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**Synthesis and characterization of
water soluble EDTA-linked chitosan
as a flocculant for the removal of heavy metal ions**

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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

1.1 Environmental pollution

Environmental pollution had been a fact of life during our history but it became a real problem after the start of the industrial age. Pollution is the introduction of chemical, physical, and biological contaminants into the environment that cause harm or discomfort to humans or other living organisms. Furthermore, it damages the environment. Pollutants such as oil, detergent, fertilizers containing nitrogen and phosphoric acid, leaving metals can have a tremendous impact on the ecosystem. For example, it can cause havoc on the ecological balance in a lake. In addition to chemical substances, pollutants involve various kinds of energy such as noise, heat or light.

Biodegradable pollutants are the ones that can be broken down and processed by living organisms, including organic waste products, phosphates, and inorganic salts. Therefore, biodegradable pollutants are only temporary substances that can be converted into harmless compounds. On the other hand, non-biodegradable pollutants are the substances that cannot be decomposed by living organisms, and they include plastics, metal, glass, some pesticides and herbicides, and radioactive isotopes. Because of their non-biodegradable property, these pollutants persist in the environment for extremely long periods of time and spread into wide range of area. When the pollutants are contaminated into a food-chain, they are accumulated in the tissues of organizer at success-fully higher levels.

1.2 Heavy metal pollutants and their conventional treatment

Among a large number of environmental pollutants, heavy metal is one of the major water pollutants and soil pollutants. Metals are introduced in aquatic systems as a

result of the weathering of soils and rocks, from volcanic eruptions, and from a variety of human activities such as mining, processing, or using metal-containing materials or products. Most of heavy metals are highly toxic or carcinogenic, and tend to readily accumulate into living organisms through food-chain. The most serious heavy metal pollutants influencing on own life are arsenic, cadmium, chromium, copper, nickel, lead, and mercury. For example, copper leads to weakness, lethargy, and anorexia,¹ and cadmium and lead are associated with nephrotoxic effects and bone damage.²

Although various methods are being used to remove heavy metal ions, for example chemical precipitation,³ ion exchange,⁴ adsorption,⁵ oxidation,⁶ electrochemical treatment,⁷ and membrane separation.⁸ Among these methods, chemical precipitation is widely used in industry, because it is relatively simple and inexpensive to operate. Fig. 1 shows flow chart of heavy metal waste water treatment by chemical precipitation. Chemical precipitation is a process that soluble metal ions are formed insoluble precipitate so as to be separated from water by sedimentation or filtration. The conventional chemical precipitation includes hydroxide precipitation and sulfide precipitation. These process use alkali as $\text{Ca}(\text{OH})_2$ and NaOH , or sulfides, respectively. However, their methods have some practical disadvantages.⁷ Hydroxide precipitate is produced as relatively low density sludge, which present dewatering and disposal problems. Complexing agents contained in waste water inhibit formation of hydroxide precipitate with metal. Hydroxide or sulfide precipitates are dispersed colloidal particle in water, which cause separation problem. In addition, there is potential danger in the use of sulfide precipitation. Heavy metal ions are known to exist in acidic condition. Because sulfide precipitation is performed under acidic condition which evolves toxic H_2S fumes, it is

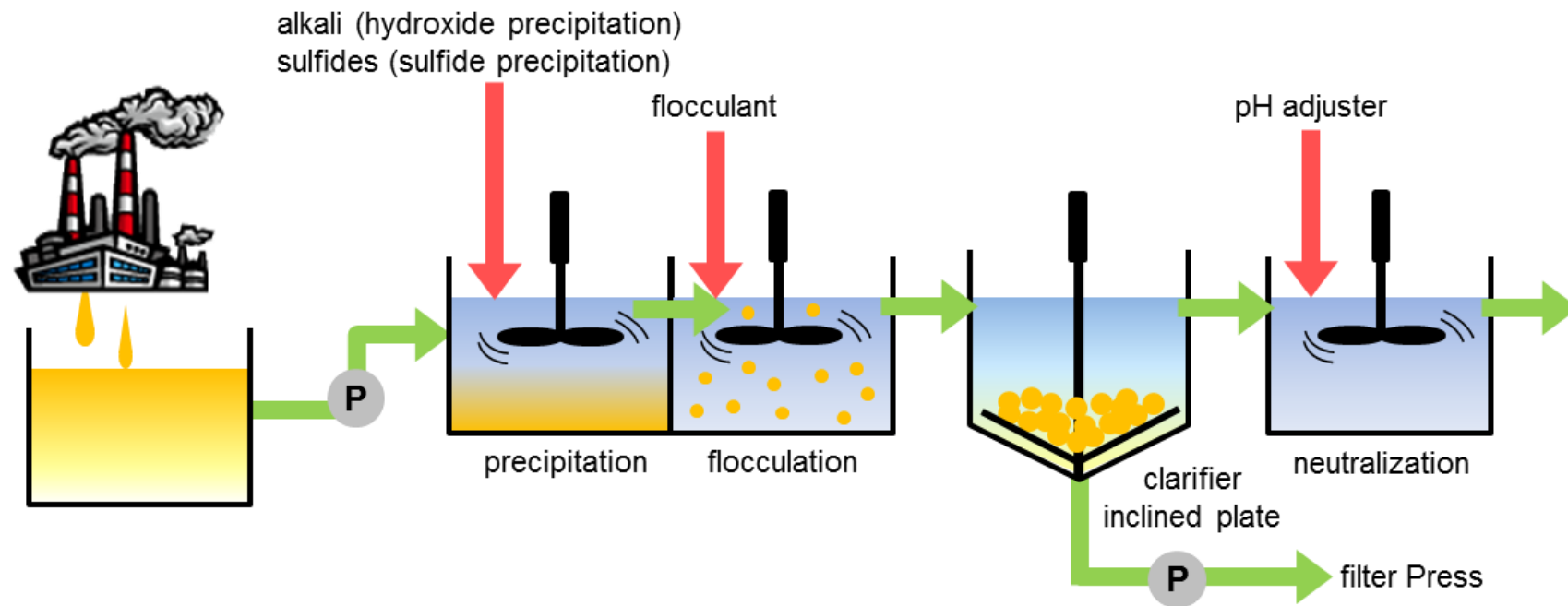


Figure 1 Flow chart of chemical precipitation, typical water treatment containing heavy metal ions.

necessary that it must be performed in natural or basic conditions.

To overcome these disadvantages, the researches have been intensified for alternative methods to remove metal ions. Because biopolymers are as environmentally friendly, biodegradable, and less toxic, many attentions have been focused on the various biopolymers such as chitosan,⁹ cellulose,¹⁰ sodium alginate,¹¹ and so on for treatment of water contaminated by heavy metal ions. Among them, chitosan is attracting an increasing interest in recent years. Because chitosan has coordination ability with transition metals,¹² chitosan based materials have been extensively investigated to remove heavy metal ions, e.g., beads,¹³ membranes,¹⁴ and chitosan immobilized onto glass beads.¹⁵ Furthermore, their chemical modification made it possible to enhance the chelation ability with heavy metal ions.^{16–18} Recently, a strong chelating agent, ethylenediaminetetraacetic acid (EDTA), was introduced into chitosan skeleton to give a crosslinked and water insoluble material. This solid-material showed remarkable adsorption ability with dissolving ions of copper,¹⁹ nickel,²⁰ and also rare earths.²¹

1.3 Flocculation/coagulation for pollutant removal

Flocculation/coagulation is a process that fine divided or dispersed particles are aggregated together to form large particles of such a size so as to cause their sedimentation and cause clarification by filtration. Thus it is an efficient and cost-effective technology for the removal of suspended insoluble organic and inorganic substances from industrial and domestic wastewater. Polyaluminum chloride (PAC) and polyferic sulfate (PFS) are commonly used coagulants that form cationic species in water, and adsorb anionic colloids, resulting in aggregation of particles (coagulation).²² Since these

aggregates are small and fragile, polymeric flocculants like polyacrylamide and polyamine are used in combination with the coagulant to enhance the formation of larger flocs bridging the destabilized particles and to facilitate sedimentation.^{23,24} Fig. 2 shows the removal of particles by coagulation/flocculation as illustration. In addition to synthetic flocculants, natural polyelectrolytes or modified biopolymers recently received much attention because they are as environmentally friendly, biodegradable, and less toxic.^{25–}

²⁶ Chitosan, a linear polysaccharide of β -(1,4)-linked 2-amino-2-deoxy-D-glucopyranose, is a semi-synthetic polysaccharide made from such abundant and renewable biomass as shells of crabs, shrimps, and insects (Fig. 3). Since chitosan is dissolved in polycationic property, chitosan and its grafted polymers have been investigated as flocculants to reduce turbidity in wastewater. As an example, the ability was revealed by removal of clay mineral such as kaolinite from water.^{5,27}

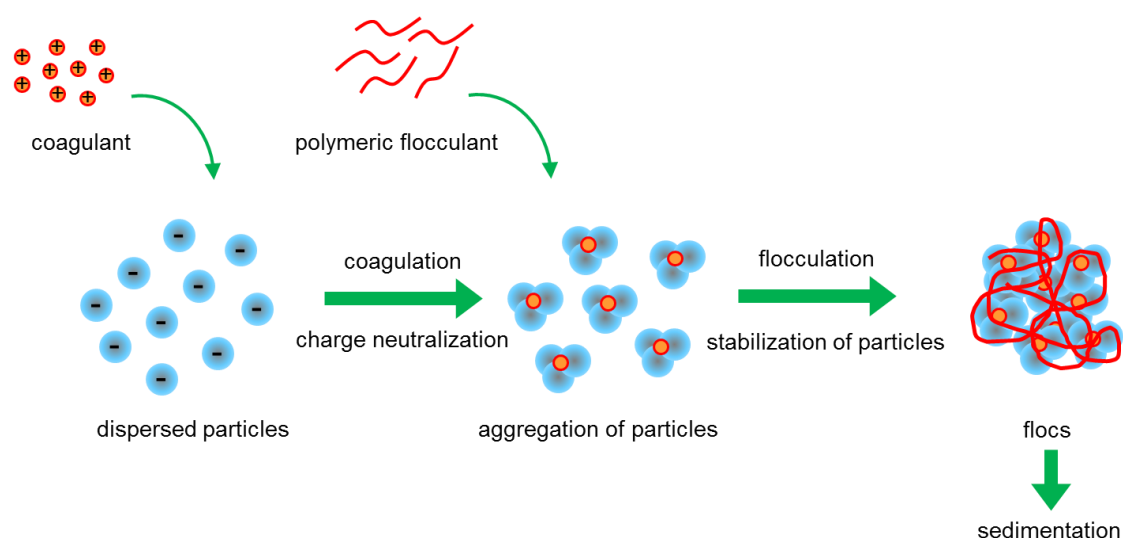


Figure 2 Coagulation/flocculation for removal of particles.

1.4 Difficulty in flocculation of heavy metal ion

Flocculation has been regarded as less effective method for removal of metal ions. It was considered to be difficult to accumulate the dissolving metal ions into large flocs. Therefore, flocculation has to be carried out in combination with another purification process. For example, conventional flocculants like PAM^{28,29} and carboxyethyl chitosan^{30,31} was used after chemical precipitation to form colloids of the heavy metal hydroxide. These combined methods result in enhanced usage of harmful reagents and running expense of wastewater treatment. To overcome this problem, investigations on new type of flocculants with pendant ligands have been reported by several research groups. Sulfur-containing functional groups such as thiourea, xanthate, and dithiocarbamate, which are known as moderately strong ligands for transition metal ions,³² were introduced into synthetic polymers³³⁻³⁵ and polysaccharide based graft copolymers.^{36,37} Their effectiveness was shown by removing Pd(II), Au(III), Cu(II), and Pb(II).

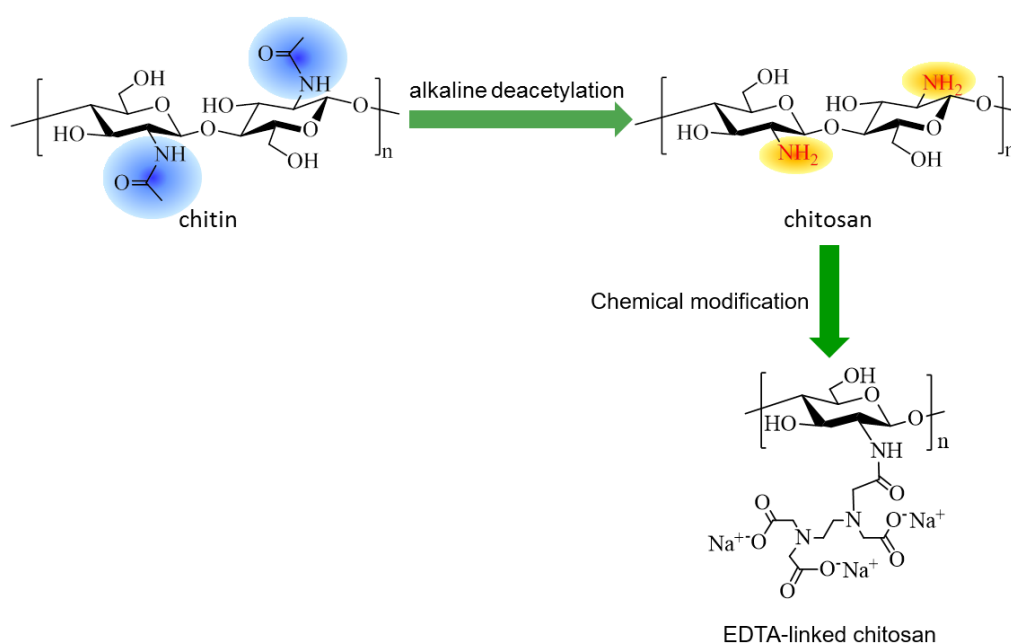


Figure 3 Chemical structure of chitin, chitosan, EDTA-linked chitosan.

1.5 Scope of this study

Since chitosan is a versatile biomaterial and extensive studies on their environmental applications have been conducted, the author's interest focused on design and synthesis of a chitosan based flocculant that has an ability to remove heavy metal ions. The success of EDTA-chitosan adsorbent prompted us to investigate on synthesis and utilization of water soluble EDTA-linked chitosan (ED-ch; Fig. 3). ED-ch has chelation site of heavy metal ions together with both anionic carboxyl group and cationic amino group in one polymer molecule. These functional groups are expected to provide an ability to flocculate dissolving heavy metal ions. Here, we would like to describe the synthesis of ED-ch through N-acylation of amino groups of chitosan using EDTA monoanhydride and its applicability for heavy metal ion removal examined by use of Cu(II) ion.

This thesis consists of six chapters. Chapter 1 describes the general introduction of wastewater treatment, flocculation, and removal of heavy metal pollutants. Purpose of the author's study and the relation to these backgrounds are also discussed. In Chapter 2, preparation of novel chitosan based material, EDTA-linked chitosan (ED-ch), is mentioned. Detail of preparation procedure, structure determination, and physical properties including water solubility are discussed. Chapter 3 deals with attempts for the removal of Cu(II) in aqueous media using ED-ch as a flocculant. Simple procedure including pH adjustment and separation of flocs by centrifuge attained almost complete removal of Cu(II). In Chapter 4, the author discuss about flocculation mechanism ED-ch for the removal of Cu(II). Chapter 5 deals with the selectivity in the removal of various heavy metal ions. Finally, the findings of each chapter and general conclusions are described.

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Chapter 2

Preparation and solubility of EDTA-linked chitosan

Abstract

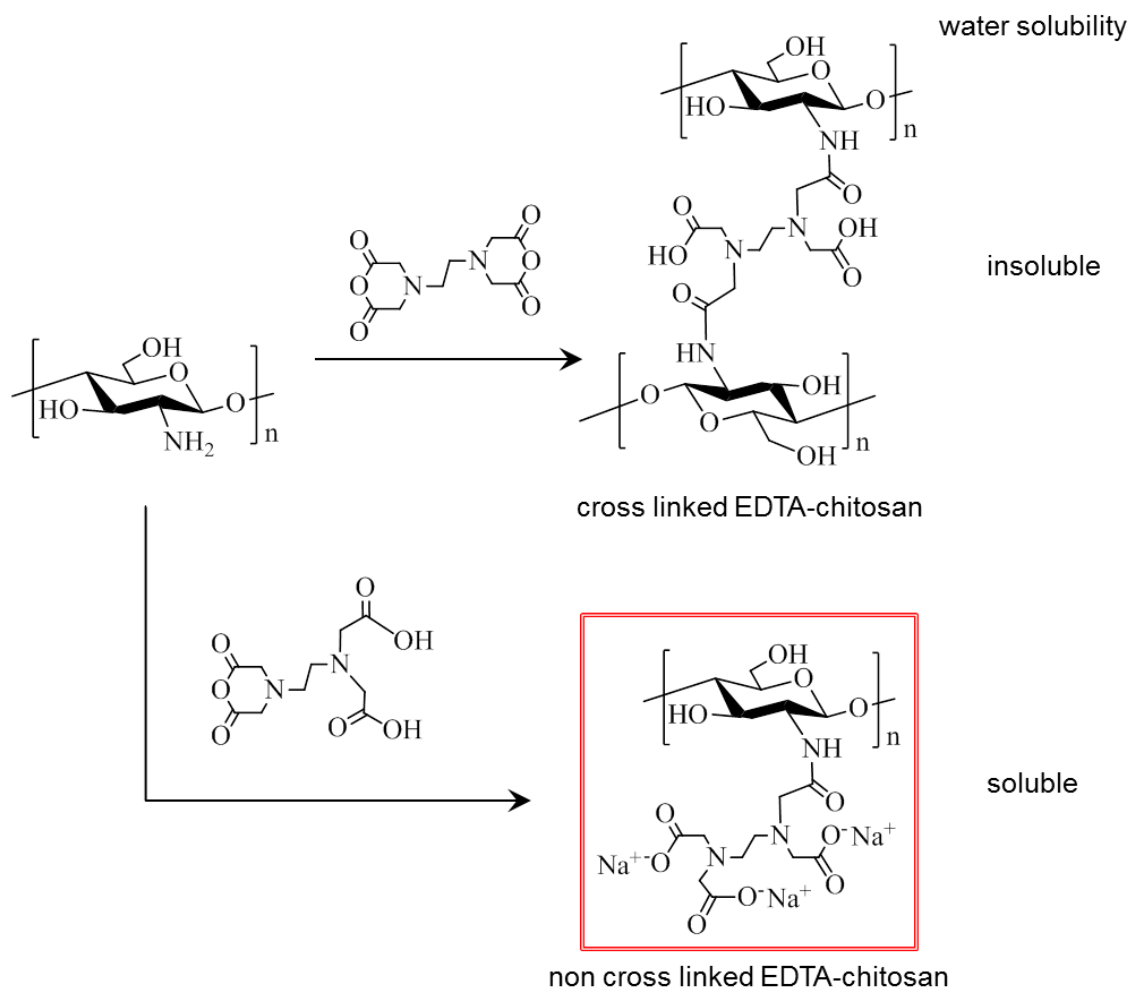
A linear and non-crosslinked polymer, EDTA-linked chitosan (ED-ch), was successfully synthesized through *N*-acylation of chitosan with EDTA (ethylenediaminetetraacetic acid) monoanhydride under from acidic to slightly basic aqueous conditions. The degree of substitution (D.S.) of EDTA residues in the product was changeable by the stoichiometry of the acylation reagent, and we obtained ED-ch with D.S. ranged from 7.5 to 79%. The structure of product was confirmed by elemental analysis, FT-IR, ^1H and ^{13}C NMR spectroscopy. The newly introduced functional group in ED-ch provided properties as an amphoteric polyelectrolyte. It was found that ED-ch had good water solubility in both acidic and basic region and precipitated in the narrow pH region.

2.1 Introduction

Chitosan is β -(1,4) linked 2-amino-2deoxy-D-glucopyranose, obtained from alkaline *N*-deacetylation of chitin. Chitosan has the reactivity of the primary amino group and the primary (O-3) and secondary hydroxyl group (O-6) in the structure of glucosamine unit.¹ A large number of chitosan derivatives have been synthesized by chemical modification of these amino or hydroxyl groups.² Furthermore, chitosan is hardly soluble in any anhydrous organic solvents or in aqueous neutral and basic solutions. However, protonation of the amino group results in polycationic property of chitosan to dissolve in aqueous solvents. Therefore, the reaction of modification of chitosan has to be carried out in aqueous acidic solution such as acetic acid and hydrochloric acid. *N*-Acylation has been frequently used because of its highest reactivity.

Using EDTA dianhydride **2** in aqueous acetic acid-methanol, EDTA linked chitosan has been synthesized by several groups, and investigated as an adsorbent for heavy metal ions.³⁻⁵ However, the chitosan material was insoluble due to crosslinking reactions occurred between chitosan chains. In the present study, the author planned to synthesize the corresponding water soluble EDTA-linked chitosan (ED-ch) derivative. ED-ch has chelation site of heavy metal ions and the presence of both carboxyl and amino groups in one polymer molecule is expected to provide flocculation ability.

In this chapter, the author would like to describe the results of the synthesis of a linear and non-crosslinked EDTA-linked chitosan (ED-ch; Scheme 1) through *N*-acylation of amino groups of chitosan using EDTA monoanhydride. Additionally, the characterization of the products was also investigated.



Scheme 1 Structure of water- insoluble and soluble EDTA-linked chitosan

2.2 Experimental

2.2.1 Materials

Low molecular weight chitosan (deacetylation degree of over 98%, average molecular weight of 1.56×10^4 g/mol) was obtained from Dainichi-seika Color and Chemicals Mtg. Co., Ltd. (Tokyo, Japan). High molecular weight chitosan (deacetylation degree of 95%, average molecular weight of $5-10 \times 10^5$ g/mol) was purchased from Hokkaido Soda Co., Ltd. (Hokkaido, Japan). Ethylenediaminetetraacetic acid (EDTA) was obtained from Dojindo Laboratories Co., Ltd. (Kumamoto, Japan). All other reagents of preparative grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and all chemicals were used without further purification. Water was purified by treatment with ion-exchanger followed by reverse osmosis with a Millipore Millex-3 apparatus.

2.2.2 Apparatus

Fourier transform infrared spectra (FT-IR) were recorded on a FT-210 spectrometer (Horiba Ltd., Japan) using a potassium bromide pellet at a resolution of 2 cm^{-1} . The samples were scanned from 4000 to 500 cm^{-1} using an average of 16 scans. ^1H and NMR spectra were recorded on an ASX-300 spectrometer (Bruker BioSpin GmbH, Germany) at 300.13 MHz and JNM-ECA600 spectrometer (Jeol Ltd., Japan) at 600.17 MHz . ^{13}C NMR spectra were recorded on JNM-ECA600 spectrometer operated at 150.91 MHz . Elemental analysis was performed by a MICRO CORDER JM-10 (J-science Co., Ltd., Japan) and CHN analyzer CE440 (Exeter Analytical Inc., USA).

Turbidity was measured with a V-560 UV-visible spectrometer (Jasco Co., Japan), using a quart cell with an optical path length of 1 cm at 600 nm. Zeta potential was conducted on a Delsa Nano HC particle analyzer (Beckman Coulter Inc., USA) with a laser light with wave-length of 658 nm at 25 °C, using the same Beckman apparatus with the standard flow cell. The zeta-potential value was calculated using Smoluchowski equation.

2.2.3 Synthesis of EDTA monoanhydride **3**

This compound was prepared by a modified procedure reported by Capretta et al..⁶ Acetic anhydride (48 ml) was added to a suspension of EDTA tetraacid (**1**: 36.0 g, 120 mmol) in pyridine (62 ml) at room temperature under N₂ atmosphere. The mixture was stirred at 65 °C for 24 h and cooled. The resulting precipitates were filtered, washed successively with acetic anhydride and diethyl ether under N₂ stream, and dried under high vacuum to give EDTA dianhydride (**2**: 30.6 g, 97% yield) as white solid.

¹H NMR (DMSO-d₆, 300 MHz): δ 2.67 (s, 4H, N(CH₂)₂N), 3.70 (s, 8H, O=CCH₂N).

The dianhydride **2** (30.0 g, 117 mmol) was dissolved in dry DMF (200 ml) at 80 °C under N₂ atmosphere. Water (2.1 ml, 117 mmol) was carefully added dropwise into the solution, and stirred at the same temperature for 3 h. Precipitate was formed during the reaction, and the turbid mixture was cooled, filtered, and washed successively with dry DMF and diethyl ether under N₂ stream. The white solid obtained was dried under high vacuum, giving the monoanhydride **3** (24.4 g, 75% yield).

¹H NMR (DMSO-d₆, 300 MHz): δ 2.59 (t, 2H, J 5.8 Hz, CH₂NCH₂C=O (carboxylate)), 2.78 (t, 2H, J 5.6 Hz, CH₂NCH₂C=O (anhydride)), 3.43 (s, 4H, NCH₂C=O (carboxylate)), 3.73 (s, 4H NCH₂C=O (anhydride)).

2.2.4 Synthesis of water soluble EDTA linked chitosan **4**

Method A: Low molecular weight chitosan (0.25 g, 1.56 mmol of D-glucosamine units) was dissolved in 2% aqueous acetic acid-methanol (100 ml, 1:1(v/v)). The monoanhydride **3** (0.21 g, 0.78 mmol, 0.5 molar equivalents to the glucosamine unit) was added to the solution, and then the mixture was stirred vigorously at room temperature. After stirring for 24 h, pH of the reaction mixture was adjusted to 8 by dropwise addition of 2% aqueous NaHCO₃, and further **3** (0.21 g, 0.78 mmol) was added to the mixture. The mixture was stirred at room temperature for 12 h, and precipitated by acidification of the mixture to pH 2-3 with 2 mol/l HCl. The resulting precipitate was separated by filtration, washed with methanol, and then dissolved in 2% aqueous NaHCO₃. The solution was subjected to ultrafiltration using a membrane (Advantec UK-10; Toyo Roshi Co., Ltd., Japan) with molecular weight cutoff 10,000 against deionized water, and lyophilized to give low molecular weight ED-ch (Na form) **4a** (0.37 g, D.S. 57%) as white hygroscopic amorphous powder.

¹H NMR (D₂O/NaOD, 80 °C, 600 MHz): δ 4.64 (s, H-1 of substituted), 4.45 (s, H-1 of unsubstituted), 3.91-3.31 (m, H3-6), 3.29 (s, NHCOCH₂N), 3.11 (s, NCH₂COONa), 2.66 (s, H-2 overlap with N(CH₂)₂N), 2.04 (s, NHCOCH₃).

^{13}C NMR ($\text{D}_2\text{O}/\text{NaOD}$, rt, 150 MHz): δ 182.5 and 182.1 (COONa), 177.5 (NHCOCH_2), 61.6 (COCH_2N), 55.0 ($\text{N}(\text{CH}_2)_2\text{N}$), 105.4, 103.9, 81.1, 77.8, 74.9, 63.1, 61.0, 59.4, and 58.1 (pyranose).

FT-IR (KBr, cm^{-1}): ν 3422 (N-H and O-H str), 2959, 2888 (C-H str), 1653 (C=O amide str), 1636 (C-O carboxylate asym), 1592 (N-H amide bend), 1405 (C-O carboxylate sym), 1324 (C-N amide str), 1151, 914 (β -(1,4) glycoside bridge), 1071, 1035 (C-O-C str).

Elemental analysis: calculate for $(\text{C}_6\text{H}_{11}\text{NO}_4)_{0.49}(\text{C}_{16}\text{H}_{22}\text{N}_3\text{O}_{11}\text{Na}_3)_{0.51}$: C 39.84, H 5.00, N 8.46%; found: C 40.37, H 5.53, N 8.57%.

Method B: ED-ch **4b** was synthesized by similar reaction with high molecular weight chitosan (2.50 g, 15.6 mmol) and **3** (0.53 g, 1.95 mmol) for 1 day and subsequent with **3** (0.53 g, 1.95 mmol) for 12 h and purified by ultrafiltration through a membrane with molecular weight cutoff 20,000, giving high molecular weight ED-ch (Na form) **4b** (2.59 g, D.S. 7.5%) as white amorphous.

^1H NMR ($\text{D}_2\text{O}/\text{DCl}$, 80 °C, 300 MHz): δ 4.91 (s, H-1 of unsubstituted), 4.71 (s, H-1 of substituted), 3.91, 3.74, 3.39 (s), 3.21 (s), 2.03 (s, NHCOCH_3).

FT-IR (KBr, cm^{-1}): ν 3430 (N-H and O-H str), 2934, 2872 (C-H str), 1641 (C=O amide str), 1636, 1598 (N-H amide bend), 1035 (O-H of primary alcohol), 1382 (C-H sym), 1326 (C-N amide str), 1151, 914 (β -(1,4) glycoside bridge), 1071, 1035 (C-O-C str).

Method C: ED-ch **4c** was synthesized by similar reaction with high molecular weight chitosan (2.50 g, 15.6 mmol) and **3** (1.06 g, 3.90 mmol) for 1 day and subsequent with **3**

(1.06 g, 3.90 mmol) for 12 h. Similar purification gave ED-ch (Na form) **4c** (3.76 g, D.S. 21%) as white amorphous.

¹H NMR (D₂O/DCl, 80 °C, 300 MHz): δ 4.91 (s, H-1 of unsubstituted), 4.71 (s, H-1 of substituted), 3.90, 3.74, 3.60, 3.39 (s), 3.21 (s), 2.03 (s, NHCOCH₃).

Elemental analysis: calculate for (C₆H₁₁NO₄)_{0.76}(C₈H₁₃NO₃)_{0.05}(C₁₆H₂₂N₃O₁₁Na₃)_{0.19}·

H₂O: C 38.92, H 6.24, N 7.93%; found: C 39.23, H 5.64, N 7.89%.

Method D: ED-ch **4d** and **4e** were synthesized by similar reaction with high molecular weight chitosan (2.00 g, 12.5 mmol) and **3** (1.71 g, 6.25 mmol) for 1 day and subsequent with **3** (1.71 g, 6.25 mmol) for 12 h. Similar purification gave ED-ch (Na form) **4d** (2.94 g, D.S. 39%) and **4e** (3.18 g, D.S. 45%) as white amorphous.

