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BIO-FUNCTIONALIZATION OF PET SURFACE VIA A FACILE CHEMICAL METHOD

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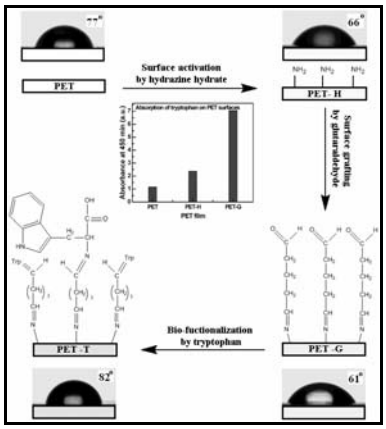
A simple approach has been adopted to chemically modify the surface of PET samples to serve as biofunctional support. The modification procedure is comprised of two main steps, first is activation of the surface using hydrazine hydrate and then grafting the activated surface with glutaraldehyde solution. Subsequently, the grafted surface has been treated with tryptophan to examine its adhesion property. Characterization of the PET surface at each step of the chemical modifications by means of FTIR, XPS and contact angle measurements confirms the successful modification and adhesion of the surface. Additionally, the bio-functionalization ability has been investigated by absorption measurements of a biological solution on the grafted sample. These have revealed enhanced adhesion properties after grafting, which confirms the bio-functionalization of the modified surface.



INTRODUCTION

Polyethylene terephthalate (PET) is a typical member of the aromatic polyester family, frequently used in many industrial applications, *e.g.* in the automotive industry, in bottles and containers, in textile and carpet applications and in the electrical industries.[1] It has a set of excellent properties, such as glass-like transparency, reasonable thermal stability, high strength, resistance to chemicals, good barrier ability, crease resistance, relatively low cost of production and so on.[2] Because of its biocompatibility and good mechanical properties, PET has found new developments in the field of medical devices, such as surgical suture material, tendon and ligament replacement material, adipose tissue-engineered construct, drug/cell delivery system, and cell culturing support.[3,4] An important issue relevant to many of these applications is to control the wetting of polymer surface. For instance, the poor surface adhesion to other materials may result in the difficulty in depositing surface coatings, printings, dying, as well as poor protein absorption, platelet adhesion and cell attachment in biomedical applications. The wetting properties of polymers surface crucially depend on the chemical sites. The hydrophilic surface is a high energy surface that has high polar components with polar groups such as –OH, –COOH. Conversely, the hydrophobic surface is a low energy surface with non-polar chemical sites, which has no possibility for establishing physicochemical interactions with the environment (–C–H and C–C). Virtually, PET has

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no chemical groups such as the hydroxyl and carboxyl (but only a very small number of terminal hydroxyls and carboxyls). Hence, surface energy of PET shows insufficient properties and creation of these groups on its surface is necessary for certain applications. Various surface modification methods and techniques can utilized for this purpose, such as chemical treatment, plasma, UV light and ion implantation.[5,6] Surface chemical treatment is considered as one of the best methods that can be utilized to enhance the surface properties, as it allows modification of the surface characteristics to both obtain better adhesion (wettability) and alter the biomedical compatibility without affecting bulk properties of the polymer.[7-9] Primary amine groups are often introduced by reaction of an organic amine agent with the ester bonds along a polymer chain. The most often used amines is hydrazine.[10] Functional groups created during modification processes can serve as anchor sites for covalent immobilization of various biomolecules. Glutaraldehyde is the most popular bis-aldehyde homobifunctional crosslinker in use today, which presents two carbonyl groups at both ends. Glutaraldehyde has been used extensively as a grafting reagent, especially for antibody–enzyme conjugations.[11] Peptides, enzyme and proteins have been successfully bonded to modified PET.[12,13] The activation and grafting methods include reaction of PET with amine, hydroxyl, carbonyl or carboxyl groups, thus incorporating corresponding functionalities onto the surface. Such action increases the hydrophilicity of the polymer and creates the anchor functionalities for subsequent reactions.[14]

In this study, PET surface has been modified by wet chemistry, firstly by an activation method based on the reaction of PET surface with hydrazine hydrate. Then, the activated surface was grafted using glutaraldehyde solution. The adhesion property and bio-functionalization of the modified surface were investigated by the reaction with both L-tryptophan solution (one of the standard amino acids and an essential amino acid in the human diet, a protein building block that can be found in many plant and animal proteins[13]) and a biological solution. The changes in chemical bonding and wetting of PET surface have been monitored by Fourier transform infrared spectroscopy (FTIR) and a water contact angle measurements respectively. The elemental composition and chemical states of the PET surface have been investigated by means of X-ray photoelectron technique (XPS).

