

Article

Coptisine Chloride: A Natural Isoquinoline Alkaloid as a Dual-Responsive Aggregation-Induced Emission Sensor for Heparin and Protamine

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Abstract

Heparin (Hep) and its clinical antidote protamine (PRO) play essential yet antagonistic roles in anticoagulant therapy, necessitating reliable analytical tools to monitor their levels and interactions. Herein, we report that coptisine chloride (COP), a natural isoquinoline alkaloid, acts as an aggregation-induced emission (AIE) sensor enabling dual-responsive fluorescence modulation toward Hep and PRO. Owing to its rigid polycyclic and intrinsically twisted molecular framework, COP displays typical AIE behavior. In a DMSO/PBS mixture (PBS fraction = 99%, *v/v*), COP forms strongly emissive aggregates with Hep through electrostatically driven complexation, allowing sensitive Hep detection with a limit of detection (LOD) of 0.70 µg/mL. Subsequent competitive binding of PRO to Hep disrupts the COP–Hep aggregates, giving rise to fluorescence quenching and reversible PRO sensing (LOD: 0.49 µg/mL). Theoretical calculations together with multiple characterization techniques reveal an aggregation–disaggregation mechanism governing the dual fluorescence modulation. Moreover, COP achieves accurate Hep quantification in spiked diluted human serum, affording satisfactory linearity and recoveries (LOD = 0.71 µg/mL; recoveries 98.3–101.6%). These results demonstrate that COP, a structurally simple natural AIE luminogen, serves as a sustainable, biocompatible, and accessible tool for reversible Hep and PRO analysis in complex media.

Keywords: coptisine chloride (COP); aggregation-induced emission (AIE); heparin; protamine; fluorescence sensing



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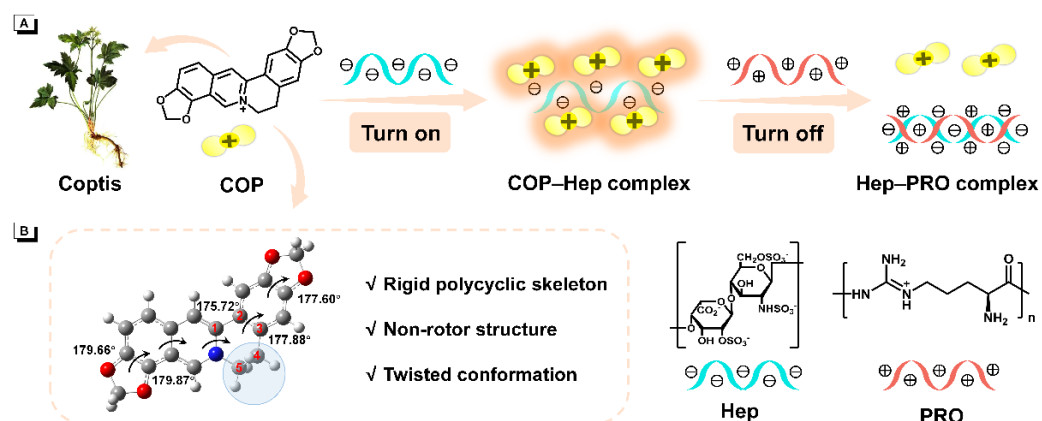
1. Introduction

Heparin (Hep) is a naturally occurring, highly sulfated glycosaminoglycan widely used as an anticoagulant [1], especially in the clinical management of myocardial infarction, cardiovascular surgery, extracorporeal circulation, and hemodialysis [2–4]. Its strong negative charge, derived from abundant sulfate groups, enables specific binding to antithrombin III, thereby significantly enhancing anticoagulant activity [5–8]. However, Hep overdose can cause various complications, including inflammation [9], bleeding [10],

thrombocytopenia [11], and hyperkalemia [12]. Protamine (PRO), the only approved antidote for Hep, is an arginine-rich, positively charged, low-molecular-weight protein that forms stable complexes with Hep to rapidly neutralize its anticoagulant effects [13–16]. However, excessive PRO administration may induce severe side effects, such as respiratory distress, pulmonary hypertension, and pulmonary edema [17,18]. Therefore, the development of a sensitive, simple, and rapid analytical method for determining levels of both Hep and PRO is of great significance.

Currently, detection strategies for Hep and PRO include electrochemical techniques [19], colorimetric assays [20], and capillary electrophoresis [21,22], which often suffer from complex procedures, high costs, or limited analytical efficiency. As a result, fluorescence spectroscopy has emerged as an attractive alternative due to its high sensitivity, excellent selectivity, and operational simplicity [23–25]. However, traditional organic fluorescent sensors are frequently compromised by aggregation-caused quenching (ACQ) and short-wavelength emissions, restricting their performance under aqueous or high-concentration conditions [26–28]. The advent of aggregation-induced emission (AIE) has effectively overcome ACQ, enabling robust fluorescence responses in aqueous environments [29–31]. Many AIE-based synthetic sensors have been explored for the detection of Hep and PRO; nevertheless, most of these systems involve complex molecular designs and multistep synthesis, which may limit their accessibility and practical application [32,33]. In contrast, natural products, notable for their structural diversity and potential biocompatibility [34–36], provide an appealing source of sustainable AIE-active sensing platforms [37–39]. Notably, natural AIE luminogens (AIEgens) capable of dual and reversible fluorescence modulation toward both Hep and PRO remain largely underexplored.

