Two-photon absorption spectra of carotenoids compounds
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Carotenoids are biosynthetic organic pigments that constitute an important class of one-dimensional \(\pi\)-conjugated organic molecules with enormous potential for application in photobiotic devices. In this context, we studied the degenerate two-photon absorption (2PA) cross-section spectra of two carotenoid compounds (\(\beta\)-carotene and \(\beta\)-apo-8'-carotenal) employing the conventional and white-light-continuum Z-scan techniques and quantum chemistry calculations. Because carotenoids coexist at room temperature as a mixture of isomers, the 2PA spectra reported here are due to samples containing a distribution of isomers, presenting distinct conjugation length and conformation. We show that these compounds present a defined structure on the 2PA spectra, that peaks at 650 nm with an absorption cross-section of approximately 5000 GM, for both compounds. In addition, we observed a 2PA band at 990 nm for \(\beta\)-apo-8'-carotenal, which was attributed to a overlapping of \(1\,^1\text{B}_\text{u}^{-}\)-like and \(2\,^1\text{A}_\text{g}^{-}\)-like states, which are strongly one- and two-photon allowed, respectively. Spectroscopic parameters of the electronic transitions to singlet-excited states, which are directly related to photophysical properties of these compounds, were obtained by fitting the 2PA spectra using the sum-over-states approach. The analysis and interpretations of the 2PA spectra of the investigated carotenoids were supported by theoretical predictions of one- and two-photon transitions carried out using the response functions formalism within the density functional theory framework, using the long-range corrected CAM-B3LYP functional. © 2011 American Institute of Physics. [doi:10.1063/1.3590157]

I. INTRODUCTION

Two-photon absorption (2PA) has been widely exploited in different fields due to its quadratic dependence on the irradiance, which makes it possible to confine the laser excitation to the focal volume.\(^1\) Such feature has led to a large number of technological applications, such as multiphoton fluorescence microscopy,\(^2\) two-photon photopolymerization,\(^3,4\) 3D optical data storage\(^5–7\) and two-photon photodynamic therapy.\(^8\) In the last decade, \(\pi\)-conjugated molecules have emerged as potential candidates for applications in photonic devices due to its easy handling, environmental stability and structural flexibility.\(^9,10\) Among these materials, carotenoids constitute an important class of linear \(\pi\)-conjugated molecules that exhibit high degree of electronic delocalization and ultrafast dynamics.\(^11–13\) \(\beta\)-carotene, for example, has an important role in the light-harvesting function of bacterial photosynthesis;\(^14,15\) all-trans retinal (analog to trans-\(\beta\)-apo-8'-carotenal) is responsible for light transduction in nervous impulses, which involves a series of biochemical events in bacteriorhodopsin.\(^16,17\)

In the last years, third-order nonlinearities of carotenoids were extensively studied, both theoretically and experimentally, via third-harmonic generation\(^18,19\) and quantum chemical calculations.\(^2,20\) Van Beek and Albrecht\(^18\) investigated the phase and magnitude of the third-order nonlinear susceptibility of \(\beta\)-carotene dissolved in benzene using the Maker fringes technique from 1030 to 1221 nm. More recently, Marder et al.\(^21\) studied the third-order optical nonlinearities in polarized carotenoids using third harmonic generation. They observed the enhancement of the optical nonlinearity as the intramolecular charge transfer increase from the polyenic chain to the acceptor moiety. Beljonne et al.\(^22\) used theoretical methods, that is configuration interaction approach, to investigate the lowest singlet excited states of the \(\beta\)-carotene. They also studied the third-order nonlinear optical response applying the sum-over-states approach. However, little is known about the 2PA cross section of these molecules. For example, the 2PA process of such carotenoid compounds employing femtosecond pulses and over a wide spectral range has not been investigated so far, what is essential for potential applications of these compounds. The ordering of the low-lying excited electronic states (\(2\,^1\text{A}_\text{g}^{-}\)-like and \(1\,^1\text{B}_\text{u}^{-}\)-like) is very important to define the photochemical properties of carotenoids. Walla et al.,\(^23\) using two-photon spectroscopy, observed a fast energy transfer from the dipole-forbidden carotenoid \(2\,^1\text{A}_\text{g}^{-}\)-like state to chlorophyll that performs an important role in photosynthesis.

