COUMARIN BASED FLUORESCENT BIOSENSORS

By

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Analytical Chemistry

Under the supervision of

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CERTIFICATE

This is to certify that the dissertation entitled, “Coumarin based Fluorescent Biosensors” is bonafide work carried out by Miss. Sanjana S. Divkar under my supervision in partial fulfilment of the requirement for the award of the degree of Master of Science in Chemistry at the School of Chemical Sciences, Goa University.

Dr. Hari K. Kadam

Dissertation Guide

Assistant Professor

Goa University

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I am extremely grateful to my parents for their immense love and support. I would like to thank each and everyone who have helped me directly or indirectly. Last but not the least I would like to thank God for his blessings.

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I. INTRODUCTION

Coumarin is a compound containing the parent nucleus of benzopyrone, an economic chromophore. Coumarin shows high intense fluorescence, good solubility, relatively high fluorescence quantum yield, ease of production, and molar absorption coefficient if an electron donating group, such as hydroxy or unalkylated amino group, is substituted at the 7^{th} position. The fluorescence intensity of coumarin has a close relationship with the substituent groups on the ring. When the intramolecular charge transfer capability was changed by modifying substituent groups, the optical properties of the whole molecule were influenced.[1] Coumarin based fluorescent sensor are also useful for Ca^{2+} and F^- detection in live cell imaging. Moreover, the modern medicine research indicates that low intake of calcium would induce many illnesses, such as growth retardation, osteoporosis and hypertension Thus, there is a great demand to develop sensor that can detect and monitor Ca^{2+} in environment and biological system therefore In the past few decades, fluorescence sensor has become a practical tool for detection and analysis of metal ions and anions in water, biochemical and imaging in living cells studies.[2] Similar to Calcium, Zinc is second most abundant transition metal ion in human body after iron, and is essential cofactor in many biological proceses such as brain function and so on. Upto now a variety of fluorescent sensors for Zn^{2+} have been developed with some sucessful applications to image Zn^{2+} in living cells.[3]

As an important group of organic hetrocycles, Counmarin derivatives possess unique photochemical and photophysical properties, making them useful for a variety of applications. Coumarin based fluorescent probe has been extensively applied for the detection of metal ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+} and so on.[4]

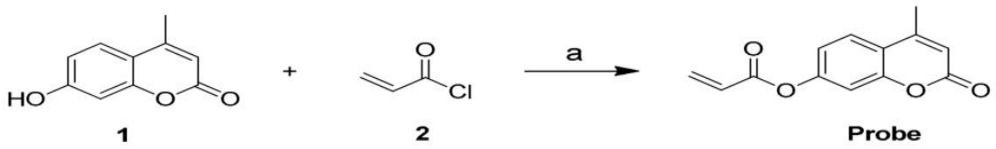
Fluorescent probes may be defined as synthetic small molecules that react specifically with analytes to induce a marked change in their fluorescence properties, on the basis of such changes, the analytes can be determined.Because of their powerful ability these probes have been extensively investigated and widely used in many fields, and to improve analytical sensitivity, and in particular to be used in in vivo imaging studies.[5]

Regarding organic dyes great improvement, coumarin has attracted enormous interest and attention from chemist/material scientists all over the world because of the large p-conjugated structure, two-photon fluorescence emission and excellent anticarcinogenic/antibiotic properties.[6] Fluorescent chemosensors which are capable of sensing biologically important analytes by different signal transduction mechanisms are of current interest.1 In this regard, sensing of certain alkaline earth metal ions such as Ca^{2+} and Mg^{2+} is significant owing to their involvements in various biological processes.[7] Coumarins (or benzopyrones) are an important class of heterocycles that find applications in various fields spanning from lasers to biomedicine. They are characterized by good photostability, high fluorescence quantum yields (although unsubstituted coumarin, namely 1-benzopyran-2-one, has a quantum yield of just 0.03% in cyclohexane), large Stokes shift, and low toxicity.[8]

II. LITERATURE REVIEW

i) A Fluorescent Coumarin-Based Probe for the Fast Detection of Cysteine with Live Cell Application

In this research paper the probe discusses about a new coumarin-based fluorescent probe, containing an allylic esters group, has been designed and synthesized for sensing cysteine in physiological pH. In this research paper, a turn-on fluorescence probe was designed for Cys based on coumarin with high selectivity and sensitivity. The allylic esters group serves as a blocker of coumarin fluorophore in the probe, which functions as a Michael receptor, and electrophile. The probe can sharply distinguish Cys from other biothiols Because Cys has lower steric hindrance to conduct Michael addition and has been successfully used in living cell imaging. Discrimination of Cys from other amino acids and biothiols, real-time monitoring of the Cys level of cells, tissues, and animals will be possible With the development of fluorescent probes.The probe selectively and sensitively detects cysteine (Cys) and has a rapid response time of 30 min and a low detection limit of 47.7 nM. The probe showed a stable, highly selective and sensitive fluorescence response towards Cys over GSH, Hcy, and other compounds. Confocal fluorescence microscopy imaging using HepG2 cells indicated that the probe can be applied for the detection of Cys in living cells. Characterization of the Probe is shown in Scheme 1.



Scheme 1. Synthesis of the probe. Reagents and conditions: (a) trimethylamine, CH2Cl2, r.t.

In this experiment they have performed Synthesis of the Probe (4-Methyl-2-oxo-2H-chromen-7-yl Acrylate), Absorption and Fluorescence Spectroscopy, Cell Culture for HepG2, Fluorescence Imaging of Cys in Living Cells. Then they got results on UV-Vis Absorption and Fluorescence Spectra, Selectivity of the Probe for Cys, Effect of pH on the Fluorescence Response of Probe, Effect of Reaction Time, reaction mechanism and application of the Probe were found out.[1]

ii) Novel coumarin-based fluorescent pH indicators, probes and membranes covering a broad pH range

