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Chitosan immobilization on polyacrylic acid grafted polypropylene monofilament

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ABSTRACT

Polypropylene (PP) suture was prepared by plasma-induced graft polymerization of acrylic acid onto polypropylene monofilament. The monofilament was treated with oxygen plasma to create hydroperoxide groups and subsequent graft polymerization was initiated on this exposed monofilament. The grafted filament was immobilized with chitosan (CS) using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) as the linking agent between amino and carboxyl groups. The chitosan content increased with the increase in the degree of grafting on the filament. However, a fraction of the carboxyl groups remained as free functional groups. The pH of the medium has significant influence over the CS immobilization. The transition in immobilization was observed at 4–4.6 pH. The characterization of the immobilized surface by attenuated total reflectance (ATR), contact angle, X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and atomic force microscopy (AFM). ATR showed that the CS is bonded to the filament surface. The contact angles of the surfaces are significantly enhanced and are the indicative of the enhanced hydrophobicity as compared to the grafted surfaces. XPS showed an increasing trend in the nitrogen content with the increasing graft levels on the filaments. The SEM/AFM revealed that the surface morphology undergoes a gradual change with the increase in the degree of grafting.

1. Introduction

Polymers are becoming important materials for drug delivery, biomedical and biotechnological applications due to their interesting physico-chemical characteristics which match with the biological systems (Göpferich, 2008). Suture is one of the important applications of polymers where both the biostable as well as biodegradable materials are used to restore wounds and tissues. Polypropylene (PP) is widely used in biomedical applications, such as hernia repair, wound dressings, sutures, biocomposites, blood contacting surfaces and thermoresponsive materials. PP offers optimum tensile strength and low level of tissue reaction in contact with wound as compared with other polymeric materials. This is where this polymer is being commercially used as Prolene suture. However, the infection at the wound site is quite common and needs to be controlled to overcome post-surgical complications. However, vicryl plus is the only suture available in the market which is biodegradable and shows antimicrobial behavior at the wound site. None of the biostable sutures is available commercially which shows activity against microbes.

It is the surface of a biomaterial that remains in contact with the tissues where surface functionality, morphology and the wettability plays an interesting role in biocompatibility (Lee, Khang, Lee, & Lee, 1998). There are several ways that a bioactive component may be introduced within a polymer matrix, such as by blending or by immobilization processes. However, the compatibility of the additive with the polymeric matrix and the stability of the bioactive component at high processing temperatures becomes a prime constraint to develop the required material by blending approach. Therefore, the most feasible way to design biopolymer is to fabricate the material with desired bulk properties and subsequently modify the surface to make it bioreceptive in nature (Kumar et al., 2007).

The radiation-induced graft polymerization of a monomer into the polymer matrix is a beautiful route to the chemical functionalization, irrespective of its shape and size. The attractive feature of the grafting process is that the extent of modification can be precisely controlled by the proper selection of the irradiation and reaction conditions, (Chapiro et al., 1977; Contreras-García, Burillo, Aliev, & Bucio, 2008; Gubler, Slaski, Wallasch, Wokaun, & Scherer, 2009; Gupta, Jain, Anjum, & Singh, 2006). However, the radiation grafting has the limitation in biomaterials science because of the fact that it modifies the bulk of a polymer and leads to loss of mechanical strength due to the high energy of the electromagnetic

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waves, (Chapiro, 1962). This is where, plasma grafting offers an advantage in terms of the nanoscale modification of the surface layers so that the inherent properties of the biomaterial remain intact while surface becomes bioreceptive in nature. The plasma grafting of an appropriate monomer followed by the immobilization of the bioactive molecules onto the modified surface makes it a formidable combination towards the development of a suture which would prevent the bacterial infection on the stitch site. Plasma pretreatment is widely recognized as a clean and effective method progressively being applied to activate the outermost surface of a polymer, without affecting its structural dimensions. (Oehr, Müller, Elkin, Hegemann, & Vohrer, 1999; Poncin-Epaillard, Chevet, & Brosse, 1994). Both ionized species and free radicals in the reactive plasma play important roles to interact with an organic surface by forming oxidative groups which may be exploited for the desirable interaction with the biomolecules.

Among the bioactive biomolecules, CS, [poly-(1-4)-D-glucosamine] is an interesting polysaccharide for its biological properties, such as water binding capacity, nontoxicity, biocompatibility, biodegradability, acceleration of wound healing and antibacterial nature (Leuba & Stossel, 1986; Liao, Lin, & Wu, 2005; Ouattara, Simard, Piette, Bégin, & Holley, 2000; Tyan, Liao, & Lin, 2003). CS is a cationic polysaccharide which is obtained by alkaline deacetylation of chitin and offers many applications in medicine, cosmetics, agriculture, biochemical separation systems, biomaterials and drug controlled release systems (Amiji, 1996; Hirano, Tobetto, Hasegawa, & Matsuda, 1980; Kaifu & Komai, 1982; Madihally & Matthew, 1999; Muzzarelli et al., 1990; Rao & Sharma, 1997). It has been observed that both chitin and chitosan exert accelerated wound healing in many clinical cases (Cho, Cho, Chung, Yoo, & Ko, 1999; Ueno et al., 1999; Yang & Lin, 2004).

