Article

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Sundaram Ellairaja, Venkatesan Subramanian, Kumaravel Velayutham, Rohini Gomathinayagam, and Vairathevar Sivasamy Vasantha

ACS Sens., **Just Accepted Manuscript** • DOI: 10.1021/acssensors.7b00822 • Publication Date (Web): 02 Mar 2018

Michael Addition based Chemodosimeter for Serum Creatinine Detection using (E)-3-(pyren-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one Chalcone

Ellairaja Sundaram[a*] Venkatesan Subramanian[b], Kumaravel Velayutham[c1], Rohini Gomathinayagam [c2] and Vairathevar Sivasamy Vasantha [a*] †

[a*] † Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai – 625 021, Tamilnadu, India.
[b]Chemical Laboratory, CSIR-Central Leather Research Institute, Adyar, Chennai – 600 020, Tamilnadu, India.
[c] Alpha Hospital and Research Center, Madurai-625 009, Tamilnadu, India.

Abstract

Firstly a simplest and highly emissive fluorescent chalcone PTP (E)-3-(pyren-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one, with an excellent quantum yield of 0.85, was synthesized instantly via simple hand shaking and proved as a stable, highly sensitive and selective biosensor for creatinine. Owing to its unique photophysical interaction with creatinine through Michael adduct formation, PTP was utilized as a mini chemodosimeter for the selective recognition of creatinine in blood serum. Under optimized condition, the creatinine was sensed broadly from 0.00000113 mg/dL to 15.8 mg/dL along with an excellent limit of detection of 0.00000065 mg/dL) (0.058 nM). This biosensor is highly reproducible even for different concentration levels of creatinine. It is the very first creatinine biosensor possessing wider linear range for clinical applications for creatinine. To ensure its clinical application, blood serum samples of people with different age group were collected from Alpha Hospital and analyzed for creatinine by using our chemodosimeter method and compared with data obtained using commercial method in the Alpha hospital. Our data show a very good agreement with clinical data. Since, clinical protocol involves tri-enzymes and tedious sample preparation; no doubt, our chemodosimeter will be a cheap and sensitive compare to the existing clinical methods.

Keywords: Chalcone, Chemodosimeter, Creatinine, Fluorescence, Biosensor, Blood serum.

Corresponding Author Email Id

vasantham999@yahoo.co.in;

Creatinine is a routine biomarker which plays a crucial role in renal and muscular dysfunctions because of its direct secretion from muscle as side product. The variation in creatinine level in blood and urine is an important parameter in clinical diagnostics. When the kidney is not properly functioned, the level of creatinine is increased in blood. In normal human fluid like urine creatinine levels are varied in the range from 0.45 to 1.69 mg/dL but under extreme pathological conditions, its level could exceed to 11.31 mg/dL and this abnormal level >5.65 mg/dL can indicate either renal malfunction in situation where creatinine concentration increases or can be a sign of muscular dystrophy for lower creatinine values. Due to its medical relevance the development of a fast and accurate assay for the determination of creatinine in human serum or urine is required. Although several techniques have been proposed for a fast and accurate quantification of creatinine in human serum or urine, most of them require expensive or complex apparatus, advanced sample preparation or skilled operators.[1-2] The earlier and frequent detection of creatinine in human blood could improve the quality of human life especially for peritoneal dialysis patients.[3-4] Generally, Jaffé's reaction has been used commonly for the quantification of creatinine based on the color change of alkaline solution of creatinine and picric acid.[5] Apart from this, a few enzymic colorimetric protocols and chromatographic tools could also be used for the detection of creatinine.[6] These protocols have been generally affected by numerous of metabolites and drugs found in biological samples and it requires high time. It is also more complicated and expensive.[7] With selectivity concern, some electrochemical creatinine sensors have also reported and having specific advantages like sensitivity, selectivity, less time consuming, cost effective etc. For example, there are lot of potentiometric biosensors, [8-9] conductometric biosensors, [10] some enzymatic [11-12] and enzyme less [13-14] creatinine biosensors and dissolved oxygen amperomatric creatinine biosensors,[15-16] nanoparticles based amperometric biosensors [17-18] and capacitive creatinine biosensors[19] are also reported with a good limit of dection. In current sensor era, fluorimetric technique has been acting as very simple analytical platform for various heavy metal ions,[20-22] biomolecules [23] and disease causing pathogens [24-25]**.** Using this simple and less time consuming technique, one could achieve a very good linear range along with an excellent Low limit of detection (LOD) towards a particular analyte.[26] A new digital camera based technology was developed and reported for creatinine detection in urine with LOD of 1 &1.25 mg/dL.[27] Recently, thioglycolic acid (TGA) capped ZnS:Mn/ZnS quantum dots (QDs) based fluorescence sensor has been reported for creatinine detection within in linear range of 0.0008 and 0.0385 mg/dL along with LOD of 0.0001 and 0.0003 mg/dL. [28] Meanwhile, our group has reported a simple fluorescence based biosensor for

the creatinine detection using Rhodamine B dye - Au^{3+} ion conjugates. [29] Currently, a novel turn-on fluorescent sensor has been reported for creatinine based on gluten stabilized gold quantum clusters along with picric acid as quencher. And the detection limit was found to be 0.00002 mg/dL within the linear range from 0.2262 to 5.8822 mg/dL. And also this reports utilized Picric acid as quencher and they have adopted the basic principle of Jaffe reaction indirectly. So, the direct detection of creatinine using an organic fluorophore/dye will be a cost effect sensitive method to substitute for clinical diagnostics of creatinine.

Chemodosimeter are molecular probes used to achieve the recognition of analyte with the irreversible process. They have been intensively studied in the anion sensing area, because they have advantage of high selectivity by the minimized interference of other anions. [30] There was plenty of fluorescent sensor so far reported which has adopted a particular mechanism like ICT, FRET, PET, ESIPT etc after the binding with specific analyte.[31] In these mechanisms, ICT has been noted as a frequent mechanism in fluorescent based chemo or biosensors. In which, based on the electron-donating character of the electron donating group and analyte binding, either blue shifts or red shifts will be expected in the absorption or fluorescence spectra. [32-34]

Peer review of above said literatures on creatinine biosensor has precisely evinced that there is no chemodosimeter based fluorescent biosensor has been reported using a simple organic fluorophore for direct detection of creatinine at normal and different diseased status. Moreover, nobody has compared their experimental data with commercially available clinical reports so far. To address these valid key notes, in our current work, we have synthesized a very simple chalcone (fluorophore) as a chemodosimeter through fissile route for the selective detection of creatinine in human serum. The structure was elucidated by NMR and ESI-Mass and its photophysical properties were analyzed using UV-Vis and fluorophotometer. As a colorimetric biosensor, the probe detected creatinine ratiometrically through ICT mechanism. This mechanism was further supported by DFT (Density Functional Theory) calculations. Under the optimized conditions, the biosensor detected the creatinine within the linear range of 1.13 to 11.31 mg/dL along with the LOD of 0.3959 mg/dL. As a fluorimetric biosensor, it could detect creatinine from 0.00000113 to 15.8 mg/dL. The performance of the biosensor implies that it could be applied for clinical diagnosis of muscle disorder (creatinine level < 0.45 mg/dL) and kidney male function (creatinine level = 11.31 mg/dL) as well. To the best of our knowledge, we are the first group applied our protocol for the determination of creatinine in clinical samples and proved as our protocol is equally good as commercial clinical protocol for the same samples. The biosensor is also seems to be cost effective for the existing clinical methods.

