

“Turn-on” type colorimetric/fluorimetric probe for selective detection of Cu^{2+} at neutral pH condition

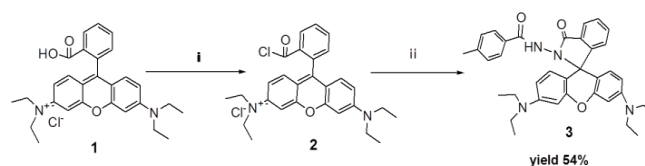
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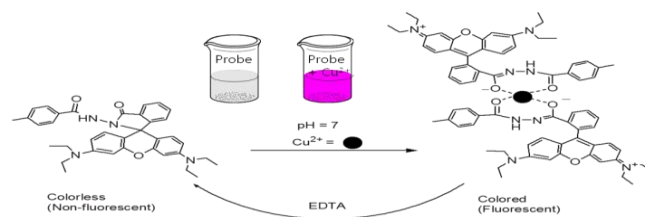
ABSTRACT: The design and development of fluorescent chemosensors have recently been intensively explored for sensitive and specific detection of environmentally and biologically relevant metal ions in aqueous solution and living cells. Herein, we report the photophysical results of rhodamine B based fluorogenic and chromogenic receptor for selective copper detection in the complete organic or mixed aqueous-organic media at neutral pH under ambient condition. The ligand exhibited the remarkable increment in the fluorescence emission and UV-visible absorption signal intensities at 587 and 547 nm, respectively, on induction of copper ion while the ligand solution remain completely silent on addition of varieties of other metal ions.

Copper ions found in the everyday body and play the important function in order to prevent tissue damage and inflammation. Beside these vital functioning in the body, the excess copper intake cause the chronic poisoning which induce the vomiting and abdominal pain. The copper can absorb into the body through drinking water and the resources of copper accumulation in the fresh water and ponds are copper plating industries, pipes and copper wires.¹⁻⁸

The present investigation deals with the synthesis of rhodamine based molecular probe **3** as signaling moiety for precise detection of copper ions in the pure as well as mixed aqueous-organic media. The probe **3** was found to sense copper ion selectively with the appreciable sensitivity of 4.35×10^{-7} M. The 2:1 ligand-copper binding stoichiometry was found by the Job's curve and the association constant of $8.11 \times 10^{-3} \text{ M}^{-1}$ was determined by the Benesi-Hilderbrand plot. In short, the selective and sensitive copper detection ability of probe **3** keep it to the frontier row of recently developed metal detecting fluorescent tools. The detailed synthetic pathway and the proposed ligand-copper binding mechanism are given in the Scheme 1 and 2, respectively.

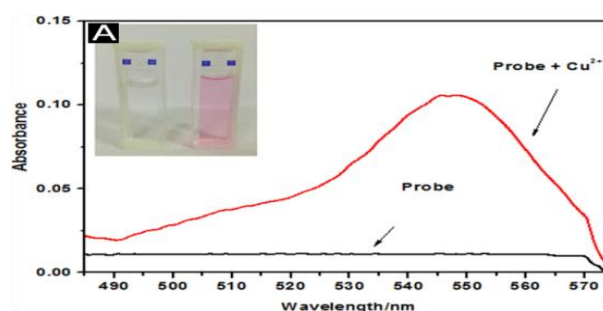


Scheme 1: Synthesis of probe **3**: reagents and conditions; (i) Rhodamine B, POCl_3 , 1,2-dichloroethane, reflux 2 h 30 min; (ii) *p*-Toluic hydrazide, TEA, acetonitrile, stirring at 0 °C, 1 h.



Scheme 2: Proposed ring opening mechanism of ligand **3** on induction of copper ion into its solution.

