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Surface modification of atmospheric plasma activated BOPP by immobilizing chitosan

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Summary

Chitosan was immobilized onto plasma activated biaxially oriented polypropylene (BOPP) films aimed at producing antimicrobially active barrier film for food packaging applications. 1% chitosan dissolved into 0.1 M acetic acid was mixed with 0.1% glutaraldehyde (cross-linking agent) and applied onto N_2-plasma + NH_3 activated BOPP film. Amount of immobilized chitosan was 1.8 g/m^2. Films had strong antimicrobial activity against both *Bacillus subtilis* and *Escherichia coli* and they reduced the oxygen transmission rate (OTR) measured in dry conditions from 1500 down to 27 cm^3/(m^2·24 h). Migration tests for determining the total amounts of substances migrating into food simulants (3% acetic acid, 95% ethanol and iso-octane) indicated, that chitosan coating was permanently immobilized onto BOPP without any leaching (total migration < 2 mg/dm^2), thus it met the requirements stipulated in Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs. The results suggest that chitosan treated BOPP films may be exploited in various food packaging applications requiring high oxygen barrier and/or antimicrobially active packaging materials.

Introduction

Antimicrobial packaging materials are interesting and promising applications of advanced active food packaging systems. They can effectively control the microbial contamination of various solid and semisolid foodstuffs by inhibiting the growth of microorganisms on the surface of the food, which normally comes into direct contact with the packaging material. Chitosan, the β-1-4-linked polymer of 2-amino-2-deoxy-β-D-glucan, is prepared by N-deacetylation of chitin, the second most abundant natural biopolymer after cellulose. Chitosan is edible and biodegradable material, which also has antimicrobial activity against different groups of microorganisms, both bacteria, yeasts and moulds [1]. As the amino group of chitosan is positively charged at below pH 6, it has a better antimicrobial activity than chitin and many other

biopolymers [2]. In addition, due to its good film-forming properties, chitosan has been successfully used in food packaging materials [3]. The preparation of chitosan films [4-9] and chitosan laminated with pectin [10] or polyethylene [11] or mixed with lipids [12] have been reported. Films have been formed by dissolving chitosan into hydrochloric, formic, acetic, lactic and citric acid solutions [13]. Chemically modified chitosan can be attached to various substrates e.g. sugars, dendrimers, cyclodextrins, crown ethers, and glass beads, forming interesting multifunctional materials [14].

Plasma treatment can be utilized in many ways for modifying surface properties of food packaging materials to improve both safety and quality of foods [15]. NH_3 and CO_2 -plasmas have been used to incorporate amino groups [29-32] and carboxyl groups [33,34] at polymer surfaces. Recently, plasma treatments have been exploited to improve the adhesion between chitosan and other polymers like PP [16] or PLLA [17]. Chitosan has also been immobilized onto acrylic acid grafted HDPE [18], PP [19], PET [20,21], glass [22] and PHB [23] using carbodiimide [19,21,22] and onto N-vinylformamide (NVF) grafted PET [20] or silk fibroin [26] via glutaraldehyde [20] as a coupling agent. Carbodiimides are generally utilized as carboxyl activating agents for amide bonding with primary amines whereas glutaraldehyde is used as crosslinking agent between amines. The aim of this study was to produce active biocoatings with barrier properties for food packaging materials by exploiting atmospheric plasma treatment. The work was carried out in EU-funded project "Functional nano-composite barrier coatings on plastic films via an aerosol assisted atmospheric plasma process" (GRD1-2001-41847).

Experimental

Materials

Chitosan, medium molecular weight, was obtained from Aldrich Chemical Company, Inc., Milwaukee, WI, USA. Glutaraldehyde, 25% solution was obtained from Merck-Schuchardt, Hohenbrunn, Germany and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) from Fluka. Acid Orange 7 was obtained from Sigma-Aldrich and Toluidine Blue O from Merck. Biaxially oriented polypropylene (BOPP) films (thickness 25 μm) were from Innovia Films, United Kingdom.

