Introducing Amine Functionalities on a Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Surface: Comparing the Use of Ammonia Plasma Treatment and Ethylenediamine Aminolysis

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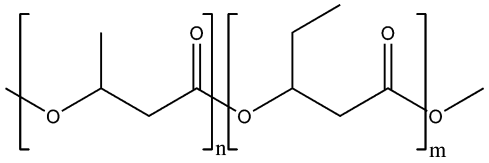
Amine functionalities were introduced onto the surface of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) films by applying radio frequency ammonia plasma treatment and wet ethylenediamine treatment. The modified surfaces were characterized by X-ray photoelectron spectroscopy (XPS) for chemical composition and Raman microspectroscopy for the spatial distribution of the chemical moieties. The relative amount of amine functionalities introduced onto the PHBV surface was determined by exposing the treated films to the vapor of trifluoromethylbenzaldehyde (TFBA) prior to XPS analysis. The highest amount of amino groups on the PHBV surface could be introduced by use of ammonia plasma at short treatment times of 5 and 10 s, but no effect of plasma power within the range of 2.5−20 W was observed. Ethylenediamine treatment yielded fewer surface amino groups, and in addition an increase in crystallinity as well as degradation of PHBV was evident from Fourier transform infrared spectroscopy. Raman maps showed that the coverage of amino groups on the PHBV surfaces was patchy with large areas having no amine functionalities.

Introduction

The design and development of novel materials for bone tissue engineering have attracted much interest in recent years. Biodegradable polyesters are probably the group of polymers most widely studied for this application. The biosynthesized polyester poly(3-hydroxybutyrate-*co*-3-hydoxyvalerate) (PHBV, Chart 1), a thermoplastic polymer that belongs to the polyhydroxyalkanoate family, has been gaining increasing attention in this respect.[1−7] It degrades slowly in vivo (15−43% in 6 months),[8] does not produce toxic degradation products,[9] and is biocompatible.[8] In addition, it has mechanical properties better than those of cancellous bone (Young's modulus of 1.0 GPa and tensile strength of 13 MPa),[10] and inclusion of a reinforcing phase can further improve these properties.[11,12]

Establishment of a stable interface between a biomaterial and the host environment is governed by both the material surface properties and the type and state of the biological tissue. The material surface properties such as topography, wettability, and surface chemistry therefore play an integral role in the success of a biomaterial in vivo as they affect the biological interactions and responses across the interface. Surface properties can be altered to suit a specific biomedical application. The most commonly used techniques include chemical etching, plasma gas treatment, ultraviolet (UV) irradiation, γ irradiation, and electron beam irradiation. Among these techniques, plasma treatment is particularly versatile because the modification is restricted to the top surface of the material, leaving the bulk unaltered.[13] Plasma treatment can be used to modify a polymer surface in a nonspecific manner by changing the wettability or in a more specific manner by introducing a variety of functional groups depending on the type of gas used.

Chart 1. Chemical Structure of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)



A number of studies have investigated plasma treatments for the modification of PHBV or poly(3-hydroxybutyrate) [PHB-(V)]. Nonspecific surface modification was performed by oxygen plasma treatment[1−3,14] and perfluorohexane plasma.[15] Specific modifications to PHBV were performed by Kang et al.,[5] who used oxygen plasma activation followed by acrylic acid grafting. The introduced carboxylic acid groups were used to immobilize insulin on the material surface. The introduction of amine functionalities on PHB surfaces has been achieved by use of allylamine[16] and ammonia plasma.[4] Both types of plasma treatments were reported to result in concurrent incorporation of other nitrogen functional groups such as amides and imines.[4,16] The origin of amide incorporation has been proposed by Mas et al.[16] to be due to residual oxygen and adsorbed water vapor in the plasma reactor, while Nitschke et al.[4] suggested that polymer degradation and subsequent reaction with ammonia was the cause.

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An alternative to plasma treatment for the introduction of amino groups on a polyester surface is chemical etching in the form of aminolysis using diamines.[17−21] It has been found that the rate of reaction depends on the type of amine used[19] and the percentage crystallinity of the polymer.[20,21] Aminolysis has proven versatile for modifying poly(lactic acid-*co*-glycolic acid) (PLGA) scaffolds[17] and poly(ϵ-caprolactone) (PCL) membranes[18] for tissue engineering applications. For both biopolymers, the free amino groups were used as a chemical linker to immobilize macromolecules such as gelatin, chitosan, and collagen.[17,18] The degree of amine functionalization was found to be dependent on the treatment time, concentration, pH, temperature, and type of solvent used.[17,18] The depth of penetration of amino groups into PCL membranes was 50 μm from the surface, although most of the amino groups were detected on the surface, decreasing in amount in the deeper part of the cross section.[18] To the best of our knowledge, this approach has so far not been successfully applied to PHB(V) materials.

In the present study, amine functionalities were introduced onto the surface of PHBV films by treatment with ammonia gas plasma and wet ethylenediamine aminolysis. The surface modified materials were characterized by X-ray photoelectron spectroscopy (XPS) and Raman microspectroscopy. The former technique was used to identify the chemical species on the PHBV surface, and the latter technique, to assess the distribution of amino groups on the surface.

Materials and Methods

Solvent Casting of PHBV Films. Poly(3-hydroxybutyrate-*co*-3-hydoxyvalerate) (PHBV) with 8.8% 3-hydroxyvalerate content and molecular weight of 600 000 g/mol was obtained from Aldrich. PHBV films were made as described previously[22,23] by dissolving 0.3 g of PHBV powder in 15 mL of chloroform (99.4% pure, Pronalys) at 50 °C. A covered glass Petri dish (70 mm i.d.) was used as casting substrate. The polymer solutions were left to stand at room temperature (~25 °C) for several days to allow for full evaporation of the solvent. The resultant films had a thickness of 50 ± 5 μm.

