Molecularly Imprinted Anisotropic Polymer Monoliths

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ABSTRACT: Anisotropic materials with selective binding properties may have potential as active components in optical sensors. A novel route for the synthesis of such materials is described and the validity of the concept has been demonstrated. To this end free radical polymerization of 2-ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate (TRIM) in the presence of a template molecule [4,4′-bis(dimethylamino)benzophenone (Michler's ketone)] and binding sites [2-(acrylamido)-2-methylpropanesulfonic acid (AMPSA) or methacrylic acid (MAA)] led to molecularly imprinted polymer monoliths. Upon irradiation using linearly polarized light, the template molecules reacted with the polymer networks to form transparent and anisotropic polymer monoliths. The origin of the dichroism is discussed and is believed to result from the incorporation of reactive template molecular species (radicals) into the polymer network. That the polymers are indeed imprinted was confirmed using competitive binding studies. The polymers are size and shape selective but not always in favor of the template molecule.

Introduction

Chemical sensors have aroused the interest of many academic and industrial research groups over the last 20 years.[1−3] The design of optical sensors in particular has attracted much attention owing to the inherently high sensitivity of optical spectroscopy.[4] Despite this advantage, the challenge remains for this, and indeed all types of chemical sensor, as to how to achieve high selectivity along with sensitivity.

Selectivity without the use of a recognition element can be introduced by using time-dependent (ultraviolet−visible) spectroscopic methods (*e.g.* fluorescence and phosphorescence decay−time measurements,[5] synchronous spectrofluorimetry[6]). The use of a recognition element, however, is the more obvious choice, allowing for the introduction of tailor-made recognition characteristics. Biomolecules, such an antibodies, DNA, receptors, and enzymes, have been applied successfully as bioselective layers.[7,8] Unfortunately, though, sensors based on these elements are highly sensitive to changes in temperature, medium, and pH, and in addition, traces of impurities can easily contaminate their active sites.[9−11] Synthetic receptors that are capable of changing their optical characteristics in a predictable way in the presence of a substrate are likely to be less prone to suffer from these shortcomings and therefore could provide a promising alternative.[1−3,12]

Here we should like to present a novel concept in the design of optical sensors that has the potential to overcome some of the problems. Figure 1 illustrates the concept. A molecularly imprinted polymer[13−16]—a type of selective polymer first introduced by Wulff *et al.*[13,14]—serves as the recognition element. It is prepared by the copolymerization in solution of a cross-linker and binding sites, in the presence of template molecules. In a subsequent step the template molecules are removed from the polymer, leaving cavities behind which are complementary to the template molecules in size and shape. The polymer can be obtained as a transparent monolith.[17] A photoreactive derivative, with molecules similar in size and shape to the original template molecules, is allowed to bind in the vacant cavities. The embedded molecules are irradiated with linearly polarized light,[18,19] forming reactive species, *e.g.* carbenes, capable of insertion into the polymer backbone. In a second extraction step, all residual, nonactivated photoreactive molecules are removed and an *anisotropic*

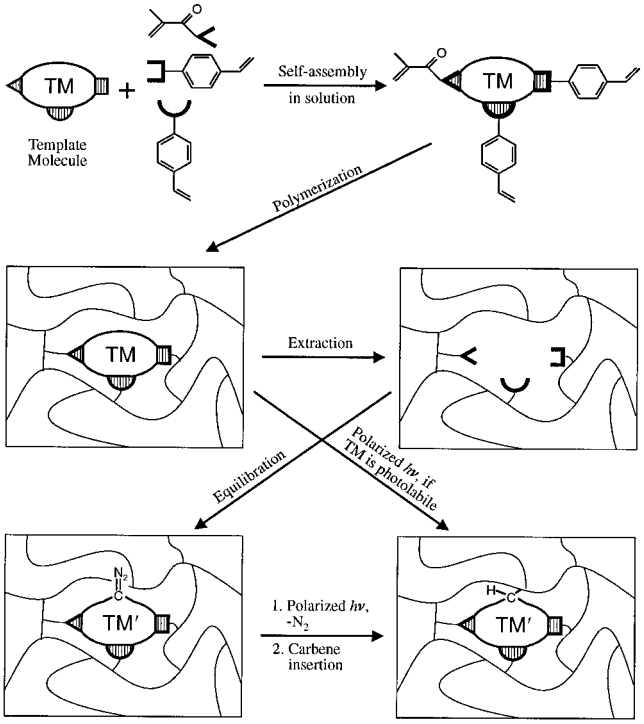

Figure 1. Steps in the formation of an anisotropic imprinted polymer. Template molecules (TM) and monomers containing appropriate binding sites self-assemble in solution and are copolymerized with a cross-linker. After extraction of the template molecules, complementary cavities remain in the polymer, which can then be equilibrated with modified template molecules (TM′), similar to the original template in binding characteristics but containing a photolabile diazo group. Irradiation of the modified template with plane polarized light preferentially photolyses TM′ molecules oriented in one direction, and the cavities containing these molecules are blocked by insertion of the resulting carbene into C−H bonds in the polymer backbone. Removal of unreacted TM′ molecules by extraction leaves a polymer containing cavities with an anisotropic distribution of orientations. Alternatively, if the original TM is photoactive, then this can be irradiated directly with plane-polarized light, without the need to remove it and replace it by TM′.

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polymer is created containing cavities which will bind preferentially to the template molecule, but which also have a net preferred orientation. If, for example, naphthalene had been chosen as the template molecule, it would potentially be possible to detect naphthalene in the presence of other similar aromatic hydrocarbons by measuring the UV absorption of the polymer parallel and perpendicular to a unique axis. Nontemplate molecules would be expected to show a random distribution in space, whereas naphthalene molecules will cause a change in the degree of anisotropy observed when bound inside the anisotropic molecularly imprinted polymer.

In practice, the chosen template molecule was 4,4′-bis(dimethylamino)benzophenone (Michler's ketone, MK) and its photoreactive analogue was to be the diazo derivative. Somewhat surprisingly, MK itself proved sufficiently photolabile to be exploited directly without the need to remove it from imprinted networks and replace it by the diazo species (Figure 1). This therefore simplified considerably the methodology that had to be followed.

