

## PREPARATION, CHARACTERIZATION AND ADHESIVE PROPERTIES OF DI- AND TRI-HYDROXY BENZOYL CHITOSAN NANOPARTICLES

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**Abstract** Modified chitosans with 3,4-di-hydroxy benzoyl groups (CS-DHBA) and 3,4,5-tri-hydroxy benzoyl groups (CS-THBA) were synthesized and their nanoparticles were prepared *via* ionic crosslinking by tripolyphosphate (TPP). The chemical structure and degree of substitution (DS) of di- and tri-hydroxy benzoyl chitosans are determined by FTIR and <sup>1</sup>H-NMR spectroscopy. The morphology of particles, size distribution and zeta potential of nanoparticles were studied using transmission electron microscopy (TEM) and dynamic light scattering (DLS), respectively. The mean diameters of particles of CS-DHBA and CS-THBA nanoparticles were 144 nm and 112 nm, respectively. It was found that the particles size decreased slightly with decreasing the degree of substitution and increasing degree of deacetylation (DD), due to increasing of ionic crosslinking of ammonium ions and polyanions of tripolyphosphate. The TEM photographs of CS-DHBA show that these particles are spherical in shape, but the particles of CS-THBA show some aggregation. In addition, the solubility and the mechanical properties of the prepared modified chitosans and their nanoparticles were evaluated for bio-adhesive and biomedical application. The results of solubility tests indicated that, the CS-DHBA and CS-THBA have higher solubility at pH > 7 comparing to CS. Also the CS-DHBA, CS-THBA and their nanoparticles showed a significant adhesive capacity and enhanced tensile strength and tensile modulus.

**Keywords:** Nanoparticles; Di- and tri-hydroxy benzoyl chitosans; Ionic cross linking; Solubility; Adhesive properties.

### INTRODUCTION

Chitosan (CS) is a  $\beta(1\rightarrow4)$  heteropolysaccharide whose chains are formed by 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose (GlcNAc) and 2-amino-2-deoxy- $\beta$ -D-glucopyranose (GlcN) units. Chitosan is generally prepared by the deacetylation of chitin which is the second most abundant polysaccharide next to cellulose on the earth<sup>[1]</sup>. Chitosan's unique physicochemical and biological properties, such as positive charge, biodegradability, biocompatibility, non-toxicity and antibacterial activity, make this macromolecule ideal for a variety of fields, such as pharmaceutical and medical applications, textiles, waste water treatment, biotechnology, cosmetics, food industry and agriculture<sup>[2–6]</sup>. In addition, more recently, authors have reported the formation of chitosan nanoparticles or microspheres for use in medical or pharmaceutical applications<sup>[7]</sup>. For example, the chitosan-tripolyphosphate (TPP) nanoparticles exhibit some attractive features which render them promising carriers for the delivery of macromolecules<sup>[8]</sup>. However, chitosan has low solubility in aqueous solvent, which limit its applications, especially in medicine and food industry. In order to improve the physicochemical properties of chitosan, such as solubility, antimicrobial activity, adhesive properties and ability to interact with other substances, chitosan derivatives have been synthesized<sup>[9]</sup>. Among chitosan derivatives, quaternary ammonium chitosan<sup>[10]</sup>, carboxyalkyl chitosans<sup>[11]</sup> and *N*-acyl chitosan and *O*-acyl chitosan<sup>[12]</sup>, are some types of modified chitosans, which have been studied intensively in order to improve solubility, biological properties and find new applications. In recent years, mussel adhesive proteins have been extensively studied<sup>[13]</sup>. One of the most salient

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characteristics for the mussel proteins is that they contain high amounts of 3,4-di-hydroxyphenylalanine (DOPA) and lysine<sup>[14]</sup>. In mussel proteins, these amino acid residues with amino or phenolic functional groups react with each other by means of a phenol-oxidative enzyme to form strong adhesive bonds. For example, synthetic polypeptides that mimic marine adhesive proteins could be used as adhesives for aluminum, steel, and plastics<sup>[15]</sup>. 3,4-Di-hydroxybenzoic acid and 3,4,5-tri-hydroxybenzoic acid are extensively used in the preparation of resins having antioxidant properties, acting as radical scavenger as well as in the preparation of composite polymer modified electrodes<sup>[16]</sup> and also, a number of studies reported about the rheological properties of chitosan solutions upon addition of diphenolic compounds and enzymes, which promote the oxidation to quinone structures, and which could lead to water-resistant adhesives<sup>[17, 18]</sup>. Therefore, many studies have focused on improving the mechanical properties of chitosan and more studies have suggested that having certain functional groups such as amino groups and phenolic hydroxyl groups is the only requirement for water-resistant adhesion and strong surface interaction, leading to improvements in mechanical properties, as well as in decreases in gas and liquid permeability<sup>[19]</sup>. In this work, chitosan derivatives with 3,4-di-hydroxy benzoyl groups (CS-DHBA) and 3,4,5-tri-hydroxy benzoyl groups (CS-THBA) were prepared and characterized by means of Fourier-transform infrared (FTIR) spectroscopy and <sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra. In addition, the formation of modified chitosan nanoparticles by ionic cross-linking with tripolyphosphate was investigated and their physicochemical properties such as size distribution, zeta potential, morphology, solubility and mechanical properties were studied.

