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# Trace and ultratrace analysis methods for the determination of phosphorus by flow-injection techniques

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**Abstract**

Trace (≤1 mg/l or 30 μM) and ultratrace (≤1 μg/l or 30 nM) analysis methods for phosphorus determination by flow-injection analysis are reviewed. Most of the methods cited in this review are fundamentally based on the reaction of orthophosphate with molybdate to form heteropoly acids, such as molybdenum yellow and molybdenum blue, and some of the methods are based on the formation of such secondary reactions as ion associates and their aggregates with bulky cations, such as cationic dyes and quaternary ammonium ions. The heteropoly acids themselves can be measured by spectrophotometry, and the ion associate formed with a cationic dye, Malachite Green (MG), can be measured based on the coloration of MG. Light scattering detection methods can be used for measuring the aggregates of ion associates formed with bulky cations. Highly sensitive detection of phosphorus can be accomplished by fluorophotometry; Rhodamine B (RB) and its analogues react with molybdophosphate to form ion associates, which shows fluorescence quenching of RB: LOD is about 5 nM. The detection method based on the chemiluminescence of luminal oxidized with molybdophosphoric acids is probably the most sensitive of all the detection methods reported so far: LOD of the method is as low as 1 nM. The LOD of the molybdenum blue method can be improved by using a liquid core waveguide: LOD is 0.5 nM.


*Keywords:* Phosphorus; Flow analysis; Trace/ultratrace analysis

## 1. Introduction

Phosphorus is one of the most essential nutrients for life on earth, and occurs in soils and sediments, in natural and waste waters, and in the bodies of plants, organisms and animals. In the natural and waste waters, phosphorus compounds are present in various states, such as dissolved states, particles, detritus, aquatic plants and organism. Phosphorus in waters is classified as orthophosphate, condensed phosphates (pyro-, meta- and polyphosphate), phosphite and other oxidation states, and organic phosphorus compounds.

The analytical chemistry of phosphorus is very important in many fields, e.g., medical and clinical science, agriculture, metallurgy and environmental sciences. In recent years, as large quantities of phosphate have been used for detergents and for the treatment of boiler waters to prevent scale formation, it has become more and more important to determine micro/trace amounts of phosphorus in waters, such as drinking waters, natural waters, waste waters and polluted waters discharged from various sources.

In recent years, in semiconductor industries, large bulks of ultrapurified water are required for manufacturing high-quality semiconductors: phosphorus existing even at trace/ultratrace amounts in the water can damage the quality of the semiconductors, and therefore the amounts must be lowered as low as possible. For a next-generation semiconductor manufacturing, phosphorus is one of the most interesting impurities that must be analyzed in ultrapurified waters, and therefore there has been a growing demand for highly sensitive, accurate and rapid determination, as well as simple

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and on-site determination, of trace and ultratrace amounts of impurities in ultrapurified water.

Most of the classical spectrophotometric methods are based on the reaction of orthophosphate with molybdate, in the absence or in the presence of vanadate or antimonate, in acidic medium, where heteropoly acids or molybdophosphoric acids, such as phosphomolybdenum yellow or molybdenum yellow and its reduction product (the so-called phosphomolybdenum blue or molybdenum blue), can be formed [1,2]. The molybdenum yellow shows the absorption maximum in UV region, where the excess amounts of molybdate also shows light absorption, and therefore the absorbance measurements must be done at longer wavelength at around 400 nm. In the presence of some reducing agents, such as ascorbic acid, hydrazine and tin (II) chloride, molybdenum yellow can be reduced to form molybdenum blue, which shows stronger light-absorption than the molybdenum yellow and the maximum absorption wavelengths are at longer wavelengths around 650–850 nm, depending on reducing agents. The molar absorptivities of molybdenum yellow and molybdenum blue are, for example, $2.4 \times 10^4\,l\,mol^{-1}\,cm^{-1}$ at 310 nm [3], and $2.3 \times 10^{-4}\,l\,mol^{-1}\,cm^{-1}$ at 725 nm [4]. Though a conventional spectrophotometry for phosphorus as the molybdenum blue is not so sensitive, the detection sensitivity can be improved by 10- to 100-fold using a long-path cell or a liquid core waveguide [86].

