Colorimetric determination of trace orthophosphate in water by using C18-functionalized silica coated magnetite

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In this study, we customized magnetic sorbents by functionalizing silica coated magnetite with octadecyl(C₁₈)silane (Fe₃O₄@SiO₂@C₁₈). This sorbent was intended for the determination of trace orthophosphate (o-PO₄³⁻) in unpolluted freshwater samples. The o-PO₄³⁻ was transformed to phosphomolybdenum blue (PMB), a known polyoxometalate ion. Then the PMB were coupled with cetyl trimethyl ammonium bromide (CTAB), cationic surfactant, in order to hydrophobically bound with the Fe₃O₄@SiO₂@C₁₈ particles through dispersive magnetic solid-phase extraction (d-MSPE) as part of sample preconcentration. The PMB–CTAB–magnetic particles are simply separated from the aqueous solution by the external magnet. The acidified ethanol 0.5 mL was used as PMB-CTAB eluent to produce an intense blue solution, which the absorbance was measured using a UV–Vis spectrophotometer at 800 nm. The proposed method (employing 2 mg of Fe₃O₄@SiO₂@C₁₈) yielded an enhancement factor of 32 with a linear range of 1.0–30.0 µg P L⁻¹. Precision at 6.0 µg P L⁻¹ and 25.0 µg P L⁻¹ were 3.70 and 2.49% (RSD, n = 6) respectively. The lower detection limit of 0.3 µg P L⁻¹ and quantification limit of 1.0 µg P L⁻¹ allowed trace levels analysis of o-PO₄³⁻ in samples. The reliability and accuracy of the proposed method were confirmed by using a certified reference material. Our method offers highly sensitive detection of o-PO₄³⁻ with simple procedures that can be operated at room temperature and short analysis time.

Abbreviations
CN-CA Cellulose nitrate and cellulose acetate
CPE Cloud-point extraction
CRM Certified reference material
CTAB Cetyl trimethyl ammonium bromide
DLLME-SFODME Dispersive liquid–liquid micro-extraction with solidified floating organic drop micro-extraction
d-MSPE Dispersive magnetic solid-phase extraction
d-SPE Dispersive solid-phase extraction
DTAB Dodecyltrimethylammonium bromide
EISE Electrostatically induced stoichiometric extraction
FT-IR Fourier-transform infrared spectrometry
HLB Hydrophilic-lipophilic balance
HPLC High performance liquid chromatography
LDHs Layered-double hydroxides
LLE Liquid–liquid extraction
LOD Limit of detection

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Phosphorus is a growth-limiting nutrient in aquatic systems. It is present at trace levels of between 10–50 µg P L⁻¹ in most natural clean water. If there are excessive amounts of phosphorus, overgrowth of algae rapidly occurs, creating an environmental problem. Orthophosphate (o-P⁰⁴⁻) is the predominant species in most water resources and is also the most bioavailable form of phosphorus. It is therefore essential to develop a quick, sensitive and reliable methods for monitoring trace o-P⁰⁴⁻ so we can prevent the problem from possible causes readily.

Quantification of o-P⁰⁴⁻ with the molybdenum blue method normally implement the preconcentration procedures in order to improve sensitivity. Various preconcentration techniques have been developed such as cloud-point extraction (CPE)¹⁴, suspended droplet micro-extraction (SDME)³⁶, dispersive liquid–liquid micro-extraction with solidified floating organic drop micro-extraction (DLLME-SFODME)⁶, electrostatically induced stochiometric extraction (EISE) via layered-double hydroxides (LDHs)⁶, vortex-assisted natural deep eutectic solvent micro-extraction (VA-NADES-ME)⁶ and, more recently, vortex-assisted based supramolecular solvents-dispersive liquid–liquid micro-extraction (VA-SS-DLLME)⁷. Even though the techniques offer low limits of detection and high enhancement factor however, the procedures are not simple and handling the liquid extractant require certain skills. Another approach that not only improve the sensitivity but also simplifies the procedures is to exploit solid-phase extraction (SPE) with different types of solid sorbent. Those solid sorbent particles can be classified into two groups: non-magnetic and magnetic.

Non-magnetic particles with C₁₈–functionalyzed surfaces have been employed in both off-line and on-line extraction for o-P⁰⁴⁻ determination in aqueous samples. First the o-P⁰⁴⁻ was converted to phosphomolybdenum blue complex (PMB) and followed by formation of a neutral ion pair complex with cationic surfactants such as cetyltrimethylammonium bromide (CTAB)¹⁰ and dodecyltrimethylammonium bromide (DTAB)¹⁵. Then the neutral ion pair complex (PMB-CTAB or PMB-DTAB) was adsorbed onto the C₁₈ particles via hydrophobic interaction before the adsorbed C₁₈ particles were collected. Finally, elution of PMB from the collected C₁₈ particles was analyzed through colorimetric detection.

Special interest has been given to the application of magnetic materials in so-called magnetic solid-phase extraction (MSPE). Commonly the small particle size of particles possesses large surface-to-volume ratio which allows high recovery of analyte. The main advantage of MSPE is that the magnetic particles can be separated from the large volume of solution efficiently and effortlessly by external magnet. One of the widely used magnetic particles is bare magnetite (Fe₃O₄), this iron oxide has strong magnetism, low toxicity, and very easy to synthesize. However, there are some drawbacks such as ease of aggregation, deterioration of the magnetic properties when exposed to air, dissolution in strongly acidic solutions and the major drawback of magnetite is lack of sorption specificity. Thus, application of bare magnetite in limited especially when the application requires strongly acidic condition (pH < 1). So, modification of Fe₃O₄ by coating with protective layers while maintaining the magnetic properties and large surface-to-volume ratio can overcome this constraint. Octadecyl (C₁₈) silane modified magnetite (Fe₃O₄@SiO₂@C₁₈) is one of the most widely used lipophilic adsorbent for extraction/prenconcentration of hydrophobic/nonpolar compounds since it provides strong hydrophobic affinity and excellent stability. However, it has not been employed for o-P⁰⁴⁻ determination.

Determination of o-P⁰⁴⁻ were diverged to analysis of o-P⁰⁴⁻ directly and transform o-P⁰⁴⁻ into polyoxometalate species (POMs) which offer many novel analysis strategies because the chemical nature of POMs is pH dependent. Since POMs are negatively charged, our strategy is to couple PMB with cationic surfactant, leading to electrostatic interactions between both chemicals and then utilizing hydrophobic interaction between surfactant and Fe₃O₄@SiO₂@C₁₈ to effectively extracted PMB from the sample solutions.
In this study, we developed a method based on dispersive magnetic solid-phase extraction (d-MSPE) for simple, fast, specificity and highly sensitive detection of trace $\text{PO}_4^{3-}$ focusing in freshwater. The $\text{Fe}_3\text{O}_4$ magnetic particles were coated by silica ($\text{SiO}_2$) layer to produce $\text{Fe}_3\text{O}_4@\text{SiO}_2$ particles which still possess magnetic property and can tolerate strongly acidic condition ($\text{pH} < 1$). To increase selectivity and affinity of the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ particles to the neutral ion pair complex, PMB–CTAB, the silica layer was functionalized with octadecyl ($\text{C}_{18}$) silane, producing $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$ particles (see Fig. 1). The structural properties of the synthesized $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$ particles, including their adsorption capacity towards PMB–CTAB, were studied and then used for developing a protocol for $\text{PO}_4^{3-}$ determination in freshwater samples. The reusability and storage durability of the synthesized $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$ particles for $\text{PO}_4^{3-}$ analysis was also investigated. Finally, the proposed method was validated and applied to trace $\text{PO}_4^{3-}$ detection in river, canal, and tap water samples.

