East European Journal of Physics. **4.** 715-719 (2025)
DOI:10.26565/2312-4334-2025-4-78 ISSN 2312-4334

ASSESSMENT OF THE SENSITIVITY OF A FLUORESCENT SQUARAINE DYE TO HEAVY METAL IONS

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Received August 30, 2025; revised September 16, 2025; accepted October 21, 2025

The contamination of natural water systems with heavy metal ions poses a significant global environmental and public health threat due to their persistence, bioaccumulation, and severe toxicological effects. Consequently, the development of rapid and sensitive detection methods is essential for effective water quality monitoring. Squaraine dyes represent a promising class of chemosensors for heavy metal detection owing to their high molar extinction coefficients, near-infrared fluorescence, and pronounced spectral responsiveness to metal binding. In this study, we evaluate the sensitivity of the symmetric squaraine dye SQ-1 toward four environmentally relevant heavy metal ions – Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+} – and explore its applicability within a β-lactoglobulin/SQ-1 nanosystem for metal sensing in aqueous media. Spectroscopic analysis revealed metal-dependent modulation of SQ-1 optical properties, driven largely by alterations in dye aggregation and metal–dye coordination. Ni^{2+} and Pb^{2+} promoted SQ-1 deaggregation and enhanced fluorescence emission, whereas Cu^{2+} induced pronounced quenching consistent with strong coordination. Our results indicate that, SQ-1 retained its responsiveness in the presence of β-lactoglobulin fibrils, exhibiting metal-specific fluorescence changes indicative of combined dye–metal–fibril interactions. Further studies are warranted to assess SQ-1 performance toward additional metal ions and to elucidate the molecular mechanisms underlying metal-induced modulation of its photophysical behavior.

Keywords: *Squaraine dye; Metal detection; Heavy metals*
PACS: 87.14.Cc, 87.16.Dg

The contamination of natural water systems with heavy metal ions remains a critical global environmental and public health issue. Metals such as mercury (Hg^{2+}), lead (Pb^{2+}), cadmium (Cd^{2+}), copper (Cu^{2+}), and iron (Fe^{3+}) persist in aquatic environments due to their non-biodegradable nature and tendency to bioaccumulate in living organisms. Exposure to these metals, even at trace levels, can result in severe toxicological effects, including neurotoxicity, carcinogenicity, kidney dysfunction, and developmental disorders [1-4]. Consequently, the development of rapid, sensitive, and cost-effective analytical methods for heavy metal detection is essential for monitoring water quality and ensuring environmental safety. In this context, organic fluorescent dyes that undergo distinct spectral changes upon interacting with metal ions have emerged as a particularly promising class of chemosensors, offering high sensitivity, fast response times, and the potential for direct visual detection [5-6].

Among the diverse families of fluorescent probes used for heavy metal detection, squaraine dyes have attracted significant attention due to their distinctive optical properties, including intense absorption and emission in the visible to near-infrared (NIR) region [7,8], high molar extinction coefficients [9], and unique donor-acceptor-donor (D-A–D) resonance-stabilized zwitterionic structure [10,11]. Numerous studies have demonstrated their high sensitivity and selectivity toward heavy metal ions, which is largely attributed to their strong electron-accepting core, extended π-conjugation, and ability to form stable complexes with metal species [12-17]. These structural features enable pronounced changes in their optical properties – such as absorption or fluorescence – upon metal binding, allowing for rapid and accurate detection even at low concentrations [12-17]. As a result, squaraine-based sensors have gained significant attention for monitoring toxic metal contaminants in biological systems, industrial wastewater, and environmental samples [12-17]. To exemplify, Chen et al. developed a novel squaraine-based chemosensor for Hg^{2+} ion selective detection, whose sensing mechanism relies on a pronounced colour change resulting from dye disaggregation upon coordination with the heavy metal [14]. A simple 1,2-squaraine derivative has been shown to provide dual colourimetric detection of Fe^{3+} and Hg^{2+} ions, with visible colour change and detection limits of 0.538 µM for Fe^{3+} and 1.689 µM for Hg^{2+} [15]. Similarly, more recently developed NIR-absorbing squaraine dyes (e.g., BBSQ) demonstrate selective response to Fe^{3+}, Cu^{2+} and Hg^{2+} even in the presence of competing metal ions, with observable colour changes and acceptable detection limits ~6 µM for Cu^{2+} [16]. Thapa et al. also developed a near-infrared squaraine-based chemosensor, SQ-68, whose solvent-dependent sensing mechanism enables selective detection of Cu^{2+} Ag^{+} in real water samples with recovery rates ranging from 73–95% for Cu^{2+} to 59–99% for Ag^{+} [17]. Shafeekh et al. reported the highly selective and sensitive colourimetric detection of Hg^{2+} ions by sulphonate group-containing unsymmetrical squaraine dyes [18].

