



Anal. Bioanal. Chem. Res., Vol. 6, No. 2, 319-328, December 2019.

Trace Analysis of Phosphate Ion by Dispersive Liquid-liquid Microextraction Based on the Ion-pair Formation with Methyltrioctylammonium Chloride

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(Received 25 August 2018 Accepted 14 February 2019)

A simple and efficient dispersive liquid-liquid microextraction (DLLME) method coupled with microvolume UV-Vis spectrophotometry was developed for the determination of trace amounts of phosphate. This method is based on the formation of phosphomolybdate due to the reaction between molybdate and phosphate followed by its reduction with stannous chloride in aqueous sulfuric acid medium. The blue product was converted into its ion-pair with methyltrioctylammonium chloride (Aliquat-336) and then extracted into an organic solvent (carbon tetrachloride) dispersed in aqueous solution. The factors affecting on the process, such as concentration of sulfuric acid, ammonium molybdate and Tin(II) chloride in the sample solution, amount of methyltrioctylammonium chloride, volume of extraction solvent and reaction time were investigated and optimized. Under optimum conditions, the linear range was found to be 50-6500 ng ml⁻¹ for phosphate, and the limit of detection was 12 ng ml⁻¹. The proposed method was applied for the determination of trace amounts of phosphate in different water and wastewater samples and satisfactory results were obtained.

Keywords: Phosphate, Dispersive liquid-liquid microextraction, Aliquat-336, Methyltrioctylammonium chloride, Phosphomolybdate

INTRODUCTION

Phosphorus is an essential element for plants and animals since it plays a key role in their growth, metabolism, and reproduction. Phosphorus in natural water can be found in three extensive classes including orthophosphates, condensed phosphates (pyro-, meta-, and poly-), and organic phosphorus. However, dissolved phosphorus mainly consists of orthophosphates [1,2]. Phosphorus is delivered to the aquatic environment mainly from industrial and municipal sources and over-fertilization. Increased concern has arisen due to the eutrophication impacts produced when phosphorus is excessively discharged into the environment [3]. So, due to human activities, inadvertent addition of phosphates to watersheds has caused eutrophication, which is commonly manifested by algal bloom formation. As a result of its impact over

aquatic systems, the monitoring of phosphorus levels in these environments is therefore important for the identification and control of anthropogenic phosphorus input and the understanding of aquatic phosphorus cycling behavior [4,5].

Several alternative methods have also been developed for the determination of phosphate concentration in a matrix, including chromatographic [6,7], electrochemical [8,9], fluorescence [10,11], enzymatic reaction [12] and spectrophotometric methods [13-15]. Nevertheless, the current standard method recommended by the Environmental Protection Agency (EPA) is colorimetric. This protocol involves the reduction of the phosphomolybdate complex (formed from reaction between reactive phosphate and acidic molybdate) to form the intensely colored "molybdenum blue" product, where ascorbic acid and potassium antimonyl tartrate are used as reducing agents. Therefore, phosphate can be determined spectrophotometrically and its concentration is proportional

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to the blue color of the sample [16,17].

However, to increase the detection sensitivity, a sample preparation step is commonly required. Thus, a preconcentration step before the detection of blue product by UV-Vis spectrophotometry is often required. Among the pretreatment methods, liquid-liquid extraction (LLE) and solid phase extraction (SPE) are often used. Although these methods show good extraction and preconcentration efficiency, SPE is time-consuming and labor intensive, and LLE requires large volumes of poisonous organic solvent. Thus, miniaturized, solvent-free, rapid and environmental friendly sample preparation techniques are needed to overcome these limitations. Recent studies oriented towards the miniaturized LLE method have led to the developed dispersive liquid-liquid microextraction (DLLME). This method developed in 2006 by Rezaee *et al.* is the miniaturized format of liquid-liquid extraction in which acceptor-to-donor phase ratio is greatly reduced compared with other methods [18,19]. In this method, after injection of an appropriate mixture of extraction and disperser solvents into the aqueous sample a cloudy solution is formed. Due to the large surface area of the interface between the two phases, the equilibrium state is achieved quickly, and therefore, the extraction time is very short. Some of the remarkable advantages of this technique are simplicity of operation, rapidity, low consumption of solvents and sample, high recovery and high enrichment factor [20].