¹H NMR (D₂O/NaOD, 80 °C, 600 MHz): δ 4.65 (s, H-1 of substituted), 4.45 (s, H-1 of unsubstituted), 3.99-3.41 (m, H3-6), 3.30 (s, NHCOCH₂N), 3.14 (s, NCH₂COONa), 2.65 (s, H-2 overlap with N(CH₂)₂N), 2.05 (s, NHCOCH₃).

FT-IR (KBr, cm⁻¹): ν 3430 (N-H and O-H str), 2912 (C-H str), 1669 (C=O amide str), 1628 (C-O carboxylate asym), 1597 (N-H amide bend), 1409 (C-O carboxylate sym), 1321 (C-N amide str), 1156, 919 (β-(1,4) glycoside bridge), 1078, 1034 (C-O-C str).

Elemental analysis: **4d**; calculate for (C₆H₁₁NO₄)_{0.54}(C₈H₁₃NO₃)_{0.05}(C₁₆H₂₂N₃O₁₁Na₃)_{0.41}·0.5H₂O: C 39.29, H 5.37, N 8.18%; found: C 39.36, H 5.37, N 8.20%.

Method F: ED-ch **4f** was synthesized by similar reaction with high molecular weight chitosan (2.50 g, 15.6 mmol) and **3** (3.18 g, 11.6 mmol) for 1 day and subsequent with **3**

(3.18 g, 11.6 mmol) for 12 h. Similar purification gave ED-ch (Na form) **4f** (4.73 g, D.S. 58%) as white amorphous.

Elemental analysis: calculate for $(\text{C}_6\text{H}_{11}\text{NO}_4)_{0.36}(\text{C}_8\text{H}_{13}\text{NO}_3)_{0.05}(\text{C}_{16}\text{H}_{22}\text{N}_3\text{O}_{11}\text{Na}_3)_{0.59}$: C 39.50, H 4.87, N 8.44%; found: C 39.30, H 5.35, N 8.33%.

Method G: ED-ch **4g** was synthesized by similar reaction with high molecular weight chitosan (2.50 g, 15.6 mmol) and **3** (4.27 g, 15.6 mmol) for 1 day and subsequent with **3** (4.27 g, 15.6 mmol) for 12 h. Similar purification gave ED-ch (Na form) **4g** (5.12 g, D.S. 71%) as white amorphous.

Method H: ED-ch **4h** was synthesized by similar reaction with high molecular weight chitosan (2.00 g, 12.5 mmol) and **3** (5.15 g, 18.8 mmol) for 1 day and subsequent with **3** (5.15 g, 18.8 mmol) for 12 h. Similar purification gave ED-ch (Na form) **4h** (4.37 g, D.S. 73%) as white amorphous.

Elemental analysis: calculate for $(\text{C}_6\text{H}_{11}\text{NO}_4)_{0.25}(\text{C}_8\text{H}_{13}\text{NO}_3)_{0.05}(\text{C}_{16}\text{H}_{22}\text{N}_3\text{O}_{11}\text{Na}_3)_{0.70} \cdot 0.5\text{H}_2\text{O}$: C 38.34, H 4.86, N 8.19%; found: C 38.82, H 5.20, N 8.29%.

Method I: ED-ch **4i** was synthesized by similar reaction with high molecular weight chitosan (2.50 g, 15.6 mmol) and **3** (6.43 g, 23.5 mmol) for 1 day and subsequent with **3** (6.43 g, 23.5 mmol) for 12 h. Similar purification gave ED-ch (Na form) **4i** (4.94 g, D.S. 79%) as white amorphous.

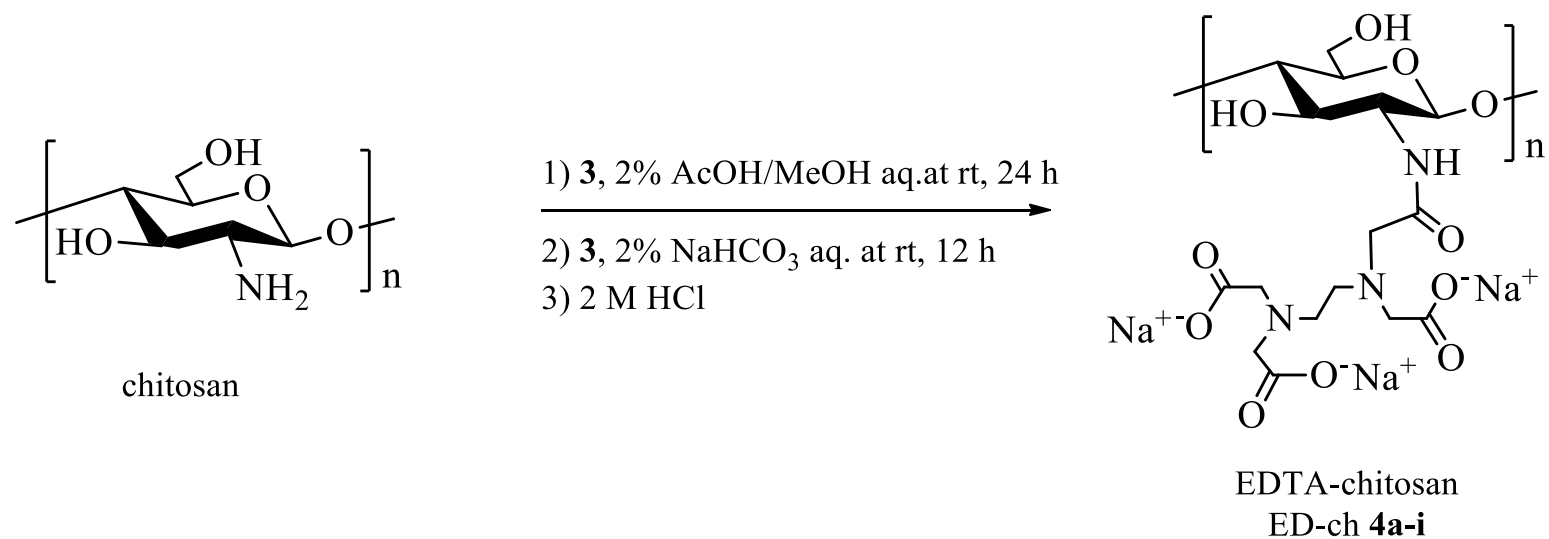
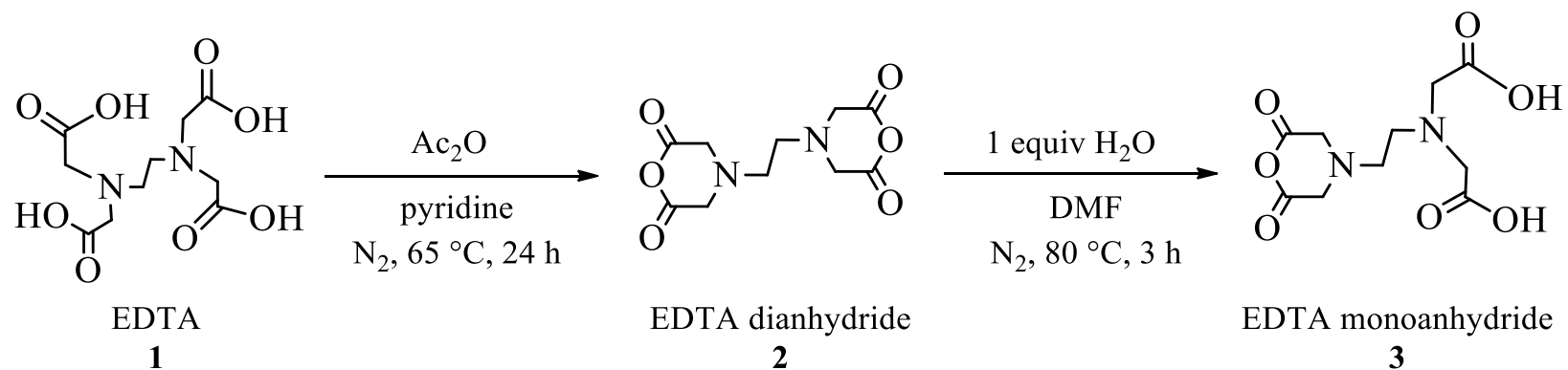
2.2.5 Solubility of EDTA linked chitosan **4**

The solubility was evaluated by turbidity measurement based on the method reported by Kubota *et al.*⁷ Fine powder of ED-ch (Na form) **4a-i** (5 mg) was mixed with deionized water (5 ml), and shook vigorously for 1 h. The optical transmittance of each sample was recorded on UV-visible spectrometer at 600 nm. The pH dependence on the solubility of ED-ch and chitosan was also estimated from the optical transmittance in a similar way. Each sample was prepared by dissolving or chitosan (20 mg) in 0.1 mol/l HCl (20 ml), and adjusted the pH by addition of small amount of 3 mol/l HCl or 3 mol/l NaOH solutions. The zeta potential value of each sample was also measured at different pH.

2.3 Results and Discussion

2.3.1 Preparation of water soluble EDTA linked chitosan **4**

Since EDTA linked chitosan so far reported was synthesized by using EDTA dianhydride **2** in aqueous acetic acid-methanol, the product was insoluble due to crosslinking reactions occurred between chitosan chains.^{3,8} In order to prevent the crosslinking reaction and to obtain the corresponding water soluble material, the author examined N-acylation by use of EDTA monoanhydride **3** instead of **2**. As shown in Scheme 2, **3** was prepared by a two-step reaction including dehydration of free EDTA **1** and selective hydrolysis.⁶ Since EDTA monoanhydride **2** is hardly soluble in the reaction solvent, DMF, pure **2** was obtained by filtration of the crystal produce. N-Acylation of chitosan of both low and high molecular weight was carried out according to a slightly modified procedure developed by Satoh et al.,⁹ under continually homogenous conditions in aqueous medium where O-acylation of chitosan would be restricted by hydrolysis of **3**. A solution of chitosan in aqueous acetic acid-methanol was treated with **3**, and then the reaction was continued under slightly basic media of pH 8. Precipitate obtained by acidification of the reaction mixture with aqueous HCl was collected by filtration. The carboxyl groups of EDTA residue were converted to sodium carboxylate groups by dissolving the product in aqueous NaHCO₃, and subsequently the solution was subjected to ultrafiltration to remove unreacted agents. Finally, lyophilization was performed to give ED-ch **4** as white cotton-like hygroscopic amorphous. ED-ch was synthesized using two types of molecular weight chitosan, low molecular weight (1.56×10^4 g/mol, **4a**) and high molecular weight ($5-10 \times 10^5$ g/mol, **4b-i**). ED-ch **4a** with low molecular weight was used mainly



Scheme 2 Preparation of EDTA monoanhydride **3** and water soluble EDTA linked chitosan **4**.

Table 1 Preparation and properties of EDTA linked chitosan **4**.

sample	Mw (g/mol)	Molar ratio of the starting material (EDTA: chitosan)	D.S. (%)		Water solubility ^c
			Elemental analysis ^a	¹ H NMR ^b	
4a	1.56×10^4	1 : 1	51	57	Soluble
4b	$5-10 \times 10^5$	0.25 : 1	—	7.5	Insoluble
4c	$5-10 \times 10^5$	0.5 : 1	19	21	Insoluble
4d	$5-10 \times 10^5$	1 : 1	41	39	Soluble
4e	$5-10 \times 10^5$	1 : 1	—	45	Soluble
4f	$5-10 \times 10^5$	1.5 : 1	59	58	Soluble
4g	$5-10 \times 10^5$	2 : 1	—	71	Soluble
4h	$5-10 \times 10^5$	3 : 1	70	73	Soluble
4i	$5-10 \times 10^5$	3 : 1	—	79	Soluble

^a calculation was based on the C/N ratio.^b calculated from integral of H-1protons.^c sample (5 mg) was dissolved in H₂O (5 ml). Transmittance at 600 nm of the sample 95% higher than deionized water referred as “soluble”.

for characterization of the structure by FT-IR, ^1H , and ^{13}C NMR spectroscopy. Reaction was conducted with various molar ratios of **3** and chitosan, as shown in Table 1.

The degrees of substitution (D.S.) of the EDTA residue in the products were depended on the molar ratio of **3** and chitosan. Reaction with equimolar amount of **3** provided ED-ch with D.S. 40-60% (**4a**, **4d** and **4e**), and use of 3 fold excess reagent gave ED-ch with D.S. approximately 70-80% (**4h** and **4i**) as summarized in Table 1.

2.3.2 Structural analysis of EDTA linked chitosan **4**

2.3.2.1 FT-IR analysis

Fig. 1 shows the FT-IR spectra of chitosan and ED-ch **4a-i**. In the spectrum of chitosan (Fig. 1 (a)), broad absorption at 3435 cm^{-1} corresponds to NH and O-H stretching vibration, and absorption band between 2922 and 2880 cm^{-1} are attributable to the C-H stretching vibration. Moreover, the characteristic absorption bands were observed at 1653 , 1597 , and 1324 cm^{-1} due to the C=O stretching vibration (amide I), N-H bending vibration (amide II), and C-N stretching vibration (amide III) from the secondary amide, respectively.¹⁰ The absorption bands at 1421 and 1384 cm^{-1} relate to O-H plane deformation of primary alcohol and C-H symmetric bending vibration in -CHOH-. The absorption bands of the β -(1,4) glycoside bridge at 1152 and 897 cm^{-1} , and C-O-C stretching vibration in the glucopyranose at 1091 and 1034 cm^{-1} were observed. Compared with the original chitosan, FT-IR spectrum of **4a** reveals several new absorption bands as shown in Fig. 1 (b). Absorption appeared at 1636 and 1405 cm^{-1} corresponds to C-O asymmetrical and symmetrical stretching vibration of the carboxylate group, respectively,^{5,11} and intensity of the amide I and II bands at 1653 and 1592 cm^{-1} increased.

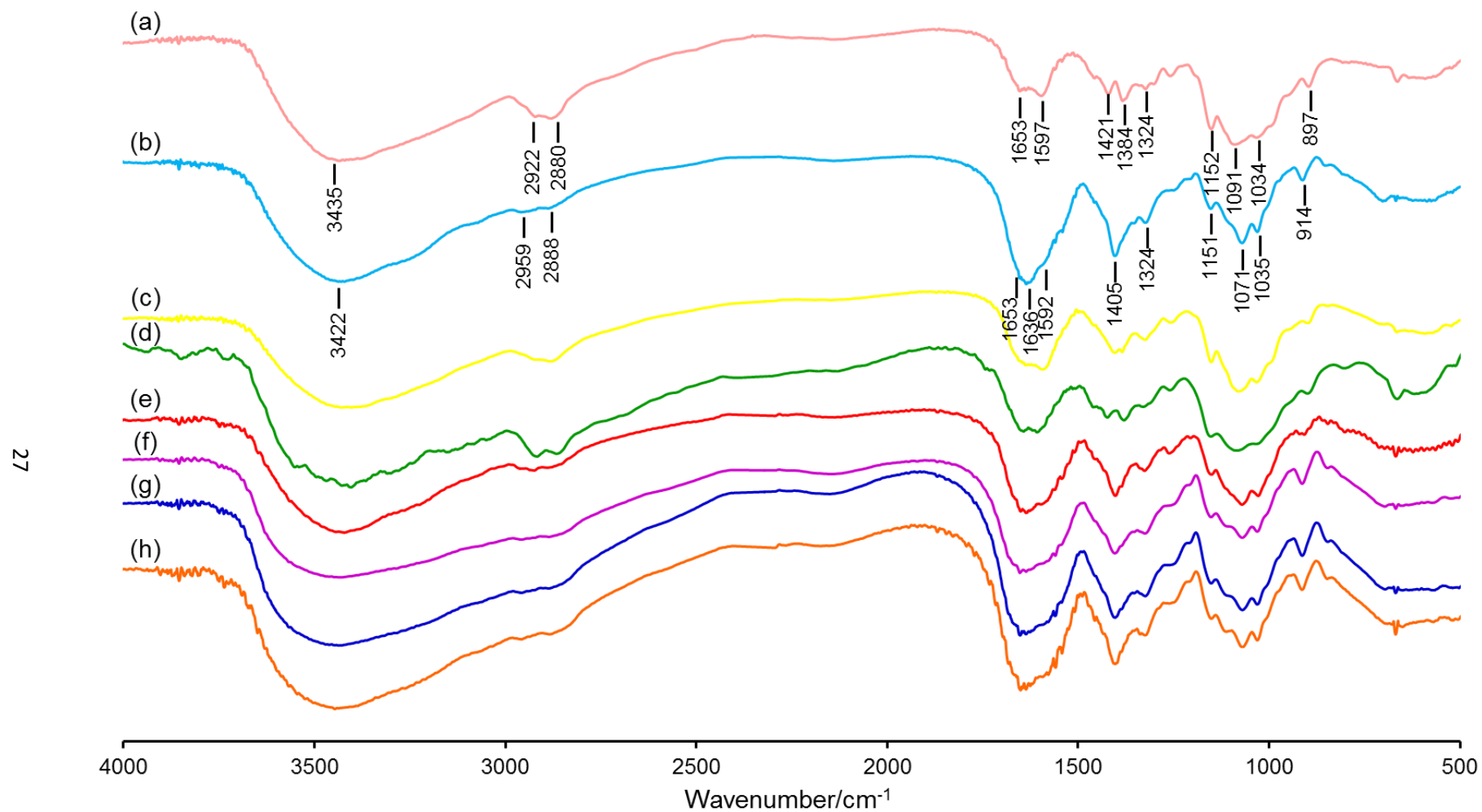


Figure 1 FT-IR spectra of (a) chitosan , ED-ch (b) **4a** (D.S. 57%), (c) **4b** (D.S. 7.5%), (d) **4c** (D.S. 21%), (e) **4d** (D.S. 39%), (f) **4f** (D.S. 58%), (g) **4h** (D.S. 73%), and (h) **4i** (D.S. 79%).

Furthermore, absorption band of ester (C=O stretch) was not observed at 1720-1770 cm^{-1} ,^{12,13} suggesting highly selective formation of the amino group by the reaction with EDTA monoanhydride **3** under the aqueous conditions.

In the spectra of ED-ch **4b** with D.S. 7.5% and **4c** with D.S. 21% (Fig. 1 (c) and (d)), the appreciable alternation was not observed comparing with that of chitosan. The spectra of **4d-i** (D.S. >39%; Fig. 1 (e)-(h)) showed that intensity of the amide I and II bands at 1653 and 1592 cm^{-1} increased with increasing D.S..

2.3.2.2 ^{13}C NMR analysis

^{13}C NMR spectra of chitosan in $\text{DCl}/\text{D}_2\text{O}$ and **4a** in $\text{NaOD}/\text{D}_2\text{O}$ are shown Fig. 2. Six signals of chitosan (Fig. 2 (a)) at 98.3 (C1), 77.8 (C4), 75.6 (C5), 70.9 (C3), 61.1 (C6) and 56.8 ppm (C2) are attributable to the pyranose carbons of the 2-amino-2-deoxy-D-glucopyranose residue.¹⁴ In the spectrum of **4a** (Fig. 2 (b)), the new signals were observed at 61.6 and 55.0 ppm, which were assigned to two kinds of methylene carbon in EDTA moiety.¹⁵ The new signals at 182.5 and 182.1 ppm were assignable to carbonyl carbons of carboxylate groups in the EDTA moiety, and the signal at 177.5 ppm corresponds to carbonyl carbon of amide group. These results also supported the assigned structure of ED-ch **4**. Relatively sharp peaks observed at 171.1 and 173.9 ppm were probably due to low molecular weight contaminants such as CO_3^{2-} in $\text{D}_2\text{O}/\text{NaOD}$.¹⁶

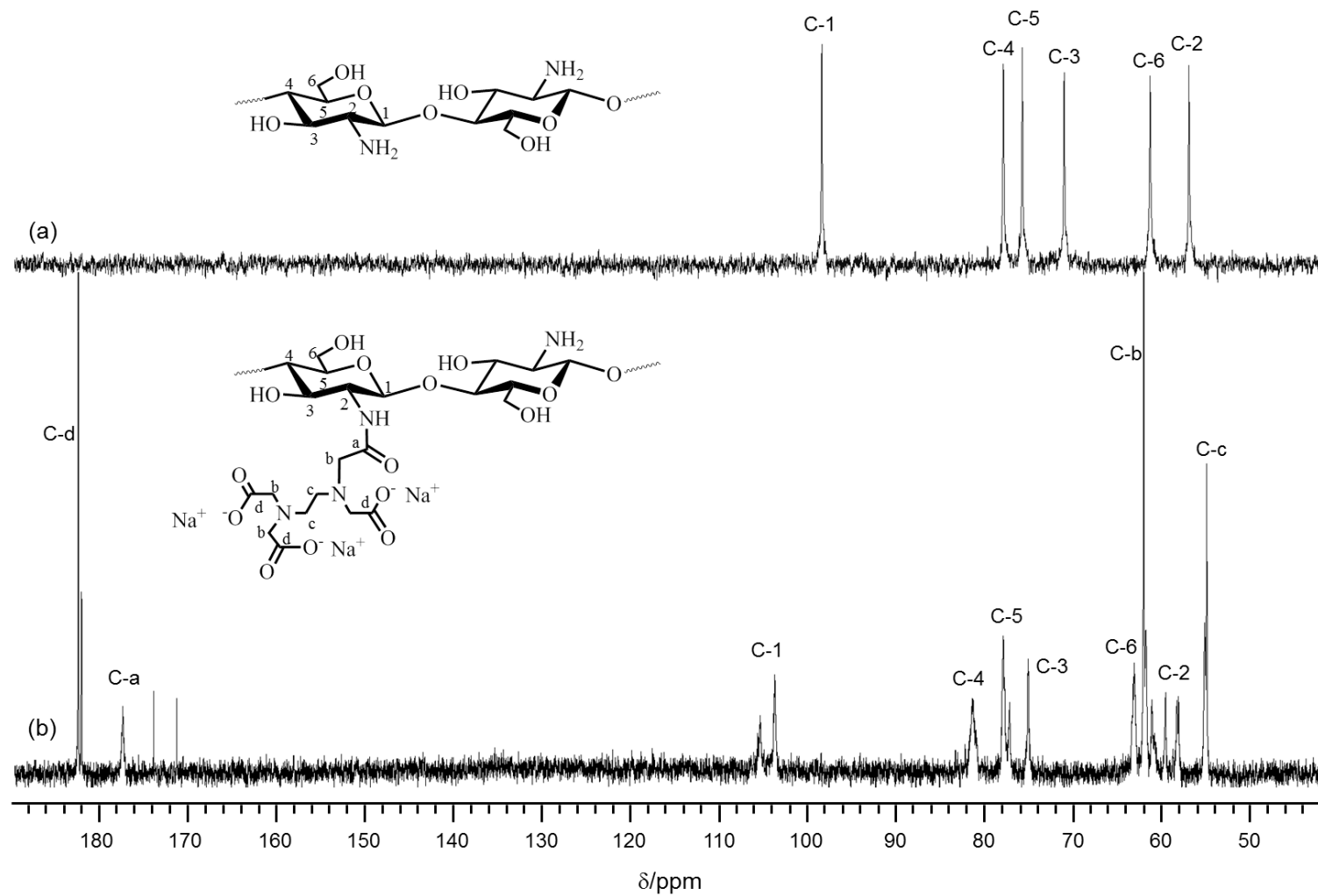


Figure 2 ^{13}C NMR spectra of (a) chitosan in $\text{DCl}/\text{D}_2\text{O}$ and (b) ED-ch 4a (D.S. 57%) in $\text{NaOD}/\text{D}_2\text{O}$ at rt.

2.3.2.3 ^1H NMR analysis

^1H NMR spectra of ED-ch **4a** in NaOD/D₂O at 5 and 80 °C are shown Fig. 3. The spectra were measured at different temperatures in order to shift the large signal of HOD. The signals at 4.64 and 4.45 ppm were attributed to the anomeric protons of *N*-substituted (H-1') and un-*N*-substituted (H-1)¹⁷ glucosamine residues, respectively. The signal at δ 3.91-3.31 ppm assigned to H-3-6 of glucosamine residues.⁸ Generally, presence of *O*-acyl groups at O-3 or O-6 positions of glucosamine residue can be determined by strong downfield shift of the corresponding α -protons.¹⁸ Observation of no signal at such lower magnetic field of δ 3.9-4.2 ppm in the spectrum (a) suggested the absence of the *O*-acyl groups. The signals at 3.29, 3.11, and 2.66 ppm correspond to protons of three kinds of methylene groups of EDTA residue. The peak at 2.66 ppm was an overlap signal of H-2 of chitosan and H-b of EDTA residue.

Fig. 4 and 5 show the spectra of chitosan and ED-ch **4b-c** in DCl/D₂O at 80 °C, and **4d-i** in NaOD/D₂O at 80 °C, respectively. Since chitosan, ED-ch **4b**, and **4c** could not be dissolved in both D₂O and NaOD/D₂O, the spectra were measured in DCl/D₂O. In the spectrum of chitosan (Fig.4 (a)), the signals at 4.89, 3.60-4.05, and 3.21 ppm were attributed to H-1, H3-6, and H-2 of glucosamine residues, respectively. Compared with the original chitosan, the spectra of **4b** and **4c** reveal three new signals at 4.71, 3.60, and 3.39 ppm, as indicated by the arrows. The new signal at 4.71 ppm was assigned to the anomeric protons of *N*-substituted (H-1') glucosamine residues. This result suggested that reaction with even less equimolar amount of EDTA monoanhydride **3** could provide ED-ch. In addition, it can be seen that the signals at 4.91 (H-1) and 3.21 ppm (H-2) in DCl/D₂O significantly shifted to high magnetic field of δ 4.45 and 2.66 ppm in NaOD/D₂O (Fig. 3 and 5).

The degree of substitution (D.S.) of the EDTA residue in the products was calculated the area ratio between the anomeric protons of substituted (H-1') and unsubstituted (H-1) glucosamine residues in ^1H NMR spectrum.

$$\text{D.S. (\%)} = \left(\frac{\text{H} - 1'}{\text{H} - 1' + \text{H} - 1} \right) \times 100$$

Moreover, D.S. was also calculated from C/N ratio of elemental analysis using the following equation:

$$\text{C/N} = \frac{73.26 + 120.1x}{14.01 + 28.02x}$$

where C/N is C/N ratio of elemental analysis and x is D.S.. The molar mass of carbon and nitrogen per mole of glucosamine unit are calculated to be 73.26 and 14.01, respectively. Those of EDTA residue are 120.1 and 28.02, respectively. The results of calculation are shown in Table 1, and D.S. values obtained from ^1H NMR spectra are in good agreement with from C/N ratio of elemental analysis. D.S. of ED-ch **4** was able to change by the molar ratio of starting chitosan and EDTA monoanhydride used.

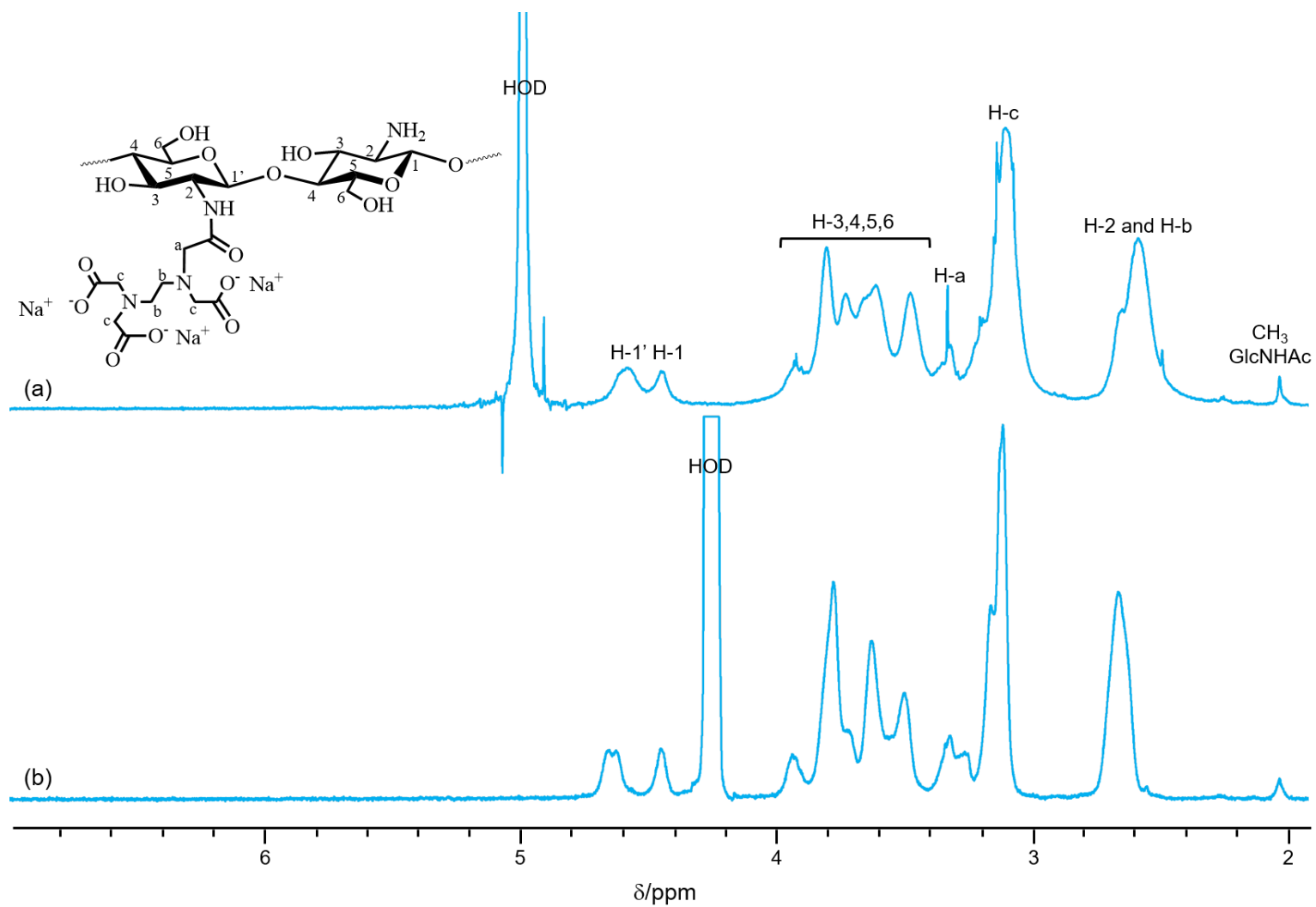


Figure 3 ^1H NMR spectra of ED-ch **4a** (D.S. 57%) at (a) 5 °C and (b) 80 °C in $\text{NaOD}/\text{D}_2\text{O}$.

