Global Tricompartamental Analysis of the Fluorescence Decay Surface of the Charged Fluorescent Probe N,N,N-Trimethyl-3-(1-pyrenyl)-1-propanaminium Perchlorate

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The kinetics of the excited-state processes of the charged fluorescent probe N,N,N-trimethyl-3-(1-pyrenyl)-1-propanaminium perchlorate (PROBE) in tetrahydrofuran are reported. At very low concentrations PROBE decays monoexponentially with a lifetime of 236 ± 1 ns, from which k0 = 1.4 ± 4.2 × 10^4 s⁻¹ is obtained. Upon addition of the quenner ammonium salt N,N,N-trimethyl-1-dodecylaminium perchlorate a bimolecular decay function is needed to describe the decay traces. The second excited state is the aggregated PROBE. This aggregation is due to dipole–dipole or ion–dipole interactions. The rate constant values of the kinetic Scheme (Scheme 4) are obtained by global bimolecular analysis: k0 = 4.2 ± 0.7 × 10^4 s⁻¹; k1 = (5.7 ± 0.1) × 10^9 s⁻¹. When the concentration of PROBE itself is varied, a triple-exponential decay function adequately describes the decay surface. The third excited-state species is a PROBE excimer, which can be formed through two different pathways: either intermolecularly when a locally excited PROBE molecule encounters a ground-state PROBE molecule or intramolecularly when an aggregate of two PROBE molecules rearranges. To resolve the kinetics of this system, global tricompartamental analysis is developed. Even after including the information available from experiments where N,N,N-trimethyl-1-dodecylaminium perchlorate is added, the information available from the global-triplexponential analysis (k0 = 0 and k1 = 0) (Scheme 5), the experimental time-resolved data do not allow one to obtain a unique solution for the rate constant values. By scanning the rate constant k1, bounds can be specified for the rate constants: 33 × 10^7 < k1 < 60 × 10^14 M⁻¹ s⁻¹, 1.5 × 10^13 < k1 < 1.7 × 10^19 M⁻¹ s⁻¹, and k2 < 1.0 × 10^11 s⁻¹. Unique values are obtained for k0, k1, and k2 = (4.25 ± 0.01) × 10^6 s⁻¹.

1. Introduction

The introduction of ionic units into neutral polymers drastically changes the properties of these materials. In polar solvents the counterions can be readily solvated, resulting in a polymer bricklike that covalently links several free charges. The behavior of these charged macromolecules in polar solvents is known in the literature as the polyelectrolyte behavior.1 In less polar solvents (< 15) the counterions will stay closer to the ionic units on the chain. Contact ion pairs and solvated separated ion pairs are more likely to be formed than free ions. These contact ion pairs can be regarded as dipoles, and ion–dipole and dipole–dipole interactions occur in these media.2 In the literature this behavior is referred to as the ion aggregate behavior.3

Since the intra- and interchain interactions seem to be important in the model describing the ion aggregate behavior, it can be expected that intra- and intermolecular excimer formation is an excellent tool to obtain more information about the ion aggregation. Stationary fluorescence measurements of a fluorescent probe attached to both ends of a polysulfone–halato telechelic polymer (HTP) have been used to discriminate between intra- and interchain association.14 It was shown that at low concentrations the intrachain dipole–dipole interactions

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**Figure 1.** N,N,N-Trimethyl-3-(1-pyrenyl)-1-propanaminium perchlorate.
weighting factors can be determined. The global compartmental analysis approach is the method of choice to unravel very complex kinetics, as is the case here.

2. Theories

2.1. Consider a causal, linear, time-invariant, photophysical system consisting of three distinct ground-state species (1, 2, 3) and three corresponding excited-state species (1’, 2’, 3’), as depicted in Scheme 1. The excited-state species can decay by fluorescence (F) and nonradiative processes (NR) (internal conversion (IC) and intersystem crossing (ISC)). The composite rate constants for these processes are denoted by $k_{1}$, $k_{2}$, $k_{3}$, $k_{4}$, $k_{5}$, $k_{6}$, $k_{7}$, $k_{8}$, and $k_{9}$. The reaction sequence is illustrated in Figure 1.

