Spectrophotometric Determination of Total Phosphorus in Fresh Water Using Ammonium Molybdate

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We developed a rapid method of total phosphorus (TP) determination in fresh water by optimizing the volumes and concentrations of the oxidant (potassium persulfate), reductant (ascorbic acid), and colorant (molybdate + antimony potassium tartrate). Instead of a UV spectrophotometer, as used in the national standard method (GB 11893-89), we used the iMark Microplate Absorbance Reader to measure the absorbance. The principle of TP determination was identical to that of the national standard method as the water sample was digested under a neutral condition and phosphorus was oxidized to orthophosphate and colored to measure absorbance. In the developed method, we adjusted the volumes of the oxidant and reductant to 80 μL and 0.1 g/mL, respectively, and the concentration of the colorant to 31.00 + 0.32 mol/mL. The water samples collected from Lake Taihu in Maoming, Guangdong, China, were used in the experiment. The feasibility and applicability of the developed method were validated by determining TP in freshwater samples. The results showed that the developed method has excellent accuracy and precision compared with the national standard method. In addition, the national standard method was simplified in terms of the digestion and determination of TP, whereby the volume of chemical reagents and the determination time were reduced. The efficiency of TP determination was improved such that the developed method can be used for monitoring water quality to prevent environmental problems.

1. Introduction

Phosphorus is an indispensable and important nutrient for the growth of organisms in natural aquatic environments. In fresh water, phosphorus is a limiting nutrient for algae;[1,2] thus, changes in phosphorus concentration in the water affect the structure of phytoplankton community.[3,4] The limiting concentration of dissolved phosphorus for the growth of phytoplankton is 0.01 mg/L. The lower its concentration, the slower the growth of phytoplankton species. Phosphorus is related to the primary productivity in natural water bodies.[5] It is

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important to monitor the phosphorus concentration in water to maintain a healthy phytoplankton community and a high water quality as excessive phosphorus causes eutrophication.

Eutrophication refers to the overpopulation of algae and zooplankton caused by excessive nutrients such as nitrogen and phosphorus in water. It degrades the water quality and decreases the transparency of the water body.[6] At present, eutrophication has become a severe environmental problem.[7] The eutrophication of water bodies under natural conditions is slow. However, with rapid urbanization and agricultural modernization, sewage discharge has increased, which raises the concentrations of pollutants and nutrients. Nitrogen and phosphorus concentrations exceeding the maximum load in a water body can cause eutrophication.[8,9] The input and behavior of phosphorus in the outer estuary region significantly affect the eutrophication of nearby lakes.[10] Qin *et al.* reported that total phosphorus (TP) affected the flux of ammonia nitrogen in the Yangtze River Basin.[11] TP is an important indicator in the long-term detection and monitoring of eutrophication and ecological changes in freshwater bodies such as rivers and lakes.[12,13]

At present, various methods including spectrophotometry, atomic spectrometry, and chromatography are used for the determination of TP in freshwater bodies.[14] The high-temperature and high-pressure digestion method using potassium persulfate is a national standard method for the determination of TP in China, as this method is simple and produces consistent results with a wider monitoring range and less cost than other methods.[15] In the national standard method, potassium persulfate is used as an oxidizing agent to dissolve and oxidize phosphorus-containing compounds.[16] Ammonium molybdate and potassium antimony tartrate under acidic conditions are added to react with orthophosphate to form phosphomolybdenum zwitterionic acid, which is reduced by ascorbic acid and generates a blue complex for the determination of TP by a colorimetric method.[17] However, this method requires digesting water samples and thus a long reaction time, which affects the efficiency of the determination. In addition, the samples need to be sealed completely to prevent the leakage of the solution during sterilization, which affects the accuracy of the experimental result and makes the determination difficult. Safety is also a concern during the digestion of phosphorus. Even if an intelligent autoclave improves the precision of pressure and temperature control during the determination of TP, the complicated process of the national standard method impacts the accuracy and precision of the determination.[18]

To solve the problems of the national standard method, such as a long determination time and possible leakage of sealed samples, we developed a small-volume reaction system to simplify the national standard method and improve the efficiency and accuracy of the TP determination method. We used a Lianhua digestion apparatus and an enzyme labeling instrument (iMark Microplate Absorbance Reader) for the digestion and determination, respectively, and adjusted the volumes and concentrations of reagents and experimental conditions. The proposed method for the determination of TP in fresh water is simple and rapid and thus can be conveniently used to monitor the phosphorus concentration in water to prevent eutrophication and ecological changes.

