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Comparison of Oxygen Plasma and Cutinase Effect on Polyethylene Terephthalate Surface

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ABSTRACT: The aim of the study is to activate inert PET surface in order to introduce the carboxyl groups and to obtain its hydrophilic character. Two advanced and environmentally friendly techniques were used for these purposes: i) oxygen plasma activation; ii) enzymatic treatment by cutinase. Differently treated PET foils were studied in terms of carboxylic group content (non-aqueous potentiometric titrations, XPS) and hydrophobic/hydrophilic character (goniometry). Moreover, the influence of both activation procedures onto chitosan adsorption was examined by XPS, zeta potential measurements and ATR-FTIR spectroscopy. Obtained results show that plasma activation gives for around 19% higher amount of carboxylic groups than cutinase treatment and is during the storage less stable. Results clearly show that the use of both surfaces activation processes increases the ability of PET foils for chitosan adsorption. Due to the fact that chitosan is an antimicrobial agent, obtained materials may be applied as an active packaging system.
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KEYWORDS: PET surface; oxygen plasma; enzyme treatment; goniometry; chitosan adsorption

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INTRODUCTION

Polyester (PES) is one of the most important polymers, used in a food industry as packaging material. Among them, PET (polyethylene terephthalate) has found the widest field of application owing to its well-known physical and chemical properties. In the last decade, there has been intensively growing interest in production of new food packaging systems, which have recently been developed as a response to trends, that dictate fresh, safe, tasty, and natural food, with prolonged shelflife time.[1,2] Owing to strict European regulations for food-contact materials, only few among new packaging systems that belong to a group of active and intelligent package in the food industry have recently been used.[3]

In the majority of applications, PET surfaces are put in direct contact with food. Therefore, it is needed to enrich the packaging materials with environmental friendly and natural substances. Among such substances, microorganisms reducing polysaccharides like chitosans are often used. Factors influencing chitosan's antimicrobial effectiveness includes its positive charge, degree of *N*-deacetylation, mean polymerization degree, and the nature of chemical modifications.[4]

To extend the shelflife and to maintain product quality and safety, chitosan is an ideal potential substance for PET coatings, with bacteriostatic function,[5] influencing elongation of the lag phase and hence reducing the growth rate of microorganisms.[6–8]

Because PET material is inert and hydrophobic, the adsorption of chitosan onto its surfaces must be enlarged using different activation procedures in order to obtain carboxylic groups on its surface as well as for turning hydrophobic character into hydrophilic.[9–13]

Carboxyl group content is extremely important due to the fact, that they represent the binding places for cationic polymers, such as chitosan.

The main objective of this work is to develop a novel, bioactive food packaging material—PET coated with chitosan. New materials represent a potential active foil, for packing fresh meat and

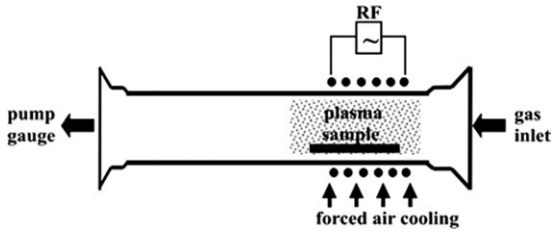

Figure 1. A schematic experimental setup for the oxygen plasma treatment.

meat products, using different conditions: controlled atmosphere and vacuum. To achieve efficient binding of chitosan onto the PET foil surface, two advanced and environmentally friendly techniques were used: oxygen plasma treatment and enzymatic–cutinase treatment.[14,15] The influence of both activation procedures on chitosan-PET attachments was analyzed.

