A New Fluorescent Probe Based on N-(2-carboxymethyl)benzoaza-15-crown-5 for Sensitive and Selective Determination of Cu^{2+}

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Copper is widely used and therefore it is a pollutant metal. It is important to develop probes that can selectively determine copper with high sensitivity. The benzoaza-15-crown-5 derivatives are used as fluorescence sensing systems and excellent spectroscopic properties are demonstrated. The "N", "O" atoms of the heterocyclic unit act as binding sites for recognizing copper ions. A new, simple, sensitive fluorescence method for the determination of Cu^{2+} ions was developed and analytical characteristics of the proposed probe were estimated. The Cu^{2+} ions can significantly quench the fluorescence intensity of N-(2-carboxymethyl)benzoaza-15-crown-5 (Cr) in ethanol/H_2O (4:6, v/v) solvent mix containing urotropine buffer (pH 7.5) at λ_{ex} = 274 nm and λ_{em} = 308 nm. The probe has high photostability. Under optimal conditions, the quenching of fluorescence intensity depends on the concentration of Cu^{2+} ions in the range of 1.70×10^{-6} - 2.38×10^{-4} M, detection limit was 0.56 μM. This method was applied for the determination of Cu^{2+} ions in drinking water. The quenching effect in the presence of copper(II) can be explained by the termination of intramolecular charge transfer from the chelate center to the aromatic part of the molecule due to chelation.

Keywords: benzoaza-15-crown-5 derivative; fluorescence intensity; quenching; Cu^{2+} ions

Новий флуоресцентний зонд на основі N-(2-карбоксиметил) бензоаза-15-краун-5 для чутливого та селективного визначення Cu^{2+}

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Мідь широко використовується і тому є металом-забруднювачем. Важливо розробити зонди, які можуть селективно визначати мідь з високою чутливістю. Похідні бензоаза-15-краун-5 використовуються як флуоресцентні системи і демонструють чудові спектроскопічні властивості. Атом азоту гетероциклічної одиниці діє як місце хелатоутворення щодо іонів металу. Розроблено та валідовано нову просту, чутливу флуоресцентну методику визначення іонів Cu^{2+}. Іони Cu^{2+} можуть значно гасити інтенсивність флуоресценції N-(2-карбоксиметил) бензоаза-15-краун-5 (Cr) у водних розчинах, що містять уротропіновий буферний розчин (pH 7.5) за довжини хвилі λ_{em} = 308 нм (λ_{ex} = 274 нм). Зонд має високу фотостабільність. За оптимальних умов, гасіння інтенсивності флуоресценції залежить від концентрації іонів Cu^{2+} в діапазоні 1.70×10^{-6} - 2.38×10^{-4} М, межа виявлення становить 0.56 мкМ. Ця методика застосована для визначення іонів Cu^{2+} у питній воді. Ефект гасіння в присутності міді(II) можна пояснити припиненням внутрішньомолекулярного переносу заряду від хелатного центру до ароматичної частини молекули внаслідок хелатування.

Ключові слова: похідне бензоаза-15-краун-5; інтенсивність флуоресценції; гасіння; іони Cu^{2+}

Copper as an important chemical element participates in the synthesis of proteins and enzymes. It is well known that excessive amounts of copper in the body is dangerous. Cu^{2+} is widely used and therefore it is a pollutant metal. According to the US Environmental Protection Agency (EPA), the permitted copper level in drinking water is 20 μM [1]. It is important to develop methods that can

selectively determine copper with high sensitivity.

Several methods for determination of Cu^{2+} ions in enviromental samples have been proposed which include electrochemical methods, mass spectrometry (MS), atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), inductively coupled plasma (ICP) and fluorometry, etc. Among of these methods fluorescent techniques have attracted more attention due to their evident advantages such as non-destructive nature, real-time detection, less expensive, high sensitivity and selectivity [2].

The fluorophores show different optical properties in its free and analyte bound state by some different photophysical mechanisms. There are well known photophysical mechanisms: photoinduced electron transfer (PET), charge transfer (CT), Förster resonance energy transfer (FRET), excimer formation [3].

