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Tailoring the surface properties of polypropylene films through cold atmospheric pressure plasma (CAPP) assisted polymerization and immobilization of biomolecules for enhancement of anti-coagulation activity

K. Navaneetha Pandiyaraj[a,*], M.C. Ram Kumar[a], A. Arun Kumar[a], P.V.A. Padmanabhan[b], R.R. Deshmukh[c], M. Bah[d], S. Ismat Shah[d], Pi-Guey Su[e], M. Halleluyah Jr[f], A.S. Halim[f]

[a] Surface Engineering Laboratory, Department of Physics, Sri Shakthi Institute of Engineering and Technology, L&T By Pass, Chinniyam Palayam (Post), Coimbatore 641062, India
[b] PSN College of Engineering and Technology, Tirunelveli 627 152, India
[c] Department of Physics, Institute of Chemical Technology, Matunga, Mumbai 400 019, India
[d] Department of Physics and Astronomy, Department of Materials Science and Engineering, University of Delaware, 208 Dupont Hall, Newark, United States
[e] Department of Chemistry, Chinese Culture University, Taipei 111, Taiwan
[f] School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

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ABSTRACT

Enhancement of anti-thrombogenic properties of polypropylene (PP) to avert the adsorption of plasma proteins (fibrinogen and albumin), adhesion and activation of the platelets are very important for vast biomedical applications. The cold atmospheric pressure plasma (CAPP) assisted polymerization has potential to create the specific functional groups such as O—C=O, C=O, C—N and S—S. on the surface of polymeric films using selective precursor in vapour phase to enhance anti-thrombogenic properties. Such functionalized polymeric surfaces would be suitable for various biomedical applications especially to improve the blood compatibility. The eventual aspiration of the present investigation is to develop the biofunctional coating onto the surface of PP films using acrylic acid (AAc) and polyethylene glycol (PEG) as a precursor in a vapour phase by incorporating specific functional groups for immobilization of biomolecules such as heparin (HEP), chitosan (CHI) and insulin (INS) on the surface of plasma modified PP films. The surface properties such as hydrophilicity, chemical composition, surface topography of the surface modified PP films were analyzed by contact angle (CA), Fourier transform infrared spectroscopy (FTIR), X-ray photo electron spectroscopy (XPS) and atomic force microscopy (AFM). Furthermore the anti-thrombogenic properties of the surface modified PP films were studied by *in vitro* tests which include platelet adhesion and protein adsorption analysis. It was found that the anti-thrombogenic properties of the PP films are effectively controlled by the CAPP grafting of AAc and PEG followed by immobilization of biomolecules of heparin, chitosan and insulin. The grafting and immobilization was confirmed by FTIR and XPS through the recognition of specific functional groups such as COOH, C—O, S—S and C—N. on the surface of PP film. Furthermore, the surface morphology and hydrophilic nature of the PP films also tailored significantly by the successful grafting and immobilization which is confirmed by AFM and CA analysis. Owing to the physico-chemical changes on the surface of PP films induced by CAPP assisted polymerization, the anti-thrombogenic properties of PP films were enhanced as confirmed by *in vitro* analysis.

1. Introduction

Owing to incredible physico-chemical properties such as high impact strength, chemical resistance, low cost, light weight, high thermal, chemical stability and so on, polypropylene (PP) plays a vital role in biomedical field for artificial organs, disposable

* Corresponding author.
E-mail address: dr.knpr@gmail.com (K. Navaneetha Pandiyaraj).

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clinical apparatus, medical devices and cardiovascular implants such as artificial heart valves, stent and sewing rings [1–6]. Nevertheless, the major hurdle in using PP as a biomedical material is its long term thrombogenic property, which may be due to inherent poor surface properties (highly hydrophobic and lower adhesion) and hence an attempt will be required to enhance anti-thrombogenic properties. Generally, anti-thrombogenic properties of the materials are determined quantitatively from the adsorption behavior of plasma proteins (albumin or fibrinogen) and inhibition of adhesion and activation of platelets on the surface when the material is exposed to blood [7]. Moreover, the surface induced thrombus formation is a major cause of patient morbidity and mortality and hence utilization of bioimplants is limited in many situations [8]. Therefore it is necessary to enhance anti-thrombogenic properties of materials through suitable surface modification technique which tailor the surface properties such as functionalities, topography, crystallinity and surface charges of the PP films without affecting intrinsic material bulk properties [9]. Thus the material requires suitable surface treatment to improve the blood compatibility and the choice of the technique should be reliant on the reproducibility, reliability and produce yield.

Recently, various techniques such as corona treatment, radiation assisted polymerization, wet chemical, phase separation, low pressure and atmospheric pressure plasma polymerization etc. have been employed for tailoring the PP film surface into precious biocompatible product [10–18]. However, the conventional surface modification methods have certain drawbacks; for instance use of solvents, emission of VOC, consume more time, huge capital cost, lack of reproducibility and stability. Past few decades have witnessed the use of low temperature plasma polymerization to deposit a thin polymeric film with specific functional groups onto the substrate polymer using various chemical precursors to improve biocompatibility, as interfacial layers in different deposition processes or as beads for active biological compounds immobilization. The product obtained by the plasma assisted polymerization has distinct properties compared with conventional polymerization techniques. The plasma polymerized polymeric films are pinhole free, conformal, highly cross linked, having unique surface chemistry and strongly adhering with the substrate [19–23]. However, major drawbacks related to vacuum process (chamber loading/unloading time, batch processing, and complexity in introducing to the in-line production, high investment costs, etc.) prevent the technique from being widely used. Hence this paper has proposed to produce such a biofunctional coating on the surface of PP films through cold atmospheric plasma assisted polymerization techniques using liquid precursors such as acrylic acid (AAc) and polyethylene glycol (PEG), which is reported only by few researchers [23–26]. However, most of the researchers have reported the development of AAc and PEG grafted surfaces by low pressure plasma polymerization technique. The cold atmospheric pressure plasma assisted polymerization has notable advantages such as high treatment effects without affecting bulk properties of the material, easy up-scaling of production processes without the need for vacuum, controllable properties of grafted polymer film, rapid kinetic reaction due to high concentration plasma particles and radicals [27,28]. Plasma polymerization of AAc can provide higher concentration of carboxylic (COOH) groups on the surface of PP films which can have great impact in biomedical applications. Moreover, PEG has been a well-known effectual water soluble polymer for biomedical applications especially blood contacting devices because it prevents adsorption of plasma protein, adhesion of platelets and formation of thrombus by the steric repulsion mechanism [7]. The improvisation of blood compatibility of PEG is mainly caused by its flexible long chain which persuade micro kinetics environment of the blood–material interface which in turn resist the formation of thrombi and adsorption of human blood proteins, etc. [8,29–32]. The AAc and PEG grafted surface provides active sites for immobilization of biomolecules such as chitosan, heparin and insulin to further improve the blood compatibility [30]. The molecules of the heparin, insulin and chitosan are the appropriate candidates to improve the anti-thrombogenic properties of PP films because they can provide high dense of amino, sulfo-amino, carboxyl groups which are desirable functional groups to improve the blood compatibility [33–37]. The changes in functionalities on the surface of PP films were analyzed by Fourier transform infra-red (FTIR) and X-ray photoelectron spectroscopy (XPS). The changes in surface topography, hydrophilicity of the surface modified films were analyzed by atomic force microscopy (AFM) and contact angle (CA) analysis respectively. The polar and dispersion components of the total surface energy of the plasma processed PP films was estimated by Fowke's approximation method using contact angle data of three testing liquids. Furthermore in order to study the durability (ageing behavior) of plasma processing, samples were stored for different durations (up to 30 days) and measurement of contact angle was carried out. The anithrombogenic properties of surface modified PP films were investigated by *in vitro* analysis which include platelet adhesion, platelet count and protein adsorption analysis.

2. Experimental

2.1. Materials

The biomolecules (insulin, chitosan and heparin), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), Triton-X100, Bovine Serum Albumin and fibrinogen were procured from Sigma-Aldrich, India. The acrylic acid, polyethylene glycol (MW 600), acetic acid etc. were obtained from MERCK and LOBA, India. Polypropylene (PP) films of 80 μm thickness were acquired from Reliance Petro Chemical Ltd, Mumbai. The PP films were cut in to the size 15 cm × 15 cm and cleaned with acetone and distilled water in an ultrasonic bath for 30 min each. Finally the films were dried in air and stored in a desiccator till the further use.