2.   Materials and Methods

2.1   Chemical reagents

The reagents used for the determination of TP in fresh water are shown in Table 1.

2.2   Optimization of method for TP determination

To find the optimal method for TP determination, we experimented with various volumes of the oxidant and reductant, oxidation times, and degrees of color development. An iMark Microplate Absorbance Reader was used to measure the absorbance of the treated samples. 20, 40, 60, 80, or 100 µL of the oxidant was added to standard phosphorus solutions of different concentrations (0, 0.04, 0.08, 0.24, 0.4, and 0.8 µg/mL). To find the optimal oxidation time, the standard phosphorus solutions were reacted with reagents for 10, 20, 30, 40, and 50 min at room temperature. Different volumes of the reductant (ascorbic acid) (0, 0.06, 0.08, 0.10, 0.12, and 0.14 g/mL) were added to oxidize the standard phosphorus solutions. Various concentrations of the colorant (molybdate + antimony potassium tartrate = 7.75 + 0.58, 15.50 + 0.12, 23.25 + 0.17, 31.00 + 0.23, and 38.69 + 0.29 mol/mL) were added. The absorbances of the standard phosphorus solutions with different volumes and concentrations of the oxidant, reductant, and colorant were measured at 700 nm. On the basis of the results, we determined the optimal volumes and concentrations of the reagents. The TP values of the collected water samples and standard

Table 1
Reagents used in the developed method.

Reagent	Concentration	Formulation
Sulfuric acid	50%	Concentrated sulfuric acid is diluted by adding it to ultrapure water at a ratio of 1:1.
Oxidant (potassium persulfate solution)	$0.05 \ /(g \cdot mL^{-1})$	5 g of potassium persulfate with an appropriate volume of ultrapure water is dissolved and transferred to a 100 mL volumetric flask.
Reductant (ascorbic acid solution)	$0.1 \ g \cdot mL^{-1}$	10 g of ascorbic acid is dissolved in an appropriate volume of ultrapure water and transferred to a 100 mL volumetric flask.
Colorant (molybdate solution)		13 g of ammonium molybdate and 0.35 g of potassium antimony tartrate are dissolved in 100 mL of ultrapure water and ammonium molybdate solution is slowly added to 300 mL of 50% sulfuric acid with constant stirring. Potassium antimony tartrate solution is added and mixed.
Turbidity-color compensation solution		50% sulfuric acid and 0.1 g/mL ascorbic acid are mixed well.
Storage solution for standard phosphorus solution	$50 \ g \cdot mL^{-1}$	An appropriate volume of potassium dihydrogen phosphate is dried at 110 °C for 2 h and cooled at room temperature in a desiccator. 0.02197 g of the dried potassium dihydrogen phosphate is dissolved in water and transferred to a 1000 mL volumetric flask. 800 mL of ultrapure water is added with 5 mL of 50% sulfuric acid and diluted to the mark with water.
Standard phosphorus solution	$2 \ g \cdot mL^{-1}$	4 mL of the phosphorus standard reserve solution is transferred to a 100 mL volumetric flask, added with ultrapure water, and diluted to the mark. The solution is good for one-day use.

phosphorus solutions were measured by the developed method and other methods, and the results were compared to validate the accuracy and availability of the developed method.

