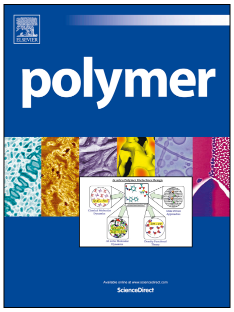

Solid fluorescence sensors obtained by functionalization of photocrosslinked water-swollen acrylic membranes with 4-piperazine naphthalimide derivatives

S. Fernández-Alonso, T. Corrales, J.L. Pablos, F. Catalina

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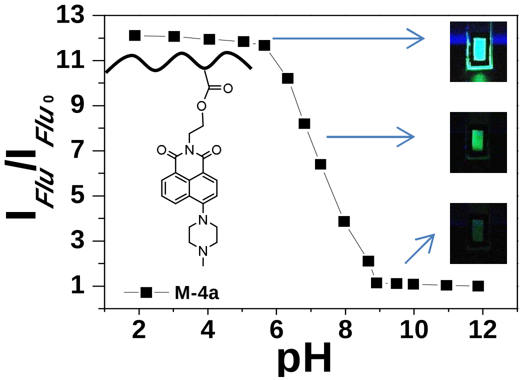
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Solid fluorescence sensors obtained by functionalization of photocrosslinked water-swollen acrylic membranes with 4-piperazine naphthalimide derivatives.

S. Fernández-Alonso, T. Corrales, J.L. Pablos, F. Catalina.*

Departamento de Química Macromolecular Aplicada, Instituto de Ciencia y Tecnología de Polímeros (CSIC). C /Juan de la Cierva, 3. 28006-Madrid, Spain.

E-mail: fcatalina@ictp.csic.es

Abstract

A photocrosslinked membrane (M-Cl) based on vinyl-pyrrolidone/butyl acrylate containing ethylene glycol dimethacrylate as a crosslinking agent and methacryloyl chloride as reactive monomer has been prepared for further functionalization. A series of hydroxy substituted piperazine naphthalimide compounds have been synthetized as pH sensitive Off-On fluorescence probes using two methods: microwave and reflux. Three of the derivatives were selected to be anchored to the M-Cl membrane through the acid chloride groups by the Schotten-Baumann reaction. All the obtained water-swollen membranes were characterized using different techniques. Photo-induced electron transfer (PET) and its pH-dependent optical changes were investigated and various photochemical parameters were determined by using pH-dependent absorption and fluorescence spectroscopies. In the pH range of 9.0-4.0, these solid sensors undergo PET process from the piperazine to the naphthalimide moiety leading to a fluorescence quenching. However, in the pH range of 4.0–1.0, the PET is inhibited to give a fluorescence enhancement. The sensitivity of the functionalized membranes to pH changes depended on the size and position of the naphthalimide substituents.

Keywords: photopolymerization, membrane functionalization, naphthalimide fluorescence probe.

1. Introduction

Polymeric membranes have been widely utilized as a support material for various types of sensors and also used in systems where the environmental information is gathered by the measurement of photons. The most commonly used method is fluorescence emission. Its applications include optical chemical [1,2] and biological [3] sensors. Hydrophilic polymer matrices are widely used for immobilization of optical indicators due to their advantages such as fast response time, low cost, flexibility and their capability for deposition into various types of substrates.

In recent years, the developments of fluorescence pH sensors have attracted considerable attention because of their potential application in environmental analytics, medical diagnostics and process control [4,5]. Compared to small organic compounds, polymer based optical sensors display several important advantages as they can be non-invasive or minimally invasive, disposable, easily miniaturized and simple to process as a membrane, coating or solid layer on adequate surfaces [6,7]. The most widely used technique amongst optical sensors is the measurement of fluorescence intensity. Among the large number of fluorescent structures that have been developed, 1,8-naphthalimide derivatives have numerous applications in a variety of different areas. They have gained increasing interest as fluorescent probes for pH [8,9], metal cations [10,11,12,13] and anions [14] because they have been recognized for excellent photophysical properties with excellent stability, visible excitation and emission exhibiting high fluorescence quantum yields and large Stokes' shift that minimize the effects of the background fluorescence.

Polymerizable naphthalimide derivatives have been copolymerized with different co-monomers such as methyl methacrylate [15] and styrene [16,17], 2-hydroxyethyl methacrylate [18] and acrylamide [19,20]. In particular alkylpiperazinyl derivatives of naphthalimides have been studied and their behavior as proton “off–on” switch sensors has been demonstrated as very sensitive [21,22] and interesting for applications [23] in complex media.

In an earlier work, we modified the surface of a film based on a copolymer of ethylene butyl acrylate with a naphthalimide derivative as an acidity sensor [24]. Even though the functionalized film was effective as an acid sensor, the hydrophobicity of the polyolefin surface modified with 4-dimethylamino-1,8-naphthalimide (water contact angle value of 92.6º) increased the time of its response to acid media.

Vinyl-pyrrolidone (VP) based materials exhibit highly interesting properties due to its unique combination of physical and chemical properties (biocompatibility, nontoxicity, chemical stability, good solubility in water and many organic solvents, affinity to both hydrophobic and hydrophilic complex substances). Also, VP copolymers with acrylic monomers such as butyl acrylate (BA) have important applications [25,26].

In this work we have prepared a photocrosslinked membrane based in vinyl-pyrrolidone/ butyl acrylate and added ethylene glycol dimethacrylate as the crosslinking agent and methacryloyl chloride as the reactive monomer for further functionalization. Through the acid chloride groups, three derivatives of naphthalimide were anchored to obtain solid fluorescence sensors. The membrane preparation and the functionalization with naphthalimides are shown in Fig. 1.

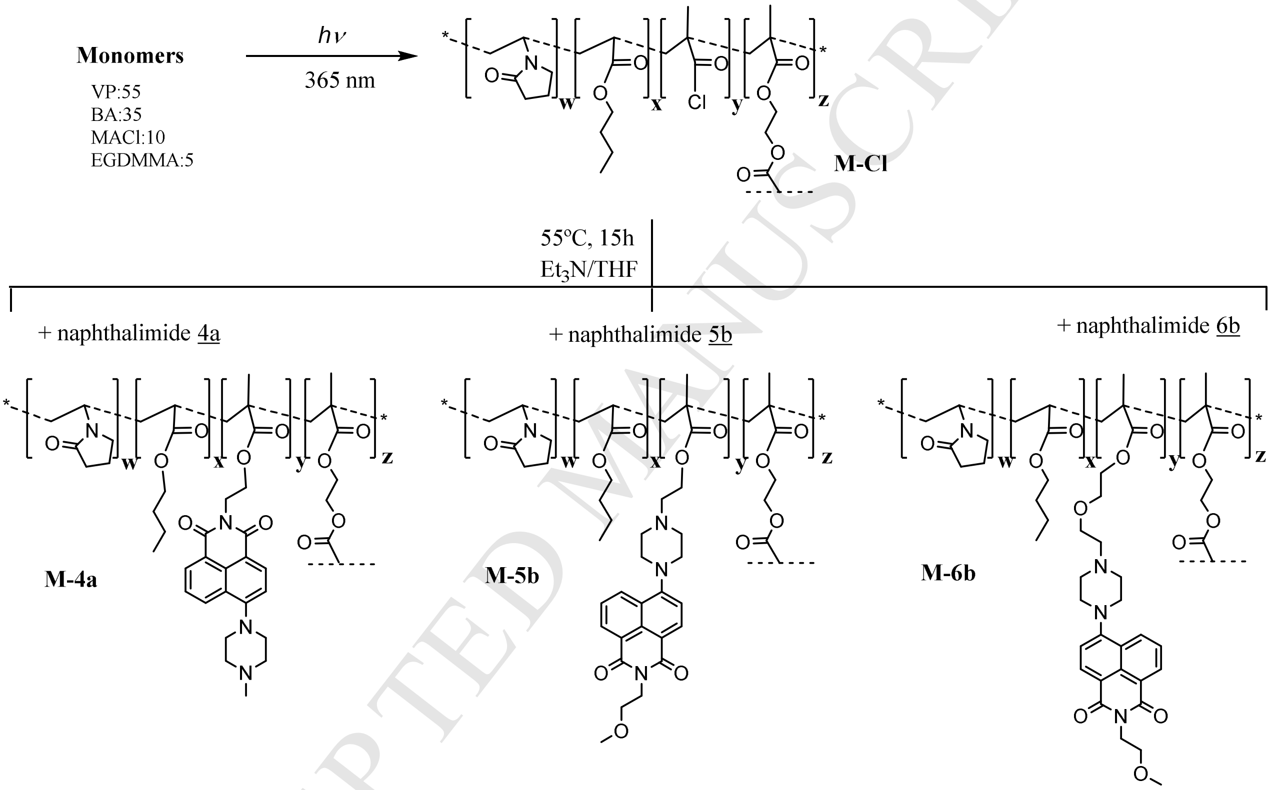


Fig.1. Synthesis of the photocrosslinked acrylic membrane containing acid chloride groups (M-Cl) and functionalization with the selected naphthalimide structures.

The naphthalimides linked to the membrane containing acid chloride (M-Cl) were selected from a series of naphthalimide derivatives previously synthetized in this work using two methods: microwave and reflux. The structures of the series (Fig. 2) are hydroxyl derivatives of piperazine 1,8-naphthalimides which have been reported to have excellent fluorescence properties [21,22]. The membranes were characterized and the spectroscopic characteristics of the naphthalimide derivatives and the membranes were studied in function of pH and of different solvent polarities using UV-vis and fluorescence spectroscopies.

