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Molecularly imprinted polymer membranes for substance-selective solid-phase extraction from water by surface photo-grafting polymerization

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Abstract

Hydrophilized polyvinylidene fluoride microfiltration membranes were surface-modified in the presence of a template (terbumeton) in methanol with a graft copolymer of a functional monomer (2-acrylamido-2-methyl-1-propane sulfonic acid, AMPS, methacrylic acid, MAA, or acrylic acid, AA) and a cross-linker (*N,N′*-methylene-bis-acrylamide) using UV irradiation and benzophenone as photoinitiator. As result, membranes covered with a thin layer of imprinted polymer selective to terbumeton were obtained. Blank membranes were prepared with the same monomer composition, but in the absence of the template. The membranes' capacity to adsorb terbumetone from aqueous solution was evaluated yielding information regarding the effect of polymer synthesis (type and concentration of functional monomer, concentration of cross-linker) on the resulting membranes' recognition properties. UV spectroscopic studies of the interactions with terbumetone revealed that AMPS forms a stronger complex than MAA and AA. In agreement with that finding, imprinting with AMPS gave higher affinities than with MAA and AA. The terbumeton-imprinted membranes showed significantly higher sorption capability to this herbicide than to similar compounds (atrazine, desmetryn, metribuzine). With the novel surface modification technology, the low non-specific binding properties of the hydrophilized microfiltration membrane could successfully be combined with the receptor properties of molecular imprints, yielding substance-specific molecularly imprinted polymer composite membranes. The high affinity of these synthetic affinity membranes to triazine herbicides together with their straightforward and inexpensive preparation provides a good basis for the development of applications of imprinted polymers in separation processes such as solid-phase extraction.

Keywords: Molecular imprinting; Photo-initiated graft copolymerization; Affinity membrane; Solid-phase extraction; Filtration; Pesticides; Terbumeton

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1. Introduction

During the last decades molecular imprinting has

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received considerable attention as an approach for introducing binding sites mimicking those of biological receptors in synthetic polymers [1,2]. A prearrangement of template molecules and functional monomers takes place in solution prior to a cross-linking polymerization which is initiated preferably at low temperature generating a highly cross-linked polymeric network. The extraction of the template results in formation of cavities in the polymer which in shape and spatial arrangement of functional groups are complementary to the template molecule. The specific interactions between the functional monomer and the template can be either reversible covalent or non-covalent in nature [1]. According to the covalent approach a polymerizable derivative of the analyte should be synthesized and used as a template. The subsequent extraction of such a template requires cleavage of covalent bonds. Since the choice of reversible covalent interactions and the number of potential templates are substantially limited, the attention is very much focused on using non-covalent interactions. In addition to the better versatility of this more general approach, it allows fast and reversible binding of the template [3].

It has been shown previously, that molecularly imprinted polymers (MIPs) possess high selectivity and sensitivity for low-molecular-mass compounds [4,5]. Synthesis of MIPs is a relatively straightforward and inexpensive procedure. Moreover, these polymers demonstrate very good thermal and mechanical stability and can be used in aggressive media [6]. MIPs have been widely studied for chromatographic separation [2,7,8], as antibody mimics [2,8,9] and as selective elements of chemical sensors [10,11]. In particular, the application of MIPs for solid-phase extraction (SPE) is a field of intense development [12,13]. However, reducing the non-specific binding of solutes to the MIPs and especially enabling applications for watery solutions are still major challenges.

Membranes, instead of columns filled with particles, become increasingly attractive for efficient affinity separations [14], including SPE [13]. Several approaches for combining imprinting and membrane technologies have been proposed to develop stable permselective or affinity membranes for the separation of special target molecules from a mixture of structurally similar compounds [15–18]. However, for MIP membranes synthesized via in situ polymerization, the small fluxes (typically not larger than 10^{-3} mol m^{-2} h^{-1}) shaded their practical application. This can be explained by the high degree of cross-linking of MIPs which is prerequisite for MIP membrane selectivity. Alternative approaches to the synthesis of MIP membranes are based on grafting or casting a layer of imprinted polymer to the surface of a stable support membrane [18–20]. In the first example [19], membranes made of a special photoreactive polymer were photografted with acrylic acid and *N*,*N*′-methylene-bis-acrylamide in the presence of theophylline yielding theophylline-specific filtration membranes. The complicated membrane formation including synthesis of a special polymer together with very long reaction times needed for MIP functionalization (24 h) and the very low membrane permeability substantially limit the feasibility of this particular method for separation applications. In another example [20], an already established MIP synthesis recipe was applied to reactively ''cast'' the MIP onto the entire surface of a polypropylene microfiltration membrane. However, the macropores of the matrix membranes were blocked, and only the application in a slow (solute flux~10^{-3} mol m^{-2} h^{-1}) diffusion-driven separation was possible.

