Electronic Supplementary Information

Fluorescein Based Dual Optical Sensor for Highly Selective and Sensitive Fluoride Detection in 100 % Water Medium

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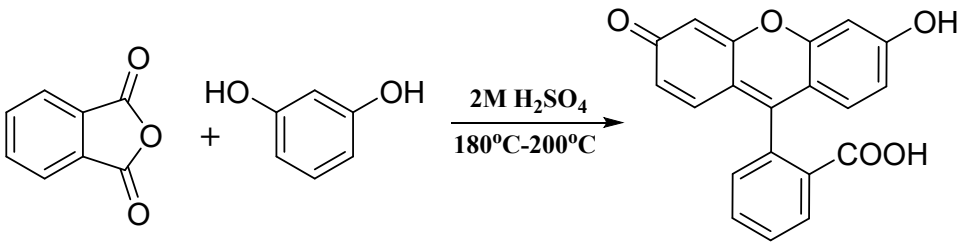
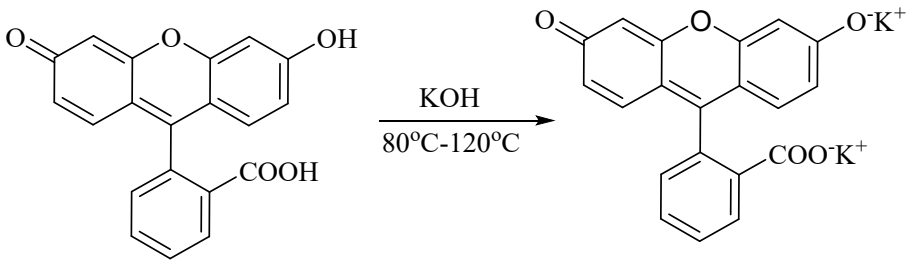


General Synthesis procedure:

Scheme 1: Synthesis of Fluorescein (**M**).

Fluorescein (**M**) was prepared by reacting 0.3 g (2.2 mmol) of phthalic dianhydride with 0.5 g (4.5mmol) of Resorcinol in presence of 6-7 drops of 2M H_2SO_4 in a round bottom flask. Later, the reaction mixture was heated at 180ºC - 200ºC for 30 min and cooled for 5 min after removing it from the reaction bath. After coming to room temperature, 10 mL of acetone was added to the reaction mixture and stirred for another 15-20 min until the solution turns yellow.

M: Reddish brown, Yield: 70%, FT-IR of **M** (cm^{-1}): υ(O-H) = 3389.73, υ(C=O) = 1590. ^1H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 7.98 (d, J = 7.9 Hz, 1H), 7.85 – 7.64 (m, 1H), 7.26 (d, J = 8.0 Hz, 1H), 6.81 – 6.47 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 207.05,



169.25, 160.06, 153.03, 152.38, 136.12, 130.59, 129.55, 126.71, 125.12, 124.58, 113.16, 110.09, 102.79. LCMS (M+H) =333.10 (Calculate: 332.10).

Scheme 2: Synthesis of Potassium salt of Fluorescein (**K_2M**).

The Potassium salt of fluorescein was synthesized by treating with aqueous KOH (Scheme 2). 100 mg of fluorescein was mixed with 100 mg of KOH in 10 mL of water and refluxed overnight. The reaction was stopped and kept idle till precipitated as reddish orange coloured crystal.

K_2M: Reddish orange, Yield: 70%, FT-IR of **M** (cm^{-1}): υ(O-H) = 3442.52, υ(C=O) = 1630.90.

General experimental procedure:

UV−Visible and Fluorescence Spectroscopy Studies:

All the sensing experiment of fluorescein (**M**) and potassium salt of fluorescein (**K_2M**) was performed in dimethyl sulphoxide and water solution respectively. The UV-Vis absorption experiments were performed with a 10 μM solution of the probe molecule (**M** in DMSO and **K_2M** in H_2O), while the corresponding fluorescence spectra were recorded with a 1 μM solution at an excitation wavelength of 520 nm at room temperature.

Calculation of Binding Constant:

The equilibrium constant for the complexation of M^{2-} and Al^{3+} is calculated from the UV-Visible titration data by considering absorbance at 475 nm with 1:1 and 1:2 binding model with the Bindfit tool accessed from the http://supramolecular.org.[1]

Calculation of limit of detection (LOD):

Limit of detection (LOD) was calculated using the formula

$$LOD = \frac{3S_E}{m},$$

where S_E is the standard error of the intercept and *m* is the slope of the calibration plot in presence of fluoride ion.

Determination of F^- in Water Samples

Different volumes of standard solution of F^- (100 ppm) were added to prepare water samples containing different concentrations of F^- required to obtain the calibration plot. The methodology was validated with different concentration of fluoride in ppm level prepared in tape water.

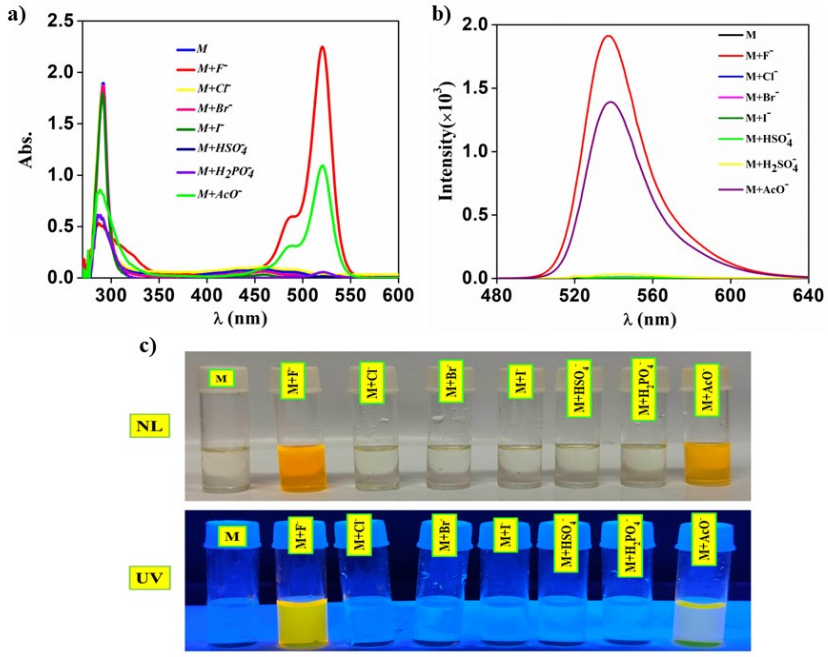


Figure S1: (a) UV-Vis spectra of **M** solution (10 μM) in DMSO in presence of different anions (1 x 10^{-2} M) as their tetrabutylammonium salt; (b) Emission spectra of **M** solution (1 μM) in DMSO in presence of different anions (1 x 10^{-3} M) as their tetrabutylammonium salt; (c) Colorimetric and fluorometric colour change of the probe molecules in presence of different anions under normal light (NL) and UV lamp (365 nm wavelength).

