



Development of Optical Biosensor for the Detection of Glutamine in Human Biofluids Using Merocyanine Dye

Vijayakumar Sathya¹ · Appadurai Deepa¹ · Lakshmi Kandhan Sangeetha¹ · Venkatesan Srinivasadesikan² · Shyi-Long Lee³ · Vediappen Padmini¹

Received: 16 November 2021 / Accepted: 24 March 2022 / Published online: 14 April 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract

Merocyanine dye based fluorescent organic compound has been synthesized for the detection of glutamine. The probe showed remarkable fluorescent intensity with glutamine through ICT (Intermolecular Charge Transfer Mechanism). Hence, it is tested for the detection of glutamine using colorimetric and fluorimetric techniques in physiological and neutral pH (7.2). Under optimized experimental conditions, the probe detects glutamine selectively among other interfering biomolecules. The probe has showed a LOD (lower limit of detection) of 9.6×10^{-8} mol/L at the linear range 0–180 μ M towards glutamine. The practical application of the probe is successfully tested in human biofluids.

Keywords Glutamine · Fluorimetry biosensor · LOD · Competitive study

Introduction

Glutamine is a typical biomarker which plays a vital role in renal and muscular dysfunctions as it is directly released from kidney [1, 2]. The variation of glutamine level in blood and urine of human body is an important parameter for clinical diagnostics. An abnormal level of glutamine in human serum causes cancer cell growth. Normally serum contains 0.8 mg/dl glutamine which is reduced under extreme

pathological conditions [3, 4]. It indicates the presence of cancer cells under metabolic stress conditions. Due to its medical compliance, the evolution of a fast and valid assay towards the determination of glutamine in human serum or urine is essential [5, 6]. Several techniques have been proposed for a fast and accurate quantification of glutamine [7–9]. Higher level of glutamine in human blood could lead to constipation or diarrhea for cancer patients [10]. It is clearly understood that glutamine is responsible for tumors in human body [11]. Glutamine is the most abundant endogenous antioxidant molecule to appreciate the cancer cell survival [12] compared to all other amino acids in our body [13]. Glutamine is also the precursor for the synthesis of various biologically important molecules including glutamate and α -amino butyrate (GABA), inhibitory neurotransmitter, excitatory neurotransmitter and glutathione (an antioxidant) [14].

Reports are available for the detection of glutamine with amperometric techniques containing various biocompatible principles [3, 15]. Fluorimetric technique is the most potent tool for the detection of various kinds of biologically important metal ions and biomolecules such as amino acids, nucleic acids and enzymes [16] as this technique provides very high sensitivity with biomolecules [17].

Though cyanine based organic fluorophore shows excellent fluorescent response towards various kinds of metal ions [18], it is not so with biomolecules, especially amino acids

Highlights

- The organic sensor of merocyanine dye was synthesized by a simple condensation method with moderate yield.
- The dye as a sensor detects the glutamine with high fluorescence intensity.
- The sensor was selectively detects glutamine while interfering other biomolecules and biologically important metals.

✉ Vediappen Padmini
padmini.chem@mkuniversity.org

¹ Department of Organic Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai 625021, Tamilnadu, India

² Division of Chemistry, Department of Sciences and Humanities, Vignan's Foundation for Science, Technology and Research, Vadlamudi, Guntur 522 213, Andhra Pradesh, India

³ Department of Chemistry and Biochemistry, National Chung-Cheng University, Chia-Yi 621, Taiwan

[19]. In general, most of the disease inducing biomolecules have a tendency to interact or bind with hydroxyl groups and amino groups [20]. Prompted by this, we have synthesized a simple cyanine based organic fluorophore, (*E*)-2-(2-(1*H*-indol-3-yl) vinyl)-1,1,3-trimethyl-1*H*-benzo[*e*] indol-3-ium iodide, which is used for the detection of glutamine. The synthesis involves a simple method with low cost and less time consumption. Various analytical techniques were used for the confirmation of the structure and photophysical properties of the synthesized molecule. Regarding the detection of glutamine, it is found that this compound is very effective in low concentration and non-toxic at high concentration [21]. This merocyanine dye is found to selectively detect glutamine in the presence of other interfering biomolecules and metal ions at neutral pH (7.2) with excellent lower limit of detection (LOD) and linear range.

Materials and Methods

Methyl iodide, 1,1,2-trimethyl-1*H*-benzo[*e*]indole, ethanol, piperidine, glutamine, creatine, creatinine, ascorbic acid, urea, proline, cysteine, histidine, tryptophan and various acetate salts of metal ions (Co^{2+} , Cd^{2+} , Ag^{+} , Ca^{2+} , Na^{+} , K^{+} , Fe^{3+} , Zn^{2+} and Cu^{2+}) were purchased from Sigma Aldrich and Alfa Aesar. ^1H and ^{13}C NMR spectra were recorded using DMSO-d_6 solvent with a trace of tetramethylsilane (TMS) as internal standard in a Bruker-300 MHz spectrometer. The chemical shifts were reported in ppm. Mass spectra were recorded by ESI ionization in MS-LCMS mass spectrometer. UV spectra were recorded using JASCO single beam UV–Vis spectrophotometer and fluorescence measurements were done with Cary Eclipse spectrophotometer having a 450 W xenon lamp.