CS confers considerable antibacterial activity against a broad spectrum of bacteria. The interaction between polycationic CS and electronegative residues at the bacterial cell surface alters cell permeability and hence, causes the leakage of intracellular electrolytes and protein (Muzzarelli et al., 1990). Due to these advantages, chitosan filaments have been reshaped into sponge-like wound dressings (Mi et al., 2002). The molecular weight of CS has significant influence on the antimicrobial nature as observed by several authors (Chatelet, Damour and Domard, 2001; Guo et al., 2008; Hernández-Lauzardo et al., 2008; No, Park, Lee, & Meyers, 2002). Hernández-Lauzardo et al. (2008) observed that low molecular weight CS was more effective for the inhibition of mycelial growth while the high molecular weight CS influenced more the development of spores (sporulation, shape, and germination). Similarly Guo et al. (2008) have demonstrated that quaternized CS derivatives are more effective antifungal agents than CS. However, they have observed that high molecular weight material has stronger antifungal behavior than low molecular weight.

Our earlier attempts on the development of antimicrobial PP sutures were followed by the radiation grafting of acrylic and vinyl monomers onto the filament surfaces so that an antimicrobial drug may be bonded to its surface which subsequently provides infection free wound healing (Gupta, Jain, & Singh, 2008; Gupta, Anjum, Gulrej, & Singh, 2007). However, incompatibility between the PP matrix and the grafted moieties led to significant loss in knot strength of the resultant suture (Gupta, Jain, Anjum, & Singh, 2004) This invesigation utilizes low pressure oxygen plasma to create hydroperoxides on the PP filament so that the grafting of acrylic acid (AAc) may be initiated, (Gupta, Saxena, & Ray, 2008). These carboxyl groups offer sites for the immobilization of the CS as the bioactive component on the filament surface. The presence of the CS would help in the development of an antimicrobial and scar preventive PP suture. In the present investigation, the influence of immobilization conditions on the CS binding to suture surface was investigated. The CS immobilized sutures were subsequently characterized by different techniques to understand physico-chemical and morphological behavior.

2. Experimental

2.1. Materials

Polypropylene, supplied by Indian Petrochemicals Limited, India was melt spun into monofilament at 230 °C under nitrogen atmosphere. The filament had a diameter of 0.26 mm and denier of 428. The monofilaments were soxhlet extracted with acetone to remove any impurity adhering on the surface.

Acrylic acid was supplied by Merck India Ltd. and was purified by distillation under vacuum. CS (medium viscous with the degree of deacetylation of 0.80) was obtained from Fluka. The molecular weight of medium viscosity chitosan has been reported to be 2.38×10^4 Da by Hernández-Lauzardo et al. (2008).

Methanol obtained from Qualigens Fine Chemicals, India, was used as received. Toluidine Blue O (TBO) was supplied by Spectrochem. Acid orange 7 was supplied by Sigma.

Citric acid, acetic acid, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium acetate trihydrate and trisodium citrate dihydrate was supplied by Merck Chemicals. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) was obtained form Sigma–Aldrich. Distilled water was used for all the experiments.

2.2. Acrylic acid grafting

The monofilament was treated with oxygen plasma for specified period of time. The system consisted of RF reactor operating at 13.6 MHz. Subsequently, acrylic acid was grafted on the plasma-treated monofilament using methanol/water as the solvent, at a constant temperature for certain period of time. After the grafting reaction, the homopolymer was removed and the sample was dried in an oven at 50 °C under vacuum and the degree of grafting was estimated by colorimetric method with Toluidine Blue O (TBO) staining, as reported earlier (Gupta, Saxena, et al., 2008). The grafted filament was placed into TBO solution of pH 10 for 6 h at 40 °C. The filament was subsequently removed and washed with sodium hydroxide solution of pH 9 to remove any noncomplexed dye adhering on the filament surface. The dye was desorbed from the filament in 50% acetic acid solution and the optical density of the solution was measured by using an UV–vis spectrophotometer at 623 nm. The polyacrylic acid content (degree of grafting) was obtained from the calibration plot of the optical density *vs.* dye concentration with the assumption of 1:1 ratio between the dye and the carboxylic acid groups.