Plasma activation

Plasma activation was carried out in a dielectric barrier discharge (DBD)-reactor at atmospheric pressure (Figure 2.). The configuration consists of two parallel electrodes (20 × 25 cm) covered with a dielectric material, in this case glass. The inter-electrode distance was set at 2 mm and the BOPP sheets were placed on the lower electrode. Standard purity nitrogen was used as inert carrier gas. The flow rate was controlled by mass flow controllers and set at 20 L/min. During activation NH_3 and CO_2 were added to the nitrogen flow at a rate of 3 L/min. An AC-field with a frequency of 2 kHz, generated by a 20 kV/200 mA AC power supply was applied to the electrodes, giving rise to a transient, spatially uniform glow with a power density of 0.5 W/m^2. The BOPP sheets were treated during 0.5 min.

Determination of surface densities of amino and carboxyl groups

Surface densities of amino and carboxyl groups were evaluated from the uptake of acidic and basic dyes [35]. For determination of amino groups, film samples (0.5 dm^2) were immersed in 20 mL of 0.01 g/mL Acid Orange 7 of pH 3 at room temperature for 5 h to yield an ion complex between the amino groups and the acidic dye, and then washed with distilled water, followed by 1 mM HCl to remove non-complexed dye molecules. The dye molecules complexed to the film surface were desorbed into 1 M NaOH and the optical density at 485 nm was measured on the resulting supernatant. Carboxyl groups were complexed with 20 mL of 0.01 g/mL Toluidine Blue O of pH 10 at room temperature for 5 h. Non-complexed dye was removed with distilled water and 1 mM NaOH and desorption of of dye molecules complexed to the carboxyl groups on the film surface was conducted with 50% acetic acid solution. The dye concentration was determined at 633 nm by using a spectrophotometer and calculated from the calibration curve. The surface densities of functional groups were calculated assuming that Acid Orange 7 and Toluidine Blue O were complexed to equivalent moles of amino and carboxylic groups.

Chitosan coating

Chitosan (1% w/v) was dissolved in 0.1 M acetic acid by stirring on a magnetic stirrer for two days. 0.1% glutaraldehyde was added after which the final coating solution was immediately applied onto BOPP films. Drying of the coated films was performed at 80 °C for two hours.

Fourier transform infrared spectroscopy (FTIR)

FTIR (Bruker Equinox 55 spectrometer with photo acoustic detector) was used to determine the chemical changes between the original and surface treated films as well as to confirm the successful immobilization of chitosan onto BOPP surface.

Measurement of water contact angle

Water contact angles of the films were measured using a CAM-200 equipment (KSV Instruments, Finland) in test conditions of 23 °C and 50 % relative humidity. Contact angle values were measured after incubation for 2 seconds.

Scanning electron microscopy (SEM)

SEM (LEO DSM 982 Gemini; FE-SEM) was used to investigate the surface topography of original and surface treated BOPP films as well as the cross-section of chitosan coated films. About 1 x 1.5 cm^2 pieces of the studied films were attached to sample holders (paper binder clips) in cross-section orientation and attached to the SEM stage with the help of douple sided carbon tape. To image the surface topography, the SEM stage was tilted about 10° degrees from horizontal position. Imaging was performed using 2 keV electron to avoid the need of additional conductive coating of the films (thus allowing direct imaging of the original surface topography). Due to high tilt of the studied surface, only a small portion in the middle of the SEM micrographs is in focus (Figure 4.).

Gas transmission determination

Oxygen transmission measurements were performed with Ox-Tran 2/20 Oxygen Transmission Rate System (Mocon, Modern Controls, Inc., USA) using the method described in the standard ASTM D3985-02. Tests were carried out at 23 °C and 0% relative humidity using 100% oxygen as test gas. Aluminium foil masks, with an inner diameter area of 5 cm^2, were used to mount film samples in the diffusion cell. Carbon dioxide and ethylene transmission measurements were performed with a gas chromatograph. Film samples were placed between cell halves, with an open area of 78.5 cm^2. One side of the cell was filled with 100% nitrogen gas and the other side with 100% carbon dioxide or ethylene gas. After 6 or 24 hours gas samples were taken from nitrogen side and the amounts of test gases were analysed gas chromatographically using thermal conductivity detector (TCD). Tests were carried out at 23 °C and 0% relative humidity.

