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3.1 Abstract:

Fluorescent molecular probe having excimer emission property templated with ϒ-cyclodextrin (ϒ-CD) including calix[4]arene as ionophore and pyrene as fluorophore has been synthesised. With ϒ-CD, pyrene substituted calixarene (PCX4) shows strong excimer formation with very high intensity at 482 nm which is the characteristic peak and is used to study interaction between them. Binding study of these fluoroionophore PCX4 with ϒ-CD has been investigated in water and the recognition process is monitored by UV-Vis, steady-state and time-resolved fluorescence, FT-IR, SEM, HR-MS techniques. In present study, depending upon the concentration of ϒ-CD excimer formation is possible from both intramolecular (two pyrene moieties of the same PCX4 i.e. 1:1 (PCX4 : ϒ-CD) complexation) and intermolecular (two pyrene moieties from two different PCX4s i.e. 2:1 (PCX4 : ϒ-CD) complexation) stacking interactions. Since the monomer emission decreases at lower concentration of ϒ-CD, the weak excimer emission could be due to the dimerization of pyrene groups between two different PCX4s, which is an intermolecular process. Beyond 1.2 mM of ϒ-CD, the intramolecular excimer formation is predominant, resulting in a strong excimer emission. On the other hand, ϒ-CD induces downward pK_a shift in PCX4 which may find applications in drug delivery.

3.2 Introduction:

Pyrene-armed calix[4]arenes are widely used as fluorescent chemosensor which are capable of selectively recognizing cationic guests and show potential analytical applications in many different fields, including chemistry, biology, and medicine[1]. Synthesis and design of molecule with new functional group can be serving as sensing of specific ion molecule in specific area of research. Now a days calixarene has become an important tool in biomedical application as it has anticoagulant, antiviral, anticancer, antithrombotic activities in protein complexation and enzyme blocking [2]. It is also used in medical application as drug delivery vehicle. Calixarene have phenolic unit, Methylene bridge and favourable binding
sites, due to these characteristic it can be modified in their architecture and used as fluorescent sensors [3]. Calixarene has hydrophilic upper and lower rim separated by hydrophobic mid region, hence it is bipolar molecule [4]. This macrocyclic molecule is flexible and capable to undergo change in conformation. Considering all the above facts, we synthesized pyrene armed calixarene which can act as good fluoroionophore with γ–CD.

3.3 Literature Review:

Literature review shows that γ-cyclodextrin is one of the macrocycle with large hydrophobic inner cavity and hydrophilic rims, and consists of 8 glucopyranose units. It forms inclusion complexes with various organic guest molecules. Modulation of photophysical properties of fluorescent dyes with macrocyclic hosts like cyclodextrin, cucurbituril has been investigated extensively [5, 6]. Evidences were found for dimer and/or excimer formation with pyrene and γ–CD [7]. It was investigated that pyrene and anionic γ-cyclodextrin [8] show different types of stoichiometries for complexation. The interaction of γ-CD and γ -CD with polyacryl amide modified with pyrenyl was studied in mixture of solvents [9]. New chemical sensors using hybrids of CDs with π-conjugated polymers, peptides, DNA, nanocarbons and nanoparticles are also described in last few years [10]. γ–CD has lipophilic cavity of 7.5 Å inner rim diameter and 8.5 Å outer rim diameter. It is readily water soluble and can form host-guest inclusion complexes with molecules and ions [11]. This makes cyclodextrin an interesting molecule because there cavities are less polar than water to hold and protect hydrophobic molecules from aqueous solution [12]. Therefore it may act as a good probe for complexation study with disubstituted pyrene derivative of calix[4]arene [PCX4–( 1)].

Among different fluorogenic units, pyrene is an important fluorophore because its monomer to excimer emission ratio (IE/IM) changes on complexation which is very sensitive and selective to different metal ions [13, 14]. Host molecules with more than one pyrenyl group exhibit both intra- and intermolecular excimer emission [15]. Intermolecular excimer formation is the unique character of ester containing pyrenyl moiety due to π-π interaction between excited state pyrene and ground state pyrene reported by Broan [16] and Jong Seung Kim [17].

