Chemically modified polysulfones for molecular imprinting. Synthesis and complexation with a fluorescent model template

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A B S T R A C T

Polysulfone (PSU) was chemically modified to prepare new molecular imprinted membranes (MIMs). Several amounts of amine and sulfonyl groups were introduced into the PSU chemical structure in order to create interactions with acid or base templates, such as biomolecules or biomacromolecules. A fluorescent dye, Acridine Orange base (AO), was used as a model template and its complexation with the prepared PSUs was monitored by spectroscopic techniques. This study showed an absence of complexation with the native PSU and a strong complexation with the aminated and the sulfonated PSUs. Partially allylated PSU bearing amine or sulfonyl groups were also synthesized. These compounds are expected to be used as precursors for designing new crosslinked molecular imprinting membranes (MIMs), exhibiting high stability of the template memory.

Keywords:
Polysulfone
Molecular imprinting
MIP
MIM

1. Introduction

Molecularly imprinted polymers (MIPs) are attractive synthetic materials mimicking the highly specific receptor properties of antibodies [1]. Nowadays, MIP polymers have found biomedical applications. First used in chromatography for the separation of specific analytes, imprinted polymers have also applications in molecular sensors [2–6], and solid-phase extraction [3,7–10]. A variation of MIP techniques, termed configurational biomimetic imprinting (CBIP), has been developed and used in novel intelligent catalytic, biomedical and drug delivery systems (DDSs) [11–16]. Imprints against small-molecule templates have been generated for decades, but attempts to prepare imprints against biomacromolecules, such as proteins, have been far less successful. However, the field has progressed rapidly and a number of molecular imprints – based on acid/base monomers – selective for protein ligands have now been reported. Given the enormous potential of replacing the antibodies used in a host of immunoassays with robust and inexpensive receptors, efforts in this area continue to intensify [17]. MIP polymerizations all follow the same general procedure. In a solution of appropriate functional monomers, a template molecule (or the molecule to be recognized) is added, and the solution is mixed. This mixing allows ''self-assembly'' of the template with the complimentary monomers to form a pre-polymerization complex. The functional monomer exhibits specific chemical moieties designed to interact with the template either by covalent chemistry [18–20], non-covalent chemistry [21–24], or both [25]. Once the pre-polymerization complex is formed, the polymerization reaction usually occurs by free radical initiation in the presence of a crosslinking monomer and an appropriate solvent. The template can then be extracted from the crosslinked polymer via diffusion, and the resulting structure is a porous matrix with specific recognition cavities for the template molecule [26,27]. These cavities not only maintain the ordered arrangement of complimentary chemical functionalities of the template, but also the overall spatial configuration of the target molecule is maintained.

Elaboration of molecularly imprinted membranes (MIMs) for separation is an emergent and promising research field [8–28]. Polymer solution film casting and subsequent phase inversion, the main approach towards technical polymeric membranes, can also be applied for molecular imprinting. Instead of an in situ polymerization, the solidification of a polymer, by solvent evaporation or by precipitation induced via contact with a non-solvent, is used. It is remarkable, that most MIM prepared via phase inversion imprinting had at least acceptable binding performance in aqueous media. However, such MIM lost their ''template memory'' when exposed to a too organic environment where swelling and chain rearrangement seemed to ''erase'' the imprinted information [29]. During the last decades, some attempts have been focused on

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http://dx.doi.org/10.1016/j.reactfunctpolym.2012.12.003

preparing MIMs based on PSUs by the phase inversion process. For example, PSU-based MIMs exhibiting specific recognition towards Rhodamine B [30], Dibenzofuran [31], Indole derivatives [32], Guanosine and Adenosine [33] have been prepared. However, except in the work of Mathias Ulbricht's team [30] performed on 10% sulfonated PSU, amino and sulfonyl groups have never been used as recognition sites in PSU-based MIMs.

Herein, we describe chemical modifications of a commercially available PSU to be used as MIMs towards acid/base templates, such as peptides and proteins. The physico-chemical interactions (complexation or self assembly) between the Acridine Orange base (AO), a fluorescent dye model template, and the modified and unmodified PSUs are investigated to determine the optimal conditions of complexation.

2. Experimental

2.1. Materials

Polysulfone (PSU) (Aldrich, France), Chlorosulfonic acid (99%, Sigma Aldrich, France), sulfuric acid (98%, Carlo Erba, France), nitric acid (65%, Carlo Erba, France), hydrochloric acid (36%, Aldrich, France), glacial acetic acid (100%, Merck, France), NaI (Sigma Aldrich, France), $SnCl_2{\cdot}2H_2O$ (cristallized, Prolabo, France), allylamine (99%, Acros Organics, France), allyl bromide (99%, Acros Organics, France), Acridine Orange base (AO) (Sigma Aldrich, France), were used as received. The solvents: chloroform ($CHCl_3$), *N*-methylpyrrolidone (NMP), methanol (MeOH) were used without further purification.

2.2. Instruments

^{1}H NMR spectra were recorded with Bruker AC200, Avance 300 instruments, in DMSO-d6 or $CDCl_3$. 1H chemical shifts were reported in parts per million (ppm) relative to $(Me)_4Si$ as external standard. ^{1}H data were reported as follows: chemical shift (multiplicity; s: singlet, t: triplet, q: quadruplet and m: multiplet; peak assignments). ^{13}C data were reported as follows: chemical shift (peak assignments). Infrared spectra were recorded on a Perkin–Elmer 1725X FTIR spectrometer. UV/visible absorption spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. Cell of 1 cm optical path length were used. The estimated experimental error was 2 nm on the band maximum and 5% on the molar extinction coefficient. Fluorescence measurements were performed on a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. The excitation source was a flash lamp filled with a mixture of nitrogen and helium (30/70). Data were collected over 200 channels with a time-base of 0.1 ns per channel. Size Exclusion Chromatography (SEC) was conducted on a Waters Associates equipment with three Styragelw HR (7 μm) 7.8 mm × 300 mm columns (10^4,10^3,100 Å pore size) thermostated at 35 °C. THF, Toluene and $CHCl_3$ were used as eluents with a flow rate of 1.0 mL min^{-1}. A differential refractometer (Waters Associates) was associated as detector with a light-scattering diffusion mini Dawn® apparatus purchased from Wyatt Technology Corporation. The *dn*/*dc* refractive index increment values were determined by the iterative method software.