Preparation of stock solution: Stock solutions (1.0×10^{-3} M) of biomolecules such as histidine, cysteine, proline, tryptophan, urea, asparagine, creatinine, creatine and ascorbic acid were prepared in 100 ml of double distilled water. Working stock solutions were obtained from these by further dilution with double distilled water/buffer solution [22].

Result and Discussion

General Procedure for the Synthesis of Merocyanine Dye

The merocyanine dye (probe) has been synthesized by a condensation reaction [23] as shown Fig. S1 in the supporting information. The synthesized probe was characterized by ^1H NMR, ^{13}C NMR and ESI-Mass spectroscopic studies (Figs. S2–S5, Supporting Information).

Photo Physical Properties of Merocyanine Dye

The UV–Vis absorption spectrum of merocyanine dye (1×10^{-6} M) was recorded at pH 7.2 in ACN (Acetonitrile)/PBS (Phosphate Buffer Solution) (v/v, 1:4) solvent. Under the optimized condition, the absorption spectrum of merocyanine dye has exhibited two characteristic peaks at 294 nm and 478 nm (Fig. S8a), which are mainly attributed to π - π^* and n - π^* transitions respectively. While varying the concentration of glutamine from 0 μM to 180 μM (Fig. 1), the intensities of absorption peaks at 294 nm and 478 nm increased gradually. The linear (Fig. 2) plot didn't show any remarkable shift in absorption position in this range, 0 μM to 180 μM .

The competitive titrations were also performed to expose the fastidious of the sensor towards glutamine in the presence of various interfering biomolecules. The absorbance spectra for the selective recognition of glutamine in presence of other interfering biomolecules are shown in Fig. 3. From these studies, it is clearly understood that the developed optical biosensor could detect glutamine selectively. Similarly, the emission studies were carried out for the merocyanine dye (1×10^{-6} M) and glutamine at neutral pH (7.2) at in ACN/PBS (v/v, 1:4) solvent. The effect of pH on sensing property of probe with glutamine was also studied with different buffer solutions (pH 2–13) (Fig. S6).

The glutamine containing reactive oxygen species is responsible for the sharp increase of the fluorescent intensity in the emission spectrum. The addition of other interfering biomolecules leads to no spectral change. It is clearly understood that the merocyanine dye detects the glutamine

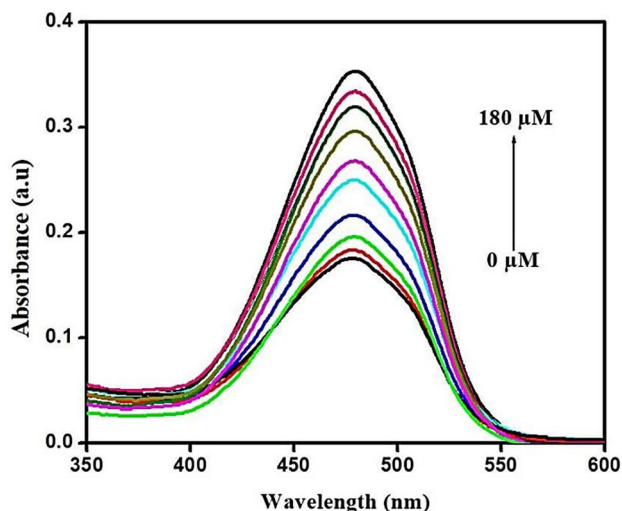


Fig. 1 Changes in absorption spectra of probe (1×10^{-6} M) in ACN/PBS (v/v, 1:4) at pH 7.2 upon addition of glutamine (0–180 μM) (λ_{ex} = 478 nm)

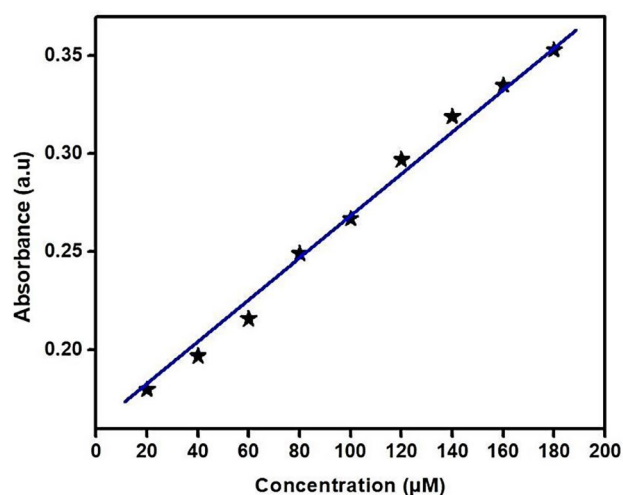


Fig. 2 The linear relationship of probe (1×10^{-6} M) toward glutamine (180 μ M), $\lambda_{\text{ex}} = 478$ nm

with high selectivity. The corresponding bar diagram shows the high selectivity towards glutamine compared to other interfering competitors.

The quantum yield of merocyanine dye has been calculated by using absorption spectral data and it is found to be 0.77. Rhodamine 6G in ethanol is used as standard in this work ($\Phi = 0.95$) (Fig. S11) [24].

Colorimetric Biosensor for Glutamine Detection

UV-Visible spectral analysis was performed with probe at concentration of 1×10^{-6} M in phosphate buffer (pH 7.2).

