Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology A: Chemistry 159 (2003) 199–206
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Sensitization of photoeffects by hyper-Rayleigh scattering (HRS)

Matthew Goertz, M.R.V. Sahyun*

Department of Chemistry, University of Wisconsin—Eau Claire, Eau Claire, WI 54702, USA

Received 8 May 2002; received in revised form 21 February 2003; accepted 20 March 2003

Abstract

We have observed fluorescence from Coumarin334 (C334) in a variety of solvents using a fundamental wavelength (840 nm) twice that of radiation actinic for excitation of this fluorescence, and we attribute this fluorescence to second harmonic, hyper-Rayleigh light scattering (HRS). Fluorescence intensity is compared to that obtained by direct excitation of fluorescence under the same conditions to provide an estimate of the efficiency of the HRS process; Φ = ca. 10^{-6}, strongly solvent dependent. Photochemical actinometry indicates a fundamental optical power of 1 mW cm^{-2} under our conditions. By mapping the action spectrum for second harmonic excitation of fluorescence, we exclude the possibility suggested by the solvent dependence of Φ, namely that the principal pathway for excitation of C334 involves second harmonic scattering by solvent, followed by absorption of actinic light by the solute. Instead, we find that the action spectrum does not correlate with the absorption spectrum of C334. Therefore, we hypothesize that second harmonic scattering is coupled to an electronic transition in the C334 chromophore, which probably involves a higher excited state than that responsible for emission.

Keywords: Hyper-Rayleigh scattering; Coumarin334

1. Introduction

Historically, photochemistry has been limited by the applicability of the Grotthus–Draper Law, one of the fundamental principles of molecular photochemistry [1]. Namely, if a molecule is to undergo photochemical change, it must absorb light of the wavelength(s) used to effect that change. Since many molecules absorb only in the blue or ultraviolet regions of the spectrum, this requirement is limiting in terms of the scope of wavelengths useful for photochemistry. The limitation was overcome early on in imaging photochemistry by the discovery of spectral sensitization [2]. Accordingly, a sensitizer may absorb light of a wavelength longer than that required to carry out the desired photochemistry, and subsequently activate the reactive species either by energy transfer [1] or electron transfer [2,3].

More recently, two-photon absorption has been proposed [4] and demonstrated [5,6] as a method for effecting useful photophysics and photochemistry with light whose frequency, ω, is one-half that which corresponds to an actinic electronic transition in the reactive molecule, i.e. the molecule absorbs light of frequency, 2ω. This technique has proved useful for fabricating microdevices [7], optical memories [8], and fluorescence microscopy of biological systems [6]. A drawback of this technique, however, is that high photon fluxes may be required, e.g. 3×10^{30} photons cm^{-2} s^{-1} [9], which corresponds to 10^{12} W cm^{-2} at 800 nm. This level of irradiation, in turn, would require a high powered light source, e.g. a 10 kW laser if the light is focused to a 1 μm spot, and may also result in considerable damage to the substrate, especially if the latter is a biological sample [10]. Most reports of two-photon fluorescence microscopy, however, utilize optical power densities in the order of MW cm^{-2}, examples of this technique are found in [11,12].

We now propose a new strategy for long wavelength sensitization of photophysical and photochemical effects. As long ago as 1965, hyper-Rayleigh scattering (HRS) in isotropic liquids was reported [13], and subsequently applied to the determination of the second-order hyperpolarizability, β, of solvents and solutes [14]. Accordingly, deformation of the electron cloud of a molecule, in response to the periodic fluctuation of the optical electric field leads to scattering, not only of the fundamental frequency of the incident light, ω, but of the higher harmonics as well, e.g. 2ω. Symmetry restrictions governing second harmonic generation do not appear to apply to second harmonic scattering [15]. In chemistry, HRS has found analytical applications, both in Raman spectroscopy [16], and in the characterization of nanocrytals [17], interrogation of interfaces [18], and the monitoring

* Corresponding author. Fax: +1-715-836-4979.
E-mail address: sahyunm@uwec.edu (M.R.V. Sahyun).

1010-6030/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1010-6030(03)00188-6

of surface reactions, again in the nanoparticle environment [19].

Our proposal is to use HRS to provide actinic light, of frequency 2ω, where irradiation is carried out at ω. We envision that it may be implemented in two ways:

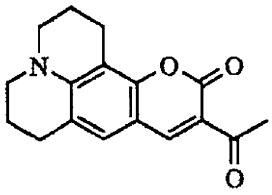
(1) The reactive species itself may exhibit a high hyperpolarizability, in which case it both scatters the second harmonic and then uses it to effect a photochemical process. This strategy may be most applicable in nanoscale systems, e.g. nano-AgBr which has already been shown to exhibit $\beta = 75 \times 10^{-30}$ esu [19].
(2) The reaction solvent scatters second harmonic, which is subsequently absorbed by a solute for which it is actinic. Such a strategy should be most applicable to dilute solutions in which the intensity of the second harmonic, $I(2\omega)$ is given by [14]

$$I(2\omega) = GI(\omega)^2[\beta_s^2 N_s + \beta_a^2 N_a] \quad (1)$$

where G is a gain factor characteristic of the experimental set-up, N is the number concentration of the solvent and solute species, designated by the subscripts s and a, respectively. For dilute solutions, the term in brackets is likely to dominate, since $N_s \gg N_a$ and the usual range of molecular β spans approximately two order of magnitude.

The purposes of the present study are reduction to practice of these strategies and evaluation of their photon efficiency, in order to project under what conditions photochemical sensitization by HRS might prove useful. Demonstration of useful sensitization of photoeffects by HRS could have interesting ramifications for the design of image capture devices, photodynamic therapy, and fluorescence microscopy, among other applications.

