Quantitative CD ATR-FTIR and CD SEM-EDX analyses of locally amino-functionalized polymer surfaces

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1. Introduction

Cold atmospheric-pressure plasmas in form of dielectric barrier discharges (DBD) have commonly been used in the industry for several decades to improve surface properties of polyolefines and other polymers by generating a multitude of chemical functional groups on their surfaces and thereby increasing their surface free energy. Frequently specific plasma-chemically generated reactive moieties can only be identified or distinguished by utilizing group selective chemical derivatization or labeling techniques. It results, for example, in the incorporation of unique elements, which are not contained in the plasma-treated material before labeling [1]. X-ray photoelectron spectroscopy (XPS) applied after chemical derivatization (CD-XPS) is one of the most often used tools for quantitative chemical analysis of plasma-modified polymer surfaces. In this contribution we present results of two new analytical methods, namely quantitative CD ATR-FTIR and CD SEM-EDX analyses for plasma-modified surfaces of polyolefines. Polymer surfaces were locally amino-functionalized by a short contact of them with microplasmas in N-containing gases. Both analytical methods are based on the selective derivatization of primary amino groups with TFBA (4-trifluoromethyl-benzaldehyde) for quantitative determination of -NH_2 area densities and their spatial distributions.

2. Experimental

2.1. Plasma printing with PDMS stamps

The experiments were carried out applying plasma stamps based on polydimethylsiloxane (PDMS) with different thicknesses of the dielectric barrier for high-voltage (HV) and low-voltage (LV) plasma excitations. Electrodes with cavities diameter of 500 µm and 1000 µm, and heights of 350 µm were utilized. Detailed description of the electrodes with their fabrication processes is given in [2]. A schematic view of the experimental so-called plasma printing setup is shown in **Fig. 1**. It consists of two vacuum chucks allowing the parallel arrangement of the microplasma stamp and the polymer substrate to be treated. During plasma printing process the polymer substrate is compressed together with the plasma stamp to generate a process gas in enclosed cavities with well controlled spatial extension of the plasma. The upper borosilicate glass chuck assembled with a transparent acrylic

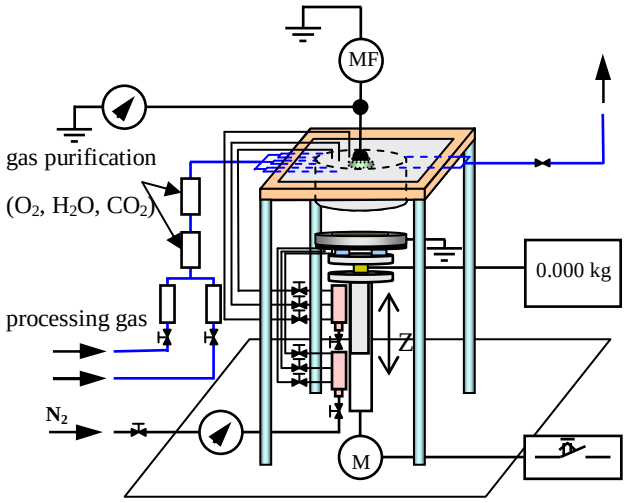


Fig. 1 Setup for plasma printing of amino groups.

compartment forms an upper part of a gas chamber. The plasma stamp is vacuum-fixed to the glass chuck. This arrangement provides good observation of the ignited plasma. The substrate to be functionalized is placed on a grounded aluminium table (lower chuck). The table serves as a counter electrode and simultaneously as a bottom part of the gas chamber. The table is arranged on the top of the electric mini slide enabling good compression between microplasma stamp and the substrate with adjustable force up to 196 N at 1 bar. Recent research has shown the very strong influence of O_2 content in the plasma gases on the amino-functionalization quality, due to the oxygen diradical competing very effectively with nitrogen species in the amination reaction [3, 4]. Therefore, plasma gas was purified in order to keep O_2, H_2O and CO_2 contents at sub-ppm levels. A process gas supply is realized on the side of the acrylic compartment just opposite the plasma stamp. Removing of the air from the setup is achieved by flushing of the gas chamber with the process gas during repeated extension and reduction of the gas volume enclosed between the acrylic compartment, the glass chuck and the substrate table.

