Chemical modification and characterization of poly(ethylene terephthalate) surfaces for collagen immobilization

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Abstract: The functionalization of poly(ethylene terephthalate) (PET) surface films by reactions with multifunctional amines such as triethylenetetramine (TETA), and tetraethylenepentamine (TEPA) was investigated. For the functionalization of PET films surface we used a new way of treatment, a “sandwich model”. Physical-chemical properties of functionalized PET films were analysed. Qualitative and quantitative determination of the introduced amine groups were examined by means of Fourier Transform Infrared Attenuated Total Reflexion (FTIR - ATR), X-ray photoelectron spectroscopy (XPS), and potentiometric titration. Gained wetting properties were determined by using contact angle measurements and thoroughly analysed by acid-base approach. In addition, surface topography was investigated by atomic force microscopy (AFM). The amount of the introduced amino groups after TETA incorporation has been found to be two times higher as compared to TEPA. Wetting properties were significantly improved after aminolysis. Surface free energy was higher for PET - TETA treated film than that observed for PET - TEPA treated which is in accordance with titration results. The collagen immobilization onto PET treated films was evidenced by using AFM and subsequently by using XPS.

Keywords: PET • Aminolysis • Wettability • AFM • Collagen immobilization

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1. Introduction

The utilization of numerous polymeric materials in various applications is drastically limited due to their inert and hydrophobic surfaces. Therefore, to overcome these limitations, the surface functionalization of these kinds of materials becomes a hot topic due to their hi-tech applications, good wettability, adhesion, adsorption, lubrication, permeability, as well as biocompatibility [1–6]. The above mentioned properties, strongly depends on several parameters such as polymer charge, hydrophilicity, etc. In order to strictly achieve the reaction on polymer surface, techniques without affecting the polymer bulk are required.
Among different of known techniques, polymer surface grafting provides a high chemical stability and prevents delamination, as the grafted chains are covalently bound to the substrate [7–10]. Poly(ethylene terephthalate) (PET) constitute a cheap, chemically stable, and nontoxic material for a wide range of technological [11,12] and biomedical applications [13–15], due to its excellent mechanical properties and moderate biocompatibility. Different methods for the PET surface modification are known such as: controlled chemical breaking of ester bonds [16–28], surface grafting polymerization [29–39] and plasma treatment [40–45] which increases the polymer’s hydrophilicity and/or introduce specific functional groups. However, the main drawback is to define the optimal parameters of such processes, avoiding high degradation and/or prevents significant decrease of the sample mechanical properties.

For biomedical applications, is extremely important to introduce nitrogen-containing groups with antimicrobial properties. Moreover, these nitrogen-containing sites could be used as binding places for specific bioactive molecules. One straight forward approach for the introduction of nitrogen-containing groups to the surface of PET is aminolysis, when amide groups are formed due to the reaction of a polyester carbonyl group with an amine. This method has already been identified as a simple approach for the surface modification of PET [46–49]. Interestingly, a regular microscale pattern on the surface of PET films was reported to be formed upon aminolysis treatments. The amines attack the electron deficient carbonyl carbon, where chain scission and amide formation occurred [50,51]. It is expected that the amide groups, incorporated via surface modification, would present a potential reactive site to form hydrogen bonds or van der Waals type bonds, resulting in improved polymer wettability. Moreover, these kind of functionalized surfaces are ideal for biomolecules immobilizations. This research focused on the developing of an optimal and simple method for attaching nitrogenous containing groups onto the polymer surface. PET samples were functionalized using triethylenetetramine (TETA) and tetraethylenepentamine (TEPA).

These two amines were chosen because we believe that other amines such as ethylenediamine have been widely used in many studies as agent aminolysis reaction. The structure of these two aliphatic amines TETA and TEPA involve increased reactivity of the polymeric support, but lower compared to the ethylenediamine due to their relatively high molecular weight. The difference in structure between these two amines is only a unit of ethyleneamina. In another study with ethylenediamina [19] a destructive action was observed with functionalization spending in the vapor atmosphere which is not desirable. This study followed a surface functionalization in optimal time and with minimal degradation.

