



Review

A review on chitosan-based flocculants and their applications in water treatment[☆]Ran Yang, Haijiang Li, Mu Huang, Hu Yang^{*}, Aimin Li

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ARTICLE INFO

Article history:

Received 27 November 2015

Received in revised form

29 February 2016

Accepted 29 February 2016

Available online 3 March 2016

Keywords:

Chitosan-based flocculants
 Structure-activity relationship
 Structural factors
 Flocculation mechanism
 Chemical modification
 Water treatment

ABSTRACT

In recent years, the use of chitosan and its derivatives as flocculants in water treatment has received considerable attention due to their many advantages, including their widespread availability, environmental friendliness, biodegradability, and prominent structural features. However, it is a significant strategy for selection and design of the high-performance materials on the basis of their structure-activity relationships. Here we describe several of the chemical modification methods commonly used to prepare chitosan-based flocculants. These methods allow convenient control and adjustment of the structures of the obtained materials to meet the different practical requirements. The influence of structural elements of the chitosan-based flocculants on their flocculation properties are emphasized in this review by examining different flocculation mechanisms and their applications in the treatment of various wastewaters containing different pollutants (insoluble suspended colloids but also dissolved matters). Above all, the chitosan-based flocculants with proper structures by precise structure control bear great application potentials in water treatment.

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[☆] Supported by the Natural Science Foundation of China (grant nos. 51438008 and 51378250) and Six Talent Peaks Project in Jiangsu Province of China (grant no. 2015-JNHB-003).

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Abbreviations

7GL	Basic Bright Yellow	LLC	Lower limit concentration
AM	Acrylamide	MO	Methyl orange
ASR	Acidic Sandolan red	MW	Molecular weight
CAN	Ceric ammonium nitrate	NCBCh	<i>N</i> -carboxybutyl chitosan
CEC	<i>N</i> -carboxyethylated chitosans	NHMFCh	reduction product of the aldimine obtained from chitosan and 5-hydroxymethyl-2-furaldehyde
CMC	Carboxymethyl chitosan	NMPC	<i>N</i> -methylene phosphonic chitosan
CD _p	Polymer charge density	<i>N</i> -MPC	<i>N</i> -methyl piperazinium chloride
CTA	3-chloro-2-hydroxypropyl trimethylammonium	NOM	natural organic material
CTS	Chitosan	OPC	<i>O</i> -phosphorylated chitosan
CTS-GMA	Glycidyl-methacrylate-modified chitosan	PAC	Poly-aluminum chloride
CTS-GMA-TMAEMC	CTS-GMA-co-poly(<i>N</i> -trimethyl aminoethylmethacrylate chloride)	PAM	Polyacrylamide
DD	Degree of deacetylation	PBB	Procion Brilliant Blue
DKG	Direct Kahi Green	PDMC	Poly(2-methacryloyloxyethyl) trimethyl ammonium chloride
DLVO	Derjguin-Landau-Verwey-Overbeek	PFS	Polymerized ferrous sulfate
DMC	(2-methacryloyloxyethyl) trimethyl ammonium chloride	PNVCL	Poly(<i>N</i> -vinylcaprolactam)
DMF	Dimethyl formamide	THM	Trihalomethane
DS	Degree of substitution	TRE	Turbidity removal efficiency
DW	Demineralized water	TW	Tap water
ETA	2, 3-Epoxypropyl trimethyl ammonium chloride	WA	Window of application
GCC	Calcium carbonate	WsCs-SDDCt	Water-soluble chitosans blended with sodium <i>N</i> , <i>N</i> -diethyl dithiocarbamate trihydrate
GlcN	<i>D</i> -glucosamine	WsCs-SSc	Water-soluble chitosans blended with sodium salicylate
GlcNAc	<i>N</i> -acetyl- <i>D</i> -glucosamine		

1. Introduction

The rapid development of modern industries throughout the world has been accompanied by the production of various wastewaters containing different types of dissolved and undissolved contaminants and therefore of increasingly severe water pollution (Schwarzenbach et al., 2006; Shannon et al., 2008). To address this problem, a broad range of technologies, such as coagulation/flocculation (Dao et al., 2016; Jiang, 2015; Lee et al., 2014; Matilainen et al., 2010), adsorption (Crini, 2005; Qu, 2008), oxidation/reduction (Arena et al., 2015; Chen, 2004; Liu et al., 2015; Oturan and Aaron, 2014), membrane filtration (Fane et al., 2015; Hillis, 1988;

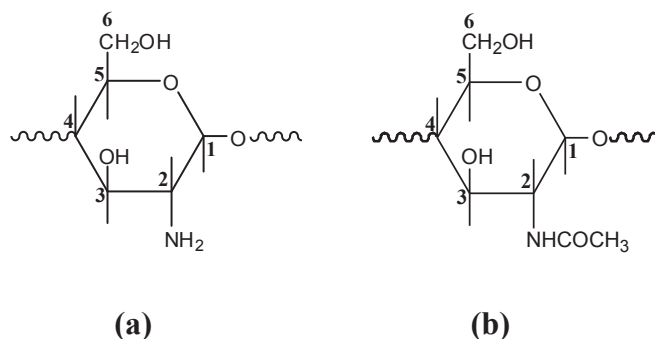


Fig. 1.1. The chemical structures of chitosan (a) and its precursor chitin (b).

Pendergast and Hoek, 2011), and biotechnology (dos Santos et al., 2007; Imran et al., 2015; Vela et al., 2015), have been developed and employed in water treatment.

Among these techniques, coagulation/flocculation is one of the most commonly used to achieve solid-liquid separation, based on its cost effectiveness and ease of operation (Dao et al., 2016; Jiang, 2015; Lee et al., 2014; Matilainen et al., 2010). In practical coagulation/flocculation processes, the small colloids suspended in wastewater are destabilized by the addition of coagulants. Coagulation is followed by flocculation, in which the destabilized particles aggregate to form larger flocs that can be effectively removed by sedimentation. It should be noted that, in some literature, the terms “coagulation” and “flocculation” as well as “coagulant” and “flocculant” are sometimes used interchangeably.

The efficiency of flocculation is highly dependent on the selected flocculants. Flocculants are mainly divided into two distinct classes: (1) inorganic coagulants, such as aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$), poly-aluminum chloride (PAC), and polymerized ferrous sulfate (PFS), and (2) organic polymeric flocculants including synthetic and natural ones. Traditional inorganic salts of multivalent metals and their polymerides have been widely used for decades as coagulants because of their advantage of low cost (Joo et al., 2007; Li et al., 2005). As reported in many studies, they are often preferred in coagulation–flocculation process for their adsorption on negatively charged colloidal particles, resulting in simultaneous surface charge reduction and formation of microflocs (Li et al., 2005, 2006). Therefore, large doses of inorganic coagulants are usually required for efficient flocculation, causing the production of large volumes of metal hydroxide sludge and subsequent disposal problem. Other drawbacks of inorganic coagulants still include highly sensitive to pH, inefficient towards very fine particles and applicable only to a few disperse systems (Bratby, 2006; Sharma et al., 2006). Moreover, an increase in metal concentration in treated water may have serious human health concerns (Walton, 2013; Ward et al., 2006).

Organic polymer flocculants have different molecular weight (MW), structure (linear versus branched), ionic nature, charge density, chemical composition, and degree of substitution (DS) of the various functional groups (Bolto and Gregory, 2007), which are highly efficient with little doses (e.g. few milligrams per litre) and generate much smaller sludge volume without consumption of alkalinity unlike inorganic coagulants. The flocs formed during flocculation are moreover bigger and stronger to endow excellent settling characteristics (Bolto and Gregory, 2007; Razali et al., 2011). On the other hand, polymeric flocculants, especially cationic polymers, can be used in direct flocculation processes, which possess dual functions of coagulation and flocculation: neutralizing the negative charges and bridging the aggregated destabilized particles (Chong, 2012; Lee et al., 2014).

However, as for the synthetic organic polymer flocculants such as polyacrylamide (PAM) and its derivatives, residual unreacted monomers including acrylamide (AM) and ethyleneimine are extremely toxic causing severe neurotoxic effects (Bolto and Gregory, 2007; Bratby, 2006; Dao et al., 2016). Therefore, the development of natural polymeric flocculants, such as chitosan (Guibal et al., 2006; Renault et al., 2009; Zeng and Yuan, 2004), starch (Krentz et al., 2006; Singh et al., 2000; Wang et al., 2013a), and cellulose (Liu et al., 2014; Sievanen et al., 2015), has been promoted, given their widespread availability, low price, non-toxicity, and biodegradability.

Chitosan has found applications in many fields, including biotechnology, biomedicine, food processing, and wastewater treatment (Bolto, 1995; Crini and Badot, 2008; Guibal et al., 2006; Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Reddy and Lee, 2013; Renault et al., 2009; Rinaudo, 2006). It is a linear

copolymer of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) synthesized by the alkaline deacetylation of chitin, the second-most-abundant natural polymer in the world, after cellulose. The structural parameters that determine chitosan's properties, including its water solubility, conformation, and chain stiffness in solution, are its MW and degree of deacetylation (DD) (Anthonsen and Smidsr, 1995; Anthonsen et al., 1993; Jiang, 2001; Lamarque et al., 2005; Muzzarelli and Muzzarelli, 2005; Rinaudo, 2006; Strand et al., 2001a; Thanou and Junginger, 2005). When the DD reaches approximately 50%, chitin usually becomes soluble in aqueous acidic medium and is called chitosan (Muzzarelli and Muzzarelli, 2005). The structures of both fully deacetylated chitosan and its precursor chitin are shown in Fig. 1.1.

Kasaai reviewed several of the methods to determine the DD of chitin and chitosan. Those most commonly used are: (1) spectroscopy (IR, ^1H NMR, ^{13}C NMR, ^{15}N NMR, and UV); (2) conventional methods (various types of titration, conductometry, potentiometry, ninhydrin assay, adsorption of free amino groups of chitosan by picric acid); and (3) destructive methods (elemental analysis, acidic or enzymatic hydrolysis of chitin/chitosan followed by DD measurement by colorimetry or high performance liquid chromatography, pyrolysis-gas chromatography, and thermal analysis using differential scanning calorimetry) (Kasaai, 2009).

Polymer MW is usually measured by static light scattering, viscosity, gel-permeation chromatography, and so on (Chu, 1991; Elias, 1984; Flory, 1953; Sperling, 2006). As for chitosan, viscosity is a commonly used method according to Mark-Houwink equation (Eq. (1.1)):

$$[\eta] = KM^\alpha \quad (1.1)$$

in which correct constants of K and α are the key to obtain accurate polymer MW. The calculated viscosity-average MW (M) from the intrinsic viscosity ($[\eta]$) is close to the weight-average MW. Rinaudo summarized the various reported K and α values under different solution conditions (Rinaudo, 2006). Wang and his colleagues (Wang et al., 1990, 1991a, 1991b, 1992) considered the effects of the DD on K and α and then determined the quantitative relationship between DD and these constants for a DD between 69% and 100%, as shown in Eqs. (1.2) and (1.3). When the buffer solution is 0.2 mol/L CH_3COOH and 0.1 mol/L CH_3COONa (pH = 4.40) at 30 °C:

$$K = 1.64 \times 10^{-30} \times \text{DD}^{14.0} (r = 0.996) \quad (1.2)$$

$$\alpha = -1.02 \times 10^{-2} \times \text{DD} + 1.82 (r = 0.998) \quad (1.3)$$

In addition to its long chain structure, chitosan has abundant free amino groups along its polymer chain backbone. These would be protonated in acidic medium, in which case chitosan would behave as a typical cationic polyelectrolyte (Guibal et al., 2006; Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Renault et al., 2009; Rinaudo, 2006). These properties endow chitosan with its good flocculation performance, including efficient charge neutralization and bridging effects, in the treatment of various contaminants in water, as most of them have a negative surface charge (Jiang, 2015; Lee et al., 2014; Matilainen et al., 2010).

However, chitosan also has several deficits that hinder its optimal use, including inactive chemical properties and poor solubility in neutral or alkaline aqueous solutions (Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Rinaudo, 2006). To improve the performance of chitosan, various physical and chemically modified forms have been synthesized and evaluated (Dao et al., 2016; Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Prashanth and Tharanathan, 2007; Rinaudo, 2006; Yang et al., 2011a; Zhang, 2006). These have allowed the use of chitosan and its derivatives

Table 1.1
Examples of different effluents treated by coagulation/flocculation using chitosan-based flocculants.

Type of effluent	Pollutants	Chitosan-based flocculants
Inorganic suspended solids-contaminated effluent	Silt, mineral dissolutions, silica, clay (kaolin, bentonite suspensions, etc.)	Chitosan (Divakaran and Pillai, 2001; Roussy et al., 2004), chitosan-g-PAM (Ali and Singh, 2009a; Wang et al., 2008; Wang et al., 2015a; Yuan et al., 2010); chitosan-g-PAM-co-PDMC (Wang et al., 2012); chitosan-CTA (Li et al., 2004a); CMC-CTA (Yang et al., 2012b); CMC-g-PDMC (Yang et al., 2014b); chitosan-g-N-MPC (Dharani and Balasubramanian, 2015); chitosan sulfate (Rios-Donato et al., 2012); chitosan/PNVCL composites (Schwarz et al., 2012)
Heavy metal ions-contaminated effluent	Hg ²⁺ , Co ²⁺ , Ni ²⁺ , Ag ⁺ , Cu ²⁺ , Zn ²⁺ , Cd ²⁺ , etc	Chitosan (Guibal et al., 2005; Lasko and Hurst, 1999; Wu et al., 2008b); mercaptoacetyl chitosan (Chang et al., 2009; Zhang et al., 2013); carboxyethyl chitosan (Bratskaya et al., 2009); chitosan/montmorillonite composites (Assaad et al., 2007)
Humic acid-contaminated effluent	Humic acid	Chitosan (Bratskaya et al., 2001; Bratskaya et al., 2002; Kvinnesland and Odegaard, 2004; Vogelsang et al., 2004); CMC-g-PDMC (Yang et al., 2014b); chitosan hydrochloride and chitosan glutamate (Bratskaya et al., 2004)
Dye-contaminated effluent	Reactive, acidic and direct dyes (PBB, ASR, DKG, AO7, MO, 7 GL, etc.)	Chitosan (Guibal and Roussy, 2007; Guibal et al., 2005; Patel and Vashi, 2012; Szyguia et al., 2009; Verma et al., 2012); CMC-g-PAM (Yang et al., 2013a); CMC-g-PDMC (Wu et al., 2015); CMC, NCBC, and NHMFCh (Delben et al., 1994; Stefancich et al., 1994); CTS-GMA-TMAEMC (Jiang et al., 2011); PAC/chitosan coagulant/flocculant (Sanghi and Bhattacharya, 2005; Zonoozi et al., 2011)
Microorganism-contaminated effluent	Bacteria (<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Enterobacter cloacae</i> , <i>Pseudomonas putida</i> , <i>Bacillus megaterium</i>), algae (<i>Chlorella</i> , <i>Protothecoides</i> , <i>Nannochloropsis salina</i> , <i>Cyanobacteria</i>)	Chitosan (Billuri et al., 2015; Rehn et al., 2013; Riaño et al., 2012; Strand et al., 2001b; Strand et al., 2002; Strand et al., 2003); CMC-g-PDMC (Yang et al., 2014a); Al ₂ (SO ₄) ₃ /chitosan coagulant/flocculant (Mehdinejad et al., 2009); hyaluronic-acid-collagen/chitosan complex film (Wu et al., 2008a); chitosan modified soil (Li and Pan, 2015; Li et al., 2015b; Zou et al., 2004; Zou et al., 2006)
Raw natural water	Suspended solids, algae, humic acids, nitrogen	Chitosan (Capelete, and Brandão, 2013; Chen et al., 2014; Kurniawati et al., 2014; Xu et al., 2013); chitosan-CTA (Huang et al., 2013); chitosan-g-PAM (Zhang et al., 2010a); chitosan-CTA-g-PAM (Lu et al., 2011); CMC-CTA (Dong et al., 2014; Yang et al., 2011b); FeCl ₃ /chitosan coagulant/flocculant (Perez et al., 2016); Al ₂ (SO ₄) ₃ /chitosan coagulant/flocculant (Garcia and Moreno, 2012; Zemmouri et al., 2012; Zemmouri et al., 2013); PAC/chitosan composite (Ng et al., 2012; Ng et al., 2013); chitosan/montmorillonite nanocomposites (Wang et al., 2015b)
Textile and paper production effluent	Cellulosic fiber fines, dissolved and colloidal carbohydrates, dye	Chitosan (Nicu et al., 2013); chitosan-CTA (Li et al., 2004b); CMC-CTA (Cai et al., 2010); chitosan/tannin coagulant/flocculant (Roussy et al., 2005a); CMC/PAM coagulant/flocculant (Chen et al., 2006); PFS/PAM/CTS composites (Zeng et al., 2012); chitosan/bentonite composites (Miranda et al., 2013)
Food industry effluent	Grease and protein, emulsified oil, saccharides	Chitosan (Ahmad et al., 2006; Cheng et al., 2005; Chi and Cheng, 2006; Gassara et al., 2015; Hwang and Srinivasan, 1995; Meyssami and Kasaiean, 2005; No and Meyers, 1989; Piontti and Zaritzky, 2001); O-carboxymethyl-N-trimethyl chitosan chloride (Li et al., 2010); chitosan-magnetite nanocomposite (Saifuddin and Dinara, 2011)
Livestock effluent	Antibiotics, inorganic nutrients (NH ₃ and PO ₄ ³⁻), bacteria, wheat dregs	Chitosan (Chung et al., 2005; Chung, 2006; de Godos et al., 2011; Johnson and Gallanger, 1984)
Electroplating and mining effluent	Heavy metal ions	Chitosan (Coughlin et al., 1990; Li et al., 2012; Tirmizi et al., 1996); WsCs-SDDCT and WsCs-SSC (Seo et al., 2002)

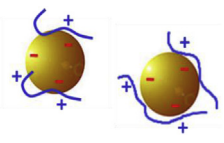
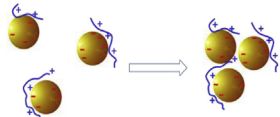
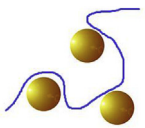
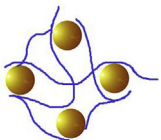
as coagulants, flocculants, coagulant aids, and as components in composite flocculants to efficiently treat wastewaters containing different types of dissolved and undissolved inorganic, organic, and biological contaminants, including suspended solids, heavy metals, humic acid, dyes, algae, and bacteria (Chen et al., 2014; Guibal et al., 2006; Renault et al., 2009; Wan et al., 2015). Table 1.1 lists the various effluents treated by coagulation/flocculation using chitosan-based flocculants.

