Electronic Supplementary Material (ESI) for RSC Advances.

Ratiometric rapid distinction of two structurally similar fluoroquinolone antibiotics by a Tb/Eu hydrogel

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Contents

1. **Materials**: Sodium cholate, terbium-acetate, europium-acetate, Norfloxacin (NFLX) and Ofloxacin (OFLX) were purchased from Sigma-Aldrich. Western blotting filter paper (thickness 0.83 mm) was purchased from Thermo Scientific. Black chart paper was locally purchased. Millipore water (18.2 MΩ.cm at 25 ºC) was used for all the studies. An ultrasonic bath sonicator (frequency: 33 kHz, 1.5 L) was used for the preparation of the gels. A TLC viewer UV lamp (365 nm) was used for observing the Tb^{3+}/Eu^{3+} luminescence on drug-doped, gel-coated paper discs.
2. **Spectroscopic methods:** Absorption spectra were recorded on a UV-3600 Shimadzu UV-Vis-NIR spectrometer. Time delayed emission from gel samples were recorded on a Varian Cary Eclipse spectrometer in phosphorescence mode (delay time: 0.2 ms, gate time: 3.0 ms). For qualitative assessment, luminescence measurements of the gel coated paper discs were done on a Varioscan® Flash Spectral Scanning Multimode Reader with TRF delay time of 200 µs and integration time of 1000 µs. 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.
3. **Ln^{3+}-Cholate Gel and Preparation of Coated Discs**: Tb^{3+} acetate (10 mM), Eu^{3+} acetate (10 mM) and sodium cholate (NaCh, 30 mM) solutions were prepared using freshly collected Millipore water. Stock solutions of NFLX (1 mM) and OFLX (3.2 mM) were prepared in DMSO/30 mM NaCh (2:98) and 30 mM NaCh, respectively. These solutions were diluted with appropriate volumes of 30 mM NaCh to prepare the final working concentrations. For the preparation of fluoroquinolone-doped Tb^{3+}-cholate gels, Tb^{3+}-acetate solution (200 µL, 10 mM) and antibiotic containing sodium cholate (Na-Ch, 30 mM) solution (200 µL) were mixed, sonicated for 12-15 s to make a transparent gel and then stabilized for 5 min. The stabilized transparent gel was sonicated for an additional 3-5 s to reduce its viscosity. Circular discs having 3.5 mm diameter were cut from Western blotting filter paper (0.83 mm thickness) with a hole-puncher. On each disc, 20 µL gel was drop casted by a micropipette, and dried for 5 min. Dropcasting the drug mixture solution over the previously coated (with mixed gel) paper disc was tried. (**Figure S20)** Each disc was coated with 20 µL of Tb/Eu/Cholate (4.5/.5/15 mM) gel, dried for 20 mins, and the 5 µL of (NFLX+OFLX) was dropcasted on dried discs, dried for 10 mins, TRF was measured.
4. **Preparation of milk samples for analysis**: Commercial toned milk or fresh (unpasteurized) cow's milk (50 µL) was diluted with Millipore water (50 µL) and then with 200 µL of 60 mM Na-Ch (containing a NFLX/OFLX). The sample (50 µL) was mixed with an equal volume Tb^{3+}-acetate (10 mM) solution, sonicated and used as above for further analysis.
5. **Preparation of blood samples for analysis:** Blood serum (50 µL) was diluted with an equal volume of Millipore water and then diluted with 200 µL 60 mM Na-Ch containing a fluoroquinolone. This sample (200 µL) was mixed with equal volume Tb^{3+}-acetate (10 mM) solution, sonicated and used as above for further analysis.

6. Photophysical characterization of the samples:

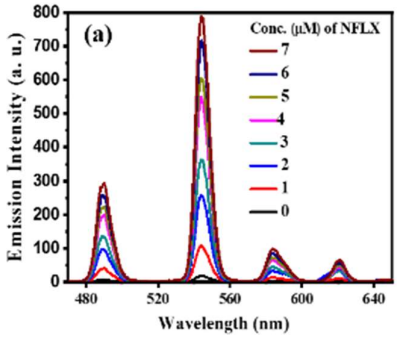


Figure S1 Tb^{3+} emission spectra of Tb^{3+}-cholate gels with increasing concentrations of NFLX with λ_{ex} 320 nm

