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Fluorescence Spectroscopic Studies on Plasma-Chemically Modified Polymer Surfaces with Fluorophore-Labeled Functionalities

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Abstract Molecular engineering of polymer surfaces that includes the attachment of functional molecules to existing or previously generated reactive chemical groups like e.g., $-OH$, $-NH_2$, or $-CHO$ requires simple strategies and tools for the controlled generation of surface functionalities and their derivatization as well as for their identification and eventually quantification. Here, we systematically investigate the plasma-chemical surface modification of polypropylene films in combination with dansyl labeling chemistry and conventional, yet costly, XPS and highly sensitive fluorescence spectroscopy for the detection of surface groups. Based on these results, the potential of and requirements on the fluorometric characterization and quantification of surfaces functionalities are discussed.

Keywords Fluorescence spectroscopy · Surface functionalization · Covalent labeling of polymer surfaces · Plasma modification · Fluorescent probe

Introduction

Chemical reactions at surface functionalities are of ever increasing importance for molecular-level engineering of surfaces and provide, e.g., the basis for the attachment of sensor and biomolecules to various supports [1,2]. Here, polymers are gaining in importance. For the fabrication of polymeric support materials for bioanalytical techniques employing fluorescence detection, problems arise from the limited number of suited polymer substrates. Only polymethyl methacrylate (PMMA), polycarbonate, cyclic olefinic copolymers (COC), and polypropylene (PP) have the necessary optical properties and the chemical and temperature resistance for the attachment of functional molecules by means of chemical reactions for the attachment of functional molecules by means of chemical reactions [3]. An elegant approach to introduce, e.g., reactive $-OH$, $_2-NH$, or $-CHO$ groups into polymers and to tailor surfaces is the application of plasma-chemical processes. Modification of polymer surfaces by low-temperature glow discharge plasmas enables the control the hydrophilicity as well as the adsorption and wetting properties of polymers [4, 5]. Plasma-chemically activated polymeric support materials can be further modified with surface-bonded functional molecules by chemical reactions. For the application of such sophisticated materials, a crucial point remains the characterization of these materials with respect to the type and density of reactive functional groups at the surface. Since typically used analytical methods (like photoelectron spectroscopy XPS, FTIR, SIMS, etc.) are costly and often fail at low concentrations of surface functionalities, the application of comparatively simple and very sensitive fluorometric the methods in combination with labeling techniques is very attractive. Despite the obvious potential of fluorometry, however, there exist only few reports on the characterization of chemically or plasma-modified polymer films [6–8] via measurement of fluorescence. Even though all authors reported the qualitative detection of characteristic fluorescence bands, the potential of fluorometry for quantitative analysis of surface-attached labels is still under debate [8, 9], as the characterization and quantification of fluorescently labeled surface functionalities is complicated by different factors [10]. This includes, e.g., nonspecific adsorption, inhomogeneous

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dye distribution, and penetration of dye molecules into the polymer as well as dye–dye interactions that typically lead to fluorescence quenching and the sensitivity of the spectroscopic properties of the fluorophore to its microenvironment.

Since many analytical methods in the life sciences rely on fluorescence measurements on surfaces of, e.g., microarrays, functionalized microplates, or sensor devices [11], the validation of fluorescence methods for surface analysis seems to be straightforward and advisable. In addition, well-characterized fluorophore-labeled surfaces can be exploited for example as fluorescence intensity standards for the microarray technology [12] and single molecule spectroscopy (SMS) [13] as well as fluorescing reference layers, as desired for image calibration in quantitative fluorescence microscopy [14].

This motivated us to perform systematic investigations of a series of plasma-chemically modified and subsequently fluorophore-labeled polypropylene films. Here, first steps toward the development of validated fluorescence methods for the evaluation of surface functionalities of model systems are presented. Different synthetic concepts are used for covalent coupling of commercially available dansyl labels to plasma-chemically generated functional groups on polypropylene surfaces. Aside from the spectroscopic investigation of surface-bound fluorophores, the emission measurements are correlated with XPS results, thereby evaluating the potential of steady-state fluorometry for the characterization and quantification of surface functionalities.