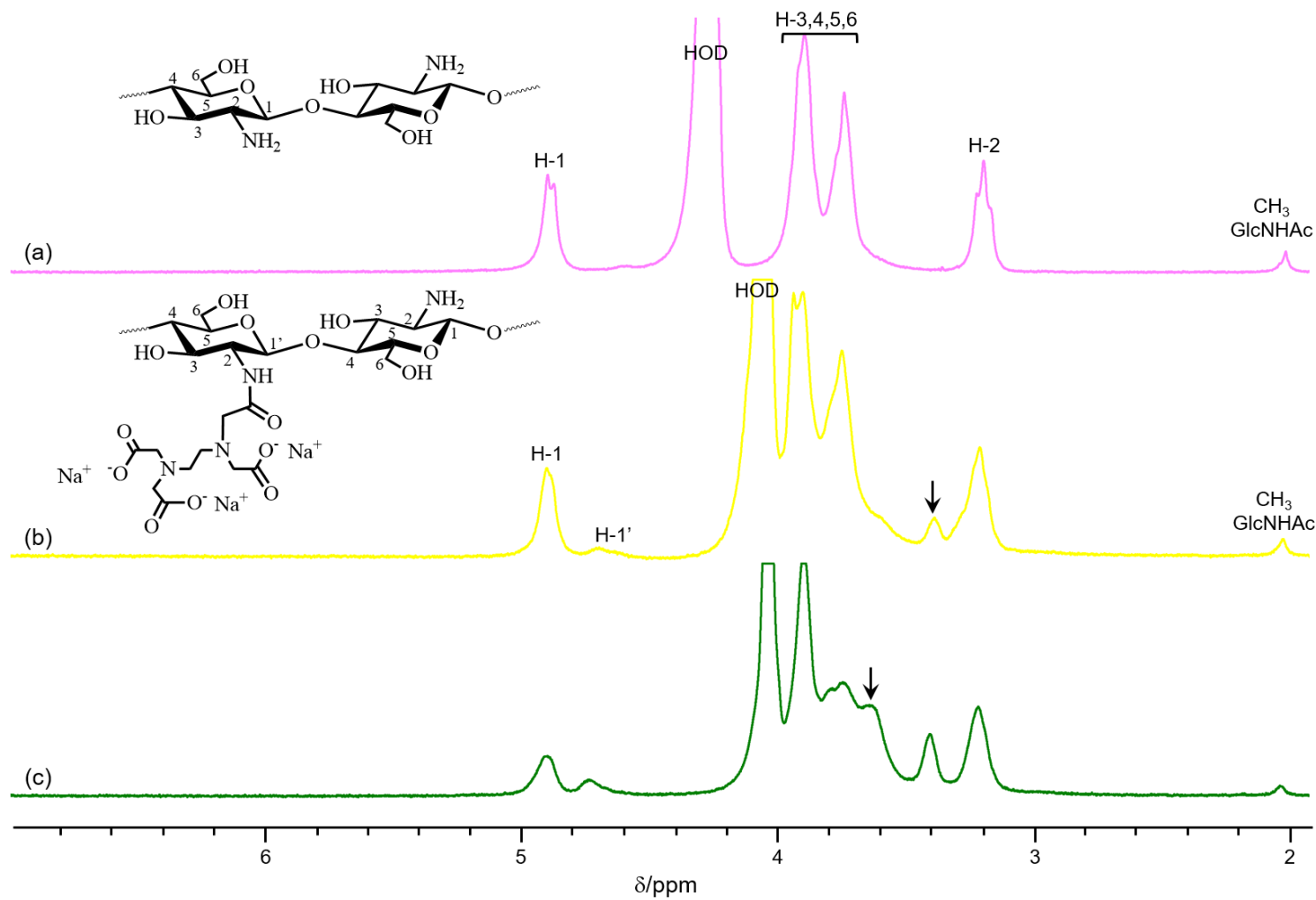


Figure 4 ^1H NMR spectra of (a) chitosan, ED-ch (b) **4b** (D.S. 7.5%), and (c) **4c** (D.S. 21%) at 80 °C in DCl/D₂O.

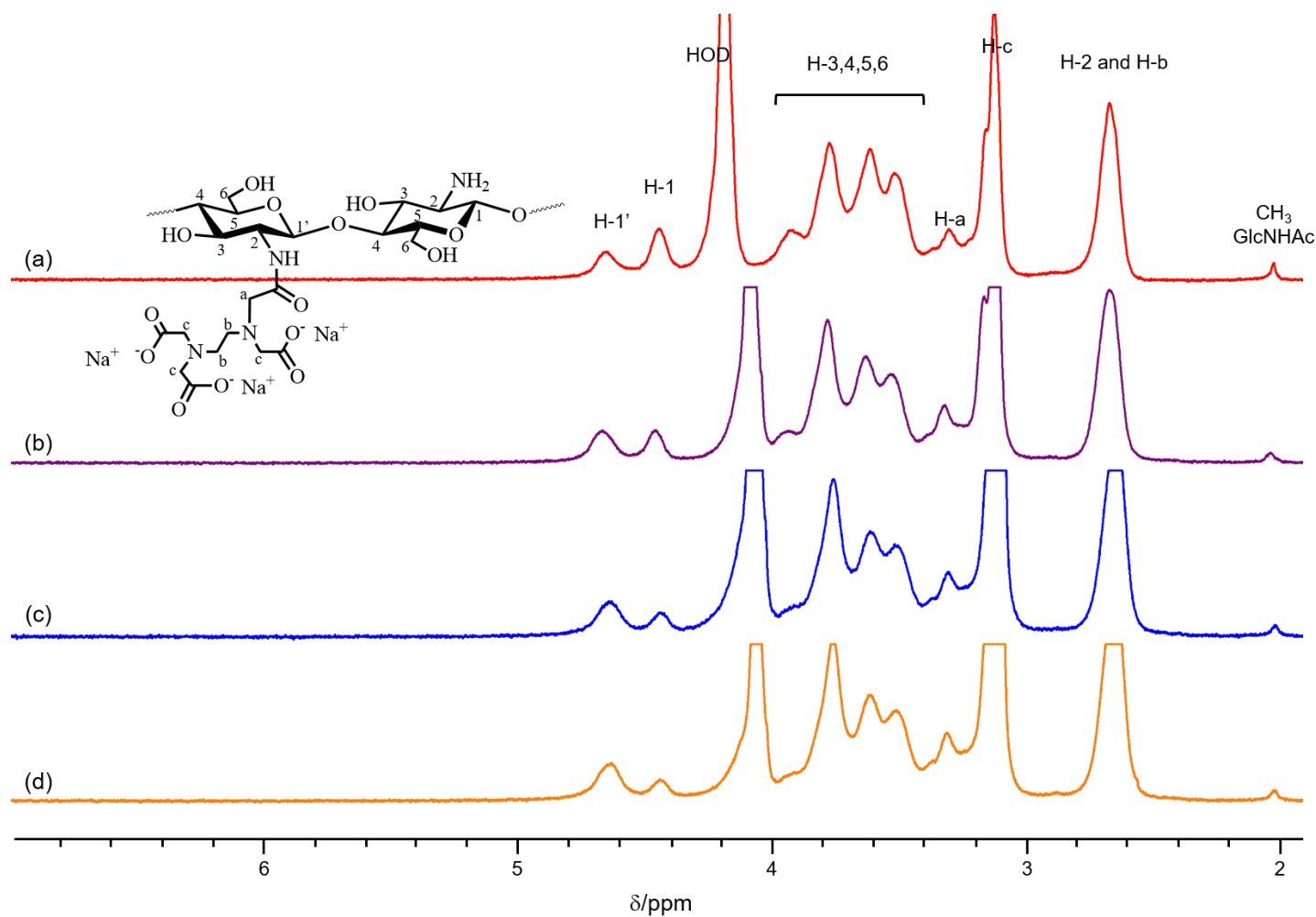


Figure 5 ^1H NMR spectra of ED-ch (a) **4d** (D.S. 39%), (b) **4f** (D.S. 58%), (c) **4h** (D.S. 73%), and (d) **4i** (D.S. 79%) at 80 °C in $\text{NaOD}/\text{D}_2\text{O}$.

2.3.3 Solubility of EDTA linked chitosan **4**

Solubility is one of the most basic characteristics in utilizing as flocculant for water treatment. We investigated water solubility by turbidity measurement, and the sample was determined soluble when the transmittance was higher than 95%, compared to that of deionized water. Fig. 6 shows the relationship between the transmittance and D.S.. The transmittance of ED-ch with D.S. 7.5 and 21% were lower than 95% and these were poorly soluble. ED-ch with over D.S. 39% was found to be dissolved readily in deionized water without adding any acid in contrast to original chitosan, and the transmittance of sample was higher than 95%. This can be caused by that inter and intramolecular hydrogen bonds of chitosan were disrupted by introduction of EDTA as bulky group, and the carboxyl groups were also enhanced affinity in water.

Next our interest focused on pH dependence on solubility of ED-ch **4**, because behavior of the polymeric amphoteric compound in aqueous media seemed to be important for application to flocculant. The pH dependence on solubility of high molecular weight ED-ch with different D.S. **4b-i**, and chitosan were investigated by turbidity measurement in aqueous solution in the pH range of 1-8. The results are shown in Fig. 8-12 and that of chitosan is shown in Fig. 7 for comparison. The transmittance of chitosan solution was close to 100% between the pH region of 1.0-6.0, whereas it rapidly decreased at pH >6.0. The solubility of chitosan in acidic region is known to be caused by the proto-nation of amino groups.¹⁹

By contrast to chitosan, the EDTA derivatives **4** were found to be the soluble in the wide range of pH region, including both acidic and basic region. These results are explainable by consideration that ED-ch is amphoteric polyelectrolyte containing amino and carboxyl groups, which can be protonated or deprotonated depending on pH of the

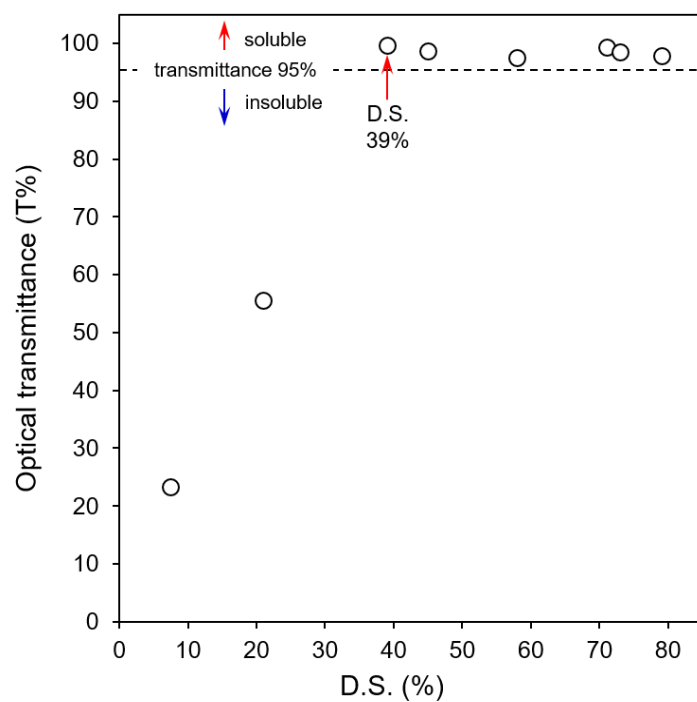


Figure 6 D.S. dependence of water solubility of ED-ch **4**. Sample (5 mg) was dissolved in H₂O (5 ml). Transmittance at 600 nm of the sample was measured at 600 nm.

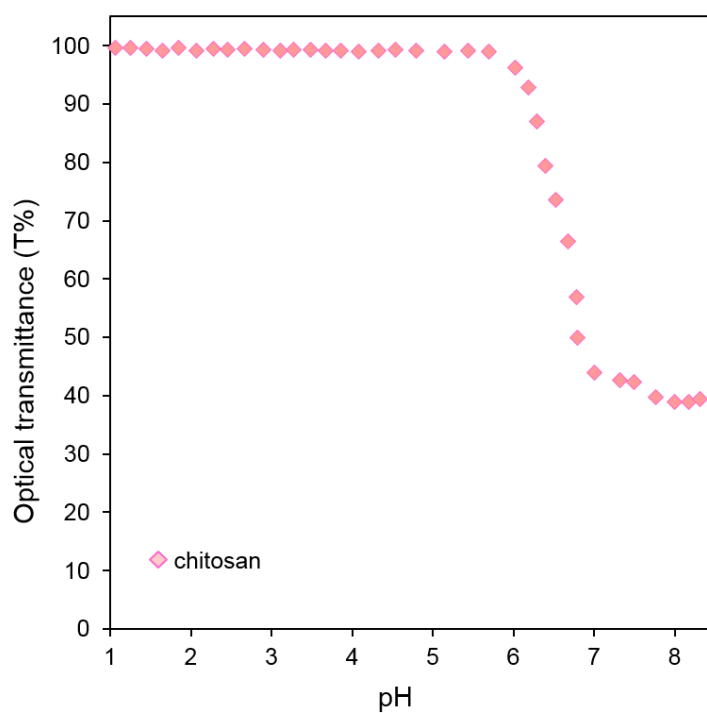


Figure 7 The optical transmittance (T%) of aqueous solutions of chitosan under various pH.

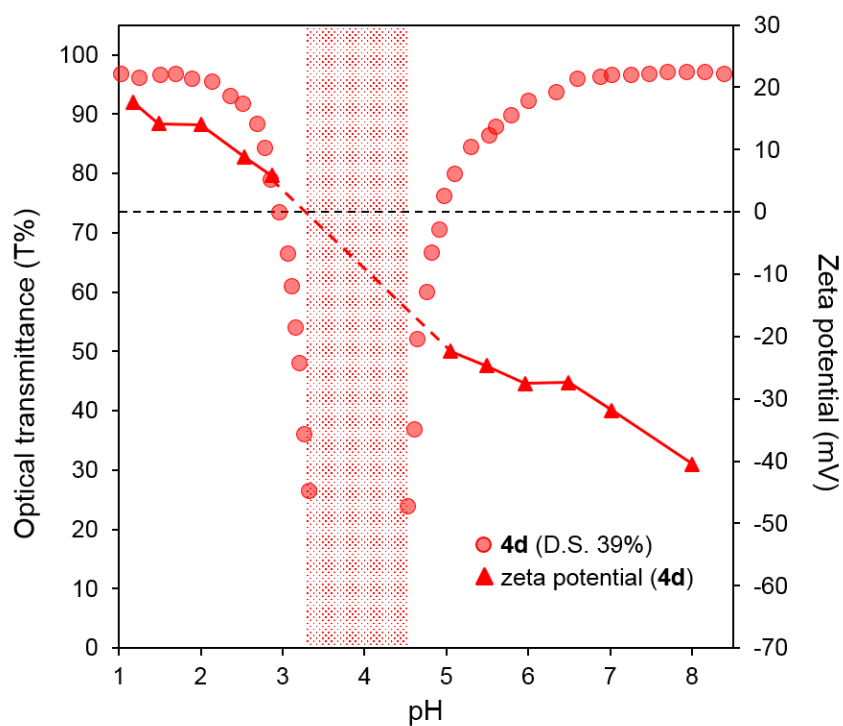


Figure 8 The optical transmittance (T%) and the zeta potential of aqueous solutions of ED-ch **4d** with D.S. 39% under various pH.

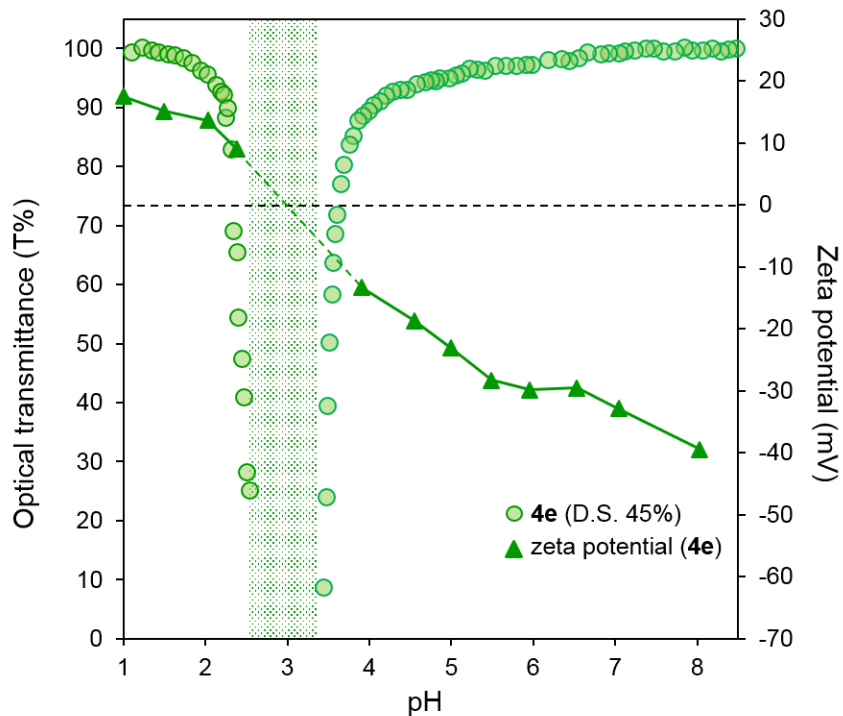


Figure 9 The optical transmittance (T%) and the zeta potential of aqueous solutions of ED-ch **4e** with D.S. 45% under various pH.

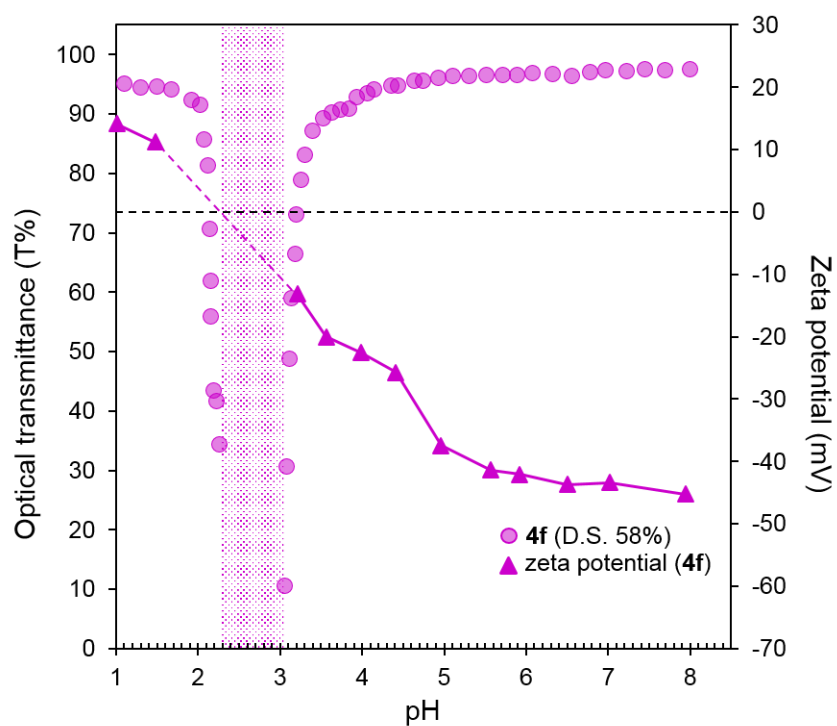


Figure 10 The optical transmittance (T%) of aqueous solutions of ED-ch **4f** with D.S. 58% under various pH.

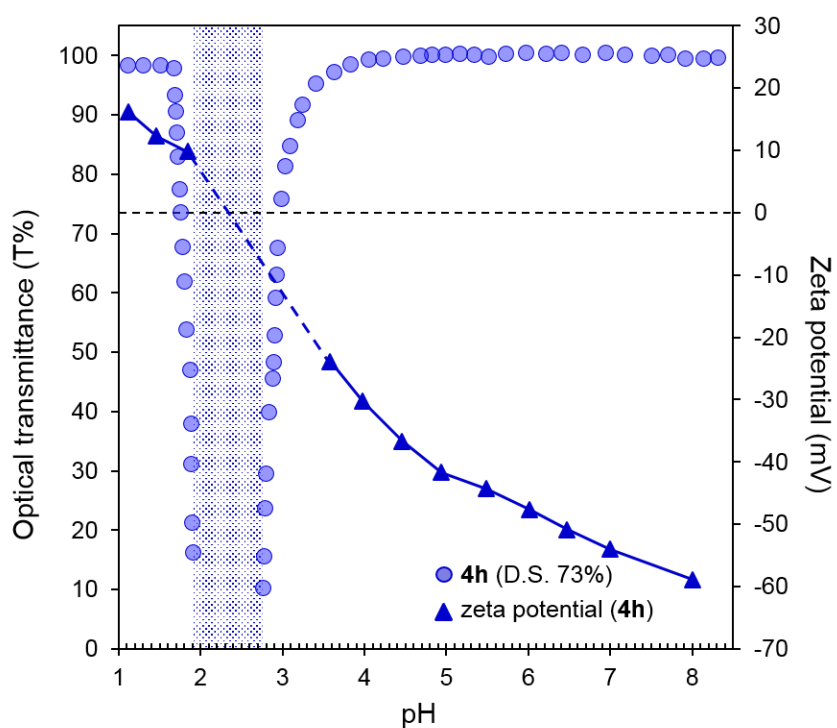


Figure 11 The optical transmittance (T%) of aqueous solutions of ED-ch **4h** with D.S. 73% under various pH.

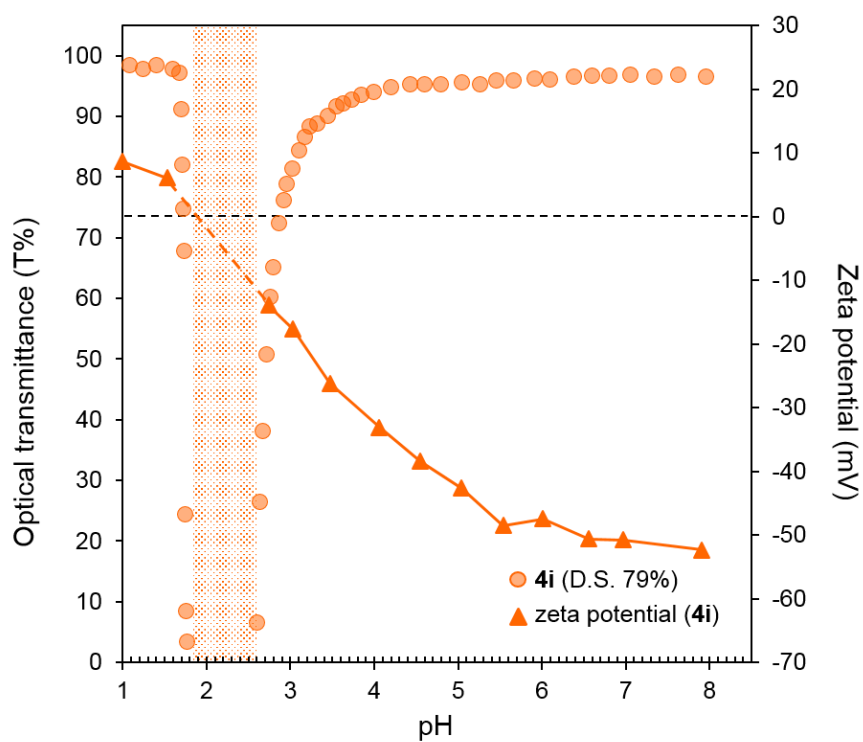


Figure 12 The optical transmittance (T%) of aqueous solutions of ED-ch **4i** with D.S. 79% under various pH.

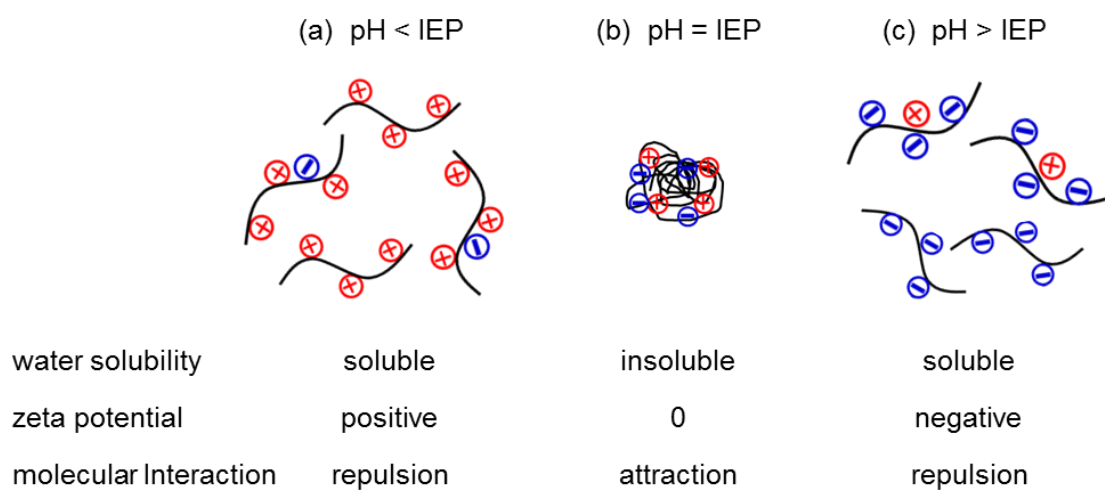


Figure 13 Schematic representation of effect of pH on solubility of ED-ch.

solution. In acidic solution, it should have unionized carboxyl groups (-COOH) but protonated amino groups ($-\text{NH}_3^+$), behaving as a cationic polyelectrolyte that causes intra- and inter-molecular electrostatic repulsive interaction. While the amino groups are deprotonated in basic solution, ED-ch can also act as an anionic polyelectrolyte due to the deprotonation of carboxyl group to carboxylate ions ($-\text{COO}^-$). The zeta potential was also corroborated these discussion (Fig. 8-12). The zeta potential of ED-ch was positive value at acidic conditions, while it changed to negative value at neutral or basic conditions, *i.e.*, ED-ch had either positive or negative surface charge at the pH region we examined, as shown in Fig. 13 (a) or (c). These results suggested that ED-ch behaved as a cationic or an anionic polyelectrolyte when it was dissolved in aqueous solution.

It was quite interesting that the transmittance of **4d**, **4e**, **4f**, **4h**, and **4i** decreased in the acidic ranges of pH 2.4-6.3, 2.1-4.8, 1.9-4.4, 1.7-3.6, and 1.6-3.7 respectively. The solution became opaque in these pH regions due to formation of precipitates. Furthermore, the observed transmittance became unstable in the narrow pH regions of 3.3-4.5 (**4d**), 2.6-3.4 (**4e**), 2.3-3.0 (**4f**), 1.9-2.7 (**4h**), and 1.8-2.6 (**4i**) which are shown by dotted bar in these figures. Leaving these samples to stand for 10 min, we found the transmittance gradually increased to maximum of approximately 95%. These tendencies of **4d** could be observed visually as shown in Fig. 14. Precipitate formation of transparent solution of pH 7.0 (Fig. 14 (a)) occurred by changing to pH 4.6, 4.0 (Fig. 14 (b), (c)). At pH 4.0, fine particles presented in the suspension (Fig. 14 (c)) gradually became larger aggregates and settled out in the clear after leaving for 10 min (Fig. 14 (d)). The zeta potential of ED-ch decreased with increasing pH. Although the potential could not measure when the sample contained precipitations, it changed from positive

value to negative value at the boundary pH regions. The zeta potential of the precipitates can be presumed to be close to zero by interpolation of these data. These ranges of pH are affected by the effect of the isoelectric point (zeta potential = 0) at which equimolar of NH_3^+ and COO^- exists in the molecule. When the pH approaches the isoelectric point (IEP), the electrostatic attractive interaction between NH_3^+ and COO^- dominates the electrostatic repulsive interaction, which makes polymer chain aggregate²⁰ (as show in Fig. 14 (b)) thus the solution exhibited low optical transmittance. These behaviors are agreed characteristic of most of amphoteric polyelectrolytes.²¹⁻²³ The solubility and IEP of ED-ch with various D.S. in aqueous solution of pH 1.0-8.0 are summarized in Table 2. IEP was approximately determined from interpolation as dotted line in the zeta potential data. The insoluble pH region narrowed and moved to the lower pH region with increasing D.S.. IEP also shifted to lower pH with increasing D.S.. It is reasonable for ED-ch with higher D.S. to precipitate in the lower pH region because it has fewer amounts of amino groups.

