



Solid Phase Extraction–Spectrophotometric Determination of Phosphate Using Modified Magnetite Nanoparticles as Extractor

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ABSTRACT

In this work, a new, simple and fast method for the solid phase extraction-spectrophotometric determination of phosphate using cetyltrimethyl ammonium bromide immobilized on alumina-coated magnetite nanoparticles, (CTAB@ACMNPs) has been developed. The determination of phosphate is based on the molybdenum blue method which was monitored at λ_{\max} of 810 nm. MNPs and ACMNPs characterized by SEM, VSM, and XRD spectroscopy. This method avoided the time-consuming column-passing process of loading large volume samples in traditional SPE through the rapid isolation of CTAB@ACMNPs with an adsorptive magnet. Under the optimal experimental conditions, the preconcentration factor (PF), detection limit (DL), linear range (LR) and relative standard deviation (RSD) of phosphate were 80 (for 400 mL of sample solution), 0.038 $\mu\text{g mL}^{-1}$, 0.8-10.0 $\mu\text{g mL}^{-1}$ and 2.5 % (for 5.0 $\mu\text{g mL}^{-1}$, $n=7$), respectively. The proposed method was successfully applied to the separation/preconcentration and determination of phosphate in different water samples and suitable recoveries were obtained.

KEYWORDS: Solid phase extraction, Phosphate; Modified ACMNPs; Molybdenum blue method.

1. INTRODUCTION

It is well known that phosphorus is major component of various soils and natural minerals. Phosphate has been used in different industries for water softening, fertilizers and many other applications. Inorganic specie of phosphate is important environmental contaminant. An increase in concentration of this anion in natural water lowers the dissolved oxygen concentration and cause the death of aquatic animals such as fishes due to the suffocation [1]. Therefore, the removal of the excess ions is important to improve the water quality. There are a relatively limited number of studies dealing with the separation/preconcentration of phosphate ions in real samples such as water [2-6].

Among separation/preconcentration methods, solid phase extraction (SPE) due to faster operation, easier manipulation, and reduction of the use of organic solvents, less stringent requirements for separation, higher preconcentration factor and easier compatibility with analytical instruments have been widely studied. At present, nano-sized materials such as Al_2O_3 and carbon nanotubes are more important in SPE [7,8], but separation of these particles from aqueous medium is difficult. SPE by using Fe_3O_4 nanoparticles (MNPs) with a nonreactive shell made of alumina as sorbent

applied recently [9-14]. The main advantage of the preparation of alumina-coated magnetite nanoparticles (ACMNPs) compared only Fe_3O_4 NPs is higher stability in acidic and basic solutions. Recently, we also reported the methods for separation/preconcentration of Ag(I), Pb(II), Hg(II), Ni(II), arsenate/arsenite and speciation analysis of Cr(IV)/Cr(VI) using ACMNPs [11-16]. To our knowledge, this is the first report of using ACMNPs for the SPE and determination of species of phosphate from real samples. In this study, ACMNPs were successfully synthesized and modified by cationic surfactant of cetyltrimethylammonium bromide (CTAB). The CTAB@ACMNPs sorbent have proved to be suitable for the extraction of phosphate ions from different water samples prior to determination by spectrophotometric technique based on molybdenum blue method.

2. EXPERIMENTAL

2.1. Chemicals and solutions

All chemicals used were as least of the analytical reagent grade. Triply distilled water was used throughout. The stock standard solution (1000 $\mu\text{g mL}^{-1}$) of phosphate was obtained by dissolving appropriate amounts of NaH_2PO_4 (Merck) in triple distilled water.

