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Review

Chitosan derivatives bearing cyclodextrin cavities as novel adsorbent matrices

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Abstract

Cyclodextrins (CDs) are cyclic oligosaccharides which have recently been recognized as useful adsorbent matrices. Due to its hydrophobic cavity, CDs can interact with appropriately sized molecules to result in the formation of inclusion complexes. These complexes are of interest for scientific research as they exist in aqueous solution and can be used to study the hydrophobic interactions which are important in the biomedical and environmental fields. The grafting of CD onto chitosan can result in the formation of a molecular carrier that possess the cumulative effects of inclusion, size specificity and transport properties of CDs as well as the controlled release ability of the polymeric matrix. In this review, different methods of CD grafting onto chitosan are discussed. In addition, the inclusion ability and the controlled release properties of CD grafted chitosan derivatives and their mechanisms of host–guest complexation with organic molecules are reviewed.

Keywords: Adsorbent matrices; Chitosan; Cyclodextrins; Grafting; Inclusion complexes

1. Introduction

CDs are cyclic oligosaccharides built from six to eight ($\alpha=6$, $\beta=7$, $\gamma=8$) D-glucose units and are formed during the enzymatic degradation of starch and related compounds (Buschmann, Knittel, & Schollmeyer, 2001). The D-glucose units are covalently linked together by 1,4 linkages to form torus-like structures (Fig. 1). All the secondary hydroxyl groups at the 2- and 3-positions of the glucose units are on one side of the torus, and all the primary hydroxyl groups at the 6-positions of the glucose units are on the other side of the ring (Loftsson & Brewster, 1996). The melting points of α-, β-, γ-CD are between 240 and 265 °C, consistent with their stable crystal lattice structure (Nash, 1994). CDs have gained prominence in recent years because their cavity, which is hydrophobic in nature, is capable of binding aromatic and other small organic molecules, and therefore provide ideal binding sites (Laine et al., 19953; Uekama, 2002). Selective functionalization at the 6-position is relatively easy. However, the secondary side is shown to be the more important side of CD in binding studies (Pablo, Isasi, & Gaitano, 2005; Rozou, Michaleas, & Vyza, 2005). The stability of the CD-inclusion complex depends on the polarity of the guest molecule and on the compatibility of the size of the host and that of the guest (Szejtli, 1988). Recent biotechnological advancements have resulted in dramatic improvements in CD production, which has lowered their production cost. This has led to the availability of highly purified CDs which are well suited as pharmaceutical excipients. CDs are widely used in basic research and industrial processes for the microencapsulation of unstable or volatile substances (Indra, Bhesh, & Bruce, 2000).

Chitin is a polysaccharide that is widely spread among marine and terrestrial invertebrate and lower forms of a plant kingdom (Yao, Peng, Yin, & Xu, 1995). Chitosan is a polyaminosaccharide, normally obtained by alkaline deacetylation of chitin (Fig. 2). Chitosan is available in a variety of useful forms and its unique chemical and biological properties make it a very attractive biomaterials. It is extensively used in many types of applications such as treatment of wastewater (Crini, 2005), chromatographic support (Shi, Jing, & Sui, 1996), enzyme immobilization (Krajewska, Leszko, & Zoborska, 1990), wound-healing dressing (Shahabeddin, Damour, & Bethod, 1991), dental application (Muzzarell, Biagini, & Paugnaloni, 1989), adhesion bandages for surgery (Costain, Kennedy, Ciona, McAlister, & Lee, 1997),

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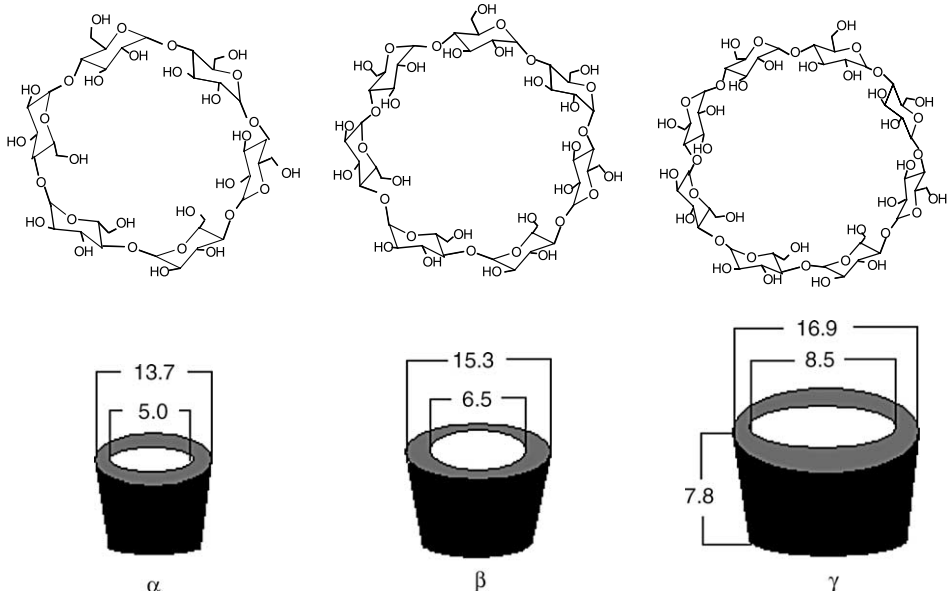


Fig. 1. Chemical structure of CDs (dimensions in Å).

and drug–delivery system (Hirano, Ohe, & Metsuda, 1978; Hirano, Tobetto, Hasagawn, & Matsuda, 1980; Miyazaki, Ishii, & Nadai, 1981; Prabaharan & Mano, 2005). In those applications, chitosan's key properties are biocompatibility, non-toxicity (its degradation products are natural metabolites) and solubility in moderated acidic aqueous solutions. Studies on chemical modification of chitosan have been extensively performed to introduce novel functions into this biopolymer (Mi, Shyu, Chen, & Schoung, 1999; Nishimura, Miyura, Ren, Sato, Yamagishi and Nishi, 1993; Schnurch, Hornof, & Zoidl, 2003; Song, Onishi, & Machida, 1992). Chitosan allows specific chemical modifications since it has primary amine groups at the C-2 position and primary alcoholic groups at the C-6 position of its monomeric units. These reactive sites enable the grafting of a large variety of properly functionalized molecules (Grcic, Voinovich, Moneghini, Lacan, Magarotto and Jalsenjak, 2000; Illum, 1998; Rossi, Ferrari, Bonferoni, & Caramella, 2000).

CD has the merit of a hydrophobic cavity, which is easy to assemble with other molecules. Chitosan has the merit of degradation slowly in organism. Therefore, grafting CD molecules into chitosan-reactive sites may lead to a molecular carrier that possess the cumulative effects of inclusion, size specificity and transport properties of CDs as well as the controlled release ability of the polymeric matrix (Azuely & Rinaudo, 2001). The products obtained by CD grafting to chitosan using different methods and their inclusion ability, sorption and controlled release properties have been studied extensively. The aim of this paper is to focus on the different types of CD grafted chitosan derivatives designed as adsorbent matrices for the association and delivery of aromatic and other small organic molecules. In this review, various kinds of strategies involved in the grafting of CDs onto chitosan are discussed in detail. In addition, the inclusion ability and the controlled release properties of CD grafted chitosan derivatives and their mechanisms of host–guest complex formation with organic molecules are reported. The final section of this review is focused on the CD–chitosan complexes and their drug release profiles and the complexation properties of CD toward the chemically modified chitosan.

