CHAPTER 2

Materials, Instrumentation and Methods

This chapter provides details of the procurement sources, synthesis and purification of the different materials used in this study. The methods of sample preparation for spectral measurements and microscopic experiments have been described. The instrumentation details, especially the time-resolved confocal fluorescence microscope and time-correlated single photon counting based on picosecond set up have been discussed in detail. The methods of data analysis in FCS and fluorescence lifetime experiments have also been discussed. Various methodologies employed in the present study such as measurement of fluorescence quantum yield of the QDs, determination of the observation volume in an FCS measurement and calculation of the size and concentration of the QDs in solution have also been described.

2.1. Materials:

Laser grade coumarin 102 (C102) dye was purchased from Exciton Inc. and used without further purification. Coumarin 153 (C153) was procured from Eastman Kodak and used as received. Rhodamine 6G (R6G) and disodium hydrogen orthophosphate (Na_2HPO_4) (anhydrous) were obtained from Loba Chemie. Fluorescein (FL) and bovine serum albumin (BSA) (≥ 96%, fatty acid-free) were purchased from Sigma Aldrich and used as received. Sodium dihydrogen orthophosphate (NaH_2PO_4) and urea were purchased from the local suppliers. Sodium chloride (NaCl) and Potassium hydroxide (KOH) were purchased from Merck. Laser grade dye, 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran (DCM), was purchased from Exciton Inc. and used as received. Rhodamine 123 (R123) was purchased from Aldrich and used as received. Azetidine and 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) for the synthesis of 4-azetidinyl-7-nitrobenz-2-oxa-1,3-diazole (4NBD) were purchased from Sigma-Aldrich. [bmim][PF_6], [bmim][BF_4], [emim][Tf_2N], [bmim][Tf_2N], and [hmim][Tf_2N] were of "Advanced Materials Research" grade from Kanto Chemicals (Japan). These ionic liquids were rigorously dried under high vacuum for 48 h prior to use. Solvents such as ethanol (EtOH), acetonitrile (ACN), methanol (CH_3OH) and chloroform ($CHCl_3$) were purchased from Merck and distilled and dried prior to use following standard procedures. Cadmium acetate dihydrate [$(CH_3COO)_2Cd\cdot 2H_2O$] and tellurium (Te) powder for the synthesis of QDs were obtained from local suppliers.

Hexadecylamine (HDA), trioctylphosphine (TOP), 3-mercaptopropionic acid (MPA), octadecene (ODE), 1-Methylimidazole and 11-bromo-1-undecanethiol used for synthesis of task-specific ionic liquid 1-(1-undecanethiol)-3-methyl imidazolium bromide (SMIMBr) were also obtained from Sigma-Aldrich. Unless stated otherwise, all the experiments were carried out at 25°C. Milli-Q water was used in the present study. The purity of the compounds was checked by single spot in thin layer chromatography (TLC) and also by the nuclear magnetic resonance (NMR), UV-vis absorption and emission spectral data.

Various drying agents such as calcium chloride ($CaCl_2$), calcium hydride (CaH_2), phosphorous pentoxide (P_2O_5), iodine (I_2), and magnesium (Mg) turnings used at different stages of solvent purification, hydrochloric acid (HCl) for cleaning Mg turnings and molecular sieves for storage of dried solvents were purchased from local companies.

2.2. Synthesis of molecules and quantum dots:

2.2.1. Synthesis of 4NBD:

NBD was synthesized following a standard procedure,[1] in which 1 mmol of 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) was dissolved in 3 mL of ethyl acetate. 1.2 mmol of azetidine was diluted in 2 mL of ethyl acetate and added dropwise to NBD-Cl solution at 0°C with stirring. After stirring for 30 min at this temperature, the reaction mixture was further stirred for another 2 hrs at room temperature. The product, a red precipitate, was filtered out and purified by column chromatography using a silica gel column. Hexane and ethylacetate were used as eluent for the purification of the compound. The purified compound was recrystallized from absolute ethanol.

