Published in final edited form as:

Spectrochim Acta A Mol Biomol Spectrosc. 1997 September ; 53A(10): 1637–1644. doi:10.1016/s1386-1425(97)00095-4.

Two-photon excitation of dioxane: time-resolved measurements of excited state complex formation with water

Joseph R. Lakowicz*, Ignacy Gryczynski, Kazmierz Nowaczyk

Center for Fluorescence Spectroscopy and Medical Biotechnology Center, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 25 W. Lombard Street, Baltimore, MD 21201, USA

Abstract

We observed emission from the non-aromatic hydrocarbon 1,4-dioxane upon illumination with ps pulses at 380 nm. The emission intensity depended quadratically on incident power at 380 nm, indicating a two-photon process. In the absence of water the intensity decay was close to a single exponential, but displayed some evidence of an excited state process. In the presence of 1% water the emission spectra shifted dramatically to long wavelength. Water also resulted in wavelength-dependent intensity decays with negative pre-exponential factors on the long wavelength side of the emission, demonstrating the presence of an excited state reaction. At this water concentration the results are consistent with a two-state model due to emission from dioxane and a dioxane-water complex.

Keywords

Fluorescence; Time-resolved fluorescence; Two-photon excitation; Alkane fluorescence; Exciplex

1. Introduction

During the past 5 years two-photon excitation has been an active area of research in time-resolved fluorescence [1–3] and in fluorescence microscopy [4–8]. The advantages of two-photon excitation (2PE) include the use of the long-wavelength fundamental output of dye and Ti:Sapphire lasers as the excitation source, localization of the excited volume at the point of focus, and the absence of photobleaching or phototoxicity outside of the region of observation.

In several recent reports we described an additional advantage of two-photon excitation, this being the ability to excite alkane fluorescence with ultraviolet (UV) rather than vacuum ultraviolet (VUV) wavelengths. Using TPE we were able to observe emission from cyclohexane [9] and tetradecane [10] using excitation at 300 nm. Our studies of alkane fluorescence were stimulated by the pioneering studies of Lipsky [11–13] and Ware [14,15] of alkane fluorescence with VUV excitation.

*Corresponding author.

While not widely known, the oxygen-containing hydrocarbon 1,4-dioxane is also fluorescent. Lipsky and co-workers [16] previously reported that dioxane was fluorescent when excited at 185 nm. Additionally, Ware and co-workers [17] reported the lifetime of dioxane to be 2.15 ns. Both groups [16,17] suggested that the emission from neat dioxane appeared to be the result from some type of complex, as suggested by the decreased dioxane quantum yield with dilution [16] and the large apparent radius of 13 Å for oxygen quenching [17]. Lipsky also reported [16] that the emission spectrum of dioxane displayed a long wavelength shift upon addition of water.

In the present report we extend these pioneering studies of dioxane fluorescence by the use of two-photon excitation, and wavelength dependent intensity decay measurements to construct the time-resolved emission spectra.

2. Materials and methods

Dioxane (1,4-dioxane) was obtained from Aldrich (99.9% HPLC Grade). Preliminary experiments revealed the presence of water, and the need for additional purification. Dioxane was purified as follows: 500 ml of dioxane was refluxed with 20 ml of 6M HCl under stream of nitrogen for 6 h. Next the mixture was neutralized with pellets of KOH. The decanted solvent was dried with NaH (sodium hydride) and distilled from NaH twice. The solvent was kept in the dark and without air contact.

Excitation of dioxane at 380 nm was accomplished using the frequency-doubled output of a Pyridine 2 dye laser, 2 MHz repetition rate, with a pulse width near 7 ps. The excitation light was focused with best form laser lens from Oriel, 2 cm focal length, to an appropriate 20 μ diameter spot. The average intensity was about $1.5 \times 10^3 \text{ W cm}^{-2}$ and the maximum peak intensity was about $1 \times 10^8 \text{ W cm}^{-2}$. Emission of dioxane was observed using interference filters with a 10 nm bandpass. Additionally, we use a short wave pass (SWP) filter to eliminate scattered light at 380 nm. Polarizers were not used in the excitation or emission paths, but the 380 nm dye lasers output was horizontally polarized. All measurements were performed at 25°C in the presence of dissolved oxygen due to equilibration with the air.

