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Magnetic multi-walled carbon nanotubes assisted dispersive solid phase extraction of nerve agents and their markers from muddy water

Deepak Pardasani[a], Pankaj K. Kanaujia[a], Ajay K. Purohit[a], Anchal Roy Shrivastava[b], D.K. Dubey[a,*]

[a] *Vertox Laboratory, Defence Research and Development Establishment, Gwalior, India*
[b] *Electron Microscopy Division, Defence Research and Development Establishment, Gwalior, India*

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ABSTRACT

The multi-walled carbon nano-tubes (MWCNT) were magnetized with iron oxide nanoparticles and were characterized by SEM and EDX analyses. These magnetized MWCNT (Mag-CNT) were used as sorbent in dispersive solid phase extraction (DSPE) mode to extract nerve agents and their markers. Mag-CNT were dispersed in water and collected with the help of an external magnet. From Mag-CNT, the adsorbed analytes were eluted and analyzed by GC-FPD in phosphorus mode. DSPE was found to be advantageous over conventional solid phase extraction (SPE) in terms of operational simplicity, speed, handling of large sample volume and recoveries. Extraction parameters such as eluting solvent, sorbent amount, pH and salinity of aqueous samples were optimized. Optimized extraction conditions included 40 mg of Mag-CNT as sorbent, chloroform as eluent, pH 3–11 and salinity 20%. Under the optimized conditions, recoveries from distilled water ranged from 60 to 96% and were comparable in tap and muddy water. Limits of quantification and limits of detection of 0.15 ng/ml and 0.05 ng/ml, respectively, were achieved. Superiority of Mag-CNT over conventional C_{18} SPE was also established.

1. Introduction

Nerve agents are chemical warfare agents (CWA), and belong to a group of organophosphorus compounds which can cause death within minutes after exposure [1,2]. Extraction, analysis and identification of nerve agents (NA) and their markers from environmental samples are an important contemporary research problem [3]. Proliferation of CWA is prohibited by an international treaty known as chemical weapons convention (CWC) [4,5]. The CWC is implemented through its strict verification program; which is executed by an organization known as organization for the prohibition of chemical weapons (OPCW) [4]. The verification regime of CWC relies on inspections, on-site analysis or sampling and off-site analysis of samples in a network of 'designated laboratories' appointed by the OPCW. The objective of verification is 'unequivocal identification' of CWA and their environmental markers, which in turn requires efficient sample preparation methods to extract and enrich the analytes from environmental samples.

Among various environmental matrices, water is an important matrix that can be contaminated by deliberate or inadvertent spread of NA. NA can react with water over a period of time; therefore, water samples are analyzed not only for the live agents themselves but also for their less-toxic environmental markers. Markers are chemicals, structurally, closely related to NA and are co-produced during their production, or produced by hydrolytic degradation of NA. The by-products of NA are di-esters of alkylphosphonic acids and their presence in water sample is indicative of prior presence of NA. Therefore, extraction, enrichment and identification of these chemicals from water samples are of utmost importance from verification point of view of CWC and gathering the forensic evidence of use of nerve agents.

Conventional methods of extraction of CWC related chemicals from water involve liquid–liquid extraction (LLE), solid-phase micro-extraction (SPME), liquid phase micro-extraction (LPME) and solid-phase extraction (SPE) [3,6]. LLE is a widely used sample pre-treatment method for liquid samples. However, LLE is not preferred when water either contains emulsifying agents or analytes are present in trace quantities. Moreover it is time consuming, requires large volume of toxic organic solvents and lacks automation. SPME is solvent free and simple technique, however suffers from some drawbacks such as high cost, memory effects and decline in performance with time [7].

SPE is another popular technique for extraction. In SPE, the sorbent plays key role in obtaining higher enrichment efficiency of analytes. For CWA and other related analytes, several kinds of

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[*] Corresponding author at: Vertox Laboratory, Defence Research and Development Establishment, Jhansi Road, Gwalior 474002, India. Tel.: +91 751 2233488; fax: +91 751 2341148.
E-mail address: dkdubey@rediffmail.com (D.K. Dubey).

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sorbent materials such as C_{18}, Oasis HLB, diatomaceous earth materials, etc. have already been optimized and reported [8,9].

In recent years, carbon nanotubes (CNT) have attracted great attention due to its high surface area and good electrical, chemical, mechanical and conducting properties [10,11]. CNT are considered as a sheet of graphite that has been rolled into a tube and can be classified as single-walled carbon nanotubes (SWCNT) and multi-walled carbon nanotubes (MWCNT). CNT have been exploited in analytical and other fields such as gas sensors, enzymatic biosensors, immunosensors, DNA probes and so on [11]. Because CNT surfaces have a strong interaction with organic molecules, they possess excellent adsorption ability and can be substituted for conventional SPE sorbents. CNT have been investigated as SPE sorbents to extract various analytes such as pesticides, antibiotics, anxiolytics, anti-inflammatories, phthalate esters, phenolic compounds, inorganic ions and organo-metallic compounds, etc. [12–14]. However, one major drawback of CNT, when used as SPE sorbent, is resistance to flow of water sample, especially if it contains suspended particles. It restricts the use of large volumes of water sample and hampers the enrichment of trace quantities of analytes. Thus, the advantage of high surface area of CNT is not fully utilized in conventional SPE mode.

The analytical capabilities of CNT can be further increased by surface modifications [15]. One such approach is the magnetization of CNT with iron oxide nanoparticles. The introduction of magnetic properties into CNT provides separation convenience of the sorbent in addition to high adsorption capacity [15]. The prepared magnetic CNT can be well dispersed in water and easily separated from the medium with the help of a magnet [16]. Thus magnetic CNT have an edge over conventional SPE in which sorbents are used in column form. The use of SPE in conventional column form becomes inconvenient when the sample is either dirty (causes plugging of sorbent column) or its volume is high (sample loading consumes lot of time).

