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Review

Synthetic methods and applications of chitosan containing pyridylmethyl moiety and its quaternized derivatives: A review

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ABSTRACT

Chitosan is a natural polysaccharide with non-toxic, biodegradable, and biocompatible properties. Subsequently, there has been much interest in chitosan and it has been widely used in many fields. However, its applications have only been shown in acidic medium because of its poor solubility in neutral and basic pH. To enhance the solubility, the physicochemical and biological properties and application, chemical modifications of chitosan were made. Quaternization is a popular means of modifying chitosan resulting in a water-soluble chitosan derivative with a wide pH range including neutral and basic conditions. This is due to a permanent positive charge on the polymer backbone. The *N*-arylation of chitosan, particularly the aromatic containing nitrogen atom, has gained increasing attention because it not only has a hydrophobic character, but it is also a hydrophilic and nucleophilic characters at nitrogen atom. Therefore, the focus was on the recent synthetic methods and applications of *N*-pyridylmethyl chitosan and its quaternized derivatives in this review. Moreover, the quaternization of chitosan and its derivatives with different synthetic routes and quaternizing agents were reviewed.

1. Introduction

Chitin is the second most abundant natural polysaccharide, generally found in the composition of crustacean shells, insects, molluscan organs, and fungi. It consists of β-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) as a repeating unit (Peter, 2005). The deacetylation of chitin yields chitosan, which is actually a copolymer of GlcNAc and β-(1 → 4)-2-amino-2-deoxy-D-glucopyranose (GlcN) with a GlcN content greater than 50% (Muzzarelli, 1997). The chemical structures of chitin and chitosan are shown in Fig. 1. Chitosan is obtained from chitin by alkaline hydrolysis with inorganic base, the process essentially hydrolyzes *N*-acetyl groups at random within the polymer backbone (Fig. 2). However, most commercially available samples of chitosan are not 100% deacetylated. Therefore, the chitosan is often represented by the degree of deacetylation (DDA). Chitin and chitosan have gained tremendous interest due to their properties as non-toxic, biocompatible and biodegradable polymers. Chitin is insoluble in water and almost all organic solvents while chitosan is soluble in dilute organic acid solutions such as acetic, formic, succinic, and lactic acids at pH below 6.5. Therefore, the applications of chitin and chitosan are limited due to less solubility in water and organic solvent. In order to improve the solubility and physicochemical and biological properties, several chemical modifications of chitosan have been reported (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Kurita, 2001; Mourya & Nazma, 2008).

Pyridine is a simple aromatic heterocyclic organic compound used as a precursor to agrochemicals and pharmaceuticals in addition to being an important solvent and reagent. Pyridine is a basic with chemical properties similar to tertiary amines. Therefore, it is easily attacked by alkylating agents to give *N*-alkylpyridinium salts (Fujimoto et al., 2006). Pyridine and its derivatives are widely used in many fields such as a ligand in coordination chemistry (Mishra, Kaushik, Verma, & Gupta, 2008), a drug for anticancer activity (Onnis, Cocco, Fadda, & Congiu, 2009), an antimicrobial agent (Riahi, Wurster, Lalk, Lindequist, & Langer, 2009), a gene carrier (Vroman, Mazza, Fernandez, Jérôme, & Préat, 2007), and so on. Pyridine derivatives were introduced into the polymer backbone in order to improve the polymer properties including the solubility, physicochemical and biological properties. The introduction of pyridine derivatives into the chitosan backbone has recently gained interest because it can be applied in metal absorption (Baba & Hirakawa, 1992; Inoue, Ohto, Yoshizuka, Yamaguchi, & Tanaka, 1997; Rodrigues, Laranjeira, de Favere, & Stadler, 1998), antimicrobial activity (Badawy, 2008; Kumar, Dutta, & Dutta, 2009; Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2008a), gene delivery (Opanasopit et al., 2008; Sajomsang, Ruktanonchai, Gonil, Mayen, & Opanasopit, 2009b), sensor application (Bao & Nomura, 2002), and biomedical application (Kumar et al., 2009; Sajomsang, Rungsardthong Ruktanonchai, Gonil, & Nuchuchua, 2009c). In this paper, the synthetic methods of *N*-pyridylmethyl chitosan and quaternized chitosan are

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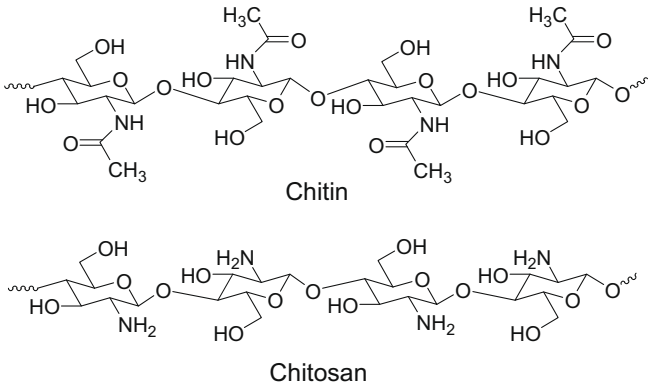


Fig. 1. Chemical structures of chitin and chitosan.

reviewed. Furthermore, the applications of *N*-pyridylmethyl chitosan and its quaternized derivatives are also summarized.

2. Synthesis of *N*-pyridylmethyl chitosan derivatives

N-Alkylation of chitosan is selectively carried out using a halogen displacement reaction or a reductive amination. The direct *N*-alkylation of chitosan was achieved by the reaction between the primary amino groups of chitosan and alkyl halide under heterogeneous conditions in the presence of a strong base (Fig. 3). This method involved vigorous reaction conditions such as high temperature and high sodium hydroxide concentration, resulting in a lower degree of substitution and much more degradation of molecular weight in the polymer backbone (Li, Liu, & Yao, 2002; Liu, Zhang, Sun, Sun, & Yao, 2003). Further selective *N*-alkylation and *N*-arylation were performed via Schiff bases intermediates. The reaction was done by reacting the primary amino groups of chitosan with aldehydes or ketones under homogeneous acidic conditions followed by reduction of the Schiff base intermediates with sodium borohydride or sodium cyanoborohydride (Fig. 4). Sodium cyanoborohydride is a versatile and mild reducing agent, ideal for reducing imines selectively in the presence of aldehydes or ketones. This makes it excellent for performing reactive aminations and this reaction is known as the Borch reaction. The reduction of the imine was performed using sodium cyanoborohydride which is more reactive and selective than the usual reducing agents, such as sodium borohydride and phenylselenol in alcoholic potassium hydroxide. The reduction of aldehydes and ketones is pH dependent, the reaction proceeding readily at pH 3–4 (Borch, Bernstein, & Durst, 1971). This is the advantage of this reducing agent which is stable in acidic media. Moreover, the reduction of imine by cyanoborohydride anion is rapid at pH values ranging from 6 to 7 and the reduction of aldehydes or ketones is negligible in this pH range (Desbrieres, Martinez, & Rinaudo, 1996). Therefore, Desbrieres et al. used this condition to synthesize the hydrophobic derivatives of chitosan. The reactive alkylation reaction was carried out by dissolving chitosan in 0.2 M acetic acid and then ethanol was added. The pH was adjusted to 5.1 before adding sodium

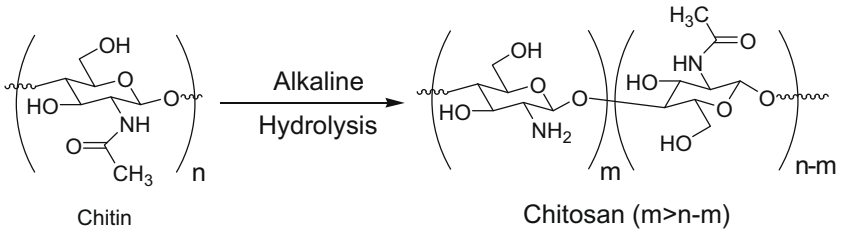


Fig. 2. Preparation of chitosan from chitin.

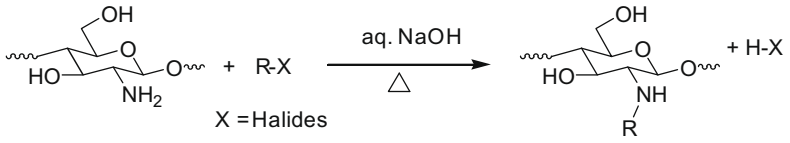


Fig. 3. Synthesis of *N*-alkyl chitosan derivatives by halogen displacement reaction.

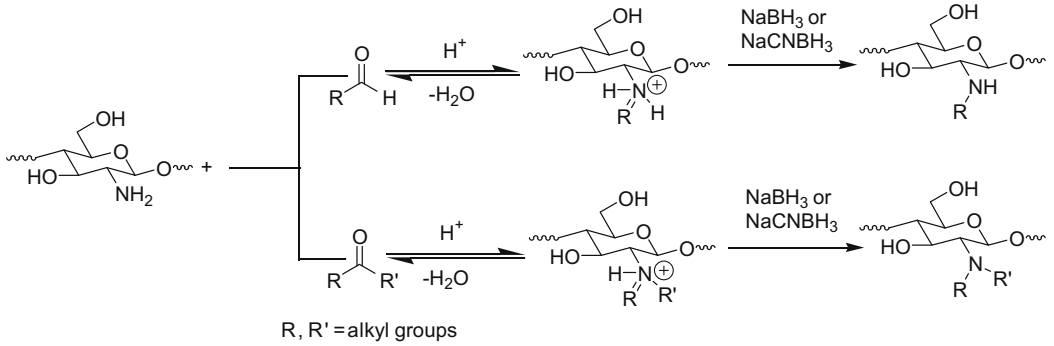


Fig. 4. Synthesis of *N*-alkyl chitosan derivatives by reductive amination reaction.

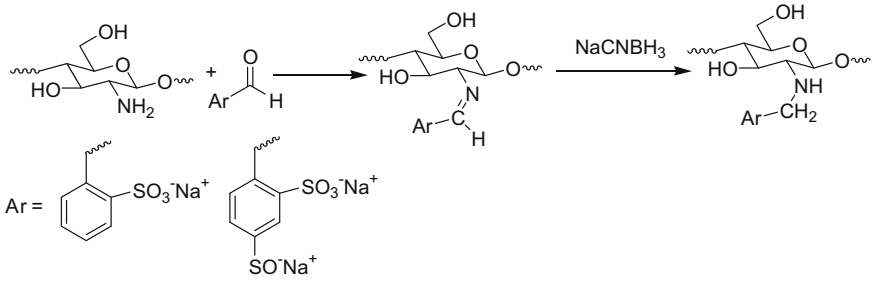


Fig. 5. Synthesis of *N*-benzyl sulfonated chitosan derivatives.

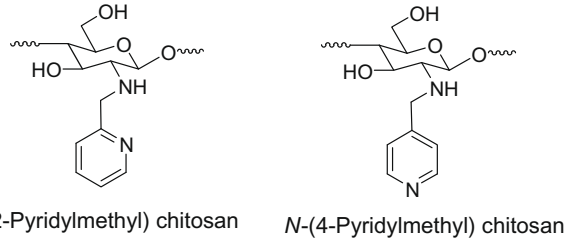


Fig. 6. Chemical structures of *N*-(2-pyridylmethyl) chitosan and *N*-(4-pyridylmethyl) chitosan.

cyanoborohydride in order to avoid the precipitation of the chitosan. In addition, an optimal reaction pH range between 4 and 8 was also suggested. The *N*-alkyl chitosan derivatives, one of the most important hydrophobic derivatives of chitosan, have been reported by several research groups (Desbrieres, 2004; Desbrieres et al., 1996; Keisuke, Satoko, Yasuhiro, & Manabu, 2002; Sashiwa & Shigemasa, 1999; Uragami, Kato, & Miyata, 1997). However, much less attention has been paid to the synthesis of *N*-aryl chitosan derivatives. The reductive arylation of chitosan with salicylaldehyde has been reported for metal absorption (Baba, Hirakawa, Yoshizuka, Inoue, & Kawano, 1994). Crini et al. synthesized *N*-benzyl sulfonated derivatives of chitosan (Fig. 5) and reported their one-dimensional and two-dimensional NMR spectra (Crini et al., 1997).

