

Figure 7.46. Time- and wavelength-dependent intensity decays of 2-naphthol in 10 mM sodium phosphate buffer, pH = 6.6. Revised and reprinted with permission from [141]. Copyright © 2001, American Chemical Society.

Hence, the decay of the initially excited state becomes a single exponential. This prediction has been verified in experimental studies of reversible and irreversible reactions. We note that the negative pre-exponential factor may be associated with either of the decay times (τ_1 or τ_2) depending on the values of the kinetic constants. The negative pre-exponential factor is always associated with the shorter decay time.¹³⁸

7.12.3. Differential Wavelength Methods

In considering excited-state reactions there is a general principle that clarifies the complex decay kinetics and results in simplified methods of analysis.^{139–140} This principle is that the population of the *R* state can be regarded as a convolution integral with the *F* state. That is, the *F*-state population is the excitation pumping function of the *R* state. Consider a measurement of the *R* state, made relative to the *F* state. The *F* state effectively becomes the lamp function, so that measurements relative to the *F* state directly reveal the decay kinetics of the *R* state. The application of this procedure requires a spectral region where the emission from the *F* state can be observed, without overlap from the *R* state. Since non-overlap of states is frequently found on the short-wavelength side of the emission, the time profile of the *F* state can generally be measured directly. Deconvolving the *R*-state emission with the *F*-state emission yields the lifetime of the *R* state that would be observed if the *R* state could be directly excited.

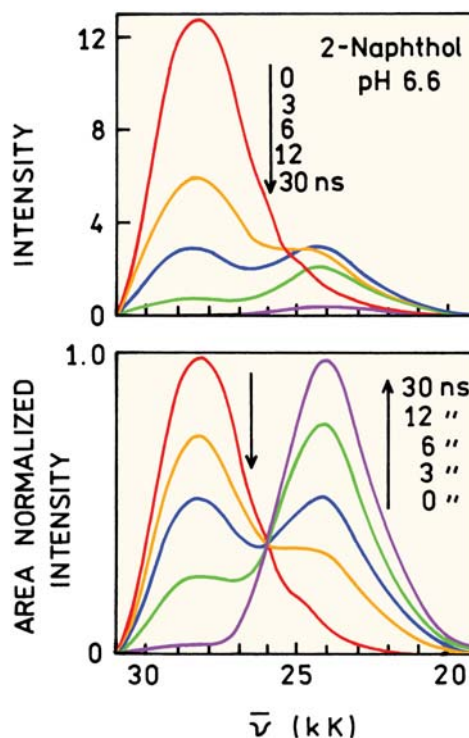


Figure 7.47. Time-resolved emission spectra of 2-naphthol in 10 mM sodium phosphate, pH 6.6. The top panel shows the actual time-resolved intensities. The lower panel shows the area-normalized intensities. Revised and reprinted with permission from [1041]. Copyright © 2001, American Chemical Society.

7.13. TIME-DOMAIN STUDIES OF NAPHTHOL DISSOCIATION

The properties of excited-state reactions can be effectively studied using time-domain methods. Typical data for the excited-state ionization of 2-naphthol are shown in Figure 7.46. The time-dependent data were collected at wavelengths across the emission spectrum ranging from 335 to 485 nm.¹⁴¹ Examination of Figure 7.41 shows the emission at 335 nm is from the unionized form of 2-naphthol and the emission at 485 nm is due to the ionized naphtholate form. The naphtholate emission starts at zero and shows a rise time during which the ionized species is formed.

Time-resolved data collected across the emission spectra can be used to calculate the time-resolved emission spectra (Figure 7.47). The top panel shows the actual time-resolved intensities, not the peak or area-normalized values. As time proceeds the emission from both species decays to zero. At $\tau = 0$ the emission is dominantly due to unionized

2-naphthol. The ionized form shows a transient increase in intensity at time from 3 to 12 ns. These TRES show two distinct emission spectra, which is different from the gradual spectral shifts seen for solvent relaxation. The lower panel in Figure 7.47 shows the TRES that are normalized to the same total area. These spectra show an isoemissive point at 389 nm. Observation of an isoemissive point in the area-normalized TRES proves that there are only two emitting species.¹³⁹ An isoemissive point is not expected for continuous spectral relaxation or if there are more than two emitting species.

