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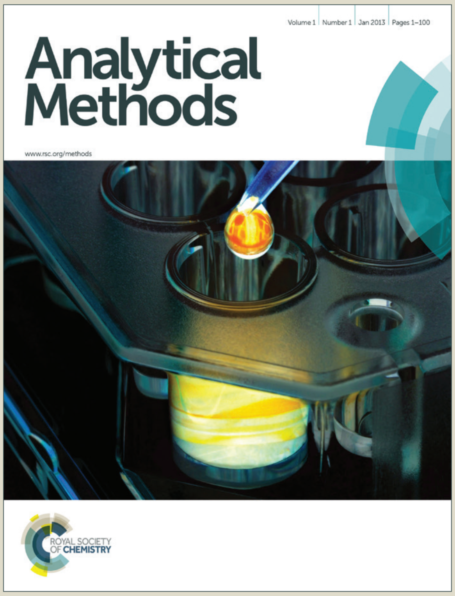

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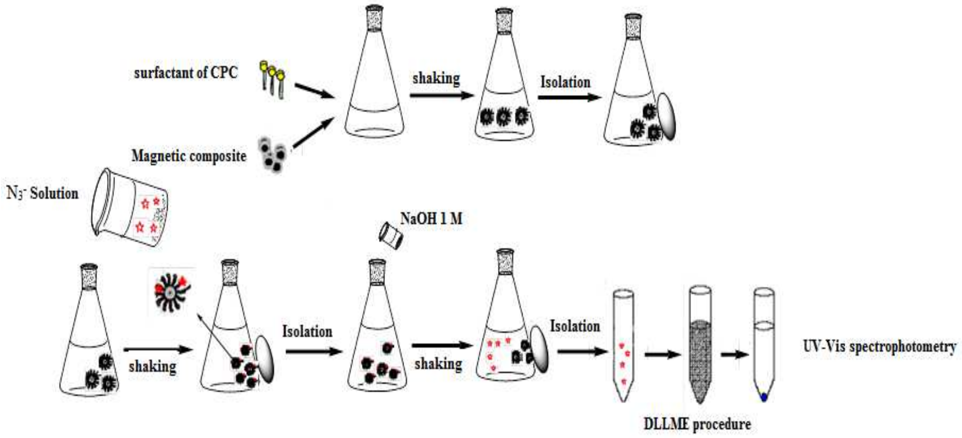
Graphical Abstract



Schematic illustration of the preparation of surfactants coated MWCNT/Nano-Fe_3O_4 composites and its application for SPE-DLLME procedure.

Solid phase extraction combined with dispersive liquid-liquid microextraction for the spectrophotometric determination of ultra trace amounts of azide ion in water samples

Ali Reza Zarei[*], **Reihaneh Hajiaghabozorgy**

Faculty of Chemistry and Chemical Engineering, Malek Ashtar University of Technology, Tehran, P.O.Box 15875-1774, Iran

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[*] Corresponding author: Fax: +9821 22938641.

E-mail address: zarei1349@gmail.com.

Abstract

In this research, the solid-phase extraction (SPE) using modified multi-walled carbon nanotube/nano-Fe_3O_4 composites combined with the dispersive liquid–liquid microextraction (DLLME) as a preconcentration technique has been developed for determination of ultra trace amounts of azide ion in water samples. In SPE–DLLME, azide ion was adsorbed from a large volume of aqueous samples (100 mL) into 20 mg modified multi-walled carbon nanotube/nano-Fe_3O_4 composites as sorbent. After the elution of azide ion from the sorbent by using sodium hydroxide, DLLME technique was performed on the desorbed solution. Finally, the azide ion in the extracted organic solvent was determined by UV-Vis spectrophotometry. Some important parameters in SPE and DLLME steps were studied and optimized. The proposed method provided a high enrichment factor 250 for azide ion. The calibration graph was linear in the range of 0.10–5.0 ng mL^{-1} with a limit of detection (LOD) 0.05 ng mL^{-1}. The proposed method was successfully applied for determination of azide ion in water samples.

Keywords: Azide ion; Solid-phase extraction (SPE); Dispersive liquid–liquid microextraction (DLLME); Preconcentration; Spectrophotometry.

Introduction

Sodium azide (NaN_3) is widely used in agricultural industries for the production of pesticides and herbicides, in biomedical sciences as preservative in diagnostic reagents, in the automotive industries as a propellant for air bags, and for the production of detonators and other explosives in military purposes.[1,2] Sodium azide is a highly toxic compound classified as a first-class poison.[3] In humans, it is rapidly absorbed through ingestion and inhalation and hydrolyzed to hydrazoic acid. In this regard, it forms strong complex with hemoglobin, that consequently blocks oxygen transport in the blood and thus it causes the death of the cells. So it is more harmful to the heart and the brain than the other organs, because the heart and the brain use a lot of oxygen. The threshold limit value (TLV) for sodium azide was reported 0.3 mg/m^3.[4,5]

Different methods have been reported for the determination of azide ion, such as titrimetry,[6,7] high performance liquid chromatography,[8-10] gas chromatography,[11] capillary electrophoresis,[12,13] ion chromatography,[14-17] amperomety,[18,19] electron paramagnetic resonance[20] and spectrophotometry.[21-25] Some of these methods are time consuming and suffer from lack of sensitivity. Therefore, the development of new microextraction techniques and low cost instrumental methods could be very interesting for the determination of ultra trace amounts of azide ion.