2. Experimental

2.1. Materials and reagents

All materials and solvents were commercially available and used as received, unless otherwise indicated. The materials included: sodium hydroxide (Panreac, 98-100.5%), hydrochloric acid (VWR Chemicals, 37%), ultrapure MilliQ water (Millipore), triethylamine (Aldrich, ≥99%), 4-bromo-1,8-naphthalic anhydride (Aldrich, 95%), potassium carbonate (Panreac, 99%), 2-aminoethanol (Aldrich, 99%), 2-methoxyethylamine (Aldrich, 99%), piperazine (Aldrich, 99%), 1-methylpiperazine (Aldrich, 99%), 1-(2-hydroxyethyl)piperazine (Aldrich, 98%), 1-[2-(2-hydroxyethoxy)ethyl]piperazine (Aldrich, 95%) ethanol (VWR Chemicals, 99.95%), dimethylformamide (Scharlau, 99.8%), Irgacure 2959 photoinitiator (BASF), ethyl acetate (Aldrich, ≥99.5%), hexane (Carlo Erba Reagents, 99%), 2-propanol (Scharlau, 99.5%), dichloromethane (Aldrich, ≥99.9%), toluene (Merck, ≥99.9%), diethyl ether (Carlo Ebra, >99.8%), 1,4-dioxan (Panreac, 99%), tetrahydrofuran (Aldrich, 99.9%), chloroform (Scharlau, 99%), acetone (Scharlau, 99.5%), acetonitrile (Aldrich, ≥99.5%), 1-butanol (Panreac, 99.5%), methanol (Aldrich, ≥99.8%), coumarin 6 (Aldrich, 98%), dimethylsulfoxide D6 (Euriso-top, 99.8% D), chloroform-d (Aldrich, 99.8% D), diiodomethane (Aldrich, 99%).

Monomers, 1-vinyl-2-pyrrolidone (Aldrich, ≥97%), butyl acrylate (Aldrich, ≥99%) and ethylene glycol dimethacrylate (Aldrich, 98%) and methacryloyl chloride (Aldrich, ≥97%) were distilled under vacuum to remove the inhibitor before use.

2.2. Spectroscopic characterization and thermal analysis

Attenuated Total Reflectance / FT-Infrared Spectroscopy (ATR-FTIR) was used to characterize naphthalimide derivatives and membranes. ATR-FTIR spectra were obtained using a PERKIN ELMER BX-FTIR Spectrometer coupled with a MIRacle^{TM}ATR accessory, from PIKE Technologies and interferograms were obtained from 32 scans.

UV spectroscopy was employed for the quantitative determination of each hydroxyl-piperazine-naphthalimide derivative anchored to acrylic membranes in a *PERKIN ELMER Lambda 35* spectrometer. The naphthalimide content was determined through measurement of absorbance at the peak maximum of the absorption band. Assessment was made in quintuplicate for each material.

Fluorescence spectra were recorded using a Perkin Elmer LS 55 Fluorescence Spectrometer. Fluorescence emission spectra of the probe were recorded in the range 490–700 nm using as the excitation wavelength the maximum of the longest wavelength absorption band. All the spectra were corrected using the response curve of the photomultiplier. The fluorescence quantum yields (ϕ_F) were measured relatively to Coumarin 6 (ϕ_F =0.78 in ethanol) [27].

Nuclear magnetic resonance, ^{1}H- and ^{13}C- NMR spectra were recorded on a *Varian-Mercury* 400 MHz and a Bruker 300 MHz Nuclear Magnetic Resonance Spectrometers using hexadeuterated dimethyl sulfoxide (DMSO-d_6) and chloroform-d ($CDCl_{3)}$ as the solvent. Chemical shifts were reported in parts per million (ppm). ^{1}H-NMR and ^{13}C-NMR chemical shifts were referenced to DMSO-d_6 (2.5 and 39.52 ppm respectively) or $CDCl_3$ (7.25 and 77.0 ppm respectively) as standard.

Mass Spectra (*MS)* were recorded on a HP 5973-MSD spectrometer.

Differential Scanning Calorimetry (DSC) was performed on a METTLER DSC-823e instrument (30–180◦C) previously calibrated with an indium standard (Tm= 429 K, ΔHm= 25.75 Jg^{-1}). Samples (10 mg) were tightly sealed onto the DSC pans and heating or cooling at 10◦C·min^{-1} rates under nitrogen. DSC was used to measure the glass transition temperature of the acrylic membranes and the melting points of the naphthalimide derivatives. The glass transition temperature of the membranes (T_g) were determined in a second scan after erasing the previous thermal history of the material in a first scan. rate of 10 ◦C/min under nitrogen flow (30 psi).

Thermogravimetric Analysis (TGA) was carried out in a TGA Q-500 TA Instruments coupled to a Pfeiffer Vacuum ThermoStar^{TM} mass spectrometer. The weight loss was measured as a function of temperature, and the evolved gas masses were directly monitored. The heating rate for the dynamic conditions was 10 ºC·min^{-1}, and the nitrogen flow was maintained constant at 60 ml·min^{-1}. The onset degradation temperature (T_{Onset}) was defined as the initial temperature of degradation, corresponding to the intersection of the tangent drawn at the decomposition step with the horizontal zero-line of the TG curve.

Elemental analysis (EA) was carried out on a Carlo Erba EA1108 elemental analyzer (% of C, H, N).

2.3. Swelling Ratio and water contact angle measurements

Swelling degree (SD) in water and THF of the crosslinked membranes were measured by immersing dried and weighed sample films (W_d) into deionized water or THF at 30ºC for 24 h. The superficial solvent was removed and the samples were immediately weighed (W_s). The SD were calculated as follows: $SD\ (\%) = ((W_s - W_d)/W_d) \times 100$, where W_s and W_d are the weight of the swollen and dry sample, respectively.

Contact angle (CA) measurements were performed at 25 °C using a KSV instruments LTD CAM200 Tensiometer and MilliQwater as wetting solvent. Surface energy was determined using two liquids (water and methylene iodide) for the measurements. On the basis of Owens–Wendt's method [28], the surface energy (γ) and its dispersive (γ^d) and polar (γ^p) parts were calculated using the CAM 200 software.

2.4. Microwave and UV irradiation equipments

The microwave equipment used in this work was an *Anton Paar Monowave™ 300* microwave synthesis reactor provided with an infrared sensor (IR pyrometer). All reactions were performed in pressure-resistant 30-mL test tubes sealed with silicon septum and using a magnetic stirring bar.

The photopolymerization reactions were carried out in a Biolink™ BLX-365 type Bio-link apparatus (Vilbert Lourmat™).

2.5. Membrane preparation by photopolymerization (M-Cl) and functionalization with HO-piperazine-naphthalimide derivatives

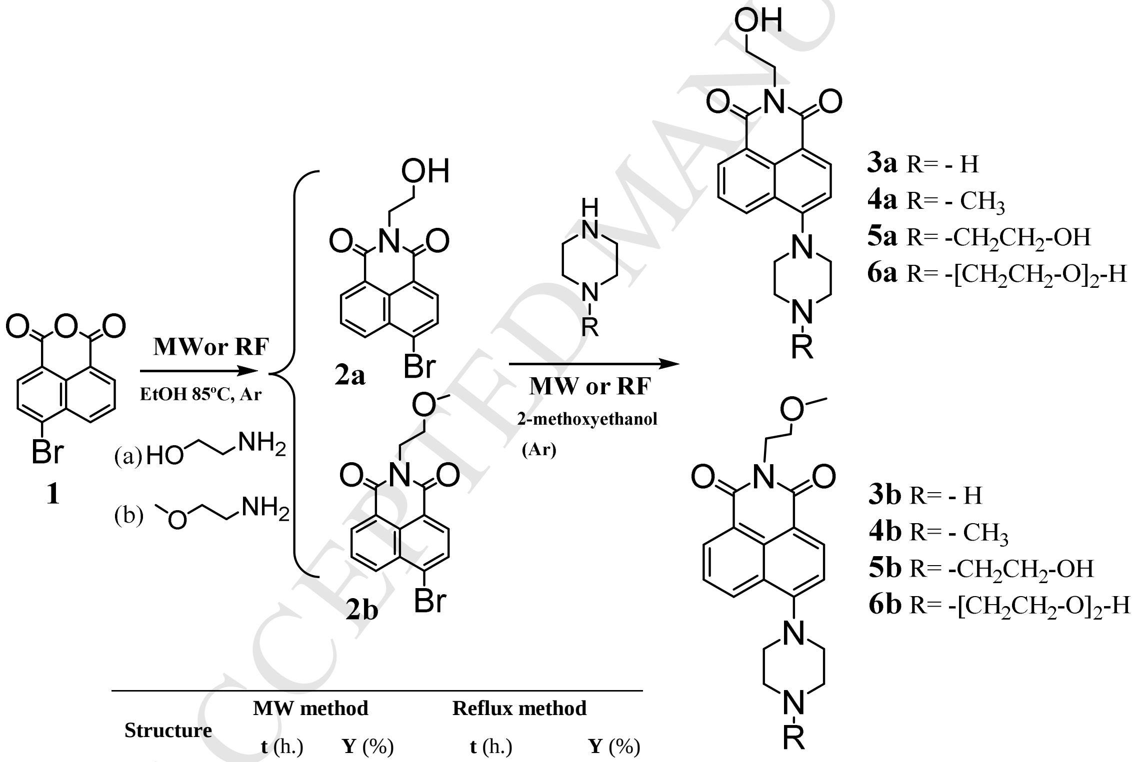
The reaction scheme for the photopolymerization reaction and the membrane functionalization with naphthalimide is shown in Figure 1.

A crosslinked membrane (M-Cl) was obtained by the bulk photopolymerization of a mixture of monomers: N-vinylpyrrolidone (VP), butyl acrylate (BA), ethylene glycol dimethacrylate (EGDMMA) as the crosslinking agent and methacryloyl chloride (MACl) as the reactive monomer. The monomer molar ratio of the feed mixture was VP:55/BA:35/ MACl:10/EGDMMA:5 and the photoinitiator (Irgacure 2959) was added to the mixture at 1% (w/w). The homogenous mixture was transferred to an ampoule, degassed by argon bubbling for 15 min and injected into a previously degassed silanized glass mould. The two glass pieces were separated by a Teflon spacer of 180 µm thick to define the reaction chamber. After 40 minutes of irradiation at 365 nm (dose 20 J cm^{-2}), the transparent crosslinked membrane (180 µm of thickness) was demoulded and conditioned (12h) at room temperature under argon atmosphere. The crosslinked membranes were then washed several times with ethanol and dried under vacuum at 40 °C to remove the un-reacted monomers. The presence of acid chloride was confirmed by ATR-FTIR (wavenumbers,cm^{-1}), $\nu_{C=O}$ acyl chloride 1795 cm^{-1}; ν_{C-Cl} 898 cm^{-1} together with the characteristic co-monomers bands of VP $\nu_{C=O}$ lactam, amide-I 1675 cm^{-1} and BA, $\nu_{C=O}$ ester 1725 cm^{-1}; ν_{C-O} (O-CH_2-) ester 1161 cm^{-1}. The quantitative determination of chlorine in the membrane (2.8%) was carried out by TGA-MS (Table 1 and Fig. 3B and C).