The photophysical properties of the probe was determined to evaluate its applicability as metal ion sensor by using varieties of in-organic metallic ions including Fe^{3+} , In^{3+} , Sr^{3+} , Hg^{2+} , Mg^{2+} , Ni^{2+} , Zn^{2+} , Pb^{2+} , Cd^{2+} , Co^{2+} , Fe^{2+} , Mn^{2+} , Pd^{2+} , Ba^{2+} , Na^+ , K^+ , Li^+ and Ag^+ in the pure as well as mixed organic-aqueous media. The probe did not show any absorption or emission signal at 547 and 587 nm on induction of varieties of metal ions except copper which concurrently turn the colorless solution of probe into light pink alongside the emergence of intense absorption band at 547 nm and emission band at 587 nm. The fluorescence emission properties of probe were also observed in the solution phase by keeping the probe solution under the UV-lamp by illuminating at higher wavelength (Fig. 1).



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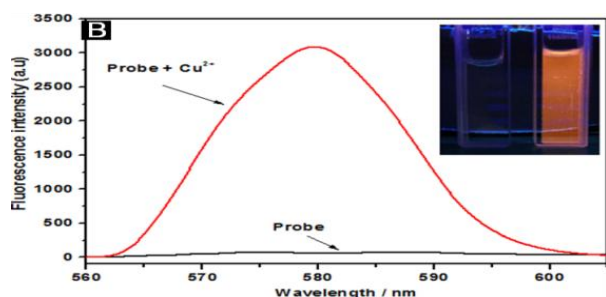


Fig. 1: (A) UV-visible absorption spectrum of probe (50 μM) in presence and absence of Cu^{2+} (50 μM) in acetonitrile at pH 7.0. Inset photograph described the colorimetric change (under ambient light) before and after CuCl_2 addition into probe solution; (B) Fluorescence emission spectrum of probe (50 μM) in the presence and absence of Cu^{2+} (50 μM) in acetonitrile at pH 7.0 (Excitation wavelength = 547 nm); Inset photograph described the colorimetric change (under UV light, 365 nm) before and after CuCl_2 addition into probe solution. Fluorescence quantum yield increment from 0.002 to 0.68.

The ligand-metal binding stoichiometry was observed by the Job's curve (Fig. 2A) as well as by the mass spectrometric analysis. The Job's plot clearly represents the 2:1 ligand-metal binding stoichiometry and the proposed chelation mechanism on the basis of Job's plot are represented in the Scheme 2. The association constant of probe upon metal binding was determined by the Benesi-Hilderbrand plot which comes to be $8.11 \times 10^{-3} \text{ M}^{-1}$ (Fig. 2B).

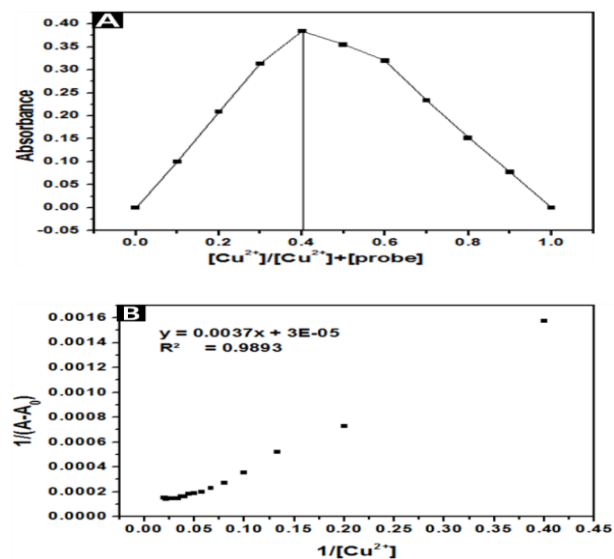


Fig. 2: Probe-metal binding stoichiometry by; (A) Job's plot (probe-metal binding 2:1) and (B) Benesi-Hilderbrand plot (association constant; $8.11 \times 10^{-3} \text{ M}^{-1}$)

In order to get deep insight about the probe metal binding, the UV-visible titration experiment was conducted by recording the changes in the probe absorption spectra upon successive addition of copper ions. The appearance of two clear isosbestic points at 262 and 273 nm as well as momentous increment in the absorption signal intensity at 547 nm clearly represent the complexation process between the probe and copper ions (Fig. 3A).

The similar results was observed in case of fluorescence emission spectral measurement which showed the linear increase in the emission signal at 587 nm up to 0.5 eq. copper induction afterward the random changes without noticeable increase in the emission signal intensity at 587 nm (Fig. 3B).