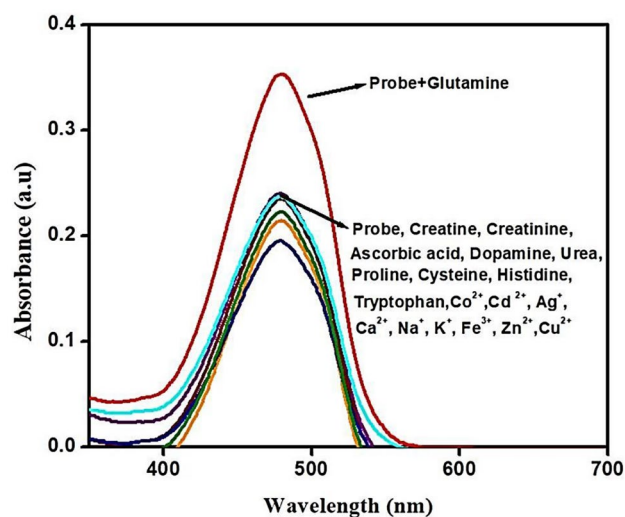


Fig. 3 Absorption spectra of merocyanine dye (1×10^{-6} M) for selective detection of glutamine in presence of other interfering biomolecules (1×10^{-5} M)

Glutamine of different concentration (0 μ M to 180 μ M) (Fig. 4) was added to the dye (1×10^{-5} M) solution which was then incubated for 5 min. The UV-Vis spectra were recorded before and after the addition of the analyte. The gradual increment of absorbance was observed while adding analyte. Also we performed the selectivity study for glutamine, with other interfering biomolecules and other essential metal ions. Glutamine shows selective absorbance in the UV spectrum. Based on the above results, it is inferred that there is an interaction between dye and glutamine at neutral medium. The dye has a mild yellow color and upon the addition of glutamine, the color has been changed to red orange as noted by naked eye. However the addition of interfering biomolecules and important metal ions do not bring any change of color. This indicates that the optical biosensor can sense glutamine colorimetrically. As the dye has indole N-H, it can interact with the reactive oxygen species of glutamine and the other biomolecules do not interact with the dye. Under the UV region, the dye does not exhibit fluorescence. But upon the addition glutamine blue colour fluorescence is observed. No change has been observed between the dye and other competitors as shown in Fig. S10.

Fluorescent Response of Dye Towards the Analyte

Under optimized condition, the synthesized dye shows a fluorescence peak at 436 nm with mild fluorescence intensity (Fig. S8b). With the addition of glutamine, the fluorescence TURN-ON response was observed (Scheme 1). While increasing the concentration of glutamine from 0–180 μ M, a gradual increase in the fluorescent intensity was observed along with a hypsochromic (blue shift) (Fig. 4).

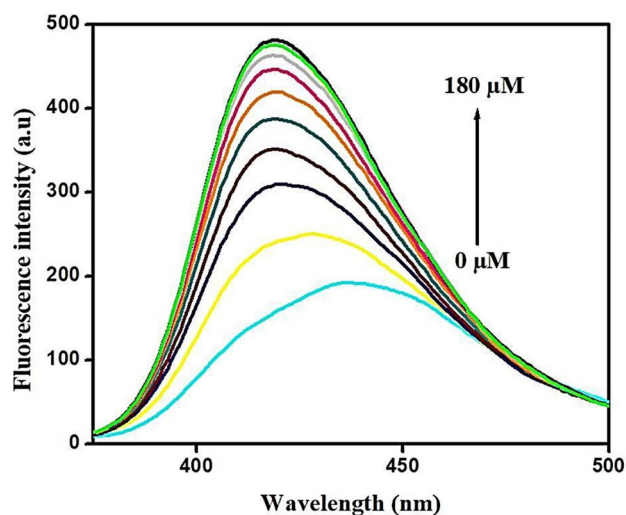
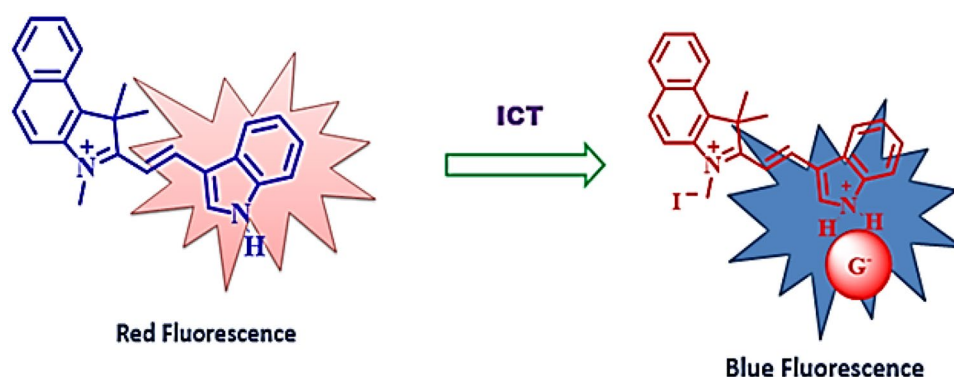


Fig. 4 Changes in emission spectra of probe (1×10^{-6} M) in ACN/PBS (v/v, 1:4) at pH 7.2 upon addition of glutamine (0–180 μ M) ($\lambda_{\text{em}} = 436$ nm)

Scheme 1 Proposed sensing mechanism of dye with glutamine



A linear range between dye and glutamine was found from 0 μM –180 μM at neutral pH (7.2) (Fig. 5). The lower detection limit (LOD) (Fig. S12) was found to be 9.6×10^{-8} mol/L at pH 7.2 (Fig. 6). For the first time, very good LOD of 9.6×10^{-8} mol/L along with great linear detection range for glutamine has been observed when compared to the previous reports (Table 1).

