Determination of Phosphate in Water Samples by Ion-Pair Extraction Combined with Molybdenum Blue Spectrophotometry

การวิเคราะห์ฟอสเฟตในตัวอย่างน้ำด้วยการสกัดแบบไอออนแพร์ร่วมกับโมลิบดินัมบลูสเปกโทรโฟโตเมทรี

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ABSTRACT

A simple ion-pair extraction procedure for preconcentration of phosphate in natural waters has been developed prior to spectrophotometric detection. The extraction of 12-molybdophosphate with ion-pair reagent, hexadecyltrimethylammonium bromide (CTAB), into dichloromethane was investigated. The organic phase was measured at 725 nm using spectrophotometry. The effect of various experimental parameters such as a choice of surfactant, the concentration and volume of surfactant, extraction solvent and its volume, sample volume and extraction time, were studied. The calibration graphs were linear in the range of 0.001–0.300 and 0.300–5.000 mg L^{-1} phosphate with the correlation coefficients of greater than 0.999. The limit of detection and limit of quantification were 7 and 25 µg L^{-1}, respectively. The recoveries for the determination of phosphate in real samples were obtained in the range of 84–102%.

บทคัดย่อ

การพัฒนาวิธีสกัดแบบไอออนแพร์เพื่อเพิ่มความเข้มข้นของฟอสเฟตในตัวอย่างน้ำธรรมชาติก่อนการตรวจวัดด้วยวิธีสเปกโทรโฟโตเมทรี โดยการสกัดโมลิบโดฟอสเฟตด้วยไอออนแพร์รีเอเจนต์ CTAB และไดคลอโรมีเทนและตรวจวัดที่ความยาวคลื่น 725 นาโนเมตร ได้ศึกษาตัวแปรที่มีผลต่อการวิเคราะห์ ได้แก่ ชนิดของสารลดแรงตึงผิว ความเข้มข้นและปริมาณของสารลดแรงตึงผิว ตัวทำละลายสกัดและปริมาตรที่ใช้ ปริมาตรตัวอย่างและระยะเวลาที่ใช้ในการสกัด กราฟมาตรฐานของการวิเคราะห์ฟอสเฟตมีความเป็นเส้นตรงในช่วงความเข้มข้น 0.001–0.300 และ 0.300–5.000 มิลลิกรัมต่อลิตร ค่าสัมประสิทธิ์สหสัมพันธ์สูงกว่า 0.999 ค่าขีดจำกัดต่ำสุดของการตรวจวัดและขีดจำกัดต่ำสุดของการหาปริมาณฟอสเฟต คือ 7 และ 25 ไมโครกรัมต่อลิตร ตามลำดับ ร้อยละการกลับคืนอยู่ในช่วง 84–102 เปอร์เซ็นต์

Key Words: Phosphate, Spectrophotometry, Ion-pair extraction

คำสำคัญ: ฟอสเฟต สเปกโทรโฟโตเมทรี การสกัดแบบไอออนแพร์

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Introduction

Phosphorus is an important element for life on earth, growth of plants and animal. It was found in soils, sediments, foods, waters, plants and animals. Dissolved species of phosphorus include orthophosphate, organic condensed phosphate and inorganic condensed phosphate (Yaqoop *et al.,* 2004). This study focuses on phosphate (PO_4^{3-}) as inorganic ion in water samples. It is used in fertilizers, detergents and industry. The classical methods for determination of phosphate in real samples e.g. water, sugarcane juice, bone, soil, detergent and food samples have been described. The malachite green molybdophosphate species were investigated at 625 nm by spectrophotometric method (Linge *et al.,* 2001) and the mixed reagent containing anionic surfactant, molybdate and malachite green (Surf-Mo-MG) was used for determination of trace phosphate at low acidicity medium, $pH_T = 1.0$ (Huang & Zhang, 2006). In addition, the molybdenum blue method based on the reaction between molybdate and phosphate was proposed. Thiourea and sodium sulfide in aqueous sulfuric acid were used as reducing reagent (Mahadevaiah *et al.,* 2007; Shyla *et al.,* 2001). However, the low concentration of phosphate in the complex environmental matrices makes it difficult to direct determine by spectrophotometry.