In this context, we present here a study of the degenerated 2PA cross-section spectra of two carotenoid molecules (\(\beta\)-carotene and \(\beta\)-apo-8'-carotenal) employing the conventional (720–1100 nm) and white-light continuum (WLC) Z-scan (590–720 nm) techniques. However, as reported by Roth et al.,\(^24\) carotenoid compounds coexist at room temperature as a mixture of isomers (\(\text{trans}\) and \(\text{cis}\)). Therefore, the
2PA spectra reported here refers to a distribution of isomers, which presents different conjugation length and molecular conformation. We model the experimental 2PA cross-section spectra using the sum-over-state (SOS) approach considering the two-photon allowed states for β-carotene and β-apo-8'-carotenal, respectively. In an effort to understand the origin of the structures observed along the 2PA spectra, a theoretical study using the response functions formalism within the DFT framework was carried out. The theoretical computations allowed to determine the two-photon allowed states and their probabilities over the experimentally studied spectral region.

II. EXPERIMENTAL

We prepared β-carotene/toluene and β-apo-8'-carotenal/toluene solutions with concentrations of $8.5 \times 10^{-6}$ and $2.4 \times 10^{-3}$ mol L$^{-1}$, for linear and nonlinear optical measurements, respectively. Both molecules were purchased from Sigma-Aldrich in powder with purity between 93%-96%. Nonlinear optical measurements were performed at a temperature of 20°C. We measured the linear absorption and fluorescence spectra before and after each nonlinear optical measurement and no degradation was observed for the temperatures and intensities used. The molecular structures of the studied carotenoid compounds are presented in Fig. 1.

The samples were placed in 2 mm thick quartz cuvettes for the optical measurements. The linear absorption spectra were recorded using a Cary 17 UV-Vis-NIR spectrophotometer. The 2PA spectrum from 720 to 1100 nm was obtained using the Z-scan technique, employing 120-fs pulses from an optical parametric amplifier (OPA). The OPA was pumped by 150-fs pulses from a Ti:sapphire chirped pulse amplifier operating at 775 nm and 1 kHz. From 590 to 720 nm, the 2PA spectrum was measured using the WLC Z-scan, since this technique provides decreased experimental error in the obtained nonlinear spectrum, allowing better determination of two-photon states. The WLC is produced by focusing the laser beam from the OPA at 1110 nm with an $f = 10$ cm lens into a 4 cm-thick quartz cell containing distilled water. Figure 2 shows the WLC spectrum obtained. A low-pass filter is used to remove the strong pump pulse and the infrared part of the WLC spectrum. The WLC beam is focused into the sample, which is scanned along the beam propagation in Z-direction, as usually done in the standard Z-scan technique. The WLC transmitted through the sample is completely collected and directed to a spectrometer with a resolution of $\sim 1$ nm. The spectra are acquired for each z position as the sample is scanned along the Z-direction and then normalized by the one obtained far from the focal plane. By selecting a particular wavelength from the whole measured spectrum, we obtain a Z-scan signature which is related to the nonlinear response of the sample at that wavelength. The WLC pulse used in our experiment presents a positive chirp of approximately 5 ps, measured using optical Kerr-gate in hexane. This chirp introduces a separation of 18 fs/nm, leading to a nondegenerated behavior of the nonlinear process, considering the $\sim 10$ nm bandwidth of 150 fs pulses. The Z-scan setup is the same as that described in a previous publication.

III. THEORETICAL SECTION

Theoretical calculations were used to scrutinize the systems at the molecular level and shed light on the experimental results. Our motivation is to explore and understand the electronic structure, relating to the one- and two-photon absorption spectra of the investigated compounds, by means of first-principles theoretical methodology for computing 2PA cross sections.

A. Two-photon absorption

In the Z-scan experiment, the two-photon absorption is measured through the dissipation of the incident light, which for a single beam 2PA experiment is twice the transition rate. In the case, the 2PA cross-section $(\sigma_{gf})$ of each excited state (two-photon resonant condition), for a degenerate process, is written as:

$$\sigma_{gf}(\omega) = \frac{16\pi^3 a_0^5 (\hbar \omega)^2}{c \pi (\Gamma_f) \langle \delta g_f \rangle},$$

