

Modification of Poly(octadecene-*alt*-maleic anhydride) Films by Reaction with Functional Amines

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ABSTRACT: Thin films of poly(octadecene-*alt*-maleic anhydride) on top of Si wafers and glass plates were modified by reactions with different functional amines to be used in future studies on the relevance of certain molecular surface properties for the covalent immobilization of proteins. For that aim, a strategy was developed and applied to convert the anhydride moieties of the copolymer by functional amines into side chains bearing hydrophilic groups of acidic (carboxylic acid, sulfonic acid), basic (amines), or neutral (poly(ethylene oxide) (PEO), glucose) character. The modification of the copolymer films was achieved through the two-step formation of a cyclic imide, which was very stable in aqueous solution. Depending on the reactivity of the applied amine, the adjustment of the reaction time was

suitable for the preparation of partially converted surfaces of the polymer film. Degrees of modification between 5 and 30% (according to X-ray photoelectron spectroscopy data) were obtained. Annealing the modified polymer films induced efficient back-formation of the anhydride groups. By reaction of the layered polyanhydrides with highly crosslinked diamines, amine-functionalized polymer films were produced that were capable of binding secondary polyanhydride layers. © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 87: 1255–1266, 2003

Key words: films; functionalization of polymers; modification; surfaces; thin films

INTRODUCTION

The surface properties of biomaterials forming interfaces with biological organisms basically determine the biocompatibility of the corresponding systems and treatments. The patterns of interactions between solid materials and dissolved proteins have to be considered as the origin of several cascade reactions, such as coagulation and immune response, that can potentially interfere with the application of biomedical devices. In turn, biomolecular surface modification (e.g., immobilization of proteins) has been proven effective to tailor the biointerfaces of new (bioactive) materials for advanced applications.^{1,2}

Studies on protein adsorption have shown that the surface properties of solid substrate materials are crucial for the adsorption process.³ On hydrophobic solid surfaces, protein adsorption has often been found to be very strong, inducing denaturation of the protein.⁴ In contrast, protein adsorption onto certain highly

dynamic hydrophilic surfaces, such as surfaces tethered with PEO chains, can be very weak.⁵ In general, hydrophobicity and the presence and redistribution of ionized groups have been confirmed to be most important when the impact of the surface properties of materials on protein adsorption phenomena is considered.

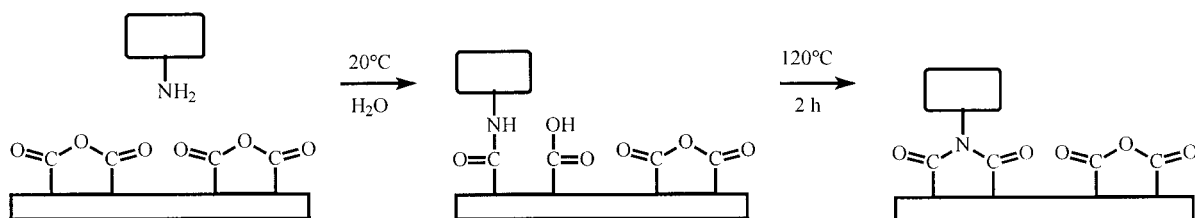
In contrast to the high interest in the origin of distinct protein adsorption patterns, the influence of substrate surface properties (hydrophilicity, surface charge, and surface mobility) on the covalent immobilization of proteins has hardly been studied thus far in systematic ways. The latter aspect, however, is very important with regard to the immobilized amount and the structural and functional conservation of the bound proteins on materials required in several applications. A suitable set of solid surfaces is required to further elucidate this question: The structural variation of the substrate has to be restricted to certain defined properties, and the surface function of the solid involved in the covalent fixation of the protein has to be available in defined quantities.

In this study, a strategy was selected and applied to achieve a combination of substrate moieties capable of covalent protein immobilization (i.e., anhydride groups) with a set of distinguished cofunctions on a given polymer substrate [i.e., poly(octadecene-*alt*-maleic anhydride) (POMA)]. The utilized maleic anhy-

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Scheme 1 Principle of the modification reaction.

dride copolymers are attractive because of the variability in their properties achieved through the introduction of different comonomers.⁶ Furthermore, these copolymers contain the reactive anhydride unit, which offers possibilities for subsequent chemical modification of the polymer. The anhydride moieties are very reactive to primary amine groups and slightly less reactive to alcohols. As ϵ -amino groups of lysine residues of proteins spontaneously react in neutral and mildly alkaline media with the anhydride groups to form amides, maleic anhydride copolymers have already been applied for covalent protein immobilization for decades.⁷⁻⁹

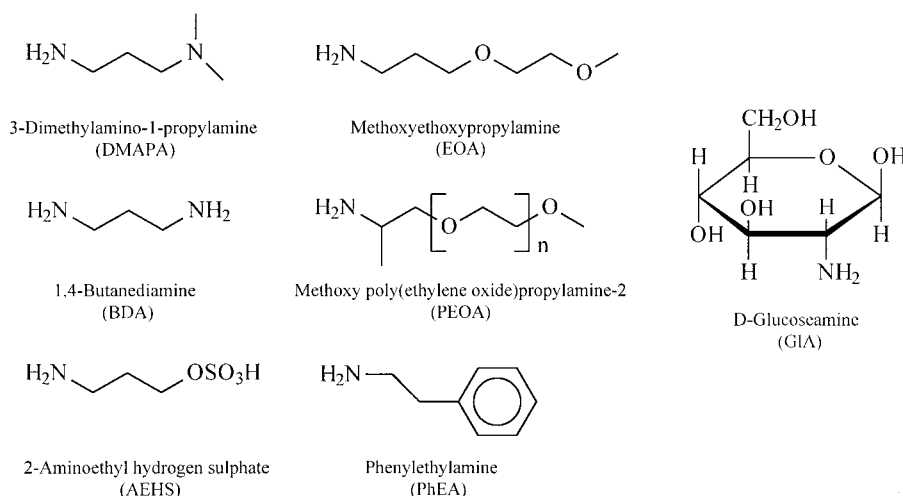
Similarly, maleic anhydride copolymers can be modified systematically by reactions of the anhydride moieties with a wide variety of functional amines. Through the choice of adequate conditions, this reaction can be restricted to a part of the anhydride groups that permits subsequent covalent protein immobilization via the remaining moieties. For systematic studies on the impact of the surface properties for covalent protein immobilization and protein adsorption, a water insoluble copolymer was required. Importantly, the polymer substrate had to form thin stable films on solid carriers for convenient surface analysis. Hydrophobic POMA was selected for that aim. It was proven to reproducibly form well-defined layers on different solid carriers (glass/wafer, polyethylene (PE), poly-

urethane (PUR), poly(ethylene terephthalate) (PET)) of different shapes (planar surfaces, membranes, fibers, tubes).¹⁰

To keep the stability of the deposited polymer films on modification and to obtain samples with distinct degrees of functionalization (vs. protein binding capacity), the conversion of the polymer was claimed to become restricted on the outermost surface of the film.

