Selective Adsorption of Dibenzothiophene Sulfone by an Imprinted and Stimuli-Responsive Chitosan Hydrogel

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ABSTRACT: A DBTS-imprinted chitosan hydrogel (IH_{DBTS}) was synthesized by cross-linking of chitosan with glutaraldehyde in the presence of dibenzothiophene sulfone (DBTS) as the template. An increase in cross-linker induced a major adsorption of DBTS until a plateau was reached at a ratio of 2.0 mol of glutaraldehyde per mole of glucosamine. The IH_{DBTS} was found to be stimuli-responsive with temperature and showed a LCST between the swollen and the collapsed phases at 50 °C. The major specific adsorption of DBTS by the hydrogel occurred at such transition and was due to the stronger ligand−gel interactions as demonstrated by the fluorometric studies where DBTS served as ligand and fluorescent probe. The fluorescence results showed that the DBTS presented a hydrophobic environment with predominance of intermolecular DBTS complexes (dimers) in the presence of the IH_{DBTS}. In contrast, the soluble chitosan promoted a higher hydrophobicity around DBTS and also favored the presence of monomeric DBTS as temperature increased. The steady-state fluorometry was found to be a useful technique to identify the molecular recognition conformation based on the ligands and not the polymers, to reveal the general interaction between ligands and MIP, and to give information concerning the nature of the environment around the ligands in the presence of MIPs.

Introduction

The molecular imprinting technique through the cross-linking of polymers contributes to set up recognition cavities inside the polymeric network[1] through the reduction of the frustration phenomenon; i.e., the number of local minimum energy conformations of the polymer around the ligand.[2] Therefore, the specificity of binding occurs at only one conformation (imprinting effect). Besides, the cross-linking is usually high enough to maintain the molecular recognition for long periods of time.[3] Nevertheless, there are at least two critical factors restraining ligand recognition and MIPs adsorption capacities. The first concerns the entrapment of the template inside the polymer matrix during the imprinting process causing both a reduction of the number of free binding cavities and ligand bleeding during the assays. Second, the high rigidity of the polymer matrix, excluding all further chain mobility, may rise to MIPs pore obstruction by adsorbed ligands which limits further ligand−cavity interaction.

The above factors may be overcome by reducing the polymer cross-linking density, allowing the existence of distinct polymer phases that favor or limit the molecular recognition and adsorption. Such behavior greatly motivated research on the designated *intelligent gels*.[4] Indeed, polymer matrices with a low cross-linking density, such as gels, can undertake conformational changes depending on external environmental conditions, i.e., swollen phase (solvent-rich) to collapsed phase (solvent-poor) as a function of temperature, pH, solvent polarity, etc. With this in mind, we undertook the study of organosulfur compounds adsorption on molecularly imprinted chitosan hydrogels.

Organosulfur compounds such as thiophenes, benzothiophenes, dibenzothiophenes (DBTs), and their alkylated derivatives represent major petroleum compounds contributing to catalyst poisoning and equipment corrosion during petroleum refining. Removal of these compounds with conventional hydrodesulfurization (HDS) requires new catalysts and extremely high pressures and temperatures as well as new reactor designs to barely reach the 15-ppm sulfur limit in diesel stated by the US Environmental Protection Agency (EPA) for 2006.[5] However, other alternative technologies have been proposed to achieve fuels desulfurization, i.e., biodesulfurization (BDS),[6] oxidation,[7] and adsorption.[8]

The oxidation of x-DBT, either by chemical or enzymatic means, produces the corresponding sulfones (x-DBTS). Such sulfones have a permanent dipole absent in others hydrocarbons currently present in fuels, which enhances their affinity for polar media through hydrogen bonding interactions. This property allowed the reduction of DBT from model solutions by coupling the oxidation of this compound, using an immobilized cytochrome *c*, to the selective adsorption of the produced sulfone on diatomaceous earth.[9] A similar approach used the selective separation of DBTS by a tailor-made adsorbent through a molecular imprinting process that selectively recognized the DBTS over other aromatic sulfur-related compounds found in diesel.[10]