Materials and methods

Chemicals and materials

Polypropylene (PP) films (thickness 100 μm (Goodfellow, U.K.)) were ultrasonically cleaned for 15 min in a diethyl ether bath. All solvents (tetrahydrofurane (THF), ethanol, methanol) were distilled and dried before use. THF was stored over molecular sieve for more than 1 week before use. Toluylene-2,4-diisocyanate (TDI), (80/20 isomer mixture, pro synthesis, Merck, DE), methylene-di-*p*-phenylene (MDI), (pro synthesis, Sigma-Aldrich, DE), hexamethylene diisocyanate (HDI), and dibutyltin dilaurate (DBTL) (>98%, Merck, DE) were used as received. The dyes dansyl chloride (DNS), dansyl cadaverine (DNS-Ca), and dansylhydrazine (DNS-Hy) (purity of all fluorescent labels >95%), provided by Sigma-Aldrich Gmbh (former Fluka AG, Switzerland) were applied as received.

Plasma-chemical surface modification

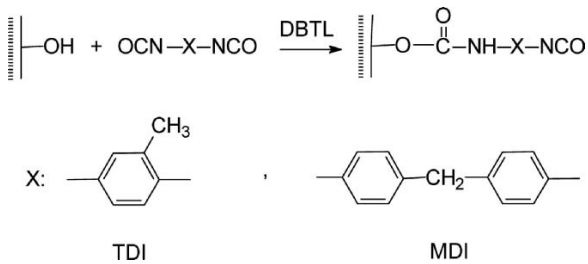
O_2-Plasma treatments were performed in a 50 dm^3 volume of a cylindrical plasma reactor. The design of the plasma reactor has been described elsewhere in detail [15]. The plasma reactor is equipped with a radio-frequency generator (r.f. 13.56 MHz) with automatic matching unit and an r.f. bar antenna (length: 35 cm), mass flow controllers for gases and a turbo molecular pump for generating an oil-free high vacuum. The polymer substrates were mounted on a grounded steel cylinder (Ø 10 cm, length 35 cm), which was rotated with a frequency of 0.5 –1 s^{-1} at a distance of 10 cm from the r.f. powered electrode. Surface functionalization of PP films in O_2-plasma was performed in the continuous-wave (cw) mode using a power input of 100 W at a standard pressure of 10 Pa.

Wet-chemical reduction of O_2 plasma-treated polypropylene films

The oxygen plasma-treated films were immersed in 12 mL dry THF and 3 mL of 1 M diborane THF complex solution (1.0 M, stabilized with 0.005 M $NaBH_4$; Fa. Aldrich) and stirred under nitrogen atmosphere at room temperature for 18 hrs. The films were removed and dipped in an alkaline mixture of H_2O_2 and THF for 2 hr. After washing with THF, water, and methanol, the modified PP–OH films were dried and stored in a desiccator.

Chemical attachment of spacer molecules

The reaction of surface-bonded OH groups with diisocyanates was performed according to Scheme 1. In order to exclude humidity, reaction vessels and hydroxyl-modified PP films (PP–OH) (5 cm × 5 cm) were dried at 30°C for about 1 hr before the reaction. The films were dipped, thereafter, in dry THF under dry N_2 flow. The THF-solution of the isocyanates and the catalyst DBTL were added with a syringe. The films were shaken various times at room



Scheme 1 Schematics of the general reaction of OH-groups at polymer surfaces with diisocyanates

temperature. Afterwards, the NCO-modified PP films (PP–NCO) were washed twice in dry THF.

Fluorophore labeling

The reaction of NCO-functionalized films with fluorescent labels containing amino groups was carried out in dry THF or ethanol. After addition of the THF-solution of the dye, the reaction was continued for 4 hrs in the dark. The films were washed with ethanol, water, and acetone. Fluorophore-modified films were stored in the dark prior to fluorescence measurements.

XPS surface analysis

The XPS data were obtained with a SAGE 150 Spectrometer (Specs, Berlin, Germany) using nonmonochromatized MgK_α radiation with 12.5 kV and 250 W settings at a pressure of ca. 10^{-7} Pa in the analysis chamber equipped with channeltron detectors. XPS spectra were acquired in the constant analyzer energy (CAE) mode at 90° takeoff angle. Peak analysis was performed using the peak fit routine from Specs as described in detail elsewhere [16].

