CHITOSAN COATINGS APPLIED TO POLYETHYLENE SURFACE TO OBTAIN FOOD-PACKAGING MATERIALS

BOGDĂNEL SILVESTRU MUNTEANU, ELENA PÂSLARU,[*] LIDIJA FRAS ZEMLJIC,[**] ANAMARIA SDROBIŞ,[*] GINA MIHAELA PRICOPE,[***] and CORNELIA VASILE[*]

"Al. I. Cuza" University, 11, Carol I blvd., Iasi, Romania; Universita degli Studi di Genova UNIGE, Genova, via Balbi 5, Italy
[*]*"Petru Poni" Institute of Macromolecular Chemistry, Physical Chemistry of Polymers Department, 41A, Gr. Ghica Voda Alley, 700487 Iasi, Romania*
[**]*University of Maribor, Faculty of Chemical Engineering, 17, Smetanova ulica, SI-2000 Maribor, Slovenia*
[***]*Veterinary and Food Safety Laboratory, Food Safety Department, Iasi, Romania*
✉ *Corresponding author: Cornelia Vasile, cvasile@icmpp.ro*

Received March 12, 2013

This paper deals with the optimization of the experimental conditions used for coating of polyethylene (PE) with chitosan, a nontoxic cationic polysaccharide, approved by the Food and Drug Administration (FDA) Agency, with good antimicrobial properties and biocompatibility. To enhance chitosan adhesion, the PE surface was previously corona treated. The uncoated and chitosan-coated polyethylene films were characterized by ATR-FTIR spectroscopy, X-ray photoelectron spectroscopy (XPS), potentiometric titration and scanning electron microscopy (SEM). The chitosan-coated PE films were also examined for oxygen permeability and antimicrobial activity against food pathogen microorganisms as Gram-positive (*Listeria monocytogenes*) or Gram-negative (*Escherichia coli*, *Salmonella*) bacteria.

Keywords: chitosan, polyethylene, surface modification, food-packaging

INTRODUCTION

Diet is a major focus of public health strategies aimed at maintaining optimum health throughout life, preventing early onset of chronic diseases, such as gastrointestinal disorders, cardiovascular disease, cancer, osteoporosis, as well as promoting healthier ageing.[1] The increasing consumer health consciousness and the growing demand for healthy foods stimulate innovation and the development of new products in the food industry and are responsible for the expanding worldwide interest in functional foods.[2]

Food coating/packaging is intended to provide some level of protection, prevent the transfer of materials from one food component to another, enhance the appearance of fruits and vegetables, and frequently contains other compounds to retard insects, microorganisms, oxidation and other intruders that would spoil the product.[3]

In consideration of this, in the last few years there has been a growing interest in innovative technological developments in the production of functional foods, whose bioactive principles and actuators are devised to be contained within packaging or coating materials.[2]

The Encyclopaedia Britannica defines surface coating as "any mixture of film-forming materials plus pigments, solvents, and other additives, which, when applied to a surface and cured or dried, yields a thin film that is functional and often decorative".[4]

Many fresh and processed foods are packed in an inert or low oxygen atmosphere (by purging air with nitrogen or carbon dioxide). This procedure is known as modified atmosphere packaging (MAP) and can increase shelf-life four-fold, by inhibiting microbial growth and, consequently, food spoilage. In most circumstances, the packaging materials used are based on polymers, which, however, have their limitations. While materials such as glass and metals are impermeable to gases, plastics are semi-permeable and undesirably affect food and drink

quality over relatively short periods of time (e.g. carbon dioxide escape from carbonated drinks, oxygen ingress to packaged foods resulting in faster decay, and ethylene spread between fruits resulting in faster ripening). Plastics can be made more impermeable to gases through coatings or through the inclusion of nanoparticles within the polymer matrix.[5]It is well known that chitosan is a biopolymer with good antimicrobial properties, because it inhibits the growth of a wide variety of fungi, pathogenic bacteria and spoilage microorganisms.[6,7] The antimicrobial activity of chitosan depends on its molecular mass, the deacetylation degree and chemical degradation.[6] In addition, chitosan is readily soluble in various acidic solvents. From acidic solutions,[8] flexible, clear and tough films can be formed, with good oxygen barriers.[9,10]

Polyethylene is the most frequently used polymer film for packaging, as it offers the advantage of being inert.[11] Low-density polyethylene is heat sealable, inert, odour free and shrinks when heated. It is a good moisture barrier, but has relatively high gas permeability, sensitivity to oils and poor odour resistance. It is less expensive than most films and is therefore widely used.

Hence, chitosan coating of polyethylene represents a procedure that can lead to obtaining new materials with decreased gas permeability and, additionally, with antibacterial properties.

The objective of this study was to improve the polyethylene quality as food packaging material by chitosan surface deposition using different coating procedures.

EXPERIMENTAL

Materials

Polyethylene (PE) with 0.02 mm thickness was purchased from SC LORACOM SRL, Roman, Romania. The polyethylene film was made of a 2/1 mixture of UV treated Low Density Polyethylene (LDPE) (Tipolen, Tiszai Vegyi, Hungary) and High Density Polyethylene (HDPE) (SIDPEC, Egypt).

Low average molecular weight chitosan (CHT) with 20-300 cP viscosity in 1% acetic acid and 75-85% deacetylation degree was purchased from Sigma-Aldrich, Steinheim, Germany.

Ethanol (96%) and glacial acetic acid (99.5%), as analysis reagents, were purchased from Chemical Company, Iasi, Romania.

