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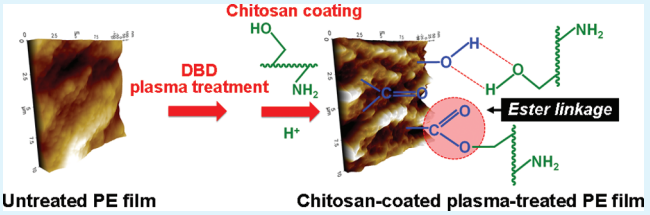
Preparation of Chitosan-Coated Polyethylene Packaging Films by DBD Plasma Treatment

Siriporn Theapsak,[†] Anyarat Watthanaphanit,[†] and Ratana Rujiravanit*[,†,‡]

[†]The Petroleum and Petrochemical College and [‡]Center for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University, Bangkok 10330, Thailand

Supporting Information

ABSTRACT: Polyethylene (PE) packaging films were coated with chitosan in order to introduce the antibacterial activity to the films. To augment the interaction between the two polymers, we modified the surfaces of the PE films by dielectric barrier discharge (DBD) plasma before chitosan coating. After that the plasma-treated PE films were immersed in chitosan acetate solutions with different concentrations of chitosan. The optimum plasma treatment time was 10 s as determined from contact angle measurement. Effect of the plasma treatment on the surface roughness of the PE films was investigated by atomic force microscope (AFM) while the occurrence of polar functional groups was observed by X-ray photoelectron spectroscope (XPS) and Fourier transformed infrared spectroscope (FTIR). It was found that the surface roughness as well as the occurrence of oxygen-containing functional groups (i.e., C═O, C−O, and −OH) of the plasma-treated PE films increased from those of the untreated one, indicating that the DBD plasma enhanced hydrophilicity of the PE films. The amounts of chitosan coated on the PE films were determined after washing the coated films in water for several number of washing cycles prior to detection of the chitosan content by the Kjaldahl method. The amounts of chitosan coated on the PE films were constant after washing for three times and the chitosan-coated PE films exhibited appreciable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Hence, the obtained chitosan-coated PE films could be a promising candidate for antibacterial food packaging.



KEYWORDS: *chitosan, polyethylene film, dielectric barrier discharge plasma, antibacterial activity, packaging film*

INTRODUCTION

One of the most commonly found problems in food products is microbial recontamination during post-processing handling step.[1] The growth of microorganisms leads to decrease in quality and shorten shelf life of food that can induce pathogenic problems. The use of packaging containing antimicrobial agents is more efficient than direct surface application of the antimicrobial substances onto food, because the agents are allowed to migrate slowly from the packaging material to control the rate of release of the active substances and thus maintain better quality of food in the packaging.[2] Nowadays, antimicrobial packaging is a food packaging concept that has received increasing interest in market trends.

Among polymers for packaging, polyethylene (PE) film is used predominantly because of its good chemical resistance, high impact strength, plentiful supply, and low cost.[3] Despite these outstanding characteristics, the PE film itself does not possess antimicrobial property. For this reason, extensive researches have been carried out in order to investigate potent methods to prepare antimicrobial PE films. Approaches to antimicrobial packaging can be classified into two types. The first can be done by incorporation and immobilization of antimicrobial agents to the polymer films and the others are by surface modification and surface coating.[2] By the first approach, several antimicrobial agents, such as sorbic anhydride[4] and nisin[5,6] have been incorporated in the PE polymer prior to fabrication of the films. However, the preparation of the PE films by this approach is limited by the thermal stability of the antimicrobial agents during extrusion or by the incompatibility of the agents with the polymer. Therefore, surface modification and coating techniques are more preferable and a polymer-based solution coating would be the most desirable way in terms of stability and adhesiveness of attaching an antimicrobial molecule to a plastic film.[7]

Chitosan, a β-1,4-linked polymer of glucosamine (2-amino-2-deoxy-β-D-glucose), is a natural antimicrobial agent used either alone or together with other polymers. It has been utilized in biomedical, chemical, and food industries due to its appreciable antimicrobial activity, high killing rate, and low toxicity.[8] In food applications, chitosan is used directly as a surface coating in meat products, fruits, and eggs, or as an additive to acidic foods.[9−11] Its protective barrier can retard ripening and water loss as well as reduce the destruction of food products.[12] Chitosan films for food packaging are also produced. Inclusion of various organic substances such as acetic acid, propionic acid, cinnamaldehyde, and lauric acid, into the chitosan matrix has

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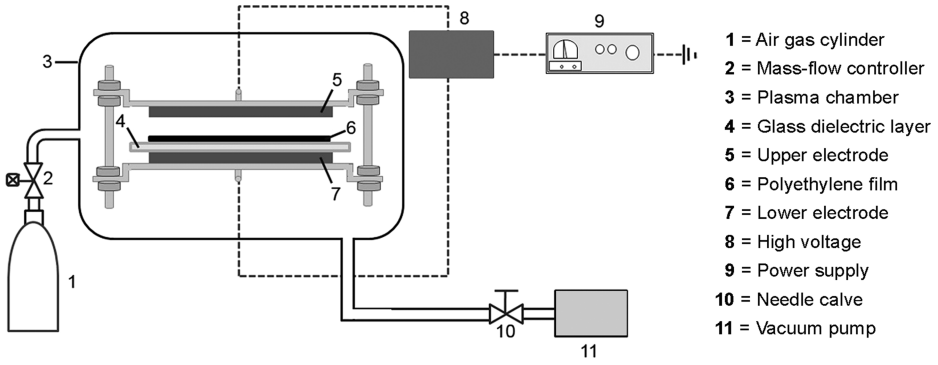

Figure 1. Schematic view of DBD setup used for surface modification of PE films.

been done before measuring the diffusion of the substances from the matrix.[13,14] Moreover, chitosan has been coated on papers for use in food packaging.[15,16] In addition to the antimicrobial property, chitosan-coated paper has been reported to prominently enhance both the gloss and the oxygen barrier properties of the paper.[16] However, among the chitosan-coated products, there are a limited number of studies on the chitosan-coated PE films.[17,18] Because the PE is long aliphatic chains of hydrocarbon consisting of only carbon and hydrogen, the PE surfaces are nonpolar and lack of active functional groups. As a result, it is difficult to utilize the PE for applications involving in adhesion such as printing and coating. According to these limitations, surface modification of the PE film prior to chitosan coating is required.