Magnetic multi-walled carbon nanotubes (Mag-CNT) filled with Fe_2O_3 nanoparticles have been prepared and employed as sorbent for the magnetic removal of dye contaminants and metal ions from water [17,18]. However, to the best of our knowledge, Mag-CNT have never been used for the extraction of CWA and their markers from water samples.

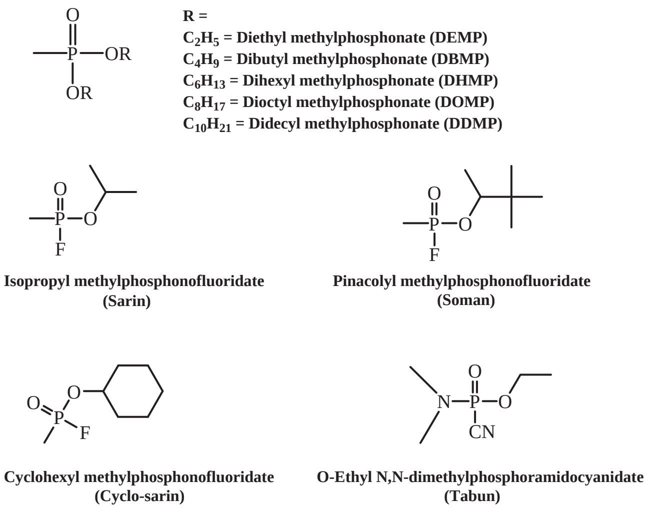
Prompted by the adsorption virtues and ease of magnetic separation, herein, we report the use of Mag-CNT for the dispersive solid phase extraction (DSPE) of nerve agents (NA) from water. The compounds selected for this study includes four toxic NA and their five non toxic markers (Fig. 1). Various extraction parameters have been optimized to recover the analytes at maximum possible level from different aqueous environmental samples such as tap and muddy water. Both the forms of extraction, i.e. DSPE and conventional column form SPE are also compared.

2. Materials and methods

2.1. Chemicals and materials

Multi-walled carbon nanotubes (o.d. 7–15 nm, i.d. 3–6 nm and length 0.5–200 μm), iron (II) chloride tetrahydrate, iron (III) chloride hexahydrate and tributyl phosphate (TBP) were obtained from Sigma–Aldrich, Mumbai, India. All the dialkyl methylphosphonates, sarin, soman, tabun and cyclosarin were synthesized in our laboratory as per reported procedures [19].

Caution: Chemical warfare agents are extremely toxic compounds; they should be prepared and handled by trained professionals in an efficient fume hood equipped with alkali scrubber. Individuals handling them should wear facemask, gloves and protective suit.



R =
C_2H_5 = Diethyl methylphosphonate (DEMP)
C_4H_9 = Dibutyl methylphosphonate (DBMP)
C_6H_{13} = Dihexyl methylphosphonate (DHMP)
C_8H_{17} = Dioctyl methylphosphonate (DOMP)
$C_{10}H_{21}$ = Didecyl methylphosphonate (DDMP)

Isopropyl methylphosphonofluoridate (Sarin)

Pinacolyl methylphosphonofluoridate (Soman)

Cyclohexyl methylphosphonofluoridate (Cyclo-sarin)

O-Ethyl N,N-dimethylphosphoramidocyanidate (Tabun)

Fig. 1. Analytes selected for DSPE experiments.

2.2. Preparation and characterization of Mag-CNT

Mag-CNT were prepared as reported by Gong et al. [18]. Typically, MWCNT were dispersed in concentrated nitric acid and stirred at 60 °C overnight. Purified MWCNT thus obtained, were washed with copious amount of water followed by ethanol and dried at 110 °C for 6 h. Subsequently, 500 mg of purified MWCNT were suspended in 250 ml water containing 700 mg (2.5 mM) of iron (II) chloride tetrahydrate and 1350 mg of iron (III) chloride hexahydrate (5 mM). The temperature of the suspension was raised to 50 °C under argon atmosphere followed by the slow addition of 5 ml of 8 mol/L ammonium hydroxide solution with stirring. The pH of the suspension was controlled in the range of 10–11. After complete addition of ammonium hydroxide solution, the temperature was raised to 80 °C and reaction was allowed to be continued for 30 min. The suspension was cooled to room temperature and Mag-CNT were isolated from the mixture with the help of a permanent magnet. Separated Mag-CNT were washed thrice with deionised water followed by ethanol. Finally, Mag-CNT were dried under vacuum. The dry weight of Mag-CNT thus obtained was measured to be 1.075 g.

The Mag-CNT were characterized by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analyses using a FEI, Quanta 400 Instrument (FEI Co., Hillsboro, OR, USA). Significant change in surface morphology was observed when the SEM micrograph of Mag-CNT was compared with that of MWCNT (Fig. 2). Clusters of nanoparticles were clearly visible onto the surfaces of Mag-CNT (Fig. 2b). Presence of iron oxide was further confirmed by EDX analysis, which showed distinct peaks of Fe and O along with some impurities. The EDX elemental microanalysis of Mag-CNT in Fig. 3b exhibited the affluence in O and Fe elements.

