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Exploring the mechanism of fluorescence quenching in two biologically active boronic acid derivatives using Stern-Volmer kinetics

H.S. Geethanjali [a], D. Nagaraja [a,*], R.M. Melavanki [b,*]

[a] Department of Physics, Bangalore Institute of Technology, Bangalore 560004, Karnataka, India
[b] Department of Physics, M S Ramaiah Institute of Technology, Bangalore 560054, Karnataka, India

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ABSTRACT

The fluorescence quenching of 5-chloro-2-methoxyphenylboronic acid (5CMPBA) and 4-fluoro-2-methoxyphenyl boronic acid (4FMPBA) has been studied at room temperature by steady state fluorescence measurements. Aniline is used as quencher. Emission spectra are corrected for inner filter effect. The positive deviation observed in Stern-Volmer (S-V) plot is analyzed using different quenching models. Various quenching parameters like Stern-Volmer constant (K_{SV}), quenching rate parameter (k_q), volume constant (V), and kinetic distance (r) are estimated using extended S-V equations. The calculated values of quenching parameters suggest that static quenching mechanism is active in the studied system provided that the reactions are diffusion limited. Finite sink approximation model is invoked in order to check whether reactions are diffusion limited.

1. Introduction

The fluorescence quenching is a mechanism by which the fluorescence intensity of a given substance decreases. There are many vital applications of fluorescence quenching. Quenching studies can reveal, for example, the diffusion rate of quenchers, localization of fluorophores in proteins and membranes and their accessibilities to quenchers. Because of many such novel applications, fluorescence quenching study has attracted many investigators in the last couple of decades [1–17]. One of the well-known experimental techniques used to study the fluorescence quenching is to determine some of the quenching parameters using Stern-Volmer plots. If the quenching mechanism is mainly due to dynamic process then experimental results follow linear Stern-Volmer relation. In some cases, the quenching is due to a combination of static and dynamic process and Stern-Volmer plots tend to display a positive deviation from the linearity [18–20]. In addition, a downward curvature or negative deviation from the linearity is also observed in Stern-Volmer plots. This happens when a system contains a fluorophore in different environments or more than one fluorophore with different accessibility to the quencher, or the occurrence of a reverse reaction in the photochemical process, or hydrogen bond complex formation with the fluorophore [18–22].

Boronic acids have emerged as one of the most useful class of organo boron molecules with application in synthesis, catalysis, analytical chemistry, biology, and medicine [23]. Highly water soluble monoboronic acid probes display the more desirable OFF-ON fluorescence response. They show a remarkable sensitivity for glucose rather than fructose and galactose [24]. A wide range of boronic acid probes for the detection and determination of monosaccharides in contact lens polymers have been developed [25]. In chemical biology, boronic acids are used in the detection and sensing of peroxides, recognition and sensing of the tetra serine motif in protein, development of new MRI contrast agents [26]. Also, boronic acid derivatives act as strong fluorescent sugar sensors. For example, Czarnik and co-workers reported the properties of anthrylboronic acid 3, which senses sugars in neutral aqueous solution via fluorescence quenching process [27]. Some of these properties of boronic acid derivatives prompted us to study the fluorescence quenching of the abovementioned samples in different solvent environments. In the present paper we report the study of fluorescence quenching of 5CMPBA and 4FMPBA by aniline in solvents of different polarity. Various quenching parameters are calculated using Stern-Volmer (SV) plots and extended Stern-Volmer equations.

2. Experimental methods

2.1. Absorption and emission spectroscopy

Boronic acid derivatives used in our present work are prepared by standard method [28] and their molecular structures are as shown in Fig. 1. Solvents pentane (PT), heptane (HP), decane (DE), tetrahydrofuran (THF) and 1,4,dioxane (DX) are of spectroscopic grade and used as they were received. They were obtained from S-D-Fine Chemicals Ltd., India. Doubly distilled aniline obtained from S-D-Fine Chemicals Ltd.,

* Corresponding authors.
E-mail addresses: nagarajdd86@gmail.com (D. Nagaraja), melavanki73@gmail.com (R.M. Melavanki).

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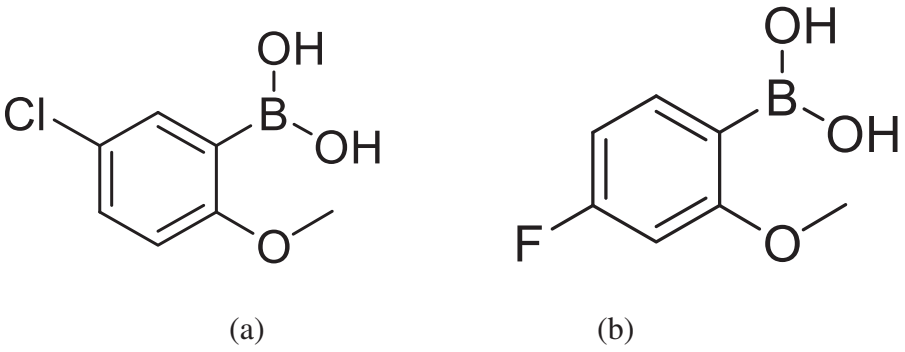

Fig. 1. Molecular structure of (a) 5CMPBA and (b) 4FMPBA.