Activation of PE films – corona treatment

Corona treatment of PE was carried out prior to chitosan deposition using Enerkon Corona Osman Onder atmospheric plasma treatment equipment. The experimental set-up of the corona treatment system is given in Figure 1. The PE film was placed between two electrodes subjected to a potential difference. The treatment station applies 50/60 Hz electrical power to the material surface, through an air gap, via a pair of electrodes. One of the electrodes is connected to a high potential source, and the other one rolls at ground potential, which also supports the material. Only the side of the material facing the high potential electrode should show an increase in surface tension. Atmospheric air was chosen as gas and the following parameters were used: a frequency of 30 kHz, inter-electrode distance of 7 mm, and a plasma treatment power of ~45 kJ/m^2.

After the corona discharge pre-treatment, the PE surface was enriched with oxygen-containing functionalities, which would assure good adhesion, as demonstrated below.

Coating procedures

Chitosan coating onto the PE surface was achieved using three different methods:

(a) Film dipping/immersion (I) into chitosan solutions of various concentrations of 1 wt%, 3 wt% and 5 wt%;

(b) Spreading (S) of the same chitosan solutions on the PE surface;

(c) Electrospraying (ES) of chitosan onto the polymeric surface.

Figure 2 illustrates the experimental set-up of the electrospraying system used for PE coating with chitosan.

The device consists of a direct current high voltage supply, a rotating metal plate collector, and a syringe oriented with the needle perpendicular to the metal plate. The high direct voltage (0 to 30 kV) is applied between the metal plate and the syringe needle. The distance between the tip of the needle and the metallic plate can be set in the 4-40 mm range.

A polymer solution (chitosan in acetic acid/water/ethanol) is loaded into the syringe. The solution is extruded from the needle tip at a constant flow rate. The flow rate is controlled by a step-by-step motor (stepper), which is a brushless, synchronous electric motor that converts digital pulses produced by a computer into mechanical shaft rotation. One revolution of the stepper motor (360°) is divided into 200 discrete steps. The motor receives a separate pulse for each step and each pulse causes the motor to rotate to a precise angle (1.8°), which is controlled by a computer. The rotation motion is converted to linear motion using a lead screw/worm gear drive system. The resolution of the lead screw system is of 0.0025 mm/step. At the point of ejection (the needle tip), a polymer jet is created as a result of the electric charge repulsion outgoing the solution surface tension.

Sample designation includes the following information: without/with corona activation

(PE/PEcor), the coating method (I/S/ES) and the chitosan solution concentration (1÷5 wt% CHT). For example, PEcor, I, 1CHT or PEcor, ES, 3CHT designate corona treated samples obtained by immersion in 1% chitosan solution or by electrospraying with a 3% chitosan solution. Chitosan solutions were prepared in 8% acetic acid and 30% ethanol and twice distilled water.

After the chitosan-coating of the surfaces, the films were dried, first at room temperature and then in vacuum at 50 °C for 24 hours.

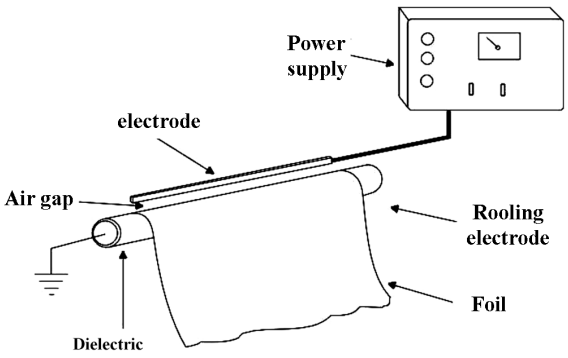


Figure 1: Experimental set-up for corona treatment

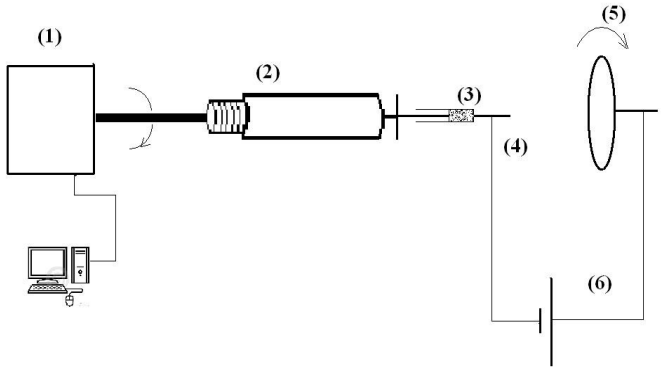


Figure 2: Experimental set-up of the electrospraying system; (1) stepper; (2) micrometer screw; (3) syringe; (4) needle; (5) rotating metal plate; (6) high direct voltage (0 to 30 kV) power supply

Investigation methods

ATR-FTIR spectroscopy

The ATR-FTIR spectra were recorded by means of a Bruker VERTEX 70 spectrometer, in the transmittance mode. The background and the sample spectra were obtained in the 600 to 4000 cm^{-1} wave number range. The processing of the spectra was achieved using the SPECVIEW program.

XPS analysis

XPS analysis was carried out by means of a TFA XPS Physical Electronics instrument. The base pressure in the chamber was about 6×10^{-8} Pa. The samples were excited with X-rays over a 400 µm spot area with a monochromatic Al $K_{\alpha1,2}$ radiation at 1486.6 eV. The photoelectrons were detected with a hemispherical analyzer positioned at an angle of 45° with respect to the normal to the sample surface. Survey-scan spectra were made at a pass energy of 187.85 eV and 0.4 eV energy step, while high resolution spectra of carbon C1s were made at a pass energy of 23.5 eV and 0.1 eV energy step. An electron gun was used for surface neutralization. The concentration of elements was determined by using MultiPak v7.3.1 software from Physical Electronics, which was supplied with the spectrometer. XPS survey spectra were taken from 2 different spots for each sample and the average value was used for data evaluation.