Experimental Section

Chemicals. All solvents used in the polymerizations were purified and dried according to standard procedures. They were stored under argon and were kept over dried molecular sieves (4 Å). 2-Ethyl-2-(hydroxymethyl)-1,3-propanediol trimethacrylate (TRIM) of technical quality (Aldrich, 90%) was fractionally distilled prior to use. Azobis(2,4-dimethylvaleronitrile) (ADVN) was of analytical grade (Polysciences Inc.). 2-(Acrylamido)-2-methylpropanesulfonic acid (AMPSA), obtained from Aldrich (99%), was recrystallized twice from a mixture of ethanol and water (70:30 v/v) and dried over P_2O_5 under vacuum. MK, supplied by Aldrich (98%), was recrystallized twice from ethanol/toluene and at least twice from pure ethanol. It was dried over P_2O_5 under vacuum. Methyl methacrylate (MMA) (Aldrich, 99%) and methacrylic acid (MAA) (Aldrich, 99%) were purified by fractional distillation. Both compounds were stored at 4 °C in the dark. 4-Aminobenzophenone (4-ABP) (Koch Light Laboratories, "pure") was recrystallized from $CHCl_3$. Dimethylaniline (DMA) and 4,4′-dipyridyl (Aldrich, 98%) were used as received. 4,4′-Methylenedianiline (Aldrich, 98%) was recrystallized from $CHCl_3$/EtOH prior to use.

3,3′-Diaminobenzophenone (3,3′-DABP) was prepared according to a literature procedure[20] from 3,3′-dinitrobenzophenone (Lancaster Synthesis, 99%).

4,4′-Bis(dimethylamino)benzophenone (2,4-dinitrophenyl)-hydrazone was prepared according to standard procedures using Brady's reagent. An excess of (2,4-dinitrophenyl)-hydrazine in concentrated H_2SO_4 dissolved in EtOH (Brady's reagent) was added to MK (1.0 g, 3.73 mmol) and stirred. The reaction mixture was heated under reflux for 14 h and then allowed to cool to room temperature. The pH of the solution was adjusted to 7 by addition of concentrated aqueous KOH, and the precipitate formed was filtered off. The crude product was recrystallized from $CHCl_3$/EtOH (1/2 v/v). The hydrazone was obtained as a fine, deep purple powder. Yield: 88%. Mp: 268−9 °C. ^{1}H-NMR ($CDCl_3$): δ 11.49 (s, 1H), 9.09 (dd, 1H), 8.31 (dd, 1H), 8.18 (dd, 1H), 7.60 (dd, 2H), 7.24 (dd, 2H), 6.88 (d, 2H), 6.74 (d, 2H), 3.09 (s, 6H), 3.05 (s, 6H).

Bis(4-(dimethylamino)phenyl)diazomethane was prepared according to the procedure of Humphreys *et al.*[21]

Spectroscopy. ^{1}H-NMR spectra were recorded on Bruker WM-250 or AMX-400 spectrometers. TMS was used as internal standard. Ultraviolet−visible spectra were recorded on a Shimadzu UV−250 spectrometer. The path length of cells used was 1.000 cm.

A Glan-Taylor UV prism polarizer was obtained from Speirs Robertson with the following specifications: 20 mm diameter; quality "B", beam deviation 0°5′; extinction ratio at 2/3 or less of full aperture 1×10^{-4}; spectral transmission 214−2300 nm. Air was used as the separating medium instead of rubber or cement film, allowing an energy density of 200 W cm^{-2}. Experiments with the prism positioned in the sample beam of the UV−visible spectrometer, with its polarization direction vertical and horizontal, demonstrated that the beam of the instrument itself was significantly polarized. When the polarization direction of the prism was set at 45° and 135° to the vertical, however, this effect was minimized and both spectra obtained were essentially identical apart from a small deviation at about 410−430 nm. Polarized UV−visible spectra were thus usually measured at these two angles.

Preparation of Imprinted Polymers Used in Competitive Binding Studies. Polymer with MAA as Binding Site (EQ-1). A polymerization tube was filled with ADVN (104 mg, 0.42 mmol) and MK (402 mg, 1.50 mmol). MAA (589 mg, 6.86 mmol) and TRIM (6.62 mL, 19.20 mmol) were added under a blanket of nitrogen followed by chlorobenzene (1.0 mL) and $CHCl_3$ (7.2 mL). The solution was sonicated for 2 min at room temperature. Nitrogen was passed through the solution, which was kept at ∼0 °C for 10 min. The polymerization tube was sealed with a tap and cooled down to −78 °C in an EtOH/dry ice slush and was then subjected to three freeze−pump−thaw cycles. Finally the monomer solution was allowed to warm to room temperature and was sonicated for a further 2 min. The monomer mixture was allowed to polymerize for 14 h at room temperature and was subsequently cured in a water bath at 60 °C for 5.5 h. Polymer EQ-1 was extracted with $CHCl_3$ for 14 h in a Soxhlet apparatus, and 56% of the template molecule was recovered. Further extractions using EtOH (14 h), toluene (16 h), and EtOH/concentrated HCl (95/5 vol) (14 h) and finally stirring of the polymer particles in EtOH/$CHCl_3$/concentrated HCl (2/10/1 vol %) (15 h) removed another 3%, 8%, 6%, and 3% of the MK, respectively. The polymer was washed with EtOH and $CHCl_3$ and then dried under vacuum.

During the preparation it was observed that the initial orange color of the monomer solution at room temperature changed to yellow when the solution was cooled down to −78 °C. When the ampule was exposed to UV light (365 nm), both the monomer mixture, and later the polymer, fluoresced green-blue. No fluorescence was observed with light of a shorter wavelength (274 nm). Even after extraction of the polymer in a Soxhlet apparatus, both the orange color and the fluorescence remained. This can probably be attributed to permanently entrapped template molecules.