Migration test

Migration tests were carried out as described in European prestandard ENV 1186-3 Materials and articles in contact with foodstuffs - Plastics - Part 3: Test methods for overall migration into aqueous food simulants by total immersion'. 3% acetic acid and 95% ethanol were used as food simulants in test conditions of 10 days at 40 °C. Iso-octane, which can be used as an alternative fatty food simulant, was used in conditions of 2 hours at 20 °C. Tests were performed by immersing test specimens in food simulant, after which the simulant was evaporated to dryness and the overall mass of the residue was determined.

Antimicrobial activity

The antimicrobial activity of the coated films against *Escherichia coli (*ATCC 11775*)* and *Bacillus subtilis* (Merck 1.10649*)* was measured using the antimicrobial drop test [24]. *Escherichia coli* culture cultivated in Trypticase soy broth (BBL) for 24 h at 37 °C and *Bacillus subtilis* spore suspension were diluted into sterile peptone-saline to contain approximately 1×10^6 cfu/mL. The films were cut to 1.5×1.5 cm^2 test pieces and each piece was placed into a Petri dish. 0.1 mL of bacterial suspension was placed on each test piece. The Petri dishes were placed on a tray containing a wetted paper sheet, covered with a lid and incubated for 24 h at 30 °C. After incubation 5 mL of peptone-saline was added in the Petri dishes and the bacteria were washed from the test pieces by shaking (Infoss AG CH 4103 orbital shaker, 100 rpm) for 5 min at 25 °C. The number of surviving bacteria was measured by plating on Trypticase soy agar (BBL) plates and incubating for 24 h at 37 °C (*Escherichia coli)* or 30 ºC (*Bacillus subtilis).*

Results and discussion

Chitosan can be immobilized onto plasma activated BOPP by exploiting either carbodiimide or glutaraldehyde chemistries (Figure 1.). Carbodiimide is capable of forming covalent bonds between carboxylic groups of the activated BOPP and amino groups of chitosan. In more detail, EDC carbodiimide activated the carboxyl group of the film to form an active *O*-acylisourea intermediate, allowing it to be coupled to the amino group of chitosan. A by-product was released as a soluble urea derivative after substituted by chitosan, thus no spacer existed between the molecules being coupled.

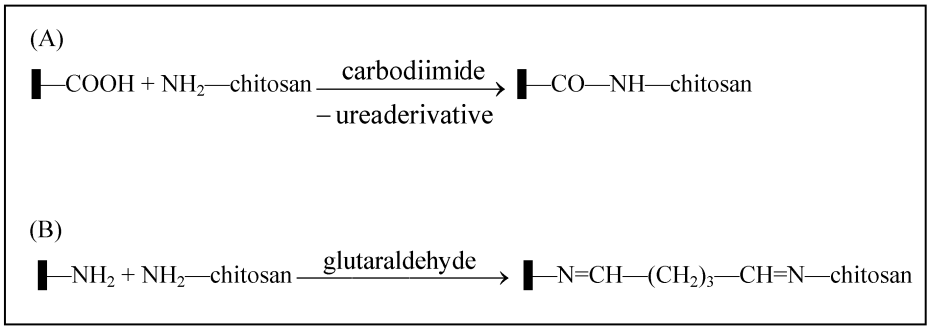

Figure 1. Schematic diagram of immobilization of chitosan onto (A) carboxyl activated and (B) amino activated food packaging film (BOPP).

The mechanism with glutaraldehyde is based on the formation of imine bonds between aldehyde groups of glutaraldehyde and amino groups of chitosan and amino activated substrate [25]. Nitrogen was chosen as the carrier gas during plasma polymerisation because of its excellent properties with respect to activation of polymers and its low price compared to other commonly used carrier gases like helium and argon. NH_3 and CO_2 were mixed with the carrier gas in order to graft amino and carboxyl functionalities, respectively, onto the polymer surface. Plasma parameters including treatment time, plasma power, frequency, flowrate and electrode distance were optimized for optimal plasma homogenity and maximum concentration of functional groups at the highest temperature acceptable for treatment of BOPP films. N_2-plasma + CO_2 and N_2-plasma + NH_3 treatments (Figure 2.) produced carboxyl group densities of 0.90 nmol/cm^2 and amino group densities of 1.7 nmol/cm^2 onto BOPP, respectively. 0.1% glutaraldehyde and 0.8% EDC (carbodiimide) were dissolved with 1% chitosan into 0.1 M HCl and further applied onto activated BOPP surfaces.

