

A photoluminescence assay with a portable device for rapid, sensitive and selective detection of Europium and Terbium

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1. Materials and methods:

Sodium cholate, Terbium(III) nitrate pentahydrate, Europium(III) nitrate pentahydrate, Yttrium(III) nitrate hexahydrate, were purchased from Sigma-Aldrich. Biphenyl-4,4'-dicarboxylic acid (**1**), and Naphthalene-2,6-dicarboxylic acid (**2**) were purchased from Alfa-aesar. Whatman blotting filter paper was locally purchased. MiliQ water (18.2 MΩ.cm at 25 °C) was used for all the experiments. An ultrasonic bath (frequency: 33 kHz) was used for the preparation the of gel. Absorption spectra were recorded a on UV-3600 Shimadzu UV-Vis-NIR spectrometer. Time-delayed emission from gel samples was recorded on the Varian Cary Eclipse spectrometer in phosphorescence mode (delay time: 0.2 ms, gate time: 3.0 ms). A Varioskan® Flash Spectral Scanning Multimode Reader was used for the luminescence measurement of gel-coated paper discs. The emission intensities from the gel-coated paper discs were recorded using a plate reader instrument with a TRF delay time of 200 μs, and an integration time of 1000 μs. The error bars reflect the standard deviations of four sets of measurements. AFM images were recorded using the JPK NanoWizard II instrument.

2. Preparation of the metallo cholate gels gel-coated paper discs:

- (a) Sodium salt of biphenyl-4,4'-dicarboxylic acid (**1**) and naphthalene-2,6-dicarboxylic acid (**2**) as sensitizers were solubilized in aqueous sodium cholate, which acted as a sensitizer upon formation of the gel with metal ions. Sensitizer (**1** & **2**) doped MCh (M = Tb³⁺, Eu³⁺, Y³⁺) gel preparation: Metal ion (10 mM) solution and sodium cholate (NaCh, 30 mM) solution containing 400 μM of sensitizer were prepared. Metal ion (10 mM, 200 μL) solution was mixed with 400 μM sensitizer doped NaCh (30 mM, 200 μL) solution and the mixture was sonicated for 4-5 s to make a translucent gel.
- (b) MCh gel was prepared by mixing an equal volume of a trivalent metal cation and NaCh solution in a 1:3 molar ratio. Using a 3 mm hole puncher, circular-shaped western blotting filter paper (thickness: 0.83 mm) was cut and put on the 96 multiwell plates.

20 μL gel was drop cast uniformly on a paper disc and air-dried for 5 mins. After 5 min, the excess water of gel moiety was found to be absorbed by the filter paper. First, the gel-coated paper discs were checked under a UV lamp (365 nm) which showed green/red luminescence. Quantitative detection of lanthanides was done using sensitizer-doped YCh gel where the additive was lanthanide metal ions.

3. Optimization of sensitizer conc. for obtaining maximum lanthanide luminescence:

The concentration of **1** was optimized in both TbCh and EuCh gel to achieve maximum luminescence intensity (Fig S1). A 200 μM concentration of **1** was found to be the optimum concentration beyond which the Tb^{3+} and Eu^{3+} phosphorescence intensity drops down. The reason is not very clear but dimer/excimer formation or reabsorption of the organic chromophore at higher concentrations might be the reason for the luminescence decrease beyond 200 μM concentration.

