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Paper-based Sensing of Phytotoxicant Gossypol in Aqueous Media through Turn-on Visible-light Emitting Lanthanide-Luminescence

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Materials and methods

Terbium acetate (99.9% trace metals basis), sodium cholate (NaCh, from bovine and/or bovine bile, ≥99% purity), gossypol (from cotton seeds, ≥95% Purity, HPLC), linoleic acid (≥99% purity), (±)-α-Tocopherol (≥95.5% purity) were purchased from Sigma Aldrich. Palmitic acid and 12-hydroxy stearic acid were purchased from S.D. Fine-Chem Ltd. Whatman 3 paper and Western Blotting filter paper were purchased from GE Healthcare. Absorption spectra were recorded on a UV-3600 Shimadzu UV-Vis NIR spectrometer using 1 cm path-length quartz cuvettes. Time-delayed emission for the gel samples was recorded on a Varian Cary Eclipse spectrometer in phosphorescence mode (delay time 0.2 ms, gate time 5.0 ms) and Varioskan® Flash Spectral Scanning Multimode Reader in time-resolved fluorescence (TRF) mode (delay time 0.2 ms, integration time 1.0 ms, bandwidth 12 nm). Emission from the gel-coated paper discs was recorded in 96 well plate on Varioskan® Flash Spectral Scanning Multimode Reader in TRF mode (delay time 0.2 ms, integration time 1.0 ms, bandwidth 5 nm). The error bars reflect the standard deviations of five to six sets of measurements (n). AFM images (dried gel on a mica sheet) were recorded on a JPK Nano Wizard II instrument. Scanning electron microscopy (SEM) was done on a Zeiss Ultra 55 microscope to determine the thickness of the gel layer coated on the paper surface. POM images were recorded on an Olympus BX 51 polarizing optical microscope. HPLC analysis was performed using a Shimadzu HPLC System (SCL-10A VP), consisting of a binary pump and a diode-array detector (DAD) and equipped with a Gemini C18 column (5 μm, 250 × 4.6 mm). Millipore water (18.2 MΩ.cm at 25 °C) was used for all the studies. An ultrasonic bath sonicator (frequency: 33 kHz, 1.5 L) was used to prepare the gels. A TLC viewer UV lamp (365 nm) was used to observe the gel-coated paper discs.

Procedure for gel-based assay

Fresh stock solutions of Gossypol were prepared in CH_3CN and stored in a glass vial wrapped in Aluminium foil. Before every experiment, it was prepared fresh and instantly diluted with 30 mM Na-Ch solution and the experiments were performed immediately. These solutions (200 µL each) were taken in a test-tube (7.5 cm × 1.2 cm) to which 200 µL of 10 mM $Tb(OAc)_3$ was added. The as-prepared Tb-Ch gels were incubated at 25 °C for 15 min in an oil bath, and then, the photoluminescence of Tb^{3+} was measured.

Paper-based sensing procedure

TbCh hydrogel (5/15 mM) was prepared by mixing 200 µL of 30 mM Na-Ch solution with 200 µL of $Tb(OAc)_3$ (10 mM) in a test tube (7.5 cm × 1.2 cm) and sonicating in a bath sonicator (1.5 L) at 5-10 °C for 5-6 s. After stabilizing the gel at RT for 10 min, it was sonicated again (5-6 s) to reduce its viscosity. This weak gel (20 µL) was drawn using a micropipette and drop casted on 3.5 mm diameter discs (discs were cut from Whatman 3 paper and Western blotting filter paper using a standard one-hole office punch). The paper absorbed the gel in 30 minutes and transferred it to a 96-well plate. Gossypol solution (10 µL, prepared in NaCh) was added on each disc, and emission measurements were made using a plate reader after 30 min of air drying.

Extraction of Gossypol from pure cold-pressed cotton seed oil:

A commercial pure cottonseed oil (*Gossypium spp*) (natural therapeutic grade cold pressed (Pure, Natural, Undiluted as claimed by the company) was purchased from the market. Gossypol in cottonseed oil was selectively separated by extracting 20 mL of the oil with 5 mL of hexane and 25 mL (N,N dimethyl formamide: water =2∶1, v/v) solvent mixture. Most triglycerides and some coloring materials went to the hexane layer, and the gossypol was partitioned to the bottom layer of the solvent mixture.