Experimental Methods

Development of biosensor using (E)-3-(pyren-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one

Typically, 2 mL of 5 μM of (E)-3-(pyren-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (PTP) solution (Ammonium buffer, pH=10) was mixed with different concentrations of creatinine. (from 1.13 to 11.31 mg/dL for UV studies and from 0.00000113 to 15.8 mg/dL for emission studies) at room temperature. The changes in absorbance were recorded at 297 and 407 nm and the changes in emission were observed at 473 and 587 nm. The linear calibration curve for creatinine was drawn between absorbance/fluorescence intensity versus the concentration of creatinine molecules.

Analysis of human blood serum by our protocol

Under optimized conditions, 2 mL of 5 μM (E)-3-(pyren-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (PTP) solution at pH=10.0 (Ammonium buffer) was thoroughly mixed with 10 μL of different blood serum samples from different persons and incubated for 10 minutes. As per report of Alpha hospital, the clinical samples were contained in between concentration of creatinine (in mg/dL). In our case, the fluorescence was linearly varied at 527 nm for this particular concentration range. So, that we have recorded the fluorescence changes at 527 nm with the excitation wavelength of 407 nm.

Analysis of human blood serum by Clinical protocol

To 10 μL of different blood serum samples, 180 μL of Reagent R1 and 60 μL of Reagents R2 was added and allowed to react for 10 minutes. And then amount of creatinine from the reaction mixture was recorded at primary wavelength of 550 nm and secondary wavelength of 700 nm using Medica Easy RA Chemistry Analyzer programmed on the RFID chip on the reagent wedge. And the calibration interval time and reagents –onboard stability is maximum for 20 days. [35]

RESULTS AND DISCUSSION

Photophysical studies

Effect of pH on photochemical properties of PTP Chalcone

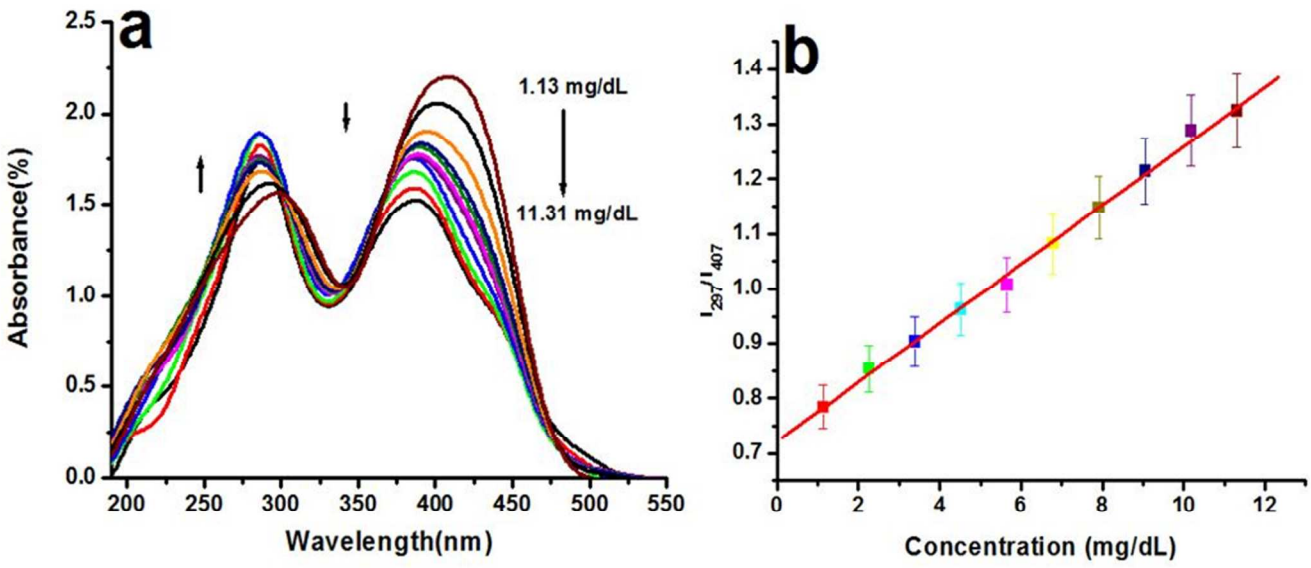
In general, the protons in the probe/fluorophore could play curial role in the absorbance or fluorescence response of the fluorescent probe. From the response, one could design a sensor based on the absorbance or fluorescence changes after binding with particular analytes. So, it is necessary to investigate the effect of pH. At low pH, the probe showed two major absorption peaks at 297 & 407 nm **(Figure S4a)**. While, the pH was being adjusted from 2 to 10, there was a gradual increment in the absorbance at both the wavelengths along with a small blue shift which is mainly attributed to the charge transfer mechanism within the PTP Chalcone (**Figure S5)**. When, the pH was further increased from 10 to 13.5, no absorbance change was observed. The PTP chalcone consists of $–OCH_3$ and –C=O groups and no effective functional groups like –OH or $–NH_2$ generally which are responsible for the change in pH of the solution. So, under acidic pH from 2 to 5, the protonation of $–OCH_3$ and –C=O groups could be taken place whereas under basic pH, the PTP chalcone might be existed in neutral form so that the absorbance intensity of the probe was high due to charge transfer mechanism. Based on above observation, the pH change didn't show any remarkable changes in peak position, but a drastic change in absorption intensity was observed in the pH range from 7 to 10. Further increase of pH from 10-14, no change in the peak position and intensity of the probe. Since, our ultimate aim was to develop the chemodosimeter for the detection of creatinine based on the simple Michael Addition reaction; we have chosen pH-10 for our study to detect the creatinine. In this aspect, our synthesized PTP chalcone could act as Michael acceptor and creatinine molecules as Michael Donor.