### Experimental Chemicals

All chemicals were of analytical grade and used without further purification. Reagents were prepared with Type I deionized water (18 MΩ·cm$^{-1}$, ELGASTAT UHQ PS, ELGA, England).

For preparation of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$ particles, the following chemicals were used: iron (II) chloride tetrahydrate ($\text{FeCl}_2\cdot4\text{H}_2\text{O}$) (Sigma-Aldrich, Germany), iron (III) chloride hexahydrate ($\text{FeCl}_3\cdot6\text{H}_2\text{O}$) (Sigma-Aldrich, Germany), 30% (w/v) ammonia solution (Carlo Erba, Italy), absolute ethanol (Carlo Erba, Italy), tetraethyl orthosilicate (TEOS) (Fluka, Switzerland), anhydrous pyridine (Sigma-Aldrich, Germany), and chloro(dimethyl)octadecylsilane (Sigma-Aldrich, USA).

A 100 mg P L$^{-1}$ stock solution was prepared by dissolving 0.5624 g of potassium dihydrogen phosphate (KH$_2$PO$_4$) (Merck, Germany) in deionized water. Mixed reagents ($R_1$) were prepared in a 50-mL volumetric flask, by sequentially dissolving 5 mL of sulfuric acid (98% (w/w), H$_2$SO$_4$) (RCI LabScan, Thailand), 0.0116 g of potassium antimony tartrate hemihydrate ($\text{C}_4\text{H}_4\text{KO}_7\text{Sb}\cdot\text{1/2H}_2\text{O}$) (Carlo Erba, Italy), and 0.4325 g of ammonium dihydrogen phosphate (KH$_2$PO$_4$) (Merck, Germany) in deionized water. Mixed reagents ($R_1$) were prepared in a 50-mL volumetric flask. The CTAB solution (0.25 g L$^{-1}$) was prepared by dissolving 0.1250 g of CTAB (Ajax Finechem, New Zealand) in 500 mL deionized water. Acidified ethanol was prepared by dissolving 30.4 mL of 98% (w/w) H$_2$SO$_4$ in absolute ethanol to a final concentration of 0.56 mol L$^{-1}$ 10.

The following chemicals were used for the interference study: sodium metasilicate anhydrous (Na$_2$SiO$_3$) (Fluka, Switzerland), sodium arsenate dibasic heptahydrate (HAsNa$_2$O$_4\cdot7\text{H}_2\text{O}$) (Sigma-Aldrich, India), sodium chloride (NaCl) (Carlo Erba, Italy), sodium hydrogen carbonate (NaHCO$_3$) (Carlo Erba, Italy), sodium nitrite (NaNO$_2$) (Ajax Finechem, New Zealand), potassium nitrate (KNO$_3$) (Carlo Erba, Italy), magnesium sulfate anhydrous (MgSO$_4$) (Panreac, Spain), and potassium dichromate (K$_2$Cr$_2$O$_7$) (Carlo Erba, Italy).

A certified reference material (CRM) for $\text{PO}_4^{3-}$ (product ID QC1166; certified value 0.752 ± 0.0140 mg P L$^{-1}$; acceptance interval 0.526–0.978 mg P L$^{-1}$; traceable to NIST SRM 3186) was purchased from Sigma-Aldrich, USA. This CRM was diluted 100 times with deionized water and employed for method validation.

### Instruments and apparatus

Fourier-transform infrared spectrometry (FT-IR) (Nicolet 6700 FT-IR, Thermo Scientific, USA), laser scattering particle size distribution analysis (Partica LA-950V2, Horiba, Japan), and transmission electron microscopy (TEM) (JEM-2010, Jeol, Japan) were employed for characterization of the synthesized $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$ particles. Saturated magnetization ($\text{MS}$) and the hysteresis curve were analyzed using Vibrating Sample Magnetometer (VSM) (VSM 7404, Lakeshore Cryotonics, USA). X-Ray diffraction (XRD) patterns were obtained from JDX 3500, Jeol, Japan. The specific surface area (SSA) measurements were performed the Quantachrome® ASIWin™ - Automated Gas Sorption Data analyser (Autosorb iQ, Quantachrome instrument, USA). The SSA values were calculated using the BET (Brunauer–Emmett–Teller) method. Orbital shaker (Innsmax 2010, Heidolph, Germany) was used in all extraction step. A UV–Vis spectrophotometer (UV-1700, Shimadzu, Japan) and a 10-mm path length optical glass micro-cuvette (700 µL internal volume) (Hellma, Germany) were used for measurement of absorbance. Unbranded rectangular magnetic bar (3.7 cm × 6.3 cm × 1.2 cm) with a magnetic flux density of 3200 G was purchased from a local distributor in Bangkok, Thailand.

### Synthesis of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$

#### Synthesis of $\text{Fe}_3\text{O}_4$

The $\text{Fe}_3\text{O}_4$ core was prepared by chemical co-precipitation$^{18}$, $\text{FeCl}_2\cdot4\text{H}_2\text{O}$ 1.0 g and 2.7 g of $\text{FeCl}_3\cdot6\text{H}_2\text{O}$ were dissolved together in 50 mL deionized water. Then the mixture was transferred into a 250-mL three-neck round bottom flask and heated to 80 °C under N$_2$ atmosphere. To produce $\text{Fe}_3\text{O}_4$ black precipitate, 25 mL of 30% (w/v) NH$_3$ solution were added into the mixture using a plastic syringe. After 20 min of reaction time, the black precipitate was washed several times with deionized water to remove excess NH$_3$, followed by ethanol and then dried at 60 °C for 24 h.

#### Synthesis of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$

First, 0.50 g of the $\text{Fe}_3\text{O}_4$ was sonicated in a mixture of ethanol and deionized water (80:20) for 10 min. Then under N$_2$ atmosphere, 1 mL of 30% (w/v) NH$_3$ solution and 1 mL of TEOS were added to the mixture under mechanical stirring. After 4 h of reaction time, the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ particles were washed several times with deionized water to remove excess reagents, followed by ethanol to remove the remaining water, and then dried at 60 °C for 24 h.

Chemical modification of the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ surface with $\text{C}_{18}$ groups was carried out under N$_2$ atmosphere by dispersing 0.50 g of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ in pyridine 40 mL$^{-2}$. Then, 1 mL of chloro(dimethyl)octadecylsilane was added to the mixture under continuous sonication for 20 min. The mixture was left at room temperature for 24 h to complete the reaction. The Fe3SO4@SiO2@C18 particles were washed several times with ethanol before drying with N2.
Adsorption capacity, reusability, and storage durability of the synthesized Fe₃O₄@SiO₂@C₁₈.

