Porous Polypropylene Membranes with Different Carboxyl Polymer Brush Layers for Reversible Protein Binding via Surface-Initiated Graft Copolymerization

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Photoinitiated surface-selective graft copolymerization onto polypropylene (PP) microfiltration membranes was performed using two different methods for coating the photoinitiator, benzophenone (BP), on the membrane surface. An already established adsorption method and a novel method based on preswelling of the PP in heptane, subsequent solvent exchange, and thus entrapping of the BP in the surface layer of the PP had been evaluated. With acrylic acid (AA) as the monomer, functional polymer brush structures on the entire membrane pore surface were obtained. Further variations of the grafted layer had been achieved by copolymerization of AA with acryl amide (AAm) and methylene bisacrylamide (MBAA). Characterization had been done mainly by detailed measurements of membrane permeability including pH dependency as well as the reversible binding of a protein (lysozyme, Lys) under membrane chromatography conditions. Compared with BP adsorption, the BP entrapping method yielded a less dense grafted layer with longer PAA chains at the same degree of functionalization (DG). This was due to somewhat lower immobilized BP amounts, but also less side reactions via nonselective photoinitiated cross-linking by dissolved BP. Unexpected properties of the PAA-*co*-AAm brush layers were their even larger swelling/deswelling as a function of a pH change (above and below the pK_a of PAA) as compared with the PAA brushes. Both PAA-*co*-AAm and cross-linked PAA-*co*-MBAA layers showed Lys binding capacities—more than 10 times higher than monolayer adsorption onto the unmodified PP membrane surface—and quantitative recoveries similar to PAA of the same DG; and the highest efficiencies of protein binding (Lys amount relative to amount graft copolymer) were achieved with the membranes prepared by the entrapping method. In general, the more controlled BP entrapping method had distinct advantages in terms of the control of grafted layer structure leading to an improved membrane adsorber performance.

Introduction

Surface modification of polymers is of great interest in many fields, ranging, for example, from the protection of surfaces from corrosion to improving their biocompatibility.[1] In bioseparation using membrane technology, functionalizations are widely used to change the character of the membrane surface from hydrophobic to hydrophilic in order to decrease the adsorption of proteins[2] or to introduce functional groups to obtain special perm-selective membranes[3] or membrane adsorbers.[4,5]

Applications in membrane technology require a permanent modification which will be best realized by a covalent grafting to the base polymer surface. Polymer brush layers will be especially suited for effectively shielding the base polymer at a minimum layer thickness.[1,6] On the other hand, it has already been demonstrated that functional brush layers can have a three-dimensional binding capacity for proteins.[7,8] Among the two strategies toward covalently anchored polymer brush structures, "grafting-to" and "grafting-from",[6] the latter using functional monomers has proven to be much more flexible and versatile.[1,2,6−8]

Among several approaches, UV-initiated graft copolymerization is a powerful technology, with low cost of operation and potentially reducing or even avoiding negative effects onto the bulk polymer.[9−11] It is also hence been increasingly used for membrane functionalization, and membranes made from different materials, for example, polyacrylonitrile,[2,12] polysulfone,[13,14] or polypropylene,[15−17] have been modified

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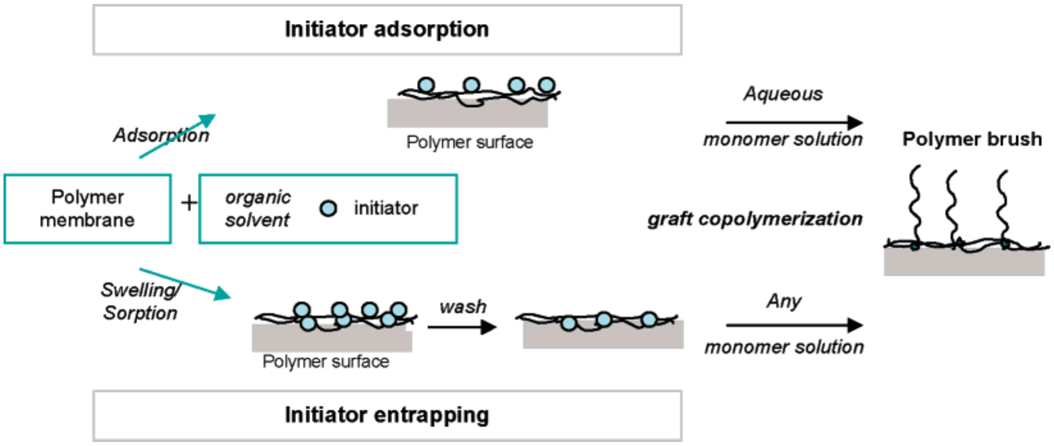
Scheme 1. Schematic Description of Surface-Initiated Graft Copolymerization via Adsorption and Entrapping Methods for Initiator Immobilization



using various hydrophilic or other functional monomers. However, controlling the architecture of the grafted layer will be very much important in order to really tailor and optimize the membrane performance. During the conventional UV-initiated graft copolymerization process, a mixture of monomer, solvent, and photoinitiator is used.[9−11] Hence, polymerization can also be initiated in solution, chain-transfer reactions occur, and the result may be a branched or even cross-linked polymer. To build a layer with well-defined brush structure, a controlled and truely surface-initiated polymerization of monomers should be preferred.

Several routes had been proposed, mainly elucidated in studies with nonporous substrates. For example, a coating of polyvinyl acetate to fix the photoinitiator on the polymer substrate surface had been used in numerous studies by the group of Kubota.[18] Also, coated hydrophobic or amphiphilic macroinitiators had been applied to subsequently attach a copolymer on the surface.[19,20] Both routes have disadvantages because an unwanted polymer is involved in the reaction, and blocking the pores of a filtration membrane by such primary polymer layer is another problem. More sophisticated attempts, introduced for planar silica or gold surfaces, had focused on covalently bound initiators on the surface for a subsequent initiation of a conventional or a controlled radical polymerization.[21−23] Because those methods require syntheses of special initiator conjugates and their coupling to finally yield a surface-bound initiator, they cannot easily be transferred to established (membrane) polymers and/or porous membranes, and therefore they are not suited for industrial applications.

For intrinsically photosensitive polymers such as polysulfones, an additional photoinitiator is not required when a direct UV excitation of the base polymer is used for the initiation of the graft copolymerization. This approach had been adapted to membrane functionalization,[13,24,25] but it suffers from a severe degradation of the base membrane's pore structure. Ma et al.[26] had described a two-step photo-initiated process for a graft-copolymer functionalization of polypropylene using benzophenone (BP): first (without monomer), the BP was grafted onto the surface, yielding a benzpinakol; second (with monomer), far-UV irradiation was used to cleave this bond again, and a "quasi-living" polymerization via recombination and photocleavage of benzpinakol had been proposed. However, due to the high-energy excitation, the conditions will also cause photodegradation of many graft copolymers.

