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Towards a new strategy of a chitosan-based molecularly imprinted membrane for removal of 4-nitrophenol in real water samples

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Keywords: molecularly imprinted membrane; chitosan; azobenzene ligand; 4-nitrophenol water removal; adsorption

Abstract

The issue of water contaminants, which affects human and environment health, is not trivial. It is thus paramount to find new cheap and user friendly ways to detect and remove them from environment.

Here, the synthesis of a green chitosan-based molecularly imprinted membrane for the detection and quantification of 4-nitrophenol (4-NO_2Ph) in aqueous media was proposed. The concentration of 4-NO_2Ph in water solution was measured by High Performance Liquid Chromatography analysis (HPLC). Chitosan (CS) as functional polymer, 4-NO_2Ph as template, 4-[(4-Hydroxy)phenylazo]benzenesulfonic acid (PABSA) as ligand, and glutaraldehyde as crosslinker in the presence of polyethylene glycol as porogen were used. The membrane was characterized by SEM and FT-IR analyses, which confirmed the CS and PEG backbone of the membrane. Kinetic studies of the detection system were performed by using pseudo-first order and pseudo-second order models. Then, the binding efficiency between 195.33 µmol L^{-1} and 9235.55 µmol L^{-1} of 4-NO_2Ph was evaluated, finding a maximum adsorption of 723.25 µmol of 4-NO_2Ph per gram of membrane consistent with Q_{max} calculated from Langmuir isotherm. The selectivity of the membrane versus three phenolic competitor

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molecules, sharing very similar molecular structure to 4-NO_2Ph, was demonstrated. Finally, the applicability of the membrane to real-world samples was evaluated, by using drinking water spiked with 7.19 µmol L^{-1} of 4-NO_2Ph, obtaining a removal efficiency of 70.6 %.

INTRODUCTION

Phenolic derivatives have intrinsic toxicity and persistence after their release as waste materials[1,2] thus the US and European environmental protection agencies jointly agreed on classifying phenolic compounds as priority pollutants.[3] Concerning the currently available methods for phenolic compounds detection, there is a broad panel of techniques such as the high performance liquid chromatography (HPLC),[4,5] the GC-MS,[6,7] together with the use of fluorescence- and electrochemical-based methods,[8,9] mass spectrometry and/or capillary electrophoresis.[10] All of these approaches, though analytically validated during the last decades, share several common drawbacks such as expansive and time-consuming sample pre-treatment steps, highly specialized research staff and expansive instruments requirements. The nowadays purification procedures also present significant disadvantages. For example, nanofiltration or reverse-osmosis membranes remove all the available ions, which can make the purification process less effective.

Therefore, new solutions for the water detoxification of phenolic compounds are required in the fields of environmental science and technology. Today researchers have been studying the potential of bio-derived materials for adsorption of pollutants.

In this context, chitosan as biomaterial, and molecular imprinting technology have been considered in this work. Molecular Imprinting Technology[11] based on the ability of mimicking natural receptors with the aim of selectively identifying a specific template, has been extensively used in biotechnological and separation processes, catalysis, and biochemical sensors development.[12-16] Molecularly imprinted polymers (MIPs) are prepared by creating a three-dimensional polymeric matrix specifically interacting with a selected target molecule, named template. The template is added during the polymerization process and removed by washing procedures, leaving

complementary cavities with respect to shape and size in the polymer network. In this way the polymer can exhibit high affinity towards the template molecule that can be selectively re-bound to the specific sites.[17-19]

In this paper, the production of a new specific category of MIPs, namely Molecularly Imprinted Membranes, was implemented for the detection of the phenolic compound 4-NO_2Ph as model organic pollutant. To date some researchers already prepared MIPs using 4-NO_2Ph 4-NP as the template.[20-22] Moreover recently Hu et al.[23] developed an imprinted electrochemical sensor based on ZnO nanoparticles/carbon nanotubes doped chitosan film with excellent selectivity towards 4-NO_2Ph detection. On the other hand the preparation of this sensor is quiet expansive and complex.

In our work we focused on a green and easy synthesis of a CS-based membrane to further improve the biocompatibility of MIP system. This membranes represent indeed a valid alternative that enable overcoming the typical MIPs-related synthetic procedures. More than that, this work aimed at creating a 'green' synthesis to further improve the biocompatibility of MIP system, thus chitosan (CS) was chosen as molecular backbone for the membrane. Chitosan is a linear polycation composed of D-glucosamine, it is obtained through deacetylation of chitin. CS combines, in fact, the great advantage of a very well established biodegradability, biocompatibility, non-antigenic and non-toxic characteristics,[24,25] with the possibility to complex metal ions. Therefore, it is often used for the purification of pollutants from wastewaters.[26] Despite there are some few studies on the preparation of MIMs with CS,[25,27,28] still none of them exploited the proposed to adsorb phenolic compounds. In detail, Ma et al. prepared a MIM for naringin recognition while Zheng et al. for L-tyrosine recognition. They used PEG as a porogen and they evaluated the membranes with permeation experiments. Thus, the system realized in our work according to the above reported examples was called molecularly imprinted membrane.

CS was used as functional polymer, 4-NO_2Ph as template molecule, 4-[(4-Hydroxy)phenylazo]benzenesulfonic acid (PABSA) as ligand, polyethylene glycol (PEG) as

porogen and glutaraldehyde as crosslinker. A combination of FT-IR and SEM analyses provided the physicochemical characteristics of the synthesized CS-MIM, while its binding capacity was investigated through batch re-binding experiments and the 4-NO_2Ph was determined by means of HPLC. The kinetic of the membrane adsorption was studied with pseudo-first order and pseudo-second order models. A corresponding non-imprinted membrane (CS-NIM), prepared using the same procedure of CS-MIM in absence of 4-NO_2Ph, was tested as control.

Selectivity experiments with three other phenolic compounds structurally similar to the template were also performed. Finally, to confirm the applicability of the prepared CS-MIM to real-world water samples, drinking water was spiked with 4-NO_2Ph and analysed before and after incubation with the membrane and the extraction recovery was calculated.