In this work, we present coptisine chloride (COP), a natural isoquinoline alkaloid found in traditional Chinese medicinal herbs *Coptis chinensis* (Huanglian) and *Phellodendron amurense* (Huangbai), as the first dual-responsive AIE sensor for reversible detection of Hep and PRO (Scheme 1A). Owing to its rigid fused polycyclic skeleton and non-rotor molecular structure (Scheme 1B), COP exhibits pronounced AIE behavior in aqueous media with a large Stokes shift of 195 nm. Electrostatic complexation between the cationic COP and negatively charged Hep triggers rapid fluorescence “turn-on” within 1 min, enabling highly selective Hep detection with a limit of detection (LOD) of 0.70 $\mu\text{g/mL}$, even in the presence of various interferents. Subsequent competitive binding of PRO disrupts the COP–Hep aggregates, leading to fluorescence quenching and reversible dual-analyte sensing. Mechanistic studies combining theoretical calculations and multiple characterization techniques reveal that the sensing behavior is governed by an electrostatically driven aggregation–disaggregation process. Furthermore, COP exhibits reliable Hep sensing performance in diluted human serum, achieving an LOD of 0.71 $\mu\text{g/mL}$ and a high recovery (98.3–101.6%). This work demonstrates a structurally simple, cost-effective natural AIEgen for Hep and PRO detection, providing a promising, sustainable, and biocompatible tool for reversible biomacromolecular fluorescence sensing in complex media.



Scheme 1. (A) Schematic illustration of the use of COP for the detection of Hep and PRO. (B) DFT-optimized geometry of COP at the B3LYP/6-31G (d, p) level, showing the numbering of carbons 1–5 in the nitrogen-containing six-membered heterocycle.

2. Experimental Section

2.1. Materials

COP, adenosine-5'-triphosphate disodium salt (ATP), bovine serum albumin (BSA), and cucurbit [7]uril (CB7) were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Heparin sodium (Hep, MW $\approx$ 17 kDa), phosphate-buffered saline (PBS, 0.1 mol/L, pH 7.2–7.4), and ultrapure water were obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Protamine sulfates (PRO), chondroitin sulfate sodium salt (CHS), heparan sulfate (HS), dermatan sulfate (DS), and hyaluronic acid sodium salt (HA) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Glucose (Glu) and Urea were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Dextran (Dex, MW $\approx$ 10 kDa) was obtained from Beijing J&K Scientific Co., Ltd. (Beijing, China). Cationic salts containing Ag^+ , Al^{3+} , Ba^{2+} , Cu^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Fe^{3+} , Fe^{2+} , K^+ , Li^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , Pb^{2+} , and Zn^{2+} ions were purchased from Xilong Scientific Co., Ltd (Shantou, China). Normal human serum was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Dimethyl sulfoxide (DMSO), methanol, ethanol, dichloromethane (DCM), acetonitrile, and tetrahydrofuran (THF) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). All reagents were of analytical grade and used without further purification.

2.2. Characterization

Ultraviolet-visible (UV-vis) absorption spectra were recorded on a Lambda 750 spectrophotometer, and fluorescence spectra were measured using a Hitachi F-4700 spectrometer. Quantum yields and fluorescence lifetimes were determined using an Edinburgh FLS 1000 fluorescence spectrometer. Scanning electron microscopy (SEM) and fluorescence images were collected using a ZEISS Sigma 300 microscope and a Leica DMi 8 inverted fluorescence microscope, respectively. Dynamic light scattering (DLS) and zeta potential measurements were performed on a Zetasizer Nano ZS90, and pH values were measured using a FiveEasy Plus digital pH meter.

2.3. Measurement of AIE Properties of COP

A 2 mM COP stock solution in DMSO was prepared. Equal aliquots of the stock solution were added to DMSO/THF mixtures with different THF volume fractions (f_{THF}) ranging from 0% to 99%, yielding a series of solutions with a final COP concentration of

20 μM . Fluorescence spectra were recorded in sealed 1 cm quartz cuvettes with an excitation wavelength of 355 nm (slit width: 5 nm for both excitation and emission).

2.4. Detection of Hep and PRO

Stock solutions (2 mg/mL) of Hep, PRO, and other interfering proteins were prepared in ultrapure water. Fluorometric titrations were carried out to detect Hep and PRO.

For Hep detection, aliquots (0.8 mM, 50 μL) of COP stock solution were diluted with PBS and mixed with varying concentrations of Hep to obtain a final COP concentration of 10 μM in a total volume of 4 mL (0–136 $\mu\text{g/mL}$ Hep). The mixtures were incubated at room temperature for 1 min before fluorescence measurement.

For PRO detection, 50 μL of 0.8 mM COP stock solution and 100 μL of Hep stock solution were mixed with varying volumes of PBS and titrated with different concentrations of PRO (0–14 $\mu\text{g/mL}$) under identical conditions (10 μM COP, 50 $\mu\text{g/mL}$ Hep).

The LOD was calculated as $\text{LOD} = 3\sigma/k$, where σ is the standard deviation of five blank measurements of the COP solution in PBS, and k is the slope of the calibration curve of fluorescence intensity versus concentration.

