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Carbaryl insecticide conjugation onto chitosan via iodochitosan and chitosan carbonyl imidazolide precursors

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Abstract

Chitosan conjugated with 1-naphthyl methylcarbamate or carbaryl (CBR), were prepared by two molecular designs, i.e. without spacer via iodochitosan to obtain chitosan-carbaryl (Type 1, CHI-CBR), and with spacer by using *N*,*N*′-carbonyldiimidazole (CDI) to obtain chitosan acetate-carbonyl imidazolide-carbaryl (Type 2, CA-CDI-CBR). The chitosan precursors of Types 1 and 2 were accomplished for 50–60% substitution as confirmed by FT-IR, NMR, and elemental analysis. The conjugation of carbaryl onto iodochitosan (Type I) was achieved via alkylation of *N*-substituted amide using NaH as a catalyst. The coupling reaction of carbaryl onto chitosan acetate was succeeded by carbonyl imidazolide which proceeded by nucleophilic substitution. The introduction of carbaryl was identified from the ester peak at 1707 cm^{-1} by FT-IR curve fitting technique.

Keywords: Chitosan; Iodochitosan; Carbonyldiimidazole; Chitosan acetate; Carbaryl; Drug conjugation; Insecticide

1. Introduction

Chitin–chitosan copolymer (Scheme 1) is the second most abundant natural occurring polymer next to cellulose, found as the major constituent of the exoskeleton of crustaceans and insects as well as the cell wall of bacteria and mushrooms. Chitin–chitosan has received much attention due to its applications in value added products in various fields owing to its specific properties of biodegradability (Xu, McCarthy, Gross, & Kaplan, 1996) and biocompatibility (Prudden, Miegel, Hanson, Friedlich, & Balassa, 1970), together with the possibility for physical (Mi, Wong, Shyu, & Chang, 1999; Sawayanagi, Numbu, & Nagai, 1982; Somorin, Nishi, Tokura, & Noguchi, 1979) and chemical modifications (Kurita, Ishii, Tomita, Nishimura, & Shimoda, 1994). Chitosan gives higher potential chemical modifications than chitin owing to an additional reactive amino group at C-2 (Fujii, Kumagai, & Noda, 1980; Hirano, Ohe, & Ono, 1976; Yalpani & Hall, 1984; Nishimura, Kongo, & Kurita, 1991) besides the secondary hydroxyl at C-3 and primary hydroxyl at C-6 (Nagasawa, Toshira, Inoue, & Tonoura, 1971; Kurita et al., 1992).

In the past decades, chitin–chitosan derivatives received a great interest as the advanced polymeric materials for drug delivery system (DDS) (Ohya, Nonomura, & Ouchi, 1995; Mi et al., 1997; Onishi, Machida, & Nagai, 1994) in medical and pharmaceutical areas. The present work is, thus, oriented on the utilization of chitosan in agriculture (Hirano & Nagao, 1989) by introducing insecticide onto chitosan via chemical conjugation. 1-naphthyl methylcarbamate or carbaryl (CBR) (Scheme 1), the short application interval but widely used insecticide, was applied as a model drug. The present work is focused on the preparation of CBR conjugated chitosan by designing into two types (Scheme 2), i.e. Type 1, to enhance the stability of carbaryl through chitosan polymer chains by connecting carbaryl directly on chitosan unit, and Type 2, to achieve the delivery system controlled by the stability of the spacer group attached between chitosan main chain and carbaryl.

2. Experimental

2.1. Materials

Chitosan with a degree of deacetylation (DD) 75.8% was provided by the Asian Institute of Technology, Bangkok, and DD 85.9% was the product from Wako Chemicals, Japan. *N*,*N*′-carbonyldiimidazole was produced by TCI, Japan. Chloroform, methanol and hydrochloric acid were purchased from J.T. Baker, USA. Acetic acid, *N*,*N*′-dimethylformamide, benzene, and sodium hydroxide were the products from UNIVAR, Australia. Sodium iodide and

