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Inner filter effect and the onset of concentration dependent red shift of synchronous fluorescence spectra

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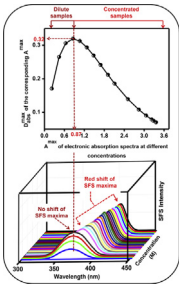
HIGHLIGHTS

- Concentration dependent red shift (CDRS) of Synchronous Fluorescence (SF) Spectra for mono and multi-fluorophoric systems.
- CDRS starts when derived absorption spectral parameter reaches 0.32 which corresponds to the absorbance value of 0.87.
- Correspondence of wavelength of derived absorption spectral maximum with wavelength of SF spectral maximum.

GRAPHICAL ABSTRACT



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ABSTRACT

The phenomenon of concentration dependent red shift, often observed in synchronous fluorescence spectra (SFS) of monofluorophoric as well as multifluorophoric systems at high chromophore concentrations, is known to have good analytical advantages. This was previously understood in terms of large inner filter effect (IFE) through the introduction of a derived absorption spectral profile that closely corresponds to the SFS profile. Using representative monofluorophoric and multifluorophoric systems, it is now explained how the SF spectral maximum changes with concentration of the fluorophore. For dilute solutions of monofluorophores the maximum is unchanged as expected. It is shown here that the onset of red shift of SFS maximum of both the mono as well as the multifluorophoric systems must occur at the derived absorption spectral parameter value of 0.32 that corresponds to the absorbance value of 0.87. This value is unique irrespective of the nature of the fluorophore under study. For monofluorophoric systems, the wavelength of derived absorption spectral maximum and the wavelength of synchronous fluorescence spectral maximum closely correspond with each other in the entire concentration range. In contrast, for multifluorophoric systems like diesel and aqueous humic acid, large deviations were noted that could be explained as to be due to the presence of non-fluorescing chromophores in the system. This work bridges the entire fluorophore concentration range over which the red shift of SFS maximum sets in; and in the process it establishes the importance of the derived absorption spectral parameter in understanding the phenomenon of concentration dependent red shift of SFS maximum.

1. Introduction

A notable shift of synchronous fluorescence (SF) spectral maximum at higher concentrations of fluorophores has earlier been observed in many cases during analysis of multifluorophoric

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systems [1–3]. This SF spectral shift, generally termed as 'concentration dependent red shift' (CDRS), has been explored for various analytical applications involving multifluorophoric systems like petroleum products and humic acids [1–9]. Prior to the report by Divya et al. [6] it was generally thought that the original concentration dependent red shift lies in various factors like energy transfer, quenching, primary and secondary inner filter effects [1,2,4,5]. The work by Divya et al. [6] showed that the most important reason for the observed CDRS is primary inner filter effect and the contributions of other effects are less significant. Using a derived absorption spectral parameter introduced by them, they were able to predict the change in synchronous fluorescence spectral maximum with concentration using UV-VIS spectrophotometer.

In conventional fluorimetric set up that uses right angle geometry configuration for fluorescence measurements, the magnitude of inner filter effect is fairly sensitive to the absorbance at the excitation wavelength [10,11]. In order to ensure an appreciable linearity of fluorescence intensity with concentration, working with dilute solutions are generally recommended such that the absorbance value at the excitation wavelength is less than 0.1 [10–12]. The literature is replete with various suggestions of handling inner filter effect at absorbance value more than 0.1 [13–19].

It is important to note that the observation of CDRS of SFS is due to the shift of the long wavelength absorption band-edge with concentration [6]. In contrast, in dilute solutions where absorbance at λ_{abs}^{max} is low, no shift in SF spectral maximum is expected and the CDRS would be absent. Thus it would be interesting to carry out a systematic study across a wide concentration range of fluorophoric solutions in order to observe and understand the onset of CDRS.

To the best of our knowledge, this important study bridging the fluorophore concentration from very dilute to concentrated range has not been carried out so far. The major objective of the present work is to examine this and to see the relevance of the 'derived absorption spectral parameter' $A_{\lambda_x} \cdot 10^{-(A_{\lambda x}/2)}$ ($A_{\lambda x}$represents absorbance at the excitation wavelength) in the expanded concentration range. Towards this, four single fluorophores have been chosen for the study with different absorption and emission characteristics. i) fluorophores with large Stokes shift (e.g. quinine sulphate (~104 nm) and 3-hydroxyflavone (~186 nm)), ii) moderate Stokes shift (e.g. coumarin-450, (~68 nm)) and iii) small Stokes shift (rhodamin-6G, (~20 nm)). Further the applicability of the derived absorption spectral parameter has been studied in complex multifluorophoric systems such as diesel and humic acid.