The introduction of aliphatic amine moieties on PET surfaces, which lead to amide functionalities, has been monitored by using FT-IR, potentiometric titration and XPS. The surface-modified PET films were supplementary characterized by using contact angle measurements, and atomic force microscopy respectively. The aminolyzed PET films with the highest content of the amino groups were then additionally chemically treated with collagen via glutaraldehyde crosslinking route. Using glutaraldehyde it is assumed that Schiff base intermediates are formed by amino groups.

PET activation by using amines will create new binding sites for collagen immobilization. The structural detail changes and spatial arrangement are elucidating by atomic microscopy, while direct detection and identification of collagen multiple components were obtained by optical imaging techniques.

Compared to other treatments studied in solution using these two amines [52], in the “sandwich model” the time is significantly reduced. The first time in the aminolysis Nissen was evaluated after 60 minutes, in the “sandwich model” the first time was after 10 minutes. This model can be operated in industrial applications. Other advantages were not reported in our study, because our interest was the surface functionalization with minimal secondary effects.

2. Experimental procedure

2.1. Materials and methods
PET biaxially oriented film with 30 mm thick were produced by TEROM Romania (Tg = 80 ± 2°C, 40% crystallinity, 1.38 g cm⁻³ density). Samples were ethanol (Sigma-Aldrich, 98%) washed before surface treatment, and then with milli-Q water. After this treatment, samples were oven dried at 40°C for 10 minutes. TETA (b. p. of 266 - 267°C, v. p. ≤ 0.01 mmHg at 20°C), TEPA (b. p. of 340°C, v. p. ≤ 0.01 mmHg at 20°C) were purchased from Aldrich and used as received.

For contact angle measurements, four different test liquids were used: milli-Q water, diiodomethane (Sigma-Aldrich, 99%), formamide (Sigma-Aldrich, 99%), and ethylene glycol (Sigma-Aldrich, ≥99.5%). All chemicals were used without purification. Milli-Q water from a Millipore water purification system (Millipore, USA) (resistivity = 18.2 Ω⁻¹ cm⁻¹) was used for contact angle and for other measurement.
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Collagen as a hydrophilic, triple-helical protein with three polypeptide chains was used to obtain biocompatible surface. Collagen was isolated from rat tail tendon (type I, Sigma - Aldrich). The collagen (type I) was dissolved in phosphate buffered (PBS, Sigma- Aldrich) solution for 20-25 min at room temperature at a concentration of 3.0 mg mL⁻¹.

2.2. Surface modification of PET films through chemical approach

2.2.1. Aminolysis

Cleaned PET films were independently treated with TEPA or TETA. The PET film samples (25x25 mm) were immersed in TETA or TEPA solutions, in the dark, at 90°C for different times: 10, 20, and 30 min respectively. The “sandwich model” was used, as follow: two PET film samples were separated by the use of amine solution, as schematically shown in Fig. 1a. By choosing the proper experimental conditions, namely: exposure time, reagents, temperature, it was possible to restrict the chemical polyester modification only to the surface of the polymer film. After exposure, the treated samples were ultrasonically rinsed with ethanol and milli-Q water, to remove the amine excess.

2.2.2. Collagen immobilization

The aminolyzed PET films were immersed in a 1% (weight) glutaraldehyde (GA) (Sigma-Aldrich, 25% solution in water) solution for 3 h at room temperature, followed by rinsing with a large amount of milli-Q water for another 24 h to remove free GA. The films were then incubated in 3 mg mL⁻¹ collagen/phosphate buffered

![Figure 1. a. Schematic drawing of the experimental chemical treatment; b. Reactions scheme of collagen immobilization on PET surface.](image-url)
solution (PBS, pH = 3.4) for 24 h at 2° to 4°C. Collagen was dissolved with 3.0 mg mL⁻¹ collagen/PBS for 20-25 min at room temperature. The collagen immobilized films were rinsed with 1% acetic acid (Sigma-Aldrich, 99.85%) solution and then rinsed with milli-Q water for 24 h to remove non-immobilized collagen.