Working standard solutions were obtained by appropriate dilution of the stock standard solution. Cetyltrimethylammoniumbromide (CTAB), ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), aluminum isopropoxide, hydrochloric acid and ammonia were used without further purification processes. Stock solution of molybdate was prepared by dissolution of ammonium molybdate and potassium antimonyl tartarate in 60 mL of 9 mol L^{-1} sulfuric acid and diluted by triple distilled water to a final volume of 100 mL in a volumetric flask and it is stable for one month. A reducing agent of ascorbic acid solution was prepared before use and stored in foil-wrapped polyethylene bottle. This solution was stable at least 24 h and therefore it was prepared daily before use. The pH adjustments of 4.0-10.0 were made by using buffer solutions of $\text{CH}_3\text{COONa}/\text{CH}_3\text{COOH}$, $\text{CH}_3\text{COONH}_4/\text{CH}_3\text{COOH}$ and $\text{NH}_3/\text{NH}_4\text{Cl}$.

2.2. Apparatus

A GBC UV-Visible Cintra 6 Spectrophotometer model, attached to a Pentium (IV) computer, with 10-mm glass cell was used to measure the absorbance of phosphate and silicate at 810 and 830 nm, respectively. A Fourier transform infrared spectrometer (FTIR Prestige-21, Shimadzu), scanning electron microscope (LEO 1455VP SEM) and vibrating sample magnetometer (VSM 7400 Model Lake-Shore) were used to characterize the structure of the prepared MNPs and ACMNPs. For magnetic separations a strong neodymium-iron-boron ($\text{Nd}_2\text{Fe}_{12}\text{B}$) magnet (1.2 T, 2.5cm \times 5cm \times 10cm) was used.

2.3. Preparation of CTAB@ACMNPs

CTAB@ACMNPs was prepared according to our previous works [15,16]. First, ACMNPs was synthesized by chemical co-precipitation procedure [11,12]. Then, the obtained ACMNPs (100 mg) was coated with admicelles of CTAB (45 mg). The pH of this suspension was adjusted to 7.5 by addition of 5 mL of $\text{CH}_3\text{COONH}_4/\text{CH}_3\text{COOH}$ buffer (0.1 mol L^{-1}) solution. The mixed solution was shaken for 5 min and then CTAB@ACMNPs separated from the reaction medium under the magnetic field, and rinsed with 10 mL pure water. This product was used as sorbent for PO_4^{3-} ions.

2.4. General procedure

A 5 mL volume of standard solutions of PO_4^{3-} was added to CTAB @ACMNPs from above section, subsequently the pH value was adjusted to 7.5 with ammonium acetate/acetic acid buffer and the solution was shaken for 2 min to facilitate adsorption of the PO_4^{3-} ions onto the NPs. The beaker was placed on the magnet and NPs were collected. After decanting the supernatant solution the collected magnetite adsorbents have been washed with 5 mL of mixture solution of 0.25 mol L^{-1} H_2SO_4 and 0.15 mol L^{-1} HNO_3 in order to desorb the adsorbed phosphate ions by stirring for 3 min. Finally, PO_4^{3-} ions were determined by visible spectrophotometry at λ_{max} of 810 nm using molybdenum blue method.

2.5. Sample preparation

Samples of different waters were filtered through filter paper (Whatman, no. 4) to remove suspended particulate matters after collection and buffered to a pH of 7.5 with ammonium acetate/acetic acid buffer prior to storage in polyethylene container for use. The SPE procedure was carried out as described in general procedure.

3. RESULTS AND DISCUSSION

3.1. Characterization of ACMNPs

SEM was conducted to characterize the surface of the ACMNPs as adsorbent (Fig. 1). The image of Fig. 1 illustrates a highly porous morphology of adsorbent with uniform size distribution of the nanospheres. The crystalline structure of ACMNPs was further examined with XRD. Figure 2 indicates the XRD pattern of synthesized ACMNPs. All the reflections of Fe_3O_4 and Al_2O_3 correspond to the software database file. In addition, the average crystalline size of as synthesized ACMNPs nanoparticles using the Debye-Scherrer formula is calculated to be 18.6 ± 2 nm [17].