It is well known that molybdophosphate reacts with cationic dyes to form ion associates, which can be separated as a precipitate from an aqueous solution [5–8], or floated between an aqueous and an organic phase [9–12], and extracted into organic solvents [13–19]. Later, Motomizu et al. reported the solvent extraction/spectrophotometric method for the sensitive determination of phosphate with Malachite Green (MG) as an ideal pairing ion [20]: the molar absorptivity of the ion associate in a mixture of toluene and 4-methylpentan-2-one (1:3 v/v) is $2.3 \times 10^5\,l\,mol^{-1}\,cm^{-1}$ with the small reagent blank of 0.05 at 630 nm.

It is very interesting with respect to sensitivity improvement for phosphate determination that MG reacts with molybdophosphate in an acidic medium to form a colored ion associate [21]. This coloration reaction was applied to the analysis of serum and urine samples [22–27]. In the presence of poly(vinyl alcohol), the ion associate can dissolve stably in acidic aqueous solutions and shows strong light-absorption at 650 nm with the molar absorptivity of $8 \times 10^5\,l\,mol^{-1}\,cm^{-1}$ and the reagent blank of 0.02 [28].

More recently, a fluorophotometric detector with a flow-through cell was used for detecting fluorescence and light-scattering changes caused by the formation of ion associates of molybdophosphate with bulky cations [29–32]. Probably, the fluorescence quenching method is most suitable and versatile for the sensitive determination of phosphate in aqueous solutions by a simple and rapid flow-injection technique.

Other flow-based methods used for the sensitive determination of phosphate are also reviewed in this article.

## 2. Trace analysis for phosphorus by flow-injection technique

Trace analysis covers the concentration ranges from about 30 nM to 30 μM or μg/l to mg/l of phosphorus in sample solutions. Trace analysis method is often used for the determination of phosphorus in fertilizers, soils and rocks, or sometimes polluted waste waters. The molar absorptivity of molybdophosphate, the so-called molybdenum yellow [33], is about or less than $10^3\,l\,mol^{-1}\,cm^{-1}$, and therefore molybdenum yellow method is suitable for the detection of phosphorus at concentration levels of 10 μM. A typical reaction of the formation of molybdenum yellow in an acidic medium is as follows:

$$H_3PO_4 + 12(MoO_3) \rightarrow H_3PMo_{12}O_{40}$$

where $MoO_3$ represents the molybdate being present as Mo(VI) in an acidic medium. In the presence of oxoacids, such as vanadate and antimonite, ternary heteropoly acids can form. The ternary heteropoly acids have larger molar absorptivities and longer maximum wavelengths than the heteropoly acid itself. In Fig. 1, the absorption spectra of ternary heteropolyacids in 2 M nitric acid are shown [34]. The molar absorptivity of $H_4PVMo_{11}O_{40}$ is the largest of all heteropoly acids, and is $3.6 \times 10^3\,l\,mol^{-1}\,cm^{-1}$ at 385 nm. The formation of the ternary heteropoly acid, vanadomolybdophosphoric acid, has often been used for the determination of trace amounts of phosphate in a batchwise method: the determinable range is 1–20 mg/l of phosphorus [35] and was used for the determination of phosphorus with a micro-flow-injection system [34]. For the flow-injection determination of phosphate, zirconomolybdophosphric acid is more recommended because of its faster formation rate. In Fig. 2, a simple flow diagram for the determination of phosphate with molybdate and Zr(IV) is shown, and the flow signals obtained are shown in Fig. 3: the limit of detection (LOD) is about 1 μM or 30 μg/l [34].

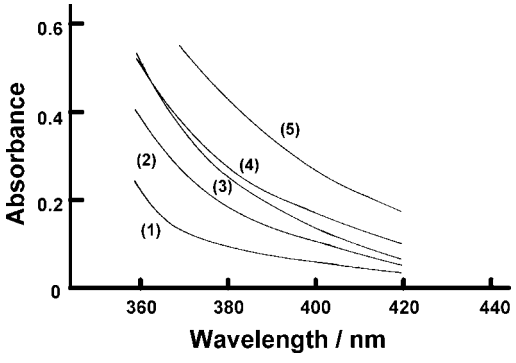


Fig. 1. Absorption spectra of ternary molybdophosphoric acids: phosphate, $1 \times 10^{-4}$ M; molybdate, $8 \times 10^{-2}$ M; $HNO_3$, 1 M; Hf(V), Ti(IV), Zr(IV) and V(V), $4 \times 10^{-3}$ M. Absorbances were measured using 10 mm path cell against reagent blanks: (1) $H_3PMo_{12}O_{40}$, (2) $H_4PHfMo_{11}O_{40}$, (3) $H_5PZrMo_{11}O_{40}$, (4) $H_5PTiMo_{11}O_{40}$, (5) $H_4PVMo_{11}O_{40}$.