A much more general approach for surface design of separation membranes by heterogeneous photografting is currently being explored [21,22]. Briefly, polymer membranes are coated with a photoinitiator, for example benzophenone, which after selective UV excitation, via a hydrogen abstraction reaction, creates radicals on the membrane polymer surface. These radicals can be used as starter of a graft copolymerization of functional monomers from the surface by this means creating a thin layer of covalently attached functional polymer covering the entire specific surface of the membrane. This approach has also already been successfully applied to the synthesis of MIP membranes as recently shown for the template desmetryn from aqueous imprinting mixtures with polypropylene as the base membrane material [23].

The aim of this study was to develop a method for achieving MIP synthesis in a thin layer on the entire surface of a porous microfiltration membrane without impairing its porosity and permeability. In contrast to previous work [23], the focus was onto syntheses from organic solvents because this increases the number of possible templates. Consequently, a rela-

tively hydrophobic triazine herbizide, terbumetone, was chosen as the template. The selection of functional monomers was based on UV spectroscopic characterization of their complex formation with the template. The impact of the reaction mixture composition onto MIP membrane structure and function was evaluated. A polyvinylidene fluoride (PVDF) membrane, already surface-modified with a thin hydrophilic polyacrylate layer, was found to be especially beneficial for efficient MIP membrane synthesis and good separation (SPE) performance. The novel thin-layer MIP composite membranes possess a high permeability matching that of conventional microfiltration (MF) membranes and a high selectivity to the analyte from aqueous solutions. Furthermore, the very low non-specific binding of MIP membranes synthesized under optimal conditions, is the precondition for a pronounced binding specificity towards the template terbumetone versus other structurally similar triazine herbicides.

2. Experimental

2.1. Materials

Hydrophobic and hydrophilized PVDF microfiltration membranes (Durapore) with a nominal pore size d_p=0.22 μm and a thickness of 125 μm were purchased from Millipore (Eschborn, Germany). Acrylic acid (AA), methacrylic acid (MAA) and 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS) were obtained from Aldrich (Deisenhofen, Germany), benzophenone (analytical-reagent grade) and *N*,*N*′-methylene-bis-acrylamide (MBAA) were purchased from Merck (Darmstadt, Germany). Terbumeton, atrazine, desmetryn and metribuzin (all Pestanal) were obtained from Riedel-de Haën (Seelze, Germany). All other chemicals and solvents (HPLC grade) were obtained from commercial sources; all materials were used as received without further purification.

2.2. Investigation of template/functional monomer complexation by UV spectroscopy

A 1-ml volume of a solution of terbumeton in methanol was titrated with aliquots of functional monomer (AA, MAA or AMPS) at 23°C. The solution was equilibrated for 3 min after injection, then the UV spectra of the mixtures were measured using a Kontron double-beam spectrophotometer (Type UVIKON 930). The data for the mixtures were then compared with the superimposition of the spectra of the single compound (terbumeton and functional monomer, respectively) solutions. The peak differences between these two data sets were calculated and plotted as a function of functional monomer concentration in order to obtain the saturation isotherms. Following the approach of Andersson and Nicholls [24], apparent complex dissociation constants, $K_{diss,a}$, were calculated from these plots using the Origin 4.0 software.

2.3. Synthesis of thin-layer MIP composite membranes

Circular PVDF membrane samples (area=4.9 cm^2) were weighed, pre-soaked in 0.15 *M* solution of benzophenone in acetone for 5 min and dried under vacuum. Membranes thus pre-coated with photoinitiator were transferred to Petri dishes filled with a methanol solution, containing 10 m*M* (or varied concentrations) of terbumeton, 50 m*M* (or varied concentrations) of functional monomer (AA, MAA, AMPS) and 300 m*M* (or varied concentrations) of MBAA. In order to limit desorption of the photoinitiator from the previously coated membranes, 5 m*M* of benzophenone was added to the monomer mixture. After 10 min, the UV irradiation on a pilot-scale UV curing system (2000 W mercury lamp; Beltron, Germany) for 10 cycles (1 cycle=1 min) followed. The membranes were then extracted with methanol in a Soxhlet apparatus to remove non-grafted polymer, residual initiator and the template. After drying, the membranes were weighed again and the degree of graft modification (DG) was calculated from mass differences. The variations of DG values for preparations repeated in triplicate were ≤10%.

2.4. Thin-layer MIP composite membrane characterization

Transmission Fourier transform (FT) IR spectra of the membranes were taken using a spectrometer FTIR-8201 PC (Shimadzu).

The membrane recognition properties were evaluated by measuring their capacity to adsorb herbicides from aqueous solution during a fast filtration (membrane-SPE). Sorption was measured using a syringe connected to a filter holder (diameter=25 mm; Schleicher & Schuell, Germany) containing the membrane. 10 ml of 10^{-5} *M* herbicide solution in water were filtered through the membranes at a rate of 10 ml/min. The filtrate was extracted with 10 ml of chloroform. The herbicide concentrations in feed and permeate both after the chloroform extraction, were determined using a gas chromatograph (column HP5MS, Hewlett-Packard GC system HP 6890 with mass-selective detector HP 5973). The variations for repeated analyses of standard and feed samples with the complete procedure were $\leq$5%.

