RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Molecularly Imprinted Polymer with Flash column Chromatography for the Selective and Continuous Extraction of Diphenyl Amine

Pankaj E. Hande, Asit B. Samui*, Prashant S. Kulkarni*

Energy and Environment Laboratory, Department of Applied Chemistry, Defence Institute of Advanced Technology (Deemed University), Ministry of Defence, Pune-411025, India

Ph: 91-20-24304161

Corresponding authors: ABS: absamui@gmail.com; PSK: ps_kulkarni@rediffmail.com

Abstract

The aim of this study was to prepare MIP for the selective recognition of DPA and its combination with flash column chromatography for the continuous extraction of DPA from ammunition waste. The DPA-MIP was prepared by free radical polymerization using methacrylic acid as a complexing monomer and EGDMA as a crosslinker. Methanol and acetic acid (9:1) were used as a leaching agent for DPA. MIP showed maximum adsorption capacity at pH 4. The maximum experimental adsorption capacity obtained for MIP and NIP particles were 31 mg g^{-1} and 10.2 mg g^{-1}, respectively. Langmuir and Freundlich adsorption isotherm models were used to analyze the experimental data of DPA adsorption on MIP. Freundlich adsorption isotherm model was very well fitted with this. Pseudo first order and pseudo second order kinetic models were used to estimate corresponding rate parameters, equilibrium capacities and correlation coefficients. Selectivity coefficients of MIP for DPA in presence of NDPA, TNT and Tetryl were 23.14, 47.07 and 52.30, respectively. Continuous extraction of DPA was done with help of flash chromatography combined with MIP and showed more than 98 % recovery. MIP

has good regeneration performance and could maintain almost same adsorption capacity even after three adsorption-desorption cycle.

1. INTRODUCTION

Diphenyl amine (DPA) is widely used as a stabilizer in nitrocellulose containing propellants (single or multi-base propellants) in missiles and other explosives.[1] In nitrocellulose based propellants, formation of degradation products such as NO, NO_2 and HNO_3 takes place, which are responsible for further degradation of propellants. DPA has ability to bind these degradation products through nitration. Thus, it results in formation of mono, di and tri nitro compounds of DPA. There are different derivatives of DPA which have been used as a stabilizer such as ‘acardites’ and ‘centralites’.[2] Apart from military applications, it is also reported to be used in production of dyes, pharmaceuticals, as a pre or post-harvest scald inhibitor in apples because of its antioxidant properties.[3]

In 1980, worldwide production of DPA was 40000 t.[3] Because of extensive use in ammunition, DPA causes contamination of soil and water. DPA is considered as toxic organic persistent because of its low solubility in water. DPA poisoning leads to bladder injury, hematuria, proteinuria, increased blood pressure and heart rate, eczema, tachycardia, hypertension, cough, sneezing, methemoglobinemia, etc.[4] DPA is toxic to the aqueous organisms as well.[5] Thus, DPA has been placed in the third Europian Union (EU) list of priority pollutants. There are several methods which have been used for the determination of DPA like Gas chromatography[6], gas chromatography mass spectroscopy[7], High performance liquid chromatography[8], spectrophotometry[9], etc. at lower concentration level. Selective extraction of DPA is a challenging task because of its low percentage in nitro explosives.

Molecularly imprinted polymer (MIP) is a technology used to create selective recognition sites in a macromolecular matrix using target molecule.[10,11] MIPs are nothing but the key and lock type of model developed by using template, monomer, crosslinker and initiator. There are two different approaches, covalent[12] and non-covalent (self assembly)[13] in development of MIP. It is a cheap material known for ease of preparation, high selectivity and good thermal stability. Therefore, imprinted polymers are reported to be used in wide applications such as sensing, extraction, preconcentration of organics as well as inorganics.[14–17] There are various modification have been done in MIP to improve its efficiency.[18–22]

Recently MIP has been used for the selective determination of DPA in apple juice samples using electrochemical cell based sensor.[23,24] There wasn't any correspondence between the equilibrium re-binding studies and potentiometric behaviour of the sensors.[23] In other article, thickness of the electrode has to be optimized which reflects its limitation.[24] The use of conducting polymer in electrode makes it sensitive to various chemicals. There is a requirement of technique which is for the determination as well as extraction of DPA simultaneously. Combination of MIP and flash column chromatography is found to be very efficient technique for the selective extraction of organic molecules.[25]

The purpose of the present study was the preparation of MIP using diphenylamine (DPA) as template and its application as an active substance for solid phase extraction in a flash column chromatography. The MIP was prepared by using previously reported template, monomer and initiator except the crosslinker and polymerization process.[23] We have extensively studied DPA-MIP including polymer synthesis, characterization, effect of pH, adsorption isotherm, kinetics and selectivity. The adsorption of DPA was observed at the same pH as reported.[23] No reports on extraction of DPA from ammunition waste using polymers imprinted with DPA were found in the literature. This is the first time, we report MIP in combination with flash chromatography technique for the continuous extraction and determination of DPA.

2. EXPERIMENTAL

2.1 Instruments and apparatus

FTIR (Bruker ALPHA) with Zn-Se ATR crystal and gold coated mirror, monitored at 4 cm^{-1}(resolution with 25 scans). All FTIR were recorded neat. To study the surface morphology, Field emission scanning electron microscopy (FE-SEM) was used (Carl Zeiss Sigma at 2.50 kv EHT and 3.6 mm of WD). Thermal stability of the polymer was analyzed by using thermogravimetric analysis (TGA) (Perkin Elmer 1061608). For the quantitative analysis of DPA, UV visible spectrophotometry (Analyticjena, SPECORD 210) (Made in Germany) was used. DPA was calibrated by using UV visible spectrophotometry as shown in **figure 1S**. Specific surface area of the polymer particles were determined by Thermo-scientific Surfer instrument at 77 K by nitrogen adsorption using Brunauer-Emmett-Teller (B.E.T) surface area analysis method.

High performance liquid chromatography (HPLC) (Hitachi, column-chromatographic reversed phase C18 column with size of 25 cm × 4.6 mm, 5 µm at 25 °C) fitted with UV detector was used at a wavelength of 225 to 300 nm range. The analytes were eluted at a flow rate of 0.4 ml min^{-1}, using acetonitrile and water mixture (80/20; v/v) as a mobile phase.

Flash column chromatography (Bonna-Agela India pvt. ltd.) with UV detector at a wavelength of 280 nm and column of 8 mm in diameter and 7 cm in length were used for the determination and extraction of DPA. Some other instruments used are table top centrifuge (KUBOTA 4200), orbital shaker (Lab companion) and pH meter (HANNA Instruments).

