

Fast and accurate analysis of molecular relaxation processes on high-intensity excitation: non-linear analysis with a convolved autoregressive model

Keiji Sasaki *, Jun-ichi Hotta, Hiroshi Masuhara

Department of Applied Physics, Osaka University, Suita, Osaka 565, Japan

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Abstract

A novel method for the analysis of the decay dynamics of densely produced, excited and ionic states is proposed, which makes it possible to estimate the rate constants of unimolecular and bimolecular processes from a transient emission or absorption curve. This method is based on a convolved autoregressive model, which is a linear form with respect to unknown model parameters, even for bimolecular excited state annihilation and ion recombination processes, so that the computation time is much shorter than that of a conventional non-linear least-squares fitting technique. The method can be extended to the analysis of a bimolecular process during irradiation by excitation light, which cannot be achieved using conventional techniques. © 1997 Elsevier Science S.A.

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1. Introduction

There have been major advances in time-resolved fluorescence and absorption spectroscopy in the last decade, which have made it possible to observe decay curves with picosecond and femtosecond time resolution and with high accuracy and precision. In order to analyse the photophysical and photochemical dynamics from the transient emission and absorption curves, and to extract quantitative information on the decay processes, novel mathematical methods are indispensable in high-performance spectroscopy systems. A variety of mathematical methods, such as non-linear least-squares fitting, moment and modulating function methods, Fourier/Laplace transform techniques and global compartmental analysis [1–3], have been widely applied to analyses of excitation energy relaxation, electron and proton transfer, molecular vibrational relaxation and isomerization occurring in various molecular systems.

These methods are normally based on linearity assumptions: (1) the phenomenon obeys a linear differential equation, so that the temporal profile is expressed as a multiexponential function; (2) the fluorescence and absorption are measured by a linear detection system, so that an

observed decay curve $x(t)$ is given as a convolution of the exponential decay with an instrumental response function $y(t)$ as follows

$$x(t) = \int_0^{\infty} \sum_{i=1}^M A_i \exp(-k_i t') y(t-t') dt' \quad (1)$$

where k_i and A_i are the rate constant and amplitude of the i th decay component respectively. Since Eq. (1) is a non-linear function of unknown parameters k_i , the least-squares fitting to an observed curve requires a non-linear iterative algorithm, which is complicated to program and takes an extremely long time to compute.

In a previous paper, we proposed a new method, based on a convolved autoregressive model, for estimating the decay parameters of transient curves [4]. The convolved autoregressive model represents a decay curve of Eq. (1) as the following expression without any approximation

$$x(n\Delta t) = \sum_{i=1}^M B_i x[(n-i)\Delta t] + \sum_{i=1}^M C_i y[(n-i+1)\Delta t] \quad (2)$$

where B_i and C_i are model parameters which uniquely correspond to k_i and A_i in Eq. (1) and Δt is the sampling interval. Eq. (2) is a linear combination of model parameters to be estimated; therefore the computation time of model fitting is

* Corresponding author. Tel.: +81 6 879 7839; fax: +81 6 879 7840; e-mail: sasaki@ap.eng.osaka-u.ac.jp

much shorter than that of non-linear least-squares fitting, and the accuracy of estimation is as high as that of non-linear fitting. In addition, the method does not require programming parameters, such as initial values for estimates, acceleration coefficients and dumping factors. Furthermore, the method can be extended to a blind deconvolution which is an estimation without the use of an instrumental response function [5].

Unfortunately, the convolved autoregressive model of Eq. (2) is applicable only to dynamic processes given by linear differential equations. When intense excitation light is used to irradiate concentrated solutions or solids to produce dense excited states, it is expected that some interactions will be induced between the excited molecules, causing deviations from a linear differential equation [6–8]. For example, laser photolysis, which is conducted with an excitation pulse of millijoules per square centimetre usually leads to S_1-S_1 and T_1-T_1 annihilation processes. In polar solvents, ionic species produced by an excitation pulse undergo homogeneous recombination in addition to geminate recombination, which are also non-linear decay processes. Another example is time-resolved microspectroscopy using laser scanning fluorescence/absorption microscopes [9], where an excitation laser is usually focused onto a wavelength-sized spot. Even a nanojoule pulse from a continuous wave (cw) mode-locked laser is condensed to an intensity of millijoules per square centimetre, which can easily induce bimolecular processes of excited and ionic states.