3. Results and discussion

The imprinting effect is generally believed to result from the complexation between template and functional monomers [1,25]. Fixation of the structure of these complexes in a rigid polymer network formed during the polymerization process produces recognition sites containing polymeric functionalities positioned to complement those of the template molecule. Here, the development of a surface functionalization of a porous membrane with a herbicide-imprinted MIP layer using photo-grafting polymerization from organic imprinting mixtures was the main objective. Another important issue was the applicability of the MIP membranes for substance-specific SPE. This requires a membrane surface with low non-specific binding. For that purpose, commercial microfiltration membranes which are mainly made from rather hydrophobic polymers [26] are often surface-modified with a thin hydrophilic polyacrylate layer [22,27]. Consequently, the non-specific binding of hydrophobic solutes (including the template terbumeton) is very low. On the other hand, such a polyacrylate layer could also increase the photo-graft polymerization efficiency [28]. Therefore, an established commercial ''low-binding'' membrane, hydrophilized PVDF, was chosen as support for the attempted synthesis of thin-layer MIP composite membranes.

3.1. Selection of the functional monomer. UV spectroscopy

When template and functional monomers form complexes in solution, the strength of these complexes is reflected in the affinity and selectivity of the imprinted polymer. Non-complexing functional monomers will yield polymers with functional groups distributed statistically throughout the polymer matrix creating non- or weakly selective binding sites contributing to non-specific binding. In contrast, functional monomers, giving high degrees of template complexation in a monomer mixture, are supposed to generate polymers which demonstrate high selectivity together with low levels of non-specific binding. Consequently, the choice of functional monomers is of significant importance for the quality of recognition sites in MIPs. For the basic, polar *s*-triazines, functional monomers with acid groups, such as MAA, had been used successfully [11,29]. To justify a comparison between monomers (here: AA, MAA, and AMPS) and their suitability for creation of recognition sites via molecular imprinting, their ability to non-covalently interact with terbumeton in a monomer mixture has been studied by UV difference spectroscopy (cf. Refs. [24,25]).

The titration of 10^{-5} *M* solution of terbumeton in methanol by increasing amounts of AA, MAA or AMPS resulted in significant changes in the UV spectra, which were not only the super-imposition of the absorbances for the single components (see Fig. 1). For every monomer, the observed shift reaches a maximum corresponding to a saturation of interaction between template and functional monomer. The UV spectrum for terbumeton saturated with AMPS (cf. Fig. 1c), corrected by subtraction of the AMPS absorbance, was almost identical with the spectrum of the protonated terbumeton, measured separately. UV data for a titration of terbumeton in water with, e.g., hydrochloric acid had shown a transition in the range of pH 4.1$\pm$0.5 between the unprotonated – λ_{max}=220 nm – and the protonated form of terbumeton – $\lambda_{max,1}$=216 nm, $\lambda_{max,2}$=245 nm. The latter result is in agreement with the pK_a value for terbumeton of about 4.2 [30]. Thus, it is assumed that AMPS as a strong acid (pK_a <1) can protonate terbumeton and that an ion-pair complex is formed between template and AMPS in methanol.