Chitosan is an aminopolysaccharide which can form gels through the Schiff base reaction between its amine groups and the aldehyde ends of glutaraldehyde used as a cross-linker. Chitosan is obtained via a deacetylation procedure from chitin, the most abundant natural structural polymer after cellulose. Chitosan has been intensely investigated for the development of novel materials and sorption systems due to the presence of free amine groups into its structure, which allow its chemical modification.[11] The presence of this functional group together with hydroxyl groups throughout the chitosan polymer should allow self-assembling with DBTS through the formation of hydrogen bonds, leading

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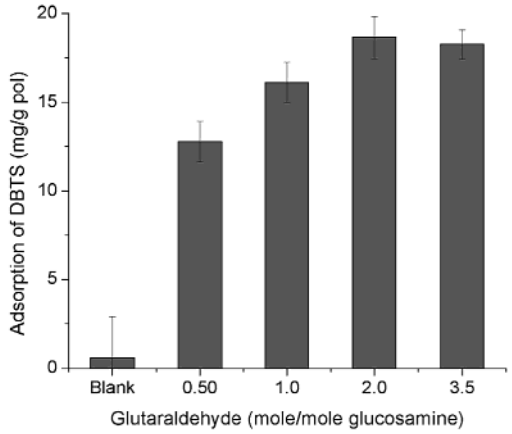

Figure 1. Effect of the cross-linker concentration on DBTS adsorption by the IH_{DBTS}. The blank is a nonimprinted hydrogel cross-linked with a 2:1 mole ratio.

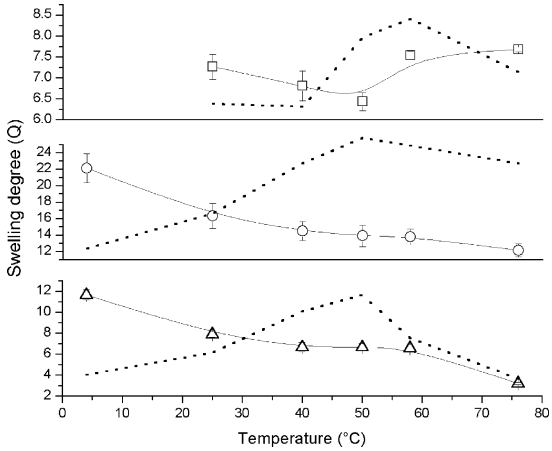

Figure 2. Change in the swelling degree (Q) of the IH_{DBTS} as a function of temperature and type of solvent: acetonitrile (△), water (○), and hexadecane (□). Each point was measured independently at least two times. Graphics are presented at different scales to be able to discern the changes in swelling degree. The dotted lines represent the first derivative and served as indicator of volume change.

to a specific conformation, which may be retained by cross-linking. A ^1H NMR study assessed the formation of hydrogen bonds between the DBTS and two secondary amines of a dicarboxamide that allowed the development of a DBTS-imprinted, high cross-linked divinylbenzene MIP (see ref 10). In addition, the molecular imprinting technique is also applied to natural materials such as cyclodextrins[4b,12] and cellulose acetate[13] resulting in stimuli-responsive and/or chiral MIPs.

In this article, we describe for the first time the synthesis of a molecularly imprinted chitosan hydrogel using DBTS as the template and glutaraldehyde as the cross-linker by a single-step procedure. The DBTS-imprinted chitosan hydrogel (IH_{DBTS}) was then used to specifically adsorb DBTS among other aromatic compounds typically found in gas oil and diesel such as fluorene (FLE), benzothiophene (BT), DBT, and 4,6-dimethyl DBT (DMDBT). We first evaluated the effect of the cross-linker concentration on DBTS adsorption, then the swelling behavior of the gel as well as the selectivity and adsorption capacity. Finally, we studied the ligand−gel interactions using steady-state spectrofluorometry in order to get some insights on the phenomena ruling the adsorption of DBTS by the IH_{DBTS}.

Results and Discussion

Cross-Linking Effect over Ligand Adsorption. The molecular imprinting technique requires the complete dissolution of both the polymer and the template to allow self-assembling through noncovalent interactions. In this study, a single-step procedure was used to obtain a miscible solution of chitosan and DBTS in acidic H_2O/acetonitrile (CH_3CN, 80:20) which was further used for cross-linking and imprinting. Here, we changed the cross-linker concentration in order to assess its effect on DBTS uptake by the IH_{DBTS}.