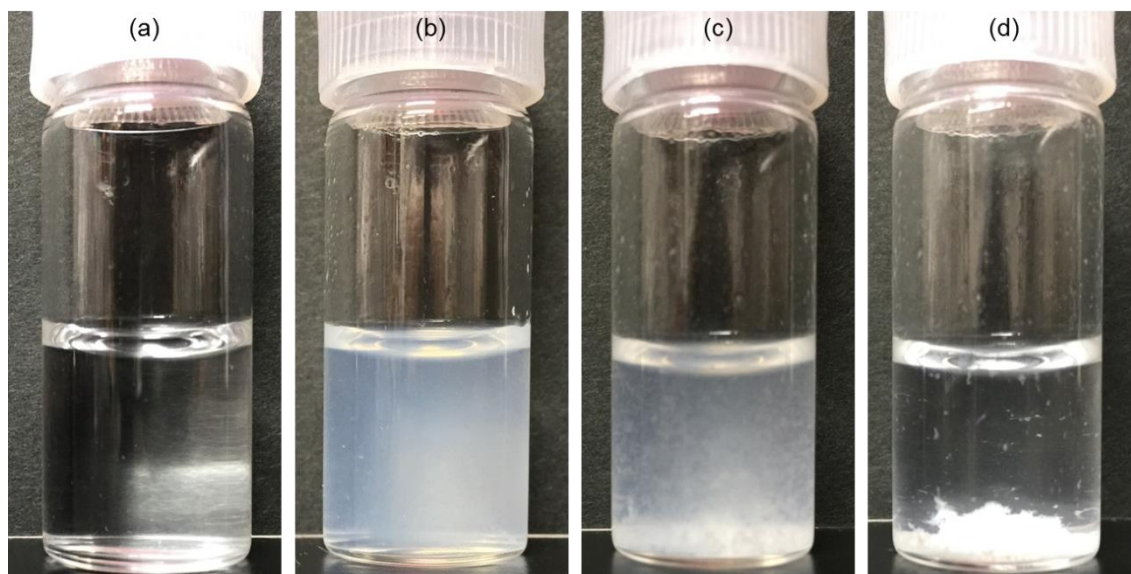


Figure 14 The photograph of ED-ch **4d** (D.S. 39%) with 0.1wt% in aqueous solution at (a) pH 7.0, (b) pH 4.6, (c) pH 4.0 immediately after adjusting pH, and (d) pH 4.0 after leaving to stand for 10 min.

Table 2 Solubility of ED-ch with various D.S. at various pH levels.

Sample	Solubility (pH) ^a								Isoelectric point (IEP) ^b
	1	2	3	4	5	6	7	8	
chitosan									—
4d (D.S. 39%)									3.3
4e (D.S. 45%)									3.0
4f (D.S. 58%)									2.3
4h (D.S. 73%)									2.3
4i (D.S. 79%)									1.9

^a opened bar means “soluble”; closed bar means “insoluble”; hatched bar means “formation of precipitation”.

^b determined from the zeta potential.

2.4 Conclusion

In the synthetic study described in this chapter, the author established method for the synthesis of a linear and non-crosslinked polymer, EDTA-linked chitosan (ED-ch), by *N*-acylation of chitosan with EDTA (ethylenediaminetetraacetic acid) monoanhydride under from acidic to slightly basic aqueous conditions. The degree of substitution (D.S.) of EDTA residues in the product was changeable by the stoichiometry of the acylation reagent, and the author obtained ED-ch with D.S. ranged from 7.5 to 79%. The structure of product was confirmed by elemental analysis, FT-IR, ^1H and ^{13}C NMR spectroscopy. The newly introduced functional group in ED-ch provided the expected property as an amphoteric polyelectrolyte. It was found that ED-ch had good water solubility in both acidic and basic region and precipitated in the narrow pH region. The characteristic property of ED-ch was expected to be used as a flocculating material for water treatment. The investigations along this line are described in the following chapters.

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Chapter 3
pH dependent flocculation property
and removal of Cu(II)

Abstract

To utilize ED-ch as a flocculant for removal of heavy metal ion from aqueous solution by flocculation process, we investigated flocculation property by using Cu(II) as a model pollutant of heavy metal ion. ED-ch was characterized for the flocculation performance against Cu(II) in terms of pH value. The flocculation performance was very sensitive to the pH of aqueous solution. ED-ch exhibited a high efficient removal toward Cu(II) with a high flocculation around the isoelectric point. The flocculation would occurred by the electrostatic attraction between NH_3^+ and COO^- . Simple procedure, mixing ED-ch with D.S. 41% and 70%, and subsequent pH adjustment (4.5 and 2.5) and centrifugal separation of the flocs formed, was shown to remove almost complete Cu(II) from aqueous solution, respectively. The residual Cu(II) concentration after these flocculation/separation process with ED-ch reached 0.03 ppm.

3.1 Introduction

Chitosan is a semi-synthetically derived amino-polysaccharides that have unique structures, various biological and physicochemical properties, and highly sophisticated functionality. Using the amino groups as the coordination sites, chitosan has been studied for heavy metal removal. Because chitosan dissolves only under acidic conditions where the coordination ability with metal ions is weakened by the protonation of amino groups, chitosan was mainly used to chelate metal ions in a variety of solid forms, such as beads,¹ membranes² and chitosan immobilized onto glass beads.³ However, use of chitosan as a chelation material involves some problems. Under acidic conditions, the amino groups are protonated to form ammonium ion, which lose the chelation ability. Furthermore, most of heavy metals form, at basic conditions, insoluble hydroxide that can not form chelate complexes with the amino groups.

Chitosan can be functionalized to improve chelation ability with heavy metal ions under even acidic conditions.⁴⁻⁶ By contrast, ethylenediaminetetraacetic acid (EDTA) has been known as strong chelating agent for various metal ions. It is well known that EDTA forms complexes with the most of heavy metal ions such as copper ion in 1:1 molar ratio. The previous studies on solid EDTA-chitosan revealed that its adsorption property was effective for the removal of various heavy metal ions.⁷⁻⁹

As mentioned in Chapter 2, water-soluble material of EDTA linked chitosan (ED-ch) with various degree of substitution has been synthesized and the water solubility was depended on its D.S. and pH conditions. To ascertain whether ED-ch can get rid of metal ion from aqueous solution by the flocculation/precipitation process, the removal of Cu(II) was next examined. Based on the findings that water-soluble EDTA-linked

chitosan is precipitated at narrow pH region, the author investigated flocculation property by using Cu(II) as a model pollutant of heavy metal ion.

It is well known the flocculation property of polyionic macromolecules is generally affected by several parameters such as pH of aqueous solution and dose of flocculant. In this chapter, examination of pH dependent flocculation property of ED-ch is first discussed in the presence of Cu(II) as a model heavy metal ion. Because Cu(II) precipitates by forming its hydroxide under high pH region, the flocculation ability was examined from acidic to neutral conditions.

3.2 Experimental

3.2.1 Materials

All ED-ch with different D.S. was prepared as described in Chapter 2. $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ of analytical grade and other reagents of preparative grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and all chemicals were used without further purification. Water was purified by treatment with ion-exchanger followed by reverse osmosis with a Millipore Millex-3 apparatus.

3.2.2 Apparatus

Concentration of Cu(II) in aqueous solution was measured by A-2000 atomic absorption spectrometer (Hitachi Ltd., Japan) which equipped with a flame atomizer (air/acetylene) and a hollow cathode lamp of Cu (wavelength of 324.7 nm).

3.2.3 Effect of pH on the removal of Cu(II) from aqueous solution

ED-ch **4d** or **4h** (1.00 g) was dissolved in deionized water (50 ml) to prepare 2wt% ED-ch solution. A Cu(II) solution (1000 ppm) prepared from $\text{Cu}(\text{NO}_3)_2$ and 0.1 mol/l HNO_3 was diluted with deionized water to give a Cu(II) stock solution (10.5 mg/l). One ml of the ED-ch solution was added to 19 ml of the Cu (II) stock solution in a 50 ml centrifuge tube, and pH of the mixture was adjusted in the range 1.0 to 6.0 by the addition of small amounts of 3 mol/l HNO_3 or 3 mol/l CH_3COONa . The initial concentration of ED-ch and Cu(II) in each sample was 1 g/l and 10 mg/l, respectively. The mixture was shaken at 120 rpm at room temperature for 24 h. The resulting precipitate was settled by centrifugation at 4,000 rpm for 15 min and the supernatant was collected

immediately. The residual Cu(II) concentration in the supernatant was determined by atomic absorption spectrometer.

The removal of Cu(II) with ED-ch was calculated according to the following equation:

$$\text{Removal (\%)} = \frac{C_0 - C_{sup}}{C_0} \times 100 \quad \text{.....Eq. (1)}$$

where C_0 is the initial Cu(II) concentration and C_{sup} is the residual Cu(II) concentration of the supernatant. The experiments were performed three times.

For comparison, the same experiments were also performed using 2wt% chitosan solution in aqueous acetic acid, which was prepared by dissolving in 1wt% acetic acid aqueous, instead of ED-ch solution.

3.2.4 Effect of contact time on the removal of Cu(II) from aqueous solution

ED-ch **4d** or **4h** (1.00 g) was dissolved in deionized water (50 ml) to prepare 2wt% ED-ch solution. A Cu(II) solution (1000 ppm) prepared from $\text{Cu}(\text{NO}_3)_2$ and 0.1 mol/l HNO_3 was diluted with deionized water to give a Cu(II) stock solution (10.5, 26.3, 52.6 mg/l). One ml of the ED-ch solution was added to 19 ml of the Cu (II) stock solution in a 50 ml centrifuge tube, and pH of the mixture was adjusted 4.5 (**4d**) and 2.5 (**4h**) by the addition of small amounts of 3 mol/l HNO_3 or 3 mol/l CH_3COONa . These pH were the optimum pH for removal of Cu(II) in section 3.3.1. The initial concentration of ED-ch and Cu(II) in each sample was 1 g/l and 10, 25, 50 mg/l, respectively. The mixture was shaken at 120 rpm at room temperature for defined times, the precipitate was separated by centrifugation at 4,000 rpm for 15 min. The residual Cu(II) concentration in the supernatant was determined by atomic absorption spectrometer. The removal of Cu(II) with ED-ch was calculated according to Eq. (1).

3.3 Results and discussion

3.3.1 pH dependent removal of Cu(II) from aqueous solution

Having data of unique behavior of ED-ch, of which solubility is highly depending on pH, the author undertook to perform preliminary removal experiment by use of Cu(II) as a model pollutant of heavy metal ion. Here interest focused on applicability of ED-ch to flocculation/precipitation process.

Since Cu(II) forms hydroxide precipitation in the higher pH region over 6.3,¹⁰ the removal experiments were conducted at pH ranging from 1.0 to 6.0 where effect of pre-precipitation of the hydroxide may be negligible. The experiments were carried out in the initial concentrations of Cu(II) and a polymer ligand (two higher molecular weight ED ch **4d** (D.S. 39%), **4h** (D.S. 73%), and chitosan) 10 ppm and 1 g/l, respectively. The calculated molar ratio of EDTA residue and Cu(II) for ED-ch **4d** with D.S. 39% and **4h** with D.S. 73% were 8.6 and 11.1, respectively.

In a preliminary experiment using ED ch **4d** (D.S. 39%), precipitate formation could be observed visually as shown in Fig. 1. The photographs (a) and (b) show almost no change after addition of **4d** in the Cu(II) solution at pH 2.5. When pH of the solution changed to pH 4.5 (the optimum conditions for precipitation as described in Chapter 2), it was observed visually formation of blue-coloured flocculent precipitates (Fig. 1 (c)). However, the precipitate formation did not observed under other pH conditions than the optimum pH. Furthermore, the blue precipitates could be readily separated by centrifugation at 4000 rpm.

According to the preliminary precipitation experiment, removal ratio of Cu(II) was determined by the experiments shown in the flow chart (Fig. 2). After contacting

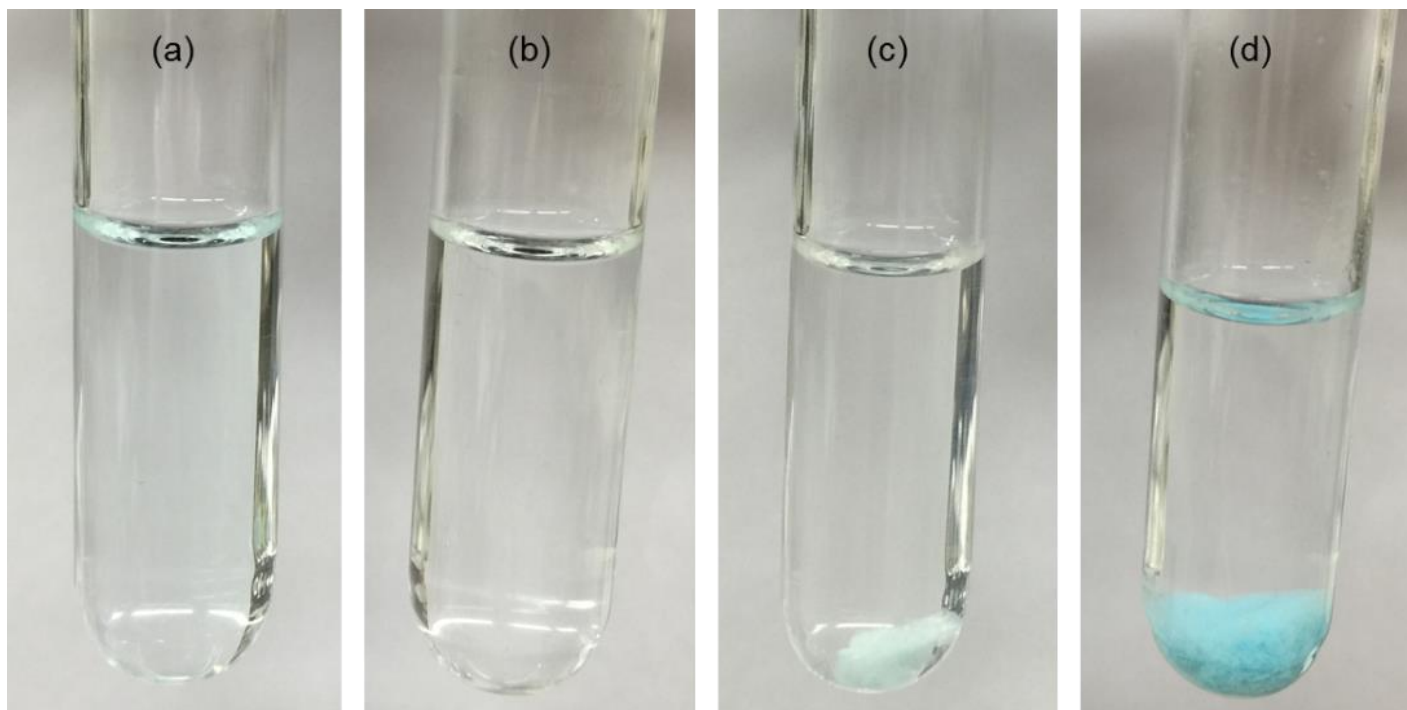


Figure 1 The photograph of Cu(II) solution (a) of 10 mg/l before, (b) after addition of ED-ch **4d** (D.S.39%) at pH 2.5, (c) pH 4.5, and (d) of 100 mg/l after adding ED-ch **4d** at pH 4.5.

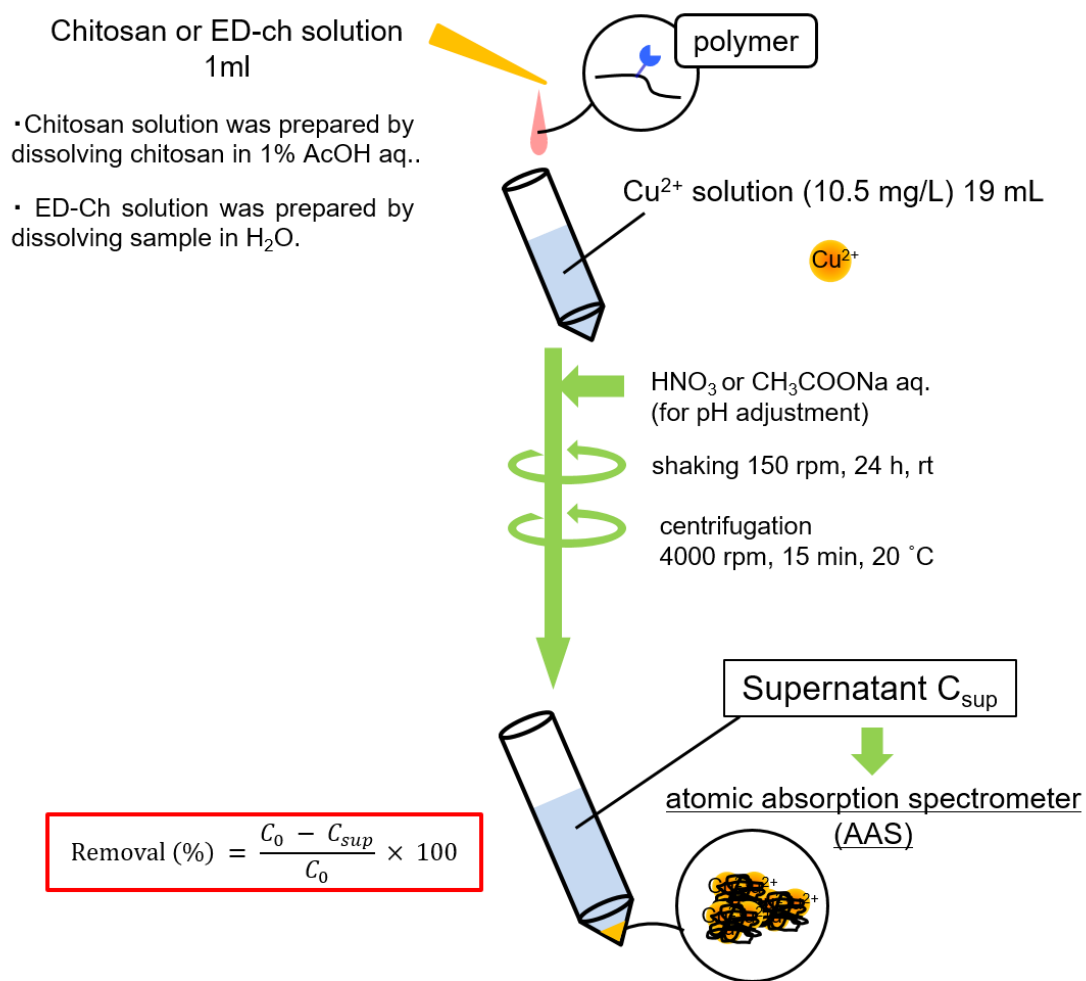


Figure 2 Flow chart of removal experiment.

Table 1 The effect of pH on the residual concentration of Cu(II) after flocculation/precipitation process.^a

pH	initial Cu(II) concentration (mg/l) ^a	residual Cu(II) concentration of supernatant ^b (mg/l) ^a		
		4d	4h	chitosan
1.0	10.38	8.49	8.77	8.46
1.5	10.58	8.92	8.50	8.15
2.0	10.41	8.50	0.49	8.31
2.5	10.14	8.50	0.03	7.71
3.0	10.63	3.86	7.72	7.38
3.5	10.21	1.44	7.54	7.28
4.0	10.40	1.08	8.98	8.16
4.5	10.38	0.03	9.04	7.88
5.0	10.34	2.17	9.18	7.97
5.5	10.20	4.38	9.19	7.85
6.0	10.31	5.75	9.07	4.88

^a concentration of initial and residual Cu(II) were measured with atomic adsorption spectrometry in ppm.

^b the resulting flocs was settled by centrifugation and the supernatant was collected immediately.

Table 2 The effect of pH on the removal of Cu(II) by flocculation/precipitation process.^a

pH	Cu(II) removal (%)		
	4d	4h	chitosan
1.0	14.4 ± 2.0	15.5 ± 2.8	18.5 ± 1.9
1.5	15.7 ± 1.5	19.7 ± 1.1	25.6 ± 2.1
2.0	18.4 ± 1.9	95.3 ± 2.0	21.5 ± 1.6
2.5	16.1 ± 1.8	100 ± 0.7	24.0 ± 2.0
3.0	63.7 ± 1.9	27.3 ± 2.7	32.3 ± 2.3
3.5	83.4 ± 1.6	26.2 ± 2.2	25.0 ± 1.7
4.0	89.6 ± 1.9	13.6 ± 1.4	21.5 ± 1.9
4.5	100 ± 0.6	13.0 ± 1.4	24.1 ± 1.9
5.0	73.6 ± 1.7	11.2 ± 2.3	22.9 ± 1.6
5.5	54.2 ± 2.4	9.9 ± 2.4	23.1 ± 1.9
6.0	44.2 ± 1.7	12.0 ± 1.5	52.6 ± 2.1

^a flocs formed by contacting ED-ch **4d** (D.S. 39%), **4h** (D.S. 73%), or chitosan were removed by centrifuge.

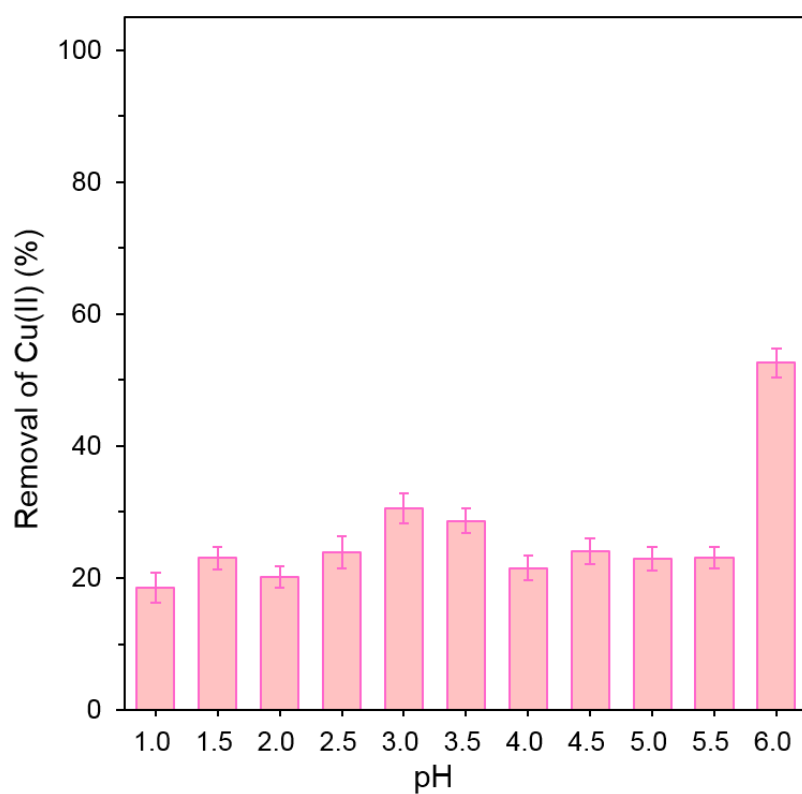


Figure 3 The effect of pH on the removal of Cu(II) contacting with chitosan by flocculation/precipitation. Initial concentration of Cu(II) 10 mg/l, dose of polymer ligand 1 g/l, contact time 24 h, shaking rate 120 rpm, rt.

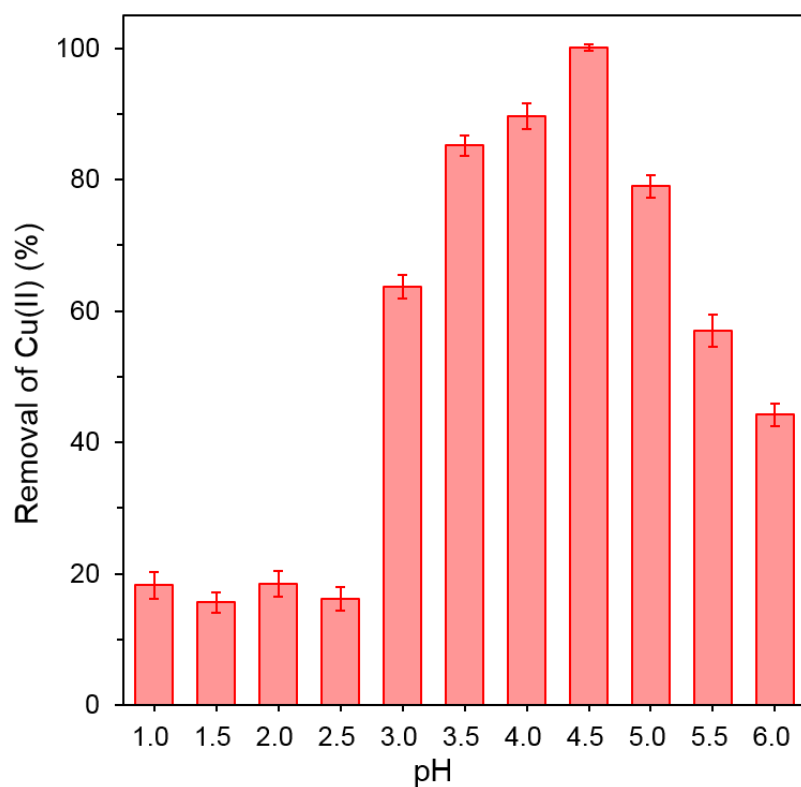


Figure 4 The effect of pH on the removal of Cu(II) contacting with ED-ch **4d** (D.S. 39%) by flocculation/precipitation. Initial concentration of Cu(II) 10 mg/l, dose of polymer ligand 1 g/l, contact time 24 h, shaking rate 120 rpm, rt.

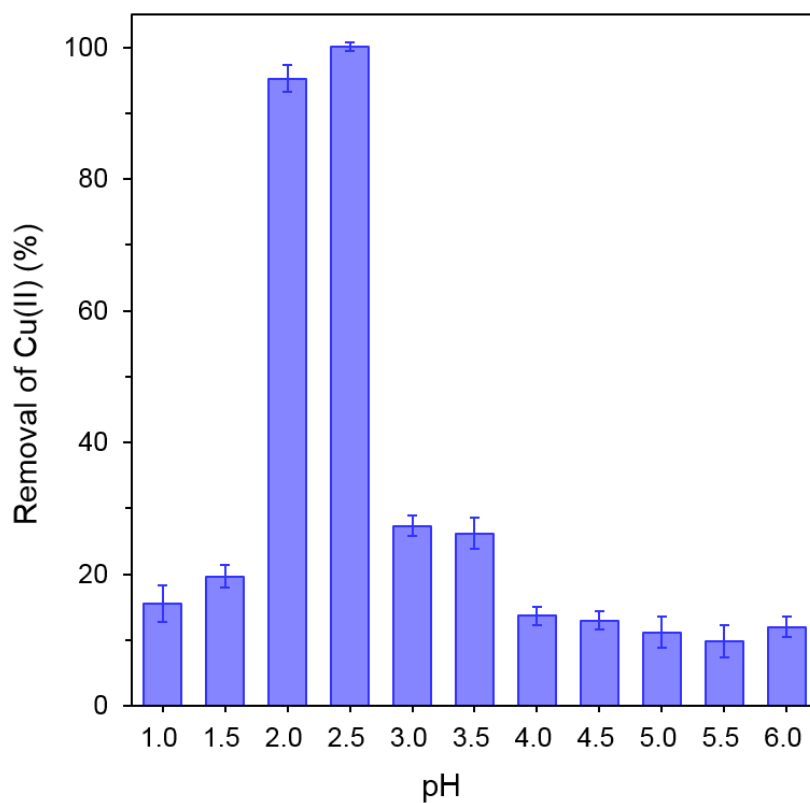


Figure 5 The effect of pH on the removal of Cu(II) contacting with ED-ch **4h** (D.S. 73%) by flocculation/precipitation. Initial concentration of Cu(II) 10 mg/l, dose of polymer ligand 1 g/l, contact time 24 h, shaking rate 120 rpm, rt.

Cu(II) and ED-ch **4d** (D.S. 39%) and **4h** (D.S. 73%) for 24 h, the experiment was performed by separation of the mixture by centrifugation at 4000 rpm for 15 min. Centrifugal separation of the mixture removed precipitate to give supernatant. Subsequent measurement of the Cu(II) concentration by atomic absorption spectroscopy of the liquid fractions gave data for the calculation of removal Cu(II) from the solution. In order to evaluate the removal efficiency of ED-ch, original chitosan was used for the control.

The results of Cu(II) removal are summarized in Table 1 and 2. Table 1 shows the residual Cu(II) concentration of the supernatant obtained by centrifugal separation of the samples of different pH. The Cu(II) removal efficiency was calculated from the concentration and are shown in Table 2. In addition, the effect of pH on the removal of Cu(II) were graphically illustrated using these results as shown in Fig. 3-5.

Chitosan showed the low Cu(II) removal of around 25% below pH 5.5 (Fig. 3). Most of the amino groups of chitosan may be protonated in these lower pH regions, and resulted in poor coordination ability with Cu(II).¹¹ Increment of pH exhibited an increase in removal efficiency of chitosan and reached the maximum that of 56.8% at pH 6.0. In contrast, the removal efficiency of Cu(II) by ED-ch **4d** with D.S. 39% (Fig. 4) and **4h** with D.S. 73% (Fig. 5) were remarkably different from that of chitosan. As shown in Table 1, the residual Cu(II) concentration treated with ED-ch **4d** (D.S. 39%) reached the lowest (0.03 mg/l) at pH 4.5, which means the dissolving Cu(II) was almost completely removed (Table 2). Fig. 4 showed that the removal Cu(II) with **4d** increased with increasing pH up to 4.5 from 3.0 and the maximum removal of 100% was obtained at pH 4.5, which was higher than that of chitosan at pH 6.0. Subsequently the removal gradually decreased with increasing pH. Furthermore, **4h** with D.S. 73% also showed

that excellent ability of removal (95.3 and 100%) at pH 2.0 and 2.5, respectively (Fig. 5). Comparing with chitosan (Fig. 4), these results show excellent flocculation ability of ED-ch toward Cu(II). These optimal pHs of ED-ch flocculation suggest that the electrostatic attraction between NH_3^+ and COO^- in ED-ch is reduced the repulsion of the polymer at around isoelectric point, which would cause a high efficient flocculation ability.

The World Health Organization (WHO) recommended that heavy metal ions in drinking water must be reduced to several mg/l (e.g., copper: less than 2 mg/l).¹² The highest removal efficiency of Cu(II) was attained at pH region where ED-ch was insoluble, and the residual Cu(II) concentration of supernatant was 0.03 mg/l (Table 1). ED-ch can removed Cu(II) to much less than the permissible level from aqueous solution at optimal pH.

