



Development of imprinted materials for the selective extraction of nitroaromatic explosives

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ABSTRACT

Molecularly imprinted sorbents were synthesized and used as selective extraction sorbents for the analysis of nitroaromatic explosives. Their synthesis by radical polymerization using organic monomers and by sol-gel approach using organosilanes was considered to develop a selective sorbent. The sol-gel approach with phenyltrimethoxysilane (PTMS) as monomer and 2,4-dinitrotoluene (2,4-DNT) as template gave the most promising results. An optimized procedure adapted to the selective treatment of aqueous samples was then developed and applied to various target explosives. For the first time four nitroaromatic compounds were retained on the molecularly imprinted silica (MIS) with extraction recoveries between 29% and 81%, while only low recoveries were obtained on the non-imprinted sorbent, thus highlighting the high degree of selectivity. The MIS was then used for the clean-up of a sample containing motor oil spiked with 2,4-DNT and 2,4,6-trinitrotoluene (2,4,6-TNT). The results were compared with those obtained using a conventional sorbent (Oasis HLB). The cleanest chromatogram obtained using the MIS emphasized the high potential of the MIS as selective sorbent.

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1. Introduction

Nitroaromatic explosives, such as 2,4,6-trinitrotoluene (2,4,6-TNT), are among the most commonly used during bomb attack. The structure of the main nitroaromatic explosives is presented in Fig. 1. The identification of the explosives residues in a post blast sample is still an analytical challenge because they are present at trace level. Moreover, the samples can be complex and of various types, depending to the bombing sites. Therefore the analysis of explosive residues at trace level requires appropriate sample clean-up. This can be achieved using solid-phase extraction (SPE) sorbents [1–6]. However, despite the optimization of the extraction, the conventional sorbent co-extracts numerous interfering compounds, thus preventing the identification of target compounds. In order to improve the selectivity of sample treatment, selective materials as molecularly imprinted sorbents can be considered. These materials possess cavities designed for a target analyte thus providing a retention mechanism based on molecular recognition. In most of cases, molecularly imprinted sorbents for SPE are synthesized by radical polymerization of organic monomers to obtain a monolith. An alternative approach for the synthesis of imprinted sorbents is sol-gel polymerization [7–9]. In this case, inorganic-organic hybrid

material is obtained using organically modified silicas (ormosils) as monomers and alkoxysilanes as cross-linker in the presence of a template. The polymerization is made in aqueous media with an acid or a basic catalyst. Concerning the nitroaromatic explosives, a few publications have reported the synthesis of molecularly imprinted materials mainly by radical polymerization of organic monomers [10–17] but also by sol-gel approach [18–21]. Concerning MIPs prepared with organic monomers, methacrylic acid (MAA) [10,14–16] and acrylamide (AA) [10,12,13,16,17] were used as monomers and the synthesis was mainly carried out in nonprotic solvent and using mainly TNT as template molecule. MIPs obtained with MAA and AA were compared. The highest selectivity obtained using AA was highlighted [10,16] when developing sensors for detecting explosives vapors. In most of the case the selectivity of the resulting MIP was proven by measuring gas vapor uptake [10,11,15,16] or by studying binding properties at the equilibrium in pure media [12,13] by comparative studies with non-imprinted polymers thus demonstrating the presence of cavities. Concerning the sol-gel approach, TNT [19] or a functionalized TNT [18,21] for non-covalent and covalent sol-gel preparation, respectively were also used for the development of selective sensors in gas phase. Recently, an organosilica imprinted sorbent was synthesized via surfactant templating, this approach favoring open pore structure [20]. To our knowledge, this work corresponds to the first demonstration of the use of an imprinted sorbent as SPE sorbent for TNT extraction from water before its direct electrochemical detection

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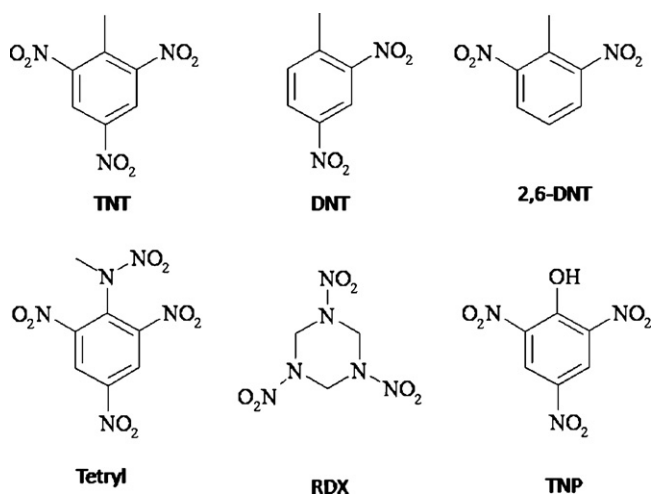


Fig. 1. Structure of nitroaromatic explosives.

without any intermediary separation step. This sorbent has shown high selectivity for 2,4,6-TNT but poor retention properties for RDX.