DBTS adsorption increased steadily with the cross-linker/glucosamine ratio until a value of 2 mol/mol (Figure 1) was reached. This is not surprising because it is well-known that the number of recognition sites in MIPs increases with network density.[14] In our case, a theoretical stoichiometric ratio of 0.5 mol of chitosan per mole of glutaraldehyde should be necessary to completely cross-link the hydrogel. Nevertheless, the increasing DBTS adsorption by the hydrogel beyond this value can be then attributed to an incomplete reticulation after 2 h of reaction. A higher cross-linker concentration (3.5 mol/mol) did not further increase DBTS adsorption. But, the use of high cross-linker concentrations is not recommended since it can cause ligand entrapment inside the gel with the above-mentioned associated problems. In subsequent experiments, a cross-linker/glucosamine ratio of 2.0 mol/mol was then used. Furthermore, it is clearly observed that DBTS adsorption was higher for all the IH_{DBTS} with respect to their nonimprinted controls. This is likely due to the conformational memory, "imprinting effect", via the formation of specific recognition sites for the DBTS inside the gel network.

Swelling Behavior of the IH. To test whether the IH_{DBTS} is stimuli-responsive toward temperature variations, we determined the swelling degree (Q) in solvents of different polarities such as water, CH_3CN, and hexadecane at different temperatures (Figure 2).

The latter becomes evident by the change in swelling degree (Q) in each tested solvent, from the swollen to the collapsed state, through the interplay of hydrophobic−hydrophilic interactions into the gel structure. We observed two different behaviors depending on solvent polarity: (a) the swelling degree diminished in polar solvents (water and CH_3CN) and (b) a minimum Q value was achieved in hexadecane which is a nonpolar solvent. The more important change on Q with temperature was observed with the polar solvents ($\Delta Q_{CH_3CN} = 5.46$; $\Delta Q_{water} = 9.96$), leading finally to the collapsing of the IH through CH_3CN expulsion.[15] Such collapsing may be referred to the increase of hydrophobic interactions throughout the carbon backbone and linking-groups of the polymer and to the weakening of the hydrogen bonds at high temperatures.[16] In the case of hexadecane, the swelling degree variation was minimal due to the poor affinity of this nonpolar solvent for the hydrophilic gel ($\Delta Q < 1.25$). Nevertheless, the IH_{DBTS} first slightly collapsed probably due to expulsion of residual CH_3CN used during the template washing, until a maximum was reached at 50 °C, followed by gel swelling presumably promoted by hexadecane absorption on hydrophobic zones. Usually, the lower critical solution temperature (LCST) of a hydrogel is defined as the temperature at which the swelling degree decreases to one-half of its value at the initial temperature.[17] Our hydrogel showed only a LCST at 50 °C in CH_3CN while the swelling in

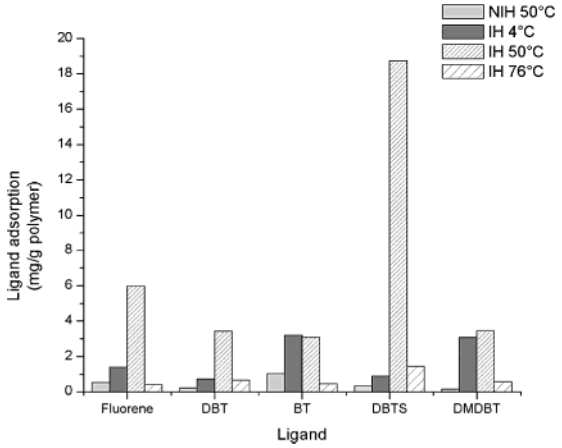

Figure 3. Effect of temperature and ligand on adsorption by a nonimprinted (NIH) and DBTS-imprinted hydrogel (IH_{DBTS}): benzothiophene (BT), dibenzothiophene (DBT), 4,6-dimethyl-DBT (DMDBT), dibenzothiophene sulfone (DBTS). Cross-linking agent ratio 2:1 mol, 50 °C. Uptake conditions: [ligand]/CH_3CN = 4 mM; 16 h; 300 rpm.