2.3. Preparation of sulfonated polysulfones (PSU—SO_3H)

1 g of PSU was dissolved in 10 mL of $CHCl_3$ at 25 °C. Then, variable amount of chlorosulfonic acid (Table 1) was added dropwise. The solution was allowed to stir for 2.5 h. The polymer recovery method was dependent on the chlorosulfonic acid amount:

(a) Chlorosulfonic acid amount < 1.4 eq: the polymer was precipitated from HCl (1 M), washed with water and dried under vacuum. The sulfonated polymer did not exhibit swelling in water and could be used for the elaboration of membranes by the phase inversion process (Table 1).

^{1}H NMR ($CDCl_3$): 7.87 (d,H7 and H21), 7.69 (m,H14), 7.27 (d,H3,H11 and H18), 7.04 and 6.97 (dd,H2,H6,H10,H17 and H20), 1.73 (s, CH_3—C—CH_3). ^{13}C NMR ($CDCl_3$): 162.01 (C5), 152.87 (C1), 147.21 (C4), 135.44 (C8), 129.98 (C3), 128.86 (C7), 119.85 (C2), 117.72 (C6), 42.45 (CH_3—*C*—CH_3), 31.00 (CH_3—C—CH_3). FTIR: ν_{O-H} = 3430 cm^{-1}; ν_{SO_3H} = 1170 cm^{-1} (asymmetric stretching); ν_{SO_3H} = 1028 cm^{-1} (symmetric stretching).

(b) 1.4 ⩽ chlorosulfonic acid amount ⩽ 3 eq: led to precipitation of the polymer. The sulfonated polymer was dissolved in NMP, precipitated from HCl (1M) and then washed with water. Consequently, this polymer could be used for the elaboration of membranes by the phase inversion process. However, this polymer was not soluble in our SEC solvents: $CHCl_3$, THF and toluene (Table 1).

(c) Chlorosulfonic acid amount > 3 eq: led also to precipitation of the polymer. However, this polymer was soluble in both water and NMP, and consequently could not be precipitated and used for membrane preparation. The sulfonated polymer was then recovered by solvent evaporation under vacuum. In addition, this polymer was not soluble in our SEC solvents (Table 1).

NMR analyses of the precipitated polymers, i.e. PSU—SO_3H (100%) and PSU—SO_3H (125%), were performed in DMSO-d6 to reach the degree of substitution (DS).

^{1}H NMR (DMSO-d_6): 7.97–7.77 (m,H7 and H21), 7.73 (m,H14), 7.27 (m,H3,H11 and H18), 7.17–6.79 (m,H2,H6,H10,H17 and H20), 1.64 (s,CH_3—C—CH_3). ^{13}C NMR (DMSO-d_6): 162.88 (C19), 161.81 (C5), 152.82 (C1), 149.41 (C16), 147.21 (C13), 146.94 (C4), 139.60 (C15), 139.60 (C22), 134.61 (C8), 130.32 (C18), 130.22 (C3 and C11), 129.72 (C14), 128.89 (C7), 127.33 (C21), 122.36 (C17), 120.23 (C2 and C10), 118.29 (C6 and C20), 42.48 (CH_3—*C*—CH_3), 31.00 (*C*H$_3$—C—CH_3). FTIR: ν_{O-H} = 3430 cm^{-1}; ν_{SO_3H} = 1170 cm^{-1} (asymmetric stretching); ν_{SO_3H} = 1028 cm^{-1} (symmetric stretching).

2.4. Preparation of aminated polysulfones (PSU—NH_2)

2.4.1. Nitration of PSU

About 1 g of PSU was dissolved in 25 mL of $CHCl_3$ at 25 °C. Then, 5 mL of a mixture of nitric acid and sulfuric acid were added dropwise at 25 °C. The volume ratio of nitric acid to sulfuric acid was varied from 1:1 to 4:1, to obtain various degrees of substitution (DS) (Table 2).

The reaction medium was allowed to stir for 30 min. The formed yellowish nitrated PSU (PSU—NO_2) was precipitated from methanol. ^{1}H NMR ($CDCl_3$): 8.28 (d, H28), 7.99 (d, H34), 7.92 (d, H7), 7.48 (d, H32), 7.27 (d, H3), 7.22–6.93 (m,H2,H6,H31 and H34), 1.75 (s,6H,CH3—C—CH3). ^{13}C NMR ($CDCl_3$): 162.01 (C5), 160.85 (C33), 153.40 (C1 and C23), 149.08 (C26), 148.88 (C4), 145.93 (C30), 141.69 (C29), 136.71 (C27), 135.57 (C36), 135.23 (C8), 133.45 (C32), 129.93 (C25), 129.87 (C3), 128.94 (C35), 128.49 (C7), 123.92 (C28), 120.30 (C31), 120.15 (C24) 119.92 (C2), 117.88 (C34), 117.74 (C6), 42.47 (CH_3—*C*—CH_3), 30.98 (*C*H3—C—CH3). FTIR: ν_{NO2} = 1534 cm^{-1} (asymmetric stretching); ν_{NO2} = 1348 cm^{-1} (symmetric stretching).

Table 1
Chemical composition and some physical properties of the sulfonated polysulfones.

Polymer code	$ClSO_3H$/PSU (eq/eq)	DS by ^1H NMR[a] (%)	$\overline{M}_n$[b]	PD	*dn*/*dc*	Film forming property[d]
PSU	0.00	0	32800	1.6	0.195	+
PSU—SO_3H (29%)	0.35	29	57500	1.4	0.140	+
PSU—SO_3H (57%)	0.70	57	52800	1.4	0.140	+
PSU—SO_3H (100%)[c]	1.40	101	/	/	/	+
PSU—SO_3H (125%)[c]	3.00	125	/	/	/	/

[a] DS is the degree of substitution.
[b] SEC analysis was performed in $CHCl_3$.
[c] Insoluble in our SEC solvents: THF, $CHCl_3$ and toluene.
[d] Determined by the ability of the material to form homogeneous film after solvent evaporation and/or after the phase inversion process.

Table 2
Chemical composition and some physical properties of the nitrated and aminated polysulfones.