For that purpose, a strategy was developed and applied to modify thin POMA films on Si/SiO₂ wafers or glass plates by controlled dipping in aqueous solutions of amines (Scheme 1). The modification reactions were monitored by Fourier transform infrared spectroscopy in attenuated total reflection (FTIR-ATR) measurements. Si-ATR crystals coated with thin POMA films were prepared by dip-coating and afterward modified by aqueous amine solutions.

The following amines (Scheme 2) were used for the modification: 1,4-butanediamine (BDA), D-glucosamine (GLA), α -methoxypoly(ethylene oxide)-1000- ω -propyl-2-amine (PEOA), methoxyethoxypropyl amine (EOA), 2-aminoethyl hydrogen sulfate (2-AEHS), and 3-dimethylaminopropyl amine (DMAPA). These functional amines were chosen for the systematic variation of several surface parameters: hydrophilicity/hydrophobicity, charge, and structural mobility of the surface. DMAPA, BDA, and 2-AEHS were used for the implementation of basic/acidic functions, whereas BDA was furthermore



Scheme 2 Amines used for the conversion of POMA films.

applied as a crosslinking reagent. The intrinsically hydrophobic polymer films could be turned hydrophilic by means of GLA, EOPA, and PEOA. PEO chains could additionally strongly influence the structural mobility of the surface in aqueous media. The freely fluctuating chains of PEO, pictorially described as *molecular cilia*, are considered one cause of the antifouling properties of PEO-modified biomaterials.⁵

With the dipping technique applied in this work, a partial conversion of the interfacial anhydride moieties of the POMA films was performed to generate samples with different amounts of functional side chains and varied reactivity.

As control of the interfacial characteristics of solid materials is crucial for a wide variety of demanding applications, the suggested approach met the important aim of current research in the field of applied polymer science: With a well-known polymer, its layered deposition and a novel strategy of controlled chemical conversion provided a very versatile means for adjustment of physicochemical properties of surfaces. This platform was mainly developed here to direct in future studies the immobilization of proteins to solid substrates more thoroughly. With this article, we report on the first step along this route, that is, on the preparation and analytical characterization of the functionalized maleic anhydride copolymer layers.

EXPERIMENTAL

Materials

POMA (molecular weight = 30,000–50,000; Poly-science, Inc., Eppelheim, Germany) was reprecipitated from tetrahydrofuran (THF) and intensively washed with hexane to remove olefin impurities and was then tempered at 120°C for 20 h. THF (Merck, Darmstadt, Germany), DMAPA (Fluka, Buchs, Germany), glucosamine hydrochloride (GLA-HCl; Fluka), EOPA (BASF, Ludwigshafen, Germany), PEOA Jeffamine M-1000 (Texaco, Haufsmann Corporation, Houston, TX), phenylethylamine (PhEA; Fluka), BDA (Fluka), and 2-AEHS (Fluka) were used as received.

Preparation of POMA films by spin-coating

For the spin-coating of POMA to prepare thin polymer films on glass or silicon substrates, a commercial RC 3 spin coater (Suss, Technique S.A., Saint Jeoire, France) was used. A defined portion of a filtered 1 wt % solution of POMA in THF was dropped onto a dust-free Si/SiO₂ wafer, which was subsequently forced to rotate on the spin-coater disk (2000 rpm). The polymer films obtained in this manner had thicknesses between 40 and 50 nm. For stabilization, the polymer films were tempered afterward for 2 h at 120°C. (For the

rationale of the selected conditions, see the Results and Discussion section.)

Modification of the POMA films

Aqueous solutions of functional amines were used to perform the desired chemical conversions of the POMA films. GLA-HCl was applied as an equimolar aqueous mixture (0.1M) with triethylamine for the release of the amine from the hydrochloride. The polymer films were dipped for different times in the amine solution followed by a rinse with deionized water, 0.5M HCl solution, and deionized water again. Finally, the samples were tempered for 2 h at 120°C for imidization.

Ellipsometry

The thickness of the POMA films was determined by variable-angle spectroscopic ellipsometry (J. A. Woolam Co. M44, Bruker, Karlsruhe, Germany). All data were collected in air at room temperature (RT; 22°C) and at a low relative humidity (40%). Ellipsometric data were collected for all samples at 44 defined wavelengths between 428 and 763.5 nm and at three angles of incidence (65, 70, and 75°). An optical three-layer model was used for evaluation. The thickness and the refractive index of the oxide layer and the polymer layer, respectively, were determined on the basis of a fitting algorithm. The thickness of the oxide layer was assumed to remain constant during deposition of the polymer layer on top of it. The Cauchy dispersion model was assumed to be valid for the wavelength dependence of the refractive index of the organic layer.

FTIR-ATR

FTIR-ATR was applied to analyze the chemical structure of the polymer films and its modification. Silicon crystals (70 × 12 × 6 mm) were dip-coated with a 2 wt % solution of POMA in THF for film preparation. The obtained layer had a thickness of about 30 nm. The POMA-coated crystals were subsequently modified in the same manner as the POMA-coated Si wafers. IR spectra were measured with a Bruker IFS 66 (Bruker, Germany) equipped with a mercury, cadmium, teller (MCT) detector. For ATR measurements, a Harrick (Ossining, NY) variable-angle ATR unit was used. One hundred scans were co-added at a resolution of 4 cm⁻¹. The uncoated ATR element was measured for collection of background spectra.