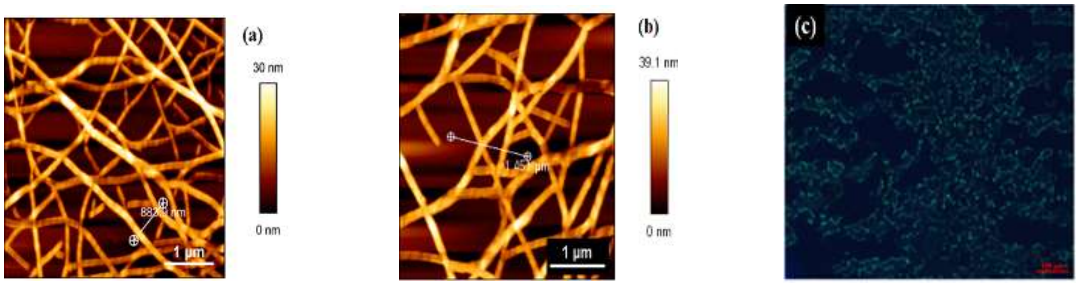


Figure S2 a) AFM image Tb^{3+}-Ch (5/15 mM) gel. b) AFM image of NFLX (5 μM) doped Tb^{3+}-Ch gel c) Fluorescence microscopic image of NFLX (5 μM) doped Tb^{3+}-Ch gel under 365 nm UV irradiation.

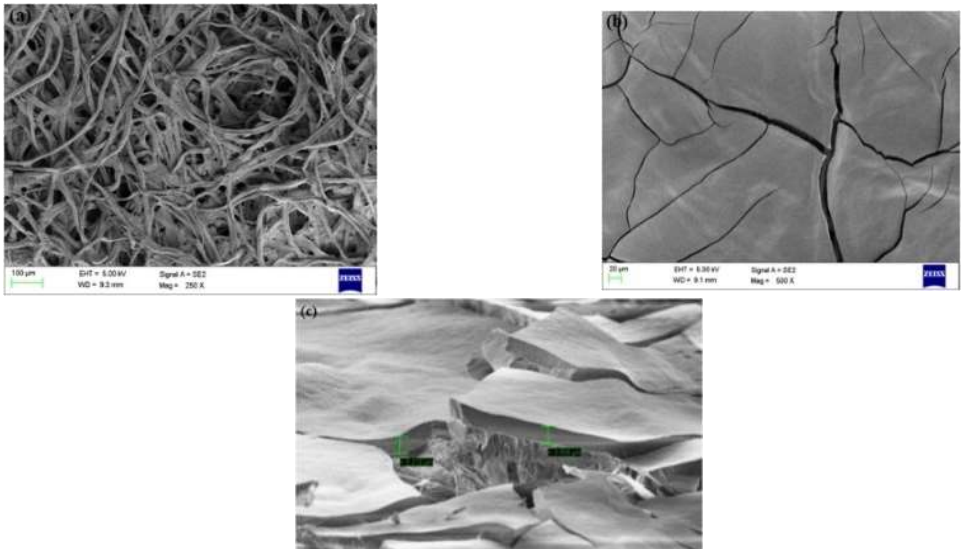


Figure S3 SEM images of (a) western blotting filter paper (scale bar 100 μm), (b) after gel coating (scale bar 20 μm)) and (c) thickness of the dried gel by tilt SEM.

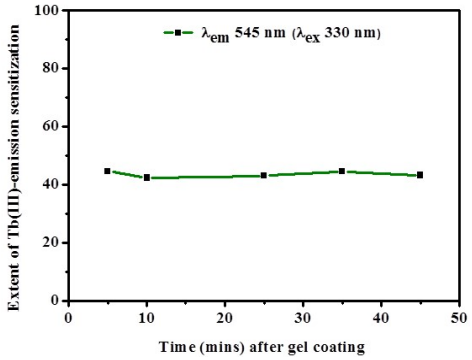


Figure S4 Variation of extent of sensitization of Tb^{3+} in cholate matrix by NFLX (4 μM) as a function of time (TRF has been recorded @λ_{ex} 330 nm)

