

**CHAPTER VI**  
**CHITOSAN CONJUGATED WITH DEOXYCHOLIC ACID AND GALLIC ACID: A NOVEL BIOPOLYMER-BASED ADDITIVE ANTIOXIDANT**

**6.1 Abstract**

Chitosan, as a novel bio-additive antioxidant for commodity polymer, is proposed. Chitosan is successfully conjugated with deoxycholic acid and gallic acid (chitosan-DC-GA) via a simple reaction using the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) / *N*-hydroxysuccinimide (NHS) conjugating agent at room temperature in a heterogeneous system. Chitosan-DC-GA shows a DC and GA content of about 40% each. Chitosan-DC-GA exhibits DPPH radical scavenging ability with  $EC_{50}$  1 mg·mL<sup>-1</sup> based on the EPR technique evaluation. A model case study of chitosan-DC-GA with LDPE confirms improvement in compatibility, as seen from the sheet clarity, as well as the good dispersion of chitosan-DC-GA in LDPE matrices, as observed by SEM.

Keywords: chitosan; deoxycholic acid; gallic acid; bio-additive; polyethylene

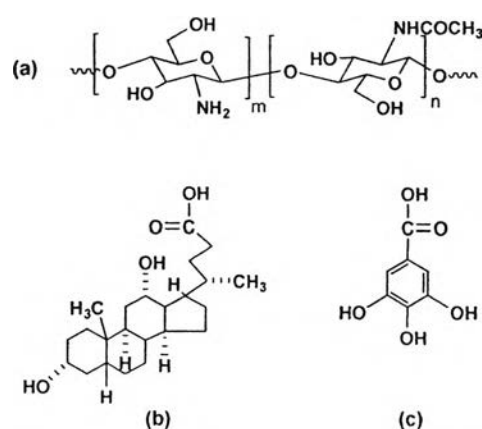
## 6.2 Introduction

An antioxidant is an important additive for polymer, as it stabilizes the polymer from resin extrusion to the molded pieces production. During processing, the antioxidant retards thermal or/and oxidative degradation. For some specific polymer products, especially medical equipment and food packaging, sterilization via radiation<sup>1</sup> is needed, and its consequent degradation, i.e. chain scission and/or crosslinking, resulting in discoloration, cracking of the surface, stiffening, and loss of mechanical properties,<sup>2</sup> has to be controlled by effective antioxidants. Hindered phenols, which contain the 2,6-di-*tert*-butylphenol functional group, and hindered amine light stabilizers (HALS), such as the derivatives of 2,2,6,6-tetramethyl piperidine, are known as primary antioxidants<sup>3,4</sup> and light stabilizers, respectively<sup>5-8</sup>

It should be noted that although these traditional antioxidants are effective, the possibility of decomposition and migration to the surface brings about problems with the chemical (e.g., amines, phenol) dissolution or vaporization during processing or use.<sup>9-11</sup> As a result, the antioxidant activity is easily diminished from the polymer products and decreases the polymer stability and may produce toxic chemicals to the surrounding system.<sup>10,11</sup> Several approaches to overcome these drawbacks, especially the use of natural antioxidants and polymer-bound antioxidants, are known as alternative choices. Vitamin E ( $\alpha$ -Tocopherol) is one of the natural antioxidants that has been studied for the stabilization of polymeric materials.<sup>12,13</sup> Its long-term performance in the polymer product, as compared to those of synthetic antioxidants (e.g., Irganox 1076), are points to be overcome.<sup>14,15</sup> Polymer-based stabilizers bring higher thermal stability, more resistance to extraction, and lower toxicity as compared to the small molecules. For example, Ranogajec and Mišak<sup>16</sup> prepared UV-protector (2-hydroxy-4-(3-methacryloxy-2-hydroxy-propoxy) benzophenone)-grafted polyethylene to increase the persistence of the stabilizer in the polymer matrix. The biopolymer functionalized with natural antioxidants is another choice when we consider the environmental friendliness or safety reasons. With this point in mind, natural starch has been studied as an additive for thermal stabilization in polyethylene.<sup>17-19</sup> Strandberg et al.<sup>20</sup> also demonstrated that starch from oats exhibits free radical stabilizing properties in polyethylene.

In this article, we focus our attention on the development of a biopolymer based-antioxidant for use as a commodity polymer (i.e., polyethylene) additive. Here, we consider chitosan as a biopolymer backbone, of which the compatibility with polyethylene and the antioxidant property have to be developed.

Chitin-chitosan (Scheme 1) is the second most naturally-occurring copolysaccharide of  $\beta$ -(1-4)-2-acetamido-2-deoxy- $\beta$ -D-glucose and  $\beta$ -(1-4)-2-amino-2-deoxy- $\beta$ -D-glucose next to cellulose, but exists in the shells of crustaceans, insects, and fungi. Its reactive amino and hydroxyl groups have received much attention for chemical modification to develop several derivatives and value-added products.<sup>21,22</sup>



**Scheme 1** Chemical structures of (a) chitin-chitosan, (b) deoxycholic acid (DC), and (c) gallic acid (GA).

Deoxycholic acid (DC, (3 $\alpha$ ,5 $\beta$ ,12 $\alpha$ )-3,12-Dihydroxycholan-24-oic acid) (Scheme 1) is considered as a hydrophobic group to functionalize the polyethylene compatibility onto chitosan. DC is a bile acid and functions as a detergent-like molecule in the biological system. It is composed of a hydrophobic steroid structure with cyclohexyl rings and an alkyl chain terminating with a carboxylic acid group, which is good for conjugating with chitosan. The chemical structure of DC favors the amidization and esterification. In the past, DC-modified chitosan was reported for self-aggregated nanoparticles and its potential as a gene-delivery carrier.<sup>23,24</sup> In

accordance with our consideration, the conjugation with DC not only obstructs the H-bond but also provides the hydrophobicity for the compatibility with polyethylene.

