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A new Schiff base and its metal complex as a fluorescent-colorimetric sensor for rapid detection of arginine
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Abstract
A novel Schiff base and its Pb²⁺-complex have been synthesized and exploited as colorimetric and fluorescent-colorimetric sensor respectively targeting the detection of arginine (Arg). Both the chemo-sensors employed here are simple, easy to synthesize and cost effective. They exhibit excellent selectivity and sensitivity towards Arg by giving dual responsive signals of visible colour change and ‘on–off’ fluorescence change within 1 min. The chemo-sensors having sufficiently low detection limits and rapid response time warrants their application in environmental analyses of Arg.

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Introduction

Amino acids being a class of organic compounds containing amino and carboxyl groups are not only the building blocks of biological macro-molecular proteins, but also the metabolic precursors of a variety of primary and secondary metabolites. Among twenty natural amino acids, arginine has attracted considerable attention because it plays a vital role in many biological functions, e.g. cell division, healing of wounds, removal of ammonia from body, functioning of immune system, erectile dysfunction, blood vessel dilation, protein production and release of hormones etc. More over arginine is the immediate precursor of NO (a key mediator in vascular homeostasis), urea, ornithine, and agmatine. Having so much influence in different biological processes occurred in a human body, the presence of arginine or some arginine-derived species in too little or too much amount can be unhealthy and even life-threatening. Thus, arginine can be used as a possible diagnostic indicator for some diseases. As a charged amino acid, arginine is the best target among the twenty amino acids for the molecular recognition of a specific side chain in a peptide. Hence it is of urgent need to develop feasible methods for detection of arginine with practical application. Till date, a variety of strategies for detecting arginine have been reported, for example, high-performance liquid chromatography (HPLC), liquid chromatography–tandem mass spectrometry (LC–MS) and molecular recognition technology. Although these approaches make great contributions to the arginine detection, most of them suffer from a number of disadvantages, such as low selectivity and sensitivity, poor precision, high costs, complexity of the equipment, complicated laboratory procedures, insufficient operating and storage stability for biosensors etc. Therefore, the development of a simple, facile, accurate and rapid method for detecting arginine is highly desirable.

Recently, great efforts have been made to develop fluorescent chemo-sensors for detection of amino acids owing to their simplicity, high selectivity and sensitivity. In spite of these, selective detection of arginine without interference from other amino acids is a challenging task and therefore there is paucity of fluorescent chemo-sensors for arginine in literature. Recently one arginine sensor have been reported by Cao et al. But it does not provide any privilege of naked eye visible sensing and the sensing of arginine is coupled with lysine and thus limits its selectivity for arginine. Pu et al. reported a colorimetric sensor based on costly gold nano-particles. Moreover it did not show any fluorescence property.
As a part of our ongoing research\textsuperscript{41,42} in the design and synthesis of chemo-sensors for anions, cations and neutral molecules, we have synthesised a novel and simple azino bis-Schiff base ligand $L$. In this work, the ligand $L$ and its Pb$^{2+}$-complex, $[\text{PbL}_2]^{2+}$ have been exploited as colorimetric and fluorescent-colorimetric chemo-sensor respectively for selective detection of arginine. The ligand exhibits change in absorbance on addition of arginine with easily discernible colour change from colourless to yellow. On the other hand Pb$^{2+}$-complex can perform rapid sensing by quenching of fluorescence with an instantaneous colour change from yellow to colourless. To the best of our knowledge, this is the first example of a Schiff base and its metal complex acting as efficient sensors for arginine detection, which provide dual responsive signals of naked-eye visible colorimetric change and fluorescence emission change.