Polymer with AMPSA as Binding Site (EQ-2). AMPSA (598 mg, 289 mmol) was dissolved in *N*-methylpyrrolidone (NMP) (4.0 mL). TRIM (18.80 mL, 54.5 mmol) was added, followed by MK (319 mg, 1.19 mmol) dissolved in $CHCl_3$ (14.0 mL). Argon was passed through the solution for 5 min, after which ADVN (168 mg) dissolved in $CHCl_3$ (1.0 mL) was added. Degassing with argon was continued for another 5 min. The polymerization tube was then stoppered and subjected to a freeze−pump−thaw cycle (−78 °C). After room temperature was reached again, argon was passed through for another 10 min, followed by sonication for 2 min. Finally the tube was sealed with a stopper and Parafilm. Polymerization was carried out at room temperature for 20 h. The polymer was cured at 60 °C for 5.5 h and then extracted with $CHCl_3$ in a Soxhlet apparatus for 120 h, resulting in the recovery of 62% of the MK. Finally it was dried in air.

Competitive Binding Studies. Polymers Containing MAA as Binding Site. A solution was prepared containing equimolar amounts of MK (6.0 mmol) and DMA (6.0 mmol) or other competitor in $CHCl_3$ (50.0 mL). *Note*: This corresponds to an excess of substrate to binding site. The dried polymer EQ-1 was suspended in one-third of the solution and the suspension was stirred for 20 h. The polymer was filtered off and washed with $CHCl_3$ (a few milliliters). The polymer was freeze-dried (1 mbar) at liquid-nitrogen temperature, but was allowed to come to room temperature twice during drying. The dried polymer was extracted with EtOH in a Soxhlet apparatus for 16 h. The amounts of DMA and MK in the polymer and in the filtrate were determined by the combined use of weight measurements and ^{1}H-NMR spectroscopy.

In a second equilibration with polymer EQ-1, MK (4.0 mmol) and 4,4′-dipyridyl (4.0 mmol) were dissolved in $CHCl_3$ (50.0

mL). The polymer (2.5 g) was suspended in 20 mL of the prepared solution. After 24 h the polymer was filtered from the equilibration solution and treated in the same way as already described above. A blank experiment was conducted to account for any possible bleeding of trapped template by suspending the polymer (2.5 g) in $CHCl_3$ (20.0 mL). The solution was also stirred for 24 h, after which 10 mg of MK was found to be extracted.

Polymers Containing AMPSA as Binding Site. A series of equilibrations were carried out on polymer EQ-2. These were essentially the same as those described for EQ-1. Equal amounts of template and competing aromatic ketone were dissolved in an appropriate solvent, and the polymer was suspended in this solution. After the equilibration the polymer was filtered off and washed with a small amount of the same solvent or the solvent mixture that used for the equilibration. The polymer was then extracted in a Soxhlet apparatus and dried in air for 5 h. The filtrate and washings were combined, and the amounts and ratios of template molecule and competing aromatic ketone were determined using UV−visible or ^{1}H-NMR spectroscopy.

Determination of Concentrations. Two methods were employed.

(i) UV Spectroscopy. The extinction coefficients for all aromatic ketones were determined in the usual way before equilibration. A defined amount of ketone was weighed and dissolved in $CHCl_3$. This solution was diluted until the measured absorption was about 0.7 absorbance units. From the concentration and the appropriate absorbance value the extinction coefficient was calculated using the Beer−Lambert law. In cases where absorption bands of MK and the competing ketone overlapped, changes in concentration were calculated by solving the appropriate set of linear equations.

(ii) ^{1}H-NMR Spectroscopy. The ratio of MK to DMA was determined from the integration of the aromatic and aliphatic proton signals separately in ^{1}H-NMR spectra. For the filtrate, the ratio DMA:MK was 1:1.1 using aromatic protons and 1:1.1 using aliphatic protons. The ratio of MK to 4,4′-dipyridyl was determined in exactly the same way.

Calculation of Binding Selectivity Factors, α-Values. These were calculated according to the following formula:

$$\alpha = \frac{K_m}{K_C} = \frac{[MK]_P/[MK]_S}{[C]_P/[C]_S}$$

K_M is the equilibrium constant of the template molecule MK distributed between the polymeric phase ($[MK]_P$) and the solution phase ($[MK]_S$). K_C is the equilibrium constant of the competing molecule, and $[C]_P$ and $[C]_S$ are its concentration in the polymer and in solution, respectively.

Preparation of Imprinted Polymer Monoliths Used in Irradiation Experiments. All steps were carried out under argon. A solution of AMPSA (8.76×10^{-4} M) and MK (3.60×10^{-4} M) was prepared in $CHCl_3$/NMP (1249/1) in two steps. First AMPSA was dissolved in NMP and then $CHCl_3$ was added. A solution of MK dissolved in $CHCl_3$ was also prepared. The former solution was added to the latter. A stream of argon was passed through the solution for 10 min. As the final step, an equal volume of the diluted solution was added to TRIM (5.00 mL). The monomer solution was purged with argon at room temperature for 30 min. After the first 15 min, the mixture was sonicated for 2 min. ADVN (116 mg) was added and allowed to dissolve during the second 15 min. At the end of the degassing step, the solution was sonicated again for 1 min.

The monomer solution was kept in the dark, while two UV cells were purged with argon. Approximately half of the 10 mL solution was placed in each cell under a blanket of argon. Care was taken to treat both mixtures equally when a final stream of argon was passed through both solutions. The cells were stoppered and sealed before they were left in a dark place at room temperature overnight.

The monomer mixture in both cells polymerized, forming a transparent polymer in which all porogen was incorporated. Curing was carried out by placing both cells in their holders and immersing the whole stand in a water bath. (The stand also served as an adjustable setup for the photochemical part of the experiment.) The desired curing temperature was reached slowly owing to the size of the water bath (Table 1). Transparency was not visibly altered after curing. The small amount of unpolymerized monomer mixture that was left before curing had polymerized completely after the curing step. The polymers were stored in a dark place until they were used for the irradiation experiment. Three polymers were prepared in this way: LPP1, LP21, and LPP3. These differed only in the time allowed for polymerization and curing (Table 1).