The ion pair extraction is a technique for partitioning of ionic compound with the aid of counter ion of opposite charge, a process in which three compartments are involved; the aqueous layer, the organic layer and the interphase. One of techniques is ion-pair liquid-liquid extraction, the method to separate compounds based on the solubility in two different phases and use for preconcentraton of the analytes. The analytes form ion-pairs with the counter ions of opposite charge (Xu *et al.,* 2010). The sensitivity of the method for determination of target analytes has been investigated in the two ways, the analytes as cationic complex species and anionic complex species. The method is simple, efficient and rapid (Farajzadeh *et al.,* 2009). The ion-pair reagent containing bulky cations are usually used for the extraction of anionic complex species such as the extraction of perchlorate from water samples (Magnuson *et al.,* 2000; Pourreza and Mousavi, 2005). The separation of palladium from water samples based on the ion-pair formation of PdI^-_4 with TTAB (Yamini *et al.,* 2005) and the determination of gold complex anion $[Au(CN)_2]$ by Astra Phloxine (Kocurova *et al.,* 2010). The extraction of cationic complex species by anionic ion-pair reagent was also presented such as the determination of metal ions. Metal complexes reacted with ion-pair reagents and extracted using liquid-liquid extraction (Oshima *et al,* 2001), (Nakai *et al,* 2005). In addition, ion-pair liquid-liquid extraction can be used for extraction and determination of the analytes such as gold (I, II) from wastewater and silver (El-Shahawi *et al.,* 2007), rosuvastatin in human plasma (Lan *et al.,* 2007) and alkyltrimethylammonium surfactants in hair conditioners and fabric softeners (Tsai *et al.,* 2004).

Objective of the study

The purpose of the present work was to develop the ion-pair liquid phase extraction for preconcentration of phosphate in water samples prior to determine by spectrophotometry. The method is based on the ion pair formation of 12-molybdophosphate with cationic surfactant, before extracting with organic solvent. Optimization of the experimental parameters influencing the extraction efficiency of the method was carried out.

Application of phosphate determination in natural water samples was also studied.

Methodology

Chemicals and reagents

All chemicals and reagents were of analytical reagent grade. Phosphate standard stock solution was prepared by dissolving disodium hydrogen phosphate (Fisher Chemical, UK) with deionized water. Molybdic acid and tin (II) chloride (Ajax Finechem, Australia) were dissolved in sulfuric acid and hydrochloric acid, respectively. Surfactants including dodecyltrimethyl- ammonium bromide (DTAB) (Sigma-Aldrich, USA), hexadecyltrimethyl ammonium bromide (CTAB) (Merck, Germany), sodium dodecyl sulfate (SDS) (BDH, England), trimethyl-tetradecylammonium bromide (TTAB) (Fluka Analytical, Denmark), Triton X-114 (Aldrich, USA), Triton X-100 (Fluka Chemika, Switzerland), tetrabutylammonium bromide (TBABr) (QReC, Newzealand) and Brij 35 (Acros Organics, USA) solutions were prepared by dissolving these reagents in water. Dichloromethane (QReC, Newzealand), chloroform and chlorobenzene (Carlo Erba, Italy) were used as extraction solvents. The solutions were prepared in deionized water from RiOs^{TM} Type I Simplicity 185 (Millipore, USA) with the resistivity of 18.2 MΩ.cm.

Instrumentation

The absorbance measurement was performed using HP 8453 UV-Vis spectrophotometer with micro cell of 1.0 cm path length. Absorbance measurement was carried out at 725 nm.

Molybdenum blue method

For the formation of phosphomolybdenum blue complex, the standard solution of 5.00 mL was mixed with 0.03 mol L^{-1} molybdic acid solution and 0.01 mol L^{-1} tin (II) chloride solution in a 25 mL volumetric flask. The volume was adjusted with water. The mixture solution was shaken and determined by UV-Vis spectrophotometer at 656 nm.

Extraction procedure

A 5.00 mL of the sample/standard solution containing various concentrations (0.001–5.000 mg L^{-1}) of phosphate, 0.03 mol L^{-1} of molybdic acid solution and 0.01 mol L^{-1} of tin (II) chloride solution were transferred into a test tube. A 0.25 mL of 5×10^{-3} mol L^{-1} of CTAB, as ion-pair reagent, was added and then the solution was shaken to form the ion-pair. A 3.00 mL of dichloromethane, as extraction solvent, was added into the mixture solution and the tube was shaken for 10 min. After separation of the layers, the lower organic phase was collected and the absorbance was measured at 725 nm against a reagent blank.

Analysis of phosphate in water samples

Natural water samples from different places were filtered through a 0.45 µm membrane filter. To examine the recovery, to each of 5.00 mL sample solution, the concentrations of standard solution in the range of 0.005–2.000 mg L^{-1} were spiked. The ion-pair liquid phase extraction and spectrophotometric measurement were investigated as the same procedure as standard solution.