The adsorption capacity (qₑ) of the synthesized Fe₃O₄@SiO₂@C₁₈ towards PMB–CTAB was studied. A PMB–CTAB solution containing 100 µg P L⁻¹ was prepared by mixing 50 µL of the stock standard o-PO₄³⁻ solution with 1 mL of each reagent (R₁, R₂, and CTAB) and made up with deionized water in a 50-mL volumetric flask. The mixture was left for 15 min to ensure equilibrium PMB–CTAB formation. The adsorption capacity determination was carried out by dispersing 2 mg of Fe₃O₄@SiO₂@C₁₈ in 20 mL of the PMB–CTAB solution for 15 min. After magnetic decantation, the concentration of o-PO₄³⁻ in the aqueous phase was quantified using external standard method. The adsorption capacity (qₑ, µg P g⁻¹) of the synthesized Fe₃O₄@SiO₂@C₁₈ was estimated by using relationship qₑ = (Cᵢ – Cₚ) V/m, where Cᵢ is the initial concentration of o-PO₄³⁻ (µg P L⁻¹), Cₚ is the concentration of o-PO₄³⁻ after magnetic decantation (µg P L⁻¹), V is the volume of the PMB–CTAB solution (L), and m is the mass of the Fe₃O₄@SiO₂@C₁₈ (g).

To investigate the reusability of the synthesized Fe₃O₄@SiO₂@C₁₈, the adsorption capacities of the Fe₃O₄@SiO₂@C₁₈ were compared after a number of uses. The Fe₃O₄@SiO₂@C₁₈ was washed with acidified ethanol, methanol, and deionized water between each usage cycle. Storage durability of the synthesized Fe₃O₄@SiO₂@C₁₈ was investigated by evaluating the adsorption capacity after storage over three months.

Water sample preparations and d-MSPE procedure. Five freshwater samples were collected: three from river, one from canal, and one from tap water. The o-PO₄³⁻ concentration of the samples was determined using the proposed d-MSPE method (Fig. 1). All samples were filtered through 0.45 µm syringe membrane filters (CN-CA, Chromex Scientific, UK) prior d-MSPE.

First, samples were pipetted into a 25-mL volumetric flask: 2.5 mL river water 1, 12.5 mL for river water 2 and 20 mL for the rest of samples, due to the difference analyte concentrations. Follow by 0.5 mL of each reagent (R₁, R₂, and CTAB) were sequentially added. Then, the mixture was left for 2.5 min at room temperature to form the PMB–CTAB ion pair complex before transferring 20 mL of the solution into a 150-mL Erlenmeyer flask which already contain 2 mg of Fe₃O₄@SiO₂@C₁₈. The mixture was shaken using an orbital shaker at 200 rpm for 5 min. The external magnet was used in order to isolate the PMB–CTAB-magnetic particles from the aqueous solution. The PMB–CTAB complex was then eluted from the particles by adding 500 µL of acidified ethanol and shaking for 1 min. After separation of Fe₃O₄@SiO₂@C₁₈ from eluate, the absorbance of PMB–CTAB complex in the acidified ethanol was measured using a UV–Vis spectrophotometer at 800 nm, which is the wavelength of maximum absorbance of the complex solution (Supplementary Materials Fig. S1).

Results and discussion

Characterization of the materials and adsorption capacity. The crystal structure of the Fe₃O₄ material was analysed using XRD. The patterns are shown in Fig. 2(a) revealing five main peaks, which were indexed to the (1 1 1), (2 2 0), (3 1 1), (4 0 0), (2 2 2), (5 1 1), and (4 4 0), confirming the characteristic diffraction peaks of magnetite (Fe₃O₄) (ICDD: PDF 01–071-6336) with a face-centred cubic magnetite structure. Surface functionalization of the Fe₃O₄@SiO₂@C₁₈ material was investigated using FT-IR (Fig. 2(b)). Absorption peaks at 588, 1073, 2852, and 2921 cm⁻¹ representing characteristic vibrations of Fe–O–Fe, Si–O–Si, and C–H bonds of –CH₃ and –CH₂, respectively. This was in good agreement with the results reported in27, and confirmed that Fe₃O₄@SiO₂@C₁₈ had been successfully synthesized.

The magnetic properties, including magnetic hysteresis loops of the Fe₃O₄, Fe₃O₄@SiO₂, and Fe₃O₄@SiO₂@C₁₈ particles, were determined using VSM at room temperature. Figure 3(a) shows the magnetization curves of all samples, with saturation magnetization (Mₛ) values of 49.35 for Fe₃O₄, 46.88 for Fe₃O₄@SiO₂ and 39.19 emu g⁻¹ for Fe₃O₄@SiO₂@C₁₈. The magnetic saturation of the Fe₃O₄ slightly decreased after coating with silica and surface functionalization with C₁₈ molecules. The hysteresis loops of all samples exhibited low coercive field and remanence values, indicating that the synthesized material had a superparamagnetic property at room temperature. The inset images in Fig. 3(a) show good dispersion of the Fe₃O₄@SiO₂@C₁₈ particles in aqueous solution and the separation of the Fe₃O₄@SiO₂@C₁₈ particles from the aqueous phase by the external magnet can simply done within a few minutes. The rapid magnetic response, simple separation from the aqueous phase, and re-dispersion of the Fe₃O₄@SiO₂@C₁₈ particles make it a suitable material for determining o-PO₄³⁻ in water.

The morphology of the Fe₃O₄@SiO₂@C₁₈ particles compared with Fe₃O₄ and Fe₃O₄@SiO₂ particles were next investigated using TEM (Fig. 3(b)). Morphology of Fe₃O₄ was found as slightly irregular shape with an average size of iron oxide about 7.5 ± 0.8 nm. After coating Fe₃O₄ by silica (SiO₂) layer using TEOS and ammonium hydroxide, the size of Fe₃O₄@SiO₂ particles was slightly increased with an average thickness of SiO₂ layer approximately 2.0 ± 0.2 nm. After C₁₈-functionalization to produce Fe₃O₄@SiO₂@C₁₈, agglomeration of the particles with irregular shape was observed. This could be caused by degradation of C₁₈-molecules by using TEM (JEOL, JEM-2010) operated with high-voltage electron (200 kV) operated with high-voltage electron (200 kV). The average median and mean of particles sizes acquired 15 min. After magnetic decantation, the concentration of o-PO₄³⁻ in the aqueous phase was quantified using external standard method. The adsorption capacity (qₑ, µg P g⁻¹) of the synthesized Fe₃O₄@SiO₂@C₁₈ was estimated by using relationship qₑ = (Cᵢ – Cₚ) V/m, where Cᵢ is the initial concentration of o-PO₄³⁻ (µg P L⁻¹), Cₚ is the concentration of o-PO₄³⁻ after magnetic decantation (µg P L⁻¹), V is the volume of the PMB–CTAB solution (L), and m is the mass of the Fe₃O₄@SiO₂@C₁₈ (g).

To investigate the reusability of the synthesized Fe₃O₄@SiO₂@C₁₈, the adsorption capacities of the Fe₃O₄@SiO₂@C₁₈ were compared after a number of uses. The Fe₃O₄@SiO₂@C₁₈ was washed with acidified ethanol, methanol, and deionized water between each usage cycle. Storage durability of the synthesized Fe₃O₄@SiO₂@C₁₈ was investigated by evaluating the adsorption capacity after storage over three months.
Figure 1. Schematic illustration of d-MSPE procedure for determination of $\text{PO}_4^{3-}$ as PMB–CTAB complex using $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$.

