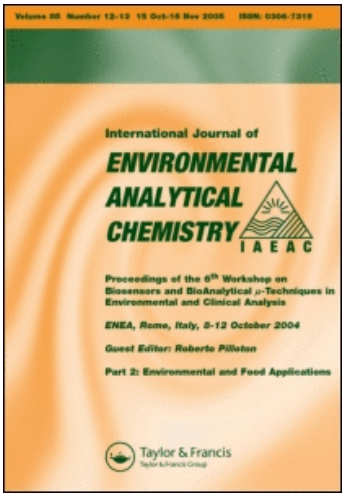

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of phosphate in freshwater samples by flow-injection with lucigenin chemiluminescence

Attiq-ur-Rehman[a]; Mohammad Yaqoob[a]; Amir Waseem[a]; Abdul Nabi[a]; Maqsood A. Khan[b]
[a] Department of Chemistry, University of Balochistan, Quetta, Pakistan [b] Department of Environmental Management and Policy, Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Pakistan

Online publication date: 22 October 2010

To cite this Article Attiq-ur-Rehman, Yaqoob, Mohammad , Waseem, Amir , Nabi, Abdul and Khan, Maqsood A.(2010) 'Determination of phosphate in freshwater samples by flow-injection with lucigenin chemiluminescence', International Journal of Environmental Analytical Chemistry, 90: 14, 1119 — 1129
To link to this Article: DOI: 10.1080/03067310903194949
URL: http://dx.doi.org/10.1080/03067310903194949

Intern. J. Environ. Anal. Chem.
Vol. 90, Nos. 14–15, 10–20 December 2010, 1119–1129

Taylor & Francis
Taylor & Francis Group

Determination of phosphate in freshwater samples by flow-injection with lucigenin chemiluminescence

Attiq-ur-Rehman[a], Mohammad Yaqoob[a]*, Amir Waseem[a], Abdul Nabi[a] and Maqsood A. Khan[b]

[a]*Department of Chemistry, University of Balochistan, Quetta, Pakistan;* [b]*Department of Environmental Management and Policy, Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Pakistan*

(*Received 24 November 2008; final version received 27 June 2009*)

Lucigenin chemiluminescence (CL) in conjunction with flow-injection analysis (FIA) is used for the determination of phosphate in freshwater samples. The procedure is based on the formation of molybdophosphoric heteropoly acid (MoP–HPA) by the reaction of phosphate and ammonium molybdate under acidic conditions. CL emission was observed as a result of oxidation of lucigenin in aqueous sodium hydroxide solution in the presence of MoP–HPA. Calibration was linear up to 500 $\mu g\,L^{-1}$ ($r^2 = 0.9998$; $n = 8$), with a detection limit ($S/N = 3$) of 0.95 $\mu g\,L^{-1}$. An injection throughput of 120 h^{-1}, and relative standard deviation (RSD; $n = 4$) of 1.3–3.2% were achieved in the concentration range studied. An on-line chelating column was used to remove interfering cations. The method was applied to freshwater samples, and the results (51 ± 1.0 – $107 \pm 2.0\,\mu g\,L^{-1}$) did not differ significantly from results obtained using a spectrophotometric method (52.5 ± 1.0 – $102 \pm 2.0\,\mu g\,L^{-1}$) at 95% confidence level (*t*-test).

Keywords: flow-injection analysis; chemiluminescence; lucigenin; molybdate; phosphate; freshwater samples

1. Introduction

Phosphorus is well known as an essential nutrient element for organisms in aquatic environments [1,2], and excessive phosphorus concentrations can result in eutrophication of natural waters [3]. The analysis of water samples are especially complex owing to the fact that phosphorus can be found in the form of different inorganic and organic species [4,5], which in turn can be present in either the dissolved, colloidal or particulate form. However, the dominant species is always orthophosphate. Usually, in the analysis of water samples, the analysis of the phosphorus content is carried out on aliquots of the whole sample and on aliquots of the sample previously filtered through membrane filters of nominal pore size. Total phosphorus concentrations in non-polluted natural waters extend over a very wide range from $<1.0\,\mu g\,L^{-1}$ in ultra-oligotropic waters, to $>200\,\mu g\,L^{-1}$ in highly eutrophic waters. However, most uncontaminated freshwaters contain between 10 and 50 $\mu g\,L^{-1}$ of total phosphorus [6]. In open ocean water, orthophosphate concentration of 5.7 $\mu g\,L^{-1}$ or lower is reported [7].