where $\alpha$ is the fine structure constant, $a_0$ is the Bohr’s radius, $c$ is the speed of light and $E = \hbar \omega$ is the photon energy (half of the transition energy). $\Gamma_f$ is the damping constant describing half width at half-maximum of the final state.
linewidth (assuming a Lorentzian line-shape) and \(\langle \delta_{gf} \rangle\) is the two-photon transition probability for the transition from the ground state (g) to a final state (f). To obtain the 2PA cross-section in G"opbert–Mayer units (1 GM = 10^{-50} \text{cm}^2 \text{s photon}^{-1}) in Eq. (1) one has to use \(a_0 = 5.291772108 \times 10^{-69} \text{cm}, \quad c = 2.99792458 \times 10^{10} \text{cm/s}\) and the values of \(E = \hbar \omega, \quad \Gamma_x, \quad \Gamma_y, \quad \text{and} \quad \langle \delta_{gf} \rangle\) in atomic units.

The degenerate two-photon transition probability in an isotropic medium, using a linearly polarized laser beam is given by\(^{32,33}\)

\[
\langle \delta_{gf} \rangle = \frac{1}{36} \sum_{x, \beta} 2 S_{xz}^{gf}(\langle \delta_{gf} \rangle^* + 4 S_{xf}^{gf}(S_{gf}^{gf})^*)
\]

in which the subscripts \(x\) and \(\beta\) represent the Cartesian coordinates and \(S_{x\beta}^{gf}\) is the two-photon matrix element, identified from the sum-over-states (SOS) expression and defined as

\[
S_{x\beta}^{gf} = \frac{1}{2\hbar} \sum_k \left[ \frac{\langle g| \hat{e} \cdot \hat{\mu}_x |k\rangle \langle k| \hat{e} \cdot \hat{\mu}_\beta |f\rangle}{\omega_{fk} - \omega} + \frac{\langle g| \hat{e} \cdot \hat{\mu}_\beta |k\rangle \langle k| \hat{e} \cdot \hat{\mu}_x |f\rangle}{\omega_{fk} - \omega} \right]
\]

for a single beam 2PA experiment.\(^{29–31}\)

**B. Computational details**

The equilibrium molecular geometries of the studied compounds were determined based on the density functional theory (DFT)\(^{34,35}\) employing the hybrid exchange-correlation B3LYP\(^{36,37}\) functional and the standard 6-311 G(d,p) basis set\(^{38}\) as implemented at the Gaussian 03 package.\(^{39}\) Subsequently, to characterize the lowest one- and two-photon allowed states of the studied carotenoid compounds, the response functions formalism,\(^{40–42}\) within the DFT framework, was used as implemented in the DALTON program.\(^{43}\) In this approach, the excitations energies and transition moments (two-photon probabilities) are analytically computed as poles and single residues of the linear (quadratic) response function of the molecular electronic density, respectively. All electronic transition computations were carried out employing the recently developed Coulomb-attenuated CAM-B3LYP hybrid functional\(^{44}\) and the 6–31 + G(d) basis set.\(^{38}\) All computations were carried out _in vacuo._

It is important to mention that linewidths of the excited states vary, both from one molecule to another, as well as for different transitions within one molecule. In this work, the excited states linewidths of the compounds were estimated by fitting the nonlinear spectra employing Eq. (4) and the values were used to determine the theoretical 2PA cross sections.

**IV. RESULTS AND DISCUSSION**

Figure 3 (left axis —gray line) illustrates the electronic absorption spectra of (a) \(\beta\)-carotene and (b) \(\beta\)-apo-8'-carotenal in toluene. Both molecules present an absorption band from 430 nm to 550 nm, with vibronic structures which are associated with the two \(\beta\)-end groups for \(\beta\)-carotene and with the keto carbonyl group for \(\beta\)-apo-8'-carotenal.\(^{45–47}\) The vibrational progressions exhibit peaks separated by 155 meV in both molecules. Moreover, these compounds are completely transparent in the region above 590 nm. Figure 3 (right axis —circles) shows the 2PA cross-section spectra of the \(\beta\)-carotene (a) and \(\beta\)-apo-8'-carotenal (b). For \(\beta\)-carotene [Fig. 3(a)] one notices a monotonic increase of the 2PA cross section as the excitation wavelength approaches the one-photon absorption region (resonance enhancement) and a band located around at 650 nm. A similar behavior is observed for \(\beta\)-apo-8'-carotenal [Fig. 3(b)], but an extra 2PA band appears at 990 nm. Such band is red-shifted by about 1100 cm\(^{-1}\) in comparison to the lower-energy state observed in the linear absorption spectrum, indicating that the state accessed by the absorption of two-photons does not correspond, necessarily, to the state accessed by one-photon. It is known that carotenoids present a low-lying \(2^1\text{Ag}^-\)like state which is allowed only by 2PA process.\(^{11,22,48}\) In this case, we attribute this 2PA band to the overlapping of \(1^1\text{Bu}^+\)like and \(1^1\text{Ag}\)-like states, which are strongly one- and two-photon allowed, respectively. Although we have not observed, within our experimental error, any band structure in the spectral region near to the \(1^1\text{Bu}^+\)-like state for \(\beta\)-carotene, we added the \(2^1\text{Ag}^-\)-like state in our sum-over-states model due the considerable 2PA cross-section values in this region.