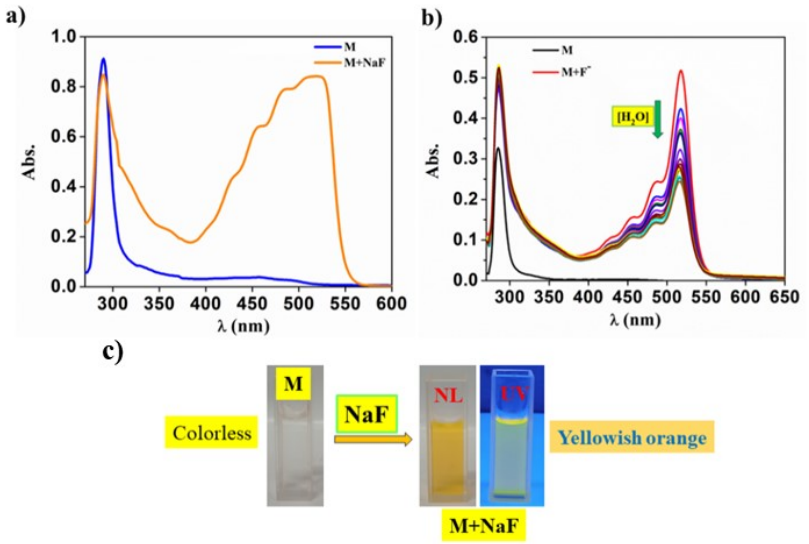


Figure S2: UV-Vis spectrum of **(**a) **M** in DMSO upon addition of 50 μL of NaF(1 × 10^{-2}M) aqueous solution; (b) Change in the UV-Vis spectrum of the (**M**+ NaF) solution in DMSO +H_2O mixture upon addition of H_2O; (c) Change in the colour of the solution of **M** in DMSO solution upon addition of NaF (aq).

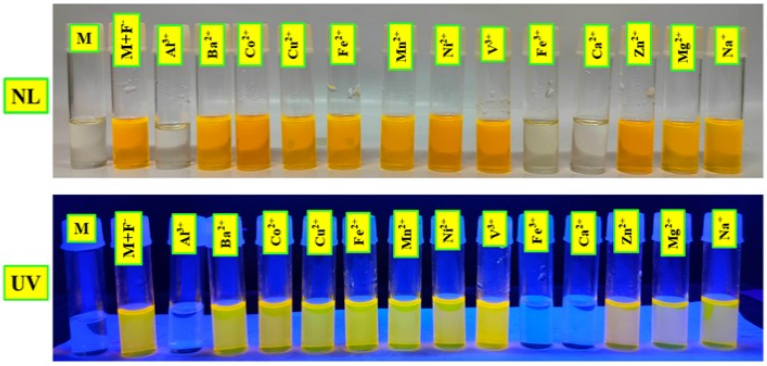


Figure S3: Images of colorimetric and fluorometric colour changes of **M** [10 μM] in DMSO solution upon addition of 10 mM aqueous solution of different metal salts (NaCl, $MgSO_4$, VCl_3, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $CaCl_2$, $BaSO_4$, $FeCl_2$, $AlCl_3$) in presence of F^- ion (1 x 10^{-2} M).

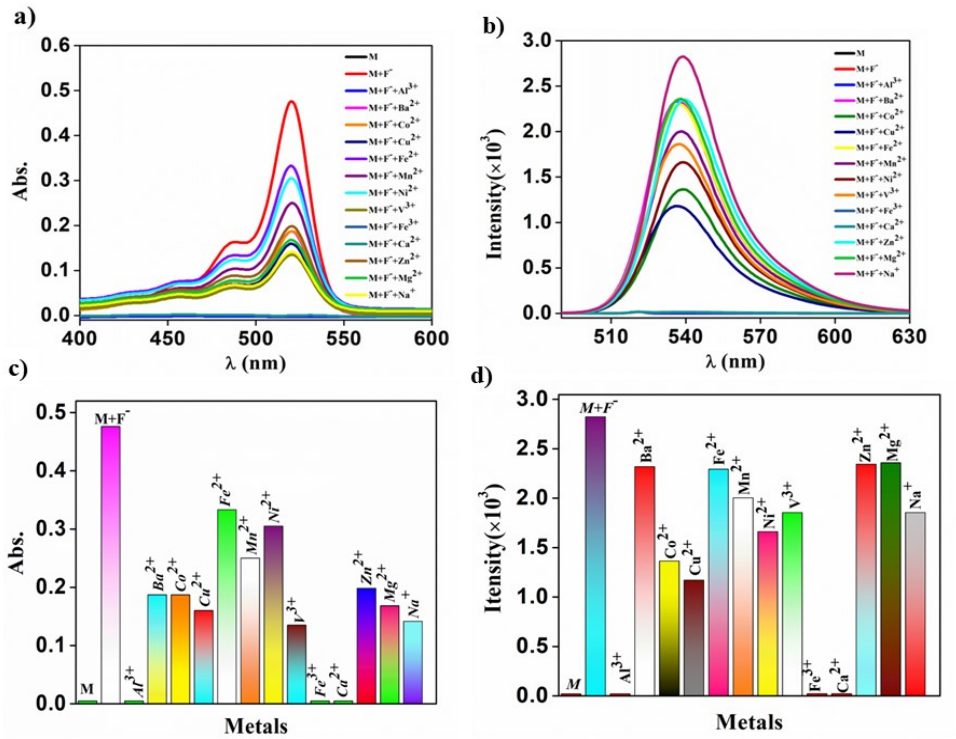


Figure S4: (a) UV-Vis spectra of **M** [10 μM] in DMSO solution upon addition of 10 mM aqueous solution of different metal salts (NaCl, $MgSO_4$, VCl_3, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $CaCl_2$, $BaSO_4$, $FeCl_2$, $AlCl_3$) in presence of F^- ion (1 x 10^{-2} M); (b) Emission spectra of **M** [2.5 μM] in DMSO solution upon addition of 1 mM aqueous solution of different metal salts (NaCl, $MgSO_4$, VCl_3, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $CaCl_2$, $BaSO_4$, $FeCl_2$, $AlCl_3$) in presence of F^- ion (1 x 10^{-2} M); (c) Bar representation of absorbance of the peak at 520 nm; (d) Bar representation of emission intensity of the peak at 540 nm.