The reductive arylation of chitosan with pyridinecarboxaldehyde was first reported by Baba and Hirakawa (1992). The *N*-(2-pyridylmethyl) chitosan and *N*-(4-pyridylmethyl) chitosan were synthesized through Schiff base intermediate by Rodrigues et al. (1998). Chitosan was dissolved in ethanolic solution then 2- or 4-pyridinecarboxaldehyde (4 meq/GlcN) was added. The solution was refluxed for 24 h. The formation of the Schiff base was observed by the color change in chitosan solution. The *N*-(4-pyridylmethyl) chitosan was yellow, while the *N*-(2-pyridylmethyl) chitosan was purple. The *N*-pyridylmethyl chitosan derivatives were purified in Soxhlet with ethanol and acetone for the removal of excess aldehyde. Afterwards, the Schiff base intermediate was reduced with sodium cyanoborohydride for 24 h. The solids were washed with water to remove the excess sodium cyanoborohydride, filtered and dried at 60 °C for 4 h. The chemical structures of *N*-(4-pyridylmethyl) chitosan and *N*-(2-pyridylmethyl) chitosan are shown in Fig. 6. The degree of *N*-substitution (DS) of *N*-(4-pyridylmethyl) chitosan and *N*-(2-pyridylmethyl) chitosan was 0.85. Sashiwa and Shigemasa synthesized a series of *N*-alkyl and *N*-benzyl chitosan derivatives (Fig. 7) (Sashiwa & Shigemasa, 1999). The *N*-(4-pyridylmethyl) chitosan was synthesized by dissolving chitosan in lactic acid. The solution was diluted with methanol. 4-Pyridinecarboxaldehyde (3 meq/GlcN) was added to the diluted solution and stirred at room temperature. After standing the mixture for the prescribed time, sodium cyanoborohydride (4 meq/GlcN) was added and stirred at room temperature for 24 h. The precipitate was collected by filtration, dispersed in water, and the pH was adjusted to 10–12 with aqueous sodium hydroxide. The solution was then dialyzed with distilled water and lyophilized. The DS of *N*-(4-pyridylmethyl) chitosan was 0.72 and it showed a lower pH of the insoluble point compared with parent chitosan. *N*-(3-Pyridylmethyl) chitosan was first synthesized by Badawy (2008). The chitosan was dissolved into 1% (v/v) aqueous acetic acid. One equivalent of 3-pyridinecarboxaldehyde was dis-

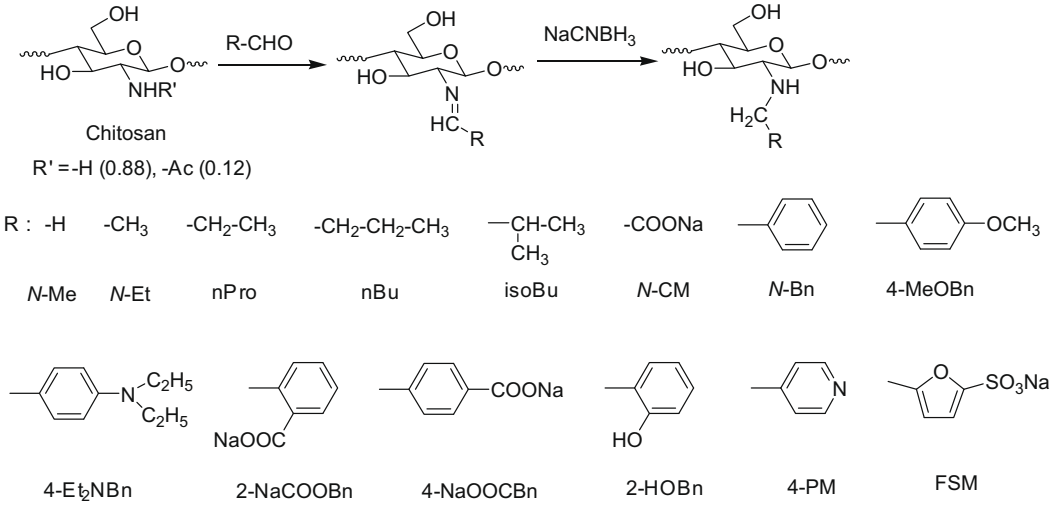


Fig. 7. Synthetic pathway of *N*-alkyl and *N*-benzyl chitosan with an aldehydes.

solved in methanol and added dropwise to the chitosan solution at room temperature. After 1 h of stirring, the pH of the solution was adjusted to 4.5 by adding 1 M sodium hydroxide solution. To this solution, 10% (w/v) sodium borohydride solution in water (1.5 meq/aldehyde) was added, and the solution was stirred for 1.5 h. Precipitates of *N*-(3-pyridylmethyl) chitosan were obtained by adjusting the pH of the solution to 10. The precipitate was washed with distilled water for neutralization and the unreacted aldehyde was Soxhlet-extracted with 1:1 (v/v) ethanol/diethyl ether for 2 days and then oven-dried overnight at 60 °C. Sajomsang et al. synthesized *N*-(4-pyridylmethyl) chitosan by varying the mole ratio of 4-pyridinecarboxaldehyde to chitosan (Sajomsang, Tantayanon, Tangpasuthadol, Thatte, & Daly, 2008b). The chitosan was first dissolved in 0.2 M acetic acid (pH 4). The solution was diluted with ethanol prior to the addition of aldehyde (0.05–0.5 meq/GlcN). The reaction mixture was stirred at room temperature for 1 h. At this point, the pH of the solution was adjusted to 5 by adding 1 M sodium hydroxide. Then, sodium cyanoborohydride (24.46 meq/GlcN) was added to the resulting solution. The solution was allowed to stir at room temperature for 24 h. After the pH was adjusted to 7, if precipitation occurred, the precipitate was continuously extracted (Soxhlet) by ethanol:ether (1:1, v/v) for 2 days and washed with ethanol several times, followed by acetone wash prior to drying at room temperature under nitrogen. In the case of no precipitation, the aqueous solution was dialyzed in distilled water for 4 days and then freeze-dried. They found that the DS of *N*-(4-pyridylmethyl) chitosan was in the range of 0.03–0.30 which depended on the mole ratio of an aldehyde. The mole ratio of an aldehyde increased with increasing DS. Kumar et al. synthesized two Schiff bases of *N*-heterocyclic chitosan derivatives (Fig. 8) (Kumar et al., 2009). Chitosan was dissolved in 1% (v/v) acetic acid and then it was filtered to remove the undissolved substances. Next, 7% (w/v) of 4-pyridinecarboxaldehyde and 2% (w/v) 2,6-pyridinedicarboxaldehyde in methanol were separately added to the chitosan solution and stirred until the chitosan solution turned into a more viscous gel. After that, the magnet bar was stopped. The prepared hydrogel was then subjected to solvent exchange into acetone, filtered and lyophilized for about 3 h.

The introduction of pyridylmethyl moiety into the chitosan backbone was successfully synthesized by reacting chitosan with pyridinecarboxaldehyde in mild acidic condition. The reaction easily occurred through the Schiff base intermediate and was followed by reduction with sodium cyanoborohydride or sodium borohydride. This was due to the homogenous reaction and higher reactive carbonyl group of aldehyde compared with carboxylic acid or acid chloride. Moreover, the obtained DS is dependent on the mole ratio of the pyridinecarboxaldehyde per the primary amino group, reaction time, temperature and the position of the N atom in the pyridinecarboxaldehyde.

3. Synthesis of quaternized chitosan

3.1. Quaternization of chitosan using iodomethane

Quaternization (methylation) of the primary amino groups of chitosan has been carried out using iodomethane in an alkaline solution of *N*-methyl pyrrolidinone (NMP). The quaternization is based on the nucleophilic substitution of the primary amino group on the C-2 position of chitosan with iodomethane and sodium iodide used as a catalyst. Muzzarelli et al. first prepared *N*,*N*,*N*-trimethyl chitosan iodide (TMI) by reacting *N*,*N*-dimethylated chitosan, which had previously been prepared by treating chitosan with formaldehyde followed by reduction with sodium borohydride, with iodomethane in acetonitrile at 35 °C for 30 h (Muzzarelli & Tanfani, 1985). The product was extracted with diethyl ether in a Soxhlet apparatus. The high degree of quaternization (DQ) was obtained as 60%, but it was not soluble in water. Domard et al. prepared the TMC by reacting chitosan, suspended in NMP, with iodomethane in the presence of sodium hydroxide and iodomethane at 36 °C for 3 h (Fig. 9) (Domard, Rinaudo, & Terrassin, 1986). The DQ with 64% was obtained after repeated quaternization. The chloride counter-ion was changed to an iodide one using ion-exchange resin in order to enhance the stability of the quaternized chitosan. It was noted that the DQ greater than 25% is soluble in water. Moreover, the chemical structure and various DQs of the TMC were determined using ^{1}H and ^{13}C-NMR spectroscopic techniques. They

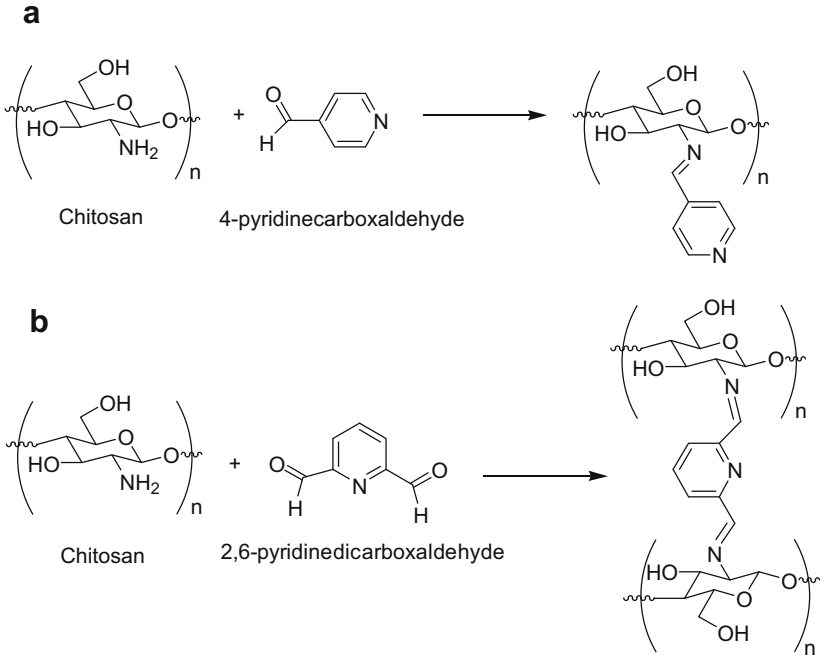


Fig. 8. Synthesis of chitosan Schiff base; (a) 4-pyridinecarboxaldehyde and (b) 2,6-pyridinedicarboxaldehyde.