2.3. Chitosan immobilization

The polyacrylic acid (PAA) grafted PP filaments with various degrees of grafting were immersed in 25 mL *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) aqueous solution (concentration 10 mg/mL), buffered to pH 4.8 (in sodium citrate) at 4 °C for 30 min. EDC was used for activating carboxyl groups on PAA-grafted samples, (Liao et al., 2005). The EDC-coupled samples were then immersed in 50 mL CS solutions, which included 0.4% CS and buffered to a certain pH using acetic acid-buffered solutions at 4 °C for 24 h. The amide bond (–CONH–) formed between amino groups in CS and C=O in carboxylic acid. The CS immobilized samples were washed with acidified water in slow stirring at 4 °C for 30 min and dried at room temperature.

2.4. Determination of the surface density of amino groups

The surface density of amino groups on chitosan immobilized samples was measured from the uptake of an acidic dye. The sample was treated in 0.1 mg/mL acid orange 7 (the pH was adjusted to 3 with 0.1 mM HCl) at 30 °C for 5 h. The sample was rinsed with double-distilled water followed by washing in 0.1 mM HCl for the removal of the adsorbed dye. The samples visibly turned orange in color. The desorption of the complexed dye was performed with 0.1 mM NaOH solution. The dye content was determined from the optical density of the solution measured by using an UV–vis spectrophotometer at 485 nm. The surface density of amino groups was then determined from the calibration plot of the optical density *vs.* dye concentration with the assumption of 1:1 ratio between the dye and the protonated amino group (Hu, Jou, & Yang, 2002).

2.5. Determination of the surface density of chitosan

The surface density of chitosan (CS) was measured from the uptake of an acidic as well as basic dye. The CS immobilized filament was treated in both acidic and basic dyes under the conditions as mentioned above. The treatment with basic dye gives the number of nonbonded carboxyl groups after CS immobilization. The treatment with acidic dye gives the number of nonbonded amino groups remaining free after binding with carboxyl groups of PAA chains. From the pre-determined carboxyl content of the grafted surface, it was possible to have total no. of NH_2 groups. Taking into consideration the degree of deacetylation of 0.80, the CS content was calculated.

2.6. FTIR-ATR measurements

FTIR–ATR measurements were performed on Bruker Alpha P (Opus 65) spectrophotometer in the range of 400–4000 cm^{-1}.

2.7. UV–vis spectrophotometer

Acidic dye and basic dye content was measured on Perkin Elmer Lambda E Z 201 spectrophotometer at 485 and 623 nm, respectively.

2.8. Contact angle

Contact angle measurements on filaments were made on DCAT 21 Tensiometer from Dataphysics using Wilhelmy method, (Huang, Wei, Wang, & Xu, 2006). The sample was mounted on the holder and the force exerted on the contact of the filament with water surface was measured. The contact angle of the sample was obtained from the force by in-built software.

2.9. X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) on filament surfaces was performed on Physical Electronics Quantun 2000 equipped with monochromatized AlKα X-ray source.

2.10. Atomic force microscopy (AFM)

Topographical studies of the sample surface were carried out in air using atomic force microscope (AFM), Nanoscope IIIa (Digital Instruments, Veeco Metrology Group) and was operated in the contact mode using an etched silicon tip attached to the end of a cantilever (115–135 μM in length). The AFM measurements were carried out at a cantilever resonant frequency of around 277.5 kHz and a scan rate of 0.5 Hz. The spring constant of the cantilever was in the range of 20–80 N/m.

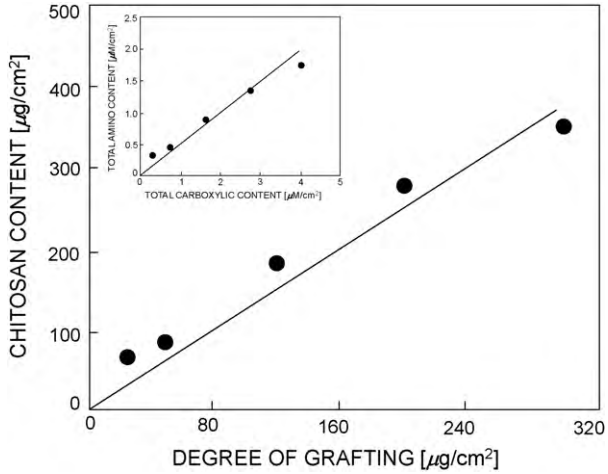

Fig. 1. Variation of chitosan content with the degree of grafting. Sample preparation conditions: plasma exposure: 180 s; power, 100 W; O_2 flow rate, 20 sccm. Grafting conditions: [M]: 40%; [methanol]: 40%; *T*: 50 °C. Chitosan immobilization conditions: EDC in citrate buffer of pH 4.8 (10 mg/mL) at 4 °C for 30 min. Chitosan in acetate buffer of pH 4.6 (0.4%) at 4 °C for 24 h.

