

# Interfering Effect of Silicate in the Quantification of Phosphate by the Molybdenum-blue Method

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**Abstract:** Phosphorus (P) in aqueous solutions is often measured as reactive orthophosphate by the molybdenum-blue method. The main drawback of this method is the presence of interfering ions, most importantly silicate ( $\text{SiO}_4$ ) when concurrently present with phosphate ( $\text{PO}_4$ ). Here, we address how  $\text{SiO}_4$  interferes in the determination of  $\text{PO}_4$  with various  $\text{SiO}_4:\text{PO}_4$  ratios. Experimental data showed that increasing the aqueous phase ratios of  $\text{SiO}_4:\text{PO}_4$  results in the overestimation of aqueous  $\text{PO}_4$ . For example, a  $\text{SiO}_4:\text{PO}_4$  aqueous ratio of 1500 can overestimate 10  $\mu\text{M}$   $\text{PO}_4$  up to ~250%. However, this interfering effect of  $\text{SiO}_4$  becomes negligible with increasing aqueous phase  $\text{PO}_4$  concentrations with similar  $\text{SiO}_4:\text{PO}_4$  ratios. For the higher concentrations of  $\text{PO}_4$  (e.g., 25, 35, and 50  $\mu\text{M}$ ), increasing the aqueous phase of  $\text{SiO}_4:\text{PO}_4$  ratios results in about 20% (maximum) overestimation of  $\text{PO}_4$  concentrations. Analysis of spectral features (UV-Vis) reveals that  $\text{SiO}_4$  also reacts with the analytical reagents and forms a molybdenum-blue complex with maximum absorbance at 812 nm beside the representative peak for  $\text{PO}_4$  at 890 nm. Accounting for the absorbance at 890 nm for various  $\text{SiO}_4$  concentrations in the absence of  $\text{PO}_4$ , we estimate the concentrations of 10  $\mu\text{M}$   $\text{PO}_4$  at various  $\text{SiO}_4:\text{PO}_4$  ratios, which are in good agreement with the measured concentrations. The feasibility of this spectrophotometric method for the quantification of aqueous  $\text{PO}_4$  with various  $\text{SiO}_4:\text{PO}_4$  ratios has also been addressed by considering the US National Water Information System database. Taken together, this study highlights the interfering effects of aqueous  $\text{SiO}_4$  in the spectrophotometric quantification of  $\text{PO}_4$  in natural waters.

**Key words:** Molybdenum-blue method, UV spectroscopy, phosphate, silicate, US NWIS database.

## Introduction

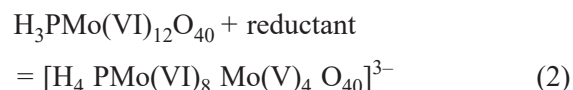
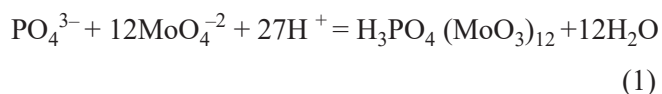
Phosphorus (P), as phosphate ( $\text{PO}_4$ ), is a crucial nutrient for phytoplankton growth and often limits productivity in freshwater systems (Filippelli & Street, 2002; Schindler et al., 2008).  $\text{PO}_4$  additions can lead to accelerated algal growth in surface waters, making regular monitoring

of  $\text{PO}_4$  concentrations essential for assessing water body health. Several methods exist for quantifying P in aqueous solutions. While ICP-OES measures total dissolved phosphorus (Worsfold et al., 2016; Yang et al., 2018), chromatographic and spectrophotometric methods specifically measure dissolved inorganic  $\text{PO}_4$ . Each method has its advantages and challenges (Table

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1). Among these, UV-Vis spectroscopy, ICP-OES, and ion chromatography are widely used (Murphy & Riley, 1962; Xie et al., 2013; Yang et al., 2018), with UV-Vis being particularly convenient due to its simple preparative steps, cost-effectiveness, and rapid analysis (Worsfold et al., 2016; Zhu & Ma, 2020).

Dissolved  $\text{PO}_4$  in natural waters is typically quantified using the molybdenum-blue method with UV-Vis spectroscopy (Murphy & Riley, 1962; Woods & Mellon, 1941). This method involves the reaction of  $\text{PO}_4$  with molybdate ions to form a blue-coloured molybdenum-blue complex. The maximum absorbance of this complex is measured using a UV-Vis spectrophotometer to determine  $\text{PO}_4$  concentrations according to Beer's-Lambert law (Murphy & Riley, 1962; Nagul et al., 2015). The formation of the blue complex occurs in two steps: first, a polyoxometalate species (e.g.,  $\text{H}_3\text{PO}_4(\text{MoO}_3)_{12}$ ) forms from dissolved  $\text{PO}_4$  and molybdate ions under strong acidic conditions (reaction 1), followed by the reduction by  $\text{SnCl}_2$  or L-ascorbic acid of this complex to create the blue phospho-molybdenum complex (reaction 2). Since L-ascorbic acid is a weak reductant for  $\text{H}_3\text{PO}_4(\text{MoO}_3)_{12}$ , potassium antimonyl tartrate (PAT) is used to enhance the reduction in strong acidic conditions (pH 0.9-1.4) and stabilise the colour of the molybdenum-blue complex (Nagul et al., 2015).