Dielectric barrier discharge (DBD) plasma, nonthermal plasma, is one of the promising methods to improve surface wetting and adhesion properties.[19,20] The speed of this method is within a few minutes or even seconds, which reduces the energy consumption. Comparing with other plasma methods, the application of DBD plasma treatment allows for continuous in-line processing, no needed special gas, and can be operated at atmospheric or medium pressure. These factors lead to the lower operational costs.[21,22] The DBD plasma can generate radicals and excited species which are able to initiate chemical and physical modifications within the depth of few nanometers on the surface of polymer films.[23,24] Earlier studies reported that the surface free energy and hydrophilicity of the PE films have been dramatically improved after the DBD plasma treatment since some oxidized species are introduced into the sample surfaces.[25−27]

In this study, PE films were first treated with dielectric barrier discharge (DBD) plasma under medium vacuum pressure in the presence of air gas. The plasma-treated films were determined for their water contact angle and mechanical properties to investigate the optimum time of the DBD plasma treatment. Effect of the DBD plasma treatment on surface property of the PE films was evaluated by means of atomic force microscopy (AFM), X-ray photoelectron microscopy (XPS), and Fourier transformed infrared spectroscopy (FTIR). In order to coat chitosan onto the polymer film, the plasma-treated films were immersed in chitosan acetate solutions with different concentrations of the chitosan. The amount of the chitosan deposited on the films was determined by the Kjeldahl method. The antibacterial property of the plasma-treated chitosan-coated PE films against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* was evaluated.

EXPERIMENTAL SECTION

Materials. Shrimp shells (*Litopeneous vannamei*) were kindly supplied by Surapon Food Public Co. Ltd. (Thailand). Chitosan (% DD = 97, M_v = 807 kDa) was prepared from chitin obtained after deproteination and decalcification of the shrimp shells. *N*-deacetylation of chitin was accomplished by alkaline treatment in an autoclave and this process was repeated for three times. The degree of deacetylation, %DD, of chitosan was determined by the method of Sannan et al.[28] It is a parameter defined as the mole fraction of deacetylated units in the chitosan chain, showing a number of acetyl groups attaching to N atom located on C2 positions of glucosamine ring, which are replaced by H atoms. The properties of chitosan, including antimicrobial property, depend considerably on %DD because such a property is functioned by amino groups ($-NH_2$) on the chitosan chain. Commercial PE film with a thickness of 0.048 ± 0.003 mm was purchased from Thantawan Industry Public Co., Ltd. (Thailand). Sodium hydroxide aqueous solution (NaOH, 50% w/v) was supplied by KTP Corporation Co., Ltd. (Thailand). Sodium acetate (CH_3COONa), sodium borohydride ($NaBH_4$), and hydrochloric acid (HCl, 37% w/w) were analytical reagent grade of Carlo Erba Co., Ltd. (Italy). Glacial acetic acid (CH_3COOH, 99.9% w/w) was analytical reagent grade and was purchased from Labscan Asia Co., Ltd. (Thailand). Sodium hydroxide anhydrous pellets (NaOH), sulfuric acid (H_2SO_4, 98%), and hydrogen peroxide (H_2O_2, 35% w/w) were purchased from Ajax Finechem Pty Ltd. (Australia). Amido Black 10B and copper(II) sulfate ($CuSO_4{\cdot}5H_2O$) were purchased from Wako Pure Chemical Industries, Co., Ltd. (Japan). Air gas used for plasma treatment was obtained from Thai Industrial Gas Co., Ltd. (Thailand).

Experimental Setup for the Dielectric Barrier Discharge (DBD) Plasma. Schematic drawing of DBD plasma experimental set up is shown in Figure 1. The DBD system contains two parallel stainless steel electrodes and a 2-mm thick of dielectric glass plate covering on the lower electrode. During the treatment, the discharge between the electrode and the polymer surface was induced by an AC high voltage power supply working with the optimum condition reported previously by Onsuratoom et al.,[29] i.e., at a voltage of 15 kV, a frequency of 350 Hz and an electrode gap of 4 mm. The flowing air gas was introduced directly through the gap of electrode.

Preparation of Chitosan-Coated PE Films. Chitosan was dissolved in 1% w/v acetic acid aqueous solution to obtain different concentrations of chitosan solutions (0.125, 0.25, 0.5, 0.75, 1.0, and 2.0% w/v, based on the volume of the acetic acid solution) and stirred overnight at room temperature. To make the PE films hydrophilic and chemically reactive, the PE films were treated with the DBD plasma. The PE films were cut into square shape (6 cm ×6 cm) before placing on the dielectric glass for the DBD plasma treatment. After that, the plasma-treated PE films were immediately immersed in the chitosan solution with constant stirring for 1 min, followed by washing with distilled water to accomplish pH neutralization. The chitosan-coated PE films were air-dried at room temperature overnight prior to characterizations.