UV spectral analysis of synthesized PTP Chalcone and PTP-CRT Conjugates

The UV studies were carried out under different conditions for PTP Chalcone and PTP-CRT Conjugates. Initially, the PTP Chalcone showed two major peaks around 297 & 407 nm which are mainly because of π-π^* and n-π^* transitions, respectively. **(Figure S4a)** These two peaks were fixed for designing chemodosimeter based the colorimetric sensor for the detection of creatinine at pH.10. There was a ratiometric response was observed while varying the concentration of creatinine from 1.13 to 11.31 mg/dL with blue shift in both spectral wavelength and the limit of detection (LOD) is calculated as 0.39 mg/dL. **(Figure 1a & 1b.).** The blue shift in both spectra clearly supports the Internal Charge Transfer (ICT) mechanism during the interaction between creatinine and probe.

Response of PTP chalcone towards creatinine in the presence of interfering molecules

Our human serum consists of so many biomolecules like glucose, urea, dopamine, ascorbic acid, cretin, uric acid, various amino acids, proteins etc. So, the selectivity study has to be done to show the specific recognition of creatinine by PTP chalcone in the presence of other competitive biomolecules. Herein, we have checked the selectivity of PTP chalcone for creatinine in both and diseased **(Figure. 1c)** and normal **(Figure. S6a)** physiological concentration (mg/dL) levels in the presence of interfering molecules like urea, uric acid, ascorbic acid, dopamine, bilirubin, glucose, creatine, serotonin, amino acids like L- Arginine, L- Cystine, L-Cystin, reduced glutothione and BSA in same physiological standards level. A very good ratiometric absorbance change with blue shift in spectra was observed only for creatinine, i.e. the peaks at 297nm and 407 nm were blue shifted by 13 nm and 23 nm, respectively. No remarkable change was observed for other biomolecules and it is clearly evinced that the developed colorimetric sensor has absolute selectivity towards creatinine. Based on the blue shift, it is concluded that the interaction between creatinine and PTP chalcone must be ICT (Intramolecular Charge Transfer) mechanism. Further, the selective recognition of the PTP chalcone for creatinine was confirmed by adding different biologically relevant metal ions like Ag^{+}, Fe^{2+}, Na^{+}, K^{+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, and Cu^{2+} ions and the results clearly reveals that the above metal ions do not show any interference**.** The corresponding color change is shown in supporting information **(Figure S7a)**.



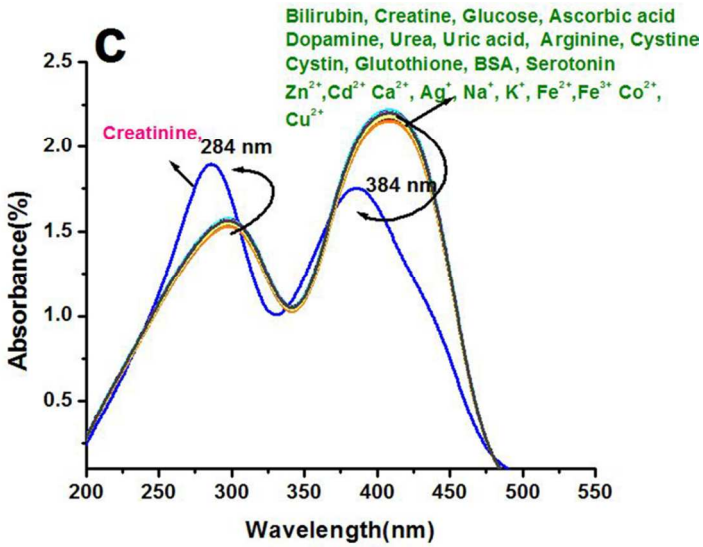


Figure 1. (a) Change in Absorbance while varying the concentration of creatinine from 1.13 to 11.31 mg/dL and (b) Corresponding linear plot. (c) Selectivity study based on the absorbance change in presence of other interfering biomolecules and metal ions [For selectivity study: Concentration of creatinine and other interferents are 11.31 mg/dL in pH 10.0 ammonium buffer medium].

Notably, we have separately done the selectivity study for sulfur containing aminoacids. Because these kind of sulfur nucleophile can also form Michael adduct by means of 1, 4 conjugate addition. But the sulfur nucleophile can form Michael adduct in very strong basic catalyst condition like amine nucleophilic catalyst like DBU, DBN, phosphates etc.[36] But we use only simple ammonium buffer pH 10.0, So that other sulfur related biomolecules could not able to form Michael adduct. So the PTP chalcone didn't show any responses to these aminoacids and only shown excellent selectivity for creatinine only.

Competitive studies in presence of all other interfering molecules and metal ions.

The selectivity recognition of creatinine by the PTP chalcone chemodosimeter was further confirmed by performing the absorbance competitive titrations in presence of various biologically important metal ions and biomolecules along with creatinine in the same experimental conditions. To this one equivalent of PTP chalcone, one equivalent of creatinine (10 μL) and two equivalents (20 μL) of various biomolecules and metal ions (concentration is 11.312 mg/dL) were added to the same solution one by one and there was no obvious absorbance change. The developed sensor has a ratiometric response at 297 and 407 nm only for creatinine **(Figure 1.).** The relevant competitive emission spectra for all those interferents are given in

supporting information **(Figure S8 & S9.)** From these studies, it is clearly understood that the developed sensor can detect creatinine molecules selectively even in presence of double the concentration of interferents.

Effect of pH on fluorescent property of PTP chalcone

In UV-Vis study, the PTP chalcone shows two absorption maxima at 297 & 407 nm, while exciting the PTP chalcone at 407 nm, two emission peaks appeared around 473 and 587 nm under optimized conditions **(Figure. S4b)** .While adjusting the pH from 2 to 12.5, no remarkable peak shift was observed as observed in UV spectra whereas gradual increase in fluorescent intensity was observed at 473 and 587 nm from pH 2.0 to 8 whereas a drastic increase in intensity was observed from pH= 8 to 10 **(Figure 2)** and then remained constant. So, pH 10.0 was chosen an optimum pH for further studies. The quantum yield PTP chalcone was calculated from the fluorescent intensity at pH=10 and found as 0.85. The above value is very close to the quantum yield of Rhodamine 6G (~ 0.93) and this excellent emitting property of PTP has been exploited for the development of fluorescent biosensor for creatinine. In addition, the stability of the PTP chalcone was tested once in a week for 5 weeks and no change in fluorescence property was observed **(Figure S10).** The results depict that the PTP chalcone is stable for five weeks [Details of quantum yield calculations and stability data are given in supporting information].

