

## Supporting Information of

**Sustainable chemistry approach for preparation of bluish green emissive copper nanoclusters from *Justicia adhatoda* leaves extract: A facile analytical approach for sensing of myoglobin and L-thyroxine**

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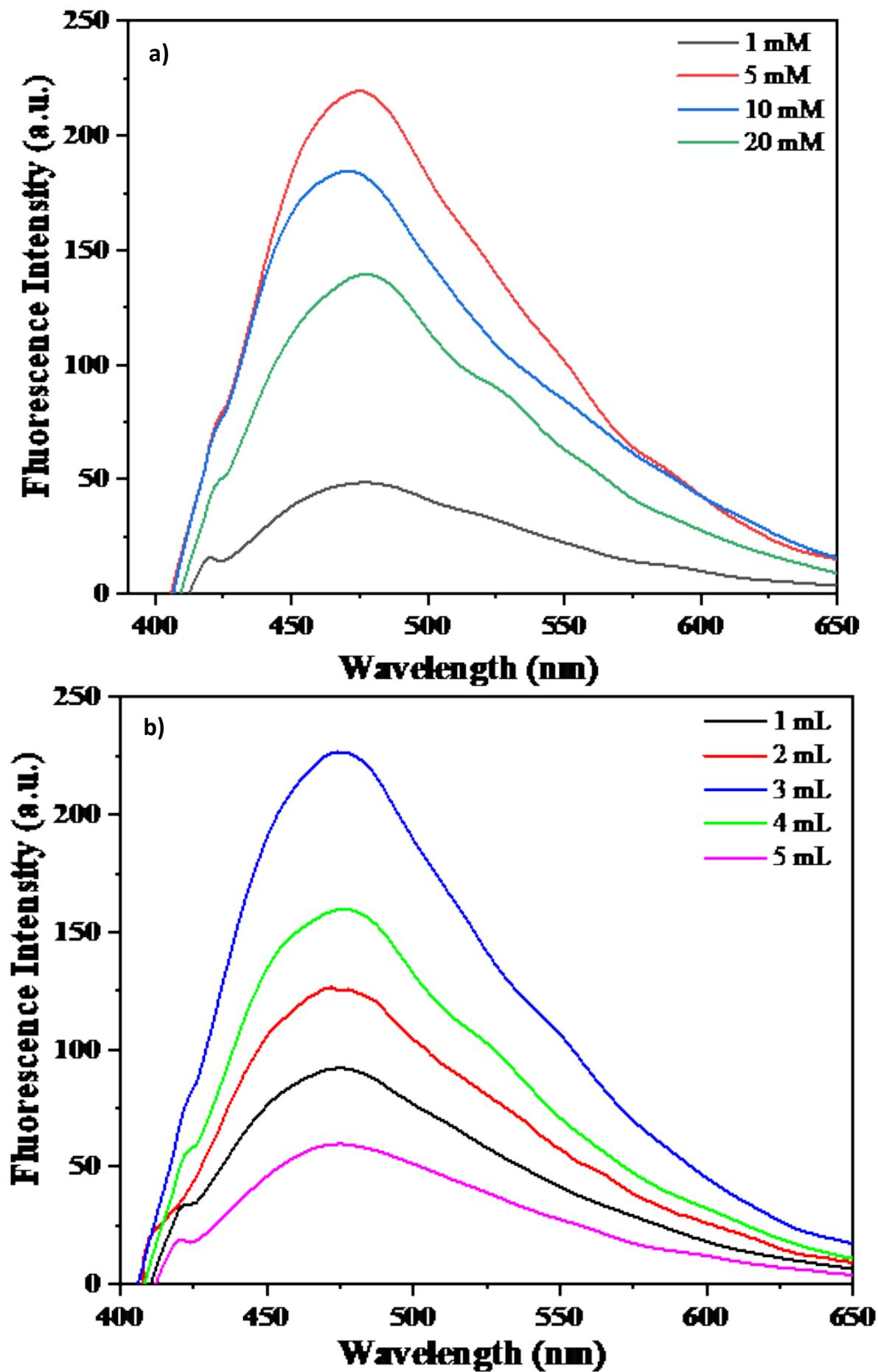


Figure S1. a) Study of fluorescence spectra of Cu NCs using different concentration of copper acetate concentration (1.0 – 20 mM). b) Effect of *J. adhatoda* extract volume (1.0 – 5.0 mL, 50 mg/10 mL) for synthesis of Cu NCs

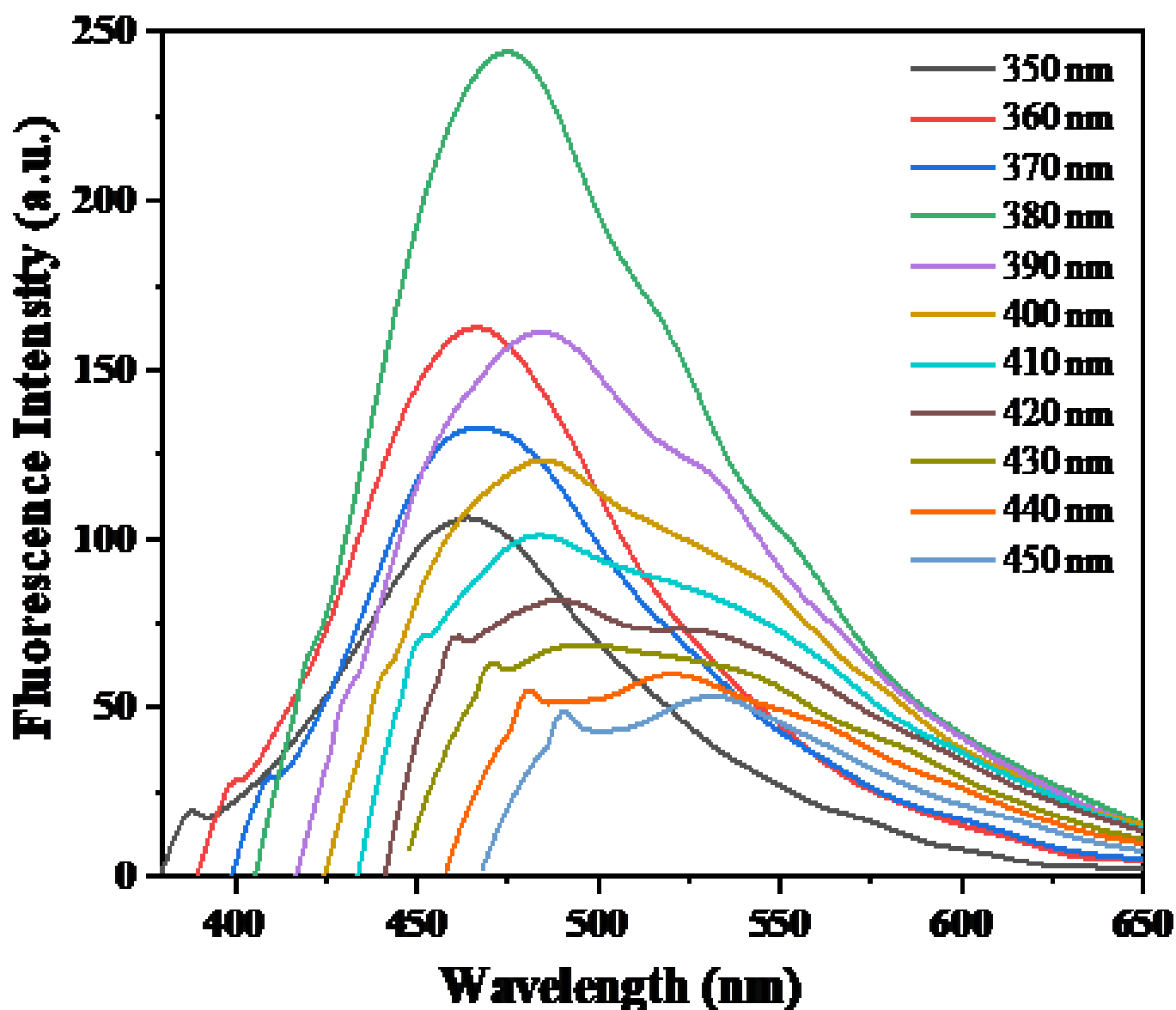


Figure S2. Study of excitation dependent emission spectra of *J. adhatoda*-Cu NCs at different wavelengths (350 – 450 nm)