EXPERIMENTAL

Materials and apparatus

Chitosan (CS) (degree of deacetylation 75-85 %, low molecular weight), polyethylene glycol 4000 (PEG), formic acid 98-100 %, chloridric acid >37 % were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium nitrite was supplied from Merck (Darmstadt, Germany). Glutaraldehyde (GA, 50 wt % content in distilled water) was supplied by Fluka (Steinheim, Germany). Potassium carbonate, analytical grade acetonitrile (MeCN) and methanol (MeOH) were obtained from J.T. Baker (Deventer, Holland). Sulfanilic acid and phenol were purchased from Alfa Aesar GmbH & Co KG (Karlsruhe,Germany). Sodium acetate was supplied from Carlo Erba (Milano, Italy).

4-Nitrophenol (4-NO_2Ph), 3-nitrophenol (3-NO_2Ph), 4-methoxyphenol (4-MeOPh) were purchased from Sigma Aldrich, phenol was purchased from Alfa Aesar. All chemicals were used without further purification. Deionized water was provided by a water purification system (Human

Corporation, Korea). For pH measurements, pHmeter Basic 20, (Crison, Alella, Barcelona, Spain, Europe, www.crisoninstruments.com) was used.

A rocking table type Rotamax 120 from Heidolph Instruments (Schwabach, Germany) was used for shaking incubated membranes. Sonication was carried out using a Sonorex RK 102H ultrasonic water bath from Bandelin Electronic. Centrifugation was carried out with a PK121 multispeed centrifuge from Thermo Electron Corporation. NMR spectra were recorded on a Bruker Advance 400 NMR spectrometer at room temperature, and chemical shifts were reported relative to tetramethylsilane. Fourier Transform Infrared Spectroscopy (FT-IR) spectra were recorded on a JASCO FT-IR 660 plus spectrometer with a resolution of 4 cm^{-1}, by 64 scans in the region between 4000 and 650 cm^{-1}. FT-IR spectra of CS and CS-MIM were obtained on KBr pellets (1 mg of dry sample and 150 mg of KBr, pressed into 13 mm diameter discs at a pressure of 10 Ton) while an acetone solution of PEG was spread directly on a ATR ZnSe crystal. The morphology of the membranes was analysed with a ZEISS EVO 40 scanning electron microscopy (SEM) in high vacuum mode without prior treatment. The thickness of the membranes was measured using a Dino-Lite digital microscope (AnMo Electronics Corporation). Elemental analyses were performed using the SEM equipped with EDS analysis (Bruker 127 eV mod. XFlash detector 5010; Bruker, Berlin, Germany). 4-NO_2Ph concentration in batch rebinding solutions were determined by using a Jasco V-660 UV-visible spectrophotometer (Jasco, Palo Alto, CA, USA) at 317 nm. Phenol compounds were quantified with HPLC analyses by using an Agilent 1100 Series LC/MSD system coupled to a photo-diode array detector. Chromatography separation was carried out on a 150 × 4.6 mm i.d., 5μm Gemini C18 column thermostated at 32 °C. The mobile phase was composed of water (0.1 % v/v formic acid) (solvent A) and methanol (solvent B). The chromatograms were acquired at the wavelength of 280 nm working at a flow rate of 0.5 $mL \cdot min^{-1}$, with the following gradient: 0 min 50 % B; 5 min 50 % B; 7 min 70 % B; 10 min 90 % B; 20 min 50 % B.

Synthesis of 4-[(4-Hydroxy)phenylazo]benzenesulfonic acid (PABSA)

4-[(4-Hydroxy)phenylazo]benzenesulfonic acid (PABSA) was synthesized following a procedure described elsewhere[29] with slight variations. Briefly, sulfanilic acid (40 mmol) and K_2CO_3 (40.6 mmol) were dissolved in 30 mL of deionized water. Then 3.0 g of $NaNO_2$ were added in 20 mL of deionized water. The mixture was cooled to 0°C and 25 mL of 5 N HCl was added dropwise. A white crystal of the diazonium salt was obtained. The resultant slurry was stirred for 1h over a salt-ice bath, and was then added dropwise to a stirring mixture of phenol (40 mmol) and K_2CO_3 (65 mmol) in 100 mL of deionized water at 0°C. The slurry was kept at a T < 3°C under stirring for a further 3 h. The phenol disappearance was confirmed by thin layer chromatography. The slurry was then neutralized by the addition of 2 N HCl. The precipitated orange product was collected by filtration and washed by cold water, chloroform and acetone, and then dried under vacuum. The crude product was recrystallized from aqueous ethanol (4/6 v/v) to give 7.0 g (yield 64 %) of purified PABSA as orange powder: 1H NMR (400 MHz, d6-DMSO): δ, ppm 10.4 (s, 1H), 7.81 (d, 2H), 7.76 (s, 4H), 6.93 (d, 2H); 13C NMR (400 MHz, d6-DMSO): δ, ppm 161.75, 152.42, 150.66, 145.90, 127.29, 125.59, 122.24, 116.63.

Preparation of 4-NO_2Ph-chitosan membranes

CS solution was prepared by dissolving 42.6 mg of CS into 3 ml of aqueous acetic acid solution (1 % v/v) and stirred until complete dissolution, then 2.5 mg of PABSA was added, followed by 5.6 mg of 4-NO_2Ph under stirring at 60°C for 1/2 h. A solution of polyethylene glycol 4000 (PEG 3 % w/w) was finally added to the CS solution, and kept stirring at 60°C for 1/2 h. Membranes were prepared by casting 2.0 g of the solution onto a polypropylene vessel with a diameter of 2 cm, allowing the water in the casting membrane to evaporate at 50°C for 18 h. After this time, the membrane was first incubated in 10 mL of a solution of GA 1 % (v/v) for 5 h to allow the reticulation, and then immersed several times in 10 mL of water to eliminate any unreacted GA and 4-NO_2Ph, and two final washings with EtOH. The 4-nitrophenol in the washing solutions was

monitored by UV-Vis spectroscopy to verify that no more template was in solution. The membrane was dried in oven at 60°C overnight.