Polymer code	Composition of nitration agent HNO_3/H_2SO_4 (v/v)	HNO_3/PSU (eq/eq)	DS by ^1H NMR (%)[a]	$\overline{M}_n$[b]	PD	*dn*/*dc*	Film forming property[d]
PSU—NO_2 (5.0%)	1:1	15	5.0	16200	1.2	0.180	+
PSU—NO_2 (11.1%)	2:1	21	11.1	12000	1.3	0.180	–
PSU—NO_2 (53.3%)	4:1	25	53.3	5400	1.2	0.165	–
PSU—NH_2 (6.9%)[c]	1:1	15	6.9	/	/	/	+
PSU—NH_2 (15.1%) [c]	2:1	21	15.1	/	/	/	+
PSU—NH_2 (54.5%)[c]	4:1	25	54.5	/	/	/	+

[a] DS is the degree of substitution.
[b] SEC analysis was performed in $CHCl_3$.
[c] Insoluble in our SEC solvents: THF, $CHCl_3$ and Toluene.
[d] Determined by the ability of the material to form homogeneous film after solvent evaporation and/or after phase inversion process.

2.4.2. Reduction of nitrated polysulfones (PSU—NO_2)

About 0.5 g of PSU—NO_2 was dissolved in 15 mL of $CHCl_3$ in a two-necked flask equipped with a reflux condenser. Then, a solution of 5 g of $SnCl_2{\cdot}2H_2O$ and 0.16 g of NaI in 12 mL of HCl–glacial acetic acid mixture (2:1) was added in a dropwise manner at 60 °C under stirring. The resulting mixture was further refluxed for 3 h and then cooled to room temperature in order to reach a polymer precipitation. The orange obtained polymers were air dried at 40 °C under vacuum. The degrees of substitution of aminated polysulfones (PSU—NH_2) were close to those of the nitrated ones (Table 2).

^1H NMR (DMSO-d_6): 7.92 (d, H7 and H49), 7.30 (m, H3 and H39), 7.05 (m, H2, H6, H26, H34), 6.91 (m, H45), 6.86 (m, H42), 6.67 (m, H46), 4–3.5 (m, NH_2) 1.63 (s, 6H, CH_3—C—CH_3). ^{13}C NMR (DMSO-d_6): 161.92 (C5), 152.73 (C1 and C37), 148.39 (C4), 147.51 (C44), 135.72 (C8), 135.57 (C41), 135.13 (C43), 130.32 (C3), 128.86 (C7), 121.86 (C45), 121.22 (C46), 120.19 (C42) 118.25, (C2 and C38), 117.48 (C6), 42.41 (CH_3—C—CH_3), 31.02 (CH_3—C—CH_3).

FTIR: ν_{N-H} = 3500 cm^{-1} (asymmetric and symmetric stretching); ν_{N-H} = 1620 cm^{-1}, N—H bend, strong.

2.5. Preparation of crosslinkable polysulfones bearing allyl groups

2.5.1. From chlorosulfonated polysulfone

To 1 g of PSU dissolved in 10 mL of chloroform, 0.1 mL of chlorosulfonic acid was added dropwise. The mixture was allowed to stir for 2.5 h at 25 °C. After, 4 mL of allylamine were added to react with the formed chlorosulfonyl groups within 12 h at 25 °C. The obtained polymer was precipitated from aqueous solution of triethylamine (1M) and then, washed with water to give partially sulfonated and allylated polysulfone.

^1H NMR ($CDCl_3$): 7.89 (d, H7 and H65), 7.73 (m, H55), 7.27 (m, H3, H52 and H59), 7.03 (d, H2, H51, H58), 6.97 (d, H6, H54, H68), 5.78 (m, H61), 5.27 and 5.17 (dd, H62), 3.45 (t, H60) 1.64 (s, CH_3—C—CH3). ^{13}C NMR ($CDCl_3$): 162.01 (C5), 152.96 (C57), 152.86 (C1), 147.20 (C4), 135.42 (C8), 129.98 (C3), 129.37 (C56), 128.84 (C7), 119.85 (C2), 117.71 (C6), 116.95 (C62), 42.62 (C60), 42.45 (CH_3—*C*—CH_3), 30.99 (*C*H3—C—CH3). FTIR: ν_{O-H} = 3430 cm^{-1}; $\nu_{C=C}$ = 1661 cm^{-1}; ν_{SO_3H} = 1170 cm^{-1} (asymmetric stretching); ν_{SO_3H} = 1028 cm^{-1} (symmetric stretching).

2.5.2. From aminated polysulfone

To 0.2 g of PSU—NH_2 (54.5%) dissolved in 5 mL of DMF, 7.57 μL of allyl bromide and a catalytic amount of NaI were added. The mixture was allowed to stir for 24 h at 25 °C. Then, the obtained partially aminated and allylated polysulfone was precipitated from NaOH (0.1M) and washed with water.

^1H NMR (DMSO-d_6): 7.89 (d, H7 and H49), 7.29 (m, H3 and H39), 7.01 (m, H2, H6, H26, H34), 6.79 (m, H71 and H45), 6.75 (m, H68 and H42), 6.46 (m, H72 and H46), 5.68 (m, H74), 5.04 and 4.98 (dd, H75) 3.61 (m, H73), 3.41 (m, NH) 1.63 (s, 6H, CH_3—C—CH_3). ^{13}C NMR (DMSO-d_6): 161.93 (C5), 152.68 (C1 and C37), 148.41 (C4), 147.69 (C44), 138.50 (C70), 136.42 (C67), 135.78 (C8), 135.57 (C41), 134.85 (C43), 130.32 (C3), 128.86 (C7), 121.86 (C45), 121.22 (C46), 120.19 (C42), 118.25 (C2, C38 and C71), 117.48 (C6 and C72), 115.51 (C68), 48.96 (C73), 42.38 (CH_3—*C*—CH_3), 31.02 (CH_3—C—CH_3). FTIR: ν_{N-H} = 3500 cm^{-1} (asymmetric and symmetric stretching); $\nu_{C=C}$ = 1672 cm^{-1}; ν_{N-H} = 1620 cm^{-1}, N—H bend, strong.