Gallic acid (GA, 3,4,5-trihydroxy benzoic acid) (Scheme 1), a naturally occurring phenolic antioxidant extractable from plants, especially green teas,<sup>25</sup> is attractive when we consider the conjugation onto chitosan to obtain a biopolymer-based antioxidant. GA is a biomolecule known for its high reducing potential due to low O-H bond dissociation enthalpy of the tri-hydroxyl groups on the benzene ring.<sup>26</sup>

The present work is thus based on a molecular design of chitosan as a novel biopolymer-based antioxidant by demonstrating the preparation steps of conjugating with DC and GA to obtain chitosan-DC-GA. The work also studies the use of chitosan-DC-GA as an antioxidant additive by showing the antioxidative potential of chitosan-DC-GA via a DPPH free radical model and the compatibility of chitosan-DC-GA with polyethylene as a commodity polymer model.

## 6.3 Experimental

### 6.3.1 Chemicals

Chitosan (degree of deacetylation (%DD) = 95 and  $M_v = 9.5 \times 10^5$  Dalton) was supplied from Seafresh Chitosan (Lab) Company Limited, Thailand. Deoxycholic acid and sodium hydroxide were purchased from Fluka Chemika, Switzerland. Gallic acid was from Aldrich Chemical Company, Inc., USA. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, hereinafter abbreviated as water soluble carbodiimide (EDC), and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from TCI, Japan. *N*-hydroxysuccinimide (NHS) was purchased from Wako (Osaka, Japan). Methanol and acetic acid were from Lab Scan, Co., Ltd, Thailand. Low density polyethylene (LDPE) was obtained from Thai Polyethylene, Co., Ltd, Thailand. All chemicals were used without further purification.

### 6.3.2 Instruments and Equipment

Qualitative FT-IR spectra were recorded on a Thermo Nicolet Nexus 670 Fourier transform infrared spectrophotometer with 32 scans at a resolution of 2  $\text{cm}^{-1}$  in a frequency range of 4000-400  $\text{cm}^{-1}$  using a deuterated triglycinesulfate

detector (DTGS). The  $^{13}\text{C}$  CP/MAS NMR spectra were taken from a BRUKER DPX-300 (300 MHz) at  $23 \pm 1^\circ\text{C}$ . Elemental analysis was carried out by using a Yanako CHN Corder MT-3, MT-5 with a combustion temperature of  $950^\circ\text{C}$  under air atmosphere with  $\text{O}_2$  as a combustion gas (flow rate 20 mL/min) and He as a carrier (flow rate 200 mL/min). Powder X-ray diffraction (XRD) patterns were recorded by a RIGAKU RINT 2000 over  $2^\circ$ - $60^\circ$   $2\theta$  using  $\text{CuK}\alpha$  as an X-ray source, and operating at 40 kV and 30 mA with a Ni filter. A Dupont thermogravimetric analyzer was applied under a  $\text{N}_2$  flow rate of 20 mL/min and a heating rate of  $10^\circ\text{C}/\text{min}$  from  $30^\circ\text{C}$  to  $600^\circ\text{C}$ . Surface morphology was observed by a JEOL JSM-6400 scanning electron microscope (SEM). The samples were prepared by dispersing freeze-dried chitosan products on sample holder. Nanoparticles were obtained by using a Hitachi H7650 zero A transmission electron microscope (TEM). The samples were prepared by diluting the colloidal solution with RO water before dispersing particles on the sample holder. EPR Spectroscopy was carried out by using a Bruker EMX equipped with a  $\text{TM}_{110}$  cavity using a quartz flat cell. The EPR spectrometer settings were: receiver gain,  $2.5 \times 10^5$ ; microwave power, 10 mW; modulation frequency, 100 kHz; frequency, 9.780 GHz; modulation amplitude, 0.10 G; time constant, 40.96 ms; the number of scan, 1 scan; and scan rate, 80G/10s.

### 6.3.3 Preparation of Chitosan-DC-GA

Chitosan flakes (1 %w/v) were dissolved in acetic acid (2 %v/v) to obtain a chitosan solution. The solution was re-precipitated in sodium hydroxide (1 %w/v) and then washed with water three times to obtain fine chitosan particles. Deoxycholic acid (DC) (5.1033 g, 1 mole) was added to the chitosan (2.1203 g, 1 mole) methanol suspension (30 ml). The mixture was stirred for 5 min and 1-ethyl-3-(3 -dimethylaminopropyl) carbodiimide (EDC) (2.4921 g, 1 mole) was added to the mixture under ice bath for 15 min. The reaction was carried out in a heterogeneous system overnight at room temperature to obtain **2**. *N*-hydroxysuccinimide, NHS (2.9923g, 2 moles) and EDC (4.9833 g, 2 moles) were added to gallic acid (GA) (4.4231 g, 2 moles) in methanol under ice bath for 1 h to obtain *O*-acylisourea, **3** and reactive ester intermediate, **4**. Compound **2** was then added to **4** and stirred for 24 h

at room temperature. The product was washed thoroughly with methanol and water before freeze-drying to obtain the white powder, **5** (Chitosan-DC-GA).

Compound **2**:  $(C_{30}H_{49}O_7N)_{0.95}(C_8H_{13}O_5N)_{0.05}$ : Calcd. (%C/N = 24.78 for DS = 100%) C 66.90, H 9.10, N 2.70, O 21.30; Found (%C/N = 11.25, DS = 45.40%) C 52.2, H 7.51, N 4.64, O 35.83. FTIR (KBr,  $cm^{-1}$ ) 2935 and 2862 (C-H stretching), 1646 (amide I), 1550 (amide II), 3452 (OH) 1200-800 (pyranose ring):  $^{13}C$  NMR:  $\delta$  = 18.8-57.0 (C of cyclohexyl groups of DC), 160.2 (C=O ester), 105.2 (C-1 of pyranose ring) 75.0-81.7 (C-3 to C-5 of pyranose ring), 57.9-60.8 (C-2 and C-6 of pyranose ring).