Steady state fluorescence measurements

The fluorescence spectra of the fluorophore-labeled polymer films were measured between two quartz windows in a 0°/90°-geometry with a Spectronics Instruments 8100 spectrofluorometer with an excitation polarizer set to 0° and an emission polarizer set to 54.7°. If not otherwise stated, all the fluorescence spectra presented are corrected for the wavelength- and polarization-dependent spectral responsivity of the detection system and for the relative spectral irradiance of the excitation channel at the sample position [26]. Prior to each fluorescence measurement, the fluorophore-labeled films underwent a Soxhlet extraction employing ethanol as solvent to remove physically adsorbed dye molecules. For each series of measurements, blank or so-called reference samples were prepared by reaction of nonfunctionalized polymer films with the respective fluorescent label using the same procedure as applied to covalently attach fluorophores to the surface-functionalized films.

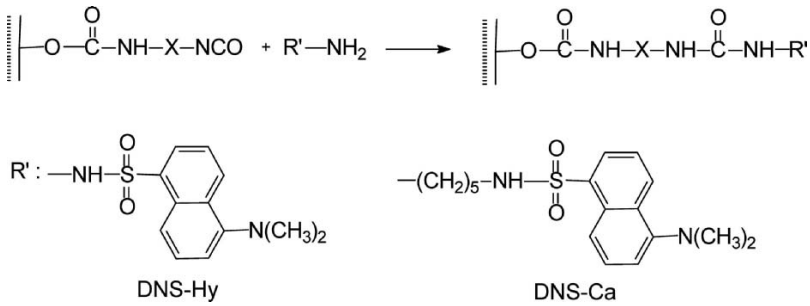
Results and discussion

Surface-linked fluorescent labels

Functional groups at polymer surfaces are a prerequisite for grafting of spacer, sensor, and biomolecules. Applying an oxygen plasma, a broad variety of different O-functional groups including carbonyl, aldehyde, carboxyl, hydroperoxide, ether, and hydroxyl groups is generated at polymer surfaces [17]. By plasma-chemical modification on pristine inert polypropylene surfaces as model polymer substrates, reactive hydroxy-functionalities have been generated with typical OH-concentrations up to 2–4 OH groups/100°C.

With the application of a previously developed additional wet-chemical reduction process to plasma-treated substrates, [18, 19] surface densities of about 10–14 OH/100 C can be realized. This equals 2.4–3.4 OH groups per nm^2 or 0.3–0.4 nmole OH per cm^2. This procedure shows moderate selectivity, whereby about 60% of all the O-functionalities are converted into OH groups [20,21]. These values are based on quantum chemical MO or MM2 calculations and the assumptions of surfaces with a low roughness and the geometric model of one propylene unit occupying about 0.12 nm^2 and accordingly eight propylene units occupying about ∼1 nm^2 (cf. six functional groups/nm^2 in Ref. [22]. The described surface functionalization allows a durable coupling of hydroxyl groups directly to the polymer backbone. Deposited allyl alcohol layers, however, show a decrease in the amount of hydroxyl groups probably caused by dissolving of oligomers from the plasma polymer deposit [23].

After combined plasma-chemical oxidation and wet-chemical reduction, the hydroxy-modified PP substrates were reacted with diisocyanates MDI and TDI as illustrated in Scheme 1. The obtained NCO-terminated polymer surfaces present useful materials for further grafting procedures. Subsequent reactions of these films with amino-terminated fluorescent labels like, e.g., dansyl cadaverine (DNS-Ca) and dansyl hydrazine (DNS-Hy) result in



Scheme 2 Schematics of the grafting reaction of amino-functionalized fluorescent labels to NCO-modified polymer surfaces

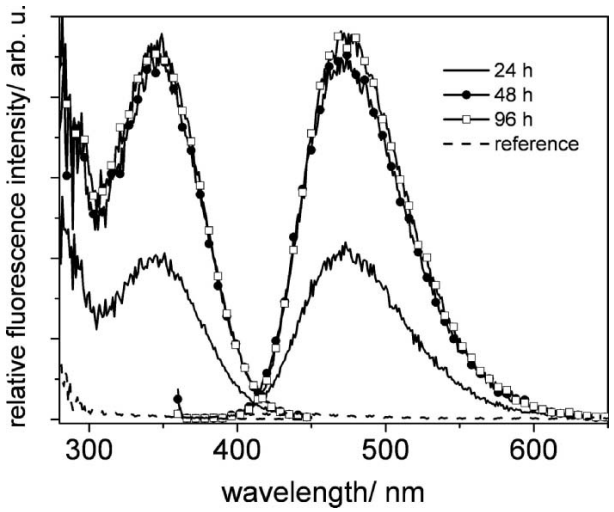


Fig. 1 Fluorescence excitation and emission spectra (uncorrected; excitation at 350 nm) of DNS-Hy labeled PP-OH films (*solid lines*) and of a reference film (*dashed lines*). Variations in fluorescence intensity are caused by different degrees of dye labeling due to prolonged reaction times with over TDI from 24 hrs (*solid lines*), 48 hrs (*solid circles*) to 96 hrs (*open squares*).

fluorescent PP surfaces (see Scheme 2). Covalent attachment of fluorophores to hydroxyl-modified polypropylene surfaces following via interposed spacer molecules synthetic concepts, adopted from well-investigated polyurethane chemistry result in fluorescent PP surfaces with an amount of about 1–3 fluorophores/100 C atoms estimated by XPS.