Stannous ion ( $\text{Sn}^{2+}$ ), from  $\text{SnCl}_2$ , can replace L-ascorbic acid with PAT to form the phosphomolybdate complex (Nagul et al., 2015). While the  $\text{SnCl}_2$  method offers faster kinetics and a lower detection limit, the Sn-substituted phosphomolybdate complex is hydrolytically unstable, potentially due to the presence of acidified  $\text{Mo}^{6+}$ . Additionally,  $\text{SnCl}_2$  methods use lower reductant concentrations, increasing the risk of re-oxidation of phosphomolybdate complex and often requiring a co-reductant. Therefore, L-ascorbic acid, in combination with PAT, remains a preferred choice for obtaining a stable blue phosphomolybdate complex with greater stability than that achieved using the  $\text{Sn}^{2+}$  reductant.

Although the spectrophotometric method of  $\text{PO}_4$  quantification is highly sensitive, the main obstacle to this method is the presence of interfering ions, e.g., arsenate, fluoride and silicate (Murphy & Riley, 1962; Woods & Mellon, 1941; Xie et al., 2013). Among these anions, the concentrations of arsenate and fluoride in natural waters are typically lower than  $\text{PO}_4$  (Edmunds & Smedley, 2013; Sabur, 2019).  $\text{SiO}_4$  concentrations, on the other hand, are much higher than that of  $\text{PO}_4$  in natural waters (Sabur, 2019) and may interfere with the formation of the phosphomolybdenum blue complex (Nagul et al., 2015).

**Table 1: Common methods for determining aqueous  $\text{PO}_4$  with advantages and limitations**

Methods	LOD ( $\mu\text{M}$ )	Advantages	Limitations
ICP-OES <sup>a,b</sup>	0.2	Low sample volume; highly sensitive; wide concentration range; minimal matrix effects.	Total-P measurement; not specific to $\text{PO}_4$ ; high instrument costs.
Ion Selective Electrode <sup>c</sup>	-	Polymer membrane electrodes for ion transfer.	Short lifespan; detection range: 0.002 to 1 mg/L; linear range: 0.16 to 3100 mg/L.
Fluorometric Method <sup>d,e</sup>	0.05	$\text{PO}_4$ -specific; measures $\text{PO}_4$ in organics; less time-consuming; higher accuracy.	Low quantification range (0.5-3 $\mu\text{M}$ ); costly operation.
HPLC <sup>f</sup>	0.001	Measures soluble reactive $\text{PO}_4$ ; small sample volume; minimal matrix effects.	High maintenance cost; precision affected by multiple factors.
Ion Chromatography <sup>g,h</sup>	0.0008 to 43.5	Reliable and precise $\text{PO}_4$ measurement; low matrix effect.	Interference from natural cations/anions; time-consuming preparation.
Molybdenum-blue Method <sup>i,j</sup>	0.3	Simple, cost-effective, widely used; requires UV-Vis spectrometer.	Susceptible to interference from other ions; moderately high LOD.

LOD, limit of detection; References: <sup>a</sup>(Worsfold et al., 2016); <sup>b</sup>(Yang et al., 2018); <sup>c</sup>(Estela & Cerdà, 2005); <sup>d</sup>(Diaz & Ingall, 2010); <sup>e</sup>(Winkler et al., 2020); <sup>f</sup>(Haberer & Brandes, 2003); <sup>g</sup>(Galceran, 2005); <sup>h</sup>(Xie et al., 2013); <sup>i</sup>(Murphy & Riley, 1962); <sup>j</sup>(Nagul et al., 2015).

Previous studies have addressed  $\text{SiO}_4$  interference using a flow-injection method, where reagents are added sequentially to slow the formation of the silicomolybdenum blue complex, rather than a single mixed reagent (Galhardo & Masini, 2000; Zhang et al., 1999). However, a simplified mixed reagent is easier to use and more practical for routine analyses. The addition of oxalic and tartaric acids with stronger inorganic acids in mixed reagents has also been used to slow silicomolybdate complex formation (Galhardo & Masini, 2000). This later method has been shown to be applicable for 2 mg/L ( $\sim 66 \mu\text{M}$ )  $\text{PO}_4$ , which is much higher than the concentrations found in natural waters (Sabur, 2019). Furthermore, however, oxalic and tartaric acids significantly reduce (25–50%) the absorbance of the phosphomolybdenum-blue complex, potentially lowering  $\text{PO}_4$  quantification sensitivity.