*Corresponding author. Email: yaqoob2001@hotmail.com

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310903194949
http://www.informaworld.com

Several procedures have been reported in the literature for the determination of phosphate in different samples. These include visible spectrophotometry [8–12], fluorescence spectrophotometry [13,14], fourier transform infrared spectrometry [15], inductively coupled plasma atomic emission spectrometry [16], and electro-analytical methods [17–19]. Comprehensive reviews for phosphorus analysis in water samples based on flow techniques have also been reported [5,20].

The analytical application of chemiluminescence (CL) detection has received a strong surge by the advent of flow systems, as exemplified in several monographs and fundamental reviews [21–24]. Chemiluminescence [25–31] and electro-chemiluminescence [32] methods have been reported for the determination of phosphate in diverse samples and, some of these FIA–CL methods are based on the oxidation of luminol with heteropoly acids for phosphate determination. The interaction of luminol with heteropoly acids are reported by examination of CL spectra, using electron spin resonance spectroscopy [33]. It has been found that heteropoly acids act as a catalyst, accelerating CL reaction of luminol with oxygen through the formation of semiquinone and superoxide radicals following the formation of aminophthalate and light emission. Thus, the reaction of luminol with oxygen and heteropoly acid is a complex multi-component, multi-stage process. Table 1 shows the comparison of various analytical methods in terms of sample matrix, linear calibration range and sensitivity for the determination of phosphate in freshwater samples.

Lucigenin (N,N′-dimethyl-9,9′-diacridinium nitrate), undergoes CL reaction in alkaline medium to yield N-methylacridone and light [34]. The excited state N-methylacridone emits at about 420 to 500 nm with a maximum at about 440 nm. The overall reaction is an oxidation, but the mechanism is complicated and involves both oxidation and reduction steps. Lucigenin can react with hydrogen peroxide or with a reductant plus oxygen. In the reaction with H_2O_2, the peroxide probably acts as both oxidant and reductant; considerably more light is emitted in the presence of catalysts like Co^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, Cr^{3+}, and Ni^{2+} [35]. In the presence of dissolved oxygen, lucigenin can undergo CL reaction with various reductants [36].

We report a simple and rapid flow injection analysis–chemiluminescence (FIA–CL) method for the determination of phosphate in freshwater samples. The method is based on the oxidation of lucigenin in aqueous sodium hydroxide medium by heteropoly acid, emitting CL intensity according to the following reaction scheme. The proposed reaction scheme could involve the oxidation of lucigenin which later reacts with air oxygen in solution to form superoxide radical producing N-methylacridone as an emitting species:

$$\text{Phosphate} + \text{Ammonium molybdate} \xrightarrow{H_2SO_4} \text{Molybdophosphoric heteropolyacid (MoP–HPA)}$$

$$\text{MoP–HPA} + \text{Lucigenin} \longrightarrow \text{N-methylacridone}^* + \text{other products}$$

$$\text{N-methylacridone}^* \longrightarrow \text{N-methylacridone} + h\nu$$

2. Experimental

2.1 *Materials and methods*

All solutions were prepared using ultra high purity (UHP) water (Elga, Purelab Option, UK), and all reagents were of analytical grade (Merck, Darmstadt, Germany), unless stated otherwise.

Table 1. Analytical performance of relevant flow-injection analysis methods applied to determination of phosphate-phosphorus in different samples.

Technique	Sample	Determination range	Detection limit	R^2 value	Sampling rate/h	Reference
FIA–Spec	Natural water	0.01–1.0 μM	10 Nm	0.9986	18	[8]
FIA–Spec	Surface and groundwater	20–500 μg L^{-1}	17 μg L^{-1}	0.9990	60	[9]
FIA–Spec	Natural water	10–50 μg L^{-1}	8.0 μg L^{-1}	0.9916	30–40	[10]
FIA–Pot	Hydroponic nutrients	15.5–310 mg L^{-1}	0.093 mg L^{-1}	NG	12	[17]
FIA–Vol	Aqueous solution	0.031–15.5 mg L^{-1}	0.062 mg L^{-1}	NG	NG	[18]
FIA–CL	River water	0.032–3.26 μg L^{-1}	0.03 μg L^{-1}	0.9880	180	[25]
FIA–CL	Freshwater	2.0–10 μM	0.2 μM	0.9985	60	[26]
MSFIA–CL	Environmental samples	5.0–50 μg L^{-1}	2.0 μg L^{-1}	0.9975	11	[27]
Luminol–CL	Power plant and river water	0.06–1.7 μg L^{-1}	0.02 and 0.1 μg L^{-1}	NG	<3	[28]
FIA–CL	River water, rice wine, seaweed	0.03–32.3 μM	0.03 μM	NG	NG	[29]
FIA–ECL	Water	2.0×10^{-10}–1.0×10^{-8} g mL^{-1}	8.0×10^{-11} g mL^{-1}	0.9954	NG	[32]
FIA–CL	Freshwater	5.0–500 μg L^{-1}	0.95 μg L^{-1}	0.9986	120	This method

Notes: CL, chemiluminescence; ECL, electrochemiluminescence; FIA, flow-injection analysis; MSFIA, multi-syringe FIA; NG, not given; Pot, potentiometric; Spec, spectrophotometric; Vol, voltammetric.