X-ray photoelectron spectroscopy (XPS)

XPS measurements were performed to determine the elemental composition of the uppermost surface layer of the polymer films. An Axis Ultra spectrometer

(Kratos Analytical, Manchester, United Kingdom) was utilized equipped with a monochromatized Al $K\alpha$ X-ray source of 300 W at 15 kV. The kinetic energy of photoelectrons was determined with a hemispherical analyzer with a constant pass energy of 160 eV for survey spectra and 20 eV for high-resolution spectra.

The *take-off angle*, here defined as the angle between the sample's surface normal and the electron-optical axis of the spectrometer, was 0°. Hence, the information depth of XPS data was not more than 5 nm. An effective charge compensation was used during all measurements. All spectra were related to the hydrocarbon reference peak C1s at a binding energy (BE) of 285.00 eV.

Quantitative elemental compositions were determined from peak areas with experimentally determined sensitivity factors and the spectrometer transmission function. The high-resolved spectra were evaluated by means of software routines. The corresponding free parameters of the component peaks were their BE, height, full width at half maximum, and Gaussian–Lorentzian ratio.

Streaming potential (U_{str}) measurements

Electrokinetic measurements were performed to analyze the interfacial charge at POMA films and modified POMA films in aqueous electrolyte solutions. For that aim, a commercial U_{str} instrument (EKA, A. Paar KG, Graz, Austria) was applied. A home-built measuring cell was used to form a rectangular streaming channel between two polymer-coated silicon wafer carriers 10 × 20 mm in size. The slit channel was adjusted to a sample distance (height) of 60 μm . Solutions of 0.003N potassium chloride were prepared from deionized water and used as a background electrolyte. The pH of the solutions was varied by the

addition of 0.1N HCl and 0.1N KOH solutions. U_{str} data were evaluated according to the Smoluchowski equation to provide the zeta (ζ) potential of the hydrodynamic slip plane at the solid/liquid interface:¹¹

$$\zeta = \frac{\eta K_B U_{str}}{\epsilon_0 \epsilon_r \Delta p}$$

where U_{str} is the streaming potential, Δp is the pressure drop across the slit channel, K_B is the specific electrical conductivity of the liquid, ϵ_0 is the permittivity of the vacuum, ϵ_r is the dielectric constant, and η the viscosity of the liquid.

Wetting measurements

The wetting properties of the samples were measured by the drop shape analysis system (DAS 10-MK2; Krüss, Hamburg, Germany). Advancing and receding contact angles were determined with MilliQ water as the test liquid.

Atomic force microscopy (AFM)

Morphological features of the polymer films were characterized by means of AFM. The experiments were performed in tapping mode under ambient conditions with a Nanoscope IIIa Dimension 3100 (Digital Instruments, Santa Barbara, CA). Pointprobe silicon cantilevers for force modulation made by Nanosensors (Tonawanda, NY) (Germany; resonance frequency = 75 kHz, force constant = 2.8 N/m) were used for height imaging.

The roughness average of the surface (S_a) was determined. S_a values can be calculated with the following equation:¹²

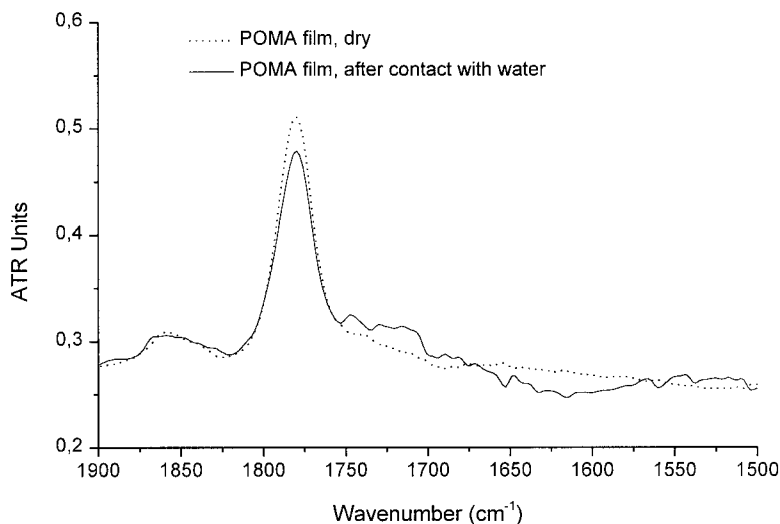


Figure 1 ATR–FTIR spectra of a native POMA film and the same POMA film immersed in water for 20 h at RT.

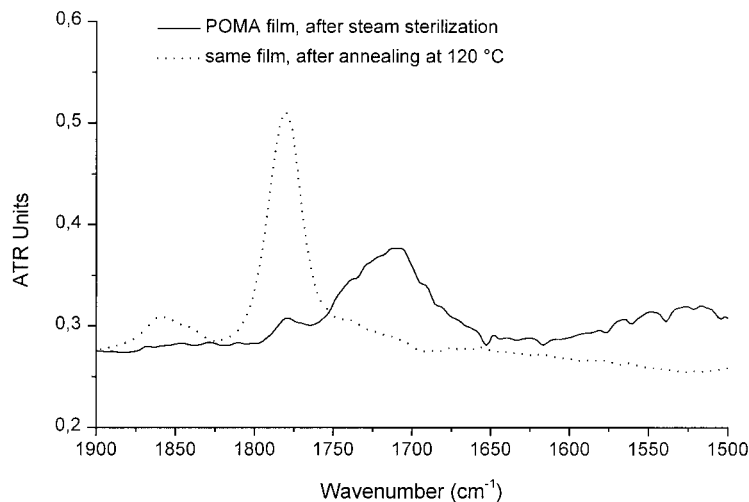


Figure 2 ATR-FTIR spectra of a POMA film hydrolyzed by steam sterilization and this film after subsequent annealing at 120°C for 2 h.

$$S_a = \frac{1}{L_x L_y} \int_0^{L_y} \int_0^{L_x} |f(x, y)| dx dy$$

where $f(x, y)$ is the surface relative to the central plane and L_x and L_y are the dimensions of the surface.

