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Study on synthesis of β-cyclodextrin linked chitosan derivatives with different linkers and removal of dyes

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A Doctoral Dissertation
Division of Environmental Materials Science
Graduate School of Environmental Science
Hokkaido University
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Chapter 1

General Introduction

General Introduction

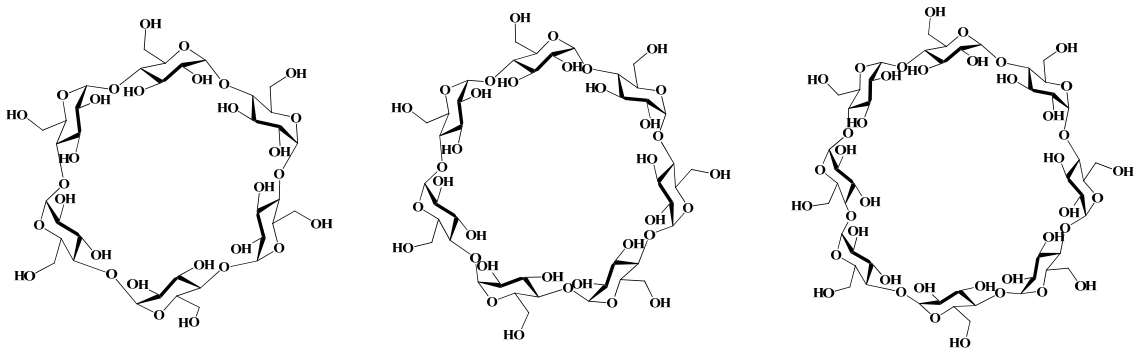
Water pollution due to toxic metals and organic compounds remains a serious environmental and public problem and also become a major source of concern and a priority for most industrial sector. Thailand's surface waters are being increasingly polluted, population growth, urbanization, agricultural activities and industrial expansion causing the quality of various water resources to continuously degenerate. Dyes, aromatic compounds and heavy metal ions are often found in the environment as a result of their wide industrial uses. The effluent dyes from different sources, e.g., textile industries, paper and printing production, leather tanning and food technology, are considered as a hazardous pollutants introduced into the natural water. Especially, the wastewaters originated from textile industry including various pollutants such as high organic materials and color depending on dyes in various structures. Most of these dyes are toxic and potentially carcinogenic, since textile industry is one of the most important industries in developing countries. The dyes removal from textile industrial effluents is one of important and unsolved environmental problem in Thailand. Recently, biomaterials have been widely used for removing dyes from wastewater due to its low cost. Therefore, the developments of more effective and easily available biomaterials are still in progress in order to improve their removal performance.

1.1 Cyclodextrin (CD)

1.1.1 Structure and properties

Cyclodextrins (CDs) are well-known as a series of cyclic oligosaccharides consisting of 6-12 D-glucopyranose units, which one covalently linked together by α-(1→4) glycosidic bonds. Each glucose unit has $^4C_1$ chair conformation and further

formed the cone-like structure of CDs. CDs are afforded by enzymatic degradation (α-1,4-glucan-glycosyltransferase for example) of starch, and various types of CDs are available by use of the particular glucosyl transferase, the nature of starch and the reaction conditions (e.g., the presence of precipitating agent). Typical native CDs contain six, seven and eight glucose units, named α-, β-, and γ-CD, respectively (Fig. 1). The primary hydroxyl groups are located at C-6 position of the glucopyranose group and at the narrow edge of the cone (primary hydroxyl rim). These hydroxyl groups can freely rotate on partially block. On the other hand, the secondary hydroxyl group at C-2 and C-3 are located at the wider edge (secondary hydroxyl rim), and are held rigidly and less prone to chemical transformation (Fig. 2) [1-3]. Several important physical parameters of those cyclodextrins are given in Table 1. The most attractive property of CDs is inclusion ability. The central cavity of the molecule is lined with skeletal carbons and ethereal oxygen of the glucose residue and hydrogen atoms at position C–3 and C–5. Therefore, the central cavity is relatively hydrophobic compared to medium aqueous solution and the glycosidic oxygen bridges possess a high electron density because of their acetal function. Since all of the hydroxyl groups are on the outside of the molecule, the outer surface is shows a hydrophilic behaviour. As a consequence of these features CDs, they can encapsulate a variety of hydrophobic molecules or part of molecules inside cavity. The affinity between the guests and CDs is non-covalent interactions which allow for the inclusion of several guest molecule types, bearing appropriate size, to form inclusion complexes of the host-guest type. Some properties of guest molecules change through the formation of inclusion complexes, e.g., the solubility and reactivity. This phenomenon constitutes the basic of most CDs applications for analytical and complexation purposes [3].



α-CD β-CD γ-CD

Fig. 1. Chemical structure of α-, β- and γ-cyclodextrins.

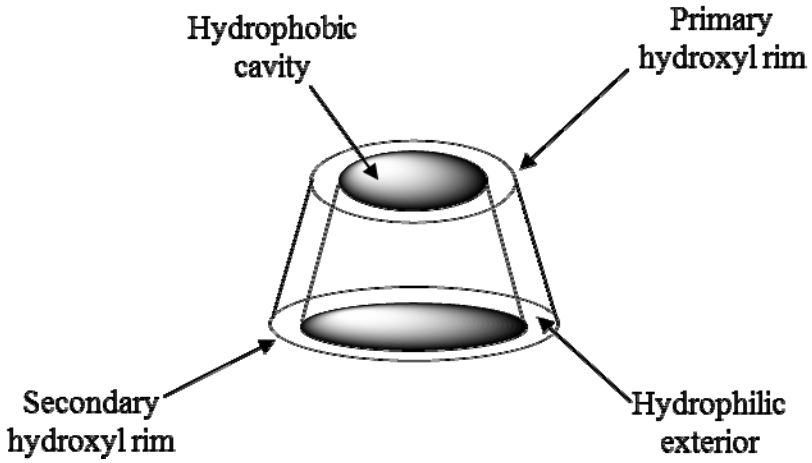


Fig. 2. Shape of the cyclodextrin molecules.

Table 1 Some physical parameters of cyclodextrins

Property	**α-CD**	**β-CD**	**γ-CD**
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Cavity diameter (Å)	4.7-5.2	6.0-6.4	7.5-8.3
Outer diameter (Å)	14.6	15.4	17.5
Height of torus (Å)	7.9	7.9	7.9
Cavity volume ($Å^3$)	174	262	427
Solubility in water at 25 °C (g/100 ml)	14.5	1.85	23.2
Water molecules in cavity	6	11	17
Crystal forms (from water)	Hexagonal plates	Monoclinic parallelograms	Quadratic prisms

1.1.2 Inclusion complex formation

The most notable feature of cyclodextrin is its ability to form solid inclusion complexes (host-guest complexes) with a wide range of solid, liquid and gaseous compounds by a molecular complexation [4]. In the complexes, a guest molecule is held within the cavity of the cyclodextrin host molecule. Complex formation is a dimensional fit between host cavity and guest molecule [5]. The lipophilic cavity of

cyclodextrin molecules provides a microenvironment which appropriately sized non-polar moieties can enter to form inclusion complexes [6]. No covalent bonds are broken or formed during formation of the inclusion complex [7]. The formation of inclusion complexes were performed by various kinds of intermolecular interactions (Van der Waals force, hydrophobic interaction, electrostatic interaction, hydrogen bonding and dipole-dipole interaction).

Inclusion in cyclodextrins exerts a profound effect on the physicochemical properties of guest molecules as they are temporarily locked or caged within the host cavity giving rise to beneficial modifications of guest molecules [8]. These properties are: enhance the solubility of highly insoluble guests, stability of labile guests against the degradative effects of oxidation, can be chromatographic separations, can be taste modify by masking off flavours and controlled release of drugs and flavours. Therefore, cyclodextrins are used in food, pharmaceuticals, cosmetics [9-10], environment protection, bioconversion, packing and the textile industry [11-12].

The potential guest list for molecular encapsulation in cyclodextrins is quite varied including the compounds with straight or branched chain aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and polar compounds such as halogens, oxyacids and amines [8]. Due to the availability of multiple reactive hydroxyl groups, the functionality of cyclodextrins is greatly increased by chemical modification. Through modification, the applications of cyclodextrins are expanded. CDs are modified through substituting various functional compounds on the primary and/or secondary face of the molecule. Modified CDs are useful as enzyme mimics because the substituted functional groups act in molecular recognition. The same

property is used for targeted drug delivery and analytical chemistry as modified CDs show increased enantioselectivity over native CDs [4].

The ability of a cyclodextrin to form an inclusion complex with a guest molecule mainly depends on two key factors. First, it depends on the relative size of the cyclodextrin and the size of the guest molecule, including the certain key functional groups within the guest. If the guest size is inappropriate, it will not fit properly into the cyclodextrin cavity. Second, the thermodynamic interactions between the different components of the system (cyclodextrin, guest, solvent) must be in agreement. There must be a favourable net energetic driving force that pulls the guest into the cyclodextrin.

As the height of the cyclodextrin cavity is the same among all three types (α-, β-, and γ-) the number of glucose units determines the internal diameter of the cavity and its volume. Based on these dimensions, α-cyclodextrin can typically complex low molecular weight molecules or compounds with aliphatic side chains, β-cyclodextrin will complex aromatics and heterocycles and γ-cyclodextrin can accommodate larger molecules such as macrocycles and steroids.

2.1 Chitosan

In recent year, removal of contaminants from wastewater was studied using various physicochemical and biological methods, adsorption has been found to be effective and interest technique for removing many contaminants from wastewater. Chitosan is a β-(1, 4)-linked polysaccharide of D-glucosamine prepared from

N-deacetylation of chitin, the second most abundant biopolymer in nature next to cellulose and a major component of the shells of crustacean organisms, such crabs and shrimps by deacetylating its acetamido group under an alkaline condition treated with boiling temperature allows the acetylated groups to be removed and the deacetylation degree to be increased. This chemical treatment leads to the formation of chitosan, a partially deacetylate from of chitin as in **Fig. 3**. Chitosan and its derivatives have been used in a wide range of application in the chemical, pharmaceutical, medical, and environmental fields, largely due to their many advantages such as being readily available, non-toxic, biocompartibility, biodegradability, anti-bacterial, and reactive as well as adsorptive [13-14]. Chitosan is recognized as effective biosorbent for various contaminants, including heavy metal ions or species, fluorides, dyes, phenol and its derivatives, and other pollutants [15-16]. Chitosan appears to be more useful as compared to chitin, since it has a large number amine and hydroxyl groups that can serve as chelating sites and can be chemically modified [17].

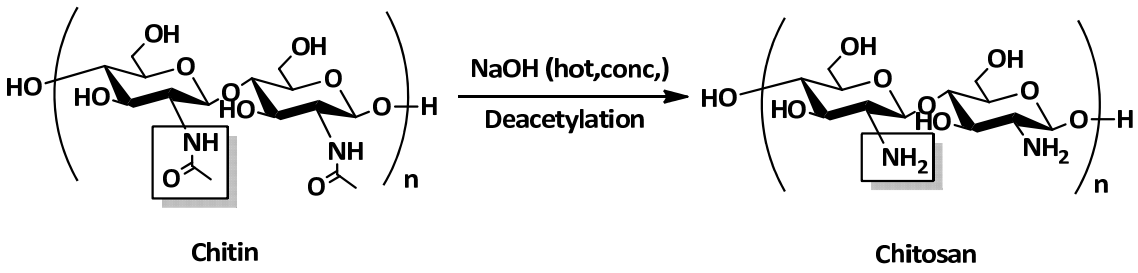


Fig. 3. Deacetylation of chitin.

1.1 Purpose of this studies

In order to create effective adsorbent material for the removal of dyes from water, β-CD linked chitosan derivatives having different linkers of C0 and C4 units were synthesized and subjected to inclusion property analysis.

This thesis describes the preparation of two difference β-CD linked chitosan derivatives, their structure analysis, and characterization of inclusion complex between both derivatives by determination of fluorescence and ^{1}H NMR spectroscopy. Furthermore, the application in adsorption process to removal dye pollutants was also studied.

Chapter 1, the literature reviews on cyclodextrin structure, properties and its inclusion complex ability with various guest molecules were described. Additionally, the application of chitosan was also described.

Chapter 2, the preparation of β-CD linked chitosan derivatives to have different linking arms of C0 and C4 units by reductive alkylation of the amino group of chitosan with β-CD aldehyde derivatives and the characterization by fluorescence spectroscopy was performed to investigate their inclusion properties with 6-(*p*-toluidino)-2- napthalene-6-sulfonate (TNS), as a guest compound, were described.

Chapter 3, an inclusion complex between β-CD linked chitosan derivatives with TNS were investigated by using ^{1}H NMR spectroscopy in aqueous solution. The stoichiometry confirmation of 1:1 inclusion complex by Job's method, were also described.

Chapter 4, the adsorption of Reactive Black5 (RB5) and Reactive Orange16 (RO16)

from aqueous solution using cross-link β-CD linked chitosan was performed by batch experiment. The adsorption isotherms, kinetics, the optimum pH, desorption, and regeneration of cross-link β-CD linked chitosan, were described.

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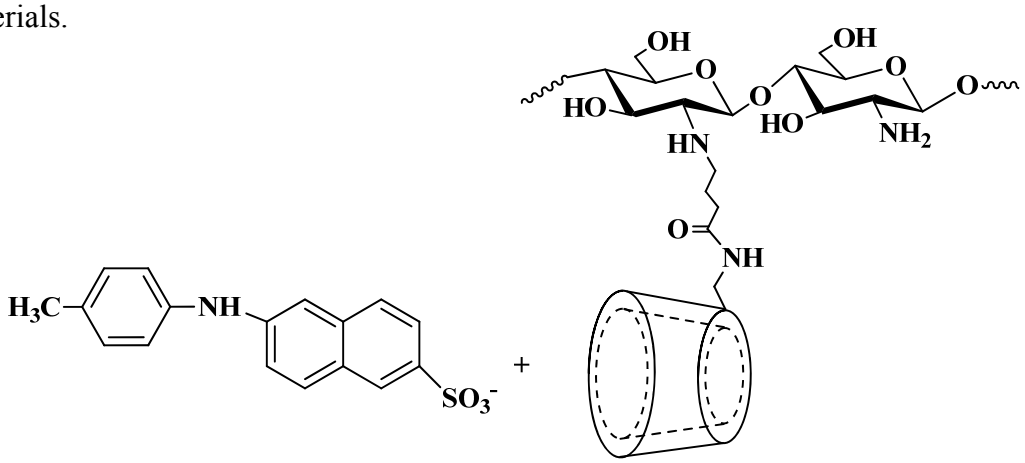
Chapter 2

Preparation and Characterization of β-cyclodextrin linked chitosan

Abstract

Reductive alkylation of the amino group of chitosan with β-cyclodextrin (CD) aldehyde derivatives, i.e., 6-deoxy-6-(4-oxobutyramido)-β-CD and 6-oxo-β-CD, gave two β-CD-linked chitosan derivatives with C4 (4-butylamido) and C0 linking arms, respectively. Degree of substitution (D.S.) of both C4-β-CD and C0-β-CD linked chitosan was controlled by the ratio of starting materials. The structures of the products were confirmed by ^{1}H and ^{13}C NMR and FT-IR spectra. Their inclusion properties of C4-β-CD (D.S. 18%) and C0-β-CD linked chitosan (D.S. 17%) with a fluorescent probe, 6-(*p*-toluidino)-2-napthalene-6-sulfonate (TNS) were investigated in acetate buffer (pH 4.3) at 25 ℃. Continuous variation of Job's method revealed that the stoichiometry of inclusion complex of C4-β-CD linked chitosan-TNS was 1:1, whereas that of C0-β-CD linked chitosan was not 1:1. The stability constant of C4-β-CD linked chitosan determined by Benesi–Hildebrand plot was 2.3×10^{3} M^{-1}. The finding of this study suggested that length of the linking arms between CD and chitosan is influenced on the inclusion property and provides further step for designing the environmental friendly materials.



6-(p-toluidino)-2-napthalene-6-sulfonate (TNS)

C4-β-CD linked chitosan

2.1 Introduction

Cyclodextrins (CDs), consisting of 6 to 8 D-glucopyranose units through α-(1→4)-glycosidic bonds, are regarded as a family of the most characterized supramolecular host compounds. Their torus molecular structure creates hydrophilic outer surfaces which results in water solubility. The lipophilic inner cavity of CDs is able to interact with a wide variety of guest molecules forming non-covalent inclusion complexes [1, 2]. Due to the rigidity and chirality of the hydrophobic cavity, CDs can form a tight inclusion complex with some guest molecules through such intermolecular interactions by van der Waals forces and hydrogen bonding. Polymers grafting CD residues represent a unique class of host materials with tunable inclusion properties, and are recently received much attention in connection with the development of drug delivery system [3], materials for nanotechnology [4], adsorbents for water-treatment [5-9], hydro gel [10], and so on.

Chitosan obtained by alkaline *N*-deacetylation of chitin, a major component of crab and shrimp shell, is a polysaccharide of β-(1→4) linked 2-amino-2-deoxy-D-glucopyranose. Chitosan has been regarded attractive and versatile biomaterials because of its unique characters such as non-toxic, biocompatible, and biodegradable. The range of its applications has recently been enormously expanded in various fields including biotechnology, water-treatment, membranes, cosmetics, food industry, and medicine [11].

Based on our research [12], we synthesized CD-linked chitosan derivatives through modification of highly reactive amino group of chitosan [13-15]. Furthermore, syntheses and investigation of the inclusion ability of various types CD-polymers have

been reported recently [16-19]. These results revealed their physical properties such as inclusion ability and water solubility are highly affected by degree of substitution (D.S.) and the structure of introduced CD moieties. Since alkylation of CDs changes their physic-chemical property, our interest focused on the linking arms between β-CD and chitosan. In this paper, we described preparation of β-CD linked chitosan derivatives having different linking arms of C0 and C4 units and investigation on their property to form inclusion complex with 6-*p*-toluidinyl-napthalene-2-sulfonate (TNS) as a guest compound, TNS structure was shown in Fig. 1.