Lucigenin stock solution (1.0×10^{-2} mol L^{-1}) was prepared by dissolving the required amount of N,N′-dimethyl-9,9′-diacridinium nitrate ($C_{28}H_{22}N_4O_6$, Fluka, Buchs, Switzerland) in UHP water. A working lucigenin solution (5.0×10^{-6} mol L^{-1}) was prepared by diluting the required volume of stock solution in 100 mL of water. A sodium hydroxide stock solution (1.0 mol L^{-1}) was prepared by dissolving 4.0 g of NaOH in 100 mL of water. From this stock solution, 4.0×10^{-2} mol L^{-1} working standard was prepared by diluting the required volume in 100 mL of water.

Phosphate stock solution (0.01 mol L^{-1}) was prepared by dissolving 0.156 g of NaH_2PO_4 in 100 mL of water. Working solutions of different concentrations were prepared by suitable dilution as required. Ammonium molybdate stock solution (0.01 mol L^{-1}) was prepared by dissolving 1.236 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 100 mL of water. A working solution (1.0×10^{-4} mol L^{-1}) was prepared by diluting 1.0 mL of stock solution in 100 mL of water containing H_2SO_4 (5.0×10^{-3} mol L^{-1}).

Stock solutions (1000 mg L^{-1}) of Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Pb^{2+}, Cr^{3+}, Cu^{2+} and Zn^{2+} (chloride/nitrate counter ions) in HCl/HNO_3 (0.01 mol L^{-1}) and anions Cl^-, SO_4^{2-}, NO_3^-, NO_2^-, HCO_3^-, F^-, SiO_4^{4-} and AsO_4^{3-} (potassium/sodium counter ions) in water, were prepared from their respective salts. Various working solutions were prepared from these stock solutions for interference studies.

2.2 *Instrumentation*

A flow-injection manifold with CL detection system shown in Figure 1 was used for the determination of phosphate. Sample and reagent streams were delivered using a peristaltic pump (four channels, Ismatec, Reglo, Glattbrugg, Switzerland) at a flow rate of 1.6 mL^{-1}min/channel. Phosphate standards were injected via an injection valve (Rheodyne 5020, Anachem, Luton, UK) into a sample carrier stream, (UHP water) which after passing through a chelating resin column (50 mm × 4.0 mm id.) [25] merged at a T-piece with a stream of ammonium molybdate (1.0×10^{-4} mol L^{-1}) in H_2SO_4 (5.0×10^{-3} mol L^{-1}). This stream was then merged at another T-piece with the lucigenin (5.0×10^{-6} mol L^{-1}) and NaOH (4.0×10^{-2} mol L^{-1}). Both streams were allowed to travel 2.0 cm before passing through a glass spiral flow cell (2.0 mm i.d., 25 mm length) placed

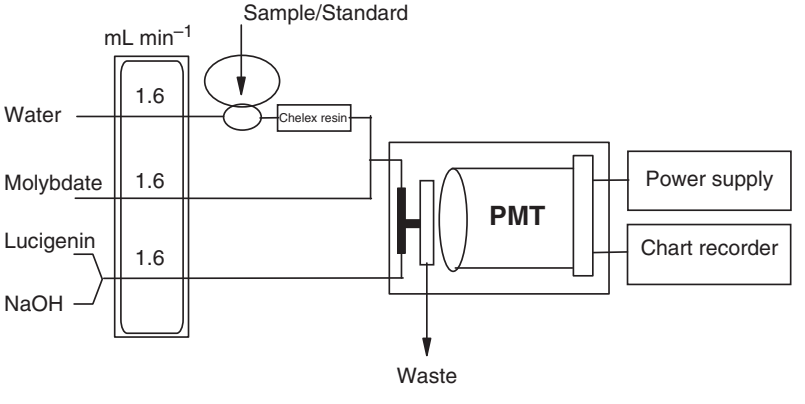

Figure 1. Flow-injection analysis chemiluminescence (FIA–CL) manifold for the determination of phosphate.

directly in front of an end of window photomultiplier tube (PMT, 9798B, Electron Tubes, Ruislip, UK). The PMT, glass coil and the T-piece are placed in a light-tight housing. The PMT was attached to a 2 kV power supply (Electron Tubes, PM20SN, Ruislip, UK). To read the output of the PMT a chart recorder (BD11E, Kipp & Zonen, Delft, The Netherlands) was used. The whole manifold was assembled by Tygon tubing (1.02 mm, id.).