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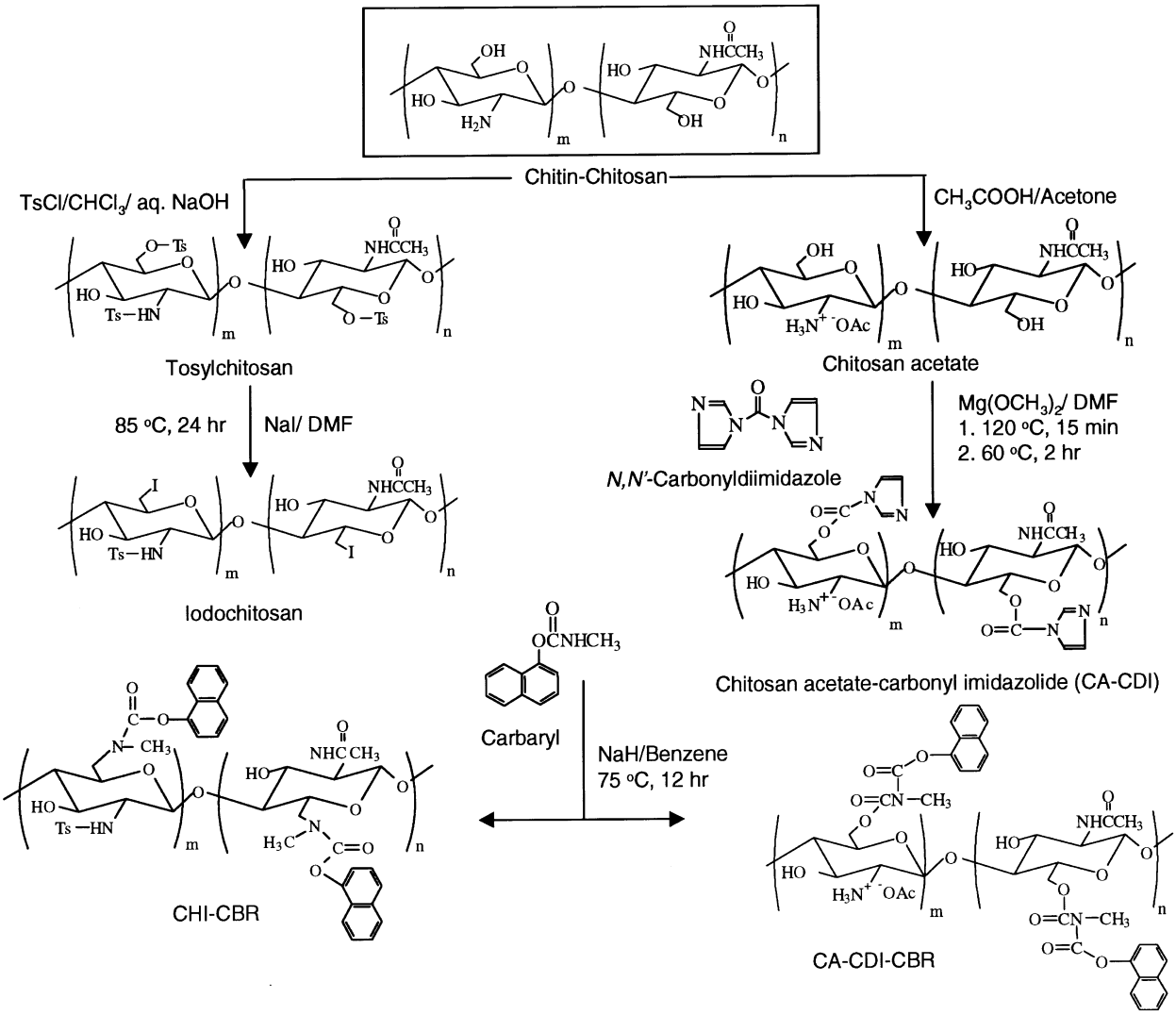
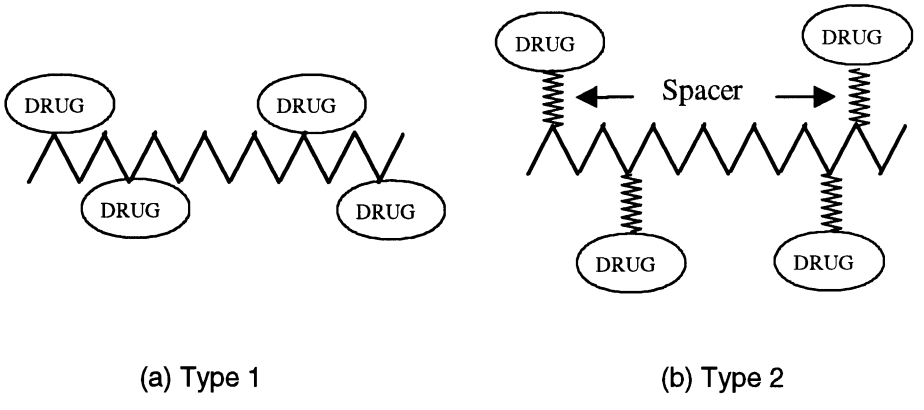


Scheme 1. Overall preparations of tosylchitosan CHI-CBR (Type1), and CA-CDI- CBR (Type 2).



Scheme 2. Molecular design for insecticide conjugated chitosan, (a) Type 1, polymeric drug molecule without spacer, and (b) Type 2, polymeric drug molecule with spacer.

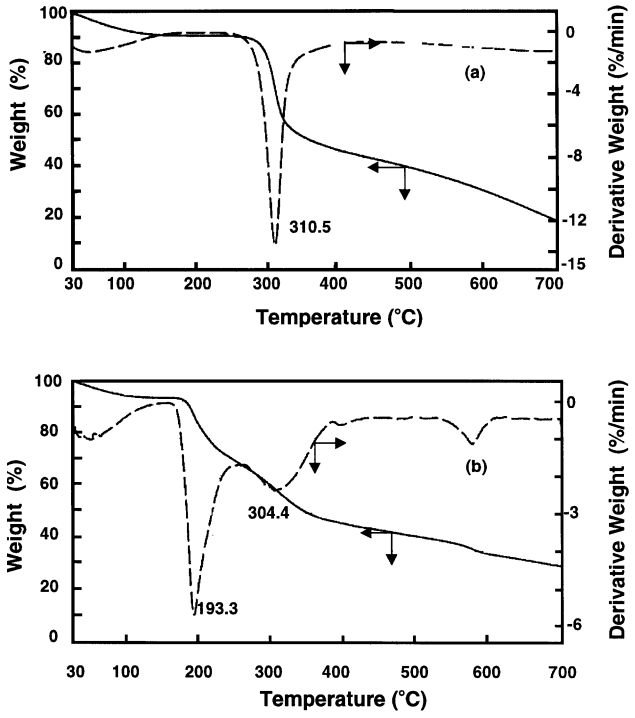


Fig. 1. TGA diagrams of (a) chitosan with degree of deacetylation 85.9% and (b) tosyl chitosan.

sodium hydride were purchased from Fluka Chemika, Switzerland. All these chemicals were used without further purification. A commercial grade of 1-naphthyl methylcarbamate was provided by AG-GRO (THAILAND) Co., Ltd. and recrystallized in acetone three times before use.