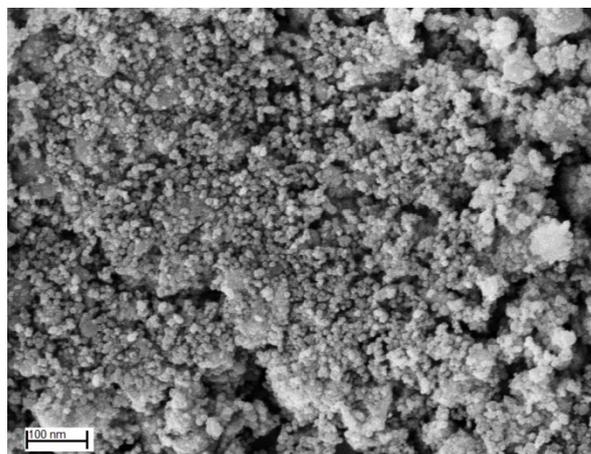


Fig. 1. SEM image of ACMNPs.

Magnetic particles may exhibit different magnetic properties due to their synthetic conditions [18]. The hysteresis loop measured for the MNPs and ACMNPs is shown in Figure 3. As can be seen, the typical characteristics of superparamagnetic behavior are observed. The saturation magnetization values from the magnetization curve in Fig. 3 for the uncoated and alumina-coated Fe_3O_4 nanoparticles were found to be 56.7 and 9.3 emu g^{-1} , respectively, at 300 K. The decrease of saturated magnetization was attributed to the formation of the alumina layer. However, the sorbent of ACMNPs is sufficient for magnetic separation with a normal magnet.

3.2. Effect of CTAB

Recently, we are reported the methods for separation and preconcentration of palladium, rhodium, arsenate and chromium using magnetite nanoparticles [13,15, 16]. These methods are based on the solid-phase extraction (SPE) of trace amounts of Pd(II), Rh(III), As(V) and Cr(VI) using MNPs and ACMNPs without

addition of a chelating agent and modification of NPs. In this paper, we investigate the application of CTAB immobilized on ACMNPs (CTAB@ACMNPs) for the separation/preconcentration of PO_4^{3-} ions. Positively charged surfactant of CTAB, can be strongly adsorb on negatively charged surfaces of ACMNPs in basic solutions (Fig. 4). Influence of various amounts of 10, 20, 30, 40, 45, 50, 60 and 80 mg CTAB (below its critical micellar concentration, $1 \times 10^{-3} \text{ mol L}^{-1}$) on adsorption of PO_4^{3-} ions through the ACMNPs substrate was investigated. The results showed that maximum adsorption was obtained when 45 mg of CTAB per 100 mg of ACMNPs used for PO_4^{3-} ions. Thus, this amount was selected as the optimum concentration of CTAB for further studies.

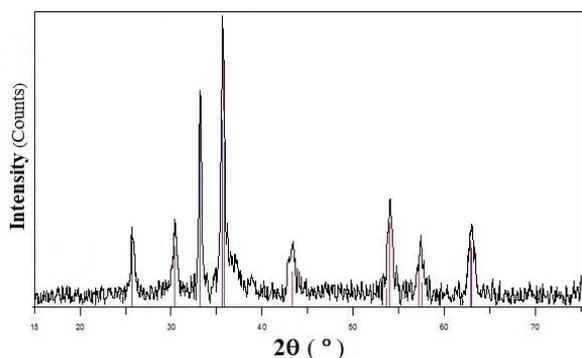


Fig. 2. XRD patterns for the naked ACMNPs.

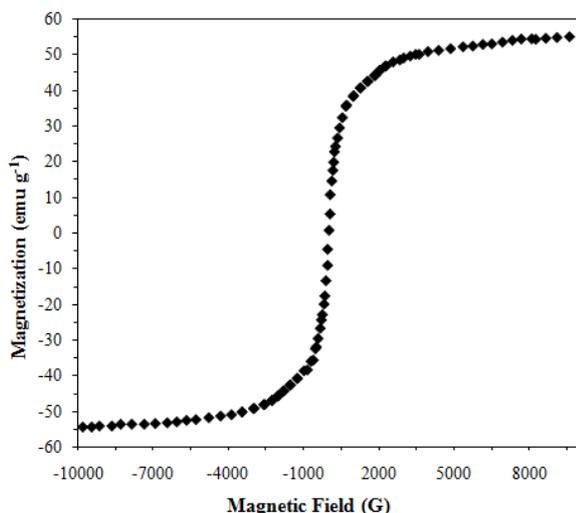


Fig. 3. The magnetic behavior of ACMNPs.

