

Applying the Yb³⁺ ion as a simple and sensitive probe to detect phosphate-containing derivatives in aqueous solution†

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A simple and sensitive detection method for HPO₄²⁻ and phosphate-containing derivatives in aqueous solution with a new ensemble which is prepared by mixing ytterbium chloride and pyrocatechol violet in a 2:1 molar ratio in 10 mM HEPES buffer at pH 7.0.

Phosphorus is one of the most important elements in life. Together with heterocyclic bases and deoxyribose, phosphates make up the nucleotides which in turn make up genes, the hereditary elements of life. In addition, phosphorus and its derivatives, particularly adenosine triphosphates (ATPs), play pivotal roles in energy utility and signal transduction in various biological processes. *In vivo*, the phosphate groups may form metallic complexes *via* coordination by taking O atoms as electron donors – this is especially true when the metal ions are hard Lewis acids, such as Mg²⁺ or Ca²⁺ ions.¹ Numerous sensors for anions, including phosphate ions, have been devised but most use organic solvents as the detection medium because these sensors rely on hydrogen-bonding and electrostatic interaction for the recognition of analytes.^{2,3} These interactions are, however, hindered drastically in a highly polar medium such as water because of the competing solvation effect.⁴ The detection for phosphate anion, and more importantly for ATP, in water is, hence, a challenging task. Among those anion sensors² in aqueous media, significant progress has been made in the detection of oxy-anions (such as HCO₃⁻, H₂PO₄⁻) with lanthanide-containing receptors [especially Eu(III) and Tb(III)] by spectrometry and NMR methods.^{5,6}

During the investigations into the biological effects of dinuclear lanthanide complexes stemmed from a phenol-based ligand, Hbbimp⁷[(2,6-bis(bisbenzimidazolymethyl)aminomethyl)-4-methyl-phenol], we found that this system is an efficient sensor for HPO₄²⁻ and ATP. It is prepared by mixing Hbbimp, YbCl₃ and pyrocatechol violet (abbreviated as PV afterwards) in a 1:2:1 molar ratio in aqueous solution. However, more interestingly, and to our amazement, we discovered an even more simple and sensitive sensor for both HPO₄²⁻ and ATP. The new ensemble is prepared by simply mixing YbCl₃ and PV in a 2:1 molar ratio in an aqueous solution (10 mM HEPES buffer, pH 7.0). Fig. 1(a) shows the UV/Vis spectra obtained when the solution of [YbCl₃] was titrated into

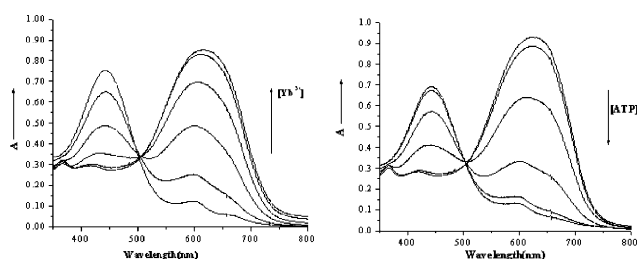


Fig. 1 (a) UV/Vis spectra: HEPES 10 μM buffer, [PV] = 50 μM, YbCl₃ was added gradually, with [YbCl₃] = 0–100 μM. (b) UV/Vis spectra: with addition of [ATP] = 0–100 μM to HEPES 10 μM buffer, [Yb₂(PV)] = 50 μM.

† Electronic supplementary information (ESI) available: Fig. 1s–9s. See <http://www.rsc.org/suppdata/cc/b315965e/>

the buffer of PV (50 μM). Upon the addition of [YbCl₃], the maximum absorption peak gradually shifted from yellow (λ_{\max} = 444 nm) to blue (λ_{\max} = 623 nm) (Fig. 2, left). With the addition of phosphate anions or ATP solution into the solution, the ensemble resulted in a change of color back from blue to yellow (Fig. 2, right), and caused obvious change of the UV/Vis absorption spectra [Fig. 1(b)], namely, the absorption peak at 623 nm decreased while the peak at 444 nm increased. The effect of different pH buffers of the system on phosphate anions and ATP sensing was also studied, and we found that the aforesaid UV/Vis absorbance changes all occurred at various pH values in the range of 6.5–7.5, most distinctively at pH 7.0 (Fig 2s, ESI†).