Selective Analysis for Glutamine Among Other Interfering Species

The interference study was taken with many biomolecules and biologically important metal ions such as creatine, creatinine, ascorbic acid, urea, proline, cysteine, histidine, tryptophan and biologically important various acetate salts of metal ions (Co^{2+} , Cd^{2+} , Ag^+ , Ca^{2+} , Na^+ , K^+ , Fe^{3+} , Zn^{2+} and Cu^{2+}). All these competitors were tested against glutamine at pH 7.2 (Fig. 7) [25]. From the spectral results, it is understood that the dye shows higher selectivity towards

glutamine. All interfering biomolecules and important metal ions do not show any change in absorption and emission properties. Two equivalents (40 μL) of interfering biomolecules and metal ions (1×10^{-5} M) was added to the solution consisting one equivalent of glutamine (20 μL). Then the corresponding emission spectrum was recorded. Results show that merocyanine dye selectively sense glutamine at pH 7.2 (Fig. 5) [26]. Based on the spectral changes in UV and emission properties, it is clear that the interaction between merocyanine dye and glutamine adopted ICT mechanism in the stoichiometry of 1:1 (Fig. S9). Job's plot has been constructed from fluorescence titration to determine the stoichiometry of Merocyanine dye: Glutamine.

The effect of pH on fluorescent nature of dye was analysed with different PBS solutions (from pH 2 to 13). The pH has been adjusted from 2 to 6 and there was a gradual increase in intensity up to pH 7.2. But, further increase of pH decreases the intensity along with a blue shift. At pH 7.2, the maximum fluorescent intensity is observed. Hence,

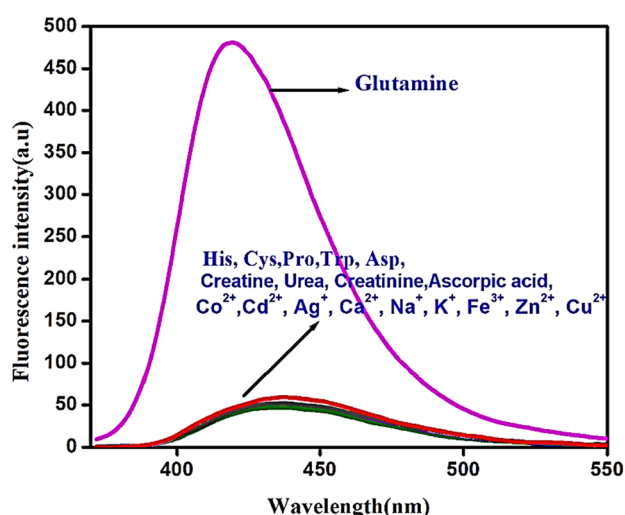


Fig. 5 Fluorescence spectra of merocyanin dye (1×10^{-6} M) for selective detection of glutamine in presence of other interfering biomolecules (1×10^{-5} M)

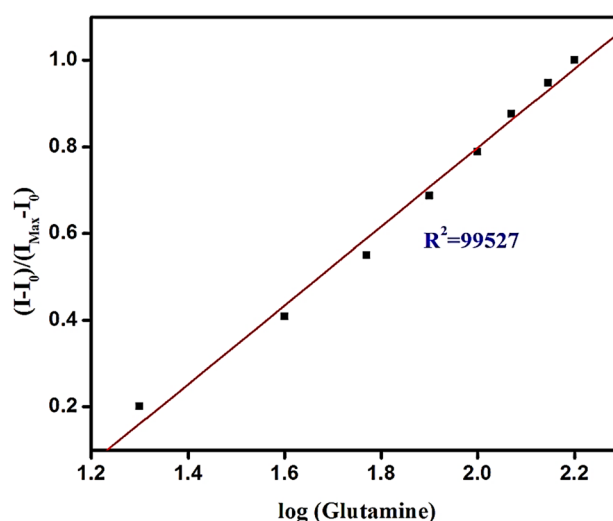


Fig. 6 The linear relationship of probe (1×10^{-6} M) toward glutamine (180 μM), $\lambda_{\text{em}} = 436$ nm

Table 1 Comparative performance for previously reported glutamine biosensors

Sl. No	Types of sensor platform	Techniques used	LOD	References
1	Porous silicon nano-technology	Optical microsensor	0.21 $\mu\text{g/L}$	[13]
2	optical protein micro sensor, based on porous silicon nanotechnology	Optical sensor	2.0–40.0 $\mu\text{g/L}$	[34]
3	Determination of glutamine in the pulsed-batch Cultivation of hybridoma cells	Amperometric biosensor	0.1–3 mM	[15]
4	L-glutamate oxidase based enzyme sensor	Amperometric biosensor	1×10^{-5} mol/L	[17]
5	Merocyanine dye based optical biosensor	Optical biosensor	9.6×10^{-8} mol/L	Present work

all spectroscopic studies were carried out in PBS at pH 7.2. This may be attributed to inhibition of fluorescent intensity by protonation of the complex at low pH. Under neutral condition, the complex emits high intensity due to the lone pair electrons of the complex. At basic pH, deprotonation may lead to poor fluorescent intensity of the complex of probe with glutamine. The stoichiometry of merocyanine dye: glutamine was determined as 1:1 as shown in Fig. S9 [27]. The competitive experiments were performed with glutamine (180 μM) in the presence of other interfering biomolecules as shown in Fig. S7.

