

## Short Communication

## Correction of emission data in quenching experiments

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Energy transfer is often analysed in terms of the Stern–Volmer equation

$$\Phi_0/\Phi = 1 + k_q \tau_0 c_Q \quad (1)$$

The ratio of the quantum yields  $\Phi_0/\Phi$  is usually taken from the ratio  $I_0/I$  of the emission intensities of the emitting molecule D with ( $I$ ) and without ( $I_0$ ) the addition of quencher Q. This is correct only under ideal quenching conditions, *i.e.* if the exciting light is solely absorbed by D and if its emission is not reabsorbed by Q. Non-ideal systems (which are not unusual) require corrections  $k_A$  and  $k_E$  to the experimental emission intensity  $I_m$  for the quencher's absorption of exciting light and for the quencher's reabsorption of the donor's fluorescence respectively:

$$I = k_A k_E I_m \quad (2)$$

$k_A$  is given in the literature [1, 2]; in this communication we present a formula for  $k_E$  which we have developed assuming the validity of the usual optical requirements [3] for 90° excitation–emission geometry.

We select a small wavenumber interval  $\Delta\tilde{\nu}$  for the evaluation of the data at, for example, the fluorescence maximum  $\tilde{\nu}_{\max}$ . This eliminates the problem of the change in shape of the fluorescence band by increasing reabsorption. The emission from the solution without Q is

$$I_0(\nu) = G f_D(\nu) \Phi_0 J'_{\text{abs,D}} = p_0 J'_{\text{abs,D}} \quad (3)$$

where  $G$  is a geometric factor of the instrument,  $f_D(\nu)$  represents the shape of the emission spectrum and  $J'_{\text{abs,D}}$  is the amount of exciting light absorbed by D in the sample volume “seen” by the detecting system.

Addition of a quencher reduces the observed emission intensity. If we assume no absorption of exciting radiation by Q we can calculate the emission intensity not reabsorbed by Q according to Fig. 1.  $I_y = p J'_{\text{abs,D}} \Delta y$  is the emission intensity directed to the analysing device from a layer of thickness  $\Delta y$  at position  $y$ . After reabsorption according to the Lambert–Beer law

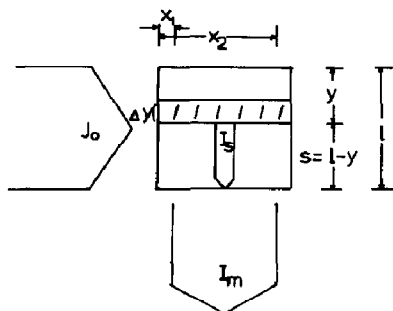


Fig. 1.

$$\frac{dI}{ds} = -I \sum \epsilon_i^{(n)} c_i$$

where  $\epsilon_i^{(n)}$  is the natural absorption coefficient of the  $i$ th substance absorbing at the emission wavelength, the layer at  $y$  contributes

$$\begin{aligned} I_s &= I_y \exp \left\{ - \int_0^{l-y} (\sum \epsilon_i^{(n)} c_i) ds \right\} \\ &= I_y \exp(-A^{(n)} + \sum \epsilon_i^{(n)} c_i y) \end{aligned}$$

to the total measured emission intensity. The latter is obtained by summation over all layers  $\Delta y$  in the infinitesimal limit by integration:

$$\begin{aligned} I_m &= pJ'_{\text{abs,D}} \int_0^l \frac{\exp(\sum \epsilon_i^{(n)} c_i y)}{\exp(A^{(n)})} dy \\ &= pJ'_{\text{abs,D}} \frac{\exp(A^{(n)}) - 1}{\exp(A^{(n)}) \sum \epsilon_i^{(n)} c_i} \\ &= pJ'_{\text{abs,D}} \frac{10^A - 1}{2.303 \times 10^A A/l} \end{aligned} \quad (4)$$

where  $A = A(\nu) = \sum \epsilon_i(\nu) c_i l$  is the absorbance at the analysing wavelength. The ratio of the experimental intensities is

$$\frac{I_0(\nu)}{I_m(\nu)} = \frac{Gf_D(\nu) \Phi_0 J'_{\text{abs,D}}}{Gf_D(\nu) \Phi J'_{\text{abs,D}} (10^A - 1) l / 2.303 \times 10^A A}$$