In a second step, the M-Cl film was cut into strips (1 × 4 cm) and functionalized in the solid state with the naphthalimide derivatives synthetized in this work (Fig 2). The procedure was as follows: the M-Cl membrane was placed in an ace round-bottom pressure flask with 20 mL of THF. 10 mg of hydroxy-piperazine naphthalimide derivative (4a, 5b or 6b) and 500 µL of triethylamine were added under argon atmosphere. The mixture was heated at 55°C for 15h. After this reaction time, the corresponding modified membranes M-4a, M-5b and M-6b were washed with cold ethanol and with water thrice in order to remove the unreacted acid chloride groups and the unreacted naphthalimide. The hydrolysis reaction of unreacted acid chloride groups was confirmed by ATR-FTIR and by TGA-MS as the characteristic peak of acid chloride was not present (Fig. 3C). After drying, the transparent functionalized membranes exhibited an intense yellow–green fluorescence and the content of naphthalimide was quantitatively determined by UV–Vis spectroscopy (Table 1). The characteristic peaks of naphthalimide structures in the membranes were not clearly observed in the ATR-FTIR spectra due to their low intensity and their overlap with the broad absorptions of the membrane functional groups.

2.6. Synthesis of the naphthalimide derivatives

Compound 2a was synthesized following the procedure (Fig. 2) described in the bibliography All the naphthalimide derivatives were synthetized using microwave (MW) and reflux (RF) methods with the same experimental conditions but differing in reaction times and yields. The RF reactions times were larger (15 h for all the reactions) and RF yields were lower than those obtained under MW irradiation. The synthesis path-ways, naphthalimide structures and reaction data are shown in Fig.2.



Structure	MW method		Reflux method	
	t (h.)	**Y** (%)	**t** (h.)	**Y** (%)
2a	2	90	15	76
2b	2	93	15	80
3a	1	84	15	64
4a	1	85	15	67
5a	1	88	15	68
6a	1	85	24	70
3b	1	84	15	69
4b	1	83	15	73
5b	1	84	15	71
6b	1	85	24	69

Fig.2. Synthesis of the naphthalimide derivatives and their corresponding reaction times and yields for microwave (MW) and reflux (RF) methods.

To synthesize the desired piperazinyl derivative of naphthalimide, two bromide derivatives of naphthalimide containing 2-hydroxyethyl (Compound 2a) and 2-methoxyethyl (Compound 2b) groups were synthesized following the procedure described in the bibliography [29, 30]. In a second step, the bromide atom was substituted by piperazinyl groups (Fig. 2) following a modified method similar to that reported by Tian et al. [19].

2.6.1. Synthesis of N-(2-hydroxyethyl)-4-bromo-1,8-naphthalimide (2a)

In a pressure-resistant microwave test tube, a mixture of 4-bromo-1,8-naphthalic anhydride (compound 1) (1.4 g, 5 mmol) and ethanol amine (0.4 g, 5 mmol) in ethanol (15 ml) was heated under argon at 85 ºC and stirred at 600 rpm for 2h. The resulting mixture was cooled at 5 °C. The solid separated was filtered and washed with cold ethanol (3 x 30 ml) and after dried was identified as N-hydroxyethyl-4-bromo-1,8-naphthalimide (compound 2a). Yield 90% (1.4 g). M.p.: 206 ± 2ºC. ^{1}H-NMR (δ_H ppm) (300 MHz, DMSO-d$_6$, Me$_4$Si): δ 8.42 (dd, *J* = 13.9, 7.9 Hz, 2H), 8.20 (d, *J* = 7.9 Hz, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.89 (t, *J* = 7.9 Hz, 1H), 4.81 (s, 1H), 4.10 (t, *J* = 6.4 Hz, 2H), 3.61 (t, *J* = 5.9 Hz, 2H). ^{13}C-NMR (δ_C ppm) (100.6 MHz, DMSO-d$_6$, Me$_4$Si): δ 162.90, 162.85, 132.41, 131.42, 131.24, 130.80, 129.61, 128.98, 128.67, 128.14, 122.70, 121.92, 57.71, 41.95. FT-IR (wavenumbers, cm^{-1}): ν_{OH} 3386 cm^{-1}; $\nu_{C\text{-}H}$ 3066 cm^{-1} aromatic stretch vibration $\nu_{C=O}$ 1692, 1658 cm^{-1}; $\nu_{N\text{-}C=O}$ 1611 cm^{-1}; $\nu_{C\text{-}C}$ 1585, 1568 cm^{-1} aromatic ring chain vibrations. EA: theoretical values: %C 52.52; %H 3.15; %N 4.38; experimental values: %C 52.42; %H 3.25; %N 4.25. EI-MS m/z calculated for $C_{14}H_{10}BrNO_3$ $(M+H)^+$ 321.0; found 321.0.

2.6.2. Synthesis of N-(2-methoxyethyl)-4-bromo-1,8-naphthalimide (2b)

Compound 2b was synthetized following the same procedure described for compound 2a from but using 2-Methoxyethylamine (0.38 g, 5 mmol) as a reactive amine. Yield

93% (1.55 g). M.p.: 163.57 ± 2ºC. ^{1}H-NMR (δ_H ppm) (300 MHz, $CDCl_3$, Me_4Si): δ 8.62 (d, *J* = 7.3 Hz, 1H), 8.52 (d, *J* = 8.4 Hz, 1H), 8.38 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 7.9 Hz, 1H), 7.85 – 7.77 (m, 1H), 4.42 (t, *J* = 5.8 Hz, 2H), 3.72 (t, *J* = 5.8 Hz, 2H), 3.37 (s, 3H). ^{13}C-NMR (δ_C ppm) (100.6 MHz, $CDCl_3$, Me_4Si): δ 163.80, 163.78, 133.38, 132.26, 131.43, 131.18, 130.68, 130.40, 129.12, 128.16, 123.09, 122.23, 69.68, 58.92, 39.50. FT-IR (wavenumbers, cm^{-1}): $\nu_{C\text{-}H}$ 3065 cm^{-1} aromatic stretch vibration; $\nu_{O\text{-}CH3}$ 2824,2832 cm^{-1}; $\nu_{C=O}$ 1698, 1655 cm^{-1}; $\nu_{N\text{-}C=O}$ 1613 cm^{-1}; $\nu_{C\text{-}C}$ 1589, 1570 cm^{-1} aromatic ring chain vibrations. EA: theoretical values: %C 53.91; %H 3.62; % N 4.19; experimental values % C 53.82; % H 3.65; % N 4.22. EI-MS m/z calculated for $C_{15}H_{12}BrNO_3$ $(M+H)^+$ 335.0; found 335.0.

Synthesis of compounds 2a and 2b were also carried out in an ace round-bottom pressure flask and conventional heating during 15 h yielded 75 and 80 % respectively.

2.6.3. Synthesis of N-(2-hydroxyethyl)-4-(piperazine-1-yl)-1,8-naphthalimide (3a)

In a pressure-resistant microwave test tube, compound 2a (0.20 g, 0.625 mmol) and piperazine (0.16 g, 1.875 mmol) were mixed in 2-methoxyethanol (5 mL) and heated at 130 ºC in argon atmosphere under constant stirring (600 rpm) for 1 h. The mixture was poured into cooled ice/water at 5 °C and the residue was filtered and washed with hot ethanol (30 mL). The solution was filtered and the solvent removed under reduced pressure in order to obtain the yellow solid compound. M.p.: 219.23 ± 2ºC. MW-yield: 84%. RF method for compound 3a yielded a 64% after 15 h of reaction time. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 – 8.41 (m, 2H), 8.38 (d, *J* = 8.1 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 4.78 (s, 1H), 4.13 (t, *J* = 6.4 Hz, 2H), 3.60 (s, 2H), 3.13 (s, 4H), 3.00 (s, 4H). ^{13}C NMR (75 MHz, DMSO) δ 163.65, 163.11, 156.20, 132.14, 130.48, 129.15, 125.78, 125.22, 122.59, 115.33, 114.79, 57.86, 54.10, 45.67, 41.60. FT-IR (wavenumbers, cm^{-1}): ν_{NH} 3248 cm^{-1}; $\nu_{C\text{-}H}$ 3066 cm^{-1} aromatic stretch vibration; $\nu_{C=O}$ 1685, 1642 cm^{-1}; $\nu_{N\text{-}C=O}$ 1613 cm^{-1}; $\nu_{C\text{-}C}$ 1586, 1571 cm^{-1} aromatic ring chain vibrations; ν_{NH} 1514 cm^{-1} flexion. EA: theoretical values: %C 66.45; %H 5.89; %N 12.91; experimental values: %C 66.55; %H 5.93; %N 12.96. EI-MS m/z calculated for $C_{18}H_{19}N_3O_3$: $(M+H)^+$ 326.1, found. 326.0 (M^+).

2.6.4. Synthesis of N-(2-hydroxyethyl)-4-(4-methylpiperazine-1-yl)-1,8-naphthalimide (4a)

The synthesis of compound 4a was similar to the preparation procedure of the compound 3a but using 1-methylpiperazine (208 µL, 1.875 mmol) as a reactive amine. The MW yield was 85% after 1 h and RF yield 67% after 15h. Compound 4a was purified by silica gel column chromatography using dichloromethane:methanol (20:1) as an eluent to obtain a yellow solid. M.p.: 188.43 ± 2ºC. Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 (dd, *J* = 11.9, 8.1 Hz, 2H), 8.37 (d, *J* = 8.2 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 4.81 (t, *J* = 5.7 Hz, 1H), 4.12 (t, *J* = 6.4 Hz, 2H), 3.59 (d, *J* = 6.2 Hz, 2H), 3.22 (s, 4H), 2.63 (s, 4H), 2.30 (s, 3H).^{13}C NMR (75 MHz, DMSO) δ 163.61, 163.07, 155.51, 132.04, 130.48, 130.31, 129.08, 125.87, 125.20, 122.59, 115.56, 114.93, 57.85, 54.63, 52.50, 45.74, 41.60. FT-IR (wavenumbers, cm^{-1}): ν_{OH} 3435 cm^{-1}; $\nu_{C\text{-}H}$ 3060 cm^{-1} aromatic stretch vibration; $\nu_{C=O}$ 1692, 1635 cm^{-1}; $\nu_{N\text{-}C=O}$ 1612 cm^{-1}; $\nu_{C\text{-}C}$ 1585 cm^{-1} aromatic ring chain vibrations. Elemental analysis, theoretical value %C 67.24; %H 6.24; %N 12.38; calculate %C 67.15; %H 6.15; %N 12.29. EI-MS m/z calculated for $C_{19}H_{21}N_3O_3$: $(M+H)^+$ 340.2, found. 340.3 (M^+).

