NJC

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d1nj02659c

A cationic on–off fluorescent sensor with AIE properties for heparin and protamine detection†

Yingxi Zhou,[a] Hongbo Jiang,[b] Yuting Wang,[a] Song Zhao,[a] Lianzhe Hu [a] and Yan Zhang *[a]

Received 31st May 2021,
Accepted 5th August 2021

DOI: 10.1039/d1nj02659c

rsc.li/njc

In this research, a distyryl-anthracene derivative (DSAI) with two quaternary ammonium groups was synthesized. It emitted strong yellow-green fluorescence after aggregating with heparin through electrostatic attraction. In the presence of protamine, the fluorescence of DSAI remarkably quenched because of the greater affinity between heparin and protamine. Based on the change of fluorescence signal, a highly sensitive and selective method is successfully constructed to detect heparin and protamine. The DSAI sensor toward heparin showed a broad linear range (0.1 to 1.1 μg mL^{-1}) and a low detection limit (down to 5.64 ng mL^{-1}). The linear detection for protamine was in a wide range (0.1 to 1.7 μg mL^{-1}) with the detection limit of 5.20 ng mL^{-1}. Moreover, DSAI was successfully applied to detect heparin and protamine in complicated goat serum samples with satisfactory results. This simple and ultrasensitive biosensor has potential applications in diagnostics, therapeutics and biological research.

Introduction

Heparin (Hep), a naturally occurring linear polymer consisting predominantly of 1–4 linked uronic acid and glucosamine subunits, is known as the densest negatively charged biological macromolecule. It is a reagent for DNA decondensation and one of the oldest biological drugs that has been widely used as an anticoagulant during clinical procedures and treatment of thrombotic diseases.[1–4] The quantification of heparin is very important in anticoagulation therapy. A series of side effects can be caused by an overdose of heparin, such as haemorrhage or thrombocytopenia.[5–7] Protamine is a vasodilator and positively charged peptide, which can bond with heparin alleviating emergency bleeding.[8–10] To reverse the effect of heparin, protamine strongly binds to the sulfonate group of the heparin molecule, forming a stable neutral salt complex.[11] However, an overdose of protamine may produce life-threatening side effects such as systemic hypotension, pulmonary vasoconstriction or allergic reactions.[12–15]

Protamine can be detected inadequately by the traditional method measuring UV-absorption at 280 nm, a standard method for the quantification of proteins, because it is composed of aliphatic amino acids with the absence of aromatic amino acids. The administration of protamine is empirically based on heparin analysis in clinical practice.[16,17] The traditional assays for heparin quantification, such as the activated coagulation time (ACT) or the activated partial thromboplastin time (APTT), are indirect and inaccurate due to their lack of specificity and potential interference.[13,14,18] However, fluorescence detection is a burgeoning detection method, which has the characteristics of high sensitivity and selectivity, easy operation, and real time detection. And during the detection process, the detection of the substance is capable of being directly identified by the fluorescence sensor. However, conventional fluorescent small molecule sensors tend to undergo π–π accumulation in aqueous solutions, resulting in aggregation-caused quenching (ACQ) effects.[19,20] Molecules with an aggregation-induced emission property (AIE), a completely opposite property to ACQ, can be used in sensing applications by amplifying the signal and provide a new option for fluorescence detection.[21,22] In addition to the common tetraphenyl ethylene,[23–26] there are reports of some AIE molecular structures, such as silole,[27] triphenylethene,[28] and distyrylanthracene (DSA).[29] Modifications on the basis of these compounds can interact directly with the substrate and then aggregate to produce fluorescence amplification for detection. Precisely because its sensing method is simple and direct, it will reduce the interference to a large extent, thus making it possible to reach a lower detection limit.[30] Moreover to the best of our knowledge, few researchers have reported that heparin and protamine are detected in one detection system with a detection limit in single-digit nanograms per milliliter.[31–33] Thus, it is meaningful to develop this sensor.

