Chapter V

Synchronous Fluorescence Scan (SFS) Spectroscopy
study of CdSe and CdSe/ZnS QDs
Synchronous Fluorescence Scan (SFS) Spectroscopy study of CdSe and CdSe/ZnS QDs

V.1 Introduction

The use of novel nanomaterials has gained considerable attention by the scientific community over the past decade [1-4]. The applications of these various nano-scale materials continue to grow and their potential to beneficially impact the medical and technological world is seemingly boundless [5-10]. However, with increased manufacturing of these diverse nanoparticles worldwide, comes an urgent need for investigations regarding their toxicity - to those involved in their production, to patients who may be treated with such particles in medicine and also to humans and the environment should these nanoparticles be exposed to our waterways, air, soil, and other surroundings [11-14]. The fact that these questions have rarely been raised poses serious concern. The realization that these materials may pose a threat, and that exploration of the subject is sorely lacking, has only just come into popular attention. Therefore determination and characterization of such nanocrystals in water and the environment becomes important [15-17].
Synchronous Fluorescence Scan (SFS) spectroscopy is one of the most powerful techniques for multi-component analysis of QDs mixture solutions without resorting to tedious separation procedures and is extremely useful for routine analysis. Fluorescence spectrum of a mixture of different sized QDs is usually broad due to spectral overlap from various sizes of the QDs. Therefore conventional single-wavelength fluorescence measurement is limited in its ability to analyze complicated multi-component mixture samples due to the overlapping emission and/or excitation spectra. Fortunately this can be overcome by using SFS method.

In conventional fluorescence spectroscopy, two types of spectra are generally measured. When a sample is excited at a fixed wavelength ($\lambda_{ex}$), an emission spectrum is produced by recording the emission intensity as a function of the emission wavelength ($\lambda_{em}$). An excitation spectrum may be obtained when $\lambda_{ex}$ is scanned while the observation is conducted at a fixed $\lambda_{em}$. The broad nature and spectral overlap of conventional fluorescence spectra can be overcome, and enhanced selectivity can be obtained using synchronous fluorescence spectroscopy (SFS). In SFS, the $\lambda_{ex}$ and $\lambda_{em}$ are scanned simultaneously. Depending on the scan rate three basic types of SFS technique are possible [18]. Constant-wavelength SFS is very simple technique as the scan rate is constant for both monochromators and, therefore, a constant wavelength interval, $\Delta \lambda$, is kept between $\lambda_{em}$ and $\lambda_{ex}$. Second technique is known as the variable-angle SFS. The excitation and emission wavelengths may be varied simultaneously but at
different rates. The third technique, constant-energy SFS, has not been used much. SFS is often considered as a convenient technique for the analysis of multi-component samples without resorting to tedious separation procedures [19-23].

The sharpness and narrowness of the peak of a SFS spectrum, compared to those of conventional spectrum, may be explained with reference to a simplified Jablonski diagram (Fig. 5.1). A molecule can be excited in the whole absorption band starting from wavelengths \( A_1, A_2, \ldots, A_9 \) and could give fluorescence at wavelengths \( F_1, F_2, \ldots, F_9 \). Generally, the fluorescence emission spectrum of a fluorophore remains unchanged, irrespective of the excitation wavelength, except for a variation in the fluorescence intensity, which depends on the probability of the electronic transition of the molecule. To get a fluorescence emission spectrum, the molecule is generally excited at its absorption maximum \( (A_5) \) and fluorescence is collected in all the emission wavelengths, i.e., \( F_1, F_2, F_3, \ldots, F_9 \).

A fluorescence excitation spectrum is obtained by exciting the molecule at all possible excitation wavelengths, e.g., \( A_1, A_2, \ldots, A_9 \), and collecting the fluorescence only at the emission maximum \( (F_5) \). But, in the case of SFS, a particular wavelength interval is chosen, so that a signal is observed only when \( \Delta \lambda \) matches the interval between an absorption band and an emission band. Therefore, initially, e.g., taking \( \Delta \lambda = A_5 - F_5 \), we will not see any fluorescence until the excitation monochromator is at \( A_5 \) and fluorescence wavelength is at \( F_5 \). In the next moment, the molecule will be excited at \( A_6, A_7, \ldots, A_9 \) and
Figure 5.1: Jablonski Diagram provide explanation of synchronous fluorescence scan
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corresponding fluorescence will be recorded at $F_6$, $F_7 \ldots \ldots F_9$, respectively. This process continues till a full spectrum is recorded. As the emission intensity is a function of the excitation wavelength (which is related to the probability/population of transition), and $\Delta \lambda$ defines the matching of absorption and emission band, we obtain a sharper peak in SFS compared to a conventional spectrum [17]. The combination of synchronous and derivative fluorometry enhances minor spectral features and allows more reliable identification of chemical species [17, 23, 24].