Ammonia Plasma Treatment. PHBV films (1 cm × 2 cm) were mounted in a metal sample holder where the film was positioned vertically to allow treatment on both sides of the sample. This sample holder was placed on top of a glass slide, which was inserted inside a plasma reactor downstream from coil (~10 cm). The plasma reactor has been described previously.[24] Briefly, it consisted of a tubular glass chamber with an external copper electrode wrapped around the central part. This copper electrode was connected to a radio frequency (RF, 27 MHz) generator, which supplied the plasma power. The reactor was sealed and the air inside was evacuated. When the pressure inside the reactor reached a base pressure (8×10^{-5} bar), the reactor was filled with ammonia gas (99.98%, Linde Gas, Australia) to the processing pressure (4×10^{-4} bar), after which the RF generator was activated at certain plasma power (2.5−20 W) and treatment time (5−20 s) combinations.

Ethylenediamine Aminolysis. Prior to treatment, the PHBV films were soaked in Milli-Q water overnight. The films were treated with aqueous ethylenediamine (ED) (99%, Merck, Germany) (5%, 10%, 15%, 20%, and 40%) at pH 10, adjusted by addition of aqueous sodium hydroxide (pellets, AR grade, Univar, Ajax Fine Chemicals, Australia). Films were treated at 23 or 50 °C with continuous stirring for various times (60, 90, or 120 min). After treatment, samples were subjected to extensive washing; the first washing solution was 10^{-4} M hydrochloric acid (37%, AR grade, Lab-scan), the subsequent five washes (1 h each) were in cold distilled water, and the final single wash (48 or 72 h) was achieved by soaking in distilled water at room temperature. Washed samples were dried in a vacuum desiccator.

Trifluoromethylbenzaldehyde Derivatization. Trifluoromethylbenzaldehyde (TFBA) (98%, Aldrich) vapor derivatization was carried out as previously described.[4,25,26] In short, untreated, freshly ammonia plasma-treated, and dried ED-treated PHBV films were exposed to TFBA vapor for a period of 1 h at room temperature (22 °C). Polymer films were subsequently allowed to outgas for 12 h to remove the excess unreacted TFBA adsorbed on the surface.

Contact Angle Measurements. Contact angle measurements of untreated and modified PHBV films were obtained by a sessile drop method[27] with the setup previously described.[22,23] For the aging studies, advancing contact angles (θ_A) were determined by placing a 5 μL drop of double-distilled water onto the PHBV surface. Duplicate measurements were obtained from different areas of each sample. The image of the drop was taken by a digital camera, which was connected to a computer. Contact angles were calculated by use of NIH IMAGE software. For all other studies, both advancing and receding (θ_R) contact angles were obtained by delivering drops of Milli-Q distilled water on the film surfaces with a minimum of three repeats for each sample. The advancing (θ_A) contact angle was measured on a 5 μL drop and subsequently after each addition of 5 μL until a total 20 μL volume was added. For receding (θ_R) contact angle, a 5 μL increment was withdrawn from a 25 μL water drop for each contact angle measurement. By use of the equation $2h/\Delta = \tan \theta/2$, contact angles ($\theta$) were calculated ($\Delta$ is the base diameter of the drop and h is the height of the drop).[27]

X-ray Photoelectron Spectroscopy. XPS survey and high-resolution spectra were collected from an Axis Ultra XPS spectrometer (Kratos Analytical) at analyzer pass energies of 160 and 20 eV, respectively. The spectrometer has a monochromatic Al Kα X-ray source operating at 15 kV, 10 mA (150 W) for all data acquisitions. Overall information depth is 3λ (where λ is the electron free path), which for Al Kα is λ = 3.5 nm, resulting in a total depth of ~10 nm. Binding energies were charge-corrected to 285.0 eV for aliphatic carbon. High-resolution spectra were resolved into individual Gaussian−Lorentzian peaks by use of a least-squares fitting program (PeakFIT, Jandell Scientific Software). Component energies, number of peaks, and peak widths (fwhm of 1.0 and 1.2 for all Cs and Ns, respectively) were fixed initially, and refinement was done only for peak heights. In a final refinement cycle, component energies and peak widths were also refined and these changed by less than 1.0%. Peak fit results were imported into a graphic software package (Origin, OriginLab Corp.) for final illustrations.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy. ATR-FTIR spectra (64 scans, 8 cm^{-1} resolution, wavenumber range 4000−500 cm^{-1}) were acquired on a Nicolet Nexus 870 with a Smart Endurance diamond ATR accessory. Peak height measurements were performed with the spectral analysis software (GRAMS/32, Galactic Industries Corp., Salem, NH).

Raman Microspectroscopy. Raman spectra were obtained from a Renishaw Raman microscope system 3000 (Renishaw, Gloucestershire, U.K.) incorporating a Leica microscope equipped with a short working distance 50× objective lens, a 1200 lines/mm grating, and a charge-coupled device (CCD) detector (578 × 385 pixels). The spectra were excited by the 785 nm line of a 200 mW Renishaw diode laser operating in line focus mode. Spectra were recorded with the grating static and centered at 1550 cm^{-1}, giving a spectral range of approximately 1800−900 cm^{-1}. Raman mapping was achieved by moving the sample with a computer-controlled translational stage. Typically, an area of 200 μm × 240 μm was scanned in a grid pattern with a step size of 3.76 μm in the x direction and 33.86 μm in the y direction. Spectra were acquired at each point on the grid for two accumulations of 60 s each to give a total map acquisition time of approximately 12 h. The 33.86 μm dimension corresponds to the 18 pixels on the CCD covered by the line focus of the laser, which was further partitioned by software into nine separate spectra to give a spectrum every 3.76 μm in both the x and y directions. Spectral information was extracted by means of spectral analysis software (GRAMS/32).

