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The determination of phosphate in seawater by reverse flow injection spectrophotometry

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Abstract

A simple, sensitive, accurate and portable reverse flow injection (rFIA) spectrophotometric method is proposed for the on-line determination of trace phosphate in seawater. In acid conditions, phosphate reacts with ammonium molybdate to form molybdophosphoric acid, which forms a soluble complex compound with ethyl rhodamine B in the presence of polyvinyl alcohol. The absorbance of the complex compounds was measured by optical detector at 585 nm. Chemical factors and rFIA variables affecting the system were also discussed. Under the optimal conditions, the calibration graph for phosphate was linear over the concentration range from 2.0 to 200.0 μg/l with a correlation coefficient of 0.9995. The detection limit was 0.05 μg/l; the relative standard deviation was 0.61% for a solution containing 50 μg/l phosphate (n=15). The recovery of phosphate from three samples ranged from 96.4 to 98.4% of the added amount. The results obtained for phosphate by the proposed rFIA spectrophotometric method were in very close agreement with those using the conventional method.

Keywords: phosphate; reverse flow injection analysis; seawater; spectrophotometry.

Introduction

Phosphorus is rich in nature and exists in different forms in natural waters, soil leachates and agricultural runoffs, with the most commonly measured fractions being dissolved reactive phosphorus, total dissolved phosphorus and total particulate phosphorus (Xi et al. 2004). Phosphorus (P) is an essential element for all life including plant growth and photosynthesis in algae, and excessive phosphorus concentration can cause stimulation leading to algae growth, resulting in a bloom, which is a very abundant dense growth of algae (He et al. 2004). The eutrophication phenomenon is one of the serious environmental problems throughout the world (Harper 1992). Therefore, the monitoring of phosphate is important for evaluating and controlling water quality.

There are a number of different methods used for the determination of phosphate, such as spectrophotometry (Harmsen 1984, He et al. 1996, Di et al. 2000, Rimmelin and Moutin 2005, Huang and Zhang 2009), ion chromatography (Colina et al. 1996, Yokyama et al. 2009), high performance liquid chromatography (Tomás et al. 2005), and so on. Of the above mentioned methods, spectrophotometric methods are still the most widely used today. Most of the conventional spectrophotometric methods are based on the reaction of orthophosphate with molybdate, in the absence or in the presence of vanadate or antimonite, in acidic medium, where heteropoly acids or molybdophosphoric acids, such as phosphomolybdenum yellow or phosphomolybdenum blue, can be formed. However, the conventional spectrophotometric methods have major shortcomings such as color instability, the long time required for full color development, a low sensitivity or absorbance of the blank. It is well-known that molybdophosphate reacts with dyes to form ion associates (Motomizu et al. 1984, Liu et al. 1988, Wang et al. 1988, Mas et al. 1990). In the presence of polyvinyl alcohol (PVA), the ion associates can dissolve stably in acidic aqueous solutions. The sensitivity is improved greatly for phosphate determination when molybdophosphate reacts with dye in an acidic medium to form a colored ion associate. FIA, which involves injection of a small volume of sample into a stream of reagent flowing through the system, is a widespread and well-established approach for the determination of phosphate (Burns et al. 1991, Benson et al. 1994, Liu and Jiang 2000, Lyddy-Meaney et al. 2002, Gimbert et al. 2007, Nakatani et al. 2008). Motomizu and Zhen (2005) reviewed trace and ultratrace analysis methods for determination of phosphorous by FIA in 2005. Different from this FIA technique is reverse flow injection analysis (rFIA). In rFIA, with the injection of reagent into the sample flow, the determination concentration in the reagent zone increases with increasing dispersion, therefore the determination is carried out with only slight dilution and higher sensitivity can be obtained. The rFIA also has advantages such as high selectivity and very low consumption of reagents. In Kenneth and Robert (1982) published the first rFIA paper about determination of phosphate in seawater with a detection limit of 0.05 μm. Recently, the rFIA method was applied to determine nanomolar soluble reactive phosphorus in seawater, the linearity and the detection limit were 0–165.0 nm and 0.5 nm (Ma et al. 2009).

In this study, a very simple and sensitive rFIA method was successfully used for the direct determination of phosphate in seawater based on the sensitive color system of ethyl rhodamine B (ERB)-molybdophosphoric acid-PVA. Operative conditions for the rFIA procedures, including flow injection variables and reagents concentration, were investigated and optimized. The proposed method has the advantages of being less reagent-consuming, more sensitive, simple and rapid.