water and hexadecane were not enough to induce a 50% change in swelling degree. It is important to notice that such a swelling transition (see Figure 2, dotted lines for the first derivative of data) is present at the temperature of cross-linking/imprinting, for instance 50 °C, in all three tested solvents. This confirmed the existence of a conformational memory, set by cross-linking/imprinting conditions, that allows DBTS recognition (see below) and probably hydrogel's temperature sensitivity as has been reported by Piletsky et al.[18] Indeed, the global molecular conformation of a hydrogel responds to external factors and is the result of multiple local conformations due to heterogeneous interactions throughout the gel. Under the influence of a specific factor during the imprinting procedure (i.e., template, temperature), the hydrogel achieved a molecular recognition conformation of minimal global energy that could be fixed by cross-linking to recognize and adsorb DBTS. Such conformation has been previously theoretically[19] and experimentally[2a,4e,18,20] discussed.

On the other side, the nonimprinted hydrogel also presented such swelling behavior for all tested solvents but the difference remained small when compared to the IH_{DBTS} (data not shown). This implies that both nonimprinted and imprinted hydrogels were sensitive to changes on temperature.

Adsorption Selectivity of the IH. The selectivity and adsorption of various organosulfur compounds were studied in CH_3CN. We observed that ligand adsorption by the IH_{DBTS} strongly depends on temperature and type of ligand (Figure 3). The single component adsorption was minimal at 4 °C for all ligands and DBTS recognition was even lost at this temperature. On the contrary, DBTS adsorption was an order of magnitude higher at 50 °C. This behavior was only found for DBTS, the template molecule used during the imprinting process, since other molecules were poorly adsorbed by the hydrogel because of the absence of specific recognition cavities for these compounds into the matrix. An easy way to prove template selectivity and give good evidence of the imprinting effect by the imprinted hydrogel is to look at the binding ratio of a template (DBTS) against a structurally similar analyte (DBT). Thus, we compared the binding ratios of three systems: (1) a nonimprinted, (2) a DBTS-imprinted, and (3) a DBT-imprinted hydrogel (Table 1, see Supporting Information). We observed a major binding ratio for the analyte used as template during the cross-linking-imprinting process, 5.49 and 2.56 for IH_{DBTS} and IH_{DBT}, respectively. Therefore, the presence of an imprinting effect in the hydrogels is proposed for DBTS recognition and adsorption (see below). Moreover, the NIH showed a higher adsorption for DBTS when compared to DBT that could be explained in terms of a better affinity with the hydrogel through hydrogen-bonding.

Table 1. Binding Ratio for Dibenzothiophene Sulfone (DBTS) and Dibenzothiophene (DBT) in (a) Nonimprinted Hydrogel (NIH), (b) a DBTS-Imprinted Hydrogel (IH_{DBTS}), and (c) a DBT-Imprinted Hydrogel (IH_{DBT})[a]

	binding ratio	
hydrogel	DBTS/DBT	DBT/DBTS
NIH	1.61	0.62
IH_{DBTS}	5.49	0.18
IH_{DBT}	0.39	2.56

[a] Cross-linking agent ratio 2:1 mol, 50 °C. Uptake conditions: [ligand]/CH_3CN = 4 mM; 16 h; 50 °C; 300 rpm.

We also observed a good correlation between the swelling degree of the hydrogel and the temperature of ligand adsorption. Indeed, the highly swollen hydrogel at 4 °C should interact mainly with the solvent while the poor ligand uptake should be related to absorption. Afterward, the swelling degree showed a LSCT value at 50 °C (inflection between 40 and 60 °C) in CH_3CN that corresponded to the highest DBTS adsorption likely due to the favored ligand−gel interactions instead of solvent-hydrogel (see next section). Moreover, the ligand adsorption dropped down quickly at temperatures higher than 50 °C and was comparable to the adsorption of the non imprinted hydrogel (Figure 3). Such gel collapsing presumably avoids ligand−gel interactions by steric hindrance and/or solvent expulsion. The influence of the cross-linking−imprinting temperature over the imprinting effect was confirmed by an earliest study for an IH_{DBTS} at 25 °C.[21] Thus, we observed the highest DBTS adsorption (16.54 ± 0.46 mg/g of polymer) coinciding with the synthesis temperature, i.e., 25 °C, but this phenomenon was not further investigated in this study (see Supporting Information).