Results and discussion

Optimization of molybdenum blue method

Effect of the concentration of molybdic acid solution

The spectrophotometric method for the determination of phosphate is based on the reaction of phosphate with molybdate in acidic medium. Tin (II) chloride was used as reducing agent to form molybdenum blue. The effect of the concentration of molybdic acid solution on the formation of molybdenum blue in the range of 0.01–0.05 mol L^{-1} were studied with 0.01 mol L^{-1} of tin (II) chloride and various concentrations of 0.1–10.0 mg L^{-1} of phosphate. Absorbances of the solutions were measured. The maximum absorbance value was achieved with 0.03 mol L^{-1} of molybdic acid solution (see Fig. 1).

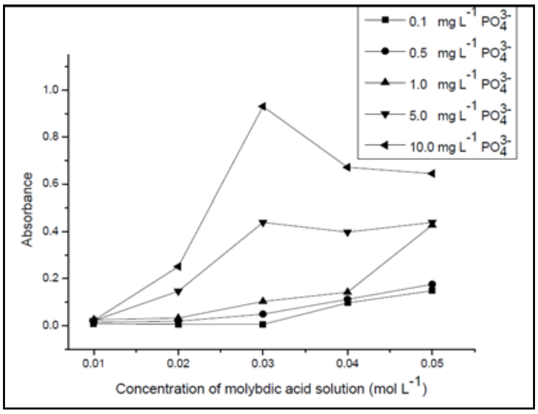

Fig. 1 The effect of molybdic acid concentration on molybdenum blue method

Effect of the concentration of tin (II) chloride solution

The effect of the concentration of reducing agent, tin (II) chloride, on the formation molybdenum blue complex was investigated. The solution containing various concentrations (0.1–5.0 mg L^{-1}) of phosphate, 0.03 mol L^{-1} of molybdic acid solution and the concentration of reducing agent in the range of 0.005–0.030 mol L^{-1} were studied. Absorbance values of the solutions were found to be increased with increasing concentration of reducing agent up to 0.010 mol L^{-1} (Fig. 2). Therefore, 0.010 mol L^{-1} of tin (II) chloride was used in the further experiments.

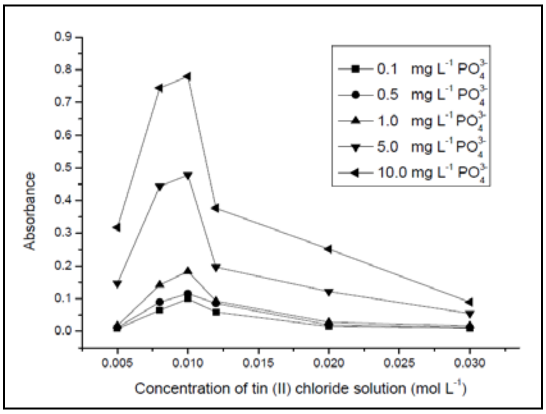

Fig.2 The effect of tin (II) chloride concentration on molybdenum blue method

Optimization of extraction condition

Effect of ion-pair reagent and its concentration

The effect of different ion pair reagents including cationic surfactant (CTAB), non ionic surfactant (Triton X-100, Triton X-114 and Brij 35) and anionic surfactant (SDS) were studied. In this work phosphomoybdate complex, as anionic species, can be formed ion-pair with cationic surfactants. Therefore, CTAB was selected. The effect of CTAB concentration on extraction efficiency was evaluated in the range of 0.05–5.00×10^{-3} mol L^{-1}. The solutions containing phosphate concentrations in the range of 0.01–5.00 mg L^{-1}, 0.03 mol L^{-1} of molybdic acid solution and 0.01 mol L^{-1} of tin (II) chloride solution were used in this study. The addition of CTAB gave rise to an increase in the absorbance up to 5×10^{-3} mol L^{-1} (Fig. 3). CTAB concentration of 5×10^{-3} mol L^{-1} was chosen. Various volumes (0.1-0.5 mL) of CTAB were studied. To achieve maximum ion-pair extraction, CTAB volume of 0.25 mL was selected (Fig. 4).