EXPERIMENTAL

Starting Materials

For experimental work, amorphous polyethylene terephthalate (PET) foil with thickness 350 μm, produced by Goodfellow was used. Other chemicals used in the experimental work were supplied by Sigma Aldrich. The foils were cut into the stripes of 20 x 100 mm^2 and cleansed in a pure ethanol bath, exposed to an ultrasound (Transsonic 825/H) for 30 min. Thereupon they were immersed in bidistilled water and air-dried to a constant weight. Once foils were treated with oxygen plasma or enzymatically using cutinase, they were placed into the 1.5% (w/w) chitosan solution for 72 h. Finally, foils were dried in a vacuum dryer (Kambič, VS–25°C) at 50°C, for 24 h. For activated and nonactivated foils, nonaqueous potentiometric titrations, XPS, and contact angle measurements were performed.

MATERIALS AND METHODS

PET Activation Methods for Carboxyl Group Formation

Cutinase Treatment. The cutinase from Humicola insolens was produced as previously described by Araujo and Müller.[16,17] Stripes of PET foil were treated using enzyme with a concentration 200 μg/mL (120 h at 50°C). After the treatment, they were washed with the phosphate buffer[16,17] and air dried to a constant weight.

Plasma Treatment. Plasma system that was used for activation of PET foils is shown in Figure 1. The plasma excitation was provided by a radiofrequency (RF) generator, which is coupled to an excitation coil that is wrapped around the discharge tube.[18] The discharge tube was a Pyrex cylinder with a length of 0.6 m and an inner diameter of 0.036 m. Plasma was formed with an inductively coupled RF generator, operating at a frequency of 27.12 MHz and an output power of about 200 W. Parameters were measured with a double Langmuir probe and a catalytic probe.[19,20] PET sample was placed into the discharge chamber at the position of the coil that is wrapped around the discharge chamber (Figure 1). The system was pumped with a two-stage oil rotary pump, with a pumping speed of 4.4 x 10^{-3} m^3 s^{-1}. Commercial oxygen was leaked into the discharger chamber, and the pressure was measured by an absolute vacuum gauge. During continuous pumping, the pressure was adjusted up to a 75 Pa, with a precise leak valve, at which it is possible to obtain the highest degree of molecule dissociation, measured by a catalytic probe. At described discharge parameters, plasma with an ion density of about 2 x 10^{15} m^{-3}, an electron temperature of 4 eV and a neutral atom density of about 4 x 10^{21} m^{-3}, has been obtained. Both sides of the PET foils were treated with oxygen plasma for 30 s.

Adsorption of Chitosan. Chitosan solution of a concentration 1.5% (w/w) had been made in bidistilled water. pH was adjusted down to a 3.6, using 37% HCl among stirring. Cleansed reference PET, O_2 plasma and cutinase treated foils were separately immersed into the chitosan solution and placed on a platform shaker (IKA KS501) for 72 hours. Afterwards, they were dried at 50°C in a vacuum to a constant weight.

Characterization of PET Foils

Activated PET foils (plasma and cutinase treatments).

Nonaqueous potentiometric titrations. The nonaqueous potentiometric titration for determination of carboxyl groups in PET, after plasma and cutinase treatment, has been done by Mettler DL 53 titrator. Before the titration, 0.1 M tetrabutyl ammonium hydroxide (TBAH) solution in isopropanol had been standardized, using 0.1 M benzyl alcohol. Reference sample, plasma and cutinase treated PET foils (0.25 g), were immersed into the 50 mL of boiling benzyl alcohol (AR grade) to be dissolved. After cooling the solution down to 80°C, the potentiometric titration of sample suspension was preformed automatically, with titration of a 0.1 M TBAH in isopropanol, using combined glass pH electrode for nonaqueous media Mettler Toledo DGi 112. The titration is typical organic acid base titration where equivalent point is determined from inflection point of the titration curve.

The amount of carboxylic groups has been calculated using the equation written below:

$$\mu\text{Eq/kg(COOH)} = \frac{(a-b)\times t \times 100}{E} \quad (1)$$

where a is consumption of mL titrating reagent for the sample, b is consumption of mL titrating reagent for the blank value, t is titrating reagent factor, and E is a sample mass in grams.