## EXPERIMENTAL

### Materials

Chitosan ( $M_w = 800$  kDa, deacetylation degree = 82%) was purchased from Fluka. Tripolyphosphate (TPP), 3,4-di-hydroxy benzoic acid (DHBA, 98%) and 3,4,5-tri-hydroxy benzoic acid (THBA, 99%) were purchased from Merck Co. *N*-(3-di methyl aminopropyl)-*N*-ethyl carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS, 98%) were obtained from Sigma-Aldrich chemical. All other chemicals were of analytical reagent grade and were purchased from Merck. All solutions were prepared using double distilled water.

### Preparation of Modified Chitosans

10 g/L chitosan solution was prepared in 1% acetic acid solution. 0.192 g of DHBA, 0.24 g of EDC and 0.04 g of NHS were dissolved in 20 mL of 1% aqueous acetic acid solution. This solution was added drop-wise to 20 mL of 1% chitosan solution and obtained mixture was stirred for 24 h at room temperature. For the synthesis of CS-THBA 0.216 g of THBA, 0.24 g of EDC and 0.04 g of NHS were dissolved in 20 mL of 1% acetic acid solution. This solution was added drop-wise into 20 mL of 1% chitosan solution and obtained mixture was stirred for 24 h at room temperature. The modified chitosans CS-DHBA and CS-THBA were precipitated as solid with the addition of large amount of acetone. After filtration, washing with acetone and drying under continuous flow of nitrogen, the obtained materials were stored under a nitrogen atmosphere.

### Preparation of CS Nanoparticles

CS nanoparticles were obtained by ionic cross-linking of CS with TPP, which was described in literatures<sup>[7, 20]</sup>. 2.5 g/L chitosan solution was prepared in 1% acetic acid solution, and TPP solution 10 g/L was prepared in deionized water, nanoparticles were spontaneously obtained by addition of 1 mL of the TPP solution into 10 mL of the chitosan solution under constant stirring at room temperature for 30 min. The prepared nanoparticles were separated by centrifugation at 10000 r/min for 1 h.

### Preparation of CS-DHBA and CS-THBA Nanoparticles

CS-DHBA and CS-THBA nanoparticles were prepared by cross-linking of CS-DHBA and CS-THBA in solutions with TPP. 2.5 g/L CS-DHBA solution was prepared in 1% acetic acid solution. 1 mL of TPP solution (1%) was added drop-wise to 10 mL of the CS-DHBA solution with magnetic stirring at room temperature for 30 min. The CS-THBA nanoparticles were spontaneously obtained in this process, and were separated by

centrifugation at 10000 r/min for 1 h and then purified and dispersed in water and freeze dried.

### **Characterization of CS, CS-DHBA, CS-THBA and Their Nanoparticles**

#### *Fourier-transform infrared (FTIR) spectroscopy*

FTIR spectra of CS, CS-DHBA, CS-THBA and their corresponding nanoparticles were recorded with a Shimadzu- 4300 spectrometer. The FTIR absorbency was recorded in the wave number range of 600–4000  $\text{cm}^{-1}$  using KBr method at room temperature.

#### *$^1\text{H}$ nuclear magnetic resonance ( $^1\text{H}$ -NMR) spectra*

$^1\text{H}$ -NMR spectra of chitosan, modified chitosans and their nanoparticles were recorded with a Bruker, Switzerland 400 MHz spectrometer at room temperature. Samples were dissolved in  $\text{D}_2\text{O}$ , which contained a small amount of DCl.

#### *Particles size analysis and zeta potential measurements*

The size distribution of CS, CS-DHBA and CS-THBA nanoparticles were determined by dynamic light scattering (DLS) with a Malvern, Autozer 4700 instrument. Surface charge and stability of the three nanoparticles were obtained by zeta potential measurements (DLS-ZP/particle sizer Nicomp<sup>TM</sup> 380 ZIS). For potential measurements, samples were diluted with 0.1 mmol/L KCl and measured in automatic mode. A bias potential of –150 mV was set in the instrument.

#### *Transmission electron microscopy (TEM)*

The morphological characteristics such as inherent matrix and shape of CS, CS-DHBA and CS-THBA nanoparticles were examined by a transmission electrons microscope LEO-906 with an accelerating voltage of 80 kV.

#### *Solubility tests*

Solubility of CS, CS-DHBA and CS-THBA was evaluated by turbidity measurements<sup>[21]</sup>. The pH dependence of the water solubility of CS, CS-DHBA and CS-THBA was estimated from measurements of transmittance of the solution. Briefly, 10 mg sample was dissolved in 2% (V/V) HCl solution (5 mL), the pH of the solution was adjusted by the addition of 10% NaOH solution and the transmittance of the solution at 600 nm as a function of pH value was recorded.