2.6. Study of the complexation of acridine orange with the prepared polysulfones

2.6.1. In the solid state

Binary mixtures of AO with 57% sulfonated PSU (PSU—SO_3H (57%)) or with the native PSU were dissolved in $CHCl_3$. The resulting solutions were cast on PTFE molds to prepare films by solvent evaporation. The polymer concentration in the starting solutions was maintained constant at 33.34 mg/mL and the molar ratio R = AO/PSU or R = AO/PSU—SO_3H (57%) was ranged from 0 to 0.64. Then, all the prepared films, exhibiting a thickness of about 50 μm, were analyzed by fluorimetry in the following conditions:

$\lambda_{excitation}$ = 405 nm; $\lambda_{emission}$ = 535 nm; slits = 2 nm; Voltage = 1000 V.

2.6.2. In organic solvents

^{1}H NMR analysis: Binary mixtures of AO with the modified or unmodified PSU, at different molar ratios R = AO/PSUs, were dissolved in DMSO-d6 or $CDCl_3$ and analyzed by ^{1}H NMR. The concentration of AO was kept constant at 37.7 mmol/L.

UV–visible analysis: Binary mixtures of AO with the modified or unmodified PSU were dissolved in NMP and analyzed by UV spectroscopy. The concentration of AO was kept constant at 9.42 μmol/L and the molar ratio R = AO/PSUs was modified as follows: 0.05, 0.5, 1, 2 and 4.

2.7. Preparation of imprinted membranes by the phase inversion process

Totally sulfonated (PSU—SO_3H (100%)) or unmodified PSU was dissolved in NMP (20 wt.%). Then, AO was added to each solution in a molar ratio R = AO/PSU of 0.05. Each solution was poured onto a glass plate and spread, with a specific knife, to obtain films with a thickness of about 350 μm. Thereafter, the plate with the cast layer was placed in a water bath for about 3 min to obtain the desired MIM.

2.8. Kinetics study of AO release from the prepared membranes

About 10 coupons (~2 cm^2) of each membrane were prepared. Each coupon (~10 mg) was then placed in a flask containing 30 mL of distilled water. Coupons were periodically picked up (i.e., after 0 min, 2 min, 6 min, 30 min, 1 h, 2 h, 4 h, 5 h, 6 h, 8 h, 24 h), dried from water and weighted.

The concentration of AO released in water was assessed by fluorimetry measurements in the following conditions: $\lambda_{excitation}$ = 245 nm; $\lambda_{emission}$ = 530 nm; excitation slit: 4 nm; emission slit: 2 nm; voltage: 1000 V. A calibration curve of AO in water was established in these conditions.

In order to determine the remaining quantity of AO within the membranes, the coupons were dissolved in 2 mL of NMP and analyzed by fluorimetry in the following conditions: $\lambda_{excitation}$ = 444 nm; $\lambda_{emission}$ = 530 nm; excitation slit: 4 nm; emission slit: 2 nm; voltage: 1000 V.

A calibration curve was established in these conditions using solutions of AO and the prepared PSUs in NMP (R = 0.05 and 0.03, at various concentrations).

3. Results and discussions

3.1. Chemical modifications of the native polysulfone

New precursors for MIMs preparation, bearing amine or sulfonyl groups, with or without allyl groups, were prepared by simple chemical modifications of the native polysulfone (PSU). Sulfonation and amination were performed in order to generate acid/base interactions with base/acid templates (Scheme 1). Allyl groups were also introduced (Scheme 2) to allow post-crosslinking of the MIMs and to enhance the stability of the template memory.

3.1.1. Sulfonation

Direct sulfonation of aromatic polymers has been investigated intensively since the pioneering work of Noshay and Robeson [34], who developed a mild sulfonation procedure for bisphenol-A-based poly(ether-sulfone). This approach has found considerable interest in the area of water desalination through reverse osmosis and related water purification applications. In this work, sulfonation process was achieved by reaction of chlorosulfonic acid with the PSU followed by an acid hydrolysis of chlorosulfonyl groups into sulfonyl groups. Four sulfonated PSU (PSU—SO_3H) were then synthesized and characterized as shown in Table 1. Solubility of the PSU—SO_3H in organic solvents was strongly dependent on their degree of substitution (DS). Indeed, for a DS $\geqslant$ 100%, the solubility changed dramatically and the polymer became insoluble in some classical solvents of the native PSU such as THF, dichloromethane and chloroform. However, the film forming property – which was defined as the ability of the material to form homogeneous solid film after solvent evaporation and/or after the phase inversion process-, was lost for an DS = 125%. Actually, the preparation of PSU—SO_3H (125%) films by evaporation or by the phase inversion process was compromised, because this polymer was not soluble in volatile solvents and was soluble in water.

Sulfonation process of the polysulfone was confirmed qualitatively by FTIR and UV–visible analyses. The FTIR revealed the presence of sulfonic groups in the polymer backbone after sulfonation reaction. The SO_3 asymmetric and symmetric stretching bands assigned to the sulfonyl groups were observed at 1170 cm^{-1} and at 1028 cm^{-1}, respectively. This result was in close agreement with those obtained by Johnson et al. [35]. The UV–visible analysis showed a slight bathochromic shift on the absorption of polysulfone after sulfonation (Fig. 1).

The degree of substitution (DS) was determined by ^{1}H NMR using the β proton from sulfonyl group (proton No. 14, Scheme 1) and the methyl protons that are present in the modified and unmodified repeating units.