Sample preparation has a direct effect on accuracy, precision, and quantitation limits and is often the rate determining step of analytical process, especially when trace determination is required.[26] Liquid-liquid extraction (LLE) is a versatile classical sample preparation technique prescribed in many standard analytical methods. However, conventional LLE uses large amounts of potentially toxic organic solvents such as halogenated hydrocarbon solvents which be toxic and-or carcinogenic and some solvents (e.g. diethyl ether) can form peroxides and are potential explosion hazardswhich are often hazardous and explosive.[27,28] To overcome these limitations, some techniques such as solid-phase extraction (SPE), solid phase microextraction (SPME), and liquid phase microextraction (LPME) have been developed.

Solid-phase extraction (SPE), a traditional sample preparation technique, has the advantages of reducing organic solvent consumption and processing time, and high reproducibility relative to liquid–liquid extraction (LLE). Nowadays, it is applied as a common sampling technique in various fields, including environmental, pharmaceutical, clinical, food and industrial chemistry.

Dispersive liquid-liquid microextraction (DLLME) is a consolidated LLE technique that uses micro-liter volumes of extraction solvent along with a few mililiter of dispersive solvents. In this method, a cloudy solution is formed when an appropriate mixture of extraction and dispersive solvents is injected into an aqueous sample containing the analytes of interest. The hydrophobic solutes are enriched in the extraction solvent, which is dispersed into the bulk aqueous solution. After centrifugation, determination of the analytes in the settled phase can be performed by conventional analytical techniques.[29-33] In most cases, DLLME has been applied directly to water samples. The main disadvantage of the DLLME is that it is not a selective extraction method. In the other hand, It is should be noted that the enrichment factoris often obtained in low amounts using of DLLME procedure (for example, <200 in DLLME-UV/Vis spectrophotometry[34-39]) which still cannot be satisfied for the requirement of the ultra trace residue analysis. In principle, SPE combined with

DLLME can provide a solution to this problem. So far, several reports about the combination of SPE with DLLME for the determination of ultra trace amounts of analytes in aqueous samples have been reported.[40-45]

Although the MWCNTs have high sorption capacity for the separation of analytes from aqueous solution, it is difficult to separate CNTs from aquatic phase because of their small size. The centrifugation method needs very high rate and the traditional filtration method may cause the blockage of filters. Compared with centrifugation and filtration methods, magnetic separation method is considered as a rapid and effective technique for the separation of magnetic particles from aqueous solutions. Magnetic separation methods, which represent a group of techniques based on the use of magnetic or magnetizable adsorbents, have been used for many applications.[46-49] To facilitate the separation and recovery of CNTs from aqueous solution, the incorporation of magnetite with CNTs can be a promising method.

In this paper, we combined SPE with DLLME as an efficient sample preparation technique with high enrichment factors for the determination ultra trace amounts of azide ion in water samples. After preconcentration, the azide ion was determined using UV-Vis spectrophotometric method.

Experimental section

Reagents and chemicals

All chemicals used were of analytical-reagent grade. Doubly distilled water was used throughout the work. A stock solution of 1000 μg mL^{-1} of azide (Merck) was prepared by dissolving 0.1548 g sodium azide in water and diluting to 100 mL in a volumetric flask. A 100 µM malachite green solution (Merck) was prepared by dissolving appropriate amount of this reagent in water and diluting to 100 ml in a volumetric flask. The acetate buffer solution (pH 5.2) was used to adjust the pH. Multi-walled carbon nanotubes (MWCNTs) were purchased from Plasmachem GmbH company. Ferric chloride ($FeCl_3.6H_2O$), Ferric sulfate ($FeSO_4 \cdot 7H_2O$) and cetyl pyridinium choloride (CPC) were purchased with high purity from Merck.

Apparatus

A Hitachi model 3310 UV-Vis spectrophotometer with 1-cm quartz micro cells was used for recording absorbance spectra. A Metrohm model 713 pH-meter was used for pH measurements. A Hettich centrifuge (EBA 20) with 10 ml calibrated centrifuge tubes was used to accelerate the phase separation process. A shaker (Heidolph) was used for stirring of solutions. The morphologies of MWCNTs and prepared magnetic nanocomposites were examined by using a scanning electron microscope (SEM-EDX, XL30, Philips, Netherland). The crystal structure of synthesized materials was determined by an X-ray diffractometer (XRD, 38066 Riva, d/G. Via M. Misone, 11/D (TN) Italy) at ambient temperature.

Preparation of MWCNT/Nano-Fe_3O_4 composites

The magnetic nanocomposites was prepared from a suspension of 1.0 g MWCNTs in a 150 mL solution of 2.98 g $FeCl_3 \cdot 6H_2O$ and 1.53 g $FeSO_4 \cdot 7H_2O$ at 70 °C under N_2 condition. NaOH solution (30 mL, 0.5 mol L^{-1}) was added dropwise to precipitate iron oxides. After the addition of NaOH solution, the mixture was adjusted to pH 11 and stirred for 1 h. The mixture was aged at 70 °C for 4 h and was washed 3 times with doubly distilled water. The obtained materials were dried in an oven at 100 °C for 3h.

