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Rhamnolipid biosurfactant adsorption on plasma treated polypropylene surface to induce antimicrobial and antiadhesive properties

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Abstract

A glycolipid kind of biosurfactant (rhamnolipid) which is obtained from *Pseudomonas aeruginosa* MA01, adsorbed on the polypropylene film to produce antimicrobial and antiadhesive polymeric surface for the first time. The polypropylene was modified using oxygen and air plasma. The effects of plasma operating condition including the plasma power and the time of plasma exposure were studied. The characteristics and hydrophobicity of the polypropylene surface evaluated by several techniques including ATR-FTIR, XPS, SEM and AFM as well as measuring water contact angles (WCA). The results, confirmed successful attachment of rhamnolipid on plasma treated surfaces, however in different degrees based on plasma condition. Antibacterial and antiadhesive performance of the rhamnolipid-adsorbed-films was investigated against pathogenic bacteria, and the results showed considerable activity of the surface to reduce the number of bacteria on the treated polymeric film. The optimum plasma condition, in which the best antimicrobial and antiadhesive surface was obtained, revealed as power of 50 W, exposure time of 6 min with air as plasma gas.

Keywords: Plasma, Rhamnolipid, Biosurfactant, Polypropylene, Antimicrobial surface

Introduction

Safety and quality of ready-to-eat "fresh" food products is one of the major new challenges in food industry.[1] Development of packaging materials with antibacterial or antiadhesive properties and ensuring their hygienic status remains a fundamental scientific, technological and industrial challenge.[2] Polymers are the most frequently used materials in food industry, especially polypropylene (PP) which often used in soft or hard packaging. Antibacterial/antiadhesive packaging materials can effectively inhibit the growth or prevent the adhesion of microorganisms to the surface. The *L. monocytogenes* is an important foodborne pathogen which can cause the serious illness, listeriosis. Furthermore, *S. aureus*, is the bacterium which causes staph infections, and a Gram negative bacterium, *K. pneumoniae*, the bacterium responsible for pneumonia.[3] These bacteria have been found in a wide variety of food products such as raw vegetables, raw meat, dairy products and ready-to-eat foods.[4]

In the recent years, many attempts have been made to functionalize the surfaces with chemical antimicrobial agents to manufacture antimicrobial films.[5, 6] However; very few studies have been reported on the use of natural antimicrobial agents produced by microorganisms. Surface modification has been performed using natural biological substances like bacteriocins, which possess antimicrobial activity.[7] Nisin is currently the only bacteriocin widely used as a food preservative.[7] This peptide, which is produced by *Lactococcus lactis* subsp. *lactis,* exerts rapid bactericidal effects against a broad spectrum of Gram-positive bacteria and food pathogens, including *L. monocytogenes*, *S. aureus*, *B. cereus*, and *C. botulinum*.[8,9]. Karam et al. [1] investigated nisin adsorption on the polyethylene surface which was previously modified using Argon/Oxygen (Ar/O_2) plasma, nitrogen (N_2) plasma and plasma-induced grafting of acrylic

acid. Maximum antibacterial activity was recorded on the Ar/O_2 plasma followed by acrylic acid and N_2 treated films, and the lowest activity was observed on the native film.

Biosurfactants are amphipathic compounds excreted by microorganisms showing surface activity.[10]The rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* is a glycolipid composed of one or two rhamnose molecules linked to one or two fatty acid alkyl chains. They are synthesized as a mixture of homologs mainly composed of di-rhamnolipids and mono-rhamnolipids.[11] Rhamnolipid biosurfactants show several properties such as surface activity, emulsification, better environmental compatibility, biodegradability and specific activity at extreme temperatures, pH and salinity.[12] These properties are very useful in the processing industries [10.] The rhamnolipid biosurfactant has demonstrated great antiadhesive and antimicrobial activity against several microorganisms such as the Gram-positive bacteria, *S. aureus*, *B. subtilis*, *C. perfringens*, the Gram-negative bacteria *S. Typhimurium*, *E. coli*, *E. aerogenes* and the fungi *P. infestans*, *P. capsici*, *B. cinerea*, *F. graminearum* and *Mucor* spp.[4] Because of their antimicrobial activity, biosurfactants are used as food preservative.[13] With regard to the above explanation, it can be a noble idea to employ biosurfactants as antimicrobial agents for active packaging.

The objective of this study is to evaluate the use of plasma treatment to modify a polymeric surface with the goal of rhamnolipid adsorption on the surface. Polypropylene, a well-known polymer in the food and biomedical sectors, was subjected to the plasma environment on different conditions. Rhamnolipid biosurfactant was then adsorbed on the plasma treated surface. The treated polymeric surfaces characterized by different methods before and after treatment. Finally, the antimicrobial and antiadhesive activity of the plasma treated polymeric surface was evaluated.

Results and Discussion

In order to simplify and clear the real parameters affecting the surfaces, primary experiments were performed to find out the optimum exposure time. This optimum time was selected for further tests.

Primary experiments for plasma exposure time optimization

The untreated PP film has hydrophobic properties ant its water contact angle (WCA) was 91.3° .The plasma exposure times of 2, 4, 6, 8 and 10 minutes using air and oxygen gases and the power of 50 W studied and the measured water contact angles (WCAs) in the case of air gas were 70.7, 66.1, 51.2, 50 and 50.5°, respectively. The most significant decrease in hydrophilicity appeared in the exposure time of 6 min and further extension of time did not change the WCA significantly. Similar trend was observed using oxygen gas. Therefore the exposure time of 6 minute was used as optimum plasma exposure time for further experiments.

Hydrophilicity and antimicrobial/antiadhesive activity of the surfaces

Experiments were carried out according to Table 1 with two types of gas (Air and Oxygen) and four different plasma RF powers (25, 50, 75 and 100 W) at a fixed exposure time of 6 min. The measured contact angles of PP films after plasma treatment and also after rhamnolipid adsorption have been presented in Table 1. The contact angles of PP surfaces dropped from original value of 91.3 ± 2.3° to 50.6 ± 1.7° for oxygen treatment with the power of 75 W (PP-O2-75) and to 50.2 ± 2.1 for air treated with the power of 50 (PP-Air-50). After rhamnolipid adsorption on the surface, the WCA dropped significantly. WCA decreased from 50.6 ± 1.7 to 15.9 ± 1.7 for PP-O2-75 and from 50.2 ± 2.1 to 12.2 ± 1.4 for PP-Air-50. The same trend was observed for remaining samples. WCA results confirmed that the plasma treatment was effective to improve the surface hydrophilicity. Moreover, the influence of rhamnolipid adsorption on the

improvement of surface hydrophilicity was more deeper than only with plasma treatment. The decrease in contact angle is likely due to the presence of polar groups such as oxygen-containing functional groups.[13,14]. Fig. 1 illustrates the selected images of water droplets for WCA measurement. The droplet shape on the PP surface changes after plasma treatment and more significantly after rhamnolipid adsorption.