The main classes of fluorescent molecular sensors for cation recognition are presented in review [4]. These classes differ by the nature of the cation-controlled photoinduced processes. Differences in each class are in the structure of the complexing moiety: chelators, podands, crown ethers, cryptands, calixarenes.

A recent review discussed on recent advances of smart fluorescent probe containing a crown ether moiety that could be developed as a sensor for metal ions, anions and other bio-molecules and can be applied to monitor the relevant biological process *in vivo* [5].

Fluorescent based sensors can detect common metal ions and can serve as agents for imaging. The well known fluorescent chemosensors for the detection of Cu^{2+} based on rhodamine, coumarin, quinoline, benzothiazole, indole, imidazole, lanthanide chelates and other fluorophores are given in the Table 1 [6 - 26].

Table 1. Overview on fluorescent chemosensors for determination of Cu^{2+}

Fluorescent Chemosensor	Ka* (M^{-1})	Linear range (M)	Limit of detection (M)	Ref
rhodamine spirolactam derivative	-	8.0×10^{-7} – 1.0×10^{-5}	3.0×10^{-8}	6
receptor with benzimidazole moieties	5.90×10^{3}	1.0×10^{-5} – 2.0×10^{-4}	2.3×10^{-5}	7
2-(2'-aminolphenyl)-4-hydroxymethylthiazole	1.43×10^{5}	1.0×10^{-6} – 1.0×10^{-5}	-	8
phenanthroline-based chemosensor	1.00×10^{5}	1.0×10^{-6} – 6.0×10^{-5}	8.7×10^{-7}	9
1,8-diaminonaphthalene	2.58×10^{4}	1.0×10^{-6} – 2.0×10^{-5}	-	10
4-(diethylamino)-salicylaldehyde rhodamine hydrazone	-	1.0×10^{-6} – 1.2×10^{-4}	-	11
rhodamine B with 3-methyl-2-benzothiazolinone hydrazone	1.36×10^{5}	5.0×10^{-6} – 6.0×10^{-5}	4.7×10^{-7}	12
triazole-based novel bis Schiff base chemosensor	4.70×10^{4}	2.0×10^{-6} – 2.0×10^{-5}	1.2×10^{-6}	13
retinal-based polyene fluorescent probe	-	2.0×10^{-7} – 2.0×10^{-5}	5.0×10^{-8}	14
coumarin derivative	-	5.0×10^{-7} – 1.0×10^{-5}	-	15
2-methyl-7-hydroxy-quinoline	-	1.0×10^{-7} – 6.0×10^{-7}	1.5×10^{-7}	16
naphthalimide derivatives	1.10×10^{10}	4.0×10^{-6} – 7.0×10^{-6}	1.5×10^{-7}	17
benzothiazole-based chemosensor	1.24×10^{4}	8.0×10^{-6} – 5.0×10^{-5}	3.3×10^{-6}	18
quinoline-based compound	3.12×10^{5}	1.0×10^{-6} – 1.0×10^{-5}	3.01×10^{-7}	19
bis-thiophene based Schiff base receptor	1.07×10^{5}	1.0×10^{-6} – 8.0×10^{-6}	8.8×10^{-8}	20
coumarin-based chemosensor	5.22 (lgK)	1.0×10^{-6} – 8.0×10^{-6}	1.97×10^{-7}	21
imidazole[2,1-b]benzothiazole	5.75×10^{5}	0 – 5.0×10^{-5}	4.32×10^{-8}	22
chemosensor Schiff base	-	2.0×10^{-6} – 1.0×10^{-5}	1.8×10^{-6}	23
indole-based chemosensor	2.63×10^{10}	2.0×10^{-6} – 5.0×10^{-5}	8.93×10^{-8}	24
ZnS:Mn/SiO2 nanoparticles	-	8.2×10^{-8} – 5.0×10^{-4}	-	25
europium tetracycline hydrogen peroxide complex	-	2.0×10^{-7} – 1.6×10^{-6}	2.0×10^{-7}	26
N-(2-carboxymethyl)benzoaza-15-crown-5	3.26×10^{2}	1.7×10^{-6} – 2.4×10^{-4}	5.6×10^{-7}	This work

Ka* - association constant

A two-component system where a 14-membered tetrathia crown (strong affinity towards Cu^{2+}) is covalently linked to an anthracene subunit has been described [27].