Figure 2. (a) XRD patterns of $\text{Fe}_3\text{O}_4$ particles and (b) FT-IR spectra of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{C}_{18}$ particles at room temperature.
314.3 ± 59.4 µg P g⁻¹ (Table S1). The relative standard deviation (RSD) of the mean $q_e$ showed acceptable HOR-RAT $r^2$ precision of 1.7, which indicated that in-laboratory preparation of the Fe₃O₄@SiO₂@C₁₈ was reproducible.

### Influential parameters of dispersive magnetic solid-phase extraction (d-MSPE).

To develop a simple but yield good sensitivity protocol for determination of trace $OPO_4^{3−}$ in freshwater, the key d-MSPE parameters were identified by optimizing one parameter at a time. Following the d-MSPE procedure shown in Fig. 1, 20 mL of standard solution containing 20 µg P L⁻¹ were used to prepare a sample solution. When optimizing the sample volume, a standard solution of 13 nmol P was used.

#### Time and temperature for PMB–CTAB formation.

The extraction efficiency of the Fe₃O₄@SiO₂@C₁₈ for PMB depended on the comprehensiveness coupled of PMB with CTAB. Since PMB is polyanion species, the mole ratio between phosphorus and CTAB was used at 1:18. Precipitation of PMB-CTAB were reported when using large amount of CTAB³⁰ but in our study at 1:18 ratio, the PMB-CTAB ion pairs were in aqueous solution. The optimum time for PMB–CTAB formation at room temperature (25 °C) was investigated by observing the change in absorbance of the extracted PMB-CTAB. Figure 4 showed the relatively steadiness absorbance of the complex over reaction times of 2.5 to 15 min. The steadiness absorbance exhibit that the PMB–CTAB complexes were form completely within 2.5 min. The presence of sufficient ascorbic acid and the trace amount of $OPO_4^{3−}$ are the main reason for the reaction quickness²⁶,³¹.

The molybdenum blue method is temperature dependent. Even though formation of the PMB complex is accelerated as the temperature is increased²⁶,³¹ however the PMB complex also can decompose under high temperature environment²⁶,³². In this work the PMB–CTAB complex is the target specie for Fe₃O₄@SiO₂@C₁₈ not the PMB. So increasing temperature above room temperature even though speed-up the formation of PMB but will not improve the stability of PMB–CTAB ion paired complex formation.

Therefore, reaction time of 2.5 min and reaction at room temperature were chosen to minimize the analysis time and to simplify the analytical procedures.
Type and concentration of eluent. Type and concentration of eluent should be optimized so it can effectively elute the PMB–CTAB complex from Fe₃O₄@SiO₂@C₁₈. Polar organic solvents have been shown to be effective for eluting⁹,¹⁰ the ion pair complex of phosphomolybdic acid and a cationic surfactant in an aqueous medium. Hence, ethanol was chosen for this work because of its compatible polarity, cost, availability and environment friendliness. Figure 5 shows the absorbance of the eluted PMB–CTAB using a mixed ethanol and water. As the concentration of ethanol was increased from 50% to ~ 99% (absolute ethanol), the absorbance, corresponding to the amount of eluted PMB–CTAB, increased. Furthermore, the addition of H₂SO₄ with a fixed final concentration of 0.56 mol L⁻¹ to the ethanol solvent was found to enhance the eluting efficiency. This could be explained by the greater stability of the PMB–CTAB complex under acidic conditions (i.e. acidified ethanol). Polar solvent, H₂SO₄ and deionized water, cannot elute PMB–CTAB complex. Consequently, acidified ethanol was utilized in this work.

Sample solution volume and amount of Fe₃O₄@SiO₂@C₁₈ particles. The effect of the solution volume on extraction efficiency was studied, since it determines the surface-to-volume ratio (contact area) between the solid sorbent and target analyte. Solutions with volumes ranging from 20 to 80 mL and containing a fixed amount of 13 nmol P, PMB–CTAB, were prepared. The quantity of Fe₃O₄@SiO₂@C₁₈ particles for each trial was 2 mg. Figure 6(a) shows that the absorbance values of the extracted PMB–CTAB changed significantly as the sample solution volume was increased. This reduction in absorbance was due to the decreasing in surface-to-volume
ratio between the solution and the Fe₃O₄@SiO₂@C₁₈. A volume of 20 mL was therefore selected to provide a high surface-to-volume ratio.

Since the concentration of \( \text{o-PO}_4^{3-} \) in clean natural water \(^1\) is lower than 50 µg P L\(^{-1}\). To investigate the minimum amount of Fe₃O₄@SiO₂@C₁₈ that adequate for detecting \( \text{o-PO}_4^{3-} \) in clean natural water, 2 mg and 6 mg of Fe₃O₄@SiO₂@C₁₈ were dispersed in 20 mL of 30 µg P L\(^{-1}\) standard \( \text{o-PO}_4^{3-} \) solution. Both 2 mg and 6 mg of Fe₃O₄@SiO₂@C₁₈ yielded not significantly difference in absorbance (Fig. S4). This result suggested that 2 mg of Fe₃O₄@SiO₂@C₁₈ was suffice for determination \( \text{o-PO}_4^{3-} \) in clean natural water sample.

**Extraction time and speed of agitation.** Extraction of PMB–CTAB complexes from sample solution with Fe₃O₄@SiO₂@C₁₈ particles can be accelerated by agitation. Using of an ultra-sonication bath can create high frequencies agitation but also generates heat, which cause decomposition of PMB. The vortex mixer is a convenient method but suitable for low extraction volumes and yield small number of sample extraction throughput unless equip with special accessories. The orbital shaker produces less heat and more practical for a sample volume of 20 mL and was therefore used in this work. The extraction time and speed of shaking were studied in the following experiments.

At 200 rpm agitation speed and extraction times of 1 to 20 min were investigated. As results displayed in Fig. 6(b), the absorbance increased significantly from 1 to 5 min, then reached a plateau. This indicated that the PMB–CTAB was completely extracted by the Fe₃O₄@SiO₂@C₁₈ after 5 min of extraction. This extraction time was selected for investigating the speed of agitation.

The agitation speed determines the dispersion of Fe₃O₄@SiO₂@C₁₈ in the sample solution and can maximize the contact area within a given extraction time. In this work, the agitation speed was in the range of 50 to 300 rpm. Figure 6(c) shows that the absorbance increased significantly as the speed of the shaker was increased from 50 to 100 rpm. Agitation speed over 100 rpm produced no further increase in absorbance, as the extraction of PMB–CTAB by Fe₃O₄@SiO₂@C₁₈ has already completed. An extraction speed of 200 rpm was therefore chosen to maintain the dispersion to maximize.

**Elution time.** To achieve a method with high sample throughput and yet high sensitivity, the optimum time for elution of the PMB–CTAB from the Fe₃O₄@SiO₂@C₁₈ was investigated. Aliquot of 500 µL acidified ethanol was used to elute the enriched PMB–CTAB. Figure 6(d) shows that the elution time of 1–3 min yielded no significantly different results. This indicated that the PMB–CTAB complex was eluted completely within 1 min. An elution time of 1 min was therefore selected.

