

Chemical reactions on plasma functionalized polymer surfaces

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Abstract

The utilization of primary functional groups on the surface of plasma treated polyethylene films is demonstrated for the coupling of complex molecules. The limited selectivity of plasma induced reactions, i.e. the formation of other than the desired functional group, can be used to bind different complex molecules via selective chemical reactions. Especially the controlled formation of carboxyl groups by an acrylic acid plasma and the following introduction of amino groups by a nitrogen/hydrogen plasma can be used to create a defined multifunctional surface.

Introduction

In order to obtain certain properties (e.g. enzymatic activity, molecular recognition) at the surface of a polymer, the coupling of complex molecules is inevitable. Many materials do not allow efficient and spatially controlled surface reactions in the framework of the traditional organic chemistry. The treatment of such materials with low pressure plasmas is a convenient way to provide their inert surfaces with reactive functional groups. The advantages of using plasma for this purpose are the high efficiency and the limitation of the chemical alterations to a very thin layer. The limited selectivity of plasma-induced reactions might be a potential disadvantage. However, if the desired amount of a coupling group can be generated this method can be applied.

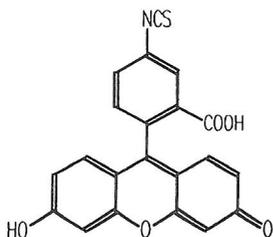
In this paper, we will demonstrate the use of primary functional groups of plasma treated polyethylene films for the coupling of functional molecules. As an example we will discuss the coupling of different dyes. The selectivity of the coupling reactions towards one or another primary functional group offers the possibility to create a defined multifunctional surface.

Experimental

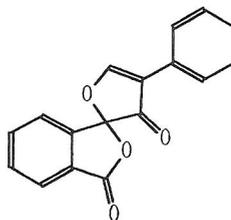
Materials

Polyethylene (PE) films (Lupolen) were supplied by BASF. Before and after treatment the films were cleaned with 96 % ethanol in an ultra sound cleaner for 5 min and dried in dynamic vacuum at 0.1 Pa for 10 min. Dansyl hydrazine (5-dimethylamino-naphthalene-1-

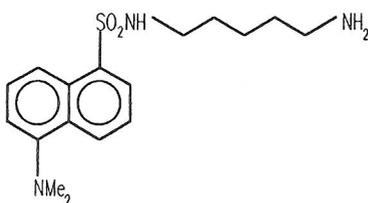
sulfonhydrazide; Fluka), dansyl cadaverine (N-[5-aminopentyl]-5-dimethylamino-1-naphthalene sulfonamide; Fluka), fluorescamine (Fluram; Fluka), and fluorescein isothiocyanate (Fluka) were used as received.



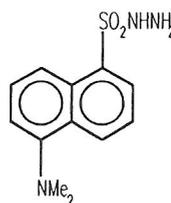
FITC



Fluram



DCa



DHy

Plasma treatment

The functionalization with the N_2/H_2 -plasma was carried out in a home made pyrex tube reactor provided with rf power (13.56 MHz, Advanced Energy RTX 600) via ring electrodes outside the tube. The substrates were located down-stream from the grounded electrode and treated for 5 minutes (50 W , 10 sccm nitrogen and 20 sccm hydrogen, 5 mbar).

The deposition of acrylic acid was carried out in a UHV chamber by a remote MW-ECR-plasma (Roth & Rau source) at a MW power of 60 W (12 sccm acrylic acid, $8 \cdot 10^{-3}$ mbar).

Coupling reactions

Carbonyl groups [-C=O] were labeled by the reaction with dansyl hydrazine (DHy, 2 hours, room temperature, in methanol). Dansyl cadaverine (DCa) was used for the coupling to carboxyl groups [-COOH] (3 hours, room temperature, in ether). Fluorescamine (Fluram) was used to label primary amino groups [-NH₂] (5 minutes, room temperature, in acetone). The reaction with fluorescein isothiocyanate (FITC) was carried out for 30 minutes (room temperature, in acetone). This reaction is not completely selective, since it proceeds with different oxygen containing functionalities [-OH,-COOH] as well as with amines.

After each coupling reaction the films were washed thoroughly and dried in air.

Fluorescence spectroscopy

The fluorescence measurements were carried out with a Fluorolog-112 spectrometer (Spex) in the front face arrangement (22.5 deg). The spectra of the films were recorded with the films located along the diagonal of a fluorescence cuvette (10 x 10 mm²), which can be filled with a buffer solution.

The concentration of the chemically bonded dansyl groups was measured by recording the fluorescence intensity at 467 nm (excitation at 350 nm). The Fluram fluorescence measurements were performed in 0.2 M NaBO₂·4H₂O buffer (pH = 9.0) at 472 nm (excitation at 420 nm). The coupled fluorescein was determined by measuring the fluorescence in n/100 NaOH at 518 nm (excitation at 490 nm).