2.2.2. Synthesis of SMIMBr:

Task-specific thiol functionalized imidazolium ionic liquid (IL), SMIMBr for the preparation of QD-IL hybrids was synthesized following a reported procedure.[2] 1-methylimidazole, which was distilled from KOH under reduced pressure prior to use, and 11-bromo-1-undecanethiol were taken in 1:1.5 mol ratio. The latter was slowly added to 1-methylimidazole under cooling conditions, and then the reaction was carried out at room temperature (298 K) for 24 hrs under N_2 atmosphere. Light yellow colored salt, SMIMBr,

obtained after the reaction was washed several times with ethylacetate to remove the unreacted starting materials and then dried under high vacuum for several hours.

2.2.3. Synthesis of QDs:

2.2.3.1. CdTe/HDA QDs:

CdTe/HDA QDs were prepared following a reported procedure.[2,3] Briefly, 5 g of HDA and 3 mL of TOP were taken in a two-necked, round-bottom (RB) flask and heated at 80°C for 15 min to bring the mixture to a liquid state. In the meantime, a separate solution was prepared in a reagent bottle containing 0.41 g of $(CH_3COO)_2Cd\cdot 2H_2O$ and 0.16 g of Te powder in 4 mL of TOP. The mixture was sonicated for 1 h to obtain an almost clear solution. This solution was then quickly injected into a RB flask containing HDA and TOP. The reaction was carried out at 180°C for several minutes so that the desired size of the CdTe core is reached. Because the luminescence of the QD depends on its size, the growth of the particle was monitored by its fluorescence at regular intervals of the reaction time. After completion of the reaction, excess starting materials were removed by washing the QDs with methanol followed by repeated precipitation and centrifugation. Then the QDs were dissolved in nonpolar solvents like $CHCl_3$.

2.2.3.2. CdTe/MPA QDs:

Water soluble CdTe/MPA QDs were prepared from CdTe/HDA by ligand exchange method as reported in the previously published literature.[3] 0.5 M methanolic solution of MPA-KOH (20 mol % excess KOH) was added dropwise to a stirring solution of CdTe/HDA QDs in $CHCl_3$ until the QDs flocculate out of the solution. The solution was then centrifuged to separate the precipitate, which was easily soluble in water as the exchange of HDA with MPA made the outer layer of the QDs polar. This transfer of the QDs from $CHCl_3$ to water was carried out under nitrogen environment. Stronger binding capability of the thiol with the surface Cd atoms helped the replacement of HDA by MPA.

2.2.3.3. QD-IL hybrid, CdTe/SMIM:

QD-ionic liquid hybrid CdTe/SMIM was synthesized according to the procedure reported by Santhosh et al.[2] A dilute solution (0.05 M in $CHCl_3$) of SMIMBr was added drop

wise to the $CHCl_3$ solution of CdTe/HDA until the QD was flocculated out of the solution. This solution was then centrifuged and dissolved in [bmim][PF_6].

2.2.3.4. CdTe/ZnS core/shell QDs:

Synthesis of core/shell QDs involve two steps.[4] Firstly, the core QDs were synthesized according to the procedure described earlier. Finally, the shell of few monolayers (typically 1-5) was grown over the core QDs through successive ion layer adsorption and reaction (SILAR) method.[5] In order to prevent self-nucleation of the shell material and uncontrolled Ostwald ripening of the core QDs, it is necessary to maintain a lower temperature for the shell growth compared to that used for the core QD synthesis. In the present study, ZnS shell is grown over CdTe QDs according to the following procedure. 8 mL 7.2 μM $CHCl_3$ solution of CdTe/HDA core QDs, 3 gm of HDA and 10 mL of ODE were taken in two-necked RB flask and kept in vacuum for 2 hours to remove chloroform and then heated to 80˚C for 1 hr to remove residual air. Then the mixture was kept at 160˚C to add the shell precursors. Zinc oleate precursor was prepared by mixing zinc oxide in required amount of oleic acid and 5 mL octadecene in a RB flask and heated at 240˚C in N_2 atmosphere until the solution became clear and then the solution was allowed to cool at 80^0C.[6] Sulphur precursor was prepared by sonicating sulphur powder in 3 mL TOP and 5 mL octadecene for 1 hr. These precursors were injected following SILAR technique[5] i.e. alternate addition of the shell precursors to the vigorously stirring mixture of the core CdTe QDs in the RB flask at 160˚C over a period of 5-10 minutes to form CdTe/ZnS core/shell QD. After the addition was complete, the mixture was monitored by eluting a fraction of the mixture at a certain time interval and comparing its luminescence. Herein, we synthesized 1-monolayer (ML) and 2 ML CdTe/ZnS QDs. On completion of the reaction, excess starting materials were removed by washing the core/shell QD with methanol and then the core/shell QD was dissolved in non-polar solvents like $CHCl_3$. Water soluble CdTe/ZnS/MPA QDs were prepared from CdTe/ZnS/HDA by ligand exchange method described earlier.