In conclusion, very different approaches of synthesis were reported for the synthesis of MIPs to be used as sensors mainly for the determination of a unique target analyte. Most of the evaluations were made in gas phase [10,11,15,16,18] or by binding in pure media [12,13,18,19].

The aim of this work was to synthesize a molecularly imprinted material to be used as SPE sorbent for the simultaneous selective extraction of several nitroaromatic explosives from post blast samples before their chromatographic analysis. The exploitation of the results previously described (sensors developments and binding experiments) is difficult to determine the best conditions to synthesize MIP for SPE purpose. Indeed, the expected selective interactions must be stronger than in gas phase (inert medium) to ensure the selective retention of the target analyte during the percolation of the sample. Moreover, the composition of real liquid samples conditions, the nature of the interactions that is different comparing to the nature of the involved interactions for a use in gas phase or in binding experiments in pure media. Several imprinted sorbents were then synthesized by radical polymerization using organic monomers and by sol-gel approaches with ormosils. These sorbents were further evaluated by studying the retention of 2,4-DNT and 2,4,6-TNT. Non-imprinted sorbents were synthesized and used in parallel for assessing the selectivity of the retention mechanism involved by the imprinted sorbents. The different parameters affecting the extraction recoveries in a SPE process such as the capacity, nature and volume of the percolation and of washing fractions were studied using the most promising sorbent. This sorbent was then applied to the clean-up of simulated samples made of motor oil to check its potential for the treatment of complex samples.

2. Experimental

2.1. Chemicals

Solid 2,4-dinitrotoluene (2,4-DNT) and 2,4-diaminotoluene (DAT) were from Sigma–Aldrich (St Quentin Fallavier, France), 2,6-dinitrotoluene (2,6-DNT) was from CIL (Courbevoie, France) and 2,4,6-trinitrophenol (TNP) was from Merck (Fontenay sous bois, France).

HPLC-grade acetonitrile (ACN), methanol (MeOH), dichloromethane (DCM) and isopropanol (IPA) were from Carlo Erba (Val de Reuil, France). Acetic acid (MeCOOH) and ammoniac

were from Merck. High purity water was obtained from a Milli-Q purification system (Millipore, Saint-Quentin en Yvelines, France).

2,4,6-Trinitrotoluene (2,4,6-TNT), 2,4,6-N-tetranitromethylaniline (tetryl) and cyclotrimethylenetrinitramine (RDX) were from Sigma–Aldrich. Solutions of 100 mg/L were obtained by dilution in acetonitrile from the standard solutions (1 g/L). These solutions were stored at 4 °C.

Methacrylic acid (MAA) was distilled under reduced pressure and ethylene glycol dimethacrylate (EGDMA) was washed consecutively with 10% NaOH, water and brine, and then dried over MgSO₄, filtered and distilled under reduced pressure. Acrylamide (AA), 2-vinylpyridine (2-VP), styrene, divinylbenzene (DVB), phenyltrimethoxysilane (PTMS), tetraethoxyorthosilane (TEOS) and azo-N,N'-diisobutyronitrile (AIBN) were used as received. All these polymerization chemicals were from Sigma–Aldrich.

2.2. Analytical conditions

The HPLC system consisted of an autosampler (715 Ultra Wisp Waters, St Quentin en Yvelines, France), a solvent delivery system (LC-10AD) and a diode array detector (SPD-M10A) (Shimadzu, Croissy-Beaubourg, France). Compounds were monitored at 247 nm for 2,4-DNT and RDX, 228 nm for 2,4,6-TNT and Tetryl, 210 nm for TNP and 235 nm for 2,6-DNT. The reversed-phase column was a Symmetry Shield RP18 (250 mm × 3 mm i.d., 5 μm, Waters) connected to a precolumn filter (2 μm frit, Interchim, Montluçon, France). The mobile phase consisted of water/MeOH mixture (40/60, v/v). The flow rate was set at 0,4 mL min⁻¹ and the sample injection volume was 20 μL.

2.3. Synthesis of MIPs by radical polymerization of organic monomers

Seven MIPs (1, 2, 3, 4, 5, 6 and 7) were synthesized by bulk polymerization with the following procedure using the reagents presented in Table 1. The template (0.5 mmol), the initiator (AIBN), the monomer (2 mmol) and the cross-linker (10 mmol) were added to 2.8 mL of solvent (except for chloroform, 5.6 mL) and the solution was transferred to a glass tube. The polymerization mixture was placed in an ice bath and degassed with nitrogen for 10 min. The tube was sealed and transferred to a water bath at 60 °C for 24 h. After polymerization, the tube was crushed and the polymer was ground and sieved to obtain particles mainly in the 25–36 μm size range. Then, a sedimentation with MeOH/water mixture (80/20, v/v) was performed to remove fine particles. 50 mg of MIP was packed into a 1 mL disposable cartridge between two frits and the template was removed from the sorbent with MeOH. Non-imprinted polymers (NIPs) were obtained by performing the same procedure in the absence of the template.