Spectroscopic properties of surface-attached dansyl chromophores

The synthetic approach shown in Scheme 2 yields fluorescent films, displaying characteristic emission features of the charge-transfer (CT) dansyl chromophore. As an example, fluorescence excitation and emission measurements of DNS-Hy, attached to PP-OH, reacted over different times with TDI, are displayed in Fig. 1. These different reaction times were used here to vary the concentration of the surface functionalities in a controlled fashion.

The fluorescence excitation spectra, which reflect the absorption spectra of the emitting species, show maxima at about 347 nm as to be expected from the absorption spectra of well-investigated dansyl chromophores in various environments and matrices. The uncorrected emission spectra of the surface-bound dansyl label display emission maxima at 473 nm independent of the reaction time, whereas the fluorescence intensity increases with prolonged reaction time. For comparability reasons, only the reaction time of PP–OH with TDI was varied in these experiments. The duration of the labeling reaction was kept constant. The measured fluorescence originates from covalently attached fluorophores only and not from physically adsorbed dye molecules, as follows from the comparison to reference emission spectra obtained for nonfunctionalized PP films immersed in dye solutions under exactly the same conditions as used for films carrying surface functionalities. For all samples, physically adsorbed dye molecules were removed by soxhlet extraction prior to fluorescence measurements as described in the materials and methods section. Table 1 summarizes the absorption and emission maxima as well as the resulting Stokes shifts of various dansyl-labeled PP films from our measurements and selected data from the literature.

As follows from Table 1, the absorption maxima λ_{abs}(max), derived from the fluorescence excitation spectra of the dansyl-labeled PP films, are in very good agreement with the data reported for PE films with directly linked dansyl labels [7], independent of the spacer molecules used. Contrary to the spacer-independent absorption maxima, the spectral position of the emission maximum λ_{em}(max), and accordingly the Stokes shift $\Delta\tilde{\nu}_{(abs-em)}$, clearly depends on the type of investigated film. The emission features of the dansyl chromophore are known to be highly sensitive to the nature of their microenvironment. They exhibit, for instance, large bathochromic shifts on going from a nonpolar to a polar environment. For that reason, as well as because of their

Table 1 Selected spectroscopic data of fluorophore-labeled polymer films

Polymer support	Label	Spacer	λ_{abs} (max)/nm	λ_{em} (max)/nm	Stokes shift[a] $\Delta\tilde{\nu}_{(abs-em)}$/cm^{-1}	Ref
PP	DNS-Hy	MDI	349	482	7900	[c]
PP	DNS-Hy	TDI	348	487	8200	[c]
PE	DNS-Hy	—	350	467	7200	[b]
PP	DNS-Ca	MDI	340	460	7700	[c]
PP	DNS-Ca	TDI	338	459	7800	[c]
PE	DNS-Ca	—	350	467	7200	[b]

[a] Stokes shift defined as $\Delta\tilde{\nu}_{(abs-em)} = \tilde{\nu}_{abs} - \tilde{\nu}_{em}$, where $\tilde{\nu}_{abs}$ is low energy absorption maximum (here, the maximum of the fluorescence excitation band) given in wave numbers and $\tilde{\nu}_{em}$ is the mixed of the emission band gave in wave numbers

[b] correction procedure not stated (cf. Ref. [7])