Additionally, we have shown that the 2PA band located at the 650 nm, for both molecules, presents cross-section
values among the largest ones reported in the literature for organic molecules (about 5500 GM for β-carotene and 4500 GM for β-apo-8’-carotenal). We observed a linear dependence of the transmittance change (ΔT) with the excitation laser irradiance for the wavelength range from 630 to 1100 nm (data not shown), which is the typical behavior of nonresonant 2PA process.49 These high 2PA cross-section values should be associated with the planar configuration of the equilibrium molecular geometry (Fig. 4) of both molecules, which is expected to favor the effective conjugation length and increase the 2PA cross section.1,50

It is known that these compounds coexist, at room temperature, as a mixture of isomers (trans and cis)54 with very low activation energies for thermal isomerization. Although the chromophores studied here were purchased as isomers trans, both the spectral behavior and the magnitude of 2PA cross-section spectra are due to a distribution of isomers with distinct conjugation length and conformation. Even with this limitation, the experimental results provide useful information about the nonlinear optical properties of this class of organic compounds, similarly to what happens for conjugated polymers, which are polydisperse.51-53

To further characterize the studied molecules and support our interpretations of the 2PA spectra, we performed theoretical calculations employing quantum-chemical methods. Due to the size of the studied compounds, all computations were carried out at the DFT level (see computational details section). Table I presents the theoretical results of the 1PA and 2PA calculations for the carotenoids compounds employing the response functions formalism with the aid of the CAM-B3LYP functional and the 6–31 + G(d,p) basis set.

As shown in Table I, β-carotene presents three two-photon allowed electronic states located at 342, 319, and 258 nm. The states accessed via two-photon transitions are dark states for the 1PA process (see Table I), what is due to its molecular symmetry (centrosymmetry) and the distinct selection rules of the one- and two-photon absorption processes.54 The theoretical results indicate four 2PA allowed states for β-apo-8’-carotenal, located at 472, 329, 317, and 273 nm. In this case, the 2PA allowed states are slightly allowed via 1PA, while the strongly 1PA allowed state S1 is only slightly allowed via 2PA, as expected due to the relaxation of the electric-dipole selection rules for noncentrosymmetric molecules,54 such as β-apo-8’-carotenal.

It has been shown that prediction of correct ordering of $2^1Ag$-like and $1^1Bu^-$-like states in conjugated polyenes is still difficult for quantum-mechanical methods, basically due to the importance of considering double excitations to obtain the correct transition energy to the $2^1Ag$-like excited state of these molecules. Only highly accurate approaches, such as CASPT2 or MR-CI, are successful in describing the correct ordering of these excited electronic states. The use of such computationally expensive methods to investigate the one- and two-photon processes of carotenoids compounds is however not feasible, in particular for the two-photon process, due to the size of the investigated compounds.

It is known that the $2^1Ag$-like state is the lowest excited state of the carotenoids compounds.55-57 In addition, previous experimental studies also confirmed that such state is allowed by 2PA11,22,48 and forbidden by 1PA. The results of DFT computations carried out in this study suggest the $1^1Bu^-$-like state as the lowest excited state in the case of both compounds, and transition energies are in good agreement with the 1PA experimental data. On the other hand, the computed transition energy of the $2^1Ag$-like state (S2 state in Table I) is overestimated by about 1 eV and, therefore, did not provide a correct ordering of the two ($2^1Ag$-like and $1^1Bu^-$-like) lowest-lying states. Nevertheless, the 2PA probability and the oscillator strength computed confirmed that the $2^1Ag$-like state of both compounds is allowed for a two-photon transition and only weakly allowed for a one-photon transition.

