Disposable paper based PET fluorescence probe linked with calix[4]arene for Lithium and Phosphate ion detection

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Chemicals and reagents

All the reagents and chemicals like DCC (N, N'-dicyclohexylcarbodiimide), DMAP (4-dimethylaminopyridine) and 4-Aminoquinoline were used of analytical grade procured from Sigma Aldrich. Silica gel (Merck, 0.040-0.063mm) was used for column chromatography. Metal salts (99-101% purity of SRL) used for the studies were their perchlorate salts (Caution: Since perchlorate salts are known to explode under certain conditions, these are to be handled cautiously!) with formula, $M(ClO_4)_2.xH_2O$. Anions used for the studies were their tetra butyl ammonium salts (99-101% purity of SRL) which were prepared in acetonitrile. Stock solutions of cations and anions (0.01 M) are prepared in acetonitrile. Further dilutions are completed as per requirement. Spectroscopic properties of L was investigated in mixed aqueous organic medium (acetonitrile/aqueous phosphate buffer (8:2, v/v; pH=7.2)).

Apparatus

Melting points were taken on Opti-Melt (Automated melting point system). FT-IR spectra were recorded as KBr pellet on Bruker TENSOR-27 in the range of 4000-400 cm^{-1}. Discover Bench Mate system-240 V (CEM Corporation) microwave synthesizer was used for synthesis of p-tertbutylcalix[4]arene. GmbH Vario Micro cube elementar analyzer was used for elemental analysis. ^{1}H NMR spectra was scanned on 400 MHz FT-NMR Bruker Avance-400 in the range of 0.5 - 15 ppm using internal standard tetramethylsilane (TMS) and deuterated DMSO as a solvent. ESI Mass spectra were taken on a Shimadzu GCMS-QP 2000A. Emission spectrum was recorded on Horiba Fluoromax Plus C. UV–vis absorption spectra were acquired on a Jasco V-570 UV–Vis. Spectrometer. Working standard solutions were prepared daily in deionized water.

Experimental
Electrochemical Measurements

Electrochemical measurements were carried out using electrochemical workstation (Model CHI600E. instruments, Inc. Austin TX) electrochemical workstation using a conventional three electrode system compromised of (WE: glassy carbon electrode (round disk diameter 3mm), RE: Ag/Ag$^+$ (0.1M AgNO$_3$ in Acetonitrile, CE: Platinum wire **(Figure S7)**. The surface of the working electrode was polished properly with 0.3μm alumina to remove any impurity and

sonicated for 15 minutes in deionized water. The electrodes were further dried and washed with pure acetonitrile (ACN) before use. For measurement the cyclic behavior of electrochemical species with supporting electrolyte was added with (0.1M) TBAP (Tetra butyl ammonium perchlorate) at scan rate of 25mV.sec^{-1}.

General procedure for ion-binding study

As acetonitrile is mixable with water, we have prepared stock solutions of analytes such as perchlorate salts of metal ions Na$^+$, K$^+$, Rb$^+$, Cs$^+$, Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Ag$^+$, Zn^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}, Hg^{2+} and tetrabutyl ammonium (TBA) salts of anions (HPO$_4^{2-}$, PO$_4^{3-}$, F$^-$, Cl$^-$, Br$^-$, I$^-$, ox^{2-}, NO$_3^-$, SO$_4^{2-}$, CH$_3$COO$^-$, CN$^-$, H$_2$PO$_4^-$) in acetonitrile with the concentration of 1×10^{-8} M. Simultaneously, the stock solution of ligand *p*-C4A was also made in acetonitrile with the concentration of 1×10^{-8} M (**Fig.2**). Then the stock solutions were diluted to desired concentration when needed. The spectroscopic properties of *p*-C4A were examined in mixed aqueous organic medium [acetonitrile/4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (6:4, v/v; pH = 7.2)]. Absorption and emission spectra of the novel synthesized probe *p*-C4A were recorded by making solution as mentioned above. The compound shows the absorption peak in the region between 240-400 nm, wherein the peak at 306 nm indicates π-π* transition of 4-aminoquinoline system.

As in real samples, various other metal ions and anions are also present along with the Li$^+$ and PO$_4^{3-}$ and can affect the sensing process, it is necessary to investigate functional properties and selective nature of *p*-C4A (1×10^{-6} M) towards Li$^+$ and PO$_4^{3-}$ in the presence of co-existing cations and anions (**Fig.5**). This interference study was carried out using 10 equivalents of other cations (1×10^{-6} M) with Li$^+$ (1×10^{-6} M) separately. In a same way, 10 equivalents of different anions (1×10^{-6} M) were taken with PO$_4^{3-}$ (1×10^{-6} M) to check whether it affects the

fluorescence intensity or not. The ratio of host molecule: guest ions was 1:2 for the interference study.