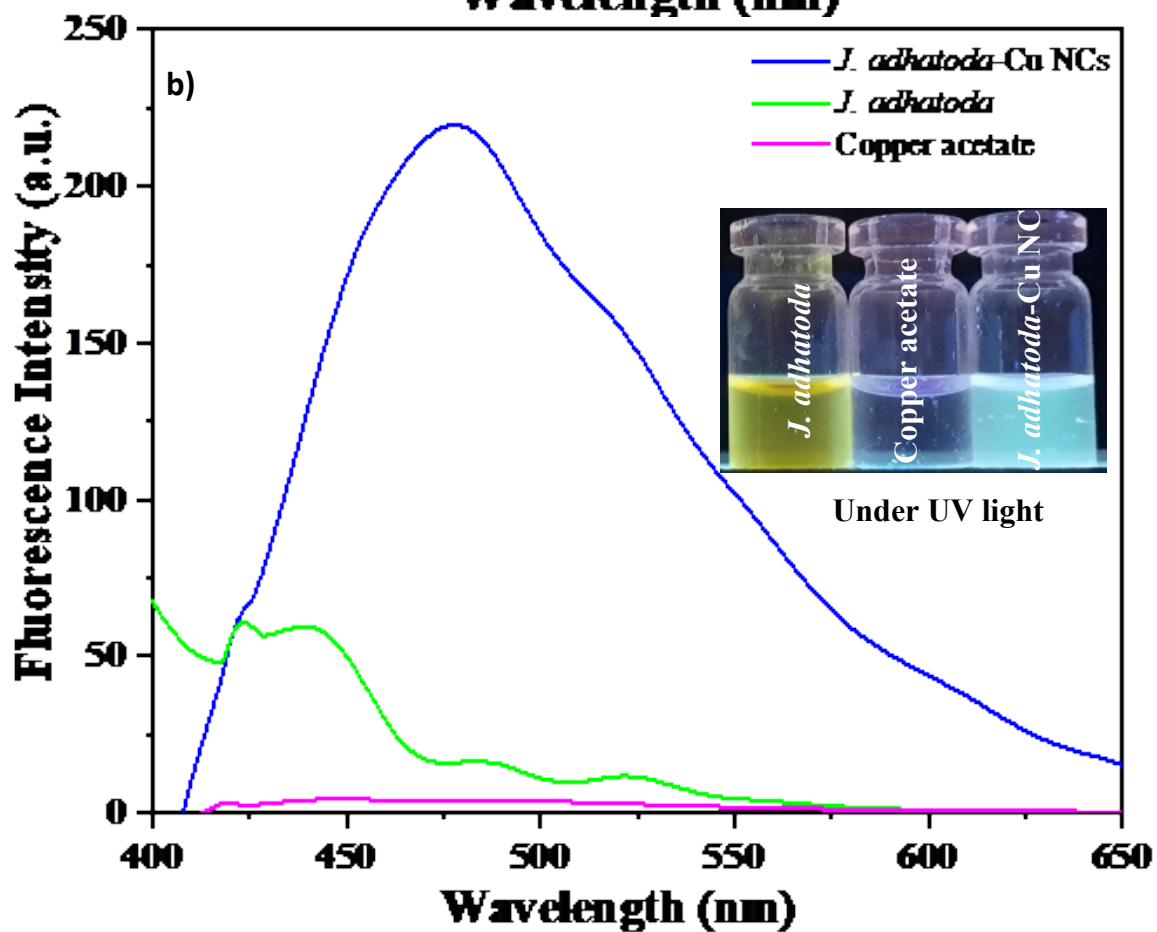
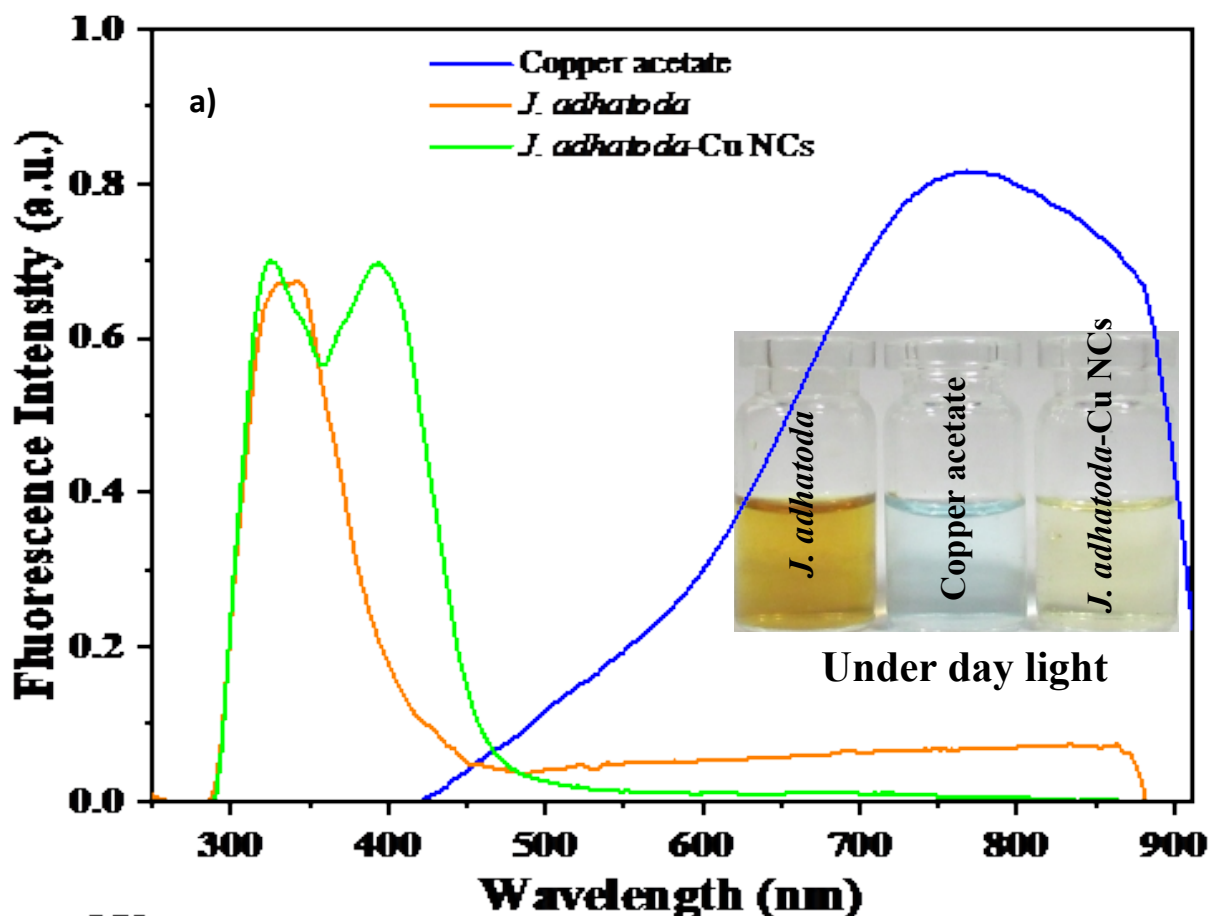


Figure S3. a) Absorption spectra of *J. adhatoda*-Cu NCs, copper acetate and *J. adhatoda* leaves extract. b) Absorption spectra of *J. adhatoda*-Cu NCs, copper acetate and *J. adhatoda* leaves extract. Insets: Photographical images of corresponding solutions in daylight and under UV light.