2.2. Instruments and equipment

Qualitative and quantitative FT-IR spectra were obtained with a VECTOR 3.0 BRUKER Spectrometer with 16 scans at a resolution of 4 cm^{-1}. A frequency range of 4000–400 cm^{-1} was observed using a deuterated triglycinesulfate detector (DTGS) with a specific detectivity, D^*, of 1×10^9 cm $Hz^{1/2}$ W^{-1}. The elements (in percent) were obtained from PE2400 SeriesII CHNS/O Analyzer with combustion temperature at 975°C and reduction temperature at 500°C. The sample of 1–2 mg was put in tin foil and analyzed under air (flow rate 60 psi) with O_2 as a combustion gas (flow rate 15 psi) using He gas as a carrier gas (flowing rate 20 psi). Solid state ^{13}C-NMR spectra were analyzed by a DPX-300 Avance 300 MHz Digital NMR Spectrometer of Bruker, Switzerland, by courtesy of the National Metal and Materials Technology Center (MTEC), Bangkok, Thailand. Perkin Elmer Thermogravimetric Analyzer was used for TGA studies. The sample (approximately 5 mg) was loaded in a platinum pan and heated from room temperature to 700°C at the rate of 10°C/min under N_2 flow rate of 20 ml/min.

2.3. Experimental procedure

The reactions for preparing Types 1 and Type 2 are summarized in Scheme 1.

2.4. Preparation of chitosan precursors

2.4.1. Preparation of tosylchitosan

Tosylchitosan was prepared as reported by Kurita et al. (1992). The product was characterized by FT-IR, solid state ^{13}C-NMR, EA, and TGA.

2.4.2. Preparation of iodochitosan (Kurita et al., 1992)

One gram of tosylchitosan was dispersed in 28 ml of *N*,*N*-dimethylformamide (DMF) and stirred in the N_2 atmosphere for 15 min. A solution of sodium iodide, 3.3 g (10 moles equivalent to pyranose rings) in 50 ml of DMF, was added and the mixture was stirred under N_2 at 85°C for 24 h. The precipitate was collected and washed thoroughly with acetone and ether. The product was dried in vacuo to obtain the tan powder.

2.4.3. Preparation of chitosan acetate-carbonyl imidazolide (CA-CDI)

Chitosan acetate was prepared by reprecipitating chitosan acetic acid aqueous solution (10% v/v) in acetone. A gram of chitosan acetate was dispersed in DMF (100 ml) under vacuum and heated to 120°C. A catalytic amount of magnesium methoxide was added in the solution. After 15 min, *N*,*N*′-carbonyldiimidazole (3.0 g, 4 moles equivalent to pyranose rings) was added and reacted at 120°C for 15 min. The temperature was then reduced to 60°C and stirring was continued for 2 h. After the reaction, the precipitate was washed thoroughly with methanol and chloroform and dried in vacuo to give pale yellow powder. The obtained product was analyzed by FT-IR, XRD, TGA, and ^{13}C-NMR. The quantitative analysis was done by EA.

2.5. Preparation of chitosan conjugated carbaryl

2.5.1. Synthesis of chitosan conjugated drug type 1: chitosan-carbaryl (CHI-CBR)

A solution of carbaryl 0.7 g (3 moles equivalent to pyranose rings) in absolute benzene 50 ml was cooled in an ice bath and stirred under vacuum for 15 min. Sodium hydride equivalent to the moles of carbaryl in the system was added and the reaction proceeded under N_2 atmosphere for 20 min. Iodochitosan, 0.5 g, was added gradually and the ice bath was replaced by an oil bath. The mixture was reacted at 75°C and refluxed under N_2 atmosphere for 12 h. The brown precipitate was collected and washed thoroughly with ethanol and acetone. The product was characterized by FT-IR, and TGA.

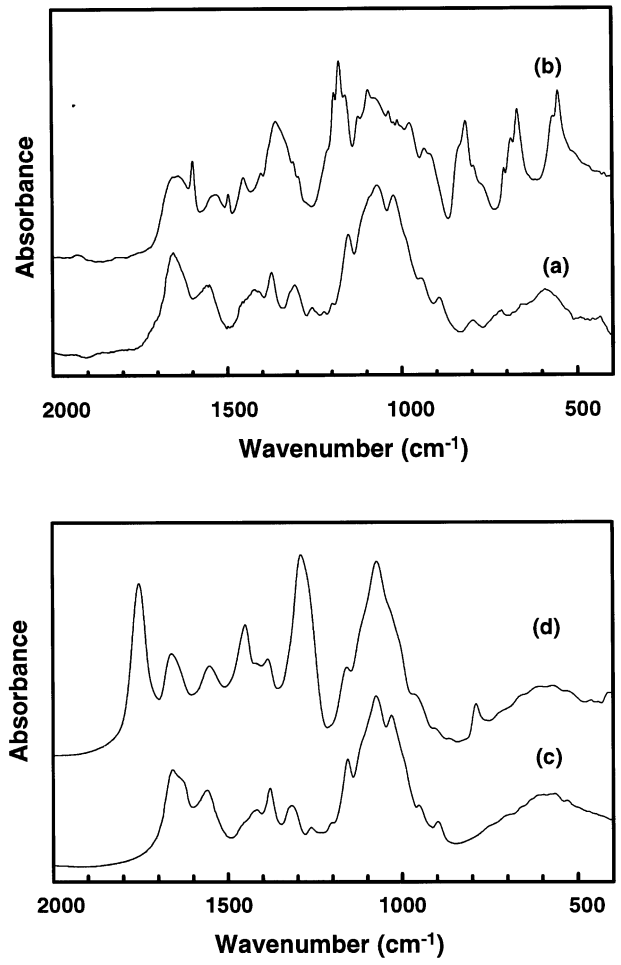
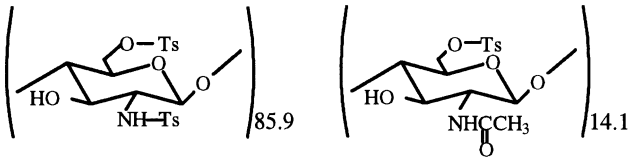