2.3. Purification of the conventional solvents:

Conventional solvents used at different stages of the experiments were purified by using standard procedures available in the literature.[7] After drying, molecular sieves were added to

protect the solvents from moisture.

Methanol and ethanol: Initially the solvents were dried over CaH_2 overnight. Further dehydration of the solvents was carried out using Mg-alkoxide. This was prepared by mixing 5 gm of clean dry Mg turnings and 0.5 gm of iodine in the RB flask, followed by 50-75 mL of alcohol and warming the mixture until all the Mg is converted into Mg-alkoxide. After then about 900 mL of alcohol was added slowly to this and refluxed for an hour and then distilled under moisture free atmospheric conditions.

Acetonitrile: Initially the solvent was refluxed for 3-4 hrs with anhydrous P_2O_5 and then distilled under dry conditions.

Chloroform: The solvent was stirred overnight with $CaCl_2$ and then distilled under moisture free conditions.

Ethyl acetate: After stirring with P_2O_5 for 3-4 hrs, the solvent was distilled out under dry atmosphere.

Water: Milli-Q water produced from Millipore, Synergy Pack was used for all the experiments.

2.4. Purification of the RTILs:

The RTILs obtained from Kanto Chemicals (Japan) were stored in a vacuum desiccator under nitrogen atmosphere. Prior to use, all the RTILs were dried under high vacuum (pressure 10^{-2} – 10^{-3} mbar), sometimes with heating at 50-60°C, for at least 8-10 hrs to minimize the water content.

2.5. Sample preparation:

2.5.1. Fluorescence spectral and temporal measurements:

For the steady state and time-resolved fluorescence measurements in conventional solvents and RTILs, the solutions were prepared such that the absorbance of the solution (1 cm pathlength) at the excitation wavelength was around 0.05-0.25, to avoid problems due to inner filter effects. Since RTILs are hygroscopic, care was taken to tightly seal the cuvettes with septum and parafilm.

2.5.2. Transmission electron microscopy (TEM) measurements:

The samples for TEM measurements were prepared by placing a drop of clear solution of the QDs (in H_2O and $CHCl_3$) on carbon-coated copper grids followed by removal of the solvent under high vacuum. The size of the QDs was determined using a Tecnai G2 FE1 F12 transmission electron microscope at an accelerating voltage of 200 kV.

2.5.3. Fluorescence correlation spectroscopy (FCS) measurements:

Samples were diluted for FCS measurements. After then the dilute sample was placed on a coverslip. To study the diffusion in BSA solution and RTILs the concentrations of the fluorophores were maintained at ~10-20 nM and ~35-40 nM, respectively. FCS measurements on QDs were carried out at different concentrations ranging from 20 nM to 400 nM.