EXPERMENTAL

Samples of PET films (1 x 3 cm) were washed in an ultrasound bath successively in water then dried in air at the room temperature. Hydrazine monohydrate NH_2-NH_2.H_2O and glutaraldehyde $CH_2(CH_2$-$CH=O)_2$ were obtained from Sigma-Aldrich. L-Tryptophan $C_{11}H_{12}N_2O_2$ (amino acid) was obtained from Merck. FTIR spectra were acquired by Thermo Nicolet 6700 spectrophotometer, operating in attenuated total reflectance (ATR) mode with a diamond crystal for analyzing the external surface of the film (the film was pressed on the crystal). The wavelength range was from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} and 20 scans.

The contact angle measurements were obtained using OCA 15 plus, Data Physics Instrument GmbH, equipped with a video CCD-camera and SCA 20 software. The measurements were performed by using the sessile drop method with 3 µL drops of double distilled water. The digital image was processed using the drop shape analysis system (± 0.1°). Each measurement was repeated five times and the average value was considered. The elemental and chemical compositions of the films have been measured by X-ray photoelectron technique. The XPS analyses were performed using the SPECS system with a hemispherical energy analyzer. The monochromated Al K_α X-ray (1486.6 eV) was used as the excitation source and operated at 250 W. The binding energies were calculated with respect to the C-(C, H) component of the C 1s peak at 284.7 eV. High resolution spectra were treated and deconvoluted with CasaXPS version 2.3.16Dev52, using a non-linear least-squares method with a Gaussian/Lorentzian peak shape GL(30) and the background was subtracted using the Shirley method.

1. Surface activation

PET films were washed several times with distilled water to remove any impurities during 15 min before use. The samples were placed in a 24-well polystyrene plate and immersed in a freshly prepared solution of hydrazine hydrate. The samples were left for 24 h at the ambient temperature, which was 37 °C. Then, the activation solution was removed by suction; subsequently the samples were washed with distilled water. The obtained activated samples were dried overnight at room temperature, and then used for the grafting reactions. These activated samples were labelled as PET-H (Fig. 1).

2. Surface grafting

The activated samples (PET-H), placed in a polystyrene plate, were incubated for 24 h at 40 ºC with a solution of glutaraldehyde. The solutions were removed by suction and the samples were washed well with distilled water. The samples were dried for 24 h at room temperature. These samples were labelled as PET-G (Fig. 1).

3. Surface treatment

The efficiency of the modified surface was tested with tryptophan. An aqueous solution of 5 mmol/L tryptophan was prepared (204 mg L-Tryptophan to 200 mL distilled water). PET-G samples were incubated into this tryptophan solution for 24 h at 30 ºC. The solutions were removed. Then, these samples (labelled as PET-T, Fig. 1) were washed with distilled water, and dried at room temperature.

4. Bio-functionalization test

The functionalized surface was further used to immobilize various biomolecules, such as proteins, on the PET samples. The PET films (PET, PET-H, PET-G) were incubated with biological solution comprises 0.1 mg/mL litmus milk in 3 % phosphate-buffered saline solution (PBS) containing 30 mg bovine serum albumin. After 60 min, the films were washed 3 times with phosphate buffered saline tween (PBST). The films were left for 1 hour in 150 µL Goat Anti Human HRP, these films were washed 3 times with PBST. Finally, for each sample, 150 µL 3,3′,5,5′-tetramethylbenzidine was added as indicator (The color of the solution turns to blue after about 10 minutes). Sulfuric acid solution (0.5 N) was used to inhibit reaction. Absorbance measurements were performed using a Thermo Labsystems Multiskan EX device at 450 nm.

