Simultaneous spectrophotometric determination of phosphate and silicate ions in river water by using ion-exclusion chromatographic separation and post-column derivatization

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Abstract

The simultaneous spectrophotometric determination of phosphate and silicate ions in river water was examined by using ion-exclusion chromatography and post-column derivatization. Phosphate and silicate ions were separated by the ion-exclusion column packed with a polymethacrylate-based weakly acidic cation-exchange resin in the H^+-form (TSKgel Super IC-A/C) by using ultra pure water as an eluent. After the postcolumn derivatization with molybdate and ascorbic acid, so-called molybdenum-blue, both ions were determined simultaneously by spectrophotometry. The effects of sulfuric acid, sodium molybdate and ascorbic acid concentrations and reaction coil length, which have relation to form the reduced complexes of molybdate and ions, on the detector response for phosphate and silicate ions were investigated. Under the optimized conditions (color-forming reactant, 50 mM sulfuric acid-10 mM sodium molybdate; reducing agent, 50 mM ascorbic acid; reaction coil length, 6 m), the calibration curves of phosphate and silicate ions were linear in the range of 50 - 2,000 μg L^{-1} as P and 250 - 10,000 μg L^{-1} as Si. This method was successfully applied to water quality monitoring of Kurose-river watershed and it suggested that the effluent from a biological sewage treatment plant was significant source of phosphate ion in Kurose-river water.

Keywords: ion-exclusion chromatography; phosphate ion; silicate ion; molybdenum-blue; river water

1. Introduction

The eutrophication phenomenon is one of the serious environmental problems throughout the world [1]. A high loading of nutrients such as phosphate ion from watershed to semi-closed water area such as lake and estuary eventually stimulate the leading to algae growth, resulting in a bloom which is a very abundant dense growth of algae. In addition to phosphate ion, silicate ion is also one of the nutrients for phytoplankton diatoms in order to build rigid diatom frustules. In recent years, the silica deficiency in freshwater was caused by the accumulation of diatom frustules in bottom sediment, leading to a changing of phytoplankton species composition in the coastal seawater [2]. Therefore, the monitoring of these nutrients in waters is important for evaluating and controlling the water quality and investigation of biogeochemical cycles of these nutrients.

The traditional method for the determination of phosphate and silicate ions in natural water and wastewater was conducted by using chemical derivatization with molybdate, that is called molybdenum-yellow and molybdenum-blue methods, followed by spectrophotometry [3]. On the basis of these chemical derivatization, the flow injection analysis (FIA) has been applied successfully to determine phosphate and silicate ions in various waters [4-6].

Ion-exclusion chromatography (IEC) has been the one of convenient method to determine weakly basic cations, weakly acidic anions and both of cations and anions [7-11]. In the case of IEC using a separation column packed with weakly acidic cation-exchange resin in the H^+-form, weakly acidic anions are separated by the penetration reaction of anions to the resin associated to Donnan's membrane effect. In general, dissolved silicate in natural waters present as non-ionized form of H_4SiO_4 ($pK_{a1} = 9.83$), while dissolved phosphate is mainly present as $H_2PO_4^-$ form ($pK_{a1} = 2.15$, $pK_{a2} = 7.20$, $pK_{a3} = 12.35$) [12]. Therefore, the ion-exclusion chromatography is effective method for separation of these ions.

Previously, Ikedo et al. reported the selective and simultaneous determination of phosphate and silicate ions in water by using IEC coupled with UV-detection after postcolumn derivatization of molybdenum-yellow [13]. This method was applied to determine phosphate and silicate ions in leaching process waters for ceramics glaze raw materials. However, more sensitive method than the previous method is required to determine simultaneously phosphate and silicate ions, due to the low concentration of these nutrients, particularly phosphate ion, in natural and waste waters.

In present study, the postcolumn chemical derivatization methods of molybdenum-blue, which are generally more sensitive method than that of molybdenum-yellow, was examined to determine phosphate and silicate ions in river water. After the separation of phosphate and silicate ions by the column of IEC with ultra pure water used as an eluent, the reduced complexes of molybdate with phosphate and silicate ions by adding ascorbic acid were determined by spectrophotometry. The present methods were also applied for the simultaneous and selective determination of phosphate and silicate ions in the Kurose-river water in Hiroshima prefecture.

