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Microtiter plate assay for phosphate using a europium–tetracycline complex as a sensitive luminescent probe

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Abstract

A new luminescent europium probe is presented for the determination of phosphate (P) in microtiter plate format. The assay is based on the quenching of the luminescence of the europium–tetracycline (EuTc) 1:1 complex by phosphate using a reagent concentration of 20.8 μmol/L. The probe is excited at 400 nm and displays a large Stokes' shift of 210 nm. The emission maximum is located at 616 nm. The system works best at neutral pH 7 and is therefore suitable for phosphate determination in biological and biochemical systems. The linear range of the calibration plot is from 5×10^{-6} mol/L to 7.5×10^{-4} mol/L of phosphate, and the limit of detection is 3 μmol/L.

Keywords: Phosphate; Microplate assay; Luminescence; Lanthanide

1. Introduction

Phosphate is an essential nutrient and an important substance in metabolism of humans, animals and plants. Therefore, methods were developed for its determination in fertilizers, plants, crops [1,2], natural waters [3,4] and other environmental samples [5]. Phosphate, also, is an important parameter in clinical chemistry [6] and in industry in boiler feed-water to prevent scaling [7].

The broad field of applications is reflected by a huge variety of methods for phosphate determination. Among them, the formation of heteropoly acid complexes between orthophosphates and molybdate is frequently used to obtain a colored species, which is optically easy to determine. Analysis can be performed spectrophotometrically either directly with the yellow heteropoly acid complex (in the UV region) [8] or after reduction to phosphomolybdenum blue with various reducing reagents [9–11]. The sensitivity of these methods depends on acidity, temperature and the reducing agent. However, the acidic pH (between 0.3 and 3) needed in the phosphomolybdenum blue method prevents its applicability to bioanalysis (where enzymes, for example, require near-neutral pH).

Other optical methods for phosphate detection include light scattering techniques (using malachite green or rhodamine B) [12] in a combination with flow injection analysis and fluorescence quenching of rhodamine 6G [13] with molybdophosphate. Thiamine was used for fluorometric determination of phosphate in HPLC at a comparatively short emission wavelength of 437 nm [14]. The procedure is laborious, because careful pH adjustment is needed throughout the analytical process and the assay is prone to interference by oxidation of background [15]. The method using the fluorescent Al^{3+}–morin complex [16] works at acidic conditions and suffers from a long incubation time of 45 min, while the spectrophotometric determination with Alizarin Red sulfonate [17] at pH 6.6 works in the millimolar concentration range, only. Recently, a spectrophotometric C_{3v} symmetric synthetic receptor [18] for tetrahedral oxyanions was employed in an indicator-displacement assay. It works best at pH 7.4 in the biological range.

Electrochemical methods mostly use ion selective electrodes for phosphate detection. The response time of some potentiometric schemes [19–21] is within minutes at pH around 9.7. Amperometric schemes [22] have also been presented. Here, the response times are fast, too (1 min), but again a fairly alkaline pH has to be used.

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Finally, a coupled enzyme system, which incorporates three enzymes together with the formation of resorufin from Amplex Red was shown for the detection of low concentrations (50 nM) of phosphate at pH 7.4. The drawbacks of this method are high costs for the reagents (three enzymes and the Amplex Red dye), an incubation time of 0.5 h, and interference of detergents like 3-(cyclohexylamino)-propanesulfonic acid (CAPS) or SDS [23]. However, this assay is very popular because it works at pH 7.

Luminescence detection along with microtiter plate formats provides a high throughput technology for clinical assays with sensitive determination of low analyte concentrations [24–26]. The unique spectral properties of lanthanide cations (long luminescence lifetimes, sharp emission bands, a large energy gap between the absorption and emission bands of the complexes) make them ideally suited as specific reporters in such applications.

We recently introduced the EuTc system for the determination of hydrogen peroxide [27–29]. Here, a 3:1 stoichiometry was employed for sensitive determination of hydrogen peroxide in microtiter plate format, in sensor membranes and in various matrices. The method showed some interference due to phosphate. From this finding, we deduced that a detection scheme for phosphate could be established. Upon changing the stoichiometry between Eu^{3+} and Tc and optimizing further parameters like pH, an assay for the luminescent determination of phosphate was developed and is presented here. It is simple, fast, and can be performed in aqueous buffer at neutral pH and is therefore suitable for phosphate determination in biological and biochemical systems. We have optimized stoichiometry, incubation time and pH and report on the effect of interferences. The assay was validated by using different natural water samples as a matrix that was spiked with different concentrations of phosphate.