As is known, the final performance of a material is determined by its structure. From a physical perspective, the structure of a macromolecule includes its short-range, long-range, and condensed structures. These are corresponded to the repeating units of the polymer chain (their type and chemical composition), the long-chain morphology (shape and size) of the polymer, and its supermolecular configuration (determined by the packing of the polymer chains) respectively. (Elias, 1984; Flory, 1953; Sperling, 2006). The multiplicity of the polymer structure accounts for the wide-ranging performance characteristics of polymer-based materials. In addition, the conformation of a polymer in solution (long-range structure) is a

particularly important determinant of the final flocculation performance besides its type and chemical composition (short-range structure), since most flocculants will be dissolved in water.

Two previous reviews related to the topic of chitosan flocculant have been published in 2006 and 2009 respectively (Guibal et al., 2006; Renault et al., 2009). Guibal and his colleagues (Guibal et al., 2006) have summarized the results mainly obtained in their own research groups about treatment of four kinds of wastewaters using chitosan: (a) bentonite suspensions, (b) organic suspensions (mushroom suspensions), (c) anionic dye solutions (Reactive Black 5 solutions), and (d) humic acid solutions. Later, Renault et al. have given an overview of the coagulation/flocculation performance of chitosan for various pollutant suspensions and solutions by chitosan including dissolved and undissolved contaminants (Renault et al., 2009). Both of them have also introduced the relevant coagulation/flocculation mechanisms and the effects of the characteristics of chitosan. However, their studies did not include chemically modified chitosan flocculants substantially, and the relationship between the structural characteristics of chitosan-

Table 2.1
Flocculation mechanisms of chitosan-based flocculants.

Mechanism	Description	Illustration
Simple charge neutralization	Efficient reduction of the thickness of the electric double layer and full charge neutralization.	
Charge patching	Unevenly distributed surface charges are incompletely neutralized.	
Bridging	Adsorption and connection of the primary flocs on soluble linear large-MW flocculants.	
Sweeping	Enmeshing and entrapping of small colloidal pollutants by large flocs or polymeric precipitates.	

based flocculants and their flocculation performance could not give in-depth and systematical discussion also.

Accordingly, this review describes the use of chitosan and its derivatives as flocculants for the treatment of different types of wastewater. The main focus is the structure-activity relationships of these polymers, as these significantly impact the selection and design of high-performance flocculants in the decontamination of targeted pollutants. In addition to the effects of external environmental factors, such as the pH, dose, ionic strength, and temperature, those of various structural factors, including DD, MW, and the degree of substitution by various functional groups, on the flocculation properties of chitosan-based flocculants are discussed in detail with respect to the flocculation mechanisms involved and the methods used to chemically modify the polymers. Finally, we present several of the chitosan-based flocculants currently under development and point out their potential applications.

2. Flocculation mechanisms

The different types of flocculants and pollutants interact via a broad spectrum of flocculation mechanisms and kinetic processes. The recognition both is needed to optimize the reaction conditions and to guide the development of novel and high-performance flocculants (Bolto and Gregory, 2007; Chang, 2011; Ghernaout and Ghernaout, 2012; Gregory, 2006; Ives, 1978; Jiang, 2015; Lee et al., 2014; Lyklema, 1985; Overbeek, 1977; Thomas et al., 1999; Verwey and Overbeek, 1948).

In general, the kinetics of flocculation by polymeric flocculants can be described as follows. After a suitable concentration of the flocculant is fed into the wastewater, the macromolecular flocculant makes contact with the suspended colloids by adsorption through electrostatic interactions, hydrogen bonding, van der Waals forces, etc. This leads to a rearrangement of the conformation of the adsorbed polymer such that the adsorbed suspended particles aggregate to form large flocs that then settle down effectively (Bolto and Gregory, 2007; Chang, 2011; Gregory and Barany, 2011). The flocculation mechanisms that underlie the activities of the various polymeric flocculants, including chitosan and its

derivatives, can be categorized as simple charge neutralization, charge patching, bridging, and sweeping (Guibal et al., 2006; Renault et al., 2009) (Table 2.1).

However, actual flocculation processes usually processed by two or more flocculation mechanisms. The predominant mechanism mainly depends on: (1) the nature of the colloidal dispersion and dissolved solutes; that is, whether they are hydrophobic vs. hydrophilic, their surface charge density, and their functional groups; and (2) the type of flocculant added to the wastewater: its ionic properties, charge characteristics, functional groups, adsorption capacity, and MW. Together these features determine whether charge neutralization or bridge formation among the suspended colloids and dissolved solutes is the dominant means of flocculation (Guibal et al., 2006; Jiang, 2015; Lee et al., 2014; Matilainen et al., 2010; Renault et al., 2009).

2.1. Charge neutralization

The flocculation mechanism referred to as charge neutralization is based on the diffuse electric double layer model and DLVO (Derjugin-Landau-Verwey-Overbeek) theory (Gregory, 2006; Hubbard, 2002). Most dispersed colloidal particles in wastewater are charged, and the electrostatic repulsion effects among them result in the relatively dynamic stability of the system. However, when flocculants with opposite charge are added to wastewater, both the ionic strength of the medium and the concentration of counterions in the diffuse double layers are increased. Even many of counterions are able to diffuse into the compact electric double layer of the dispersed contaminants. The result is a reduction in the particle charge and a decrease in the zeta potential, leading to a greater collision frequency and a higher collision efficiency among the suspended colloidal particles and thus the instability of the system (Gregory, 2006; Hubbard, 2002).

2.1.1. Simple charge neutralization

When the surface charges of the dispersed pollutants are neutralized completely by a given flocculant dose, the electrostatic repulsions are reduced to a minimum. Under these conditions, the

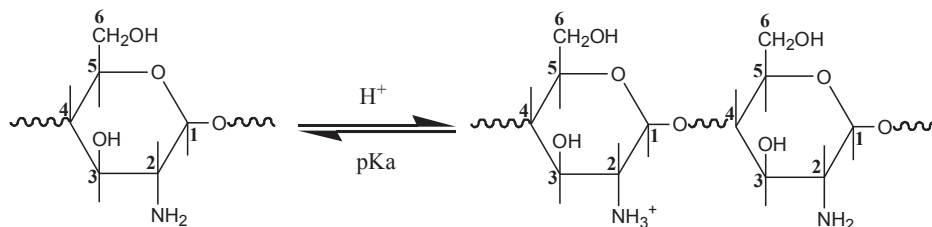


Fig. 2.1. Protonation of chitosan in acidic medium.

suspended particles aggregate to form large flocs and therefore settle, thus allowing their effective removal.

This flocculation mechanism, referred to as “simple charge neutralization” (Duan and Gregory, 2003; Guibal and Roussy, 2007; Rios-Donato et al., 2012; Yang et al., 2012b, 2012c), usually requires an optimal dose of the flocculant in the flocculation system. Following the addition of an optimal concentration of flocculant, destabilization and aggregation occur continuously, such that large sized flocs are formed and precipitated. However, when flocculants are fed into the wastewater in excess, the suspended particles are completely surrounded, and the zeta potential of the colloids thus deviates from zero. The recharged particles are therefore again stabilized by the electrostatic repulsion effects and the flocculation efficiency is accordingly reduced. To avoid this “re-stabilization effect,” the optimal flocculants concentration should be determined. Moreover, if simple charge neutralization is the dominant mechanism in a flocculation process, the optimal dose of the flocculant will increase linearly depending on the initial turbidity of the wastewater (Guibal et al., 2006; Yang et al., 2011b).

2.1.2. Charge patching

However, in many real-life wastewater treatments, the overdosed flocculants do not obviously reduce the flocculation efficiency; rather, there is a broad “flocculation window” in that efficient flocculation is achieved with a wide range of flocculant doses, without the aforementioned re-stabilization effect. In this case, the zeta potential of wastewater is far away from zero at optimal dose. These observations challenge simple charge neutralization as the dominant flocculation mechanism and instead suggest charge patching. Thus, when a flocculant is added to wastewater, the polymer easily and rapidly adsorbs onto the surface of the oppositely charged suspended colloids, resulting in heterogeneous coverage. Accordingly, various micro-regions on the surface of the suspended particles bear different charges. The unevenly distributed positive and negative surface charges allow direct electrostatic attraction among the suspended particles. Collisions among them lead to their eventual aggregation and the formation of large, highly compact flocs. Therefore, the zeta potential of the medium does not reach zero at the optimal flocculant dose, resulting in a wide “flocculation window” (Bratskaya et al., 2006; Li et al., 2004a; Roussy et al., 2005b; Yang et al., 2012c).

In flocculation, simple charge neutralization dominates when the surfaces of the suspended particles are quickly and completely neutralized by the flocculant. Conversely, charge patching prevails when primary flocs collide and aggregate before charge neutralization is complete. The two processes can be roughly distinguished from one another based on the synchronous changes in the zeta potential of the medium as a function of the flocculant dose. In details, if the zeta potential of supernatant is near zero at optimal dose, simple charge neutralization is dominant. On the contrary, charge patching play a vital role in flocculation when that is far away from zero.

2.1.3. Charge neutralization by chitosan flocculants

Chitosan is a typical cationic polyelectrolyte whose amino groups are protonated in acidic aqueous medium (Fig. 2.1). Protonation allows the interaction of chitosan with the negative surface charges found on most pollutants, through charge neutralization effects (Guibal et al., 2006; Renault et al., 2009). For polyelectrolytes, charge neutralization effects depend on the charge density, defined as the distance between two charge units along the macromolecular chain. In the case of chitosan, its charge density depends on the number of protonated amino groups on the chain backbone. Although increasing the DD would be expected to increase the number of free amino groups and thus the positive charge density, resulting in enhanced charge neutralization effects, this is not the case sometimes. Rather, protonation of amino groups is an equilibrium process that is simultaneously associated with deprotonation, and the reversibility of these reactions is a function of the pK_a . The intrinsic pK_a of chitosan is about 6.0–6.5, depending on the pH of the medium (Chiappisi and Gradzielski, 2015; Rinaudo, 2006; Rinaudo et al., 1999; Sorlier et al., 2001). Thus, the degree of chitosan protonation will be much lower at pH 7.0 than at pH 5.0.

By influencing the protonation equilibrium of chitosan in water, the pH also determines the charge density. Thus, together with the control of the DD, the charge density can be altered over a large range. However, the relationship between protonation degree, pH, and DD is complicated by the fact that the pK_a varies to some extent with the protonation degree and the DD (Sorlier et al., 2001). Yet another consideration is the charge distribution along the polymer chain.

Moreover, the charge density of a polyelectrolyte is also affected by the counterions in the medium, i.e., by the ionic strength. The ionized groups on a polyelectrolyte in a solution are not uniformly dispersed, due to macromolecular connectivity and the distance between any two charge units, which cannot be changed with further dilution. It is fully different from the situation of a low-MW electrolyte, which solution can achieve an infinite dilution state. According to the counterion condensation theory (Manning, 1974; Oosawa, 1971), when the charge density of an ionic polymer is high, so is the free energy of the mutual repulsion; thus, the counterions gather in the vicinity of the ionized groups of the polyelectrolyte and the actual charge density is accordingly reduced, but the dissociation state is maintained. The contribution of counterion condensation can be estimated using the Manning criterion (ξ_M), defined in Eq. (2.1) as:

$$\xi_M = L_B/b \quad (2.1)$$

where L_B is the Bjerrum length, at which the electrostatic repulsion energy of the two basic charges is equal to the thermal energy; and b is the axial distance between the adjacent charge units along the polymer chain. The Bjerrum length is defined as $L_B = e^2/Dk_B T$, where e is the elementary charge, D is the dielectric constant, k_B is the Boltzmann constant, and T is the absolute temperature. At room

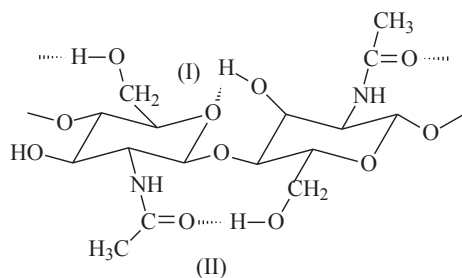


Fig. 2.2. Intra-chain hydrogen bonds (I) and (II) of chitosan.

temperature, the L_B in aqueous medium is 0.7 nm (Russel et al., 1989). At $\xi_M < 1$, there is no counterion condensation, while at $\xi_M > 1$ a fraction of the counterions $(1 - 1/\xi_M)$ is condensed. Thus, L_B is the smallest distance between adjacent charge units and the maximal linear charge density of a polyelectrolyte (q_{\max}) is equal to e/L_B .

At least theoretically, chitosan carries a maximum of one charge every ~ 0.5 nm, estimated on the basis of complete deacetylation (DD = 100%) and full protonation (Chiappisi and Gradzielski, 2015). According to the counterion condensation theory (Manning, 1974; Oosawa, 1971), when the linear charge density is higher than q_{\max} , i.e., when the DD of chitosan is $> 80\%$, counterion condensation occurs and the charge density is reduced to q_{\max} . Under these conditions of a higher DD, the dependence of the final flocculation performance of chitosan on the DD becomes insignificant or limited (Chen et al., 2003; Guibal et al., 2006; Roussy et al., 2005c), as discussed in the following section.

2.2. Bridging flocculation

2.2.1. Bridging effect

When polymeric flocculants with a long chain conformation are absorbed onto the surface of suspended colloids, they attempt to form loops, but the ends may also dangle in the aqueous medium. During flocculation, the loops and dangles contact with and are attracted to other pollutant particles. A bridge is thereby formed among the contaminants that allows their connection and subsequent aggregation into large flocs. Accordingly, this type of flocculation is called “bridging flocculation” and it is another important mechanism of flocculation (Bolto and Gregory, 2007; Gregory and Barany, 2011; Lyklema, 1985). Various interactions between flocculants and pollutants are involved, depending on the different situations (Bolto and Gregory, 2007; Gregory and Barany, 2011; Lyklema, 1985; Strand et al., 2003; Yang et al., 2012b, 2013a). However, there is no evident “bridging effect” when low-MW flocculants are used; rather, the bridging mechanism is typical only for large-MW flocculants.

Accordingly, the bridging effect is strongly related to the long-range structure (i.e., the chain morphology and conformation) of the polymer chains in water, which would reveal different sharp, hydrodynamic size and viscosity of the polymeric flocculants in solution. With a more extended conformation of the polymer chain, bridging effects will be enhanced (Cho et al., 2006). Increasing the MW of the polymer usually results in a larger hydrodynamic size and better bridging effects. As demonstrated by the Flory-Krigbaum equation (Elias, 1984; Flory, 1953), the scaling exponent between the hydrodynamic size of a polymer (\bar{R}) and its molecular weight (MW) in a good solvent is 0.6, as shown in Eq. (2.2):

$$\bar{R} \propto MW^{0.6} \quad (2.2)$$

During an actual flocculation process using polymeric flocculants, the particles suspended in the wastewater are first destabilized by charge neutralization, via a coagulation process, then they aggregate to form large flocs through bridging, i.e. a flocculation effect. Thus, polymeric flocculants, including chitosan and its derivatives, are sometimes used as coagulant aids of traditional low-MW inorganic coagulants to promote the final flocculation performance (Chang, 2011; Jiang, 2015; Lee et al., 2014).

2.2.2. Bridging flocculation using chitosan flocculants

As for chitosan, there are a number of factors affecting its chain flexibility and conformation in solution including DD, pH, and ionic strength, besides the MW. However, there is both a trivial and a complicated correlation between the aforementioned parameters and the persistence length (l_p), which describes the structural character of a semi-rigid polymer chain such as chitosan (Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Rinaudo, 2006).

The reported effects of the DD on the l_p of chitosan are contradictory: some authors have reported a decrease in stiffness, and thus of the l_p , with increasing DD (Brugnerotto et al., 2001; Skovstrup et al., 2010), whereas others found that the two are unrelated (Rinaudo et al., 1993; Schatz et al., 2003). The difference may be due to the fact that the actual l_p at a given ion concentration contains an intrinsic contribution, $l_{p,0}$, and an electrostatic contribution, $l_{p,e}$, calculated according to the method of Odijk and shown in Eq. (2.3) (Odijk, 1979).

$$l_p = l_{p,0} + l_{p,e} \quad (2.3)$$

The intrinsic contribution $l_{p,0}$ is strongly related to the stability of the intra-chain hydrogen bonds (II) of the *N*-acetylglucosamine (GlcNAc)-GlcNAc segments, which are greatly affected by the DD, as shown in Fig. 2.2 (Rinaudo, 2006). With increasing DD, the intra-chain hydrogen bonds (II) are broken and the flexibility of the chitosan chain improves; thus, its $l_{p,0}$ decreases. However, the electrostatic contribution $l_{p,e}$ is also influenced by the DD, with a higher DD usually resulting in a higher $l_{p,e}$. These contradictory effects likely account for the different conclusions reached in the various studies.

Rinaudo (Rinaudo, 2006) reported that the l_p of chitosan depends moderately on its DD. According to experimental results and theoretical predictions: (a) At $75 < DD < 100\%$, l_p is ~ 11 nm and remains nearly constant, based on counterion condensation theory (Manning, 1974; Oosawa, 1971). In this case, the Bjerrum length is larger than the average spacing between neighboring charged units. (b) down to 40% deacetylation, the stiffness of chitosan is not greatly influenced by the DD, and the l_p increase only slightly, to 15 nm. The polymer becomes increasingly hydrophobic and the intra-chain hydrogen bonds (II) have been enhanced, resulting in a stiffer chitosan chain. (c) Finally, as the DD approaches 0, the chitin chain becomes insoluble.

Another important factor that adds further complexity to the effect of the DD on chain conformation is the distribution of the GlcNAc units along the backbone (Skovstrup et al., 2010). Monte Carlo simulations of chitosan oligosaccharides have shown that flexibility is mostly determined by the number of GlcN-GlcNAc sequences, whereas GlcNAc-GlcNAc, GlcNAc-GlcN, and GlcN-GlcN sequences stabilize the extended conformation via a number of inter-chain hydrogen bonds (Skovstrup et al., 2010). The importance of the primary sequence in determining polymer stiffness might be another reason for the contradictory reports in the literature (Chiappisi and Gradzielski, 2015).

Bridging flocculation in combination with charge neutralization effects has been invoked to comprehensively explain the various kinds of practical flocculation processes induced by polymeric

flocculants. Since, as discussed above, the DD influences the charge density of chitosan as well as charge neutralization effects and bridging, its impact on the flocculation performance of chitosan flocculants has been difficult to predict, as discussed in the following section.

Moreover, as noted above, the conformation of chitosan in solution is also highly dependent on the pH and the ionic strength, which directly influence the degree of ionization ($pK_{a,eff} \sim 6.0-6.5$) and charge screening effects respectively (Chiappisi and Gradzielski, 2015). Changes in pH alter the protonation equilibrium of chitosan in water and therefore its charge density and chain conformation, according to the counterion condensation theory (Manning, 1974; Oosawa, 1971). As for ionic strength, the dose of chitosan typically used in a flocculation process is quite low, which implies an extremely dilute concentration. Consequently, an increase in the ionic strength of the medium will cause a decrease in the l_p , the radius of gyration, and the intrinsic viscosity, because of charge screening.