Non-imprinted 4-NO_2Ph-chitosan membrane was also prepared as a control, following the same method, but without the addition of 4-NO_2Ph.

Adsorption kinetic

The kinetic adsorption experiment was conducted as following: 3 mg of 4-NO_2Ph-chitosan imprinted membrane was immersed into 3 mL of 4-nitrophenol water solution in a quartz cuvette at a known concentration (230.90 µmol L^{-1}), and kept under constant stirring at room temperature. The concentration of 4-NO_2Ph in solution was monitored at UV-Vis spectrophotometer at different times (t) from t 0 until 23 h, following the absorbance at 317 nm (taking care to do not let the beam interfacing with the membrane deposited on the bottom of the cuvette). The amount of 4-NO_2Ph adsorbed at time t Q_t (µmol g^{-1}), was determined from equation (1) by the difference between the initial concentration of 4-NO_2Ph in the solution (C_i) at t=0 and the residual concentration at t adsorption time (C_t) as:

$$Q_t = (C_i - C_t)\frac{V}{m} \quad (1)$$

Where V is the volume of incubation solution (L), and m is the weight of the membrane (g).

Two widely used kinetic models including pseudo-first order and pseudo-second order equations were employed to investigate the kinetic of 4-NO_2Ph adsorption on the imprinted membrane. The pseudo-first order Lagergren model was described by equation (2):

$$\log(Q_e - Q_t) = \log(Q_e) - \frac{K_1}{2.303}t \quad (2)$$

where Q_e is the amount of the template adsorbed onto the imprinted membrane at equilibrium time and K_1 (min^{-1}) is the rate constant of the first-order adsorption, while, the pseudo-second order model was given by equation (3):

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_e^2} + \frac{1}{Q_e} t \qquad (3)$$

where K_2 (g μmol^{-1} min^{-1}) is the rate constant of the pseudo-second order adsorption.

Adsorption experiments

The adsorption capacity of both the chitosan imprinted and chitosan non-imprinted membrane was evaluated through batch adsorption experiments. In particular, the membrane was incubated in a glass tube to different known concentrations of 4-NO_2Ph in water. In all experiments, membrane concentration was kept constant at 1 mg ml^{-1} with a 4-NO_2Ph concentration in the range between **1**95.33 - 9235.55 μmol L^{-1}. The mixture was shaken on a rocking table for 23 h at room temperature, followed by filtration through a 0.20 μm porosity filter and finally 4-NO_2Ph concentration after incubation was analyzed by HPLC analysis. The binding capacity, expressed as μmol of 4-NO_2Ph per gram of membrane, was calculated according to the following equation:

$$Q = (C_i - C_e)\frac{V}{m} \qquad (4)$$

where C_i and C_e are the initial and the equilibrium concentration of 4-NO_2Ph, respectively (μmol L^{-1}), V is the volume of 4-NO_2Ph water solution (L), and m is the weight of the membrane (g).

The imprinting factor (α), was calculated as follows:

$$\alpha = \frac{Q_{CS-MIM}}{Q_{CS-NIM}} \qquad (5)$$

where $Q_{CS\text{-}MIP}$ is the binding capacity of MIP on the template molecule and $Q_{CS\text{-}NIP}$ is the binding capacity of NIP on template molecule.

The adsorption isotherm was fitted with a Langmuir model. Langmuir isotherm equation is represented in the linear form according to the following equation:

$$\frac{1}{Q_e} = \frac{1}{Q_{max} C_e K_L} + \frac{1}{Q_{max}} \quad (6)$$

where Q_e and K_L are the amount of 4-NO_2Ph adsorbed at equilibrium (μmol g^{-1}) and the Langmuir constant (L μmol^{-1}), respectively. Q_{max} (μmol g^{-1}) is the maximum adsorption capacity that will be determined from the linear plot of $1/Q_e$ against $1/C_e$. All the experiments of the binding processes and measurements were performed in triplicate.

Selectivity studies

To study the selectivity of 4-NO_2Ph-chitosan imprinted membrane for 4-nitrophenol, competitive adsorptions of phenol, 3-nitrophenol and 4-methoxyphenol were evaluated. In particular a mixture of 200 μM of 4-NO_2Ph, phenol, 3-NO_2Ph, and 4-MeOPh was used for the experiments, and 1 mg of membrane was incubated for each volume of solution. After 23 h of incubation time, when the binding equilibrium was reached, the concentrations of 4-nitrophenol, phenol, 3-nitrophenol and 4-methoxyphenol in the solution was determined by means of high performance liquid chromatography (HPLC) at the wavelength of 280 nm. Selectivity data were evaluated with a known literature method as below described. [30]

The distribution coefficients of phenol, 3-nitrophenol and 4-methoxyphenol were calculated by equation (7):

$$K_d = \frac{Q_e}{C_e} \quad (7)$$

where, K_d (μmol g^{-1}) indicates the distribution coefficient; Q_e (μmol g^{-1}) is the equilibrium binding amount while Ce (μmol L^{-1}) is the equilibrium concentration.

The selectivity coefficient of 4-NO_2Ph-chitosan imprinted membrane for 4-NO_2Ph with respect to the competitor species (assigned as B) can be obtained from the equilibrium binding data according to equation (8):

$$k = \frac{K_d(4\text{-nitrophenol})}{K_d(B)} \quad (8)$$

where, k is the selectivity coefficient, and B represents the 3-nitrophenol, phenol or 4-methoxyphenol. The value of k allows an estimation of selectivity of CS-MIM for 4-nitrophenol. A relative selectivity coefficient k' can be defined as expressed in equation (9), and the value of k' can indicate the enhanced extent of binding affinity and selectivity of CS-MIM for the template with respect to CS-NIM.

$$k' = \frac{k_{CS-MIM}}{k_{CS-NIM}} \quad (9)$$

where, $k_{CS\text{-}MIM}$ and $k_{CS\text{-}NIM}$ is the selectivity coefficient of 4-NO_2Ph - chitosan imprinted membrane and 4-NO_2Ph - chitosan non imprinted membrane for 4-nitrophenol, respectively.

