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Stopped-flow injection simultaneous determination of phosphate and silicate using molybdenum blue[☆]

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Abstract

Kinetic information for the phosphate–molybdate–ascorbic acid reaction can be obtained by making use of a very simple manually operated stopped-flow injection (FI) system. Various parameters (concentrations of reagents, flow rate, mixing coils, and volume of flow cell) were investigated for determination of phosphate. A stopped-FI system should be arranged for low degree of mixing (of reactants) and low dispersion so that good signals of rate changes will be observed. Simultaneous determination of phosphate and silicate by the stopped-FI technique is proposed, using a laboratory-made semi-automatic stopped-FI Analyzer with LED-based photometer. It is based on kinetic separation of phosphate and silicate using molybdenum blue. The proposed procedure has been demonstrated for the application to water samples. The results obtained agree with that of a standard method.

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Keywords: Stopped-FI; Simultaneous determination; Phosphate; Silicate; Molybdenum blue; Kinetics

1. Introduction

Since the first application by Ruzicka and Hansen [1] for the determination of phosphate using molybdenum blue, a number of flow injection (FI) techniques have been reported for phosphate and silicate. They involve a continuous mode, such as on-line microwave digestion [2], determination of phosphate in silicate rocks by adding tartaric acid [3], on-line column separation [4–7]. Sequential injection analysis (SIA) has been applied to determine phosphate and silicate simultaneously by forming vanadomolybdo complexes [8], or using the molybdenum blue [9]. SIA with lab-on-valve using a stopped flow mode for determination of phosphate has been proposed [10,11].

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Kanaya and Hiromi [12] used stopped-flow to record the progress curve for the formation of the colored complex of 12-molybdophosphate with malachite green. Linares et al. [13] described the fluorometric differential kinetic determination of silicate and phosphate using two measurements at different times. Yoza et al. [14] determined ortho-, di-, and triphosphates using stopped-flow, based on substitution reactions with colored metal complexes. Lacy et al. [15] used sorbent extraction on a hydrophobic sorbent and optosensing measurement to determine mixtures of phosphate and silicate, based on differences in the reduction rates of the heteropoly complexes, using partial least squares analysis.

In this work, we employ stopped-FIA with simple instrumentation for kinetic information on phosphate and silicate using the molybdate and ascorbic acid reactions. Simultaneous determination of phosphate and silicate based on kinetic separation, using a simple semi-automatic stopped-FI Analyzer is proposed.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were analytical grade unless otherwise stated. Deionized water was used throughout. A stock phosphate standard solution ($1000 \mu\text{g ml}^{-1}$) was prepared by dissolving potassium dihydrogen phosphate (Merck, 0.4390 g) in a portion of water before making up to a volume of 100.00 ml. A commercial silicate standard solution of $1000 \mu\text{g ml}^{-1}$ (Merck) was used. Working phosphate and silicate standard solutions were obtained freshly by appropriate dilutions of the stock solutions.

A sodium molybdate solution (0.6% w/v) was prepared by dissolving sodium molybdate (Merck, 1.512 g) in 100 ml water, and 3.25 ml of conc. HNO_3 before making to a volume of 250.0 ml with water.

An ascorbic acid solution (0.5% w/v) was prepared freshly from 2.5 g of L-ascorbic acid (Fluka) which was dissolved in 500-ml water.

2.2. FI manifolds with manual operation

Two FI manifolds for manual operation were designed, as depicted in Fig. 1. In manifold I, a standard/sample solution (S) was injected via an injection valve (FIA-lab, USA, with a sample loop of $100 \mu\text{l}$) into a molybdate line with a mixing coil (MC1) before merging with the ascorbic acid stream, passing through another mixing coil (MC2) before entering into a flow cell in a spectrometer (Spectronic 21, Spectronic Instruments, USA), connected to a chart recorder (R100 A, Perkin-Elmer, USA). Manifold II represents a different arrangement, in which a standard/sample solution (S) was injected into a merged stream of molybdate and ascorbic acid via an injection valve placed between mixing coils (MC3 and MC4).