Antimicrobial assay was performed after adsorption of rhamnolipid on the PP surfaces. The results were compared with the antimicrobial activity of untreated PP and reported as area (mm^2) of inhibition zone. Two Gram-positive (i.e. *S. aureus B. subtilis*) and two Gram-negative (i.e. *P. aeruginosa and K. pneumonia*) bacteria were used for this assay. Fig S1 shows a schematic example of the test.

Table 1 shows that no inhibition zone formed around the PP films placed on Gram negative bacteria. The antimicrobial agents interact with the cell membrane of Gram negative bacteria and may serve as a barrier to the entry of antimicrobial molecules. The difference between the structure of the cell membrane of Gram positive and Gram negative bacteria is the main reason for differences in their susceptibility towards antimicrobial agents.[15,16]

In low RF power of 25 W (Table 1), a lower antimicrobial activity of the film was observed. In general, PP-O2-75 and PP-Air-50 samples, with 595 and 681 mm^2 clear zones, respectively, resulted in best antimicrobial activities. This can be explained on the base of rhamnolipid immobilization on the surface. This indicates that rhamnolipid biosurfactant have immobilized more efficiently through hydrophilic interactions or hydrogen bonds on the samples surfaces. In addition the antimicrobial agent is well oriented on the surface to inactivate Gram positive bacteria deposited over them. Comparatively the air plasma treatment (PP-air-50) resulted in better final antimicrobial activity than oxygen treated plasma (PP-O2-75).

The rhamnolipid biosurfactants have a great antiadhesive activity. This activity may help the rhamnolipid-adsorbed-surface to prevent bacterial adhesion. The histograms shown in Fig. S2 of the supplementary data, present antiadhesive activity of the rhamnolipid-adsorbed-films. The results showed that almost all treated samples could prevent bacterial adhesion to the surface for both Gram positive and negative bacteria. However, the number of Gram negative *K. pneumonia* adhered onto the films was generally higher than that of for *B. subtilis,* which may be due to the difference between the physicochemical characteristics of the bacteria and materials.[17] The antibacterial samples did not fully inhibit the formation of the bacterial biofilm after 18 h incubation, which is in agreement with Zhang et al. [18]. However, over 60% inhibition is observed for some samples suggesting the capability of rhamnolipid to inhibit bacterial adhesion.

Surface topology and morphology analysis

Fig. 2 shows the two-dimensional and three-dimensional AFM surface morphology of the films before and after plasma treatment. Table 2 also shows the values for the most common surface parameters for the plasma treated samples before rhamnolipid adsorption. These parameters include average roughness (R_a), root mean roughness (R_q), Skewness (R_{sk}) and Kurtosis (R_{ku}).[19,20] The untreated PP film is quite smooth, with an average roughness (R_a) value of 9.92 ± 1.13 nm and root mean roughness (R_q) value of 10.01 ± 1.33 nm. Experimental evidences have shown that the surface morphology of the polymer films turned rougher after the plasma treatment.[21] In some cases, however, the plasma treated surface turned smoother.[2] In this study, R_a was increased almost for all treated samples except for PP-O2-25 sample. In the case of PP-O2-25 the R_a showed a slight decrease from 9.92 to 9.11 nm. In addition, for PP-Air-25 the R_a has a slight increase from 9.92 to 11.09 nm. It can be say that in low powers, no appreciable change in roughness occurs. The R_a values for PP-O2-75 and PP-Air-50 have been increased

more than two and three folds, respectively. It is hard to draw some direct conclusions between roughness parameters and rhamnolipid adsorption to the surface, however, rougher surfaces have been generally led to better rhamnolipid adsorption (so better antimicrobial performance).

The 3D AFM images of air treated (B, C, D, E) and O_2 treated (F, G, H, I) plasmas, are presented in Fig 2. A considerable differences between the formed patterns are observed. "Lay" is the term used to indicate the direction of the dominant pattern of texture on the surface. For samples which have been treated with O_2 gas, the lay is in the front-to-back direction. However, in the case of air plasma, an irregular pattern was formed. In general, the surfaces with irregular structure (Air plasma films) showed higher amount of antimicrobial activity (Table 1).

This kind of structure may provide more anchoring or filling sites for rhamnolipid adsorption[1] and further higher antimicrobial activity. 3D image of AFM analysis for a representative sample (PP-Air-50) after rhamnolipid adsorption has been presented in Fig. 2 J. In this sample, all peaks have been covered with the antimicrobial material. In fact, it seems that rhamnolipid molecules filled the valleys and causes the surface to be smooth. This observation can boost the hypothesis that valleys may serve as anchoring or filling sites for adsorption of other molecules.

The SEM images of the sample PP-Air-50 (which showed the best antimicrobial activity) after plasma treatment and after rhamnolipid adsorption along with the untreated PP are illustrated in Fig. 3. In Fig 3 A, a relatively smooth and uniform morphology was observed for the untreated film. After undergoing some alterations in the plasma chamber (Fig. 3 B), the modification leads to the presence of some diagonal patterning and irregular shape surface texture. This topography is beneficial for the subsequent coupling processes due to the surface area and roughness increase.[22] In fact, the generated pattern on the plasma treated specimen is

due to both ablation and functionalization of the surface which leads to surface restructuring.[23] However, these changes in surface morphology are not appreciable. On the other hand, the surface after rhamnolipid adsorption has entirely different structure. As can be seen in Fig. 3 C, a granular like structure is formed after rhamnolipid adsorption.

Surface chemistry analysis, ATR-FTIR and XPS

Fig. 4 shows the ATR-FTIR pattern of the untreated PP along with the PP-Air-50 sample after plasma treatment and after rhamnolipid adsorption. In the IR spectra of the untreated PP, special interest is focused on the following absorption peak: 973 cm^{-1}, rocking vibration (-CH_2-); 997 cm^{-1}, rocking vibration (-CH_2-); 1167 cm^{-1} anti-symmetric deformation (-CH_3-); 1455 cm^{-1} symmetric deformation (-CH_2-); 1167 cm^{-1}, Symmetric deformation (-CH_3-); 1167 anti-symmetric deformation (-CH-); 2917 symmetric stretching (-CH_3-). However, as can be observed in Fig 4, upon exposure of the untreated sample to plasma discharge, almost no considerable change was detected in ATR-FTIR of PP-Air-50 sample. Only a weak broad peak between 3400-3500 cm^{-1} corresponding to OH group can be taken into consideration. This is not only because of signals overlapping, but also due to the plasma modification depth being limited to solely top layers of the surface and cannot be well evidenced by ATR–FTIR. The crystals used in ATR cells (Zinc selenide (ZnSe)) for polymers have a low solubility in water and very high refractive index(≈2.4) and average sampling depth of ≈4 μm.[24] This sampling depth exceeds the normal thickness of plasma modified layers on a substrate (<100nm). Nonetheless, ATR–FTIR is still widely used to provide semi-quantitative information on the chemistry of the near-surface region.