The amount of carboxylic groups was determined for treated and nontreated foils from five parallels. The method was also used for monitoring the influence of both activation procedures to the storage time.

XPS (X-Ray Photoelectron Spectroscopy). XPS spectra of PET foils were recorded using the PHI model TFA XPS spectrometer. The atomic composition was measured after chitosan adsorption onto differently activated PET, and then compared to the elemental chemical composition of nontreated materials surface. The base pressure in the XPS analysis chamber was about 6 × 10^{-10} mbar and the samples were excited with X-rays over a specific 400 -μm area, using monochromatic Al $K\alpha_{1,2}$ radiations at 1486.6 eV. The photoelectrons were detected by a hemispherical analyzer, positioned at an angle of 45° with respect to the sample's surface normal. Energy resolution was about 0.6 eV. Spectra were recorded from at least two locations on each sample, using an analysis area of 400 -μm. Surface elemental concentrations were calculated from the survey-scan spectra, which were recorded at a

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Table I. Carboxylic Group Content of Plasma and Enzymatically Modified PET, Determined with Nonaqueous Potentiometric Titrations

Pretreatment	n COO- (mmol/kg)
Reference material	0
Plasma	32.72 ± 5.74
Within 1 day	
After 3 days of storage	17.25 ± 1.25
After 1 week of storage	0
Cutinase within 1 day	27.40 ± 0.80
After 3 days of storage	27.40 ± 1.00
After 1 week of storage	27.40 ± 1.20

pass energy of 187.85 eV with 0.4 eV energy step. Individual high-resolution spectra of C1s were taken at a pass energy of 23.5 eV and a 0.1 eV energy step. Since the samples were insulators, an additional electron gun was used to allow for surface neutralization during the measurements. Two repetitions of measurement were done for each sample.

Goniometry. Contact angle measurements on differently treated PET foil surfaces were carried by sessile drop method, using goniometer Data Physics OCA35 (DatPhysics, Germany) apparatus. For the measurements, MilliQ water (Millipore, USA) with volume drops fixed to a 3 μL was used. Measurements have been done at room temperature with at least five repetitions, obeying ± 2% experimental error.

Adsorption of Chitosan onto PET Foils

ATR-FTIR. Besides XPS (as described above), characterization with ATR-FTIR was used to examine the influence of activation procedures onto chitosan adsorption. In this way, surface chemical composition of PET foils was being studied. Analysis of dry samples was performed on a PerkinElmer Spectrum GX spectrometer, equipped with a diamond crystal ATR attachment. The depth analysis was ~0.75 μm. Spectra were recorded at 2 cm^{-1} spectral resolution within the range (4000–650) cm^{-1}, using an average of 16 scans. The detailed description of technique is given in Skoog et al.[21]

Zeta Potential Measurements

Zeta potential was measured by detecting streaming potential, using Anton Paar electrokinetic analyzer. For the measurements, 0.001 M KCl electrolyte solution was used. One gram of differently treated PET foil was maintained in a cell, while the electrolyte was forced to flow through the membrane at varying pressures. Three measurements were carried out on each sample for pH values of the electrolyte solution from pH 3 to pH 10.

RESULTS AND DISCUSSION

Characterization of Activated PET Foils

Nonaqueous Potentiometric Titrations. The amount of carboxylic groups in reference PET foils and foils activated by oxygen plasma and cutinase treatment, respectively, is presented in Table I. It is clearly seen that both activation procedures introduce carboxyl groups to PET foils. Plasma activation gave for 19.4% higher amount of carboxyl groups in comparison to cutinase-treated samples. However, the advantage of cutinase treatment is in the stability of carboxyl groups. The storage of PET foils within 1 month did not influence the amount of carboxyl groups, while plasma treatment did not provide constant amount of carboxyl groups as a function of storage time. After 4 days of plasma activated PET foils storage, the amount of carboxyl groups is reduced on half of the initial carboxyl group amount. After 1 week, no carboxyl groups were detected in plasma activated PET foils. This is due to ageing effects of plasma treated surface that is not in equilibrium. Because of the substantial increase of the surface energy after plasma treatment, which is not energetically favorable, the surface tends to rearrange. Surface rearrangement includes different mechanisms like reorientation of the functional groups into the bulk of the polymer and reactions of the groups between themselves or with the environment.[22] Therefore, their concentration at the plasma-activated polymer surface is decreasing with time.[18]