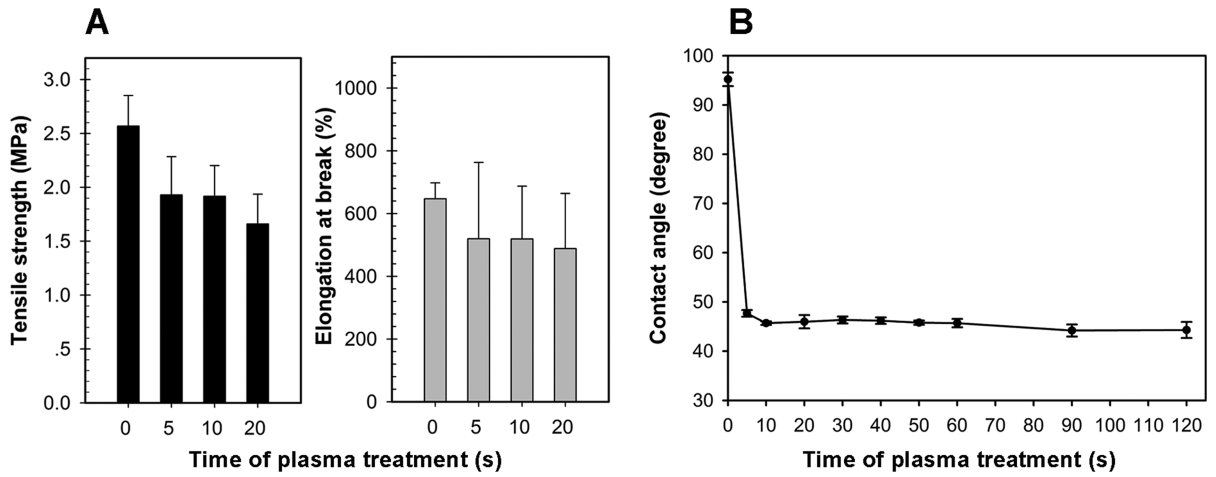

Figure 2. Effect of plasma treatment time on (A) mechanical properties and (B) water contact angle of the plasma-treated PE films.

Characterization Technique. Mechanical properties in terms of tensile strength and elongation at break of the untreated and the plasma-treated PE films were detected by a Universal Testing Machine (Lloyd, Model LRX) at 25 °C. The films were cut into square shape (15 cm ×15 cm) and equipped with a 500 N load cell. A strain rate of 10 mm min^{-1} and a gauge length of 50 mm were employed according to ASTM D882−91 standard test method.

Water contact angle of the untreated and the plasma-treated PE films was evaluated by using contact angle analyzer system G10 (Krüss, DSA10 MK2), according to the sessile drop technique. All measurements were performed at room temperature using deionized water. A water droplet of 10 μL was placed on the film surface and the diameter of the droplet was noted after 10 s of the application. The drop image was then stored via a video camera. The contact angle values were obtained using Laplace Young curve fitting based on the image of water drop. The value of the statistic contact angle is an average of ten values.

Atomic force microscope (AFM, XE-100, Park systems) was used to determine surface roughness of the films. The Root Mean Squared (rms) roughness and the topographic profiles measured on 10 μm × 10 μm images were evaluated. For each sample, the roughness value was obtained from ten different areas.

Surface chemical composition of the untreated and the plasma-treated PE films was observed by an attenuated total reflection-Fourier transform infrared spectroscope (ATR-FTIR, Thermo Nicolet Nexus 670) and X-ray photoelectron spectroscope (XPS, JEOL, JPS-9000MX). The ATR-FTIR spectra were investigated between the wavenumber ranging from 4000 to 650 cm^{-1} with 64 scans at a resolution of 4 cm^{-1}. For XPS analysis, excitation was via the Mg K_α radiation ($h\nu$ = 1253.6 eV) with an emission voltage and a current of the source equal to 12 kV and 10 mA, respectively. The hydrocarbon component of C1s spectrum at 285.0 eV was used as an internal standard of the energy scale. The C1s peaks were deconvoluted using Gaussian−Lorentzian component profile.

To confirm the existence of chitosan deposited on the PE films, the chitosan-coated PE films were immersed in 0.01% w/v Amido Black 10B aqueous solution for 12 h. The films were then washed with distilled water to remove an excess dye, followed by observing the dispersion and distribution of the deposited chitosan by an optical microscope. The amount of chitosan coated on the untreated and the plasma-treated PE films was determined by Kjeldahl nitrogen analysis. A film with a precise size of 6 cm ×6 cm was put into the digestion flask. Concentrated H_2SO_4 (5 mL) and $CuSO_4{\cdot}5H_2O$ (0.05−0.1 g) were subsequently added into the digestion flask before heating it on a heating mantle for 2 h. After heating, decomposition of the film was indicated by visual observation of color change into dark black. Then five drops of H_2O_2 was added into the decomposed sample followed by further heating until the solution became transparent and colorless. The resulting solutions were subjected to the distillation step of the Kjeldahl method. Twenty mL of 0.01 M HCl aqueous solution was added into an Erlenmeyer flask (200 mL) and set to the end of the condenser. NaOH aqueous solution (40% w/v) was added into the digested sample through the distillation column in the closed system. The ammonium ions from chitosan were distilled in the form of ammonia gas by a stream. The ammonia gas was allowed to pass through a trapping solution (0.01 M HCl aqueous solution) where it dissolved and became an ammonium ion once again. Finally, the amount of the ammonia was determined by titration with a standard solution (0.01 M NaOH aqueous solution). Chitosan content in the chitosan-coated PE films was calculated by the following equation:

$$\text{amount of chitosan (g)} = ((V_1M_1 - V_2M_2)/1000) \times 161.06\ \text{g/mol of chitosan} \quad (1)$$

Where V_1 and V_2 are volume of HCl solution and NaOH solution, respectively, and M_1 and M_2 are concentration in molarity (M) of HCl solution and NaOH solution, respectively, ($V_1M_1 - V_2M_2$ = mmol of consumed HCl solution = mmol of nitrogen).

Antibacterial Evaluation. Antibacterial activity of the neat and the chitosan-coated PE films was evaluated based on the colony count method against Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*), according to the standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions (ASTM E 2149−01). Briefly, a broth solution was prepared by mixing beef extract (0.3 g) and peptone (0.5 g) in 100 mL of water. An inoculum was prepared by transferring one colony of each microorganism into 20 mL of a broth solution. The mixture was cultured at 37 °C in a shaking incubator at a speed of 150 rpm for 24 h. The cell suspension of each microorganism was then diluted with 0.85% sterile NaCl aqueous solution by a factor of 10^6 for *S. aureus* and 10^5 for *E. coli*. Sample with a precise shape of 3 cm ×3 cm was added into the cell suspension. The suspension was shaken in a shaking incubator under a controlled temperature of 37 °C at a shaking speed of 150 rpm. After the incubation time of 3 h, 100 μL of the suspension was dipped and spread on the sterilized nutrient agar in Petri dishes (circular disk: 15 cm in diameter). Bacterial growth was visualized after incubation at 37 °C for 24 h. The percentage of bacterial reduction was calculated by the following equation:

$$\text{bacterial reduction rate (\%)} = ((\text{CFU}_{\text{in control}} - \text{CFU}_{\text{in chitosan-coated sample}})/\text{CFU}_{\text{in control}}) \times 100 \quad (2)$$

Where control is the neat plasma-treated PE film. The experiments were carried out in triplicate for each formulation.