The aim of the present study is to assess the potential of synchronous fluorescence scanning spectroscopy using multivariate data analysis methods to differentiate mixed cluster of different size CdSe and CdSe/ZnS QDs samples.

V.2 Materials and methods

V.2.1 Quantum dots

CdSe (480, 520, 560, 590 nm) core and CdSe/ZnS (530, 560, 590, 610 nm) core-shell QDs were purchased from Sigma-Aldrich (USA) and used as received. To prepare the stock solutions a known amount of the QDs was dissolved in a desired volume of HPLC grade Toluene solvent. The individual reference QDs solution and the mixture of the QDs samples were prepared from the respective stock solution by taking different QDs of various sizes. The spectroscopic measurements were performed on the fresh samples at room temperature.
V.2.2 Instrumentation

Absorbance measurements were done using a uv/vis Spectrophotometer (Hitachi, Model U-2800). Fluorescence measurements were obtained employing spectrofluorimeter (JOBIN YVON Horiba Fluoromax-4). The excitation and emission slits width were fixed at 2 nm. The excitation source was a 100W Xenon lamp. The detector used was R-928 operating at a voltage of 950 V. The synchronous fluorescence scan (SFS) spectra were measure at $\Delta \lambda = 10$ nm, 25 nm, 50 nm, 75 nm, 100 nm, 125 nm and 150 nm for the analytical technique development, because of its high sensitivity (SFS intensity) in the excitation wavelength range $\lambda_{ex} = 250$-700 nm. The excitation emission matrix fluorescence (EEMF) spectra were collected in the excitation wavelength range $\lambda_{ex} = 250$-50 nm and in the emission wavelength range $\lambda_{em} = 400$-700 nm within an interval of 4 nm. The spectral data were collected using Fluorescence software and OriginPro 8.0 software was used for further data analysis.

V.3 Results and discussion

Fig. 5.2 shows the absorption spectrum of CdSe and CdSe/ZnS QDs various size from blue to redder region. Because of the size quantization effect, the absorption is shifted to lower energies with a maximum at around 440 nm (for CdSe 480), 485 nm (for CdSe 520), 526 nm (for CdSe 560) and 557 nm (for CdSe 590), similarly for CdSe/ZnS QDs maximum occurred at around 510 nm (for CdSe/ZnS 530), 540 nm (for CdSe/ZnS 560), 570 nm (for CdSe/ZnS 580) and 600 nm (for CdSe/ZnS 610). Likewise fluorescence spectra of these QDs also showed a
Figure 5.2: Absorption and Fluorescence spectra of various size (A) CdSe and (B) CdSe/ZnS QDs
red shift as the particle size increased. Notice from the Fig. 5.2 that high extinction coefficient of these QDs at shorter wavelength range irrespective of their sizes. This results in higher fluorescence intensity in the excitation emission matrix fluorescence (EEMF) spectra of these QDs and their mixtures as shown in Fig. (5.3 & 5.4). In the mixture sample, the contour map presented a broad emission wavelength region in the range $\lambda_{em} = 500$-700 nm for the whole excitation wavelength range $\lambda_{ex} = 250$-500 nm, which makes it difficult to identify the individual QDs from EEMF spectra.