Time-dependent Tb^{3+} emission sensitization:

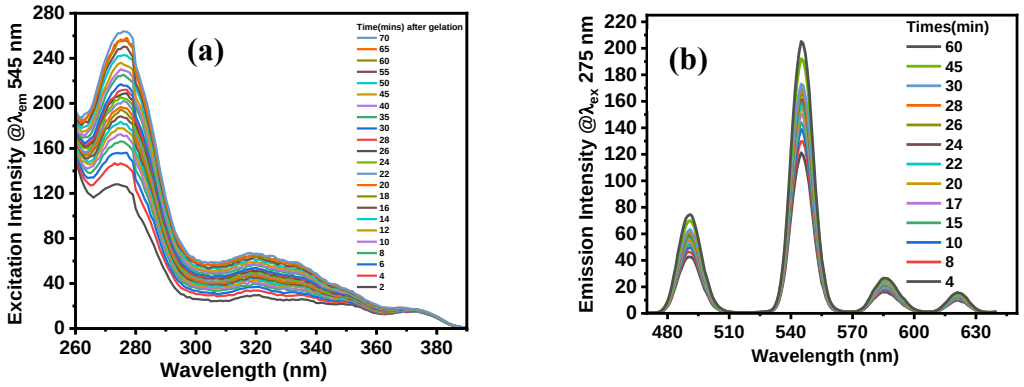

Figure S1 Tb^{3+} -luminescence (a) excitation spectra (λ_{em} 545 nm) (b) emission (λ_{ex} 275 nm) with increase of time doping 1 µM Gossypol in cholate hydrogel.

Importance of Gel Matrix:

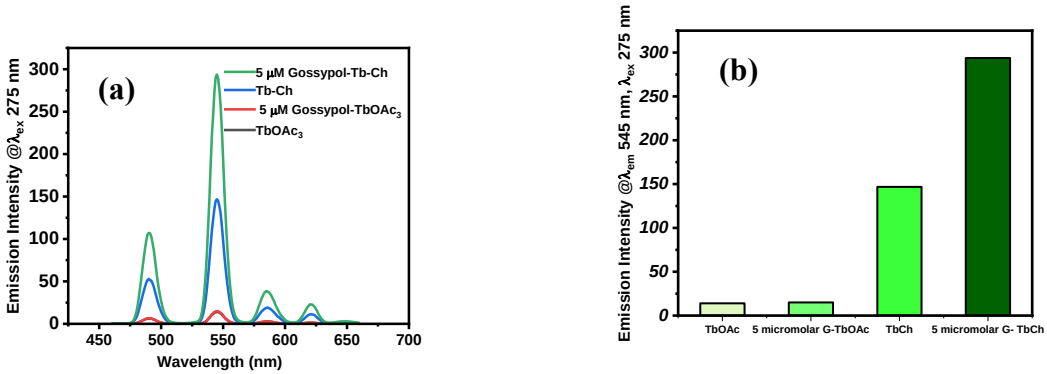

Figure S2: Tb^{3+} -luminescence emission spectra (λ_{ex} 275 nm) by Gossypol (5µM) in cholate hydrogel (a) spectra (b) bar-plot

Tb^{3+}-luminescence in cholate hydrogel with increasing concentration of Gsp:

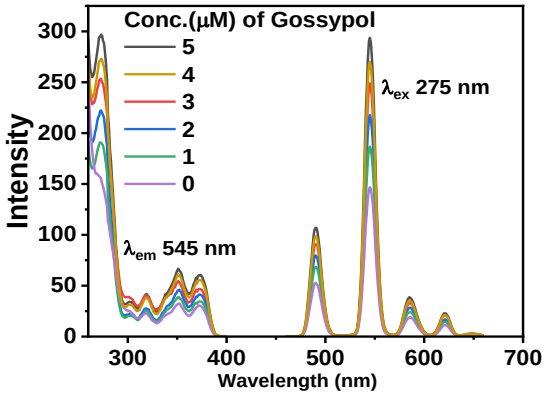

Figure S3: Excitation Spectra @ λ_{em} 545 nm and Emission Spectra @ λ_{ex} 275 nm Tb-Ch gel doped with different concentrations of Gossypol