![Figure 6. Effect of d-MSPE parameters; (a) sample volume, (b) extraction time, (c) agitation speed, and (d) elution time on absorbance signals. Absorbance ± SD, n = 3.](image-url)
addition of thiosulphate \((S_2O_3^{2−})\) pretreatment which has colorless solution 36, false negatives might arise when these two species are present in the sample. Nonetheless, interference from the \(AsO_4^{3−}\) might be tolerated by the proposed d-MSPE tolerate the presence of \(SiO_3^{2−}\) up to 0.001 mg L\(^{−1}\) 34. The limits of interference from \(NaCl\) and \(SO_4^{2−}\) were examined in the studied range. These levels of concentrations are found in polluted waters rather than in unpolluted waters 37,38.

When alkalinity of sample is very low, the pH adjustment of sample could be neglected (pH range of unpolluted freshwater are 6–8). Strong alkali sample might neutralize some of the acid in the reagent solution which placed in highly acidic condition. Hence the acid amount in reagent solution had been compromised for the analysis of \(-PO_4^{3−}\) and \(AsO_4^{3−}\) species using the proposed method, compared with other typical and recommended levels in different types of water.

Silicate (\(SiO_3^{2−}\)) and arsenate (\(AsO_4^{3−}\)) are two major interference ions of molybdenum blue method, because their chemical behavior and formation conditions are similar to those of \(PO_4^{3−}\). The results showed that the proposed d-MSPE tolerated the presence of \(SiO_3^{2−}\) up to 6 mg Si L\(^{−1}\), which is higher than the level found in most natural waters 39. However, in this study \(AsO_4^{3−}\) was found to seriously hinder the analysis when presented even at the very low level of 0.001 mg As L\(^{−1}\) 35. Nonetheless, interference from the \(AsO_4^{3−}\) can be eliminated by addition of thiosulphate (\(S_2O_3^{2−}\)), reducing As (V) to As (III) which is not reactive with molybdenum blue 3,13,26.

The oxidizing potential of hexavalent chromium (Cr (VI)) and nitrite ions (\(NO_2^{−}\)) have been reported to be one of the possible interferences to the PMB reaction 2. The proposed method can tolerate at least 100 mg \(NO_3^{−}\) L\(^{−1}\), which is much higher than the typical level in most fresh waters 39. Interference from the other common species, carbonate (\(CO_3^{2−}\)) 39, the dissolved sodium chloride (\(NaCl\)) 41 and sulfate (\(SO_4^{2−}\)) 42 had been studied. A level of 500 mg L\(^{−1}\) of \(CO_3^{2−}\) did not alter the analytical results. Tolerance limits of interference from \(NaCl\) and \(SO_4^{2−}\) salinities were found to be 2300 mg NaCl L\(^{−1}\) and 750 mg \(SO_4^{2−}\) L\(^{−1}\), respectively. The tolerance levels of \(NaCl\) and \(SO_4^{2−}\) interference in this study indicate our d-MSPE method allow the quantification of \(PO_4^{3−}\) in freshwater but in high salinity waters such as brackish and seawaters, sample pretreatment i.e., dilution, precipitation with Ag\(^+\) will be needed before analysis.

Alkalinity of sample has been considered in the proposed d-MSPE method, since the PMB formation take place in highly acidic condition. Strong alkali sample might neutralize some of the acid in the reagent solution and effect the formation of PMB. Hence the acid amount in reagent solution had been compromised for the alkalinity of sample so the pH adjustment of sample could be neglect (pH range of unpolluted freshwater are between pH of 6–8).

Reusability and storage durability of the \(Fe_3O_4@SiO_2@C_{18}\) particles. The synthesized \(Fe_3O_4@SiO_2@C_{18}\) could be reused at least three times without loss of PMB-CTAB adsorption capability (Fig S5(a)). After three cycles, the adsorption remarkably decreased because of the hydrolysis of the silica bonded phase in the extremely acidic solutions (pH < 1) of the extraction and elution steps 43.

### Table 1. Typical and recommended level of the interference species in comparison with tolerance levels analysed by using the proposed method.

| Species       | Typical/recommended level | Type of water         | References | Examined range | Tolerance level of this work |
|---------------|---------------------------|-----------------------|------------|----------------|-----------------------------|
| Silicate      | 0.368–3.68 mg Si L\(^{−1}\) | River and lake water  | 35         | 4–6 mg Si L\(^{−1}\) | 6 mg Si L\(^{−1}\)         |
|               | 0.184 mg Si L\(^{−1}\)   | Sea water             |            |                |                             |
| Arsenate (VI) | 0.001–0.1 mg As L\(^{−1}\) | Unpolluted water      | 37         | 0.001–0.01 mg As L\(^{−1}\) | 0.001 mg As L\(^{−1}\) |
| Chromium (VI)| 0.0005–0.002 mg Cr L\(^{−1}\) | Surface water        | 37         | 0.05–0.5 mg Cr L\(^{−1}\) | 0.05 mg Cr L\(^{−1}\) |
| Nitrate       | < 0.02 mg NO\(_3^−\) L\(^{−1}\) | Unpolluted water | 38         | 10–70 mg NO\(_3^−\) L\(^{−1}\) | 30 mg NO\(_3^−\) L\(^{−1}\) |
| Nitrate       | < 1 mg NO\(_3^−\) L\(^{−1}\) | Surface water         | 40         | 10–100 mg NO\(_3^−\) L\(^{−1}\) | No interference in the studied range |
| Carbonate     | 25 to 400 mg CO\(_3^2−\) L\(^{−1}\) as alkalinity | Ground and surface water | 40 | 50–500 mg CO\(_3^2−\) L\(^{−1}\) | No interference in the studied range |
| Sulfate       | 0–230 mg SO\(_4^2−\) L\(^{−1}\) | Groundwater         | 35         | 250–1000 mg SO\(_4^2−\) L\(^{−1}\) | 750 mg SO\(_4^2−\) L\(^{−1}\) |
| Sulfate       | 2–250 mg SO\(_4^2−\) L\(^{−1}\) | Lake water          | 41         | 1000–35,000 mg NaCl L\(^{−1}\) | 2300 mg NaCl L\(^{−1}\) |
| Sulfate       | 0–630 mg SO\(_4^2−\) L\(^{−1}\) | River water         | 41         |                |                             |
| Sulfate       | 2700 mg SO\(_4^2−\) L\(^{−1}\) | Sea water           | 41         |                |                             |
| Sodium chloride | Up to 1000 mg NaCl L\(^{−1}\) | Fresh water        | 41         | 1000–35,000 mg NaCl L\(^{−1}\) | 2300 mg NaCl L\(^{−1}\) |
| Sodium chloride | 1000–3000 mg NaCl L\(^{−1}\) | Fresh to brackish water | 41 |                |                             |
| Sodium chloride | 3000–5000 mg NaCl L\(^{−1}\) | Brackish water      | 41         |                |                             |
| Sodium chloride | 5000–35,000 mg NaCl L\(^{−1}\) | Saline             | 41         |                |                             |
| Sodium chloride | 35,000 mg NaCl L\(^{−1}\) and above | Hyper-saline | 41 |                |                             |

**Interference studies.** To determine the tolerance limit for common ions of the d-MSPE system in real sample matrices, different concentrations of possible interferences were spiked into a standard \(o-PO_4^{3−}\) solution (10 µg P L\(^{−1}\)). The absorbance values after addition of these species were compared with the control absorbance obtained from pure standard \(o-PO_4^{3−}\). The tolerance limits in this work were defined as the change in ± 3SD of absorbance of the \(o-PO_4^{3−}\) standard (10 µg P L\(^{−1}\)). Table 1 shows the tolerance levels of various interference species using the proposed method, compared with other typical and recommended levels in different types of water.