Potentiometric titration

Potentiometric titration was used for direct determination of the amino groups on the PE surface and was carried out using a Mettler Toledo T70 two-burette instrument (equipped with a combined glass electrode), within inert atmosphere (N_2 bubbling). The titrations were performed with 0.1 M HCl (Fluka, analytical) and 0.1 M KOH (Baker, Dilut-it). All the solutions were prepared with deionised water with very low carbonate content, which was achieved through boiling and subsequent cooling under nitrogen atmosphere. The samples were titrated in forward and back runs between pH = 2.8 and pH = 11, at constant ionic strength (0.1 M) set to its appropriate value with KCl (Riedel-de-Häen, Germany). The titrant was added at varied preset intervals of [0.001-0.25] mL. The stability criterion for taking a reading after each addition was set to dE/dt = 0.1 mV/30 s, where 30 s was the minimum time to reach equilibrium conditions between two additions of the titrant, and the maximum time was set to 180 s. Subsequently, the blank HCl-KOH titration was carried out under the same conditions as above.

From the potentiometric titration data, the molar concentration Q relating to the overall charge of the weak ions was calculated, a method described in detail by Čakara *et al.*[12] All the reported values are the average values from triplicate determinations.

Scanning Electron Microscopy (SEM)

The sample examination was performed on a VEGA II TESCAN Microscope (USA). Before microscope observation, the specimens were fixed on a sample holder and their surface was covered in

vacuum with a fine layer of gold. SEM images were recorded at different magnifications. Magnification is given on photos.

Oxygen permeability tests

Permeability tests were performed with a PERME™ OX2/231 Permeability Tester from Labthink Instruments CO., LTD (Jinan, China), using oxygen as test gas (RH ~50%), at a temperature of 23 °C. Nitrogen was used as oxygen carrier. The oxygen flow rate was fixed at 20 mL/min, while that of nitrogen was 10 mL/min.

Antimicrobial tests

Antimicrobial tests have been carried out by well-known standard methods such as:

- SR ISO 16649-2/2007 – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* – Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. The most probable number of β-glucuronidase-positive *E. coli* is determined according to the number of tubes of Minerals Modified Glutamate Broth (Cat. 1365), whose subcultures produced blue or blue-green colonies on tryptone bile glucuronide agar; inoculation and incubation at a temperature of 44 °C for 20-48 hours.
- SR EN ISO 11290-1:2000/A1:2005 – Part 1: Detection method – Amendment 1 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes.*
- SR EN ISO 6579/2003/AC/2004/AC/2006, Amd.1:2007 – Horizontal method for detection of *Salmonella spp.* bacteria, approved by CEN as EN ISO 6579:2002.

RESULTS AND DISCUSSION

To appreciate the efficiency of the coating methodologies and the influence of chitosan concentration on the modification of surface properties of the polyethylene films, different investigation methods have been used.

ATR-FTIR results

Figures 3 and 4 present the characteristic ATR-FTIR spectra corresponding to polyethylene surfaces coated with different chitosan solutions, of 1 wt%, 3 wt% and 5 wt% concentration, by immersion (Figure 3(a)), spreading (Figure 3(b)) and electrospraying (Figure 4). The spectra recorded for chitosan-coated PE were compared to the spectrum recorded for pure low molecular mass chitosan powder (Figure 3 – spectrum 8). The broad band centred around 3400 cm^{-1}, both in chitosan-coated PE and in pure chitosan spectra, is assigned to –OH functional group. The presence of a primary aliphatic amino group in chitosan is evidenced by the deformation vibration band at around 1650 cm^{-1} and 1590$cm.^{-1}$ The slight shift of this band toward lower wavenumbers, observed in the case of chitosan coated corona treated PE sample, was ascribed to the covalent interaction between the carboxyl functional group and the amino group during the last step of functionalization.

The IR spectra reveal that the chitosan absorption bands (the –OH stretching and the aliphatic C-H stretching bands at 3550-2830 cm^{-1} and the -NH_2 vibration band at 1597 cm^{-1}) have smaller intensities when PE is not pre-activated by corona discharge. In this case, some viscous chitosan solution sticks only physically to the surface after drying. After corona activation, the chitosan characteristic IR bands are much more intense and well-defined. Furthermore, the intensities of these vibration bands tended to increase with an increase in the concentration of chitosan solution deposited on the PE films and the coating appeared significant only after corona activation. The results are similar with those obtained by Theapsak *et al.*[13]

The IR spectra of corona untreated and treated PE and coated with 5 wt% chitosan by electrospraying are presented in Figure 4. The chitosan characteristic bands were observed only for two samples, which were previously corona treated and coated with chitosan using the following parameters: d = 5 cm, V = 26 kV, t = 20 min (Figure 4 – spectrum 7) and d = 11 cm, V = 30 kV, t = 20 min, respectively. Taking into account the IR results, the above mentioned experimental conditions were considered as optimal for electrospraying chitosan onto the PE surface and were used for further investigation.

From the chitosan IR band intensities corresponding to the samples obtained from the same concentration of the chitosan solution, it can be concluded that the most efficient method was the immersion of the PE film into the chitosan solution, when compared with the spreading method. On the other hand, electrospraying is a more versatile method, because it allows a more precise control of the chitosan content deposited onto the surface by varying the deposition time.

