Determination of Phosphate Ion Based on Fluorimetric Quenching of Eu^{3+} and TTA Complex in Brij 58 Surfactant

Ki-won Cha,* Chan-il Park, Young-bae Jung, and Kwang-won Park†

Department of Chemistry, Inha University, Incheon 402-751, Korea
†Korea Atomic Energy Research Institute, Taejon 305-600, Korea
Received June 19, 1999

Phosphorus is essential to all living organisms and is believed to be involved in the metabolism of life itself. Hence, this element is distributed widely in plant and animal tissues.[1] Phosphorus occurs in nature as orthophosphate, in its most stable oxidation state and the simplest form of phosphates.[2] Phosphate is widely used commercially to produce fertilizers and other applications. The overuse of fertilizers brings about eutrophication, causing red tide in the seas since phosphate is the limiting nutrient for microbial growth.[3] Polyphosphates (chain form) are hydrolized phosphate ions. Condenced phosphates (chain and ring structures) can be converted to the orthophosphate form by the addition of HNO_3. In this sense, the determination method for trace amounts of phosphate ion is important for the seas. The general procedure for spectrophotometric determination of phosphate is based on the reaction with molybdate ion.[4,5] Several spectrophotometric methods based on this reaction have been reported, however, their detection limits were not adequate to test clean water, which has normally a 10^{-7} M level of phosphate.

In this work, we developed a spectrofluorimetric determination method for phosphates based on the fluorescence quenching of Eu^{3+}-thenoyltrifluoroacetone (TTA) complex. The complex[6-8] of Eu^{3+}-TTA gives a strong fluorescence by intra-molecular energy transfer, where TTA absorbs light first and transfers energy to the excited energy level of the emitting ion.[9,11] The addition of phosphate to the Eu^{3+}-TTA system shows a quenching effect. This phenomenon enables the rapid and sensitive determination of phosphates. This method has been used to determine the level of phosphates in natural, tap and lake water.

Experimental Section

Apparatus and reagents. Absorption spectra were measured with a Perkin-Elmer 552S Spectrophotometer. All fluorescence measurements were done with a Shimatzu RF-5301PC Spectrofluorophotometer using 1 cm quartz cell. The band passes was at 10 nm for excitation and emission monochromaters. The light source was a 150W Xenon lamp. All pHs were measured with a Mettler Toledo MP220 pH meter.

Eu^{3+} stock solution (1.0×10^{-2} M) was prepared by dissolving Eu_2O_3 with a small amount of hydrochloric acid, which was then diluted with water. A standard solution thenoyltrifluoroacetone (TTA) (1.0×10^{-2} M) was prepared by dissolving the TTA in 30% ethanol. The working standard solutions of phosphate were prepared by diluting a 1.0×10^{-2} M KH_2PO_4 stock solution. A surfactant solution (1.0×10^{-2} M) was prepared by dissolving the appropriate amount of Brij 58 in water by gentle heating. Hexamethylenetetramine (hexamine) solution (0.1 M) was prepared as a buffer solution and the pH adjusted to 6.8 with either hydrochloric acid or sodium hydroxide.

Analytical chemicals and deionized distilled water were used throughout the experiment. All glassware was washed thoroughly with concentrated hydrochloric-nitric acid at 70-80 °C and rinsed with deionized water to minimize phosphate contamination.

Precedure. 10 mL pH 6.8 buffer solution (1.0 M hexamethylenetetramine-HCl), 10 mL 1.0×10^{-4} M TTA, 2 mL 1.0×10^{-4} M Eu^{3+}, 10 mL 1.0×10^{-3} M Brij 58 and an appropriate amount of phosphate (from KH_2PO_4) were added to a 100 mL volumetric flask and diluted to the mark. The fluorescence intensity of the mixed solution was measured at 615 nm when excited at 343 nm. All experiments were conducted at room temperature (23 ± 2 °C) and blank corrected.