3.3. Effect of pH

The sorption of CTAB on ACMNPs is highly dependent on the solution pH. Negatively charged ACMNPs surfaces effectively adsorb positively charged CTAB at upper pH values. Therefore, retention of PO_4^{3-} ions on CTAB@ACMNPs occurs. When solution was basified, CTAB would form hemi-micelles on ACMNPs by strong sorption and these micelles could trap PO_4^{3-} ions. The sorption behavior of PO_4^{3-} ions on the modified ACMNPs in water samples was studied in the pH range of 4.0–10.0. Results show that the maximum removal on the

adsorbents was observed at pH of 7.0 to 8.0 (Fig. 5). At the pH values below 7.0 and above 8.0, the recovery decreased, due to diminish in the formation of negative charges of ACMNPs surfaces (reduction of CTAB adsorption) and competition of OH^- ions with PO_4^{3-} ions, respectively. Thus, the mean pH value (7.5) was used as optimum pH for further works.

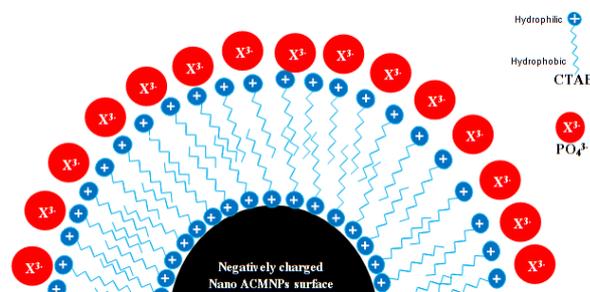


Fig. 4. A suggested schematic for adsorption of PO_4^{3-} to the CTAB@ACMNPs.

3.4. Effect of ACMNPs amount

The amount of CTAB@ACMNPs for complete extraction of 50 mL solution containing $10.0 \mu\text{g PO}_4^{3-}$ at pH 7.5 was studied. The results showed that the recovery percent increased up to 80 mg and remained constant. Therefore, 100 mg of CTAB@ACMNPs was used in all subsequent experiments.

3.5. Desorption conditions

The influences of various eluents on the recoveries of PO_4^{3-} ions from CTAB@ACMNPs were also examined. Different eluents such as HNO_3 , H_2SO_4 , HCl , ethanol and acetonitrile were investigated. Quantitative recoveries of the analytes were obtained with 5.0 mL of mixture solution of $0.25 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ and $0.15 \text{ mol L}^{-1} \text{ HNO}_3$.

3.6. Effect of sample volume

For study the influences of the sample volume on the recovery of analytes, the sample solutions in the range of 50–400 mL containing $5.0 \mu\text{g}$ of PO_4^{3-} ions were operated according to the general procedure and eluted using 5.0 mL of mixture solution of $0.25 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ and $0.15 \text{ mol L}^{-1} \text{ HNO}_3$. Analyte species were quantitatively (>95%) recovered at the sample volumes lower than 400 mL for PO_4^{3-} . With respect to eluent volume (5.0 mL), preconcentration factor of 80 (the ratio of the highest sample volume to the lowest eluent volume) for the PO_4^{3-} ions, were obtained.

3.7. Magnetic separating and standing time

In the experiment, CTAB@ACMNPs possessed large saturation magnetization and super-paramagnetism properties, which enabled them to be completely isolated at the less than 1 minute by a strong magnet. When the CTAB@ACMNPs was isolated immediately without a standing process, the recovery of PO_4^{3-} ions was only 70%. But, when the standing time was adjusted to 2, 5, 10 and 15 min, recoveries was improved to 95.0, 97.0, 97.0 and 97.5 %, respectively.