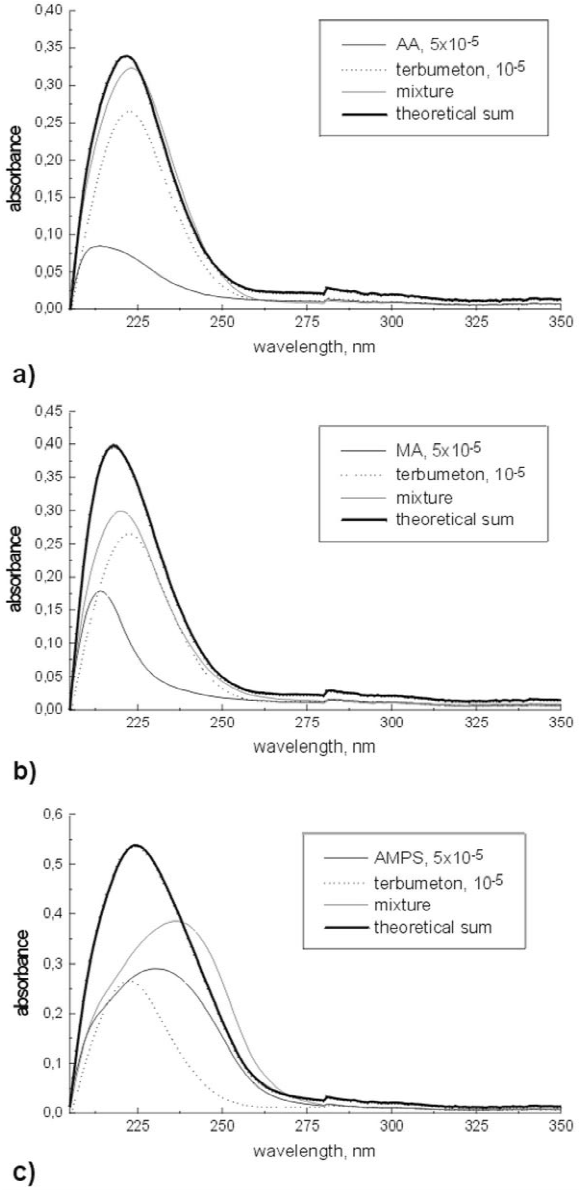


Fig. 1. UV spectra for terbumeton (10^{-5} *M*) and functional monomers (AA, MAA and AMPS; $5 \cdot 10^{-5}$ *M*) in methanol related to non-covalent complex formation in the reaction mixture used for MIP synthesis.

For the weaker acidic monomers MAA ($pK_a = 4.65$) and AA ($pK_a = 4.2$) this mechanism is less effective. Consequently, the calculated $K_{diss,a}$ values also indicated that the complex AMPS–terbumeton is stronger as compared with AA and MAA (see Table 1). Using the same spectroscopic method, Andersson and Nicholls [24] obtained $K_{diss,a}$ values in the range of 0.05–0.1 *M* for MAA and a peptide as template. A tentative explanation for the much lower K_{diss} values (higher complex stability) in the present study is the formation of an ion-pair as outlined above. It should, however, be kept in mind, that the absolute values also involve a significant experimental error. The difference between AA and MAA which does not correlate with the pK_a differences may indicate the additional contribution of hydrophobic interactions [25].

Table 1
Template complexation with different functional monomers (terbumeton in methanol; cf. Fig. 1 and Experimental)

Monomer	$K_{diss,a}$ (*M*)
AA	$2.0 \cdot 10^{-4}$ ($\pm 0.3 \cdot 10^{-4}$)
MAA	$8.0 \cdot 10^{-5}$ ($\pm 1.0 \cdot 10^{-5}$)
AMPS	$3.0 \cdot 10^{-5}$ ($\pm 0.3 \cdot 10^{-5}$)

3.2. Influence of functionalization parameters on photo-graft copolymerization

Modification of the PVDF membranes with imprinted polymers was performed using AA, MAA, AMPS as functional monomers, terbumeton as a template and MBAA as cross-linker (see Table 2). No attempts were made to exclude oxygen because: (i) significant and reproducible DG values were achieved, and (ii) the photoinitiator coating of the surface along with the inhibiting action of oxygen in the solution could even increase the probability of a thin layer modification of the membrane surface. In fact, no polymerization was observed after UV irradiation of the complete reaction mixtures but without a membrane. Hence, the main contribution to the functionalization of the membrane is due to the photoinitiation on its surface.

There was a pronounced difference between the hydrophobic and hydrophilized PVDF membrane: for the hydrophobic, not pre-modified PVDF graft modification could not be achieved under the selected conditions. For hydrophilized PVDF, in the studied range, DG was almost not affected by varied parameters, except for the highest AMPS concentration (cf. Table 2c). Further increasing either

Table 2
Degrees of modification (DG, in $\mu g/cm^2$) for hydrophilized PVDF membranes obtained by a photo-graft copolymerization with (MIP) or without (Blank) the template terbumeton: (a) variation of the functional monomer at 50 m*M* and with 300 m*M* MBAA; cf. Fig. 3, (b) variation of cross-linker concentration with 50 m*M* AMPS; cf. Fig. 4, (c) variation of functional monomer concentration with 300 m*M* MBAA; cf. Fig. 5; in MIP experiments 10 m*M* terbumeton, in all experiments 5 m*M* BP.

(a)	AA	MAA	AMPS				
MIP 1a	380	360	340				
Blank 1a	320	310	340				
(b)	MBAA concentration (m*M*)						
	200	225	250	275	300	325	350
MIP 1b	350	300	310	370	340	340	360
Blank 1b	390	350	360	360	340	330	320
(c)	AMPS concentration (m*M*)						
	0	20	40	50	60		
MIP 1c	350	370	370	340	410		
Blank 1c	370	390	370	340	420		

monomer concentration(s) or/and UV irradiation time yielded increasing DG values, but the tendency for gelation of the entire reaction mixture was much higher, and it was hard to control the modification. On the other hand, without MBAA, i.e., with the functional monomers alone at a concentration of 50 m*M*, only very low DG values in the range of 20 $\mu g/cm^2$ were obtained. Remarkably, the differences for DG between MIP and Blank preparations under the same conditions were not significant (cf. Table 2).

The IR spectra (see Fig. 2) allow the following conclusions:

(i) The hydrophilized PVDF membrane indeed contains a polyacrylate layer (indicated by the strong carbonyl band at around 1720 cm^{-1} which is not present in the case of the hydrophobic PVDF).