Removal of 4-NO_2Ph from drinking water

Batch experiments were conducted to explore the application of the prepared membrane to real-world samples. In particular, the extraction capabilities of CS-MIM in drinking water was evaluated. Two milligrams of the membrane were dipped in 2 ml of drinking water spiked with 7.19

μmol L^{-1} of 4-NO_2Ph. The mixture was shaken on a rocking table for 23 h at room temperature, filtered and 4-NO_2Ph concentration after incubation was analyzed by HPLC analysis. The experiment was carried out in triplicate.

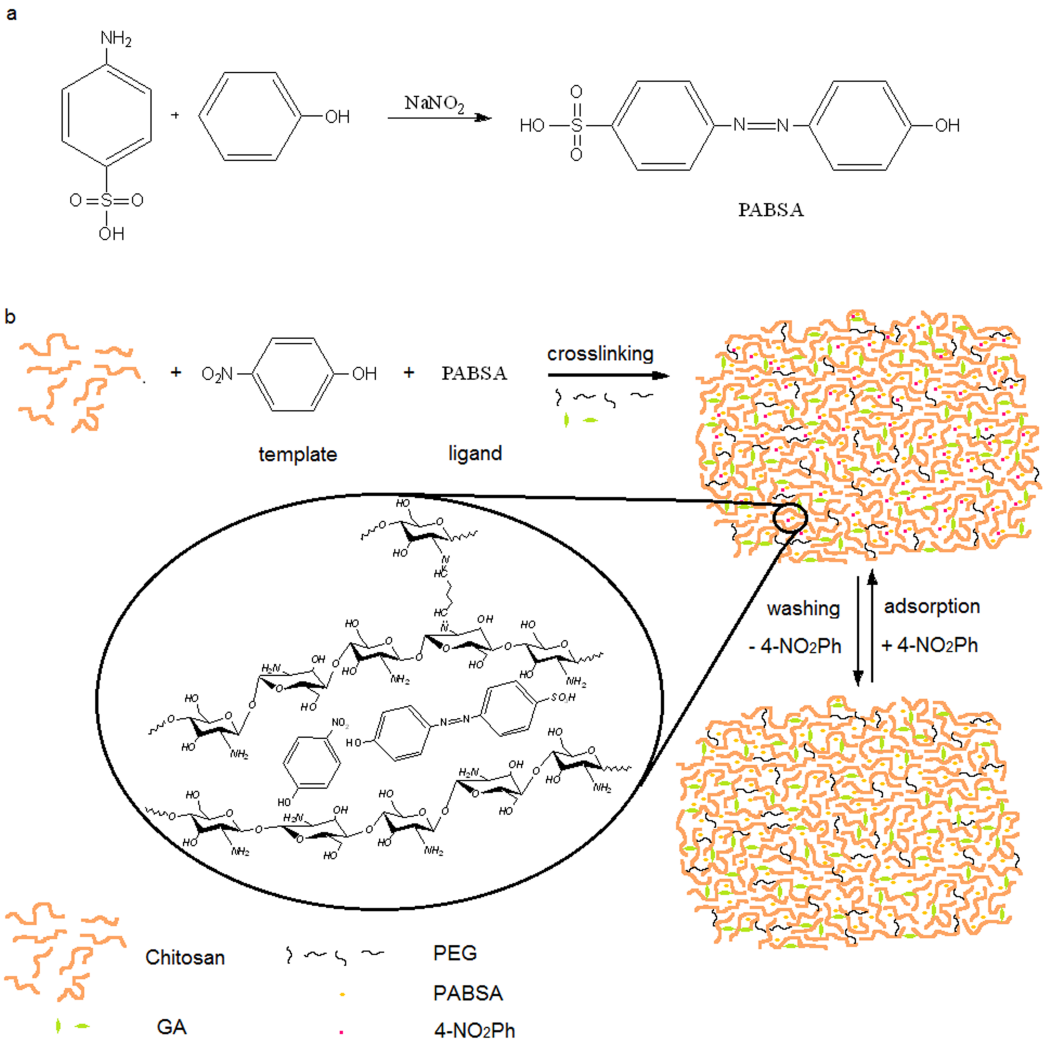
RESULTS AND DISCUSSION

In the context of water contaminants, that dramatically affects the human health and the environment, phenolic compounds represent one of the most wide-spread pollutants. Even though MIPs have been extensively studied as selective sorbents, especially for environmental applications, their preparation needs to be still improved as it is mainly based on conventional synthesis techniques.[31-33] Some of these include bulk precipitation-, emulsion- and seed polymerization. Herein a green chitosan-based membrane able to selectively adsorb 4-NO_2-Ph molecules in water samples was proposed.

4-NO_2Ph- chitosan imprinted membrane synthesis

Firstly the ideal conditions for the membrane preparation were attempted. The synthetic route included the use of PABSA as ligand because such Azo-type compound has high water solubility (see materials and methods section for the PABSA synthesis and Scheme 1a) and this is an important aspect of the synthetic strategy as the chitosan membranes preparation was obtained from an acidic water solution of chitosan. To increase the template-polymer interaction the azo-derivative was trapped into the polymeric network. PABSA was chosen because it is a water friendly compound with various potential interaction sites with both the chitosan backbone and the 4-NO_2Ph (e.g., the-SO_3H, -OH and -N=N- functional groups) allowing a physical enclosing into the polymeric matrix. In order to trap the PABSA together with the template within the CS membrane, a glutaraldehyde-based crosslink reaction was used. The crosslinking procedure has also the great

advantage to make the resulting product (the CS membrane in our approach) highly insoluble in water. This aspect is essential, as the CS membrane is expected to work and detect the phenolic pollutants in water, where it should not be dissolved. The template has been then removed through several washing steps in water (Scheme 1b).



Scheme 1. Synthesis of PABSA (a) and proposed schematic process for preparation of 4-NO_2Ph imprinted CS-MIM (b).