3. Results and discussion

3.1. Chitosan immobilization

The biofunctionalization of PP filament has been carried out to develop surfaces which show innovative features, such as antimicrobial and scar preventive nature. Chitosan (CS) has such interesting characteristics and may provide the suture with desired features. The process requires activation of the filament and subsequent binding of CS on its surface. Oxygen plasma activation of PP leads to the generation of hydroperoxides as the oxygenated species within a very short exposure time of 60–240 s so that the grafting of acrylic acid may be initiated. The plasma treatment parameters and reactions conditions influence the degree of grafting significantly, which subsequently governs the physicochemical structure and architecture at the surface (Saxena, Ray, & Gupta, 2010). The grafting introduces carboxyl groups on the filament surface for the binding of CS *via* amino groups where EDC acts as the coupling agent for the covalent bonding between amino group of CS and carboxyl group of PAA chains. It is observed that the CS bonding to the surface is significantly influenced by the amount of grafting and the pH of the medium.

The variation of CS content with the degree of grafting is presented in Fig. 1. The CS content increases with the increase in the degree of grafting. This is the indication of the enhanced interaction of amino groups of CS with the carboxyl groups on the filament surface. However, not all the carboxyl groups are bonded to the amino groups. The molar variation of amino groups with the carboxyl groups shows that a large fraction of carboxyl groups still remains nonbonded depending on the degree of grafting (inset). From the slope of the plot in Fig. 1 amino/carboxyl ratio comes out to be 0.51 which indicates that half of the carboxyl groups still remain nonbonded after the immobilization process. This suggests that the accessibility of CS chains to the carboxyl groups into the surface grafted layer is limited. The fraction of carboxyl groups bonded as –CO–NH– (amide) linkage and as free carboxyl is presented in Fig. 2. The amide fraction is much higher (0.94) at low graft level of 24 μg/cm^2. As the carboxyl content increases subsequently to 290 μg/cm^2, the amide fraction diminishes to 0.43. The

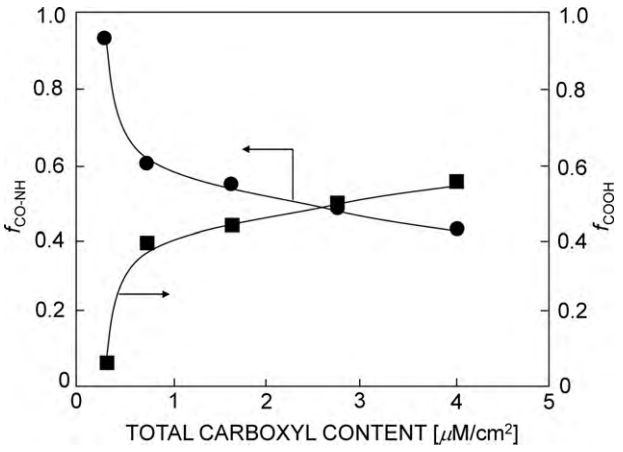

Fig. 2. Variation of the amide fraction (f_{CO-NH}) and free carboxyl (f_{COOH}) with the total carboxyl content.

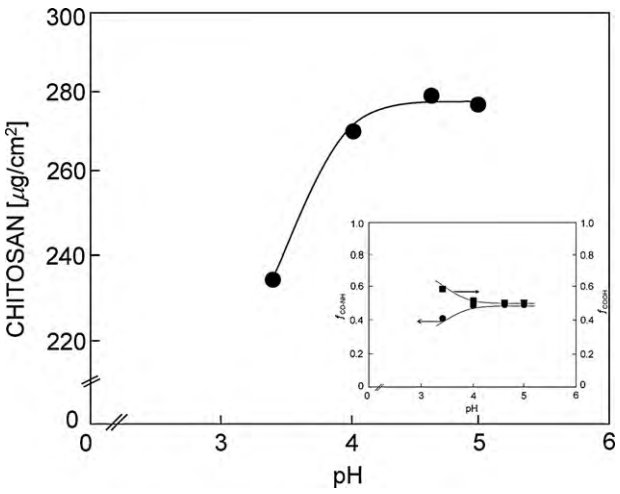

Fig. 3. Variation of chitosan content with the pH of immobilizing medium. Sample preparation conditions: plasma exposure: 180 s; power, 100 W; O_2 flow rate, 20 sccm. Grafting conditions: [M]: 40%; [methanol]: 40%; *T*: 50 °C. Chitosan immobilization conditions: EDC in citrate buffer of pH 4.8 (10 mg/mL) at 4 °C for 30 min; Chitosan in acetate buffer of varying pH (0.4%) at 4 °C for 24 h.

free carboxyl, consequently, varied in the range of 0.06–0.57. This observation reflects that the interaction of CS molecules with the grafted chains becomes difficult as the graft level increases.