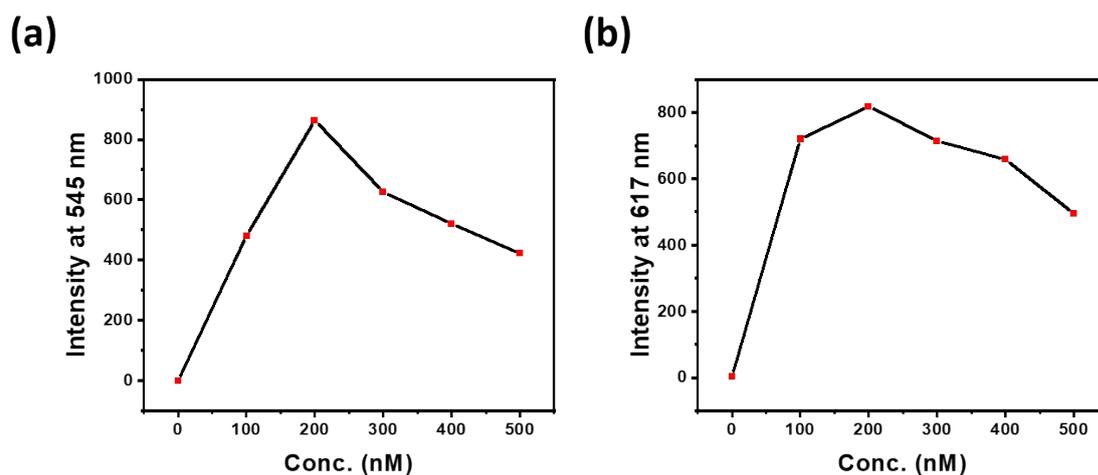


Fig. S1 Optimization of sensitizer (**1**) concentration for achieving highest luminescence in (a) (5/15 mM) TbCh and (b) (5/15 mM) EuCh hydrogel (λ_{ex} for **1** = 295 nm, λ_{em} for Tb^{3+} = 545 nm, and λ_{em} for Eu^{3+} = 617 nm).

4. Lifetime of sensitizer doped TbCh and EuCh gel:

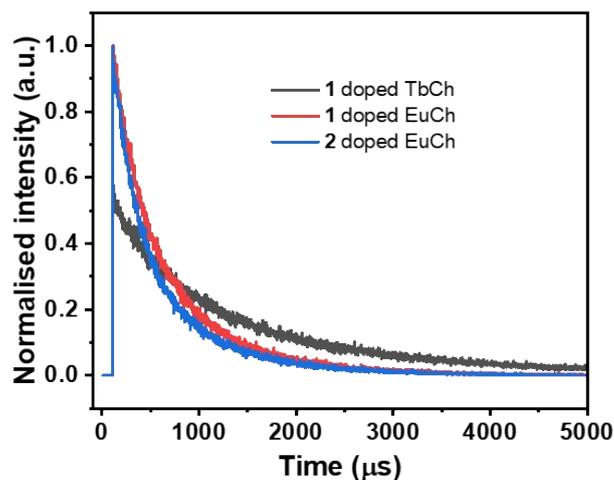


Fig. S2 Luminescence decay profile of sensitizers (200 μM) doped TbCh and EuCh gel (λ_{ex} for **1**= 295 nm, and λ_{ex} for **2**= 297 nm; λ_{em} for Tb^{3+} = 545 nm, and λ_{em} for Eu^{3+} = 617 nm).

Table S1 Lifetime values of sensitized TbCh and EuCh gels

Sample	Lifetime (μs) Contribution of each component	Average lifetime (μs)
1 doped TbCh gel	596 (22.5%) + 1874 (77.5%); $\chi^2 = 1.04$	1869
1 doped EuCh gel	305 (30.2%) + 840 (69.8%); $\chi^2 = 1.07$	767
2 doped EuCh gel	260 (33.3%) + 817 (66.7%); $\chi^2 = 1.02$	740

5. Quantum efficiency measurement:

Table S2 Quantum efficiencies of sensitized TbCh and EuCh gels

Sample	Quantum Efficiency (%)
1 doped TbCh gel	27.2
1 doped EuCh gel	9.5
2 doped EuCh gel	9.2

6. Optimization of metallochololate hydrogel matrix for lanthanide sensing:

The highest sensitivity was observed in lanthanum and yttrium chololate gels. The experiment is performed on a paper disc. 200 μM **1** doped metallochololate ($\text{M}=\text{Zn}^{2+}$, Ag^{1+} , Y^{3+} , Yb^{3+} , Nd^{3+} , La^{3+}) gels were prepared by adding 0.5 mM of lanthanides into it. 20 μL gel was drop cast on the paper discs and luminescence intensity was checked.