2.3 *Validation of method*

The FIA–CL method for the determination of phosphate was validated by comparing with a spectrophotometric method [40]. 5.0 mL of H_2SO_4 (0.1 mol L^{-1}), 2.0 mL of ammonium molybdate (5.0%) and 1.0 mL of hydroquinone were added to 10 mL of a solution containing 25 to 125 μg of phosphate. After standing for 15 to 20 min, 1.0 mL of the sulfite-bisulfite mixture (15 g of sodium bisulfite and 20 g of sodium sulfite per 100 mL of water) was added to the solution and was left for a further 10 to 15 min. The volume was then made up to 25 mL and the absorbance was monitored at 720 nm using a UV/Vis spectrophotometer (Model 6505, Jenway, Essex, England). Water samples were analysed for phosphate by taking appropriate volume of the samples and treated according to the procedure described above. The concentration was determined from the calibration graph prepared from the standards, and was compared with FIA–CL method.

3. Results and discussion

3.1 *Optimisation of FI manifold*

FIA lucigenin CL method for the determination of phosphate was optimised with respect to various experimental parameters such as sodium hydroxide, sulfuric acid, lucigenin, and ammonium molybdate concentrations (Figure 2), flow rate, sample volume and PMT voltage. For optimisation studies phosphate standard solution (95 μg L^{-1}, 60 μL) was used and in all experiments, four replicate measurements were performed for each parameter under study.

3.1.1 *Effect of sodium hydroxide concentration*

Lucigenin CL reaction occurs in alkaline medium. The effect of NaOH concentration on the CL reaction was examined in the range of 1.0×10^{-2}–5.0×10^{-2} mol L^{-1}. The CL response was increased up to 4.0×10^{-2} mol L^{-1} after which the CL intensity decreased. Therefore, NaOH concentration of 4.0×10^{-2} mol L^{-1} was selected as optimum for rest of the experiments. The effect of NaOH was studied by using a separate channel, as mixing with lucigenin may result in background signal and degradation of reagents.

3.1.2 *Effect of sulfuric acid concentration*

Sulfuric acid concentration was investigated over the range 1.0×10^{-3}–10×10^{-3} mol L^{-1}. The CL intensity increased with increasing H_2SO_4 concentration up to 5.0×10^{-3} mol L^{-1}, above which the CL intensity decreased. Therefore, 5.0×10^{-3} mol L^{-1} H_2SO_4 was finally chosen as the reaction medium for subsequent studies. These conditions (low acidity) are different from those commonly used for spectrophotometric phosphorus determinations