In this research paper we have seen synthesis of new family of coumarin-based pH indicators which are sensitive to pH in either weakly acidic or weakly basic solution. This indicators possess moderate to high brightness, excellent photo stability and

compatibility with light-emitting diodes. In figure 1 we have seen the chemical structures of dyes which were synthesied. In our day to day life many biological and geochemical processes occurring in freshwater, seawater and marine sediments involve strong pH changes. The output of many biotechnological processes (e.g., bacterial growth or sour fermentation of milk) depends on the pH, which is important to be known therefore pH indication are needed. For pH measurements glass electrode is the established tool but they have drawbacks that is electrodes are limited to single point measurements and are not suitable for obtaining information on pH distribution. Moreover, electrodes are bulky and invasive, and potentially create the risk of electric shock during in vivo measurements therefore a fluorescent pH sensor is used as an alternative tool as they can be easily produced in various sizes and shapes. The concentration of an analyte is continuously and reversibly sensed by an ideal optical sensor through changes in the optical properties of an indicator. Most fluorescent optical sensors, however also suffer from several drawbacks compared with a glass pH electrode. Like, the signal is dependent on the ionic strength (IS) of the sample solution. For some indicators the performance is compromised even with small changes in IS; therefore, such sensors can be used at constant IS only

The synthesis of these indicators is complicated Similar to optical pH sensing based on absorbance, measurement of slightly acidic pHs with fluorescent indicators is often problematic because few fluorescent indicators meet the requirements of brightness and photo stability. Here we describe the syntheses and characterization of novel pH-sensitive imino coumarin derivatives. Indicators covalently coupled to an amino-modified polymer surface which can be used successfully in pH sensing in different pH ranges.[9]

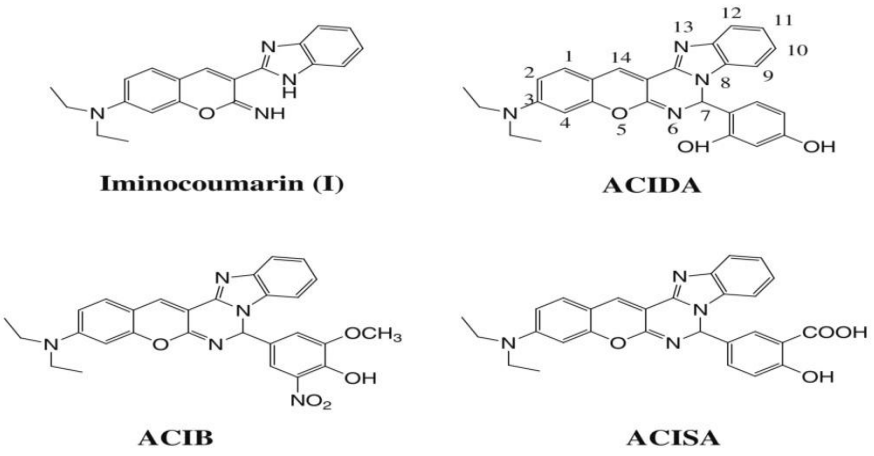


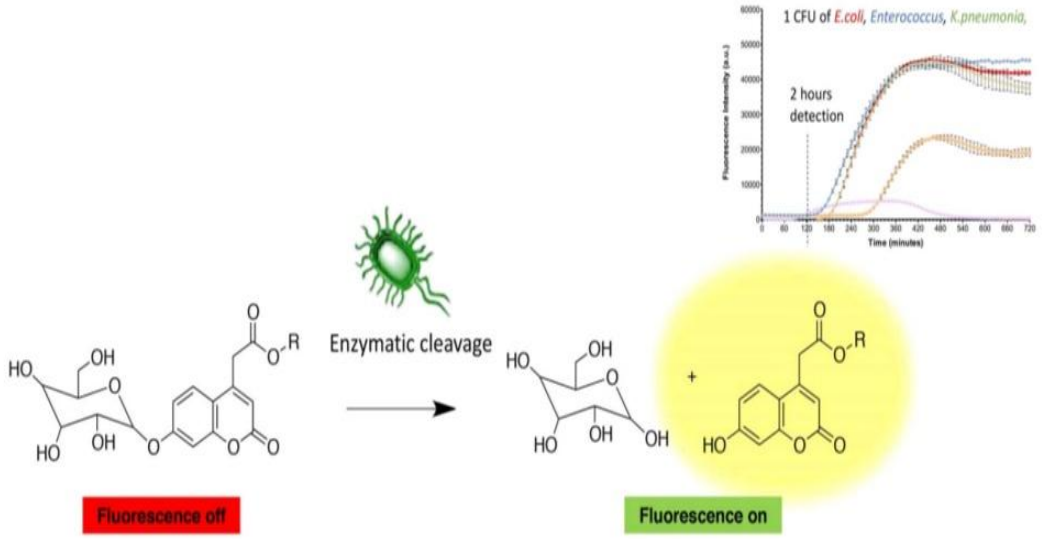
Fig. 1 Chemical structures of the dyes used

iii) Coumarin-based, switchable fluorescent substrates for enzymatic bacterial detection

Infectious diseases cause millions of deaths and hospitalizations each year because these infections are often misdiagonised and delay in diagnosis. The above topic talks about Enzymatically-switchable fluorescent substrates, such as the commercially available 4-

methyl umbelliferones (4-MU) are used as standard indicators of enzymatic activity for selective detection and identification of bacterial pathogens.

Figure 1 shows us new method for enzymatically-switchable, fluorescent substrates with improved photo- physico/chemical properties. The lead derivative, 4-AAU, shows excellent solubility in acqueous media (0.81mg/mL) when compared to 4-MU (0.16mg/mL), which improve quantum yield and wider dynamic range of its fluorescence properties. The corresponding bacterial substrate β-4-AAUG showed superior selectivity in the detection of clinically relevant amounts of E. coli, Enterococcus and K. pneumonia (1 CFU).