In this paper, the miniaturization of the spectrophotometric method for phosphate determination in water samples is proposed by combination of DLLME and microvolume UV-Vis spectrophotometry. The proposed methodology is based on the ion pair formation between molybdophosphate and cationic counter ion provided by methyltriethylammonium chloride, which also acts as a dispersive agent, and its subsequent extraction onto a microdrop of high-density organic solvent. The parameters affecting on the extraction efficiency are studied and optimized.

EXPERIMENTAL

Apparatus

Recording the spectra and the absorbance measurements

were made by a model Lambda-35 spectrophotometer (Perkin Elmer) using quartz microcells with capacity of 350 μ l. A Metrohm 632 (Switzerland) pH-meter was used to measure pH with a combined glass electrode. To separate the phases, an ELE centrifuge (Ieccentra-CLD) was used.

Reagents

All chemicals were of analytical grade, and double distilled water was used throughout. A stock solution of 1000 mg l⁻¹ of phosphate was prepared by dissolving 0.72 g of potassium dihydrogen phosphate (Merck, Germany) in water and diluting to 500 ml in a volumetric flask. Working standard solutions were prepared daily by successive dilutions of this stock solution. A 2.5% (w/v) solution of ammonium molybdate was prepared by dissolving 2.50 g of ammonium heptamolybdate tetrahydrate (Merck) in warm water and diluting to 100 ml. A solution of 1.0% (w/v) of stannous chloride was prepared by dissolving 1.0 g of SnCl₂ (Merck, Germany) in 2 ml of concentrated hydrochloric acid and diluting to 100 ml with distilled water. The Sulfuric acid solution was prepared by appropriate dilution of concentrated sulfuric acid (Fluka, 98%, d = 1.84 g ml⁻¹). 2.0% (w/v) solution of methyltriethylammonium chloride (Merck) was prepared by dissolving 0.2 g of methyltriethylammonium chloride (Aliquat 336s) in carbon tetrachloride and diluted to 10 ml.

Dispersive Liquid-liquid Microextraction Procedure

Under the optimized conditions, an aliquot of the solution containing of phosphate, 1.0 ml of 2.5% (w/v) of ammonium molybdate and 0.5 ml of 1.0% (w/v) of stannous chloride were added to a 10 ml volumetric flask and diluted to the mark with water and mixed thoroughly. After 20 min the resulting solution was transferred to a glass test tube with a conical bottom, and subsequently 300 μ l of carbon tetrachloride containing methyltriethyl ammonium (2.0% (w/v)) was rapidly injected into the produced solution by a microsyringe to induce the formation of cloudy solution and the mixture was shaken for several seconds manually. At this step, ion pair of molybdophosphate-methyltriethyl ammonium was extracted into the fine droplets of carbon tetrachloride. The mixture was then centrifuged at 6000 rpm for 5 min. Accordingly, the dispersed fine droplets of the

extraction phase were settled to the bottom of the conical test tube as a blue phase. After elimination of the aqueous phase, the settled phase was removed using a microsyringe and placed into the quartz microcell and its absorbance was measured at 740 nm against the blank. A blank solution was also run under the same procedure without adding any phosphate.

Collection and Preparation of Samples

The determination of phosphate in three water samples including Karoon River, tap water (Ahvaz, Iran) and Persian Gulf water (Khuzestan Province, Iran) was performed. The water samples were collected and filtered using a filter paper (Whatman, No. 1 Quantitative Filter Papers, 110 mm) and stored in the dark at 4 °C. Aliquot of water samples were subjected to the proposed DLLME procedure.

RESULTS AND DISCUSSION

The developed DLLME technique is based on the formation of molybdophosphate-methyltrioctylammonium ion pair and its extraction onto a microvolume of organic solvent followed by the spectrophotometric detection. Unlike the conventional DLLME, there is no need to use a dispersive solvent because methyltrioctylammonium chloride also plays the role of a disperser agent which is injected along with extraction solvent into the aqueous solution. In order to find the appropriate conditions for DLLME, different experimental parameters were studied and optimized.