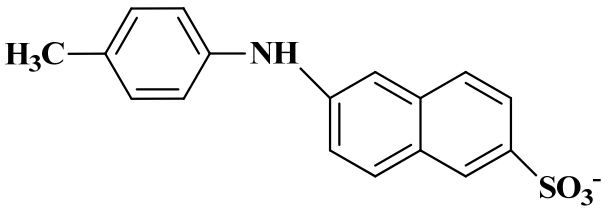


Fig. 1. Structure of 6-*p*-toluidinyl-napthalene-2-sulfonate (TNS)

2.2 Experimental

2.2.1 Materials and methods

β-Cyclodextrin (β-CD) with purity of ≥ 98% was purchased from Nihon Shokuhin Kako Co. Ltd (Tokyo, Japan). Chitosan with average molecular weight of 70 kDa and degree of deacetylation of 85% was purchased from Hokkaido Soda Co. Ltd (Tomakomai, Japan). 6-(*p*-Toluidino)-napthalenesulfonic acid potassium salt (TNS) was purchased from Sigma-Aldrich Japan G.K. (Shinagawa, Japan). All other reagents were preparative grade, and purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). These materials were used without further purification. Water was purified by treatment with ion-exchanger and subsequent reverse osmosis with a Millipore Millex-3 apparatus. ^{1}H and ^{13}C NMR spectra were recorded in D_2O on a Bruker ASX-300 spectrometer operated at 300.13 and 75.3 MHz, respectively. Fourier transform IR spectra were recorded on a Horiba FT-210 spectrophotometer using KBr pellets. Fluorescent spectra were recorded on a Hitachi F-4500 spectrometer in a standard quartz cell.

2.2.2 *Preparation of 6*-O-*tosyl-β-CD* ***2***

6-*O*-Tosyl-β-CD **2** was prepared by the methods previously described by [20]. Fine-powdered *p*-toluenesulfonic anhydride (Ts_2O: 10 g, 31.62 mmol) was added to a solution of β-CD (**1:** 18.9 g, 16.65 mmol) in water (500 ml) at room temperature, and the mixture was stirred vigorously at room temperature overnight. An aqueous solution of NaOH (10% w/v, 100 ml) was added dropwise to the mixture. The reaction mixture was stirred at room temperature for 20 min, and filtered unreacted Ts_2O with a

glass filter. The filtrate was adjusted to pH 8 with NH_4Cl and stored overnight in a refrigerator at 4 °C. The precipitate was filtered and recrystallized at room temperature from *n*-BuOH-EtOH-H_2O (5:4:3 v/v/v) to give 6-Ts-β-CD (**2**: 6.31 g, 29% yield) as white solid.

^{1}H NMR (DMSO-d_6) δ: 2.4 (s, 3H, CH_3), 3.41-3.74 (m, H-3, 5, 6) overlap with HOD, 4.12-4.53 (m, 6H, C6–OH), 4.75 (s, 7H, H–1 of CD), 7.38-7.80 (dd, 4H, aromatic tosyl group).

2.2.3 Preparation of 6-amino-6-deoxy-β-CD **4**

A solution of 6-Ts-β-CD (**2**: 2.33 g, 1.80 mmol) and sodium azide (1.50 g, 23.07 mmol) in water (25 ml) was stirred at 80 °C for 5 h. The resulting solution was added dropwise into acetone (150 ml) with stirring. The precipitate formed was filtered and dried in vacuum at 60 °C for 24 h to give 6-azido-6-deoxy-β-CD **3**, which was dissolved in DMF (25 ml). After addition of triphenylphosphine (0.75 g, 2.86 mmol), the mixture was stirred at room temperature for 2 h. Aqueous ammonium hydroxide (25% w/v, 5 ml) was added dropwise to the solution, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into ethanol (100 ml) with vigorous stirring. The solid obtained was purified by column chromatography on CM Sephadex C-25 (NH_4^+ form) with gradient elution from water to 1 M NH_4OH to give 6-amino-6-deoxy-β-CD (**4:** 1.00 g, 49% yield).

^{1}H NMR (DMSO-d_6) δ: 6.08-5.52 (m, 14H, OH–2, 3), 5.11-4.72 (m, 7H, H–1), 4.67-4.25 (m, 6H, OH–6), 3.89-3.49 (m, H–3, 5, 6), 3.10-3.49 (m, H–2, 4 overlap with HDO), 3.02-2.63 (m, $–NH_2$).

2.2.4 Preparation of 6-deoxy-6-(4-pentenoyl)amido-β-CD ***5***

1-Hydroxybenzotriazole monohydrate (HOBt: 0.14 g, 0.91 mmol), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (WSC: 0.11 g, 0.57 mmol), and 4-pentenoic acid (0.25 ml, 2.497 mmol) were successively added to dimethylsulfoxide (DMSO: 2 ml), and the mixture was stirred at room temperature for 10 min to get a reagent solution. Subsequently, 6-amino-6-deoxy-β-CD (**4:** 0.28 g, 0.25 mmol) was added to this reagent solution and stirred at room temperature overnight. The reaction mixture was precipitated by adding drop wisely to BuOH-EtOAc (1:1 v/v, 100 ml) with stirring. The precipitate formed was filtered and washed with acetone. The product was subjected to ion exchange chromatography on CM Sephadex C-25 (NH_4^+ form) with gradient elution from water to 1 M aqueous NH_4OH. Further purification was performed with reversed phase chromatography using Diaion HP-20 with gradient elution water-methanol to obtain 6-deoxy-6-(4-pentenoyl)amido-β-CD (**5:** 0.275 g, 92% yield).

^{1}H NMR (DMSO-d_6) δ: 7.74 (s, 1H), 5.91-5.60 (m, 1H), 5.07-4.74 (m, 9H), 3.80-2.99 (m, 41H), 2.30-2.04 (s, 4H).

*2.2.5 Preparation of C4-β-CD linked chitosan **8***

Ozone was bubbled through a solution of 6-deoxy-6-(4-pentenoyl)amido-β-CD (**5:** 1.5 g, 1.23 mmol) in 20% aqueous methanol (15 ml) with stirring at 0 °C for 1 h. After bubbling oxygen gas for several minutes to purge dissolving ozone, dimethyl sulfide (1 ml) was added to the solution and the solvent was removed by evaporation under reduced pressure. The crude aldehyde **6** (1.5 g, 1.23 mmol) was dissolved in chitosan solution (280 mg, 1.74 mmol) of 2% v/v aqueous acetic acid (15 ml) and methanol (5 ml). To the mixture was added sodium cyanoborohydride (150 mg), and stirred at room temperature overnight. The resulting solution was subjected to ultrafiltration using a membrane with molecular weight cut-off 20,000, washed with water, and lyophilized to give the C4-β-CD linked chitosan (**8:** 0.175 g, 37%yield) as white cotton-like hygroscopic amorphous (D.S. 18%).

^{1}H NMR (D_2O/CD_3COOD, 60 °C) δ: 5.50-5.10 (m, H–1 of β-CD), 4.75 (s, H–1 of chitosan overlap with HDO), 4.10-3.50 (m, H–2-H–6 of β-CD overlap with H–3-H–6 of chitosan), 3.00 (s, H–2 of chitosan).

^{13}C NMR (D_2O/CD_3COOD, 60 °C) δ: 178.92, 100.55, 78.93, 76.12, 72.78, 61.71, 57.49, 22.53. FT-IR (KBr, cm^{-1}): 3424 (O–H), 2926, 2878 (C–H str), 1635 (H–O–H), 1155 (C–O–C str), 1026 (C–O str).

Elemental analysis: calculate for $[C_6H_{11}O_4N]_{0.82}\cdot[C_{52}H_{85}O_{39}N_2]_{0.18}\cdot 1.5H_2O$: C 42.41, H 6.81, N 4.08%; found: C 42.23, H 7.31, N 3.95%.

2.2.6 Preparation of 6-oxo-β-CD ***9***

A solution of 6-Ts-β-CD (**2:** 500 mg, 0.38 mmol) and γ-collidine (1 ml) in DMSO (5 ml) was stirred at 135 °C for 2 h. After cooling to room temperature, the mixture was poured into acetone (100 ml), stirred and then the precipitate was collected by glass filter. The obtained Crude 6-oxo-β-CD (**9:** 400 mg, ca. 0.35 mmol) was dried in vacuum and used in the downstream reaction without further purification.

2.2.7 Preparation of C0-β-CD linked chitosan ***11***

Crude 6-oxo-β-CD (**9:** 300 mg, ca. 0.26 mmol) was treated with chitosan (150 mg, 0.93 mmol) and $NaBH_3CN$ (24 mg, 0.38 mg) in the same protocols as described for preparation of C4-β-CD linked chitosan **8** to give C0-β-CD linked chitosan (**11:** 160 mg) as hygroscopic amorphous (D.S. 17%).

^{1}H NMR (D_2O/CD_3COOD, 60 °C) δ: 6.19-5.78 (m, H–1 of β-CD), 5.31 (s, H–1 of chitosan), 4.80-4.20 (m, H–2-H–6 of β-CD overlap with H–3-H–6 of chitosan and HDO), 3.64-3.33 (s, H–2 of chitosan).

^{13}C NMR (D_2O/CD_3COOD, 60 °C) δ: 102.63, 82.03, 79.09, 74.36, 74.04, 72.99, 61.33, 57.44. FT-IR (KBr, cm^{-1}): 3440 (O–H), 2928 (C–H str), 1639 (H–O–H), 1155 (C–O–C str), 1026 (C–O str).

Elemental analysis: calculate for $[C_6H_{11}O_4N]_{0.83}\cdot[C_{48}H_{79}O_{38}N]_{0.17}\cdot 2.4\ H_2O$: C 40.02, H 6.99, N 3.55%; found: C 40.05, H 6.74, N 3.50%.

2.2.8 Fluorescence spectroscopic analysis

All sample solutions for fluorescent analysis were prepared by using 0.1 M acetate buffer at pH 4.3. A guest stock solution (3.18×10^{-5} M) was prepared by dissolving 1.17 mg of TNS in 100 ml the buffer solution and a guest dilution solution of TNS (1.06×10^{-5} M) was prepared by mixing the stock solution and the buffer solution. Three host stock solutions (5×10^{-3} M) of β-CD, C0-, and C4-β-CD linked chitosan were prepared by dissolving 57 mg of β-CD, 104 mg of C0- (**11**, D.S. 17%) and 106 mg of C4-β-CD linked chitosan (**8**, D.S. 18%) in 10 ml of the buffer solution, respectively. An analytical sample was prepared by mixing 3.4 ml of the guest stock solution and 6.6 ml stock solution of. Fluorescence titration for inclusion property was performed by use of samples prepared by mixing the analytical sample with the appropriate amount of the dilution solution. All samples were freshly prepared before fluorescence measurement. Fluorescence spectra were measured in a conventional quartz cell ($10 \times 10 \times 45$ mm) at 25 °C on a Hitachi F-4500 spectrometer at excitation wavelength of 360 nm.

2.2.9 Determination of complexation stoichiometry

The stoichiometry of inclusion complex was determined by the continuous variation of Job's method [21]. Samples were prepared from the stock solutions, keeping total TNS and β-CD derivatives constant (1.06×10^{-5} M) and varying their molar ratio from 0 to 1. After stirring the solutions for 10 min, the fluorescence emission was measured in the same wavelength as described above.

2.3 Results and discussion

2.3.1 Preparation of C4- and C0-β -CD linked chitosan

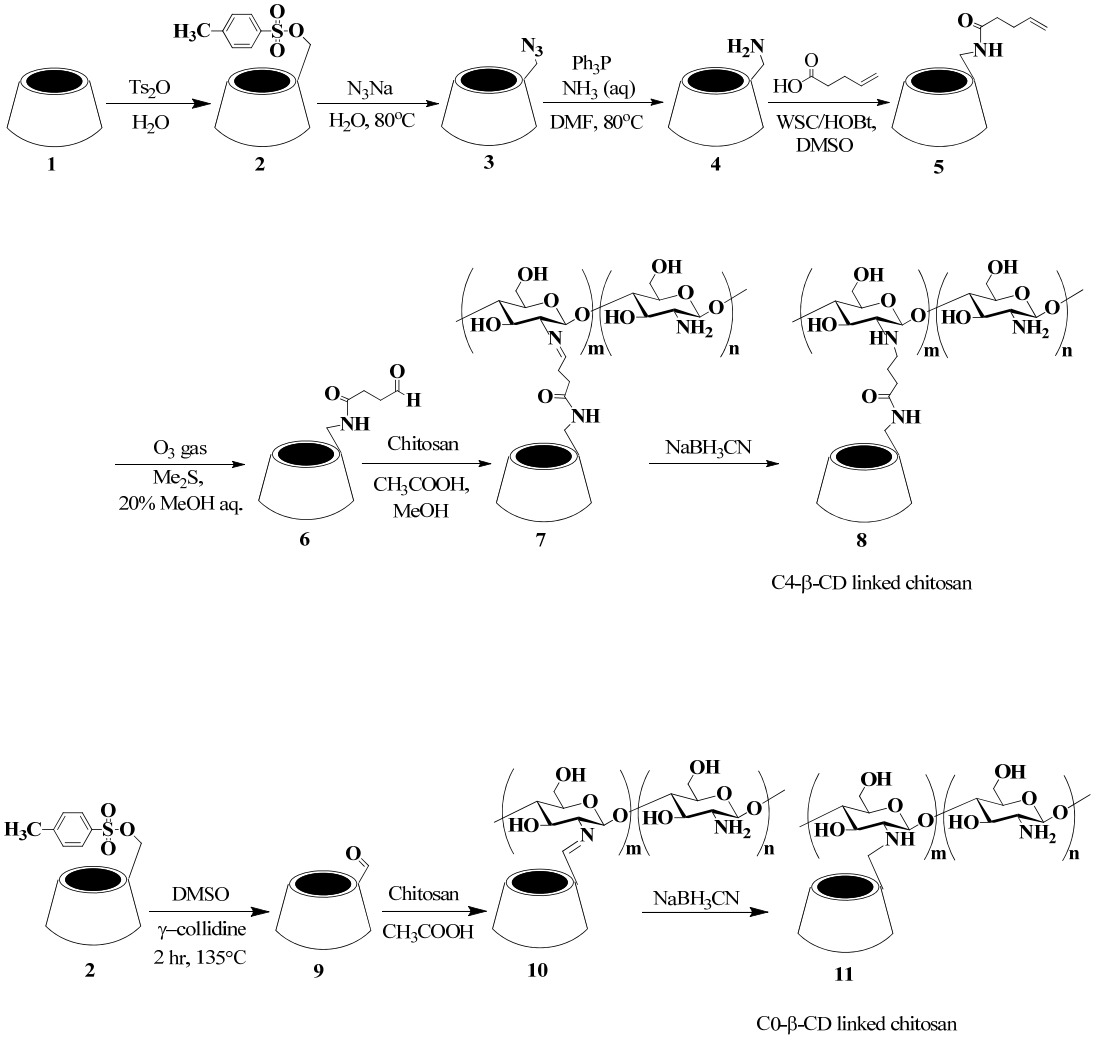
Synthesis of β-CD linked chitosan derivatives with and without a linking moiety of C4 unit (**8** and **11**) were performed using reductive *N*-alkylation as shown in Scheme 1. C4-β-CD linked chitosan **8** has a 4-butylamido group linking between β-CD and chitosan, whereas C0-β-CD linked chitosan **11** linked directly both residues. Since 6-*O*-*p*-toluenesulfonyl-β-CD (**2**: TsCD) is one of the most versatile derivatives for the modifications on the primary hydroxyl groups of β-CD, was used as a common starting material for **8** and **11** in our study. According to a slightly modified procedure of the method described [20], β-CD **1** was treated with *p*-toluenesulfonic anhydride and in aqueous sodium hydroxide to give crystalline TsCD **2** in 29% yield. The first step of the modification of 6-position was initiated by nucleophilic substitution of **2** with azide ion to give the mono(6-azido-6-deoxy)-β-CD **3**, which underwent Staudinger reaction [22]. Thus, **3** was treated with triphenylphosphine to give a phosphoimine derivative, which underwent basic hydrolysis in aqueous ammonia to give 6-amino-6-deoxy-β-CD **4**. The amino group in **4** was converted into 6-deoxy-6-(4-pentenoyl)amido derivative **5** by WSC-mediated condensation with 4-pentenoic acid. Ozonolysis of the alkene function in **5** and subsequent reductive work-up with dimethylsulfide gave the aldehyde **6**. On the other hand, preparation of 6-oxo-β-CD **9** was performed by an oxidation procedure of TsCD **2** reported by [23]. Thus, TsCD **2** was heated in DMSO at 135 °C in the presence of γ-collidine providing the 6-oxo-β-CD **9**. Because both aldehyde intermediates **6** and **9** were unstable, these intermediates were precipitated from the reaction mixture in acetone and used for the next coupling without further purification.

Having key β-CD intermediates with an aldehyde function, we conducted reductive *N*-alkylation of chitosan, which is known to be carried out under slightly acidic and homogeneous conditions [24-26]. An aqueous acetic acid solution of chitosan and the aldehydes **6** or **9** was treated with sodium cyanoborohydride, giving C4-β-CD linked chitosan **8** and C0-β-CD linked chitosan **11** (Scheme 1). The products were purified by ultrafiltration through a membrane with molecular weight cut-off 20,000 and lyophilization.

The structure of chitosan, β-cyclodextrin and the present of derivatives were characterized by FT–IR and ^{1}H–NMR spectroscopy. Fig. 2 shows the FT–IR spectra of β-CD, Chitosan, C4-β-CD and C0-β-CD linked chitosan. Fig. 2a shows the spectrum of β-CD at wavenumber 3546–3206, 2929 and 1634 cm^{-1} corresponding to O–H stretching, C–H stretching vibration and H–O–H bending, respcetively. The spectrum band at wavenumbers 1154, 1083 and 859 cm^{-1} assigned to strong C–O–C, C–O stretching vibration and skeleton vibration involving α-(1→4)-glucopyranose linkage in β-CD, respectively. Fig. 2b shows the spectrum of 85% deacetylated chitosan at wavenumber 3437 cm^{-1} assigned to O–H and the aliphatic C–H stretching between 2900 and 2854 cm^{-1}. The spectrum band at wavenumber 1649 cm^{-1} represents acetylated amino group of chitin, which confirms that this chitosan is not fully deacetylated and 1383 cm^{-1} assigned to C–O stretching of primary of alcoholic (CH_2–OH). The spectrum band at wavenumber 1159–1016 and 667 cm^{-1} assigned to free primary amino group (–NH_2) at C-2 position and pyranoside ring stretching vibration, respectively [27]. Fig. 2c shows the spectrum of C4-β-CD linked chitosan demonstrated stronger band at wavenumbers 2926 and 2878 cm^{-1} assigned to C–H stretching of the methyl group than that of C0-β-CD linked chitosan (Fig. 2d). The O–H stretching was shown at wavenumber

3424 cm^{-1} for C4-β-CD, while the spectra band of C0-β-CD exhibited at 3440 cm^{-1}. Additional, the characteristic spectrum of the α-pyranyl vibration of β-CD at 942 cm^{-1} and the characteristic spectrum of the β-pyranyl vibration of chitosan at 850 and 861 cm^{-1} both appeared in the IR spectra of C4-β-CD and C0-β-CD linked chitosan, respectively.

Scheme 1. Synthesis of C4 linked chitosan **8** and C0-β-CD linked chitosan **11**.