RESULTS AND DISCUSSION

1. FTIR

Figure 2 shows the FTIR spectra of polyethylene terephthalate film before treatment (PET) and after consecutive treatments with hydrazine monohydrate (PET-H), glutaraldehyde (PET-G) and tryptophan (PET-T). The FTIR spectrum of PET film exhibits the expected IR bands the expected characteristic IR band of PET film at around 872 cm^{-1}, 1080 cm^{-1}, 1232 cm^{-1}, 1408 cm^{-1}, 1707 cm^{-1} and 2889 cm^{-1}-2958 cm^{-1} due to C = H bending, out of plane C–H bending, in plane C–H bending, aromatic skeleton stretching, C=O stretching and aliphatic CH_2 – stretching in PET respectively.[15]

The FTIR spectrum of yellow precipitate (PET-H) shows two additional IR transmittance bands at around 1618 cm^{-1} and 3315 cm^{-1} that are attributed to N-H bend and N-H stretch respectively, and it is characteristic of primary amides. This result confirms the activation of surface with hydrazine as shown in Figure 1.

After grafting the PET-H surface with glutaraldehyde, two peaks associated with C-H stretch of the CH=O group in aldehydes were shown (Figure 2, b). One near 2727 cm^{-1}, the other around 2866 cm^{-1}. This gives the evidence of grafting of glutaraldehyde on the surface (PET-G). After surface treatment with tryptophan, the relative intensity of N-H bend at around 1618 cm^{-1} is significantly enhanced. In addition, IR spectrum of PET-T compound revealed two bands between 3500-3200 cm^{-1} correspond with N-H stretching vibrations for tryptophan.[16] The measurements have been repeated after a long time of treatment (about 6 months), indicating the stability of the treated surface. FTIR results indicate the efficiency of adopted method in modification and bio-functionalization of the PET surface, which will be also confirmed using XPS.

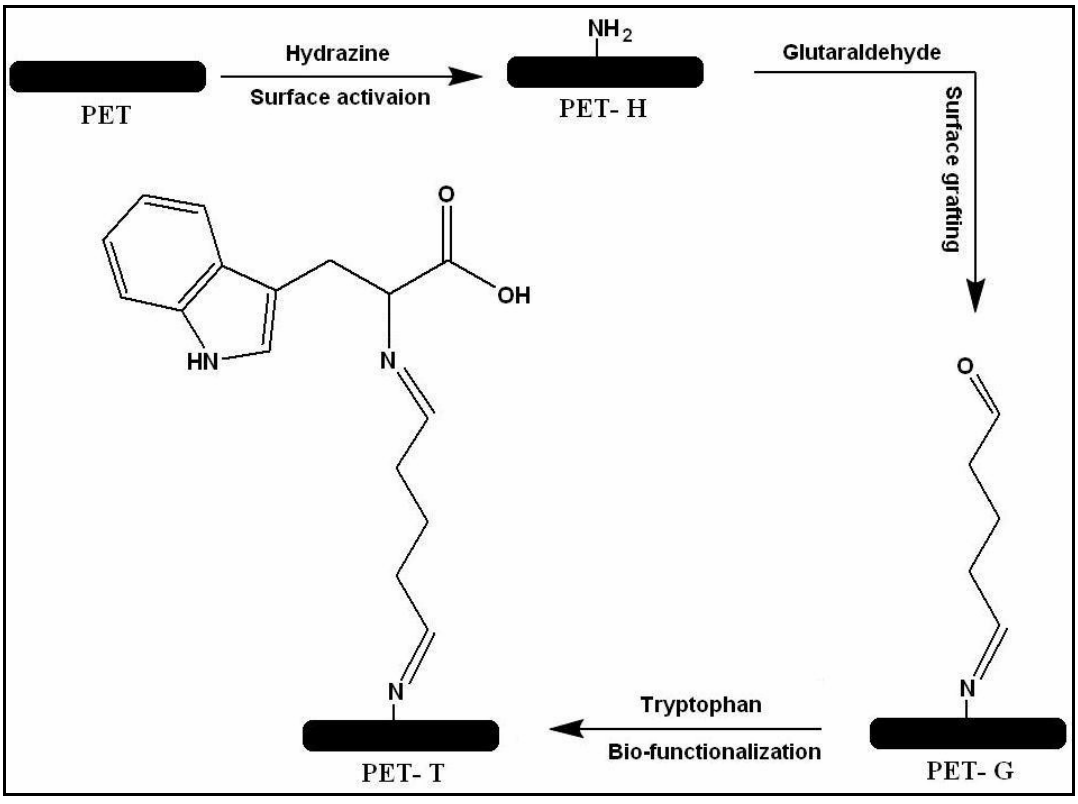

Fig. 1 – Drawing presents different PET films (PET, PET-H, PET-G and PET-T).