**Experimental**

**General information**

UV-Visible spectra were recorded on a Shimadzu UV 1800 spectrophotometer using a 10 mm path length quartz cuvette. Fluorescence spectra were recorded on a Hitachi spectrophotometer. $^1$H and $^{13}$C NMR spectra of ligand $L$ were recorded on a Bruker Ultrashield 400 using CDCl$_3$ and NMR spectra of Pb$^{2+}$-complex were carried out in solvent mixture of DMSO-d$_6$ and D$_2$O respectively at room temperature and the chemical shifts are reported in $\delta$ values (ppm) relative to TMS. High resolution mass (HRMS) spectra and ESI were recorded on a Waters mass spectrometer using mixed solvent HPLC methanol and triple distilled water. All the chemicals and metal salts were purchased from Merck.

**Synthesis and characterisation of $L$ and $[\text{PbL}_2](\text{NO}_3)_2$**

Benzildihydrazone (1.190 g, 5 mmol) is dissolved in 100 mL of anhydrous methanol. To this solution, 1.82 g (10 mmol) of solid syringaldehyde is added with constant stirring. The resulting bright yellowish solution is refluxed for 12h, maintaining dry condition. Then the reaction mixture is cooled to room temperature. Light yellow crystalline solid (suitable for X-ray analysis) precipitates out. It is filtered off, and dried in air. Yield, 2.35 (72%); mp, 202$^\circ$C. $^1$H NMR (400 MHz, CDCl$_3$, TMS): $\delta$ 8.30 (s, -HC=N, 2H), 7.89 (d, 4H), 7.39 (dd, 6H), 6.75 (s, 4H), 3.77 (s, -OCH$_3$, 12H) (Fig S1). $^{13}$C NMR (400 MHz, CDCl$_3$, $\delta$ ppm, TMS): 165.1, 159.4, 147.10, 137.8, 135.3, 130.6, 128.7, 127.7, 125.9, 105.6 and 56.4 (Fig. S2). FTIR/cm$^{-1}$ (KBr): 692(w), 729 (w), 756 (w), 1114(vs), 1157(m), 1216(s), 1246(m), 1317(s), 1355(s), 1421(m), 1458(s), 1512 (vs), 1597(vs, -C=N), 3408 (w) (Fig. S3). ESI MS: 567.50 (LH+,}
(Fig. S4). UV-VIS $\lambda_{\text{max}}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$) (CH$_3$OH): 345 (40 970). Anal. Calc. for C$_3$H$_{30}$N$_4$O$_6$: C, 67.83; H, 5.34; N, 9.89. Found C, 67.77; H, 5.39; N, 9.85%.

To a methanol solution of $L$ (0.566 g, 1 mmol), solid Pb(NO$_3$)$_2$ ( 0.165 g, 0.5 mmol) was added. The reaction mixture was stirred for 2 h under reflux condition. Then the reaction mixture was cooled at room temperature. A yellow solid precipitated on evaporation of the solvent, was filtered. The solid mass was washed subsequently with diethyl ether for several times and dried in air. Yield, 0.475 (65%). FTIR/cm$^{-1}$ (KBr): 503(w), 572 (m), 618 (w), 648(w), 690(s), 725(s) 756 (m), 1114(vs), 1157(m), 1216(s), 1246(m), 1310(vs, NO$_3$), 1363(s), 1421(m), 1440(m), 1508 (vs), 1600 (vs, -C=N), 3408(w) (Fig. S3). $^1$H NMR (400 MHz, DMSO-d$_6$, TMS): $\delta$ 9.0 (s, -OH, 2H), 8.32 (s, -HC=N, 2H), 7.75 (d, 4H), 6.70 (s, 4H), 3.67 (s, -OCH$_3$, 12H) (Fig S5). HRMS: 1339.50 ([PbL$_2$]$^{2+}$) (Fig. S6). UV-VIS $\lambda_{\text{max}}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$) (CH$_3$OH): 340 (2950), 243 (2420). Anal. Calc. for C$_{64}$H$_{60}$N$_{10}$O$_{18}$Pb: C, 52.49; H, 4.13; N, 9.56. Found C, 52.45; H, 4.16; N, 9.61%.