Experimental

Apparatus

A model HYY3-1 automatic nutrients analyzer (made by Prof. Xinshen Zhang, Chengdu, P.R. China) equipped with a six-way automatic injection valve, optical detector and optical flow cell has the function of flow injection analysis. The six-way automatic injection valve was controlled by a time-delay device. The flow system employed one four-channel peristaltic pump (Shanghai Huxi Analytical Instrument Plant, P.R. China), which delivered all flow streams. PTFE tubing (0.5 mm i.d.) was used to connect all components in the flow system. The color liquid (R) was injected into samples and working standard solutions (S) by a six-way injection valve. The absorbance of solutions was detected by the optical detector when they flowed into the optical flow cell. Data acquisition and processing were performed with HW-2000 Chromatography software (Version No. 2.13, Qianpu Software Co. Ltd., Shanghai, P.R. China) running under Windows XP. Ultraviolet and visible spectra were obtained using a Spectrumlab UV/VIS (UV-2800, Unic instrument Co. Ld., Shanghai, P.R. China). The absorbance intensity was recorded at 585 nm on a personal computer (PC).

Reagents and solutions

All reagents (Chengdu Kelong Chemical Reagent Factory, Sichuan, P.R. China) used included potassium dihydrogen orthophosphate, ERB, ammonium molybdate, PVA and nitric acid. All reagents were of analytical grade and all solutions were prepared with deionized water from ultrapurification of the molecular system.

A 100 mg/l PO_4^{3-}-P stock solution was prepared by dissolving 0.4384 g of potassium dihydrogen orthophosphate (oven-dried for 2 h at 110°C) in 1000 ml ultrapure water. Working standards were prepared by suitable dilution of the stock solution.

Mixed chromogenic reagent solution (R_1) was 14.3 ml of 3.5 mol/l nitric acid, 3 ml of 40.00 g/l ammonium molybdate and 1 ml of 10.00 g/l PVA were mixed and the mixture solution was diluted to 100 ml. Chromogenic reagent solution (R_2) was prepared by dissolving 0.10 g ERB in 1000 ml deionized water; 0.5 mol/l nitric acid was used as the carrier (C).

Artificial seawater was obtained by dissolving 26.519 g sodium chloride, 2.447 g magnesium chloride, 3.305 g magnesium sulfate and 0.725 g calcium chloride in 1 l deionized water.

Flow injection procedure

A series of working standard solutions with different concentrations were prepared by diluting PO_4^{3-}-P stock solution. As shown in Figure 1, flow paths were inserted into samples or standard solutions (S), C, R_1 and R_2, respectively. With the six-way automatic injection valve in the load position, the pump was started to wash the whole flow system until a stable baseline was recorded. When the switching valve was in the analyzing position, the sample solution or standard solution was mixed with reagent solution R_1. In the reaction coil L_1, there was formation of molybdophosphoric acid between ammonium molybdate and phosphate. Then the color reaction of ERB with molybdophosphoric acid occurred in the reaction coil L_2. Finally, ERB-molybdophosphoric acid was carried into the flow cell. The absorbance intensity of ERB-molybdophosphoric acid was determined at 585 nm by a detector and transformed to a signal of the peak which was recorded by HW-2000 Software on a PC.

Results and discussion

Detection wavelength

The proposed method is based on the color-forming reaction between ERB and phosphomolybdate ion. Figure 2 shows the absorbance spectrum of the reaction solution: 2.0 ml PO_4^{3-}-P solution (50 μg/l), 4 ml R_1 and 4 ml R_2. The wavelength of maximum absorption of the color formed was obtained at 586 nm, which was chosen for further work.

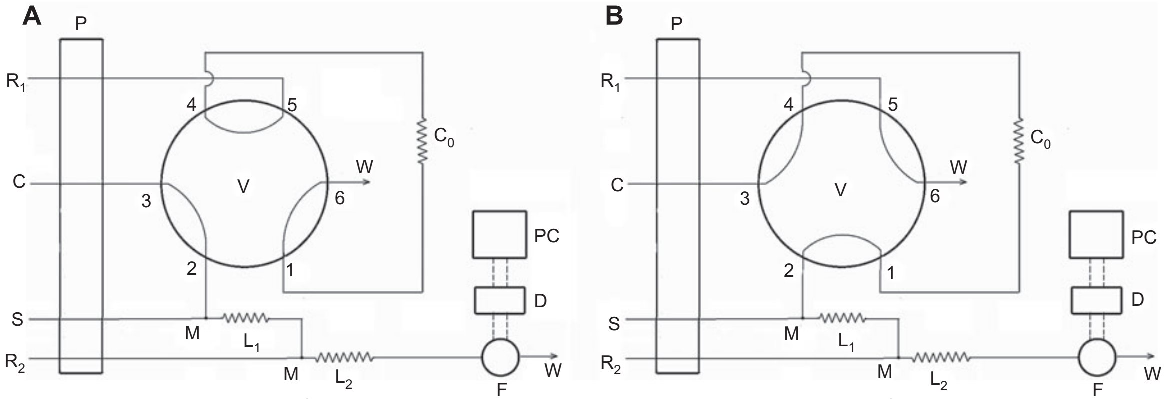
Optimization of flow injection variables