2.3. Other flocculation mechanisms

2.3.1. Sweeping flocculation

Sweeping flocculation mechanism was established from inorganic coagulants. Besides adsorption of soluble hydrolysis products and consequent reduction in particle charge, aluminum and iron salts still have an important effect, i.e. formation of hydroxide precipitates initially as a fine colloidal dispersion, which would further aggregate and produce hydroxide flocs. These flocs could efficiently enmesh and sweep the colloidal particles originally present in the water (Duan and Gregory, 2003; Ives, 1978).

Similarly, some polymeric flocculants with limited solubility could physically entrap small colloids from water in the precipitated mass (Parazak et al., 1988; Strand et al., 2001b). Moreover, polymeric flocculants and suspended solids could usually form large sized flocs with a three-dimensional net-like structure in flocculation. Because of its large surface area and strong adsorption capacity, the residual pollutants in water are able to be efficiently captured and swept out by hydrodynamic collisions with those large flocs then settle together (Yang et al., 2013a).

Therefore, sweeping mechanism is quite different from bridging flocculation. The former one is that the small colloidal pollutants could be enmeshed and entrapped by insoluble large metal hydroxide or polymeric precipitates; while the later one is that the soluble linear large-MW flocculants connect the primary flocs by adsorption causing aggregation into large flocs and consequent precipitation.

2.3.2. Special interactions

In addition, the chain backbones of polymeric flocculants usually contain many active functional groups. These are able to chemically react or bind with the corresponding functional groups on pollutants, especially for organic contaminants and bacteria, through some highly specific forces. The polymeric flocculants carrying the bound treated pollutants are then integrated into large flocs, which eventually precipitate. As for chitosan, its available amino and hydroxyl groups can effectively chelate metal ions, such as Hg^{2+} and Cu^{2+} , and other contaminant components to form insoluble complexes, allowing efficient removal of the pollutants (Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Rinaudo, 2006).

2.4. External factors affecting the mechanism of flocculation

As noted above, many external environmental factors effectively influence the structure and solution properties of polymeric flocculants, and thus both the mechanism and performance of

flocculation (Bolto and Gregory, 2007; Guibal et al., 2006; Renault et al., 2009; Rojas-Reyna et al., 2010). In this section, the effects of several of these factors, including flocculant dose, pH, ionic strength, and temperature, on the efficiency of flocculation are briefly described from a mechanistic perspective.

2.4.1. Dose

Flocculant dose is one of the most important determinants of flocculation performance. For each flocculation process, there is usually an optimal dose; however, as also discussed above, in many practical flocculation processes the flocculation window (effective range of flocculant dose) is wider than expected, mainly because of the involvement of several flocculation mechanisms. The dominance of simple charge neutralization reflects an optimal flocculant dose and a relatively narrow flocculation window. Excessive flocculants re-stabilize the suspended pollutants and thereby reduce flocculation performance (Guibal and Roussy, 2007; Li et al., 2013b; Rios-Donato et al., 2012; Yang et al., 2012b, 2012c). Conversely, when a charge patch mechanism dominates, overdosing of the flocculants does not usually result in a decrease in flocculation efficiency and the flocculation window is accordingly wide (Bratskaya et al., 2006; Guibal et al., 2006; Li et al., 2004a; Roussy et al., 2005b; Yang et al., 2012c).

2.4.2. pH

The impact of pH on flocculation performance is most significant in ionic flocculants. Chitosan is a weakly cationic polyelectrolyte when dissolved in acidic media. Both the pK_a of chitosan and its charge density change as a function of pH. Although, for chitosan-based flocculants, the flocculation efficiency is sensitive to many other parameters besides pH, in general, their efficiency improves at lower pH values. This is due to better charge neutralization and bridging effects, which in turn are related to the enhanced charge density and the more extended chain conformation of chitosan and its derivatives in aqueous solutions (Guibal et al., 2006; Li et al., 2013a, 2013b; Renault et al., 2009; Roussy et al., 2005c; Strand et al., 2001b).

2.4.3. Ionic strength

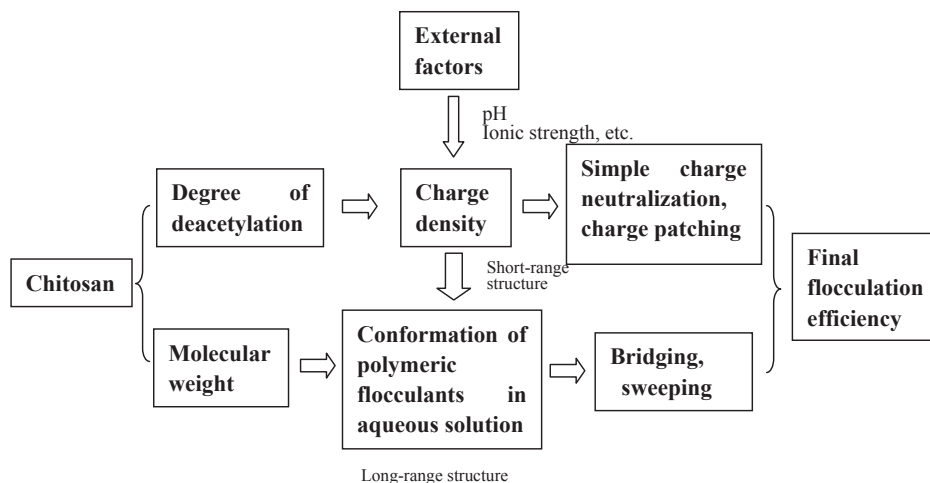
The ionic strength of the medium influences both the charge density and the chain conformation of ionic flocculants, in accordance with the counterion condensation theory (Manning, 1974; Oosawa, 1971). However, the increased ionic strength also effectively reduces the electric double layers of pollutants colloids. In the case of chitosan-based flocculants, suitable increases in ionic strength usually improve their flocculation efficiency (Guibal et al., 2006; Li et al., 2013a, 2013b; Roussy et al., 2005b; Yang et al., 2012c).

2.4.4. Temperature

The need for a lower flocculant dose at higher temperatures (Chen et al., 2007; Lu et al., 2011; Zhang et al., 2010a) can be ascribed to two effects: The first is the fact that the viscosity of the carrier liquid decreases with increasing temperature, by increasing the Brownian movements of the colloids and therefore their collision frequency, such that larger particles are more easily formed. This is explained by the flocculation kinetics described in Eq. (2.4) (Von Smoluchowski, 1916; Von Smoluchowski, 1917):

$$k_1 \propto \alpha \times \beta \quad (2.4)$$

where k_1 is the aggregation rate, α denotes the collision efficiency, and β is the collision frequency. The second effect of an increase in temperature is a more extended conformation of polymeric flocculants (Elias, 1984; Flory, 1953; Sperling, 2006), due to their



Scheme 3.1. Effects of molecular structural elements on the final flocculation efficiency of chitosan flocculants.

Table 3.1

Characteristics of chitosan preparations used in the flocculation of a bentonite suspension.

Sample no.	MW (g/mol)	DD (%)	pH of flocculation	Ionic strength	Brief description	Reference
1	1.62×10^6 – 4.70×10^6 ^a	~48–86	Not clear	NaClO ₄ , 0.01 mol/L	The optimal chitosan dose decreased linearly with increasing DD, but the MW was a more important determinant.	(Huang et al., 2000)
2	2.79×10^4 – 3.00×10^5 ^a	~69–100	~6.5–7.5	NaCl, 0.001 mol/L	MW was a more important determinant than DD.	(Chen et al., 2003)
3	3–5100 cP ^b	~83–91	~5–9	Not clear	DD and MW had only slight effects on the coagulation/flocculation performance.	(Roussy et al., 2004)
4	4.51×10^4 – 3.08×10^5 ^c	~78–95	5 or 7	Demineralized water (DW) and tap water (TW)	Coagulation was better in TW than in DW; chitosans with a higher MW were more efficient.	(Roussy et al., 2005b)
5	0.15×10^4 – 2.33×10^5 ^c	~54.6–95.3	~3–9	DW and TW	Coagulation was better in TW than in DW; flocculation efficiency depended on not only the DD and MW, but also the ionic strength, pH, and the flocculant dose.	(Li et al., 2013b)

^a Viscosity-average molecular weight.

^b Centipoise, as a direct measure of viscosity.

^c Weight-average molecular weight.

improved solubility, and therefore better flocculation. Accordingly, in water treatment, a reduced flocculant dose in summer and a larger dose in winter are required (Zhang et al., 2010a).

3. Chitosan-based flocculants and the effects of structural factors

Knowledge of the structure-activity relationship of a material can facilitate precise control of its molecular structure and thus improve its performance (Elias, 1984; Flory, 1953; Sperling, 2006). This is also the case for flocculants, as their careful design and selection, based on an understanding of the relationship between their structural characteristics and their flocculation performance, will result in the efficient flocculation in aqueous media of different contaminants with diverse features.

The flocculation efficiency of chitosan-based flocculants in wastewater treatment is likewise highly dependent on their structures. As mentioned above, the DD and the MW are two very important structural factors for chitosan. When chitosan is used as a flocculant, the DD is related to the charge density (short-range structure) and greatly influences the charge neutralization efficiency, which together with the MW accounts for the long-range

structure, i.e., the morphology and conformation of the polymer chain in aqueous medium. The latter features are intrinsically related to bridging and sweeping flocculation mechanisms. As for chemically modified chitosan-based flocculants, the degree of substitution of the functional groups and grafting ratio also determine their final flocculation efficiency. In addition, all of these structural factors interact with the various environmental factors (dose, pH, ionic strength, and temperature) to influence the performance of the flocculants.

The effects of the molecular structural factors of chitosan-based flocculants on the final flocculation efficiency in target wastewaters containing inorganic, organic, and bacterial pollutants are described in this section. However, field operation data are limited, especially for modified chitosan flocculants.

3.1. Chitosan flocculants

3.1.1. Structural factors: degree of deacetylation and molecular weight

In relation to flocculation mechanisms, the effects of molecular structural factors on the final flocculation efficiency of chitosan flocculants were discussed in detail in Sect. 2 and are summarized

in Scheme 3.1. As shown in this scheme, increasing the DD increases the number of free amino groups which usually results in the improved positive charge density, thereby enhancing both the charge neutralization effects and bridging flocculation by extended conformations due to intramolecular electrostatic repulsion. Moreover, external factors such as pH and ionic strength also affect the positive charge density on the chitosan backbone, by altering both the protonation equilibrium of chitosan in solution and the counterion condensation effects. The final DD effects should therefore combine various factors.

As for MW, in high-MW forms of chitosan, the more extended conformation of the macromolecular chain in water based on Eq. (2.2) improves the polymer's bridging and sweeping effects. This accounts for the preferential use of high-MW polymeric flocculants in actual flocculation processes.

The effects of DD and MW on the final flocculation performance are usually complimentary and synergistic. The relationship between the two of chitosan and the actual flocculation efficiency is very complicated and highly dependent on the characteristics of the wastewater and the relative contributions of the diverse flocculation mechanisms.

3.1.2. Effects of structural factors on flocculation of various wastewater

3.1.2.1. Inorganic suspended pollutants. Bentonite suspensions are frequently used in as an experimental, synthetic inorganic wastewater. Table 3.1 summarizes the results obtained with various chitosan preparations in the flocculation of bentonite suspension, as reported by different researchers.

Huang et al. deacetylated chitin powder using a NaOH solution (Huang et al., 2000). They obtained several chitosan preparations with DDs ranging from 48% to 86% and then tested their ability to coagulate a bentonite suspension. The authors found a linear relationship between the DD and the charge density (Fig. 3.1a). The optimal chitosan dose decreased linearly with increasing DD (Fig. 3.1b). The amino groups on the chitosan chains were shown to play important roles in coagulating bentonite solids. However, chitosan with lower DD but higher MW had a lower optimal coagulation dose than that with higher DD but lower MW. The latter resulted in poor coagulation, higher residual turbidity, smaller floc diameters, and a slower settling velocity. These observations suggested that MW is a more important determinant than DD. Chen et al. (Chen et al., 2003). Also flocculated bentonite colloid using various chitosan preparations with different MWs and DDs, performing the reaction under near-neutral conditions. The pyrene-fluorescence spectra of the flocculations showed the key role played by MW rather than the DD and that bond bridging rather than charge neutralization was the dominant mechanism of the chitosan-mediated flocculations.

Roussy et al. obtained satisfactory coagulation/flocculation efficiencies using various chitosan samples at pH 5 (Roussy et al., 2004). The flocculation kinetics of a chitosan flocculant (no. 261) with a low MW and the lowest DD were the slowest. However, at neutral pH, the efficiency of a preparation with a high MW and the highest DD was only slightly better. The authors concluded that DD and MW have only slight effects on coagulation/flocculation performance.

In another study (Roussy et al., 2005b), the same authors tested ten chitosan samples with different MWs and DDs on the coagulation of suspensions of 5 g of bentonite/L, both at pH 5 and at pH 7 as well as in demineralized water (DW) and in tap water (TW). Coagulation was better in TW than in DW regardless of the pH, due to the improved attachment of the flocculant to bentonite. This affinity was ascribed to the presence of sulfate and other counterions in TW as well as to the increased coiling of the chitosan

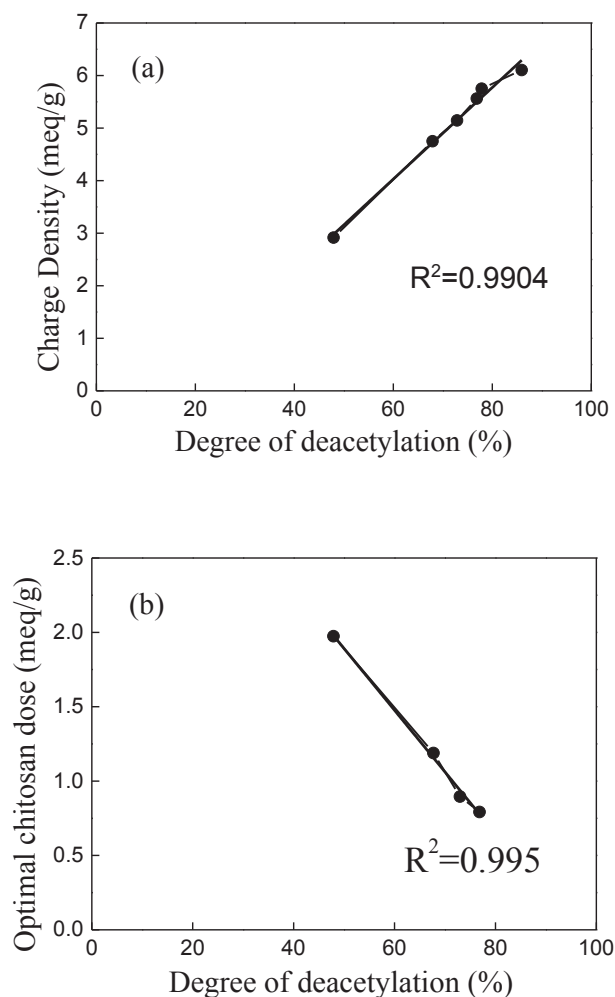


Fig. 3.1. (a) The relationship between the charge density and degree of deacetylation; (b) The relationship between the optimal chitosan dose and degree of deacetylation (Huang et al., 2000).

molecules, which resulted in more effective electrostatic patch destabilization. Coagulation was typically more effective using higher-MW chitosans, both in TW and in DW, because of the enhanced bridging effect at pH 5 and pH 7, although the efficiency tended to level off at MWs $>1.0 \times 10^5$ g/mol.

Li et al. used chitosans with various DDs and MWs to adsorb and flocculate bentonite in DW and TW at pH values ranging from 3 to 9 (Li et al., 2013b). For every chitosan tested, the coagulation/flocculation efficiency of a bentonite suspension was higher in TW than in DW. They also showed the importance of the ionic strength of TW on the properties of the bentonite particles and the chitosan solution. Specifically, in the low ionic strength of TW, the bentonite suspension aggregated more easily than in DW, due to compression of the electric double layer. In addition, in low ionic strength solutions, third electroviscous effects dominate, such that the chitosan molecules assume an extended conformation (Avena and De Pauli, 1998). The effects of DD and MW on the residual turbidity in both TW and DW were shown to be strongly related to the chitosan dose and to the pH. From Figs. 3.2 and 3.3, at an optimal dose of 5 mg chitosan/L in TW and 20 mg chitosan/L in DW, the DD and MW only minimally affected the coagulation/flocculation efficiency, except for chitosans with a MW $<3.5 \times 10^3$ g/mol, under all investigated conditions. When the chitosan dose was insufficient, increasing the DD or MW reduced the residual turbidity, while

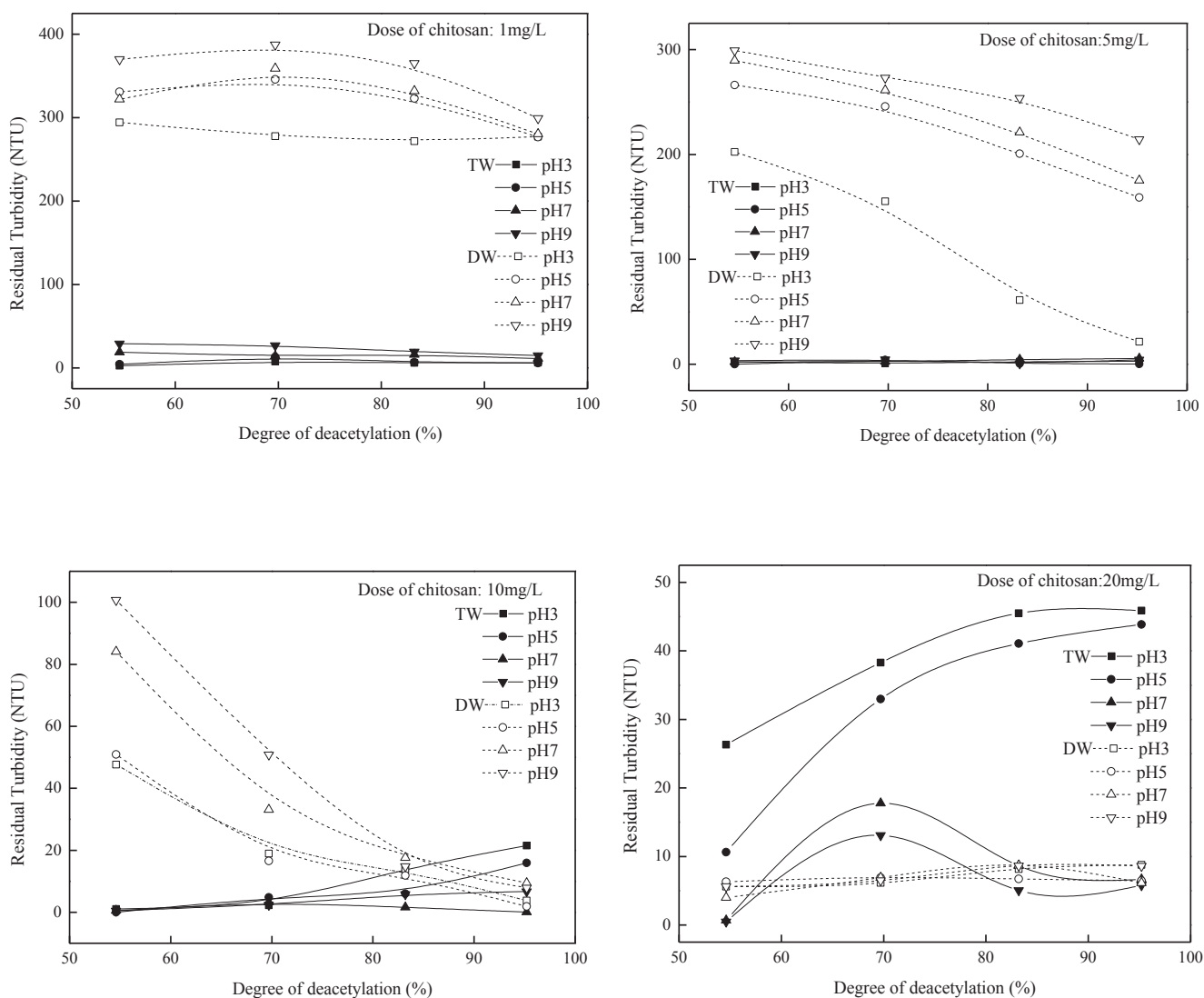


Fig. 3.2. Influence of the degree of deacetylation and chitosan dose on the coagulation–flocculation of the bentonite suspension at different pH in DW and TW. Initial turbidity of bentonite suspension 500 NTU, settling time 10 min (Li et al., 2013b).

lowering the pH enhanced the charge density and thereby increased the flocculation efficiency. When the chitosan dose was higher than the optimal one, the effects were the opposite and restabilization appeared. Under the conditions of this experiment, charge neutralization associated with bridging effects was the main flocculation mechanism. The structural characteristics of chitosan, i.e. DD and MW, interact and compensate to enhance the final flocculation. Furthermore, the mechanism type depends on not only those molecular parameters but also the ionic strength, pH of the surrounding medium, and flocculant dose.