2.2 Reagents

Diphenyl amine (DPA), 2-nitro diphenyl amine, (NDPA), methacrylic acid (MAA), Ethylene glycol dimethacrylate (EGDMA), azobisisobutyronitrile (AIBN), acetonirtile (LR grade and HPLC grade), methanol acetic acid and buffer tablets (pH 4 and 7), all the chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Deionized water was used in preparation of all solutions.

2.3 Polymer synthesis

Molecularly imprinted Polymer was synthesized by using free radical polymerization method.[26] For the synthesis of polymer, DPA was used as a template, methacrylic acid (MAA) as a monomer, Ethylene glycol dimethacrylate (EGDMA) as a crosslinker, AIBN as a radical initiator and acetonitrile as a solvent. 1 mmol of DPA and 4 mmol of MMA were mixed together in 40 ml of acetonitrile for half an hour. Then 20 mmol of EGDMA and 100 mg of AIBN were added into it. The reaction mixture was heated at 70 °C under nitrogen atmosphere. After precipitation, polymer particles were separated by using filtration. Polymer particles were ground in a mortar and dried in an oven at 50 °C for two hours. About 4 g of the resultant polymer particles were subjected to Soxhlet extraction to remove template by using a solvent mixture of methanol and acetic acid (9:1, v/v). Around 200 ml of methanol in acetic acid was used for the leaching study. Leaching was done until DPA could not be detected by using U.V. visible spectroscopy. For the complete removal of template it took around 24 hours. By using same method non imprinted polymers (NIP) have been synthesized in absence of DPA. All the studies of MIP and NIP were done at room temperature.

2.4 Optimization of pH and batch adsorption experiments

Adsorption of template on MIP depends on pH. Thus, adsorption of DPA on MIP was studied at various pH. Therefore, 4.85 mg L^{-1} solution of DPA (35% ethanol in water) with different pH from 3 to 9 were prepared. Buffer solutions of pH 4 and 7 were used to prepare different pH solutions of DPA with the help of 0.1 M HCl and 0.1 N NaOH. Effect of pH on adsorption of DPA on MIP was studied at room temperature. Extraction efficiency (E) was calculated at different pH using following equation 1.

$$E(\%) = \frac{(Ci - Ce)}{Ci} \times 100 \qquad (1)$$

where Ci and Ce are the initial and equilibrated concentrations of DPA, respectively

Adsorption of DPA on MIP and NIP were studied in batch experiments. For this study 10 mg of sorbent was taken in a 50 ml of different concentration solutions of DPA in the range of 0.97 to 116.4 mg L^{-1}. The pH of all DPA solutions was maintained at 4. Adsorption was performed by using orbital shaker at 150 rpm. The adsorption studies were carried out for 48 hours at room temperature. After shaking, the concentration of DPA was measured by using HPLC. The amount of DPA adsorbed on sorbent, was determined by using following equation 2.

$$q = \frac{(Ci - Ce)V}{M} \qquad (2)$$

where q (adsorption capacity) is the amount DPA (mg) adsorbed on the MIP (g); Ci and Ce are the initial and equilibrated concentrations of DPA respectively; V is the volume of the solution (ml); M is the mass of the MIP (mg).

2.5 Adsorption kinetics experiment

Kinetic study of adsorption of DPA on MIP and NIP were studied at room temperature. For this study 10 mg of sorbent was taken in a 50 ml of 10 mg L^{-1} concentration solution of DPA of pH 4. Adsorption of DPA on MIP and NIP was studied after regular interval of time. Samples were then taken out at time intervals of 5, 10, 30, 60, 90, 120, 150, 180, 240, 300 min, respectively and were centrifuged in order to separate the adsorbent with a centrifuge at 5000 rpm for 10 min.

2.6 Selectivity experiment

Selectivity of MIP for DPA was studied in presence of 2-nitrodiphenyl amine (NDPA), Tetryl and trinitrotoluene (TNT). All the solutions were of 10 mg L^{-1} concentration. Tetryl and TNT solutions were prepared in water and NDPA solution was prepared using 35 % ethanol in water. In selectivity studies, 10 ml solutions of each DPA, NDPA, Tertyl and TNT were mixed together and pH of the solution was maintained at 4. Then 20 mg of MIP was added into it and kept for stirring on an orbital shaker for half an hour. Same procedure has been used for the selectivity studies of NIP. All the samples were analyzed by using HPLC. Following equations (3, 4, and 5) were used to evaluate selectivity of MIP and NIP.

$$Static\ distribution\ coefficient\ K_d = \frac{q_e}{C_e} \tag{3}$$

$$Selectivity\ coefficient\ \alpha = \frac{K_{d1}}{K_{d2}} \tag{4}$$

$$Relative\ selectivity\ coefficient\ \beta = \frac{\alpha_1}{\alpha_2} \tag{5}$$

where q_e represents adsorption capacity and C_e is the equilibrium concentration; K_{d1} and K_{d2} represents distribution coefficients of DPA and competitors molecules; α is the selectivity coefficient; β is the relative selectivity coefficient; α_1 and α_2 are the relative selectivity coefficient of MIP and NIP, respectively.

2.7 Extraction of DPA from ammunition waste using MIP modified column in a flash chromatography

Flash column chromatography is the most widely used technique by synthetic chemist for purification of organic compounds.[27] It has also been applied for the fast extraction and purification of natural products.[28] Separation using flash chromatography has advantages such as high loading capacity, speed of operation and ease of automation, which is ideal for large-scale separation. For the extraction of DPA, we have prepared different samples of nitrocellulose containing 1, 2 and 3% DPA. The resultant mixtures were soaked with 100 ml solution of 35 % ethanol in water, and sonicated for one hour. Nitrocellulose was removed by simple filtration method as it is insoluble in ethanol-water system. Filtrate containing DPA was analyzed by using HPLC to measure the concentration of DPA. Then, flash chromatographic technique was employed in combination with column cartridges containing MIP for the continuous extraction of DPA. MIP column cartridges were prepared by filling 1 g of MIP powder inside the cartridge. Initially the column of flash chromatography was eluted by using 35% ethanol in water for 5 minutes at the flow rate of 5 ml min^{-1}. Then, DPA containing solution was allowed to pass through the MIP cartridge column. The flow rate for the elution was kept at 2 ml min^{-1}. Acetonitrile was used as a desorption agent for DPA. Flow rate of acetonitrile was maintained at 5 ml min^{-1}. Samples containing DPA was collected in test tubes and analyzed with the help of HPLC to find out % extraction of DPA. Complete removal of DPA was confirmed by using flash chromatography. Same procedure was followed for the 1% and 2 % samples of DPA. All the samples were quantitatively analyzed by using HPLC.