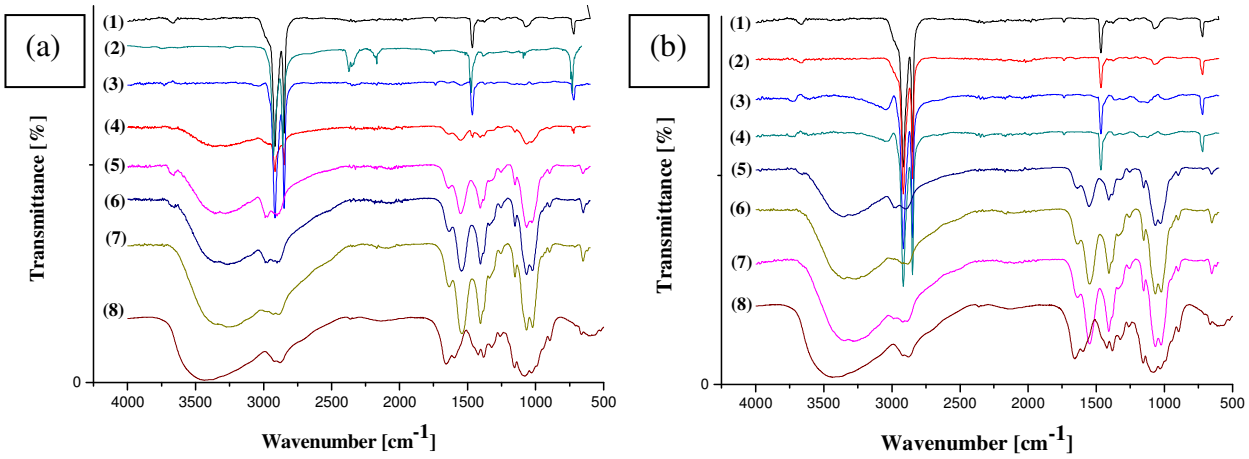


Figure 3: ATR-FTIR spectra of native polyethylene (1), and chitosan-coated PE surfaces by immersion (a): (2) PE, I, 1CHT; (3) PE, I, 3CHT; (4) PE, I, 5CHT; (5) PEcor, I, 1CHT; (6) PEcor, I, 3CHT (7) PEcor, I, 5CHT; CHT and by spreading (b): (2) PE, S, 1CHT; (3) PE, S, 3CHT; (4) PE, S, 5CHT; (5) PEcor, S, 1CHT; (6) PEcor, S, 3CHT (7) PEcor, S, 5CHT

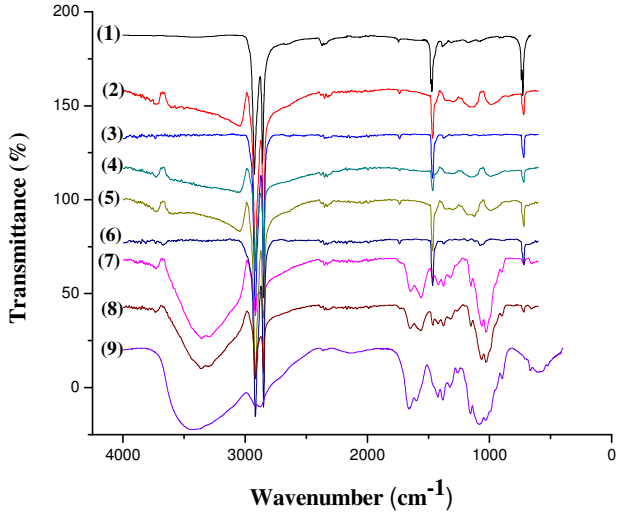


Figure 4: ATR-FTIR spectra of chitosan coated PE by electrospraying under varying experimental conditions (distance, voltage and time); (1) PE; (2) PE, 5CHT_5 cm, 25 kV, 30 min; (3) PE, 5CHT_11 cm, 29 kV, 10 min; (4) PEcor, 5CHT_5 cm, 25 kV, 30 min; (5) PE, 5CHT_11 cm, 30 kV, 20min; (6) PEcor, 5CHT_8.5 cm, 25 kV, 17 min; (7) PEcor, 5CHT_5 cm, 26 kV, 20 min; (8) PEcor, 5CHT_11 cm, 30 kV, 20 min; (9) Chitosan

Table 1
Amount and thickness of chitosan layers deposited onto corona treated PE surface

Sample	CHT mass deposited onto surface [μg/cm^2]	CHT layer thickness [μm]
PEcor, I, 1CHT	279.4	10
PEcor, S, 1CHT	343.8	20-30
PEcor, I, 3CHT	293.5	30-40
PEcor, S, 3CHT	347.7	10-20
PEcor, I, 5CHT	399.0	40-80
PEcor, S, 5CHT	489.1	30-90
PEcor, ES, 5CHT (11cm_30kV_20min)	1.7	8.5×10^{-3}

Chitosan layer thickness

The initial thickness of the polyethylene film was 0.02 mm. The average mass and thickness of the chitosan layer deposited on the PE surface was determined both by high precision weighing (gravimetric method) and automatic micrometer measurements. The results are listed in Table 1.

The mass and thickness of the chitosan layer increased with increasing concentration and were higher after corona discharge treatment of the polymeric substrate. As expected, the chitosan coating applied by the electrospraying method and determined by examination of the cross section, through fracture analysis, using scanning electron microscopy, was the thinnest one.