It has been shown recently by Hsu, Hirata, and Head-Gordon58 that for trans-1,3-butadiene, trans-trans-1,3,5-hexatriene, all-trans-1,3,5,7-octatetraene, and all-trans-1,3,5,7,9-decapentaene, the value of the excitation energy corresponding to the transition to the $2^1Ag$-like state can be satisfactorily determined applying the TD-DFT method, with the aid of some commonly employed DFT functionals (e.g., B3LYP), while the transition energy of the $1^1Bu^-$-like state is underestimated (by about 0.5–0.7 eV) and, therefore, TDDFT in combination with such functionals also provides an incorrect ordering of the two lowest-lying states arising from the presence of polyenic chains. The authors also noted that the deviation from experimental results (1PA spectra) is slightly larger for the longer polyenes and concluded that the systematic underestimation of excitations energies of the $1^1Bu$ state apparently indicates a deficiency in the exchange-correlation functionals employed in the study. However, in the present study we observed a quite distinct performance of the response functions (within the DFT framework) for carotenoid compounds using the long-range corrected CAM-B3LYP functional. The transition energy of the $1^1Bu^-$-like state in our computations is slightly overestimated for both compounds (<0.1 eV) and this small difference could even be attributed to solvent effects, which slightly shift this transition to the infrared region, not taken into account in this study. On the other hand, as already mentioned the transition energy of the $2^1Ag$-like state is overestimated by about 1 eV in our computations for both compounds. Therefore, we

![FIG. 4. Ground-state equilibrium geometry of (a) all-trans β-carotene and (b) trans-β-apo-8’-carotenal optimized at the DFT level of theory [B3LYP/6-311G(d,p)].](image-url)
and 317 nm for trans-electronic state located at 319 nm for all-trans for both molecules (Fig. 3) corresponds to the 2PA allowed transition probabilities, in agreement with the experimental theoretical results revealed that these states possess high 2PA formation (transition energies and one- and two-photon amplitudes) and further clarified the experimental 2PA spectrum of both carotenoid compounds.

should point out that the incorrect orderings determined by the DFT based methods for the two lowest-lying states of the polyenic chairs\textsuperscript{46,58} and the carotenoids compounds (present study) in these two studies have distinct reasons and basically reflects the fundamental importance of the DFT functional choice.

The experimental 2PA band located around at 650 nm for both molecules (Fig. 3) corresponds to the 2PA allowed electronic state located at 319 nm for all-trans \(\beta\)-carotene and 317 nm for trans-\(\beta\)-apo-8'-carotenal (Table I). The theoretical results revealed that these states possess high 2PA transition probabilities, in agreement with the experimental results. However, the differences observed between the experimental (Table II) and theoretically determined (Table I) 2PA cross-section values are probably due to imprecisions in the determination of the linewidths and transition energy. Even though, theoretical calculations provided useful information (transition energies and one- and two-photon amplitudes) and further clarified the experimental 2PA spectrum of both carotenoid compounds.

| All-trans-\(\beta\)-carotene |  |
|---|---|---|---|---|
| States | Nature transition | 1PA | 2PA |  |
| | | Energy (cm\(^{-1}\)) | Oscillator strength | Energy (cm\(^{-1}\)) | Transition probability | 2PA cross-section/2 (GM) |  |
| \(S_2 (1^1\text{Bu}^-)\) | (HOMO-1 \(\rightarrow\) LUMO +1) | 3\% | 20919 | 4.126 | 20919 | 79 | 0.04 |  |
| | (HOMO \(\rightarrow\) LUMO) | 47\% | (478 nm) | (478 nm) |  |
| \(S_3 (2^1\text{Ag}^-)\) | (HOMO-2 \(\rightarrow\) LUMO +1) | 3\% | 29239 | 0.000 | 29239 | 1.58E6 | 1677 |  |
| | (HOMO-1 \(\rightarrow\) LUMO) | 45\% | (342 nm) | (342 nm) |  |
| \(S_3 (3^1\text{Ag}^-)\) | (HOMO-1 \(\rightarrow\) LUMO +2) | 3\% | 31381 | 0.000 | 31381 | 2.96E6 | 2589 |  |
| | (HOMO-1 \(\rightarrow\) LUMO +1) | 44\% | (319 nm) | (319 nm) |  |
| | (HOMO-3 \(\rightarrow\) LUMO) | 2\% | (319 nm) | (319 nm) |  |
| | (HOMO \(\rightarrow\) LUMO +4) | 1\% | 38758 | 0.000 | 38758 | 3.22E6 | 7008 |  |
| | (HOMO \(\rightarrow\) LUMO) | 11\% | (258 nm) | (258 nm) |  |
| \(S_2 (4^1\text{Ag}^-)\) | (HOMO \(\rightarrow\) LUMO +7) | 25\% | (258 nm) | (258 nm) |  |
| | (HOMO \(\rightarrow\) LUMO+14) | 3\% | (258 nm) | (258 nm) |  |