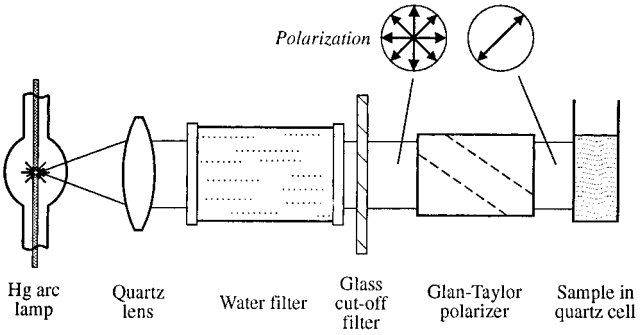

Figure 2. Optical arrangements for irradiating polymer samples with plane-polarized UV light.

Table 1. Preparation of Imprinted Monoliths for Irradiation Experiments

polymer	polymerization time (h)/T (°C)	curing time (h)/T (°C)
LPP1	17/20	1/50; 4/53; 5/50; 1/45
LPP2	24/20	5/50
LPP3	14/20	2/50; 2.5/55; 5.5/60; 2.5/50

Irradiation Using Linearly Polarized Light. The irradiation experiment is depicted schematically in Figure 2. An Oriel 200 W high-pressure Hg lamp was the light source. The light was approximately collimated by a quartz lens and then passed through two filters: first a water filter of 10 cm path length with quartz windows to eliminate IR radiation and then a glass long-wave pass filter (λ > 330 nm). Finally, a Glan−Taylor prism, set with its polarization plane at an angle of 45° with respect to the vertical axis of the sample, produced a linearly polarized light beam incident on the sample. Stray light was excluded from the sample by covering the area around the prism with aluminium foil. For this reason the reference cell was protected from light during irradiation as well. After all the components had been properly aligned, irradiation was carried out by removing an aluminium foil shutter from in front of the light source for given time intervals. For polymer LPP1 the time intervals were 2, 10, 106 and 1086 min; for polymer LPP2, 10, 30, 50 and 80 min; and for polymer LPP3, 5, 10, 15, 30, 150 and 1110 min.

Measurement of Dichroism. The setup used to measure the dichroism of the polymers is shown schematically in Figure 3. The irradiated polymer sample was placed in the sample beam of the UV−visible spectrometer, and the Glan−Taylor prism was positioned behind it as the last optical component before the detector. An identical sample of nonirradiated polymer was placed in the reference beam. Usually the UV−visible spectra were recorded with the prism set at 45° and 135° to the vertical and the absorption of MK at $\lambda_{max} \sim 351$ nm noted (A_{11} and A_1, respectively, at 45° and 135°) for various irradiation times. In order to ensure that the observed dichroism was genuine and not a result of an unfortunate arrangement of optical components, measurements were also carried out at prism settings of 225° and 315°. In addition, any dichroism of the sample was also measured by turning it around its vertical axis in 90° increments. In all cases it was found that any dichroism found was consistent regardless of the experimental setup used in each particular measurement. In some of the measurements an interchange of the components within the beams was made necessary by the specifications of the UV−visible spectrometer.

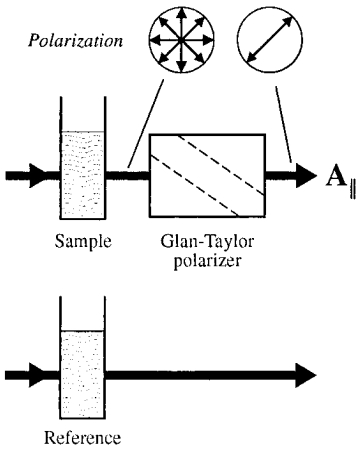

Figure 3. Optical arrangements for measuring dichroism in the UV−visible spectra of polymer samples irradiated with plane-polarized light. With the Glan−Taylor prism parallel to the plane of polarization of the photolysis beam, as shown, the measured absorbance is $A_\parallel$; rotating the prism 90° will give $A_\perp$.

Table 2. Dichroism Data for Polymers LPP2 and LPP3 following Extraction after Irradiation

	ΔA		
		after extraction (mL, h)	
polymer	before extraction	$CHCl_3$	$CHCl_3$/EtOH/concentrated HCl (250/5/0.1)
LLP2	+0.040	+0.010 (300, 5)	+0.000 (240, 4)
LLP3	+0.030	+0.015 (670, 14)	

UV−visible spectra were recorded for LPP1, LPP2, and LPP3 at the time intervals given in the previous section. In all cases at least three or four sets of spectra were recorded to eliminate any possibility of optical artifacts or maladjustment of the angle of the prism. Each set comprises two UV−visible spectra, one with the prism set at 45° and a second with the prism set at 135°. For each set the prism was separately adjusted to eliminate any methodological error. Additionally, after irradiation, polymers LPP2 and LPP3 were extracted with $CHCl_3$ and acidified $CHCl_3$, and the dichroism measurement was repeated. The results are shown in Table 2.

After extraction with $CHCl_3$, polymer LPP3 was kept at 55 °C for 17 h, when its dichroism was found to be +0.010. The margin of error was found to be 0.005 absorbance units, determined by placing the non-irradiated reference cell in the sample beam and recording the UV−visible spectra with the polarizer at 45° and 135°.

Results and Discussion

General Remarks. The polymer systems under investigation are complex in nature. In order to provide the basis for a viable chemical sensor using optical detection, they would have to fulfill the following requirements.

(i) To be useful as a recognition element, the polymer has to exhibit discrimination for the analyte in question [*i.e.* for one particular molecule (the template), or at least a set of structurally related molecules].

(ii) The recognition process has to be fast, so that changes in the concentration of the molecules of interest can be detected quickly. In addition, the polymer structure also has to be easily accessible to allow for swift diffusion of the analyte toward the recognition sites.

(iii) The polymer has to be transparent for two reasons: firstly, to guarantee that photochemical reactions leading to an anisotropic network are unobstructed and of a defined nature (minimal scattering, *etc.*), and, secondly, to ensure maximum sensitivity and reproducibility when used as a sensing element.

(iv) The final polymer matrix should be reasonably inert to changes in temperature, pH, and analyte composition.

