

# Pyrophosphate Selective Recognition in Aqueous Solution Based on Fluorescence Enhancement of a New Aluminium Complex

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**Abstract** A novel and simple fluorescence enhancement method for selective pyrophosphate (PPi) sensing was proposed based on a 1:1 metal complex formation between bis(8-hydroxy quinoline-5-solphonat) chloride aluminum(III) (Al(QS)<sub>2</sub>Cl), (L) and PPi in aqueous solution. The linear response range covers a concentration range of  $1.6 \times 10^{-7}$  to  $1.0 \times 10^{-5}$  mol/L of PPi and the detection limit of  $2.3 \times 10^{-8}$  mol/L. The association constant of L-PPi complex was calculated  $2.6 \times 10^5$  L/mol. L was found to show selectively and sensitively fluorescence enhancement toward PPi over than  $I_3^-$ ,  $NO_3^-$ ,  $CN^-$ ,  $CO_3^{2-}$ ,  $Br^-$ ,  $Cl^-$ ,  $F^-$ ,  $H_2PO_4^-$  and  $SO_4^{2-}$ , which was attributed to higher stability of inorganic complex between pyrophosphate and L.

**Keywords** Fluorescence enhancing · Sensor · Pyrophosphate · (Al(QS)<sub>2</sub>Cl)

## Introduction

Development of optical sensors has become a rapidly expanding area of analytical chemistry, because they offer advantages of simple preparation, reasonable selectivity and sensitivity and no need for separate reference devices [1–5]. During the past decade, an extensive effort has been devoted to developing artificial receptors that are able to transform binding of ionic species into spectroscopic signals [6–9]. The recognition and sensing of anions have received considerable attention for their important roles in biological, industrial and environmental processes [10–12]. However, there are only a few artificial receptors that allow the recognition of anions in aqueous solution [13, 14]. The reason is that hydrogen bond and ion pairing interaction between host and guest molecules for molecular recognition would be weakened significantly by the competitive influence of protic solvents. Therefore, the receptors reported so far for the effective recognition of target molecules in water all need a combination of multiple non-covalent interactions, such as multiple hydrogen bonds, [15–17] ion pairing interaction or metal coordination [18–20] to overcome the competitive influence of water.

Phosphates play an essential role in human body and other living systems. Among them, pyrophosphate (PPi) and adenosine triphosphate (ATP) are involved in energy transduction, metabolic processes, extra cellular signal mediations, DNA polymerisation and cyclic adenosine monophosphate synthesis, etc. [21, 22]. Selective sensing and discrimination of different phosphate anions are

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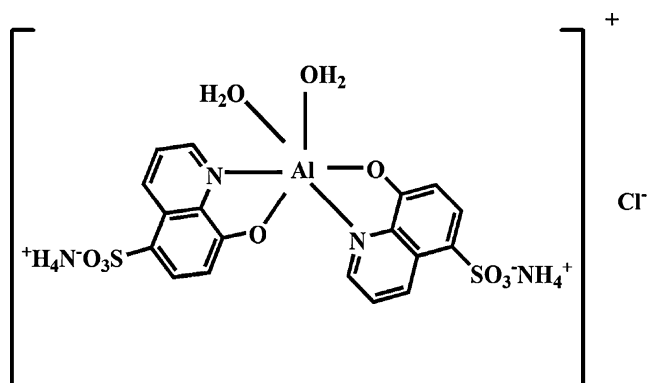
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**Scheme 1** Chemical structure of Al(QS)<sub>2</sub>Cl (L)

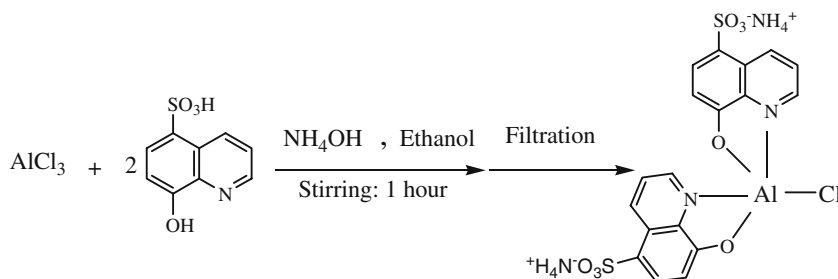
essential for clarifying their roles in these processes and has been extensively studied [10, 23–27]. The fluorescent sensing strategy *via* metal coordination-altered emission is found to be more effective than that of hydrogen bonding-altered emission, since metal coordination leads to a higher phosphate affinity in aqueous solution [10, 20–24]. Up to now, some examples of selective recognition sensors for PPI have been reported, but only a few examples can display turn-on in emission spectra [18, 19, 28, 29]. With this in mind, herein we introduce a novel fluorescent turn-on PPI sensor Al(QS)<sub>2</sub>Cl (Scheme 1) with two 8-hydroxyquinoline moieties (Scheme 2).