XPS. Modification of the PET foil surface composition, after oxygen plasma and cutinase treatment, was also determined by XPS. In Figure 2 comparison of high resolution carbon C1s spectra for nonactivated, plasma-activated, and cutinase-activated PET foil is shown. Nonactivated PET foil (reference PET) consists of three peaks due to —C=C bonds in the phenyl ring, ether bonds —C—O and ester groups O=C—O. After both treatments, the relative concentrations of different bonds are changed and furthermore a new peak due to C=O or O—C—O group may appear as well as it is expected for plasma treatment [18]. Here we should also mention, that the last peak at high binding energies represents all types of —O=C—O groups, involving not just ester groups from original polymer but also carboxylic —COOH groups. This value significantly increased after the plasma treatment due to the formation of new carboxylic groups. Cutinase treatment shows negligible increase in concentration of —O=C—O groups.

The relative amounts of different carbon functional groups as determined by fitting the carbon C1s peaks presented in Figure 2 are given in Table II. Obviously, plasma treatment gives more surface-limited carboxylic groups in comparison to cutinase

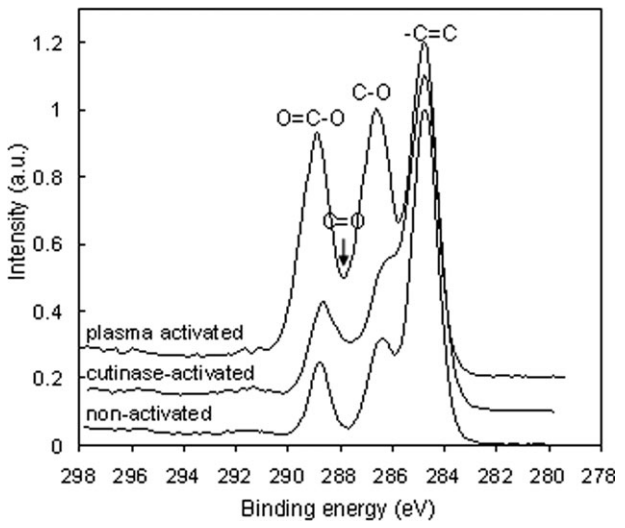

Figure 2. Comparison of high resolution carbon C1s spectra for non-treated, plasma-treated, and cutinase-treated PET foil.

Table II. Comparison of a Carbon Bond Concentration on PET Surface (in %)

			O—C—O	
Bond type	C—C	C—O	C=O	O=C—O
PET blank	68.9	19.9	0	11.2
PET plasma 30 s	35.8	33.9	2.3	28.1
PET cutinase	59.1	22.5	5.5	12.9

treatment. When results are compared to titration techniques, it can be suspected that plasma treatment provides more carboxylic groups in thinner surface layer (up to 10 nm; sensitive for XPS), whilst cutinase treatment forms —COOH groups in deeper surface layers of PET material.