Results and Discussion

Excitation and emission spectra. The excitation and emission spectra of the Eu^{3+}-TTA in the absence and presence of phosphate are shown in Figure 1. Maximum excitation and emission wavelengths are approximately 343 and 615 nm, respectively. The presence of phosphate results in a decrease in the intensity of excitation and emission spectra although

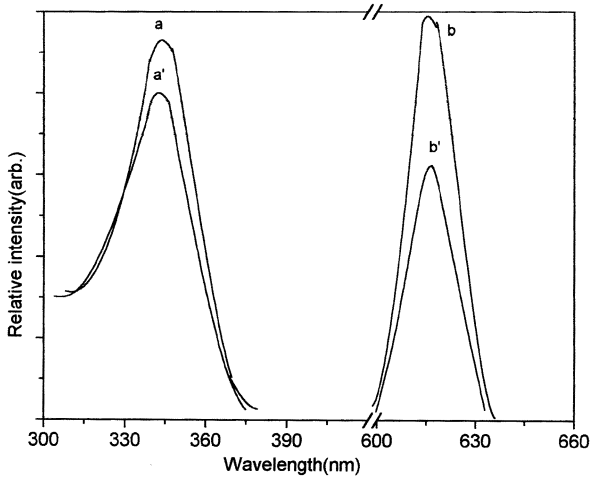

Figure 1. Excitation (a, a') and emission (b, b') spectra of Eu^{3+}-TTA in the absence and presence of phosphate. a, b: $[Eu^{3+}] = 2.0 \times 10^{-6}$ M, $[TTA] = 1.0 \times 10^{-5}$ M, $[Brij\ 58] = 1.0 \times 10^{-4}$ M; pH = 6.8. a', b': adding 8×10^{-7} M phosphate to a, b respectively.

*Corresponding author: Fax: +82-32-872-2520; E-mail: kwcha@inha.ac.kr

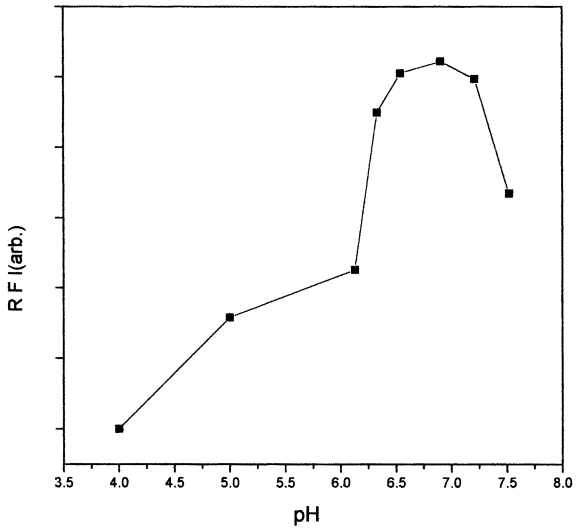

Figure 2. pH effect on the fluorescence intensity of Eu^{3+}-TTA. $[Eu^{3+}] = 2.0 \times 10^{-6}$ M; $[TTA] = 1.0 \times 10^{-5}$ M, $PO_4^{3-} = 8 \times 10^{-7}$ M. [hexamethylenetetramine-HCl buffer] = 1.0×10^{-1} M. RFI: Relative fluorescence intensity.

there is no change in maximum wavelengths of excitation and emission spectra by adding phosphate.

The pH influence on the relative fluorescence intensity of Eu^{3+}-TTA complex in the presence of phosphate was investigated in the range of pH 4.0-7.7. The results are shown in Figure 2. Maximum fluorescence intensity occured at pH 6.5-7.2. Among several buffer systems, such as H_3BO_3-citric acid, NH_3-NH_4Cl, NaB_4O_7-HCl and hexamethylenetetramine-HCl, hexamethylenetetramine-HCl (pH 6.8) was chosen as the optimum buffer system for the determination of phosphate based on fluorescence quenching of Eu^{3+}-TTA complex.