2. Experimental

2.1. Apparatus

The ion chromatograph consisted of two dual pumps (DP-8020, Tosoh), on-line degasser (SD-8022, Tosoh), oven for separation column (CO-8020, Tosoh) and UV/VIS spectrophotometric detector (UV-8020, Tosoh). These apparatuses were controlled by chromatographic workstation (LC-8020 Model-II, Tosoh). To flow a reducing agent of ascorbic acid solution, a HPLC pump (PU-980, JASCO) was used. The injection volume was 100 μL. A separation column used in this study was TSKgel Super IC-A/C (150 mm × 6.0 mm i.d., Tosoh), packed with a

polymethacrylate-based weakly acidic cation-exchange resin in the H^+-form (0.2 mequiv. mL^{-1}). The flow lines and reaction coil were made from polyetheretherketone (PEEK) tube with 0.25 mm of inner diameter. Both of column and coil were kept in the column oven operated at 45°C. The wavelength of the detector was set at 700 nm.

2.2. Reagents

All reagents of analytical reagent grade (Wako Co., Japan) were used as received. The commercial standard solutions of phosphate ion (1000 mg L^{-1} as P) and silicate ion (1000 mg L^{-1} as Si) were used as stock standard solutions. As a color-forming reactant for the molybdenum-blue method, a mixture of sulfuric acid and sodium molybdate was prepared. A reducing agent of ascorbic acid solution was prepared before use and stored in foil-wrapped polyethylene bottle. This solution was stable at least 24 hours. The ultra pure water (>18 MΩ cm, Millipore Co.) was used as an eluent and for the preparation of the standard solution and the reagents.

2.3. River water

River water samples were collected from the Kurose-river watershed (*n* = 8, pH 7.2 - 8.0) in Higashi-hiroshima city, western Japan on 28 September, 2007. This river is a small river (238.8 km^2 of flow passage area, 50.6 km of river channel length) that flows through the center of Higashi-hiroshima city, where the population has increased steadily with in last decade and the pollution from a sewage treatment plant significantly contributes to the river [14, 15], into the Seto Inland Sea. After filtration through a hydrophilic polytetrafluoroethylene (PTFE) syringe filter with 0.2 μm pore size (DISMIC®-25$_{HP}$, Advantec Toyo Kaisha, Ltd.), the water samples were stored in

the dark room at 5 $^{\circ}$C until the analysis.

3. Results and discussion

3.1. Optimization of molybdenum-blue method for simultaneous determination of phosphate and silicate ions after ion-exclusion chromatographic separation

To optimize the analytical conditions for molybdenum-blue, the effects of sulfuric acid and sodium molybdate concentrations in the color-forming reactant on the detector responses for phosphate and silicate ions were examined. As shown in Fig. 1 for the reactant containing 10 mM sodium molybdate, it indicated that the detector response for phosphate ion dramatically increased with the concentration of sulfuric acid until at 15 mM and it remained unchanged. For silicate ion, the detector response gradually increased due to increasing the concentration of sulfuric acid. The highest and steady detector response for both phosphate and silicate ions were obtained at the concentration of *ca.* 50 mM sulfuric acid. Therefore, the optimized sulfuric acid concentration in the reactant was judged to be 50 mM.

In Fig. 2, it shows the effect of concentration of sodium molybdate in the color-forming reactant containing 50 mM sulfuric acid on the detector response for phosphate and silicate ions. The detector response for phosphate ion remains almost unchanged while increasing the concentration of sodium molybdate in the reactant, whereas that of silicate ion increased noticeably due to increasing the concentration of sodium molybdate. On the other hand, the baseline was gradually noisy due to increasing the concentration of sodium molybdate, especially above 10 mM sodium molybdate. The highest signal-to-noise ratio (*S/N*) of silicate at 1,000 μg L^{-1} was obtained at the concentration of *ca.* 10 mM sodium molybdate. Therefore, the optimized sodium molybdate

concentration was judged to be 10 mM.

In this study, ascorbic acid was used as reducing agent. The detector response for phosphate ion slightly increased by increasing the concentration of ascorbic acid, whereas that for silicate ion increased with the concentration of ascorbic acid until 50 mM then. From this result, 50 mM ascorbic acid was selected as the reducing agent.