2. Experimental

2.1. Chemicals and buffers

All chemicals used were of analytical grade. Tetracycline hydrochloride was purchased from Serva (Heidelberg, Germany), europium trichloride hexahydrate ($EuCl_3 \cdot 6H_2O$) from Alfa (Karlsruhe, Germany), 3-morpholino-propanesulfonic acid (MOPS) from ABCR (Karlsruhe, Germany) and sodium dihydrogenphosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) from Merck (Darmstadt, Germany). Water was doubly distilled.

All solutions were prepared in 13 mmol/L MOPS buffer. This buffer was obtained by dissolving 5.92 g of 3-morpholino-propanesulfonic acid in 1900 mL of doubly distilled water. Before being filled up to 2000 mL, the solution was adjusted to pH 7 using 70% perchloric acid. Desired concentrations of phosphate were obtained by dissolving the $NaH_2PO_4 \cdot H_2O$ salt in MOPS buffer.

2.2. EuTc reagent

The EuTc reagent solution was obtained by dissolving 4.9 mg of tetracycline hydrochloride and 3.8 mg of $EuCl_3 \cdot 6\ H_2O$ in 250 mL of MOPS buffer to give a concentration of 41.5 μmol/L. The EuTc stock solution was allowed to stay at 4 °C in the dark overnight before use. The EuTc solution is stable for at least 1 month at 4 °C.

2.3. Instrumentation

Luminescence spectra were acquired on an Aminco Bowman AB2 luminescence spectrometer (from SLM Spectronic Unicam, Rochester, New York, USA). Luminescence was excited at 400 nm and emission was collected at 616 nm. Bandpasses were set to 4 nm for both, the excitation and emission slit. A PMT voltage of 905 V was applied. Measurements were carried out after an incubation time of 20 min at 30 °C.

Luminescence measurements in microtiter plates (MTP) were performed using 96-well flat bottom black microplates (from Greiner Bio-One GmbH, Frickenhausen, Germany) on a Genios Plus microplate reader (from Tecan, Grödig, Austria). Unless otherwise described, the instrumental parameters of the MTP reader were as follows: excitation and emission wavelengths at 405/612 nm, gain: 131 V, 10 flashes per well, lagtime 0 μs, integration times 40 μs (or lagtime 60 μs and integration time 100 μs for better sensitivity between 3 and 10 μmol/L of phosphate, respectively); time gap between move and flash: 200 ms. The plates were thermostatted to 30 °C and luminescence was read after 1200 s of shaking.

2.4. Preparation of solutions

Solutions for the acquisition of the emission spectra were prepared as follows: 2.5 mL of the solution in the cuvettes had the concentration of EuTc 20.8 μmol/L and contained final concentrations of phosphate between 25 and 500 μmol/L. The samples were allowed to thermostat at 30 °C for 20 min before measurement.

Solutions for the determination of the pH effect were prepared as follows: 250 μL of an 20.8 μmol/L EuTc solution of different pH (from 5.5 to 8.2) were pipetted into a 96-well microplate. Two rows of the wells contained solutions of the same pH. To each second row were added 5 μL of a 250 μmol/L phosphate solution. Here, a PMT voltage of 120 V was used.

Solutions for the calibration plot in the concentration range of 0.2–1000 μmol/L of phosphate were prepared in columns of eight replicates for each concentration of phosphate in 96-well microplates. After the first 50 μL of MOPS buffer solution in each well, 100 μL of phosphate solutions of different concentration (from 0.5 to 2500 μmol/L) were added. Finally, 125 μL of EuTc reagent solution was added.

Different concentrations of conceivable interferents were additionally prepared to be checked for their effect on the average luminescence intensity of EuTc in the presence of 25 μmol/L of phosphate. Each concentration of interferent was tested in eight replicates. The concentrations of the interferent were varied in the range between 0.01 and 1000 μmol/L.