Preparation of CPC coated MWCNT/nano-Fe_3O_4 composites

For coating the magnetic composites, 0.2 g MWCNT/nano-Fe_3O_4 composites was added to 25 mL of 100 μM CPC solution in a beaker. The solution was shaken for 5 min on the shaker. Then, CPC-coated MWCNT/nano-Fe_3O_4 composites (CPC/MWCNT/ Fe_3O_4) was separated using the magnet and washed with distilled water for several times.

SPE–DLLME procedure

For SPE procedure, in a 100- mL beaker containing different amounts of azide ion, 0.2 g of CPC/MWCNT/Fe_3O_4 composites was added. The pH of the solutions adjusted at 6.5 using HNO_3 and/or NaOH diluted solutions. The solution was shaken at room temperature for 20 min. Then, the magnetic nanocomposites after adsorption of azide ion were separated from the mixture via a strong hand-held magnet. After decanting the supernatant solution, the collected magnetic nanocomposites was washed with 0.5 mL of 1.0 mol L^{-1} NaOH solution and accumulated into a 10-ml centrifuge tube.

For DLLME step, 1.0 mL of 50 μM malachite green solution and 0.5 mL of the acetate buffer solution (pH 5.2) were added to obtained solution from SPE procedure. The contents were diluted with distilled water up to 3.0 mL and then 400 μL of acetone (as disperser solvent) contains 100 μL of 1,2-dicolorobenzene (as extraction solvent) was injected rapidly into a sample solution by using a 1 ml syringe. A cloudy solution was formed in the test tube. In this step the ion pair product was extracted into the fine droplets of 1,2-dicolorobenzene. The mixture was centrifuged at 3500 rpm for 2 min and fine droplets of 1,2-dicolorobenzene were sedimented in the bottom of test tube. The supernatant aqueous phase was readily decanted with a sampler. The remained organic phase was diluted to 400 µL with ethanol and the absorbance measured at 620 nm against blank. The blank solution was prepared as sample solution except that distilled water was used instead of azide ion solution. The entire scheme of the SPE-DLLME procedure is shown in Scheme 1.

Results and Discussion

Characterization of magnetic nanocomposites

Figure 1 shows the XRD patterns of the MWCNTs and the magnetic nanocomposites. Two peaks corresponding to the structure of MWCNTs exist in the XRD pattern of the magnetic nanocomposites, which indicates that the MWCNT structures are not destroyed during the processes of the chemical coprecipitation of iron oxides on MWCNTs. Figure 2 shows the SEM images of MWCNTs and the magnetic nanocomposites, respectively. SEM image of the composites depicts an entangled network of MWCNTs with clusters of iron oxides attached to them, which indicates the formation of MWCNT/iron oxide magnetic composites.

Parameters affecting the SPE

Effect of pH

The pH of the sample solution plays a key role in the SPE procedure. An appropriate pH value can improve the adsorption efficiency, and also reduce interference from the matrix. The influence of pH on the adsorption of azide ion on magnetic nanocomposites was investigated. The pH of each solution was adjusted to values ranging from 4 to 9 by NaOH and HNO_3 to find the optimum pH value. The obtained results are shown in Figure 3. Based on the obtained results, the extraction efficiency of azide ion increased by increasing pH up to 6.5 and decrease at higher pH. Therefore, the optimum pH was 6.5 for extraction of azide ion from the aqueous solution.

Effect of CPC concentration

It was observed that magnetic nanocomposites without modification did not adsorb azide ion from aqueous solution, while magnetic nanocomposites modified by CPC adsorbed azide ion efficiently. The effect of CPC amount in modification of magnetic nanocomposite on the adsorption efficieny was studied in the range of 0–500 μM CPC. The result revealed that adsorption amount of azide ion increased by increasing the amount of surfactant up to 100 μM, and remained nearly constant at higher concentrations. The suggested mechanism for the adsorption of the azide ion by magnetic nanocomposites modified by CPC is due to that cetyl pyridinium choloride (CPC) is a cationic quaternary ammonium surfactant and tends to interact with surface magnetic nanocomposite via hydrophobic chains and coats them and hydrophilic ammonium group in CPC adsorb azide ion in solution. Therefore, 100 μM of CPC was chosen as the suitable concentration for further experiments.

Effect of amount of adsorbent

The dependence of the adsorption of azide ion on the amount of magnetic nanocomposites was studied at room temperature and at pH 6.5 by varying the adsorbent amount from 5.0-50 mg in contact with 100 mL solution of 3.0 ng mL^{-1} of azide ion. The results showed that the percentage recovery of azide ion increased by increasing adsorbent due to the availability of higher adsorption sites. The adsorption reached to a maximum with 20 mg of adsorbent and remained nearly constant at higher values. Thus, that maximum percentage recovery was about 95% for azide ion.

Influence of contact time

To achieve maximum efficiency of the extraction, contact time and stirring speed were studied in solution simultaneously. Therefore, 100 mL of azide solution (3.0 ng mL^{-1}) in the range of 5-30 min stirring with variable speed 100 to 600 rpm was extracted. It should be noted that increasing the stirring speed can achieve maximum extraction recovery and minimum adsorption time. Therefore, an agitation time of 20 min and stirring speed of 400 rpm was selected for further works.