2.3   Standard phosphorus solution

By the national standard method, a standard working curve for the determination of TP was created. The absorbances of the standard phosphorus solutions were measured using the iMark Microplate Absorbance Reader. The standard phosphorus solutions were prepared as follows. In a 100 mL volumetric flask, 4 mL of the phosphorus standard solution was added with pure water to the mark. Then, standard solutions of 0, 0.1, 0.2, 0.6, 1, and 2 mL were added into two groups of six round-bottomed and screw-capped 18–250 mm ablution tubes and diluted to 5 mL with ultrapure water. 80 μL of the potassium persulfate solution was added as an oxidant and reacted at 120 °C for 30 min. Then, 20 μL of the ascorbic acid solution was added as a reductant, mixed well, and left for 30 s. 40 μL of the molybdate solution was then added as the colorant, mixed well, and left for 15 min for a full reaction. If the solution showed turbidity or chromaticity, 60 μL of a turbidity–chromaticity compensation solution was added. The concentration of the blank solution was measured and subtracted from the absorbances of the sample solutions. When the concentration of arsenic was higher than 0.002 mg/mL, sodium thiosulfate was used to remove interference from arsenic. When the concentration of sulfide was higher than 0.002 mg/mL, nitrogen gas was introduced to purge sulfur. When the concentration of sulfide chromium was greater than 0.05 mg/mL, sodium sulfite was used to remove interference. After sufficient reaction, the absorbances of the standard solutions were measured at 700 nm and used to draw a standard working curve. To verify the accuracy and reliability of the standard curve, TP was determined by the developed and national standard methods. In the two methods, linear regression was used to construct a standard curve of the absorbance and concentration.

2.4   Sampling method

Five freshwater samples were collected in three locations: domestic and agricultural wastewater (No. 1, located near Kechuang Road, Dapang Village, Maoming City), a freshwater aquaculture pond [No. 2, located near Xicheng Campus of Guangdong Institute of Petrochemical Technology (GIPC), No. 1, Kechuang Road, Maoming City], and small lakes (Nos. 3, 4, and 5, located in the Xicheng Campus of GIPC as well as in the West Lake of Guandu Campus) (Fig. 1). The water samples were collected using plastic bottles made of polyethylene terephthalate glycol (PETG) and stored at 4 °C after collection. After the samples were moved to the laboratory, TP in the samples was determined by the developed and the national standard methods. The results were analyzed for feasibility, accuracy, and reliability using the *F*- and *t*-tests.

The scale of the map of Guangdong Province is 1:3500000, and that of the Maonan District, Maoming City, is 1:83000. Maps were downloaded in March 2021 from the Standard Map Service (http://bzdt.ch.mnr.gov.cn/). The review numbers were GS(2019)3333 and Yue S(2018)038.
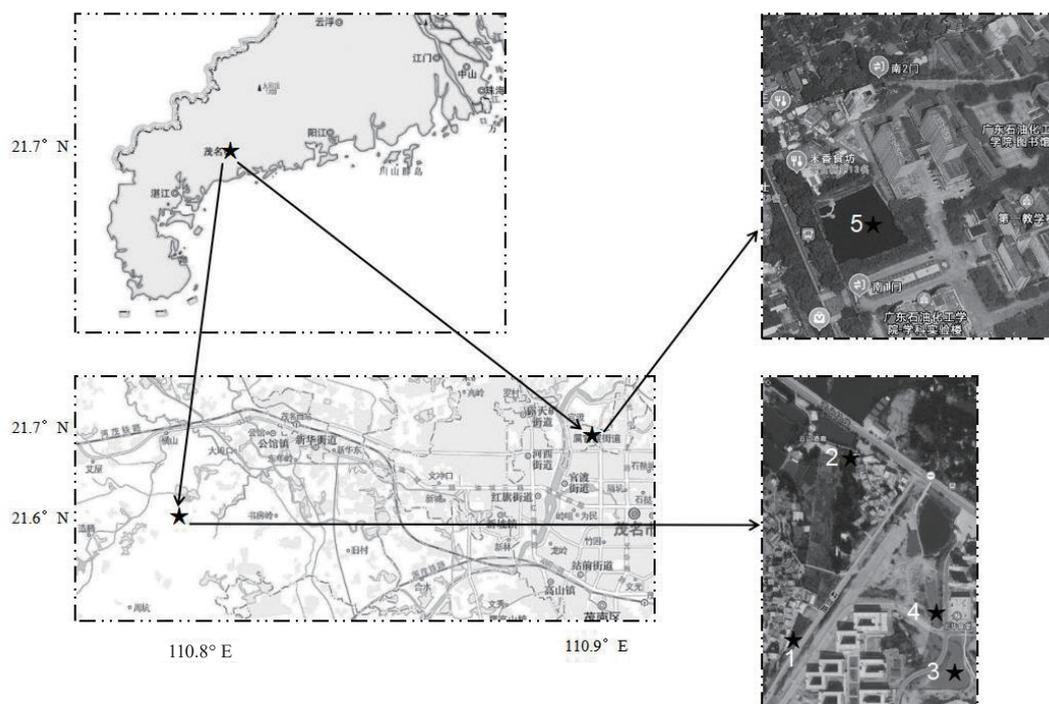
Fig. 1.   Map of sampling locations.