Herein, we report a simple method to detect heparin and protamine. A DSA derivative with two quaternary ammonium

[a] Chongqing Key Laboratory of Green Synthesis and Applications, College of Chemistry, Chongqing Normal University, Chongqing, China
[b] Bashu Secondary School, Chongqing, China

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1nj02659c

groups, 4,4′-(1*E*,1′*E*)-2,2′-(anthracene-9,10-diyl) bis(ethene-2,1-diyl) bis(*N*,*N*,*N*-trimethylbenzenaminium (DSAI), was synthesized. DSAI was combined with heparin due to electrostatic attraction and aggregated. Then the as-prepared DSAI was used as a fluorescent indicator and heparin was used as protamine recognition units to construct a DSAI/Hep complex probe for ''turn-off'' assay for protamine. The limit of detection of heparin and protamine was 5.64 ng mL^{-1} and 5.20 ng mL^{-1}, respectively. It also showed a good detection effect in goat serum. This simple and ultrasensitive heparin biosensor has potential uses in diagnostics, therapeutics and biological research.

Experimental

Reagents and chemicals

Heparin (Hep) and protamine (Pro) were purchased from Sigma-Aldrich (Missouri, USA) with average molecular weights of 6117 and 4070, respectively. All chemicals were of analytical grade and purchased from Aladdin (Beijing, China), J&K (Beijing, China) unless stated otherwise. Prior to use, all solvents were purified by fractional distillation.

NMR spectra were recorded using a Bruker Avance 300 (Rheinstetten, Germany). Mass spectra were recorded on an Agilent 1100 LC-MS system (California, America). UV-Vis absorption spectra were recorded using a UV-2550 UV-Vis spectrophotometer (Kyoto, Japan). Fluorescence spectra were measured using a PerkinElmer LS-55 spectrophotometer (Massachusetts, America). HRMS was recorded using a Bruker ultraflextreme MALDI-TOF system (Rheinstetten, Germany).

Synthesis of DSAI

Synthesis of compound 1 (4,4′-(1*E*,1′*E*)-2,2′-(anthracene-9,10-diyl) bis(ethene-2,1-diyl) bis(*N*,*N*-dimethylaniline)). Tetraethyl anthracene-9,10-bis (methylene)phosphonate (2.40 g, 5.00 mmol), which was synthesized according to known procedures, was stirred with *t*-BuOK (1.68 g, 15.00 mmol) in THF (50 mL) under nitrogen. Compound **4**-(dimethylamino) benzaldehyde (1.79 g, 12.00 mmol) in THF (50 mL) was added to the solution that was kept in an ice bath and the mixture was stirred for 18 h at room temperature. The resultant precipitate was washed successively with MeOH and filtered off to yield a yellow coloured compound. ^{1}H NMR (300 MHz $CDCl_3$) δ (TMS, ppm): 8.44–8.41 (m, 4H), 7.75–7.69 (d, J = 16.5 Hz, 2H), 7.60–7.57 (d, J = 8.5 Hz, 4H), 7.45–7.42 (m, 4H), 6.87–6.82 (d, J = 6.8 Hz, 2H), 6.83–6.80 (d, J = 3.3 Hz 4H), 3.03 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (TMS) 150.5, 137.3, 133.0, 129.7, 127.6, 126.6, 126.1, 124.8, 120.7, 112.6, 40.5.

Synthesis of DSAI (4,4′-(1*E*,1′*E*)-2,2′-(anthracene-9,10-diyl) bis(ethene-2,1-diyl) bis(*N*,*N*,*N*-trimethylbenzenaminium)iodide). An improved method based on the literature was used to prepare DSAI (Scheme S1, ESI†).[34] Compound **1** (0.20 g, 0.43 mmol) was dissolved in 15 mL of trichloromethane, followed by the addition of 5 mL methyl iodide, and refluxed for 14 h under N_2. After cooling to room temperature, the mixture was filtered and washed with trichloromethane, and then dried overnight *in vacuo* at 50 °C. ^{1}H NMR (300 MHz DMSO-d_6) δ (TMS, ppm): 8.41–8.33 (m, 6H), 8.38–8.33 (d, J = 16.5 Hz, 2H), 8.12–8.04 (m, 8H), 7.61–7.58 (m, 4H) 7.08–7.02 (d, J = 16.5 Hz, 2H), 3.68 (s, 18H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (TMS) 146.5, 138.5, 135.2, 132.1, 128.9, 128.0, 127.9, 127.8, 126.1, 125.8, 120.8, 56.5; HRMS (ESI) 249.1514 (Calcd: 249.1539 for $C_{36}H_{38}N_2^{2+}$) (Fig. S1–S3, ESI†).

