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Ion-pair single-drop microextraction with ATR-FTIR determination of phosphate in water samples

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A simple, rapid and green ion-pair single-drop microextraction procedure followed by attenuated total reflectance-Fourier transform infrared technique has been developed for the analysis of phosphate in water samples. This method is based on the extraction of the phosphate-cetyltrimethylammonium bromide ion-pair by the single-drop extraction procedure. The linear range for calibration plot of phosphate is 1-900 ng mL^{-1}, with good correlation coefficient (r^2 = 0.998). The limit of detection, limit of quantification, standard deviation and relative standard deviation of six replicate measurements are respectively 0.34 ng mL^{-1}, 1.12 ng mL^{-1}, 0.001 and 0.94-3.36%. The significant parameters such as selection of solvent, their volume, ion-pair reagent and their concentration, extraction time, stirring rate, sample pH, extraction temperature and effect of salt concentration are studied and optimized. The present method is successfully applied for the quantification of phosphate in water samples with minimal solvent consumption and sensitivity as compared with the conventional methods.

Keywords: Analytical chemistry, Phosphate, Ion-pair complex, Ion-pair, Single drop microextraction, Attenuated total reflectance spectroscopy, Fourier transform infrared spectroscopy, Water analysis

Phosphorus is an essential nutrient for plants and animals because it plays an important role in their growth, metabolism and reproduction. It is present at low concentrations in the earth's crust[1]. Phosphorus can exist in three forms when present in water, viz., orthophosphate, condensed phosphate (pyro-, meta- and poly-) and organic phosphorus. Dissolved phosphorus is mainly present in the form of orthophosphate[2]. Excess phosphate leads to over-enrichment and nutrient pollution in the water body due to the excessive growth of aquatic plants and algae. A reduction in dissolve oxygen in water bodies is caused by algal bloom and leads to eutrophication[3], which results in increase of biomass, disruption of aquatic life cycles and fish death. It may be harmful for human beings and animals, as some algal blooms produce toxins, contaminating water and sea products[4-6].

There are natural sources of phosphate such as phosphate ore (phosphorites and apatites), sediments which are naturally found in surface water[7]. In addition to the above sources, various anthropogenic sources such as agricultural effluents (fertilizer, animal feed run off), industry (detergents) and sewage[8,9], effluents containing phosphate from wastewater treatment plants and industrial plants[10, 11] also lead to excess of phosphates in the water. All effluents run off in water body and cause eutrophication due to excess phosphate levels and reduced water quality[12]. In view of the above, a number of different analytical methods have been applied for the analysis of phosphate. Colorimetric technique has been used as a standard method for measuring soluble phosphate in water, in which blue color heteropoly complex (phosphomolybdenum complex) is formed in acidic medium by reduction with various reducing agents[13,14]. A colorimetric analyzer based on the mobile phone camera has been also applied in 2015 by Moonrungsee[15]. This method is difficult to adopt for on-line measurements, in which specific reagents are used and require safe disposal. Therefore, many electrochemical sensor methodologies have been used for the sensing of phosphate ions in various systems including cysteine-capped cadmium sulfide quantum dots and silver nanoparticles[16], nanocomposite of immobilized magnetic nanoparticles on cationic polymer (Fe_3O_4-NPs)[17], carbon black nanoparticle modified screen printed electrode sensor[18], gold nanoparticles with luminescence probe ($[Tb\text{-}(EDTA)]^-$)[19].

Fluorescence sensing[20, 21], ion chromatography[22], cross injection analysis[23], ion exchange chromatography[24] and chemiluminescence[25] have also been carried out for phosphate determination. Various extraction techniques such as liquid-liquid extraction[26], directly suspended droplet microextraction (DSDME)[27], cloud point extraction[28], electrostatically induced stoichiometric extraction (EISE)[29] and dispersive liquid-liquid microextraction based on the solidification of a floating organic drop (DLLME-SFO)[30] have been used for the phosphate extraction in various samples.

However, these methods possess some drawbacks such as high cost, carry-over effects and high solvent volume. Therefore, a simple, rapid, cost effective, low solvent volume method ion-pair single-drop microextraction (SDME) has been developed with attenuated total reflectance-Fourier transform infrared (ATR-FTIR) technique for the determination of phosphate. In this method, the ion-pair formed of the analyte with ion-pair (IP) reagent in the aqueous sample was extracted into the single-drop of ethyl acetate as the organic solvent and the extraction solvent enriched with ion-pair was analyzed by direct ATR-FTIR technique. The various parameters such as selection of IP reagent and their concentration, solvent and their volume, pH, temperature of solution, stirring rate, stirring time and salt addition, have been optimized.