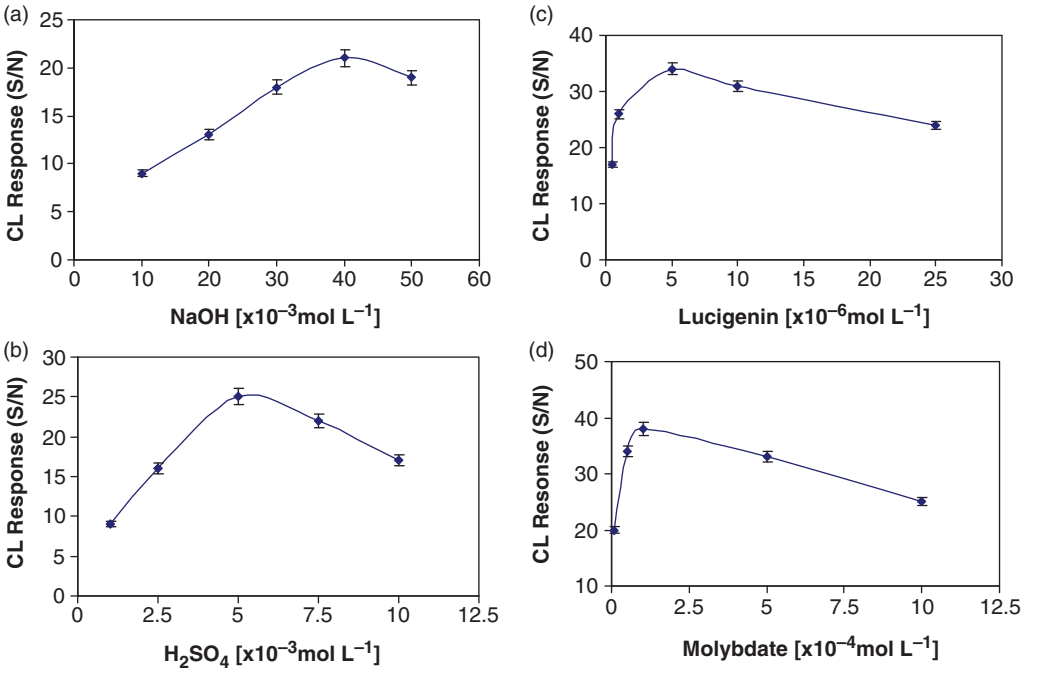

Figure 2. Variation of CL intensity with: (a) sodium hydroxide; (b) sulfuric acid; (c) lucigenin; and (d) ammonium molybdate concentrations.

[37,38], but is a compromise with the optimum conditions required for the subsequent CL reaction.

3.1.3 *Effect of lucigenin concentration*

Lucigenin concentration on the CL reaction was examined in the range 5.0×10^{-7}–2.5×10^{-5} mol L^{-1}. The results showed that both the CL response gradually increased as lucigenin concentration increased within the studied concentration range, but the background response also increased with increased lucigenin concentration. The optimum S/N and higher CL response were obtained when using 5.0×10^{-6} mol L^{-1} lucigenin. Therefore, lucigenin concentration of 5.0×10^{-6} mol L^{-1} was used subsequently.

3.1.4 *Effect of ammonium molybdate concentration*

Ammonium molybdate concentration on the formation of molybdophosphoric heteropoly acid complex was examined over the range of 1.0×10^{-5}–1.0×10^{-3} mol L^{-1}. The CL intensity increased up to 1.0×10^{-4} mol L^{-1}, and above which the response decreased due to low acid/molybdate ratio [37,39]. Therefore, 1.0×10^{-4} mol L^{-1} ammonium molybdate solution was used for subsequent experiments.

3.1.5 *Effect of key physical parameters*

Flow rate and sample volume on the CL response was studied in terms of sensitivity, speed and reagent consumption. Flow rates of three channels were examined over the range of

1.0–3.0 mL min^{-1}. Maximum CL intensity was obtained at a flow rate of 1.6 mL min^{-1}/channel with steady baseline and was used for further studies. The optimisation of flow rate was also carried out independently for each channel by changing peristaltic tubes of different diameters. However, no significant difference in terms of sensitivity was observed. Similarly, the effect of sample injection volume on the sensitivity of the flow system was examined in the range 60–240 μL, with a maximum CL response at 120 μL, and was used for further studies. The effect of PMT voltage was also examined in the range 700–950 V for maximum CL intensity. The CL output increased up to 850 V and further increase in PMT voltage resulted in noise and high background signal. Therefore, a voltage of 850 V was used to cope with the background signal.

3.1.6 *Analytical figures of merit*

Under the selected conditions described above, the calibration graph for CL intensity versus phosphate concentration was linear in the range 5.0–500 μg L^{-1}. The regression equation was $y = 45.424x + 0.2438$ (where y = CL response in mV and x = concentration in μg L^{-1}) with a coefficient of determination 0.9986 ($n = 8$). The limit of detection ($S/N = 3$) was 0.95 μg L^{-1} and the relative standard deviation was 1.3–3.2% ($n = 4$) in the range studied. An injection throughput of 120 h^{-1} was achieved.

3.2 *Interference studies*

The effect of foreign species on the determination of phosphate (95 μg L^{-1}) was studied by preparing standards of major cations and anions in the range of their environmentally relevant concentration in freshwater samples. The effect of cations and anions on the blank response (in the absence of phosphate) and on the determination of phosphate was examined and are shown in Table 2. Cations including Co^{2+}, Cu^{2+}, Cr^{3+}, Fe^{2+} and Ni^{2+} had the enhancing effect on the lucigenin CL, while Ca^{2+}, Pb^{2+}, Mg^{2+} and Mn^{2+} had suppressive effect on the blank response and the phosphate signal, while Zn^{2+} had no effect on phosphate determination. Cations were removed with on-line micro-column (50 mm × 4.0 mm id.) containing an iminodiacetate chelating resin (Chelex 100, 50–100 mesh, sodium form, Sigma). The life of column was checked by injecting 5.0 mg L^{-1} Fe^{2+} (50 replicate injections) and did not show any breakthrough or have any deleterious effect on the phosphate response for 20 replicate injections of phosphate standard (95 μg L^{-1}). The anions including; SO_4^{2-}, Cl^-, NO_3^-, NO_2^-, HCO_3^-, F^-, and SiO_4^{4-} had no effect on the blank response and the phosphate signal while AsO_4^{3-} has a suppressive effect on the phosphate signal. In the previously reported method [25], nitrite (10 mg L^{-1}) had suppressive effect on the luminal CL system and silicate (0.1 mg L^{-1} Si) had enhancement effect on phosphate response.