2.6.5. Synthesis of N-(2-hydroxyethyl)-4-(4-(2-hydroxyethyl)-piperazine-1-yl)-1,8-naphthalimide (5a)

The synthesis of compound 5a was similar to the preparation procedure of the compound 3a but using 1-(2-Hydroxyethyl)piperazine (230 µL, 1.875 mmol) as a reactive amine. Compound 5a was purified by silica gel column chromatography using dichloromethane:methanol (20:1) as an eluent to obtain an yellow solid. M.p.: 178.36 ± 2ºC. The MW yield was 88% after 1 h and RF yield 68% after 15h. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.49 – 8.41 (m, 2H), 8.39 (d, *J* = 8.1 Hz, 1H), 7.84 – 7.76 (m, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 4.81 (t, *J* = 5.9 Hz, 1H), 4.49 (t, *J* = 5.3 Hz, 1H), 4.13 (t, *J* = 6.5 Hz, 2H), 3.65 – 3.51 (m, 6H), 3.23 (s, 4H), 2.74 (s, 4H). ^{13}C NMR (75 MHz, DMSO) δ 163.94, 163.41, 155.88, 132.38, 130.82, 130.67, 129.42, 126.19, 125.51, 122.92, 115.85, 115.17, 60.57, 58.95, 58.19, 53.50, 53.00, 41.94. FT-IR (wavenumbers, cm^{-1}): ν_{OH} 3260, 3146 cm^{-1}; $\nu_{C\text{-}H}$ 3052 cm^{-1} aromatic stretch vibration; $\nu_{C=O}$ 1695, 1644 cm^{-1}; $\nu_{N\text{-}C=O}$ 1613 cm^{-1}; $\nu_{C\text{-}C}$ 1589, 1576 cm^{-1} aromatic ring chain vibrations. EA: theoretical values %C 65.03; %H 6.28; %N 11.37; experimental values: %C 65.00; %H

6.25; %N 11.31. EI-MS m/z calculated for $C_{20}H_{23}N_3O_4$: $(M+H)^+$ 370.17, found 370.2 (M^+).

2.6.6. Synthesis of N-(2-hydroxyethyl)-4-(4-[2-(2-hydroxyethoxy)ethyl]-piperazine-1-yl)-1,8-naphthalimide (6a)

The synthesis of compound 6a was similar to the preparation procedure of the compound 3a but using 1-[2-(2-hydroxyethoxy)ethyl]piperazine (308 µL, 1.875 mmol) as a reactive amine. After completion of the reaction, the solvent was evaporated under reduced pressure and the crude product was then diluted in 50 ml of water. The precipitated product was then filtered off. The aqueous part was extracted with dichloromethane (3 x 30 mL) and the organic layer was dried over K_2CO_3. The solvent was evaporated under reduced pressure to obtain a yellow solid. M.p.: 170.20± 2ºC. For compound 6a the MW yield was 85% after 1 h and RF yield 70% after 24h. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 – 8.39 (m, 2H), 8.37 (d, *J* = 8.1 Hz, 1H), 7.79 (t, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 4.79 (t, *J* = 5.8 Hz, 1H), 4.63 (t, *J* = 5.0 Hz, 1H), 4.13 (t, *J* = 6.5 Hz, 2H), 3.62 – 3.54 (m, 4H), 3.54 – 3.48 (m, 2H), 3.45 (d, *J* = 4.6 Hz, 2H), 3.22 (s, 4H), 2.75 (s, 4H), 2.65 – 2.58 (m, 2H).^{13}C NMR (75 MHz, DMSO) δ 163.63, 163.09, 155.52, 132.07, 130.51, 130.35, 129.08, 125.88, 125.18, 122.58, 115.53, 114.88, 72.29, 68.31, 60.32, 57.88, 57.28, 53.15, 52.64, 41.62. FT-IR (wavenumbers, cm^{-1}): ν_{OH} 3374 cm^{-1}; ν_{C-H} 3066 cm^{-1} aromatic stretch vibration; $\nu_{C=O}$ 1690, 1650 cm^{-1}; $\nu_{N-C=O}$ 1616 cm^{-1}; ν_{C-C} 1588 cm^{-1} aromatic ring chain vibrations. EA: theoretical values %C 63.91; %H 6.58; %N 10.16; experimental values: %C 63.97; %H 6.62; %N 9.60. EI-MS m/z calculated for $C_{22}H_{27}N_3O_5$: $(M+H)^+$ 414.20, found 414.2 (M^+).

2.6.7. Synthesis of N-(2-methoxyethyl)-4-(piperazine-1-yl)-1,8-naphthalimide (3b)

Compound 2b (0.20 g, 0.598 mmol) and piperazine (0.155 g, 1.794 mmol) were mixed in 2-methoxyethanol (5 mL) and heated under MW radiation at 130 ºC in an argon atmosphere under constant stirring (600 rpm) for 1 h. After completion of the reaction, the solvent was evaporated under reduced pressure and the crude was washed with water and filtered to obtain the compound as a yellow solid. M.p.: 145.60 ± 2º The MW yield was 84% after 1 h and by conventional heating, RF yield 69% after 15h. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.48 – 8.41 (m, 2H), 8.40 (s, 1H), 7.83 – 7.75 (m, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 4.23 (t, *J* = 6.1 Hz, 2H), 3.57 (t, *J* = 6.1 Hz, 2H), 3.26 (s, 3H), 3.15 (s, 4H), 3.00 (s, 4H). ^{13}C NMR (75 MHz, DMSO) δ 163.50, 162.94, 156.29, 132.25, 130.58, 129.09, 125.75, 125.17, 122.33, 115.00, 114.77, 68.67, 57.94, 54.08, 45.66, 38.33. FT-IR (wavenumbers, cm^{-1}): ν_{NH} 3248 cm^{-1}; $\nu_{C\text{-}H}$ 3069 cm^{-1} aromatic stretch vibration; $\nu_{O\text{-}CH3}$ 2828, 2808 cm^{-1} $\nu_{C=O}$ 1690, 1650 cm^{-1}; $\nu_{N\text{-}C=O}$ 1613 cm^{-1}; $\nu_{C\text{-}C}$ 1588, 1572 cm^{-1} aromatic ring chain vibrations; ν_{NH} 1514 cm^{-1} flexion. EA: theoretical values %C 67.24; %H 6.24; %N 12.38; experimental values: %C 67.20; %H 6.18; %N 12.31. EI-MS m/z calculated for $C_{19}H_{21}N_3O_3$: $(M+H)^+$ 340.1, found. 340.0 (M^+).

2.6.8. Synthesis of N-(2-methoxyethyl)-4-(4-methylpiperazine-1-yl)-1,8-naphthalimide (4b)

Compound 4b was synthetized by mixing compound 2b (0.2 g, 0.598 mmol), 1-methylpiperazine (200 µL, 1.7964 mmol) in 5 mL of 2-methoxyethanol and following the same procedure as for compound 3b. M.p.: 139.44 ± 2ºC. The MW yield was 83% after 1 h and by conventional heating, RF yield 73% after 15h. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (dd, *J* = 12.3, 8.0 Hz, 2H), 8.36 (d, *J* = 8.1 Hz, 1H), 7.83 – 7.73 (m, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 4.22 (t, *J* = 6.1 Hz, 2H), 3.57 (t, *J* = 6.1 Hz, 2H), 3.25 (s, 3H), 3.22 (s, 4H), 2.63 (s, 4H), 2.30 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 163.51, 162.97, 155.66, 132.21, 130.64, 130.51, 129.08, 125.93, 125.21, 122.40, 115.29, 114.99, 68.67, 57.94, 52.49, 45.73, 38.36. FT-IR (wavenumbers, cm^{-1}): $\nu_{C\text{-}H}$ 3067 cm^{-1} aromatic stretch vibration; $\nu_{C=O}$ 1695, 1650 cm^{-1}; $\nu_{N\text{-}C=O}$ 1613 cm^{-1}; $\nu_{C\text{-}C}$ 1586, 1574 cm^{-1} aromatic ring chain vibrations. EA: theoretical values %C 67.97; %H 6.56; %N 11.89; experimental values %C 67.87; %H 6.56; %N 11.80. EI-MS m/z calculated for $C_{20}H_{23}N_3O_3$: $(M+H)^+$ 354.17, found 354.2 (M^+).

2.6.9. Synthesis of N-(2-methoxyethyl)-4-(4-(2-hydroxyethyl)-piperazine-1-yl)-1,8-naphthalimide (5b)

The synthesis of compound 5b was similar the preparation procedure of the compound 3b but using 1-(2-Hydroxyethyl)piperazine (220 µL, 1.796 mmol) as a reactive amine. Compound 5b was purified by silica gel column chromatography using dichloromethane:methanol (20:1) as an eluent to obtain an yellow solid. M.p.: 124.17 ± 2ºC. Yield: 84%.^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.57 (d, *J* = 7.2 Hz, 1H), 8.50

(d, *J* = 8.0 Hz, 1H), 8.39 (d, *J* = 8.4 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 4.41 (t, *J* = 5.6 Hz, 2H), 3.71 (t, *J* = 5.3 Hz, 4H), 3.36 (s, 3H), 3.30 (s, 4H), 2.84 (s, 4H), 2.75 – 2.65 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.94, 164.45, 156.25, 133.07, 131.63, 130.66, 130.32, 126.53, 126.05, 123.55, 117.09, 115.33, 70.09, 59.83, 59.18, 58.31, 53.48, 53.41, 39.49. FT-IR (wavenumbers, cm^{-1}): ν_{OH} 3140 cm^{-1} ; $\nu_{C\text{-}H}$ 3052 cm^{-1} aromatic stretch vibration; $\nu_{C=O}$ 1696, 1645 cm^{-1}; $\nu_{N\text{-}C=O}$ 1613 cm^{-1}; $\nu_{C\text{-}C}$ 1589, 1576 cm^{-1} aromatic ring chain vibrations. Elemental analysis, theoretical value %C 65.78; %H 6.57; %N 10.96; calculate %C 65.87; %H 6.68; %N 11.04. EI-MS: m/z calculated for $C_{21}H_{25}N_3O_4$ $(M+H)^+$ 384.18; found 384.2 (M^+).

