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SPECTROSCOPIC STUDY OF THE INTERACTIONS OF METAL IONS AND PROTEINS WITH BENZANTHRONE DERIVATIVES

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The present work describes the characterization of complexes between various metals and three benzanthrone derivatives bearing structurally similar substituents. Within this investigation, the obtained complexes were meticulously examined using spectroscopic techniques. The absorption spectra of solutions of the obtained complexes in the UV-visible region showed a small bathochromic or hypsochromic shift in the visible region compared to the initial compounds. In the emission spectra of these complexes, significant changes in the positions and intensities of the fluorescence bands were observed as a function of the dyes' chemical structures. Interaction with metal ions in the presence of β-lactoglobulin amyloid fibrils showed metal-specific effects, including fluorescence enhancement with Zn^{2+} and pronounced quenching with Cu^{2+}, suggesting a combined contribution of dye–metal and fibril–metal interactions. These findings highlight the potential of benzanthrone derivatives, both free and fibril-bound, as sensitive and selective fluorescent probes for heavy metal detection, providing a foundation for the development of hybrid biomaterial-based sensing platforms.

Keywords: *Benzanthrone dyes; Metal complex; Optical spectroscopy; β-lactoglobulin*
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With the expansion of industrial and agricultural activities and the consequent direct and indirect anthropogenic impacts on the environment, heavy metals have emerged as a global concern due to their contamination of aquatic systems, soils, and other ecosystems, posing significant environmental and health risks. Previous studies have identified Zn, Pb, Hg, Cr, and Cd as exhibiting notable toxicity and potential ecological hazards [1]. In particular, Pb^{2+}, Cr^{3+}, and Cd^{2+} ions are associated with prolonged detrimental effects on human health and environmental quality [2]. While certain heavy metals play essential roles in biochemical processes at trace levels, elevated concentrations can pose significant risks to human health [3,4].

Chemical sensors play a vital role as devices designed to detect, measure, and monitor specific chemical compounds in a given environment, providing critical data for a wide range of scientific, industrial, medical, and environmental applications [5-7]. These sensors operate by interacting with a sensing material that generates a signal interpreted to determine the concentration of the target compound. Their ability to detect analytes at minimal concentrations has led to their widespread use in areas such as environmental monitoring, clinical diagnostics, food safety, and the pharmaceutical industry. As demand for accurate and reliable data in these areas grows, the development of highly selective sensors has become a research priority.

The rapid advancement of fluorescent probe technology enables faster and more efficient real-time detection of important molecules and ions in complex matrices. The advancement of contemporary science also increasingly highlights the need for comprehensive investigation of biologically relevant small molecules, biomarkers, and proteins. This demand has stimulated the development of biosensors capable of selectively recognizing cellular and biomolecular targets. The investigation of biologically significant proteins increasingly requires the development of novel and highly efficient fluorescent probes. In particular, real-time monitoring of β-lactoglobulin is of considerable importance due to its extensive application in the modern food and beverage industry [8,9].

Therefore, the development of sensitive and selective methods for heavy metal detection is critical to effective environmental monitoring. Benzanthrone derivatives exhibit strong potential as heavy-metal sensors for environmental monitoring owing to their multifunctional characteristics, including broad fluorescence emission and high photostability [10,11]. In this study, we evaluate the sensitivity of benzanthrone-based fluorescent dyes for detecting Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^{2+} ions, and investigate the interactions between the dye molecules and β-lactoglobulin fibrils.

METHODS

Materials

Bovine β-lactoglobulin (βlg), copper (II) chloride dihydrate, nickel (II) chloride, lead (II) nitrate, and zinc chloride were purchased from Sigma, USA. Benzanthrone dyes A, B, and C (see Fig. 1) were synthesized by previously described procedures [12-14]. All other reagents were of analytical grade and used without further purification.

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Preparation of working solutions

The absorption and steady-state fluorescence measurements were carried out in ethanol. Dye concentration was 32 μM and 23 μM for the absorbance and fluorescence measurements, respectively. Heavy metal concentration was 414,3 μM.