Compound **5**:  $(C_{30}H_{47}O_7N)_{0.454}(C_6H_8O_4N)_{0.496}(C_8H_{11}O_5N)_{0.050}$ : Calcd. (%C/N = 28.31 for DS = 100%) C 57.22, H 5.14, N 2.02, O 35.62; Found (%C/N = 12.58, DS = 44.44%) C 52.34, H 7.55, N 4.16, O 35.95. FTIR (KBr,  $cm^{-1}$ ) 1730 (C=O ester), 2935 and 2862 (C-H stretching), 1646 (amide I), 1550 (amide II), 3452 (OH), 1200-800 (pyranose ring):  $^{13}C$  NMR:  $\delta$  = 18.8-57.0 (C of cyclohexyl groups of DC), 142 (aromatic), 160.2 (C=O ester), 104.5 (C-1 of pyranose ring) 74.8-81.9 (C-3 to C-5 of pyranose ring), 56.2-59.9 (C-2 and C-6 of pyranose ring).

#### 6.3.4 Antioxidant Potential of Chitosan-DC-GA

Compound **5** (0.5, 1.0, 1.5, 2.0, and 2.5 mg) was reacted with a DPPH methanolic solution (75  $\mu$ M, 1 mL). The mixture was stirred and allowed to react for 30 min before filtering out the **5** from the DPPH solution. The DPPH supernatant was transferred to quartz flat cell. The intensity of the remaining DPPH was measured by an EPR spectrometer. The radical scavenging capacity (%RC) is defined as  $\%RC = 100 \times (h_0 - h_c)/h_0$ , where  $h_c$  and  $h_0$  are the peak heights of the DPPH spectrum with and without **5**, respectively. Similarly, the antioxidant potential of chitosan and **2** were studied.

#### 6.3.5 Effect of Temperature on Antioxidant Potential of Chitosan-DC-GA

Compound **5** (0.5, 1.0, 1.5, 2.0, and 2.5 mg) was incubated in aerobic conditions at 190°C in a hot-air oven for 5 and 15 min before mixing with a 1 ml

methanolic solution of DPPH (75  $\mu\text{M}$ ). The antioxidant potential of the incubated compound was evaluated as described above.

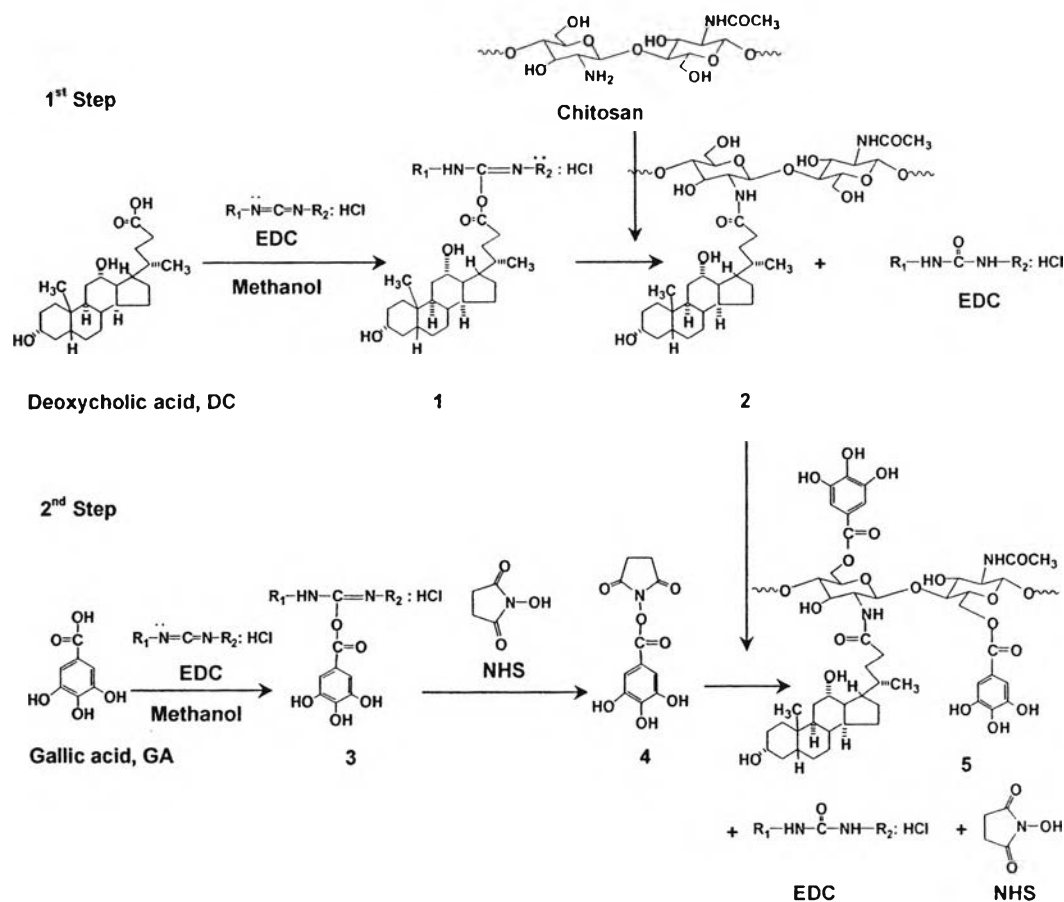
#### 6.3.6 Compatibility of CS-DC-GA with Polyethylene

Compound **5** (0, 1, 2, 3, 4, 5, and 7% w/w) was blended with low density polyethylene (LDPE) at 160°C using twin screw extruder. The blended PE resin was prepared as a 1 mm thick sheet using compression mold. The compatibility of CS-DC-GA was determined by SEM. The blended sheet was cut in liquid N<sub>2</sub> to observe the cross section.

