A Novel Process to Develop Modified Polymeric Surfaces for the Analysis of Bacterial Adhesion: Surface Properties and Adhesion Test

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ABSTRACT: Surface of polypropylene (PP) sheets were modified by radiation induced graft polymerization of a mixture of two monomers, a quaternary ammonium salt ([2-(methacryloyloxy)ethyl]-trimethyl ammonium chloride) (QAS) and acrylic acid(AA) to control the bacterial adhesion. Electron beam radiation was used to activate PP sheets prior to the grafting reaction. Depth concentration profile of nitrogen on modified PP up to 10 nm was performed by XPS. However, XPS has revealed no significant changes in the concentration profile of nitrogen for the grafted PP prepared under different parameters. Energetic characteristics (Lifshitz-van der Waals (γ^{LW}), electron acceptor (γ^+), and electron donor (γ^-) components) of these modified surfaces (with different ratio of comonomer mixtures) were calculated by using contact angle measurements. The PP surfaces prepared under one specific ratio of comonomer mixture (AA : QAS) 20 : 40 (M-4) has been compared with the surfaces modified with 20% AA (AA-20) and 40% QAS (QAS-40). The surface charge and isoelectric point have been determined by zeta potential measurements. AA-20 and comonomer mixture modified PP (M-4) surfaces exhibit negative charges of -27 and -16, respectively, while QAS-40 modified PP surfaces show positive charge of $+16$. The physicochemical properties of *Listeria monocytogenes* (LM) were obtained by microbial adhesion to solvent method. Finally, adhesion of LM on unmodified and modified PP surfaces was observed by electron microscopy. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 1746–1756, 2008

Key words: polypropylene; radiation grafting; contact angle; surface energetic characteristics, zeta potential; bacterial adhesion

INTRODUCTION

Food-borne bacterial infections are starving to develop novel processes to inhibit the growth of microorganisms in the food products. However, it is very important to maintain the quality, safety, and freshness of food. Bacteria tend to associate with surfaces. Once bacteria adhere on the surface, a multi-step process begins, which leads to the formation of a complex and heterogeneous biofilm.[1,2] Biofilms deteriorate food and food related equipments and therefore it is also important to inhibit the adhesion of bacteria as well as the formation of an infectious and pathogenic biofilm. Mainly, biofilm formation is a two-step process, where the first step may be reversible, involving physicochemical forces followed by the second irreversible chemical step, which leads to the synthesis of an extracellular polymeric film.[1] One of the ways to tackle this problem is to control the physicochemical interactions between the bacteria and the substratum surface. In food industries, antimicrobial food packaging acts to reduce, inhibit or retard the growth of microorganism that may be present in the packed food or packaging material itself.[3] Antimicrobial polymers are widely used in various food related applications like active packaging. However, polymer surface modification has been a significant issue in many fields[4,5] over the years.

A number of surface modification techniques have been used to develop surfaces with antimicrobial properties. Some of them are silver coated, surface immobilized polyethylene oxide, surface thiocyanation, surface modification by various gas plasma (such as oxygen and argon) and plasma immersion ion implantation.[6–12] Unlikely, radiation-induced graft polymerization has also been proved efficient to introduce different functionalities onto polymer surface as well as into bulk.[13] The advantage of radiation induced graft polymerization to promote or reduce bacterial adhesion is that it enables the graft-chain-containing interfaces bearing functional groups into various polymeric backbones.[14] Other researchers reported that nitrifying bacteria show a high adhesiveness to a membrane whose surface is modified with positively charged graft polymer chains.[15] Likewise Lee et al. found that a hollow fiber

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membrane modified by radiation induced graft polymerization exhibits a significantly high capturing rate for *Staphylococus aureus*, indicating efficient bacterial recovery for water purification.[16,17]

Radiation grafting has also been projected as one of the most convenient routes for the modification of the polypropylene (PP) to achieve a desired chemical functionality.[18] In our previous investigation, surface modification of PP sheets has been carried out by radiation induced graft polymerization of a mixture of two monomers, a quaternary ammonium salt (QAS) and acrylic acid (AA).[19] The synergistic effect caused by different reactivity ratio of both monomers mixture has been described earlier.[19] QASs are synthetic organic chemicals and are widely used in a variety of areas such as environmental disinfection, cosmetics, ophthalmic solution, pharmaceutical preparation, and against fungal infection.[20] QASs have an antimicrobial effect by damaging the cytoplasmic membrane.[21] However, such an antimicrobial effect is due to the presence of alkyl chain and their surfactant properties.[22] QASs have strong antibacterial effect towards gram positive bacteria than gram negative bacteria, since they have an extra protective membrane, which inhibits diffusion of many chemical products.[23]

In the food industries, numerous studies have shown that the contamination of products by pathogenic organisms and/or by alteration generally results from their adhesion to surfaces. This adhesion is the first step in the formation of biofilms, a permanent source of biocontamination in the industry. Among the undesirable microorganisms, *Listeria monocytogenes* (LM), an ever-present bacteria involved in food poisoning, occupies a preponderant place at the same level as *Salmonella* Typhimurium or even *Escherichia coli*. These gram-positive or gram-negative bacilli can survive and develop in particularly difficult conditions (i.e., low temperatures, acid pH levels, highly saline environments)[24] and adhere to a wide variety of surfaces.[25,26] They can be found throughout the food chain, particularly in rinsing water and on equipment surfaces.[27] It has been reported that the mechanism of bacterial adhesion to solid surfaces is governed by electrostatic interactions i.e., surface charge on substrate as well as on microorganism.[28–32] However, it is also important to take into account the nonelectrostatic interactions such as Lifshitz-van der Waals (LW) and Lewis acid–base (AB).[33,34] On the other hand, Speranza et al. have described that the bacterial adhesion is also influenced by the chemical properties of polymeric surfaces.[35] In the present investigation, we have determined the surface properties of previously prepared grafted PP surfaces[19] and their effect on bacterial adhesion. On the basis of contact angle measurements with polar and apolar liquids, surface free energy of different grafted PP surfaces were calculated with the help of Lifshitz van der Waals and Lewis acid–base approaches.[36] Zeta potentials of ungrafted and grafted PP were also determined by streaming potential measurements to get surface charge.[32,37,38] The present studies also reveal the nitrogen concentration depth profile in QAS grafted samples by XPS.