3.3.2 Effect of contact time on removal of Cu(II)

The previous investigation described in section 3.3.1 revealed that ED-ch **4d** and **4h** almost completely remove Cu(II) at pH 4.5 and 2.5, respectively. In this experiment, ED-ch was shaken in a Cu(II) solution for 24 h at room temperature. The contact time was thought to be too long for adsorption or precipitation equilibrium. In order to shorten the required treatment time for the removal of heavy metal ions, the author's interest next turned to the effect of contact time.

By changing the contact time with ED-ch, removal ratio of Cu(II) was examined in Cu(II) solutions of initial concentration in range of 10-50 mg/l under these optimum pH. The molar ratio of EDTA/Cu(II) for ED-ch **4d** with D.S. 39% was 8.6 (10 mg/l),

3.4 (25 mg/l), and 1.72 (50 mg/l), respectively. That of EDTA/Cu(II) for ED-ch **4h** with D.S. 73% was 11.1 (10 mg/l), 4.4 (25 mg/l), and 2.2 (50 mg/l), respectively.

The experimental results were shown in Fig. 6. Surprisingly, the removal of Cu(II) with ED-ch was extremely quick and highly efficient, and the removal ratio reached approximately 100%. Among 6 series of experiment shown in Fig. 6, 5 reached plateau in 1 min. Although one exception was that of ED-ch **4d** in Cu(II) solution of 50 mg/l, it took only 15 min to be equilibrium.

The solid EDTA-chitosan needed 4 h to attain equilibrium conditions using Co(II) and Ni(II) aqueous solution (100 mg/l).⁸ The extremely short contact time necessary for the water-soluble ED-ch is explainable by consideration of homogeneity of the reaction. When both solutions of ED-ch and Cu(II) are mixed, chelation of Cu(II) with EDTA and charge-neutralization occur quickly in the homogeneous conditions. As a result, complex of Cu(II) and ED-ch is precipitated as the flocs.

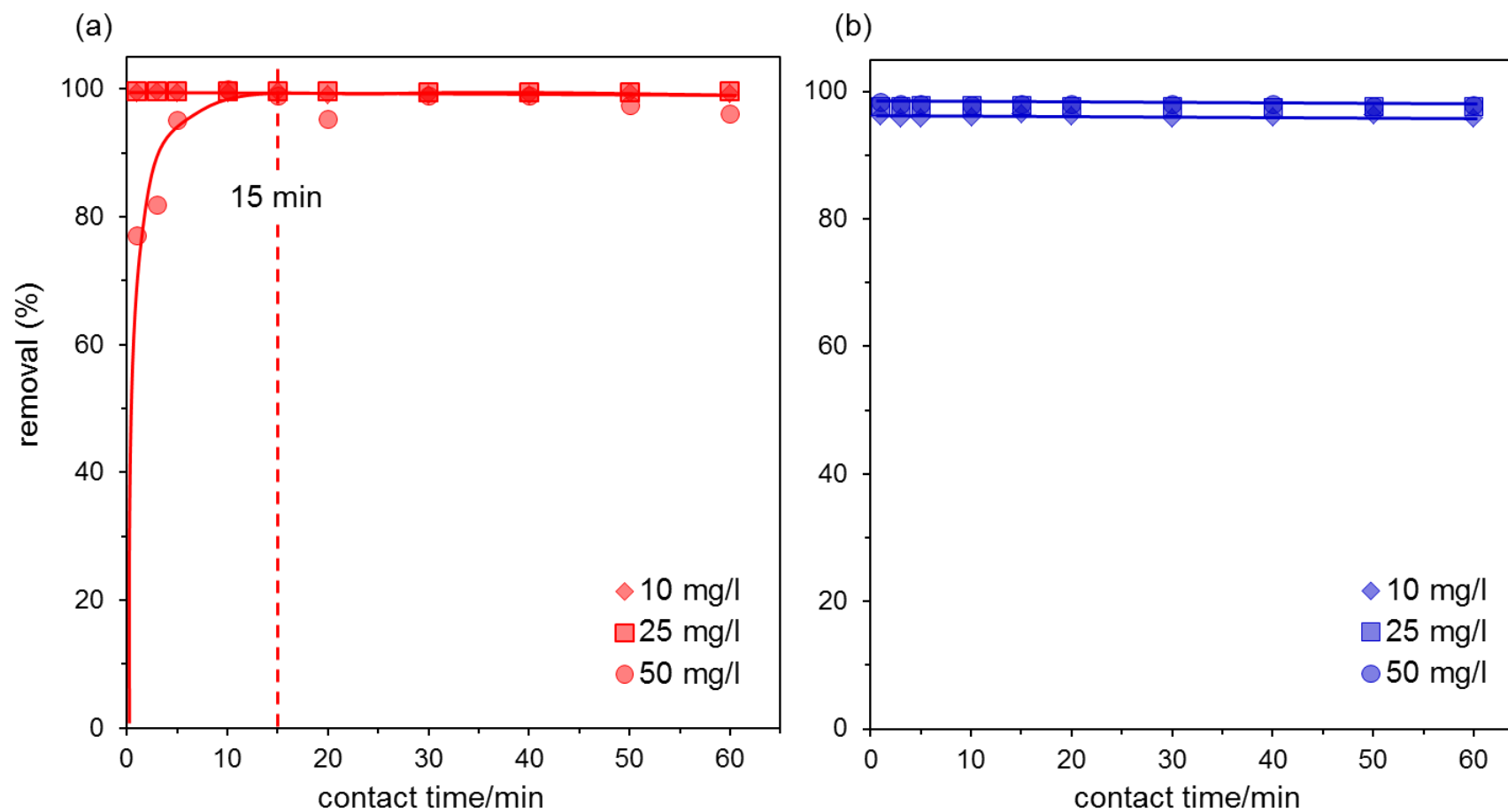


Figure 6 The effect of contact time on removal of Cu(II) by ED-ch (a) **4d** (D.S. 39%) at pH 4.5 and (b) **4h** (D.S. 73%) at pH 2.5. Initial concentration of Cu(II) 10-50 mg/l, dose of polymer ligand 1 g/l, shaking rate 120 rpm, rt.

3.4 Conclusion

ED-ch was characterized for the flocculation performance against Cu(II) in terms of pH value. The flocculation performance was very sensitive to the pH of aqueous solution. ED-ch exhibited a high efficient removal toward Cu(II) with a high flocculation within the narrow pH range. The removal of heavy metal ions by flocculation was proven using 10 ppm Cu(II) solution, i.e., centrifugal separation of the flocs formed either with ED-ch **4d** (D.S. 39%) at pH 4.5 or with ED-ch **4h** (D.S. 73%) at pH 2.5 was shown to remove almost complete Cu(II) from aqueous solution. The residual Cu(II) concentration after these flocculation/separation process with ED-ch reached 0.03 ppm. The results demonstrated that ED-ch was superior to original chitosan for application as flocculant for removal of heavy metal ion.

In order to shorten the required treatment time for the removal of heavy metal ions, the removal efficiency was examined by changing the contact time with ED-ch. It was found that the removal of Cu(II) with ED-ch was extremely quick and highly efficient, and the removal ratio reached approximately 100% in less than 15 min. The extremely short contact time necessary is an advantage of the water-soluble ED-ch.

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Chapter 4

Mechanistic investigation of flocculation removal of Cu(II) with EDTA-linked chitosan

Abstract

The flocculation mechanism of ED-ch for the removal of Cu(II) was investigated to clarify the influence of the chelation of EDTA residues. In the presence of ED-ch, distribution of Cu(II) was examined at various pH. As a result, most of Cu(II) was found to be chelated with the EDTA residue of dissolving or precipitated ED-ch at pH region of 1.0-6.0. The complexation stoichiometry of Cu(II) and EDTA residue of ED-ch was determined by UV-vis titration, and was 1:1 or 1:1.2. In order to perform flocculation removal at wide region of pH, effects of molar ratio of EDTA/Cu(II) and pH were carefully examined. ED-ch was characterized for the flocculation performance against Cu(II) at various dose and various pH conditions. At lower pH than isoelectric point (IEP) of ED-ch, ED-ch has no flocculation ability, whereas, the flocculation of ED-ch was found to occur at higher pH than IEP. The optimum condition for high removal efficiency was depended on both molar ratio of EDTA/Cu(II) and pH. Higher pH of the solution required higher concentration of Cu(II) to attain efficient removal. Zeta potential measurement was also supported the flocculation occurred by charge neutralization.

4.1 Introduction

Flocculation is widely used as one of method for treatment of life sewage, industrial effluents and mineral water. Flocculant is divided into two major types, inorganic additives such as polyaluminum chloride (PAC), polyferric sulfate (PFS) and organic macromolecules like polyacrylamide.¹ The above three flocculants are commonly used, and cause aggregation of dispersed particles and hydrophobic colloids into larger flocs so as to can be easily settled and separated.^{2,3} They are used mainly for removal of insoluble organic and inorganic compounds from contaminated water. It is well known the flocculation property is affected by several parameters, pH of aqueous solution and dose of flocculant which are the two most notable ones.⁴⁻⁶ In general, it is known that flocculants have flocculation ability through adsorption bridging, charge neutralization and sweep floc mechanism.⁷

In this chapter, four experiments are carried out to clarify flocculation mechanism of ED-ch for removal of Cu(II). (i) The distribution of Cu(II) is first examined at various pH after contacting with chitosan material and Cu(II). Cu(II) in the mixture is considered to be chelated with the polymer ligand, which is presented either in precipitate or in solution. The separation of the precipitate form mixture is performed by 2 different ways, the distribution of Cu(II) is estimated. (ii) The complexation stoichiometry of Cu(II) and EDTA residue of ED-ch is determined by UV-vis titration. (iii) The effects of molar ratio of EDTA/Cu(II) on removal of Cu(II) and pH are carefully examined. (iv) The zeta potential was measured at varying molar ratio of ED-ch to Cu(II).

4.2 Experimental

4.2.1 Materials

$\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ of analytical grade and other reagents of preparative grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and all chemicals were used without further purification. Water was purified by treatment with ion-exchanger followed by reverse osmosis with a Millipore Millex-3 apparatus.

4.2.2 Apparatus

Concentration of Cu(II) in aqueous solution was measured by A-2000 atomic absorption spectrometer (Hitachi Ltd., Japan) which equipped with a flame atomizer (air/acetylene) and a hollow cathode lamp of Cu (wavelength of 324.7 nm). UV spectra were measured with a V-560 UV-visible spectrometer (Jasco Co., Japan), using a quartz cell with an optical path length of 1 cm. Zeta potential was conducted on a Delsa Nano HC particle analyzer (Beckman Coulter Inc., USA) with a laser light with wave-length of 658 nm at 25 °C, using the same Beckman apparatus with the standard flow cell. The zeta-potential value was calculated using Smoluchowski equation.

4.2.3 Effect of pH on the distribution of Cu(II)

ED-ch **4d** or **4h** (1.00 g) was dissolved in deionized water (50 ml) to prepare 2wt% ED-ch solution. A Cu(II) solution (1000 ppm) prepared from $\text{Cu}(\text{NO}_3)_2$ and 0.1 mol/l HNO_3 was diluted with deionized water to give a Cu(II) stock solution (10.5 mg/l). One ml of the ED-ch solution was added to 19 ml of the Cu (II) stock solution in a 50 ml centrifuge tube, and pH of the mixture was adjusted in the range 1.0 to 6.0 by the addition of small amounts of 3 mol/l HNO_3 or 3 mol/l CH_3COONa . The initial concen-

tration of ED-ch and Cu(II) in each sample was 1 g/l and 10 mg/l, respectively. The mixture was shaken at 120 rpm at room temperature for 24 h. The resulting precipitate was settled by centrifugation at 4,000 rpm for 15 min and the supernatant was collected immediately. Part of the supernatant was filtrated by ultrafiltration tube (vivaspin 6; Sartorius Stedim Biotech GmbH, Germany) with molecular weight cutoff 10,000 to rid ED-ch, and then the filtrate was taken. The residual Cu(II) concentration in the supernatant and the filtrate was determined by atomic absorption spectrometer.

The removal of Cu(II) with ED-ch was calculated according to the following equation:

$$\text{Removal (\%)} = \frac{C_0 - C_{sup}}{C_0} \times 100 \quad \text{.....Eq. (1)}$$

where C_0 is the initial Cu(II) concentration and C_{sup} is the residual Cu(II) concentration of the supernatant. The experiments were performed three times. For comparison, the same experiments were also performed using 2wt% chitosan solution in aqueous acetic acid, which was prepared by dissolving in 1wt% acetic acid aqueous, instead of ED-ch solution.

4.2.4 UV-vis spectroscopic titration of ED-ch with Cu(II)

Complexation stoichiometry was determined by UV-vis titration. Three different pH (1.5, 4.5, and 5.5) of ED-ch stock solutions (3.49×10^{-3} mol/l) and Cu(II) solutions (2.36×10^{-3} mol/l) were prepared in a 50 ml measuring flask as follows. ED-ch **4i** with D.S. 79% (75 mg) was dissolved in appropriate quantities of deionized water, pH of the solution was adjusted to 1.5, 4.5, or 5.5 by the addition of small amounts of 3 mol/l HNO_3 or 3 mol/l CH_3COONa , and the solutions was diluted in to 50 ml with deionized water. $\text{Cu}(\text{NO}_3)_2$ (58 mg) was dissolved in appropriate quantities of deionized

water, pH of the solution was adjusted to 1.5, 4.5, or 5.5, and then the solution of Cu(II) was diluted to 50 ml. Analytical sample was prepared by mixing the Cu(II) stock solution (1 ml) and appropriately diluted ED-ch stock solution (2 ml). All analytical samples had the same Cu(II) concentration, and the molar ratio of EDTA residue and Cu(II) was varied from 0 to 2.0. After each freshly prepared analytical sample was stirred for 30 min at room temperature, UV-vis spectrum was measured at wavelength of 200-900 nm.

For comparison, the same titration experiments were also performed using EDTA instead of ED-ch.

4.2.5 Effect of complexation stoichiometry on the removal of Cu(II) from aqueous solution

ED-ch **4e** with D.S. 45% (1.00 g) was dissolved in deionized water (50 ml) to prepare 20 g/l of ED-ch stock solution. The ED-ch stock solution was diluted with deionized water to provide various concentrations of ED-ch solution. A Cu(II) solution (1,000 ppm), which was prepared from Cu(NO₃)₂ and 0.1 mol/l HNO₃, was diluted with deionized water to give a Cu(II) stock solution (10.5 mg/l). One ml of the ED-ch solution was added to 19 ml of the Cu (II) stock solution in a 50 ml centrifuge tube, and pH of the mixture was adjusted in the range 1.0 to 6.0 by the addition of small amounts of 3 mol/l HNO₃ or 3 mol/l CH₃COONa. The initial concentration of Cu(II) in each sample was 10 mg/l. The molar ratio of EDTA residue and Cu(II) for ED-ch was 0.09-2.7. The mixture was shaken at 120 rpm at room temperature for 15 min. The resulting precipitates were settled by centrifugation at 4,000 rpm for 15 min and the supernatant was collected immediately. The residual Cu(II) concentration in the supernatant was deter-

mined by atomic absorption spectrometer. The removal of Cu(II) with ED-ch was calculated using the equation (1).

4.2.6 Measurement of zeta potential

In a 50 ml of measuring flask, ED-ch **4e** with D.S. 45% (0.25 g) was dissolved in appropriate quantities of deionized water, pH of the solution was adjusted to 2.0, 5.5, or 6.0 by the addition of small amounts of 3 mol/l HNO₃ or 3 mol/l CH₃COONa, and then the solution was diluted to 50 ml with deionized water, giving three different pH of ED-ch stock solutions (5.0 g/l). Three different pH of Cu(II) stock solutions (175 mg/l) were prepared by dissolving 67 mg of Cu(NO₃)₂ in appropriate quantities of deionized water, pH of the solution was adjusted to 2.0, 5.5, or 6.0, and then the solution of Cu(II) was diluted in 50 ml of measuring flask to 50 ml with deionized water. Analytical samples, which contain in varying molar ratio of EDTA residue and Cu(II) from 0 to 3.0, was prepared by mixing of the ED-ch stock solution (0.5 ml) and appropriately diluted Cu(II) stock solution (6.5 ml). All analytical samples had the same ED-ch concentration. After freshly prepared analytical solution was stirred at room temperature for 30 min, zeta potential value of each sample was measured.

4.2.7 Effect of Cu(II) initial concentration and D.S. on the removal of Cu(II) from aqueous solution

ED-ch **4e-i** (1.00 g) were dissolved in deionized water (50 ml) to prepare 20 g/l of ED-ch stock solutions. ED-ch stock solution was diluted with deionized water to provide various concentrations of ED-ch solution. A Cu(II) solution (1000 ppm) prepared from Cu(NO₃)₂ and 0.1 mol/l HNO₃ was diluted with deionized water to give a

Cu(II) stock solution with varying concentration. Five ml of the ED-ch solution was added to 15 ml of the Cu (II) stock solution in a 50 ml centrifuge tube, and pH of the mixture was adjusted in the range 5.5 by the addition of small amounts of 3 mol/l HNO_3 or 3 mol/l CH_3COONa . The initial concentration of Cu(II) in each sample was 10, 25, 50, 100 mg/l, respectively. The mixture was shaken at 120 rpm at room temperature for 15 min. The resulting precipitate was settled by centrifugation at 4,000 rpm for 15 min and the supernatant was collected immediately. The residual Cu(II) concentration in the supernatant was determined by atomic absorption spectrometer. The removal of Cu(II) with ED-ch was calculated according to the equation (1).

4.3 Results and discussion

4.3.1 The effect of pH on the distribution of Cu(II)

As described in Section 3.3.1, ED-ch exhibited a high removal efficiency of Cu(II) by flocculation within the narrow pH range, where the solubility of ED-ch was extremely low. The pH range is close to the isoelectric point of ED-ch. It is well known that EDTA form chelate complexes with Cu(II) in 1:1 molar ratio at various pH region. If the EDTA residue linked with ED-ch chelate with Cu(II) in 1:1 molar ratio under other pH conditions, ED-ch was consider to have a possibility to remove Cu(II) at different pH. Therefore, the distribution of Cu(II) was first examined because there should be at least three kind of Cu(II) presented in the removal experiment, i.e., free Cu(II) in solution, captured Cu(II) with the flocs, and chelated Cu(II) with dissolving ED-ch.

In addition to the centrifugal separation, the author attempted ultrafiltration as another separation procedure of ED-ch bounded Cu(II), as shown in Fig. 1. Centrifugal separation of the mixture removed the precipitate ED-ch containing Cu(II) to give supernatant, which was then subjected to ultrafiltration to remove dissolving ED-ch with Cu(II). Subsequently, measurement of the Cu(II) concentration of the liquid fractions was carried out by atomic absorption spectroscopy. From these data, amount of three kinds of Cu(II), Cu(II) in ED-ch flocs, Cu(II) bound dissolving ED-ch, and non-chelated Cu(II), were calculated, and the distribution of Cu(II) was estimated. Fig. 2-4 show the distribution of Cu(II) after contacting with the three polymer ligands in various pH.

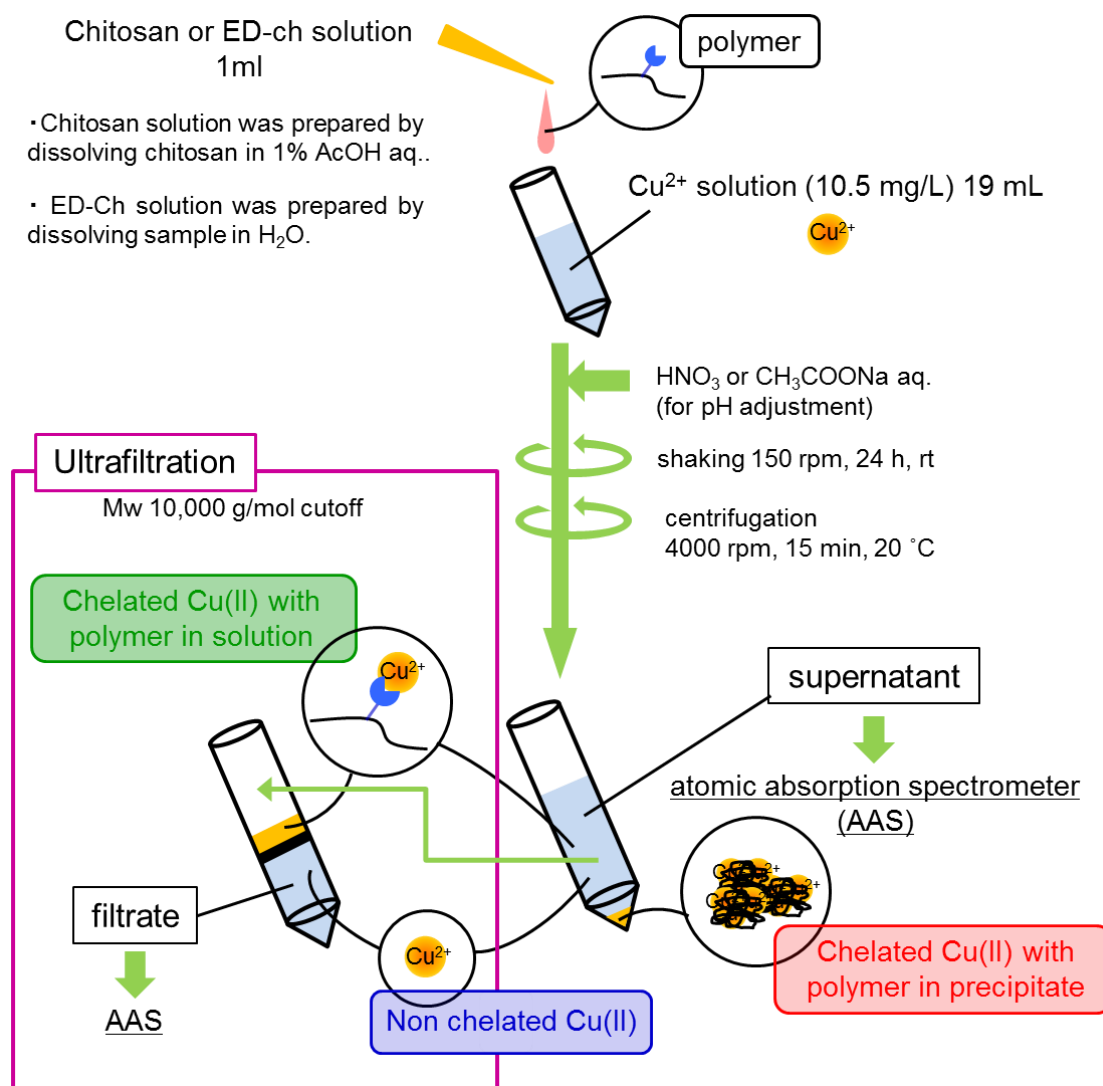


Figure 1 Flow chart of removal experiment.

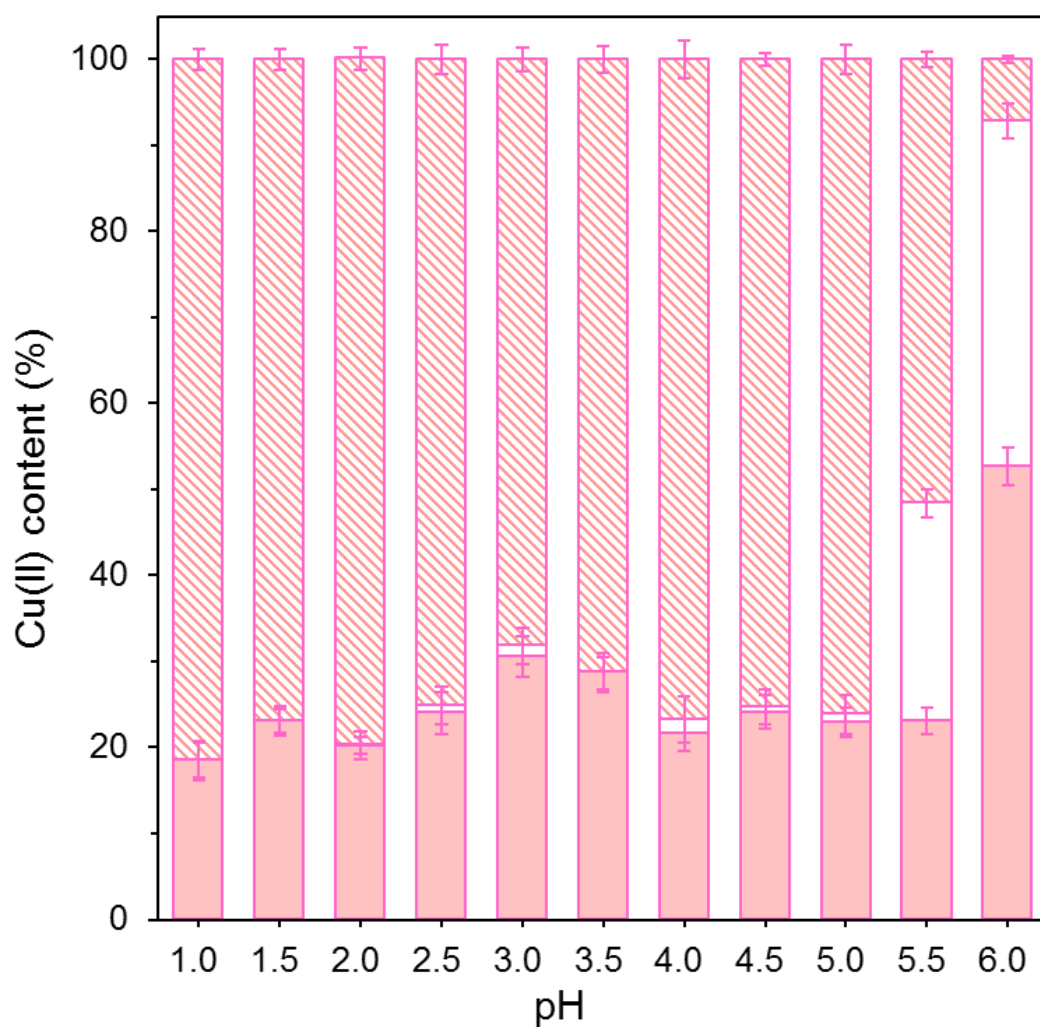


Figure 2 The effect of pH on the distribution of Cu(II) contacting with chitosan. Chelated Cu(II) with polymer ligand in precipitate (closed column), in solution (open column), and non-chelated Cu(II) (hatched column). Initial concentration of Cu(II) 10 mg/l, dose of polymer ligand 1 g/l, contact time 24 h, shaking rate 120 rpm, rt.

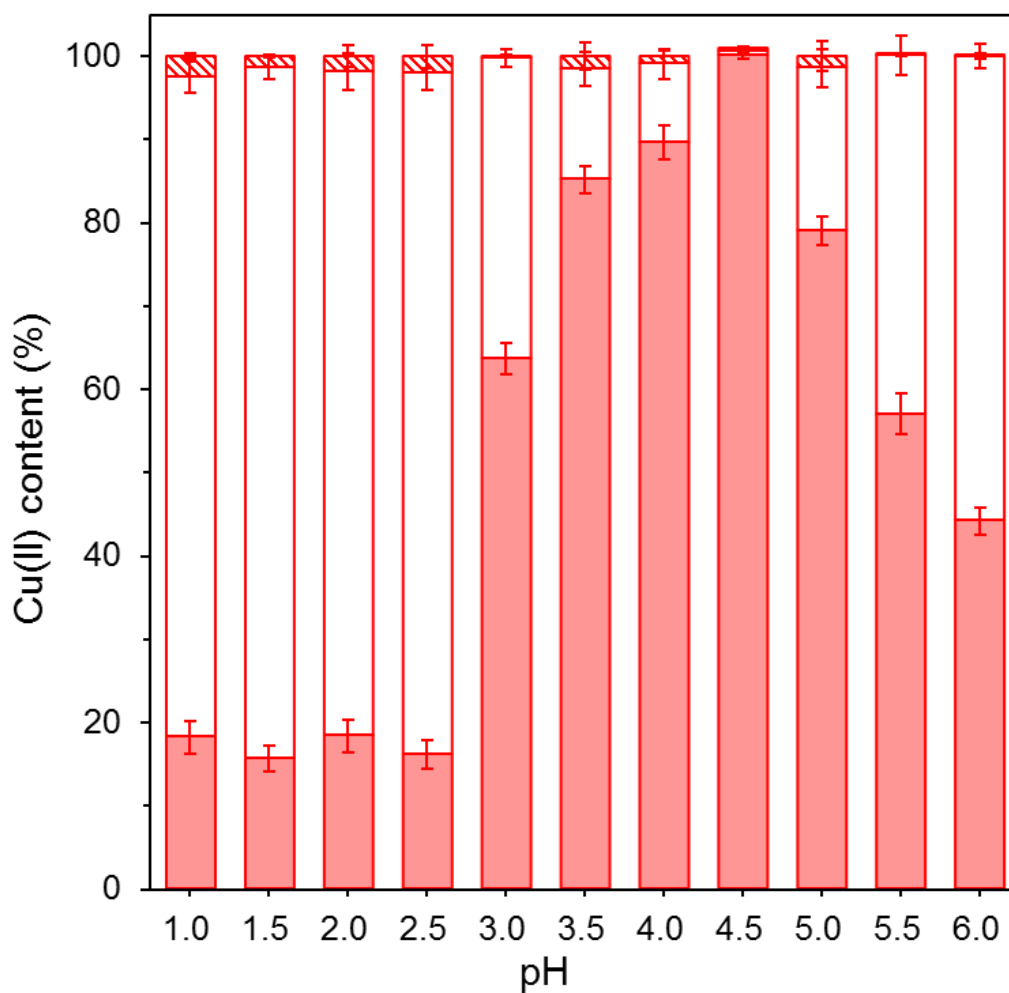


Figure 3 The effect of pH on the distribution of Cu(II) contacting with ED-ch **4d** (D.S. 39%). Chelated Cu(II) with polymer ligand in precipitate (closed column), in solution (open column), and non-chelated Cu(II) (hatched column). Initial concentration of Cu(II) 10 mg/l, dose of polymer ligand 1 g/l, contact time 24 h, shaking rate 120 rpm, rt.