Scheme 1

![Scheme 1](image1)

Defining the normalized elements $b_{ij}$ and $c_{ij}$,

$$b_{ij} = \frac{b_{ij}}{b_{nj}} \quad \text{for} \quad i = 1, 2, 3$$

$$c_{ij} = \frac{c_{ij}}{c_{nj}} \quad \text{for} \quad i = 1, 2, 3$$

Equation 1 can be written as

$$f(k_{1}, k_{2}, k_{3}, \ldots) = e^{(k_{1} + k_{2} + k_{3})t}$$

2.2. Theoretical considerations

Assuming that the interconversions $1 \rightarrow 2$ and $1 \rightarrow 3$ are bimolecular and therefore have to be described by the second-order rate constants $k_{2}$ and $k_{3}$, respectively, and the processes $2 \rightarrow 3$ and $3 \rightarrow 3'$ are also nonradiative, the first-order rate constants $k_{4}$ and $k_{5}$ are negligible (see Scheme 1). Scheme 1 simplifies to Scheme 2.

Scheme 2

![Scheme 2](image2)

This scheme describes the kinetic behavior of PROBE in tetraethylammonium, as will be evident from the analysis. In this case matrix (A) can be simplified to

$$A = \begin{pmatrix}
-k_{2} & -k_{3} & 0 \\
-k_{4} & k_{5} & -k_{5} \\
-k_{6} & k_{7} & -k_{8}
\end{pmatrix}
$$

The exponential factors $\gamma_{i}$ are related to the decay times

$$\gamma_{i} = 1/t_{i}$$

and are explicitly given by

$$\gamma_{i} = 1/t_{i}$$

with

$$\gamma_{1} = 1/t_{1}$$

$$\gamma_{2} = 1/t_{2}$$

$$\gamma_{3} = 1/t_{3}$$

3. Results and Discussion

3.1. Fluorescence Decay Surface of PROBE

The fluorescence decay surface of PROBE is given by

$$f(k_{1}, k_{2}, k_{3}, \ldots) = \frac{b_{ij} e^{(k_{1} + k_{2} + k_{3})t}}{b_{nj} e^{(k_{1} + k_{2} + k_{3})t}}$$

2.3. Equation 2 (4)

$$\epsilon_{i} = \frac{c_{ij}}{c_{nj}} \quad \text{for} \quad i = 1, 2, 3$$

From eq. 9–11 it is clear that only one decay time (see eq. 9).
Fluorescence Decay Surface of PROBE

$$k_{3a} = k_{0}B + k_{d}D = F = 0$$ (24)

The combination of eqs 16, 19, and 22 gives

$$k_{0} = \frac{2}{C/k_{d} + E/A} = 0$$ (25)

Since eq 24 and 25 do not divide, the common root of both equations allows one to assign a unique value to $k_{d}$.

Analogously, for $k_{s}$ and $(k_{3c} + k_{4})$ from eqs 17, 20, and 23 we have

$$k_{3c} = k_{0}B + k_{0}D = F = 0$$ (26)

$$(k_{3c} + k_{4}) = (k_{3c} + k_{4})B + (k_{3c} + k_{4}) +$$

$$k_{0}D = F = 0$$

Solution of eq 24, 26, and 27 yields three roots, one of which can be uniquely assigned to $k_{0}$. However, we are not able to unequivocally assign the other values to $k_{0}$ and $(k_{3c} + k_{4})$ without additional information. The concentration dependence (Figures 2–4) may provide the lacking information. If the two variable decay times decrease with increasing [M], which can be the case for type B, we have that $(k_{3c} + k_{4}) > k_{0}$. This automatically establishes the proper assignment. One decay time increases, the assignment cannot be made and two sets of solutions are mathematically possible. However, as $[M] = 0$ the monoexponential decay yields the unique value for $k_{0}$.