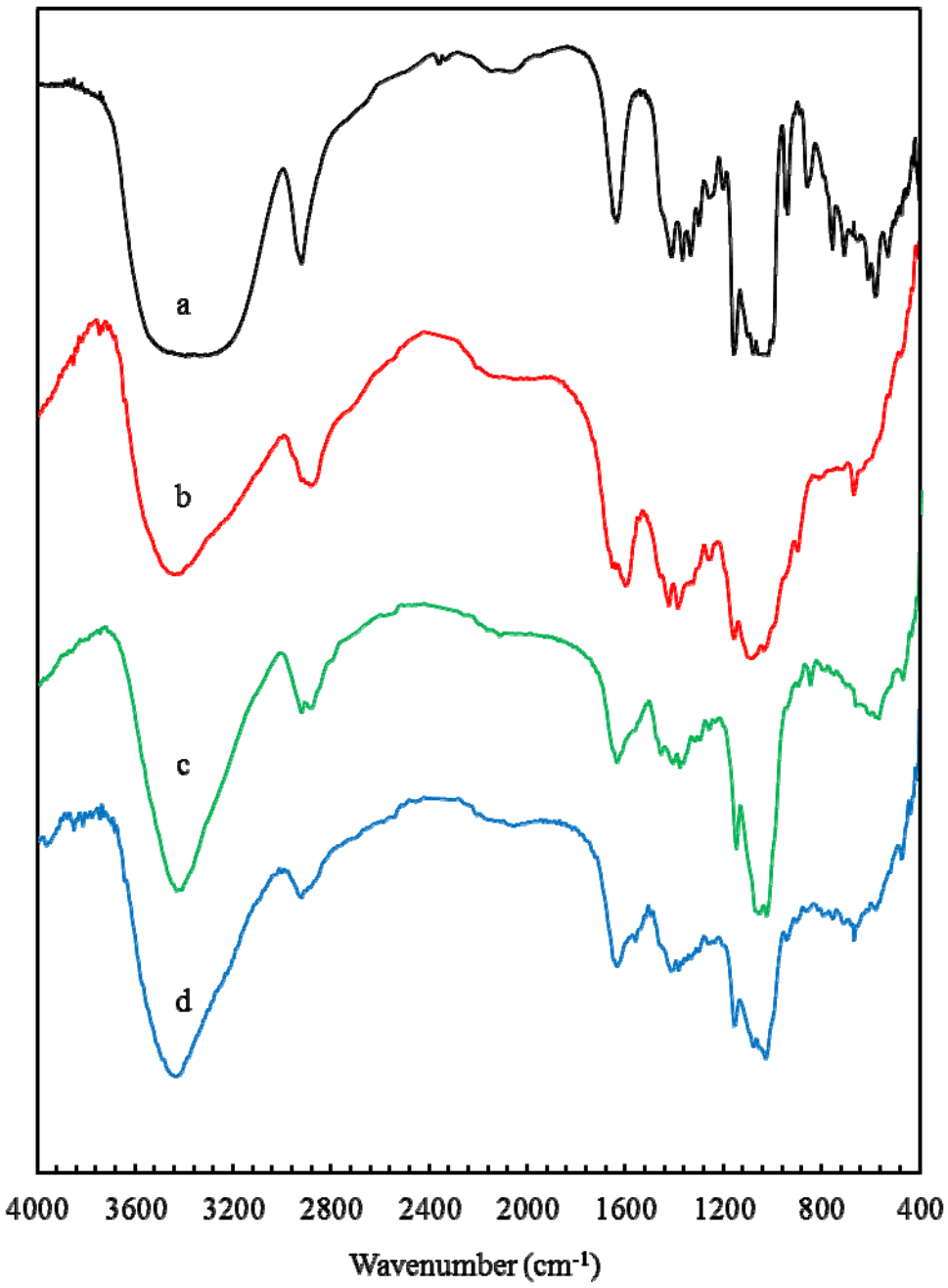


Fig. 2. FT-IR spectra of (**a**) β-CD, (**b**) chitosan, (**c**) C4-β-CD linked chitosan **8** (D.S.18%) and (**d**) C0-β-CD linked chitosan **11** (D.S.17%).

Fig. 3 shows the ^{1}H and ^{13}C NMR spectrum of 6-*O*-*p*-toluenesulfonyl-β-CD **2** (TsCD) and C0-β-CD linked chitosan **11**. Fig. 3a shows ^{1}H spectra of TsCD in DMSO-d_6. The double of doublet peak at δ 7.38–7.80 ppm was assigned to the aromatic protons of the tosyl group. The peak at 4.75 ppm was attributed to H–1' proton of the substituted CD. The multiplet peak at 4.12–4.53 ppm was corresponded to the methylene protons on C6–OH, that substituted by tosyl group. The multiplet peak at 3.41- 3.74 ppm assigned to H–3, H–5, H–6 of CD. The singlet peak at 2.4 ppm corresponded to the methyl protons from aromatic moiety at para–position. Fig. 3b shows ^{1}H spectra of C0-β-CD linked chitosan **11** in D_2O/CD_3COOD at 60 °C. The multiplet peak at δ 5.78–6.19 ppm was assigned to anomeric proton H–1 of α-D-glucopyranosyl residues in the β-CD moiety. The singlet peak at 5.31 ppm was attributed to H–1' of 2-amino-2-deoxy-β-D-glucopyranosyl residues in the chitosan skeleton. The multiplet peak at 3.33–3.64 ppm was corresponded to H–2' of glucosamine units. The disappearance of aromatic protons peak of the tosyl group at 7.38–7.80 ppm was suggested that oxidation of OH group at C-6 position of β-CD completely proceeded and that β-CD moiety was successfully introduced by reductive *N*-alkylation. Although similar C0-β-CD linked chitosan was synthesized by nucleophilic substitution of **2** with amino group of chitosan showing the presence of unreacted tosyl group [28], the purity of our product **11** suggested the advantage reductive *N*-alkylation route. Fig. 4 shows the ^{1}H and ^{13}C NMR spectrum of C4-β-CD linked chitosan **8** in D_2O/CD_3COOD at 60 °C. Anomeric protons of glucopyranose moieties of β-CD were appeared at 5.1–5.5 ppm as multiplet peaks, and that of chitosan was shown at 4.75 ppm as a broad singlet peak.

The degree of substitution (D.S.) of the CD residue was elucidated from C/N of elemental analysis and from the area ratio between anomeric protons of CD and CS in ^{1}H NMR spectrum. The D.S. results are summarized in Table 1 and indicated D.S. values obtained from elemental analysis. Although these results of C0-β-CD, and C4-β-CD linked chitosan showed good agreement, peak broadening and overlapping signal made it difficult to elucidate by NMR spectroscopy. The D.S. of C4-β-CD linked chitosan **8** was changeable by the molar ratio of starting chitosan and β-CD derivatives used. When 10 molar equivalent excess of β-CD derivative **6** was used to the glucosamine residue, D.S. reached to 82%. Similar results were obtained in synthesis of C0-β-CD linked chitosan. In our previous studies, C2-β-CD chitosan that has an ethylene link showed that β-CD linked chitosan with D.S. 20% was swelling in water and that compound with D.S. more than 36% was soluble in water [14]. By contrast, C0-β-CD linked chitosan with D.S. less than 40% was swelling and that with D.S. 68% was soluble in water. These results are agreed with the results reported by [29] that the D.S. increased with an increasing of mole ratio of Ts-CD. On the contrary, solubility of C4-β-CD linked chitosan was found to be markedly increased. Thus, C4-β-CD linked chitosan with D.S. 9% was swelling and all compounds with D.S. more than this ratio were soluble in water. These experiments suggested that chain length of the linking arm between chitosan and CD moiety was influenced on the physical property of the products. Moreover, the conformational flexibility and distance of the CD residue probably changed its water solubility.

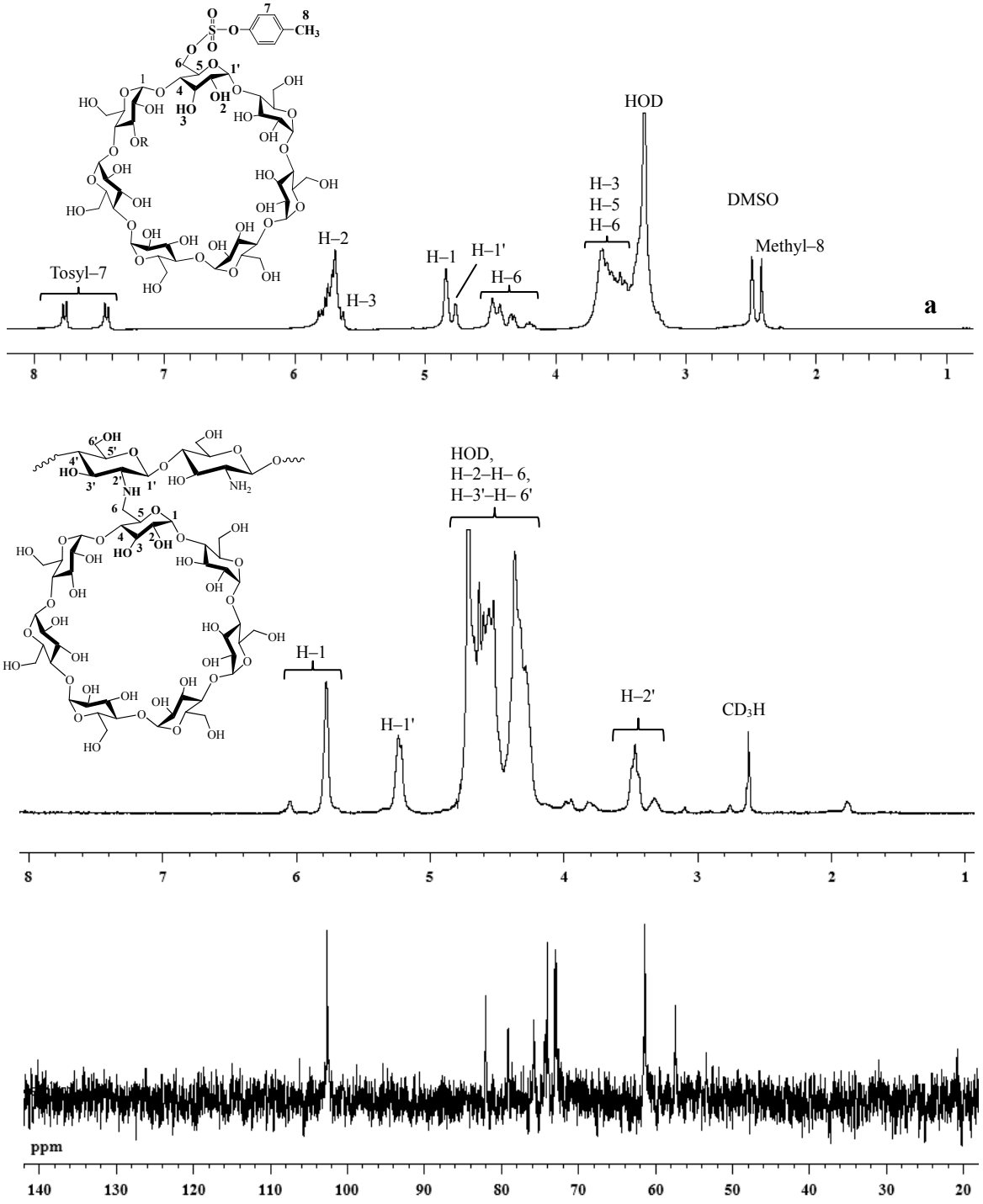


Fig. 3. ^{1}H NMR spectra of (**a**) 6-*O*-p-toluenesulfonyl-β-CD **2** (TsCD) in DMSO-d_6, (**b**) ^{1}H and ^{13}C NMR spectra of C0-β-CD linked chitosan **11** (D.S.17%) in D_2O/CD_3COOD at 60℃.

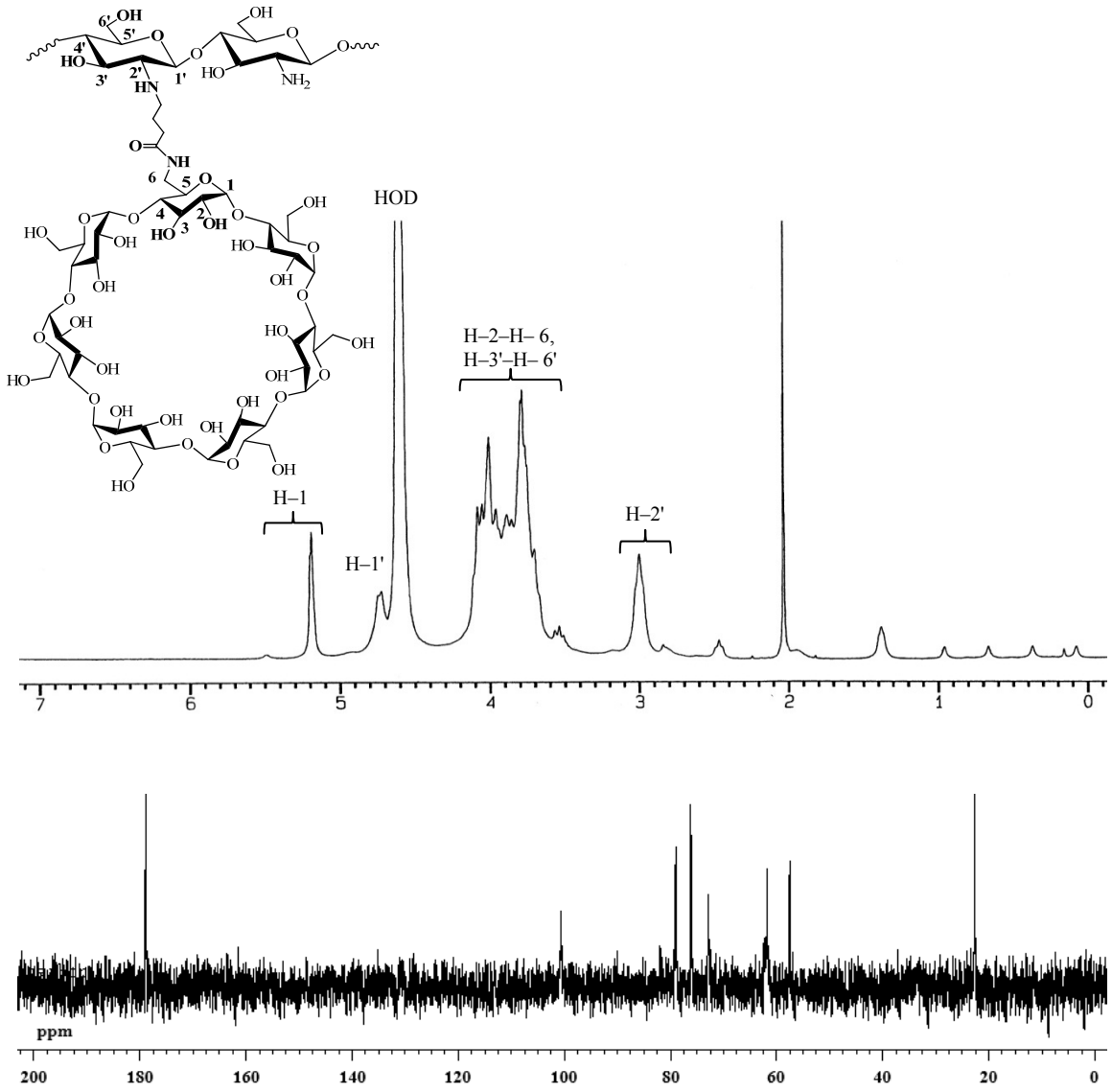


Fig. 4. ^{1}H and ^{13}C NMR spectra of C4-β-CD linked chitosan **8** (D.S.18%) in D_2O/CD_3COOD at 60℃.

Table 1 Preparation of C0-β-CD and C4-β-CD linked chitosan

Bridging residue	**Molar ratio of the starting materials, CD : chitosan**	**DS% [a)]**
C4	1:3	9
	1:2	11
	1:1.4	18
	1:1	41
	10:1	82
C0	0.2:1	9
	0.28:1	17
	0.4:1	27
	0.8:1	42
	1.2:1	68

a) Degree of substitution was determined by elemental analysis

2.3.2 Fluorescence spectroscopic analysis of inclusion complex

To confirm inclusion complex behavior of synthesized β-CD derivatives, the inclusion complex property of C0-β-CD linked chitosan (D.S. 17%) and C4-β-CD linked chitosan (D.S. 18%) was investigated using a fluorescence probe, 6-(*p*-toluidino)-2-napthalene-6-sulfonate (TNS) compared with the original β-CD. The fluorescence intensity of TNS is known to be quite low in aqueous solution but strongly increased in hydrophobic environment [28]. The fluorescence spectral titration of the inclusion complex formation was carried out in acetate buffer (pH 4.3) at 25 °C. As shown in Fig. 5, the addition of parent β-CD, C0-β-CD, and C4-β-CD linked chitosan leads to the fluorescence intensity of TNS increased; moreover, a strong increase of TNS fluorescence was recorded in the presence of C0-β-CD and C4-β-CD linked chitosan. The emission maximum wavelength of TNS showed significant blue shifts to 455, 445 and 443 nm in addition of β-CD, C0-β-CD and C4-β-CD linked chitosan, respectively. These results can be attributed to the cooperative binding of the β-CD and its derivatives on inclusion complexes. Consequently, the environment of TNS molecules was changed from polar aqueous solution to embed into hydrophobic cavity of β-CD and its derivatives. As shown in Fig. 6, the addition of β-CD (Fig. 6a) leads the intensity of TNS increased gradually, while the relative fluorescence intensity of TNS was higher when the concentration of C4-β-CD linked chitosan (D.S. 18%) (Fig. 6b) increased compared to the intensity obtained from C0-β-CD linked chitosan (D.S. 17%) (Fig. 6c), as the water solubility of both C0-β-CD and C4-β-CD linked chitosan in the inclusion complex with TNS was equalized in acidic condition. This indicated that the inclusion complex behavior might dependent on the structural feature of derivatives and the hydrophobic part of TNS preferentially insert to the hydrophobic cavity of C4-β-CD

linked chitosan rather than the C0-β-CD linked chitosan.

