

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/221748698>

[Ru(bpy)(3)](2+) as a reference in transient absorption spectroscopy: differential absorption coefficients for formation of the long-lived (MLCT)-M-3 excited state

ARTICLE *in* PHOTOCHEMICAL AND PHOTOBIOLOGICAL SCIENCES · JANUARY 2012

Impact Factor: 2.27 · DOI: 10.1039/c2pp05333k · Source: PubMed

CITATIONS

9

READS

47

2 AUTHORS, INCLUDING:



Pavel Müller

Atomic Energy and Alternative Energies Co...

26 PUBLICATIONS 441 CITATIONS

SEE PROFILE

[Ru(bpy)₃]²⁺ as a reference in transient absorption spectroscopy: differential absorption coefficients for formation of the long-lived ³MLCT excited state

Pavel Müller^{a,b} and Klaus Brettel^{*b}

Received 4th October 2011, Accepted 23rd November 2011

DOI: 10.1039/c2pp05333k

Transient absorption spectroscopy and other time-resolved methods are commonly used to study chemical reactions and biological processes induced by absorption of light. In order to scale the signal amplitude or to compare results obtained under different conditions, it is advisable to use a reference system, a standard of convenient and well-defined properties. Finding Tris(bipyridine)ruthenium(II), [Ru(bpy)₃]²⁺, a suitable candidate for a transient-absorption spectroscopy reference due to its favourable photochemical properties, we have determined accurate relative values of differential molar absorption coefficients ($\Delta\epsilon$) for light-induced formation of the metal-to-ligand charge transfer (MLCT) excited triplet state at several relevant wavelengths (wavelengths of commercially available lasers) in the UV and visible regions. We have also attempted to determine the absolute value of $\Delta\epsilon$ close to the wavelength of maximum bleaching (~ 450 nm) and we propose to narrow down the interval of conceivable values for $\Delta\epsilon_{450}$ from the broad range of published values ($-0.88 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ to $-1.36 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$) to $-1.1 \times 10^4 \text{ M}^{-1}\text{cm}^{-1} \pm 15\%$. Having ourselves successfully applied [Ru(bpy)₃]²⁺ as a standard in a recent time-resolved study of enzymatic DNA repair, we would like to encourage other scientists to use this convenient tool as a reference in their future spectroscopic studies on time scales from picoseconds to hundreds of nanoseconds.

Introduction

Transient absorption spectroscopy is a powerful method for studies of dynamic photochemical and photobiological processes. It monitors absorption changes induced by flash excitation of the sample as a function of time and wavelength. Various set-ups appropriate for different time scales (from femtoseconds to seconds) and spectral ranges (from UV to infrared) have been described.¹

For quantitative applications like determination of reaction yields and difference absorption coefficients, and for comparison of amplitude data obtained using different set-ups or in different labs, it is desirable to have a reference compound that yields flash-induced absorption changes of well known and reproducible amplitudes in a wide spectral range. In 2004, an IUPAC Technical report² suggested the use of [Ru(bpy)₃]²⁺ for “laser pulse actinometry *via* standard transients”. We applied this standard successfully for amplitude adjustments between different set-ups and quantum yield determinations in DNA photolyase.^{3,4} It was also used for testing the time response of optical detection systems.^{5,6} We believe that a wider use of [Ru(bpy)₃]²⁺ as a

standard would be beneficial for quantification and comparability of transient absorption studies.

[Ru(bpy)₃]²⁺ in aqueous solution and polar organic solvents is extremely photostable. It absorbs light from the UV up to ~ 550 nm with prominent absorption bands centred at 243 nm (molar absorption coefficient $\epsilon = 2.72 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$), 286 nm ($8.61 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$) and 452 nm ($1.46 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$).^{7,8} Upon absorption of a photon, the initially formed singlet excited metal-to-ligand charge transfer state (¹MLCT) undergoes an ultrafast (~ 15 fs) intersystem crossing with a near-unity probability to a long-lived (~ 600 ns lifetime in water at ambient temperature) triplet state ³MLCT (ref. 9 and references therein). Formation of the ³MLCT state from the ground state is accompanied by significant absorption changes (positive or negative depending on the wavelength) at least from 230 to 800 nm.^{10,11} Hence, excitation of [Ru(bpy)₃]²⁺ by a short flash anywhere from the UV to 550 nm should produce absorption change signals in the 230 to 800 nm range that are essentially constant in time from less than 1 ps up to nanoseconds, and are still detectable up to a few hundred nanoseconds. These features together with its commercial availability make [Ru(bpy)₃]²⁺ an ideal reference for many applications of transient absorption spectroscopy.