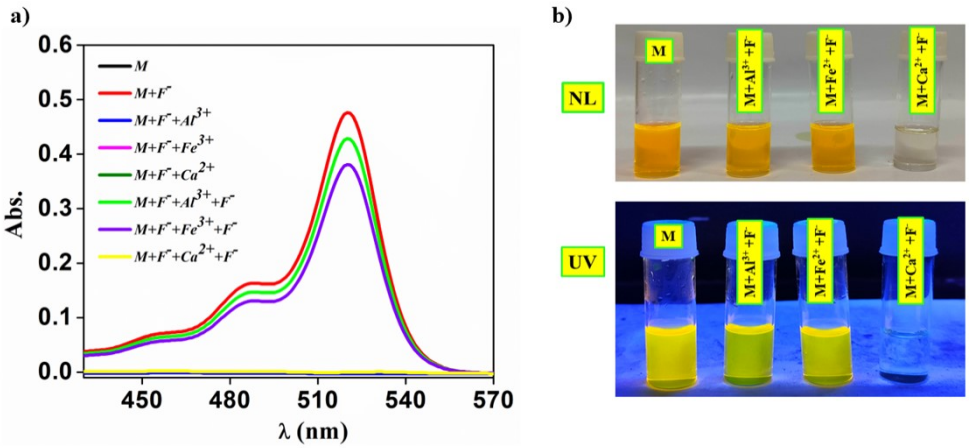


Figure S5: (a) UV-Vis spectra of **M**-metal complex [**M** (DMSO) + F^- (DMSO) + Al^{3+} (aq)] solution upon re-addition of aqueous NaF (1×10^{-2} M) (**M:** Ca, Fe and Al); (b) Images of the corresponding colorimetric and fluorometric colour changes.

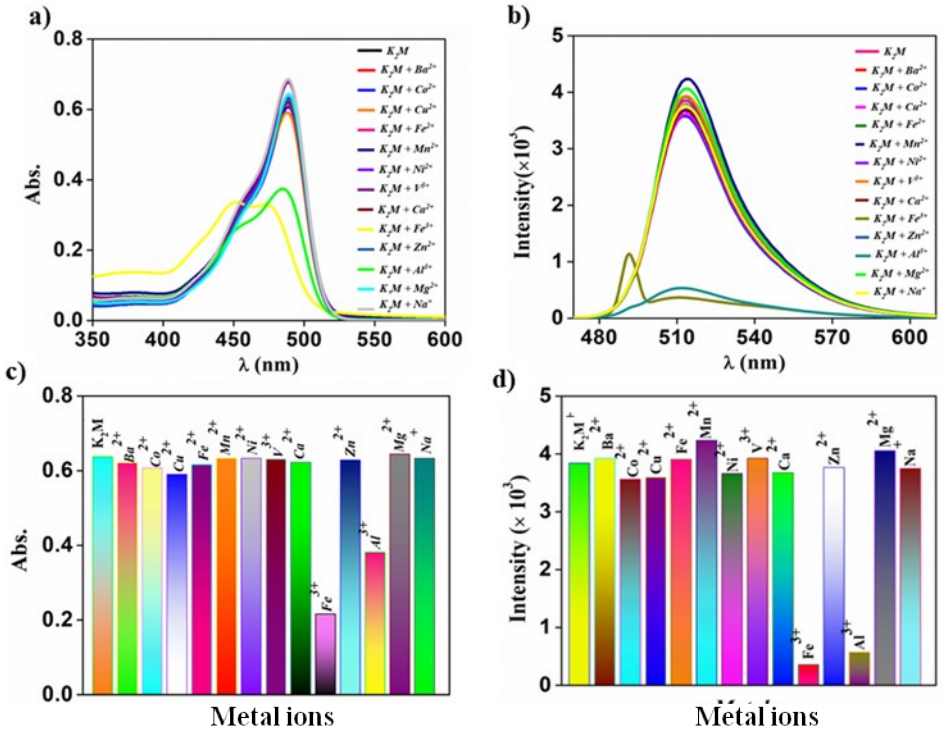


Figure S6: (a) UV-Vis spectra of $\mathbf{K_2M}$ [10 μM] in water solution upon addition of 10 mM aqueous solution of different metal salts (NaCl, $MgSO_4$, VCl_3, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $CaCl_2$, $BaSO_4$, $FeCl_2$, $AlCl_3$) in presence of F^- ion; (b) Emission spectra of $\mathbf{K_2M}$ [2.5 μM] in water solution upon addition of 1 mM aqueous solution of different metal salts (NaCl, $MgSO_4$, VCl_3, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $CaCl_2$, $BaSO_4$, $FeCl_2$, $AlCl_3$) in presence of F^- ion; (c,d) Bar representation of above two titrations.

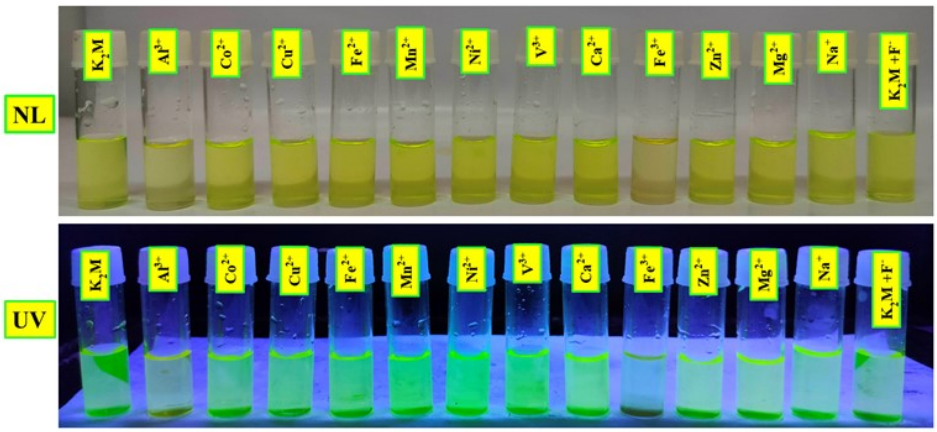


Figure S7: Images of the colorimetric and fluorometric colour change (NL: Normal light, UV: UV light) of **K_2M** [10 μM] in water solution upon addition of 10 mM aqueous solution of different metal salts (NaCl, $MgSO_4$, VCl_3, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $CaCl_2$, $BaSO_4$, $FeCl_2$, $AlCl_3$) in presence of F^- ion.
in absorbance; (d) Bar representation of the change of emission intensity.