### **Mechanical Tests**

An Instron Universal Testing Machine (Model 200, HIWA) was applied to determine tensile strength (TS), according to the ASTM standard method (ASTM D882). For the tensile testing, 200 mg of the CS, CS-DHBA, CS-THBA and their nanoparticles distinctly were added in 1 mL of de-ionized water and mixed with a spatula until a viscous homogeneous paste was obtained. Within about 1–2 min, the adhesive materials to be examined were applied. The strain rate used for tensile strength experiments was 50 mm/min. Tensile properties were expressed in MPa and were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of the original length). Shear strength (SS) experiments were performed to evaluate the efficiency of the CS, CS-DHBA and CS-THBA nanoparticles to adhere two surfaces together. For the shear strength tests, beef skin was used as the substrate following the ASTM D3164 standard for measuring the strength properties of tissue adhesives in lap-shear by tension loading. The beef skin samples were obtained from Tabriz shambles and were immediately excised after slaughter and stored at –5 °C in isotonic buffered saline at pH 7.4 (2.38 g of  $\text{Na}_2\text{HPO}_4$ , 0.19 g of  $\text{KH}_2\text{PO}_4$ , and 8.00 g of NaCl in 1000 mL deionized water). The peeling was carefully washed and the substrates were cut in to pieces 12.7 mm (0.5”)  $\times$  25.4 mm (1”) in size. The surface size layered film for adhesive nanoparticles was 12.7 mm  $\times$  12.7 mm squares, and each one was sandwiched between the two beef skin. Figure 1 shows a schematic of lap shear joint specimen for adhesion test. Average values of tensile strength and maximum strain were determined after repeating the test five times.

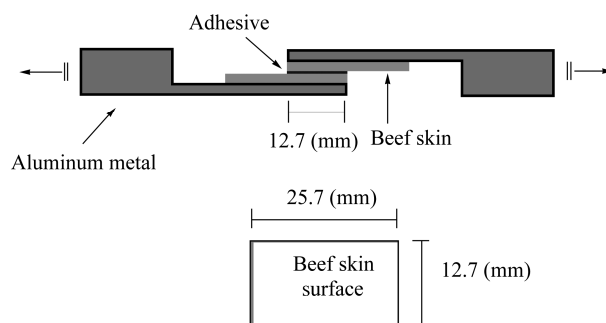


**Fig. 1** Simplified schematic of lap shear joint for shear testing

## RESULTS AND DISCUSSION

### Preparation of CS-DHBA and CS-THBA

The synthesis schemes of CS-DHBA and CS-THBA were represented in Fig. 2. Modified chitosans were prepared under homogenous conditions in 1% acetic acid solution. The reaction occurs between the amino groups of chitosan and carboxyl groups of benzoic acid derivatives in the presence of EDC and NHS. EDC and NHS have been used as the activating agents for the amide bond formation<sup>[22]</sup>.

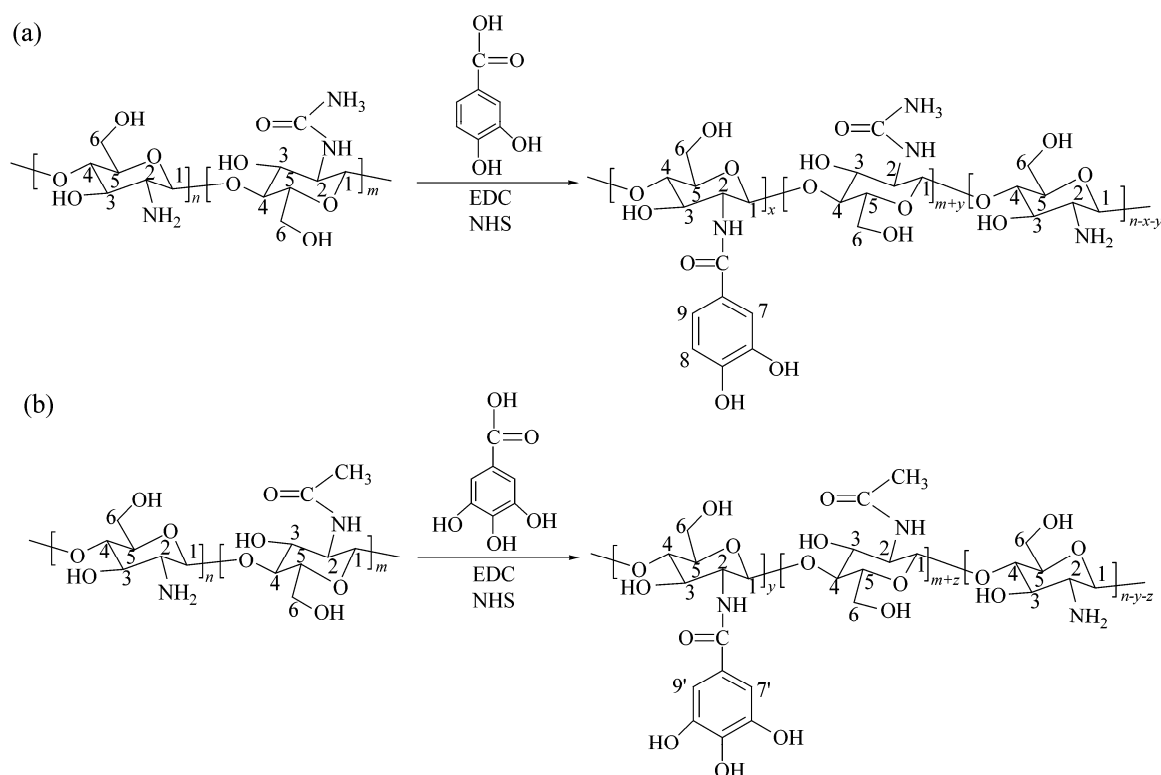


**Fig. 2** Synthesis procedure of modified chitosans (a) CS-DHBA and (b) CS-THBA  
The reactions were performed in 1% AcOH, at room temperature, stirring, for 24 h.