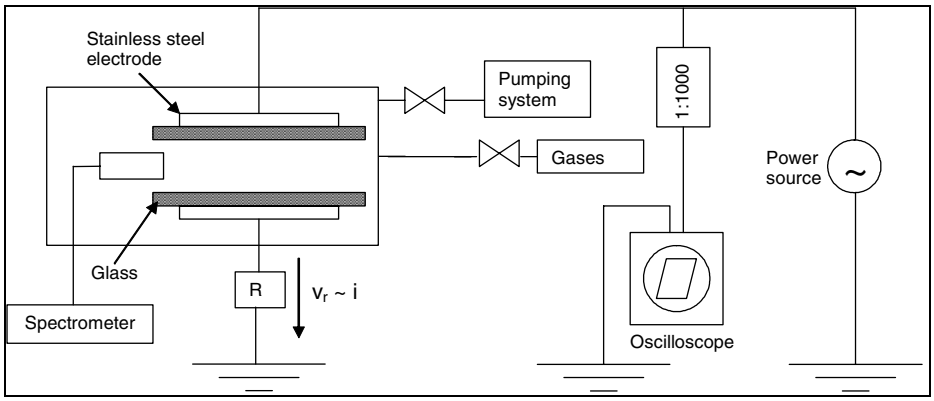

Figure 2. Experimental setup of dielectric barrier discharge (DBD) plasma configuration.

In the case of glutaraldehyde, the amount of immobilized chitosan was 1.64 g/m^2, whereas with carbodiimide only 0.58 g/m^2 of chitosan was attached. The coating solutions without any linking agents as well as the BOPP surfaces without plasma activation treatments were incapable of forming permanent chitosan coatings

Table 1. Amount of immobilized chitosan on activated BOPP film. Coating solution (1% chitosan in 0.1 M HCl).

Coupling agent	Chitosan g/m^2		
	BOPP (without plasma)	BOPP (N_2-plasma + NH_3)	BOPP (N_2-plasma + CO_2)
no coupling agent	0.02	0.14	0.0
0.1% glutaraldehyde	0.04	1.64	*
0.8% carbodiimide	0.0	*	0.58

Means (n=3)
*not measured

(Table 1.). Due to the better performance of glutaraldehyde combined with N_2-plasma + NH_3, these were used in further tests. By using 0.1 M acetic acid as a solvent for 1% chitosan and 0.1% glutaraldehyde, the immobilization yield of 1.75 g/m^2 was reached. Thus acetic acid was chosen as most applicable solvent for immobilization experiments.

As can be seen in Figure 3., all film samples had absorption peaks at 1377, 1455, 2840, 2921 and 2959 cm^{-1} assigned to the hydrocarbon structure of BOPP. In addition, chitosan coated samples had a broad peak between 3200 and 3500 cm^{-1} corresponding to the hydroxyl groups of chitosan and also peaks between 1000 and 1200 cm^{-1} typical for saccharide structures. Chitosan coated samples had also strong amino characteristic peak at 1590 cm^{-1}. N_2-plasma + NH_3 activated BOPP samples had higher density of both amino and hydroxyl groups as compared with untreated BOPP indicating the successful surface activation by plasma pretreatment.

Water contact angles of the films decreased because of the amino activation and chitosan immobilization (Table 2.). BOPP, as other synthetic polymers, is hydrophobic, whereas the amino functionalized surfaces and especially the cationic polysaccharide structure of chitosan are very hydrophilic.