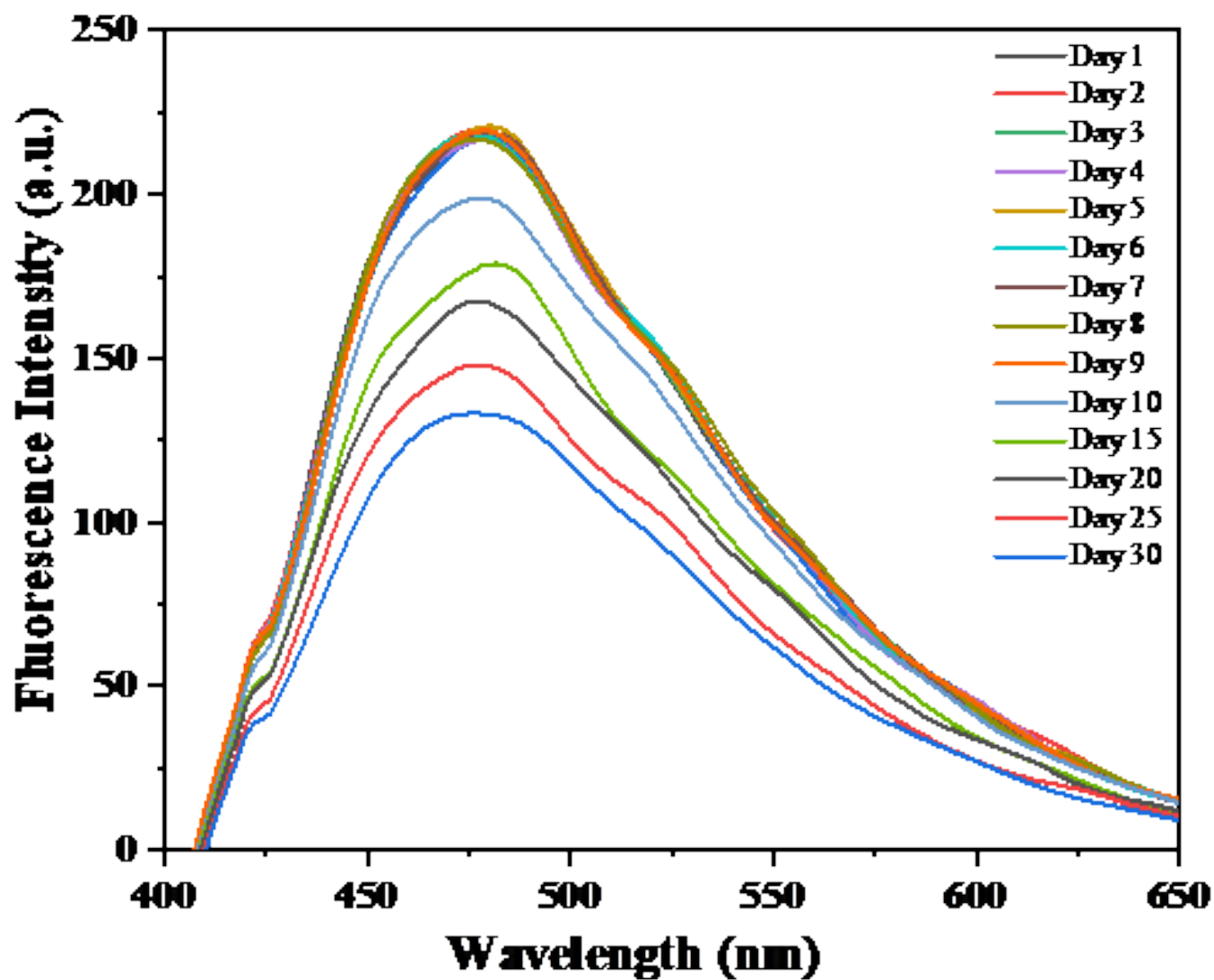


Figure S4. Stability study of *J. adhatoda*-Cu NCs from day 1 to day 30

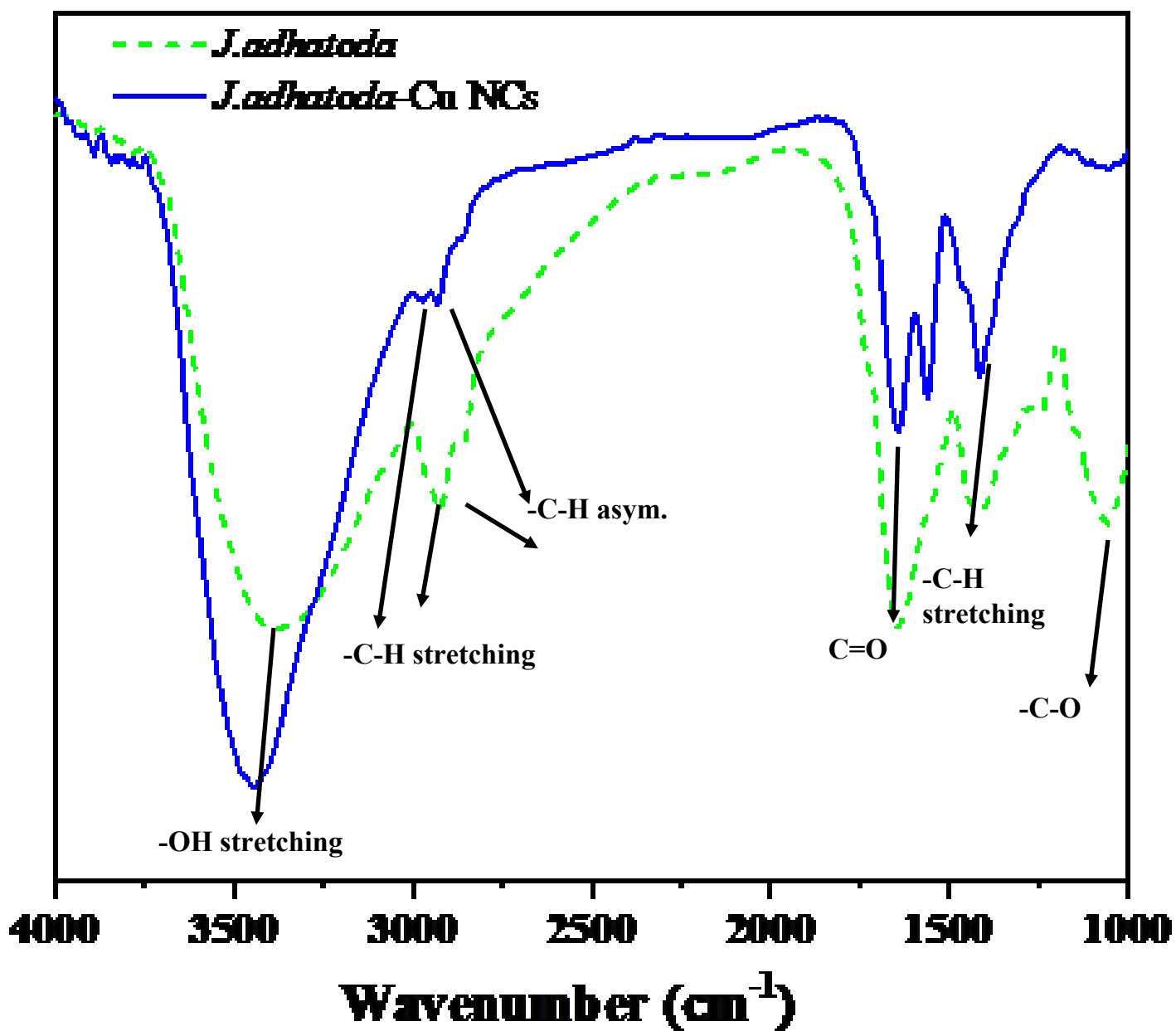
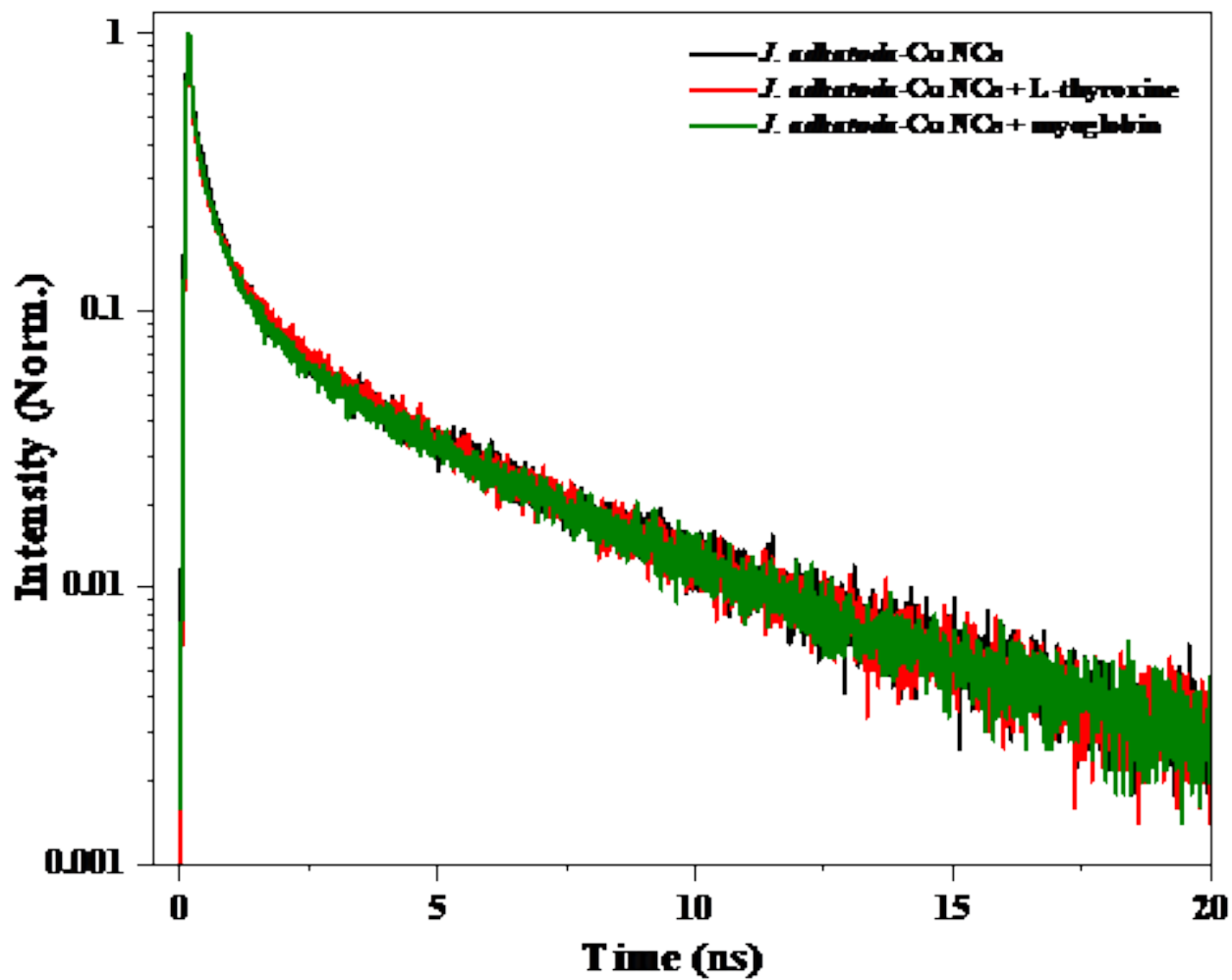