| trans-\(\beta\)-apo-8'-carotenal |  |
|---|---|---|---|---|
| States | Nature transition | 1PA | 2PA |  |
| | | Energy (cm\(^{-1}\)) | Oscillator strength | Energy (cm\(^{-1}\)) | Transition probability | 2PA cross-section/2 (GM) |  |
| \(S_1 (1^1\text{Bu}^-)\) | (HOMO-1 \(\rightarrow\) LUMO +1) | 2\% | 21212 | 3.560 | 21212 | 9.74E4 | 63 |  |
| | (HOMO \(\rightarrow\) LUMO) | 46\% | (472 nm) | (472 nm) |  |
| | (HOMO-2 \(\rightarrow\) LUMO +1) | 1\% | (472 nm) | (472 nm) |  |
| | (HOMO-1 \(\rightarrow\) LUMO) | 44\% | (472 nm) | (472 nm) |  |
| \(S_2 (2^1\text{Ag}^-)\) | (HOMO-4 \(\rightarrow\) LUMO) | 22\% | 30407 | 0.081 | 30407 | 9.51E5 | 989 |  |
| | (HOMO-4 \(\rightarrow\) LUMO +1) | 15\% | (329 nm) | (329 nm) |  |
| | (HOMO-4 \(\rightarrow\) LUMO +2) | 7\% | (329 nm) | (329 nm) |  |
| \(S_3 (3^1\text{Ag}^-)\) | (HOMO-1 \(\rightarrow\) LUMO +2) | 2\% | 31553 | 0.091 | 31553 | 1.68E6 | 1836 |  |
| | (HOMO-1 \(\rightarrow\) LUMO +1) | 44\% | (317 nm) | (317 nm) |  |
| | (HOMO-3 \(\rightarrow\) LUMO) | 2\% | (317 nm) | (317 nm) |  |
| \(S_2 (4^1\text{Ag}^-)\) | (HOMO-3 \(\rightarrow\) LUMO +1) | 1\% | 36620 | 0.070 | 36620 | 4.04E5 | 778 |  |
| | (HOMO-2 \(\rightarrow\) LUMO +1) | 36\% | (273 nm) | (273 nm) |  |
| | (HOMO-2 \(\rightarrow\) LUMO +1) | 36\% | (273 nm) | (273 nm) |  |
| | (HOMO-1 \(\rightarrow\) LUMO +1) | 1\% | (273 nm) | (273 nm) |  |
| | (HOMO \(\rightarrow\) LUMO+14) | 4\% | (273 nm) | (273 nm) |  |