The system selected for investigation was the laser dye, Coumarin334 (C334), which fluoresces with near unit quantum efficiency, dissolved in a variety of solvents.



Unless otherwise specified, the fundamental wavelength was 840 nm. We hypothesized that under these conditions the solvent should scatter some of this radiation as 420 nm light by the HRS mechanism. This latter wavelength is close to the absorption maximum of C334. In so far as C334 may be hyperpolarizable, second harmonic scattering should be resonant with the principal electronic transition of the molecule. In this case, the dispersion of HRS should exhibit a maximum as well, though self-absorption should preclude experimental confirmation of this expectation through measurement of the scattered second harmonic itself.

Fluorescence from C334 was then monitored at its maximum wavelength, 490–505 nm depending on solvent, in order to detect the HRS. Detection of the fluorescence would thus implicate one of the two mechanisms of HRS sensitization outlined above. In so far as mechanism (2) may be operative, the measured fluorescence intensity, $I_f(2\omega)$, should correlate with $I(2\omega)$ for the solvent, measured directly at 420 nm. The absolute efficiency of HRS, Φ_{HRS} may be evaluated in these experiments by comparing the intensity of C334 fluorescence sensitized by HRS to that measured under conditions of direct excitation at 420 nm, $I_f(\omega)$.

2. Experimental detail

2.1. Materials

Laser grade C334 was obtained from the Eastman Kodak Co., and used as received ($\varepsilon = 3.3 \times 10^4\ cm^{-1}\ M^{-1}$). Solvents were of the best available grade, and used as received: spectrograde methanol, acetonitrile, and toluene (Spectra Chemicals, Gardena, CA), ethylene glycol (Fisher Certified™, Fisher Chemical Co.), along with dimethylsulphoxide (DMSO) and acetone, both >99.5% purity (Aldrich "gold label", Aldrich Chemical Co.). Water was glass distilled. C334 was dissolved in these solvents at a concentration of 1.0×10^{-5} M ($N_a = 1 \times 10^{15}\ cm^{-3}$), by addition of 25 μl of a 1.0×10^{-3} M methanol solution of the dye to 2.5 ml of the solvent being studied. Diphenyliodonium hexafluorophosphate, used in the actinometry experiments, was obtained from Avocado Chemicals, Ltd. (UK), and used as received.

2.2. Methods

Our optical set-up has been described in detail in our previous papers [19,20]. Optical components were derived from the optical table of a Perkin-Elmer model MPF44b spectrofluorimeter. We use the output of a 150 W Xe-arc lamp, from which the desired fundamental wavelength is isolated by a grating monochromator and a 470 nm cut-off filter, chopped, and focussed to an 0.05 cm^2 spot in the center of a 1 cm quartz fluorescence cuvette (2.5 ml sample volume). The emission (HRS or fluorescence) is detected by a Hamamatsu model 933 photomultiplier with lock-in amplification, at right angles to the fundamental beam, with isolation from stray light by a second monochromator and a polarizing filter. (The polarizing filter was found empirically to improve signal-to-noise in HRS detection, presumably by excluding stray light reflected off optical surfaces.)

Adequate signal-to-noise for signal quantification required that the output of the emission monochromator be compared with the output from a second, matched photomultiplier monitoring the fundamental beam intensity.

This procedure corrected for time-based fluctuations in the Xe-arc lamp output power. The recorded signal was thus

$$I_{obs} = \frac{I(2\omega)}{I(\omega)} \quad (2)$$

where I_{obs} is a dimensionless ratio, measured as the potential (mV, reported as arbitrary units) required to balance the bridge circuit in which the two photomultipliers are incorporated. Note that the two photomultipliers are measuring radiation at different wavelengths, hence a correction factor has to be included, below, in the estimation of Φ_{HRS}. No correction is applied for difference in lamp output at 420 and 840 nm; examination of the output spectra of typical Xe-arc lamps [21] indicates that we should expect comparable output (order of magnitude, at least) at both wavelengths.

Another correction is required for the fact that a cutoff filter may not be absolutely efficient in the exclusion of stray light from the fundamental beam. Stray, adventitious second harmonic, e.g. scattered off the optical surfaces, may thus be Rayleigh scattered into the detection path. We controlled this source of error by measuring the fluorescence intensity with and without the cutoff filter in place. Experimentally, we found that the filter exhibited ca. 0.82% transmission in the bandpass of interest, 400–460 nm. Therefore, we reduced the fluorescence intensity measured with the cutoff filter in place by 0.82% of the signal measured in its absence. This correction, of course, represents an upper limit to the contribution of stray light to measured $I_f(2\omega)$, insofar as not all the 420 nm light exciting C334 fluorescence is stray light, even in the absence of the cutoff filter. The magnitude of the correction is reflected in the confidence limits shown below for the estimates of $I_f(2\omega)$. A similar correction was applied to the estimation of solvent HRS intensity $I(2\omega)$, detected at 420 nm in the absence of C334. No stray light in the 490–505 nm bandpass (region of C334 fluorescence) could be detected in our system, with the fundamental monochromator set for either 420 or 840 nm.

Additional tests for the authenticity of the HRS signal in our experimental set-up are described elsewhere [22].

3. Results

3.1. Hyperpolarizability of C334

The non-linear optical properties of C334 do not appear to have been characterized. The scattered second harmonic intensity was accordingly characterized for a fundamental wavelength of 680 nm for a series of concentrations of C334 in methanol. Under these conditions, absorption of second harmonic (340 nm) by the C334 is minimized, although it remains finite. Accordingly, Eqs. (1) and (2) become

$$I_{obs} = GI(\omega)[\beta_s^2 N_s + \beta_a^2 N_a]\exp(-2.303\varepsilon N_a) \quad (3)$$

where ε is an effective extinction coefficient for C334, with units of ($l\,mol^{-1}$), when N_a is expressed in molar units.