Choice of Polymer Network. Methacrylate esters were chosen for the network because they are well-studied polymer matrices and have been widely used in molecular imprinting.[13−16,22] Chirally imprinted polymers derived from ethylene glycol dimethacrylate (EDMA) or TRIM exhibit long-term and temperature stability.[13−16,23] Furthermore, they have been the materials of choice to obtain imprinted polymers, showing favorable characteristics in terms of selectivity and mass transfer when used as chiral stationary phases.[13−16,22−28] In terms of reaction (binding and desorption) kinetics, TRIM is considered to be the slightly better choice.[24]

For a completely different reason, methacrylate networks were indispensable to this work. They can be obtained as macroporous transparent polymer monoliths via precipitation polymerization in a variety of porogens. This will be reported elsewhere.[17] As pointed out earlier, transparency is vital in the crucial, linearly polarized photochemical step.

Choice of Template Molecule. MK was selected as the template molecule for a number of reasons. Although subsequently not required, it can be converted into its (photoreactive) diazo derivative via its hydrazone.[20] The difference in size and shape between the template molecule and its photoreactive derivative is small, so that the modified template molecule is expected to bind similarly to the imprinted polymer. MK provides three sites that are capable of hydrogen-bond formation. It has been shown that at least two binding interactions are required to obtain molecularly imprinted polymers exhibiting sufficient preference toward their template molecule.[13−16,29] Lastly, little is known about using flat aromatic template molecules for molecular imprinting.[27,28] MK is almost flat and is of interest in gaining a better understanding of how to prepare molecularly imprinted polymers that exhibit selectivity toward aromatic hydrocarbons. This might prove useful for their online selective detection.

Choice of Binding Site. Although covalent interactions between the template molecule and the binding site lead to well-defined cavities, they have been found to be slower in chromatographic applications than weaker interactions such as hydrogen-bonding or ionic interactions.[13−16] MAA has been shown to be most favorable when the template molecule contains functional groups like amides, aliphatic alcohols, or aliphatic amines.[29,30] Preliminary results obtained in preparing imprinted polymers using MAA and MK showed, however, that MAA is too weak an acid to interact sufficiently with an aromatic amine ketone. This was also confirmed by ^{1}H-NMR studies, which showed that the presence of MAA in chloroform has no effect on the chemical shift of the protons of the template molecule. AMPSA, however, is able to interact strongly with MK. ^{1}H-NMR and UV−visible evidence suggests that MK in the presence of equimolar amounts of AMPSA forms hydrogen bonds to one, two, or all three of its basic centers and is at least partially protonated as well. If AMPSA is present in excess, further changes in chemical shifts occur and can be interpreted as the occurrence of diprotonation together with hydrogen-bonding interactions. This view is in accordance with literature data.[31−35] and in particular with studies carried out in

toluene/trifluoroacetic acid media.[32] The interaction of MK and AMPSA has to be looked at as a multistage equilibrium involving protonation and hydrogen bonding at the same time. Disagreement arises, however, regarding the preference for N-protonation versus O-protonation. It seems that the type and concentration of acid, among other reaction parameters, determine whether N- or O-protonation prevails.[31,32,36−39] Literature data and our results from ^{1}H-NMR and UV−visible spectroscopic studies suggest that MK forms hydrogen bonds with all its basic sites, and the balance between either mono- and diprotonation of the N atoms or O-protonation lies very much on the side of N-protonation, accompanied by a small percentage of O-protonation. The degree of monoprotonated ion versus diprotonated ion is unknown.

Competitive Binding Studies. Two types of polymers have been prepared: one in which MAA acts as a polymerizable binding site (EQ-1) and one in which AMPSA replaces MAA (EQ-2). Both polymers were clear and orange before they were taken out of the polymerization ampule. Once the solvent started to evaporate, cracks formed, which led to the disintegration of the monolith into small, random-sized particles. The molar ratio of binding site (functional monomer) to template molecule was 4.57:1 for EQ-1 and 2.43:1 for EQ-2. The total of template molecule recovered by extraction of EQ-1 was 76%. The total extracted from EQ-2 was 62%. In contrast to EQ-1, EQ-2 was partially dried in air after extraction, prior to its use in binding studies.

Polymer EQ-1 (MAA as Binding Site). Comparing the acid strength of MAA and the basicity of MK, it is expected that hydrogen bonding will provide the major if not sole contribution of binding interactions in this system. It has been established in imprinting with a mixture of ion pair interaction and hydrogen bonding that a ratio of 1:1 of binding sites to available basic centers, or an even higher ratio (1.5:1), produced polymers with maximum selectivity.[26,40−42] A slight excess is favorable to shift the equilibrium of the formation of hydrogen bonds to the side of the complex. This and other literature data was used to optimize the conditions for achieving templating.

Despite this the α-value for the equlibration between MK and dimethylaniline (DMA) is 0.49, while equilibration of MK and 4,4′-dipyridyl yielded an α-value of 0.56. Polymer EQ-1 is therefore more selective toward binding the competing molecules than it is to binding the template. Relative size and basicity may explain this. Not only are the competing molecules much smaller than MK, they are also the stronger bases. The acid groups will therefore bind preferentially to the molecule which is the stronger base. Nevertheless, if this factor was alone important, it would imply that DMA and 4,4′-dipyridyl should bind exclusively to the polymer each in competition with MK, but this is not the case. It strongly suggests therefore that the cavities do indeed have some influence on the selectivity, but probably only as a result of their shape and not because they possess a spatially defined arrangement of functional groups inside. Further evidence for this argument is the fact that although 4,4′-dipyridyl is the slightly stronger base, DMA is more strongly incorporated into the polymer. The difference in size therefore seems to be the determining factor in this particular case.

Polymer EQ-2 (AMPSA as Binding Site). A different ratio of binding site to template molecule and a different porogen mixture had to be adopted to accommodate AMPSA as binding site. AMPSA dissolves only in much more polar solvents than does MAA (solvents such as NMP). Fortunately NMP has also been shown to be a good porogen to obtain transparent polymer monoliths.[17] A combination of $CHCl_3$ and NMP was favored, in which the amount of NMP was kept as small as possible to minimize its effect as a "buffer", interfering in the equilibrium (hydrogen bonding) between AMPSA and MK.[43] The ratio of binding site to porogen was reduced to 2.4:1 because strong acids can catalyze the decomposition of diazo compounds[44] and this was to be minimized in the planned irradiation experiments. The ratio of 2.4:1 was a compromise. The factor 2 is based on the assumption that both nitrogen atoms of MK interact with AMPSA more strongly than the oxygen, and the 0.4 excess is the contribution to push the overall equilibrium toward the complexed species. The polymer was extracted only with $CHCl_3$, this time to avoid undesirable changes of the polymer network by using eluents of different polarities and strongly acidified eluents as found for EQ-1. Polymer particles were not dried prior to their use in competitive binding studies.

