Application of total phosphorus sequential injection system in Qingdao coastal area

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Abstract. An integrated system was developed for automatic determination of total phosphorus in seawater based on sequential injection analysis combined with spectrophotometric detection. The experimental parameters of digestion were optimized based on a univariate experimental design. The upper limit of the linear range of the proposed method was 1000 μg/L. The relative standard deviations (RSDs) of accuracy were below ±2% (n=3) and the RSD of precision was 2.18% (n=6). This system has been successfully applied to determine total phosphorus in Qingdao Coastal area. The F test and t test implied the results of the system had no evident difference with those detected by nutrient flow analyzer in the laboratory.

1. Introduction
In the ocean, phosphorus is an essential nutrient for the growth of marine organisms. Because of the photosynthesis of zooplankton on the surface of the ocean, phosphate in some sea areas is consumed in large quantities, resulting in the decrease of phosphorus concentration, and even become the limiting element of marine primary productivity[1]. In recent years, with the deepening of the study of phosphorus biogeochemical cycle, scientists have found that when dissolved inorganic phosphorus is exhausted in water environment, phytoplankton can further use dissolved organophosphorus and particulate phosphorus for their own metabolism[2-7]. Marine microorganisms can also decompose dissolved and particulate nutrients in seawater into bioavailable products through autotrophic, heterotrophic and other nutritional methods. For this reason, the study of total phosphorus in seawater is of great significance. In addition, the change of total phosphorus concentration in seawater can reflect the pollution and eutrophication of seawater to a certain extent, which truly reflect the quality of marine environment and the degree of pollution.

At present, there are many commonly used methods for the determination of total phosphorus in water. After digestion, the samples can be analyzed by spectrophotometry[8], ion chromatography[9] or electrode method[10]. In recent years, some novel methods have been developed, such as liquid core waveguide optical detection[11], inductively coupled plasma emission spectrometry[12], software sensor estimation method[13] and so on, which improve the sensitivity and accuracy of detection. However, all the above methods need to take seawater samples to the laboratory for analysis. These methods have some shortcomings, such as poor data timeliness, long measurement cycle, high labor cost, and easy to be polluted in the process of sample transportation and preservation[14]. Therefore, it is difficult to monitor the total phosphorus in the marine environment effectively, and forecast the marine pollution.

In this paper, the performance test of the sequential injection system was carried out, and the developed system was applied to detect total phosphorus in Qingdao coastal area.
2. Experimental

2.1. Standard solutions and reagents
All used chemicals were of analytical grade or better, and purchased from Sigma Chemical Reagent Co., USA, unless stated otherwise. All solutions were prepared with ultrapure water (resistivity ≥ 18.2 MΩ cm) obtained from a Millipore water purification system (Millipore Co., USA). The artificial seawater was 31 g/L of sodium chloride, 10 g/L magnesium sulfate and 0.05 g/L sodium hydrogen carbonate made up to 1000 mL with ultrapure water.

The β-glycerophosphate (100 mg/L, P) was prepared as stock solutions. Working standards were obtained from appropriate dilution of stock solutions with artificial seawater. The oxidation reagent (OR) was 30 g/L of potassium peroxodisulphate made up to 1000 mL with ultrapure water. Acidic molybdate was prepared by dissolving 4.44 g of ammonium molybdate in 120 mL of ultrapure followed by the addition of 20 mL of concentrated sulfuric acid, then added 0.096 g of potassium antimony tartrate before dilution to 200 mL with ultrapure water (R1). The reductant was 54 g/L ascorbic acid with ultrapure water (R2).

2.2. Instrumentation
A system (5200 S1) for determination total phosphorus was developed by the Institute of Oceanographic Instrumentation, Qilu University of Technology (Shandong Academy of Sciences), and was equipped with 10 μm filter and reagent warehouse. The San++ nutrient flow analyzer used in the comparative experiment was purchased from Skalar, Netherlands.