### 6.4 Results and Discussion

#### 6.4.1 Conjugation of Deoxycholic Acid and Gallic Acid onto Chitosan (Chitosan-DC-GA)

Chitosan was chosen as a polymer backbone to be functionalized with both the hydrophobic group of the deoxycholic acid (DC) and the antioxidative group of the gallic acid (GA) to obtain a bio-additive-based antioxidant. The conjugation of DC and GA on chitosan is possible at either C-2, to obtain the amide linkage, or C-3 and C-6 to obtain the ester linkage.<sup>27</sup> For example, the reaction mechanism can be shown as Scheme 2 to represent the conjugation of DC in the first step and GA in the second step to form a bi-functional polymer in a one-pot reaction. For the first step, DC is reacted with the EDC conjugating agent to form *O*-acylisourea, **1**. At this step, the fine chitosan particles are dispersed in **1** to create a nucleophilic reaction that possibly occurs between **1** and the amino (at C-2) or hydroxyl group (at C-3 and C-6) of the chitosan to obtain **2**. In the first step, the conjugation of DC with chitosan via amide linkage is significant, as seen from the peak at 1646  $\text{cm}^{-1}$  (Fig. 1(b)). The peaks at 2935 and 2826  $\text{cm}^{-1}$  (C-H stretching) also confirm the successful conjugation of deoxycholic onto chitosan to obtain **2**. In the second step, to facilitate the coupling reaction with primary amine groups in chitosan, the carboxylic group of GA is activated by converting the carboxylic acid group into NHS-ester, as reported previously.<sup>28</sup> For this step, EDC/NHS was used to



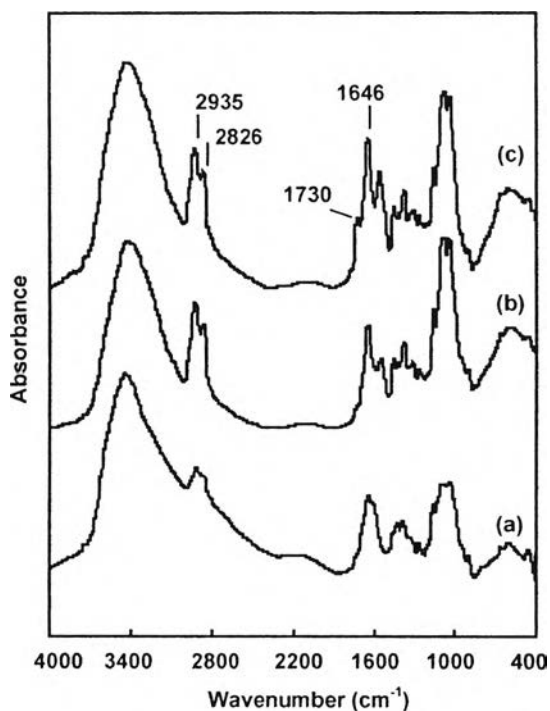
**Scheme 2** Suspected conjugation mechanism of chitosan with deoxycholic acid and gallic acid.

activate the gallic acid, **4** (succinimido gallate, GA-NHS), and to conjugate the GA onto the remaining amino and/or hydroxyl group of **2**. The un-reacted species and the by-products were removed by washing thoroughly with good solvents, i.e. methanol and water, several times. The suspension obtained was centrifuged and freeze-dried to obtain the white powder, **5** (chitosan-DC-GA). Fig. 1(c) shows the new ester peak at  $1730\text{ cm}^{-1}$  as well as the significant amide peak at  $1646\text{ cm}^{-1}$ . This confirms the successful conjugation of gallate group onto chitosan.

To confirm **2**,  $^{13}\text{C}$  CP/MAS NMR was also used. Compound **2** shows the new ester at 160.2 ppm and a series of methylene carbon species at 28.8-57.0 ppm, belonging to the cyclohexyl carbons of DC (Fig. 2(B)). This also confirms the successful conjugation of the chitosan with the DC.