Insights on Ligand Binding. To further confirm the presence of a conformational memory for molecular recognition in the IH_{DBTS}, we undertook the study of ligand−gel interactions through the monitoring of DBTS excimers by steady-state fluorometry.[22] We evaluated the emission and excitation spectra of four systems: (1) DBTS/CH_3CN as a blank, (2) DBTS/soluble chitosan/CH_3CN, (3) DBTS/non imprinted hydrogel/CH_3CN and (4) DBTS/imprinted hydrogel/CH_3CN.

For these studies, we chose a DBTS-imprinted hydrogel obtained at 25 °C because of the easily to work at ambient temperature with the fluorometer at our disposition.[23] The excimers exist as singlet (^{1}) or triplet (^{3}) state (paired and unpaired electrons, respectively) stabilized by resonance and van der Waals forces:[22b,c]

$$^1M + h\nu \rightarrow {}^3M^* + {}^1M \rightarrow {}^3E^* \rightarrow {}^1E + h\nu'_p$$

where M is the monomer molecule in its ground state, M* corresponds to the excited monomer molecule, E* is the intermolecular excited dimer or excimer, ^{1}E is the ground state of the bimolecule complex (dimer), and $h\nu'_p$ is the excimer phosphorescence.

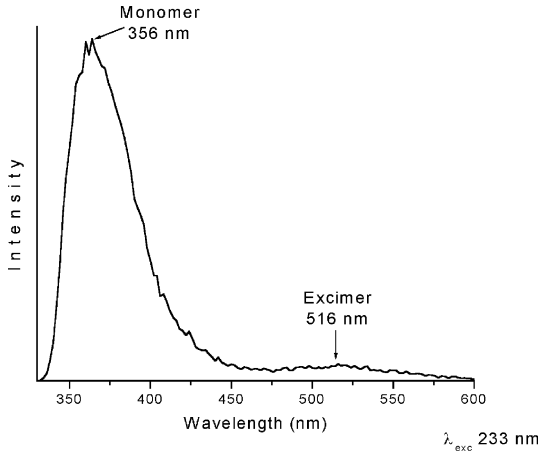

Figure 4. Emission spectrum of DBTS showing monomer fluorescence and excimer phosphorescence. Conditions: 25 °C, [DBTS]/CH_3CN = 0.10 mM; λ_{ems} 233 nm.

This approach involves the presence of two populations of excimers in the assay solution, one interacting with the chitosan and one in bulk solution that cannot be separately quantified but only averaged by steady-state fluorometry.

The emission spectra of DBTS shows a vibronic band at 356 nm corresponding to the monomer fluorescence emission whereas the excimer phosphorescence presented a characteristic broad featureless band at 516 nm (Figure 4). The presence of soluble chitosan (**2**) increased the I_E/I_M ratio (0.0527),[24] when compared to the free-chitosan blank (**1**) with a value of 0.0500, and revealed either an enhanced monomer-hydrogel interaction with a major presence of the dimer in the solution[22e,25] or a better solvatation of monomeric DBTS. On the other hand, NIH and IH_{DBTS} interacted mainly with the dimer as shown by the reduction of the I_E/I_M value, 0.0486 and 0.0459, respectively. The formation of ground-state dimers, and not the quenching of the monomer's intensity,[26] provoked this decrease as shown below by the excitation spectra.

The excitation spectra of the monomer and excimer (λ_{ems} 356 and 516 nm, respectively) allows the calculation of two parameters, $\Delta\lambda$ and $P_M - P_E$, related to the preformed ground-state dimers (Figure 5).[27] The excimer of DBTS yielded a red-shifted displacement (bathochromic) with altered vibronic structure because of its more polar nature respecting to the monomer. The presence of noncollisional excimers for each system was demonstrated by a $\Delta\lambda$ value equal to 8.