For the validation test, three different natural water samples were tested in microtiter plates. To 50 μL of each water sample was added 75 μL of phosphate solution of different concentrations and 125 μL of EuTc to obtain final concentrations of

phosphate between 10 and 375 μmol/L. The samples were analyzed on the basis of the calibration plot established, and its slope.

3. Results

3.1. Spectral properties of EuTc in the presence of phosphate

The EuTc probe shows an excitation maximum at 400 nm, and the emission maximum is at 616 nm. The line-like emission peak is typical for a lanthanide emission. The probe displays a ∼210 nm Stokes' shift. On addition of phosphate in MOPS buffer of pH 7, the luminescence intensity of EuTc decreases. This is shown in Fig. 1 for the concentration range from 25 to 500 μmol/L of phosphate. We assume that phosphate anions coordinate to EuTc due to electrostatic interactions with the metal center and contribute to lower the energy transfer from tetracycline to Eu^{3+}, thus quenching its emission intensity.

3.2. Effect of pH

Previous studies of the effect of pH on the luminescence intensity of the EuTc complex in various molar ratios had revealed [30] that its intensity is highly pH dependent. If the stoichiometry of Eu:Tc is 3:1, the emission intensity of EuTc is strongest in the pH range of 6.6–7.2 [27]. Fig. 2 shows the pH dependence of the emission intensity of EuTc in a 1:1 molar ratio in absence and presence of phosphate. The error bars represent standard deviations from eight replicate samples. Here, the luminescence intensity is increasing from lower (5.5) to higher pH (8.2) in the absence of phosphate (solid squares). After the addition of phosphate (5 μmol/L), the overall signal decreases. On going from pH 6.5 to 8.2, the luminescence decreases by between 33 and 38%. Since the signal decrease at pH 7 is not distinctly smaller than at more alkaline pH, we chose pH 7.0 for the following experiments in order to make the assay useful for physiological studies.

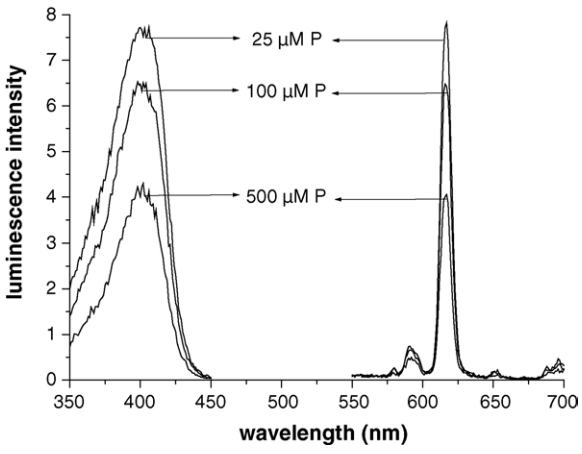

Fig. 1. Excitation and emission spectra of EuTc in presence of various concentrations of phosphate (P) in MOPS buffer of pH 7 (λ_{exc} 400 nm, λ_{em} 616 nm).

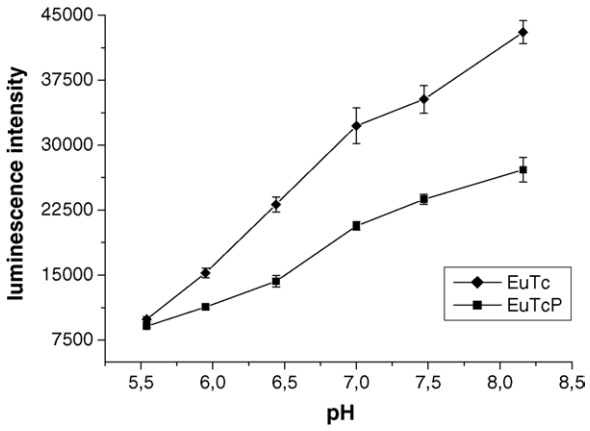

Fig. 2. Effect of pH on the luminescence intensity of EuTc in the absence and presence of phosphate (5 μmol/L).