Frequency-domain measurements of the intensity decays were performed using the 10 GHz instrument described previously [18]. Emission spectra were collected with a monochromator (2 nm bandpass) and a Hamamatsu R928 PMT.

Intensity decays were analyzed on terms of the multi-exponential model

$$I(\lambda, t) = \sum_{i=1}^2 \alpha_i(\lambda) e^{-t/\tau_i} \quad (1)$$

where $I(\lambda, t)$ is the intensity decay at each wavelength λ , and $\alpha_i(\lambda)$ are the wavelength-dependent amplitudes, $\alpha_1(\lambda) + \alpha_2(\lambda) = 1.0$. Two decay times (τ_i) were found to be adequate to explain the data at all wavelengths. The frequency-domain data were analyzed to recover the parameters in Eq. (1) as described previously [19,20]. The data at all wavelengths were analyzed globally [21] with the decay time the same at all wavelengths, and the $\alpha_i(\lambda)$ values different for each wavelength.

The wavelength-dependent intensity decays were used to construct the time-resolved emission spectra (TRES), that is, the emission spectra which would be observed through an instantaneous time-window at the chosen time. These TRES were calculated from the $I(\lambda, t)$, and the steady state emission spectrum, as described by Brand and co-workers for time-domain data [22] and by this laboratory for frequency-domain data [23].

In the presence of an excited state process one expects the spectra to shift to longer wavelengths at longer times. The rate of spectral relaxation and the nature of the intensity process can be understood by examination of the time-dependent emission centers of the time-dependent emission center of gravity ($\nu_{cg}(t)$) and the time-dependent half-width ($\nu_{cg}(t)$). These time-dependent parameters were calculated from the FD data as described previously [24].

3. Results

Emission spectra of neat dioxane are shown in Fig. 1 for excitation at 380 nm. Dioxane shows a surprising intense emission with a maximum near 250 nm. Since dioxane does not absorb above 280 nm, the excitation must be a multi-photon process. We examined the effect of attenuation the 380 nm excitation intensity on the emission intensity of dioxane. This dependence was found to be precisely quadratic (Fig. 1, insert), demonstrating two-photon excitation of dioxane at 380 nm. Observation of two-photon excitation of dioxane near 380 nm is in agreement with previous studies of resonance-enhanced two-photon ionization of dioxane at this wavelength [25].

We examine the effects of a small amount of water on the emission spectrum of dioxane. Addition of 1% water (0.555 M) resulted in a dramatic shift of the dioxane emission to longer wavelengths (Fig. 2, bottom). This effect of water was observed previously by Lipsky [16]. It is interesting to notice that the effect of water on dioxane fluorescence is distinct from the effects of water on alkane fluorescence. In the case of cyclohexane and tetradecane, we found that emission was collisionally quenched, but not shifted, by water and alcohols [9,10].

A priori, the effect of water in dioxane could be the result of solvent polarity effects, ground state complex formation, or formation of an excited state complex. General solvent effects seem unlikely because such effects require a change in the overall polarity on the solvent, which are not significant for a small volume addition of water. Distinction between ground state complex formation or an excited state process can be revealed by time-resolved measurements. In particular, formation of a new emitting species in the excited state can be demonstrated by the presence of a negative preexponential factor in the intensity decay [26], or phase angles in excess of 90° [27,28]. Hence we measured the wavelength-dependent intensity decays across the emission spectra of dioxane.

Fig. 2 shows the frequency-domain intensity decay of neat dioxane observed on the blue (220 nm) and red (290 nm) sides of the emission. Compared to similar data in the presence of water (Fig. 3, below) there is only a modest difference between these wavelengths. Nonetheless, these differences are experimentally significant, and suggest the presence of an

excited state process in neat dioxane. The occurrence of an excited state process is consistent with the one-photon experiments of Hirayama et al. [16] who found that the emission spectrum of dioxane shifts to shorter wavelengths, and the quantum yield decreases, when dioxane is diluted in isooctane.