Fig. 2. FT-IR spectra of (a) chitosan (DD 85.9%), (b) tosylchitosan, (c) chitosan acetate (CA), and (d) CA-CDI.

2.5.2. Synthesis of chitosan conjugated drug type 2: chitosan acetate-carbonyl imidazolide-carbaryl (CA-CDI-CBR)

Carbaryl catalyzed by sodium hydride was prepared by the same procedures as for for Type 1. To the obtained solution, CA-CDI, 0.3 g, was added and stirred for 15 min. The ice bath was replaced by an oil bath and the mixture was allowed to react at 75°C and refluxed under N_2 atmosphere for 12 h. The brown precipitate was collected and washed thoroughly with ethanol and acetone. The product was characterized qualitatively and quantitatively by FT-IR, and TGA.



Scheme 3. Complete substitution of tosylchitosan to make the S/N ratio 4.25 for chitosan with degree of deacetylation 85.9%.

3. Results and discussion

Chitosan with the degree of deacetylation 85.9 and 75.8% showed the weight loss at 107.4 and 86.3°C, and at 310.5 and 304.4°C, respectively. Fig. 1(a) shows TGA diagram of chitosan (DD 85.9%). It can be mentioned that the broad peak starting from 60–110°C refer to the loss of moisture and/or water content while the sharp peaks at 310.5°C imply the degradation of chitosan after the loss of intramolecular hydrogen bonding and glycoside linkage.

3.1. Preparation of chitosan precursors

In order to achieve the designed structures of Types 1 and 2, in the present work, chitosan was changed to reactive precursors by focusing on those chemically modified at C-6 position. In the case of Type 1, chitosan precursor was prepared by two steps, i.e. tosylchitosan followed by iodochitosan. Iodochitosan allows carbaryl conjugated directly onto chitosan chain. In the case of Type 2, chitosan precursor was achieved by the reaction of chitosan acetate with carbonyldiimidazole (CA-CDI).

3.1.1. Chitosan precursor for Type 1

3.1.1.1. Preparation of tosylchitosan Chitosan with degree of deacetylation 85.9% was used as a starting material. The reaction was achieved via a heterogeneous condition and found to be dependent on temperature with rapid reaction at the interface.

The characterization by FT-IR is shown in Fig. 2. As compared to chitosan (Fig. 2(a)), the tosylchitosan (Fig. 2(b)) shows a characteristic peak at 1176 cm^{-1} attributed to tosyl groups including the absorption bands at 1598 and 815 cm^{-1} due to *p*-phenylene groups.

The elemental analysis (EA) result is shown as follows.

Anal. Calcd. for $(C_{23}H_{23}NS_2O_8)_{0.859}(C_{15}H_{19}NSO_7)_{0.141}$: %C, 51.09; %H, 4.95; %N, 3.09; %S, 13.13. Found: %C, 47.53; %H, 5.97; %N, 4.38; %S, 10.84.

Elemental analysis reveals a significant sulfur content. The quantitative analysis can be determined from the S/N ratio to evaluate the success of the substitution. The S/N ratio from the elemental analysis was equal to 2.47 while the calculation from the ideal structure assuming 100% tosylation (Scheme 3) is about 4.25. This implies that the reaction occurred around 58% at either hydroxyl or amino group of chitosan.

TGA diagram of tosylchitosan is shown in Fig. 1(b). The percent weight loss at the initial step until 62.0°C refers to the loss of moisture and water. Comparing to that of chitosan (Fig. 1(a)), the peak at around 193.3°C (in Fig. 1(b)) may reveal the loss of tosyl groups. The latter peak at 304.4°C should be due to the breaking of intermolecular hydrogen bonding and the glycoside linkage as seen in the case of chitosan.