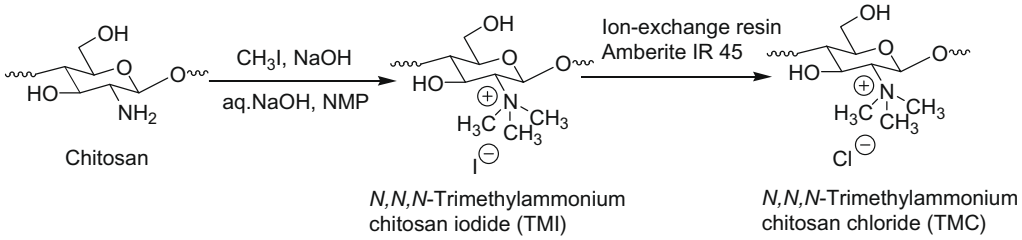


Fig. 9. Synthesis of *N*,*N*,*N*-trimethyl chitosan chloride.

found that not only were the primary amino groups quaternized, but so were the hydroxyl groups of chitosan (Domard, Gey, Rinaudo, & Terrassin, 1987). Dung et al. prepared the TMC by single treatment with iodomethane (Dung, Milas, Rinaudo, & Desbrieres, 1994). The procedure was similar to the method used by Domard et al. (1986) but it was different in sodium hydroxide concentration and the reaction time. The base concentration was changed from 1.4 M to 15% (w/v) at 60 °C while the reaction time was increased from 30 to 180 min. They found that the DQ with 53% was obtained without any detectable *O*-methylation. Misinterpretation concerning the ^{1}H-NMR spectrum of the TMC, particularly with respect to the chemical shifts attributed to the protons of *N*,*N*-dimethyl and *N*,*N*,*N*-trimethyl groups, was highlighted by Dung et al. (1994). Therefore, Sieval et al. proposed new assignments for the above-mentioned signals. They found that the execution of this single treatment with iodomethane produced a poorly water-soluble chitosan derivative with DQ ranging from 10% to 15% (Sieval et al., 1998). They also concluded that this derivative was mainly a *N*,*N*-dimethylated chitosan. Therefore, it was necessary to carry out repeated quaternization in order to obtain TMC with the DQ close to 60% which was completely soluble in water. Repeated quaternization through subsequent additional steps resulted in a still DQ higher than 85%, but it produced a poorly water-soluble TMC due to a large amount of *O*-methylation. Hamman and Kotźe studied the effects of the type of base, the amount of quaternization on the DQ and the molecular weight of the TMC (Hamman & Kotźe, 2001). The reaction was performed using 15% (w/v) sodium hydroxide as a base at 60 °C for 45, 15, and 30 min with single, double and triple treatments with iodomethane, respectively. Increases in the DQ ranged from 21% to 59% with an increase in the amount of quaternization. The intrinsic viscosity value showed that the dimethylaminopyridine used as a base did not cause polymer degradation compared to the sodium hydroxide. However, the disadvantage of the dimethylaminopyridine is that it provided low DQ ranging from 7% to 10%, even though the amount of quaternization was increased. In addition, the DQ can be slightly increased from 10% to 34% if the two bases, dimethylaminopyridine and sodium hydroxide, can be used together. Snyman, Hamman, Kotze, Rollings, & Kotźe (2002) synthesized the TMC with various conditions based on the methods of Sieval et al. (1998), and Hamman and Kotźe (2001). They found that the DQ was in the range of 22–59% depending on the number of the repeated reaction steps. The DQ was increased by increasing the number of the repeated reaction steps. Moreover, the decrease in intrinsic viscosity and the molecular weight of the starting chitosan correlated with the increase in the number of the repeated reaction steps. This was due to the effects of time, alkali and temperature. Curiti et al. studied the effect of the quaternization condition of chitosan, particularly in sodium hydroxide concentration. The reaction was performed at room temperature by fixing a volume of sodium hydroxide but varying the sodium hydroxide concentrations, 15%, 20%, 30%, and 40% (w/v) and time, 9 or 24 h (Curiti, Britto, & Campana-Filho, 2003). The average DQ was obtained ranging from 10% to 45%. The chemoselectivity of the *N*-methylation of chitosan was affected by adding excess sodium hydroxide and iodomethane. Therefore, *O*-methylation was favored when the larger excess of these reagents was used. Polnok et al. investigated the effects of the quaternization of the chitosan process and types of base (Polnok, Borchard, Verhoef, Sarisuta, & Junginger, 2004). A high DQ with low *O*-methylation was prepared by employing single treatment with iodomethane, single treatment with iodomethane and one subsequent addition, double treatment with iodomethane, and double treatment with iodomethane and one subsequent addition. The alkaline environment of the mixture reaction controlled all experiments. They found that the DQ higher than 75% was necessary to repeat the reaction steps. However, an increase in the number of the reaction steps provided high *O*-methylation which would decrease the aqueous solubility of the TMC. Runarsson et al. synthesized the TMC by changing the solvent system from NMP to *N*,*N*-dimethylformamide/water mixture (50:50) and performed the reaction without the aid of a catalyst-sodium iodide (Rúnarsson et al., 2007). The reaction was carried out using chitosan:iodomethane:sodium hydroxide in a molar ratio of 1:6 or 12:1.5 to 9 and varying the time from 0.5 to 48 h and temperature at 21, 50, and 75 °C. This significantly reduced *O*-methylation since *N*,*N*-dimethylformamide/water seems to lower the reactivity of the hydroxyl group enough to keep the *O*-methylation down. They found that the DQ was in the range of 0–74% depending on the reaction conditions accompanied by *N*-monomethylation, *N*,*N*-dimethylation and *O*-methylation. Based on this solvent system, they also recently claimed to get a high DQ ranging from 81% to 88% by the '*one pot*' synthesis procedure. The reaction was done by using chitosan:iodomethane:sodium hydroxide in a molar ratio of 1:6:3 at room temperature for 48 h. In addition, they suggested a protection group strategy for more selective *N*-quaternization (sequence of *N*-phtahloylation, *O*-tritylation, *N*-deprotection, *N*-methylation, and *O*-deprotection) (Rúnarsson, Holappa, Jónsdóttir, Steinsson, & Másson, 2008). Recently, Verheul et al. synthesized the TMC without *O*-methylation using two steps (Fig. 10) (Verheul et al., 2008). In the first step, a formic acid–formaldehyde methylation (Eschweiler–Clarke) was used to synthesize the *N*,*N*-dimethylated chitosan (DMC). The quaternization of the DMC was done by using iodomethane in NMP without the assistance of a catalyst for the last step. Moreover, they found that the molecular weight of the TMC slightly increased with increasing DQ, implying that no chain scission occurred during synthesis.

3.2. Quaternization of chitosan using glycidyl trimethylammonium chloride

Glycidyl trimethylammonium chloride (GTMAC) was selected as a quarternizing agent because it has a quaternary ammonium group itself. When a primary amino group at C-2 of chitosan reacted with GTMAC, the chain of quaternary ammonium group obtained was longer than that of the TMC. Lang et al. synthesized *N*-(2-hydroxy)propyl-3-trimethylammonium chitosan chloride (HPTC) by reacting chitosan with GTMAC, however, they did not

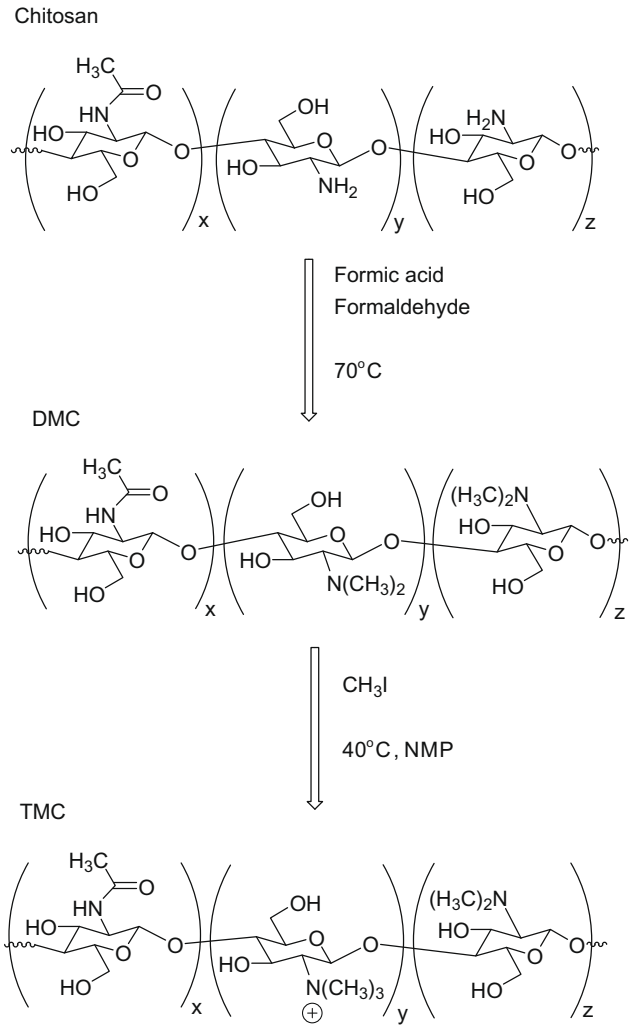


Fig. 10. Two-step synthetic pathway for the preparation of *N*,*N*,*N*-trimethyl chitosan avoiding *O*-methylation.

characterize the resulting derivatives (Lang, Wendel, & Konrad, 1990). Loubaki et al. synthesized and characterized GTMAC-modified chitosan (Fig. 11) (Loubaki, Ourevitch, & Sicsic, 1991). The structure of HPTC was confirmed using elemental analysis, IR, and NMR spectroscopic techniques. The reaction was performed in water at 60 °C for 15 h. The complete DQ was obtained by using the molar ratio of GTMAC:GlcN of chitosan as 6:1. They found that the *N*-monoalkylation was obtained under this condition. Daly and Manuszak-Guerrini developed a method for the synthesis of HPTC (Daly & Manuszak-Guerrini, 2001). Chitosan was quaternized using commercially available Quat-188 salt, 3-chloro-2-hydroxypropyl trimethylammonium chloride, under basic condition. This product was called chitosan Quat-188. Under this condition, Quat-188 readily generated the corresponding epoxide which reacted in both the primary amino groups and hydroxyl groups of the chitosan via a nucleophilic substitution pathway to introduce the quaternary ammonium substituent (Fig. 12). Moreover, Quat-188 has been used as etherifying agent in order to quaternize polysaccharides (Geresh, Dawadi, & Arad, 2000; Yu, Huang, Ying, & Xiao, 2007) including starch (Heinze, Haack, & Rensing, 2004) and cellulose (Hashem, Hauser, & Smith, 2003) under the catalytic action of sodium hydroxide. It is important to note that the sodium hydroxide concentration affects to the generation of the epoxide formed of Quat-188. If a high sodium hydroxide concentration is used, it will not only activate the polysaccharide but it will also hydrolyze Quat-188 and generate the diol at a high amount (Geresh et al., 2000; Song, Sun, Zhang, Zhou, & Zhang, 2008; Yu et al., 2007). Recently, Sajomsang et al. quaternized the *N*-aryl chitosan derivatives which contained different electron donating and electron withdrawing substituents using Quat-188 (Fig. 13) (Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2009d). The iodine was used as a catalyst and the pH of the reaction condition was controlled at 8 at room temperature for 48 h. In order to obtain complete quaternization, the reaction was heated up to 50 °C for 24 h. Even though the reaction was performed at room temperature and the pH was adjusted to 8, *O*-alkylation could be occurred in this condition. Nam et al. synthesized HPTC using GTMAC and then blended with polyacrylonitrile (Num, Kim, & Ko, 1999). The reaction was carried out using $Zn(BF_4)_2$ as a catalyst at 100 °C for 20 h. The complete DQ was obtained using the molar ratio of GTMAC:GlcN of chitosan as 4:1. Seong et al. synthesized *N*-(2-hydroxy)propyl-3-trimethylammonium chito-oligosaccharide chloride (HPTCOS) (Seong, Whang, & Ko, 2000). The reaction was performed in acetic acid at 80 °C for 18 h. The complete DQ was obtained using the molar ratio of GTMAC:GlcN of chito-oligosaccharide (COS) as 4:1. Kim et al. used a similar procedure to Seong et al. (2000) for the synthesis of HPTC but with different reaction conditions. In this case, the procedure was performed in water at 70 °C for 24 h (Kim, Nam, Choi, & Jang, 2003b). Kim, Lee, Lee, & Park (2003a) used a similar procedure to Kim et al. (2003b) for the synthesis of HPTCOS. Lim et al. synthesized HPTC by using GTMAC (Lim & Hudson, 2004). The reaction was performed in water at 85 °C with the GTMAC being added in three portions (7.1 mL each) at 2 h intervals over 10 h. The complete DQ was obtained using the molar ratio of GTMAC:GlcN of chitosan as 3:1. Li et al. synthesized HPTC for potential retention-aids in alkaline papermaking (Li, Du, Wu, & Zhan, 2004). The GTMAC was prepared from trimethylamine and concentrated hydrochloric acid at 4 °C and followed by adding epoxy chloropropane at 31 °C in a basic condition as shown in Fig. 14. The reaction was performed in aqueous sodium hydroxide (pH 9) at 75 °C for 8 h. The complete DQ was obtained using the molar ratio of GTMAC:GlcN of chitosan as 6:1.