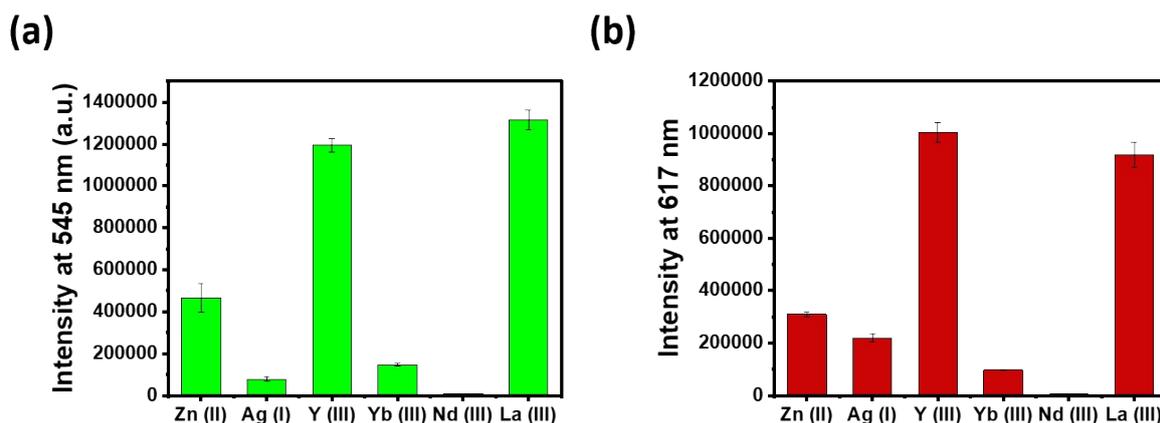


Fig. S3 Optimization of the several metallochololate gels [$\text{MCh} = (7.5/15 \text{ mM}) \text{ZnCh}$, $(15/15 \text{ mM}) \text{AgCh}$, $(5/15 \text{ mM}) \text{YCh}$, $(5/15 \text{ mM}) \text{YbCh}$, $(5/15 \text{ mM}) \text{NdCh}$ and $(5/15 \text{ mM}) \text{LaCh}$] as host matrix for (a) Tb^{3+} detection (500 μM) and, (b) Eu^{3+} detection (500 μM), where **1** (200 μM) was used as sensitizer (with λ_{ex} for **1** = 295 nm, and λ_{ex} for **2** = 297 nm; λ_{em} for Tb^{3+} = 545 nm, and λ_{em} for Eu^{3+} = 617 nm).

7. Lanthanide detection:

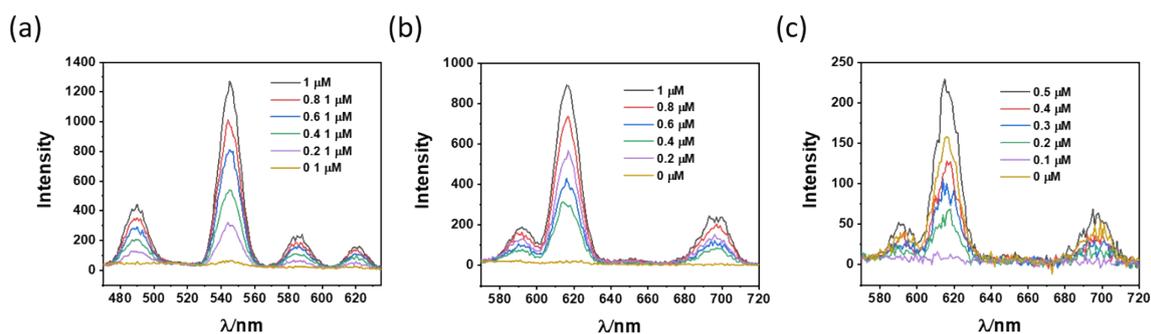


Fig. S4 Time-delayed emission spectra of gel coated paper discs, (a) 20 μL 200 μM **1** doped YCh gel for Tb^{3+} detection. (b) 20 μL 200 μM **1** doped YCh gel for Eu^{3+} detection. (c) 20 μL 200 μM **2** doped YCh gel for Eu^{3+} detection (with λ_{ex} for **1** = 295 nm, and λ_{ex} for **2** = 297 nm; λ_{em} for Tb^{3+} = 545 nm, and λ_{em} for Eu^{3+} = 617 nm).