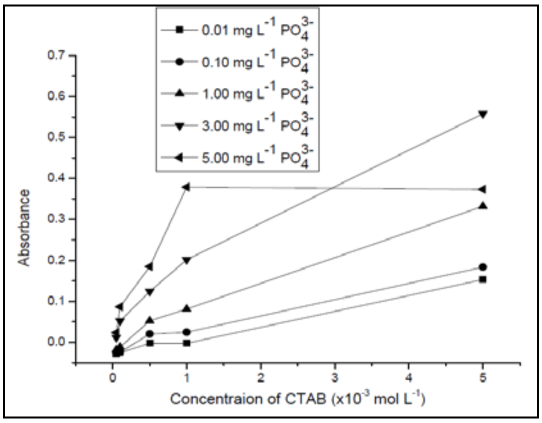

Fig. 3 The effect of CTAB concentration on the extraction of phosphate

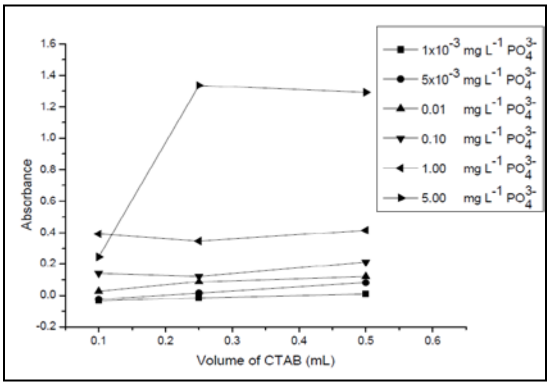

Fig. 4 The effect of CTAB volume on the extraction of phosphate

Effect of organic solvent

Comparative test of various organic solvents such as dichloromethane, chloroform and chlorobenzene were studied for extraction of ion-pair complex. Extraction were carried out from 8 mL solutions containing phosphate concentrations in the range of 0.001–5.000 mg L^{-1}, 0.03 mol L^{-1} of molybdic acid solution and 0.01 mol L^{-1} of tin (II) chloride solution. Dichloromethane could be used as a good extraction solvent due to it gave the highest absorbance for the extracted molybdenum blue complex. At room temperature, the extraction was completed by shaking for 10 min. The Effect of dichloromethane volume in the range of 1.00–3.00 mL was investigated, and 3.00 mL of dicholomethane was selected as the extraction solvent.

Method validation

Under the optimized experimental condition, two calibration graphs were established for phosphate concentration in the range of 0.001–0.300 and 0.300–5.000 mg L^{-1} with the correlation coefficients of greater than 0.999. The enrichment factor of 15 was achieved, leading to lower limit of detection and limit of quantification of 7 and 25 µg L^{-1}, respectively. The precision of the method, expressed by the relative standard deviation, is found to be less than 4.07% (n=3).

Analytical application

The performance of the proposed method was tested by applying it to the determination of phosphate in ten different natural water samples. The concentrations of phosphate in water samples were found in the range of 0.038–0.705 mg L^{-1}. The recovery of phosphate from natural water samples, spiked with standard solution of phosphate at five different concentrations (0.005, 0.010, 0.500, 1.000 and 2.000 mg L^{-1}) levels, was investigated. Recoveries for spiked phosphate standards were in range of 84–102% (Table 1). The results indicated that the proposed method was helpful for phosphate determination in water sample.

Conclusion

In this study, an analytical method employing ion-pair liquid phase extraction combined with molybdenum blue spectrophotometry for phosphate preconecentration was developed. Dichloromethane was used as extractant for extraction the complex between molybdophosphate with CTAB as ion-pair reagent. The reducing reagent, tin (II) chloride was employed to reduce phosphomolybdate to phosphmolybdenum blue. The spectrophotometric procedure is very simple, low cost and rapid for preconcentraton of phosphate in water samples. The method provided the low detection and quantification limits, and good recovery.

Table 1 Application of the proposed method for determination of phosphate in water sample

Sample	Found (mg L^{-1})	Spiked (mg L^{-1})	Recovery (%)	Sample	Found (mg L^{-1})	Spiked (mg L^{-1})	Recovery (%)
Water 1	0.041	0.005	102	Water 6	0.320	0.005	84
		0.010	97			0.010	95
		0.500	98			0.500	96
		1.000	94			1.000	96
		2.000	98			2.000	97
Water 2	0.268	0.005	98	Water 7	0.158	0.005	93
		0.010	99			0.010	97
		0.500	98			0.500	95
		1.000	96			1.000	95
		2.000	99			2.000	98
Water 3	0.135	0.005	101	Water 8	0.038	0.005	101
		0.010	99			0.010	97
		0.500	98			0.500	98
		1.000	97			1.000	97
		2.000	99			2.000	99
Water 4	0.041	0.005	84	Water 9	0.587	0.005	85
		0.010	95			0.010	95
		0.500	99			0.500	96
		1.000	97			1.000	98
		2.000	98			2.000	99
Water 5	0.101	0.005	95	Water 10	0.705	0.005	93
		0.010	97			0.010	95
		0.500	96			0.500	98
		1.000	98			1.000	96
		2.000	98			2.000	98

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