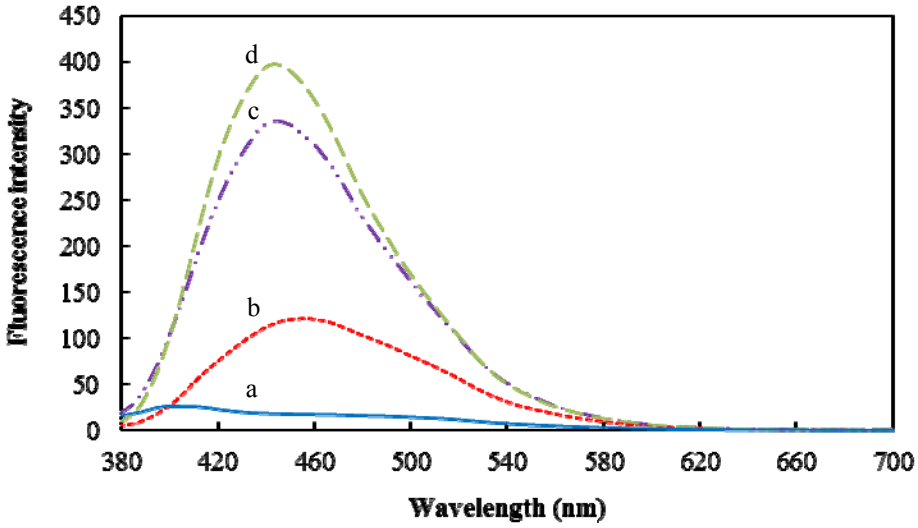


Fig. 5. Fluorescence spectra of TNS with β-CD and derivatives in acetate buffer (pH 4.3) at 25°C. [TNS] = 1.60×10^{-5} M and [β-CD], [C0], and [C4-β-CD linked chitosan] = 2.22×10^{-3} M; (**a**) Fluorescence spectra of TNS; (**b**) in the presence of β-CD; (**c**) in the presence of C0-β-CD linked chitosan (D.S.17%); and (**d**) in the presence of C4-β-CD linked chitosan (D.S.18%).

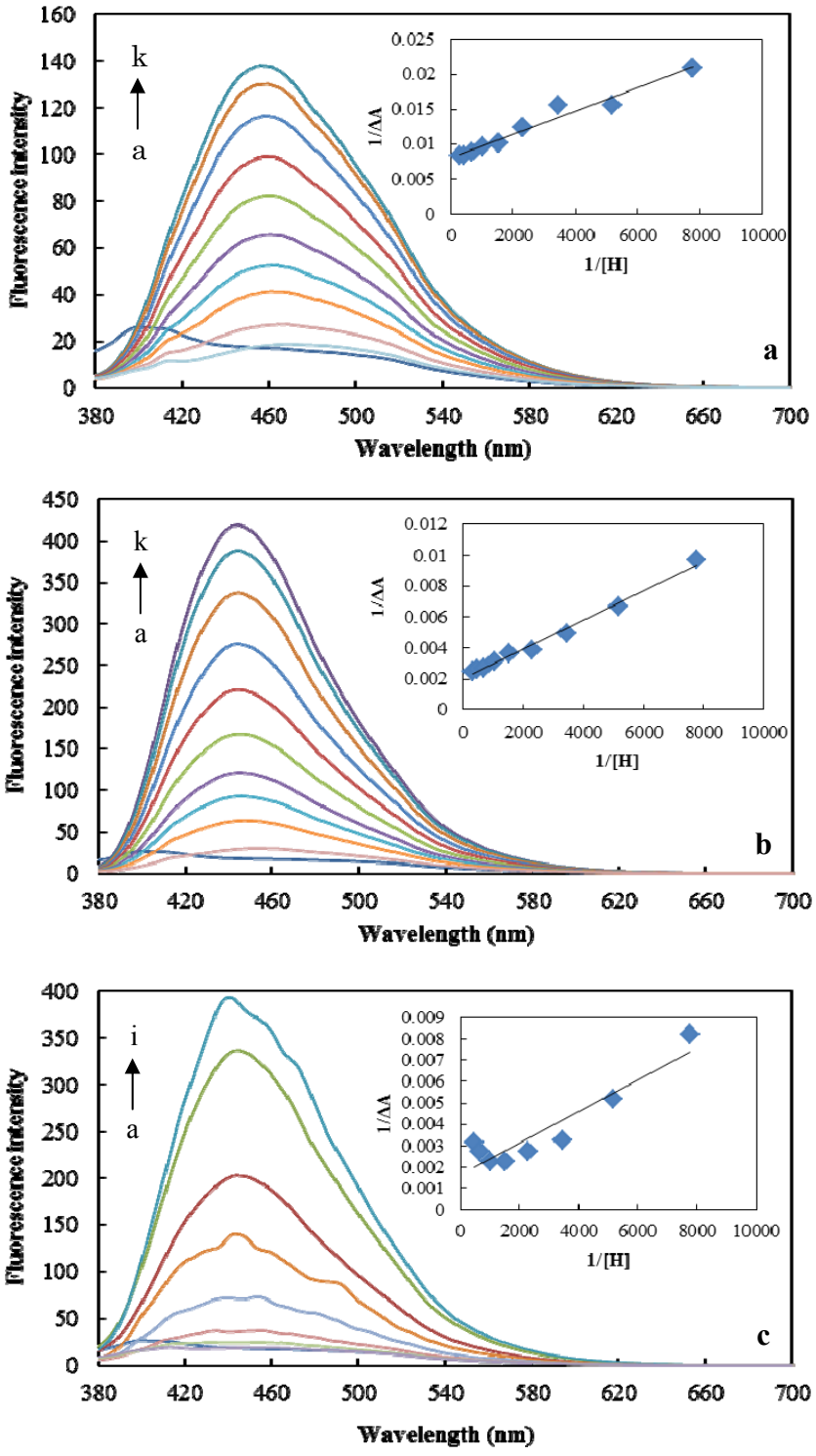


Fig. 6. Fluorescent titration of TNS (1.06×10^{-5} M) in acetate buffer (pH 4.3) at 25 °C on addition of (**a**) β-CD, (**b**) C4-β-CD linked chitosan (D.S.18%) and (**c**) C0-β-CD

linked chitosan (D.S.17%) with various concentration: (from a to k = 0, 2.54×10^{-5}, 5.72×10^{-5}, 8.60×10^{-5}, 1.30×10^{-4}, 1.93×10^{-4} , 2.90×10^{-4}, 4.35×10^{-4}, 6.52×10^{-4}, 9.78×10^{-4}, and 1.47×10^{-3} M). Inset: Benesi–Hildebrand plot for the complexation of (**a**) β-CD, (**b**) C4-β-CD linked chitosan (D.S.18%) and (**c**) C0-β-CD linked chitosan (D.S.17%) for the complexation of with TNS.

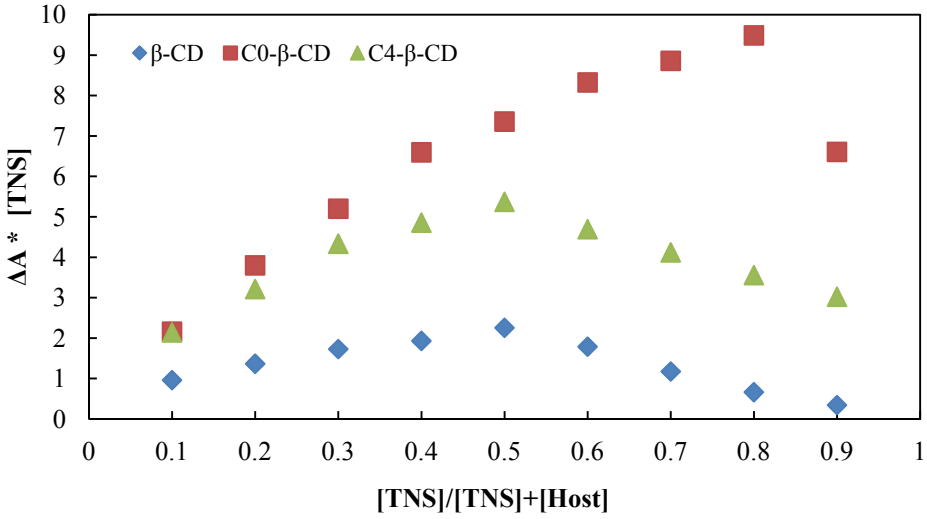


Fig. 7. Continuous variation Job's plot for the complexation of TNS with β-CD, C0, and C4-β-CD linked chitosan in acetate buffer (pH 4.3) at 25 °C. The total concentration of TNS and β-CD, C0, and C4-β-CD linked chitosan was 1.06×10^{-5} M.

2.3.3 Stoichiometry of the inclusion complex

The stoichiometry of the inclusion complex between β-CD, C0-β-CD, and C4-β-CD linked chitosan with TNS were determined by using the Job's method of continuous variation [21]. Fig. 7 shows Job's plot for the complexation of β-CD and its derivatives with TNS, where R = [TNS]/([TNS] + [Host]) was varied from 0–1 and plotted against difference in fluorescence intensity ($\Delta A \times$ [TNS]). As shown in Job's plot, the maximum of parent β-CD and C4-β-CD linked chitosan was obtained at R = 0.5, which indicates that the formation of parent β-CD and C4-β-CD linked chitosan with TNS is 1:1 inclusion complex in acetate buffer. In contrast, the maximum of C0-β-CD linked chitosan was obtained at R = 0.8, indicating that the formation between C0-β-CD linked chitosan and TNS is 1:4 inclusion complex in acetate buffer. These results can be explained by the flexibility of the CD residues that are linked to rigid chitosan backbone. C4-β-CD linked chitosan with longer linking arm may form 1:1 complex with TNS similarly to original β-CD. However the rigid structure of C0-β-CD linked chitosan may inhibit the inclusion ability of most of the CD residues, and most of CD residues lose the inclusion ability. Consequently, host-guest stoichiometry becomes 1:4. These results suggest that the length of linking arm plays an important role on the formation of inclusion complex.

2.3.4 Determination of the stability constant

The stability constant (K_s) of the inclusion complexes of TNS with β-CD and C4-β-CD linked chitosan was determined by using Benesi–Hildebrand method plot in term of emission intensities as given by Eq. 1 for 1:1 complex stoichiometry or Eq. 2 for 2:1 complexation [30, 31].

$$\frac{1}{I-I_0} = \frac{1}{I'-I_0} + \frac{1}{K_S(I'-I_0)[H]} \quad (1)$$

$$\frac{1}{I-I_0} = \frac{1}{I'-I_0} + \frac{1}{K_S(I'-I_0)[H]^2} \quad (2)$$

When I_0 is the initial fluorescence intensity of TNS in the absence of host, I is the intensity with presence concentration of host, I' is the intensity at the maximum concentration of host, [H] is the concentration of host and K_s is the stability constant. As shown in previous studies [32, 33] TNS is able to form 1:1 and 2:1 host-guest complex with β-CD, depending on the β-CD and its derivatives concentration. Fig. 6 inset figure shown the plot of 1/[I-I_0] vs. 1/[H] for the inclusion complex of C0-β-CD linked chitosan (Fig. 5a) and C4-β-CD linked chitosan (Fig. 5b). The good linear correlation of C4-β-CD linked chitosan was obtained, whereas a poor straight line was obtained from C0-β-CD linked chitosan. No linear plot was observed in the plot of 1/[I-I_0] vs. 1/[H]^2 according to Eq. 2 and consequently the possibility of 2:1 inclusion complex formation is ruled out (data not shown). The obtained linearity of C4-β-CD linked chitosan from Benesi–Hildebrand agreement with the result that obtained from Job's plot confirming that the stoichiometry of 1:1 inclusion complex, while the poor linearity of C0-β-CD linked chitosan indicated disagreement with 1:1 inclusion complex, according to the

result obtained from Job's plot. The results suggested that C4-β-CD linked chitosan (D.S. 18%) with the 4-butylamido linking shows ability to form 1:1 inclusion complex with TNS, whereas C0-β-CD linked chitosan (D.S. 17%) with the linking alkyl chain of C0 was unable to form 1:1 inclusion complex. This may probably due to the conformational flexibility and distance of linking arm between chitosan and CD moiety. The stability constant (K_s) was calculated by dividing the slope-intercept of the straight line from Benesi-Hildebrand plot for 1:1 complex. The Gibbs free energy change (–ΔG°) of the inclusion complexes formation of TNS with β-CD and C4-β-CD linked chitosan were shown in Table 2. The ΔG° of C4-β-CD linked chitosan (–19.21 KJ mol^{-1}) suggested that the complex formation of TNS is spontaneous encapsulated into C4-β-CD linked chitosan cavity at 25 °C. Considering the structure characteristics of host and guest suggested that the toluidinyl group is more hydrophobic than the napthalenesulfonate group of TNS. Therefore, the binding of TNS to parent β-CD and C4-β-CD linked chitosan could occur with toluidine moiety and the hydrogen bonding and hydrophobic effect are important factors in the formation of inclusion complex [34].

Table 2 Stability constant (K_s and log K_s) and gibbs free energy change (-ΔG°) for the inclusion complex of *β*-CD, C0-β-CD, and C4-*β*-CD linked chitosan with TNS in 0.1 M acetate buffer (pH 4.3) at 25°C.

Host	**D.S.**	**Solubility in acetate buffer (pH 4.3)**	K_s/M^{-1}	**Log K_s**	**–ΔG°/KJ mol^{-1}**
β-CD	-	soluble	4000	3.60	20.55
C4-*β*-CD linked chitosan	18	soluble	2333	3.37	19.21

2.4 Conclusion

The two difference linking arms of β-CD linked chitosan have been synthesized and their characterization, inclusion complex behavior and binding ability with TNS were investigated. In the present study, C0-β-CD and C4-β-CD linked chitosan were synthesized by using 6-*O*-*p*-toluenesulfonyl-β-CD as a common starting material. The synthesis was performed by reductive alkylation of the amino group of chitosan with β-CD derivatives bearing an aldehyde group that soluble in water under acidic conditions. Formation of the inclusion complex with TNS led to an enhancement in the fluorescence intensity with blue shift of spectra. Job's plot and Benesi–Hildebrand plot showed 1:1 complexation stoichiometry for C4-β-CD linked chitosan with D.S. 18%, whereas C0-β-CD linked chitosan with D.S. 17% was not occurred. This could be due to the effect of the flexibility and chain length of linking arm of β-CD moieties and chitosan that highly influenced on water solubility and inclusion complex of derivatives. Our synthetic procedure can be application to prepare various CD linked chitosan with different linking arms by choosing unsaturated carboxylic acid, and could provide further step for designing more environmental friendly host materials in a wide variety of fields. Further studies along this line are now in progress.

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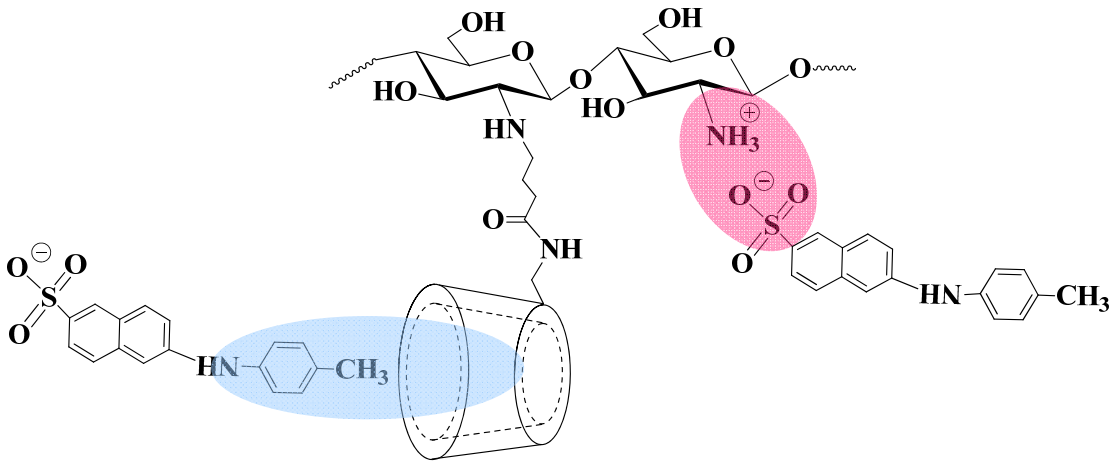
Chapter 3

NMR investigation of inclusion complex

β-cyclodextrin linked chitosan

Abstract

Inclusion complex between hosts and guest compounds were determined by using ^{1}H NMR spectroscopic. The inclusion complexation of 6-(*p*-toluidino)-2-napthalene- 6-sulfonate (TNS), guest fluorescence probe with original β-cyclodextrin (β-CD), C0-β-CD and C4-β-CD linked chitosan were compared using Job's method. The original β-CD was shown 1:1 inclusion complex with the toluidine moiety of TNS, while C4-β-CD linked chitosan, that the structure compose of CD residue and chitosan moiety was also shown inclusion complex with toluidine moiety same as original β-CD, in addition the chitosan structure was shown ability form ionic interaction with naphthalenesulfonate moiety of TNS. On the contrary, C0-β-CD linked chitosan was shown only the ionic interaction with TNS moiety. The effect of the flexibility and change length of linking arm between β-CD moiety and chitosan related to the formation of host provided more highly water solubility and then offer better inclusion complexation.



3.1 Introduction

Cyclodextrins (CDs) are truncated cone-shape molecules, a class of cyclic oligosaccharides with 6-8 D-glucose units linked by α-1,4-glucose bonds. The structure of CDs is consisting of hydrophobic cavity that is capability to form inclusion complex with various organic or inorganic molecules in both aqueous solution and the solid state, depending on the size, shape and polarity of the CD. The complexation driving forces have been attributed to hydrophobic interactions, van der Waals-London dispersion forces, and hydrogen bonds [1-3]. β-cyclodextrin is the most largely produced cyclodextrin used in many fields including pharmaceuticals, foods, cosmetics, chemical products and technologies [4-5]. Chitosan is a natural polysaccharide, obtained from *N*-acetylation of chitin. Because of its attractive non-toxic, biocompatibility, and biodegradable, chitosan have been used in various field such as biomedical field, cosmetic, food, and textile industries as adsorbents for removal or separation of various pollutants (i.e. dye, heavy metals, among others) from aqueous solution through ionic interaction and electrostatic forces [6-8]. Due to advantages of both β-CD and chitosan, the β-CD linked chitosan was synthesized. In the previous study [9], β-cyclodextrin linked chitosan derivatives having different linkers were synthesized and the results from fluorescence spectroscopy shown that the length of linking arms have effect on their solubility and the formation of inclusion complex. In this study, we focus on the confirmation of inclusion complex formation by using different characterization techniques. The interaction, stoichiometry and structure conformation of TNS with β-CD, C0-β-CD linked chitosan, and C4-β-CD linked chitosan are investigated through NMR techniques.

3.2 Experimental

3.2.1 Materials and Methods

6-(*p*-Toluidino)-napthalenesulfonic acid potassium salt (TNS) was purchased from Sigma-Aldrich Japan G.K. (Shinagawa, Japan). All other reagents were preparative grade, and purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). These materials were used without further purification. Water was purified by treatment with ion-exchanger and subsequent reverse osmosis with a Millipore Millex-3 apparatus. ^{1}H NMR spectra was recorded on a Bruker ASX-300 spectrometer operated at 300.13 MHz.