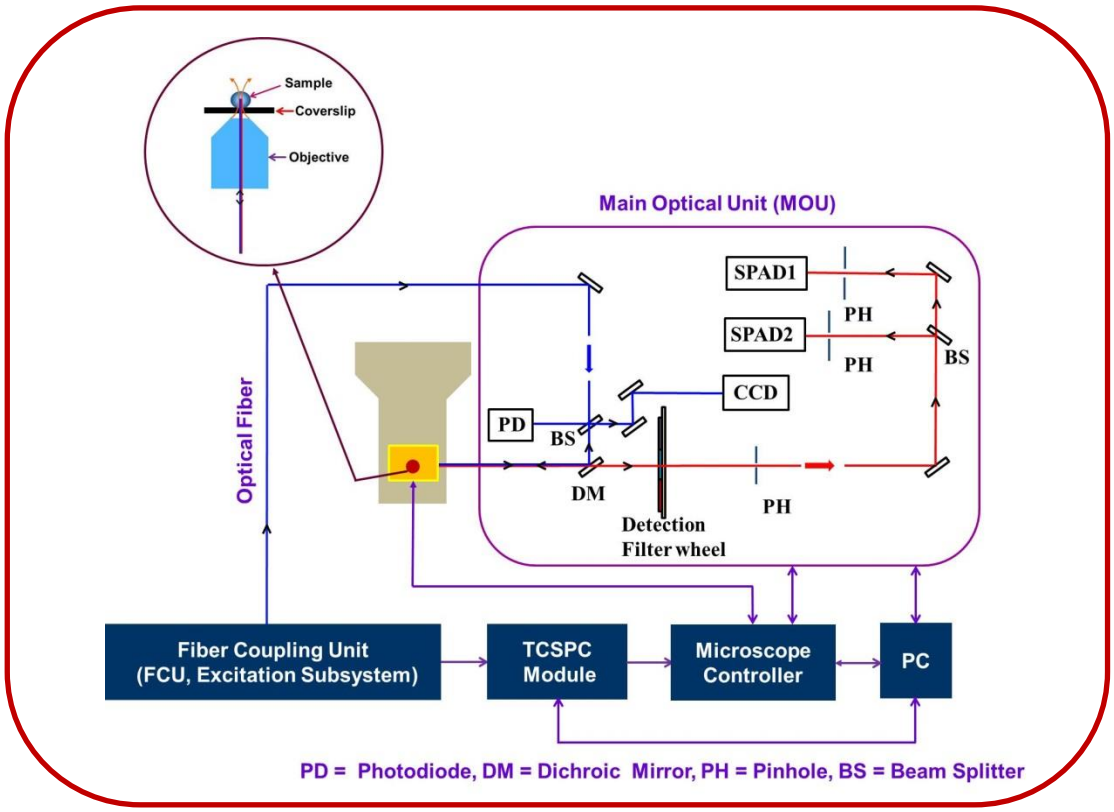
2.6. Instrumentation:

NMR spectra were recorded using Bruker AVACE 400 MHz NMR spectrometer for the characterization of the compounds. Steady-state absorption and fluorescence spectra were recorded on a UV-vis spectrophotometer (Cary100, Varian) and a spectrofluorometer (FluoroLog-3, Jobin Yvon), respectively. The viscosities of the RTILs were measured by a LVDV-III Ultra Brookfield Cone and Plate viscometer (accuracy: 1% and repeatability: 0.2%). Tecnai G2 FE1 F12 transmission electron microscope at an accelerating voltage of 200 kV were used to examine the size and shape of the QDs. EDX spectra were captured in the transmission electron microscope mentioned above Equipped with an energy dispersive X-ray spectrometer. Photoirradiation of the QDs was carried out using a 8 W fluorescent tube lamp (FL8 D daylight, Toshiba) for different exposure time prior to recording the absorption and fluorescence spectra. Intensity of this exposure is 5 mW/cm^2. The details of the other instrumental set up employed in the present study are given in the following section.

2.6.1. Time-resolved confocal fluorescence microscope:

FCS measurements were carried out using a time-resolved confocal fluorescence microscope (MicroTime 200, PicoQuant). An inverted microscope (Olympus IX71) Equipped with a water immersion objective (UPlansApo NA 1.2, 60 X) served as the microscope body (Scheme 2.1). The samples were excited at 485 nm and 405 nm using pulsed diode lasers with full width half maximum of 176 ps (405 nm) and 144 ps (485 nm) respectively. The pulse repetition rate of the laser was 20 MHz. The output of this pulsed