### Preparation of CS, CS-DHBA and CS-THBA Nanoparticles

Nanoparticles of CS, CS-DHBA and CS-THBA were synthesized by ionic cross-linking with tripyrophosphate (TPP). CS nanoparticles obtained as a result of the ionic cross-linking between the positively charged protonated amine groups of chitosan and negatively charged phosphate groups of TPP. In the CS-DHBA and CS-THBA

protonation of amino groups took place at the unsubstituted amines of CS-DHBA and CS-THBA. Nanoparticles were obtained by reaction between protonated unsubstituted amine groups of CS-DHBA and CS-THBA with TPP cross-linker<sup>[23]</sup>.

#### <sup>1</sup>H-NMR Studies of CS-DHBA and CS-THBA

The structure of the CS, CS-DHBA and CS-THBA was identified by <sup>1</sup>H-NMR. The technique of <sup>1</sup>H-NMR has been used by many authors to determine deacetylation degree (DD) of chitosan. Briefly, the degree of deacetylation was calculated from Eq. (1):

$$DD(\%) = \left[ 1 - \left( \frac{2I_{CH_3}}{I_{H-2,H-3,H-4,H-5,H-6}} \right) \right] \times 100 \quad (1)$$

where  $I_{CH_3}$  is the intensity of the methyl proton and  $I_{H-2,H-3,H-4,H-5,H-6}$  is the intensity of all protons from the cyclic glucose structure except the proton on the anomeric carbon<sup>[24, 9]</sup>. The <sup>1</sup>H-NMR spectra of CS, CS-DHBA and CS-THBA are shown in Fig. 3. The obtained results are summarized in Table 1. In the <sup>1</sup>H-NMR spectra of CS-DHBA and CS-THBA, in addition of the protons of repeating unit of chitosan, protons of aromatic ring (H-arom) appear at 6.8 (H-8), 7.5 (H-7,9) and 7.2 (H-7',9'), respectively. This results indicate that the chitosan contained 3,4-di- and 3,4,5-tri-hydroxy benzoyl residues. In addition, in the <sup>1</sup>H-NMR spectrum of chitosan, H<sub>1</sub> peak intensity of chitosan is much higher than that of other two derivatives. The low intensity of two derivatives, probably is due to the modified chitosans structure and effects of 3,4-di-hydroxy benzoyl groups and 3,4,5-tri-hydroxy benzoyl groups. The degree of substitution (DS) was calculated by comparing the integral ratio between proton of H-2 and the protons of substituted groups<sup>[25]</sup>. During the reaction of chitosan with DHBA and THBA, acetylation also occurred with aqueous acetic acid which is the solvent for the homogeneous acetylation reaction thus, a decrease in degree of deacetylation and an increase of degree of acetylation (DA) in CS-DHBA and CS-THBA are observed<sup>[26]</sup>.

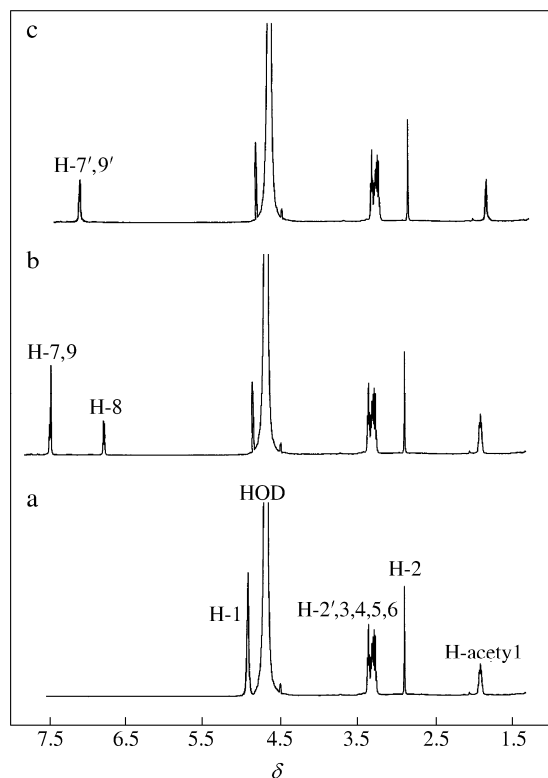


Fig. 3 <sup>1</sup>H-NMR spectra of (a) chitosan, (b) chitosan-DHBA and (c) chitosan-THBA

**Table 1.** Chemical shifts  $^1\text{H}$  ( $\delta$ ) in CS, CS-DHBA and CS-THBA

|         | Chemical shifts $^1\text{H}$ ( $\delta$ ) |            |             |         |         | DS (%) | DD (%) | DA (%) |
|---------|-------------------------------------------|------------|-------------|---------|---------|--------|--------|--------|
|         | H (acetyl)                                | H-2 (GlcN) | H-2,3,4,5,6 | H-1     | H-arom  |        |        |        |
| CS      | 1.9–2.0                                   | 2.9        | 3.2–3.4     | 4.5–4.8 | –       | –      | 82     | 18     |
| CS-DHBA | 1.9–2.0                                   | 2.9        | 3.2–3.4     | 4.5–4.8 | 6.8–7.4 | 38     | 39     | 23     |
| CS-THBA | 1.9–2.0                                   | 2.9        | 3.2–3.4     | 4.5–4.8 | 7.2     | 29     | 46     | 25     |