The surface plasma-amination was carried out at 1, 3, 5, 7 and 10 s of plasma exposure in virtually oxygen-free N_2 + 4 % H_2 and N_2 + 10 % NH_3 gas atmospheres with 85-100 N of contact pressing force. Solvent-cleaned biaxially oriented polypropylene (BOPP, Goodfellow

GmbH, Germany), low-density polyethylene (LDPE, additives-free, Goodfellow GmbH, Germany) foils and grinded carbon-filled minidiscs (PP/C204, produced from CESA® conductive granulate, Clariant GmbH, Germany) of 75 µm, 50 µm and 1.5 mm thickness, resp., were utilized as the substrates. The substrates were pre-cleaned by washing three times in isopropanol, three times in acetone, and drying in a stream of pure N_2. The required power for plasma printing was supplied by a medium-frequency generator 7010 R and high-voltage transformer AT 7010 R (Softal electronic GmbH, Hamburg, Germany) operated in the continuous wave mode (peak voltage 10.8 kV, 23 kHz). To avoid, as far as possible, the formation of plasma-etching products formed from the PDMS stamp, prior to the first ignition of all the stamps in a controlled atmosphere, passivation of the stamp walls by plasma-oxydation in air (8.5-9 kV, 23 kHz) with 2 min of plasma exposure and 85-100 N of contact pressing force was performed.

2.2. Chemical derivatization (CD) for quantitative analyses

Gas-phase derivatization with TFBA (4-trifluoromethyl-benzaldehyde, Sigma-Aldrich GmbH, Germany) was utilized according to the reaction demonstrated in **Fig. 2** to label primary amines in order to evaluate their area densities and spatial distributions within functionalized microspots:

Locally aminated samples were placed in a closed glass

Fig. 2 Scheme of the derivatization reaction.

vial to be exposed to vapours of 0.5 ml TFBA under Ar protective atmosphere at room temperature for 2 h in the case of BOPP and LDPE foils, and for 1 h in the case of PP/C204 minidiscs. To remove the physisorbed products from the polymer surfaces the specimens were flushed overnight with the very low stream of pure N_2.

2.3. Quantitative chemical derivatization ATR-FTIR analysis (CD ATR-FTIR)

Quantitative attenuated total reflectance mode spectroscopy after selective chemical derivatization (CD ATR-FTIR) has been applied to evaluate area densities of surface-bond -NH_2 groups introduced to the polymer surfaces [3, 4]. ATR measurements were performed on a Nicolet 5700 FTIR spectrometer equipped with an MCT detector and a DuraSamplIR single reflection 45° diamond ATR crystal. Unpolarized light and a spectral resolution of 1 cm^{-1} were utilized. There are three relatively strong absorptions bands in the wave number region between 1120 and 1350 cm^{-1} related to the presence of CF_3 groups attached to a benzene ring of TFBA. These bands appear at 1323 to 1325, 1169 and 1134 cm^{-1} and can be detected by ATR-FTIR on the polymer surface with TFBA-derivatized microspots. The strongest C-CF_3 stretching absorption band located at 1323 to 1325 cm^{-1} and associated with the TFBA moiety was used to determine the area densities of primary amines on the surface of polyolefines. The quantitative CD ATR-FTIR measurements are based on a comparison of ATR spectra of plasma printed TFBA-derivatized polymer substrates and suitable reference solutions, containing small molecules with the same functional groups in an environment similar to the polymer. Measurement calibrations were performed with the dilute solutions of three low-molecular imines dissolved in hexadecane and reacted with TFBA [3].