The effect of various parameters, including the flow injection variables and the reagents concentration were investigated and optimized based on univariate experimental design. During the optimization of these parameters, a 50.0-μg/l standard solution was used as testing sample. Each sample was determined three times and the result was shown as the average value. To evaluate the optimum parameters for the proposed method, the sensitivity, analysis time and baseline noise were the most important. The optimized results of the flow injection variables are shown in Table 1.

Effect of flow rate

The flow rate of C, S, R_1 and R_2, which affected peak height by affecting the reaction of sample and reagent, were adjusted by changing the internal diameter of the pump tube. The flow rate of C, S, R_1 and R_2 were varied from 0.15 ml/min to 0.85 ml/min through a univariate approach. The test results showed that the highest peak height was obtained when the flow rate of C, S, R_1 and R_2 were 0.40 ml/min, 0.75 ml/min, 0.30 ml/min and 0.25 ml/min.

Effect of injection volume In the rFIA system, the color liquid was injected into the flowing system mixing and



The injection flow path

The analyzing flow path

Figure 1 Schematic diagram of the rFIA system.
S, samples or working standard solutions; C, carrier; R, color liquid; P, four-channel peristaltic pump; V, six-way automatic injection valve; C_0, injection coil; M, mixer; L, reaction coil; F, optical flow cell; D, optical detector; PC, computer; W, waste.

reacting with the sample in the reaction coil. An injection volume from 200 to 400 µl was studied to determine the optimum value. Figure 3 showed the absorbance signals obtained in this study. The best result was obtained with the 250 µl injection volume. Beyond this volume the absorbance decreased with increasing injection volume, thus 250 µl was chosen as the injection volume for subsequent measurements.

Effect of reaction coil lengths The length of reaction coil was an important factor affecting the result of rFIA. If reaction coil was too short, the reagents would react with analyte incompletely. If reaction coil was too long, it would be time consuming. Thus, reaction coil lengths of L_1 and L_2 were tested, which was in the range from 0.25 to 1.0 m and from 0.25 to 2.0 m, respectively. As illustrated in Figure 4, the 0.50 m of L_1 led to the highest absorbance signals and was chosen for further tests. As illustrated in Figure 5, the absorbance signals had no clear difference in the length of L_2, but the least noise of baseline was obtained when a 2-m length of L_2 was used. Therefore, the 2-m of L_2 was chosen for further study.

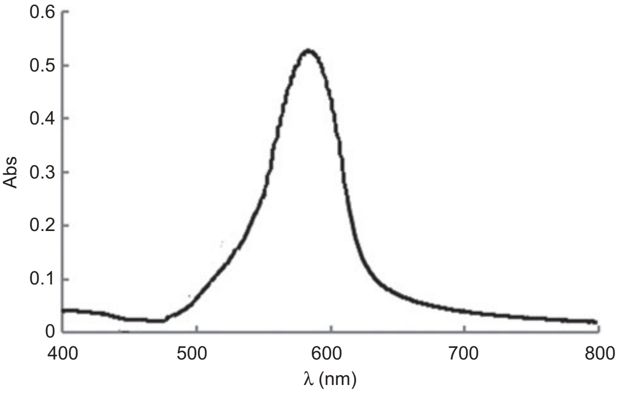


Figure 2 Absorption spectrum of the reaction of solution.

Optimization of chromogenic reagent solution

Effect of the ERB concentration The reagent concentration was an important factor for sensitivity in rFIA. The ERB concentration from 0.05 to 0.20 g/l was studied (Figure 6). As can be seen, the absorbance signals increased with increasing concentration of the ERB. Higher concentration of ERB was beneficial to the sensitivity. However, baseline noise increased with increasing concentration of ERB. Therefore, a concentration of 0.10 g/l of this reagent was used from here on in as a balance between sensitivity and baseline noise.