2. Grafting of CD onto chitosan

2.1. By using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)

The water soluble EDC is a so-called 'zero-length' crosslinker because the amide linkages are formed without leaving a spacer molecule. EDC reacts with carboxyl group of carboxymethylated β-CD to form an active ester intermediate. The intermediate can react with a primary amine of chitosan to form an amide linkage. Furusaki, Ueno, Sakairi, Nishi, and Tokura (1996) described the preparation of a β-CD grafted chitosan by coupling carboxymethylated β-CD and a partially deacetylated chitin oligomer using water soluble EDC. Coupling carboxymethylated β-CD and a partially deacetylated chitin (M_w = 7300) afforded a new type of functional chitosan derivative having an ability to form an inclusion complex. The ability to form an inclusion complex was studied using a fluorescent dye, 6-(*p*-toluidino)-2-naphthalene-6-sulfonate (TNS), as the guest molecule. It was found that the presence of the CD grafted onto chitosan enhanced the relative intensity of TNS fluorescence significantly (3.6 times as much

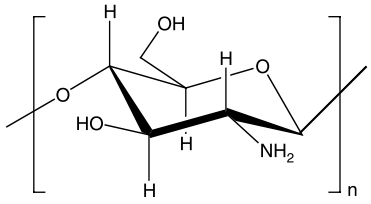


Fig. 2. Chemical structure of chitosan.

as that of β-CD). In order to examine the effect of chitosan on the fluorescence intensity of TNS, the fluorescence spectrum was measured by adding chitosan to the solution of β-CD-TNS. The mixture revealed almost the same spectrum as that of β-CD-TNS, suggesting that chitosan had no influence on the fluorescence enhancement. Fluorometric titration revealed that the 1:1 stoichiometrical complex was formed and that the equilibrium constant was $1.13\text{-}1.68\times10^{3}\,M^{-1}$. Ionic interaction between the sulfonic acid moiety of TNS and the amino group of chitosan was suggested from experiments which involved changing the pH and ionic strength of the buffer solution as well as adding a surface-active agent. The polymer guests having free amino groups on the chitosan backbone were found to recognize a TNS molecule through an ionic interactions as well as host–guest complexation.

The preparation of polymers having a CD moiety are based on the interest in the synthesis of artificial receptors (Asanuma, Akiyama, Kajiya, Hishiya, & Kimiyama, 2001), the removal of undesired compounds from food (Asanuma, Shibata, Kakazu, Hishiya, & Kimiyama, 1997) or the removal of toxic compounds from water (Szejtli, 1988). Normally, the chitosan derivatives having a CD moiety are soluble in water and it is necessary to crosslink the chitosan before connecting the CD for the application as an adsorbent in water (Ishigami, Okemoto, Arai, Yoshida, Sato and Yamamoto, 1995). In studies aimed at obtaining insoluble CD–chitosan from previously crosslinked chitosan, the CD contents were found to be relatively low. The main reason for the limited CD content can be assumed to that the reactions for binding CD to chitosan were carried out in heterogeneous systems. To avoid this problem, an insoluble crosslinked chitosan bearing CD moieties was prepared by a one-step procedure with *N*-succinylated chitosan and mono-6-amino-mono-6-deoxy-β-cyclodextrin in the presence of the water soluble EDC under homogeneous conditions (Fig. 3) (Aoki, Nishikawa, & Hattori, 2003). It was considered that the product obtained in this study was crosslinked between the carboxyl groups and amino groups on the succinyl chitosan skeleton. The degree of substitution by the CD moiety achieved 0.27 with the addition of *N*,*N*-dimethylformamide (DMF) to the reaction solution. Results of the adsorption experiments using bisphenol A (BPA) and *p*-nonylphenol (NP) are shown in Fig. 4(a) and (b), respectively (Aoki et al., 2003). The initial concentration of BPA and NP in Fig. 4 were different because the solubility of NP was quite low. Fig. 4(a) showed that chitosan bearing CD moiety adsorbed 80% of the BPA in the solution while only a small amount of BPA was adsorbed to succinyl chitosan crosslinked without the CD moiety. This indicated that the adsorption mainly occurred with the CD moiety and that the adsorption onto the hydrophilic chitosan skeleton was negligible. The amount of adsorbed BPA at equilibrium onto chitosan bearing a CD moiety was consistent with that expected from the amount of the CD moiety in chitosan with a molar ratio, CD/BPA = 0.85:1. In the case of the dilute NP solution (1.23×10^{-5} M), the chitosan bearing CD moiety adsorbed most of the NP because CD was in excess to the NP (molar ratio, CD/NP = 33:1). Fig. 4 also showed that the initial adsorption rates of the chitosan bearing CD moiety with BPA and NP were larger than that of activated carbon. The kinetic parameter (k_1) for the adsorption of BPA onto chitosan bearing CD moiety was 8.6 times larger than that of NP onto chitosan bearing CD moiety while the k_1 for the adsorption of BPA onto activated carbon was 0.28 times than for NP onto activated carbon. These results showed that the chitosan bearing CD moiety has some selectivity for the adsorption of BPA.

2.2. By the nucleophilic substitution

Monochlorotriazinyl groups are often encountered in textile chemistry, where they are used as functional groups of reactive dyes for the finishing of wool and cellulose. Their reactivity is based on the ready nucleophilic displacement of the chlorine atom by the amino or hydroxyl groups carried by the polymer that builds the fiber. Martel, Devassine, Crini, Weltrowski,

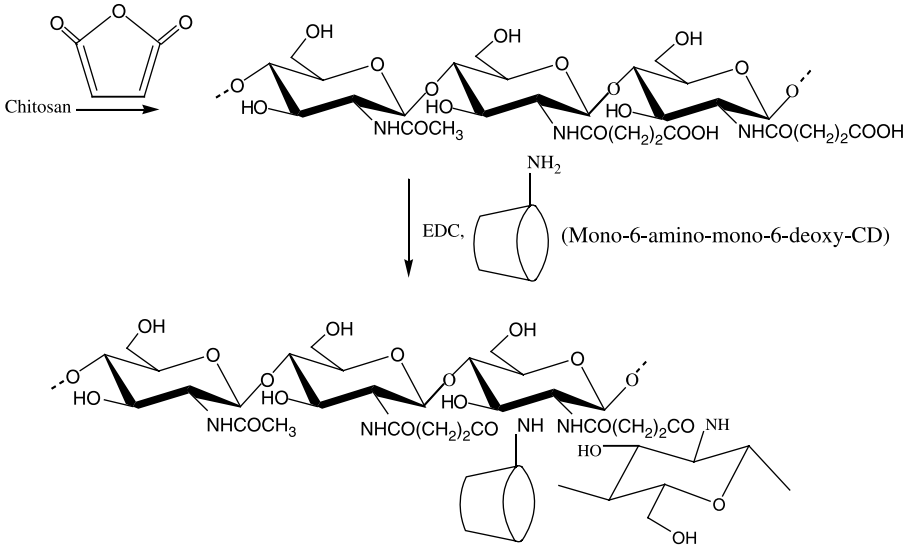


Fig. 3. Preparation of chitosan-*graft*-β-CD from *N*-succinyl chitosan (Aoki et al., 2003).

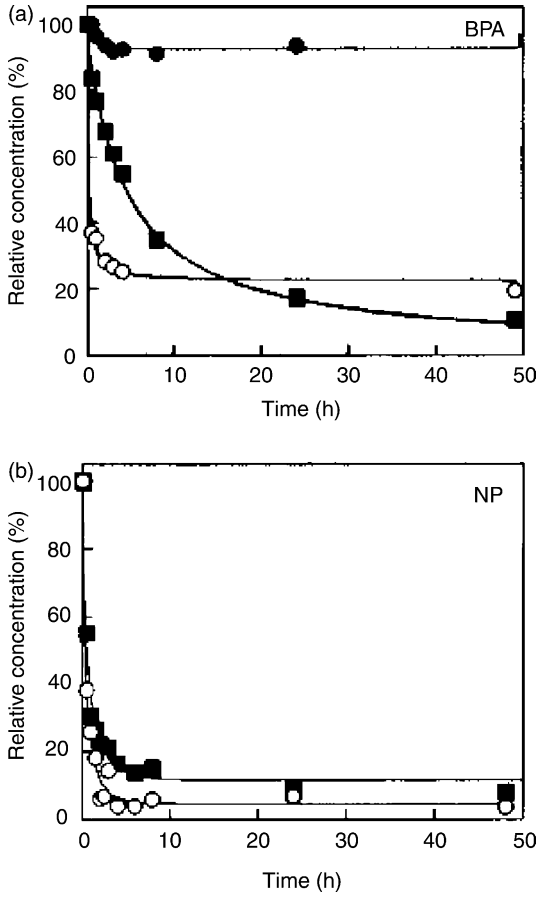


Fig. 4. Adsorption of (a) 4.8×10^{-4} M BPA and (b) 1.23×10^{-5} M NP. ●: succinyl chitosan (4.8×10^{-5} M), ○: β-CD-*graft*-chitosan, ■: activated carbon (Aoki et al., 2003).

