation and separation for organic liquid mixtures are significantly governed by the solubility of permeants into the polymer membranes and the diffusivity of permeants in the polymer membranes. The former factor is dependent on an affinity of the permeant for the polymer membrane and the latter on the molecular size of the permeant. If the swelling of polymer membranes based on the former factor is depressed, both the sorption selectivity and diffusion selectivity are raised and consequently polymer membranes having high permeability and high selectivity for the separation can be given. From such a viewpoint, in order to design polymer membranes for the separation of benzene/cyclohexane mixtures, chitosan is selected as a membrane material source. Because chitosan molecules have many reactive

functional groups such as hydroxyl and amino groups, chemical modifications of the chitosan molecules are very easy and an introduction of substituent groups having a high affinity for a component in the feed mixtures to those functional groups is possible. Also since the chitosan molecule chains don't have an affinity for benzene and cyclohexane, it is possible to control the depression of swelling of the membrane.

In this study, chitosan was modified by benzoylation as one of hydrophobic chitosan derivatives and the permeation and separation characteristics of the benzene/cyclohexane mixture through benzoylchitosan membranes by PV were studied. And also the relationship between the permselectivity and the membrane structure is discussed by the solution-diffusion model [1,2].

Experimental

Materials

Chitosan with a degree of deacetylation of 100 % and an average molecular weight of $3x10^5$ - $4x10^5$ was supplied by Koyo Chemical Co. Ltd., Japan. The other reagents used in this study were supplied by commercial sources.

Synthesis of benzoylchitosan

Syntheses of benzoylchosans have been reported in a previous paper [3]. In this study, the benzoylchitosans with the degree of benzoylation of 0.7, 1.0, 2.2, 2.3 and 2.5 were synthesized.

Preparation of benzoylchitosan membrane

The benzoylchitosan membranes with the degree of benzoylation of more than 2.2 were prepared by casting a chloroform solution of 1.6 wt% benzoylchitosan on a rimmed glass plate and evaporating the solvent completely for 24 hr at 25 °C. On the other hand, the benzoylchitosan membranes with the degree of benzoylation of less than 1.0 were prepared by casting a dimethyl formamide solution of 1.6 wt% benzoylchitosan on a rimmed glass plate and evaporating the solvent for 15 hr at 70 °C. The chitosan membrane was prepared according to a previous paper [4].

Measurements of characteristics of benzoylchitosan membrane

Contact angle, density, crystallinity, and degree of swelling of the benzoylchitosan membranes were determined by the methods reported in previous papers [5,6].

Composition sorbed in membrane and PV apparatus

The composition of benzene/cyclohexane sorbed in the membrane was determined by a previous method [3,5,6]. The sorption selectivity, $\alpha_{sorp,B/C}$, was calculated from eq. (1),

$$\alpha_{sorp,B/C}=(M_B/M_C)/(F_B/F_C) \quad (1)$$

where F_B and F_C are the weight fractions of benzene and cyclohexane in the feed solution and M_B and M_C are those in the benzoylchitosan membranes, respectively.

The PV cell and PV apparatus have been described in previous papers [3-6]. The separation factor, $\alpha_{sep,B/C}$, was calculated from eq. (2),

$$\alpha_{sep,B/C}=(P_B/P_C)/(F_B/F_C) \quad (2)$$

where F_B and F_C are the weight fractions of benzene and cyclohexane in the feed solution and P_B and P_C are those in the permeate, respectively.

Results and discussion

Characteristics of benzoylchitosan membrane

The contact angles to methylene iodide on the surface of the benzoylchitosan membranes were lower than that of the chitosan membrane. Therefore, the benzoylchitosan membranes are more hydrophobic than the chitosan membrane. The chitosan membrane did not almost sorbed benzene and cyclohexane. Therefore, the chitosan membrane was not swollen by the benzene/cyclohexane mixtures and consequently not shown a permeability for those mixtures. On the other hand, since the benzoylchitosan membrane could sorb the benzene/cyclohexane mixtures and had an affinity for them, a permeability in PV was observed. The density and correlation crystallinity index of the benzoylchitosan membranes were smaller than those of the chitosan membrane. The decreases in the density and correlation crystallinity index suggest that the structure of benzoylchitosan membrane became more opened. This phenomenon is due to that intermolecular hydrogen bonds between the chitosan molecule chains are broken by benzoylation of the chitosan molecule. These results support that the benzoylchitosan membranes have a permeability. Consequently, it was recognized that the benzoylchitosan membranes can be applied to the permeation and separation for the benzene/cyclohexane mixtures.