Selectivity study:

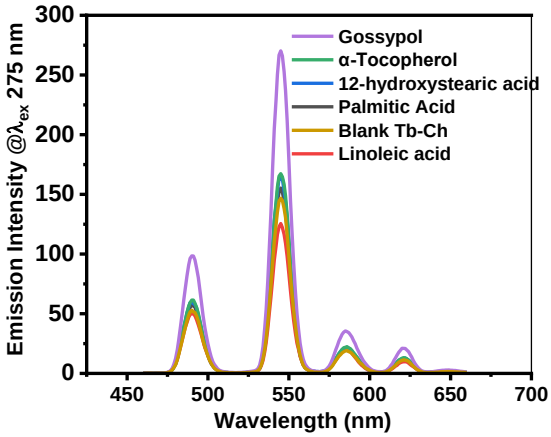

Figure S4: Emission Spectra with λ_{ex} 275 nm of Tb^{3+}-cholate gel doped with different components of cottonseed oil.

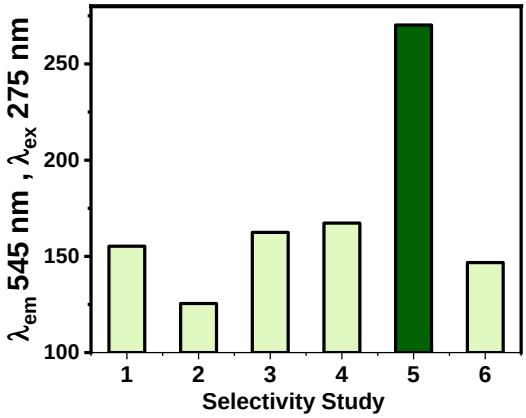

Figure S5: Intensity at λ_{em} 545 nm with λ_{ex} 275 nm of Tb^{3+}-cholate gel doped with different components of cottonseed oil. **1**: palmitic acid, **2**: α-tocopherol, **3**: linoleic acid, **4**: 12-hydroxystearic acid, **5**: Gossypol, **6**: Blank

Paper based Assay of Gossypol:

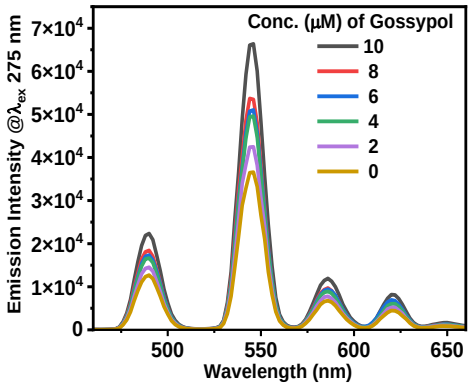

Figure S6 Emission spectra at λ_{ex} 275 nm (on Whatman #3 filter paper discs).

Sensing of Gossypol in cotton seed oil extract:

UV-Vis Spectra:

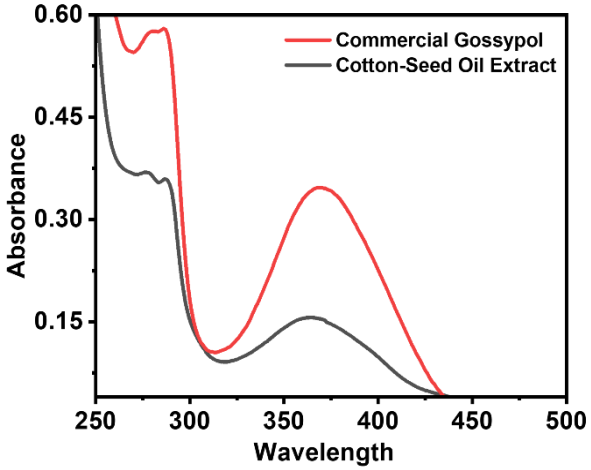

Figure S7: UV-Vis Spectra of Cotton Seed Extract and commercial Gossypol in Na-Ch solution

HPLC Chromatogram:

We carried out HPLC analysis of commercial Gossypol, an extract of cotton seed oil, and the same extract spiked with commercial Gossypol. The analysis was done on a C18 analytical column with 90 % MeOH-Water (0.5 % AcOH) as the mobile phase. The chromatogram shows that cottonseed oil extract contains Gossypol (evident from the peak having retention time 9.4 min, which gets enhanced with external spiking of Gossypol (**Figure S11)**.