Concerning the extent of dimer formation ($P_M - P_E$ value), we employed instead the ground-state ratio (GSR)[28] to facilitate its interpretation and comparison. The GSR value increased in the presence of IH_{DBTS} throughout the temperature range tested when compared to the other assays (Figure 6a). This means that the IH_{DBTS} interacted mainly with DBTS dimers and confirms the I_E/I_M values previously obtained. DBTS adsorption occurred preferentially with the dimeric form of DBTS, i.e., an IH−DBTS dimer, rather than with its monomeric form. The GSR further diminished at 40 °C corresponding to hydrogel collapsing and reduction of DBTS adsorption as previously mentioned. Such behavior might be attributed to the decrease of dimer's polar interaction as explain below. The NIH interacted also with dimers as shown by the slightly increment in GSR values with temperature but it was not sensitive to temperature changes. Besides, the GSR values for

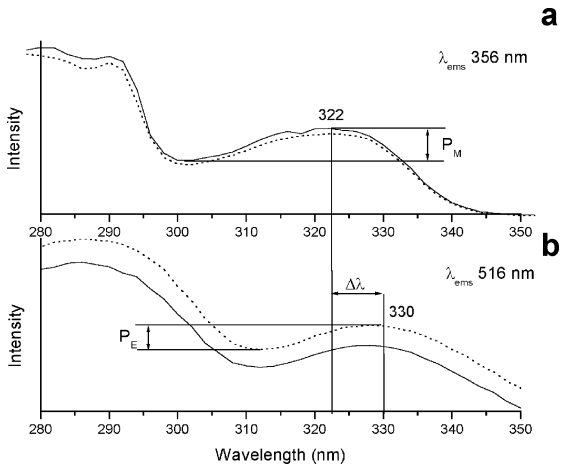

Figure 5. Excitation spectra of DBTS: (a) monomer (λ_{ems} 356 nm) and (b) excimer (λ_{ems} 516 nm), in the blank (absence of chitosan, dark line) and in the presence of the imprinted chitosan hydrogel (dashed line). See the text for parameter explanation of $\Delta\lambda$, P_M, and P_E. Conditions: 10 mg of IH_{DBTS}, [DBTS]/CH_3CN = 0.10 mM; 25 °C.

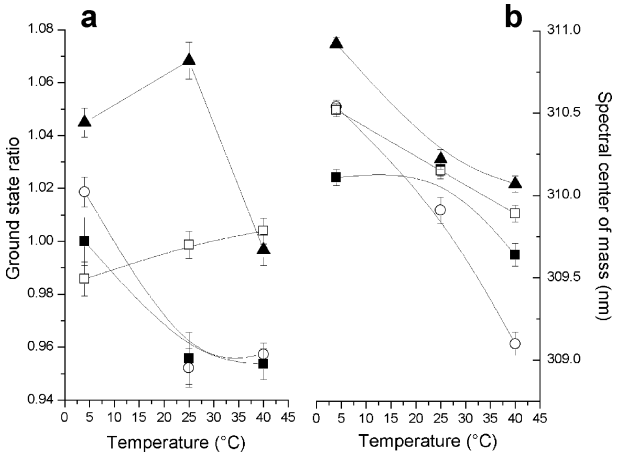

Figure 6. Effect of temperature on (a) the ground-state ratio (GSR) of DBTS and (b) the excimer's spectral center of mass (CM) in the blank (■), the soluble chitosan (○), the non imprinted hydrogel (□) and the imprinted hydrogel (▲). Each point represents the average of three-fluorescence excitation spectra. Imprinting/cross-linking conditions: 25 °C, 2 h; DBTS/glucosamine, 0.1 mol; glutaraldehyde/glucosamine, 2 mol.

DBTS in the blank and in the presence of soluble chitosan diminished with temperature and remained constant between 25 and 40 °C. Such diminution can be explain in terms of an increasing presence of monomeric DBTS in solution due to the weakening of polar interactions stabilizing the DBTS dimer. Moreover, soluble chitosan did not further stabilize the dimer as the GSR values were similar to the blank. Nonetheless, it caused the largest changed on the spectral center of mass suggesting an important interaction with DBTS excimer. Excimer destabilization and excimer-soluble hydrogel interaction were confirmed by the blue shift of the spectral center of mass (vide infra).