The present study aims to expand the application of squaraine dyes for heavy metal ion detection by evaluating the sensitivity of the squaraine dye SQ-1 (Figure 1) toward Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+} ions. Specifically, the study had two main objectives: first, to assess the sensitivity of SQ-1 to these metal ions, and second, to explore the potential development of a β-lactoglobulin/SQ-1 nanosystem for detecting heavy metals in water.

Cite as: U. Malovytsia, O. Zhytniakivska, K. Vus, V. Trusova, G. Gorbenko, East Eur. J. Phys. 4, 715 (2025), https://doi.org/10.26565/2312-4334-2025-4-78

EXPERIMENTAL SECTION

Materials

Bovine β-lactoglobulin (βlg), copper(II) chloride dihydrate, nickel(II) chloride, lead(II) nitrate, zinc chloride and thioflavin T (ThT) were purchased from Sigma, USA. The symmetric squarylium derivative SQ-1 (Figure 1) was synthesized according to previously reported procedures [10] and was kindly provided by Professor A. Vasilev. All other reagents were of analytical grade and used without the further purification.

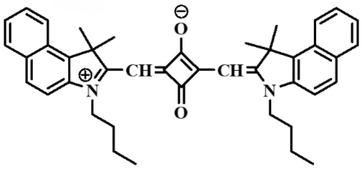

Figure 1. Structural formula of the symmetric squarylium dye SQ-1.

Spectroscopic measurements

SQ-1 stock solution was prepared in ethanol, whereas ThT was dissolved in 10 mM Tris buffer (pH 7.4). The β-lactoglobulin stock solution (10 mg/mL, βlgF) was prepared in distilled water and adjusted to pH 2.0 using HCl. Fibrillization was induced by incubating the protein solution at 90 °C for 2 days. Amyloid formation kinetics were monitored by the Thioflavin T (ThT) assay [19]. Working protein solutions were obtained by diluting the fibrillar β-lactoglobulin stock in distilled water (pH 6.07). All fluorimetric measurements were performed in distilled water at pH 6.07.

The absorption spectra of the examined dyes were recorded with a Shimadzu UV-2600 spectrophotometer (Japan) at 25ºC. The dye concentrations were determined spectrophotometrically using the extinction coefficients $\varepsilon_{663}^{EtOH} = 2.3\cdot10^{5}$ $M^{-1}cm^{-1}$ and $\varepsilon_{412}^{water} = 3.6\cdot10^{4}$ $M^{-1}cm^{-1}$ for SQ-1 and ThT, respectively. Steady-state fluorescence spectra were recorded with an RF-6000 spectrofluorimeter (Shimadzu, Japan). Fluorescence measurements were performed at 25°C using 10 mm pathlength quartz cuvettes. Fluorescence spectra were recorded in the range 620–825 nm with an excitation wavelength of 600 nm. The excitation and emission slit widths were set at 10 nm**.**

RESULTS AND DISCUSSION

Squaraine dye SQ-1 features a symmetric zwitterionic structure, comprising a central squarate core flanked by two butyl-substituted heterocyclic chromophores [10]. This intrinsic electronic symmetry gives rise to a sharp and intense absorption spectrum, characterized by a dominant peak at 662 nm and a secondary shoulder at 614 nm in ethanol [10]. SQ-1 has demonstrated remarkable versatility across a broad range of applications [10,20–24]. Notably, the dye exhibits high sensitivity to protein-induced modifications in the structural and physicochemical properties of lipid bilayers [10], serves as an effective fluorescent probe for detecting reactive oxygen species [9,20], and provides a powerful platform for both detecting and structurally analysing amyloid fibrils [21]. Owing to its near-infrared fluorescence, SQ-1 has proven highly suitable for the development of photoluminescent amyloid-based nanomaterials, achieved by functionalizing insulin nanofibrils with the dye, where SQ-1 acts as a long-lasting fluorescent sensor within the cascade system [22, 23].

