Preparation of molecularly imprinted membranes and evaluation of their performance in the selective recognition of dimethoate

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\textbf{ABSTRACT}

Molecularly imprinted membranes with affinity properties for the pesticide dimethoate were prepared using polyacrylonitrile and its copolymers with two different functional monomers (methacrylic acid and acrylamide) \textit{ad hoc} synthesized. Membranes were prepared via phase inversion technique using N-methylpyrrolidone as the solvent and adding the dimethoate to the casting solution. Membranes without the template were also prepared and used as reference (blank). To evaluate the influence of the two different functional monomers on the binding capacity of membranes toward the template, binding experiments in a dead-end filtration cell were performed. All imprinted membranes made from acrylonitrile copolymers exhibited good recognition properties. Among them, the membrane prepared with the copolymer containing methacrylic acid as functional group, showed the highest binding capacity. In all cases, blank membranes only showed a poor non-specific binding. The selectivity of the imprinted membranes was investigated performing binding experiments in the same operating conditions using the structural analogue triclorphon.

\textbf{Keywords:} Organophosphorus pesticides; Acrylic copolymers; Imprinted membranes; Binding capacity; Selectivity

\section{1. Introduction}

Organophosphorus pesticides have played an important role in increasing agricultural productivity. They are generally biodegradable and do not tend to accumulate in the food chain. On the other hand, elevated amounts of these pesticides in wastewaters coming from manufacturing processes are produced. Consequently, the direct discharge of these waters can cause harmful effects for health and the environment, due to their toxic nature. In particular, dimethoate (DMT), was identified as one type of organophosphorus pesticides listed in guidelines for drinking water by World Health Organization [1]. This pesticide exhibits insecticide and acaricide activity which kills acari by contact and stomach action. It is now widely used against a large range of insects and acari of vegetables, tea, mulberry, cotton, oil plants and corns [2]. Dimethoate is emerging as contaminant in water. In fact, it is highly soluble in water and adsorbs very weakly to soil particles. Although the minute concentration of dimethoate in water, its chronic effect to the livings can cause oncogenicity, mutagenicity, fetotoxicity and reproductive effects [3,4]. As a result

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of the toxic effect of the pesticides used in agrochemical industry, efficient methods suitable to reduce their content in wastewaters are requested.

Traditionally, removal of pesticides from water was done by activated carbon filtration but this method was expensive and required frequent regeneration. Over the past few years, nanofiltration membranes were studied as useful means of pesticides removal [3,5,6]. Recently, molecular imprinting technique received attention as an approach to prepare sorbent materials to be used in solid-phase extraction for removal of contaminants from different matrices [7–9]. The most important aim of the researchers is the improvement of selectivity of these materials since many of them are not specific for target molecules. In this perspective, molecularly imprinted polymeric materials possess higher selective affinity for target substances and could be used as specific preconcentration elements or detection platform for analyte of interest.

Molecular imprinting involves arrangement of functional monomers around a template molecule, subsequent polymerization results in trapping template in highly cross-linked polymer matrix. Removal of the template molecules with suitable solvents allows the formation of specific recognition sites in the polymer network which are complementary to the template in terms of shape, size and functionality [10–12]. The combination of imprinting and membrane technologies allowed to prepare molecularly imprinted membranes (MIMs) with specific selectivity for the separation of target compounds and thus making the MIMs posses the advantages of both molecular imprinting and membrane technology [13–15]. MIMs are highly attractive due to their promising properties of ease and low energy of operation. First MIMs were prepared by Piletsky et al. in 1990 [16] via in situ bulk polymerization of acrylate monomers. Others prepared MIMs via photo-copolymerization [17,18] and phase inversion technique [19–21].

In this work the potential application of molecularly imprinted membranes in the removal of the organophosphorus pesticide dimethoate from water was investigated. To our knowledge, this is the first paper in which the removal of dimethoate from water samples is included in the development of a MIMs-based method. Membranes were prepared via phase inversion technique in the presence of dimethoate using the polyacrylonitrile (PAN) homopolymer and its copolymers poly(acrylonitrile-co-methacrylic acid) (P(AN-co-MAA)) and poly(acrylonitrile-co-acrylamide) (P(AN-co-Aamide)). All the polymers were synthesized by the water-phase precipitation polymerization method.