The behavior in Fig. 2 may be understood from the fact that CS is a long chain polysaccharide and its mobility within the grafted domain on the filament surface would be a key factor in its bonding ability with the carboxyl groups. The grafted PAA chains are hydrophilic in nature and swell in the immobilization medium comprising of water, leading to the hydrogel matrix. Since a hydrogel swollen in a solvent is the medium of very high viscosity, the CS chains may find it difficult to move within the hydrogel system and hence restrict the interaction with the groups. At low graft levels, the chains are shorter and allow CS to access PAA chains. As the degree of grafting increases, the PAA chain become longer and offer high viscosity within the surface layer. Consequently, this hinders the mobility of the CS chains deep into the surface layer. May be, a large number carboxyl group in the deeper section are rendered nonbonded. For the CS component, almost all amino groups are bonded to the carboxyl groups and a very small fraction of 0.01–0.02 is left as free amino groups. This indicates that whatever the CS is immobilized, it is covalently bonded to the filament surface *via* –CO–NH– linkage. The nonbonded CS is washed away from the surface during the washing step.

3.2. Influence of the pH of the medium

The pH of the medium plays a very crucial role in CS immobilization. A sharp transition in CS content takes place at the pH of 4–4.6. A 25% increase in the CS content was observed for the increase in the pH from 3.4 to 4.6 (Fig. 3). The fraction of amide linkage shows an increasing trend with the increase in the pH of the medium. Consequently, the fraction of the free carboxyl groups tends to decrease as the pH increases (inset). It is evident that the variation in carboxyl fraction as amide and as free carboxyl does not change much beyond 4.6. This observation suggests that the surface structure of the filament undergoes significant transition which makes the surface more ameanable to the CS interaction. Looking at the dissociation constant of –COOH groups, it may be proposed that the ionization of carboxyl groups takes place at a pH of 4.6 which enhances the CS interaction within the grafted layer. Based on the results in Fig. 3, a schematic representation of CS interaction with the carboxyl groups may be presented as in Fig. 4. At lower pH of 3.4, the carboxyl groups remain in the non-ionized –COOH form. This leads to the strong hydrogen bonding among the groups and close association within the grafted chains. As a result, PAA chains remain collapsed and tightly bonded onto the surface. The CS chains therefore are unable to penetrate into the grafted layer leading to a significant fraction of carboxyl groups as nonbonded. At a pH of 4–4.6, carboxyl groups have tendency to dissociate into COO^- state creating repulsion among themselves and hence, CS molecules are able to move deeper into the grafted layer and interact with the carboxyl groups in an efficient manner which leads to higher amount of CS being immobilized at the higher pH.

3.3. ATR-FTIR

Fig. 5 shows the ATR spectra of the virgin PP filament, CS powder, polyacrylic acid grafted PP with the degree of grafting 290 μg/cm^2 and CS immobilized on grafted filament. A systematic evaluation of peaks and shifting of peak positions takes place in the samples during each step of modification *i.e.* grafting and CS immobilization. Fig. 5(a) shows the characteristic peaks of PP in the region

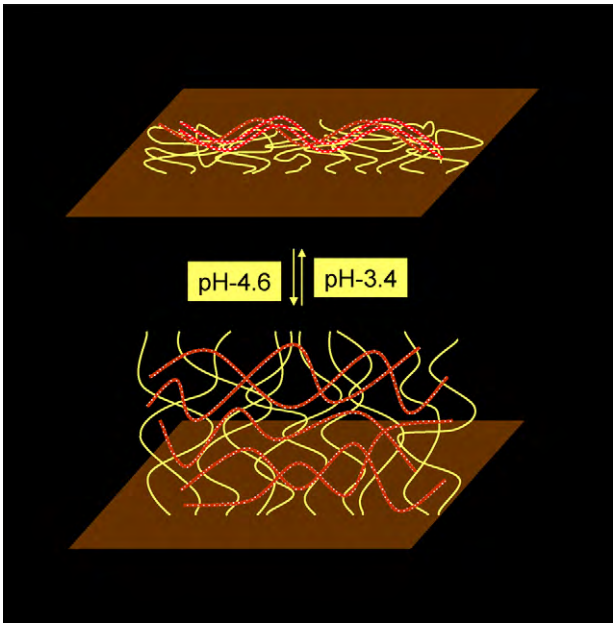

Fig. 4. Schematic representation of the change in polyacrylic acid brushes on the PP suture with the change in the pH of the chitosan immobilization.