2.5. Detection of Hep in Diluted Human Serum

Human serum was diluted with PBS to 0.05% (v/v). The COP stock solution was mixed with Hep solutions of varying concentrations (0–40 $\mu\text{g/mL}$) in the diluted human serum to obtain a final COP concentration of 10 μM . The mixtures were incubated at room temperature for 1 min before fluorescence measurement. For recovery tests, known amounts of Hep stock solution (16–48 μL , 2 mg/mL) were spiked into the diluted serum containing COP stock solution (2 mM, 20 μL). Fluorescence intensities were measured in triplicate, and recoveries were calculated using the following equation:

$$\text{Recovery (\%)} = (\text{Found/Added}) \times 100\%$$

2.6. Theoretical Calculations

Density functional theory (DFT) calculations were carried out with Gaussian 16 [40]. The geometry of COP was optimized at the B3LYP/6-31G (d, p) level using the SMD implicit solvent model and Grimme's D3 dispersion correction. The absorption and emission spectra were obtained by time-dependent DFT (TD-DFT) at the same level with the SMD solvent model in DMSO. The electrostatic potential distribution and molecular orbitals were further analyzed and visualized with Multiwfn.

3. Results and Discussion

3.1. AIE Characteristics of COP

The photophysical behavior of COP was first investigated in various solvents. Its UV–vis absorption spectra showed negligible changes, whereas fluorescence spectra displayed weak emission in DMSO (Figure S1). Due to the extremely low solubility of COP in water, leading to uncontrolled precipitates and unstable fluorescence signals (Figure S2), DMSO and THF were chosen as good and poor solvents, respectively, to construct mixed systems for studying solvent-dependent fluorescence behavior. In pure DMSO, COP emitted at 550 nm. As f_{THF} increased from 10% to 90%, the emission intensity rose significantly, reaching approximately 3.2-fold that in pure DMSO at $f_{\text{THF}} = 90\%$ (Figure 1A). This pronounced enhancement confirmed the typical AIE effect, as further quantified by the I/I_0 plot (Figure 1B). Notably, excitation at 355 nm under aggregation-inducing conditions led to a large Stokes shift of 195 nm. At $f_{\text{THF}} = 99\%$, the intensity slightly decreased, likely due to excessive aggregation forming oversized particles that reduced light transmission (Figure S3). The Commission Internationale de l'Éclairage (CIE) chromaticity diagram

further demonstrated the emission color in the aggregated state, with color coordinates (0.3910, 0.4727) (Figure 1C). DLS analysis revealed the particle size increased from 1.3 nm to 9.6 nm as the f_{THF} increased from 0% to 60% (Figure 1D–F), consistent with the aggregation behavior observed by inverted fluorescence microscopy (Figure S4). The fluorescence quantum yield also rose from 2.1% in pure DMSO to 5.1% at $f_{\text{THF}} = 60\%$ (Figure S5). These findings confirm COP as a typical AIE-active fluorophore.