The supramolecular approach can be applied in fluorescent sensors design for detection of transition metal due to photo-induced electron transfer mechanism. The selection of the appropriate receptor fragment is rather difficult. Selectivity can be achieved through the electronic and stereochemical requirements. Thus, the metal binding subunit can be constructed and changed through the nature and number of donor atoms, the size of the coordinating cavity and the fluorescent fragment.

Crown compounds are able to bind metal cations via multidentate coordination with heteroatoms which are part of the macrocycle. Azacrown compounds are interesting due to their unusual complexing properties, intermediate between the properties of crown ethers, which strongly bind ions of alkali and alkaline-earth metals, and the properties of cyclams, which form stable complexes with ions of heavy and transition metals. Optical sensors for metal cations can be developed on the base of these compounds.

The molecules of benzoaza-15-crown-5 derivatives possessed a large electron delocalization might lead to a sufficient fluorescence, meanwhile, the "N", "O" atoms of the molecule could act as binding sites for recognizing copper ions, but there was no report using these derivative as copper sensitive chemsensor.

The aim of this study is to develop the fluorescent probe - N-(2-carboxymethyl)benzoaza-15-crown-5 - for Cu^{2+} determination with good selectivity in ethanol/H_2O (4:6, v/v) solvent mix using urotropine buffer solution (pH = 7.5).

Materials and Methods

Apparatus. All fluorescence measurements are carried out on Cary Eclipse (Varian, Australia) fluorescence spectrophotometer within the range (220-700 nm) equipped with a 150-W xenon lamp, 1.0 cm quarts cell. The excitation and emission monochromator band widths were 5 nm. The excitation wavelength was set at 274 nm and the fluorescence was measured using the peak height at 308 nm. All measurements were performed at room temperature (21–23 °C).

A pH meter (Lab 850, Schott Instruments GmbH, Germany) was used for pH adjustment.

Absorption spectra were recorded with a UV-2401 PC (Shimadzu, Japan) spectrophotometer.

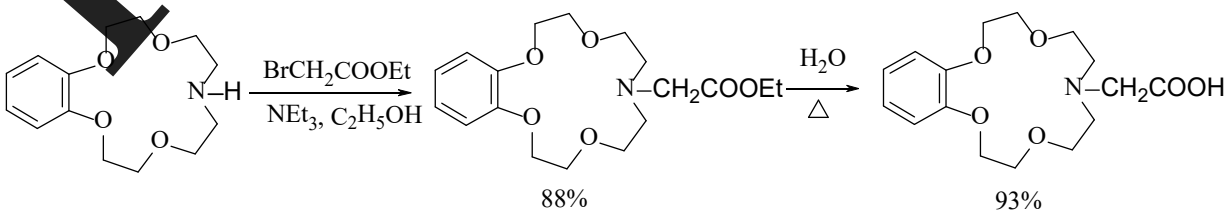
FTIR spectra were recorded using FTIR-8400S (Shimadzu) spectrometer with Specac Quest ATR diamond accessory High throughput diamond. Electrospray ionization (ESI) mass spectra were recorded on a 6530 Accurate Mass Q-TOF (Agilent Technologies, USA) mass spectrometer.

Synthesis of the probe (Cr). To a solution of 5.35 g (0.02 moL) of benzoaza-15-crown-5 and 5.56 mL (0.04 moL) of triethylamine in 50 mL ethanol was added 3.2 mL (0.03 moL) of ethyl bromoacetate and the reaction mixture was boiled for 24 hours (Scheme 1).