The changes in ATR-FTIR pattern after adsorption of antimicrobial rhamnolipid on PP film, is clearly detectable (Fig. 4). The O–H stretching of free hydroxyl groups of rhamnose rings

around 3385–3390 cm^{-1}, the stretching bands of the methylene and terminal methyl groups of the acyl chains between 2850 and 2930 cm^{-1}, the stretching band of the carbonyl C=O groups at approximately 1744 cm^{-1}, the free −COO− band (free carboxyl group of the second fatty acid) around 1560–1580 cm^{-1}, and the C–O–C vibrations (rhamnose rings) at about 1045 cm^{-1} are some characteristic peaks of adsorbed antimicrobial agent on the surface. The FTIR pattern of pure mono-rhamnolipid produced by *Pseudomonas aeruginosa* MA01 can be observed in Fig. S3 B in supplementary file.

The FTIR pattern cannot tell us in detail about the changes in surface chemistry of the plasma treated film. Therefore, XPS analysis has been performed on plasma treated PP films, in combination with a labeling technique for better understanding on the functionalities introduced to the polymer surface. Figure 5 presents the XPS scans of the untreated PP as well as for PP-O2-75 and PP-Air-50 samples with best antimicrobial activity. The elemental composition expressed as atomic concentrations of the selected samples have been presented in Table 2. The scan of the untreated PP shows only one peak, which is attributed to the C1s of the aliphatic carbon bonds or carbon–hydrogen bonds (C-C, C-H) (Fig. 5). It should be noted that the carbon C1s peaks of the untreated sample as well as PP-O2-75 are shown in Figs S4 and S5. The XPS records reveal that no traces of any contaminant element were found on the surface of untreated PP. After oxygen or air plasma an increase in oxygen concentration was observed on the films (Table 2 and Fig. 5). This can be associated to the created oxygen functional groups.[25] Furthermore, when air was used as a plasma gas, nitrogen element (N1s) was also detected on the surface. Surprisingly, small amount of chlorine (Cl2p) was observed on the surface of PP film for all plasma treated samples, which may come from plasma parts as a contaminant.

The amount of carbon content was reduced after plasma treatment (Table 3). The decrease of carbon content and the increase of oxygen content on both samples can be contributed to the introduction of oxygen-containing polar groups (C-O, C=O, -O-C=O, -COH) on the surface of polypropylene. The incorporation of oxygen containing polar groups in PP surface may be the main reason for the hydrophilic improvement of PP-O2-75 and PP-Air-50 samples as described in Table 1. On the other hand the presence of these oxygenic group can improve the linkage between antimicrobial molecules and the surface, during adsorption process.

Film stability

In order to investigate the stability of the antimicrobial surfaces under likely application conditions, PP-O2-75 and PP-Air-50 samples were submitted to relatively soft cleaning conditions such as immersion in water for 24 h and in a non-ionic detergent solution. The results showed that both PP-O2-75 and PP-Air-50 samples were stable and kept their antibacterial activity after contacting with water. In case of emerging in non-ionic detergent solution the activity of the films reduced by 22 and 13%, respectively.

Materials and Methods

Chemicals and Microorganisms

All chemicals were purchased from Merck (Germany) unless otherwise stated. *Pseudomonas aeruginosa* MA01, which had been isolated from spoiled apple in our previous work,[11] was used for production of rhamnolipid biosurfactant. *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, and Pseudomonas aeruginosa* were kindly provided by the university of Tehran and used for antimicrobial or antiadhesive assays.

Production, extraction and purification of the rhamnolipid biosurfactant

Pseudomonas aeruginosa MA01 (isolated and identified in the previous work[11]) was used for rhamnolipid production. This microorganism was pre-cultured in nutrient broth medium at 30 °C and 200 rpm for 14-16 h. The amount of 4% inoculation was performed from this seed culture to the production medium. The medium used for rhamnolipid biosurfactant production includes (g/L): sunflower oil 20, yeast extract 1, $NaNO_3$ 3, $MgSO_4.7H_2O$ 0.25 and KH_2PO_4 0.25. After 5-6 days cultivation at 30 °C and 200 rpm, the biosurfactant was extracted from the cell free supernatant using the method of precipitation followed by solvent extraction. The biosurfactant was then purified using column chromatography as explained in the previous work.[11] The slurry of silica gel 60 in chloroform was poured into glass column chromatography. Crude biosurfactant was dissolved in chloroform and loaded onto the column. The purification was carried out by washing the column with chloroform (to elute neutral lipids), followed by chloroform:methanol. During the purification steps, analytical TLC (using chloroform:methanol:H_2O (65:15:2) as mobile phase) was used to check the purity of the fractions. The purified biosurfactant was then characterized by TLC, FTIR and ES-MS techniques (Fig S3). Finally, pure mono-rhamnolipid was kept in appropriate condition for further studies.

Film preparation

The polypropylene (PP) films, with the thickness of 0.2 mm, were initially cut into 1.5 × 1.5 cm^2. The films were then washed with ethanol in an ultrasonic bath to remove possible dusts, oily compounds or any other chemicals and wetting agents absorbed on the film surface. The films were dried in an oven at 55 °C for 3 h and stored in a desiccator before use.

Plasma treatment

Plasma treatment of polymeric films was carried out in a plasma chamber (Nano-LF-RF-PC, Diener electronic Technology, Germany) using RF generator (13.56 MHz, max 100 W). The schematic of the plasma system and plasma equipment are illustrated in Figs. S6 and S7. The polymeric sample was placed into the chamber (made of quartz glass) and the pressure was reduced to 0.1 mbar by means of a vacuum pump (Trivac, Germany). Process parameters such as gas type, power and exposure time were varied to optimize for the best treatment condition. In order to reduce the number of experiments and so reducing the costs, the films were initially plasma treated with air and oxygen plasma and a medium power of 50 W at different exposure times to select the optimum exposure time. Since low contact angle corresponds to formation of oxygenic groups, the surface hydrophilicity was employed as response. After this primary experiment, a comprehensive study was performed to see the effect of gas type and power strength on plasma treatment procedure and finally rhamnolipid adsorption to the surface. Therefore, using each oxygen or air plasma, RF powers of 25, 50, 75 and 100 W in optimized exposure time were studied. It should be noted that the gas flow in all experiments was set to 20 sccm.

Adsorption of rhamnolipid on the surface

The plasma treated substrates were immersed into 10 ml of 5 g/L biosurfactant solution and left in a shaker incubator (Kühner, Germany) for 16-18 h at 8 °C and 100 rpm. The samples were rinsed with appropriate buffer (5 times, 5 min with 5 ml of buffer), and then with deionized water (5 times, 5 min with 5 ml of water). Samples were dried and stored at room temperature for further analyses.

Surface characterization

Surface chemistry studies- ATR-FTIR

ATR-FTIR analysis was used for surface chemistry examination. The test was performed on a Bruker IFS 66 spectrometer equipped with a Specac Golden Gate diamond ATR accessory. Spectra were acquired over a 700–4000 cm^{-1} wavelength range.

Surface morphology studies- SEM

Scanning electron microscopy (SEM, VEGA3, TESCAN, Germany) was employed to investigate the morphology of the samples. The 10000× magnification was used. Samples were stuck with a conducting adhesive on the SEM metallic substrate holder and directly introduced into the chamber. The analytical chamber is equipped by fully motorized sample manipulator.