Wavelength Selection

In order to perform the quantification analysis by UV-Vis spectrophotometry, the wavelength of maximum absorbance was established. In the preliminary experiment, the sample solution containing different concentrations of phosphate was examined according to the recommended procedure of DLLME and corresponding spectra of sedimented phase were recorded in the range of 500-900 nm. The absorption spectra are shown in Fig. 1. The results showed that maximum absorption wavelength is at 740 nm for sedimented phase, and upon increasing of phosphate concentration the absorbance at this wavelength is increased. Therefore, 740 nm was selected for measuring

the absorbance of the extracted phase throughout this study.

Effect of Ammonium Molybdate Concentration

The effect of ammonium molybdate concentration on the resultant blue product and therefore, absorbance of sedimented phase after DLLME procedure was studied. To this end, the influence of ammonium molybdate concentration in the range of 0.025-0.375% on the DLLME of phosphate was investigated. As can be seen in Fig. 2, the absorbance of sedimented phase increased by increasing the ammonium molybdate concentration up to 0.25% (w/v) and it was almost constant at values upper than 0.25%. Therefore, a concentration of 0.25% of ammonium molybdate in the final solution was selected as an optimum value for further experiments.

Effect of Sulfuric Acid Concentration

The preliminary experiments showed that the highest absorbance of sedimented phase was achieved in acidic medium. It is well known that formation of phosphomolybdenum blue in the presence of reductant agent can only occur in acidic media [4]. To achieve suitable acidic media, the effect of different concentrations of sulfuric acid was investigated. On the basis of the results, the maximum absorbance was in the range of 0.15-0.35 M of sulfuric acid. Thus, a concentration of 0.25 M of sulfuric acid in the final solution was used as the optimum for further experiments.

Effect of stannous chloride concentration

Reduction of phosphomolybdic acid to phosphomolybdenum blue requires a reducing agent which performs by SnCl_2 . Therefore the effect of SnCl_2 concentration on the blue product was investigated. As can be seen in Fig. 3, maximum absorbance of sedimented phase was obtained when the SnCl_2 concentration was 0.05% (w/v) in final solution. It is worth mentioning that by addition of stannous chloride concentration, even in the absence of phosphate in solution, self-reduction of molybdate may occur. So, by using the SnCl_2 concentration higher than 0.05% (w/v), the signal of blank was increased and finally the absorbance difference between sample and blank was decreased. Therefore a concentration of 0.05% (w/v) was chosen as the optimum value for further

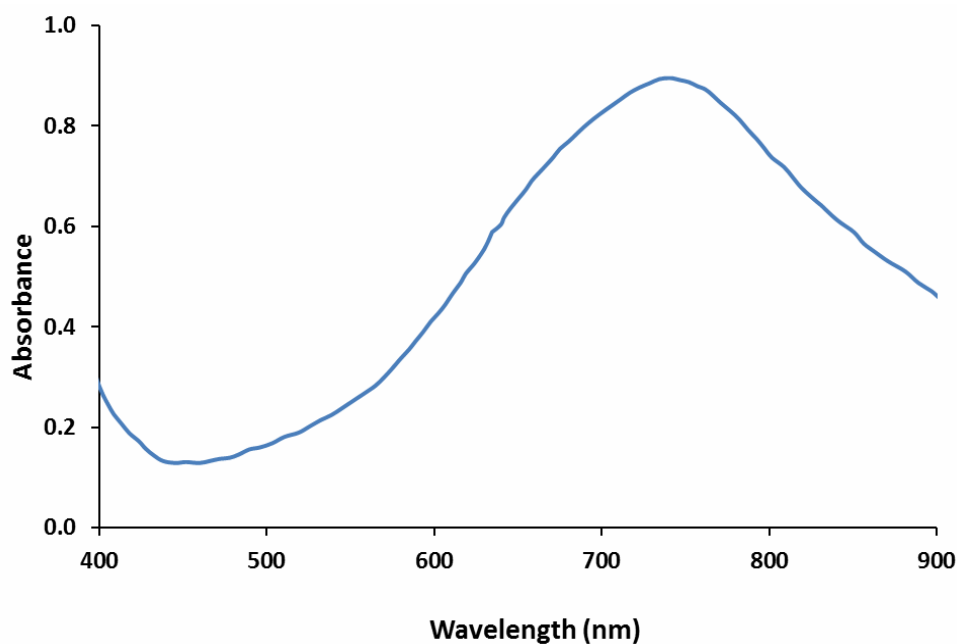


Fig. 1. UV-Visible absorbance spectra of molybdophosphate after DLLME procedure.