Bourdonneau and Morcellet (2001) used a monochlorotriazinyl derivative of β-CD as a reagent for the grafting of β-CD onto chitosan. In this approach, as shown in Fig. 5, β-CD was coupled to chitosan by the intermediate of its monochlorotriazinyl derivative through the nucleophilic substitution of the chloride atom by the amino groups. The resulting products were not soluble, but did swell, in water, nor were they soluble in the numerous organic solvents. Because the average degree of substitution of the monochlorotriazinyl derivative of β-CD was 2.8, the reaction yielded crosslinked insoluble products due to both intra- and intermolecular bondings occurred with the polymer chains. Decontamination of water containing textile dyes were carried out with the crosslinked derivatives. These chitosan derivatives were characterized by a rate of sorption and a global efficiency of these derivatives was found to be superior to that of the parent chitosan polymer and of the well-known CD-epichlorohydrin gels. Different mechanisms of sorption such as host–guest complexation occurred in β-CD grafted chitosan, hydrophobic and electrostatic interactions mainly involved in chitosan were reported as the reason for the superior sorption efficiency. It was observed not only that these products have the chemical properties derived from the initial chitosan and β-CD, but also that their covalent bonding yielded materials with an improved sorption capacity.

2.3. By using tosylated β-CD

Chen and Wang (2001) synthesized β-CD-*graft*−2-chitosan by reacting β-CD with *p*-toluenesulfonyl chloride, then grafting with chitosan. The reaction scheme for the synthesis is shown in Fig. 6. The products obtained by this method were found to be soluble in water, methanol, dimethylsulfoxide (DMSO), and DMF. The polymer inclusion complex of β-CD-*graft*-2-chitosan with iodine was prepared and its inclusion ability was studied. The experimental results showed that a substantial amount of iodine was included with β-CD-*graft*−2-chitosan and formed a stable inclusion complex, while chitosan alone had little ability to adsorb iodine. The stronger inclusion ability of β-CD-*graft*-2-chitosan with iodine was caused by the special hydrophobic cavity structure of β-CD-*graft*−2-chitosan. The absorption of iodine was considered to be caused by $n-\delta$ charge transfer between amino groups of chitosan and iodine molecules (Shigeno & Takemot, 1980). After the subcutaneous implantation of the polymer inclusion complex of β-CD-*graft*-2-chitosan with iodine in rats, iodine exhibited the property of slow release. The amount of iodine in the blood decreased slowly and maintained approximately half of maximum for 70 days later, and maintained much higher radioactivity in the organs of rats compared to the inclusion complex of β-CD with iodine.

Immobilization of photo or electro-active molecules onto thin films to form highly ordered architectures is one of the important approaches to prepare functional films (Akamatsu, Takei, Mizuhata, Kajinami, Deki and Takeoka, 2000; Choi, Lee, Park, Ha, & Yoon, 2000). This technology has been the focus of intense interest of many scientists due to their potential applications in creation of novel electronic/photonic devices and sensing materials (Ohring, 1992; Zhai, Wei, & Huang, 2000). A series of work on sensing films based upon the principle of the supramolecular behavior, that is association

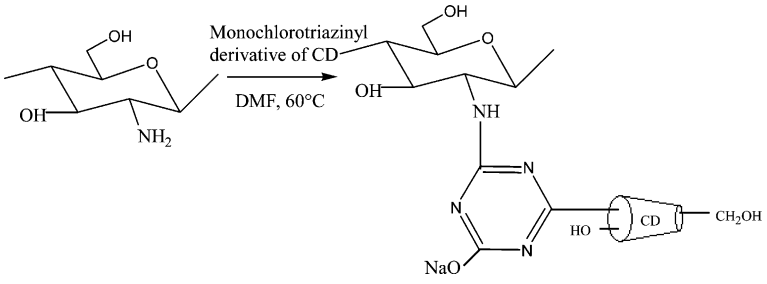


Fig. 5. Reaction scheme for the synthesis of β-CD-*graft*-chitosan by the nucleophilic substitution reaction (Martel et al., 2001).

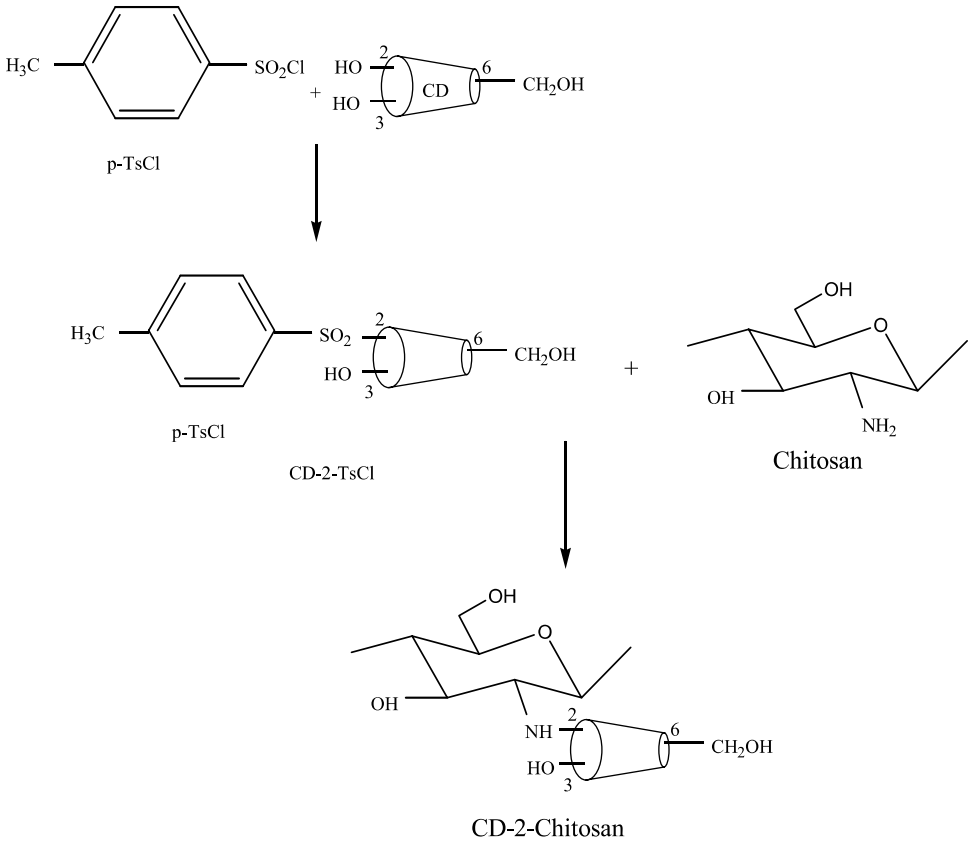


Fig. 6. Reaction scheme for the synthesis of β-CD-*graft*−2-chitosan (Chen & Wang, 2001).

and dissociation of aromatic molecules in immobilized state have been reported (Fang, Ning, Lu, & Hu, 2000; Wang, Fang, Hu, Cui, & Gao, 2002). In these studies, pyrene was chemically immobilized onto chitosan thin films in order to prepare chemical sensors for solvent polarity measurement. Recently, a novel nitromethane sensing film was prepared by chemical immobilization of pyrene and β-CD onto chitosan thin films coated on quartz plate (Wang, Fang, Ding, Gao, & Hu, 2003). In this work, mono [6-*O*-(*p*-toluene-sulfonyl)]-2-β-CD (CD-2-TsCl), which was prepared by reacting β-CD with *p*-toluene-sulfonyl chloride in dry pyridine under nitrogen atmosphere was grafted with chitosan film in DMF. The β-CD grafted film was again reacted with 1-pyrenesulfonyl chloride in the mixture of pyridine and ethanol. The chemical structure of the film is schematically shown in Fig. 7. It might be expected that when both CD and pyrene were co-immobilized on chitosan film, it was possible to form intra-molecular CD–pyrene inclusion complexes. The fluorescence behavior of this film was found to be very different from that of the corresponding chitosan-1-pyrenesulfonyl chloride film, due to the presence of β-CD. Fluorescence studies revealed that the immobilized pyrene was mainly trapped within the cavity of the β-CD. It was found that the addition of common fluorescence quenchers, including copper, cobalt and some other transition metal salts, KI and acrylamide, had little effect upon the emission of the film. Addition of nitromethane, however, quenched the emission dramatically. This special feature of the film was found to be useful for sensing nitromethane in methanol, ethanol and water.