Once a value for $k_{0}$ is assigned, the value for $k_{2}$ is known, bounds on the rate constants ($k_{1}$, $k_{2}$, $k_{3}$, $k_{4}$) can be specified as follows. Equation 17 yields a value for $B^*$:

$$B^* = B - k_{0} - k_{3c} - k_{4}$$ (28)

Combining eqs 16, 17, and 19 yields

$$C = A(B - k_{0}) = C = k_{3c}k_{4}$$ (29)

Equations 16, 28, and 29 provide the basis for the determination of the interconversion rate constants ($k_{1}$, $k_{2}$, $k_{3c}$, $k_{4}$). Thus, only three independent equations are available to determine four unknown rate constants. Therefore, a unique set of values for those rate constants cannot be obtained. It is possible, however, to obtain information from these data. For each value assigned to one of the rate constants $k_{1}$, $k_{2}$, $k_{3c}$, and $k_{4}$, the corresponding values of the remaining rate constants can be determined within the following limits:

$$0 < k_{2} < A - C*B$$

$$C*/A - k_{1} < A$$

$$0 < B^* < C*A$$

$$C*A < k_{4} < C*A$$ (31)

3. Experimental Procedure

3.1. Materials

The fluorescence decay curves were obtained using a Spectra-Physics model 64000, synchronously pumped, cavity-dumped, frequency-doubled DCM dye laser as excitation source (excitation wavelength: 578 nm) with a detection system. A detailed description of the apparatus has been given elsewhere. All decay curves were fitted to a sum of exponential functions using the nonlinear least-squares fitting program. The fluorescence quantum yields of the probes were determined with quinine sulfate in 0.1 N sulfuric acid as a reference. The correction for the refractive index was applied.

3.2. Analysis

The analysis of the fluorescence decay surface of species undergoing excited-state processes was performed in the existing global analysis program32 based on Marquardt’s34 algorithm.

Consider the excited-state processes depicted by Scheme 2.
The global fitting parameters are \( k_1, \Delta_0, \) and \( k_2 \) \((k = 1, 2)\). The only local fitting parameters are the scaling factors. Using this approach, experiments done at different excitation/emission wavelengths and at multiple timing calibrations and PROBE and ammonium salt concentrations are linked by all rate constants defining the system.

The fitting parameters were determined by minimizing the global reduced \( \chi^2 \):

\[
\chi^2 = \sum_i \left[ \frac{W_i (y_{i0} - y_{i})}{\sigma_i} \right]^2
\]

where the index \( i \) sums over \( q \) experiments, and the index \( i \) sums over the appropriate channel limits for each individual experiment. \( y_i \) and \( \chi_i \) denote respectively the observed (experimentally measured) and calculated (fit) values corresponding to the \( i \)th channel of the \( i \)th experiment, and \( W_i \) is the corresponding statistical weight. \( \sigma_i \) represents the number of degrees of freedom for the entire multidimensional fluorescence decay surface.

The statistical criteria to judge the quality of the fit include both graphical and numerical tests. The graphical methods comprised plots of surfaces of the autocorrelation function values vs. experiment number and of the weighted residuals vs. channel number vs. experiment number. The numerical statistical tests incorporated the calculation of \( \chi^2 \) and its corresponding \( Z_\text{cr} \):

\[
Z_\text{cr} = (\sum W_i (y_{i0} - y_i)^2)^{1/2}
\]

The additional statistical criteria to judge the quality of the fit are described elsewhere.  

4. Results and Discussion

4.1. The Properties of PROBE at Very Low Concentration.

Since PROBE covalently links a quaternary ammonium salt to a pyrene moiety, a 1:1 stoichiometry between the ammonium salt and the fluorophore is established irrespective of the concentration. Several authors have mentioned interactions between aromatic chromophores and quaternary ammonium groups. A close contact between the salt and the aromatic moiety, which can be established at the surface of a micelle or at very high concentrations in solution, is believed to be necessary to obtain quenching by the ammonium salt. The exact mechanism of this quenching process, however, has not been established.