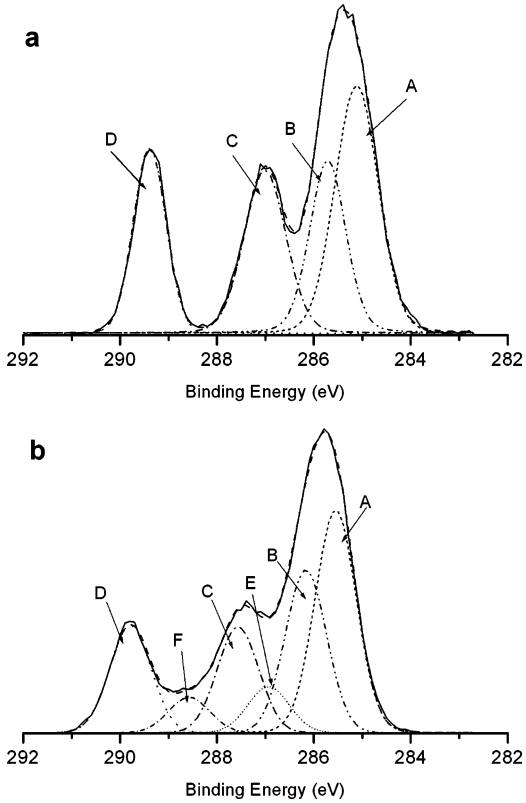


Figure 1. XPS C 1s core level spectra of (a) untreated and (b) ammonia plasma-treated (10 W, 20 s) PHBV.

Results and Discussion

The surface of the PHBV films were functionalized by two different methods, ammonia plasma treatment and wet chemical aminolysis, in order to introduce amino groups. The former technique has previously been investigated for the related polymer PHB, where it was shown that a mixture of nitrogen species were introduced.[4] Aminolysis with ED has been shown to be a versatile method for introducing amino groups on the entire (external and internal) surface of a 3D porous PLGA scaffold.[17] This procedure has also been applied to the hydrolytically more stable polyester PCL,[18] where only very small amounts of amino groups (below the detection limit of XPS) were introduced. Treatments of our PHBV films with aqueous ED solutions at room temperature did not lead to any detectable amine functionalities as assessed by XPS either, nor did we observe any changes in contact angle. However, harsher conditions of 5−40% aqueous ED at 50 °C did lead to the introduction of nitrogen functionalities. In this paper we present data related to samples treated with 40% ED at 50 °C.

XPS of Untreated PHBV. The XPS survey spectrum of the untreated PHBV film shows the characteristic peaks of the C 1s and O 1s at 285 and 535 eV, respectively. The observed carbon to oxygen (C/O) ratio was 2.65, which is significantly higher than the theoretical value (2.04); however, in our previous study high values have also been observed.[20] The XPS multiplex scan of the carbon region along with the curve fit (Figure 1a) shows four peaks corresponding to the different oxidation states of the carbon element: A (285.0 eV), B (285.6 eV), C (286.9 eV), and D (289.3 eV), due to C*−C, C*−C(═O)−O, C*−O, and C*(═O)−O components, respectively. From the C 1s curve fit it can be seen that the C*−C component at 285.0 eV (peak A in Figure 1a) is larger (1.6:1.2:1.1:1) than the theoretical value (1.1:1:1:1), thus indicating the presence of hydrocarbon impurities.

XPS of Ammonia Plasma-Treated PHBV. After ammonia gas plasma treatment, an N 1s peak at 400 eV was observed in the survey spectrum. The XPS multiplex scan of the carbon region gave a spectrum that requires two additional peaks for the curve fit, E (286.4 eV) and F (288.0 eV) (Figure 1b). Nitschke et al.[4] have also found these carbon species after ammonia plasma treatment of PHB films and have assigned these peaks to C−N (amino) and N−C═O (amide) groups, respectively. During plasma treatment, a variety of reactive species are produced in the gas plasma and they can all interact with the exposed material surface. As a result, not only can functional groups be incorporated on the surface but also removal of functional groups can occur. In this case, a decarboxylation reaction has occurred on the PHBV surface since peaks D and C have decreased relative to peaks A and B as compared to the untreated PHBV (Figure 1).[4,16]

The high-resolution spectrum of the N 1s peak confirmed the addition of different nitrogen functionalities to the surface during plasma treatment. The assignments of different nitrogen groups under the N 1s peak found in the literature are as follows: amines (398.9−399.3 eV),[28−31] nitriles (399.6 eV),[28,32] amides (399.8 eV),[28,29,31,32] imides (400.5 eV),[29,32] and quaternary amines (401.3−401.5 eV).[28,32,33] After a 10 W, 20 s ammonia plasma treatment, three peaks were required for the peak fit and these peaks can be assigned to amines (peak A at 398.9 eV), amides (peak B at 399.8 eV), and imides (peak C at 400.5 eV) (Figure 2a). Thus, in comparison to the C 1s peak, the N 1s peak is more valuable in evaluating the different nitrogen functionalities introduced onto the PHBV surface. We do not see a separate peak corresponding to nitrile groups on the surface. This can be due to the absence of nitrile groups or due to its peak position being too close to other peaks, making it impossible to resolve. If it is assumed that the amine peak is not overlapping with a possible nitrile peak, the calculated amine percentage from the curve fit to the N 1s peak is 21% ± 2%.