(ii) Photo-grafting yields an additional functionalization with a poly acrylate/acrylamide (indicated by the carbonyl band at around 1720 cm^{-1} along with a amide I band (carbonyl) at 1665 cm^{-1} and a amide II band (N–H) at 1535 cm^{-1}).

(iii) For this added poly acrylate/acrylamide, MIP and Blank cannot be distinguished by IR spectra.

The first result, (i), is in agreement with the

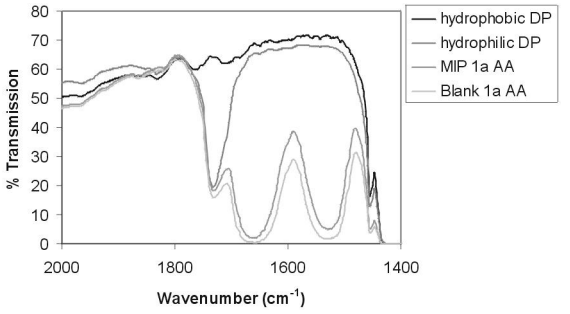


Fig. 2. IR spectra for hydrophobic and hydrophilized PVDF membranes compared with the imprinted (MIP 1a AA) and reference (Blank 1a AA) membranes (cf. Table 2).

assumption that the pre-modification of the PVDF membranes is performed via synthesis of a thin cross-linked hydrophilic polyacrylate (''*layer I*'') covering the entire surface of the porous membrane [27]. The latter two observations are further supported by results of previous XPS studies where MIP and Blank surfaces prepared by photo-grafting surface modification had the same composition of a functional acrylate copolymer [23]. In addition, by staining with the cationic dye toluidine blue the presence of cation-exchange groups (from AMPS) could be qualitatively verified for MIP and Blank, but not for the unmodified membranes.

Hence, as expected, the pre-modification of PVDF with the polyacrylate *layer I* improved the photo-grafting efficiency very much. This could be due to either:

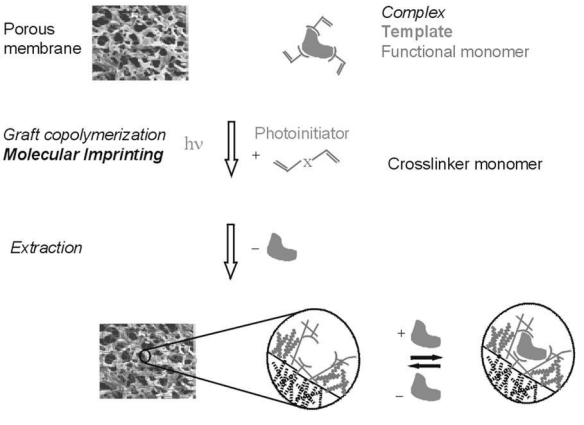
·Grafting on a graft (*layer I*) having a higher reactivity than the base polymer (PVDF) or/and;

·Formation of an ''inter-penetrating network'' (IPN) where the new polymer is entrapped and entangled in the one already present.

Because the hydrophilic *layer I* will be swollen by the reaction mixture in methanol, the second mechanism will work in this case. The almost constant DG values over a wide range of polymerization parameters (cf. Table 2) support that *layer I* is ''filled'' with the new polymer. Further functionalization seems to exceed the ''capacity'' of *layer I*. A contribution of the graft-on-graft mechanism, i.e., an additional attachment between the two polymers by chemical links formed via UV excitation of benzophenone and hydrogen abstraction from the *layer I* polymer (cf. Refs. [21,22,28]) followed by a graft copolymeriza-

tion of the MIP or Blank monomer mixture or/and by radical recombination, is highly probable. This will enforce the desired stability of the novel composite membranes.

With scanning electron microscopy, no changes of the membrane pore structure (cf. Scheme 1) due to functionalization could be detected. Hence, for all preparations shown in Table 2, the coverage of the entire membrane surface with a very thin, probably mainly IPN layer can be assumed without membrane pore blocking by excess polymer. Note that, for a polypropylene microfiltration membrane with similar pore structure (d_p=0.2 μm) but without a *layer I* and at similar DG values, a slight increase of the specific surface area measured by nitrogen adsorption/desorption was detected indicating that in addition to the membrane porosity the MIP and Blank layers possess a specific microporosity; furthermore, the estimated added MIP or Blank layer thickness was only 10 nm [23]. When compared to the work of Dzgoev and Haupt [20], who ''filled'' the pores of a polypropylene microfiltration membrane with about 15 mg/cm^2 and who consequently obtained a MIP membrane with negligible permeability, the novel surface-functionalized PVDF materials can certainly be considered as thin-layer composite membranes. Consequently, the high permeability of the PVDF microfiltration membrane (about 20 ml/min cm^2 at 1 bar) was not affected by the modification, allowing a very fast filtration. Therefore, various sets of MIP and Blank membranes were tested in filtration experiments, a fast membrane-SPE through one 125-μm thick membrane (see Figs. 3–5).