3. RESULTS AND DISCUSSION

The preparation of MIP using diphenylamine (DPA) as template and its application as an active substance for solid phase extraction in a flash column chromatography is the goal of the study. The representative process sequence of it is outlined in **fig. 1**.

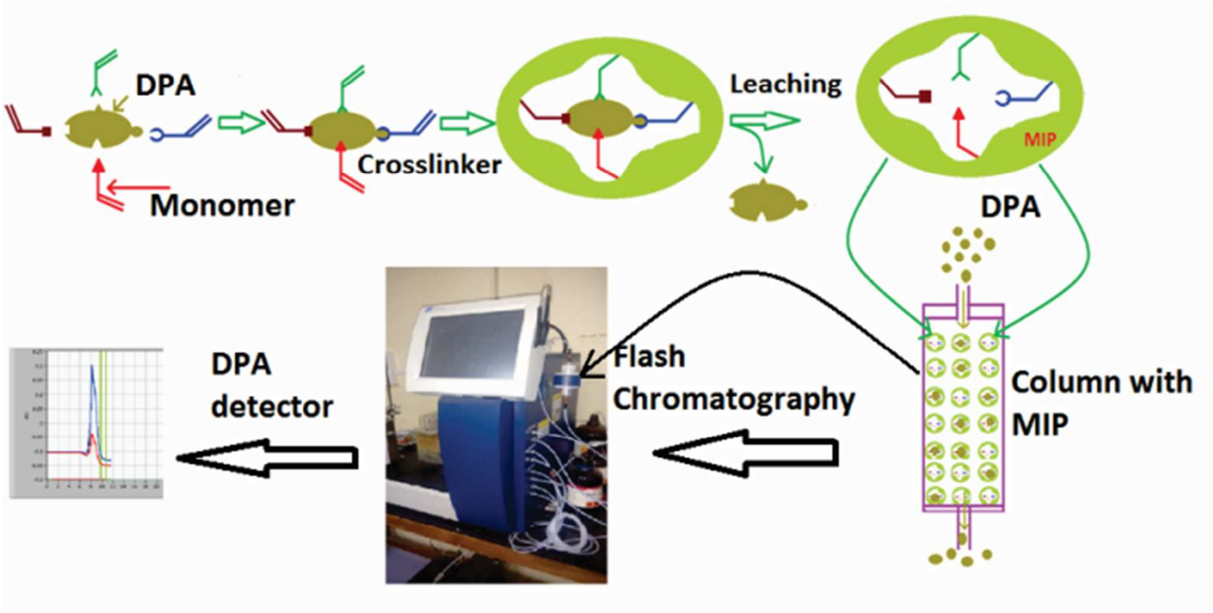

Fig. 1 Schematic for the development of DPA-MIP and its combination with flash chromatography

3.1 Characterization of polymer

The polymerization of monomer and crosslinker is confirmed by using FTIR spectroscopy. FT-IR spectrum of the EGDMA and MAA shows -C=C- stretching frequency at 1630 cm^{-1}, which has disappeared in MIP as shown in **fig. 2.** Thus, the polymerization is confirmed and further, the unreacted monomer/crosslinker does not exist in the product. The –C=O stretching for MAA and MIP were observed at 1690 cm^{-1} and 1730 cm^{-1} which can be assigned to the hydrogen bonded carboxylic dimmers and free carboxylic group, respectively. FTIR spectroscopy was used to study complete leaching of DPA from polymer matrix to get MIP. DPA shows stretching frequencies at 3378 cm^{-1}(secondary amine) and 1580 and 1486 cm^{-1}(aromatic –C=C-). It also shows aromatic –C-H bending frequencies at 683 cm^{-1} and 748 cm^{-1} respectively. None of these DPA peak is observed in MIP indicating complete leaching of DPA form the polymer matrix. From the FTIR studies of MIP, MIP-DPA and NIP it is shown that all shows –C=O stretching frequency at 1730 cm^{-1}, -C-O stretching at 1150 cm^{-1} indicating similarity of polymer nature (**fig. 3**). In case of MIP-DPA, the peak at 1580 cm^{-1} is characteristic of DPA, which is absent in both MIP and NIP respectively as shown in **fig. 3.**

