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Dispersive micro-solid phase extraction of *ortho*-phosphate ions onto magnetite nanoparticles and determination as its molybdenum blue complex

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ABSTRACT

A direct microextraction method, employing dispersive micro-solid phase extraction (μ-SPE) of *ortho*-phosphate (o-PO_4^{3-}) anions onto ferromagnetic nanoparticles (MNPs) is described in this work for the first time. The method exploits the complexation of phosphate ions on the surface of positively charged magnetite nanoparticles through the formation of an inner sphere complex, which are separated from the bulk aqueous phase with the application of an adscititious magnetic field. Phosphates are eluted with sodium bicarbonate and determined spectrophotometrically as their phosphomolybdenum blue complex. The method is generally free from common interferences, likely to affect the measurement of phosphate, since it alleviates their presence already from the extraction step, thus they are absent during detection. The detection limits are as low as 0.01 μM with very satisfactory precision ranging from 3.68% (intra-day) to 8.5% (inter-day) and accuracy between 91.5% and 104.8%.

1. Introduction

In recent years, nanoscale ferrite materials have been receiving particular attention due to their vast potential in many state-of-the-art industrial, biological and medicinal applications [1,2]. Beyond their high surface area, a unique attribute of these materials is their superparamagnetic response allowing them to be monitored and manipulated in the presence of an adscititious magnetic field. These attributes hold great promise in analytical chemistry since they afford improved adsorption capacity and simplification of the time-consuming and laborious enrichment process of loading large sample volumes through rapid removal by an external magnetic field [3].

The deployment of iron oxide magnetic nanoparticles (MNPs) has been the springboard for the development of novel analytical methods for the determination of organic and inorganic compounds in various matrices of environmental, biological and food origin [4–8]. In the greatest majority of these articles, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) nanoparticles are commonly employed coupled to post-synthetic modifications in order to stabilize nanoparticles and introduce desired functionalities onto their surface, thus facilitate analyte partitioning onto the modified NP surface [5–10]. On the other hand, the utilization of uncoated MNPs for analytical applications are less frequent [8,11,12] since ferrite nanomaterials, due to their large surface area-to-volume ratio, tend to agglomerate and are generally subject to oxidation [2,13]. However, unmodified magnetic NPs have been successfully proposed as a vehicle for the efficient removal of hexavalent chromium [14], arsenic and arsenate [15,16], cationic dyes [17], salicylic acid [9], copper ions [18] and recently phosphate ions [19].

The adsorption of phosphate by iron hydroxides, is well documented and has proven to be an effective method for removing phosphorus from water and wastewater [20,21]. However, despite the large number of research on the topic, the interaction of magnetic iron oxide nanoparticles with phosphate ions has been focused on the influence of phosphation on the magnetic properties of superparamagnetic maghemite (γ-Fe_2O_3) nanoparticles and on the coupling interaction between magnetic nanoparticles [22,23]. Magnetite, on the other hand, has been subjected to far less investigations mainly the past few years [19,24]. Interestingly, the utilization of iron oxide nanomaterials for the extraction and analytical determination of phosphate ions has not been reported.

In this sense, this work describes the deployment of magnetic NPs, made up of magnetite (Fe_3O_4) as a medium for the microextraction of *ortho*-phosphate (o-PO_4^{3-}) anions from aqueous samples. The method exploits the complexation of phosphate ions onto the positively charged surface of magnetite NPs, which are separated from the bulk aqueous phase with magnetic decanting afforded by an adscititious magnetic field. The determination of phosphates is based on the typical formation of a heteropolyacid between phosphates and acidic molybdate, which is reduced by ascorbic acid towards the formation of phosphomolybdenum blue complex.