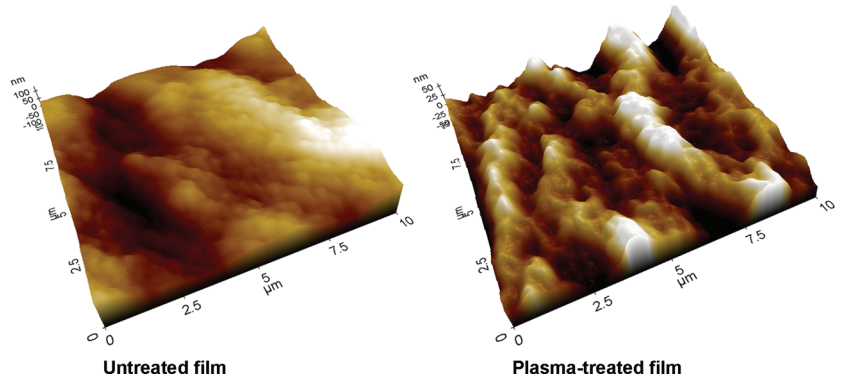

Figure 3. Three-dimensional AFM images of the untreated and the plasma-treated (after the plasma treatment time of 10 s) PE film surface.

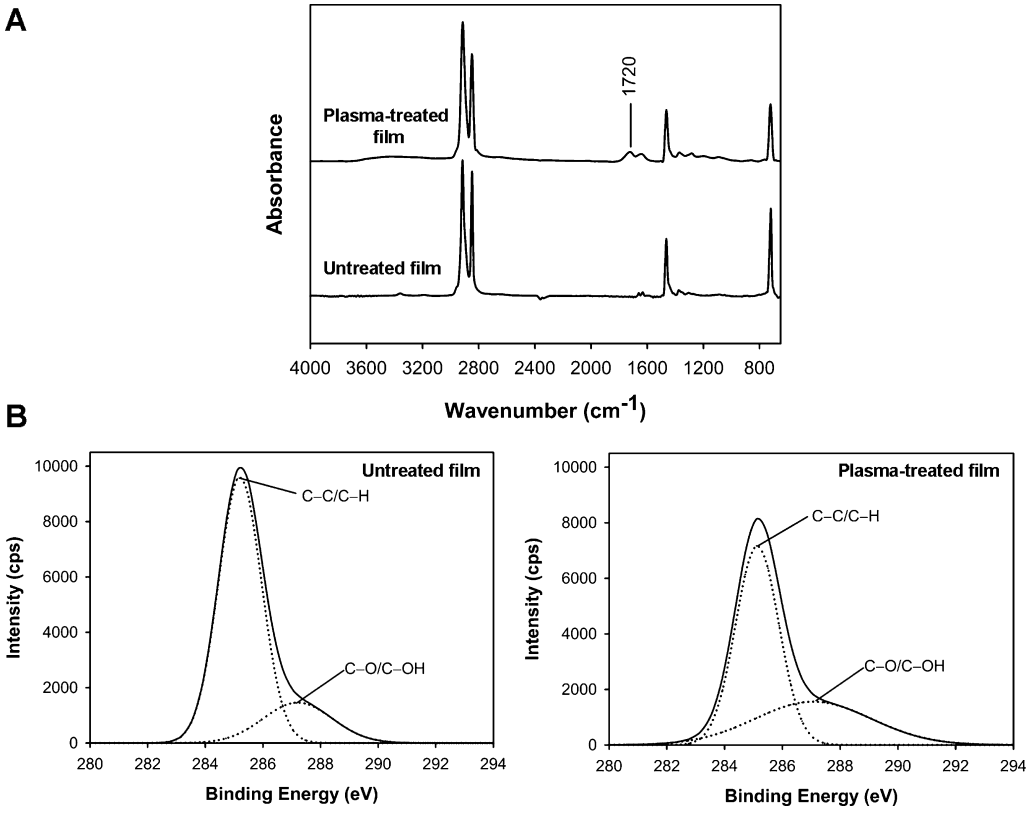

Figure 4. (A) ATR-FTIR and (B) XPS spectra of the untreated and the plasma-treated PE films after the plasma treatment time of 10 s.

RESULTS AND DISCUSSION

Effect of Plasma Treatment Time on Mechanical Property and Water Contact Angle. Tensile strength and elongation at break of the untreated and the plasma-treated PE films with different plasma treatment times are shown in Figure 2A. At the plasma treatment time of 5 s, both the tensile strength and the elongation at break of the plasma-treated PE films slightly decreased from those of the untreated one. However, when the plasma treatment time was prolonged to 10 and 20 s, no statistically significant difference ($P > 0.05$) in the mechanical properties was found. Reduction of the mechanical properties in terms of tensile strength and elongation at break of the PE film after the film was treated with plasma is in agreement with the work reported by Shin et al.,[17] who concluded that mechanical properties of polymeric films are influenced mainly by energy from the plasma source not from the treatment time.