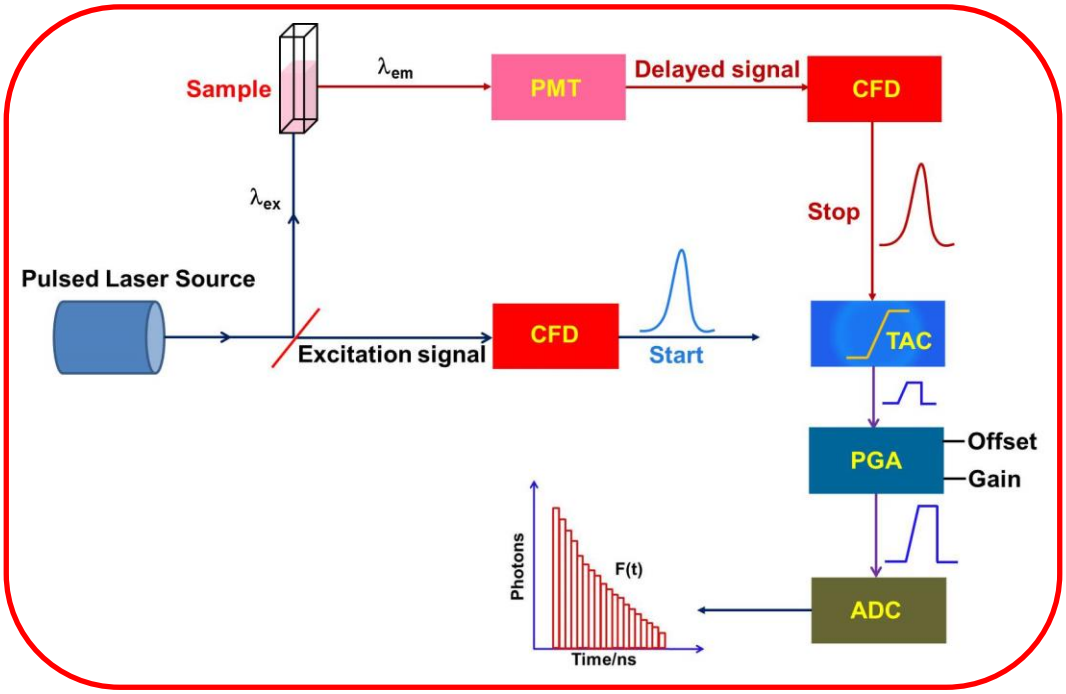
diode laser was coupled to the main optical unit by a polarization maintaining single mode optical fiber. In the main optical unit the excitation light was guided through a dichroic mirror, which reflected the excitation light onto the entrance port of the microscope. The sample was placed on a cover-slip and the directed laser beam was focused onto the sample using the water immersion objective. Fluorescence was collected by the same objective, passed through a dichroic mirror, 430 nm long pass filter (for 405 nm) or 510 nm long pass (for 485 nm) to block the excitation light. After then the signal was spatially filtered by focusing onto a 50 µm diameter pinhole to cut the out-of-focus signals, re-collimated, and directed onto a (50/50) beam splitter prior to entering into two single photon avalanche photodiodes (SPADs). The fluorescence correlation traces were generated by cross-correlating signals from the two SPAD detectors. The data acquisition was performed with PicoHarp 300 TCSPC module in a time-tagged time-resolved (TTTR) mode.



Scheme 2.1. Schematic diagram of the time-resolved confocal fluorescence microscope set up (adapted from PicoQuant MicroTime 200 user manual).

2.6.2. Time-correlated single photon counting setup:

Fluorescence lifetime measurements were carried out using a time-correlated single-photon counting (TCSPC) spectrometer (Horiba Jobin Yvon IBH). Nano LED (λ_{ex}= 439 nm, FWHM = 150 ps) was used as the excitation source and a MCP photomultiplier (Hammatsu R3809U-50) as the detector. The pulse repetition rate of the laser source was 1 MHz. The TCSPC experiment starts with the excitation pulse that simultaneously excites the samples and sends a signal to the electronics (Scheme 2.2). This excitation signal is received by a constant fraction discriminator (CFD), which accurately measures the arrival time of the pulse and then diverts the signal towards the time to amplitude convertor (TAC) to start the voltage ramp. This voltage ramp is a voltage that increases linearly with time on the nanosecond timescale. The second channel (CFD) which accurately measures the arrival time of the emitted photon sends a signal to stop the voltage ramp in TAC. This voltage in TAC is proportional to the time delay (Δt) between the excitation and emission signals. This voltage is amplified by a programmable gain amplifier (PGA) and later converted to a numerical value by the analog-to-digital converter (ADC). This numerical value is stored as a single event with the measured time delay. A histogram of the fluorescence intensity decay can be constructed by repeating this process numerous times with a pulsed-light source. The lamp profile was recorded by placing a dilute solution of Ludox in water as a scatterer (dilute solution of Ludox in water) in place of the sample. The analysis of the fluorescence decay curves is covered in the later section.