RESULTS AND DISCUSSION

Preparation and characterization of partially modified POMA films

The alternating, water-insoluble POMA copolymer was used here as a midsize macromolecule with a molecular weight of about 50,000. To prepare thin solid films out of this copolymer on Si/SiO₂ wafers or

glass plates, we applied the spin-coating method. Spin-coating of 1 wt % solutions (in THF) was optimized to produce smooth, tight films about 50 nm thick on wafers and glass plates. Subsequent annealing of the films at 120°C [i.e., above the glass-transition temperature (T_g) = 87°C]¹³ substantially stabilized the polymer layer and reactivated any hydrolyzed anhydride moieties. Analysis of the films by AFM showed very smooth surfaces with an S_a of about 1 nm. The films were stable in water for more than 4 weeks.

Hydrolysis of the anhydride moieties at RT in water was studied by FTIR-ATR spectroscopy (Fig. 1). During a period of 20 h, only a minor decay of the high intensity antisymmetric carbonyl stretching band of the cyclic anhydride at 1778 cm⁻¹ was measured.

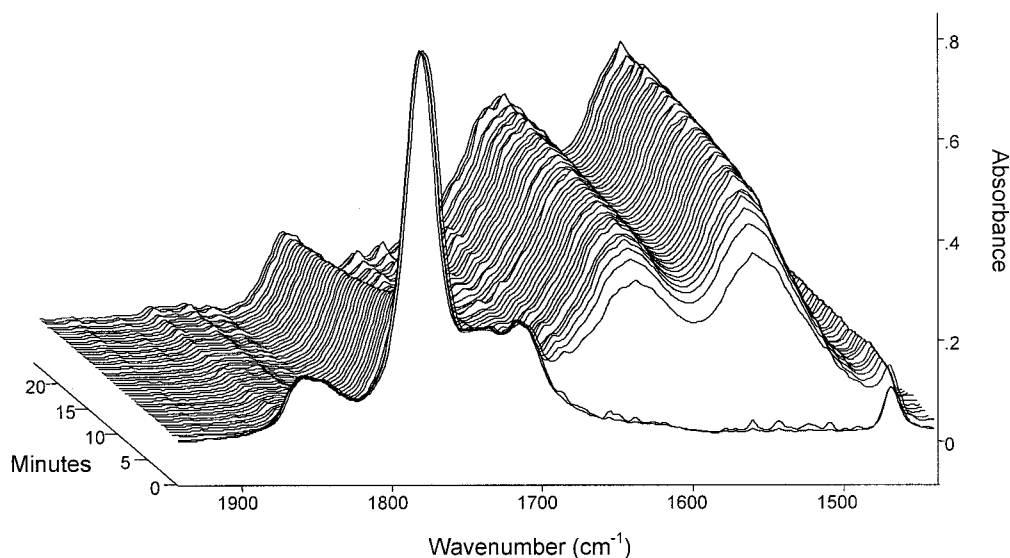


Figure 3 ATR-FTIR spectra of the reaction of a 30-nm POMA film on a Si-ATR crystal with BDA (2 wt % aqueous solution, RT).

TABLE I
Conditions of the Reactions: Amine Reagents, Reaction Times, and Concentrations of the Amine Solution

Modification reagent	Reaction time	Concentration
EOPA	3 min	0.01M
DMAPA	1 min	0.1M
DMAPA	1 min	0.01M
PEOA	6 h	0.01M
PhEA	1 h	0.1M
BDA	5 min	0.1M
2-AEHS	0.5 h	0.1M
GLA-HCl	1 h	0.1M

However, the drastic conditions of steam sterilization (20 min, 120°C, 2 bar) led for similar samples to rapid hydrolysis obvious from the decay of the anhydride band and the simultaneous appearance of a new carbonyl stretching band of the maleic acid at 1710 cm^{-1} . Annealing of the hydrolyzed polymer film at 120°C for 2 h led to a back-formation of the anhydride (Fig. 2).

In contrast to the rather low reactivity of the polymer with water at neutral pH and ambient conditions, Figure 3 shows the fast reaction occurring with primary amines (e.g., BDA).

Within the first 5 min of contact, a sharp decay of the anhydride band at 1778 cm^{-1} occurred, which was associated with the appearance of the amide I and amide II bands at 1652 and 1544 cm^{-1} .

These results are supported by an earlier kinetic investigation,¹⁴ indicating the amidization reaction of maleic anhydride copolymers to be about 100 times faster than an even base-catalyzed hydrolysis. The findings of this study coincide with the conclusions drawn in ref. 14 because the observed conversions in the presence of an excess of water require significantly

higher rates of amidization as compared to the rates of hydrolysis.

On the basis of this promising general reactivity pattern of the POMA films, the spin-coated layers were expected to be accessible to modifications with functionalized amines in aqueous solutions. Hydrolysis did not seriously interfere the aimed modification as a side reaction because partial modification was required. The limited conversion of the anhydride was considered to be useful to provide sufficient stability of the modified film when very hydrophilic amines (e.g., PEO-containing molecules) were used and to permit subsequent protein coupling after the back-formation of the anhydride. Films on Si/SiO₂ wafers were dipped in aqueous solutions of the different amines (see Table I). Depending on the reactivity of the amine, the required reaction time varied between 1 min (DMAPA) and 6 h (PEOA). After rinsing with 0.5M HCl for the elimination of ammonia salts, the samples were annealed for 2 h at 120°C for imide formation.

Imides are distinguished from amides by a substantially increased stability to hydrolysis. Because the films were prepared here for subsequent protein immobilization in aqueous buffer solutions, imide formation was considered to be necessary for sufficient film stability. As an indication of the formation of a five-membered cyclic imide, two characteristic bands of the symmetric (1770 cm^{-1}) and antisymmetric (1700 cm^{-1}) carbonyl vibration were observed in the FTIR spectra (Fig. 4).¹⁵

FTIR-ATR spectra of the modified surfaces showed beside the amide bands and imide bands (after annealing) significant anhydride bands at 1778 and 1858 cm^{-1} . If the applied amine was very reactive, the conversion of the anhydride was not limited to the