What is the stoichiometric relation about our system? To answer this, we carried out experiments of isothermal spectrophotometric titration (IST) and plotted the curve of [Yb³⁺] against ΔA , the absorbance differences of the ensemble before and after the addition of [Yb³⁺], the curve of [ATP] vs. ΔA , and also [HPO₄²⁻] vs. ΔA .⁸ From the data, the coordination ratio of Yb to PV was found to be 2:1, whereas for ATP, Yb:ATP is consistent with being 1:1 which is also suitable for Yb:HPO₄²⁻. We also calculated the stability constants of the complexes related to the following reactions:

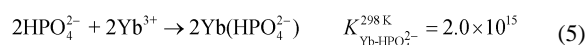
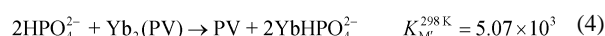
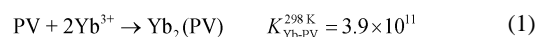


Table 1 summarizes the thermodynamic parameters and stability constants for the metal complexes derived from PV and analytes by IST. From the data one can find that phosphate anions and ATP bind with Yb³⁺ much more tightly than with PV, quantitatively, over four orders of magnitude. We think that the coordination reactions are driven both enthalpically and entropically.

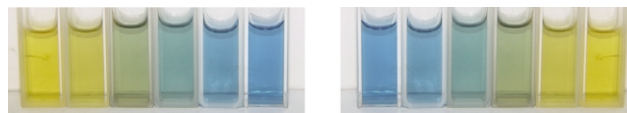


Fig. 2 Left: color of the ensemble containing PV (50 μM) in HEPES (10 mM) changed from yellow to blue with the addition of [Yb³⁺] = 0–100 μM. Right: color of the ensemble changed back to yellow with the addition of [ATP] = 0–100 μM.

Table 1 Thermodynamic parameters and stability constants (*K*) for the binding of PV, ATP and phosphate anions to Yb³⁺ in an aqueous solution

Data from IST	PV	ATP	HPO ₄ ²⁻
$\Delta H/\text{kJ mol}^{-1}$	-40.29	-35.37	-53.38
$\Delta G/\text{kJ mol}^{-1}$	-66.12	-89.94	-85.88
$\Delta S/\text{J K}^{-1} \text{mol}^{-1}$	86.67	183.1	109.1
$K_{298\text{K}}/\text{M}^{-1}$	3.9×10^{11}	5.85×10^{15}	2.0×10^{15}

The measurement for ATP obeys the Beer–Lambert absorption law very well within the concentration range of 10–320 μM , with a $\epsilon = 6993 \text{ (mol/L)}^{-1}\text{cm}^{-1}$.⁹ Linear regression with least squares fitting gives a correlation coefficient of 0.9992. The ensemble exhibits excellent selectivity towards phosphate anions and ATP over other common anions, including Cl^- , SO_4^{2-} , CH_3COO^- , HCO_3^- and ClO_4^- as shown in Fig. 3 (for further details see ESI†, Fig. 4s). To our knowledge the number of efficient analytical methods for phosphate anions with good selectivity over other common anions in aqueous solution is much fewer than for those in organic solvents.¹⁰

The detection course can even be observed with the naked eye (Fig. 2). It should be pointed out here that in our simpler ensemble, Yb^{3+} is the best candidate among the metal ions for the measurement. Other lanthanide ions are incapable of working as well as Yb^{3+} does, although they can play a similar part. Specifically, when La^{3+} or Pr^{3+} were added to the detection ensemble, the UV/Vis spectrum did not change distinctly and regularly (Fig 5s, ESI†). As for ions such as Nd^{3+} , Sm^{3+} , Gd^{3+} , Er^{3+} and Ho^{3+} , although the UV/Vis spectra gave rise to some variations [Fig. 1(a)], precipitations were produced quickly. It is a pity that Eu^{3+} and Tb^{3+} do not form stable complexes in aqueous solutions. As for the transition metal ions, they cannot do the same job (Fig 5s).