Standing time of 5 min was sufficient to achieve satisfactory adsorption and better recovery of PO_4^{3-} ions.

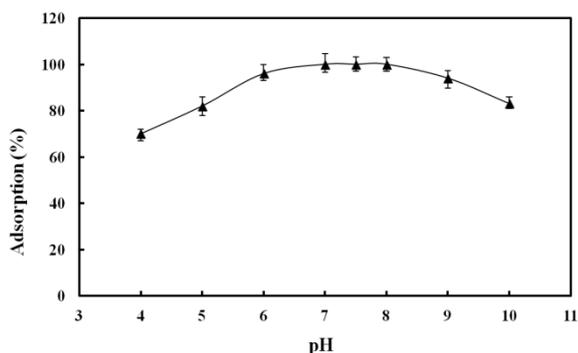


Fig. 5. Effect of pH on adsorption of PO_4^{3-} . Conditions: CTAB@ACMNPs (100 mg), PO_4^{3-} solution ($5.0 \mu\text{g mL}^{-1}$).

3.8. Interference study

The influences of some potentially interfering ions that interfere with the separation/preconcentration method and determination of analyte ions was examined. The tolerable limit was defined as the highest amount of potentially interfering ions that produced an error within 5% in the determination of the PO_4^{3-} ions investigated. This study was performed by binary mixtures containing $5.0 \mu\text{g}$ of PO_4^{3-} ions and a certain amount of one of the foreign ions. The following excesses of ions do not interfere (i.e., caused a relative error of less than 5%): less than a 1000-fold (largest amount tested) amount of Na^+ , K^+ , Ag^+ , NO_3^- , F^- , Cl^- , Br^- ; a 500-fold amount of NH_4^+ , Ba^{2+} , Co^{2+} , Zn^{2+} , Co^{2+} ; a 200-fold amount of Ni^{2+} , Zn^{2+} , Cu^{2+} , Fe^{2+} , Mn^{2+} , Cd^{2+} , Fe^{3+} , Al^{3+} ; a 50-fold amount of Ca^{2+} , Mg^{2+} , Pb^{2+} , Hg^{2+} ; and a 1-fold amount of AsO_4^{3-} and SiO_3^{2-} . The results showed that most of the investigated ions do not interfere in the adsorption-desorption and determination of traces of PO_4^{3-} in real samples. Only species of AsO_4^{3-} and SiO_3^{2-} appeared to interfere with PO_4^{3-} for molybdenum blue method, that these interferences can be eliminated by reduction with thiosulfate in an acidic medium and using a reagent composed of ammonium molybdate in nitric acid, containing oxalic acid to avoid the formation of molybdosilicic acid, respectively [19-21].

3.9. Adsorption capacity

This method was used to investigate the adsorptive capacity of CTAB@ACMNPs. 100 mg of CTAB@ACMNPs was added to the 50 mL of solution containing 5.0 mg of PO_4^{3-} ions at pH 7.5. After shaking for 1 h, the beaker was placed on the magnet and NPs were collected. After decanting the supernatant solution, the collected magnetite adsorbents have been washed with 5 mL of mixture solution of $0.25 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ and $0.15 \text{ mol L}^{-1} \text{ HNO}_3$ in order to desorb the adsorbed phosphate ions by stirring for 3 min. Finally, PO_4^{3-} ions were determined by molybdenum blue method. The static sorption

capacities of CTAB@ACMNPs were found to be 9.7 mg g^{-1} for PO_4^{3-} ions.

3.10. Analytical figures of merit

The detection limit (DL), linear range (LR) and relative standard deviation (RSD) of phosphate were $0.038 \mu\text{g mL}^{-1}$, $0.8\text{-}10.0 \mu\text{g mL}^{-1}$ and 2.5 % (for $5.0 \mu\text{g mL}^{-1}$, $n=7$), respectively. The regression equations were $A=0.01186C_{\text{phosphate}}+0.1117$ ($n=7$), where A is the absorbance and $C_{\text{phosphate}}$ is the concentration of PO_4^{3-} ions in $\mu\text{g mL}^{-1}$.