3.3. Time dependence of complexation between EuTc and phosphate

Fig. 3 shows the time-dependent decrease of the luminescence intensity of EuTc upon addition of 350 μmol/L of phosphate at pH 7 in MOPS buffer. The luminescence intensity of the probe in absence of phosphate is stable over time (30 min). Following addition of phosphate, the change of the luminescence signal of EuTc was followed over 1800 s. The signal decreases for 1200 s and lowers very modestly using longer incubation times. After a 20 min incubation time, the relative signal change is smaller by only 10% compared to 1800 s. Since this difference is small, we chose an incubation time of 1200 s (20 min) for the phosphate assay. A 20 min incubation time turned out to be optimal, even for lower concentrations of phosphate. The reliability of this incubation time was confirmed by minimized standard deviations at 1200 s incubation time in the subsequent experiments in microtiter plates.

3.4. Effect of stoichiometry

The stoichiometry of the EuTc complex has a large effect on its luminescence intensity. According to our measurements and

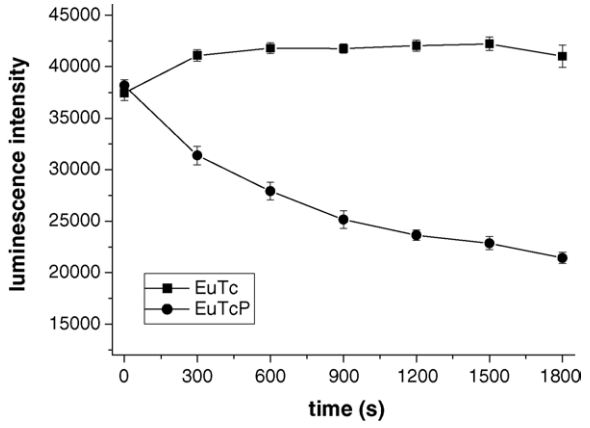

Fig. 3. Time-dependent changes in luminescence intensity of EuTc at 616 nm (λ_{exc} = 400 nm) in MOPS buffer of pH 7 in the absence (top) and in the presence (lower plot) of phosphate (350 μmol/L).

previous studies [27–29], an excess of Eu^{3+} over Tc results in a weak enhancement of the luminescence intensity of the EuTc in the region of neutral pH upon addition of phosphate. At a molar ratio Eu:Tc = 1:1, however, the addition of phosphate results in a pronounced decrease of EuTc luminescence intensity. On the other hand, no significant cross-sensitivity towards <100 μM concentrations of hydrogen peroxide is found under these conditions (see interference study). Therefore, a stoichiometry of EuTc 1:1 was used in all experiments.

3.5. Calibration plot

The assay shows high accuracy for the determination of phosphate within the linear range of the calibration plots. For better comparability of the two different detection methods, we show F/F_0 (normalized luminescence intensity against luminescence signal in absence of phosphate) in Fig. 4. In semilogarithmic representation, the plot with 0 μs lagtime detection can be described by an equation of the type $y = A + B\log(c_{\text{phosphate}} \times 10^6)$, with $A = 1.033 \pm 0.018$ and $B = -0.224 \pm 0.007$, for which an r^2 of 0.993 is found (Fig. 4, squares). The analytically useful range spans more than two decades (from 5 to 750 μmol/L of phosphate). The limit of detection is 5 μmol/L of phosphate. The shape of the calibration plot extends linearly over a relatively wide range (5–750 μmol/L). This is interpreted by an overlap of two plots, one for primary phosphate (the predominant species at pH < 7.3) and secondary phosphate (the predominant species at pH > 7.3). At pH 7.0, both species are present in substantial fraction. In fact, distinctly different calibration plots (not shown) are found for determinations performed at pH 6 and 8, respectively. Since, however, the effects described before are largest at pH 7.0, we think that this is the most appropriate pH for determination of phosphate.

Upon using a lagtime of 60 μs and an integration time of 100 μs, both the LOD and the sensitivity of the method can be improved. This is reflected well by the much steeper slope of the calibration plot (Fig. 4, circles). In semilogarithmic representation, the plot with 60 μs lagtime and 100 μs integration time detection can be described by an equation of the type $y = A + B\log(c_{\text{phosphate}} \times 10^6)$, with $A = 0.955 \pm 0.081$ and $B = -0.586 \pm 0.091$, for which an r^2 of 0.954 is found. The analytically useful range is between 3 and 10 μmol/L of phosphate. The limit of detection is 3 μmol/L of phosphate.