Several absorption coefficient difference spectra ($\Delta\epsilon = \epsilon(^3\text{MLCT}) - \epsilon([\text{Ru}(\text{bpy})_3]^{2+})$) have been published,^{10–13} however, there are certain ambiguities in the data. Firstly, the spectrum has a very steep zero crossing close to 266 nm (wavelength of Nd: YAG 4th harmonic and an important wavelength for studies on

^aPCMP, Université Pierre et Marie Curie, F-75005 Paris, France.

E-mail: pavel.muller@upmc.fr; Fax: +33 1442 76151;

Tel: +33 1460 51596

^bCEA, iBiTec-S, Laboratoire de Photocatalyse et Biohydrogène, F-91191

Gif-sur-Yvette, France and CNRS, URA 2096, F-91191 Gif-sur-Yvette,

France. E-mail: klaus.brettel@cea.fr; pavel.muller@cea.fr;

Fax: +33 1690 88717; Tel: +33 1690 89869

DNA).³ Therefore, based on the published spectrum,¹¹ it is difficult to determine not only the value of $\Delta\epsilon_{266}$, but also its sign (\pm). Furthermore, the published data above 500 nm^{10,12} have a poor amplitude resolution. And finally, the absolute scaling of the published differential absorption spectra differs significantly (e.g., the value of $\Delta\epsilon_{450}$, i.e., $\Delta\epsilon$ at the wavelength of maximum bleaching, varies from $-0.88 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$, obtained by one of the methods in ref. 12 to $-1.36 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ in ref. 11).

Here we report on the determination of accurate relative values of $\Delta\epsilon$ for the formation of the ³MLCT state at several important wavelengths (wavelengths of commercially available lasers) and review the validity of the formerly published absolute values. We also discuss the advantages and limitations of the use of $[\text{Ru}(\text{bpy})_3]^{2+}$ as a standard for transient absorption spectroscopy.

Experimental

Apparatus

Determination of relative $\Delta\epsilon$ values at different wavelengths. The samples contained in a $2 \times 2 \times 10 \text{ mm}$ (height \times width \times length) cell were excited at 355 nm by a Nd:YAG laser (Continuum Leopard SS-10, pulse duration of 100 ps, repetition rate 2 Hz). The laser beam was passed through attenuation filters and a quarter-wave plate (in order to change the polarization from linear to circular and thus achieve a more homogeneous excitation of the ensemble of statistically oriented Ru complexes) and shaped by a cylindrical lens so that the cross section of the beam covered the whole $2 \times 10 \text{ mm}$ cell window.

Monitoring light was provided by the following continuous wave (cw) lasers:

266 nm–20 mW ASS laser Sony UW-1020A,
448 nm–50 mW laser diode Nichia NDHB510,
473 nm–50 mW DPSS laser Oxxius 473 L-50-COL-PP),
488 nm–20 mW DPSS laser Picarro Cyan-20,
561 nm–25 mW DPSS laser Oxxius 561-25-COL-002,
690 nm–5–10 mW external cavity diode laser system EOSI 2010.

The monitoring light beam was perpendicular to the excitation beam and shaped such that the central part of the beam passed through the $2 \times 2 \text{ mm}$ cell window along the 10 mm path. In order to minimize sample excitation by monitoring light, a rotating blade with a small opening was introduced between the light source and the sample, chopping the monitoring light to rectangular pulses of 140 μs duration. The system was synchronized so that the excitation pulse came 30 μs after the rise of the monitoring light.

The position of the cell with respect to the excitation beam was unchanged for all experiments in order to guarantee identical excitation conditions. The amplitudes of the absorbance changes at different wavelengths were corrected for minor laser energy fluctuations, which were determined independently by an energy meter (Gentec QE25SP-H-MB-D0) measuring a reflected fraction of the excitation pulse.

The detection system for the monitoring light consisted of a photodiode (Alphas UPD-500-UP, rise time 500 ps, spectral range 170–1100 nm, sensitive area 1 mm^2), connected via an electronic signal amplifier (Femto HCA; DC-325 MHz, 28 dB)

to a digital oscilloscope (Tektronix DSA602A, with 11A52 plug-in set to DC-20 MHz; this rather low bandwidth limit was chosen to improve the signal-to-noise ratio). The detection system was triggered by a small fraction of the excitation pulse through a fast photodiode. Interference filters with transmittance maxima at the wavelengths of monitoring light were used to eliminate the detection of stray light and sample fluorescence induced by the excitation pulse.