Figure S8: Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the data for the UV-Vis titration of **K_2M** with Al^{3+} ion for the absorption at 490 nm vs. the data fitted to the 1:1 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

Figure S9: Screenshot of the summary window of http://app.supramolecular.org/bindfit/. This screenshot shows the data for the UV-Vis titration of $\mathbf{K_2M}$ with Al^{3+} ion for the absorption at 490 nm vs. the data fitted to the 1:2 UV binding model, the corresponding residual plot and the association constants with the calculated asymptotic standard errors.

Table S1: Summary of association constants between $\mathbf{K_2M}$ and Al^{3+} according to different binding models.

Binding Models	
1:1	**1:2**
$2.1\times10^5(\pm 64.23\%)$	$K_{11} = 5.55\ (\pm 13.25\%)$ $K_{12} = 3.08\times10^7\ (\pm 17.66\%)$

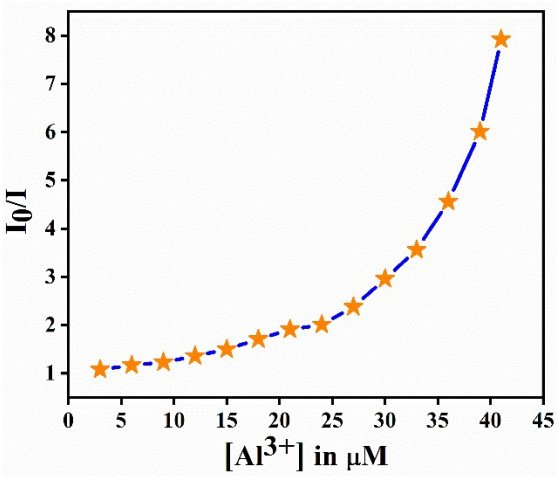


Figure S10: Change in the fluorescence intensity of the **K_2M** (aq) solution upon incremental addition of Al^{3+} (aq) solution.

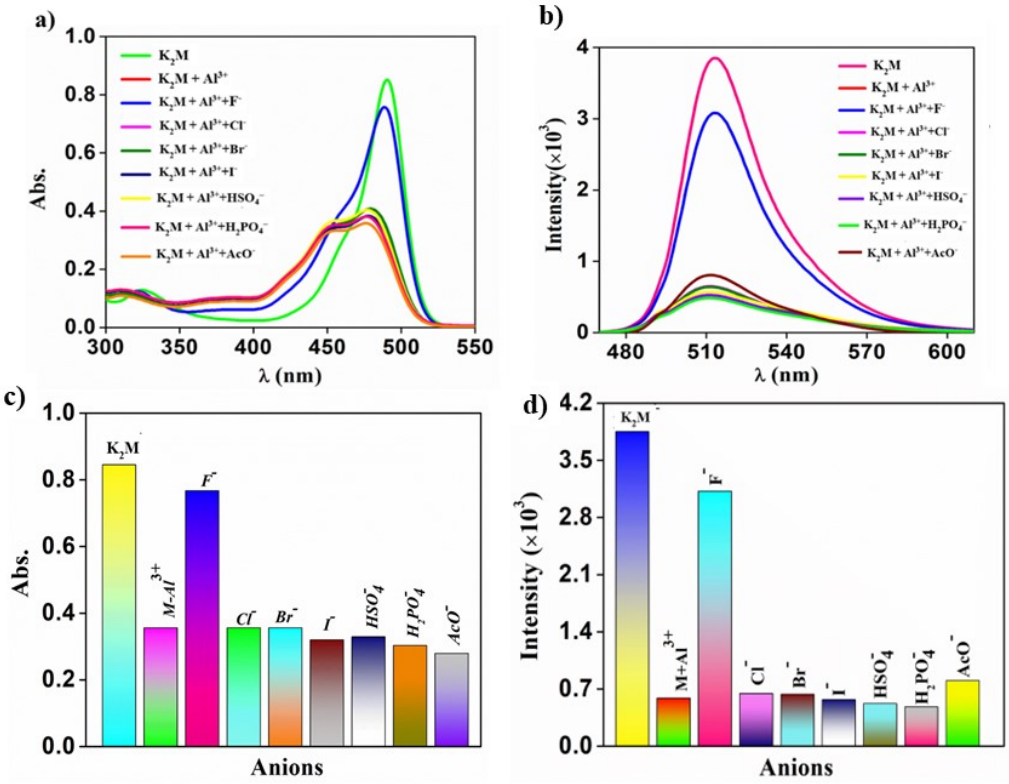


Figure S11: (a) UV-Vis spectra of probe **K_2M** (10 μM) in water upon addition of different anions (1 x 10^{-2} M)in presence of Al^{3+} (1 x 10^{-2} M); (b) Emission spectra of probe **K_2M** (2.5 μM) in water upon addition of different anions (1 x 10^{-3} M)in presence of Al^{3+} (1 x 10^{-3} M); (c) Bar representation of the change

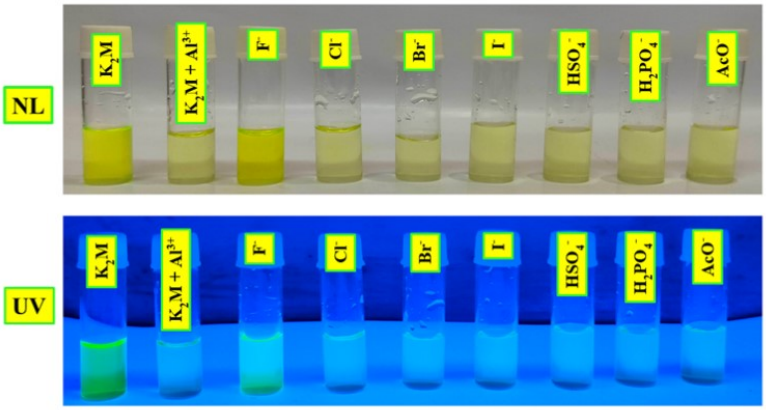


Figure S12: Images of colorimetric and fluorometric changes of **K_2M** (10 μM) in water solution upon addition of different anions (1 x 10^{-2} M)in presence of Al^{3+} (1 x 10^{-2} M).