Due to its chemical structure the dimethoate is able to interact via hydrogen bonds with the functional monomers of the synthesized copolymers. The donors of hydrogen bonds were the amide and the carboxyl groups of the own copolymers and the acceptors were oxygen and sulphur groups of dimethoate.

The binding capacity of the imprinted membranes prepared using these two copolymers was evaluated and compared performing binding tests in a dead-end-filtration cell. For comparison experiments were also carried out using blank membranes prepared in the same conditions but without template molecule. In addition, the selectivity of P(AN-co-MAA) dimethoate-imprinted membranes vs. the structural analogue trichlorphon (TCF) was investigated. The chemical structure of the monomers acrylonitrile, methacrylic acid, acrylamide and of the two organophosphorus pesticides is shown in Fig. 1.

![Fig. 1. Chemical structure of acrylonitrile (a), methacrylic acid (b), acrylamide (c), dimethoate (d) and trichlorphon (e).](image-url)
2. Experimental

2.1. Materials

Acrylonitrile (AN), methacrylic acid (MAA), acrylamide (Aamide), potassium persulfate, ferrous sulfate, sodium bisulfite, N-methylpyrrolidone (NMP), n-hexane, dimethylacetamide (DMA), acetonitrile (HPLC grade), methanol (MeOH) and trichlorphon were purchased from Sigma (Italy) and used as received. Dimethoate was gently supplied by Product Manager Insecticides, Isagro SpA (Italy).

2.2. HPLC measurements

The quantitative determination of pesticides was performed on a LaChrom D7000 HPLC system (Hitachi) equipped with L-7400 UV detector. Analysis were carried out using the column Prevail C18, 5 μm, 250 4.6 mm (Alltech, Italy). The mobile phase consisted of water/acetonitrile with volume ratio of 65:35. The operating conditions were: flow rate of 1.2 ml/min, temperature of 25°C, pressure of 125 bar and wavelength of 190 nm.

2.3. Polymer preparation and characterization

The synthesis of polyacrylonitrile homopolymer and its copolymers with 10% of the different monomers (methacrylic acid and acrylamide) was made by water-phase precipitation polymerization method. The reaction was carried out at 50°C for 2 h under inert atmosphere and stirring conditions in a reactor vessel of 1 liter (l). The amount of total monomer was 50 g in all cases. The water/total monomer ratio was 6:1. The couple K₂S₂O₈-NaHSO₃ was used as catalyst in the presence of Fe²⁺ (1 ppm of the total monomer), K₂S₂O₈ and NaHSO₃ were 4.7 × 10⁻² and 1.6 × 10⁻² wt.% of the total monomer, respectively. At the end of each reaction the synthesized polymer was rinsed several times with de-ionized water in order to remove the un-reacted monomers and the residual catalysts. Afterward, the polymer was dried under vacuum at 90°C overnight. The molecular weight of the different products of synthesis was determined by intrinsic viscosity measurements on the basis of the following equation [21–24]:

\[ [\eta] = 2.75 \times 10^{-2} M^0.767 \] (1)

where \([\eta]\) is the intrinsic viscosity and \(M\) is the viscosity average molecular weight.

Polymers were also characterized by FT-IR analysis using a Perkin-Elmer Spectrum One FT-IR spectrometer.

2.4. Membrane preparation and characterization

The membranes were prepared via the phase inversion technique using the solvent evaporation method. Polymer solutions, based on 9.0 wt.% of each polymer and 1.5 wt.% of DMT as the target molecule in NMP, were cast onto a glass plate with a casting knife having a gap of 400 μm and membranes were formed by the evaporation of the solvent at 60°C for 2 h in an oven. Next, membranes were immersed in n-hexane overnight in order to remove the residual solvent. For comparison, blank membranes were prepared without adding the template to the casting solutions. The membrane morphology was investigated by scanning electron microscopy (SEM) using a FEI QUANTA 200 F microscope at 20 kV. Membrane permeability (\(L_p\)) was determined by water flux measurements (\(J\)) at different transmembrane pressure (TMP) in a dead-end filtration cell connected to a N₂ gas cylinder. The working diameter of the membrane was 7 cm. The plot of \(J\) vs TMP directly gives the membrane permeability.