Characteristics of permeation and separation

Effect of the benzene concentration in the feed solution on the permeation rate and

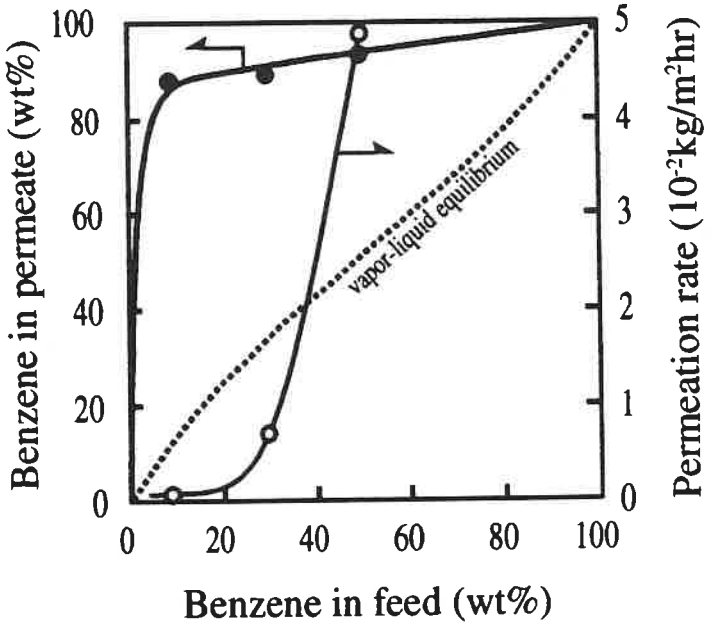

Fig. 1 Effect of the benzene concentration in the feed on the permeation rate and benzene concentration in the permeate of the benzoylchitosan membrane in PV. Degree of benzoylation: 2.2.

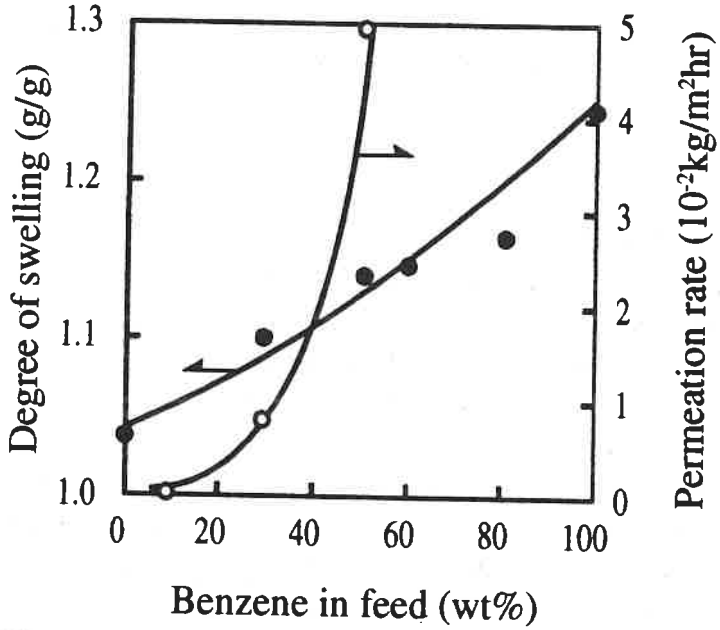

Fig. 2 Permeation rate and degree of swelling of the benzoylchitosan membrane for the benzene/ cyclohexane mixtures. Degree of benzoylation: 2.2.

benzene concentration in the permeate through the benzoylchitosan membrane with a degree of benzoylation of 2.2 is shown in Fig. 1. The permeation rate remarkably increased and the benzene concentration in the permeate also increased with increasing benzene concentration in the feed. The benzene concentrations in the permeates were higher than those in the feed. These results suggest that the benzoylchitosan membrane is a benzene-permselective. In order to reveal the above characteristics of permeation and separation, the degree of swelling of the benzoylchitosan membrane and the composition of a benzene/ cyclohexane mixture sorbed in the benzoylchitosan membrane were measured.