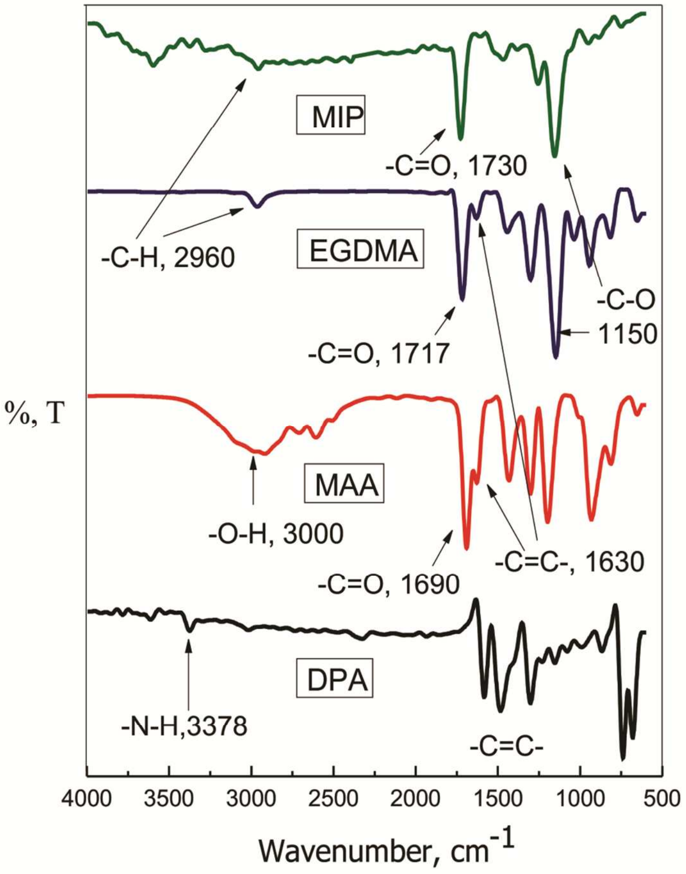

Fig. 2 FTIR spectra of DPA, MAA, EGDMA and MIP

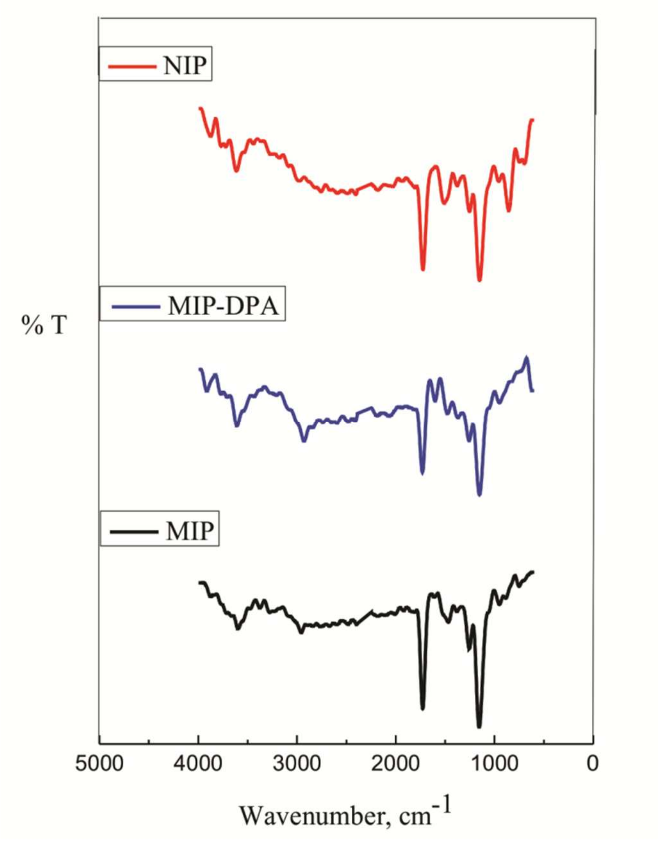

Fig. 3 FTIR spectra of MIP, Polymer matrix (MIP-DPA) and NIP

Morphology of polymers was studied by using FE-SEM. Both MIP and NIP exhibit globular morphology. From the FE-SEM images **(fig. 4)** we can clearly observe increase in porosity due to leaching of DPA. It is also observed from the micrograph that pore size of leached polymer was in the nano range. In case of NIP defects are very small in size and porosity is not defined. The sizes of both the imprinted and non imprinted polymer particles are nearly same around 30 nm. There is always disagreement in literature considering the effect of template on particle size of the polymers. In some cases both MIP and NIP with same particles size have been observed[29] while some reported NIP with larger particle size as compared with the MIP.[23,30] In the present case, presence of template doesn't affect the particle size of the polymer. In fact it is not the particle size, rather it is the presence of cavity in MIP, which is not there in NIP. BET analysis shows MIP with larger specific surface area of 100.4 m^2 g^{-1} which is far greater than NIP (23.69 m^2 g^{-1}). Pore specific volume for the MIP and NIP were 0.3176 and 1317 cm^3 g^{-1}, respectively. Thus, it could be concluded that formation of imprinted sites increases the surface area and pore volume of the MIP.

Thermogravimetric analysis (TGA) was used to investigate thermal stability as well as complete leaching of MIP as shown in **fig. 5.** TGA plot shows nearly identical properties for both leached and unleached MIP particles. Leached and unleached MIP particles show decomposition temperature in the range of 280-420 °C. DPA has boiling point at 302 °C. There is an additional degradation in the temperature range of 260-310 °C for unleached MIP. It indicates the exit of DPA from MIP matrix.

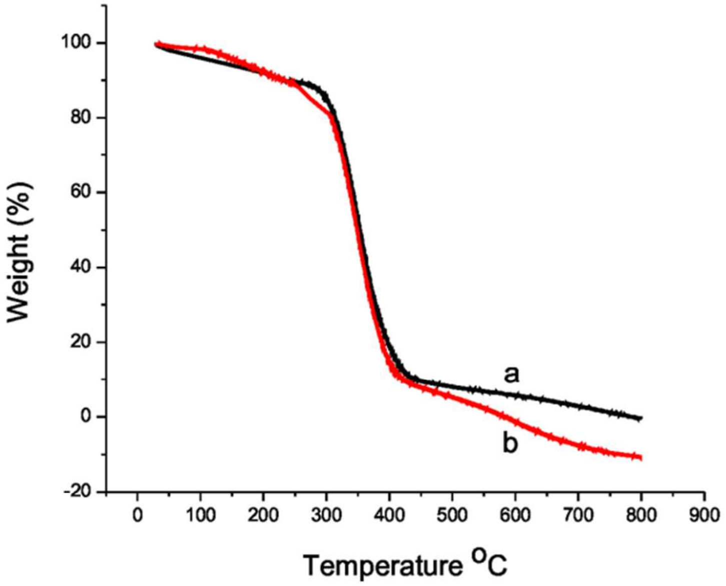

Fig. 5 TGA plots of (a) leached and (b) unleached MIP polymers

From calculated values considering 100% polymer formation and 100% DPA incorporation in MIP, the theoretical amount of DPA is 3.77%. From TGA curve it is approximately 4 wt%, which conforms well with the calculated one. This degradation peak is absent in leached MIP. Thus, it could be concluded from the TGA study that DPA was incorporated in the MIP which got removed from the MIP.

3.2 Effect of pH on adsorption of DPA

Initial pH is one of the essential parameters in selective extraction of DPA. The pH of the feed phase solution was 5.2. Adsorption capacities of MIP and NIP have been studied as a function of pH. At pH 4, MIP showed maximum adsorption capacity and it goes on decreasing with increase in pH as shown in **fig. 6**.

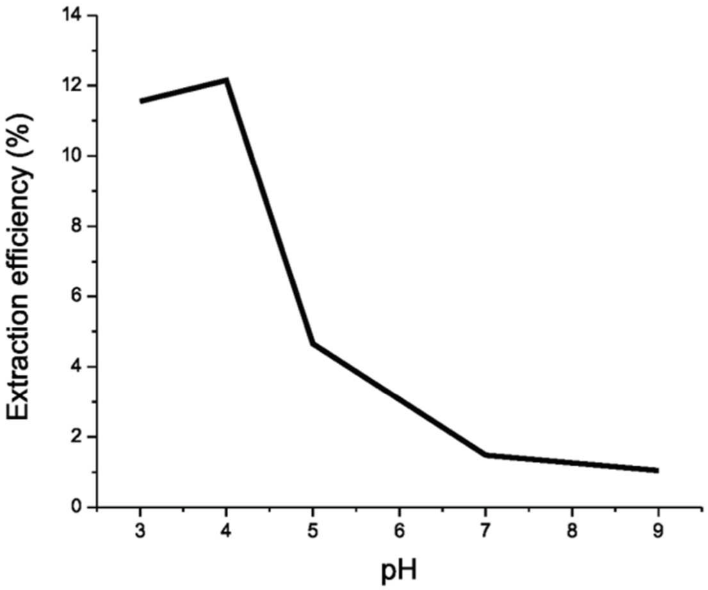

Fig. 6 Effect of pH on adsorption of DPA on MIP, (DPA concentration- 4.85 mg L^{-1})

There was no apparent adsorption observed above pH 9. It may be due to the interference of hydroxide ions in MIP cavities. With decrease in pH below 4, adsorption capacity decreases. This can be due to the protonation of the MAA at lower pH as well as easy protonation of DPA via lone pair making the rebinding difficult. Thus, pH 4 was used for the further studies of DPA-MIP.