Scheme 1. General scheme of the photo-graft cross-linking copolymerization surface modification of a microfiltration membrane with a thin selective MIP layer.

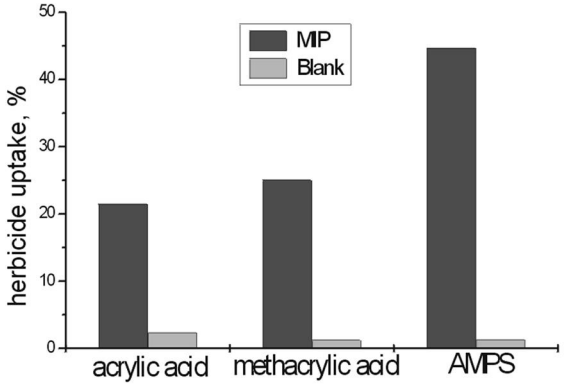


Fig. 3. Influence of the functional monomer on the imprinted (MIP) and reference (Blank) membrane's sorption capability. A reaction mixture, containing 10 m*M* of terbumeton, 50 m*M* of functional monomer, 300 m*M* of MBAA and 5 m*M* of BP in methanol was used for membrane modification. 10^{-5} *M* solutions of terbumeton in water were used in (SPE) filtration experiments.

3.3. Influence of MIP synthesis and functional monomer on membrane sorption capability

The membranes imprinted with terbumeton using the different functional monomers were able to bind 20 to 48% of this herbicide from aqueous solution

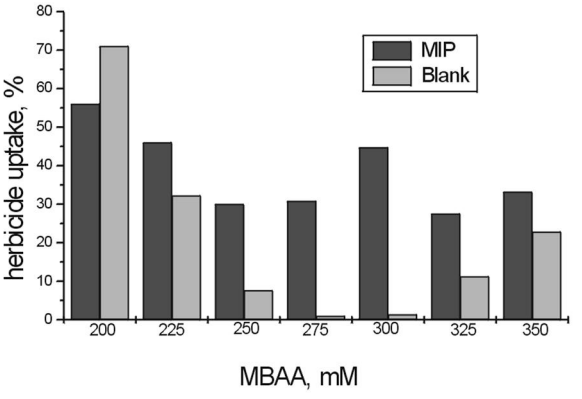


Fig. 4. Sorption capability of MIP and Blank membranes depending on the concentration of cross-linker. The reaction mixture, containing 10 m*M* of terbumeton, 50 m*M* of AMPS, 200 to 350 m*M* of MBAA and 5 m*M* of BP in methanol was used for membrane modification. 10^{-5} *M* solutions of terbumeton in water were used in (SPE) filtration experiments.

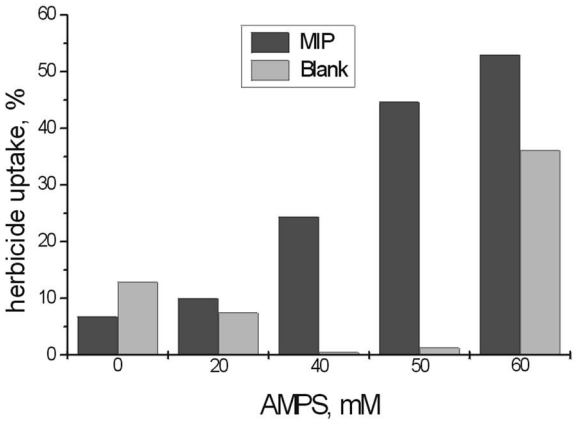


Fig. 5. Sorption capability of MIP and Blank membranes depending on the concentration of functional monomer. The reaction mixture, containing 10 m*M* of terbumeton, 0 to 60 m*M* of AMPS, 300 m*M* of MBAA and 5 m*M* of BP in methanol was used for membrane modification. 10^{-5} *M* solutions of terbumeton in water were used in (SPE) filtration experiments.

(see Fig. 3). This corresponds to up to 30 nmol/cm^2 for one 125-μm thick membrane. The sorbed terbumeton could be released from the MIP membrane by elution with 0.1 *M* hydrochloric acid in water; in preliminary experiments with 1 ml eluent about 90% recovery could be achieved. This confirms the contribution of the AMPS cation-exchange groups to the template binding. At the same time, Blank membranes of the same composition demonstrated only negligible binding of terbumeton (1 to 3%). This is a most remarkable result, especially when compared with the previous preparations of MIP membranes via surface graft copolymerization onto polypropylene where pronounced MIP selectivities were accompanied with relatively high ''background'' sorption to the Blank membranes (50 to 60% in an identical membrane-SPE protocol) [23].

Furthermore, as it was expected from the results of the titration experiments (cf. Section 3.1), AA and MAA were found to be less effective as functional monomers than AMPS. Thus, a correlation between the structure and the properties of the monomer, the complex formation with the template (cf. Table 1) and the resulting MIP recognition properties (cf. Fig. 3) could be established experimentally. Consequently, further investigations regarding the impact of other MIP synthesis parameters were done with AMPS.