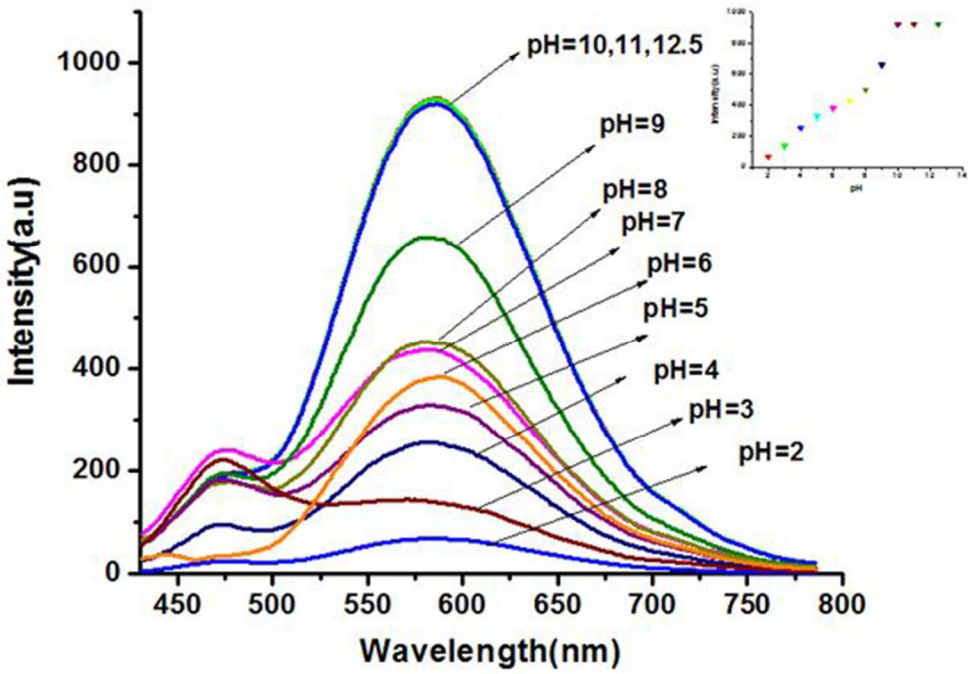


Figure 2. Effect of pH on fluorescent property of PTP chalcone while adjusting the pH from 2 to 12.5. [The concentration of PTP chalcone is 5 μM in different buffer solutions. The stock solution was prepared by using 0.001 M of PTP chalcone in DMSO and then diluted to 5 μM by using various buffers]

Selective fluorescent response of PTP chalcone towards creatinine

In sensors, selective detection towards a particular target analyte is the one of the essential criteria to extend its application for real sample analysis. Our human biofluids consist of so many biomolecules and metal ions. So, we have already carried out the selectivity study of the PTP chalcone for creatinine in the presence different interfering biomolecules and metal ions **(Figure. 1b)** using UV-Vis spectroscopy. **T**he same experiments were repeated using Fluorimetry technique**.** We do not observe any fluorescent change of PTP chalcone in presence of interfering biomolecules like urea, uric acid, creatine, glucose, ascorbic acid, dopamine, bilirubin serotonin, amino acids like L- Arginine, L- Cystine, L-Cystin, reduced glutothione, BSA and metal ions like Na^{+}, K^{+}, Ag^{+}, Ca^{2+}, Cd^{2+} , Zn^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+} and Cu^{2+} ions at diseased **(Figure. 3a & 3b)** and normal **(Figure. S6b & 6c)** physiological concentration level of creatinine. For creatinine, a very good blue shift (60 nm) has been observed from the original wavelength of PTP chalcone i.e from 587 nm to 527 nm. From this scrutiny, the selective response of the PTP chalcone for creatinine has once again been proved. The corresponding fluorescent color changes were shown in supporting information **(Figure S7b)**.

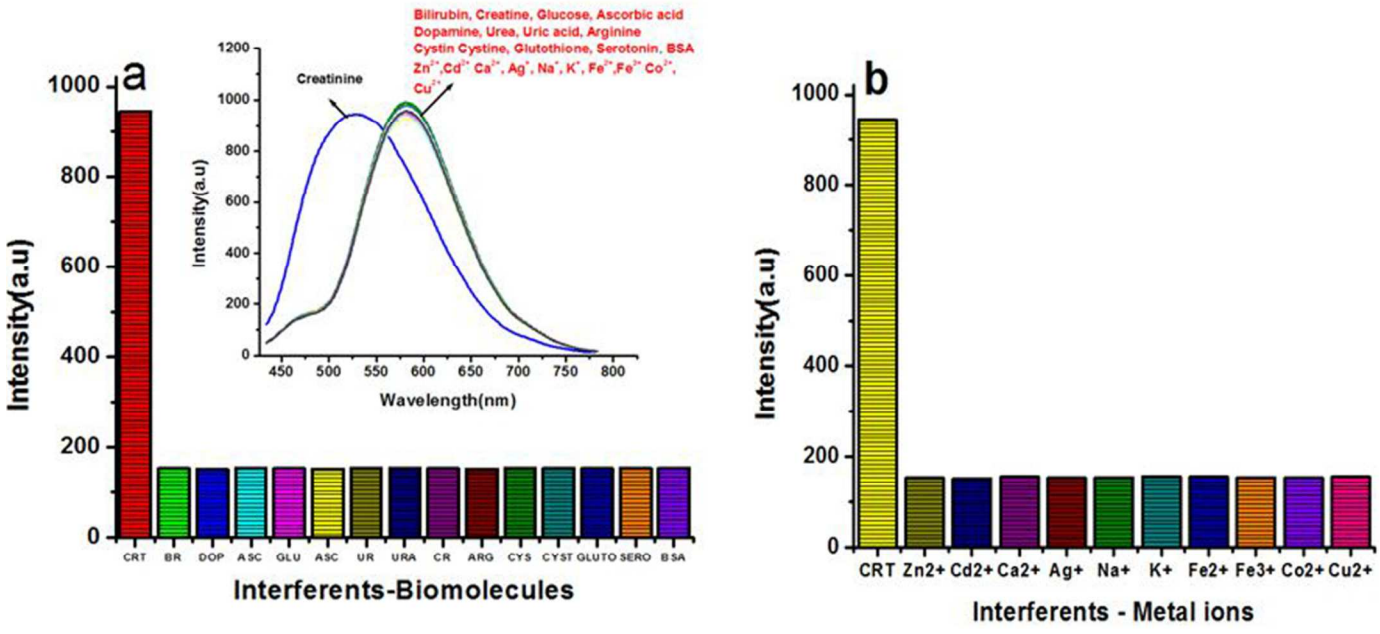


Figure 3. Bar diagram: Selective recognition of creatinine in presence of other interfering biomolecules (a). Inset of (a): Corresponding emission spectra for selectivity study. Selective recognition of creatinine in presence of other interfering metal ions (b) [For selectivity study: Concentration of PTP chalcone and creatinine is 5 μM and 15.8 mg/dL; other biomolecules and metal ions are expressed in 11.31 mg/dL in pH 10.0 ammonium buffer medium].