Analytical procedure

Procedure for the detection of heparin. Aliquots of 8 μL volume of prepared DSAI solution were added to 1.5 mL centrifuge tubes, and then various concentrations (0 to 84 μg mL^{-1}) of heparin solution were added. The final volume of the mixtures was maintained at 1.0 mL by phosphate buffer (0.2 M, pH 7.0). The resulting solutions were adequately mixed and incubated for 15 min. The fluorescence of the mixtures was measured at 408 nm excitation. All measurements were performed at room temperature and there is no need for the removal of oxygen or the protection of nitrogen.

Procedure for the detection of protamine. 8 μL methanol solution of DSAI (0.1 mmol L^{-1}) was first added to a heparin aqueous solution (0.12 mg mL^{-1}, 100 μL) in 1.5 mL centrifuge tubes, and then different amounts of protamine (from 0 to 20 μg mL^{-1}) were added. The mixture solution was diluted to 1.0 mL with phosphate buffer (0.2 M, pH = 7.0), sonicated for more than 5 minutes and mixed thoroughly. Then their fluorescence measurement was performed under excitation at 408 nm.

Selectivity to heparin and protamine. 8 μL methanol solution of DSAI and phosphate buffer solution (0.2 M, pH = 7.0) were added to a 1.5 mL centrifuge tube. They were mixed into a solution with a final volume of 1 mL and pH 7.0, and then the fluorescence intensity was measured. Different substances with the same concentration of 10 μg mL^{-1}, sodium phosphate (Na_2HPO_4), adenosine triphosphate (ATP), sodium citrate, vitamin C, hyaluronic acid (HA), sodium cholate hydrate, bovine serum albumin (BSA), and heparin (Hep), were added to the DSAI phosphate buffer solutions separately under the same conditions above. The total volume was kept at 1 mL, and their fluorescence intensity was measured.

8 μL methanol DSAI and 100 μL heparin (12 μg mL^{-1}) were mixed with 0.2 M phosphate buffer (pH = 7.0) to make a 1 mL solution with pH 7.0, and the fluorescence intensity was measured. Under the same DSAI and heparin concentration conditions above, sodium chloride (NaCl), calcium chloride ($CaCl_2$), melamine, cysteine (Cys), glutathione (GSH), tryptophan, and protamine (10 μg mL^{-1}) were mixed separately and the total volume was kept at 1 mL.

Heparin detection in goat serum. For the assay of heparin in goat serum samples, 30 μL of goat serum sample and 8 μL of prepared DSAI were added to 1.5 mL centrifuge tubes, and the final volume of the mixtures was maintained at 1.0 mL. The resulting mixture was adequately mixed and incubated at room temperature for 15 min, and then the fluorescence of the mixture was measured at 408 nm excitation. After that, the goat serum samples with different concentrations of spiked

heparin (0.36, 0.48, 0.60 and 0.72 μg mL^{-1}) were measured under the same conditions.

Protamine detection in goat serum. For the recovery measurement, 30 μL of goat serum sample and 8 μL of DSAI were added to 1.5 mL centrifuge tubes. The final volume of the mixture was maintained at 1.0 mL, and the mixture was reacted with ultrasound at room temperature for 5 min. Then the fluorescence of the mixture was measured at 408 nm excitation. After that, the goat serum samples with different concentrations of spiked protamine (0.50, 0.70, 0.80, and 1.00 μg mL^{-1}) were measured under the same conditions.

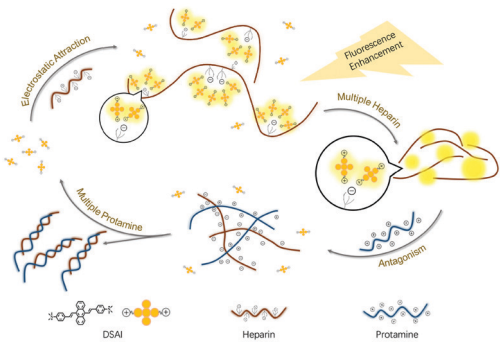
Results and discussion

Probe detection mechanism

In recent years, AIE molecules have been used as fluorescent probes for protein detection, bioimaging, DNA analysis, and biomolecular detection. When AIEgen is aggregated with biomolecules, the fluorescence quantum yield can be improved. As a derivative of DSA with typical AIE properties, DSAI can produce an AIE effect to achieve the purpose of detection.