3.11. Real sample analysis

In order to check the feasibility of the analytical method, determination of PO_4^{3-} in water samples was carried out after SPE onto CTAB@ACMNPs. Good recovery results after spiking ranging from 94.6 to 98.8% are shown in Table 1. The results show that the proposed method could be successfully applied to the separation/preconcentration and determination of phosphate.

Table 1. Application of the proposed method to the separation/preconcentration and determination of PO_4^{3-} ions in different water samples (sample volume: 50 mL, $N=3$). The results are mean of three measurements \pm standard deviation.

| Sample | PO_4^{3-} ($\mu\text{g mL}^{-1}$) | | Recovery (%) |
|---------------------------------|--|---------------------|--------------|
| | Added | Found | |
| Tap water from Ardakan city | 0.0 | N.D | --- |
| | 2.5 | 2.54 (± 0.22) | 101.6 |
| River water from Hajiabad river | 0.0 | N.D | ---- |
| | 2.5 | 2.46 (± 0.30) | 98.4 |
| Spring water from Sirjan city | 0.0 | 0.34 (± 0.16) | ---- |
| | 2.5 | 2.96 (± 0.28) | 104.8 |
| Sea water from Mazandaran sea | 0.0 | 0.12 (± 0.06) | ---- |
| | 2.5 | 2.57 (± 0.25) | 98.0 |

4. CONCLUSION

In this work SPE carried out using ACMNPs to extract the phosphate anion and determine by spectrophotometric method. It has been demonstrated that the modified ACMNPs provides a new and fast route for separation/preconcentration and determination of PO_4^{3-} ions. This method shortened the analysis time of the method. This sorbent was successfully applied for efficient enrichment of trace amounts of phosphate ions from real samples. The main benefits of this methodology are: simplicity and high capacity of sorbent, preconcentration factor, fast adsorption and low cost.