Scheme 2.2. Schematic diagram of the TCSPC setup

2.7. Measurement of Fluorescence Quantum Yield:

Fluorescence quantum yields (QYs) of the CdTe core and CdTe/ZnS core/shell QDs were determined by comparing the integrated emission of the QDs in $CHCl_3$ and H_2O to the emission of a solution of C153 in acetonitrile (QY=0.56)[8] of identical optical density at the excitation wavelength by using the following Equation:

$$QY\ (QD) = QY\ (C153) \times (I/I_R) \times (OD_R/OD) \times (n^2/n_R^2) \qquad (2.1)$$

R indicates here reference, I is the integrated fluorescence intensity, OD is optical density at the excitation wavelength and n is the refractive index of the solvent. OD was kept at less than 0.05.

2.8. Data Analysis:

2.8.1. FCS Measurements:

Data analysis of the individual correlation curves was performed by using the SymPhoTime software of PicoQuant. Following models were used to fit the correlation functions (defined in Equation 1.3). The quality of the fit was judged by the residuals and χ^2 values.

(1) 3-D diffusion with triplet state contribution:

$$G(\tau) = 1 - T + T\exp\left(-\frac{\tau}{\tau_{tr}}\right)\sum_{i=1}^{k}\rho_i\left(1+\frac{\tau}{\tau_i}\right)^{-1}\left(1+\frac{\tau}{\tau_i\kappa^2}\right)^{-\frac{1}{2}} \tag{2.2}$$

Where, ρ_i is given by,

$$\sum_{i=1}^{k}\rho_i = \frac{1}{\langle N\rangle(1-T)} \tag{2.3}$$

Which implies,

$$\rho_i = \frac{\alpha_i}{\langle N\rangle(1-T)} \tag{2.4}$$

In the above expressions, the diffusion time, τ_i, denotes the average time a dye molecule resides in the confocal volume, τ_{tr} is the lifetime of the triplet state of the molecule, τ is the delay or lag time, N is the average number of fluorescent molecules in the observation volume, T is the fraction of the molecules in the triplet state, α_i is the fraction of the molecules having diffusion time, τ_i. Here $i = 1$ and 2 corresponds to a 1-component a 2-component diffusion model respectively. κ is the structure parameter of the observation volume and is given by $\kappa = r_0/z_0$, where, z_0 and r_0 are the longitudinal and transverse radii, respectively, of the observation volume (Scheme 1.3). τ_i is related to r_0 by the following Equation

$$\tau_i = \frac{r_0^2}{4D_t} \tag{2.5}$$

Here, D_t is the diffusion coefficient of the fluorescent probes.

(2) Simple 3-D diffusion model:

$$G(\tau)=\frac{1}{N}\sum_{i=1}^{n}\frac{\alpha_i}{\left(1+\frac{\tau}{\tau_i}\right)\left(1+\frac{\tau}{\kappa^2\tau_i}\right)^{\frac{1}{2}}} \qquad (2.6)$$

$i=1$ and 2 correspond to 1- and 2-component diffusion model, respectively.

(3) 1-component 3-D diffusion model with a stretched exponential term:

$$G(\tau)=\left[1+\frac{T}{1-T}\exp\left(-\frac{\tau}{\tau_t}\right)^{\beta}\right]\frac{1}{N}\left(1+\frac{\tau}{\tau_D}\right)^{-1}\left(1+\frac{\tau}{\kappa^2\tau_D}\right)^{-\frac{1}{2}} \qquad (2.7)$$

Where, τ_D is the diffusion time of the QDs. N is the average number of molecules undergoing reversible transition between on and off state in the observation volume. T is fraction of the off state. τ_t is the dark state relaxation time, β is the stretching exponent with value between 0 and 1, and this is related to the distribution of τ_t.