## Experimental

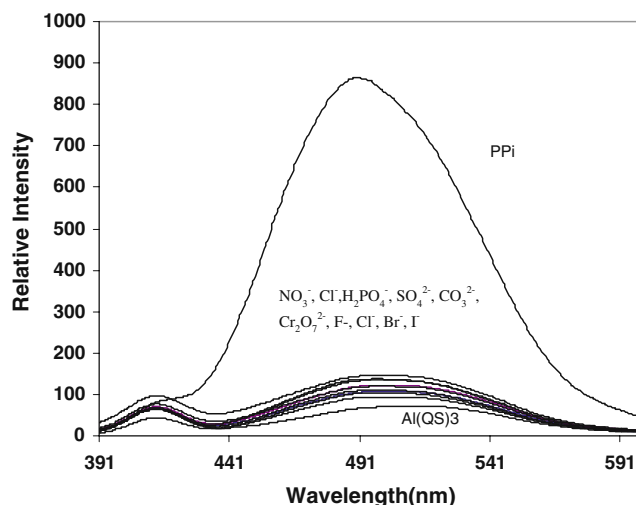
### Reagents

All Chemicals were of the reagent-grade from Fluka and Merck chemical companies. The fluorogenic reagent Al(QS)<sub>2</sub>Cl was prepared as follows:

The Al(QS)<sub>2</sub> was prepared according to previous literature [30]. Aqueous solution of aluminum chloride was mixed with a weakly alkaline solution of 8-HQS in ethanol (1:1.05). The pH of solution was adjusted to 7.0 by adding a 2N ammonium hydroxide solution. The precipitates were filtered and washed with ethanol to remove excess 8-HQS. This product was dried prior using.



**Scheme 2** Procedure of Al(QS)<sub>2</sub>Cl synthesis



**Fig. 1** Fluorescence responses of L (3 ml  $1 \times 10^{-6}$  mol/L) upon addition of different anions (50  $\mu$ lit) ( $5.0 \times 10^{-3}$  mol/L) in aqueous solution ( $\lambda_{\text{ex}}$ : 360 nm)

<sup>1</sup>H NMR (DMSO, 500 MHz):  $\sigma_{\text{H}}$  6.16 (m, 1 H), 7.8 (m, 3 H), 8.6 (m, 1 H), 9.19 (m, 1 H).

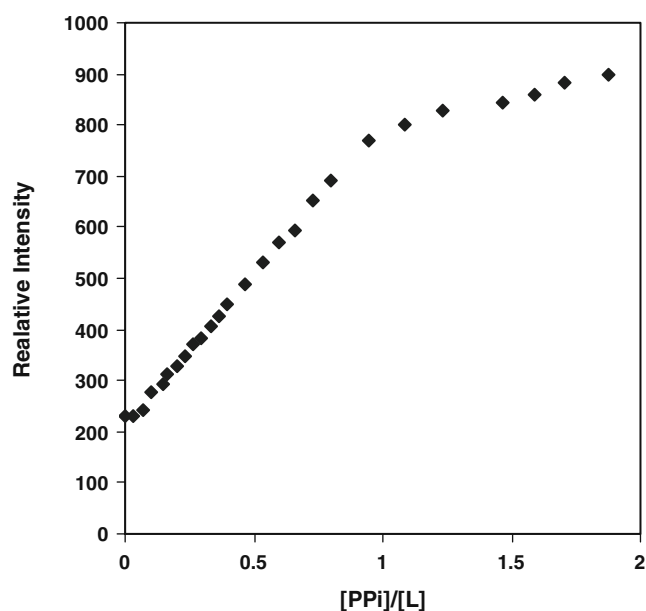
IR data:  $\nu_{\text{max}}$  (KBr pellets)/ $\text{cm}^{-1}$  3,175 (C-H str.), 1,323, 1,370, 1,402, 1,460 (C-C, C-N, CO str. and C-H bend.) 1,498, 1,577, 1,604 (C-C str.) 754 (Al-N str.)

### Apparatus

All fluorescent measurements were carried out on a Perkin-Elmer LS50 luminescence spectrometer. Absorption spectra were studied on a Shimadzu UV 2100 PC UV-Visible spectrometer. FTIR spectra were obtained on a Vector 22 Bruker spectrometer with samples prepared as KBr pellets.