Fig. 2 shows the degrees of swelling of the benzoylchitosan membrane in the benzene/ cyclohexane mixtures as a function of the benzene concentration in the feed and also includes the permeation rates for the benzene/cyclohexane mixtures. The degree of swelling of the benzoylchitosan membrane increased with an increase of the benzene concentration in the feed mixture. This result suggests that an affinity of benzene for the benzoylchitosan membrane is greater than that of cyclohexane. The increase in the permeation rate with an increase of the benzene concentration is attributed to the increase in the degree of swelling of the benzoylchitosan membrane.

Composition in membrane

The effect of the benzene concentration in the feed on the benzene concentrations in the benzoylchitosan membrane and permeate is shown in Fig. 3. As can be seen from this figure, the benzene concentrations in the benzoylchitosan membrane were higher than those in the feed mixtures. These results suggest that the benzoylchitosan membrane has a higher affinity for benzene than for cyclohexane. These facts are not contradictory to the results for the degree of swelling as shown in Fig. 2. Also, the benzene concentration in

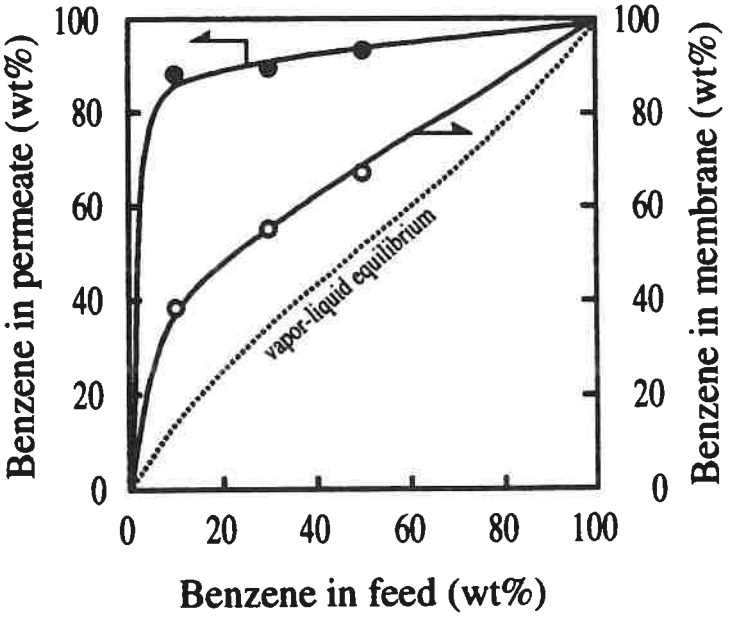

Fig. 3 Effect of the benzene concentration in the feed on the benzene concentrations in the permeate and benzoylchitosan membrane. Degree of benzoylation: 2.2.

the permeates were greater than those in the benzoylchitosan membrane. These results imply that the benzoylchitosan membrane shows a benzene-permselectivity for the benzene/cyclohexane mixture. However, a mechanism of the permeation and separation for the benzene/cyclohexane mixture through the benzoylchitosan membrane in PV can not be explained by an only high affinity of the benzoylchitosan for benzene. Then, in order to clarify a mechanism of the permeation and separation in the benzene-permselectivity, the separation characteristics for the benzene/cyclohexane mixture through the benzoylchitosan membrane were analyzed by the solution-diffusion model [1,2].

Permselectivity of benzoylchitosan membrane

In general, the separation mechanism of liquid mixtures through polymer membrane in pervaporation is due to the difference of the solubility of permeants into polymer membranes in the sorption process and of the diffusivity of permeants in polymer membranes in the diffusion process.

In the results in Fig. 3, the benzene concentrations in the permeants were higher than those in the membrane. These facts suggest that the benzene-permselectivity of the benzoylchitosan membrane depends on both the sorption process and diffusion process in the solution-diffusion model. Because benzene has a high affinity for the benzoylchitosan membrane and the molecular size of benzene is smaller than that of cyclohexane. Thus, it is very important to determine both the sorption selectivity and diffusion selectivity to discuss the separation mechanism for the benzene/cyclohexane mixtures through the benzoylchitosan membranes in PV in detail. The sorption selectivity, $\alpha_{sorp,B/C}$, was determined by eq. (1) using the data in Fig. 3 and the diffusion selectivity, $\alpha_{diff,B/C}$, was calculated from the separation factor, $\alpha_{sep,B/C}$, obtained by applying the results in Fig. 1 to eq. (2) and sorption