In another study (Li et al., 2013a), a kaolin suspension was flocculated as in their above-described experiment. The final flocculation efficiency was again related to the structural characteristics of chitosan and to several external factors. MW was shown to play a more important role than DD when the flocculation was carried out in TW and bridging rather than charge neutralization dominated the flocculation process. The ionic strength of TW was important in reducing the residual turbidity in the flocculation process. In DW, the influence of chitosan's MW and DD on the coagulation/flocculation efficiency was very limited.

Taken together, these studies show that for most inorganic colloid suspensions, which typically include clay compounds such

as bentonite and kaolin, chitosans with a high MW and a high DD are usually more efficient in their flocculation performance, because of the improved charge neutralization and bridging effects. The greater significance of MW than DD (Chen et al., 2003; Huang et al., 2000) may be intrinsically due to the minimal impact of changes in both the charge density and the conformation of chitosan flocculants at higher DDs, because of counterion condensation effects (Manning, 1974; Oosawa, 1971).

3.1.2.2. Organic pollutants. The effects of DD and MW on the flocculation of organic pollutants, including dissolved and undissolved forms, have also been extensively investigated. Those studies are summarized in Table 3.2. Common examples of dissolved pollutants are dyes, humic substances, soluble proteins in surimi wash water, and soluble inks from packaging wastewater. Undissolved ones include polystyrene latex, mushroom powder, aquaculture, and papermaking wastewaters.

Guibal and Roussy investigated the coagulation/flocculation of an anionic dye (Reactive Black 5) using two chitosan samples with the same DD but far different MWs (Guibal and Roussy, 2007). In acidic media, the protonated amine groups of chitosan were expected to effectively attract the sulfonic groups of Reactive Black 5,

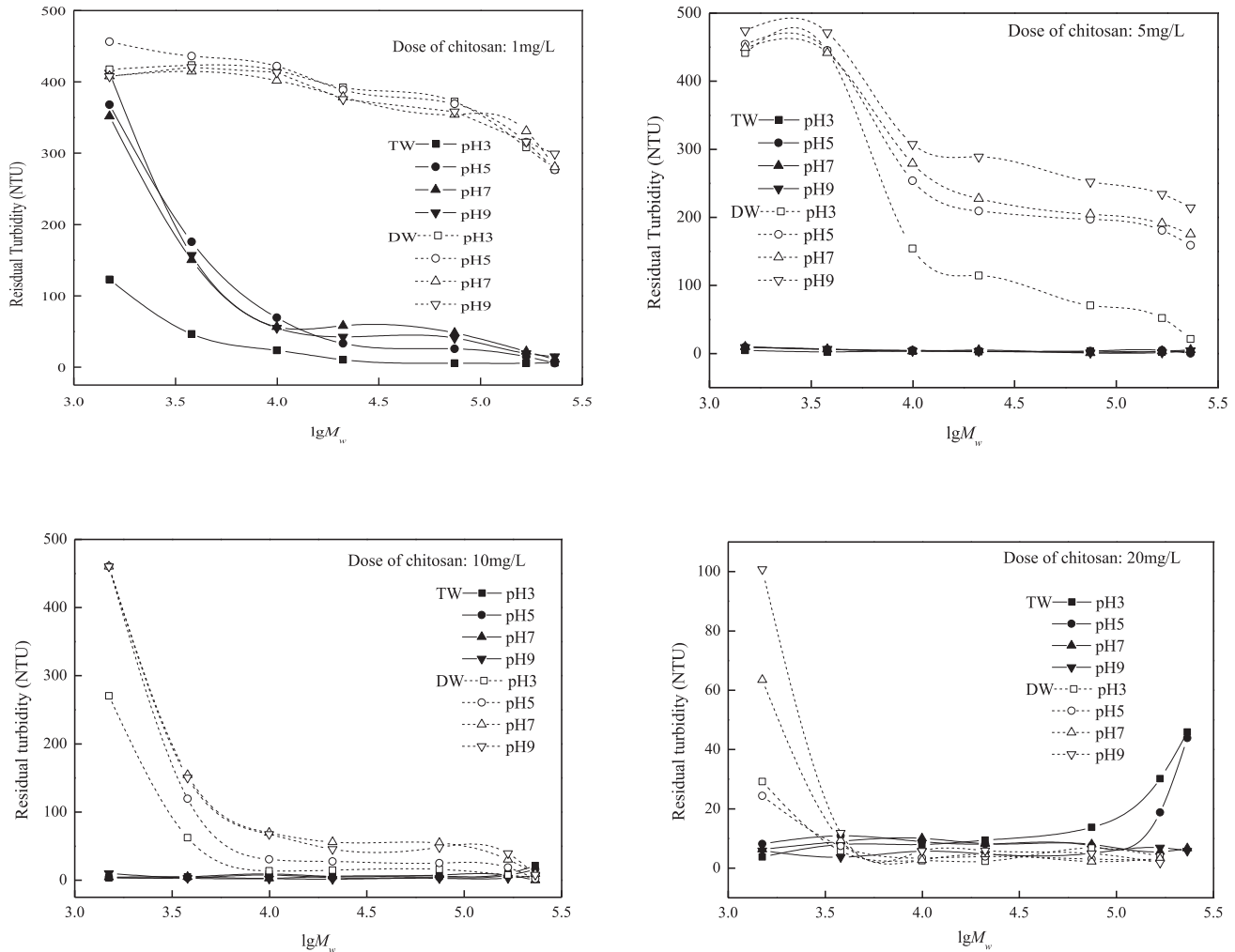


Fig. 3.3. Influence of the molecular weight and chitosan dose on the coagulation–flocculation of the bentonite suspension at different pH in DW and TW. Initial turbidity of bentonite suspension 500 NTU, settling time 10 min (Li et al., 2013b).

through electrostatic interactions. The molar ratio between dye molecules and amine groups ($[n]$) was set up to respect the stoichiometry between the sulfonic groups and the protonated amine groups at the pH of the initial solution (pH 5). However, the presence of chitosan in excess of the optimal dose resulted in re-stabilization of the particles, consistent with simple charge neutralization as the main flocculation mechanism of dye coagulation when chitosan is added under acidic conditions. They (Guibal and Roussy, 2007) found that the coefficient $[n]$ depended not only on the pH of the medium but also on the MW of chitosan. However, chitosan with a higher MW slightly decreased the process efficiency due to the reduced availability of amine groups and the polymer's less flexible structure (Guibal and Roussy, 2007).

Vogelsang et al. reported the flocculation behavior of chitosan during the removal of humic substances from water (Vogelsang et al., 2004). Whereas chitosan efficiently removed high-MW humic substances, the removal efficiency of medium-MW humic substances was enhanced only after the addition of small amounts of Fe^{3+} , which also lowered the optimal chitosan dose. From Fig. 3.4, increasing the DD of chitosan enhanced the charge density on the chain backbone and improved flocculation efficiency, whereas changes in the MW of chitosan had no significant influence on the maximum removal of the humic substances. This result was confirmed by Guibal et al. (Guibal et al., 2006), who also found that the MW of chitosan did not affect the coagulation/flocculation

of humic acid. Furthermore, they (Guibal et al., 2006) have also compared the effects of DD and MW on various types of wastewater. MW had more effect on bentonite suspensions and dye solutions than on kaolin and humic acid suspensions.

Wibowo et al. used chitosan-alginate complexes to recover soluble proteins from surimi wash water (Wibowo et al., 2007). The mixing ratio of chitosan and alginate in complexes was kept constant at 0.2. The superior effectiveness of the complexes was confirmed by the recovery of soluble proteins. However, the differences in protein recovery efficiency using chitosans differing in their MWs and DDs were not significant.

In the study of Roussy et al., chitosan and tannin were used as coagulant and flocculant, respectively, to remove soluble inks from industrial effluents generated during packaging (Roussy et al., 2005a). The authors compared two chitosan samples with the same DDs but very different MWs. Though the process could be slightly improved using high-MW chitosan, the overall difference in coagulation/flocculation performance was not substantial, which suggested that charge neutralization, rather than bridging, was the dominant mechanism in this system.

Ashmore et al. used a series of chitosan preparations with different DDs and MWs to flocculate model polystyrene latex particles varying in both their size and the charge densities of their sulfate groups (Ashmore and Hearn, 2000; Ashmore et al., 2001). Flocculation efficiency increased with increasing MW or DD but the

Table 3.2
Characteristics of chitosan preparations used in the flocculation of organic pollutants.

Sample no.	Organic pollutant	MW (g/mol)	DD (%)	pH of the flocculation	Brief description	Reference
1	Anionic dye (Reactive Black 5)	8.01×10^4 , 3.08×10^5 ^a	89.5	3, 5	High-MW chitosan slightly decreased the process efficiency due to the reduced availability of amine groups and the polymer's less flexible structure. The molar ratio between dye molecules and amine groups ([n]) respected the stoichiometry between the sulfonic groups and the protonated amine groups at the initial pH of 5.	(Guibal and Roussy, 2007)
2	Humic substances	-3.00×10^3 – 3.99×10^5 ^b -4.51×10^4 – 3.08×10^5 ^a	–51–99 78–95	–3–7 –4–9	The MW of chitosan did not significantly influence the maximum removal of humic substances. Chitosans with the highest DDs were the most efficient coagulants. The MW of chitosan did not affect the coagulation/flocculation of humic acid.	(Vogelsang et al., 2004) (Guibal et al., 2006)
3	Soluble proteins in surimi wash water	-2.23×10^4 – 3.83×10^6 ^c	–75–94	5, 7	Chitosan-alginate complexes were used as flocculants at a constant mixing ratio of 0.2. The superior effectiveness of the complexes was confirmed but differences among chitosan types did not correlate with MW and DD.	(Wibowo et al., 2007)
4	Ink-containing packaging wastewater	8.01×10^4 , 3.08×10^5 ^a	89.5	5, 7.5	The MW had no major effects on coagulation/flocculation performance.	(Roussy et al., 2005a)
5	Surfactant-free polystyrene latex	1.90×10^4 – 1.92×10^6 ^c	62–98	–3–7	Flocculation efficiency increased with increases in either MW or DD, but the effects were slight.	(Ashmore and Hearn, 2000; Ashmore et al., 2001)
6	Mushroom powder suspended in TW	-4.51×10^4 – 3.08×10^5 ^a	78–95	5, 7, 9	MW and DD had a limited effect on flocculation performance, which is related to pH and flocculant dose.	(Roussy et al., 2005c)
7	Organic compounds, inorganic nutrients, and bacteria in aquaculture wastewater	-3.0×10^5 – 6.0×10^5 ^d -6.21×10^3 – 3.62×10^5 ^d	80–98 90	–3–10 –3–10	A high DD and low pH improved the flocculation performance of chitosan. High-MW chitosan was best at removing turbidity and suspended solids as well as at lowering biological and chemical oxygen demand (BOD and COD). Low-MW chitosan was best at removing NH ₃ and PO ₄ ³⁻ from wastewater.	(Chung, 2006) (Chung et al., 2005)
8	Pulp/calcium carbonate from papermaking	-7.70×10^4 – 4.44×10^5 ^d	–85.7–87.5	Not clear	Flocculation efficiency increased with increasing MW.	(Nicu et al., 2013)

^a Weight-average molecular weight.

^b Number-average molecular weight.

^c Viscosity-average molecular weight.

^d No details.

effects were slight. At high DD, the optimal dose of chitosan was independent of the MW but the required dose was reduced because of the higher charge density on the chitosan backbone at lower pH. In this system, charge neutralization was the dominant mechanism. The authors therefore recommended high DD under acidic conditions to obtain an optimum coagulation efficiency. However, using chitosan with a low DD, the flocculation efficiency improved with the MW of the polymer increased, indicating the predominance of a bridging effect. In addition, increasing the ionic strength of the dispersion medium could broaden the flocculation concentration range.

Roussy et al. compared several chitosan preparations with different DDs and MWs in a synthesized organic suspension comprising mushroom powder and TW (Roussy et al., 2005c). The influence of MW on chitosan's removal efficiency was reduced substantially by slightly increasing the polymer concentration. And the DD effect was insignificant, especially at DDs >90% possibly due to counterion condensation effects (Manning, 1974; Oosawa, 1971). However, the flocculation process was very sensitive to pH. At pH 5, the chitosan concentration needed to achieve acceptable flocculation was less than half that at a pH close to neutral. Under the acidic conditions, the DD and MW had limited effects on the flocculation efficiency of chitosan; however, at lower chitosan concentrations, the removal efficiency increased with increasing MW. At a near-neutral pH, at which a higher chitosan concentration was required, the coagulation performance was slightly improved by increasing the DD and decreasing the MW of chitosan. Generally,

charge neutralization was the dominant flocculation mechanism in this case.

Chung used various chitosans with different DDs and MWs to remove a mixture of organic compounds and inorganic nutrients from aquaculture wastewaters (Chung, 2006; Chung et al., 2005). For chitosans with similar MWs, both DD and pH significantly affected the flocculation performance. The final results showed improved treatment efficiency at a high DD and low pH (Chung, 2006). At a constant DD of 90%, greater removal efficiencies of turbidity, suspended solids (SS), BOD, and COD were obtained with high-MW chitosans, whereas NH₃ and PO₄³⁻ were better-removed using low-MW forms of the polymer. Moreover, high-MW chitosan was better than low-MW forms in the elimination of SS of various particle sizes (Chung et al., 2005).

Nicu et al. compared the ability of three chitosan preparations with different MWs to remove calcium carbonate (GCC) and pulp/GCC suspensions in papermaking (Nicu et al., 2013). The flocculation behavior of chitosan against the pulp/GCC suspension was very different than determined against the GCC suspension alone, due to the additional presence in the former of cellulose fibers; however, chitosan's performance was close to that of a traditional cationic flocculant, polyethylene imine. Chitosans with a higher MW had a greater efficiency and stronger affinity towards cellulose fibers and therefore greater flocculation efficiency.

In summary, the structural features of chitosan play a larger role in the flocculation of organic than inorganic pollutants in water. This can be explained by the more complicated surface structure of

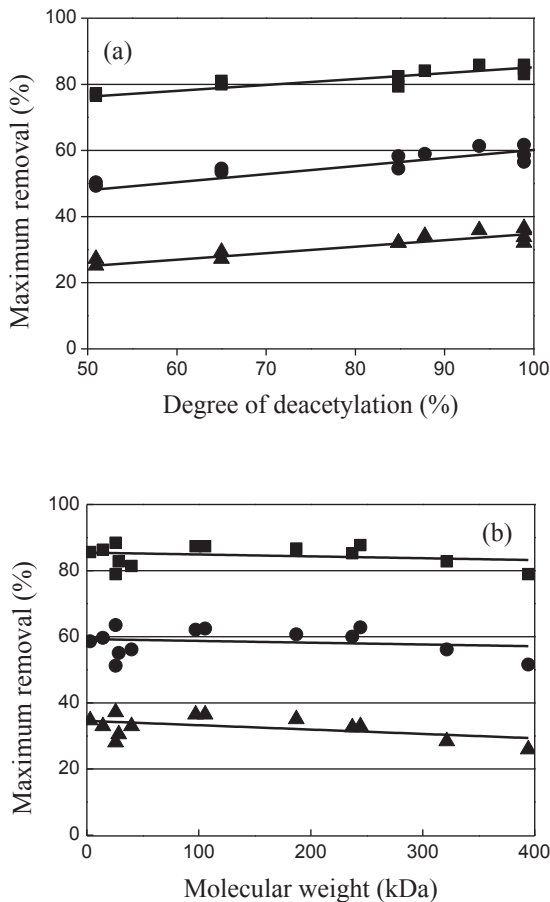


Fig. 3.4. The apparent maximum removal ratios of color (■), UV absorbance (●), and TOC (▲) from artificial raw water at pH 5 using different chitosans plotted against (a) the chitosans' degree of deacetylation and (b) number-average molecular weight (Vogelsang et al., 2004).