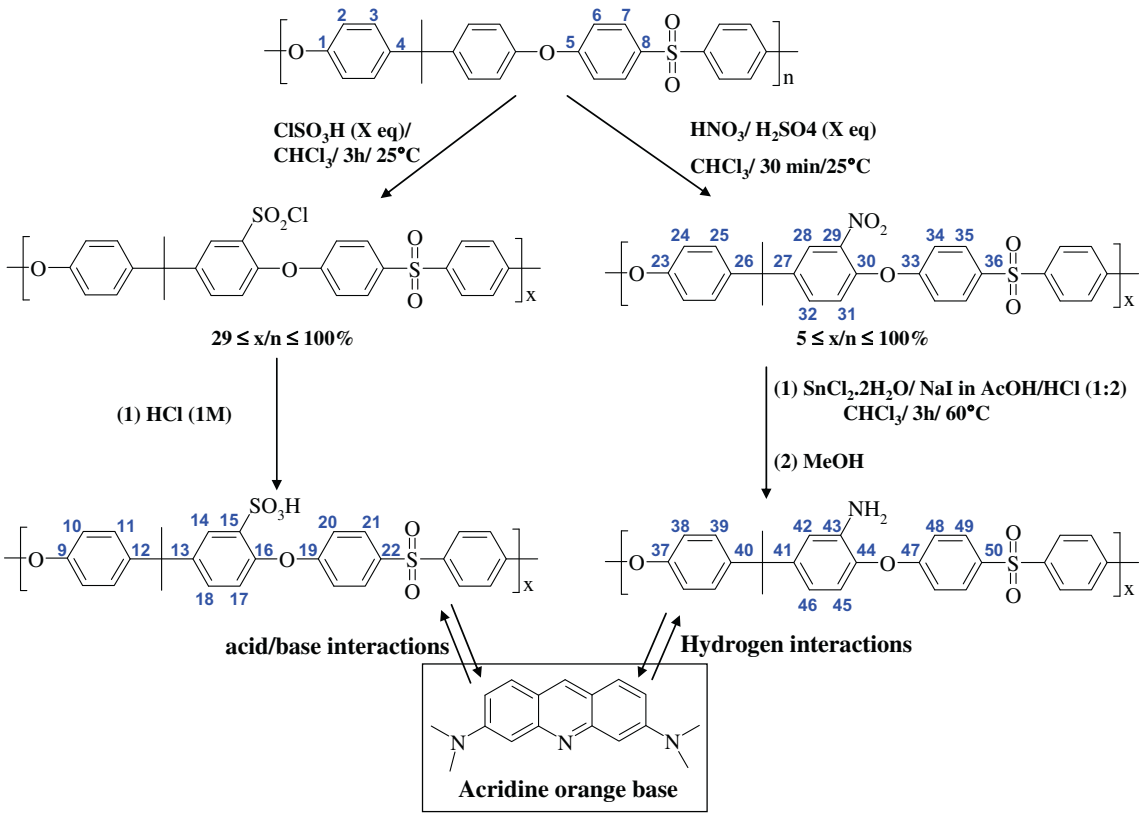
3.1.2. Amination

Amination of PSU was performed in two steps, i.e. nitration followed by reduction of nitro groups into amine groups. The nitration is known to be restricted to a level of monosubstitution of phenyl ring. Actually, the nitro group (NO_2) deactivates the aromatic ring towards further electrophilic substitution and strongly inhibits the introduction of a second nitro group [36]. The nitration of PSU was achieved while varying volume ratio of nitrating mixture (HNO_3:H_2SO_4) from 1:1 to 4:1 (Table 2). The reaction time (30 min) and temperature (25 °C) were carefully monitored to limit the polymer degradation. However, during the nitration, a decrease of molecular weight as a function of HNO_3 concentration was observed (Table 2). Bhole et al. have obtained similar results when performing nitration on poly(phenylene-oxide) [36]. Above 10% of substitution, the nitrated polysulfones had a powder form and lost their film forming properties.

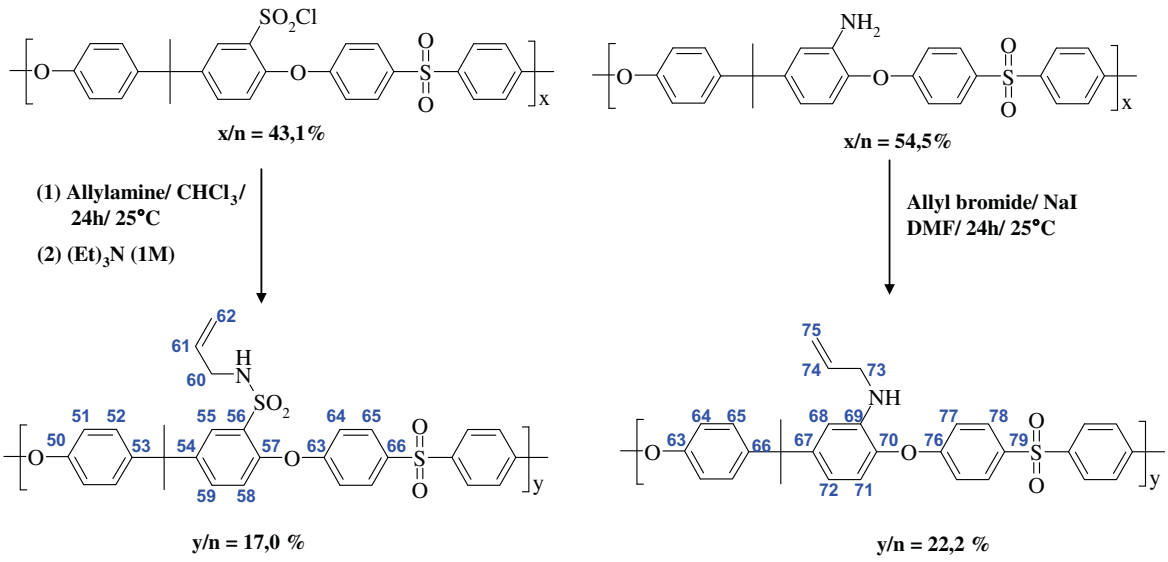
Then, reduction of NO_2 groups led to the desired aminated PSU (PSU—NH_2) (Table 2). Independently on the DS, the PSU—NH_2 exhibited different solubility properties compared to the native PSU.

The complete conversion of —NO_2 to —NH_2 was revealed by FTIR and ^{1}H NMR. Actually, in comparison with the native PSU, the FTIR spectra of the PSU—NO_2 exhibited two additional absorption bands at 1534 cm^{-1} and 1348 cm^{-1}, ascribed to anti-symmetric and symmetric stretching vibrations of the aromatic NO_2, respectively. The FTIR spectrum of PSU—NH_2 revealed the disappearance of the NO_2 bands. In addition, it exhibited a large absorption band at 3500 cm^{-1} corresponding to the N—H antisymmetric and symmetric stretches, and a band at 1620 cm^{-1} corresponding to the NH_2 deformation. The ^{1}H NMR spectrum of PSU—NH_2 revealed a disappearance of nitrated units and exhibited a new browse peak between 3.5 and 4 ppm, which was ascribed to the amine protons. The degree of substitution (DS) in both PSU—NO_2 and PSU—NH_2 was calculated using signals of the aromatic protons of the modified and unmodified repeating units.

The UV–visible analysis showed a bathochromic shift on the absorption of PSU after substitution by nitro or amino groups. Thus, two bands corresponding to the unmodified and the modified repeating units were observed when the DS was less than 100% (Fig. 1).