The contact angle (θ) is a variable that determines the wettability of a surface. The tendency of a liquid drop to spread out over a flat surface increases as the contact angle decreases. Thus, high contact angle indicates the poor wetting. The contact angle is determined by the force balance between adhesive (the forces between liquid and solid) and cohesive (the forces within the liquid). Therefore, a water-wettable surface may indicate its hydrophilic property.[30] Figure 2B is the variation curve of water contact angle versus DBD plasma treatment time of the PE films. As can be seen in Figure 2B, the water contact angle dramatically decreased from approximately 95 to 48° when the plasma treatment time was 5 s. The value of water contact angle still decreased slightly until the plasma treatment time of 10 s was reached. After that the contact angle remained constant at 45.7°, even the plasma treatment time was prolonged to 120 s. The result suggests that the plasma treatment time of 10 s provided a saturation state of air DBD

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plasma treatment of the PE film and thus was selected for further experiments. The decreasing of the water contact angle demonstrates that the DBD plasma treatment increases the hydrophilicity of the PE film, which might be explained by the appearance of polar functional groups on the surface of the PE film after the DBD plasma treatment.

Effect of DBD Plasma Treatment on Surface Morphology of PE Film. To investigate the change of the film surface before and after the plasma treatment, we used AFM observation to present a three-dimensional surface view. Figure 3 shows the AFM images of the untreated and the plasma-treated PE surface after the plasma treatment time of 10 s. Clearly, the DBD plasma treatment significantly altered the surface morphology of the PE films. While most areas of the untreated PE surface were quite smooth, the prominent parts appeared on the surface of the plasma-treated PE films. Furthermore, the change in the surface roughness can be quantified by the Root Mean Square (rms) roughness value, which refers to the average size of peaks and valleys within the interest area. Lower rms numbers indicate a smoother surface. It could be calculated from Figure 3 that the rms of the untreated PE film was 29.35 ± 8.94 nm, whereas this value increased to 37.33 ± 9.03 nm after the plasma treatment time of 10 s. The results indicate that DBD plasma species strongly impact on the PE surface by removing the top layer of the surface. This phenomenon may relate with the physical or chemical removal of molecules, chain scission, and degradation process.[31]

Chemical Composition of the DBD Plasma-Treated PE Surface. Surface chemical modification induced by the DBD plasma treatment in air was characterized by ATR-FTIR and XPS. Figure 4A shows the ATR-FTIR spectra of the untreated and the plasma-treated PE films after the plasma treatment time of 10 s. Because the chemical structure of PE is composed almost completely of methylene (CH_2) groups, infrared spectrum of the untreated PE film composed of four sharp peaks including the peaks corresponding to the methylene stretching at 2920 and 2850 cm^{-1} and to the methylene deformations at 1464 and 719 cm^{-1}. After the plasma treatment, new peaks at 1720 cm^{-1} corresponding to C═O stretching vibration and at the region of 3200−3800 cm^{-1} corresponding to hydroxyl group (−OH) vibration appeared.[32]

Figure 4B shows deconvoluted C1s of XPS spectra of the untreated and the plasma-treated PE films after the plasma treatment time of 10 s. The XPS spectra of both the untreated and the plasma-treated PE films could be fitted into two components: (1) a component at 285.0 eV assigned to carbon linked to carbon itself or to hydrogen (C−C/C−H); and (2) a component at 286.7 eV assigned to carbon linked to single oxygen (C−O/C−OH).[31] The corresponding quantitative atomic composition and atomic ratio of the PE films determined by XPS are given in Table 1.

According to Table 1, although the O/C atomic ratio of the untreated PE film was 0.26, this value increased to 0.39 after the plasma treatment. Furthermore, an increase in the N/C atomic ratio from 0.037 to 0.040 was observed for the untreated and plasma-treated PE films, respectively. The increments of oxygen-containing carbon peak area and of the O/C atomic ratio reveal that, oxygen in air participates in chemical reaction to form new oxygen-containing groups on the surface of the plasma-treated PE films.

Ren et al.[27] had also studied on surface modification of the PE film by the DBD plasma treatment in air and found the similar oxygen-containing groups on the PE film surface after the plasma treatment. However, in their XPS result, they found two new peaks in addition to the peaks at 285.0 and 286.7 eV. The new peaks were at 288.0 eV assigned to ketone [−(C═O)−] and/or acetal [−(O−C−O)−], and at 289.2 eV assigned to carboxyl [−(C═O)−O−]. The loss of these peaks in our result could be a result from the difference in the operational condition. Compared with our study, higher voltage and frequency (i.e., 16 kV and 4 kHz), lower electrode gap (i.e., 3 mm), and longer treatment time (i.e., 20 s) were operated. However, because the ATR-FTIR spectra of our result also exhibited a peak corresponding to C═O, another reason for the loss of this peak in the XPS spectrum could be the tendency of this active species to be quickly neutralized by atmospheric contaminants before the XPS observation.

It was found that oxygen-containing components including C−O, C═O, and −OH occurred after the DBD plasma treatment. The introduction of the new oxygen-containing groups in the polymer surface is the main reason for the increase in the hydrophilicity of the PE film. As a consequence, it can be confirmed that DBD plasma treatment is an effective method to generate hydrophilic groups on the PE surfaces.

Effect of DBD Plasma Treatment on Surface Coating of Chitosan on the PE Film. The effect of the DBD plasma treatment on surface coating of chitosan on the PE film was determined by comparing the amounts of the chitosan coated on the untreated and the plasma-treated PE films. Both the untreated and the plasma-treated PE films were coated with chitosan by immersing the PE films in the chitosan acetate solutions having different chitosan concentrations. The amounts of chitosan coated on the PE films were then determined by the Kjeldahl nitrogen analysis. Before this step, suitable number of washing cycle was performed after chitosan coating in order to remove the loosely bound and unbound chitosan from the film surface. The PE films immersed in 2% chitosan acetate solution were used in this study. Figure 5A shows relation between the number of washing cycle and the amount of chitosan deposited on the PE films as characterized by the Kjeldahl method. It was found that the amounts of chitosan deposited on the PE films slightly decreased with the increase in the number of washing cycle and became constant after washing for three times. Therefore, the chitosan-coated PE films were washed three times before determination of the amounts of chitosan coated on the PE films. Figure 5B shows the comparison on the amounts of coated chitosan on the untreated and plasma-treated PE films immersed in different chitosan concentrations. For the untreated PE films, chitosan could not be deposited on the film surface at any chitosan concentrations. On the other hand, the amount of chitosan coated on the plasma-treated PE films increased with the increase in the chitosan concentrations. These results suggest that the DBD plasma treatment of the PE films could enhance the interaction between chitosan and the plasma-treated PE film.