Effect of the type and concentration of the desorbing solution

Various desorbing reagents were used to find the best desorbing solution for the adsorbed azide ion. Among different solutions used, NaOH solution provided higher recovery. The concentration of the NaOH solution was optimized. The results indicated that highest recovery was obtained at 1 mol L^{-1} NaOH solution. Subsequent experiments showed that 0.5 mL of 1 mol L^{-1} solution of NaOH was the optimum volume for desorbing of azide ion from CPC-MWCNT/Nano-Fe_3O_4. The effect of contact time on the desorption of azide ion was studied. It can be seen that after about 10 min, almost all the azide ion became desorbed.

Effect of sample volume

Effect of sample volume on the adsorption of azide ion was studied in the range 25–200 mL. In order to study the effect of sample volume, 15 mL of 3.0 ng mL^{-1}azide ion was diluted to 25, 50, 100, 150 and 200 mL with distilled water. Then adsorption process was performed under the optimum conditions (pH 6.5) using 20 mg magnetic nanocomposites as described in experimental section. It can be seen that the azide ion present in the volumes up to 100 mL was completely adsorbed with magnetic nanocomposites and decreased at higher volumes. Therefore, for determination of trace amounts of azide ion in samples, a sample volume of 100 mL was selected in order to increase the preconcentration factor.

Parameters affecting the extraction efficiency of DLLME

To spectrophotometric determination of trace amounts of azide ion in solution, we used from malachite green as ion pairing reagent. In acetate buffer, azide ion reacts with malachite green to form ion pair complex that can be extracted into 1,2-dichlorobenzene. Therefore, it can be a suitable method for separation and preconcentration of azide ion by DLLME. The visible absorption spectra of the ion pair complex of azide ion with malachite green after DLLME exhibit a maximum absorbance at 620 nm (Figure 4) . For achieving the highest efficiency and sensitivity in DLLME of azide ion, the influence of effective variables was investigated and optimum conditions were obtained.

Effect of the malachite green concentration

For ion association formation of azide ion, some of cationic dyes as malachite green (MG), methylene blue (MB) and crystal violet (CV) were investigated, which extraction of MG^+:N_3^- ion pair into 1,2-dichlorobenzene was high efficiency. The effect of malachite green concentration on the absorbance of the system was investigated within the range 2.5-15 μM. The results revealed that the absorbance increased by increasing reagent concentration up to 10 μM, and remained nearly constant at higher concentrations (Figure 5). Therefore, a concentration of 10 μM malachite green was applied in the proposed method.

Effect of pH

The pH of the sample solution is one of the important factors affecting the selectivity of ion association formation reaction and the subsequent extraction. The effect of pH was studied over the pH range 3.0 to 8.0. As can be seen in Figure 6, the maximum absorbance was achieved over the pH range of 5.0 to 5.5. Therefore, a pH 5.2 was selected as pH optimum and adjusted by acetate buffer solution.

Selection of the extraction and disperser solvent type

The type of extraction solvent used in DLLME is an important factor for extraction efficiency. The solvent should be denser than water, which make it possible to separate extraction solvent from aqueous phase by centrifugation. Moreover, it should have more capability for the extraction of interested compounds and lower solubility in water. Thus, chloroform, dichloromethane, chlorobenzene, 1,2-dichlorobenzene, 1,2-dichloroethane and nitrobenzene were studied as extraction solvents. The main point in choosing the disperser solvent in DLLME is its miscibility in organic phase (extraction solvent) and also in aqueous phase (sample solution) which enabling extraction solvent to be dispersed as fine particles in aqueous phase to form a cloudy solution (water/disperser solvent/extraction solvent). The selection of a disperser solvent is limited to solvents such as methanol, ethanol, acetonitrile and acetone, that are miscible with both water and extraction solvents. In this study, all combinations of chloroform, dichloromethane, chlorobenzene, 1,2-dichlorobenzene, 1,2-dichloroethane and nitrobenzene (100 μL) as extraction solvents and methanol, ethanol, acetonitrile and acetone (400 μL) as disperser solvents were examined. In the case of 1,2-dichlorobenzene with acetone, a stable two-phase system and higher signal was observed (Figure 7). Therefore, 1,2-dichlorobenzene and acetone were selected as extraction and disperser solvents, respectively.

Influence of the extraction and disperser solvent volume

The effect of the volume of the extraction solvent on the analytical signal was investigated. Optimization were performed with different volumes of 1,2-dichlorobenzene in the range of 20-200 μL as the extraction solvent by fixing the volume of the acetone at 400 μL. The results revealed that that the absorbance increased by increasing the volume of the 1,2-dichlorobenzene to 100 μL and then remained approximately constant by further increasing of its volume between 100-200 μL. Then, 100 μL of 1,2-dichlorobenzene was selected as volume optimum. In order to examine the effect of the disperser solvent volume, solutions containing different volumes of acetone in the range of 200-800 μL containing 100 μL of 1,2-dichlorobenzene were subjected to the same DLLME procedure. The results indicated that the absorbance reached to maximum value at 400 μL of acetone and then gradually decreased by further increasing of volume. It probably can be due to increment of the dissolution of the extraction solvent in water and thus lower extraction efficiency of the analyte is acquired.