SEM and EDS analyses

Before SEM analysis the specimens were analysed with a digital optical microscope obtaining with enlargements at around 200x a thickness for both membranes in the range of 47–57 μm.

Fig. 1 shows a preliminary SEM morphological characterization of the membranes. The pictures clearly demonstrate a significant structural change in the dried membranes texture between CS-MIM (Fig. 1a) and CS-NIM (Fig. 1b) after washing steps. In particular, the CS-MIM presents a highly porous surface, which is likely to be ascribable to the template that influences the crosslinking process. On the other side, the CS-NIM membrane obtained after the crosslinking reaction shows a more uniform, though still roughened, surface with a lower porous structure.

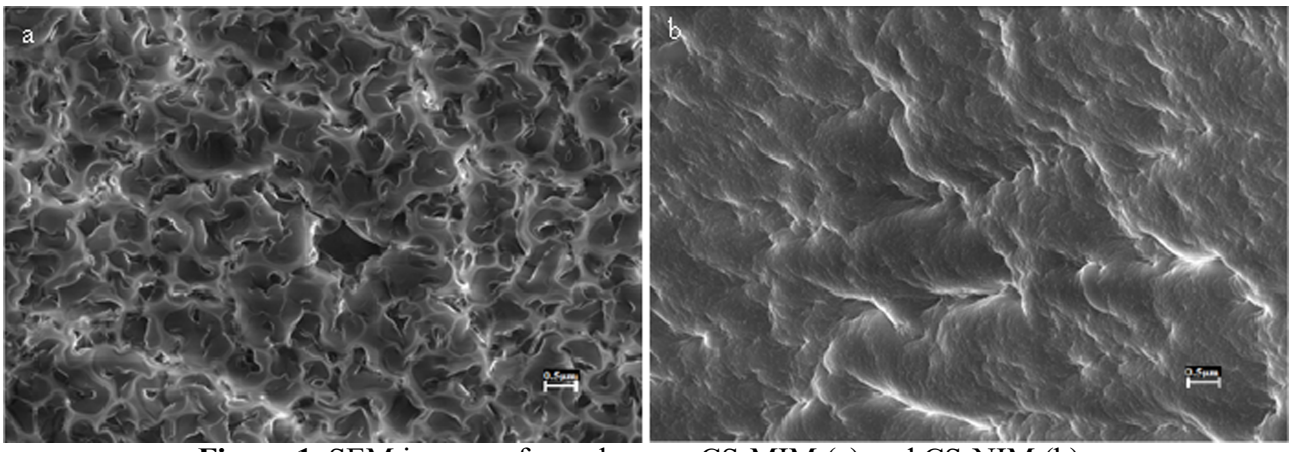


Figure 1. SEM images of membranes: CS-MIM (a) and CS-NIM (b).

To go deeper insight into the characterization steps, we carried out EDS-based elemental analyses upon templates removal. Fig. 2 demonstrates that there is not differences in the percentage of chemical elements of the membranes as expected since after 4-NO_2Ph removing both membranes have similar chemical composition. In particular, the spectrum highlights the presence of C (57.80 % wt), N (7.20 % wt), O (34.60 % wt) and S (0.40 % wt), this latter representing the trapping of PABSA within the membrane.

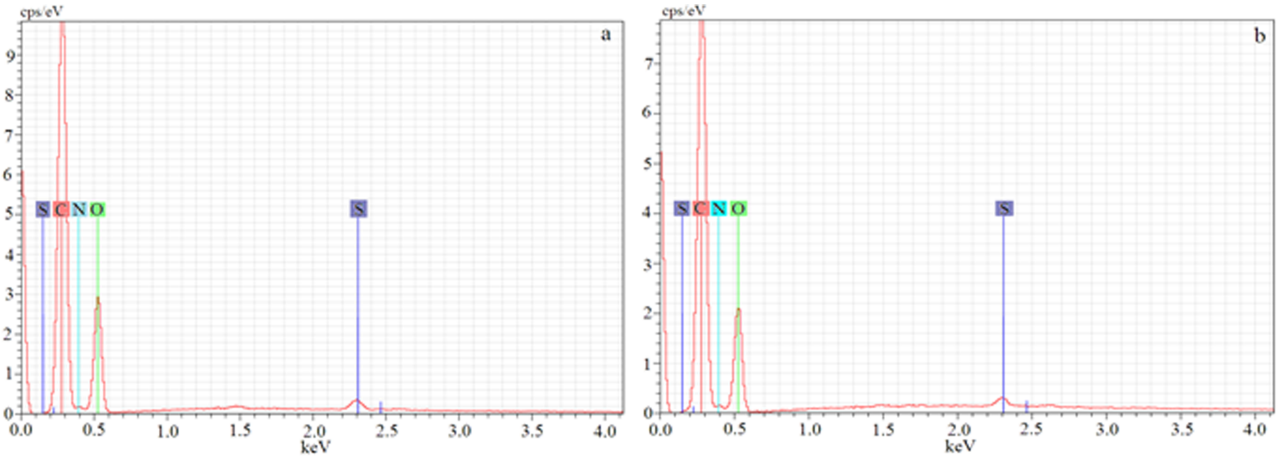


Figure 2. EDS analysis of CS-MIM (a) and CS-NIM (b).