2.3. Sample collection and testing
Seawater was collected from three locations along the coastline of Qingdao City, Shandong Province, China. The samples were preserved in closed, plastic buckets at 4 ℃ and then detected within 24 h. The sampling was conducted in three locations (L1: Zhongyuan Wharf; L2: May 4th Square; L3: Shilaoren Beach) once a day for a week. These samples were divided into two parts. Some of the samples were filtered through a 10 μm pore-size membrane filter immediately after collection, and then were manually digested and measured with the nutrient flow analyzer. The other samples were directly tested with a 5200 S1 type total phosphorus in-situ system.

2.4. Principles, operating procedure, and structure of the system

2.4.1 Principle of chemical reactions. The used method for total phosphorus determination was modified phosphomolybdenum blue (PMB) method, and PMB was detected at 880 nm with dual beam method in order to correct baseline drift.

2.4.2 Operating procedure. The sample and OR solution were drowned by an ejector and mixed evenly in the syring. Then the mixed solution was injected into high temperature digestion module, which was heated to 125 ℃ to convert the phosphorus compounds in the sample into orthophosphate. After digestion, the sample was drowned from the digestion tank mixed with R1 solution to form phosphomolybdate yellow. Then R2 solution was added to form blue complex, which was injected into the optical flow detection module to determine the absorbance. The concentrations of total phosphorus was calculated from the absorbance of the output result using the prepared calibration curve.

2.4.3 Structure of the system. The system includes four parts: sampler module, digestion module, detection module and control module. All modules of the system were supported by a metal bracket, and besides control module, other modules were connected through PTFE tubes. The system housing was made of PVC and was equipped with a reagent rack. (shown in figure 2).
3. Results and discussion

3.1. Optimization of digestion conditions

Based on Specification for Oceanographic Survey\(^{[15]}\), \(\beta\)-glycerophosphate was chosen as the model compounds to evaluate the effect of digestion condition.

3.1.1 Optimization of OR’s concentration. Because the average annual water temperature in Qingdao coastal area is only 8.4 °C, the high concentration of OR may lead to the precipitation of potassium persulfate at low temperature, which will affect the accuracy of the determination results. According to the Specification for Oceanographic Survey\(^{[15]}\) and other literatures\(^{[14, 16]}\), different concentrations of persulfate varied from 10 g/L to 50 g/L were chosen and put in the refrigerator overnight at 4 °C. The results showed that the potassium persulfate of 10g/L, 20g/L and 30g/L did not precipitate at low temperature. In order to reduce the amount of reagent as much as possible, we finally selected 30g/L potassium persulfate as OR.

3.1.2 Optimization of digestion temperature. The digestion temperature also have a great impact on the accuracy and stability of the determination results. In theory, the higher the digestion temperature is, the greater the digestion efficiency of total phosphorus. However, the excessive digestion temperature may affect the safety of the digestion module. In this paper, 120 °C, 125 °C and 130 °C were chosen as the digestion temperature, and 20 min was used as the digestion time. As shown in Figure 2, the maximum relative digestion percentages of \(\beta\)-glycerophosphate was reached at 20 min. The result shows that the maximum relative digestion percentages of \(\beta\)-glycerophosphate appeared when the digestion temperature is 125 °C.

3.1.3 Optimization of digestion time. Besides, we investigate the different digestion time, in order to reduce the analysis time and energy consumption of the whole instrument as much as possible on the basis of satisfying the complete digestion of total phosphorus. Different digestion time varied from 10 min to 30 min were selected. As shown in Figure 2, the maximum relative digestion percentages of \(\beta\)-glycerophosphate was reached at 20 min, and no significant increase in the digestion percentage was observed with further increase in the digestion time of 25 min and 30 min. And Under the digestion time of 20 minutes, the results are stable, with the RSD of 4.33%. Hence, 20 min is chosen as the final digestion time.