XPS of Ethylenediamine-Treated PHBV. The total amount of nitrogen groups introduced on the surface of ED-treated films was at most 4 at. %. Broadening of the N 1s peak was observed and this is similar to that observed for aqueous ED treatment of PLGA substrates.[17] Nonlinear least-squares fitting of the N 1s high-resolution peak for an ED-treated PHBV film (40% ED at 50 °C for 120 min followed by a 72 h wash) required three peaks (Figure 2b) with a relative ratio of 3.3:3.3:1, corresponding to amines (peak A at 399.3 eV), amides (peak B at 400.0 eV), and quaternary amines (peak C at 401.7 eV). The theoretical ratio of amine to amide moieties is 1:1 (Scheme 1a), which is what we observe in this and all other samples; however, the content of quaternary amine groups varied between samples. In none of these samples was any chlorine detected by XPS. The presence of quaternary amine might therefore be due to ion pair formation between protonated ED and carboxylate groups formed by hydrolysis (rather than aminolysis) of the ester bond (Scheme 1b). This suggests that even after extensive washing procedures some unreacted ED remains trapped within the material. We have previously shown[23] that diffusion of acrylic acid in methanol can occur throughout the entire cross-section of solvent-cast PHBV films during γ irradiation. It is therefore likely that also ED in water will diffuse into the PHBV substrate during the aminolysis reaction as a consequence of changes in the wettability of the substrate.

TFBA Derivatization. To verify the relative amount of amino groups on the PHBV surface of treated samples, vapor derivatization with TFBA was performed prior to XPS analysis. Derivatization XPS is an acceptable procedure used to determine

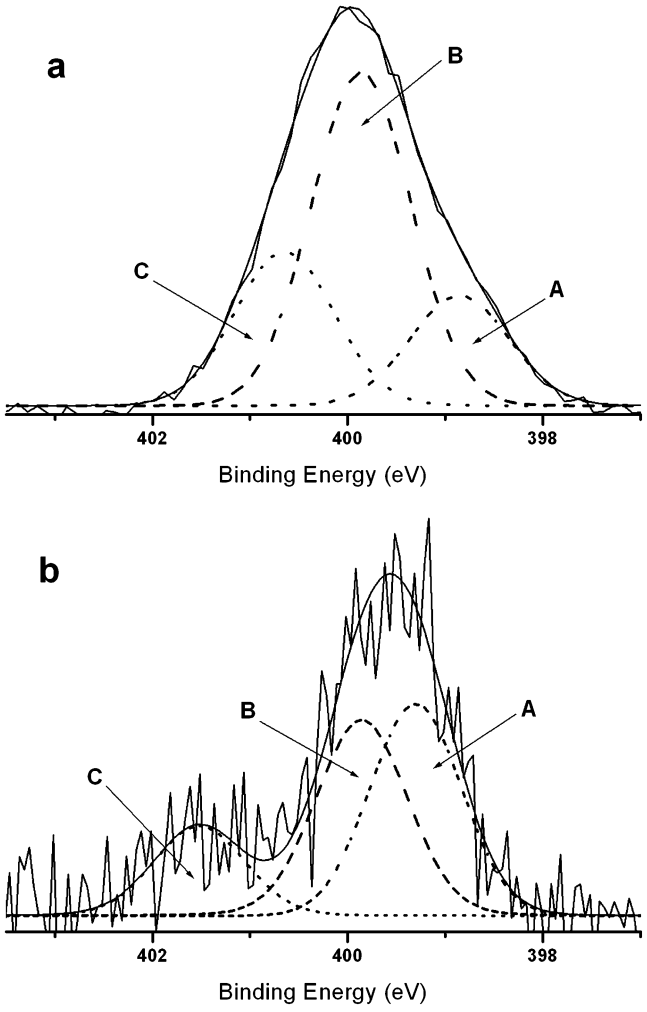
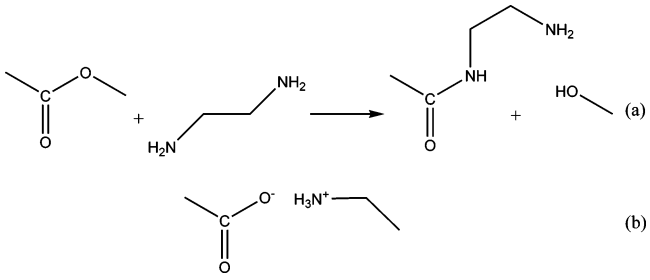
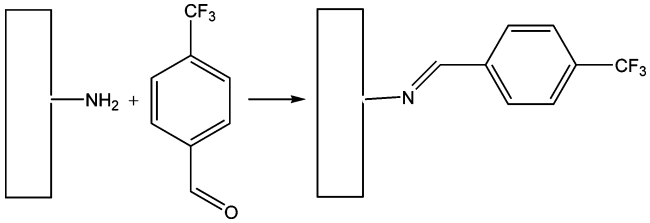


Figure 2. XPS N 1s core spectra of (a) ammonia plasma-treated (10 W, 20 s) and (b) ethylenediamine-treated PHBV (120 min ED treatment followed by 72 h wash).

Scheme 1. Reaction Scheme for (a) Aminolysis and (b) Ion Pair between Protonated Amine and Carboxylate Groups



Scheme 2. Reaction Scheme for TFBA Derivatization of Amine Functionalities



the type and relative amount of functional groups present on a surface.[34] For a successful derivatization process, selectivity of the reagent toward a particular functional group, its detectivity, kinetics of the reaction, and stability of the derivatized species are some of the factors necessary to identify.[34] During ammonia plasma treatment we find from the N 1s narrow scan that amines, amides, and imides are the main nitrogen containing functional groups on the surface of the polymer. For the ED-treated samples we find amine, amide, and quaternary amine groups. TFBA has been found to satisfy the criteria mentioned above[4,25,26] and was used in our study to quantify the number of amino groups introduced during the various treatments (Scheme 2). For all the treated samples an F 1s peak at 680 eV was observed in the XPS survey spectrum after TFBA derivatization. From the relative atomic ratios of fluorine to nitrogen, the relative amount of amino groups to the total amount of nitrogen can be calculated.