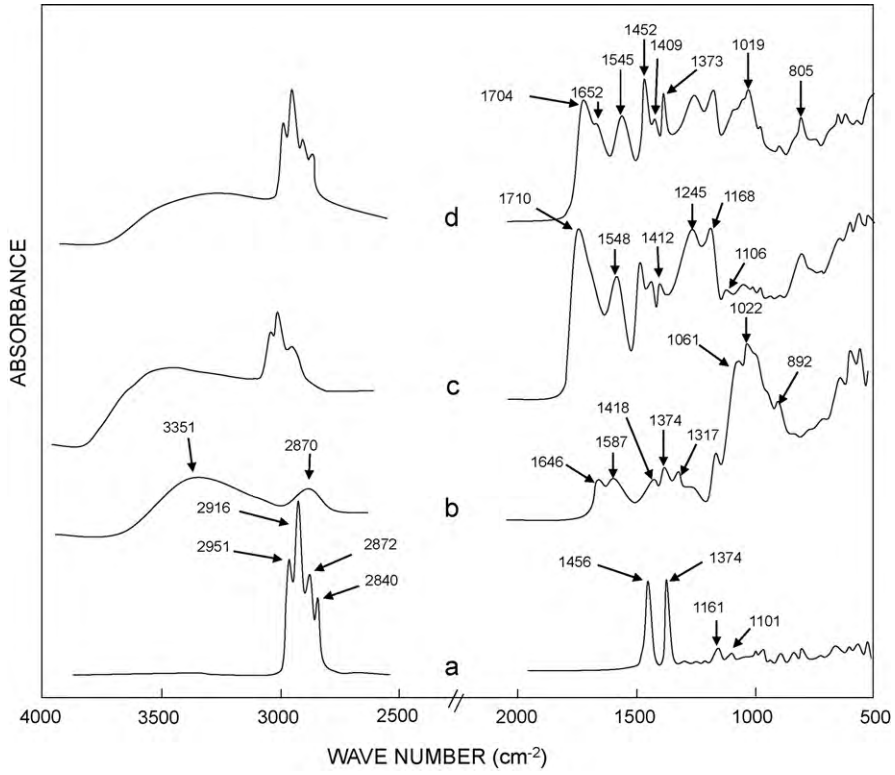

Fig. 5. ATR of (a) virgin PP (b) chitosan powder (c) PP-*g*-PAA monofilaments with the degree of grafting 290 μg/cm^2 (d) sample c with chitosan immobilization.

2800–3000 cm^{-1} (2951, 2916, 2872, 2840 cm^{-1}). The peaks at 2951 and 2872 cm^{-1} are due to the asymmetric and symmetric stretching vibrations of methyl group, respectively. While the peaks at 2916 and 2840 cm^{-1} may be ascribed to the –CH_2 asymmetric and symmetric stretching vibrations respectively (Sciarrtta, Vohrer, Hegemann, Muller, & Oehr, 2003; Scorates, 2001). The original PP shows two distinct peaks at 1456 and 1374 cm^{-1} which is due to the asymmetric and symmetric bending vibrations of methyl groups, respectively (Morent, Geyter, Leys, Gengembre, & Payen, 2008). The grafting of acrylic acid results in the origin of new peaks which are evident from the Fig. 5(c). A new broad peak arises between 3000 and 3600 cm^{-1} due to –OH stretching vibrations from the carboxyl groups (Sun, Zhang, Chai, & Chen, 2006). The broad peak is the indication of hydrogen bonding among the carboxyl groups. The origin of the peak at 1710 cm^{-1} may be attributed to the carbonyl stretching vibration of the carboxyl group while the peak at 1245 cm^{-1} may be assigned to the C–O stretching vibrations (Scorates, 2001). This is the indication of the grafted polyacrylic acid chains on the filament surface. There is another peak originating at 1412 cm^{-1}. This peak is related to the in plane O–H bending vibration. The peak at 1168 cm^{-1} is the indication of C–C asymmetric stretching which is present in the original polypropylene at 1161 cm^{-1}. The presence of a peak at 1548 cm^{-1} in the grafted sample may be attributed to the carboxylate ion, the intensity of this peak is quite high.

The functional groups in pure CS were observed in a broad peak at 3351 cm^{-1} for –OH, –NH and NH_2; 1061 cm^{-1} for C–O–C stretching vibration in glucosamine ring while amide I C=O in O=C–NH at 1646 cm^{-1}, amide II NH in O=C–NH at 1587 cm^{-1} and amine at 1317 cm^{-1} (Fig. 5(b)). For the CS immobilized surface, the IR absorbance of carbonyl in carboxylic acid was found at 1704 cm^{-1} which is significantly lower as compared to the 1710 cm^{-1} in polyacrylic acid grafted surface (Fig. 5(d)). This might be an indication of loss of some free carboxyl groups due to the formation of amide bond with deacetylated sites of CS molecules. The CS immobilized surface also shows the characteristic peaks at 1652 cm^{-1} for amide I and amide II peak at 1545 cm^{-1} which is sufficient indication of CS immobilization on the filament. The presence of a new absorption band at 1061 cm^{-1} may be ascribed to the ether group of pyranose ring in CS. The peak at 1022 cm^{-1} is slightly shifted to 1019 cm^{-1}. The inherent peaks of PP at 1456 cm^{-1} and 1374 cm^{-1} remain evident in CS immobilized samples almost at the identical places. All these observations are indicative of the linking of CS with the carboxyl groups *via* covalent bonding.