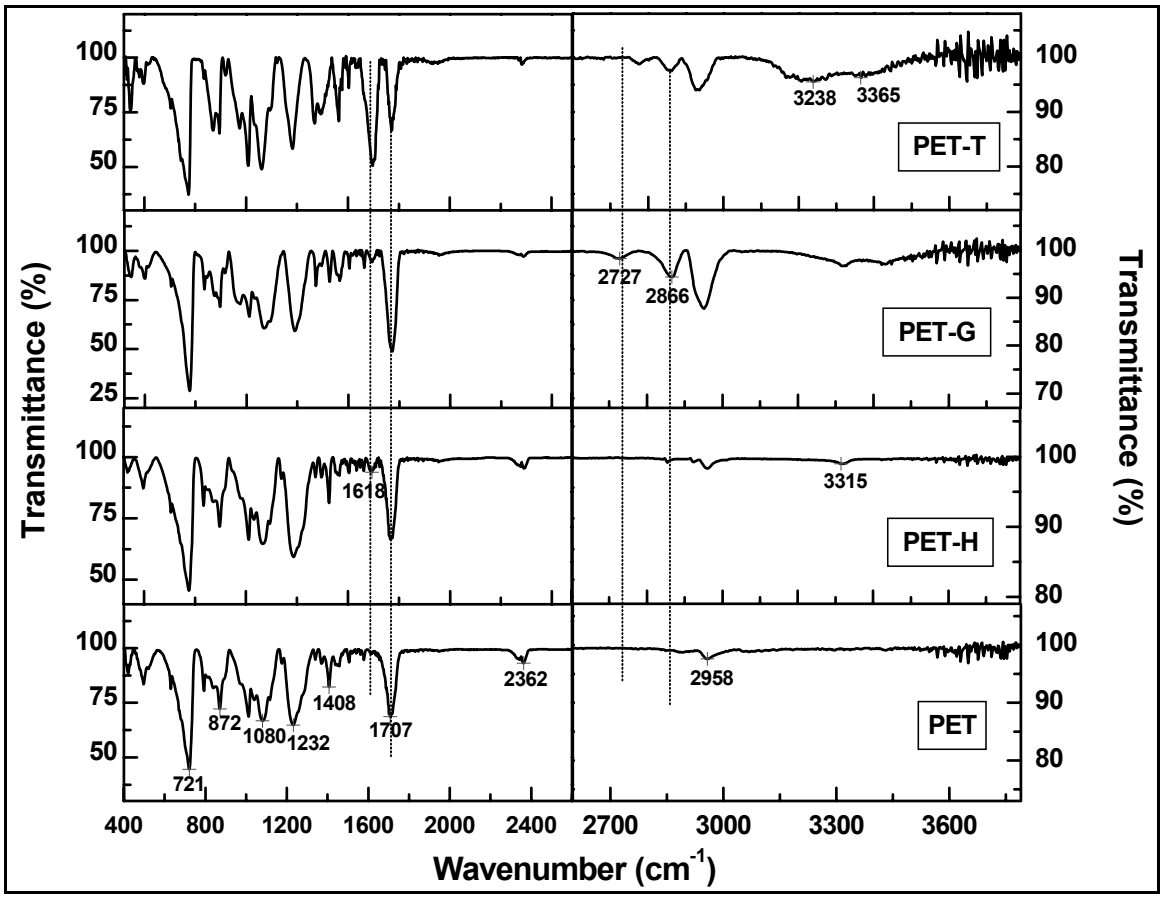

Fig. 2 – FTIR spectra of PET films (PET, PET-H, PET-G and PET-T).

2. Contact angle

Table 1 shows the water contact angle of surface before and after modification. The contact angle value (CA) of the non-treated PET was about 77º. In comparison with PET, contact angle of the activated sample (PET-H) was decreased by about 11º. This indicates that the surface has become more hydrophilic. This result may be correlated to the presence of N-H polar groups on the surface. Upon grafting of the sample surface (PET-G), the lowest contact angle values were observed, where CA decreased by 13º compared to non-treated film, due to the increase of polar groups (O functionalities) on the grafted surface. Therefore, the surface energy and hydrophilicity of PET surface turn into higher values. Thus, the surface becomes more effective to reaction and adhesion process. However, CA value increases after treatment of the surface with tryptophan, which indicates a decrease of the surface hydrophilicity. This increase of CA could be attributed to the presence of a hydrophobic aromatic ring in tryptophan.