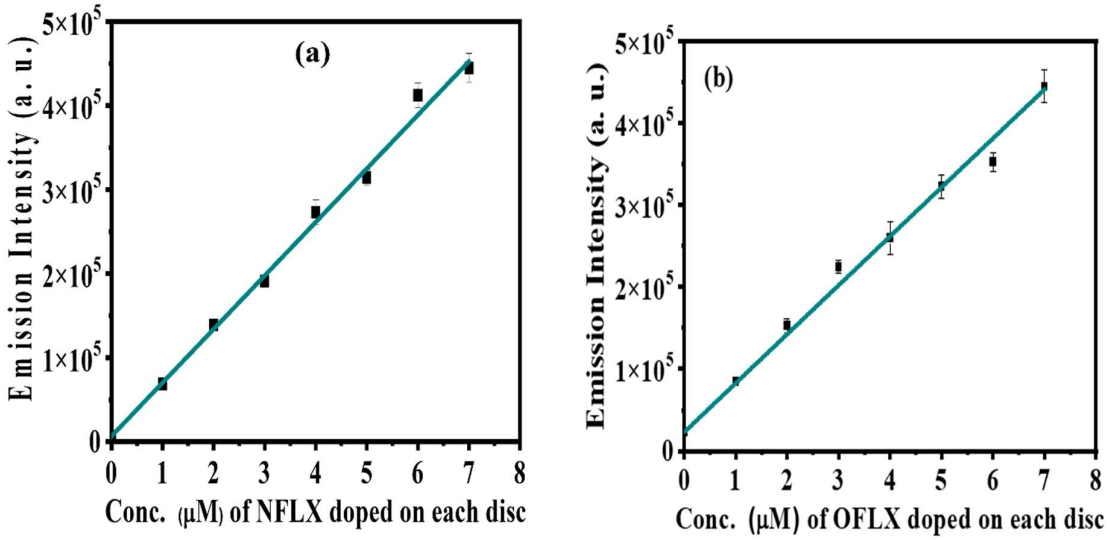


Figure S5 Linear LOD plot of Tb^{3+}-cholate gel coated discs with increasing concentrations of (a) OFLX with λ_{ex} 300 nm (b) NFLX with λ_{ex} 330 nm

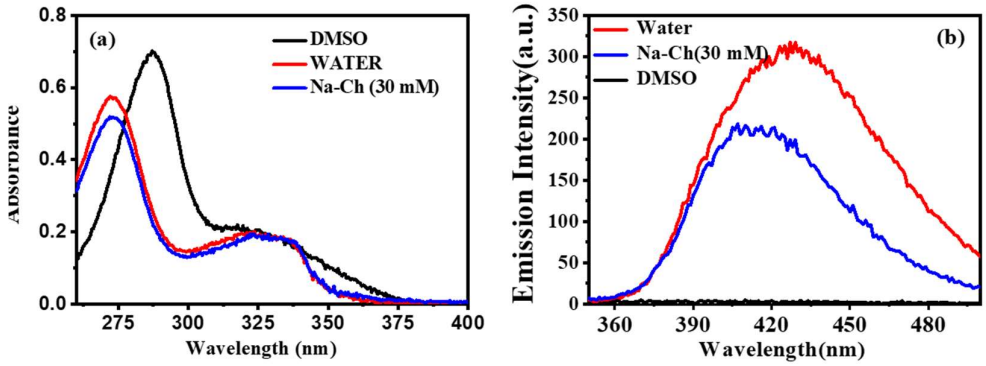


Figure S6 (a) Absorption spectra and, (b) Emission spectra (λ_{ex} 330 nm) of 16 µM NFLX in different solvents.