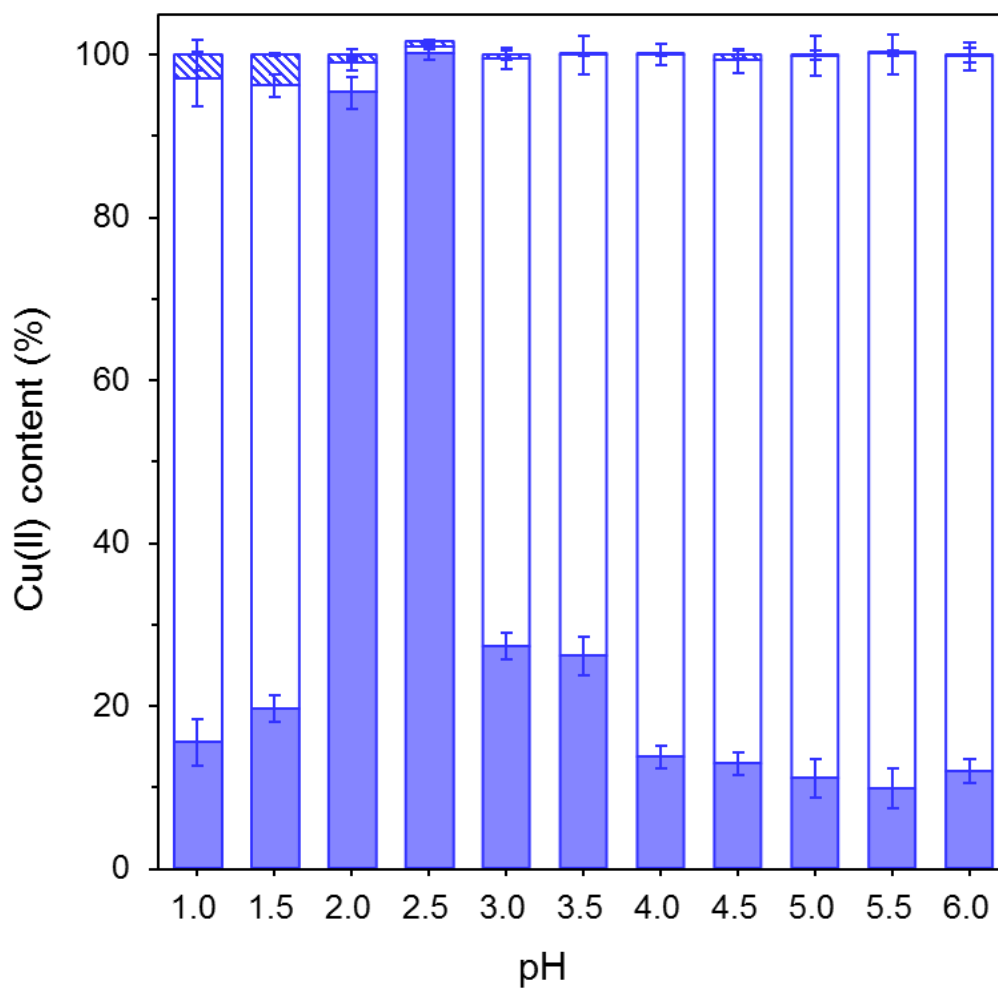


Figure 4 The effect of pH on the distribution of Cu(II) contacting with ED-ch **4h** (D.S. 73%). Chelated Cu(II) with polymer ligand in precipitate (closed column), in solution (open column), and non-chelated Cu(II) (hatched column). Initial concentration of Cu(II) 10 mg/l, dose of polymer ligand 1 g/l, contact time 24 h, shaking rate 120 rpm, rt.

Experiment with chitosan (Fig. 2) showed that, most of Cu(II) was non-chelated with chitosan and presented in solution in acidic solution of pH below 5.0, and very small amount of Cu(II) was bound with chitosan. Most of the amino groups of chitosan should be protonated in these pH regions, and resulted in poor coordination ability with Cu(II).⁸ While chitosan exhibited an increased coordination ability toward Cu(II) and decreased solubility in upper pH region (pH 5.0-6.0), and Cu(II) contents in both soluble chitosan and precipitated chitosan were increased.

By contrast, the distribution profiles of ED-ch **4d** with D.S. 39% (Fig. 3) and **4h** with D.S. 73% (Fig. 4) were remarkably different. Amount of non-chelated Cu(II) in solution was very small (less than 5%) in the all pH region examined (pH 1.0-6.0). These results indicate that ED-ch has a chelation ability with Cu(II) whether flocculation is occurred or not, and ED-ch chelating Cu(II) is still remained in solution after separation of the flocs. The author expected that this dissolving complex of polymer ligand and Cu(II) can be removed by novel flocculation conditions other than pH control.

4.3.2 Determination of complexation stoichiometry of ED-ch and Cu(II)

The author had another question about stoichiometry of the complex of ED-ch and Cu(II). As described in Section 3.3.1, ED-ch exhibited a high removal efficiency of Cu(II) by flocculation within the narrow pH range, where the solubility of ED-ch was extremely low. The pH range is close to the isoelectric point of ED-ch. However, the molar ratio of EDTA residue and Cu(II) for ED-ch **4b** with D.S. 41% and **4c** with D.S. 70% were 8.6 and 11.1, respectively. In the research described in the previous section,

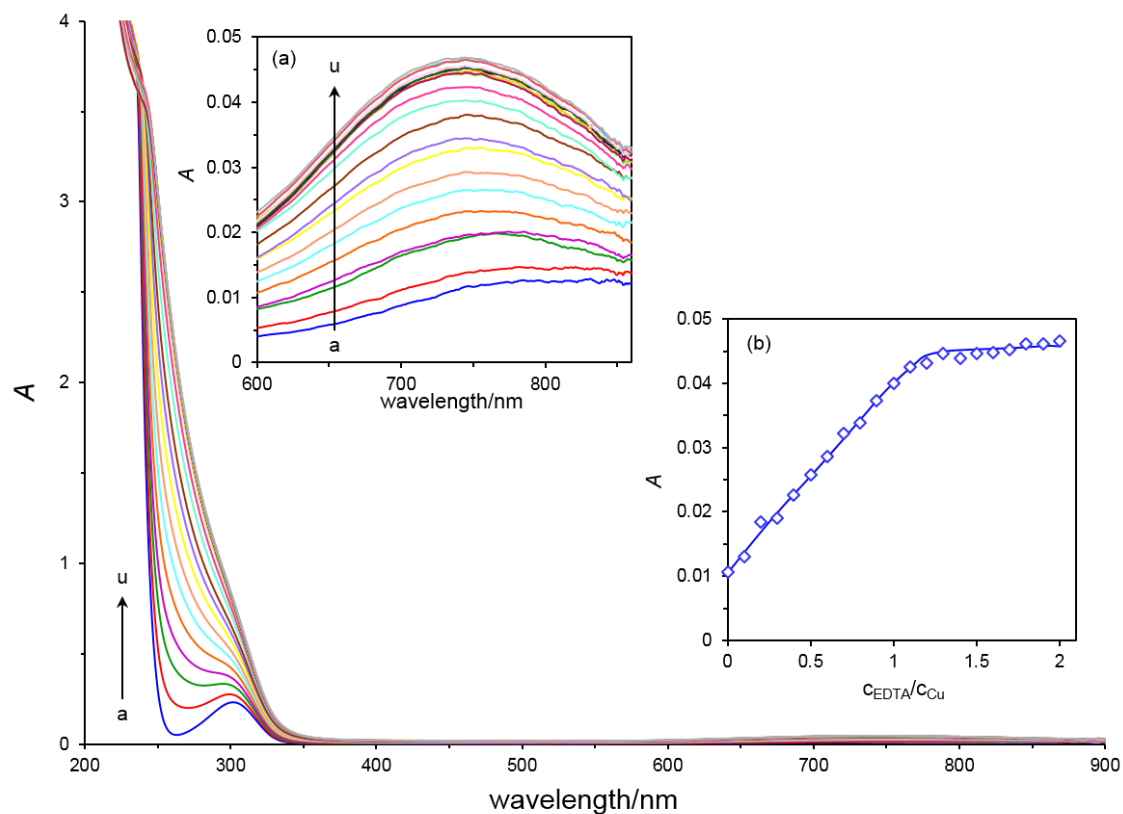


Figure 5 UV titration of Cu(II) (7.87×10^{-4} mol/l) at pH 1.5 on addition of ED-ch **4i** with D.S. 79% (from a to u= 0, 9.96×10^{-5} , 1.99×10^{-4} , 2.99×10^{-4} , 4.00×10^{-3} , 4.98×10^{-4} , 5.97×10^{-4} , 6.97×10^{-4} , 7.97×10^{-4} , 8.96×10^{-4} , 9.96×10^{-4} , 1.10×10^{-3} , 1.19×10^{-3} , 1.29×10^{-3} , 1.39×10^{-3} , 1.49×10^{-3} , 1.59×10^{-3} , 2.69×10^{-3} , 1.79×10^{-3} , 1.89×10^{-3} , 1.99×10^{-3} mol/l mol/l). Inset: (a) the magnified figure at range of wavelength 600-860 nm, (b) A vs $C_{\text{EDTA}}/C_{\text{Cu}}$ at wavelength of 730 nm.

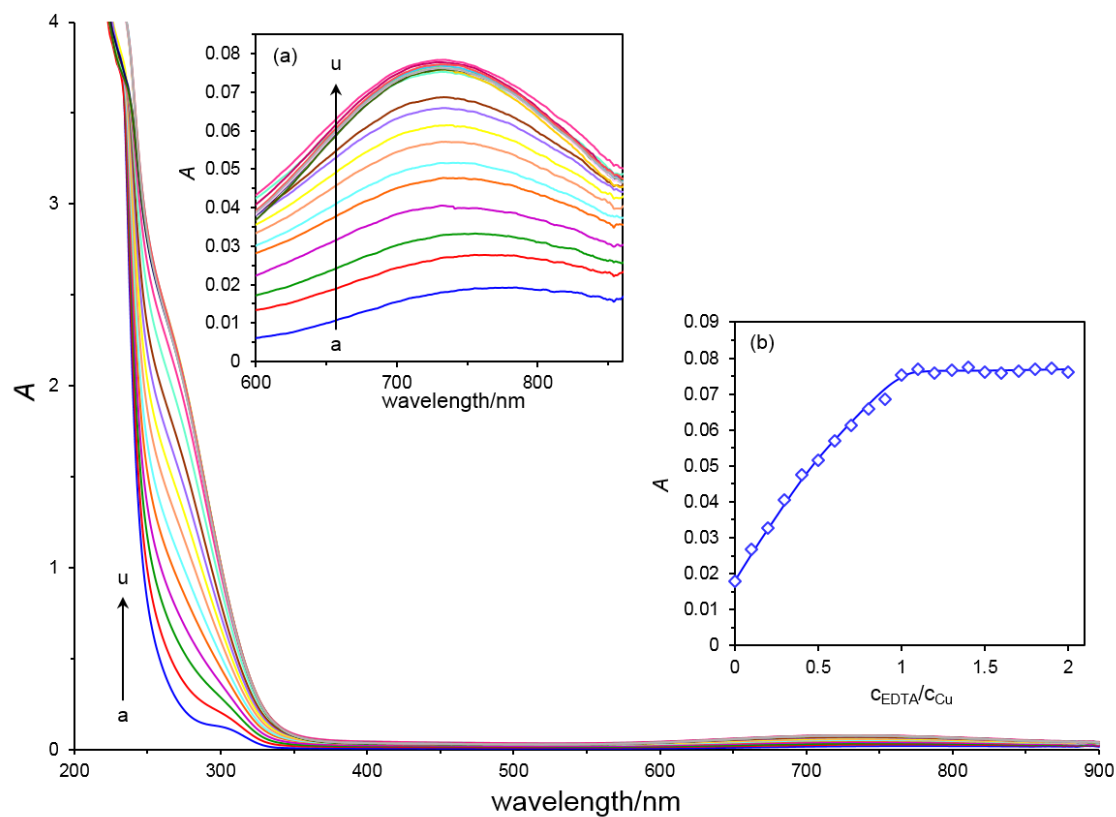


Figure 6 UV titration of Cu(II) (7.87×10^{-4} mol/l) at pH 5.5 on addition of ED-ch **4i** with D.S. 79% (from a to u= 0, 9.96×10^{-5} , 1.99×10^{-4} , 2.99×10^{-4} , 4.00×10^{-3} , 4.98×10^{-4} , 5.97×10^{-4} , 6.97×10^{-4} , 7.97×10^{-4} , 8.96×10^{-4} , 9.96×10^{-4} , 1.10×10^{-3} , 1.19×10^{-3} , 1.29×10^{-3} , 1.39×10^{-3} , 1.49×10^{-3} , 1.59×10^{-3} , 2.69×10^{-3} , 1.79×10^{-3} , 1.89×10^{-3} , 1.99×10^{-3} mol/l mol/l). Inset: (a) the magnified figure at range of wavelength 600-860 nm, (b) A vs $C_{\text{EDTA}}/C_{\text{Cu}}$ at wavelength of 730 nm.

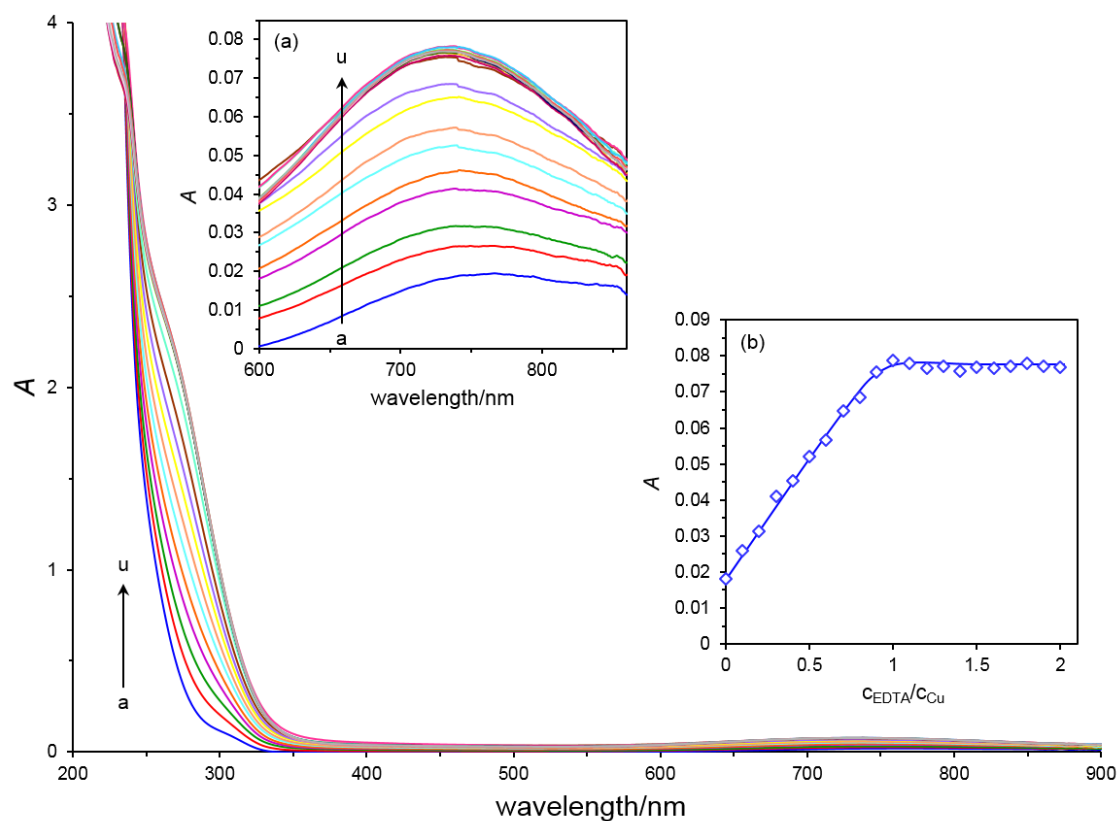


Figure 7 UV titration of Cu(II) (7.87×10^{-4} mol/l) at pH 5.5 on addition of ED-ch **4i** with D.S. 79% (from a to u= 0, 9.96×10^{-5} , 1.99×10^{-4} , 2.99×10^{-4} , 4.00×10^{-3} , 4.98×10^{-4} , 5.97×10^{-4} , 6.97×10^{-4} , 7.97×10^{-4} , 8.96×10^{-4} , 9.96×10^{-4} , 1.10×10^{-3} , 1.19×10^{-3} , 1.29×10^{-3} , 1.39×10^{-3} , 1.49×10^{-3} , 1.59×10^{-3} , 2.69×10^{-3} , 1.79×10^{-3} , 1.89×10^{-3} , 1.99×10^{-3} mol/l). Inset: (a) the magnified figure at range of wavelength 600-860 nm, (b) A vs $C_{\text{EDTA}}/C_{\text{Cu}}$ at wavelength of 730 nm.

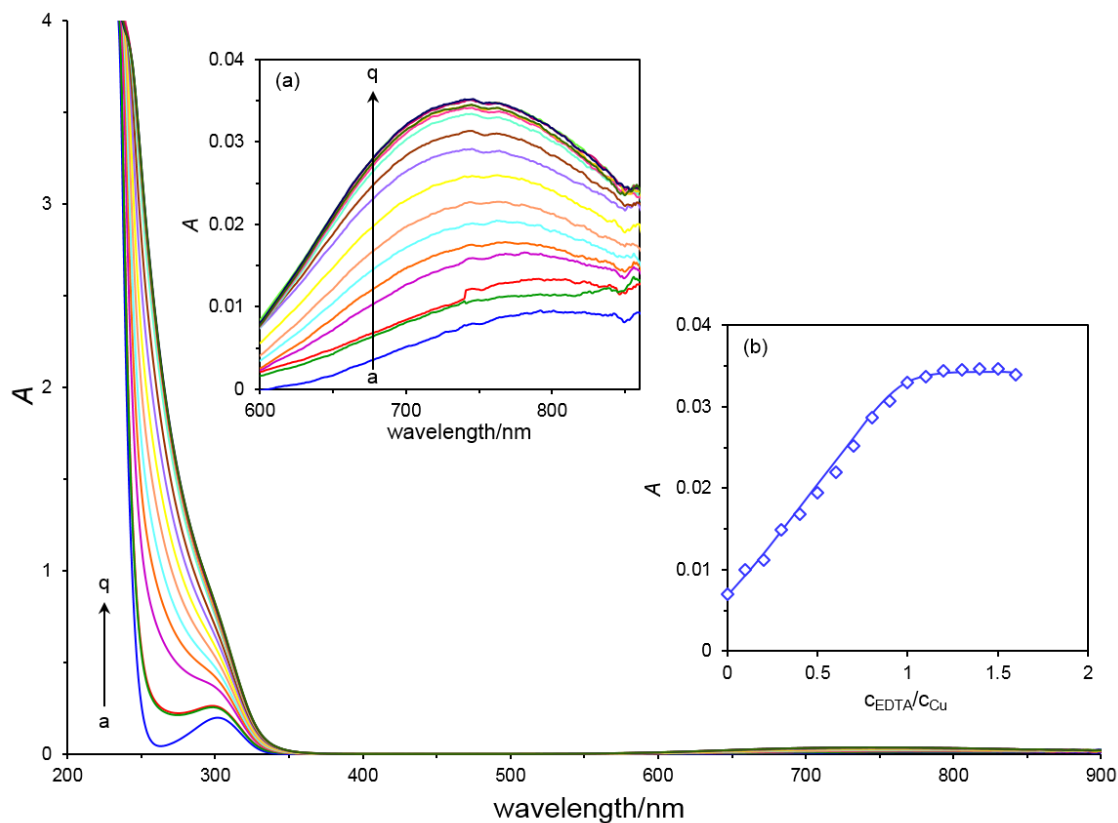


Figure 8 UV titration of Cu(II) (7.87×10^{-4} mol/l) at pH 1.5 on addition of EDTA (from a to q= 0, 7.87×10^{-5} , 1.57×10^{-4} , 2.36×10^{-4} , 3.15×10^{-4} , 3.93×10^{-4} , 4.72×10^{-4} , 5.51×10^{-4} , 6.29×10^{-4} , 7.08×10^{-4} , 7.87×10^{-4} , 8.65×10^{-4} , 9.44×10^{-4} , 1.02×10^{-3} , 1.10×10^{-3} , 1.18×10^{-3} , 1.26×10^{-3} mol/l). Inset: (a) the magnified figure at range of wavelength 600-860 nm, (b) A vs $C_{\text{EDTA}}/C_{\text{Cu}}$ at wavelength of 730 nm.

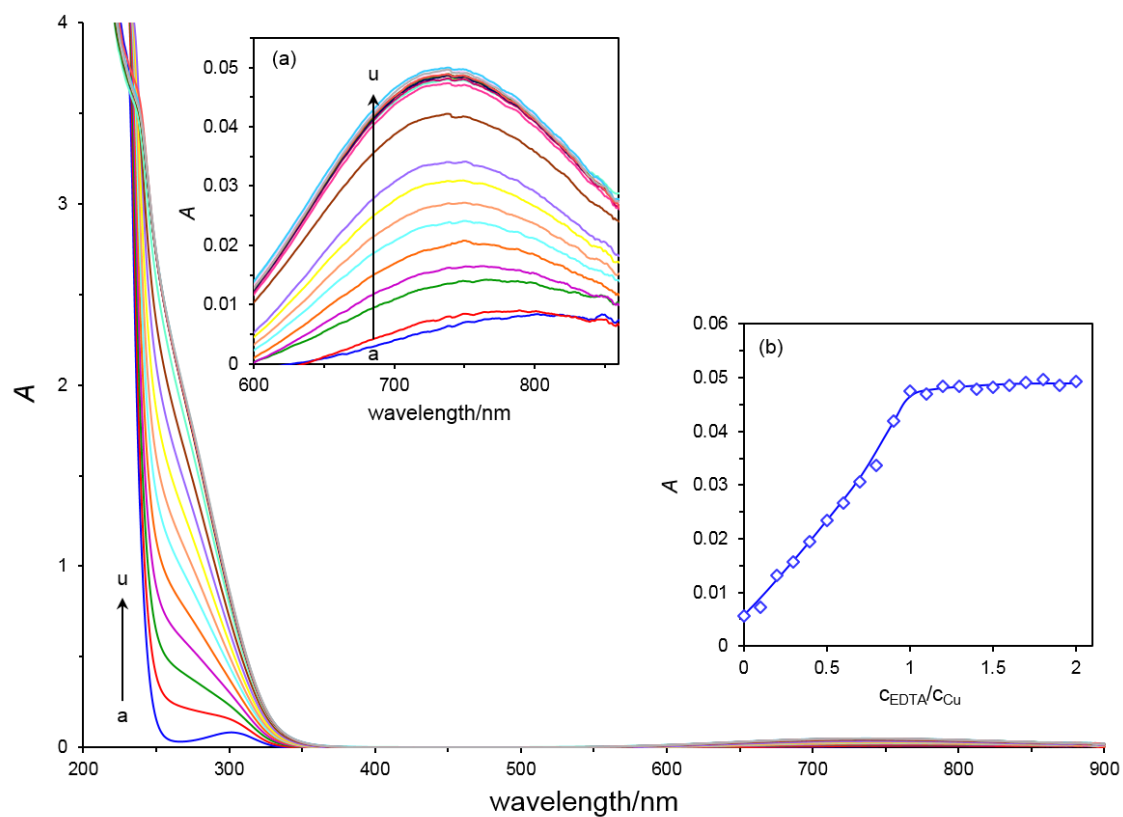


Figure 9 UV titration of Cu(II) (7.87×10^{-4} mol/l) at pH 4.5 on addition of EDTA (from a to u= 0, 7.87×10^{-5} , 1.57×10^{-4} , 2.36×10^{-4} , 3.15×10^{-4} , 3.93×10^{-4} , 4.72×10^{-4} , 5.51×10^{-4} , 6.29×10^{-4} , 7.08×10^{-4} , 7.87×10^{-4} , 8.65×10^{-4} , 9.44×10^{-4} , 1.02×10^{-3} , 1.10×10^{-3} , 1.18×10^{-3} , 1.26×10^{-3} , 1.34×10^{-3} , 1.42×10^{-3} , 1.49×10^{-3} , 1.57×10^{-3} mol/l). Inset: (a) the magnified figure at range of wavelength 600-860 nm, (b) A vs $C_{\text{EDTA}}/C_{\text{Cu}}$ at wavelength of 730 nm.

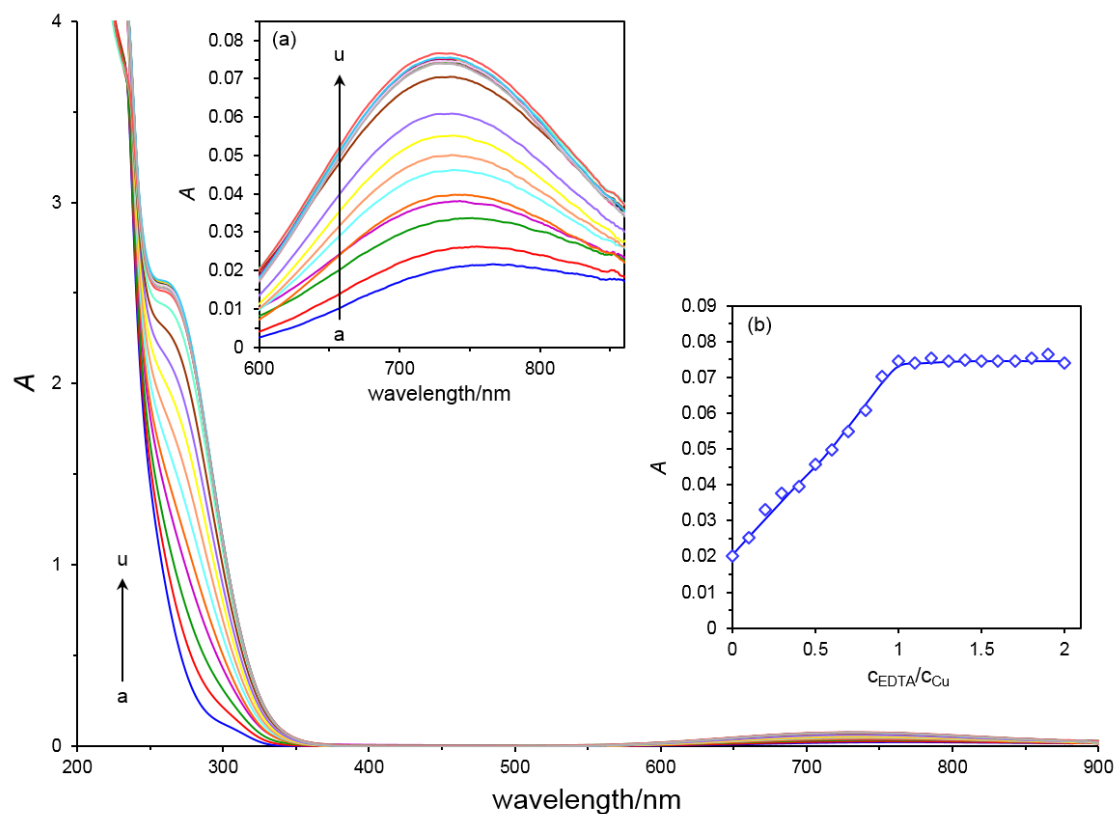


Figure 10 UV titration of Cu(II) (7.87×10^{-4} mol/l) at pH 5.5 on addition of EDTA (from a to u= 0, 7.87×10^{-5} , 1.57×10^{-4} , 2.36×10^{-4} , 3.15×10^{-4} , 3.93×10^{-4} , 4.72×10^{-4} , 5.51×10^{-4} , 6.29×10^{-4} , 7.08×10^{-4} , 7.87×10^{-4} , 8.65×10^{-4} , 9.44×10^{-4} , 1.02×10^{-3} , 1.10×10^{-3} , 1.18×10^{-3} , 1.26×10^{-3} , 1.34×10^{-3} , 1.42×10^{-3} , 1.49×10^{-3} , 1.57×10^{-3} mol/l). Inset: (a) the magnified figure at range of wavelength 600-860 nm, (b) A vs $C_{\text{EDTA}}/C_{\text{Cu}}$ at wavelength of 730 nm.

it was confirmed that most of Cu(II) was formed chelation with ED-ch, when such excess amount of EDTA residue against Cu(II) was added. Since EDTA forms 1:1 chelation complex with Cu(II) and its stability constant is extremely high, the question was whether such excess ED-ch is necessary for flocculation.

To determine complexation stoichiometry, UV-vis spectrophotometric titration^{9,10} of Cu(II) with ED-ch was carried out. Since precipitate formation influence on the absorbance of sample, the experiment was carried out using ED-ch **4i** (D.S. 79%), which is soluble in the wide range of pH region. The UV-vis spectral titration of Cu(II) with ED-ch was performed under three different pH conditions of 1.5, 4.5, and 5.5. The concentration of Cu(II) in all of sample was fixed 7.87×10^{-4} mol/l. In addition, the complex stoichiometry was also investigated using original EDTA for comparison.