EXPERIMENTAL

Materials and modification of PP surfaces

Materials

PP of 1 mm thickness was received from Goodfellow Cambridge, UK.

Electron beam (linear electron accelerator CIRCE II) was used for the irradiation of PP sheets. The energy of electron beam was 10 MeV and the power was 20 kW with the speed of 0.44 m/min (IONISOS laboratory, Orsay, France). The irradiation was carried out in air for the dose of 100 kGy. Samples were exposed twice for the irradiation (dose of 50 kGy each). After the irradiation, PP sheets were kept at $-80°C$ prior to the grafting reaction.

Grafting reaction

Grafting reaction was carried out in a closed reactor.[19] The monomer solution was prepared by mixing a mixture of two monomers AA and QAS (received from Aldrich, Germany) in distilled water (monomers were used without any purification). We have used different ratio of each monomer (AA : QAS) such as 50 : 10, 40 : 20, 30 : 30, 20 : 40, 10 : 50, and denoted as M-1, M-2, M-3, M-4, and M-5, respectively. The required amount of monomer solution was added to the glass reactor along with the Mohr's salt (Aldrich, Germany) as homopolymer inhibitor. The reactor was placed in an oven at 70°C. Electron beam irradiated PP sheet (100 kGy) was placed in the monomer solution in a closed reactor. Argon was continuously purged into the reaction mixture to create inert atmosphere. After desired period, grafted PP sample was taken out and washed with distilled water in ultrasonic water bath at 40°C for 15 min to avoid any traces of homopolymer. Grafted PP samples were dried overnight in an air over at 40°C. The degree of grafting was calculated according to the following equation.

$$\text{Degree of grafting } (\%) = \frac{W_g - W_o}{W_o} \times 100 \quad (1)$$

where, W_o and W_g are the weights of ungrafted and grafted PP sheets, respectively.

Surface properties of modified surfaces

X-ray photoelectron spectroscopy

The nitrogen concentration depth profile was determined using ESCALAB 250 equipped with a non-chromatized AlK(X-ray source from Electron Corp. The pass energy for general spectrum was 100 eV. The analysis was carried out under UHV of 6×10^{-9} mbar. During analysis X-ray arrives on sample surface with an angle of $45°$, however the angle in the detection column was $90°$ with the surface of the sample. The spectra were collected at a photoelectron take-off angle 45 and $90°$, which corresponds to sampling depths of ~ 0–5 nm and 0–10 nm, respectively.

Determination of the energetic characteristics of modified PP surfaces

Contact angle measurements were carried out on KRUSS (France) G 40 goniometer with three pure liquids of known surface properties i.e., high purity water (Millipore milliQ), formamide and α-bromo-naphthelene (supplied by Sigma France). Samples were mounted on platform and a drop of appropriate liquid was placed on the surface. The contact angle was measured within 30 s of placing the drop on the PP surface and an average of seven measurements was reported.

The surface energetic properties of the ungrafted and grafted PP were determined by using the following equation of Young-van Oss:[36,39–41]

$$(1 + \cos \theta)\gamma_L = 2[(\gamma_S^{LW}\gamma_L^{LW})^{1/2} \\ + 2(\gamma_S^+\gamma_L^-)^{1/2} + 2(\gamma_S^-\gamma_L^+)^{1/2}] \quad (2)$$

where γ_L is the total surface tension, γ^{LW} is the Lifshitz-van der Waals and γ^+ and γ^- are the electron-acceptor/donor components of the surface of unmodified and modified PP (S) and were estimated by measuring contact angles (θ) with above-mentioned three pure liquids (L). The energetic characteristics of all three pure liquids are presented in Table I. The solid surface free energy was expressed in mJ/m^2.

Zeta potential measurements

Zeta potentials (ζ) of unmodified and modified PP were determined using a Zetacad from CAD Instrumentation, France. The concentration of NaCl solution was 1.5×10^{-3} M. The pH of the solution was adjusted within the range of 2–10 by adding KOH or HNO$_3$. Zeta potential was measured through streaming potential method described by Wagenen et al.[38] Streaming potential ΔE was measured at a driving pressure ΔP, which varies from 50 to 110 mBar. The measurements were repeated six times for each pres-

TABLE I
Values of Energetic Characteristics Components of Pure Liquids

Pure liquids	γ^{LW}	γ^+	γ^-	γ^{AB}	γ^T
Water	21.8	25.5	25.5	51.0	72.8
α-Bromonaphthalene	40.0	0.0	0.0	0.0	40.0
Formamide	39.0	2.3	39.6	19.0	58.0

sure that means three times for one flow direction and three times in the reverse flow direction. The streaming potential depends both on the surface charge in the diffuse layer and the electrolyte properties i.e., conductivity K_{sol}, viscosity η, and dielectric constant D. For flat surface like PP sheets, streaming potential (ΔE) is related to zeta potential, so by knowing ΔE, zeta potential (ζ) can be calculated by the following equation:

$$\zeta = \frac{4\pi\eta}{D}\left(K_{sol} + \frac{L_{surf}}{b}\right)\frac{\Delta E}{\Delta P} \quad (3)$$

where, L_{surf} represents the specific conductance at the surface of shear and b the half distance between the two samples. The value of b was 0.05 mm in our experimental setup.