Calixarene has been widely used as host for ions and other neutral guest molecules [18]. Study involving pyrene based calixarene as a sensor is not only
important for practical point of view but also interesting to study the complexation behaviour. In the present study, pyrene based calixarene shows significant increase in excimer intensity with γ-CD in aqueous media as compared with that of bare pyrene.

Therefore, here we present pyrene based calixarene with spacer as fluorophore which can give characteristic strong excimer emission due to both inter and intramolecular π-π stacking. Due to longer spacer, intermolecular interaction between two pyrene molecules of two PCX4 may be established. Figure 3.1 shows chemical structure of γ-CD and PCX4.

![Chemical structure of PCX4 and γ-cyclodextrin.](image)

Figure 3.1: Chemical structures of PCX4 and γ-cyclodextrin.

3.4: Materials and Method:

3.4.1: Chemical Reagent:

γ-cyclodextrin used in the experiment was purchased from TCI Mark, Tokyo, with 98% purity. Acetonitrile used as solvent in experiment is also 99.8% pure. Perchloric acid and sodium hydroxide were purchased from Sigma Aldrich. All reagents employed for the synthesis and extraction were of AR grade.

3.4.2 Measurement:

UV-Vis absorption spectra were performed using Shimadzu 1800 UV-Vis spectrophotometer. Fluorescence spectra were recorded on Jasco spectrofluorometer 8300. The fluorescence spectra were corrected whenever necessary. Data were collected with 1 nm interval and excitation slit width 2.5 nm and emission slit width 5 nm. All measurements were taken with PCX4 concentration (1 x 10^{-6} M) in
acetonitrile and titration was carried out in water. Life time measurement was carried out by collecting fluorescence decays using picoseconds resolution equipment time correlated single photon counting (TCSPC) setup (Horiba Jobin Yvon IBH, U.S.A.). Binding curve was plotted by Origin 8 software for graphing.

$^1$H NMR spectra were recorded on a Varian Mercury YH-300 (350 MHz) spectrophotometer in DMSO-d$_6$ at room temperature (298 K) with TMS as an internal standard. FT-IR of compound was recorded on Shimadzu 8400 FT IR. Solid sample for FT-IR was prepared by slow evaporation of mixture of PCX4: Y-CD (1:1 equivalent) in acetonitrile: water (1:9). HR-MS spectra were recorded on Bruker Daltonik GmbH, Germany, Impact II UHR-TOF Mass Spectrometer System, and Ionisation source: ESI (Electron Spray Ionization), APCI (Atmospheric Pressure Chemical Ionization).

3.4.3 Synthesis of Calixarene:

As per our knowledge, we are the first to report the novel synthesis of pyrene derivative of calix[4]arene (PCX4) with butyric acid chain as spacer responsible for inter and intramolecular excimer formation. PCX4 (1) has been synthesized by DCC coupling reaction as mentioned in the procedure reported by Biswadip Banerji et al [19]. Synthetic route is shown in Scheme 1. To an ice cold stirred solution of butyric acid (0.223 gm, 0.5 equiv, 1.0 mmole) and dry dichloromethane (5 mL), EDC(3-dimethylaminopropyl)-N-ethylaminocarbide (1 equiv, 1.0 mmole) is added followed by 1-hydroxybenzotriazole HOBT (1 equiv, 1.0 mmole). The resulting mixture was stirred vigorously for 30 minutes and then p-ter. butyl calix[4]arene (0.5 g, 1 equiv, 1.0 mmole) was added followed by 1 equivalent of triethyl amine and mixture stirred for overnight then washed thoroughly with citric acid solution and then with water (10 mL x 3). Drying and concentration in vacuum yielded the ester derivative of calix[4]arene. Further column chromatography is carried out by petroleum ether and ethyl acetate mixture (8:2) gives the desired pale yellow product of ester derivative of pyrene calix[4]arene. Yield is 60%. Purity is checked by TLC, melting point. PCX4 (1) obtained is in partial cone confirmation, which is proved by $^1$H NMR. It shows two singlets and two pairs of doublet for aromatic proton as well as one pair of doublet for bridging –CH$_2$ reported by C. David Gutsch [20, 21] further split singlet of tertiary butyl group at 1.25 ppm proves partial cone confirmation of PCX4(1).
$^1$H-NMR (500 Hz, CDCl$_3$): 1.25 (singlet split ter. butyl 36H), 1.95 (m 4H), 2.4 (t 4H), 3.4(t 4H), 4.06 (t, 8H), 7.4-7.9 (two singlets and two pairs of doublet Ar-H 8H), 8.0-8.4 (m 8H Py), 9.5(s 2H) (Figure 3.2).