3.   Results

3.1 Volume of oxidant

The absorbances of TP in the standard solutions with different oxidant volumes were measured and are shown in Fig. 2. Even with the increasing volume of added potassium persulfate, the absorbance did not change significantly. That is, the different oxidant volumes did not affect the results of TP determination. To prevent insufficient oxidation, a volume of 80 μL was selected for the developed method.

3.2   Digestion time

Digestion time is important in the conversion of phosphorus to orthophosphate. The absorbance of the standard solution for TP with different digestion times is shown in Fig. 3. The absorbances of the standard solutions decreased with increasing digestion time from 10 to 20 min. However, a higher absorbance was observed at the digestion time of 30 min than at 20 min, and the absorbance did not change significantly thereafter. A digestion time of 10 min was very short for sufficient oxidation, and intermediate products still existed in the solution, which caused a high absorbance at 700 nm. After 20 min, the intermediate products were completely reacted and thus the absorbance decreased. After 30 min, phosphorus was completely oxidized to orthophosphate and the absorbance increased. Therefore, the optimal digestion time was determined as 30−40 min. Thus, we chose 30 min as the digestion time.
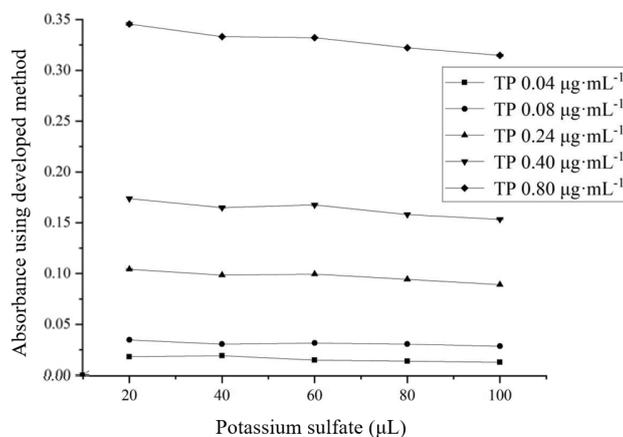
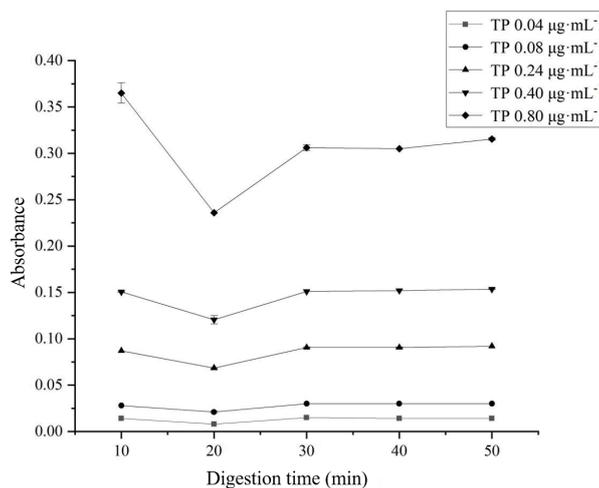Fig. 2.	Effect of oxidant (potassium persulfate) on absorbance of TP of different concentrations.

Fig. 3.	Effect of digestion time on absorbance of TP of different concentrations.