Experimental

All the reagents used were of analytical reagent grade. A 1000 ng mL^{-1} phosphate stock solution was prepared by dissolving an appropriate amount of sodium dihydrogen phosphate in ultrapure water. The working solutions were prepared by the appropriate dilution of the standard solution. The stock solution of CTAB (1 mM) was prepared by dissolving the appropriate amount of CTAB in ultrapure water, and the several standard solutions were prepared by the dilution of stock solution of CTAB. High quality organic compounds, viz., butanol, carbon tetrachloride (CCl_4), ethyl acetate, methyl isobutyl ketone (MIBK), octane and toluene (Merck, Darmstadt, Germany) was tested as solvent in the present work. The different surfactants employed in the present work were purchased from Sigma Aldrich (AR grade, ≥ 99%).

The attenuated total reflectance-Fourier transform infrared spectrometer (ATR-FTIR) equipped with zinc selenide (ZnSe) crystal (model: Nicolet iS10, Thermo Fisher Scientific Instrument, Madison, USA) was used for the determination of phosphate in the water samples. The detector used in the present work was deuterated L-alanine doped triglycine sulfate (DLaTGS). The spectral scans were recorded in the range of 4000-400 cm^{-1} with nominal spectral resolution at 4 cm^{-1}. Sartorius electronic balance (model CP225D, AG Gottingen, Germany) was used for weight measurements of all chemicals. Micropipette, GalaxoSmithKline Pharmaceutical Ltd, Finland was employed for measuring liquid volumes. Ultra-pure water from Thermo Fisher Scientific Barnstead Smart2pure ultrapure water system with conductivity 18.2Ω was used for solution preparation. All glasswares were cleaned by ultrasonic cleaning bath (PCI analytics Pvt. Ltd, India, model 3.5L100H/DIC) using mild detergent to reduce the possible errors. Systronics digital pH meter was used for the measurement of the pH value. The reaction solution was mixed using 5 MLH magnetic stirrers from Remi Equipment Pvt. Ltd India.

The water samples such as ground water, tap water and agricultural water from the different sampling sites were collected for the ion-pair SDME-ATR-FTIR determination of phosphate. The above water samples were collected from the different sampling points of the industrial areas of the Raipur city. The collected water samples were stored in a Teflon screw capped bottles in cool and dry place to avoid any contamination. All water samples were filtered using a 0.45 µm membrane filter before to use.

Aliquot of 2 mL standard solution containing 10 ng mL^{-1} of phosphate was placed in a 10 mL extraction vial for SDME procedure. The pH was adjusted by using 0.1 N HCl and 0.1 N NaOH solution. To this 2 mL IP reagent (CTAB) was added and the extraction vial was sealed with a polytetrafluoroethylene (PTFE) coated silicon septum and placed on a magnetic stirrer. The microsyringe (Hamilton manual injection microsyringe, 10 µL) was rinsed with the extracting organic solvent (ethyl acetate) several times for removal of contaminants and air bubbles, and then, 10 µL of extracting organic solvent was taken in it. The ion-pair between phosphate and cetyltrimethylammonium bromide was formed due to interaction between the electron pair of PO_4^{3-} and positively charged part of cationic surfactant, CTAB. In the experimental setup the syringe was kept at a fixed height with the help of clamps and stand. The needle tip was then inserted into the stirring solution through the septum of the vial, and 4 µL of extracting solvent was squeezed out forming an organic drop at the tip of the needle of microsyringe. The solution was stirred for 15 min (500 rpm) at room temperature for the complete ion-pair. After 15 min, the microdrop was drawn back into the microsyringe and the needle tip was wiped with tissue to remove contamination. The microdrop containing the analyte extract was taken directly for the ATR-FTIR analysis. The schematic representation of SDME setup with ATR-FTIR is shown in Fig. S1 (Supplementary data).

The FTIR was purged with >99.99% analytical grade dry nitrogen gas using the iS10 iZ10 external

purge kit (Thermo Fisher Scientific) to minimize atmospheric obscure peaks of water vapor and carbon dioxide. Then the extracted microdrop containing ion-pair of phosphate with cationic surfactant was put directly on the zinc selenide (ZnSe) crystal of ATR-FTIR accessory. Spectral scanning was recorded after the vaporization of solvent applying optimum instrumental conditions the optimum instrumental conditions are given in Table S2 (Supplementary data).