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REFERENCES

- [1] C.W. Randall, L.J. Barnard, H.D. Stensel, Design and retrofit of wastewater treatment plants for biological nutrient removal, Technomic Publishing, USA, 1992.
- [2] B. Saha, S. Chakraborty, G. Das, Trimesic acid coated alumina: An efficient multi-cyclic adsorbent for toxic Cu(II), *J. Colloid Interf. Sci.* 331 (2009) 21-26.
- [3] A. Alcázar, P.L. Fernández-Cáceres, M.J. Martín, F. Pablos, A.G. González, Ion chromatographic determination of some organic acids, chloride and phosphate in coffee and tea, *Talanta* 61 (2003) 95-101.
- [4] T. P. Mawhinney, Separation and analysis of sulfate, phosphate and other oxyanions as their tert.-Butyldimethylsilyl derivatives by gas-liquid chromatography and mass spectrometry, *J. Chromatogr. A* 257 (1983) 37-44.
- [5] Y. Yokoyama, T. Danno, M. Haginoya, Y. Yaso, H. Sato, Simultaneous determination of silicate and phosphate in environmental waters using pre-column derivatization ion-pair liquid chromatography, *Talanta* 79 (2009) 308-313.
- [6] N. Nakatani, D. Kozaki, W. Masuda, N. Nakagoshi, K. Hasebe, M. Mori, K. Tanaka, Simultaneous spectrophotometric determination of phosphate and silicate ions in river water by using ion-exclusion chromatographic separation and post-column derivatization, *Anal. Chim. Acta* 619 (2008) 110-114.
- [7] T. Taghizadeh, F. Shemirani, M.A. Karimi, K. Abdi, M. Ezoddin, Modified nanoalumina sorbent for sensitive trace cobalt determination in environmental and food samples by flame atomic absorption spectrometry, *Int. J. Environ. Anal. Chem.* 92 (2012) 1264-1273.
- [8] S.G. Ozcan, N. Satiroglu, M. Soylak, Column solid phase extraction of iron(III), copper(II), manganese(II) and lead(II) ions food and water samples on multi-walled carbon Nanotubes, *Food Chem. Toxicol.* 48 (2010) 2401-2406.
- [9] L. Sun, X. Sun, X. Du, Y. Yue, L. Chen, H. Xu, Q. Zeng, H. Wang, L. Ding, Determination of sulfonamides in soil samples based on alumina-coated magnetite nanoparticles as adsorbents, *Anal. Chim. Acta* 665 (2010) 185-192.
- [10] L. Sun, C. Zhang, L. Chen, J. Liu, H. Jin, H. Xu, L. Ding, Preparation of alumina-coated magnetite nanoparticle for extraction of trimethoprim from environmental water samples based on mixed hemimicelles solid-phase extraction, *Anal. Chim. Acta* 638 (2009) 162-168.
- [11] M.A. Karimi, A. Hatefi-Mehrjardi, S.Z. Mohammadi, M.R. Hormozi Nezhad, A. Askarpour Kabir, M. Mazloun-Ardakani, A. Mohadesi, Solid phase extraction of trace amounts of Pb(II) in opium, heroin, lipstick, plants and water samples using modified magnetite nanoparticles prior to its atomic absorption determination, *J. Iran. Chem. Soc.* 9 (2012) 171-180.
- [12] M.A. Karimi, A. Hatefi-Mehrjardi, S.Z. Mohammadi, A. Mohadesi, M. Mazloun-Ardakani, A. Askarpour Kabir, M. Kazemipour, N. Afsahi, Solid phase extraction of trace amounts of silver(I) using dithizone-immobilized alumina-coated magnetite Nanoparticles prior to determination by flame atomic absorption spectrometry, *Int. J. Environ. Anal. Chem.* 92 (2012) 1325-1340.
- [13] S.Z. Mohammadi, M.A. Karimi, H. Hamidian, Y.M. Baghelani, L. Karimizadeh, Determination of trace amounts of Pd(II) and Rh(III) ions in Pt-Ir alloy and road dust samples by flame atomic absorption spectrometry after simultaneous separation and preconcentration on non-modified magnetic nanoparticles, *Scientia Iranica* 18 (2011) 1636-1642.
- [14] M.A. Karimi, M. Kafí, Removal, Preconcentration and determination of Ni(II) from different environmental samples using modified magnetite nanoparticles, *Arabian J. Chem.* In Press.
- [15] M.A. Karimi, A. Mohadesi, A. Hatefi-Mehrjardi, S.Z. Mohammad, J. Yarahmadi, A. Khayrkah, Separation/preconcentration and speciation analysis of trace amounts of arsenate and arsenite in water samples using modified magnetite nanoparticles and molybdenum blue method, *J. Chem.* In Press.
- [16] M.A. Karimi, R. Shahin, S.Z. Mohammadi, J. Hashemi, A. Hatefi-Mehrjardi, J. Yarahmadi, Speciation analysis of Cr(III) and Cr(VI) after solid phase extraction using modified magnetite nanoparticles, *J. Chin. Chem. Soc.* 60 (2013) 1339-1346.
- [17] R.Y. Hong, T.T. Pan, Y.P. Han, H.Z. Li, J. Ding, S.J. Han, Magnetic field synthesis of Fe₃O₄ nanoparticles used as a precursor of ferrofluids, *J. Magn. Magn. Mater.* 310 (2007) 37-47.
- [18] A.K. Gupta, M. Gupta, Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications, *Biomaterials* 26 (2005) 3995-4021.
- [19] D.L. Johnson, M.E.Q. Pilson, Spectrophotometric determination of arsenite, arsenate, and phosphate in natural waters, *Anal. Chim. Acta* 58 (1972) 289-299.
- [20] P.D. Goulden, P. Brooksbank, Automated phosphate analysis in the presence of arsenic, *Limnol. Oceanogr.* 19 (1974) 705-707.
- [21] C.X. Galhardo, J.C. Masini, Spectrophotometric determination of phosphate and silicate by sequential injection using molybdenum blue chemistry, *Anal. Chim. Acta* 417 (2000) 191-200.