Chemometrics-assisted cross injection analysis for simultaneous determination of phosphate and silicate

Kanchana Uraisin\textsuperscript{a,b}, Supavita Janya\textsuperscript{a,b}, Chutima Phechkrajang\textsuperscript{a,c}, Nathawut Choengchan\textsuperscript{a,d}, Warawut Tiyapongpattana\textsuperscript{a,e}, Víctor Cerdà\textsuperscript{f} and Duangjai Nacapricha\textsuperscript{a,b}

\textsuperscript{a}Flow Innovation-Research for Science and Technology Laboratories (FIRST Labs.); \textsuperscript{b}Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand; \textsuperscript{c}Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand; \textsuperscript{d}Department of Chemistry, Faculty of Science, King Mongkut’s Institute of Technology Ladkrabang, Bangkok, Thailand; \textsuperscript{e}Department of Chemistry, Faculty of Science and Technology, Thammasat University, Rangsit Center, Prathum Thani, Thailand; \textsuperscript{f}Department of Chemistry, University of the Balearic Islands, Palma de Mallorca, Spain

\textbf{ABSTRACT}

This work presents a novel method for simultaneous spectrophotometric determination of phosphate and silicate by using a cross injection analysis (CIA) coupled with the use of partial least squares (PLS) for data evaluation. The detection principle is based on the well-known ‘molybdenum blue’ method. The molybdate ions in the presence of stannous chloride in acidic medium give phosphomolybdenum blue and silicomolybdenum blue as products. In this work, all the liquids, including sample and reagents were simultaneously introduced into a CIA platform by using two peristaltic pumps for controlling the x-channel and y-channel flow which was automatically manipulated by using in-house control board. Crossflow provides sufficient mixing inside the platform prior detection of the absorption spectra of blue complexes in the wavelength of 400–900 nm. Since spectra of the blue colour product of phosphate and silicate are resemblant, these two analytes therefore reciprocally interfere with one another. This results in difficulty in simultaneous analysis of phosphate and silicate. In this work, PLS was utilised as assistor of CIA system for simultaneous analysis of phosphate and silicate using molybdenum blue reaction without using any modification of reagents and addition of selective masking agent. The calibration ranges are 0.1–6 mgP L\textsuperscript{−1} and 5–100 mgSi L\textsuperscript{−1} for phosphate and silicate, respectively. By using CIA coupled with PLS for data evaluation, the analysis of two analytes was achieved within 1.5 min with only single injection. The developed system was applied to natural water samples and the system was validated with the conventional methods. By statistical paired t-test, there was no evidence of significant difference at 95% confidence level ($t_{\text{stat}} = 2.28$, $t_{\text{critical}} = 2.31$ and $t_{\text{stat}} = 0.62$, $t_{\text{critical}} = 2.31$ for phosphate and silicate, respectively). This implied that the chemometrics-assisted CIA system was successfully developed for simultaneous spectrophotometric determination of phosphate and silicate.

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1. Introduction

Phosphate and silicate are key factors in an environmental monitoring that affect the aqueous ecosystem. The problem has serious consequences due to the extensive use of phosphate and silicate in synthetic detergents which later discharge as sewage effluents. Excess quantities of both ions in the effluent result in lower quality of water and cause of death to living organisms by stimulating the growth of alga in aquatic system or so-called eutrophication.

Several different analytical technologies have been proposed for quantification of phosphate and silicate. Liquid chromatography techniques including reversed-phase high performance liquid chromatography [1,2] and ion-exclusion chromatography coupled with precolumn derivatisation have been reported for concurrent determination of both analytes [3,4]. Voltammetric method has also been widely reported for simultaneous analysis of phosphate and silicate by monitoring molybdophosphate and molybdosilicate complexes [5–7]. Analysis of phosphate in an organic medium was also feasible using amperometric detection [8].

The simple and common approach for quantitative analysis of phosphate and silicate has relied on the formation of molybdophosphoric acid and molybdosilicic acid and then subsequently reduces to a molybdenum blue complexes with spectrophotometric detection. The mutual interferences of each component were eliminated by using different acid concentrations that affected the formation rates (kinetics) of phosphomolybdenum and silicomolybdenum blue [9,10]. Tartaric acid is an effective masking agent of silicate for phosphate determination, whereas oxalic acid can suppress formation of phosphomolybdenum blue for analysis of silicate [11,12].