This study aims to investigate the effect of aqueous  $\text{SiO}_4$  in the determination of aqueous  $\text{PO}_4$  spectrophotometrically by molybdenum-blue method with L-ascorbic acid as a reductant. UV-Vis spectra that appear for phosphate-molybdenum complex in the absence and presence of  $\text{SiO}_4$  are assessed to evaluate the mechanistic insights into the effect of  $\text{SiO}_4$ . We also present a potential solution to address the interfering effect of  $\text{SiO}_4$  in the quantification of  $\text{PO}_4$  in natural waters. Finally, the concentrations of  $\text{PO}_4$  and  $\text{SiO}_4$  in various aquatic environments are extracted from the US NWIS database (NWIS, 2017) to understand the suitability of the molybdenum-blue method for the determination of dissolved  $\text{PO}_4$ .

## Materials and Methods

### Preparation of $\text{PO}_4$ and $\text{SiO}_4$ Solutions

Concentrated solutions of  $\text{PO}_4$  and  $\text{SiO}_4$  were prepared by dissolving sodium phosphate ( $\text{NaH}_2\text{PO}_4$ ) and sodium metasilicate nonahydrate ( $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ ) in a 10 mM NaCl solution. These solutions were then diluted with 10 mM NaCl to create individual ( $\text{PO}_4$  or  $\text{SiO}_4$ ) and combined ( $\text{PO}_4 + \text{SiO}_4$ ) solutions of desired concentrations. To investigate  $\text{SiO}_4$  interference in  $\text{PO}_4$  determination, four sets of solutions were prepared, each containing specific  $\text{PO}_4$  concentrations (10, 25, 35, or 50  $\mu\text{M}$ ), while varying the aqueous phase  $\text{SiO}_4$  ratios from 0 to 1500. For the highest  $\text{SiO}_4\text{:PO}_4$  ratio with 10  $\mu\text{M}$   $\text{PO}_4$ , the  $\text{SiO}_4$  concentration was 15,000  $\mu\text{M}$ , which increased to 37500, 52500, and 75000  $\mu\text{M}$  for 25, 35, and 50  $\mu\text{M}$   $\text{PO}_4$ , respectively. Additionally, solutions of  $\text{SiO}_4$  at concentrations of 100, 2500, and 5000  $\mu\text{M}$  without  $\text{PO}_4$  were prepared to examine the

interference of  $\text{SiO}_4$  with molybdate ions under the given analytical conditions.

### Preparation of Reagents

Spectrophotometric quantification of  $\text{PO}_4$  by molybdenum-blue method requires a mixture of individual reagents. Here, the reagents are prepared according to Murphy and Riley (1962) by mixing 2.5 M  $\text{H}_2\text{SO}_4$ , 0.0324 M Ammonium molybdate heptahydrate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ , 1235.86 g/mol), 0.1 M ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ , 176.12 g/mol), and 4.5 mM potassium antimony (III) tartrate trihydrate ( $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$ , 667.87 g/mol) at a ratio of 10:3:6:1. Solution of individual reagents are prepared by dissolving the appropriate amount of chemicals in deionised water.

### Phosphate Quantification

Using a micropipette, 4000  $\mu\text{L}$  of  $\text{PO}_4$  standard solutions (0 to 100  $\mu\text{M}$ ) were transferred to 10 mL Teflon vials. Next, 1000  $\mu\text{L}$  of a mixed reagent (composed of  $\text{H}_2\text{SO}_4$ ,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ,  $\text{C}_6\text{H}_8\text{O}_6$ , and  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$ ) was added to each vial. The vials were wrapped in Al-foil and shaken for about 10 minutes at a constant speed. Then, 2500  $\mu\text{L}$  of the reaction mixture was transferred to a UV quartz cell, and spectra were collected in the 400 to 1000 nm range using a UV-1800 Shimadzu spectrophotometer. The molybdenum-blue complex is stable at room temperature for only a few hours, so spectra were acquired within an hour.  $\text{PO}_4$  concentrations in the solutions at various  $\text{SiO}_4$  concentrations were estimated based on the absorbance measured at 890 nm. Additionally, standard  $\text{SiO}_4$  solutions (100 to 5000  $\mu\text{M}$ ) were also allowed to react with the  $\text{PO}_4$  determination reagents.