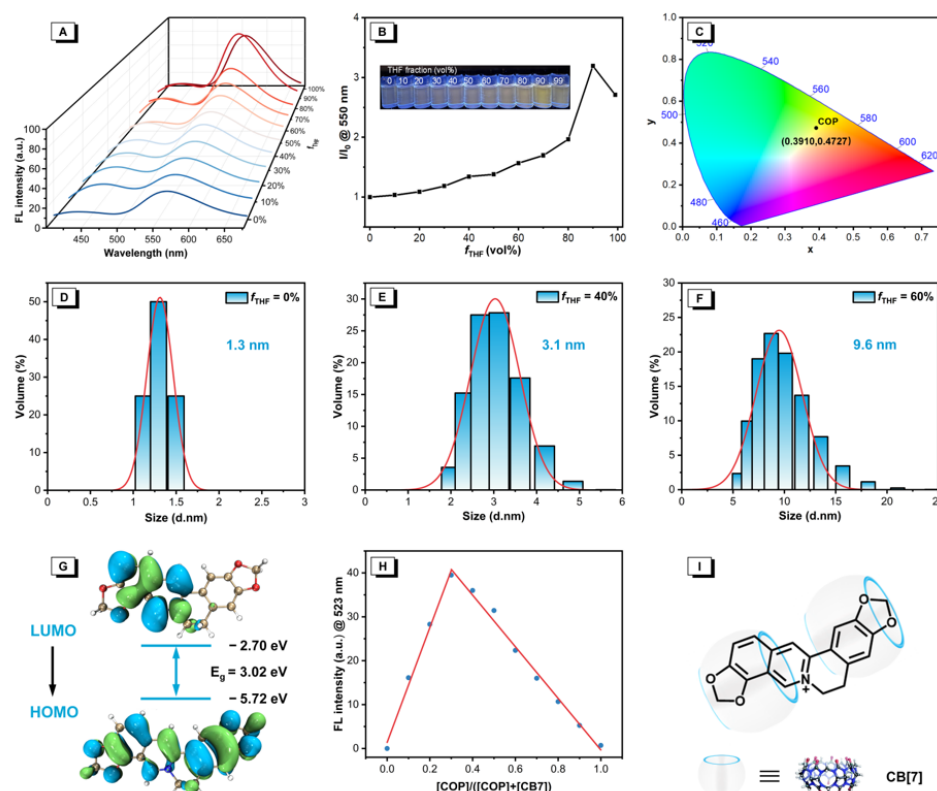


Figure 1. (A) Fluorescence spectra of COP (20 μM) in DMSO/THF mixtures with different THF fractions (f_{THF}). (B) Change in the fluorescence intensity ratio (I/I_0) with f_{THF} (inset: photographs of COP solutions under UV illumination at different f_{THF} ; $\lambda_{\text{ex}} = 355$ nm, slit = 5 nm). (C) CIE chromaticity diagram of COP at 90% f_{THF} . (D–F) DLS profiles of COP in DMSO/THF mixtures at different f_{THF} . (G) HOMO–LUMO distributions and energy of COP in DMSO based on DFT/B3LYP/6-31G (d, p) calculations. (H) Job’s plot of COP and CB7 ($[\text{COP}] + [\text{CB7}] = 20$ μM ; $\lambda_{\text{ex}} = 355$ nm, slit = 2.5 nm). (I) Schematic illustration of the host–guest complex between COP and CB7 at a ratio of 1:2.

Unlike classical synthetic AIE luminogens such as tetraphenylethene, COP is a naturally occurring isoquinoline alkaloid featuring a rigid polycyclic skeleton and a non-rotor nature. DFT-optimized geometry reveals a slightly twisted conformation, with a dihedral angle of $\sim 175^\circ$ between the isoquinoline plane and the adjacent fused ring (Scheme 1B). The nitrogen-containing six-membered heterocycle exhibits a distinct non-planar configuration, where the saturated C4 and C5 atoms (circled in blue) are displaced above the mean ring plane, thereby mitigating π – π stacking. In addition, the two benzodioxole units embedded in the backbone enhance molecular rigidity and steric bulk, impeding close intermolecular packing. Notably, the oxygen atoms in these units can engage in weak interactions with the positively charged nitrogen centers of neighboring molecules, restricting local molecular motions in the aggregated state (Figure S6). These structural characteristics highlight the rigid, non-rotor skeleton as the fundamental origin of the AIE behavior of COP. Subsequently, frontier molecular orbital analysis (Figure 1G) demonstrates that the highest occupied molecular orbital (HOMO) is delocalized across the entire molecule, while the

lowest unoccupied molecular orbital (LUMO) is mainly localized on the rigid isoquinoline moiety. This localization indicates that the excited-state electron density is confined to a structurally rigid region, thereby limiting excitation-induced conformational relaxation and suppressing nonradiative decay pathways, which accounts for the experimentally observed AIE behavior [41]. Moreover, excitation and emission wavelengths calculated by TD-DFT are in good agreement with the experimental data, supporting the assignment of the electronic transitions and the reliability of the proposed photophysical interpretation (Table S1 and Figure S7).

To gain deeper mechanistic insights into the photophysical behavior of COP, viscosity-, temperature-, and concentration-dependent fluorescence measurements were carried out. In DMSO/glycerol mixtures, COP exhibited gradual aggregation; concurrently, the emission intensity increased markedly with increasing glycerol volume fraction (f_{Gly}), reaching ~150-fold higher intensity at $f_{\text{Gly}} = 99\%$ compared with that in pure DMSO (Figures S8 and S9). This behavior suggests that increased viscosity promotes aggregation and restricts intramolecular motion, thereby suppressing nonradiative decay. Temperature- and concentration-dependent experiments carried out in DMSO/THF mixtures further supported this interpretation, as lower temperature and higher concentration facilitated stronger fluorescence emission (Figure S10). In addition, cucurbit [7]uril (CB7) encapsulation enhanced COP fluorescence, with emission intensity reaching a maximum at the 2:1 CB7:COP ratio (Figure S11). Job's plot confirmed the 2:1 binding stoichiometry (COP:CB7), indicating the formation of a stable host–guest complex (Figure 1H). The confinement of COP within the macrocyclic cavity leads to enhanced emission (Figure 1I). These results indicate that environmental constraints on molecular motion play an important role in modulating the fluorescence of COP, consistent with its AIE characteristics.

3.2. Spectral Response and Selectivity of COP Toward Hep

Building on the established AIE mechanism, we next investigated the fluorescence response of COP to Hep in a DMSO/PBS mixture (PBS fraction, $f_{\text{PBS}} = 99\%$, v/v), where DMSO ensured sufficient dissolution of COP under biologically relevant conditions. DLS measurements confirmed the formation of COP aggregates in the DMSO/PBS system with increasing f_{PBS} (Figure S12). As a positively charged natural AIEgen, COP may bind to highly negatively charged Hep through electrostatic interactions, thus modulating its fluorescence. To test this interaction, the COP emission spectrum in the presence of Hep was recorded, and a significant fluorescence enhancement at 550 nm was observed (Figure 2A), corresponding to bright yellow emission under 365-nm UV excitation (inset of Figure 2A). This enhancement arose from strong electrostatic interactions between the quaternary ammonium cation of COP and the sulfate or carboxylate groups of Hep, leading to molecular aggregation and triggering AIE. To further understand the COP–Hep interaction, we evaluated the influence of pH on the fluorescence response (Figure 2B). In the absence of Hep, COP fluorescence remained relatively stable across pH 1.0–12.0. Upon addition of Hep, the fluorescence intensity increased at pH 1.0–4.0, which can be attributed to strong electrostatic interactions between cationic COP and anionic Hep, promoting molecular aggregation and favoring AIE-type emission behavior. The fluorescence remained relatively stable at pH 5.0–7.0. A slight decrease observed at pH 8.0–12.0 may result from reduced stability of the quaternary ammonium cation under basic conditions, weakening the electrostatic interaction [42,43].