Consequently, a simple and, nevertheless, effective and nondegradative method for surface-initiated polymerization will be appreciated. Such an easy alternative is to immobilize the photoinitiator on the polymer surface through physical adsorption prior to polymerization.[12,14,15] However, limitations may result from the reversibility of the immobilization under the polymerization conditions. An advanced way could be entrapping the initiator into a thin surface layer of the polymer. Depending on the solvent property of the initiator solution, the immobilization mechanism may be changed from adsorption to entrapping; the immobilized initiator amount will depend on swelling/sorption time and initiator concentration, as well as the subsequent washing with solvents (Scheme 1).

In this work, polypropylene (PP) microfiltration membranes were coated with benzophenone (BP) using the already established simple adsorption method and the novel entrapping method (cf. Scheme 1). Graft copolymerization was performed using acrylic acid (AA) as a functional monomer, and copolymerizations of AA with acryl amide (AAm) and the cross-linker monomer methylene bisacrylamide (MBAA) were also done. The degree of grafting (DG) had been measured gravimetrically. Detailed analyses of membrane permeability including its pH dependency as well

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as the reversible ion-exchange binding of a protein (lysozyme) yielded information about the structure of the functional polymer brush layers. The resulting porous carboxyl polymer brush composite membranes showed a very promising membrane adsorber performance.

Experimental Section

Materials. Polypropylene (PP) membranes (Accurel PP 2EHF, cutoff pore size ~0.4 μm, membrane thickness 150 μm) were purchased from Membrana GmbH, Wuppertal, Germany. Methanol (HPLC grade) and benzophenone (BP; p.a.) were from Merck; acrylic acid (AA; 99%), acrylamide (AAm; 99+%), and *N*,*N*′-methylene bisacrylamide (MBAA; 99%) were from Aldrich; HEPES (min. 99.5%) was from Sigma; sodium chloride (biochemica) was from AppliChem; lysozyme (Lys; from hen egg white, 36 000 units/mg) as well as all other salts and titer solutions were from Fluka. All chemicals were used as received. Water purified with a Milli-Q system (Millipore) was used for all experiments.

Photoinitiated Graft Copolymerization. A UV illumination system (UVAPrint, Hoenle AG, Gräfelfing, Germany) equipped with a high-pressure mercury lamp and a glass filter ($\lambda > 300$ nm), providing homogeneous illumination of up to 100 cm^2 area with an intensity of about 50 mW/cm^2 (measured with the UVA meter, Hoenle AG), was used. The UV grafting method had been described elsewhere.[2,15−17] In this study, circular membrane samples with a diameter of 25 mm (always 4 samples per batch) were presoaked for 15 min (or 60 min) in 5 mL of a solution of 1 mM (or 10 mM) BP in methanol (“adsorption”) or heptane (“entrapping”). Thereafter, the samples were taken out and immediately immersed for 1 min into 5 mL of methanol (“adsorption”) or 2 times for 1 min in 5 mL of methanol (“entrapping”). Then, the samples were quickly wiped with filter paper in order to remove the adhering solvent and were then immediately immersed into 8 mL of the solution of the respective monomer or monomer mixture in water (deaerated by bubbling with nitrogen for 10 min) in a Petri dish (diameter 90 mm) and fixed separately between two sheets of filter paper (diameter 90 mm; no. 593, Schleicher & Schuell). After 5 min of equilibration, UV irradiation for 15 min followed. Then the samples were taken out immediately and then washed sequentially with 80 mL of water under stirring, first for 30 min at room temperature, second for 60 min at 60 °C, and third for 30 min at room temperature. Then the membranes were dried at 50 °C overnight. The degree of grafting (DG) was determined gravimetrially from the weight of each sample before and after functionalization.

Control experiments for the gravimetric determinations had been performed in advance. For the coating method blank effects were not significant. For the entrapping method, the effects of extraction from the membranes by heptane (weight loss) and of BP uptake without photografting were smaller than ± 10 $\mu g/cm^2$. Hence, neither pre-extraction of the membranes with heptane or numeric correction of the DG data were performed.

Determination of Photoinitiator Amount on/in Membranes. Membrane samples (4.9 cm^2) with adsorbed or entrapped BP were put for 2 h into 4 mL of methanol or heptane in a tightly closed vessel on a shaker (100 rpm). Then the BP concentration was determined based on the UV absorption at 250 nm with using a spectrometer Cary 50 Probe (Varian).

Heterogeneous Carboxyl Titration (Back-titration). Membrane samples (4.9 cm^2) were fixed in a syringe filter holder (Swinnex, Millipore). They were equilibrated for 1 h in 15 mL of a 100 mM HCl solution, facilitated by two filtrations of this solution through the membrane during this period. Then, three washing steps by filtration of 5 mL of water within 5 min through the membranes followed. Thereafter, the membranes were taken out of the filter holder and were immersed into 25.0 mL of a 3 mM NaOH in a tightly closed vessel and kept on a shaker (100 rpm) for 24 h; a blank NaOH sample without membrane was run in parallel. Thereafter, aliquots of 3.5 mL were taken from the NaOH solution and were titrated against a 1 mM HCl solution using phenolphthalein as the indicator; all titrations were done in quadruplicate. The carboxyl amounts were calculated from the average values, including the results of titer determinations for the NaOH and the HCl solutions as well as the respective blank values. The variation coefficient (VK) of the data from at least 4 independent membrane samples was <20% for membranes with higher DG values (>200 $\mu g/cm^2$), but even larger for samples with lower DG.

Water Permeability Measurement. Before flux measurements, all membrane samples were wetted with acetone or methanol and then exchanged with water. The water flux as a function of transmembrane pressure up to 2.5 bar was measured using the capillary flow porometer CFP-34RTG8A-X-6-L4 (PMI Inc., Ithaca, NY). Always, the pressure was stepwise increased in intervals of 0.05 bar at a waiting time of 30 s at each point. The water flux at different pH values, adjusted by the addition of HCl or NaOH, respectively, was measured using an ultrafiltration cell (Amicon model 8010, Millipore) with an effective membrane area of 3.14 cm^2, connected to a water reservoir and a nitrogen tank for adjusting a pressure of 0.1 or 1.5 bar, respectively.