To better quantify the excited state processes in dioxane we measured the frequency-domain intensity decay at six wavelengths across the emission spectra (Fig. 1, top, arrows). These data were analyzed globally to obtain the wavelength-dependent intensity decays (Table 1). The data could not be fit to single exponential intensity decay, which resulted in a goodness-of-fit χ^2_R value of 24.2. However, two decay times were found adequate to account for the data at all emission wavelengths from 220 to 290 nm, yielding $\chi^2_R = 1.2$. Importantly, this analysis shows that the intensity decays at 280 and 290 nm displays a negative amplitude, which proves the presence of an emitting species formed following excitation [22,26]. Hence, these results confirm the speculation of Lipsky [16] and Ware [17] that the emission from neat dioxane is due, at least in part, to some type of excited state complex between dioxane molecules. It seems likely that the emission from dioxane monomers and higher aggregates display overlapping emission spectra, which is consistent with the $\alpha(\lambda)$ values being less than equal and opposite [22,26–28]. Further studies are needed to clarify the nature of this complex.

Frequency-domain intensity decay data were also obtained for dioxane containing 1% water. In this case the FD intensity decay data are dramatically dependent on emission wavelength (Fig. 3). At short wavelengths (225 nm) a complex frequency response was observed with evidence of subnanosecond and nanosecond decay time components. On the long wavelength side of the emission (335 nm) the phase angles are as large as 100°, which proves the existence of an excited state reaction [27,28]. Global analysis of these data reveal the existence of at least two decay times, 0.21 and 2.97 ns (Table 2). In the presence of 1% water there is a large negative pre-exponential at 315 and 335 nm. Hence these data unambiguously demonstrate that dioxane, or a dioxane aggregate, reacts with water to yield a long wavelength emitting species.

The rate and nature of the excited state process can be determined by examination of the time-resolved emission spectra. More specifically, an excited state spectral shift can be due to a continuous relaxation process in which a constant emission spectral shape shifts to longer wavelengths with time following pulsed excitation [29]. Alternatively, the emission can shift due to the decay of one spectral shape and the appearance of a second emission spectrum. Such spectral shifts are often observed for excited state protonation reactions, exemplified by acridine [26] or naphthol [30]. For continuous spectral relaxation, the spectral shape is invariant with time [29] and one expects the half-widths of the emission to be time-invariant. In contrast, for a two-state model, two emission spectra are expected at intermediate times, so the half-width is expected to increase at times when both species contribute [31].

We used the wavelength-dependent intensity decays in Tables 1 and 2 to construct the time-resolved emission spectra. For neat dioxane we observed a small spectral shift from 0 to

1000 ps (Fig. 4, top). This spectral shift appears to occur with a relaxation time near 0.5 ns (Fig. 5, top). The spectrum may become slightly more narrow as relaxation proceeds (Fig. 6, top), but this effect is minor. In any event, there was no evidence of a transient increase in spectral half width.

The presence of 1% water in dioxane resulted in remarkably different TRES (Fig. 4, bottom). The emission spectra were found to shift dramatically to longer wavelengths from 30 to 1000 ps. The spectral relaxation time is near 400 ps, as estimated from the time-resolved emission center-of-gravity (Fig. 5, bottom). Examination of the TRES reveals that the emission spectrum appears wider at 300 ps and more narrow at shorter (30 ps) or longer (1000 and 3000 ps) times. A detailed examination of the TRES shows the widest emission near 120 ps. This transient increase in half-widths is seen explicitly in Fig. 6 (bottom). These results are analogues to those observed previously for a two-state process [31], and suggest the presence of two or more species with different emission spectra.

The TRES reveal the time-dependent shifts, but do not reveal the emission spectra of the individual species. Hence we examine the decay associated spectra (DAS). These spectra are given by

$$I_i(\lambda) = \alpha_i(\lambda)I(\lambda) \quad (2)$$

where $I(\lambda)$ is the steady state emission spectrum. In the case of mixtures of non-interacting fluorophores the DAS represent the emission spectra of the individual species [32]. DAS can also be constructed for an excited state process [33], but in this case the DAS typically do not represent emission spectra of the individual species.

Decay associated spectra of neat dioxane are shown in Fig. 7 (top). These spectra indicate the presence of an excited state reaction as indicated by the values of $\alpha_1(\lambda)$ which are both positive and negative [33]. In favorable cases, where the number of emitting species are known, such data may be used to recover the emission spectra of the emitting species [34].