Binding studies were conducted as for the polymer EQ-1. The amount of template extracted was 62% of MK using $CHCl_3$ for 120 h. Template molecule (MK) and competitors [4-aminoacetophenone (4-AAP), 4-ABP, benzophenone (BP), 3,3′-DABP, and the (2,4-dinitrophenyl)hydrazone of MK] were present in equimolar amounts and thus in a 2.4-fold excess to the number of theoretically available binding sites. The solvent was $CHCl_3$ or $CHCl_3$/EtOH, depending on the solubility of the individual aromatic ketones. After 24 h the polymer was filtered off, and the filtrate was analyzed. The residual polymers were extracted for 72 h with $CHCl_3$, and their filtrates also analyzed. The α-values were calculated with the inclusion of an allowance for leaching of the polymer, estimated by running a blank under identical conditions. The results are shown in Table 3.

Table 3. α-Values from Equilibration of MK and Various Competitors (C) with Polymer EQ-2

equilibration mixture (MK/C)	α
MK/BP	14.7
MK/4-AAP	0.25
MK/4-ABP	0.36
MK/3,3′-DABP	0.14
MK/MK 2,4-DNP[a]	2.1

[a] (2,4-Dinitrophenyl)hydrazone of MK.

Polymer EQ-2 binds 4-AAP, 3,3′-DABP, and 4-ABP selectively in the presence of MK, despite MK being the template. However, MK is bound in preference to BP ($\alpha = 14.7$) and the 2,4-dinitrophenyl hydrazone of MK ($\alpha = 2.1$). The latter is most closely related to MK. The main difference is the bigger size of this molecule because of its (dinitrophenyl)hydrazine moiety. The shape, size, number, and basicity of interacting groups are almost identical to those of MK if the hydrazone substituent is ignored.

If the (2,4-dinitrophenyl)hydrazone of MK were able to embed itself in a similar manner to MK inside a cavity, the total binding interactions would not be expected to differ too much from those with MK itself, with one C═O−AMPSA interaction being replaced by one C═N-NHR−AMPSA interaction. The favorable binding of MK must therefore reflect the exclusion of its (2,4-dinitrophenyl)hydrazone derivative primarily because of its size and shape.

Table 4. Recovery of Template Molecules (MK) and Competitors (C) after Equilibration with EQ-2 and Subsequent Extraction with $CHCl_3$

equilibration mixture (MK/C)	substrate recovery (%) MK	C	extraction medium
MK/BP	92	98	$CHCl_3$
MK/4-AAP	90	51	$CHCl_3$/EtOH (2.8/1)
MK/4-ABP	89	86	$CHCl_3$
MK/3,3′-DABP	77	84	$CHCl_3$/EtOH (2/1)
MK/MK 2,4-DNP[a]	76	99	$CHCl_3$

[a] (2,4-Dinitrophenyl)hydrazone of MK.

Clearly, when the size and shape are of secondary importance, however, as they are when 3,3′-DABP is the competing molecule, other factors take over. The amino groups of 3,3′-DABP are far more basic than the dimethylamino substituents on MK, because of their positions *meta* to the electron-withdrawing carbonyl group (as opposed to *para* in MK). The overall sizes and shapes of both molecules are very similar and even the distances between the nitrogen atoms are essentially the same. Thus, the difference in basicity of the amino groups on MK and 3,3′-DABP is likely to be the major factor accounting for the highly selective incorporation of the 3,3′-DABP ($\alpha = 0.14$).

The influence of basicity and shape are also manifest in the selectivity of EQ-2 toward 4-AAP ($\alpha = 0.25$) and 4-ABP ($\alpha = 0.36$). Although both molecules are smaller in size than 3,3′-DABP, they compete against MK less effectively than 3,3′-DABP. This is probably a reflection again of the relative basicity of the interacting amino groups, which are in *para* positions to the carbonyl group in 4-AAP and 4-ABP (*i.e.* less basic) but *meta* in 3,3′-DABP. Shape, however, is still important as well: 4-AAP is bound with a higher selectivity to EQ-2 than 4-ABP, suggesting that the smaller 4-AAP fits better in the cavity, although 4-AAP is probably slightly more the basic as well.

The apparent lack of influence of the second amino group on MK in terms of selectivity in the equilibration with 4-ABP (and 4-AAP) is an unexpected result. It may be explained by the absence of a second binding interaction between MK and AMPSA. If indeed the MK template is only monoprotonated during polymerization, the resulting cavities will contain only one binding acid site. The presence of the second amino group on MK during equilibration with 4-ABP and 4-AAP would therefore offer no advantage and, indeed, might provide a minor steric disadvantage. That at least one binding site is present in the cavities is shown, however, by the large incorporation of MK when equilibrated in competition with BP, which is a neutral molecule ($\alpha = 14.7$).

The data in Table 4 reveal the extent to which the template molecule and its competitor can be recovered from the polymer EQ-2 after equilibration and then extraction with chloroform. Smaller molecules, like 4-AAP in particular, seem to become entrapped to a high level. The high percentage of the (2,4-dinitrophenyl)-hydrazone of MK recovered, however, agrees nicely with the argument that the hydrazone is either only weakly bound inside a cavity or simply excluded, either of which would certainly simplify recovery. Competitors similar in shape to MK and possessing two amino substituents seem to reduce the amount of MK which can be recovered. An explanation for this phenomenon is not at hand. Extractions with more polar solvents have not been carried out.

A number of interesting color changes took place during equilibration. The orange of the initial polymer EQ-2 changed toward a darker red-orange during equilibration with 4-AAP. The presence of BP left the color unchanged, but 3,3′-DABP caused a similar change to 4-AAP, but less pronounced. Equilibration with 4-ABP also gave rise to a very similar color change and the polymer turned almost red. The (2,4-dinitrophenyl)-hydrazone of MK is strongly colored, and it was therefore not possible to detect a color change during equilibration. After the polymer was filtered, and washed twice with $CHCl_3$, the polymer was still dark red, with the filtrate of the second wash only faintly colored. Given the good solubility of the hydrazone, the persistance of the dark red of the polymer indicates that the hydrazone is actually deep inside the polymer matrix and not simply adsorbed on the surface.