2.2. Methodology

The CAPP assisted polymerization was accomplished by atmospheric pressure AC excited dielectric barrier discharge plasma reactor as shown in Fig. 1a which consists of square type of plasma chamber with the dimension of 40 cm × 40 cm × 20 cm. Two electrodes with dimension 30 cm × 30 cm were fixed parallel to each other inside the chamber. Moreover, both electrodes are covered by 3 mm polypropylene sheet which act as dielectric layer to avoid arcing and passage of high current. The distance between the electrodes was kept 6 mm throughout the experiment. The plasma was generated between the two electrodes using high voltage AC power supply (V_{max} = 40 kV, I_{max} = 40 mA and ν = 50 Hz). The upper electrode is a live electrode and the lower electrode was grounded (Fig. 1a). The sample was kept on the lower electrode. The gas inlet system enables gas mixing controlled by gas flow controller.

Before polymerization, PP samples were subjected to the atmospheric pressure argon plasma for 60 s which removed adsorbed contaminants from the surface of PP films which is called plasma cleaning. For plasma cleaning, argon gas was fed between two electrodes with the flow rate of 3 lpm. An ac potential (14 kV) was applied between two electrodes and adjusted to get a stable glow discharge (Fig. 1b). Argon plasma induces chain session and surface roughness onto the PP film surface. After surface treatment, the oxygen gas was fed into the plasma chamber for 10 min in the

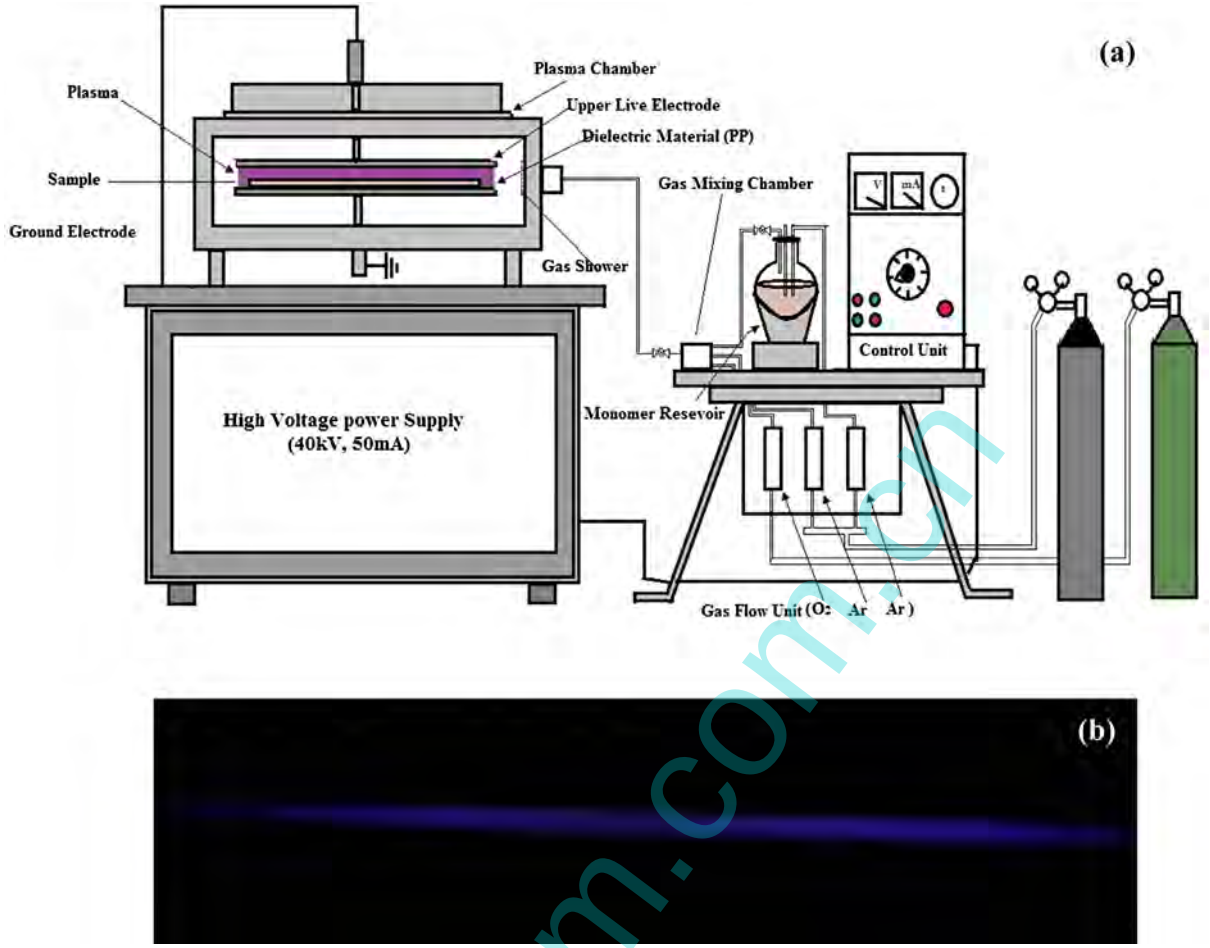

Fig. 1. (a) Schematic diagram of non thermal atmospheric pressure plasma reactor and (b) discharge plasma during plasma polymerization.

absence of plasma to create oxygen containing polar groups onto the PP surface. After this, again the plasma was ignited using argon with the fixed flow rate of 3 lpm, at the same time AAc vapours were fed into the plasma regime along with argon as a carrier gas. In order to get sufficient vapour pressure, AAc reservoir was maintained at 80 °C and AAc flow rate was kept at 10 mg/min. Since it is atmospheric pressure plasma, the system was held at atmospheric pressure due to continuous passage of argon gas. The continuous ablation and polymerization results in the formation of AAc coated PP film surface. The deposition was carried out for 5 min and such films were designated as AAc-PP. The AAc-PP surface contains high dense carboxylic groups which is further confirmed by functional group analysis and the same is reported in the section 3. Finally the obtained AAc-PP films were cleaned by 0.1 wt% of Triton X-100 which removes the volatile AAc molecules from the AAc-PP surface. Furthermore, PEG molecules were grafted on the surface of AAc-PP films so that the molecules of PEG can act as spacer for immobilization of biomolecules molecules such as heparin, chitosan and insulin as well as it helps to retain the bioactivity of the PP films. In this process the cleaned AAc-PP films were again placed on the surface of lower electrode. After that the argon plasma was generated by the above mentioned processing parameters. At the same time the PEG vapour was produced by heating the reservoir at 220 °C temperature (PEG vapour flow rate was 1 mg/min) and it was allowed in plasma regime by carrier gas (Ar) which leads to produce PEG layer on the surface AAc-PP films (deposition time was maintained at 5 min). The obtained sample was designated as PEG-AAc PP. The typical operating parameters are listed in Table 1.

Table 1
Typical operating parameters for plasma processing.

Main gas	Ar
Carrier gas	Ar
Precursor monomer	AAc, PEG
Ar plasma exposure time	60 s
Electrode separation	6 mm
Working pressure	Atmospheric pressure
Main gas flow (Ar)	3 lpm
Carrier gas (Ar)	0.5 lpm
Deposition time	5 min
Discharge potential	14 kV
Biomolecules	Chitosan, insulin, heparin

2.2.1. Immobilization of biomolecules

The PEG-AAc-PP films were further immersed in INS, HEP and CHI containing solutions separately. HEP and INS containing solution prepared by dissolving 20 mg of heparin or 4 mg of insulin in a 20 ml of EDC solution (mixture of 80 ml of sodium citrate buffer solution and 80 mg of *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC)) respectively. The reactions were carried at 24 °C for 24 h in order to produce heparin- or insulin immobilized PEG-AAc-PP films. Furthermore, the molecules of chitosan were immobilized on the surface of PEG-AAc-PP films by simple chemical route. Initially 500 mg of chitosan was dissolved in 50 ml of acetic acid and mixture was stirred vigorously for 12 h at 30 °C to get 10% (w/v) chitosan solution. Thereafter, the 3 cm × 3 cm of PEG-AAc-PP film was subsequently reacted with that chitosan containing solution for 30 min. Finally, the obtained HEP, INS and CHI separately immobilized PEG-AAc PP films were washed with

Table 2
Surface energy parameters of the testing liquids.