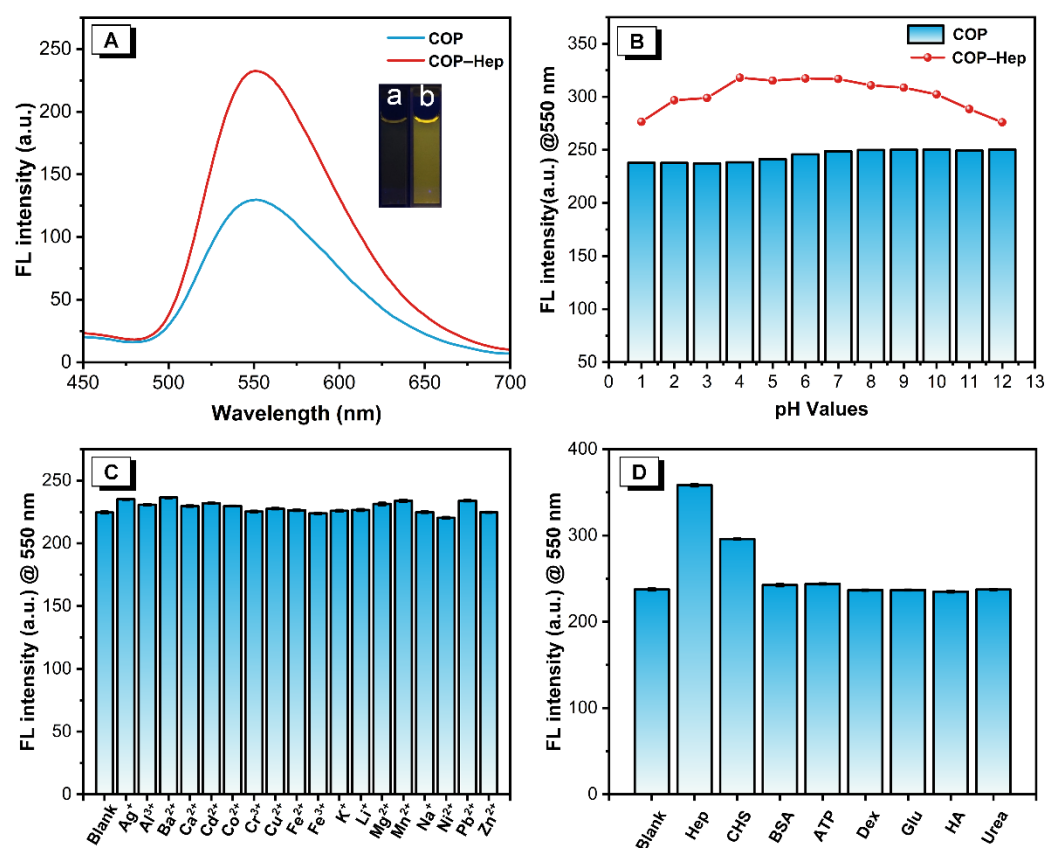


Figure 2. Fluorescence response of COP in a DMSO/PBS mixture ($f_{\text{PBS}} = 99\%$; $\lambda_{\text{ex}} = 355$ nm, slit = 10 nm). (A) Emission spectrum of COP (5 μM) in the absence and presence of Hep (100 $\mu\text{g/mL}$). Inset: Photographs of (a) COP solution and (b) COP-Hep solution under 365 nm UV illumination. (B) Fluorescence intensity of COP (10 μM) and COP-Hep complexes at different pH values. (C) Fluorescence intensity of COP (10 μM) before and after addition of metal ions (25 μM). (D) Fluorescence response of COP (10 μM) to various biomolecules (20 $\mu\text{g/mL}$).

Selectivity tests were then performed under near-physiological pH (7.2–7.4). The potential interference of 18 metal ions (Ag^+ , Al^{3+} , Ba^{2+} , Cu^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Fe^{3+} , Fe^{2+} , K^+ , Li^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , Pb^{2+} , Zn^{2+}) was found to be negligible, demonstrating remarkable anti-interference performance (Figure 2C). Further experiments were conducted using other biomolecules, including CHS, BSA, ATP, Dex, Glu, HA, and Urea (Figure 2D). Among these, only Hep induced a significant fluorescence increase, which was ~ 1.5 -fold that of COP alone. Notably, CHS, despite structural similarity to Hep, produced a less pronounced fluorescence enhancement, likely due to a lower negative charge density relative to Hep, and hence weaker electrostatic interactions with COP [44]. Additionally, for DS, CHS, HA, and HS, four polysaccharides structurally similar to Hep, COP showed no noticeable fluorescence response across the investigated concentration range, in sharp contrast to the pronounced response observed for Hep (Figure S13). Overall, COP exhibited excellent specificity for Hep and strong anti-interference capability against other species.