**X-ray data collection and structural determination**

X-ray single crystal data were collected using MoK$\alpha$ ($\lambda = 0.7107$ Å) radiation on a BRUKER APEX II diffractometer equipped with CCD area detector. Data collection, data reduction, structure solution/refinement were carried out using the software package of SMART APEX.$^{43}$ The structures were solved by direct methods (SHELXS-97) and upgraded by using ShelXL2013 and standard Fourier techniques, and refined on F2 using full matrix least squares procedures (SHELXL-97) using the SHELX-97 package$^{44}$ incorporated in WinGX.$^{45}$ In most of the cases, non-hydrogen atoms were treated anisotropically. Hydrogen atoms were fixed geometrically at their calculated positions following riding atom model. The crystallographic data are listed in Table 1. Structural information has been deposited at the Cambridge Crystallographic Data Center (CCDC 1410686).

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Table 1 Crystallographic data and structure refinement parameters for receptor L

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<tr>
<th>Parameter</th>
<th>Value</th>
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General method of UV-vis and fluorescence titrations

For UV-vis and fluorescence titrations, stock solution of the sensors L and [PbL$_2$]$^{2+}$ were prepared (c = 1x10$^{-5}$ ML$^{-1}$) in CH$_3$CN : H$_2$O (1 : 2, v/v). The solution of the guest metal
cations, anions and various amino acids were prepared (c = 1x10^{-4} ML^{-1}) in CH$_3$CN : H$_2$O (1 : 2, v/v). The original volume of the receptor solutions are 3 mL each. Solutions of the sensors L and [PbL$_2$]$^{2+}$ of various concentrations and increasing concentrations of metal cations, anions and amino acids were prepared separately. The spectra of these solutions were recorded by means of UV-vis and fluorescence methods after mixing them for a few seconds at room temperature.

**Colorimetric test kit**

Chemosensors L and [PbL$_2$]$^{2+}$ (0.01 mmol) were dissolved in methanol (10 mL) to get 1mM solution. Test kits were prepared by immersing filter-papers into this solution (1 mM), and then dried in air to get rid of the solvent. Arginine and other amino acids were dissolved in water (10 mL) to prepare 0.1 mM solution. The test kits prepared above were dipped into the aqueous solution of arginine and other amino acids and then dried at room temperature.

**Computational details**

The GAUSSIAN-09 Revision C.01 program package was used for all calculations.\(^{46}\) The gas phase geometries of the compounds L and [PbL$_2$]$^{2+}$ were fully optimized without any symmetry restrictions in singlet ground state with the gradient-corrected DFT level coupled with the hybrid exchange-correlation functional that uses Coulomb-attenuating method B3LYP.\(^{47,48}\) Basis sets 6-31G and LANL2DZ were found to be suitable for the molecule L and [PbL$_2$]$^{2+}$ respectively.

**Result and discussion**

**Syntheses and structures of L and [PbL$_2$]$^{2+}$**

Receptor L was obtained by the condensation reaction of benzil dihydrazone and syringaldehyde in methanol with 72% yield (Scheme 1) and characterized by $^1$H NMR, $^{13}$C NMR, IR, mass spectrometry, elemental analysis and X-ray crystallography and SEM imaging. The IR spectra of azino bis-Schiff base L showed a broad band at 3408 cm$^{-1}$ for OH stretching vibration. In addition, the band appeared at 1597 cm$^{-1}$ is due to $\gamma$(C=N) stretching frequency.

[PbL$_2$]$^{2+}$ complex was obtained in good yield as a yellow solid by refluxing the mixture of the ligand L and Pb(NO$_3$)$_2$ in 2:1 molar proportion in methanol and it was characterized by IR, UV-Vis, HRMS-mass spectrometry, elemental analysis and SEM imaging. In the IR spectra the band appeared at 1310 cm$^{-1}$ is due to $\gamma$(NO$_3^-$) stretching
frequency. In SEM image studies framework like structure results due to strong interaction of the Pb$^{2+}$ ion with the ligand L (Fig. S7).