The more detailed spectral characteristics of DSAI were measured. The absorption, excitation and emission of compound **1** were measured in Fig. S4 (ESI†), which were at 405 nm, 430 nm, and 520 nm respectively. As shown in Fig S5 (ESI†), the wavelengths of DSAI formed after interaction with methyl iodide were measured to be 415 nm, 408 nm, and 519 nm, respectively. After being converted to a quaternary ammonium salt, its optical properties were not changed significantly, but it has been given a positive charge and an ability to electrostatically bond. Furthermore, the remarkable AIE property of DSAI (a tenfold increase in fluorescent strength)[34] proves that DSAI can be used as a sensor for detecting heparin.

The detection mechanism of heparin and protamine is shown in Scheme 1. Due to the presence of two quaternary ammonium groups, DSAI has good solubility in methanol or a mixed solution of methanol/water and emits faint fluorescence. DSAI aggregated on heparin by electrostatic attraction between the positively charged quaternary ammonium groups in DSAI and the negatively charged sulfates or carboxylates in heparin. Then, the probe combined with heparin to form long chain composites, and at this point, the composites could emit strong fluorescence. The composites have become a new probe, DSAI/Hep complex. Due to the greater affinity between heparin and protamine, when protamine is added, DSAI is separated from heparin and re-dispersed, resulting in a decrease in the fluorescence intensity.



Scheme 1 Schematic illustration of the heparin and protamine detection mechanism for the fluorescence sensor.

The feasibility of the above-mentioned principle is proved by the experiment. As shown in Fig. 1A, DSAI emitted dim fluorescence, but the fluorescence intensity increased remarkably after adding heparin. An apparent fluorescence change is clearly observed under irradiation at 365 nm (Fig. 1A, inset). When protamine was added, an obvious fluorescence quenching phenomenon appeared.

The above changes are caused by the aggregation and dispersion of DSAI with AIE properties. As described in Fig. 1B, DSAI was dispersed in distilled water and the energy of the excited state is dominated by non-radiative transition due to vibrations and rotations of molecules. The aggregation (greater than 100 nm) occurred after the interaction between DSAI and heparin and the radiation channel is opened. The re-dispersion of DSAI could be found from the DLS data and quenched the fluorescence in the presence of protamine. The binding between the sensor and heparin and heparin and protamine is due to electrostatic attraction, which can be proved by the potential changes after heparin and protamine are added to DSAI. With the addition of heparin, the initially positive surface potential of DSAI gradually decreased and even overturned to a negative potential (Fig. S6, ESI†). In contrast, the addition of protamine recovered the potential to a positive charge. This result illustrated that there are electrostatic interactions between the probe, heparin and protamine.

The detection of heparin and protamine

Heparin is directly recognized using DSAI and is also the medium for subsequent testing. For obtaining the optimal experimental conditions, the pH was optimized first. The stronger fluorescence of DSAI could be observed when DSAI methanol solution was added with heparin at the optimal phosphate buffer solution (pH = 7.0), as shown in Fig. S7 (ESI†). Moreover, the reaction time between DSAI and heparin is optimized. As shown in Fig. S8 (ESI†), the fluorescence

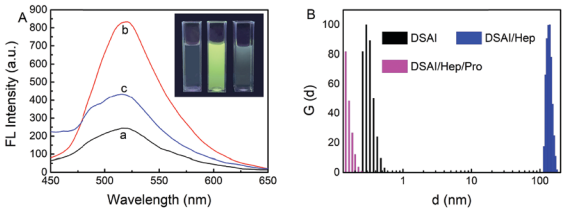


Fig. 1 (A) Fluorescence spectra of (a) DSAI, (b) DSAI/HEP complex, (c) DSAI/Hep/Pro complex. [Inset: From left to right are DSAI, DSAI/hep, and digital photos illuminated at 365 nm.] (B) DLS of DSAI in the presence or absence of heparin and protamine (10 μg mL^{-1}).