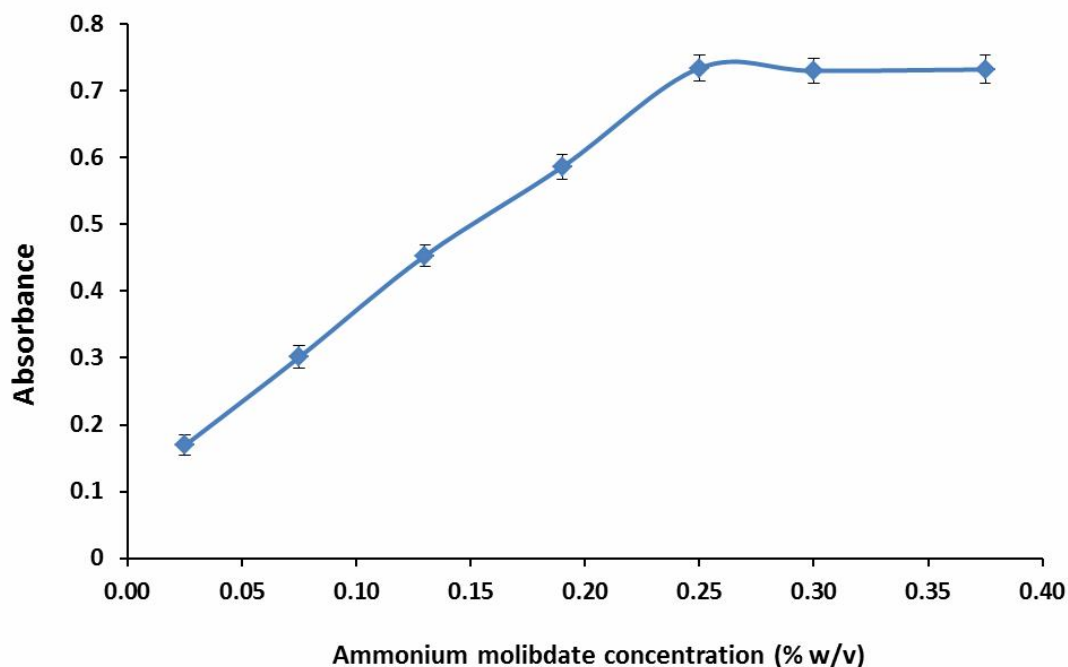


Fig. 2. Influence of ammonium molybdate concentration on the determination of phosphate by using DLLME procedure. Extraction conditions: aqueous sample volume, 10 ml; phosphate concentration, 500 ng ml⁻¹; H₂SO₄ concentration, 0.25 M; SnCl₂ concentration, 0.05% (w/v); reaction time, 20 min; volume of CCl₄ containing 2.0 % (w/v) methyltrioctylammonium chloride, 300 µl; centrifugation time, 5 min.