The membrane thickness was determined by a digital micrometer (MAHR 40E, Germany) as the average of at least ten measurements.

2.5. Binding experiments

The prepared membranes were previously extracted in a dead-end filtration cell by prolonged washing with methanol till no dimethoate was detected in the permeate stream by HPLC analysis. Membrane permeability was measured as described above and finally binding tests were performed. The binding capacity of the blank and imprinted membranes was evaluated by measuring the sorption of DMT during the filtration of 300 mL of water sample containing \(2.2 \times 10^{-5}\) mol/L of pesticide. The amount of dimethoate retained by the membrane was calculated from the mass balance of the following equation:

\[ Q_{DMT} = \frac{V(C_0 - C_e)}{m} \] (2)

where: \(Q_{DMT}\) is the mass of the retained dimethoate per gram of membrane; \(C_0\) is the initial concentration of dimethoate; \(C_e\) is the concentration at the end of the test; \(V\) is the volume of the used solution; \(m\) is the mass in grams of the membrane. The concentration of DMT at the beginning and the end of the filtrations was measured by HPLC as above described. All the experiments were carried out in duplicate. After each binding test membrane was re-extracted with MeOH (in order to recover the retained template) and re-used for one more time.
The selectivity of dimethoate-imprinted membranes was investigated performing binding experiments in the same operating conditions using the structural analogue trichlorphon. The selectivity factor ($\alpha$) was calculated as the ration between the amount of dimethoate and the amount of the other organophosphorus pesticide specifically bound on the membrane as the following equation:

$$\alpha = \frac{[S_{\text{template}}]}{[S_{\text{analogue}}]}$$

where $\alpha$ is the selectivity factor, $[S_{\text{template}}]$ is the template amount (µmol./g memb.) specifically bound on the membrane and $[S_{\text{analogue}}]$ is the analogue amount (µmol./g memb.) specifically bound on the membrane [25].

3. Results and discussion

3.1. Polymer preparation and characterization

The synthesis of PAN homopolymer and its copolymers P(AN-co-MAA) and P(AN-co-Aamide) was accomplished by a water-phase precipitation polymerization process. Due to the solubility of the used monomers in water, the polymerization took place in water. On the opposite, being the synthesized polymer insoluble in water it precipitated from the water phase. Results of the polymerizations are reported in Table 1, which shows the yield of the process, the intrinsic viscosity and the molecular weight of the synthesized polymers.

The presence of the functional monomers in the PAN polymer chain was confirmed by the FT-IR spectra reported in Fig. 2: PAN homopolymer (a) only exhibits the typical nitrile stretching at 2,244 cm$^{-1}$ and all the aliphatic CH and CH$_2$ bands at about 2,900 cm$^{-1}$. In addition to the cited bands, in (c) spectrum there is a large band at 1,731 cm$^{-1}$ due to C=O stretching of the methacrylic carboxyl group. P(AN-co-Aamide) (b) shows a large band at 1,682 cm$^{-1}$ due to stretching of the carbonyl group in the acrylamide monomer. Moreover, this copolymer shows large N–H stretching at 3,365 and 3,463 cm$^{-1}$. These bands further confirm the presence of the acrylamide units in the synthesized copolymer.

3.2. Membrane preparation and characterization

The solvent evaporation method used for membrane preparation allowed to obtain flat sheet samples with dense homogeneous structure in all cases. Fig. 3 shows SEM images of the cross section of P(AN-co-Aamide) blank and imprinted membranes. The other membranes show similar morphology without any substantial difference between blank and imprinted membranes.

All membranes exhibited water permeability values (ranging from 2.0 to 4.5 l m$^{-2}$ h$^{-1}$ bar$^{-1}$) typical of nanofiltration. No reduction in flux was observed for each sample before and after removal of the template with methanol and during binding tests. Table 2 reports permeability values and thickness of the prepared membranes.