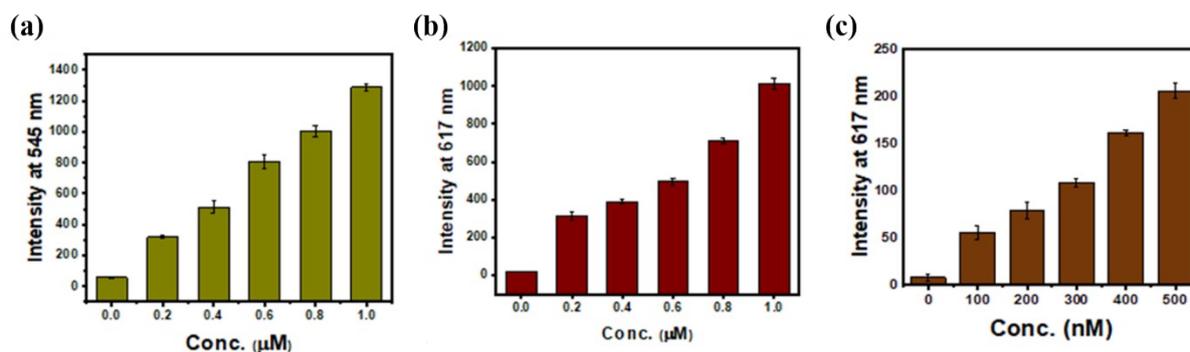


Fig. S5 Time-delayed emission intensity ($n = 4$) of gel-coated paper discs, (a) 20 μL 200 μM **1** doped YCh gel for Tb^{3+} detection. (b) 20 μL 200 μM **1** doped YCh gel for Eu^{3+} detection. (c) 20 μL 200 μM **2** doped YCh gel for Eu^{3+} detection (with λ_{ex} for **1** = 295 nm, and λ_{ex} for **2** = 297 nm; λ_{em} for Tb^{3+} = 545 nm, and λ_{em} for Eu^{3+} = 617 nm).

8. Selectivity study:

Table S3 The assay errors in the presence of each interfering metal (calculated from Fig. 4).

Metal	% Error
Fe(III)	8 (± 8)
Pr(III)	15 (± 14)
Al(III)	29 (± 7)
Sr(II)	22 (± 17)
Ni(II)	8 (± 13)
Cu(II)	10 (± 6)
Ag(I)	1 (± 3)
Co(III)	1 (± 29)
Mn(II)	11 (± 3)
K(I)	4 (± 9)
Ce(III)	0.2 (± 2)
Zn(II)	15 (± 2)
Cd(II)	13 (± 20)
La(III)	17 (± 10)

Selective sensing of Eu^{3+} from $\text{Tb}^{3+}/\text{Eu}^{3+}$ mixture

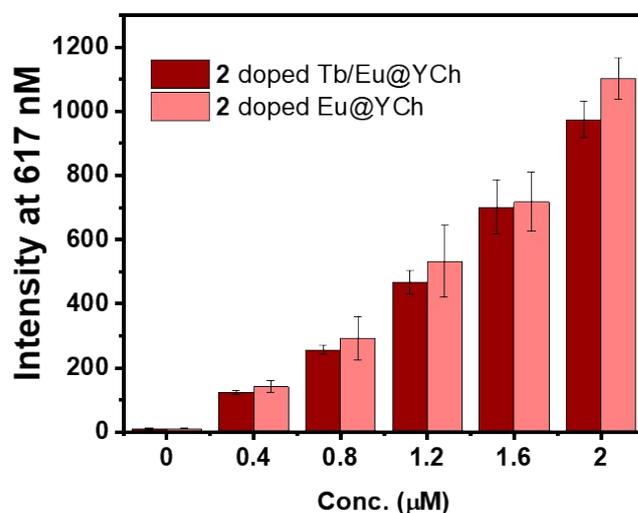


Fig. S6 Time-delayed emission intensity ($n = 4$) of paper discs for selective sensing of Eu^{3+} from $\text{Tb}^{3+}/\text{Eu}^{3+}$ mixture using **2** (200 μM) doped (5/15 mM) YCh gel, with $\lambda_{\text{em}} = 617$ nm and $\lambda_{\text{ex}} = 297$ nm.