$^{13}$C-NMR: 27, 33, 125,135,137,174. (Figure 3.3).

HRMS Spectra (Acetonitrile): 1238, 1192, 1076, 975, 833,671. (Figure 3.4).

FT-IR $\nu$ max (KBr, cm$^{-1}$): 3321, 2956, 1680, 1593, 1481, 842 cm$^{-1}$(Figure 3.5).

![Scheme 1](image.png)

Scheme 1: Synthetic route for the PCX4 (1).
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Figure 3.2: $^1$H-NMR of PCX4 (1).

Figure 3.3: $^{13}$C-NMR Spectra of PCX4 (1).
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Figure 3.4: Enlarged portion of HR-MS spectra of PCX4.

Figure 3.5: FT-IR spectra of PCX4 (1).
3.5. Results and discussion:

3.5.1 Absorption properties of PCX4 in presence of Y-Cyclodextrin:

The UV-Vis study of 1 with Y-CD was carried out in aqueous medium. Absorption spectra for 1 show the strong and sharp peak of pyrene at 312, 328 and 345 nm. The addition of Y-cyclodextrin initially shows decrease in the absorbance and later on increase in the absorbance with slight bathochromic shift by 2-3 nm.

The decrease in absorbion initially at lower concentration (<1.2 mM) may be due to intermolecular interaction [22] of two pyrene moieties of different PCX4s. Later on increase in absorbance may be due to the inclusion of both pyrene moieties in the cavity of Y-cyclodextrin and shows π-π* transition due to stacking. On addition of more amount of Y-cyclodextrin upto 22 mM, gradual increase in OD is observed [23]. This binding of 1 (PCX4) with Y-cyclodextrin is due to hydrophobic interaction as pyrene is probe for hydrophobic region and the cavity of CD is hydrophobic in nature. There is no isosbestic point obtained after addition of Y-CD.

The changes in absorption spectra is further supported by steady-state and time-resolved fluorescence measurements. Upon addition of Y-CD, the absorption peak underwent a hypochromic shift with slight change in peak position which indicates the formation of complex between Y-CD: PCX4. At higher concentrations of Y-CD, noticeable change in the spectra is observed. The scattering in the 400-450 nm regions could be due to the formation of higher order complexes which are insoluble in water. Figure 3.6 (A, B and C) shows absorption spectra of 1 with Y-CD.

The absorbance data is fitted using following equation [25].

\[
A = \frac{A_0 + A_{CD,PY}K_1[Py]_0 + A_{(CD)_2:PY}K_1K_2[Py]^2_0}{1 + K_1[Py]_0 + K_1K_2[Py]^2_0}
\]

3.5.2 Effect of pH on the absorption spectra of complex 1 (PCX4) with Y-CD:

Absorption spectra of PCX4 and Y-CD are also pH dependent. At low pH, absorbance of complex is very low. However with increase in pH, absorbance goes on increasing and gets saturated at pH 10. Critical point is observed at pH 4-4.5 which is suitable for highest complexation [26] (Figure 3.6).
Figure 3.6: A & B) UV-Visible absorption spectra of PCX4 (1) in H₂O at different concentrations of Υ-CD in mM a) 0, b) 0.24, c) 0.48, d) 0.91, e) 1.21, f) 1.61, g) 2.82, h) 5.1, i) 7.94, j) 11.67, k) 15.91 and l) 20.62.