Physicochemical properties of bacteria

Bacterial strains and growth conditions

The strain of LM CIP 103574 (N° UBHM 152) used in this study was provided by UBHM (INRA, France). Bacterial cells were stored in a biofreezer at $-80°C$ prior to the experiments. The strains were subcultured twice, and then cultivated for 24 h at $20°C$ in BHI (brain heart infusion, Oxoid) under oxygenated conditions until the stationary stage was reached. For the preparation of bacterial suspension, the cells were harvested by centrifugation for 10 min at $7000 \times g$ and $4°C$ and then washed twice with, and resuspended in the sterile suspending liquid (1.5×10^{-3} mol L^{-1} NaCl).

Microbial adhesion test to solvents

The partitioning method has originally been reported by Bellon-Fontaine et al.[39] It is based on the comparison between microbial cell affinity to a monopolar solvent and an apolar solvent. The monopolar solvent can be electron—acceptor or electron—donor, but both solvents must have similar van der Waals surface tension components. We therefore used the following pairs of solvents:

- Chloroform, an acidic and electron acceptor-solvent with hexadecane, an apolar *n*-alkane.

- Ethyl acetate, a basic and strong electron-donor solvent with decane, an apolar n-alkane.

The difference in the results between chloroform/hexadecane and ethyl acetate/decane (due to their surface tension properties) enabled to determine the surface properties at the surface of LM: electron-donor/acceptor interactions as well as hydrophobic/hydrophilic properties.

In the experiment, a suspension containing $\sim 10^8$ cells in 2.4 mL suspending liquid (NaCl 1.5×10^{-3} M) was vortex-mixed for 60 s with 0.4 mL of the solvent under investigation. The mixture was allowed to stand for 15 min to ensure complete separation of two phases before a sample (1 mL) was carefully removed form the aqueous phase and the optical density measured at 400 nm. The percentage of bound cells to each solvent was subsequently calculated using the following equation:

$$\% \text{ Affinity} = (1 - A/A_0) \times 100 \qquad (4)$$

where A_0 is the optical density measured at 400 nm of the bacterial suspension before mixing and A is the absorbance after mixing.

Electrophoretic mobility and zeta potential

For electrophoretic mobility measurements, bacteria were suspended in 1.5×10^{-3} M NaCl solution at a concentration of $\sim 10^7$ cells mL^{-1}. The pH of the suspension was adjusted to vary over the range 2–7 by adding HNO$_3$ or KOH. Measurements were taken in an electric field of 50 V with a Laser Zetameter (Zêtaphoremetre II, Société d'Etude Physicochimiques, France).

Observation of bacterial adhesion by electron microscopy

LM cells adhering to inert surfaces were visualized by field emission scanning electron microscopy (FESEM). Before analysis, each sample was rinsed with 150 mL of demineralised water, fixed with 3% glutaraldehyde for 1 h and washed three times with sodium cacodylate (0.2 mol L^{-1}, pH 7.4). Further fixing with 1% osmium tetroxide for 1 h at 4°C was the performed. The samples were washed with sodium cacodylate and then dehydrated by passage through a graded series of ethanol/water solutions from 50 to 100%. The samples then remained desiccated before gold/platinum sputtering and viewing as secondary electron images (8 kV) with a Hitachi S4500 FESEM.
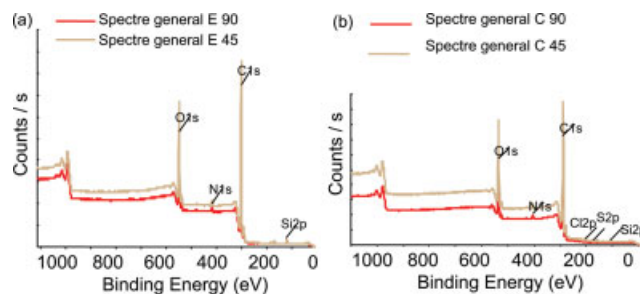

Figure 1 Spectra of nitrogen concentration depth profile by XPS. (a) Grafted PP with comonomer mixture, AA : QAS (20% : 40%), (b) Grafted PP with QAS (40%). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

RESULTS AND DISCUSSION

Surface properties of modified surfaces

Nitrogen concentration depth profile by XPS

To evaluate the concentration depth profile of nitrogen (N) from the surface of grafted PP up to the core, nitrogen concentration depth profile (NCDP) experiments were carried out using XPS. XPS has proven to be a valuable tool for determining the elemental composition on the polymer surface as well as to study the concentration depth profile of different atoms from surface to the core of the materials. NCDP of PP grafted with comonomer mixture M-4 and QAS are presented in Figure 1(a,b). These two spectra were collected at a photoelectron take-off angle 45 and 90°, which corresponds to sampling depths of $\sim$ 0–5 nm and 0–10 nm, respectively. Figure 1(a) indicates that the relative amount of N is slightly different as a function of the surface region being analyzed. However, the relative amounts of N concentrations given by Figure 1 are difficult to analyze due to the weak N$_{1s}$ peak intensities. The N/C atomic ratio (0.031) in Figure 1(a) until 5 nm (at 45°) is almost similar to that calculated (0.029) up to 10 nm (at 90°). These data suggest that the N concentration is almost identical from the surface (modified PP) up to 10 nm. Spectra in Figure 1(b) also illustrated the same trend of N/C atomic ratio. However, the difference between N/C atomic ratios (0.037–0.027 up to 5–10 nm) was slightly higher in spectra 1b. Nevertheless, there is no significant difference between the two. The only difference is the presence of an extra peak intensity of Cl in 1b spectra. Cl is present as a counter ion in QAS. All the above data reveal that in both modified PP samples the corresponding decrease (not significant) in the amount of N has been observed with the increase photoelectron take off angle. That means the N concentration has found to be almost similar from the surface up to 10 nm depth in both modified surfaces.
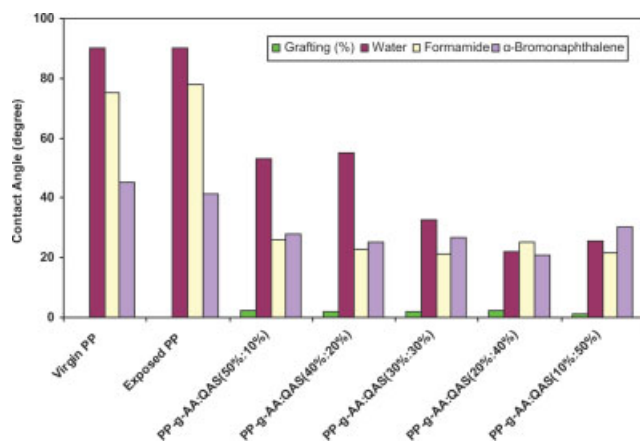
Figure 2 Variation of the contact angle measurements by using three pure liquids (water, formamide and α-bromo-naphthelene) on ungrafted and grafted PP at different ratio of comonomer mixtures. [Color figure can be viewed in the online issue, which is available at www.interscience. wiley.com.]