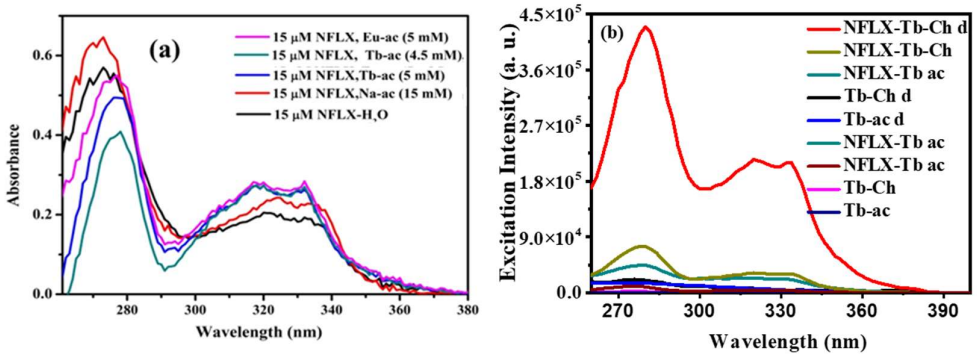


Figure S7 (a) Absorption spectra 15 µM NFLX in water, 15 mM Sodium acetate, 5 mM Terbium-acetate, 5 mM Europium acetate, Terbium/Europium (4.5/0.5 mM)-acetate solution (b) Tb^{3+} -excitation spectra (λ_{em} 545 nm) from NFLX (4 µM) doped solution/gel coated paper discs (Ac, Ch, D: acetate solution, cholate matrix and coated paper disc, respectively). Tb-Ac, Tb-Ac D, Tb-Ch, Tb-Ch D, NFLX-Tb Ac, NFLX -Tb Ac D, NFLX-Tb Ch, NFLX-Tb Ch D

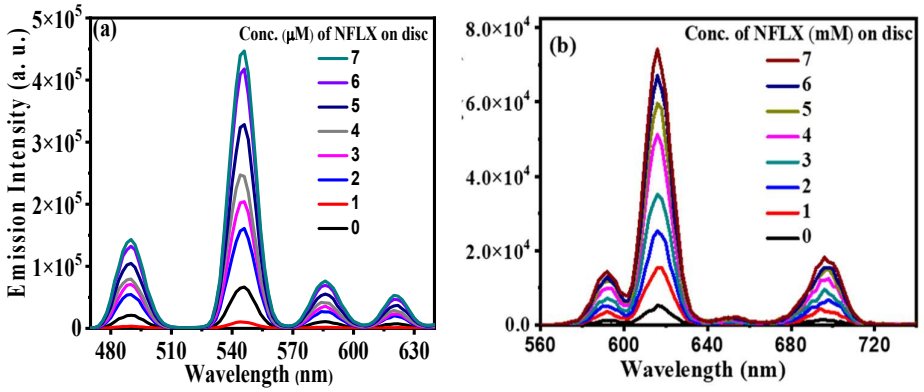


Figure S8 (a) Tb^{3+} emission spectra (b) Eu^{3+} emission spectra λ_{ex} 330 nm with increasing NFLX conc. on Tb^{3+} -Ch and Eu^{3+} -Ch gel coated disc respectively

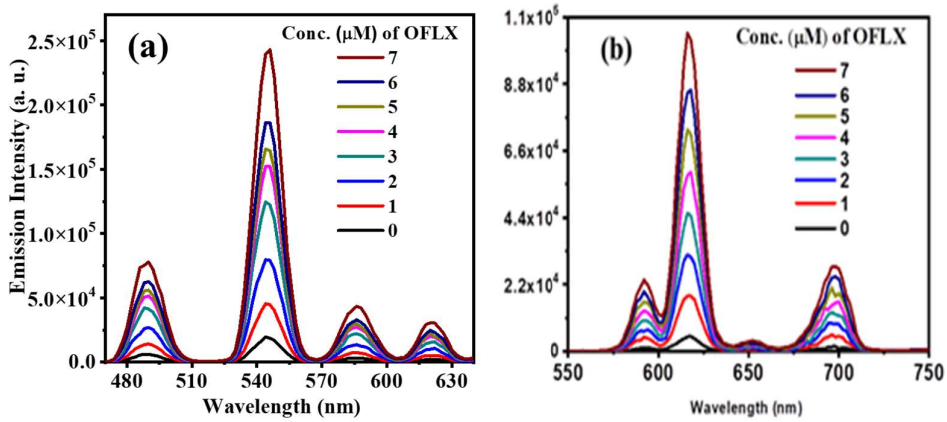


Figure S9 (a) Tb^{3+} emission spectra b) Eu^{3+} emission spectra λ_{ex} 330 nm with increasing OFLX conc. on Tb^{3+} -Ch gel and Eu^{3+} -Ch gel coated disc respectively.