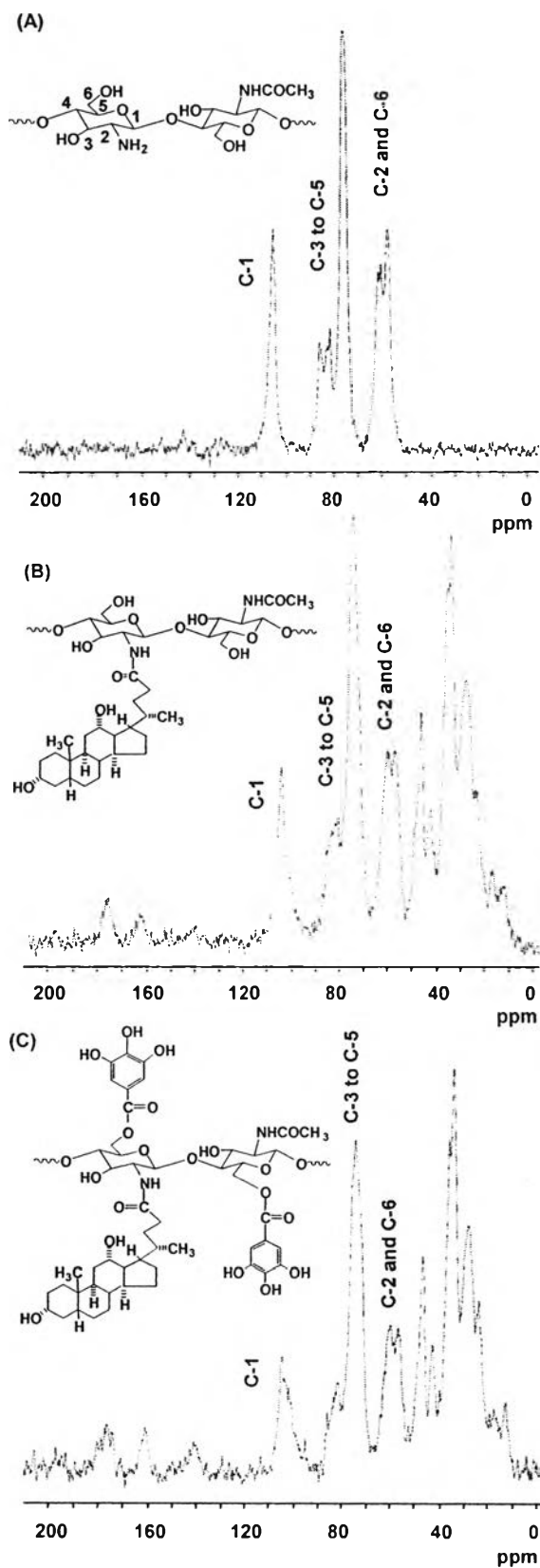


The substitution degree (%DS) was evaluated via the C/N ratio, by elemental analysis. An ideal substitution (100%) of the DC on the chitosan was assumed for the conjugation only at the amino group on the C-2 position, based on the fact that the amide linkage is clearly observed in the FT-IR spectrum (Fig. 1(b)).



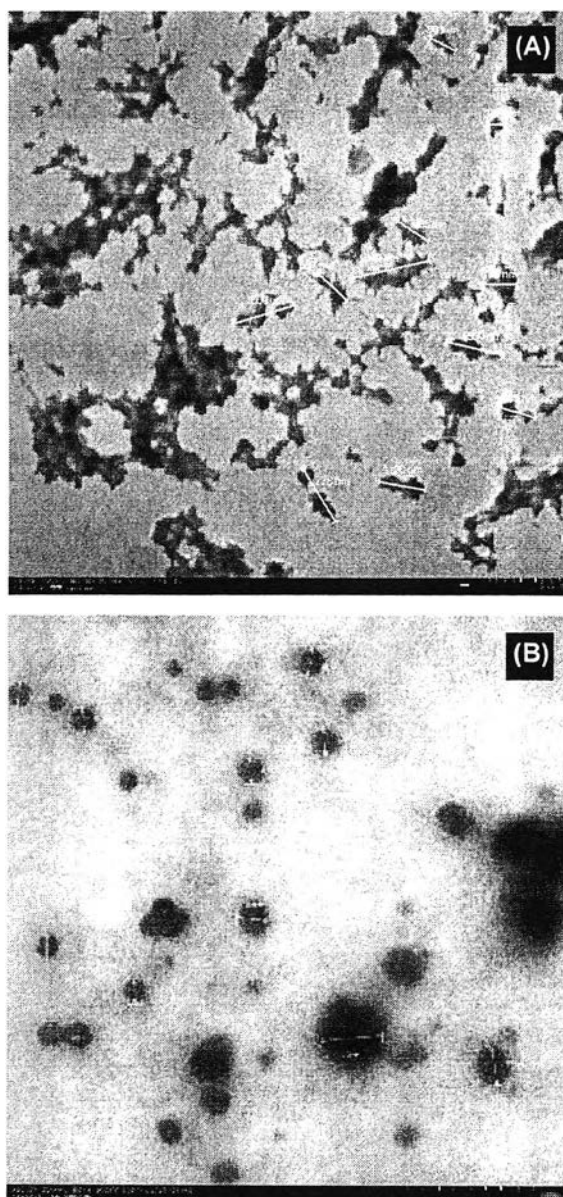
**Figure 1** FT-IR spectra of (a) chitosan, (b) **2**, and (c) **5**.

As C/N ratios for the ideal substitution is 24.78, whereas our derivative was 11.25, we suspected that the %DS of **2** was 45.40%. From the %DS of GA on the chitosan-DC and the degree of deacetylation of chitosan (%DD = 95%), we could further calculate the C/N ratio of the ideal structure of **5**. At that time, the substitution may possibly occur at C-3 and C-6 for chitin and the chitosan-DC units and at C-2, C-3, and C-6 for the chitosan unit. The %DS of GA on **2** was then found to be 44.44 (C/N = 12.58). The high substitution degree might come from the fact that chitosan was re-precipitated into the colloidal solution and dispersed in methanol before carrying out the reaction. Figure 3(A) shows the re-precipitate chitosan particle with an average size of 400-1400 nm. After DC was conjugated onto re-precipitated chitosan, the



**Figure 2**  $^{13}\text{C}$ -NMR spectra of (A) chitosan, (B) **2**, and (C) **5**.

particle size was reduced to 60-140 nm. This may be due to the spherical formation in colloidal aqueous solution of **2**. (Fig. 3(B)). In this way, the conjugating sides for the GA on **2** are on the surface of spheres.