## Results and Discussion

### Effect of $\text{SiO}_4$ in the Determination of $\text{PO}_4$

In the present study, the effect of  $\text{PO}_4$  and  $\text{SiO}_4$  concentrations as well as  $\text{SiO}_4\text{:PO}_4$  ratios in the quantification of aqueous  $\text{PO}_4$  were assessed. Figure 1a shows the UV spectra of the phosphate-molybdate complex at room temperature for the determination of  $\text{PO}_4$  in the absence of  $\text{SiO}_4$ . We observed that the reaction between the mixed reagent (mixture of  $\text{H}_2\text{SO}_4$ ,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ,  $\text{C}_6\text{H}_8\text{O}_6$  and  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$ ) and the aqueous  $\text{PO}_4$  results in the formation of the phosphate-molybdate complex with an absorption maxima at  $\sim 890$  nm which is consistent with the past studies (Murphy & Riley, 1962). Thus, the absorbance at 890 nm was considered for the construction of a

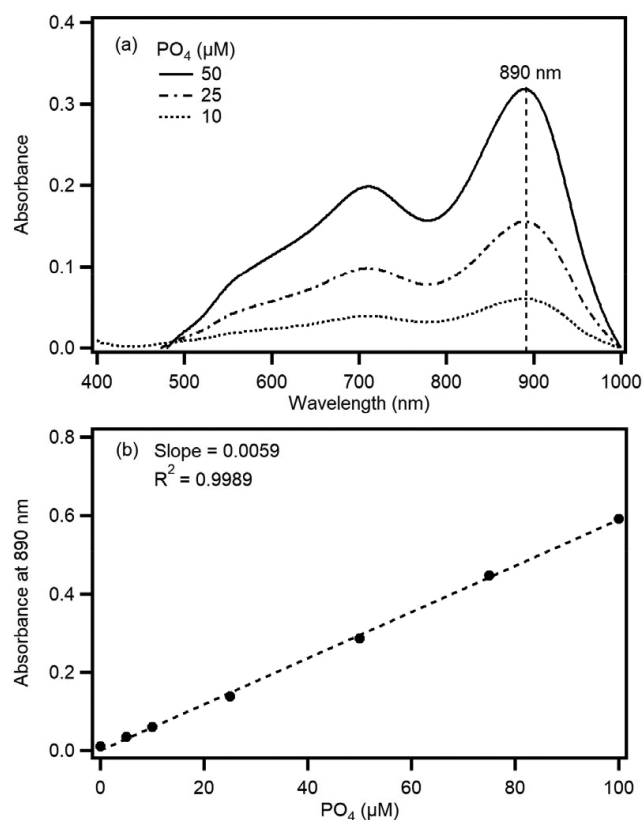
calibration curve for the standard  $\text{PO}_4$  solutions. The absorbance at 890 nm obtained for all the standard  $\text{PO}_4$  solutions is plotted against the known concentrations of  $\text{PO}_4$  solutions which provide a straight line with intercept zero that follows Beer's-Lambert's law (Figure 1b).

Figure 2 illustrates the changes in  $\text{PO}_4$  concentrations as influenced by the  $\text{SiO}_4:\text{PO}_4$  ratio, highlighting the impact of this ratio on  $\text{PO}_4$  determination using the molybdenum-blue method. The results reveal that higher  $\text{SiO}_4:\text{PO}_4$  ratios significantly contribute to the overestimation of  $\text{PO}_4$  concentrations. For instance, the  $\text{SiO}_4:\text{PO}_4$  aqueous ratio of 1500 can overestimate 10  $\mu\text{M}$   $\text{PO}_4$  up to 250% (Figure 2). However, this overestimation becomes insignificant whenever the concentrations of  $\text{PO}_4$  in the aqueous phase increase from 10  $\mu\text{M}$  to 25–100  $\mu\text{M}$ . For example, if an aqueous sample contains high concentrations of  $\text{PO}_4$  (e.g., 25, 35 and 50  $\mu\text{M}$ ), the interfering effect of  $\text{SiO}_4$  becomes minor with increasing  $\text{SiO}_4:\text{PO}_4$  ratios, resulting in a maximum 20% overestimation of  $\text{PO}_4$  concentrations. Note that the concentrations of  $\text{SiO}_4$  for the set of 50  $\mu\text{M}$   $\text{PO}_4$  solutions are 5 times higher than the

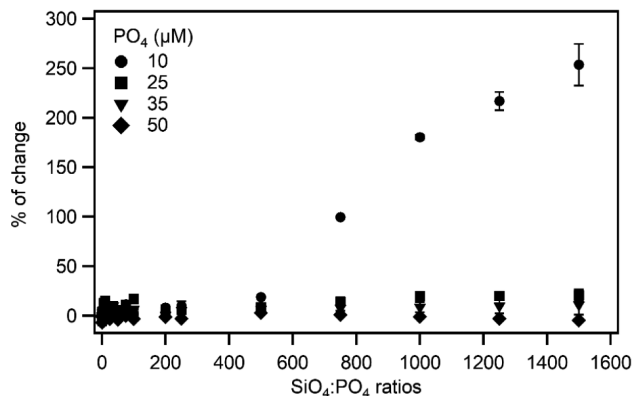
$\text{SiO}_4$  concentrations present in the set of 10  $\mu\text{M}$   $\text{PO}_4$  solutions.