Table 1. Contact Angle and XPS Data for ED-Treated PHBV Samples[a]

treatment time (min)	washing time (h)	θ_A[b] (deg)	θ_R[c] (deg)	% NH_2 from N1s curve fit	% NH_2 from TFBA derivatization
120[d]	72	83	69		
60	72	66	41	*e*	*e*
90	48	76	55	51	20
90	72	58	39	41	12
120	48	70	41	37	18
120	72	58	36	42	7

[a] All samples were treated with 40% ED in water at 50 °C. [b] Error on θ_A is ±5°. [c] Error on θ_R is ±9°. [d] Treatment in water only. [e] Not determined.

A selection of the ED-treated PHBV films were subjected to TFBA derivatization. The amount of amine functionalities found by this method ranged from 7% to 20%, which is significantly lower than the amine content obtained from the N 1s curve fit (Table 1). Thus, for the film treated with 40% ED at 50 °C for 120 min followed by a 72 h wash, the amount of amine functionalities was found to be 7% from the TFBA derivatization experiment compared to 42% obtained from the N 1s curve fit. A possible explanation for this discrepancy is a limited availability of the amino groups[25] introduced by ED treatment. This is in accordance with the study on aminolysis of PCL membranes, where the depth of penetration of amino groups was found to be 50 μm.[18] Since our PHBV films are 50 ± 5 μm in thickness, it is likely that functionalization has occurred throughout the material.

The amount of amino groups determined from TFBA derivatization of ammonia plasma-treated samples are shown in Figure 3. Whereas the amount of total nitrogen increases to a plasma power of 15 W (for a constant treatment time of 10 s) the maximum amount of amino groups that can be introduced is independent of plasma power (Figure 3a). Similarly, for a constant treatment power of 10 W, a treatment time of 15 s is optimal for introducing nitrogen groups on the surface whereas a maximum amount of amino groups is found at shorter treatment times of 5 and 10 s (Figure 3b). Thus, a high amount of total nitrogen does not necessary impart a high amount of amino groups on the polymer surface. This result is similar to that found by other workers who have investigated ammonia gas plasma treatment of polymer surfaces.[4] The amount of amino groups on the PHBV surface determined by TFBA derivatization of ammonia plasma-treated samples compares well to the findings from the peak fitting of the N 1s peak. Thus, for a 10 W, 20 s sample 21% ± 2% amino groups were found by the N 1s curve fit (see above) and for the TFBA derivatization the same sample yielded 18% ± 2% amino groups. This suggests that within experimental error the TFBA derivatization of amino groups is 100% for ammonia plasma-treated samples and verifies that ammonia plasma treatment occurs exclusively in the outermost molecular layer, where good availability of the amino groups is expected.[37]

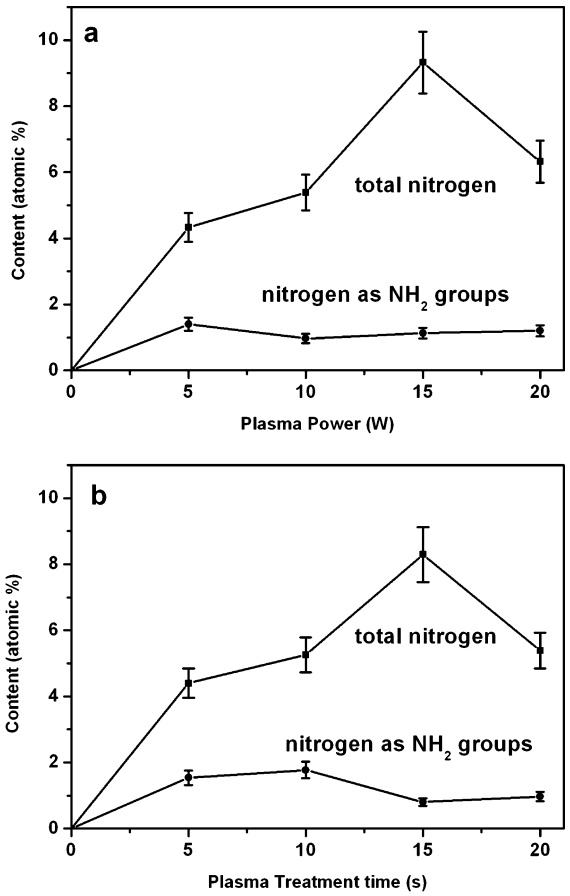


Figure 3. Atomic percent of total nitrogen and nitrogen as amino groups after different treatment power (panel a; constant time = 10 s) and different plasma treatment time (panel b; constant power = 10 W).

In summary, the maximum amount of amino groups introduced onto the outermost surface of PHBV and accessible for TFBA derivatization is for plasma treated samples 1.6 at. % and for ED-treated samples 0.08 at. %.

Surface Wettability of Ammonia Plasma-Treated Samples. The advancing water contact angle of the PHBV film was found to be 83° ± 5°, which compares well with previous studies.[22,23] Ammonia plasma treatment of PHBV resulted in production of an increasingly more hydrophilic surface with increasing plasma power at constant treatment time of 10 s. At low treatment power of 2.5 or 5 W, the advancing contact angle remained unchanged within experimental error. As the plasma power was increased to 7.5 and 10 W, the advancing contact angle decreases to 66° ± 5° and 39° ± 5°, respectively. Surfaces treated for increasing time at constant plasma power of 10 W showed no direct correlation between time and advancing contact angle (θ_A values at 5, 10, 15, and 20 s were found to be 51° ± 5°, 39° ± 5°, 43° ± 5°, and 53° ± 5°, respectively). These trends follow the amount of amino groups as determined after TFBA derivatization rather than the total nitrogen as obtained from XPS.