Figure S5. FT-IR spectra of *J. adhatoda* extract and *J. adhatoda*-Cu NCs



**Figure S6.** Life time analysis of *J. adhatoda*-Cu NCs and *J. adhatoda*-Cu NCs with myoglobin and L-thyroxine

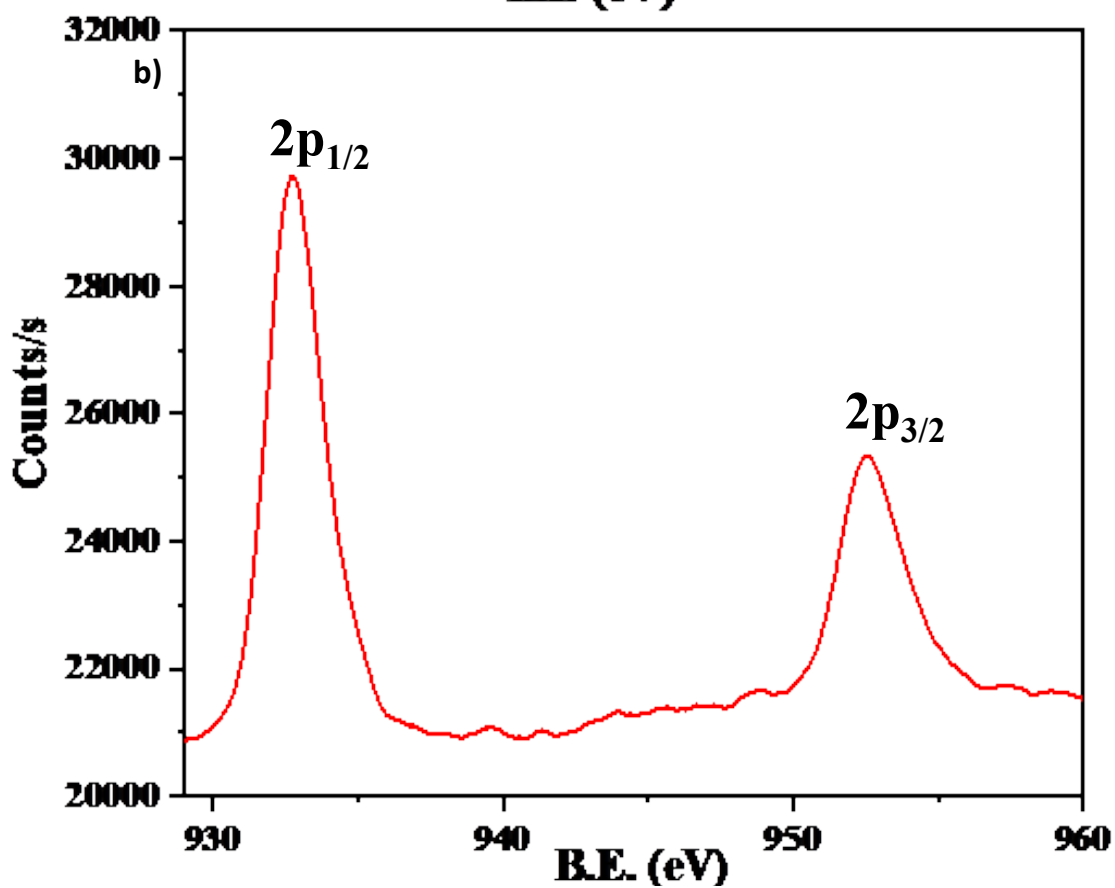
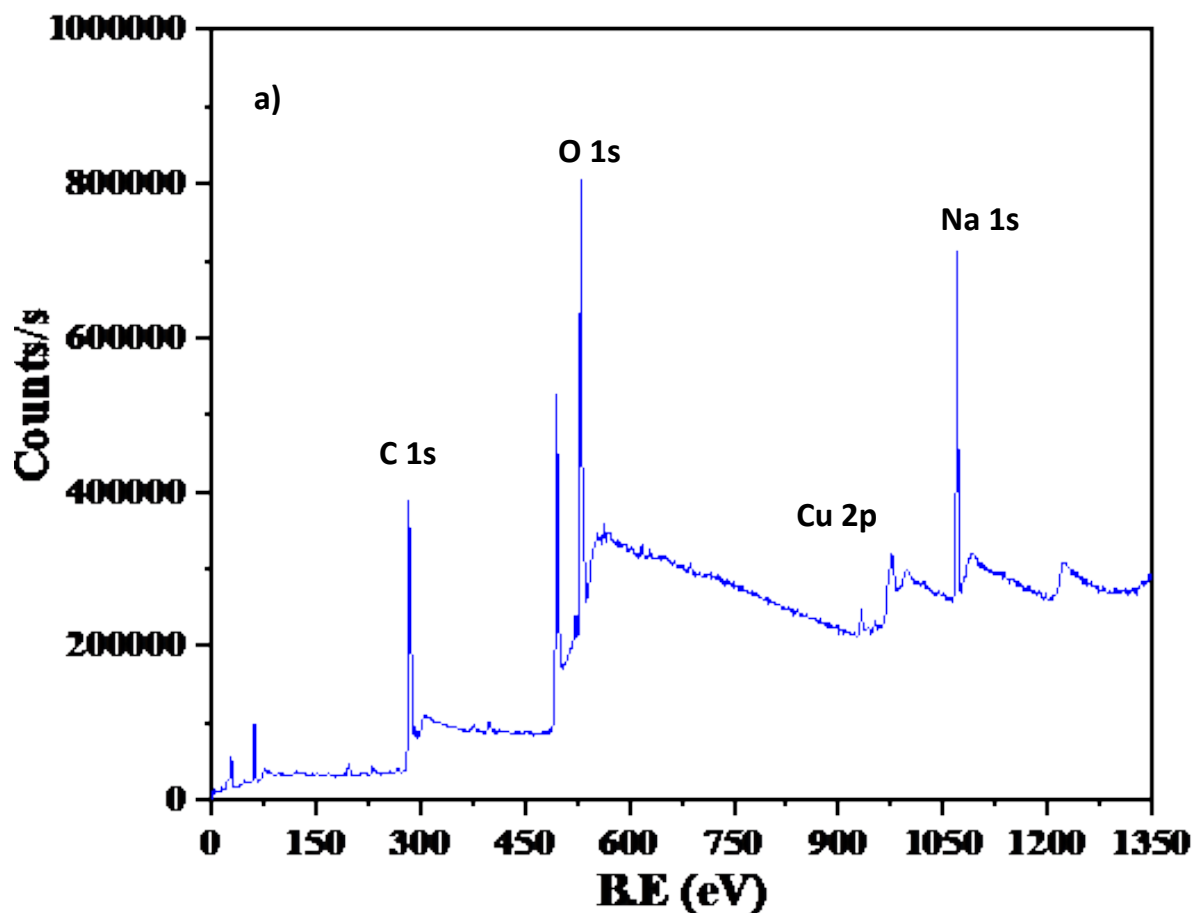


Figure S7. XPS analysis of *J. adhatoda*-Cu NCs, a) XPS survey of *J. adhatoda*-Cu NCs and b) XPS graph showing two oxidation state ( $\text{Cu } 2p_{1/2}$  and  $\text{Cu } 2p_{3/2}$ ) of Cu in *J. adhatoda*-Cu NCs.