The oxidizing potential of hexavalent chromium (Cr (VI)) and nitrite ions (\(NO_2^{−}\)) allows them to interfere in the analysis of \(o-PO_4^{3−}\). Since Cr (VI) can oxidize the ascorbic acid 35 and \(NO_2^{−}\) can oxidizes PMB to PMB product which has colorless solution 36, false negatives might arise when these two species are present in the sample. It was determined that Cr (VI) above 0.05 mg Cr L\(^{−1}\) and \(NO_2^{−}\) above 30 mg NO\(_2^{−}\) L\(^{−1}\) caused significant reduction of absorbance. These levels of concentrations are found in polluted waters rather than in unpolluted waters 37,38. Nitrate ions (\(NO_3^{−}\)) have been reported to be one of the possible interferences to the PMB reaction. The proposed method can tolerate at least 100 mg NO\(_3^{−}\) L\(^{−1}\), which is much higher than the typical level in most fresh waters 39.

Interference from the other common species, carbonate (\(CO_3^{2−}\)) 39, the dissolved sodium chloride (\(NaCl\)) 41 and sulfate (\(SO_4^{2−}\)) 42 had been studied. A level of 500 mg L\(^{−1}\) of \(CO_3^{2−}\) did not alter the analytical results. Tolerance limits of interference from \(NaCl\) and \(SO_4^{2−}\) salinities were found to be 2300 mg NaCl L\(^{−1}\) and 750 mg \(SO_4^{2−}\) L\(^{−1}\), respectively. The tolerance levels of \(NaCl\) and \(SO_4^{2−}\) interference in this study indicate our d-MSPE method allow the quantification of \(o-PO_4^{3−}\) in freshwater but in high salinity waters such as brackish and seawaters, sample pretreatment i.e., dilution, precipitation with Ag\(^+\) will be needed before analysis.
The storage durability of the Fe$_3$O$_4$@SiO$_2$@C$_{18}$ particles was measured from the adsorption capacity of a representative batch. Fig S5(b) shows the adsorption in five cycles conducted over three months. These results clearly demonstrated the stability of the particles at least three months when stored in the desiccator at ambient temperature, as no reduction in adsorption capability was observed.

**Analytical performance.** Under optimal conditions, the linear range of the proposed d-MSPE method was 1.0–30.0 µg P L$^{-1}$ with a determination coefficient ($r^2$) of 0.9925 (Fig. S6). The range covered the target o-Po$_4^{3−}$ levels in unpolluted water samples. The limit of detection (LOD) and limit of quantification (LOQ), calculated using 3 times and 10 times the standard deviation (SD) of the reagent blank divided by the slope of the calibration curve, were 0.3 and 1.0 µg P L$^{-1}$, respectively. The attained LOD was satisfactory for detection of low levels of o-Po$_4^{3−}$ in unpolluted waters. The sensitivity enrichment was attained by calculating the slopes ratio of the calibration curve before and after d-MSPE (Absorbance = (0.0007 ± 0.0000)[P] + (0.0024 ± 0.0003); $r^2$ 0.999 and Absorbance = (0.0224 ± 0.0007)[P] + (0.0976 ± 0.0148); $r^2$ 0.998). A 32-fold enhancement was found after preconcentration using the proposed method. Reliability of the proposed method was evaluated by using the relative standard deviation (RSD) among six extraction batches, the results were 3.70% at 6.0 µg P L$^{-1}$ and 2.49% at 25.0 µg P L$^{-1}$. These %RSDs results indicated that the proposed method will yield sufficiently analytical precision.

We compared our method with the thirteen previously reported shown in Table 2. Comparison with d-MSPE method, LOD and linear range are comparable, but our method used shorter analysis time. Comparison when using bare Fe$_3$O$_4$ as sorbent even though much simpler than Fe$_3$O$_4$@SiO$_2$@C$_{18}$ but lack of acid tolerance has made the number of reusability become limited not to mention less reproducibility. Moreover, since bare Fe$_3$O$_4$ lack of specificity hence substantial amount of particles was needed and require extensive time for extraction. Compare with the nonmagnetic SPE methods and others, our method offers shorter analysis time than that of many methods. Relatively simpler than most methods, since our method doesn’t require heating or cooling during the blue phase complex formation or the extraction and preconcentration steps. The magnetic property of the Fe$_3$O$_4$@SiO$_2$@C$_{18}$ delivers fast collection of the solid phase not only with very simple procedures but also minimum loss of analytes.

**Method validation and application to water samples.** The developed d-MSPE method was validated by analysis of a certified reference material (CRM). The experimental mean value of 0.764 ± 0.0319 mg P L$^{-1}$ (n = 3) was obtained for the CRM values of 0.752 ± 0.0140 mg P L$^{-1}$. Statistical analysis $t$-test shows insignificantly difference between the certified value and the experimental mean value at 95% confidence level ($t_{	ext{stat}}$ Table 2. Comparison of analytical performance of the proposed d-MSPE method with other extraction/preconcentration methods for colorimetric determination of o-Po$_4^{3−}$ in water samples. LLE: liquid–liquid extraction; CPE: cloud-point extraction; SDME: suspended droplet micro-extraction; DLLME: dispersive liquid–liquid micro-extraction-solidified floating organic drop micro-extraction; EISE: electrostatically induced stoichiometric extraction; VA-NADES-ME: vortex-assisted natural deep eutectic solvent micro-extraction; V A-SS-DLLME: vortex-assisted based supramolecular solvents-dispersive liquid–liquid micro-extraction; SPE: solid-phase extraction; d-SPE: dispersive solid-phase extraction; TEPA-NCMs: Tetraethylenepentamine-functionalized nano-size meterials; FIA: Flow injection analysis; SIA: Sequential injection analysis; FA: flow analyzer; MIBK: Methyl isobutyl ketone; NADES: natural deep eutectic solvents; NP4EO: non-ionic nonylphenol tetra-ethoxylate; HLB: hydrophilic-lipophilic balance; HPLC: high performance liquid chromatography; ODS: octadecylsilane; LOD: limit of detection; n.r.: Not reported; $*$: in-laboratory synthesized sorbent; $\S$: commercial sorbent; $\S$: requires heating to prepare NADES.