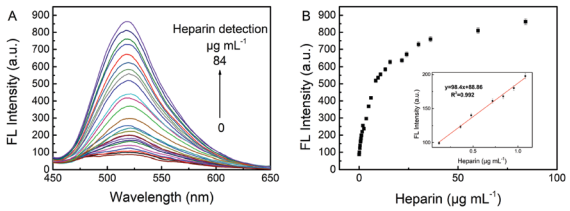


Fig. 2 (A) and (B) Fluorescence spectra (λ_{ex} = 408 nm) of DSAI [0.1 mmol L^{-1} in phosphate buffer solution (0.2 M), pH = 7] in the presence of different amounts of heparin (from 0 to 84 μg mL^{-1}). [Inset: The relationship between fluorescence intensity and the concentration of the heparin from 0 to 1.1 μg mL^{-1}.].

intensity did not change after 15 minutes. Therefore, 15 minutes was chosen as the optimal reaction time for the following experiments.

The fluorescence intensity change of DSAI with the increase of the concentration of heparin is shown in Fig. 2A and B. The fluorescence intensity increased with the increase of heparin concentration. A linear plot ($y = 98.4x + 88.86$, $R^2 = 0.992$) of the fluorescence intensity at 519 nm was obtained, with a linear range of 0.1 to 1.1 μg mL^{-1} and the detection limit reached 5.64 ng mL^{-1}. Compared to previously reported heparin fluorescence assays, the present method using DSAI as a fluorescent probe based on the electrostatic attraction can have comparable or even lower detection limits (Table 1).

We also investigated the response of DSAI to possible competitors. The turn-on responses of DSAI towards dibasic sodium phosphate (Na_2HPO_4), adenosine triphosphate (ATP), sodium citrate, vitamin C, hyaluronic acid (HA), sodium cholate hydrate, bovine serum albumin (BSA), blank and heparin were evaluated. Interestingly, only heparin has a stronger negative charge triggering the AIE response of DSAI (Fig. 3), which suggests that the probe could selectively detect heparin.

Protamine is a positively charged peptide showing a high binding affinity for heparin. DSAI would be freed from the heparin after the addition of protamine and then turned off the AIE fluorescence. To keep the detection consistent, the pH of the buffer for protamine should be the same as the heparin detection. In order not to affect the result of protamine detection by the mixing time, the fluorescence response at different times was measured (Fig. S9, ESI†). When performing detection, heparin was used as the identification unit of protamine, and its concentration was also very important. Fig. S10 (ESI†) shows that the largest fluorescence quenching effect was at 12.0 μg mL^{-1}. After configuring the DSAI/Hep complex sensor,

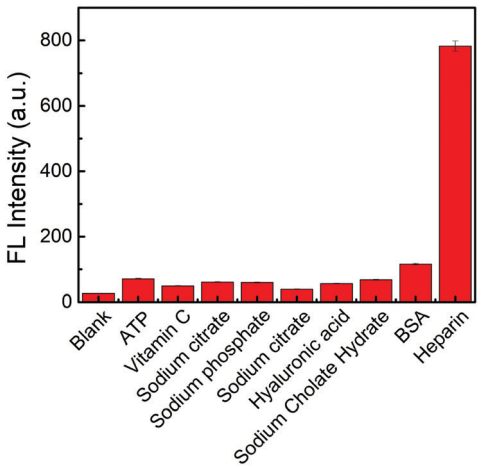


Fig. 3 Fluorescence intensity of DSAI in 9 equivalents of some micromolecules and common less sulfated glycosaminoglycans.

the fluorescence intensity decreases as the concentration of protamine increases (Fig. 4A and B). A splendid linearity of the fluorescence intensity at 519 nm ($y = 568.21 - 71.1x$) was fitted in the concentration range of 0.1 to 1.7 μg mL^{-1}, with a linear correlation coefficient (R^2 = 0.995). The detection limit for heparin with this method could reach 5.20 ng mL^{-1}. These measurement results clearly show that the DSAI/Hep complex can be used as a more sensitive sensor for protamine compared to previous reports (Table 1).