2.3. Standards and spiking solutions

Stock standard solutions (1000 μg/ml) were prepared in acetonitrile (ACN) separately by accurately weighing 10 mg of analyte into 10 ml volumetric flasks and diluting to volume. Intermediate mix standard solutions of 10 μg/ml were prepared by diluting the stock standard solutions in ACN. Stock and intermediate standard solutions of internal standard, tributylphosphate (TBP) were prepared in the same way in ACN. The final concentration of internal standard was kept at 200 ng/ml. Water samples were spiked at a concentration of 3 ng/ml with standard solutions of analytes and were used for the extraction experiments. To avoid the loss of

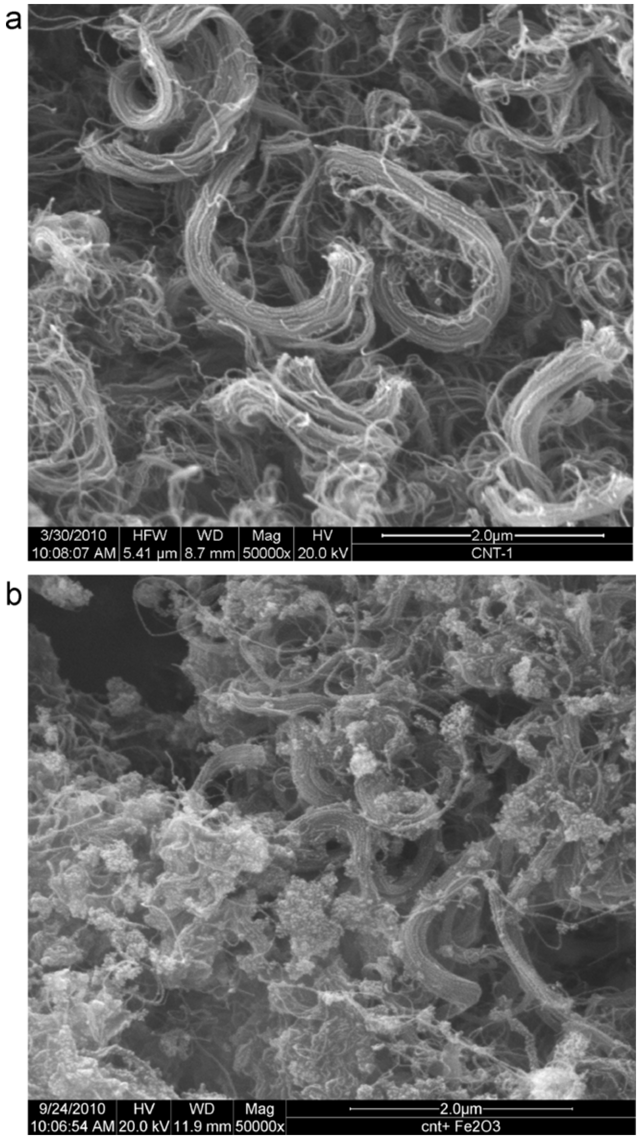
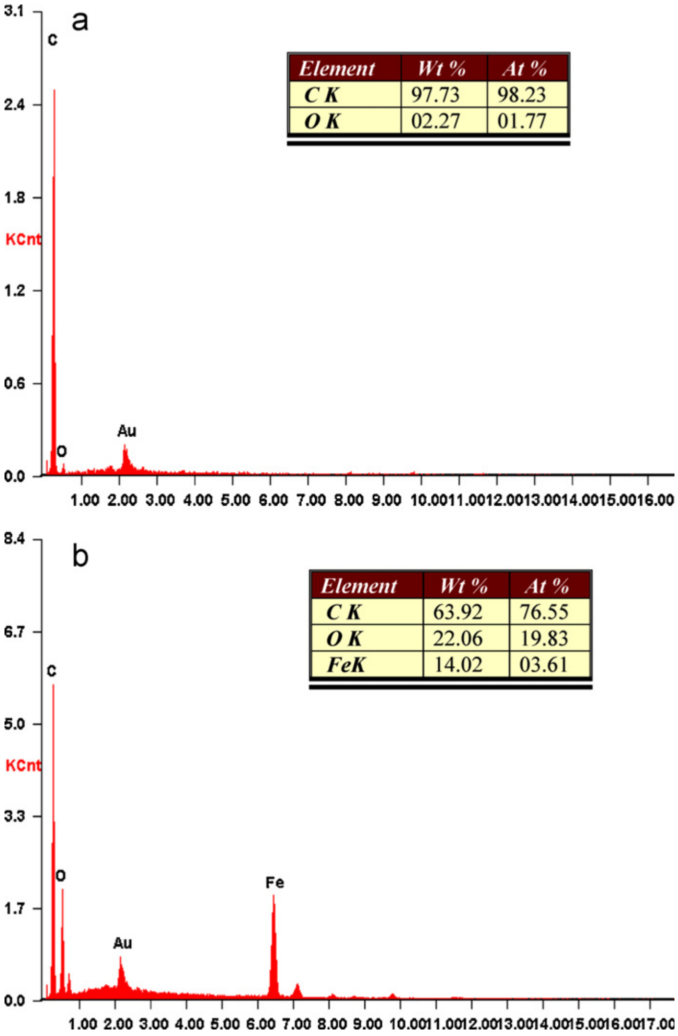


Fig. 2. SEM images of (a) non-magnetized MWCNT and (b) magnetized MWCNT (Mag-CNT).



Element	Wt %	At %
C K	97.73	98.23
O K	02.27	01.77

Element	Wt %	At %
C K	63.92	76.55
O K	22.06	19.83
FeK	14.02	03.61

Fig. 3. EDX analyses of (a) non-magnetized MWCNT and (b) magnetized MWCNT (Mag-CNT).

analytes by hydrolysis, water samples were prepared freshly every day.