2.3. Semi-automatic FI Analyzer

The laboratory-made system (Fig. 2) consists of a controller via a microprocessor to control a peristaltic pump (S-mini, Alitea) to propel re-

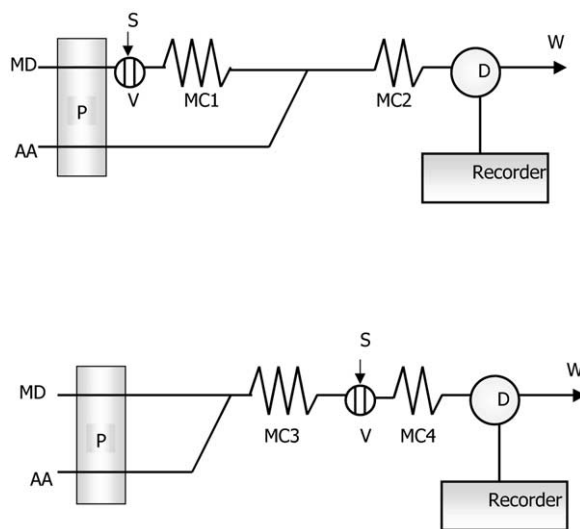


Fig. 1. FI manifolds with manual operation for stopped-FIA for phosphate, *top*: manifold I, S injected into MD before merging with AA; *bottom*: manifold II, S injected into a stream of mixed MD and AA; MD = molybdate solution, AA = ascorbic acid solution, P = peristaltic pump, MC = mixing coil, D = spectrometer, V = injection valve, W = waste.

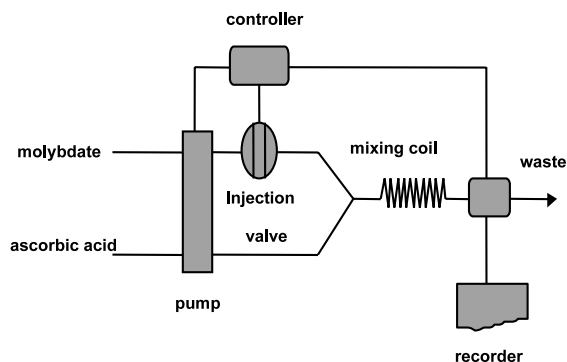


Fig. 2. Semi-automatic FI Analyzer.

agents, to switch an injection valve (V-451, Upchurch), and to set a photometer for data acquisition and to evaluate the signals. The photometer is a laboratory-design, using a LED red light source, with a peak height holding feature, similar to the ones previously reported [16–18]. The output signals can be via built-in digital read-out and/or a chart recorder (Philips, PM 8251, Holland). Signal profiles are illustrated in Fig. 3. The controller can be used to preset the traveling time (T), the period of flow between the point of injection to the point at which the flow is stopped for monitoring the reaction development at the flow-cell; the stopping time (S), the period during the flow stopped; and the washing time (W), the period when the stream is re-started to flow (after stopping) until this operation cycle ends and is ready for the next cycle. I and D , the signals at the first and last stopped points, respectively, will be given as digital read-outs on the photometer. Fig. 3(b–d) demonstrate stopped-FI recordings for phosphate, silicate, and mixtures of the standard solutions.

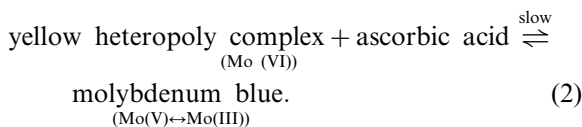
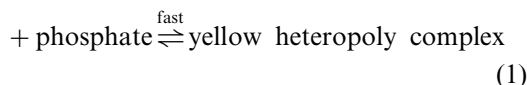
3. Results and discussion