3.3. Quaternization of chitosan using other quaternizing agents

Another alternative for the introduction of a quaternary ammonium group into the chitosan backbone has been reported. Britto et al. synthesized TMC by using a dimethylsulfate as the methylating agent. They suggested the dimethylsulfate is less expensive and less toxic than iodomethane. Moreover, it has a higher boiling point than iodomethane and no solvent is required for the reaction, unlike for NMP. The quaternization of chitosan was done in the mixtures of sodium hydroxide solution and sodium chloride and

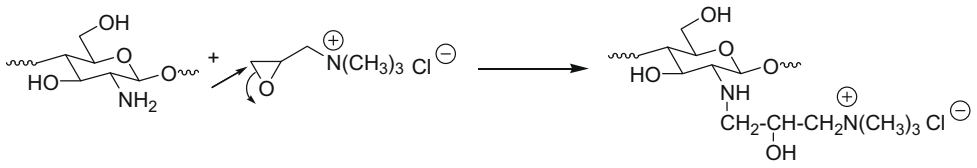


Fig. 11. Reaction of chitosan with glycidyl trimethylammonium chloride.

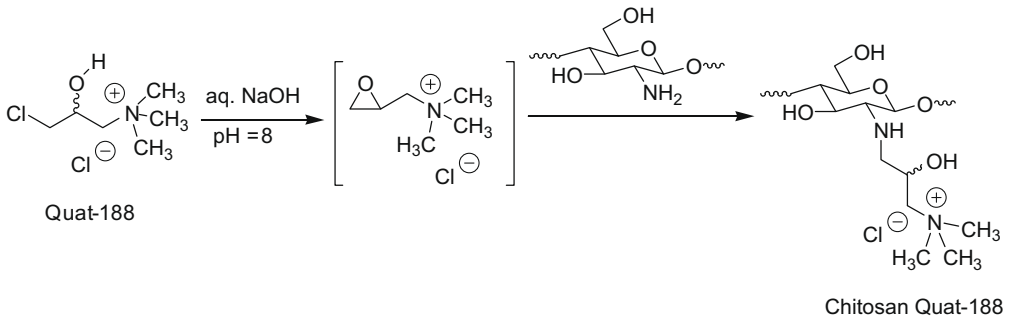


Fig. 12. Reaction of chitosan with 3-chloro-2-hydroxypropyl trimethylammonium chloride (Quat-188).

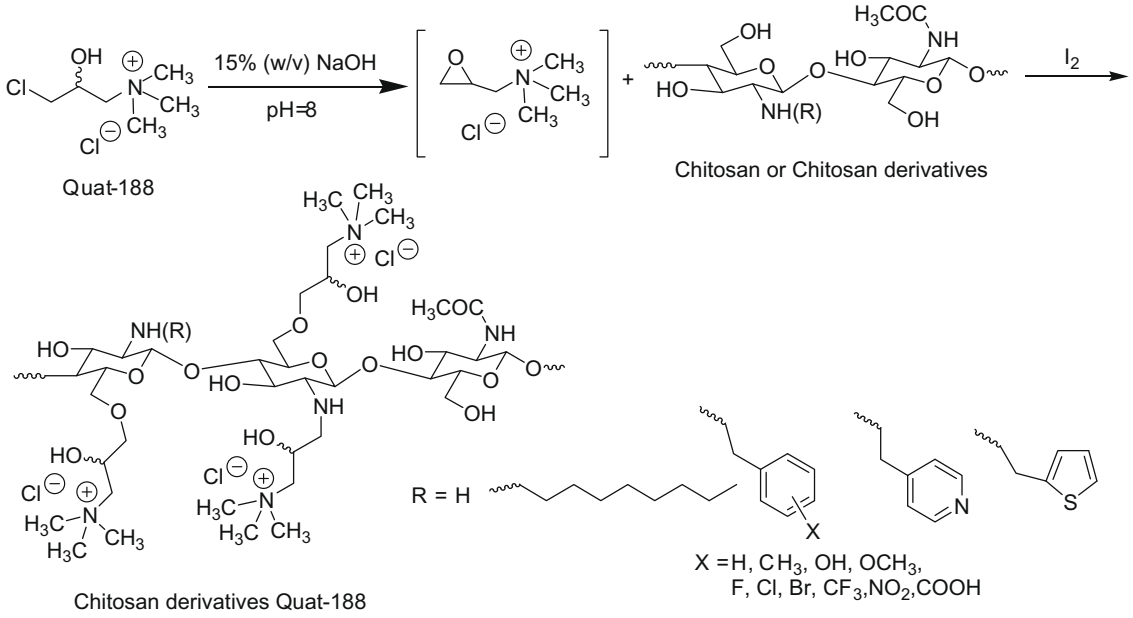


Fig. 13. Synthesis of quaternized chitosan and quaternized *N*-aryl chitosan derivatives using Quat-188.

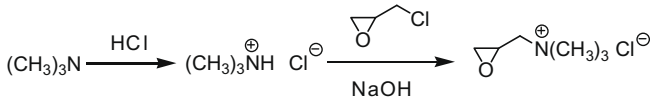


Fig. 14. Synthetic pathway of glycidyltrimethylammonium chloride.

refluxed with a methylating agent at room temperature or at 70 °C (De Britto & Assis, 2007). They found that the DQ was in the range of 15–52% depending on reaction time and temperature. Moreover, undesirable *O*-methylation and polymeric degradation were also observed to have taken place in the reaction.

The quaternization of chitosan was carried out using iodomethane in the presence of sodium hydroxide, sodium iodide, and NMP. There are various conditions and synthetic roughs for preparation of the TMC. Furthermore, the dimethylsulfate was proposed as another alternative methylating agent for the quaternization of chitosan. However, the TMC obtained from dimethylsulfate still has low DQ and provides the *O*-methylation. So far, researchers have tried to control the degree of quaternization, degree of *N*,*N*-dimethylation, degree of *N*-monomethylation, degree of *O*-methylation and molecular weight of the TMC in order to apply the TMC to the right job. In this review, the researchers were able to synthesize the TMC that they required by using the proper synthetic methods. In addition, it is important to note that molecular weight should not be ignored during the quaternization. The molecular weight might be increased or decreased after quaternization depending on various factors such as type and concentration of base, temperature, reaction time, the number of reaction steps, synthetic route, type of quaternizing agent and so on. Therefore, similar molecular weight should be compared beside similar DQ. Besides iodomethane, glycidyl trimethylammonium chloride (GTMAC), or 3-chloro-2-hydroxypropyl trimethylammonium chloride (Quat-188) was used to quaternize the chitosan with and without the catalyst in both acidic and basic conditions. The quaternized product has the longer chain length of the quaternary ammonium group than the TMC. This might have led to the differences in the physicochemical and biological properties. The chemoselectivity of quaternized chitosan prepared with the GTMAC was dependent on the pH in the reaction medium. The acidic condition was provided only in *N*-alkylation while the basic condition was provided in both *N*-alkylation and *O*-alkylation. The DQ was dependent on the mole ratio of the GTMAC or Quat-188 per primary amino group of chitosan, temperature, pH and the reaction time.

3.4. Quaternization of chitosan derivatives

Alkylation of TMC produces the amphiphilic polymeric molecules since it possesses both charged groups and non-polar linear hydrocarbon branches into chitosan backbone. Kim et al. prepared

quaternized *N*-alkyl chitosan derivatives containing alkyl substituents of different chain lengths (Kim & Choi, 2002; Kim, Choi, Chun, & Choi, 1997). The reaction was carried out in two steps: *N*-alkylation and quaternization. In the first step, the chitosan reacted with formaldehyde, butyraldehyde, *n*-octylaldehyde, and *n*-dodecylaldehyde. Then the resulting Schiff bases were reduced with sodium borohydride. In the last step, the *N*-alkyl chitosan derivatives were quaternized with iodomethane in the presence of sodium hydroxide as base and NMP (Fig. 15). Jia et al. synthesized quaternized *N*-alkyl chitosan derivatives by reacting chitosan with different molecular weights and propylaldehyde or furfuraldehyde (Jia, Shen, & Xu, 2001). The resulting Schiff bases were treated with sodium borohydride. The quaternized chitosan derivatives were obtained by the quaternization of *N*-alkyl chitosan derivatives with iodomethane. The yields, DQ and water solubility of the quaternized chitosan derivatives were influenced by the molecular weight of the initial chitosan. Avadi et al. prepared *N*,*N*,*N*-diethylmethyl chitosan chloride (DEMC) based on a modified two-step process (Avadi et al., 2004). The *N*-alkyl chitosan derivative was first prepared by introducing a methyl group from formaldehyde into chitosan via a Schiff base, followed by reducing with sodium borohydride. Finally, the *N*-methyl chitosan reacted with iodoethane to produce DEMC. Moreover, the *N*-triethylated chitosan chloride (TEC) was prepared using iodoethane as an ethylating agent based on a modified one-step process via a 2^2 factorial design (Avadi et al., 2003). It was found the DQ was in the range of 36–66% depending on the sodium hydroxide concentration and the amount of iodoethane. The optimized condition was 1.5% chitosan, 12% iodoethane, and 3.1% sodium hydroxide at 60 °C for 6 h, which provided the highest DQ. The trimethylated and triethylated 6-NH_2-6-deoxy chitosans were synthesized by Sadeghi et al. (2008). The 6-amino-6-deoxy chitosan was prepared in four steps, namely the phthaloylation, tosylation, amination, and deprotection of the phthaloyl group. Then methylation and ethylation were carried out at both C2 and C6 of the 6-amino-6-deoxy chitosan using iodomethane and iodoethane in the presence of sodium hydroxide and NMP, respectively. The DQs of the trimethylated and triethylated 6-NH_2-6-deoxy chitosans were 65% and 51%, respectively. The chemical structures of DEMC, TEC, trimethylated 6-NH_2-6-deoxy chitosan, and triethylated 6-NH_2-6-deoxy chitosan are shown in Fig. 16. Holappa et al. synthesized chitosan *N*-betainates with various degrees of substitution (Holappa et al. 2004). An efficient five-step synthetic route – *N*-phthaloylation, 6-*O*-triphenylmethylation, removal of the *N*-phthalimido moiety, addition of the *N*-betainate, and chitosan *N*-betainates – was developed for the full *N*-substitution of chitosan (Fig. 17). Previously, *N*-acylation of chitosan with betaine was performed in aqueous acidic solutions, but it did not obtain sufficient substitution degrees. To overcome this problem, an organo-soluble 6-*O*-triphenylmethyl chitosan intermediate was used as a starting material for *N*-acylation reactions to enable reactions in homogeneous reaction mixtures in organic solvents. This was due to the high DS and good control of the modification reaction. The DS was obtained in the range of 0.4–0.9 depending on the equivalents of *N*-chlorobetainyl chloride in the acylation reaction. Furthermore, novel quaternary ammonium chitosan

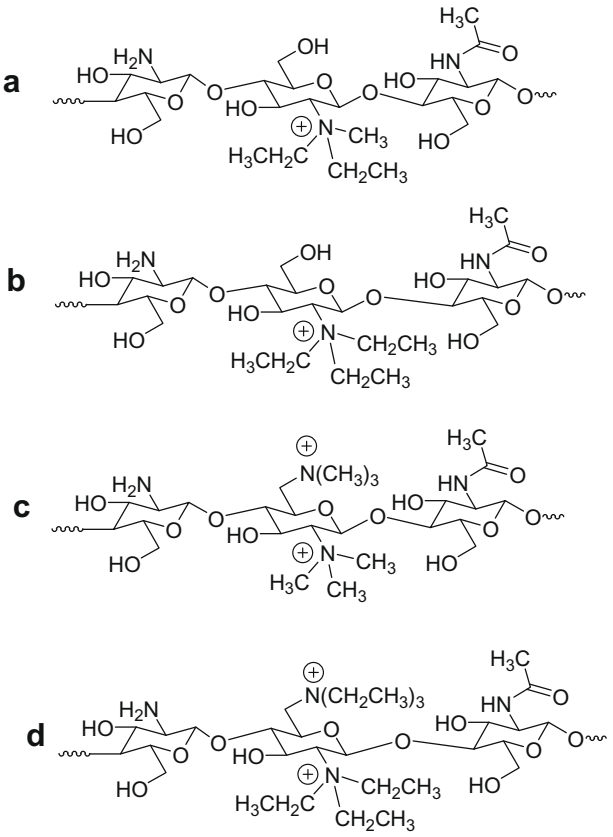
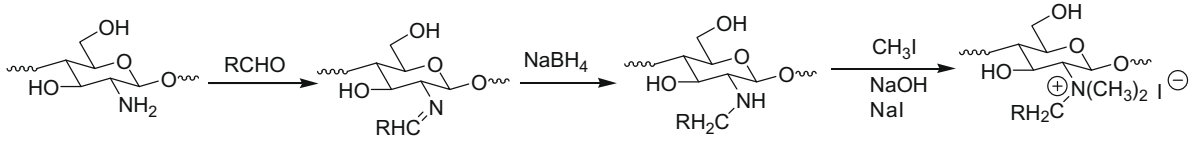