Association constants[49] for fluoroionophore were calculated by using fluorescence titration data following the previously reported articles. Here, we have displayed representative spectra showing the changes observed in emission intensities upon the addition of increasing concentration of ions. According to this procedure, the fluorescence intensity (F) scales with the metal ion/anion concentration ([M]) through $(F_0-F)/(F-F_\infty) = ([M]/K_{diss})_n$. The binding constant (Ks) is obtained by plotting $Log[(F_0-F)/(F-F_\infty)]$ vs Log[M], where F_0 and F_∞ are the relative fluorescence intensities without addition of guest metal ions and with maximum concentration of metal ions (when no further change in emission intensity takes place), respectively. The value of Log [M] at $Log[(F_0-F)/(F-F_\infty)] = 0$ gives the value of $log(K_{diss})$, the reciprocal of which is the binding constant (Ks).

The quantum yield was calculated by following equation

$$\phi = \phi_{std}\frac{(F \times A_{std} \times \eta)}{(F_{std} \times A \times \eta_{std})}$$

where F and F_{std} are the areas under the fluorescence emission curves of the Li^+ and PO_4^{3-} ions complexes with p-C4A and only standard p-C4A respectively. A and A_{std} are the areas under relative absorbance of the Li^+ and PO_4^{3-} ions complexes with p-C4A and standard p-C4A at the excitation wavelength, respectively. η and η_{std} are the refractive indices of solvent (acetonitrile) used for the Li^+, PO_4^{3-} and standard (p-C4A), respectively.


Real Sample Preparation

To assess the applicability of the method in real media, we have taken waste water for Li^+ and soil samples for PO_4^{3-}. For soil sample preparation, we collected soil samples from industrial