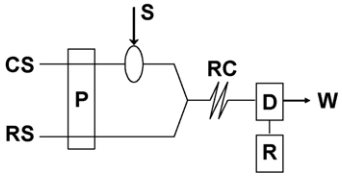


Fig. 2. Flow diagram for phosphate determination: CS: carrier ($H_2O$); RS: reagent solution; P: double plunger micropump (each flow rate: 50 μl/min); S: sample (30 μl); RC: reaction coil (1 m); R: recorder; D: detector (385 nm); W: waste.

The molybdenum yellow method was used for rock analysis [36]. Recently, a ternary heteropolyacid, vanadomolybdophosphoric acid, was used for the detection of phosphate in a microfluidic manifold with a photometry using a UV-LED as a light source [37].

The molybdenum yellow method is very simple and the reagent solution is very stable for 1 year in storage [37]. For practical use, however, samples may contain some organic substances, which show light absorption in a UV region and can give positive errors for analytical results. Therefore longer wavelengths are favorable for diminishing the interferences from coexisting substances.

Molybdophosphoric acid, molybdenum yellow, can be easily reduced with some reducing agents, such as ascorbic acid, tin(II) and hydrazine, to form reduced molybdophosphoric acid, molybdenum blue [38], in which some parts of molybdenum(VI) can be reduced to Mo(V) and whose absorption maximum wavelengths and molar absorption coefficients can be changed on the dependence of reducing agents used; that is, the molybdenum blue shows their absorption maximum at about 650–850 nm, and the molar absorptivity, for example, at 725 nm is $2.3 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ [39]. In the spectrophotometric determination of phosphorus

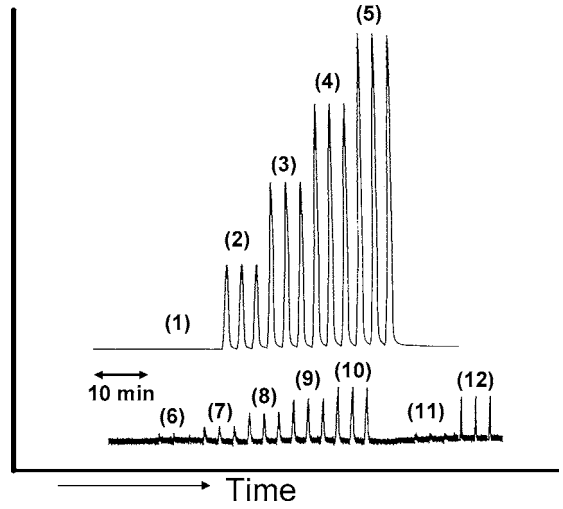


Fig. 3. Flow signals for phosphate determination: flow system: Fig. 2; phosphate ($10^{-4}$ M): (1) 0; (2) 2; (3) 4; (4) 6; (5) 8; (6) 0; (7) 0.02; (8) 0.04; (9) 0.06; (10) 0.08. Sample: (11) tap water (<LOD); (12) river water ($6 \times 10^{-6}$ M).

in water samples, the molybdenum blue method has often been used [40,41]. This is because of its relatively high sensitivity and less interference from coexisting ions, compared with the molybdenum yellow method. However, as is described above, the absorption maximum wavelengths of the molybdenum blue and their molar absorptivities are much varied depending on the conditions of reducing reactions, such as reducing agents, reaction temperature and reaction time, which results in low reproducibility of phosphate determination, and requires delicate, tedious and time-consuming procedures by classical batchwise methods.

The molybdenum blue method was, for the first time, applied to the determination of phosphate at mg/l levels by flow-injection analysis [42], where ascorbic acid was used as a reducing agent, and the absorbances were measured at 660 nm: this was the dawn of a flow-injection analysis method. Using a flow-injection technique, the disadvantages of the molybdenum blue method were all overcome, and furthermore the detection sensitivity was improved. Soon later, the method was applied to the practical samples, such as plant materials [43,44] and blood serum [45].

The flow-injection analysis by the molybdenum blue method with ascorbic acid is included in the standard methods for water and waste water analysis [46].

In these days, the molybdenum blue method has been frequently used for the flow-based determination of micro amounts of phosphorus as a conventional detection reaction. For example, the method was used for the validation of on-line UV radiation/persulfate digestion method for the determination of total phosphorus [47], for the simultaneous determination of phosphate and silicate in biological materials with on-line cation-exchange pretreatment and anion-exchange separation [48], for the simultaneous determination of phosphate and silicate by stopped-flow-injection spectrophotometry [49] and by sequential-injection spectrophotometry [50].