Fig. 5-10 show the UV spectra of ED-ch-Cu(II) and EDTA-Cu(II) chelates at different pH. The spectra show maximum adsorption peaks at 730 nm after forming complex of Cu(II) with different concentration of ED-ch. Absorbance was increased with increasing concentration of ED-ch or EDTA. To determine the complex stoichiometry, the $C_{\text{EDTA}}/C_{\text{Cu}}$ was plotted against absorbance of complex at 730 nm (Fig. 5-10(b)). Where C_{EDTA} is concentration of EDTA residue of ED-ch in analytical sample, C_{Cu} is that of Cu(II). Absorbance of complex with ED-ch and Cu(II) increased in a nearly linear manner with increasing $C_{\text{EDTA}}/C_{\text{Cu}}$, when $C_{\text{EDTA}}/C_{\text{Cu}}$ was less than 1.0 at pH 4.5 and 5.5, respectively. Absorbance was reached plateau when $C_{\text{EDTA}}/C_{\text{Cu}}$ was more than 1.0 at pH 4.5 and 5.5. The plateau of the plot, (that is, absorbance was not changed with increasing $C_{\text{EDTA}}/C_{\text{Cu}}$) indicated that the chelation reaction had reached saturation. The intersection at the turning point in the plot occurred at $C_{\text{EDTA}}/C_{\text{Cu}} = 1.0$,

suggesting that EDTA residue of ED-ch form chelation complex with Cu(II) in 1 : 1 molar ratio at pH 4.5 and 5.5. The results of UV-vis titration with original EDTA showed similar stoichiometry (Fig 9 and 10).

By contrast, the intersection at the turning point at pH 1.5 was $C_{\text{EDTA}}/C_{\text{Cu}}=1.2$, which indicating that the complexation stoichiometry between Cu(II) and EDTA residue of ED-ch is 1:1.2. These results are explainable by consideration of protonation of EDTA residue. EDTA is known to have four dissociation constants, and exist as the structure as shown in Fig. 11, at pH lower than 2.0,¹¹ where EDTA forms chelate complex with Cu(II) (coordination number is 4) as shown in fig. 11(a), two of the carboxylate ion are form coordination with Cu(II). Considering this model, chelate complex of ED-ch with Cu(II) can be expected the structure as shown in Fig. 11(b) or coordination with carboxylate ion of another EDTA residue (Fig. 11 (c)). These EDTA complexes in strongly acidic solution thought to have lower stability constant than normal

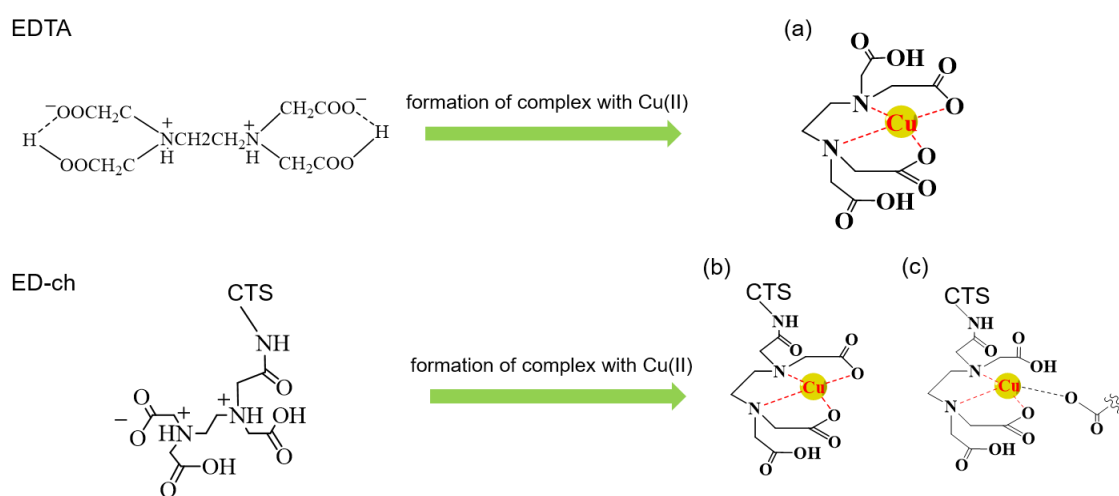


Figure 11 The structure of complex with Cu(II) at lower pH.

hexagonal EDTA complex, and consequently, complexation stoichiometry becomes 1:1.2 at pH 1.5.

4.3.3 Effect of complexation stoichiometry on the removal of Cu(II) from aqueous solution

As described in Section 3.3.1, ED-ch exhibited a high removal efficiency of Cu(II) by flocculation within the narrow pH range, where the solubility of ED-ch was extremely low. The pH range is close to the isoelectric point of ED-ch. However, the molar ratio of EDTA residue and Cu(II) for ED-ch **4b** with D.S. 41% and **4c** with D.S. 70% were only 8.6 and 11.1, respectively. In contrast to this, the experiments described in the previous section showed that the EDTA residue of ED-ch forms chelation complex with Cu(II) in 1:1 molar ratio at neutral to weakly acidic solution. Therefore, the author expected that discovery of new flocculation method other than pH control would increase remarkably the removal efficiency of ED-ch. Here, removal ability under higher Cu(II) concentration was investigated to clarify the effect of complexation stoichiometry on the flocculation.

Similar removal experiment of Cu(II) with ED-ch **4e** (D.S. 45%) by flocculation was carried out by changing molar ratio EDTA/Cu(II) and the results are shown in Fig. 12-14. Experiments were performed The theoretical value, shown by dashed line in the graphs, was calculated on the assumption that EDTA residue of ED-ch forms chelation with Cu(II) in molar ratio 1:1 and ED-ch chelated with Cu(II) is completely separated from aqueous solution.

Removal profile at lower pH region than the isoelectric point of ED-ch **4e** of 3.0 was shown in Fig. 12. ED-ch exhibited the low Cu(II) removal of around 15%. After contacting for 15 min, the precipitate of ED-ch was not observed visually at these pH. Unexpectedly, removal efficiency at pH 2.5 (close to the isoelectric point) was less than 40% under higher Cu(II) as shown Fig. 13. Surprisingly, at pH 4.0-6.0 (Fig 13 and 14), the removal increased with increasing molar ratio of EDTA/Cu and the maximum removal was almost 100%. These results showed that flocculation of ED-ch **4e** occurred much higher pH region than its isoelectric point. Subsequently the removal efficiency gradually decreased with increasing the molar ratio. The highest removal efficiency was depended on both molar ratio of EDTA/Cu(II) and pH, *i.e.*, the molar ratio is 1.44-1.89, 1.35-1.44, 1.35-1.44, 1.26-1.35, and 0.99-1.35 at pH 4.0, 4.5, 5.0, 5.5 and 6.0, respectively. The optimum molar ratio at the each of pH are summarized in Table 1, suggesting that increasing the Cu(II) concentration result in increasing optimum pH for the flocculation of ED-ch..

Moreover, the zeta potential of the mixture solution of ED-ch **4e** and Cu(II) was measured at pH 2.0, 5.5, and 6.0 (Fig. 15). At lower pH of 2.0 (Fig. 15 (a)) where almost no flocs were observed, the zeta potential value did not exhibit significant change with increasing the molar ratio of EDTA/Cu, and was around +20 mV. At higher pH of 5.5 and 6.0 (Fig. 15 (b) and (c)), the zeta potential increased with increasing the molar ratio of EDTA/Cu(II), and reached approximately zero when the molar ratio was 1.2 at pH 5.5 and 1.35 at pH 6.0, respectively. These conditions were almost same as the optimum condition of high removal efficiency. These results indicated that the flocculation process is controlled by the charge neutralization mechanism at higher pH than

IEP.^{12,13} Subsequently, when the removal decreased with increasing molar ratio (above the optimum molar ratio), the zeta potential also decreased to negative value, preventing from flocculating.¹³⁻¹⁶ These charges should increase repulsion among particles, thereby hindering flocs are growing into larger ones.

Next, the author thought about the possible factor of the charge neutralization effective for the flocculation of ED-ch. EDTA is known to have four dissociation constants, and the structures at various pH are shown in Fig. 16. The EDTA residue of ED-ch is assumed to have similar five structures as shown in Fig. 17, in aqueous solution at various pH. The chelation complex with Cu(II) is expected to have as two structures (protonated form and carboxylate form) as illustrated. Therefore, coordination with Cu(II) should result in decreasing the negative charges of the EDTA residue in ED-ch.

The phenomenon shown in Table 1 is explainable by this hypothesis. Since the number of protonated amino group in ED-ch increase with decreasing pH, it require more carboxylate ion is required for charge neutralization, as shown in Fig. 18. Finally, the charge neutralization is assumed to cause by balance of the following 2 factors; (i) chelation between Cu(II) and EDTA residue that decreases negative charge of ED-ch, and (ii) protonation that increases positive charge (NH_3^+) and decreases negative charge (COO^-).¹⁴ The charge neutralization reduces the electrostatic repulsion of the polymer chains. From the results above, the mechanism of flocculation for Cu(II) removal can be elucidated as illustrated in Fig. 19.

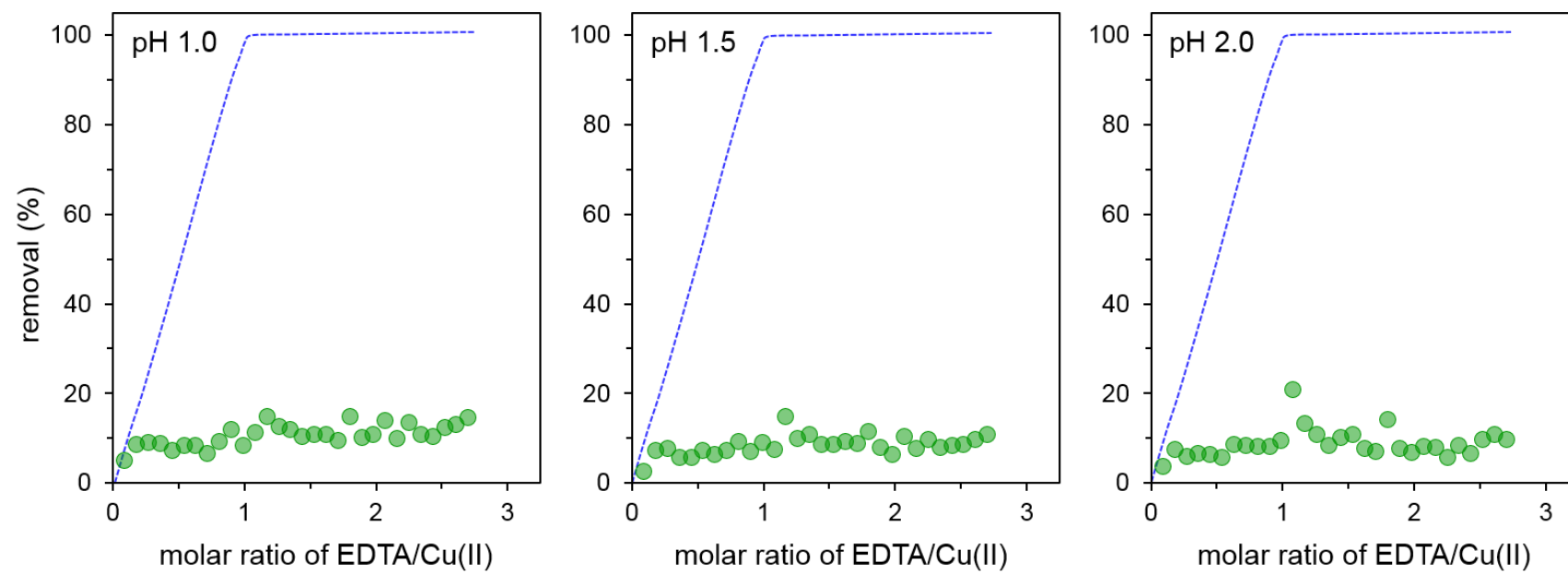


Figure 12 The effect of molar ratio EDTA/Cu(II) on removal of Cu(II) by ED-ch **4e** (D.S. 45%; ●) and the theoretical value (dashed line) at pH 1.0-2.0. Initial concentration of Cu(II) 10 mg/l, shaking rate 120 rpm, rt.

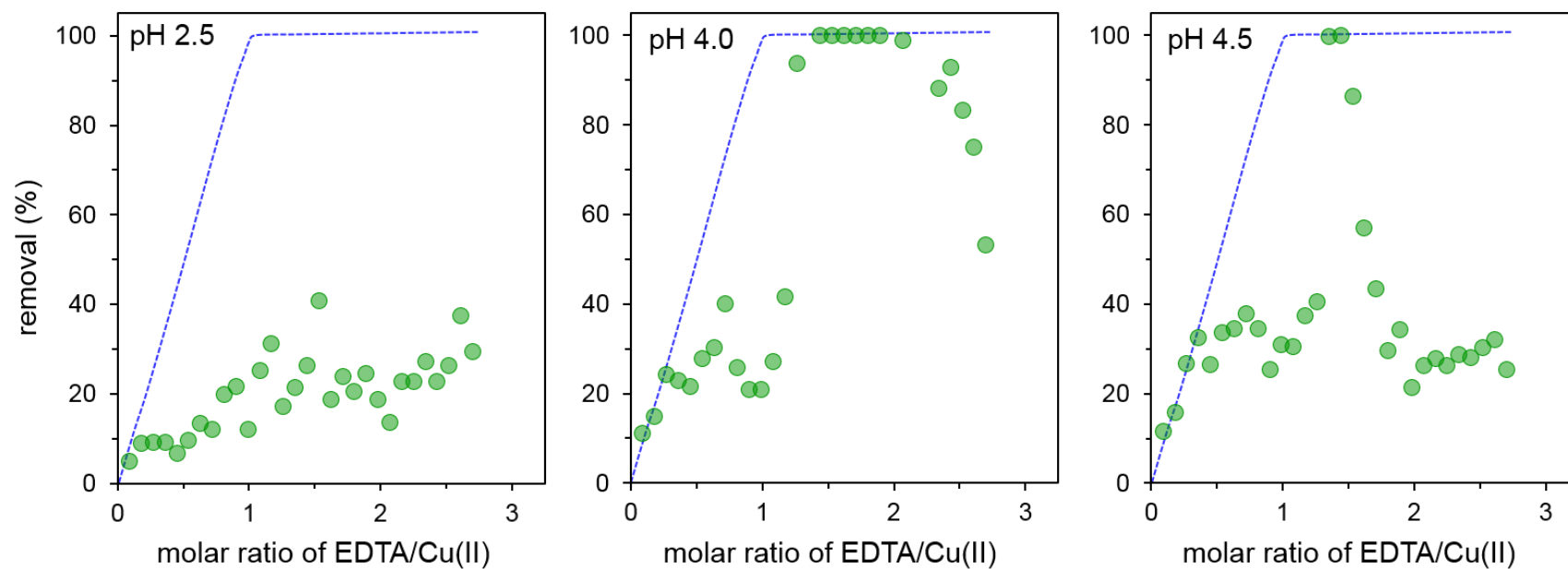


Figure 13 The effect of molar ratio EDTA/Cu(II) on removal of Cu(II) by ED-ch **4e** (D.S. 45%; ●) and the theoretical value (dashed line) at pH 2.5, 4.0 and 4.5. Initial concentration of Cu(II) 10 mg/l, shaking rate 120 rpm, rt.

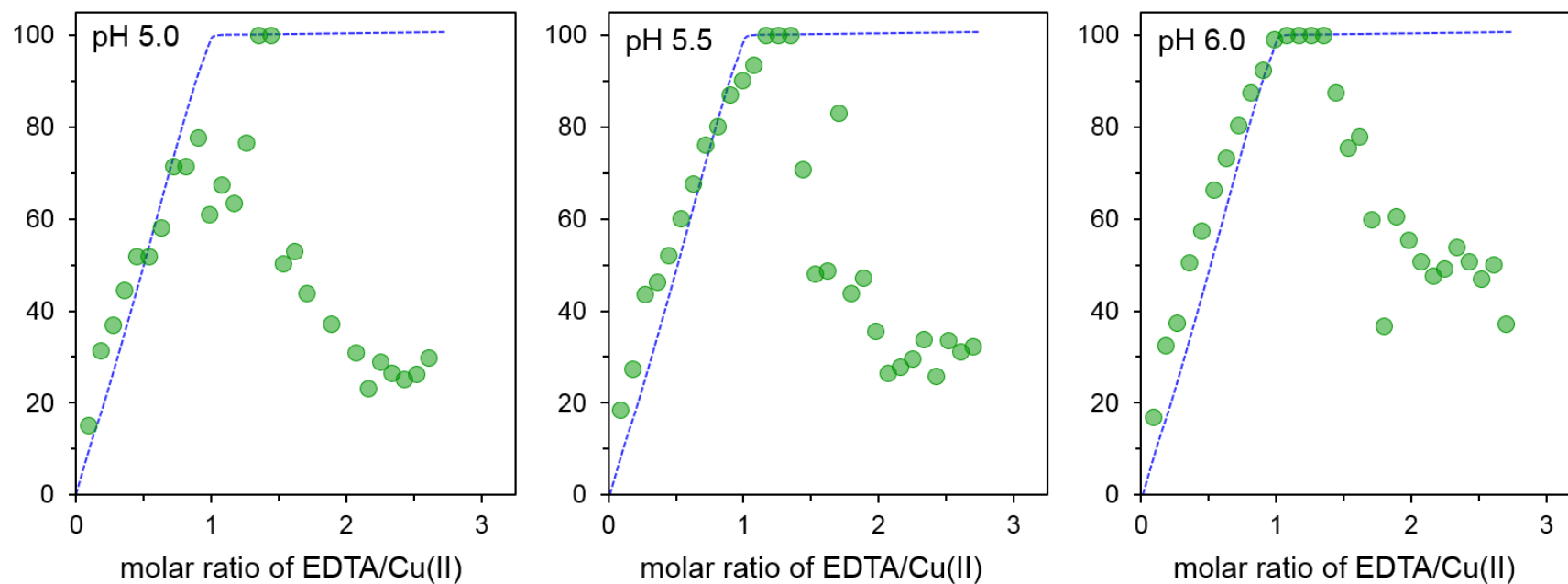
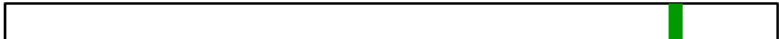
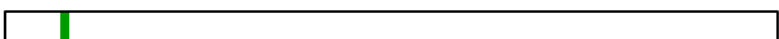
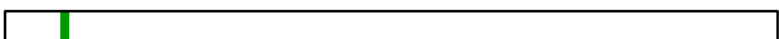


Figure 14 The effect of molar ratio EDTA/Cu(II) on removal of Cu(II) by ED-ch **4e** (D.S. 45%; ●) and the theoretical value (dashed line) at pH 5.0-6.0. Initial concentration of Cu(II) 10 mg/l, shaking rate 120 rpm, rt.

Table 1 The relationship between molar ratio of EDTA/Cu(II) and removal of Cu(II).

Molar ratio EDTA/Cu(II)	pH				
	4.0	4.5	5.0	5.5	6.0
0.99					
1.17					
1.26					
1.35					
1.44					
1.53					
1.71					
1.89					

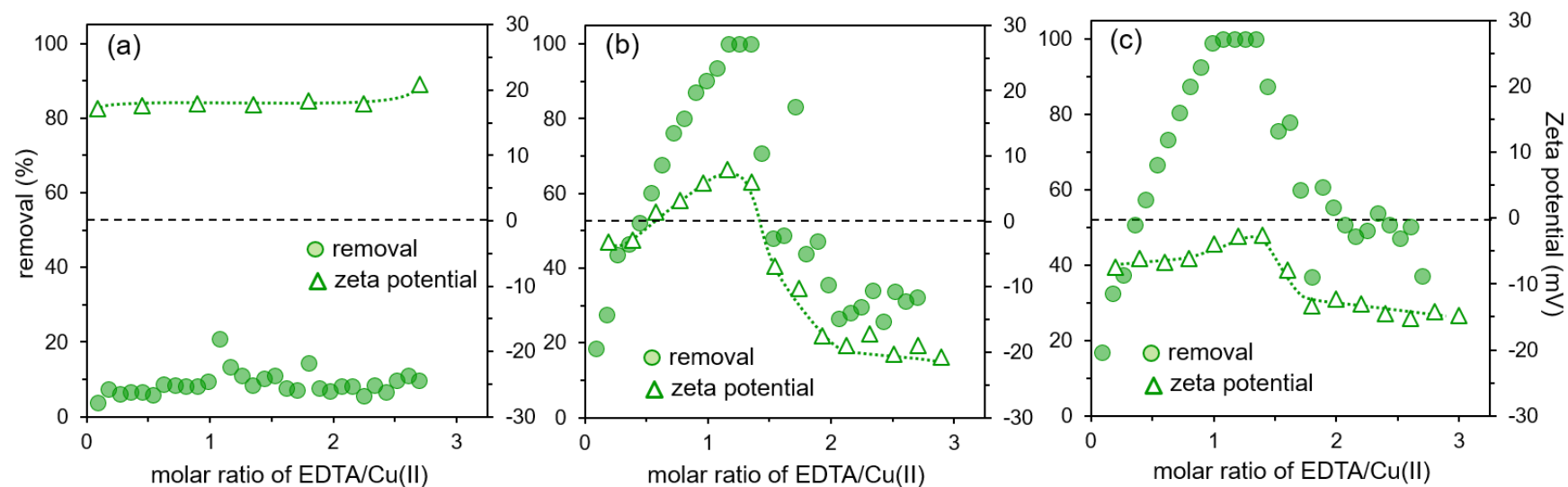


Figure 15 The effect of molar ratio on removal of Cu(II) by ED-ch **4e** (D.S. 45%) and zeta potential of the mixture solution of ED-ch **4e** and Cu(II) at (a) pH 2.0, (b) pH 5.5 (c) pH 6.0. Initial concentration of Cu(II) 10 mg/l, shaking rate 120 rpm, rt.

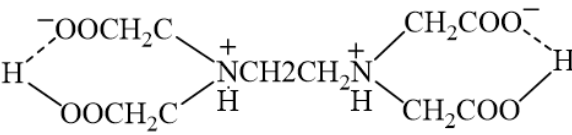
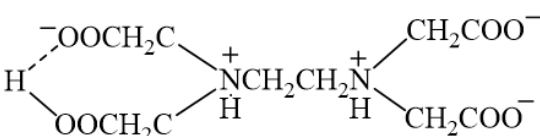
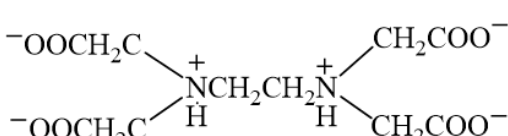
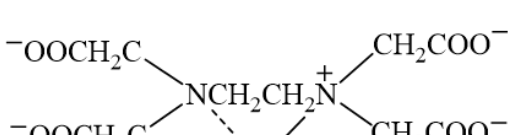
		pKa
	H_4Y	
	H_3Y^-	2.0
	H_2Y^{2-}	2.67
	HY^{3-}	6.16
	Y^{4-}	10.26

Figure 16 The structure of EDTA and pKa value.

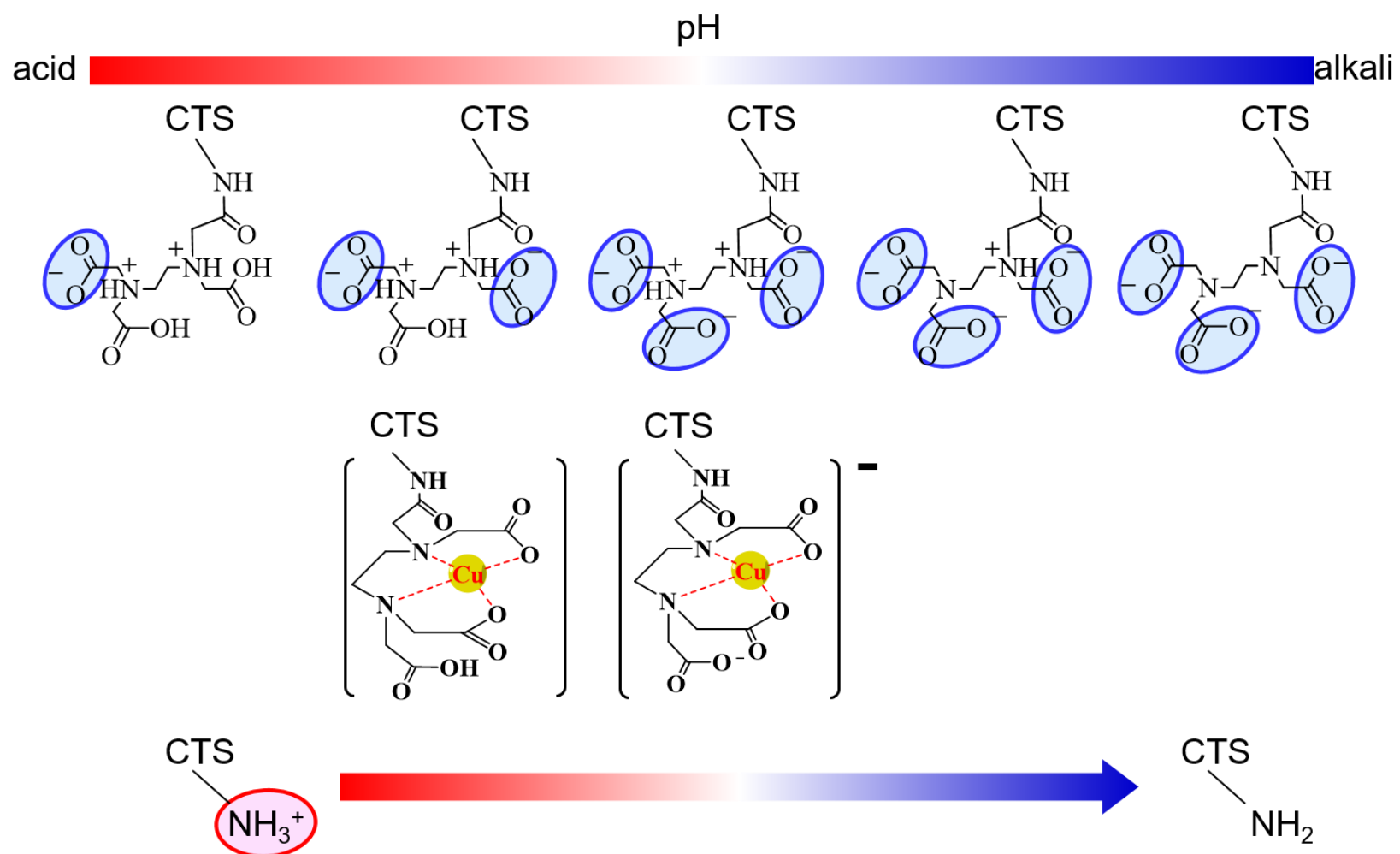
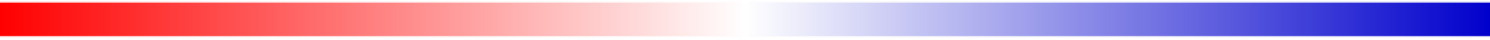
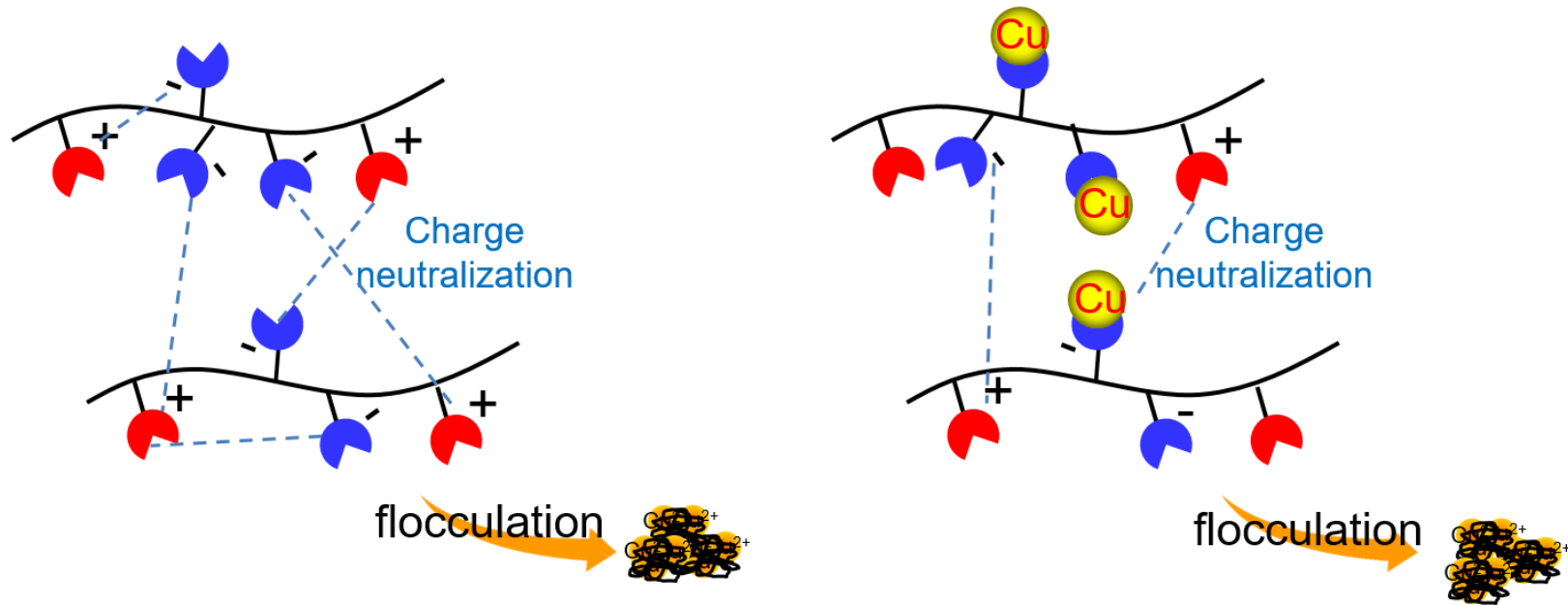


Figure 17 The structures of EDTA residue of ED-ch at various pH and its chelation complex with Cu(II) .

acid  alkali

NH_3^+  NH_2



The molar ratio of EDTA/Cu at optimum dose

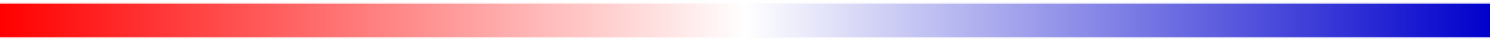
high  low

Figure 18 The effect of the molar ratio of EDTA/Cu(II) on flocculation ability.