2.6.10. Synthesis of N-(2-methoxyethyl)-4-(4-[2-(2-Hydroxyethoxy)ethyl]-piperazine-1-yl)-1,8-naphthalimide (6b)

Compound 2b (0.2 g, 0.5988 mmol), 2-[2-(1-Piperazinyl)ethoxy]ethanol (295µL, 1.7964 mmol) was mixed in 5 mL of 2-methoxyethanol and heated at 130 °C in argon atmosphere. The mixture was stirred at 600 rpm for 1 h. After completion of the reaction, the solvent was evaporated under reduced pressure and the crude product was then diluted in 50 ml of water. The precipitated product was then filtered off. The aqueous part was extracted with dichloromethane (3 x 30 mL) and the organic layer was dried over K_2CO_3. The solvent was evaporated under reduced pressure to obtain a yellow solid. M.p.: 219.73 ± 2ºC. The MW yield was 85% after 1 h and by conventional heating, RF yield 69 % after 24 h. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.43 (dd, *J* = 11.0, 8.0 Hz, 2H), 8.37 (d, *J* = 8.1 Hz, 1H), 7.82 – 7.75 (m, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 4.64 (s, 1H), 4.22 (t, *J* = 6.1 Hz, 2H), 3.58 (q, *J* = 6.0 Hz, 4H), 3.53 – 3.48 (m, 2H), 3.45 (d, *J* = 4.7 Hz, 2H), 3.25 (s, 3H), 3.22 (s, 4H), 2.75 (s, 4H), 2.62 (t, *J* = 5.8 Hz, 2H).^{13}C NMR (75 MHz, DMSO) δ 163.54, 162.99, 155.68, 132.24, 130.67, 130.55, 129.08, 125.94, 125.20, 122.39, 115.27, 114.94, 72.28, 68.70, 68.31, 60.30, 57.97, 57.26, 53.13, 52.64, 38.39. FT-IR (wavenumbers, cm^{-1}): $\nu_{O\text{-}H}$ 3356 cm^{-1}; $\nu_{C\text{-}H}$ 3065 cm^{-1} aromatic stretch vibration; $\nu_{C=O}$ 1687, 1648 cm^{-1}; $\nu_{N\text{-}C=O}$ 1613 cm^{-1}; $\nu_{C\text{-}C}$ 1585 cm^{-1} aromatic ring chain vibrations. EA: theoretical value %C 64.62; %H 6.84; %N 9.83; experimental values %C 64.60; %H 6.93; %N 9.76. EI-MS: m/z calculated for $C_{23}H_{29}N_3O_5$ $(M+H)^+$ 428.2; found 428.3 (M^+).

2.7. pH measurements

Measurements of pH were obtained with a Mettler Toledo SevenGo Duo Pro meter with an InLab Expert Pro ISM-ID67 electrode at room temperature (23ºC) and previously calibrated with standard buffers. The sensing measurements determined from the solutions of naphthalimide derivatives and functionalized membranes were performed using HCl and NaOH solutions for pH variation in the mixture water:ethanol (4:1) as solvent.

The acid titration by UV/Vis and fluorescence in aqueous solution was performed as follows. The titration in solution with naphthalimide derivatives (water:ethanol 4:1, naphthalimide concentration 10^{-4} M and pH=12.0 fixed by addition of NaOH solution) was carried out by increasing the acidity from pH 12.0 to pH 2.0 by adding aliquots of the diluted hydrochloric acid. After each addition, the pH was measured after the solutions were allowed to equilibrate for 10 min and the UV/Vis and fluorescence spectra were recorded.

In the case of functionalized crosslinked membranes, films were cut into strips of 1x4 cm and fixed in a homemade support that can also be slotted into the cell holder of the spectrophotometers. To study the effect of the pH in the spectroscopic properties of the membranes, film strips were immersed in previously prepared vials (50 mL) containing solutions from pH 12.0 to pH 2.0. The UV/Vis and fluorescence spectra for each pH were taken after 20 min. of conditioning time.

3. Results and discussion

3.1. Synthesis of naphthalimide derivatives

The synthesis route [3,4] to obtain N-hydroxy alkyl piperazine naphthalimides is presented in Fig. 2. In the first step, the condensation of 4-bromo-1,8-naphthalic anhydride with 2-hydroxyethylamine (series a) or 2-methoxyethylamine (series b) under microwave irradiation in ethanol at 85 °C afforded 2a and 2b respectively in high yield at a shorter reaction time than conventional conditions.

In the second reaction step (Fig. 2), compounds 2a and 2b reacted with 3 equiv. of each piperazine derivative as detailed in the experimental description (section 2.6.) to obtain the piperazine-naphthalimides derivatives shown in Fig. 2. Hence, we have prepared piperazine substituted derivatives with hydrogen and methyl groups [19] as substituents on the amine groups (structures 3 and 4 respectively), but also with bigger substituents (structures 5 and 6) in order to study comparatively their PET processes and their protonation in acid media.

The best synthesis conditions were noted under MW irradiation for 1 h at 850W and a ceiling temperature of 130 °C in 2-methoxyethanol. In contrast, the best conditions for the synthesis carried out under reflux by conventional heating required reaction times of 15 h and 24 h for compounds 6a and 6b when 2-[2-(1-Piperazinyl)ethoxy]ethanol was used as reactive amine.

In all the cases, MW irradiation allowed higher yields at shorter reaction times than the conventional RF method. These results are in agreement with those reported in the literature [24,19].

3.2. Membrane preparation, functionalization with piperazine naphthalimides sensors and characterization

The membrane prepared containing acid chloride functionalities (M-Cl) as a base material for further functionalization was obtained by bulk photopolymerization of a mixture of monomers combining the hydrophilic character of VP (55%) with the hydrophobic nature of BA (35%) and adding MACl (10%) as the reactive monomer for functionalization. A 5% of EGDMMA as the crosslinking agent of the mixture controlled the crosslinking degree of the final membrane. This produced a reversible water-swollen membrane (Fig. 1). After the bulk photopolymerization, all the experiments reached >98 % of conversion (determined by weight differences), and the resulting crosslinked acrylic membrane contained 2.8% of chlorine in the structure (Fig. 3B).

In a second step, the M-Cl membrane was functionalized with the three naphthalimide derivatives from the series synthetized in this work (Fig. 1) that contained mono hydroxy alkyl piperazine, derivatives 4a, 5b and 6b. This functionalization was accomplished successfully by conventional heating in THF at 55ºC for 15h under very low stirring speed. This selected reaction temperature is 10°C below the THF boiling point to be sure there isn't any damage of the membrane in the heterogeneous reaction, such as breaks, wrinkles and warps that could decrease the optical quality of the membrane. Under these experimental conditions, the Schotten-Baumann reaction used to graft the naphthalimide derivatives to the M-Cl membrane proceeded very slowly. This allowed a good control in homogeneity of functionalization without physically damaging the membrane. The unreacted acid chloride group disappeared after hydrolysis when the membranes were washed as shown in Fig 3C. The amount of naphthalimide grafted to the membranes (Table 1) was determined from the UV-Vis absorbance using the absorption coefficient of each derivative (Table 3) previously calculated in water-ethanol (4:1, v/v).

Table 1. Content of chlorine and naphthalimide derivative in the membranes and thermal properties determined by DSC and TGA.

Membrane	**Cl Content**[(a)] (%)	**Naphthalimide Content**[(b)] (mol·L^{-1}) x10^3	T_g [(c)] (ºC)	T_5 [(d)] (°C)	T_{10} [(d)] (°C)
M-Cl	2.8	-	46.9	214	337
M-4a	0	2.73	45.3	326	369
M-5b	0	3.27	45.4	337	371
M-6b	0	4.72	45.6	334	372

[a] Determined by TGA peak at 175ºC corresponding to Cl, EI-MS m/z 36 (M^+); [b] Determined from the membrane (180 microns of thickness) by UV spectroscopy; [c] Measured by DSC second heating run at a heating rate of 10ºC·min^{-1}; [d] Temperatures corresponding to 5% and 10% weight loss.

After 15 hours of reaction time the content of the naphthalimide derivative in the membrane was very low (Table 1) but the concentration is adequate for sensing applications by UV-vis and fluorescence spectroscopies.

The good physical characteristics of the functionalized membranes are similar than those observed by other authors [25] with copolymers of VP/BA. All DSC thermograms of the membranes revealed the presence of a single glass transition temperature for all the membranes confirming copolymer miscibility. The characteristic T_g values for the membranes are shown in Table 1 and the DSC thermograms (second scan) obtained for

M-Cl and M-5b samples are plotted in Fig. 3A. The observed step for M-Cl appeared at a similar temperature (46.9ºC) than the corresponding steps for the functionalized membranes (Table 1) due to the low content of C-Cl dipoles present in the M-Cl membrane.

The thermal stability of the membranes was evaluated using TGA and Table 1 shows the thermal data, in terms of characteristic weight loss (T_5 and T_{10} respectively).