Surface energetic characteristics of modified surface

To control bacterial adhesion, surface modification of PP has been done by radiation induced graft polymerization of a mixture of two monomers, AA and QAS.[19] To analyze the surface wettability, contact angle measurements were carried out on unmodified (virgin and exposed PP) and previously modified (grafted) PP surfaces[18,19] and are presented in Figure 2 and Table II. Water contact angle is the most convenient parameter for analyzing the surface hydrophilic/hydrophobic properties of modified polymeric surface.[40] Moreover on the basis of contact angle, one can also determine the energetic characteristics of unmodified and modified surfaces by using polar and apolar liquids. Three pure liquids, two polar and one apolar were used to measure contact angles as described in the experimental part (2.2.2). Most of the liquid used to measure contact angles, were capable of interacting via hydrogen bonds, while modified surfaces may interact via polar, apolar and hydrogen bond attractive forces. Interestingly,

through these attractive forces, it is possible to check the interaction behavior of polar and apolar liquid simultaneously. According to Figure 2, water contact angles of virgin and exposed PP were found to be higher than the grafted PP surfaces, showing their hydrophobic nature. However, decreasing trend of contact angle has been observed with water as the ratio of QAS increases in the comonomer mixtures. The decrease in the water contact angle with the increase in the ratio of QAS has been discussed elsewhere in details.[19]

According to our earlier investigation,[19] we chose one ratio of comonomer mixture i.e., M-4 [20 : 40(AA : QAS)] for other investigations. The samples grafted with the above concentration ratio exhibit low water contact angle of 22° and we have appropriate amount of both monomers too. However, we have also observed the individual effect of each monomer on the degree of grafting[19] and contact angle. Figure 3 shows the variation in the contact angle, using polar and apolar liquids on the surface of PP modified with a mixture of comonomer M-4 and individual monomer such as QAS-40 (40% QAS in distilled water), and another one with AA-20 (20% AA in distilled water). QAS-40 modified PP surfaces show almost similar wettability as M-4 modified samples. In reverse AA-20 grafted PP surfaces exhibit higher water contact angle as compare to M-4 and QAS-40 modified PP surfaces. However formamide contact angle shows complete hydrophilicity for the PP modified with QAS and AA individually. It is to be noted that we put 20°, as the value of contact angle to show complete hydrophilicity, as the real value was too small or even was not possible to measure the contact angle (Table III).

Surface energetic characteristics of unmodified and modified PP were evaluated by using Young-van Oss equation[36,39–42] and presented in Table IV. The contact angle values of α-bromonaphthalene with γ^- and $\gamma^+ = 0$ was used to derive γ^{LW} while water and formamide contact angles were inserted in eq. (2). to get electron donor γ^- and electron acceptor γ^+ components of the surface free energy respectively.

TABLE II
Values of Contact Angle Measurements by Using Three Pure Liquids
(Water, Formamide, and α-Bromonaphthelene) on Ungrafted and Grafted PP at
Different Ratio of Comonomer Mixtures

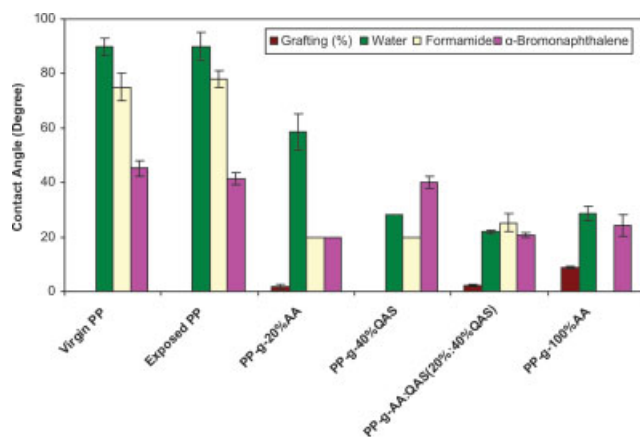| Sample	Grafting (%)	Contact angle (°)		
		Water	Formamide	α-Bromonaphthalene
Virgin PP	0.0	90.0 ± 3.4	75.1 ± 5.8	45.2 ± 3.3
Exposed PP	0.0	90.0 ± 3.6	78.1 ± 3.5	41.4 ± 2.3
PP-g-AA : QAS (50% : 10%)	2.4 ± 0.3	53.2 ± 2.2	26.0 ± 3.1	28.1 ± 2.8
PP-g-AA : QAS (40% : 20%)	1.9 ± 0.1	55.0 ± 2.3	23.0 ± 2.8	25.1 ± 3.4
PP-g-AA : QAS (30% : 30%)	2.0 ± 0.1	32.6 ± 2.0	21.2 ± 3.7	26.9 ± 2.1
PP-g-AA : QAS (20% : 40%)	2.3 ± 0.2	21.9 ± 0.7	25.2 ± 3.3	20.7 ± 1.0
PP-g-AA : QAS (10% : 50%)	1.2 ± 0.1	25.5 ± 2.6	21.5 ± 3.0	30.5 ± 2.2

Figure 3 Variation of the contact angle measurements by using three pure liquids (water, formamide and α-bromo-naphthelene) on ungrafted and grafted PP with AA (20 and 100%), QAS (40%) and a specific ratio of comonomer mixture, AA : QAS (20% : 40%). [Color figure can be viewed in the online issue, which is available at www. interscience.wiley.com.]