India is used as quencher. Before preparation of the solutions, all the solvents were degassed by purging with inert gas like nitrogen and the same solvents were used for the preparation of solutions. However the quantity of aniline added to the solution is too less to degas. This is done to avoid the interference of oxygen in quenching process as oxygen is also a good quencher.

The absorption spectra are measured at room temperature using double beam UV–VIS Spectrophotometer (Model: Shimadzu UV-1800) with a wavelength accuracy of 0.5 nm. The concentration of the solution is maintained at 1×10^{-4} M in order to avoid self-absorption process and aggregation formation. The solutions are prepared by varying the quencher concentration (0.00 M–0.10 M). Each time fresh solution is taken in an air tight rectangular quartz cell. The fluorophores are excited at 281 nm and fluorescence spectra are recorded using fluorescence spectrophotometer (Model: Hitachi F-2700) with standard quartz cuvettes at room temperature with perpendicular geometry. The problem of attenuation in the excitation beam, commonly called as inner filter effect (IFE), arises from the situation when the absorption maxima of aniline (280 nm) is close to the excitation wavelength for 5CMPBA (281 nm) and 4FMPBA (279 nm). Consequently there is a reduction in the intensity of the emitted beam. This can be mistaken for quenching. Hence the IFE must be corrected before proceeding with the quenching data analysis. There are many reports available which outline IFE problem and how to correct it [29,30]. M.M. Puchalski et al. suggests three correction methods out of which we have used the correction factor introduced by Parker [29] given in Eq. (1)

$$I_{real}/I_{meas} = [2.303D(d_2-d_1)]/\left[10^{-Dd}{}_2-10^{-Dd}{}_1\right] \quad (1)$$

where I_{real} and I_{meas} are the real and measured fluorescence intensities respectively, D ($=\varepsilon Q$) is the product of molar absorption coefficient (ε) of aniline and the quencher concentration (Q), d_1 and d_2 are the cuvette dimensions.

2.2. Lifetime measurements

Fluorescence lifetimes (τ_0) of two boronic acid derivatives in different solvents and in the absence of the quencher are measured using TCSPC nanosecond fluorescence lifetime spectrometer (Model/Make: Chronos BH, USA). The samples are excited at 280 nm using short pulsed LED light source and PMT is used as detector. Fluorescence lifetimes (τ) are also measured for different quencher concentration for both the fluorophores in 1,4,dioxane solvent. The analysis of fluorescence lifetime data is carried out using Vinci Fluorescence Spectroscopy Analysis software (ISS, Champaign, IL), a user-friendly, Windows-based software package [31]. All the measurements are carried out at room temperature.

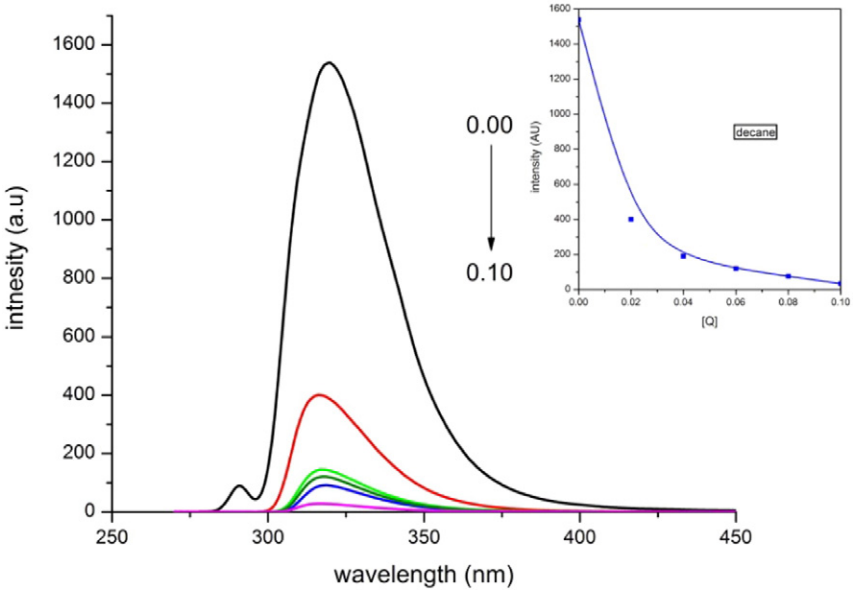

Fig. 2. Emission spectra of 5CMPBA in decane (DE) with varying quencher concentration [0.00, 0.02, 0.04, 0.06, 0.08 0.10 M]. (Inlet: variation of intensity with respect to quencher concentration).