area of Anand GIDC, Gujarat, India for analysis. The soil samples were first dissolved in concentrate HNO_3 and stirred for 1 hour after then we took 10 mL of solution and diluted upto 100 mL into volumetric flask. From this solution, we took 2 mL of solution and 2 mL of our synthesized ligand *p*-C4A for fluorometric analysis by maintaining pH 6-8 with phosphate buffer. This experiment carried out by using in situ generated PO_4^{3-} complex of *p*-C4A with various soil samples. All the waste water samples collected were simply filtered and showed that no Li^+ was present. Our compound was soluble in chloroform as well as in acetonitrile but as acetonitrile is miscible with water, chloroform was preferred to prepare the solution of the compound. Then in a separating funnel, we procured 60 ml of *p*-C4A (10 µM) solution and 40 ml of water sample by shaking for half an hour. Then we separated organic layer and dehydrated organic phase with anhydrous Na_2SO_4. The extraction was repetitive three to four times. Then different concentrations of Li^+ (10 µM, 20 µM, 30 µM, 40 µM, 50 µM and 100 µM) were spiked to the solution. Then the fluorescence intensity for each sample was recorded.
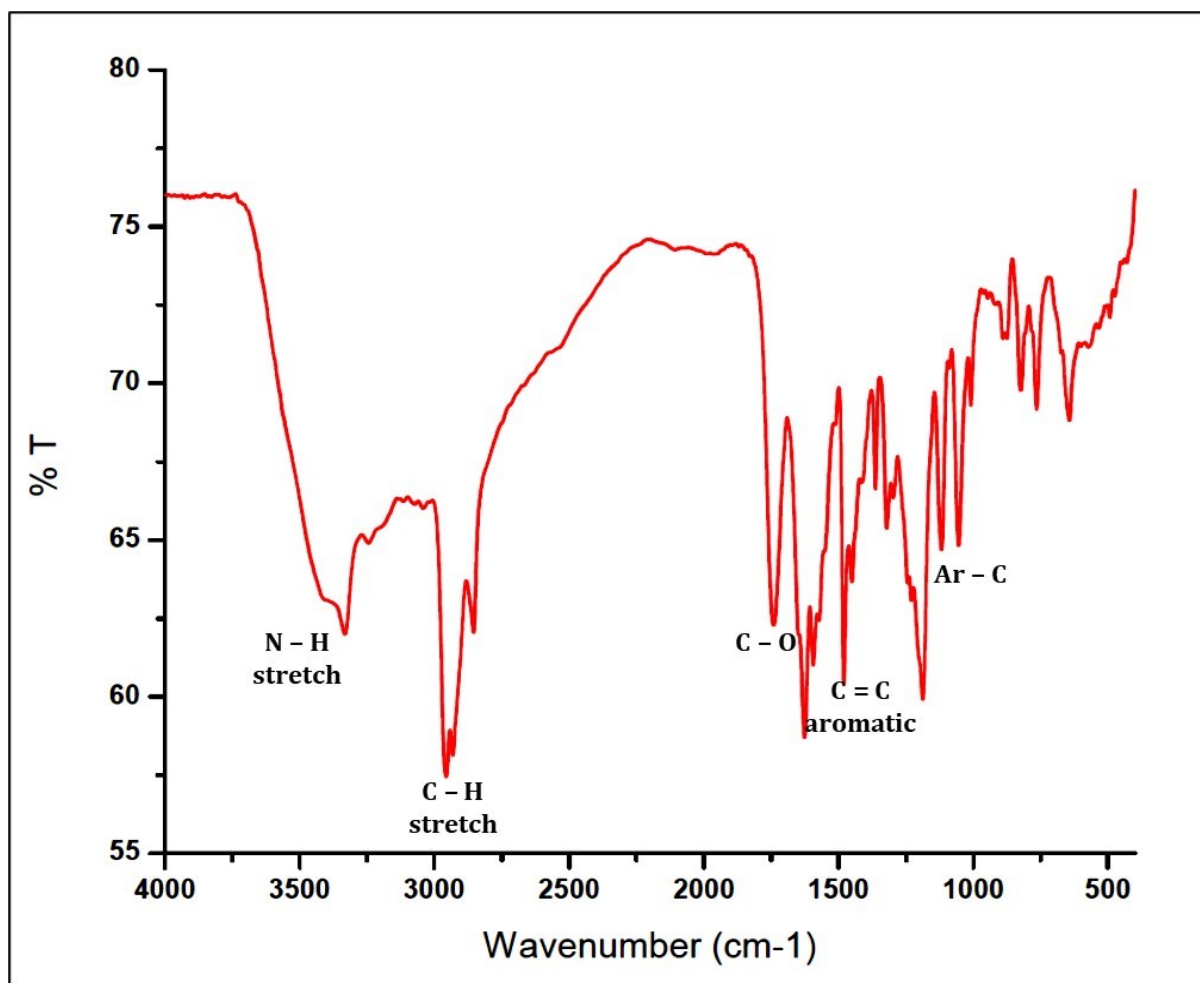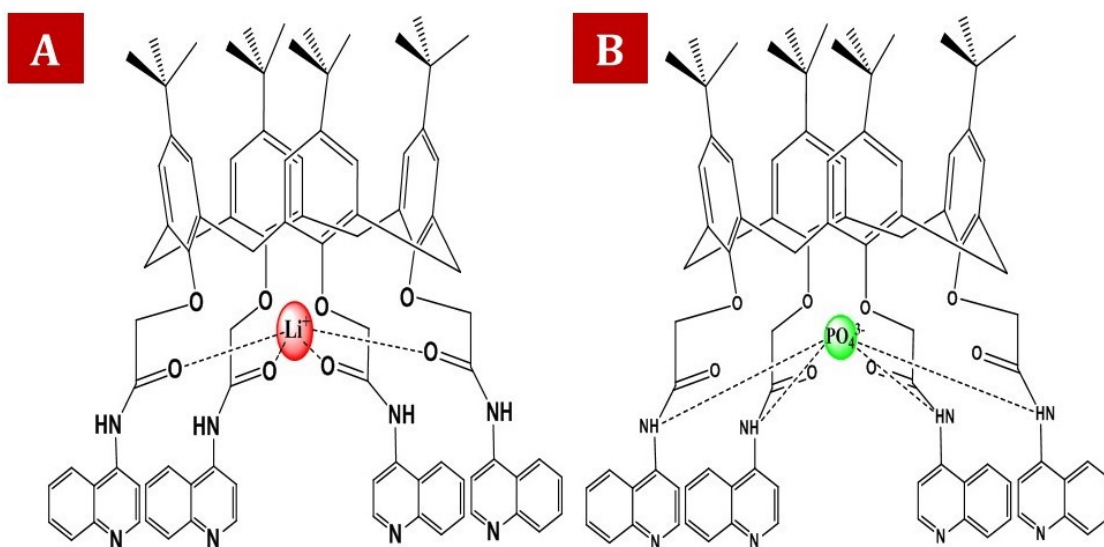
Figure S1: FT-IR spectra of *p*-C4A.
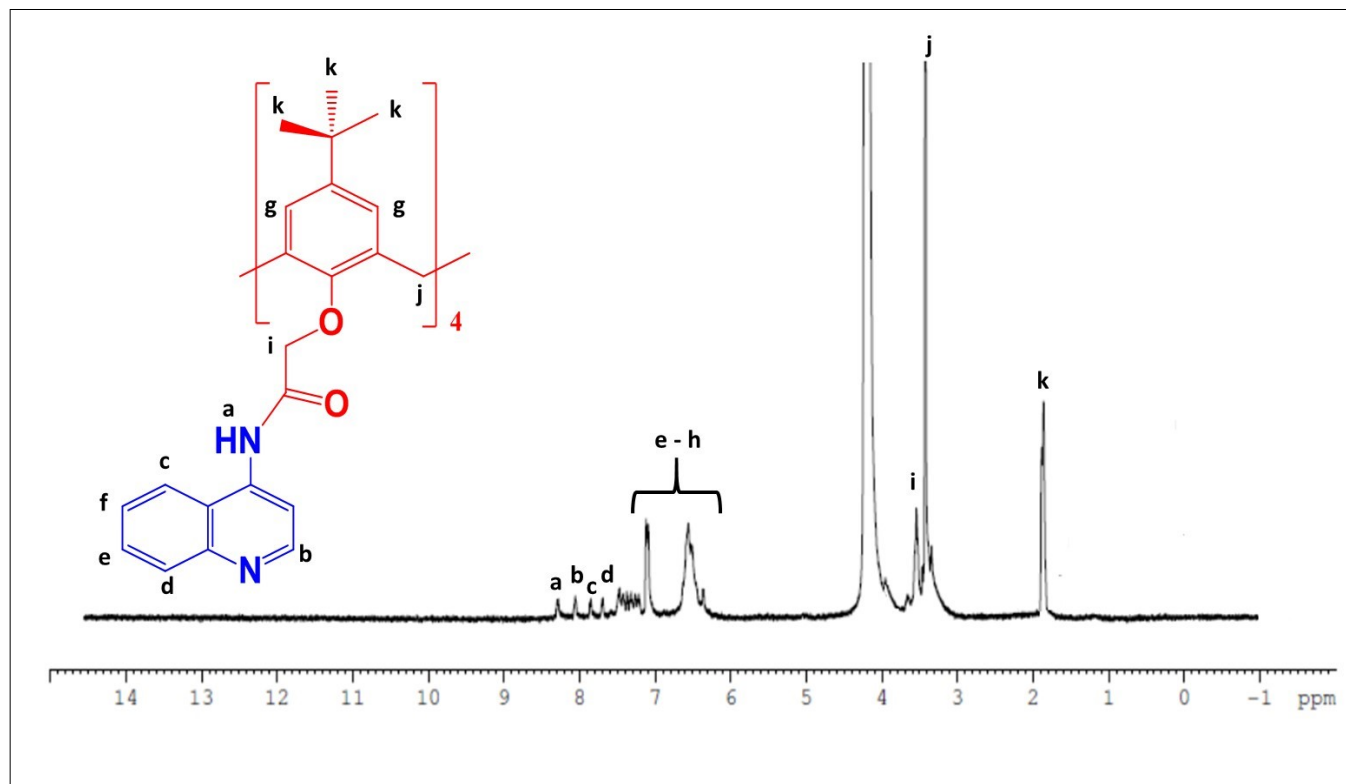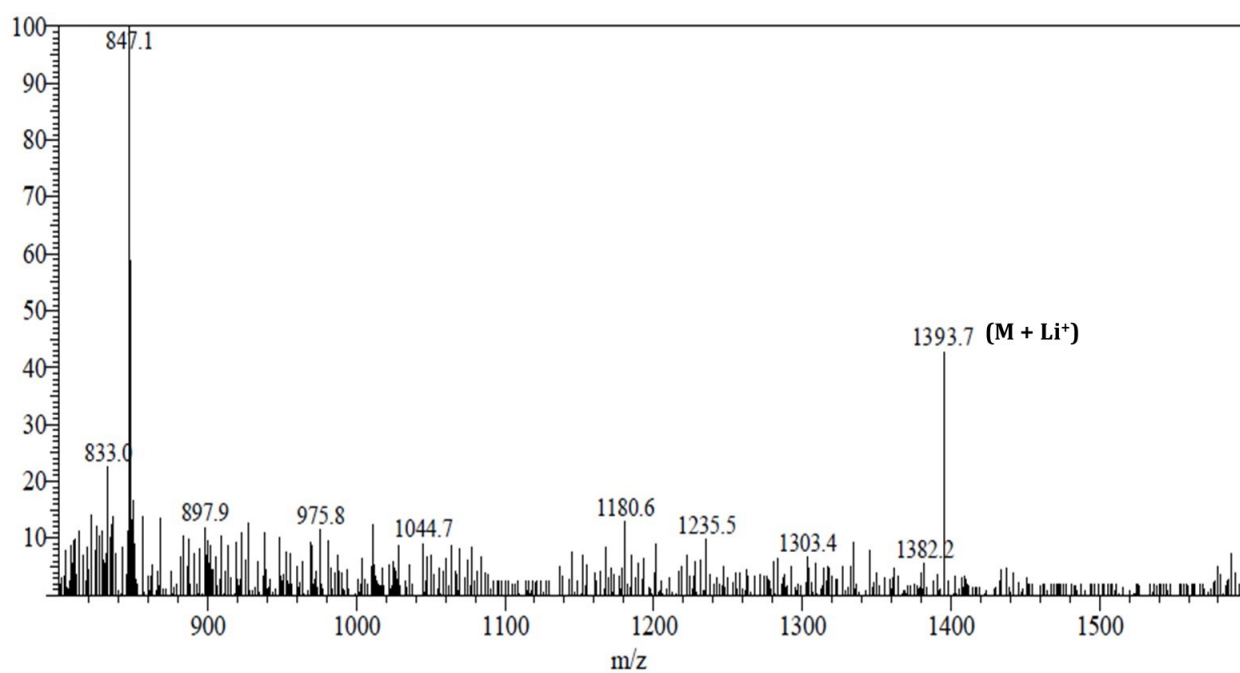
Figure S2: ^{1}H NMR spectra of *p*-C4A
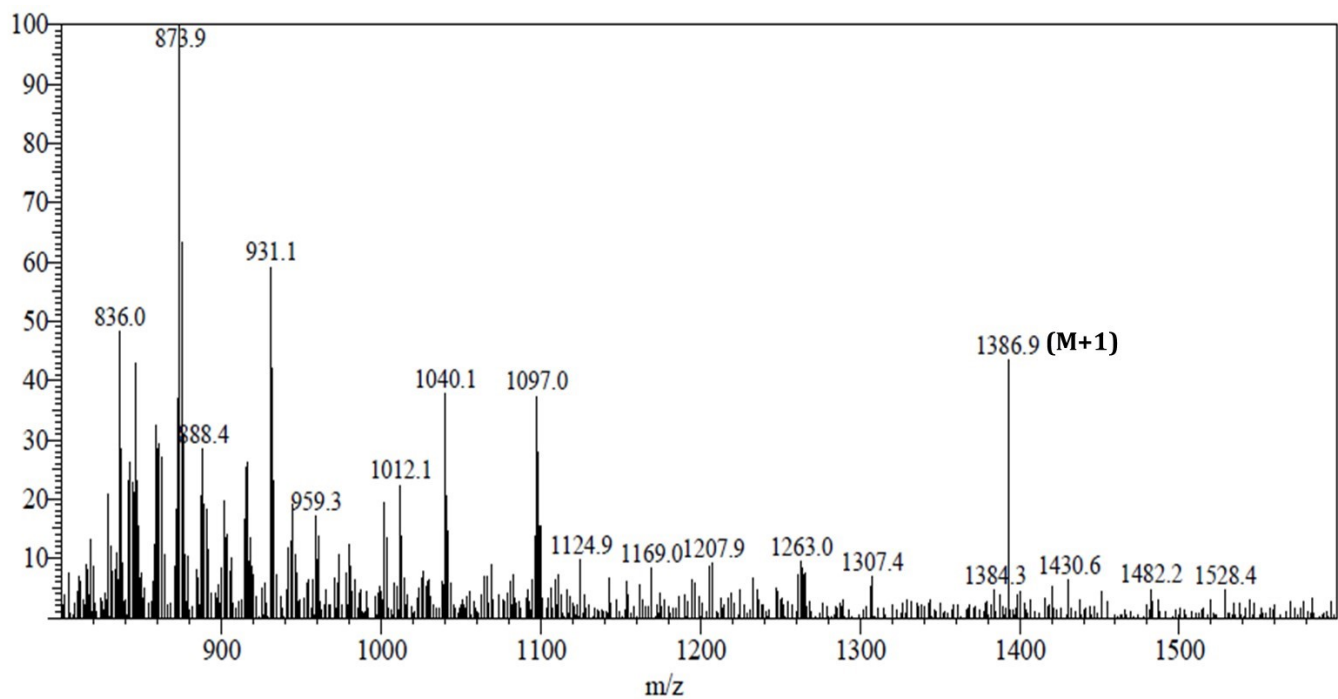
Figure S3: ESI-MS spectra of *p*-C4A