Scheme 1 Synthetic procedure of the receptor L and PbL$_2$.

To elucidate the structures of the chemosensors L and [PbL$_2$]$^{2+}$, we employed density functional theory (DFT) calculations using the Gaussian 09 software package. Chemosensors L and [PbL$_2$]$^{2+}$ were subjected to energy optimization using B3LYP/6-31G and B3LYP/LANL2DZ, respectively. The global minima structures for L and [PbL$_2$]$^{2+}$ are shown in Figs. 1 and 2 respectively. The optimized distance between the lead atom and the nitrogen atoms occupying 63, 64, 133 and 134 in [PbL$_2$]$^{2+}$ are 2.87, 2.75, 2.75 and 2.68 Å, respectively.
Crystals of \( L \) were obtained by slow evaporation of methanol solution. Single crystal-XRD analysis of \( L \) reveals that the molecule crystallizes in \( P2_1/c \) space group and the ‘z’ value is 4. The ORTEP diagram of \( L \) is shown in Fig. 3. The molecular structure is based on the \(-C^1-N^1-N^2-C^8-N^3-N^4-C^{10}\)- backbone where two unsubstituted phenyl rings are attached to the middle C-atoms (\( C^8 \) and \( C^9 \)) and two substituted phenyl rings are attached to terminal C-atoms (\( C^1 \) and \( C^{10} \)). Even after numerous attempts we could not able to get single crystal of the \([\text{Pb}L_2]^{2+}\) complex.
Absorption studies of L towards different amino acids

The photo-physical properties of probe L were investigated by monitoring the absorption behaviour with the addition of different analytes including competing amino acids (Gly, Ala, Lys, Trp, Arg, Val, His, Leu, Ph ala, Isoleu, Ser, Asp, Cys, Tyr and Glu), different metal cations and anions in solvent mixture of CH$_3$CN-H$_2$O (1:2, V/V). Only the addition of Arg induced distinct spectral changes while other amino acids did not induce any spectral change.

![ORTEP diagram of the L molecule (H atoms are omitted for clarity).](image1)

**Fig. 3** ORTEP diagram of the L molecule (H atoms are omitted for clarity).

![Changes in the absorption spectra of L (10 mM) in the presence of 10 eq. of different amino acids.](image2)

**Fig. 4** Changes in the absorption spectra of L (10 mM) in the presence of 10 eq. of different amino acids.

The probe L without Arg exhibited a strong UV-vis absorption band at 345 nm. Addition of arginine to a solution of L led to an obvious absorption enhancement at 430 nm with
concomitant decrease in absorption intensity at 345 nm (Fig. 4). Consistent with this change in the UV-Vis spectra, the solution of L resulted in an immediate colour change from colourless to yellow with the Arg (Fig. 5), indicating that receptor L can serve as a ‘naked-eye’ Arg indicator in aqueous solution.

**Fig. 5** The colour changes of L (10 µM) upon addition of various amino acids (10 eq.) in CH₃CN-H₂O (1:2, v/v) at room temperature.

The selectivity behaviour over a wide range of competing amino acids is obviously a matter of importance for an excellent sensing material. The exclusive selectivity of chemo-sensor L towards Arg was shown in bar graph (Fig. 6). However, the absorption study carried out with common metal cations and anions showed no significant change, indicating their non-interactive nature with L (Figs. S8 and S9). This indicates that under signalling conditions, the possible interference by them is not a practical problem in sensing of Arg by L.

The recognition ability of probe $L$ towards Arg was established upon titration of the probe solution with variable concentration of Arg followed by subsequent measurement of absorption spectra (Fig. 7).

![Absorption spectra of $L$](image)

**Fig. 7** Change in the absorption spectra of $L$ after the addition of Arg up to 30 eq. (inset plot shows the change of relative intensity on addition of equivalence of Arg).