Solid state ^{13}C-NMR spectrum of tosylchitosan (Fig. 3)

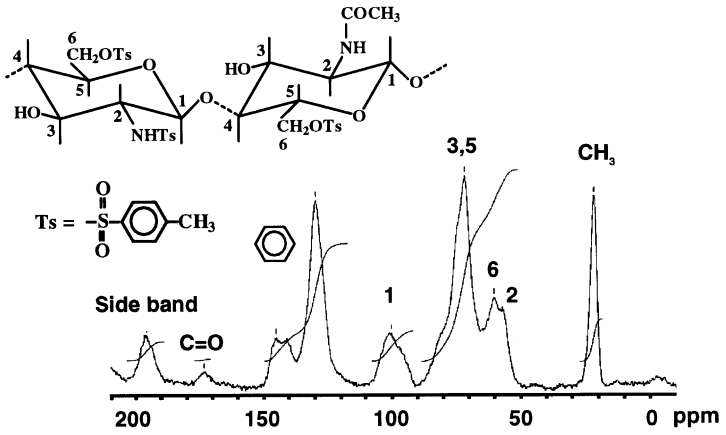


Fig. 3. Solid state ^{13}C NMR spectrum of tosylchitosan.

shows the success of the preparation. Here, the carbon peaks, i.e. acetyl methyls and tosyl methyls, at 21.9 ppm, pyranose rings at 54–100.3 ppm, aromatic rings at 129.9–145.2 ppm, and carbonyl at 173.3 ppm, were observed.

3.1.1.2. Preparation of iodochitosan Iodochitosan was prepared by the same procedures as iodochitin (Kurita et al., 1992). The reaction was operated under heterogeneous condition at high temperature to enhance the efficiency of the reaction. The tosyl groups are substituted via the iodide ion of sodium iodide. The substitution of alkyl tosylate is known to take place by either S_N1 or S_N2 depending on the substrate (Wade, 1991). Nucleophilic substitution proceeds under S_N2 because tosyl group is a good leaving group. In this work, the S_N2 substitution of tosylchitosan (Scheme 4 (b)) could be prior owing to the primary alkyl group at C-6 position. However, when we consider that the chitosan unit is a bulky group, that nucleophilic substitution proceeded to the intermediate via S_N1 (Scheme 4 (a)) is another possibility.

The success of the reaction should be evaluated by the characteristic peaks of organohalogen compounds appearing below 700 cm^{-1}, but in the present case we could not clarify those peaks owing to the overlapping with the broad characteristic peaks of chitosan. Thus, we used FT-IR qualitatively with a curve fitting technique to identify the decrease of tosyl group at 1176 cm^{-1} (Fig. 4(a) and (b)) after the reaction of tosylchitosan with sodium iodide. It was found that the tosyl peak had decreased drastically after the reaction, which implied proceeding of the iodination. In addition, it should be noted that there is a significant peak appearing at 1699 cm^{-1}, as identified from curve fitting technique (Fig. 4(c)). Kurita et al. (1992) indicated that the iodination leads to the acetyl migration of chitin. In the present case, we think that the peak observed at 1699 cm^{-1} might be due to the acetyl migration.

The TGA diagram of iodochitosan shows the degradation temperature at 216.6°C. This implies that iodination leads to the decrease of thermal stability, which may be due to the decrease of inter and intramolecular hydrogen bonding after the introduction of the bulky iodo group and the repulsion among each chitosan chain. Structural analysis by XRD pattern gives the board peak at 22° while the chitosan starting material shows the strong peak at 20° with a shoulder at 22° and a weak peak at 10°. This implies that the iodination changes the packing structure of chitosan with an increase of the amorphous part.

3.1.2. Chitosan precursor for Type 2

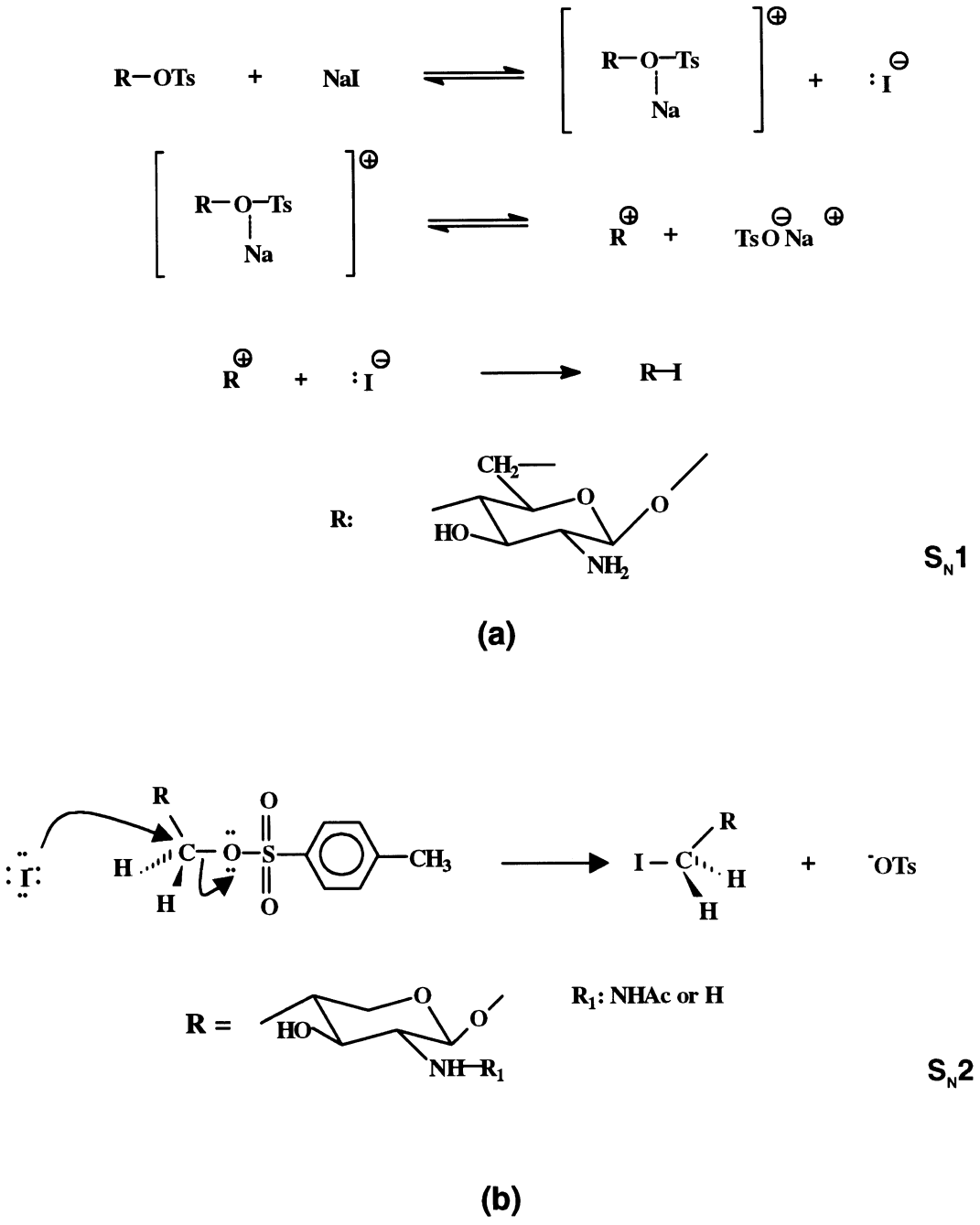
3.1.2.1. Preparation of chitosan acetate (CA) Chitosan acetate is known as a product with most of the amino groups at C-2 protected by acetate groups. This allows the reaction to occur selectively at the hydroxyl group of the C-6. The acetate salt pathway is considered to be the easiest, inexpensive and able to avoid the decrease of molecular weight owing to the mild conditions of preparation. Since both chitosans, i.e. DD 75.8 and 85.9%, can be changed to acetate salt easily, here we chose the lower DD chitosan (75.8%) as a starting material for the model reaction.