### FT-IR Analysis

The FT-IR spectra of CS, CS-DHBA, CS-THBA and their corresponding nanoparticles are shown in Fig. 4. In the FT-IR analysis of chitosan, specific peaks of chitosan are observed at  $1659$ ,  $1630$  and  $1564\text{ cm}^{-1}$ , which are attributed to the  $\text{C}=\text{O}$  stretching vibration of amid I,  $\text{N}-\text{H}$  bending vibration of amine and  $\text{N}-\text{H}$  bending vibration of amide II, respectively<sup>[27]</sup>. Compared to the FT-IR spectrum of chitosan, the spectrum of CS-DHBA showed new peaks at  $1656\text{ cm}^{-1}$  and at  $1552\text{ cm}^{-1}$ , attributed to the carbonyl stretching vibration of amide I and  $\text{N}-\text{H}$  bending vibration of amide II, respectively, for CS-THBA these peaks appeared at  $1642$  and  $1556\text{ cm}^{-1}$  respectively. These results and other evidences obtained from  $^1\text{H}$ -NMR spectra confirmed that the CS is substituted with 3,4-di- and 3,4,5-tri-hydroxy benzoic acid. In the spectra of chitosan nanoparticles, the shoulder peak of  $1659\text{ cm}^{-1}$  disappears and a new sharp peak appears at  $1636\text{ cm}^{-1}$ , and the peak of  $-\text{NH}_2$  bending vibration at  $1630\text{ cm}^{-1}$  shifts to  $1535\text{ cm}^{-1}$ , indicating that the linkage between the phosphate groups of TPP and ammonium ions of chitosan<sup>[28, 29]</sup>. In addition, the peak of  $3449\text{ cm}^{-1}$  becomes wider indicating that hydrogen bonding is enhanced, and a new peak appears at  $1219\text{ cm}^{-1}$  due to  $\text{P}=\text{O}$  stretching in chitosan nanoparticles, which is absent in the case of chitosan<sup>[30]</sup>. In the spectra of CS-DHBA and CS-THBA nanoparticles new peaks appear at  $1638$  and  $1639\text{ cm}^{-1}$  respectively, indicating that the linkage between the phosphate groups of TPP and remaining ammonium ions of CS-DHBA and CS-THBA. Thus, these results show the tri-polyphosphate groups of TPP were linked with ammonium groups ( $-\text{NH}_3^+$ ) of CS, CS-DHBA and CS-THBA, and their nanoparticles were formed.

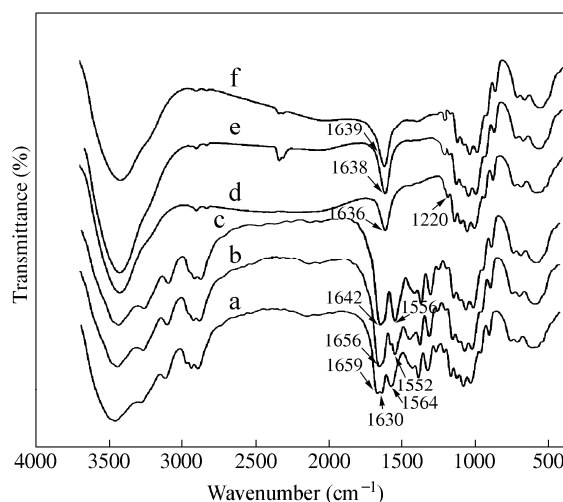


**Fig. 4** FTIR spectra of (a) CS, (b) CS-DHBA, (c) CS-THBA, (d) CS nanoparticles, (e) CS-DHBA nanoparticles and (f) CS-THBA nanoparticles

### Particles Size of CS, CS-DHBA and CS-THBA Nanoparticles

The chitosan nanoparticles were synthesized by ionic cross-linking of positively charged chitosan and their derivatives with negative charged TPP. Particle size distribution and zeta potential of CS, CS-DHBA and CS-THBA nanoparticles depend on concentration, molecular weight and other conditions<sup>[31, 32]</sup>. In this work, the chitosan nanoparticles were prepared with the fixed CS, CS-DHBA and CS-THBA concentration at  $2.5\text{ g/L}$ . In

addition nanoparticles were formed at selected CS/TPP, CS-DHBA/TPP and CS-THBA/TPP ratio of 2.5:1. Figure 5 shows the histograms of the size distribution of CS, CS-DHBA and CS-THBA nanoparticles by DLS. The average diameters for the CS, CS-DHBA nanoparticles were found to be 136 and 176 nm, respectively. But there are two separate peaks in the histogram of CS-THBA, a small number of particles are around 146.6 nm in size and the majority of particles have sizes around 768.3 nm that were believed to be formed by aggregation of the small particles<sup>[31]</sup>. Before this, Liu *et al.* reported that for nanoparticles based on linoleic acid modified chitosan having a hydrophobic group, a small number of large particles were formed due to aggregation<sup>[32]</sup>. We suppose this result may probably be due to the increasing of hydroxyl groups (three hydroxyl groups) in CS-THBA that are hydrophilic groups. However, particle size depends on degree of substitution and degree of deacetylation, because as mentioned above, chitosan nanoparticles are formed by the ionic crosslinking between oppositely charged ammonium ions and TPP. When the reactions are performed using DHBA and THBA to form of CS-DHBA and CS-THBA, amine groups of chitosan is decreased, therefore particle size of CS nanoparticles is less than that of CS-DHBA and CS-THBA nanoparticles. Also the degree of substitution of CS-DHBA is more than that of CS-THBA, therefore the mean size of CS-DHBA nanoparticles is bigger than that of the CS-THBA ones, since in the case of high degree of substitution, there are no enough protonated amine groups remaining for ionic cross-linking to occur. In addition this result is possibly due to the lower degree of deacetylation of CS-DHBA (Table 1).