We have mentioned that our new sensor exhibits excellent selectivity towards phosphate anions and ATP over other common anions. But how about its selectivity relative to GTP, CTP, TTP, UTP and to AMP, ADP? One would speculate that all NTPs would bring about a similar color variety with the sensor. This is indeed the case. One fact is sure, that is, AMP was totally incapable of causing to the color change, even where its quantity is 10 times as much as that of ATP. As for ADP, at double quantity of ATP, it was able to give rise to the color change gradually, resulting in a final color of yellow-green.¹¹

In our experiment, the color variation is very distinct. What is the possible structure of species formed by Yb^{3+} ions with the ligands? It is known that the yellow color of PV (the indicator) at neutral pH may change to blue when the molecules bind to metal ions.¹² In aqueous solution, the molecule of PV would be present in an acid/base equilibrium (forms I and II of Fig. 3s, ESI†). Form I would initiate a complexation reaction with metal ions and give the deprotonated form II with two catechol groups in favor of forming the complex when encountering another metal ion.

As a strong Lewis acid, Ln^{3+} ions prefer O atoms as their coordination sites and the most common coordination numbers are 8 or 9 with the favored structures.^{13,14} Since it was found by experiment that the best performance occurred when a 1:2 molar ratio of PV: Yb^{3+} was adopted, we proposed a structure on the left of Fig. 4 when Yb^{3+} ions coordinate with PV. With the addition of ATP, competition between ATP and PV for the Yb^{3+} ions will take place. It is most possible that PV is released from the complex when

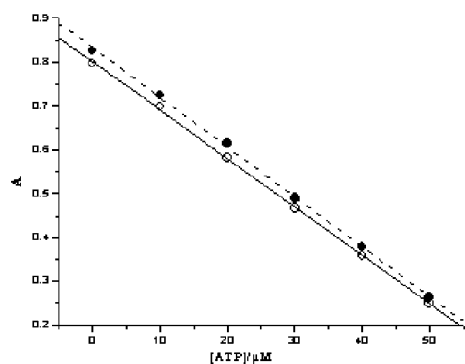


Fig. 3 Plot of absorbance (at 623 nm) against concentration of ATP in HEPES solution (pH 7.0) in the absence (—○—) and presence of other anions (---●---) (Cl^- , CH_3CO^- , HCO_3^- , ClO_4^- and SO_4^{2-}).

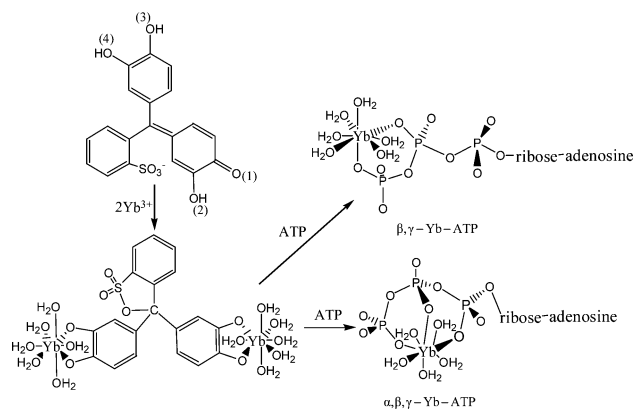


Fig. 4 Proposed structure organized by the Yb^{3+} ion when it coordinates with PV (left) and with ATP (right) to form $\beta,\gamma\text{-Yb-ATP}$ or $\alpha,\beta,\gamma\text{-Yb-ATP}$ complexes.

ATP coordinates to Yb^{3+} , forming a new structure (Fig. 4, on the right). It is known that ATP can form two kinds of complexes with Mg^{2+} , *i.e.* $\beta,\gamma\text{-Mg-ATP}$ and $\alpha,\beta,\gamma\text{-Mg-ATP}$.¹ The extrication of PV should be the cause of the color changing back to yellow (Fig. 2).

In summary, we have developed a simple and efficient colorimetric measuring method for phosphate anions by simply mixing YbCl_3 and PV, a commercially available dye, in water at neutral pH. The reversibility of the anion recognition is demonstrated in Fig. 8s, ESI†. Yb^{3+} as a sensor can detect phosphate anions and ATP both spectrophotometrically and visually, with high selectivity over a variety of mono- and di-valent anions. This sensor also allows a quantitative assay of phosphate-containing derivatives in aqueous solution down to the concentration range around 10^{-5} M, which is more sensitive than 10^{-4} M as presented by Miranda and co-workers.¹⁵ Compared with the method of Parker's group,^{5,6} our sensor is simpler and more selective in recognizing phosphate-containing derivatives in aqueous solution.

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- See Fig. 9s(a–c) in the ESI†.
- See ESI† Table 1s and Fig. 1s.
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