3.3 Adsorption Isotherm

Adsorption isotherm of MIP and NIP towards DPA at pH 4 is shown in **fig. 7**. It has been observed that the equilibrium adsorption capacity increased sharply at lower concentration, then increased slowly and finally reached maximum equilibrium adsorption capacity. Maximum adsorption capacity of MIP and NIP were found to be 31 mg g^{-1} and 10.2 mg g^{-1}, respectively. Obviously, MIP shows more adsorption capacity as compared to NIP.

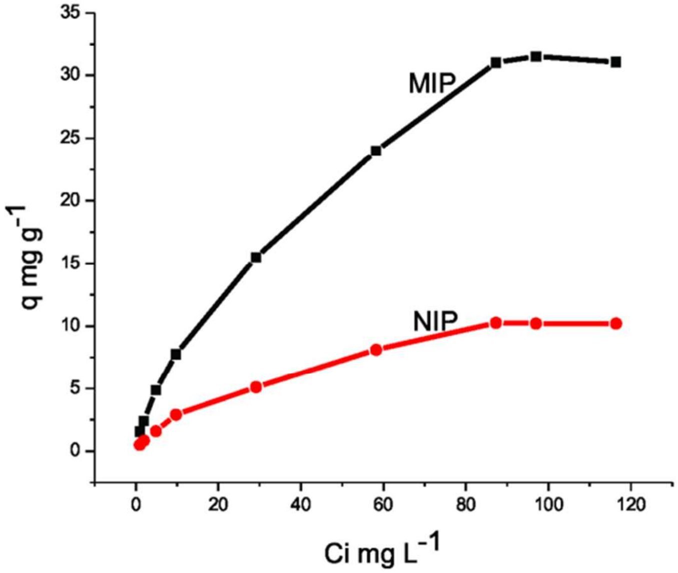

Fig. 7 Adsorption of DPA (mg g^{-1}) on MIP and NIP at different initial concentrations, (0.97 to 116.4 mg L^{-1} (Ci) of DPA)

Saturation for DPA in MIP was observed for 87.3 mg L^{-1} solution of DPA. Further increase in concentration of DPA results in consistency in adsorption capacity. Thus, there is no adsorption of DPA, which means all the sites in MIP are occupied by it. Langmuir model was used to study the distribution of DPA between the liquid and adsorbent phases and achieve the maximum adsorption uptake. Langmuir adsorption isotherm model assumes that the molecules are adsorbed at a fixed number of well defined sites, in which each site holds one molecule.[31] The respective non-linearized and linearized Langmuir isotherms are described by the following equations (6 and 7).

$$q_e = \frac{Q.K.Ce}{1 + K.Ce} \qquad (6)$$

$$\frac{1}{q_e} = \frac{1}{Q} + \frac{1}{KQ}.\frac{1}{Ce} \qquad (7)$$

where q_e is the amount of adsorbed DPA on the adsorbents (mg g^{-1}); Ce is the equilibrium DPA concentration in solution (mg L^{-1}); K the Langmuir constant (L mg^{-1}) and Q is the maximum adsorption capacity (mg g^{-1}). Langmuir adsorption isotherm parameters were estimated by plotting 1/ Ce versus 1/q_e as shown in **fig. 8 (a).**

The obtained experimental results were also fitted with Freundlich model as shown in following equations (8 and 9). It describes multilayer adsorption on the heterogeneous surface of sorbent.

$$q_e = K_f C_e^{\frac{1}{n}} \qquad (8)$$

$$log q_e = log K_f + \frac{1}{n} log C_e \qquad (9)$$

where K_f and n are the Freundlich constants, which affects the adsorption capacity and the intensity of the adsorption, respectively. Freundlich isotherm parameters were estimated by plotting $log q_e$ versus $log C_e$ as shown in **fig. 8 (b).** All the estimated data obtained from Langmuir and Freundlich isotherm model is shown in table 1. As seen in **fig. 8 (b)**, it is suggested that Fraundlich adsorption isotherm model with R^2 0.998 is very well fitted as compare to the Langmuir model for the adsorption of DPA on MIP. Thus, Freundlich isotherm model suggests multilayer chemisorption of DPA on MIP.

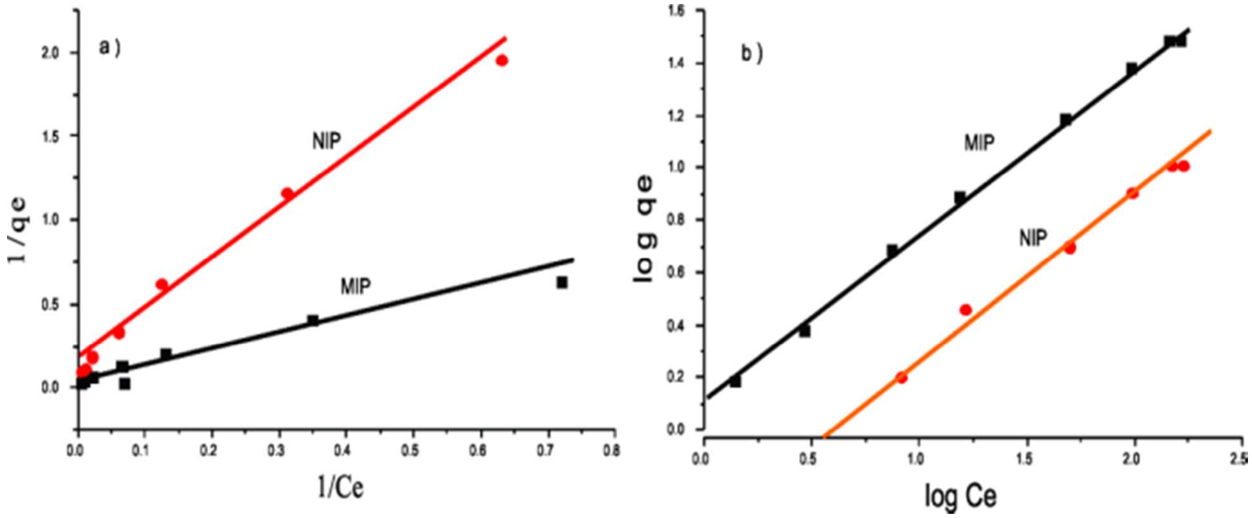

Fig. 8 a) Langmuir and b) Freundlich adsorption isotherm plots for adsorption of DPA on the MIP and NIP particles