Liquids	γ_l (mJ/m^2)	γ_l^p (mJ/m^2)	γ_l^d (mJ/m^2)
Distilled water (W)	72.8	51.0	21.8
Formamide (F)	58.2	18.7	39.5
Ethylene glycol (EG)	48.0	19.0	29.0

0.1 wt% Triton X-100 for 10 min ultrasonically to remove excess amount of unreacted biomolecules from the surface of PEG-AAc-PP films and dried at room conditions [34,37,38].

2.3. Characterization of surface modified PP films

The extent of hydrophilicity of the surface modified PP films was investigated by measurement of contact angle using sessile drop method [39] using three testing liquids (distilled water, formamide and ethylene glycol) of known surface energy parameters (Table 2) which is described in detail elsewhere [40]. The contact angles of these liquids with the films are precisely measured. An average of 10 independent measurements of contact angle at different points on the surface of modified films is reported here. The experimental error in the measurement of CA was found to be ±2°. The contact angle measurements were made under controlled temperature and humidity conditions (24 °C and R_H = 55%). Few samples were stored in air at ambient condition for determining the stability of the hydrophilicity of modified PP films. For this purpose CA was measured with regular interval up to 30 days. The advantage of Fowke's method is that it minimizes error involved in the measurement of CA.

Furthermore the surface energy of surface modified PP films was evaluated by Fowke's equation extended by Ownes–Wendt.

According to Fowke's, the solid surface energy can be determined by the sum of two major components such as polar and dispersion components. Hence,

$$\gamma_s = \gamma_s^d + \gamma_s^p \quad (1)$$

where the superscript d and p represents the dispersion and polar contribution of the total surface energy. The polar components can be further split up in to various independent components which may be given as

$$\gamma_s^p = \gamma_s^h + \gamma_s^i + \gamma_s^{ab} + \gamma_s^o \quad (2)$$

where γ_s^d, γ_s^p, γ_s^h, γ_s^i, γ_s^{ab} and γ_s^o are dispersion, polar, hydrogen, induction, acid-base and other components of solid surface energy respectively. The polar and dispersion component of the total surface energy of the PP films was calculated by the following relation [41,42]

$$\left[\frac{1+\cos\theta}{2}\right] \times \left[\frac{\gamma_l}{\sqrt{\gamma_l^d}}\right] = \sqrt{\gamma_s^p} \times \sqrt{\frac{\gamma_l^p}{\gamma_l^d}} + \sqrt{\gamma_s^d} \quad (3)$$

Eq. (3) is in the form of Y (LHS) = $m{\cdot}X$ (RHS) + C.

The plot of LHS vs RHS gave a straight line with intercept on Y-axis. Slope and intercept obtained from the plot were squared and added up to get the total surface energy. The results obtained by the above equations are highly reliable and it is based on the geometric mean approach. The added advantage of this method is that it gives polar and dispersion components of the total surface energy.

The changes in chemical composition of the surface modified PP films were analyzed by FTIR at a resolution of 4 cm^{-1} in the range of 700–4000 cm^{-1} and XPS (Omicron Surface Science Instruments with EAC2000-125 Energy Analyzer). For XPS, Monochromatic AlKα X-rays (1486.7 eV) were used. Typical operating conditions for the X-ray source were a 400-μm nominal X-ray spot size (full width at half-maximum) at 15 kV, 8.9 mA, and 124 W for both survey and high resolution spectra. The C1s and O1s envelopes were analyzed and peak-fitted using a combination of Gaussian and Lorentzian peak shapes obtained from XPSPEAK4.1 software package.

The surface topography of the untreated and surface modified PP films was assessed using AFM by Seiko Instruments Scanning force microscopy (AFM, Ben-Yuan, CSPM 4000). The AFM was operated in tapping mode with horizontal and vertical resolution of 0.26 nm and 0.10 nm respectively. The values of Ra and RMS are an average from five independent measurements on regions of 1 μm × 1 μm.

2.4. Platelet adhesion and protein adsorption tests

Platelet-rich plasma (PRP) was prepared by collecting human blood in plastic syringes containing anticoagulation agents. The blood was centrifuged at 1300 rpm for 10 min at 4 °C and the supernatant was collected. Polymer disks (15 mm in diameter prepared using punch) were washed with phosphate buffered saline (PBS) for 24 h and placed in the bottom of the wells of a multiwell tissue culture plate; the PBS solution was removed from the multiwell tissue culture plate by pipetting. PRP (1 ml) was then seeded and incubated at 37 °C for 30 min. After incubation, the disks were recovered and rinsed three times with PBS to remove any weakly adsorbed platelets. After fixation in 2.5% glutaraldehyde PBS solution, the morphology of the adsorbed platelets was observed using a scanning electron microscopy (SEM) (FEI-QUANTA FEG 450).

Human albumin and fibrinogen were used to study the adsorption behavior of proteins on the film surfaces. Polymer disks were immersed in 1 mg/ml protein solutions in phosphate-buffered saline (PBS, pH 7.3–7.4) at 37 °C for 1 h. The disks were then recovered, and changes in the protein concentrations of the solution were determined using a UV-spectrophotometer (at 280 nm).

3. Results and discussion

3.1. Functional group analysis

3.1.1. XPS results

The apparent functional changes induced by cold atmospheric pressure plasma assisted polymerization on the surface of PP films were further investigated and calculated by deconvolution of XPS C1s core level spectra (Fig. 2a–g) using Gaussian–Lorentzian non-linear curve fits by XPSPEAK4.1 software and the quantification of each functional group is given in Table 3. It was clearly exhibited that the major contribution of C1s core level spectra of untreated PP films was found at 285.0 eV which may be attributed to carbon singly bounded to carbon or hydrogen (C—C/C—H) and subsequently found minor contribution of the component C—O at 286.4 eV which is mainly caused by incorporation of oxygen atoms on the surface of PP films while cleaning the substrate with acetone and de-ionized water or adsorbed oxygen atoms from the surrounding (Fig. 2a). The C1s core level spectra of the argon plasma treated PP films also exhibit the same peaks at 285.0 eV and 286.4 eV. However the intensity of C—O was found to be increased whereas the component C—C got decreased. Besides, one new addition peak was found at 288.0 eV which may be assigned to C═O/O—C—O [32,43–47] (Fig. 2b). Hence the argon plasma treatment creates significant extent of polar functional groups on the surface of PP films. The changes may be due to argon plasma particles that have created vast amount of free radicals on the surface of PP films via abstraction of hydrogen bonds in the polymer network. The free radicals thus obtained are able to interact with oxygen atoms when oxygen gas was passed in the plasma chamber leading to the formation of oxygen containing polar groups on the surface of PP films which initiate polymerization process.