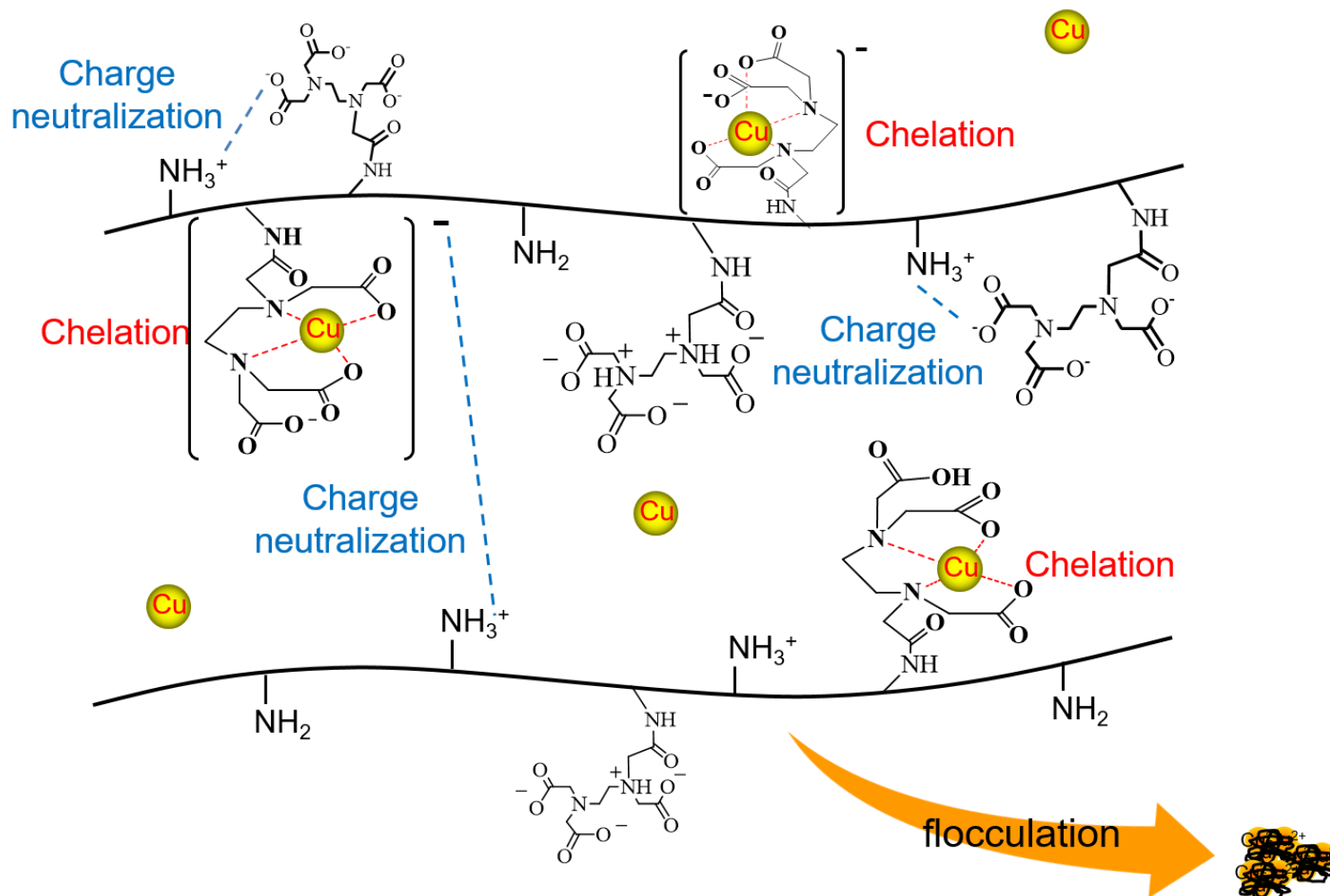


Figure 19 The mechanism for flocculation of ED-ch with Cu(II).

4.3.4 The effect of Cu(II) concentration and degree of substitution of ED-ch **4** on removal of Cu(II)

Investigation described in the previous section revealed that the optimum conditions of flocculational removal was depended on both molar ratio of EDTA/Cu(II) and pH. To confirm this hypothesis, the effects of Cu(II) initial concentration and degree of substitution of ED-ch were investigated in this section.

The experiments were carried out in using four ED-chs with different D.S. (**4e**; D.S. 45%, **4f**; D.S. 58%, **4h**; D.S. 73%, and **4i**; D.S. 79%) at pH 5.5. The initial concentration of Cu(II) was 10, 25, 50, and 100 mg/l. These results are shown in Fig. 20. ED-ch **4e** showed that the removal efficiency increased with increasing molar ratio of EDTA residue for Cu(II). And the maximum removal of almost 100% was obtained at the optimum molar ratio of EDTA/Cu(II) at all initial concentration of Cu(II). The optimum molar ratio was 1.17-1.35, 1.35, 1.35 and 1.26 for initial concentration of Cu(II) 10, 25, 50, and 100 mg/l, respectively. The ratios of EDTA/Cu(II) found to be almost same even though the concentrations of Cu(II) were different.

The maximum removal of **4f** (D.S. 58%) was lower than that of **4e** (D.S. 45%), but the optimum molar ratio was approximately 1.02 for all initial concentration of Cu(II). The flocculation ability of **4f** was lowered at pH 5.5. Furthermore, the higher D.S. materials, ED-ch **4h** and **4i**, exhibited the low removal of around 30%, even though the molar ratio increased to be near 1:1.

These removal experiments showed that the removal efficiency of Cu(II) decreased with increasing D.S. at pH 5.5. These results also suggested that the charge

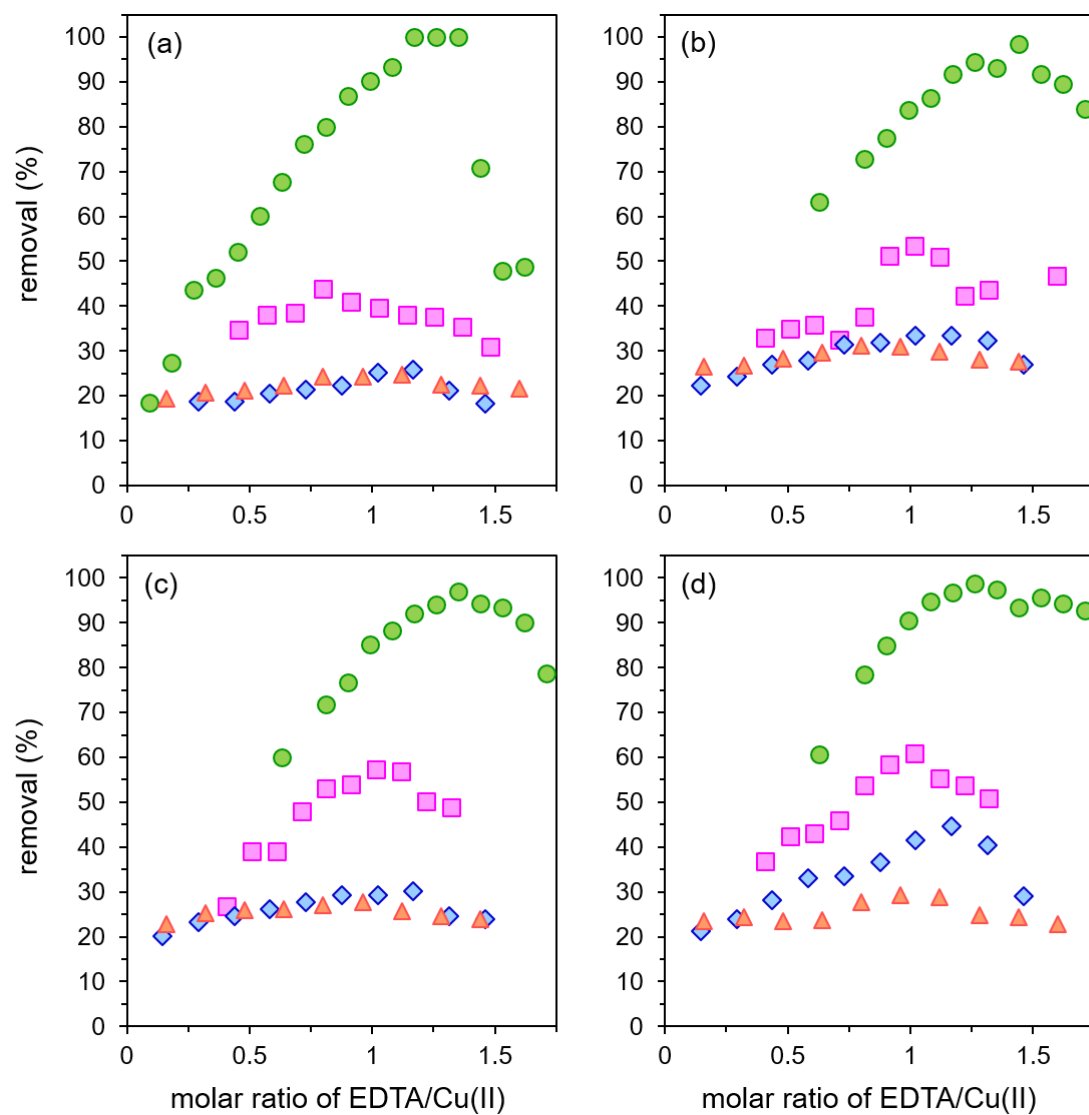


Figure 20 The effect of complexation stoichiometry on removal of Cu(II) by ED-ch **4e** (D.S. 45%; ●), **4f** (D.S. 58%; ■), **4h** (D.S. 73%; ◆), and **4i** (D.S. 79%; ▲) with Cu(II) concentration (a) 10 mg/l, (b) 25 mg/l, (c) 50 mg/l, (d) 100 mg/l at pH 5.5. Shaking rate 120 rpm, rt.

neutralization occur mainly between NH_3^+ and COO^- to cause flocculation as shown in Fig. 19. Because there are less amount of amino groups and larger amount of carboxyl groups in higher D.S. materials, anionic property due to COO^- surpasses cationic property at pH 5.5, even though the number of COO^- is decreased by chelation with Cu(II) . Therefore, the charge neutralization of higher D.S. materials hardly occurs and causes a low flocculation ability.

4.4 Conclusion

The flocculation mechanism ED-ch for the removal of Cu(II) was discussed to the influence of the chelation of EDTA residues in this chapter. From the experiment of Cu(II) distribution, it was confirmed that most of Cu(II) was formed chelation with ED-ch, when such excess amount of EDTA residue against Cu(II) was added. The complexation stoichiometry of Cu(II) and EDTA residue of ED-ch was determined by UV-vis titration, and was 1:1 or 1:1.2. Therefore, the effect of molar ratio of EDTA/Cu(II) on removal of Cu(II) was carefully examined. At lower pH than isoelectric point (IEP) of ED-ch, ED-ch has no flocculation ability. While, at higher pH than IEP, each of pH has the optimum molar ratio of EDTA/Cu(II) for high removal of Cu(II) (almost complete removal of Cu(II)). When the high removal for Cu(II) exhibited, the zeta potential is close to 0 which suggested that flocculation occurred by charge neutralization. The charge neutralization is assumed to cause by balance of the following 2 factors; (i) chelation between Cu(II) and EDTA residue that decreases negative charge of ED-ch, and (ii) protonation that increases positive charge (NH_3^+) and decreases negative charge (COO^-).

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Chapter 5
Selective removal of heavy metal ions
by EDTA-linked chitosan

Abstract

The removal of other heavy metal ions was investigated. The preliminary experiments with ED-ch to the removal for such toxic metal ions as Cd(II), Pb(II), and Cr(VI) from aqueous single metal solution. The low efficiency removal was observed for Cr(VI) that exists oxyanion, whereas Cd(II) and Pb(II) were found to be removed effectively. Next, the selectivity for removing metal ion was also examined by using aqueous solution which contained various metal ions, Ag(I), Cu(II), Cd(II), Pb(II), Zn(II), Mg(II), Hg(II), Mn(II), Ca(II), Fe(III), and Cr(VI). ED-ch has a high efficient removal for transition metal ions.

5.1 Introduction

In Chapter 3 and 4, we described the flocculation property of ED-ch and removal of metal ion by using Cu(II) as a model pollutant of heavy metal ion. However, many heavy metal ions exist as pollutants in water and solid, e.g., Cr(VI), Cd(II), and Pb(II) and so on. In addition, it is well known that the amino groups of chitosan shows to serve as selective chelating site for transition metal ions,^{1,2} and EDTA has the different of stability constant for each metal ion.³⁻⁵ Table 1 shows the stability constants for various metal ions. In this chapter, we examined the selectivity for removing metal ion from aqueous solution which contained various metal ions, Ag(I), Cu(II), Cd(II), Pb(II), Zn(II), Mg(II), Hg(II), Mn(II), Ca(II), Fe(III), and Cr(VI).

Table 1 Stability constants of various metal-EDTA complexes.

metal ion	log K
Fe(III)	25
Hg(II)	22.1
Cu(II)	18.7
Pb(II)	17.9
Zn(II)	16.4
Cd(II)	16.4
Mn(II)	13.8
Ca(II)	10.7
Mg(II)	8.9
Ag(I)	7.32
Cr(VI)	—

5.2 Experimental

5.2.1 Materials

$\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Ag}(\text{NO}_3)$, $\text{K}_2\text{Cr}_2\text{O}_7$, $\text{Fe}(\text{NO}_3)_2 \cdot 9\text{H}_2\text{O}$, HgCl_2 , $\text{Mn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ of analytical grade and other reagents of preparative grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and all chemicals were used without further purification. Water was purified by treatment with ion-exchanger followed by reverse osmosis with a Millipore Millex-3 apparatus.

5.2.2 Apparatus

Concentration of Cu(II), Cd(II), Pb(II), and Cr(VI) in aqueous single metal solution was measured by A-2000 atomic absorption spectrometer (Hitachi Ltd., Japan) which equipped with a flame atomizer (air/acetylene) and a hollow cathode lamp (wavelength of 324.7, 228.8, 217.0, and 357.9 nm, respectively). Concentration of metal ions in aqueous solution contains various metal ions measured by ICPS-7000 inductively coupled plasma atomic emission spectroscopy (Shimadzu Corp., Japan).

5.2.3 The removal of Cd(II), Pb(II), and Cr(VI) from aqueous single metal solution

ED-ch **4d** (1.00 g) was dissolved in deionized water (50 ml) to prepare 2wt% ED-ch solution. Each of Cd(II), Pb(II), and Cr(VI) solution (1000 ppm) prepared from $\text{Cd}(\text{NO}_3)_2$, $\text{Pb}(\text{NO}_3)_2$, $\text{K}_2\text{Cr}_2\text{O}_7$, and 0.1 mol/l HNO_3 was diluted with deionized water to give stock solutions (10.5 mg/l). One ml of the ED-ch solution was added to 19 ml of the Cu (II) stock solution in a 50 ml centrifuge tube, and pH of the mixture was adjusted

4.5 by the addition of small amounts of 3 mol/l HNO₃ or 3 mol/l CH₃COONa. The initial concentration of ED-ch and metal ion in each sample was 1 g/l and 10 mg/l, respectively. The mixture was shaken at 120 rpm at room temperature for 24 h. The resulting precipitate was settled by centrifugation at 4,000 rpm for 15 min and the supernatant was collected immediately. The residual metal ion concentration in the supernatant was determined by atomic absorption spectrometer. The removal of metal with ED-ch was calculated according to the following equation:

$$\text{Removal (\%)} = \frac{C_0 - C_{sup}}{C_0} \times 100 \quad \text{.....Eq. (1)}$$

where C₀ is the initial Cu(II) concentration and C_{sup} is the residual metal concentration of the supernatant.

5.2.4 The selectivity for removing metal ion form aqueous solution which contained various metal ions

ED-ch **4d** or **4h** (1.00 g) was dissolved in deionized water (50 ml) to prepare 2wt% ED-ch solution. Each of metal ion stock solutions (1000 ppm) prepared from Cu(NO₃)₂, Cd(NO₃)₂, Pb(NO₃)₂, Ca(NO₃)₂, Zn(NO₃)₂, Ag(NO₃), K₂Cr₂O₇, Fe(NO₃)₂, HgCl₂, Mn(NO₃)₂, Mg(NO₃)₂ and 0.1 mol/l HNO₃. Test solution prepared by diluting of 0.2 ml of each of metal stock solutions with deionized water (16.8 ml). One ml of the ED-ch solution was added to 19 ml of the test solution in a 50 ml centrifuge tube, and pH of the mixture was adjusted 5.5 by the addition of small amounts of 3 mol/l HNO₃ or 3 mol/l CH₃COONa. The initial concentration of ED-ch and each of metal ions in test solution was 1 g/l and 10 mg/l, respectively The mixture was shaken at 120

rpm at room temperature for 24 h. The resulting precipitate was settled by centrifugation at 4,000 rpm for 15 min and the supernatant was collected immediately. The residual metal ion concentration in the supernatant was determined by inductively coupled plasma atomic emission spectroscopy except for Pb(II) which was analyzed by AAS. The removal of metal with ED-ch was calculated according to the following Eq. (1).

5.3 Results and discussion

5.3.1 The removal of Cd(II), Pb(II), and Cr(VI) from aqueous single metal solution

Next the author's interest focused on removal of other heavy metal ions. The preliminary experiments with ED-ch **4d** to examine applicability to the removal for such toxic metal ions as Cd(II), Pb(II), and Cr(VI). In the same manner as described for Cu(II) using the each metal aqueous solution (10 mg/l) at pH 4.5 (Chapter 4). The results are summarized in table 2. Although low efficiency removal (14.8%) was observed for Cr(VI) that exists oxyanion form in aqueous solution,⁶ Cd(II) and Pb(II) were found to be removed effectively and the removal ratios were 88.0 and 93.5%, respectively. These results would be indicative of usefulness of ED-ch as a flocculation material which can get rid of harmful metal ions from wastewater.

Table 2 The effect of pH on the removal of metal ions by ED-ch **4d** (D.S. 39%).

metal	initial metal concentration (mg/l) ^a	residual metal concentration of supernatant ^b (mg/l) ^a	removal(%)
Cu(II)	10.38	0.03	100
Cd(II)	9.79	1.17	88.0
Pb(II)	9.82	0.64	93.5
Cr(VI)	10.50	8.95	14.8

^a concentration of initial and residual metal ion were measured with atomic adsorption spectrometry in ppm.

^b the resulting flocs was settled by centrifugation and the supernatant was collected immediately.

5.3.2 The selectivity for removing metal ion from aqueous solution which contained various metal ions

In 5.3.1, the successful results encouraged to exam other metal ions. Next we examined the selectivity for removing metal ion from aqueous solution which contained various metal ions, Ag(I), Cu(II), Cd(II), Pb(II), Zn(II), Mg(II), Hg(II), Mn(II), Ca(II), Fe(III), and Cr(VI). The experiments were performed by using a solution contained various metal ions at concentration of 10 mg/l for each metal ion. The results in Fig. 1 showed that Ag(I), Cu(II), Cd(II), Pb(II), Zn(II), Hg(II), and Fe(III) were selectivity removed more than 80%. The amino groups of chitosan is formed selectivity chelation with these metal ions, and EDTA also has the relatively high stability constants for these metal ions except of Ag(I). The stability constant of Ag(I) with EDTA is the lowest value in these metal ions,⁵ indicating that ED-ch may be mainly formed the coordination between Ag(I) and amino groups of chitosan. Thus ED-ch showed the selectivity removal toward these metal ions. In contrast, ED-ch exhibited a low effectively removal for Mg(II) (less than 5%). This is because chitosan and EDTA have low chelation ability for alkaline earth metal ions, Mg(II) and Ca(II).^{4,5,7} For Cr(VI), it exists several forms of oxyanion in aqueous solution depending on pH. At lower pH than 6.8, it is predominantly presented as HCrO_4^- .⁶ The removal of Cr(VI) caused by the electrostatic attraction between negative charged HCrO_4^- and protonated amino group of chitosan.⁸ These results suggested that ED-ch possessed selectivity chelation ability toward transition metal ions.

From these results, the mechanism of flocculation for metal ions removal can be elucidated as illustrated in Fig. 2. As described in Chapter 4, EDTA residue of ED-ch form chelation

complex with metal ions. Moreover, the amino group form coordination with metal ion, and the protonated amino group form an ionic bond with oxyanion metals.

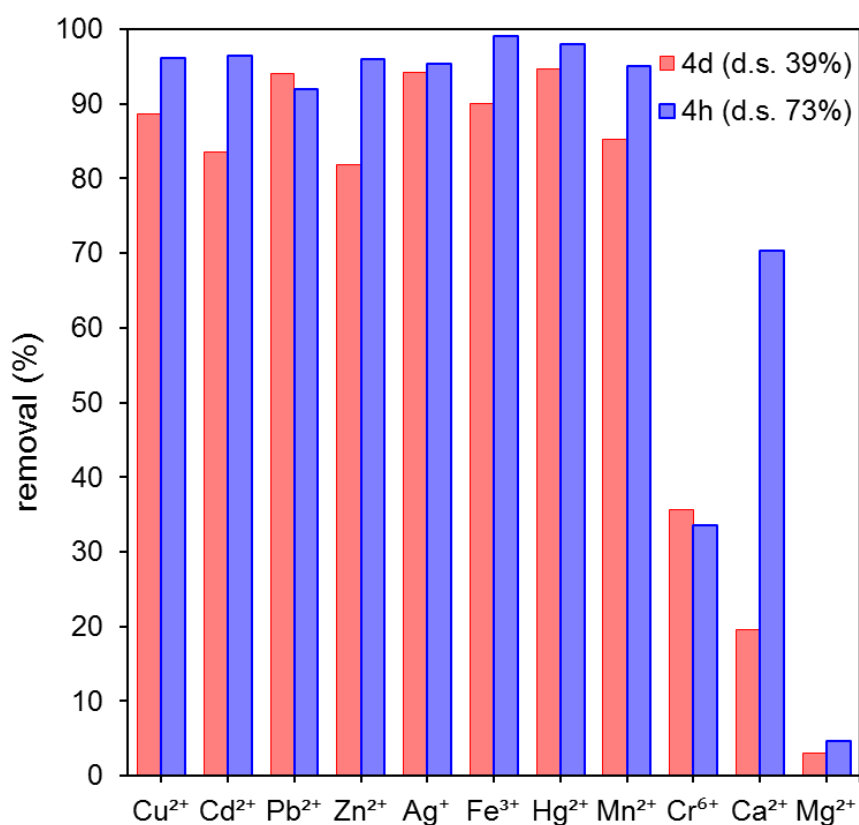


Figure 1 The removal of various metal ions by ED-ch **4d** (D.S. 39%) and **4h** (D.S. 73%). The initial concentration of each metal ion 10 mg/l, dosage of polymer ligand 1 g/l, pH 5.5, contact time 24 h, shaking rate 120 rpm, rt. Precipitate was separated by centrifugation (4000 rpm, 15 min).

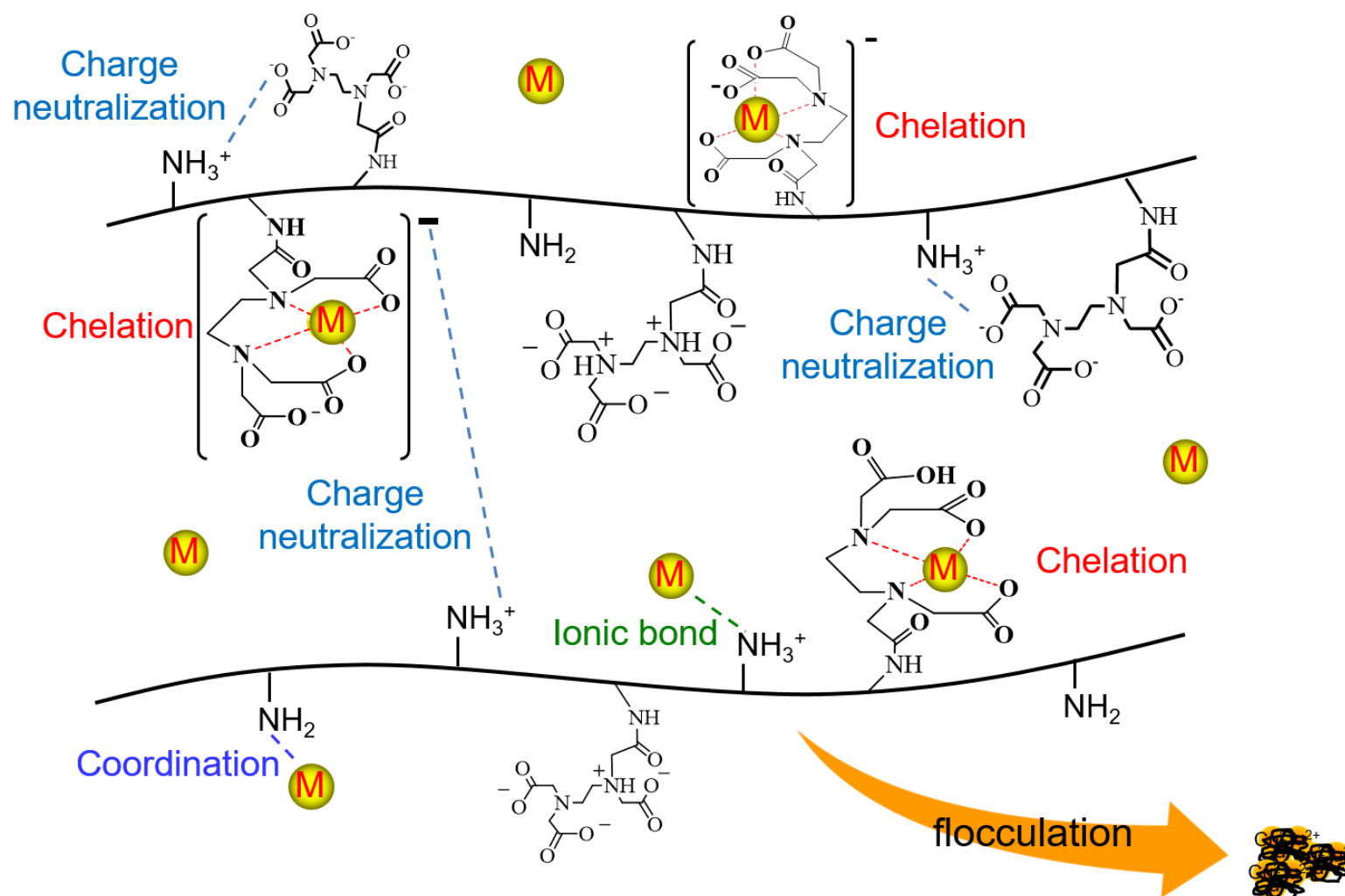


Figure 2 The mechanism for flocculation of ED-ch with metal ions.

5.4 Conclusion

Utilizing the chelation ability of water soluble EDTA-linked chitosan (ED-ch) synthesized *N*-acylation of chitosan with EDTA monoanhydride, removal various heavy metal ions was examined. In order to remove transition metal contaminants, flocculation conditions were examined by use of Cu(II). The preliminary experiments with ED-ch **4b** to examine applicability to the removal for such toxic metal ions as Cd(II), Pb(II), and Cr(VI). It was found that Cd(II) and Pb(II) were removed effectively, and the removal ratios were 88.0 and 93.5%, respectively. On the other hand, removal ratio of Cr(IV) was only 14.8%. Furthermore, 11 metal ions were tested for the similar flocculation and various transition metal ions, e.g., Ag(I), Cu(II), Cd(II), Pb(II), Zn(II), Hg(II), and Fe(III), were found to be removed by ED-ch with 80-100% removal ratio. ED-ch exhibited a high efficient removal for transition metal ions, Ag(I), Cu(II), Cd(II), Pb(II), Zn(II), Hg(II), and Fe(III). Metal ions form not only chelation with EDTA residue of ED-ch but also coordination and ionic bond with amino group.

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Chapter 6

Conclusion and future prospects

This research aimed to develop a chitosan based flocculant for removal of heavy metal ions from aqueous solution. The synthesis and property of EDTA-linked chitosan as flocculant for removal of metal ions have been performed as described in Chapter 2. Water soluble EDTA-linked chitosan (ED-ch) was successfully synthesized through N-acylation of chitosan with EDTA monoanhydride under aqueous acidic conditions followed by basic conditions. Having property as an amphoteric polyelectrolyte, ED-ch has good water solubility in both acidic and basic region and precipitated in narrow pH region. As described in Chapter 3, the flocculation property of ED-ch was investigated by using Cu(II) as a model pollutant of heavy metal ion. The flocculation performance was very sensitive to the pH of aqueous solution. ED-ch exhibited a high efficient removal toward Cu(II) with a high flocculation within the narrow pH range. The results demonstrated that ED-ch was superior to original chitosan for application as flocculant for removal of heavy metal ion. As described in Chapter 4, we investigated the flocculation mechanism of ED-ch toward Cu(II). It was found that the flocculation of ED-ch occurred by charge neutralization. The charge neutralization is assumed to cause by balance of the following 2 factors; (i) chelation between Cu(II) and EDTA residue that decreases negative charge of ED-ch, and (ii) protonation that increases positive charge (NH_3^+) and decreases negative charge (COO^-). The charge neutralization reduces the electrostatic repulsion of the polymer chains, which assisted the flocculation. In chapter 5, the sensitive for removing metal ion were described. ED-ch exhibited a high efficient removal for transition metal ions, Ag(I), Cu(II), Cd(II), Pb(II), Zn(II), Hg(II), and Fe(III). These results would be indicative of usefulness of ED-ch as a flocculation material which can get rid of harmful metal ions from wastewater.

For the future prospect, we suggest that this synthesized approach can be applied and prolonged in the field of remediation and treatment system of wastewater contained heavy metal ion.

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