[c] spectrally corrected data, this work

comparative insensitivity to fluorescence quenching dye–dye interactions, these labels are extensively used as fluorescent probes for environmental effects such as e.g. polarity and viscosity [25]. From the steady-state emission data in Table 1, rough conclusions can be drawn on the local environment of the polymer-linked labels. Comparing the spectroscopic properties of the two polymer supports, the Stokes shift increase, going from 7200 cm^{-1} for fluorophores directly linked to PE [7] to values of ≥ 7700 cm^{-1} for the spacer-linked fluorophores of the polypropylene systems. The Stokes shifts between 7700 and 8200 cm^{-1} measured for the latter correspond to values of 7920 cm^{-1} reported for dansyl-labeled polymer-coated glass fibers in water. The dried fibers show Stokes shifts of about 7000 cm^{-1} [24]. Evaluating the spectroscopic data in Table 1—which are measured on non-dried polymer films in ambient atmosphere—the increase in Stokes shift, observed for PP films compared to directly labeled PE suggests a more polar environment of the dansyl moiety, attached to PP surfaces via the spacer molecules TDI and MDI. The more polar environment of the last-mentioned fluorophores points to a better accessibility of the spacer-linked dansyl group to water molecules derived from air humidity. The formed "pseudo-aqueous" microenvironment dominates the spectroscopic properties of the attached dansyl moiety, almost independent of the type of spacer molecules.

Uncertainty of fluorescence measurements at polymer surfaces

For the evaluation of spectroscopic data from the literature, it should be kept in mind, however, that reported spectroscopic data frequently descend from raw or technical fluorescence spectra. [25–27] This is often not clearly indicated. For the comparability of fluorescence measurements, these uncorrected spectra have to be corrected for the spectral characteristics of the respective fluorescence instrument. The impact of spectral correction on fluorescence data is exemplary illustrated in Fig. 2. Here, the fluorescence excitation and emission spectra of polypropylene, labeled with DNS-Ca via TDI before and after proper spectral correction are shown. For comparison of the data in Table 1 it is advantageous, that the emission maxima of the dansyl chromophore are located between 450 and 500 nm. In this wavelength region, the relative spectral responsivity of typical fluorescence instruments is normally only slightly wavelength-dependent, resulting in comparatively minor deviations between the band maxima of uncorrected and corrected fluorescence spectra. As illustrated in Fig. 2, the correction-induced deviation in band position of the fluorescence spectra of dansyl cadaverine linked to PP by TDI accounts for 4 nm a spectral shift of only.

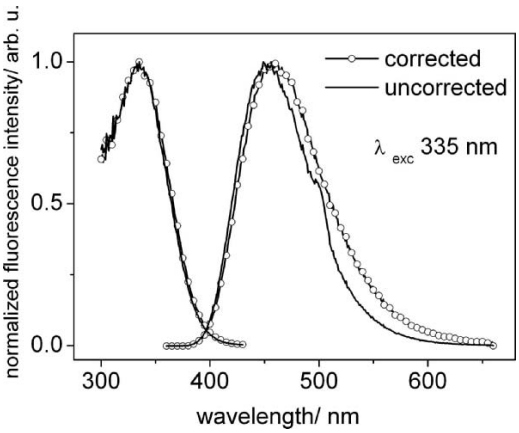


Fig. 2 Effect of spectral correction on excitation and emission spectra as represented by comparison of normalized uncorrected (*solid line*) and corrected (*open circles*) spectra of polypropylene, labeled with DNS-Ca via TDI. The bump observed at about 500 nm for the uncorrected emission spectrum is caused by Wood anomalies of the emission monochromator gratings of the instrument used

Despite of the impact of fluorescence measurements on solid supports, to the best of our knowledge, there are no data available on the uncertainty of this method. Aiming at the reliable spectroscopic investigation of surface-functionalized polymer materials, the many typical fluorescence-inherent sources of error [25–27] need to be considered. In addition to the factors given in the introduction that were minimized by us by proper choice of the fluorescent label and washing procedures, inhomogeneous functionalization/labeling and, in the case of solid substrates like, e.g., films, the reproducibility of sample positioning can critically influence the uncertainties of fluorescence measurements. For commonly performed cuvette-type fluorescence measurements of transparent dilute solutions, the relative uncertainty of recording fluorescence spectra is better than 2%, whereas for analogue measurements of solid films with a routine fluorometer, the achievable uncertainty is inferior [27]. The magnitude of the relative uncertainty of fluorescence measurements i.e., with fluorophore-functionalized polymer films on polymer surfaces has been determined by repeatedly recording spectra of polymer films. From the variation of the signal intensities these at the emission maximum of 10 fluorescence spectra, we obtained a relative uncertainty for fluorescence measurements of covalently attached fluorophores of about 15% (see Fig. 3). Most likely, this uncertainty arises from inhomogeneous labeling.