Reversible Protein Adsorption (Membrane Chromatography). The chromatography system was an ÄKTA purifier (Amersham Pharmacia Biotech) with a UV detector set at 280 nm as well as conductivity and pH detectors. The following solutions were used: Buffer A, 10 mM HEPES buffer (pH 7.0) as the running buffer; Buffer B, 10 mM HEPES buffer + 1 M sodium chloride (pH 7.0) as the elution buffer. Both solutions were filtered through a 0.2 μm membrane filter before use. For each evaluation three circular membrane samples (diameter 12 mm) were first wetted in 1 mL of Buffer A for at least 1 h and then used as a stack in a CIM module (BIA Separations, Ljubljana, Slovenia). For all evaluations, the following protocol had been used: (i) stack conditioning with Buffer A for 3 min at a flow rate of 1.0 mL/min and for 1 min at 0.5 mL/min; (ii) injection of 1.0 mL of a Lys solution (5 mg/mL in Buffer A) and separation for 8 min with Buffer A at 0.5 mL/min; (iii) elution by gradient from Buffer A to Buffer B in 0.4 min and for 4 min Buffer B at 0.5 mL/min; (iv) reconditioning by switching to Buffer A and washing for 5 min at 1.0 mL/min; then the sequence (i) to (iv) was repeated. Quantification was based on the integration of the areas for the breakthrough and the elution peaks. Calibrations were done by injection of Lys solutions in Buffer A with different concentrations, from 1 to 5 mg/mL, at different sodium chloride concentrations and using the CIM module without membrane stack. No significant effect of sodium chloride had been observed. The estimated accuracy limit of this method is 0.01 mg.

Results and Discussion

The surface functionalization of polypropylene membranes using photoinitiated graft copolymerization had already been explored and used in several studies.[15−17] With BP, a “type 2” photoinitiator, the initiation of a heterogeneous graft polymerization relies on a hydrogen abstraction reaction from the base (membrane) polymer.[10,12,14] However, different from the work about other membrane polymers such as polyacrylonitrile[12] or polysulfone and polyethersulfone,[14] detailed investigations of the interactions of BP with the hydrophobic

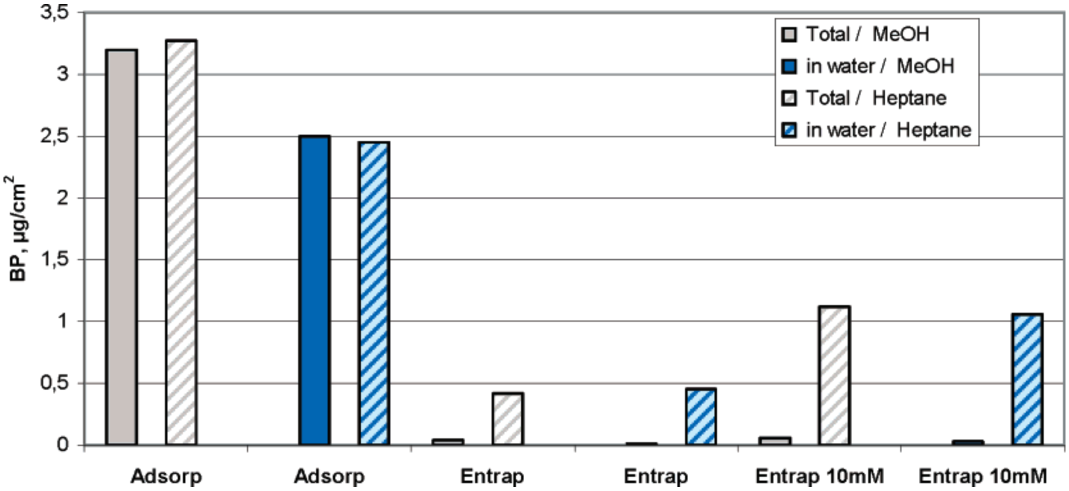

Figure 1. Amounts of BP on/in PP membranes after adsorption or entrapping method (using 1 mM or 10 mM BP concentration) determined by extraction with methanol or heptane: *total* ... samples after coating via adsorption or entrapping method and subsequent drying; *in water* ... samples after adsorption or entrapping method and subsequent transfer into water for 2 h.

membrane polymer PP had not yet been performed. In particular, the functionalization toward brush or "tentacle" membrane adsorbers for reversible protein binding and separation (cf.[4,5,7]) will require a good control because of the following:

(i) The surface of the PP should be shielded to avoid unspecific protein adsorption and thus product loss.

(ii) The three-dimensional binding capacity should be high, and reversible binding (recovery) should be quantitative.

(iii) The permeability of the porous membrane should not be reduced too much.

Photoinitiator Immobilization. For the coating with the photoinitiator, relatively low BP concentrations (1−10 mM) had been used because BP precipitation either after the evaporation of the solvent or during the exchange of the BP solution with the aqueous monomer solutions could occur as a consequence of a BP accumulation in the membrane pores. This would result in an uneven BP distribution and subsequently in inhomogeneous grafting. Note that in some earlier work 100 mM had been used, and attempts had been made to control the redistribution of BP into the monomer solution during grafting by further addition of BP to the solution.[17]

Heptane can moderately swell PP, and the heptane sorption in PP films at room temperature had been estimated experimentally between ∼12 wt %[27] and 7.6 wt %[28] while the value for ethanol had been only 0.015 wt %.[28] Therefore, heptane was used for the "entrapping method", while methanol was the solvent for the "adsorption method" (cf. Scheme 1). In general, it should be possible to control the density and depth of immobilized photoinitiator in the PP surface layer by choosing solvents of different affinity as well as different contact times and photoinitiator concentrations. The amounts of BP—before and after immersion of the coated membranes into water—were determined, and a polar and a nonpolar solvent were used to elucidate the extent and mechanism of BP immobilization on/in the PP surface (Figure 1).

The immobilized BP amounts were significantly higher for the adsorption than for the entrapping method. However, the effects of equilibrating the coated membrane with water and of the solvents for re-extraction were markedly different for the two methods. The identical values using methanol and heptane for re-extraction after the adsorption method confirmed that both solvents can quantitatively remove the BP from the membrane surface. After immersion in water, a significant fraction of the BP had been desorbed. Note that this fraction will be even larger when using an aqueous monomer solution. In contrast, for the entrapping method less than 10% could be removed with the polar methanol as compared with the nonpolar heptane. Also, there was no release into the water. These two findings confirm a permanent entrapping of the BP under polar solvent conditions.

When a higher BP concentration (10 mM) at longer contact time (60 min) is used, the amounts of entrapped BP could reproducibly be increased to about 1.1 μg/cm^2. For the adsorption method, larger variations of the data, especially for the total amount, were observed (not shown); after equilibration with water a BP loading of ∼3 μg/cm^2 could be achieved.