The β-lactoglobulin stock solutions 10 mg/ml (βlgF) was prepared in distilled water with HCl (pH 2.0). The protein (stock solutions) fibrillization reaction was conducted at 90°C for 2 days. The working solutions of protein were prepared by dissolving a stock solution of the fibrillar β-lactoglobulin in distilled water (pH 6.07). The fluorometric measurements were carried out in distilled water (pH 6.07).

Spectroscopic measurements

The absorption and steady-state fluorescence spectra were recorded using a Shimadzu UV-2600 spectrophotometer and an RF-6000 spectrofluorimeter (Shimadzu, Japan) at 25°C. All measurements were conducted in 10-mm path-length quartz cuvettes.

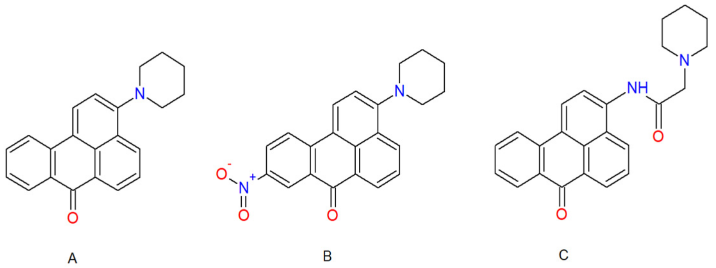

Figure 1. Structure of studied dyes A, B, C.

RESULTS AND DISCUSSION

Our previous studies have shown that amidine derivatives of benzanthrone tend to form complexes with chromium ions [15], and amino derivatives of benzanthrone form complexes with heavy metal ions, showing pronounced spectral changes in solution upon binding of Cu^{2+}, Zn^{2+}, Ni^{2+} and Pb^{2+} [16].

In this study three highly luminescent benzanthrone derivatives A, B, C were used for study of complexation with copper (II), nickel (II) lead (II), and zinc ions. All substances have benzanthrone residue with a piperidine moiety.

In the initial phase of our study, we evaluated dye's ability to detect heavy metal ions. The absorption spectrum of the dye A shows a band in the UV-visible region at 460 nm; the ethanol solutions of the obtained complexes with metal ions showed a bathochromic shift of 10-30 nm from the initial compound (see Fig. 2).

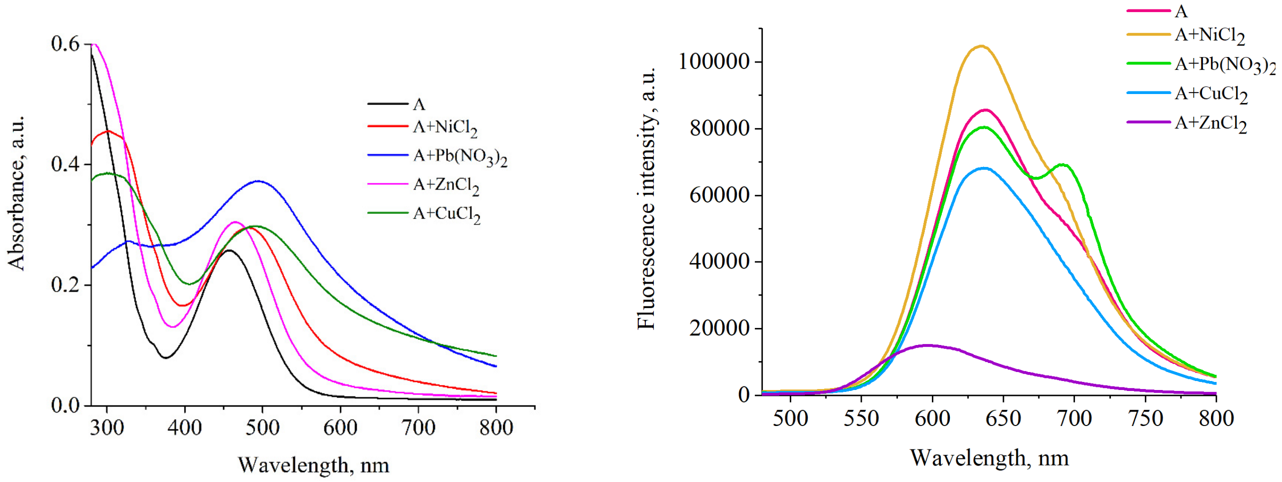

Figure 2. Absorption (left) and emission spectra (right) of dye A in the presence of heavy metal ions. Concentrations of 32 μM and 23 μM were used for absorbance and fluorescence measurements, respectively