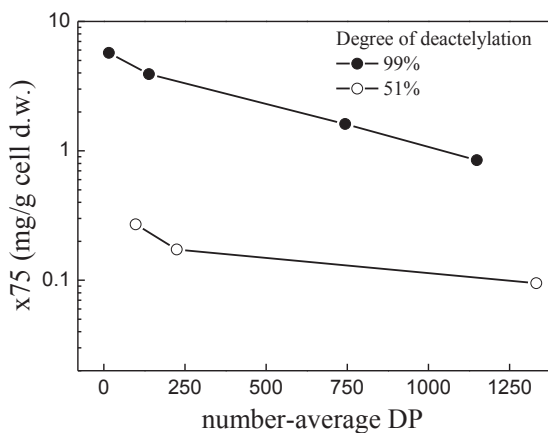


Fig. 3.5. Chitosan-HCl concentration at 75% flocculation ($\times 75$) as a function of number-average degree of polymerization (DPn) for chitosans with the degree of deacetylation 99% and 51% respectively, after 24 h of sedimentation. Bacteria ($3-4 \times 10^9$ cells/mL) were resuspended in PBS with pH 6.8 and ionic strength 0.1 mol/L (Strand et al., 2001b).

organic pollutants, which carry an abundance of functional groups. The more efficient flocculation performance is due to a broad range of highly specific flocculation mechanisms that are independent of MW and DD. However, in real wastewater systems, the diverse contributions from the different compounds in the wastewater

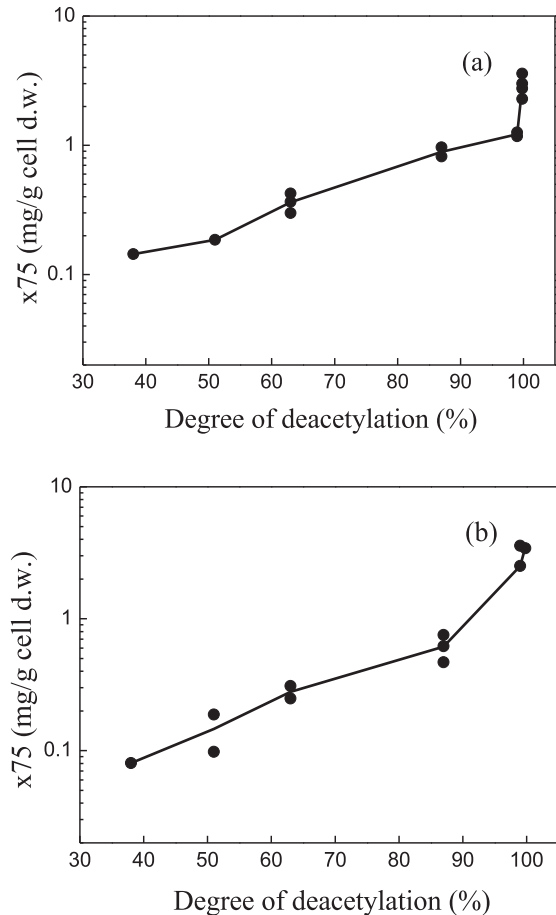


Fig. 3.6. Chitosan-HCl concentration at 75% flocculation ($\times 75$) as a function of the degree of deacetylation, after 24 h of sedimentation. Bacteria ($3-4 \times 10^9$ cells/mL) were resuspended in PBS pH 6.8 (a) and acetate/NaCl pH 5.0 (b) with ionic strength 0.1 mol/L (Strand et al., 2001b).

result in complex interactions that remain to be dissected through in-depth investigations.

3.1.2.3. Bacterial suspensions. The efficient treatment of biological colloids, such as bacteria, is required in many biotechnological processes, including separation and downstream processing. Studies of the ability of chitosan-based flocculants to flocculate various species of bacteria from water are date back to the 1980s (Eriksson and Hardin, 1987; Lazarenko et al., 1986; Medvedev et al., 1985). Hughes et al. reported that chitosan was effective in flocculating and separating *Escherichia coli* and *Bacillus subtilis* from broth by hydrogen bonding between chitosan and cell surface polymers, in addition to electrostatic interactions (Hughes et al., 1990).

Hydrogen bonding as the main non-electrostatic interaction promoting the flocculation of *E. coli* cells by chitosan was confirmed by Agerkvist (Agerkvist, 1992) in a study of the benefits of urea addition to the test system. The author tested four different chitosan preparations with different DDs and MWs in the selective flocculation of *E. coli* cell debris generated during β -galactosidase purification. High-MW chitosan produced large and shear-resistant flocs whereas low-MW forms resulted in small and shear-sensitive flocs; the former was ascribed to "non-equilibrium" bridging effects. In that study, changes in the MW only slightly affected the chitosan dose, although the flocculation window was much broader with low-MW than with high-MW polymers. By contrast, the dose was significantly influenced by the charge density on the chitosan

backbone, as a decrease in the DD from 93% to 39% increased the optimal flocculation dose by 100–150%. Rehn et al. similarly found that the flocculation of *E. coli* cells with high-MW and high-DD chitosan yielded good floc stability and rapid sedimentation (Rehn et al., 2013).

Strand et al. investigated various chitosan preparations with different MWs and with DDs ranging from 38 to 99.8%, in their small-scale assay of the flocculation of *E. coli* suspensions, performed in tubes (Strand et al., 2001b). In agreement with other studies (Agerkvist, 1992; Rehn et al., 2013), their results showed that the coagulation of *E. coli* was improved with increasing MW (Fig. 3.5). Flocculation occurred over a wide MW range, even at a degree of polymerization much lower than 200. However, the effects of DD were the opposite based on Fig. 3.6, as an increase in the fraction of acetylated units resulted in better flocculation efficiency and lower chitosan doses. The decrease in DD from 99.8% to 40% allowed a dose reduction of approximately 10-fold, at both pH 5 and pH 6.8. The mechanisms were suggested to be related to contributions from segment-segment attraction, due to the presence of the acetyl units, and/or specific interactions between the *N*-acetylglucosamines of chitosan and certain surface components of *E. coli* (Strand et al., 2003). The authors also investigated the effects of pH and ionic strength. There was little pH effect in the range of pH 4–7.4, whereas at pH > 7.4 a higher chitosan concentration was required, in part because of the polymer's insolubility under weakly alkaline conditions. An increase in ionic strength enabled a decrease in the effective chitosan concentration and led to a broadening of the flocculation range attributable to decreased electrostatic repulsions among the double layers of the bacterial colloids at higher ionic strengths (Strand et al., 2001b).

In another study (Strand et al., 2002), three types of well-characterized chitosans with various MWs and DDs were used to flocculate eight different gram-positive and gram-negative bacterial species. The authors found that gram-negative bacteria had the highest flocculation efficiencies with the lowest DD (51%) chitosan sample in their measured range, while, for the three gram-positive species, highly charged chitosan type with DD approximately 99% seemed to be better. They could not obtain the evidence of a correlation between the surface charge or hydrophobicity of the bacteria and the optimal structural characteristics of chitosan. The authors concluded that purely electrostatic interactions do not play a dominant role in the flocculation of gram-negative bacteria and the presence of GlcNAc residues on chitosan was clearly beneficial. It was therefore suggested that, in studies of bacterial flocculation mechanisms, investigation of the surface morphology of the target bacteria, especially at the nanometer scale, would be more productive than classical determination of surface charges and hydrophobicity.

Kaseamchochoung et al. studied the influence of the DD and MW of chitosan on the flocculation of anaerobic sludge in a granulation process which is significant for the performance of upflow anaerobic sludge bed systems (Kaseamchochoung et al., 2006). The key bacterial populations were acetogenic bacteria and *Methanosaeta* species. Chitosan with an 85% DD was shown to be more effective than chitosan with a 70% DD at all studied pH values, but the enhancement of flocculation was greater with low-MW than with high-MW chitosan. The efficiency of chitosan in flocculating bacterial suspensions may thus depend on the type of bacteria. An additional consideration is that anaerobic granulation is a complex process in which not only physical but also various biological and microbiological factors are involved.

The flocculation of bacterial colloids may destroy the cells directly since the normal nutrients transfer has been inhibited after chitosan adsorption on the cell wall (Goy et al., 2009). However, the detailed synergistic mechanisms still need to further study. In fact,

chitosan, especially chitosan oligomers, has good antimicrobial effects. These are also a function of the structural characteristics of chitosan (Goy et al., 2009; Rabea et al., 2003; Yalpani et al., 1992). In their sterilization by chitosan, gram-positive and gram-negative bacteria have opposing MW dependencies (Zheng and Zhu, 2003), whereas the antimicrobial action of chitosan is usually better with increasing DD. Nonetheless, the influence of the DD on antimicrobial activity is weaker than that of MW (Goy et al., 2009).

In summary, the final performance of chitosan in the flocculation of different target wastewaters containing inorganic, organic, and bacterial pollutants or a mixture thereof is clearly related to its structural characteristics. But, as pointed out for organic pollutants, the relationship between structural factors (DD and MW) and the flocculation of bacterial suspensions by chitosan is an irregular one, given the diverse structural characteristics and abundant functional groups of bacterial cells (Strand et al., 2001b). In these cases, flocculation will predominantly involve some highly specific forces between the chitosan flocculants and the bacteria. Systematic investigations of the surface structure of common bacterial contaminants, especially at the molecular level, are needed to better understand the mechanisms of flocculation and thus to improve the performance of chitosan-based flocculants.

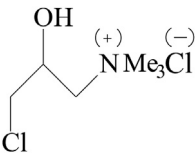
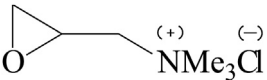
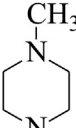
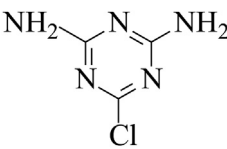
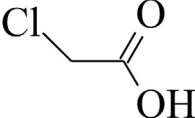
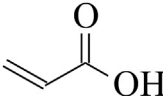
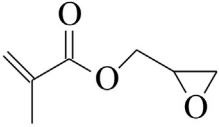
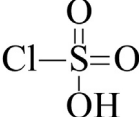
3.2. Modified chitosan flocculants

As mentioned above, although chitosan has been used directly as a flocculant, its low MW, inactive chemical properties, and poor water solubility (Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Rinaudo, 2006) considerably weaken its efficiency. To further improve its performance, chemically modified forms have been synthesized (Dao et al., 2016; Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Prashanth and Tharanathan, 2007; Rinaudo, 2006; Yang et al., 2011a; Zhang, 2006) by taking advantage of the abundance of the free amines and hydroxyl groups on the chitosan backbone to introduce various functional groups. These modified forms exhibit improved water solubility, MW ranges, charge density, and multi-functionality, allowing their use in a much wider range of applications (Jiang, 2001; Muzzarelli and Muzzarelli, 2005; Rinaudo, 2006).

It is a significant strategy to chemically modify chitosan on the basis of the characteristics of the target pollutants and structure-activity relationships, e.g., by introducing certain functional groups (Jiang, 2001; Rinaudo, 2006; Yang et al., 2011a). In the case of most inorganic suspended particles and many organic pollutants, their negative surface charge has been targeted by introducing cationic functional groups, such as quaternary ammonium salts, onto the chitosan backbone. The enhanced charge neutralization effects have resulted in a more efficient flocculation performance.

Among the methods used to modify chitosan, the most common are etherification/amination (Bratskaya et al., 2009; Chang et al., 2009; Li et al., 2004a; Yang et al., 2011b), graft copolymerization (Laue and Hunkeler, 2006; Wang et al., 2008; Yuan et al., 2010), acylation (Hsien and Rorrer, 1995; Lee et al., 2005), esterification (Jiang, 2001; Rinaudo, 2006), and oxidation (Jiang, 2001; Rinaudo, 2006). In applications of chitosan-based flocculants in wastewater treatment, the two main chemical modification methods are etherification/amination (Bratskaya et al., 2009; Chang et al., 2009; Li et al., 2004a; Yang et al., 2011b) and graft copolymerization (Laue and Hunkeler, 2006; Wang et al., 2008; Yuan et al., 2010). Thus, in the following section we describe both modification techniques, focusing on the conditions used in their preparation and methods to control their structural properties, including the degree of substitution by various functional groups and the grafting ratio. We then conclude this section by considering the effects of these modifications on the flocculation efficiencies in various target

Table 3.3
Examples of modifying agents used in etherification/amination reactions for preparation of chitosan-based flocculants.

Types	Modifying agents	Structure	References
Cationic groups	3-Chloro-2-hydroxypropyl trimethyl ammonium chloride (CTA)		(Ali et al., 2010; Ali and Singh, 2009b; Antonopoulou et al., 2013; Cai et al., 2010; Dong et al., 2014; Huang et al., 2013; Lu et al., 2011; Yang et al., 2011b; Yang et al., 2012b)
	2, 3-Epoxypropyl trimethyl ammonium chloride (ETA)		(Cheng et al., 2013; Li et al., 2004a; Li et al., 2004b; Lin et al., 2012; Liu et al., 2011; Rojas-Reyna et al., 2010; Zhang et al., 2010b)
	N-methyl piperazine		(Dharani and Balasubramanian, 2015)
	2,4-bis(dimethylamino)-6-chloro-(1,3,5)-triazine		(Jia et al., 2016)
Carboxyalkyl groups	Monochloroacetic acid		(Cai et al., 2010; Chen and Park, 2003; Chen et al., 2006; Hebeish et al., 2006; Li et al., 2010; Lu et al., 2011; Mourya et al., 2010; Sun et al., 2008; Yang et al., 2011b; Yang et al., 2012b; Yang et al., 2013a; Yang et al., 2014a)
	Acrylic acid		(Bratskaya et al., 2009; Pestov et al., 2007; Zhang et al., 2014b)
Others	Glycidyl methacrylate		(Jiang et al., 2011)
	Chlorosulfonic acid		(Rios-Donato et al., 2012)

wastewaters.

3.2.1. Etherified/N-alkylated chitosan flocculants

3.2.1.1. Etherification/amination. Etherification/amination are common and simple methods for introducing functional groups onto chitosan, via its abundant free hydroxyl and amine groups, respectively. The proton in the $-OH$ or $-NH_2$ of chitosan is activated and then can be displaced via a nucleophilic reaction with the modifying agents summarized in Table 3.3. Etherification consists of the transformation of an $-OH$ group to form a $-C-O-C-$ structure, yielding etherified chitosan. Amination involves the substitution of $-NH_2$ with a $-C-N-C-$ structure, yielding N-alkylated chitosan. In most cases, the reactivity of $-NH_2$ is higher than that of $-OH$ (Cheng et al., 2013; Jiang, 2001; Li et al., 2004a, 2004b; Lin et al., 2012; Liu et al., 2011; Rinaudo, 2006; Rojas-Reyna et al., 2010; Zhang et al., 2010b). Among the various hydroxyl groups on the glucosamine ring of chitosan, C6-OH is the most reactive, because of the reduced steric hindrance and higher electronegativity of its oxygen. Thus, etherification reaction mostly

take place on C6-OH groups (Jiang, 2001; Lu et al., 2011; Rinaudo, 2006; Yang et al., 2011b, 2012b, 2013a, 2014a).

Table 3.3 lists several commonly used modifying agents, most of which are either positively or negatively charged. The former include 3-chloro-2-hydroxypropyl trimethylammonium chloride (CTA), 2, 3-epoxypropyl trimethyl ammonium chloride (ETA), etc. These cationic groups are generally introduced onto chitosan to enhance its charge neutralization effects. CTA can be easily converted to ETA under alkaline treatment (Ali and Singh, 2009b; Ali et al., 2010). The negatively charged agents are those that add carboxyalkyl (carboxymethyl, carboxyethyl, and carboxybutyl) groups to improve the water solubility and chelating effects of chitosan in wastewater containing high concentrations of positively charged pollutants. In addition, besides containing-oxygen organic acids, others such as sulfate could be also introduced onto chitosan by similar methods. However, the types of modifying agents used in actual applications listed in Table 3.3 are still limited due to complicated synthesis techniques and increased cost.

An example of the preparation of a quaternary chitosan using

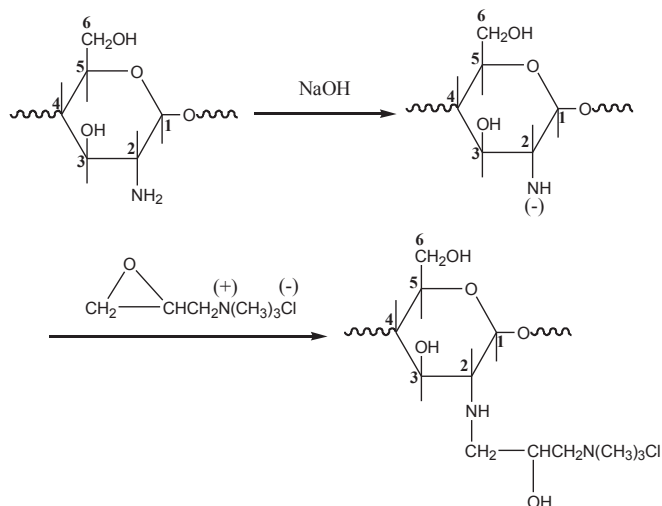


Fig. 3.7. Details of an amination reaction.

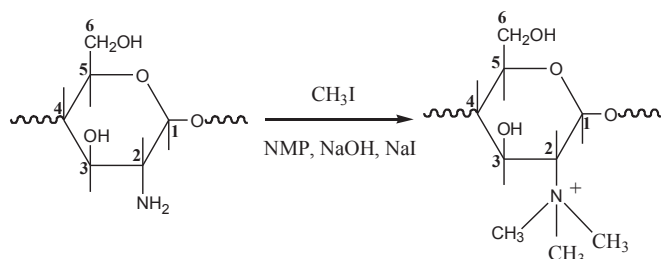


Fig. 3.8. Direct amination.

CTA as the modifying agent is shown in Fig. 3.7. After activation by a strong base, i.e. sodium hydroxide, the H^+ in the nucleophilic center of $-NH_2$ is displaced by $-CH_2CH(OH)CH_2N^+(CH_3)_3$. Quaternary ammonium salts can be also formed directly from the amino groups of chitosan in the special type of amination reaction (Li et al., 2010) shown in Fig. 3.8.

The introduction of negatively charged carboxyl groups onto the chitosan backbone is most frequently achieved using monochloroacetic acid as the modifying agent. In addition, carboxyethyl chitosan has been synthesized using acrylic acid under different conditions (Bratskaya et al., 2009; Pestov et al., 2007; Zhang et al., 2014b). Among the acidic groups that can be added, chitosan sulfate was synthesized using chlorosulfonic acid as the modifying agent (Rios-Donato et al., 2012). The complex mixture of chlorosulfonic acid and dimethyl formamide (DMF) was dissolved in toluene and then added to pretreated chitosan in an etherification reaction. The obtained chitosan sulfate product has been used in the

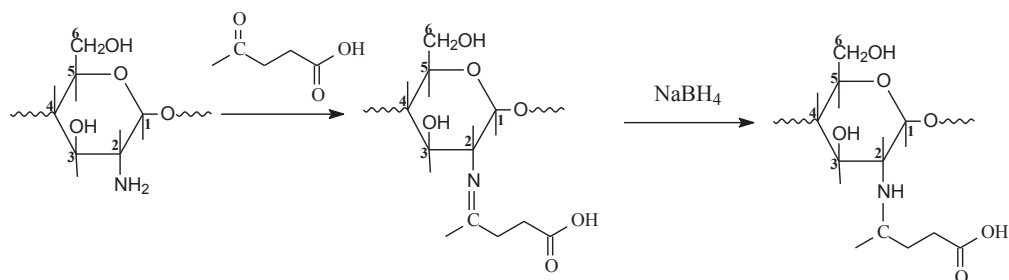


Fig. 3.9. Preparation of *N*-carboxybutyl chitosan.

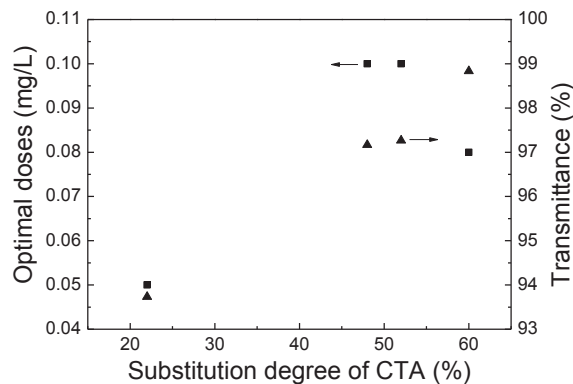


Fig. 3.10. Optimal doses of various CMC-CTA samples (■) and their corresponding transmittance (▲) of the supernatant as a function of the substitution degree of CTA (Yang et al., 2012b).

coagulation–flocculation of colloidal suspensions of kaolinite, bentonite, and alumina.