Fig. 16. Chemical structures of *N*,*N*,*N*-diethylmethyl chitosan (a), *N*-Triethylated chitosan (b), Trimethylated 6-NH_2-6-deoxy chitosan (c), and Triethylated 6-NH_2-6-deoxy chitosan (d).

derivatives were synthesized by the same group (Holappa, Nevalainen, Soininen, & Järvinen, 2006b). The *N*-chloroacyl-6-*O*-triphenylmethylchitosan was used as the starting material for the synthesis of the quaternary ammonium chitosan derivatives through reaction with four tertiary amines, pyridine, *N*-methylpyrrolidone, triethylamine, and tributylamine (Fig. 18). The quaternary piperazine derivatives of chitosan, monoquaternary *N*-[1-carboxymethyl-2-(1,4-dimethylpiperazinium)]chitosan chloride, monoquaternary *N*-[1-carboxymethyl-2-(4,4-dimethylpiperazinium)] chitosan chloride, and diquaternary *N*-[1-carboxymethyl-2-(1,4,4-trimethylpiperazi-1,4-dium)]chitosan dichloride, were synthesized in two ways (Holappa et al. (2006a). Firstly, 1,4-dimethylpiperazine reacted with the *N*-chloroacyl-6-*O*-triphenylmethylchitosan in the presence of potassium iodide and NMP under argon at 60 °C for 72 h (Fig. 19a). Secondly, 4-carboxymethyl-1,1-dimethylpiperazinium iodide or 1-carboxymethyl-1,4,4-trimethylpiperazi-1,4-dium diiodide reacted with 6-*O*-triphenylmethylchitosan using a coupling agent (Fig. 19b). They found that the quaternary ammonium moiety can be selectively inserted into either one or both of the piperazine nitrogens, yielding struc-



R = Methyl, Butyl, Octyl, and Dodecyl groups

Fig. 15. Synthesis of quaternized *N*-alkyl chitosan derivatives using iodomethane as quaternizing agent.

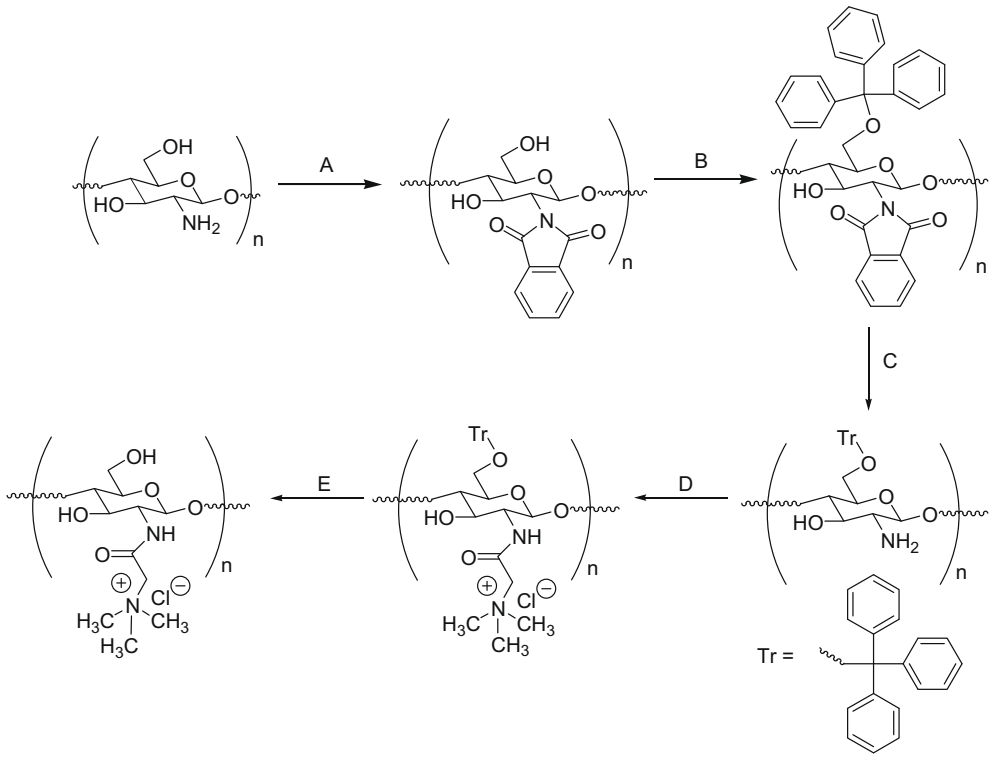


Fig. 17. Synthetic route for the preparation of chitosan *N*-betainates; (A) Phthalic anhydride, DMF/water, 120 °C, (B) Triphenylchloromethane, pyridine, 90 °C, (C) hydrazine monohydrate, water, 120 °C, (D) *N*-Chlorobetainyl chloride (1, 2, and 4 equivalents), pyridine, room temperature and (E) Aq. HCl, room temperature.

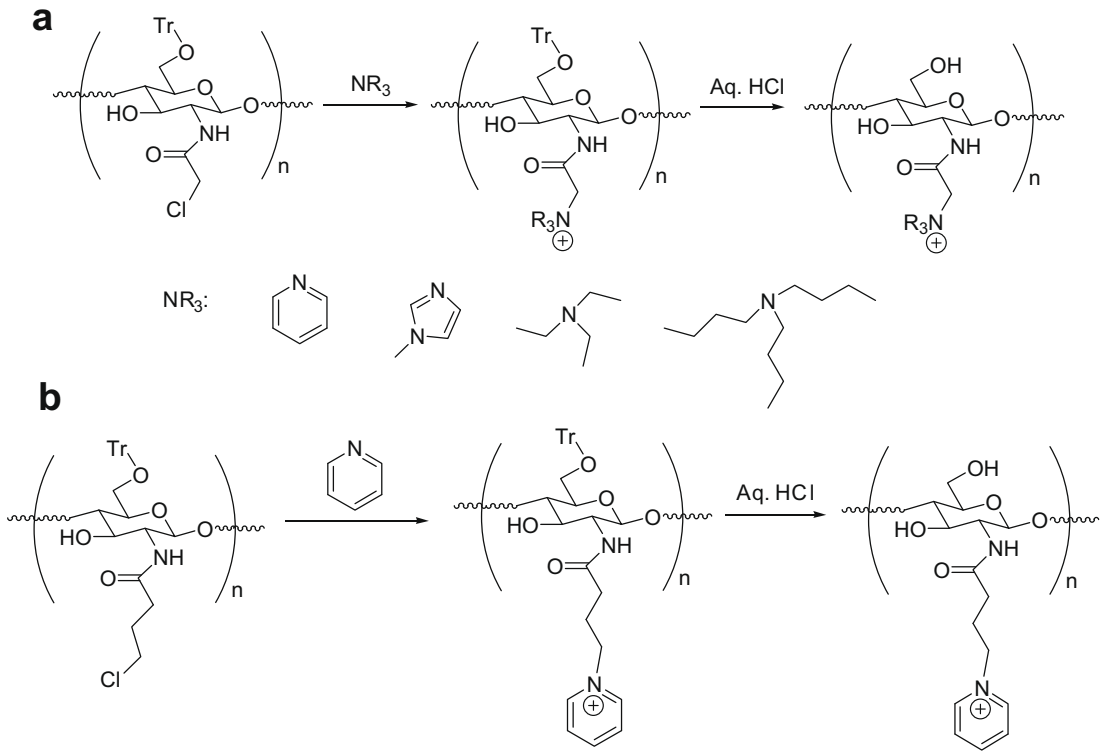


Fig. 18. Synthetic route for the preparation of various quaternary ammonium chitosan derivatives via *N*-chloroacyl-6-triphenylchitosans (a and b).

turally uniform chitosan derivative structures. Sajomsang et al. (Sajomsang, Gonil, & Saesoo, 2009a; Sajomsang et al., 2008a) synthesized quaternary ammonium chitosan containing aromatic moieties, particularly aromatics bearing *N*,*N*-dimethylaminobenzyl and *N*,*N*-dimethylaminocinnamyl groups, based on the method of Curiti et al. (2003). In addition, quaternized *N*-(4-pyridylmethyl) chitosan was also synthesized. Quaternization occurred among *N*,*N*-dimethylaminobenzyl, *N*,*N*-dimethylcinnamylamino, *N*-pyri-

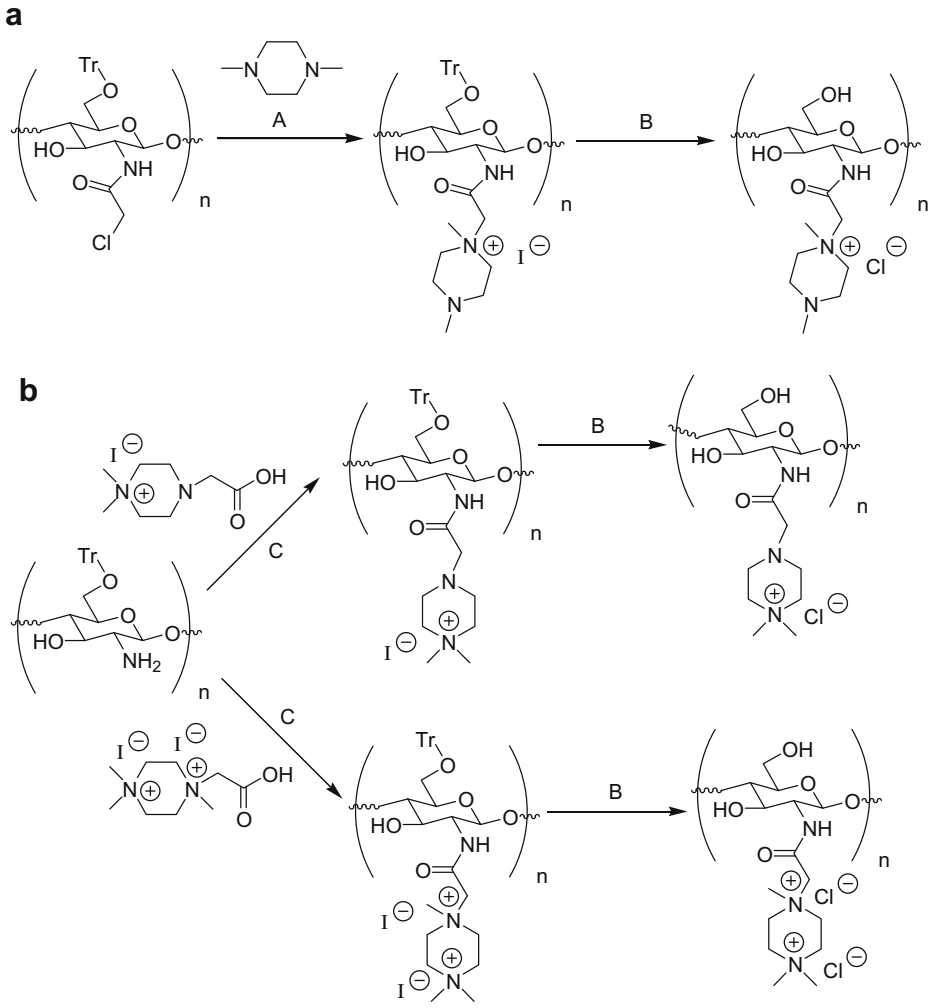


Fig. 19. Synthetic route for the preparation of quaternary piperazine derivatives of Chitosan; (A) KI, NMP, 60 °C, (B) Aq. HCl, room temperature, and (C) DCC, HOBt, NMP, room temperature.

dylmethyl groups and the primary amino groups of chitosan (Fig. 20). The total DQ of each chitosan derivative varied depending on the DS and the sodium hydroxide concentration used in quaternization. Increasing DS increased the total degree of quaternization while *N*,*N*-dimethylation and *N*-methylation at the primary amino group of chitosan decreased at higher DSs. Higher total DQ and degrees of *O*-methylation resulted when higher concentrations of sodium hydroxide were used. Zambito et al. synthesized *N*,*O*-[*N*,*N*-diethylaminomethyl(diethyldimethylene ammonium)*n*]methyl chitosan (Fig. 21) (Zambitoa, Uccello-Barrettab, Zainoa, Balzanob, & Di Coloa, 2006). Chitosan or chitosan hydrochloride microparticles were dissolved in aqueous hydrochloric acid or water. Then 2-diethylaminoethyl chloride (DEAE-Cl) and 15% (w/v) of sodium hydroxide were added in sequence to the chitosan solution under vigorous stirring at 60–65 °C. The molar ratio between DEAE-Cl and the chitosan unit was 2 or 4. After purification, no polymeric material was found in the internal phase when the DEAE-Cl/chitosan molar ratio of 2 was used. The DQ and the n value were dependent on the DEAE-Cl/chitosan repeating unit molar ratio. Previously, the reaction of chitosan with DEAE-Cl has been reported by Lee, Kim, and Kim (1999). However, a suspension of chitosan free base was used. Therefore, 18 h were required to obtain a derivatization even though a DEAE-Cl excess much larger than the present ones was used. This confirms the importance of starting from an acidic chitosan solution to speed up the derivatization. Numerous quaternization studies have been carried out with an alkyl iodide or glycidyltrimethylammonium chloride. Unfortunately, these reactions usually proceed at basic pH and relatively high temperatures, leading to a non-random distribution of quaternary groups into the chitosan backbone. Therefore, Cardile et al. synthesized water-soluble chitosan tetraalkylammonium salts, namely TMC, DEMC, *N*-carboxymethyl-*N*,*N*-dimethyl chitosan, and *N*-[*N*,*N*-diethylaminomethyl(diethyldimethylene ammonium)*n*]methylchitosan (Fig. 22) under power ultrasound using a closed sonochemical reactor (Cardile et al., 2008). The advantage of power ultrasound is that besides cutting down reaction times, the experimental procedure did not lead to blockwise modification.