3.4. Influence of the cross-linker concentration on membrane sorption capability

As widely recognized, for effective performance imprinted polymers should be highly cross-linked thus enabling the selective cavities to retain their shape after removal of the template. However, a compromise should be found between the degree of cross-linking needed for polymer stability and a certain degree of polymer chain's flexibility which provides rapid equilibration with the template to be bound. Hence, the sorption capability of the MIP composite membranes depending on the amount of cross-linker in the monomer mixture was investigated (see Fig. 4). In the studied range, the DG values were almost independent of MBAA concentration and thus independent of the degree of chemical cross-linking (cf. Table 2b). The membrane sorption properties, however, were clearly affected by a change in MBAA content. While the sorption data of MIP showed a slight decline with rising MBAA concentration, for the Blank membranes a pronounced minimum was observed at MBAA concentrations of 275 and 300 m*M*.

These data indicate, that the MIPs with too low and too high degrees of cross-linking have lower binding capability in comparison with better performing MIP (300 m*M* MBAA concentration), although the degree of their modification (quantity of the grafted polymer) is similar. For too low cross-linking (here at 200 m*M*), the ion-exchange groups of the functional monomer in the grafted copolymer are too mobile causing high level of non-specific binding by both ''MIP'' and Blank. Increasing cross-linking density reduces flexibility and thus improves the contribution of specific binding to imprinted receptor sites. Too high MBAA concentration generates a higher percentage of non-specific binding sites, and repulsing forces between the negatively charged polymer groups might again increase the mobility of functional AMPS units or domains. Furthermore, the increasing fraction of excessively cross-linked domains will also reduce the number of MIP receptor sites. This can explain the slight decay of specific binding of the template to MIP membranes with increasing MBAA content.

Consequently, at MBAA concentrations of 275 and 300 m*M* an optimum of MIP affinity was

obtained, with a high binding capability of the MIP and negligible binding of the Blank membranes (cf. Fig. 4).

3.5. Structure and function of MIP binding sites in thin-layer MIP composite membranes

The concentration of AMPS was varied in order to optimize the template/functional monomer ratio in the monomer mixture. Although the DG was not changed upon addition of AMPS (cf. Table 2c), the terbumeton binding was very different for the membranes synthesized in the absence and presence of this monomer (see Fig. 5). An increase of the AMPS concentration up to 50 m*M* resulted in a significant increase of the MIP membrane binding capability, while the non-specific sorption of terbumeton on Blank membranes was constantly low. However, membranes modified in the presence of 60 m*M* of AMPS in the monomer mixture demonstrated significant values of non-specific binding (cf. Fig. 5). Hence, the optimum AMPS concentration was found to be at 50 m*M*.

It is important to note that the sorption/desorption processes were very fast in the case of MIP membrane and could be performed within 1 to 2 min. The reason for that lies in the thin porous structure of the polymer layer, favorable for the diffusion.

In addition, a series of triazine analogues, herbicides of related chemical structure (atrazine, desmetrine, metribuzine), was used to examine the selectivity of the terbumeton-imprinted membranes, again in membrane-SPE filtration experiments. Remarkably, the novel MIP composite membranes as well as the Blank materials had very low non-specific binding of the terbumeton analogues. In contrast to that, very efficient sorption of the template terbumeton was observed, exclusively in the case of MIP (see Fig. 6).

These findings can be interpreted as follows (cf. Section 3.4). An increasing AMPS content at constant DG (cf. Table 2c) creates an increasing number of specific template binding sites in the MIP membranes. In contrast, for the Blank membranes, the AMPS functional groups of the graft copolymer may be entrapped in the IPN within *layer I*, introduced by the manufacturer during the pre-modification. By these means, even if the hydrophilized PVDF membranes are further surface-modified in the absence of template producing Blank membranes, they still possess low binding ability. When the DG exceeds the value (about 390 $\mu g/cm^2$), necessary for ''filling'' *layer I* with the photo-grafted copolymer (cf. Section 3.2), AMPS groups ''outside'' *layer I* having higher mobility will increase non-specific binding via ion-exchange. For the MIP membranes, similar to previous studies with other *s*-triazines and acidic monomers [11,23], the optimum terbumeton/AMPS ratio in the monomer mixture was found to be 1:5 (at 50 m*M* AMPS; cf. Fig. 5). Hence, the structure of the complex to be fixed in the MIP will be more complex than an ion-pair (cf. Section 3.1), a larger number of AMPS molecules may be involved. Following this line of arguments, it is speculated that this large, pre-organized complex will not fully penetrate into the hydrophilic and cross-linked *layer I* before the UV polymerization is started. This size-exclusion effect will explain that specific MIP sites, well accessible during a fast filtration, are formed ''ontop'' of an otherwise low-binding *layer I* (see Scheme 2). Therefore, the novel MIP composite membranes have not only higher affinity for the