2.3. Characterization

2.3.1. FTIR-ATR spectroscopy
The PET modified surface films were examined by FTIR-ATR spectroscopy in the 600 - 4000 cm⁻¹ range. The spectra were recorded on a BRUKER VERTEX 70 spectrometer at a resolution of 2 cm⁻¹ at a 45° incidence angle. The signal-to-noise ratio was improved by co-adding 64 scans per spectrum. All measurements were carried out at room temperature under ambient conditions.

2.3.2. Potentiometric titration
The pH potentiometric titration of PET film suspension was carried out with a two-burette instrument Mettler Toledo T70, in an inert atmosphere (N₂ bubbling). The burettes were filled with 0.1 M HCl and 0.1 M KOH. All solutions were prepared in Mili-Q water with low carbonate content (<10⁻⁶ M). This was achieved by boiling and cooling under a nitrogen atmosphere. The suspension was titrated in a forth and back manner between the initial pH = 2.8 to the preset pH = 11. The titration experiments were carried out at 0.1 M ionic strength, set to its appropriate value with KCl. The pH potentiometric titration of PET film suspension was carried out with a two-burette instrument Mettler Toledo T70, in an inert atmosphere (N₂ bubbling). The burettes were filled with 0.1 M HCl and 0.1 M KOH. All solutions were prepared in Mili-Q water with low carbonate content (<10⁻⁶ M). This was achieved by boiling and cooling under a nitrogen atmosphere. The suspension was titrated in a forth and back manner between the initial pH = 2.8 to the preset pH = 11. The titration experiments were carried out at 0.1 M ionic strength, set to its appropriate value with KCl. The titration was added dynamically within a preset interval of [0.001 – 0.25] mL. The equilibrium criteria for the timed addition was set to dE/dt = 0.1/60 s, where 60 s was the minimum time to reach equilibrium conditions between two additions of the titrant, the maximum time was set to 1800 s. The pH value was measured with a Mettler Toledo DG-117 combined glass electrode. A blank HCl-KOH titration was carried out under the same conditions as above. Three parallels measurements for each sample were performed.

2.3.3. Contact angle and surface free energy determination
In order to determine the wettability of pure and treated with TETA and TEPA PET films, static contact angle (SCA) measurements were performed using the SCA20 contact angle measurement system from Dataphysics (Germany). Static contact angle measurements were carried out using four different test liquids: Milli-Q water, diiodomethane, ethylene glycol, and formamide. All measurements were conducted at room temperature on two independent surfaces with a test liquid volume of 4 µl. Each SCA value was the average of at least six drops of liquid per surface. The surface free energy (SFE) of the films was calculated from contact angle values of the test liquids by the van Oss and Good method. The values of the surface tension and its components of the probe liquids used for SCA measurements were taken from the literature [53,54]. The total SFE \( (\gamma_{i}^{TOT}) \) was calculated using the acid-base approach of van Oss and Good, which divides the total SFE into the dispersive Lifshitz-van der Waals interaction \( (\gamma_{i}^{LW}) \) and the polar Lewis acid-base interactions \( (\gamma_{i}^{LW}) \) (Eqs.1 and 2). A detailed description of the theory can be found elsewhere [55]. In short, the Lewis acid base interactions are subdivided into electron donor \( (\gamma_{i}^{+}) \) (Lewis base) and electron acceptor \( (\gamma_{i}^{-}) \) (Lewis acid) parts.

\[
\gamma_{i}^{TOT} = \gamma_{i}^{LW} + \gamma_{i}^{AB}
\]

\[
\gamma_{i}^{AB} = 2\sqrt{\gamma_{i}^{+}\gamma_{i}^{-}}
\]

Using the Young’s equation, it is possible to relate the work of adhesion to the measureable contact angle of a liquid on a solid substrate of interest (Eq. 3).