The dye A displays a broad emission spectrum with a prominent peak at 630 nm and a shoulder at approximately 700 nm. Upon binding to Ni^{2+} ions, the emission band at 700 nm of dye A diminishes, while the fluorescence around 630 nm increases by approximately 1.3-fold. In contrast, exposure to Pb^{2+}, Cu^{2+}, and Zn^{2+} leads to a decrease in overall fluorescence intensity, with Zn^{2+} producing the most pronounced effect—over sixfold quenching of the 630 nm band—indicating a particularly strong affinity of dye A for zinc ions. The observed shifts in emission peaks are likely due to the formation of metal–ligand charge-transfer complexes, involving electron transfer from the metal ions to nitrogen- and oxygen-containing groups within the dye. Interestingly, the absorption spectrum of dye A shows minimal changes upon complexation with Zn^{2+}, suggesting that the interaction primarily involves the electron transfer switching moiety, which modulates fluorescence without significantly perturbing the fluorophore's ground-state electronic structure [17]. To explore the influence of electron-withdrawing substituents on metal binding and electron transfer modulation, a derivative of dye A (dye B) containing a nitro group was synthesized, and its spectral behaviour in the presence of metal ions was investigated.

The spectral study of the dye B shows an absorption band at 468 nm and a broad emission peak at 657 nm in ethanol. Unlike dye A, derivative B does not change the position of absorption and fluorescence maxima in the presence of metal ions (see Table 1 and Fig. 3). Binding with metal ions results in about a 1.3-fold reduction in fluorescence intensity. Thus, dye B exhibits a lower affinity for metal ions than dye A. Notably, dye B shows an even lower affinity for metal ions than the compound without a nitro group. Apparently, the presence of the nitro group causes a redistribution of electron density that ultimately hinders intracomplex charge transfer.

Table 1. The basic photophysical characteristics of benzanthrone dyes binding with metal ions.

	Dye A				Dye B				Dye C			
	λ_A, nm	I, a.u.	λ_F, nm	I, a.u.	λ_A, nm	I, a.u.	λ_F, nm	I, a.u.	λ_A, nm	I, a.u.	λ_F, nm	I, a.u.
Dye	457	0.28	636	85441	468	0.3810	657	18945	423	0.2426	566 687	14360 16350
Ni^{2+}	484	0.29	634	103930	468	0.3563	657	14540	420	0.2513	548	44780
Cu^{2+}	495	0.29			468	0.3454	657	14220	407	0.2592	543	83500
Zn^{2+}	488	0.301	598	14878	468	0.3492	657	14400	423	0.2435	557 682	21670 15980
Pb^{2+}	517	0.37	635 693	80087 69349	468	0.3478	657	14170	417	0.2399	544	103900

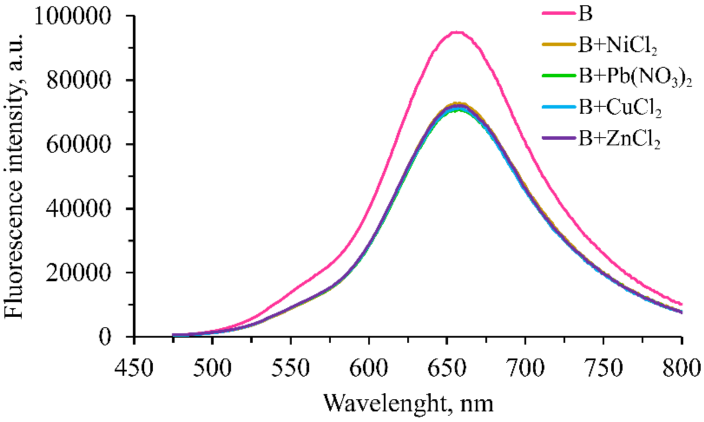

Figure 3. Emission spectra of dye B in the presence of heavy metal ions. Concentration of 23 μM was used for fluorescence measurements