The sampling depth of the ATR method is roughly 1 µm. The evaluation of the area density of -NH_2 groups contained within the ultra thin selectively plasma-modified surface region was based on Eq. (1) using the assumption of equal molar absorption coefficients for the characteristic vibrations in the polymer and the reference solution:

$$\rho_{NH2} = A_{Pq} c d_p / 2 A_R \qquad (1)$$

where A_{Pq} - the hypothetical absorbance of a uniform film, covering the complete sampled area with the same thickness as the (average) thickness on the microspots; c - known concentration of CF_3 groups in the reference solution; d_p - penetration depth; A_R - the absorbance of the reference solution. A_{Pq} is used in Eq. (1) because the microspots cover only a fraction q of the area of the ATR crystal (1.87 mm^2); A_{Pq} and reflectivity R_{Pq} are calculated from the measured absorbance of a polymer A_P using Eq. (2):

$$A_{Pq} \equiv \log(1/R_{Pq}) = -\log[(10^{-A_P} - 1 + q)/q] \qquad (2)$$

2.4. Quantitative chemical derivatization SEM-EDX analysis (CD SEM-EDX)

Quantitative X-ray microanalysis of fluorine introduced to the plasma-treated and TFBA-derivatized microspots was performed by energy dispersive spectrometry in a scanning electron microscope (CD SEM-EDX) in order to obtain quantitative information about area densities and spatial distributions of primary amino groups on the polymer surfaces. CD SEM-EDX analysis was carried out using a SEM Leo 1530 (primary electron energy E_0 = 1.5 kV, Oxford EDX-system with Ge detector). The depth of the analysis is given by the ultimate depth of X-ray emission (d_e). In case of not too strong X-ray absorption it is almost identical with the maximum depth of X-ray generation (d_{max}), which can be calculated from Castaing's formula [5]:

$$d_{max}[\mu m] = (0.033 / \rho) \cdot (E_0^{1.7} - E_C^{1.7}) \cdot (A / Z) \qquad (3)$$

where ρ [g/cm^3], A [g/mol] and Z are the density, mean atomic weight and the mean atomic number, resp., of the investigated material; E_C [keV] is the critical excitation energy of the X-ray line. For E_0 = 1.5 keV the maximum X-ray emission depth $d_e \approx d_{max}$ for F Kα (E_C = 0.67 keV) in a typical polymer is about 50 nm. The evaluation of the CD SEM-EDX measurements is based on the comparison of the net intensities of F Kα and C Kα (I_F, I_C), obtained from TFBA-derivatized specimens and a reference sample

of PTFE serving as a standard, resp. K-ratios are calculated from these intensities according to Eq. (4):

$K \equiv K_F/K_C \equiv (I_{F(sample)}/I_{F(standard)}) / (I_{C(sample)}/I_{C(standard)})$ (4)

The standard and the samples were coated with evaporated carbon film with a defined thickness between 8 and 18 nm, controlled by the distance d between evaporation source and the polymer. The C coating makes the surfaces not only electrically conductive but simultaneously prevents fluorine loss by the radiation damage during the analysis. X-ray reflectivity measurements (XRR) were carried out using PANalytical's X'Pert PRO MRD under ambient conditions to obtain the thicknesses of the C coatings deposited with a MED 020 high vacuum coating system (Bal-Tec, now Balzers AG, Liechtenstein) at d = 5.5 and 7.5 cm, resp. The thicknesses of the C coatings determined on Si wafers for these distances were 15.0 ± 1.0 nm and 9.0 ± 1.0 nm, resp., with a layer density of 2.1 g/cm³. The same C-coated Si wafers were investigated by means of electron probe micro-analysis (WDS-EPMA, Cameca SX-100) at 20 keV. The values of C coating thicknesses calculated from EPMA measurement were 18.0 ± 1.5 and 10.0 ± 1.0 for d = 5.5 and 7.5 cm resp. EPMA measurements were also applied to determine the chemical composition of the PTFE standard (53 at.% F, 47 at.% C). CD SEM-EDX measurements were accomplished with the simulations (STRATAgem program, SAMx, France) of X-ray production and absorption in multilayer stacks corresponding to TFBA-derivatized specimen performed for different C coating thicknesses in the range of 6 - 20 nm in order to derive the K-ratios (K_F/K_C) by changing the fluorine mass thicknesses (see **Fig. 3**). A description of the SAMx-STRATAgem program is given in the manual [6]. The simulations results of K-ratios were used to calculate the density of fluorine atoms from SEM-EDX measurements, which is three times the original amino group density. Spatial resolutions of 10 - 15 µm and 100 - 110 µm for CD SEM-EDX measurements were utilized although a much higher, sub-µm resolution can be achieved with the method in principle.