Decay associated spectra in the presence of 1% water are shown in Fig. 7 (bottom). Since some of the pre-exponential factors are negative (Table 2) the DAS also show negative amplitudes. The characteristic shape of $\alpha_2(\lambda)$ indicates an excited state process in which the rate of deactivation of the initially excited state is more rapid than deactivation of the reacted state [33]. Also, the DAS of the long wavelength component is expected to represent the emission spectrum of the reactive product [33]. This was found to be approximately correct, as seen by comparison of the DAS and TRES of the 2.97 ns component in Fig. 7 (bottom).

It is of interest to consider the rate of reaction of dioxane with water. From Tables 1 and 2 one can estimate that the decay time of neat dioxane is near 2 ns, and this decay time is decreased to about 0.2 ns in the presence of 0.555 M water. These decay times can be analyzed in terms of the Stern–Volmer equation

$$\frac{\tau_0}{\tau} = 1 + k_q \tau_0 [\text{H}_2\text{O}] \quad (3)$$

where τ_0 and τ are the unquenched and quenched dioxane lifetimes and k_q is the biomolecular quenching constant. Hence, the values of k_q for dioxane-water is near $9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. This value is comparable to that found for collisional quenching of fluorophores by low molecular weight quenchers [35] and suggests that the interaction with water is diffusion controlled. This value of k_q does not suggest the presence of dioxane aggregates, as suggested from the oxygen quenching data [17].

4. Discussion

Dioxane displays interesting photophysical properties in neat solution and in the presence of water. However, relatively few studies have appeared due to the difficulties of excitation near 185 nm, which require removal of all surrounding oxygen and the use of special optics. The growing availability of ps and fs lasers makes it possible to use two-photon excitation, and thus avoid the difficulties of VUV excitation.

The present result should be regarded as preliminary, and indicative of the need for additional studies of dioxane emission. For instance, to the best of our knowledge, there have been no reports of the anisotropy on dioxane or alkane emission. Also, it seems possible that higher concentrations of water may result in formation of additional species and/or a distribution of dioxane aggregates.

Finally, it should be noted that derivatives of dioxane could be used as extrinsic probes in proteins or membranes. One can imagine amino acid analogues of dioxane, or lipids which contain the dioxane ring. The high sensitivity of dioxane to water could provide a sensitive indicator of water penetration into proteins or membranes.

Acknowledgements

This work was supported by grants from the National Institutes of Health (RR-10416) and the National Center for Research Resources (RR-08119). The authors thank Dr A. Razynska for dioxane purification.

Abbreviations:

TRES	time-resolved emission spectra
1PE and 2PE	one- or two-photon excitation
FD	frequency-domain
DAS	decay associated spectra
UV	ultraviolet
VUV	vacuum ultraviolet