The effect of several surfactants on the fluorescence intensity of Eu^{3+}-TTA-PO_4^{3-} complex was examined. As shown in Table 1, the non-ionic surfactant Brij 58 has the greatest enhancement effect among the surfactant. The use of the surfactant Brij 58 was most effective in improving solubility. Thus Brij 58 was recommended for the further study. The optimum concentration of Brij 58 was also studied (Figure 3). When the concentration of Brij 58 is less than 1.0×10^{-4}

Table 1. Effect of various surfactant on the fluorescence of Eu^{3+}-TTA

Surfactant[a]	Concentration (M)	R F I (%)[b]
None	–	1.00
CTAB	1.0×10^{-3}	0.00
CPC	1.0×10^{-3}	0.00
SDS	1.0×10^{-3}	0.00
MTAB	1.0×10^{-3}	–[c]
Triton X-100	1.0×10^{-3}	177
Brij 35	1.0×10^{-3}	75.7
Brij 58	1.0×10^{-3}	214

$[Eu^{3+}] = 2.0 \times 10^{-6}$ M, $[TTA] = 1.0 \times 10^{-5}$ M. [a]CTAB = cetyltrimethyl ammonium bromide, CPC = cetylpyridium chloride, SDS = dodecyl sulfate sodium salt, MTAB = myristyl-trimethyl ammonium bromide, Triton X-100 = octylphenol polyoxyethylene isooctylphenol, Brij 35 = polyoxyethylene lauryl ether, Brij 58=polyoxyethylene cetyl ether. [b]Relative fluorescence intensity. [c]precipitation occur.

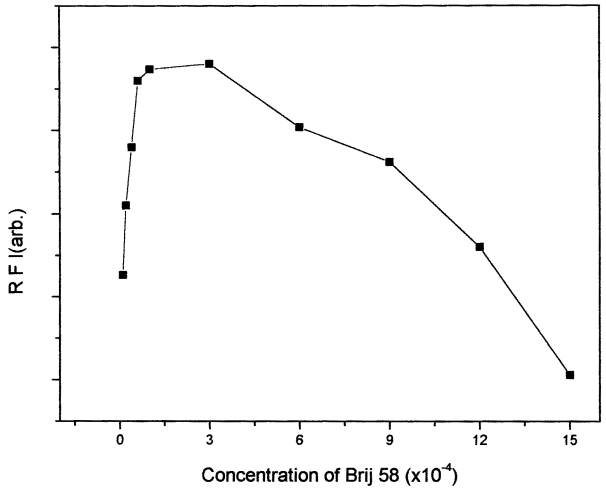

Figure 3. Effect of Brij 58 on the fluorescence intensity of Eu^{3+}-TTA. $[Eu^{3+}] = 2.0 \times 10^{-6}$ M, $[TTA] = 1.0 \times 10^{-5}$ M, pH = 6.8, $PO_4^{3-} = 8 \times 10^{-7}$ M.

M, fluorescence intensity increases significantly with increasing Brij 58 concentration, and decreases slowly when the Brij 58 concentration is above 3.0×10^{-4} M. Brij 58, 1.0×10^{-4} M was used throughout.

Calibration curve of phosphate. The decrease in the fluorescence intensity of the Eu^{3+}-TTA complex was a linear function of the phosphate ion concentration. The linear range was 1.0×10^{-7}-1.2×10^{-6} M phosphate ion when the concentrations of Eu^{3+}, TTA and Brij 58 were 2.0×10^{-6} M, 1.0×10^{-5} M and 1.0×10^{-4} M, respectively (r=0.999). The detection limit was 1.0×10^{-7} M phosphate ion (S/N=3). The linear range can be changed according to the concentrations of Eu^{3+} and TTA. By adjusting the concentration of Eu^{3+} and TTA only, different linear ranges can be formed. For example, when the concentrations of Eu^{3+} and TTA were 1.0×10^{-5} M and 5.0×10^{-5} M, respectively, the calibration range of the phosphate ion was 8.0×10^{-7} M-9.6×10^{-6} M. The detection limit of this method are compared with those of other spectrophotometric methods in Table 2. The present method is more sensitive than the other methods.