**TABLE II.** Spectroscopic parameters used/obtained employing the SOS approach.

<table>
<thead>
<tr>
<th>Spectroscopic Parameters</th>
<th>All-trans-(\beta)-carotene</th>
<th>All-trans-(\beta)-apo-8'-carotenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\nu_{\text{G}}) (cm(^{-1}))</td>
<td>20200 (495 (\pm) 5 nm)</td>
<td>20200 (495 (\pm) 5 nm)</td>
</tr>
<tr>
<td>(\nu_{\text{G}}) (cm(^{-1}))</td>
<td>21280 (470 (\pm) 2 nm)</td>
<td>21280 (470 (\pm) 2 nm)</td>
</tr>
<tr>
<td>(\nu_{\text{G}}) (cm(^{-1}))</td>
<td>30303 (330 (\pm) 2 nm)</td>
<td>30770 (325 (\pm) 2 nm)</td>
</tr>
<tr>
<td>(\nu_{\text{G}}) (cm(^{-1}))</td>
<td>35358 (283 (\pm) 2 nm)</td>
<td>35358 (283 (\pm) 2 nm)</td>
</tr>
<tr>
<td>(\Gamma_{\text{G}}) (cm(^{-1}))</td>
<td>4000 (98 (\pm) 10 nm)</td>
<td>3335 (83 (\pm) 5 nm)</td>
</tr>
<tr>
<td>(\Gamma_{\text{G}}) (cm(^{-1}))</td>
<td>3860 (85 (\pm) 2 nm)</td>
<td>4260 (94 (\pm) 2 nm)</td>
</tr>
<tr>
<td>(\Gamma_{\text{G}}) (cm(^{-1}))</td>
<td>5340 (58 (\pm) 5 nm)</td>
<td>4366 (45 (\pm) 5 nm)</td>
</tr>
<tr>
<td>(\Gamma_{\text{G}}) (cm(^{-1}))</td>
<td>3335 (26 (\pm) 1 nm)</td>
<td>3335 (26 (\pm) 1 nm)</td>
</tr>
<tr>
<td>(\mu_{\text{G}}) (Debye)</td>
<td>1.5 (\pm) 1</td>
<td>1.5 (\pm) 1</td>
</tr>
<tr>
<td>(\mu_{\text{G}}) (Debye)</td>
<td>14.8 (\pm) 1</td>
<td>14.0 (\pm) 1</td>
</tr>
<tr>
<td>(\mu_{\text{G}}) (Debye)</td>
<td>19.0 (\pm) 1</td>
<td>16.5 (\pm) 1</td>
</tr>
<tr>
<td>(\mu_{\text{G}}) (Debye)</td>
<td>15.5 (\pm) 1</td>
<td>15.5 (\pm) 1</td>
</tr>
<tr>
<td>(\Delta\mu_{\text{G}}) (Debye)</td>
<td>20.0 (\pm) 5</td>
<td>25.0 (\pm) 5</td>
</tr>
</tbody>
</table>
To better understand the 2PA spectrum and its connection with the molecular properties of the compounds, we employed the sum-over-states (SOS) approach. To model the 2PA spectra of carotenoids we considered an electronic states diagram (show in Fig. 5) based on the excited states energies, obtained from the one- and two-photon absorption spectra and theoretical calculation results. In this case, the SOS expression used to fit the 2PA spectra is given by

$$\delta(\nu) = \frac{4}{5\pi}\left(\frac{\mu_0^2}{\hbar c}\right)^2 \left\{ \frac{\mu_{11}^2}{(\nu_{11} - 2\nu_0^2)^2 + \Gamma_{11}^2} + \frac{\nu^2}{(\nu - \nu_0)^2 + \Gamma_0^2} \times \left[ \frac{\mu_{02}^2}{(\nu_{02} - 2\nu_0^2)^2 + \Gamma_{02}^2} + \frac{\mu_{12}^2}{(\nu_{12} - 2\nu_0^2)^2 + \Gamma_{12}^2} \right] \right\}$$

(4)

where $\hbar$ is the Planck’s constant, $c$ is the speed of light and $\nu$ is the excitation laser frequency. $\nu_{nm}$, $\Gamma_{nm}$, and $\mu_{nm}$ represent, respectively, the transition frequency, damping constant describing half width at half-maximum of the final state linewidth and transition dipole moment corresponding to the $n \rightarrow m$ transition. In this expression, $\Delta \mu_{01} = \mu_{11} - \mu_{00}$ is the difference between the permanent dipole moment of first excited ($|\mu_{11}\rangle$) and the ground ($|\mu_{00}\rangle$) states. According to this model, the 2PA spectra is described by a dipolar two-photon transition to $|S_1\rangle$ and terms related to transitions to higher electronic states, $|S_2\rangle$ and $|S_3\rangle$, which are affected by a resonance enhancement of the nonlinearity. For the resonance enhancement factor (term multiplying the parenthesis in the square brackets) we assumed that only one intermediate state contributes to the optical nonlinearity. Equation (4) can be directly used to model the results observed for $\beta$-carotene. However, for $\beta$-apo-8’-carotenal the term $|\mu_{01}\rangle^2$ is substituted by $\mu_{01} \cdot \mu_{02}$ due the overlapping between the states $|S_1\rangle$ and $|S_2\rangle$.