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2. Experimental

2.1. Materials

All reagents were of analytical-grade and they were used without further purification. For the synthesis of magnetic iron oxide nanoparticles (MNPs) the appropriate amount of iron (III) chloride ($FeCl_3$) (Sigma-Aldrich. Athens, Greece) and iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) (Merck, Darmstadt, Germany) was used. Standard solutions of phosphate anions ($1000\ \mu g\ mL^{-1}$) were prepared from di-potassium hydrogen phosphate (Riedel-de Haen, Sleeze, Germany). Working solutions of $100\ \mu g\ mL^{-1}$ were prepared weekly by appropriate dilution. The determination of phosphate was accomplished by a strongly acidic reagent mixture composed of ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, Mallinckrodt Baker, Inc., Phillipsburg, USA), ascorbic acid (Merck, Darmstadt, Germany) and potassium antimonyl tartrate ($C_4H_4KO_7Sb \cdot 0.5H_2O$, Fluka Chemie AG, Buchs, Switzerland). Adjustment of the pH of the solutions was accomplished by the addition of dilute HCl (Merck, Darmstadt, Germany) or NaOH (Mallinckrodt Baker, Inc., Phillipsburg, USA). Standard solutions of metal ions used for the interference study were prepared by dilution of $100\ mg\ L^{-1}$ stock solutions prepared by dissolving the appropriate amounts of $Cr(NO_3)_3 \cdot 9H_2O$ ($>99.99\%$), silicon dioxide (SiO_2 $>99.99\%$), V_2O_5 (99.99%) purchased from Sigma-Aldrich (Athens, Greece) and $As_2O_5 \cdot xH_2O$ (Merck, Darmstadt, Germany). Standard solutions of anions were prepared by dilution of $1000\ mg\ L^{-1}$ stock solutions prepared by dissolving the appropriate amounts of NaCl, Na_2SO_4, $NaHCO_3$ (Merck, Darmstadt, Germany), NaBr, KNO_2 and KNO_3 (Fluka Chemie AG, Buchs, Switzerland). Na_2SO_3 (Merck, Darmstadt, Germany) and $COOK(CHOH)_2COONa \cdot 4H_2O$ (Carlo-Erba, Italy) were used as masking agents for arsenate and silicate, respectively.

2.2. Apparatus

UV–vis absorption measurements were performed with matched quartz cells of 1 cm path length in a Jenway (Essex, UK) 6405 UV/Vis spectrophotometer. FT-IR spectra of the magnetite nanoparticles were taken on a Perkin-Elmer Spectrum GX FT-IR spectrophotometer.

2.3. Synthesis of magnetite nanoparticles

Preparation of magnetic iron oxide nanoparticles was based on alkaline hydrolysis of concentrated mixed solutions of ferric and ferrous salts. Briefly, 4.9 g $FeCl_3$ (1.6873 g Fe (III)) and 4.2 g $FeSO_4 \cdot 7H_2O$ (0.84368 g Fe(II)), at a Fe(II)/Fe(III) ratio of 0.5, were added to deoxygenated distilled water (100 mL) at room temperature and the solution was stirred for 5 min under continues flow of nitrogen. Then, 25 mL of sodium hydroxide (25% w/v) was gradually added and a black precipitate of magnetite NPs was formed, according to the reaction:

$$FeCl_3 + FeSO_4 + 8NaOH \rightarrow Fe_3O_4 + 6NaCl + Na_2SO_4 + 4H_2O$$

MNPs were washed several times with distilled water until obtaining a neutral solution ($pH=7$) and re-dispersed in distilled water. The concentration of MNPs in solution, calculated from difference between the weight of distilled water and MNPs solution ($n=10$), was $7.5 \pm 0.4\ mg\ mL^{-1}$.

2.4. Preparation of reaction reagent

For the determination of phosphate anions as their phosphomolybdenum blue complex, a strongly acidic mixture was prepared by mixing 25 mL of sulfuric acid (5 M), 5 mL of potassium antimonyl tartrate ($2.74\ g\ L^{-1}$), 15 mL ammonium molybdate ($60\ g\ L^{-1}$) and 30 mL ascorbic acid ($40\ g\ L^{-1}$) under continues stirring. The mixture was stirred for 10 min protected from light and used within 4 h.

2.5. Point of zero charge

For the determination of point of zero charge, 25 mg of MNPs were placed in degassed 0.01 M $NaNO_3$ aqueous solutions and the pH was adjusted in the range of 2–9. The initial pH was recorded, the vials were air-sealed with snap caps and shaken for 24 h in an end-over-end mechanical shaker. The pHpzc is the point where the curve pH final versus pH initial intersects the straight line corresponding to pH final=pH initial.

2.6. FT-IR spectroscopy

For FT-IR spectra acquisition the samples were initially dried at 373 K for 24 h, ground to fine powder and mixed with KBr crystals to prepare KBr wafers.