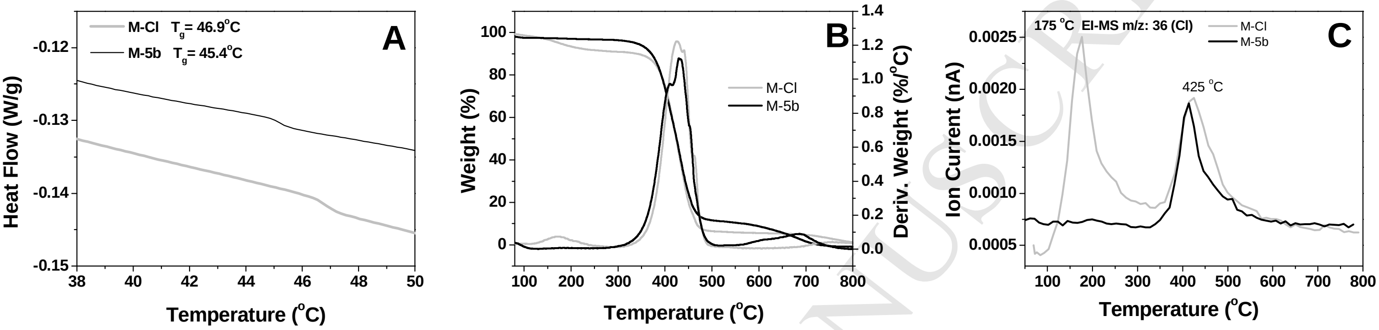


Fig.3. DSC thermograms (A), TGA and DTGA curves (B) and evolved gas analysis by MS detector (C) of the M-Cl crosslinked membrane and M-5b membrane functionalized with naphthalimide derivative 5b.

Figure 3B shows the TGA thermograms obtained for the M-Cl and M-5b membranes and their corresponding first derivative curves. The M-Cl membrane exhibits a weight loss of 2.8% that was identified by MS analysis of evolved gas at 175ºC as chlorine (EI-MS m/z: 36 (Cl)), as it is shown in fig 3C. After functionalization, the chlorine weight was not observed confirming the total hydrolysis of unreacted acid chloride. For all functionalized membranes two peaks were observed, as it is shown for M-5b in Fig. 3B. The highest weight loss rate that was observed at 410 °C corresponds to the degradation of acrylic components of the membrane and the second one observed at 431 °C has been attributed [7] to the lactam subgroup degradation.

The swelling properties of the membranes were examined by measuring the swelling (%) after immersion in water and THF and are detailed in Table 2.

Table 2. Swelling degree (SD) in water and THF, advancing contact angle (CA) and total (γ), polar (γ^p), and disperse (γ^d) surface energy data for the crosslinked membranes.

Membrane	SD (%)[a]		Static CA (°)		Surface Energy ($mN.m^{-1}$)[b]		
	Water	THF	Water	Diiodomethane	γ	γ^p	γ^d
M-Cl	29.8	45.4	59.9	41.6	51.64	13.48	38.16
M-4a	29.9	61.2	69.6	42	45.43	9.25	36.18
M-5b	30.4	62.0	71.7	38.6	45.75	6.56	39.19
M-6b	29.9	63.5	71	46.1	46.22	6.88	39.34

[a]±0.3; [b] Calculated using Owens-Wendt´s method

The SD in water (approx. 30 %) and in THF (approx. 60 %) confirms the hydrophilic character of the membranes and the adequate swelling for solid sensors in water and organic media. Also, the hydrophilicity of the membrane surfaces was characterized by static contact angle determination. The measured CAs were then used to calculate the polar, disperse, and total surface energies of the membranes. The change in the total surface energy (γ) and its polar (γ^p) and dispersive (γ^d) components of the M-Cl and functionalized membranes with naphthalimides (M-4a, M-5b and M-6b) was calculated by the Owens–Wendt's method [28] (Table 2). The variation in the values of the total, polar, and disperse solid surface energies before (M-Cl) and after functionalization (M-4a, M-5b and M-6b), shows that the total surface energy of the functionalized membranes decreases from 51.64 $mN \cdot m^{-1}$ for M-Cl to 45-46 $mN \cdot m^{-1}$ for the membranes containing naphthalimide moieties and, particularly for the polar component.

The functionalized membranes with piperazine-naphthalimide groups were easily handled materials and exhibited convenient properties for solid sensor applications, such as swelling in water, thermal stability, flexibility, optical transparency and dimensional stability to be reused. The obtained three-dimensional cross-linked hydrophilic polymer networks are capable of swelling or de-swelling reversibly in water and retaining a controlled and reproducible volume of liquid in the swollen state. This behaviour, as will be seen in the next section, allowed controllable responses to external environmental changes of pH.

3.3. Effect of pH on the absorption and fluorescence properties of piperazine naphthalimides derivatives and membranes

Piperazine substituted naphthalimides such as the structures synthetized in this work (Fig. 2) exhibit interesting fluorescence properties, hence 4-amino-1,8-naphthalimide fluorophore can be quenched by the PET process that occurs from the alkylated amine donor to the 4-amino-1,8-naphthalimide fluorophore through the piperazinyl ring. The fluorescence of the 4-amino-1,8-naphthalimide fluorophore is quenched. The PET path can be switched off either by the protonation or quaternization of the amine and the fluorescence of the naphthalimide fluorophore is then recovered as is shown for the derivative 4a anchored to the membranes in figure 4.

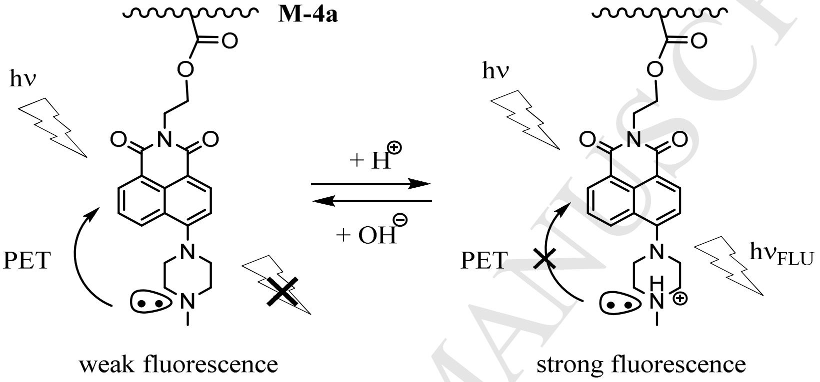


Fig.4. Fluorescence response of piperazine-naphthalimide structure to pH though PET mechanism.

The effect of pH variation on the absorbance and fluorescence properties of these derivatives is illustrated in figure 5 for the naphthalimide derivative 4a in solution and the corresponding membrane M-4a under the same conditions.

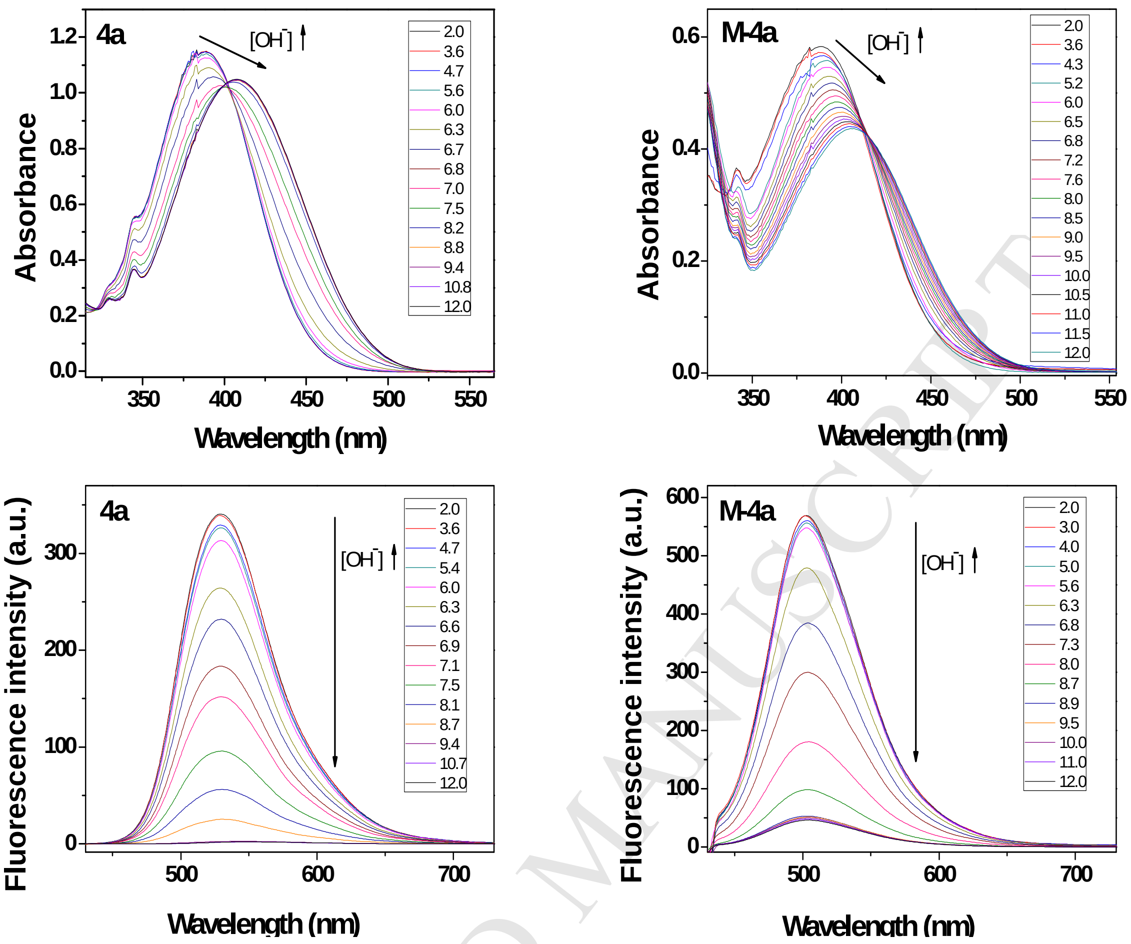


Fig. 5. Spectroscopic characteristics as a function of pH (2.0-12.0) of absorption and fluorescence of naphthalimide derivative 4a and M-4a membrane.

All the absorption spectra of the naphthalimide derivatives and membranes exhibit (as solution or as 180μm-membrane) similar band shapes in the absorption range of 350-500 nm. As it is shown in figure 5, for the compound 4a and membrane M-4a, their absorption peaks were red-shifted from 388 to 408 nm with a clear isosbestic point at 402 nm when pH values were changed from 2.0 to 12.0 in water:ethanol (4:1) by adjusting pH values with NaOH and HCl aqueous solutions. As expected, the fluorescence intensities originating from 4a and M-4a fluorophores were varied upon different pH values (Figure 5), resulting in a strong enhancement in acid media due to the protonation of the piperazine and its subsequent PET blocking effect (Fig. 4). In the case of compound 4a the factor of enhancement (FE) was about 25 times in fluorescence intensity when adjusting the pH from 12.0 to 2.0 (Table 3). Moreover, the wavelength emission maxima were red-shifted by around 20 nm (Table 3) by changing the pH values from 2.0 to 12.0 for all the naphthalimide derivatives in solution except for naphthalimide 3b and 4b that do not have hydroxyl substituents in their structures and the red-shift for them was about 2-3 nm. For the naphthalimide fluorophores bonded to the membranes though the hydroxyl groups, the bathochromic shift of the fluorescence maxima with pH was also 2-3 nm.