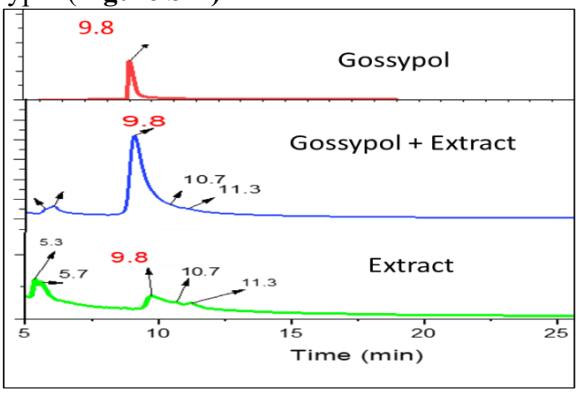

Figure S8: HPLC Studies of Gossypol, Cotton seed oil extract and Gossypol spiked cotton seed oil extract.

Detection of Gossypol in cotton seed oil extract:

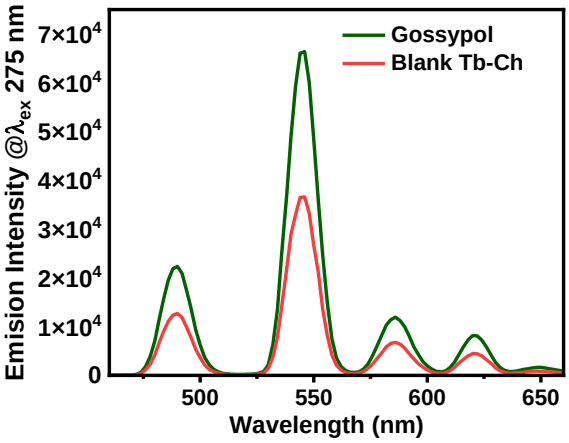

Figure S9: Emission Spectra @ λ_{ex} 275 nm paper discs coated with Tb-Ch gel in presence and in absence of cotton seed oil extract.

Microscopic Investigations:

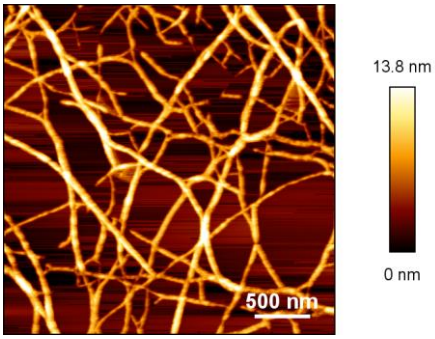

Figure S10: AFM Images of Tb^{3+} cholate gel,

Figure S11: POM images of the (a) uncoated, (b) gel coated paper.