2.7. Analytical procedure

For the extraction of phosphate ions, 4.85 mL of water samples, containing up to 1 μM of $o\text{-}PO_4^{3-}$, were added in a vial and the pH was adjusted to the value of 3. Subsequently, MNPs aqueous solution ($7.5\ mg\ mL^{-1}$) was intensively churned to re-disperse MNPs. 50 μL (0.375 mg) of this solution were added to the sample or standard solution, and the mixture was mechanically stirred for 3 min. Separation of MNPs from the bulk aqueous phase was rapidly accomplished under the influence of a strong magnetic field provided by a neodymium magnet (NdFeB, 5500 Gauss). The supernatant solution was discarded and the collected MNPs were treated with 1.6 mL of 0.75 M $NaHCO_3$ solution for 5 min by mechanical shearing.

Subsequently, the extract was recuperated with magnetic decantation and 400 μL of reaction reagent were added in order to initiate the reaction of phosphates towards the formation of the phosphomolybdenum blue complex. After 15 min of incubation at room temperature, samples were measured spectrophotometrically at 880 nm, against reagents blank.

3. Results and discussion

3.1. Characterization of magnetic nanoparticles

3.1.1. Point of zero charge (PZC)

Magnetite is an amphoteric solid, which can develop charges in the protonation ($Fe\text{–}OH + H^+ \leftrightarrow Fe\text{–}OH_2^+$) and deprotonation ($Fe\text{–}OH \leftrightarrow Fe\text{–}O^- + H^+$) reactions of Fe–OH sites on its surface. Thus, the nature of charge on their surface can be predicted at a given pH of the solution [25]. Which mechanism prevails depends on the PZC of the surface; at the PZC the surface charge is neutral and it becomes positively or negatively charged at pH values below or above the PZC, respectively. The PZC of the synthesized magnetite NPs was close to 7.0 which lies within the reported range of values for magnetite [24,26,27].

3.1.2. FT-IR spectra

The FT-IR spectra of fresh and aged MNPs showed the typical Fe–O stretching at approx. 575–580 cm^{-1} which is concurrent with previous reports on the IR bands of magnetite nanoparticles [28–30].

3.2. Dispersive micro-solid phase extraction of phosphate ions

3.2.1. pH profile

pH is a parameter of paramount importance on the adsorption of phosphate anions on magnetite NPs because it determines the surface charge of magnetite NPs. As a rule of thumb, as pH increases, the surface charge of MNPs varies from positive to negative according to the PZC due to deprotonation of surface hydroxyls [24]. At the same time, phosphate species in solution change from mono- to di- and tri-basic ($H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}). According to the profile of absorption versus pH illustrated in Fig. 1, extraction efficiency decreases with increasing pH, as a result of the increased repulsion between the more negatively charged PO_4^{3-} species and negatively charged surface sites [20]. At pH values below 2 the solution turns dark brown indicating partial decomposition of MNPs by the excess of HCl while at too alkaline conditions ($pH > 12$) NPs were found to lose their magnetic properties. Based on these findings, a pH value of 3 was defined as an optimum, a value which is in absolute agreement with the previous reports concerning phosphate absorption by magnetite [24]. At this pH, the dominant phosphate species is $H_2PO_4^-$ which form an inner sphere complex (consisting of Coulombic and Lewis acid–base interactions) with magnetite surface groups involving interaction with both positively charged groups ($Fe–OH_2^+$) and hydroxyl sites at the surface of magnetite forming phosphate complexes [31–34].

3.2.2. Effect of MNPs concentration

The amount of the magnetic nanoparticles necessary to afford quantitative extraction of phosphate anions is a critical parameter that determines the efficiency of the overall method. Increasing amounts of MNPs, ranging from 0.18 to 37.5 mg, were examined for that purpose yielding maximum signal at 0.375 mg. Evidently, at lower MNP concentrations, the available surface area is inadequate to afford quantitative recovery of phosphates while higher concentrations have a trivial effect since there is an excess of reactive sites which are not involved in the extraction of phosphates.

3.2.3. Desorption conditions

Desorption of phosphate ions grafted at the surface of magnetite NPs was studied with various aqueous based solvents. In order to enable direct comparison among the various elution solvents, the composition of the reagent mixture was prepared according to Standard Methods [35].

Various elution mixtures composed of distilled water, dilute NaOH solutions, methanol:water (50:50), acidic molybdate, sodium fluoride and sodium bicarbonate were tested. The selection of these elution solvents was based on literature reports while distilled water was used as a reference. Solutions of NaOH have been used to desorb phosphate anions from iron oxide surfaces [19,24]; organic solvents, have been shown to be effective in removing hydrogen-bonded phosphate [24] while fluoride and carbonate anions are extensively utilized to remove Fe-bound phosphorous from soils and sediments [36,37]. Finally, acidic molybdate was considered since sulfate ions in acid solution can dissolve Fe-phosphates [36] while they could also trigger the formation of the colored phosphomolybdenum blue complex.