Table 1. Relative chemical composition and atomic ratio of the PE films determine by XPS

	chemical composition (%)			atomic ratios	
samples	C1s	O1s	N1s	O/C	N/C
untreated	77.13	20.03	2.84	0.26	0.037
plasma-treated	69.78	27.42	2.81	0.39	0.040

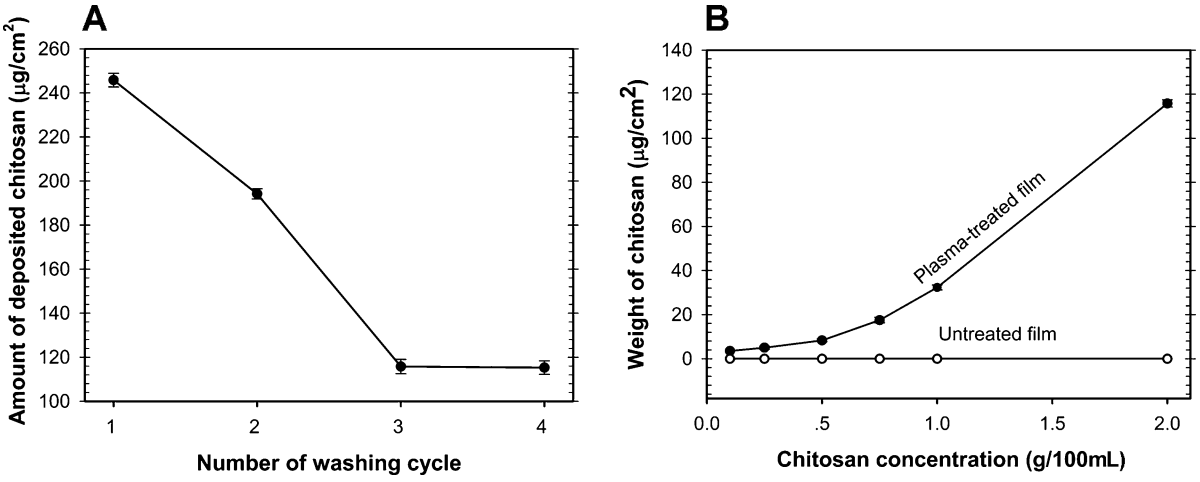

Figure 5. (A) Effect of number of washing cycle on amount of chitosan deposited on the PE films and (B) comparison on the amounts of coated chitosan on the untreated and plasma-treated PE films immersed in different chitosan concentrations.

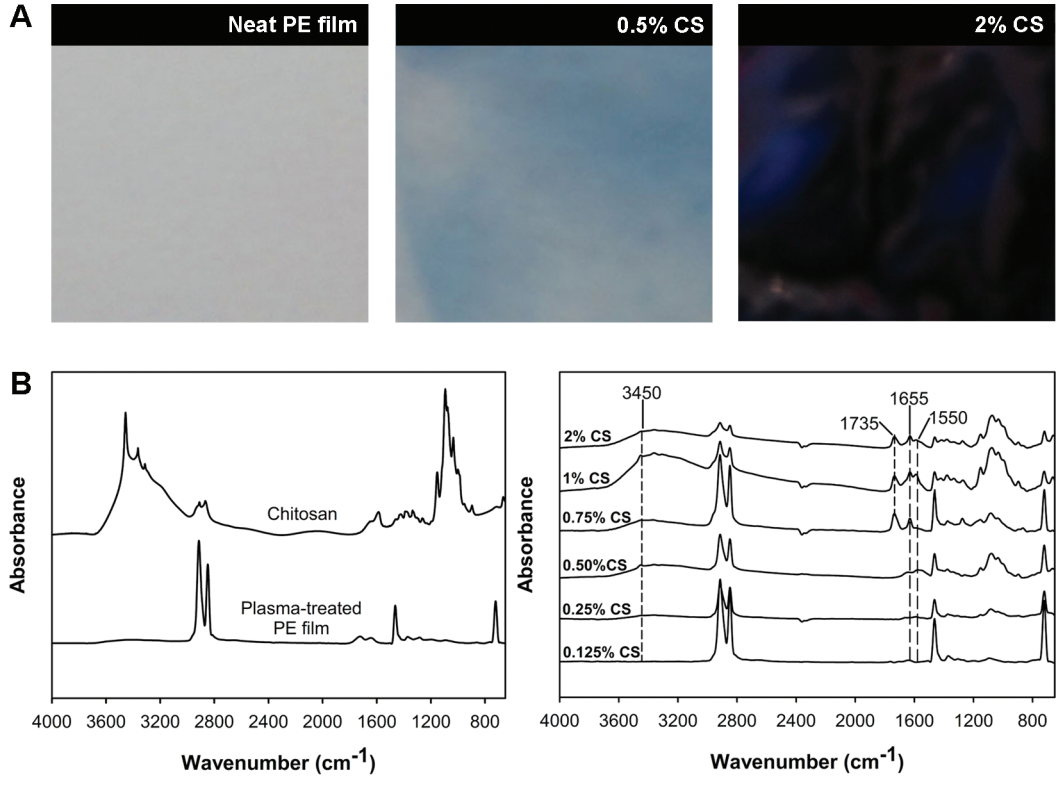

Figure 6. (A) Photographic images of the neat PE film and the plasma-treated PE films coated with 0.5 and 2% w/v chitosan acetate solutions, obtained after staining in Amido Black 10B aqueous solution for 12 h. (B) ATR-FTIR spectra of the plasma-treated PE film, the neat chitosan, and chitosan-coated plasma-treated PE films having different chitosan contents.