3.2.2 Preparation of C4-β-CD linked chitosan

C4-β-CD linked chitosan was prepared by the methods previously described by [9]. Ozone was bubbled through a solution of 6-deoxy-6-(4-pentenoyl) amido-β-CD (1.5 g, 1.23 mmol) in 20% aqueous methanol (15 ml) with stirring at 0 °C for 1 h. After bubbling oxygen gas for several minutes to purge dissolving ozone, dimethyl sulfide (1 ml) was added to the solution and the solvent was removed by evaporation under reduced pressure. The crude aldehyde (1.5 g, 1.23 mmol) was dissolved in chitosan solution (280 mg, 1.74 mmol) of 2% v/v aqueous acetic acid (15 ml) and methanol (5 ml). To the mixture was added sodium cyanoborohydride (150 mg), and stirred at room temperature overnight. The resulting solution was subjected to ultrafiltration using a membrane with molecular weight cut-off 20,000, washed with water, and lyophilized to give the C4-β-CD linked chitosan (**8:** 0.175 g, 37%yield) as

white cotton-like hygroscopic amorphous (D.S. 18%).

^{1}H NMR (D_2O/CD_3COOD, 60 °C) δ: 5.50-5.10 (m, H–1 of β-CD), 4.75 (s, H–1 of chitosan overlap with HDO), 4.10-3.50 (m, H–2-H–6 of β-CD overlap with H–3-H–6 of chitosan), 3.00 (s, H–2 of chitosan).

3.2.3 Preparation of C0-β-CD linked chitosan

C0-β-CD linked chitosan was prepared by the methods previously described by [9]. Crude 6-oxo-β-CD (300 mg, ca. 0.26 mmol) was treated with chitosan (150 mg, 0.93 mmol) and $NaBH_3CN$ (24 mg, 0.38 mg) in the same protocols as described for preparation of C4-β-CD linked chitosan to give C0-β-CD linked chitosan (160 mg) as hygroscopic amorphous (D.S. 17%).

^{1}H NMR (D_2O/CD_3COOD, 60 °C) δ: 6.19-5.78 (m, H–1 of β-CD), 5.31 (s, H–1 of chitosan), 4.80-4.20 (m, H–2-H–6 of β-CD overlap with H–3-H–6 of chitosan and HDO), 3.64-3.33 (s, H–2 of chitosan).

3.2.4 Preparation and determination of inclusion complexes

For stoichiometry determination by Job's method the samples of hosts and guest were prepared by dissolving 8.5 mg of TNS and 27.5 mg of β-CD in 6 ml of D_2O. the solution of C0- and C4-β-CD linked chitosan were prepared by dissolving 50 mg and 51 mg in 6 ml of D_2O/CD_3COOD, respectively. Keeping total guest and host concentration constant at 4 mM and varying guest-host molar ratio from 0 to 1. The measurements

were carried out after at least 5 h dark storage. The signal at 4.80 ppm of HOD was used as an internal reference for β-CD inclusion complex with TNS and the signal at 2.04 ppm of CD_3COOD was used as an internal reference for C0- and C4-β-CD linked chitosan inclusion complex with TNS.

3.3 Results and discussion

3.3.1 ^{1}H NMR spectroscopy

At present, ^{1}H NMR spectroscopy give one of the most powerful evidence, is convenient and informative method for studying host-guest chemistry in solution , and the information of the chemical shifts has been used to establish inclusion modes [10], [11]. Chemical shifts variations of selected host and guest protons reflect the formation of a complex between them. The screening environment should be sensed by magnetic nuclei inside the cavity, the entry of a nonpolar part of the guest into the lipophilic cavity of the host induce a shielding of the inner protons of the glucose units of β-CD. H–3 and H–5 protons of β-CD are located in the interior of the cavity, and the H–3 protons are close to the wide side while the H–5 protons are near the narrow side of the cavity [12]. In consequence, H–3 and H–5 chemical shift variation to higher fields are the direct evidence of inclusion. NMR experiments can provide information about stoichiometry and structure of CD complexes [13]. Especially Job's plot [14], which describes the dependence of chemical shift on ratio between host and guest. This method has been widely used to determine complex stoichiometry [15-16].

The chemical shift change (Δδ) is defined as the difference in chemical shifts in the presence and absence of the other guests and calculated by the following equation: Δδ = Δδ complex – Δδ free. According to [17], positive sign shows a downfield shift and negative sign shows an upfield shift. The chemical shifts of β-CD in the absence and presence of guest in D_2O are shown in Table 1.

From Table 1 and Fig. 1, the NMR chemical shifts and assignments of TNS, β-CD and TNS/ β-CD are shown, after the inclusion complex with TNS was formed, the

upfield shift of the H–3 located on the inner surface at the secondary hydroxyl group side (Δδ = -0.137 ppm), followed by the H–5 proton located on the inner surface at the primary hydroxyl group side (Δδ = -0.129 ppm), and the H–6 proton located at the rim of the narrow side of the β-CD (Δδ = -0.065 ppm). In consequence of the significantly shifted upfield of the H–3 proton, this indicates that TNS was inserted into the cavity from the wider side, confirmed the inclusion complexation between TNS and β-CD cavity. Fig. 2 shown $\Delta\delta_H$ of β-CD protons plotted versus the mole fractions of TNS for the complexation with β-CD.

However, the displacement of the inclusion complex of C0-β-CD and C4-β-CD linked chitosan with TNS were difficult to determine due to the complication in the overlapping of CD molecules and chitosan moiety. Therefore, the displacement of C0-β-CD and C4-β-CD linked chitosan with TNS were obtained by observing the changes in the chemical shifts of TNS for ring protons, as well as, for H–8 and H–1 of the naphthalenesulfonate moiety and the protons of methyl group in the toluidine moiety. As shown in Table 2, the displacement of TNS with C4-β-CD linked chitosan shown the significant shifts upfield, follow by H–1 (Δδ = -0.071 ppm), CH_3 protons (Δδ = -0.065 ppm) and H–8 (Δδ = -0.063 ppm) of TNS. Whereas the displacement of C0-β-CD linked chitosan shown change in chemical shifts by H–1 (Δδ = -0.001 ppm), CH_3 protons (Δδ = 0.009 ppm) and H–8 (Δδ = 0.003 ppm) of TNS, these chemical shift changes were relatively small. This indicated that the insertion of TNS protons into C4-β-CD linked chitosan cavity is deeper than C0-β-CD linked chitosan.

Table 1 ^{1}H NMR chemical shifts corresponding to β-CD in the presence and absence of TNS.

β-CD	**H–1**	**H–2**	**H–3**	**H–4**	**H–5**	**H–6**
δ free	5.043	3.649	3.947	3.564	3.831	3.859
δ complex	4.993	3.598	3.810	3.547	3.702	3.794
Δ δ[a]	-0.05	-0.051	-0.137	-0.017	-0.129	-0.065

Δ δ[a] = δ free – δ complex

Concentration of both compounds is 4 mM.

Table 2 Variation of ^{1}H NMR chemical shifts (ppm) of TNS protons in the presence of C0-β-CD and C4-β-CD linked chitosan in D_2O/CD_3COOD 60 °C.

TNS protons	**Δδ C0-β-CD linked chitosan**	**Δδ C4-β-CD linked chitosan**
H–1	-0.001	-0.071
H–8	0.009	-0.063
CH_3	0.003	-0.065

Concentration of both compounds is 4 mM.

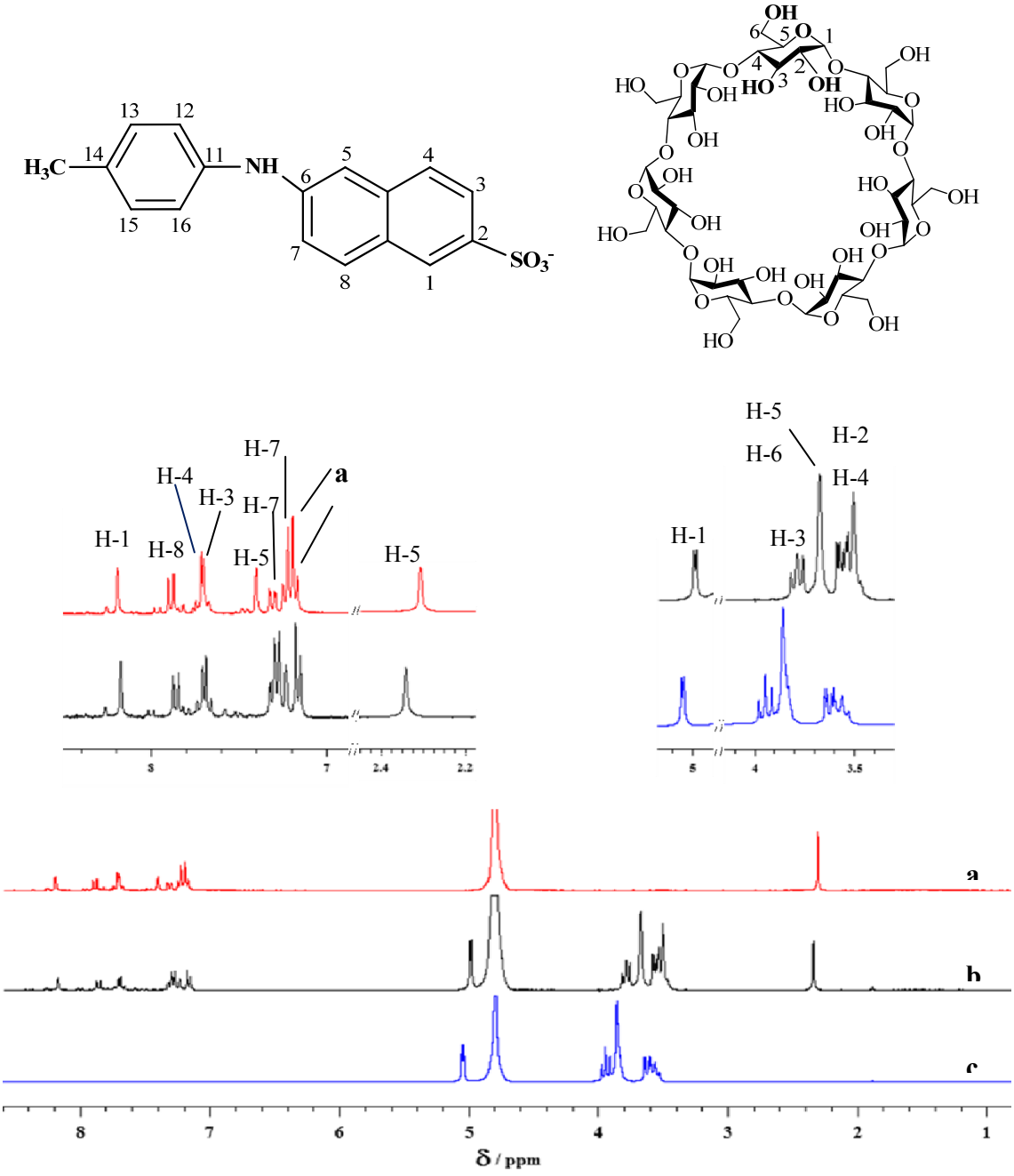


Fig. 1. ^{1}H NMR spectra of β-CD in the absence and presence of TNS in D_2O at 25 °C (**a**) TNS, (**b**) TNS–β-CD inclusion complex and (**c**) β-CD.

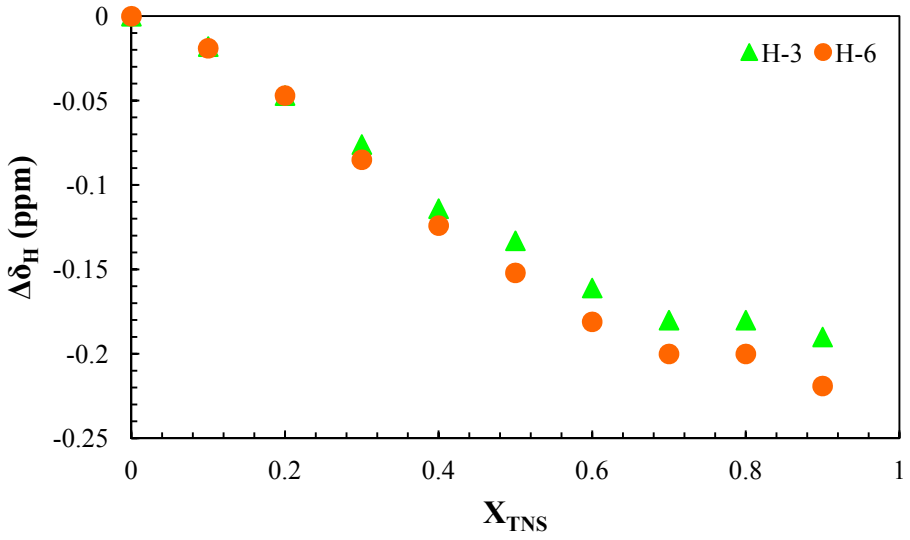


Fig. 2. ^{1}H NMR chemical shift titration ($\Delta\delta_H$) of TNS with H–3 and H–6 protons of β-CD versus mole fraction of TNS.

3.3.2 Stoichiometry of inclusion complexes

The continuous variation method, Job's plot [18] was performed in order to confirm the conformation of the complex based on the ^{1}H NMR chemical shifts between TNS with β-CD, C0- and C4-β-CD linked chitosan. The sum of the concentrations of both components was kept constant ([Guest]+[Host] = 4 mM) and the molar fraction of each component (r=[Guest]/([Guest]+[Host])) ranged between 0 to 1. In order to calculate the stoichiometry, only several protons of TNS have been selected to measurement of their chemical shifts variations. As shown in Fig. 3, the Job's plots of chemical shift displacements of methyl group in the toluidine moiety and H–1 of the naphthalenesulfonate moiety of TNS in the free and complex state with β-CD, at the

same time observed that the CH_3 proton of TNS shown the most significant changes in chemical shifts than H–1 of TNS. This could be suggested that the insertion of CH_3 proton into the β-CD cavity and the maximum value at 0.5, indicating the 1:1 stoichiometry of the complexes between β-CD and CH_3 proton of TNS as shown in Fig. 4.

Different results were observed for CH_3 group of TNS in the free and complex state with C0-β-CD and C4-β-CD linked chitosan as shown in Fig. 5. The chemical shift displacements of CH_3 group for both derivatives were shown at least two peaks. This can be described by the structure of derivatives that compose of CD moiety and chitosan molecules, therefore two peaks obtained from results could be occurred by the hydrophobic interaction of β-CD cavity and the ionic interaction of chitosan molecules. At the same time observed from Fig. 5 and Fig. 6, at TNS volume less than 0.5, the CH_3 and H–1 of TNS were embedded into the CD cavity of C4-β-CD linked chitosan by the hydrophobic interaction, while the ionic interaction of chitosan was become dominant when increased the volume of TNS more than 0.5. In contrast, the Job's plots of chemical shift displacements of methyl group of TNS in the free and complex with C0-β-CD linked chitosan was shown equality of the both interactions and the chemical shift displacements of H–1 of TNS was shown in Fig. 6, the chemical shift of C0-β-CD linked chitosan was shown a slightly shift when increasing TNS volume.

From the different chemical shift displacements of TNS with C4-β-CD linked chitosan and C0-β-CD linked chitosan could be suggested that the ionic interaction between chitosan and TNS molecules is stronger than the hydrophobic interaction of β-CD cavity. C4-β-CD linked chitosan was shown both ability of CD and chitosan,

hydrophobic interaction and ionic interaction with TNS molecules. However for C0-β-CD linked chitosan, the ionic interaction is controlled effect in this studied. These results consistent to previous studied [9] that the formation of 1:1 stoichiometry only occurred in C4-β-CD linked chitosan.

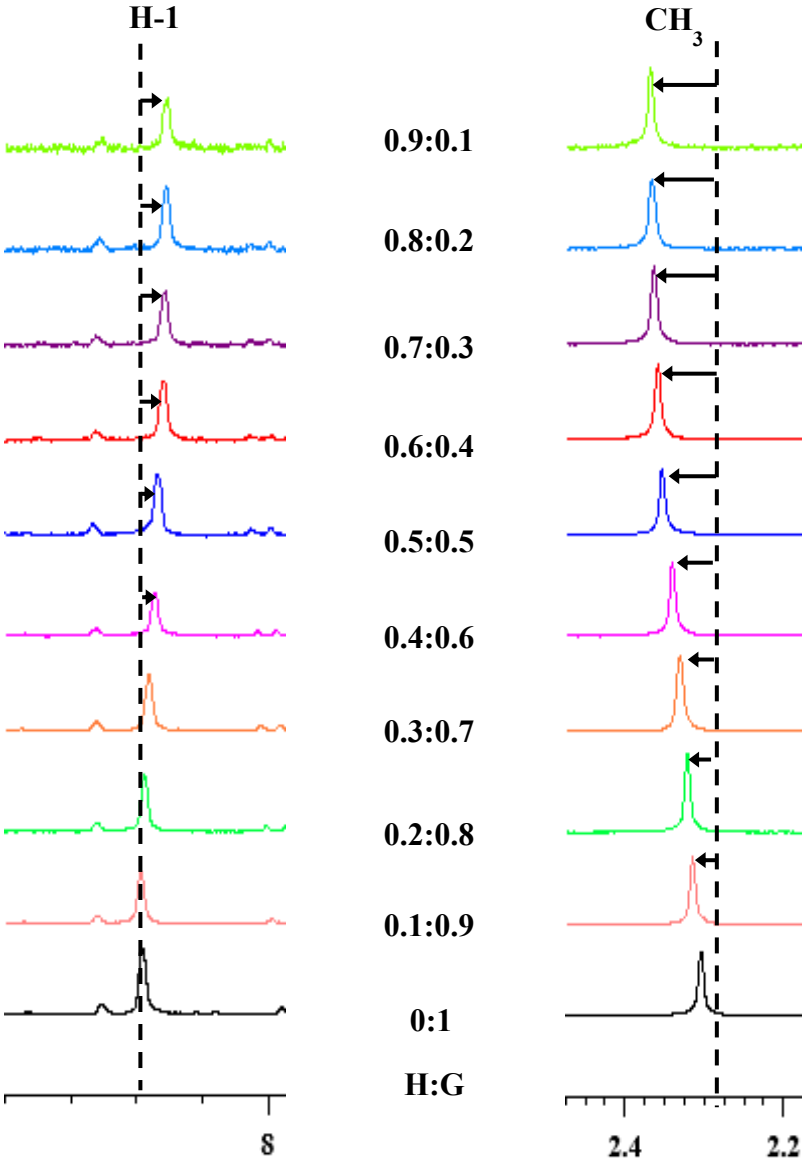


Fig. 3. The chemical shift displacements of methyl group (CH_3) and H–1 of TNS in the presence and absence of β-CD obtained by ^1H NMR titration spectra.