- **Method**
  - CPE
  - SPE
  - DLLME
  - EISE
  - VA-NADES-ME
  - VA-SS-DLLME

- **Extractant phase**
  - CPE: Triton X-45
  - SPE: non-magnetic sorbent
  - DLLME
  - EISE
  - VA-NADES-ME
  - VA-SS-DLLME

- **Sample solution volume (mL)**
  - CPE: 10
  - SPE: 10
  - DLLME: 1
  - EISE: 10
  - VA-NADES-ME: 0.05
  - VA-SS-DLLME: 0.06

- **LOD (µg P L$^{-1}$)**
  - CPE: 80.7
  - SPE: 0.5
  - DLLME: 0.19
  - EISE: 10
  - VA-NADES-ME: 0.05
  - VA-SS-DLLME: 0.06

- **Enhancement factor**
  - CPE: n.r.
  - SPE: 32.6
  - DLLME: 325
  - EISE: 50
  - VA-NADES-ME: 71
  - VA-SS-DLLME: 50

- **Linear range (µg P L$^{-1}$)**
  - CPE: 80.7–970.5
  - SPE: 1–125
  - DLLME: 1.55–46.6
  - EISE: 5–200
  - VA-NADES-ME: 2–80
  - VA-SS-DLLME: 0.5–28.0

- **Need of cooling or heating?**
  - CPE: Yes
  - SPE: Yes
  - DLLME: No
  - EISE: No
  - VA-NADES-ME: Yes
  - VA-SS-DLLME: No

- **Analysis time (min)**
  - CPE: > 20
  - SPE: 20
  - DLLME: 18
  - EISE: 18
  - VA-NADES-ME: 13
  - VA-SS-DLLME: 10

- **References**
  - CPE: Triton X-45
  - SPE: non-magnetic sorbent
  - DLLME: suspended droplet micro-extraction
  - EISE: electrostatically induced stoichiometric extraction
  - VA-NADES-ME: vortex-assisted natural deep eutectic solvent micro-extraction
  - VA-SS-DLLME: vortex-assisted based supramolecular solvents-dispersive liquid–liquid micro-extraction
  - d-SPE: dispersive solid-phase extraction
  - TEPA-NCMs: Tetraethylenepentamine-functionalized nano-size materials
  - FIA: Flow injection analysis
  - SIA: Sequential injection analysis
  - FA: flow analyzer
  - MIBK: Methyl isobutyl ketone
  - NADES: natural deep eutectic solvents
  - NP4EO: non-ionic nonylphenol tetra-ethoxylate
  - HLB: hydrophilic-lipophilic balance
  - HPLC: high performance liquid chromatography
  - ODS: octadecylsilane
  - LOD: limit of detection
  - n.r.: Not reported
  - $*$: in-laboratory synthesized sorbent
  - $\S$: commercial sorbent
  - $\S$: requires heating to prepare NADES.
Table 3. Analyzed concentrations and recovery percentages of $\text{o-P}_3\text{PO}_4$ in water samples. n.d.: not detectable (< LOD 0.3 µg P L$^{-1}$).

| Sample (pH) | Analyzed o-P$3\text{PO}_4$ concentration ± SD, µg P L$^{-1}$ (n = 3) | Recovery, % |
|-------------|-------------------------------------------------|-------------|
| River water 1 (pH 7.52) | 60.5 ± 2.4 | 95.5 ± 21.5 |
| River water 2 (pH 6.68) | 12.0 ± 0.8 | 95.5 ± 14.6 |
| River water 3 (pH 6.72) | 2.3 ± 0.6 | 101.5 ± 15.6 |
| Canal water (pH 8.53) | n.d | 100.0 ± 1.8 |
| Tap water (pH 7.38) | 1.1 ± 0.1 | 89.1 ± 12.0 |

0.64 < $t_{\text{crit}}$ < 4.30. This confirmed accuracy of the developed d-MSPE method. The method was finally used to analyze o-P$3\text{PO}_4$ in the five real samples (three river, one canal, and one tap water). Table 3 shows the o-P$3\text{PO}_4$ concentrations in the water samples and recovery percentages analyzed using the proposed method. High recovery percentages ranging from 89.1 to 101.5% were obtained (Supplementary Materials Table S2), indicating that no matrices affected detection in real applications.

Conclusions

In this work, we developed a simple, fast, and highly sensitive method for trace analysis of o-P$3\text{PO}_4$ in unpolluted freshwater by using silica coated magnetite functionalized with octadecyl (C$_{18}$)silane (Fe$_3$O$_4$@SiO$_2$@C$_{18}$). The Fe$_3$O$_4$@SiO$_2$@C$_{18}$ was synthesized and characterized to confirm the final product. The synthesized particles were used for extraction and preconcentration of o-P$3\text{PO}_4$ through hydrophobic interaction with PMB–CTAB ion pair complexes in the water. The proposed Fe$_3$O$_4$@SiO$_2$@C$_{18}$ d-MSPE method was operated at room temperature, using generic glassware and apparatus, short analysis time, simple extraction procedures, high sensitivity and detecting o-P$3\text{PO}_4$ at concentrations as low as 0.3 µg P L$^{-1}$, with a limit of quantification of 1.0 µg P L$^{-1}$. The analysis was highly precise (RSD < 3.70%) and accurate (recovery 89.1–101.5%). Interference studies suggested the proposed method can tolerate a range of species commonly found in ordinary unpolluted freshwater samples. The Fe$_3$O$_4$@SiO$_2$@C$_{18}$ can store in desiccator at ambient temperature for over three months without impairing the adsorption capacity. These results emphasis the potential of our method for trace analysis of o-P$3\text{PO}_4$ that could apply for various environmental samples.

Received: 20 June 2021; Accepted: 16 November 2021
Published online: 29 November 2021

References

1. Rehman, A., Yaqoob, M., Waseem, A., Nabi, A. & Khan, M. A. Determination of phosphate in freshwater samples by flow-injection with lucigenin chemiluminescence. Int. J. Environ. Anal. Chem. 90, 1119–1129 (2010).
2. Worsfold, P., McKelvie, I. D. & Monbet, P. Determination of phosphorus in natural waters: A historical review. Anal. Chim. Acta. 918, 8–20 (2016).
3. Katsaounos, C. Z., Giokas, D. L., Vlessidis, A. G., Paleologos, E. K. & Karayannis, M. I. The use of surfactant-based separation techniques for monitoring of orthophosphate in natural waters and wastewater. Sci. Total Environ. 305, 157–167 (2003).
4. Alkhani, A. & Norooz-aal, R. Cloud point extraction for the spectrophotometric determination of phosphorus(V) in water samples. J. Hazard. Mater. 167, 752–755 (2009).
5. Pena-Pereira, E. et al. Directly suspended droplet microextraction in combination with microvolume UV–vis spectrophotometry for determination of phosphate. Talanta 85, 1100–1104 (2011).
6. Zaruba, S., Vishnikin, A. B. & Andrushc, V. Application of solidification of floating organic drop microextraction for inorganic anions: Determination of phosphate in water samples. Microchem. J. 122, 10–15 (2015).
7. Gissawong, N., Sanwut, S. & Srijaranai, S. The alternative use of layered double hydroxides as extraction medium coupled with microcomplexation for determination of phosphate in water samples. Spectrochim. Acta. A 173, 994–1000 (2017).
8. Najafi, A. & Hashemi, M. Vortex-assisted natural deep eutectic solvent microextraction using response surface methodology optimization for determination of orthophosphate in water samples by molybdenum blue method. J. Sep. Sci. 40, 3102–3109 (2019).
9. Najafi, A. & Hashemi, M. Feasibility of liquid phase microextraction based on a new supramolecular solvent for spectrophotometric determination of orthophosphate using response surface methodology optimization. J. Mol. Liq. 297, 117454 (2020).
10. Zhang, Y., Pan, S., Shen, H. & Hu, M. Amino-functionalized Nano-size Composite Materials for Dispersive Solid-Phase Extraction of Phosphate in Water Samples. Analy. Sci. 28, 887–892 (2012).
11. Asaoka, S. et al. An online solid phase extraction method for the determination of ultratrace level phosphate in water with a high performance liquid chromatograph. Chem. Geol. 380, 41–47 (2014).
12. Wu, W., He, Q. & Jiang, C. Magnetic Iron Oxide Nanoparticles: Synthesis and Surface Functionalization Strategies. Nanoscale Res. Lett. 3, 397–415 (2008).
13. Su, C. Environmental implications and applications of engineered nanoscale magnetite and its hybrid nanocomposites: A review of recent literature. J. Hazard. Mater. 322, 48–84 (2017).