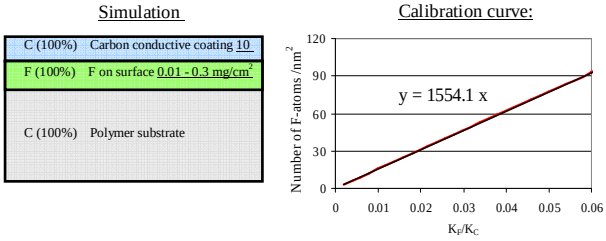


Fig. 3 Determination of K-ratios (K_F/K_C) with simulation STRATAgem varying the area density of F atoms from 3.2 up to 96 per nm^2.

2.5. Qualitative fluorescence surface characterization

Fluorescence labeling with fluorescamine (Fluram™, Fluka) was employed to indicate the presence of primary amines. Fuorescamine was dissolved in dry acetone at the concentration of 25 mg/100 ml. The treated samples were immersed into the solution for 5 min, subsequently washed twice in dry acetone and once in ethanol in order to hydrolyze unreacted fluorescamine, and then dried with a stream of pure N_2. Fluorescence intensity of the samples was measured with a Biochip Reader (Biodetect, Fraunhofer IPM, Freiburg, Germany) with filter setting: λ_{ex}= 380 nm to 410 nm, λ_{em}= 440 nm to 480 nm.

Bioassays were carried out on peptide arrays synthesized at Helmholtz Centre for Infection Research GmbH (HZI, Braunschweig) with two blocks of β-alanine coupled to plasma-introduced amino groups. The peptides were N-terminally biotinylated. Binding to the biotine moiety was probed using Cy5-labeled streptavidine. Cy5-fluorescence was quantified using a Biochip Reader with filter setting: λ_{ex}= 635 nm to 645 nm, λ_{em}= 671 nm to 693 nm.

3. Results and discussion

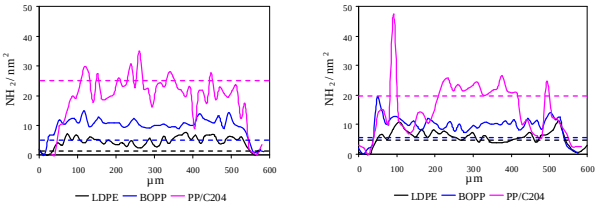
PDMS electrodes were tested and proved to be very efficient for local functionalization with primary amines of all applied polymers. Since fabrication of the LV-design electrodes is a time demanding process, our experiments were carried out only with HV-design electrodes.

Plasma-treated PP/C204 conductive specimens were exposed to TFBA vapours for 1 h instead of 2 h of exposure as was applied for LDPE and BOPP. A noticeable signal of fluorine was registered by SEM-EDX not only on plasma treated but also on untreated areas of composites after 2 h of exposure to TFBA vapours, due to a strong physisorption of TFBA on the graphite added to PP/C204 to make it conductive. It causes an additional calculation of the net intensities of fluorine signal on plasma treated spot by subtraction of the "background" fluorine signal on untreated areas from it. After 1 h of exposure of the PP/C204 specimens to TFBA vapours the fluorine signal on untreated areas was insignificant and could be ignored.