The next object of study was a derivative of benzanthrone in which the piperidine ring is attached to benzanthrone via an acetylamide group (dye C). Such a structure with additional nitrogen and oxygen atoms should presumably facilitate the possible complexation of the dye and metal ions. The performed spectral study confirms this assumption. Observed changes in the absorption and luminescence spectra confirm the formation of complexes between dye C molecules and metal ions. In particular, binding to Ni^{2+}, Cu^{2+} and Pb^{2+} results in a hypsochromic shift of the absorption band by 3–16 nm compared to the free ligand C.

Even more pronounced changes are observed in the luminescence spectra. The dye C displays a broad emission spectrum with a two broad peaks at 566 nm and 687 nm (see Fig. 4). When this dye binds to metal ions, the emission at 566 nm is enhanced, while the band at 687 nm is weakened and disappears completely in the presence of copper and lead ions. Dye C exhibits the highest affinity for lead ions, which is confirmed by a significant increase in fluorescence (more than 7 times). Apparently, when interacting with dye molecules, Pb^{2+} ions stabilize the excited state of the dye, reducing nonradiative decay and leading to high emission intensity at a wavelength of 544 nm.

In recent decades, protein-derived nanomaterials have attracted growing interest as versatile platforms for heavy metal remediation, driven by the chemical diversity of amino acid side chains and their inherent ability to self-assemble into well-defined supramolecular architectures, including fibrillar networks, hydrogels, and nanoscale condensates [18-22]. For instance, hydrogels derived from soy protein have demonstrated selective removal of Cu(II) even in the presence of competing ions [20]. Particularly promising are composite filtration membranes that combine activated carbon with amyloid fibrils obtained from proteins such as β-lactoglobulin or soy protein. These hybrid systems have been shown to efficiently remove a wide range of metal ions—including Au, Hg, Pb, Pd, As, Cr, and Ni—from both model solutions and real wastewater streams [18–23]. Beyond their established adsorption performance, amyloid fibrils exhibit outstanding mechanical stability, high specific surface area, and a dense distribution of functional binding sites, rendering them highly suitable scaffolds for post-functionalization. Despite these advantageous properties, the incorporation of amyloid fibril architectures with fluorescent probes for heavy metal ion sensing remains largely unexplored. Integrating these two approaches could enable the development of hybrid sensing platforms that combine the adsorption capacity and structural robustness of amyloid frameworks with the high sensitivity of fluorescence-based detection. Achieving this, however, requires a detailed understanding of fluorophore–metal interactions as well as the binding mechanisms between metal ions and β-lactoglobulin amyloid fibrils.