Irradiation Using Linearly Polarized Light. Exploratory studies into the thermal stability of the diazo derivative of MK revealed that it was not sufficiently stable to be kept for several hours (the time required for equilibration) in solution at room temperature in the absence or presence of acid. The search for a way out of this impasse, however, ultimately turned into a short cut. It was surmised that MK itself might be sufficiently photoreactive, so that the use of its diazo analogue could be avoided altogether. The MK template might therefore be irradiated *in situ* without the need to remove it from the polymer. Data on the photolability of MK are available.[45−47] MK irradiated at low concentrations (10^{-5} M) forms the corresponding pinacol, whereas at higher concentrations (10^{-2} M) an asymmetric dimer of MK is observed.[47] The photochemical behavior of MK in the presence of acid is not known, but 4-ABP, a molecule very similar to MK, is photoreduced to its pinacol upon irradiation in acidic media.[45−52]

Control irradiation experiments with MK and 4-ABP in a variety of solvents, at different wavelengths, and in the presence of small amounts of sulfonic acids, confirmed that MK and 4-ABP behave very similarly upon irradiation. While these experiments did not allow detailed conclusions to be drawn concerning the molecular process during irradiation, they did show that MK and 4-ABP have similar reactivity and most likely undergo photoreduction upon irradiation.

Two identical UV cells for sample and blank, tailormade to allow for continuous extraction and equilibration, were filled under identical conditions with the same monomer solutions, containing TRIM, AMPSA, MK, ADVN, and $CHCl_3$/NMP (1249/1 v/v). After polymerization and curing, transparent, almost colorless polymers were obtained. The irradiation of each sample with linearly polarized light employed an optical setup and a procedure designed to cancel any polarizaton properties in the system itself.[53] Figure 4 shows the differences in absorbance of the polymers LPP1-3 at ~351 nm at the prism settings of 45° and 135° ($\Delta A = A_{\perp} - A_{||}$) vs irradiation time. For polymer LPP1 the linear dichroism observed increased with increasing time. After 2 min a slight difference of 0.010 absorbance units was found which rose to a value of 0.030 after 1086 min, well outside the margin of error as indicated. Linear dichroism also increased with time in the case of polymer LPP2. For unknown reasons, the dichroism did not change between 10 and 30 min of irradiation. Only after 50 min did the value increase from 0.020 to 0.030, reaching 0.040 after 80 min. The experiment was not continued any further. The values are consistent

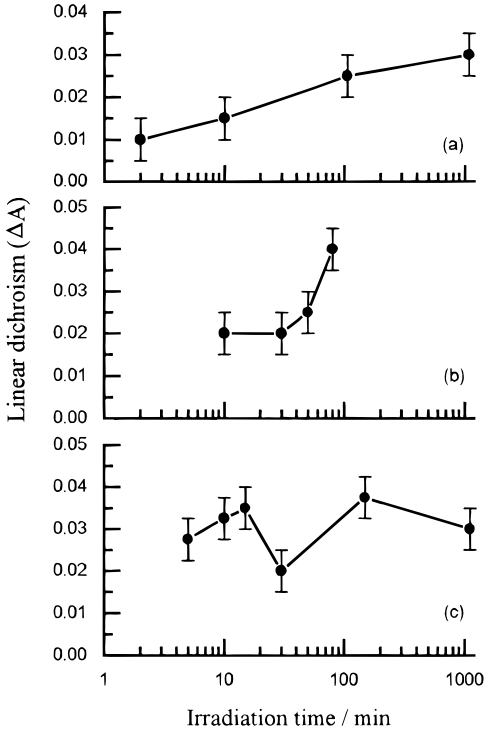

Figure 4. Linear dichroism ($\Delta A = A_{\perp} - A_{\parallel}$) at 351 nm induced by irradiation of polymers LPP1-3 with plane polarized light (λ > 330 nm).

with those for LPP1 if one considers the margin of error. The findings for LPP3 on the dependence of dichroism versus the irradiation time are similar to the results for LPP1 and LPP2. The observed dichroism increased with increasing irradiation time up to 150 min, but appeared to decrease slightly thereafter. The dichroism at short irradiation times (5, 10, and 15 min) was found to be almost twice that for LPP1 and LPP2. The values toward longer irradiation times were in very good agreement for all three polymers. Excellent agreement has been found in all other cases by using various combinations of experimental setups. The absorbance differences were measured at least three times per sample with the *smallest* value being taken as representative.

It was also shown that possible dark reactions after irradiation were not responsible for changes in the dichroism. Polymer LPP3 was kept in the dark for about 15 h after being irradiated for 30 min. No subsequent change in dichroism was observed during the dark period.

Extraction of both LPP2 and LPP3 with $CHCl_3$ led to a decrease in the measured dichroism. It dropped from 0.040 to 0.010 for LPP2 and from 0.030 to 0.015 for LPP3. Further extraction of LPP2 using a mixture of $CHCl_3$/EtOH/concentrated HCl (250/5/0.1 (v/v/v)) eliminated the dichroism in LPP2. LPP3 was not extracted for a second time but instead was heated for 17 h to 55 °C, whereupon the difference in absorbance was reduced again to 0.010−0.005 absorbance units. The flexibility of the polymer backbone must be quite high to allow these changes to occur. Movement of the template molecule, which is assumed to have reacted photochemically with the polymer backbone, is part of the structural rearrangements (movements of flexible polymer chains) occurring during extraction and thermal treatment. The randomness of the process destroys the imprinted anisotropy of the polymer, at least partially and, under more severe conditions, completely. Nevertheless, ^{13}C-MAS-CP solid state NMR spectra of samples of LPP2 before irradiation and after 80 min of irradiation revealed that the dry polymer is a rather rigid network. This, however, does not seem to apply to the solvent-swollen state. The solid state ^{13}C-NMR spectra show good agreement with published spectra of poly(TRIM),[54,55] and the proportion of residual double bonds was approximately l0%, determined from the ratio of the two types of carbonyl groups present in the polymer. The ^{1}H-NMR spectra of LPP2 before and after irradiation are almost indistinguishable. This suggests that the polymerization of residual double bonds via irradiation is negligible under the conditions of the irradiation experiments (filter cutoff at 330 nm).