For biological sensing and imaging biosensors which are fluorescence-based are used and they exploit switchable, 'on-off' fluorogenic probes. Coumarin-derivatives have been largely explored and have become very popular commercially available 'on-off' fluorescent probes to detect metal ions, anions, small molecules biological material (proteins, DNA, RNA etc.) and enzymes. For detection of bacterial enzymes, such as b-galactosidase, b-glucuronidase and b –glucosidase reference compound, 4-methyl-umbelliferone (4-MU), and its glycoside derivatives have been used. In this paper they have prepared seven, new umbelliferone derivatives and compared their photo-physico/chemical properties to 4-MU as the gold standard. Here the starting material used was 7-hydroxy-4-coumarin acetic acid (4-AAU) for synthesis of the umbelliferone derivatives. 4-AAU has two obvious reactive sites: the phenolic group at position 7 and the carboxylic acid group at position 4 of the benzopyrone ring (Figure 1a). Because changes at position 4 do not alter the switchable properties of the compound, all derivatives have been synthesised by modifying the carboxyl group. The only exception is compound 7, for which the umbelliferone ring was synthesised via Pechmann condensation between resorcinol with ethyl 4-bromoacetoacetatein in 70% aqueous sulfuric acid (Figure 1b).[10]

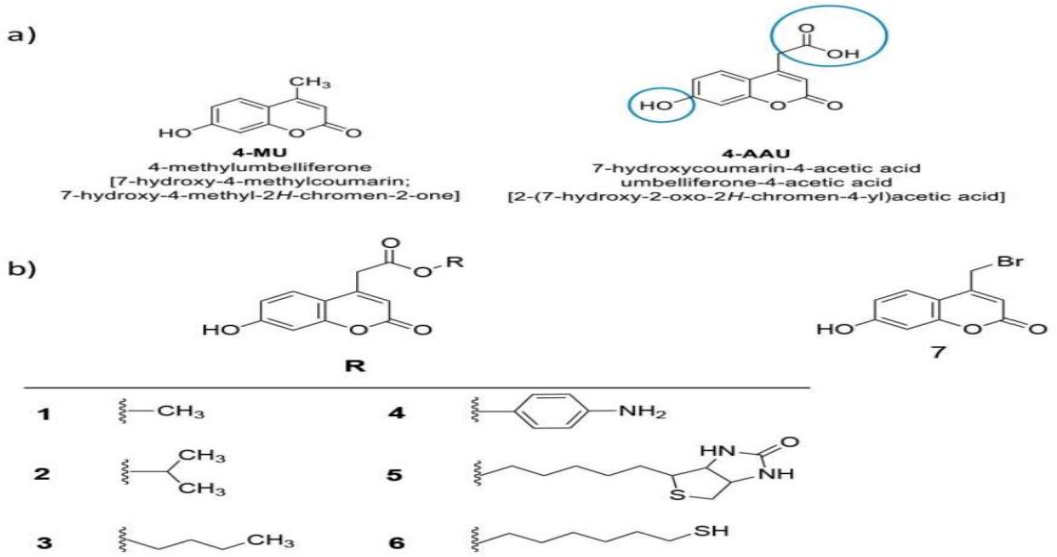
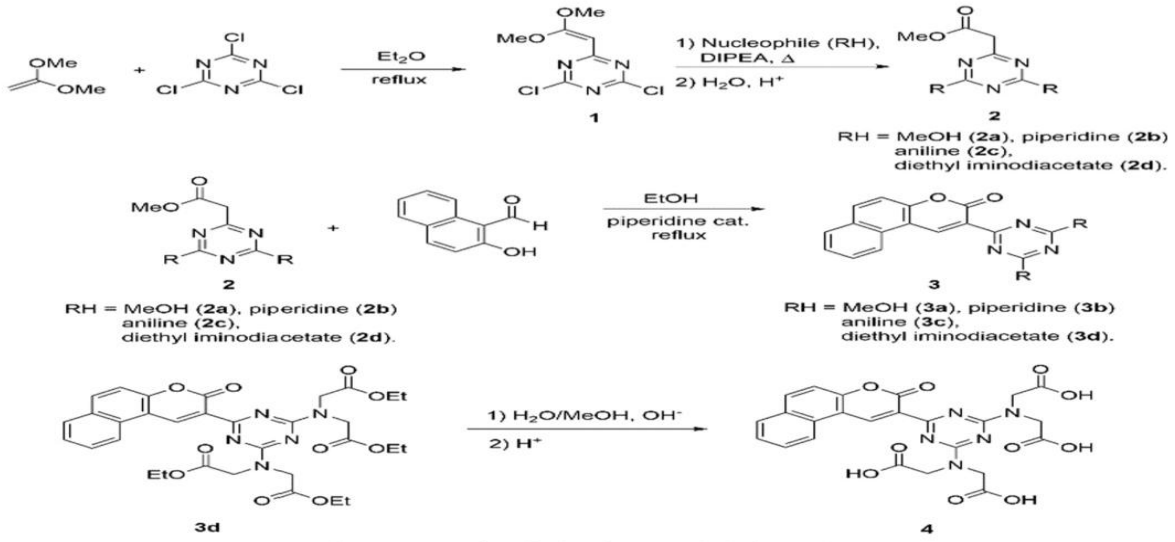


Figure 1: a) Structures of 4-MU and 4-AAU with highlighted reactive sites in positions 4 and 7. b) 4-AAU was used as starting material for the synthesis of umbelliferone derivatives 1-6 except for compound 7

iv) Coumarin-based fluorescent biosensor with large linear range for ratiometric measurement of intracellular pH

Here we describe a fluorescent pH biosensor based on a conjugated coumarin-triazine scaffold that is excitable in the visible range, shows pseudo-linear ratiometric response over more than 6 pH units with a single fluorogenic unit, and allows imaging of the whole endo-lysosomal pH landscape of living cells with a single acquisition. The probe can discriminate, on the basis of intracellular acidity, between physiologic and tumor cells, being potentially suitable in perspective as diagnostic tool.

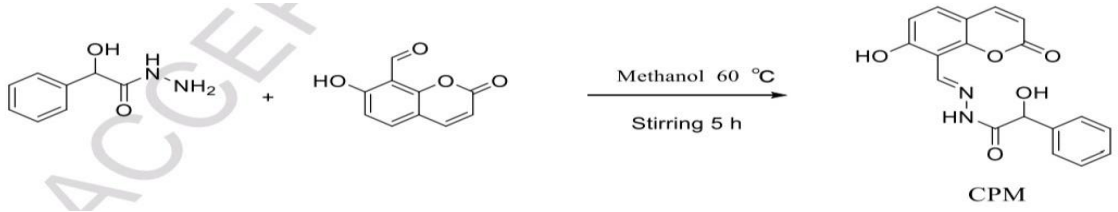


Scheme 1. General synthetic pathway to triazinyl coumarins.