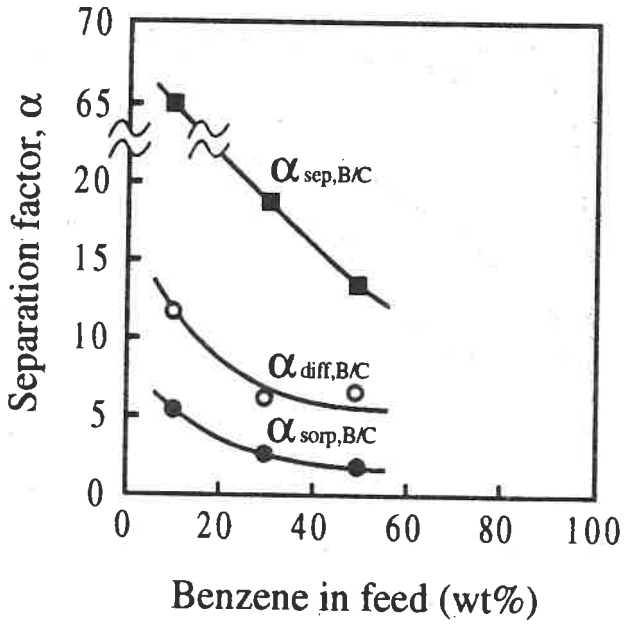

Fig. 4 Separation factor, $\alpha_{sep,B/C}$, sorption selectivity, $\alpha_{sorp,B/C}$, diffusion selectivity, $\alpha_{diff,B/C}$ for the benzene/cyclohexane mixtures through the benzoylchitosan membrane with degree of benzoylation of 2.2 in PV as a function of the benzene concentration in the feed.

selectivity by eq. (1), using eq. (3):

$$\alpha_{diff,B/C} = \alpha_{sep,B/C}/\alpha_{sorp,B/C} \quad (3)$$

In Fig. 4, the effect of benzene concentration in the feed on the separation factor, sorption selectivity and diffusion selectivity are shown. In all benzene concentrations in the feed, the diffusion selectivity was greater than the sorption selectivity. This result suggests that the separation mechanism for the benzene/cyclohexane mixtures of the benzoylchitosan membrane is mainly governed by the diffusion process. The fact that benzene concentrations in the permeates were higher than those in the membrane shown in Fig. 3 can be understood by the above discussion, namely the total of the sorption selectivity and diffusion selectivity significantly influences the benzene-permselectivity. On the other hand, the separation factor decreased with an increase of the benzene concentration in the feed mixture. This result is dependent on both the decrease in the sorption selectivity and diffusion selectivity, which are attributed to more opened structure of the membrane based on the increase in the swelling of membrane with an increase of the benzene concentration.

The degree of swelling of the benzoylchitosan membranes was higher than that of chitosan membrane and increased with increasing degree of benzoylation. This result for hydrophobic solvent mixtures suggest that the benzoylchitosan membranes were more hydrophobic than the chitosan membrane and the increase of the benzoylation enhanced the hydrophobicity of these membranes.