To assess the impact of  $\text{SiO}_4$  on developing the peak intensity at 890 nm during  $\text{PO}_4$  determination, UV-Vis spectra of  $\text{PO}_4$  solutions at varying  $\text{SiO}_4:\text{PO}_4$  ratios were analyzed. At low  $\text{PO}_4$  concentrations (10  $\mu\text{M}$ ), increasing  $\text{SiO}_4:\text{PO}_4$  ratios result in a gradual increase in absorbance at 890 nm (Figure 3a). In contrast, with higher  $\text{PO}_4$  concentrations (50  $\mu\text{M}$ ), the overestimation of  $\text{PO}_4$  was minimal (Figure 2), as reflected in the spectral features (Figure 3b). These results suggest that elevated  $\text{PO}_4$  concentrations may inhibit  $\text{SiO}_4$  from reacting with the mixed reagent. It is important to note that the same  $\text{SiO}_4:\text{PO}_4$  ratios were used across all four  $\text{PO}_4$  solution sets (10, 25, 35, and 50  $\mu\text{M}$ ), though the  $\text{SiO}_4$  concentrations varied. For example, at a  $\text{SiO}_4:\text{PO}_4$  ratio of 1500,  $\text{SiO}_4$  concentration in the 10  $\mu\text{M}$   $\text{PO}_4$  solution was 15000  $\mu\text{M}$ , which is 5-times higher than in the 50  $\mu\text{M}$   $\text{PO}_4$  solution. This indicates that while higher  $\text{SiO}_4$  concentrations are associated with higher  $\text{PO}_4$  concentrations, the  $\text{PO}_4$  concentration primarily governs the complexation between  $\text{SiO}_4$  and molybdate ions under the given experimental conditions.

We also analysed UV-Vis spectral features to evaluate how silica overestimates the determination of  $\text{PO}_4$ . To achieve this, standard solutions of  $\text{SiO}_4$  were reacted with the mixed molybdate reagent (mixture of  $\text{H}_2\text{SO}_4$ ,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ,  $\text{C}_6\text{H}_8\text{O}_6$  and  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2\cdot 3\text{H}_2\text{O}$ ), in the absence of  $\text{PO}_4$ . Interestingly, the most intense peak in the UV-Vis spectra for  $\text{SiO}_4$  solutions in  $\text{PO}_4$  reagents appears at 812 nm, instead of at 890 nm (Figure 4). Moreover, the gradual increase in absorbance at 812 nm with the increasing concentrations of  $\text{SiO}_4$  was observed which might be due to the formation of silicate-molybdate complex under the given experimental



**Figure 1:** UV spectra of the phosphate-molybdate complex (a), and the calibration curve for standard  $\text{PO}_4$  solutions measured at 890 nm (b).



**Figure 2:** Changes (%) of  $\text{PO}_4$  concentrations with increasing  $\text{SiO}_4:\text{PO}_4$  ratios. The symbols indicated the concentrations of  $\text{PO}_4$ .