To sum up, the optimum digestion condition of seawater total phosphorus in situ sensor is that the sample is digested in 30 g/L potassium persulfate at 125 °C for 20 min.
3.2. Analytical figures of merit
The analytical figures of merit for the proposed method are summarised in Table 1. The system and method display excellent sensitivity and linearity over the calibration range used in these experiments which resulted in a quantification limit of 12 μg/L. An RSD of 2.18% was obtained when measuring a sample collected from Qingdao coastal area (n=6), which indicated that the conversion of different forms phosphorus in sea water to orthophosphate is reasonably precise. The accuracy of the system is obtained by measuring different concentrations (100 μg/L, 500 μg/L, 800μg/L) of β-glycerophosphate standards, and the RSD was less than ±2.00%.

Table 1. The analytical figures of merit for the sequential injection system

| Parameters                  | Results                      |
|-----------------------------|------------------------------|
| Standard curve              | y=0.0008x-0.0101             |
| Calibration range           | 12-1000μg/L                  |
| Linearity                   | 0.9992                       |
| Limit of quantification     | 12 μg/L                      |
| 100 μg/L                    | -1.13%                       |
| Accuracy                    | 500 μg/L                     |
| 800 μg/L                    | 1.84%                        |
| Precision                   | 2.18%                        |

3.3. The application of total phosphorus sequential injection system
The seawater samples collected from three sampling locations along the coastline of Qingdao City were examined with the 5200 S1 total phosphorus sequential injection system and nutrient flow analyzer.

Figure 3 shows the concentrations of total phosphorus in seawater using different analytical instruments for seven days at different sampling sites. The results measured by 5200 S1 total phosphorus sequential injection system are close to those measured by nutrient flow analyzer.
The differences of results between the two instruments are verified by F test and t test. It can be seen from Table 2 that the calculated F value is 2.27, which is less than the value of F (0.05, 6, 6), so there is no significant difference in the precision of the two methods. And then the t test (see Table 3) result shows that t < t (0.05, 12), which indicate there is no significant difference between the two methods. Therefore, it can be considered that the 5200 S1 total phosphorus sequential injection system can used as a replacement of nutrient flow analyzer to detect seawater sample.

Table 2. F test of the two instruments

| Sample Location | Sample Size | Instrument | S²  | F    | F (0.05,6,6) | Conclusion          |
|-----------------|-------------|------------|-----|------|--------------|---------------------|
| Zhongyuan Wharf | 7           | 5200 S1    | 1.86| 2.27 |              | No evident difference |
|                 |             | San++      | 4.22|      |              |                     |
| May 4th Square  | 7           | 5200 S1    | 8.54| 1.34 | 4.28         |                     |
|                 |             | San++      | 11.49|     |              |                     |
| Shilaoren Beach | 7           | 5200 S1    | 15.39| 3.47 |              |                     |
|                 |             | San++      | 4.44|      |              |                     |

Table 3. t test of the two instruments

| Sample Location | Sample Size | Instrument | Average value | Standard error | t      | t (0.05,12) | Conclusion          |
|-----------------|-------------|------------|---------------|---------------|-------|------------|---------------------|
| Zhongyuan Wharf | 7           | 5200 S1    | 28.17         | 0.93          | 1.67  |            | No evident difference |
|                 |             | San++      | 26.62         |               |       |            |                     |
| May 4th Square  | 7           | 5200 S1    | 31.21         | 1.69          | 1.06  | 4.28       |                     |
|                 |             | San++      | 29.41         |               |       |            |                     |
| Shilaoren Beach | 7           | 5200 S1    | 29.03         | 1.68          | 0.34  |            | No evident difference |
|                 |             | San++      | 28.45         |               |       |            |                     |
4. Conclusion
A sequential injection analysis combined with spectrophotometric method for total phosphorus determination was established. The proposed method had the advantages of a high precision and accuracy, wide linear range and easy to operate. There was no significant difference between the results obtained with the proposed method and reference methods.

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