Finally, the temperature and the chitosan (soluble, non imprinted or imprinted hydrogel) had little influence on the monomer's excitation spectra between 280 and 350 nm, expressed as the spectral center of mass (CM).[29] Hence, the CM value slightly diminished from 303.5 to 302.5 nm as temperature increased and not significant difference were found between the four assays (data not shown). This suggests that the mono-

mer of DBTS was largely solvated for a hydrophobic environment with none or poor interaction with the soluble chitosan or hydrogel. On the other hand, a hypsochromic shift occurred as temperature increased, which indicates an increasing nonpolar medium around the excimer for all four systems (Figure 6b). The environment surrounding DBTS in the blank (**1**) became more hydrophobic at 40 °C, which coincided with the decrease of the GSR value shown above. It is important to notice that soluble chitosan (**2**) caused the largest change on the CM value of DBTS, which could be attributed to a better solvatation but mainly to the different and more hydrophobic environments adopted by the lineal polysaccharide structure around DBTS. The CM value of DBTS in the presence of NIH (**3**) showed a slightly diminution with temperature suggesting a better excimer stabilization in comparison with the blank because of the hydrophobic interaction between DBTS and the surrounding environment established by NIH. Concerning the IH_{DBTS} (**4**), the CM value diminished steadily with temperature and showed the highest stabilization of the DBTS excimer between 25 and 40 °C. This closely corresponds to the highest adsorption of DBTS, the LCST value, and the maximum interaction with DBTS dimers as shown by the GSR value. The latter let us propose the existence of a conformational memory to recognize and adsorb specifically the DBTS by the IH_{DBTS}.

Conclusions

The IH_{DBTS} selectively adsorbed DBTS from an acetonitrile solution when compared to other organosulfur compounds found in diesel. Such selectivity was attributed to the conformational memory obtained by cross-linking with glutaraldehyde in the presence of DBTS as the template and commonly referred to as the "imprinting effect".

Moreover, the DBTS molecule specifically adsorbed as dimer complexes on the IH_{DBTS}. Monomeric DBTS remained for all four systems mainly solvated and scarcely interact with the soluble chitosan and hydrogels. The presence of a conformational memory together with the simultaneous presence of dimer complex favors the recognition and adsorption of DBTS into the IH_{DBTS}. The latter showed a conformational memory at the temperature of transition (LCST), between the swollen-collapsed states, which coincides with the temperature of synthesis of the material. This finding is currently under study and is the subject of future work.

DBTS selective adsorption by the IH_{DBTS} compared to other ligands was probably due to the inability of these ligands to form dimer complexes matching the minimal global energy conformation of the IH at the tested temperature. Further studies will be undertaken to elucidate the monomeric or dimeric ligand binding to chitosan hydrogel and its effect on adsorption.

Although a lot of studies reported stimuli-responsive gels and MIPs, this is the first example that brings some insights into the complexity of the ligand binding on a DBTS-imprinted hydrogel. The steady-state fluorometry was found to be a useful technique (1) to identify the molecular recognition conformation based on the ligands and not the polymers, (2) to reveal the general interaction between ligands and MIP, and (3) to give information concerning the nature of the environment around the ligands in the presence of MIPs. The present strategy can be used for the design and synthesis of other responsive gels based on fluorometric data with promising application in analytical and industrial separations.

Experimental Section

Chemicals. Food Grade 90 chitosan (30kD) with an acetyl content of 6% was kindly donated by PRIMEX and Dra. K. Shirai from the UAM Iztapalapa. Glacial acetic acid was acquired from Baker. Glutaraldehyde (50% w/w in water), Triton X-114, reagent grade methanol, HPLC grade acetonitrile, dibenzothiophene (DBT) 99%, dibenzothiophene sulfone (DBTS) 97%, 4,6-dimethyldibenzothiophene (DMDBT) 97%, thianthrene (TNE) 97%, and fluorene (FLE) 98% were purchased from Aldrich Co and used without further purification.

Cross-Linking and Molecular Imprinting of Chitosan. Chitosan was cross-linked with glutaraldehyde and molecularly imprinted with DBTS as the template following a single-step procedure. First, 100 mg of chitosan (dry basis) was dispersed in 5 mL of a 1% solution of acetic acid in distilled water. Then 3 mL of H_2O was then added to the solution and maintained at the selected temperature for 30 min at 300 rpm to allow chitosan dissolution. Afterward, 40 μL of Triton X-114 (surfactant agent) and 2 mL of a DBTS solution (0.1 mol ligand/mol glucosamine) in acetonitrile (CH_3CN) were dropwisely added and kept under stirring for 30 min. The surfactant agent facilitated the homogeneous dispersion of DBTS into the H_2O/CH_3CN solution. The cross-linking and molecular imprinting of chitosan proceeded by the addition of glutaraldehyde (0.5−3.5 mol/mol glucosamine) and the reaction was carried out during 2 h at 50 °C. The imprinted chitosan hydrogel (IH) was then collected by filtration and washed with 2 × 25 mL CH_3CN. Removal of DBTS from the IH was performed by Soxhlet extraction with CH_3CN during 16 h (removal yield 66%). Finally, the hydrogel was slowly dried at 50 °C during 12 h. The preparation of the nonimprinted hydrogel (NIH) followed exactly the above procedure but no template was added.