2.4. By using 1,6-hexamethylene diisocyanate (HMDI)

HMDI is generally utilized as a strong cross-linker of amino or hydroxyl groups since it possesses two isocyanate groups (–N=C=O). Under the suitable conditions (pH < 6), the hydroxyl groups of chitosan reacts with an isocyanate to form a urethane product (–NH–COO–) due to the transfer of proton from hydroxyl to nitrogen atom of isocyanate. In addition, isocyanate also reacts with hydroxyl groups of β-CD to form a product the same with urethane (Wade, 1999). It is assumed that the crosslinking of the hydroxyl groups of chitosan with HMDI resulted in chitosan–HMDI complex,

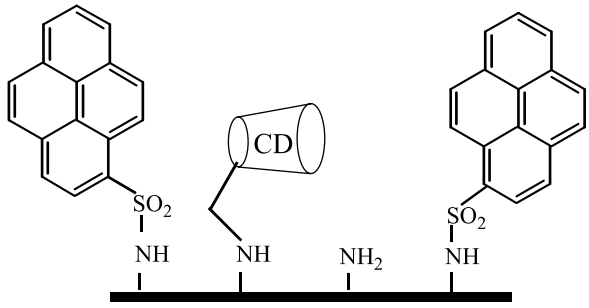


Fig. 7. Schematically representation of chemical structure of the film (Wang et al., 2003).

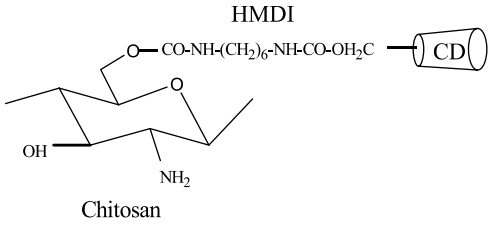


Fig. 8. Hypothetical illustration of β-CD immobilized to chitosan using HMDI (Chiu et al., 2004).

which then binds with the hydroxyl groups of β-CD to form β-CD-*graft*-chitosan. HMDI cannot bind to amino groups of chitosan due to the lower affinity for amino groups as compared to hydroxyl groups under low pH value (Wade, 1999). Due to these facts, Sreenivasan (1998) reported an adsorbent matrix synthesized by coupling β-CD to chitosan using HMDI. The matrix obtained in this study was found to be insoluble in organic as well as acidic or alkali media. The extent of cholesterol removal by this matrix from the solution was studied. The results indicated that nearly 21% of the cholesterol was removed from the solution. In this study, the cholesterol adsorbed by the polymer matrix was removed by rinsing the polymer with an organic solvent like chloroform, which indicated the feasibility of using the modified chitosan as a reusable sorbent. It was reported that the factors involved in the complex formation of β-CD-*graft*-chitosan with cholesterol are the van der Waals interaction between the hydrophobic moiety of the guest molecules and the CD cavity, hydrogen bonding between the polar functional groups molecules and the hydroxyl groups of CD, release of high-energy water molecules from the cavity during complex formation, etc.

In another study, immobilization of β-CD to chitosan beads by cross-linking with HMDI was developed for cholesterol adsorption from egg yolk. The behavior of cholesterol adsorption was investigated using the Langmuir isotherm (Chiu, Chung, Giridhar, & Wu, 2004). The hypothetical illustration of β-CD immobilized to chitosan using HMDI is shown in Fig. 8. The efficiency of immobilization was found to depend on the concentrations of HMDI and β-CD, time of cross-linking and immobilization, and reaction temperature. The effect of these variables on β-CD binding was investigated and the reaction conditions were optimized. Under the optimum conditions, a maximum β-CD loading of 0.43 g/g-chitosan was obtained using 5% (v/v) HMDI with a cross-linking time of 40 min and using 2% (w/v) β-CD for immobilizing for 1 h at 25 °C. The experimental data on cholesterol adsorption by immobilized β-CD fitted well in the Langmuir isotherm equation with a maximum adsorption capacity of cholesterol 0.33 g cholesterol/g-adsorbent. This result showed that 1 mol of β-CD has complexed with more than 2 mol of cholesterol. The chelation of more cholesterol molecules in the gap between chitosan and β-CD was found to be responsible for an increased cholesterol adsorption. For the cholesterol adsorption from yolk, 92% of cholesterol was removed with 1% chitosan immobilized β-CD in 2 h and at a yolk to water ration of 1:30. In the case of desorption, 96% of the cholesterol in the chitosan immobilized β-CD was dissociated using 95% ethanol with an agitation rate of 75 rpm and at 50 °C. In addition, the chitosan immobilized β-CD retained 84% adsorption capacity after 12 reuses. A comparison of the efficiencies of cholesterol adsorption using various adsorbents reported in the literature is listed in Table 1. This table showed that the cholesterol adsorption capacity of 0.33 g/g achieved with chitosan immobilized β-CD is higher than that obtained by using other adsorbents. In addition, cholesterol adsorption by chitosan immobilized β-CD has the lowest energy consumption since the reaction is carried out at room temperature.

2.5. By the reductive amination

Reductive amination is one of the major reactions applicable to the modification of chitosan. Introduction of CD residue into chitosan has been successfully attained in a homogeneous system through a reductive amination strategy. CD derivatives with aldehyde functional groups are useful to graft CD into chitosan by the formation of Schiff's base. Tanida, Tojima, Han, Nishi, Tokura and Sakairi (1998) reported the synthesis of β-CD grafted chitosan by the formation of Schiff's base between 2-*O*-formylmethyl-β-cyclodextrin and chitosan in acetate buffer at pH 4.4, followed by reduction with sodium cyanoborohydride. The product, which had a degree of substitution of 37%, was found to be soluble in water at neutral and alkaline conditions. The inclusion ability of the β-CD grafted chitosan was examined in terms of UV–visible and circular dichroism spectroscopy using *p*-nitrophenolate in a phosphate buffer at pH 8.7. Similar to the parent β-CD, a bathochromic shift was observed in

Table 1
Comparison of the efficiency of cholesterol removal by different adsorbents

Adsorbent	Reaction time	Temperature (°C)	Cholesterol removal (mg/g-matrix)	References
Alumina/CO_2	4 h	40	2.4	Mohamed, Neves, and Kieckbusch (1998)
Alumina/methane	6 h	40	3	Mohamed, Saldana, Socantaype, and Kieckbusch (2000)
Terpolymers	24 h	37	17	Sellergren, Wieschemeyer, Boos, and Seidel (1998)
β-CD	10 min	50	92	Awad and Smith (1996)
β-CD	40 min	50	43.8[a]	Mine and Bergougnoux (1998)
Chitosan-β-CD	50 min	25	330	Chiu et al. (2004)

[a] Cholesterol is from low density lipoprotein.