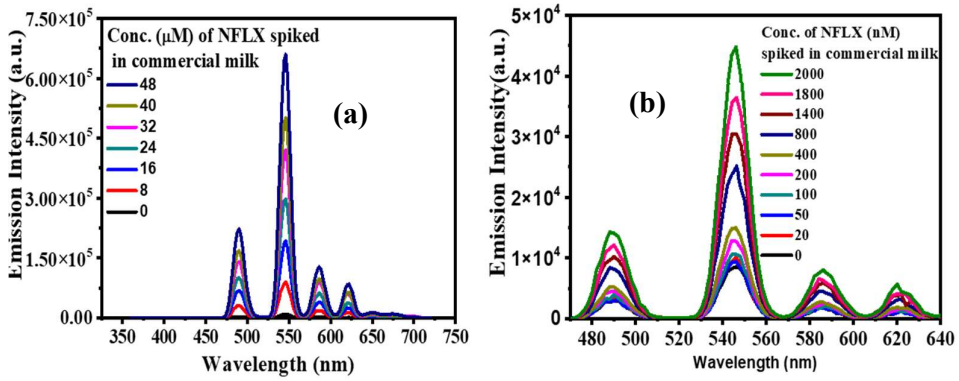


Figure S10: Emission spectra of Tb^{3+}-cholate gel coated discs doped with NFLX spiked commercial homogenized milk @λ_{ex} 330 nm (a) higher (b) lower concentration range

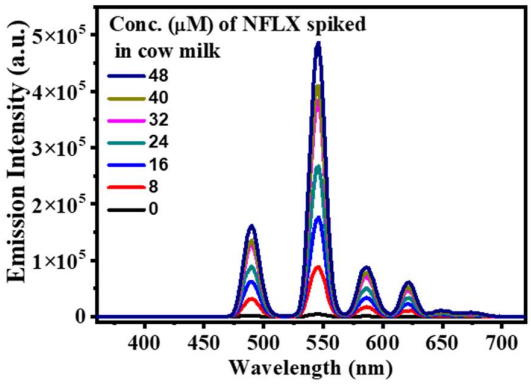


Figure S11 Emission spectra of Tb^{3+}-cholate gel coated discs with increasing concentrations of NFLX spiked raw cow milk (λ_{ex} 330 nm).