The results of quantitative measurements of area densities and spatial distributions of primary amino groups, introduced to surfaces of polyolefines are shown in **Fig. 4**. They were obtained by microplasma treatment of polymers in N_2 + 4 % H_2 and N_2 + 10 % NH_3 gas mixtures applying plasma stamps with cavities diameter of 500 µm. Derivatization with TFBA was carried out directly after plasma-functionalization. The average densities of -NH_2 introduced area-selectively on LDPE, BOPP and PP/C204 surfaces were measured to be 2, 5.3 and 26.2 -NH_2 per nm^2, resp., by CD ATR-FTIR analysis. The average values of -NH_2 area densities obtained from CD SEM-EDX were 5.2, 11.2 and 22.2 -NH_2 per nm^2 for LDPE, BOPP and PP/C204 substrates resp. The latter were calculated as average value for the C layers of 8, 10 and 12 nm taking into account the insufficient reproducibility of C coating thicknesses by vacuum deposition at defined distance d and the difference of the C coating thickness values taken from XRR and WDS-EPMA. A

spatial resolution of 10 - 15 µm for CD SEM-EDX analysis was used.

All the values presented in **Fig. 4** include low-molecular weight components. Utilizing N_2 + 4 % H_2 plasmas, sufficiently uniform amination over the diameter of the spots has been achieved on all polymer substrates. Significant departures from the uniformity are visible for the conductive PP/C204 substrate applying NH_3-containing atmosphere.



a) b)

Fig. 4 Distributions of primary amino group densities across the diameters of plasma-printed 500 µm spots on LDPE, BOPP, and PP/C204 composite, resp., obtained (a) in N_2 + 4 % H_2 and (b) in N_2 + 10 % NH_3 by CD SEM-EDX. Average values calculated from CD ATR-FTIR measurements are shown for comparison as dotted lines.

The results of CD SEM-EDX measurements are in a reasonable agreement with average amino group densities determined by CD ATR-FTIR. Especially good correlation between two independent analytical methods was achieved for PP/C204 composites. The fraction q used for the area density determination by CD ATR-FTIR can be easily calculated for PP/C204 from light microscope measurements. The plasma-treated microspots on the PP/C204 surface are clearly seen. After plasma-functionalization of LDPE and BOPP foils the treated microspots cannot be seen. The average value of -NH_2 area densities for LDPE and BOPP were obtained applying maximum and minimum values of fraction q determined for PP/C204 from 10 measurements. The correlation between CD ATR-FTIR and CD SEM-EDX can be improved by regular measurement of the C coating thickness on a Si-wafer vacuum deposited together with the specimens prepared for CD SEM-EDX analysis.