From the bar plots of Fig. 2 it is evident that $NaHCO_3$ offers significantly higher extraction than other elution solvents. The low recovery of aqueous solutions is attributed to the low alkalinity of the solution. In general, strongly alkaline media (1 M) are required for almost quantitative desorption of phosphates in combination with prolonged incubation times (≥ 24 h) [19,24]. For analytical purposes, neither was feasible without increasing experimental effort or analysis time. On the other hand, organic solvents are more efficient in removing hydrogen-bonded phosphate, thus amending extraction [24], but it was still not satisfactory. Fluoride anions were also insufficient since their utility in dissolving Fe-bound phosphates is amended by HCl which was not used since too acidic conditions deter the formation of the phosphomolybdenum blue complex [38]. For the same reason, direct addition of the reaction reagent (i.e. acidic molybdate) did not yield any color unless dilution has been performed in order to reduce acidity and initiate the formation of the phosphomolybdenum blue complex. However, even in that occasion the extraction efficiency was noticeably inferior to that of $NaHCO_3$.

Based on these observations the optimum concentration of $NaHCO_3$ was seeked in the range of 0.1–1.5 M. The results (Fig. 3a) indicate a significant increase of the absorbance signal with increasing concentrations up to 0.75 M. Subsequently, the optimum volume of elution solvent ($NaHCO_3$) and reaction reagent (acidic molybdate) was investigated in parallel to a final volume of 2 mL by increasing the amounts of reaction reagent from 100 to 1000 μL and adjusting the volume of $NaHCO_3$,

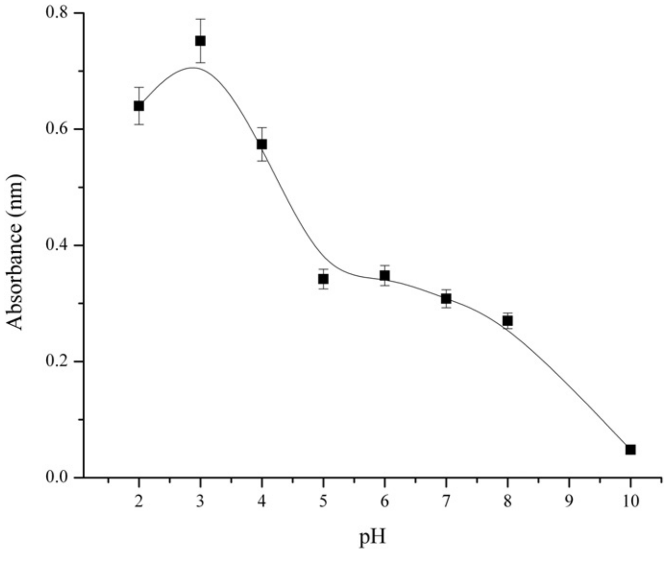

Fig. 1. Effect of solution pH on the extraction of phosphate ions onto magnetite NPs.

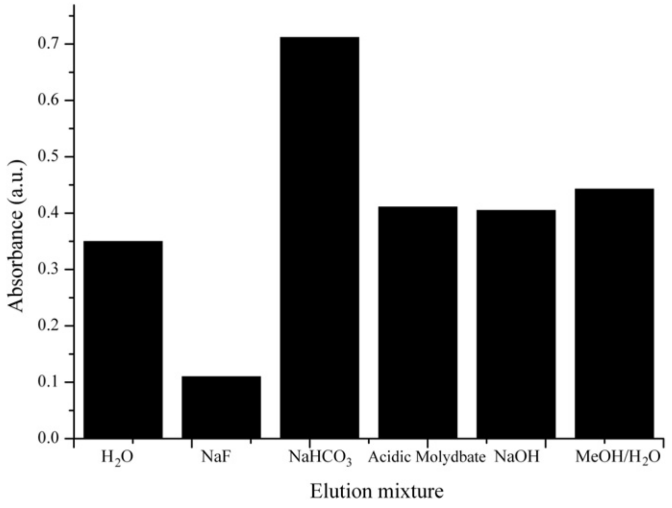

Fig. 2. Influence of different wash solvents on the desorption of phosphate. (NaF=0.05 M, $NaHCO_3$=0.5 M, NaOH=0.1 M, acidic molybdate as reported in Standard Methods [36]).