Abbasian et al.[43] suggested that apart from the parameters such as drop volume, molecular weight, surface roughness, etc., which affect the contact angle measurements and to analyze surface free energy of polymers, the film thickness in nanometric scale can also be considered. However we did not take into account the film thickness parameter in our experimental procedure.

The value of γ^{LW} was found to be 29.0 and 30.6 mJ/m^2 for virgin and exposed PP respectively, while the electron acceptor component γ^+ exhibited very low value of 0.01 and 0.35 mJ/m^2 for the same samples. For the grafted samples, the value of γ^{LW} was calculated in the range of 31.1–37.5 mJ/m^2, which are the close values of unmodified PP samples. This indicates that there is no difference for the influence, according to van der Waals attractive forces between grafted and ungrafted samples. Guillemot et al. have also reported almost the same value (26.3 mJ/m^2) of γ^{LW} for PP.[44] The electron donor component γ^- was

shown to vary greatly from 5.5 mJ/m^2 for unmodified to 10.18–47.28 mJ/m^2 for modified PP surfaces. This suggested that unmodified PP samples are hydrophobic in nature, however the PP samples grafted with AA-20 show moderately hydrophilic character, while grafting with QAS-40 exhibited hydrophilic and basic character. For the other grafted samples the value of γ^- increases with the increase of QAS ratio in the comonomer mixture [except for the comonomer ratio 40 : 20 (AA : QAS)] indicating the increase in the hydrophilicity and basic nature. The hydrophilicity in all cases may be due to the presence of QAS and carboxylic groups on the surface of PP, which is not identified on unmodified PP surfaces. The hydrophilicity can be attributed to the basic character of the modified PP. Furthermore, in case of QAS an increase in the basic nature may be attributed to the effect of counter ion (Cl$^-$) present in the QAS.

Zeta potential measurements

Zeta potential is the average potential in the surface of shear and denoted as ζ. Zeta potential measurements reveal the acidity or basicity of solid surfaces quantitatively. To investigate the surface charged properties of unmodified and modified PP, the streaming potential were measured as a function of pH in 1.5×10^{-3} M NaCl solution followed by the calculation of zeta potential by using eq. (3). Figure 4 illustrated the effect of pH on the zeta potential of unmodified and modified PP surfaces. The variation of zeta potential with pH indicates that H$^+$ and OH$^-$ ions are potential determining ions. They are ionic species of the aqueous medium, which actually interact with the surface.[45] Depending on the acid–base character of the surface, whatever the group present on the surface may gain or lose a proton according to the pH value of the aqueous phase. Table V presented the values of zeta potential at neutral pH and isoelectric point (iep) of different PP samples. Iep is the point where the value of zeta potential is zero, that means the total positive charges are

TABLE III
Values of Contact Angle Measurements by Using Three Pure Liquids
(Water, Formamide, and α-Bromonaphthelene) on Ungrafted and Grafted PP
with AA (20 and 100%), QAS (40%), and a Specific Ratio of Comonomer
Mixture, AA : QAS (20 : 40%)

			Contact angle (°)	
Sample	Grafting (%)	Water	Formamide	α-Bromonaphthalene
Virgin PP	0.0	90.0 ± 3.4	75.1 ± 5.8	45.2 ± 3.3
Exposed PP	0.0	90.0 ± 3.6	78.1 ± 3.5	41.4 ± 2.3
PP-g-20% AA	1.8 ± 0.2	59.0 ± 6.6	20.0 ± 0	20.0 ± 0
PP-g-40% QAS	0.07 ± 0.06	28.0 ± 1.0	20.0 ± 0	40.0 ± 2.1
PP-g-AA : QAS (20% : 40%)	2.3 ± 0.2	21.9 ± 0.7	25.2 ± 3.3	20.7 ± 1.0
PP-g-100% AA	8.0 ± 1.4	28.6 ± 1.6	20.0 ± 0	24.2 ± 4.0

TABLE IV
Surface Energetic Characteristic Components of Ungrafted and Grafted PP

Sample	γ^{LW} (mJ/m^2)	γ^+ (mJ/m^2)	γ^- (mJ/m^2)	γ^{AB} (mJ/m^2)
Virgin PP	29.0	0.01	5.55	0.57
Exposed PP	30.6	0.35	7.56	3.27
PP-g-20% AA	37.5	4.36	10.18	13.24
PP-g-40% QAS	31.1	3.29	43.46	23.92
PP-g-100% AA	36.56	1.81	43.55	17.79
PP-g-AA : QAS (50 : 10%)	35.43	3.36	17.57	15.4
PP-g-AA : QAS (40 : 20%)	36.3	3.78	14.63	14.89
PP-g-AA : QAS (30 : 30%)	35.72	2.1	39.56	18.2
PP-g-AA : QAS (20 : 40%)	37.5	1.42	47.28	16.43
PP-g-AA : QAS (10 : 50%)	34.82	1.94	47.5	19.21

equal to the total negative charges and beyond iep the zeta potential increases due to the adsorption of potential determining ions, which can also reveal the surface acidity–basicity character qualitatively.