Figure S4: Proposed binding of p-C4A with lithium and phosphate with electrostatic interaction and hydrogen bonding.
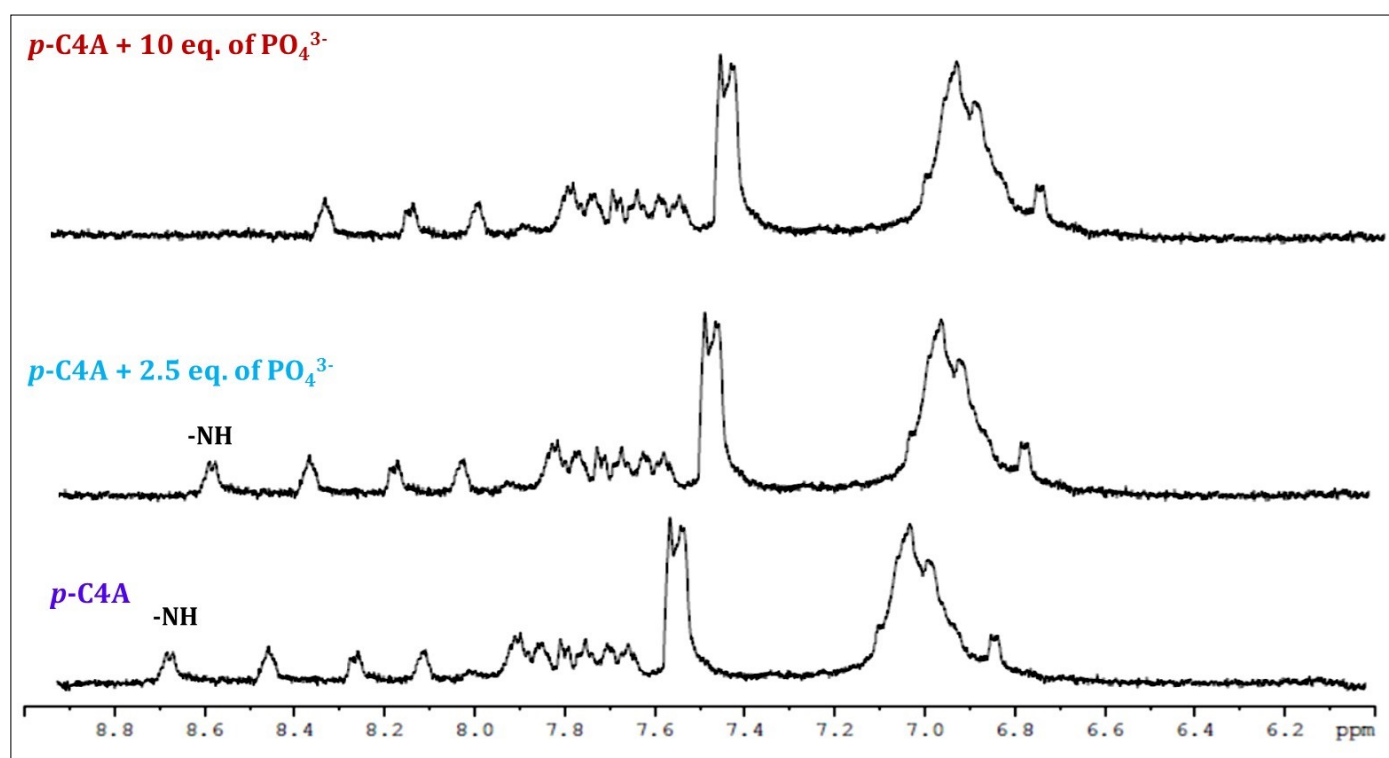
Figure S5: ESI-MS study of *p*-C4A with Li⁺ ion.