To determine the selectivity of detection of protamine, the effect of interference from other potentially competing species including positively charged ions and related physiological molecules was investigated. For this purpose, the changes in the fluorescence intensity of the system upon the addition of common biological species including sodium chloride (NaCl), calcium chloride ($CaCl_2$), melamine, cysteine (Cys), glutathione (GSH), tryptophan, and protamine were observed and recorded. The sharply fluorescent quenching in the system containing protamine demonstrated that the heparin–protamine complex was formed and DSAI redistributed in solution. In contrast, when other competing species and molecules were used in the system, almost no change in fluorescence intensity was observed (Fig. 5). As a result, a simple fluorescence sensing system is successfully developed for the rapid determination of protamine.

The cyclability of this assay for detecting heparin and protamine is also important. For this purpose, quantitative

Table 1 List of different sensors to detect the performance of heparin and protamine

Serial number	Sensor	Heparin detection limit	Protamine detection limit	Ref.
1	Pyrene-based ratiometric fluorescent sensor	153 ng mL^{-1}	107.8 ng mL^{-1}	35
2	Ratiometric scheme for the fluorescent detection	—	7.7 ng mL^{-1}	36
3	Accelerated etching of gold nanorods	10 ng mL^{-1}	—	37
4	AIE-based fluorescence probe	37 ng mL^{-1}	30 ng mL^{-1}	38
5	AIE-based fluorescence probe	0.18 ng mL^{-1}	47 ng mL^{-1}	39
6	DNA-templated gold nanoclusters	0.15 μg mL^{-1}	—	40
7	Perylene diimide derivative	—	90 ng mL^{-1}	41

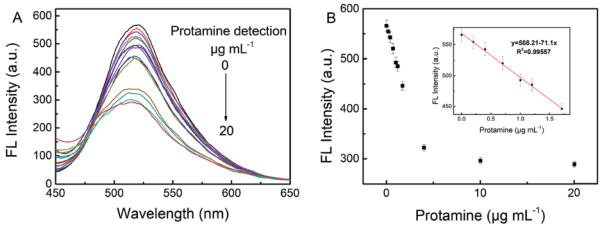


Fig. 4 (A) and (B) Fluorescence spectra (λ_{ex} = 408 nm) of DSAI [0.1 mmol L^{-1} in phosphate buffer solution (0.2 M), pH = 7] and heparin (12.0 μg mL^{-1}) in the presence of different amounts of protamine (from 0 to 20 μg mL^{-1}). [Inset: The relationship between fluorescence intensity and the concentration of the protamine from 0 to 1.7 μg mL^{-1}.].

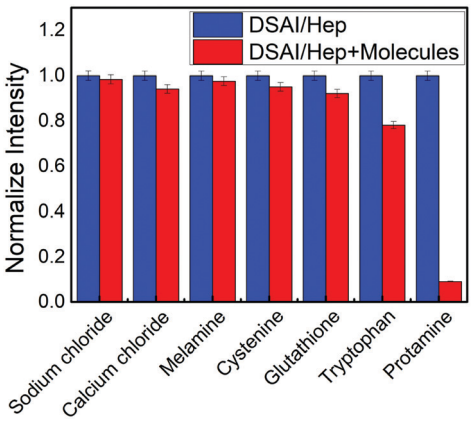


Fig. 5 Fluorescence intensity of the DSAI/Hep complex in 7 equivalents of different positively charged ions and physiological protein molecules.

heparin and protamine were alternately added into the solution system to observe the increase and decrease of fluorescence. Although the previous fluorescence intensity could not be fully restored, significant changes in fluorescence intensity were observed even after five cycles (Fig. S11, ESI†). This indicated that this assay has the ability of reproducibility and repeatability.

In addition, in a real sample, there would be an adequate concentration of heparin or protamine. Therefore, the determination of a high concentration of heparin and protamine is also meaningful. After increasing the concentration of the DSAI, the response difference between the fluorescence intensity of heparin at two high concentrations increased (Fig. S12, ESI†). But unfortunately, a similar result was not observed in high concentrations of protamine detection. This result revealed that only a high concentration of heparin (without diluting) could be detected by increasing the concentration of DSAI.