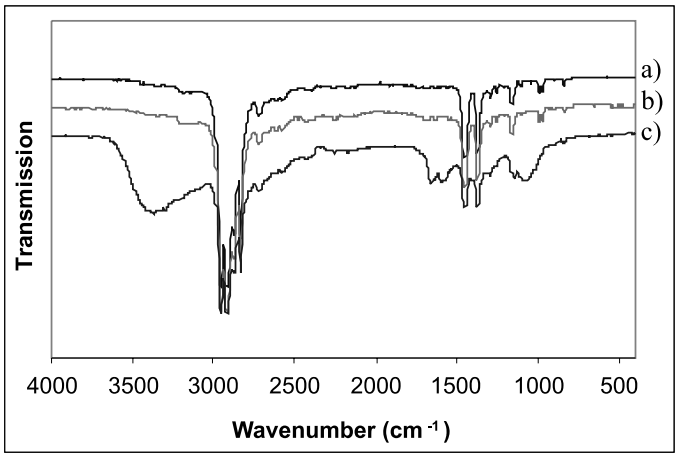

Figure 3. FTIR spectra of the original and surface modified BOPP films. a) BOPP (without plasma), b) BOPP (N_2-plasma + NH_3), c) BOPP (N_2-plasma + NH_3) + 1% chitosan in 0.1 M acetic acid + 0.1% glutaraldehyde.

Table 2. Water contact angles (θ_{water}) of BOPP films.

Film	θ_{water} (°)
BOPP (without plasma)	96
BOPP (N_2-plasma + NH_3)	66
Chitosan coated BOPP (N_2-plasma + NH_3)[a]	51

Means (n=4)
[a]1% chitosan in 0.1 M acetic acid + 0.1% glutaraldehyde

As shown in the SEM pictures (Figure 4.), the amino activation slightly smoothed the surface of untreated BOPP. The surface roughness did not increase after plasma treatment because of the homogeneous nature of the dielectric barrier discharge. In contrast to commonly used corona treatments, no streamers are formed in this type of plasma. Said streamers can cause local destruction of the polymer substrate, which gives rise to an increased surface roughness. In each case, the observed surface roughness was below 1 µm. As the cross-section picture indicates (Figure 4d.), the bonding between chitosan and BOPP was relatively strong and the thickness of the chitosan layer was about 2 µm.

Oxygen transmission rates fell from 1500 to 27 $cm^3/(m^2 \cdot 24\ h)$ because of chitosan barrier layer. In addition, chitosan formed an effective barrier against both carbon dioxide and ethylene. Good gas barrier properties of chitosan in dry conditions are due to the high amount of hydrogen bonds and crystallinity. Both gas solubilities and