The reaction coil length was investigated for 0, 2, 4, 6 and 8 m in terms of the peak resolution and the detector responses for both phosphate and silicate ions. As a result, the peak resolutions between phosphate and silicate ions were almost unchanged. However, the highest detector response of phosphate ion was obtained for a 6 m of coil length, while that of silicate increased by increasing the length of reaction coil. Therefore, the 6 m of reaction coil length was chosen.

Under the optimized separation and detection conditions, a good simultaneous IEC separation of phosphate and silicate ions was obtained, as shown in Fig. 3A.

3.2. Analytical performances

Under the optimized conditions, the linear calibration curves of phosphate and silicate ions using the present IEC coupled with molybdenum-blue were obtained in the range of 50 - 2,000 µg L^{-1} as P for phosphate ion and 250 - 10,000 µg L^{-1} as Si for silicate ion (r^2 = 0.9998 and 0.9993, respectively).

The detection limits of phosphate and silicate ions based on 3 times the standard deviation of a lowest standard solution (50 µg L^{-1} for phosphate ion and 250 µg L^{-1} for silicate ion) injected 10 times were 1.5 and 8.7 µg L^{-1}, respectively. This detection limit of phosphate ion was superior to that by the previous system [13] with molybdenum yellow (4.75 µg L^{-1}), while that of silicate ion

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was inferior (2.58 μg L^{-1}). It is enough to determine the silicate ion using this system due to the concentration of silicate ion is observed at several mg L^{-1} levels in river water [16].

The relative standard deviation (RSD) of peak area in the 10 times-repeated of chromatographic runs for phosphate ion at 50 μg L^{-1} and silicate ion at 250 μg L^{-1} were 1.05% and 1.15%, respectively, and that of retention time was 0.41% and 0.30% using IEC coupled with molybdenum-blue.

3.3. Application to water quality monitoring of river water

For the application of present system to monitor the water quality, the concentrations of phosphate and silicate ions were determined in river water samples collected from Kurose-river watershed, Higashi-hiroshima city, western Japan. As shown in Fig. 3B, phosphate and silicate ions in the authentic river water samples could be selectively and simultaneously determined. When adding 500 μg L^{-1} of phosphate ion and 2,500 μg L^{-1} of silicate ion in the river water, the recoveries were 99.0% and 97.1 %, respectively.

The summary of determination of phosphate and silicate ions in the Kurose-river water collected from upstream to downstream are shown in Fig. 4. The concentration of phosphate ion was gradually increased from upstream to downstream which area was more urbanized. Especially, the concentration was dramatically increased after the discharge from a biological sewage treatment plant (between point F and G), indicating that the discharge from the treatment plant was significant source of phosphate ion in Kurose river watershed. On the other hand, the silicate ion in river water except point A of pond as a river source was slightly decreased from upstream to downstream. It can be considered that the silicate ion is insusceptible to human activity such as discharge from a household and a waste treatment plant, though further investigation is need to confirm this.

Thus, the present IEC coupled with molybdenum-blue of derivatization system was successfully applied to simultaneous determination of phosphate and silicate ions in authentic river water and will be used as the monitoring method for water quality.

4. Conclusions

The IEC coupled with postcolumn derivatization using molybdate and ascorbic acid followed by spectrophotometry provided the simultaneous and selective determination of phosphate and silicate ions under the optimized analytical conditions (color-forming reactant, 50 mM sulfuric acid-10 mM sodium molybdate; reducing agent, 50 mM ascorbic acid; reaction coil length, 6 m). The effectiveness of the present system for the simultaneous determination of phosphate and silicate ions was demonstrated in the practical applications to the water quality monitoring.

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Figure captions

Fig. 1. Effect of sulfuric acid concentration in the color-forming reactant on detector responses (peak areas) for phosphate and silicate ions.

Analytical conditions: separation column, TSKgel super IC-A/C; reaction coil, PEEK tubing (0.25 mm i.d. x 6 m); column oven temperature, 45°C; eluent, ultra pure water; color-forming reactant, sulfuric acid-10 mM sodium molybdate; reducing agent, 50 mM ascorbic acid; eluent flow, 0.4 mL min^{-1}; flow rate of color-forming reactant and reducing agent, 0.2 mL min^{-1}; both of phosphate and silicate ion concentration, 1,000 μg L^{-1}. Lines: ○, phosphate ion: ●, silicate ion.