Scheme 1. Synthesis of sulfonated and aminated polysulfones.



Scheme 2. Allylation of aminated and sulfonated polysulfones.

3.1.3. Grafting of allyl groups

New crosslinkable PSUs bearing reactive allyl groups were synthesized using two pathways (Scheme 2). In a first way, allylamine was reacted with 43.1% chlorosulfonated PSU. Then, a smooth hydrolysis of the remaining chlorosulfonyl groups was performed to give allylated PSU—SO_3H. The second pathway involved a nucleophilic substitution between the amine groups of PSU—NH_2 (54.5%) and allyl bromide, in presence of a catalytic amount of NaI, to afford allylated PSU—NH_2. FTIR spectra of the allylated PSU—SO_3H and of the allylated PSU—NH_2 revealed new bands at 1661 cm^{-1} and 1672 cm^{-1}, respectively, assigned to the symmetric stretching vibration of carbon–carbon double bonds.

The percentage of allylation was assessed by ^1H NMR using the allyl and the methyl signals. Results showed 17.0% and 22.2% of allylation for PSU—SO_3H and PSU—NH_2, respectively.

3.2. Study of interactions between AO and the different PSUs

The presence of interactions between the polymer matrix and the target molecule is a key element for molecular imprinting.

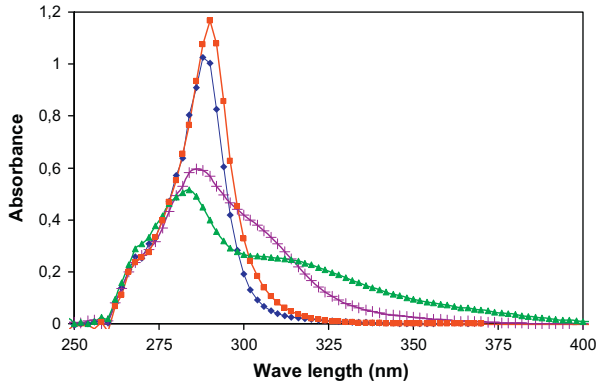


Fig. 1. UV spectra in NMP of: (◆) PSU, (▲) PSU—NO_2 (53.3%), (+) PSU—NH_2 (54.4%) and (■) PSU—SO_3H (100%).

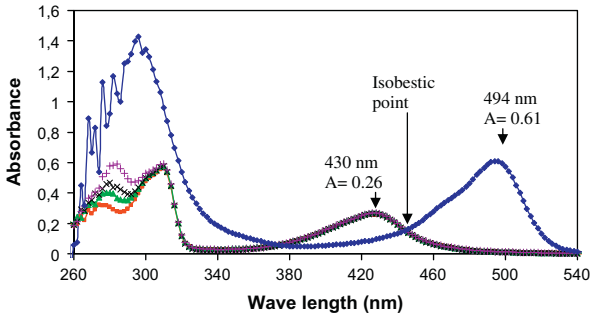


Fig. 3. UV spectra of mixtures based on PSU—SO_3H (100%) and AO in NMP (9.42 μmol/L) at various ratios R = AO/PSU: (■) AO, (▲) R = 2, (×) R = 1, (+) R = 0.5 and (◆) R = 0.05.

Therefore, a study of interaction between AO and the different PSUs was performed in the solid state as well as in organic solutions. For the solid state study, transparent films based on PSU or PSU—SO_3H (57%) containing AO, at different R ratio, were prepared by $CHCl_3$ evaporation process. Transparent films based on PSU—NH_2 could not be obtained since this polymer was insoluble in volatile solvents and because the phase inversion process gave porous opaque white films unsuitable for fluorimetry analysis. AO is a fluorescent dye which is used in various applications. Its fluorescence properties are due to its nitrogen and π free electrons leading to numerous states of resonance. AO exhibits fluorescence in organic solvents and in neutral or basic aqueous solution, but AO is not fluorescent in acid solutions, which is due to a decrease of the resonance states by ammonium salt formation. Similarly, AO loses its fluorescence in the solid state because of intermolecular interactions leading to auto-association and aggregation.

Fig. 2 shows the evolution of fluorescence intensity of the prepared films versus the R ratio. In the case of PSU, one can observe that fluorescence intensity decreased linearly with R until an AO composition of 23%. Indeed, for $R \geqslant 0.23$, macro-phase segregation as well as fluorescence extinction appeared. This suggests an absence of interactions between AO and the PSU. Actually, the presence of fluorescence at $R < 0.23$ suggests that AO was well dispersed within the PSU matrix and that its nitrogen and π electrons were free from strong interaction that leads to the fluorescence extinction. The linear decrease of fluorescence with AO content is assigned to the increase of AO auto-associations, which became very high for $R > 0.23$ leading to the macro-phase segregation. On the other hand, independently on R values, films made of

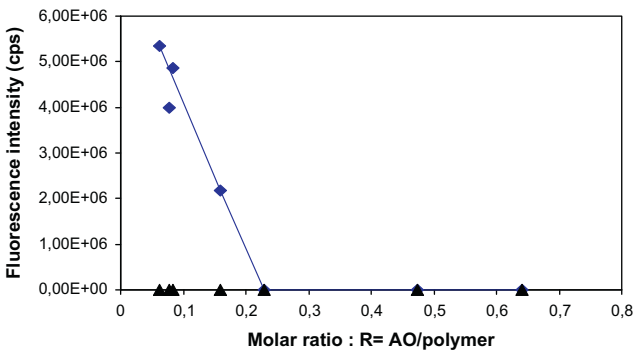


Fig. 2. Fluorescence intensity versus AO content within: PSU films (◆, R = AO/PSU) and PSU—SO_3H (57%) films (▲, R = AO/PSU—SO_3H (57%)).

the PSU—SO_3H (57%) and AO are all homogeneous, transparent and not fluorescent. This result was expected because PSU—SO_3H is an acid polymer which reacts with the basic part of AO leading to ammonium salt formation and fluorescent extinction.