Surface topology studies- AFM

Atomic Force Microscopy (AFM) analysis was carried out using DME equipment (Danish Micro Engineering, DS 95, Denmark). The equipment works in contact mode, in air conditions, and at room temperature. In this mode, during scanning over the surface, the cantilever/tip assembly is sinusoidally vibrated by a piezo mounted above it, and the oscillating tip slightly taps the surface. We have used silicon probes with a rectangular cantilever and a pyramid tip with 450 micron length and 50 micron width and the thickness of about 2 micron. The curvature radius is in the order of 10 nm. The force constant of the cantilever and the resonance frequency are 0.42 N/m and 10 kHz. All images were collected with a resolution of 512×512 pixels and a scan rate of 1 Hz on two different regions of the films. Roughness parameters were calculated with the DME software (Spain).

Surface elemental composition studies- XPS

X-ray photoelectron spectroscopy (XPS) measurements were made with an X-Ray 8025-BesTec spectrometer (Germany) with an AlKα X ray source. The operation conditions were set to 15 kV. The binding energy scale was fixed by assigning a binding energy of 285.0 eV to the –CH_2– carbon (1s) peak. The samples were analyzed at a takeoff angle of 0° relative to the normal of the surface. The C1s, O1s, and N1s envelopes were analyzed and peak-fitted using a combination of Gaussian and Lorentzian peak shapes obtained from the XPSpeak 4.1 software.

2.6.5. Surface hydrophilicity studies- WCA

Water contact angles (WCA) were measured by a contact angle system (Data physics, Germany) equipped with a camera monitor at 25 °C. Ten separate readings were averaged to obtain one representative contact angle. The contact angle is referred as an angle between the solid/liquid and liquid/vapor interface. The water drop volume was 3 µl. Measurements were done 10 s after drop deposition. Distilled water was dropped onto the sample surface at 25 °C. Contact angles of four drops were analyzed for each sample and means were reported.

Antimicrobial and antiadhesive assays

Assessment of the antibacterial activity

The antimicrobial assay was carried out against two Gram positive (*S. aureus, B. subtilis)* and two Gram negative (*P. aeruginosa* and *K. pneumonia*). Antibacterial test was performed using disk diffusion assay.[14] Briefly, samples were placed on the surface of solidified Müller–Hinton agar, inoculated with microorganisms (10^7 CFU/plate) facing coated side down and incubated at 37 °C for 16 h. Next, the zone of growth inhibition around the films were measured as area (mm^2). The more the clear zone area, the higher the antimicrobial activity.

Assessment of the antiadhesive activity

Antiadhesive activity of the surface was assayed according to the method described by Asadinezhad et al.[26] Briefly, bacterial strain (*B. subtilis* and *K. pneumonia)* was inoculated in 10 ml of sterile water solution of nutrient broth in test tubes to reach $\approx 10^8$ CFUml^{-1} and left at room temperature for 30 min. The samples (1.5×1.5 cm^2) were then inserted into the test tubes. After 18 h incubation at 37 °C under continuous shaking at 100 rpm, the test tubes were opened and the samples were carefully removed from the medium, rinsed with sterile distilled water to remove loosely adhered bacteria and placed into other test tubes containing 2 ml of sterile deionized water. The bacteria adhered on the surface of the samples were removed by vigorous shaking of the test tube at 2000 rpm for 30 s and quantified by serial dilutions and spread plate technique. A 1 ml aliquot of the suspension was diluted decimally and from each dilution, 0.1 ml was transferred to a nutrient agar plate and the surviving bacteria were counted after 18 h of cultivation at 37 °C reported as CFU/cm^2. Each experiment was repeated in triplicate.

Wash durability test

Rhamnolipid adsorbed films were subjected to the cleaning conditions. The samples were immersed in water or in a neutral pH, non-ionic detergent (1%) overnight. The stability of the surfaces was indirectly evaluated by antimicrobial testing before and after stability tests.

Statistical Analysis

Antimicrobial and antiadhesive data were analyzed using the SPSS 11.5 statistical analysis system. A one-way analysis of variance was used to determine whether a significant difference existed between the treated groups and controls. Data were expressed as mean values of three replicates and differences were considered statistically significant if $P<0.05$.

Conclusion

In this study, we demonstrated the effects of plasma treatment to polypropylene surface properties and then rhamnolipid adsorption on the surface. Both oxygen and air plasma treatment introduced oxygen-containing polar groups to the surface of PP, and the hydrophilicity of PP was improved. More improvement in hydrophilicity was obtained after rhamnolipid adsorption. The morphology of PP was significantly influenced by the type of plasma treatment conditions as confirmed by AFM and SEM analysis. The overall results of the study confirmed the successful adsorption of rhamnolipid to the surface and consequently appropriate antimicrobial and antiadhesive properties were induced to the surface. Antimicrobial PP film which has been obtained in this study could be a useful choice for usage in food and pharmaceutical industries as packaging material.

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Figure captions

Fig. 1. Contact angle images of deionized water droplets over the film surfaces: (a) untreated PP, (b) PP-O2-75 (c) PP-Air-50 (d) PP-O2-75 after adsorption (e) PP-Air-50 after adsorption

Fig. 2. Atomic force microscopy schemes: A) untreated PP B) PP-Air-25 C) PP-Air-50 D) PP-Air-75 E) PP-Air-100 F) PP-O2-25 G) PP-O2-50 H) PP-O2-75 I) PP-O2-100 and J) PP-Air-50 after rhamnolipid adsorption.

Fig. 3. SEM images of A) untreated PP B) PP-Air-50 C) PP-Air-50 after rhamnolipid adsorption

Fig. 4. FTIR patterns of the polymeric film for untreated PP, PP-Air-50 sample, and PP-Air-50 after rhamnolipid adsorption

Fig. 5. XPS spectra of untreated PP along with the samples PP-O2-75 and PP-Air-50

Table 1. Plasma treatment conditions, contact angle and antimicrobial results

Sample name	Plasma gas	Power (W)	Time (min)	WCA (Θw) after plasma	WCA (Θw) after adsorption	Area of inhibition zone (mm^2)**			
						B. subtilis	*S. aureus*	*K. pneumonia*	*P. aeruginosa*
Untreated PP	—	—	—	*	80.1 ± 3.3	225	225	225	225
PP-O2-25	O_2	25	6	68.3 ± 2.5	50.1 ± 2.5	326.61	331.24	225	225
PP- O2-50	O_2	50	6	57.9 ± 2.1	33.3 ± 2.1	380.25	420.25	225	225
PP- O2-75	O_2	75	6	50.6 ± 1.7	15.9 ± 1.7	595.36	529	225	225
PP- O2-100	O_2	100	6	55.9 ± 1.1	17.8 ± 1.1	580.81	538.24	225	225
PP-Air-25	Air	25	6	60.2 ± 3.3	27.8 ± 3.3	542.89	316.84	225	225
PP-Air-50	Air	50	6	50.2 ± 2.1	12.2 ± 1.4	681.21	501.76	225	225
PP-Air-75	Air	75	6	51.1 ± 2.1	14.1 ± 2.1	630.01	475.24	225	225
PP-Air-100	Air	100	6	51.2 ± 1.6	13.8 ± 1.6	650.25	484	225	225

* WCA of untreated PP is 91.3°, ** area of untreated film is 15×15=225 mm^2 which shows no inhibition

Table 2. The mean surface properties of the samples after plasma treatment.