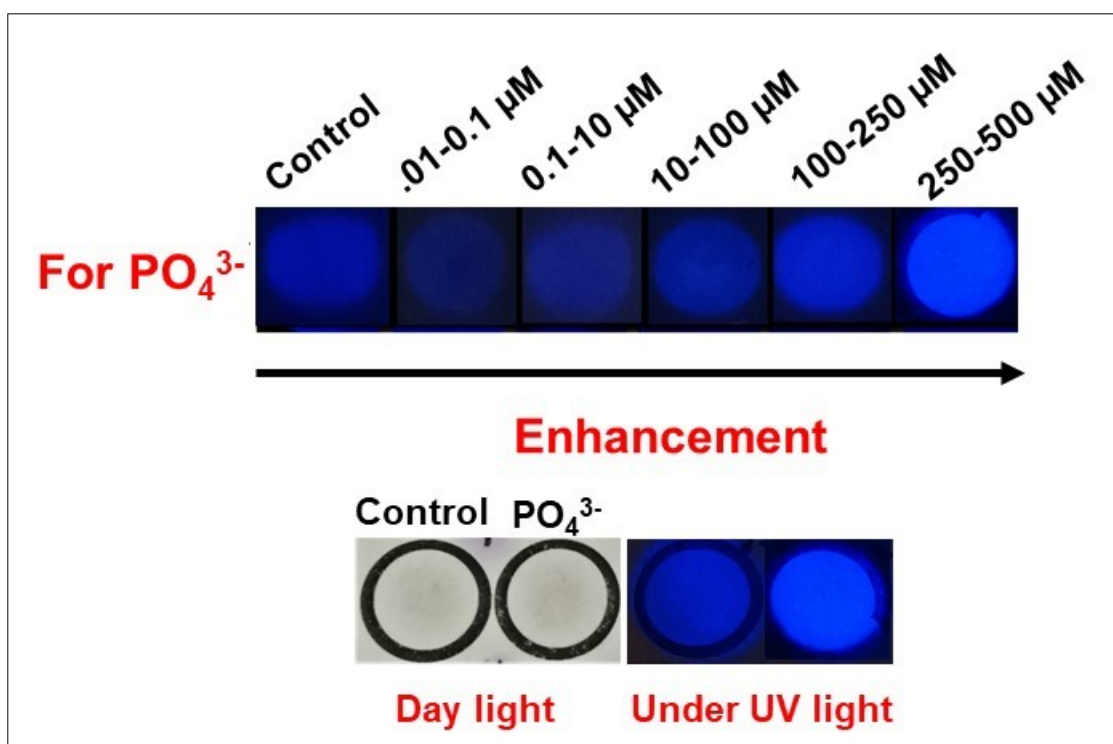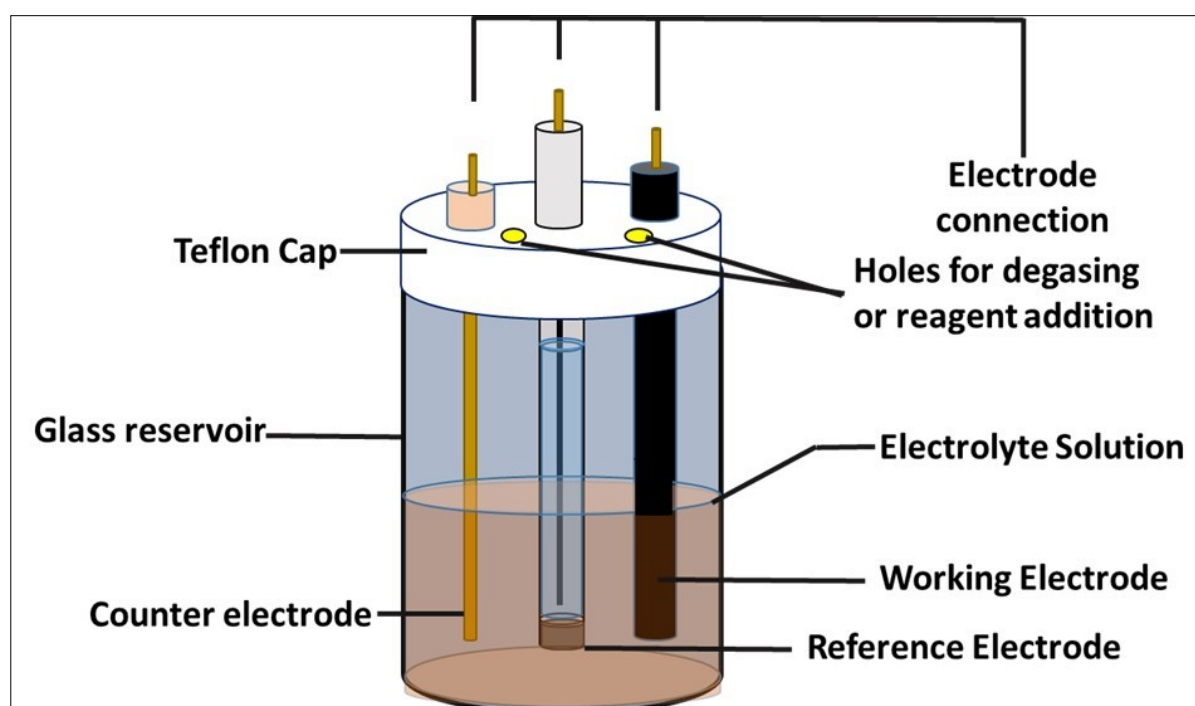**Figure S6: ^{1}H NMR study of *p*-C4A in the presence of PO_4^{3-}.**