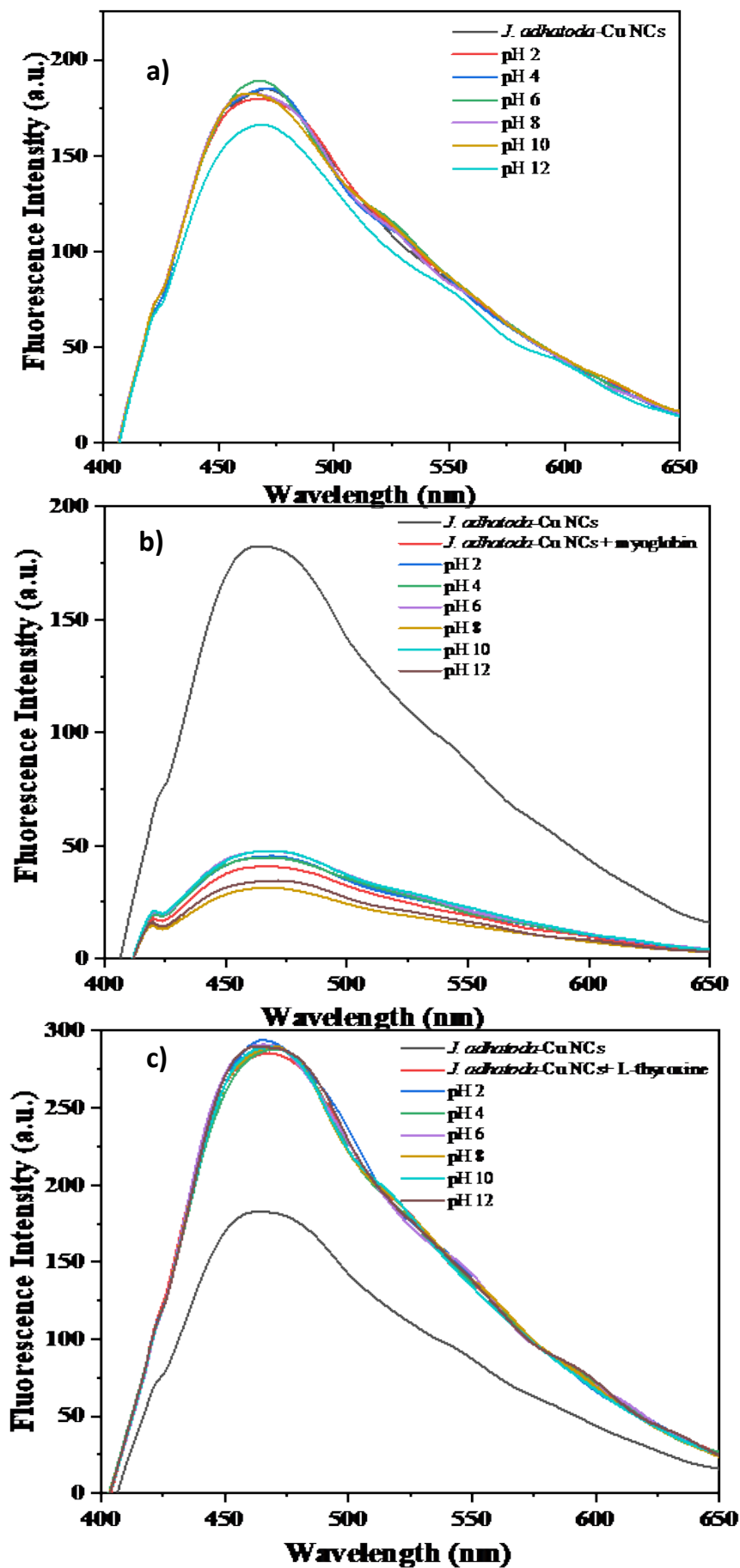
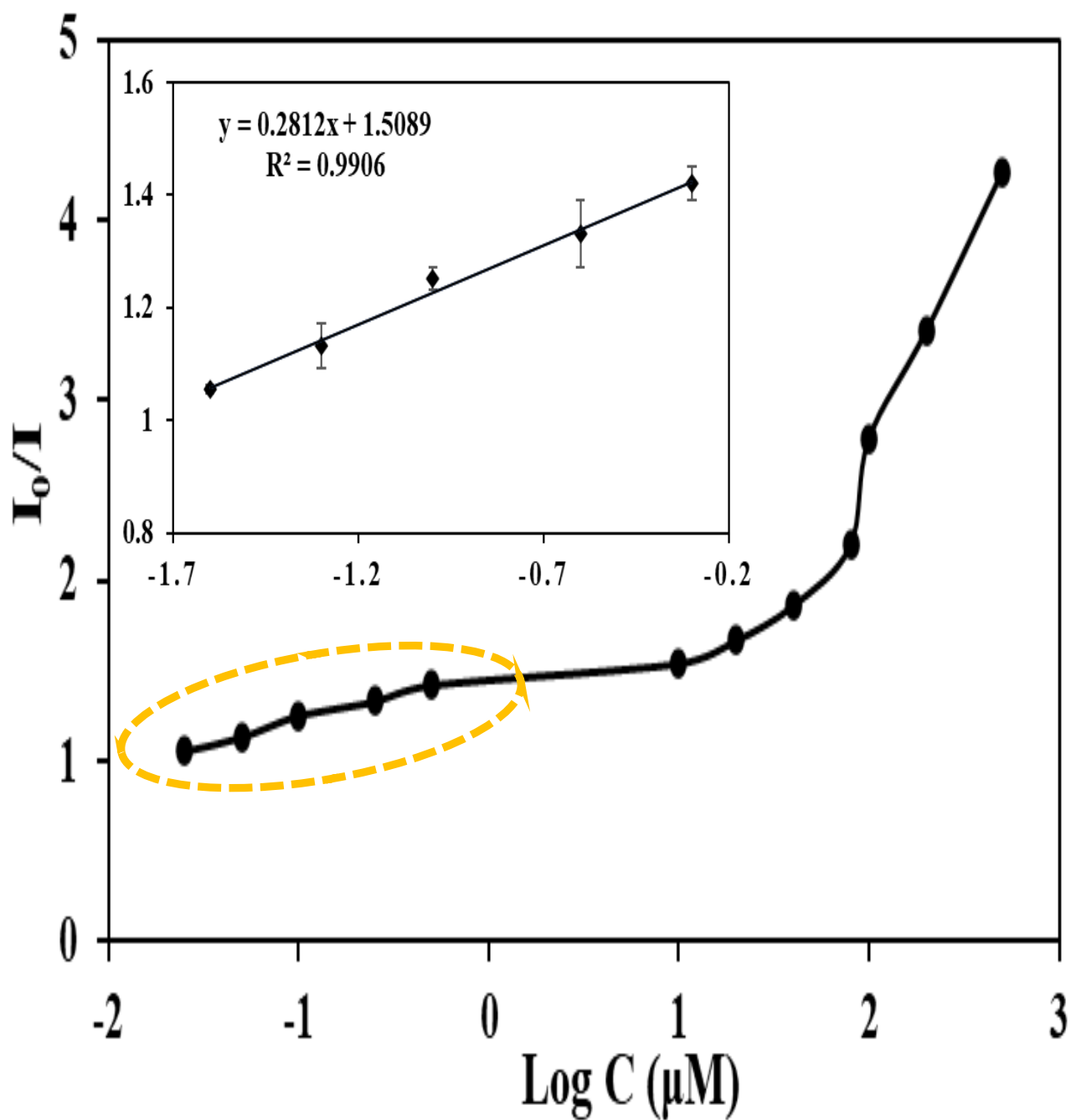
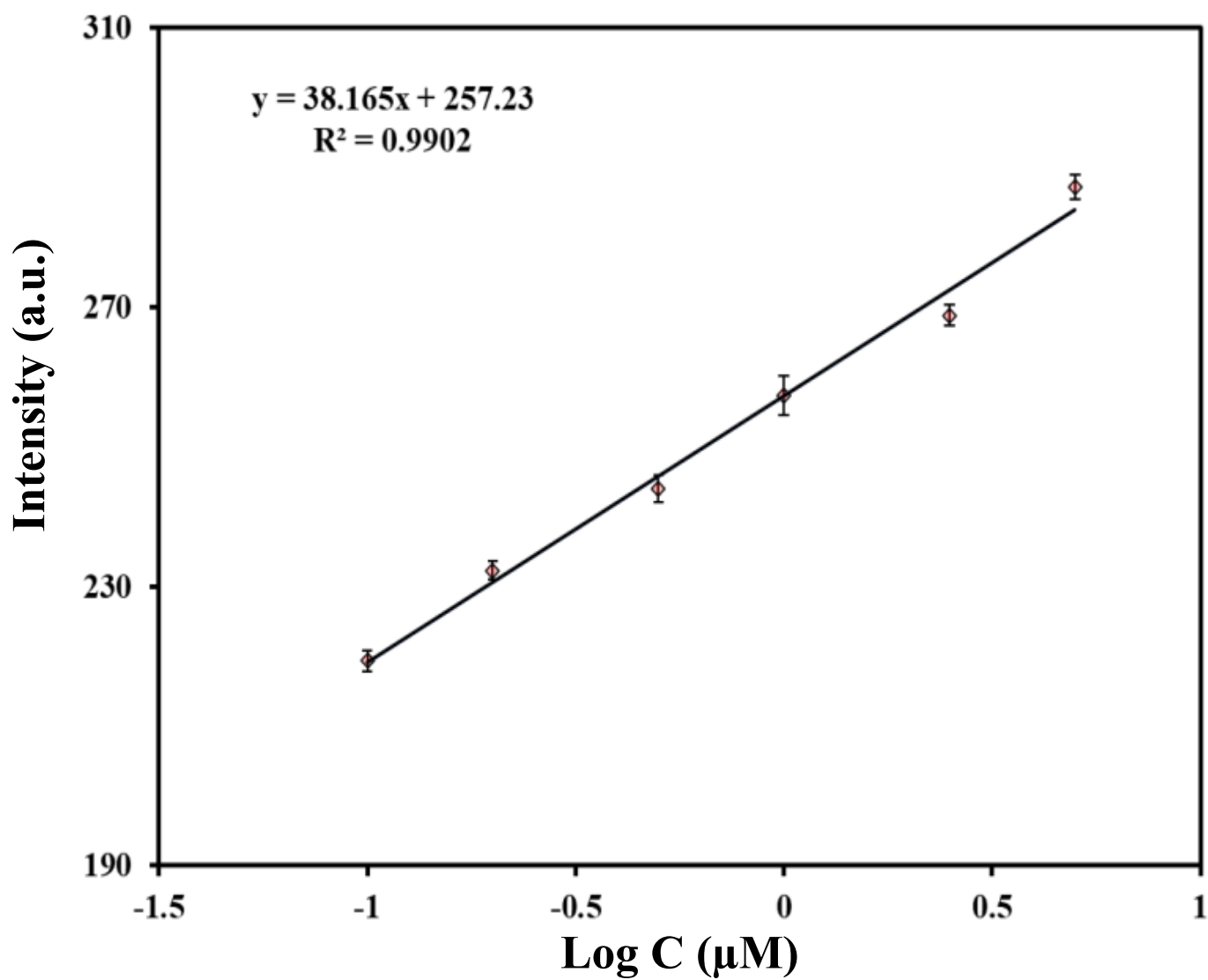