Sensing of Creatinine using PTP **chalcone by Fluorimetry**

The fluorescent change of the PTP chalcone was studied during the addition of different concentration of creatinine. As we have already discussed in the introduction, the detection for creatinine was focused in the normal and diseased states so that the senor could be applied to identify the various health issues associated with creatinine in clinics. So, the concentration of creatinine was varied from 0.00000113 to 15.8 mg/dL **(Figure 4).**Very interestingly, the peak intensity at 587 nm gradually decreased whereas the peak intensity at 473 nm gradually increased for 0.00000113 to 0.0001 mg/dL concentration range of creatinine in a ratiometric manner. When the creatinine level was increased from 1.13 to 15.8 mg/dL the peak at 587 nm was blue shifted (60 nm) from 587 nm to 527 nm. This might be due to the formation of Michael adduct at higher concentration of creatinine under basic condition.[37] This remarkable blue shift is also may be due to the creatinine induced ICT mechanism between donor and acceptor part of PTP chalcone. The formation of Michael adduct was further confirmed by observing the emission peak for isolated adduct and the emission was recorded at the excitation wavelength of 407 nm and the similar emission spectra was obtained at 524 nm **(Figure S11).** Due to the solubility problem, NMR spectra for this Michael adduct could not be measured. Under optimized condition, the developed biosensor could detect creatinine with a broad detection range as mentioned above, It would be very useful to diagnosis various diseases associated with creatinine level in our human body when compare to the previously reported optical and electrochemical creatinine biosensors **(Table 1)**. The reproducibility experiment was carried out for three different concentrations of creatinine from different diseased range and our chemodosimeter shows a very good reproducibility even for 6 trails towards creatinine at three different concentrations ranges 0.00000113, 5.65 & 15.8 mg/dL. The related standard deviation values were calculated as 0.73 %, 0.13 % and 0.85 % at respective concentration ranges of creatinine, respectively. (Calculations' were given in supporting information). Corresponding data are given in supporting information **(Figure S12).**

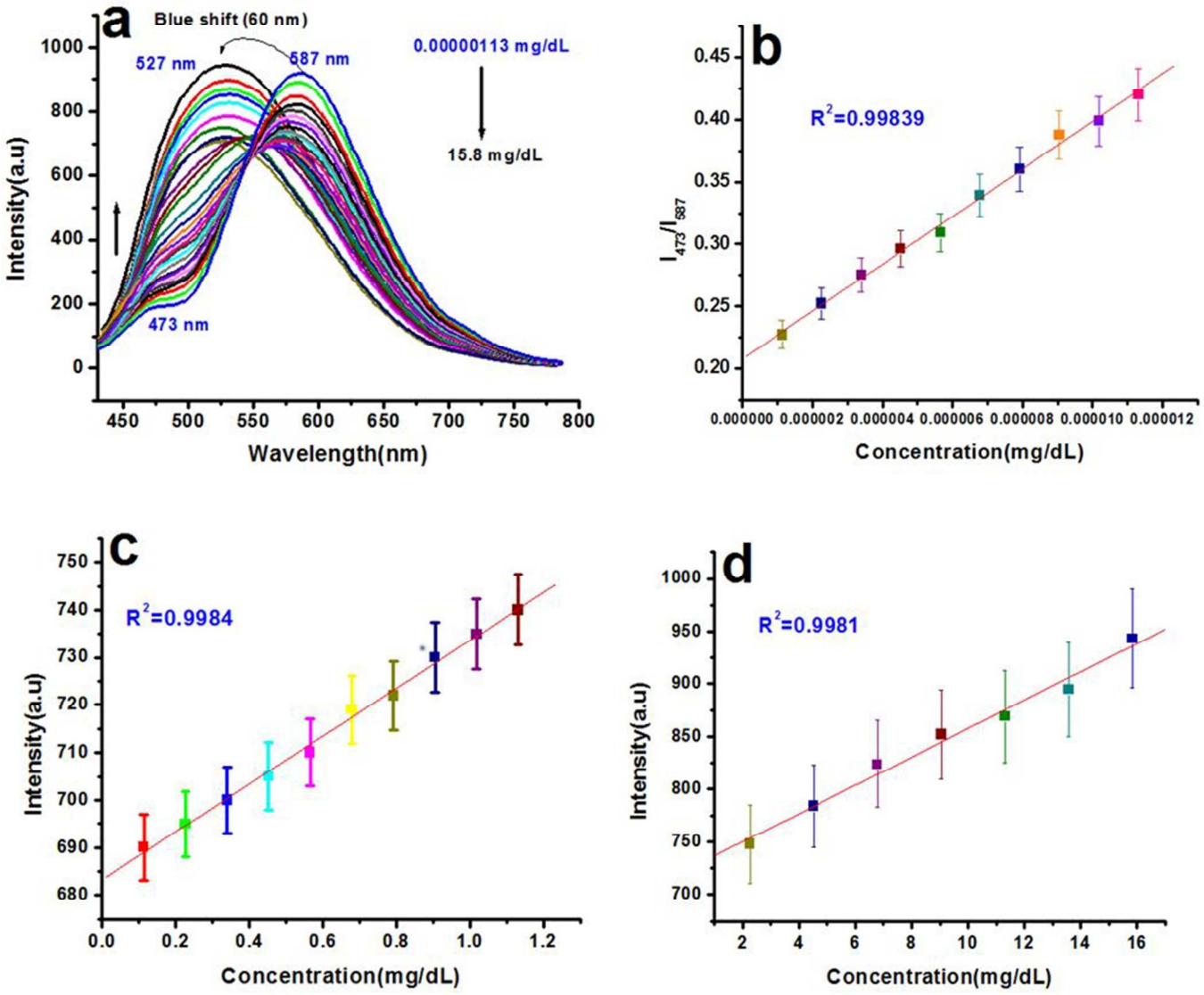


Figure. 4 (a) Fluorescence emission change of the PTP chalcone while adding different concentrations of creatinine from 0.00000113 to 15.8 mg/dL. (b), (c) & (d) corresponding linear plots for different concentration levels respectively. [Concentration of PTP chalcone is 5 µM and different concentration solutions of creatinine were made from stock creatinine solution of 11.312 mg/dL using ammonium buffers, pH 10.0]