the UV–visible spectrum with increasing concentration of the host molecule. Isobestic points were observed at 334, 400 and 455 nm. Using the Benesi–Hildebrand equation, the dissociation constant of the host–guest complex of β-CD grafted chitosan and *p*-nitrophenolate was calculated to be 1.49×10^{-3} M, which was comparable to that of the parent β-CD ($K_D = 1.3\times10^{-3}$ M at pH 10). Similarly, the inclusion ability of α-CD grafted chitosan towards *p*-nitrophenolate was reported (Tojima, Katsura, Han, Tanida, Nishi and Tokura, 1998). In this study, 2-*O*-formylmethyl-α-CD was linked into chitosan by reductive amination. 2-*O*-formylmethyl-α-CD was prepared by selective allylation of α-CD and subsequent ozonolysis of the C–C double bond in the resulting mono-allylated derivative. The reaction scheme and conditions are given in Fig. 9. All the derivatives obtained were found to be soluble in water and in alkaline solvents such as aqueous ammonia and aqueous sodium hydroxide, except for that with degree of substitution 11%, which was soluble only in acidic solution. The inclusion ability of the α-CD grafted chitosan with degree of substitution of 50% was examined in terms of UV–visible spectroscopy using *p*-nitrophenolate as a guest compound in a phosphate buffer at pH 11 (Tojima et al., 1998). A bathochromic shift was observed in the UV–visible spectrum with increasing concentration of the host molecule, and the isosbestic points were at 397 and 451 nm. Using the Benesi–Hildebrand equation, the dissociation constant of the host–guest complex of α-CD grafted chitosan and *p*-nitrophenolate was calculated to be 7.54×10^{-4} M, which was comparable to that of the parent α-CD ($K_D = 2.21\times10^{-4}$ M).

Since chitosan beads have been widely used in various fields such as metal ion adsorptive material and immobilization of enzymes, water-insoluble porous beads having an ability to form inclusion complexes with specific substrates were synthesized by adding an aqueous acetic acid solution of chitosan into ethanolic aqueous sodium hydroxide and subsequent crosslinking with HMDI in DMF (Tojima, Katsura, Nishiki, Nishi, Tokura and Sakairi, 1999). The resulting beads were further treated with 2-*O*-formylmethyl-α-CD in the presence of sodium cyanoborohydride in acetate buffer at pH 4.4, giving the CD grafted chitosan beads (Fig. 10). Their inclusion ability was examined by the use of *p*-nitrophenol and 3-methyl-4-nitrophenol as model compounds. Although these two guest molecules have closely resembling structures, the methyl group of the latter strongly inhibited the formation of an inclusion complex due to steric hindrance of the methyl group. The potent inclusion ability was observed on α-CD grafted chitosan beads toward *p*-nitrophenol while 3-methyl-4-nitrophenol was not adsorbed on the beads. Controlled release study suggested that *p*-nitrophenol entrapped with α-CD grafted chitosan beads was released slowly into the buffer and that equilibrium was reached after 15 h. In contrast to these results, chitosan beads, which have little ability to form inclusion complexes, released almost all of the *p*-nitrophenol within several hours. These experiments suggested that α-CD grafted chitosan may serve as an adsorbent for controlled release of drugs.

In terms of CD inclusion complexes, the introduction of a guest molecule in the cavity classically takes place from the wider secondary hydroxyl groups side although the other situation may also be encountered, depending on the guest. It has been shown that steric hindrance effects due to substitution of CD could result in an important decrease of the association

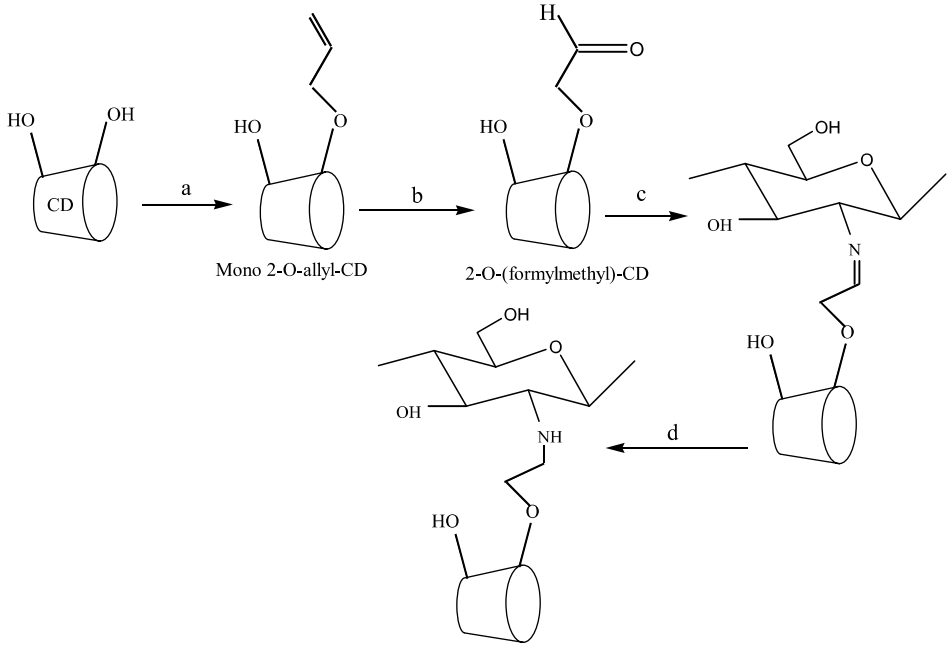


Fig. 9. Reaction scheme and conditions for the synthesis of α-CD-*graft*-chitosan: (a) LiH-LiI, DMSO; and then allyl bromide, 60 °C, overnight; (b) O_3 in 50% aqueous MeOH, 0 °C, 4 h; and then Me_2S, 25 °C, over night; (c) chitosan in acetate buffer (pH 4.4), 25 °C, 1 h; (d) $NaBH_3CN$ in acetate buffer (pH 4.4), 25 °C, 4 days (Tojima et al., 1998).

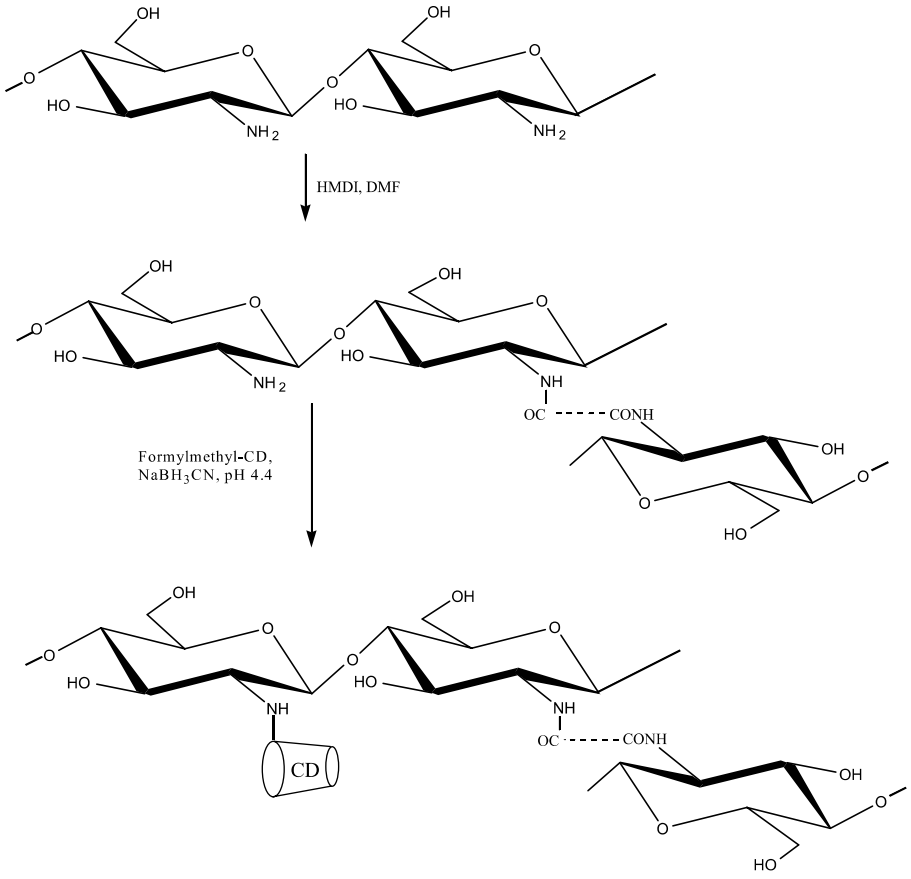
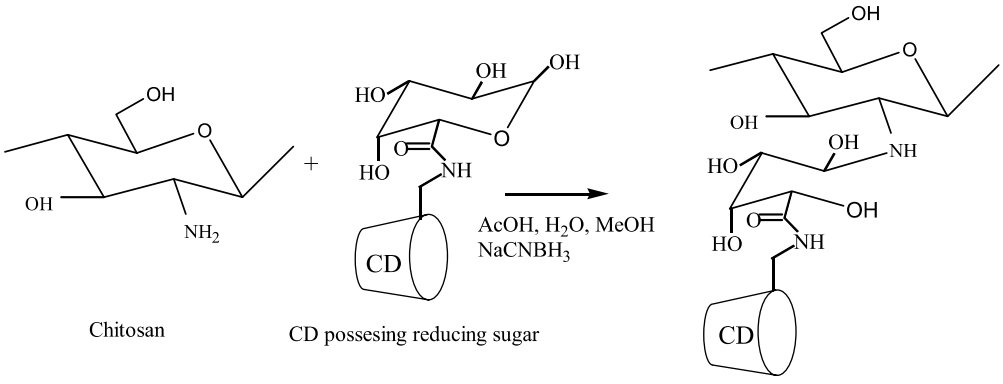