In addition, the NMR titration was also carried out and it is clearly exhibited that the interaction between probe and analyte. The NH proton of probe appeared at 13.10 ppm and it was shifted to 11.46 ppm in the ^1H NMR spectrum (Fig. S13) during the addition. All these experimental studies confirm the formation of 1:1 binding [28]. The binding constant of the analyte with probe has been calculated from the emission plot as $13.2 \times 10^6 \text{ M}^{-1}$ (Fig. S16).

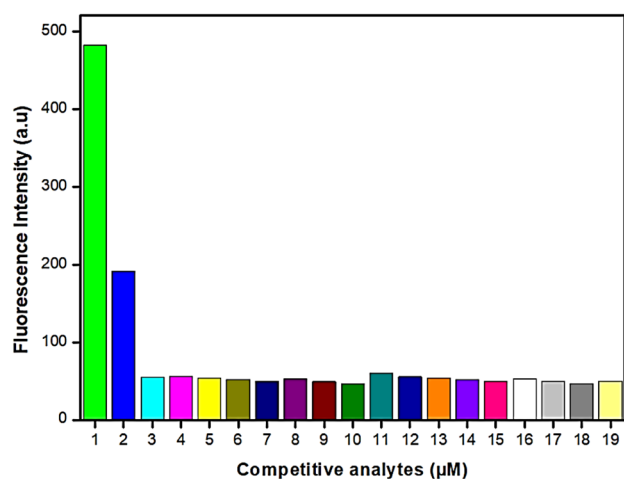


Fig. 7 Fluorescence intensity of probe ($1 \times 10^{-6} \text{ M}$) upon addition of glutamine (180 μM) with in the presence of other competitors (180 μM) in ACN/PBS (v/v, 1:4), $\lambda_{\text{em}}=436 \text{ nm}$. [1=Probe + glutamine, 2=Probe, 3=Creatinine, 4=Creatine, 5=Tryptophan, 6=Cysteine, 7=Urea, 8=Proline, 9=Ascorbic Acid, 10=Histidine, 11= Co^{2+} , 12= Cd^{2+} , 13= Ag^+ , 14= Ca^{2+} , 15= Na^+ , 16= K^+ , 17= Fe^{3+} , 18= Zn^{2+} , 19= Cu^{2+}]

Sensitivity of Glutamine in Human Biofluids

The quantitative evaluation of glutamine in human serum and urine (Figs. 8 and 9) has been performed using the standard addition method. In brief, a known concentration of merocyanine molecule (5 μM) was added into human serum. Then, different concentrations of glutamine (20 μM , 40 μM , 60 μM , 80 μM & 100–180 μM) were spiked in to blood sample along with above solution and incubated for 15 min. After the incubation period, the fluorescence studies were carried out under optimized conditions and the spectral changes were noted in Table S15.1. Then, the recovery of the biosensor was calculated based on the change in fluorescent intensity of merocyanine molecule while spiking known concentrations of glutamine into human blood serum and buffer solution containing $1 \times 10^{-6} \text{ M}$ of merocyanine as shown in Fig. 8.

The known concentration of merocyanine (5 μM) was taken with the urine sample like 20 μM , 40 μM , 60 μM ,

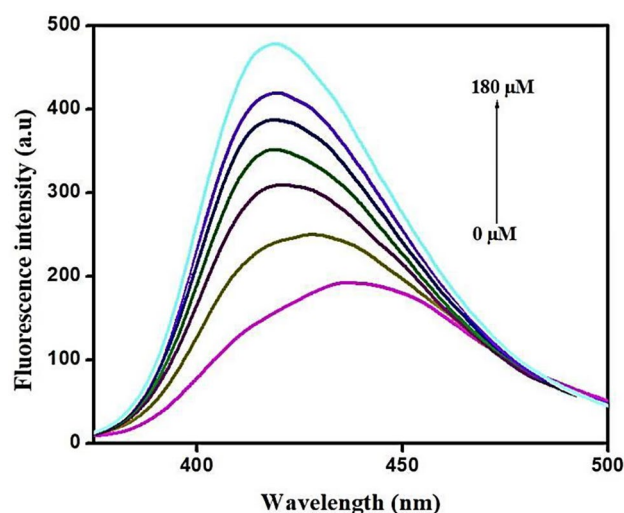


Fig. 8 Changes in emission spectra of probe ($1 \times 10^{-6} \text{ M}$) in ACN/PBS (v/v, 1:4) at pH 7.2 upon addition of glutamine in blood serum (0–180 μM) ($\lambda_{\text{em}}=436 \text{ nm}$). [Concentration of glutamine spiked: 20 μM , 40 μM , 60 μM , 80 μM & 100–180 μM]