Determination of the absolute value of $\Delta\epsilon_{448}$. In order to reach higher excitation photon fluences and to allow for precise determination of the excitation energy, the setup described above was slightly modified. An $8 \times 2 \times 10 \text{ mm}$ (height \times width \times length) cell was oriented with the $8 \times 2 \text{ mm}$ window towards the excitation beam. The monitoring light passed through a $2 \times 2 \text{ mm}$ mask placed onto the $8 \times 10 \text{ mm}$ window at central height but laterally displaced to the side where the excitation beam entered the cell so that only the $2 \times 2 \times 2 \text{ mm}$ volume most exposed to the excitation beam was probed by the monitoring light.

The excitation beam was focused ca 10 cm in front of the cell by a converging spherical lens so that it was slightly divergent ($\sim 1^\circ$) at the entrance window of the cell and had a diameter of $\sim 3.5 \text{ mm}$, assuring the excitation of the whole sample volume to be probed. The laser intensity was attenuated to different degrees by filters of well-defined transmittance at 355 nm.

To determine the absolute excitation laser energy seen by the sample, the $8 \times 2 \times 10 \text{ mm}$ cell was replaced by a $2 \times 2 \times 10 \text{ mm}$ cell filled with pure water and oriented with the $2 \times 2 \text{ mm}$ window towards the excitation beam. The pulse energy leaving the cell through the back window was measured by the energy meter (specifications above) placed behind the cell. Corrections were made for reflections on the water/quartz and quartz/air interfaces and the calculated light attenuation by $[\text{Ru}(\text{bpy})_3]^{2+}$. The output of the excitation laser was stable throughout the whole series of experiments (drift $\pm 1\%$ for the averaged energy of each signal accumulation).

Reagents and materials

$[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ (99.95%) was purchased from Sigma-Aldrich Co. Water was de-ionized ($\geq 18.2 \text{ M}\Omega\text{cm}$ specific resistance) by Elga ultrapure water purification system.

Quartz cells with self-masking solid black walls and sample chambers of $2 \times 2 \times 10 \text{ mm}$ (height \times width \times length) and $8 \times 2 \times 10 \text{ mm}$ and with 3 and 4 clear windows, respectively, were obtained from Starna and used aerobically.

Procedures

Signals were recorded as averages ($4\times$ or $16\times$ for the determination of the absolute value of $\Delta\epsilon_{448}$, and $64\times$ or $256\times$ for the determination of relative $\Delta\epsilon$ values, depending on the signal-to-noise ratio for a single trace). Voltage vs time traces were converted to $\Delta A(t)$ according to $\Delta A(t) = -\log(U(t)/U_0)$; $U(t)$ being the recorded voltage in time t and U_0 the voltage prior to excitation. In order to correct for a slight loss of the initial signal amplitude due to the instrument response time ($t_{10-90} \sim 20 \text{ ns}$), signal decays were fitted starting at $t = 60 \text{ ns}$ and extrapolated

Table 1 Relative values of $\Delta\epsilon = \epsilon(^3\text{MLCT}) - \epsilon([\text{Ru}(\text{bpy})_3]^{2+})$ at studied wavelengths

λ/nm	266	448	473	488	561	690
$\Delta\epsilon_{\text{rel}}^a$	1.314	-1.000	-0.568	-0.101	0.083	0.140

^a Normalized to the value at 448 nm.

back to time zero, defined as the time at which the signal rise reached its half-maximum. Signal amplitudes obtained by this method were found to be in good agreement with the amplitudes obtained directly using 2 ns response time (for bandwidth limit of the oscilloscope set to 600 MHz).

Results and discussion

Determination of relative $\Delta\epsilon$ values

Keeping the cell position and the excitation beam unchanged, we have used several commercially available cw lasers to monitor the absorption changes due to excitation of $[\text{Ru}(\text{bpy})_3]^{2+}$ at their output wavelengths. Kinetic traces showing the formation of the $^3\text{MLCT}$ state and its decay back to ground state are shown in Fig. 1. The relative $\Delta\epsilon$ values for given wavelengths extracted from the signal amplitudes are summarized in Table 1. The data were normalized to the signal at 448 nm.