If there is no absorption of the exciting light by Q ( $k_A = 1$ ), the Stern-Volmer ratio is given by

$$\frac{\Phi_0}{\Phi} = \frac{I_0}{I_m} \frac{2.303 \times 10^A A}{(10^A - 1) l} = \frac{I_0}{k_E I_m}$$

Thus the correction factor for reabsorption of the donor's emission by the quencher is

$$k_E = \frac{2.303 \times 10^4 A}{(10^{-A} - 1)l} \quad (5)$$

For practical use it is necessary to calculate the absorption correction factor [1]

$$k_A = \frac{2.303 \sum \epsilon_i' c_i (x_2 - x_1)}{10^{-x_1 \sum \epsilon_i' c_i} - 10^{-x_2 \sum \epsilon_i' c_i}} \quad (6)$$

for the instrument used.  $x_1$  and  $x_2$  are the limits for the segment of the emitting sample seen by the analysing system. Sometimes  $x_1$  and  $x_2$  can be taken from the slit widths. It is preferable, however, to determine  $k_A$  empirically. The plot of the measured emission intensity of a non-self-quenching fluorescent molecule *versus* the concentration is non-linear. By proper adjustments of the limits  $x_1$  and  $x_2$  a correction factor  $k_A$  is found that transforms the experimental curve to a straight line with the same slope at low concentrations. This correction differs considerably between instruments having the focus of the optical system in the sample (*e.g.* Aminco) and those having the focus at the monochromator slits (*e.g.* Farrand). In Fig. 2 the system 1-aminonaphthalene–azobenzene in 66 vol.% sulphuric acid is presented as an extreme example [4]. The excitation wavelength was 268 nm where azobenzene- $H^+$  absorbs very weakly. The fluorescence of the protonated amine was evaluated at 333 nm. The fluorescence of the protonated amine seems to be quenched by azobenzene- $H^+$ . This is wrong; there is no singlet–singlet energy transfer.

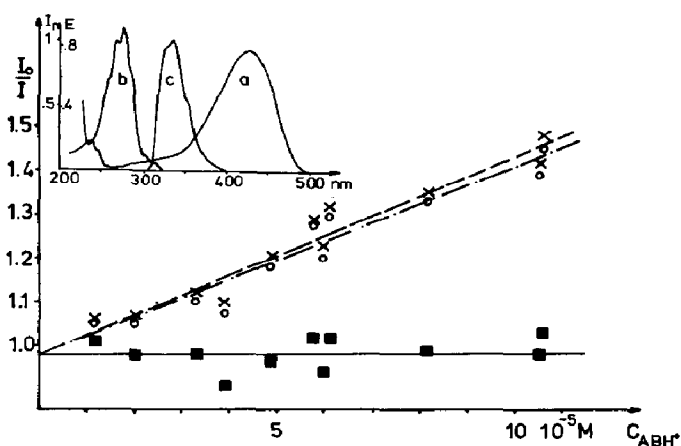


Fig. 2. The 1-aminonaphthalene- $H^+$  ( $NAH^+$ )–azobenzene- $H^+$  ( $ABH^+$ ) system. Stern–Volmer plot of the quenching experiments:  $-\cdot-x-\cdot-$ ,  $I_0/I_m$ ;  $-\cdot-o-\cdot-$ ,  $I_0/k_A I_m$ ;  $-\blacksquare-$ ,  $I_0/k_A k_E I_m = \Phi_0/\Phi$ . Inset: curve a, absorption spectrum of *trans*- $ABH^+$ ; curve b, absorption spectrum of  $NAH^+$ ; curve c, fluorescence spectrum of  $NAH^+$ .

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- 3 H. Mauser, *Formale Kinetik*, Bertelsmann Universitätsverlag, Düsseldorf, 1974, p. 167.
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