Apart from modification of reagents or addition of selective masking agents for simultaneous analysis of phosphate and silicate, evaluation of spectral data of polyheteromolybdenum blue by first-derivative spectrophotometry has been found to be the useful method for direct analysis of mixture without any separation steps and chemical treatments [13]. Moreover, multivariate calibration methods are alternative manner for multicomponent determination. Among various chemometrics approaches for multicomponent analysis (i.e., multilinear regression analysis and neural networks), principal component regression (PCR) and partial least squares (PLS) applied to spectrophotometric signals have been successfully adopted in many quantitative assays of pharmaceutical formulation [14–16]. Both of these chemometrics methods were employed for resolving the mixture of different compounds whose absorption spectra are overlapped. PLS and PCR require a calibration step, where the relationship between the spectra and the component concentrations is interpreted from a set of standard, followed by a prediction step, which is the result of the calibration step, employed for estimation of component concentrations in sample [17]. However, the difference between the PCR and PLS is that PCR spectral decomposition is based entirely on variations in spectral response data without the relevance to analyte concentrations, whereas PLS decomposition is influenced by the concentration data matrix [18]. Batchwise analysis couples with multivariate calibration methods have been reported for phosphate and silicate determination. Afkhami and Tarighat [19] presented chemometrics analysis on visible spectra of phospho- and silico-molybdenum blue complexes. They reported the use of numerous chemometrics tools including PCR for the determination of phosphate and silicate in detergent and river water sample without any separation steps. In 1998, Pettersson and Karlberg [20] used PLS regression based on molybdenum blue reaction
for simultaneous determination of phosphate and silicate in brackish water. This method enables for simultaneous determination of the two analytes using only one spectral recording per sample. The calibration ranges are up to 20 µmol L$^{-1}$ for phosphate and up to 40 µmol L$^{-1}$ for silicate which cover the natural variations in the most brackish environmental samples. However, the batchwise procedure is time-consuming, which has to equilibrate the sample after the addition of reagents for 30 min prior recording of the spectrum.

The flow-based systems including flow injection analysis (FIA) and sequential injection analysis (SIA) are alternative approaches for rapid chemical analysis since the detection in flow-based systems is not relied on steady-state conditions. Reproducible results could be achieved even though the measurement of signals were taken prior to the reaction process reach to equilibrium. With the general basis of the flow system, dispensing of sample and reagents is exactly controlled by high performance liquid delivery system (peristaltic pump and syringe pump) that allows accurate reaction timing resulting in reproducible signals. In order to achieve rapid and automated procedure, the spectrophotometric detection of phospho- and silico-molybdenum blue coupled with FIA and SIA have been extensively proposed for the simultaneous analysis of phosphate and silicate. Similar to batchwise protocols, instantaneous determination of phosphate and silicate was also carried out by using typical chemical treatments including (i) modification of acid concentration that results in different formation rates of phosphomolybdenum and silicomolybdenum blue [21,22] and (2) utilisation of appropriate masking agents in order to suppress mutual interferences of each component [23–26].

Recently in 2013, our group proposed a new flow analysis technique called ‘cross-injection analysis (CIA)’ [27]. The CIA platform is made of an acrylic block and drilled out to have one channel as the analytical flow path and several channels perpendicular to the former channel for sample and reagent lines. In general, sample and reagents are introduced as stacked layers into analytical flow path that is similar to SIA. However, introduction of liquids in CIA is performed simultaneously, contradictory to SIA technique that is carried out by sequential aspiration. With cross-flowing of sample and reagents inside CIA platform, mixing in CIA system is enhanced, leading to a significant improvement in sensitivity. Moreover, CIA technique is a simple system with low maintenance costs because the system does not require any sophisticated pumping system as the SIA.

This work aims to develop an alternative cost-effective technique in flow analysis, namely the CIA technique, for simultaneous determination of phosphate and silicate coupled with chemometrics-assisted spectrophotometric method ‘PLS’ for data evaluation. The colorimetric approach used in this work is based on the well-known molybdenum blue reaction. The proposed method offers an alternative technique with a simple and rapid procedure. The method does not require any separation steps, masking agents and special instrument for simultaneous analysis of phosphate and silicate, and it is applicable in water samples.

2. Experimental

2.1. Reagents and sample

All chemicals used in this work were analytical reagent grade and deionised distilled water was utilised for preparation of all standard solutions and reagents. Standard stock
solution of phosphate (100 mgP L\(^{-1}\)) was prepared by dissolving an accurate weight of potassium dihydrogen phosphate (Merck, Germany), 0.11 g with 250.0 mL of water. A 10-times dilution of 100 mgP L\(^{-1}\) standard phosphate was employed for preparation of second standard stock solution of phosphate (10 mgP L\(^{-1}\)). Then, this solution was used for further dilution with water to obtain the desired concentration of working standard phosphate solution. Standard silicate solution of 1000 mgSi L\(^{-1}\) was prepared by dissolving an accurate weight of anhydrous sodium metasilicate (21.75 g) (Fluka, Switzerland), in water and making up to 500.0 mL with water. Working standard solutions of silicate were prepared from this stock solution by diluting with water.

Reagent stream (R\(_1\)): ammonium molybdate solution was a solution of 12 g L\(^{-1}\) ammonium molybdate in 0.4 mol L\(^{-1}\) sulphuric acid. The solution was obtained by dissolving 6 g of ammonium heptamolybdate tetrahydrate (Fluka) in 200 mL of deionised distilled water. Approximately 10 mL of concentrated sulphuric acid (Merck) was slowly added and diluted to 500 mL with water. This solution was stable for several months.