We performed plasma printing at 1, 3, 5, 7 and 10 s utilizing the electrodes with cavities diameter of 1000 µm in N_2 + 4 % H_2 gas atmosphere to find out the influence of the plasma treatment duration on the area density of introduced -NH_2 to the PP/C204 surface followed by TFBA-derivatization directly after plasma-functionalization. The spots size was chosen to manufacture bioassays with two blocks of β-alanine coupled to plasma induced amino group. The average values of -NH_2 area densities calculated from CD SEM-EDX measurement with spatial resolution of 100 - 110 µm were 2.8, 3.2, 3.6, 9.9 and 25.5 -NH_2 per nm^2 for the mentioned treatment duration, resp. Such noticeable rising of the density values of -NH_2 with increasing treatment time can be explained with growing number of low-molecular weight components accumulated on the polymer surface containing primary amino-groups derivatized with TFBA. In order to appreciate the stability of the plasma-introduced primary amino groups we exposed specimens treated at 7 and 10 s to dry acetone for 5 min corresponding to the routine fluorescamine-labeling technique and to DMF (Merck KGaA, Germany) for 3 h. Subsequently derivatization with TFBA was applied. The results of CD SEM-EDX measurement showed considerable decreasing of -NH_2 area densities from 9.9 and 25.5 -NH_2 per nm^2 for 7 and 10 s of treatment duration to 3.3 and 2.8 -NH_2 per nm^2 after 5 min of exposure in acetone; and to 1.6 and 2.0 -NH_2 per nm^2 after 3 h of exposure in DMF resp. Spatial distribution of primary amines introduced to the PP/C204 surface at 10 s treatment duration and remaining -NH_2 after exposure in above mentioned solution are demonstrated in **Fig. 5**. It can be concluded, that not all of the plasma-introduced amino groups are strongly attached on to the polymer surfaces. The lightly attached -NH_2 have already been washed after the immersion of microplasma-treated samples in to the fluorescamine solution for 5 min by the labeling procedure. After 3 h of exposure in DMF the number of primary amines is reduced negligibly in comparison with the exposure of specimens to dry acetone for 5 min. The number of remaining -NH_2 after 3 h of exposure in DMF changes insignificantly with increasing treatment time between 3 and 10 s.

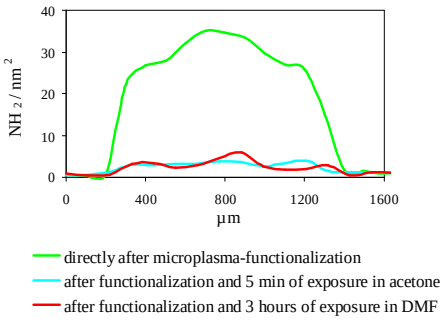


Fig. 5 Distributions of primary amino group densities across the diameters of plasma-printed 1000 µm spots on PP/C204 composite obtained in N_2 + 4 % H_2 at 10 s of microplasma-treatment duration.

To evaluate qualitatively the stability of -NH_2 labeling with fluorescamine was performed in two steps. The first labeling step was made directly after amino-functionalization of the specimens. The second step to label the specimens was made after amino-functionalization followed by 3 h of their exposure in DMF. The results of the first labeling step demonstrated, that the fluorescence signal corresponding to -NH_2 remaining after 5 min of exposure in fluorescamine solution grows with the increasing treatment duration from 1 up to 10 s. The average value of fluorescence intensity signal to be measured with the Biochip Reader was 1026 and 1748 units for 1 and 10 s of plasma expo-

sure resp. The results of the second labeling step showed a considerable decrease of the fluorescence intensities for all treatment durations after 3 h of exposure in DMF. Moreover, the intensities of labeled -NH_2 insignificantly rise with increasing treatment time between 3 and 10 s. The latter was proved by amino-functionalization of the PP/C204 specimens at 1, 3, 5, 7 and 10 s of plasma exposure followed by manufacturing of peptide arrays carrying biotine at the N-terminal amino groups. Biotine was subsequently probed using Cy5-streptavidine in a bioassay. Producing the peptide arrays involves 3 h of specimen exposure in DMF and is described in detail elsewhere [7]. Fluorescence intensity distribution on the specimens confirmed an insignificant increase of the florescence intensities corresponding to remaining -NH_2 after 3 h of exposure in DMF with increasing treatment time between 3 and 10 s. The average values of fluorescence intensity signal of 30693 and 35692 units were determined for 3 and 10 s resp.

Spatial distribution of -NH_2 within plasma modified areas obtained in the absence of NH_3 appeared to be sufficiently uniform in spite of generally non-uniform plasma intensity distribution (see **Fig. 4** and **Fig. 5**) thanks to the crucial role of metastable N(4S) and N_2(A) species [3].

4. Conclusions

Newly developed CD ATR-FTIR and CD SEM-EDX analytical techniques are promising methods to be used for quantitative evaluation of area densities and spatial distributions of functional groups (here -NH_2).

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