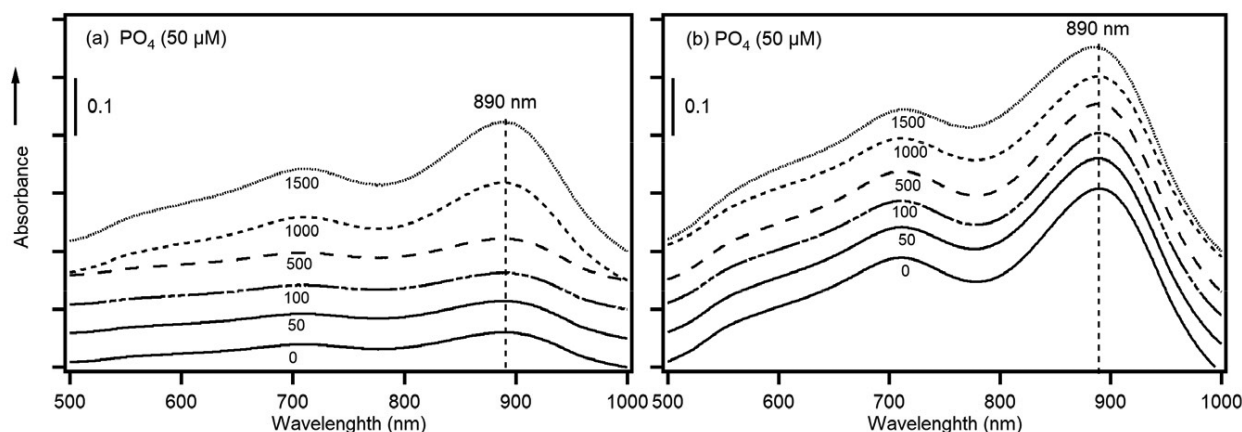


Figure 3: UV-Vis spectra of the molybdenum-blue complex (a) 10  $\mu\text{M}$  and (b) 50  $\mu\text{M}$   $\text{PO}_4$  solutions with varying  $\text{SiO}_4:\text{PO}_4$  ratios. The number below each spectrum represents the aqueous phase  $\text{SiO}_4:\text{PO}_4$  ratio.

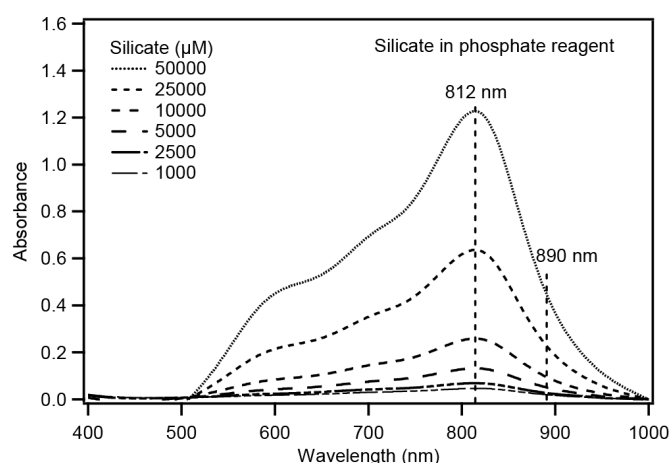


Figure 4: UV-Vis spectra of  $\text{SiO}_4$  solutions in  $\text{PO}_4$  reagents in the absence of  $\text{PO}_4$ .

conditions in the mixed reagent of  $\text{PO}_4$  determination (Ringuelet et al., 2011).

The reagents for the spectrophotometric determination of  $\text{SiO}_4$  by the molybdenum-blue method consist of ammonium molybdate, oxalic acid and L-ascorbic acid (Murphy & Riley, 1962; Nagul et al., 2015). The main difference between the reagents of  $\text{PO}_4$  and  $\text{SiO}_4$  is the strength of an acid used to create the necessary acidic conditions for forming the polyoxometallate complex (Nagul et al., 2015). Silicate forms silicate-molybdate complex under slightly lower acidic conditions compared to phospho-molybdate complex, which requires stronger acidic conditions (Nagul et al., 2015). Thus, oxalic acid is preferably used for  $\text{SiO}_4$  determination using the molybdenum-blue method, rather than sulphuric acid used in  $\text{PO}_4$  determination. However, it is important to note that the complex of  $\text{SiO}_4$  and molybdate ions can absorb a small fraction of UV light, which may contribute to the absorbance at 890 nm during  $\text{PO}_4$  determination.

The absorbance at 890 nm for the silicate-molybdate complex, formed in the absence of  $\text{PO}_4$  but with  $\text{PO}_4$  reagents, increases linearly with  $\text{SiO}_4$  concentration (Figure 5a). This suggests that higher  $\text{SiO}_4$  concentrations can lead to false positive  $\text{PO}_4$  concentrations in the molybdenum-blue method. For instance, 1000  $\mu\text{M}$   $\text{SiO}_4$  may result in 13.05  $\mu\text{M}$   $\text{PO}_4$ , and 2000  $\mu\text{M}$   $\text{SiO}_4$  in 14.24  $\mu\text{M}$   $\text{PO}_4$ , even in the absence of  $\text{PO}_4$ . Adjusting for these false positives,  $\text{PO}_4$  concentrations are estimated for different  $\text{SiO}_4:\text{PO}_4$  ratios (0 to 1500) with 10  $\mu\text{M}$   $\text{PO}_4$  (Equation 3).

$$[\text{PO}_4]_{\text{estimated}} = [\text{PO}_4]_{\text{actual}} + [\text{PO}_4]_{\text{false}} \quad (3)$$