Fig. 2. Effect of sodium molybdate concentration in the color-forming reactant on detector responses (peak areas) for phosphate and silicate ions.

Analytical conditions: color-forming reactant, 50 mM sulfuric acid-sodium molybdate; both of phosphate and silicate ion concentration, 1,000 μg L^{-1}. The other conditions are as in Fig. 1. Lines: ○, phosphate ion: ●, silicate ion.

Fig. 3. Typical chromatogram of phosphate and silicate ions obtained using optimized chromatographic conditions.

Analytical conditions: color-forming reactant, 50 mM sulfuric acid-10 mM sodium molybdate. The other conditions are as in Fig. 1. (A) Standard solution of 2,000 μg L^{-1} as P for phosphate ion and 10,000 μg L^{-1} as Si for silicate ion, (B) Kurose river water. Peaks: 1, phosphate ion; 2, silicate ion.

Fig. 4. Trends of phosphate and silicate ion concentration in river water collected from Kurose river

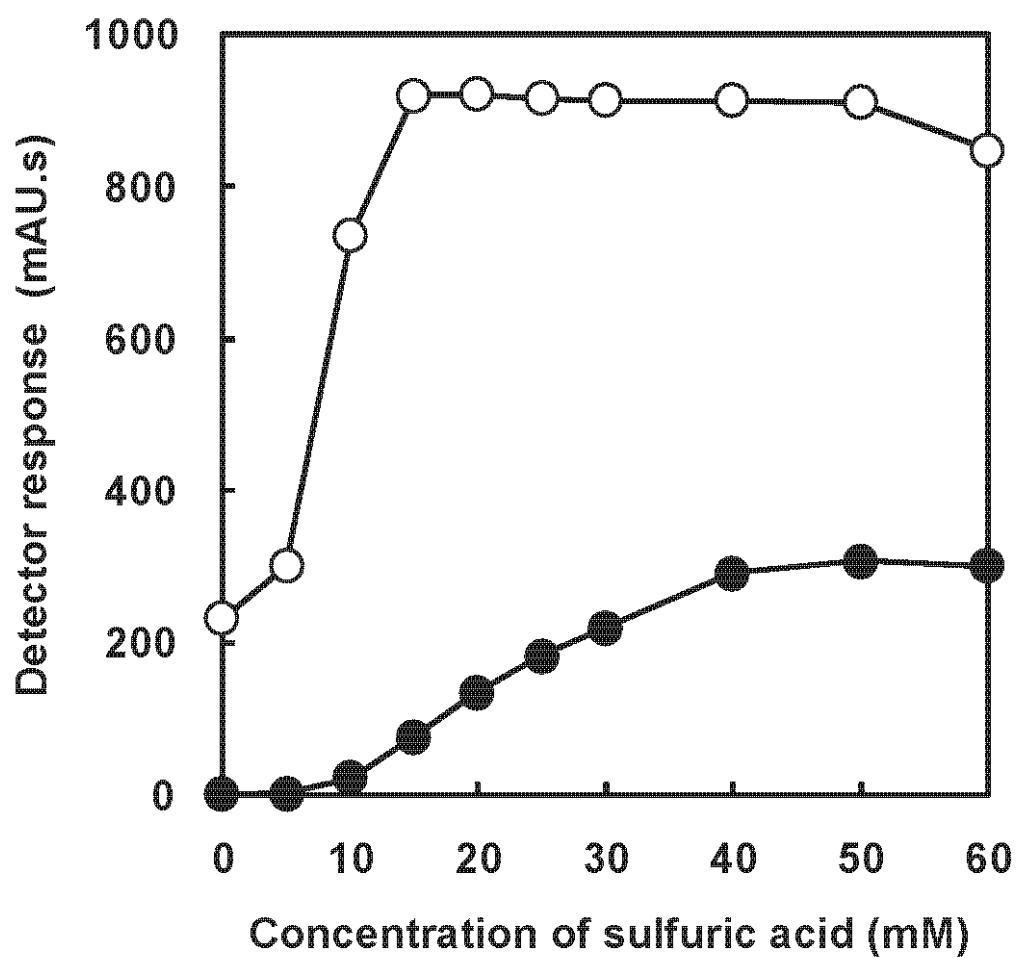watershed from upstream to downstream. A biological sewage treatment plant is located between F and G.