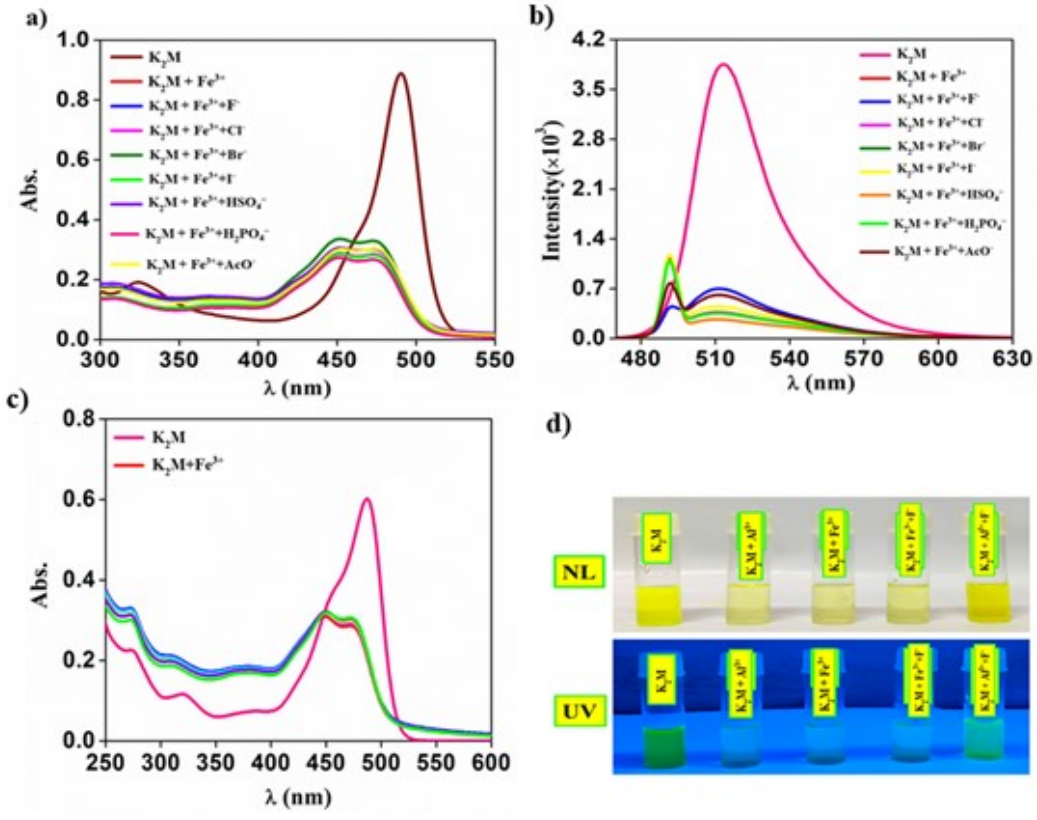


Figure S13: (a) UV-Vis absorption spectra of **K_2M**-Fe^{3+} solution upon addition of different anions in water medium; (b) Emission spectra of **K_2M**-Fe^{3+} upon addition of different anions in water medium; (c) UV-Vis absorption spectra of **K_2M**-Fe^{3+} solution upon gradual addition of F^- in water medium; (d) Images of colorimetric and fluorometric changes.

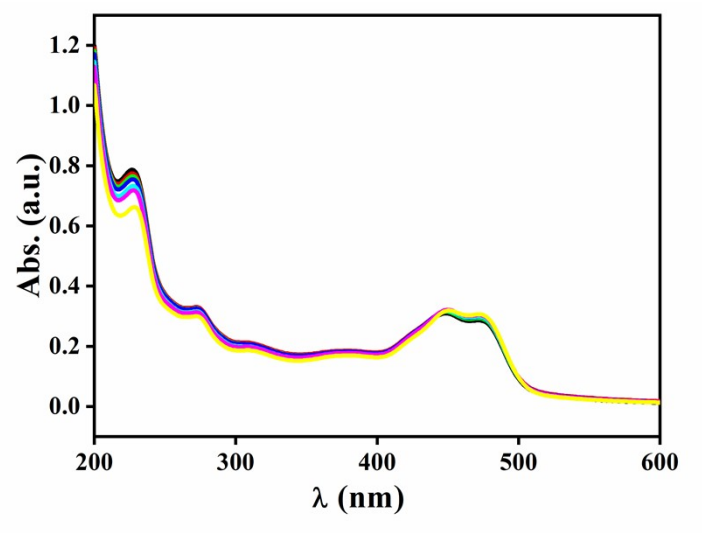


Figure S14: (a) UV-Vis absorption spectra of $\mathbf{K_2M}$-Fe^{3+} solution upon addition of different equivalents of NaF in water medium.

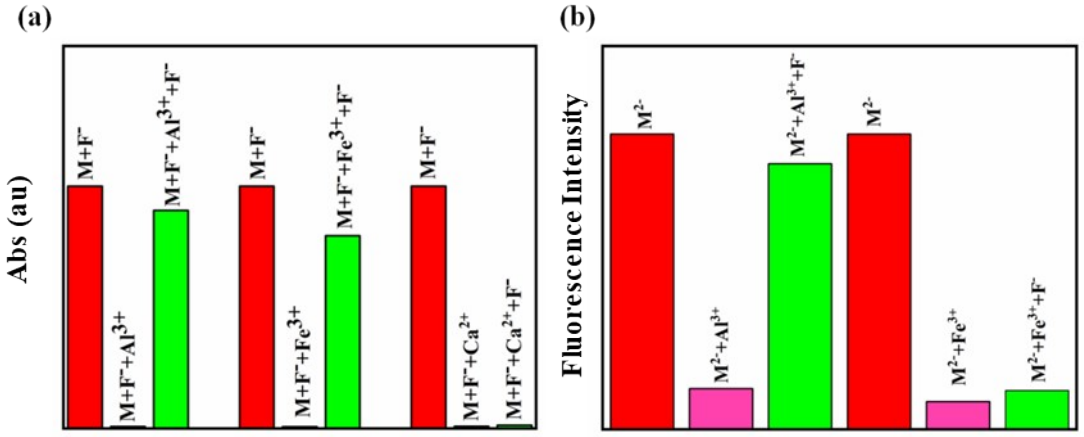


Figure S15: Fluoride sensing affinity of different metal complexes generated (a) insitu generated (**M** + F^-) in DMSO-H_2O medium; (b) $\mathbf{M^{2-}}$ in H_2O medium.