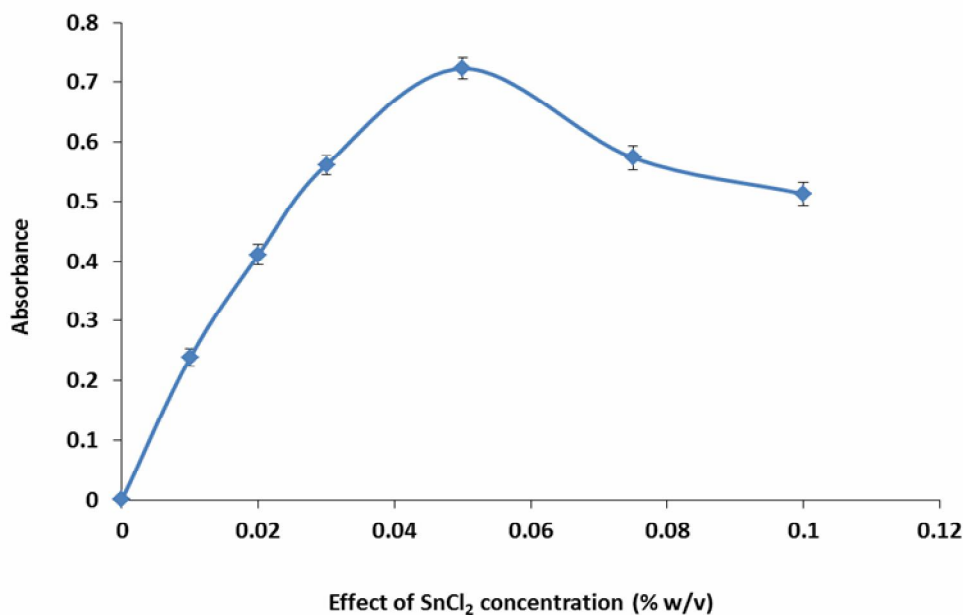


Fig. 3. The Effect of SnCl₂ concentration on the determination of phosphate by using DLLME procedure. Extraction conditions: aqueous sample volume, 10 ml; phosphate concentration, 500 ng ml⁻¹; ammonium molybdate concentration, 0.25 (w/v); H₂SO₄ concentration, 0.25 M; reaction time, 20 min; volume of CCl₄ containing 2.0% (w/v) methyltrioctylammonium chloride, 300 μl; centrifugation time, 5 min.

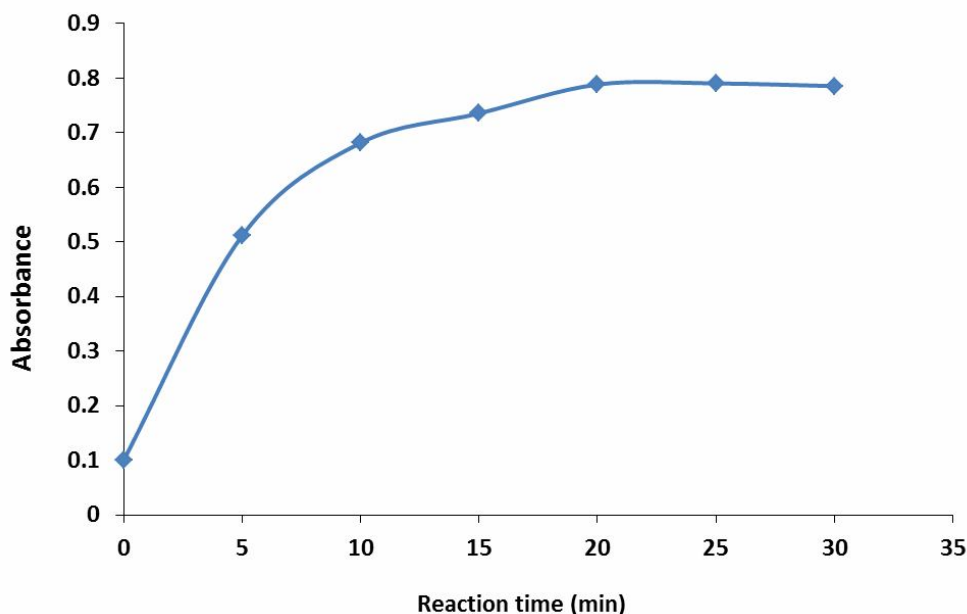


Fig. 4. Effect of reaction time on the determination of phosphate by using DLLME procedure. Extraction conditions: aqueous sample volume, 10 ml; phosphate concentration, 500 ng ml⁻¹; ammonium molybdate concentration, 0.25% (w/v); H₂SO₄ concentration, 0.25 M; SnCl₂ concentration, 0.05% (w/v); volume of CCl₄ containing 2.0% (w/v) methyltrioctylammonium chloride, 300 μl; centrifugation time, 5 min.

experiments.

Effects of Reaction Time

Optimization of incubation time of the aqueous solution before injection step is necessary for the complete reaction of phosphate in aqueous medium. Hence, the effect of reaction time on DLLME of phosphate was investigated in the range of 0-30 min Fig. 4 shows that the absorbance value increased rapidly with the increase in time up to 20 min and remained nearly constant after that. Accordingly, a reaction time of 20 min was chosen for use in the next experiments.