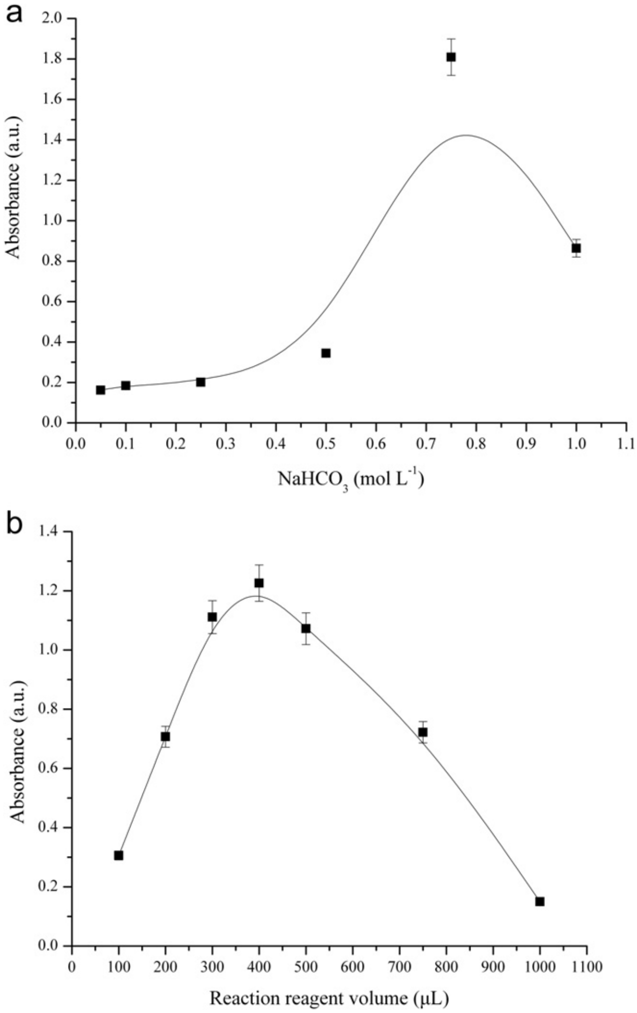

Fig. 3. (a) Optimization of NaHCO$_3$ concentration (b) Effect of reaction reagent (acidic molybdate) volume of the analytical signal.

accordingly. From the graph of Fig. 3b it is blatant that the optimum signal is attained at 400 μL gradually leveling off with increasing volume. Obviously, lower volumes are insufficient for complete color formation while larger volumes result in increased acidity of the final mixture, which is not favorable for the formation of the phosphomolybdenum blue complex, as previously discussed [38].

Finally, the composition of the elution mixture was assessed for its influence on the analytical response. Acidity, ammonium molybdate, ascorbic acid and antimonyl concentrations were varied in the range of 2–6 M H_2SO_4, 10–100 g L^{-1} ammonium molybdate, 5–60 g L^{-1} ascorbic acid and 1–8 g L^{-1} antimonyl. From the results obtained (Fig. S1 supplementary information) it was revealed that the optimum composition of the reaction reagent is 5 M H_2SO_4, 60 g L^{-1} ammonium molybdate, 40 g L^{-1} ascorbic acid and 2.75 g L^{-1} antimonyl.

3.2.4. *Analysis time*

In the proposed procedure, several steps are time depended. Mixing of MNPs with the aqueous sample solution was the first parameter investigated between 0.5 and 15 min. From the results illustrated in the dotted line of Fig. 4, it is made clear that extraction is amended up to 2 min, reaching a plateau up to 5 min, and gradually flattens out with increasing time. Based on this observation, 3 min of mixing was decided. Interestingly, periodic mixing of the sample with interim standing had a beneficial effect in the extraction yield as compared to continuous mixing. Similar observations have been reported elsewhere [3].

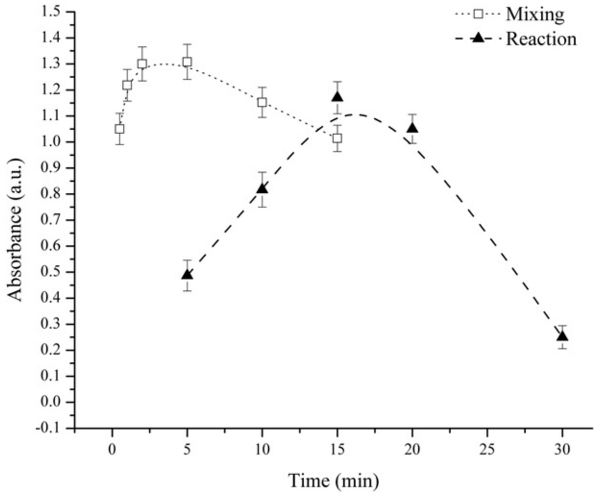

Fig. 4. Effect of mixing and desorption time on the extraction of phosphates from magnetite NPs. (–□—Mixing time; –▲—Reaction time).