Orthophosphate reacts with molybdate in acidic medium to yield 12-molybdophosphoric heteropoly acid; subsequently, detection is undertaken either on the molybdophosphate reduction product or on the yellow vanadomolybdate complex [5,20]. Blomqvist *et al.* [41] reported the interference from arsenate, fluoride and silicate when determining phosphate in water by the phosphoantimonylmolybdenum blue method and suggested that interfering effects of these species can be reduced by dilution of the sample prior to reagent addition. Moreover, Blomqvist and Westin [42] reported interference from chromate, germinate, tungstate and vanadate when determining phosphate in aqueous solution using a molybdenum blue method. They reported that the effects found fall into

Table 2. Effect of various ions on the determination of phosphate. All readings are the mean of four replicate injections (blank value = 0.1 mV).

Interferents	Concentration ($mg\,L^{-1}$)	CL signal without phosphate (mV)	CL signal with phosphate (mV)	Standard values* ($mg\,L^{-1}$)
PO_4^{3-}	0.095	0.1	43	0.1
Co^{2+}	0.001	100	210	<0.001
Cr^{3+}	0.1	10	48	0.05
Cu^{2+}	0.1	4.0	53	2.0
Fe^{2+}	0.0056	150	240	<0.3
Ni^{2+}	0.1	10	50	0.02
Zn^{2+}	1.0	4	43	3.0
Ca^{2+}	150	0.5	20	100
Mg^{2+}	30	10	24	30
Pb^{2+}	1.0	0.2	9.0	0.01
Mn^{2+}	0.1	0.2	10	<0.05
SO_4^{2-}	200	0.3	43	250
Cl^-	200	0.4	45	250
NO_3^-	20	0.1	41	<10
NO_2^-	0.1	0.2	42	0.1
HCO_3^-	20	0.3	43	–
F^-	1.0	0.2	44	1.5
SiO_4^{4-}	5.0	0.2	44	2.0
AsO_4^{3-}	0.01	0.4	35	0.01

Note: *World Health Organization and European Commission Council Directive (98/83/EC) guidelines for drinking water quality, 1993 and 1998, respectively.

two types: (1) Formation of additional blue molybdenum complexes, causing elevated strength of absorption and possible over-estimation of phosphate concentrations, which is true for germinate and high concentrations of tungstate and (2) reduced blue colour and an accompanying hypsochromic shift, possibly resulting in under-estimation of phosphate concentrations, which is valid for chromate, vanadate and low concentrations of tungstate. The authors described in detail the effect of these ions compared with previous reports as well as environmental considerations. Grace *et al.* [43] reported a FIA–spectrophotometric method for phosphate determination using molybdenum blue with ascorbic acid as the reductant. Sulfide interference caused major errors in filterable reactive phosphorus analyses and was removed on-line using potassium permanganate as the carrier. Kowalenko and Babuin [44] conducted a critical evaluation of potential chemical interference on a molybdenum based phosphorus colorimetric method that has been used widely for soil, plant, and water research. A wide variety of elements including aluminum, manganese, iron, potassium, calcium, magnesium, sodium, nitrate, and ethylenediaminetetraacetic acid commonly found in these materials or extracting solutions used on them were shown to cause interferences.

Silicate and arsenate are the major potential interferents when determining phosphorus in natural waters, since they form heteropoly acid species with ammonium molybdate in acidic medium, and causes CL emission from the oxidation of luminol [28–30]. Silicate and arsenic concentrations in freshwaters are typically $2.0\,mg\,L^{-1}$ or lower and $0.02\,mg\,L^{-1}$ or lower, respectively [45,46]. Silicate interference can be reduced or eliminated using lower temperatures or by reducing the pH [39,47]. Masking agents

Table 3. Determination of phosphate ion in freshwater samples by the proposed FIA–CL method and a spectrophotometric method [40]. All readings are the mean of four injections.