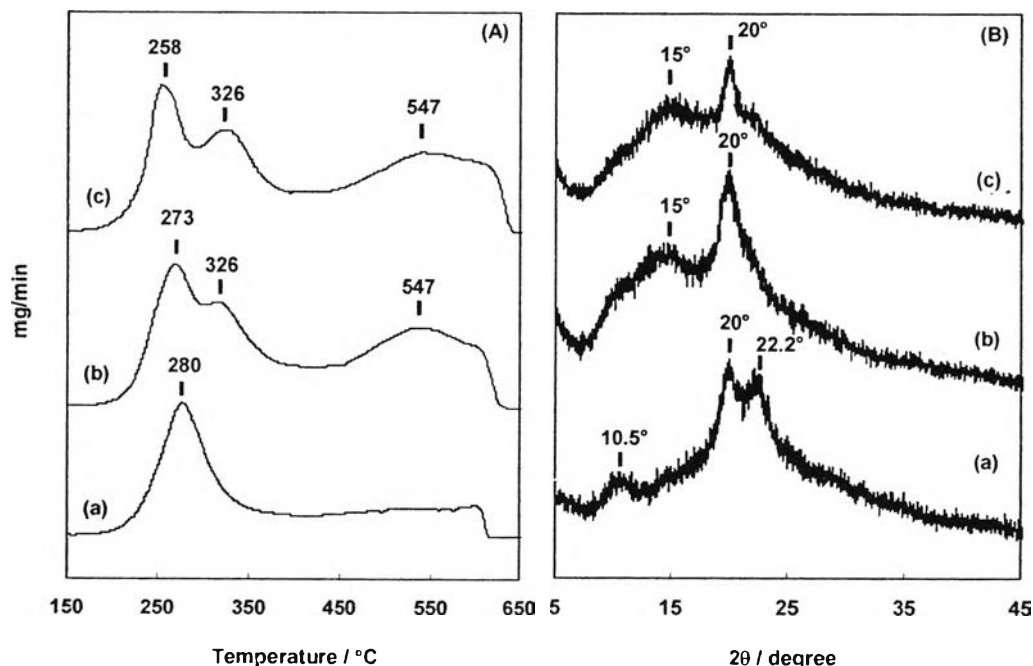


**Figure 3.** TEM micrographs, taken from each colloidal aqueous solution, at 100 kV of (a) re-precipitated chitosan (average size = 400-1400 nm) and (b) **2** (average size = 60-140 nm). The average particle size was randomly chosen for ten particles in the micrograph.

#### 6.4.2 Thermal and Morphological Properties of Chitosan-DC-GA

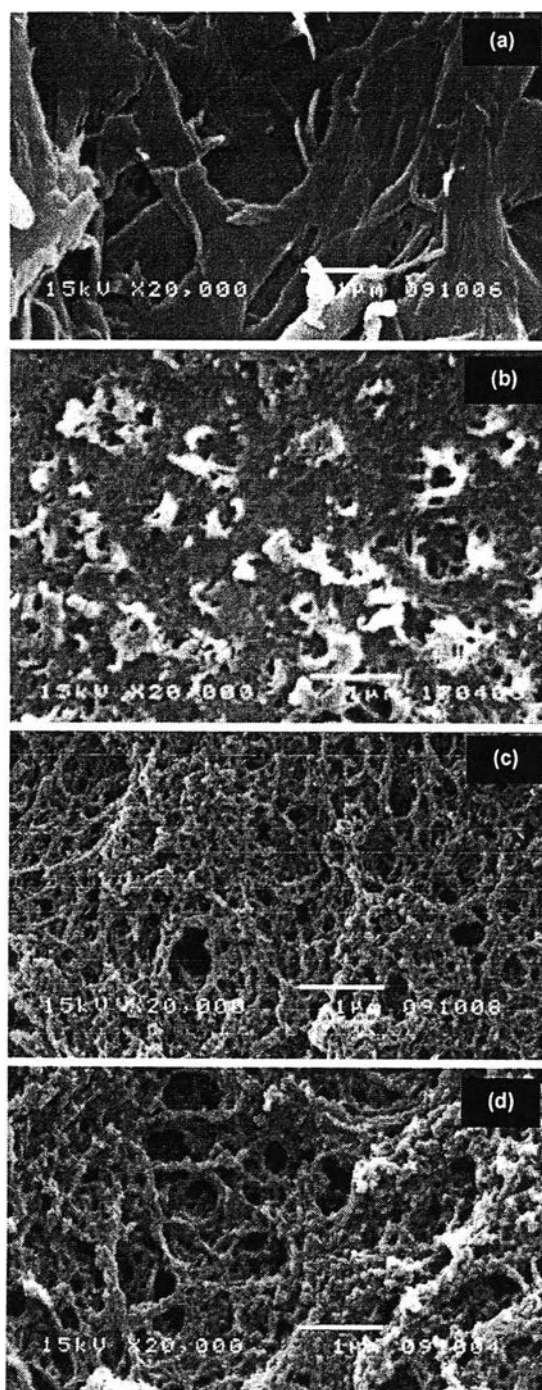
The conjugation of both DC and GA may result in a change of thermal properties and packing structure. Figure 4 shows the DTG curves and XRD patterns of chitosan, **2**, and **5**. As shown in Figure 4(A), the re-precipitated chitosan (curve (a)) shows a step of weight loss, implying a degradation temperature ( $T_d$ ) at 280°C. In the cases of **2** and **5** (curves b and c, respectively), there are three steps of weight loss. For **2**, the first degradation, at 273°C, can be interpreted as the loss of the chitosan chain; the second degradation, at 326°C, as the degradation of the amide linkage. The weight loss starting from 450°C can be referred to as the degradation of DC. In the case of **5**, the shift of the first degradation temperature of chitosan to the lower temperature (258°C) might be due to the loose chain packing of the chitosan after conjugating with both DC and GA.

The XRD pattern (Fig. 4(B)) also reflects the looseness in chain packing after conjugating the bulky group onto the chitosan chains. The XRD pattern of chitosan (curve a) gives the peaks at 10.5°, 19.8°, and 21.5°  $2\theta$ , whereas those of **2**



**Figure 4** (A) DTG curves and (B) X-ray diffractograms of (a) chitosan, (b) **2**, and (c) **5**.

and 5 (curves b and c, respectively) show broad peaks from  $10^{\circ}$ -  $20^{\circ}$ , and the peak at  $22.5$  has disappeared. The consequent increase of amorphous phase confirms the