The solvent was evaporated to dryness under reduced pressure, the resulting N-(ethoxycarbonylmethyl)-benzoaza-15 crown-5 residue was extracted with heptane (3×75 mL) and then recrystallized from heptane. Yield: 5.4 g (88%). mp 53–54 ºC. To 5.4 g (0.0153 moL) of the crown-ether obtained was added 60 mL of distilled water, and the mixture was boiled for 24 hours. After cooling, the reaction mixture was extracted with 40 mL of CH_2Cl_2. The extract was discarded, and the aqueous layer was evaporated to dryness under reduced pressure. The resulting residue was dried in vacuo to constant weight at 80 ºC. An obtained oily compound was crystallized on standing. Yield: 4.6 g (93%), mp 75.0–75.5 ºC. m/z (LC-MS) 326 $[M+H]^+$. Anal. Calcd for $C_{16}H_{23}NO_6$: C 59.06, H 7.13; Found C 59.01, H 7.04. IR ν_{max} cm^{-1}: 3260 (OH), 1645, 1622 (C=O), 1119 (C-O-C). ^1H NMR ($CDCl_3$) δ_H, ppm: 3.03 (br.s, 4H, CH_2N), 3.47 (br.s, 2H, CH_2), 3.74 (br.s, 4H, CH_2O), 3.85 (t, *J*=3.5 Hz, 4H, CH_2O), 4.13 (t, *J*=3.4 Hz, 4H, CH_2O), 6.87–6.92 (m, 4H, Ar), 9.82 (br.s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ_C, ppm: 55.70 (2C, CH_2N), 58.76 (1C, CH_2), 68.84 (2C, CH_2O), 69.12 (2C, CH_2O), 69.58 (2C, CH_2O), 113.91 (2C, Ar), 121.51 (2C, Ar), 148.81 (2C, Ar), 172.43 (1C, COO).

Reagents. All of the used chemicals were of analytical grade or chemically pure; doubly-distilled water was used.

The standard solution of N-(2-carboxymethyl) benzoaza-15-crown-5 (1×10^{-3} M) was prepared by dissolving accurate weights of the solid compound in ethanol.



Scheme 1. Synthetic strategy of the probe N-(2-carboxymethyl)benzoaza-15-crown-5 (Cr).

The working solutions of copper(II) nitrate (1.7×10^{-4} M, 1.7×10^{-3} M) was prepared from standard solution of copper ions (1.7×10^{-1} M) RS 143-2020 (Ukraine). All metal salts were used as either their nitrate or their chloride salts.

A solution (2.4×10^{-4} M) of L-Tryptophan («Merck», CAS 73-22-3) was prepared in distilled water. An urothropine buffer was prepared by dissolving 40.0 g of urothropine in 100 mL volumetric flask with water.

Measurement procedures of absorption and fluorescence spectra of Cr in presence of different concentration of Cu^{2+}. 0, 0.1, 0.5 mL of Cu^{2+} working solution (1.7×10^{-4} M) and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 0.9, 1.0, 1.1, 1.3, 1.4 mL of Cu^{2+} working solution (1.7×10^{-3} M) were placed into volumetric flasks. 4.0 mL of Cr standard solution (1×10^{-3} M) and 1.0 mL of urotropine buffer (40 %) were added to each of these volumetric flasks. The solutions were diluted with water up to 10 mL and stirred. In 5 minutes the fluorescence intensity of solutions at $\lambda_{ex}/\lambda_{em}$ = 274 / 308 nm and the optical density at λ = 231 nm and 273 nm are measured.

Measurement procedures of the quantum yield of probe. The fluorescence quantum yield of Cr was estimated by integrating the area under the fluorescence curves with the equation:

$$\Phi_i = \Phi_{ref} \frac{A_{ref} \cdot S_i \cdot \mathrm{n}_\mathrm{i}^2}{A_i \cdot S_{ref} \cdot \mathrm{n}_\mathrm{ref}^2}$$

where *S* is the area under the fluorescence spectral curve, *A* is the optical density of the compound at the excitation wavelength and *n* is the refractive index of the solvent used. The standard used for the measurement of fluorescence quantum yield was L-Tryptophan (Φ = 0.13 in water) [28].

Estimation of analytical characteristics. How to evaluate some analytical characteristics such as selectivity, recovery and precision in the validation of the method is described in the Guidelines [29]. For example, the values of the limit of detection (LOD) and the limit of quantification (LOQ) are calculated from the values of the standard deviation of the intercept (s_a) and the slope (b) of the calibration curve:

$LOD = 3.3 \cdot s_a / b$, $LOQ = 10 \cdot s_a / b$.

Results and discussion

Spectral characteristics. The absorption spectra of the Cr in solutions are characterized by the presence of two bands in the UV with peaks around 231 nm and 273 nm. The molar absorbtivity (1175, 1917 $L \times mol^{-1} \times cm^{-1}$) of these bands enable effective absorption of excitation light energy.