## 3.3	Reductant

The absorbance of the standard solution with different volumes of ascorbic acid solution is shown in Fig. 4. The absorbance decreased when the volume of the reductant was increased from 0.06 to 0.08 g/mL. Such an increase was evident in the solution with a TP of 0.04 μg/mL. It did not change significantly with a reductant of more than 0.06 g/mL. When the volume of ascorbic acid was 0.06 g/mL, the reduction was insufficient, and intermediate products resulted in high absorbances at 700 nm as chlorine ions might exist in the solution owing to insufficient reduction. More investigation is necessary to understand such an insufficient reduction. When the volume of ascorbic acid was increased to 0.08 g/mL, phosphorus was completely reduced, and the absorbance decreased. Even with a higher volume, the absorbance did not decrease. To ensure the complete reduction of phosphorus, the volume of ascorbic acid was chosen to be 0.1 g/mL.
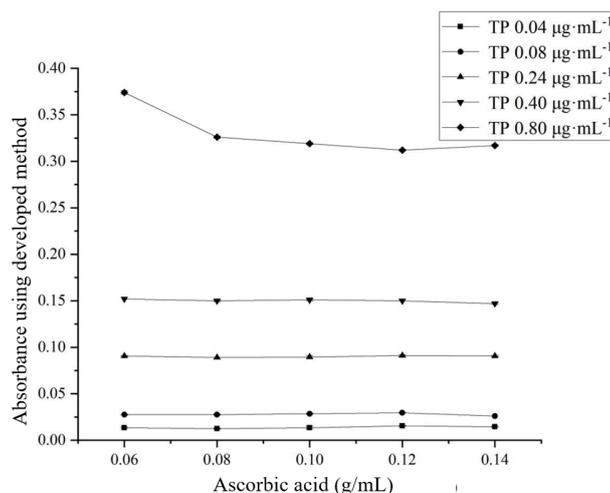
Fig. 4.   Effect of reductant (ascorbic acid) on absorbance of TP of different concentrations.

3.4   Colorant

The colorant is essential for the determination of TP in fresh water. The effect of using different volumes of the molybdate solution (ammonium molybdate + antimony potassium tartrate) on the determination of TP is shown in Fig. 5. The absorbance of the standard solution increased when the concentrations of the colorant were 7.75 + 0.058 and 15.50 + 0.12 mol/mL. The higher volume of the molybdate solution did not affect the absorbance. With the colorant concentration of 7.75 + 0.058 mol/mL, the color did not develop sufficiently to react with orthophosphate, resulting in a low absorbance. When the concentration exceeded 15.50 + 0.12 mol/mL, the orthophosphate reacted with the ammonium molybdate sufficiently to produce the phosphomolybdenum heteropolymeric acid in the presence of antimony salts that were reduced by ascorbic acid to produce a blue complex. Therefore, the absorbance increased. To ensure color development in the solution, we selected the colorant concentration of 31.00 + 0.32 mol/mL.

3.5   Standard curve fitting

On the basis of the experimental results, the optimal conditions for the developed method were determined to be as follows: 80 μL of oxidant, 30 min of digestion time, 0.1 g/mL of reductant, and 31.00 + 0.32 mol/mL of colorant. The curve of the absorbance of the standard phosphorus solution prepared by the developed method is shown in Fig. 6. A linear relationship was observed between absorbance and TP, and regression equations were determined for the developed [Eq. (1)] and national standard [Eq. (2)] methods. For the two regression equations, the coefficient of determination, R^2, was 0.9998.

$$Absorbance = 0.3991 \times concentration - 0.0018 \tag{1}$$

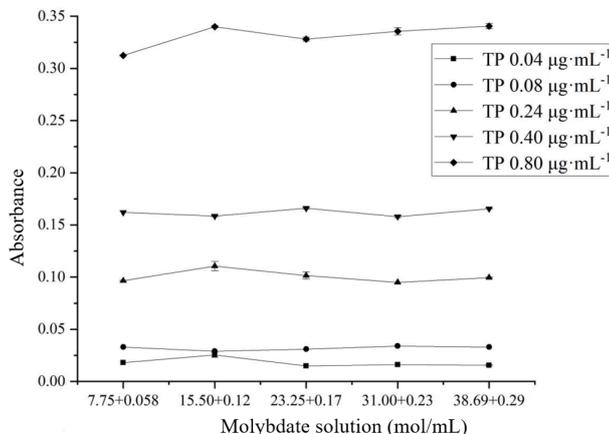$$Absorbance = 0.3965 \times concentration - 0.0028 \tag{2}$$