Chitosan also readily reacts with aldehydes or ketones to form Schiff's bases under neutral conditions. After the reduction of chitosan by sodium borohydride, many useful *N*-alkylated products can be easily synthesized (Jiang, 2001; Muzzarelli et al., 1982; Muzzarelli and Muzzarelli, 2005; Rinaudo, 2006). For example, *N*-carboxybutyl chitosan is prepared using levulinic acid as the modifying agent (Fig. 3.9) (Delben et al., 1994; Muzzarelli et al., 1989; Stefanchich et al., 1994).

3.2.1.2. Effects of the degree of functional group substitution on wastewater flocculation. The degree of functional group substitution in chitosan-based flocculants is a very important structural factor affecting the final flocculation performance. Accordingly, during etherification/amination reactions, the dose of the modifying agent, the alkalization time, alkalization temperature, and the proportions of alkali and solvents in the reaction medium can influence the final structure of the chitosan derivatives. Among them, adjusting the DS of introduced functional groups can be easily accomplished by carefully controlling the mass feed ratio of chitosan and the modifying agent (Huang et al., 2013; Li et al., 2004b; Lu et al., 2011; Yang et al., 2012b).

3.2.1.2.1. Chitosan-CTA flocculants. As mentioned above, CTA or ETA modified chitosan (chitosan-CTA) is a kind of popular quaternary chitosan flocculants. Li et al. prepared a series of chitosan-CTA flocculants differing in their degrees of CTA substitution and MWs using ETA as the modifying agent (Li et al., 2004a). The effects of the degree of CTA substitution and the MW on the adsorption and flocculation properties of the quaternary chitosans were then studied for potential retention-aids with respect to $CaCO_3$ fillers in alkaline papermaking. The optimum flocculation concentration

Table 3.4

The flocculation experimental results by different CMC-CTA flocculants at the pilot scale (Yang et al., 2012b).

Sample names	Substitution degree of CTA (%)	Water temperature (°C)	Average turbidity (NTU)		
			Before sand filtration	After sand filtration	Raw water
CMC-CTA1	22.0	23.1	8.79	1.32	44.8
CMC-CTA 2	48.0	23.3	7.78	0.89	36.8
CMC-CTA 3	52.0	22.5	6.68	0.71	40.7
CMC-CTA 4	60.0	22.6	5.03	0.47	43.2

decreased with the increasing degrees of CTA substitution. Increasing the charge density of the polyelectrolyte strengthened the electrostatic interactions between quaternary chitosans and negatively charged CaCO_3 particles; thereby improving CaCO_3 flocculation. However, higher-MW chitosan-CTA was less efficient. Charge patching was suggested to be the predominant mechanism of CaCO_3 flocculation by quaternary chitosans.

Ali et al. also evaluated the effects of CTA substitution and MW on the flocculation performance of CTA-modified chitosan (Ali et al., 2010). They prepared a series of chitosan-CTA polymers with different degrees of CTA substitution and different MWs by altering the molar ratio of CTA to chitosan in the reaction mixtures. They then compared the efficiency of the various chitosan-CTA preparations vs. native chitosan in the flocculation of kaolin and iron-ore powder suspensions. The results indicated that not all of the modified chitosans had a better flocculation performance than native chitosan. Among the different chitosan-CTAs, those with a moderate MW and a moderate charge density had the best flocculation performance against either of the model suspensions. The authors concluded that both polymer bridging and charge neutralization contributed to the flocculation performance. The differences between the results of Li et al. (Li et al., 2004a). And those of Ali (Ali et al., 2010) can be ascribed to the different characteristics of the treated wastewaters. In addition, the linear charge density of chitosan-CTAs with a much higher degree of CTA substitution would be lower, according to counterion condensation theory (Manning, 1974; Oosawa, 1971), resulting in a reduction of the flocculation efficiency.

Yang et al. successively modified chitosan with CTA and monochloroacetic acid in typical etherification and amination reactions (Yang et al., 2011b, 2012b). A series of amphoteric chitosan-based flocculants (CMC-CTA) with different degrees of CTA substitution but a consistent content of carboxymethyl groups was obtained by controlling the mass feed of CTA to chitosan. The degree of CTA substitution of the final products increased with an increase of the mass feed of CTA, before gradually reaching a plateau. CMC-CTA with a higher CTA content had a better flocculation performance and the dose requirement was lower when the flocculants were tested both at laboratory scale using a kaolin suspension (Fig. 3.10) and in a pilot-scale study using raw water from the Zhenjiang part of the Yangtze River of China (Table 3.4). Moreover, the rate

constant (k) calculated from a flocculation kinetics model was shown to increase as the degree of CTA substitution increased, due to the more efficient charge neutralization and bridging flocculation effects conferred by the larger number of cationic ions on the chain backbone of CMC-CTA (Yang et al., 2012b). Furthermore, we obtained similar findings in two other systems: CTA-modified chitosan (chitosan-CTA) (Huang et al., 2013) and CTA-modified chitosan-graft-polyacrylamide (chitosan-CTA-g-PAM) (Lu et al., 2011), discussed below. Our results showed that chitosan-based flocculants with a higher cationic content have better water solubility, higher flocculation performance, and a lower dose requirement.

3.2.1.2.2. Other modified chitosans. Chitosan can also be modified by the addition of anionic groups to improve the flocculation of positively charged pollutants. Bratskaya et al. prepared a series of *N*-carboxyethylated chitosans (CECs) in which the DS of carboxyethyl groups ranged from 0.7 to 1.6 per glucosamine unit of chitosan (Bratskaya et al., 2009). The polymers were used to flocculate positively charged heavy metals hydroxides at $\text{pH} > 7.5$, i.e., higher than their isoelectric points. The flocculation performance of these CECs was highly dependent on the pH of the medium and the DS of the CEC derivatives. The higher the pH and the DS, the narrower the flocculation window and the stronger the negative effect of flocculant overdosing on heavy metal removal. According to the estimated floc settling rates and the residual metal concentrations, the optimal DS of the CEC derivatives in the precipitation of metal hydroxides was 0.7–1.0. Moreover, flocculation by the CEC derivatives rather than chelating was the predominant mechanism of metal removal under the tested conditions, as evidenced by the strong correlation between the electrokinetic properties of the metal colloids and the efficiency of their removal (Bratskaya et al., 2009).

In addition to the DS and MW, the fine structure of the functional groups introduced onto the glucosamine ring of chitosan has been studied with respect to the flocculation efficiency. Zhao et al. obtained two phosphorylated chitosan derivatives with a similar DS (~0.5 per glucosamine unit of chitosan) but different structures, i.e., *O*-phosphorylated chitosan (OPC) and *N*-methylene phosphonic chitosan (NMPC) (Zhao et al., 2012). The compounds were synthesized, respectively, by the esterification of the hydroxyl group of chitosan using P_2O_5 in methanesulfonic acid and by the

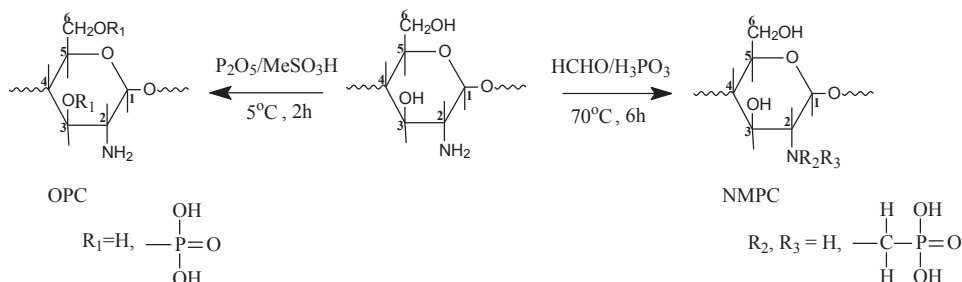
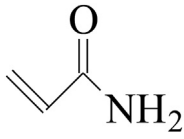
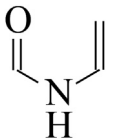
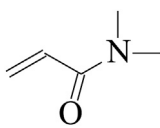
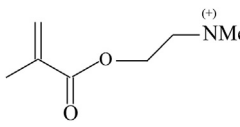
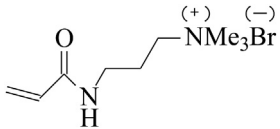
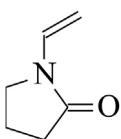
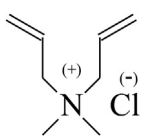
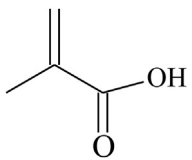
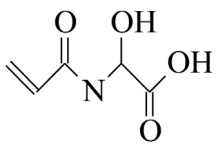
**Fig. 3.11.** Preparation of *O*-phosphorylated and *N*-phosphorylated chitosans (Zhao et al., 2012).

Table 3.5
Examples of monomers used in graft copolymerization reactions for preparation of chitosan-based flocculants.

Monomer type	Monomers	Structure	Reference
Non-ionic	Acrylamide		(Ali and Singh, 2009a; Jia, 2012; Laue and Hunkeler, 2006; Lu et al., 2011; Pal et al., 2012; Wang et al., 2008; Wang et al., 2011; Wang et al., 2012; Yang et al., 2011b; Yang et al., 2012c; Yuan et al., 2010; Zhang et al., 2010a; Zhang et al., 2012; Zhu et al., 2012)
	<i>N</i> -vinyl formamide		(Mishra et al., 2008)
	<i>N,N</i> -dimethylacrylamide		(Tripathy et al., 2010)
Cationic	(2-Methacryloyloxyethyl) trimethyl ammonium chloride		(Laue and Hunkeler, 2006; Jiang et al., 2011; Wang et al., 2007; Wang et al., 2009; Wang et al., 2011; Wang et al., 2012; Yang et al., 2014a; Yang et al., 2014b; Zhu et al., 2012)
	3-(Acrylamide)propyl trimethylammonium bromide		(Serita, 1995)
	<i>N</i> -vinyl-2-pyrrolidone		(Srivastava et al., 2010)
Anionic	diallyl dimethyl ammonium chloride		(Yang et al., 2015)
	(Meth)acrylic acid		(Izvozhikova et al., 2003; Mishra et al., 2009)
	2-Acrylamidoglycolic acid		(Yadav et al., 2012)

selective incorporation of methylene phosphonic groups on the amino groups of chitosan using formaldehyde/H₃PO₃ in water (Fig. 3.11). In experiments measuring turbidity removal from kaolin suspensions, the removal efficiency of OPC was much higher than that of NMPC (~90% vs. 60%). The lower flocculation efficiency of NMPC was attributed to the decreased acid strength of its *N*-methylene phosphate groups. These results highlighted the importance of the acidity of the phosphate groups introduced onto the modified chitosan in determining flocculation efficiency.

The inconsistent effects of the DS on the flocculation performance of etherified or *N*-alkylated chitosan flocculants can be

attributed to several factors such as the different characteristics of various wastewaters and the fine structures of flocculants (Ali et al., 2010; Li et al., 2004a). Generally, increasing the DS within a suitable range can improve the flocculation of pollutants containing opposite surface charges, via enhanced charge neutralization effects. An additional consideration is the effects of the distribution of introduced functional groups on the chitosan backbone. These have not been fully investigated because of the difficulty in accurately determining and characterizing the fine structures of modified chitosan at the molecular level.

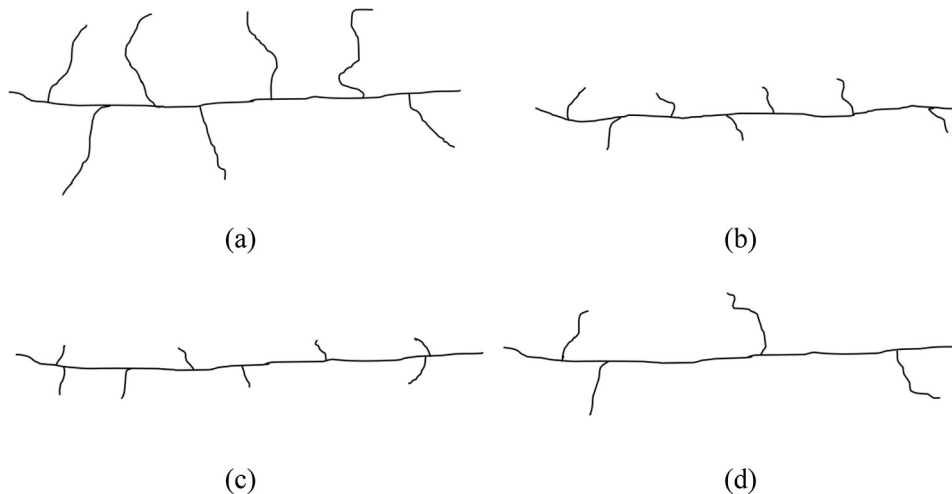


Fig. 3.12. Different morphologies of graft copolymers. (a) Longer and (b) shorter but similar number of grafted chains prepared by high and low concentrations of monomers, respectively, but a constant amount of chitosan and a constant irradiation/initiator dose. (c) Shorter but more numerous and (d) longer but fewer grafted chains obtained by a high and low total irradiation/initiator dose, respectively, but a constant amount of chitosan and a constant monomer dose.

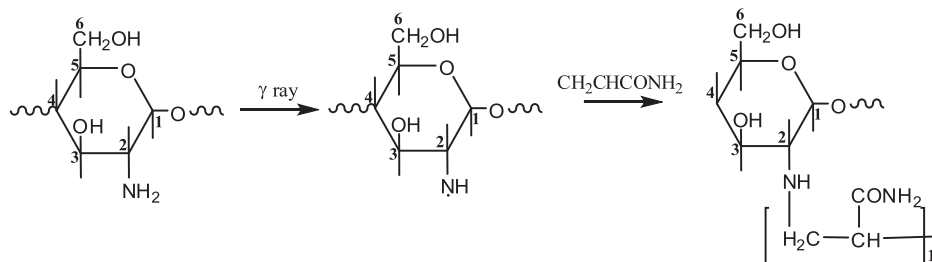


Fig. 3.13. Graft copolymerization of chitosan with acrylamide (Wang et al., 2008).

3.2.2. Grafted chitosan flocculants

3.2.2.1. Graft copolymerization. Graft copolymerization is a rapidly advancing field in the chemical modification of polymers (Ceresa, 1973; Flory, 1953). Grafting consists of the introduction of synthetic functional polymers as side chains on the polymer backbone, thus enabling various molecular designs.

Chitosan-grafted copolymers have been widely used in wastewater treatment (Lee et al., 2014; Wang et al., 2008; Yang et al., 2014a; Yuan et al., 2010). A graft copolymerization reaction for chitosan usually involves three steps: the dissolution of chitosan into a homogenous aqueous solution, initiation of the reaction, and grafting after feeding of the desired amount of the selected monomer. The whole reaction is performed under an inert gas atmosphere. The dissolving medium is typically a dilute acidic medium, such as acetic acid or hydrochloric acid, and the graft reaction is initiated by radiation, either gamma ray or microwave (Singh et al., 2012), or ceric ammonium nitrate (CAN) and persulfates. The monomers that can be fed into the system may be nonionic, cationic, or anionic. Several examples are listed in Table 3.5.

The nonionic monomer most commonly grafted onto chitosan is acrylamide, since the utility of polyacrylamides (PAMs) in water purification is well-established. These chitosan-graft-polyacrylamides (chitosan-g-PAMs) are discussed in detail in Sect. 3.2.2.2.1. However, because the majority of pollutants are negatively charged, the most commonly grafted chitosans bear cationic groups, such as quaternary ammonium salts, for improved charge neutralization effects. Moreover, anionic groups are also introduced via etherification/amination reactions, as discussed above, and using graft copolymerization. The resulting modified chitosans by anions have improved charge density, greater intramolecular

electrostatic repulsion properties, a more extended conformation, and enhanced bridging flocculation effects. Thus anionic chitosan-based flocculants exhibit better water solubility and chelating effects in wastewater mostly containing positively charged pollutants.

3.2.2.2. Effects of grafting ratio on flocculation of various wastewater. The properties of graft copolymers are mostly determined by their structural features, such as the grafting ratio, charge density, and the length and number of the attached polymer chains, which are affected by the synthesis conditions, especially the total irradiation, the initiator dose, and the amount of the fed monomer. Graft copolymerization is thus one of the most attractive approaches for constructing versatile molecular environments (Ceresa, 1973; Rinaudo, 2006). At constant amounts of chitosan and irradiation/initiator dose, the grafting ratio usually increases with increasing amounts of monomers (Wang et al., 2007; Yuan et al., 2010). When both the amount of chitosan and the monomer dose are held constant, the graft copolymers contain fewer but longer side chains when the total irradiation dose or initiator concentration is reduced (Ali and Singh, 2009a). Fig. 3.12 shows the different morphologies of the graft copolymers prepared under the various conditions.

3.2.2.2.1. Chitosan-g-PAM flocculants. As noted in Sect. 3.2.2.1, chitosan-g-PAM are widely used, nonionic grafted chitosan flocculants. Wang et al. synthesized chitosan-g-PAM initiated by gamma ray in acid-water solution (Fig. 3.13) and investigated the effects of acetic acid concentration, total irradiation dose, dose rate, and monomer concentration on the grafting percentage (Wang et al., 2008). The results showed the negligible effect of the acetic acid concentration whereas the grafting ratio increased with

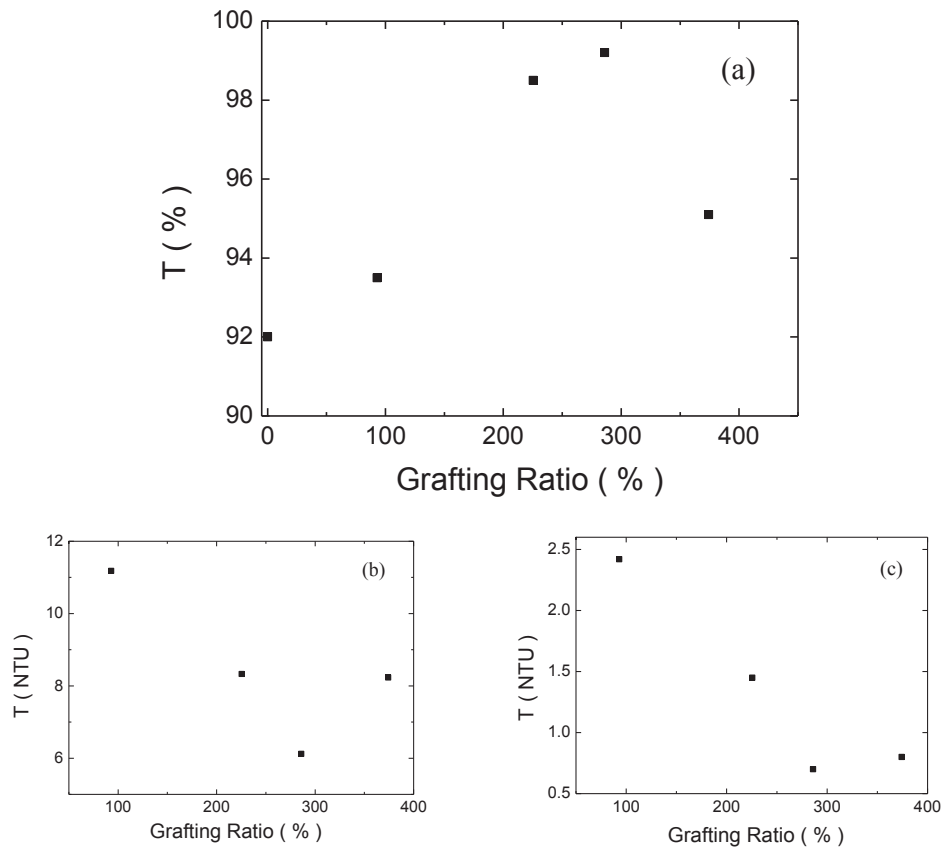


Fig. 3.14. The effects of grafting ratio of various chitosan-g-PAM samples on the flocculating properties, (a) in a laboratory-scale study using a kaolin suspension and (b, c) in a pilot-scale study of raw water from the Zhenjiang part of the Yangtze River of China (b) before and (c) after sand-filtrating (Yuan et al., 2010; Zhang et al., 2010a).