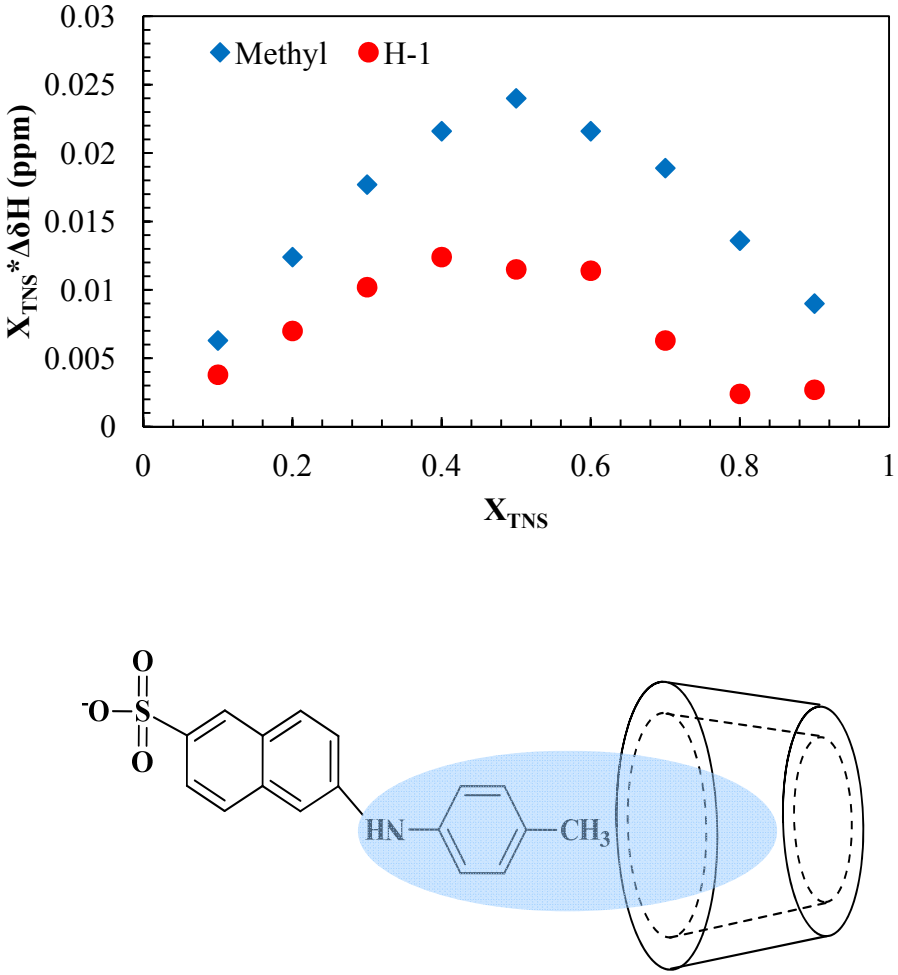


Fig. 4. Job's plots corresponding to chemical shift of the methyl group and H–1 of TNS in the presence of β-CD and simplified schematic of possible inclusion complexation.

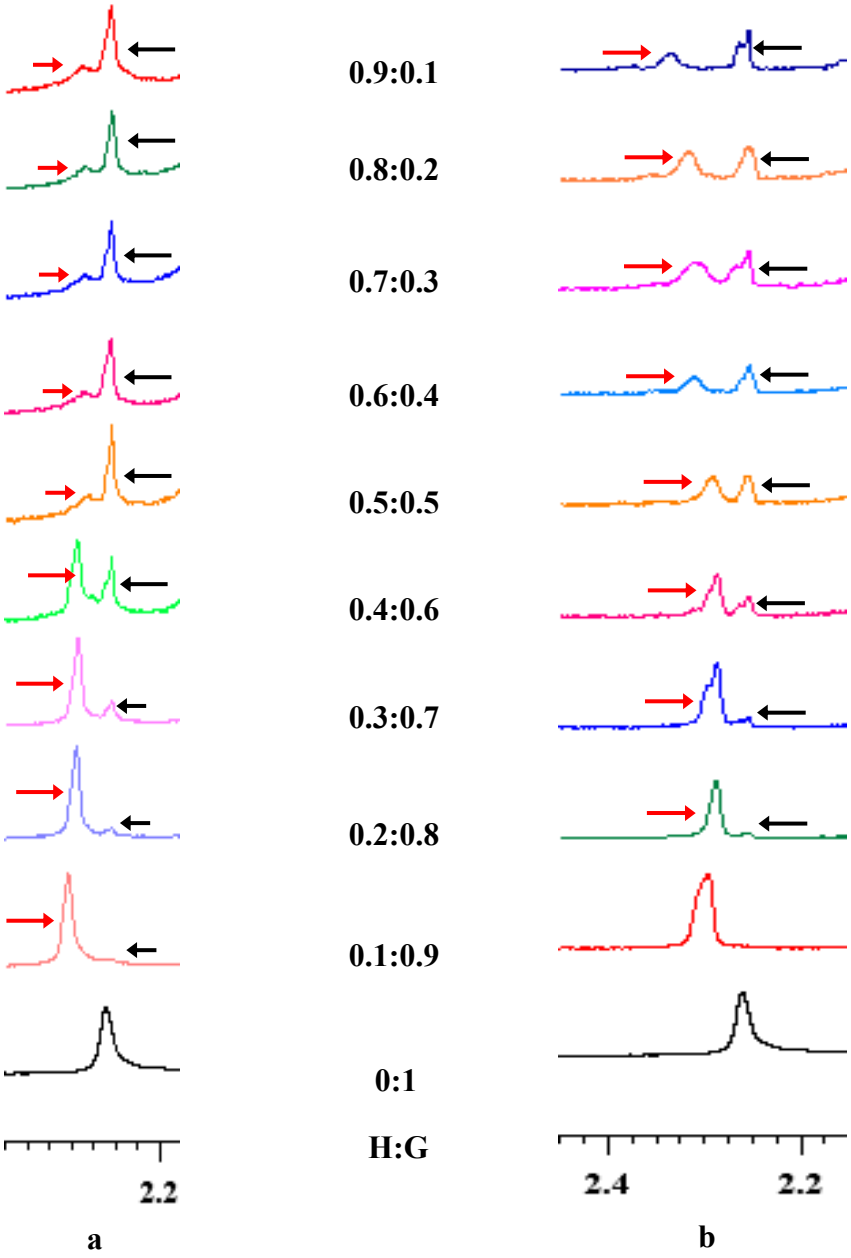


Fig. 5. The chemical shift displacements of methyl group (CH_3) of TNS in the presence and absence of (a) C4-β-CD linked chitosan (D.S. 18%) and (b) C0-β-CD linked chitosan (D.S. 17%) obtained by ^{1}H NMR titration spectra.

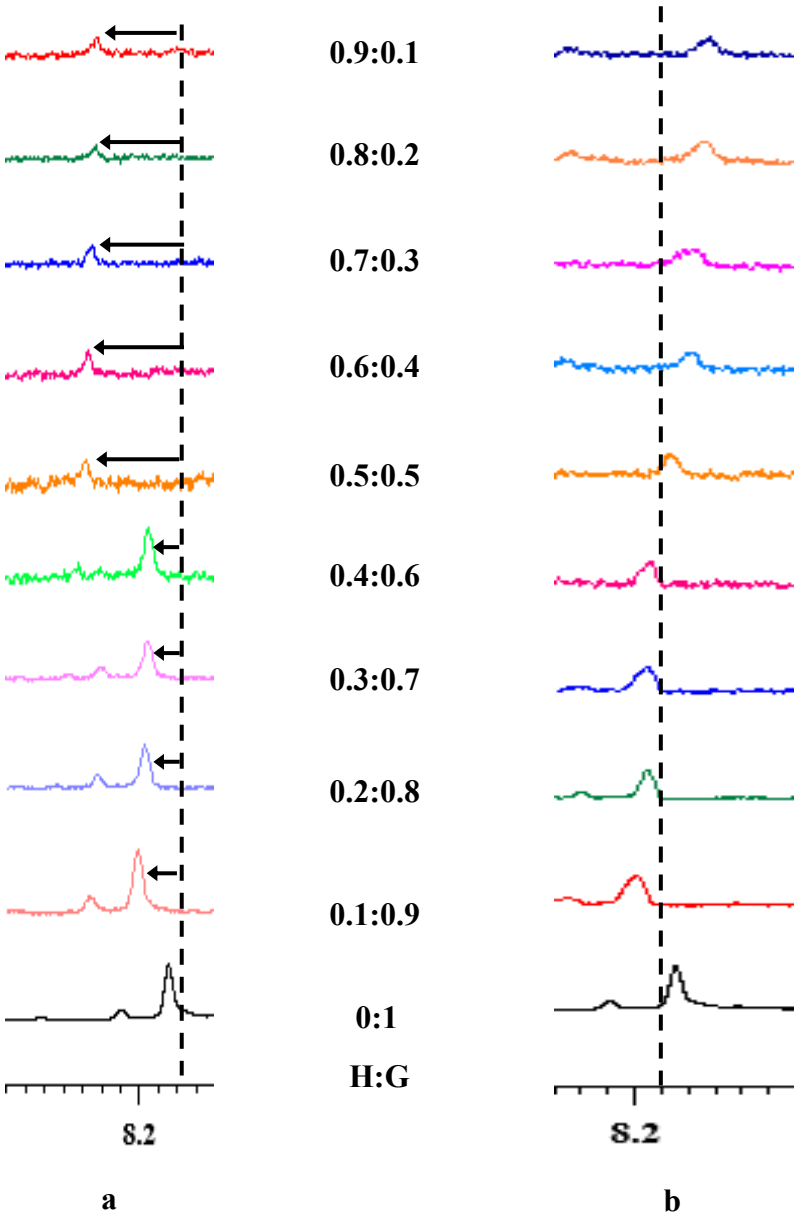


Fig. 6. The chemical shift displacements of H–1 of TNS in the presence and absence of (a) C4-β-CD linked chitosan (D.S. 18%) and (b) C0-β-CD linked chitosan (D.S. 17%) obtained by ^{1}H NMR titration spectra.

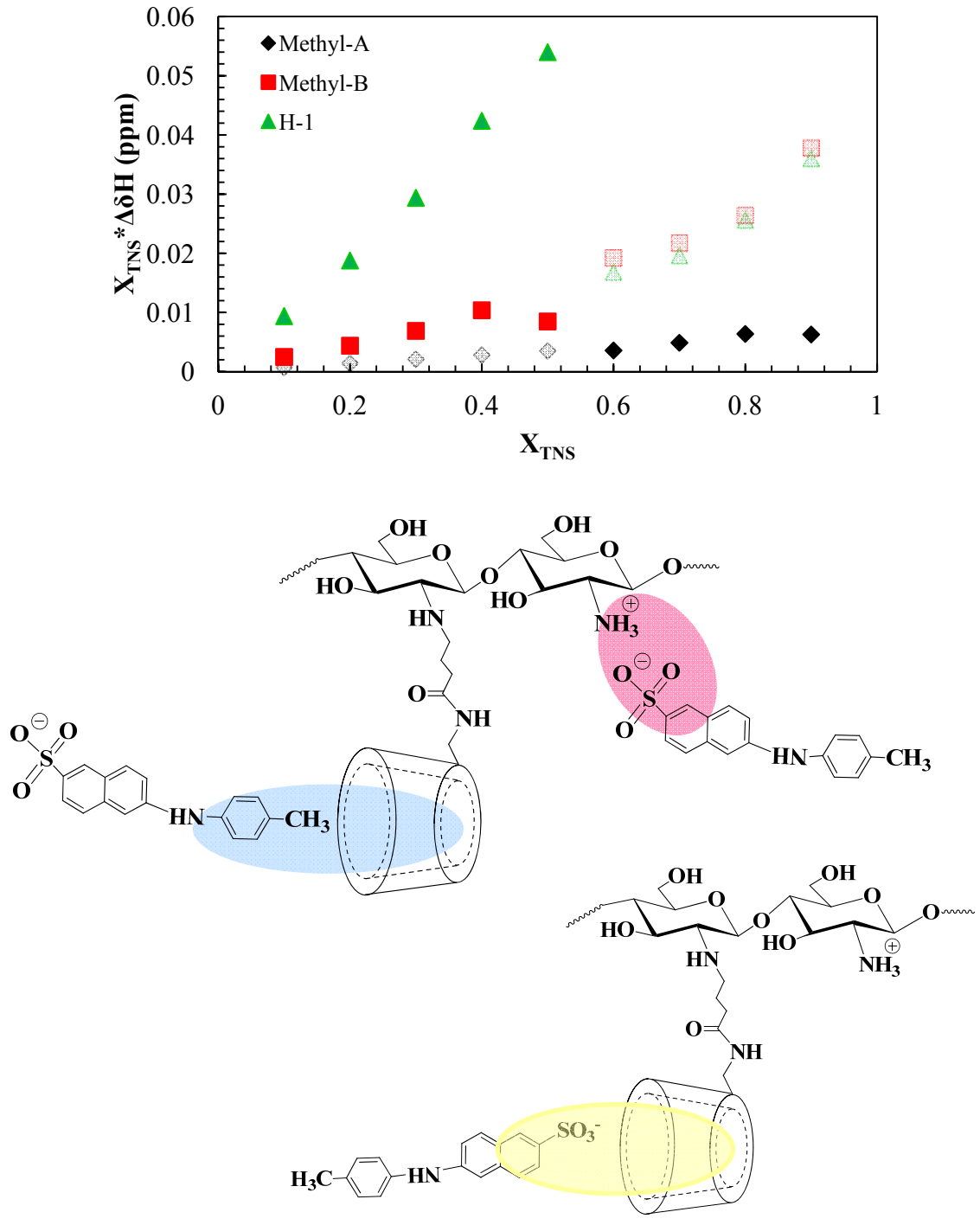


Fig. 7. Job's plots corresponding to chemical shift of the methyl group and H–1 of TNS in the presence of C4-β-CD linked chitosan (D.S. 18%) and simplified schematic of possible inclusion complexation.

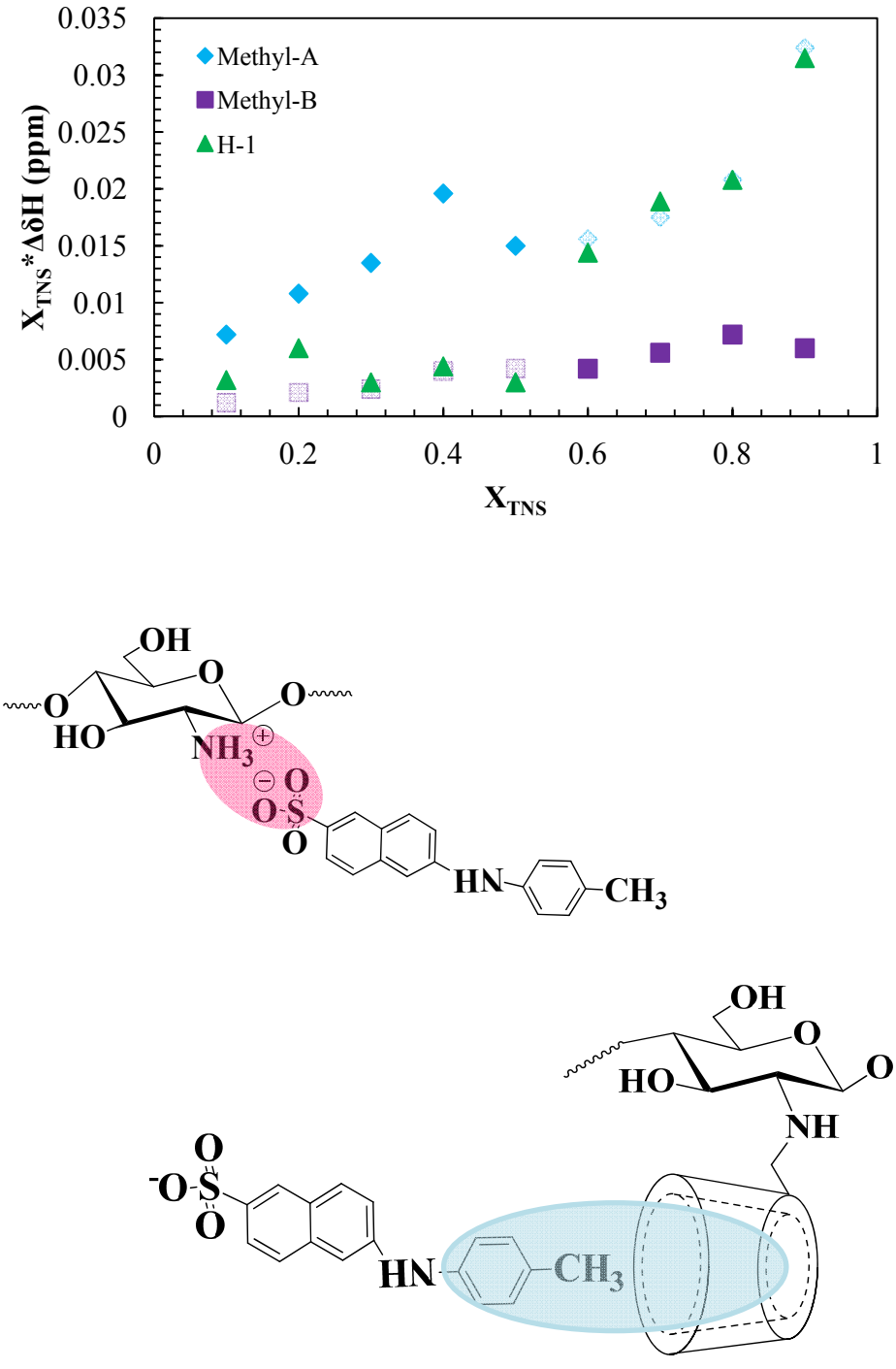


Fig. 8. Job's plots corresponding to chemical shift of the methyl group and H–1 of TNS in the presence of C0-β-CD linked chitosan (D.S. 17%) and simplified schematic of possible inclusion complexation.

3.4 Conclusion

The inclusion complex conformation between TNS with β-CD, C4-β-CD linked chitosan and C0-β-CD linked chitosan were investigated by ^{1}H NMR spectroscopy. The insertion of guest molecule into the CD cavity was demonstrated by changes in NMR proton chemical shift values. The experiments performed by Job's methods. The results obtained suggested that, the CH_3 of toluidinyl group is more probably included into the hydrophobic β-CD cavity than H–1 of naphthalene group of TNS, while C4-β-CD linked chitosan and C0-β-CD linked chitosan could be attributed to not only hydrophobic inclusion complex within β-CD cavity, but also ionic interaction with chitosan molecule.