In the present study, we extend the application of the squaraine dye SQ-1 by assessing its sensitivity to Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+} ions, while also exploring the potential development of a β-lactoglobulin/SQ-1 nanosystem for detecting heavy metals in water. Figure 1 shows the absorption and fluorescence spectra of the fluorophore in aqueous solution and in the presence of these metal ions. The absorption spectrum of SQ-1 in water exhibits two peaks at 614 nm and 660 nm, with the shorter-wavelength component showing higher absorbance than the longer-wavelength peak. Assuming that in ethanol the short-wavelength component appears as a minor spectral shoulder, the dominant 614 nm peak observed in aqueous solution suggests dye aggregation due to π–π stacking interactions. The tendency of squaraine dyes to aggregate in water has been reported in previous studies and is typically dependent on dye concentration [7, 14]. The addition of heavy metal ions led to a pronounced decrease in the absorbance of H-aggregates, with the effect diminishing in the order $Ni^{2+} > Zn^{2+} > Pb^{2+} > Cu^{2+}$. Importantly, the monomer peak (~660 nm) predominated in all systems investigated, except Zn^{2+}, where the absorbance of the monomer and H-aggregates remained comparable, indicating partial retention of aggregation in the presence of this ion. Moreover, as shown in Figure 1, binding with Cu^{2+} and Pb^{2+} induces approximately a 1.6-fold decrease in absorbance at 614 nm, accompanied by a reduction in monomer absorption, a 28 nm red shift of the monomer maximum, and notable band broadening.

In aqueous solution, SQ-1 displays a broad emission spectrum with a prominent peak at 715 nm. The addition of heavy metal ions leads to pronounced changes in the dye's fluorescence intensity, with the magnitude of these effects depending on the specific metal ion. Notably, binding with Ni^{2+} and Pb^{2+} induces an approximately 1.4-fold increase in fluorescence intensity at 715 nm, accompanied by a 3 nm red shift of the emission maximum and an enhanced shoulder at 787 nm, with the effect being most pronounced for Ni^{2+}. In contrast, complexation of SQ-1 with Cu^{2+} leads to a decrease in fluorescence intensity at 715 nm, along with a 2 nm red shift of the emission maximum.

Numerous studies indicate that the sensing mechanism of squaraine dyes presumably relies on coordination between the dye molecule and the metal ion, which can induce significant changes in both the dye's electronic structure and its aggregation behavior [12-17]. In general, probe molecules exhibit selectivity for specific metal ions based on how effectively ligand atoms with lone-pair electrons, such as nitrogen, oxygen, and sulfur, can coordinate with those ions. Therefore, to interpret the aforementioned changes in the absorption and fluorescence spectra of the dyes, it is necessary to discuss the possible mechanisms underlying the squaraine dye–metal interactions. Numerous studies examining the sensing behavior of squaraine dyes indicate that, upon interaction with a target metal ion, they may (i) form coordination complexes through electron-rich sites on the dye, or (ii) disrupt dye aggregates, which are commonly present in aqueous environments. These processes enable squaraine dyes to function as either "turn-on" or "turn-off" sensors, depending on the specific metal ion and dye structure [12–17].