3.1. Continuous FIA for phosphate

In preliminary investigation, it was found that the reactions to form the molybdenum blue product are reversible and will be in equilibrium. For continuous FI determination of phosphate, manifold II should be employed. Manifold II

resulted in higher FI responses compared to the peaks obtained by using manifold I. Calibrations (a plot of peak height vs concentration) were: $y_1 = 65.08x_1 + 9.76$, $r^2 = 0.998$ and $y_2 = 91.93x_2 + 15.39$, $r^2 = 0.991$ for the manifolds I and II, respectively ($0.5\text{--}3\text{ }\mu\text{g P ml}^{-1}$). The reactions for molybdenum blue may involve [19]:

heptomolybdate



Using manifold II with a premixed stream of molybdate and ascorbic acid, the reactive intermediate of the reduced form of molybdate will be readily available and reactive to the injected phosphate, to yield the molybdenum blue very fast (on the order of seconds), whereas in the manifold I, phosphate injected into molybdate stream will form an observed yellow product of phosphomolybdate which is not as reactive, with slow reduction to yield molybdenum blue (on the order of minutes). This was found in the batch molybdenum blue method for determination of phosphate. It should be noted that for batch analysis, if molybdate was premixed with ascorbic acid before adding phosphate, no molybdenum blue was observed at all.

Using manifold I with MC1 and MC2 being 25 and 50 cm, respectively, and ascorbic acid solution being 0.5% w/v, molybdate concentrations were varied (0.1–1.2% w/v). It was observed (Fig. 4(a)) that the same steady state of the system should be established in the molybdate concentrations in the range of 0.5–1% w/v. Similar observation was found for the stopped-FIA studies (Fig. 4(b)). A lower molybdate concentration would cause slower approach to equilibrium. Disproportionation of Mo(VI) and Mo(III) yielding Mo(V) may occur if a higher concentration of molybdate is used. This shifts the equilibrium in Eq. (2), resulting in less phosphomolybdenum(V) blue.

Similarly, a study on the effect of ascorbic acid concentrations (Fig. 5(a and b)) indicated that the

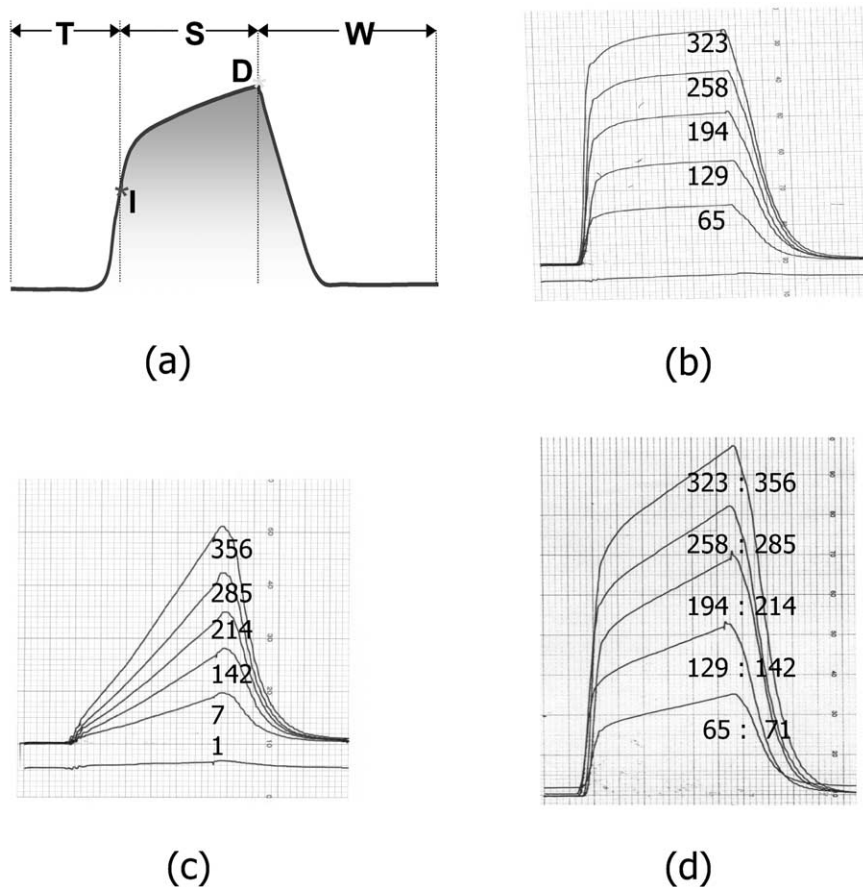


Fig. 3. Stopped-FI profiles obtained (a) in general, (b) phosphate, (c) silicate, (d) mixture of phosphate and silicate: T = traveling time, S = stop time, W = washing time, I = signal at the first stopped point, D = signal at the last stopped point before resuming flow. The numbers refer to concentrations [μM].

system with 0.3–0.8% w/v ascorbic acid resulted in reaction rates at steady state for the molybdate used (0.8% w/v). With lower concentrations of ascorbic acid, different rates were observed.