4. Applications of *N*-pyridylmethyl chitosan and its quaternized derivatives

4.1. Metal absorption

Chitosan is a sorbent material because the primary amino groups on the polymer backbone serve as the coordination sites (Guibal, Saucedo, Jansson-Charrier, Delanghe, & Le Cloirec, 1994; Mitani, Fukumuro, Yoshimoto, & Ishii, 1991; Onsoyen & Skaugrud, 1990). The adsorption of transition metals on the chitosan is known to be mainly affected via coordination with the unprotonated amino groups (Monteiro & Airoldi, 1999). Chemical modifica-

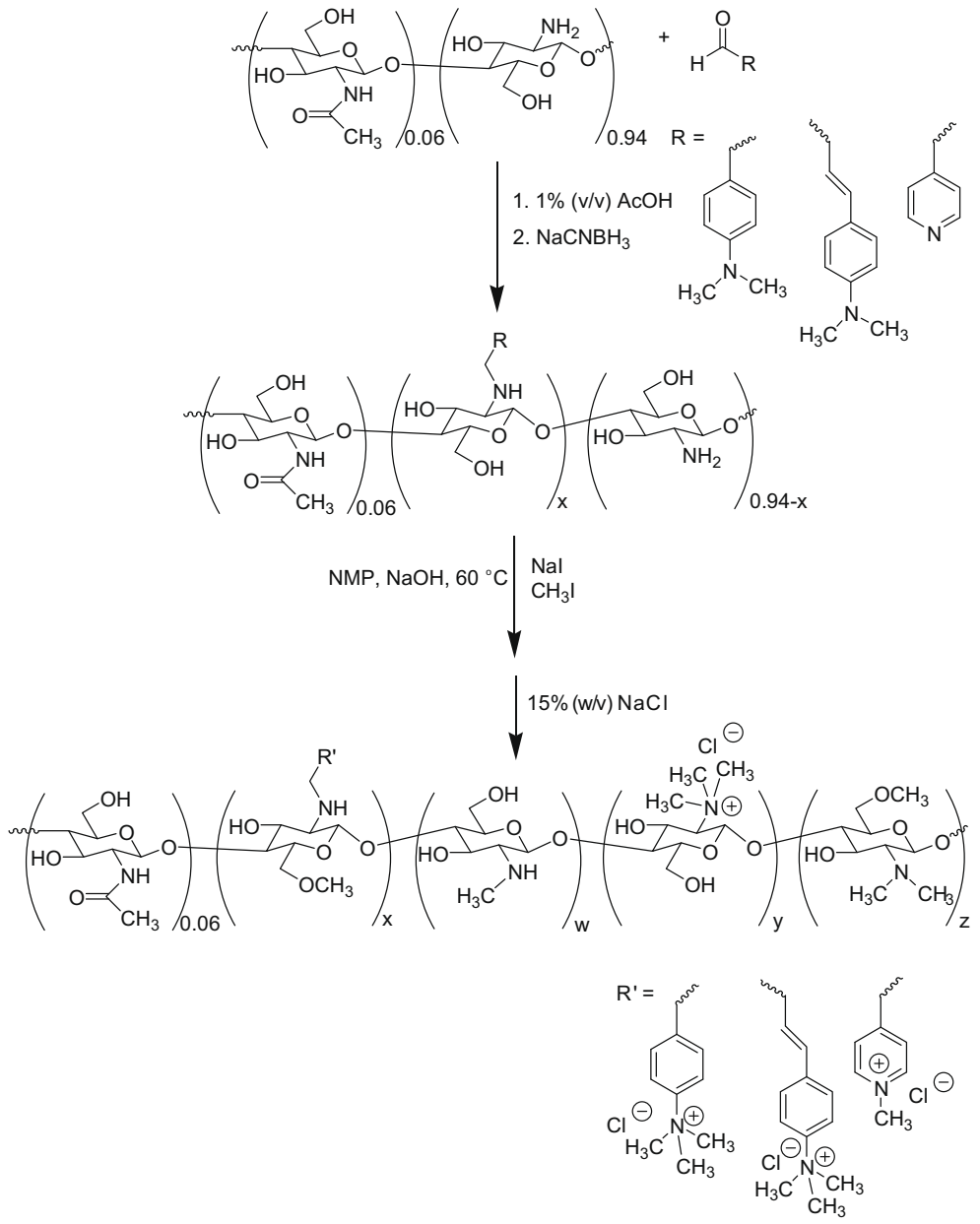


Fig. 20. Synthesis of the quaternary ammonium chitosan containing aromatic moieties.

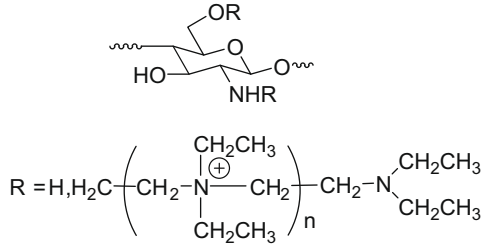


Fig. 21. Chemical structure of *N,O*-[*N,N* diethylaminomethyl(diethyldimethylene ammonium)*n*]methyl chitosan.

tions of chitosan such as carboxyalkyl-substitution, aldehyde-crosslinking, ligand-crosslinking, and polyamination are accessible to prevent it from dissolution in acidic media (pH < 2) or to enhance adsorption ability, or both (Guibal et al., 1994; Juang & Ju, 1997; Wu, Tseng, & Juang, 1999). The *N*-(2-pyridylmethyl) chitosan was selective for Pd(II) over base metals in a low pH range and for Cu(II) over iron in aqueous ammonium nitrate solutions (Baba & Hirakawa, 1992; Inoue et al., 1997). Moreover, the *N*-(2-pyridylmethyl) chitosan adsorbed selectively Ni(II) and Pd(II) which formed planar complexes at a lower pH compared to crosslinked chitosan (Baba, Masaaki, & Kawano, 1998a; Baba, Matsumara, Shiomori, & Kawano, 1998b). The metal ions, which form octahedral complexes such as Cd(II), Zn(II), and Co(II), were adsorbed at almost the same pH range as crosslinked chitosan, and Hg(II) could be selectively adsorbed on the *N*-(2-pyridylmethyl) chitosan in dilute hydrochloric acid solution (Baba et al., 1998a, 1998b). The results revealed that the *N*-(2-pyridylmethyl) chitosan has selectively adsorption of some transition metal ions than crosslinked chitosan and chitosan. Rodrigues et al. determined the selective capacity of chelating *N*-(2-pyridylmethyl) chitosan and *N*-(4-pyridylmethyl) chitosan to form complexes with Cu(II) (Rodrigues et al., 1998). The adsorption parameters *K* (adsorption constants Langmuir) and $\{R_T\}$ (maximum value of adsorbed Cu(II))

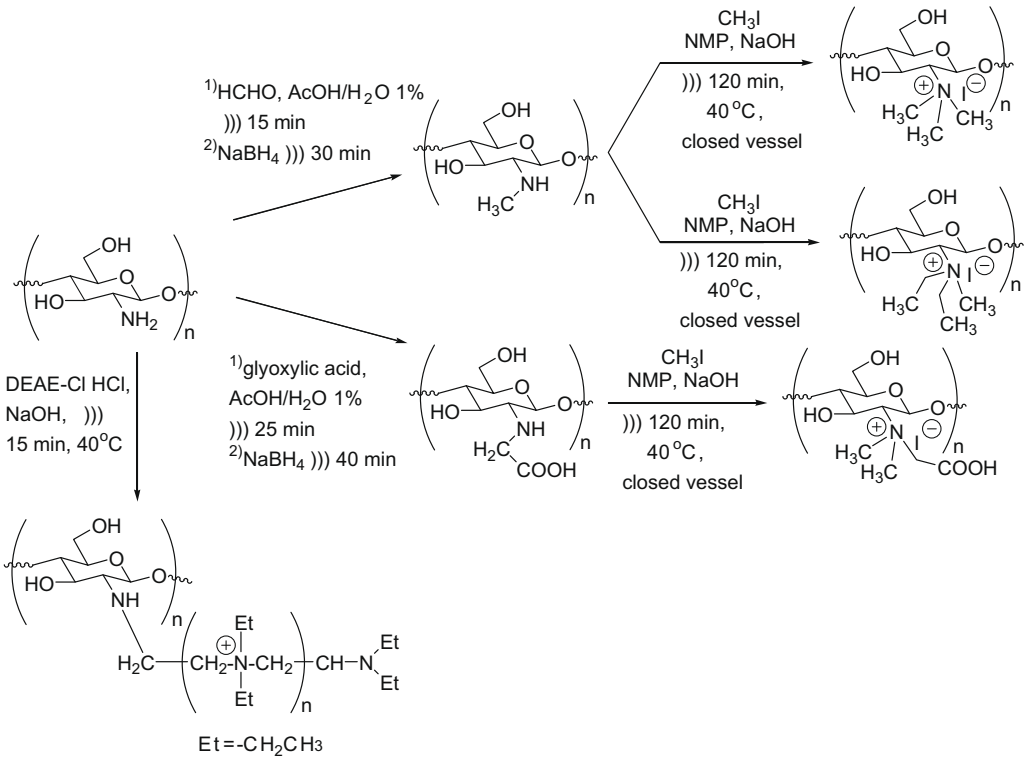


Fig. 22. Synthesis of *N*,*N*,*N*-trimethyl chitosan, *N*,*N*,*N*-diethylmethyl chitosan, *N*-carboxymethyl-*N*,*N*-dimethyl chitosan and *N*-[*N*,*N*-diethylaminomethyl(diethyldimethylene ammonium)*n*]methylchitosan under sonochemical conditions.