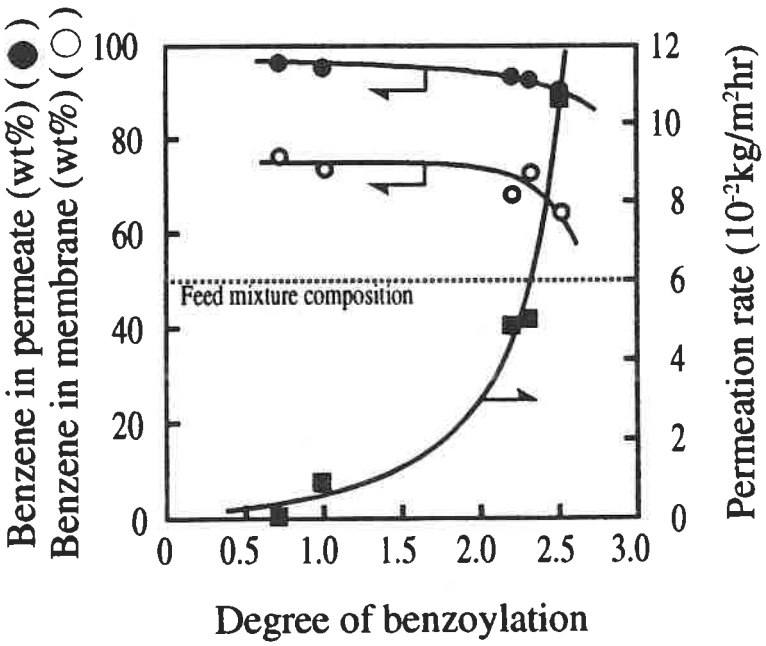

Fig. 5 Permeation rate and benzene concentrations in the permeate and membrane for a benzene/cyclohexane mixture of 50 wt% benzene through the various benzoylchitosan membranes in PV as a function of the degree of benzoylation of chitosan. Dotted line is the feed mixture composition.

Effect of degree of benzoylchitosan

The effect of the degree of benzoylation on the permeation rate and benzene concentration in the permeate through the benzoylchitosan membranes for a benzene/cyclohexane mixture of 50 wt% benzene by PV is shown in Fig. 5. The permeation rate remarkably increased with increasing degree of benzoylation. It is well known that a remarkable swelling of polymer membranes leads to an opened membrane structure and consequently an enhancement of the diffusivity of the permeants in the polymer membranes. Therefore, this increase in the permeation rate is attributed to the increase in the degree of swelling of the benzoylchitosan membranes with an increase of the degree of benzoylation as shown in Fig. 2.

The benzoylchitosan membranes exhibited a high benzene-permselectivity, namely benzene concentrations in the permeate were higher than that in the feed mixture and more than about 90 wt%. The benzene-permselectivity of the benzoylchitosan membranes was slightly lowered with increasing degree of benzoylation. A lowering of the benzene-permselectivity is mainly dependent on an increase in the degree of swelling of the benzoylchitosan membranes for the benzene/cyclohexane mixtures. Fig. 5 also includes the benzene concentration in the benzoylchitosan membranes. Comparing the benzene concentrations in the permeates and in the benzoylchitosan membranes as a function of the degree of benzoylation reveals the benzene concentrations in the permeates were greater than those in the benzoylchitosan membranes. These differences mean that the benzene-permselectivity is not dependent on only difference of solubility of the permeants into the

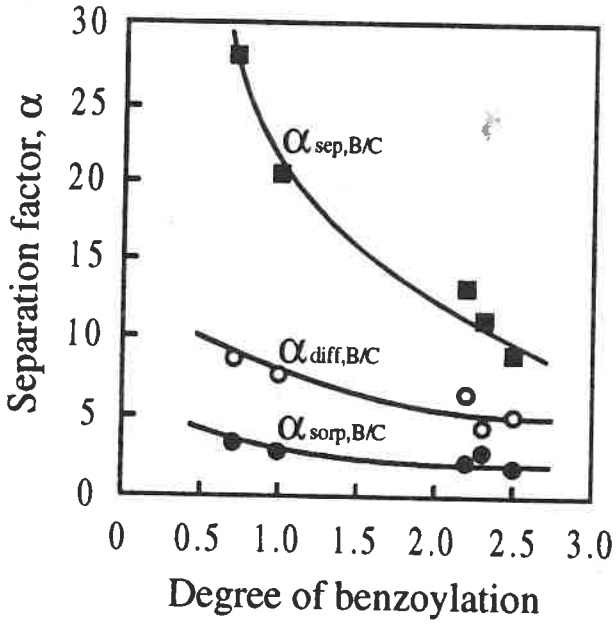

Fig. 6 Effect of the degree of benzoylation on the separation factor, $\alpha_{sep,B/C}$, sorption selectivity, $\alpha_{sorp,B/C}$, diffusion selectivity, $\alpha_{diff,B/C}$ for the benzene/cyclohexane mixture through the benzoylchitosan membranes in PV. Feed mixture: benzene/cyclohexane=50/50 (w/w).

benzoylchitosan membrane.

In Fig. 6, the separation factor, sorption and diffusion selectivities for a benzene/cyclohexane mixture with 50 wt% benzene are shown as a function of the degree of benzoylation. The separation factor, sorption and diffusion selectivities decreased with increasing degree of benzoylation. These decreases are mainly caused by an increase in the degree of swelling of the benzoylchitosan membranes. On the other hand, the diffusion selectivities were higher than the sorption selectivities in all degree of benzoylation. These results suggest that the benzene-permselectivity is strongly governed by the diffusion separation process.

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