Acetate salt formation of chitosan (DD 75.8%) was studied by elemental analysis. The elemental analysis of chitosan acetate is as follows.

Anal. Calcd. for $(C_8H_{13}NO_5)_{0.242}(C_8H_{15}NO_6)_{0.758}$: %C, 44.31; %H, 6.70; %N, 6.46. Found: %C, 38.89; %H, 7.56; %N, 5.87.

The acetate salt substitution can be evaluated from the (C/N) ratio of the found and the calculated values. The salt formation was achieved at 96.65%. Thus, chitosan acetate in this present work may have small amounts of amino groups remaining in the chitosan chain. The FT-IR spectrum of chitosan acetate is shown in Fig. 2(c).

The TGA of chitosan acetate reveals the moisture absorbed in the derivatives as confirmed from the wide range of weight loss from 73.2 to 100°C, while the degradation peak at 292°C appeared similar to that of the chitosan starting material.



Scheme 4. Possible mechanisms of iodination onto tosylchitosan, (a) S_N1, and (b) S_N2.

3.1.2.2. Synthesis of chitosan acetate carbonyl imidazolide (CA-CDI) The use of a coupling agent is one of the interesting approaches for polymer–drug conjugation. In that case, the spacer formed by the coupling agent is a key factor for a controlled release system. *N,N′*-carbonyldiimidazole is known to show high reactivity with various functional groups, such as alcohols, carboxylic acids, and amines (Stabb, 1962). In this work, an effective spacer at either the amino or the alcohol group in the chitosan chain was expected. However, the amino group at the C-2 position of chitosan was already protected by changing to acetate salt in the previous step. This should make the reaction proceed only at the C-6 position. The reaction also has to be performed in a non-aqueous system due to the degradation of CDI by water. Organic solvents such as *N,N*-dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMAc) were considered. However, owing to the low solubility of chitosan in these solvents, the reaction was carried out in the system where chitosan was swelling. At the start, an excess amount of CDI

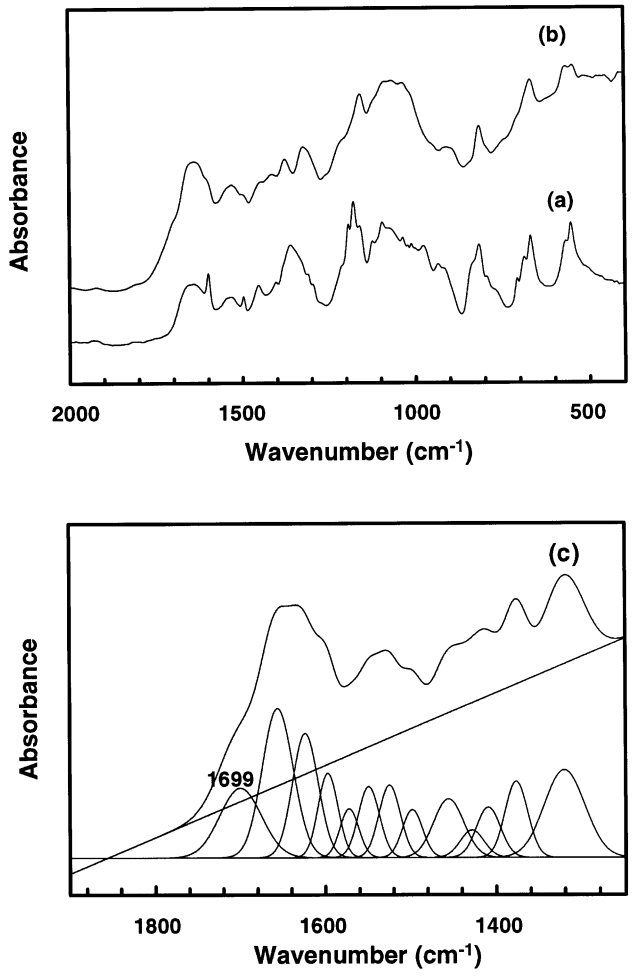


Fig. 4. FT-IR spectra of (a) tosylchitosan, (b) iodochitosan, and (c) qualitative curve fitting of iodochitosan.