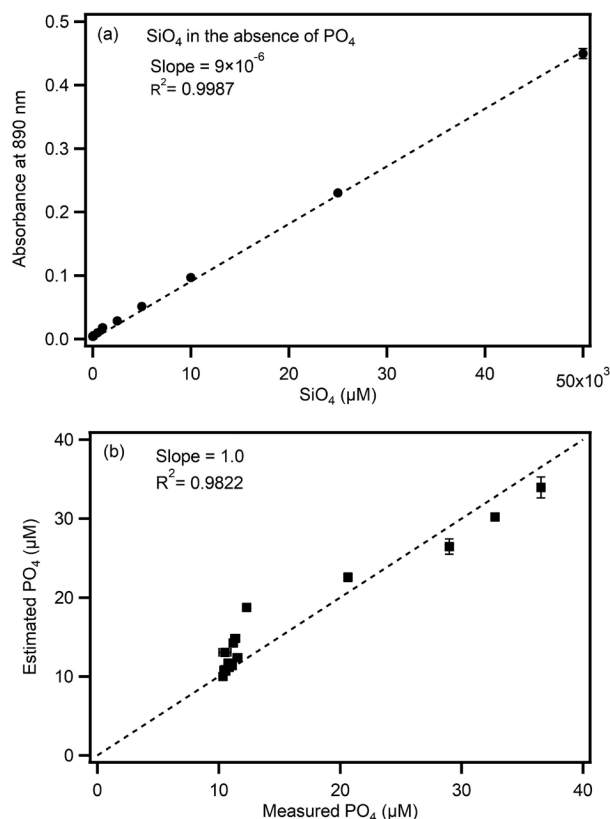
Here,  $[\text{PO}_4]_{\text{estimated}}$  is calculated by summing the actual  $\text{PO}_4$  concentrations originally present in the solution  $[\text{PO}_4]_{\text{actual}}$ , e.g., 10 mM) with  $[\text{PO}_4]_{\text{false}}$ , which represents the  $\text{PO}_4$  concentrations unexpectedly measured due to the presence of  $\text{SiO}_4$ .  $[\text{PO}_4]_{\text{false}}$  is derived from a calibration curve at 890 nm constructed using the standard solutions of  $\text{SiO}_4$  (prepared in the absence of  $\text{PO}_4$ ) in the  $\text{PO}_4$  determination reagents.

The results show a strong correlation between measured and estimated  $\text{PO}_4$  concentrations, closely matching the 1:1 line (Figure 5b). Therefore, excess  $\text{PO}_4$  due to  $\text{SiO}_4$  interference in the molybdenum-blue method can be calculated by subtracting  $[\text{PO}_4]_{\text{false}}$  from the measured values. For example, with 10  $\mu\text{M}$   $\text{PO}_4$  and 500  $\mu\text{M}$   $\text{SiO}_4$  ( $\text{SiO}_4:\text{PO}_4$  ratio of 50), the estimated  $\text{PO}_4$  is 11.7  $\mu\text{M}$ , a 17% overestimation. These overestimations for  $\text{SiO}_4:\text{PO}_4$  ratios of 100 and 500 are 31% and 50%, respectively. Recalibrating the curve (Figure 5a) at 890 nm with standard  $\text{SiO}_4$  solutions and measuring  $\text{SiO}_4$  before  $\text{PO}_4$  quantification can improve accuracy, especially at high  $\text{SiO}_4$  ratios.

### Environmental Significance

The typical concentration of  $\text{PO}_4$  and  $\text{SiO}_4$  in natural waters such as groundwater (well), streams and rivers plus standing water bodies including lakes, reservoirs and impoundments according to the US NWIS database are shown in Figure 6. The data, extracted from 2010 to 2016, showed that the average concentrations of  $\text{PO}_4$  and  $\text{SiO}_4$  are highly varied in various types of natural waters. However, the concentration of  $\text{PO}_4$  is generally very low compared to that of  $\text{SiO}_4$ . For example, the average concentrations of  $\text{PO}_4$  in groundwater (well), streams and/or rivers, and standing waters are  $3.11 \times 10^{-3} \pm 8.85 \times 10^{-3}$  (n= 2011),  $3.12 \times 10^{-3} \pm 8.85 \times 10^{-3}$  (n= 11881), and  $3.16 \times 10^{-3} \pm 8.5 \times 10^{-3}$  mM (n= 1527), respectively.