As is described above, the molybdenum blue method coupled with spectrophotometric flow-injection technique is one of the most suitable methods for the trace analysis for phosphorus as ortho-phosphate with respect of its simplicity, rapidity, enough sensitivity, good selectivity and less interferences from coexisting and matrix substances.

Trace analysis at the concentration ranges from 0.1 to 10 μM, which correspond to 3 μg/l to 0.3 mg/l of phosphorus, is most versatile for the determination of phosphorus in real samples. The molybdenum blue method has often been used for this purpose by coupling with a basic flow-injection system [46]. At the first stage of FIA researches [42–45], sample solutions were injected into a reagent stream containing molybdate, ascorbic acid and a strong acid, and absorbances were measured at 660 nm. The manifold is very simple, and rapid measurement is possible. However, in a single line flow system, where samples are injected into a reagent stream, a system peak occurs because of the blank value of the reagent solution and the difference in the diffraction in-

dex between the reagent solution and the sample solution, and sample peaks can often overlap on the system peak, which is unable to improve the sensitivity of the measurement. Such demerits of the single-line manifold can be overcome by using a double-line manifold, where a carrier and a reagent stream merge at the downstream of the injection point, and the mixture flows into a reaction coil; sample solutions are injected into the carrier stream. By using the double-line manifold, phosphorus at 0.1 μM could be detected [51]. The molybdenum blue method has been most widely used for the trace analysis for phosphorus by FIA [52–54], and as a reducing agent, ascorbic acid is most suitable because of the high sensitivity and the stability of the reagent mixture of molybdate, ascorbic acid and a strong acid. As a reagent mixture, for example, two kinds of mixed solutions, A and B, are prepared and they are mixed before use: the solution A contains ammonium molybdate, antimonyl potassium tartrate and sulfuric acid, and the solution B contains ascorbic acid and sodium dodecyl sulfate. Such reagent mixtures are stable and can be used for at least 3 months if they are stored in a refrigerator [55].

Recently, the molybdenum blue method was applied to the simultaneous determination of phosphate and silicate in boiler water in power plants [56]: the determinable ranges of phosphate and silicate were 0.05–22 mg/l P and 0.1–24 mg/l Si, respectively.

In these days, various kinds of light-emitting diodes (LED) as a light source for easily assembling a simple and low-cost photometric detector are commercially available, and a red LED (strongest wavelength: about 660 nm) can be used for the detection of the molybdenum blue. A portable micro flow-type flow-injection system with an LED detector was proposed: the system is a one-box type, about 8 kg and works with a 12 V battery. This system was applied to the on-site determination of phosphate in river water samples: LOD was 5 μg/l of phosphorus [51]. A compact FIA system with a gas-pressure propelling system and an LED (650 nm) detector was developed and used for surface mapping of phosphate in marine waters [57]. A micro-FIA system using a fabricated manifold on a borosilicate glass was developed: the elctroosmotic flow was used for propelling solutions. The determinable range is 1–10 μg/l of phosphorus [58].

The molybdenum blue method can be applied to the total phosphorus determination after the decomposition of organic and inorganic phosphorus compounds, organic and inorganic condensed phosphorus compounds, and solid and dissolved phosphorus compounds to orthophosphate by heating at high temperature (120–160 °C) [59,60], at high temperature with a platinum wire in a reaction coil [61], or by irradiating UV-light to aqueous samples containing persulfate [47,55]. By using a reaction coil, which is wound on two low-pressure mercury lamps (14 mm o.d., 134 mm length, 4 W germicidal use), most organic phosphorus compounds were decomposed to orthophosphate at 70 °C: LOD was 1 μg/l of phosphorus at 830 nm [55].

More sensitive spectrophotometric method for phosphate determination was developed: the method is based on the ion association reaction of phosphomolybdate with a cationic dye, Malachite Green ($MG^+$). This coloration reaction proceeds in an acidic medium as in the following equations:

$$\underset{\text{(yellow)}}{H_3PMo_{12}O_{40}} + \underset{\text{(yellow, } \lambda_{max}=446\text{ nm)}}{HMG^{2+}} \rightarrow \underset{\text{(green, } \lambda_{max}=650\text{ nm)}}{(MG^+)(H_2\,PMo_{12}O_{40}{}^-)} + 2H^+ \quad (1)$$

$$(MG^+)(H_2PMo_{12}O_{40}{}^-) + HMG^{2+} \rightarrow (MG^+)_2(HPMo_{12}O_{40}{}^{2-}) + 2H^+ \quad (2)$$

$$(MG^+)_2(HPMo_{12}O_{40}{}^{2-}) + HMG^{2+} \rightarrow (MG^+)_3(PMo_{12}O_{40}{}^{3-}) + 2H^+ \quad (3)$$

In the presence of large excess of MG, the reactions (1)–(3) can occur, and the (3:1) ion associate formed in Eq. (3) can easily precipitate in the acidic aqueous solution, can be extracted into an organic phase [20] and can adsorb and be collected on a hydrophobic membrane like a cellulose nitrate filter [62–65].