The next time-depended step is desorption time required to accomplish complete extraction of phosphate ions from the surface of MNPs. The analytical signal increases slightly up to 5 min but is quenched thereafter, dictating the application of 5 min as desorption time for maximum recovery.

The last step of the experimental procedure is related to the reaction kinetics for the formation of the phosphomolybdenum blue complex. Typically, the determination of phosphates using the phosphomolybdenum blue chemistry is feasible in a time span from 10–30 min [35]. Monitoring of the absorbance values from 1–30 min (in 5 min intervals) showed that the signal increases with time up to 15 min (Straight line-Fig. 4), which was adopted as an optimum.

3.2.5. *Effect of temperature*

The influence of temperature on the adsorption of phosphate ions on MNPs was investigated at various temperatures from 10 to 80 °C at 10 °C intervals. According to the line graph of Fig. S2 (supplementary information) the absorption signal reaches a plateau between 20 and 50 °C leveling off thereafter, which is probably related to the acceleration of oxidation of magnetite nanoparticles [39]. This notion is supported by the findings of previous studies which recorded a strong dependence of magnetite oxidation with increasing temperature through a two stage process involving a relatively fast process at the beginning and slow towards the end [39]. Based on these observations, and for the simplicity of the experimental procedure, room temperature was decided as an optimum.

Temperature during the reaction of acidic molybdate with phosphates was intentionally maintained at room temperature, since increasing temperature favors the formation of silicomolybdenum blue which causes overestimation of phosphate levels [38].

3.2.6. *Effect of Ionic strength*

According to previous studies, the number of charged surface sites increases with increasing ionic strength due to the screening effect of the electrolyte [40]. In this context, the influence of ionic strength on the performance of the method was assessed with increasing $NaNO_3$ concentrations from 0.01 to 5 M. The absorbance pattern with increasing ionic strength (Fig. S3—Supplementry information) suggests that phosphate extraction efficiency is reduced possibly due to competition from the salt counter-anion. Based on this observation, experiments were conducted in the absence of ionic strength regulators. However, the influence of salt on the applicability of the method in seawater analysis was further evaluated and the results are presented in Section 3.4 below.

3.2.7. *Maximal extraction volume*

The maximal sample volume that can be employed without impairing the extraction efficiency was determined using a series of standard solutions (5–40 mL) spiked with phosphate ions under the experimental conditions specified above, in the presence of 0.075 mg mL^{-1} MNPs. Insufficient recovery was considered to occur when recovery decreased below 90%. This condition was satisfied for sample volume up to 40 mL, which was the maximum sample volume examined. A local increase at 10 mL was observed but all values were within ±6% of the maximum signal. Therefore, for the convenience of analysis, 5 mL were employed during the method application in real samples.

3.2.8. *Ageing and preservation of nanoparticles*

Spontaneous processes known as ageing, take place in time in colloidal dispersions of magnetite nanoparticles, which mainly involve aggregation (i.e., the adhesion due to Van der Waals attraction) and chemical transformation of magnetite to maghemite through a process involving reaction of the surface Fe^{2+} cations with oxygen.

Aggregation and precipitation of MNPs in neutral aqueous solution was readily observed. As a result direct sampling of MNPs from the solution was not feasible dictating the application of vigorous mixing, in order to re-disperse MNPs. The reproducibility of this procedure was examined with two different methods. In the first method, 250 μL of aqueous MNPs solution was collected after intense mixing and their weight was measured in an analytical balance subtracting the weight of water. The reproducibility of the measurements for 10 consecutive samplings was 5.85% which is very satisfactory for analytical extraction. The second method concerned the influence of this sampling method on the analytical method. To this end, 6 samples were analyzed for their content in phosphate according to the experimental protocol. Recoveries were above 95% and reproducibility below 4.8%. Based on these cross-validated results, it was inferred that re-dispersion of MNPs by intense mechanical mixing immediately before sampling can safely be applied without jeopardizing the quality of the analytical results.