Fig. 10. Reaction scheme for the preparation of α-CD-*graft*-chitosan beads.

constant of complexes (Laine, Sarguet, Gadelle, Defaye, Perly and Pilard, 1995). In view of these data, Auzely and Rinaudo (2001, 2002, 2003) synthesized β-CD-*graft*-chitosan based on the preparation of a monosubstituted β-CD derivatives possessing a reducing sugar on the primary face in order to specifically attach it on chitosan from the side which is 'less involved' in the inclusion of guests followed by its reductive amination as described in Fig. 11. The synthesis of monosubstituted β-CD was based on the carbodiimide-mediated coupling of 6-amino-6-deoxycyclomaltoheptaose with the intermediate 1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranuronic acid. The complexation ability of the grafted CD was analyzed by NMR spectroscopy and calorimetric titration. It was demonstrated that the specific chelation properties of CD were not strongly modified by grafting on the polymer. For this purpose, different techniques were used to determine the association constant with some hydrophobic small molecules from which *tert*-butylbenzoic acid and (+)-catechin. Using the continuous variation technique, the inclusion complex of these molecules with CD derivative was found to be 1:1. In the case of *tert*-butylbenzoic acid, it was shown from Rotating Frame Overhauser Effect Spectroscopy data and due to the bulkiness of the *tert*-butyl group that inclusion proceeds through the wider side of β-CD (Fig. 12). It was reported that the CD cavity was specific for encapsulation of hydrophobic molecules and might stabilize them against oxidation or increase their



Chitosan CD possesing reducing sugar

Fig. 11. Synthesis of β-CD-*graft*-chitosan based on monosubstituted β-CD (Auzely & Rinaudo, 2001).

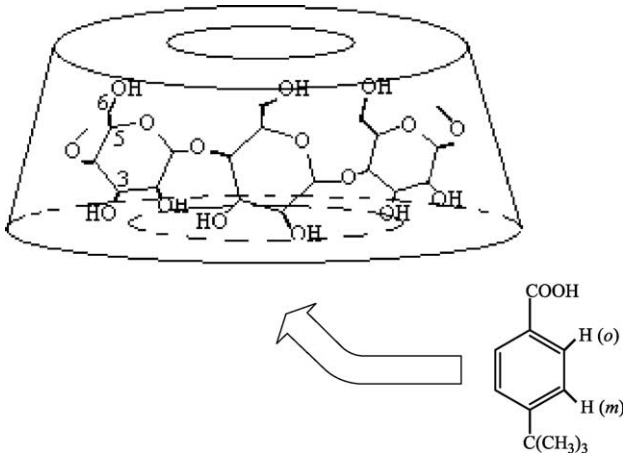


Fig. 12. Model for the inclusion process of 4-*tert*-butylbenzoic acid in β-CD in aqueous solution (Auzely & Rinaudo, 2001).

solubility in aqueous solution. It was concluded that the affinity remained nearly unchanged for the grafted CD compared to free CD (Auzely & Rinaudo, 2001, 2002, 2003).

The study on inclusion ability of free- and chitosan grafted CD towards 6-thiopurine was also performed. Here, the β-CD grafted chitosan was synthesized by reductive coupling reaction of mono-fuctionalized β-CD with chitosan (Jimenez, Belmar, & Alderete, 2003). The mono-fuctionalization of β-CD was performed by using 1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide as oxidizing agent in DMSO. The results showed that β-CD retained its inclusion ability after its incorporation into chitosan chains and, in the particular case of 6-thiopurine, an increase was observed in the association constant when comparing native β-CD with polymer grafted β-CD. The inclusion performance of polymer grafted β-CD with 6-thiopurine was examined by using UV–visible spectroscopy. The results revealed that as the polymer concentration increased, larger absorbance variations were observed and the polymer addition induced a hypochromic effect on the absorption band. The determination of the association constant between 6-thiopurine and grafted β-CD was found to be based on the absorbance variations observed during inclusion assays. In this work, a new method was proposed for the determination of association constants in CD-polymer systems. This new procedure has provided more accurate and reliable results than traditional procedures.

A large variety of polymeric networks, in particular chemical hydrogels, have been described in the literature in recent years, demonstrating the interest of the scientific community in basic and applied studies on such innovative materials. Chitosan and its derivatives are well known for their binding properties toward transition metal ions. Recently, a great deal of attention has been paid to the grafting of CD on chitosan for manufacturing new metal ion sorbents using a Schiff's base reaction. Paradossi, Cavalieri, and Crescenzi (1997); Paradossi, Chiessi, Cavalieri, Moscone, and Crescenzi (1997); Crescenzi, Paradossi, Desideri, Dentini, Cavalieri and Amici (1997) prepared a chemical hydrogel based on chitosan using a polyfunctionalized β-CD as crosslinking agent. In these studies, chitosan was crosslinked with oxidized β-CD using reductive amination method (Fig. 13). The structural and catalytic features of this hydrogel loaded with copper (II) ions were studied in aqueous medium. Stability of the complex and its structural characteristics were determined by isothermal microcalorimetry and EPR spectroscopy, respectively. The catalytic properties of the copper-loaded hydrogel were assessed toward the oxidation of (D,L)-adrenaline and L-adrenaline by molecular oxygen in aqueous media. The advantages of working with a heterogeneous system were found to be coupled with the preservation of both structural and catalytic features of copper (II) site in the matrix with

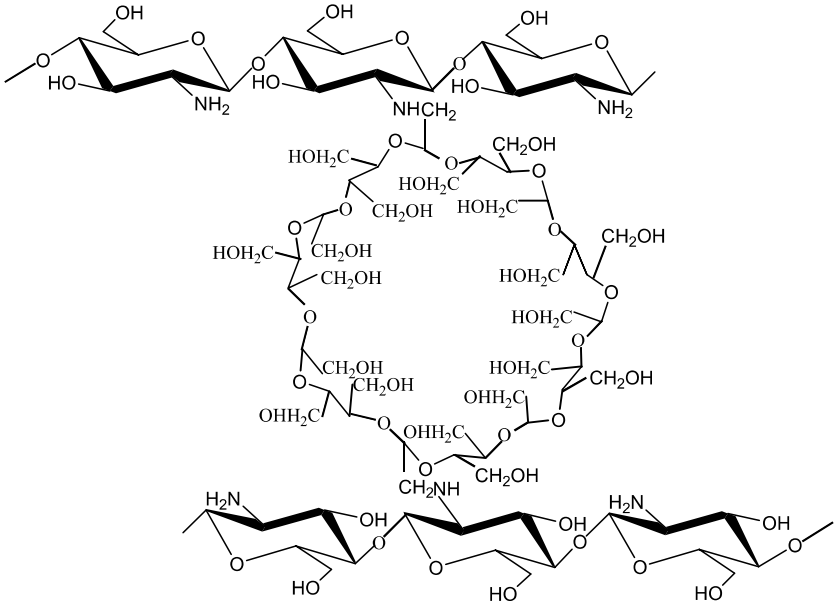


Fig. 13. Structure of chitosan crosslinked by oxidized β-CD (Paradossi, Cavalieri, et al., 1997; Paradossi, Chiessi, et al., 1997).