Sample	Roughness, Ra (nm)	Root mean squared roughness, R_q (nm)	Roughness skew, R_{sk} (nm)	Roughness kurtosis, R_{ku}
Untreated PP	9.92±1.13	10.01±1.33	0.11±0.06	2.21±0.41
PP-O2-25	9.11±0.31	10.21±0.67	0.21±0.01	3.13±0.31
PP-O2-50	19. 22±1.11	23.18±1.19	0.14±0.04	3.45±0.27
PP-O2-75	20.88±3.35	24.41±2.21	-0.04±0.03	4.13±0.56
PP-O2-100	19.78±3.67	25.45±4.01	0.05±0.01	3.72±0.61
PP-Air-25	11.09±2.11	13.56±3.13	0.25±0.07	3.26±0.32
PP-Air-50	27.98±1.88	31.76±2.17	0.11±0.03	3.91±0.48
PP-Air-75	29.55±4.50	32.04±3.45	0.15±0.03	3.67±0.45
PP-Air-100	30.10±3.12	38.11±2.12	-0.09±0.03	3.48±0.17

Table 3. Surface elemental composition and ratios of the selected samples obtained from XPS analysis (mean ± standard deviation).

Sample	C	O	N	Cl	O/C	N/C	Cl/C
Untreated PP	100	0	0	0	0	0	0.00
PP-O2-75	85.4±0.9	13.7±1.1	0	2.1±0.5	0.16	0.00	0.02
PP-Air-50	76.3±1.1	15.1±1.2	5.6±0.2	3.1±0.9	0.19	0.07	0.04

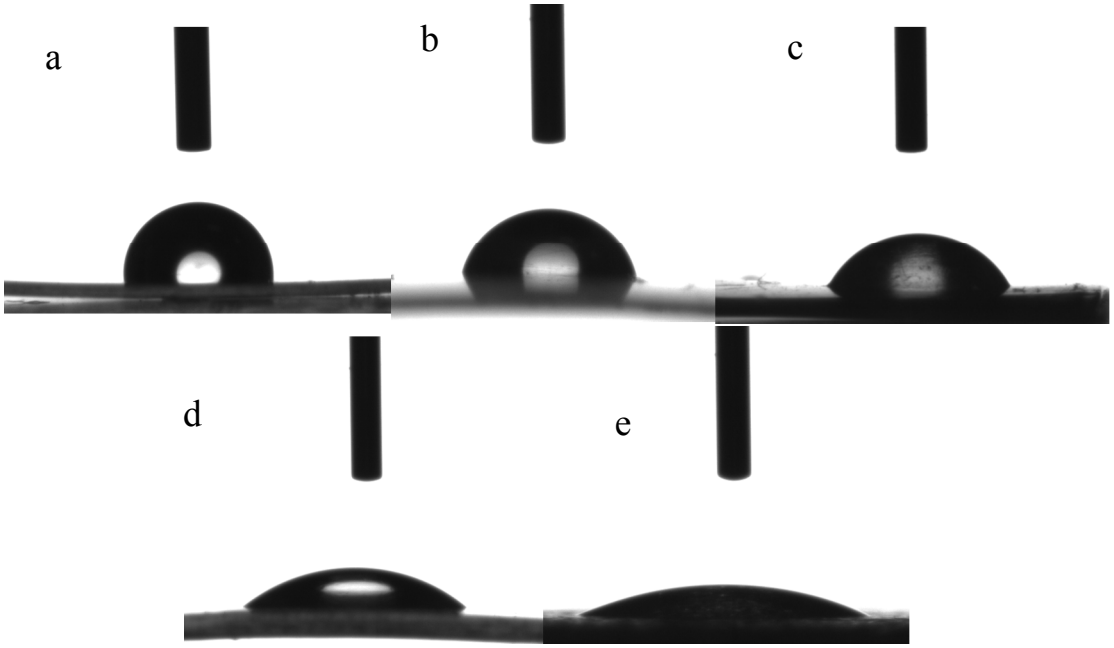
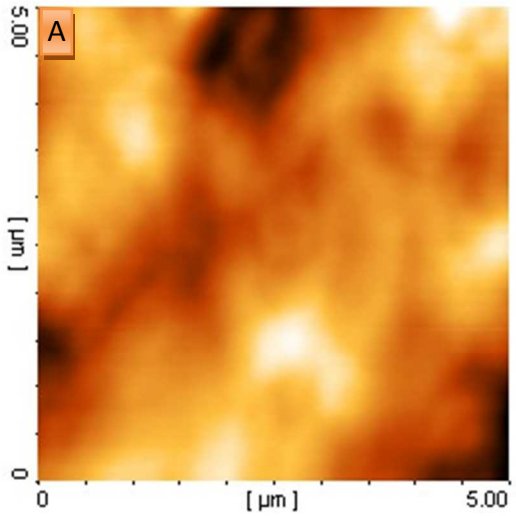
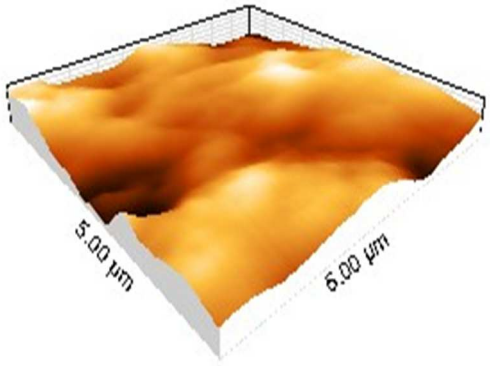
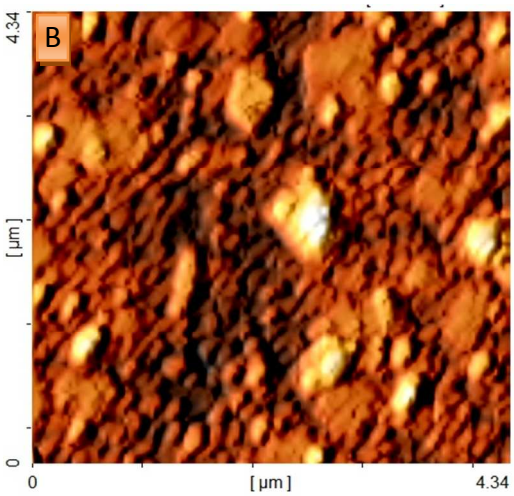
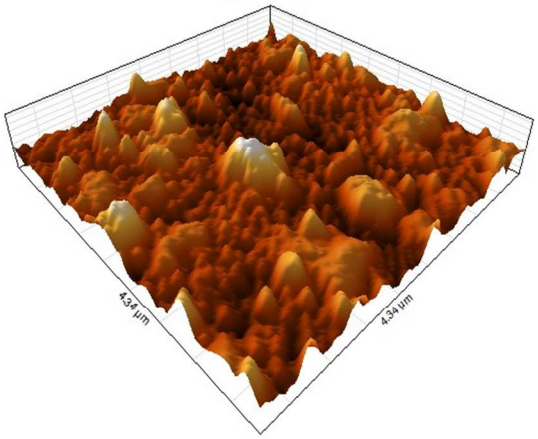