3.2. Stopped-FI determination of phosphate

At the flow-cell, in the stream, a bolus of the mixture (phosphate, molybdate and ascorbic acid) is stopped, and an increase in signal is observed for a period before gradually becoming constant (Fig. 3(b)). The signal represents the rate of the reactions, which come into equilibrium as discussed earlier.

A manifold used for stopped-FIA should provide both a low degree of axial mixing of reactants and low dispersion, to provide a suitable bolus of reaction mixture stopped at the flow cell for monitoring the reaction progress.

It was found that the manifold I (Fig. 1) without mixing coil and using a 8- μl flow cell is more suitable, compared to manifold II for stopped-FI determination of phosphate (Fig. 6). A dispersion study using a dye (bromothymol blue) [20] for manifold I is summarized in Table 1. As expected, apart from the effect of mixing coil length on dispersion, a higher volume flow cell creates more dispersion. It should also be noted that a signifi-

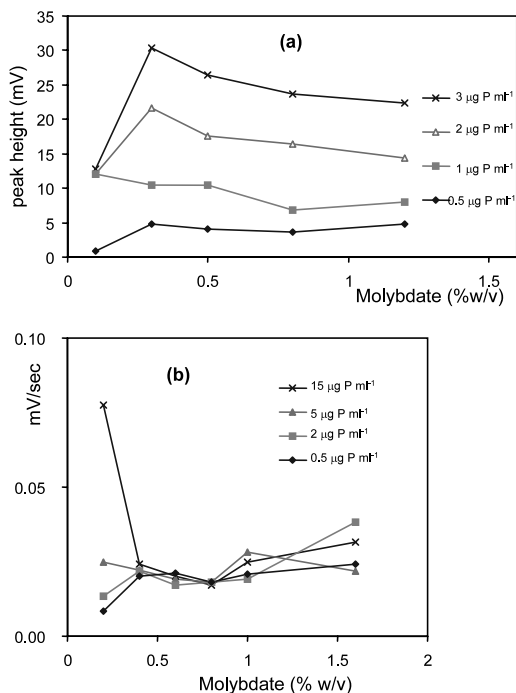


Fig. 4. Effect of molybdate: (a) continuous-FI; (b) stopped-FI.

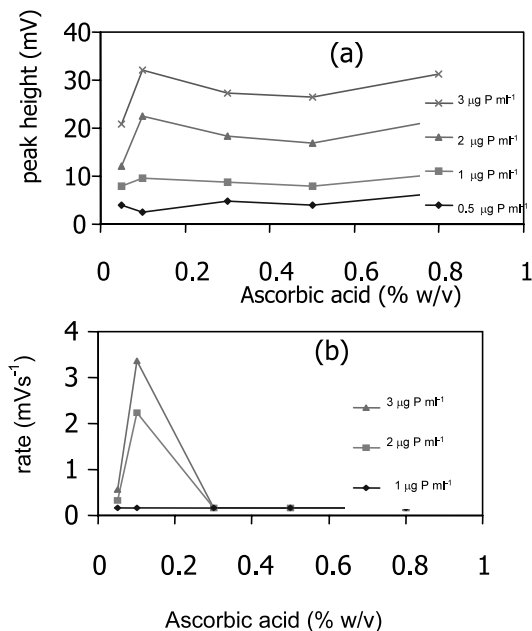


Fig. 5. Effect of ascorbic acid, (a) continuous-FI; (b) stopped-FI.