Theory of the Origin of Dichroism. The following observations are in support of the assumption that the photogenerated decomposition of MK is responsible for the observed dichroism.

(i) The UV−visible absorption spectra of the sample cell taken after each irradiation step showed a gradual decrease of the absorption band of MK at 351 nm.

(ii) The maximum dichroism was always found to coincide with the maximum of the absorption band of MK. To both sides of this band the differences between the spectra recorded parallel and perpendicular to the polarization plane of the light used for irradiation gradually decreased.

(iii) Analysis of the extracts from the irradiated polymers LPP1-3 showed that the template molecules had decomposed. However, the quantity of material recovered was simply too small to allow proper identification and quantification of the fragments.

(iv) A fall in the dichroism after extraction of the irradiated polymer (LPP2 and LPP3) with $CHCl_3$ and a further reduction after using more severe elution conditions or heat treatment can be attributed to a random rearrangement of the swollen polymer network resulting from the flexibility of the polymer chains.

That the observed dichroism is not due to photopolymerization of residual double bonds using linearly polarized light is assured by the following arguments.

(i) The maximum absorption would be expected at a wavelength corresponding to the absorption maximum of the band for the residual double bond. This is not the case in practice.

(ii) ^{13}C-MAS-CP solid state NMR spectra of polymer LPP2 before and after irradiation for 80 min were essentially identical. The proportion of residual double bonds (about 10%) remained unchanged after irradiation.

(iii) A reduced dichroism was found when the polymer sample was turned around its vertical axis by 90°. This was as expected. Again the maximum degree of dichroism was found at ∼350 nm.

The Introduction of Dichroism Interpreted at the Molecular Level. The concentration of MK in the monomer mixture of LPP1-3 is approximately 10^{-4} M, and therefore decomposition of MK has to be expected to proceed via hydrogen abstraction (from the polymer network surrounding MK) from the lowest excited triplet state, $^{3}(n-\pi)$, as was confirmed by quenching experiments.[46] Eventually a covalent bond with the polymer backbone is formed (Figure 5) or, rather less likely, two ketyl radicals of MK form the pinacol. Molecules of MK with their transition dipole moments parallel or nearly parallel to the electric vector of the linear polarized light are most likely to absorb the

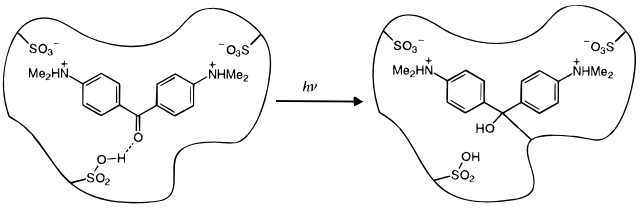

Figure 5. Photolysis of MK [4,4′-bis(dimethylamino)benzophenone] in an imprinted polymer gives the triplet biradical as a reactive species, which can insert into a C−H bond of the polymer backbone.

polarized light and eventually react. Those with their transition moments perpendicular to the polarization plane of the light will not react. In solution molecular motion will ensure that molecules originally misaligned become aligned and undergo photoreaction as well. Molecules inside the cavities of an imprinted polymer network, however, are believed to be restricted in their rotational and translational motion and therefore realignment can occur to a much lesser extent. It cannot be excluded that molecules of MK completely entrapped within the polymer network can also react with the polymer backbone after irradiation and thus contribute to the overall dichroism. If they were the sole species responsible for the observed dichroism, however, extraction should not lead to a decrease in anisotropy, as observed in practice. The changes in the polymer network introduced by a reaction of entrapped molecules of MK with their environment would be expected to influence the swollen network only slightly. Extraction with $CHCl_3$ of a polymer already swollen in $CHCl_3$/NMP (1250/1 v/v) should essentially have no effect on the polymer network which is in its equilibrium state already.

Conclusions

Assemblies of oriented molecules can be created through the use of a polymer matrix. Anisotropic alignment can be achieved, for example, by their incorporation into the polymer and subsequent orientation by stretching the polymer evenly in one direction.[56−59] A stretched polymer film can also be used as a preoriented environment in which reactions are carried out. Tran-Cong *et al.*, for example, studied an intramolecular photodimerization of 9-(hydroxymethyl)-10-[(naphthylmethoxy)methyl]anthracene in a uniaxially oriented PMMA film.[56−58] Other examples of assemblies of oriented molecules are liquid crystals, which without any "post-functionalization" are oriented owing to their inherent geometry.[60] In this paper we have described an entirely novel way of preparing selective, anisotropic polymer networks by the molecular imprinting of polymers and subsequent irradiation of the template molecules with linearly polarized light. The dichroism achieved is comparable in magnitude to that achieved in irradiated pre-orientated polymer films.[56−58] The selective binding properties of the polymers were investigated by competitive equilibration studies. It was shown that the polymers are size and shape selective but not necessarily in preference for their template molecules. This result was attributed to lack of sufficiently strong and numerous interactions between template molecules and binding sites during imprinting.

The subsequent irradiation of these polymers using linearly polarized light led to dichroic polymer monoliths. The anisotropic nature of the irradiated polymers is attributed to the photodecomposition of the template molecules. The measured dichroism increased at first with increasing irradiation time, but prolonged irradiation could lead to a decrease thereafter. The polymers were not only dichroic in the direction of irradiation but exhibited a smaller degree of anisotropy perpendicular to the direction of propagation of the linearly polarized light. The anisotropy of the polymers decreased significantly after extraction and vanished after prolonged heating at 55 °C. The degree of anisotropy introduced into the polymer network, although small, agrees well with literature data obtained from photoreactions of small molecules entrapped in aligned polymer matrices.[56−59] The development of chemical sensors based on these results now appears feasible in principle, but sensitivity would be low with the small degrees of anisotropy so far achieved.

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