2.4. Synthesis of MIS by sol-gel approach

A MIS sorbent was also synthesized. For this, the template (2,4-DNT, 0.5 mmol), PTMS (0.5 mmol) and TEOS (2.4 mmol) were added to 1 mL of water with 200 μL of ammonia. The tube was placed in an

Table 1
Reagents used to synthesized MIPs.

MIP	Template	Monomer	Cross-linker	Solvent
MIP 1	DNT	2-VP	EGDMA	ACN
MIP 2	DNT	MAA	EGDMA	ACN
MIP 3	DNT	AA	EGDMA	ACN
MIP 4	DNT	MAA	EGDMA	DCM
MIP 5	DNT	AA	EGDMA	Chloroform
MIP 6	DNT	MAA	DVB	DCM
MIP 7	DAT	Styrene	DVB	MeOH

oil bath at 40 °C for 24 h. After polymerization, the tube was placed in an oven at 120 °C for 18 h. The sorbent was ground, sieved and the sedimentation was performed in the same conditions as previously described for MIPs. 50 mg of the resulting MIS was packed into a 1 mL disposable cartridge between two frits and the template was removed from MIS by several washing solvents: MeOH (45 mL), acetonitrile (40 mL), dichloromethane (10 mL) and IPA (20 mL) and then 30 mL of MeOH/MeCOOH (95/5, v/v) to totally remove the template molecule. A non-imprinted silica (NIS) was obtained by performing the same procedure in the absence of template.

2.5. Evaluation of the imprinted materials

For MIPs 1–6, a conditioning step with 5 mL of porogen solvent was done before each use. 1 mL of the porogen solvent spiked with 2 µg of 2,4-DNT for MIPs 1, 2 and 4, and 500 ng for MIPs 3, 5 and 6, was percolated through the cartridges. 1 mL of porogen/MeOH (90/10, v/v) mixture was then percolated through the sorbent for the washing step. The target analyte was then eluted from the MIPs with 1 mL of MeOH. Percolation and washing fractions were directly analyzed by LC, except the dichloromethane and chloroform fractions, which were concentrated to dryness under a nitrogen stream and dissolved in 500 µL of a water/MeOH (50/50, v/v) mixture. Elution fractions were dissolved in 1 mL of water before injection in LC.

MIP 7 was conditioned with 5 mL of water before each use. Then, 1 mL of water spiked with 500 ng of 2,4-DNT or 2,4,6-TNT was percolated through the MIP. The washing step was carried out with 1 mL of a water/MeOH mixture (40/60, v/v). The elution step consisted of the percolation of 1 mL of MeOH followed by 1 mL of MeOH/MeCOOH mixture (95/5, v/v). The resulting fractions were directly injected in LC except the elution fractions, which were previously diluted in 1 mL of water.

The MIS was conditioned with 5 mL of water. 1 mL of water spiked with 500 ng of 2,4-DNT and 2,4,6-TNT was percolated and washing steps were carried out with 500 µL to 1 mL of water/MeOH (40/60) mixture. Finally, the elution step was achieved with 2 mL of MeOH. The percolation and washing fractions were directly analyzed in LC except the elution fractions, which were diluted in 2 mL of water before LC injection.

2.6. Selective clean-up of motor oil extract by MIS

Four drops of motor oil were withdrawn from a clean piece of aluminum foil using a cotton swab previously wetted with a water/MeOH mixture (50/50, v/v) in order to obtain a simulated sample. This cotton swab was further extracted with 10 mL of the same water/MeOH mixture as the procedure previously described [6]. The resulting extract was filtered, spiked with 50 ng of 2,4-DNT and 50 ng of 2,4,6-TNT and further diluted with 40 mL of water.

15 mL of the extract were collected and percolated through the MIS. After a washing step using 750 µL of water/MeOH mixture (40/60, v/v), the MIS was dried for 5 min under vacuum before the elution step using 2 mL of MeOH. The eluate was evaporated to dryness under a nitrogen stream, dissolved in 300 µL of a water/MeOH mixture (50/50, v/v) and injected in LC/UV.

To assess the selectivity of the MIS, the same aqueous extract was percolated through a conventional sorbent. The SPE procedure involving the use of an Oasis HLB polymer (Waters) was previously described [6]. 60 mg of sorbent was conditioned with 1 mL of acetonitrile, 1 mL of MeOH and 1 mL of water. After this conditioning step, 15 mL of the aqueous extract was percolated through the cartridge followed by 1 mL of water/MeOH (50/50, v/v) mixture as washing step. The sorbent was dried for 5 min under vacuum before the elution of explosive compounds with 1.5 mL of acetonitrile. The elution fraction was evaporated to dryness under a nitrogen

stream, dissolved in 300 µL of a water/MeOH mixture (50/50, v/v) and injected in LC/UV.

3. Results and discussion

3.1. Characterization of MIPs obtained using organic monomers

Selective retention on imprinted material depends on the structural complementarity between the cavities formed during the polymerization step and the target analyte. It also depends on the nature and the strength of the interactions that take place between this analyte and the monomer residues. Most MIPs used in SPE have been prepared using basic or acid monomers in order to form strong hydrogen bonds or electrostatic interactions with acid or basic analytes, respectively. For nonpolar analytes, neutral monomers have also been used to form strong hydrophobic interactions [22]. Taking into account the lack of functionalities in the structure of nitroaromatic explosives allowing well defined and strong interactions with monomers, different types of monomers were used in order to determine which types of selective interactions can be favored.