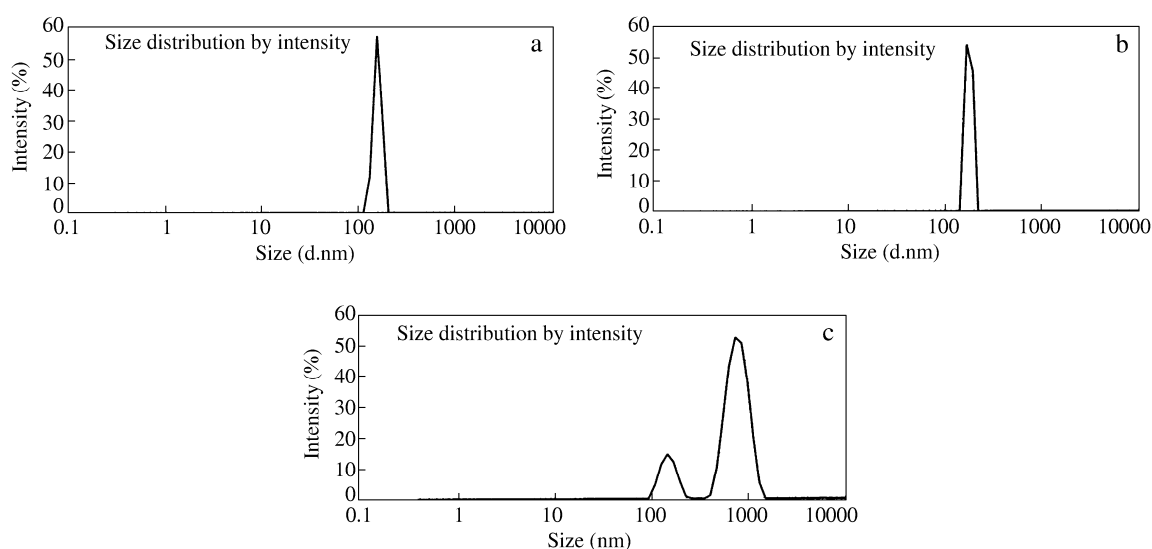


**Fig. 5** Size distribution of (a) CS, (b) CS-DHBA and (c) CS-THBA nanoparticles

#### **Zeta Potential of CS, CS-DHBA and CS-THBA Nanoparticles**

Surface charge and their stability of the prepared nanoparticles were determined by zeta potential measurements. Zeta potential values for the CS, CS-DHBA and CS-THBA nanoparticles were found to be  $(28.1 \pm 1.8)$ ,  $(17.5 \pm 1.3)$  and  $(24.6 \pm 1.4)$  mV, respectively. The CS, CS-DHBA and CS-THBA nanoparticles are all positively charged which is a typical feature of chitosan/TPP particles. This can be explained by the particle formation mechanism, the positively charged amine groups neutralized by their interaction with the negative charge in tripolyphosphate molecules<sup>[33]</sup>. The residual amino groups in CS-DHBA and CS-THBA would be responsible for the positive potential. It was observed that CS-DHBA and CS-THBA nanoparticles had a lower zeta potential than CS nanoparticles. This result is possibly due to the low degree of deacetylation of CS-DHBA and CS-THBA. The CS-DHBA and CS-THBA have a lower number of free amine groups which can be protonated in an acidic condition. In addition zeta potential of CS-DHBA is lower than CS-THBA, because the degree of deacetylation of CS-DHBA is lower than that of CS-THBA, and also the aggregation behavior of CS-THBA creates the high value of electrical double layer thickness.

### TEM of CS, CS-DHBA and CS-THBA Nanoparticles

The morphology of CS, CS-DHBA and CS-THBA nanoparticles was investigated by the transmission electron microscopy method. The TEM photographs of CS, CS-DHBA and CS-THBA nanoparticles are shown in Fig. 6. The CS and CS-DHBA nanoparticles are mostly spherical in shape (Figs. 6a and 6b), but the CS-THBA nanoparticles are not completely spherical in shape and tend to aggregate, therefore the majority of the particles are not separated from each other (Figs. 6c and 6d). We suppose this aggregation is due to the three hydroxyl groups of THBA attached to CS and the increasing intermolecular hydrogen bonds (H-bond) between the ammonium ions and the electronegative oxygen atom from hydroxyl groups. The size of nanoparticles of CS, CS-DHBA and CS-THBA were 98, 144.4, and 112 nm, respectively. These average sizes for CS, CS-DHBA and CS-THBA are smaller than those measured by DLS. The particle sizes of CS, CS-DHBA and CS-THBA nanoparticles measured by using DLS and TEM are presented in Table 2. This difference is due to the measurement conditions used in two techniques. In DLS the particles are dispersed in water and are stayed in swelled condition and the hydrodynamic diameter is measured, but in TEM the nanoparticles are prepared in dried condition, and there is no swelling effect present<sup>[34, 35]</sup>.