When densely formed, excited or ionic states decay by diffusion-limited bimolecular interaction; the decay curve of the emission intensity or transient absorbance $x(t)$ obeys a rate equation [5] as

$$\frac{dx(t)}{dt} = -k_u x(t) - k_b x(t)^2 \quad (3)$$

where k_u and k_b are the rate constants of a unimolecular transition and bimolecular decay respectively. A rate constant of an excited state annihilation or ion recombination process can be converted from k_b with a knowledge of the initial concentration of the excited or ionic molecules. A solution of Eq. (3) is given by

$$x(t) = \frac{1}{(x_0^{-1} + k_b k_u^{-1}) \exp(k_u t) - k_b k_u^{-1}} \quad (4)$$

where x_0 is an abbreviation for $x(0)$. Usually, the rate constant of a unimolecular transition k_u is estimated from the tail of a decay, where the curve approaches an exponential function, or from a decay curve observed on low-intensity excitation; a plot of $x(t)^{-1}$ against $\exp(k_u t)$ is fitted to a straight line and k_b is given by the intercept [5]. However, even a small error in the given k_u value drastically degrades the accuracy of the estimate of k_b . Without any doubt, direct fitting of Eq. (4) to an observed decay curve is superior to the above method for estimating both k_u and k_b with high accuracy. Unfortunately, the non-linear least-squares fitting

requires a long computation time, as mentioned above. Furthermore, Eqs. (3) and (4) are valid only for a decay process after irradiation with excitation light; however, a bimolecular decay process on high-intensity excitation sometimes reduces the excited and ionic state decay times to values comparable with or shorter than the excitation pulse duration. A pumping term should be added to the right-hand side of Eq. (3) during excitation. Unfortunately, the differential equation with the pumping term is analytically impossible to solve, so that the bimolecular process during excitation cannot be analysed by analytical methods.

In this paper, we propose a new method for analysing the decay dynamics of densely formed, excited states and ionic species. A convolved autoregressive model is modified to represent the non-linear bimolecular decay process, which is still linear with respect to unknown model parameters. A simple linear least-squares technique is used to fit the convolved autoregressive model to a decay curve. In addition, the method has the ability to analyse a bimolecular decay process during irradiation by excitation light.

2. Theory

2.1. Analysis of a decay process after intense excitation

When the time-resolved measurement of the relaxation processes of dense excited and ionic states is performed with a time interval of Δt , Eq. (4) can be rewritten in a discrete form as

$$x(n\Delta t) = \frac{1}{(x_0^{-1} + k_b k_u^{-1}) \exp(k_u n\Delta t) - k_b k_u^{-1}} \quad (5)$$

We now show that $x(n\Delta t)$ given by Eq. (5) can also be expressed as the following convolved autoregressive model

$$x(n\Delta t) = Bx[(n-1)\Delta t] + Cx(n\Delta t)x[(n-1)\Delta t] \quad (6)$$

where B and C are model parameters which uniquely correspond to k_u and k_b as follows

$$B = \exp(-k_u \Delta t) \quad (7)$$

$$C = k_b k_u^{-1} (B - 1) \quad (8)$$

Eq. (6) shows that a decay curve x at the n th time is given by a linear combination of a value x at one-past time (the first term on the right-hand side) and a product of x at the present and one-past times (the second term on the right-hand side). The derivation of this convolved autoregressive model is described below.

First, we define a curve $u(n\Delta t)$ as an inverse of $x(n\Delta t)$ as

$$u(n\Delta t) = x(n\Delta t)^{-1} = (x_0^{-1} + k_b k_u^{-1}) \exp(k_u n\Delta t) - k_b k_u^{-1} \quad (9)$$

Using z transform [10], Eq. (9) becomes

$$U(z) = \frac{x_0^{-1} + k_b k_u^{-1}}{1 - \exp(k_u \Delta t) z^{-1}} - \frac{k_b k_u^{-1}}{1 - z^{-1}} \quad (10)$$

where $U(z)$ represents a z transform of $u(n\Delta t)$. Rearrangement of Eq. (10) yields

$$\{1 - \exp(k_u \Delta t)z^{-1}\}U(z) = x_0^{-1} + \frac{k_b k_u^{-1} \{\exp(k_u \Delta t) - 1\}}{1 - z^{-1}} \quad (11)$$

An inverse z transform of Eq. (11) for $n > 1$ is given by

$$u(n\Delta t) - \exp(k_u \Delta t)u[(n-1)\Delta t] = k_b k_u^{-1} \{\exp(k_u \Delta t) - 1\} \quad (12)$$

This regressive equation of $u(n\Delta t)$ can be rewritten by the following expression with respect to $x(n\Delta t)$

$$x(n\Delta t) = \exp(-k_u \Delta t)x[(n-1)\Delta t] + [k_b k_u^{-1} \{\exp(-k_u \Delta t) - 1\}]x(n\Delta t)x[(n-1)\Delta t] \quad (13)$$

This is just the convolved autoregressive model given by Eqs. (6)–(8).