The water contact angle of the ammonia plasma-treated surfaces changed upon contact with air (Figure 4). Within the first 3 h the advancing contact angle increased significantly for all samples investigated (Figure 4, inset). After 1 day of storage in air, the contact angle of the samples had reached a value similar to that of an untreated surface (within experimental error). This phenomenon has been observed by several workers who have used ammonia plasma treatment of other polymers.[35−37] Chain relaxation processes and post-reaction with the storage environment were some of the contributing factors suggested

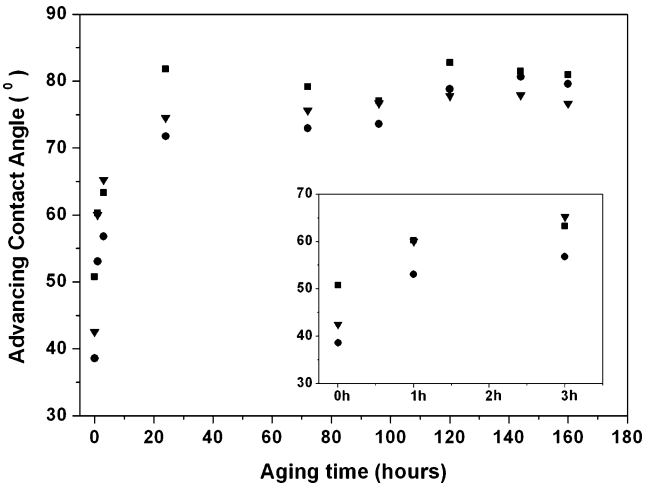


Figure 4. Effect of aging times on the advancing water contact angle of ammonia plasma-treated PHBV; the error of each measurement is ±4°. Plasma treatment conditions: (■) 10 W, 5 s; (●) 10 W, 10 s; (▼) 10 W, 15 s.

to explain the effect of wettability reversal.[36−38] We observed that plasma-treated surfaces that had been stored in air for 10 days and subsequently soaked in water for 3 days displayed a contact angle similar to that of the original value obtained immediately after plasma treatment. Thus, the contact angle for a sample treated for 5 s at 10 W initially decreased by 20° ± 5° but reverted back to the original value after storage in air. Subsequently, after soaking in water, it again showed a value 20° ± 5° less than that of the original surface. This indicates that hydrophilic groups introduced on the PHBV surface become buried when stored in air but upon water exposure can revert back to the surface. It highlights the fact that ammonia plasma treatment introduces functionalities only in the outermost molecular layer of a polymer film.[37]

Surface Wettability of ED-Treated Samples. The surface wettability of ED-treated samples (40% ED at 50 °C followed by a 72 h wash) was found to decrease with treatment time between 0 and 90 min; thus at 0 min θ_A = 83° ± 5°, after 60 min θ_A = 66° ± 5°, and after 90 min θ_A = 58° ± 5°, after which they remained constant (at 120 min θ_A = 58° ± 5°) (Table 1). This is similar to contact angles measured for ED-treated PLGA samples, which were found to initially decrease with treatment time (between 0 and 20 min) after which they remained constant at longer treatment times.[17] For our PHBV samples much longer treatment times and harsher conditions were necessary, possibly as a consequence of PHBV being more hydrolytically stable.

It was found that increased washing time of the samples yielded lower contact angles (both advancing and receding). Thus, for the 90 min ED treatment 48 h washing gave θ_A = 76° ± 5° and θ_R = 55° ± 9°, whereas 72 h washing yielded θ_A = 58° ± 5° and θ_R = 39° ± 9°. Likewise, for the 120 min ED treatment 48 h washing yielded θ_A = 70° ± 5° and θ_R = 41° ± 9°, whereas 72 h washing resulted in θ_A = 58° ± 5° and θ_R = 36° ± 9° (Table 1). This observed decrease in contact angle with increased washing time does not correlate with an increase in amine groups on the surface. The decrease in contact angle might therefore be attributed to hydrolytic degradation during the washing procedure producing an increased number of carboxylic acid end groups (see further evidence from FTIR investigation below).

It is expected that aminolysis will preferentially occur in the amorphous regions of the semicrystalline PHBV polymer,