Previous works [10,12,13,15,16] have already shown the possibility of forming cavities by using the 2,4,6-TNT as template with the MAA or the AA as monomer, EGDMA as cross-linker and acetonitrile or chloroform as solvent. A first series of experiments consisted of the bulk synthesis of various MIPs with organic monomers favoring the formation of polar interactions with the target analyte selected. As mentioned in Table 1, MIP 1, MIP 2 and MIP 3 were synthesized with 2-VP, MAA and AA as monomers, with 2,4-DNT as template and EGDMA as cross-linker. The porogen, acetonitrile, is a nonprotic and moderately polar solvent that was selected to favor the formation of polar interactions between the monomers and the template. Concerning the development of the SPE procedure, the porogen was chosen as percolation solvent to favor the establishment of the same selective polar interactions previously developed during the synthesis. Acetonitrile spiked with 2,4-DNT was then percolated on 50 mg of the imprinted material. A washing solution containing acetonitrile with 10% of MeOH was applied to remove the nonspecific interactions, i.e. to disrupt polar interactions formed out of cavities. Finally, the elution step was carried out with pure MeOH to totally disrupt the interactions between the analyte and the polymer. These experiments were carried out in parallel on the NIP to assess the selectivity of the imprinted material. Unfortunately, the application of this procedure to MIPs 1, 2 and 3 led to a loss of 80% of 2,4-DNT during the percolation step. The same behavior was observed with the corresponding NIPs, thus demonstrating the lack of specific cavities in both MIPs but also the weakness of the polar interactions formed between the functional group of the 2,4-DNT and the monomers thus preventing the formation of well-designed cavities during the polymerization.

Others MIPs (MIPs 4 and 5) were prepared with MAA and AA as monomer in dichloromethane and chloroform, respectively, because these nonprotic solvents are less polar than acetonitrile, thus favoring more the formation of polar interactions during the polymerization. Once again, the same elution profiles were obtained, thus confirming the difficulty of forming selective cavities when favoring polar interactions during the polymerization step. Those results also confirm that, despite the possibility to develop imprinted material for sensors purposes with monomers such as MAA and AA, those conditions are not adapted to develop SPE sorbents. The strength of the interactions should be very high to ensure a sufficient retention during the percolation step.

Another attempt was made for MIP 6 by replacing the EGDMA by DVB to favor π - π interactions during retention on the polymer. When applying the same extraction procedure, 2,4-DNT was only slightly retained on the MIP: a loss of 2,4-DNT of around 60% for the

MIP and around 80% for the corresponding NIP was observed during the percolation step. The use of DVB therefore allows a slightly higher retention of 2,4-DNT on the MIP than EGDMA and involves the formation of specific cavities due to the presence of π - π interactions. However, the degree of selectivity obtained with MIP 6 was still insufficient to consider further experiments.

As nitroaromatic compounds have an aromatic cycle involving hydrophobic properties as well, another MIP (MIP 7) synthesized with hydrophobic reagents was prepared. Styrene and DVB were then chosen as monomer and cross-linker, respectively to favor the development of hydrophobic interactions associated to π - π interactions. It is important to note that the nitroaromatic compounds can inhibit styrene polymerization due to the nitro groups on the aromatic cycle which can form radicals. Polymerization of styrene using AIBN as initiator in the presence of nitroaromatic compounds is delayed [23]. Therefore, a structural analog as template was preferred to synthesize this MIP 7 using styrene as monomer. The dummy molecule should be similar to 2,4-DNT in terms of shape, size and functionalities. 2,4-Diaminotoluene (DAT) was chosen to replace 2,4-DNT because it also possesses an aromatic ring to favor hydrophobic interactions. Obviously, the synthesis of MIP 7 was achieved in a polar solvent, i.e. methanol, to favor the formation of hydrophobic interactions between the template and monomers. To evaluate the selectivity of this MIP, 1 mL of water spiked with 2,4-DNT or 2,4,6-TNT was percolated through MIP 7 followed by a washing step with 1 mL of water/MeOH mixture (40/60, v/v). Two elution steps using MeOH and MeOH/MeCOOH mixture (95/5, v/v) were finally carried out. 2,4-DNT extraction recovery was 91% on MIP 7, compared with 59% on NIP. 2,4,6-TNT extraction recovery was 78% on MIP and only 30% on NIP. These results prove that cavities formed with DAT allow selective retention of 2,4-DNT and 2,4,6-TNT by hydrophobic interactions. However, a second synthesis was carried out in the same conditions and a very poor repeatability was obtained for the elution profile. To obtain the same profile, lower elution strength was required for the washing step, i.e. 50% of MeOH instead of 60%, thus highlighting the difficulty of creating well-defined cavities using these synthesis conditions. In conclusion, these results highlight the difficulty for obtaining an imprinting effect with the target analytes, i.e. 2,4-DNT, or a dummy molecule, i.e. DAT. MIPs made with MAA or AA as used for sensors or binding experiments did not lead to a sufficient selective retention. Only the use of styrene and DVB for the synthesis of MIP allowed a slight selective retention. Therefore, another strategy was investigated by changing the polymerization mode. Indeed, a approach by sol-gel mode was considered.