Film Staining. Deposition of chitosan on the PE films was confirmed by staining the PE films with Amido Black 10B aqueous solution. Amido Black 10B is an anionic dye that can interact with amino groups of chitosan. Owing to the positively charged nature of chitosan, the anionic dye will selectively be adsorbed by chitosan, not by PE. Figure 6A illustrates photographic images of the neat PE film and the plasma-treated PE films coated with 0.5 and 2% w/v chitosan acetate solutions. It was evident that no specific interaction between the neat PE film and the anionic dye was observed. On the other hand, blue color was seen on the chitosan-coated PE films, indicating the presence of chitosan on the plasma-treated PE films. In addition, the intensity of the dye color increased with the increase in the chitosan concentrations. The appearance of the dye color on the chitosan-coated plasma-treated PE films resulted from an occurrence of specific interaction between the coated chitosan and the dye molecules, confirming a successful coating of chitosan on the plasma-treated PE films. It is clearly demonstrated that the DBD plasma treatment in air could improve the adhesion between chitosan and the PE film.

Figure 6B shows ATR-FTIR spectra of the plasma-treated PE film, the neat chitosan, and chitosan-coated plasma-treated PE films obtained by using different concentrations of chitosan.

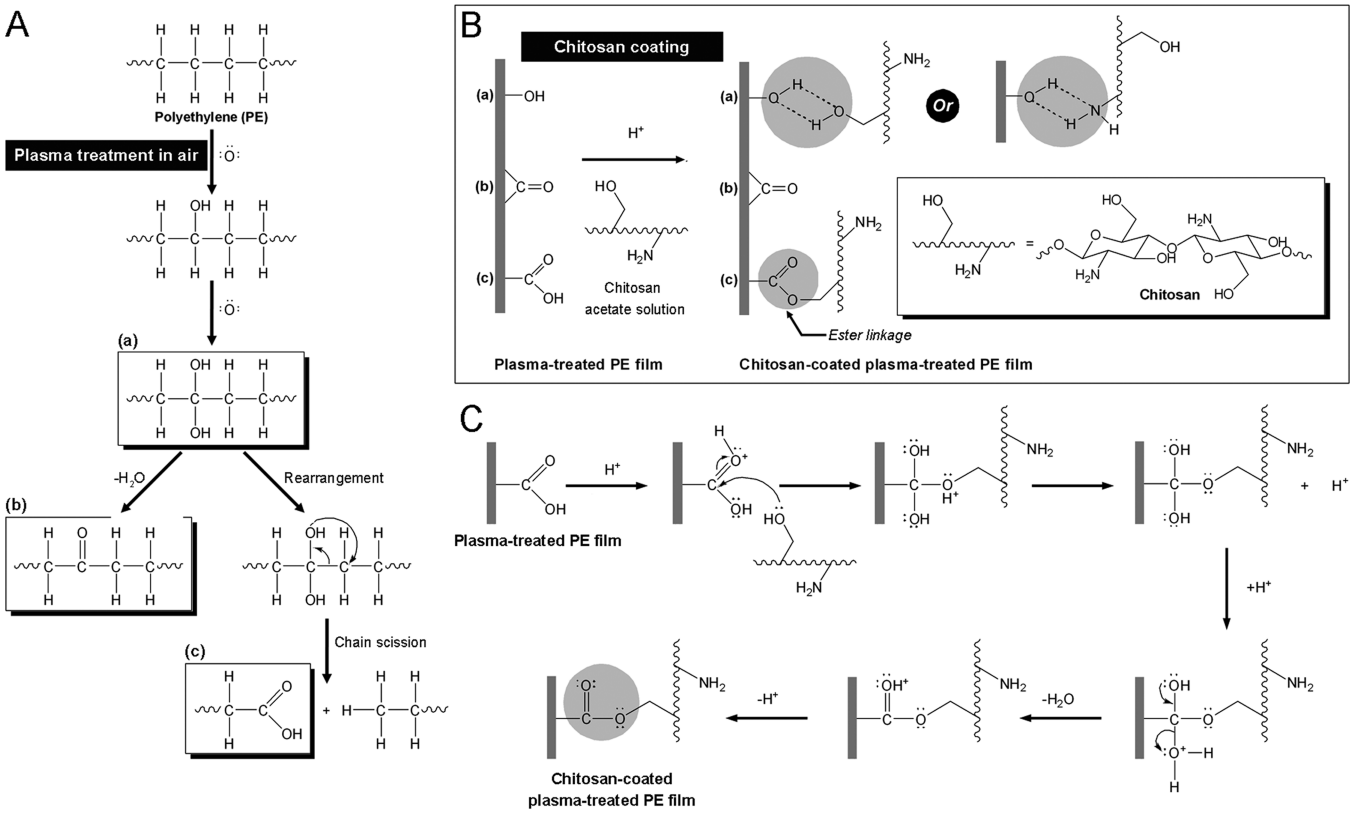

Figure 7. (A) Mechanism for the atmospheric plasma oxidation proposed by Gonzalez and Hicks,[32] (B) proposed scheme illustrates chitosan coating site on the plasma-treated PE film, and (C) possible mechanism for the chitosan coating on the plasma-treated PE film via ester linkage formation.

The neat chitosan displays characteristic absorption bands at 1655 and 1550 cm^{-1}, corresponding to the vibrations of amide I and amide II, respectively. The overlap of N−H and O−H stretching of the carbohydrate ring was observed in a large band covering the range of 3250 to 3460 cm^{-1}.[33] For the chitosan-coated plasma-treated PE films, the characteristic peaks of chitosan at 3450 cm^{-1} (N−H and O−H stretching) and at 1655 and 1550 cm^{-1} (amide I and amide II) were observed. Moreover, the intensities of these peaks tended to increase with an increase in the chitosan content coated on the PE films. In addition to the characteristic peaks of chitosan, a new peak at 1735 cm^{-1} representing C═O stretching vibration of ester group[32] was evidenced when the chitosan content on the PE film reached to 0.75%. It might be concluded that chitosan was coated on the plasma-treated PE films by the formation of covalent bonds occurring via ester linkages.