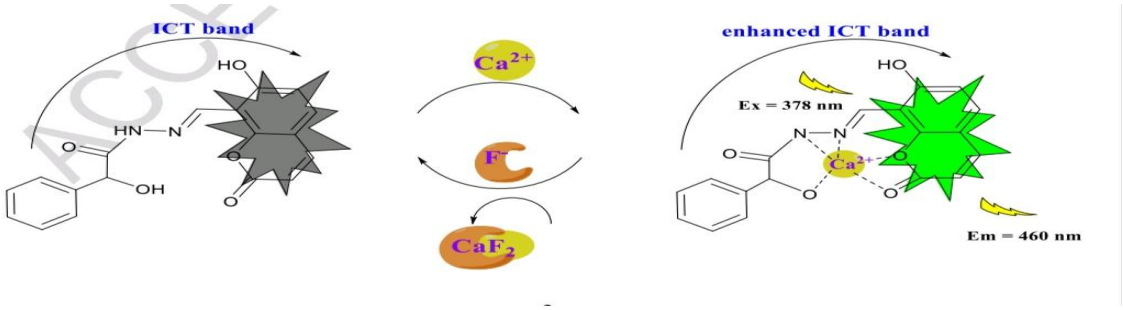
Here we see how the general synthetic pathway was carried on to get triazinyl coumarins firstly the Synthesis of 2,4-dichloro-6-(2,2-dimethoxyethenyl)-1,3,5-triazine(1) was done and product 1 was obtained with 90% yield. After this the Synthesis for 3a-d was done and product 2 was obtained, the product obtained was dissolved in methanol and further synthesis was carried out to get 3a-d product. Then the characterisation of 3a product was done and 87% of yield was obtained. After this in same waythe characterisation of 3b, 3c, and 3d product was done and 82%, 78% and 84% of yield was obtained. After this the synthesis of 4 product was done and finally the characterisation of product 4 was done with 85% of yield. After all this process was done Spectroscopic characterisation was done followed by Cell viability assay. Then Live cell imaging and intracellular pH measurements were done in that pH calibration, cell culture, imaging in living cells, imaging of cells buffered at different pH and Colocalization analysis was done. In this way we obtained a universal fluorescent pH sensor based on triazine-derivatized coumarins that provides linear ratiometric response to pH changes.[11]

v) A novel coumarin-based fluorescent sensor for Ca^{2+} and sequential detection of F^- and its live cell imaging

Synthesis of sensor CPM was done In a 100 mL round bottom flask, compound mandelohydrazide (0.25 g, 1.50 mmol) was dissolved in hot methanol (10 mL) and heated to reflux. Then, a solution of 7-hydroxy-8-formylcoumarin (0.34 g, 1.80 mmol) in 10 mL methanol was added dropwise to the solution in 0.5 h. Then the reaction kept stirring at 60 ℃ for 5 h. Then the reaction was filtered and washed with methanol to give 0.43 g of compound CPM, as a pale yellow solid (yield, 85 %)



Scheme 1: Synthesis of compund CPM

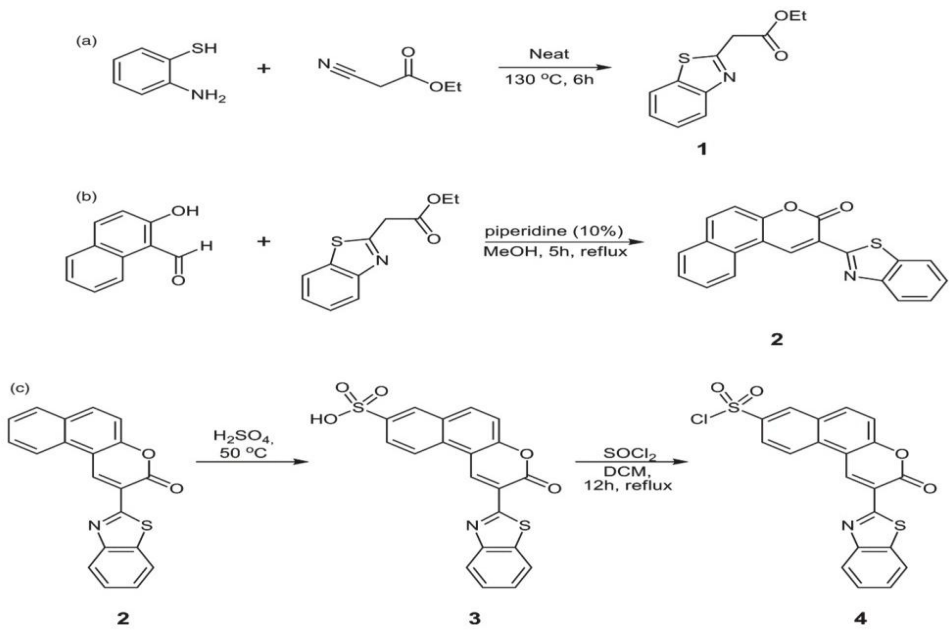


Scheme 2. Proposed mechanism for Ca^{2+} and F^- ions detection by sensor CPM.

Here we developed a novel coumarin-based fluorescence sensor CPM for the sequential detection of Ca^{2+} and F^- from other metal ions and anions in aqueous solutions. Based on the ^1H NMR spectroscopy, UV-vis, fluorescence spectra and the DFT calculations, the ICT mechanism of the CPM toward Ca^{2+} was illustrated. The sensor CPM exhibited an INHIBIT logic function to monitor the emission mode at 460 nm with Ca^{2+} and F^- as chemical inputs. Furthermore, the sensor CPM could be used for the detection of Ca^{2+} in different environmental waters and monitor Ca^{2+} and F^- in living cells. Given above is the synthesis and mechanism for CPM sensor.[2]

vi) A Novel Coumarin Fluorescent Sensor to Probe Polarity around Biomolecules

In this paper, we describe the development of a new solvatochromic biosensor. The probe is structured as a "push-pull" system, bearing an electron-rich naphtyl ring and an electron poor benzothiazene group, conjugated to a central coumarin core. These features confer interesting spectroscopic and solvatochromic properties to the fluorophore. The dye was derivatized with a versatile sulfonyl chloride functional group. We also demonstrated that solvatochromic properties are nearly maintained when the fluorophore is covalently linked to proteins usually employed for nanoparticle coatings (bovine serum albumin or streptavidin). Finally, we proved the efficiency of our polarity-sensitive probe by obtaining a statistically significant difference between the fluorescence signals of fluorophore-labeled streptavidin before and after addition of BSA-labelled biotin. Given below is the strategy for preparation of biosensor 4.[12]