Analytical performance

The calibration graph was linear in the range of 0.10-5.0 ng mL^{-1} of azide ion in the initial solution by applying the optimized conditions. The regression equation was

$A= 0.2135C+0.0732$ with correlation coefficient (r) of 0.9995 (n=10) where A is the absorbance and C is the concentration of azide ion in ng mL^{-1}. The limit of detection (LOD), defined as $C_L=3S_B/m$, where C_L, S_B, and m are the limit of detection, standard deviation of the blank, and slope of the calibration graph, respectively, was 0.05 ng mL^{-1}. S_B was obtained from five determinations of the blank solutions. The calibration graph by the conventional LLE was $A= 0.00108C + 0.0135$ where A is the absorbance and C is the concentration of azide ion in ng mL^{-1}. The enrichment improvement factor was calculated as the ratio of the slope of the calibration graph for the SPE-DLLME method to that of the slope of calibration graph of conventional LLE. According to this concept, enrichment improvement of 197.7 was obtained for azide ion.

Selectivity

Prior to the application of proposed method on real samples it was vital to investigate the effect of some of the interfering ions on the recovery percentage of azide ion. The tolerance limit was defined as the amount of the foreign ion causing a change of ±5% in the absorbance reading. The effect of some common ions coexisting with azide ion on the adsorption of 3.0 ng mL^{-1} azide ion with 20 mg adsorbent at pH 6.5 was investigated and the results are shown in Table 1. It was found that most of the investigated species did not interfere. Therefore, the results presented in this table show the good selectivity of the procedure.

Application

Finally, the applicability and reliability of this sample preparation and preconcentration method for the analysis of azide ion was investigated in water samples.

For studying the matrix effect on the separation efficiency, they were spiked with azide ion at variable concentrations and after preparation, were analyzed according to recommended procedure. The analytical data summarized in Table 2 suggests that good recoveries for azide ion added to samples are achieved.

Conclusions

This study proposes the use of SPE-DLLME as a method for separation and preconcentration of azide ion as a prior step to its determination by spectrophotomety. The SPE-DLLME is versatile, simple and provides good enrichment factors and efficient separation. Furture, in comparison to solvent extraction methods, it is much safer, since only a small amount of the solvent is used. The limit of detection of the method (LOD) is better or comparable to some of the previously reported techniques. A comparison of the results is given in Table 3. The results of this study clearly show the potential and versatility of this method, which could be applied to monitoring of azide ion in water samples. Finally, the coupling of SPE-DLLME with UV-Vis spectrophotometry, gave a fast and low-cost procedure for determination of azide ion without require to sophisticated instruments such as electrophoresis, GC and HPLC.

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Table 1 Tolerance limit of diverse ions on the determination of 3.0 ng mL^{-1} of azide ion

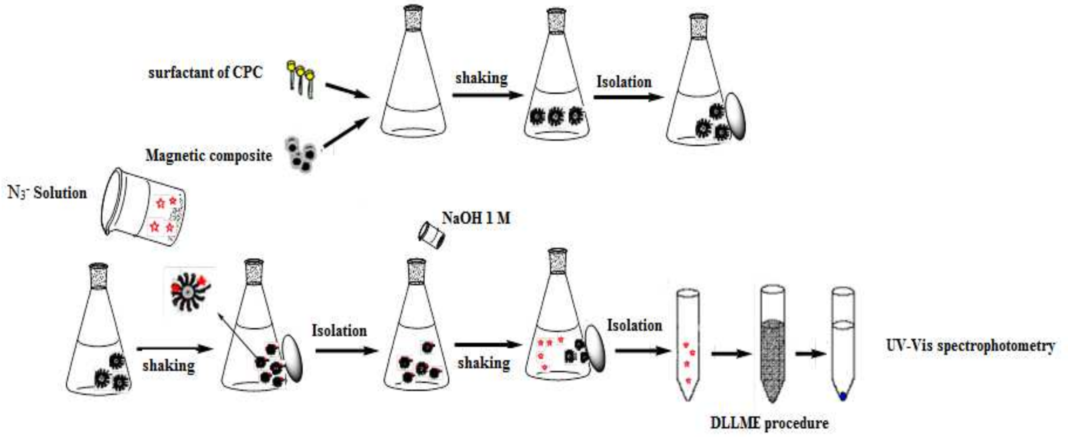
Species	Tolerane ratio (w_{ion}/w_{azide})
NO_2^-	62500:1
CO_3^{2-}	30000:1
CN^-,NO_3^-, Cl^-	20000:1
SO_4^{2-}	3000:1
Br^-	1000:1
Fe^{2+}	1000:1
Fe^{3+}	3000:1

Table 2 Determination of azide ion in water samples by proposed method

Samples	Azide ion (ng mL^{-1})		
	Spiked	Found	Recovery (%)
Tap water	0.0	Not detected	-
	0.5	0.49	98
	1.0	1.03	103
	3.0	2.95	98.3
Well water	0.0	Not detected	-
	0.5	0.51	102
	1.0	0.95	95
	3.0	2.85	95
Mineral water	0.0	Not detected	-
	0.5	0.48	96
	1.0	1.01	101
	3.0	2.9	96.7

Table 3 Comparison of the limit of detection (LOD) of the proposed method with some of the methods reported in literature.