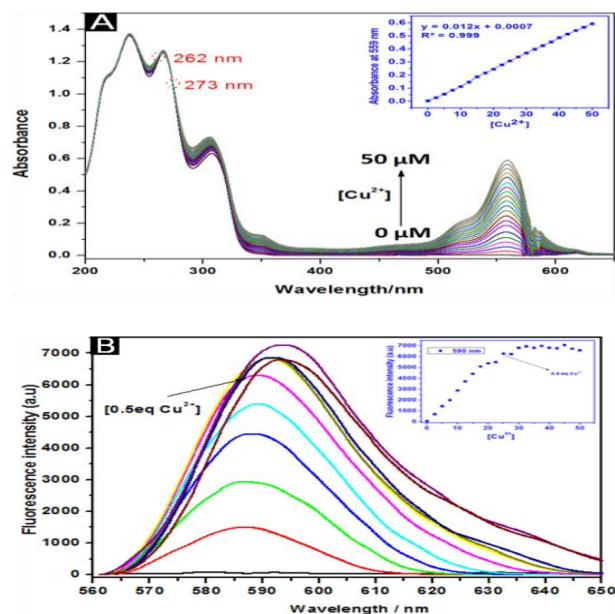


Fig. 3 : (A) Absorption spectrum of probe (50 μM) with increasing copper concentration, the inset shows titration curve by absorbance at 547 nm which give the limit of detection of $4.35 \times 10^{-7} \text{ M}$. The isosbestic points is 262 nm and 273 nm suggest the inter conversion of probe into probe-metal complex; (B) Fluorescence titration of probe (50 μM) with copper (0-50 μM), the inset described the fluorescence enhancement at emission maxima of 587 nm as a function of copper concentration.

The probe selectivity was observed by the addition of several metal ions into the probe solution and only the copper ions brings the turn-on change in the probe absorption and emission spectra indicate the good selectivity of probe toward the copper ions as shown in the Fig. 4.

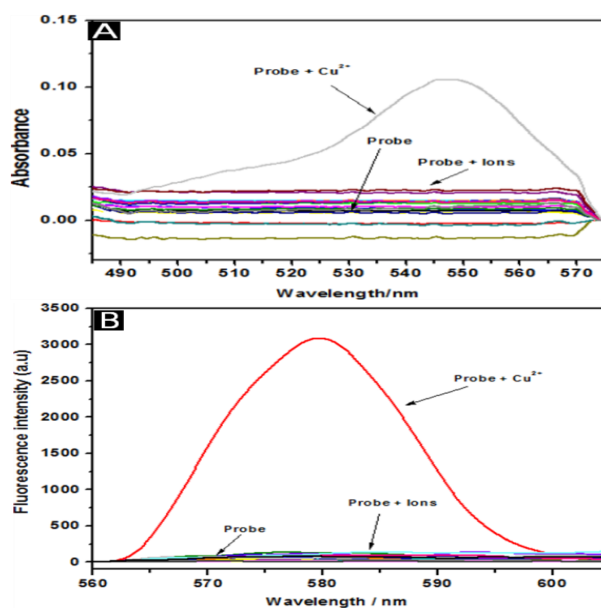


Fig. 4: (A) UV-vis and (B) fluorescence selectivity of probe (50 μM) over varieties of other metal ions (50 μM) (Fe³⁺, In³⁺, Sr³⁺, Hg²⁺, Mg²⁺, Ni²⁺, Zn²⁺, Pb²⁺, Cd²⁺, Co²⁺, Fe²⁺, Mn²⁺, Pd²⁺, Ba²⁺, Na⁺, K⁺, Li⁺, Ag⁺).

From acidic to neutral pH condition, the probe operate well while the basic pH cause slow death in the absorption and emission signal as shown in the Fig. 5A. The appearance of high intensity signal under acidic condition was due to acid induced ring opening of spirolactam of probe molecule.

The quick response of probe upon metal binding is a prerequisite of a competitive probe and the presently reported probe fulfil the criteria of facile and quick detection of copper ions. The Fig. 5B represents the changes in the absorption signal intensity over variable time interval which showed that the probe absorption signal intensity approaches near to maximum within one minute after metal addition while the absorption signal intensity saturated after 5 minutes of initial metal induction.