According to Figure 4, zeta potentials of Virgin PP and exposed PP are −40 mV and −43 mV at neutral pH respectively, and −33 mV at the pH of physiological water. The iep (Table V) of virgin and exposed PP are 3.3 and 2.6. We have also measured the zeta potential on virgin PP after the extraction of additives to neglect the effect of additive on the surface charge. However, no significant difference has been observed in the values of zeta potential (−35 at the pH of physiological water) and iep (3.0) after extracting the additives. Thus the negative zeta potential may be due to the processing of PP at elevated temperature, which causes the thermal degradation or partial oxidation of PP during storage and contamination on the PP surface. In that case, the behavior of negative charge on exposed PP would be different than the negative charge of grafted PP caused by carboxylic groups. Aranberri-Askargorta et al.[46] has also reported the same value of iep (3.3)

of pure PP fibers. Grafting of PP samples vary the zeta potential greatly. Grafting with AA-20 slightly increased the iep to 3.7. However the zeta potential for the AA-20 grafted sample at neutral and physiological water pH was found to be −27 and −23, respectively. The decrease in negative zeta potential may be assigned to the change in basic character due to the presence of carboxylic groups. Kuehn et al. has suggested that for polymeric materials, an increase in the hydrophilic functional groups on the surface decreases the negative zeta potentials as ion adsorption occurs in concurrence to water adsorption at hydrophilic sites.[47] However an increase in negative zeta potential may occur if no water adsorption takes place, which causes an increased number of dissociable surface groups.[46] The curve of QAS-40 grated PP clearly shows that the surface charge is positive throughout the entire pH range (2–10). Hence QAS-40 grafted PP surface does not show iep. This is due to the presence of basic functional groups on the QAS-40 modified PP surfaces. In QAS-40, nitrogen may be considered as the source of the positively charged surface. The iep of comonomer mixture (M-4) grafted PP surfaces is 5.2. These surfaces exhibit positive and negative charges below and above their iep. At neutral and physiologic water pH, the values of zeta potentials are −16 and −15. Here, one can assume that above the iep the negatively charged surfaces are generated by acidic
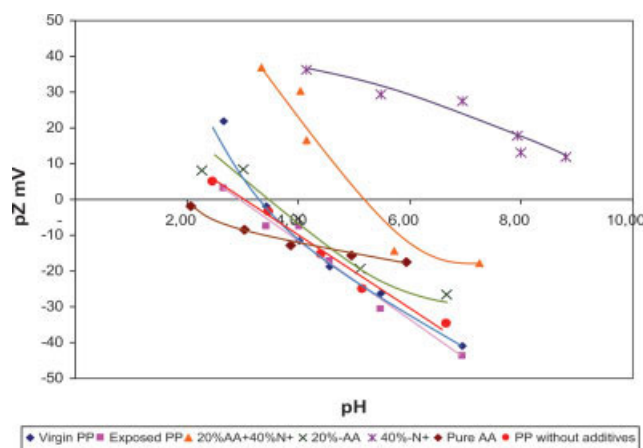

Figure 4 Zeta potential of ungrafted and grafted PP with AA (20 and 100%), QAS (40%) and a specific ratio of comonomer mixture, AA : QAS (20% : 40%). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE V
Values of Zeta Potential at Neutral and Physiologic Water pH and iep of Ungrafted and grafted PP with AA (20 and 100%), QAS (40%), and a specific ratio of comonomer mixture, AA : QAS (20 : 40%)

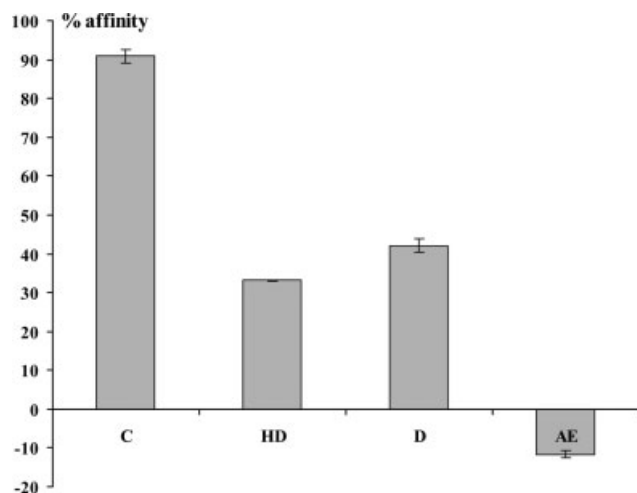| Sample	ζ (mV) at neutral pH	ζ (mV) at physiologic water pH	iep
Virgin PP	−40.0	−33.0	3.3
Exposed PP	−43.0	−33.0	2.6
PP-g-20% AA	−27.0	−23.0	3.7
PP-g-40% QAS	+27.5	+30.0.	−
PP-g-AA : QAS (20% : 40%)	−16.0	−15.0	5.2

Figure 5 Percentage of affinity of LM to solvents. C, chloroform; HD, hexadecane; D, decane; AE, ethyl acetate.

functional group such as carboxylic groups while below the iep, presence of basic functional group is responsible for positive charge. However, the above modified surfaces have much stronger positive charge at acidic conditions (+36) than negative charge at alkaline conditions (−18). This could suggest that the modified surfaces may have a higher density of QAS than carboxylic groups. These results can be correlated with the contact angle measurements, where the PP grafted with comonomer mixture (M-4) and QAS-40, exhibit almost identical wettability, whereas PP grafted with AA-20 shows different behavior of wettability. Moreover, PP surfaces grafted with comonomer mixture (M-4) and QAS-40 also have the identical maximum value of zeta potential (+36) in acidic pH.

We have also measured the zeta potential of pure AA grafted PP samples (as in case of pure AA grafted PP most of the carboxyl groups are present on the surface[18]). The introduction of dissociable carboxylic groups into hydrophobic PP influences the zeta potential due to the change in surface hydrophilicity. The presence of carboxylic groups on the surface, subsequently increase the hydrophilicity, which results in the extension of the swelling layer on the modified PP surface and lead to a shift of shear plane towards the bulk solution. As a result of which, we observed the relatively low negative value of zeta potential due to the decrease in the potential between the charged surface and the bulk solution as a function of the distance from the charged surface.