Analytical method	Linear range (µg mL^{-1})	Detection limit (µg mL^{-1})	Relative standard deviation (RSD) (%)	Reference
High performance liquid chromatography (HPLC)	0.2-100	-	1.4	[10]
Gas chromatography-mass spectrometry	0.042-8.40	0.021	<10	[11]
Capillary electrophoresis based on derivatization with 3,5-dinitrobenzoyl derivative	6.5–323	1.9	0.24	[13]
Ion chromatography	0.02-0.80	0.02	-	[17]
Amperometry biosensor based on catalase enzyme	67-342	-	4.23	[18]
Electron paramagnetic resonance (EPR)	2.1-84	0.63		[20]
Spectrophotometry based on based on the reduction of Ce(IV) to Ce(III) with N_3^-	0.05-1.0	-	<4	[21]
Spectrophotometry based on reaction with with Fe^{3+} in HCl	10-1000	3.0	<20	[24]
SPE-DLLME -Spectrophotometry	0.0001-0.0050	0.00005	1.7	Proposed method



Scheme 1. Schematic illustration of the preparation of surfactants coated MWCNT/Nano-Fe_3O_4 composites and its application for SPE-DLLME procedure.

Figure captions:

Figure 1. XRD patterns of the MWCNT/Nano-Fe_3O_4 composites.

Figure 2. (a) SEM images of oxidized MWCNTs and (b) the MWCNT/Nano-Fe_3O_4 composites.

Figure 3. The effect of pH on the recovery percentage in SPE step, Conditions: Azide ion, 3.0 ng mL^{-1}; magnetic nanocomposites, 20 mg; CPC, 100 μM.

Figure 4. Absorption spectra of the ion pairing product of azide ion with malachite green after DLLME, Conditions: Azide ion, (1) 0.20 (2) 0.40 (3) 0.60 (4) 1.0 (5) 2.0 (6) 3.0 (7) 4.0 (8) 5.0 ng mL^{-1}; Malachite green, 10 μM; pH 5.2.

Figure 5. Effect of malachite green concentration on the absorbance of the system, Conditions: Azide ion, 10 ng mL^{-1}; pH 5.2.

Figure 6. Effect of pH on the analytical signals, Conditions: Azide ion, 10 ng mL^{-1}; Malachite green, 10 μM.

Figure 7. Effect of the type of extraction and dispersant solvent on the analytical signals, Conditions: Azide ion, 10 ng mL^{-1}; Malachite green, 10 μM; pH 5.2.

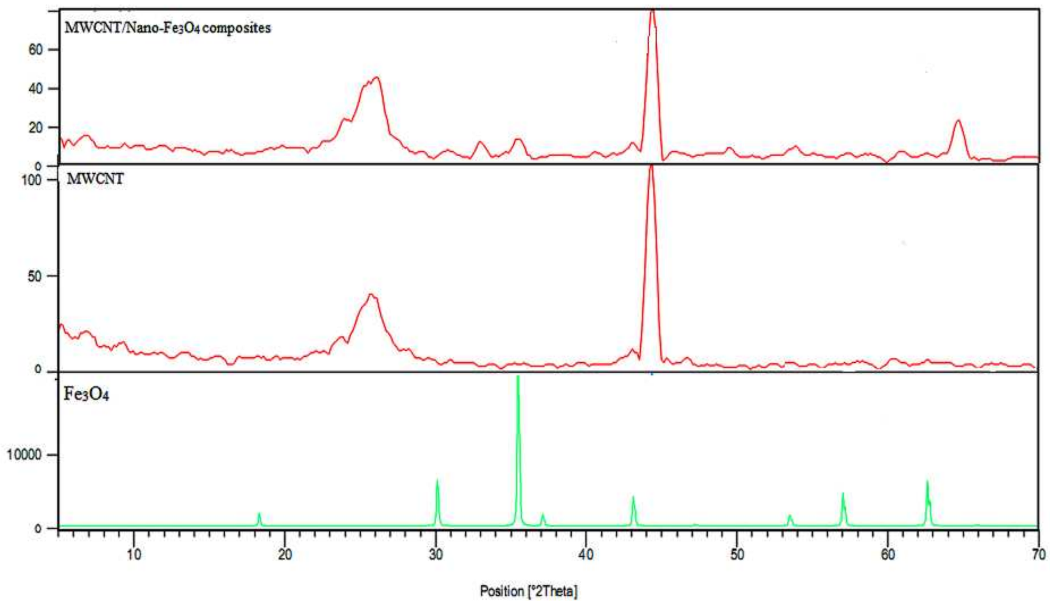

MWCNT/Nano-Fe3O4 composites
60
40
20
0
100
MWCNT
50
0
Fe3O4
10000
0
10
20
30
40
50
60
70
Position [°2Theta]