3.3. Sensitivity Detection of Hep and PRO

The response time of COP toward Hep was first examined. A stable fluorescence signal was achieved within 1 min at room temperature (Figure S14); thus, all subsequent experiments were conducted under identical conditions. With the gradual addition of Hep, the fluorescence intensity of COP at 550 nm increased steadily, reaching approximately 2.1 times that of COP alone at a Hep concentration of 136 $\mu\text{g/mL}$ (Figure 3A). A good linear correlation ($R^2 = 0.9984$) was observed between fluorescence intensity

and Hep concentration in the range of 0–131.75 $\mu\text{g/mL}$, with an LOD of 0.70 $\mu\text{g/mL}$ (Figure 3B). Given the efficient sensing of Hep, we next examined whether COP could also respond to its clinical antagonist, PRO. The addition of PRO to the COP–Hep system induced gradual fluorescence quenching (Figure 3C). It should be noted that the fluorescence intensity does not fully return to its original level, which may be attributed to incomplete disaggregation of COP aggregates or residual electrostatic interactions during the switching process. Within the range of 0–10 $\mu\text{g/mL}$ PRO, the fluorescence intensity decreased markedly with increasing PRO concentration, showing a strong linear relationship ($R^2 = 0.9962$) with an LOD of 0.49 $\mu\text{g/mL}$ (Figure 3D). Additionally, the reversibility of the COP was evaluated through repeated Hep/PRO switching experiments, with the fluorescence response remaining stable over 5 cycles, indicating good fatigue resistance (Figure S15). Its long-term stability was confirmed by COP stored for one month, which still exhibited well-defined fluorescence responses toward Hep/PRO (Figure S16). These results demonstrate that COP enables sensitive and reversible detection of both Hep and PRO. Although the LOD for Hep is slightly higher than that reported in some previous systems (Table S2) [4,44–50], COP, being a naturally AIE-active molecule, exhibits distinct advantages, including rapid response, low toxicity, and excellent biocompatibility. Its dual detection capability toward Hep and PRO underscores its potential in monitoring of anticoagulant therapy.

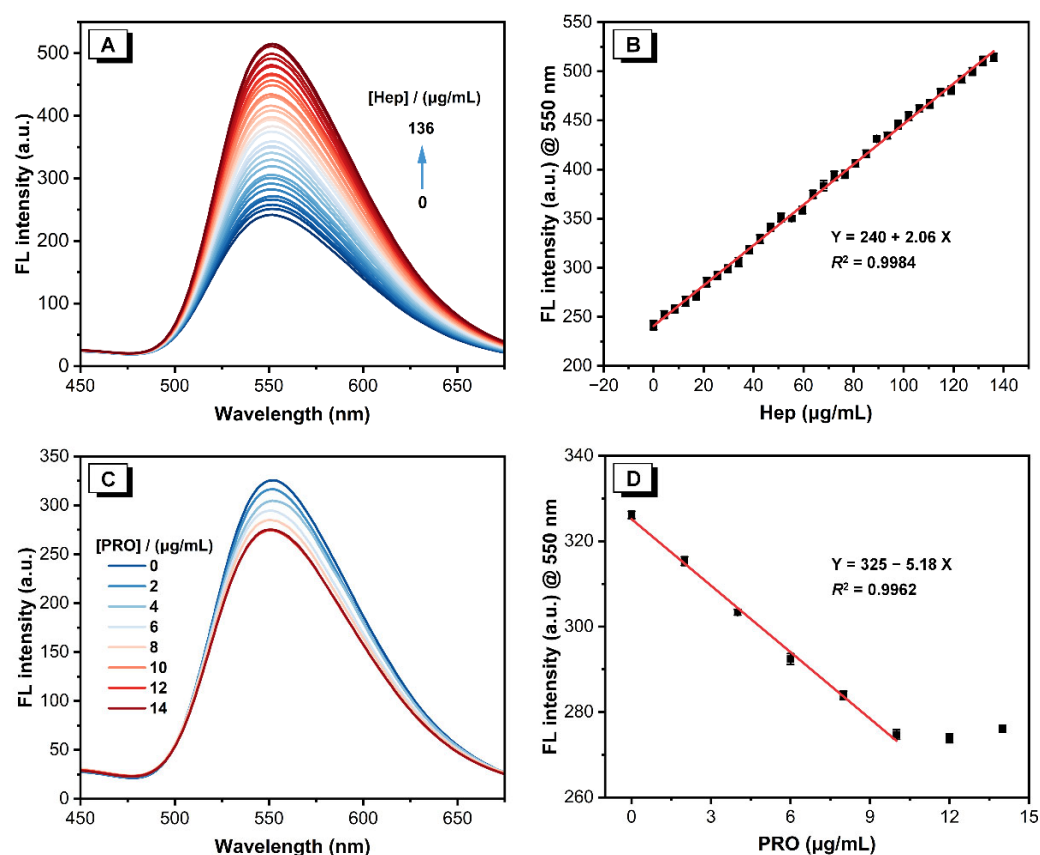


Figure 3. (A) Fluorescence spectra of COP (10 μM) with varying Hep concentrations in a DMSO/PBS mixture ($f_{\text{PBS}} = 99\%$). (B) Linear relationship between fluorescence intensity and Hep concentration. (C) Fluorescence spectra of COP–Hep complex with varying PRO concentrations. (D) Linear relationship between fluorescence intensity and PRO concentration ([COP] = 10 μM , [Hep] = 50 $\mu\text{g/mL}$). Measurement conditions: $\lambda_{\text{ex}} = 355 \text{ nm}$, slit = 10 nm.