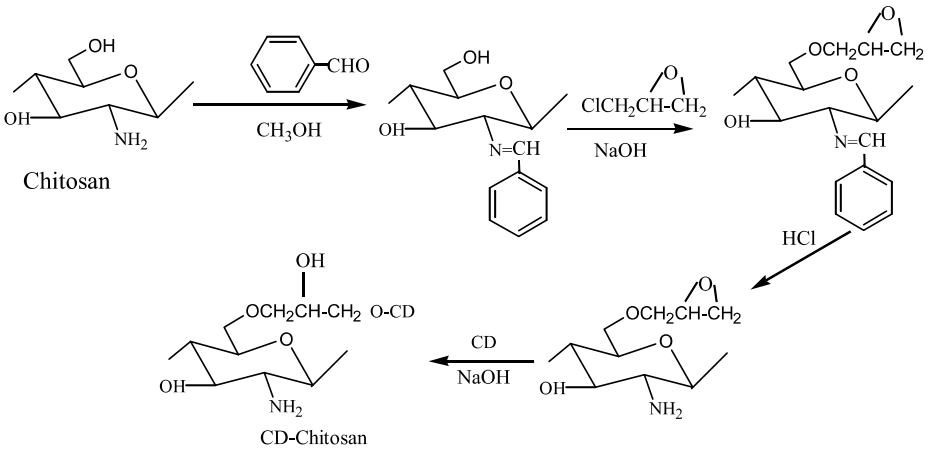


Fig. 14. Reaction scheme for the synthesis of β-CD-*graft*-chitosan via epoxy-activated chitosan (Zhang et al., 2004).

respect to copper (II) ions bound to chitosan derivatives in solution.

2.6. Via epoxy-activated chitosan

Recently, a new synthetic route was reported to graft β-CD onto chitosan using epoxy-activated chitosan as shown in Fig. 14 (Zhang, Wang, & Yi, 2004). The adsorption properties of this product for *p*-dihydroxybenzene were studied and compared with that of chitosan and β-CD-*graft*−6-chitosan that was synthesized by the reaction of mono [(6-*O*-(*p*-toluenesulfonyl)]-6-β-CD with chitosan. The grafting of β-CD onto chitosan resulted in higher adsorption capacities for *p*-dihydroxybenzene than that of chitosan. The results indicated that the adsorption of *p*-dihydroxybenzene by polymeric adsorbents was greatly affected by the increase in *p*-dihydroxybenzene concentration. The adsorption capacity of *p*-dihydroxybenzene increased at the beginning with the *p*-dihydroxybenzene concentration. However, after the *p*-dihydroxybenzene concentration reached 200 mg/L, the adsorption capacity decreased. This was due to the higher *p*-dihydroxybenzene concentration inhibited the diffusion of *p*-dihydroxybenzene into the adsorbent. The experimental results showed that these adsorbents exerted adsorption on the carefully chosen target. The highest saturated capacity of *p*-dihydroxybenzene of β-CD-*graft*-chitosan and β-CD-*graft*-6-chitosan were found to be 51.68 and 46.41 mg/g, respectively.

2.7. By using redox initiator

Graft copolymerization of vinyl monomers onto chitosan and other natural polymers can introduce desired properties and enlarge the field of potential applications by choosing various types of side chains. In recent years, a number of initiator systems have been developed to initiate grafting copolymerization. Redox systems, such as ceric ammonium nitrate (CAN) and potassium persulfate have been usually used to produce free radical sites on many kinds of polymers. Recently, β-CD-*graft*-chitosan was prepared by reacting β-CD itaconate vinyl monomer with chitosan using CAN (Gaffar, Rafie, & Tahlawy, 2004). In this work, β-CD itaconate was prepared by esterification of β-CD with itaconic acid in a semidry process and then the pendent double bonds of β-CD itaconate were utilized in graft copolymerization onto chitosan (Fig. 15). The resultant product was then subjected to crosslinking using different concentrations of glutaraldehyde. This crosslinked chitosan derivative was evaluated as a new adsorbent for three classes of dyes (acid, basic, and hydrolyzed reactive), since it has three different active groups such as carboxyl groups, amino groups and CD-ring hosting molecule. The adsorption experiments were conducted under different conditions with a view to establish the appropriate conditions for dye adsorption. The results showed that the optimum pH for anionic dyes (acid, and hydrolyzed reactive) uptake was at pH of 6. At this pH, the β-CD annuli present in the chitosan backbone formed a stable host–guest inclusion complex and anion–cation interaction with anionic dye molecules thereby enhanced the anionic dye uptake. The alkaline medium favoured the higher percent of basic dye uptake due to the interaction β-CD-*graft*-chitosan anions and dye cations. It was shown that the adsorption percent of the used dye increased significantly by increasing the duration up to 75 min, and then leveled off. This was attributed to the higher availability of active sites at the initial stage. It was also shown that the adsorption percent of acid and basic dye decreased by increasing the extent of crosslinking. The decrease in active site, accessibility, and swellability of the adsorbent by increasing the level of crosslinking were found to be the responsible for this observation. In the case of hydrolyzed reactive, the adsorption percent was enhanced slightly by increasing the extent of crosslinking. This result showed that not only reactive sites play an important role in the sorption mechanism, but also other interactions, probably physical adsorption and hydrogen bond interactions, due to the crosslinking agent, and hydrophobic guest–guest interactions.

3. CD–chitosan complexes

CDs have been widely used in drug delivery applications due to their capability of forming inclusion complexes with

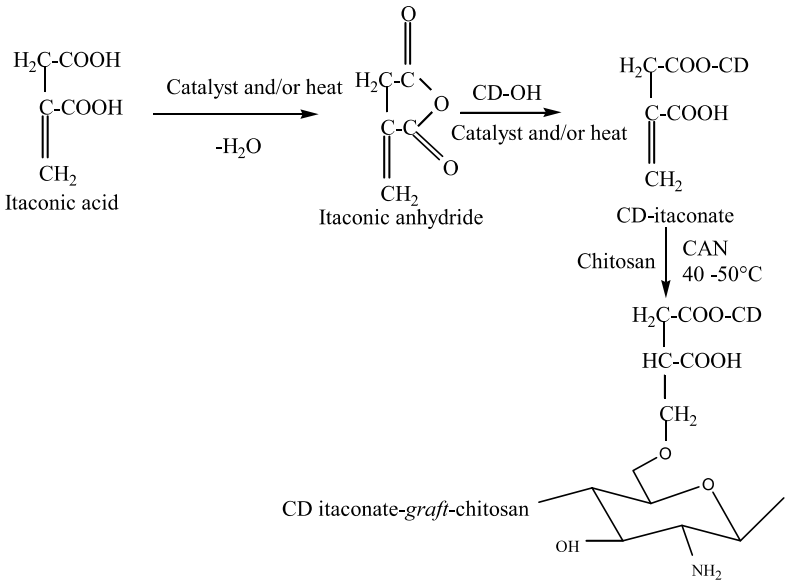


Fig. 15. Reaction scheme for the synthesis of β-CD itaconate-*graft*-chitosan using CAN.