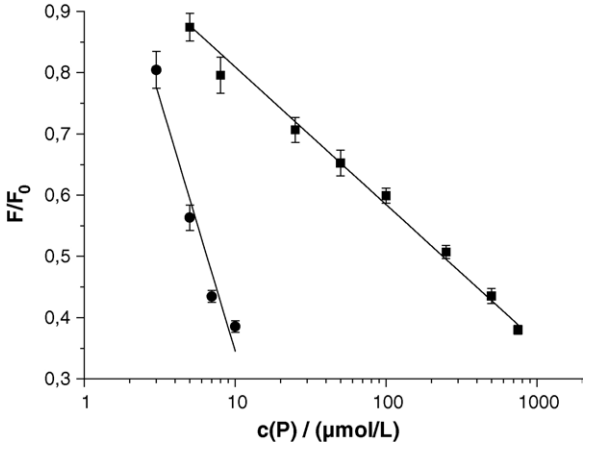

Fig. 4. Calibration plots for the phosphate assay with 0 μs lagtime and 40 μs integration time (squares) and 60 μs lagtime and 100 μs integration time (circles).

3.6. Interferences

The luminescence of the EuTc 1:1 complex is not interfered by K^+, Na^+ and Ca^{2+} in concentrations up to 1 mmol/L, while Mg^{2+} and Al^{3+} have a weak effect (Table 1). Mg^{2+} increases the luminescence signal of EuTc at concentrations >20 μmol/L, whereas Al^{3+} increases the luminescence signal when present in concentrations >100 μmol/L. Fe^{3+} is tolerated up to 15 μmol/L and Tb^{3+} (as the only other strongly fluorescent lanthanide) up to 33 μmol/L.

Ligands containing oxygen with negative charge and anions have to be considered in the interference study because they may coordinate (like phosphate) the Eu^{3+} ion and affect its emission. However, sulfate, nitrate, acetate and carbonate do not interfere in concentrations up to 1 mmol/L, silicate can be present up to 500 μmol/L. The AsO_4^{3-} ion is tolerated up to 100 μmol/L and AsO_3^{3-} up to 90 μmol/L. Oxalic acid weakly interferes if present at concentrations >250 μmol/L. Tartaric acid increases the signal at above 160 μmol/L, while citric acid increases the luminescence significantly at above 0.8 μmol/L. Ammonium ion does not interfere up to 1 mmol/L. Among the halides tested, F^- increases the luminescence at concentrations >100 μmol/L, and Cu^{2+} is found to act as a quencher if present in concentrations >0.8 μmol/L.

Table 1
Concentrations of interferences tolerated in the presence of phosphate (25 μmol/L)

Interferent	Concentration (μmol/L)
Citric acid	0.8
Cu^{2+}	0.8
Tartaric acid	160
H_2O_2	100
K^+	1000
Tb^{3+}	33
Fe^{3+}	15
Ca^{2+}	1000
Mg^{2+}	20
Al^{3+}	100
Na^+	1000
F^-	100
Cl^-	1000
Br^-	1000
I^-	1000
NO_3^-	1000
SO_4^{2-}	1000
NH_4^+	1000
CH_3COO^-	1000
CO_3^{2-}	1000
AsO_4^{3-}	100
AsO_3^{3-}	90
SiO_3^-	500
$C_2O_4^{2-}$	245

Table 2
Slopes of calibration plots and recovery rates of phosphate (P) in different natural water samples

Water sample	Slope	Recovery of 10.5 mM P (%)	Recovery of 25.5 mM P (%)	Recovery of 52.5 mM P (%)	Recovery of 120 mM P (%)	Recovery of 375 mM P (%)
S1	−10371.9 ± 853	101.3	97.7	102.6	96.1	102.5
S2	−7177.4 ± 1235	103.6	96.1	100.0	96.3	104.1
S3	−4848.4 ± 1150	103.6	97.3	97.8	98.1	103.3

Sources: S1, Regensburg university lake; S2, Danube river at Regensburg; S3, Laaber river.

It is important to note that the effect of hydrogen peroxide is comparatively low. Although EuTc in 3:1 stoichiometry was used in previous studies to determine H_2O_2 in water samples or in enzymatic reactions in which hydrogen peroxide was involved as a substrate [27] or as a product [28,29], H_2O_2 does not interfere in concentrations up to 100 μmol/L, if the stoichiometry of Eu:Tc is 1:1.