\[
W_{ad} = (1 + \cos \theta)\gamma_{i}^{TOT} = 2\sqrt{\gamma_{i}^{LW}\gamma_{i}^{-} + \gamma_{i}^{LW}\gamma_{i}^{+} + \gamma_{i}^{LW}\gamma_{i}^{-} + \gamma_{i}^{LW}\gamma_{i}^{+}}
\]

Where \( \theta \) is the contact angle, \( \gamma_{i}^{TOT} \) is the liquid’s total surface tension, and \( \gamma_{i}^{LW} \) and \( \gamma_{i}^{LW} \) are the apolar Lifshitz-van der Waals components of the liquid and the solid, respectively. Whereas \( \gamma_{i}^{+} \) and \( \gamma_{i}^{-} \) are the Lewis acid-base contributions of either the solid or the liquid phase as indicated by the subscripts. In order to solve the Eq. 3, it is essential to use at least three different test liquids (two polar and one apolar) with known \( \gamma_{i}^{TOT}, \gamma_{i}^{LW}, \gamma_{i}^{+}, \gamma_{i}^{-} \). The SFE values obtained from contact angle measurements by the method of van Oss are relative ones. They are based on assumed values for a reference compound. Water with \( \gamma_{i}^{TOT} = \gamma_{i}^{LW} = 25.5 \text{ mJ m}^{-2} \) was chosen by van Oss and co-workers [55]. It is worth to mention that depending on the arbitrary chosen reference values of test liquid for SFE calculation, the surfaces with a dominating or low polarity \( \gamma_{i}^{+} \) can be obtained. Although there is no generally accepted reference substance, the results obtained from three component approaches are suitable for most practical purposes and can be compared with available literature data [52].

2.3.4. Atomic force microscopy (AFM)
Topographical features of the sample surfaces were characterized by atomic force microscopy (AFM) in tapping mode with an Agilent 5500 AFM multimode scanning probe microscope (Digital Instruments, Santa
Barbara, CA). The images were scanned using silicon cantilevers (ATEC-NC-20, Nanosensors, Germany) with a resonance frequency of 210-490 kHz and a force constant of 12-110 N m⁻¹. All measurements were performed at room temperature under ambient conditions. Image sizes were set to 1×1 µm².

Fluorescence and light polarization measurements were carried out with an optical microscope Leica DM 2500M.

2.3.5. X-ray photoelectron spectroscopy (XPS)
The compositional analysis of the studied samples was carried out by X-ray photoelectron spectroscopy (XPS) using a PHI-5000 VersaProbe photoelectron spectrometer (Φ ULVAC-PHI, INC.) with a hemispherical energy analyzer (0.85 eV binding energy resolution for organic materials). A monochromatic Al Kα X-ray radiation (hv = 1486.7 eV) was used as excitation source. The standard take-off angle used for analysis was 45°, producing a maximum analysis depth in the range of 3-5 nm. Spectra were recorded from at least three different locations on each sample, with a 1×1 mm area of analysis. Low-resolution survey spectra were recorded in 0.5eV steps with 117.4 eV analyzer pass energy. The XPS data were acquired using the PHI SUMMIT XPS for Versa Probe software.

3. Results and discussion

3.1. Chemical changes of amino modified PET surfaces
The stretching vibrations ν(C=O) of the ester structure of polyethylene terephthalate is localized at 1712 cm⁻¹ and in fact is an envelope of several bands belonging to the carbonyl group.

The macromolecular chain of PET-COO⁻ ester structure has different symmetry planes than the aromatic rings and allows the formation of several structures.

IR spectrum evidences the shift of carbonyl wavelength toward lower values, this phenomenon being correlated with the formation of a more stable system, is in agreement with the quantum chemistry theory that explains the involvement of COO⁻ bonds in conjugation with p and π electrons belonging to aromatic nucleous.

In the aminolysis functionalization method, we expect that the reaction with the aliphatic polyester amines involves carbonyl groups which are outside of the aromatic plane, present on the polymer surface.