Even with the possibility to select both the excitation and emission wavelengths, conventional fluorescence methods have limited practical applicability for simultaneous determination of the QDs. Observing the Fig 5.5. Emission spectrum of mixture samples cannot be resolved adequately. For individual component analysis, mixture sample needs pre-separation of all the solutes. However, this can be overcome by using some special techniques, such as SFS [20]. In SFS the choice of appropriate scanning interval ($\Delta \lambda$) is mainly dictated by the different spectra requirements of resolution and sensitivity. For selecting the appropriate $\Delta \lambda$, the spectra have been recorded for four different sizes of CdSe and CdSe/ZnS QDs at $\Delta \lambda = 10$ nm, 25 nm, 50 nm, 75 nm, 100 nm, 125 nm and 150 nm. The best $\lambda_{SFS}^{max}$ have been obtained for CdSe 480 at $\Delta \lambda = 10$ nm, CdSe 520 at $\Delta \lambda = 125$ nm, CdSe 560 at $\Delta \lambda = 150$ nm and CdSe 590 at $\Delta \lambda = 100$ nm (Fig. 5.6), similarly for CdSe/ZnS 530 at $\Delta \lambda = 75$ nm, CdSe/ZnS 560 at $\Delta \lambda = 150$ nm, CdSe/ZnS 590 at $\Delta \lambda = 150$ nm and CdSe/ZnS 610 $\Delta \lambda = 150$ nm (Fig. 5.7).
Figure 5.3: Excitation Emission Matrix Fluorescence (EEMF) spectra of (A) CdSe 480, (B) CdSe 520
Figure 5.3: Excitation Emission Matrix (EEMF) fluorescence spectra of (C) CdSe 560, (D) CdSe 590 and (E) their mixtures
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Figure 5.4: Excitation Emission Matrix (EEMF) fluorescence spectra of (A) CdSe/ZnS 530, (B) CdSe/ZnS 560 QDs

**Figure 5.4:** Excitation Emission Matrix (EEMF) fluorescence spectra of (A) CdSe/ZnS 530, (B) CdSe/ZnS 560 QDs
Figure 5.4: Excitation Emission Matrix Fluorescence (EEMF) spectra of various QDs of different sizes (C) CdSe/ZnS 590, (D) CdSe/ZnS 610 and (E) their mixtures
Figure 5.5: Synchronous fluorescence scan spectra of different sizes of (A) CdSe, (B) CdSe/ZnS QDs and their respective mixture at $\Delta \lambda = 150$ nm.
Figure 5.6: Synchronous fluorescence scan (SFS) spectra of the various CdSe QDs of different sizes at $\Delta \lambda = 10$ nm, 25 nm, 50 nm, 75 nm, 100 nm, 125 nm and 150 nm. (A) CdSe 480, (B) CdSe 520,
Figure 5.6: Synchronous fluorescence scan (SFS) spectra of the various CdSe QDs of different sizes at \( \Delta \lambda = 10 \text{ nm}, 25 \text{ nm}, 50 \text{ nm}, 75 \text{ nm}, 100 \text{ nm}, 125 \text{ nm} \) and 150 nm. (C) CdSe560, (D) CdSe 590.
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Figure 5.7: Synchronous fluorescence scan (SFS) spectra of the various CdSe/ZnS QDs of different sizes at $\Delta \lambda = 10$ nm, 25 nm, 50 nm, 75 nm, 100 nm, 125 nm and 150 nm. (A) CdSe/ZnS 530, (B) CdSe/ZnS 560.
Figure 5.7: Synchronous fluorescence scan (SFS) spectra of the various CdSe/ZnS QDs of different sizes at $\Delta \lambda = 10$ nm, 25 nm, 50 nm, 75 nm, 100 nm, 125 nm and 150 nm. (C) CdSe/ZnS 590, (D) CdSe/ZnS 610
This can be easily explained by taking into consideration that these QDs are characterized by a strong excitation spectral band in uv region and a $\Delta \lambda$ of above mentioned value is used for this study, so as to obtain the maximum signal.

In semiconductor quantum dots, there are two kinds of emission bands, the first one is the band edge or near band edge emission, which is size dependent and excitation wavelength independent in certain wavelength range. The second one is the deep trap emission and surface state emission in the longer wavelength range and is less size-dependent. For CdSe 480 nm QDs the $\lambda_{\text{sfs max}}$ spectrum showed a broad red tail (Fig.5.6 [A]), which is present in the single wavelength fluorescence /conventional fluorescence emission spectrum also. We presume here that the surface states of CdSe QDs play a major role. CdSe QDs with their surface geometry where shift of surface states maintain the interior character of the absorption mechanism, often exhibit fluorescence spectra with red tail towards longer wavelength, the so called deep trap emission [25]. The synchronous fluorescence scan technique allows the stronger peaks to be increased selectively by the use of suitable $\Delta \lambda$ [26-29]. Hence, for the CdSe 480 QDs the surface state emission or deep trap emission at the offset of $\Delta \lambda = 10 - 100$ nm was less pronounced and even disappeared at $\Delta \lambda = 125$ and150 nm.