was added and the reaction was operated at 120°C with magnesium methoxide as a catalyst.

The FT-IR curve fitting technique was applied to evaluate the coupling reaction of CDI (Fig. 2(d)). The curve fitting peaks show the ester peak at 1752 cm^{-1}, while the amide II band appears as a shoulder on 1659 cm^{-1}. The pyranose band appearing on 1077 cm^{-1} suggested that the saccharide unit did not degrade after the reaction.

The elemental analysis (EA) result is shown below.

Anal. Calcd. for $(C_{12}H_{17}N_3O_7)_{0.733}$ $(C_{14}H_{15}N_5O_6)_{0.025}$ $(C_{12}H_{15}N_3O_6)_{0.242}$: %C, 46.42; %H, 5.29; %N, 13.71. Found: %C, 41.06; %H, 5.38; %N, 8.23.

The calculation from the complete substitution structure gives the C/N ratio to be 3.39; however, the found value is 4.98. The result implies that carbonyl imidazole is introduced for approximately 68%, at either hydroxyl or residue amino unit remaining from the acetate substitution in the previous step.

Thermal property studies revealed that the packing structure of chitosan was changed after the reaction. The weight loss peak at 222.8°C may refer to decrease of crystallinity

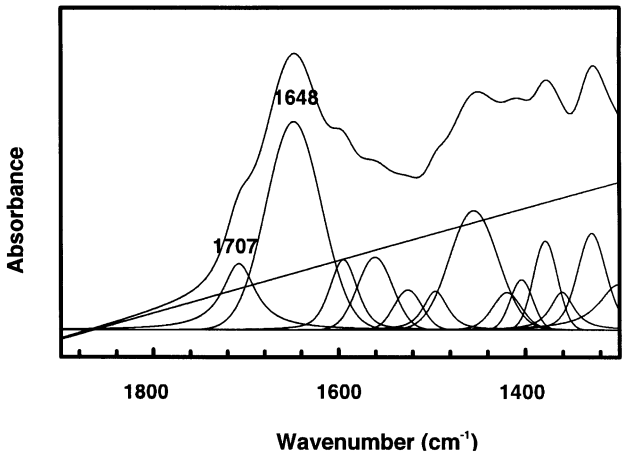


Fig. 5. FT-IR spectrum and qualitative curve fitting of Type 1: CHI-CBR.

after introduction of the ester moiety group while the latter peak at 306.5°C could be the cleavage of intermolecular hydrogen bonding and glycoside linkage. This implies the successful introduction of CDI onto the chitosan chain.

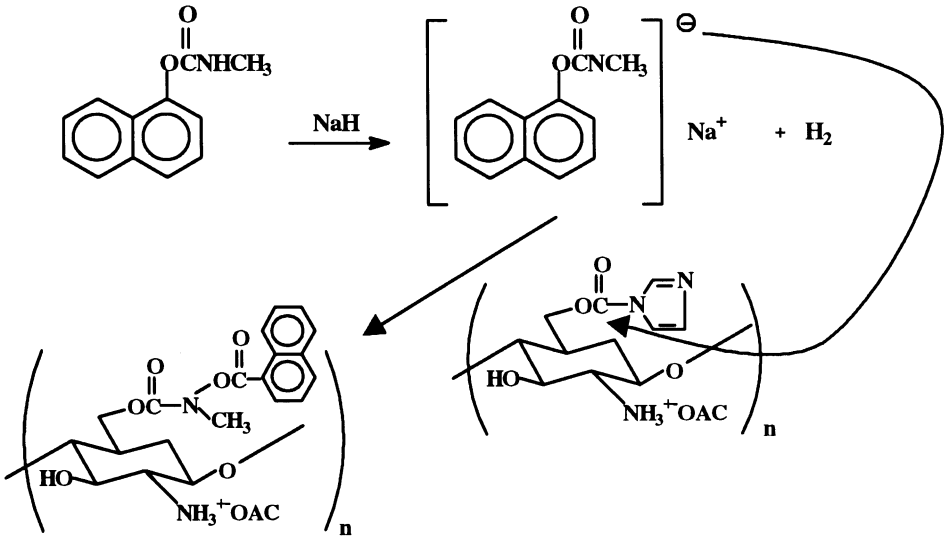
Solid state ^{13}C-NMR was also applied for qualitative analysis of the obtained product. The carbon peaks can be assigned as follows; acetyl methyls at 23.3 ppm, pyranose rings at 56.1–103.7 ppm, and carbonyl imidazole and chitin carbonyl at around 150–170 ppm. Moreover, the peaks at around 60 ppm of the C-6 are found to be shifted to the lower field, reflecting the result of imidazolide conjugation.