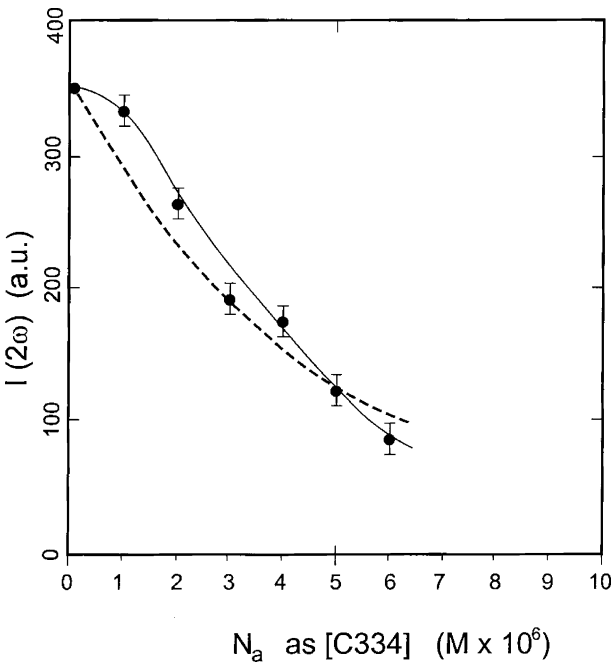


Fig. 1. Detection of HRS (I_{obs}) at 340 nm for various concentrations (N_a) of C334 in methanol; data are fit to Eq. (3) (solid line) and compared to Beer's Law fit (dashed line).

Note that it is not numerically equivalent to the usual molar extinction coefficient for C334 at this wavelength, as the optical path for scattered light is not necessarily linear. The data obtained, plotted as I_{obs} versus N_a are shown in Fig. 1. If β_a is zero, i.e. C334 is non-hyperpolarizable, then Eq. (3) reduces to Beer's Law; under these circumstances we obtain a poor fit to the data, with $\varepsilon = 9 \times 10^4\,l\,mol^{-1}$ with a correlation coefficient, r, of only 0.976. Best-fit values of $GI(\omega)\beta_a^2 = (175 \pm 45) \times 10^6\,l\,mol^{-1}$ and $\varepsilon = (2.0 \pm 0.5) \times 10^5\,l\,mol^{-1}$ yield $r = 0.994$. Direct measurement under the same conditions of $GI(\omega)\beta_a^2 = (17 \pm 1) \times 10^5\,l\,mol^{-1}$ ($r = 0.995$) for the reference compound *p*-nitroaniline, for which $\beta = 35 \times 10^{-30}$ esu in methanol [14], allows estimation of $\beta_a = (350 \pm 50) \times 10^{-30}$ esu for C334 in this solvent.

It is well-known that hyperpolarizability of a solute reflects the dielectric properties of the solvent [14], and may be thought of as a property of the solute and its solvation shell [20], in light of current theories of solvatochromism [23]. Accordingly, we attempted to estimate the hyperpolarizability of C334 in toluene. Under these circumstances, we obtained data for which the two-parameter fit according to Eq. (3) provided no improvement over the one-parameter fit, with $\beta_a = 0$ and $\varepsilon = 5.4 \times 10^4\,l\,mol^{-1}$ ($r = 0.997$). These results indicate that we should expect a large solvent dependence of the C334 hyperpolarizability, hence of HRS observed from it as a solute. Similar results were previously obtained in our laboratory [20] with cyanine dyes as solutes. In that study, we proposed that β is a property of the molecule and its solvent coordination shell. In this connection, it has been reported that coumarin laser dyes in

hydroxylic solvents may exist in the form of hydrogen-bonded solvent–coumarin complexes [24]. The fact that there are conditions under which we do not detect HRS, and that it would be unexpected under these circumstances, provides additional confidence that out observed signals are not biased by adventitious stray light.

3.2. Estimation of Φ_{HRS}

The emission spectra of C334 in methanol, excited at 420 and 840 nm are shown in Fig. 2. Note that in order to obtain these data, the amplifier gain, G, was 300× higher for recording the spectrum excited 840 nm than for the spectrum excited at 420 nm. It is clear, however, that the spectral distribution of emission in both cases is the same.

The emission intensity at 495 nm, $I_f(2\omega)$ was measured and corrected for C334 in methanol with and without a neutral density filter ($D = 0.50$) in place in the fundamental beam path. Experimentally, we found that this filter reduced the intensity of the fundamental beat at 840 nm by a factor of 3.1. A photomultiplier signal of 178 ± 12 mV was obtained without the filter in place; it was attenuated to 17 ± 4.5 mV with the filter inserted. Thus, attenuation of $I(\omega)$ by a factor of 3.1 reduces the detected fluorescence intensity by a factor of 10.5 ± 2.7, as expected for a biphotonic process. Thus, the observed fluorescence, whose wavelength distribution is shown in Fig. 2, is most likely attributable either to two-photon excitation or to one of the two proposed mechanisms involving HRS.

Estimates of $I_f(2\omega)$, measured in a series of solvents, with excitation at 840 nm and corrected as described above, $I_f(\omega)$ measured in the same solvents with 420 nm excitation, and solvent HRS intensity, $I_f(2\omega)$, are given in Table 1. Solvent HRS was in all cases found to be monochromatic, with a peak-width at half-maximum corresponding to 2 nm, the monochromator slit width. Note that G is 10× greater for measurement of $I_f(2\omega)$ than for $I_f(\omega)$.