2.4. Muddy water sample

Muddy water sample was collected from the fast flowing drainage system of our campus immediately after a heavy rain fall. It was allowed to stand for 1 h. The settled sand particles as well as the debris floating on the water surface were discarded and brownish muddy water thus obtained was used in further studies. The measured pH of the water was 6.5. Total suspended particulate matter was ~0.75%, calculated on the basis of dry weight of the total solid particulates obtained after the centrifugation of 100 ml of water sample at 3000 rpm for 20 min.

2.5. Dispersive solid phase extraction (DSPE) procedure

200 ml of spiked water sample was taken in a 250 ml capped bottle and 40 mg of Mag-CNT were dispersed into it with sonication for 2 min. It was allowed to stand for 20 min. The Mag-CNT were forced to settle by placing a strong magnet near the bottle and the water was decanted (Fig. 4). Thus separated Mag-CNT were re-dispersed in 2 ml of distilled water and transferred into a 4 ml screw cap vial with the help of a pipette. Mag-CNT were again forced to settle in the bottom and the supernatant water was decanted and discarded. Wet Mag-CNT were then washed twice with 2 × 400 μl methanol followed by extraction with 3 × 1000 μl of chloroform. All the methanol washings and chloroform extracts were combined, shaken vigorously and allowed to stand for few minutes. Thus separated upper aqueous layer (~250 μl) was discarded and remaining organic layer was dried over anhydrous sodium sulfate. In a pre-calibrated auto sampler vial, the organic fraction was concentrated to 500 μl by gentle stream of dry nitrogen. Internal standard was added just before the GC-FPD (P) analysis.

2.6. SPE procedure

Conventional SPE taking MWCNT as sorbent material was carried out as reported by Ravelo-Perez et al. [13]. Typically, 40 mg of sorbent column was packed into a 3 ml empty SPE tubes with the help of PTFE frits. SPE column was preconditioned with 10 ml ACN followed by 10 ml of distilled water. 200 ml of water sample was passed through the preconditioned SPE column under gravity. In case of muddy water samples, suspended particulate matter was removed by centrifugation at 3000 rpm for 20 min. After loading, SPE column was washed with 2 ml of distilled water and after

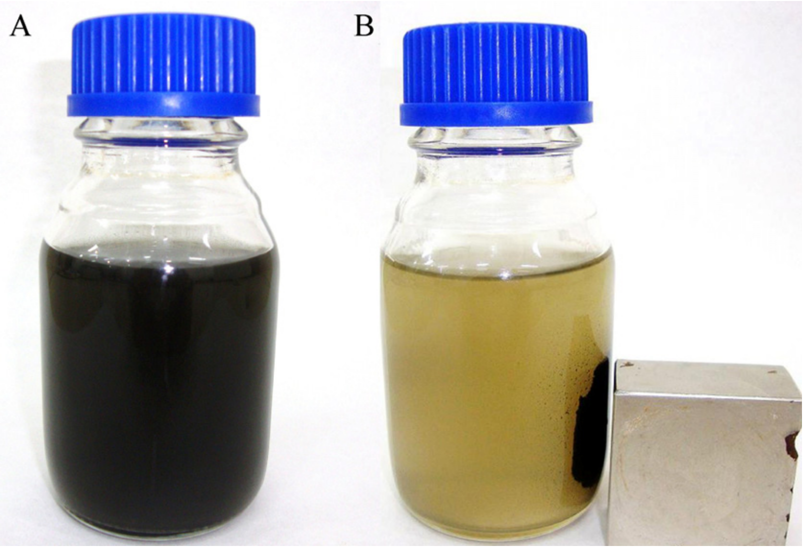


Fig. 4. DSPE procedure: (a) Mag-CNT dispersed in muddy water sample and (b) magnetic separation of dispersed Mag-CNT.

which, the cartridge was dried under a vacuum of 10 mm Hg for 20 min. The retained analytes were eluted with 800 μl of methanol followed by 3 ml of chloroform (as in case of DSPE). Organic extracts were dried over anhydrous sodium sulfate and concentrated to 500 μl by dry nitrogen stream. Internal standard was added just before the GC-FPD (P) analysis.

For C_{18} cartridges, reported procedures were used [9].

2.7. GC-FPD (P) analysis

The GC-FPD (P) analyses were carried out on an Agilent 7890 GC equipped with a DB-5 capillary column of 30 m length, 0.25 mm i.d. and 0.25-μm film thickness. GC oven was operated at a temperature program of 50 °C (hold for 2 min) to 280 °C (hold for 3 min) at a rate of 20 °C/min. Helium was used as the carrier gas at a constant flow of 1 ml/min. For analyses, 1 μl of samples was injected in splitless mode at injection temperature of 250 °C and detector temperature of 250 °C.

3. Results and discussion

The study was focused on optimization of DSPE parameters for extraction of NA from water using Mag-CNT. Initially, the extraction parameters from distilled water (DW) were optimized with non toxic markers; subsequently NA were extracted from different water samples employing optimized conditions. Recoveries of analytes were calculated from the ratios of peak areas of analytes to internal standard, compared in control versus spiked samples. Control was processed in parallel to the sample and prepared by adding analytes in post extract aliquot exactly at the concentration corresponding to the maximum theoretically possible recovery. All extractions were performed in triplicate and the values shown in figures are the mean of triplicate runs. Error bars in the figures indicate relative standard deviations in percentage. Fresh Mag-CNT were used for each extraction experiment to avoid sample carry over and memory effect in the GC-FPD (P) analyses.