XPS determination of surface chemical composition

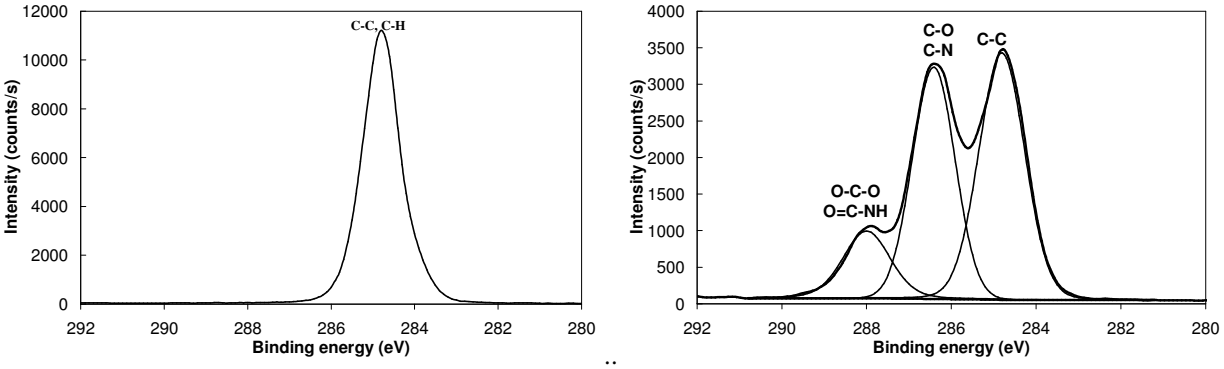
XPS investigation method was used complementary to ATR-FTIR spectroscopy. Using XPS survey-scan spectra allowed obtaining the surface chemical composition and atomic concentrations (at. %) of the native PE film, corona activated and chitosan coated samples. Table 2 presents the average values for the elemental surface atomic composition of the samples, recorded for two different spots on the investigated surfaces.

Polyethylene surface shows, besides carbon, as a major chemical component, a small percentage of oxygen, possibly due to surface oxidation or impurities. Corona treatment of PE brings an increase in the oxygen percentage (5.6 atomic %) by introducing onto the surface new chemical groups containing oxygen. The new functionalities introduced by corona treatment and air exposure are expected to improve chitosan adhesion.

The XPS spectra of PE corona treated and chitosan coated samples confirmed the presence of carbon, oxygen and nitrogen on the surface, as according to the elemental composition of chitosan, in accordance with the above-presented investigation methods (ATR-FTIR and gravimetric measurements). The highest amount of chitosan on surface unit was obtained when using a solution with 5 wt% concentration. Consequently, the highest content of oxygen (26.7 atomic %) on the surface was recorded for PEcor, I, 5CHT, while the highest nitrogen atomic percentage was determined for PEcor, S, 5CHT.

Table 2
Experimental elemental composition (atomic %) obtained by analysis of the XPS survey spectra for corona activated and chitosan coated PE

Sample	Atomic % C	Atomic % O	Atomic % N
PE	99.2 ± 0.3	0.8 ± 0.1	-
PEcor	94.2 ± 0.3	5.6 ± 0.1	-
Immersion method			
PE, I, 1CHT	97.4 ± 1.0	2.2±1.0	-
PEcor, I, 1CHT	89.0 ± 1.6	6.7 ± 1.0	1.8 ± 0.1
PE, I, 3CHT	98.9 ± 0.1	1.1 ± 0.1	-
PEcor, I, 3CHT	67.8 ± 0.9	26.0 ± 1.1	5.7 ± 0.2
PE, I, 5CHT	99.2 ± 0.1	0.8 ± 0.1	-
PEcor, I, 5CHT	67.8 ± 0.2	26.7 ± 0.2	5.5 ± 0.03
Spreading method			
PE, S, 1CHT	98.4 ± 1.0	1.6±1.0	-
PEcor, S, 1 CHT	78.3 ± 6.8	18.6 ± 4.5	3.1 ± 2.3
PE, S, 3CHT	99.1 ± 0.1	0.9 ± 0.1	-
PEcor, S, 3 CHT	69.4 ± 0.3	25.5 ± 0.1	4.6 ± 0.2
PE, S, 5CHT	99.0 ± 0.1	1.0 ± 0.1	-
PEcor, S, 5 CHT	67.9 ± 0.1	26.3 ± 0.4	5.9 ± 0.4
Electrospraying			
PEcor, ES, 5CHT (11cm_30kV_20min)	85.7 ± 3.2	13.3 ± 2.9	1.0 ± 0.4



(a) (b)

Figure 5: High-resolution carbon peak (C1s) of PE (a) and PEcor, I, 3CHT (b)

The C1s spectrum of PE (Figure 5a) shows a single peak at 284.8 eV, while the C1s spectra of the chitosan coated corona treated PE surface can be curve-fitted with three peak components (Figure 5b), derived from chemically non-equivalent carbon atoms mainly bonded to oxygen and nitrogen. The shape of the C1s peaks does not change depending on the coating method or chitosan concentration used, showing that the coating is relatively uniform.