## Results and Discussion

In order to evaluate whether L could be used as a selective fluorescent chemosensor for anions, in preliminary experiments the complexation of L with a number of anions was investigated spectrofluorometrically in an aqueous solution at  $25.0 \pm 0.1^\circ\text{C}$ . A  $5.0 \times 10^{-6}$  mol/L solution of L in an



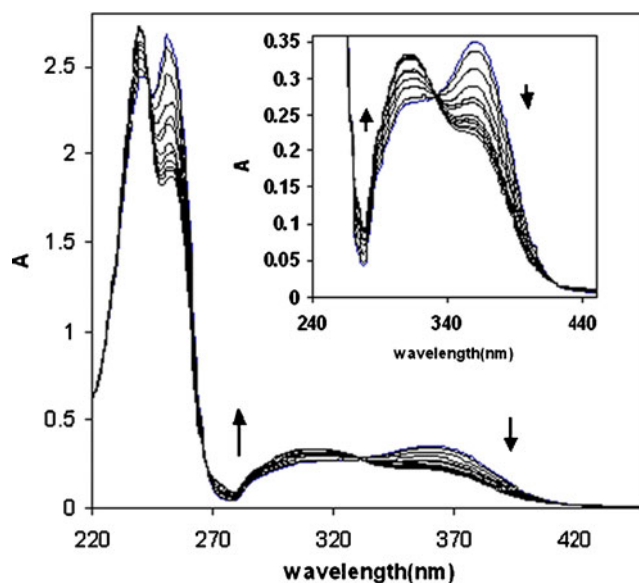
**Fig. 2** Fluorescence intensity vs. [PPi]/L mole ratio plot in aqueous solution for PPi ions

aqueous solution was titrated with microliter amounts of  $1.0 \times 10^{-4}$  mol/L solutions of anions spectrofluorometrically. Fluorescence titration experiments were recorded on excitation at 360 nm. As shown in Fig. 1, when L is titrated with PPi a significant enhancement in the fluorescence intensity was observed. No significant fluorescence changes were obtained when  $F^-$ ,  $H_2PO_4^-$  and other ions tested.

The resulting mole ratio plot of PPi anion is shown in Fig. 2. As it is obvious, L as a receptor forms a 1:1 complex with anions in aqueous solution. For the evaluation of formation constants of the resulting 1:1 complexes from fluorescence intensity vs. mole ratio data, a non-linear least-squares curve fitting program KINFIT was used [31] and the results are given in Table 1. It is seen that, in aqueous solution, the stability of L complexes with anions decreased in the following order:  $PPi > H_2PO_4^- > F^- > CO_3^{2-} > Cl^- > SO_4^{2-} > NO_3^-$ . Electron-deficient Lewis-acidic centers are able to

**Table 1** Association constants for various anions toward receptor L in aqueous solution

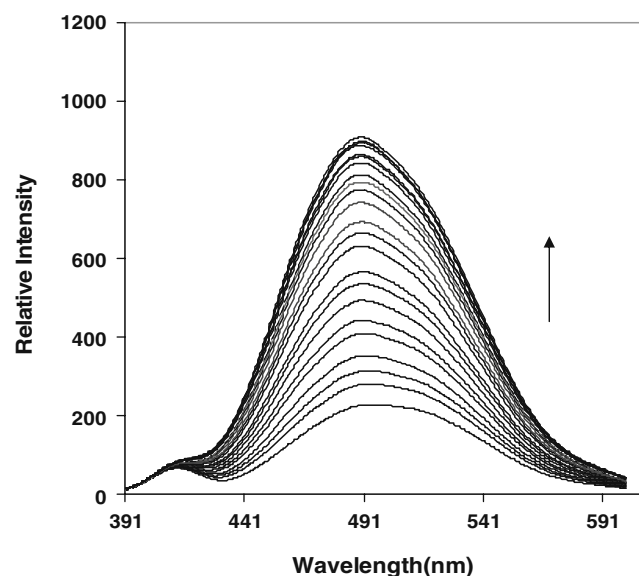
Cation	$\log K_f$
$AcO^-$	$2.15 \pm 0.10$
$H_2PO_4^-$	$3.15 \pm 0.11$
$F^-$	$2.85 \pm 0.10$
PPi	$5.41 \pm 0.11$
$Br^-$	$< 2.0$
$CO_3^{2-}$	$< 2.0$
$BrO_3^-$	$< 2.0$
$NO_3^-$	$< 2.0$
$SO_4^{2-}$	$1.95 \pm 0.17$
$Cr_2O_7^{2-}$	$2.08 \pm 0.17$
$Cl^-$	$2.15 \pm 0.11$