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Chapter 4

Adsorption of dyes from aqueous solution using Cross-link β-cyclodextrin linked chitosan

Abstract

Cross-link β-CD linked chitosan were synthesized and used as a sorbent for the removal of reactive black5 (RB5) and reactive orange16 (RO16) from aqueous solution. The dye adsorption experiments were carried out by using batch procedure. The effects of initial solution pH, contact time and initial dye concentration were investigated. Results showed the effective pH was 3 for the both of dyes adsorption. The maximum adsorption capacities were 340 mg g^{-1} for RO16 and 693 mg g^{-1} for RB5. The equilibrium adsorption data were determined using Langmuir and Freundlich models. The adsorption kinetics showed that the adsorption behaviour of crosslink β-CD linked chitosan followed the pseudo-second order kinetic model. Repeated RO16 adsorption and desorption cycles showed that the cross-link β-CD linked chitosan could be used repeatedly. The efficiency adsorption by cross-link β-CD linked chitosan suggested that these biomaterial may be a way towards highly-efficient for removal dyes in wastewater treatment.

4.1 Introduction

The remaining dyes from different sources, *e.g*., textile industries, leather tanning, paper printing production, and plastic industry, are considered as hazardous pollutants introduced into natural water [1-2]. Dyes are a relatively large group of organic chemicals classified based on their different chromophoric groups such as azobenzene, triphenylmethane, or phthalocyanine dyes [3]. The azo dyes represent about half of the dyes used in the textile industry, characterised by having an azo group consisting of two nitrogen atoms (–N=N–) that form covalent bonds with OH^-, NH^-, or SH^- groups in fibres. Inside the azo dyes have a widely types of dyes, *i.e*., acid, reactive, disperse, vat, metal complex, mordant, direct, basic, and sulphur dyes. Among these, the reactive dyes are extensively used for dyeing process in textile because of their solubility, low expense, stability and variety of colour. The release of these dyes wastewaters into the environment are causing pollution problems to aquatic life, interfering in transmission of sunlight, reducing photosynthesis, furthermore, allergy, irritation, mutagenic, and potentially carcinogenic to human [4-7].

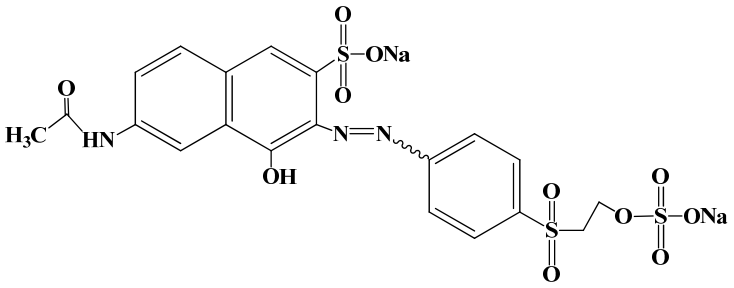
Conventional wastewater treatment methods, such as chemical precipitation, chemical oxidation and reduction, ion exchange, reverse osmosis, adsorption [8], Fenton process, electro-chemical degradation, and ultrasonic irradiation [9] have been available for the dyes removal. Particularly, adsorption techniques represent a popular technology for ease of operation and insensitivity to pollutants, flexibility and simplicity of design, high efficiency, and no result in the formation of harmful substances [10].

Adsorption techniques employing solid adsorbents are effective methods for water decontamination. Most commercial systems currently use activated carbons and

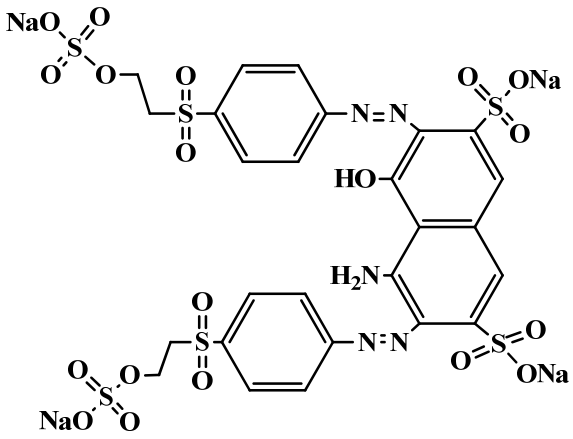
organic resins as adsorbents to remove dye in wastewater because of their excellent adsorption abilities [11]. A large variety of non-conventional adsorbent materials have been also proposed and studied for their ability to remove dyes. Some of the reports adsorbents include clay material, zeolites, siliceous materials [12], agricultural wastes [13], industrial by-products [14], and biomass [15].

Much effort has recently been focused on various materials based on polysaccharides that can be obtained in large quantities and that are harmless to nature [16]. Special attention has been given to cyclodextrin (CDs) which are natural molecules derived from starch [17]. These substances, particularly β-cyclodextrin, and its derivatives have remarkable capacity to form inclusion complexes in solution or in the solid state with other molecules through host-guest interactions [18], so CDs complexation is a procedure of choice for adsorption techniques. Chitosan is β(1-4)-2-amino-2-deoxyglucan prepared by *N*-acetylation of chitin. Chitosan has two types of highly reactive functional groups, hydroxyl groups and amino groups. Due to its intrinsic characteristics, the natural polymer is widely used as an effective biosorbent for the removal or the recovery of hazardous dyes, heavy metals and so on [19-20].

In this chapter, the author described the application of insoluble biomaterial cross-link β-CD linked chitosan for the removal of Reactive Orange16 (RO16) and Reactive Black5 (RB5) from aqueous solution, their chemical structures and physical properties are presented in Fig. 1 and Table 1. The effects of pH, initial dye concentrations, kinetics, equilibrium, desorption and regeneration ability were determined. This information would be useful for further applications in the treatment of wastewaters process.



Reactive Orange16 (RO16)



Reactive Black 5 (RB 5)

Fig. 1. Chemical structures of RO16 and RB5.

Table 1 Physical properties of reactive dyes.

Parameters	**RO16**	**RB5**
Molecular weight (g/mol^{-1})	617.54	991.82
Molecular formula	$C_{20}H_{17}N_3Na_2O_{11}S$	$C_{26}H_{21}N_5Na_4O_{16}S_6$
λ max (nm)	494	597

4.2 Experimental

4.2.1. Materials and methods

Reactive Orange16 (RO16) and Reactive Black 5 (RB5) were purchased from Sigma-Aldrich Co. LLC. (St. Louis, USA.). All reagents were preparative grade, purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). These materials were used without further purification. Water was purified by treatment with ion-exchanger and subsequent reverse osmosis with a Millipore Millex-3 apparatus. Adsorption and desorption experiments were conducted on a reciprocal shaker NANOMIZER SR-1. The UV-vis spectra were recorded on a JASCOV-560 spectrometer in a standard quartz cell.

4.2.2 Preparation of crosslink β-CD linked chitosan

β-CD (50 g, 44.05 mmol) was dissolved in dimethylsulfoxide (DMSO; 500 ml) and lithium hydride (0.252 g) was added portion-wise to the solution. The mixture was stirred under nitrogen atmosphere at room temperature for 20 h, and then ally bromide (3.81 ml) and lithium iodide (170 mg) were added to the resulting solution. The mixture was stirred for 3 h at 55 ºC, and quenched with methanol (20 ml). Solvent was evaporated under reduced pressure and precipitated with acetone (500 ml) to give a mixture of allylated β-CD (45 g). Ozone gas and oxygen gas were successively bubbled through a solution of the CD derivative (2.68 g) in 20% aqueous methanol (20 ml) at room temperature for 1.5 h and 1 h, respectively. After addition of dimethyl sulfide (0.5 ml), the mixture was stirred overnight, and evaporated to half volume, and

mixed with a solution of chitosan (1.5 g) in 5% aqueous acetic acid. The mixture was stirred at room temperature for 1 h, sodium cyanoborohydride (0.291 g) was added, and stirred for 2 d. Solvent was removed by freeze dry to give solid material, which was washed with water and filtered and dried.

4.2.3 Effect of pH on the adsorption of reactive dyes

The effect of pH on dyes adsorption was investigated by using 100 mg L^{-1} of aqueous dye solutions. Then 0.02 g of β-CD linked chitosan was added to 20 ml of dye solutions. The mixture was shaken at 130 rpm using reciprocal shaker for 60 min to ensure that equilibrium were reached. The pH of the solutions was adjusted in the range 2 to 9 by using 0.1 mol L^{-1} solutions of NaOH or HCl. Blanks without sorbent were used to establish the initial dye concentration. The mixture was collected and centrifuged. The supernatant was analysed using UV-vis spectrophotometer and the maximum adsorption wavelengths were determined by scanning from 300 to 700 nm.

$$Percent\ removal\ (\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (1)$$

Where C_0 and C_e are the initial and equilibrium concentration of reactive dyes (mol/L), respectively.

4.2.4 Adsorption studies

Adsorption capacities and kinetics were conducted using the batch experiment. The sorption studies were carried out by varying the concentration of dye in aqueous solution from 50 to 300 mg L^{-1}. In each experiment, 0.04 g of the β-CD linked chitosan was added to 40 ml of dye concentration at a known initial concentration. The pH was adjusted to 3 using either 0.1 mol L^{-1} solutions of NaOH or HCl. The mixtures were shaken at 130 rpm using reciprocal shaker for 24 hr. The samples were collected at different intervals and centrifuged. The concentration of dye in the supernatant solution was analyzed by UV-vis spectrophotometer. The amount of dye equilibrium adsorption was calculated according to Eq. (2):

$$q_e = \frac{(C_0 - C_e)\mathrm{V}}{\mathrm{m}} \qquad (2)$$

where q_e is the equilibrium adsorption amount (mg Dye/g β-CD chitosan), C_0 is the initial dye concentration (mg dye/L), C_e is the equilibrium dye concentration (mg dye/L), V is the solution volume (L) and m is the adsorbent mass (g β-CD chitosan)

4.2.5 Desorption studies and regeneration of cross-link β-CD linked chitosan

For desorption study, the adsorbent utilized for the adsorption of initial concentration 100 mg L^{-1} was separated from RO16 and Methylene Blue (MB) solution by centrifugation. The dye-loaded biomaterial was washed gently with water to remove excess unadsorbed dye. Then the spent adsorbent was shaking with 20 mL of distilled water at various pH for 180 min, one by one and the desorbed dye was determine using

Eq. (3).

For regeneration study, cross-link β-CD linked chitosan 0.02 g suspended in 20 mL of 100 mg L^{-1} RO16 solution. After adsorption equilibrium, the dye-loaded biomaterial was separated from the aqueous solution by centrifugation, and then washed gently with water to remove unadsorbed dyes. Then, the dye-loaded biomaterial was transferred to 20 ml of 50% ethanol and shaken at 120 rpm for 5 h. after that wash with deionized water for 60 min. The reusability of cross-link β-CD linked chitosan in removing RO16 from aqueous solution, 3 cycles of the adsorption-desorption are repeated and after each cycle percentage removal was calculated as mentioned before.

$$Percent\ desorption\ (\%) = \left(\frac{Concentration\ of\ desorbed\ dye}{Concentration\ of\ dye\ loaded\ on\ Biomaterial}\right) \times 100\% \quad (3)$$

Desorption study is helpful to elucidate the nature of adsorption mechanism and to regenerate the biomaterial.

4.3 Results and discussion

4.3.1 Effect of pH on the adsorption of reactive dyes

The pH of the initial dye solution is one of the most important parameters in the controlling in the adsorption process, particularly in the adsorption capacity. The effects of pH on the dye removal was investigate at different pH conditions ranging from 2 to 9, and the results are shown in Fig. 2, the maximum removal percentage of RO16 and RB5 onto cross-link β-CD linked chitosan observed at pH 3 attributed to 84.34% and 74.71%, respectively. This may be described that at a lower pH solution, the protonation of functional groups such as amino groups of chitosan to form $–NH_3^+$ groups, which increases the electrostatic forces between the protonated amino groups ($–NH_3^+$) of chitosan and the anionic groups ($–SO_3^-$ and $–OSO_3^-$) of the dye, causing as increase in dye adsorption. However, a lower dye adsorption at higher pH solution was due to the presence of excess hydroxide ions (OH^-) and deprotonation of functional groups, which reduces the interactions between the the adsorbent and adsorbate. A negatively charged surface site on the chitosan dose not favors the adsorption of anionic RO16 and RB5 molecules due to the electrostatic repulsion. From the results suggesting that, the electrostatic forces could be the main interaction in adsorption process. Additional, possible other driving force from β-CD also included such as Van der Waals and hydrophobic interactions [21-22].

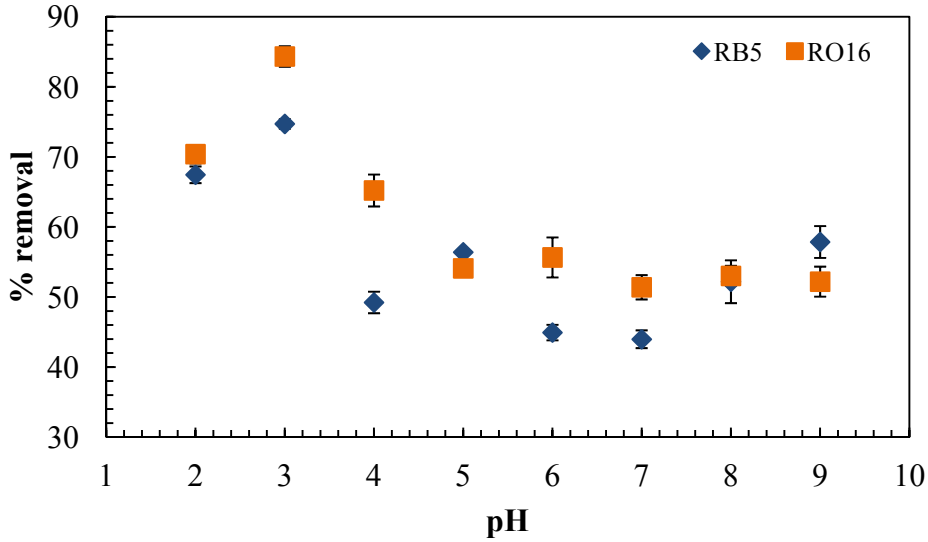


Fig. 2. % removal of RO16 and RB5 by various pH in the solution at equilibrium onto cross-link β-CD linked chitosan. [RO16] & [RB5] = 100 mg g^{-1}, mass of biomaterial= 0.02 g, T = 25 °C.

4.3.2 Adsorption isotherms

Adsorption isotherms describe the relation between the amount of pollutant adsorbed on sorbent materials and its concentration remained in solution. In order to evaluate the sorption capacity of cross-link β-CD linked chitosan, the adsorption isotherm experiment were performed at pH 3 with various initial dye concentration (50-300 mg/L) and 0.04 g of cross-link β-CD linked chitosan. The equilibrium data were fitted by Langmuir and Freundlich isotherm equations [23-25]. The Langmuir equation is expressed by following:

$$q_e = \frac{q_{max} b C_e}{1 + b C_e} \quad (4)$$

where q_e is the equilibrium capacity of dye on the adsorbent (mg/g), C_e is the equilibrium concentration of dye in the solution (mg/L), q_{max} is the maximum capacity of adsorbent (mg/g), b is the Langmuir constant.

Freundlich equation is expressed by following:

$$q_e = K_F C_e^{1/n} \quad (5)$$

where K_F is the adsorption capacity ($mg^{(1-n)}L^n/g$) and $1/n$ is the Freundlich constant. The Langmuir model assumes that adsorbent surface to be homogeneous with negligible interaction between the adsorbate molecules. By contrasting, the Freundlich model used to describe characterization of multilayer adsorption process, an exponential distribution of adsorption sites and energies, and the possible of mutual interaction between

adsorbate molecules. Fig. 3 illustrated the comparison of Langmuir and Freundlich isotherm models for RB5 and RO16 adsorption onto cross-link β-CD linked chitosan and the adsorption isotherm parameters are given in Table 1. From the high value of correlation coefficient (r^2), the data obtained from RB5 was fitted to the Langmuir model, while the data of RO16 was better fitted to the Freundlich model than the Langmuir model. The maximum dye adsorption capacity was calculated to be 693 mg dye/g for RB5 and 340 mg dye/g for RO16. Values of 1/n suggested that RB5 and RO16 adsorption onto cross-link β-CD linked chitosan was favourable.

Table 1 The Langmuir and Freundlich isotherms parameters with their correlation coefficients of dyes adsorption onto cross-link β-CD linked chitosan.

Dyes	Langmuir parameters			Freundlich parameters		
	Q_{max} (mg Dye/g)	b (L/mg)	r^2	K_F ($mg^{(1-n)}L^n/g$)	$1/n$	r^2
RB5	693	1.3091	0.9940	446	0.7136	0.9792
RO16	340	0.9044	0.9206	149	0.438	0.9713

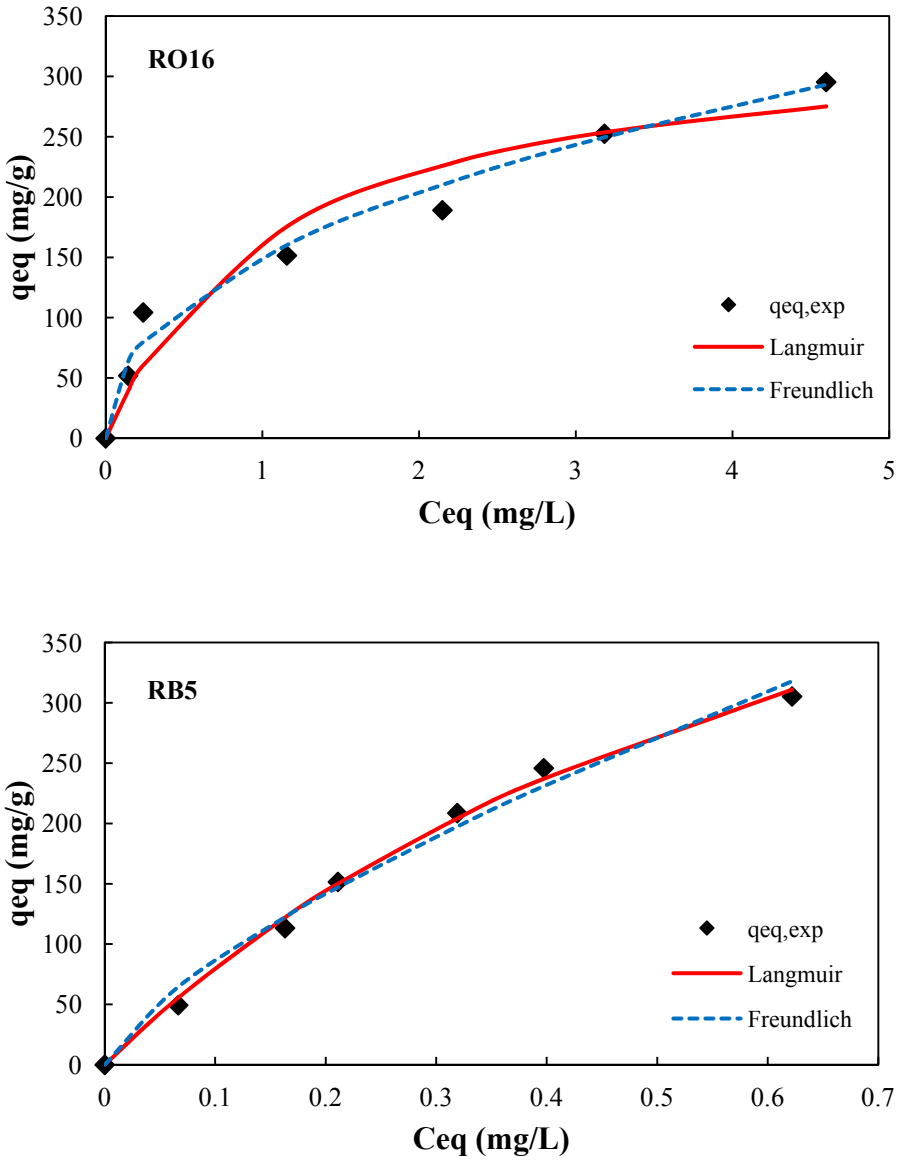


Fig. 3. Comparison of isotherm models for the adsorption of RO16 and RB5 onto cross-link β-CD linked chitosan. The initial dye concentrations ranged from 50 to 300 mg dye/L. Dotted and solid lines are predictions calculated using Freundlich and Langmuir adsorption isotherm models, respectively.