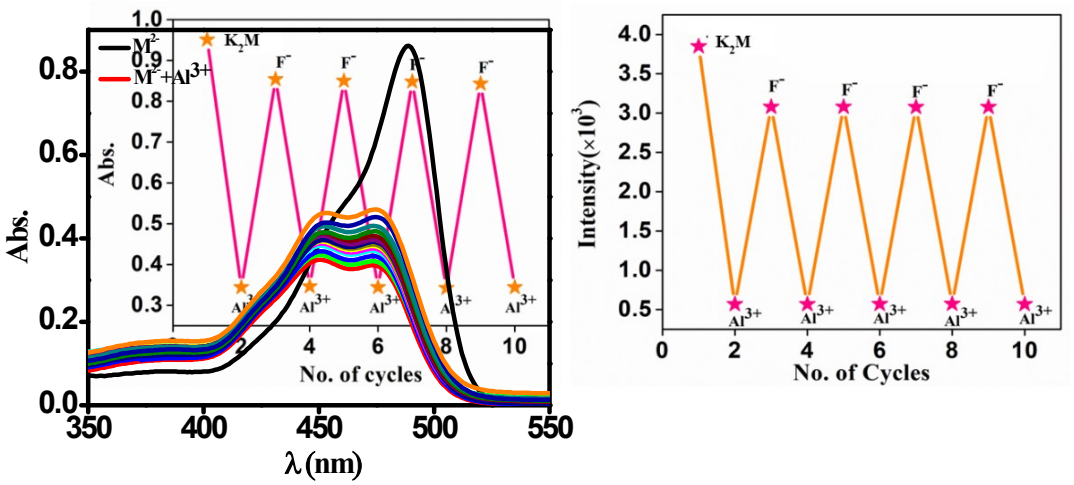


Figure S16: Change in the UV-Vis absorption spectra of $\mathbf{K_2M}$-Al^{3+} solution upon addition of F^- (upto 10 ppm) in water medium; (Note: A slight bathochromic shift of the peak is observed above addition of 10 ppm of F^-).

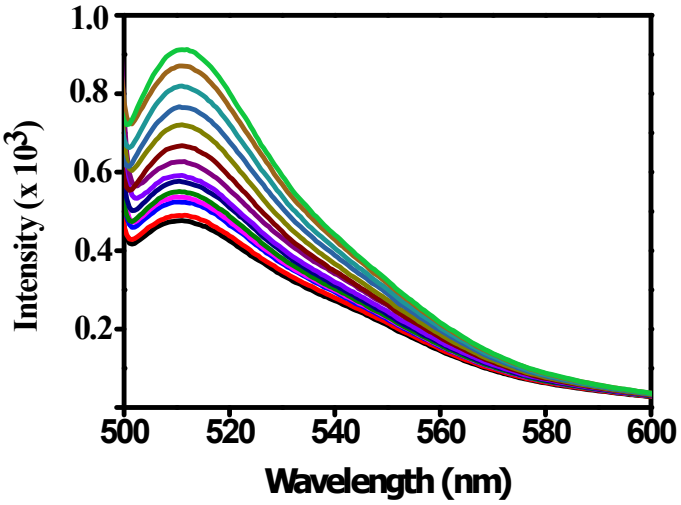
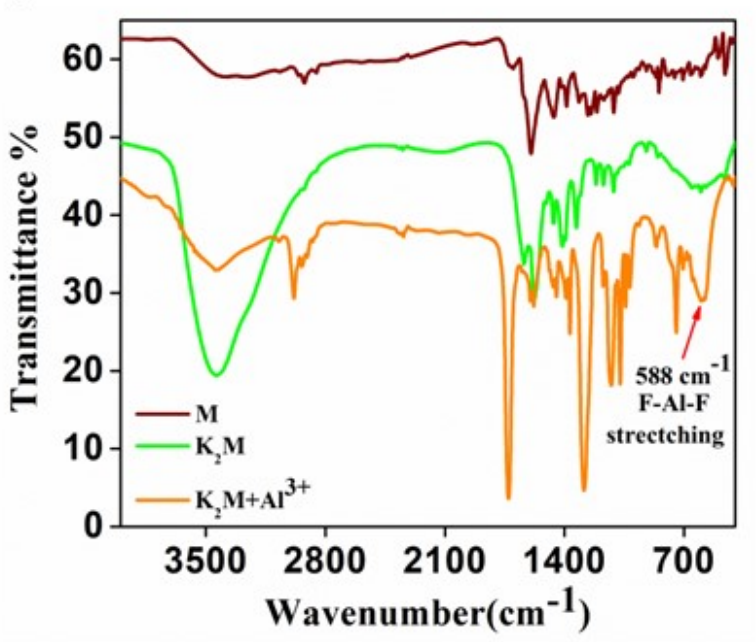
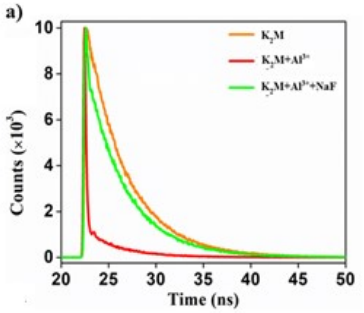


Figure S17: (a) Change in the emission spectra of $\mathbf{K_2M}$-Al^{3+} aqueous solution upon gradual addition of F^- (upto 10 ppm) in water medium.