	Phosphate ion found ($\mu g\,L^{-1}$)	
Samples	Proposed FIA–CL method	Spectrophotometric method
1	51 ± 1.0	52.5 ± 1.0
2	67.3 ± 0.7	65 ± 2.0
3	107 ± 2.0	102 ± 2.0
4	60.5 ± 1.2	58 ± 1.5
5	70.8 ± 2.1	72 ± 0.8
6	90.5 ± 2.5	92 ± 0.5
7	98.5 ± 0.9	95 ± 1.0
8	75.9 ± 0.8	76.5 ± 1.2

such as tartaric acid can also be effective when they are added before the molybdophosphate or molybdosilicate species formed [48]. One advantage of automated analyses over manual ones is a precise timing and reagent mixing. The shorter interval between reagent addition and absorbance/CL measurement in automated analysis also reduces the extent of interference given the slower formation of molybdosilicate.

Considering that arsenate can positively interfere when measuring phosphate, reduction of As(V) to As(III) prior to measurement might eliminate the interference [49] when using sodium thiosulfate as reducing agent to mask the interfering effect from As(V) [32].

3.3 *Application to freshwater samples*

Freshwater samples were collected from various locations in the Quetta Valley, Pakistan and placed into acid (10%, v/v, HCl) washed high density polyethylene bottles. After collection, samples were filtered through a cellulose membrane filter (pore size 0.45 μm, 47 mm diameter, Whatman, Maidstone, UK), kept refrigerated in the dark at 4°C, and analysed. The samples were directly injected into the proposed FIA–CL manifold and the results obtained for the samples (range 51 ± 1.0 to $107 \pm 2.0\,\mu g\,L^{-1}$) together with a spectrophotometric method [40] are shown in Table 3. The comparison of two methods for measuring phosphate concentration in eight freshwater samples was performed using student's *t*-test based on individual differences between results for each sample. It was found that $t_{calculated}$ (1.13) is less than t_{table} (2.356) for 95% confidence and 7 degrees of freedom. The two methods are not significantly different at the 95% confidence level.

4. Conclusions

This paper describes a flow-injection method based on the lucigenin–molybdophosphoric heteropoly acid reaction for the determination of trace levels of phosphate in freshwater samples. The method is simple, rapid ($120\,h^{-1}$ injection throughput), with a limit of detection $0.95\,\mu g\,L^{-1}$. Common interfering cations present in freshwaters were removed by

an on-line chelating resin micro-column. The attraction of the reported method was that no interference from nitrite and silicate was observed on the determination of phosphate using lucigenin CL system as was observed in case of luminol–CL system [25]. The method was applied to natural freshwater samples, and the results obtained were in reasonable agreement with a spectrophotometric method.

Acknowledgements

The authors are grateful to the Higher Education Commission of Pakistan for financial support in the form of an Indigenous PhD Scholarship (PIN-042-210 012-Ps2-323).

References

[1] D.L. Correll, J. Environ. Qual. **27**, 261 (1998).
[2] S.L. Young, T. Seiki, T. Mukai, K. Takimoto, and M. Okkada, Water Res. **30**, 1490 (1996).
[3] T.C. Daniel, A.N. Sharpley, and J.L. Lemunyon, J. Environ. Qual. **27**, 251 (1998).
[4] I.D. McKelvie, D. Peat, and P.J. Worsfold, Anal. Proc. **32**, 437 (1995).
[5] J.M. Estela and V. Cerda, Talanta **66**, 307 (2005).
[6] R.G. Wetzel, *Limnology* (Academic Press, New York, 2001), p. 1006.
[7] Y. Liang, D. Yuan, Q. Li, and Q. Lin, Anal. Chim. Acta **571**, 184 (2006).
[8] L.J. Gimbert, P.M. Haygarth, and P.J. Worsfold, Talanta **71**, 1624 (2007).
[9] M.S.A.C. Neves, M.R.S. Souto, I.V. Toth, S.M.A. Victal, M.C. Drumond, and A.O.S.S. Rangel, Talanta **77**, 527 (2008).
[10] O.N. Omaka, M. Keith-Roach, I.D. McKelvie, and P.J. Worsfold, Intern. J. Environ. Anal. Chem. **88**, 91 (2008).
[11] J. Murphy and J.P. Riley, Anal. Chim. Acta **27**, 31 (1962).
[12] W.J. Williams, *Handbook of Anions Determination* (Butterworth, London, 1979).
[13] T.P. Ruiz, C.M. Lozano, V. Tomas, and A. Sanz, Analyst. **121**, 477 (1996).
[14] T. Taniai, M. Sukegawa, A. Sakuragawa, and A. Uzawa, Talanta. **61**, 905 (2003).
[15] R. Vonach, B. Lendl, and R. Kellner, Analyst. **122**, 525 (1997).
[16] C.W. McLeod, I.G. Cook, P.J. Worsfold, and J.E. Davies, J. Queay, Spectrochim. Acta. B: **40**, 57 (1985).
[17] R. De Marco and C. Phan, Talanta. **60**, 1215 (2003).
[18] A.G. Fogg and N.K. Bsebsu, Analyst. **107**, 566 (1982).
[19] S. Hinkamp and G. Schwedt, Anal. Chim. Acta **236**, 345 (1990).
[20] P.J. Worsfold, L.J. Gimbert, U. Mankasingh, O.N. Omaka, G. Hanrahan, P.C.F.C. Gardolinski, P.M. Haygarth, B.L. Turner, M.J. Keith-Roach, and I.D. McKelvie, Talanta **66**, 273 (2005).
[21] I.P.A. Morais, I.V. Toth, and A.O.S.S. Rangel, Talanta. **66**, 341 (2005).
[22] A.C. Calokerinos and L.P. Palilis, in *Chemiluminescence in Analytical Chemistry*, edited by A.M. Garcıa-Campana and W.R.G. Baeyens (Marcel Dekker, New York, 2001), pp. 321–348.
[23] P. Fletcher, K.N. Andrew, A.C. Calokerinos, S. Forbes, and P.J. Worsfold, Luminescence **16**, 1 (2001).
[24] Y.F. Mestre, L. Zamora, and J.M. Calatayud, Luminescence **16**, 213 (2001).
[25] M. Yaqoob, A. Nabi, and P.J. Worsfold, Anal. Chim. Acta **510**, 213 (2004).
[26] M. Yaqoob, M. Anwar, and A. Nabi, Intern. J. Environ. Anal. Chem. **85**, 451 (2005).
[27] I.P.A. Morais, M. Miro, M. Manera, J.M. Estela, V. Cerda, M.R.S. Souto, and A.O.S.S. Rangel, Anal. Chim. Acta **506**, 17 (2004).
[28] O.V. Zui and J.W. Birks, Anal. Chem. **72**, 1699 (2000).