Aqueous potentiometric titrations

It is known from literature[14] that the protonation and the number of amino groups existing on chitosan backbones, which are essential in electrostatic interaction, play an important role in enhancing the antibacterial activity. Hence, to explain the antibacterial activity of the chitosan coated PE sample, it was important to analyze the charging behaviour of the prepared samples and this was investigated by potentiometric titration. The experimental charging isotherms, normalized to the mass of the film, are given in Figure 5, and the corresponding average charges and pKa values are listed in Table 3. The surface charge amount was calculated from the plateau level of the charging isotherms (Figure 6 a-b). In this case, the main source for the positive charge is the protonation of chitosan amino groups at acidic pH. It should be noted that the charge amount detected on corona untreated PE surface was very low (Table 3), mainly because the chitosan had no adhesion to that surface, even when using a 5 wt% concentration of biopolymer. The average charge increased after corona discharge treatment and chitosan coating and further increased with an increasing concentration of the chitosan solution. In the case of 1 wt% chitosan concentration, the total charge was 113.04 mmol/kg and increased by about 20 times (reaching a value of 2252.06 mmol/kg) when the solution concentration was 5 wt%. The average surface charge was lower for electrosprayed PE surfaces (80.32 mmol/kg) than for the surfaces obtained by immersion. However, the very thin electrosprayed layer proved to be sufficient for inhibiting the antimicrobial activity (as shown further).

The pK_a value of $-NH_3^+$ group was estimated from the midpoint of the titration curve (half neutralization value method).[15]

The experimental pKa of chitosan was calculated as being 6.5. It was observed that with increasing chitosan concentration, the pKa values of chitosan-treated samples approached that of neat chitosan (e.g. for PE^Tcor, I, 5CHT, the calculated pK_a was 6.2). In conclusion, a thicker biopolymer coating on the PE films was achieved with higher chitosan concentration.

Scanning electron microscopy

SEM images of the polyethylene films corona treated and coated with chitosan, using different methods: immersion, spreading and electrospraying, are given in Figure 7. It can be observed from the SEM micrographs that the chitosan layer applied onto the PE surface through the first two methods is uniform and compact, while the chitosan layer deposited by electrospraying contains spherical microparticles randomly distributed over the surface.

Table 3
Average charge and pKa values determined from charging isotherms

Sample	Charge per mass [mmol/kg]	pKa
PE	Undetectable	-
PEcor	Undetectable	-
Chitosan	5250.0	6.55
PE, I, 1CHT	18.34	3.8
PEcor, I, CHT	113.04	6.0
PE, I, 5CHT	33.30	5.7
PEcor, I, 5CHT	2252.60	6.20
PE, ES, CHTm	Undetectable	-
PEcor, ES CHT	80.32	6.15

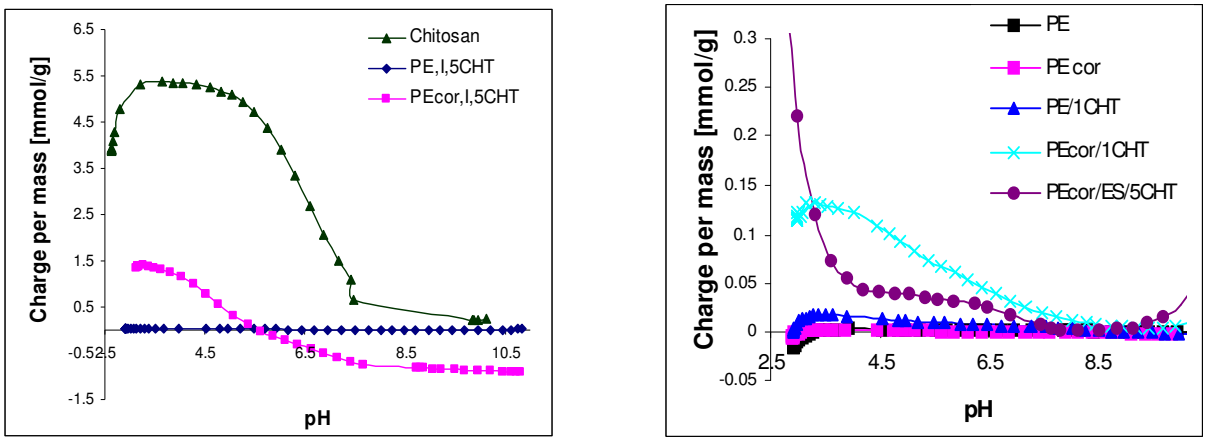
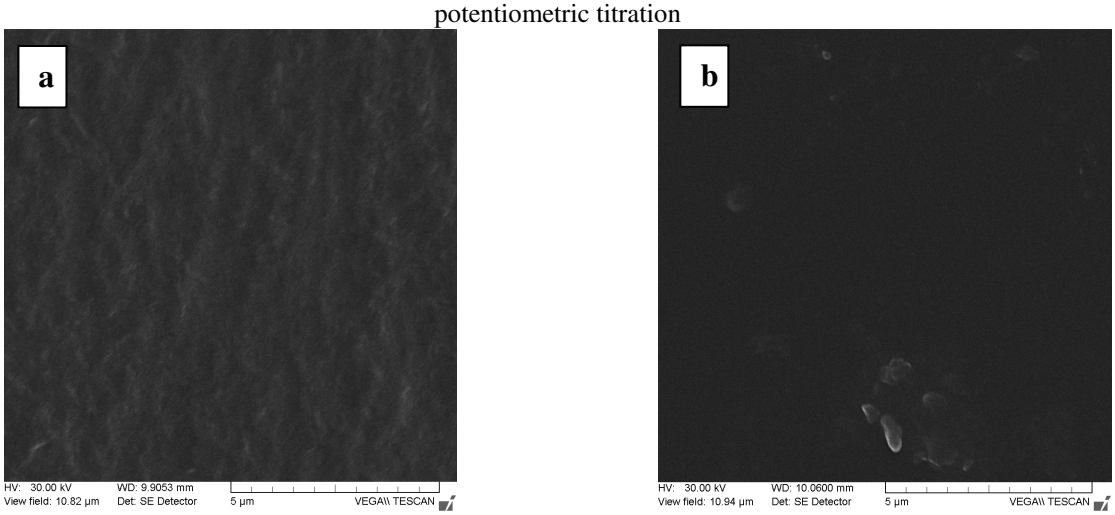