3.3. Binding experiments

After removal of DMT, recognition capacities of imprinted membranes were evaluated by sorption experiments carried out in a dead-end filtration cell as described above. For comparison experiments using blank membranes prepared in the same conditions were performed. A water volume of 300 ml containing $2.2 \times 10^{-5}$ mol/L of DMT was filtered through the membrane at pressure of 10 bar. As expressed by Eq. (2), the difference between the amount of template present in the initial solution and that one present in the collected permeate at the end of the filtration allowed to determine the amount of pesticide retained by the membrane. The difference in dimethoate retention between an imprinted membrane and the corresponding blank gives the specific binding capacity. As it is shown in Fig. 4, PAN homopolymer and blank membranes exhibit similar dimethoate retention (ranging from 1.8 to 2.5 µmol/g memb.) which can be attributed to the non-specific interaction between target molecules and nitrile groups of the base polymer. On the other hand, all the imprinted membranes were found to possess affinity towards dimethoate, retaining them in different extent. In particular, P(AN-co-MAA) membrane exhibits the highest overall (10 µmol/g memb.) and specific (7.5 µmol/g memb.) binding capacity. P(AN-co-Aamide) membrane retained 6.5 µmol/g memb. and exhibited a specific binding of 4.2 µmol/g memb. Both, methacrylic acid and acrylamide functional monomers recognized dimethoate via hydrogen bonds. The donors of hydrogen bonds were the amide and the carboxyl groups of the own

Table 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Yield (%)</th>
<th>$[\eta]$ (ml/g)</th>
<th>$M_0 \times 10^{-3}$ (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN</td>
<td>82</td>
<td>279</td>
<td>167</td>
</tr>
<tr>
<td>P(AN-co-MAA)</td>
<td>72</td>
<td>413</td>
<td>279</td>
</tr>
<tr>
<td>P(AN-co-Aamide)</td>
<td>64</td>
<td>274</td>
<td>163</td>
</tr>
</tbody>
</table>
Fig. 2. FT-IR spectra of PAN (a), P(AN-co-Amide) (b) and P(AN-co-MAA) (c).

Fig. 3. SEM images of the cross-section of blank (a) and imprinted P(AN-co-Aamide) membrane.
copolymers and the acceptors were oxygen and sulphur groups of dimethoate. So, the specific recognition properties of the imprinted membranes resulted from the formation of recognition sites complementary to the dimethoate in the shape and in the positioning of the functional groups as the effect of the imprinting process during the membrane formation step. In fact, dimethoate was absent during the preparation of blank membranes, in which there were almost no specific binding sites.

P(AN-co-MAA) dimethoate-imprinted membrane was chosen to evaluate its selectivity properties performing binding experiments (carried out in duplicate) with aqueous solutions of trichlorphon, which is a structural analogue of dimethoate. The amount of trichlorphon retained and the selectivity factor (calculated according to Eq. (2)) are reported in Table 3.

Blank membrane exhibits similar retention for both dimethoate and trichlorphon which mean no selective binding sites were present in blank membranes. On the opposite, the retention of dimethoate onto DMT-imprinted membrane was higher than the structural analogue. The selectivity factor was 2.3.

4. Conclusions
Molecularly imprinted membranes using poly(acrylonitrile-co-methacrylic acid) and poly(acrylonitrile-co-acrylamide) copolymers were prepared via phase inversion technique using the organophosphorus pesticide dimethoate as template molecule. All membranes showed significant recognition properties compared with PAN homopolymer and blank membranes which only showed a poor non-specific binding. In particular, P(AN-co-MMA) dimethoate-imprinted membrane exhibited the highest overall (10 μmol/gₘemb.) and specific (7.5 μmol/gₘemb.) binding capacity. These results indicates that the carboxyl groups was more effective compared with the acrylamide functional monomer of the synthesized copolymers.

Furthermore, P(AN-co-MMA) dimethoate-imprinted membranes showed significant higher binding capacity to their corresponding template as compared with the structurally analogue compound trichlorphon, via the binding experiments. The selectivity factor was 2.3.

Results of this work suggest that the dimethoate-imprinted membranes could potentially be used for the removal of this pesticide from water.

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References