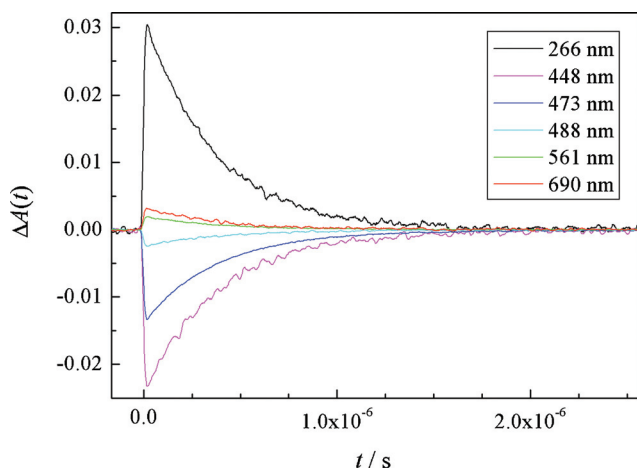


Fig. 1 Kinetic traces showing the formation of the $^3\text{MLCT}$ state and its decay back to the ground state monitored at different wavelengths. $c([\text{Ru}(\text{bpy})_3]\text{Cl}_2) = 20 \mu\text{M}$. $\lambda_{\text{exc}} = 355 \text{ nm}$. Excitation flash intensity $\sim 2 \text{ mJ cm}^{-2}$; optical path length of 2 and 10 mm for the excitation and the monitoring light, respectively.

Determination of $\Delta\epsilon_{448}$

Under the assumption of a simple monophotonic process $[\text{Ru}(\text{bpy})_3]^{2+} \xrightarrow{h\nu} ^3\text{MLCT}$ and a homogeneous isotropic excitation (at 355 nm in our case), the concentration of the $^3\text{MLCT}$ excited state right after the excitation pulse is given by eqn (1),¹⁴ where $[[\text{Ru}(\text{bpy})_3]^{2+}]_0$ is the initial concentration of ground state,

Φ is the quantum yield of this photoreaction, σ stands for the absorption cross section (in cm^2) of $[\text{Ru}(\text{bpy})_3]^{2+}$ molecules at the excitation wavelength, and I' for the intensity of the excitation light (in photons/cm^2).

$$[^3\text{MLCT}] = [[\text{Ru}(\text{bpy})_3]^{2+}]_0 (1 - e^{-\Phi\sigma_{355}I'}) \quad (1)$$

According to this equation, $[^3\text{MLCT}]$ rises linearly at low intensities ($\Phi\sigma_{355}I' \ll 1$) and approaches $[[\text{Ru}(\text{bpy})_3]^{2+}]_0$ at high intensities.

Since $\Delta A_\lambda = \Delta\epsilon_\lambda l [^3\text{MLCT}]$, where l is the optical path length and $\Delta\epsilon_\lambda$ the difference $\epsilon(^3\text{MLCT}) - \epsilon([\text{Ru}(\text{bpy})_3]^{2+})$ at the given wavelength, and since $\Phi = 1$, eqn (2) is applicable.

$$\Delta A_{448} = [[\text{Ru}(\text{bpy})_3]^{2+}]_0 \Delta\epsilon_{448} l (1 - e^{-\sigma_{355}I'}) \quad (2)$$

Derivation of eqn (2) with respect to light intensity yields eqn (3).

$$\frac{d(\Delta A_{448})}{dI'} = [[\text{Ru}(\text{bpy})_3]^{2+}]_0 \Delta\epsilon_{448} l \sigma_{355} e^{-\sigma_{355}I'} \quad (3)$$

Since $e^{-\sigma_{355}I'} = 1$ for $I' = 0$, the initial slope becomes

$$\left(\frac{d(\Delta A_{448})}{dI'} \right)_{I' \rightarrow 0} = [[\text{Ru}(\text{bpy})_3]^{2+}]_0 \Delta\epsilon_{448} l \sigma_{355} \quad (4)$$

Fig. 2 shows transient absorption changes at 448 nm at low excitation energies (inset) and a plot of amplitudes (extrapolated to time zero) against excitation flash intensity. The data are well described by a straight line through origin with a slope of $-2.266 \times 10^{-18} \text{ photons}^{-1} \text{ cm}^2$. Inserting this value and σ_{355} , $[[\text{Ru}(\text{bpy})_3]^{2+}]_0 = 2.243 \times 10^{-17} \text{ cm}^2$ (calculated from ϵ_{355} , $[[\text{Ru}(\text{bpy})_3]^{2+}]_0 = 5865 \text{ M}^{-1} \text{ cm}^{-1}$ from ref. 7 and 8 and using¹⁵ $\sigma = \epsilon \times 3.825 \times 10^{-24} \text{ mol}$), $[[\text{Ru}(\text{bpy})_3]^{2+}]_0 = 4.47 \times$