Interestingly, probe $L$ exhibits two well-defined isosbestic points at 240 and 390 nm in the absorption spectra upon increasing concentration of Arg. In the UV–visible titration experiment, the blue-shifted band around 345 nm went down while the intensity of the newly formed band at 430 nm triggered by Arg continuously increases with successive increment of arginine (0–30 eq.) into the probe solution as shown in Fig. 7. The isosbestic point at 390 nm in the absorption spectra indicates an equilibrium between two species—the free ligand (colourless) and its deprotonated form (yellow). Among the 20 amino acids, arg with guanidino group has the highest pI value at about 10.8. Sensing of arg occurs here through the deprotonation of two terminal phenolic OH groups of the chemo-sensor by dint of the basic nature of Arg in aqueous solution. Though some cations are also basic in nature but as evidenced from UV spectra, Fig. S8 the receptors $L$ is insensitive to these cations. At the concentration of measurements Arg is more basic than those cations. Upon the increasing amount of Arg, a new absorption band at lower energy (430 nm) appears which may led us to propose the transition of the intramolecular charge transfer (ICT) band through deprotonation of the chemo-sensor $L$ by Arg, based on Bhattacharya’s proposal. To identify the ICT
property of \(L\), we have checked the change of its absorption as well as emission spectra in both polar and non-polar solvents such as dimethyl sulfoxide, methanol, chloroform and acetonitrile as it has been reported that the solvent dipole can relax the ICT excited by polar solvents.\(^{54}\) As shown in Fig. S10 and Fig. S11 and summarized in Table 2, the absorption spectra of \(L\) featured marginal red-shift of the absorption maxima (\(\Delta\lambda_{\text{abs}} = 9\) nm), and emission maxima (\(\Delta\lambda_{\text{em}} = 13\) nm) indicating an apparent solvent dependence of the absorption and emission band. Therefore, this solvatochromic behaviour demonstrates the occurrence of the ICT transition in receptor \(L\). The inset plot of Fig. 7 which highlights the ratiometric change of absorption intensity of the probe \(L\) on addition of Arg in narrow range shows that the curve becomes a plateau at [Arginine] / [\(L\)] ratio over 2.

To further elucidate the binding interaction of receptor \(L\) with Arg, \(^1\)H NMR experiments were carried out in solvent mixture \(d_6\)-DMSO and \(D_2O\) (Fig.8).

![Fig. 8 \(^1\)HNMR Spectra of \(L\) and \(L+Arg\).](attachment:image.png)

Upon addition of 2 eq. of the Arg to the receptor \(L\), the proton signals of –OH at 9.05 ppm disappeared completely and the aromatic protons- 3, 4, 5 and 6 shifted to upfield, and OCH\(_3\) protons shifted to downfield. This results indicate that the Arg participates in the deprotonation of the –OH protons and the negative charges developed from deprotonation of \(L\) by Arg are delocalized through the whole receptor molecule.\(^{55}\) The proton signals from Arg appear at 3.4 and 1.5 ppm. Amine protons are not discerned properly. From the titration profile, the detection limit calculated was found to be 0.67 \(\mu\)M (Fig. S12a).
Table 2 Absorption properties of L in various solvents

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<tr>
<td>Chloroform</td>
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<tr>
<td>Acetonitrile</td>
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<tr>
<td>DMSO</td>
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Fluorescence and absorption studies of [PbL$_2$]$^{2+}$ toward different amino acids

The ligand L employed is emission silent due to the photo-induced electron transfer (PET) transition in the molecule. But intense yellow coloured complex [PbL$_2$]$^{2+}$ in solvent mixture CH$_3$CN–H$_2$O (2/1, v/v), was found to be strongly fluorescent upon excitation at 345 nm at room temperature. This is because of the fact that the presence of Pb$^{2+}$ ion causes PET blocking through selective coordination to Pb$^{2+}$ with the inner imino nitrogen atoms of L. In addition, the stable chelate formation of Pb$^{2+}$ with L renders rigidity to the resulting complex thereby generating efficient chelation enhanced fluorescence (CHEF). The fluorescence response of [PbL$_2$]$^{2+}$ complex towards above-mentioned amino acids were investigated in acetonitrile-water (2/1, v/v) as shown in Fig. 9. It was observed that the fluorescence emission at 442 nm completely disappeared after the addition of Arg. Under the same condition, the addition of the other amino acids caused small decrease in fluorescence intensity with hardly perceptible colour changes of the solution.