Table 3.6

Comparison of the flocculation performance of the different chitosan-based flocculants, in both laboratory- and pilot-scale studies.

Flocculant (reference)	Chain architecture	DS with CTA ^a (%)	DS with carboxymethyl groups ^a (%)	Grafting ratio of PAM ^b (%)	Laboratory scale ^c		Pilot scale ^d	
					Optimal dose (mg/L)	TRE ^e (%)	Optimal dose (mg/L)	TRE ^f (%)
Chitosan-g-PAM (Yuan et al., 2010; Zhang et al., 2010a)		—	—	286	0.10	93.2	1.0	90.1
Chitosan-CTA-g-PAM (Lu et al., 2011)		44.7	—	286	0.10	92.1	1.3	90.0
Chitosan-CTA (Huang et al., 2013)		89	—	—	0.05	93.3	1.1	90.5
Carboxymethyl chitosan-CTA (Yang et al., 2011b; Yang et al., 2012b)		60	48.3	—	0.80	90.4	1.1	88.4

^a DS (%) = $W_{\text{substituent}}/W_{\text{chitosan}} \times 100\%$; here, $W_{\text{substituent}}$ and W_{chitosan} are the mass weights of the substituent and chitosan, respectively.

^b Grafting ratio (%) = $W_{\text{grafted chain}}/W_{\text{chitosan}} \times 100\%$; here, $W_{\text{grafted chain}}$ and W_{chitosan} are the mass weights of the grafted chain and chitosan, respectively.

^c The initial turbidity of synthetic water (kaolin suspensions) is 75 NTU (measured at 25 °C and pH 7.0).

^d The initial turbidity of raw water (the Zhenjiang portion of the Yangtze River of China) is between 20 and 60 NTU depending on when it is measured.

^e Turbidity removal efficiency (TRE, %) = $(T_{\text{untreated}} - T_{\text{treated}})/(100 - T_{\text{untreated}}) \times 100\%$; here, $T_{\text{untreated}}$ and T_{treated} are the transmittances of untreated and treated water, respectively.

^f Turbidity removal efficiency (TRE, %) = $(T_{\text{raw}} - T_{\text{treated}})/T_{\text{raw}} \times 100\%$; here, T_{raw} and T_{treated} are the turbidity of raw water and water treated by flocculation but before sand filtration, respectively.

increasing total irradiation dose. A lower irradiation dose rate improved the grafting ratio at a fixed total irradiation dose, whereas a higher monomer concentration resulted in a higher grafting ratio. The authors found that chitosan-g-PAM was better than chitosan alone and that chitosan-g-PAM efficiently flocculated kaolin suspensions, especially under alkaline conditions. They also demonstrated shorter settling rates at various pH values when chitosan-g-

PAM was synthesized with a grafting ratio of 166% vs. 56%.

Ali and Singh also prepared chitosan-g-PAM in acid–water solution but used CAN as the initiator (Ali and Singh, 2009a). They obtained various chitosan-g-PAM preparations with different grafting ratios and distributions of the grafted chain by controlling the amounts of CAN feeding and acrylamide. In flocculation experiments in which kaolin, iron ore, silica, and bentonite power

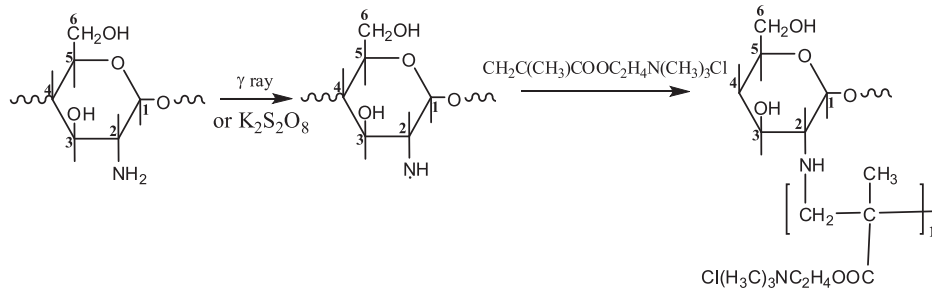


Fig. 3.15. Graft copolymerization of chitosan with a cationic monomer (2-methacryloyloxyethyl) trimethyl ammonium chloride (DMC).

suspensions were evaluated in settling and jar tests, the chitosan-g-PAM flocculants with the fewest but longest PAM side chains and the highest intrinsic viscosity showed the best settling performance and were able to bridge the largest number of colloidal particles before their flocculation.

We prepared a series of chitosan-g-PAM also using CAN as the initiator (Yuan et al., 2010; Zhang et al., 2010a). In our system, the grafting ratio increased with increasing acrylamide monomer concentrations. The effects of the grafting ratio on the efficiency of flocculation were investigated in a laboratory-scale study using a kaolin suspension and in a pilot-scale study of raw water from the Zhenjiang part of the Yangtze River of China shown in Fig. 3.14. The addition of branched PAM chains efficiently improved bridging flocculating but shielded the cationic groups on the chitosan backbone, thus reducing charge neutralization effects. However, the final flocculating effects reflected the cooperation of these two mechanisms. These results demonstrate the importance of a proper grafting ratio in the preparation of chitosan-g-PAM flocculants with optimal flocculating properties.

Table 3.6 compares the flocculation performance of four chitosan-based flocculants differing in their chain architectures and charges: chitosan-g-PAM (Yuan et al., 2010; Zhang et al., 2010a), chitosan-CTA-g-PAM (Lu et al., 2011), chitosan-CTA (Huang et al.,

2013), and carboxymethyl chitosan-CTA (CMC-CTA) (Yang et al., 2011b, 2012b). In the laboratory-scale experiments, chitosan-CTA, with its high positive charge density, showed the best flocculation efficiency, the lowest optimal dose and the highest turbidity removal efficiency (TRE), indicating the importance of charge neutralization effects in the purification of a kaolin suspension. However, in the pilot scale experiments using raw water, there was little difference in the flocculation efficiencies of the four flocculants. This result probably reflects the more complicated mixture of compounds present in the water samples but also their efficient removal by all of the tested flocculants. Although the grafted PAM chains on chitosan-g-PAM and chitosan-CTA-g-PAM partially shield the positive charges distributed on their chain backbones, the branch structure of polymeric flocculants results in enhanced bridging effects. As for the amphoteric flocculant of CMC-CTA containing both cationic and anionic groups, it may more suit the real water due to its notably improved solubility and salt-resistance in the wide pH range.

3.2.2.2.2. *Chitosan-g-PDMC flocculants.* As discussed above, quaternary ammonium salts can be grafted onto chitosan to improve its positive charge density. Wang et al. grafted the cationic monomer 2-methacryloyloxyethyl trimethyl ammonium chloride (DMC) onto chitosan using gamma ray initiation (chitosan-g-

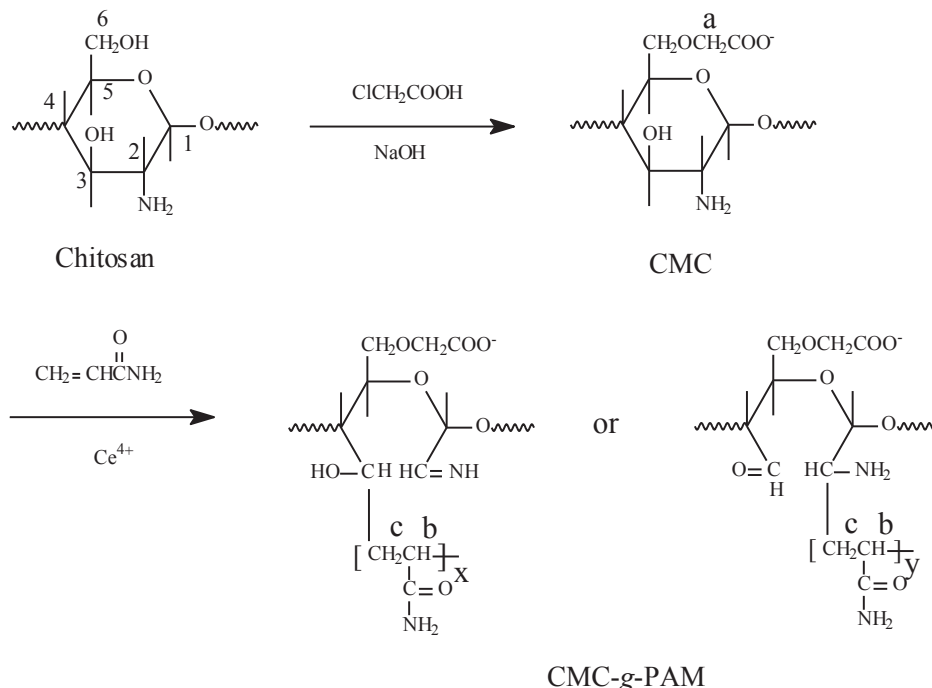


Fig. 3.16. Preparation of carboxymethyl chitosan-graft-polyacrylamide (CMC-g-PAM) (Yang et al., 2012c).

PDMC; Fig. 3.15) (Wang et al., 2007). A systematic investigation of the effects of the total irradiation dose, dose rate, monomer concentration, and temperature on the grafting ratio showed that the grafting ratio increased with an increasing total irradiation dose up to 450 Gy and with increasing reaction temperature. An increase in the monomer concentration increased the availability of DMC molecules to react with the chitosan macroradicals, leading to an increase in the grafting ratio.

The same research group also tested potassium persulfate as an initiator in the synthesis of chitosan-g-PDMC (Wang et al., 2009). Temperature and monomer concentration were shown to be the most important factors influencing the graft percentage. The water solubility of chitosan-g-PDMC increased with increasing grafting ratio and the flocculant was completely dissolved in water when the grafting ratio exceeded 236.4%. This chitosan-g-PDMC with a higher grafting ratio showed better flocculation in acidic, neutral, and alkaline conditions when used to treat pulp mill wastewater (Wang et al., 2009). Serita prepared grafted chitosan flocculants using 3-(acrylamide)propyl trimethylammonium bromide and tested their efficiency in the flocculation of a 5% kaolin suspension. The flocculating ability of the graft copolymers increased with increasing graft chain length and graft ratio (Serita, 1995).

3.2.2.2.3. Other modified chitosans. Graft copolymerization and etherification/amination reaction can be applied in succession to chemically modify chitosan by introducing various functional groups. Yang et al. used this combined approach, synthesizing carboxymethyl chitosan (CMC) onto which they grafted PAM (Fig. 3.16), yielding the novel, amphoteric, chemically bonded composite flocculant CMC-g-PAM (Yang et al., 2012c, 2013a). The graft reaction was initiated by CAN in acid-water. CMC-g-PAM was tested in the flocculation of both an anionic (Methyl Orange) and a cationic (Basic Bright Yellow) dye in water (Yang et al., 2013a). The results indicated that the introduction of excess PAM chains in CMC-g-PAM increased the optimal flocculant dose, decreased the color removal efficiency, and worsened the floc properties. These findings reflected the fact that although the PAM chains improved bridging and sweeping effects, excess amounts screened the charges on the chitosan backbone and thus reduced charge neutralization by CMC-g-PAM. Therefore, the grafting ratio of PAM should be controlled such that it remains in a suitable range.

Besides a single monomer, two or more different monomers can be used to prepare bi- or multi-graft chitosan copolymer flocculants with diverse functionality (Wang et al., 2011, 2012). Wang et al. prepared a series of graft chitosan flocculants in aqueous solution using acrylamide and DMC as co-monomers and CAN as

the initiator (Wang et al., 2011). Graft chitosans containing more DMC groups had a better flocculation performance, because of the increased number of positive charges and greater charge neutralization effects.

Laue and Hunkeler prepared a series of chitosan copolymers also grafted with DMC and AM and using CAN as the initiator (Laue and Hunkeler, 2006). The flocculation performance of these grafted chitosans in the purification of model kaolin suspensions was more robust than that of PAM and completely synthetic polycations with the same charge density. This was most likely due to the long chain branches and rigid nature of the backbone, which may have provided a more extended conformation of the chitosan in solution and a higher effective charge density.

The effects of the length and number of grafted chains, the charge density, and the ionic strength on flocculation performance have been investigated quantitatively (Laue and Hunkeler, 2006). The length and number of graft chains was estimated from the number-average MW, determined by size exclusion chromatography, and the composition of the polymer by Fourier transform infrared spectroscopy. The authors created a mathematical expression linking the window of application (WA) for flocculants with the number of branches of the grafted polymer and the salt concentration. From Fig. 3.17, increasing both the number of branches and the ionic strength of the aqueous solution increased the WA. Moreover, a novel parameter (ξ), defined as the critical charge ratio for flocculation, was introduced. It was obtained from the product of the lower limit concentration of flocculants for a given WA (lower bound of the WA, LLC) and the polymer charge density (CD_p) divided by the surface charge density of the suspended particles (σ_{Kaolin}) as shown in Eq. (3.1).

$$\xi = \frac{LLC \cdot CD_p}{\sigma_{Kaolin}} \quad (3.1)$$

At the point at which flocculation begins, i.e., the LLC, this ratio of charges (ξ) is equal to or close to 1, in which case simple charge neutralization is the operative mechanism. Smaller values of this ratio indicate bridging and charge patching neutralization effects. In the authors' work (Laue and Hunkeler, 2006), ξ is $\ll 1$ at the point of incipient flocculation (LLC), which strongly implicated bridging and/or charge patching neutralization as the dominant flocculation mechanisms.

However, as is the case for the modifying agents used in the preparation of etherified/N-alkylated chitosan flocculants, the types of graft monomers used in actual applications are limited by the complicated synthesis techniques involved and the high costs. Consequently, there have been few studies on the effects of structural factors on the flocculation performance of grafted chitosan flocculants. Moreover, the flocculation properties of polymeric flocculants are determined not only by the grafting ratio but also by the distribution of the introduced chemical substituents along the chitosan backbone, which also has not been systematically investigated. Furthermore, there are far different chain architectures: linear and branching forms respectively, for etherified/n-alkylated and grafted polymeric flocculants even containing the same chemical components, which may result in distinct hydrodynamic properties in water and different flocculation performance. However, very few work related to their comparisons has been reported until now. In addition, most of the relevant studies have been qualitative rather than quantitative, due to the complicated characteristics of both the polymeric flocculants and the various contaminants in the target wastewaters.

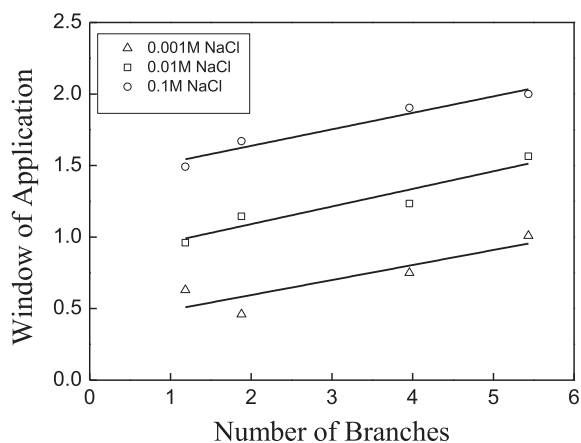


Fig. 3.17. Window of application (WA) as a function of the number of exclusive PDMC branches per chitosan chain (Laue and Hunkeler, 2006).

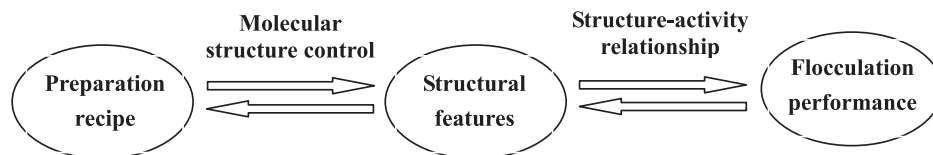


Fig. 4.1. The relationship between the preparation conditions, structural features, and flocculation performance of a flocculant.

4. Perspectives

4.1. In-depth investigations of chitosan-based flocculants

Chitosan and its derivatives are known as effective, safe, and cost-effective flocculants with applications in a wide variety of settings, including the treatment of industrial effluents as well as potable water. However, maximizing the applications of chitosan-based flocculants first requires in-depth and molecular-level investigations of their mechanisms of action with respect to their structural characteristics.

4.1.1. Various available studies

Most research into the mechanisms underlying chitosan-based flocculation and their relationship to the structural characteristics of the respective flocculants has been qualitative rather than quantitative (Laue and Hunkeler, 2006). The challenge to such studies is the difficulty in analyzing the structural features of chitosan-based flocculants, including their short- and long-range structures, and the complex interactions with the surface of the targeted pollutants. The forces involved in these interactions include covalent bonds, electrostatic interaction, hydrogen bonding, van der Waals forces, and other highly specific forces resulting in unusual flocculation mechanisms. Besides, polymer materials still own far different chain architectures, including linear, grafting/branching, and star-like forms, resulting in diverse application performance (Elias, 1984; Flory, 1953; Sperling, 2006). Thus, more precise characterization awaits the development of advanced technologies and more sophisticated analytic instruments.

Because chitosan and its derivatives are typical polyelectrolytes, their long-range structure in solution is critical to their final flocculation performance. Moreover, the optimal dose of chitosan-based flocculants is usually in the range of 0.1–100 mg/L, which according to polymer solution theory is extremely dilute (Flory, 1953; Elias, 1984; Qian, 2002). Therefore, besides traditional polyelectrolyte theories (Fuoss and Cathers, 1949; Fuoss and Strauss, 1948; Manning, 1974; Oosawa, 1971; Radeva, 2001), an understanding of the efficient performances achievable with much wider polymer concentration ranges is needed to study the hydrodynamic structure of polymers under these conditions (Cheng, 1997; Yang et al., 2012a). Such studies are also currently lacking.