Reagent stream (R\(_2\)): stannous chloride solution was a solution of 0.3 g L\(^{-1}\) stannous chloride and 0.015 mol L\(^{-1}\) hydrazinium sulphate in 0.4 mol L\(^{-1}\) sulphuric acid. This solution was prepared by dissolving 0.15 g of stannous chloride dihydrate (BDH, England) and 1 g of hydrazinium sulphate (Merck) in 200 mL of deionised distilled water. Approximately 10 mL of concentrated sulphuric acid was slowly and cautiously added into the solution and then diluted to 500 mL with water. This solution was stable for at least 1 week when stored at 4°C.

Natural water samples were collected from canals around Bangkok area. Cellulose nitrate filters (Sartorious AG. 37070, Germany) were used for filtering all water samples before simultaneous determination of phosphate and silicate by using the CIA system with chemometrics-assisted spectrophotometric detection.

### 2.2. CIA system

#### 2.2.1. CIA platform

The CIA platform is a simple flow-based platform with a flow conduit inside. The platform was made of a small piece of Perspex\textsuperscript{TM} with dimensions of 15 × 40 × 30 mm (depth × length × height). As depicted in Figure 1S (Supplemental data), there was a centre cylindrical channel for horizontal flow (x-channel) for the carrier solution and other three channels that were drilled perpendicularly to the centre channel, for sample (or standard solution) and reagents by vertical cross flow (y-channels). All the channels were connected to a pump tube with screw nuts (Upchurch, USA) as connector.

#### 2.2.2. The manifold

An arrangement of the CIA manifold is shown in Figure 1. Two peristaltic pumps were employed in the system. A six-channel peristaltic pump (P1; Ismatec, model ISM843A-00078, Switzerland) was used for propelling sample/standard (S) and reagents (R1 and R2) through y-channels of the CIA platform. Single-channel peristaltic pump (P2; Ismatec, model ISM827B) was used for controlling the flow in the x-channel which was the water carrier. All peristaltic pumps were automatically operated by using in-house control board for starting/stopping the pumps. The CIA system was also furnished with a switching valve (SV, 1/4-28 Upchurch, model V-101T, USA) for protecting the reaction
zone in loading step propels to the detector. Polytetrafluoroethylene (PTFE) tubings with 1.0 mm i.d. (Cole Parmer, USA) were employed for the connecting tubings and mixing coil (MC). An Agilent UV-visible spectrophotometer with photodiode array detector (Model 3851, Germany) equipped with a 5-cm path length flow-through cell (Hellma, USA) was utilised for absorption spectra measurement in the range of 400–900 nm. The absorption spectra were used in chemometrics approach for simultaneous analysis of phosphate and silicate.

2.2.3. Operation procedure
The operation procedure is summarised in **Table 1**. Step 1, pump P2 introduced the carrier solution of water for 10 s into the flow line, whereas SV was at OPEN position. Step 2, mixed standard of phosphate and silicate, all reagents and carrier solution were simultaneously introduced into the CIA platform using time-based approach by turning on the two peristaltic pumps (P1 and P2) for 20 s with SV at CLOSE position. In this step, the minute zones (µL level) of standard and reagents reside inside the crossing space of the conduit. Step 3, SV is set at OPEN position and the carrier solution now flows along x-direction to flush the reacted zone into flow-through cell. Further, pump P2 was then turned off when the middle of product zone reached the detector (at a duration of 20 s). Absorption spectrum of blue products was recorded between 400 and 900 nm with wavelength interval of $\Delta \lambda = 1$ nm.

2.3. Preparation of ‘calibration set’, ‘test set’ and software for data analysis
Multivar version 7.0 software program (Universidad Nacional de Rosario, Argentina) was utilised for calculating PLS for simultaneous analysis of phosphate and silicate. PLS was employed in order to evaluate the spectral data and concentrations matrices which consist of several variables based on multivariate calibration.
According to statistical treatment of data by PLS, spectral data of various concentration matrices of the standard mixture of phosphate and silicate were required. Two series of mixed standards which were ‘calibration set’ and ‘test set’ (or ‘validation set’) were prepared by using a standard stock solution of phosphate (10 mgP L\(^{-1}\)) and silicate (1000 mgSi L\(^{-1}\)). Central composite design (CCD), introduced by Box and Wilson [28], was employed using concentrations of phosphate and silicate mixed standards for the calibration set (12 mixtures; C1-C12) whereas solutions of both analytes were selected by random approach for the validation set (6 mixtures; T1-T6). The composition of these analytes is summarised in Table 1S (Supplemental data).