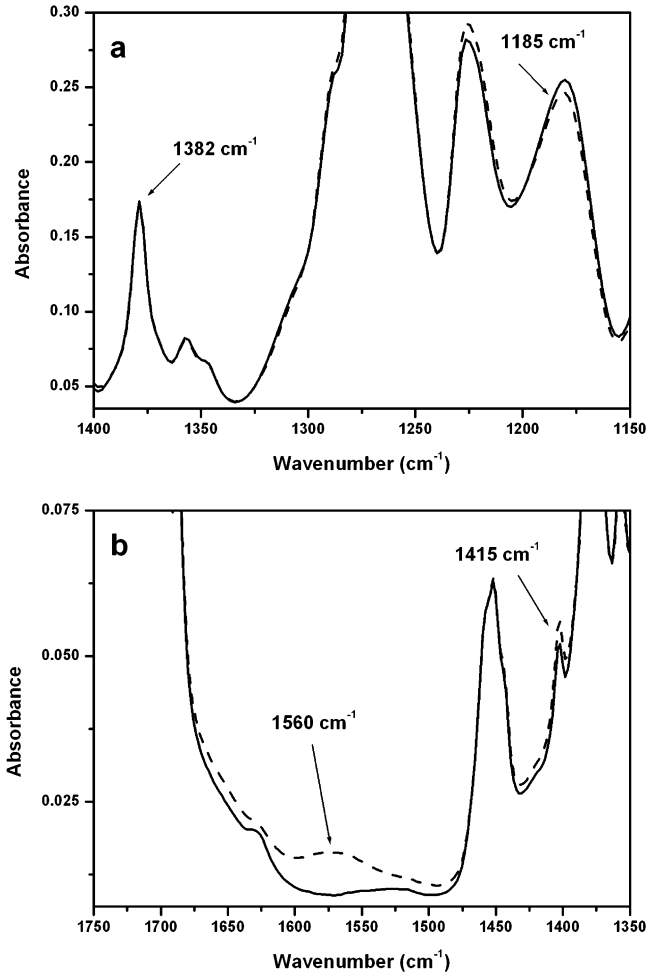


Figure 5. ATR-FTIR spectra of PHBV samples: solid line (untreated; 120 min water treatment at 50 °C follow by 72 h wash at room temperature) and dashed line (ED-treated; 120 min ED treatment at 50 °C followed by 72 h wash at room temperature) showing (a) 1400−1100 cm^{-1} and (b) 1750−1350 cm^{-1} spectral regions.

similar to what was observed for PET fibers.[19−21] If such a heterogeneous modification of the surface had occurred, increased roughness could be expected; however, in our samples no increase in contact angle hysteresis was observed (untreated $\theta_A - \theta_R = 18° \pm 10°$ and 120 min ED-treated $\theta_A - \theta_R = 22° \pm 10°$, Table 1).

Long-Term Stability of ED-Treated PHBV Samples. It was noticed that after 3 weeks of storage the ED-treated samples became brittle, indicating an increase in crystallinity. Changes in FTIR spectral features—band shape or position—can reveal bands that are sensitive to the change in crystallinity.[39,40] Bloembergen et al.[40] observed from the infrared spectrum of PHBV that the relative intensity of the band at 1185 cm^{-1} displayed the largest difference in intensity between crystalline and amorphous states. Crystallinity index, CI, was defined by normalizing the 1185 cm^{-1} band to that of the 1382 cm^{-1} band, which is insensitive to the degree of crystallinity. Applying a similar analysis to FTIR spectra of samples of this study clearly showed a decrease in the 1185 cm^{-1} band (Figure 5a) when an untreated but washed sample (120 min water treatment follow by 72 h wash) was compared with an ED treated sample (120 min ED treatment followed by 72 h wash). The calculated crystallinity indices from each of these samples were found to be 0.89 and 0.95, respectively.

Further inspection of the FTIR spectra of the ED-treated samples showed the presence of a new band at 1560 cm^{-1} (Figure 5b) and a weak increase in the intensity of the band at 1415 cm^{-1}, which can be attributed to the antisymmetric and symmetric COO^- stretching vibration, respectively, of the carboxylate group. The formation of carboxylate groups in the ED-treated samples indicates degradation of the polymer and is in agreement with the findings from contact angle measurements (above).

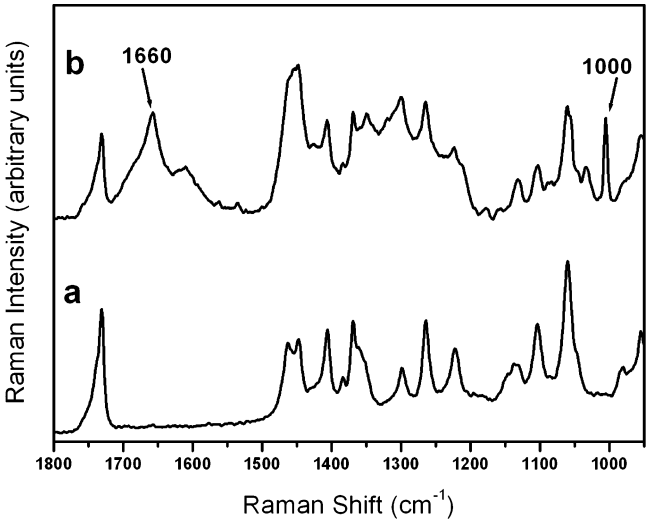


Figure 6. Raman spectra of (a) ammonia plasma-treated (10 W, 20 s) PHBV and (b) after TFBA derivatization.

The long-term stability of the ED-treated PHBV samples (even when lower concentrations of ED were used) was poor even though the samples were subjected to rigorous washing after treatment. This clearly inhibits the use of ED aminolysis to introduce amino groups onto the surface of PHBV materials.

Spatial Distribution of Amino Groups. Raman microspectroscopy of modified PHBV samples was used to assess the spatial distribution of amine functionalities on the substrates. Figure 6 shows the Raman spectra of the region 1800−900 cm^{-1} of an ammonia plasma-treated PHBV film (a) and a TFBA-derivatized plasma-treated PHBV film (b). The derivatization of amino groups with TFBA produces stable azomethine groups (Scheme 2), which can be seen as a broad Raman band at 1660 cm^{-1} corresponding to the C═N stretching vibration. In addition, a sharp, intense band at 1000 cm^{-1} can also be detected, and this is attributed to the breathing mode of the aromatic ring of TFBA.

To investigate the spatial distribution of TFBA groups on the PHBV surface, spectra from the Raman maps were assessed for the presence of TFBA functional groups. The integrated ratio of the 1660 cm^{-1} band (characteristic band of TFBA) and the 1750 cm^{-1} band (characteristic band of PHBV) of the accumulated Raman spectra were calculated and these ratios were collated as a function of *x* and *y* position to produce the Raman maps of TFBA. From our observations described above it seems that TFBA reacts only with amino groups presented on the surface and the Raman maps will therefore give an indication of the distribution of these surface amino groups, which in turn will be accessible for biomolecule attachment.