3.4. COP Recognition of Hep and PRO

To elucidate the recognition mechanism of COP toward Hep and PRO, we combined theoretical calculations with multiple characterization techniques, including zeta potential, DLS, inverted fluorescence microscopy, and SEM. DFT calculations revealed that COP is predominantly positively charged, suggesting it could bind negatively charged Hep through electrostatic interactions (Figure 4A), which was confirmed by zeta potential measurements. The zeta potential of free COP was 6.46 mV, shifted to -35.9 mV upon Hep binding and recovered to -6.37 mV after PRO addition, indicating the disassembly of the COP–Hep complex, likely accompanied by Hep–PRO complex formation (Figure 4B). Consistently, DLS analysis revealed an increase in particle size from 593.7 nm to 699.9 nm upon Hep addition, followed by a decrease to 376.4 nm with PRO, supporting a reversible aggregation–disaggregation process (Figure 4C). To visually confirm these changes, morphological alterations were examined using inverted fluorescence microscopy and SEM. Free COP exhibited a cross-shaped structure (Figure 4D,G), which transformed into compact block-like aggregates upon Hep binding (Figure 4E,H) and reverted to the cross-shaped morphology after PRO addition, consistent with reversible complex disassembly (Figure 4F,I). Additionally, the fluorescence lifetime of the COP–Hep complex decreased from 0.92 ns to 0.78 ns after PRO addition (Figure S17), further confirming disassembly of the complex. Collectively, these results indicate that Hep induces COP aggregation via electrostatic interactions, enhancing fluorescence, whereas PRO competitively binds Hep, triggering reversible disassembly and fluorescence quenching. This competitive binding mechanism accounts for the observed fluorescence switching and dual detection capability of COP.

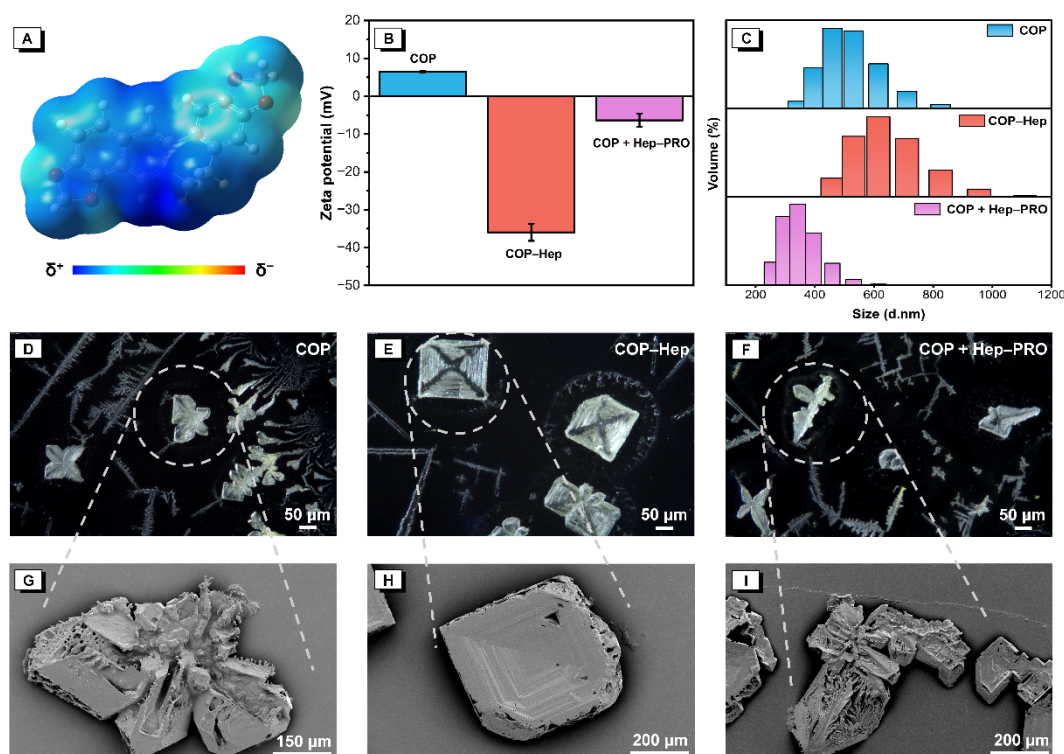


Figure 4. (A) Electrostatic potential of solid-state COP calculated by DFT at the B3LYP/6-31G (d, p) level. (B) Zeta potential and (C) DLS profiles of COP, COP–Hep complexes, and COP–Hep complexes with PRO in a DMSO/PBS mixture ($f_{\text{PBS}} = 99\%$) ($[\text{COP}] = 10 \mu\text{M}$, $[\text{Hep}] = 50 \mu\text{g/mL}$, $[\text{PRO}] = 10 \mu\text{g/mL}$). (D–F) Inverted fluorescence microscopy and (G–I) SEM images of dried COP sample, COP–Hep complexes, and COP–Hep complexes with PRO.