### 2.4. PLS protocol for simultaneous determination of phosphate and silicate

PLS is a chemometrics tool used for spectral analysis of mixtures containing two or more compounds with overlapping spectra. The calibration of the methods involved absorption spectra and concentration data matrices of phosphate and silicate. Generally, PLS-1 employs a unique model (calibration) for each analyte to achieve the optimisation of the number of factors (or calibration model) for only one component at a time and predicts the concentration simultaneously in samples [29]. In this work, all absorption spectra of phospho- and silico-molybdenum blue were achieved by the proposed CIA system. By using the chemometrics manipulation, the procedure of the PLS method for analysis of phosphate and silicate is illustrated in [Scheme 1](#).

| Step | Process | Duration (s) | P1 | P2 | SV |
|------|---------|--------------|----|----|----|
| 1    | Carrier loading | 10 | OFF | ON | OPEN |
| 2    | Carrier loading + sample and reagent loading (x-direction and y-direction flow) | 20 | ON | ON | CLOSE |
| 3    | Zone flushing to detector (x-direction flow) | 20 | OFF | ON | OPEN |
| 4    | Stop the reacted zone inside the detector and scan spectrum | 5 | OFF | OFF | OPEN |
| 5    | Zone flushing to waste (x-direction flow) | 35 | OFF | ON | OPEN |

Repeat step 2–5 for next sample

Table 1. Operation step of CIA system for simultaneous analysis of phosphate and silicate.

According to statistical treatment of data by PLS, spectral data of various concentration matrices of the standard mixture of phosphate and silicate were required. Two series of mixed standards which were ‘calibration set’ and ‘test set’ (or ‘validation set’) were prepared by using a standard stock solution of phosphate (10 mgP L\(^{-1}\)) and silicate (1000 mgSi L\(^{-1}\)). Central composite design (CCD), introduced by Box and Wilson [28], was employed using concentrations of phosphate and silicate mixed standards for the calibration set (12 mixtures; C1-C12) whereas solutions of both analytes were selected by random approach for the validation set (6 mixtures; T1-T6). The composition of these analytes is summarised in Table 1S (Supplemental data).
submitted for analysis of both analytes in test set. The models have to give comparable predicted and true concentrations of phosphate and silicate in test set. Finally, the optimum calibration models were applied to the analysis of phosphate and silicate in real samples.

2.5. Method validation

In this work, the conventional batch method for determination of phosphate and silicate in water samples was utilised as reference method. The procedures were modified according to the standard method for analysis of water and wastewater [11]. The method for phosphate analysis called ‘ascorbic acid method’ was based on the principle that ammonium molybdate and antimony potassium tartrate react in acid medium with orthophosphate to form heteropolyacid–phosphomolybdic acid that was later reduced to intensely coloured molybdenum blue by ascorbic acid. The absorbance was measured at 880 nm. The ‘molybdosilicate method’ was used for silicate analysis. Basically, ammonium molybdate reacts with silica and any present phosphate to produce heteropoly acids at pH 1.2. Oxalic acid was added to extinguish the molybdophosphoric acid whereas the molybdosilicic acid remains in the solution. The intensity of yellow colour was proportional to the concentration of molybdate-reactive silica. The absorbance was monitored at 410 nm.

3. Results and discussion

Analysis of phosphate and silicate were carried out with well-known spectrophotometric detection of molybdenum blue method by forming the complex of
phosphomolybdenum blue and silicomolybdenum blue, respectively. In this work, a flow analysis, so-called CIA with chemometrics approach, PLS, was employed for simultaneous analysis of phosphate and silicate. Normally, PLS algorithms have been developed for the model that provides linear relationships between signal responses and concentrations. In this section, some of chemical and physical parameters of CIA system have to be optimised in order to achieve the individual dynamic ranges of phosphate and silicate. In addition, each linear range was further utilised for specifying the appropriate calibration concentrations of mixed components prior to applying PLS approach for data evaluation of the spectrophotometric signals (Section 3.3).

3.1. Optimisation of the CIA system

In order to find the linear response between absorbance and concentration of phosphate or silicate, the optimisation of CIA system (Figure 1) was examined by using individual standard phosphate and silicate. The reacted zones of phosphate or silicate were continuously propelled through the flow-through cell where phospho- and silicomolybdenum blue complexes were recorded at their maximum absorption wavelength at 715 nm and 810 nm, respectively.

3.1.1. Chemical parameters

In molybdenum blue method, phosphate and silicate react with molybdate ion in acid solution to form molybdophosphoric and silicomolybdic acid, which were later reduced to the blue complex by stannous chloride. Normally, the molybdate and Sn (II) ion are prepared in H₂SO₄ solution. Therefore, all the above-mentioned chemicals have to be optimised. A study of these chemical parameters was carried out by repetitive individual injections of 100 mgSi L⁻¹ silicate and 0.2 mgP L⁻¹ phosphate.

The optimisation of molybdate concentration was studied in the range of 6–36 g L⁻¹. The signal of silicate increased with increasing molybdate concentration from 6 to 12 g L⁻¹ because a higher amount of silicomolybdic acid was formed (Figure 2(a)). However, increment of the reagent concentrations results in light refraction appearing at liquid interface (the Schlieren effect) in the flow line. This phenomenon affected the increase of blank signals which interfered the signal measurement using spectrophotometric detection. Furthermore, sensitivity reduction was observed when 24–36 g L⁻¹ of molybdate was used. This may be the cause of the high blank signal due to light refraction of reagent that is higher than the absorption of blue colour products when using spectrophotometric detection. For phosphate analysis, the signal increased with increasing molybdate concentration from 6 to 24 g L⁻¹ and extremely decreased at the concentration of 36 g L⁻¹ (Figure 2(b)). However, the increase of blank signal was obtained, as found in the silicate experiment. In order to make a compromise between the absorbance signal of phosphate and silicate and the blank value, the concentration of molybdate was selected at 12 g L⁻¹.