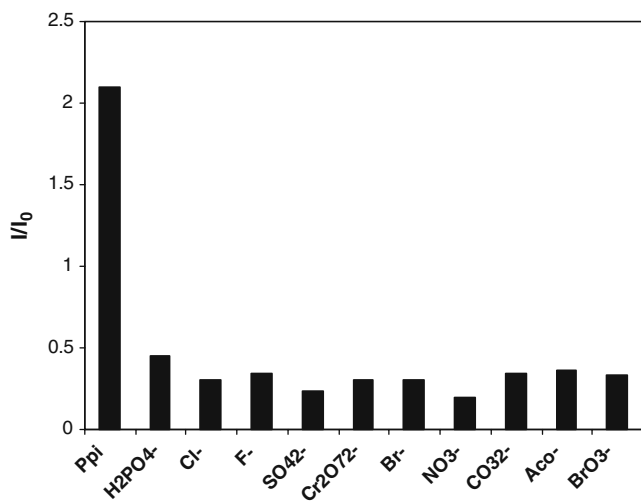
**Fig. 3** Changes in the UV-vis spectra of L ( $5 \times 10^{-5}$  mol/L) upon addition of PPi ( $5 \times 10^{-3}$  mol/L) in aqueous solution

bind to anions by an orbital overlap that causes a bonding interaction. This has led to the production of many new chelating atoms such as boron, mercury, silicon, germanium and tin [11]. The association constants of L with PPi and dihydrogen phosphate anions are relatively high because they have stronger basicity that allow them to coordinate more strongly to Lewis acidic metal (Aluminium).

Thus, based on the relative stabilities of resulting complexes, L molecule was expected to act as a selective fluoroionophore in the preparation of an optical sensor for PPi.



**Fig. 4** Fluorescence titration of L with PPi in an aqueous solution,  $L = 5 \times 10^{-6}$  mol/L,  $[PPi] = 5 \times 10^{-4}$  M,  $\lambda_{ex} = 360$  nm



**Fig. 5** Fluorescence responses of L (3 ml  $1 \times 10^{-6}$  mol/L) containing 30  $\mu$ M PPI and the background anions (120  $\mu$ M) ( $\lambda_{\text{ex}}$ : 360 nm)

The effect of PPI anion (sodium salt) on the absorption spectrum of L ( $1 \times 10^{-5}$  mol/L) was examined in an aqueous solution at 25°C (Fig. 3).

Obviously seen from Fig. 3, compound L was characterized by a broad absorption band centered at 250 nm and 360 nm which can be contributed to  $\pi \pi^*$  and transition.

Notably, titration of L against PPI led to a pronounced and two blue-shifted shoulders in the UV spectra with a well defined three isosbestic point at 244, 267 and 332 nm. Respectively, indicating that the stable complex having a certain stoichiometric ratio between the receptor L and PPI formed. Sensor L did not show any obvious spectral change upon addition of H<sub>2</sub>PO<sub>4</sub><sup>-</sup> as well as other monovalent anions such as CH<sub>3</sub>CO<sub>2</sub><sup>-</sup>, F<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup> even up to an excess of 10 equiv.

As it can be seen from Fig. 4, the fluorescence emission spectra of L are sensitive to PPI. In the presence of various concentration of PPI ion ranging from  $1.6 \times 10^{-7}$  to  $1.0 \times 10^{-5}$  mol/L, significant fluorescence enhancement of fluorescent probe was observed. The fluorescence intensity is enhanced with increasing PPI concentration, which constitutes the basis for the recognition of PPI with fluorescent probe proposed in this work

The significant fluorescence enhancement might be resulting from the electrostatic interaction between PPI and L, in which two oxygen atoms of PPI coordinate with the center of Al<sup>3+</sup> to reduce the magnitude of the electron withdrawal via partial neutralization of the charge on the Al<sup>3+</sup> ion, thus increasing the electron-donating character of the QS moiety and finally resulting in an increased efficiency of ICT [18, 19].

The detection limit of L as a fluorescent sensor for the analysis of PPI was also determined from the plot of the

fluorescence intensity as a function of the concentration of added anion ions. It was found that L has a detection limit of  $2.5 \times 10^{-8}$  mol/L for PPI ions, which is quite low for the detection of PPI ions found in many chemical and biological systems.

The selectivity behavior is obviously one of the most important characteristics of a chemosensor, that is, the relative sensor response for the primary ion over other ions present in solution. To examine the selectivity of L, we investigated its affinity in the presence of PPI at 30  $\mu$ M mixed with 120  $\mu$ M background another anions such as F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, HSO<sub>4</sub><sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, P<sub>2</sub>O<sub>7</sub><sup>4-</sup>. The influence intensity of the solutions containing both background anions and PPI is shown in Fig. 5. As it can be seen, other background anions had small or no obvious interference with the detection of PPI. All these results indicated that L could be used as a potential candidate of fluorescent chemosensor for PPI with very high selectivity.

## Conclusion

In conclusion, a novel enhancement fluorescent chemosensor, L, for PPI in aqueous solution was synthesized and investigated. It showed selective and sensitive fluorescence response to PPI in aqueous solution. The fluorescence enhancing of L is attributed to the 1:1 complex formation between L and pyrophosphate which has been utilized as the basis for the selective detection of pyrophosphate. Receptor L exhibits enhance fluorescent probe along a wide concentration range of PPI with no interferences of background anions.

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