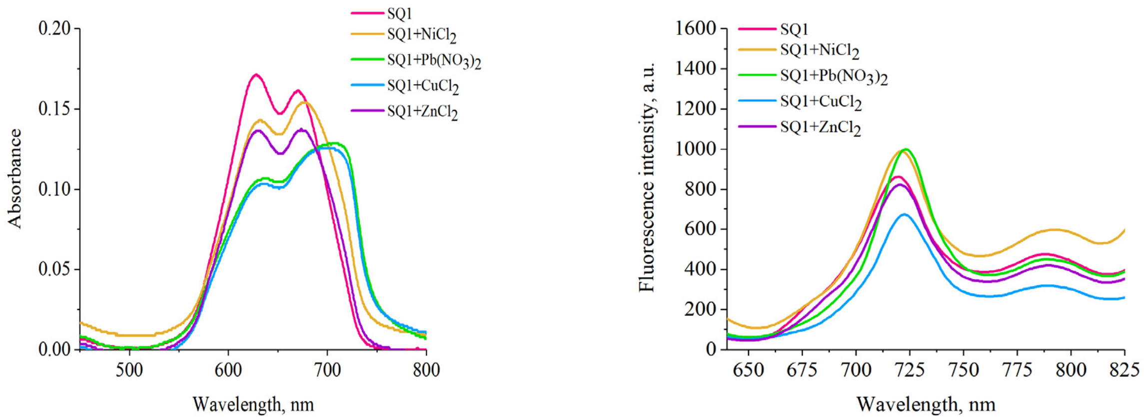

Figure 1. Absorption (left) and emission spectra (right) of SQ-1 in water and in the presence of heavy metal ions. Heavy metal concentration was 423 μM. SQ1 concentration was 2 μM.

One possible explanation for the aforementioned changes in the absorption and fluorescence spectra of the dyes lies in the interplay between metal–dye interactions and the aggregation state of SQ-1. In aqueous solution, the dominant absorption band at 614 nm indicates the presence of H-type aggregates formed through π–π stacking of squaraine molecules. Upon addition of heavy metal ions, these aggregates are disrupted to varying extents, leading to a decrease in aggregate absorbance and a corresponding increase in the monomeric band near 660 nm. The differing degrees of aggregate disruption ($Ni^{2+} > Zn^{2+} > Pb^{2+} > Cu^{2+}$) likely reflect variations in the binding affinity and coordination geometry of each metal ion with the squaraine dye. Zn^{2+} appears to partially preserve the aggregated state, as suggested by the comparable intensities of the monomer and H-aggregate peaks, possibly due to weaker or more sterically hindered interactions. Notably, a study by Chen et al. reported that among multiple tested metal ions, only Hg^{2+} produced a significant sensing response for a related squaraine dye, underscoring the strong dependence of sensing behavior on dye structure and metal-binding characteristics [14].

The fluorescence response of SQ-1 further supports this interpretation. The enhanced emission observed upon interaction with Ni^{2+} and Pb^{2+} may arise from deaggregation, which reduces nonradiative decay pathways and thus promotes more efficient radiative emission. The observed red shifts and increases in shoulder intensity suggest subtle modifications of the dye's electronic environment, potentially caused by metal-induced conformational adjustments or stabilization of specific dye orientations. In contrast, Cu^{2+} induces fluorescence quenching, likely due to stronger coordination with the squaraine core that facilitates nonradiative pathways—such as electron or energy transfer—or promotes structural perturbations that favor aggregation.

In the next phase of our study, we examined the response of SQ-1 to heavy metal ions in the presence of amyloid fibrils. Figure 2 presents the emission spectra of SQ-1 in systems containing β-lgF and various metal ions. In the absence of metals, SQ-1 exhibits a strong emission peak at 660 nm with a weaker shoulder around 760 nm. The addition of heavy metal ions modulates the relative intensities of these bands in a metal-specific manner, reflecting differential interactions among SQ-1, β-lgF, and the metal ions. Specifically, the addition of Zn^{2+}, Ni^{2+}, and Pb^{2+} did not substantially alter the overall shape of the emission spectra but did affect the peak intensities. Compared to the SQ-1–β-lgF system, an enhancement in fluorescence intensity was observed in the presence of these ions, with the effect being most pronounced for Zn^{2+}. In contrast, the addition of Cu^{2+} to the SQ-1–β-lgF system resulted in a 1.4-fold decrease in emission intensity.

A possible explanation for these observations is that metal ions interact with both the dye and the amyloid fibrils, altering the local environment of SQ-1. Zn^{2+}, Ni^{2+}, and Pb^{2+} may weakly bind to the fibrils or interact with the dye in a way that restricts nonradiative relaxation, leading to enhanced fluorescence. On the other hand, Cu^{2+} likely binds more strongly to the fibrils and/or the dye, inducing conformational changes or promoting quenching interactions that decrease emission intensity. These results suggest that the fluorescence response of SQ-1 is determined not only by direct dye–metal interactions but also by how metal ions influence the structural and electronic environment of the fibril-bound dye.The influence of metal ions on aggregation plays a critical role in determining the absorption and emission behavior of SQ-1. However, further studies are needed to evaluate SQ-1's sensitivity to additional metal ions and to elucidate the molecular mechanisms underlying metal–dye and metal–dye–fibril interactions.