7.14. ANALYSIS OF EXCITED-STATE REACTIONS BY PHASE-MODULATION FLUOROMETRY

Frequency-domain fluorometry provides a number of interesting opportunities for the analysis of excited-state reactions. One measures the frequency responses across the emission spectra. These data can be used to construct the TRES. However, the data can also be analyzed in terms of the kinetic constants, which leads to insights into the meaning of the measured phase and modulation values. Additional detail can be found elsewhere.^{142–144}

For simplicity we consider the irreversible reaction ($k_2 = 0$). Assume that by appropriate optical filtering the emission from the F and R states can be individually observed. Then, relative to the phase and modulation of the excitation, the phase and modulation of the F and R state are given by

$$\tan \phi_F = \omega / (\gamma_F + k_1) = \omega \tau_F \quad (7.33)$$

$$m_F = \frac{\gamma_F + k_1}{\sqrt{(\gamma_F + k_1)^2 + \omega^2}} = \frac{1}{\sqrt{1 + \omega^2 \tau_F^2}} \quad (7.34)$$

Several aspects of these equations are worthy of mention. Since we have initially assumed that F and R are separately observable, and the reverse reaction does not occur, the decay of F is a single exponential. Hence, for the F state we find the usual expressions for calculation of lifetimes from phase and modulation data. In the absence of any reaction ($k_1 = 0$) the lifetime is $\tau_{0F} = \gamma_F^{-1}$. In the presence of a reaction, the lifetime of F is shortened to $\tau_F = (\gamma_F + k_1)^{-1}$. Thus, for an irreversible reaction, the observed values of ϕ_F and m_F can be used to calculate the true lifetime of the unreacted state.

The phase and modulation of the R state, when measured relative to the excitation, are complex functions of the various kinetics constants. These values are given by

$$\tan \phi_R = \frac{\omega(\gamma_F + \gamma_R + k_1)}{\gamma_R(\gamma_F + k_1) - \omega^2} \quad (7.35)$$

$$m_R = m_F \frac{\gamma_R}{\sqrt{\gamma_R^2 + \omega^2}} = m_F m_{0R} \quad (7.36)$$

In contrast to the F state, the measured values for the R state cannot be directly used to calculate the R state fluorescence lifetime. The complexity of the measured values (ϕ_R and m_R) shows why it is not advisable to use the apparent phase (τ^ϕ) and modulation (τ^m) lifetimes of the relaxed state.

Closer examination of eqs. 7.33–7.36 reveals important relationships between the phase and modulation values of the F and R states. For an excited-state process, the phase angles of the F and R states are additive, and the modulations multiply. Once this is understood, the complex expressions (eqs. 7.35–7.36) become easier to understand. Let ϕ_{0R} be the phase angle of the R state that would be observed if this state could be excited directly. Of course, this is related to the lifetime of the directly excited R state by $\tan \phi_{0R} = \omega \tau_{0R}$. Using eq. 7.36, the law for the tangent of a sum, and $\tan(\phi_F + \phi_{0R}) = \tan \phi_R$, one finds

$$\phi_R = \phi_F + \phi_{0R} \quad (7.37)$$

Hence, the phase angle of the reacted state, measured relative to the excitation, is the sum of the phase angle of the unreacted state (ϕ_F) and the phase angle of the reacted state, if this state could be directly excited (ϕ_{0R}). This relationship (Figure 7.48) may be understood intuitively by recognizing that the F state is populating the R state. Of course, these are the same considerations used to describe differential-wavelength deconvolution. For the irreversible reaction, measurement of $\Delta\phi = \phi_R - \phi_F = \phi_{0R}$ reveals directly the intrinsic lifetime of the reacted fluorophore, unaffected by its population through the F state.

The demodulation factors of the two states display similar properties. From eq. 7.36 one finds that the demodulation of the relaxed state (m_R) is the product of the demodulation of the unrelaxed state (m_F) and that demodulation due to the intrinsic decay of the R state alone (m_{0R}). That is,

$$m_R = m_F \frac{1}{\sqrt{1 + \omega^2 \tau_R^2}} = m_F m_{0R} \quad (7.38)$$