3.2. Evaluation of MIS

A MIS was prepared using an organically modified organosilane monomer that possesses a phenyl group to form hydrophobic bonds and π - π interactions with the target analyte during the synthesis in aqueous media. 2,4-DNT was used as template molecule

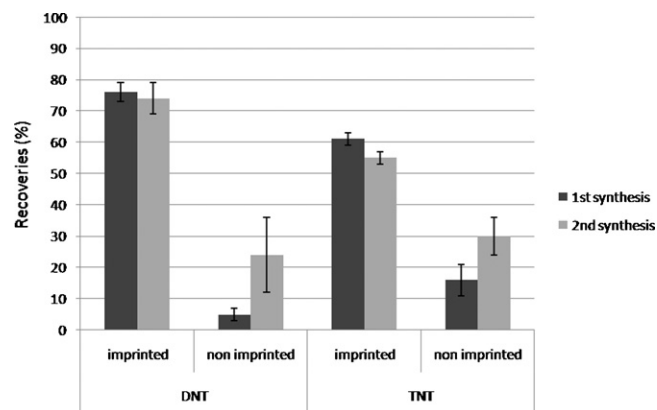


Fig. 3. Elution profiles obtained for 50 mg of MIS and of NIS for two distinct syntheses (see figure 2 for the extraction procedure, $n = 5$).

and an alkoxysilane (TEOS) was used as cross-linker. The catalysis of the reaction was achieved in basic conditions. A NIS was synthesized in parallel using the same procedure in the absence of the template. The evaluation was made in aqueous media to favor a retention mechanism based on hydrophobic interactions. Water spiked with 500 ng of 2,4-DNT or 2,4,6-TNT was percolated through the MIS. After a washing step with 1 mL of water/MeOH (40/60, v/v) mixture, the elution of 2,4-DNT or 2,4,6-TNT was carried out with 1 mL of MeOH. The same experiments were carried out in parallel on the NIS. The elution profile obtained for 2,4-DNT is reported in Fig. 2A. An extraction recovery of $76 \pm 3\%$ ($n = 5$) was obtained for 2,4-DNT on the MIS, while only $5 \pm 2\%$ ($n = 5$) was retained on the NIS. These results emphasize the high potential of the MIS as selective sorbent. The low relative standard deviation (RSD) demonstrates the good repeatability of the extraction procedure. For the 2,4,6-TNT, in the same conditions, the extraction recoveries were around 40% on the MIS and 4% on the NIS (results not shown), thus demonstrating the ability of the MIS to selectively recognize this related structural compound. The use of a reduced volume of washing (500 μ L instead of 1 mL) led to $61 \pm 2\%$ ($n = 5$) extraction recovery for 2,4,6-TNT on MIS and only $16 \pm 5\%$ ($n = 5$) on the NIS as illustrated in Fig. 2B. So, the MIS prepared with 2,4-DNT as template allows the selective extraction of the 2,4,6-TNT, but with a lower retention, which can be explained by the steric hindrance caused by the additional nitro group in the TNT structure. Consequently, 2,4,6-TNT ($\log K_{ow} = 1.6$) is slightly more polar than 2,4-DNT ($\log K_{ow} = 1.98$), which can also explain the lower retention of 2,4,6-TNT, which forms weaker hydrophobic interactions than 2,4-DNT.

To confirm the selective potential of the MIS for 2,4-DNT and 2,4,6-TNT, a second MIS (MIS') was synthesized at a different time and evaluated in the same conditions. Fig. 3 shows the comparison of the average extraction recoveries obtained after five extractions on both imprinted sorbents (MIS and MIS') and on the correspond-

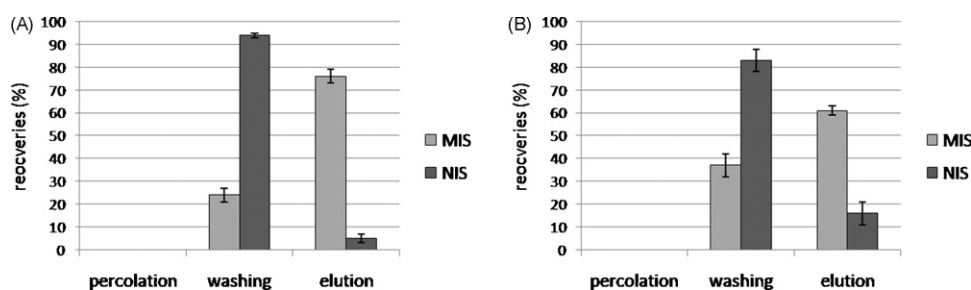


Fig. 2. Elution profiles obtained for 50 mg of MIS and of NIS, after the percolation of 1 mL of water spiked with 500 ng of 2,4-DNT (A) or of 2,4,6-TNT (B); washing step: 1 mL or 500 μ L of water–MeOH (40/60, v/v) for 2,4-DNT (A) or 2,4,6-TNT (B), respectively; elution: 2 mL of MeOH ($n = 5$).