2. Derived absorption spectral parameter

Based on the path length dependence of the fluorescence intensity, Divya et al. [6] showed that for right angle geometry, the fluorescence intensity at a particular excitation wavelength (λ_{ex}) at the center of the cuvette (path length = $l/2$) is given by Equation (1).

$$F_{l/2,\lambda_x} = K\, A_{\lambda_x} \cdot 10^{-(A_{\lambda_x}/2)} = K \cdot D_{abs} \quad (1)$$

where K is a constant. The parameter ($A_{\lambda_x} \cdot 10^{-(A_{\lambda_x}/2)} = D_{abs}$) was termed by them as "derived absorption spectral parameter".

Details of this derivation are given in Appendix at the end of the paper for ready reference.

Divya et al. [6] used concentrated samples to show that the variation of fluorescence intensity with excitation wavelength at the center of the cuvette can be estimated using D_{abs}.

For highly concentrated samples, the difference between λ_{der}^{max} (wavelength of derived absorption spectral maximum) and λ_{em}^{max}(wavelength of fluorescence spectral maximum) yields optimum $\Delta\lambda$ that gives most intense fluorescence signal.

$$\Delta\lambda = \lambda_{em}^{max} - \lambda_{der}^{max} \quad (2)$$

It was demonstrated that, for fluorophoric solutions at high concentrations, the observed SFS maximum matches nicely with the maximum obtained from the plot of D_{abs} parameter with λ. A CDRS based spectral data acquisition protocol was also introduced which was found to be applicable for both single as well as multifluorophoric systems.

3. Materials and methods

3.1. Sample preparation

Quinine sulphate and rhodamin-6G were purchased from S. D. Fine Chemicals having 99% and 95% purity respectively. 3-hydroxy flavone and coumarin-450 were obtained from Aldrich chemical company, USA with ~99% purity. Ethanol was purchased from Hayman, UK and it had 99.9% purity. All chemicals were used as such without further purification. Diesel was procured from the authorized local vendor in Chennai, India. Humic acid was obtained from Patsen Biotee Pvt. Ltd., Chennai, India. The stock solutions (10^{-3} M) of rhodamin-6G, 3-hydroxyflavone and coumarin-450 were prepared in ethanol. The stock solution (10^{-3} M) of quinine sulphate was made in 0.1 N sulphuric acid. Samples spanning the range from dilute to concentrated were required for our study. Therefore further dilution of all stock solution was done with their respective solvents depending on the molar absorptivity ($\in$) of each individual molecule.

Diesel samples were prepared by varying the relative fraction of diesel from 0.2%–10% (in %, v/v) in hexane. Commercially available Humic acid sample was first centrifuged at 6000 rpm for 5 min and the supernatant was collected. The decanted humic acid was used to prepare the stock solution of 500 mg L^{-1} and further dilution (4–200 mg L^{-1}) was made with triply distilled water.

3.2. Experimental

Shimadzu UV-2600 spectrophotometer was used for collecting the absorbance spectra of all selected fluorophores. Fluoromax 4 Spectrofluorometer (Horiba Jobin Yuon Inc.) equipped with 150 W Xenon lamp was used for synchronous fluorescence measurement for all sample. All fluorescence spectra obtained as corrected spectra. Cuvette of 1 cm path length was placed in a sample holder perpendicular to the source and the detector was used for SF scanning. The band pass of excitation and emission monochromator was kept at 2 nm. OriginPro 8 was used to generate and plot the derived absorbance data.

3.3. Selection of offset wavelengths ($\Delta\lambda$) for SFS measurements

For monofluorophoric systems the SF spectra were collected by choosing suitable offsets ($\Delta\lambda$) following the protocol developed earlier by Divya et al. [6]. According to the protocol, the difference between the wavelength of derived absorption spectral maximum and corresponding wavelength of fluorescence spectral maximum gives optimum SFS intensity (Equation (2)). For multifluorophoric systems, the offset wavelength ($\Delta\lambda$) of a dilute sample ($A_{\lambda_x}^{max} < 0.87$) obtained by the same protocol. This $\Delta\lambda$ was kept constant for the entire concentration range.