The effect of Sn(II) concentration was investigated in the range of 0.15–1.25 g L⁻¹. The results in Figure 2(c) and (d) show that the signals of silicate and phosphate depict the similar trend. The signals increased by increasing Sn(II) concentration from 0.15 to 0.6 g L⁻¹. This could be due to the greater reducing agent concentration affecting higher product formation, but it also resulted in elevation of blank signal. Moreover, a dramatic decrease of signal at the concentration of 1.25 g L⁻¹ Sn(II) was observed, which is possible due to the similar effect observed at higher concentrations of molybdate concentration.
Concentration of stannous (II) chloride at 0.3 g L$^{-1}$ was selected as a compromise between the signals of phosphate, silicate, and the blank value. In this work, H$_2$SO$_4$ concentration of reagent R1 and R2 were varied from 0.2 to 1.2 mol L$^{-1}$. Signals of silicate and phosphate significantly decreased with increasing concentration of sulphuric acid (Figure 2(e,f)). Moreover, at lower concentration of acids, the blank signal was extremely high. This could be explained by the self-reduction of molybdate [30] resulting in the formation of blue colour in mixed reagent, especially in the presence of stannous chloride. This lead to shift in the baseline, which was not desirable. In contrast, at higher concentrations of H$_2$SO$_4$, there was no signal of silicate due to the masking of silicate at lower pH. For phosphate, similar effect was obtained at lower concentrations of H$_2$SO$_4$; however, it still emitted absorbance signals of phosphomolybnenum blue when concentrations of sulphuric acid exceed 0.8 mol L$^{-1}$. In order to optimise the sensitivity of phosphate and silicate, the concentration of sulphuric acid in reagent lines of molybdate and stannous chloride at 0.4 mol L$^{-1}$ was selected in the further studies.

**Figure 2.** Effect of molybdate concentration (a, b), stannous (II) chloride concentration (c, d) and sulphuric acid concentration (e, f) on the detection of 100 mgSi L$^{-1}$ silicate (a, c, and e) and 0.2 mgP L$^{-1}$ phosphate (b, d, and f) using CIA system.
3.1.2. Physical parameters

Study of physical parameters was carried out by using repetitive individual injections of 100 mgSi L\(^{-1}\) silicate and 1 mgP L\(^{-1}\) phosphate. Loading time of carrier, sample and reagents (in step 2 of Table 1) was determined by comparing the absorbance signal obtained from various loading time periods in the range of 5–25 s. The signal increased with increase in loading time for both silicate and phosphate detection, which is possible due to the effect of larger sample and reagents' volume. However, besides this observation, it also resulted in higher blank signal. Loading time at 20 and 25 s provided higher sensitivity, however, lower blank signal was obtained at 20 s. Hence, loading time of 20 s was selected for further studies because of the satisfactory sensitivity for both analytes.

Effect of MC length was investigated at 50, 100, 150 and 200 cm (PTFE with 1.0 mm i.d.). The results showed that increase in the signals of silicate, with the coil length of 50–150 cm, was achieved. This may be because the formation rate of silicomolybdenum blue was slower than phosphomolybdenum blue. Therefore, with longer MC length, silicate was held in the system for longer period that resulted in a higher signal. However, at extensive coil length, the sensitivity decreased because the sample zone was diluted and dispersed in the flow channel. For phosphate analysis, a decrease in the signal from 50 to 200 cm was achieved. This may be due to the effect of dilution and dispersion at longer MC length. In order to compromise between sensitivity of silicate and phosphate analysis, 150 cm of MC was selected for further investigation.

3.2. Analytical features of CIA system for determination of phosphate and silicate

Under optimum conditions, a linear response was obtained for phosphate concentration in the range of 0.1–6 mgP L\(^{-1}\) (Abs\(_{715}\) = (3.89 ± 0.08) \times 10\(^{-1}\)C\(_{P}\) + (8.53 ± 1.73) \times 10\(^{-3}\)) with a correlation coefficient of 0.997. For silicate, a linear response was obtained in the range of 5–100 mgSi L\(^{-1}\) (Abs\(_{810}\) = (4.41 ± 0.08) \times 10\(^{-3}\)C\(_{Si}\) + (1.15 ± 0.05) \times 10\(^{-1}\)) with a correlation coefficient of 0.999. Detection limit (3\(\sigma\) of blank) of phosphate and silicate in the CIA system was found to be 0.05 mgP L\(^{-1}\) and 3.5 mgSi L\(^{-1}\), respectively. Sample throughput was 40 injections h\(^{-1}\). Repeatability of the CIA method was obtained from 10 injections of 1 mgP L\(^{-1}\) and 10 mgSi L\(^{-1}\) standard solutions. The relative standard deviations (RSD) obtained were 1.76 and 1.53, respectively. Moreover, inter-day reproducibility of the method was 2.85 and 2.49 %RSD for phosphate and silicate, respectively. These considerably low RSD values represent satisfactorily good reproducibility of the CIA system.