The band located at 1410 cm⁻¹ attributed to phenyl ring vibrations (C-H bend coupled with ring C-C stretch) [56-58] is considered to be a reference band, since it is known as non-sensitive to both orientation and conformation. FTIR spectrum evidences the presence of characteristic PET absorption peaks located at 1713 cm⁻¹ (C=O stretching band characteristic of an ester group), the vibration of aromatic ring was assigned at 1410, 1018, and 865 cm⁻¹, the bending vibration characteristic of -CH₂ groups assigned at 1340 and 1180 cm⁻¹, the stretching vibration characteristic of C-O bonds at 1240 and 964 cm⁻¹ and 1124 and 1100 cm⁻¹ the stretching vibration of C-O bonds resulting from to amorphous and crystalline structure of PET. It is well known that in the amorphous state, PET has a gauche structure in ethylene glycol fragment, contrary to the crystalline state where the ethylene glycol fragment has a trans structure.

During aminolysis reaction, the nitrogen atom from the amine group attack the electron deficient carbonyl carbon atom of PET chain where chain scission and subsequent amide group formation occurred in Fig.1b. The formed amide group is the main cause of the molecular weight diminution. It was assumed that the ordered or crystalline regions are non-reactive sites. Therefore the amines predominantly react with the noncrystalline (amorphous) regions. As a result of the chemical treatments of PET with aliphatic amines, obvious changes in FTIR-ATR spectra could be observed, Figs. 2a, 2b. In the infrared spectroscopy, the amide group is a complex vibrational unit that involves stretching and bending vibrations. During aminolysis, both amide and primary amine units are generated, depending on the chain mobility and polymer film permeability. Spectra were normalized to the 1410 cm⁻¹ peak before any data processing.

For both PET treated samples, it was demonstrated that functional groups containing nitrogen were introduced onto the PET films surface. Moreover, the amount of nitrogen containing groups increased by prolonging the treatment time. The formation of amide I and II bands was confirmed by the appearance of two absorption bands located at 1648 cm⁻¹ (stretching vibration, \(\langle\rangle\)C=O) and 1545 cm⁻¹ (the NH bending vibration \(\delta\)NH), respectively. In this study, during the PET treatment that peak intensity for amide I band and amide II increased significantly.

The position of amide I and amide II bands revealed that hydrogen bonding involving amide C=O is present. This result suggests that the carbon chains in the amine molecule that are oriented towards the film surface also are changed. Longer exposure time to the amine increases the NH stretch vibration of the associated bands.
Potentiometric titration was used to determine the amount of protonated amino groups. Table 1 presents the amount of protonated amino group as determined by potentiometric titration. Increasing the reaction time, the amount of amino groups increased as well. This increase is more obvious in the case of TETA functionalization as compared to TEPA. It can be stated that in the case of TETA treated films, aminolysis starts within 10 minutes and reach a maximum of the amino group content after 30 minutes. In the case of TEPA treated films, the amino group content after 10 or 20 minutes of exposure, are rather insignificant. After 30 minutes, PET-TEPA films, exhibit as much as a half of the total amount of amino groups as compared to PET-TETA films. TETA possess two primary and two secondary amino groups, while TEPA possesses two primary and three secondary amino groups; therefore, one could expect that at any reaction time, TEPA would introduce a higher amount of amino groups onto PET surface. As Table 1 revealed, the experimental results do not confirm this supposition. A reasonable explanation could be that TETA, a tetramine with shorter alkyl-chains could react much faster with PET surface as compared to TEPA, an alkyl-pentamine, and therefore, more TETA could be

Figure 2. ATR - FTIR measurements for PET film normalized to the band at 1410 cm⁻¹ (ref. band): a. modified PET by TEPA; b. modified PET by TETA.
grafted onto the surface. This view is supported by the work of Nissen et al. [52] who observed an enhanced reactivity between PET surface and diethylenetriamine in comparison with reaction between TETA and PET. In addition, lower adsorption of TEPA onto PET surface as compared to TETA may be assigned also to the steric hindrance effect, due to higher amino groups number content. However, it seems that process is dependent on chain mobility and permeability of polymer film [53]. Interestingly, the change in SCA (H2O) after 30 min reaction time (Fig. 3), denoting that most of the reaction has occurred at longer reaction time. However, it is interesting to note that the contact angle values of all test liquids have decreased with increasing reaction time, proving that the surface wettability has increased after the aminolysis.