Figure 1

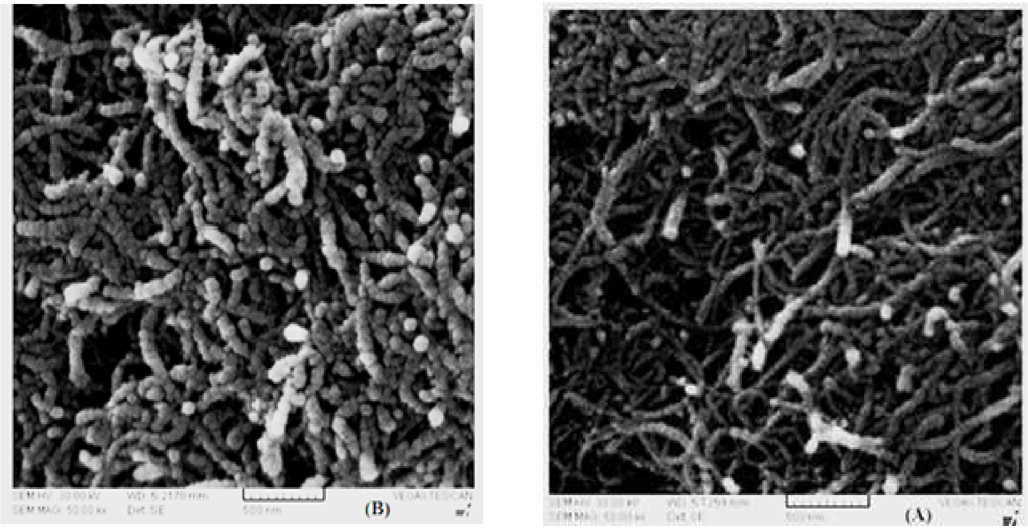

SEM HV: 30.00 kV
WD: 5.2170 mm
VEGA\\ TESCAN
SEM MAG: 50.00 kx
Det: SE
500 nm
(B)
(A)