Results and discussion

Single drop microextraction (SDME) is an analytical technique in which the analyte is transferred from aqueous phase (donor phase) to a microdrop of organic phase (acceptor phase). In order to perform the ion-pair single-drop microextraction of phosphate, many parameters such as IP reagent and their concentration, selection of solvent and their volume, effect of extraction time, pH, stirring rate, extraction temperature and salt concentration were studied and optimized for best extraction efficiency (Fig. S2, Supplementary data).

The IP reagent forms an ion-pair with oppositely charged species in the SDME process. The oppositely charged species enables higher partition coefficient in comparison with the native species. In this work, various cationic surfactants including decyltrimethylammonium bromide (DTAB), dodecyltrimethylammonium bromide (DDTAB), myristyltrimethylammonium bromide (MTAB) and cetyltrimethylammonium bromide (CTAB) with different carbon chain length and geometry were tested as IP reagent. Ion-pair formation of analyte with surfactant depends on the hydrophobicity nature of it. CTAB was found to have the highest relative peak area due to strongest hydrophobicity nature from longest alkyl chain at the nitrogen atom[31]. Thus, CTAB was taken as an IP reagent in all experiments. The effect of concentration of IP reagent was investigated for maximum SDME efficiency in the range 0.1–1 mM CTAB. The high extraction efficiency was observed at 0.7 mM concentration. Hence, 0.7 mM CTAB concentration was applied for further experiments.

The effect of extraction time on the extraction efficiency was studied in the range of 2–20 min. With the increase in extraction time absorbance increased up to 15 min., after which there was no further increase. Maximum sensitivity is obtained in SDME after equilibrium between aqueous and organic phase. However, in this method, for determining the optimum extraction time, drop dislodgement (depletion), and not the equilibrium, must be considered. At higher time (i. e., >15 min.), the drop depletion increased. Hence, 15 minutes was chosen as the optimized extraction time. The stirring speed is a significant factor for extraction efficiency. The extraction efficiency is increased with the increasing of the stirring speed. The stirring speed was performed 50-600 rpm. After 600 rpm the drop fell down. Therefore, 500 rpm was selected for the experimental stirring rate.

The ion-pair of phosphate with cationic surfactant is dependent on the pH of the solution. To study the effect of pH on the complex formation, the experiments were carried out at pH levels ranging from 1–7 using 0.1 N HCl and 0.1 N NaOH. It was found that the best SDME efficiency was obtained at pH 4 (Fig. S2(e), Supplementary data). At higher pH, the absorbance was decreased and reduced extraction efficiency at more alkaline pH range. Thus, on the basis of extraction efficiency, a pH 4 was used.

The extraction efficiency increases on increasing temperature due to fast mass transfer of analyte. In this work, extraction in the temperature range of 20–60 ºC was studied. The extraction efficiency increased with temperature, but high temperature caused evaporation of solvent drop and led to formation of air bubbles. Hence, the room temperature was applied for all analysis.

The effect of the ionic strength on the SDME efficiency was investigated by the addition of NaCl in the range of 0-3 g to the sample solution. The extraction of analyte was restricted by the addition of salt, because it changed the physical properties of extraction film and diffusion rates of analyte into the drop was reduced (Fig. S2(f), Supplementary data). The effect of addition of NaCl significantly affects the SDME of phosphate. Hence, no salt was added in further experiments.

The selection of suitable solvent is a key parameter in the sensitivity, selectivity, precision and accuracy of the SDME procedure. The solvent should have the following properties, viz., low water solubility, high affinity towards analyte, proper viscosity, good drop stability when stirred and free from toxicity. Towards this, six organic solvents, butanol, carbon tetrachloride (CCl_4), ethyl acetate, methyl isobutyl ketone (MIBK), octane and toluene were tested. The final selection of solvent was based on extraction

efficiency of the solvent (Fig. S2(g), Supplementary data). With respect to high absorbance, ethyl acetate has higher extraction efficiency, and hence was chosen for further experiments.

The instability of the microdrop is a weakness of SDME technique; hence the effect of drop volume (1-5 µL) on the extraction efficiency was also evaluated. Generally, analyte gets into the drop through the diffusion process. When the drop volume is large, the analyte takes longer time to reach the equilibrium. When the drop size exceeds a certain volume, it falls due to its dislodgement due to gravity. Figure S2(h) (Supplementary data) shows the drop volume of 4 µL to be optimum for this study.