### 3.2. Surface free energy (SFE) determination

The surface energy values of untreated and aminated PET surfaces at different reaction times are shown in Fig. 4. For untreated PET surface, the \( \gamma_{s}^{TOT} \) of 36.4 ± 0.8 mJ m² and apolar Lifshitz-van der Waals interaction, \( \gamma_{s}^{LV} \) of 31.7 ± 0.2 mJ m² were obtained. A very low value of Lewis acid (\( \gamma_{s}^{LA} \)) and Lewis base (\( \gamma_{s}^{LB} \)) component is observed for original PET surface which could originate from the impurities or the type of reference test liquids chose for the SFE determination. The surface energy values and its components of all amine treated PET surfaces increased dramatically with advancing reaction time compared with that of untreated one. This increase was higher for TETA than that observed for TEPA treated surface, which reflects a greater reactivity of TETA with PET surface. The PET surface treated for 30 min with TETA shows the highest degree of \( \gamma_{s}^{TOT} \) = 55.3 ± 0.5 mJ m² and Lewis-basicity (\( \gamma_{s}^{LB} \) = 38 ± 0.5 mJ m²) than those of shorter reaction times while the similar trend could already be seen with TEPA-treated PET surface, but the values are lower. The results (e.g. \( \gamma_{s}^{TOT} \)) obtained in this study, are comparable with the values obtained in the literature for TETA-treated PET surface [55]. In this study, compared to similar treatments for surface modification using a “sandwich” model, the reaction time decreased. Increasing the exposure time of the PET films to TETA or TEPA lead to a regular increase in Lewis-base character, \( \gamma_{s}^{LB} \) and dispersive component, \( \gamma_{s}^{LV} \) as compared to original PET surface which can be explain by to the incorporation of a longer hydrocarbon chain containing polar nitrogen groups onto the PET surface. Furthermore, this observation could be supported from the charge titration values (see Table 1).

### 3.3. Surface topography

The changes in the surface topography of the PET samples after reaction with amine compounds were characterized by atomic force microscopy. A
relatively smooth and parallel oriented crystalline surface with root mean square (RMS) roughness values of 1.2 nm were obtained for untreated PET film (Fig. 5).

AFM analysis of PET films exposed to TETA or TEPA shows that the surface roughness increases by increasing the reaction time; this is a clear indication of the successfully grafted amino groups onto the PET surface. Since the time of aminolysis is relatively short (10 min), the RMS roughness value is lower than that obtained for the blank sample. In order to explain this decrease, the uniformity of the surface topography irregularities during the film (commercial film) processing could be taken into consideration. In this first stage of the aminolysis, the surface etching is a frequently phenomenon. In the same time we can assert that the process inertia plays an important role, the amine reactivity influencing the course of the reaction. The surface morphology is completely different, a particle-like structure formation (Fig. 6) is observed and a high surface roughness (RMS = 2.8 nm) were noticed for PET films treated for 30 min with TETA (Fig. 6b). Moreover, increasing the reaction time between PET and TETA, a gradual increase in roughness values (Fig. 6b) due to higher amount of bonded amines can be observed. Covalently bonded molecules are well distributed across the surface and their number is increasing with longer reaction time. Similar results were obtained for films that were reacted with TEPA.

3.4. Collagen immobilization
The samples were quantified by fluorescence measurements with an optical microscope. The PET films sample treated at 30 min were immersed in a large amount of glutaraldehyde. The reaction between NH$_2$ and OHC - (CH)$_3$ - CHO yielded a bonding via – N = CH - (CH)$_3$ – CHO, and one free aldehyde group could react with NH$_2$ groups existing in collagen. Collagen molecules were immobilized chemically on functionalized films via glutaraldehyde.