Results

Amino- and carbonyl-groups on PE-surfaces

In a previous paper [1] we described the formation of primary amines [-NH₂] on the surface of a PE-film by the treatment with a N₂/H₂-plasma. Due to oxygen residues in the plasma reactor, we can expect the formation of oxygen containing functionalities too (e.g. carbonyls [-C=O]). Amines and carbonyls can be detected by fluorescence labeling with good reproducibility on the plasma treated surface (Table 1, rows 2 and 3). It is important to note, that both coupling reactions do not seem to influence each other (Table 1, rows 4 and 5), that is, the fluorescence intensities of each of the dyes are the same even when they were coupled one after the other.

The coupling of FITC to a surface which already has bonded DHy and Fluram (Table 1, last row) clearly demonstrates the existence of functional groups other than amines and carbonyls, e.g. hydroxyls [-OH]. Using a reaction sequence as indicated in the last row Table 1, the detected amount of carbonyls is higher than the amount determined before on the unlabeled surface. This excess can most probably be attributed to the reaction of the DHy with the carbonyl group of FITC.

Table 1 Fluorescence intensities (in 1000 counts per second) at the surface of N₂/H₂-plasma treated PE after reactions with different fluorescence labels

reactions	coupling group		
	[-NH ₂]	[-C=O]	[-OH, -COOH]
Fluram	155±7	-	-
DHy	-	205±9	-
Fluram ----> DHy	159 (1,03)*	212 (1,03)*	-
DHy ----> Fluram	151 (0,97)*	198 (0,97)*	-
Fluram ----> FITC ----> DHy	150 (0,97)*	264 (1,29)*	456

* - relative intensities compared to rows 2 and 3, respectively.

Carboxyl- and Carbonyl-Groups on PE-Surfaces

In order to create carboxyl groups on the surface of PE, we treated a film in an acrylic acid plasma. Under certain conditions [2], we found an efficient carboxylation of the surface (Table 2, column 2). Beside carboxyls also carbonyl and other oxygen containing groups are formed by this treatment. Again, both carboxyl and carbonyl groups can be used for independent coupling reactions.

Table 2 Fluorescence intensities (in 1000 counts per second) of different labels at the surface of acrylic acid plasma treated PE as a function of the plasma treatment time

time, sec	coupling group		
	[-COOH]	[-C=O]	[-OH, -COOH]
10	780	264	7
30	694	245	26
60	679	279	31

Please note that the numbers listed in Table 2 are fluorescence intensities which do not always reflect real concentrations. For [-COOH] and [-C=O] the same label (the dansyl group) is used. Therefore we can assume that the calibration factors are similar for these

groups. The measured intensities for the couples FITC can not be compared to the other two. Moreover, the fluorescence efficiency of the fluorescein group depends strongly on the pH of the environment. We found indications that it is influenced by the concentration of the surface carboxyl groups.

The amount of the two coupling groups can be increased if the plasma functionalized surface is reacted with FITC first. The carboxyl and carbonyl groups of this dye are then used for further coupling reactions. In that case, the coupling capacity for DHy as well as for DCa can be increased considerably (Table 3). The fact, that the amount of the dansyl groups coupled via the carboxyl functionality increases with plasma treatment time while it decreases when it is coupled via the carbonyl group of FITC is not completely understood yet. A possible explanation might be the different steric conditions in DCa and DHy. With longer plasma treatment times more FITC is coupled to the surface. If this dye is packed more dense, the NH₂ group of DCa, which is attached via a C₅ spacer to the dansyl unit, can access the FITC more easily than the -NHNH₂ group in the DHy molecule.

Table 3 Fluorescence intensities (in 1000 counts per second) of DCa and DHy at the surface of acrylic acid plasma treated PE which was labeled with FITC as a function of the plasma treatment time

time, sec	reactions	
	FITC ----> DCa	FITC ----> DHy
10	768 (0,98)*	437 (1,66)*
30	885 (1,28)*	371 (1,51)*
60	1260 (1,86)*	305 (1,09)*

* - relative to the corresponding intensities in Table 2

Amino-groups on a plasma deposited acrylic acid film

Increasing the duration of the treatment in an acrylic acid plasma, a thin film with a high content of carboxyl groups can be deposited on a substrate. We deposited a 50 nm layer on a PE substrate which was then treated with a N₂/H₂-plasma (as described in the first section).

Amino groups can be introduced into the surface of this plasma polymerized acrylic acid film in a concentration which is in the same order of magnitude as observed after the treatment of a PE surface (Table 4, compare with Table 1, column 2). The formation of amino groups is accompanied by only a slight decrease in the concentration of the carboxyl groups as determined by DCa.coupling.

Table 4 Fluorescence intensities (in 1000 counts per second) of DCa and Fluram coupled to the surface of an acrylic acid plasma polymer layer on PE

reactions	coupling group	
	[-NH ₂]	[-C=O]
DCa (untreated)	-	718
DCa ----> Fluram	209	532
Fluram ----> DCa	124	350

Conclusions

Complex organic molecules as fluorescence dyes can be coupled specifically to a plasma functionalized polymer surface. Different functional groups and coupling reactions can be utilized to equip the surface with different fluorescence labels.

References

- [1] J. Behnisch, A. Holländer, H. Zimmermann, Proceedings of the 11th Intern.Symp.Plasma Chem., Vol.3(1993)1221-1223.
- [2] F. Mehdorn, J. Behnisch, A. Holländer, H. Zimmermann, to be published.