Figure S18: Recyclability of the probe studied by (a) absorbance data at 520 nm, and (b)



emission data at 540 nm.



b)

Observed properties	K_2M	K_2M+Al^{3+}	$K_2M+Al^{3+}+F^-$
Quantum Yield (%)	92	13	90
Average life time (ns)	26.71	22.81	25.93
Radiative recombination rate, $K_r(s^{-1})$	0.176×10^{11}	0.024×10^{11}	0.172×10^{11}
Non-radiative recombination rate, $K_{nr}(s^{-1})$	0.015×10^{11}	0.166×10^{11}	0.019×10^{11}

Figure S19: (a) Time resolved photoluminescence spectra of **K_2M** in presence of Al^{3+} and F^-; (b) characteristic photophysical parameters.

Figure S20: FTIR spectra of **M, K_2M** and **K_2M** in presence of Al^{3+} and F^-.

Method	**Slope of the calibration plot**	**Intercept of the calibration plot**	**LOD ($= \frac{3S_E}{m}$)**

UV-Visible	0.0119 (SE:0.0058)	0.013 (SE:0.0026)	0.72 ppm
Fluorescence	0.088 (SE: 0.0021)	0.0176 (SE: 0.0112)	0.04 ppm

Table S2: Calculation of LOD

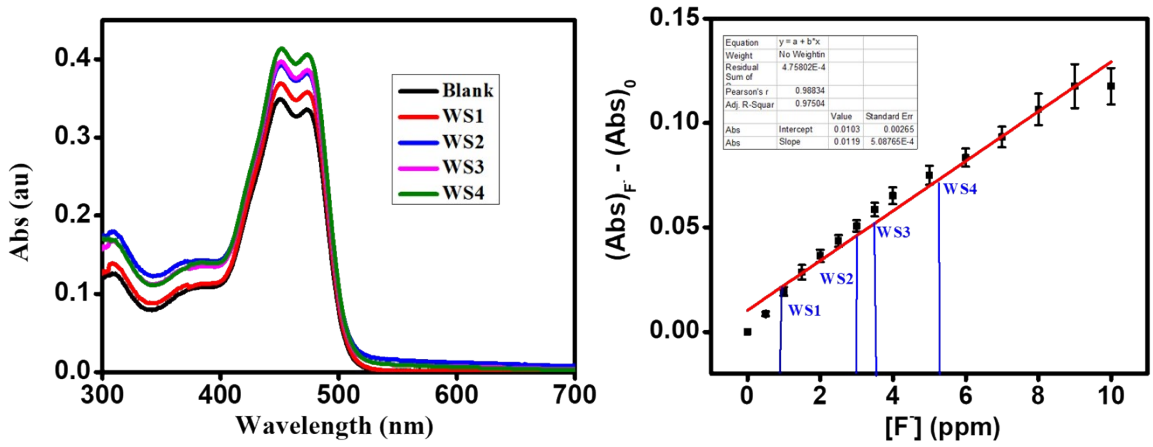


Figure S21: (a) UV-Vis spectra of **K_2M (**3 mL of 10 μM in water**)** and Al^{3+} (80 μL of 0.01 M in water) mixture upon addition of the 50 μL of the fluoridated water samples; (b) the concentration of fluoride samples w.r.t. the calibration plot. The absorbance at 490 nm is correlated for quantification.

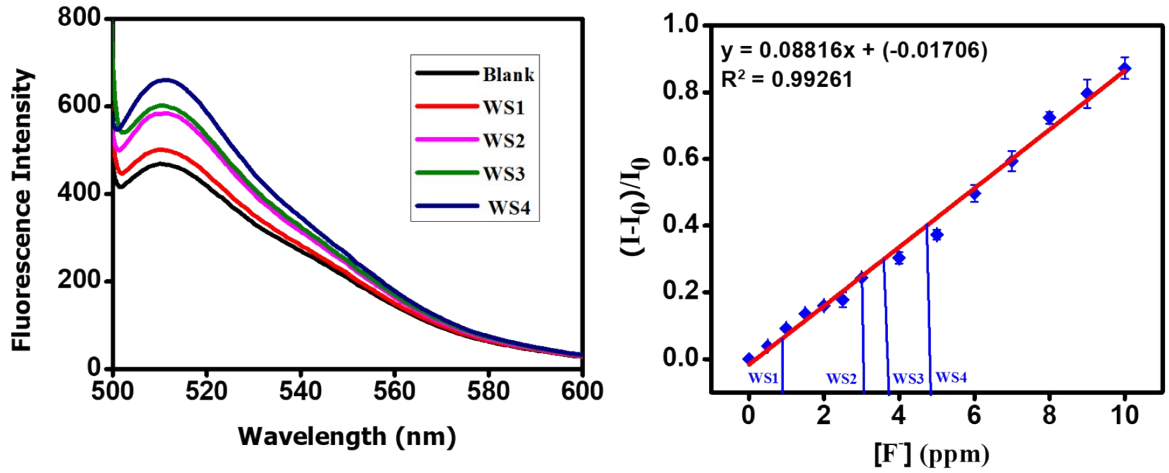


Figure S22: (a) Fluorescence spectra of **K_2M (**3 mL of 2.5 μM in water**)** and Al^{3+} (80 μL of 0.001 M in water) mixture upon addition of the 50 μL of the fluoridated water samples; (b) the concentration of fluoride samples w.r.t. the calibration plot. The emission intensity at 510 nm is correlated for quantification.

Samples	Concentration of fluoride in ppm in water		
	UV-Visible spectroscopy	**Fluorescence spectroscopy**	**Fluoride Selective Electrode**
WS1	1.02 ± 0.071 (RSD: 6.96 %)	0.90 ± 0.084 (RSD: 9.3 %)	
WS2	2.92 ± 0.314 (RSD: 10.7 %)	3.03 ± 0.147 (RSD: 4.85 %)	
WS3	3.71 ± 0.13 (RSD: 3.5 %)	3.50 ± 0.044 (RSD: 12.5 %)	
WS4	5.07 ± 0.16 (RSD: 6.6 %)	4.63 ± 0.158 (RSD: 3.4 %)	