Physicochemical properties of bacteria

Microbial adhesion test to solvents

The results of this microbial adhesion to solvent test of the breakdown between solvents and a suspension of LM (Fig. 5) showed that LM had a greater affinity for chloroform (an electron acceptor solvent) than for hexadecane (an apolar solvent), indicative of an electron donor character of this hydrophilic bacteria.

Electrophoretic mobility and zeta potential

Measurements of the electrophoretic mobility of LM in a pH range 2–7 is shown in Figure 6. It can be noted that LM was highly negatively charge and no isoelectrical point could be determined in the range of pH explored.

Observation of LM by electron microscopy

Adhesion of LM was observed by electron microscopic images on unmodified and modified polymeric surfaces having different surface characteristics. Bacterial adhesion with solid substrate is thought to be governed as a result of different parameters present in the suspension medium such as positively–negatively charged ions in solution, the charge on the bacterial wall and the acid/base sites on the polymeric surface. Therefore, it is important to know the surface characteristics of LM and polymeric surfaces that we used for bacterial adhesion test. It can be seen that, LM exhibits highly negative charge and electron donor (γ^-) character i.e., hydrophilic nature. According to characteristics of LM, we have modified the PP surface by radiation grafting of comonomer mixture. The surface properties of unmodified and modified PP have already been explained in the previous section (see section 3.1.2–3.1.3). The EM images of LM adhesion experiments performed on unmodified and modified PP surfaces are shown in Figure 7(a,b). The extent of bacterial adhesion seems to be higher in the case of exposed than the PP, which is grafted with comonomer mix-
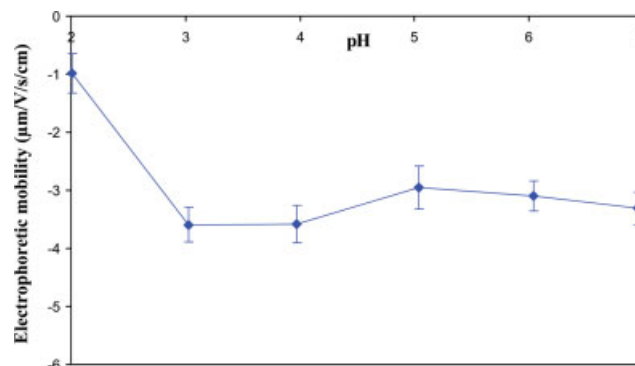

Figure 6 Electrophoretic mobility of LM suspended in NaCl $1.5 \times 10^{-3} M$ in a range 2–7. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
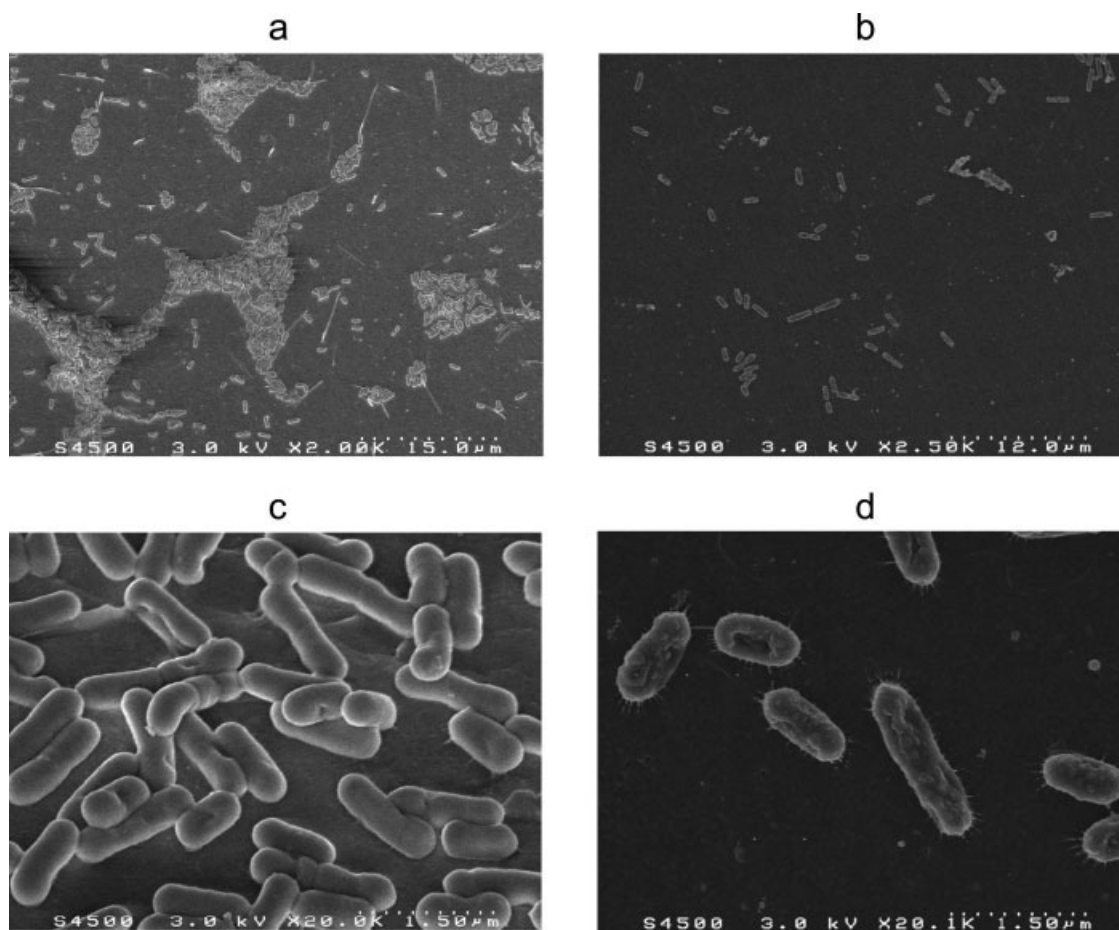
Figure 7 Scanning Electron microscopy images of ungrafted and grafted PP showing adhesion of LM. (a,c) Exposed PP, (b,d) Grafted PP with comonomer mixture, AA : QAS (20% : 40%). (a,b) LM observed under ×2 objectif. (c,d) LM observed under ×20 objectif.

ture (M-4). The different adhesion trend may be explained taking into account the hydrophilicity and the presence of an acid–base interaction as the van der Waals attractive forces have very little influence between ungrafted and grafted samples.