Interactions between AO and the PSUs in organic solvents was monitored by UV–visible and ^1H NMR. The UV–visible study was performed in NMP using a constant AO concentration of 9.42 μmol/L. NMP was chosen because it is commonly used for the preparation of PSU membranes by the phase inversion process. Spectra of binary mixtures of AO with PSU, PSU—SO_3H (100%) (Fig. 3) or PSU—NH_2 (54.5%) were recorded. The UV absorption spectra of AO in NMP exhibited three bands at 280, 310 and 430 nm. The presence of PSU in various R ratios did not change the UV absorption profile spectrum of AO. This suggests that no interactions between PSU and AO were present in these conditions. For R values of 0.5; 1 and 2, similar results were obtained with PSU—SO_3H (100%) (Fig. 3) and PSU—NH_2 (54.5%). Thus, it could be assumed that the ability of these polymers to form a complex or to establish any interaction with AO was inhibited by the solvent interactions and by the dilution effect, in comparison with the solid state.

On the other hand, the UV absorption spectra of AO changed dramatically when these sulfonated or aminated PSUs were in large excess (R = 0.05) (and higher concentration). An example is shown in Fig. 3. Indeed, bathochromic as well as hyperchromic shifts were observed since the maximal wavelength (λ_{max}) and the molecular extinction coefficient (ε) of AO absorption bands increased. This result confirmed the assumption mentioned above, suggesting that concentrated media or solid state enable much better complexation of AO than in diluted media. In addition, the wavelength at 444 nm seems to be an isosbestic point between the free and complex states of AO.

The ^1H NMR study of the interaction between PSUs and AO was first performed in $CDCl_3$, which is a good solvent of PSU and PSU—SO_3H. DMSO-d6 was used as the only deuterated solvent common to PSU—SO_3H and PSU—NH_2 as well as their complexes with AO. The concentration of AO was kept constant at 37.7 mmol/L.

In $CDCl_3$, the ^1H NMR spectra of the native PSU mixed to AO, at different ratios (R = AO/PSUs = 0.5, 2), did not reveal any change in chemical shifts. Once again, this suggests an absence of interactions between the native PSU and AO. However, the addition of PSU—SO_3H (57%) to AO in $CDCl_3$ led to precipitation owing to complex formation. In DMSO-d6, the mixture of PSU—SO_3H (100%) or PSU—NH_2 with AO exhibited ^1H—NMR spectra that were dramatically different compared to those of the mixture components (Figs. 4 and 5). Indeed, one can observe that all AO signals shifted in presence of the modified PSUs. The signal shift was dependent on the nature of the interacting polymer (i.e. on acid–base, hydrogen

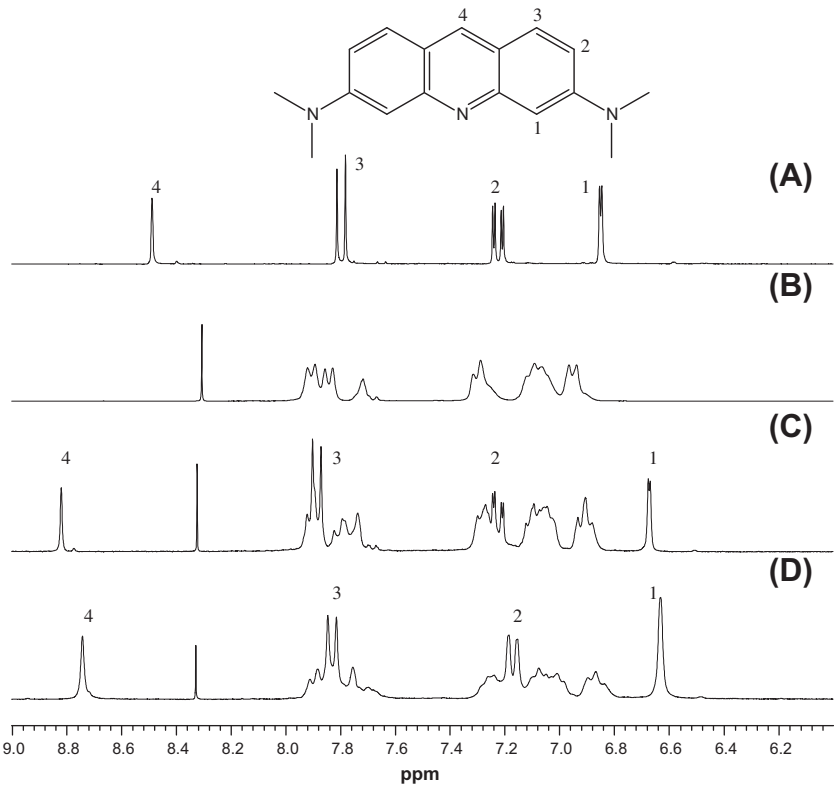


Fig. 4. ^{1}H NMR spectra of mixtures based on PSU—SO_3H (100%) and AO in DMSO-d6 ([AO] = 37.7 mmol/L) at various ratios R = AO/PSU—SO_3H (100%): (A) AO, (B) PSU—SO_3H (100%), (C) R = 0.5 and (D) R = 2.

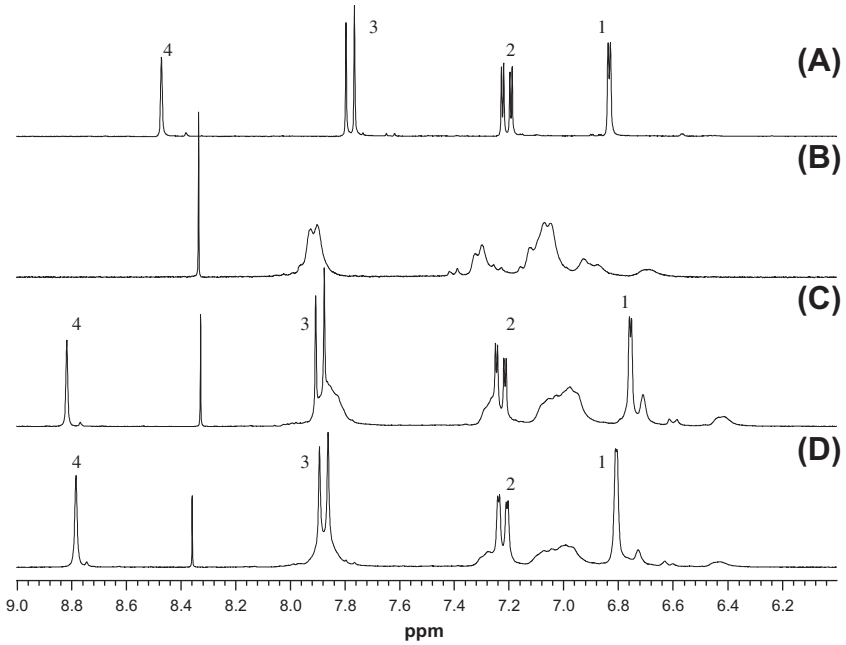


Fig. 5. ^{1}H NMR spectra of mixtures based on PSU—NH_2 (54.5%) and AO in DMSO-d6 ([AO] = 37.7 mmol/L) at various ratios R = AO/PSU—NH_2 (54.5%): (A) AO, (B) PSU—NH_2 (54.5%), (C) R = 0.5 and (D) R = 2.

and/or π staking interactions) and on the mixture composition. Actually, these parameters influence the complex geometry and its magnetic environment.