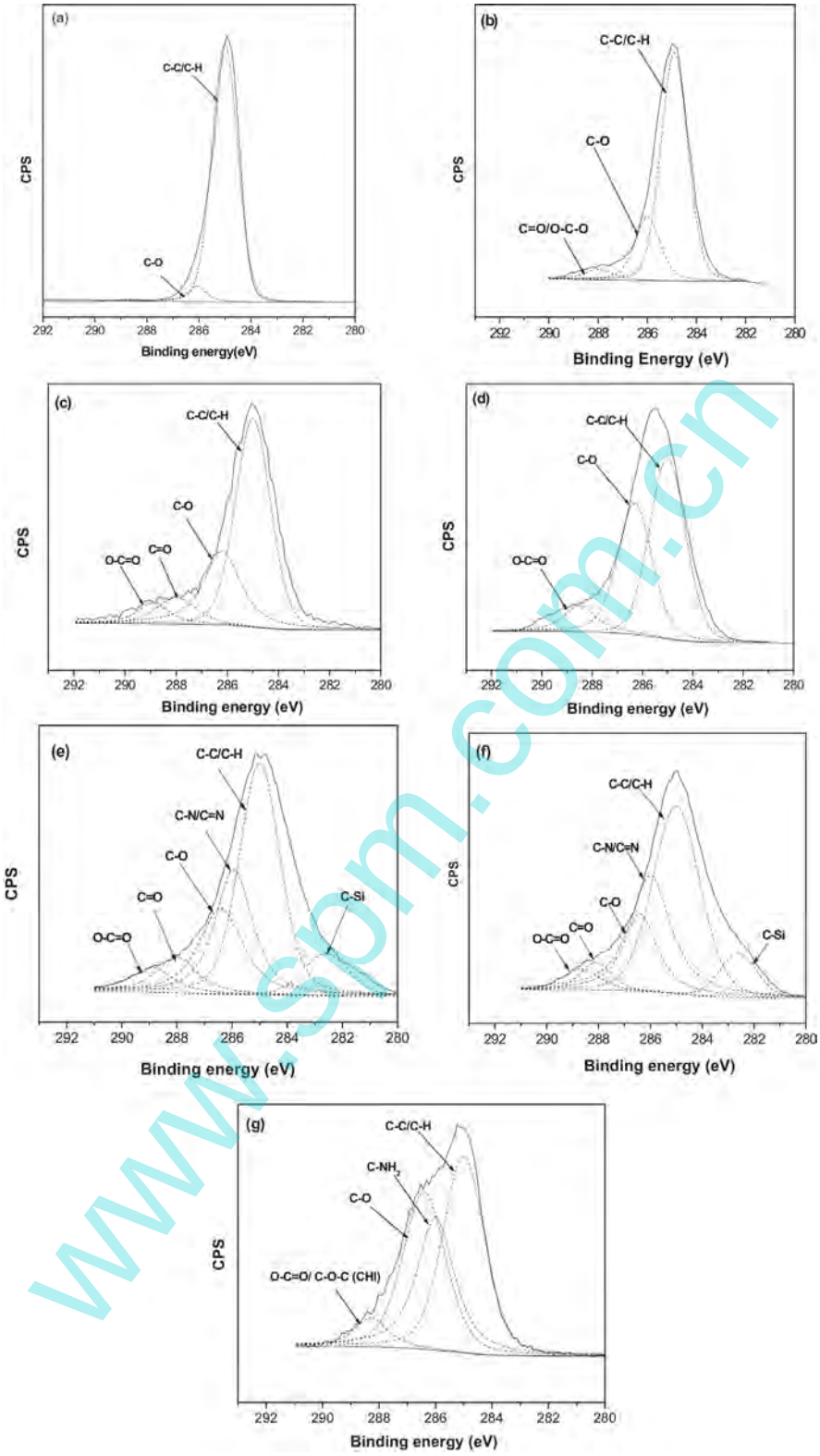

Fig. 2. High resolution C1s spectra of (a) untreated, (b) Ar plasma treated (c) AAc, (d) PEG, (e) HEP (f) INS and (g) CHI immobilized PP films.

After grafting AAc on the surface of such PP films in the plasma environment, one additional peak was observed at 288.8 eV which may be attributed to the formation of an ester or carboxylate groups. Also, AAc grafting increases the intensity of other existing peaks at 286.4 and 288 eV whereas significant attenuation of C—C/C—C (285 eV) components (Fig. 2c) was observed. Hence presence of carboxylate groups evidently confirms the successful grafting of acrylic acid on the surface of PP film. The high resolution spectra of PEG grafted AAc-PP films reveals only three major components at 285.0, 286.4 and 288.9 eV which may be attributed to presence of C—C, C—O and C═O groups respectively [31,32,47]. However, the component C—O majorly dominate the surface due

Table 3
Contribution C1s component of surface modified PP films.

Samples	% of C1s contribution in surface modified PP films					
	C—C/C—H (285.0 eV)	C—N (286.0 eV)	C—O (286.4 eV)	C=O (288.0 eV)	O—C=O (288.9 eV)	C—Si (282.6 eV)
PP	94.61	–	5.39	–	–	–
PP-Plasma	74.22	–	21.35	4.43	–	–
PP-AAc	57.08	–	27.27	9.14	6.51	–
PP-PEG	52.27	–	39.06	–	8.67	–
PP-Hep	38.24	24.40	18.34	5.16	7.28	6.58
PP-Ins	38.83	27.97	15.40	6.38	4.13	7.29
PP-CHI	37.07	26.8	31.04	5.09	–	–

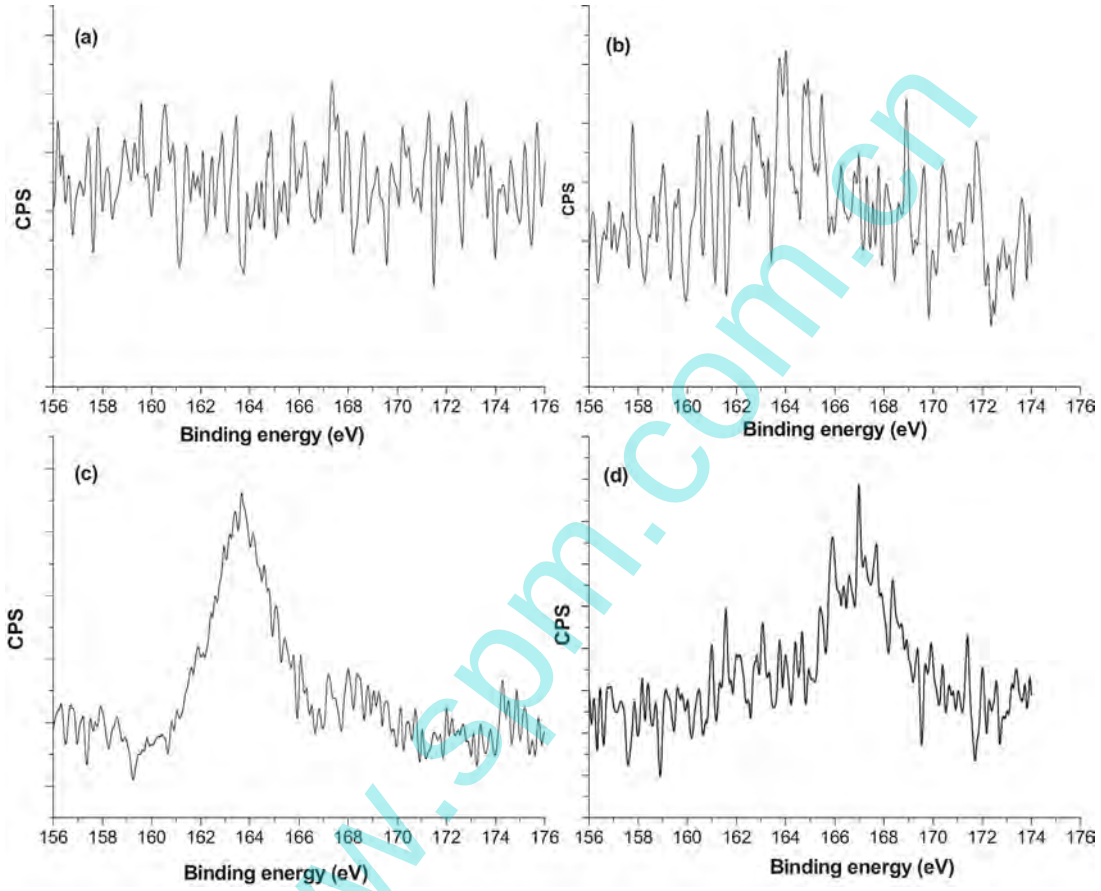

Fig. 3. Si2p spectra of the (a) untreated (b) PEG (c) HEP and (d) INS immobilized PP films.

to the incorporation of PEG molecules onto the PEG grafted AAc-PP film. Nevertheless, intensity of peak due to O—C=O increased slightly whereas peak due to C—C/C—H and C=O suppressed significantly compared with acrylic grafted surface (Fig. 2d). Heparin and insulin immobilized PEG-AAc-PP films reveals three additional peaks at 288.0, 286.0 and 282.6 eV which is assigned to C=O, C—N and C—Si respectively [47,48]. The presence of C—Si at 282.6 eV may be due to the incorporation of Si impurities on the surface of immobilized PP films from the plasma chamber and/or stored in the desiccator. The intensity of the peaks corresponding to C—O, O-C=O and C—C/C—H decreased slightly due to dominance of amide (C—N) bonds which clearly corroborated that the molecules of heparin and insulin are immobilized successfully onto the surface of PEG-AAc-PP films (Fig. 2e and f). The chitosan immobilized PEG-AAc-PP films reveals two additional peaks at 286.0 and 288.0 eV which can be assigned to C—N corresponding to chitosan molecules and C=O corresponding to anomeric carbon in the glucosic-like unit (Fig. 2g). However, the intensity of the peak at 286.4 eV (C—O) corresponding to polysaccharides such as cellulose and chitosan was found to be increased as compared to AAc and PEG grafted PP films. The relative intensity of the peak at 285.0 eV due to CH_3 group in the acetylated chitosan moieties and also to carbons in the PP underneath film decreased significantly compared with other surface modified PP films. This confirms that the chitosan molecules are successfully immobilized on the surface of PEG-AAc-PP films [47]. The conjugation of surface with insulin and heparin molecules exhibits the peaks at 163 eV and at 168 eV in the PEG-AAc-PP film surface which may be attributed to presence of disulfide groups (S—S) of insulin and sulphinate ($-SO_3Na$) groups of immobilized heparin (Fig. 3c and d). The expected peak due to immobilized biomolecules has not been detected in the untreated and other modified surfaces (Fig. 3a and b). The above results have confirmed the immobilization of molecules of heparin and insulin on the surface of PEG-AAc-PP films [47,48].