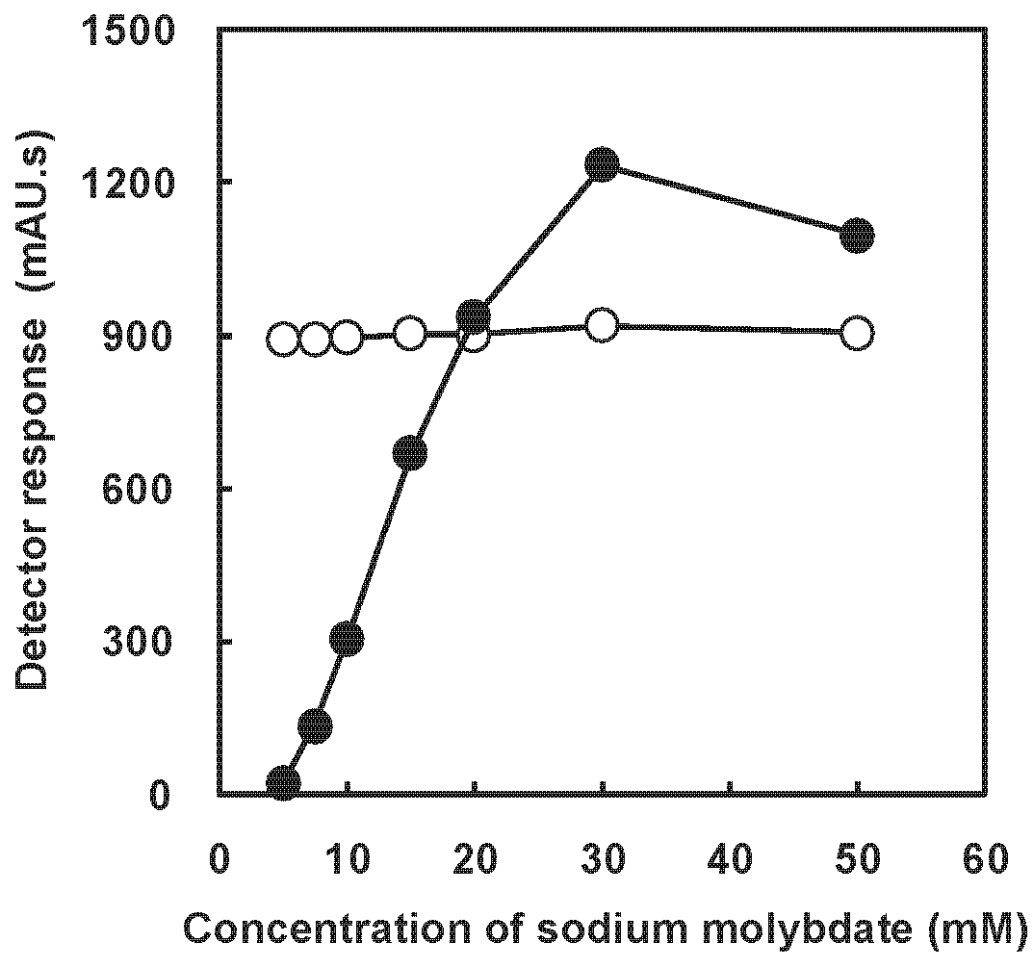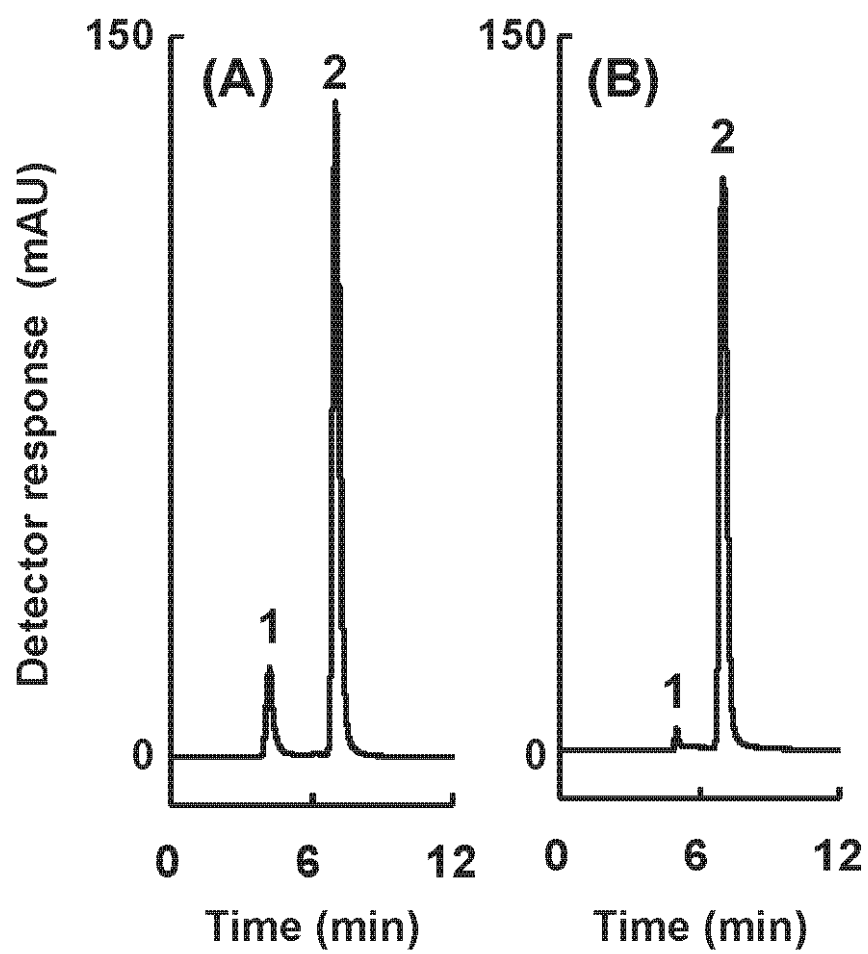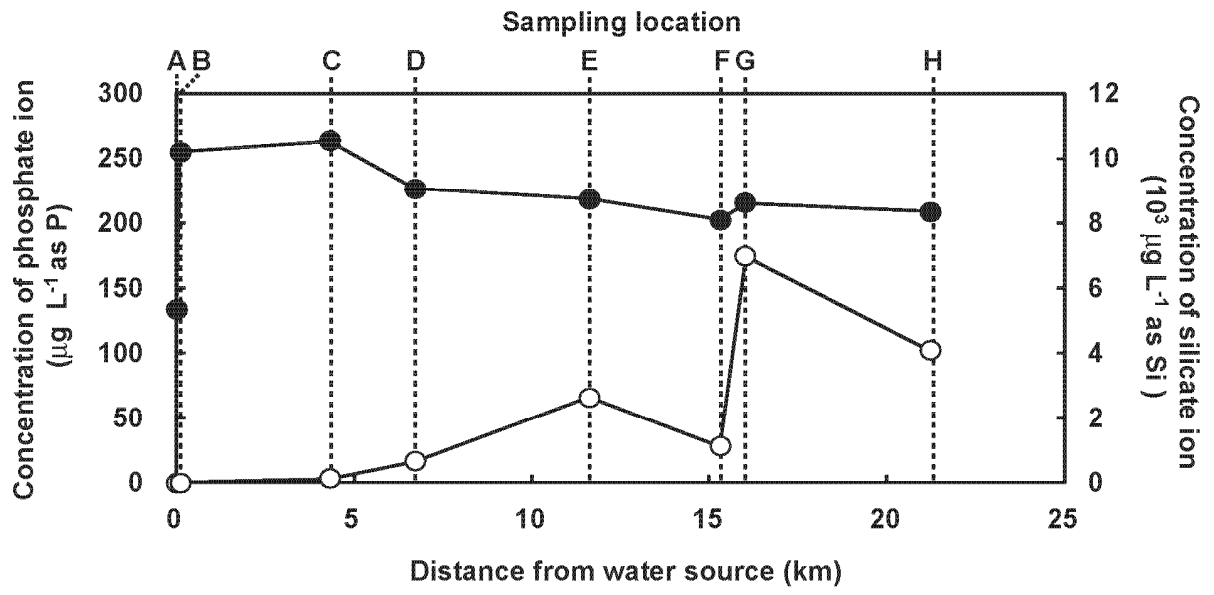Lines: ○, phosphate ion: ●, silicate ion.

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Fig. 4.