ing non-imprinted sorbent (NIS and NIS'). The extraction recoveries obtained on the imprinted materials of each synthesis were similar, thus confirming that both MISs present similar performances. Despite the higher recoveries obtained on NIS' than on NIS, the recoveries on the non-imprinted sorbent are still low (lower than 30%). Additional syntheses will be required to confirm the real reproducibility of the MIS synthesis but those first encouraging preliminary results confirm the selectivity of the obtained MIS.

Previous results have shown that 2,4-DNT and 2,4,6-TNT can be selectively retained on MIS with high extraction recoveries. However, different conditions of washing were applied depending on the analyte: 500 μL of washing solution for 2,4,6-TNT and 1000 μL for 2,4-DNT. To allow the simultaneous extraction of both nitroaromatics, the washing procedure was optimized. The elution profiles obtained on MIS and on NIS using 750 μL of water/MeOH mixture (40/60, v/v) as washing solvent and 2 mL of MeOH as elution solvent are shown in Fig. 4. Extraction recoveries were $80 \pm 4\%$ and $46 \pm 5\%$ on MIS and $15 \pm 1\%$ and $8 \pm 1\%$ on NIS for 2,4-DNT and 2,4,6-TNT, respectively ($n = 3$).

This procedure was also applied to the other nitroaromatic explosives, i.e. 2,6-DNT, tetryl, RDX and TNP. Indeed, the aim of this work was to provide a sensitive and selective method to detect several nitroaromatic explosives after a terrorist attack. Elution profiles for those compounds are also reported in Fig. 4. For 2,6-DNT, extraction recoveries of about $68 \pm 3\%$ ($n = 3$) on the MIS and $26 \pm 5\%$ ($n = 3$) on the NIS were obtained. Concerning tetryl, extraction recoveries of $29 \pm 3\%$ ($n = 3$) were obtained on the MIS and only $8 \pm 2\%$ ($n = 3$) on the NIS. These results confirm the presence of cavities designed for 2,4-DNT that allow the selective retention of 2,6-DNT and tetryl. However, the lower retention of tetryl on MIS and NIS can be explained by the size and polarity of this compound. Tetryl is larger and slightly more polar ($\log K_{ow} = 1.64$) than 2,4-DNT. RDX was retained during the percolation but was recovered in the washing step, and TNP was not retained. Almost 100% of TNP was recovered in the percolation and washing steps. The low retention of RDX ($\log K_{ow} = 0.87$) and TNP ($\log K_{ow} = 1.10$) on the MIS can be explained by the size and by the weak hydrophobic properties of these compounds compared with 2,4-DNT. In conclusion, this sol-gel allows the selective extraction of tetryl, 2,4,6-TNT and its manufacturing intermediates, i.e. 2,6-DNT and 2,4-DNT. These first results are very promising because until now no imprinted

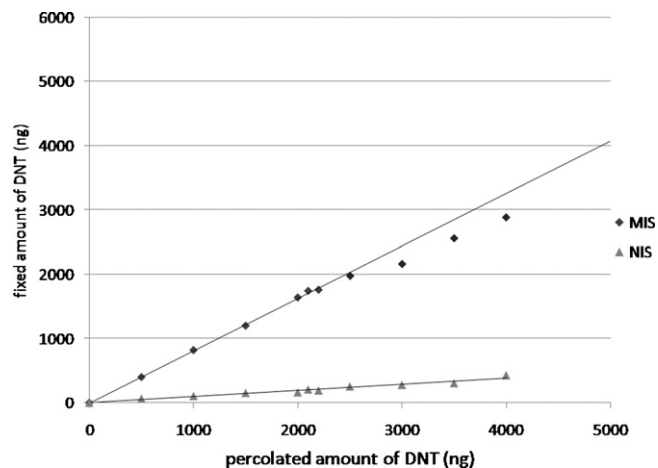


Fig. 5. Calibration curves obtained after the percolation of 1 mL of water spiked with increasing amounts of 2,4-DNT on the MIS and on the NIS (50 mg).

materials have been developed for the selective extraction of these compounds and then for the clean-up of complex samples before LC/MS analysis.