Effect of the ammonium molybdate concentration The effect of the ammonium molybdate concentration on the peak height was studied from 0.8 to 2.0 g/l (Figure 7). The results showed that the peak height increased with increasing concentration of ammonium molybdate. However, the baseline drifted up clearly when the concentration of the ammonium molybdate was up to 1.6 g/l. Therefore, 1.2 g/l of the ammonium molybdate was selected as the optimal concentration.

Effect of the nitric acid concentration Acidity is a very important factor in a colored system experiment. The effect of the nitric acid concentration on the sensitivity was studied from 0.25 to 0.75 mol/l. The results indicated that the peak height obviously decreased with increasing concentration of nitric acid. Lower concentration of the nitric acid was beneficial to the sensitivity. However, baseline noise was high at the concentration of 0.25 mol/l. Hence, 0.5 mol/l of nitric acid was chosen as a compromise between sensitivity and steady baseline.

Effect of PVA concentration PVA was chosen for the dispersing agent in this study. It not only enhanced the solubility of the complex compound but also kept the colored system in a clear and transparent manner. The effect

Table 1 Optimization of rFIA variables.

Variables		Studied range	Optimum value
Pump tube flow rate (ml/min)	S	0.25–0.85	0.75
	C	0.25–0.75	0.40
	R_1	0.25–0.75	0.30
	R_2	0.15–0.75	0.25
Reactor coil length L_1 (m)		0.25–1.0 (i.d. 0.5 mm)	0.50 (i.d. 0.5 mm)
Reactor coil length L_2 (m)		0.25–2.0 (i.d. 0.5 mm)	2.0 (i.d. 0.5 mm)
Injection volume (μl)		200–400	250
Sampling time (min)		Calculated according to the flow rate, the immobile tubes	1
Analyzing time (min)		Calculated according to the flow rate, the volume of reactors and immobile tubes	1

of PVA concentration on the sensitivity was tested from 0.05 to 0.20 g/l. The results showed that the peak height increased when the PVA concentration was in the range of 0.05–0.10 g/l. However, the peak height was almost changeless when the PVA concentration changed from 0.10 to 0.20 g/l. Therefore, 0.10 g/l of PVA was selected as the optimal concentration.

Research of calibration curve using different matrices

There were massive ions in seawater, and these ions possibly affect the color reaction. To find out whether ions would affect the color reaction and obtain a high sensitivity of the proposed methodology, four solutions were investigated at the optimal conditions achieved from the above experiments: deionized water, 3.09% sodium chloride, artificial seawater, seawater without phosphate (Lai and Li 2008). The results showed that the calibration curve using water as matrix had a lower sensitivity compared with the other three mediums. Only a slight difference in 3.09% sodium chloride, artificial seawater and seawater were without phosphate. This behavior occurs due to a lens effect which arises due to a combination

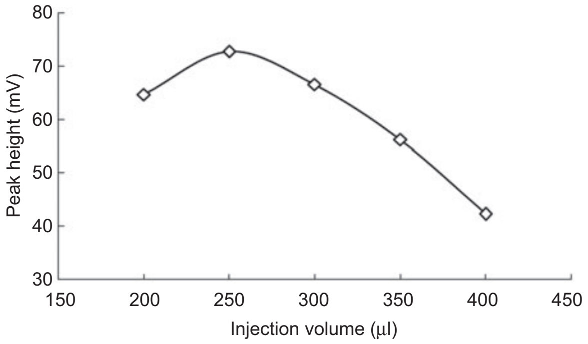


Figure 3 Effect of injection volume.

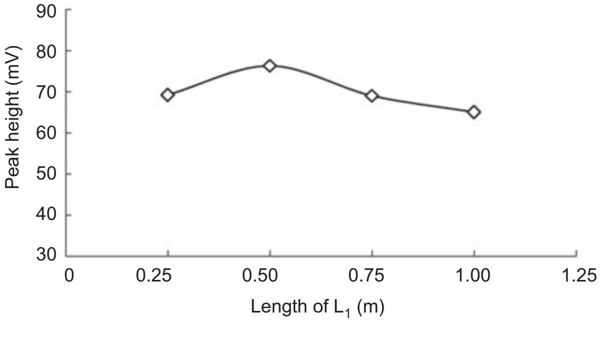


Figure 4 Effect of reaction coil (L_1) length.