The quantification of C1s component of the unmodified and surface modified PP films is evaluated from C1s high resolution spectra (Table 3). It can be seen that a major contribution of the C1s component of the untreated PP film is due to C—C groups (94.61 at%)

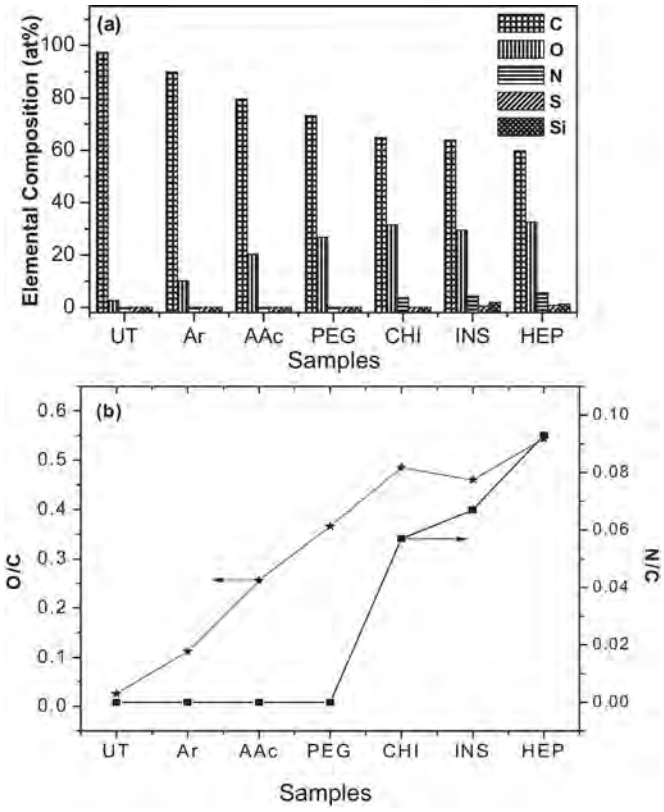

Fig. 4. (a) Elemental composition and (b) elemental ratio of the surface modified PP films.

and the remaining is due to C—O groups (5.39 at%). Subsequently the contribution of C—C decreased when the sample was treated by argon plasma and grafting of acrylic acid due to formation of new functional groups such as C—O, C=O,O—C=O on the surface of PP film. However, the functional group of C=O disappeared on the surface of PEG immobilized surface due to incorporation of C—O on the surface of AAc-PP films. The contribution of C—O, O—C=O and C—C/C—H got deceased on the surface of HEP or INS or CHI immobilized-PEG-AAc-PP films whereas incorporation of C—N and C=O groups was observed. Moreover the incorporation of the Si containing impurities (as indicated by C—Si peak) was observed for both insulin and heparin immobilized PP films. The above results clearly confirmed the modification of functional changes on the surface of PP films through atmospheric pressure assisted polymerization.

The elemental composition of the surface modified PP films was acquired from XPS wide scan spectra as shown in Fig. 4a. It clearly evident that the carbon component of the untreated PP film was decreased after the Ar plasma treatment and the same was further decreased in the order of UT > Ar > AAc > PEG > CHI > INS > HEP while incorporation of oxygen component on the surface of PP films. Meanwhile the nitrogen was perceived on the surface of biomolecule immobilized PP films which indicates existence of amino groups of immobilized biomolecules (Fig. 4a). Furthermore, we observed component of sulfur on the surface of HEP/INS-PP films which manifests for successful immobilization of the molecules of heparin and insulin on the surfaces of PEG-AAc-PP film and also found small amount of Si component on the surface of HEP/INS-PP films which may be due to incorporation of impurities from the plasma chamber and/or stored in the desiccator. Fig. 4b shows the O/C and N/C ratio of the surface modified PP films. It was found that the untreated PP films exhibit least value of O/C (%) due to residual amount of oxygen on the surface of untreated PP films and it was increased in the order of

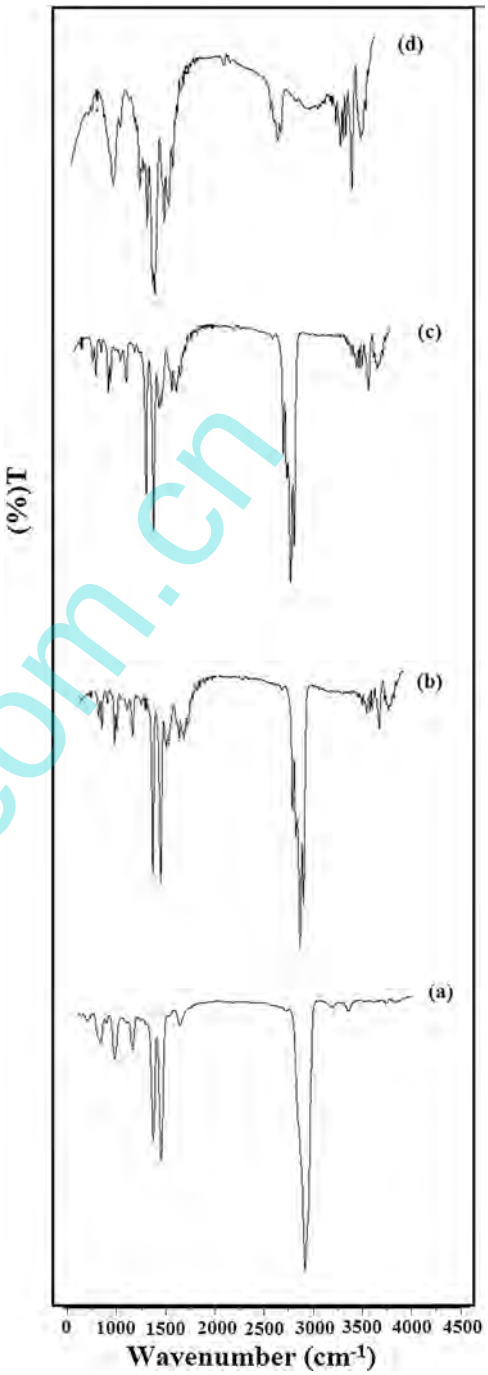

Fig. 5. FTIR spectra of (a) untreated (b) HEP (c) INS and (d) CHI immobilized PP films.

UT < Ar < AAc < PEG < CHI < INS < HEP. Furthermore, no N1s component was observed on untreated, Ar plasma treated, AAc and PEG grafted PP films surfaces. However the N/C ratio of the PP film increased due to immobilization of biomolecules and is in the order of CHI < INS < HEP. Hence the above results clearly confirmed that the introduction of oxygen and nitrogen content on the surface of modified PP films. Finally, the functional groups which are introduced by plasma assisted polymerization and immobilization have great potential to improve the hydrophilicity as well as hemocompatibility of the PP films.