Nature of the Extraction Solvent and Disperser Agent

The selection of a suitable extracting solvent, as an essential parameter in the DLLME techniques, is of primary importance. The extraction solvent should be denser than water and, in addition, it should have low volatility, low water solubility and high capability for extracting interested compound. Thus, carbon tetrachloride, trichloromethane and dichloromethane were chosen as the extraction solvents. Hence, the study was carried out utilizing different volumes of these extraction solvents containing the constant amount of methyltrioctylammonium chloride [2.0% (w/v)] to achieve 0.3 ml of sedimented phase. We have previously reported that the cloudy solution and two-phase system appeared by applying carbon tetrachloride or trichloromethane but did not appear by using dichloromethane. However, carbon tetrachloride was chosen, because in this case the highest and reproducible signal was observed, in addition, the smaller volume of extraction solvent was needed in the injection step [20].

Although in this DLLME method, methyltrioctylammonium chloride dispersed CCl_4 in the aqueous sample, similar to our previous research [21,22], the influence of several conventional disperser solvents such as methanol, ethanol, acetonitrile and acetone was investigated. Accordingly, extensive studies were performed via injection of a mixture containing an appropriate amount of CCl_4 , 2.0% (w/v) of methyltrioctylammonium chloride, and mentioned previously disperser solvents to achieve 0.3 ml of sedimented phase. The results indicated that carbon tetrachloride is partially dissolved in disperser solvent and

migrates into aqueous phase, and the dissolved carbon tetrachloride is not sedimented down well and consequently the extraction efficiency is decreased. Finally, the suggested method was carried out only by injecting the extraction solvent (without disperser solvent) containing appropriate amount of methyltrioctylammonium chloride to the sample solution. Moreover, unlike the routine disperser solvents, methyltrioctylammonium chloride could dissolve the smallest volume of carbon tetrachloride in aqueous solution, therefore, volume of sedimented phase (0.3 ml) is almost equal to the volume of injected carbon tetrachloride, which is an advantage for the proposed method.

Effect of Methyltrioctylammonium Chloride Amount

The investigation of methyltrioctylammonium chloride amount has an important role in the proposed method because it acts as a counter ion for phosphomolybdenum blue anion and disperser agent too. Therefore, the study of the amount of methyltrioctylammonium chloride on the extraction of phosphate is very important. For this purpose, studies were performed using solutions of carbon tetrachloride containing different amounts of methyltrioctylammonium chloride (in the range of 0.5-3.5% (w/v)). 300 μl of these solutions were injected into the sample solutions. The obtained results revealed that the dispersion of CCl_4 in aqueous phase is increased by increasing the methyltrioctylammonium chloride concentration. The experimental results (Fig. 5) showed that the maximum absorbance of sedimented phase is achieved in 2.0% (w/v) of methyltrioctylammonium chloride solution. Thus, 2.0% (w/v) of methyltrioctylammonium chloride solution was chosen for the purpose of this study.

Effect of Injection Solution Volume

The volume of extraction solvent was another crucial parameter affecting the extraction efficiency, enrichment factor, and sensitivity, due to its effect on volume of the sedimented phase. To examine the effect of this parameter, different volumes of CCl_4 containing methyltrioctylammonium chloride (2.0% w/v) were subjected to the same DLLME procedure (in the range of 100-400 μl). Using injection volumes less than 150 μl of the mixture, low volume of organic phase was obtained, so that the