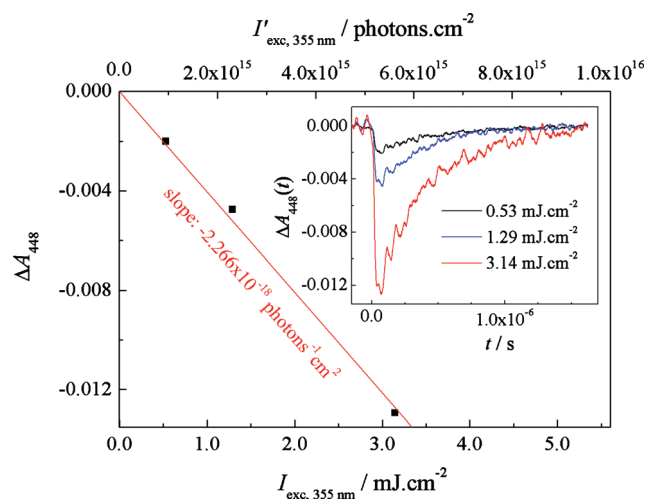


Fig. 2 ΔA_{448} plotted against excitation flash intensity. $c([\text{Ru}(\text{bpy})_3]\text{Cl}_2) = 44.7 \mu\text{M}$. $\lambda_{\text{exc}} = 355 \text{ nm}$. Optical path of the monitoring light: 0.2 cm. Low excitation flash intensities ($\sigma_{355}I' < 0.15$) were used for this experiment in order to keep the probability of double hits negligible. ΔA values result from an extrapolation of the signal decays to time zero. Experimental traces are shown in the inset.

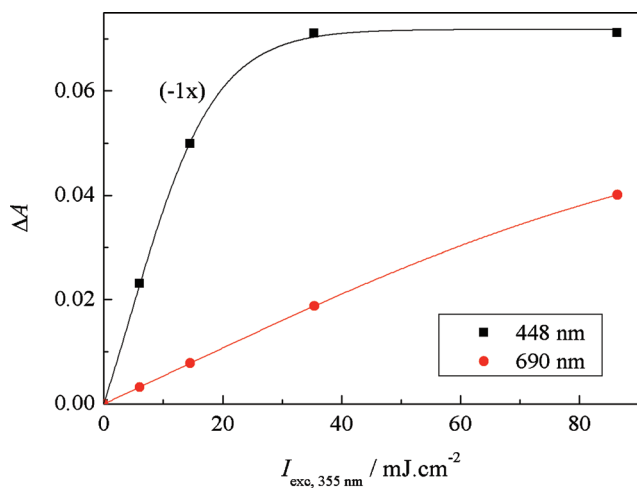


Fig. 3 ΔA_{448} and ΔA_{690} plotted against excitation flash intensity. c ($[\text{Ru}(\text{bpy})_3]\text{Cl}_2$) = 44.7 μM . λ_{exc} = 355 nm. Optical path of the monitoring light: 0.2 cm. ΔA values result from an extrapolation of the signal decays to time zero. The lines only serve to guide the eyes.

10^{-5} M, and $l = 0.2$ cm into eqn (4), one obtains $\Delta\epsilon_{448} = -1.13 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$.

In principle, $\Delta\epsilon_\lambda$ could be directly derived from the value of ΔA_λ at very high excitation energies, assuming that all $[\text{Ru}(\text{bpy})_3]^{2+}$ molecules in the sample volume are converted to the $^3\text{MLCT}$ state (see eqn (1)). This would eliminate possible sources of errors associated with the determination of excitation flash intensities within the sample volume. In practice, however, the situation becomes much more complicated by the fact, that the excited $[\text{Ru}(\text{bpy})_3]^{2+}$ can absorb a second photon (at some wavelengths, e.g., at 355 nm, the $^3\text{MLCT}$ state absorbs even significantly stronger than $[\text{Ru}(\text{bpy})_3]^{2+}$ in the ground state) to form $[\text{Ru}(\text{bpy})_3]^{3+}$ and a hydrated electron (e_{aq}^-), both having a non-negligible absorbance throughout the whole visible spectrum. These two species can then recombine back to $[\text{Ru}(\text{bpy})_3]^{2+}$. Further possible reactions include reduction of the remaining $[\text{Ru}(\text{bpy})_3]^{2+}$ by e_{aq}^- and oxidation of water to H^+ and $\cdot\text{OH}$ upon absorption of another photon by $[\text{Ru}(\text{bpy})_3]^{3+}$.^{9,13}

We examined the transient absorption behaviour of $[\text{Ru}(\text{bpy})_3]^{2+}$ also at high intensities of our excitation flash (Fig. 3).

Two monitoring light wavelengths were used, namely 448 nm, where bleaching of the $[\text{Ru}(\text{bpy})_3]^{2+}$ ground state is expected to be dominating, and 690 nm, where e_{aq}^- absorb strongly ($\epsilon_{690} = 2.3 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$).¹⁶ ΔA_{448} ceased to grow with increasing excitation energy at $\sim 50 \text{ mJ cm}^{-2}$ (corresponding to ~ 2 photons per absorption cross section of the ground-state $[\text{Ru}(\text{bpy})_3]^{2+}$ at 355 nm). ΔA_{690} , however, kept on growing further, indicating that an increasing amount of e_{aq}^- was formed in a multiphotonic process, i.e., double excitation resulting in a formation of $[\text{Ru}(\text{bpy})_3]^{3+}$ and e_{aq}^- .