3.1. Optimization of extraction procedure

In SPE, the sorbent is first exposed to the water sample containing desired analytes and subsequently to organic solvent (mostly water immiscible) for the extraction of adsorbed analytes. However, the extraction step essentially requires the complete removal of water adsorbed onto the sorbent surface. The most common method to remove the adsorbed water from sorbent column is to flush with dry nitrogen or air, which is carried out after sample loading and washing steps. However, in cases, where the sorbent is CNT, this method of water removal is not effective. Water molecules diffuse into the nanotubes and are strongly retained via hydrogen bonding [20]. Complete removal of adsorbed water on CNT requires high vacuum treatment [13,14]. This not only incorporates one additional step in the extraction protocol, but also increases extraction time.

In present study, an alternative approach was adopted to remove the adsorbed water from Mag-CNT. After magnetic separation, Mag-CNT were first washed twice with small aliquots of methanol. This step removes the adsorbed water from Mag-CNT, and makes them compatible for subsequent chloroform extraction. These methanol washings, containing small amount of water, were preserved in a separate vial. Methanol washed Mag-CNT were then extracted with the chloroform and chloroform extracts were combined with methanol washings. Initial additions of chloroform to methanol washings resulted in a clear solution, which separated into two phases with further addition of the former. Thus separated water layer was discarded and organic layer was subjected to chromatographic analysis after concentration.

This methodology of water separation was found to be much simpler than the vacuum drying technique, as it does not require any vacuum drying equipment and can be adopted in field conditions also.

3.2. Selection of eluting solvent

Extraction of the analytes from sorbent is an important parameter which determines their overall recoveries. For the selection of extracting solvent, Mag-CNT were first spiked with the selected phosphonates at a concentration of 200 ng/g. For spiking, calculated amount of analytes were dissolved in 5 ml of dichloromethane and 500 mg of Mag-CNT were dispersed into it. Solvent was evaporated via gentle nitrogen stream. 30 mg of spiked Mag-CNT were extracted with 3 × 1 ml of extracting solvent. The final volume was reduced to 500 μl by nitrogen stream and analyzed after the addition of internal standard. Extractions were carried out in triplicate and the average value was taken as the final recovery. Various extracting solvents and corresponding recoveries of all the analytes are summarized in Fig. 5. Among the selected solvents, chloroform

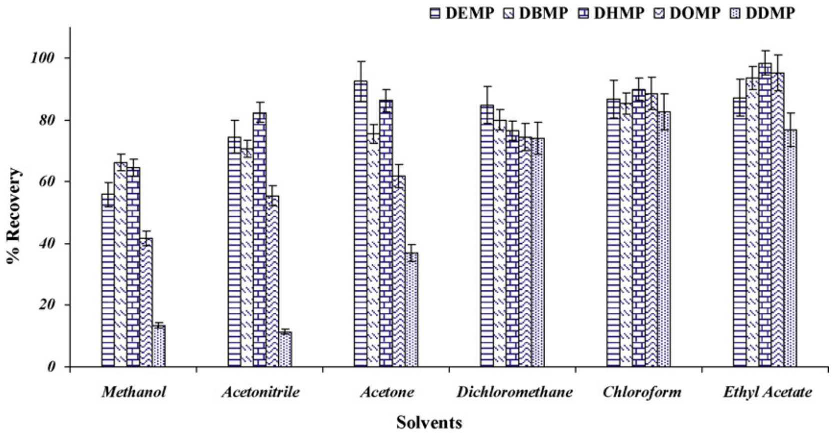


Fig. 5. Selection of eluting solvent for DSPE experiments.

and ethyl acetate clearly showed superiority over others as recoveries of all analytes were more than 80%. However, chloroform provided an additional advantage over the ethyl acetate in 'phasing out' the aqueous layer from combined organic extracts (discussed in previous section). Hence, chloroform was taken as solvent of choice and used in the further studies for extraction purposes.

3.3. *Optimization of amount of Mag-CNT and water sample*

In SPE, the sample volume is determined by typical concentrations of potential analytes and degree to which they will need to be concentrated from the sample prior to analysis. It also depends upon the detection limits and the linearity range of the instrument used for the analysis. Considering the real world application where the concentrations of the analytes selected may lie at the lowest possible level, spiking level of 3 ng/ml was selected for the present study. Arbitrarily assuming 50% recoveries of the selected analytes from spiked water and taking into account the linearity range obtained for the analytes in GC-FPD (P) analysis, a sample volume of 200 ml was considered to be sufficient and used for the optimization of various parameters.

The amount of sorbent is little bit more critical than the sample amount. Insufficient amount of sorbent will cause the breakthrough of the analytes whereas higher amounts will increase the cost and time of the analytical procedure. Higher amounts of sorbent material may also adversely affect the final recoveries if the back extraction (elution) of the analytes from sorbent is not quantitative.

For the determination of optimum sorbent amount, 200 ml of water containing 3 ng/ml of analytes, was extracted with varying amounts (20–100 mg) of Mag-CNT as per procedure described in experimental section. Results summarized in Fig. 6, show that the recoveries of DBMP and DHMP were not much affected by the variation in sorbent amount. The recoveries of DOMP and DDMP decreased, while that of DEMP increased with the increasing amount of sorbent (Fig. 6). However, the recoveries of all the analytes were higher (ranging from 69 to 96%) at the sorbent amount of 40 mg, hence this amount was taken as optimum for the present study.

Variation in the recoveries, which could be attributed to relative hydrophobicities of selected analytes, is discussed in detail in subsequent section.

3.4. *Recoveries of the analytes*

In reversed phase SPE of small polar analytes from aqueous phase, adsorption of the analytes onto the sorbent surface becomes dominant as the interaction between analyte and sorbent is generally weak. Once adsorbed, these can be eluted efficiently with organic solvents. On the other hand, large hydrophobic analytes exhibit strong interaction with hydrophobic sorbent surface making elution step more critical.