References

- [1]. Lakowicz JR, Kusba J, Danielsen E, Two photon-induced fluorescence intensity and anisotropy decays of diphenylhexatriene in solvents and lipid bilayers, *J. Fluoresc.* 2 (1992) 241–258.
- [2]. Lakowicz JR, Gryczynski I, Tryptophan fluorescence intensity and anisotropy decays of human serum albumin resulting from one-photon and two-photon excitation, *Biophys. Chem* 45 (1993) 1–6.
- [3]. Lakowicz JR, Gryczynski I, Fluorescence intensity and anisotropy decay of the 4',6-diamidino-2-phenylindole DNA complex resulting from one-photon and two-photon excitation, *J. Fluoresc* 2 (1992) 117–122. [PubMed: 24243275]
- [4]. Denk J, Strickler JH, Webb WW, Two-photon laser scanning fluorescence microscopy, *Science* 248 (1990) 73–76. [PubMed: 2321027]
- [5]. Denk W, Piston DW, Webb WW, Two-photon molecular excitation in laser-scanning microscopy, in: Pawley JB (Ed.), *Handbook of Biological Confocal Microscopy*, Plenum Press, New York, 1995, pp. 445–458.
- [6]. Lindek S, Stelzer EHK, Hell SW, Two new high-resolution confocal fluorescence microscopies (4Pi, Theta) with one- and two-photon excitation, in: Pawley JB (Ed.), *Handbook of Biological Confocal Microscopy*, Plenum Press, New York, 1995, pp. 417–430.
- [7]. Hell SW, Lindek S, Stelzer EH, Enhancing the axial resolution in far-field microscopy: two-photon 4Pi confocal fluorescence microscopy, *J. Mod. Optics* 41 (1994) 675–681.
- [8]. Denk W, Two-photon excitation in functional biological imaging, *J. Biomed. Optics* 1 (3) (1996) 296–304.
- [9]. Gryczynski I, Lakowicz JR, Quenching of methylcyclohexane fluorescence by methanol, *Photochem. Photobiol* 62 (3) (1995) 426–432.
- [10]. Gryczynski I, Razynska A, Lakowicz JR, Two-photon induced fluorescence of linear alkanes: a possible intrinsic lipid probe, *Biophys. Chem* 57 (1995) 291–295.
- [11]. Rothman W, Hirayama F, Lipsky S, Fluorescence of saturated hydrocarbons: III, effect of molecular structure, *J. Chem. Phys* 58 (4) (1973) 1300–1317.
- [12]. Hirayama F, Lipsky S, Fluorescence of saturated hydrocarbons, *J. Chem. Phys* 51 (1969) 3616–3617.
- [13]. Hirayama F, Rothman W, Lipsky S, Fluorescence of saturated hydrocarbons: II, the effect of alkyl substituents, *Chem. Phys. Lett* 5 (1970) 296–298.
- [14]. Ware WR, Lyke RL, Fluorescence lifetimes of saturated hydrocarbons. *Chem. Phys. Lett* 24 (2) (1974) 195–198.
- [15]. Ware WR, O'Connor DV, Fluorescence lifetimes of saturated hydrocarbons in the vapour phase, *Chem. Phys. Lett* 62 (1979) 595–599.
- [16]. Hirayama F, Lawson CW, Lipsky S, Fluorescence of *p*-dioxane, *J. Phys. Chem* 74 (11) (1970) 2411–2413.
- [17]. Halpern AM, Ware WR, Fluorescence of *p*-dioxane: lifetime and oxygen quenching, *J. Phys. Chem* 74 (11) (1970) 2413–2414.
- [18]. Laczko G, Lakowicz JR, Gryczynski I, Gryczynski Z, Malak H, A 10 GHz frequency-domain fluorometer, *Rev. Sci. Instrum* 61 (1990) 2331–2337.
- [19]. Lakowicz JR, Gratton E, Laczko G, Cherek H, Limkerman M, Analysis of fluorescence decay kinetics from variable-frequency phase shift and modulation data, *Biophys. J* 46 (1984) 463–477. [PubMed: 6498264]
- [20]. Gratton E, Lakowicz JR, Maliwal B, Cherek H, Laczko G, Limkerman M, Resolution of mixtures of fluorophores using variable-frequency phase and modulation data, *Biophys. J* 46 (1984) 479–486. [PubMed: 6498265]
- [21]. Beechem JM, Ameloot M, Brand L, Global and Target analysis of complex decay phenomena, *Anal. Instrum* 14 (3/4) (1985) 379–402.
- [22]. Easter JH, DeToma RP, Brand L, Nanosecond time-resolved emission spectroscopy of a fluorescence probe adsorbed to L-egg lecithin vesicles, *Biophys. J* 16 (1976) 571–583. [PubMed: 945086]