The detection of heparin and protamine in practical applications

To evaluate the practical application of the proposed sensor, we detected heparin in goat serum samples. The goat serum was diluted 33-fold and the heparin concentrations are in the linear range of this sensor and the potential interference can be reduced. The corresponding results based on the four duplicated measurements are displayed in Table S1 (ESI†). It could be seen that the values measured using our proposed method were consistent with the spiked values for the concentration of heparin. These results indicate that this sensor has great potential applications for the assay of heparin in real samples.

Moreover, to demonstrate the practical application of the DSAI/Hep complex sensor for protamine, the detection of protamine in the goat serum matrix was performed. The determination results are illustrated in Table S2 (ESI†). As can be seen, the recovery of protamine detected in serum samples ranged from 98% to 105%, which was satisfactory for performing quantitative assays in biological samples.

Conclusion

In conclusion, a simple and highly sensitive fluorescent sensor DSAI was synthesized. Based on its AIE property, the emission intensity of DSAI could be enhanced by heparin and detection of heparin was realized. The DSAI/Hep complex sensor implemented highly sensitive detection of protamine because of the stronger affinity between heparin and protamine. The limits of detection for heparin and protamine both reached single-digit nanograms per millilitre. Meanwhile, we also demonstrated the analysis mechanism of DSAI for heparin and protamine detection. Furthermore, the as-developed DSAI sensor was also used for the assay of heparin and protamine in goat serum samples with satisfactory results. This work provides a simple, highly sensitive and selective method for efficient sensing of heparin and protamine.

Conflicts of interest

There is no conflict to declare.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (No. 21604006) and the Youth Talent Development Program of the Chongqing Normal University (No. 15XLB019).

Notes and references

1 B. Casu, *Adv. Carbohydr. Chem. Biochem.*, 1985, **43**, 51–134.
2 I. Capila and R. J. Linhardt, *Angew. Chem., Int. Ed.*, 2002, **41**, 390–412.
3 S. M. Bromfield, E. Wilde and D. K. Smith, *Chem. Soc. Rev.*, 2013, **42**, 9184–9195.
4 S. Pratihar, Y. V. Suseela and T. Govindaraju, *ACS Appl. Bio Mater.*, 2020, **3**, 6979–6991.
5 A. V. M. K. M. Einhäupl, S. Mehraein, C. Garner, M. Pellkofer, R. L. Haberl, H.-W. Pfister, P. Schmiedek and W. Meister, *Lancet*, 1991, **338**, 597–600.