The results obtained in the present study substantiated above facts. The recoveries of the analytes obtained from triplicate trials, using 40 mg of Mag-CNT are depicted in Table 1. These values exhibited an increasing trend from DEMP to DHMP (entries 1–3, Table 1) and then adopted a negative slope towards DDMP (entries 4 and 5, Table 1). DEMP being the least hydrophobic, preferred to be in aqueous phase rather than the hydrophobic Mag-CNT surface. Thus its recovery was minimum among the selected analytes (~69%). On the other hand, DOMP and DDMP, which are highly hydrophobic in nature, were strongly adsorbed by the Mag-CNT. The hydrophobic interaction between these analytes and Mag-CNT surface was so strong that their back extraction (desorption) from sorbent surface was not quantitative; which ultimately lead to the average recoveries (86% and 78% for DOMP and DDMP, respectively). DBMP and DHMP, having intermediate hydrophobicities showed maximum recoveries.

Above arguments were further substantiated by the results obtained with varying sorbent amounts. Increasing the amount of Mag-CNT, the recovery of DEMP also increased from 56 to 73%, which is attributed to its increased adsorption (Fig. 6). On the contrary, high sorbent amount decreased the recovery of DOMP and DDMP, which is ascribed to their strong retention on the sorbent (Fig. 6).

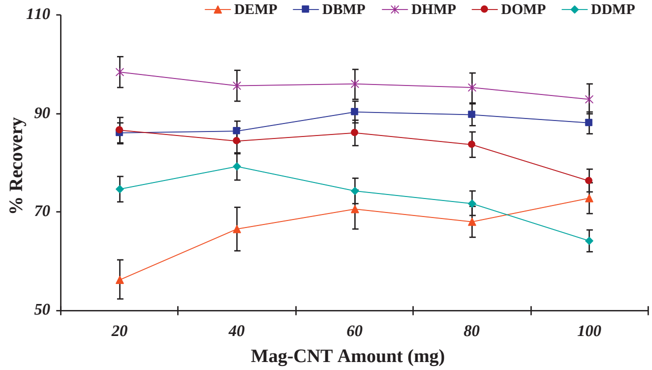


Fig. 6. Variation in the mean recoveries of the selected analytes with respect to the change in Mag-CNT amount.

Table 1
Recoveries, LOD and validation results of DSPE experiments.

S. no.	Analyte	Recovery[a] (%)			LOD (ng/ml)			LOQ[b] (ng/ml)	Correlation coefficient (r^2)	Precision[b] (% RSD)	
		Distilled water	Tap water	Muddy water	Distilled water	Tap water	Muddy water			Intra-day	Inter-day
1	DEMP	68.8 ± 4.4	70.2 ± 3.2	69.8 ± 4.9	0.5	0.5	0.5	1.75	0.9834	6.4	7.1
2	DBMP	88.5 ± 2.8	91 ± 2.8	86.2 ± 4.1	0.05	0.05	0.05	0.15	0.9956	3.2	3.1
3	DHMP	96.3 ± 3.2	92.8 ± 2.1	90.6 ± 5.0	0.05	0.1	0.1	0.15	0.9925	3.3	3.1
4	DOMP	86.1 ± 3.0	88.6 ± 3.5	82.1 ± 3.8	0.05	0.05	0.25	0.15	0.9893	3.5	2.9
5	DDMP	78 ± 3.5	72.9 ± 2.9	71.9 ± 5.7	0.25	0.5	0.5	1.0	0.9863	4.5	4.8
6	Sarin	59.7 ± 5.2	58.3 ± 3.4	55.5 ± 6.8	1.0	1.0	1.0	5.0	0.9810	8.7	9.2
7	Soman	85.9 ± 5.3	86.6 ± 2.4	82.8 ± 2.9	0.25	0.25	0.5	1.0	0.9973	6.2	8.5
8	Cyclosarin	85.2 ± 2.6	89.1 ± 1.9	85.7 ± 3.1	0.25	0.25	0.25	1.0	0.9939	3	4.6
9	Tabun	64.7 ± 4.8	60.5 ± 3.9	59.7 ± 4	0.75	0.75	1.0	2.25	0.9882	7.4	8.8

[a] Based on triplicate runs.
[b] In distilled water.

3.5. Effect of salinity and pH

It was imperative to study the effect of salt concentration and pH on the overall recoveries of the analytes. These two factors may vary from sample to sample and can have profound effect on the final results.

Presence of salt in water sample can disrupt the solvation cage of the water soluble analytes (salting out effect) or may cause alteration in the diffusion rate of analytes from aqueous to solid phase. To see the effect of salinity in the present study, the concentration of sodium sulfate in the water sample was varied from 0 to 20% and was plotted against the recoveries of selected analytes. Results are depicted in Fig. 7. Except DEMP, which showed a positive response towards the salt addition, all other analytes remained unaffected by the sample salinity. The increase in the DEMP response was due to salting out effect which enhanced its adsorption on solid surface by decreasing its solubility in aqueous phase. This salting out effect was less pronounced in rest of the hydrophobic analytes.

Sample pH also plays an important role in partitioning of the analytes especially those, having ionisable polar groups. It also determines the hydrolytic stability of the analytes and thus the extraction efficiency. To evaluate the effect of sample pH in the present study, it was varied from 3 to 11. The results demonstrated stable recoveries of all the analytes in the entire pH range (data not shown). Dialkyl methylphosphonates exist as neutral molecules under ordinary conditions and therefore their recoveries were independent of the sample pH. However, point to be noted is that the extraction of analytes from water sample was carried out just after the spiking. Prolonged storage of spiked water sample may lead to lower recoveries as the phosphonate esters as well as NA are known to hydrolyse at higher pH values [21].