Correlation of fluorescence measurements and XPS characterization

Despite of the suitability for the qualitative detection of fluorophore-labeled surface functionalities, their

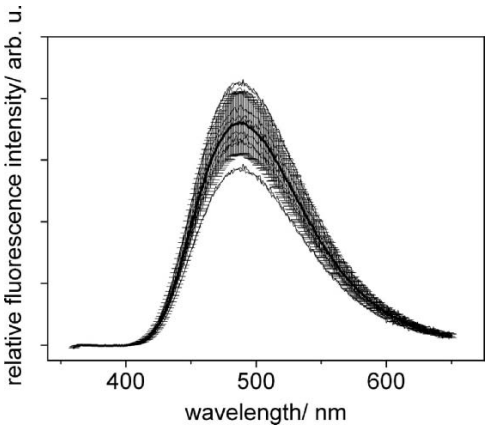


Fig. 3 Relative uncertainty of fluorescence measurements on polymer films. Ten repeatedly recorded spectra of DNS-Hy attached to plasma-chemically modified PP via TDI (excitation wavelength 350 nm) are shown

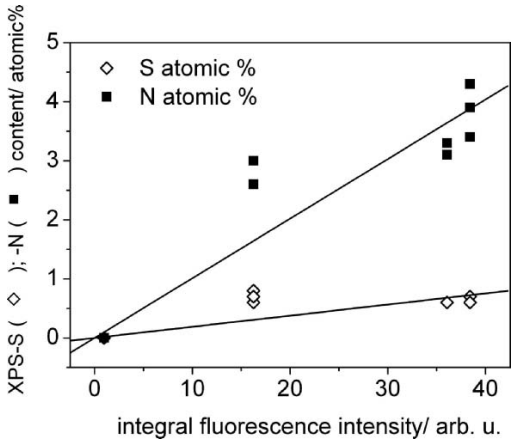


Fig. 5 Correlation between integral fluorescence intensities (excitation wavelength 350 nm) and the N (*solid squares*) as well as the S (*open diamonds*) content, respectively, obtained from XPS measurements of TDI-linked DNS-Hy

quantification by means of fluorescence spectroscopy is still a challenging task. Quantitative fluorescence analysis is commonly based on calibration curves generated from known analyte concentrations in solutions [7, 8]. Similarily, a calibration step is required to determine the concentration of fluorophore-labeled functional groups on polymer surfaces. Due to the inherent sensitivity of fluorescence to the dye's microenvironment, different surroundings of dissolved and polymer-linked chromophores can cause significant variations in the spectral characteristics of absorption and emission. The effect of surface-linking on the fluorescence spectra of the bound fluorophores is measurable (see Table 1), but typically, the influence of the dye attachment on absorption coefficients and quantum yields remains unknown

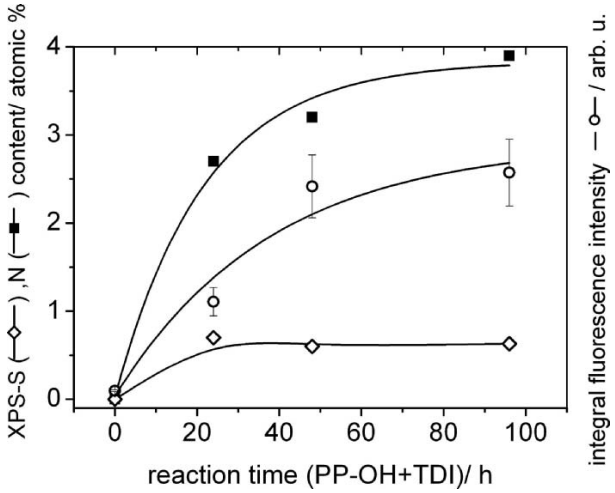


Fig. 4 Integral fluorescence intensity (*open circles*; excitation wavelength 350 nm) as well as the N (*solid squares*) and S (*open diamonds*) content, respectively, obtained from XPS measurements of DNS-Hy, linked to PP via TDI

[28]. To eliminate errors in quantification, the chromophore to be specified and the calibration standard have to be in a comparable microenvironment to guarantee identical fluorescence spectra, molar absorption coefficients, and fluorescence quantum yields, respectively. Because this precondition is often not fulfilled for the comparison of dissolved and polymer-attached fluorescent labels, the use of such calibration data yields increased uncertainties.