Table 1

Evaluation of contact angle for PET surfaces.

Sample	CA (degree)
PET	77.0 ± 3.8
PET-H	65.8 ± 3.1
PET-G	61.2 ± 2.5
PET-T	81.9 ± 3.8

3. XPS analysis

The atomic and chemical compositions of the samples were studied by XPS. The survey spectra showed the presence of the following elements, carbon (C1s at 285 eV), oxygen (O1s at 532 eV) and nitrogen (N1s at 400 eV) [16]. The elemental composition of the studied samples surfaces has been measured and the C/O atomic ratio were calculated and presented in table 2.

The C and O contents of non-treated PET were found to be 77 and 23 % at. Conc., respectively (C/O is 3.4). After surface activation with hydrazine (PET-H), the nitrogen atoms were created on the surface and the C/O ratio was slightly decreased. The presence of N atoms leads to an increase of polar groups (N-H) of the surface. This confirms the activation process and augmentation of hydrophilicity of surface that has been shown by previous techniques (FTIR and CA).

Following grafting, the decrease of N %, for PET-G sample was expected, due to the liaison of glutaraldehyde on the surface (Table 2). Also, this bond increases the oxygen amount. Consequently, the decreasing of C/O ratio on the surface suggest increase of polar group after surface grafting.

The adhesion of L-tryptophan was investigated by XPS analysis for PET-T. The increasing of nitrogen atoms on the surface (up to 6.7 %) is an indicator to the reaction with tryptophan that contains N atoms. The drastic decrease of oxygen atoms (polar groups) and the subsequent augmentation of C/O ratio should explain the increased CA value (Table 1) and decreased hydrophilicity.

The evaluation of chemical state of the polymers surfaces has been studied by analyzing high-resolution scan of the C1s peaks. The deconvolution of C1s spectrum of the non-treated sample gives three components (Figure 3, PET), that correspond to 284.7 eV, 286.2 eV, and 288.6 eV, which may be assigned to –C–C– (or C–H), –C–O (or C–OH) and O=C– (or COOH), respectively[17, 18, 19]

The surface activation, as shown in Figure 3 (PET-H), led to considered changes in the C1s spectrum. C-C peak at 284.7 eV decreases and new peak at 285.5 eV that might attribute to C-N component has appeared. These results indicate that some of the C–C bonds in polymer surface may be broken by the activation, and the broken C–C bonds recombine with nitrogen atoms, forming nitrogen groups on the PET film surface. This finding explains clearly the reason of the increase of the wettability discussed above.

Figure 3 (PET-G) demonstrates a successful grafting of the surfaces by glutaraldehyde. The large increase of C=O component indicate dominant presence of carbonyl groups on the surface. The C=N component at 287.0 eV emerged as a result of reaction between the carbonyl groups of glutaraldehyde and N components on the PET-H surface. The decrease of the nitrogen percentage is attributed due to the deposition of glutaraldehyde on the PET-H surface layer. The incorporation of new oxygen bonds onto the surface leads to the formation of additional functional groups. These polar groups contribute to increase the hydrophilicity of the PET-G film. This modification can lead to the creation of anchor functionalities for subsequent reactions.

The high-resolution C1s spectrum of PET-T (Fig. 3) confirms the surface treatment by tryptophan. The increase of C-C, C-N and C=N components suggests adhesion of the tryptophan on the surface.

It is noteworthy to say that the composition and chemical components of the samples, investigated by XPS, are well correlated with FTIR and contact angle measurements.

To demonstrate the stability of the surface of grafted samples with the time, FTIR, CA and XPS measurements were repeated after three months of storage. The obtained results revealed stable modified surfaces.

Based on the above results, reactions that have possibly occurred during the PET surface modification are depicted in Fig. 4.