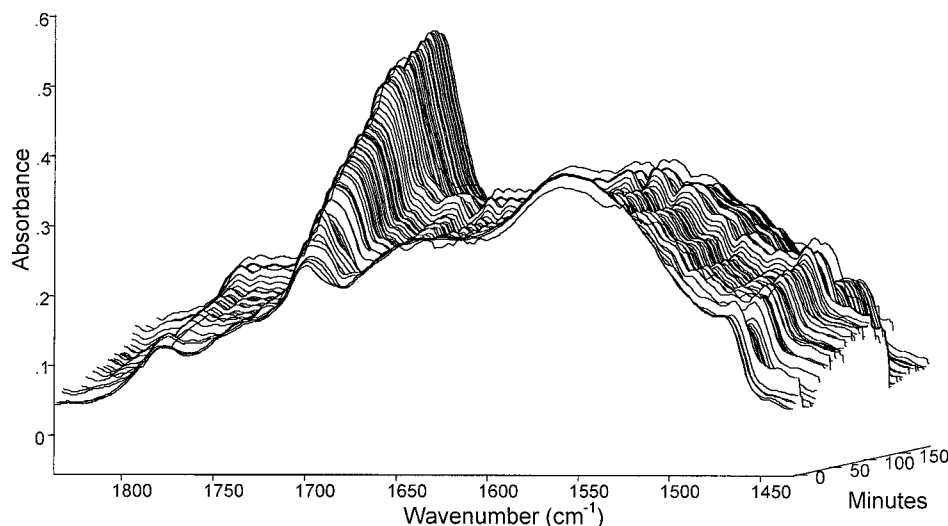


Figure 4 ATR-FTIR spectra of the imidization of POMA/BDA film by annealing at 130°C.

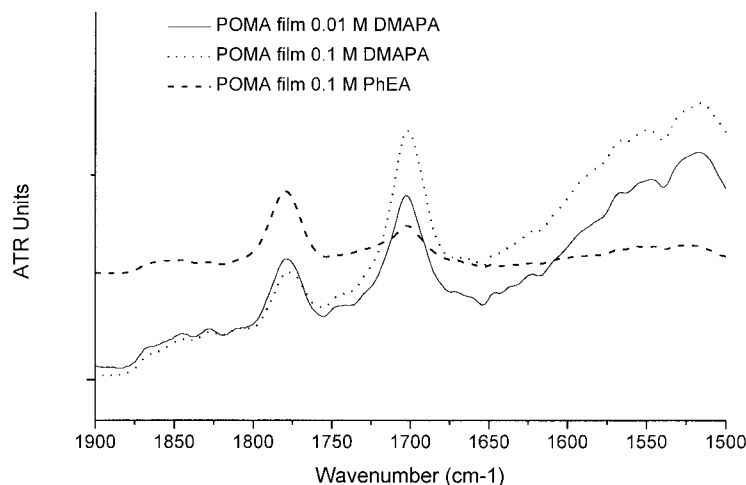


Figure 5 ATR-FTIR spectra of a POMA film after conversion with 0.01M, 0.1M DMAPA, and 0.1M PhEA for 1 min at RT.

surface, as shown in Figure 3 for BDA. As shown in Figure 5, DMAPA reacted within a few minutes with POMA. An increase in the concentration of the amine or an increase in the reaction time led to a further decrease of the remaining anhydride bands up to about 70–80%. In contrast, the reaction with PhEA caused a minor decay of the anhydride bands only. Increasing the amine concentration or reaction time did not alter this behavior.

The remaining band at 1778 cm^{-1} after the modification of POMA films could be considered a rough estimate of anhydride groups at the surface because FTIR spectra accumulated information about the whole polymer layer.

XPS is, with an analysis depth up to 3–4 nm, a more surface sensitive method. In contrast to unmodified POMA, all modified samples showed in the XPS survey spectrum a XPS N1s signal. From the C/O and C/N ratios, we calculated an approximate value of the degree of modification for the layer within the XPS sampling depth. It varied from about 10% for PhEA up to about 30% for GLA (Table II). In the case of modification with PEOA, the dramatic increase in the C—O intensity of the high-resolution XPS C1s spectrum (Fig. 6), together with an overall increase in

oxygen content, indicated the successful attachment of the PEO chains.^{16,17}

Preparation of POMA films with reactive amino groups and the formation of a secondary polyanhydride layer

For the preparation of crosslinked polymer layers bearing reactive amino groups, POMA was modified with BDA. As shown in Figure 2, a major decay of the anhydride band was detected by FTIR spectroscopy. Therefore, a nearly quantitative amidization of the anhydride groups through the whole layer was assumed. On this account and from the C/O ratio obtained by XPS (C/O = 7.55, C/N = 17.87), we concluded a nearly quantitative reaction with high degrees of crosslinking (more than 50%) and a majority of BDA bound as imide.

On the basis of this analysis, the presence of reactive amino groups on the crosslinked layer of POMA was obvious. These groups can be used for further modification reactions, including covalent protein immobilization.¹⁸ Because of the crosslinked structure of the POMA film, it is stable in THF, which permits reactions in organic solvents.

This layer can be further used for the covalent binding of a second layer of a more hydrophilic maleic anhydride copolymer by reaction of the amino groups with the anhydride of the second copolymer (Scheme 3). Corresponding reactions of poly(styrene-*alt*-maleic anhydride) and poly(propene-*alt*-maleic anhydride) (PPMA) were performed in THF; the modification with poly(*N*-vinylpyrrolidone-*alt*-maleic anhydride) was carried out in water.

The FTIR spectra in Figure 7 prove the formation of the two layered films through the decrease and increase of the anhydride bands.