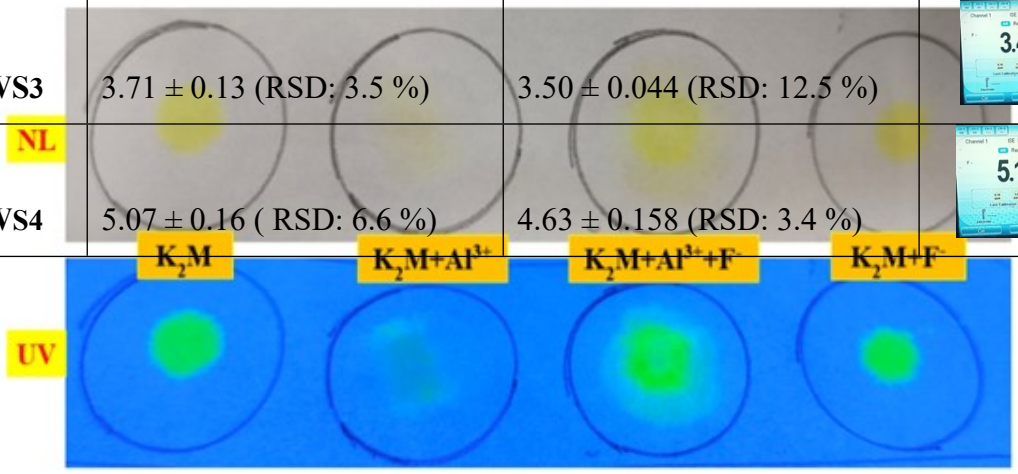


Table S3: Concentration of fluoride in the water sample as per the different mode of measurement.

Figure S23: Validation of the methodology in cellulose paper strip.

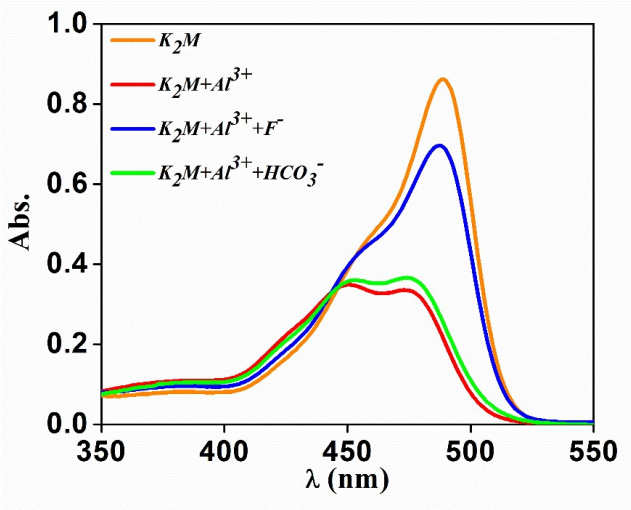


Figure S24: Change in the absorbance of the **K_2M+Al^{3+}** aqueous solution upon addition of F^- and HCO_3^- ion.

Table S4: Comparison of the performance of the proposed methods with some of the reported methods

S. No.	**Article title**	**F^- Salt used**	**Solvent used for study**	**LOD**	**Reference**
1	Spectroscopic Determination of Fluoride Using Eriochrome BlackT (EBT) as a Spectrophotometric Reagent from Groundwater	NaF	Water	0.19 ppm	A. G. Mariam, A. Diro, T. G. Asere and D. Jado, F. Melak, *International Journal of Analytical Chemistry*, *2021*(1), 2045491.
2	Ultrasensitive colorimetric detection of fluoride and arsenate in water and mammalian cells using recyclable metal oxacalixarene probe: a lateral flow assay	NaF	Water	21 ppb	S. Dey, A. Kumar, P. K. Mondal, D. Chopra, R. Roy, R., S. Jindani and V. K. Jain. *Scientific Reports*, 2022, *12*(1), 17119.
3	Highly Sensitive Optical	NaF	Water	0.05	A. Chatterjee, N. Pan, T. K.

	Sensor for Selective Detection of Fluoride Level in Drinking Water: Methodology to Fabrication of Prototype Device			ppm	Maji, S. S. Pasha, S. Singh, S. A. Ahmed, J. T. Al-Thakafy and S. K. Pal *ACS Sustainable Chemistry & Engineering* 9, no. 20 (2021): 7160-7170.
4	A reversible switch as highly selective sequential chemosensor for Al^{3+} cation followed by F^- anion	NaF	Water	1.6 µM	Y. Mi, Z. Cao, Y. Chen, S. Long, Q. Xie, D. Liang and J. Xiang, *Sensors and Actuators B: Chemical* 2014, 192,164-172.
5	Near-Infrared Fluoride Sensing Nano-Optodes and Distance-Based Hydrogels Containing Aluminum-Phthalocyanine	TBAF	THF (gel system at different pH)	0.1µM	L. Wang, Y. Zhang, L. Wang, Y. Cheng, D. Yuan, J. Zhai, and X. Xie, *ACS sensors*, **2023**, *8*(11), 4384-4390.
6	Fluoride ion detection in aqueous medium: Colorimetric and turn-off fluorescent Schiff base chemosensor	NaF	DMSO-Water	2.35×10^{-8} M	B. Devi, A. K. Guha and A. Devi, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2024, *305*, 123448.
7	Highly sensitive and selective fluoride detection in water through fluorophore release from a metal-organic framework	NaF	Water	15 ppb	F. M. Hinterholzinger, B. Rühle, S. Wuttke, K. Karaghiosoff and T. Bein *Scientific reports*, **2013**, *3*(1), 2562.
8	Highly Sensitive Ratiometric Fluorescent Paper Sensors for the Detection of Fluoride Ions	NaF	Water	73 nM	X. Wu, H. Wang, S. Yang, H. Tian, Y. Liu, Y. and B. Sun, *ACS omega*, 2019, *4*(3), 4918-4926.
9	Perylene tetracarboxylate dye-based colorimetric and fluorometric sensor for ppb-level fluoride detection in water	NaF	Water	1 ppb	S. P. Mahanta, B. C. Mushahary, D. Bora, C. Goswami and R. Das, *New Journal of Chemistry*, 2025,**49**, 5444-5450.
10	Selective fluoride ion sensing using novel quinoline chemosensor insights into kinetics and molecular logic gate	NaF	Water	5.52 nM	A. V. Ashwathi and S. M. Basheer, *Scientific Reports*, **2025**, *15*(1), 1859.

	functions				
11	Fluorescein Based Dual Optical Sensor for Highly Selective and Sensitive Fluoride Detection in 100 % Water Medium	NaF	Water	0.04 ppm	This Work

References:

1. D. B. Hibbert and P. Thordarson, The death of the Job plot, transparency, open science and online tools, uncertainty estimation methods and other developments in supramolecular chemistry data analysis. *ChemCommun*, 2016, ***52***(87), 12792-12805.