Proposed Mechanism for the Interaction between the Plasma-Treated PE Films and Chitosan. Previously, Gonzalez and Hicks[32] proposed a mechanism for the atmospheric plasma oxidation of high density polyethylene (HDPE). In the proposed mechanism, oxygen molecules presenting in air insert across C−H bonds to form hydroxyl (−OH) species. These species may subsequently pass through two possible pathways; (1) they may lose water to form a ketone or (2) they may undergo rearrangement and cause chain scission, leading to the formation of a carboxylic group at the chain end. The formation of the functional groups as described in the above-mentioned mechanism after the plasma treatment was also found in this study, including the appearance of C═O (of ketone or carboxylic acid), C−O, and −OH groups on the plasma-treated PE films.

As evidenced in the ATR-FTIR spectra of the chitosan-coated plasma-treated PE films, the oxygen-containing polar functional groups formed on the PE films after plasma treatment may interact with hydroxyl groups (−OH) of chitosan by the formation of ester linkages. The active position on the plasma-treated PE film, where ester linkages occur, may be at the carboxylic groups (−COOH). Briefly, the simplest method of the ester formation is the Fischer method, in which a hydroxyl group and a carboxylic group are reacted in an acidic medium.[34] Since chitosan dissolved in acetic acid solution was used in the coating step, therefore, the acid solution could be act as an acid catalyst for the ester formation. In addition, intermolecular hydrogen bonds between hydroxyl groups on the plasma-treated PE film and hydroxyl groups or amino groups ($-NH_2$) of chitosan may occur. Figure 7 shows the mechanism for the atmospheric plasma oxidation proposed by Gonzalez and Hicks,[32] proposed scheme illustrates chitosan coating site on the plasma-treated PE film, and our proposed mechanism for the chitosan coating on PE films via the formation of ester linkage. According to Figure 7C, after the plasma-treated PE film was immersed in the chitosan in acetic acid solution, carboxylic groups of the film will be protonated by acetic acid. Subsequently, −OH groups or $-NH_2$ groups of chitosan will exhibit as nucleophiles by attacking C atom of the protonated carboxylic groups on the plasma-treated PE film. As a result, ester linkages will be formed and caused the chitosan to chemically bond on the plasma-treated PE films.

Antibacterial Activity Test. Packaging plays a vital role in food preservation. Microbial contamination is one of the most important factors affecting the shelf life of food. Accordingly, antimicrobial packaging is a promising form of active food

packaging. Antibacterial property of chitosan depends on several factors such as its concentration, molecular weight, degree of deacetylation, and type of bacteria.[35,36] To evaluate the antibacterial activity of the chitosan-coated plasma-treated PE films, we tested the films with different chitosan contents (i.e., 0.25, 0.75, and 2.0%) against two commonly studied microbes, i.e., Gram-positive *S. aureus* (TISTR no. 1466) and Gram-negative *E. coli* (TISTR no. 780), by using the colony count method. The result is shown in Figure 8. It was found

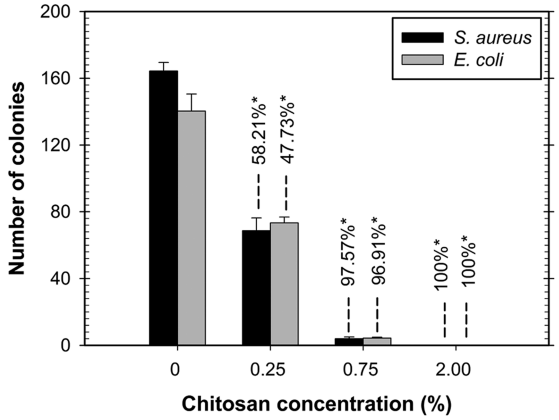

Figure 8. Number of colonies of the neat PE film and the chitosan-coated plasma-treated PE films containing 0.25, 0.75, and 2% w/v chitosan and the corresponding bacterial reduction rate (BRR*, %) against *S. aureus* and *E. coli*.

that after the films were in contact with the bacterial cells for 3 h, the number of colonies of both bacteria decreased with the increase in the chitosan content in the films. The values of bacterial reduction rate (BRR) of the chitosan-coated plasma-treated films containing 0.25% chitosan against *S. aureus* and *E. coli* were 58 and 48%, respectively, and the BRR against both bacteria reached 100% when the chitosan content in the PE films was 2%. It might be implied that the chitosan coated on the PE films is responsible for the antibacterial activity of the films. Mechanism for the antimicrobial activity of chitosan relies on the interaction between the positively charged molecules of chitosan and the negatively charged molecules of bacterial cell membrane. Specifically, the interaction is mediated by electrostatic forces between protonated NH_3^+ groups of the chitosan and phosphate groups in phospholipid bilayer of the bacterial cell membrane. This interaction results in deformation of the cell membrane and consequently disrupts its functions including internal osmotic balance and cell permeability, leading to the leakage of intracellular electrolytes such as potassium ions and other low-molecular-weight substances such as nucleic acid and glucose. As a result, the growth of the bacteria is inhibited and eventually causing cell death.[37,38]

CONCLUSION

Chitosan was successfully coated on the PE films by increasing the surface activity of the PE films with the DBD plasma treatment in air before chitosan coating. The modification of the film surface by the DBD plasma effectively increased the surface roughness and generated oxygen containing polar functional groups, including C═O, C−O, and −OH, on the plasma-treated PE film surface. As a result, hydrophilicity of the film surface increased and thus coating of chitosan on the PE film was achieved. The amount of chitosan coated on the PE films was determined after removal of the loosely bound chitosan by washing the chitosan-coated PE films in water for three times. Therefore, only chitosan that was chemically bonded on the PE surface was remained on the PE films. Our findings remark that DBD plasma treatment is an effective technique for enhancing the adhesion between chitosan and the PE films. The chitosan-coated plasma-treated PE films exhibited strong antibacterial activity against both Gram-negative *E. coli and* Gram-positive *S. aureus.*

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel.: (662)2184132, Fax: (662)2154459. E-mail: ratana.r@chula.ac.th.

Notes

The authors declare no competing financial interest.

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