Based on porosity and nitrogen adsorption (BET) data for the membrane material (∼20 m^2/g[17]), BP amounts of 3 μg/cm^2 (relative to the outer membrane area) correspond to 47 μg/m^2 (relative to the specific surface area); i.e., the area per BP molecule is ∼600 Å^2. In an adsorbed monolayer, BP has an area of ∼90 Å^2. Hence, for the adsorption method the coverage of the specific membrane surface with BP can be estimated to be about 15%. The main reason is the relatively low BP concentration used for the coating. Higher densities of covalently immobilized initiators had been used for the synthesis of dense brush layers.[8,23] On the other hand, the average cross-sectional area of a stretched polyacrylate chain has been estimated to about 180−200 Å^2.[23] Also, for experiments at maximum surface densities of initiator groups a rather lower grafting initiation efficiency had been ob-

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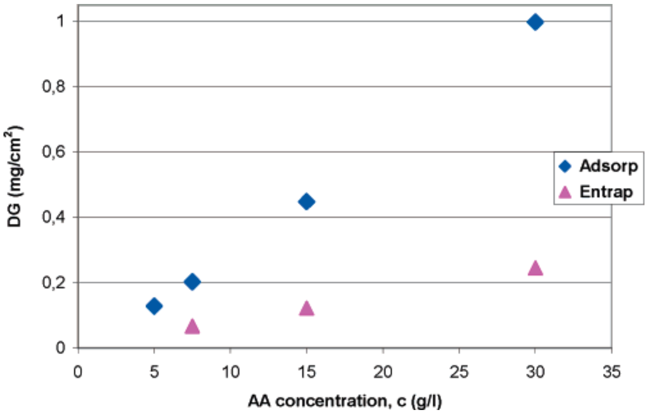

Figure 2. Degree of functionalization (DG) with grafted poly(acrylic acid) (*g*-PAA) as a function of monomer concentration (1 mM BP for adsorption and entrapping method).

served.[23] Note also that especially in the micro- and mesopore fraction of the PP membranes, the growth of the grafted layer will be hindered sterically. During grafting, the release of adsorbed BP into the reaction mixture is not suppressed completely. In contrast, after the entrapping method the BP is efficiently fixed in the surface layer of the PP when the membranes are exposed to polar, including aqueous solutions. However, accessibility could be reduced by the entrapping in the PP surface layer, and the BP surface densities were lower by a factor of 3−5 than estimated for the adsorption method.

Surface Photoinitiated Graft Copolymerization and Grafted Polymer Layer Structure. Figure 2 shows the effects of monomer concentration using the two photoinitiator immobilization methods. In both cases, an almost linear increase of the DG as a function of the AA concentration was observed. The DG values for the entrapping method were between 33 and 24% compared to the ones for the adsorption method, while the relative BP amounts for these coating conditions (1 mM) and equilibration with water was only 18% (cf. Figure 1). This indicates the following:

(i) The entrapped BP is indeed accessible for the initiation of a heterogeneous graft copolymerization.

(ii) The initiation efficiency of the adsorbed BP seems to be lower, e.g., caused by further BP desorption from the PP surface during the reaction. Nevertheless, the grafting density for the entrapping method will still be lower than that for the adsorption method, but for the adsorption method side reactions initiated by desorbed BP in the monomer solution cannot be ruled out.

The graft functionalization of the PP could be unambiguosly characterized by ATR-IR spectra (not shown); characteristic peaks for PAA (1710 cm^{-2}) could be identified along with the fingerprint peaks of the substrate PP. The wetting of the membranes with water improved very much due to the graft modification, and beyond a DG of ∼300 $\mu\text{g/cm}^2$ the membranes were hydrophilic (water was spontaneously taken up by those membranes while this was not possible for the unmodified PP membranes). Very similar modification efficiencies were achieved for both outer surfaces of the membranes, the UV exposed and remote surfaces, as judged from the intensity ratios PAA vs PP in the ATR−IR spectra or the wetting with water. Previous work with PP microfiltration membranes having the same thickness had also confirmed that a rather homogeneous distribution of the grafted functional polymer on the entire pore surface throughout the membrane cross section can be achieved via photoinitiated "grafting-from" under optimized conditions.[15−17]

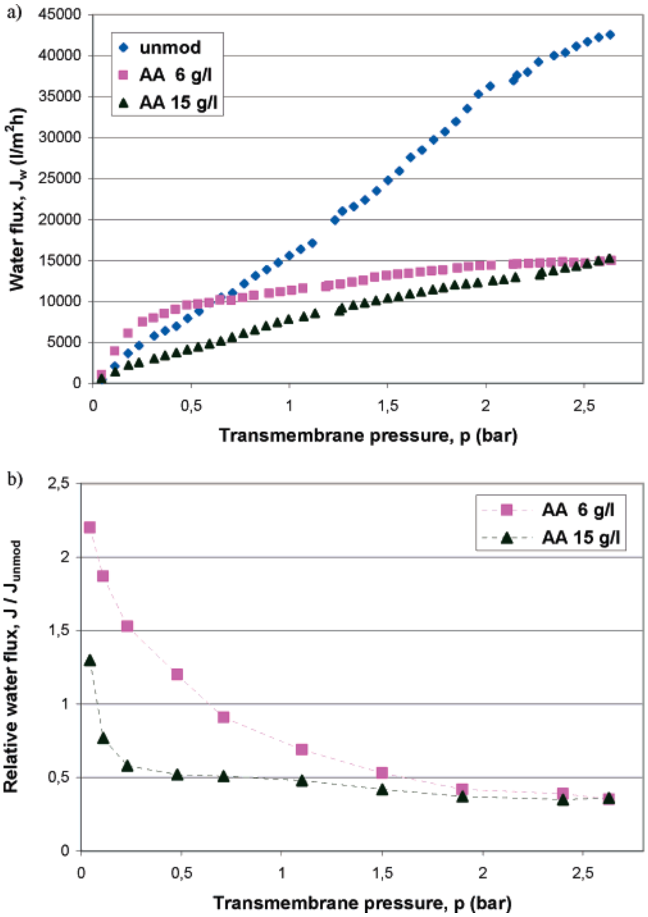

Figure 3. (a) Water flux through unmodified and *g*-PAA functionalized PP membranes (adsorption method; cf. Figure 2) as a function of transmembrane pressure; (b) normalization of the fluxes of the *g*-PAA functionalized membranes relative to the flux of the unmodified PP membrane. The DG values were 160 $\mu\text{g/cm}^2$ and 440 $\mu\text{g/cm}^2$, respectively, for the membranes prepared with 6 g/L and 15 g/L AA, respectively. The pH of water was 5.7, i.e., above the $\text{p}K_a$ of PAA.