In Fig. 3, the solid black line represents the fitting obtained using Eq. (4), with $\nu_{02}$, $\nu_{03}$, and $\Gamma_0$ taken from the linear absorption spectrum, and $\nu_{01}$, $\Gamma_{11}$, and $\nu_{04}$ obtained from the non-linear spectra and from results of the quantum chemical calculations. From the fitting we were able to determine the linewidths ($\Gamma_{01}$, $\Gamma_{03}$, and $\Gamma_{04}$), the transition dipole moments ($\mu_{23}$ and $\mu_{24}$) and the dipole moment changes $\Delta \mu_{01}$. The transition dipole moment $\mu_{01}$ was fixed in 1.5 D for both compounds, since this transition is weakly allowed by 1PA.

Table II summarizes the spectroscopic parameters used/obtained by the SOS model, with a five-level energy diagram. In summary, the energy diagram proposed for $\beta$-carotene is described by three two-photon allowed states, while for $\beta$-apo-8’-carotenal four 2PA allowed states were employed to describe the nonlinear spectrum, since this molecule present a overlapping of the states $|S_1\rangle$ and $|S_2\rangle$. Such diagrams are in agreement with the general model to describe optical nonlinearities in conjugated systems, proposed by Beljonne et al.22

It is observed that both molecules studies here present a significant change in transition dipole moment due to its large

FIG. 5. Energy level diagram of lowing single-excited states of (a) $\beta$-carotene and (b) apo-8’-carotenal used to fit the 2PA spectrum utilized in the SOS approach. The gray region represents the overlapping of states $2^1\text{Ag}^-$-like and $1^1\text{Bu}^+$-like.

FIG. 6. (Color online) Representation of the highest occupied (HOMOs) and lowest unoccupied (LUMOs) molecular orbitals of (a) all-trans $\beta$-carotene and (b) trans-$\beta$-apo-8’-carotenal.
charge redistribution in the $|S_2\rangle$ excited state.\textsuperscript{21,22,56} In addition, the 2PA cross-section of $\beta$-carotene in the region between 590 and 700 nm is about 1.25 times the value obtained for the $\beta$-apo-8'-carotenal. This result seems to be related to the increase of the conjugation length and the difference between the electron-acceptor and -donor character of the end-groups of the compounds. In fact, from an analysis of the molecular orbitals of these two carotenoids compounds, Fig. 6, one can observe that the electronic density of both compounds in mainly distributed over their polyenic chains, while their ending groups play only a secondary role. Therefore, we point out the long conjugation length of these compounds as the main factor for the high 2PA cross-section measured, than intramolecular charge transfer effects enhanced by ending-groups.\textsuperscript{60} On the other hand, the molecular orbitals are also an additional evidence for the different character of the ending-groups of the trans-$\beta$-apo-8'-carotenal, which is responsible for an effective break of symmetry and, therefore, explains its lowest 2PA allowed transition, a dipolar transition (HOMO $\rightarrow$ LUMO).

V. CONCLUSION

We presented a wide spectral range (590–1100 nm) analysis of the 2PA cross-section spectra of $\beta$-carotene and $\beta$-apo-8'-carotenal using the conventional and the white-light-continuum Z-scan techniques and quantum chemistry calculations. Large 2PA cross-sections were found, with values comparable to the best ones presented in the literature for organic compounds specially designed to improve the nonlinear absorption. It is important to mention that the nonlinear optical measurements reported here are due to a distribution of isomers of each molecule, which presents distinct conjugation length and conformation. The theoretical study carried out using the response functions formalism within the DFT framework determined the two-photon allowed states of these carotenoid compounds over the wide spectral range investigated, and supported the interpretations of the experimental spectra. The theoretical predictions for the values of the excitation energies corresponding to the transition to the $1^1$Bu'-like and $3^1$Ag'-like states, respectively strongly one- and two-photon allowed states, are in good agreement with the linear and nonlinear spectra of both compounds. Although the excitation energy of the $2^1$Ag'-like state is overestimated and the ordering of the $2^1$Ag'-like and $1^1$Bu'-like states is not corrected, the 2PA probability and the oscillator strength computed confirmed that the $2^1$Ag'-like state of both compounds is two-photon allowed and only weakly allowed for a one-photon transition. We believe that based on the results presented herein, possible applications of carotenoids molecules as 2PA-based biophotonic devices in the visible region could be outlined.

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