To eventually overcome these difficulties, the applicability of XPS measurements as an independent calibration tool has been tested. For the XPS experiments, a further chemical derivatization step could be elegantly circumvented by using the sulfur-atoms of the SO_2-moiety and the N-atoms of the isocyanate-coupled dansyl fluorophores as internal XPS tags. Figure 4 displays the data for the atomic content of the S and N hetero atoms of the dansyl-labeled films as determined by XPS and the fluorescence intensities from the measurements shown in Fig. 1. All the data are plotted as a function of the reaction time of hydroxy-modified PP films with TDI. The error bars for the emission curve are related to the previously derived relative uncertainty of the fluorescence data, see Fig. 3.

The correlation between the atomic contents from surface-sensitive XPS measurements and integral fluorescence intensities of labeled PP surfaces is depicted in Fig. 5. The linear correlation reveals a good agreement between both analytical methods for the plasma-chemically surface functionalized and dansyl-labeled polymer films.

Assuming that the labeling reactions are more or less quantitative with respect to the conversion of plasma-chemically generated surface functionalities, we estimate fluorophore concentrations of about 0.3–0.4 nmole/cm^2 on the PP surfaces. This fluorophore content lies within the detection limit of about $\sim 10^{-3}$ nmol/cm^2 reported for the

fluorometric characterization of dansyl-labeled polymer surfaces [29] and concentrations >1 nmol/cm^2, where fluorescence quenching due to dye–dye interaction can occur [7]. Within the examined concentration range, fluorescence spectroscopic methods can be esteemed as a supplementing technique for the characterization of surface-functionalized solid supports such as e.g. the plasma-modified, fluorophore-labeled polymer films exemplary investigated by us and other solid.

Conclusion

Strategies of synthesis for the functionalization of polypropylene films and the subsequent covalent attachment of dansyl fluorophores to these surface groups either directly or via spacers of varying length have been developed by application of plasma-chemical processes and concepts adopted from well-investigated polyurethane chemistry. As a first step toward the development of simple and standardized methods for the characterization and quantification of surface functionalities, the potential of fluorescence spectroscopy—in combination with labeling techniques—for the characterization and quantification of surface groups has been evaluated. The chosen dansyl fluorophore that contains a sulfur heteroatom enables a direct comparison of fluorescence spectroscopy with results derived by the commonly used surface characterization technique XPS. The good correlation between both methods, found for the spacers MDI and TDI, reveal the potential of fluorescence spectroscopy as a complementary and highly sensitive tool for the characterization of surface groups within the examined concentration range. With the choice of a fluorescent probe, that has a comparatively small tendency toward aggregation and fluorescence quenching dye-dye interactions and proper removal of physically adsorbed nonreacted dye molecules, the relative uncertainty of the fluorescence analysis is determined to maximum 15% from the measured variations in emission intensity. The limiting factors here seem to be the reproducibility of sample, i.e., film positioning, and the spatial inhomogeneity of the label concentration.

Moreover, the developed strategies for molecular engineering and fluorophore labeling can be most likely exploited for the fabrication of fluorescence standards. Such fluorophore-labeled polymer films are interesting candidates as fluorescence intensity standards, for example, for microscopic techniques, SMS, or the microarray area.