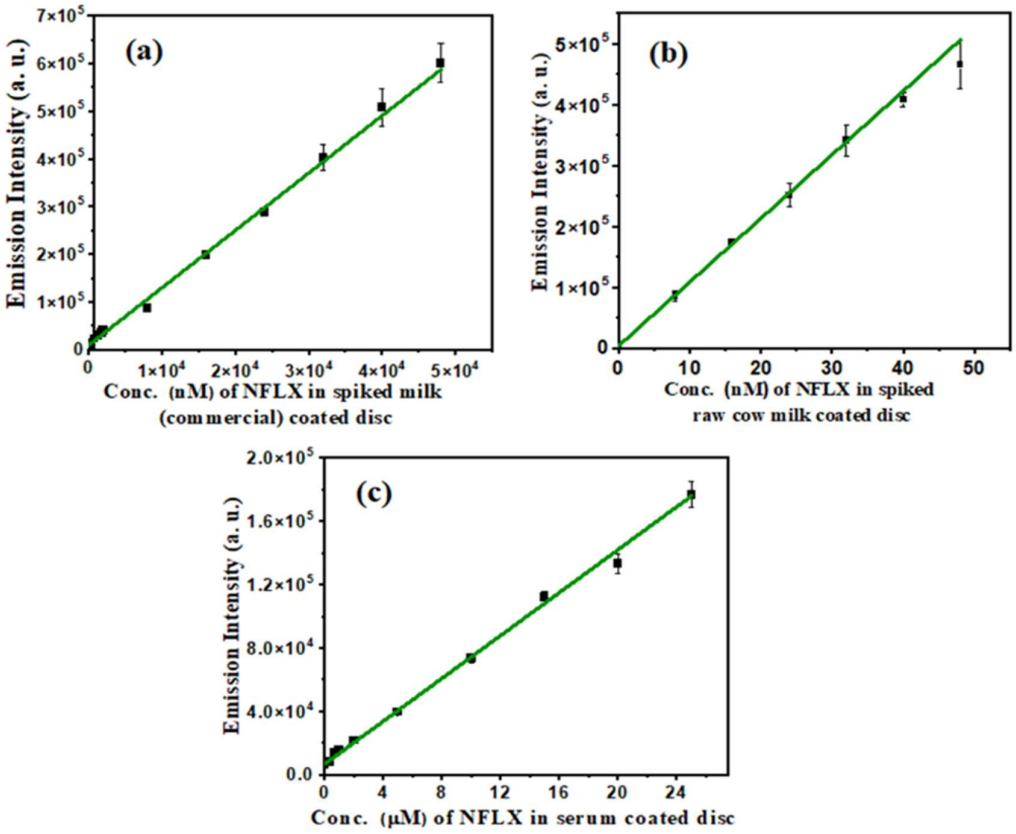


Figure S12 Tb^{3+} emission intensity at 545 nm (λ_{ex} 330 nm) in Tb^{3+} cholate gel coated discs with increasing concentrations of NFLX in (a) spiked commercial milk, (b) raw cow milk, (c) human blood serum.

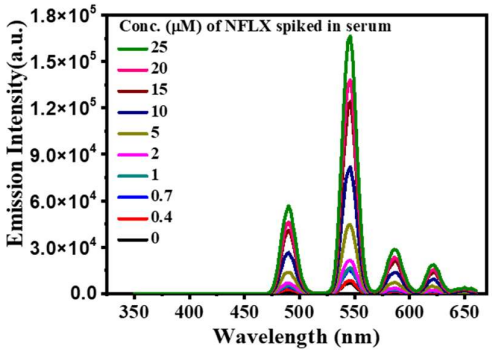


Figure S13 Emission spectra of Tb^{3+}-cholate gel coated discs with increasing concentrations of NFLX spiked human blood serum (λ_{ex} 330 nm).

7. Detailed investigation of the ratio-metric bis-lanthanide ensemble:

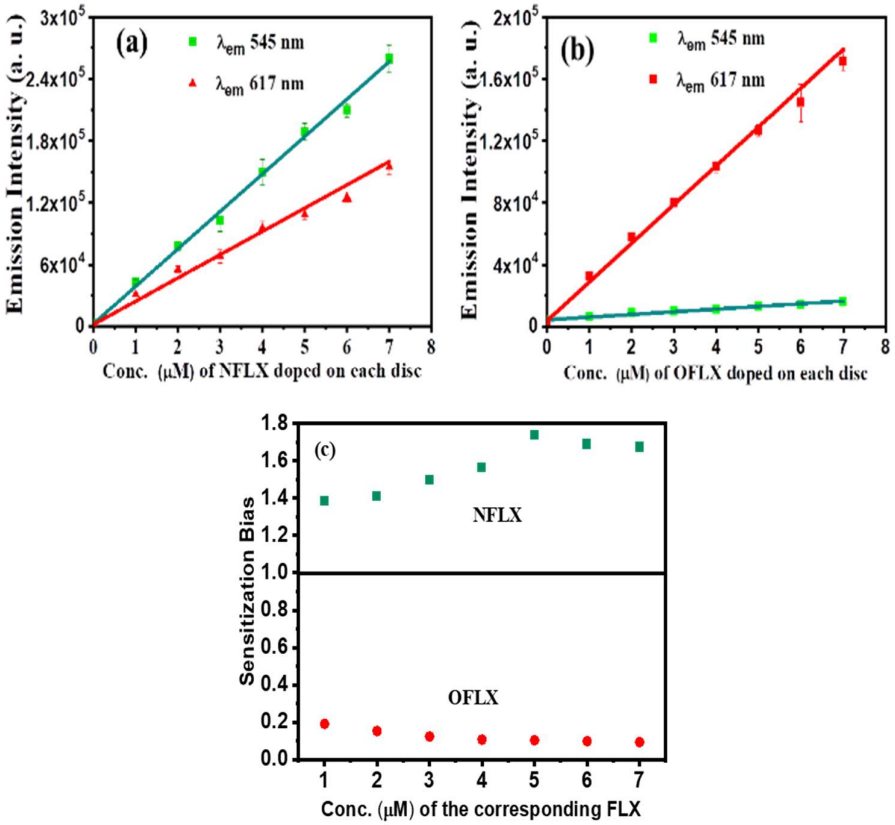


Figure 14 Variation of intensities of Tb^{3+} (545 nm) and Eu^{3+} (617 nm) emission ((λ_{ex} 330 nm) in Tb^{3+}/Eu^{3+}cholate (4.5 mM/ 0.5 mM/15 mM) gel coated discs with increasing concentrations of (a) NFLX and (b) OFLX, (c) sensitization bias of both the drugs.