The interaction between the probe and Cu^{2+} ions was thoroughly examined with UV absorption spectra. For the UV experiments, the absorption spectrum of free probe solution was first measured, as shown in Fig. 1. When Cu^{2+} solution was gradually added into the probe solution, absorption bands at 231 nm and 273 nm gradually increased as the amount of Cu^{2+} ions increased. When more than 1 equiv. of Cu^{2+} ions was added, the new absorption band at 269 nm appears, suggesting the formation of a 1:1 stoichiometric copper(II) complex.

To determine the association constant of Cr with Cu^{2+}, the obtained absorption data were processed using the method [30]. The results clearly suggest that this probe has a high sensitivity for Cu^{2+} ions in 1:1 stoichiometric reaction due binding affinity (binding constant 3.26×10^2 M^{-1}) of the probe to the Cu^{2+} ions.

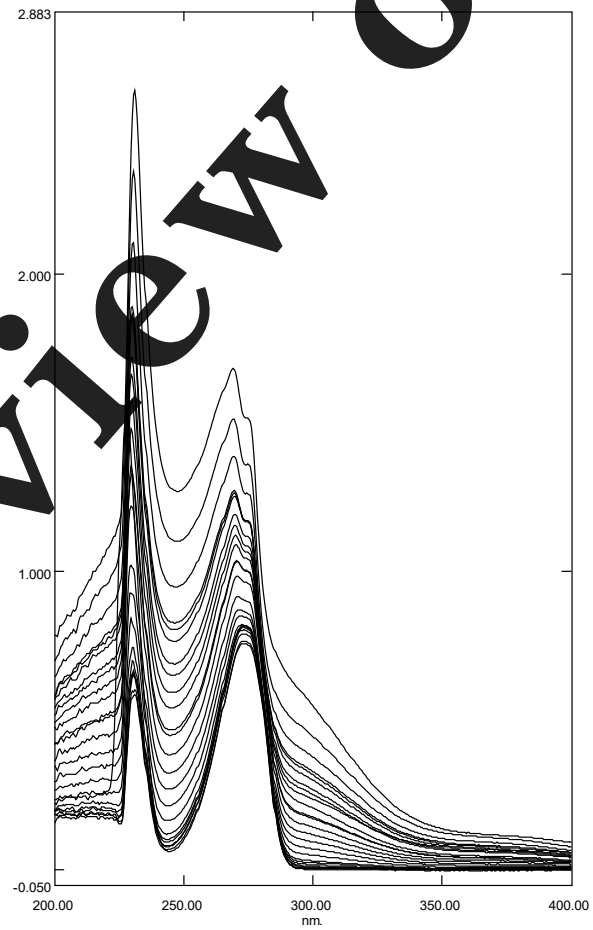


Fig. 1. The absorption spectrum of the probe in the presence of different concentration of Cu^{2+} ($C_{Cr} = 4 \times 10^{-4}$ M).

The excitation and emission spectra of Cr were recorded. Fig. 2a shows the excitation spectrum of the Cr monitored at 308 nm. The emission bands of the Cr (Fig. 2b) are located at 308 nm with fluorescence quantum yield (0.15 ± 0.01), which was measured in urotropine buffer of pH 7.5 relative to L-Tryptophan. The fluorescence intensity of Cr is greatly quenched in the presence of Cu^{2+} (Fig. 2b).