https://doi.org/10.1038/s41598-021-02516-4
18. Jang, J. H. & Lim, H. B. Characterization and analytical application of surface modified magnetic nanoparticles. Microchem. J. 94, 148–158 (2010).
19. Caon, N. B., Cardoso, C. D. S., Faita, F. L., Vitali, L. & Parize, A. L. Magnetic solid-phase extraction of tricosanol from water using n-octadecyl modified silica-coated magnetic nanoparticles. J. Environ. Chem. Eng. 8, 104003. https://doi.org/10.1016/j.jece.2020.104003 (2020).
20. Pilnaj, D. et al. C18-functionalized Fe3O4/SiO2 magnetic nano-sorbent for PAHs removal from water. Environ. Technol. Innov. 24, 101905. https://doi.org/10.1016/j.eti.2021.101905 (2021).
21. Sha, Y., Deng, C. & Liu, B. Development of C18-functionalized magnetic silica nanoparticles as sample preparation technique for the determination of ergosterol in cigarettes by microwave-assisted derivatization and gas chromatography/mass spectrometry. J. Chromatogr. A 1198–1199, 27–33 (2008).
22. Yu, P. et al. Development of superparamagnetic high-magnetization C18-functionalized magnetic silica nanoparticles as sorbents for enrichment and determination of methylprednisolone in rat plasma by high performance liquid chromatography. Anal. Chem. Acta. 678, 50–55 (2010).
23. Jiang, C. et al. Removal of sudan dyes from water with C18-functional ultrafine magnetic silica nanoparticles. Talanta 89, 38–46 (2012).
24. Jiang, C. et al. Application of C18-functional magnetic nanoparticles for extraction of aromatic amines from human urine. J. Chromatogr. B 947–948, 49–56 (2014).
25. Giakissikli, G. & Anthemidis, A. N. Automated magnetic sorbent extraction based on octadecylsilane functionalized magnetite magnetic particles in a sequential injection system coupled with electrothermal atomic absorption spectrometry for metal determination. Talanta 110, 229–235 (2013).
26. Nagul, E. A., McKelvie, I. D. & Kolev, S. D. The nature of the salt error in the Sn(II)-reduced molybdenum blue reaction for determination of dissolved reactive phosphorus in saline waters. Anal. Chem. Acta. 896, 120–127 (2015).
27. Li, Z. et al. Preparation of magnetic core mesoporous shell microspheres with C18-modified interior pore-walls for fast extraction and analysis of phthalates in water samples. J. Chromatogr. A 1218, 6232–6239 (2011).
28. Stenn, K. & Bahr, G. E. Specimen damage caused by the beam of the transmission electron microscope, a correlative reconsideration. J. Ultrastruct. Res. 31, 526–550 (1970).
29. AOAC. AOAC Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals. https://members.aoac.org/AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf. (2002).
30. Ibnul, N. K. & Tripp, C. P. A solventless method for detecting trace level phosphate and arsenate in water using a transparent membrane and visible spectroscopy. Talanta 225, 122023. https://doi.org/10.1016/j.talanta.2020.122023 (2021).
31. Sjosteen, A. & Blomqvist, S. Influence of phosphate concentration and reaction temperature when using the molybdenum blue method for determination of phosphate in water. Water Res. 31, 1818–1823 (1997).
32. Pai, S., Wang, T., Fang, T. & Jiann, K. Effect of heating on the color formation reaction in the Murphy and Riley method for the determination of phosphate in natural waters. J. Environ. Anal. Chem. 2, 139–142 (2015).
33. Zaporozhets, O. A., Bas, J. P., Kachan, I. A., Zinko, L. S. & Davydov, V. I. Solid-phase spectrophotometric and test determination of silicate in natural water. Talanta 90, 85–90 (2012).
34. Shuvaeva, O. V., Koschcheeva, O. S. & Beisel, N. F. Arsenic speciation in water by high-performance liquid chromatography with electrothermal atomic absorption detection. J. Anal. Chem. 57, 1037–1041 (2002).
35. Xu, X., Li, H., Li, X. & Gu, J. Reduction of hexavalent chromium by ascorbic acid in aqueous solutions. Chemosphere 57, 609–613 (2004).
36. Zatar, N. A., Abu-Eid, M. A. & Eid, A. F. Spectrophotometric determination of nitrate and nitrite using phosphomolybdenum blue complex. Talanta 50, 819–826 (1999).
37. WHO. Chromium in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. http://www.who.int/water_sanitation_health/dwq/chemicals/chromium.pdf. (2003).
38. Yaspoob, M., Nabi, A. & Worsfold, P. J. Determination of nanomolar concentrations of phosphate in freshwaters using flow injection with luminol chemiluminescence detection. Anal. Chim. Acta. 510, 213–218 (2004).
39. US EPA. 5.7 Nitrates. https://archive.epa.gov/water/archive/web/html/vms57.html. (2012).
40. Khan, N., Hussain, S. T., Saboor, A., Jamila, N. & Kim, K. S. Physicochemical investigation of the drinking water sources from Mardan, Khyber Pakhtunkhwa, Pakistan. J. Phys. Sci. 8, 1661–1671 (2001).
41. South Australia EPA. Salinity. http://www.epa.sa.gov.au/environmental_info/water_quality/threats/salinity. (2015).
42. WHO. Sulfate in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality http://www.who.int/water_sanitation_health/dwq/chemicals/sulfate.pdf. (2004).
43. Claessens, H. A. & van Straten, M. A. Review on the chemical and thermal stability of stationary phases for reversed-phase liquid chromatography. J. Chromatogr. A 1060, 23–41 (2004).

Acknowledgements
The authors gratefully acknowledge facilities support from the Central Scientific Instrument Center (CISC) and Department of Chemistry, Faculty of Science and Technology, Thammasat University, and financial support from the Thailand National Nanotechnology center (NANOTEC), NSTDA [P 1450172 and P 1750205] and Thammasat University Research Fund [Contract No. SciGR18/2563]. Special acknowledgement is given to Prof. Supapan Seraphin (NSTDA Professional Authorship Center, PAC) for her fruitful comments.

Author contributions
Authors’ contributions Conceptualization, funding, and acquisition: P.P. and K.S. Writing: V.P., P.P. and K.S. Review: S.B. and K.S. Analysis: V.P., S.M. and D.V. All authors agreed position and publication.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-02516-4.

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