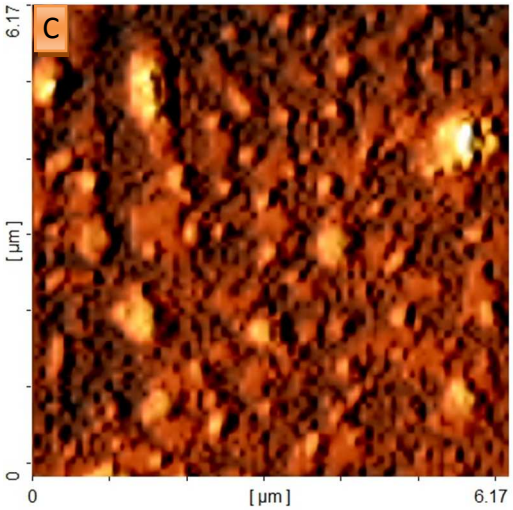
Fig. 1.

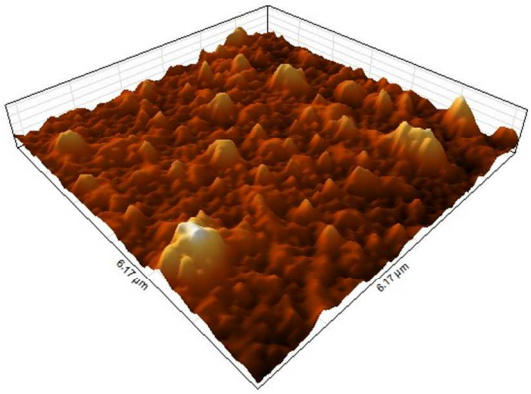


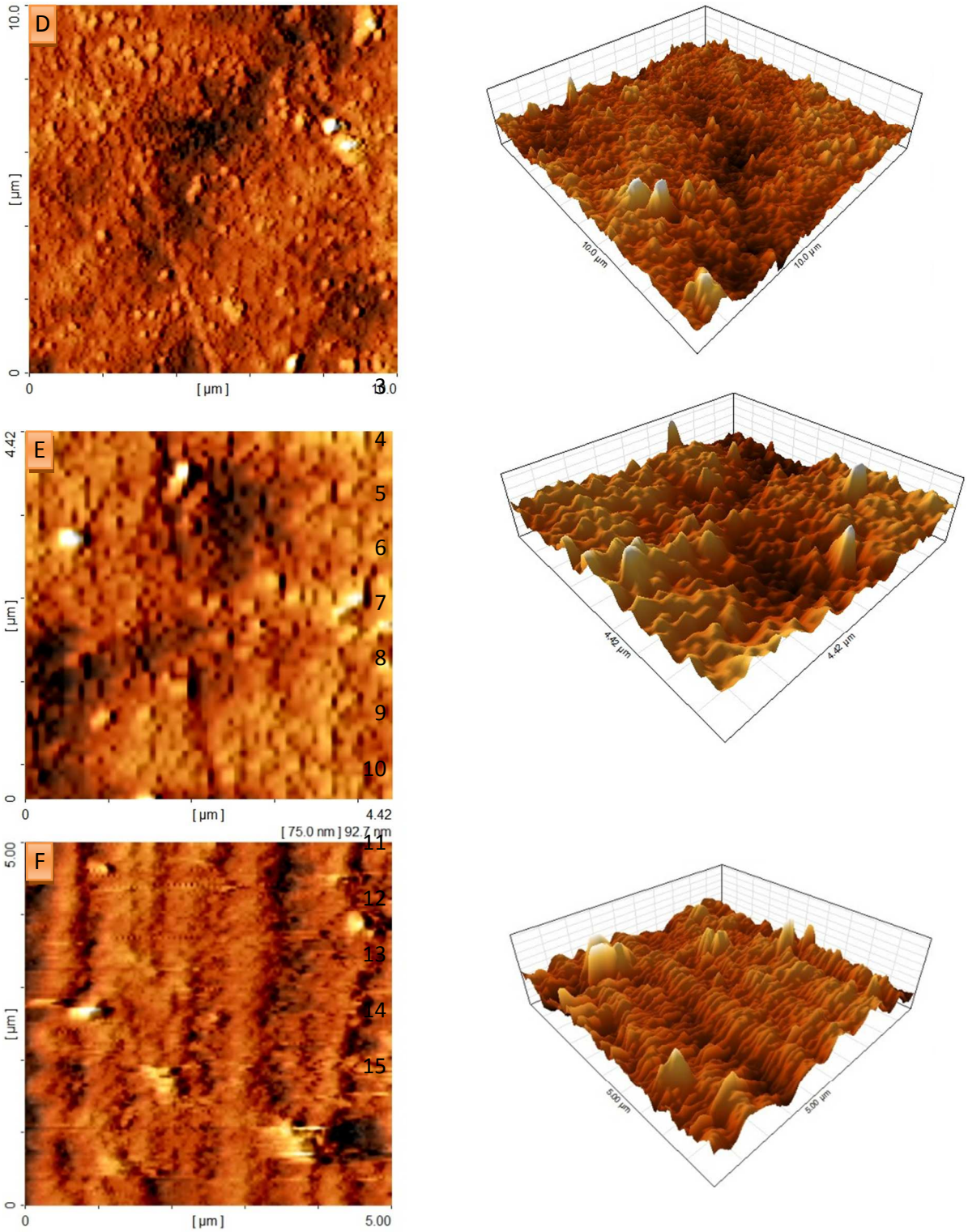


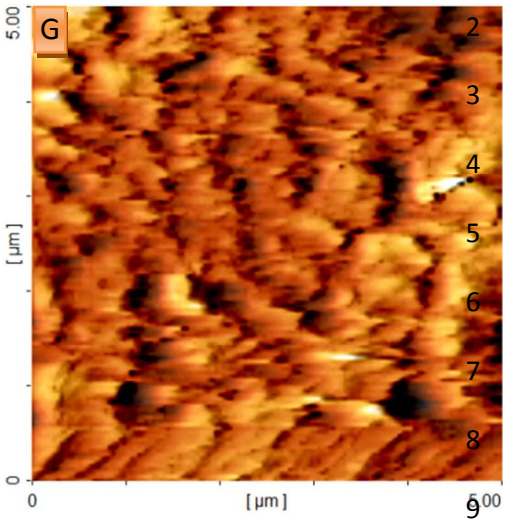


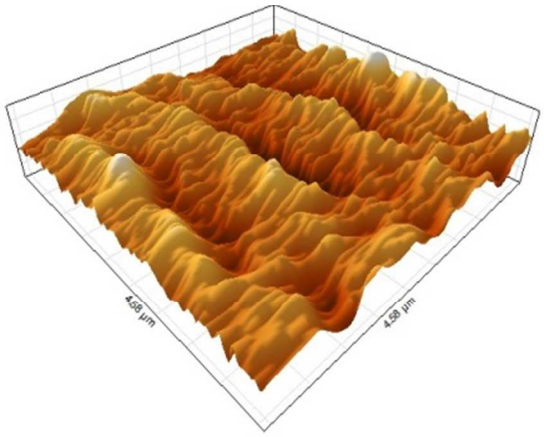