FT-IR analysis

A comparative FT-IR spectra characterization of chitosan, 4-NO_2Ph-chitosan imprinted membrane, and PEG is shown in Fig. 3. Fig. 3a shows a typical IR spectrum of CS. The absorption peak at 3438 cm^{-1} can be assigned to –OH and –NH stretching vibrations, while the bands 2921 cm^{-1} and 2868 cm^{-1} are observed for the C-H stretching vibration of methane in residual-sugar group.[28,34] The distinct amide I band and amide II band at 1650 cm^{-1} and 1597 cm^{-1}, respectively, refer to the higher degree of deacetylation (75-85 %) of pure chitosan. The absorption bands at 1154 cm^{-1} (asymmetric stretching of C-O-C bridge), 1078 cm^{-1} and 1025 cm^{-1} (C-O stretching) are also typical of the CS saccharine structure.[35] Fig. 3c shows typical peaks of PEG: the peak at 2873 cm^{-1}, which is due to the aliphatic C-H stretching and the peaks at 1144 cm^{-1}, 1096 cm^{-1}, 1059 cm^{-1} due to C-O-H stretching. Fig. 3b shows the peaks of the 4-NO_2Ph-chitosan imprinted membrane. The membrane spectrum appears quite similar to the chitosan one since it is the major component of the polymer even if various little differences can be noted because of chemical modification. Among them, the peaks at 2920 cm^{-1} and 2854 cm^{-1} for aliphatic C-H stretching show a change in shape e wavenumber that can be ascribable to the addition of PEG peak. Also in the region of amide peaks, between 1650 cm^{-1} and 1600 cm^{-1}, where PEG does not absorb, there is an evident change that can be due to glutaraldehyde linkage to the chitosan backbone and finally between 1160 cm^{-1} and 1000

cm^{-1} there is a strong absorption with four peaks that can be due to the overlapping of PEG and chitosan absorption.

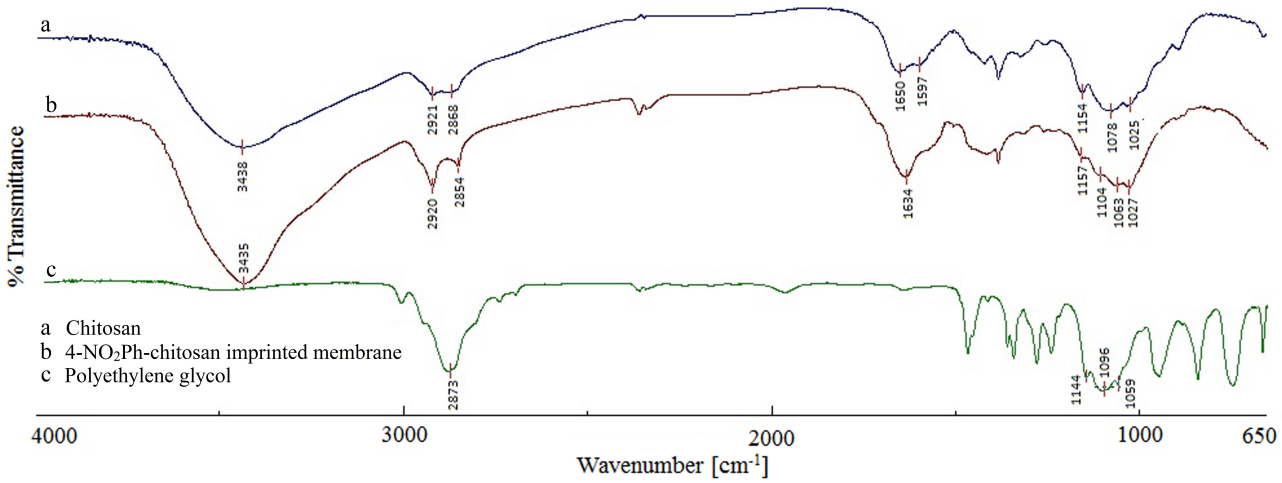


Figure 3. FT-IR spectra of chitosan (a), 4-NO_2Ph-chitosan imprinted membrane (b) and polyethylene glycol (c).

Adsorption kinetic

In this section, the kinetics of adsorption that describe 4-NO_2Ph uptake rate were evaluated in order to define the detection efficiency of our system. To do this, the imprinted CS membrane was immersed in a quartz cuvette containing a solution of 230.90 µmol L^{-1} of 4-NO_2Ph. Then several UV-Vis measurements of the solution at different time intervals were carried out. Since the membrane not interfere with the measurement, we decided to measure directly the absorbance into the cuvette avoiding several withdrawal and dilution steps. Fig. 4 displays the sorption kinetic curve of 4-NO_2Ph binding capacity of chitosan membrane (Q) as function of time.

In Fig. 4 is evident how the level of adsorption is significantly high and fast within the first 8 h of incubation time, and then it slows down reaching a plateau phase. In particular, only a 9.49 µmol g^{-1} (14 %) of sorption was quantified after 1 h post incubation. This level rapidly rises up to 53.00 µmol g^{-1} (79 %) after 5 h, and reaches 59.81 µmol g^{-1} (89 %) within 8 h. In the range between 8 h and 23 h the plateau phase of 67.00 µmol g^{-1} (100 %) was observed.

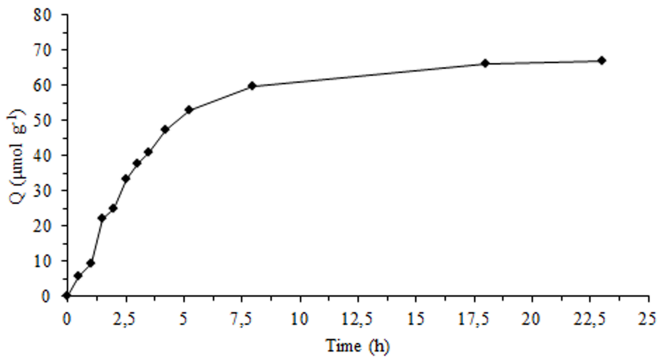


Figure 4. Adsorption kinetic curve of 4-NO_2Ph-chitosan imprinted membrane.

To insight the mechanism of adsorption, two kinetic models were used to test the experimental data illustrated in Fig. 4. One was pseudo-first order described by the equation $\log(Q_e-Q_t) = \log(Q_e)-(K_1/2.303)t$, and the other was pseudo-second order explained by $t/Q_t = (1/K_2Q_e^2)+(1/Q_e)t$, that are based on different assumptions. The pseudo-first order equation relies on the assumption that one molecule adsorbs within the active site of the membrane. On the other side, the pseudo-second order equation is based on the assumption that one adsorbate molecule interacts with two active sites.[36] The above equations were plotted in Fig. 5.