3.3. Chemometrics approach with CIA system for simultaneous analysis of phosphate and silicate in water sample

The colorimetric method used in this work for simultaneous analysis of phosphate and silicate is molybdenum blue method as shown in the following equations:

\[
\text{PO}_4^{3-} + 12\text{MoO}_4^{2-} + 24\text{H}^+ \rightarrow [\text{PMo}_{12}\text{O}_{40}]^{3-} + 12\text{H}_2\text{O}
\]

\(\text{(molybdophosphoric acid)}\)
As shown in Figure 3, the absorption spectra of phospho- and silico-molybdenum blue overlapped over the wavelength of 400–900 nm (Figure 3(a,b)). There was no any selective wavelength for individual analysis. Therefore, determination of phosphate and silicate by spectrophotometric methods with univariate calibration, especially using molybdenum blue reaction, was very difficult due to mutual interference with each other. In this work, multivariate calibration, so-called PLS, was utilised to assist the spectrophotometric method for multicomponent analysis of phosphate and silicate using molybdenum blue reaction.

3.3.1. PLS with molybdenum blue method for simultaneous determination of phosphate and silicate

According to the PLS protocol for simultaneous analysis of phosphate and silicate discussed in Section 2.4, the synthetic calibration set containing different concentrations of phosphate and silicate were introduced to the CIA system to produce mixed complex of phospho- and silico-molybdenum blue. Absorption spectra were recorded at the wavelength between 400 and 900 nm. The spectra were submitted to PLS process for the construction of the calibration model.

In the calibration procedure for PLS method, the first step was the selection of the optimum number of factors (or calibration models). In order to select the number of factors in the PLS algorithms, a cross-validation method was applied by using calibration set of 12 calibration spectra. These standards were used to predict and find the internal...
error of the model. Each component (phosphate and silicate) was independently mod-
elled by PLS, employing the optimum wavelength (for phosphate from 420 to 634 nm
and for silicate from 805 to 900 nm). The optimum number of factors (calibration
models) for PLS components was chosen for each analyte by selecting the minimal
value of predictive residuals sum of squares (PRESS) and standard error of prediction
(SEP), which later was expected to provide accurate prediction (lowest value of %REP,
relative error of prediction). Table 2 shows statistic value of the PLS models of phosphate
and silicate. It was found that the lowest PRESS and SEP values were obtained for factors
5 and 7, for phosphate and silicate, respectively. Therefore, these factors were selected
as optimal calibration models.

The ability of the selected model to predict the concentration of standard in calibration
set was verified as shown in Table 2S (Supplemental data). It was found that the predicted
concentrations of both analyses agreed well with standard concentration in calibration
set with average recovery of 100.7 ± 10.4% and 100.2 ± 6.8% (n = 12) for phosphate and silicate,
respectively. It might be inferred that the selected number of factors was satisfactory.
Moreover, in order to confirm the suitability of the selected calibration model for determi-
nation of phosphate and silicate, the test sets were subjected to PLS analysis. Then, a second
validation was carried out through a test set (six mixture solutions obtained from random
design) whose concentration levels were different from those used for calibration set. The
purpose of the test set was to predict the concentration of the samples, in order to find
errors and predictive ability of the selected models. The amounts obtained in the determi-
nation of the test samples were compared with their actual values. The results in Table 3
show that the models permit the analysis of this binary mixture with good recoveries in the
range from 92.7 to 114.7%. This indicates that the chemometrics approach was successfully
applied for simultaneous analysis of phosphate and silicate.

### 3.3.2. Application to water samples

By using the optimum models discussed in Section 3.3.1, CIA system with data
evaluation by PLS was applied for simultaneous analysis of phosphate and silicate

**Table 2. Statistic values for the selection of the optimum number of factors of phosphate and silicate.**

| No. of factors | PRESS | SEP | REP (%) | \( r^2 \) | PRESS | SEP | REP (%) | \( r^2 \) |
|---------------|-------|-----|---------|---------|-------|-----|---------|---------|
| 0             | 7.130 | 0.71| 85.46   | −0.148  | 18000 | 35.9| 80.59   | −0.148  |
| 1             | 1.520 | 0.33| 39.49   | 0.755   | 3150  | 15.0| 33.72   | 0.799   |
| 2             | 1.520 | 0.33| 39.28   | 0.757   | 2490  | 13.3| 29.96   | 0.841   |
| 3             | 0.268 | 0.14| 16.58   | 0.957   | 429   | 5.53| 12.43   | 0.973   |
| 4             | 0.062 | 0.07| 7.97    | 0.990   | 270   | 4.4 | 9.88    | 0.983   |
| 5             | 0.049 | 0.06| 7.08    | 0.992   | 248   | 4.21| 9.46    | 0.984   |
| 6             | 0.052 | 0.06| 7.29    | 0.991   | 244   | 4.17| 9.38    | 0.984   |
| 7             | 0.054 | 0.06| 7.42    | 0.991   | 241   | 4.15| 9.33    | 0.985   |
| 8             | 0.054 | 0.06| 7.41    | 0.991   | 242   | 4.16| 9.34    | 0.985   |
| 9             | 0.054 | 0.06| 7.41    | 0.991   | 242   | 4.16| 9.34    | 0.985   |
| 10            | 0.054 | 0.06| 7.41    | 0.991   | 242   | 4.16| 9.34    | 0.985   |