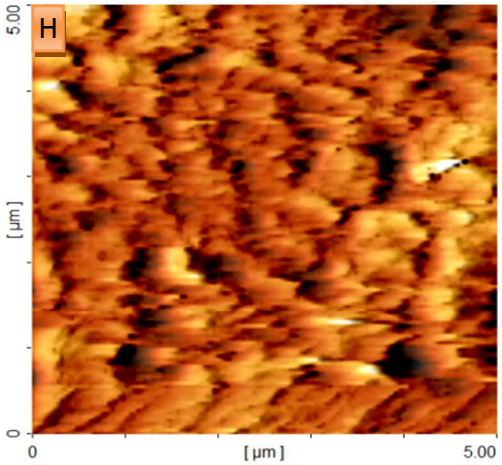


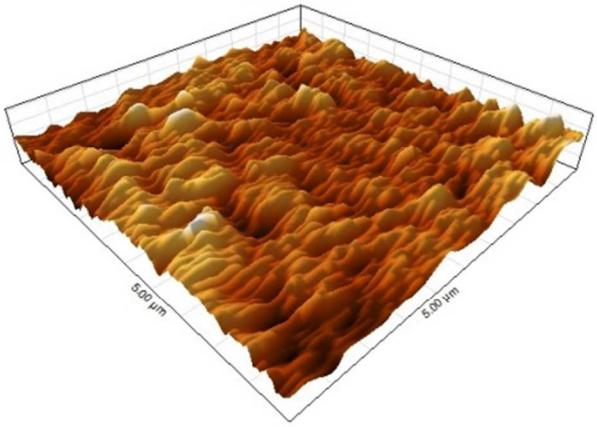


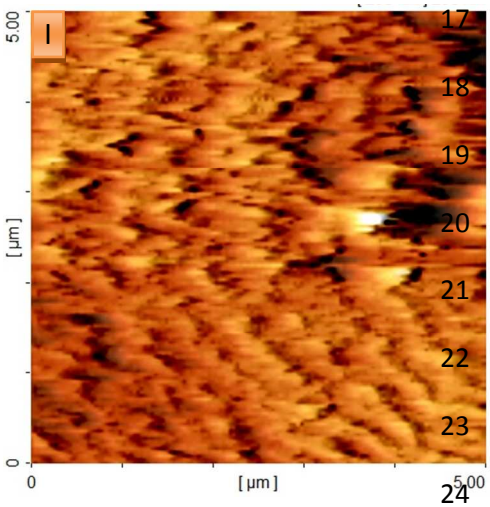


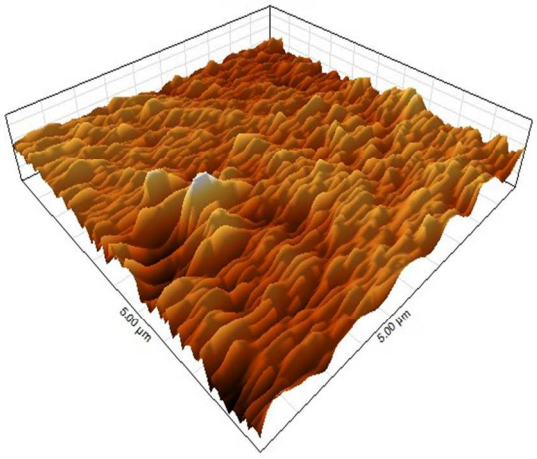












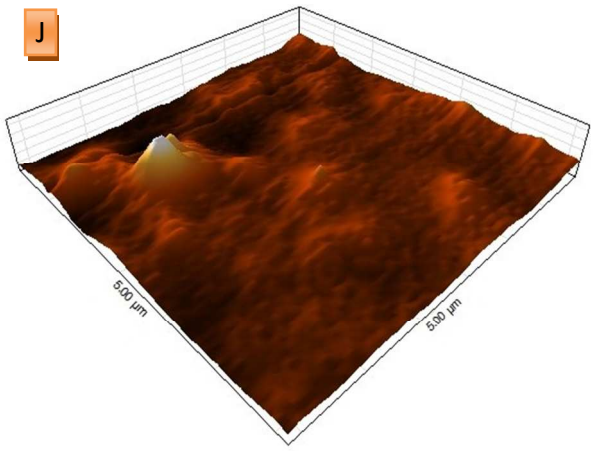


Fig. 2.