Influence of Surface Initiation onto the Grafted Polymer Layer Structure. After the functionalizations, the membranes were macroscopically unchanged; even with high-magnification scanning electron microscopy the morphology of the pore surfaces (in dry state) could not be distinguished from the unmodified PP (cf.[15−17]). However, the influence of functionalization conditions onto the structure (in solvent) of polymer layers grafted to the pore walls can conveniently be studied by membrane filtration experiments. Detailed water permeability studies were performed with membranes at different degrees of functionalization; characteristic data are shown in Figure 3. The unmodified membrane showed a linear increase of flux with pressure; i.e., the permeability was constant. The water flux of functionalized membranes with higher DG (beyond ∼300 $\mu\text{g/cm}^2$) was lower than that of the original PP membrane, except for very low pressures; and the permeability was slightly decreasing with increasing trans-membrane pressure. However, for membranes with lower DG (up to 250 $\mu\text{g/cm}^2$), the flux in the lower pressure range was higher than that of the original PP membrane; and with increasing pressure a very pronounced decrease of permeability, reaching values lower than those for the unmodified membrane, was observed (cf. Figure 3b).

Considering the average pore diameter of the unmodified membrane (cutoff 0.4 μm) and the rather even functionalization of the entire pore surface over the membrane thickness (cf.[15,17]), a reduced water permeability ($J/J_{\mathrm{unmod}} < 1$) can be explained by a reduction of the average pore size. However, the wettability of the hydrophobic membrane pores is much enhanced by the grafted PAA. This additional effect on flux ($J/J_{\mathrm{unmod}} > 1$) can only be detected at low pressure *and* when the effect of the grafted polymer layer onto pore blocking is small. Furthermore, an increased shear at higher pressure/flux caused a deformation of the layer. Such effects onto polymer brushes in membrane pores had first been studied with adsorbed block copolymers in cylindrical membrane pores,[29] and more recently with poly(vinylpyrrolidone) grafted onto a silica microfiltration membrane.[30] Depending on grafting density and chain length, a more or less pronounced shear-induced reduction of layer thickness had been deduced from the flux data. In contrast, a further reduction of membrane permeability, i.e., an apparent "thickening", had been observed in this study. This could be explained by the uneven pore structure of the spongelike PP membrane along with a high brush flexibility because the effect was more pronounced for membranes with low DG. Similar findings, i.e., a complex influence of a flexible and responsive grafted polymer brush layer onto permeability, had been reported by Peng and Cheng.[31]

The conformation of grafted PAA chains changes as a function of the ionization below and above its $\mathrm{p}K_a$ value of about 4.[32] If the PAA is attached to a pore surface, the effect is also known as membrane permeability switch or valve effect.[15,32] Consequently, the susceptibility of the trans-membrane flux to pH will be related to the structure of the grafted layer. The magnitude of the effect will increase with (average) chain length. Further, compared with a random, branched, or even cross-linked structure, brush layers with linear chains will have a more pronounced response.

Data measured at low trans-membrane pressure where the shear effects (cf. above) were minimized are shown in Figure 4. For the membranes prepared by both methods, the same dependency of permeability on DG was seen at low pH. At lower DG values, the effect of enhanced hydrophilicity dominated clearly; only at values beyond 3 mg/cm^2 did the pore blocking become dominating. However, at high pH, the permeability was much lower than that for the unmodified membranes even at the lowest DG values. Furthermore, a significant difference between the two different photografting methods could be observed: The membranes prepared by the entrapping method showed a larger susceptibility toward pH changes. A complete blocking of the hydraulic water flow through 0.4 μm pores was achieved at a DG of 1.2 mg/cm^2 for the entrapping method while the same effect was obtained only at DG larger than 2.5 mg/cm^2 for the adsorption method.

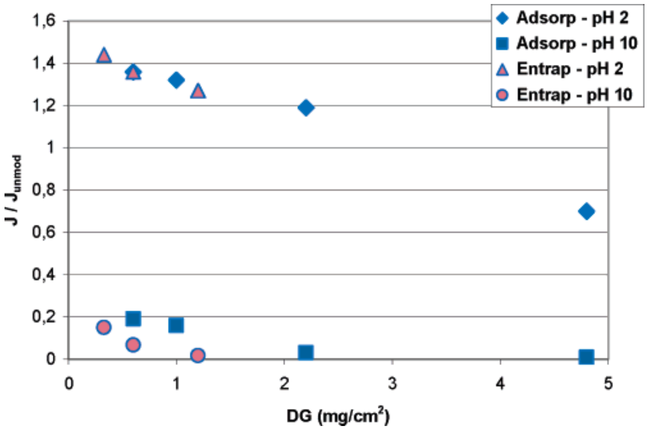

Figure 4. Water flux ratio J/J_{unmod} for *g*-PAA functionalized PP membranes at different pH value and low trans-membrane pressure (0.1 bar; 1 mM BP for adsorption and entrapping method; the membranes with the two highest DG values in both series were prepared using 30 g/L AA and longer UV times; i.e., 30 and 60 min; cf. Experimental Section).

The relative permeabilities at higher trans-membrane pressure (1.5 bar) for the high pH were identical to the data shown in Figure 4, while at the low pH a small but significant reduction compared with low pressure had been observed (data not shown). Hence, the shear-induced deformation yielding an additional pore blocking occurred also with the grafted layer in its collapsed conformation. This deformation seems to require a minimum flow rate which had not yet been reached for the pores almost completely blocked by the grafted chains in their stretched conformation (at high pH). However, during the water flux measurements at pH 5.7, i.e., for the partially ionized state of PAA, this deformation could be observed (cf. Figure 3).

The larger switch effect at the same DG and low pH permeability for the membranes prepared using the entrapping method can be explained by the lower amount of immobilized BP and its tighter fixation, which leads to a lower grafting density and longer grafted chains compared to the adsorption method (note also that with the entrapping method the same DG had been obtained at higher monomer concentration which will also facilitate a larger average grafted polymer chain length).

Varied Grafted Copolymer Layer Structure. Further preparations were done with the aim to introduce variations into the grafted structure at the same overall degree of functionalization. The copolymerization with AAm should introduce spacers between the carboxyl functional monomer units; the cross-linking should reduce the flexibility of the grafted layers. The higher BP concentration was used in the photoinitiator coating step, and conditions were identified, making possible a comparison of both methods. The DG values for the same reaction conditions (here 15 g/L AA) were not significantly higher after entrapping immobilization using 10 mM BP (cf. Table 2) as compared with 1 mM BP (120 μg/cm^2; cf. Figure 2). The larger amount of entrapped BP (cf. Figure 1) seemed to have been used less efficiently as a photoinitiator for the heterogeneous graft copolymerization. Presumably, due to the longer sorption time (cf.