Using absorption coefficients from the literature (references in the legend of Fig. 4) and $\Delta\epsilon_{448}$ from this study and assuming formation of e_{aq}^- and $[\text{Ru}(\text{bpy})_3]^{3+}$ at equal concentrations, the transient absorption amplitudes at 448 and 690 nm (Fig. 3) allow deducing the concentrations of $^3\text{MLCT}$, e_{aq}^- and $[\text{Ru}(\text{bpy})_3]^{3+}$ at each excitation energy (Fig. 4).

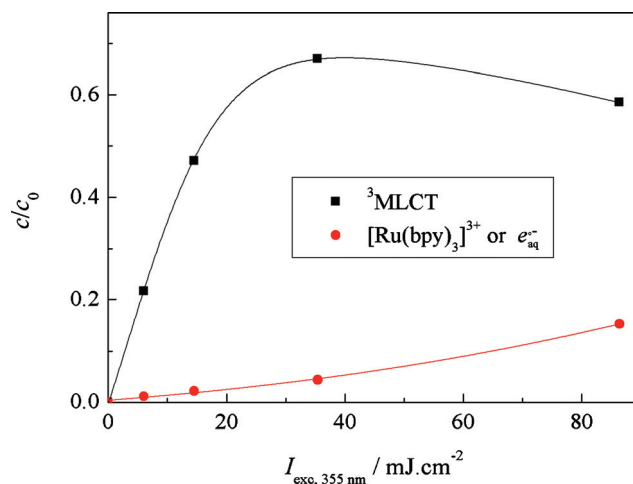


Fig. 4 Relative concentrations of the $^3\text{MLCT}$ state and $[\text{Ru}(\text{bpy})_3]^{3+}$ or e_{aq}^- calculated from data points in Fig. 3 using molar absorption coefficients: ϵ_{448} (e_{aq}^-) = 5000 $\text{M}^{-1}\text{cm}^{-1}$ and ϵ_{690} (e_{aq}^-) = 23 000 $\text{M}^{-1}\text{cm}^{-1}$ (ref. 16), ϵ_{448} ($[\text{Ru}(\text{bpy})_3]^{2+}$) = 14 283 $\text{M}^{-1}\text{cm}^{-1}$ and ϵ_{690} ($[\text{Ru}(\text{bpy})_3]^{2+}$) = 36 $\text{M}^{-1}\text{cm}^{-1}$ (values from ref. 8 normalized to ϵ_{452} ($[\text{Ru}(\text{bpy})_3]^{2+}$) = 14 600 $\text{M}^{-1}\text{cm}^{-1}$ according to ref. 7), ϵ_{448} ($[\text{Ru}(\text{bpy})_3]^{3+}$) = 375 $\text{M}^{-1}\text{cm}^{-1}$ and ϵ_{690} ($[\text{Ru}(\text{bpy})_3]^{3+}$) = 315 $\text{M}^{-1}\text{cm}^{-1}$ (ref. 17), $\Delta\epsilon_{448}$ ($^3\text{MLCT}-[\text{Ru}(\text{bpy})_3]^{2+}$) = 11 280 $\text{M}^{-1}\text{cm}^{-1}$ (from this study), $\Delta\epsilon_{690}$ ($^3\text{MLCT}-[\text{Ru}(\text{bpy})_3]^{2+}$) = 1580 $\text{M}^{-1}\text{cm}^{-1}$ (from $\Delta\epsilon_{448}$ and the coefficient in Table 1). The lines only serve to guide the eyes.

Qualitatively similar to a previous study,¹³ the slope of the response curves for formation of e_{aq}^- and $[\text{Ru}(\text{bpy})_3]^{3+}$ increased with increasing intensity, and the population of the $^3\text{MLCT}$ state reached a maximum well below 100% and then decreased significantly, indicating that this state is depopulated by another photoreaction.