All the spectral data measured as a function of pH values for the naphthalimide derivatives and the three functionalized membranes are summarized in Table 3.

Table 3. Various parameters for piperazine-1,8-naphthalimide derivatives (a and b structures) in solution$^{(a)}$ and bonded to the polymer membrane (M) determined according to pH dependence by UV–Vis absorption and fluorescence spectroscopy.

Parameter	**3a**	**4a**	**5a**	**6a**	**3b**	**4b**	**5b**	**6b**	**M-4a**	**M-5b**	**M-6b**
$\lambda_{ABS\text{-}acid}$	389	388	388	388	389	389	389	388	388	388	390
$Log\ \varepsilon_{acid}$ $^{(b)}$	4.07	4.06	4.05	4.06	4.06	4.06	4.056	4.06	-	-	-
$\lambda_{Isobestic}$	403	402	402	402	404	402	402	402	403	402	402
$\lambda_{ABS\text{-}base}$	413	408	408	408	410	409	409	409	407	408	408
$Log\ \varepsilon_{base}$ $^{(b)}$	4.04	4.02	4.01	4.02	4.01	4.02	4.02	4.02	-	-	-
pK_a $^{(c)}$	8.24	7.37	6.99	6.65	8.62	7.30	6.82	6.71	7.62	7.25	6.40
$\lambda_{FLU\text{-}acid}$	533	529	530	530	531	529	530	529	502	503	503
$\phi_{FLU\text{-}acid}$ $^{(d)}$	0.097	0.330	0.184	0.238	0.084	0.274	0.170	0.197	-	-	-
$\phi_{FLU\ pH=7}$ $^{(d)}$	0.093	0.304	0.101	0.099	0.079	0.249	0.095	0.091	-	-	-
$\phi_{FLU\ ethanol}$ $^{(d)}$	0.017	0.025	0.022	0.026	0.026	0.020	0.017	0.024	water:ethanol (4:1, v/v)<		
$\phi_{FLU\ hexane}$ $^{(d)}$	0.203	0.389	0.285	0.293	0.213	0.365	0.270	0.275	< Ethanol <<< hexane		
$\lambda_{FLU\text{-}base}$	543	548	550	550	532	532	554	545	505	506	504
$\phi_{FLU\text{-}base}$ $^{(d)}$	0.010	0.013	0.010	0.009	0.005	0.010	0.009	0.007	-	-	-
pK_a^{*} $^{(c)}$	7.1	6.93	6.95	6.4	7.15	7.11	7.04	6.48	7.15	6.71	6.5
$\Delta\nu$ $^{(e)}$	6329	6258	6070	6061	6293	6123	6070	5998	5515	5618	5539
FE $^{(f)}$	8.5	25.3	19.1	22.0	18.1	17.9	22.0	27.1	12.3	8.0	9.5

(a) Measured at 10^{-4} M in water-ethanol (4:1, v/v) unless specifically stated otherwise. The subscripts “acid” and “base” refer to the limiting value of a given parameter when the acid or base condition is increased until pH=2.0 or pH=12.0 respectively. Fluorescence emission spectra were obtained by excitation at $\lambda_{Isobestic}$; (b) ε, molar absorptivity in $M^{-1}cm^{-1}$; (c) Obtained [31] by analyzing the pH dependence of the absorbance (A) or emission intensity (I) at a given wavelength according to the equation $\log[(A_{acid} - A)/(A - A_{base})] = pH - \mathbf{pK_a}$ or $\log[(I_{FLU\text{-}acid} - I)/(I - I_{FLU\text{-}base})] = pH - \mathbf{pK_a^{*}}$ (d) Relatively to coumarin 6 (ϕ_F=0.78 in ethanol), uncertainty = 0.001 or 10%, whichever is the larger; (e) Stokes shift ($\nu_{FLU} - \nu_{ABS}$) in frequency (cm^{-1}); (f) Acid- induced fluorescence enhancement factor FE= $\mathbf{A}_{FLU\text{-}acid}/\mathbf{A}_{FLU\text{-}.base}$

The quantum yields of fluorescence in acid media (Table 3) for the hydroxy alkyl substituted piperazine naphthalimides derivatives ($\phi_{FLU\text{-}acid}$ =0.33-0.084) are lower than that of the non-hydroxylated derivative N-butyl-4-N′-methylpiperazino-1,8-

naphthalimide ($\phi_{FLU-acid}$ =0.55) [22]. Also, the quantum yields of fluorescence in basic media (Table 3) are higher ($\phi_{FLU-base}$ = 0.005-0.010) than that measured for N-butyl-4-N′-methylpiperazino-1,8-naphthalimide ($\phi_{FLU-base}$ = 0.001) [22]. The quantum yield of fluorescence for the membranes couldn't be calculated due to the difference in geometry between the fluorescence standard in solution and the membranes. The factor of fluorescence enhancement for the membranes in acid media was calculated (Table 3) by comparing the increase of intensities from basic to acid media and the enhancement was around 8-12 times. This enhancement in acid media (FE = 8-12.3) is higher than that obtained by other authors [23], where an enhancement of 4.7 was reported with a membrane obtained by copolymerization of *N*-allyl-4-(4´-methyl-piperazinyl)-1,8-naphthalimide with 2-hydroxyethyl methacrylate and acrylamide.

The presence of hydroxyl groups in the naphthalimide derivatives studied here and the ester functionality as a linking group of the naphthalimides in the membranes reduced the factor of enhancement of fluorescence in acid media with respect to that for other similar structures having only N-alkyl substituents [21,22]. Even though FE values obtained with the membranes confirm their interesting proton "off–on" switch behaviour as an optical pH solid sensor.

The pK_a values of naphthalimide derivatives and the functionalized membranes were calculated [31] from the curves of absorption changes in the presence of increasing acidity (from pH= 12.0 to 2.0) and the obtained values are compiled in Table 3.

The smaller substituents on the amine groups (structures 3 with hydrogen or 4 with methyl as substituents) enabled a higher pK_a as they are more easily protonated than the bigger substituents (structures 5 and 6). The pK_a values obtained for the functionalized membranes are close to those values measured for the corresponding piperazine derivatives (Table 3) confirming a similar sensitivity to acid media. The pK_a of the functionalized membranes is close to the pH of 7.0 which makes it adequate for sensors in biological and environmental applications [19].

The fluorescence response of the piperazine derivatives and the functionalized membranes at different pH was compared and the results are plotted in figure 6.

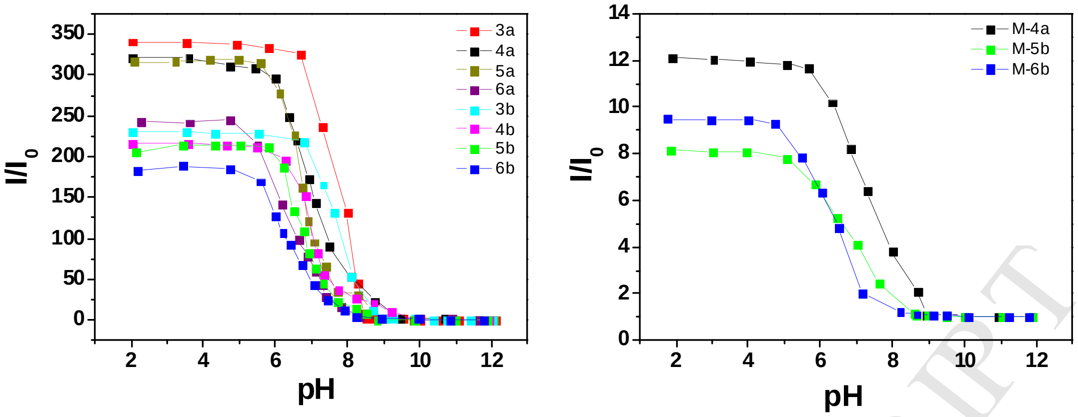


Fig.6. Comparison of the fluorescence intensity changes (I/I_o) with pH (2.0-12.0) for the piperazine-naphthalimide derivatives in solution (A) and for functionalized membranes (B). (I_0 and I are the fluorescence intensities of initial (pH=12.0) and that after acidification respectively).

All the synthesized naphthalimides (Fig. 6A) exhibited a sensitive fluorescence change towards pH but with different intervals and slopes of changes depending of the size of the amino substituents. For the functionalized membranes (Fig. 6B) the fluorescence intensity increased as the pH values decreased from 9.0 to 5.0 for M-4a and from 8.5 to 4.0 for the membranes obtained by linking the naphthalimides to the substituent of the piperazine structure, M-5b and M-6b. In the acidic pH range, especially for pH lower than 4.0, the fluorescence intensity attained its maximum and kept unchanged as well as in the basic pH range up to 9.0 where the fluorescence intensity reached its minimum. The pK_a* values (Table 3) for the excited state do not differ strongly from the corresponding pK_a for the ground state except for the structures 3a and 3b where the substituent of the piperazine is a hydrogen. For the rest of the structures and membranes the position of the inflection points in the fluorometric titration is similar to those obtained from the absorbance changes with pH.

The fluorescence quantum yields of the naphthalimides derivatives studied here are relatively low in polar solvents (water, ethanol) in comparison to those values obtained in non-polar solvents such us hexane (Table 3). This fact is probably due to the photo-induced electron transfer from the piperazine to the fluorophore and the fact that in polar solvents the orbit energy of HOMO becomes lower facilitating the PET process.

The polarity effect on the photophysical properties of the naphthalimide derivatives was studied by measuring their absorption and fluorescence spectra in solvents of increasing solvent polarity parameter $E_T(30)$. All the naphthalimide derivatives exhibited similar solvathochromic UV-Vis and emission spectra. As an illustrative example in figure 7, the values of absorption and emission maxima are plotted for different solvents.