Table 2

Percentage atomic concentration of elements and C/O ratio for samples surfaces

Sample	C %	O %	N %	C/O
PET	77.3 ± 0.5	22.7 ± 0.5	-	3.4
PET-H	74.2 ± 0.4	22.9 ± 0.8	3.0 ± 0.4	3.2
PET-G	72.8 ± 0.8	26.4 ± 0.9	0.8 ± 0.3	2.8
PET-T	79.3 ± 0.9	14.1 ± 1.0	6.7 ± 0.5	5.6

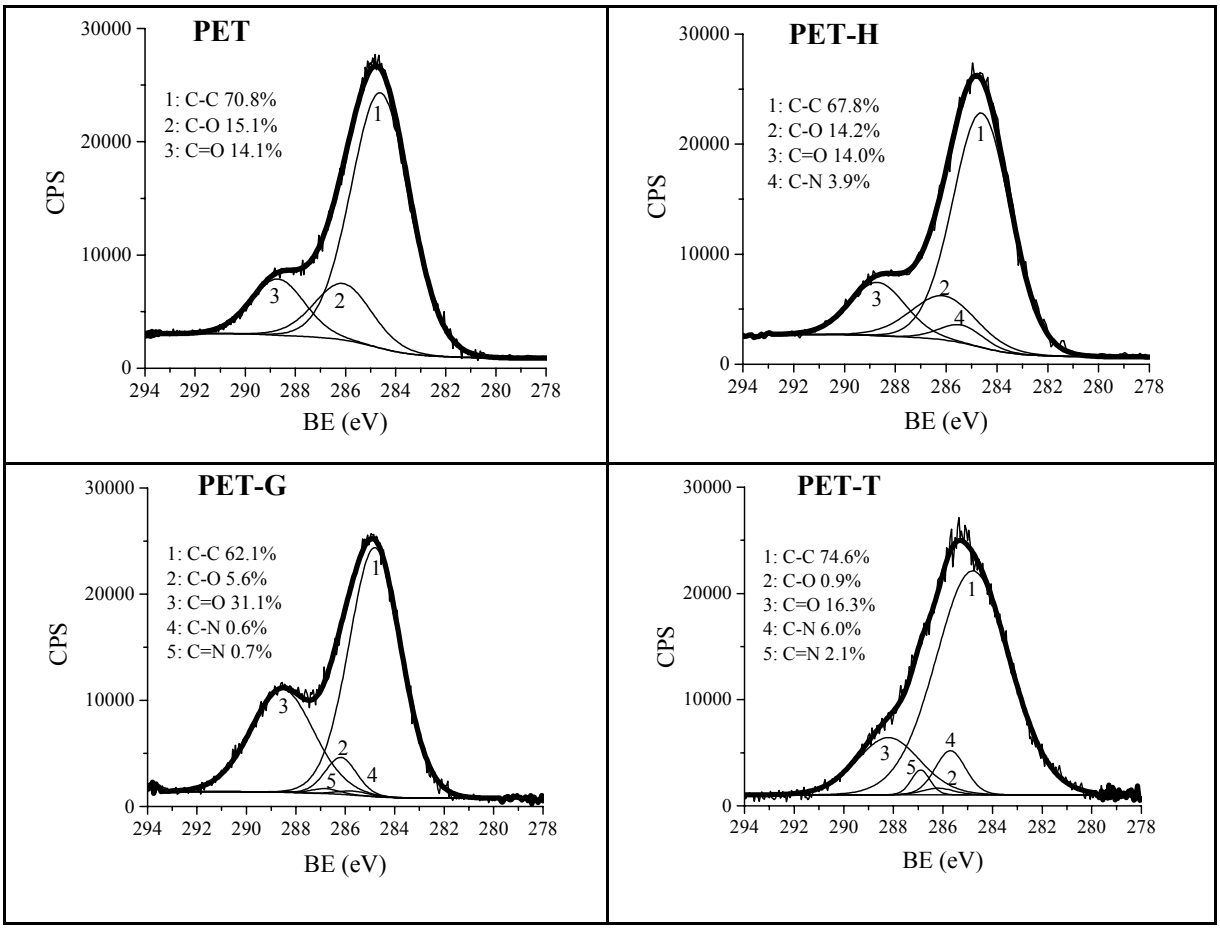

Fig. 3 – C1s XPS spectra of samples surfaces (PET, PET-H, PET-G and PET-T).