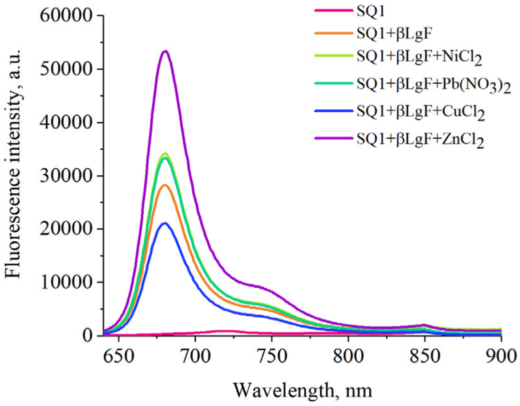

Figure 2. Emission spectra of SQ-1s in water, in the presence of 6.64 µM β-lgF (B), and in the presence of heavy metal ions. Heavy metal concentration was 423 µM. SQ1 concentration was 2 µM

CONCLUSIONS

In the present study, the sensitivity of the fluorescent squaraine dye SQ-1 toward four environmentally relevant heavy metal ions—Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+}—was evaluated. The results demonstrated that the dye's optical properties are metal-dependent, primarily influenced by changes in aggregation state and metal–dye coordination. Ni^{2+} and Pb^{2+} promoted SQ-1 deaggregation and enhanced fluorescence emission, whereas Cu^{2+} induced fluorescence quenching, consistent with strong coordination interactions. In the presence of β-lactoglobulin fibrils, SQ-1 retained its responsiveness and exhibited metal-specific fluorescence changes, indicating the potential for developing protein-based nanosystems for heavy metal detection in aqueous media. Further studies are required to assess SQ-1 performance toward additional metal ions and to elucidate the molecular mechanisms underlying metal-induced modulation of its photophysical behaviour.

Acknowledgements

This work was supported by the Ministry of Education and Science of Ukraine (the project "Development of economically affordable nanosystems for rapid identification and purification of water from heavy metal ions based on carbon nanoallotropes and amyloids from organic waste" No. 0124U000968).

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ОЦІНЮВАННЯ ЧУТЛИВОСТІ ФЛУОРЕСЦЕНТНОГО СКВАРАЇНОВОГО БАРВНИКА ДО ІОНІВ ВАЖКИХ МЕТАЛІВ

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Забруднення природних водних систем іонами важких металів становить серйозну глобальну екологічну та медико-біологічну загрозу через їхню стійкість, здатність до біоакумуляції та виражені токсикологічні ефекти. Відповідно, розроблення швидких і чутливих методів детектування є необхідним для ефективного моніторингу якості води. Сквараїнові барвники є перспективним класом хемосенсорів для виявлення важких металів завдяки їхнім високим молярним коефіцієнтам поглинання, ближньоінфрачервоній флуоресценції та вираженій спектральній чутливості до зв'язування з металами. Дана робота спрямована на дослідження чутливості симетричного сквараїнового барвника SQ-1 до чотирьох екологічно значущих іонів важких металів - Cu^{2+}, Zn^{2+}, Ni^{2+} та Pb^{2+} — і дослідження можливість його застосування у β-лактоглобулін/SQ-1 наносистемах для сенсингу металів у водному середовищі. Спектроскопічний аналіз виявив модулювання оптичних властивостей SQ-1 залежно від типу металу, зумовлене переважно змінами у ступені агрегації барвника та координації барвник–метал. Іони Ni^{2+} і Pb^{2+} сприяли дезагрегації SQ-1 та підсиленню флуоресценції, тоді як Cu^{2+} викликав виражене гасіння, можливе при координації. Наші результати свідчать, що SQ-1 зберігає свою чутливість і у присутності фібрил β-лактоглобуліну, демонструючи метал-специфічні зміни флуоресценції, характерні для комбінованих взаємодій барвник–метал–фібрила. Подальші дослідження є необхідними для оцінки ефективності SQ-1 щодо інших іонів металів і для з'ясування молекулярних механізмів, що лежать в основі модулювання його фотофізичної поведінки під впливом металів.

Ключові слова: *сквараїновий барвник; детектування металів; важкі метали*