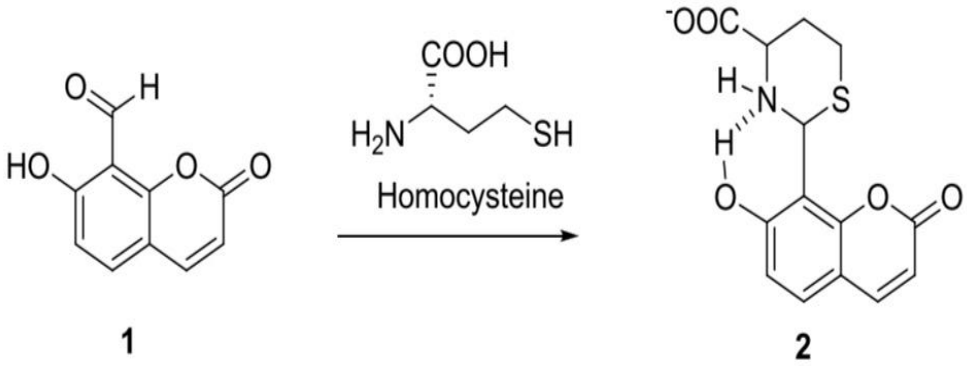


Scheme 1. Synthetic strategy for preparation of biosensor 4

vii) A Coumarin-Based Fluorescent Probe as a Central Nervous System Disease Biomarker

In this paper we have seen the application of coumarin-based fluorescent probe 1 to determine the level of homocysteine, an important biomarker in the Parkinson's disease patients using the simple and cost effective fluorescence spectroscopy technique. In addition, we have also established a rapid determination of homocysteine and methylmalonic acid (another important biomarker) in the serum of Parkinson's disease patients using LC-MS. Although the fluorescent analysis method indicated higher levels of homocysteine in the PD patients when compared to that of the LC-MS analysis method, the trend of results are similar with the highest homocysteine levels recorded in Group 1 (PD patients not treated with levodopa) and the lowest homocysteine levels in Group 3 (healthy subject), with Group 2 (PD patients treated with levodopa) in the middle. The overall cost and ease of analysis suggest that the fluorescence analysis method is more effective compared to the LC-MS analysis.

Elevated levels of homocysteine and/or methylmalonic acid in blood serum are important indicators to assist in the identification of patients suffering from neurological and psychiatric diseases such as Parkinson's disease, Alzheimer's disease, chronic heart failure, multiple sclerosis, depression, epilepsy, and also vitamin B_{12} deficiency. The methods presented herein allow for the rapid identification of the homocysteine and methylmalonic acid levels in the blood serum, able to distinguished between the healthy subjects and PD patients, and can be extended to other patients suffering from the above mentioned diseases. The methods presented herein will hopefully be exploited for standard routine analysis in a high-throughput clinical setting.[13]



Scheme 2. Reaction scheme of coumarin-based fluorescent probe 1 with homocysteine.

Table 1. Comparison of data from fluorescence and LC-MS detection of homocysteine and methylmalonic acid in PD patient sera.

	Homocysteine (Fluorescence) (μM)	Homocysteine (LCMS) (μM)	Methylmalonic Acid (LCMS) (μM)
Group 1			
Average	70.24	38.48	2.46
Standard deviation	20.68	19.40	1.29
Median	69.58	33.66	2.33
Max.	101.38	83.16	4.83
Min.	36.12	14.29	0.85
Group 2			
Average	58.74	41.01	4.11
Standard deviation	12.76	20.65	2.01
Median	53.79	40.36	3.60
Max.	86.52	68.28	7.71
Min.	44.33	12.44	2.03
Group 3			
Average	54.63	19.06	2.75
Standard deviation	12.76	11.10	0.98
Median	58.12	19.26	2.46
Max.	67.87	34.51	4.32
Min.	30.28	3.18	1.78

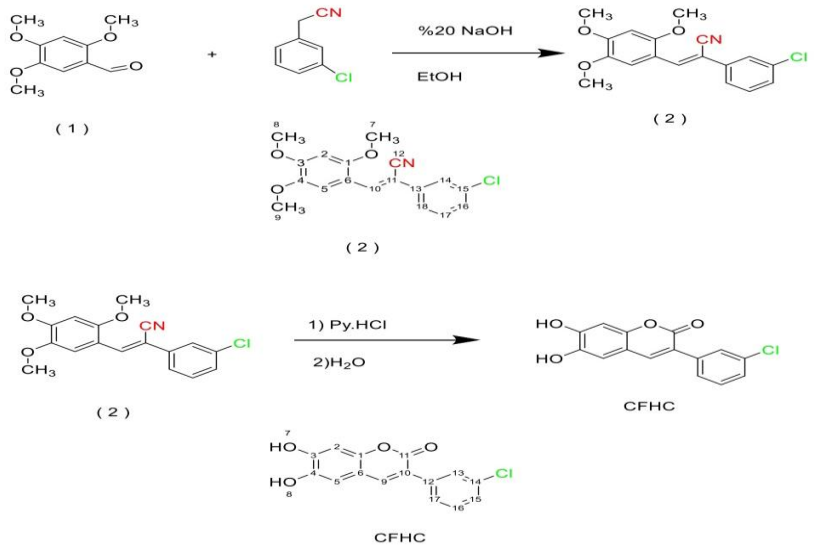
viii) V-shaped bis-coumarin based fluorescent probe for detecting palladium in natural waters