3.1.2. FTIR results

The chemical structure of the surface modified PP films was examined by FTIR spectra as shown in Fig. 5. The spectra of

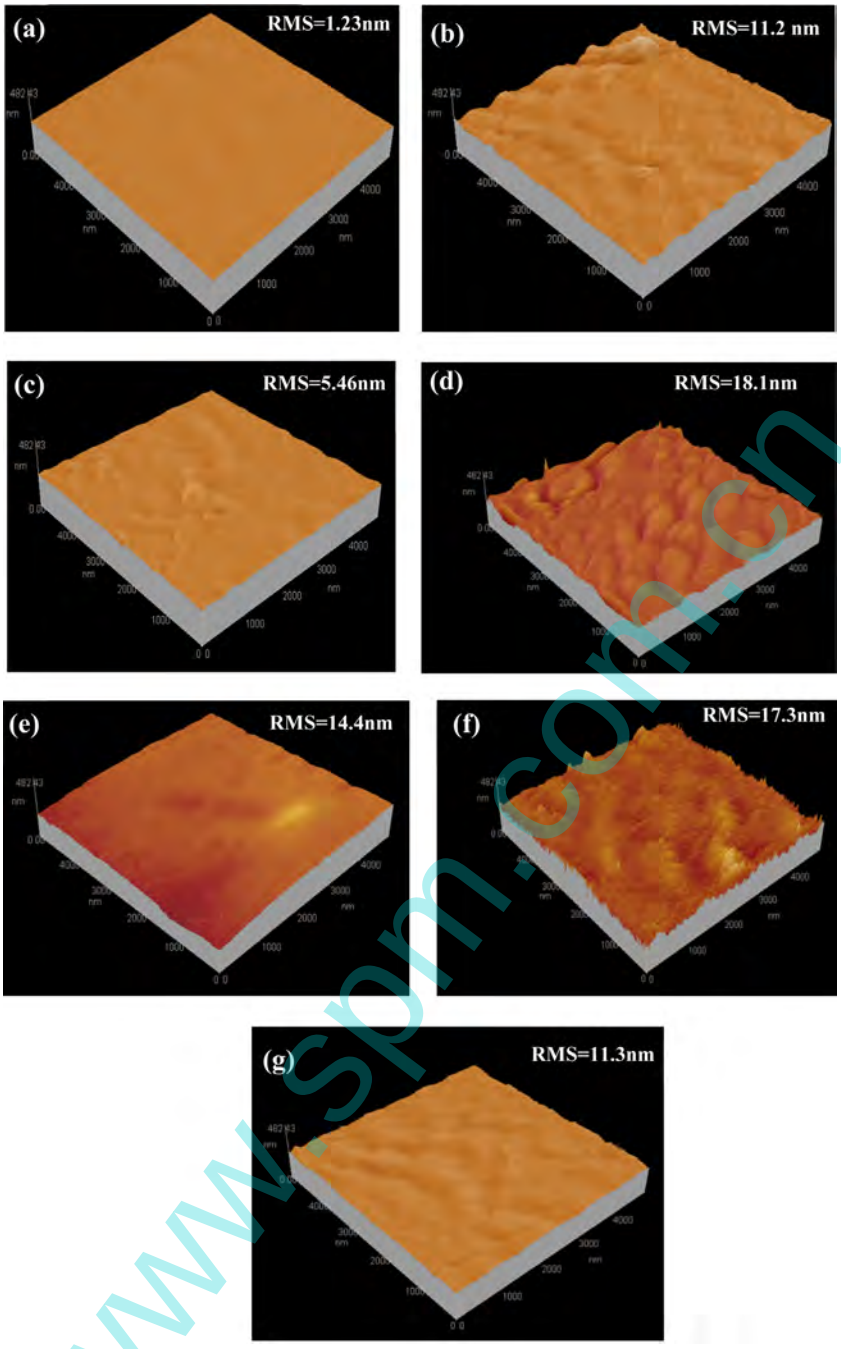

Fig. 6. AFM image of surface modified PP films (a) untreated (b) Ar plasma (c) AAc (d) PEG (e) HEP (c) INS and (e) CHI immobilized PP films.

the untreated PP film exhibits major peaks corresponding to asymmetric and symmetric stretching vibration of CH_3 and CH_2 groups around 2900 cm^{-1}. Also found two major peaks at 1460 and 1380 cm^{-1} attributed to asymmetric and symmetric bending vibration of CH_3 and CH_2 groups (Fig. 5a). The FTIR spectrum of heparin/insulin immobilized PEG-AAc-PP film also exhibits similar peaks; however, they exhibits various new peaks which may be attributed to presence of N—O symmetric/asymmetric stretching, C=O and OH stretching vibration. Among them, the presence of SO_3^- at 1250 and 1100 cm^{-1} in the saccharide group of heparin and NH—CO—NH at 1560 cm^{-1} in the amide groups of insulin confirms the immobilization of molecules of heparin and insulin on the surface of PEG-AAc-PP films (Fig. 5b and c) [34,37,43]. Furthermore, chitosan immobilized PEG-AAc-PP film reveals four new peaks at 1740, 1545, 1650 and 1091 cm^{-1} attributed to C=O stretching of the ester or in a carboxylate group, N—H bending in O=C—NH, C=O in OC—NH and C—N stretching vibration (Fig. 5d) [44,45]. Hence the occurrence of new peaks clearly corroborate the successful immobilization of molecules of heparin, insulin and chitosan on the PEG-AAc-PP film surface via covalent bonding. FTIR data is in good agreement with XPS results.

3.2. Topographical changes: AFM results

The mechanism of atmospheric pressure plasma assisted polymerization is based on the surface activation and fragmentation of monomer molecule through the interaction of charged species in the plasma environment and as a consequence it results in grafting of monomer along with specific functional groups onto the surface of polymeric films. This mechanism also modifies the surface

topography (roughness) which contributes to improve the surface properties such as hydrophilicity, adhesion etc. of the PP film. Hence, the extent of change in quantification of surface topography of the surface modified PP films was evaluated by AFM (Fig. 6a–g) and the same is given in terms of RMS roughness value. The surface of untreated PP films was relatively smooth with RMS surface roughness 1.23 nm (Fig. 6a). Consequently, the surface morphology of the PP film changed significantly (RMS value increased to 11.2 nm) by short duration (one minute) of argon plasma treatment, which may be due to the impact of heavy Ar ions on the surface of PP films (Fig. 6b). The surface of the AAc grafted PP films exhibits relatively flattened morphology (RMS = 5.46 nm), which may be due to filling in the asymmetries of the Ar plasma treated surface by plasma polymerization of AAc (Fig. 6c). The grafting of PEG on the surface of AAc-PP films led to the increase in the surface roughness substantially and the RMS value of the same is 18.1 nm which may be attributed to PEG molecules forming its own domains on the surface of PP films (Fig. 6d). However, the surface roughness of biomolecules (such as heparin, chitosan and insulin) immobilized PEG-AAc-PP films is relatively inferior to that of PEG-AAc -PP films which may due to homogenization of biomolecule content on the surface (Fig. 6e–g). The above morphological changes indicate successive grafting of AAc and PEG and immobilization of biomolecules onto the surface of PP films.

3.3. Hydrophilic analysis: contact angle and surface energy analysis

The precise information about the tailoring of hydrophilicity on the surface of modified PP films was evaluated by contact angle measurement using three testing liquids such as distilled water (W), formamide (F) and ethylene glycol (EG) which provide the information about the formation of extent of polar functional groups on the surface of polymer film. The variation in contact angle of surface modified films is shown in Fig. 7. It was found that the surface of the untreated PP films exhibits hydrophobic (θ = 92.2° for W, 87.34° for F and 83.4° for EG) nature due to dominance of C—H/C—C groups in the polymer network which considerably changed to hydrophilic one, i.e. decreased contact value when the sample was treated by Ar plasma. The hydrophilic modification induced by the plasma treatment is mainly due to an increase in effective area of contact and breaking of C—C/C—H bonds due to impact of plasma particles on the surface of PP films resulting in formation of free radicals on PP surface. The radicals which are formed by the atmospheric pressure argon plasma treatment interact with oxygen atoms (O_2 gas was purged into the chamber) which leads to the formation of polar functional groups such as C—O, C═O onto the film surface. The results obtained by the Ar plasma treatment are in support with the information obtained by the XPS. Subsequently the contact angle values were further decreased by grafting of AAc and PEG on the surface of PP films. However, the value of contact angle was decreased drastically by the immobilization of molecules of INS, HEP and CHI which may attributed to incorporation of C—O, C═O, C—N and O—C═O groups on the surface of PEG-AAc-PP films. The increase in hydrophilicity of the PP films can be related to the tailoring of morphological and chemical properties at the interface. Finally the hydrophilicity of the PP films is not related to the materials bulk; nevertheless it is dependent on the specific functional groups on the surface of the materials.

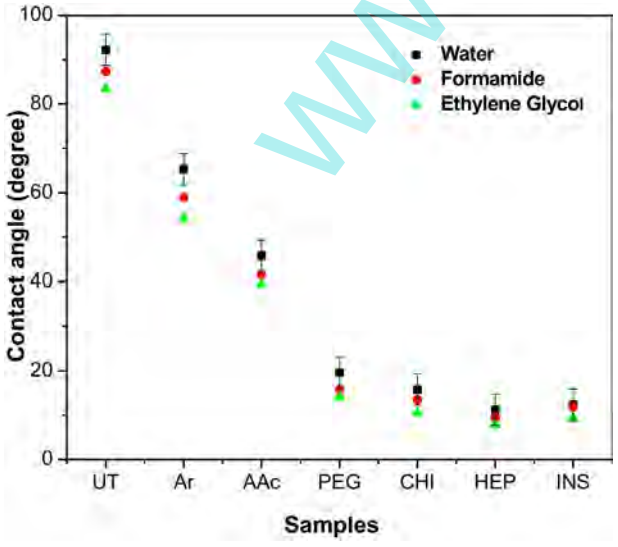

Fig. 7. Contact angle of the surface modified PP films.