According to the correlation coefficients, the experimental data fitted very well with the pseudo-first order kinetic equation (Fig. 5a) which showed a correlation coefficient (R^2) of 0.9931, and an apparent adsorption rate constant K_1 value of 0.0044 min^{-1}. The theoretical Q_e value estimated from pseudo-first order kinetic model was 64.33 µmol g^{-1} very close to the experimental value. On the other hand, the pseudo-second order kinetic equation (Fig. 5b) was not suitable to describe the kinetic of the adsorption on the sorbent since the straight line gave a correlation coefficient of only 0.907.

In conclusion, the kinetic results suggest that the pseudo-first order adsorption mechanism was predominant in the adsorption process of 4-NO_2Ph on the imprinted membrane system developed in this work.

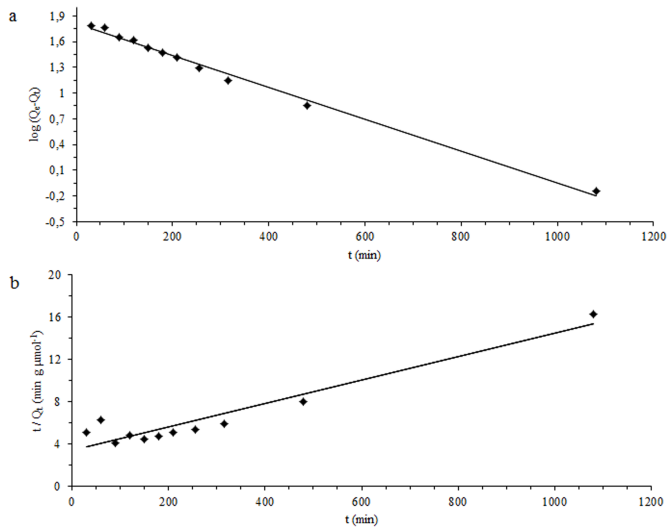


Figure 5. Adsorption kinetics of 4-NO_2Ph on 4-NO_2Ph-chitosan imprinted membrane evaluated by pseudo-first order model (a) and pseudo-second order model (b).

Binding Isotherm of CS-MIM

Another important parameter to further validate binding capacity of MIM is the binding isotherm of chitosan imprinted membrane. The binding behaviour was tested by batch rebinding experiments, 3 mg of CS-MIM membrane were immersed in 3 mL of a solution of 4-nitrophenol at different concentrations (from 195.33 µmol L^{-1} to 9235.55 µmol L^{-1}) for 23 h. The binding capacity of 4-NO_2Ph (Q) was calculated according to the equation 4 (see materials and methods). Binding equilibrium isotherm of CS-MIM and CS-NIM is shown in Fig. 6. The graphic clearly shows that the binding capacity of the imprinted membrane increases as a function of the increasing concentration of 4-NO_2Ph reaching a plateau with a maximum binding capacity (Q_{max}) of 723.25 µmol g^{-1}. On the other hand, the binding capacity curve of the non-imprinted membrane reached a plateau with a maximum binding capacity (Q_{max}) of 517.69 µmol g^{-1}, underlining an higher adsorption of the CS-MIM of about 205 µmol g^{-1}.

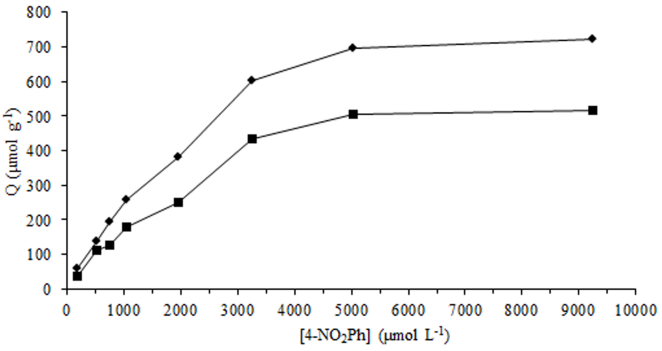


Figure 6. Binding equilibrium isotherm of CS-MIM (♦) and CS-NIM (■).

An important parameter to evaluate the imprinting effect is the ratio of adsorption capacities of chitosan imprinted and chitosan non-imprinted membranes. To this aim, the incubation of both membranes at 1968.46 μmol L^{-1} of 4-NO_2Ph were considered. In Table 1 the imprinting factor of CS-MIM calculated as the ratio between Q value of CS-MIM and CS-NIM was reported.

Table 1

The binding data were processed by Langmuir analyses to evaluate the binding characteristics of the prepared membrane. The Langmuir isotherm model assumes that the adsorption is done in a monolayer; adsorption sites located on the surface of the adsorbent are uniform and they all have the same adsorbing ability.[37] The adsorption of 4-NO_2Ph on 4-NO_2Ph-chitosan imprinted membrane was fitted by Langmuir model as given in equation 5. Consistently with the straight line shown in Fig. 7, a high R^2 value of Langmuir isotherm of 0.9978 was obtained, for 4-NO_2Ph adsorption onto the CS-MIM, which indicates that the adsorption behaviour fit the Langmuir model well. The Langmuir constant (K_L) is 5.035x10^{-4} L μmol^{-1}. The maximum adsorption capacities (Q_{max}) of the imprinted membrane as calculated from the Langmuir model is 909.09 μmol g^{-1}, it is a theoretical value close to the experimental value.

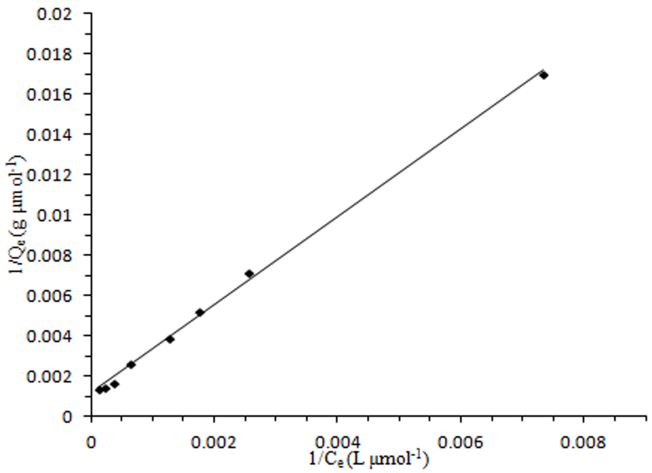


Figure 7. Langmuir isotherm of 4-NO_2Ph adsorption on CS-MIM.