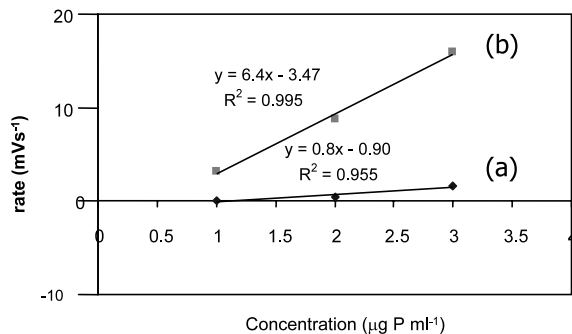


Fig. 6. Plot of reaction rates and phosphate (as P) concentrations using manifold I: (a) with and (b) without mixing coil.

cant effect of flow rate on dispersion was not observed.

3.3. Kinetic information obtained by the stopped-FIA

For a general reaction:



with a rate expression: $r = d[C]/dt = k[A]^n$, where r = rate, k = rate constant, n = order of reaction, or $\ln r = \ln(k[A]^n)$, then:

$$\ln r = \ln k + n \ln[A] \quad (4)$$

From Eq. (4), a plot of $\ln r$ vs $\ln[A]$, the order of the reaction can then be obtained from the slope (n), and the rate constant (k) can be evaluated from the y -intercept.

From the stopped-FI experiments for phosphate (Table 2), the rate (mV s^{-1}) was obtained from each stopped-FI recording for each phosphorous (phosphate) concentration (i.e., slope of the signal). By assuming that the reagents (molybdate and ascorbic acid) were in large excess, then the rate of product formation should be proportional to the phosphorous (phosphate) concentration. A plot of $\ln(\text{rate})$ and $\ln(\text{phosphorous concentration})$ was found to be linear: $y = 1.3x - 3.99$, $r^2 = 0.992$ (Fig. 7). The slope of 1.3 reflects the order of the reaction under the experimental conditions to be close to unity, in agreement with other reports [21,22]. The rate constant obtained was $1.9 \times 10^{-2} \mu\text{M s}^{-1}$.

Table 1
Studies on dispersion in the manifold I

Total flow rate (ml min ⁻¹)	Dispersion (C_0/C_{\max})			
	With mixing coils ^a		Without mixing coil ^b	
	8- μ l flow-cell	80- μ l flow-cell	8- μ l flow-cell	80- μ l flow-cell
2	3.1	4.9	1.2	2.8
3	2.9	4.7	1.3	2.8
4	2.7	4.1	1.1	2.8

^a MC1 = 25 cm, MC2 = 50cm.

^b No MC1 and MC2.

Table 2
Experiments for kinetic information on the reactions of phosphate with molybdate and ascorbic acid

Phosphate (P) concentration		ln P	Rate of reaction ^a		Ln (rate)
(μ g P ml ⁻¹)	(μ M)		(mV s ⁻¹)		
0.5	16.4	2.80	8.0×10^{-1}		-2.2×10^{-1}
1	32.8	3.49	1.6		4.70×10^{-1}
2	65.6	4.18	4.4		1.48
3	98.4	4.59	8.0		2.08

^a d[Product]/dt.

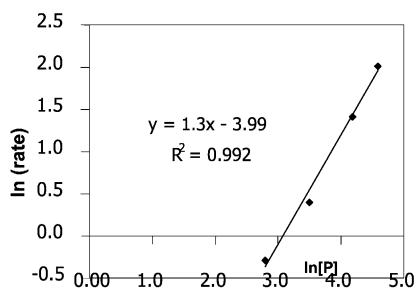


Fig. 7. Plot of ln(rate) vs ln[Phosphate].

3.4. Simultaneous determination of phosphate and silicate by stopped-FIA

The rate of the phosphate–molybdate–ascorbic acid reaction is faster than that of the silicate–molybdate–ascorbic acid reaction. It is then possible to determine phosphate and silicate simultaneously using the kinetic separation approach.

Fig. 3(b–d) show stopped-FIA recordings of phosphate, silicate, and a mixture of the two. By using the semi-automated stopped-FI Analyzer (Fig. 2), concentrations of phosphate and silicate in a mixture can be evaluated from I_P , I_{Si} and I_{mix} (signals at the first stopped points for phosphate, silicate and mixture, respectively, see Fig. 3), and D_P , D_{Si} and D_{mix} (signals at the last stopped points for phosphate, silicate and mixture, respectively, see Fig. 3).