drug molecules. These complexes are capable of altering the release pattern, changing the solubility and increasing the stability of the drugs (Rajewski & Stella, 1996; Schipper, Romeijn, Verhoef, & Merkus, 1993). In order to improve the safe delivery, increased bioavailability, and enhanced absorption of the drug, various attempts have been made to complex drug with polymer matrix (Hari, Chandy, & Sharma, 1996; Schnurch, 1998). Recently, Moses, Dileep, and Sharma (2000) attempted to complex insulin with β-CD and encapsulate in the chitosan and calcium alginate matrix. For drug release studies, insulin complexed with β-CD for 20 min and that complexed with β-CD for 150 min were used for encapsulation in the chitosan and calcium alginate matrix. The results showed that these two matrices have different drug release profiles in simulated intestinal medium (pH 7.4). Insulin complexed with β-CD for 20 min behaved like an uncomplexed system in the in vitro drug release kinetics while the 150 min complexed system had a faster release. The change in the loading character of the matrix was found to be inversely related to the concentration of β-CD when it was above the stoichiometric equivalent of the drug. In an attempt to increase the payload of the drug in the matrix, the pH of the processing medium consisting of calcium chloride and chitosan was varied. It was found that the encapsulation efficiency increased as the pH was decreased from 6 to 4. Loading efficiency was also increased by reducing the concentration gradient between the crosslinking medium and the alginate solution containing the drug.

In recent years, physical hydrogels have received much attention in the biomedical field, especially for the delivery of therapeutic drugs and proteins because of their useful properties (Hoffman, 2002). Such hydrogels can be obtained via non-covalent cohesive interactions including hydrophobic interactions, stereocomplex formation, ionic complexation, crystallinity, etc. CDs are able to accommodate a number of organic or inorganic guest molecules. The guest molecules, not only of low molecular weight, but also of polymeric molecules, can interact with one or more CD molecules, forming supramolecular-structured complexes such as rotaxanes or (pseudo)polyrotaxanes (Harada, Okada, & Kamachi, 1995). Over the past few years, various types of CD-based polymer inclusion complexes have been extensively investigated because of their importance in basic studies such as the non-covalent binding behavior based on macromolecular recognition and the properties of isolated single polymers as well as their potential applications (Huh, Tomita, Ooya, Lee, Lee and Sasaki, 2002; Ichi, Watanabe, Ooya, & Yui, 2001; Li, Harada, & Kamachi, 1994). Recently, supramolecular hydrogels were prepared on the basis of polymer inclusion complex formation between poly(ethylene glycol)-modified chitosans and α-CD (Huh, Cho, Chung, Kwon, Jeong and Ooya, 2004). Poly(-ethylene glycol)-modified chitosans were synthesized by coupling reactions between chitosan and monocarboxylated poly(ethylene glycol) using water soluble EDC as coupling agent. With simple mixing, the resultant supramolecular assembly of the polymers and α-CD molecules led to hydrogel formation in aqueous media. The poly(ethylene glycol) side-chains on the chitosan backbones were found to form inclusion complexes with α-CD, creating hydrophobic micro-domains with channel-type crystalline structure, which play an important role as physical junctions in the hydrogels. The gelation property was found to be affected by several factors including the poly(ethylene glycol) content in the polymers, the solution concentration, the mixing ratio of host and guest molecules, temperature, pH, etc. All the hydrogels in acidic conditions exhibited thermo-reversible gel–sol transitions under appropriate conditions of mixing ratio and poly(ethylene glycol) content in the mixing process. The transitions were induced by supramolecular association and dissociation (Huh et al., 2004). The internal structures of the hydrogels under different conditions are schematically presented in Fig. 16. These supramolecular inclusion complexes were found to have pH-dependent properties because of the pH-dependent

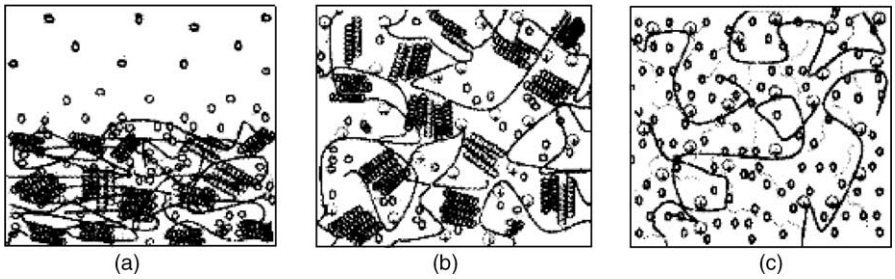


Fig. 16. Schematic illustration of proposed structure of hydrogel inclusion complexes in aqueous media; precipitation (a) in neutral or alkaline condition, and gelation (b) in an acidic medium by creation of phase-separated structures consisting of hydrated chitosan matrices and crystalline polymer inclusion complex microdomains (below T_{gel}), or gel melting (c) by supramolecular dissociation (above $T_{gel\text{-}m}$) (Huh et al., 2004).

water-solubility of chitosan. As depicted in Fig. 16(a), these supramolecular inclusion complexes were obtained as precipitates under neutral and alkaline conditions. But in acidic conditions, these inclusion complexes formed hydrogels with phase-separated structures, consisting of hydrophobic crystalline polymer inclusion complex domains, which were formed by the host–guest interaction between α-CD and poly(ethylene glycol), and hydrated chitosan matrices (Fig. 16(b)). When the hydrogels were heated up to above $T_{gel\text{-}m,}$ the crystalline polymer inclusion complex domains were found to be solubilized with dissociation of the supramolecular assembly (Fig. 16(c)). It was reported that these supramolecular hydrogels could be useful for biomedical applications because of their biocompatible constituents and supramolecular functionality, such as a thermo-reversible gel–sol transition property.

There is currently great interest on β-CD dimers because favorable cooperative binding effects, due to multivalency, may occur, leading to significantly higher binding constants compared to the monomeric species. The complexation properties toward adamantane-grafted chitosan of two β-CD homodimers, containing two CD moieties that are linked through their primary sides by one or two aliphatic spacers, were studied by rheological and dynamic light scattering measurements (Lecourt, Sinay, Chassenieux, Rinaudo, & Auzely, 2004). Very different behaviors were observed between these two CD molecules due to different molecular flexibilities and inclusion properties of their cavities. These results showed that a subtle change in the molecular architecture of such CD dimers dramatically changes their ability to physically cross-link modified chitosan chains.

4. Conclusions

CDs have received much attention because of their unique ability to form host–guest complexes with various organic compounds. Grafting CD molecules into chitosan leads to a molecular carrier exhibiting promising properties because of the cumulative effects of size specificity and the transport properties of CDs. Due to the CD moiety present in the chitosan backbone, it was found that β-CD grafted chitosan has some selectivity for the adsorption of TNS, bisphenol A, *p*-nonylphenol, and cholesterol and has the stronger inclusion and slow release ability with iodine. This CD grafted polymer has also confirmed the host–guest complex with *p*-nitrophenol, *p*-nitrophenolate, *tert*-butylbenzoic acid, 6-thiopurine, *p*-dihydroxybenzene, and copper ions. Due to the inclusion properties, the CD grafted chitosan was found to be useful in drug delivery, cosmetics, decontamination of waters containing textile dyes and metal ions, and analytical chemistry. It is clear from this review that the grafting of CD onto chitosan can result in an increase in the extent of complexation ability, sorption and controlled release properties. In addition, the grafting of CD onto chitosan can offer a variety of physicochemical advantages over the free CD including the possibility for increased stability, recoverability and reutilization. Owing to the increasingly globalized nature of the CD-related science and technology, development of the CD-based adsorbent matrices is also rapidly progressing. In addition to the role of the currently applied CD-linked chitosans, newer derivatives are constantly being developed and reported.

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