To check whether there is an influence of the quaternary ammonium salt on the pyrene moiety in THF, spectrometric measurements of PROBE were compared with those of 1-methylpyrene.

4.1.1. Stationary Measurements. The absorption spectrum of PROBE shows a small deviation, especially the \( \lambda_{\text{a}} \) band, compared to the absorption band of 1-methylpyrene (Figure 5). The fact that the \( \lambda_{\text{a}} \) rather than the \( \lambda_{\text{b}} \) band is influenced is also seen in the normalized emission spectra (Figure 7) and the normalized excitation spectra (Figure 8). The emission spectra show a shift in intensity demonstrating the influence from the ammonium group on the \( \lambda_{\text{b}} \) band. The excitation spectra, however, show no clear shift, suggesting that there is no influence on the \( \lambda_{\text{a}} \) band.

4.2. Fluorescence Measurements of PROBE as a Function of Added Quaternary Ammonium Salt. An increase in the PROBE concentration automatically induces a change in two important parameters, namely, the salt concentration (which relates to the ammonium salt) and the pyrene concentration (which is relevant to the excimer formation). One way to study the salt influence on the probe behavior is to add a simple quaternary ammonium salt to the solution. Solutions of the reference probe, 1-methylpyrene, were prepared at a low concentration (1 \( \times 10^{-4} \) M) and with increasing concentrations of \( \lambda_{\text{b}} \)TUTAP. The concentration range of the added salt extended from 1 \( \times 10^{-4} \) M to 2 \( \times 10^{-3} \) M. Solutions of PROBE were prepared in an analogous way. The added salt in this case was DOPAT (2 \( \times 10^{-4} \) M PROBE, 2 \( \times 10^{-3} \) M, 1 \( \times 10^{-3} \) M, 5 \( \times 10^{-4} \) M, and 1 \( \times 10^{-3} \) M DOPAT).

4.2.1. Stationary Measurements. The fluorescence emission spectra of PROBE did not change upon adding DOPAT to the solution (Figure 9). The emission spectra of PROBE and 1-methylpyrene in the two different concentrations were used in the fluorescence decay measurements: 1.204 nM channel and 121 ps/channel. All decay data of 1-methylpyrene could be described by standard global analysis by a monoexponential function \( \alpha = 36 \) with \( n = 1 \) with the decay time linked over the different \( \lambda_{\text{b}} \)TUTAP concentrations. This means that the addition of \( \lambda_{\text{b}} \)TUTAP does not influence the kinetic and spectroscopic behavior of 1-methylpyrene.

The decay data of PROBE in the presence of DOPAT could not be described by a monoexponential function: a biexponential decay function \( \alpha = 36 \) with \( n = 2 \) was needed to get satisfactory statistics of the fits.

4.2.2. Time-Resolved Measurements. For PROBE and 1-methylpyrene, two timing calibrations were used in the fluorescence decay measurements: 1.204 nM channel and 121 ps/channel. All decay data of 1-methylpyrene could be described by standard global analysis by a monoexponential function \( \alpha = 36 \) with \( n = 1 \) with the decay time linked over the different \( \lambda_{\text{b}} \)TUTAP concentrations. This means that the addition of \( \lambda_{\text{b}} \)TUTAP does not influence the kinetic and spectroscopic behavior of 1-methylpyrene.

The decay data of PROBE in the presence of DOPAT could not be described by a monoexponential function: a biexponential decay function \( \alpha = 36 \) with \( n = 2 \) was needed to get satisfactory statistics of the fits.

One decay time was found to be independent of the salt concentration, because it could be linked over the data surface of several experiments performed at different salt concentrations. Scheme 3 represents the linking scheme applied in the analysis. In this scheme boxed parameters represent the linked decay times \( \tau_i \), while \( \alpha \) denotes the preexponential factors. The long decay time \( \tau_i \) was linked over all experiments at each concentration at different wavelengths. Table 1 shows the decay parameters estimated by standard global biexponential analysis. For an intermolecular two-state excited-state process, there are only two cases where one of the decay times can be independent of the concentration. This is discussed in the Appendix. Since \( k_1 \) is known and a shorter decay time appears upon addition of the salt, we have that \( k_2 = k_3 \).