In order to stop the formation of the ion association reactions shown in Eqs. (2) and (3), and to stabilize the ion associate in the aqueous solution, poly(vinyl alcohol) (PVA) was added to the solution in a batchwise method [28]. The molar absorptivity in the aqueous solution was $7.8 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ at 650 nm, as is shown in Fig. 4. The advantages of the method based on the formation of the ion associate with MG, MG method, are the fast coloration reaction without heating, the higher sensitivity than those of the molybdenum blue methods and the longer maximum wavelength than those of the molybdenum yellow methods.

The MG method was applied to the determination of phosphate in water samples by FIA, where the mixed reagent solution containing molybdate, MG, sulfuric acid and ethanol

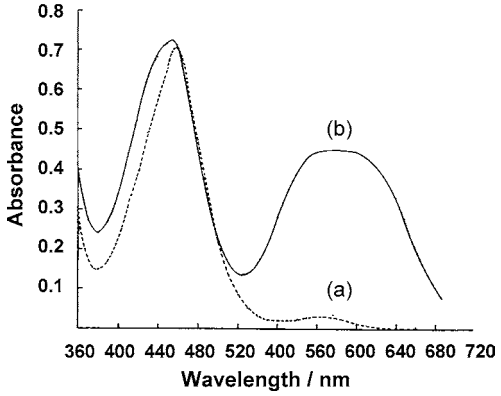


Fig. 4. Absorption spectra of the ion associate of molybdophosphate with Malachite Green in aqueous solution (Malachite Green method) (a) reagent blank, (b) phosphate $4 \times 10^{-6}$ M.

(20%) was used [66,67]: LOD is 1 μg/l of phosphorus at 650 nm. Later, the mixed reagent solution was improved by the addition of an anionic surfactant together with ethanol [68]. The MG method was applied to the determination of total phosphorus in industrial waste waters by on-line digestion/spectrophotometric FIA [61].

An orthophosphate enzyme electrode with a hybrid membrane of trienzyme film and poly(1,2-diaminobenzene) film was used for the selective detection of both endogenous orthophosphate and orthophosphate by coupling with an acid phosphatase immobilized reactor and a 16-way switching valve [69]: the determinable range is 0.5 μM to 0.8 mM. The fluorescent reactions of vanadomolybdophosphoric acid [70] and molybdophosphoric acid [71] with thiamine were used for the determination of phosphate by FIA, where the reactions were based on the oxydation of thiamine with heteropoly acids: the determinable range is 0.015–0.6 mg/l and 0.02–20 mg/l of phosphorus, respectively. Molybdophosphate reacts with a bulky cation to form aggregates of ion associates. As a cation, a porphyrin derivative, tetrakis (1-methylpyridinium-4-yl) porphyrin (TMPyP), was used, and the light-scattering was measured at 475 nm [31]. Also, Rhodamine B and a chloro derivative of MG were investigated: the determinable range was 0.2–1 μM, and LOD was 60 nM [32].

A continuous phosphine generation coupled with inductively coupled plasma atomic emission spectrometry was investigated: LOD was 2 μg/l of phosphorus [72]. An amperometric detection method was investigated and used for the flow-through detector of FIA; the determination of phosphorus is based on the formation of molybdophosphoric acid and its reduction on a glassy carbon electrode [73,74]: the LODs were 20 and 60 nM, respectively.

Phosphorus is an essential nutrient for all lives including phytoplankton in an aquatic system. However, excessive inputs of phosphorus from domestic and industrial wastes, as well as terrestrial soils, can lead to the eutrophication of lakes and coastal sea, and to the occurrence of a red tide, which is often accompanied by abnormal growth of toxic algae. Orthophosphate is the most bioavailable of all phosphorus forms, and the criterion for phosphorus concentrations in non-eutrofic waters is considered to be less than 20 μg/l of phosphorus [75] and phosphorus at concentrations above 20 μg/l can accelerate eutrophication; therefore the lowest determinable concentration is required to be several μg/l of phosphorus. The MG method coupled with spectrophotometric FIA can satisfy this requirement of the eutrophication research. Also, the molybdenum blue method coupled with spectrophotometric FIA can satisfy it, if the manifold is well assembled with a sensitive and stable visible detector, a longer-path flow-through cell (20–50 mm path length) and a pulseless pumping system, and a well-refined reagent mixtures is prepared [55]. A red LED is very useful for assembling a stable detector, because of very stable light emitting and lower consumption of energy [51,57].