Table 1
Estimation of Φ_{HRS} in various solvents

Solvent	$I_f(2\omega)$[a]	$I_f(\omega)$[b]	Φ_{HRS}[c]	$I(2\omega)$
Toluene	1.5 ± 1.0	780	$(3.00 \pm 2) \times 10^{-7}$	0.5
DMSO	5.2 ± 2.0	618	$(1.30 \pm 0.3) \times 10^{-6}$	4.5
Ethylene glycol	5.3 ± 1.0	582	$(1.35 \pm 0.3) \times 10^{-6}$	6
Acetonitrile	6.8 ± 3.0	652	$(1.50 \pm 0.3) \times 10^{-6}$	7
Acetone	9.5 ± 1.5	712	$(2.00 \pm 0.3) \times 10^{-6}$	7.5
Methanol	8.3 ± 2.0	705	$(1.80 \pm 0.3) \times 10^{-6}$	9
Water	7.0 ± 2.0	499	$(2.10 \pm 0.3) \times 10^{-6}$	10

[a] Amplifier gain: 10×.
[b] Amplifier gain: 1×.
[c] Eq. (4).

From these data, we can estimate Φ_{HRS}, where

$$\Phi_{HRS} = F\left[\frac{I_f(2\omega)}{I_f(\omega)}\right] \cdot \left[\frac{G_\omega}{G_{2\omega}}\right] \quad (4)$$

where F is the correction factor for relative photomultiplier response at the fundamental wavelength and 495 nm, respectively, experimentally measured using the "white card" method, equal to 0.015. Use of this correction factor is required insofar as the signal from the output photomultiplier, detecting light of 495 nm, is ratioed with that from the fundamental photomultiplier, which detects 840 nm light, for purposes of noise suppression. Gain factors, G_ω and $G_{2\omega}$ correspond to the lock-in amplifier gain settings used to record the signals with 420 and 840 nm excitation, respectively. Values thus obtained for Φ_{HRS} are also reported in Table 1.

Regression of Φ_{HRS} on $I(2\omega)$ is shown in Fig. 3. The least-squares line has a correlation coefficient, $r = 0.959$, and a possibly non-zero intercept of 0.3 ± 0.3. These data, taken by themselves, do not necessarily support an inference

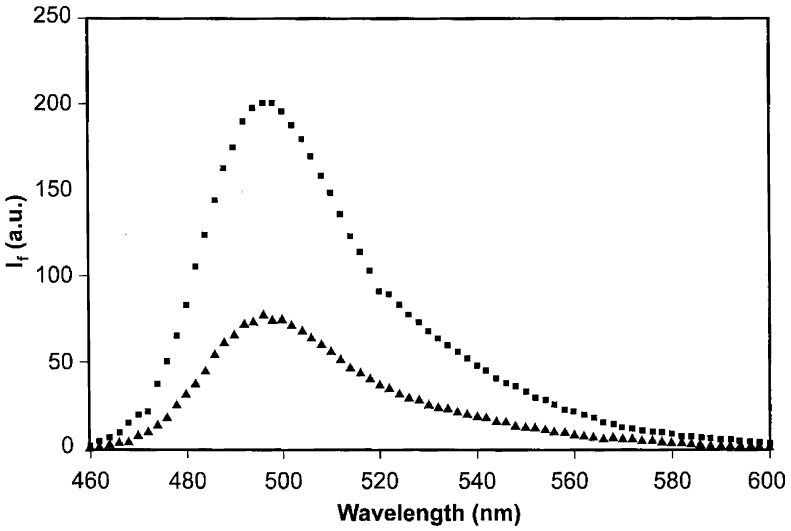


Fig. 2. Emission spectra of Coumarin334 (C334) excited at 420 nm (upper curve; amplifier gain = 0.3×) and 840 nm (lower curve; amplifier gain = 100×).

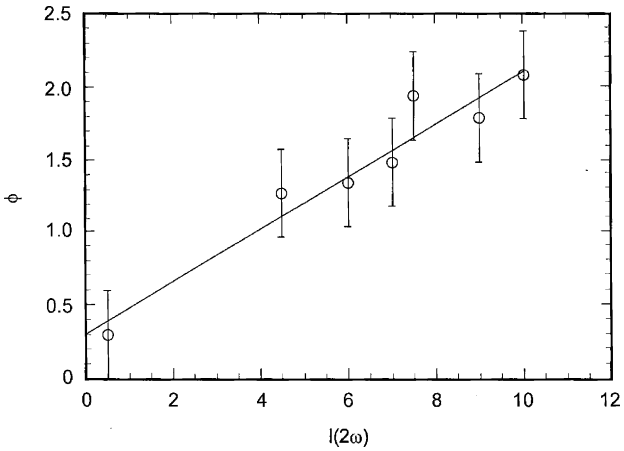


Fig. 3. Regression of Φ_{HRS}, the estimated efficiency of HRS sensitization of C334 fluorescence, on $I(2\omega)$, the measured intensity of solvent HRS.

that all observed fluorescence results from solvent-scattered second harmonic, re-absorbed by the C334. As noted above, the hyperpolarizability of C334, which should enable fluorescence directly when the second harmonic is resonant with a principal electronic transition of the molecule, is also strongly solvent dependent. It should scale with solvent polarity [14], which would provide more-or-less the ordering seen in Table 1. Thus, the correlation seen in Fig. 3, despite its statistical significance, is not diagnostic for the origin of the observed fluorescence in solvent-scattered second harmonic.