Goez and coworkers¹³ have reached a maximum of $\sim 85\%$ $^3\text{MLCT}$ at $\sim 100 \text{ mJ cm}^{-2}$ excitation at 355 nm (compared to $\sim 70\%$ at $\sim 40 \text{ mJ cm}^{-2}$ in our study). These apparent discrepancies may be related to the difference in pulse lengths (8 ns in ref. 13 vs 100 ps in our case). As the rotational diffusion time of $[\text{Ru}(\text{bpy})_3]^{2+}$ in water at room temperature is approximately 150 ps,⁹ molecules hit by a photon will have a much higher probability to turn during the 8 ns pulse than during the 100 ps pulse, which may result in a decrease of the effective cross section for the absorption of a second photon (of the same polarization as the first one) within the 8 ns pulse. Remarkably, in a study using high intensity pulses of 135 fs at 400 nm,⁹ the maximal population of the $^3\text{MLCT}$ state was only $\sim 50\%$.

The data clearly show that $\Delta\epsilon$ for formation of the $^3\text{MLCT}$ state cannot be determined in a straightforward manner from transient absorption experiments at saturating excitation, at least not with 355 nm flashes (for excitation at 532 nm, no significant deviation from eqn (1) was reported¹³ for intensities up to 460 mJ cm^{-2} , corresponding to 3.7 photons per absorption cross section of $[\text{Ru}(\text{bpy})_3]^{2+}$ at 532 nm). Therefore, if $[\text{Ru}(\text{bpy})_3]^{2+}$ is to be used as a standard for transient absorption spectroscopy, multiple excitations and significant formation of e_{aq}^- and $[\text{Ru}(\text{bpy})_3]^{3+}$ should be avoided (this, however, is not a substantial drawback since the system gives sufficiently strong signals at most wavelengths even at very low excitation energies). Data in

Fig. 4 indicate that up to $\sim 15 \text{ mJ cm}^{-2}$ of our 100 ps excitation flash at 355 nm, formation of the $^3\text{MLCT}$ state is very well linear with flash intensity and the yield of $\epsilon_{\text{aq}}^{2+}$ and $[\text{Ru}(\text{bpy})_3]^{3+}$ relative to that of $^3\text{MLCT}$ state is below 3%.

The value of $\Delta\epsilon_{448} = -1.13 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ obtained here from the initial slope of the saturation curve upon excitation at 355 nm is similar to the reported $\Delta\epsilon_{450}$ and $\Delta\epsilon_{460}$ values obtained from the high energy part of the saturation curve upon excitation at 532 nm ($-1.06 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ and $\sim -1.05 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$, respectively).^{12,13} Note that according to ref. 11 the bleaching is maximal at 452 nm and decreases by $\sim 2\%$ and $\sim 5\%$ for 448 and 460 nm, respectively. We estimate our experimental error to $<15\%$. This error results primarily from possible inaccuracy in absolute energy measurements (essential for the determination of the slope in Fig. 2); $<10\%$. The error due to the amplitude determination by our fitting procedure is estimated to be at most 5%. The value of $-1.36 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ obtained in one study¹¹ from the saturation level upon excitation at 530 nm and the value of $-0.88 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ obtained by benzophenone actinometry¹² upon excitation at 355 nm are outside this error margin. In the benzophenone actinometry study, the empirical correction for the inner filter effect due to $^3\text{MLCT}$ and the neglect of $\epsilon_{\text{aq}}^{2+}$ absorption might have introduced systematic errors. The authors of ref. 11 used an unusually high value of $\epsilon_{452}([\text{Ru}(\text{bpy})_3]^{2+}) = 15\,700 \text{ M}^{-1}\text{cm}^{-1}$. Rescaling their data to $\epsilon_{452}([\text{Ru}(\text{bpy})_3]^{2+}) = 14\,600 \text{ M}^{-1}\text{cm}^{-1}$ (from ref. 7 and 8) would yield a smaller $\Delta\epsilon_{452}$ value ($-1.26 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$), exceeding our $\Delta\epsilon_{448}$ value by $\sim 10\%$.

In many cases, the knowledge of accurate relative values of $\Delta\epsilon$ of the reference system is sufficient (e.g., for scaling of signal amplitudes at different wavelengths or comparison of signals obtained using different setups). In cases where an absolute amplitude reference is required (e.g., determination of absolute quantum yields), we suggest to use $\Delta\epsilon_{450} = -1.1 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ rather than $-1.0 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ as recommended previously¹² (absolute $\Delta\epsilon$ at other wavelengths can be calculated using this value and the factors from Table 1).

Conclusions

We have determined accurate relative differential molar absorption coefficients for the formation of the $^3\text{MLCT}$ state of $[\text{Ru}(\text{bpy})_3]^{2+}$ at several important wavelengths and reviewed their absolute scaling. Our data should now enable scientists to use $[\text{Ru}(\text{bpy})_3]^{2+}$ for normalization of signal amplitudes and as a standard for direct comparison of results obtained on different setups or in different laboratories. We hope this contribution will promote the use of $[\text{Ru}(\text{bpy})_3]^{2+}$ as a reference in transient absorption spectroscopy.