3.5. Hep Detection in Diluted Human Serum

To evaluate the performance of the COP sensor in biologically relevant environments, we conducted Hep spiking experiments in 0.05% (*v/v*) diluted human serum. This dilution was chosen to minimize interference from high concentrations of proteins and electrolytes, while still providing a protein-containing environment representative of conditions under which Hep interacts with antithrombin in the bloodstream [51]. The fluorescence intensity of COP in diluted serum (without Hep) was comparable to that in normal aqueous solution, suggesting that other serum proteins, especially negatively charged serum albumin [45], might not significantly perturb the intrinsic fluorescence of the free sensor (Figures 3A and 5A). Upon addition of Hep, the fluorescence intensity at 550 nm increased steadily (Figure 5A), and the large Stokes shift (195 nm) helps minimize interference from serum autofluorescence. A strong linear relationship ($R^2 = 0.9961$) was observed between fluorescence intensity and Hep concentration over the range of 0–28 $\mu\text{g/mL}$, with an LOD of 0.71 $\mu\text{g/mL}$ (Figure 5B). Although the LOD is moderate compared with some reported synthetic sensors (Table S2), COP exhibits stable and reproducible responses in the serum matrix. Recovery experiments confirmed the reliability of the method, with recoveries of 98.3–101.6% and relative standard deviations below 1.20% (Table 1). Thus, the COP sensor exhibits effective performance for Hep detection in a protein-containing biological environment. Under the current experimental conditions (0.05% human serum), the measured LOD is not directly comparable with clinical Hep concentrations in plasma, and further optimization would be required for detection in more complex plasma conditions.

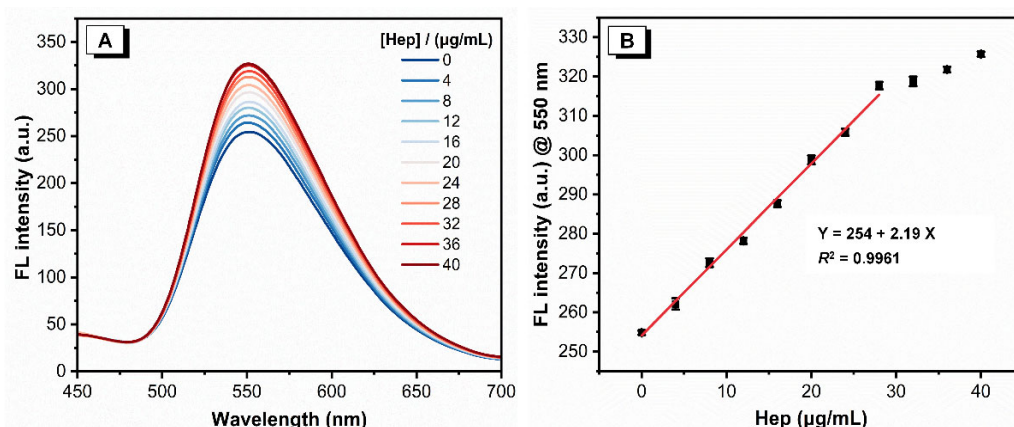


Figure 5. Quantitative analysis of Hep in 0.05% human serum. (A) Fluorescence spectra of COP with Hep at different concentrations. (B) Linear relationship between the COP fluorescence intensity and Hep concentration ([COP] = 10 μM , λ_{ex} = 355 nm, slit = 10 nm).

Table 1. Determination of Hep in diluted human serum.

Added ($\mu\text{g/mL}$)	Found ($\mu\text{g/mL}$)	Recovery (%)	RSD ($n = 5$, %)
8.0	8.1 ± 0.8	101.6	1.2
16.0	15.9 ± 0.7	99.1	1.0
24.0	23.6 ± 0.8	98.3	0.7

4. Conclusions

This study demonstrates that COP, a natural isoquinoline alkaloid, can serve as a dual-responsive AIE sensor enabling reversible detection of Hep and PRO. COP exhibits typical AIE behavior due to its rigid polycyclic skeleton and twisted conformation that prevents π – π stacking. It electrostatically binds to negatively charged Hep, producing a “turn-on” fluorescence response (LOD = 0.70 $\mu\text{g/mL}$, linear range 0–131.75 $\mu\text{g/mL}$), whereas subsequent addition of PRO disrupts the COP–Hep complex, yielding a “turn-off”

signal (LOD = 0.49 $\mu\text{g/mL}$, linear range 0–10 $\mu\text{g/mL}$). The dual-responsive fluorescence arises from an aggregation–disaggregation mechanism, as revealed by theoretical and experimental analyses. In diluted human serum, COP maintains reliable fluorescence responses toward Hep, achieving an LOD of 0.71 $\mu\text{g/mL}$, recoveries of 98.3–101.6%, and good linearity over 0–28 $\mu\text{g/mL}$. These findings establish COP as a structurally simple, sustainable, and biocompatible natural AIEgen capable of dual-mode Hep and PRO detection, providing a basis for its further evaluation in more complex or clinically relevant matrices.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/chemosensors14020051/s1>, Figures S1–S17 and Tables S1 and S2: UV–Vis absorption and fluorescence studies of COP in various solvent systems, aggregation-induced emission behavior in mixed solvents, fluorescence quantum yield and viscosity-dependent emission analyses, TD-DFT calculated and simulated spectra, supramolecular interaction models, and supplementary sensing performance data for Hep and PRO, including fluorescence response toward structurally similar polysaccharides, intensity changes during repeated addition cycles, and fluorescence responses after storage for different time periods [4,44–50].

Author Contributions: Conceptualization, X.D.; investigation, N.M., R.L., C.D. and R.R.; data curation, Y.W.; software, N.M., J.B. and D.Z.; validation, J.B. and D.Z.; visualization, X.L.; writing—original draft, N.M. and X.D.; writing—review and editing, X.D. and H.Z.; supervision, X.D. and H.Z. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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