Accordingly, we undertook to establish the action spectrum for fluorescence excitation. If, grosso modo, the observed fluorescence results from C334 absorption of solvent-scattered second harmonic, then a plot of $I_f(2\omega)$ versus λ corresponding to 2ω should be congruent with the absorption spectrum of C334. The experimental data for $I_f(2\omega)$ at various fundamental wavelengths, recorded in methanol, were wavelength corrected, i.e. as $I_f(2\omega)F$, insofar as the data were collected over a spectral regime in which there is large variation in response of the photomultiplier monitoring the fundamental beam. These data are plotted and compared with the absorption spectrum of C334 in methanol in Fig. 4. It is clear that the two distributions are not congruent; the action spectrum is shifted to shorter wavelength compared to the absorption spectrum by nearly 60 nm (3400 cm^{-1})[1]. From this result, we infer that mechanism (2), proposed in the Section 1, cannot be principally responsible for the observed fluorescence.

3.3. Computational studies

On the other hand, the mechanism of fluorescence excitation may involve direct excitation of C334 by the fundamental, owing to its own hyperpolarizability, i.e. HRS by C334 itself, with the second harmonic self-absorbed. In the usual two-level approximation [25], the hyperpolarizability couples to a charge-transfer electronic transition in the C334. Accordingly, β should exhibit a maximum at a second harmonic wavelength resonant with this electronic transition. The wavelength dispersion of C334 could not be probed by direct experiment in the wavelength regime of interest, owing to the large uncertainties in the estimation of β by the curve-fitting procedure employed above, and the extreme degree of self-absorption of the second harmonic as the condition of resonance is approached.

Accordingly, we undertook semi-empirical computational studies of C334 at the AM1 level of approximation, as found in the SPARTAN 4.0 toolkit [26]. The AM1 level has been used successfully to obtain vertical and O–O excitation energies, as well as structural characterization of ground and first excited states of other coumarin dyes [27–29]. Fig. 5 shows density plots for the HOMO, LUMO and LUMO(+1), the next-lowest unoccupied molecular orbital, for C334. From these plots, it can be seen that there is a somewhat similar distribution of electron density between the HOMO and LUMO, corresponding to a primarily π–π^* S_1–S_0 electronic transition suggesting little charge-transfer character to this transition. This interpretation is consistent with the relatively minor degree of solvatochromism observable in either the principal absorption band or the fluorescence of C334. Thus, we observe a red shift in the absorption and emission maxima of ca. 10 nm for C334, going from hydrocarbon solvent to DMSO, whilst Nad and Pal [30] report a 40 nm red shift under the same circumstances for Coumarin151 (C151).

On the other hand, the LUMO(+1) has a very different, more localized, electron density distribution, which implies significant intramolecular charge-transfer character for the S_2–S_0 electronic transition. This transition also has significant π–π^* character, not only involving charge transfer from the julolidyl nitrogen, but also, to a certain extent, from the exocyclic carbonyl group, to the π-cloud of the heteroaromatic ring system. We propose that this is the electronic transition that is likely to be coupled to second harmonic scattering and which, accordingly, determines the wavelength dispersion of β. Recently, density functional theory calculations and ZINDO calculations including configuration interaction have been reported for the related dyes, Coumarin120 (C120) and C151 [31]. In both compounds these studies find that S_2 lies about 0.4 eV above S_1, corresponding to occurrence of S_0–S_2 ca. 70 nm to the blue of S_0–S_1, i.e. at 380 nm for C334.

These authors, however, find comparable dipole moment changes on excitation to either S_1 or S_2. In both C120 and C151 rehybridization at *N* is found to be a major component of relaxation upon excitation into the S_1 state [30,31]. This relaxation pathway is not accessible in C334 owing to the immobilization of *N* in the julolidyl fused ring system. This restriction may account, in part, for the reduced solvatochromism of C334 (vide supra).

[1] We thank one of the reviewers of this paper for suggesting this important experiment.

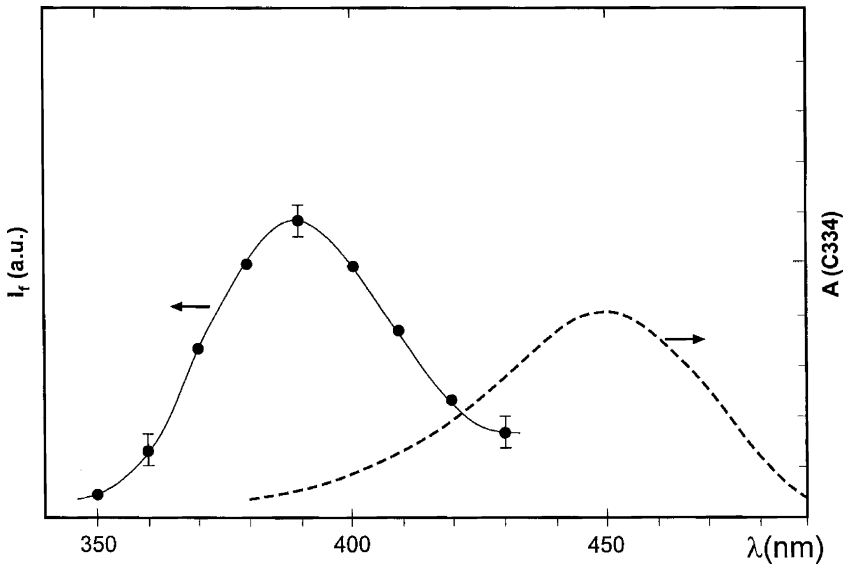


Fig. 4. Wavelength-response corrected action spectrum for second harmonic excitation of C334 fluorescence, presented as a function of the second harmonic wavelength (data points with solid line to guide the eye), and compared to the absorption spectrum of 1.0×10^{-5} M C334 in methanol (dashed line).