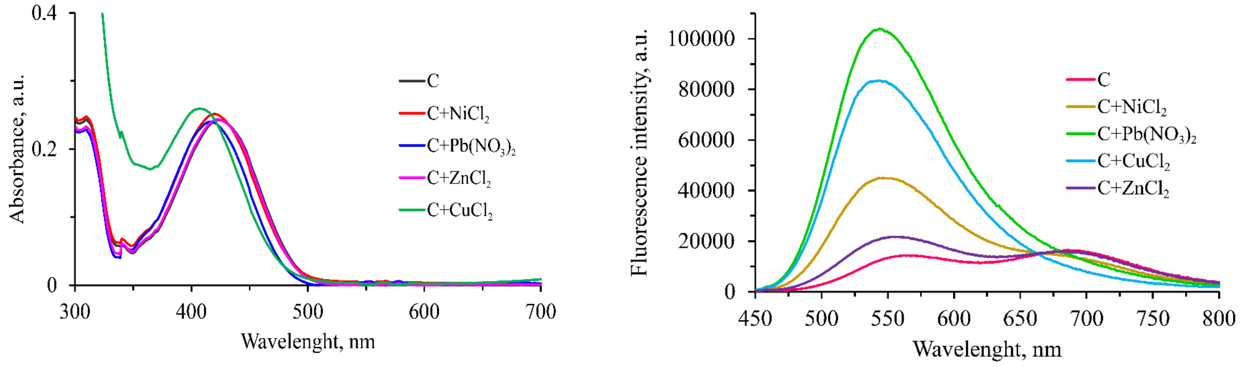

Figure 4. Absorption (left) and emission spectra (right) of dye C in the presence of heavy metal ions. Concentrations of 32 μM and 23 μM were used for absorbance and fluorescence measurements, respectively

Therefore, in the next phase of our study, we investigated the sensitivity of dye A to heavy metal ions in the presence of β-lactoglobulin amyloid fibrils (β-lgF). Fig. 5 illustrates the emission spectra of dye A in systems containing β-lgF (Fig. 5A) and β-lgF +heavy metals (Fig. 5B). As shown in Fig. 5, the free dye in aqueous solution exhibits an emission maximum at 636 nm. Upon addition of fibrillar β-lactoglobulin to dye A in water, a pronounced enhancement in fluorescence intensity was observed, together with an approximately 25 nm hypsochromic shift of the emission maximum, consistent with dye relocation into a more hydrophobic fibrillar environment. The introduction of heavy metal ions modulated the fluorescence intensity of this band in a metal-specific manner. In particular, the addition of Ni^{2+} and Pb^{2+} produced no significant changes in either the emission maximum or the overall fluorescence intensity. In contrast, relative to the dye A–β-lgF system, the presence of Zn^{2+} led to an enhancement in fluorescence intensity, whereas the addition of Cu^{2+} produced a pronounced quenching of the emission. A plausible explanation for these observations is that the metal ions interact simultaneously with the dye and the amyloid fibril scaffold, thereby modifying the local microenvironment surrounding dye A and influencing its photophysical behaviour.

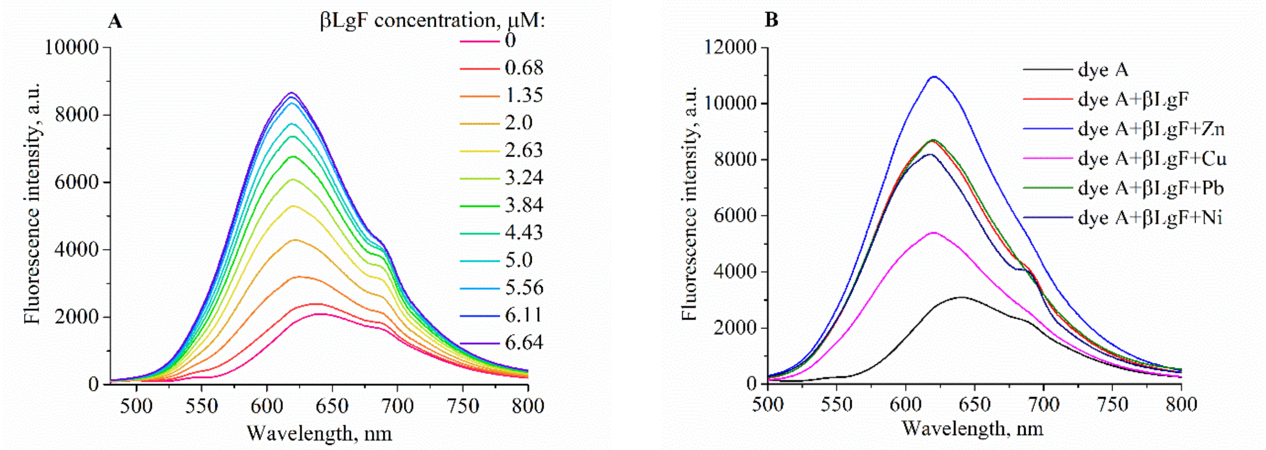

Figure 5. Emission spectra of dye A in the presence of β- lactoglobulin amyloid fibrils (A) and in the systems containing β-lgF and metal ions (B). Dye A concentration was 1 μM