3.2. Preparation of chitosan conjugated drug

In order to study drug conjugation with chitosan precursors, the model drugs should have the reactive functional groups for the reaction, be easy to handle, and give clarity of characterization by analytical methods. The present work is concerned with application in insecticides, as a model drug for a controlled release system in agricultural use. For the requirements as a model drug, carbaryl (CBR) (Worthing, 1979) was chosen for its functional group, including the naphthyl ring as a chromophore for UV detection.

3.2.1. Chitosan conjugated drug type 1: chitosan-carbaryl (CHI-CBR)

The polymer drug conjugation without spacer can be obtained by alkylation of alkylhalide. Actually, the carbamate group cannot react with chitosan or iodochitosan due to the low activity of the secondary amide. Fones (1949) exhibited the alkylation of N-substituted amides using sodium hydride (NaH) or sodium metal as a catalyst. The employment of sodium hydride instead of sodium metal is due to the mild reaction for the chitosan precursor. However, the reaction has to be carried out in a non-hydrolytic solvent. Normally, anhydrous benzene is used as a solvent. In the present work, to avoid the degradation of carbaryl, which occurs easily at high temperature and high



Scheme 5. Proposed mechanism of carbaryl conjugation onto CA-CDI.

basicity, the reaction was cooled at the beginning and NaH was added gradually in a mild condition.

The successive drug conjugated compound also can be confirmed from the ester group belonging to CBR. The FT-IR spectrum (Fig. 5) shows the shoulder around 1700 cm^{-1} and the peaks of pyranose rings still remain. This confirms that there is no degradation of chitosan, even after the reaction that has NaH as a catalyst. Comparing Fig. 4 with Fig. 5, the curve fitting indicates the sharp peaks at 1707 and 1648 cm^{-1} owing to the ester of carbamate and tertiary amide, respectively.

The TGA diagram of CHI-CBR shows weight loss at 221.5°C referred to thermal degradation. This indicates the crystalline parts of chitosan polymer drug are less than that of the chitosan starting material. In this case, the bulky groups of the drug molecules may interfere with the molecular packing leading to the decrease of thermal stability.

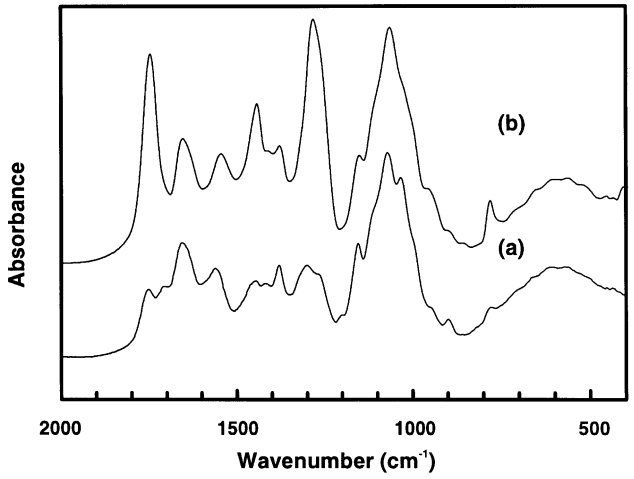


Fig. 6. FT-IR spectra of (a) CA-CDI, and (b) CA-CDI-CBR.

The XRD pattern of CHI-CBR shows two broad peaks at 2θ of 15 and 23°. This confirms that the introduction of carbaryl in the chitosan main chain was successful and, as a result, the packing structure changed.

3.2.2. Synthesis of chitosan conjugated drug type 2: carbonyl imidazolide chitosan acetate-carbaryl (CA-CDI-CBR)

Due to the low activity of the secondary amide of the carbamate ester, the reaction for carbaryl conjugated with chitosan via spacer cannot be achieved easily even with the high reactivity of chitosan acetate carbonyl imidazolide. To overcome this problem, sodium hydride was applied as a catalyst (Fones, 1949) for changing carbaryl into a reactive nucleophile before reacting with chitosan imidazolide precursor as in the mechanism shown in Scheme 5.

Fig. 6 shows the FTIR of CA-CDI before and after conjugation with carbaryl. The absorbance at 1752 cm^{-1} of imidazolide is decreased after the reaction, whereas the new peak belongs to carbamate ester at 1708 cm^{-1}. The characteristic peaks of pyranose rings remaining indicated that the reactive ester CA-CDI was partially substituted by carbaryl without degradation of the chitosan main chain.

Thermal stability studies implied that CA-CDI-CBR gave a wide range of degradation temperature from 220 to 300°C. Compared to Type 1, Type 2 showed a higher degradation temperature, somewhat closer to that of chitosan. This may be related to the partial hydrogen bonding between the ester moiety and the chitosan main chain in the system.

4. Conclusions

Chitosan conjugated carbaryl insecticide as a polymer drug was achieved by two approaches. CHI-CBR (Type 1) was obtained by coupling carbaryl directly onto chitosan at

C-6 position via iodochitosan precursor. Type 2 was prepared by conjugation carbaryl via reactive ester spacer obtained from *N*,*N*′-carbonyldiimidazole coupling agent. The conjugation of carbaryl onto chitosan effected the changing of packing structure, which induced a decrease in thermal stability. In the future work, we are studying the release behavior of the conjugated carbaryl, which is expected to be the drug stability enhancement model for Type 1 and to be the drug delivery model for Type 2.

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