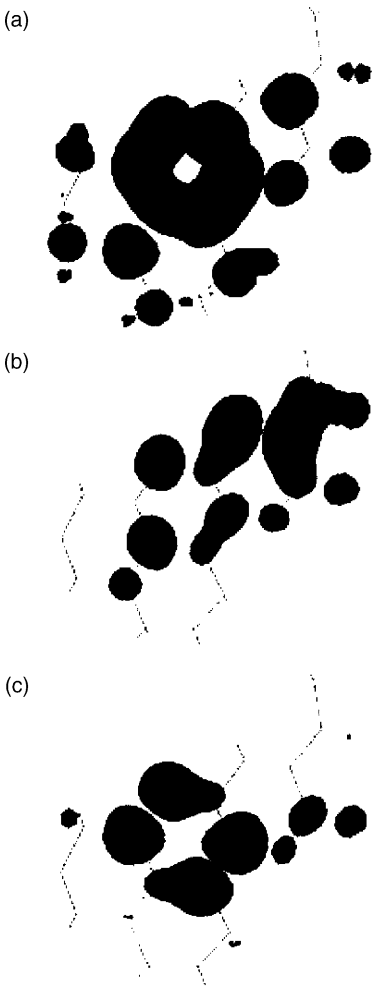


Fig. 5. Molecular orbitals of C334 according to AM1 calculation: (a) HOMO; (b) LUMO; (c) LUMO(+1).

3.4. Actinometry

Rearrangement of Eq. (1), neglecting the second term, yields:

$$\Phi_{\mathrm{HRS}} = \frac{I(2\omega)}{I(\omega)} = GI(\omega)\beta_{\mathrm{s}}^2 N_{\mathrm{s}}, \tag{5}$$

i.e. Φ_{HRS} should be intensity dependent. Our experimental configuration does not allow direct demonstration of this dependence as with full lamp power, we are operating so close to the limit of quantification of our system. However, Eq. (4) does imply that we should measure $I(\omega)$ under our conditions in order to be able to extrapolate conditions under which useful levels of Φ_{HRS} might obtain. To this end, we undertook actinometric experiments.

The reaction system chosen for actinometry was the one-electron photo-oxidation of C334 using diphenyliodonium cation (Ph_2I^+) as oxidant in methanol. The chemistry of solution phase photo-oxidations using this reagent has been elucidated in detail [32]. By analogy to these other systems,

$$\mathrm{C334} + h\nu \rightarrow \mathrm{C334^*} \tag{6a}$$

$$\mathrm{C334^*} + \mathrm{Ph_2I^+} \rightarrow \mathrm{C334^{\bullet +}} + \{\mathrm{Ph_2I^\bullet}\} \tag{6b}$$

$$\{\mathrm{Ph_2I^\bullet}\} \rightarrow \mathrm{PhI} + \mathrm{Ph^\bullet} \tag{6c}$$

$$\mathrm{Ph^\bullet} + \mathrm{C334^{\bullet +}} \rightarrow \mathrm{Ph\text{–}C334} + \mathrm{H^+} \tag{6d}$$

Accordingly, the rate of disappearance of C334 is given by

$$-\frac{\mathrm{d[C334]}}{\mathrm{d}t} = \frac{\Phi I f_{\mathrm{abs}}}{v} \tag{7}$$

where Φ is the quantum yield for disappearance of C334, I is the incident light intensity, f_{abs} the fraction of the incident light absorbed by the sample, easily estimated from

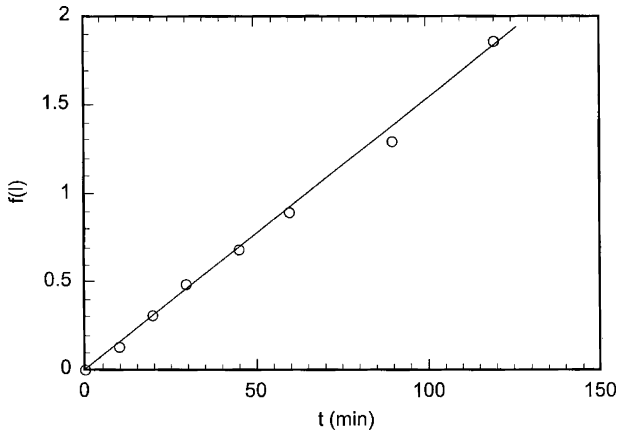


Fig. 6. Pseudo-first-order disappearance of C334 in photo-oxidation with diphenyliodonium salt at 420 nm, monitored as decrease in intensity of C334 fluorescence excited at 380 nm (actinometric experiment).

the absorption spectrum of the sample, and v is the sample volume. The quantum yield, Φ, is determined only by step (6b), as step (6c) is known to occur so rapidly (k = ca. $5 \times 10^9\,s^{-1}$) [33] that back electron-transfer is unimportant. It can be estimated therefore from the extent of C334 fluorescence quenching obtained on addition of Ph_2I^+.

Our experiments were carried out in methanol with 1×10^{-5} M C334 and 0.010 M Ph_2I^+. Irradiation was at 420 nm under the same conditions as for measurement of $I_f(\omega)$, above. Disappearance of C334 was monitored as the decrease in fluorescence excited at 380 nm, a wavelength at which C334 but not the reaction products, e.g. Ph–C334, absorbed light. Under these conditions, $f_{abs} = 0.50$ and $\Phi = 0.25$. The latter estimate was obtained from a Stern–Volmer plot for C334 fluorescence quenching by Ph_2I^+ in methanol, which yielded a slope, $K_{SV} = 32.6\,M^{-1}$, with $r = 0.998$. From these data and the measured fluorescence lifetime of 3.7 ± 0.5 ns for C334 [34], we obtain a second-order quenching rate constant corresponding to step (6b) above of $(9 \pm 1) \times 10^9\,M^{-2}\,s^{-1}$, close to the diffusion limit.