Swelling Degree (*Q*). For each determination, 30 mg of hydrogel (d.b.) were added to 3 mL of the selected solvent in 25 mL capacity vials. The vials were exposed at a temperature between 4 and 76 °C during 120 min. The polymer was then removed by filtration through a Büchner filter at constant weight and weighted. The swelling degree (Q) of the polymer in the hydrogel is as follows:

$$Q = \rho_p[(Q_m/\rho_s) + (1/\rho_p)]$$

Here ρ_p is the polymer density (1.36 g/cm^3 for cross-linked chitosan[11]), ρ_s is the solvent density at the specified temperature and Q_m is the swelling ratio, defined as the ratio of solvent mass over the dry hydrogel mass.

Adsorption Studies in Model Solutions. In the adsorption assays, 30 mg of hydrogel were added to an CH_3CN solution (1 mL) containing the specified organosulfur compound (4 mM). The screw-capped vials with the former suspensions were incubated at the specified temperature during 16 h under mechanical agitation (300 rpm). The polymers were then removed by filtration and the resulting solutions were analyzed by HPLC (Hewlett-Packard Series 1100 equipped with a diode array detector) using a C18 ODS reverse phase column. CH_3CN/water (4:1 v/v) was used as the mobile phase at a flow rate of 1.2 mL min^{-1}. TNE was used as an internal standard in all of the analyses.

Steady-State Fluorescence Spectrofluorometry. The steady-state fluorescence measurements were performed on a RF-5301PC Shimadzu spectrofluorometer, with a 150 W Xe lamp using DBTS as ligand and fluorescent probe. The emission spectra of DBTS were measured in the wavelength range 320−620 nm with an excitation wavelength of 233 nm. Excitation spectra of the monomer and excimer were measured between 280 and 350 nm with emission wavelengths of 356 and 516 nm, respectively. The measurements were repeated at least three times. In a typical experiment, 10 mg of hydrogel (d.b.) in 2 mL of DBTS/CH_3CN (0.10 mM) were added to 3 mL

capacity quartz cells. The cells were maintained at a temperature between 4 and 40 °C during 15 min before spectra recording.

The CM values of the monomer and excimer were calculated between 280 and 350 nm from the excitation spectra (λ_{ems} 356 and 516 nm, respectively) in according to

$$CM = \frac{\sum(I_{(\lambda)}\lambda)}{\sum I_{(\lambda)}}$$

where λ is the excitation wavelength and $I_{(\lambda)}$ represents the fluorescence intensity at every λ.

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Supporting Information Available: Table of adsorption data for IH_{DBTS} and IH_{DBT} at 50 °C and a figure showing the DBTS adsorption profile vs temperature for a IH_{DBTS} cross-linked and imprinted at 25 °C (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) The GSR relates every value of $P_M - P_E$ with that obtained for the blank at 4°C and represents the relation of ground-state complexes in the system.

(29) The spectral center of mass (CM) is a probe of chromophore's environment because it changes with solvent polarity and temperature. It relates the change of intensity with wavelength throughout an established range in contrast with the more utilized wavelength of maximum intensity (λ_{max}) or maxima intensities ratio (I_1/I_3). A bathochromic or hypsochromic shift is associated with a polar and nonpolar environment, respectively. For calculation and interpretation of the center of mass, see: (a) Chapeaurouge, A.; Johansson, J. S.; Ferreira, S. T. *J. Biol. Chem.* **2002**, *277*, 16478−16483. (b) Bortoleto, R. K.; Ward, R. J. *FEBS Lett.* **1999**, *459*, 438−442. Literature for I_1/I_3, see: (c) Koussathana; Lianos, P. *Macromolecules* **1997**, *30*, 7798−7802.

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