Table 1. Langmuir and Freundlich isotherm constants for adsorption of DPA using MIP and NIP particles

<table>
<tr><td colspan="12">Langmuir isotherm constants</td></tr>
<tr><td colspan="2">Polymer</td><td colspan="2">Q_{exp}(mg g^{-1})</td><td colspan="3">Q_{max}(mg g^{-1})</td><td colspan="3">K (L mg^{-1})</td><td colspan="2">R^2</td></tr>
<tr><td colspan="2">MIP</td><td colspan="2">31.0</td><td colspan="3">22.72</td><td colspan="3">0.050</td><td colspan="2">0.953</td></tr>
<tr><td colspan="2">NIP</td><td colspan="2">10.25</td><td colspan="3">7.37</td><td colspan="3">0.045</td><td colspan="2">0.985</td></tr>
<tr><td colspan="12">Freundlich isotherm constants</td></tr>
<tr><td colspan="2">Polymer</td><td colspan="4">K_f</td><td colspan="3">n</td><td colspan="3">R^2</td></tr>
<tr><td colspan="2">MIP</td><td colspan="4">1.8</td><td colspan="3">1.56</td><td colspan="3">0.998</td></tr>
<tr><td colspan="2">NIP</td><td colspan="4">0.408</td><td colspan="3">1.55</td><td colspan="3">0.995</td></tr>
</table>

3.4 Adsorption Kinetics

For more evaluation of adsorption mechanism, amount of DPA adsorbed on MIP and NIP was studied as a function of contact time as shown in **fig. 9.**

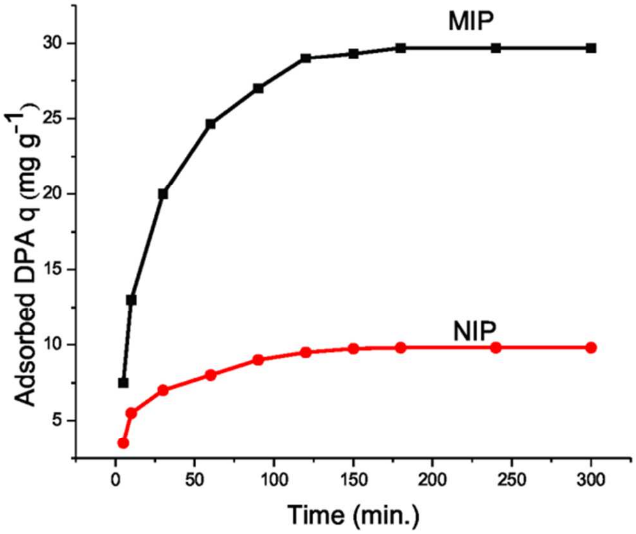

Fig. 9 Effect of contact time on uptake of DPA by MIP and NIP (initial concentration-10 mg L^{-1}; shaking rate-150 rpm; adsorbent-10 mg; pH-4)

Two different kinds of kinetic models were employed for this study. Pseudo first order and pseudo second order models were utilized to treat obtained experimental data as shown in **fig. 10** (a) and **10 (b).** Mathematical expression for the pseudo first order and pseudo second order models are presented by eq. 10 and eq. 11

$$\frac{1}{q_t} = \frac{K_1}{q_e t} + \frac{1}{q_e} \qquad (10)$$

where q_e and q_t are the amounts of DPA adsorbed, in mg g^{-1},at equilibrium and at time t (min), respectively, and K_1 is the pseudo first order rate constant (min^{-1}).

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \qquad (11)$$

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

Kinetic parameters obtained from the above model have been mentioned in table 2. Standard deviation and correlation coefficients (R^2) were used to investigate most appropriate model by comparing it with experimental data.

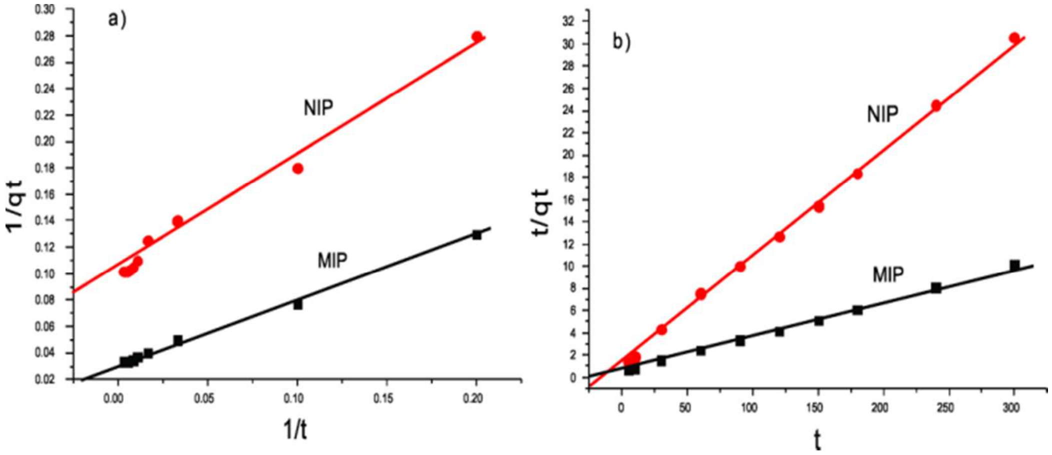

Fig. 10 a) Pseudo first-order and b) Pseudo second-order plots for the adsorption of DPA onto MIP and NIP particles

Pseudo second order model exhibits higher correlation coefficients (R^2) and lower standard deviation. Thus, it can be concluded that the pseudo second order model is well fitted for the experimental kinetic data obtained from the adsorption process of DPA on MIP as well as NIP. It reveals that chemical adsorption is the rate determining step without involvement of mass transfer in the solution.[32]

Table 2. Calculated kinetic parameters of pseudo-first and pseudo-second orders for initial DPA concentration of 10 mg L^{-1}

polymer	Experimental q_e(mg g^{-1})	Pseudo first-order			Pseudo second-order		
		k_1 (min^{-1})	q_e(mg g^{-1})	R^2	k_2 (min^{-1})	q_e(mg g^{-1})	R^2
MIP	31	15.45	31.816	0.9973	2.1×10^{-3}	31.55	0.999
NIP	10.2	8.754	9.95	0.9871	8.7×10^{-3}	10.25	0.987