The influence of humic acids (HAs) on phosphate determination is difficult to estimate for because of the polydispersity of complexing carboxy groups and the large range of molecular weight (10^2–10^5 g/mol) of HAs. The log k of the formation constants of EuTc at pH 7 is about 10 [30] whereas log k of Eu(III) with Aldrich humic acid as a model substance varies between −1.75 and −5.25 [31]. From those values, the ability of HAs to complex europium is small. However, a model humic acid was used in [31] and the formation constants depend on pH, $c_{Eu^{3+}}$, c_{HA}, mole fraction of the HA chosen and polydispersity of complexing groups. Consequently, the log k of the formation constants may vary significantly between different samples containing HAs. Therefore, we recommend setting up a calibration plot with spiking (see Section 3.7.) for samples containing HAs to estimate their possible effect on phosphate determination.

3.7. Application to spiked water samples

The assay was tested for its validation using three different natural water samples (Table 2). First, three water samples (S1, S2 and S3) were spiked with different concentrations of phosphate (10.5, 25.5, 52.5, 120 and 375 μmol/L). After the luminescence intensity was measured, the slopes of the resulting calibration plots were compared to the slope of the standard phosphate assay (−10 350 ± 350). The value of the slope is different from the one in Fig. 4 because here luminescence intensity (F) was taken as a function of $\log(c_{phosphate} \times 10^6)$ whereas in Fig. 4 (F/F_0) was a function of $\log(c_{phosphate} \times 10^6)$. The lake water S1 gave the most similar slope (−10 370 ± 850). This corresponds to a deviation of only 2.6% from the phosphate assay slope. The recovery rates of the lake water samples (Table 2) are within the tolerable range. Therefore, the assay is suitable for phosphate determination in lake water samples. The two river water systems (S2 and S3) gave different slopes (−7180 ± 1240 and −4850 ± 1150). This corresponds to a reduced sensitivity of the assay. At present, we suppose that there are some contents present in water samples, which increase the luminescence background resulting in a decreased dynamic range of the determination as shown by the reduced slope.

The assay also was performed in time-resolved mode. The application of different lagtimes (10, 20 and 40 μs) had no significant effect on the assay performance when different phosphate concentrations were determined in natural water samples. The advantage of a lower luminescence background was compensated for larger standard deviations of a series of replicate measurements.

4. Discussion

EuTc represents a promising new fluorescent probe that displays specific advantages over established procedures for phosphate determination. Table 3 gives figures of merit of common methods for phosphate determination, in particular analytical ranges, LODs, and specific features.

The luminescence properties of EuTc include a comparatively longwave excitation, a Stokes' shift of >200 nm and a sharp emission band at 616 nm. This makes it most useful for determination of phosphate in biological material where fluorescence background in the UV and near visible usually is strong. None of the fluorometric methods [13,14,16,23] offers such a longwave detection. Except from the two molybdenum-blue methods [9,10] the spectrophotometric determinations use shorter detection wavelengths. Here, significant background interference due to scatter or other biomaterial is likely to occur.

The incubation time is in the same time range as most of the other methods, except for the methods based on thin film electrodes [19–21] and on the reduced molybdophosphate complex [10] which show a remarkably faster response. The method with morin [16] requires incubation times, which are more than twofold longer than in case of EuTc. The same is true for the assay using the enzymatic assay with Amplex Red [23], where time-consuming pipetting steps (five reagents) are required, followed by a 30 min incubation.