It is anticipated that the surfaces with flexible bound maleic anhydride copolymers have advantages for

TABLE II
Elemental Ratios of Unmodified and Modified POMA Films Prepared according to the Conditions Given in Table I

Surface	C/O	C/N	C/S
POMA	7.32		
POMA/DMAPA	8.62	50	
POMA/PEOA	4.22	38.85	
POMA/PhEA	6.85	216.5	
POMA/2-AEHS	6.09	58	227
POMA/GLA	5.54	66.7	

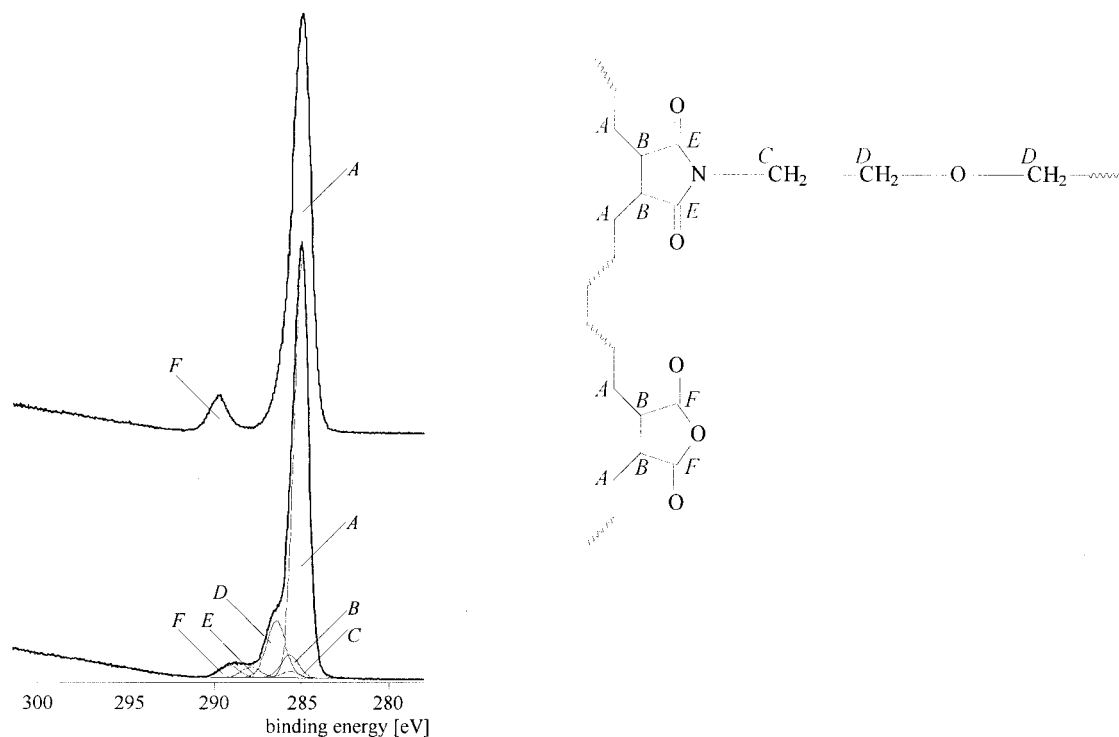


Figure 6 High-resolution XPS C1s spectra of native POMA film (upper line) and a POMA film after modification with PEOA.

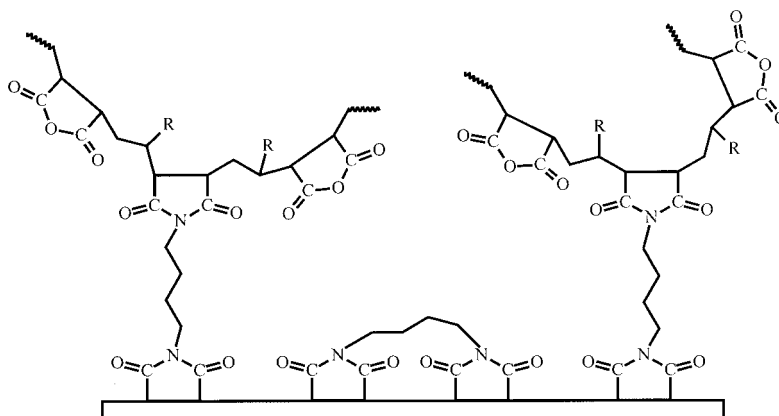
protein immobilization because a high number of anhydride groups are available for a covalent coupling in a highly adaptable interfacial structure.

Properties of the modified POMA films

The swelling behavior of the unmodified and modified POMA films was characterized *in situ* by ellipsometric measurements. For the unmodified POMA films, no increase of the layer thickness was observed within the first 6 h after immersion in phosphate buffered saline (PBS) buffer. Subsequently, swelling of the layer up to 10% was observed within the next 12 h. As

an explanation of this behavior, we assumed that in the first period, because of the high hydrophobicity of POMA,¹⁹ no water penetrated the polymer layer. Then, because of the hydrolysis of anhydride groups at the outermost surface of the POMA film, a decrease of the surface hydrophobicity might have been induced, and the related formation of carboxylic groups may have facilitated the subsequent penetration of water molecules into the layer, which resulted in the observed increase in layer thickness.

In contrast to the unmodified POMA, the modified samples showed immediate swelling of the polymer layer from 5 to 15%, depending on the type of modi-



Scheme 3 Covalent immobilization of maleic anhydride copolymers on partially crosslinked POMA films.

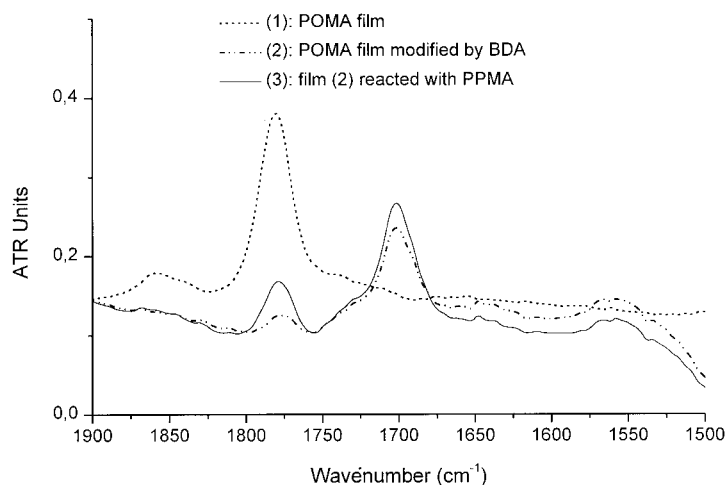


Figure 7 ATR-FTIR Spectra of (1) the native POMA film, (2) a POMA film after subsequent conversion with BDA, and (3) a POMA film after conversion with BDA and after subsequent conversion with PPMA.

fication. This behavior was supported by the increased hydrophilicity of the modified films (see Fig. 8).