Tb^{3+} to Eu^{3+} energy transfer has been reported in various systems such as coordination polymers, lanthanide-based polymers, supramolecular gels, lanthanide nanoparticles, etc.^{1,2} As **2** shows selective sensitization towards Eu^{3+} , so **2** doped YCh gel was preferentially used for the selective detection of Eu^{3+} from the $\text{Eu}^{3+}/\text{Tb}^{3+}$ mixture. Tb^{3+} and Eu^{3+} were mixed with a 1:1 ratio and 20 μL of $\text{Tb}^{3+}/\text{Eu}^{3+}$ mixture was added to the **2** doped YCh gel during gel formation. Europium luminescence was checked in the presence of terbium metal ions. No significant luminescence change was observed which implies the selective sensing of Eu^{3+} metal ion from the $\text{Tb}^{3+}/\text{Eu}^{3+}$ metal mixture.

9. Limit of detection (LOD) determination:

A calibration curve was constructed from the plot of time-delayed emission ($\lambda_{\text{em}} = 545$ & 617 nm, $\lambda_{\text{ex}} = 295$ nm for **1** and 297 for **2**) intensity, from gel-coated paper discs, versus the concentration of analyte (Fig. 2). The LOD was calculated using the following equation,

$$\text{LOD} = 3 * \text{S.D.} / m$$

Where S.D. is the standard deviation for the time-delayed fluorescent intensity of gel-coated paper discs without analyte, and m is the slope of the curve. For example, the equation of linear fit for the Tb^{3+} sensing is,

$$y = 1231x + 58 \quad (R^2 = 0.999)$$

So,

$$\text{LOD} = 3 \times 2.7 / 1231 = 0.00658 \mu\text{M} = 6.6 \text{ nM}$$

10. Sensitization of Tb^{3+} and Eu^{3+} with sensitizers **1** and **2**:

The sensitization of lanthanide luminescence is possible through energy transfer from the triplet excited state of the organic chromophore to the Ln excited state.^{3,4} Energy flow takes place from organic S_1 to organic T_1 to Ln^* . The organic chromophore's singlet and triplet states may both transfer energy to the metal ion, and this transfer can be phonon-assisted. However, since the singlet state is short-lived, this process is often not efficient. The lowest triplet state energy of the organic chromophores was examined from the reported literature better to understand the process of sensitization of lanthanide luminescence.⁵ The lowest triplet state energies of the chromophores were evaluated and presented in Fig. S7 and Fig. S8.

The lowest excited triplet state of **1** can transfer its energy to both the excited state of terbium and europium, however, the lowest excited triplet energy of **2** is smaller than the excited state of terbium, therefore it cannot transfer its energy to terbium. As a result, **2** is a selective sensitizer for europium.

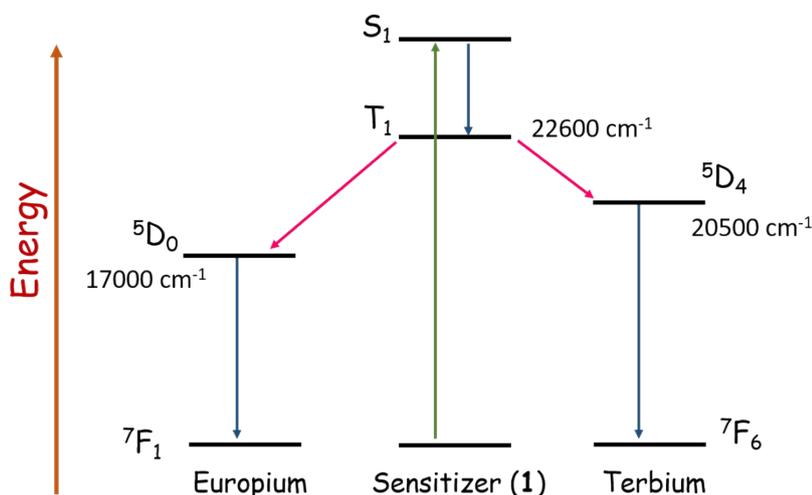


Fig. S7 Schematic representation of the energy transfer process from the lowest triplet excited state of the sensitizer (**1**) to the resonance level of Tb^{3+} and Eu^{3+} ions.