3.5 Selectivity studies

DPA separation studies have been done in presence of NDPA, TNT, and Tetryl. NDPA has been used as a competitor molecule because it is one of the stabilizer in nitrocellulose based propellants. TNT and Tetryl are the secondary explosives and can be seen in large quantity from ammunition waste. All these compounds were analyzed by using HPLC. The retention time of the DPA, NDPA, TNT, and Tetryl are obtained at 13.3, 15.6, 8.9, and 4.1, respectively as shown in **fig. 2S**. The calibration of DPA gave linear regression curve, which was obtained by plotting milli absorbance units (Y, mAU) against the concentration (X, mg L^{-1}) as shown in **fig. 3S**. Selectivity Coefficients and other parameters are listed in table 3. It is obvious that MIP showed higher selectivity towards DPA as compared with the other competitor molecules. It can be concluded that the formation of complimentary recognition sites in MIP which matches the geometry of DPA.

Table 3. Selective adsorption of DPA from multicomponent mixtures of NDPA, TNT and Tetryl by MIP and NIP (initial concentration- 10 mg L^{-1}; adsorbent- 20 mg; shaking rate- 150 rpm; solution pH-4.0)

DPA competitor	Distribution ratio (ml g^{-1})		Selectivity coefficient, $k_{DPA}/k_{Competitor}$		Relative selectivity cefficient, *k'*
	MIP	NIP	MIP	NIP	
DPA	1921	197.80	-	-	-
NDPA	83	150.53	23.14	1.31	17.66
TNT	40.81	30.45	47.071	6.49	7.25
Tetryl	36.73	40.81	52.30	4.84	10.80

3.6 Extraction of DPA using flash chromatography

To study the effectiveness of DPA extraction in a continuous method, flash chromatography technique was employed. DPA is highly UV active compound and shows strong absorbance peak at 280 nm. Flash column chromatography technique is well suited for UV active compounds. The extraction was repeated three times without significant decrease in adsorption capacity. Complete adsorption of DPA on MIP can be confirmed from the chromatogram as shown in **fig. 11 (a)** as there wasn't any peak observed. In case of desorption using acetonitrile, absorbance peak belonging to DPA is observed as shown in **fig.11 (b)**. It was further analyzed by using HPLC as shown in **fig. 12.** Thus, it could be concluded that all DPA has been desorbed from the MIP. In the present case more than 98% recovery of DPA is achieved as shown in table 4.

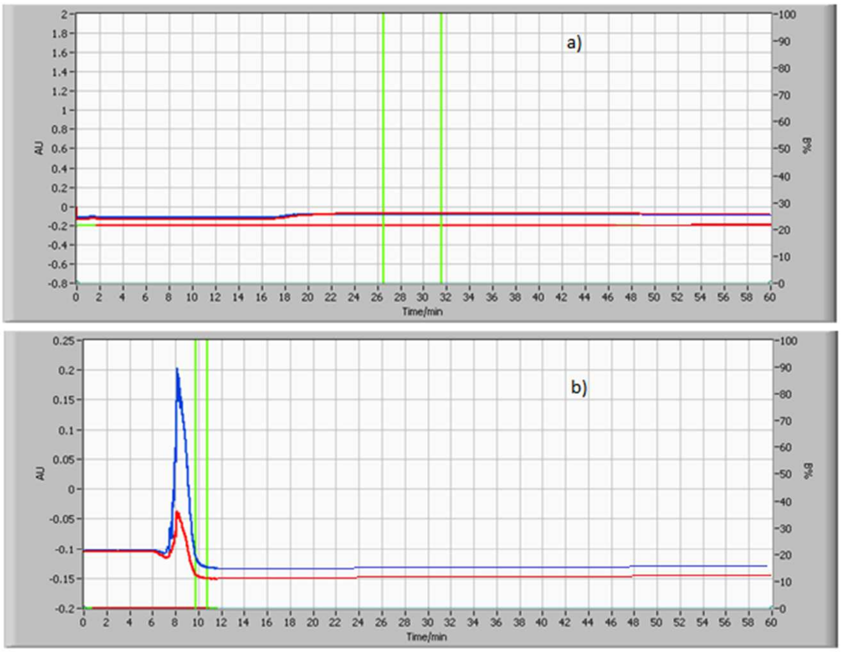

Fig. 11 a) Chromatogram of eluent after passing DPA containing solution (35% Ethanol in water) through column of flash chromatography containing MIP; flow rate- 2 ml min^{-1}; absorbance- 280 nm; b) Chromatogram of eluent after leaching MIP column containing DPA with acetonitrile; flow rate- 5 ml min^{-1}; absorbance- 280 nm.

Table 4. Repeated adsorption of DPA by MIP in flash column chromatography (1 g MIP, pH 4.0, flow rate- 2 ml min^{-1}(adsorption), flow rate- 5 ml min^{-1}(desorption))

Cycle	% of DPA in nitrocellulose	Recovery (%)
1	3 % (30 mg DPA)	99%
2	2 % (20 mg DPA)	98.5%
3	1 % (10 mg DPA)	98.3%

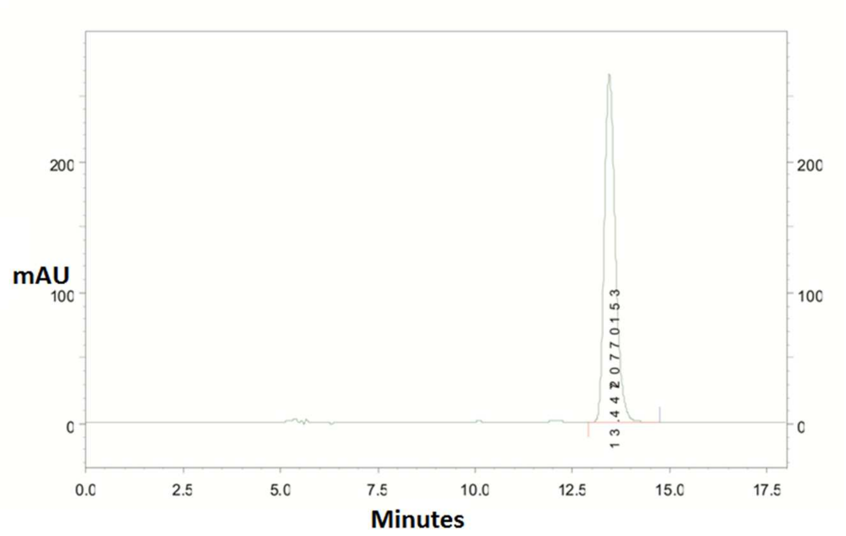

Fig. 12 HPLC Chromatogram of diphenyl amine (2 % mixture of DPA with nitrocellulose) after desorption using acetonitrile in optimum mobile phase (80:20, acetonitrile:water) with 0.4 ml min^{-1} flow rate and absorbance at 280 nm