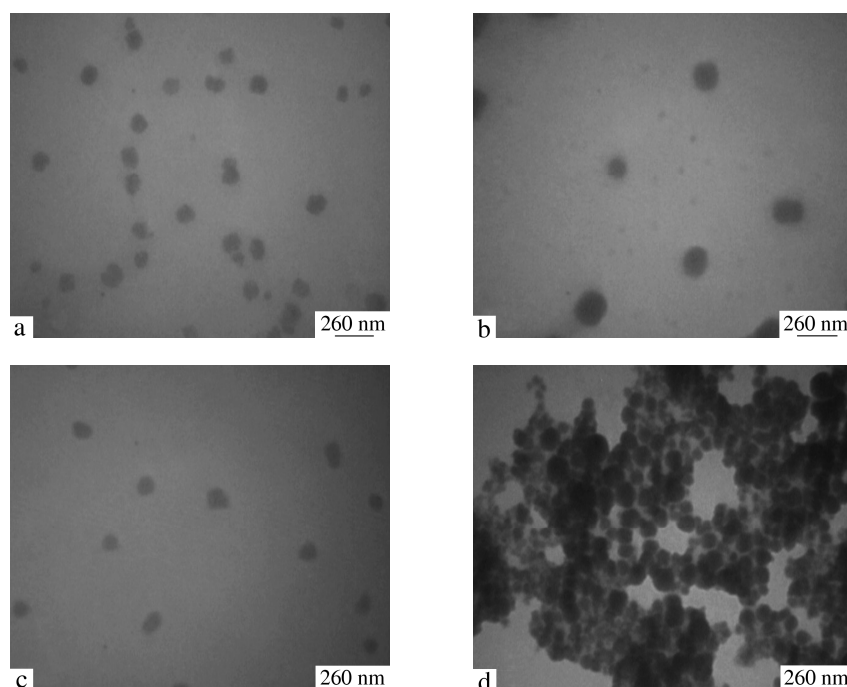


**Fig. 6** TEM photographs of (a) CS, (b) CS-DHBA, (c) CS-THBA without aggregation and (d) CS-THBA aggregated

**Table 2.** Comparison of particle size of CS, CS-DHBA and CS-THBA nanoparticles using DLS and TEM

| Nanoparticles | Particle size (nm) |                 | Zeta potential (mV) |
|---------------|--------------------|-----------------|---------------------|
|               | Measured by DLS    | Measured by TEM |                     |
| CS            | 136                | 98              | $28.1 \pm 1.8$      |
| CS-DHBA       | 176                | 144             | $17.5 \pm 1.3$      |
| CS-THBA       | 146–768            | 112             | $24.6 \pm 1.4$      |

### Solubility of CS, CS-DHBA and CS-THBA

Figure 7 shows the solubility of CS, CS-DHBA and CS-THBA in a wide pH range. CS is soluble at acidic pH, and precipitates at neutral and alkaline solutions<sup>[36]</sup>. The solubility of CS is caused by the protonation of free amine groups ( $-\text{NH}_2$ ). The CS-DHBA and CS-THBA show the improved solubility as compared with CS at neutral pH and alkaline pH. The solubility of CS-DHBA and CS-THBA in neutral water are due to *N*-acylation

of CS introducing hydroxyl ( $-OH$ ) groups to CS<sup>[37]</sup>. However the solubility of CS-DHBA and CS-THBA depends on the degree of deacetylation (DD) and the degree of substitution (DS). It could be seen from Fig. 7 the solubility of CS-THBA is higher than the solubility of CS-DHBA in the low pH range, because the DD of CS-THBA is higher than the DD of CS-DHBA. But at pH = 8.5 approximate of  $pK_2$  of hydroxyl groups of CS-DHBA the solubility of CS-DHBA is higher than the solubility of CS-THBA, because the DS of CS-DHBA is higher than the DS of CS-THBA. These results indicate that, the CS-DHBA and CS-THBA have higher solubility at pH > 7 comparing to CS. Therefore the solubility of modified chitosans depends on the variety of groups, the degree of deacetylation and the degree of substitution. Moreover the solubility of CS-DHBA and CS-THBA in the high pH range depends on the  $pK_2$  of hydroxyl groups of 3,4-di-hydroxy benzoic acid and 3,4,5-tri-hydroxy benzoic acid that are 8.9 and 10.1, respectively. Recently, water-soluble chitosan derivatives had been used to increase their stability in biological solution and decrease the cytotoxicity induced by acidic solution<sup>[38]</sup>.