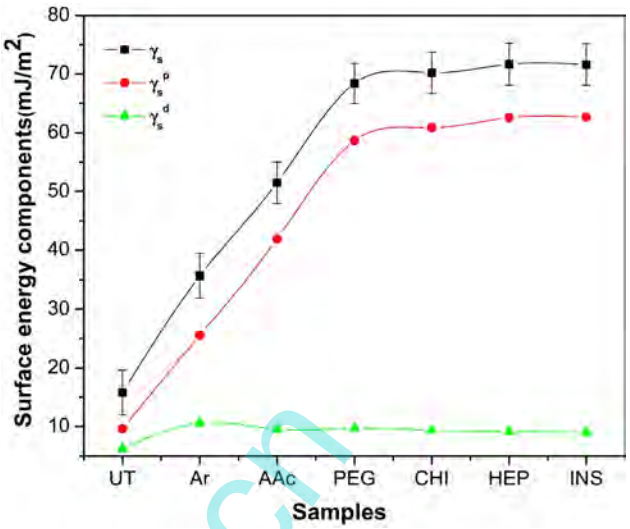

Fig. 8. Surface energy components of the surface modified PP films.

Estimation of solid surface free energy of the material is important from biomedical application point of view. The existence of unbalanced intermolecular forces such as dipole–dipole interactions and hydrogen bonding among the molecules at the interface is the origin of surface energy. Moreover the solid surface energy is one of the thermodynamical quantities which is

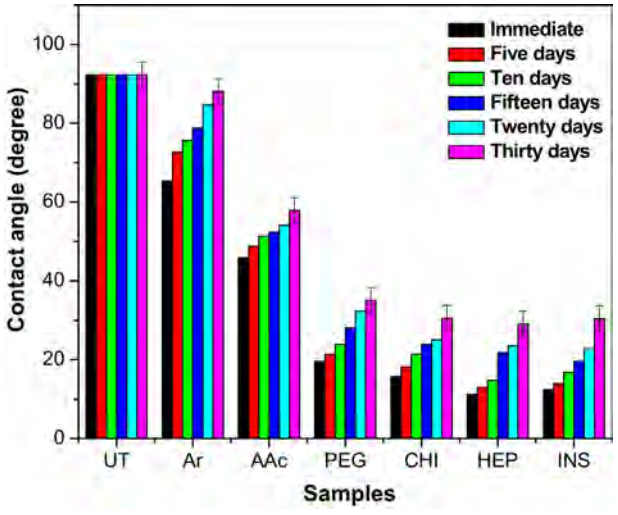

Fig. 9. Estimation of the hydrophobic recovery of the surface modified PP film.

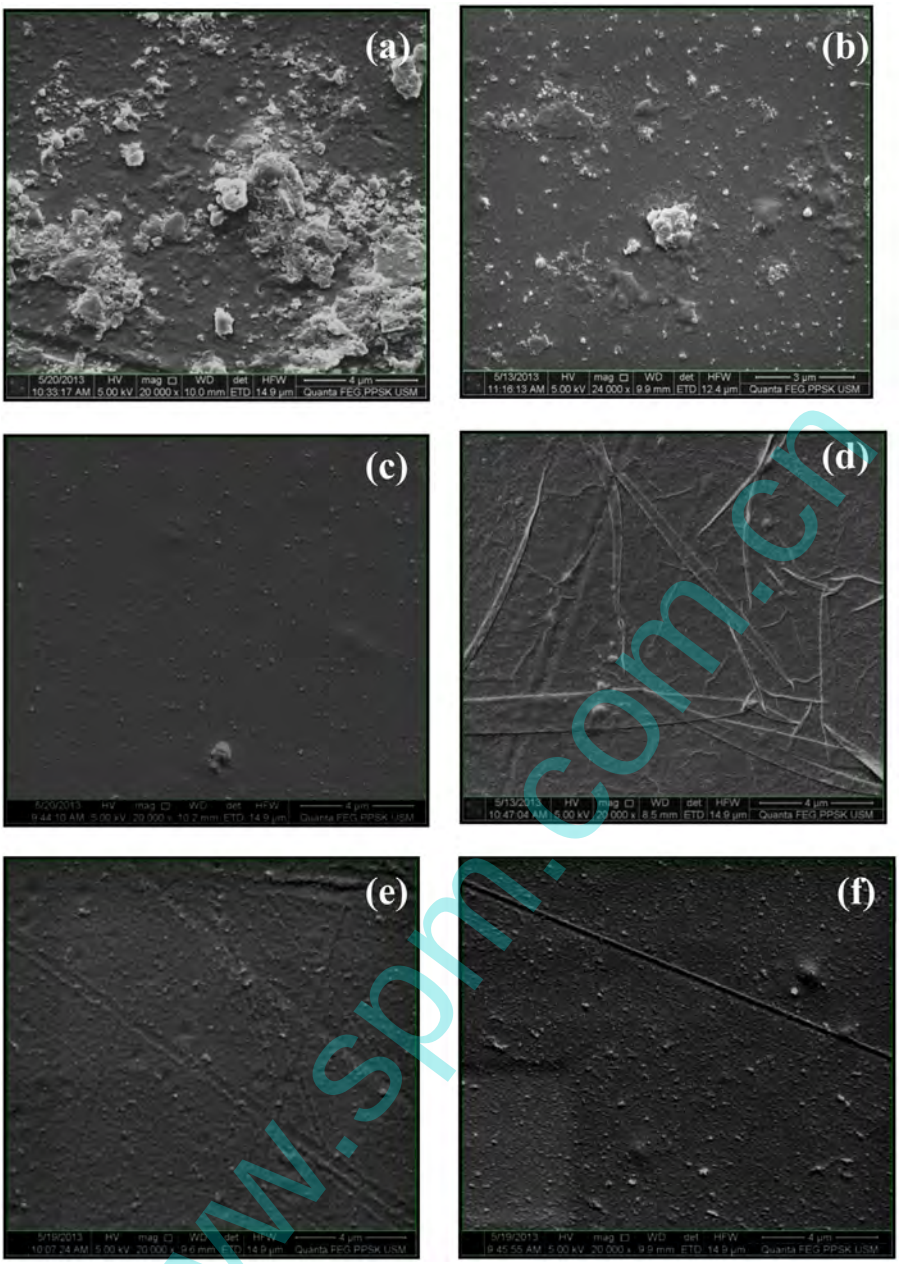

Fig. 10. Morphology of the platelet on the surface PP films (a) untreated (b) Ar plasma treated (c) PEG (d) HEP (e) INS and (f) CHI immobilized.

a measure of hydrophilicity/hydrophobicity of the surface and influencing many technological applications. The surface energy of plasma processed PP films was estimated by Fowke's approximation extended by Ownes and Wendt. Fig. 8 exhibits the changes in surface energy of the PP film by plasma assisted polymerization.

Fig. 8 shows the surface energy of modified PP films and it was found that the surface energy of the untreated PP films was just 15.8 mJ/m^2 and it increased significantly after Ar plasma treatment followed by exposure to the oxygen gas, which results in the formation polar functional groups such as C—O, C═O on the PP films. After that the surface energy of PP films increased further by the grafting of AAc and PEG. However, marginal changes were observed in the surface energy of PP films when immobilized by biomolecules of HEP, CHI and INS. Similar effect was observed for the polar components of the surface modified PP films. Furthermore, dispersion component of the PP film remained almost constant. Hence, the increase in total surface energy is mainly due to the incorporation of polar components such as C—O, C═O, C—N and O—C═O and also significant morphological changes on the surface of modified PP films. According to the Wenzel, surface roughness is one of the important factors to facilitate to improve the surface energy (decease in CA) of the materials due to increase in the effective area for contact, thus increasing the hydrophilicity of the modified surfaces [40].