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Table 1. Degrees of Functionalization for PP Membranes Photofunctionalized Using Varied Monomer Mixtures (Average Values from 4 to 15 Independent Samples; 10 mM BP, 60 min)

	DG (μg/cm^2)	
monomer solution	adsorption	entrapping
AA, 6 g/L	120 ± 10	
AA, 15 g/L	390 ± 60	110 ± 20
AA/AAm, 3/3 g/L	120 ± 10	
AA/AAm, 10/10 g/L	400 ± 40	140 ± 10
AA/MBAA, 15/0.75 g/L	280 ± 10	110 ± 10

Table 2. Water Permeabilities for Photofunctionalized PP Membranes (Measured at 0.1 bar /pH 2/ and 1.5 bar /pH 10/; between 4 and 12 Independent Samples, cf. Table 1; VK < 10%)

	$J/\Delta p$ (L/m^2·h·bar)			
	adsorption		entrapping	
monomer solution	pH 2	pH 10	pH 2	pH 10
unmod	11000	10600		
AA, 6 g/L	13000	6100		
AA, 15 g/L	12700	1180	13000	990
AA/AAm, 3/3 g/L	11700	7400		
AA/AAm, 10/10 g/L	13300	380	13400	1600
AA/MBAA, 15/0.75 g/L	12400	1900	12900	3200

Experimental Section), some BP had penetrated deeper into the PP matrix, so that it could not contribute to the initiation of "grafting-from" the PP surface. Again, the PP functionalization could be characterized by ATR-IR spectra (not shown) by characteristic peaks for PAAm (1660 and 1615 cm^{-2}). Degrees of graft functionalizations are shown in Table 1.

The data for higher total monomer concentration indicate a lower reactivity of AAm in the copolymerization with AA as well as a reduced grafting efficiency by the presence of the cross-linker monomer. These effects were less pronounced for the entrapping method and at the lower total monomer concentration. For the membranes prepared via the coating method, carboxyl titration data were compared with a theoretical carboxyl amount for the respective DG under the assumption that the graft copolymer composition would be identical to the composition of the monomer solution (carboxyl content: 100%). The results were 4.8 μmol/cm^2 (89%) for the *g*-PAA membrane with a DG of 390 μg/cm^2, 2.2 μmol/cm^2 (40%) for the *g*-PAA-*co*-AAm membrane with a DG of 400 μg/cm^2, and 3.2 μmol/cm^2 (87%) for the *g*-PAA-*co*-MBAA membrane with a DG of 280 μg/cm^2. It should be noted that the experimental error had been rather high (cf. Experimental Section), so that the data for the other membranes with lower DG had not been used. Nevertheless, considering that the data for the homo-PAA graft were also somewhat smaller than the theoretical value, it may be concluded that under the functionalization conditions the composition of the copolymer grafts of AA and acrylamides had been similar to the composition of the monomer mixture. Hence, the graft composition seemed to be much less sensitive toward monomer solution conditions than reported for the very similar photografting system PP/BP/AA/AAm.[33]

The permeabilities at different pH values are given in Table 2. While for all functionalized membranes the data at low pH were similar and always somewhat higher than for the unmodified membranes (cf. Figure 4), the data at high pH revealed pronounced variations. An overview is given in Figure 5 where the permeability ratios for the various membranes are plotted as a function of DG, and three main effects of the grafted copolymer layer structure could be identified:

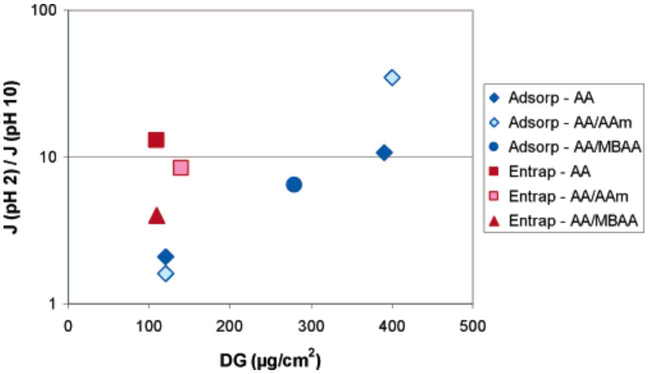

Figure 5. Water permeability ratio pH 2 vs pH 10 for photofunctionalized PP membranes as a function of the degree of functionalization (cf. Table 2).

(i) All membranes prepared via the entrapping method showed a larger switch effect, i.e., lower permeabilities at high pH, as compared with their couterparts prepared via the adsorption coating method and having the same DG.

(ii) The copolymerization of AA with the cross-linker MBAA yielded a significantly reduced switch effect for both preparation methods.

(iii) Irrespective of the lower AA content due to the copolymerization with AAm, for both preparation methods the switch effects were similar to the data for the homo PAA grafts; for the highest DG this effect was even larger.

Effect (i) can be interpreted by a lower grafting density and/or less branching due to photoinitiated side reactions achieved by the entrapping method. As a consequence, the average grafted chain length is larger, so that a more efficient pore blocking is achieved at the same DG. This is in agreement with the other data shown and discussed above.

Effect (ii) can be explained by restricting the swelling of the grafted layer through chemical cross-linking. For example, Saito et al. had reported an analogous effect, an increased permeability with increasing chemical cross-linking density at the same degree of functionalization with sulfonic acid functional brush layers on the pore surface of polyethylene hollow-fiber membranes prepared by radiation initiated graft copolymerization.[34]

The explanation of effect (iii) is less straightforward because the copolymer brushes had only about half of the (average) carboxyl density as compared with the homopolymer brushes (as deduced from the titration data). Thus, both driving forces for brush swelling, the osmotic pressure by the counterions in the brush layer,[8] and the electrostatic repulsion between the repeating units of the grafted chains[32] will be reduced. However, the following hypothesis may provide an explanation: At high pH, the solvation of the PAAm segments will be strong and thus similar to the ionized PAA segments; i.e., either the driving forces for chain

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stretching are still sufficiently high or/and the blocking is caused by the swelling of the grafted layer. Note that the swelling of weak polyelectrolyte hydrogels for intermediate degrees of ionization is indeed almost independent from the degree of ionization; for PAA-*co*-AAm solutions and gels, a constant degree of swelling had been observed beyond 35% ionization,[35] and for cross-linked PAA-*co*-AAm-MBAA hydrogels even a slight decrease of swelling at increasing AA content had been observed.[36] At low pH, the PAA segments are protonated, and hence the driving force for stretching or swelling of these segments is reduced anyway. However, in addition hydrogen bonding between PAA segments (donor) and PAAm (acceptor) are now also possible (cf.[37]). Note that via this physical cross-linking mechanism, a maximum suppression of PAAm segment swelling (as compared with the higher pH) will only be possible for equal amounts of AA and AAm and their statistical distribution in a flexible layer structure. Both preconditions seem to be fulfillled for the PAA-*co*-AAm grafts in the present study (cf. above). Nevertheless, the verification of this hypothesis will require further investigations which are currently underway by a systematic variation of the grafted layer composition and preparation conditions.