Employing the conditions summarized in Table 3, calibration plots were obtained from the experimental results (Fig. 3). For phosphate standards (up to 15 μ g P ml⁻¹):

$$I_P = 13.31[P] + 9.20, \quad r^2 = 0.986 \quad (5)$$

Table 3
Conditions for simultaneous determination of phosphate and silicate with molybdate using the semi-automatic stopped-FI Analyzer

Condition	Value
Molybdate concentration, % w/v in 0.18 M HNO ₃	0.6
Ascorbic acid concentration, % w/v	0.5
Injection volume, μ l	35
Mixing coil length, cm	50
Flow rate (each line), ml min ⁻¹	3.2 ± 0.2
Travel time, s	2.5
Stop time, s	20
Wash time, s	15
Light source	Red LED (630 nm)

Table 4

Determination of phosphate and silicate by the proposed stopped-FI method and the standard method [23]

Water sample	Phosphate ($\mu\text{g P ml}^{-1}$)		Silicate ($\mu\text{g Si ml}^{-1}$)		Phosphate added ^a ($\mu\text{g P ml}^{-1}$) (C)	% Recovery of phosphate ^b	
	Stopped-FI method (A)	Standard method (B)	Stopped-FI method	Standard method		Stopped-FI method (D)	Standard method (E)
Reservoir	n.d.	n.d.	8.2	8.6	–	–	–
	1.6	1.8 ^a	9.4	8.7	2.0	80	90
Pond	n.d.	n.d.	7.6	7.9	–	–	–
	5.7	3.5 ^a	7.5	8.0	4.0	143	88
Irrigation canal	n.d.	n.d.	8.3	8.2	–	–	–
	6.5	5.7 ^a	7.8	8.3	6.0	108	95
Moat	n.d.	n.d.	8.1	9.0	–	–	–
	6.3	7.9 ^a	7.3	8.9	8.0	79	99
Drainage (from a dormitory)	1.1	1.2	11.9	13.9	–	–	–
	3.1	3.1 ^a	11.2	13.9	2.0	100	95

^a Total of present + added.^b % Recoveries evaluated by: $D = (A/C) \times 100$ and $E = (B/C) \times 100$.

For correlation of D_P (mV) and I_P (mV):

$$D_P = 4.65I_P - 3.38, \quad r^2 = 0.998, \quad (6)$$

For silicate standards (up to $15 \mu\text{g Si ml}^{-1}$):

$$D_{\text{Si}} = 35.82[\text{Si}] + 30.55, \quad r^2 = 0.999. \quad (7)$$

The concentration of phosphate in a mixture or a sample is obtained from the calibration plot of phosphate standards (Eq. (5)) by using I_{mix} from the mixture or the sample for I_P (as at the first stopped point, there should be no contribution due to silicate). Then D_P is calculated from Eq. (6). In the signal profile of the mixture/sample, calculation for signal due to silicate at the last stopped point, $D_{\text{Si,mix}}$ is made from the correlation:

$$D_{\text{Si,mix}} = (D_{\text{mix}} - D_{P,\text{mix}}) + I_P. \quad (8)$$

$D_{P,\text{mix}}$ is the contributed signal due to phosphate in the mixture and can be evaluated from the Eq. (6). The last term (I_P) is for contribution from the initial (background) signal due to phosphate. Substituting the value of $D_{\text{Si,mix}}$ for D_{Si} in Eq. (7), one obtains the concentration of silicate in the mixture/sample.

The proposed procedure has been applied to several natural water samples. The results are compared to that obtained by the standard method [23], as summarized in Table 4.

4. Conclusion

A stopped FI system offers kinetic information on the phosphate–molybdate–ascorbic acid reaction. Simultaneous determination of phosphate and silicate in a mixture/sample is proposed by employing kinetic separation using molybdenum blue, and using a laboratory-made semi-automatic stopped-FI Analyzer with a microprocessor to control the system and for signal read-out. The proposed procedure has been demonstrated for its application to water samples by validation with a standard method.

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