Fig. 5.   Effect of colorant (ammonium molybdate + antimony potassium tartrate) on absorbance of TP of different concentrations.
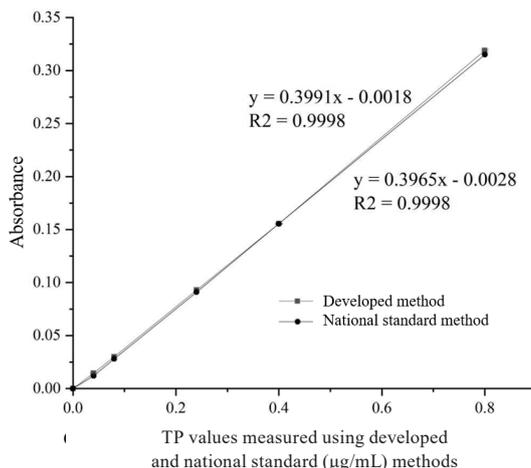

Fig. 6.   Linear regression of absorbance and TP using proposed and national standard methods.

To determine the accuracy of TP determination, we used the orthogonal validation method, and the result is shown in Fig. 7. The linear equation between the absorbances obtained by the developed and national standard methods is

$$y = 0.9934x - 0.0011, \tag{3}$$

where y is the absorbance obtained by the developed method and x is that obtained by the national standard method. The coefficient of determination, R^2, was 0.9999. The result of ANOVA confirmed the consistency of the result between the developed and national standard methods ($F = 0.001 < 5.318$ and the confidence level was 95%). Thus, the results of the developed method were found to be identical to those of the national standard method and reliable for the determination of TP in fresh water.
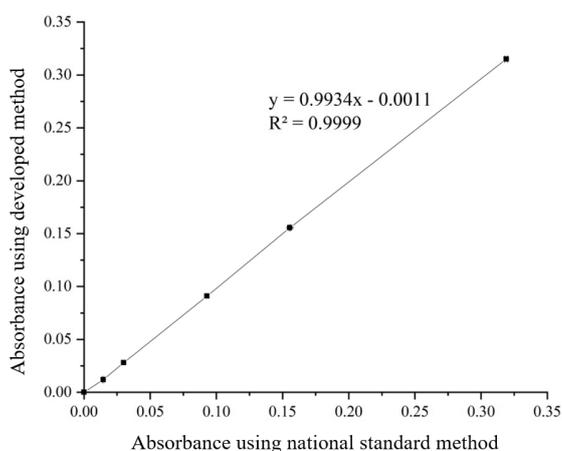
Fig. 7.   Linear regression of absorbance of standard solutions using developed and national standard methods.

3.6   Detection range of developed method

The detection range of the developed method was determined, and the result is shown in Fig. 8. When TP was in the range of 0–2 µg/mL, it had a linear relationship with absorbance (R^2 = 0.9968). In the concentration range higher than 2 µg/mL, the absorbance exceeded 1, implying that the solution should be diluted. Thus, we regarded the optimal detection range of the developed method as below 2 µg/mL, which is nearly four times higher than that of the national standard method (0–0.6 µg/mL). The developed method showed a higher upper detection limit in TP determination in fresh water than the national standard method. Reagents and time can be saved with the enhanced efficiency of the determination of a large number of water samples.