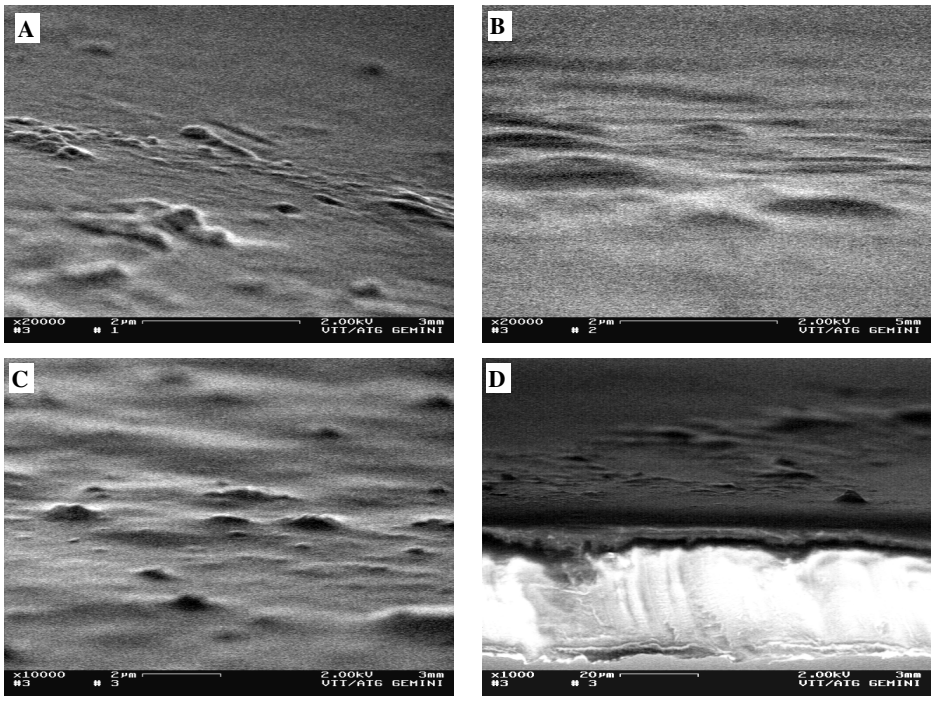

Figure 4. Surface topography of (a) untreated, (b) N_2-plasma + NH_3 treated and (c) chitosan coated, N_2-plasma + NH_3 pretreated BOPP films and (d) cross-section of chitosan coated sample.

Table 3. Gas transmissions of BOPP films expressed as $cm^3/(m^2 \cdot 24\ h)$.

Film	Oxygen	Carbon dioxide	Ethylene
BOPP (without plasma)	1500	4000	430
BOPP (N_2-plasma + NH_3)	1500	4600	390
Chitosan coated BOPP (N_2-plasma + NH_3)[a]	27	790	53

Means (n=2)
[a]1% chitosan in 0.1 M acetic acid + 0.1% glutaraldehyde

diffusion coefficients are extremely low [36]. N_2-plasma + NH_3 treatment increased the permeability to carbon dioxide as compared with the untreated BOPP. NH_3 plasma introduced basic amino groups, which have capability of interacting with dissolved carbon dioxide resulting in an increased transmission [37] (Table 3.).

The reaction between amino group of chitosan and glutaraldehyde involves the formation of a Shiff base and it can be easily noticed by colour formation [26]. Indeed, the chitosan coatings containing glutaraldehyde were slightly yellowish. The colour formation is probably due to a three-dimensional network structure of cross-linked chitosan [27].

As the total migration test results indicated (Table 4.), the cross-linked chitosan was permanently immobilized onto BOPP without any notable leaching. The amounts of dissolved substances in 3% acetic acid, 95% ethanol and iso-octane were below 2 mg/dm^2, thus the material met the requirements set for the total migration of substances migrated from the packaging materials into foodstuffs stipulated in Directive 2002/72/EC.

As chitosan immobilized BOPP films had strong activity against both *Escherichia coli* and *Bacillus subtilis* (Figure 5.), they may be exploited in various food packaging applications including antimicrobially active vacuum packages and oxygen barrier films.

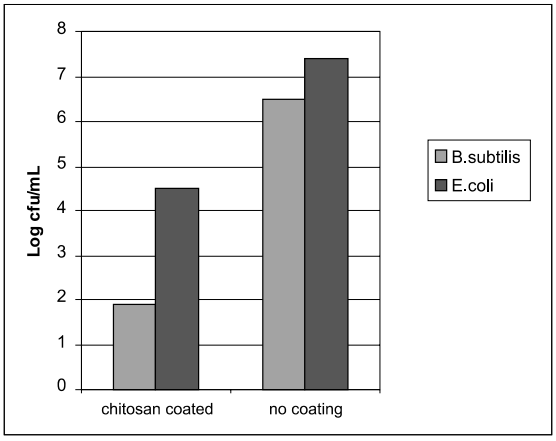

Figure 5. Effect of plasma activated BOPP film with or without surface immobilized chitosan (0.1% glutaraldehyde as linking agent) on survival of *E. coli* and *B. subtilis* (24 h in peptone saline at 30 ºC). Means (n=3).

Table 4. Overall migration of BOPP films in three different simulants expressed as mg/dm^2.

Coating solution	Overall migration mg/dm^2		
	iso-octane 2 h at 20 °C	3% acetic acid 10 days at 40 °C	95% ethanol 10 days at 40 °C
BOPP (without plasma)	<1	1	<1
BOPP (N_2-plasma + NH_3)	<1	<1	<1
Chitosan coated BOPP (N_2-plasma + NH_3)[a]	1	<1	1

Means (n=3)
[a]1% chitosan in 0.1 M acetic acid + 0.1% glutaraldehyde

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