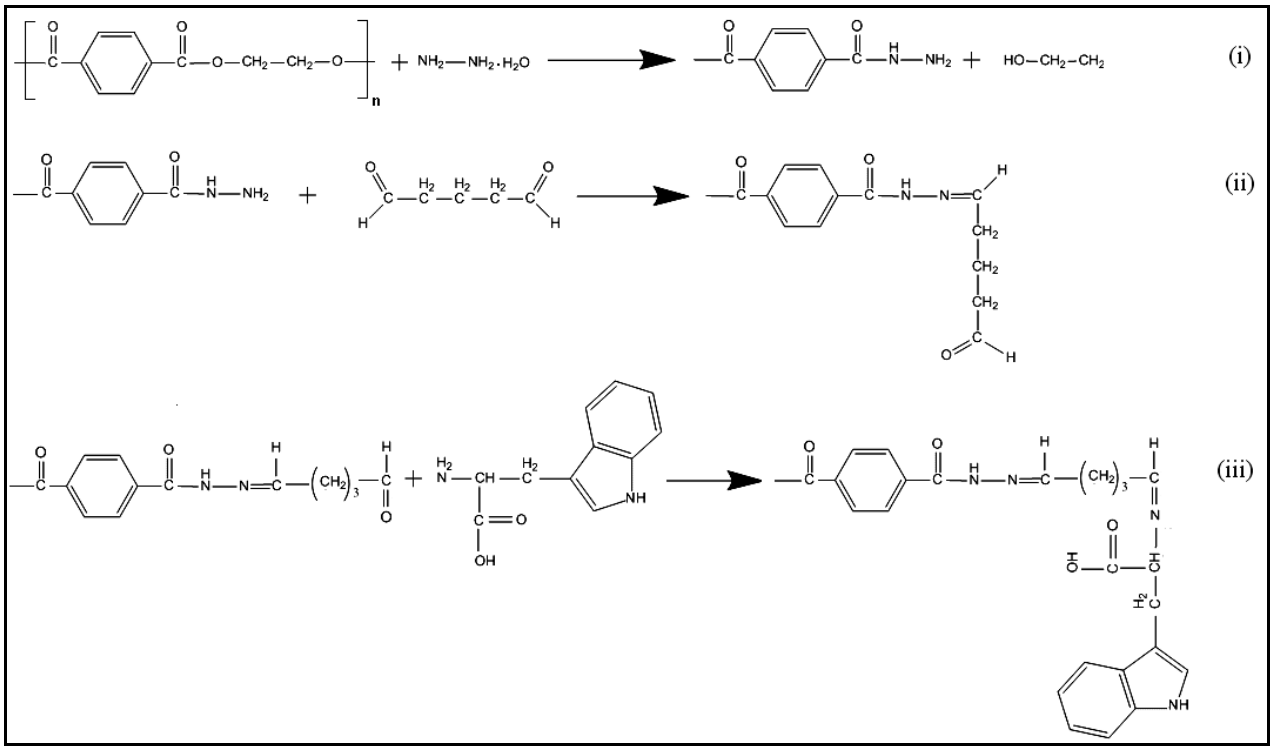

Fig. 4 – Scheme of reactions occurring during PET surface modification: (i) reaction of PET surface with hydrazine hydrate, (ii) reaction of PET-H surface with glutaraldehyde and (iii) reaction of PET-G surface with tryptophan.

4. Bio-functionalization test

The adhesion of biomolecules and proteins on the samples surface is expressed as absorption of biomolecules. Table 3 presents the biomolecules absorption on the PET (non-treated, activated and grafted). After surface activation, the absorption was increased by a factor of 2 only. While grafting of the surface lead to dramatic improvement of the absorption (more than 6 time). This result confirms that the applied grafting process in this work gives more additional functional groups on PET surface, that can play good role in bio-functionalization application.

Table 3

Evaluation of absorption of biomolecules on samples surfaces

Sample	Absorbance (a.u.)
PET	1.15 ± 0.01
PET-H	2.37 ± 0.01
PET-G	7.05 ± 0.01

CONCLUSION

The grafting of polymer surface for biocompatibility application, using PET type, is investigated. Firstly, the PET surface is activated by hydrazine hydrate to improve the following grafting procedure using glutaraldehyde solution. The FTIR spectroscopy, contact angle measurements and XPS technique confirm that the wettability, composition and chemical state of the polymer surface are significantly changed after modification. The hydrophilicity of the treated surfaces increases due to the grafting of carbonyl groups. The adhesion efficiency of modified surface is investigated using tryptophan solution. The bio-functionalization of surface is tested with a biological solution. The results reveal excellent adhesion and successful grafting.

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