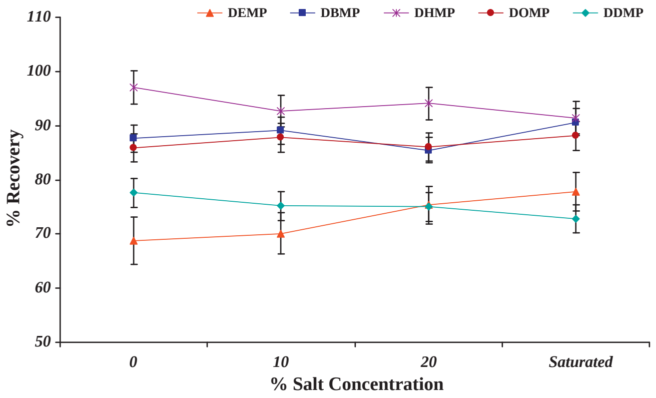


Fig. 7. Effect of salt concentration on the mean recoveries of the selected analytes in DSPE experiments.

3.6. Comparison of Mag-CNT with MWCNT and C_{18}

In the next set of experiments, the extraction efficiency of Mag-CNT in dispersive form was compared with that of MWCNT and octadecyl silane-SPE (C_{18}) in column form. Analytes were spiked at a concentration of 3 ng/ml in DW and were extracted as per procedures given in experimental sections. To avoid the loss of analytes by hydrolysis, water samples were prepared just before the experiments.

Results depicted in Table 2, substantiated our previous findings [22] and demonstrated the superiority of MWCNT and Mag-CNT over conventional C_{18} sorbent. With MWCNT and Mag-CNT, high surface area, smaller particle size and better partitioning of analytes from water were responsible for effective mass transfer even at lower analyte concentrations.

Non-magnetized MWCNT produced slightly better recoveries than Mag-CNT, which could be attributed to the less available surface area due to magnetic coating. In Mag-CNT, Fe_2O_3 nanoparticles spread over the surface of MWCNT (Fig. 2) and thereby reduce the surface area available for the interactions with the analytes (inorganic Fe_2O_3 do not contribute much in adsorption or retention of analytes). However, it is worth noticing that from 200 ml sample volume, DSPE with Mag-CNT could be performed quickly and conveniently; the column form SPE consumes much longer time in sample loading. Moreover, frequent plugging of CNT-bed (in SPE mode) with dirty water samples essentially requires centrifugation step prior to sample loading. Thus operational simplicity, faster analysis and relatively better performance of Mag-CNT in DSPE mode outweigh them from normal SPE mode.

3.7. Analysis of environmental water samples

To assess the applicability of the developed method to the real world samples, the analytes were spiked in tap and muddy water.

Table 2
Comparison of extraction efficiencies of Mag-CNT in DSPE mode and that of MWCNT and C_{18} in SPE mode.

S. no.	Analyte	Recoveries (in distilled water)		
		Mag-CNT (DSPE)	MWCNT (SPE)	C_{18} (SPE)
1	DEMP	68.8 ± 4.4	69.2 ± 3.8	12.8 ± 4.8
2	DBMP	88.5 ± 2.8	102.6 ± 3.3	21.9 ± 3.8
3	DHMP	96.3 ± 3.2	102 ± 2.9	54.8 ± 2.9
4	DOMP	86.1 ± 3.0	99.6 ± 3.1	57.6 ± 3.6
5	DDMP	78 ± 3.5	95.7 ± 4.1	57.9 ± 3.0
6	Sarin	59.7 ± 5.2	62.1 ± 3.4	nd
7	Soman	85.9 ± 5.3	94.5 ± 2.8	17.8 ± 6.9
8	Cyclosarin	85.2 ± 2.6	102.2 ± 3.6	22.7 ± 4.4
9	Tabun	64.7 ± 4.8	90 ± 4.7	nd

nd, not detected.