Figure 2

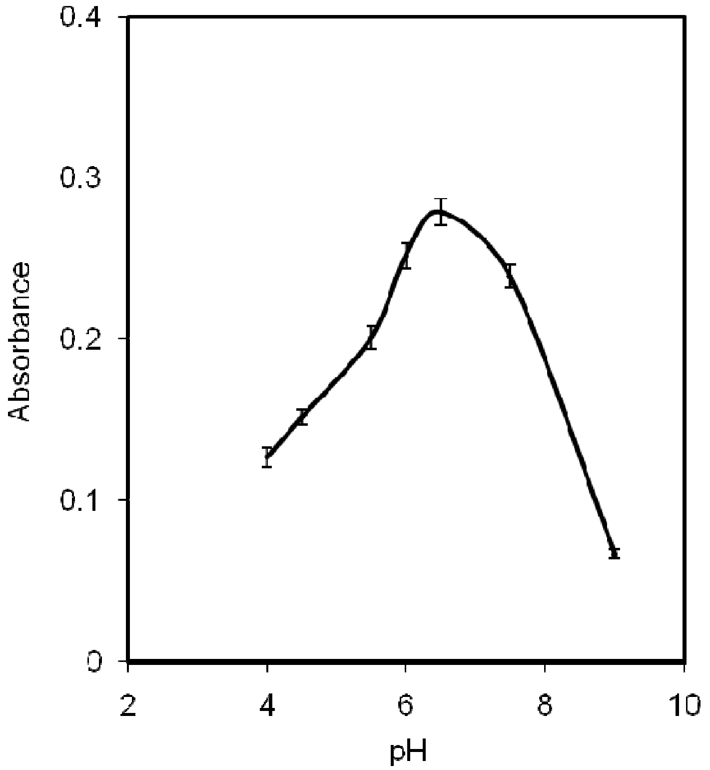


Figure 3

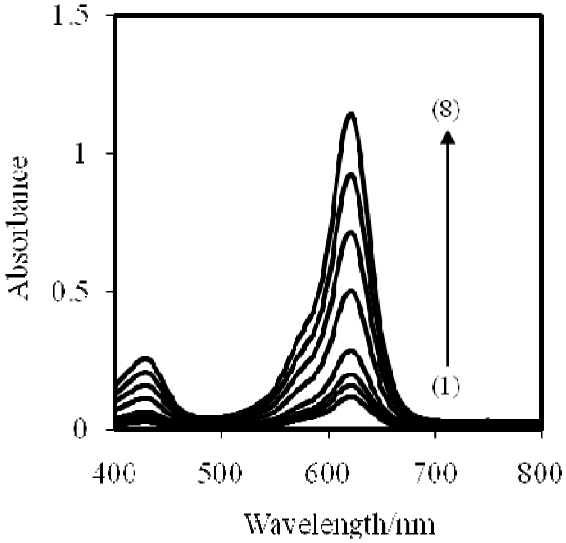


Figure 4

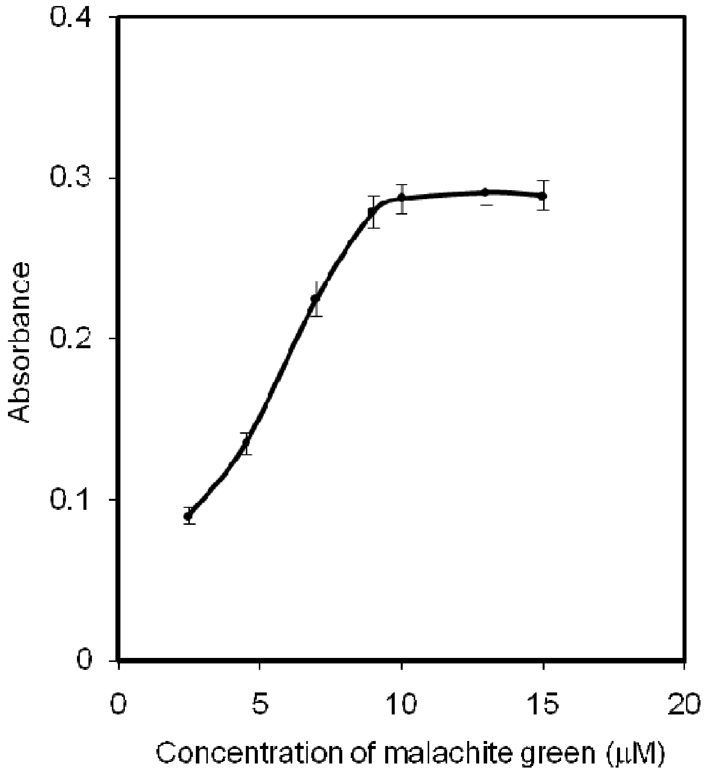

0.4
0.3
0.2
0.1
0
Absorbance
0
5
10
15
20
Concentration of malachite green (μM)

Figure 5

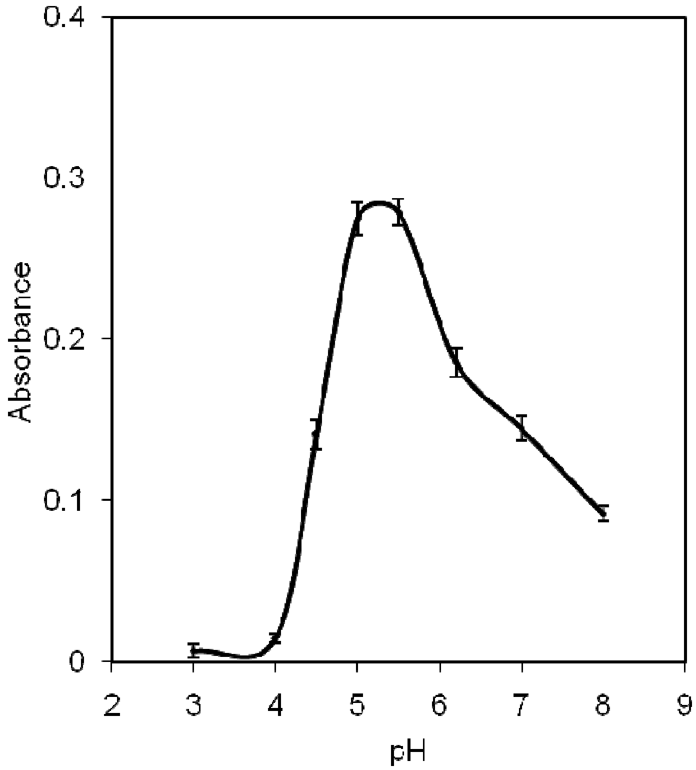


Figure 6

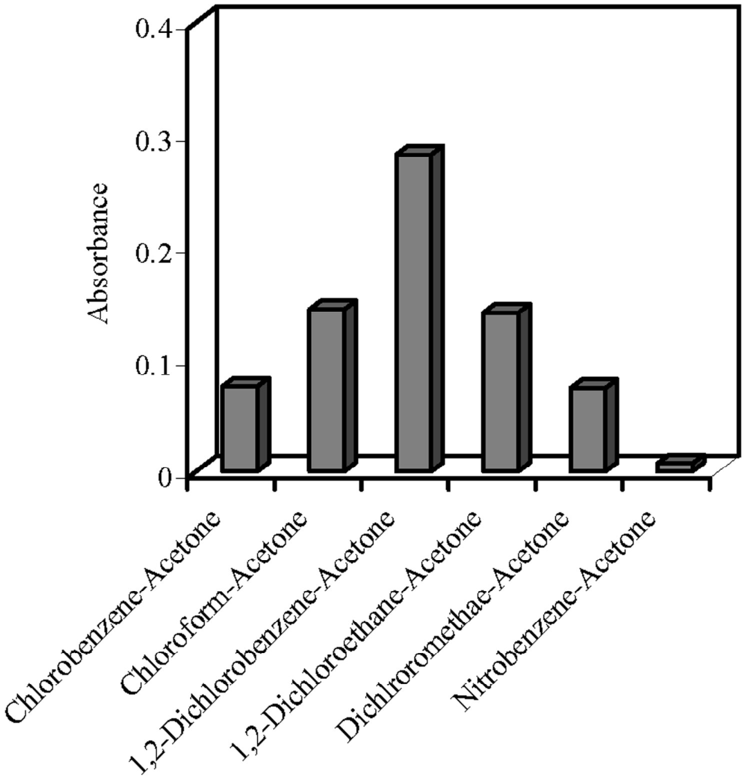

0.4
0.3
0.2
0.1
0
Absorbance
Chlorobenzene-Acetone
Chloroform-Acetone
1,2-Dichlorobenzene-Acetone
1,2-Dichloroethane-Acetone
Dichlroromethae-Acetone
Nitrobenzene-Acetone

Figure 7