The characteristic IR absorption bands for phosphate ion available in literature[32,33], were used for the qualitative detection of phosphate by ATR-FTIR spectra in the present work. The characteristic IR absorption bands for phosphate ion were checked by standard samples of phosphate salts of sodium, potassium and magnesium. The vibration peaks for phosphate are 1092-1048 cm^{-1} (υ_3) assigned to the triply degenerated anti-symmetric P-O stretching, 963 cm^{-1} (υ_1) corresponding to non-degenerate symmetric P-O stretching, 603 cm^{-1} and 571 cm^{-1} (υ_4) corresponding to triply degenerated O-P-O bending and 474 cm^{-1} (υ_2) assigned to the compounds of doubly degenerate O-P-O bending mode without giving any information about associated cations.

Phosphate (PO_4^{3-}) has tetrahedral symmetry with T_d point group. When PO_4^{3-} is protonated and form HPO_4^{2-}, the symmetry is reduced from T_d to $C_3\upsilon$, therefore triply degenerate υ_3 vibration splits into two bands at 1078 and 990 cm^{-1} and υ_1 at 850 cm^{-1}. In addition, the formation of $H_2PO_4^-$, reduces the symmetry from $C_3\upsilon$ to $C_2\upsilon$. Therefore, υ_3 vibration splits into three bands at 1159.06, 1077 and 940 cm^{-1}, and υ_1 at 875 cm^{-1}. Thus four bands appear for this species (NaH_2PO_4), which has been used as a standard compound for this method[34].

The IR spectra of pure form of phosphate in the form of sodium dihydrogen phosphate and cetyltrimethylammonium bromide were recorded by ATR-FTIR. The spectra of phosphate extracted with cetyltrimethylammoniumbromide were also recorded (Fig. 1a, b & c). No change is seen in the position of spectral peaks of phosphate in the extracted form, apart from the few spectral peaks due to the presence of cetyltrimethylammoniumbromide. The peaks at 1240.62, 1159.06, 1077.2, 1037.68, 940.42 and 904.26 cm^{-1}

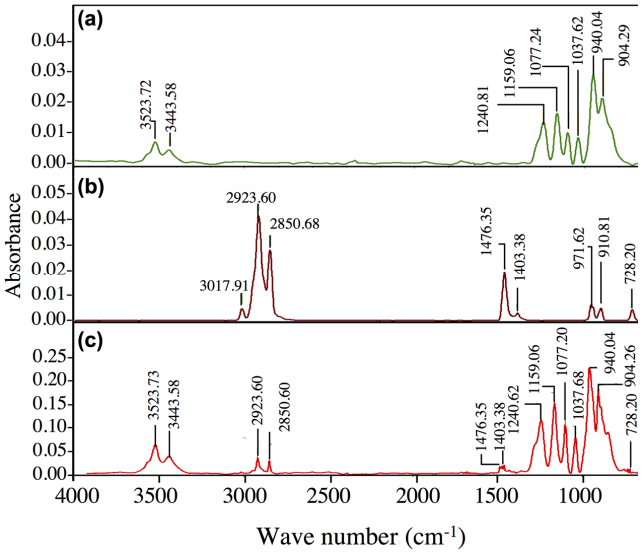

Fig. 1 — ATR-FTIR spectra of (a) phosphate in pure form, (b) cetyltrimethylammonium bromide, and, (c) ion-pair of phosphate with CTAB formed in SDME.

observed for the pure phosphate were seen exactly at the same position for the PO_4^{3-}-CTA^+ ion-pair.

The strongest (sharp and intense) absorption band at 1159.06 cm^{-1} for the asymmetric P-O stretching (υ_3) was chosen for the phosphate determination in this method. The spectral range for the base correction of qualification of phosphate was 1250-900 cm^{-1}. The analyses have been carried out by calibration curve method, with a regression equation representing the relationship between the peak intensity (peak height) or peak area in absorbance mode of the target ion and concentration from spectra of standard samples whose concentration are already known.