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References

1. Chu PK, Chen JY, Wang LP, Huang N (2002) Plasma-surface modification of biomaterials. Mater Sci Eng R36:142–206
2. Knoll W, Yu F, Neumann T, Schiller S, Naumann R (2003) Supramolecular functional interfacial architectures for biosensor applications. Phys Chem Chem Phys 5:5169–5175
3. Hawkins KR, Yager P (2003) Nonlinear decrease of background fluorescence in polymer thin-films—a survey of materials and how they can complicate fluorescence detection in μTAS. Lab Chip 3:248–252
4. Hollahan JR, Stafford BB, Falb RD, Payne ST (1969) Attachment of amino groups to polymer surfaces by radiofrequency plasmas. J Appl Polym Sci 13:807–816
5. Friedrich JF, Gähde J, Frommelt H, Wittrich H (1976) Modifizierung von Feststoffoberflächen in einer HF-Entladung III: Plasmachemisches Aufbringen funktioneller Gruppen und selektiver Abbau teilkristalliner Polymere. Faserforsch Textiltechn/Z Polymerenforsch 27:604–608
6. Holmes-Farley SR, Whitesides GM (1986) Fluorescence properties of dansyl groups covalently bonded to the surface of oxidatively functionalized low-density Polyethylene films. Langmuir 2:266–281
7. Ivanov VB, Behnisch J, Holländer A, Mehdorn F, Zimmermann H (1996) Determination of functional groups on polymer surfaces using fluorescence labelling. Surf Interf Anal 24:257–262
8. Henneuse-Boxus C, De Ro A, Bertrand P, Marchand-Brynaert J (2000) Covalent attachment of fluorescence probes on the PEEK-OH film surface. Polymer 41:2339–2348
9. McArthur EA, Ye T, Cross JP, Petoud S, Borguet E (2004) Fluorescence detection of surface-bound intermediates produced from UV photoreactivity of alkylsiloxane SAMs. J Am Chem Soc 126:2260–2261
10. Wang L, Gaigalas AK, Reipa Y (2005) Optical properties of Alexa 488 and Cy 5 immobilized on glass surface. Biotechniques 38:127–132
11. Pirrung MC (2002) How to make a DNA chip. Angew Chem Int Ed 41:1276–1289
12. Adelhelm K, Ermantraut E, Kaiser T, Tuchscheerer J (2002). Smart chip for array experiment standardization. News Drugs 5:22–23
13. Biju V, Takeuchi M, Umemura K, Gad M, Ishikawa M (2002) Fabrication of standard samples for single molecule fluorescence imaging. Jpn J Appl Phys 41(3A):1579–1586
14. Zwier JM, Van Rooij GJ, Hofstraat JW, Brakenhoff GJ (2004) Image calibration in fluorescence microscopy. J Microsc 216(1):15–24
15. Friedrich JF, Retzko I, Kühn G, Unger WES, Lippitz A (2001) In: Mittal KL (ed) Metal doped plasma polymer films 7. Metallized plastics 7. VPS Utrecht, pp. 117–142
16. Retzko I, Friedrich JF, Lippitz A, Unger WES (2001) Chemical analysis of plasma-polymerized films: The application of X-ray photoelectron spectroscopy (XPS), X-ray absorption spectroscopy (NEXAFS) and fourier transform infrared spectroscopy (FTIR). J Electr Spectr Relat Phenom 121:111–129
17. Rossmann K (1956) Improvement of bonding properties of polyethylene. J Polym Sci 19:141–144
18. Nuzzo RG, Smolinsky G (1984) Preparation and characterization of functionalized polyethylene surfaces. Macromolecules 17:1013–1019
19. Kühn G, Weidner S, Decker R, Ghode A, Friedrich JF (1999) Selective surface functionalization of polyolefins by plasma treatment followed by chemical reduction. Surf Coat Technol 116–119:796–801

20. Friedrich JF, Kühn G, Mix R, Unger WES (2004) Formation of plasma polymer layers with functional groups of different type and density at polymer surfaces and their interaction with Al atoms. Polymer Process and Plasmas 1:28–51
21. Kühn G, Retzko I, Lippitz A, Unger WES, Friedrich JF (2001) Homofunctionalized polymer surfaces formed by selective plasma processes. Surf Coat Technol 142–144:494–500
22. Oehr C (2003) Plasma surface modification of polymers for biomedical use. Nucl Instrum Methods Phys Res B 208:40–47
23. Mix R, unpublished results
24. Gonzalez-Benito J, Aznar A, Baselga J (2001) Solvent and temperature effects on polymer-coated glass fibers: Fluorescence of the dansyl moiety. J Fluoresc 11(4):307–314
25. Lakowicz CJR (1999) Principles of fluorescence spectroscopy, 2nd edn. Kluwer Academic/Plenum, New York
26. Resch-Genger U, Pfeifer D, Monte C, Pilz W, Hoffmann A, Spieles M, Rurack K, Hollandt J, Taubert D, Schönenberger B, Nording P (2205) Traceability in fluorometry—part II: Spectral fluorescence standards. J Fluoresc 15(3):315–336
27. Resch-Genger U, Hoffmann K, Nietfeld W, Engel A, Ebert B, Neukammer J, Nitschke R, Macdonald R (2005) How to improve quality assurance in fluorometry: Fluorescence-inherent sources of error and suited fluorescence standards. J Fluoresc 15(3):337–362
28. Noble JE, Wang L, Cole KD, Gaigalas AK (2005) The effect of overhanging nucleotides on fluorescence properties of hybridizing oligonucleotides labeled with Alexa-488 and FAM fluorophores. Biophys Chem 113:255–263
29. Holländer A (2004) Labelling techniques for the chemical analysis of polymer surfaces. Surf Interf Anal 36:1023–1026