Possible quenching mechanism. The fluorescence of the europium ion is weak due to the low oscillatory strength of its absorption.[11] A fluorescence increase for Eu^{3+} can be achived by energy transfer from the triple state of a ligand to the Eu^{3+} in the complex. TTA, one of the most commonly used ligands, was used as a ligand for the intramolecular energy transfer. The composition of Eu^{3+}-TTA chelate in the water is Eu^{3+}

Table 2. Comparison of the phosphate detection limit with other methods

Method	Detection limit (μg/mL)	Analytical wavelength (nm)		Reference
		Ex	Em	
Vanadomolybdo-phosphoric acid	0.2	470	–	11
Malachite green	0.008	650	–	12
Molybdenum blue	0.01	880	–	13
Present method	0.0031	343	615	–

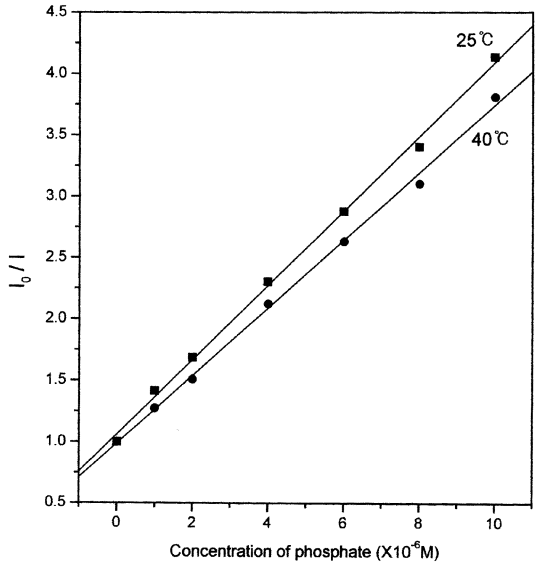

Figure 4. Stern-Volmer plot for the quenching of the Eu^{3+}-TTA by phosphate (a) 25 °C, (b) 40 °C; $[Eu^{3+}] = 2.0 \times 10^{-6}$ M; [TTA] = 1.0×10^{-5} M, [Brij 58] = 1.0×10^{-4} M; pH = 6.8.

Table 3. Tolerance limits of foreign ions

Ions	Tolerance	Mole ratio[a]
CO_3^{2-}	2.0×10^{-5} M	25
SO_4^{2-}	2.0×10^{-4} M	250
BO_3^{3-}	8.0×10^{-3} M	10,000
$C_2O_4^{2-}$	8.0×10^{-7} M	1
$B_4O_7^{2-}$	8.0×10^{-6} M	10
AsO_2^-	2.0×10^{-5} M	25
CrO_4^{2-}	3.0×10^{-6} M	3.75
F^-	4.0×10^{-5} M	500
SiO_3^{2-}	2.0×10^{-5} M	25

$[Eu^{3+}] = 2.0 \times 10^{-6}$ M, [TTA] = 1.0×10^{-5} M, [Brij 58] = 1.0×10^{-4} M, $[KH_2PO_4] = 8 \times 10^{-7}$ M. [a]Mole ratio against phosphate added.