The high hydrophobicity of POMA was verified by water contact angle measurements with an advancing angle of 105° . As known from *N*-alkyl maleimides of poly(styrene-*alt*-maleic anhydride) or PPMA copolymers, the wettability¹⁹ is dominated by the *N*-alkyl side chain if the chain has more than six C atoms. With increasing length of this side chain, the wettability approximates to ideal $-\text{CH}_3$ surfaces with a water advancing angle of about 110° . Thereby, the advancing angle of 105° of POMA also indicates a strong influence of the alkyl side chain of the octadecen comonomer. The formation of amide and acidic groups and the implementation of hydrophilic side chains by the modification of the anhydride moieties led to an increase of the surface hydrophilicity. This was detected in the receding angles of the nonan-

nealed samples. The advancing angles did not show this effect because of the dominating influence of the octadecen comonomer with the exception of long hydrophilic PEO chains. Annealing of the samples resulted in an increasing hydrophobicity by imide formation, recyclization of the anhydride, and rearrangement processes of the alkyl side chains above T_g (see Table III).¹⁹

ζ potential versus pH plots (Fig. 9), obtained by U_{str} measurements, revealed the interfacial charge formation of the polymer films in aqueous electrolyte solutions. As discussed in ref. 21, sign and magnitude of the ζ potential, the isoelectric point (i.e., the pH value of vanishing ζ potential), and the overall trend of the ζ potential versus pH plot indicate the origin of electrical surface charge and further give *in situ* information on structural variations of the interface related to the redistribution of charge carriers. The ζ potential

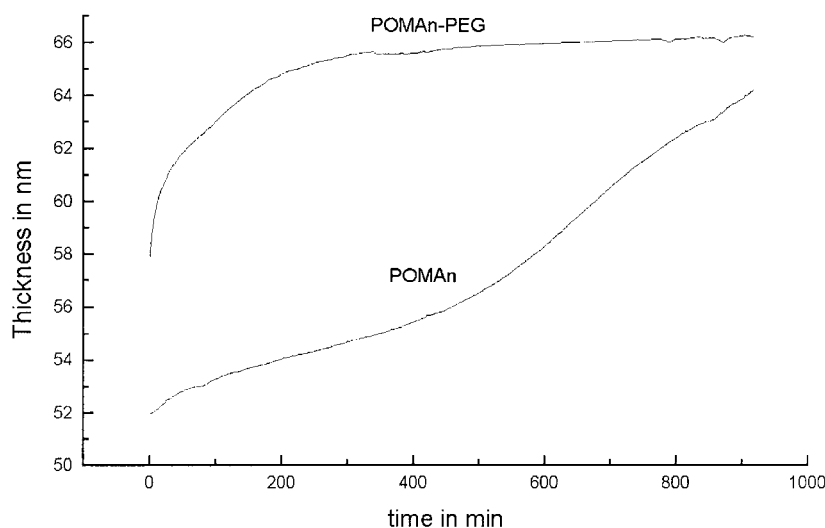


Figure 8 Swelling of a POMA film and a POMA film after conversion with PEOA (monitored by *in situ* ellipsometry).

TABLE III
Water Contact Angles of POMA Film and Modified POMA Films Before and After Annealing at 120°C, Respectively

POMA film	Advancing angle (°)		Receding angle (°)	
	106		57	
Functionalized amines	After modification	Annealed	After modification	Annealed
2-AEHS	95	103	30	42
DMAPA	97	98	23	42
BDA	92	102	17	52
PEOA	88	99	37	60
EOPA	94	103	32	45
GLA	102	102	14	55
PhEA	93	102	43	50

The accuracy of the applied method for the determination of contact angles was recently reported to be in the range of $\pm 2\text{--}3^\circ$.²⁰

versus pH plot of the unmodified POMA film initially showed isoelectric points at about pH 3.2 and a plateau of the negative ζ potential at alkaline pH values. This behavior is to be expected for surfaces bearing acidic groups; the isoelectric point corresponded to the intermediate acidity of the carboxylic acid, which clearly dominated the interfacial charge formation in this case. The magnitude of the negative ζ potential in the alkaline pH range (~ 70 mV) corresponded to a smooth, rather hydrophobic material. However, when the pH-dependent measurement was repeated, a significant decrease in the ζ potential was observed over time (Fig. 9). The latter observation was attributed to the swelling of the polymer related to the proceeding hydrolysis of the anhydride groups and penetration of water in the layer volume phase.²² Surprisingly, a shift

of the isoelectric point toward higher values occurred in parallel. This was unexpected because the density of dissociated acidic groups was increasing during hydrolysis. As the polymer film was found to be stable and not affected by any chemical conversion beyond the hydrolysis, we attributed the evident variation of net acidity to the shift in the pK value of the carboxylic acid groups occurring at higher local densities of these groups. This effect was described earlier for self-assembled monolayers of carboxylic-acid-terminated alkane thiols on gold on the basis of several experimental techniques and attributed to the electrostatic forces arising between neighboring charged functionalities.²³

ζ potential versus pH plots were further collected and evaluated for the modified POMA films described

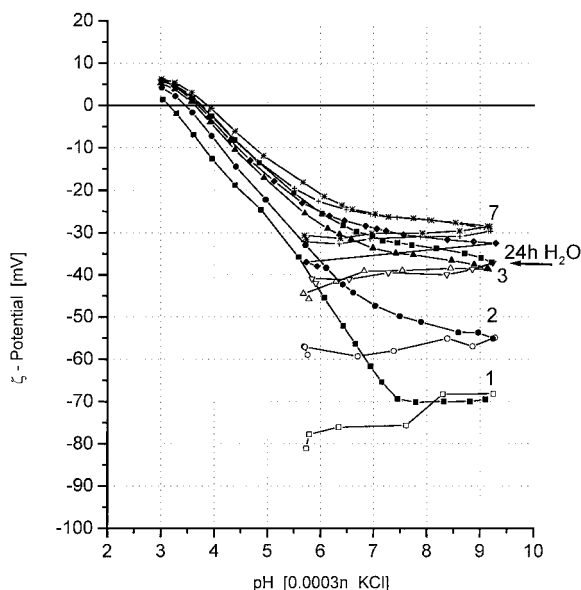


Figure 9 ζ potential versus pH plots for a POMA film: (1) first measurement, (2,3) second set of measurements, and (4–7) measurements after 24 h storage in H_2O .