3.4. Contact angle measurements

The surface behavior of CS immobilized filaments was monitored by contact angle measurement (Fig. 6). The surface shows

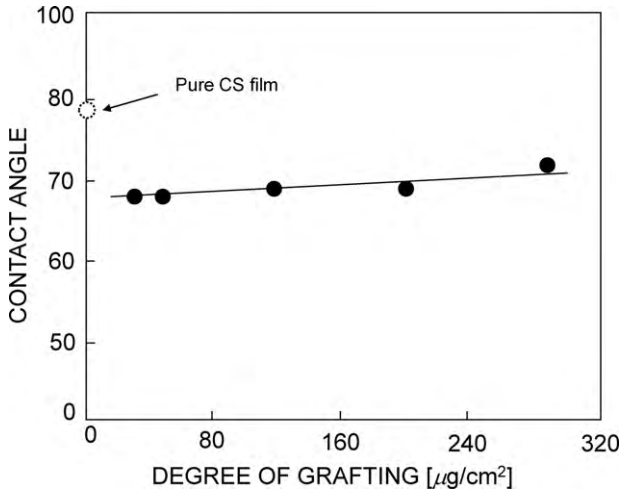

Fig. 6. Variation of water contact angle of chitosan immobilized samples with the degree of grafting.

higher level of hydrophobicity but still has a lower contact angle than pure CS films. Pure CS has contact angle of 78° while CS immobilization produces surfaces with CA of 68–71°. It is interesting to see that the CS immobilized surfaces exhibit much higher CA as compared to the grafted ones where the CA range was 30–36° (Gupta, Saxena, et al., 2008). It seems that this behavior is due to the loss of carboxyl groups in –CO–NH– formation which adds to the diminishing hydrophilicity on the surface leading to the higher contact angle. However, the lower contact angle of grafted surfaces as compared to pure CS film suggests that polyacrylic acid chains contribute partially to the hydrophilicity and hence lowers contact angle of the surfaces to some extent.

3.5. XPS analysis

The XPS analysis was carried out to study the atomic composition of the surface layers on CS immobilized filaments (Fig. 7). The CS-bonded surfaces show an increase in the nitrogen content with the increase in the CS content. The increase is very slow up to 119 μg/cm^2 PAA, beyond which it increases very fast. These values are still lower than that of pure CS film (5.6%). These results are indicative of the slow build up of CS layer on within the grafted domain on the filament surface. Therefore, the surface is not composed of pure CS, instead it comprises of a network of CS-PAA chains which enforces the dilution of CS component on the surface and leads to the nitrogen content less than that of pure CS.

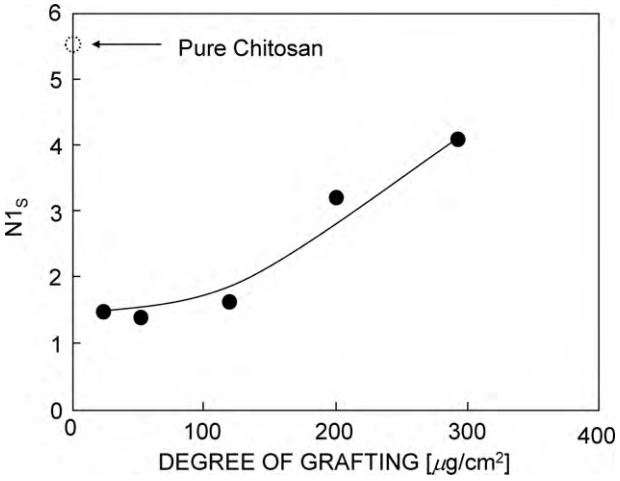

Fig. 7. Variation of N_{1s} with the degree of grafting.

3.6. Surface morphology

The surface morphology of CS immobilized PP sutures was investigated by AFM (Fig. 8). It may be mentioned that the CS immobilized surfaces were washed under acidic conditions to remove any physically trapped CS on the filament surface. Whatever the CS is left behind that should be covalently bonded to the surface. The plasma exposed surface shows hill-valley structure with significant nonhomogeneity. However, we observed that the grafting of acrylic acid is very much responsible for the flattening of the surface due to the incorporation of the PAA chains within the valleys (Saxena, Ray, & Gupta, 2010; Saxena, Ray, Mindemart, Hilborn & Gupta, 2010; Saxana et al., 2010). The CS immobilization subsequently introduces independent structures but a significant reorganization on the surface takes place. The surface at the higher graft levels becomes very much flat and is the reflection of the higher extent of homogenization as the more CS content on the