Goniometry. Changes in surface chemical composition and formation of new functional groups after cutinase and especially after oxygen plasma treatment may have a substantial influence on the surface hydrophilicity. Therefore, contact angles of water drop have been measured to observe hydrophilic character of PET surface. Results are presented in Figure 3. Between both chosen techniques for PET surface activation, plasma treatment gives the most hydrophilic character, due to the fact that a contact angle decreased from 75° to 45°. This is in accordance with the introduction of new polar —COOH groups after plasma activation. In the case of cutinase treatment, the contact angle of the sample decreased for ~14% in comparison with the reference material. The reason for that may be in —COOH groups' formation in inner parts of PET material, which does not totally influence limited surface wettability determined by goniometry. Besides hydrophilic polar group formation, plasma treatment increases surface roughness. Described changes lead to increased PET surface wettability.

Characterization of Chitosan Adsorption Onto PET Foils

The amount of nitrogen as a consequence of chitosan adsorption was determined by XPS and ATR-FTIR.

XPS. XPS results show that significant amounts of nitrogen were detected only in the samples treated with chitosan. The reference PET foils and both activated PET foils did not contain any nitrogen on the surfaces. Thus, the detected nitrogen (in at.%) was due to the amino groups introduced onto the PET

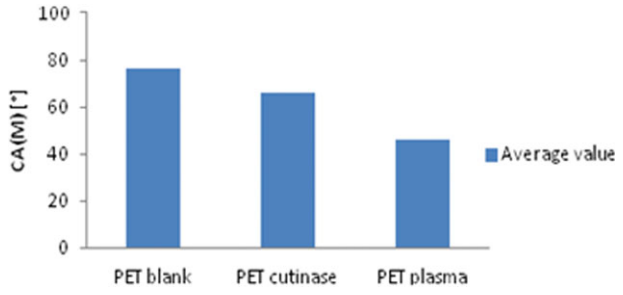

Figure 3. Average values of measured contact angles between water and differently treated PET (reference, cutinase, and plasma treated). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

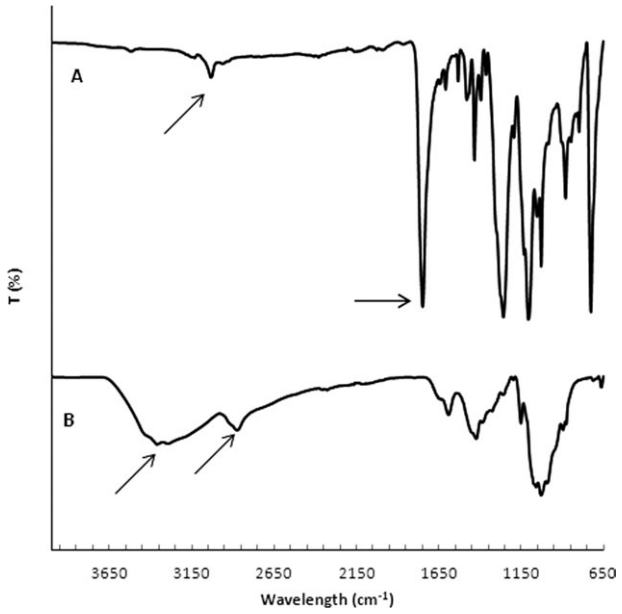

Figure 4. ATR-FTIR spectra of (A) reference PET foil and (B) freeze dried 1.5% chitosan solution. Traced peaks are marked with arrows: $-CH_2$ (A, B; 2950–3010 cm^{-1}), $-C=O$ (A; 1630–1720 cm^{-1}) and $-NH_2$ (B; 3300–3340 cm^{-1}).

foils by the adsorption of chitosan, i.e., XPS confirms that chitosan was adsorbed on all PET surfaces treated with chitosan solutions.; i.e., PET reference material, plasma, and cutinase-activated samples, respectively.

The reference PET material (nonactivated) coated by 1.5 w/w of chitosan solution contained 5.1 at. % of nitrogen, while sample activated by cutinase and further adsorbed by chitosan contained 6.6 at.% of nitrogen. Plasma activated PET foil, which was coated with chitosan, contains 5.9 at.% of nitrogen. These results clearly show that plasma and cutinase treatment increase the ability of the foils to absorb chitosan. These XPS results are in agreement with ATR-FTIR and zeta potential measurements presented below.