In this paper we have seen how a fluorescent probe based on V- shaped bis-coumarin was designed and synthesized.The mechanism by which Cp detects Pd^0 is the specific palladium-catalyzed Tsuji–Trost allylic reaction. After the addition of reductant $NaBH_4$, Pd^{2+}/Pd^{4+} can be detected. Thus, the probe can distinguish and detect $Pd^0$and Pd^{2+}/Pd^{4+} under different test conditions.At the same time, Cp has good selectivity and high sensitivity, and the probe can rapidly detect palladium in 10 min at room temperature with a low LOD (40.0 nM, 4.2 ng/g). Besides, Cp was successfully applied to the quantitative detection of environmental water samples.To sum up, the probe has excellent fluorescence properties, which provides a possibility for quantitative detection of residual palladium in chemistry and pharmacy and palladium concentration in the natural aqueous environment.[14]

ix) Coumarin Based Highly Selective Boff-on-off^ Type Novel Fluorescent Sensor for Cu^{2+} and S^{2-} in Aqueous Solution

This research tells us about how the preparation of fluorescent sensors for the recognition of heavy metal ions with high sensitivity and selectivity has received considerable attention because they play important role in environment and living systems.Detection of the metal ions in aqueous is also essential. Copper is a very important trace element for the life of organisms. However, copper ions in abnormal levels can lead todiseases

including Alzheimer's, Parkinson's, Menkes, Wilson's disease due to its oxidative and toxic effect. So, there are many methods using several instruments for detection of copper ions including AAS, electrochemical, colorimetric method, etc. In this experiment we saw Solvent free synthesis of 6,7-dihydroxy-3-(3-chlorophenyl) coumarin (CFHC) was designed and obtained by the interaction of 2-(2,4,5-trimethoxyphenyl)-1-(3-chlorophenyl)acrylonitrile with pyridinium hydrochloride in the presence of silica gel by using microwave irradiation. The characterization of CFHC was confirmed by FT-IR, ^{1}H, ^{13}C, ^{13}C–APT and 2D HETCOR spectroscopy methods. The optical behavior of CFHC towards metal ions was investigated by UV-visible and fluorescence spectroscopy. CFHC showed Bon–off^ type fluorescence response towards Cu^{2+} with high selectivity in aqueous solution (CH3CN/H2O, 9/1, v/v). Once binding with Cu^{2+}, CFHC-Cu^{2+} complex also displayed high selectivity for sulfide, resulting in Boff–on^ type sensing of sulfide anion.[15]



Scheme 1 The synthesis of 6,7-dihydroxy-3-(3-chlorophenyl)coumarin (CFHC)

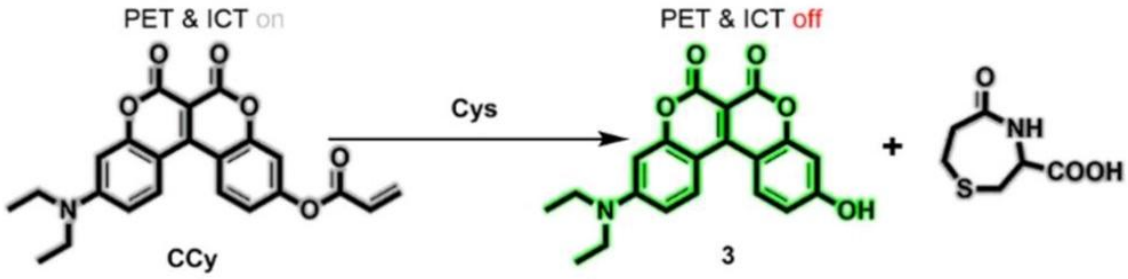
x) Novel coumarin-based pH sensitive fluorescent probes for the highly alkaline pH region

In this reseach paper we studied The development of reliable techniques to monitor pH has received significant attention because of the importance of pH measurement in various areas of scientific research and other practical applications. The determination of pH is routinely performed using glass electrodes. However, glass electrodes have several disadvantages and thus are not suitable for certain applications.pH sensing using optical methods provides an attractive alternative for measuring pH due to the advantages offered in terms of immunity to electrical interference, enhanced safety and the possibility of remote sensing. Therefore a number of pH indicators have been designed or

investigated for this application. In this paper they reported synthesis and spectroscopic properties of novel polymerizable coumarin-based using pH indicators. Novel coumarin-based fluorescent pH indicators vinylbenzylamino imidazolylmethyl coumarin (VIC), acrylamido imidazolylmethyl coumarin (AIC) and styryl imidazolylmethyl coumarin (SIC) have been synthesized. Their fluorescence behaviours have been investigated in aqueous solution at different pHs. All coumarins exhibited a significant decrease in fluorescence intensity with increasing pH in the highly alkaline region. Photophysical properties of the indicators have been postulated to explain their fluorescence properties and behaviours. The indicators were covalently immobilized to polymer supports by co-polymerising with methacrylic acid co-monomer and 1,4-bis(acryloyl)piperrazine cross-linker. These polymers are sensitive to pH in the similar response range of the free dyes and were highly photo-stable, showing no photo-bleaching, when immersed in a pH 10 buffer solution, after 60 minutes of continuous illumination using a high power Xe lamp. The sensing materials developed in this study have been designed for use with optical fibre sensors and their characteristics shown indicate how well they are suited for use in optical pH sensors for measuring pH in various alkaline media. Discussion of the design and performance of such sensors will be the subject of future publications.[16]

xi) A novel coumarinocoumarin-based two-photon fluorescent cysteine biosensor for targeting lysosome

In this paper we see Coumarinocoumarin, one of the coumarin derivatives which are easy to synthesize and have rich modification sites. The large conjugate plane of coumarinocoumarin make it has more excellent optical property than conventional coumarin, for example, the two-photon fluorescence property. So, the coumarinocoumarin-based probe (CCy) has been designed and synthesized, which is the first lysosomal targeting fluorescent biosensor for cysteine. This probe was prepared by a 3-step procedure as a latent fluorescence probe to achieve high sensitivity and fluorescence turn-on response toward cysteine (Cys) over homocysteine (Hcy), glutathione (GSH) and other various natural amino acids under physiological conditions. Upon addition of Cys to the solution of CCy, remarkable enhancement on 520 nm of fluorescence spectra can be monitored.[17]