*Obtained from the optimum wavelength of 420–634 nm.
Obtained from the optimum wavelength of 805–900 nm.
PRESS: Predictive Residuals Sum of Squares.
SEP: Standard Error of Prediction.
REP: Relative Error of Prediction.
in natural water samples. Water samples were collected from the canals around Bangkok area, which were analysed by the CIA with PLS methods. Recovery test of phosphate and silicate in water samples was studied by spiking six samples of water samples with standard mixture of phosphate, 0.2 mg L\(^{-1}\), and silicate, 10 mg L\(^{-1}\). As demonstrated in Table 3S (Supplemental data), recoveries were evaluated for the CIA system and were found to vary from 91\% to 108\%. The results indicated that matrices in water sample did not interfere with the determination. In order to test the reliability of the proposed method, the system was employed for the determination of phosphate and silicate in water samples collected from three sites; the results were compared with the conventional batchwise method as shown in Figure 4. By using paired \(t\)-test, the results obtained from CIA system agreed significantly well with the results of batchwise analysis (95\% confidence, \(t_{\text{stat}} = 2.28\), \(t_{\text{critical}} = 2.31\) and \(t_{\text{stat}} = 0.62\), \(t_{\text{critical}} = 2.31\) for phosphate and silicate, respectively). Moreover, linear relationship between the two sets of concentration values obtained from batchwise and the proposed method are \(\text{Conc}_{\text{batchwise}} = (0.98 \pm 0.03) \text{ Conc}_{\text{CIA}} - (0.01 \pm 0.06)\) for phosphate and \(\text{Conc}_{\text{batchwise}} = (1.18 \pm 0.23) \text{ Conc}_{\text{CIA}} - (0.35 \pm 0.42)\) for silicate. These results indicate that the determination of phosphate and silicate by CIA system using the chemometrics approach with spectrophotometric detection was well satisfied. The verified method was successfully applied for analysis of phosphate and silicate in water samples collected from the canals around Bangkok area.

Beside the results in Figure 4, samples can be divided into three groups according to the sampling areas (X, Y and Z). Group ‘X’ was collected from the canal at suburb place of Bangkok with agriculture area. Group ‘Y’ and group ‘Z’ were collected from the canals in modulate community of Bangkok and the canals from the polluted canals in centre of Bangkok, respectively. Sample in group ‘X’ showed phosphate concentrations lower than the other groups. However, all of samples contained phosphate levels above the limit of problematic excessive algal growth in water (0.1 mgP L\(^{-1}\)) [31]. As expected, group ‘Z’ sample contained surplus amounts of phosphate and silicate, which can be categorised as contaminated water. It is inferred from this result that group ‘Z’ samples is the effluence from the area that has high consumption of synthetic detergent. It should be noted that water treatment is required for domestic wastewater. According to our knowledge, constructed wetlands water treatment system is the simplest and green approach for improvement of water quality. The system is a natural process involving wetland vegetation, soil and their associated microbial assemblages to improve water quality without utilisation of any chemicals [32]. We would suggest that the constructed wetlands might be an alternative remarkable approach for domestic wastewater treatment in Bangkok city.

### Table 3. Predicted concentration of phosphate and silicate in test set by using the selected models.

| Test no. | Phosphate (mgP L\(^{-1}\)) | Silicate (mgSi L\(^{-1}\)) |
|----------|-----------------------------|-----------------------------|
|          | True Value | Predicted value ± SD\(^{a}\) | Recovery (%) | True Value | Predicted value ± SD\(^{a}\) | Recovery (%) |
| T1       | 0.5        | 0.52 ± 0.01 | 103.2        | 20         | 22.9 ± 0.6 | 114.7        |
| T2       | 0.5        | 0.53 ± 0.01 | 106.6        | 50         | 49.8 ± 4.4 | 99.7         |
| T3       | 1.5        | 1.39 ± 0.14 | 92.7         | 30         | 33.4 ± 1.3 | 111.4        |
| T4       | 0.3        | 0.30 ± 0.05 | 100.0        | 70         | 74.3 ± 1.3 | 106.2        |
| T5       | 1.0        | 0.99 ± 0.03 | 99.0         | 50         | 52.0 ± 1.9 | 104.0        |
| T6       | 1.5        | 1.40 ± 0.01 | 99.3         | 60         | 61.4 ± 0.1 | 102.3        |

\(^{a}\) Standard deviation (SD) obtained from three replicated injections.
3.3.3. Comparison of performance of the proposed method with previous methods for analysis of phosphate and silicate