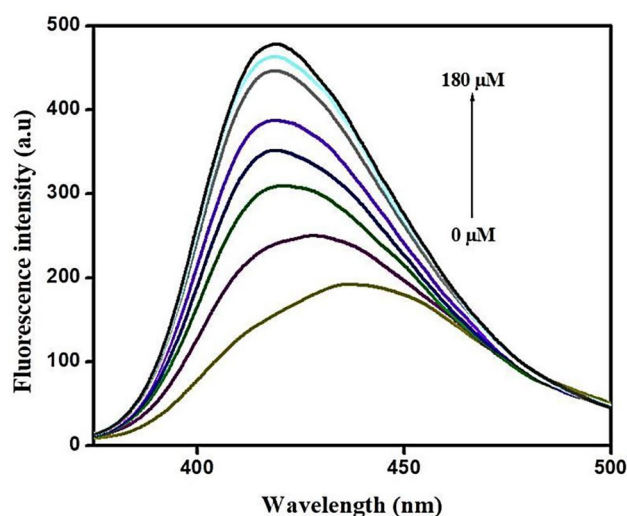


Fig. 9 Changes in emission spectra of probe (1×10^{-6} M) in ACN/PBS (v/v, 1:4) at pH 7.2 upon addition of glutamine in urine sample (0–180 μ M) ($\lambda_{em} = 436$ nm). [Concentration of glutamine spiked: 20 μ M, 40 μ M, 60 μ M, 80 μ M & 100–180 μ M]

80 μ M & 100–180 μ M and incubated for 15 min. After the incubation period, the fluorescence studies were carried out under optimized conditions and the spectral changes are noted in Table S15.2. Then the recovery of the biosensor was calculated based on the change in fluorescent intensity of merocyanine molecule while spiking known concentrations of glutamine into human urine and buffer solution containing 1×10^{-6} M of merocyanine as shown in Fig. 9. From these results, it is clearly seen that the biosensor under

study can be used for the detection of glutamine in human urine [29].

The test strip method for the detection of glutamine was tested under UV lamp by dye. Initially, the test strip was soaked into the probe solution and it was dried and analyzed for the color nature of the strip by UV lamp. Color change was observed from mild violet to blue fluorescence by UV lamp during the addition of the analyte to the probe. There is no color change for competitors (Fig. S14) [30, 31]. The test strip method has shown that the probe is a good transducer for glutamine.

DFT Studies

To understand the binding mechanism of glutamine with merocyanine, the *state-of-the-art* density functional theory (DFT) calculations were carried out. All the DFT calculations have been effected using the Gaussian 09 [32] software package. Optimization of Merocyanine, Merocyanine-glutamine complexes and its Frontier Molecular Orbital, ESP (Electron Spin Polarization) and TDDFT (time-dependent density functional theory) calculations were computed at dispersion corrected B97D [33] level using pople basis set 6-31 g(d) as basis set in the gas phase. Furthermore, it is confirmed that there are no negative vibrational frequencies observed for the optimized structures.

The optimized structure of Merocyanine is shown in Fig. 10a and that of Merocyanine ligand and its Glutamine complex is shown in Fig. 10b. The hydrogen bonding length between merocyanine and Glutamine has been observed to be 2.004 Å. The binding energy of the Merocyanine-Glutamine complex is calculated as -22.36 kcal/mol, exothermic.

Fig. 10 (a) Optimized structures of Merocyanine and (b) Merocyanine-Glutamine complex at B97D/6-31 g (d,p) in gas phase. The hydrogen bond length (Å) is shown by dotted lines. (c) optimized electrostatic potential of Merocyanine at B97D/6-31 g(d) in the gas phase. (Grey: Carbon, Blue: Nitrogen, White: Hydrogen, Red: Oxygen)

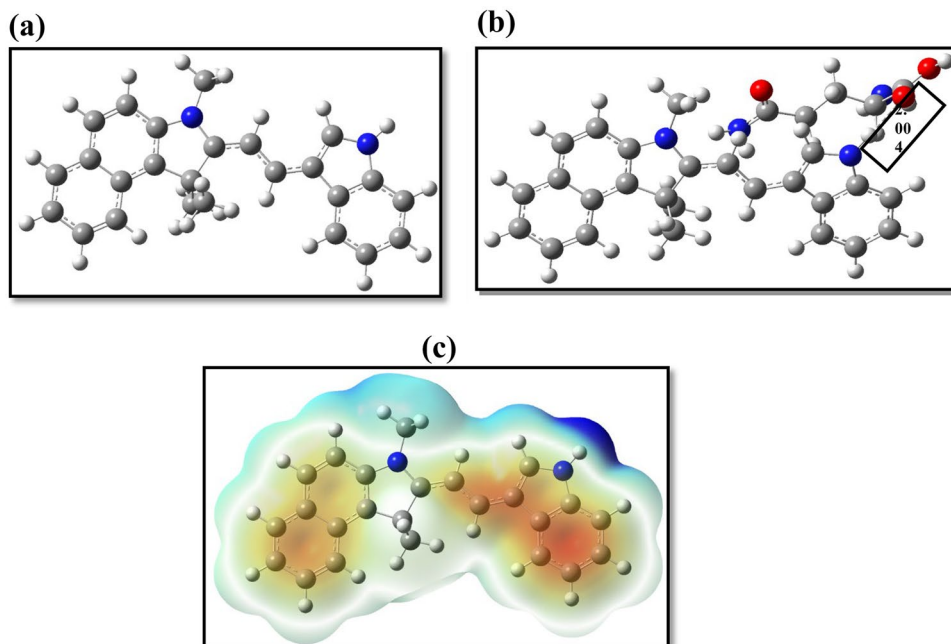
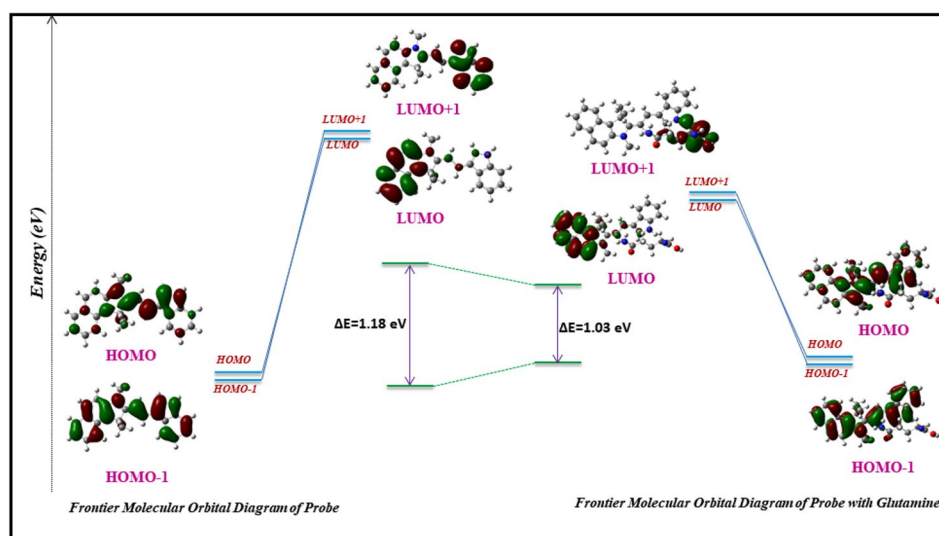