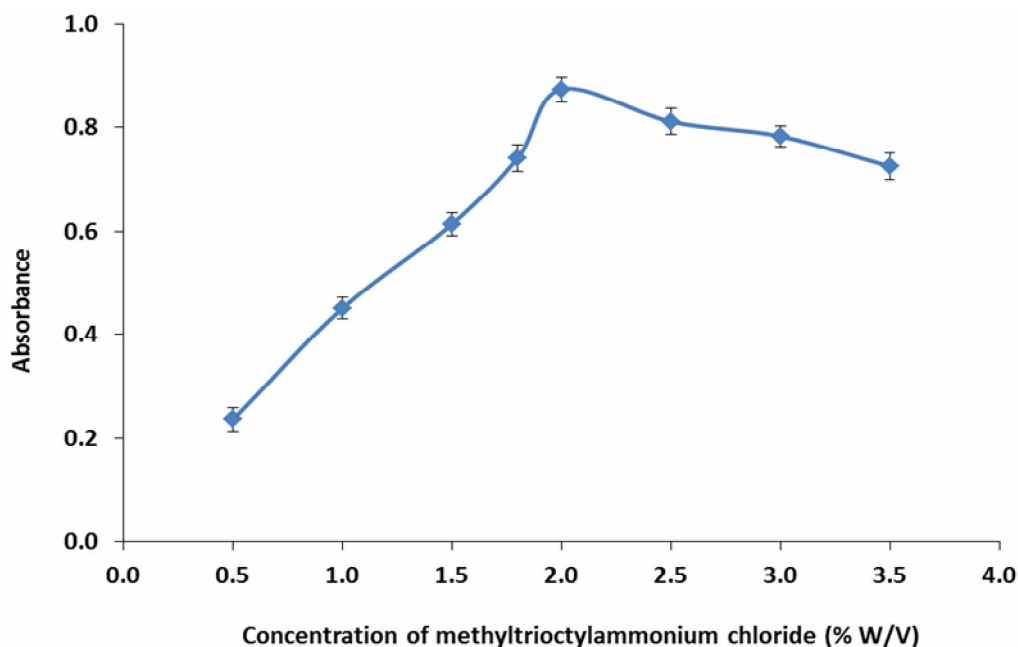


Fig. 5. Effect of methyltrioctylammonium chloride concentration on the determination of phosphate by using DLLME procedure. Extraction conditions: aqueous sample volume, 10 ml; phosphate concentration, 500 ng ml⁻¹; ammonium molybdate concentration, 0.25% (w/v); H₂SO₄ concentration, 0.25 M; SnCl₂ concentration, 0.05% (w/v); volume of CCl₄ containing different amounts of methyltrioctylammonium chloride, 300 μl; centrifugation time, 5 min.

Table 1. The Influence of Interfering Ions on the Determination of 500 ng ml⁻¹ Phosphate Using DLLME Method

Ions	Tolerance ratio
	(w/w)
Na ⁺ , K ⁺ , Mg ²⁺ , Zn ²⁺ , Al ³⁺ , NO ₃ ⁻ , Acetate	1000
NH ₄ ⁺ , Ag ⁺ , Cd ²⁺ , Ca ²⁺ , Pb ²⁺ , Mn ²⁺ , CO ₃ ²⁻	800
Au ³⁺ , Cr ³⁺ , SO ₄ ²⁻ , HCO ₃ ⁻	500
Hg ²⁺ , Cu ²⁺ , Cl ⁻ , NO ₂ ⁻ , F ⁻ , Br ⁻	100
Cu ²⁺ , Fe ³⁺	10

absorption signal could not be measured by spectrophotometer fitted with microcells. The obtained results showed that the absorbance of organic phase was

decreased by increasing the carbon tetrachloride volume. This could be due to the dilution effect which decreases the concentration of the extracted species in sedimented phase.

Thus, in order to achieve high enrichment factor and low detection limit, 300 μl of carbon tetrachloride containing 2.0% (w/v) methyltrioctylammonium chloride was selected as the optimum volume of extraction solvent.

Effect of Extraction and Centrifugation Time

In DLLME methods, the extraction time is usually a key parameter defined as the interval time between the injection of extraction solution and beginning of centrifugation [18]. Therefore, this effect was examined in the range of 1-5 min and the obtained results indicated that the extraction time does not have a significant influence on the extraction efficiency of blue product. This reveals that the extraction process is time-independent and very fast, due to the large surface of contact between the extraction solvent and the aqueous phase. This is the most important advantage of the DLLME technique.

The influence of centrifugation time was also studied. The results indicated that for a complete separation of organic and aqueous phase the mixture should be centrifuged for 5 min at 6000 rpm.

Study of Interferences

The potential interferences in the present method were investigated. The effect of some cations and anions co-exist in natural samples was studied. In these experiments, different amounts of other ions were added to a solution containing 500 ng ml^{-1} of phosphate and the recommended procedure was applied. An error of $\pm 5\%$ in the absorbance reading was considered tolerable. The results, summarized in Table 1, show suitable selectivity of the procedure.