The immobilized collagen allows the formation of a crosslinking surface. The immersion of the surfaces in deionized water has led to the removal of the unbound proteins, the collagen immobilized films being then rinsed with acetic acid and afterwards with deionized water to remove the free collagen.
Chemical modification and characterization of poly(ethylene terephthalate) surfaces for collagen immobilization

Fig. 7a reveals the process of collagen immobilization onto the PET surface film treated with TETA. The obtained AFM height image shows a surface morphology with randomly distributed large grains with an irregular sphere-like appearance. Their aspect is probably due to some overlap of the collagen molecules to each other. For surface film treated by TEPA (Fig. 7b) the AFM image for collagen immobilization indicate a surface structure with small grains, collagen forms homogeneous layers with small surface features.

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Fig. 5. AFM root mean square (RMS) roughness of the untreated and functionalized PET with TETA or TEPA, at different reaction time.

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3.5. X-ray photoelectron spectroscopy: aminolysis validation

The XPS technique is extensively used as a powerful tool to monitor the surface changes appeared after various physical-chemical treatments [63,64]. Fig. 9 shows the XPS survey spectra of original PET and TEPA treated PET film (for 30 minutes) before and after collagen immobilization. Also the XPS survey spectra are presented for the TETA treated PET film (30 minutes) before and after collagen immobilization, Fig. 10.

For the untreated PET film the only atoms detected are carbon and oxygen. The O/C atomic ratio has been found to be 0.32. Once the PET films are treated with TEPA or TETA, the XPS detect as we expected, the presence of other element, i.e., nitrogen, see Table 2. After collagen immobilization onto TEPA treated PET film for 30 min and TETA treated PET film for 30 min, the content of nitrogen as determined by XPS considerably increased, from 3.22% to 7.55% in the case of former and from 3.91% to 11.29% in the case of the latter films. Another important indication that
Figure 6. AFM height images (1×1 µm²; z-scale, 15 nm) of (a) original PET surfaces (b) functionalized PET surfaces with TETA (upper row) or TEPA (bottom row), for different reaction time.

Figure 7. AFM images analysis of collagen immobilized on (a) PET films 30 min chemical treated by TETA; (b) PET films 30 min chemical treated by TEPA.
Chemical modification and characterization of poly(ethylene terephthalate) surfaces for collagen immobilization

Collagen has been successfully immobilized onto PET treated films is the detection of the sulphur atoms on the surface of analysed PET films after collagen immobilization, see Table 2. The sulphur content founded for the both PET treated films has been around 0.30%.

4. Conclusions

PET surface was chemically activated by aminolysis using two different amines: TETA and TEPA. For the aminolysis of poly(ethylene terephthalate) (PET) surface films we used a new way of treatment, a

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**Figure 8.** Fluorescence Microscopy images of immobilized collagen on (a) TETA-PET treated (30 min) and (b) TEPA-PET treated (30 min) films.

**Figure 9.** XPS survey spectra for untreated PET, 30 min TEPA treated PET film and collagen - 30 min TEPA treated PET film.

**Figure 10.** XPS survey spectra for untreated PET, 30 min TETA treated PET film and collagen - 30 min TETA treated PET film.
The values obtained for activation are comparable with different procedures of treatments in the literature, but the reaction time is reduced. The first time in the aminolysis Nissen was evaluated after 60 minutes, in the “sandwich model” the first time was after 10 minutes. This model can be operated in industrial applications.

Surface changes after this treatment were analyzed by using FT-IR, potentiometric titration, contact angle and XPS. The specific analyses such as XPS, AFM reveals that the activated PET surface could successfully improve the biomedical applications of untreated PET. Therefore, the immobilization of biologically active molecules (i.e., collagen) was studied by using the PET aminolyzed samples with the highest content of nitrogen after TETA and TEPA reactions via glutaraldehyde crosslinking. The experiments show that effective attachment of biomolecules to a modified PET surface depends on the molecule being attached. In conclusion, aminolyzation of PET showed a great potential for biomedical applications due to minimal changes in surface and bulk properties and the ability to immobilize bioactive agents such as collagen.

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