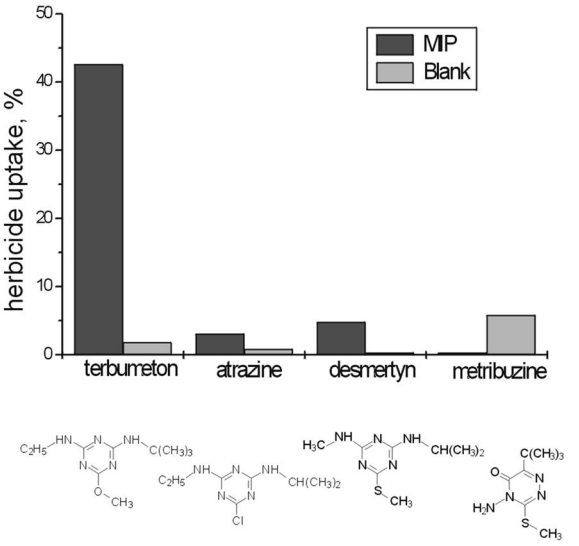
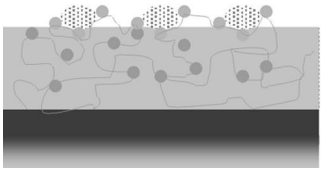


Fig. 6. Selectivity of the terbumeton-imprinted thin-layer MIP composite membrane to other herbicides of related chemical structure. The membranes were synthesized with AMPS under the conditions described in Fig. 3. 10^{-5} *M* solutions of herbicide in water were used in (SPE) filtration experiments.



Scheme 2. Model for the surface structure of thin-layer MIP composite membranes having high template affinity at low non-specific binding: The matrix for the surface modification is a membrane with a hydrophilic, low binding *layer I* which had been introduced in a pre-modification step of the base membrane material. Functional groups of the functional monomer (grey dots) in the reaction mixture, which before polymerization had been involved in the pre-organized complex (dark grey dots) with the template (large spotty dots), which could not fully penetrate into *layer I*, were during the cross-linking polymerization in *layer I* (IPN formation) fixed in ''molecular imprints'' predominately on top of the hydrophilic, low binding *layer I*.

template as compared with the respective Blank membranes, but they also recognize this template with a very high selectivity compared to other very similar substances.

4. Conclusions

A new type of composite membranes with artificially introduced affinity to terbumeton was prepared by photo-grafting of 2-acrylamido-2-methyl-1-propane sulfonic acid and *N*,*N*′-methylene-bis-acrylamide in the presence of terbumeton as a template on benzophenone-coated hydrophilized polyvinylidene fluoride membranes with a nominal pore size of 0.22 μm. Remarkably, the novel terbumeton-imprinted membranes demonstrated much higher sorption capability to this herbicide than to structurally very similar compounds which were essentially not sorbed.

The method of UV spectroscopic analysis of the molecular imprinting ''pre-polymerization stage'' provided a valuable tool for the screening of different imprinting systems. By systematic variation of crucial synthesis parameters such as the type and the concentration of functional monomer as well as the concentration of the cross-linker, the composition could be identified, able to generate a MIP membrane with high affinity and low non-specific binding as compared with control blank membrane. The novel approach, MIP synthesis as an IPN formation with a hydrophilic polymer already pre-coated on the surface of the base membrane proved to be very efficient. It can have significant advantages compared with attempts to coat MIP sorbents with an additional layer after synthesis in order to reduce non-specific interactions [31]. Furthermore, as had been shown before [23], using a polymeric membrane as a support for a MIP prepared by surface grafting is beneficial because it contributes to MIP stability and affinity. The small MIP layer thickness improves the accessibility of individual receptor sites. Furthermore, due to the porous membrane structure, high filtration rates can be applied, what dramatically reduces internal mass transport limitations within the sorbent (cf. Ref. [14]). This makes applications in separation, e.g., for membrane-SPE (cf. Ref. [13]), feasible. As for other membrane adsorbers (cf. Ref. [14]), the capacity can be scaled up easily by using stacks of the novel high-flux MIP composite membranes.

The high affinity of the novel MIP composite membranes to triazine herbicides together with their simple and inexpensive preparation, provides a good basis for applications of imprinted polymers in practical applications such as a pre-concentration step for the determination of photosynthesis-inhibiting herbicides in water.

5. Nomenclature

AMPS	2-Acrylamido-2-methyl-1-propane sulfonic acid
MAA	Methacrylic acid
AA	Acrylic acid
MBAA	*N*,*N*′-Methylene-bis-acrylamide
UV	Ultraviolet
MIP	Molecularly imprinted polymer
SPE	Solid-phase extraction
PVDF	Polyvinylidene fluoride
m*M*/*M*	mmol/l/mol/l (concentration)
DG	Degree of graft modification
IR	Infrared
XPS	X-Ray photon spectroscopy
IPN	Inter-penetrating network

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