Only Lys has some influence in quenching of fluorescence emission of the solution of complex albeit negligible in comparison to that observed in case of Arg (Fig. 10). These results indicated that the presence of other amino acids including Lys did not obviously affect the Arg detection under the same experimental condition.
Fig. 9 Fluorescence spectra of [PbL₂]²⁺ complex (10 µM) before and after addition of various amino acids (10 eq.).


Sensitivity is an important criterion for the fluorescence sensor. To study the sensitivity of the chemo-sensor [PbL₂]²⁺ with respect to Arg, the probe was titrated by varying the concentration of Arg in aqueous solution. Upon gradual addition of Arg, the
fluorescence intensity of the complex gets decreased gradually. The fluorescence changes of \([\text{PbL}_2]^{2+}\) upon addition of Arg is shown in Fig. 11. The inset picture in Fig. 11 which depicts the relationship between the relative fluorescence intensity \((I/I_0)\) and Arg concentration shows good linearity (linearly dependent coefficient \(R^2 = 0.987\)) in low concentration range (0 - 2 µM) which was over the Arg concentration range of 0.6 – 0.8 × 10\(^{-4}\) mol L\(^{-1}\) in healthy body plasma samples.\(^{56}\) This demonstrates the potential application of \([\text{PbL}_2]^{2+}\) complex as an efficient indicator which shows the plasma sample is healthy or not. From the titration profile the detection limit calculated is 0.1 nM (Fig. S12b).

\[\text{Fig. 11} \quad \text{Change in the fluorescence spectra of } [\text{PbL}_2]^{2+} \text{ after the addition of Arg upto 30 eq. (inset plot shows the change of relative intensity on addition of equivalence of Arg).}\]

To further investigate the selectivity of the \([\text{PbL}_2]^{2+}\) towards Arg, the possible interferences by other amino acids were also conducted through competitive experiments in aqueous media. The fluorescence emission intensity of \([\text{PbL}_2]^{2+}\) complex in acetonitrile-water (2/1, v/v) were monitored after adding 10 eq. of Arg and other interfering amino acids, as shown in Fig. 12. Under the same concentration, the tested interfering analytes showed no obvious interference to the detection of Arg except His and Lys showing only a little interference. The response of \([\text{PbL}_2]^{2+}\) complex toward the interfering analytes revealed a high selectivity to Arg against other analytes under the same conditions.

The results obtained in the entire fluorescence spectra are well consistent with the UV-visible spectral behaviour of [PbL2]^{2+} complex towards Arg. The colorimetric selective sensing abilities of complex [PbL2]^{2+} with various amino acids in a mixture of CH3CN–H2O (2: 1, v/v) were also investigated by UV-Vis absorption spectrometry (Fig.S13).

Only the addition of Arg induced distinct spectral changes while other amino acids did not induce any spectral changes. A UV-visible spectrometric titration of [PbL2]^{2+} in acetonitrile-water (2/1, v/v) with aqueous solution of Arg revealed that the absorption band at 380 nm decreased and two new bands at 290 nm and 450 nm emerged resulting an isosbestic point at 415 nm (Fig.13) with an immediate colour change from yellow to colourless (inset picture of Fig. 13).
Fig. 13 Change in the absorption spectra of $[\text{PbL}_2]^2+$ after the addition of Arg up to 30 eq. (inset plot shows the colour change on addition of Arg).