Fig. 11 Molecular orbital distribution plots of HOMO, HOMO-1, LUMO and LUMO+1 states in the ground state for Merocyanine receptor at B97D/6-31 g(d) level in the gas phase

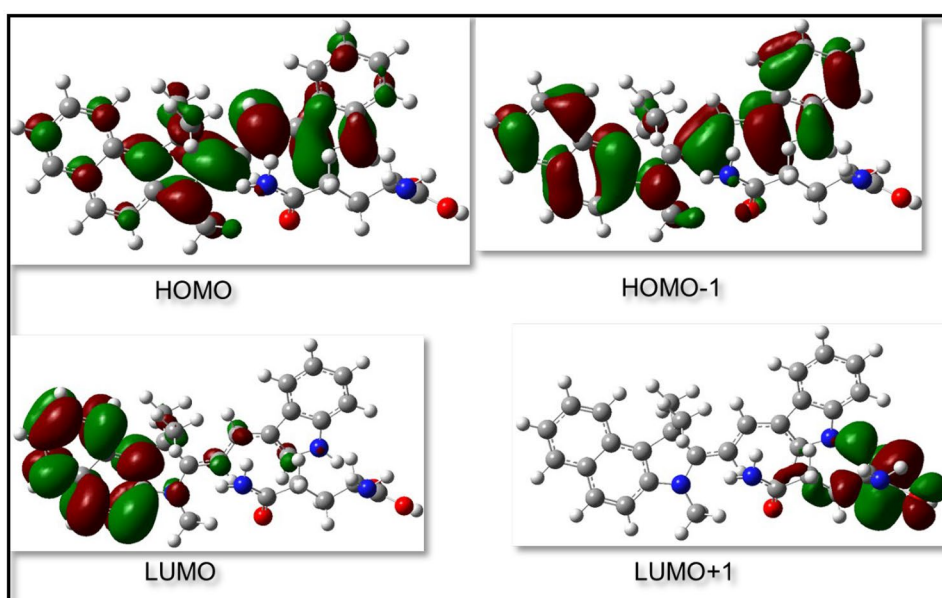


The electrostatic potential (ESP) structure of merocyanine monomer has been optimized at B97D/6-31 g(d) level in the gas phase and revealed in Fig. 10c. The ESP structure clearly shows the position of binding site for glutamine. The Frontier Molecular Orbital for the monomer and complex were computed and shown in Figs. 11 and 12. The HOMO-LUMO (Highest Occupied Molecular Orbital-Lowest Unoccupied Molecular Orbital) gap of merocyanine and merocyanine complex is 1.18 eV and 1.03 eV, respectively. The reduction of HOMO-LUMO gap particularly for the merocyanine-Glutamine complex shows the binding of Glutamine with the Merocyanine sensor. In particular, as shown in Fig. 11, the electron localization is distributed

on benzopyrrole and naphthopyrrole moiety in HOMO and HOMO-1 levels. Moreover, the electronic localization was migrated from benzopyrrole to naphthopyrrole during HOMO to LUMO excitation in merocyanine.

For the merocyanine-Glutamine complex, the localization of electron was noticed on the merocyanine and Glutamine in HOMO and HOMO-1 levels. The electron localization migrated towards the naphthopyrrole in LUMO state. However, in LUMO+1 state the electron localization has observed mostly on glutamine. Furthermore, in the beginning of electron transfer from HOMO to HOMO-1 may restrict the electron transfer from HOMO-1 to LUMO level. However, as soon as the glutamine bind with the merocyanine (Merocyanine-Merocyanine*), complex

Fig. 12 Molecular orbital distribution plots of HOMO, HOMO-1, LUMO and LUMO+1 states in the ground state for Merocyanine-Glutamine complex at B97D/6-31 g(d) level in the gas phase



shows different way of electron density localization compared with the monomer of Merocyanine. It clearly shows that the electron transfer from HOMO-1 to HOMO is restricted whereas HOMO to LUMO electron transfer was on and responsible for experimentally observed blue light while sensing the Glutamine.