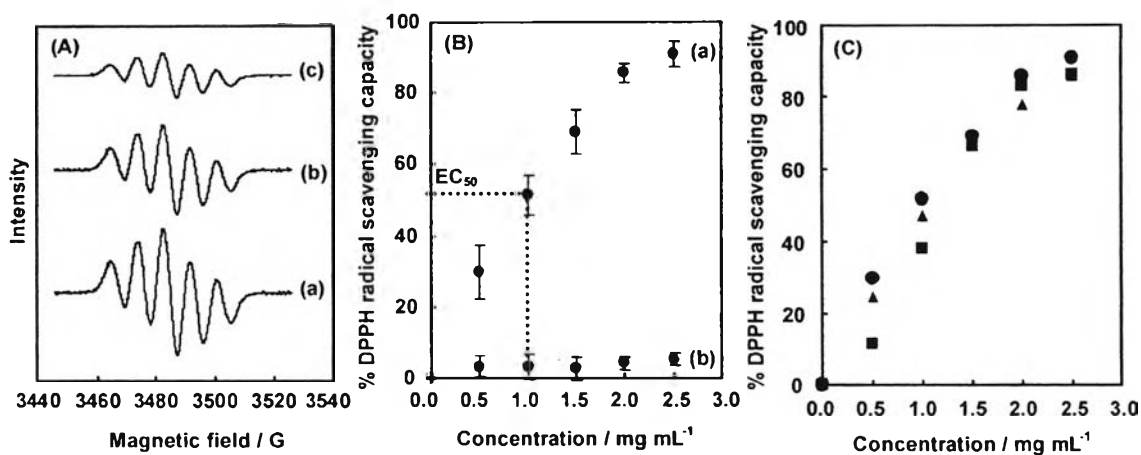
**Figure 5** Scanning electron micrographs (SEM) at 15 kV of (a) chitosan flakes, (b) re-precipitated chitosan, (c) 2, and (d) 5, after freeze-drying.

looseness in packing or, in other words, the successful conjugation of the gallate group onto the chitosan.

SEM micrographs (Fig. 5) show significant changes relevant to the results mentioned for Figure 4. As chitosan exhibits a high packing structure network, owing to its strong inter- and intramolecular hydrogen bonds, in general, chitosan appears as a dense flake-like sheet material (Fig. 5(a)). The freeze dried re-precipitated chitosan (Fig. 5(b)) was used as a reference to compare with the conjugated product. After DC and GA were introduced onto the chitosan chain, **2** and **5** after freeze-drying exhibit the porous network (Fig. 5(c) and (d)). The porous appearances, which are totally different from that of the re-precipitated chitosan, imply how the surface of chitosan was changed after the modification with the hydrophilic group (GA) and the hydrophobic group (DC).

#### 6.4.3 Antioxidant Capacity of Chitosan-DC-GA

It is important to clarify whether chitosan-DC-GA shows the ability to scavenge free radicals or not. Comparative studies on the antioxidant capacity of **2** and **5** were carried out by using DPPH<sup>•</sup> model free radicals. The decrease of the DPPH<sup>•</sup> intensity (Fig. 6(A)) indicates the ability of **5** to scavenge DPPH<sup>•</sup> related to the concentration. The scavenging capacity of **5** for DPPH<sup>•</sup> increased as its concentration increased and was close to 90% when the concentration reached 2.5 mg·mL<sup>-1</sup>. Here, it should be noted that in the case of sulfated chitosans reported by Xing et al.<sup>29</sup>, most of the derivatives obtained showed a DPPH<sup>•</sup>-scavenging ability of less than 20% at concentrations as high as 0.40 mg·mL<sup>-1</sup>, whereas the chitosan-DC-GA required 0.30 mg·mL<sup>-1</sup>. The EC<sub>50</sub> value, which expresses the effective concentration of antioxidant to reduce the radicals by 50%, is a good indicator to quantify the antioxidant capacity. Figure 6(B) shows that **5** exhibits antioxidant capacity, whereas no significant antioxidant capacity is observed in the case of **2**. This verifies that the conjugated gallate group of **5** assists chitosan in stabilizing free radicals. The EC<sub>50</sub> of **5** is about 1 mg·mL<sup>-1</sup>. Based on a previous report,<sup>29</sup> only one type of sulfated chitosan derivative, i.e. TSCTS, the sulfated chitosan of C<sub>3,6</sub> sulfonation, showed good radical scavenging ability, with an EC<sub>50</sub> of 0.025 mg·mL<sup>-1</sup>.



**Figure 6** (A) EPR spectra of DPPH<sup>•</sup>: (a) without **5**, (b) with 1 mg mL<sup>-1</sup> of **5**, and (c) with 2 mg mL<sup>-1</sup> of **5**. (B) DPPH<sup>•</sup> scavenging capacity of: (a) **5** and (b) **2**. (C) Effect of blended temperature of **5** to DPPH<sup>•</sup> scavenging capacity: (●) room temperature, (▲) 190°C for 5 min, and (■) 190°C for 15 min.

However, it is important to note that the antioxidant capacity of chitosan-DC-GA could only be evaluated in the solid form, since it is insoluble in most solvents.

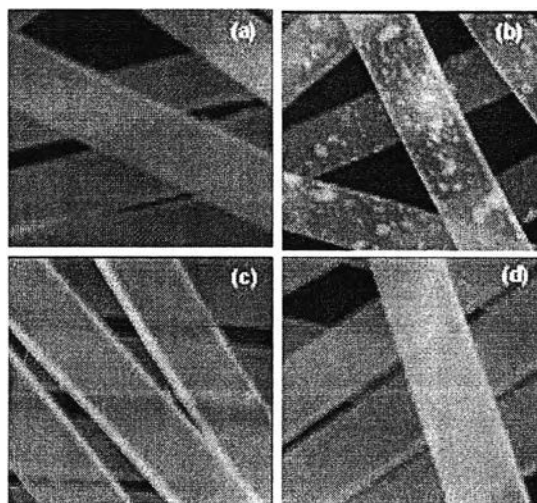
#### 6.4.4 Effect of Temperature on Antioxidant Potential of Chitosan-DC-GA

When we consider the use of chitosan-DC-GA as an additive for medical grade LDPE, we need to confirm the stability and the functioning of the antioxidant at high temperature (as high as 160°C, the processing temperature). Compound **5** was incubated at 190°C for 5 and 15 min before treating with DPPH<sup>•</sup>. Figure 6C indicates that there is no change in the DPPH<sup>•</sup> scavenging capacity of **5**, even after thermal treatment, as compared to **5** without incubation. This implies the durability of the antioxidative function of **5**.