## 3. Ultratrace analysis for phosphorus by flow-injection technique

Ultratrace analysis covers the concentration ranges of less than 30 nM or 1 μg/l. Such ultratrace analyses are required in the field of advanced industries, such as semiconductor industries, ultrapurified water plants and ultrapure chemicals suppliers, where phosphorus at nanomolar levels is required to be determined. Also, ultratrace analysis is necessary for the determination of phosphorus at nanomolar levels in surface waters, where the biological uptake of phosphate depletes its concentrations. However, most of the spectrophotometric and electrochemical methods developed so far without preconcentration procedures cannot satisfy the sensitivity required for the ultratrace analysis. In order to improve the sensitivity of phosphorus determination, flow-injection techniques coupled with on-line and off-line preconcentration procedures have been investigated. The MG method coupled with solvent extraction/FIA enabled to determine phosphate at several nanomolar levels of phosphorus, and was applied to river water analysis [76]: LOD was 0.1 μg/l of phosphorus. The MG ion associate was enriched on-line on a solid phase, such as Shephadex (cyclodextrin derivatives) packed in a flow-through cell [77], molybdenum blue on polymer beads packed in a syringe [78] and styrene-divinylbenzene polymer packed in a short desk (1.6 mm) [79]. A signal improvement factors of 30–50 could be achieved. However, the detection limit was not so improved: LOD was 0.2 μg/l of phosphorus [79].

The ion associate of molybdophosphate with MG was collected on a small membrane (cellulose nitrate; pore size 1.0 μm, diameter 5–9 mm, effective filtering diameter 2 mm) by filtration, dissolved together with the filter in 0.5–1.0 ml of methyl cellosolve. Then the absorbance of the methyl cellosolve solution was measured at 627 nm by using a single-line flow-through cell system equipped with an autosampler: the carrier stream was methyl cellosolve, and the path length of the flow cell was 10 mm [63,64]. The absorbance, the standard deviation and the relative standard deviation of the reagent blank were 0.0270, 0.0005 and 1.8%, respectively, and the calibration graph was linear over the range from 0.018 to 1.0 μg/l of phosphorus using 40 ml of sample solutions: LOD corresponding to two times of the standard deviation of the reagent blank was 3 ng/l or 0.1 nM of phosphorus [64]. The reason why the proposed method is very sensitive is that the enrichment factor of 40 or more is possible, and very small absorbances can be measured reproducibly with the flow-through cell system. The method is probably the most sensitive of all the methods reported so far.

The method coupled with off-line enrichment is very sensitive. However, ultratrace amounts of phosphorus in ultrapurified waters cannot be determined because the absorbance of the reagent blank may include the absorbance of the reagent ($MG^+$) and the phosphorus in the reagents and the water used for the preparation of solutions, and a phosphate-free water

or the standard phosphorus solution, whose accurate concentrations at ng/l levels are certified, is not available now.

A new method, the slope comparison method, was proposed for the determination of phosphorus in ultrapurified waters, in which the enrichment method of MG-ion associate was coupled with an evaporation/enrichment procedure and the flow-through cell system [65]. The evaporation/enrichment procedure is requisite for cancelling the reagent blank values. The LOD of the slope comparison method for phosphorus was 8 ng/l of phosphorus, and the method was applied to the determination of phosphorus in ultrapurified waters; the phosphorus concentrations were 70–90 ng/l of phosphorus.