ATR-FTIR. Figure 4 represents ATR-FTIR spectra of raw materials: PET and freeze dried chitosan solution, while Figure 5 show spectra of PET (reference and activated with plasma and cutinase, respectively) functionalized by chitosan. With ATR-FTIR method, the efficiency of chitosan impregnation had been traced. Results show that in all of the samples (nonactivated PET foils and both activated PET foils), chitosan was successfully distributed on their surfaces, which is proved by the presence of —NH stretching peak in the area between 3300 cm^{-1} and 3340 cm^{-1}. With the appearance of amino peak, the —CH wagging peak in the area between 2950 cm^{-1} and 3010 cm^{-1} has increased. As ATR-FTIR method detects functional groups down to the depth of 0.75 μm, the additional prove for successful impregnation is a reduction of —C=O stretching peak in the area from 1630 cm^{-1} to 1720 cm^{-1}.

Zeta Potential Measurements. Zeta potential is calculated from measurements of streaming potential, as a function of pH. When all functionalized samples are compared (Figure 6) it is

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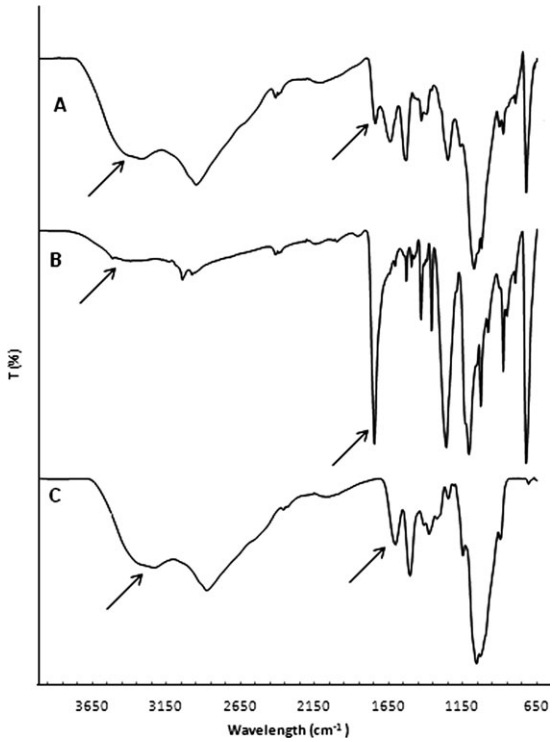

Figure 5. ATR-FTIR spectra of chitosan functionalized PET foils: (A) reference, (B) O_2 plasma pretreated, and C) cutinase pretreated. Traced peaks are marked with arrows: $-CH_2$ (A, B, C; 2950–3010 cm^{-1}), $-C=O$ (A, B, C; 1630–1720 cm^{-1}) and $-NH_2$ (A, B, C; 3300–3340 cm^{-1}).

obvious that plasma and cutinase treated samples adsorbed chitosan more extensively than nonactivated PET sample. Shifting of isoelectric point to the higher pH is observed, demonstrating the high degree of chitosan adhesion. In the case of plasma treated sample, isoelectric point is around 6.7 which is typical pH value of pure chitosan[23] and thus indicated efficient covering of plasma treated sample by chitosan. In addition, both of the preactivated samples showed increasing of positive plateau level of zeta potential after chitosan adsorption, which is qualitative indication for protonated amino groups as a consequence of chitosan binding. When both activated samples are compared, it is due to more intensive shifting of isoelectric point into alkaline region obvious, that higher amount of chitosan was adsorbed onto plasma-activated PET sample. XPS results showed opposite; i.e., the highest amount of nitrogen was detected onto cutinase PET-chitosan adsorbed sample. However, this is explainable due to the different nature of techniques; XPS gave information about the atomic concentration in the dry thin film of external polymer surfaces (around 10 nm) only, while zeta potential give information about protonated amino groups in a swelling form of PET foil (inner part of PET; higher total accessibility). Another explanation is that due to a higher amount of adsorbed chitosan onto cutinase-treated samples, there is a higher probability of electrostatic interactions formation between carboxyl PET groups and chitosan's amino groups. Consequently less accessible amino groups are available to be protonated and were detected by zeta potential measurements.