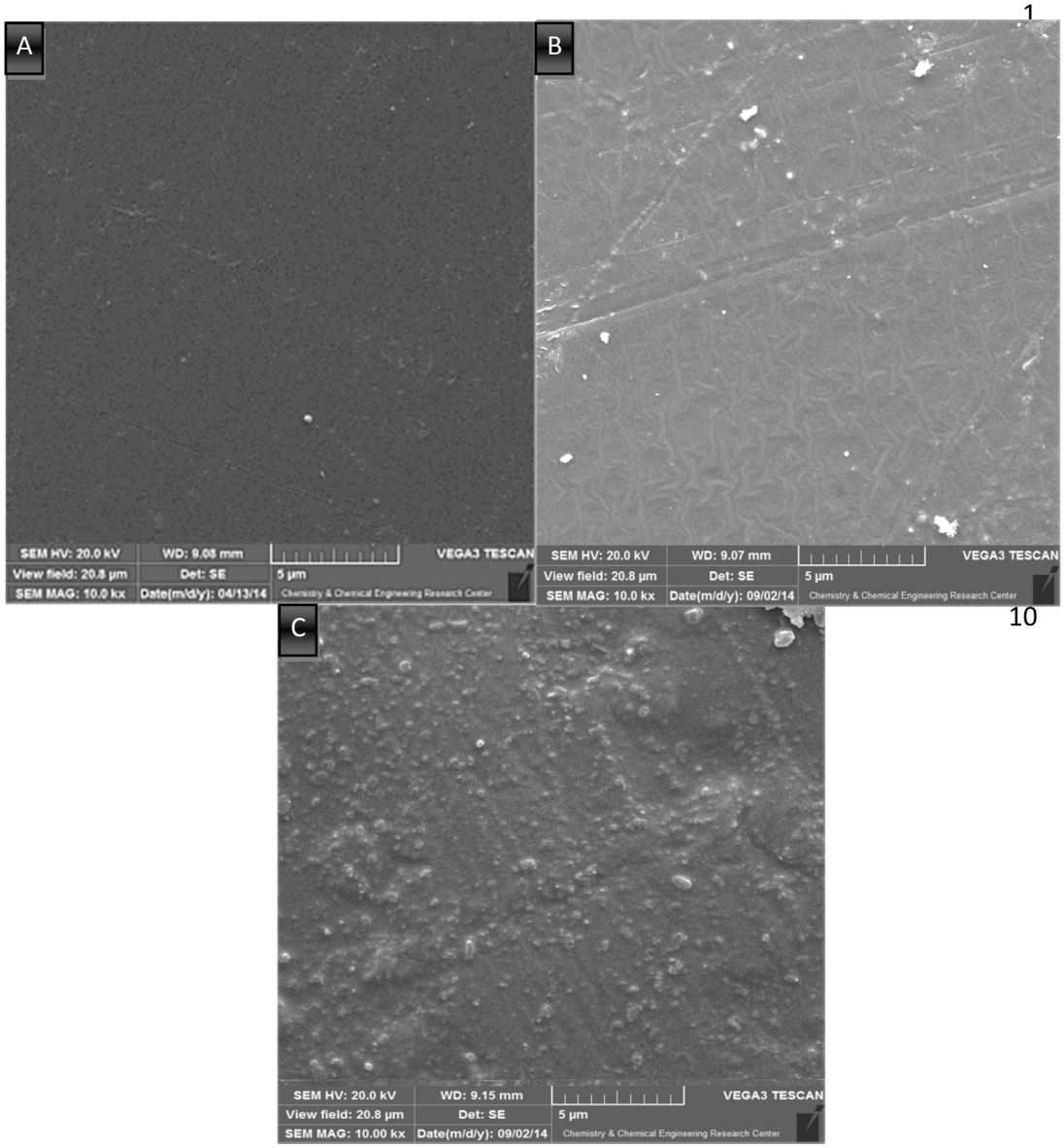


Fig. 3.

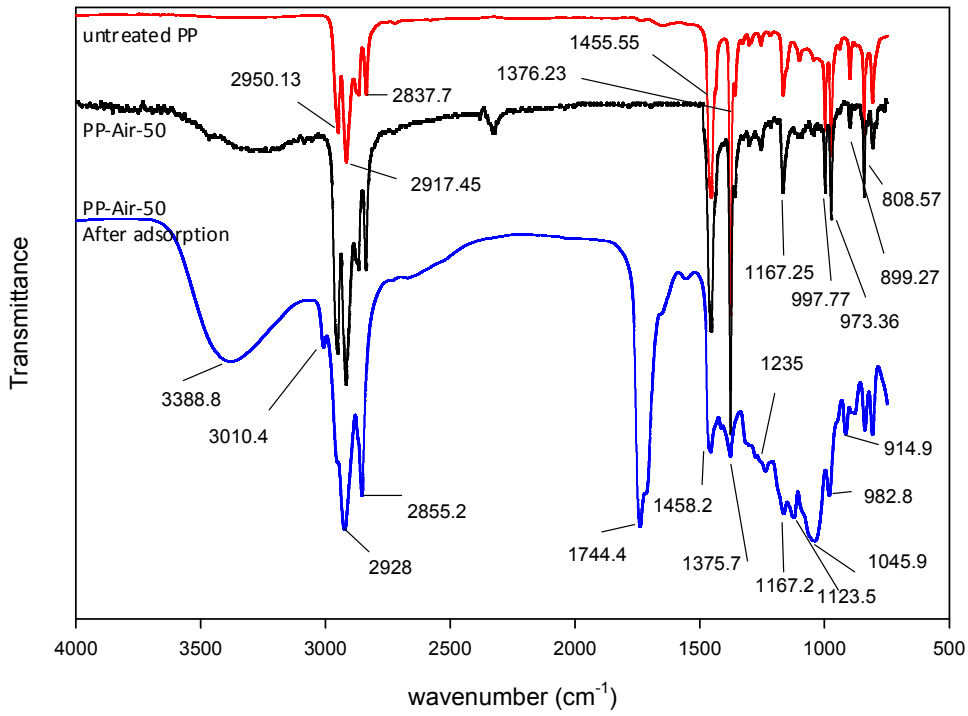


Fig. 4.

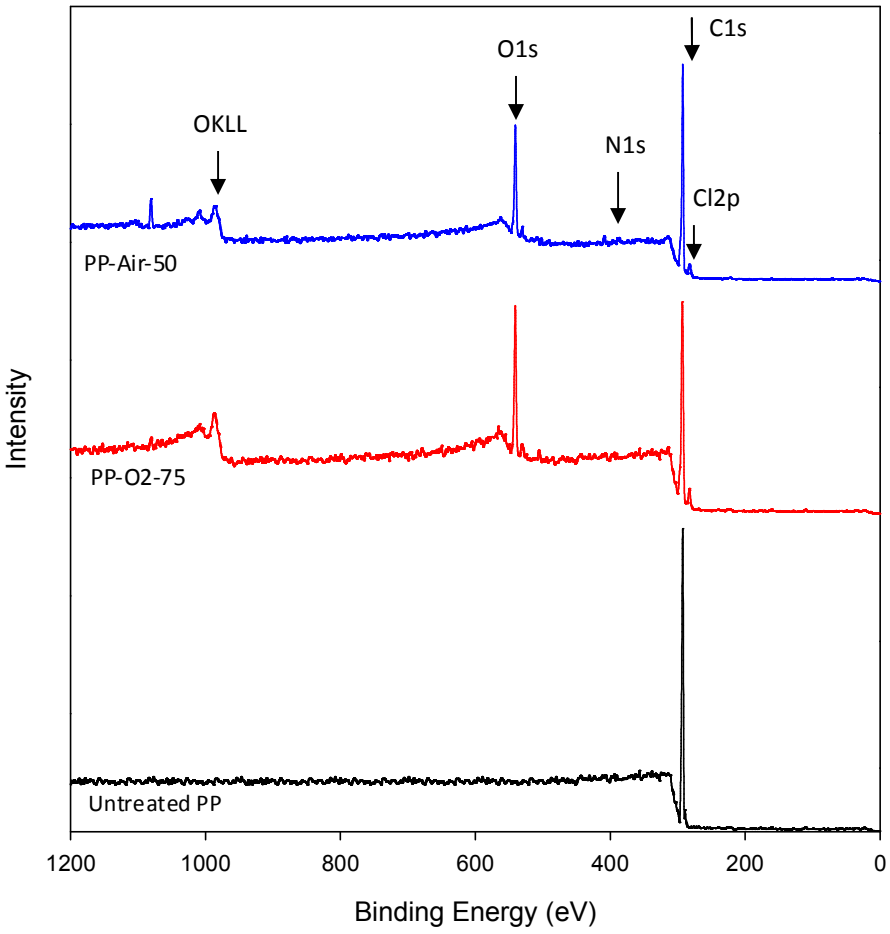


Fig. 5.