Eq. (6) is a linear form with respect to model parameters B and C even though the bimolecular decay is governed by a non-linear differential equation. Thus the unknown parameters can be estimated by linear least-squares fitting, where Eq. (6) is fitted to an observed curve so that the sum of the squared residuals between left- and right-hand sides at N sampling points is minimized. The least-squares estimates are given in a matrix form as

$$p = ([X]'^[X])^{-1}[X]'x \quad (14)$$

where

$$p = [B, C]' \quad (15)$$

$$x = [x(1), x(2), \dots, x(N-1)]' \quad (16)$$

$$[X] = \begin{bmatrix} x(0), & x(1), & \dots, & x(N-2) \\ x(1)x(0), & x(2)x(1), & \dots, & x(N-1)x(N-2) \\ y(1), & y(2), & \dots, & y(N-1) \\ y(1)x(0), & y(2)x(1), & \dots, & y(N-1)x(N-2) \end{bmatrix}' \quad (17)$$

$x(n)$ is an abbreviation of $x(n\Delta t)$. The rate constants of unimolecular and bimolecular decay, k_u and k_b , are determined from the given B and C values using Eqs. (7) and (8).

2.2. Analysis of a decay process during intense excitation

Eq. (6) is a regressive form so that $x(n\Delta t)$ can be determined from $x[(n-1)\Delta t]$ as

$$x(n\Delta t) = \frac{Bx[(n-1)\Delta t]}{1 - Cx[(n-1)\Delta t]} \quad (18)$$

Unfortunately, this relation is not valid for a bimolecular process during irradiation by excitation light, since the density of the excited or ionic state is increased by excitation compensating for the decay process. If it is assumed that the increase in the number of excited or ionic molecules during a time period from $(n-1)\Delta t$ to $n\Delta t$ is proportional to the

intensity of excitation light at that time, the decay curve $x(n\Delta t)$ during excitation is given by

$$x(n\Delta t) = \frac{Bx[(n-1)\Delta t]}{1 - Cx[(n-1)\Delta t]} + Ay(n\Delta t) \quad (19)$$

where $y(t)$ represents the temporal intensity profile of an excitation pulse and A is a constant relating to the quantum yield. Rearrangement of Eq. (19) results in

$$x(n\Delta t) = Bx[(n-1)\Delta t] + Cx(n\Delta t)x[(n-1)\Delta t] + Ay(n\Delta t) - ACy(n\Delta t)x[(n-1)\Delta t] \quad (20)$$

This is a convolved autoregressive model of a bimolecular decay process during excitation.

A linear least-squares fitting of this model to an observed curve is given by the same expression as Eq. (14), while Eqs. (15) and (17) are replaced by

$$p = [B, C, A, D]' \quad (21)$$

$$[X] = \begin{bmatrix} x(0), & x(1), & \dots, & x(N-2) \\ x(1)x(0), & x(2)x(1), & \dots, & x(N-1)x(N-2) \\ y(1), & y(2), & \dots, & y(N-1) \\ y(1)x(0), & y(2)x(1), & \dots, & y(N-1)x(N-2) \end{bmatrix}' \quad (22)$$

respectively, where $D = -AC$, which is not used to estimate A and C . By substituting a decay curve $x(n\Delta t)$ and an excitation pulse shape $y(n\Delta t)$ into Eqs. (16) and (22), model parameters A , B and C are given by Eqs. (14) and (21), and rate constants k_u and k_b are determined using Eqs. (7) and (8).

3. Numerical and experimental examples

3.1. Analyses of computer-simulated decays

We have analysed the computer-simulated decay curves to demonstrate the effectiveness of the present method. Since a bimolecular decay process during irradiation with excitation light cannot be simulated from an analytical expression, artificial decay curves were computed from Eq. (5) for decay processes after excitation. Fig. 1(a) and (b) show simulated decays with a common rate constant k_u of $1.0 \times 10^7 \text{ s}^{-1}$ and different k_b values of 1.0×10^5 and $1.0 \times 10^4 \text{ s}^{-1}$ counts $^{-1}$ respectively. The initial intensity x_0 and the sampling interval Δt were set to 10^3 and 0.4 ns respectively. The number of sampling points was 1024. The curves were contaminated with Poisson noise, whose standard deviation was equal to the square root of the intensity. Least-squares fitting of the convolved autoregressive model given by Eq. (6) was performed with a noise reduction technique proposed previously [4]. The calculation was executed on a work station (HP Series 700).