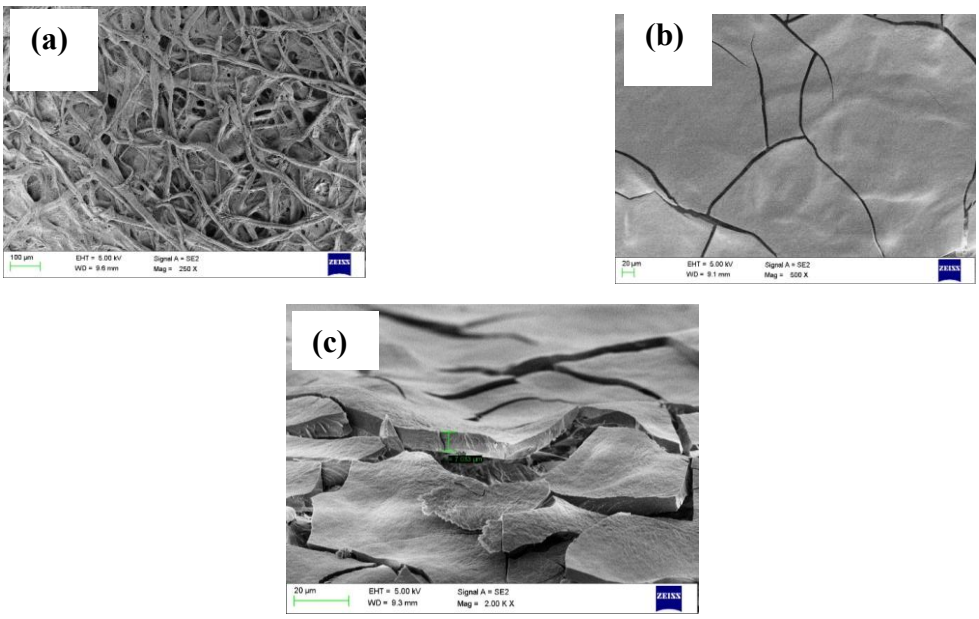

Figure S12: SEM images of the western blotting filter paper before (scale bar 100 µm) (a), and after gel coating (scale bar 20 µm) (b), Tilt-SEM for the thickness of the gel-layer coated on paper (scale bar 20 µm) (c).

Material Cost for Each Disc:

Table S1: Calculation of cost

Material	Cost for a Single Disc (INR)
Western Blotting Filter paper (38 mm^2)	0.03
Sodium cholate (0.267 mg)	0.017
Tb (III) acetate (0.067 mg)	0.140
Total	**0.187**

Interaction between gel and Gossypol:

The entrapment of hydrophobic Gossypol molecule in the hydrophobic gel pocket brings the donor gossypol and acceptor Tb^{3+} are brought in close proximity facilitating to subsequent energy transfer.

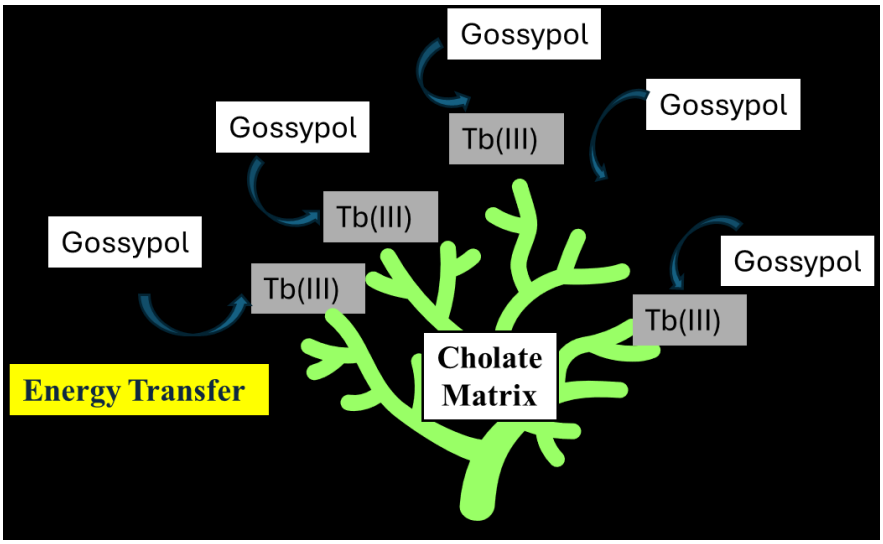

Figure S13: Interaction in Tb(III)-Cholate gel

Calculation of Sensitivity Constant of the assay:

K_{sv} (Stern-Volmer constant) values of luminescence "turn-off" sensor are estimated using the following equation:

I_0/I = Constant + K_{sv}[Gsp]
I represents luminescence intensity at a given Gsp concentration of [Gsp].
I_0 is the signal intensity in the absence of Gsp.

K_{sv} values of the lanthanide luminescence "turn-on" sensors in this work were calculated by fitting luminescence data using the following equation:

I/I_0 = Constant + K_{sv}[Gsp]

I represents luminescence intensity at a given Gsp concentration of [Gsp]. I_0 is the signal intensity in the absence of Gsp.

Ref. T. Y. Luo, P. Das, D. L. White, C. Liu, A. Star and N. L. Rosi, *J. Am. Chem. Soc.*, 2020, **142**, 2897–2904.

Version 2 of Illuminator:

Figure S14: Image of Version 2 of Illuminator