The first-order plot for the disappearance of C334 is shown in Fig. 6, where $f(I) = \ln[I_f(0)/I_f(t)]$, the ratio of the coumarin fluorescence intensities measured at times 0 and t. From these data, a pseudo-first-order rate constant, $k = 2.5 \times 10^{-4}\,s^{-1}\,M^{-1}$ was estimated ($r = 0.998$), where $k = -d\ln[C334]/dt$. From this estimate and Eq. (7), we obtain $I = 1 \times 10^{14}$ photons s^{-1}. For 420 nm radiation focused to an 0.05 cm^{-2} area on the sample, this irradiance, $I(\omega)$, corresponds to an optical power of 1 mW cm^{-1} in the fundamental beam. Comparable, or slightly greater, optical power is expected at 840 nm, on the basis of published output spectra of typical Xe-arc lamps [21].

4. Discussion

A plausible explanation for the observation of C334 fluorescence on irradiation at 840 nm is direct, two-photon excitation. The efficiency for production of fluorescence under steady-state conditions may be approximated as

$$\Phi_f = \tau\delta I^2 \tag{8}$$

where δ is the cross-section for two-photon absorption, and I is the incident photon flux. For typical values of $\delta = 10^{-50}\,cm^4\,s$ [6], $\Phi_f = 10^{-6}$ as measured here, and the experimental value of τ for C334 [34] $I = 1.6 \times 10^{26}$ photons $cm^{-2}\,s^{-1}$. This estimate corresponds to the threshold levels for two-photon excitation of fluorescence reported in [9]. Under our conditions, actinometry, above, has shown $I = 2 \times 10^{15}$ photons $cm^{-2}\,s^{-1}$, clearly insufficient by orders of magnitude for this mechanism of excitation. We have, however, showed that $I_f(2\omega)$ is proportional to $I(\omega)^2$, characteristic of biphotonic processes. We therefore conclude that HRS is responsible for the observed fluorescence. This inference is reasonable, given that HRS has been observed under our conditions directly from the solvents employed in this work, and from various other substrates in previous studies in our laboratory [19,20,22].

We account for the observation that the action spectrum for HRS sensitized fluorescence does not correspond to the absorption envelope assigned to the S_1–S_0 transition of C334 as follows. From the general two-level expression for hyperpolarizability [25],

$$\beta = \frac{3\mu_{12}^2\,\Delta\mu_{12}\,(h\omega_0)^2}{2h^4\,(\omega_0^2 - \omega^2)\,(\omega_0^2 - 4\omega^2)} \tag{9}$$

where μ_{12} is the dipole moment for the electronic transition coupled to hyperpolarizability, $\Delta\mu_{12}$ the change in dipole moment, and $h\omega_0$ is the energy of the coupled electronic transition. It can be seen from Eq. (5) that β undergoes a discontinuity when $2\omega = \omega_0$, and accordingly exhibits a maximum at corresponding wavelengths. Thus, if C334 itself is the principal source of the second harmonic scattering, the efficiency of HRS sensitized fluorescence should be greatest when $2\omega = \omega_0$, and we have inferred, on the basis of the molecular orbital calculations, that ω_0 should be that of the S_2–S_0 electronic transition. Accordingly, $h\omega_0$ for this transition must be 3.20 eV, about 0.42 eV greater than for S_1–S_0.

From Eq. (4), we project that a useful efficiency for HRS sensitization of photochemical and photophysical effects, $\Phi_{HRS} = 0.1$, should be achievable at optical powers 10^5-fold greater than used in our experiments, e.g. ca. 100 W cm^{-2}. However, it is problematical that such high levels of Φ_{HRS} can be observed in practice, owing in part to the onset of other, competing non-linear optical effects, such as stimulated Raman and Brillouin scattering, and self-focusing [14]. On the other hand, in representative studies where the dependence of $I(2\omega)$ on $I(\omega)$ has been characterized over a wide range of optical powers, no saturation of HRS response has been observed up to ca. 250 W cm^{-2} [17].

Two-photon fluorescence has been reported under conditions of fluorescence microscopy using a 150 μW laser at

720 nm, focused to a ca. 1 μm spot, i.e. ca. 2×10^4 W cm^{-2}, by Hänninen et al. [10]. This optical power density is nearly eight orders of magnitude below the saturation level for two-photon photophysics, as discussed in the Section 1, and substantially below that at which two-photon excitation of coumarin dyes, similar to the Coumarin138 used by Hänninen, has been observed, 6×10^7 W cm^{-2} (2×10^{26} photons cm^{-2} s^{-1}) and above [34]. Given the results obtained in the present study, we suggest that HRS sensitization may contribute significantly to emission assigned as biphotonic excitation in this and, perhaps other, similar scanning fluorescence microscopy studies.