Selectivity study

In addition to the binding isotherm, a crucial aspect of the imprinted membrane is its binding selectivity. A chitosan imprinted membrane that recognize only the analyte of interest, and avoid the aspecific binding of competitor molecules with similar structures is desired. To verified selectivity behaviour, CS membranes were also tested with three other phenolic compounds structurally analogous to 4-NO_2Ph: 3-NO_2Ph, Ph and 4-MeOPh. We opted for one competitor isomer, and two different molecules sharing similar structure but with different functional groups.[20,30] In particular, the membranes were incubated with a solution containing the four phenols at a concentration of 200 µM. The binding capacities of the CS-MIM and CS-NIM for 4-NO_2Ph, 3-NO_2Ph, Ph and 4-MeOPh are displayed in Fig. 8.

At a first glance, the CS-MIM exhibited remarkably higher binding capacity for the template molecule than the CS-NIM. In addition, it is evident that both the CS-MIM and CS-NIM exhibit a good selectivity for the analyte of interest (4-NO_2Ph), even though the molecular structures are significantly similar each other.

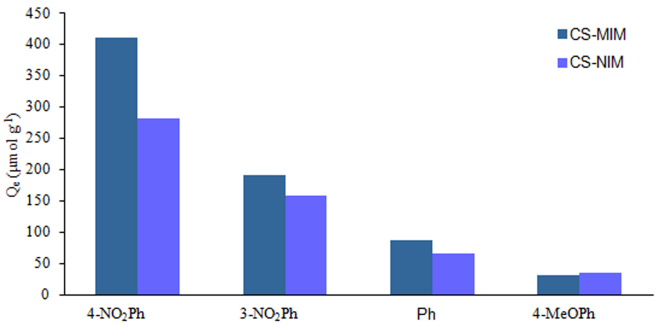


Figure 8. Binding selectivity of CS-MIM and CS-NIM in a mixture solution of 4-NO_2Ph and analogous compounds at 200 μmol L^{-1} under optimized conditions.

A quantitative evaluation of the selectivity data were carried out. To this aim, the distribution coefficients (K_d), the selectivity coefficients (*k*) and the adsorption affinities (*k'*) of 4-NO_2Ph on CS-MIM and CS-NIM were calculated and the corresponding results are summarized in Table 2.[30]

Table 2

According to the data of Table 2, the distribution coefficient of 4-NO_2Ph is higher for CS-MIM than CS-NIM. Moreover the distribution coefficient of 4-NO_2Ph is higher than that of the similar compounds. This can be ascribed to the presence of the template molecule during the CS-MIM synthesis that allows an efficient imprinting effect to the membrane that shows sites specific in shape, size and functional groups to bind 4-NO_2Ph. The results showed that the selectivity coefficients of 4-NO_2Ph than those of 3-NO_2Ph, Ph and 4-MeOPh are 2.46, 5.75 and 16.19 respectively. Among all the 4-NO_2Ph competitors, the 4-MeOPh allowed to obtain the best selectivity, probably because its chemical structure is slightly different comparing to the other phenols.

Analysis to real water samples

To evaluate the feasibility of the application of the chitosan membrane to real samples, the removal of 4-nitrophenol from drinking water was tested. CS-MIM was incubated with drinking water samples spiked with 7.19 μmol L^{-1} of 4-NO_2Ph. The membrane demonstrated in drinking water a satisfactory removal efficiency of 70.6 % (RSD 4.5 %) suggesting an interesting 4-NO_2Ph uptake in environmental water samples.

CONCLUSION

In the present work, a new biocompatible and low cost chitosan-based molecularly imprinted membrane for the detection of 4-N0$_2$Ph as model of organic contaminant was proposed with the aim to overcome some limits, such as high costs and low selectivity of traditional pollutants detecting and removing techniques.

Membrane characterization confirmed the CS and PEG backbone with PABSA ligand trapped into the matrix of CS-MIM, which shows a highly porous surface that we assumed is due to the template influence on the crosslinking process. The kinetic results suggested that the pseudo-first order adsorption mechanism was predominant in the adsorption process of 4-NO_2Ph on the imprinted membrane system. This membrane was found to be highly effective in binding the target, with a maximum adsorption of 723.25 μmol of 4-NO_2Ph per gram of membrane consistent with Q_{max} calculated from Langmuir isotherm. In addition, it displayed a good selectivity, when it was tested against three different phenolic compounds sharing similar molecular structures. Thus, CS-MIM could be an useful system to reveal the presence of 4-NO_2Ph in aqueous media.

The membrane here developed could provide a tool for solving the phenols-related environmental pollution. This will match the characteristics of a very low cost of production, together with high biodegradability, and the possibility to be used even by non-specialised users.

In conclusion, a novel chitosan-composite membrane based on the molecular imprinting technique can be a potential innovative system, in comparison to the conventional synthetic MIP, in the field

of environmental detection and/or removing of organic pollutants. To this aim, more trials need to be realized in order to test the synthesized polymer as a filtration device, for instance carrying out permeation experiments in which MIM can be placed between two rooms filled with two different solutions, a feed solution and a stripping phase.

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TABLE CAPTIONS

Table 1

Imprinting Factor (α).

C (μmol L^{-1})	Q CS-MIM (μmol g^{-1})	Q CS-NIM (μmol g^{-1})	α, imprinting factor
1986.46	381.16	249.24	1.52

Table 2

Selectivity parameters of CS-MIM and CS-NIM.

Samples	CS-MIM			CS-NIM	
	K_d (μmol g^{-1})	*k*	K_d (μmol g^{-1})	*k*	*k′*
4-NO_2Ph	2.59		1.64		
3-NO_2Ph	1.05	2.46	0.86	1.91	1.29
Ph	0.45	5.75	0.34	4.82	1.19
4-MeOPh	0.16	16.19	0.18	9.11	1.77