4. CONCLUSION

In the present study we have reported MIP for the selective extraction of organic persistant DPA from ammunition waste. BET analysis shows higher surface area and pore volume for MIP than NIP. It could be attributed to the presence of templtae in MIP synthesis. The MIP has shown optimum adsorption capacity at pH 4. It exhibited very high adsorption capacity (31 mg g^{-1}). Selectivity for DPA in presence of TNT, Tetryl and NDPA was relatively very high. Here we reported that for continuos extraction of DPA from ammunition waste, flash chromatography can be a better option. By this technique we can directly analyse from the chromatogram, whether DPA is completely desorbed from MIP or not. Desorption time was very less when acetonitrile was used as a desorbing agent. Thus, in this system the adsoption-desorption process is very fast. Combination of MIP and flash column chromatography technique can be directly used in actual applications for the treatment of ammunition waste. This technique can also be used for the determination of DPA in apple juice samples.

ACKNOWLEDGMENT

The authors wish to acknowledge the financial support from DRDO (ERIP/ER/1003883/M/01/908/2012/D, R&D/ 1416, dated, 28-3-2012) New Delhi, India. P.E.H. acknowledges Defence Institute of Advanced Technology-DRDO, Pune for the Ph.D. research scholarship.

REFERENCES

1 S. Vyazovkin, I. Dranca and A. J. Lang, *Thermochim. Acta*, 2005, **437**, 75–81.

2 R. Meyer, Explosives. Verlag Chemie, Weinheim, Germany.,1977.

3 O. Drzyzga, *Chemosphere*, 2003, **53**, 809–818.

4 European Food Safety Authority, *Eur. Food Saf. Auth. J.*, 2012, **10**, 1–59.

5 K. L. E. Kaiser and J. M. Ribo, *Toxic. Assess.*, 1988, **3**, 195–237.

6 J.S. Warner, T. M. Engel and P. J. Moudron, National Service Center For Envioonmental Publication(NSCEP) 1983, EPA method 620, 1–39.

7 L. Yu, R. Schoen, R. Dunkin, M. Firman, and H. Cushman, J. Agric. Food Chem., 1997, 45, 748–752.

8 B. Saad, N. H. Haniff, M. I. Saleh, N.H. Hashim, A. Abu, and N. Ali, *Food Chem.*, 2004, **84**, 313–317.

9 A. Ahmad, M. Qureshi, I. A. Khan and K. Z. Alam, *Anal. Lett.*, 1990, **23**, 1139–1157.

10 M. Komiyama, T. Takeuchi, T. Mukawa and H. Asanuma, *Molecular imprinting: from fundamentals to applications*, WILEY-VCH Verlag GmbH, 2003.

11 J.D. Marty and Mauzac M., 'Molecular imprinting: state of art and perspectives' Adv. Polym. Sci., 2005, 172, 1–35.

12 G. Wulff and A. sarhan, *Angew. Chemie*, 1972, **46**, 334–342.

13 K. Mosbach, *Die Makromol Chem*, 1981, **182**, 687–692.

14 F. G. Tamayo, E. Turiel and A. Martín-Esteban, *J. Chromatogr. A*, 2007, **1152**, 32–40.

15 P. E. Hande, A. B. Samui and P. S. Kulkarni, *Environ. Sci. Pollut. Res.*, 2015, **22**, 7375–7404.

16 D. Kriz, O. Ramstroem, A. Svensson and K. Mosbach, *Anal. Chem.*, 1995, **67**, 2142–2144.

17 S. A. Piletsky and A. P. F. Turner, *Electroanalysis*, 2002, **14**, 317–323.

18 A. A. Pebdani, A. M. H. Shabani, S. Dadfarnia and S. Khodadoust, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, 2015, **147**, 26–30.

19 Y. Yang, Y. Cao, X. Wang, G. Fang and S. Wang, *Biosens. Bioelectron.*, 2015, **64**, 247–254.

20 M. T. Jafari, M. Kamfirozi, E. Jazan and S. M. Ghoreishi, *J. Sep. Sci.*, 2014, **37**, 573–579.

21 M. M. Moein, M. Javanbakht and B. Akbari-adergani, *Talanta*, 2014, **121**, 30–36.

22 Y. Zhou, Z. Qu, Y. Zeng, T. Zhou and G. Shi, *Biosens. Bioelectron.*, 2014, **52**, 317–323.

23 V. L. V Granado, A. Rudnitskaya, J. A. B. P. Oliveira and M. T. S. R. Gomes, *Talanta*, 2012, **94**, 133–139.

24 V. L. V Granado, M. Gutiérrez-capitán, C. Fernández-sánchez, M. T. S. R. Gomes, A. Rudnitskaya and C. Jimenez-jorquera, *Anal. Chim. Acta*, 2014, **809**, 141–147.

25 M. Xue, Y. Wang, Z. Meng, W. Zhang, Y. Wu, S. Jiang and M. Engineering, *J. Liq. Chromatogr.*, 2013, **36**, 2677–2686.

26 F. Lanza and B. Sellergren, *Anal. Chem.*, 1999, **71**, 2092–2096.

27 A. P. Breksa III and K. Dragull, *Food Chem.*, 2009, **113**, 1308–1313.

28 J. W. Blunt, V. L. Calder, G. D. Fenwick, R. J. Lake, J. D. McCombs, M. H. G. Munro and N. B. Perry, *J. Nat. Prod.*, 1987, **50**, 290–292.

29 K.-C. Ho, W.-M. Yeh, T.-S. Tung and J.-Y. Liao, *Anal. Chim. Acta*, 2005, **542**, 90–96.

30 K. Yoshimatsu, K. Reimhult, A. Krozer, K. Mosbach, K. Sode and L. Ye, *Anal. Chim. Acta*, 2007, **584**, 112–121.

31 S. Hasan, A. Krishnaiah, T. K. Ghosh, D. S. Viswanath, V. M. Boddu and E. D. Smith., *Sep. Sci. Technol.*, 2003, **38**, 3775–3793.

32 M. Yurdakoç, Y. Seki, S. Karahan and K. Yurdakoç, *J. Colloid Interface Sci.*, 2005, **286**, 440–446.