Figure 3.6 C) The variation of OD of 1 at 355 nm with different concentrations of Υ-CD.
Figure 3.6 D) UV-Visible absorption spectra of 1: Υ-CD in H₂O at different pH 1) 2.96, 2) 3.69, 3) 4.01, 4) 4.24, 5) 4.36, 6) 4.63, 7) 5, 8) 5.56, 9) 6.01, 10) 6.56, 11) 7.13, 12) 7.52 and 13) 8.71. Inset shows the variation of OD of 1: Υ-CD at 340 nm with pH.

3.5.3 Comparison of absorption properties of pyrene with β-Cyclodextrin:

β-cyclodextrin is the lower analogue of Υ-CD with 7 glucose units having internal diameter 0.78 nm. Absorption properties of PCX4 with β-CD are studied. It is observed that with addition of β-CD absorption of pyrene goes on decreasing and get saturated till 2-3 mM concentration of β-CD. These observations are different from Υ-CD. Since, the cavity size of β-CD is smaller than Υ-CD, only one pyrene moiety of PCX4 can be accommodated in the cavity of β-CD (Figure 3.7).
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Figure 3.7: Absorption spectra of PCX4 with varying concentration of β-CD 1) 0 2) 0.049 3) 0.145 4) 0.238 5) 0.327 6) 0.454 7) 0.575 8) 0.762 9) 0.934 10) 1.241 11) 1.503 mM. Inset shows the variation of OD of 1: β-CD at 340 nm.

3.5.4 Fluorescence properties of PCX4 with γ-CD:

Small changes in the absorbance spectra are observed for 1 with γ-CD. However significant changes are observed for the complex when studied by steady state fluorescence. Fluorescence spectra were recorded in the presence and absence of γ-CD at 355 nm excitation. Due to the partial cone shape of PCX4, the pyrene moieties remain in two different directions and PCX4 shows emission peaks at 378 and 398 and 420 nm in aqueous solution which corresponds to the pyrene monomer emission (Figure 3.8A, 3.8B, 3.8C). Upon gradual addition of γ-CD to the PCX4 solution, there is decrease in the monomer emission, initially along with the appearance of weak excimer emission at 482 nm. Beyond a particular concentration of γ-CD (>1.2 mM), PCX4 shows strong excimer emission along with the increase in the monomer emission (Figure 3.8A, B, C). Owing to its large cavity size (7.5 Å inner rim diameter and 8.5 Å outer rim diameter), γ-CD has a capability to encapsulate two guest molecules. Therefore, γ-CD-templated excimer emission from PCX4 could be rationalised. Such an excimer formation is possible from both intramolecular (two pyrene moieties of the same PCX4) and intermolecular (two pyrene moieties from two different PCX4s) stacking interactions. Since the monomer...
emission decreases at lower concentration of ϒ-CD, the weak excimer emission could be due to the dimerization of pyrene groups between two different PCX4s [23]. Beyond 1.2 mM of ϒ-CD, the intramolecular excimer formation is predominant, resulting in a strong excimer emission [27,28]. Due to the π-π stacking of pyrene moieties in the hydrophobic cavity of ϒ-CD, both inter- and intramolecular excimer emission intensity increase with the addition of ϒ-CD to the PCX4 solution.

Excimer are of two types depending upon their origin: dynamic excimer and static excimer [29]. Dynamic excimer formed in the excited state of pyrene while static excimer arises from pyrene dimer in ground state [30]. Depending on the distance between two pyrene units dynamic or static excimer formation takes place [29]. In this case initially formed excimer (intermolecular) is static excimer with low intensity and later formed excimer (intramolecular) may be dynamic excimer.

Binding constants for this system is calculated by fluorescence titration method. It was carried out by varying concentration of ϒ-CD and keeping concentration of PCX4 constant till saturation point is reached. The higher binding constant for excimer, indicates that there is very strong binding between PCX4 and ϒ-CD by inter as well as intramolecular π-π stacking [31].