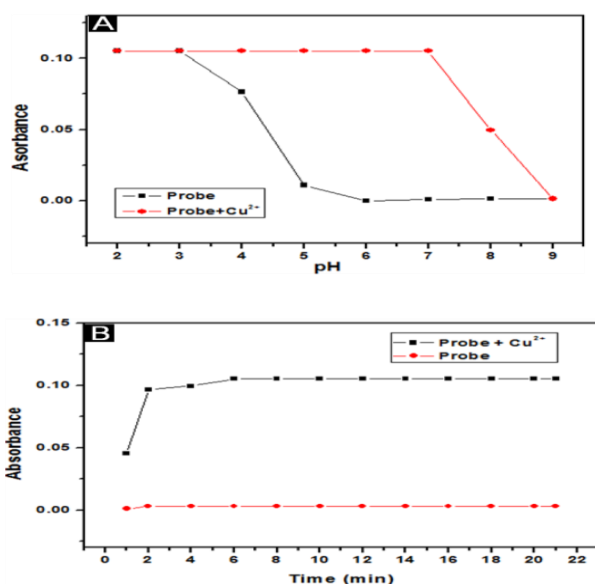


Fig. 5: (A) The effect of pH and (B) time on the probe-metal binding.

The probe was insoluble in the pure aqueous media while on increasing the percentage of organic solvent, there was constant increase in the absorption signal intensity as shown in the Fig. 6.

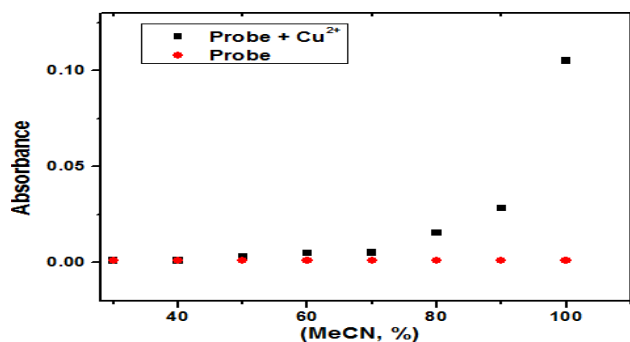


Fig. 6: Solvent effect over the optical properties of probe-metal complex.

The maximum molar absorption co-efficient value was observed in acetonitrile solvent while the absorption signal intensity slightly decreased by using the protic solvent. However, the moderate results were observed in the mixed aqueous-acetonitrile solvent system as shown in the Table 1. Meanwhile, the probe showed the very good separation of emission and excitation spectra with the Stoke's shift values of 1245 cm⁻¹ and high stokes shift believed to be more suitable for in-vivo experiments and cell imaging by retarding the auto-fluorescence.

S. no.	Solvent	Absorption maxima (nm)	ϵ ($10^4 M^{-1} cm^{-1}$)	Emission maxima (nm)	Stokes shift (cm ⁻¹)
1	MeOH	547	1.92	587	1245
2	EtOH	547	0.73	587	1245
3	MeCN	547	2.105	587	1245
4	MeCN : H ₂ O (9 : 1)	547	0.571	587	1245

Table 1: Solvent effect on the photophysical properties of probe-copper complex solution.

In summary, we developed a new colorimetric and fluorimetric chemosensor, which can detect Cu²⁺ selectively at neutral pH condition. The sensor showed sensitivity and selectivity toward Cu²⁺ ions. The 2:1 ligand-copper binding stoichiometry was confirmed by the UV-visible and fluorescent measurement by plotting Job's curve while the binding tendency was estimated by the Benesi-Hilderbrand plot which exhibited the association constant values of $8.11 \times 10^{-3} M^{-1}$. The absorption titration experiment represents the precise sensitivity of $4.35 \times 10^{-7} M$. Thus these experimental results verified the high sensitivity and Off-On colorimetric and fluorimetric utility of prepared probe for copper ions.

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KEYWORDS: rhodamine; copper; fluorogenic; chromogenic; sensor

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