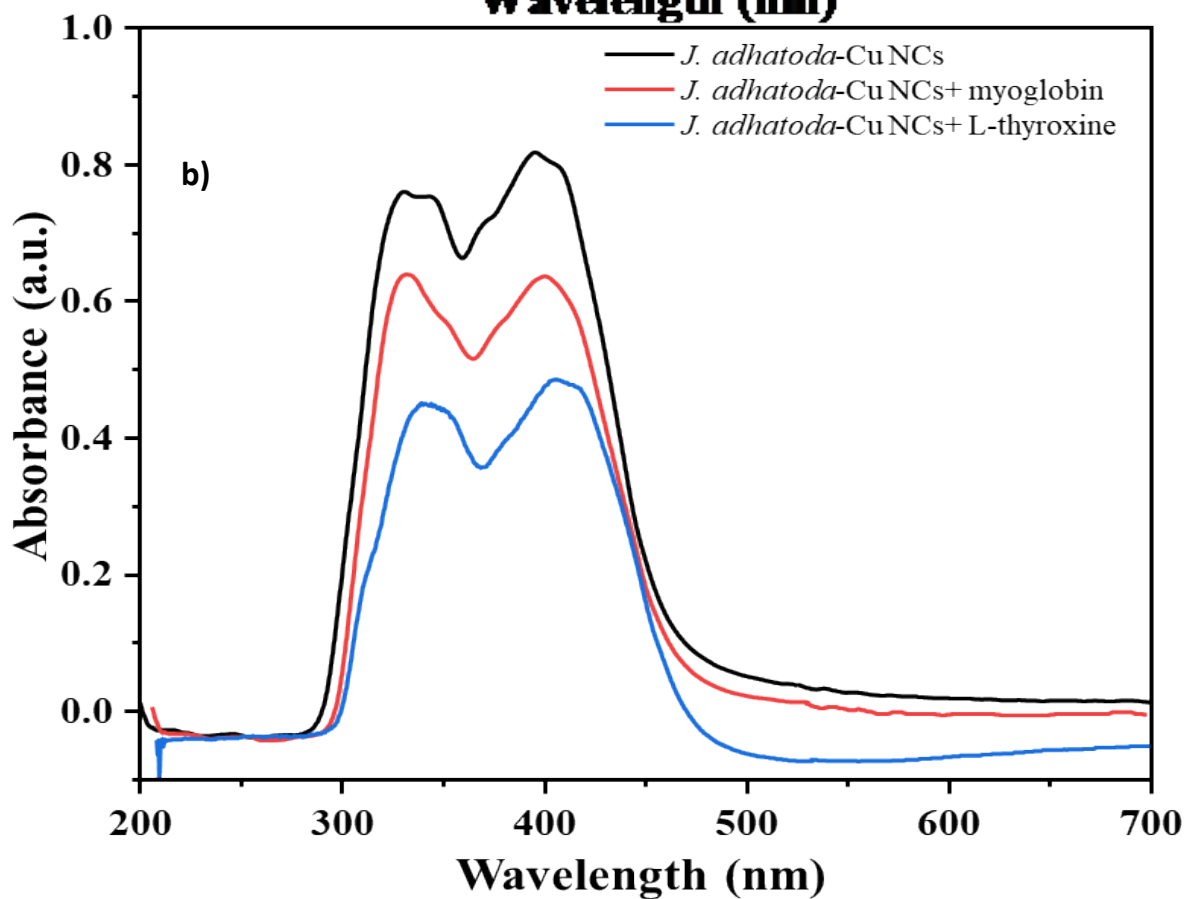
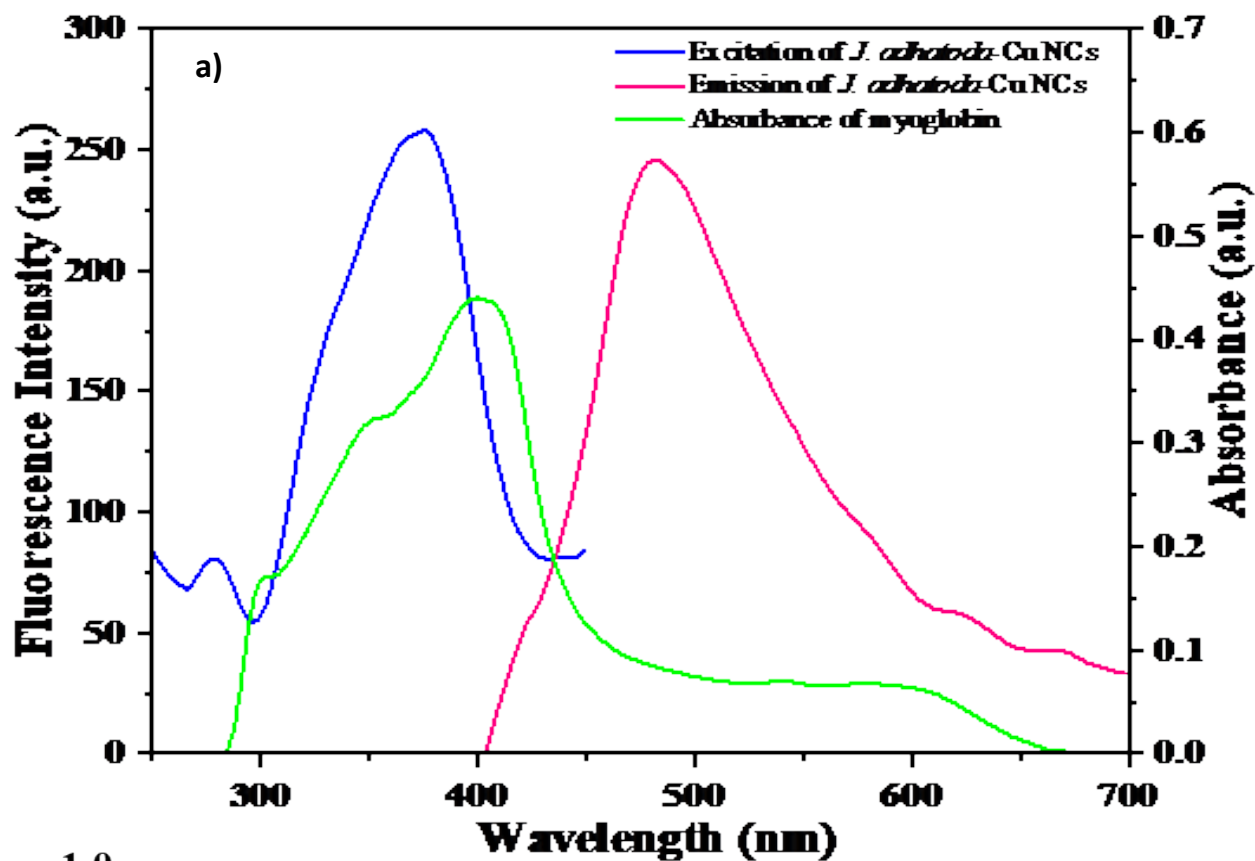
Figure S8. Effect of PBS pH on emission spectra of a) *J. adhatoda*-Cu NCs, in presence of b) myoglobin and c) L-thyroxine



**Figure S9.** Calibration curve constructed from the ratio of emission intensity of *J. adhatoda*-Cu NCs with increasing concentration of myoglobin (0.025 – 500  $\mu\text{M}$ ) ( $n=3$ ).