**Sensing mechanism of $[\text{PbL}_2]^2+$**

$[\text{PbL}_2]^2+$ complex in acetonitrile-water (2/1, v/v) shows amplified fluorescence owing to hindered PET and CHEF effect. Subsequently, when Arg was added into the $[\text{PbL}_2]^2+$ complex solution, the interactions of its stronger alkaline centres with Pb$^{2+}$ promoted the dissociation of $[\text{PbL}_2]^2+$ complex and thereby produced the free ligand L (Scheme 2).

Scheme 2 Sensing mechanism of $[\text{PbL}_2]^2+$

This resulted in the quenching of fluorescence intensity. In order to obtain better insight into the sensing mechanism, mass spectral studies of $[\text{PbL}_2]^2+$ complex have been carried out after addition of Arg. This study stands in support of regeneration of free ligand L.
in sensing mechanism, which is confirmed by the appearance of a peak at m/z 567, assignable to [L+ H+] in the ESI/MS (Fig. 14).

Fig. 14 Mass Spectra of [PbL₂]²⁺ after addition of Arg.

The quick response time for a sensing material is a prerequisite for practical applicability. To understand the dissociation time of [PbL₂]²⁺ complex in presence of Arg, the fluorescence spectra of probe [PbL₂]²⁺ complex was recorded by variable time interval and the results revealed that the response of change in fluorescence intensity started immediately upon addition Arg and almost completed within 1 min as shown in the fluorescence spectra at different time intervals (Fig. 15). It was observed that fluorescence intensity remained almost constant up to 15 min on addition of 10 eq. of Arg.

Fig. 15 Time evolution of for Arg.
Application of chemosensor L

To check the practical application, the test kits were utilized to sense Arg among different competing amino acids. As shown in Fig. 16, when the test kits coated with L were added to aqueous solution of different amino acids, the obvious colour change was observed only with Arg. Therefore, the test kits coated with the chemo-sensor L solution would be convenient for detecting Arg. These results showed that chemo-sensor L could be a valuable practical sensor for environmental analyses of Arg. For practical application of the \([PbL_2]^{2+}\) we have performed similar type colorimetric test kit experiment. But it has been found that the \([PbL_2]^{2+}\) solution cannot stain the filter paper.

![Fig. 16 Photographs of the test kits with L (0.5 mM) for detecting Arg among other amino acids.]

**Conclusion**

In this work, we have synthesized a novel, exceptionally simple and cost effective bis Schiff base ligand L and its Pb\(^{2+}\)-complex, \([PbL_2]^{2+}\) and exploited them for expeditious detection of arginine. The ligand can be successfully applied as a colorimetric sensor for arginine detection whereas \([PbL_2]^{2+}\) has the potential to detect arginine in fluorescent-colorimetric way. Both the chemo-sensors exhibit excellent selectivity and sensitivity towards arginine in aqueous solution. The detection limits calculated 0.67 \(\mu\)M (for L) and 0.1 nM (for \([PbL_2]^{2+}\)) are fairly below in comparison to those found in literature. To the best of our knowledge, this is the first time to report a simple Schiff base and its metal complex acting as an efficient chemo-sensor for Arg.

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References

55. S. Park, K. Hong, J. Hong and H. Kim, Sens. Actuators, B, 2012, 174, 140-144
A new Schiff base and its metal complex as a fluorescent-colorimetric sensor for rapid detection of arginine

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A new Schiff base and its Pb²⁺-complex have been utilized, for the first time, for the detection of arginine in aqueous medium. Receptor L exhibits an excellent selective colorimetric response towards arginine whereas Pb²⁺-complex has been exploited as a highly selective fluorescent-colorimetric probe for rapid detection of arginine. The sensitivity of the fluorescent based assay for arginine is sufficiently low (0.1 nM). The geometry of L has been optimized both by DFT calculation and single crystal X-ray studies.