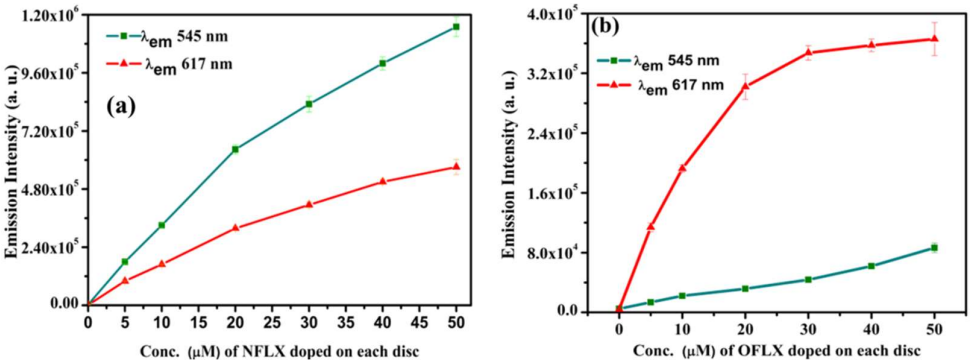
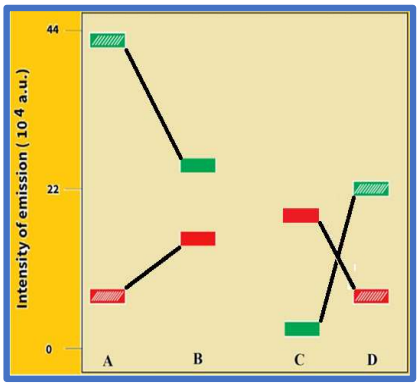


Figure S15 Increase in Tb^{3+} emission at 545 nm and Eu^{3+} emission at 617 nm (λ_{ex} 330 nm) in gel coated discs with increasing concentrations of (a) NFLX (b) OFLX. Gel composition: Tb^{3+}/Eu^{3+}/cholate (4.5 mM/ 0.5 mM/15 mM).



A	**NFLX (7 μM)**	**Tb^{3+} or Eu^{3+}-Ch (5 mM /15 mM)**
B	**NFLX (7 μM)**	**Tb^{3+}/Eu^{3+}/cholate (4.5 mM/ 0.5 mM /15 mM)**
C	**OFLX (7 μM)**	**Tb^{3+}/Eu^{3+}/cholate (4.5 mM/ 0.5 mM /15 mM)**
D	**OFLX (7 μM)**	**Tb^{3+} or Eu^{3+}-Ch (5 mM /15 mM)**

Figure S16 Green and Red boxes denote Tb^{3+} (λ_{em} 545 nm) & Eu^{3+} (λ_{em} 617 nm) emissions respectively, λ_{ex} 330 nm.

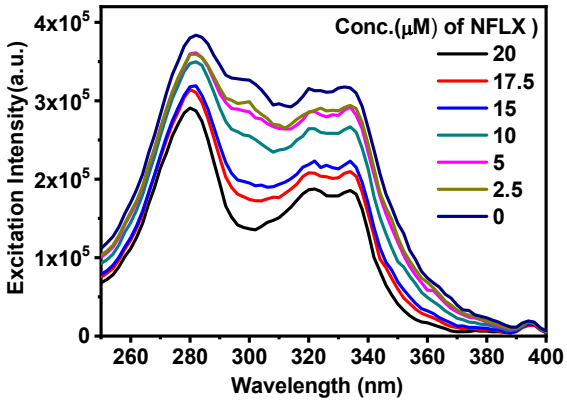


Figure S17 Excitation spectra of NFLX and OFLX mixture doped Eu^{3+}/cholate (5/15 mM) gel coated paper discs (λ_{em} 617 nm)