**Figure S10.** Calibration curve constructed from the ratio of emission intensity of *J. adhatoda*-Cu NCs with increasing concentration of L- thyroxine (0.1-2.5 μM) (n=3).



**Figure S11.** a) Excitation & emission spectra of *J. adhatoda*-Cu NCs and absorption spectrum of myoglobin b) UV spectra of *J. adhatoda*-Cu NCs and *J. adhatoda*-Cu NCs with L-thyroxine and myoglobin.

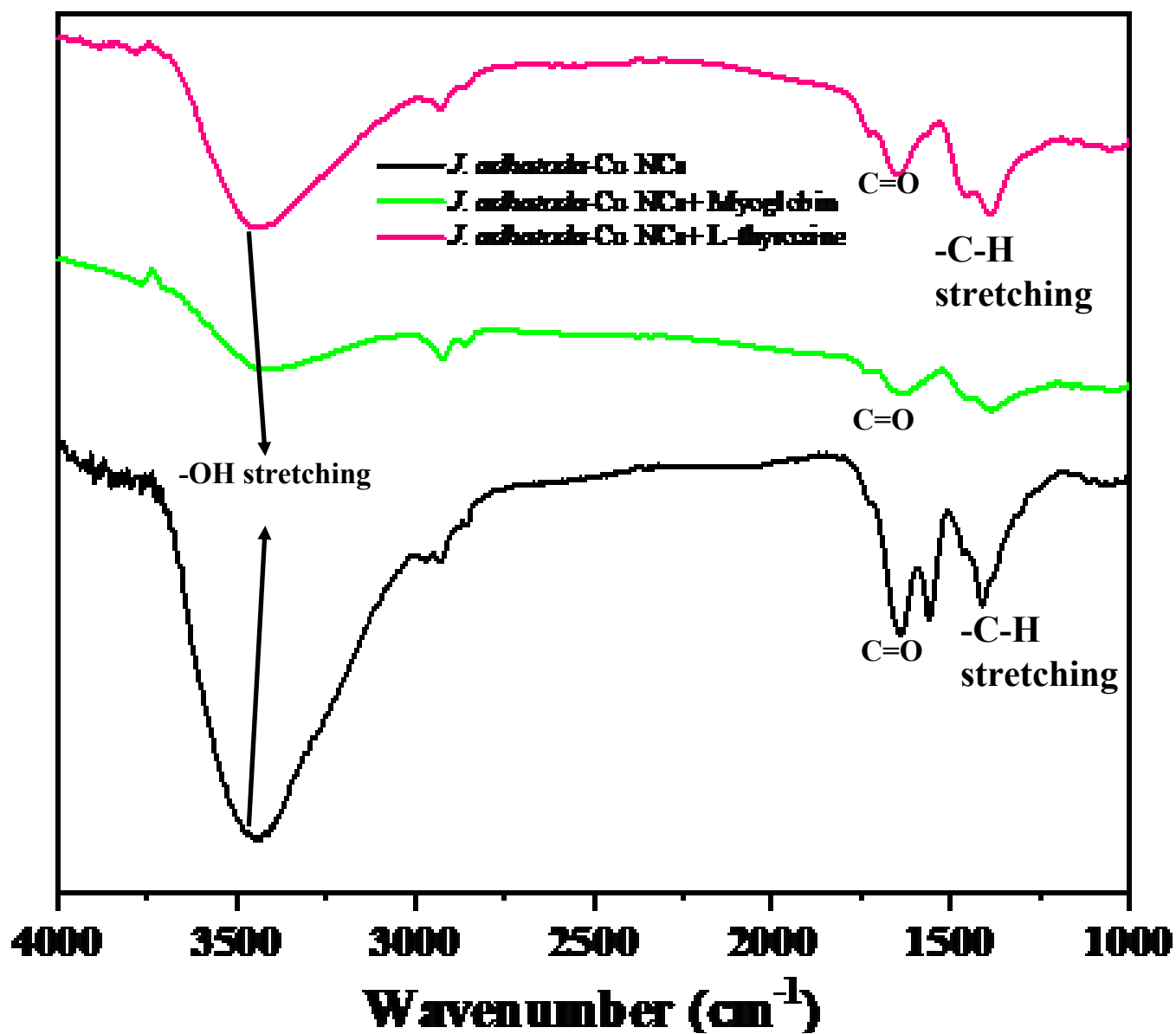
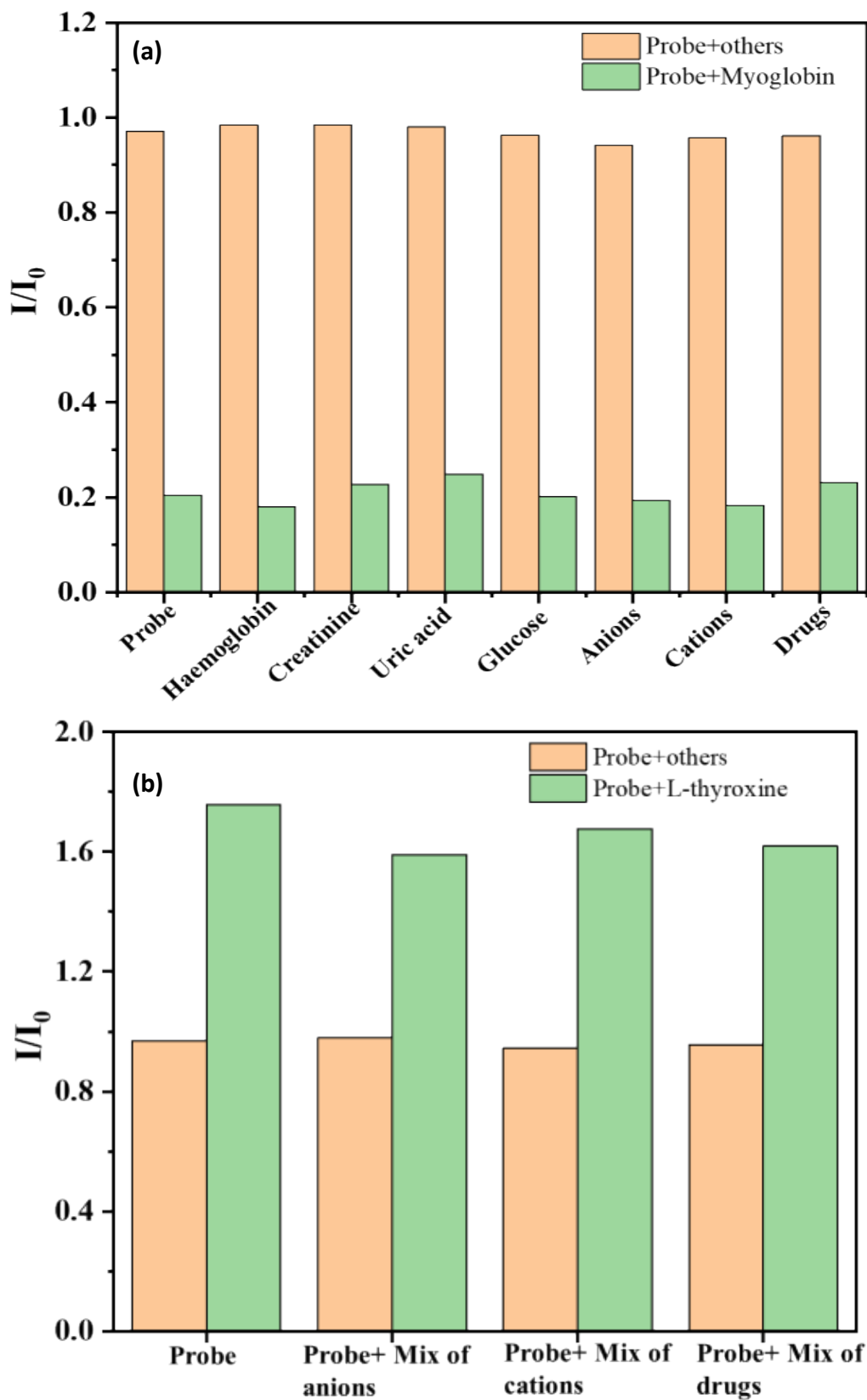
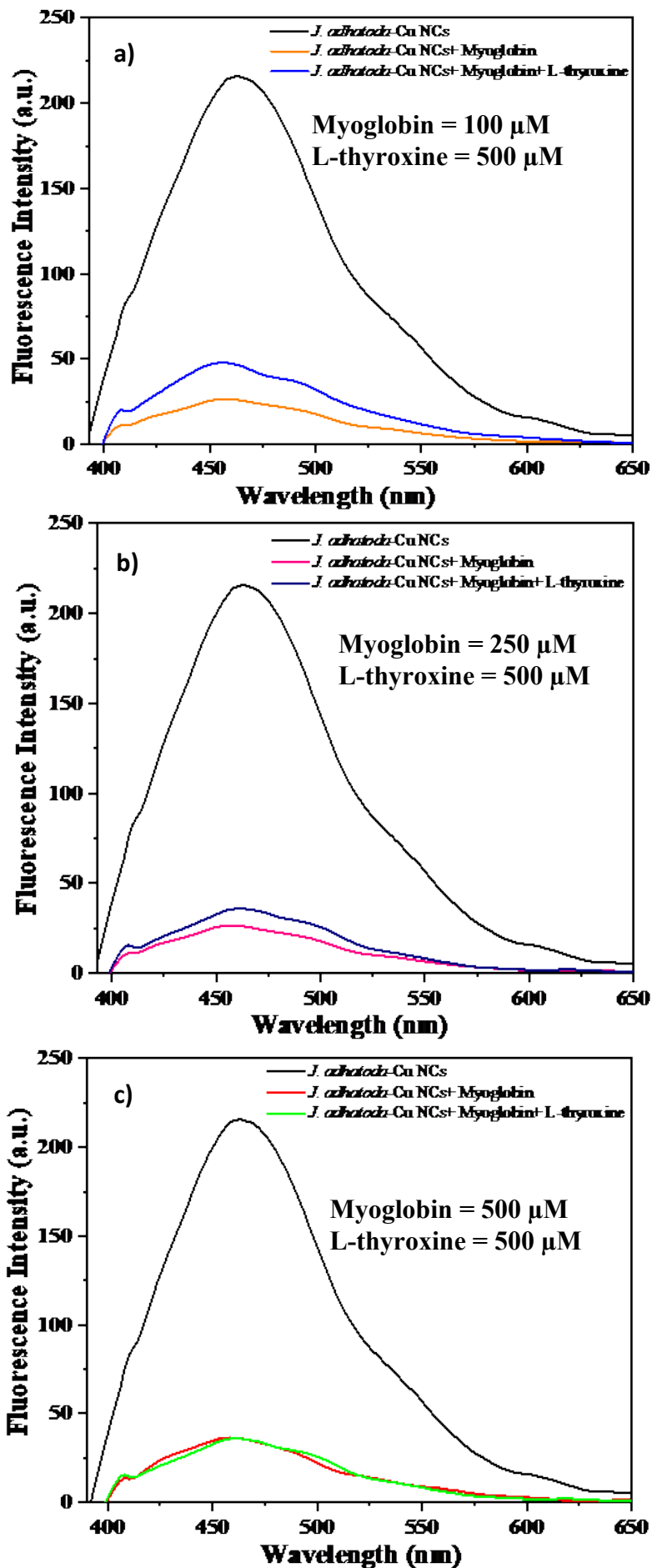