The complete characterization of the MIS requires the evaluation of the capacity value, i.e. the maximum amount of a compound that can be selectively retained by the imprinted sorbent. This was done by measuring the amount of 2,4-DNT retained on the MIS and on the NIS after the percolation of aqueous samples spiked with various amounts of 2,4-DNT, when applying the extraction procedure previously described (750 μL of the washing solution). Fig. 5 presents the resulting curves obtained with the MIS and the NIS. The curve related to the use of the MIS is characterized by a linear portion up to 2500 ng of spiked 2,4-DNT which corresponds to the concentration range that allows a constant extraction recovery of 82% for 2,4-DNT (slope of 0.82). The low retention of 2,4-DNT on the NIS is confirmed by a constant of recovery of 13% (slope of 0.13) for the whole studied mass range, which highlights again the real specificity of the binding sites already illustrated by the elution profiles of Fig. 4. The extraction recoveries obtained on the MIS slightly decreased for samples spiked with amounts higher

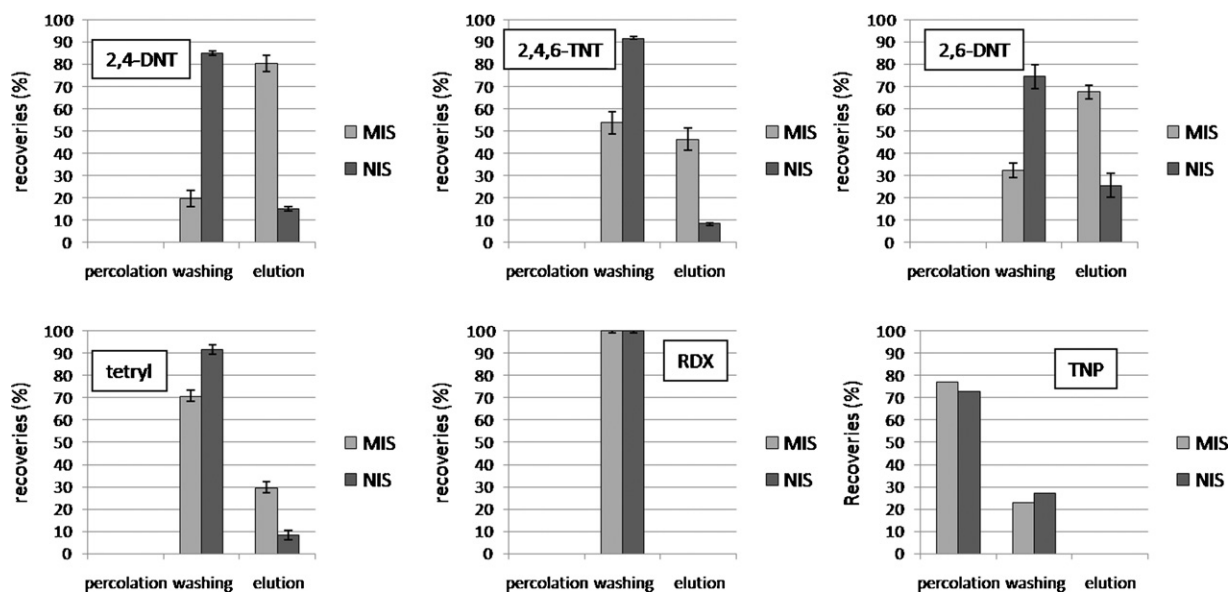


Fig. 4. Elution profiles obtained for 50 mg of MIS and of NIS, percolation of 1 mL of water spiked with 500 ng of 2,4-DNT, 2,4,6-TNT, 2,6-DNT, tetryl, RDX or TNP; washing step: 750 μL of water–MeOH (40/60, v/v); elution: 2 mL of MeOH ($n = 1$ for TNP and $n = 3$ for the other analytes).

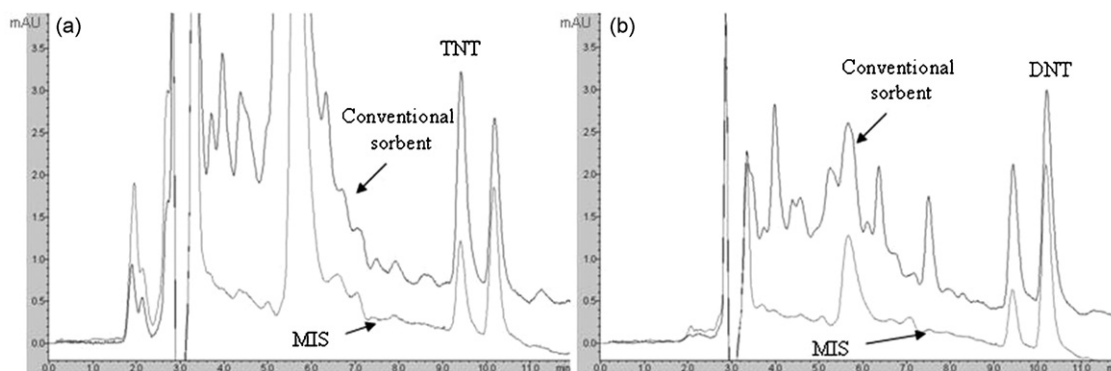


Fig. 6. Chromatograms corresponding to the LC/UV analysis of the elution fraction obtained after percolation on MIS or on Oasis HLB of a simulated sample contaminated by motor oil. (a) Detection at 228 nm. (b) Detection at 247 nm.

than 2500 ng on 50 mg of MIS, i.e. 50 μg of 2,4-DNT per gram of MIS. This decrease is explained by the saturation of the specific binding sites. Because of the heterogeneous sites distribution of the polymeric material, other recognition sites of lower energy and lower specificity are involved in the extraction processes when the spiked amount is higher than 2500 ng of MIS. The high capacity, i.e. 50 $\mu\text{g/g}$, demonstrates also the potential for using MIS in miniaturized devices.