5. Conclusion

We have observed fluorescence from C334 in a variety of solvents using a fundamental wavelength (840 nm) twice that of radiation actinic for excitation of this fluorescence, and we attribute this fluorescence to second harmonic HRS. Fluorescence intensity can be compared to that obtained by direct excitation of fluorescence under the same conditions to provide an estimate of the efficiency of the HRS process. After application of appropriate corrections to our raw data, we find $\Phi = \text{ca.}\ 10^{-6}$; it is strongly solvent dependent. Photochemical actinometry indicated a fundamental optical power of 1 mW cm^{-2} under our conditions. By mapping the action spectrum for second harmonic excitation of fluorescence, we exclude the possibility suggested by the solvent dependence of Φ, namely that the principal pathway for excitation of C334 involves second harmonic scattering by solvent, followed by absorption of acitinic light by the solute. Instead, we find that the action spectrum does not correlate with the absorption spectrum of C334. Therefore, we hypothesize that second harmonic scattering is coupled to an electronic transition in the C344 chromophore, which probably involves a higher excited state than that responsible for emission.

We infer from our experimental data and a consideration of the basic phenomenology of HRS that it may provide a useful method of long-wavelength sensitization of various photoeffects at readily available optical powers, e.g. ca. 100 W cm^{-2}. HRS may also contribute significantly to biphotonic fluorescence observed under typical conditions for multiphoton fluorescence microscopy of biological specimens.

Acknowledgements

Matthew Goertz thanks the University of Wisconsin—Eau Claire Office of University Research for a research studentship from the Don and Pat Cheney Research Fund. Instrumentation was, in part, a gift of 3M Co., St. Paul, MN. This work was presented in preliminary form at the Inter-American Photochemistry Conference, January 2–5, 2002, in Tempe, AZ. The authors thanks members of the photochemistry community assembled on that occasion for their helpful discussion and useful suggestions.

References

[1] N. Turro, Modern Molecular Photochemistry, Benjamin-Cummings, Menlo Park, CA, 1978.
[2] W. West, Photogr. Sci. Eng. 18 (1974) 35, and references cited therein.
[3] N. Serpone, R. Terzian, Adv. Electron Transfer Chem. 3 (1993) 33, and references cited therein.
[4] M. Goeppert-Mayers, Ann. Phys. 9 (1931) 273.
[5] W. Kaiser, C.B.G. Garrett, Phys. Rev. Lett. 7 (1961) 229.
[6] W. Denk, J.H. Strickler, W.W. Webb, Science 248 (1990) 73.
[7] S. Kawata, et al., Nature 412 (2001) 697.
[8] D.A. Parthenopoulos, P.M. Rentzepis, Science 245 (1989) 843.
[9] R.M. Williams, D.W. Piston, W.W. Webb, FASEB J. 8 (1994) 804.
[10] P.E. Hänninen, E. Soini, S.W. Hell, J. Microsc. 176 (3) (1994) 222.
[11] W. Denk, J. Biomed. Opt. 1 (3) (1996) 296.
[12] G. Chirico, C. Fumagalli, G. Baldini, J. Phys. Chem. B 106 (2002) 2508.
[13] R.W. Terhune, P.D. Maker, C.M. Savage, Phys. Rev. Lett. 14 (1965) 681.
[14] K. Clays, A. Persoons, Phys. Rev. Lett. 66 (1991) 2980.
[15] S. Kielich, J.R. LaLanne, F.B. Martin, Phys. Rev. Lett. 26 (1971) 1295.
[16] W.J. Schmid, H.W. Schrotter, Chem. Phys. Lett. 45 (1977) 502.
[17] M. Jacobsohn, U. Banin, J. Phys. Chem. B 104 (2000) 1.
[18] F.W. Vance, B.I. Lemon, J.A. Ekhoff, J.T. Hupp, J. Phys. Chem. B 102 (1998) 1845.
[19] A. Rassbach, M.R.V. Sahyun, J. Imaging Sci. Technol. 45 (2001) 254.
[20] A.F. Marks, A.K. Noah, M.R.V. Sahyun, J. Photochem. Photobiol. A: Chem. 139 (2001) 143.
[21] R.S. Bergman, in: W. Thomas (Ed.), Handbook of Photographic Science and Engineering, IS&T, Springfield, VA, 1997, p. 37.
[22] M.R.V. Sahyun, Spectrochim. Acta 58 (2002) 3149.
[23] B. Boldrini, E. Cavalli, A. Painelli, F. Terenziani, J. Phys. Chem. A 106 (2002) 6286.
[24] B.A. Pryor, Y. Chen, M.R. Topp, Chem. Phys. Lett. 318 (2000) 440; B.A. Pryor, Y. Chen, M.R. Topp, Chem. Phys. Lett. 321 (2000) 62, and references cited therein.
[25] J.L. Oudar, D.S. Chemla, J. Chem. Phys. 66 (1977) 2664.
[26] W.J. Hehre, SPARTAN, Version 4.0, Wavefunction, Inc., Monterey Park, CA, 1997.
[27] P.K. McCarthy, G.J. Blanchard, J. Phys. Chem. 97 (1993) 12205.
[28] A. Muhlpfordt, R. Schenz, N.P. Ernsting, V. Farztdinov, S. Grimme, Phys. Chem. Chem. Phys. 1 (1999) 3209.
[29] K. Rechthaler, G. Koehler, Chem. Phys. 189 (1994) 99.
[30] S. Nad, H. Pal, J. Phys. Chem. A 105 (2001) 1097.
[31] R.J. Cave, K. Burke, E.W. Castner Jr., J. Phys. Chem. A 106 (2002) 9294.
[32] R.J. DeVoe, P.M. Olofson, M.R.V. Sahyun, Adv. Photochem. 17 (1989) 313, and references cited therein.
[33] R.J. DeVoe, M.R.V. Sahyun, E. Schmidt, D.K. Sharma, Can. J. Chem. 68 (1990) 612.
[34] M.R.V. Sahyun, D.K. Sharma, Chem. Phys. Lett. 189 (1992) 571.