CONCLUSIONS

Two different treatment procedures were used to introduce carboxyl groups onto PET surfaces in order to obtain successful attachment of antibacterial chitosan coatings to the surface of PET foils that may be used as a packaging material for meat products. These two activation procedures include oxygen plasma treatment and cutinase treatment. The modification of PET surface after activation with both procedures and successfulness of chitosan adhesion was monitored with different methods. Nonaqueous potentiometric titrations showed significant differences in carboxylic group amount between PET reference material and plasma as well as cutinase-activated PET material, where higher amount of carboxylic groups was detected.

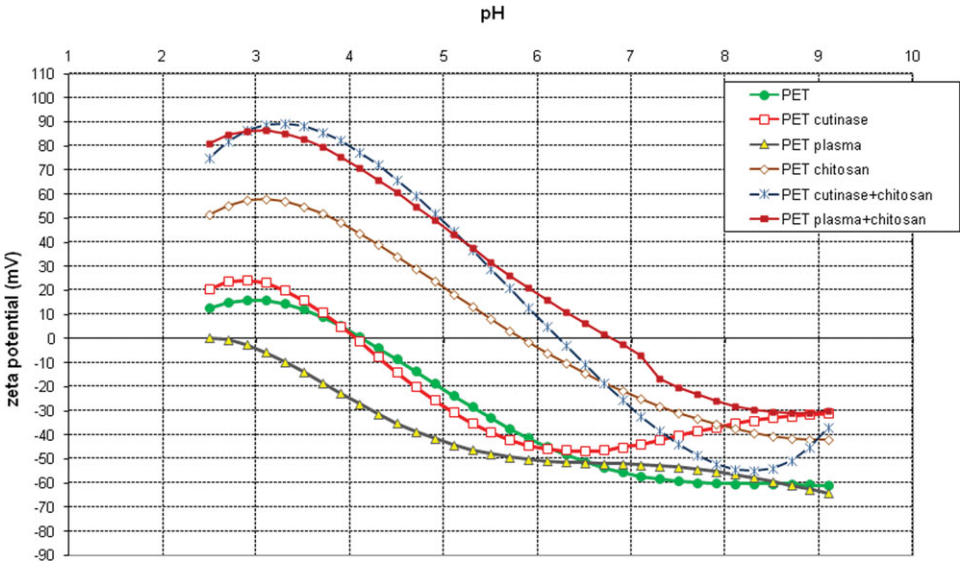

Figure 6. Zeta potential (ζ) of PET foils as a function of pH [$\zeta = f(pH)$]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

XPS, which is surface highly sensitive method, showed higher amount of carboxylic groups mostly for plasma-activated PET surface, meaning that in the case of plasma treatment the carboxylic groups are concentrated more near the surface of polymer foil than in the case of cutinase treatment. After plasma treatment samples became much more hydrophilic. Cutinase treatment gives a small increase of surface hydrophilicity. The storage of cutinase activated PET foils within 1 month did not influence the amount of carboxyl groups, while by plasma treatment after 1 week no carboxyl groups were detected

XPS, zeta potential measurements and ATR-FTIR results pointed out the effective chitosan treatment in pretreated and nonpretreated PET samples. XPS results and zeta potential measurements showed that both plasma and cutinase treatment increase the ability of the foils to absorb chitosan in comparison to nontreated PET samples. Therefore, it can be concluded, that due to successful adsorption of antimicrobial agent as chitosan is, the functionalized PET foil may be used as a potential active packaging material.

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