- [23]. Lakowicz JR, Cherek H, Maliwal B, Laczko G, Gratton G, Gratton E, Determination of time-resolved fluorescence emission spectra and anisotropies of a fluorophore-protein complex using frequency-domain phase-modulation fluorometry, *J. Biol. Chem* 259 (1984) 10967–10972. [PubMed: 6469993]
- [24]. Lakowicz JR, Cherek H, Laczko G, Gratton E. Time-resolved fluorescence emission spectra of labeled phospholipid vesicles, as observed using frequency-domain fluorometry, *Biochem. Biophys. Acta* 777 (1984) 183–193.
- [25]. Cornish TJ, Baer T, Pedersen LG, The $n + 3s$ Rydberg transition of jet-cooled tetrahydropyran, 1,4-dioxane, and 1,4-dioxane-d, studied by $2 + 1$ resonance-enhanced multiphoton ionization, *J. Phys. Chem* 93 (1989) 6064–6069.
- [26]. Gafni A, Brand L, Excited state proton transfer reactions of acridine studied by nanosecond fluorometry, *Chem. Phys. Lett* 58 (3) (1978) 346–350.
- [27]. Lakowicz JR, Balter A, Theory of phase-modulation fluorescence spectroscopy for excited state processes, *Biophys. Chem* 16 (1982) 99–115. [PubMed: 7139052]
- [28]. Lakowicz JR, Baiter A, Analysis of excited state processes by phase-modulation fluorescence spectroscopy, *Biophys. Chem* 16 (1982) 117–132. [PubMed: 7139044]
- [29]. Bakhshiev NG, Mazurenko Yu.T., Piteriskaya IV, Luminescence decay in different portions of the luminescence spectrum of molecules in a viscous solution. *Opt. Spectrosc* 21 (1966) 307–309.
- [30]. Laws JR, Brand L, Analysis of two-state excited-state reactions: the fluorescence decay of 2-naphthol, *J. Phys. Chem* 83 (1979) 795–802.
- [31]. DeToma RP, Brand L, Excited state solvation dynamics of 2-anilinonaphthalene, *Chem. Phys. Lett* 47 (1977) 231–236.
- [32]. Beechem JM, Ameloot M, Brand L, Global and target analysis of complex decay phenomena, *Anal. Instrum* 14 (34) (1985) 379–402.
- [33]. Davenport L, Knutson JR, Brand L, Excited-state proton transfer of equilenin and dihydroequilenin: interaction with bilayer vesicles, *Biochemistry* 25 (1986) 1186–1195. [PubMed: 3964666]
- [34]. Beechem JM, Ameloot M, Brand L, Global and target analysis of complex decay phenomena, *Anal. Instrum* 14 (3,4) (1986) 379–402.
- [35]. Lakowicz JR, Weber G, et al., Quenching of fluorescence by oxygen-A-probe for structural fluctuations in macromolecules, *Biochemistry* 12 (1973) 4161–4170. [PubMed: 4795686]

Fig. 1. Emission spectra at neat dioxane (top) and dioxane with 1% water (bottom) for excitation at 380 nm. The insert (top) shows the dependence of the 250 nm emission on the excitation intensity at 380 nm. The maximal intensity at 380 nm was 5 mW and focused to approximately $300 \mu^2$. The arrows show the wavelength used to collect the wavelength-dependent intensity decays.

Fig. 2.
Frequency-domain intensity decays of neat dioxane with 2PE at 380 nm, for observation at 220 or 290 nm.

Fig. 3.

Frequency-domain intensity decays of dioxane-water (99:1, V:V) with 380 nm excitation. The observation wavelength were varied from the short (bottom) to long (top) wavelength sides of the emission.

Fig. 4.
Time-resolved emission spectra of neat dioxane (top) and dioxane, containing 1% water (bottom) with 2PE at 380 nm.

Fig. 5. Time-resolved emission center-of-gravity for neat dioxane (top) and dioxane with 1% water (bottom) with 2PE at 380 nm.

Fig. 6.
Time-resolved spectral half-width of neat dioxane (top) and dioxane with 1% water (bottom) with 2PE at 380 nm.

Fig. 7. Decay associated emission spectra of neat dioxane (top) and dioxane with 1% water (bottom) with 2PE at 380 nm.

Table 1

Multiwavelength analysis of the intensity decay of neat dioxane

Wavelength (nm)	α_1 ($\tau_1 = 0.46$ ns)	α_2 ($\tau_2 = 2.11$ ns)	χ_R^2
220	0.246	0.754	
230	0.211	0.789	
250	0.052	0.948	
260	0.008	0.992	
280	-0.048	0.952	
290	-0.155	0.845	$1.2^a (24.2)^b$

^aValue of χ_R^2 for the global two decay time fit using $\delta\phi = 0.4^\circ$ and $\delta m = 0.01$.

^bValue of χ_R^2 for the global single decay time fit.

Table 2

Multiwave analysis of the intensity decay of a dioxane-water mixture, containing 1% of water

Wavelength (nm)	α_1 ($\tau_1 = 0.21$ ns)	α_2 ($\tau_2 = 2.97$ ns)	χ_R^2
255	0.863	0.137	
275	0.681	0.319	
295	0.283	0.717	
305	0.152	0.848	
315	-0.224	0.776	
335	-0.361	0.639	1.5^a (765.0) ^b

^aValue of χ_R^2 for the global two decay time fit using $\delta\phi = 0.4^\circ$ and $\delta m = 0.01$.^bValue of χ_R^2 for the global single decay time fit.