Acknowledgements

This work was supported by Agence Nationale de la Recherche (grant #ANR-09-BLAN-0248-03) and a grant from Human Frontiers (#RGP0045/2008-C).

Notes and references

- N. V. Tkachenko, *Optical Spectroscopy (Methods and Instruments)*, Elsevier, 2006, ISBN: 978-0-444-52126-2.
- H. J. Kuhn, S. E. Braslavsky and R. Schmidt, Chemical actinometry (IUPAC Technical Report), *Pure Appl. Chem.*, 2004, **76**, 2105–2146.
- V. Thiagarajan, M. Byrdin, A. P. M. Eker, P. Müller and K. Brettel, Kinetics of cyclobutane thymine dimer splitting by DNA photolyase directly monitored in the UV, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 9402–9407.
- M. Byrdin, A. Lukacs, V. Thiagarajan, A. P. M. Eker, K. Brettel and M. H. Vos, Quantum yield measurements of short-lived photoactivation intermediates in DNA photolyase: Toward a detailed understanding of the triple tryptophan electron transfer chain, *J. Phys. Chem. A*, 2010, **114**, 3207–3214.
- M. Byrdin, V. Thiagarajan, S. Villette, A. Espagne and K. Brettel, Use of ruthenium dyes for subnanosecond detector fidelity testing in real time transient absorption, *Rev. Sci. Instrum.*, 2009, **80**, 043102-1-5.
- K. Brettel and E. Schlöder, $\text{Ru}(\text{bipy})_3\text{Cl}_2$ luminescence as optical step signal for detector testing, *Rev. Sci. Instrum.*, 1988, **59**, 670–671.
- K. Kalyanasundaram, Photophysics, photochemistry and solar energy conversion with $\text{Tris}(\text{bipyridyl})\text{ruthenium(II)}$ and its analogues, *Coord. Chem. Rev.*, 1982, **46**, 159–244.
- H. Du, R. A. Fuh, J. Li, A. Corkan and J. S. Lindsey, PhotochemCAD: A computer-aided design and research tool in photochemistry, *Photochem. Photobiol.*, 1998, **68**, 141–142.
- A. N. Tarnovsky, W. Gawelda, M. Johnson, C. Bressler and M. Chergui, Photoexcitation of aqueous ruthenium(II)-tris-(2,2'-bipyridine) with high-intensity femtosecond laser pulses, *J. Phys. Chem. B*, 2006, **110**, 26497–26505.
- K. Miedlar and P. K. Das, $\text{Tris}(2,2'\text{-bipyridine})\text{ruthenium(II)}$ -sensitized photooxidation of phenols. Environmental effects on electron transfer yields and kinetics, *J. Am. Chem. Soc.*, 1982, **104**, 7462–7469.
- U. Lachish, P. P. Infelta and M. Grätzel, Optical absorption spectrum of excited ruthenium tris-bipyridyl $[\text{Ru}(\text{bpy})_3]^{2+}$, *Chem. Phys. Lett.*, 1979, **62**, 317–319.
- A. Yoshimura, M. Z. Hoffman and H. Sun, An evaluation of the excited state absorption spectrum of $[\text{Ru}(\text{bpy})_3]^{2+}$ in aqueous and acetonitrile solutions, *J. Photochem. Photobiol., A*, 1993, **70**, 29–33.
- M. Goetz, D. von Ramin-Marro, M. H. O. Musa and M. Schiewek, Photoionization of $[\text{Ru}(\text{bpy})_3]^{2+}$: a catalytic cycle with water as sacrificial donor, *J. Phys. Chem. A*, 2004, **108**, 1090–1100.
- U. Lachish, A. Shafferman and G. Stein, Intensity dependence in laser flash photolysis experiments: hydrated electron formation from ferrocyanine, tryptophan and tryptophan, *J. Chem. Phys.*, 1976, **64**, 4205–4211.
- S. E. Braslavsky and K. N. Houk, Glossary of terms used in photochemistry (Recommendations 1988), *Pure Appl. Chem.*, 1988, **60**, 1055–1106.
- P. M. Hare, E. A. Price and D. M. Bartels, Hydrated electron extinction coefficient revisited, *J. Phys. Chem. A*, 2008, **112**, 6800–6802.
- A. J. McCaffery, S. F. Mason and B. J. Norman, Optical rotatory power of co-ordination compounds. Part XII. Spectroscopic and configurational assignments for the tris-bipyridyl and -phenanthroline complexes of the di- and tri-valent iron-group metal ions, *J. Chem. Soc. A*, 1969, 1428–1441.