Additionally, differences in metal ion coordination chemistry and binding strength likely contribute to the observed metal-specific effects. Peydayesh et al. [22] reported that the adsorption of chromium, nickel, silver, and platinum ions onto β-lactoglobulin amyloid fibrils is highly exothermic and strongly ion-specific, highlighting the complex and selective nature of these interactions. As can be seen from Fig. 5B, in the presence of Ni^{2+} and Pb^{2+}, no significant changes in emission maximum or intensity are observed. This suggests that these ions likely bind to fibrillar sites located away from the dye and therefore have minimal impact on the local microenvironment of the fibril-bound fluorophore or on the efficiency of the electron transfer process. Moreover, Fan et al. showed that β-lactoglobulin amyloid fibrils exhibit a notably higher adsorption capacity for Cu^{2+} compared to Ni^{2+} [24]. Cu^{2+} is a paramagnetic d^9 ion capable of facilitating photoinduced electron transfer, enhanced intersystem crossing, and static or dynamic quenching when coordinated near the dye [25-27]. Its strong affinity for amyloid fibrils likely positions Cu^{2+} in close proximity to the dye, facilitating quenching of the dye's fluorescence. Similar quenching phenomena have been reported for other PET-active dyes, such as rhodamine B and naphthalimide derivatives, where proximity of Cu^{2+} leads to effective suppression of emission via both static and dynamic quenching mechanisms [26-29].

CONCLUSIONS

The results show that two of the studied benzanthrone dyes exhibit metal-specific fluorescence responses in ethanol solutions. In particular, dyes A and C undergo pronounced spectral changes in ethanol solution upon binding Cu^{2+}, Zn^{2+}, Ni^{2+}, and Pb^2. It was found that binding dye A to Cu^{2+}, Zn^{2+}, and Pb^{2+} ions leads to fluorescence quenching, whereas complexation of dye C with metal ions leads to increased emission. In contrast, compound B with a nitro group demonstrates a lower affinity for metal ions. The results indicate that benzanthrone dyes interact specifically with metal ions in the presence of β-lactoglobulin amyloid fibrils, exhibiting effects characteristic of each metal, including enhanced fluorescence with Zn^{2+} and pronounced quenching with Cu^{2+}. These results demonstrate the potential of using benzanthrone derivatives as sensitive and selective fluorescent probes for heavy metal detection, laying the foundation for the development of biomolecular-based hybrid sensing platforms.

Acknowledgements

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СПЕКТРОСКОПІЧНЕ ДОСЛІДЖЕННЯ ВЗАЄМОДІЇ ІОНІВ МЕТАЛІВ ТА БІЛКІВ З ПОХІДНИМИ БЕНЗАНТРОНУ

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У даній роботі охарактеризовано комплекси різних металів із трьома похідними бензантрону, що містили структурно подібні замісники. Отримані комплекси були детально досліджені за допомогою спектроскопічних методів. У спектрах поглинання комплексів бензантронів з металами виявлено незначні батохромні чи гіпсохромні зсуви у видимій області порівняно зі спектрами за відсутності металів. В спектрах флуоресценції цих комплексів спостерігалися суттєві зміни в положенні та інтенсивності спектральних смуг, які залежали від хімічної структури досліджуваних барвників. При вивченні взаємодії бензантронів з іонами металів в присутності амілоїдних фібрил β-лактоглобуліну виявлені метал-специфічні ефекти, зокрема, зростання флуоресценції в присутності іонів Zn^{2+} та значне гасіння флуоресценції іонами Cu^{2+}, що вказує на внесок обох типів взаємодій – барвник-метал та фібрили-метал. Ці дані свідчать про потенціал похідних бензантрону, як у вільному, так і у фібрил-зв'язаному стані як чутливих та селективних флуоресцентних зондів для детектування важких металів, що створює підґрунтя для розробки гібридних сенсорних платформ на основі біоматеріалів.

Ключові слова: *бензантронові барвники; комплекси металів; оптична спектроскопія; β-лактоглобулін*