For free pyrene also, binding constant are calculated as K1 = 532 M⁻¹ and K2 = 410 M⁻¹ at 374 nm. The lower binding constant (K1 = 415 M⁻¹) for free pyrene as compared to higher binding constant (K1 = 823 M⁻¹) for the excimer formation indicates PCX4 with long chain spacer is good probe for more intense and strong excimer emission with ϒ-CD than that of free pyrene. Figure 3.9 shows expected mechanism for different types of excimer formation.

\[
I_f = \frac{I_f^0 + I_{py,CD} * K_1 * [CD]_0 + I_{py(CD)2} * K_1 * K_2 * [CD]^2}{1 + K_1 * [CD]_0 + K_1 * K_2 * [CD]^2}
\]
Figure 3.8: A) Steady state fluorescence spectra of PCX4 in H$_2$O with addition of different concentration of Y-CD in mM 1) 0, 2) 0.48, 3) 0.91, 4) 1.61, 5) 2.82, 6) 5.1, 7) 7.94, 8) 11.67, 9) 15.91 and 10) 20.62.

Figure 3.8 (B). The change in the fluorescence intensity of PCX4 with the addition of Y-CD at 387 nm.
Figure 3.8 (C). The change in the fluorescence intensity of PCX4 with the addition of γ-CD at 478 nm.

3.5.5 Possible mechanism for excimer formation:

Intermolecular weak excimer formation

Intramolecular strong excimer emission
3.5.6 Comparison of fluorescence properties of PCX4 with β-CD:

Fluorescence titration is carried out by keeping concentration of PCX4 constant and varying concentration with β-CD at $\lambda_{ex}$ 355 nm (Figure 3.10). In the fluorescence spectra, there is only monomer peak at 377 nm (there is no excimer emission as observed in case of γ-CD). With addition of β-CD there is gradual increase in monomer intensity till 2-3 mM concentration. The increase in monomer emission of PCX4 with the gradual addition of β-CD clearly indicates that, there is formation of 1:1 inclusion complex between pyrene moieties of PCX4 with β-CD. Unlike γ-CD, it encapsulates only one pyrene unit due to the lower cavity size of β-CD.
3.5.7 Effect of pH on monomer and excimer emission of PCX4:

pH dependent study shows that appropriate pH range required for complexation. pH dependent fluorescence titration of PCX4 and \( \beta\)-CD are carried out. It is observed that fluorescence intensity of monomer is higher for acidic pH and lower for basic pH. Whereas, with increase in the pH of the solution excimer intensity at 480 nm increases (Figure 3.11). It may be because of formation of phenoxide ion in basic pH in the PCX4 which promotes the bending mobility of butyric acid chain due to that two pyrene moieties come closer to one another gives intramolecular excimer formation. There is sudden decrease in monomer intensity and increase in excimer intensity at pH 5. However there is no significant change in the fluorescence intensity of monomer at pH range 2-4 and 7-10. In case of excimer there is gradual increase in intensity from pH range 3-6 where there is little change in intensity from pH 6 onwards. This indicates that pH 5 is suitable for maximum complexation of PCX4 with \( \beta\)-CD.

Figure 3.10: Steady state fluorescence spectra of PCX4 in H\(_2\)O with addition of different concentration of \( \beta\)-CD in mM 1) 0 2) 0.045 3) 0.145 4) 0.238 5) 0.327 6) 0.455 7) 0.575 8) 0.762 9) 0.935 10) 1.24 11) 1.50.
Figure 3.11: Effect of pH on the fluorescence intensity of monomer (378 nm) and excimer (480 nm) of \( \gamma \)-CD: PCX4 complex.