A fluorophotometric detection method was developed for the determination of phosphorus by using a double-line FIA system: a carrier was a purified water stream, and a reagent stream contained molybdate, Rhodamine 6G and hydrochloric acid [29]. The method was based on the fluorescence quenching of a cationic dye, Rhodamine 6G, due to the formation of an ion associate of molybdophosphate with Rhodamin 6G: LOD was as low as 0.1 μg/l of phosphorus, and 0.5 M NaCl did not interfere with the determination. The method was applied to the analysis of real waters, such as river and sea waters. Later, the method was improved by developing a more stable reagent system with Rhodamine B (RB), instead of Rhodamine 6G, and poly(vinyl alcohol) (Fig. 5) [30]: LOD was about 10 nM. Recently, the RB/fluorescence quenching method was brushed up by reexamining the experimental conditions; LOD was improved to be 5 nM [80]. This method was applied to the determination of phosphorus in ultrapurified waters by coupling with an off-line evaporation/enrichment procedure: 50 ml of sample waters was evaporated on a hot plate to 5 ml, and the enriched samples were injected into the carrier stream of the double-line flow system: LOD was about 15 ng/l of phosphorus [80]. In this method, the enrichment of the ultrapurified water samples was requisite because of the same reasons as described in the paper [65].

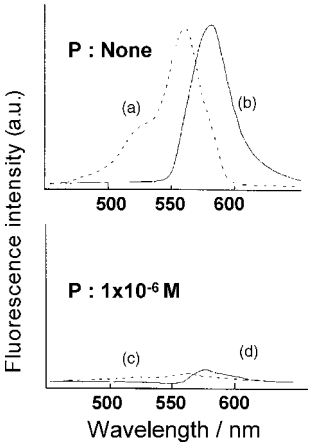


Fig. 5. Excitation and emission spectra of Rhodamine B method: (a and c) excitation spectra; (b and d) emission spectra.

The oxidation reaction of thiamine with molybdophosphoric acid was applied to the determination of total dissolved phosphorus in waters: the phosphorus compounds were decomposed by using a simple UV photoreactor in the presence of persulfate: LOD was 10 nM [81]. The thiamine method is very sensitive; however, the reaction is less selective to phosphorus, and the presence of oxidizing agents may interfere with the determination and may cause positive errors.

Chemiluminescence (CL) detection systems were developed with immobilized enzymes, and a biosensor was used for phosphate determination in drinking waters: LOD was 0.96 nM [82]. Similarly, enzymatic flow-injection CL detection methods were developed and applied to the determination of phosphorus in blood serum and water samples; purine nucleosidephosphorylase, xanthine oxidase and urate oxidase were immobilized on glass beads. In the enzymatic reactions, hydrogen peroxide was produced, and detected by CL using luminal-microperoxidase: LOD was 10 nM in blood serum [83]. The same enzymatic reactions was used for CL detection of phosphorus with bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitorophenyl]oxalate: LOD was 39 nM [84].

A luminol chemiluminescence detection system was developed. The method is based on the oxidation reaction of luminol with the molybdophophoric acid: LOD was 30 ng/l or 1 nM of phosphorus [85]. Though the method needs the removal of some metal ions with a chelating resin column and the adjustment of pH to an alkaline region after the formation of molybdophosphoric acid, the method is the most sensitive of all the methods proposed so far, except for the off-line enrichment/FIA measurement method and a spectrophotometric method with a liquid core waveguide.

A spectrophotometric method with a long-path cell or a liquid core waveguide (liquid waveguide capillary flow cell: LWCFC, 2-m quartz capillary with a 550 μm i.d.) was developed on the basis of the gas-segmented continuous flow analysis method coupled with the molybdenum blue formation, and used for the determination of phosphorus in surface water of a bay at 1.6–64 nM: LOD was 0.5 nM [86].

## 4. Conclusion

In general, flow-injection methods can enable to improve the detection sensitivity by 10–100 times, compared with corresponding batchwise methods using similar detection reactions, because flow-through cells are fixed in detectors, sample solutions are injected reproducibility and dispersions of sample zones are reproducible. Furthermore, reaction times can be set up quite reproducibly, which leads to the possibility of detecting quite small differences in physical properties of analytes between a base line (reagent blank) and a sample, though the flow-injection manifold must give full play to its ability. Such improvement of detection sensitivities can be utilized more efficiently in the detection using fluorescence and chemiluminescence, catalytic and enzymatic