Reversible Protein Binding (Membrane Chromatography). Porous membrane adsorbers have a very large potential as tailored high-performance separation materials, e.g., for the biotechnology industries. On the other hand, the evaluation of functionalized microfiltration membranes in the flow-through adsorber mode is a convenient method to obtain detailed information about the functionality of the grafted layers. In both aspects, the influence of slow diffusion processes in the porous material can be reduced or even eliminated because of the pore structure of the membrane.[4,5,17]

A chromatography system along with a module for flat-sheet porous adsorbers (monoliths or membranes[4]) had been used. Lysozyme had been selected as a typical model protein, which due to its small size (MW 13.9 kDa; 30*30*45 Å^3), high isoelectric point (pI 11.9), and rigid conformation[38,39] is suited to evaluate the binding capacity of weak cation exchange layers with a three-dimensional structure. A moderate flow rate had been selected (the permeability of most of the membranes would allow higher flows, but for some types, e.g., AA/AAm-10 (Adsorp) as the extreme case, the permeabilities at pH > pK_a are very much reduced so that the back-pressure could rise too much; cf. above). Binding was done at low salt concentrations and elution was achieved by increasing the salt concentration; a typical chromatogram is shown in Figure 6. A significant asymmetry had been observed for the elution peak, suggesting a delayed response of fractions of the bound protein to the rapid increase of salt concentration; the detailed influences of chromatographic conditions and membrane structure onto separation resolution are under investigation. Here, the binding capacity and the recovery of bound protein as well as the reusability of the membranes (from comparison of two subsequent runs) had been considered as the main criteria. An overview on the data is given in Table 3.

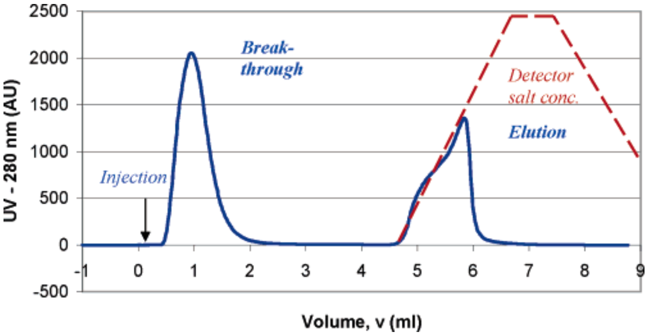

Figure 6. Typical chromatogram for the evaluation of reversible protein binding to photofunctionalized PP membrane adsorbers—here membrane "AA/MBAA", prepared via the entrapping method (for detailed conditions cf. Table 3 and Experimental Section).

Table 3. Reversible Protein Binding from Membrane Chromatography Experiments for Photofunctionalized PP Membranes (Stack of 3 Membranes, 5.0 mg Lys Injected, Flow Rate 0.5 mL/min− 10 mM Buffer pH 7, after 8 min Gradient to 1 M NaCl, pH 7)[a]

photografted membrane		DG (μg/cm^2)		Lys bound (mg)	Lys eluted (mg)	recovery (%)
unmod			first run	0.27	0.05	19
			second run	0.04	0.05	125
adsorp	AA-6	120	first run	1.41	1.13	80
			second run	1.17	1.15	98
adsorp	AA-15	390	first run	2.86	2.82	99
			second run	2.87	2.93	102
entrap	AA-15	110	first run	1.06	0.78	74
			second run	0.78	0.79	101
adsorp	AA/AAm-3	120	first run	0.94	0.70	74
			second run	0.68	0.70	103
adsorp	AA/AAm-10	400	first run	1.73	1.36	79
			second run	1.51	1.38	91
entrap	AA/AAm-10	140	first run	2.29	2.23	97
			second run	2.32	2.36	102
adsorp	AA/MBAA	280	first run	2.66	2.67	100
			second run	2.64	2.67	101
entrap	AA/MBAA	110	first run	2.28	2.16	95
			second run	2.16	2.17	100

[a] *Lys bound*, from breakthrough peak; *Lys eluted*, from elution peak; *recovery*, (Lys eluted/Lys bound) × 100%. Data shown are average values from 2 or 3 experiments with different membrane stacks from the respective preparations, cf. Table 1; variation < 5%).

The unmodified PP membrane bound significant amounts of Lys, but this binding was mostly irreversible. Also, the binding capacity was much lower in the second run (due to the very low values for Lys bound and eluted, the apparent recovery of 125% is considered to be due to the experimental error). This behavior can be explained by adsorption of Lys on the internal surface of the membrane. With the porosity and BET surface area for the PP membrane (cf. above), the irreversibly bound Lys amount (0.22 mg; cf. Table 3) corresponds to 3.3 mg/m^2. This is very close to data for adsorbed Lys monolayers on plane surfaces functionalized by SAMs with octadecyl (1.4 to 2.3 mg/m^2)[38] or dodecyl groups (2.6 mg/m^2)[39]; the irreversible adsorption of Lys on

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the hydrophobic alkyl surfaces proceeded along with its denaturation.[38] In the second run, only a small amount of reversibly bound Lys could be detected (cf. Table 3), and this will presumably be due to ionic, and thus reversible, Lys/Lys interactions.

For all functionalized membranes, the bound Lys amounts were much higher, and both reversibility (recovery) and reusability were significantly better than those for the unmodified membrane (cf. Table 3). The highest values, obtained for PP-*g*-PAA at a DG of 390 μg/cm^2, correspond to 300 μg/cm^2—relative to the outer surface area of the membranes—or to 20 mg/mL—relative to the membrane/bed volume. This is in the same range compared with other membrane adsorbers in the scientific literature.[4] For one of the first commercial membrane adsorbers with a weak cation-exchange functionality, a protein binding capacity of 20 mg/mL has been reported.[40] Furthermore, based on the estimation above, the binding capacity of the grafted layers is more than 10 times higher than that due to monolayer adsorption onto the entire pore surface of the PP membrane. "Degrees of multilayer binding" for Lys of up to 36 had been measured for sulfonic acid functional brush layers prepared via radiation-initiated grafting-from on porous hollow-fiber membranes.[7] Note that also three other membrane types in the present study showed binding capacities in the same range, and the lowest data were still 3 times higher than monolayer capacity (cf. Table 3). Hence, the photografted copolymer layers can be used for protein binding in the three-dimensional brush layer. Reversibility indicates that binding takes place exclusively or mainly to the functional groups of the graft copolymer and that the PP surface is effectively shielded. Note that the amounts of reversibly bound protein can only be explained by a grafted functional polymer on the entire specific surface area of the membranes. This confirms indirectly that a rather homogeneous functionalization of the entire pore surface had been achieved (cf. above).