4.1.2. Molecular structure control

Nonetheless, by taking advantage of the flocculation mechanisms recognized thus far, the tailored structural features of chitosan-based flocculants, and their actual flocculation performance, the structure-activity relationship could be well built and effectively exploited. Accordingly, the interdependencies of the preparation condition, structural features, and flocculation performance can be obtained and are described in Fig. 4.1.

As diagrammed in Fig. 4.1, the selection or design of the optimal flocculant relies on precise molecular control to achieve the desired flocculation performance and optimize the flocculation process. Guided by these principles, we developed two types of amphoteric

starch-based flocculants that effectively flocculate both positively and negatively charged colloidal contaminants in water (Yang et al., 2014c, 2014d). This same strategy can also be applied to the preparation of high-performance chitosan-based flocculants.

4.1.3. Flocs properties analysis

In addition to the molecular structures of the flocculants and the treated pollutants, the properties of the flocs formed in the flocculation process, including floc size, fractal structure, regrowth ability, and settling rate, must be considered in any detailed analyses of flocculation mechanisms. These properties can be studied by image analysis or light-scattering technique in combination with fractal theory (Mandelbrot, 1983; Serra, 1992; Wei et al., 2009). Among them, fractal dimension is one of the most important and powerful quantitative parameters in the fractal concept (Zhou and Franks, 2006), which indicates the space-filling capacity (Thomas et al., 1999), i.e., the compactness of the floc. Larger fractal dimensions signify more compact flocs. There are two commonly used fractal dimensions: two- (D_2) and three-dimensional (i.e., mass fractal dimension, or D_F) presentations. D_2 is defined by the power law relationship between the projected area (A) and the characteristic length (l) (Eq. (4.1)), which is usually measured by image analysis (Chakraborti et al., 2000; Cai et al., 2013). D_F is the power law relationship between mass (m) and l , which can be obtained by light-scattering since light intensity (I) is directly proportional to m and scatter vector (Q) is inverse to l (Eq. (4.2)) (Jarvis et al., 2005).

$$A \propto l^{D_2} \quad (4.1)$$

$$I \propto Q^{-D_F} \quad (4.2)$$

Many studies on this aspect of flocculation have made use of traditional flocculants such as $Al_2(SO_4)_3$, PAC, $FeCl_3$, and PAM (Chakraborti et al., 2000, 2003; Fabrizi et al., 2010; Jarvis et al., 2005; Rasteiro et al., 2008; Yu et al., 2006, 2010), whereas much less is known about natural polymer-based flocculants (Li et al., 2015a; Yang et al., 2012c; Yang et al., 2014b).

We recently studied the flocculation performance of CMC-g-PDMC including flocs properties for kaolin suspension, humic acid solution and kaolin/humic acid mixed suspension under various pH levels (Yang et al., 2014b). Flocs produced by CMC-g-PDMC had larger sizes, denser structures and rapid settling velocity than those by PAC under most corresponding conditions, resulting from both strong charge attraction and enhanced approachability of its comb-like copolymer. However, floc strength based on calculated floc breakage coefficient was dependent on the interaction force among primary particles in flocs, and flocculation systems following patching mechanism had stronger floc strength than those obeying simple charge neutralization (Yang et al., 2014b).

Moreover, the kinetics of flocculation are a function of floc formation and breakage, which together result in changing floc structures that affect the final flocculation performance. From a simple yet widely applied modified Smoluchowski model (Eq. (4.3)) (Blanco et al., 2005; Chen et al., 2007), an updated equation

combining with the fractal dimension of D_F (Eq. (4.4)) was developed and used to study the flocculation processes of kaolin suspensions quantitatively (Yang et al., 2013b). From the obtained kinetic parameters of flocs aggregation and breakage (k_1 and k_2), the influences of flocculant type (inorganic (PAC), synthetic organic (PAM) and natural polymer flocculants (CMC-g-PAM)), dose, pH, and mechanical mixing rate on flocculation kinetics were thoroughly discussed (Yang et al., 2013b).

$$\frac{d(N_t/N_{initial})}{dt} = -N_{initial}k_1 \left(\frac{N_t}{N_{initial}}\right)^2 + k_2 \frac{N_t}{N_{initial}} \quad (4.3)$$

$$\frac{N_t}{N_{initial}} = \frac{\sum_i x_{i,initial} d_{i,initial}^{(D_F)_{initial}}}{\sum_j x_{j,t} d_{j,t}^{(D_F)_t}} = \frac{k_2 \cdot e^{k_2 \cdot t}}{(k_1 \cdot N_{initial}) \cdot e^{k_2 \cdot t} + k_2 - (k_1 \cdot N_{initial})} \quad (4.4)$$

Here, $N_{initial}$ and N_t are the number concentrations of particles at the initial time and time t , respectively; $x_{i(j),t}$ is the fractional number of particles classified as type $i(j)$, which has a floc size of $d_{i(j),t}$.

However, a real flocculation process is still associated with the aspects of collision efficiency, hydrodynamic interactions, and particle surface characteristics (Kusaka et al., 2011; Li et al., 2011; Smoczynski et al., 2009; Thomas et al., 1999) besides flocs properties. Given the complexity of the flocculation process and its importance not only in water treatment but also in many industrial fields, including mining operations, oil extraction, and paper production, a comprehensive understanding of flocculation is needed to achieve its wider and more effective implementation. Acquiring this knowledge requires close multidisciplinary cooperation among researchers in environmental science and engineering, polymer chemistry and physics, colloidal chemistry, theoretical chemistry, etc.

4.2. Cost performance of chitosan-based flocculants

4.2.1. Cost of chitosan-based flocculants

One of the most important bottlenecks restricting practical applications of chitosan-based flocculants is their cost. The unit price of chitosan is still much higher than that of traditional inorganic flocculants. Hwang and Srinivasan used chitosan to remove lipids from cheese whey by chitosan (Hwang and Srinivasan, 1995). A preliminary economic analysis indicated that the additional cost involved in the manufacture of fat-free whey protein isolate using chitosan was about \$0.50/kg of whey protein. Xu et al. estimated that the cost of chitosan for dewatering of cultures of the microalga *Chlorella sorokiniana* is ~\$200/ton (Xu et al., 2013), which is not commercially viable, although chitosan has highly effective. Gupta et al. compared the recovery cost of *Scenedesmus* sp. biomass using chitosan vs. a polyamine polymer (Gupta et al., 2014). The proximate cost of harvesting 1 kg of algal biomass was approximately \$51 for chitosan but only \$0.5 for the polyamine polymer. However, chitosan is still used in some settings to obtain high additional value (Cheng et al., 2005; Chi and Cheng, 2006).

Reducing the cost of chitosan applications also requires novel strategies in the extraction and deacetylation of chitin. Nonetheless, simple, effective, and inexpensive modification techniques can be used to improve the functionality, application efficiency, and final cost performance of chitosan-based flocculants. Farid et al. analyzed the cost of harvesting the microalga *Nannochloropsis* sp. using chitosan nanopolymer (Farid et al., 2013). The harvesting cost in the production of 1 kg of dry biomass was ~\$0.0246: \$0.0225 for chitosan and \$0.0021 for acetic acid, which demonstrated the feasibility of using nanochitosan as a flocculation agent. Dong et al.

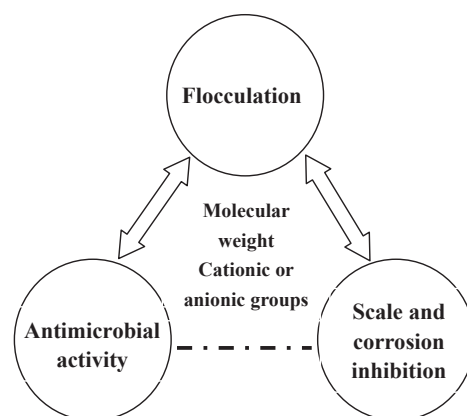


Fig. 4.2. The relationship between flocculation, antimicrobial activity, and corrosion and scale inhibition in chitosan-based multifunctional water treatment agents.

flocculated algal water in different pH solutions using quaternized carboxymethyl chitosan (CMC-CTA) (Dong et al., 2014). Although the unit price of CMC-CTA is higher than that of PAM, $Al_2(SO_4)_3$, and $FeCl_3$, the optimal dose of chitosan is much lower, making CMC-CTA a competitive and feasible coagulant.

There are also other, more affordable natural polymer materials, such as cellulose (Cai et al., 2013, 2015; Liu et al., 2014; Sievanen et al., 2015), starch (Krentz et al., 2006; Singh et al., 2000, 2013; Wang et al., 2013a), and tannin (Wang et al., 2013b), which, after appropriate modification, may provide efficient alternatives. The optimal molecular design of these other potential flocculants and their use as an alternative to chitosan-based flocculants will depend on the characteristics of the target water body.

4.2.2. Combined flocculation with chitosan-based flocculants

Chitosan-based flocculants can be also combined with other materials such as PAC, $Al_2(SO_4)_3$, $FeCl_3$, montmorillonite, soils and PAM in water purification as coagulant aids or one of main components in composite flocculants (Assaad et al., 2007; Lee et al., 2012, 2014; Li and Pan, 2015; Ng et al., 2012; Petzold and Schwarz, 2014). This strategy could obviously improve their flocculation efficiency and reduce the cost. More importantly, the applications of direct flocculation using only polymeric flocculants are mostly limited to organic-based wastewater with high concentration of suspended and colloidal solids; such as food, paper and pulp, and textile effluents (Lee et al., 2014). Thus, combined flocculation process is still preferable and widely employed by most of the industries (Chong, 2012).

Zemmouri et al. compared the flocculation performance of chitosan used individually and as coagulant aid of $Al_2(SO_4)_3$ for treating the raw water from Beni-Amrane dam (Zemmouri et al., 2013). The turbidity removal by latter strategy was 97%, but that by the former was only 87%. Zonoozi et al. also assessed of the application chitosan as a coagulant aid in conjunction with PAC towards dye-containing solutions. Chitosan could noticeably improve the dye removal efficiency of PAC. Small amounts of chitosan (3 or 5 mg/L) enhanced the dye removal efficiency of PAC up to 2.5 times for Acid Blue 292 (Zonoozi et al., 2011). Assaad et al. used chitosan-montmorillonite system to remove metal ions (Co^{2+} , Ni^{2+} , and Cu^{2+}) by coagulation-flocculation. The result showed that when chitosan and montmorillonite were used simultaneously, they would display a synergy phenomenon (Assaad et al., 2007). Perez et al. also used a combined flocculation technique by $FeCl_3$ and chitosan for harvesting of marine microalgae *Chaetoceros gracilis* (Perez et al., 2016), which is feasible and highly efficient even at

very low doses of chitosan and FeCl_3 . Furthermore, salinity was the variable with the greatest influence on this flocculation process.

Besides as coagulant aids, chitosan and its derivatives can be combined with other materials and used as composite flocculants. Ng et al. obtained a novel composite coagulant of chitosan with PAC and evaluated its coagulation process for natural organic material (NOM) removal (Ng et al., 2012). At a low Al dose (2.16 mg/L), a much higher NOM removal from synthetic water was achieved by the composite coagulants in comparison to that removed by PAC or PAC and chitosan added separately (Ng et al., 2012). Zeng et al. prepared a composite flocculant consisted of chitosan, PAC and silicate to treat the sewage of Tsinghua University (Zeng et al., 2008). The percentage of removing COD, SS and Al^{3+} in the treated water using this composite flocculant were enhanced by 1.8–23.7%, 50% and 61.2–85.5%, respectively in comparison to PAC, but its cost was cut down 7–34%. They also prepared another composite flocculant by chitosan, PFS and PAM to treat paper-making wastewater (Zeng et al., 2012). At optimal conditions, the removal of COD and SS reached 72.5% and 98.5%, which increased 15.2% and 6.4% respectively compared with traditional flocculant PAM/PAC. While input cost was cut down by 18.4%. Liu et al. synthesized a quaternized chitosan/organo-montmorillonite nanocomposite by microwave irradiation-assisted method to flocculate CaCO_3 suspensions (Liu et al., 2011). The optimal dose of this composite was only 0.005 mg/L, which is much lower than 15 mg/L of cationic starch, a common flocculant. In Pan's group, they composite chitosan with many clays, minerals, and local soils into highly effective flocculants for removal of harmful cyanobacterial blooms by increasing their netting and bridging effects (Li and Pan, 2015; Li et al., 2015b; Zou et al., 2004; Zou et al., 2006). In short, combined flocculation is an economic and efficient flocculation process.

4.2.3. Multi-function chitosan-based flocculants

Traditional water treatment agents are of single functionality, which has resulted in a wide variety of agents and the need for high doses, complicated devices, and labor-intensive operations. The development of multifunctional water treatment agents containing flocculation, bacteriostasis/sterilization, and corrosion and scale inhibition activities would have obvious advantages, given the complex mixtures of pollutants that are often encountered (Xiao, 2008). Moreover, combining the various functionalities into one agent would reduce the actual cost of treatment and greatly expand the application range of these multifunctional compounds. In 1971, Redmore designed one of earliest multifunctional water treatment agents made of imidazoline phosphoramidate (Redmore, 1971). Its functionalities included flocculation as well as scale and corrosion inhibition, but the feeding dose varied according to the application.

Besides their use as flocculants, chitosan and its derivatives can be employed under various conditions as bacteriostatic/bactericidal agents (Goy et al., 2009; Yang et al., 2014a) as well as corrosion and scale inhibitors (Zhang et al., 2014a). In tests of our recently synthesized CMC-g-PDMC, its flocculation performance in treating various raw water containing dyes (Wu et al., 2015), high turbidity (Yang et al., 2014b), high contents of humic substances (Yang et al., 2014b), and bacteria (*E. coli*) (Yang et al., 2014a) was satisfactory. The test results indicated that, besides flocculation, CMC-g-PDMC is an effective bactericidal agent. Similarly, the carboxymethyl quaternized oligochitosan evaluated by Zhang et al. exhibited good scale inhibition and bactericidal properties (Zhang et al., 2014a).

Fig. 4.2 summarizes the structural relationships of chitosan-based multifunctional water treatment agents with respect to flocculation, antimicrobial activity, and corrosion and scale inhibition. From the viewpoint of molecular structure, good flocculation is often achieved with higher MW chitosans; however, corrosion

and scale formation are best prevented by chitosans with a lower MW and wider MW distribution due to their rapid molecular migration, effective lattice distortion, and dispersion effects. Introduced quaternary ammonium salt groups typically improve both flocculation and antimicrobial activity, because of their strong electrostatic interactions with the mostly negatively charged colloidal and bacteria suspensions (Goy et al., 2009); conversely, additional anionic groups are effective in scale inhibition (Hasson et al., 2011; Martinod et al., 2008), besides abundant amine and hydroxyl groups on chitosan.

Chitosan-based multifunctional water treatment agents with improved functionality and final cost performance can be manufactured using currently available methods. However, there are few reports describing their use in field operations, including in-depth investigations of the synergy between flocculation, corrosion and scale inhibition, and antimicrobial activity. Such studies would provide a theoretical basis for the development of multifunctional water treatment agents with well-founded structure-activity relationships.

4.3. Safety of chitosan-based flocculants

4.3.1. Toxicity of chitosan-based flocculants

Safety is an important factor in the use of any water treatment agent and especially in the treatment of potable water. Natural polymers, including chitosan, are generally regarded as nontoxic and safe. As described above, chitosan was used to remove lipids from cheese whey (Hwang and Srinivasan, 1995). Toxicological studies have also shown chitosan to be nontoxic, including as a food additive. Sekine et al. treated turbid river water on-site using chitosan, followed by acute toxicity testing using the larva of *Oryzias latipes* (Sekine et al., 2006). The chitosan-treated supernatant water was shown to be very safe, but the broader effect of chitosan on living organisms is not yet clear. To test the safety of CMC-g-PDMC, Yang et al. carried out acute toxicity assays in which they assessed the risk of secondary pollution posed by the grafted flocculant and by the effluents of the treated water under optimal conditions (Yang et al., 2014b). The test organism was *Daphnia magna* and the assays were performed according to Chinese National Standard GB/T 13266-91. The 24-h half-maximal effective concentration of CMC-g-PDMC was one log-unit larger than that of PAC, indicating its much lower toxicity. The effluents treated by CMC-g-PDMC had no effect on immobilization of *Daphnia magna* provided further evidence of the safety and potential applicability of this chitosan-based flocculant.

4.3.2. Risk of the disinfection by-products

Besides the possible toxicity of the residual flocculants in the effluents, the secondary pollution risk of the disinfection by-products from the disinfection process must also be taken into account. However, conflicting results have been reported thus far. Rizzo et al. evaluated the disinfection by-products in toxicity tests of *Daphnia magna* (Rizzo et al., 2008). They found a higher toxicity of chlorinated water coagulated with chitosan than with metal coagulants (ferric chloride and aluminum sulfate). The toxicity of coagulated/chlorinated surface waters with complex mechanisms derives from the characteristics of the surface water and the chemicals used in the oxidation/disinfection step. By contrast, Vasyukova et al. reported that total trihalomethane (THM) formation was lower in chitosan-treated chlorinated water than in chlorinated raw water (Vasyukova et al., 2010), suggesting that chitosan is a suitable coagulant for drinking water treatment. Capelete and Brandão evaluated the use of chitosan as a coagulant in the treatment of eutrophic water contaminated with *Microcystis aeruginosa* cells and measured THM formation by chlorine

oxidation (Capelete and Brandão, 2013). Compared to $\text{Al}_2(\text{SO}_4)_3$, chitosan more effectively removed turbidity, apparent color, absorbance at 254 nm, and *Microcystis aeruginosa* cells. Moreover, no THM was detected after chlorine oxidation. However, more detailed evaluations of the acute toxicity of both the residual flocculants in the effluents of treated water and the disinfection by-products following chlorine oxidation are needed to confirm the safe use of chitosan and its derivatives.

5. Conclusion

Above all, chitosan and its derivatives have many notable advantages that warrant further studies leading to their broad-ranging application. In the field of water treatment, several important issues regarding the cost and safety of chitosan-based flocculants must be resolved before they can be widely used in practical applications. More importantly, the optimal structure-activity relationships of chitosan-based flocculants should be well built, since the final flocculation performance of chitosan-based flocculants is highly dependent on their structural factors. Besides those normal parameters including DD, MW, degree of functional groups substitution, and grafting ratio, the fine structural information such as the distribution of substituted functional groups or grafted chains on the chitosan backbone and chain architectures (linear, branching and star-like forms) are seriously important for further understanding of their distinct performance to various target contaminants. However, the work related to this topic is not systematical and even very limited yet. Due to the complexity of both polymer structures with multiple levels, short- and long-range, and distinct characteristics of various pollutants in water, a multidisciplinary investigation is precisely necessary for a comprehensive understanding of flocculation.

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