3.3. Application of the MIS to the clean-up of simulated samples

In order to complete the evaluation of the selective potential of this MIS, it was applied to the clean-up of a contaminated sample. Simulated samples were used because no samples collected on real bombing sites were available. Motor oil was chosen to simulate the post blast sample [2,6] because it is one of most complex matrices that can be found on bombing sites. Moreover this matrix is commonly used as binder during the conception of the bomb therefore it is often contained in the samples. Four drops of motor oil were withdrawn from aluminum foil with a cotton swab previously wetted with a water/MeOH (50/50, v/v) mixture. This cotton swab was further extracted with 10 mL of the same mixture. The solution resulting from this treatment was then spiked with 2,4-DNT and 2,4,6-TNT in order to consider only the extraction recoveries of the SPE procedure and not the recoveries of the whole procedure including the contribution of the cotton swab extraction. Finally, this spiked solution was diluted in 40 mL of water. As the resulting extract contains 10% of MeOH, the possible negative effect of MeOH on the extraction recoveries of the MIS was first studied. Elution profiles on MIS and NIS were studied by percolating 1 mL of a water/MeOH (90/10, v/v) mixture spiked at 1 μg of 2,4-DNT and by applying the extraction procedure previously described. The resulting elution profiles obtained in these conditions were exactly the same than those obtained after the percolation of pure aqueous samples (data not shown). The degree of selectivity was therefore unchanged in the presence of 10% of MeOH. We also checked whether a higher volume of water/MeOH (90/10, v/v) mixture could be percolated on the MIS without affecting its performance in terms of extraction recoveries. Various volumes of the water/MeOH mixture (1, 5, 10, 20, 30, 40 and 50 mL) were percolated through the MIS. Extraction recovery was $81 \pm 3\%$ for all experiments, meaning that the breakthrough volume was not reached after the percolation of up to 50 mL of aqueous sample containing 10% of MeOH. In conclusion, a large volume of extract can be percolated on the MIS without any loss of retention or selectivity.

To save time, only 15 mL of the 50 mL extract of the simulated sample were percolated on MIS. In parallel, 15 mL of the same extract was extracted on a conventional polymeric sorbent in order to evaluate the real performance of the MIS for the clean-up

complex samples by comparing the results. Oasis HLB was chosen because in a precedent study [6] some conventional sorbent were compared (a C18 silica sorbent and several polymers of various specific surface areas) and it was shown that this sorbent was the most efficient to concentrate and clean-up the organic explosives. The SPE procedure applied to this sorbent was previously optimized [6] and consisted of the percolation of 15 mL of the samples through the Oasis HLB sorbent followed by a washing step with a water/MeOH mixture (50/50, v/v) to remove interfering compounds and an elution step with acetonitrile. Fig. 6 shows the chromatograms obtained by LC/UV analysis using Oasis HLB and the MIS. The recoveries obtained on the MIS for 2,4-DNT and 2,4,6-TNT were close (85% and 55%, respectively) to the recoveries obtained by percolating pure water/MeOH mixtures. Therefore, the matrix components do not affect the retention of the two analytes. Moreover, the comparison of the chromatograms of Fig. 6 shows that a cleaner baseline is observed for the MIS than when using the conventional SPE sorbent. This result confirms the high potential of the MIS as selective sorbent. Therefore, quantification by mass spectrometry to improve the sensitivity of the method is easily conceivable after sample pre-treatment by MIS, while ion suppression may affect quantification of target analytes after sample treatment by conventional sorbent due to numerous interfering compounds, despite the higher recovery obtained for 2,4,6-TNT [6]. The MIS results for the extraction of 2,4-DNT and 2,4,6-TNT from complex matrices are therefore very promising.

4. Conclusions

Several syntheses of molecularly imprinted materials are presented in this study. Acidic or basic organic monomers did not allow selective retention by polar interactions. Selective cavities were obtained by using a hydrophobic monomer. However, the sorbent synthesized by sol-gel approach gave the best results in terms of selectivity. This MIS synthesized with 2,4-DNT as template, PTMS as hydrophobic monomer and TEOS as cross-linker in basic aqueous media was the most selective sorbent obtained during this work. This sorbent showed high selectivity for 2,4-DNT, 2,4,6-TNT and 2,6-DNT. Finally, the extraction of a simulated sample on MIS allows the removal of more interfering compounds than an extraction on a conventional sorbent. Therefore, the use of MIS for real samples is very promising and may constitute a very helpful selective sorbent for LC/MS analysis. This work shows the possibility to simultaneously extract the nitroaromatic explosives. However, the optimization of the MIS synthesis will certainly improve the selectivity and the cross-reactivity for the extraction of all nitroaromatic compounds on the MIS. The optimization can be also combined with the control of the pore size distribution in order to ensure a high reproducibility of the sol-gel process.

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