Dissolved  $\text{SiO}_4$  occurs ubiquitously in natural waters in the form of silicic acid ( $\text{H}_4\text{SiO}_4$ ), which forms due to the dissolution of diatom frustules, plant phytoliths, and detrital  $\text{SiO}_4$  minerals (Iler, 1979; Struyf et al., 2009). The typical concentrations of  $\text{SiO}_4$  in natural waters are high as reflected in the  $\text{SiO}_4:\text{PO}_4$  ratios

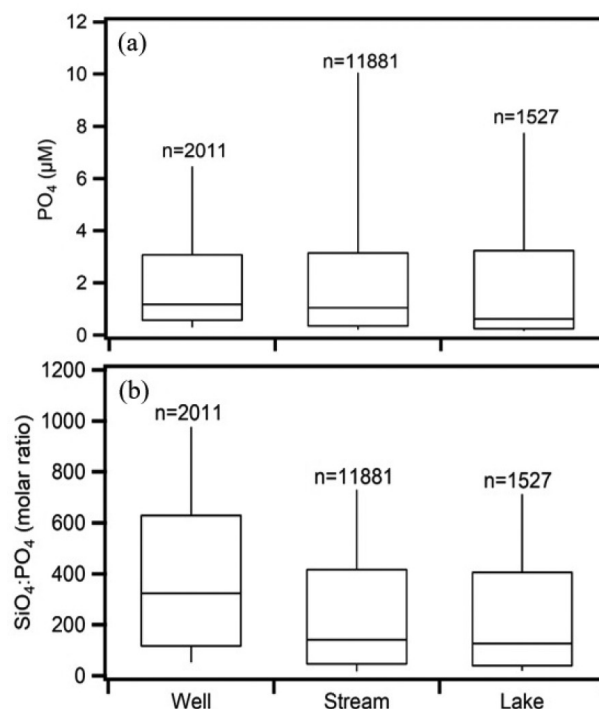


**Figure 5:** Maximum absorbance at 890 nm for different  $\text{SiO}_4$  concentrations, without  $\text{PO}_4$ , using a mixture of  $\text{PO}_4$  reagents (a). Estimated  $\text{PO}_4$  concentrations based on  $\text{SiO}_4$  quantification in the absence of  $\text{PO}_4$ , plotted against measured  $\text{PO}_4$  concentrations using the molybdenum-blue method (b). The dashed line represents the least-squares linear fit.

(Figure 6b). Therefore, the potential overestimation of  $\text{PO}_4$  by the molybdenum-blue method is anticipated. Since  $\text{PO}_4$  concentrations in most natural waters are below  $10 \mu\text{M}$  (Figure 6a), careful analysis of UV spectra in the presence of  $\text{SiO}_4$  may help address this overestimation. Given the variability in  $\text{SiO}_4:\text{PO}_4$  ratios across natural waters, further research should compare  $\text{PO}_4$  quantification, as proposed in this study, with known  $\text{SiO}_4$  concentrations using methods such as ion chromatography.

### Conclusions

The present study evaluates the effects of  $\text{SiO}_4:\text{PO}_4$  ratios in the spectrophotometric (UV-Vis) determination of  $\text{PO}_4$  concentrations by the molybdenum-blue method. Results show that  $\text{SiO}_4$  being present concurrently with  $\text{PO}_4$  leads to an overestimation of  $\text{PO}_4$ , particularly at low  $\text{PO}_4$  concentrations and at higher  $\text{SiO}_4:\text{PO}_4$  ratios. Similar to  $\text{PO}_4$ ,  $\text{SiO}_4$  at high concentrations also reacts with the molybdate reagent used for  $\text{PO}_4$  determination with the maximum absorbance at 812 nm instead of 890 nm which appears for  $\text{PO}_4$  in UV-Vis spectra. However, the amount of excess  $\text{PO}_4$  can be estimated



**Figure 6:** Dissolved  $\text{PO}_4$  concentrations in various aquatic environments and (b)  $\text{SiO}_4:\text{PO}_4$  molar ratios from US NWIS data (2010-2016). Numbers on each box indicate data points. Box boundaries represent the 25th and 75th percentiles, with the median shown by the line inside. Whiskers extend from the 10th to the 90th percentile.

by carefully assessing the absorbance at 890 nm in the UV-Vis spectra of standard  $\text{SiO}_4$  solutions with no  $\text{PO}_4$  in  $\text{PO}_4$  determination reagents. Because  $\text{SiO}_4$  exerts no or very minor effect on the determination of relatively higher concentrations of  $\text{PO}_4$  (25 to 50  $\mu\text{M}$  in this study), this method of corrections might be applicable for the determination of  $\text{PO}_4$  concentrations (e.g., <10  $\mu\text{M}$ ), which are the typical  $\text{PO}_4$  concentrations in natural waters according to US NWIS database. This study highlights the mechanistic insights into the  $\text{SiO}_4\text{:PO}_4$  ratios and their absolute concentrations for  $\text{PO}_4$  quantification in natural waters by the molybdenum-blue method and provides a potential method to deduct  $\text{SiO}_4$ -induced  $\text{PO}_4$  overestimation.

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### Disclosure Statement

No potential conflict of interest was reported by the authors.

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