3.6 FT-IR study of complex:

The complexation behaviour of ligand with \( \gamma \)-CD was examined with IR spectroscopic technique (Figure 3.12). The characteristic -C=O stretch of PCX4 (1) is observed at 1735 cm\(^{-1}\) and 1693 cm\(^{-1}\). After complexation -C=O stretch decreases to 1683 cm\(^{-1}\). The peak of C-H stretch of tertiary butyl group at 2955 cm\(^{-1}\) decreases up to 2926 cm\(^{-1}\). In aromatic region there is considerable decrease in intensity of band from 1479 cm\(^{-1}\), 1429 cm\(^{-1}\) to 1452 cm\(^{-1}\), 1413 cm\(^{-1}\) (Ar-H stretch). Thus the change in stretching frequency of aromatic functional group indicates that complexation in aromatic ring as well as –C=O stretch of PCX4 clearly indicates strong complexation between \( \gamma \)-CD and PCX4.
Figure 3.12 a: FT-IR spectral analysis of PCX4

Figure 3.12 b: FT-IR spectral analysis complex of PCX4-$\gamma$-CD.
3.7 SEM study of complex:

To explain the morphology of PCX4 with its complex Y-CD SEM images are shown in Figure 3.13 respectively. The particle size of PCX4 is 5 μM while that of complex is 10 nm. Morphology of PCX4 and complex shows that it is completely distinct from each other. PCX4 displays a regular uniform pattern of needle shape morphology while this morphology got disturbed in complex.

![PCX4](image1.png)  ![PCX4 with Y-CD](image2.png)

**Figure 3.13: SEM micrograph images of PCX4 and its complex with Y-CD.**

3.8 Effect of complexation on fluorescence lifetime:

The time resolved data give more information about the type of interaction. For distinguishing contribution of monomer emission in excimer region (also *vice versa*), we collected the emission decays in extreme region of each intensity band. i.e. 380-400 nm (monomer) and 480-510 nm (excimer). Sample was excited by using 339 nm LED.

Fluorescence decay curves for PCX4 and its complex with Y-CD monitored at the monomer region (370-400nm) and the excimer region (450-500 nm) in H₂O. Figure 3.14 shows emission decay in the presence and absence of Y-CD. For PCX4 biexponential decay traces are observed for monomer at 380 nm and triexponential decay traces for excimer at 480 nm. For the fitted data $\chi^2$ values are lower than 1.2. Upon addition of 1 mM Y-CD there is increase in lifetime for monomer at $\lambda_{em} = 390$
nm from 96.1 ns to 103.7 ns. The increase in monomer lifetime of pyrene may be due to pyrene experiences more rigidity in the cavity of \( \gamma \)-CD than free form.

Excimer formation depends on the distance between two pyrene units and length of spacer attached to pyrene [29]. Figure 3.14 b shows rise curve with – ve value of preexponential factor which indicate that there may be formation of excimer takes place via dynamic process [32]. The mean life time of excimer for PCX4 in H\(_2\)O is 94 ns (at \( \lambda_{em} = 510 \) nm), then it increases from 94.8 ns to 99.6 ns with addition of \( \gamma \)-CD. This increase in life time may be because of time taken by mobility of the pyrene molecules to achieve a specific excimer confirmation in the cavity of \( \gamma \)-CD. This represent that there is complexation between PCX4 and \( \gamma \)-CD. With further addition of 22 mM \( \gamma \)-CD, there is increase in life time up to 108 ns for monomer and 100.8 ns for excimer. This increase in life time shows complete complexation takes place with formation of strong excimer. This life time value matches approximately with the reported values [33].

![Fluorescence decay traces of PCX4 monitored at 380 and 480 nm in the a) absence and b) presence of \( \gamma \)-CD.](image)

**Figure 3.14:** Fluorescence decay traces of PCX4 monitored at 380 and 480 nm in the a) absence and b) presence of \( \gamma \)-CD.
3.9 Effect of Inclusion complexation on acidity constant:

Macrocycles like cucurbituril, cyclodextrin and calixarene show differential binding affinities with organic molecules depending upon the nature of their portals and significantly modulates the molecular properties such as fluorescence properties, prototrophic equilibrium, etc. of the guest molecule. Here Υ-CD shows good binding affinity with PCX4 (1). Such a binding interaction of cyclodextrin with PCX4 (1) may lead to significant modulation of acid base properties of guest (PCX4). For the dye-CD system prototrophic equilibrium is shown in Scheme 2.