Sensor platform	Technique	LOD (mg/dL)	Linear range (mg/dL)	References
Phosphomolybdic-polypyrrole film modified glassy carbon	Cyclic voltammetry	0.0006	0.0113-1.13	**[14]**
c-MWCNT/PANI	Amperometric	0.0011	0.1131-8.48	**[18]**
ZnO-NPs/CHIT/c MWCNT/PANI	Amperometric	0.0057	0.1131-7.35	**[19]**
Fe_3O_4-NPs/CHITg-PANI	Amperometric	0.0113	0.0113-9.04	**[20]**
Picric acid-Jaffe reaction	Digital Camera Based	1 & 1.25	1.809 – 18.09	**[21]**
ZnS:Mn/ZnS quantum dots	Fluorimetric	0.0001 &0.0003	0.008 – 0.03	**[22]**
Polyyrole/CRT/HRP-CRT Ab	Amperometric	0.4525	11.31	**[38]**
Creatinine-Peroxidase Conjugate Membrane – ELISA	Amperometric	0.0005	0.0001 – 11.31	**[39]**
Carboxylated-polyvinyl Chloride	Potentiometric	0.0011	0.67-1.35	**[34]**
Y-cut quartz crystal resonator (QCR).	Calorimetric	--	0.000011 - 0.0003	**[40]**
QD-CaR Conjugate	Fluorimetric	--	0.000001 - 0.0001	**[41]**
Rhodamine B –Au^{3+} ions	Colorimetric	0.054	0.0011 – 0.16	**[29]**
	Fluorimetric	0.0001	0.0001 – 13.57	
AuQC@gluten clusters	Fluorimetric	0.0226	0.2262 - 5.88	**[42]**
PTP Chalcone based Chemodosimeter	Colorimetric	0.39	1.13 – 11.31	**Present Work**
	Fluorimetric	0.00000065	0.0000011- 15.8	

Table 1. Comparison of performance of our biosensor with previous biosensors

Fluorescent spectral changes of PTP chalcone in presence of competitive analytes

Before going for real sample analysis, it is mandatory to check the competitive response of the PTP chalcone in presence of all interfering molecules in the same solution. We have followed the same protocol as in the case UV competitive studies. From the data analysis, it's clearly evincing that the PTP chalcone has absolute selectivity for creatinine even in presence of all the interferents **(Figure 5).** The corresponding emission spectra for competitive experiments of biomolecules and metal ions are given in supporting information **(Figure S13 & S14).**

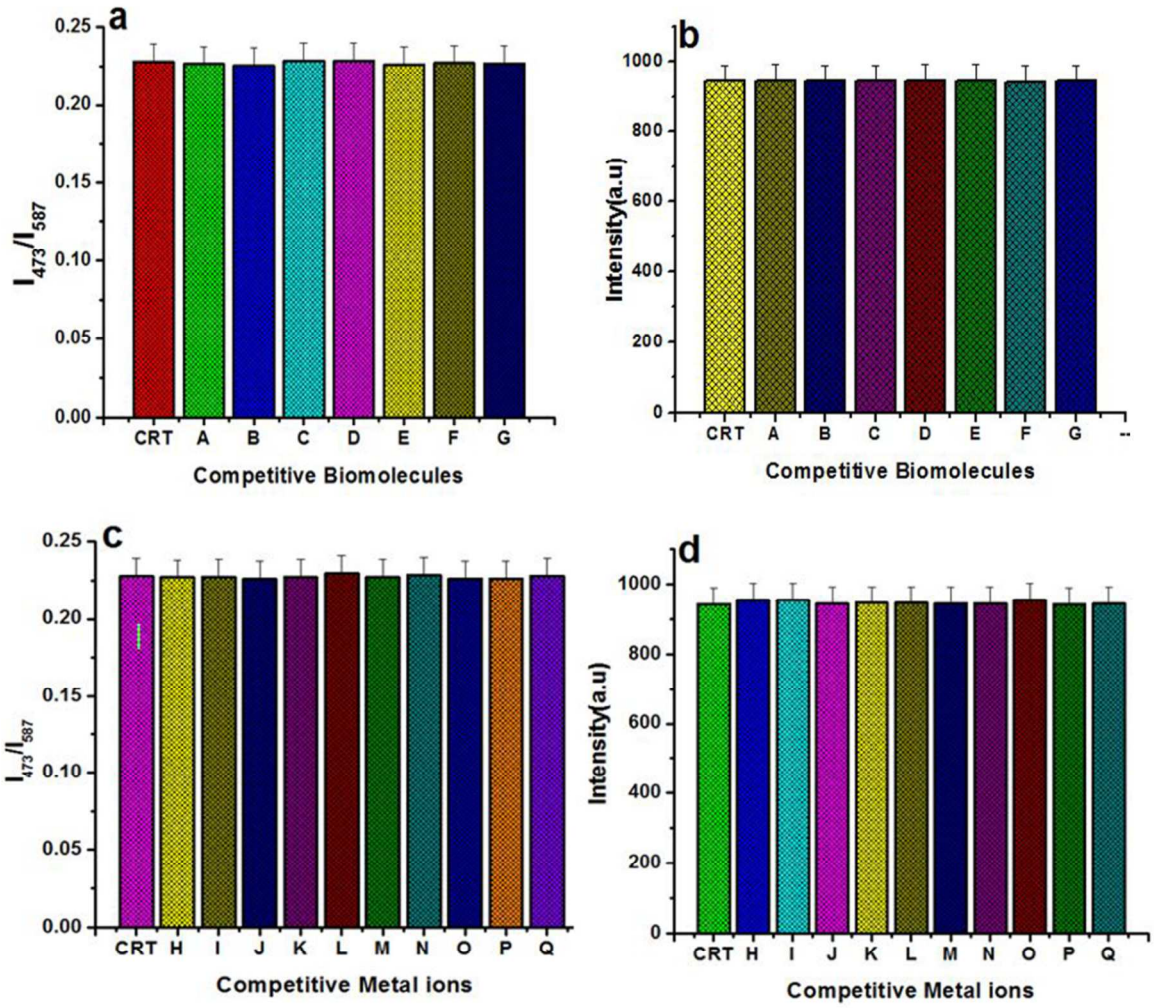
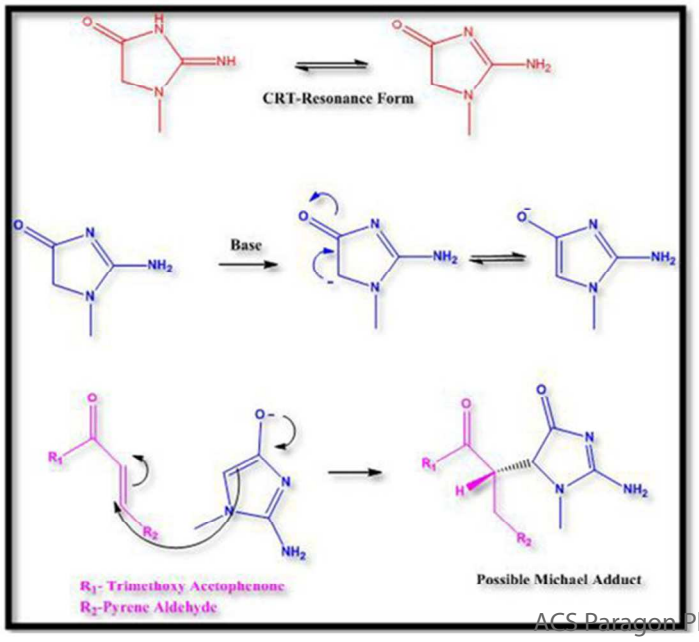
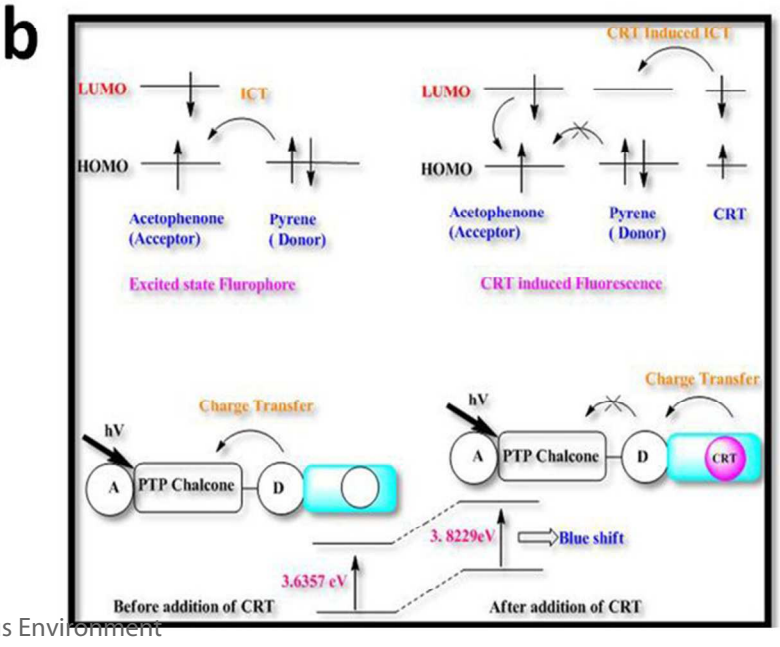