The improvement in surface energy and decrease in contact angle of the PP film was mainly due to the formation of hydrophilic functional groups which play a vital role to improve biocompatibility. The incorporation of hydrophilic groups onto the PP films contributes an inferior interfacial tension with blood components. Hence such a material can resist the adhesion and activation of platelets and suppression of adsorption of plasma protein on the surface of PP films when come in contact with blood.

3.4. Investigation of durability: ageing results

Owing to plasma assisted polymerization, high density of polar functional groups are incorporated on the surface of PP films. Nevertheless, the material may lose some of the polar functional groups obtained by the plasma processing during the ageing process. The instability of the plasma effect on the surface of PP films is mainly due to reorientation of polar functional groups in the material bulk and mobility of small polymer chain segment into matrix. Moreover the reorientation of the polar functional groups (ageing) in to the bulk may be hindered due to cross-linking or presence of voluminous of hydrophilic groups on the material surfaces. Hence durability of the plasma effect on the surface of materials is one of the important factors in determining their long term performance *in vivo* [49–51].

Fig. 9 shows the stability of plasma effect on the surface of PP films which is evaluated by the measuring contact angle with respect to water for different storage time (0–30 days). As it can be seen that the Ar plasma treatment reduces the contact angle of untreated PP films from 92.2° to 65.3°. The lower contact angle value obtained by the Ar plasma treatment starts to increase with time due to its ageing effect (hydrophobic recovery). Thus, it is suggested that the Ar plasma treated PP films should be immediately used for the subsequent reaction such as plasma polymerization and immobilization of biomolecules for avoiding ageing effect. Furthermore the AAc and PEG grafted and molecules of CHI, INS and HEP immobilized PP films show relatively less ageing effect during their storage time of 30 days which may be due to functional groups produced by the atmospheric pressure plasma assisted polymerization are efficiently cross linked with the surface of Ar plasma treated PP films which rescue migration of polar segment into the material bulk, leading to retaining surface hydrophilicity. Hence, the atmospheric pressure plasma assisted plasma technology can be more suited to biomedical industry to produce voluminous biomedical material [52–55].

3.5. In vitro *analysis: platelet adhesion and protein adsorption results*

The interaction between artificial material and platelets is an incredibly intricate sequence of dynamic action. The aggregation of platelets and formation of thrombus subsequently causes potential hazard *in vivo*. Hence, the investigation of adhesion and activation of the platelets on the surface is one of the important features in determining the materials blood compatibility. Fig. 10 shows SEM images of untreated and surface modified PP films after being in contact with platelet rich plasma prepared from whole human blood. Adhesion, accumulation and activation of platelets on the surface of untreated PP films were found because of strong adsorption of plasma protein due to its poor surface properties. However the adhesion and activation of platelet are reduced significantly by the grafting of AAc and PEG on the surface of PP films. Subsequently, there is no sign of adhesion and activation of platelets on the surface of HEP, INS and CHI immobilized PP films. Furthermore, quantitative adherence of platelets study indicates that there are 52 platelets (approx) adhered on the surface of 1 μm^2 area of untreated PP (Fig. 11).

The aggregation of platelets decreased slightly after Ar plasma treatment and decreased markedly by the grafting of AAc and PEG. Moreover, the molecules of CHI, INS and HEP immobilized surface suppressed platelets aggregation/adhesion up to 96% compared with untreated PP film. The above anti-platelet adhesion properties may be due to the incorporation of specific functional groups onto the surface of PP films through immobilization of biomolecules which inhibit adsorption of plasma proteins leading to resist adhesion and activation of platelets on the surface of polymeric films.

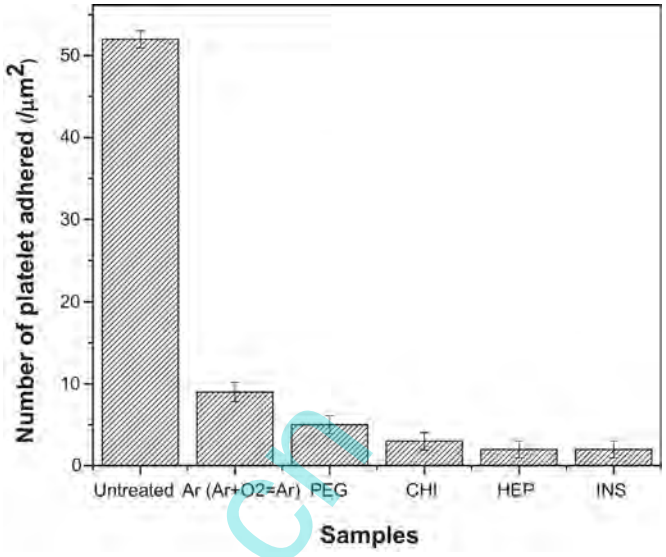

Fig. 11. Quantitative of adherence of platelets on the surface of modified PP films.

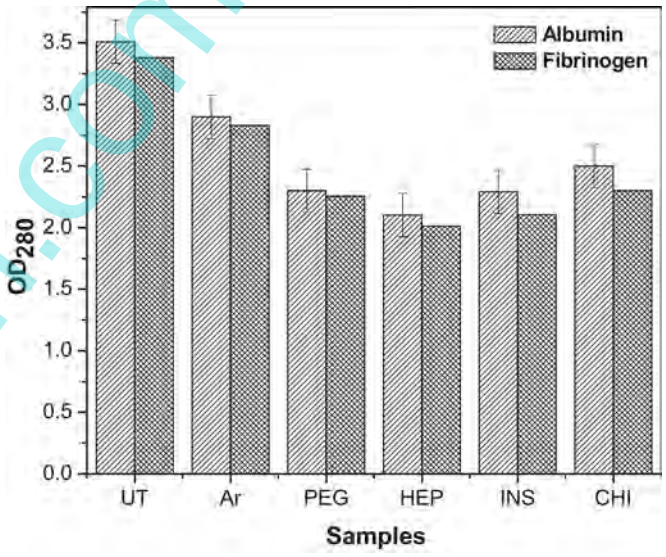

Fig. 12. Adsorption of plasma proteins on the surface of modified PP films.

Estimation of adsorption of human blood protein such as albumin and fibrinogen on the surface of polymeric material is one of the imperative key factors to reveal the materials blood compatibility. Fig. 12 exhibits the adsorption of albumin and fibrinogen onto the surface of untreated and surface modified PP films which is evaluated by optical density value at 280 nm (OD_{280}) and the magnitude of the same is proportional to the adsorbed amount protein on the surface of PP films. It is clearly seen that OD_{280} (absorbance) values of albumin and fibrinogen of the unmodified PP film are 3.51 and 3.38, respectively. However the surface modified PP film films exhibit marked anti-adsorption behavior, i.e. decreased OD_{280} value. Hence the above results clearly reveal that the surface modified PP films suppressed the adsorption of human blood proteins. Moreover, the information obtained through the protein adsorption and platelet adhesion study can be helpful to improve the anticoagulation properties of the materials when come in contact with blood [56–58].

4. Conclusion

The efficacy of anti-thrombogenic properties of the PP films are enriched by cold atmospheric pressure assisted

polymerization technique using AAc and PEG as a precursor in vapour phase and also successful immobilization of biomolecules of heparin, chitosan and insulin. Due to plasma polymerization and successful immobilization, the functional groups such as C═O, —O—C═O, C—N and S—S are incorporated on the surface of PP films which lead to improved hydrophilicity (lower contact angle value and higher surface free energy) of the PP films. The FTIR and XPS results clearly reveal the incorporation of higher concentration of oxygen containing functional groups on the surface of AAc and PEG grafted and biomolecules immobilized PP films; while decreasing carbon content. The identification of new elements such as nitrogen and sulfide on surface of modified PP films confirms the successful immobilization of HEP, INS and CHI. Moreover, the high resolution XPS spectra clearly identified the formation of high dense of above mentioned polar functional groups on the surface of modified PP films. The AFM results clearly showed different surface morphology with respected grafting of AAc and PEG as well as immobilization biomolecules. The above results clearly confirmed the successful grafting and functionalization of precursors (AAc and PEG) and immobilization of biomolecules (heparin, chitosan and insulin). Finally the *in vitro* blood compatibility analysis clearly demonstrated that the significant eradication of adhesion, activation of platelets and adsorption of plasma proteins (albumin and fibrinogen) takes place which is mainly caused by significant changes in physico-chemical properties obtained by atmospheric pressure plasma assisted polymerization and immobilization. Therefore, we suggest that this novel low cost cold atmospheric pressure plasma assisted polymerization technique has prospective for production of various biomedical materials.

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