Analytical figures of merit for the present method for the determination of phosphate were evaluated under optimized conditions. The large analytical concentration range (1-900 ng mL^{-1}) of phosphate was applied through ion-pair SDME method with ATR-FTIR technique. The ratio between the minimum and maximum phosphate concentration range was 1:900. The absorbance and peak area are important parameter for the quantification of phosphate because concentration was directly proportional to both parameters. Firstly, the calibration curve was plotted between the concentration versus absorbance of the full range of the analysis data, (calibration curve no. 1 (CCn1)) by using software Table Curve 2Dv5.01.01. This plot shows the straight line with excellent correlation value of 0.997 The slope and intercept were found to

be 0.004 and 0.070, respectively. The three different concentration ranges, low (1-300 ng mL^{-1}, CCn2), medium (300-600 ng mL^{-1}, CCn3) and high (600-900 ng mL^{-1}, CCn4) were also plotted (Fig. 2a). The results in Table S2 (Supplementary data) indicate that there is excellent correlation between concentration and absorbance values.

Similarly, curve plotted between concentration versus peak area at 1159.06 cm^{-1} for all range analysis data (calibration curve no. 5, CCn5) was obtained with excellent linearity with correlation coefficient, slope and intercept value for the straight line equation, 0.995, 0.004 and 0.436, respectively. The three different concentration range low (1-300 ng mL^{-1}, CCn6), medium (300-600 ng mL^{-1}, CCn7) and high (600-900 ng mL^{-1}, CCn8) were also plotted (Fig. 2b). These parameters for all range data are shown in Table S2 with other important statistical data for CCn1–CCn8 using Table Curve 2D software. When r^2-value approaches 1.0, the straight line fit shows the more ideal fit while zero represent a complete lack of fit. Thus, the data shown in Table S2 verify the ideal rank of the calibration curves. The LoD and LoQ were calculated to be 0.34 ng mL^{-1} and 1.12 ng mL^{-1}, respectively for phosphate by this method. The repeatability of the present method, expressed as a percentage of the relative standard deviation (% RSD), ranged between 1.28% and 3.34%

The effect of foreign ions (cations and anions) was carried out for the determining the selectivity of the present work, by the addition of various amounts of foreign species in the solution of 10 ng mL^{-1} of phosphate. Interference was observed by change in either the extraction efficiency or signal intensity or position of analyte peak. The monoatomic cations and anions do not posses dipole change; therefore do not interfere in this method. For the multiatomic ions the tolerance limits are as follows: foreign ions (Tolerence limit, ng mL^{-1}): BrO_3^-, AsO_3^{2-}, AsO_4^{3-}, CrO_4^{2-}, MoO_4^{2-}, $Cr_2O_7^{2-}$, MnO_4^-, SeO_3^{2-}, FeO_4^{2-}, NH_4^+ (2000); CO_3^{2-}, BrO_3^-, IO_3^-, IO_4^-, OH^-, SiO_4^{2-}, formate, acetate, succinate, cinnamate, citrate (1000); BO_3^{3-}, $B_4O_7^{2-}$, CN^-, SCN^-, HCO_3^-, (600); $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}(500); ClO_2^-, ClO_3^-, ClO_4^-(100).

The ion-pair SDME-ATR-FTIR technique was used to determine phosphate in various water samples without any pretreatment. The present method was compared with reference method to evaluate the efficiency of the developed method and examine its robustness. The results shown in Table 1 reveal good agreement between present method and reference method The concentration of phosphate in tap water, ground water and agricultural water sample ranged from 96.82-402.86 ng mL^{-1}, 460.92-560.80 ng mL^{-1} and 602.82-850.08 ng mL^{-1}, respectively with the RSD value of 0.94-3.36 % for the present method and 0.98-3.28 % for the reference method (Table 1). These values show good precision and accuracy of method. The calculated values of F-test shown in Table 4 are less than the tabulated value for the degree of freedom (5.05, υ=N-1, 6-1=5), indicating no significance difference in the precision of the two methods. The t-test