Further, ZnS capped CdSe core QDs has been found to significantly enhance the synchronous fluorescence intensity (Fig. 5.7[A-D]), the capping of ZnS effectively passivates the surface states and suppresses the non-radiative recombination at surface vacancies, leading to enhanced intensity [30-37].
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In a mixture sample, quantification of contribution of individual QDs to the SFS intensity cannot be overcome completely, because spectral overlap of different size QDs results in difficult to recognize/distinguish quantity of contribution. However, the observed SFS can still be improved and increased by taking the derivatives of the SFS spectrum. The combination of SFS and its derivative spectrum is more advantageous in terms of sensitivity in simultaneous identification and estimation of complex mixtures, because the amplitude of the derivative signal is inversely proportional to the band width of the original spectrum [38, 39].

Therefore, to avoid the spectral overlap of the different QDs, we applied second derivative SFS spectra for determination of individual QDs. The second derivative spectra were obtained by mathematical treatment of the data (OrignPro). The second derivative spectra of each sample of CdSe and CdSe/ZnS QDs show two maxima and a minimum (Fig. 5.8 & 5.9). With this maxima and minimum can be used for identification of the individual component [40]. In the second derivative spectra noise levels can be expected to be high, therefore identification of individual band of QDs might be difficult to distinguish from the noise level in similar derivative spectrum unless special care is taken during data analysis [41].

In this study the first maximum (maximum in the lower wavelength range) is for the identification of each component. From Fig. 5.8 & 5.9, one can observe precisely each of CdSe and CdSe/ZnS QDs with their respective clearly resolved
Figure 5.8: Second derivative synchronous fluorescence scan spectra for the identification of CdSe (A: 480, B: 520, C: 560 nm) QDs and the mixture of all CdSe QDs
Figure 5.8: Second derivative synchronous fluorescence scan spectra for the identification of CdSe (D: 590 nm) QDs and the mixture of all CdSe QDs; (E) increased concentration of CdSe 590 QDs in mixture solution.
Figure 5.9: Second derivative synchronous fluorescence scan spectra for the identification of CdSe/ZnS (A: 530 nm, B: 560 nm) QDs and in the mixture of all CdSe/ZnS QDs.
Figure 5.9: Second derivative synchronous fluorescence scan spectra for the identification of CdSe/ZnS (C: 590 and D: 610 nm) QDs and in the mixture of all CdSe/ZnS QDs
mixture sample. CdSe(480, 520, 560 and 590 nm) shows the first SD^max_SFS at 468, 503, 549 and 588 nm, respectively. Likewise, CdSe/ZnS (530, 560, 590 and 610 nm) shows SD^max_SFS at 514, 553, 592 and 617 nm, respectively. Very poor identification for CdSe 590 in the mixture sample is due to the poor signal to noise ratio at this wavelength region. If we increase the concentration of CdSe 590 QDs in the mixture, it will lead to the energy transfer from the small size to larger size (Fig. 5.8[E]). This energy transfer can easily be overcome by diluting the solution to the μg mL⁻¹ concentration range. Our results are supported by the recent observations made by Patra et. al., [37], who reported the size dependent analysis of CdTe QDs and their mixture using Synchronous Fluorescence Scan spectroscopy along with second derivative in identifying the individual QDs in mixture.

The second derivative peaks for the respective QDs are also shown in Table 1 and were used for the identification of these QDs in their mixture samples for simultaneous analysis. With these results constructing the calibrated curve for determining the individual QDs in mixture, by measuring the synchronous fluorescence intensity at synchronous fluorescence maxima at their respective Δλ for different concentrations will give linear dynamic range of mixed QDs (Table 2).
Table 5.1: photophysical properties of various size CdSe and CdSe/ZnS quantum dots.