Figure 5. Bar diagram: Emission spectra for the competitive studies of PTP chalcone containing 1equivalent (nM concentration) of creatinine along with 1equivalent of biomolecules (a). metal ions (c).And 1equivalent (μM concentration) of creatinine along with 1equivalent of biomolecules (b) and metal ions (d). [Concentration of PTP chalcone is 5 μM; creatinine was made into two different concentration levels 0.0000011 mg/dL and 15.8 mg/dL respectively. [Concentration of other biomolecules is 0.0000011 mg/dL and 15.8 mg/dL, metal cations are 0.001 M in pH 10.0 ammonium buffers. (A) Bilirubin, (B) Creatine, (C) Glucose, (D) Ascorbic acid, (E) Dopamine, (F) Urea, (G) Uric acid, (H) Zn^{2+}, (I) Cd^{2+}, (J) Ca^{2+}, (K) Ag^{+}, (L) Na^{+}, (M) K^{+}, (N) Fe^{2+}, (O) Fe^{3+}, (P) Co^{2+}, (Q) Cu^{2+}]

Mechanism involved during sensing of creatinine by PTP chalcone

Based on all the UV and Emission data, it is understood that the chemodosimeter has adopted ICT mechanism. In general, if a probe with a conjugated couple of electron-donating/electron-withdrawing groups (donor/acceptor, **D/A**), undergo ICT mechanism it will normally display large Stokes shift, visible light excitability and metal coordination-induced emission shift. Modification of **D** or **A** by any analyte will be induced blue or red shift in excitation/emission spectra along with ratiometric responses by means of altering the photo-induced internal charge transfer excited state. In our present study also we have observed a very good blue shift (23 nm) in UV- vis absorbance spectra as well as in fluorescence emission spectra (60 nm) along with a ratiometric response. From this, we clearly suggest that the chemodosimeter has followed the ICT mechanism. After the addition of creatinine at 1,4 position of PTP chalcone there may be electronic charge cloud repulsion would arise and it may increase the energy levels in PTP chalcone and finally may lead to blue shift. Hence the developed chemodosimeter has adapted ICT mechanism which is further supported by DFT calculations. In general, Michael acceptors are α, β-unsaturated compounds which having electron deficient center at which the donor will be added to form Michael adduct. Since Michael donors have acidic hydrogen atoms or having delocalization of electrons, they produce electrons to form Michael adduct. In our sight, the creatinine could act as Michael donor due to the presence of acidic hydrogen atom and PTP chalcone could act as a Michael acceptor because of the α, β-unsaturated carbonyl part. Hence, under basic condition, the creatinine could form a very stable active methylene carbanion [37] due to its resonance structure with the nearby carbonyl group present in the five member ring **(Scheme. 1a)**





Scheme 1. Plausible Mechanism for the formation of PTP-CRT adduct (a) and energy level diagram for proposed biosensor (b).

This active methylene resonance stabilization is not possible in other above biomolecules. Even creatinine and other biomolecules like urea, uric acid, dopamine and aminoacid contains amino group, the possibility of Aza Michael Reaction doesn't occur, because it need high basic or acidic condition. [43-44] Hence, this stable carbanion nature has making the creatinine to interact more strongly and specifically with the PTP chalcone via normal Michael Addition reaction pathway when comparing with other above biomolecules. This is the main reason for high selectivity of our biosensor. The stoichiometry of the PTP chalcone with CRT was found to be 1:1 approximately by continuous variation/Jobs method.[45-47] **(Figure S15)**

DFT-calculations

In order to analyze the charge transfer mechanism between PTP chalcone and creatinine, DFT calculations were performed at B3LYP/6-31G and 6-311G levels by using Gaussian 09 programme. The corresponding energy level diagram is shown in **figure S16.** The geometries of PTP chalcone optimized using the above said basis sets. TD-DFT calculations of PTP chalcone reveals that HOMO is spread over the pyrene unit and the LUMO spread over from the pyrene to acetophenone moiety with energy difference of 3.6357 eV. After binding of creatinine molecule, HOMO is only localized on the pyrene moiety whereas the charge density at LUMO was spread from acetophenone moiety to pyrene moiety with energy difference of 3.8229 eV. From the data it is well understood that the initial charge transfer has been occurred between the donor pyrene moieties to the acceptor acetophenone moiety which is responsible for an intense peak at 587 nm in emission spectra. In which, α and β unsaturated carbonyl group could act as a spacer which assist the charge transfer between the two moieties. In the excited state, after the addition of creatinine molecule, the α, β unsaturated double bond, the initial charge transfer among the molecule was insisted and now the charge was distributed highly towards the pyrene moiety which results the increased in the energy levels and finally showed a very good blue shift of 60 nm. At this stage, creatinine could acts as donor and Pyrene could act as acceptor. From this DFT visualization, it clearly supports that the developed chemodosimeter has followed the intra

molecular charge transfer mechanism and the proposed energy level diagram was shown in **scheme 1b.**