The 24 curves which were fitted by standard global biexponential analysis were subsequently analyzed in a single step by global compartmental analysis (Scheme 4). Since the fluorescence was recorded at three emission wavelengths and four different salt concentrations, the compartmental system is theoretically identifiable. During the analysis the rate constants \( k_{1}, k_{2}, k_{3}, \) and \( k_{4} \) were linked over the whole fluorescence decay surface, the \( k_{5} \) parameters were linked at the same \( \lambda_{\text{a}} \) (DOPAT), while the \( k_{6} \) parameters were linked at the same \( \lambda_{\text{b}} \) (PROBE). In Tables 2 and 3 the values for the decay parameters obtained by this analysis are shown. The results confirm that \( k_{5} \) and \( k_{6} \) are equal.
TABLE 1: Globally Estimated Decay Parameters of PROBE (2 x 10^-4 M) in THF as a Function of the Concentration of Added DOTAP

<table>
<thead>
<tr>
<th>(L^\alpha) (nm)</th>
<th>[DOTAP] (M)</th>
<th>(k_0) (ns/ct)</th>
<th>(\epsilon_0)</th>
<th>(\epsilon_1)</th>
<th>(\gamma_1)</th>
<th>(\tau_1) (ns)</th>
<th>(\gamma_2)</th>
<th>(\tau_2) (ns)</th>
<th>(Z_2)</th>
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<td>400</td>
<td>1 x 10^-4</td>
<td>1.208</td>
<td>-0.04 ± 0.01</td>
<td>13.5 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>223.3 ± 0.1</td>
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<td>400</td>
<td>5 x 10^-4</td>
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<td>0.84 ± 0.01</td>
<td>12.06 ± 0.01</td>
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<tr>
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<td>0.1 x 10^-4</td>
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<td>0.85 ± 0.01</td>
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<td>0.66 ± 0.01</td>
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<td>0.05 ± 0.01</td>
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*Experiments were performed at two time increments per channel (5): 1208 and 121 ps. Excitation wavelength was at 320 nm. For the analysis of the 24 decay a Z_2 value of 4.512 was obtained.

TABLE 2: Rate Constant Values Estimated by Global Compartmental Analysis of the 2 x 10^-4 M Decay Curves of Table 1

| \(k_0\) | 4.46 ± 0.01 (10^9 s^-1) |
|\(k_1\) | 4.48 ± 0.01 (10^9 s^-1) |
|\(k_2\) | 4.5 (7 ± 0.1) (10^9 s^-1) |

TABLE 3: Spectral Parameter Values Estimated by the Global Compartmental Analysis of Table 2

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<thead>
<tr>
<th>(b)</th>
<th>(L^\alpha) (nm)</th>
<th>(\epsilon_0)</th>
<th>(\epsilon_1)</th>
<th>(\gamma_1)</th>
<th>(\gamma_2)</th>
<th>(\tau_1) (ns)</th>
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<td>0.05 ± 0.02</td>
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<td>5 x 10^-4</td>
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<tr>
<td>1 x 10^-4</td>
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<td>0.35 ± 0.02</td>
<td>0.36 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>0.38 ± 0.02</td>
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<td>2 x 10^-4</td>
<td>0.65 ± 0.01</td>
<td>0.49 ± 0.02</td>
<td>0.44 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.46 ± 0.02</td>
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Figure 9. Decay times of PROBE as a function of the concentration in THF.