Analytical Feature of the Method

Under the aforementioned optimum conditions, the analytical characteristics of developed method for determination of phosphate (*i.e.*, linear range, repeatability, limit of detection and maximum preconcentration factor) were investigated. The calibration graph was linear in the range of 50-6500 ng ml^{-1} of phosphate with a correlation coefficient (R^2) of 0.9963. The limit of detection (LOD) calculated based on $3S_b/m$ was 12 ng ml^{-1} . The maximum preconcentration factor, defined as the volume ratio of the aqueous sample solution (10 ml) and sedimented phase (0.3 ml), was 33.3. The precision of the method determined by

analyzing samples at two concentration levels (100 and 3000 ng ml^{-1}) were 4.3% and 2.1%, respectively. A comparison between the present DLLME-UV-Vis spectrophotometry method and other sample preparation techniques for determination of phosphate from the viewpoint of linearity, LOD, %RSD, and EF (Enrichment factor) are shown in Table 2. As seen in this table, LOD, enrichment factor, and precision of suggested method are better or comparable to many of the reported techniques.

Application to Real Samples

The analytical usefulness of the proposed method was demonstrated by its application in determination of phosphate in different water samples. The accuracy of the method was performed by the analysis of the samples spiked with known amounts of the phosphate ions. The analytical results, along with the recovery for the spiked samples of this investigation are given in Table 3. As shown, recoveries for analyte ranged from 94.5% and 104.6%. Therefore, the matrix effect from river water, tap water, and seawater on the DLLME for determination of phosphate was not significant.

CONCLUSIONS

The aim of this work was to establish an efficient, simple and cheap DLLME method combined with UV-Vis spectrophotometry for the preconcentration and determination of trace amount of phosphate in environmentally water samples. Miniaturization of toxic organic solvent using dispersive liquid-liquid microextraction combined with microvolume UV-Vis spectrophotometry allows the development of a green method which is environment-friendly. In addition, simplicity of operation, rapidity, low sample volume, low cost, and high preconcentration factor are some advantages of the suggested method. This DLLME procedure is based on the formation of an ion pair and its further extraction into a microvolume of carbon tetrachloride. In this method, there is no need for additional dispersive solvent because methyltrioctylammonium chloride used for providing ion pair with phosphomolybdenum blue anion acts as a disperser too. The proposed method is practical and suitable for phosphate determination in different water samples.

Table 2. Comparison of the Proposed Method with some of the Previously Reported Methods for the Preconcentration and Determination of Phosphate

Enrichment procedure	Analytical technique	Enrichment factor	Linear range (ng ml ⁻¹)	LOD (ng ml ⁻¹)	RSD (%)	Ref.
EISE ^a	UV-Vis	14	5-200	5.0	< 8.1	[1]
PIM ^b	UV-Vis	674	0.5-1000	5.0	3.2	[4]
DSDME ^c	UV-Vis	325	5-140	0.6	2.7	[5]
IEC ^d	UV-Vis	NG ^e	50-2000	1.5	1.05	[13]
μHSI ^f	LED/LDR colorimetry	NG	100-6000	100	1.4	[14]
FIA ^g	UV-Vis	NG	200-2000	40	2.6	[15]
FI	Amperometry	NG	50-1000	3.4	1.3	[16]
DLLME	UV-Vis	28.5	50-6500	12	2.1	This work

^aElectrostatically induced stoichiometric extraction. ^bPolymer inclusion membrane. ^cDirectly suspended droplet microextraction. ^dIon-exclusion chromatography. ^eNot given. ^fMicrofluidic hydrodynamic sequential injection. ^gFlow injection analysis.

Table 3. Determination of Phosphate in Water Samples by the Proposed Method

Sample	Added (mg l ⁻¹)	Found (mg l ⁻¹) ^a	Recovery (%)
Tap water	0	0.13 ± 0.01	-
	2.0	2.04 ± 0.04	95.5
	3.0	3.27 ± 0.07	104.6
River wate	0	0.50 ± 0.01	-
	2.0	2.39 ± 0.05	94.5
	3.0	3.42 ± 0.07	97.3
Seawater	0	1.31 ± 0.03	-
	2.0	3.23 ± 0.07	96.0
	3.0	4.41 ± 0.09	103.3

^aMean ± standard deviation (n = 3).

ACKNOWLEDGMENTS

The authors are grateful to Khorramshahr University of Marine Science and Technology (KMSU) Research Council for financial support of this work (Grant1397).

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