Analytical methods requiring highly acidic or alkaline pH are hardly applicable to bioanalysis. EuTc works best at a neutral pH 7, which is often a necessity if samples contain enzymes, proteins or other biomaterials. The molybdenum reagents, for example, require highly acidic media (pH 0.9–2.5) [7–10]. This is also true for the methods using malachite green [12], rhodamine [12,13] or thiamine [14]. The determination using thiamine is even more complicated, because careful and long lasting pH adjustment procedures are needed (starting with pH 2, ending with pH 8) [15]. The determination of phosphate using the fluorescent Al^{3+}–morin complex works best at (pH 3.53), [16], whereas the electrochemical thin film sensor methods use basic reaction conditions (pH 9.7) [19–21]. The spectrophotometric

Table 3
Figures of merit of methods for (optical) determination of phosphate

References	Method	Reagent	Analytical range (mol/L)	LOD (mol/L)	Remarks
[8]	Spectrophotometry at 370 nm	Molybdenum yellow	2.6×10^{-6}–3.1×10^{-5}	2.6×10^{-6}	Surfactant (CTAB)-based separation technique, high acidity (0.27 M sulfuric acid solution); 5–10 min incubation time at 30–40 °C
[9]	Spectrophotometry at 865 nm	Molybdenum blue	5.3×10^{-6}–7.4×10^{-4}	5.3×10^{-6}	Application to flow injection analysis (FIA); 2 min incubation time; pH 0.9 and 2.5
[10]	Spectrophotometry at 880 nm	Molybdenum blue	6×10^{-8}–3×10^{-6}	n.d.	In aqueous-organic solution (formation of the reduced 12-molybdo-phosphate complex); pH 0.36–1.06; 0.8–1.5 min incubation time at RT
[17]	Spectrophotometry at 478 nm	Alizarin Red sulfonate	3.2×10^{-7}–6.3×10^{-5}	3.2×10^{-7}	pH 6.6
[12]	Light scattering at 460 nm	Molybdenum + malachite green	2×10^{-7}–1×10^{-6}	6×10^{-8}	Application to flow injection (FIA); ion associate aggregates with MG; high acidity (0.8 M sulfuric acid); 3–10 min incubation time
[12]	Light scattering at 460 and 580 nm	Molybdenum + rhodamine B	2×10^{-7}–1×10^{-6}	6×10^{-8}	Application to flow injection (FIA); ion associate aggregates with RB; high acidity (0.8 M sulfuric acid); 3–10 min incubation time
[13]	Fluorescence at 550 nm	Molybdenum + rhodamine 6G	$\leq 1.1 \times 10^{-6}$	2×10^{-8}	Fluorescence quenching of R6G by molybdophosphate; 200 nm Stokes' shift
[14]	Fluorescence at 437 nm	Thiamine	3.2×10^{-7}–3.2×10^{-6}		Application to HPLC; 65 nm Stokes' shift; high acidity; 5 min retention time
[16]	Fluorescence at 510 nm	Al–morin	5.3×10^{-7}–1.1×10^{-6}		Fluorescence quenching of Al–morin by phosphate; pH 3.53; 45 min incubation time
[23]	Fluorescence at 590 nm	Enzymatic system + Amplex Red	5×10^{-8}–5×10^{-6}	5×10^{-8}	35 nm Stokes' shift, microtiter plate assay possible, three-coupled enzymatic reactions, pH 7.4, 5 reagents required, 30 min incubation
[19]	Transmittance at 620 nm	Co_3O_4 thin film electrode	10^{-6}–10^{-2}		pH 9.3; 90% response time after 50 s at RT
[20]	Transmittance at 530 nm	NiO thin film electrode	10^{-5}–10^{-2}		pH 9.3; 90% response time after 40 s at RT
[21]	Spectrophotometry at 500 nm	$MnCo_2O_4$ thin film electrode	10^{-6}–10^{-2}		pH 9.7; 90% response time after 40 s at RT
This method	Luminescence at 616 nm	EuTc complex	5×10^{-6}–7.5×10^{-4}	5×10^{-6}	Luminescence quenching; pH 7; 210 nm Stokes' shift; 20 min incubation time, microtiter plate assay possible

determination with Alizarin Red sulfonate [17] is performed at pH 6.6, and the three-enzyme method using Amplex Red [23] at pH 7.4.

The EuTc assay has a comparatively wide linear detection range using no lagtime and a short integration time or significantly enhanced sensitivity with 60 μs lagtime in the 3–10 μM concentration range.

5. Conclusion

A new method for simple and rapid determination of phosphate in microplate format has been developed employing the EuTc probe. It is particularly useful for phosphate detection in biological and biochemical systems because it works best at neutral pH and the linear range of the calibration plot is in the μM-range. Future work will focus on monitoring enzymatic action like phosphatase activity with this lanthanide-based fluorescent assay.

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