It is well reported that the adhesion should be higher on hydrophobic than on hydrophilic surfaces.[48–50] We can correlate these assumptions with our contact angle results, which show that exposed PP has hydrophobic while the above grafted samples exhibit highly hydrophilic surface. These results can also be explained that when the interacting surfaces are highly hydrophilic, the acid–base interactions lead to hydrophilic repulsion, whereas in the case of strongly hydrophobic interacting surface long-rang hydrophobic attraction occurs.[51,52] On the other hand, in the case of hydrophobic exposed PP the later assumption can not be taken into account as the LM possesses hydrophilic nature. This is why we have observed higher adhesion of hydrophilic LM on hydrophobic exposed PP, while comonomer grafted PP and LM have shown hydrophilic nature,

leading to the repulsive interaction, which results less adhesion.

Another factor, which can be involved in the bacterial adhesion, is electron acceptor (γ^+) and electron donor (γ^-) components of the support and bacterium. Exposed PP shows fewer values for γ^+ and γ^-, so that we can consider it neutral. The comonomer grafted PP and LM have presented higher value of electron donor character. Therefore, we can assume the repulsive interaction between them, which lowers the bacterial adhesion on the grafted PP. Additionally, Figure 7(a,c) shows that LM colonized the exposed PP sheets evenly in a monolayer, rod shape structure with high density. In contrast, very little LM adhesion has been observed on comonomer grafted PP sheets [Fig. 7(b,d)]. Furthermore, the bacterium seems to be damaged on modified surfaces. This can be described as the comonomer modified PP has QAS and carboxyl groups; therefore they show antibacterial and repulsive (antiadhesion) activity against LM. However, at present the exact mechanism of adhesion or antibacterial activity on

grafted PP is not known to us. We can only describe the obtained results on the initial bacterial adhesion to some extent from the physicochemical aspect, but it is difficult to generalize the results and this is why it is needed to be further investigated in future.

CONCLUSIONS

The present work attempts to control the surface properties of PP such as hydrophilicity, surface charge, iep, etc. by using cationic and anionic molecules and their mixture (different ratio of each) to avoid bacterial adhesion. Hence, positively and negatively charged PP surfaces can be generated in the pH range below and above iep by basic and acidic functional groups present on the PP surface. To achieve that, surface modification of PP sheets was done by radiation induced graft polymerization of a mixture of two monomers, QAS, and AA. We have determined the energetic characteristics of these modified surfaces (with different ratio of comonomer mixtures) and used three different pure liquids to measure the contact angle, subsequently calculation of Lifshitz-van der Walls (γ^{LW}), electron acceptor (γ^+), and electron donor (γ^-) components. We have also compared the PP surfaces prepared under one specific ratio of comonomer mixture i.e., M-4 [20 : 40 (AA : QAS)] with the surfaces modified with AA-20 and QAS-40. All the above modified PP surfaces exhibited strong electron donor component and hydrophilic nature accept the AA-20 grafted PP, which showed moderately hydrophilic character. However, we have found very little difference for the influence of van der Waals attractive forces between grafted and ungrafted samples.

The zeta potential measurements have been performed to determine the surface charge and iep. The zeta potentials were calculated on the basis of the streaming potential measurements and were used to reflect the charge state of unmodified and modified PP surfaces. The surface charge on virgin and exposed PP was found to be −40 and −43 mV, respectively. After the grafting of AA (20 and 100%), the magnitude of zeta potential was reduced from −40 to −17, whereas in the presence of QAS-40 zeta potential was positive throughout the entire pH range (2–10). However, with comonomer mixture M-4, zeta was reversed from positive (+36) in acidic pH to negative (−16) in basic pH. NCDP was performed on modified PP surfaces by XPS but no significant changes have been observed in the NCDP for the surfaces prepared under different parameters.

In the case of surface modified by pure monomer, the degree of grating has to be very high to get hydrophilic surfaces, however, it is well known that high degree of grafting affects the physical structure of PP notably the cristallinity. In the presence of monomer mixtures [20 : 40 (AA : QAS)] for the same surface hydrophilicity (30°), the grafting rates are significantly lower with a good reproducibility. Furthermore, by using specific ratio of the two monomers, it is possible to modulate energetic surface properties of the substrate and may be to control bacterial adhesion.

Adhesion of LM was observed on exposed and comonomer grafted PP by electron microscopy. The electron microscopic images illustrated that the extent of bacterial adhesion is higher in the case of exposed than the PP, which is grafted with comonomer mixture M-4. The damage of the cells on the M-4 modified surfaces could be due to the graft molecules present on the modified surfaces. Furthermore, if we consider the surface properties of LM such as electron acceptor (γ^+) and electron donor (γ^-) components and hydrophilicity, we can suggest that in the absence of electrostatic interaction, adhesion could be due to the attractive van der Waals forces. These van der Waals forces can be counter balanced by the repulsive interaction of lewis acid–base. We have explained the adhesion of LM on the basis of physicochemical aspects, however at the time being, we do not know the exact mechanism of adhesion and therefore it is difficult to generalize the results. This is why further research work is needed to elucidate the relationship between bacterial cells and the prepared surfaces in detail.

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