Conclusion

Simple imine based fluorescent merocyanine dye was synthesized by simple condensation method and it is characterized by NMR and ESI mass spectral techniques. Merocyanine dye is a highly selective and sensitive biosensor towards glutamine at pH 7.2. LOD is found to be 9.6×10^{-8} mol/L at pH = 7.2 within linear range for glutamine concentration level from 0–180 μ M. Merocyanine dye shows a good quantum yield of 0.87.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10895-022-02937-y>.

Acknowledgements The author VS acknowledged DST, New Delhi for the INSPIRE fellowship (IF180132). Authors thank for financial support under DST-IRHPA, FIST, RUSA-MKU and PURSE for instrument facilities.

Author Contributions Vijayakumar Sathya: Conceptualization, Writing-original draft. Appadurai Deepa: Revising the article. Lakshmi Kandhan Sangeetha: Revising the article. Venkatesan Srinivasadesikan: Software resources. Shyi-Long Lee: Software resources. VEDIAPPEN PADMINI: Supervision, Writing-review.

Funding The authors received no specific funding for this work.

Data Availability All relevant data are within the paper and its Supporting Information files.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflicts of Interest The authors have no conflict of interest in this research.

References

- Alil J, Najeeb J, Alil MA, Aslam MF, Raza A (2017) *J Biosens Bioelectron* 8:1–9
- Stetter JR, Hesketh PJ, Hunter GW (2006) *Electrochem Soc Interface* 15:66–69
- Fadel FI, Elshamaa MF, Essam RG, Elghoroury EA, Saeed GS, Toukhy SE, Ibrahim MH (2014) *Int J Biomed Sci* 10:36–42
- Choi YK, Park KG (2018) *Biomol Ther* 26:19–28
- Shimmura C, Suda S, Tsuchiya KJ, Hashimoto K, Ohno K, Matsuzaki H, Iwata K, Matsumoto K, Wakuda T, Kamenoy Y, Suzuki K (2011) *PLoS One* 6:25340–25346
- Watford M (2018) *J Nutr* 138:2003–2007
- Counihan JL, Grossman EA, Nomura DK (2018) *Chem Rev* 118:6893–6923
- Zhang J, Pavlova NN, Thompson CB (2017) *EMBO* 36:1302–1315
- Lukey MJ, Wilson KF, Cerione RA (2013) *Future Med Chem* 5:1685–1700
- Valeur B, Santos MNB (2011) *J Chem Educ* 88:731–738
- Still ER, Yuneva MO (2017) *Br J Cancer* 116:1375–1381
- Mehrotra P (2016) *J Oral Biol Craniofac Res* 6:153–159
- Jin L, Alesi GN, Kang S (2016) *Oncogene* 35:3619–3625
- Stefano LD, Rotiroli L, Rendina I, Moretti L, Scognamiglio V, Rossi M, Auria SD (2006) *Biosens Bioelectron* 21:1664–1667
- Cattaneo MV, Luong JHT, Mercille S (1992) *Biosens Bioelectron* 7:329–334
- Forni A, Lucenti E, Bottab C, Cariati E (2018) *J Mater Chem C* 6:4603–4626
- Boyr F, Botre C, Lorenti G, Mazzei F, Porcellio F, Scibonao G (1993) *J Pharm Biomed Anal* 11:679–686
- Pejic FB, Marco RD, Parkinson G (2006) *Analyst* 131:1079–1090
- Tsujino L, Miyagi K, Sampson RW, Sieber F (2006) *Photochem Photobiol* 82:458–465
- Guo Z, Kim G-H, Shin I, Yoon J (2012) *Biomaterials* 33:7818–7827
- Su B, Chen FZ, Wang QM (2013) *J Org Chem* 78:2775–2779
- Cuihua X, Venkat RRD, Haiying L (2006) *Macromolecules* 39:5747–5752
- Ponnuvel K, Ramamoorthy J, Sivaraman G, Padmini V (2018) *ChemistrySelect* 3:12150–12154
- Adak AK, Dutta B, Manna SK, Sinha C (2019) *ACS Omega* 4:18987–18995
- Han J, Burgess K (2010) *Chem Rev* 110:2709–2728
- Lin X, Chun L, Hua J (2009) *Org Lett* 11:1655–1658
- Senthilkumar T, Asha SK (2015) *Macromolecules* 48:3449–3461
- Ellairaja S, Shenbagavalli K, Ponmariappan S, Vasantha VS (2017) *Biosens Bioelectron* 91:82–88
- Liu L, Lin H (2014) *Anal Chem* 86:8829–8834
- Ao H, Feng H, Zhao M, Zhao M, Chen J, Qian Z (2017) *ACS Sens* 2:1692–1699
- Udhayakumari D, Naha S, Velmathi S (2017) *Anal Methods* 9:552–578
- Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA Jr, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ (2009) *Gaussian 09, Revision A.02*, Gaussian Inc., Wallingford, CT
- (a). Grimme S (2006) *J Comput Chem* 27:1787–1799 (b). Deepa A, Padmini V (2019) *J Fluoresc* 29:813–818
- Stefano LD, Rossi M, Staiano M, Mamone G, Parracino A, Rotiroli L, Rendina I, Rossi M, Auria SD (2006) *J Proteome Res* 5:1241–1245

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.