Except aggregation, transformation of magnetite to maghemite is another parameter that needs to be taken into consideration. Magnetite is sensitive to oxidation in the presence of oxygen, according to the reaction:

$$Fe_3O_4 + 2H^+ \rightarrow \gamma Fe_2O_3 + Fe^{2+} + H_2O$$

Beyond oxidation, various electron or ion transfers depending upon the pH of the suspension are involved. According to the above equation, under acidic and anaerobic conditions, surface Fe^{2+} ions are desorbed as hexa-aqua complexes in solution, whereas, under basic conditions, the oxidation of magnetite involves the oxidation–reduction of the surface of magnetite [41].

To investigate the stability and potential aging of MNPs, method application was conducted for 15 consecutive days and the results were used to evaluate stability of the magnetite NPs aqueous suspension over time. The first interesting observation was that MNPs solution should be aged for 24 h prior to its use since direct use of MNPs on the day of their preparation produced relatively lower analytical signals which were restored to normal values from the second day and thereafter. The other finding was that MNPs were stable for the time period examined, verified also by comparative projection of the FT-IR spectra of fresh and aged MNPs (not shown). This can be attributed to two reasons. The first is the neutral pH of the solution used to preserve NPs, which also lies near the observed PZC; therefore, neither desorption of Fe^{2+} surface ions nor oxidation–reduction of the surface of magnetite is favored. In addition, the influence of oxygen on the oxidation of magnetite is diminished due to the storage at room temperature. These results agree with previous findings, where the conversion fraction of magnetite to maghemite, monitored by the absorption at near-IR, was about three months at room temperature [40].

3.3. *Interferences*

The competitive or synergistic effect of metallic species and other inorganic anions on the performance of the method was examined individually for each potential interferant using the method of mixed solutions. In this effort, aqueous solutions containing 1 μM of phosphate anions were fortified with increasing amount of potential interfering ions, selected on the basis of their common occurrence in natural waters. Interference was considered to occur when the measured signal varied more than ±5% of that of phosphate solution.

From the results obtained it was inferred that metallic elements like Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+}, Sb^{3+}, Mn^{2+}, Na^+ and K^+ had no influence on the analytical signal for concentrations up to the maximum concentration levels examined (100 μg mL^{-1}). With regards to inorganic anions like SO_4^{2-}, Cl^-, NO_3^- and Br^- no interference was observed up to the maximum concentration level investigated (100 μg mL^{-1}). This is reasonable since phosphate anions are attracted on the surface of magnetic NPs by the combined presence of Coulombic and Lewis acid–base interactions [31] while other anions by Coulombic interactions only, thus they do not afford strong competition. The only exception was NO_2^- which was found to reduce the signal slightly below the threshold of ±5% (−6.30%). However, dealing with natural water samples that contain such elevated levels of NO_2^- is highly unlike, especially in oxygenated waters due to their low thermodynamic stability which results in rapid oxidation to NO_3^-.

The above findings came as no surprise and are in concurrence with previous studies using the formation of heteropolyacids for the determination of phosphate [42]. However, of particular concern was the reactivity of anions such as CrO_4^{2-}, VO_3^-, SiO_4^{4-} and AsO_4^{3-} with molybdate, towards the formation of heteropolyacid complexes, similar to phosphate anions.

Under the optimum experimental conditions CrO_4^{2-} was not found to exert any effect up to 20 μg mL^{-1}, while VO_3^- could be tolerated at concentrations up to 1.5 μg mL^{-1}. At higher concentrations of VO_3^- the signal decreases and further tolerance can be achieved indirectly by the addition of oxalic acid which destroys the molybdophosphoric acid complex thus enabling to quantify the contribution of VO_3^- in the measured signal [43]. Nevertheless, such high levels of VO_3^- are not to be found in natural water samples.

Arsenate (AsO_4^{3-}) on the other hand, was found to increase sample absorbance when present at concentrations higher than phosphate, a scenario that can seldom be satisfied. Nevertheless, in the worst case scenario, AsO_4^{3-} interference can be mitigated by the addition of Na_2SO_3, a mild reducing agent that transforms AsO_4^{3-} to As^{3+} which exhibits no reactivity for molybdate. In the presence of $0.06-0.08$ M Na_2SO_3, AsO_4^{3-}concentrations up to 20 μg mL^{-1} can be tolerated.

The most common oxyanion known to interfere with the formation of the phosphomolybdenum blue complex is silicate, due to its abundance in natural waters ranging between 0 and 160 μM [44]. Under the specified experimental conditions silicate were found to interfere at concentrations as low as 20 μM.