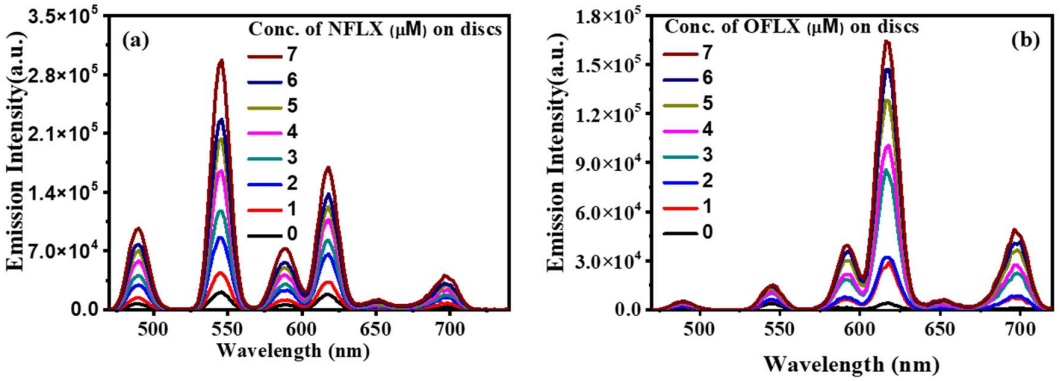


Figure S18 Emission spectra of Tb^{3+}/Eu^{3+}cholate (4.5 mM/0.5 mM/15 mM) gel coated paper discs with increasing concentrations of (a) NFLX and (b) OFLX (λ_{ex} 330 nm)

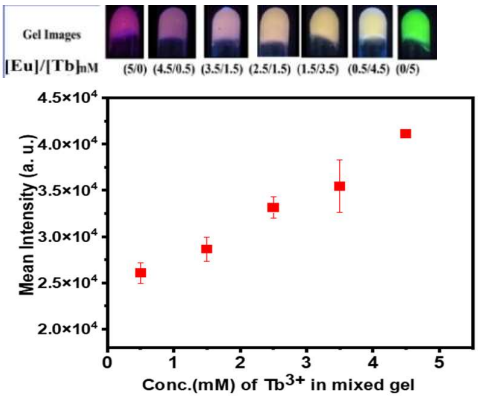


Figure S19 Variation of the intensity of Eu^{3+} emission at 690 nm (λ_{ex} 300 nm) of discs coated with 15 μM of NFLX doped Tb^{3+}/Eu^{3+} mixed-cholate gel {$[Tb^{3+}] + [Eu^{3+}] = 5mM$} as a function of Tb^{3+} concentration.

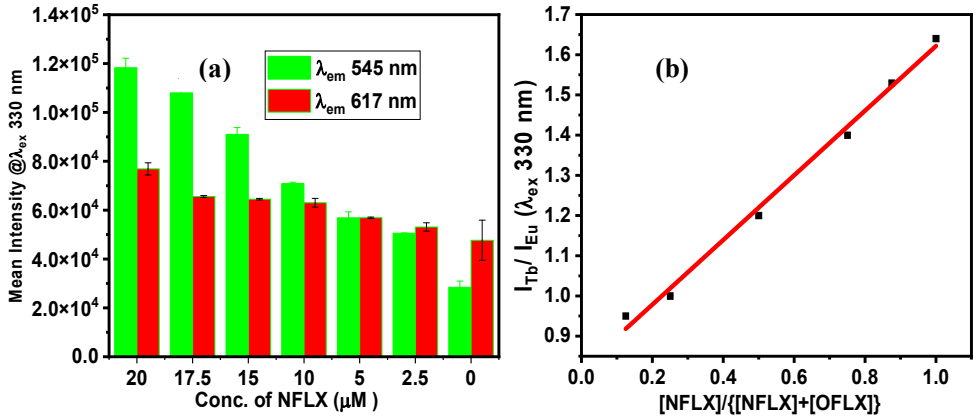


Figure S20 (a) Tb^{3+} emission at 545 nm and Eu^{3+} emission 617 nm (b) Tb^{3+} sensitization bias in Tb^{3+}/Eu^{3+}/cholate (4.5 mM/ 0.5 mM /15 mM) gel coated discs with the ratio of NFLX concentration to the total drug concentration in the mixture (λ_{ex} 330 nm)