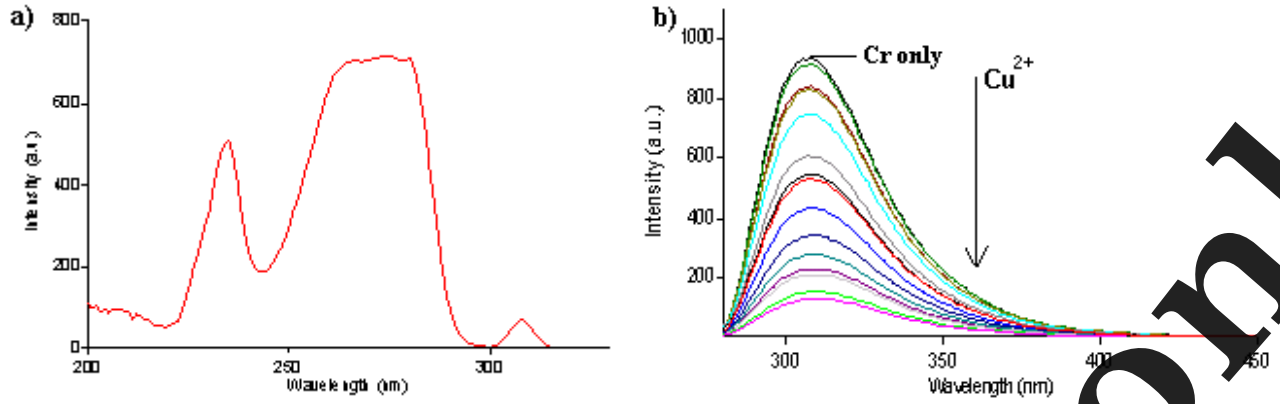


Fig. 2. Excitation spectra of Cr (a) and fluorescence spectra of Cr in the presence of different concentration of Cu^{2+} (b) (C_{Cr} = 4 × 10^{-4} M; $\lambda_{ex}/\lambda_{em}$ = 274/308 nm).

Stability on fluorescence of the probe. To evaluate the practical applicability of the probe (Cr), the effect of pH on the fluorescence intensity was tested at the pH range from 2 to 12. It was found that the optimum range of sensing Cu^{2+} ion by Cr is between pH 4 and 9.

The photostability was also investigated by irradiating the probe solution under a xenon light source (Fig. 3). After excitation at 274 nm (30 s intervals for each time), no apparent change in fluorescence intensity was observed, suggesting the good photostability of the probe which ensures the reliability of the fluorescence measurements.

Selectivity study

Selectivity is one of the most important criteria for potential applications of a cation probe. Therefore, the 15 different metal ions (K^+, Na^+, Li^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Al^{3+}) (4.25 × 10^{-4} M) were separately added into Cr solution (4 × 10^{-4} M), and the complexation was studied using fluorescence emission spectroscopy (Fig. 4). None of the cations, except Cu^{2+}, led to prominent fluorescence quenching of the peak at 308 nm upon binding with Cr. The experimental data show that the probe Cr has a high selectivity for copper ions over other metal ions.

Analytical performance. The proposed sensing probe was validated in terms of linearity, accuracy, inter and intra-day precision and specificity (Table 2).

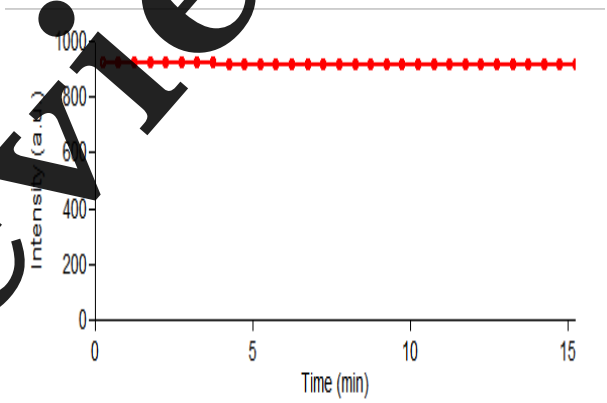


Fig. 3. Dependence of the fluorescence intensity of the probe on time by irradiation ($\lambda_{ex}/\lambda_{em}$ = 274/308 nm; C_{Cr} = 4 × 10^{-4} M).