In summary, the prepared modified polysulfones exhibited strong interaction with AO template both in bulk and organic solvents under certain conditions.

3.3. First studies of the influence of complexation on the kinetics of AO release from imprinted membranes based on the prepared polysulfones

The preparation and shaping of PSU membranes is generally made by the phase inversion process, in which the PSU is

Table 3
Kinetics data of AO release from modified and unmodified PSU membranes.

Time (min)	PSU membrane		PSU—SO_3H (100%) membrane	
	Released AO (%)	Residual AO (%)	Released AO (%)	Residual AO (%)
0	4.4 ± 0.2	19.1 ± 0.1	3.6 ± 0.5	91.4 ± 0.6
2	7.3 ± 0.3	17.9 ± 0.2	3.0 ± 0.4	92.0 ± 0.6
6	9.3 ± 0.3	12.4 ± 0.1	3.2 ± 0.4	91.9 ± 0.8
30	20.2 ± 0.8	6.8 ± 0.2	2.6 ± 0.3	92.9 ± 0.5
60	21.0 ± 0.7	5.3 ± 0.3	2.6 ± 0.3	92.3 ± 0.6
120	25.6 ± 0.9	6.7 ± 0.2	3.0 ± 0.4	92.2 ± 0.4
240	29.2 ± 0.9	6.0 ± 0.2	2.4 ± 0.2	92.7 ± 0.6
300	29.4 ± 0.9	7.0 ± 0.2	2.8 ± 0.3	92.1 ± 0.8
480	29.1 ± 0.9	5.5 ± 0.3	/	/
1440	25.1 ± 0.8	5.5 ± 0.3	3.1 ± 0.4	91.9 ± 0.8

solubilized in a water miscible solvent, such as NMP, and then precipitated in water [37]. Actually, the successful preparation of MIM membranes by this process needs stability of the complex formed between the target and the membrane, especially if the target is water soluble. Sulfonated PSU being the most known and investigated polymer in the literature, membranes based on the PSU—SO_3H (100%) blended with AO in a ratio R = AO/PSU—SO_3H (100%) = 0.05 were prepared by this process. Membranes based on the native PSU and AO were also prepared in the same conditions to be used as controls. The stability of the polymer/AO complex was estimated by the quantity of AO lost in water during the phase inversion process and by the kinetics of AO release in water from the prepared membranes. Fluorimetry analysis was used to measure the quantity of AO released from the membrane and the quantity of AO remaining within the membrane, using suitable calibrations curves.

The percentage of residual AO within the membrane was calculated as follows:

$$\text{Residual AO } (\%) = (n_t/n_0) \times 100 \quad (1)$$

where n_t was the quantity of AO remaining in the membrane as a function of time; n_0 was the theoretical quantity of AO within the membrane at t = 0 min. n_0 was calculated from the initial membrane weight (m_0), the molar ratio R = AO/polymer = 0.05 and the molecular weights of AO (M_{AO}) and of the polymer repeating unit (M_{RU}), as follows:

$$n_0 = m_0/[M_{AO} + (M_{RU})/R)] \quad (2)$$

The percentage of released AO in water was calculated as follows:

$$\text{Released AO}(\%) = (C_t/C_{TR}) \times 100 \quad (3)$$

where C_t was the concentration of AO released in water versus time and C_{TR} was the theoretical concentration of totally released AO.

The percentage of AO lost during the phase inversion process was deduced as follows:

$$\%\text{ of lost AO} = 100 - (\%\text{ of released AO} + \%\text{ residual AO}) \quad (4)$$

The kinetics results are reported in Table 3. Membrane based on the native PSU exhibited a fast kinetics of AO release since the released and residual AO percentages showed a plateau after about 60 min of contact with water. Moreover, one can observe that the percentage of AO lost during the phase inversion process is in average above 65%. This result was consistent with the spectroscopic results and evidenced once again that the native PSU and AO was not in the complex state.

Independently on time contact with water, membranes based on the PSU—SO_3H (100%) showed a quasi constant percentages of released (~3%) and residual (~92%) AO. Hence, the percentage of AO lost during the phase inversion was about 5%. This result suggest a high stability in water of the complex formed between sulfonatd PSU and AO, which make this polymer suitable for MIM preparation by the phase inversion process.

4. Conclusion

In this work, precursors for designing molecular imprinting membranes (MIMs) were elaborated by chemical modifications of a native polysulfone (PSU). Sulfonyl and amine groups were introduced in various ratios on the chemical structure of PSU in order to create physical interactions – a key element for molecular recognition – between the polymer matrix and acid/base templates such as biomolecules or biomacromolecules. The Acridine Orange base (AO) was used as a fluorescent dye model template. The study of the interaction of AO with the modified and unmodified PSU was performed by fluorimetry in the solid state as well as by UV–visible and ^{1}H NMR analyses in organic solutions. No complexation between the native PSU and AO was observed. On the other hand, strong complexation of AO has been evidenced with the aminated and sulfonated PSUs. In NMP media, i.e. in the conditions of the phase inversion process, complexation occurred only when the polymer was in large excess regarding AO (R = 0.05). Thus, membranes based on the native PSU (control) or PSU—SO_3H (100%) blended with AO (R = 0.05) were prepared by the phase inversion process. kinetics studies of AO release from the membranes showed a high stability in water of the complex formed between the sulfonated PSUs and AO, during and after the phase inversion process. Finally, allyl groups were successfully introduced into the sulfonated PSUs and into the aminated PSUs to be used as crosslinking agents for an enhancement of the MIMs template memory.

Acknowledgement

Authors thank The "Ministère de l'Enseignement Supérieur et de la Recherche" for the financial support.

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