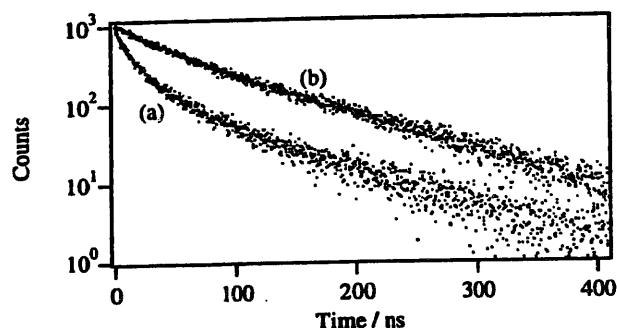


Fig. 1. Simulated decay curves with rate constants $k_u = 1.0 \times 10^7 \text{ s}^{-1}$ and $k_b = 1.0 \times 10^5 \text{ s}^{-1} \text{ counts}^{-1}$ (a) and $k_b = 1.0 \times 10^4 \text{ s}^{-1} \text{ counts}^{-1}$ (b).

The first column of Table 1 shows the rate constants estimated for the decay curves of Fig. 1 (a) and (b) by the present method. We prepared 100 curves for each decay with random noise, and the estimates for individual curves were averaged. Statistical errors were also calculated as a measure of the accuracy. For comparison, the rate constants k_b were estimated by fitting a straight line to a plot of $x(t)^{-1}$ against $\exp(k_u t)$, where k_u was assumed to be known exactly [5] (the second column of Table 1). The results show that the accuracy of the straight line fitting is lower than that of the proposed method in spite of the use of a priori information on k_u . A non-linear least-squares method was also applied to the decay analyses of Fig. 1 (a) and (b), where the theoretical curve of Eq. (5) was fitted to the data. The program was written with reference to Bevington's work [11], which was refined to shorten the computation time. By comparing the results obtained using the proposed method with those obtained using non-linear least-squares fitting, no appreciable difference in the accuracy of estimation was found, but the computation time of the proposed method is approximately 16 times shorter than that of the non-linear least-squares method.

3.2. Analyses of fluorescence decays

We have applied the present method to the analysis of the excited state annihilation processes of the pyrene excimer. It has been reported that pyrene forms an excimer readily in a concentrated solution of a low-viscosity solvent and exhibits diffusion-controlled bimolecular interaction on high-intensity excitation [5].

Table 1

Estimated rate constants and computation time for the fitting of the data of Fig. 1 (a) and (b) by the convolved autoregressive (CAR), straight line (SL) and non-linear least-squares (NLLS) methods

	CAR	SL	NLLS
$k_u \text{ (s}^{-1}\text{)}^a$	$(0.999 \pm 0.012) \times 10^7$	–	$(1.000 \pm 0.022) \times 10^7$
$k_b \text{ (s}^{-1} \text{ counts}^{-1}\text{)}^a$	$(1.001 \pm 0.021) \times 10^5$	$(0.890 \pm 0.015) \times 10^5$	$(1.000 \pm 0.017) \times 10^5$
$k_u \text{ (s}^{-1}\text{)}^b$	$(1.000 \pm 0.006) \times 10^7$	–	$(0.999 \pm 0.012) \times 10^7$
$k_b \text{ (s}^{-1} \text{ counts}^{-1}\text{)}^b$	$(1.003 \pm 0.030) \times 10^4$	$(0.926 \pm 0.017) \times 10^4$	$(1.001 \pm 0.039) \times 10^4$
Computation time (ms)	45	5	727

^a Fig. 1(a).

^b Fig. 1(b).

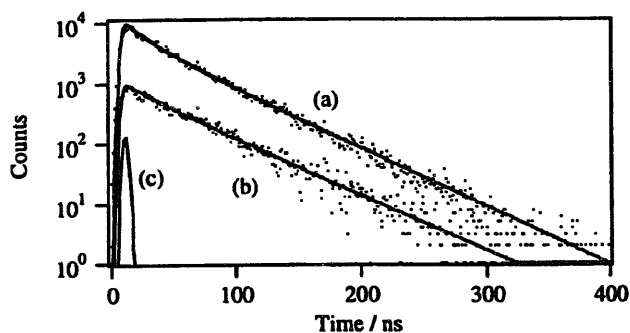


Fig. 2. Fluorescence decay curves of pyrene excimer in toluene (0.25 mol dm^{-3}) observed on excitation with intensity of approximately 3.2 mJ cm^{-2} (a) and approximately 1.3 mJ cm^{-2} (b). Excitation and emission wavelengths were 355 and 480 nm respectively. (c) Temporal profile of an excitation pulse.