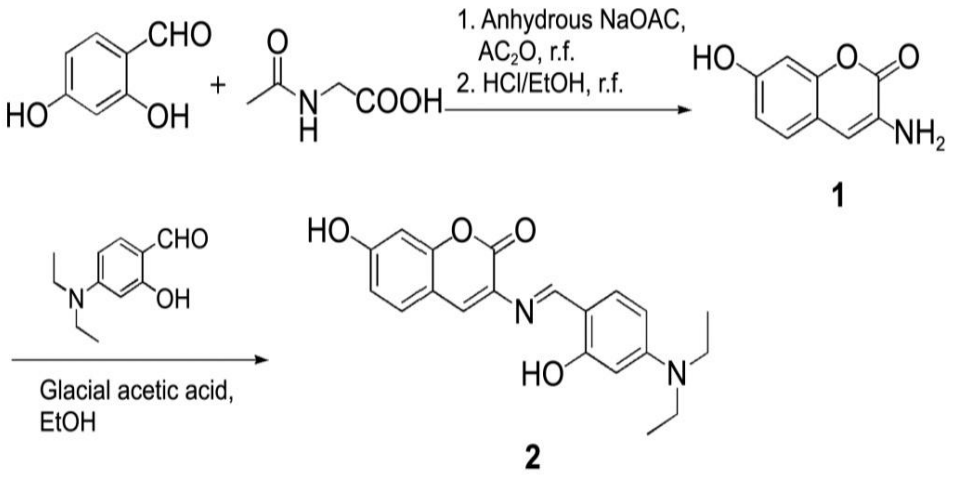
Scheme 1. Design concept of CCy for Cys.

xii) Synthesis and solvent-dependent photophysics of a novel fluorescent triazole-coumarin-based dye

Due to increasing attention of novelty and variety-oriented synthesis has become important for the examination of fluorescent dye molecules, especially coumarins. Due to their low toxicity, excellent photophysical, and structural features, coumarin and coumarin derivative molecules have an important place in fluorescent dyes.These privileged properties enable the coumarin compounds to be used in biological systems such as anti-cancer, antioxidant, and antimicrobial activity as well as optical dye laser systems and spectroscopic applications . Therefore here we saw the synthesis of a new coumarin-triazole-based dye and its photophysical parameters such as absorption, fluorescence emission, and fluorescence quantum yield were investigated. Studies have shown that the present dye has symmetry with a mirror image, especially in the ethanol solvent, with respect to the absorption and fluorescence spectra. As a result of the UV-vis and fluorescence spectroscopy techniques used, it was determined that absorption and emission spectra were shifted to the red with increasing solvent polarity.In addition, the spectral data of the synthesized compound exhibited that the stokes shifts are small, usually less than 50 nm, and the quantum yields are significantly high.In accordance with the results obtained, it can be stated that this novel dye synthesized here can offer an insight into application in sensor applications as analytical or biosensors, optoelectronic devices, and medicine industry.[18]

xiii) A Coumarin-based Fluorescent Sensor for Selective Detection of Copper (II)

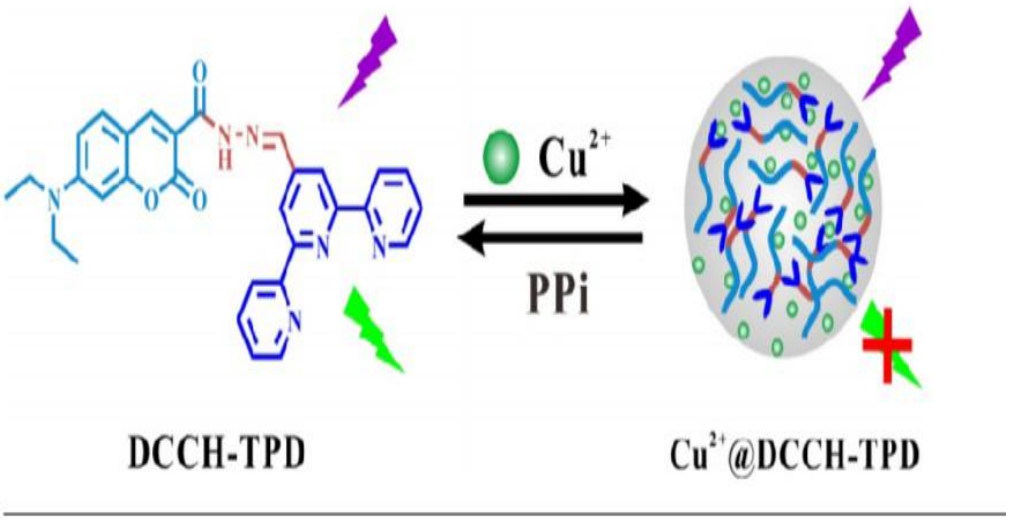
In this report, they had developed a novel coumarin-based chemosensor bearing the salicylaldimine unit (2) for Cu^{2+} selective detection. The results from fluorescence spectra demonstrated that the sensor could selectively recognize Cu^{2+} over other metal cations and the detection limit is as low as 0.2 μM. Moreover, the confocal fluorescence imaging in HepG2 cells illustrated its potential for biological applications.[19]



Scheme 1. Synthetic route of chemosensor 2.

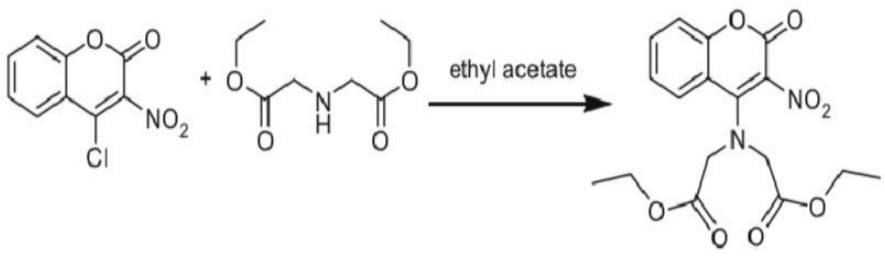
xiv) Highly sensitive fluorescent sensor based on coumarin organic dye for pyrophosphate ion turn-on biosensing in synovial fluid