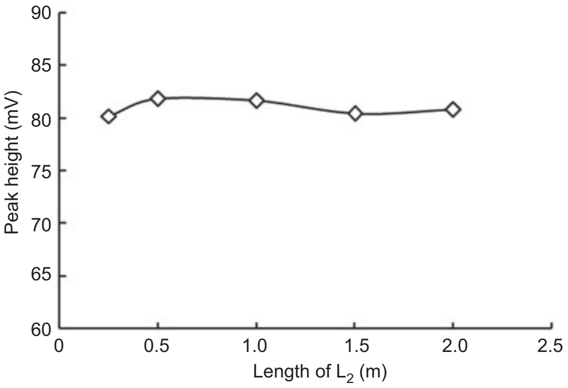


Figure 5 Effect of reaction coil (L_2) length.

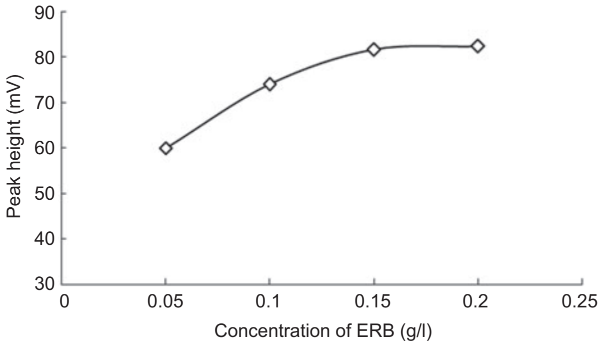


Figure 6 Effect of the ERB concentration.

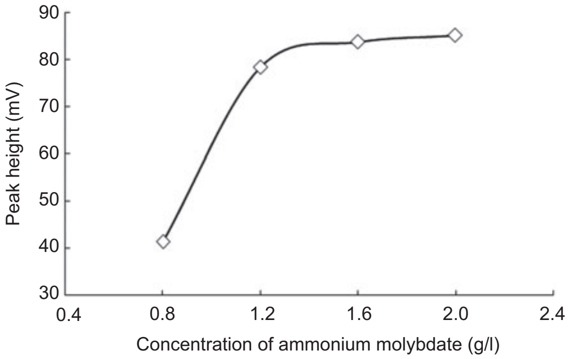


Figure 7 Effect of the ammonium molybdate concentration.

of the parabolic geometry of the sample zone under laminar-flow conditions and the refractive index difference that exists between the sample and carrier or reagent streams (McKelviea et al. 1997). Therefore, 3.09% sodium chloride was selected as matrix (Figure 8).

Effect of salinity

It is well-known that different seawater has different salinity. Salinity is the key parameter affecting seawater physicochemical properties, thus the difficulty in analyzing the trace substance in seawater is overcoming the salinity disturbance. To study the effect of salinity, work standards for phosphate were made by serial dilution of phosphate stock solutions with salinity of 35.0, 30.0, 20.0 and 10.0 sodium chloride solutions. As shown in Table 2, the salinity of 35.0, 30.0, 20.0, and 10.0 were very similar in their slope and intercept. The normal seawater salinity is from 10 to 35, so the proposed method can overcome the salinity interference in the seawater.

Table 2 Comparison of calibration equations for phosphate standards prepared at salinities from 10 to 35.0.

Salinity	Calibration equation H=b (μg/l PO_4^{3-}-P)+a	R^2
10.0	H=1.388 (μg/l PO_4^{3-}-P)+0.321	0.999
20.0	H=1.395 (μg/l PO_4^{3-}-P)+0.235	0.999
30.0	H=1.406 (μg/l PO_4^{3-}-P)+0.739	0.999
35.0	H=1.409 (μg/l PO_4^{3-}-P)+0.741	0.999

Effect of foreign ions

Possible interferences concerning the determination of phosphate caused by common ions in seawater were investigated. The tolerance limit was defined as the concentration of added solutions causing < ±5% relative error during the determination of 50 μg/l of PO_4^{3-}-P. The tolerance limits of various foreign ions that were likely to interfere during the analysis of phosphate in seawater are listed in Table 3. K^+, Na^+, Mg^{2+}, Ca^{2+}, F^-, HCO_3^-, Cl^- and Br^- did not interfere even when present in concentrations much higher than commonly found in seawaters (Riley and Skirrow 1975); 5.6 mg/l silicate did not influence the determination of phosphate in the measurement conditions used, owing to the acidity in the proposed method it did not suit the formation of the ERB-molybdosilicate complex. The interference of Fe^{3+} could be eliminated by adding 0.1% NaF. Arsenate interfered with the determination of phosphate and 0.05% hydroxylamine hydrochloride could eliminate the interference of it. As arsenate concentration is at a much lower level in most natural waters, for example, 20 nM in the open ocean (Karl and Tien 1997), the influence of arsenate could be ignored in most cases.