$(TTA)_3(H_2O)_2$[12]. The presence of phosphate as a synergetic ligand seems to remove water molecules from the coordination sphere of the lanthanide ion, possibly forming a ternary complex, and then acting as the quencher of the luminescence of the Eu^{3+}-TTA complex.[13,14]

In general, the quenching efficiency can be described by the Stern-Volmer equation.[15]

$$I_0/I = 1 + K_{sv}[Q],$$

where I_0 and I are the fluorescence intensities in the absence and the presence of the quencher, respectively. K_{sv} is the quenching constant or Stern-Volmer constant, and [Q] is the quencher concentration. In dynamic quenching, quenching efficiency increases with increasing temperature. But in the case of static quenching, the quenching effect is lower at higher temperature. Figure 4. is the Stern-Volmer plot of the Eu^{3+}-TTA in the presence of phosphate. In Figure 4, the I_0/I term is lower at higher temperature, which can be interpreted to mean that the above quenching mechanism is a static quenching (ternary complex formation between Eu^{3+}-TTA and phosphate).

Interference of foreign ions. The tolerance limit was calculated by the concentration of a foreign ion, resulting in less than 5% deviation of the fluorescence of Eu^{3+}-TTA in the prescence of phosphate (Table 3). In other spectrophotometric methods of phosphate, arsenate is often regarded as the interfering ion even though Table 3 shows that arsenate does not interfere with this method. Most anions have a relatively high tolerance limit, except $C_2O_4^{2-}$ and CrO_4^{2-}.

Table 4. Determination of phosphate in natural, tap and lake water

Sample	Found (10^{-7} M)		
	This method	RSD[a]	molyb. blue method
natural water (Bonghwa Mt.)	3.21	3.36%	no detection
tap water (laboratory)	36.0	3.23%	36.3
lake water (Inkyoung lake)	106	2.67%	106

[a]Relative standard deviation (n=7).

Application. The method was applied to the determination of phosphate in natural, tap and lake water. Tap and lake water were diluted 10 fold then the 50 mL sample of each was filtered and analyzed. The molybdenum blue method,[16] which has been used widely for phosphate determination, was compared with this method. The results in Table 4 are in good agreement with those obtained by the molybdenum blue method.

Acknowledgment. This work was supported by 98 Research Foundation of Inha University.

References

1. Furman, N. H. *Standard Methods of Chemical Analysis*; D. Van Nostrand Company Inc.: Princeton, 1968; p 798.
2. Jung, M. S.; Jung, M. H.; Lee, J. H.; Kim Y. K. *Environmental Chemistry*; Shinkwang Munhwa Company: Korea, 1994; p 99.
3. Munoz, A.; Torres, F. M.; Estela, J. M.; Cerda, V. *Anal Chim. Acta* **1997**, *350*, 21.
4. *Iso, Method No. 2998*, 1974; *prEN (CEN), Method No. 1189*, 1996.
5. U.S. EPA *Methods for Chemical Analysis of Water and Wastes*, 1983.
6. Cha, K. W.; Park, K. W.; Ha, Y. G.; Kim, H. S. *J. Korean Chem. Soc.* **1994**, *38*, 653.
7. Halvwrson, F.; Birnen, J. S.; Leto, J. R. *J. Chem. Phys.* **1964**, *41*, 157.
8. Bryden, L. C.; Reilley, C. N. *Anal. Chem.* **1982**, *54*, 610.
9. Tran, C. D.; Zhang, W. *Anal. Chem.* **1990**, *62*, 835.
10. Cha, K. W.; Park, K. W. *Analytical Science & Technology* **1997**, *10*, 433.
11. Crosby, G. A.; Whan, R. E.; Alire, R. M. *J. Chem. Phys.* **1966**, *34*, 848.
12. Halvwrson, F.; Birnen, J. S.; Leto, J. R. *J. Chem. Phys.* **1964**, *41*, 157.
13. Horrocks, W. W.; Sudnick, W. *J. Am. Chem. Soc.* **1971**, *101*, 334.
14. Jianzhong, L.; Zhujun, Z. *Analytica Chimica Acta* **1996**, *318*, 178.
15. Wolfbeis, O. S. *Fluorescence Spectroscopy*; Springer: Berlin, 1993; pp 80-81.
16. Research Society of Water Analytical Chemistry, *New Methods of Water Analytical Chemistry III*; Munji Company: Korea, 1994; pp 228-230.