were calculated using Langmuir's equations by non-linear regression methods. They found that the highest value of Cu(II) adsorption was 1.63 ± 0.05 mmol/g for the *N*-(2-pyridylmethyl) chitosan and 0.71 ± 0.04 mmol/g for the *N*-(4-pyridylmethyl) chitosan. It was noted that the adsorption constant of the *N*-(2-pyridylmethyl) chitosan is about 2.5-fold higher than that of the *N*-(4-pyridylmethyl) chitosan. This was due to the difference of the nitrogen position at the pyridinic ring since the DS is the same in the two polymers. The structures of Cu(II)–*N*-(2-pyridylmethyl) chitosan complex and Cu(II)–*N*-(4-pyridylmethyl) chitosan complex are shown in Fig. 23. The *N*-(2-pyridylmethyl) chitosan is a *N*,*N*-bidentate ligand (Fig. 23a), while the *N*-(4-pyridylmethyl) chitosan is a monodentate one (Fig. 23b). The presence of bidentate ligand in *N*-(2-pyridylmethyl) chitosan makes the complex formed between Cu(II) ion and the polymer more stable than the complex formed between the Cu(II) and *N*-(4-pyridylmethyl) chitosan. Hu et al. immobilized the Co(II) complex of bis(salicylideneethylene diamine) (CoSalen) on the *N*-(4-pyridylmethylidene) chitosan (Fig. 24) used as a catalyst for the oxidation of 3,4-dihydroxyphcnylalanine using oxygen as an oxidizer (Hu, Cui, Dong, & Fang, 2001). They suggested that immobilization of CoSalen onto *N*-(4-pyridylmethylidene) chitosan may be realized through the binding of pyridyl group in the *N*-(4-pyridylmethylidene) chitosan to cobalt in CoSalen. The sixth coordination site of *N*-(4-pyridylmethylidene) chitosan–CoSalen may be occupied by molecular oxygen when it is exposed to air. Furthermore, the *N*-(4-pyridylmethylid-

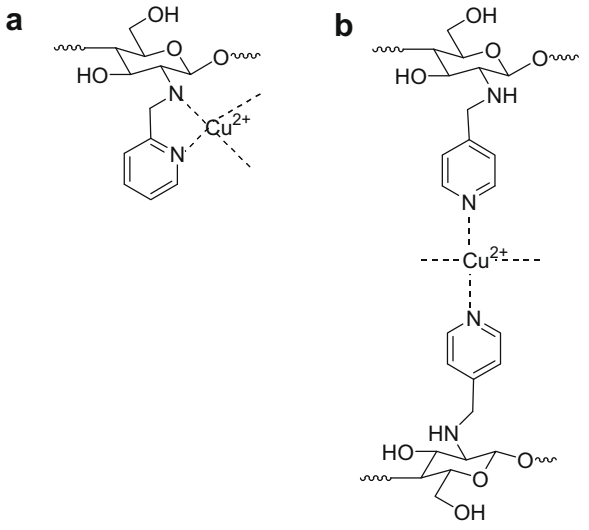


Fig. 23. Structures of Cu(II)–*N*-(2-pyridylmethyl) chitosan complex (a) and Cu(II)–*N*-(4-pyridylmethyl) chitosan complex (b).

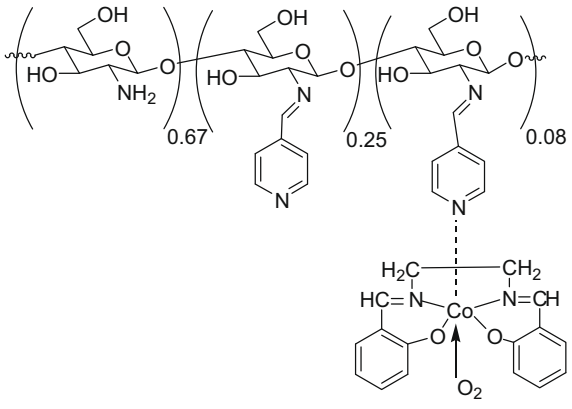


Fig. 24. Structure of Co(II) complex of bis(salicylideneethylene diamine) on the *N*-(4-pyridylmethylidene) chitosan.

ene) chitosan supported cobalt complex is more efficient than any of the controls including CoSalen, chitosan, and CoSalen with pyridine in the catalysing oxidation of 3,4-dihydroxyphcnylalanine. Rodrigues et al. prepared and characterized the complex formed between pentacyanoferrate(II) and *N*-(4-pyridylmethylidene) chitosan (Fig. 25), that can be used in sensor and biosensor electrochemistry (Rodrigues, Laranjeira, Stadler, & Drago, 2000). Dhakal et al. synthesized the *N*-(2-pyridylmethyl) chitosan and then complexed it with Ni(II) and Cu(II) to imprint their own planar geometry, followed by crosslinking with 2-(chloromethyl)oxirane (Fig. 26) (Dhakal, Oshima, & Baba, 2008). The corresponding metal ions were finally stripped out by hydrochloric acid and ethylenediaminetetraacetic acid (EDTA)/hydrochloric acid, respectively. The imprinted *N*-(2-pyridylmethyl) chitosan adsorbed to the corresponding metal ions at a much lower pH than the crosslinked *N*-(2-pyridylmethyl) chitosan does. In addition, the *N*-(2-pyridylmethyl) chitosan has also enhanced the planarity recognition properties of the imprinted materials as suggested by the selective adsorption of Pd(II), Au(III), Cu(II), and Ni(II) away from other metal ions compared to non-imprinted *N*-(2-pyridylmethyl) chitosan.

4.2. Antimicrobial activity

Native chitosan exhibits much more pronounced activity compared to chitin (Chen, Liau, & Tsai, 1998). This is due to the greater availability of primary amino groups in chitosan. Under mildly acidic conditions (pH < 6.5), the primary amino groups of chitosan can be protonated to form a positive charge. Chitosan has shown excellent antimicrobial properties (Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003) and it is generally more active against Gram-positive and Gram-negative bacteria than its corresponding monomers. This effect is believed to be due to the adsorption of the polymers onto the bacterial cell surface and membrane with subsequent disruption of membrane integrity. The monomer of chitosan, 2-amino-2-deoxy-D-glucopyranose as its hydrochloride salt, was demonstrated. It did not exhibit any antibacterial activity against several bacteria, including *Escherichia coli* and *Staphylococcus aureus* (Tanigawa, Tanaka, Shashiwa, Saimoto, & Shigemasa, 1992). Furthermore, chitosan has several advantages over other types of disinfectants because it possesses a higher antimicrobial activity, a broader spectrum of activity, a higher killing rate, and a lower toxicity toward mammalian cells (Franklin & Snow, 1981). Generally, the antimicrobial action is influenced by intrinsic factors such as the type of chitosan, the degree of chitosan polymerization, the host, the natural nutrient constituency, the chemical or nutrient composition of the substrates, and the environmental conditions such as substrate water activity or moisture (Rabea et al., 2003). To date, the exact mechanism of the antimicrobial action of chitosan and its derivatives is still debatable. However, the mechanism that appears to be the most viable candidate is the disruption of the cell membrane. Interaction between a positively charged chitosan backbone and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents (Chung, Su, Chen, & Jia, 2004; Helander, Nurmiabo-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Je & Kim, 2006; Liu, Du, Wang, & Sun, 2004; Muzzarelli et al., 1990). This evidence can be confirmed by scanning with an electron microscope (Chung & Chen, 2008; Muzzarelli et al., 1990). However, chitosan showed antimicrobial activity only in acidic medium because of its poor solubility in neutral and basic pH. To increase the solubility in water, a quaternary ammonium moiety was introduced into the chitosan backbone. The quaternary ammonium chitosan derivative which is a new derivative of chitosan can then be resolved in physiological condition. TMC is one of the original water-soluble chitosan derivatives soluble over a wide pH range (Domard et al., 1986; Muzzarelli & Tanfani, 1985). TMC has been shown to be an effective agent against an array of common bacteria (Gram-positive and Gram-negative) as well as fungi (Muzzarelli, 1997; Thorsteinsson et al., 2003). Besides the positive charge of the chitosan and its quaternized derivatives, the hydrophobicity was effective on the antibacterial activity. Even though permanent positive charges may provide an impetus for antibacterial activity, the amphiphilic nature of the bacterial cell wall is important in enhancing the hydrophobic–hydrophobic interaction between the bacterial cell wall and the chitosan derivative that contained hydrophobic moiety. Kim et al. found that the antibacterial activity increased with the increasing chain length of the alkyl substituent on the quaternized chitosan derivatives. These results clearly demonstrated that hydrophobicity and cationic charge play an important role on the antibacterial activity of quaternized chitosan derivatives. Furthermore, the antibacterial activity of quaternized chitosan derivatives in acetic medium was stronger than in water (Kim & Choi, 2002; Kim et al., 1997). Their antibacterial activities increased as the concentration of acetic acid was increased. *N*-(3-pyridylmethyl) chitosan showed fungicidal activity in acetic acid solution against soil-borne pathogenic fungi, *Pyricularia grisea*, *Pythium debrianum*, and *Fusarium oxysporum*, with half maximal effective concentrations (EC_{50}) of 2.18, 0.80, and 2.23 mg/mL, respectively (Badawy, 2008). Moreover, the *N*-(3-pyridylmethyl) chitosan showed a growth inhibition and an antifeedant against the larvae of the cotton leafworm, *Spodoptera littoralis*, since the concentration measured 3 mg/g. When compared with chitosan, the *N*-(3-pyridylmethyl) chitosan showed higher fungicidal and insecticidal activities. However, *N*-(3-pyridylmethyl) chitosan showed no effect on the larval mortality of *S. littoralis* at concentrations ranging from 1 to 5 mg/g. It was suggested that this might be due to the effect of the typically intoxicated larvae and the normal ecdysis process on the inhibition of feeding and weight gain, and the fact that the larvae were very small compared to the control. Moreover, an incomplete shedding of the old cuticle was also affected. However, the mechanism of action in insects for the *N*-(3-pyridylmethyl) chitosan still remains unknown. Previously, Rabea et al. synthesized the *N*-alkyl and *N*-aryl chitosan derivatives, which could be useful as biologically active substances against pest insects and fungi, and showed moderate to good insecticidal activity. It is important to note that the *N*-alkyl chitosan derivatives had lower activity than *N*-aryl derivatives. This is probably due to the more hydrophobic properties of the aryl substituents (Rabea et al., 2005, 2006). Kumar et al. reported that the chitosan Schiff base, chitosan-4-pyridinecarboxaldehyde derivative and chitosan-2,6-pyridinedicarboxaldehyde derivative, showed antibacterial and antifungal activities in acetic acid solution against *E. coli* (ATCC 9637), *Pseudomonas aeruginosa* (ATCC BAA-427), *S. aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 27736), and *Candida albicans*, *Cryptococcus neoformans*, *Sporothrix schenckii*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, and

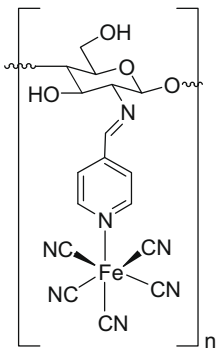


Fig. 25. Structure of [*N*-(4-pyridylmethylidene) chitosan$Fe(CN)_5]^{3-}$.