This behavior is probably related to the use of bicarbonate, which effectively desorbs silicate species from the surface of MNPs [45] and to the low working range which increases the relative importance of silicate on the measured signal [46]. On the other hand, under the specified experimental conditions (especially pH and temperature during reaction) high phosphate concentrations favor the formation of the phosphomolybdenum blue complex over silicomolybdenum blue [38,47]. In any case, during the analysis of natural water samples no interferences were observed and satisfactory recoveries were obtained (Section 3.4).

Beyond the above, further efforts were devoted to mitigate the importance of silicate by examining alternative extraction media. The best results were observed by direct addition of the molybdenum blue reagent followed by distilled water to reduce acidity and enable the formation of the molybdenum blue complex. Using this method and by addition of 0.06 M tartrate in the initial solution, in order to deter silicate absorption on MNPs surface, silicate could be tolerated up to 300 μM which is far above the usual levels in natural waters. However, using this approach absorbance signal was reduced and the enhancement factor was deteriorated reflecting the requirement for optimization of the relative concentration of acidic molybdate and the volume of water in the final extract. Since the importance of silicate is more significant in unpolluted waters poor in phosphate and becomes trivial in polluted waters rich in phosphorus [46], the application of each elution method can be decided depending on needs.

3.4. Analytical figures of merit and method performance

Analytical parameters, such as linear range, correlation coefficient, limits if detection, accuracy and precision were evaluated by preconcentrating 5 mL of standard aqueous solutions. The calibration curve was rectilinear in the range of 0.025–1.04 μM fitted to a regression line represented by the formulae $\mathrm{Abs}_{(880\ \mathrm{nm})}=0.875\times C_{\mathrm{PO}_4^{3-}}+0.390$ ($r=0.9895$). The lowest concentration of phosphates which can be distinguished from the blank with a reasonable confidence was 0.01 μM, calculated according to the DG SANCO decision, as the mean absorbance of the blank sample plus k times the standard deviation of the absorbance obtained on the blank sample, where k is a constant related to the statistical level of confidence [48]. Despite the fact that low sample volume was employed, the detection limit is better or comparable to other methods [37,49,50]. Further improvement can be achieved using a longer light path [35], capillary flow cells [44,51] or by preconcentrating larger sample volume. By preconcentrating only 5 mL of sample volume an enhancement factor (EF) close to 40 was observed, determined by the ratio of slopes of the calibration curves of preconcentrated samples to that obtained without preconcentration.

The intra-day precision of the method, expressed as the relative standard deviation (RSD) of 7 measurements was 3.68% and 4.11% at 0.1 μM and 0.8 μM, respectively. Inter-day precision was calculated from the analysis of 0.8 μM standard solutions for 15 d and was close to 8.5%, which was deemed as satisfactory. Finally, the accuracy of the method was evaluated by fortifying a series of waters samples, with known amounts of phosphate. The results gathered in Table 1 show that the method affords good recoveries between 91.5% and 104.8%.

The applicability of the method in seawater samples was also assessed by analyzing aqueous standard solutions containing 35‰ NaCl fortified with increasing concentrations of phosphates. Evidently, and in concurrence with the influence of inorganic salts discussed above, the absorbance signal was lower but enabled the determination of phosphates in the range between 0.15 and 3.5 μM.

Table 1
Determination and recoveries of phosphate in real water samples.

Sample	Measured (μM)	Spiked (μM)	Found (μM)	Recovery (%)
Tap water	0.62	0.2	0.85	104.8
Bottled water 1	0.52	0.1	0.59	94.2
Bottled water 2	n.d.	0.2	0.19	95.0
Bottled water 3	n.d.	0.5	0.49	98.0
River water 1	0.75	0.1	0.82	96.0
River water 2	0.72	0.2	0.89	95.8
River water 3	0.83	0.2	0.96	91.5

n.d. not detected.

4. Conclusions

In this study, an analytical method employing dispersive micro-solid phase extraction of phosphate anions onto the surface of magnetic nanoparticles was developed. Positively charged magnetite nanoparticles were used to attract phosphate ions from aqueous solutions and separated by magnetic decantation. Phosphates were re-dissolved by sodium bicarbonate and determined as their phosphomolybdenum blue complex, free from common interferences likely to affect the analytical signal. The method is straightforward, offers satisfactory accuracy and good analytical features rendering it attractive for the analysis of real samples, especially when potential interfering species co-exist in the sample.

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.05.021.

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