#### 6.4.5 Compatibility of CS-DC-GA-blended-LDPE

The present work aimed to design a chitosan bio-additive antioxidant for medical grade LDPE. Clarification of its compounding with LDPE, especially its compatibility, is important. Figure 7 is images, obtained by an ordinary digital camera, of the blended LDPE with chitosan, **2**, and **5** as compared to that of pure

LDPE. In the case of LDPE with chitosan powder, white traces along the samples can be observed, indicating phase separation. For the blends of **2** and **5** with chitosan, the samples obtained show homogeneous PE sheets, implying the miscibility of the system (Fig. 7(c) and (d), respectively). Although it is difficult to obtain direct

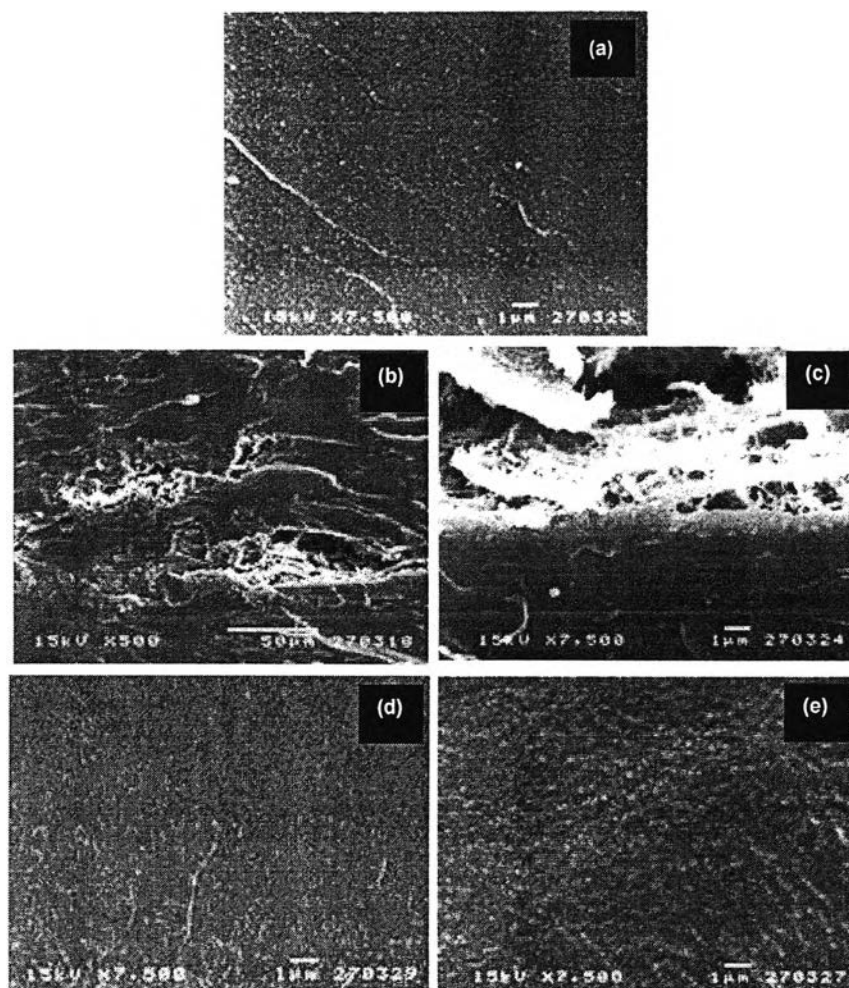


**Figure 7** Images of (a) pure LDPE, (b) 2 wt% chitosan-*blended*-LDPE, (c) 2 wt% **2**-*blended*-LDPE, and (d) 2 wt% **5**-*blended*-LDPE.

evidence about the mechanism for how **5** is miscible with LDPE, we suspect that the steroid structure with the high hydrophobicity of the DC may bring the hydrophobic site to the chitosan and initiate the miscibility between the **5** and the LDPE.

SEM was also used to confirm the miscibility of the blend. In the case of LDPE with 2 wt% of chitosan, Figure 8(b) and (c) clearly show the phase separation between the LDPE and the chitosan, whereas 2 wt% of **5** shows the miscibility with LDPE. The miscibility between **5** and LDPE is still observed when the concentration of **5** is increased to 7 wt% (Fig. 8(e)).





**Figure 8** Scanning electron micrographs (SEM) of cross sections of (a) pure LDPE, (b) 2% (w/w) chitosan-blended-LDPE (X500), (c) 2 wt% chitosan-blended-LDPE (X7500), (d) 2 wt% 5-blended-LDPE (X7500), and (e) 7 wt% 5-blended-LDPE (X7500).

## 6.5 Conclusions

A biopolymer-bound natural antioxidant for PE compounding was successfully prepared by conjugating deoxycholic acid (DC) and gallic acid (GA) onto a chitosan chain. The conjugation was performed via an EDC/NHS conjugating agent at room temperature in a heterogeneous system. The degree of substitution of DC and GA on chitosan is as high as 40%. Chitosan-DC-GA showed antioxidative

activity in stabilizing the model free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>), owing to the H-atom donor group of the GA. The EC<sub>50</sub> of chitosan-DC-GA in reducing DPPH<sup>•</sup> is 1 mg·mL<sup>-1</sup>. Based on the preliminary studies on blending and the miscibility of the LDPE blended with chitosan-DC-GA, this bi-functional chitosan derivative is a good model of a bio-polymer antioxidant for commodity polymer, especially biomedical grade polyethylene. We expect that 5 blended with LDPE may suppress the proliferation of bacteria, when used for medical purposes, and we will extend our work in the future to a study of the antibacterial effect of chitosan-blended LDPE.

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