[29] T. Fujiwara, K. Karahashi, T. Kumamaru, and H. Sakai, Appl. Organomet. Chem. **10**, 675 (1996).
[30] N.M. Lukovskaya and V.A. Bilochenko, Ukr. Khim. Zh. **43**, 756 (1977).
[31] H. Jiang, Z. Wang, J. Li, and J. Lu, Fenxi Shiyanshi **16**, 64 (1997).
[32] Y. Xue, X. Zheng, and G. Li, Talanta. **72**, 450 (2007).
[33] O. Zui, H. Takahashi, T. Hori, and T. Hinoue, Talanta. **78**, 1185 (2009).
[34] F. McCapra and K.D. Perring, in *Chemi- and Bioluminescence*, edited by J.G. Burr (Marcel Dekker, New York, 1985), p. 259.
[35] L.A. Montano and J.D. Ingle Jr, Anal. Chem. **51**, 919 (1979).
[36] R.L. Veazey and T.A. Nieman, Anal. Chem. **51**, 2092 (1979).
[37] L. Drummond and W. Maher, Anal. Chim. Acta **302**, 69 (1995).
[38] S.-C. Pai, C.C. Yang, and J.P. Riley, Anal. Chim. Acta **229**, 115 (1990).
[39] J.Z. Zhang, C.J. Fischer, and P.B. Ortner, Talanta **49**, 293 (1999).
[40] *Colorimetric Determination of Elements; Principles and Methods*, edited by G. Charlot (Elsevier Publishing Company, New York, 1964), p. 343.
[41] S. Blomqvist, K. Hjellström, and A. Sjösten, Intern. J. Environ. Anal. Chem. **54**, 31 (1993).
[42] S. Blomqvist and S. Westin, Anal. Chim. Acta **358**, 245 (1998).
[43] M. Grace, Y. Udnan, I. McKelvie, J. Jakmunee, and K. Grudpan, Environ. Chem. **3**, 19 (2006).
[44] C.G. Kowalenko and D. Babuin, Comm. Soil Sci. Plant Anal. **38**, 1299 (2007).
[45] M. Yaqoob, A. Nabi, and P.J. Worsfold, Anal. Chim. Acta **519**, 137 (2004).
[46] Attiq-Ur-Rehman, M. Yaqoob, A. Waseem, and A. Nabi, Intern. J. Environ. Anal. Chem. **88**, 603 (2008).
[47] C. Ciavatta, L.V. Antisari, and P. Sequi, J. Environ. Qual. **19**, 761 (1990).
[48] P.R. Freeman, I.D. McKelvie, B.T. Hart, and T.J. Cardwell, Anal. Chim. Acta **234**, 409 (1990).
[49] K.L. Linge and C.E. Oldham, Anal. Chim. Acta **450**, 247 (2001).