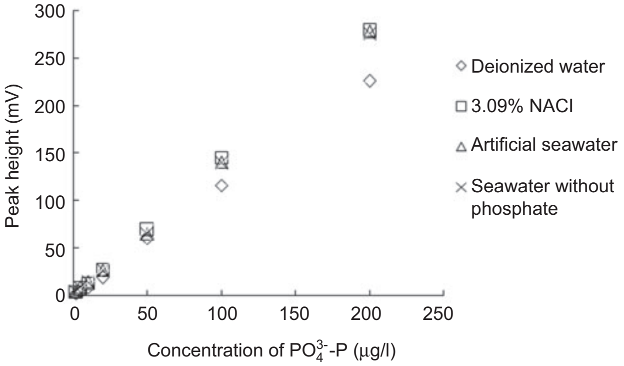


Figure 8 The calibration curves prepared at different matrices.

Analytical figures of merit

Under optimized parameters, a calibration curve was obtained over the concentration range 2.0–200.0 μg/l PO_4^{3-}-P (R^2=0.999, n=7). The regression equation is H=1.406C+0.869, where H is the peak height and C is the concentration of PO_4^{3-}-P, a limit of detection of 0.05 μg/l was achieved (obtained using a signal of three multiples of baseline noises). The relative standard deviation (RSD) for repetitive determination of the PO_4^{3-}-P sample at 50.0 μg/l was 0.61% (n=15). In 1 h, 10 samples or standards could be analyzed in triplicate. The value of the molar absorptivity was 1.6×10^6 mol/l/cm. Compared to the ERB spectrophotometric method (He et al. 1996), the proposed method was simple and sensitive, with no need of heating and cooling, and saving reagent. Mas et al. (1990) developed a high-sensitivity determination method for phosphate using FIA, which was based on a color system similar to that used here. Its limit of detection was 4 μg/l and Al^{3+}

Table 3 Effect of foreign ions on the determination of phosphate (50 μg/l).

Foreign ions	Tolerance limit (mg/l)	Foreign ions	Tolerance limit (mg/l)
K^+, Na^+, Cl^-	35,000	Zn^{2+}, Cu^{2+}	50
Mg^{2+}, Ca^{2+}, HCO_3^-	5000	SiO_3^{2-}	5.6
SO_4^{2-}, Br^-, F^-	4000	Fe^{3+}	2
Pb^{2+}, Co^{2+}, Ni^{2+}	100	As^{5+}	0.5

Table 4 Determination of PO_4^{3-}-P in seawater by the proposed method.

Sample	Measured (µg/l)	Official method (µg/l)	Added (µg/l)	Found (µg/l)	Recovery (%)
1	5.16	5.65	5.00	9.85	96.4
2	32.18	32.46	30.00	61.45	97.6
3	56.71	55.98	50.00	105.92	98.4

was a serious interference. Therefore, the proposed method was more sensitive and selective with higher accuracy. Thus, by a simple change of injecting the reagent, rather than the sample, sensitivity can be increased relative to conventional FIA (Kenneth and Robert 1982).

Application to seawaters

The proposed method was further applied to determine PO_4^{3-}-P in seawater samples, which were filtrated with 0.45 mm filtration membrane to eliminate suspended solids. To prove applicability of the method, a test of recovery was conducted by accurately adding known concentration of PO_4^{3-}-P into each sample whose original PO_4^{3-}-P concentration had been determined. The recoveries of the different concentration levels varied from 96.4% to 98.4%, as shown in Table 4. The results obtained for phosphate by the proposed method were in very close agreement with those using the conventional method (Standard Press of China). Therefore, the proposed method was suitable for the determination of PO_4^{3-}-P in seawater.

Conclusion

This study described a simple, sensitive and rapid reverse flow injection assay for the determination of trace phosphate in seawater. The proposed method enabled accurate and precise determination of phosphate at low levels, using a signal spectrophotometric detector, without the need of any additional time-consuming preconcentration step prior to the final measurement. The model HYY3-1 automatic nutrients analyzer employed in this study is cheap, small and offers fully automated analysis, which is very suitable for on-line determination of phosphate in seawater.

Acknowledgments

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