In this report we have we successfully prepared a novel organic dye (DCCH-TPD) comprising coumarin fluorophore, hydrazide group, and terpyridine moiety, and we further displayed its utilization in development of highly sensitive sensor for Cu^{2+} and PPi analysis. DCCH-TPD emitted green and bright fluorescence, and the quantum yield and lifetime were calculated to be 0.35 and 0.5555 ns, respectively. Under the exposure to Cu^{2+}, it underwent the generation of Cu^{2+} @ DCCH-TPD associated with decreased fluorescence due to the PET effect initiated by Cu^{2+} in Cu^{2+} @ DCCHTPD. On the basis of the decrease in FL intensity, sensitive detection of Cu^{2+} was readily achieved. Further, DCCH-TPD was applied in analysis of PPi due to the target-triggered competitive coordination reaction. The DCCH-TPD-based sensor presented high sensitivity and wide linear range toward PPi, which are superior to that of the methods reported previously. Moreover, DCCH-TPD implemented the assessment of concentration of PPi in synovial fluiD with acceptable recovery. Therefore, this work not only provides a way to highly sensitive and selective detection of PPi, but also enriches the PPi sensor development.[6]



xv) New coumarin-based sensor molecule for magnesium and calcium ions

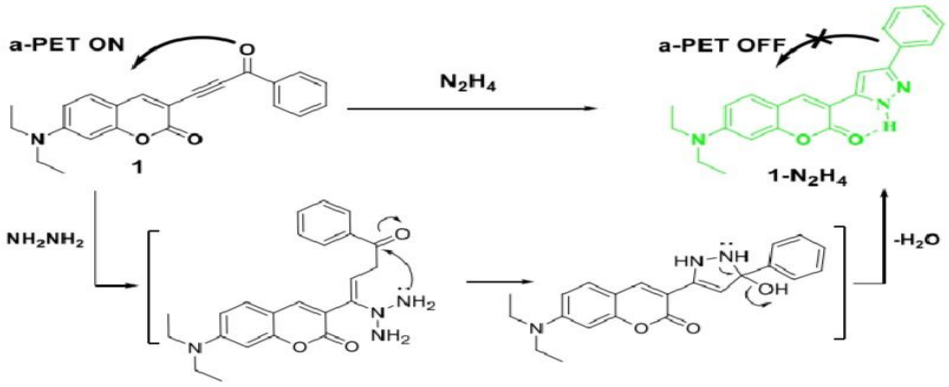
In this paper we have seen a new coumarin-based sensor molecule (L1) has been synthesized and this was found to bind calcium and magnesium ions more effectively as compared to other alkali/alkaline earth/lanthanide and certain transition metal ions. A significant enhancement in fluorescence intensity was observed on binding to Ca^{2+} and Mg^{2+} ions; while a minor quenching was observed for weakly bound Hg^{2+}, Ni^{2}+, Fe^{3+}, and Co^{2+} ions. PET process, coupled with the ICT process, is proposed to explain the observed spectral response.[7]



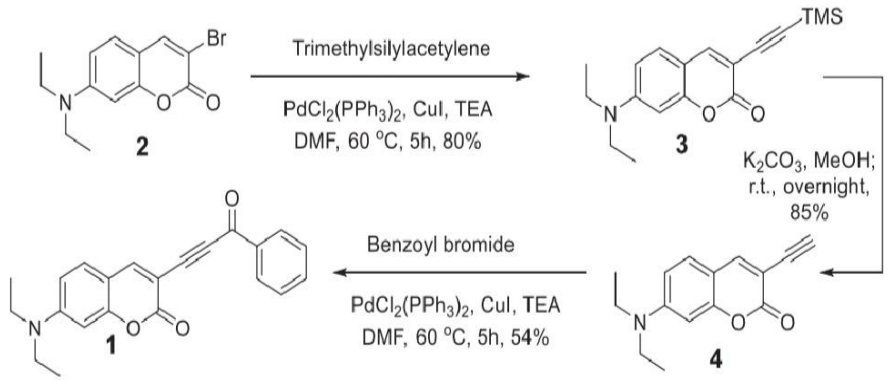
Scheme 1. Methodology followed for the synthesis of L1.

xvi) A coumarin-fused 'off-on' fluorescent probe for highly selective detection of hydrazine

Hydrazine is a kind of widely used industrial raw material and a toxic biochemical reagent. Due to its toxic to organisms, hydrazine has been classified to be a hazardous environmental pollutant. It is urgent to develop fluorescent probe tools for selective sensitivity detection of hydrazine in the environment and the body. We developed here a new coumarin-based fluorescent probe for hydrazine detection In this report They reported a coumarin-fused hydrazine fluorescent probe 1 using the pyrazole forming strategy. Coumarin was used asfluorophore because of its high fluorescence quantum yield and excellent light stability. The ynone group in the 3-position of coumarin can not only quench fluorescence by photo-induced electron transfer (PET) effect but also serve as a reactive unit of hydrazine. The selectivity and sensitivity of the probe were examined in the PBS buffer solution, the sensing mechanism was confirmed by HRMS and DFT calculation methods, and the bioimaging of hydrazine in HeLa cells using probe 1 was finally evaluated.In this paper, we developed here a coumarin-fused fluorescence probe for the detection of hydrazine. The coumarin moiety with high fluorescence quantum yield was served as the fluorophore, and the ynone part was chosen as hydrazine reactive group. The probe showed high selectivity (fluorescence off-on response was approx. 600 fold) and sensitivity (the detection limit was 267 nM). The sensing mechanism was confirmed by DFT calculation and HRMS spectra. The probe can be successfully used to image hydrazine in cells and detect hydrazine in real water samples.We further expect that the probe could be applied in tissue imaging.[20]



Scheme 1. The sensing mechanism of probe 1 with hydrazine.



Scheme 2. Synthetic route of probe 1.

III. CONCLUSION

In this report we have seen how Coumarin based Fluorescent Biosensors are used for detection of Cysteine with live cell applications then we saw how Coumarin based, switchable fluorescent substrates for enzymatic bacterial detection then we saw how Coumarin based fluorescent sensors for Ca^{2+} and sequential detecton of F^- are used. Next we also studied how Coumarin fluorescent pH indicators, probes and membranes used for covering a broad pH range.Then we saw how coumarin based pH sensitive fluorescent probes are used for highly alkaline pH region. Then we also saw how Coumarin based fluorescent probes are used for dectecting Palladium in natural waters, for central Nervous system disease Biomaker and also for detecting highly alkaline pH region.

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