\[
\begin{align*}
H^+ + PCX4-OH + g-CD & \rightleftharpoons gCD:OPCX4 + 2H^+ \\
PCX4-O^+H_2 + g-CD & \rightleftharpoons g-CD:H_2^+OPCX4
\end{align*}
\]

\[K_{eq}^1 \quad K_a \quad K_{eq}^2 \quad K_a'
\]

**Scheme 2:** Four state thermodynamic model to determine the pKₐ value of complexed guest molecule.

Effect of Υ-CD on the prototropic properties of dye (PCX4) has been studied by pH dependent changes on the fluorescence spectra of dye (PCX4) (4 μM) in the presence of Υ-CD (20 mM). pH titration curves are constructed by monitoring the fluorescence intensity of PCX4 in the absence and presence of the Υ-CD at 378 nm and is shown in Figure 3.15. The pKₐ values obtained from the inflection points of these plots are 6.2 and 5.0 for PCX4 and Υ-CD-PCX4, respectively. Downward pKₐ shift about (1.2 unit) in the presence of cyclodextrin is reported in the literature [34].
3.10 Mass Spectroscopy:

To study the composition and stoichiometry of the complex, mass spectra measurements of PCX4 (1) at two different concentrations of Y-CD (1mM and 22 mM) in acetonitrile and water mixture have been carried out. The relevant portion of mass of PCX4 with Y-CD is shown in Figure 3.16. HR-MS spectra of Y-CD, we observed mass fragment at m/z 1845.02 which is characteristic mass of Y-CD with two pyrene units (from different PCX4s) with their spacer chain of butyric acid and 2H⁺ at low concentration of Y-CD (1 mM). This proves the formation of intermolecular excimer from two pyrene groups of two different PCX4. Along with these peaks, there is strong signal of m/z 1319.41 for [Y-CD +Na⁺]. The obtained m/z values for complex are in good agreement with the calculated values.

While HR-MS data taken for PCX4 with 22 mM Y-CD shows that complex is formed by general formula [PCX4-Y-CD +Na⁺]. It shows that deprotonation of OH groups of ionophore takes place and it gives neutral 1:1 complex at m/z value of 2510.53 that exhibits signal in positive mass spectra with Na. This proves that there is intramolecular excimer formation for the complex PCX4-Y-CD (22 mM). Whatever weak excimer formed, it is due to weak intermolecular interaction of two molecules of

**Figure 3.15:** Variation of normalized intensity of PCX4 (4 μM) at 378 nm with pH a) in the absence and b) in the presence of Y-CD (22 mM).
PCX4. This proves 1:1 (PCX4:γ-CD) complexation for intermolecular excimer formation.

**Figure 3.16**: (A) HR-MS spectra of PCX4(1) with γ-CD (1mM) and inset shows magnified spectra of mass showing fragment of γ-CD π-π interaction with two pyrene units along with their butyric acid chain.

(B) **Figure 3.16** (B) HRMS spectra of PCX4 (1) with γ-CD (22mM) in acetonitrile-water mixture (inset shows magnified spectra of 1:1 complex).
3.11 Conclusion:

In this work, synthesis and characterisation of a new compound calixarene containing pyrene units (PCX4) have been undertaken. Host-guest interaction of PCX4 and cyclodextrin has been investigated. From photophysical studies, it is found that PCX4 forms weak intermolecular excimer up to ~1 mM concentration of \( \gamma \)-CD and strong intramolecular excimer above this concentration. The noncovalent interaction of PCX4 with cyclodextrin leads to significant change in protolytic equilibrium and shows downward pK\(_a\) shift. The low binding constant value indicates that the complex is stabilised by hydrophobic interaction between the probe pyrene with hydrophobic cavity of \( \gamma \)-CD. Such pK\(_a\) shift in neutral species has found great interest in drug delivery and sensor applications. Again increase in excimer emission with \( \gamma \)-CD has been demonstrated which can be applicable in molecular on/off sensor.

3.12 References:

2. A.Dhir; V. Bhalla; M. Kumar; Org. Lett. 2008, 10, 21, 4891-4894.