3.7   Accuracy of developed method

TP in the collected freshwater samples was determined using the developed and national standard methods. As shown in Fig. 9, R^2 of TP determined using the two methods was 0.9995, showing an excellent linear relationship. The *F*- and *t*-test results of the concentrations determined using the two methods showed that the *F*-value was 0.001 (<5.318) at a confidence level of 95% and the *t*-value at the same confidence level was 0.981 (>0.05). The results indicated that there were no significant differences in precision and accuracy between the developed and national standard methods. Therefore, the developed system was feasible, applicable, and reliable in measuring TP in fresh water.
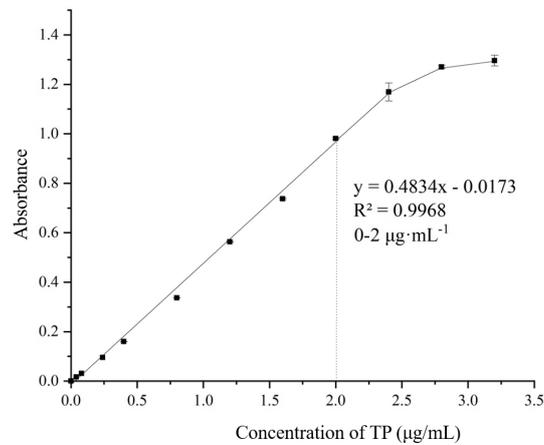
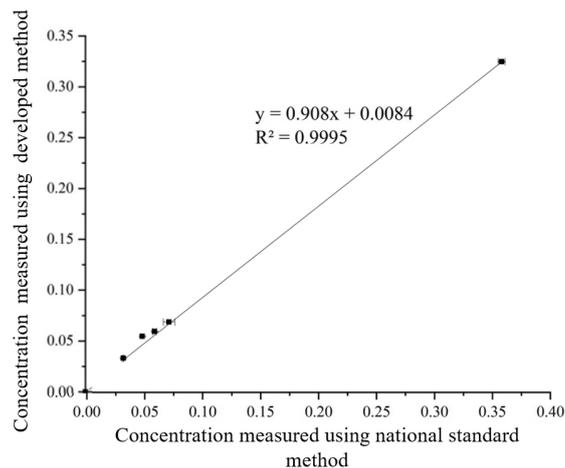Fig. 8. Detection range of TP using developed method.

Fig. 9. TP values measured using developed (y-axis) and national standard (x-axis) methods.

4. Discussion

Lake Taihu is famous for aquaculture and is an example of the successful reform of the fishery industry in China.[19–21] However, the small-scale aquaculture, decentralized operation, and high-density aquaculture increase the discharge of nitrogen and phosphorus into the environment, causing a high chemical oxygen demand (COD). Thus, ecological and environmental problems such as eutrophication occur and affect the sustainable development of the regional economy.[21–24] To prevent such problems, the real-time monitoring of pollution and the rational development of water resources are important for economic development and the environment.[25,26] TP has been regarded as an important indicator of environmental problems, and the national standard method of TP determination requires high-temperature and high-pressure digestion using potassium persulfate.[16] The sample volume for the national standard

method is 50 mL, whereas that for the developed method is 25 mL. The effective reaction volume for the determination of TP was decreased from 32 to 6.4 mL. The iMark Microplate Absorbance Reader was used instead of the UV spectrophotometer. With the increasing need for environmental monitoring and ecological research in fresh water, the developed method enables a more efficient and rapid determination of TP in fresh water than the national standard method. The developed method reduces the consumption of chemical reagents and the volume of wasted solutions after determination. It also minimizes the cost of TP determination. Its upper detection limit is lowered compared with that of the national standard method. The iMark Microplate Absorbance Reader can be used, which is conducive to an efficient and rapid determination and monitoring of TP in fresh water. The developed method can be used for the monitoring and prevention of the eutrophication of lakes, rivers, and other water bodies.

5.   Conclusions

For the effective determination of TP in fresh water, we developed a new method of using potassium persulfate as the oxidant, ascorbic acid as the reductant, and ammonium molybdate + antimony potassium tartrate as the colorant. The iMark Microplate Absorbance Reader was used for measuring the absorbance of the colored complex of TP in water samples. The optimal concentration of the reagents was determined in experiments using standard phosphorus solutions and water samples collected from Lake Taihu in Maoming, Guangdong, China. The optimal volume of the oxidant was 80 μL, the digestion time was 30 min, the volume of the reductant was 0.1 g/mL, and the concentration of the colorant was 31 + 32 mol/mL. A round-bottomed, screw-capped digestion tube of 18 × 250 mm was used as the reaction vessel instead of the 50 mL digestion tube used in the national standard method. The effective reaction volume for TP determination was reduced from 32 to 6.4 mL The absorbances obtained by the developed and national standard methods coincided well with R^2 close to 1. The detection limit was expanded to be 0−2 μg/mL, far exceeding the higher detection limit of 0.6 μg/mL of the national standard method. The developed method can be used to effectively monitor TP in fresh water, which helps prevent eutrophication and other environmental problems.

Acknowledgments

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