Figure 6: Experimental charging isotherms, normalized to the mass of the PE film, resulted from potentiometric titration



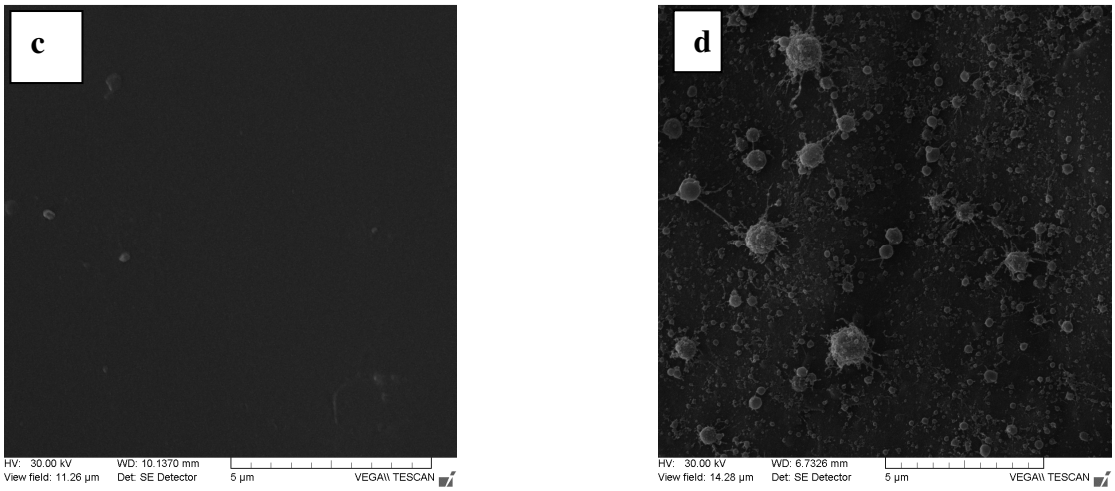


Figure 7: Selected SEM images of chitosan-coated polyethylene films: (a) PE; (b) PEcor, S, 5CHT; (c) PEcor, I, 5CHT; (d) PEcor, ES, 5CHT

The chitosan coating morphology may possibly influence the antibacterial activity. The high surface-to-volume ratio of the small electrosprayed chitosan particles intensifies the micro and nano-effects, which includes mainly increased surface reactivity and high strength to mass ratio.[16] Taking into account the advantages mentioned above of using chitosan under the form of microparticles, it can be concluded that electrospraying is a more efficient coating method, compared with immersion and spreading procedures. It offers the advantage of a lower consumption of substances, while producing very thin coatings with controlled thickness and high free surface area.

Antimicrobial tests

The inhibitory activity of the chitosan-coated PE films was investigated against two Gram-negative bacteria, namely *Salmonella enteritidis* and *Escherichia coli*, and one Gram-positive bacterial strain, *Listeria monocytogenes* – Figure 8 and Table 4. All chitosan-coated samples exhibited antibacterial activity, and a slight concentration influence was observed in the case of *Listeria monocytogenes*. This behaviour can be explained by the fact that chitosan or its derivatives have been proven more effective for Gram-negative bacteria than for Gram-positive bacteria.[17]

Table 4
Antibacterial activity of chitosan-coated PE surfaces

Sample composition	*Salmonella enteritidis* inhibition ATCC 25922 48 h (%)	*Escherichia coli* inhibition ATCC 25922 48 h (%)	*Listeria monocytogenes* Inhibition ATCC 25922 48 h (%)
PE	39	14	25
PE, S, 1CHT	95.3	89.4	92.59
PE, S, 3CHT	98.4	92.86	98.15
PE, S, 5CHT	98.4	100.00	92.59
PEcor, S, 1CHT	100.0	100.00	100.00
PEcor, S, 3CHT	100.0	100.00	100.00
PE cor, S, 5CHT	100.0	100.00	100.00
PEcor, I, 5CHT	100.0	100.00	100.00
PEcor, ES, 5CHT (11 cm, 30 kV, 20 min)	100.0	96.43	90.74

Table 5
Oxygen permeability for chitosan-coated PE films

Samples	Oxygen Transmission Rate (mL/m^2*day)
PE	3833.36
Immersion	
PE, I, 1CHT	3762.2
PEcor, I, 1CHT	2150.04
PE, I, 3CHT	3626.14
PEcor, I, 3CHT	1135.12
PE, I, 5CHT	3612.32
PEcor, I, 5CHT	778.54
Spreading	
PE, S, 1CHT	3800.25
PEcor, S, 1CHT	2142.52
PE, S, 3CHT	3714.23
PEcor, S, 3CHT	1310.00
PE, S, 5CHT	3723.56
PEcor, S, 5CHT	1065.66
Electrospraying	
PEcor, ES, 5CHT	2952.43

Oxygen permeability tests

The chitosan coating of polyethylene, having improved adhesion after corona treatment, reduced the oxygen permeability, compared to that of neat PE, a drastic reduction being observed in the case of PEcor, I, 5CHT. The thicker the chitosan layer, the lower the oxygen permeability.

The oxygen barrier properties are influenced by the thickness of the chitosan layer applied. Consequently, the deposition made through electrospraying has a lower oxygen transmission rate when compared with the native PE, but not as significant as that of the PEcor, I, 5CHT sample.