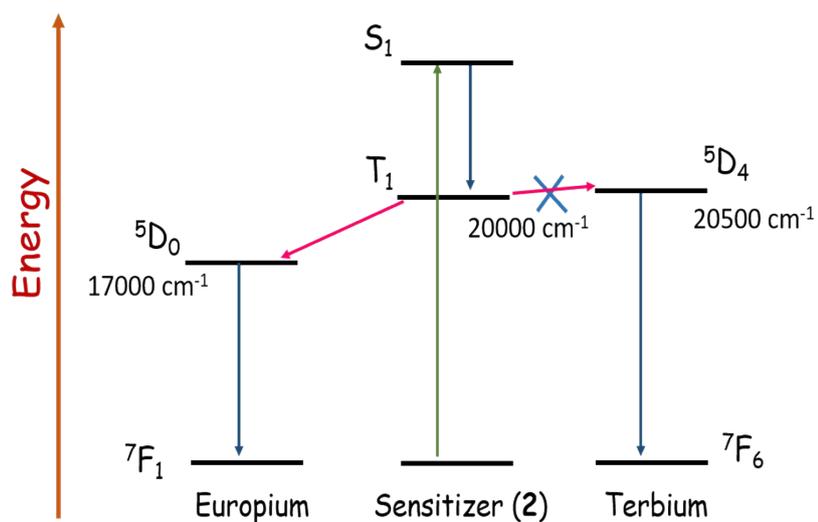


Fig. S8 Schematic representation of the energy transfer process from the lowest triplet excited state of the sensitizer (2) to the resonance level of Tb^{3+} and Eu^{3+} ions

11. Sensing on a paper-based platform

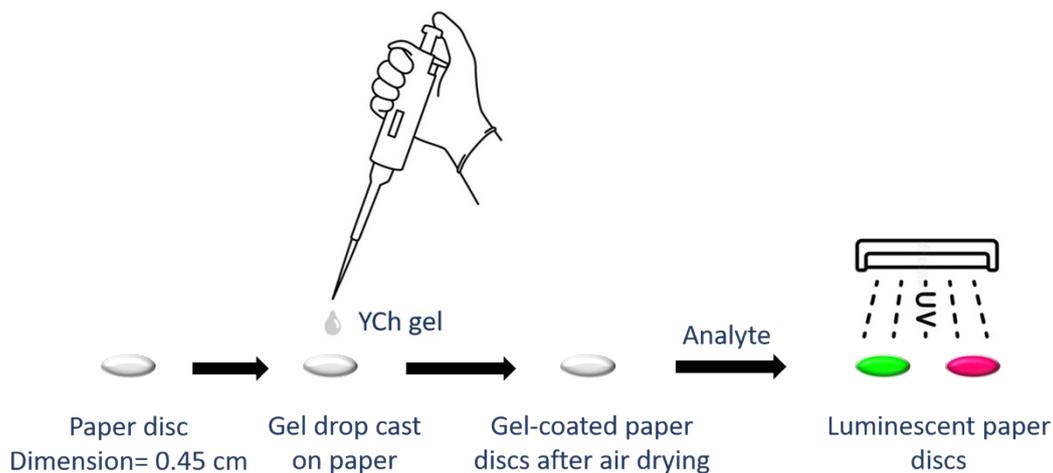


Fig. S9 Schematic representation of gel drop casting on paper discs.

12. Specifications of the imaging device:



This is a portable device that has multiple ultraviolet excitation sources of wavelengths $\lambda = 275 \text{ nm}$, 310 nm , and 340 nm , respectively, that excite the samples, mounted through the sample loading interface, in sequence and measure the photoluminescence after a delay of 100 microseconds. Two emission filters with center frequencies at 545nm and 620 nm are incorporated into the device. The unfiltered and the filtered photoluminescence are captured by an onboard camera. The embedded SoC on the device subsequently processes these images through the application of appropriate thresholds. The device provides a touchscreen display user interface through which the user can carry out all the intended functions. Besides, the device is also equipped with an on-board rechargeable battery and power module.

12. References:

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