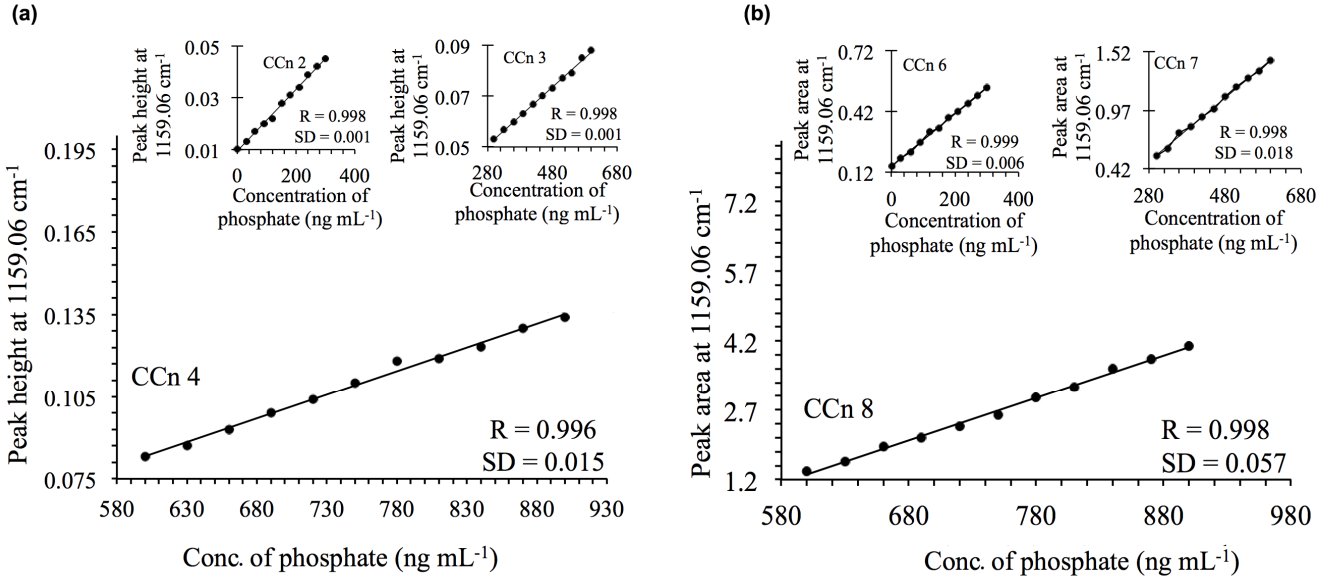

Fig. 2 — Calibration curve for concentration versus (a) relative absorbance, and, (b) peak area at 1159.06 cm^{-1}.

Table 1 — Determination of phosphate in water samples by the ion-pair SDME-ATR-FTIR technique (N = 06)

Samples		Conc. of phosphate (ng mL^{-1})				F-value[b]	t-value[c]
		Present method	RSD (%)	Ref. method[a]	RSD (%)		
Tap water	1	190.24	1.92	190.30	2.02	1.08	1.24
	2	284.16	3.18	284.08	3.16	1.14	1.28
	3	402.86	2.75	402.94	2.60	1.40	1.62
	4	120.45	1.80	120.42	1.69	1.24	1.29
	5	96.82	0.94	96.94	0.98	1.65	0.89
	6	210.14	3.11	210.28	3.08	1.19	1.72
Ground water	1	508.04	2.42	508.19	2.40	1.78	0.98
	2	560.80	1.89	560.64	1.68	1.19	1.87
	3	554.34	1.68	554.06	1.82	1.23	1.20
	4	498.20	3.12	498.18	3.06	1.65	1.02
	5	502.18	2.04	502.20	2.24	1.14	0.92
	6	460.92	1.76	460.76	1.70	1.28	1.08
Agricultural water	1	610.65	1.10	610.52	1.22	1.34	1.80
	2	850.08	1.84	850.16	1.89	1.11	1.07
	3	790.38	1.98	790.32	1.92	1.36	1.76
	4	660.26	3.36	660.40	3.28	1.15	1.28
	5	602.82	2.74	602.66	2.52	1.17	1.26
	6	826.62	1.82	826.49	1.88	1.16	1.59

[a]Ref 35. [b](sd_1^2/sd_2^2, $sd_1>sd_2$). [c]$\pm t= (\bar{X}-\mu)\frac{\sqrt{N}}{s}$

values (Table 4) are less than the tabulated t-test value of 2.571 at the 95% confidence level for the degree of freedom (6-1=5), hence there is no statistical difference in the proposed method and reference method[35].

The analytical features such as linearity, LoD, sample types, sample volume, analysis time and % RSD value of the present work were compared with other methods. The comparison results are presented in Table S2 (Supplementary data). As compared to other methods, LoD is lower and less organic extraction solvent was required in the present method. High sample volume is required in spectrophotometric and turbidimetric methods. The main disadvantage of molybdenum blue method is the interferences caused by the arsenate, silicate and germaniate[36]. These interferences can come from soils treated with pesticides containing arsenic and mine spoils, which enter the water sources. Thus, the present ion-pair SDME-ATR-FTIR method is suitable for the determination of phosphate in various water samples with high sensitivity and selectivity.

Supplementary data

Supplementary data associated with this article are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_57A(02)168-174_SupplData.pdf.

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