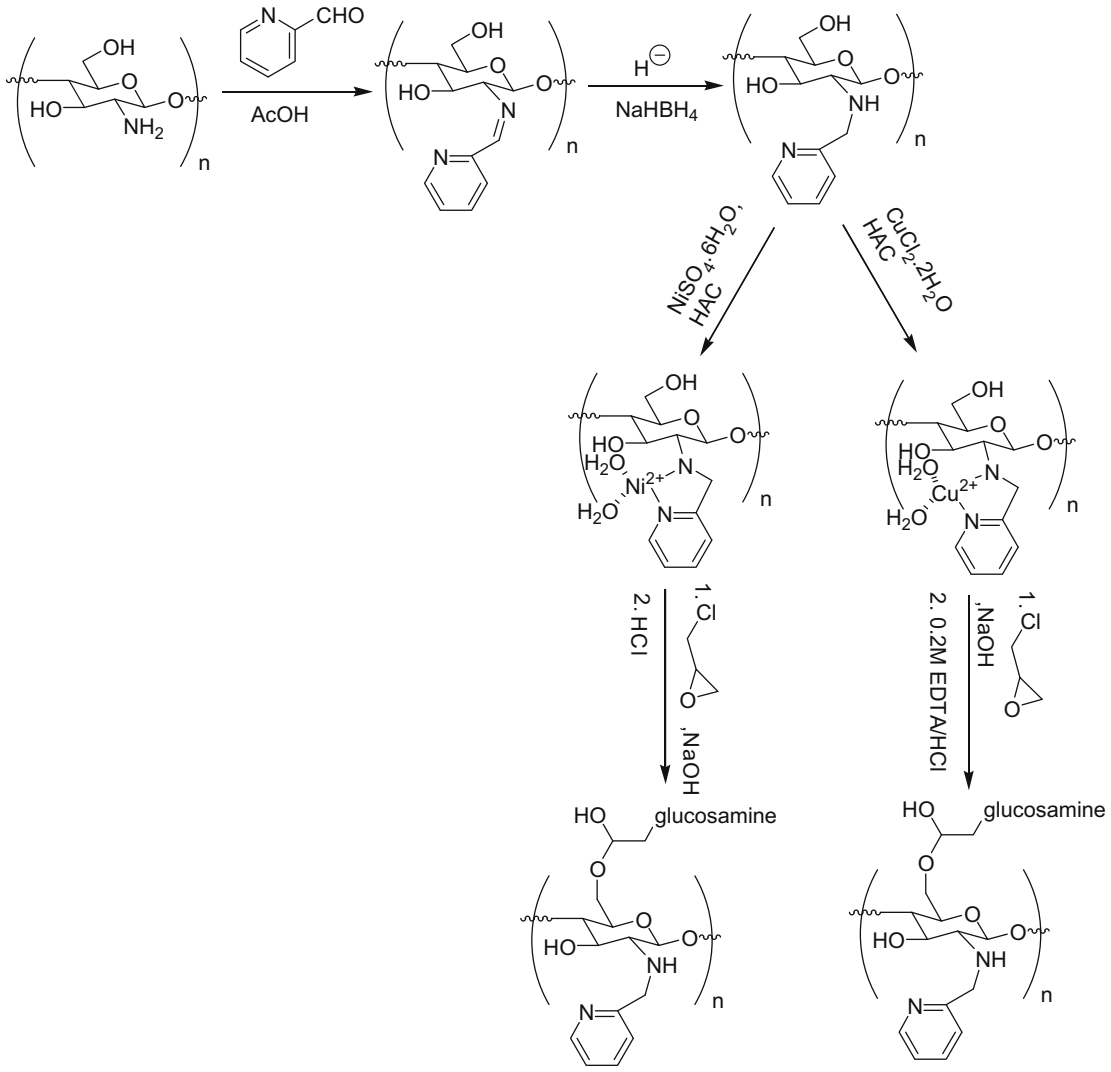


Fig. 26. Synthetic scheme for imprinting of metal ions onto *N*-(2-pyridylmethyl) chitosan.

Candida parapsilosis (ATCC 22019) with minimum inhibitory concentration (MIC_{50}) ranging from 50 to >50 μg/mL (Kumar et al., 2009). Sajomsang et al. reported that quaternized *N*-(4-pyridylmethyl) chitosan chloride (QPyMeC) showed antibacterial activity against *S. aureus* and *E. coli* bacteria in water with MIC_{90} ranging from 64 to 128 μg/mL (Sajomsang et al., 2008a). The presence of the *N*-pyridylmethyl substituents on the quaternized chitosan backbone did not enhance the antibacterial activity against *S. aureus* compared to TMC. They suggested that this was attributed to the effect of the chemical structure and the reduction of molecular weight after quaternization (Sajomsang et al., 2009a).

4.3. Gene delivery

Gene therapy is the insertion of genes into an individual's cells and tissues to treat a disease by replacing defective genes, substituting missing genes, or silencing unwanted gene expression. Basically, there are two approaches to gene delivery: viral and non-viral. Viral delivery is the more conventional approach because viruses have evolved to infect cells with high efficacy. Non-viral gene delivery systems make use of physical methods or a synthetic chemical vector or both to deliver the gene of interest. Because of limitations with viral gene delivery such as small cargo capacity, resistance to repeated infection, difficulty in production and quality control, and low safety, non-viral gene delivery can be potentially overcome. To date, numerous carriers have been studied as potential non-viral gene delivery vectors to enable improved DNA stability and uptake including inorganic surfaces, cationic lipids, polysaccharides, cationic polymers, and dendrimers (Merdan, Kopeček, & Kissel, 2002; Putnam, 2006). These carriers either bind to, complex with, or encapsulate DNA into systems that are comparatively easier to manufacture and scale-up than viral systems, although they have orders of magnitude lower efficacy. Chitosan is biocompatible, biodegradable, and non-toxic; therefore, it has been proposed as a safer alternative to other non-viral vectors. The main drawback of chitosan is its poor water solubility at physiological pH, and its low transfection efficiency. Several chitosan derivatives have been synthesized in the last few years to obtain a modified carrier with altered physicochemical characteristics. In order to improve the water solubility and gene transfection efficiency, chemically modified chitosan, such as TMC (Thanou, Florea, Geldof, Junginger, & Borchard, 2002), chitosan *N*-betainates (Gao, Zhang, Chen, Gu, & Li, 2009), quaternized *N*-(4-*N*,*N*-dimethylaminobenzyl) chitosan chloride (Rojanarata et al., 2008), and deoxycholic acid-conjugated chitosan oligosaccharide (Chae, Son, Lee, Jang, & Nah, 2005) have been reported. Although much research has synthesized novel chitosan derivatives as alternatives for gene carriers, there has been little success in increasing the transfection effi-

ciency. The QPyMeC was one of the quaternized chitosan derivatives which showed excellent water solubility across the pH range. (Sajomsang et al., 2009c). The gene transfection efficiency of the QPyMeC with various DQs on human hepatoma cell lines (Huh 7 cells) was reported by Opanasopit et al. (2008). In this study, the plasmid DNA (pDNA) encoding green fluorescent protein (pEGFP-C2) was used as a DNA model. The QPyMeC/DNA nanocomplexes were prepared by the ionic gelation method. This method is based on the electrostatic interaction between positively charged QPyMeC and negatively charged DNA. The factors affecting the transfection efficiency such as the DQ, DS and weight ratio of the QPyMeC/DNA nanocomplexes were investigated. The results revealed that the QPyMeC was able to condense with pDNA which was investigated by agarose gel electrophoresis. It is important to note that the complex formation found at different weight ratios depends on the DQ of derivatives. These derivatives at lower DQ can lead to a complete complex formation at higher weight ratio. This is due to the higher charge density of the QPyMeC required to neutralize the pDNA phosphate group compared to those of higher DQ. The QPyMeC/DNA complex (DQ 69%) showed the highest transfection efficiency at a weight ratio of 4. The results indicated that the improved gene transfection was possibly due to a 4-pyridylmethyl substituent on chitosan that promoted the interaction and condensation with pDNA as well as *N*-quaternization which increased chitosan water solubility. Recently, Sajomsang et al. studied the role of polymer architecture in the gene transfection efficiency of the quaternized chitosan containing different aromatic moieties, quaternized *N*-(4-*N*,*N*-dimethylaminocinnamyl) chitosan chloride (QDMCMC), quaternized *N*-(4-*N*,*N*-dimethylaminobenzyl) chitosan chloride (QDMBzC), and QPyMeC compared to TMC (Sajomsang et al., 2009b). They found that the rank of gene transfection efficiency on Huh 7 cells was QPyMeC > QDMBzC > TMC > QDMCMC, respectively. It is possible that the positive charge in the pyridine ring can be delocalized by the resonance effect, which would enhance DNA condensation compared to other quaternized chitosan derivatives. The difference in gene transfection efficiency between QPyMeC and TMC could be due to the lowest binding affinity of TMC. Therefore, it is postulated that the chemical structure and the positive charge location play an important role in the binding affinity between quaternized chitosan derivatives and pDNA.

4.4. Sensor application

N-(2-Pyridylmethylidene) chitosan was modified onto an electrode-separated piezoelectric quartz crystal by the intervention of silane and glutaraldehyde as reported by Bao and Nomura (2002). This electrode can be used in acidic and basic solutions. Silver(I) can be selectively determined from the coexisting metal ions in an ammonium chloride buffer solution containing EDTA. The frequency shifts caused by the adsorption of silver(I) were proportional to the concentration over the range 10–80 nM of silver(I). The detection limited was 6 nM. Moreover, this method was simple and showed high sensitivity and selectivity.

4.5. Biomedical application

Mucoadhesion is an important property of the polymer used in drug delivery systems. The mucoadhesive drug delivery system prolongs the residence time of the dosage form at the site of application or absorption and facilitates an intimate contact of the dosage form with the underlining absorption surface and thus contributes to an improved and/or better therapeutic performance of the drug. Recently, Sajomsang et al. studied the mucoadhesion of the quaternized *N*-(4-pyridylmethyl) chitosan using the mucin particle method (Sajomsang et al., 2009c). It was assumed that the surface property of the mucin particles would change with an adhesion of the polymer if the polymer had a mucoadhesive property (Takeuchi et al., 2005). The zeta-potential of the mucin particles was a negative value before the mixing. By increasing the amount of the quaternized *N*-(4-pyridylmethyl) chitosan, the zeta-potential of the mucin particles slowly changed from negative to positive value. The aggregation occurred after the zeta-potential of the mucin exceeded the critical zeta-potential of the mucin (ca. −10 mV). The higher the concentration of the quaternized *N*-(4-pyridylmethyl) chitosan, the more pronounced were the changes found in zeta-potential. It was found that the mucoadhesive property increased with an increase in the DQ. However, the mucoadhesive property is also dependent on the polymer structure. The polymer which has steric hindrance might shield the positive charges of the quaternary ammonium, resulting in reduction of mucoadhesive property. Besides the DQ and the polymer structure, the effect of molecular weight on the mucoadhesive property is very interesting and still very much debatable. Indeed, it is need of further study. Kumar et al. studied the rheological properties of chitosan Schiff base prepared from the reaction between chitosan and 4-pyridinecarboxaldehyde or 2,6-pyridinedicarboxaldehyde based gels at room temperature (Kumar et al., 2009). These hydrogels were subjected to solvent exchange to remove water. They found that the viscosity decreased rapidly with increasing shear rate in the range of 0–20,000 s^{-1} for both cases. This effect is called shear-thinning and the fluid or polymer solution that exhibits shear-thinning is called pseudoplastic. In the case of chitosan derivatives, it can be explained that the inter- and extra-molecular hydrogen bonds of the chitosan backbone are destroyed by force from shear rate leading to the deformation of the chitosan backbone and the reduction of the viscosity. The storage modulus (G') and loss modulus (G'') of both increased steeply with the increase of frequency resulting in the flexibility of chitosan-4-pyridinecarboxaldehyde or 2,6-pyridinedicarboxaldehyde derivative gels due to the electrostatic force of the attraction between chitosan and 4-pyridinecarboxaldehyde or 2,6-pyridinedicarbaldehyde. Moreover, the G' and G'' increased gradually with the increase of sweep frequency. The rheological study reveals satisfactory behavior as shown by the ratio $G'/G'' = \tan(\delta)$ becoming constant which could be considered to show the better fluidity as well as moderate viscoelastic strength of the prepared gel.

5. Conclusions

In this review, the synthetic methods and applications of *N*-pyridylmethyl chitosan and its quaternized derivatives were summarized from the author's own previous research work and that of others. Moreover, the quaternization of chitosan and its derivatives using different synthetic routes and quaternizing agents was compiled. The quaternization of chitosan was successfully carried out by using iodomethane, iodoethane, dimethylsulfate, or grafting with a compound that containing the quaternary ammonium moiety itself. To avoid the *O*-alkylation, the *N*,*N*-dimethylated chitosan was synthesized as a starting material by Eschweiler–Clarke reaction and followed by quaternization with iodomethane in NMP without the assistance of a catalyst. In case of quaternized chitosan synthesized by using glycidyl trimethylammonium chloride, it would be done under acidic condition. The chemoselectivity of quaternized chitosan was achieved by introducing the protecting group either the primary amino groups or hydroxyl groups, changing the solvent system from NMP to *N*,*N*-dimethylformamide/water mixture, increasing the number of quaternization step, or choosing the selective quaternizing agent. Other quaternized chitosan derivatives can be able to synthesize by modification of the hydroxyl groups of chitosan to the amino groups or introduction of novel amine compound including aliphatic, cyclic, and aro-

matic amines into either the primary amino group or hydroxyl group of chitosan, and followed by methylation.

At present, quaternized chitosan and its derivatives are widely applied in many fields because they have two major advantages over the parent chitosan: (i) they are water-soluble over a wide pH range including neutral and basic conditions and (ii) they have a permanent positive charge on the polymer backbone. Even though various synthetic routes for the quaternization of chitosan and its derivatives have been proposed, researchers have tried to synthesize novel quaternized chitosan derivatives for the specific application and improvement of their biological properties. It is the author's hope that this review will be helpful for researchers who are interested in synthesizing or applying quaternized chitosan and its derivatives in the future.

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