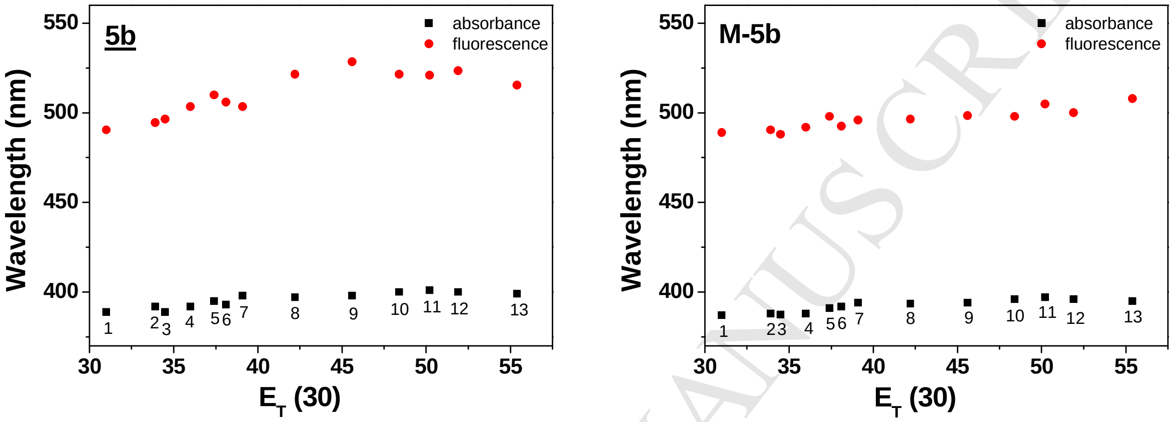


Fig. 7. Dependence of absorbance and Fluorescence emission wavelengths maxima of naphthalimide derivative 5b and the membrane M-5b on the empirical solvent polarity parameter, $E_T(30)$. Solvents: 1. Hexane, 2. Toluene, 3. Diethyl ether, 4. Dioxane, 5. THF, 6. Ethyl acetate, 7. Chloroform, 8. Acetone, 9. Acetonitrile, 10. 2-propanol, 11. Butanol, 12. Ethanol, 13. Methanol.

The maximum wavelengths of the absorption and fluorescence emissions are slightly red shifted with increasing solvent polarity except for the H-bonding solvents in the case of naphthalimide 5b. This is due to the presence of hydroxyl groups in the structure that provoke specific solute-solvent interaction. In general, the absorption maxima are less sensitive to the influence of solvent polarity than the fluorescence maximum. In the membranes, as shown in Fig. 6 for M-5b, the low bathochromic shifts in the fluorescence maximum with an increase of solvent polarity are consistent with small differences between the dipole moments of the ground and excited states.

3.4. Response time to pH of the functionalized membranes

The reversibility of the pH induced fluorescence signal was evaluated by determining the absorbance and fluorescence of the membranes by alterning their immersion in solutions of pH 2.0 and pH 12.0. Several cycles of successfull enhancement/quenching of fluorescence were carried out with each membrane without loss of fluorescent emission. The protonation of the aliphatic amine group involved seems quite reversible and no noticeable hysteresis effect was observed in the solid sensors.

The pH response of the membranes is dependent upon the proton permeability into the photocrosslinked material. Hence, the response time of the absorption and fluorescence spectra of the functionalized membranes was studied and the obtained results are plotted in Fig. 8.

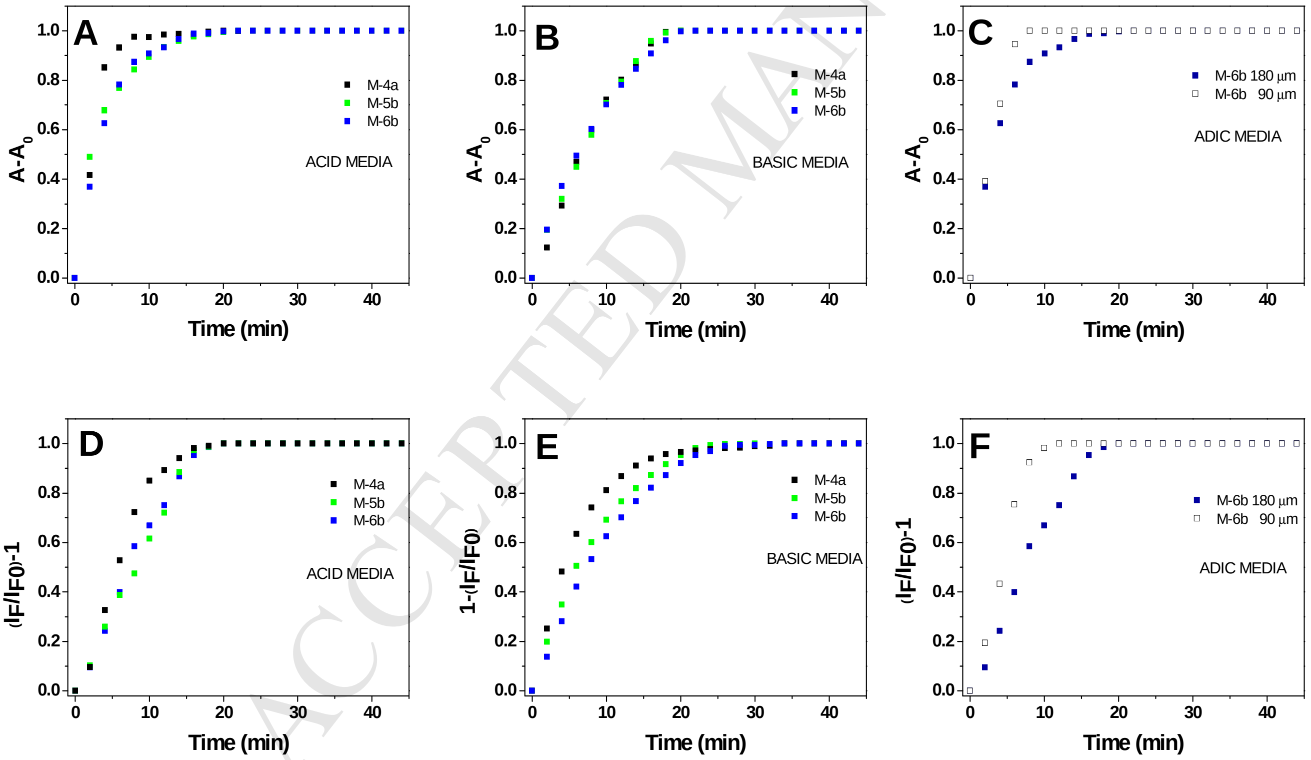


Fig. 8. Time response to pH of the functionalized membranes: Absorbance (A) and fluorescence (D) to acid media (pH=2.0), absorbance (B) and fluorescence (E) to basic media (pH=12.0) and effect of the membrane thickness in time response to acid media in absorbance (C) and fluorescence (F).

The pH response of the membranes is dependent upon the proton permeability into the crosslinked matrix and the three membranes exhibited a high sensitivity to pH in water. The absorbance and fluorescence of the membranes to acidic media (Fig. 8A and 8D respectively) reached the equilibrium in less than 15 min. The sensitivity to the basic medium of the membranes (Fig. 8B and 8E) is lower than that observed in acid media showing a slow response of around 20 min. Hence, the protonation process of the aliphatic piperazine group involved seems to be slower than the deprotonation process.

The time response of the membrane M-4a is shorter that those of the membrane b type. In the membrane M-4a the naphthalimide sensing functionality is linked to the polymer matrix through the imide group and the piperazine group is far from the polymer main chain. Hence, the interaction of the amino group of the piperazine with a proton is much easier than those of M-5b and M-6b where the piperazine is closer to the polymer main chain that can impair the mobility and hence the interactions of the sensing moiety.

The sensitivity of the solid sensor is directly related to the thickness of the pH sensitive membrane. To see the influence of the membrane thickness in the time response to the pH of the sensor, we prepared a membrane under the same experimental conditions but with 90 microns of thickness that was also an easily handled material and with good physical properties. As expected, the time response of this M-6b 90 µm-membrane (Fig. 8C and 8F) was notably shorter than that of the corresponding M-6b 180 µm-membrane.

In this study, we have described a useful procedure to obtain a water-swollen photocrosslinked membrane containing acid chloride groups capable of reacting with sensing molecules containing adequate functionalities, such as hydroxyl groups. The functionalization of M-Cl membrane was carried out using three derivatives of piperazine naphthalimide selected from a series of compounds that had been previously synthetized and spectrophotometrically characterized as a function of pH variations. The dependence of the pH sensitivity was related to the size and structure of the piperazine-naphthalimide substituents linked to the membrane. The solid sensors functionalized with naphthalimides acted as an acid sensor with interesting ranges of sensitivity to pH changes, from 9.0 to 4.0, which are adequate for medical and environmental sensing applications.

4. Conclusions

In summary, we have developed a procedure to obtain, using a photopolymerization reaction, water-swollen membranes capable of functionalization with naphthalimide fluorescence sensors and with a pH response.

The obtained results in the synthesis of the series of substituted piperazine naphthalimide derivatives carried out in this work suggested that microwave-assisted syntheses led to higher yields within very short reaction times in comparison to the conventional methods.

The new solid sensors exhibited controlled swelling in water (30%) and sensitive fluorescence changes towards pH between 9.0 and 4.0, and showed a very strong fluorescence at pH < 5.0.

The time response of the functionalized membranes to pH is dependent on the thickness of the membrane, the size of the substituents and the naphthalimide position of linkage to the membrane. The pH induced fluorescence signal of the solid sensors is reversible and several cycles of enhancement/quenching of fluorescence were successfully carried out without a noticeable hysteresis effect.

The present functionalized membranes could be applied as “Off-On” pH-fluorescence sensor in medical and environmental applications with satisfactory results.

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Photocrosslinked membranes have been functionalized with naphthalimide derivatives.

A series of hydroxyl-piperazine-naphthalimide derivatives have been synthetized.

The water-swollen functionalized membranes were characterized by different techniques

The new functionalized membranes could be used as “Off-On” pH-fluorescence sensors