SCHEME 5

Figure 10. Global compartmental analysis of 99 decay traces of PROBE in THF collected at six emission wavelengths between 375 and 500 nm. Seven different PROBE concentrations were used: 1 x 10^-4, 5 x 10^-4, 2 x 10^-4, 5 x 10^-3, 1 x 10^-3, and 6 x 10^-3 M, respectively. The excitation was at 360 nm with different excitation intensities (a). Z_2 values (35) as a function of log([PROBE]/[THF]) (B) (i.e., A vs. log([THF]/[PROBE])); (ii) B vs. log([THF]/[PROBE]); (iii) A vs. log([THF]/[PROBE]); (iv) B vs. log([THF]/[PROBE]); (v) A vs. log([THF]/[PROBE]); (vi) B vs. log([THF]/[PROBE]); (vii) A vs. log([THF]/[PROBE]); (viii) B vs. log([THF]/[PROBE]).
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Table 4: Rate Constant Values Estimated by Global

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<td>$k_0$</td>
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<td>$k_7$</td>
<td>$k_8$</td>
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<td></td>
<td>(2.38 ± 0.01)</td>
<td>(1.95 ± 0.01)</td>
<td>(1.95 ± 0.01)</td>
<td>(1.55 ± 0.01)</td>
<td>(1.95 ± 0.01)</td>
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Table 5: Fluorescence Decay Surface of PROBE

Figure 11. $x_2$ values (eq 9) from a global compartmental analysis as a function of log(k0/k2).

Figure 12. Decay times as a function of -log[M] calculated with $k_0$, $k_1$, and $k_2$ uniquely determined values were used. For the other rate constants, an arbitrary value of 0.1 was used together with the corresponding value of $k_0$, $k_1$, and $k_2$. The decay times estimated by the global compartmental analysis are also shown.

PROBE decays monoeXponentially, with $k_0$ = (4.2 ± 0.1) $10^{-3}$ $s^{-1}$, whereas $k_1$ of monomethyleneglycine equals (5.2 ± 0.1) $10^{-3}$ $s^{-1}$.

Upon increase of the quaternary ammonium salt concentration an association occurs due to ion-dipole or dipole-dipole interactions. A bieXponential decay is needed to fit the data. A bieXponential scheme can be presented describing their processes. The rate constant values describing the kinetic scheme were obtained by global bieXponential analysis: $k_0$ = $k_2$ = (4.2 ± 7) $10^{-3}$ $M^{-1} s^{-1}$, and $k_2$ = (5.5 ± 0.1) $10^{-3}$ $s^{-1}$.

Increase of the PROBE concentration, so that pyrene mojety and salt concentration increase simultaneously, leads to a tripolar exponential decay. Three excited-state species are present, and a kinetic scheme describing the processes between the three compartments is proposed (Scheme 5). To obtain information from the fluorescence decay surface, we developed a bieXponential analysis. Even after including the informative variables from experiment where DOTAP was added (eq $k_0$), and the information available from the global tripolar exponential analysis ($k_3 = 0$ and $k_2 = 0$), the exponential time-resolved data do not allow one to obtain a unique solution for the rate constant values. By applying the scanning technique, upper and lower bounds could be specified for the rate constants: $53 < k_0 < 60$, $60 < k_1 < 80$, $7 < k_2 < 10$, $5.5 < k_3 < 10$, $1.5 < k_4 < 2$, $1.7 < k_5 < 1.9$, and $1.6 < k_6 < 2$ $s^{-1}$.

The maximum value of the rate constant of excitation formation, $k_6$, is much lower than the value of the rate constants, $k_1$, which describes the aggregation. The values of $k_0$, $k_2$, and $k_3$ of the bieXponential system can be compared with those of the bieXponential system with DOTAP as added salt. Unique values were obtained for $k_0$, $k_2$, and $k_3$ of $k_6$ = (2.5 ± 0.01) $10^{-3}$ $s^{-1}$, $k_6 = (1.2 ± 0.03) $10^{-3}$ $s^{-1}$.

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6. Appendix

Consider a causal, linear, time-invariant, intermolecular system consisting of two distinct types of ground-state species (1,2) and two corresponding excited-state species (1',2'), depicted for example, in Scheme 4. In that case the compartmental matrix $A$ is given by $A_1$ whereas the rate constants have the same meaning as before.

References and Notes

(22) Liddel, J.; Ameloot, J.; co. 1985, 14, 403.