Figure 7 depicts Raman maps of the TFBA-derivatized ED-treated (a), TFBA-derivatized ammonia plasma-treated (b), and untreated (c) PHBV surfaces. The different shades of gray coloration are indicative of the value of the Raman band ratio. For the untreated PHBV sample the range observed is 0.002−0.018, whereas for ED-treated samples the range is 0.004−0.035 and for ammonia plasma-treated samples the range is 0.001−0.05. Thus, for areas of intensities higher than 0.018 the presence

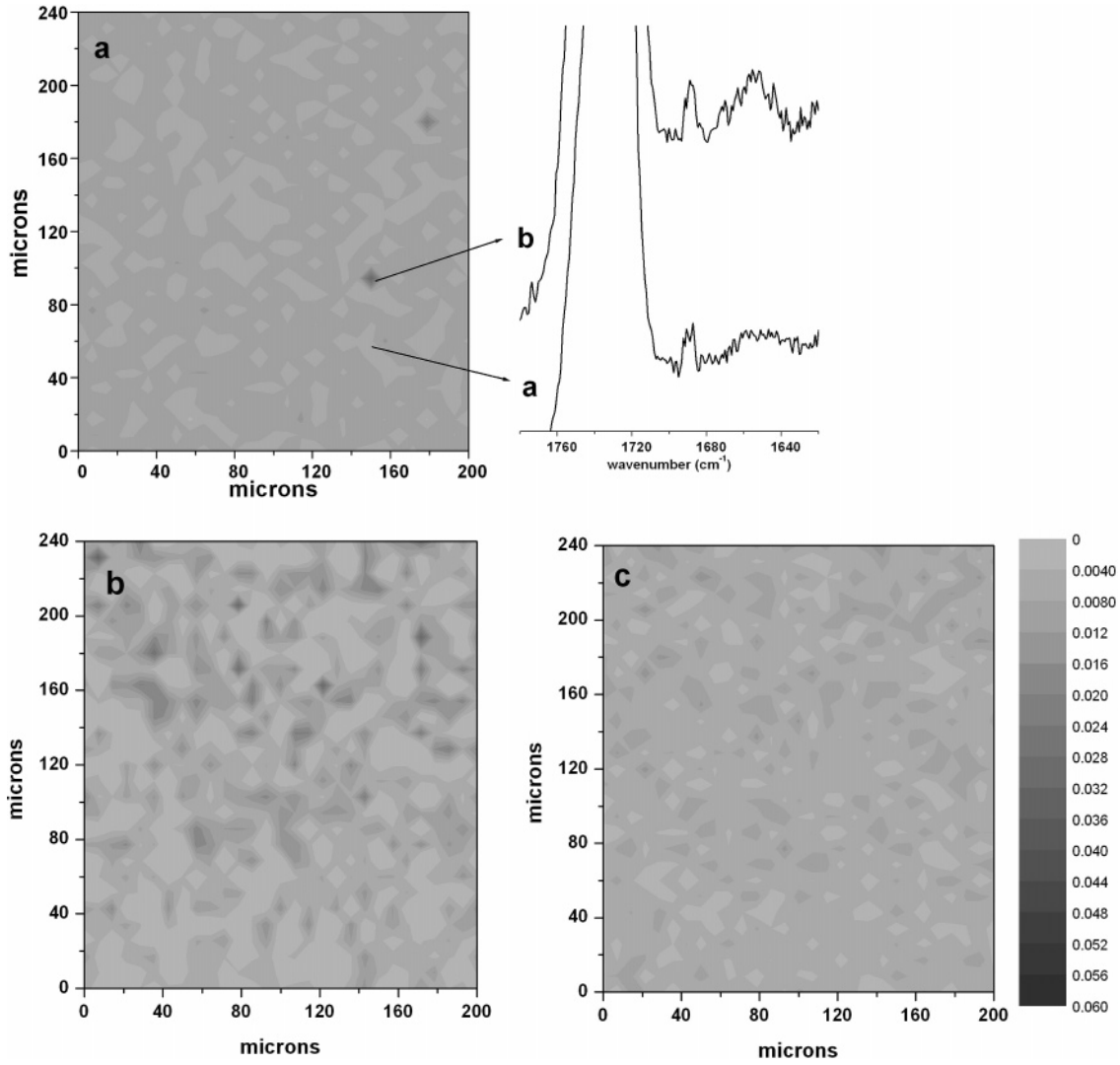


Figure 7. Raman map of TFBA-derivatized (a) ED-treated (90 min ED treatment followed by 48 h wash), (b) ammonia plasma-treated (10 W, 20 s), and (c) untreated PHBV across an area of 200 μm $\times$ 240 μm.

of TFBA (and thus amino groups) is inferred and the darkest shade of gray denotes the highest concentration (Figure 7, inset scale). Thus, not only is there a larger area on the ammonia plasma-treated sample with an intensity higher than 0.018 compared to the ED-treated sample, but the maximum intensity is also higher on these samples. This indicates that more amino groups are available for derivatization in ammonia plasma-treated samples compared to ED-treated samples. This is in accordance with the amino group atom percent calculated from XPS data (above). From the Raman maps it can be seen that the spatial distribution of TFBA is patchy and uneven, suggesting the presence of large areas having no amine functionalities.

Conclusions

Creating amine functionalities on a PHBV surface can be achieved by ammonia plasma treatment or ED aminolysis. It was found that ammonia plasma gave a larger amount of amino groups on the surface as assessed by XPS after TFBA derivatization. The ED aminolysis method was considered less advantageous as the modified films suffered from degradation and an increase in crystallinity during storage. The spatial distribution of the amino groups found by Raman mapping on the substrates treated by the two methods was patchy and uneven.

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