Chitosan coating improved the oxygen barrier properties of PE and also conferred it antimicrobial characteristics, making it very promising as food packaging material.

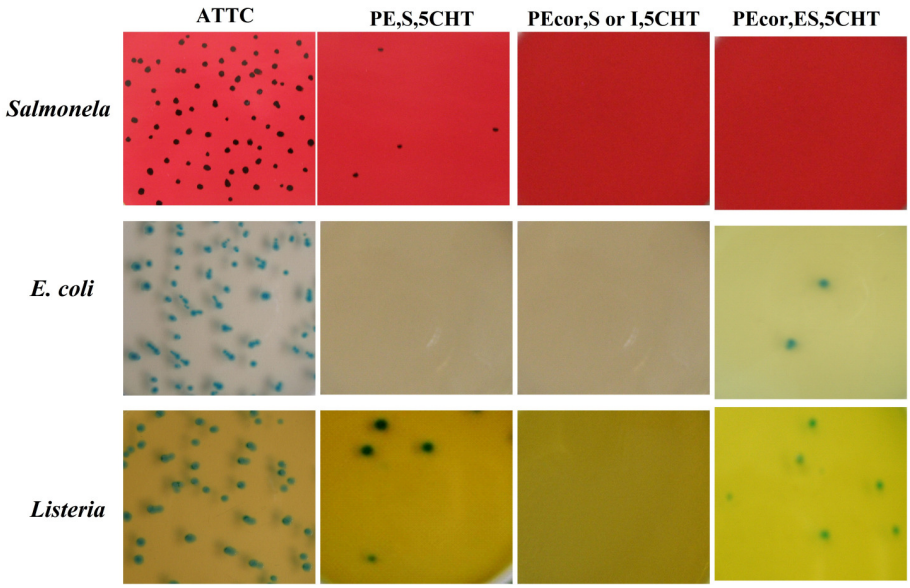


Figure 8: Microscopic images of bacterial colonies grown in the absence (ATCC) and in the presence of PE films coated with chitosan

CONCLUSION

A two-step procedure has been developed to obtain food packaging materials based on polyethylene coated with chitosan, consisting in corona treatment followed by different coating procedures, such as immersion, spreading and electrospraying. Chitosan deposition onto the polyethylene surface aims to improve barrier properties and provides antibacterial activity. Corona pre-treatment of PE has a very important role in achieving biopolymer adhesion.

Some of the investigated properties, like elemental composition, surface charge and oxygen permeability, depend on the chitosan concentration tested. All chitosan coated foils proved to have antibacterial activity. In terms of efficiency and lower substances consumption, the electrospraying method is by far the most appropriate coating procedure.

ACKNOWLEDGMENTS: Bogdănel Silvestru Munteanu acknowledges the financial support of this study through the Sectorial Operational Programme for Human Resources Development, project "Transnational network for integrated management of post-doctoral research in Science Communication, Institutional framing (postdoctoral school)" and the scholarship program (CommScie) "POSDRU/89/1.5/S/ 63663", and the other authors acknowledge the financial support by UEFISCDI through research project BIONANOMED 164/2012.

REFERENCES

[1] R. H. Liu, *Am. J. Clin. Nutr.*, **78** (Suppl.), 517S (2003).
[2] A. Lopez-Rubio, R. Gavara, J. M. Lagaron, *Trends Food Sci. Tech.*, **17**, 567 (2006).
[3] A. L. Brody, *Food Tech.*, **56**, 65 (2005).
[4] http://www.britannica.com/EBchecked/topic/575029/surface-coating.
[5] D. K. R. Robinson and M. J. Morrison, Nanotechnologies for food packaging: Reporting the science and technology research trends, Report for the ObservatoryNANO, August 2010, www. observatorynano.eu.
[6] S. Tokura, K. Ueno, S. Miyazaki, N. Nishi, *Macromol. Symp.*, **120**, 1 (1997).
[7] G. J. Tsai, W. H. Su, H. C. Chen, C. L. Pan, *Fish. Sci.*, **68**, 170 (2002).
[8] A. Begin and M. Van Calsteren, *Int. J. Biol. Macromol.*, **26**, 63 (1999).
[9] T. Bourtoom, *Int. Food Res. J.*, **15**, 237 (2008).
[10] C. Caner, P. J. Vergano, and J. L. Wiles, *J. Food Sci.*, **63**, 1049 (1998).
[11] S. Nur Dirim, H. Özlem Özden, A. Bayındırlı, A. Esin, *J. Food Eng.*, **63**, 9 (2004).
[12] D. Čakara, L. Fras Zemljič, M. Bračič, K. Stana-Kleinschek, *Carbohyd. Polym.*, **1**, 36 (2009).
[13] S. Theapsak, A. Watthanaphanit, R. Rujiravanit, *Appl. Mater. Interfaces*, **4**, 2474 (2012).
[14] M. Kong, X. G. Chen, K. Xing, H. J. Park, *Int. J. Food Microbiol.*, **144**, 51 (2010).
[15] Y. Cheng, X. Luo, J. Betz, S. Buckhout-White, O. Bekdash *et al.*, Supplementary Material (ESI) for Soft Matter, (2010).
[16] F. M. Gutierrez, P. L. Olive, A. Banuelos, E. Orrantia, N. Nino *et al.*, *Nanomedicine*, **6**, 681 (2010).
[17] Y. M. Chen, Y. C. Chung, L. W. Wang, K. T. Chen, S. Y. Li, *J. Environ. Sci. Health A*, **37**, 1379 (2002).