Pyrene (Nacali Tesque Inc., GR grade) was recrystallized twice from ethanol after chromatography on silica gel–*n*-hexane. A toluene solution of pyrene (0.25 mol dm^{-3}) was degassed by a freeze–pump–thaw method and placed in a quartz cell (optical path length, 1 mm). The sample was placed on an optical microscope (Nikon Optiphot-2) and excited with the third harmonic pulse of a Q-switched Nd:YAG laser (Spectron SL282G, 355 nm). The laser pulses were introduced into the microscope and focused into a spot of approximately $50 \mu\text{m}$ by an objective lens ($\times 10$, NA = 0.12). Fluorescence from the sample was collected by the same objective lens and detected with a polychromator-attached streak camera (Hamamatsu Photonics C4334; temporal resolution, 15 ps).

Fig. 2 shows the fluorescence decay curves of pyrene in toluene, measured at 480 nm, where pyrene excimer emission only is observed. The intensities of the excitation pulse were approximately 3.2 and approximately 1.3 mJ cm^{-2} for the data in Fig. 2(a) and (b) respectively. Fig. 2(c) shows an excitation pulse profile obtained by measuring the scattered light (duration, approximately 5 ns). The whole curve, including the initial time region during irradiation with the excitation light, was analysed with the convolved autoregressive model of Eq. (20); this cannot be performed by straight line fitting or the non-linear least-squares method. Table 2 shows the decay constants of Fig. 2(a) and (b) estimated using the pulse shape of Fig. 2(c). The computation time for each decay curve was 25 ms. The rate constants of the uni-

Table 2

Estimated rate constants for the data of Fig. 2(a) and (b) fitted by a convolved autoregressive model

k_u (s^{-1})	k_b (s^{-1} counts $^{-1}$)	k_{ss} (s^{-1} mol $^{-1}$ dm 3)
2.2×10^7 ^a	1.9×10^3	1.4×10^{10}
2.1×10^7 ^b	7.3×10^3	1.3×10^{10}

^a Fig. 2(a).

^b Fig. 2(b).

molecular decays k_u agree well with a reported value [5]. On the other hand, the bimolecular decay rate k_b is dependent on the excitation intensity. The rate constant of the annihilation process of the pyrene excimer k_{ss} can be obtained from k_b using the estimated model parameter A and the concentration of the excited molecules. The concentrations were determined to be 1.4×10^{-3} and 5.7×10^{-4} mol dm $^{-3}$ for 3.2 and 1.3 mJ cm $^{-2}$ excitation respectively using the molar extinction coefficient of ground state pyrene at the excitation wavelength ($\epsilon \sim 300$ cm $^{-1}$ mol $^{-1}$ dm 3). The estimated rate constants k_{ss} are 1.4×10^{10} and 1.3×10^{10} s $^{-1}$ mol $^{-1}$ dm 3 for the decays of Fig. 2(a) and (b) respectively, i.e. the rate constant is almost independent of the excitation intensity and consistent with the diffusion-limited bimolecular reaction rate [12], which was calculated to be 1.1×10^{10} s $^{-1}$ mol $^{-1}$ dm 3 using the viscosity of toluene ($\rho = 5.9 \times 10^{-4}$ kg m $^{-1}$ s $^{-1}$) at room temperature (293 K).

4. Conclusions

A new method has been proposed for the analysis of the relaxation dynamics of excited states and ionic species on high-intensity excitation so that the rate constants of uni-

molecular and bimolecular decays can be estimated from a transient curve. An advantage of the present method over the conventional non-linear least-squares technique is its extremely short computation time, which is due to the linear algorithm based on a convolved autoregressive model. The analysis can be performed on a desk top computer in a tolerable time, while the conventional method requires a high-performance computer. Furthermore, this advantage is indispensable for analyses of many decay profiles, e.g. multidimensional decay curves such as time-resolved spectra and dynamic imaging data [13]. The method can be extended to the analysis of a bimolecular decay process which overlaps excitation.

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