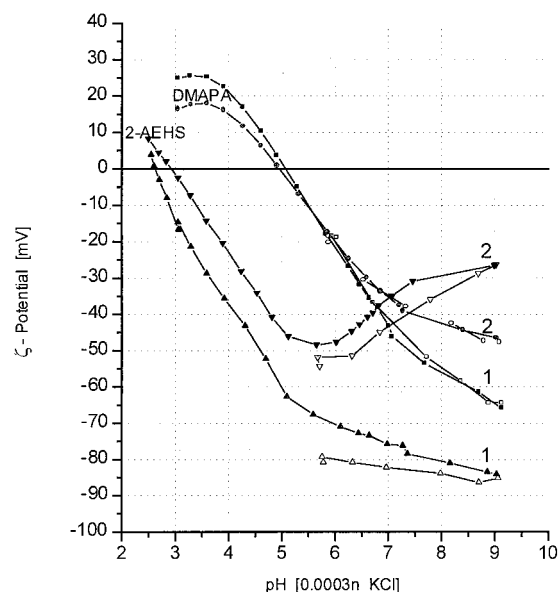


Figure 10 ζ potential versus pH plots for a POMA film after conversion with AEHS (triangles) and DMAPA (squares and circles): (1) first measurement and (2) second measurement.

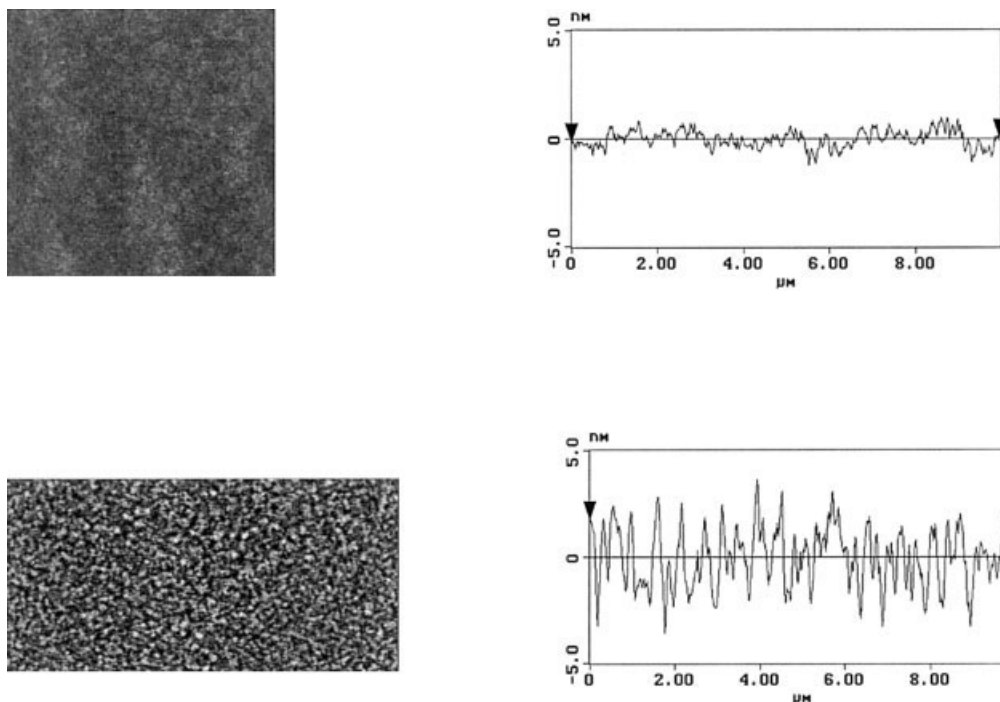


Figure 11 AFM image and section analysis of (top) a POMA film and (bottom) a POMA film after conversion with GLA.

previously. However, for those samples, the presence of a variety of different functional groups in the polymer surface interfered the straightforward interpretation. Two examples are given in Figure 9. For the POMA film partially modified by reaction with 2-AEHS, isoelectric point values of about pH 2.5 were observed, which corresponded to the strongly acidic character of the introduced function. When the results for the freshly prepared and the hydrolyzed sample were compared, similar effects as discussed previously for the pure POMA films were obtained.

For the partially amine-modified POMA film (obtained by reaction with DMAPA,) isoelectric points at about pH 5.0 were observed. Because surfaces without any dissociating sites exhibit isoelectric points at 4.0 due to preferential anion adsorption,²² this value clearly proved the presence of basic groups in the modified surface. The shape of the ζ potential versus pH plot rather indicated an amphoteric characteristic of the polymer surface in this case, caused by the interplay of amine and carboxylic acid groups. For all analyzed samples, the occurrence of unsymmetrical (preferential) ion adsorption may have caused minor, additional contributions to the observed net charge at the polymer films in aqueous solutions, as this phenomenon was concluded to be ubiquitous at polymers in aqueous environments.¹⁹

As obvious from the AFM image and section analysis in Figure 11 (top), the unmodified POMA surface exhibited a regular morphology characterized by S_a ($S_a = 0.4$ nm), which also was retained after surface

modification with the hydrophobic PhEA. The section analysis in Figure 11 (bottom) revealed a surface roughness change after modification with GLA. The modification with hydrophilic amines such as GLA or EOPA caused serious increases in the surface roughness and the formation of a close-grained morphology. It may be assumed that this observation reflects the separation tendency in the polymer induced by the very different characteristics of the introduced functional amines and the polymer film. In line with this explanation, the PEOA-modified surfaces showed the most pronounced surface roughness with an S_a of 2 nm.

CONCLUSIONS

Partially and completely modified films of POMA were prepared to be used in subsequent studies on the covalent immobilization of proteins. The introduction of a wide variety of different functional side chains into the surface-bound copolymer layer was demonstrated to provide a versatile means for the adjustment of relevant surface properties (e.g., hydrophobicity, electrical charge, interfacial dynamics). Advanced modification strategies for the used polyanhydride layers include reactions with diamines and secondary polyanhydride coatings to generate sandwich structures.

Ongoing experiments will make use of this platform to analyze covalent and noncovalent protein immobilizations at solid/liquid interfaces bearing different

combinations of interfacial functions. A set of globular model proteins will be applied in this approach to unravel the interplay of protein and substrate characteristics.

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