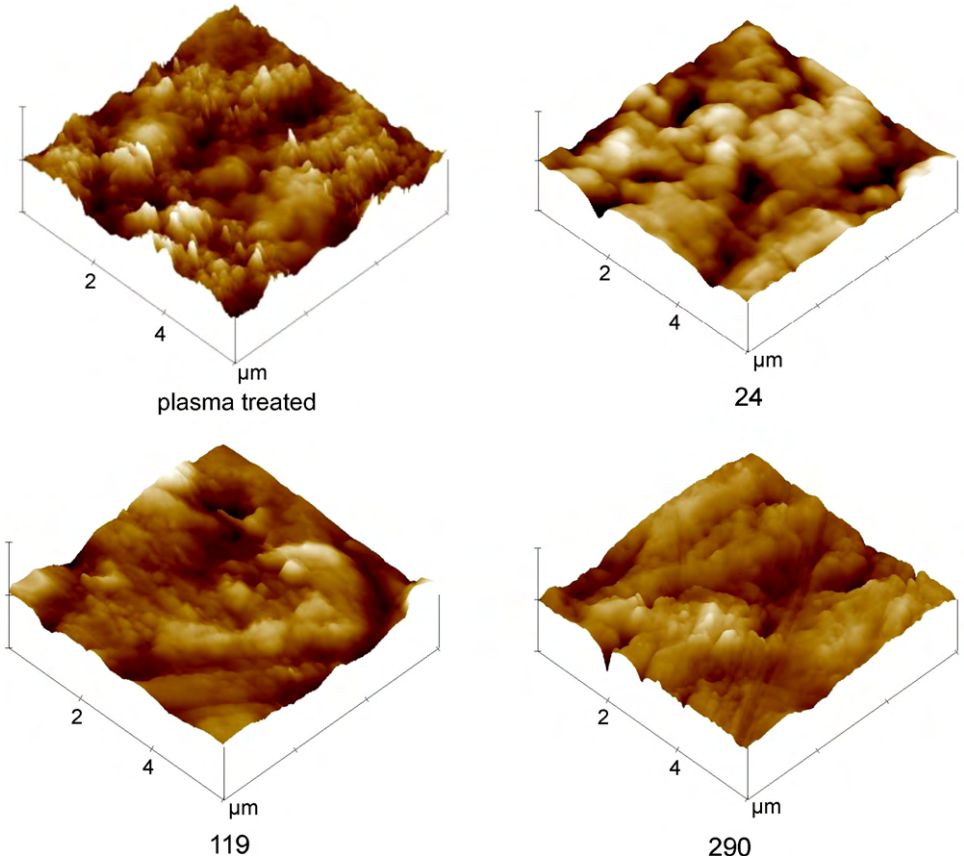

Fig. 8. AFM Images of (a) plasma treated PP and chitosan immobilized PP-*g*-PAA monofilaments with various degrees of grafting. (b) 24 μg/cm^2 (c) 119 μg/cm^2 (d) 290 μg/cm^2.

surface is bonded. Similar trend of morphological arrangements has been observed for the collagen immobilization on polyacrylic acid grafted PET surfaces (Gupta, Hilborn, Plummer, Bisson, & Frey, 2002). The CS immobilized filaments have been subsequently used for the immobilization of antimicrobial drug and nanosilver to enhance antimicrobial properties of the suture (Saxena, Ray, & Gupta, 2010; Saxena, Ray, Mindemart, et al., 2010; Saxana et al., 2010). The modified suture was tested for tissue compatibility, toxicity and wound healing in rat model (Saxena, Ray, & Gupta, 2010; Saxena, Ray, Mindemart, et al., 2010; Saxana et al., 2010).

4. Conclusion

The plasma-induced graft polymerization of acrylic acid on PP filament leads to the surface with carboxyl groups. This surface offers sites for the covalent bonding of CS molecules using EDC as the linking agent. The amount of CS increases as the PAA content increases due to the availability of the more carboxyl groups for the amide linkage. It has been observed that nearly half of the carboxyl groups remain nonbonded to CS molecules. This suggests that the CS molecules find it difficult to percolate throughout the grafted layer which is swollen in water and is of the hydrogel nature. The CS immobilization shows strong dependence over the pH of the medium. At a pH of 4–4.6, the carboxyl groups have a tendency to ionize and form a highly repulsive structure. This leaves behind enough space for the movement of CS chains and accessibility to the carboxyl groups in the grafted layer. ATR has shown that CS is bonded to the surface. At the same time, nitrogen content increases with the increase in the degree of grafting as revealed by XPS. Even at a graft level of 290 $\mu g/cm^2$, the nitrogen content is 4.2% which is less than 5.6% for pure CS. This is because of the fact that even if CS amount of 352 $\mu g/cm^2$ would mean the surface layer of 1.5 μm. This should be enough for the nitrogen content of 5.6%. This is the indication of the dilution of the CS content by the presence of the PAA chains so that the surface behaves more as the blended one and leads to lower N content than for the pure CS.

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