<table>
<thead>
<tr>
<th>Quantum Dots</th>
<th>( \lambda_{\text{fi}}^{\text{max}} ) (nm)</th>
<th>( \lambda_{\text{Abs}}^{\text{max}} ) (nm)</th>
<th>Size (nm)</th>
<th>( \lambda_{\text{SFS}}^{\text{max}} ) (nm)</th>
<th>SD ( \lambda_{\text{SFS}}^{\text{max}} ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdSe 480</td>
<td>480</td>
<td>440</td>
<td>2.1</td>
<td>467</td>
<td>470</td>
</tr>
<tr>
<td>CdSe 520</td>
<td>520</td>
<td>485</td>
<td>2.5</td>
<td>512</td>
<td>502</td>
</tr>
<tr>
<td>CdSe 560</td>
<td>560</td>
<td>526</td>
<td>3.3</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>CdSe 590</td>
<td>590</td>
<td>557</td>
<td>4.0–4.3</td>
<td>585</td>
<td>579</td>
</tr>
<tr>
<td>CdSe/ZnS 530</td>
<td>530</td>
<td>510</td>
<td>3.3</td>
<td>520</td>
<td>514</td>
</tr>
<tr>
<td>CdSe/ZnS 560</td>
<td>560</td>
<td>540</td>
<td>3.4</td>
<td>546</td>
<td>554</td>
</tr>
<tr>
<td>CdSe/ZnS 590</td>
<td>590</td>
<td>570</td>
<td>4.0</td>
<td>584</td>
<td>592</td>
</tr>
<tr>
<td>CdSe/ZnS 610</td>
<td>610</td>
<td>580</td>
<td>5.2</td>
<td>608</td>
<td>616</td>
</tr>
</tbody>
</table>
Table 5.2: Linear dynamic range and calibration curve obtained for simultaneous estimation of CdSe and CdSe/ZnS QDs.

<table>
<thead>
<tr>
<th>Quantum dots</th>
<th>LDR (µg/ml)</th>
<th>( y = mx + c )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( m )</td>
</tr>
<tr>
<td>CdSe 480</td>
<td>1-3</td>
<td>( 8.44 \times 10^6 )</td>
</tr>
<tr>
<td>CdSe 520</td>
<td>3-8</td>
<td>( 6.43 \times 10^6 )</td>
</tr>
<tr>
<td>CdSe 560</td>
<td>3-9</td>
<td>( 9.12 \times 10^6 )</td>
</tr>
<tr>
<td>CdSe 590</td>
<td>2-8</td>
<td>( 1.92 \times 10^6 )</td>
</tr>
<tr>
<td>CdSe/ZnS 530</td>
<td>1-3</td>
<td>( 1.09 \times 10^6 )</td>
</tr>
<tr>
<td>CdSe/ZnS 560</td>
<td>3-8</td>
<td>( 7.43 \times 10^6 )</td>
</tr>
<tr>
<td>CdSe/ZnS 590</td>
<td>3-9</td>
<td>( 3.40 \times 10^6 )</td>
</tr>
<tr>
<td>CdSe/ZnS 610</td>
<td>2-8</td>
<td>( 6.75 \times 10^6 )</td>
</tr>
</tbody>
</table>
V.4 Conclusion

Fluorescence spectroscopy is simple, non-destructive, non-invasive and relatively inexpensive analytical method, which can be used for the analysis of fluorescent compounds at very low concentration levels, because fluorescence is very sensitive to the microenvironment of the sample and interference from external quencher molecules and photo-physical processes e.g., ground state complex formation, excimer formation, exciplex formation, Forster energy transfer, self quenching, etc. Diluting the sample to a very low level may overcome such problem.

A new simple and sensitive method was explored for the simultaneous determination of QDs and its binary mixtures. The proposed SFS method allows the selective determination of each QDs in presence of other without any interference proving its selectivity and ability to resolve a mixture of the two or more QDs without prior separation steps. Moreover, the proposed method is time saving. The synchronous spectrofluorometric method, by virtue of its high sensitivity, could be applied for the investigation of the interaction between QDs and biomolecules, estimating the effect of the concentration of composite materials, coexisting substances can be studied.
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V.5 REFERENCES

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