2.8.2. TCSPC measurements:

Fluorescence decay curves were analyzed by nonlinear least-squares iteration procedure using IBH DAS6 (Version 2.2) decay analysis software. The quality of the fit was assessed by the χ^2 values and the distribution of the residuals.

2.9. Determination of the observation volume in the FCS measurements:

FCS can measure absolute diffusion coefficients and concentration values only when the exact size and shape of the observation volume is known. The diffusion time (τ_D) depends on the transverse radius (r_0) of the observation volume (Equation 1.15). The size and elongation of the observation volume is determined by calibration measurement with a known diffusion coefficient. In the present study, calibration was performed using aqueous solution of rhodamine 6G and rhodamine 123 with known diffusion coefficients of 426 and 460 $\mu m^2/s$ respectively.[9,10] The estimated excitation volume was found to be ~0.8 fL for 485 nm excitation and ~0.4 fL for 405 nm excitation.

2.10. Estimation of size and concentration of the CdTe QDs in solution:

The concentrations of the QDs in the solution were calculated following the method suggested by Yu et al.[11] First, the diameter (D, nm) of the CdTe QDs was estimated from the following Equation:

$$D_{CdTe} = \left(9.8127 \times 10^{-7}\right)\lambda^3 - \left(1.7147 \times 10^{-3}\right)\lambda^2 + \left(1.0064\right)\lambda - 194.84 \qquad (2.8)$$

Where, λ (nm) is the wavelength corresponding to the first excitonic absorption peak. The calculated D values correlated well with the experimental values obtained from the TEM measurements. The D value was used to find out the molar extinction coefficients (ε) of CdTe QDs using the following relation

$$\varepsilon_{CdTe} = 10043\left(D\right)^{2.12} \qquad (2.9)$$

The concentration (C) of the QDs in solution was then estimated using the relation,

$$C = \frac{A}{\varepsilon l} \qquad (2.10)$$

Where, A is the optical density and *l* is the optical path length of the solution.

2.11. Calculation of the amount of the shell precursors required for the preparation of core/shell QDs:

The amounts of shell precursors required to grow a shell of desired thickness were calculated according to a published report.[4] In order to grow a shell of certain thickness one needs to know the concentration of the core QDs. The concentration of the core QD was calculated by first estimating the molar extinction coefficient from the measured size of the QD using the empirical formula of Peng and co-workers,[11] and then using the measured optical density of the solution. With the knowledge of the QD's size and molar quantity, the required amount of shell precursors to obtain a shell thickness of x monolayers (CD) on the surface of the core QDs AB can be calculated, using the bulk crystal parameters of the shell materials as follows

$$V_{CD}\,(ML_x) = 4/3 \times \pi \times ((r_{AB} + x \times d)^3 - r_{AB}^3) \qquad (2.11)$$

$$n_{CD}(ML_x) = \rho_{CD} \times V_{CD}(ML_x) \times 10^{-27} / m_{CD} \quad (2.12)$$

$$n_{CD} = n_{AB} \times n_{CD}(ML_x) \quad (2.13)$$

V_{CD} (ML_x) is the volume of the shell containing x monolayers (nm^3), r_{AB} is the radius of the core (nm) and d is the thickness of one monolayer of the shell (nm). n_{CD} (ML_x) is the number of CD monomer units per nanocrystal contained in x monolayers of the shell (dimensionless), ρ_{CD} is the density of the bulk shell material (kg m^{-3}), m_{CD} is the mass of a shell monomer unit (kg), n_{CD} is the molar quantity of precursor CD needed for the growth of x monolayers (mmol), and n_{AB} is the molar quantity of core QDs used for the synthesis of the core shell system. The term monomer indicates smallest subunit of the shell material consisting of one cation and one anion.

2.12. Standard error limits:

Standard error limits involved in the experimental results were

λ_{max} (abs./flu.)	± 1 nm
Φ_f	± 10%
τ_f	± 5%
Viscosity	± 2%
QD size (D / nm)	± 5 – 10%

The error limits of other quantities related to FCS measurements are given in the subsequent chapters while reporting the experimental results.

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