Figure S12. FTIR spectra of *J. adhatoda*-Cu NCs in presence of myoglobin and L-thyroxine



**Figure S13.** Selectivity study of *J. adhatoda*-Cu NCs with different biomarkers, cation, anion, drugs in presence of (a) myoglobin and (b) L-thyroxine (at 1:3 ratio)



**Figure S14.** Investigation of selectivity of *J. adhatoda*-Cu NCs in the presence of myoglobin and L-thyroxine co-existed in one sample a) 100 :500  $\mu$ M b) 250: 500  $\mu$ M c) 500 : 500  $\mu$ M respectively.

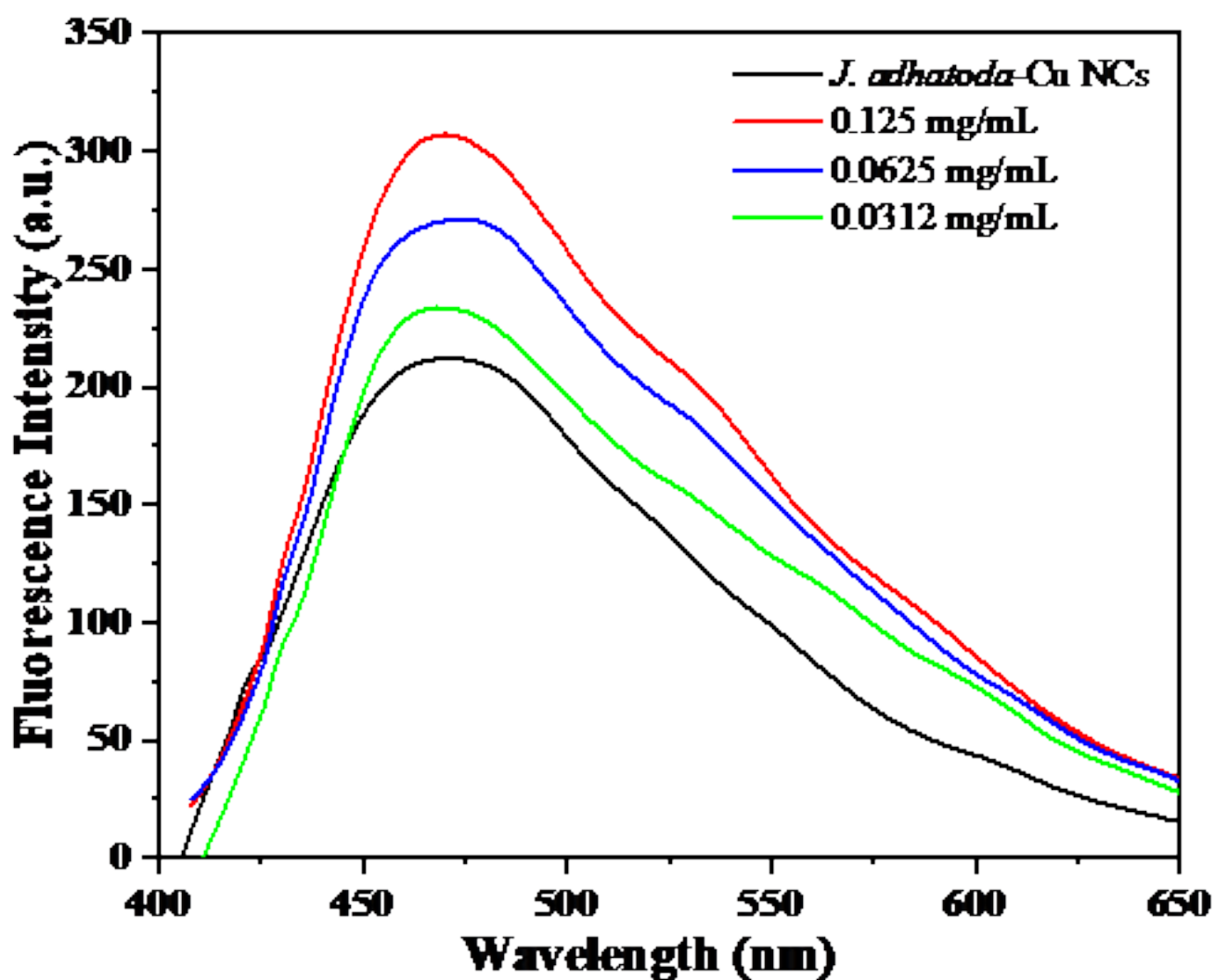


Figure S15. Emission spectra of *J. adhatoda*-Cu NCs with the addition of difference concentrations of Thyronorm 125 mcg tablet.



**Table S1.** Analysis of myoglobin and L-thyroxine in spiked serum samples by using *J. adhatoda* Cu NCs as a probe.

<b>Biomarker</b>	<b>Sample</b>	<b>Added concentration (μM)</b>	<b>Found concentration (μM)</b>	<b>Recovery (%)</b>	<b>% RSD (n=3)</b>
Myoglobin	Human serum	0.1	0.098	98.14	0.20
		0.25	0.24	97.63	0.15
		0.5	0.50	100.85	0.31
L-Thyroxine	Human serum	0.1	0.99	99.76	0.10
		0.2	0.19	99.74	0.15
		0.5	0.49	99.76	0.10

**Table S2.** Real sample (pharmaceutical composition) analysis of L-thyroxine by using *J. adhatoda* as a probe

<b>Biomarker</b>	<b>Tablet</b>	<b>L-thyroxine (Thyronorm 125 mcg) crushed tablet (mg/mL)</b>	<b>Found concentration (<math>\mu\text{M}</math>)</b>	<b>Recovery % (n=3)</b>	<b>RSD% (n=3)</b>
L-Thyroxine	Thyronorm-125 mcg	0.125 (160 $\mu\text{M}$ )	156.48	97.80	0.49
		0.0625 (80 $\mu\text{M}$ )	76.14	95.18	0.28
		0.0312 (40 $\mu\text{M}$ )	35.84	89.62	0.50