Analysis of Human Blood Creatinine

As already mentioned in the introduction itself elevation in creatinine level in our human body will results so many health issues like renal malfunction, muscle disorder etc. Till date costlier clinical methodology has been used in order to quantify the creatinine in our human blood serum and urine. There is no simple tool available as a substitute for clinical diagnosis. Keeping this need in mind, we have developed a simple chemodosimeter based biosensor for the quantification of creatinine. To highlight the outcome of this current project, we have made collaboration with Alpha Hospital & Research Center, Institute of diabetes & Endocrinology, Madurai and collected 10 human blood serum samples of different aged peoples. The creatinine level in these serum samples has been analyzed based on the fluorescence responses of the PTP chalcone. Meanwhile in the hospital, the serum creatinine for the above same blood samples has also been analyzed simultaneously using Medica instrument model name of Esyra. The detailed clinical protocol was given in supporting information. The data have been comparable clinical results for the same serum sample and given in table. **(Table. 2)** To further support our method, we have also cross checked our data for the neat creatinine of different concentrations with the help of clinical method also and these results were also showed a very good agreement **(Table. S1)** and corresponding emission data were given in supporting information **(Figure S17).**

Serum Sample Code	**Our value (mg/dL)**	**Clinical value (mg/dL)**
P1	0.9615	0.92
P2	0.9050	0.90
P3	0.6262	0.71
P4	0.4751	0.51
P5	0.55	0.57
P6	1.1086	1.09
P7	0.9502	0.94
P8	0.6787	0.72
P9	0.6891	0.73
P10	0.54	0.53

Table. 2 Analysis and comparison of blood creatinine level in serum samples collected from different persons by the proposed method and clinical methods.

Conclusions

Since creatinine is a main biomarker for renal malfunction, our group has given much more intension and more clinical sound to quantify the creatinine by using a very simple and first fluorescence chemodosimeter. The synthesizing of chalcone PTP is highly facile as it can be synthesized by simple hand shaking method. Its quantum yield is 0.85. It could able to quantify the creatinine from 1.13 to 11.31 mg/dL with LOD of 0.39 mg/dL base on absorption changes. For clinical quantification of creatinine, we have successfully achieved two the linear ranges is varying from 0.11 – 1.13 mg/dL and 2.26 – 15.8 mg/dL for normal as well as renal infection state along with an excellent LOD of 0.00000065 mg/dL. This is the very first broad detection clinical range for detection of creatinine. To stand the outcome of this developed bioassay with clinical creatinine tools, the human blood serum samples were collected and analyzed. Results were firmly supported with clinical methods and it has turned into a new opening/substitute for clinical channels for creatinine bioassay.

Acknowledgement:

This work is financially supported by DST-INSPIRE Fellowship Scheme, and DST-SERB New Delhi, India. So, the author thanks DST for offering the fellowship for pursuing his Ph.D program. The author specially thanks for additional financial support for the same. The author sincerely thanks Mr. M. Karuppasamy, CLRI Chennai for helping the DFT calculations

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website

Characterization of PTP: NMR & ESI-Mass Spectra, UV and Fluorescence Optimization studies for the developed biosensor and Abbreviations used in the Manuscript

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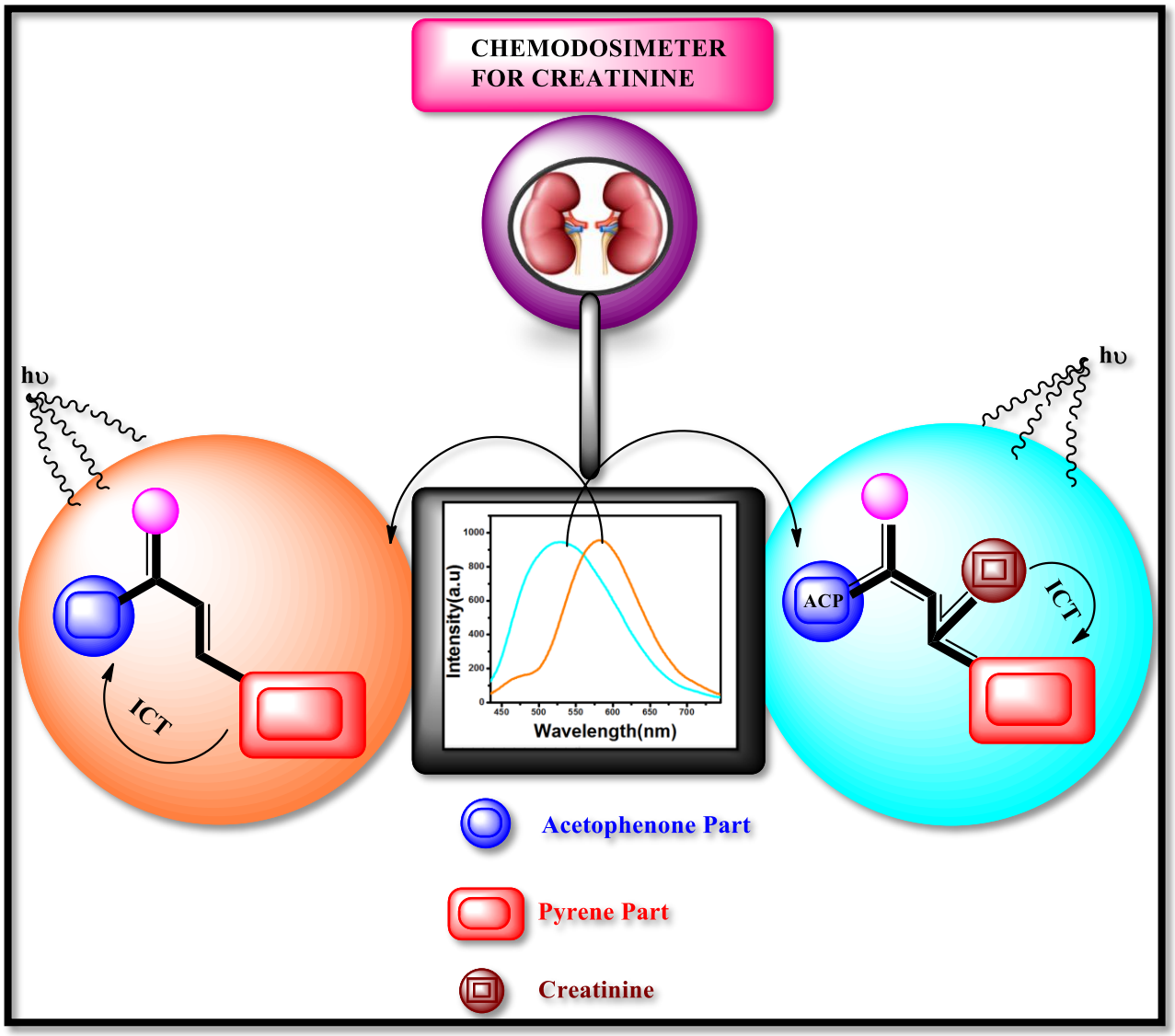
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