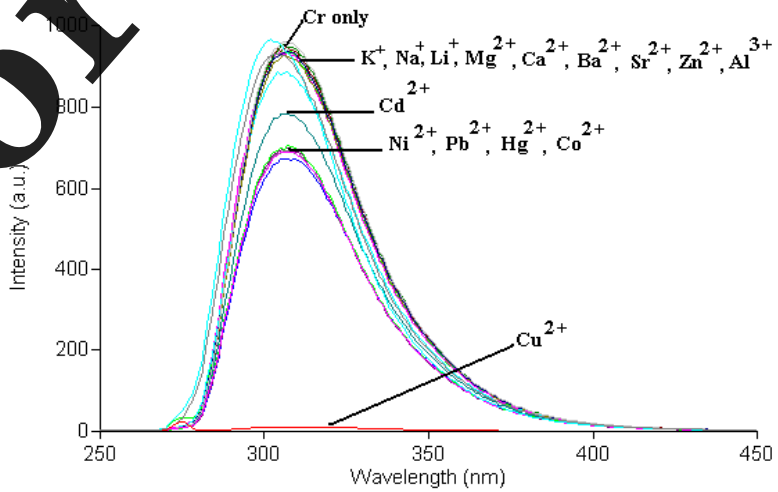


Fig. 4. Fluorescence spectra of Cr (4 × 10^{-4} M) in the presence of different metal ions (C_{Me} = 4.25 × 10^{-4} M; $\lambda_{ex}/\lambda_{em}$ = 274/308 nm).

Table 2. Summary of estimation of analytical characteristics of the proposed probe.

Parameter	Result
Linear range (M)	1.70×10^{-6}-2.38×10^{-4}
LOD (M)	5.6×10^{-7}
Correlation coefficient (r)	0.99734
Accuracy (n=5) (%)	100
Precision	
Inter-day (n=6) (%)	2.9
Intra-day (n=6) (%)	3.1
Specificity	specific

The different concentrations of Cu^{2+} were added to probe. The plot of Stern-Volmer was obtained (Fig. 5a). F_0 and F were measured at λ_{ex} = 274 nm and λ_{em} = 308 nm. The plot of Stern-Volmer was obtained $F_0/F = 1.19 - 3161.4\ C_{Cu} + 1.16\ C_{Cu}^2$; correlation coefficient is 0.99434; where F_0 and F the relative fluorescence intensities of the system without and with Cu^{2+}, respectively, C_{Cu} is concentration of Cu^{2+} (M). As can be seen from Fig. 5a, the Stern-Volmer plot is found to be non-linear with an upward curvature and is described by the polynominal equation.

When F_0/F was modified by logarithm, a linear relationship between the log (F_0/F) and concentration of Cu^{2+} was obtained: log $(F_0/F) = - 0.00941 + 3557.07\ C_{Cu}$; correlation coefficient is 0.99734 (Fig. 5b). The calibration curve is linear in the 1.70×10^{-6} – 2.38×10^{-4} M range of Cu^{2+}. The LOD and LOQ for Cu^{2+} were found to be 0.56 μM and 1.82 μM respectively.

Accuracy of the analysis was evaluated by carrying out a recovery study at three different concentrations. The results of recovery study indicate that the proposed method is accurate for estimation of Cu^{2+} in drinking water (Table 3).

In terms of sensitivity and selectivity, this probe is at the level with those presented in the literature (Table 1). The advantage of this probe is that it works in a neutral media and has good analytical characteristics as well as high selectivity. However, this probe has a low association constant.

The precision of the method was established by testing the analytical signal corresponding to a Cu^{2+} concentration of 1.02×10^{-4} M. For a series of 12 measurements, the relative standard deviation was 2.9% for the intra-days and 3.1% for the inter-days analysis for Cu^{2+}.

Fluorescence quenching mechanism. The fluorescence quenching of Cr by addition of Cu^{2+} ions could be caused by the binding of Cu^{2+} ions at the recognition unit (Scheme 2).