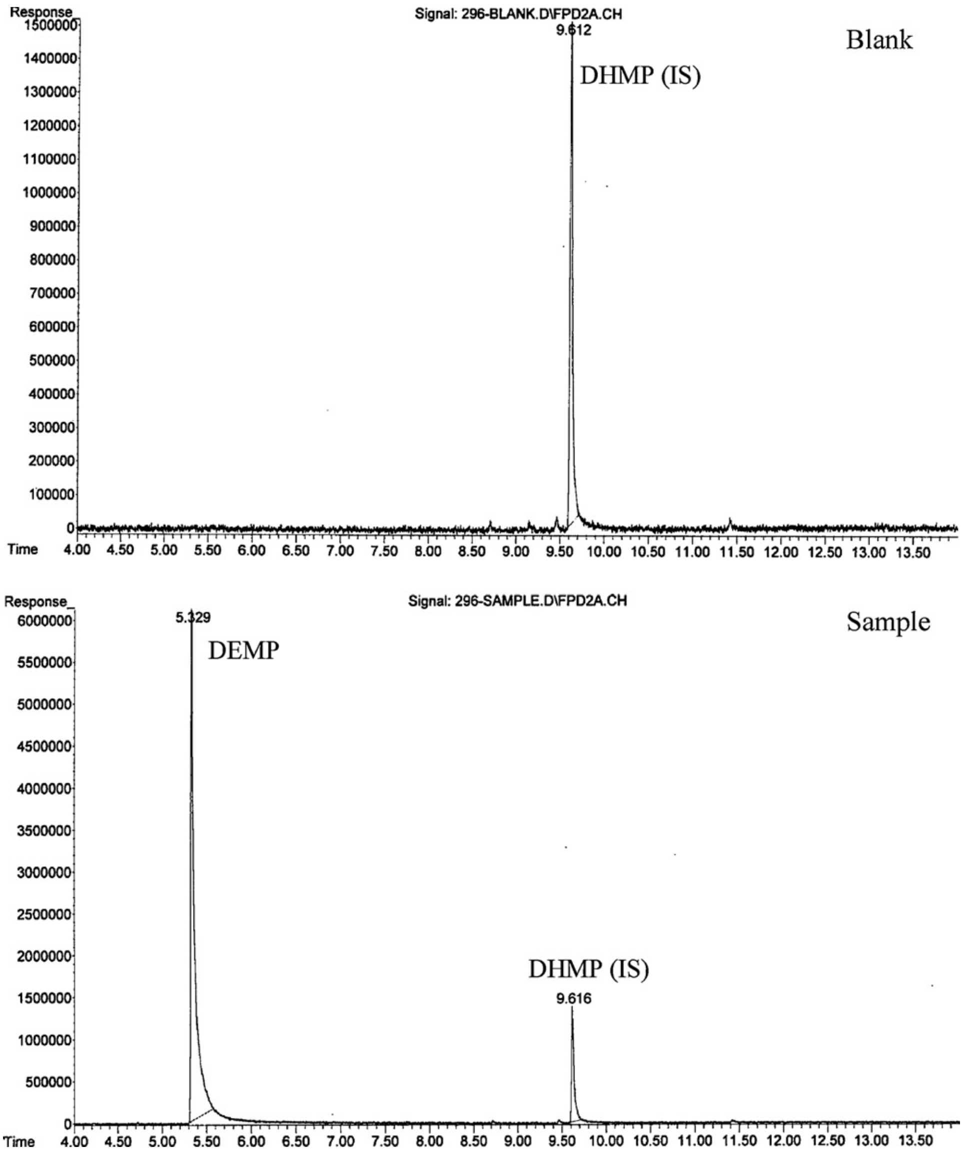


Fig. 8. Chromatograms obtained from Mag-CNT assisted DSPE and GC-FPD (P) analysis of a sample provided during the 29th OPT (sample code 296) and its corresponding blank.

Extractions were carried out under optimized conditions and final recoveries were compared to those obtained in distilled water. The results are summarized in Table 1. No noticeable variation in the final recoveries of the analytes was observed.

The applicability of the method was also investigated on the OPCW official proficiency test (OPT) samples. These samples provide a good platform to validate the methods developed for the analysis of CWA (and related compounds) as they mimic the real world samples. In 29th OPT which was conducted recently during March–April 2011, polymeric strips of flooring material (sample codes 294, 295 and 296, weighing ~1 g each) were provided for the analysis of scheduled chemicals and/or their degradation/reaction products [23]. One polymer strip of sample 296 containing diethyl methylphosphonate was processed with the developed Mag-CNT method. The sample strip was first extracted with 1 ml methanol followed by equal amount of water. Aqueous and methanolic extracts were combined and 1 ml of this solution was diluted to 50 by distilled water. DHMP was added as method internal standard to achieve the final concentration of 0.1 μg/ml. Its corresponding blank was also generated by diluting the 0.5 ml of methanol to 50 ml in distilled water. These samples were then extracted with Mag-CNT under the optimized conditions and the resulting GC-FPD (P) chromatograms are shown in Fig. 8. The presence of DEMP in sample chromatogram proclaims Mag-CNT as an efficient sorbent for the rapid enrichment of NA and their markers from large volumes of environmental samples.

3.8. *Analytical figures of merit*

To evaluate the practical applicability of DSPE, repeatability, reproducibility, linearity, and limit of detection of the selected analytes were investigated utilizing standard solutions in distilled water. Results of analytical parameters are enumerated in Table 1. The calibration curves for studied compounds were linear from 0.25 μg/ml to 1.5 μg/ml with coefficient of determination (r^2) between 0.9810 and 0.9973. Within- and between-day precision were tested with water samples spiked at concentration of 1 ng/ml of analytes. The relative standard deviations of intraday repeatability and inter-day reproducibility (three consecutive days triplicate extractions every day) were in range of 3.0–8.7 and 2.9–9.2%, respectively. For determination of limits of detection (LOD) and limits of quantification (LOQ) in distilled, tap and muddy water, analytes were spiked at 10 ng/ml and extractions were carried out under optimized conditions. LOD and LOQ were calculated as the minimum concentration providing chromatographic *S*/*N* ratio of 3 and 10, respectively. LOD were slightly better in the DW as compared to the muddy water (Table 1). The sensitivity and enrichment achieved by Mag-CNT for NA and phosphonates from water surpass the requirement set by the OPCW for verification analysis of CWC [24].

4. Conclusion

Present study dealt with the surface magnetization of MWCNT and their application for the rapid enrichment of NA and their markers from large volumes of environmental water samples. Large surface area and high adsorption capacity of the Mag-CNT resulting in high adsorption capacity and recoveries of the selected analytes. Their use in disperse solid phase extraction (DSPE) mode offered various advantages over conventional 'column form' SPE; most important being the liberty to disperse the sorbent material directly into the water samples. Subsequent magnetic separation and elution of sorbent material consumed much less time as compared to conventional process. Applicability of the method was proven in different environmental water samples and the final recoveries of the analytes were found to be unaffected by the presence of dirt particles, pH and salinity of sample. The simplicity and least requirement of laboratory equipments qualify this technique as simple and most suited sample preparation method for the off-site as well as on-site analyses of nerve agents and their markers.

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