4.3.3 Adsorption kinetics

The adsorption kinetics of RB5 and RO16 onto cross-link β-CD linked chitosan were analyzed using the Langergren pseudo-first-order and pseudo-second-order models [26-28]. The Langergren rate equation was used to describe for the adsorption of solute from a liquid solution. The pseudo-first-order kinetics model can be expressed by following:

$$log(q_e - q_t) = log(q_e) - \frac{k_1}{2.303} t \quad (6)$$

The pseudo-second-order equation can be expressed by following:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (7)$$

where q_e and q_t is the amount of dye adsorbed per unit weight of adsorbent (mg/g) at equilibrium and any time, t (min), respectively, and k_1 and k_2 are the equilibrium rate constant of pseudo-first-order $(min)^{-1}$ and pseudo-second-order rate constant (g/mg min).

The kinetics parameters calculated according to the pseudo-first- and pseudo-second order equations are shown in Table 2 together with the correlation coefficient. The experimental data were better fitted to the pseudo-second order model than the pseudo-first order model based on the correlation coefficient (r^2). The good fit of r^2 obtained from the pseudo-second order indicating that the adsorption of RB5 and

RO16 onto cross-link β-CD linked chitosan conformed to chemical reaction mechanisms. Fig. 4 shown the adsorption kinetics of RO16 and RB5 onto cross-link β-CD linked chitosan at different initial dye concentrations.

Table 2 The pseudo-first order and pseudo-second order kinetic constants for dyes adsorption onto cross-link β-CD linked chitosan.

Dyes	$q_{e,\,exp}$	Pseudo-first order			Pseudo-second order		
	(mg/g)	k_1	$q_{e,\,cal}$	R^2	k_2	$q_{e,\,cal}$	R^2
		$(min)^{-1}$	(mg/g)		(g/(mg min))	(mg/g)	
RB5	49.43	0.0149	20.46	0.8437	1.26×10^{-3}	51.28	0.9987
	113.34	0.0195	154.74	0.8616	1.42×10^{-4}	126.58	0.9933
	151.65	0.0218	182.55	0.944	2.01×10^{-4}	161.29	0.9965
	208.73	0.0122	250.84	0.9114	5.43×10^{-5}	238.09	0.9851
	245.93	0.0082	215.13	0.9955	8.01×10^{-5}	263.16	0.9885
	305.41	0.0085	256.57	0.9961	7.58×10^{-5}	322.58	0.9915
RO16	51.90	0.0156	3.00	0.7358	1.27×10^{-2}	53.19	0.9999
	104.40	0.0230	27.66	0.9058	1.93×10^{-3}	107.53	0.9999
	151.59	0.0115	97.50	0.9500	2.61×10^{-4}	161.29	0.9984
	189.11	0.0181	121.73	0.9211	2.04×10^{-4}	204.08	0.9969
	252.55	0.0059	138.13	0.8486	1.72×10^{-4}	263.16	0.9987
	295.40	0.0073	171.24	0.8254	8.46×10^{-5}	322.58	0.9951

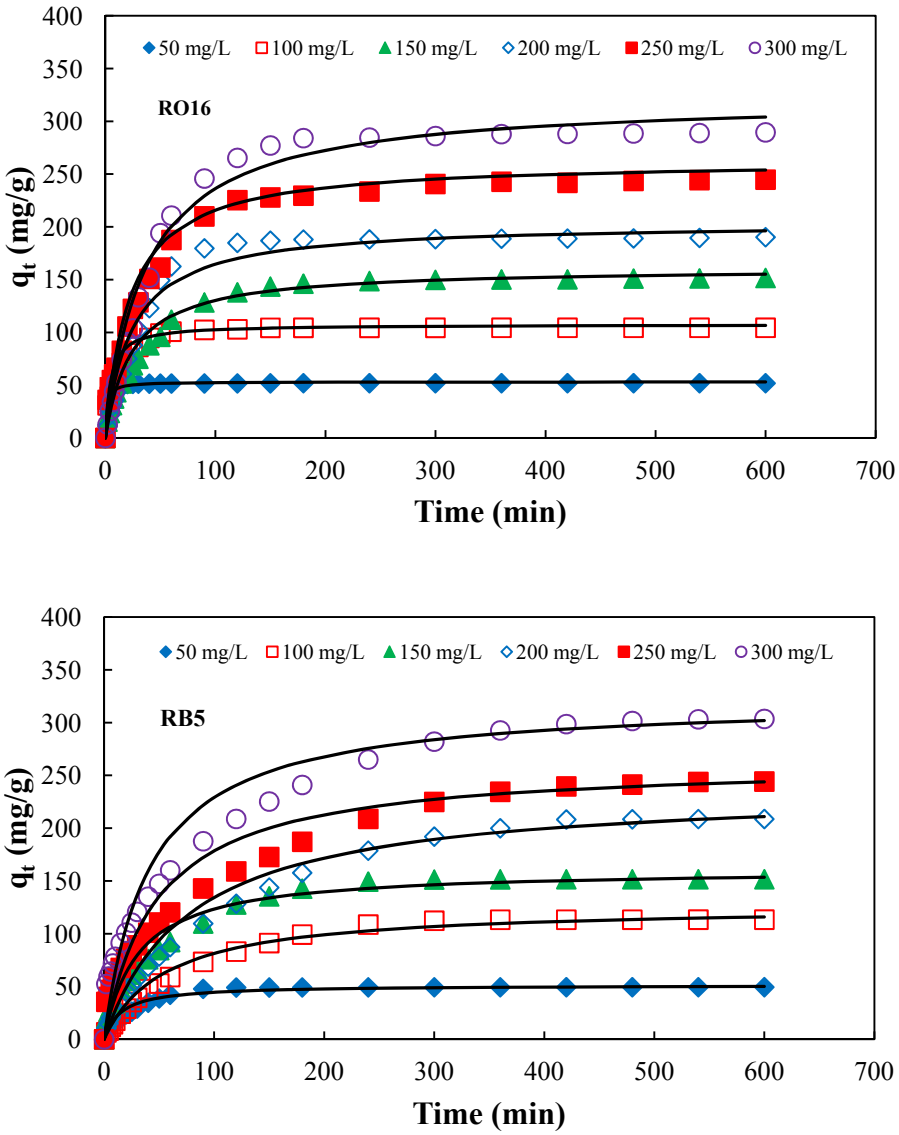


Fig. 4. The adsorption kinetics of RO16 and RB5 onto cross-link β-CD linked chitosan at different initial dye concentrations. Symbols represent the observed kinetics experimental data. Solid lines are predictions calculated using pseudo-second order kinetics.

4.3.4 Desorption and regeneration ability

Desorption studies can help elucidating the mechanism of an adsorption process. If the dye adsorbed onto the biomaterial can be desorbed by water, it can be said that the attachment of the dye onto the biomaterial is by weak bond. If the strong acids, such as HCl can desorb the dye, it can be due to the attachment of the dye by ionic interaction or electrostatic interaction [29]. Therefore, deionized water of different pH was used in the elution of dyes from the crosslink β-CD linked chitosan. Effect of pH on desorption percentage of dye from dye-loaded biomaterial is shown in Fig. 5 for initial concentration of methalene blue (MB), the cationic dye solution and RO16, the anionic dye solution: 100 mg g^{-1}, mass: 0.02 g, T = 25 °C, t = 120 min. Apparently, the desorption percentage of MB decrease with increasing the pH of deionized water. Low desorption was obtained with pH 4.0, while pH 2.0 shown the highest desorption percentage. Whereas, the desorption process of RO16 by pH of solution were not occurred. These could be describes that the interaction between adsorption process of MB and RO16 were not similar, This probably due to the structure of adsorbent compose of CD and chitosan, give the different interaction between dye molecule and adsorbent. Effect of ethanol solution on desorption percentage of dye from dye-loaded biomaterial is shown in Fig. 6, the results shown that the desorption percentage of RO16 was increasing when increased the percentage of ethanol, while the desorption percentage of MB was slightly increased.

In this study, the ability of the adsorbent to be regenerated is crucial for its practical application. Three consecutive adsorption-desorption cycling experiments were carried out, exposing crosslink β-CD linked chitosan to a test dye and then

washing with 50% ethanol for 3 h in each cycle, and the results are shown in Fig. 7. The adsorption capacity decreased slowly in each subsequence cycle, which due to that the RO16 are not completely desorbed. However, the crosslink β-CD linked chitosan could be recycled and reused to adsorb RO16 from aqueous solution a number of time and still give acceptable performance. Results confirming that this biomaterial can be recycled and reused to adsorb the anionic dyes RO16.

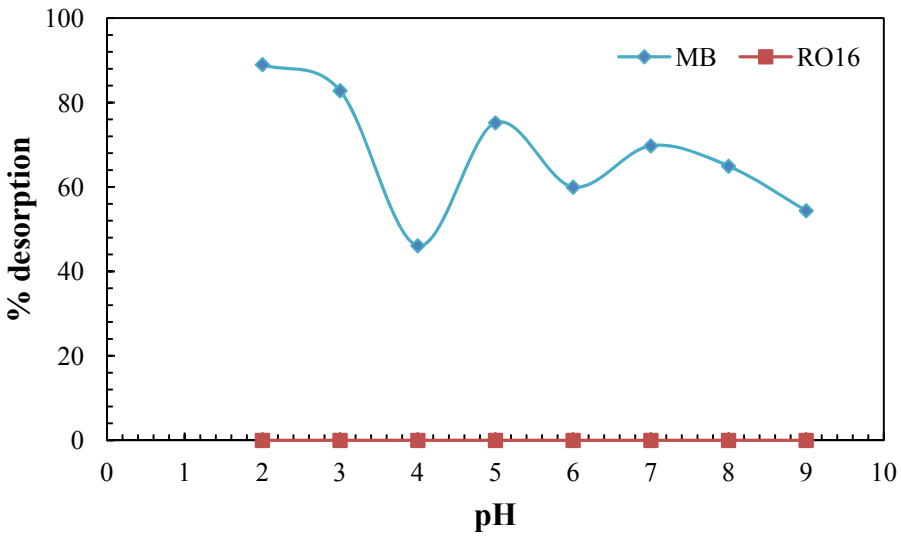


Fig. 5. Effect of pH on desorption percentage of MB and RO16 from dye-loaded cross-link β-CD linked chitosan. Adsorption experiments; [MB] and [RO16] = 100 mg g^{-1}, mass of cross-link β-CD linked chitosan = 0.02 g, T = 25 °C, t = 120 min.

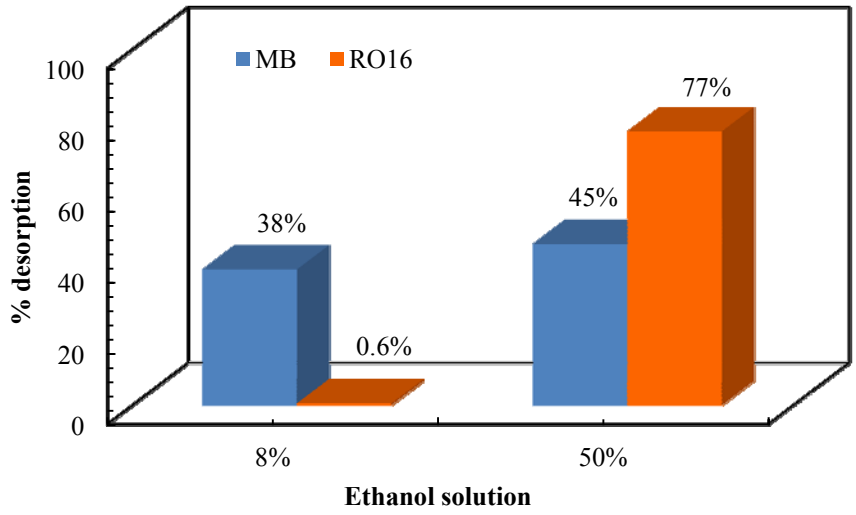


Fig. 6. Effect of ethanol solution on desorption percentage of MB and RO16 from dye-loaded cross-link β-CD linked chitosan. Adsorption experiments; [MB] and [RO16] = 100 mg g^{-1}, mass of crosslink β-CD linked chitosan = 0.02 g, T = 25 °C, t = 120 min.

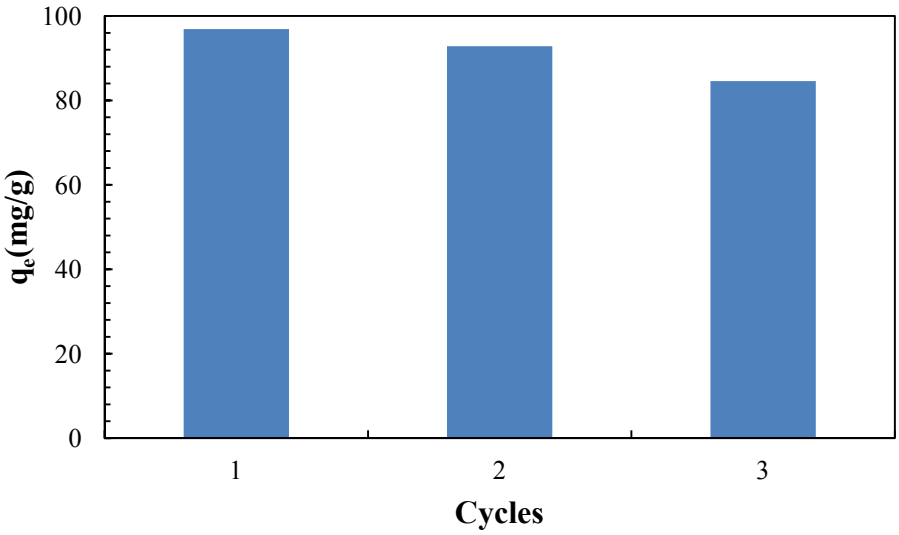


Fig. 7. Reusability studies of cross-link β-CD linked chitosan on RO16 with three cycles. [RO16] = 100 mg g^{-1}, mass of cross-link β-CD linked chitosan = 0.02 g, T = 25 °C, the contact time was 300 min for each cycle.

4.4 Conclusion

The synthesized insoluble biomaterial cross-link β-CD linked chitosan was used as an adsorbent for dye removal from water. Batch experiments showed that optimum pH for adsorption of RO16 and RB5 was obtained at pH 3. The probable mechanism for adsorption RB5 is an ionic interaction of ammonium groups in chitosan structure with the sulfonate groups of dye structure, whereas the probable mechanism for adsorption RO16 is the hydrophobic interaction of β-CD molecules with methyl groups of RO16. In addition, hydrogen bonding and acid-base interactions between the adsorbent and dyes as well as the physical sorption due to the chitosan structure are all involved. The adsorption kinetics followed the pseudo-second order model, which provided the best correlation with the experimental data. The Langmuir equation was fitted with RB5 and the Freundlich equation was fitted with RO16. The maximum adsorption capacity was 693 mg dye/g for RB5 and 340 mg dye/g for RO16. The desorption was performed by 50% ethanol solution. The reusability results obtained in this study demonstrating that cross-linked β-CD linked chitosan could be used for removal textile effluents from treatment process.

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Chapter 5

Conclusion and future prospects

This research aimed to develop effective adsorbent material for the removal of dyes from water, the attractive of β-CD is the characteristic feature to form inclusion complexes with various aromatic guests in aqueous solution and also the including of a versatile biomaterial, unique poly-cationic property of chitosan. For this reason, β-CD linked chitosan derivatives having different linkers of C0 and C4 units were synthesized by reductive alkylation of amino group of chitosan with β-CD aldehyde derivatives, as described in Chapter II. The length of linking arm between chitosan and CD moiety was influenced on the physical property of these biomaterials. Moreover, the conformational flexibility and distance of CD residue probably changed its water solubility. The inclusion properties of C0- and C4-β-CD linked chitosan were investigated using TNS, fluorescent probe as a model guest and C4-β-CD linked chitosan shown ability to form inclusion complex same as original β-CD. As described in Chapter III, the inclusion ability of original β-CD and both of biomaterials were confirmed the existence using ^{1}H NMR spectroscopy. The chemical shift change of protons of TNS suggested conformation of the inclusion complex and possibility of ionic interaction between chitosan amino group and sulfonate group of TNS. As described in Chapter IV, the cross-linked insoluble materials of β-CD linked chitosan was of advantageous to use as an adsorbent for application in dye removal because the effective adsorption against RB5 and RO16 in aqueous solution. The adsorption-desorption of RO16 and methylene blue (MB), using as indicator could be described the discrimination of the interaction between the inclusion complexation from β-CD and the ionic interaction from chitosan. The cross-linked β-CD linked chitosan could be used repeatedly in the regeneration cycle.

For the future prospect, we suggest that this β-CD linked chitosan, biomaterial can be applied and prolonged in the field of wastewater treatment as adsorbent for removal of dyestuffs from water bodies. Furthermore, this is possible to apply for removal other organic pollutants due to their hydrophobic and ionic interaction can be use in decontamination of wastewater.

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Wanvisa Buranaboripan