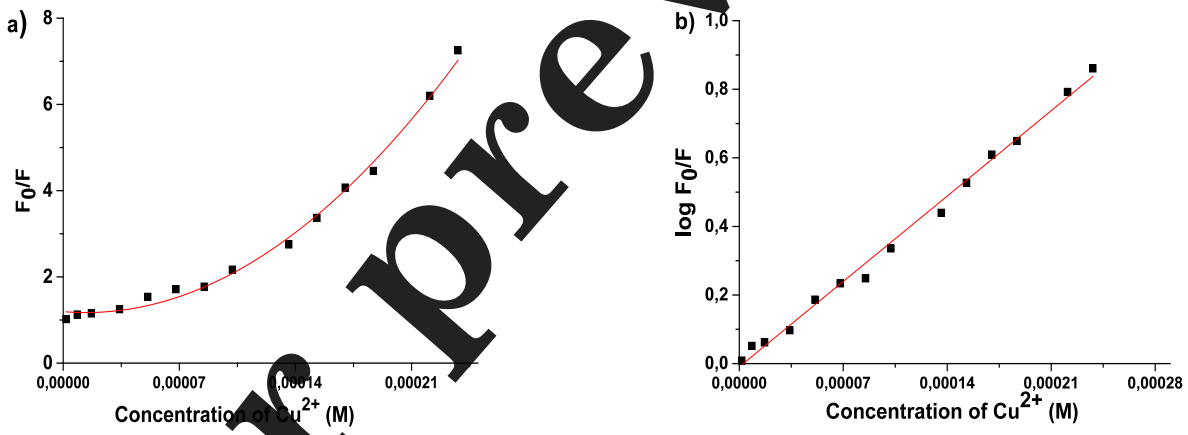
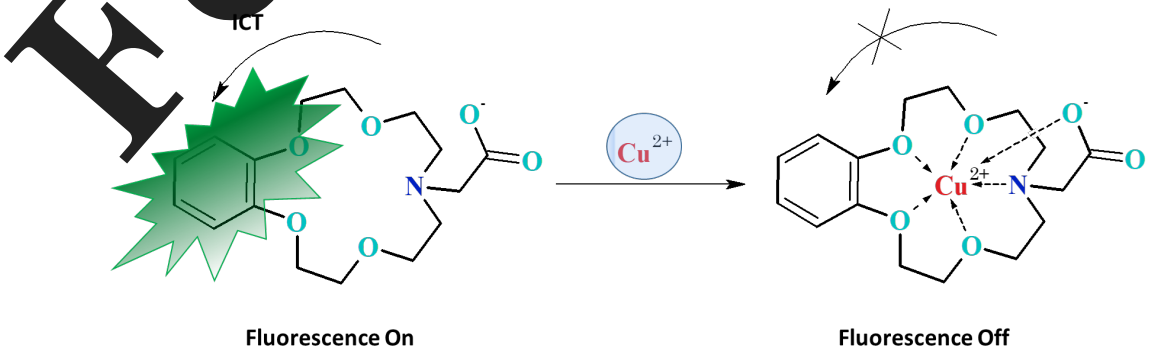


Fig. 5. Non-linear Stern-Volmer plot (a) and modified Stern-Volmer plot (b) for Cu^{2+} determination ($C_{Cr} = 4 \times 10^{-4}$ M; $\lambda_{ex}/\lambda_{em}$ = 274/308 nm).



Scheme 2. Proposed sensing mechanism of Cr with Cu^{2+} ion.

Table 3. Recovery of Cu^{2+} concentration in model solutions (n = 5, P = 95 %).

Concentration added (M)	Concentration found (M)	Recovery (%)	RSD (%)
8.50×10^{-6}	$(8.58 \pm 0.62)\times10^{-6}$	101	5.85
1.02×10^{-4}	$(1.01 \pm 0.06)\times10^{-4}$	99	4.78
2.04×10^{-4}	$(2.05 \pm 0.07)\times10^{-4}$	101	2.70
		Average recovery: 100	

Therefore intramolecular charge transfer (ICT) may be the dominant reason for the fluorescence quenching. The chelated copper(II) centre may involve electrons from the excited benzene moiety and quench the fluorescence. The quenching effect in the presence of copper(II) can be explained by the termination of intramolecular charge transfer from the chelate center to the aromatic part of the molecule due to chelation.

The results suggest that both static and dynamic quenching processes are responsible for the observed positive deviation in the Stern-Volmer plot (Fig. 5a).

Conclusions

A new photostable benzoaza-15-crown-5 derivative (Cr) as a fluorescent probe was synthesized.

It is characterized by selective sensitivity of Cu^{2+} ions over other metal ions in ethanol/H_2O (4:6, v/v) solvent mix using urotropine buffer solution (pH = 7.5) at 25 ºC. The detection limit of the probe for Cu^{2+} ions was found to be 0.56 μM. The proposed method is simple, accurate and easy to perform and can be used for the routine determination of Cu^{2+} ions in drinking water.

Acknowledgements

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