A comparison of analytical features of the present CIA–PLS method with other spectrophotometric methods for analysis of phosphate and silicate is summarised in Table 4. Multivariate calibration (principal component analysis ‘PCA’ and PLS) methods coupled with spectrophotometric measurement of molybdenum blue complexes have been reported for simultaneous analysis of phosphate and silicate [19,20]. However, the methods are not automated, since they are operated using batchwise approach. Moreover, these methods are very time-consuming (analysis time of 30 min) and requires large amounts of sample and reagents. The SIA method has also been reported for the purpose of automation [25,26]. In the SIA method proposed by Masina et al. [25], phosphate and silicate were analysed consecutively using different acidity and masking agents. In comparison with our present method, the two analytes were completely analysed with single injection using CIA system coupled with PLS for data evaluation. The SIA method for simultaneous analysis of phosphate and silicate was proposed by Cerda et al. using the concept of large sample volume, in such way that each species is
Table 4. Comparison of experiment characteristics and analytical performance of the proposed CIA–PLS method towards previously reported method for analysis of phosphate and silicate.

| Ref. | [19] | [20] | [25] | [26] | Present work |
|------|------|------|------|------|--------------|
| 1. System | Batch | Batch | SIA | SIA | CIA-PLS |
| 2. Detection compounds | Phospho- and silico-molybdenum blue | Phospho- and silico-molybdenum blue | Phospho- and silico-molybdenum blue | Vanadomolybdo phosphate and molybdosilicate | Phospho- and silico-molybdenum blue |
| 3. Method for analysis of two analytes | Multivariate calibration (PCA) | Multivariate calibration (PLS) | Using different acidity and oxalic acid as masking agent | Using different acidity and oxalic acid as masking agent coupled with the concept of large sample volume | Multivariate calibration (PLS) |
| 4. Type of sample | Detergent and river water | Brackish water | Water and sediment | Wastewater | Natural water |
| 5. Sample consumption (mL) | 1–10 | 40 | 0.275 | 3 | 0.03 |
| 6. Total reagents consumption (mL) | 3.5 | 4 | 0.8 | 0.3 | 0.06 |
| 7. Working range | 0.01–3 mgP L⁻¹ | 0–0.62 mgP L⁻¹ | 0.2–7 mgP L⁻¹ | 0–12 mgP L⁻¹ | 0.1–6 mgP L⁻¹ |
| | 0.02–6 mgSi L⁻¹ | 0–1.12 mgSi L⁻¹ | 5–50 mgSi L⁻¹ | 0–3.62 mgSi L⁻¹ | 5–100 mgSi L⁻¹ |
| 8. Limit of detection | - | - | 0.1 mgP L⁻¹ | 0.2 mgP L⁻¹ | 0.05 mgP L⁻¹ |
| | - | - | 1.0 mgSi L⁻¹ | 0.9 mgSi L⁻¹ | 3.5 mgSi L⁻¹ |
| 9. Sample throughout (h⁻¹) | 2 (for 2 analytes) | 2 (for 2 analytes) | 75 (for PO₄³⁻ analysis) | 23 (for 2 analytes) | 40 (for 2 analytes) |

CIA, cross-injection analysis; PLS, partial least squares.
analysed at the end of the sample zone [26]. It was observed that our present work has advantages of a small sample volume with high sample throughput for the simultaneous determination of phosphate and silicate (40 h⁻¹). In the view of a comparison of system operation between SIA and CIA, the introduction of liquids into the analytical flow path of CIA is also different to that of the SIA. When we simultaneously aspirate the sample and reagents through the y-channels, we also aspirate at the same time the carrier flow through the x-channel. By using this manipulation, cross-flow was achieved inside the CIA platform, which results in premixing in analytical flow path (horizontal channel). It can be said that mixing of sample and reagents inside the analytical flow path in CIA is superior to those obtained by SIA system, leading to a significant improvement in the sensitivity of CIA system [27].

4. Conclusions

A new alternative method for simultaneous determination of phosphate and silicate by using CIA system coupled with data evaluation based on multivariate calibration techniques, PLS, was developed. The ‘CIA’ flow-based technique has a major difference with ‘SIA’ in terms of mixing inside the analytical flow path. In SIA, all samples and reagents are aspirated sequentially into the flow path. The mixing is performed only at the radial interface between the aspirated zones. However, mixing in SIA is enhanced by using flow reversal several times in the holding coil prior to enter to the detector. However, this reversal procedure decreases the sample throughput of the SIA techniques. Unlike the SIA, liquid introduction in CIA is simultaneous. The employment of cross-flow mode results in premixing inside the platform, which results in improvement in sensitivity and higher sample throughput. Moreover, based on the general benefit of CIA system, low reagent consumption with the automated system was achieved. The CIA system together with chemometrics-assisted spectrophotometric method provides a simple and rapid procedure for the simultaneous analysis of phosphate and silicate without prior separation steps. Moreover, the analysis of two parameters can be completed within 1.5 min with only single injection.

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Disclosure statement

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