Table 1
Rapid and simple methods used for the determination of trace and ultratrace amounts of phosphorus as phosphate in water samples, and their limit of detection (LOD) and determinable concentration

| Methods | LOD (μM) | Determinable ranges (μM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 30 (1000)* | 3 (100)* | 0.3 (10)* | 0.03 (1)* | $3 \times 10^{-3}$ (0.1)* | $3 \times 10^{-4}$ (0.01)* |
| Spectrophotometric methods | | | | | | | |
| Molybdenum yellow method | | | | | | | |
| Ref. [34] | 1 | ◯ | ◯ | ◯ | | | |
| Molybdenum blue method | | | | | | | |
| Ref. [46] | 0.1 | ◯ | ◯ | ◯ | △ | | |
| Ref. [55] | $3 \times 10^{-2}$ | ◯ | ◯ | ◯ | △ | | |
| Ref. [57] | 0.15 | ◯ | ◯ | ◯ | | | |
| Molybdenum blue method with a long-path cell | | | | | | | |
| Ref. [86] | $5 \times 10^{-4}$ | | | ◯ | ◯ | ◯ | △ |
| Malachite Green method | | | | | | | |
| Ref. [66] | $3 \times 10^{-2}$ | | ◯ | ◯ | △ | | |
| Malachite Green method with solvent extraction | | | | | | | |
| Ref. [76] | $3 \times 10^{-3}$ | | | ◯ | ◯ | △ | |
| Malachite Green method with off-line enrichment | | | | | | | |
| Ref. [64] | $1 \times 10^{-4}$ | | | ◯ | ◯ | ◯ | ◯ |
| Fluorophotometric methods | | | | | | | |
| Rhodamine B method | | | | | | | |
| Ref. [30] | $1 \times 10^{-2}$ | | | ◯ | ◯ | △ | |
| Ref. [80] | $5 \times 10^{-3}$ | | | ◯ | ◯ | △ | |
| Thiamin method | | | | | | | |
| Ref. [81] | $10 \times 10^{-3}$ | | | ◯ | ◯ | | |
| Chemiluminescence method: Luminol method | | | | | | | |
| Ref. [85] | $1 \times 10^{-3}$ | | | ◯ | ◯ | ◯ | △ |

(◯) Determinable concentration; (△) approximate determinable limit.
* Phosphate concentration: μg/l.

reactions, and the reactions requiring some pretreatment procedures.

Now, the most sensitive detection method for the determination of phosphorus is the chemiluminescence detection of luminol oxidized with molybdophosphoric acid, and as a whole, the spectrophotometric method based on the formation of the molybdenum blue with a long-path cell is the best LOD for phosphorus determination. However, even the most carefully purified waters contain phosphorus at concentrations of nanomolar levels or more. Therefore, essentially, the real detection sensitivities cannot be improved at concentrations below 30 ng/l (=1 nM), though the very small differences in physical properties can be detected by flow-injection methods.

Only by coupling the flow-injection detection method with some enrichment procedures, which do not require any chemicals, the detection sensitivities can be improved, as is shown in the method with the evaporation enrichment/ion-associate collection method [65,80].

One serious problem is that molybdate may contain relatively large amounts of phosphorus as molybdophosphate, which is very difficult to be removed. In the MG and the RB method, however, the mixed reagent solutions were filtered with a cellulose nitrate membrane filter before use. This filtration procedure can eliminate the molybdophosphate sufficiently as the ion associates from the reagent mixtures.

In Table 1, trace and ultratrace analysis method for phosphorus determination in real samples are summarized with respect of their determinable concentrations and limit of detection. For the trace analysis covering the concentration range from 30 nM to 30 μM, flow-injection spectrophotometric methods coupled with the formation of the molybdenum blue (longer-path cell) and of the ion associate of the molybdenum yellow with Malachite Green (10 mm path cell) are the most convenient. For the ultratrace analysis covering the concentrations below 30 nM, the fluorescence quenching method with Rhodamine B, the Chemiluminescence method with luminol and the spectrophotometric method with such a long-path cell as a liquid core waveguide are utilized; the ultrapure water, which is purified very carefully, must be used for the preparation of reagents and a carrier stream.

Though the LOD can be improved by using a well-assembled FIA system and a sensitive and stable detector, it must be noticed that only differences in the physical properties between a background (the carrier) and samples can be measured by flow-injection analysis or other flow-based analyses. This means that the real concentration of an analyte in a given sample must be corrected by the summation of the measured value and the concentration in the carrier after the accurate concentrations of the standard working solutions used for the preparation of a calibration graph are corrected using the concentration in the water used. Therefore, the estimation of the phosphorus contents in water used for the carrier and the preparation of reagents solutions and the standard working solutions must be requisite before the measurement of analytes. With respect of phosphorus, ultrapure waters pu-

rified with a commercially available purification system may contain several nanomolar levels of phosphorus.

In these days, a highly sensitive detection method for phosphorus is strongly demanded in semiconductor industries. The method must enable to determine phosphorus as low as nanomolar levels without any batchwise preconcentration procedures and any contamination from experimental circumstances. Probably, such methods will be developed only by using or integrating flow-injection techniques into analysis systems.

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