6 D. L. Ram Sasisekharan, R. Godavarti, G. Venkataraman, C. L. Cooney and R. Langer, *Biochemistry*, 1995, **34**, 14441–14448.
7 G. Arepally and T. Ortel, *Ann. Thorac. Surg.*, 2003, **76**, 638–648.
8 A. Lutton, *Lancet*, 1964, **283**, 768–769.
9 G. Blaise, *Can. J. Anaesth.*, 1998, **45**, 1144–1147.
10 E. Sokolowska, B. Kalaska, J. Miklosz and A. Mogielnicki, *Expert Opin. Drug Metab. Toxicol.*, 2016, **12**, 897–909.
11 J. C. J. A. Carr and N. Silverman, *Surg.*, 1999, **40**, 659–666.
12 S. Taylor and J. Folkman, *Nature*, 1982, **297**, 307–312.
13 D. J. Murray, W. J. Brosnahan, B. Pennell, D. Kapalanski, J. M. Weiler and J. Olson, *J. Cardiothorac. Vasc. Anesth.*, 1997, **11**, 24–28.
14 P. D. Raymond, M. Ray, S. N. Callen and N. A. Marsh, *Perfusion*, 2003, **18**, 269–276.
15 L. Zhou, H. Matsumura, M. Mezawa, H. Takai, Y. Nakayama, M. Mitarai and Y. Ogata, *Gene*, 2013, **516**, 228–237.
16 N. U. Khan, C. K. Wayne, J. Barker and T. Strang, *Eur. J. Anaesthesiol.*, 2010, **27**, 624–627.
17 J. S. Cuenca, P. G. Diz, F. G. Sampedro, J. M. G. Zincke, H. R. Acuña and M. M. F. Fontanillo, *J. Extra-Corpor. Technol.*, 2013, **45**, 235–241.
18 M. Levine, M. J. Hirsh, M. Gent, A. Turpie, M. Cruickshank, J. Weitz, D. Anderson and M. Johnson, *Arch. Intern. Med.*, 1994, **154**, 49–56.
19 Z. Xie, C. Chen, S. Xu, J. Li, Y. Zhang, S. Liu, J. Xu and Z. Chi, *Angew. Chem., Int. Ed.*, 2015, **54**, 7181–7184.
20 L. Zong, Y. Xie, C. Wang, J.-R. Li, Q. Li and Z. Li, *Chem. Commun.*, 2016, **52**, 11496–11499.
21 X. Zhang, K. Wang, M. Liu, X. Zhang, L. Tao, Y. Chen and Y. Wei, *Nanoscale*, 2015, **7**, 11486–11508.
22 N.-H. Xie, C. Li, J.-X. Liu, W.-L. Gong, B. Z. Tang, G. Li and M.-Q. Zhu, *Chem. Commun.*, 2016, **52**, 5808–5811.
23 M. Faisal, Y. Hong, J. Liu, Y. Yu, J. W. Y. Lam, A. Qin, P. Lu and B. Z. Tang, *Chem. – Eur. J.*, 2010, **16**, 4266–4272.
24 M. P. Aldred, C. Li, G.-F. Zhang, W.-L. Gong, A. Li, Y. Dai, D. Ma and M.-Q. Zhu, *J. Mater. Chem.*, 2012, **22**, 7515.
25 A. Rananaware, D. D. La and S. V. Bhosale, *RSC Adv.*, 2015, **5**, 63130–63134.
26 Y. J. Wang, Z. Li, J. Tong, X. Y. Shen, A. Qin, J. Z. Sun and B. Z. Tang, *J. Mater. Chem. C*, 2015, **3**, 3559–3568.
27 Z. Zhao, B. He and B. Z. Tang, *Chem. Sci.*, 2015, **6**, 5347–5365.
28 Z. Yang, Z. Chi, T. Yu, X. Zhang, M. Chen, B. Xu, S. Liu, Y. Zhang and J. Xu, *J. Mater. Chem.*, 2009, **19**, 5541–5546.
29 Z. Wang, B. Xu, L. Zhang, J. Zhang, T. Ma, J. Zhang, X. Fu and W. Tian, *Nanoscale*, 2013, **5**, 2065–2072.
30 J. Cui, S. Zang, W. Shu, H. Nie, J. Jing and X. Zhang, *Anal. Chem.*, 2020, **92**, 7106–7113.
31 X. Wang, Q. Jiang, Y. Man, S. Feng, Y.-I. Lee and H.-G. Liu, *Sens. Actuators, B*, 2018, **261**, 233–240.
32 Y. Li, S. Teng, M. Wang, B. Duan and Z. Huang, *Sens. Actuators, B*, 2021, **330**, 129290.
33 X. Peng, Q. Long, H. Li, Y. Zhang and S. Yao, *Sens. Actuators, B*, 2015, **213**, 131–138.
34 K. M. Xing Li, S. Zhu, S. Yao, Z. Liu, B. Xu, B. Yang and W. Tian, *Anal. Chem.*, 2014, **86**, 298–303.
35 W. Gong, S. Wang, Y. Wei, L. Ding and Y. Fang, *Spectrochim. Acta, Part A*, 2017, **170**, 198–205.
36 S. P. Pandey and P. K. Singh, *J. Mol. Liq.*, 2020, **303**, 112589.
37 J. Wang, H. Z. Zhang, J. J. Liu, D. Yuan, R. S. Li and C. Z. Huang, *Anal.*, 2018, **143**, 824–828.
38 R. Jiang, S. Zhao, L. Chen, M. Zhao, W. Qi, W. Fu, L. Hu and Y. Zhang, *Int. J. Biol. Macromol.*, 2020, **156**, 1153–1159.
39 S. P. Pandey, P. Jha and P. K. Singh, *J. Mol. Liq.*, 2020, **315**, 113625.
40 L. Ou, F. Yang, J. Luo, J. Duan, A. Sun, L. Chen and L. Wang, *Can. J. Chem. Eng.*, 2021, DOI: 10.1002/cjce.23945.
41 H. Cheng, Y. Zhao, H. Xu, Y. Hu, L. Zhang, G. Song and Z. Yao, *Dyes Pigm.*, 2020, **180**, 108456.