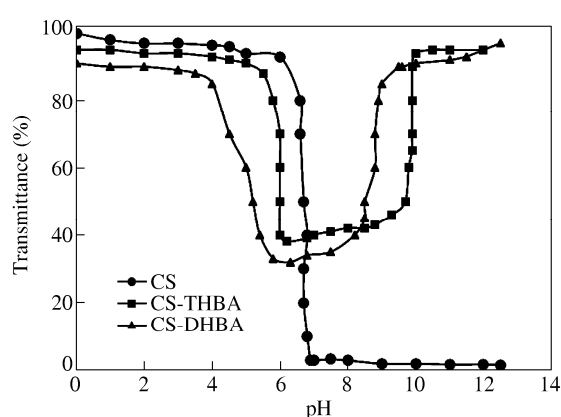


Fig. 7 Solubility of CS, CS-DHBA and CS-THBA

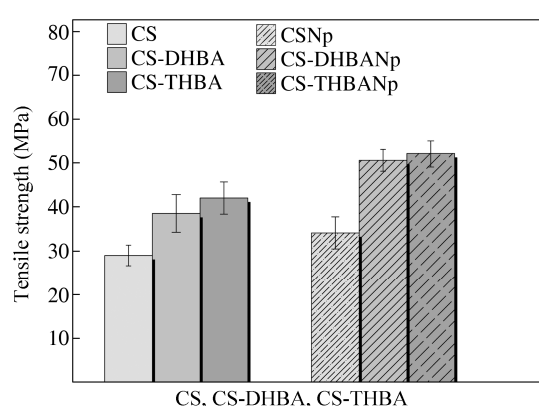


Fig. 8 Tensile strength of CS, CS-DHBA, CS-THBA and their nanoparticles

#### Mechanical Properties of Adhesives Based on CS, CS-DHBA and CS-THBA Nanoparticles

The results for tensile strength (TS), for CS, CS-DHBA, CS-THBA and their nanoparticles adhesives were reported in Fig. 8. The tensile strength of CS, CS-DHBA and CS-THBA were  $(29.12 \pm 2.73)$ ,  $(38.51 \pm 1.60)$  and  $(42.42 \pm 1.42)$  MPa, respectively. These results show that modified chitosans with 3,4-di-hydroxy benzoyl groups (CS-DHBA) and 3,4,5-tri-hydroxy benzoyl groups (CS-THBA) have improved mechanical properties compared to pure chitosan. Tri- and di- adjacent hydroxyl groups in these compounds can form stronger hydrogen bonds than a single phenolic hydroxyl group, which could be one of the explanations for the high viscosities of the adhesive systems containing a phenolic compound with three or two phenolic hydroxyl groups<sup>[18]</sup>. In addition the CS, CS-DHBA and CS-THBA nanoparticles also show improved tensile strength adhesive abilities (Fig. 8). Previous studies have shown that addition of nanoparticles resulted in a stiffer material confirming the reinforcing effect of the nanoparticles in the polymeric matrix<sup>[39]</sup>. Moreover, it was revealed that the increased surface area of smaller size CS/TPP nanoparticles improved the tensile properties by forming a polyelectrolyte complex. The results for shear strength (SS) and tensile modulus (TM) are shown in Table 3. Modified chitosan exhibits an increase in TM on beef skin due to the development of non-covalent interactions (hydrogen bonds) between chemical groups of modified chitosan and several groups of mucus chains and covalent bonds between hydroxyl groups of the CS-DHBA and CS-THBA surface and cysteine residues of the mucus glycoproteins<sup>[40]</sup>. These results indicate that CS-DHBA and CS-THBA and their nanoparticles have increased tensile strength and tensile modulus properties.

**Table 3.** Tensile modulus (TM) and shear strength (SS) of CS, CS-DHBA, CS-THBA and their nanoparticles

| Compounds   | TM (GPa)        | SS (MPa)        |
|-------------|-----------------|-----------------|
| CS          | 1.21 $\pm$ 0.31 | 5.71 $\pm$ 0.82 |
| CS-DHBA     | 1.30 $\pm$ 0.18 | 7.40 $\pm$ 0.58 |
| CS-THBA     | 1.32 $\pm$ 0.15 | 8.13 $\pm$ 0.49 |
| CS Np*      | 1.23 $\pm$ 0.22 | 5.92 $\pm$ 0.70 |
| CS-DHBA Np  | 1.31 $\pm$ 0.14 | 7.84 $\pm$ 0.39 |
| CS- THBA Np | 1.34 $\pm$ 0.15 | 8.72 $\pm$ 0.24 |

\*Np: nanoparticles

## CONCLUSIONS

Modified chitosans with 3,4-di-hydroxy benzoyl groups (CS-DHBA) and 3,4,5-tri-hydroxy benzoyl groups (CS-THBA) were synthesized, and their chemical structure and degree of substitution (DS) were determined by FTIR and <sup>1</sup>H-NMR spectroscopy. The CS, CS-DHBA and CS-THBA nanoparticles were prepared by ionic cross-linking with tripolyphosphates. The morphology of particles, size distribution and zeta potential of nanoparticles were studied using transmission electron microscopy (TEM) and dynamic light scattering (DLS). The mean sizes of CS-DHBA and CS-THBA nanoparticles were 144 and 112 nm, respectively. It was found the particle size was dependent on the degree of substitution and degree of deacetylation. Zeta potential values for the CS, CS-DHBA and CS-THBA nanoparticles were found to be (28.1  $\pm$  1.8), (17.5  $\pm$  1.3) and (24.6  $\pm$  1.4) mV, respectively. The CS, CS-DHBA and CS-THBA nanoparticles are all positively charged which is a typical feature of chitosan/TPP particles. In TEM studies the CS and CS-DHBA nanoparticles were mostly spherical in shape, but the CS-THBA nanoparticles were not completely spherical in shape and tended to aggregation, therefore the majority of the particles were not separated from each other. The results of solubility tests indicated that the CS-DHBA and CS-THBA have higher solubility at pH > 7 comparing to CS. In addition, the results of mechanical properties show that modified chitosans with 3,4-di-hydroxy benzoyl groups and 3,4,5-tri-hydroxy benzoyl groups have improved physicochemical and mechanical properties compared to chitosan. The CS, CS-DHBA, CS-THBA nanoparticles also exhibited improved tensile strength adhesive abilities.

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