Figure S7: Fluorescence effect of *p*-C4A-PAD (Under Day and fluorescence light) and after detection of PO_4^{3-} at the concentration ranging from 0.01-500 µM.

Figure S8: Diagram of liquid based cyclic voltammetry.

Sample	Spiked ions	Spiked amount (nM)	Found by AAS (nM)	Found by proposed sensor (nM)	Recovery (%)	No. of observations
Waste Water Sample 1	Li$^+$	10	9.72	9.53	98 ± 0.4	5
Waste Water Sample 2		20	19.58	19.25	98 ± 0.3	5
Waste Water Sample 3		30	28.92	28.23	97 ± 0.6	5
Waste Water Sample 4		40	39.21	39.19	99 ± 0.9	5
Waste Water Sample 5		100	98.81	98.66	99 ± 0.8	5
Soil Sample 1	PO$_4$$^{3-}$	10	-------	9.74	97 ± 0.4	5
Soil Sample 2		20	-------	19.68	98 ± 0.4	5
Soil Sample 3		30	-------	29.40	98 ± 0.1	5
Soil Sample 4		40	-------	38.61	96 ± 0.5	5
Soil Sample 5		100	-------	98.23	98 ± 0.2	5

Table S1: Results of the determination of Li$^+$ and PO$_4$$^{3-}$ in different waste water and soil samples.

Method	Recognized ion	Linear range	Limit of detection	Reference
ZnO nanoflake	Phosphate ion	--------	1×10^{-6} to 1×10^{-3} M	**[51]**
CDs@ZIF-90 fluorescence sensor	Phosphate ion	$1.0 - 50.0 \times 10^{-6}$ M	$0.23\ 10^{-6}$ M	**[52]**
Portable colorimeter	Phosphate ion	$0.02 - 9.5 \times 10^{-3}$ M	0.009×10^{-3} M	**[53]**
Fluorescence sensor	Phosphate ion	$0 - 6 \times 10^{-6}$ M (C1) $0 - 8 \times 10^{-6}$ M (C2) $0 - 6.5 \times 10^{-6}$ M (C3)	0.029×10^{-6} M (C1) 0.048×10^{-6} M (C2) 0.079×10^{-6} M (C3)	**[54]**
Aggregation-induced emission sensor	Phosphate ion	$10 - 150 \times 10^{-6}$ M	6.56×10^{-8} M	**[55]**
Fluorescence sensor	Phosphate ion	$0 - 175 \times 10^{-6}$ M	--------	**[56]**
Ion-imprinted polymeric nanoparticles	Li^+	$10 - 300 \times 10^{-6}$ M	3.29×10^{-6} M	**[57]**
ion-imprinted fluorescent polymer	Li^+	$0.5 - 50 \times 10^{-6}$ M	16×10^{-6} M	**[58]**
Recognition by [2.2]Ferrocenophanes	Li^+	--------	--------	**[59]**
Fiber-based Lithium sensor	Li^+	--------	--------	**[60]**
Fluorescence sensor	$H_2PO_4^-$	$1-10 \times 10^{-6}$ M	1.5×10^{-8} M	**[61]**
Our fluorescence probe **p-C4A**	Li^+ PO_4^{3-}	$5 - 170 \times 10^{-9}$ M $5 - 80 \times 10^{-9}$ M	6.15×10^{-9} M 9.0×10^{-9} M	**This Research work**

Table S2: Comparison of proposed *p*-C4A fluorescence sensor with various previously reported determination methods for Li^+ and PO_4^{3-}.