It is most remarkable that such high and reversible binding capacities can also be achieved for membranes at low DG values such as 100 μg/cm^2. The efficiency of protein binding had been expressed as the ratio between the amounts of protein and graft copolymer (see Figure 7). The main findings were as follows:

(i) Membranes with a DG between 100 and 150 μg/cm^2—except the two membranes prepared by the entrapping method mentioned under (iv)!—and the *g*-PAA-*co*-AAm membrane prepared by the adsorption method showed a significantly lower recovery after the first protein binding run.

(ii) The data for the membranes prepared via the adsorption method indicate that the binding efficiency is reduced with increasing DG.

(iii) The copolymer grafted layers containing AAm or MBAA have a somewhat (adsorption method) or a significantly (entrapping method) higher binding efficiency than the homo PAA grafted layers.

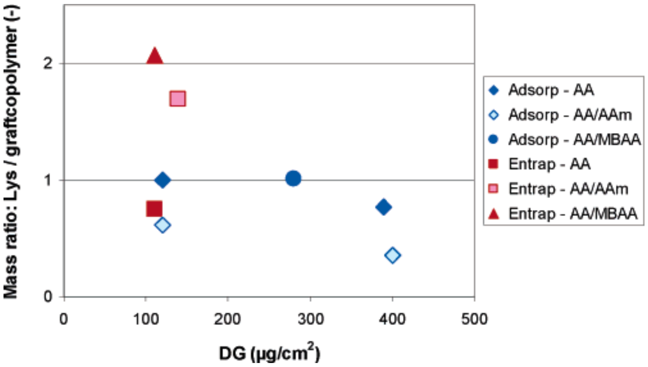

Figure 7. Efficiency of protein binding: ratio between Lys (mg Lys eluted in first run) and amount of graft copolymer on the photofunctionalized PP membranes (mg per stack from DG). Note that the overall performance is fully acceptable only for four membranes, showing >95% recovery already on the first run (cf. Table 3).

(iv) The membranes *g*-PAA-*co*-AAm and *g*-PAA-*co*-MBAA prepared via the entrapping method showed the largest binding efficiencies, both in combination with an excellent recovery.

Effect (i) for the membranes at low DG can be interpreted with irreversible Lys binding to PP, i.e., for these preparations the surface coverage of PP may have been incomplete. For the *g*-PAA-*co*-AAm membrane the Lys loss is larger than what may be adsorbed to the unmodified PP surface. Hence, Lys entrapment in the layer will be more probable. Furthermore, the accessibility of binding sites in extended or dense grafted layers will be lower (cf. (ii)). The comparison of Lys binding for PAA and PAA-*co*-PAm of similar DG prepared via adsorption method also confirms the conclusions from the carboxyl titration that the composition of the graft copolymer is similar to that of the monomer mixture (cf. above and (iii)). All data indicate that the amount or density of carboxyl groups is not the limiting factor for protein binding by cation exchange. In particular, the improvement of efficiency for the two types of copolymers, with "dilute" carboxyl groups or with a cross-linked structure, support this statement (cf. (iii)).

Finally, and along with the other conclusions regarding suited compositions and DG values, there is a clear influence of the method of surface photoinitiation also on protein binding efficiency (cf. (iv)). Therefore, it is proposed that an even grafting density, sufficient for shielding of the PP surface, in combination with long and very flexible (and thus responsive) grafted polymer chains make the membranes superior to the other materials in terms of all criteria (including a comparatively high permeability at a pH above the pK of PAA; cf. Table 2). Hence, the membranes *g*-PAA-*co*-AAm and *g*-PAA-*co*-MBAA prepared via the entrapping method are the most promising starting points for further tailoring of membrane adsorber properties.

Conclusions

The potential of rather simple and robust methods to improve the efficiency of photoinitiation by controlling photoinitiator location (surface adsorption vs entrapping in the surface layer) and amount (surface density) has been demonstrated. Both the complete photoinitiator fixation under

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(40) *Sartobind Membrane Adsorbers for Rapid Purification of Proteins*; Product information of Sartorius AG, Göttingen Germany, 2004, www.sartorius.com.

aqueous reaction conditions and the accessibility of UV-induced PP starter radicals for monomer grafting have been shown. The entrapping method may also be useful for grafting reaction from other polar organic solvents or for other types of functionalizations via “grafting-from” such as the synthesis of thin-layer MIPs.[16]

Membrane permeability and protein binding under membrane chromatography conditions have been demonstrated to be versatile tools for a detailed characterization of grafted brush layers in membrane pores. Effects of grafting density and grafted chain length as a function of the initiation and polymerization conditions as well as a grafted structure variation by copolymerization can be elucidated. Using the same methodology with membranes having a more regular pore geometry such as track-etched membranes will allow further quantitative estimations, e.g., of brush layer thicknesses.[41]

Compared with BP adsorption, the BP entrapping method yielded a less dense grafted layer with longer PAA chains at the same degree of functionalization. This was due to somewhat lower immobilized BP amounts, but also less side reactions via nonselective photoinitiated cross-linking of grafted chains by dissolved BP. Unexpected properties of the PAA-*co*-AAm brush layers were their even larger swelling/deswelling as a function of a pH change (above and below the pK_a of PAA) as compared with those of the PAA brushes. Both PAA-*co*-AAm and cross-linked PAA-*co*-MBAA layers showed Lys binding capacities—more than 10 times higher than monolayer adsorption onto the unmodified PP membrane surface—and quantitative recoveries similar to PAA of the same DG; and the highest efficiencies of protein binding (Lys amount relative to amount of graft copolymer) were achieved with the membranes prepared by the entrapping method. Hence, the more controlled BP entrapping method had distinct advantages in terms of the control of grafted layer structure leading to an improved membrane adsorber performance.

The results provide the basis for the design of improved membrane adsorbers. In particular, the inter-relationships between stretching and collapse of the carboxyl functional brush layers as a function of pH and salt concentration and their protein capturing efficiency, both as a function of flow rate through the pores, are under investigation in more detail. Proteins with larger size than the model (Lys) used in this work will also be studied, with a particular focus on the accessibility of binding sites. Furthermore, using other functional monomers will allow extension of the range of membrane adsorber binding mechanisms (cf.[17]). However, the work also has relevance for the better understanding and further development of responsive (“smart”) membranes and other polymeric materials, reacting to stimuli such as change of pH, temperature, or solute binding.[42]

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