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Chirality-Specific Nonlinear Spectroscopies in Isotropic Media

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Sum or difference frequency generation (SFG or DFG) in isotropic media is in the electric-dipole approximation only symmetry allowed for optically active systems. The hyperpolarizabilty giving rise to these three-wave mixing processes features only one isotropic component. It factorizes into two terms, an energy (denominator) factor and a triple product of transition moments. These forbid degenerate SFG, i.e., second harmonic generation, as well as the existence of the linear electrooptic effect (Pockels effect) in isotropic media. This second order response also has no static limit, which leads to particularly strong resonance phenomena that are qualitatively different from those usually seen in the ubiquitous even-wave mixing spectroscopies. In particular, the participation of two (not the usual one) excited states is essential to achieve dramatic resonance enhancement. We report our first efforts to see such resonantly enhanced chirality specific SFG.

All optical spectroscopies may be classified as even-wave mixing (eWM) or odd-wave mixing (oWM) processes depending on the number of fields that participate. This includes incoherent absorption and emission phenomena. The wave count in $(n + 1)$-wave mixing is made of the n incident waves that give rise to the n'th order polarization of the medium and the $(n + 1)$'th wave that emerges from it.

In the electric dipole approximation (EDA) the expression for the macroscopic polarization at n'th-order involves an appropriate average over altogether $(n + 1)$ direction cosines that relate the corresponding hyperpolarizabilities evaluated in the molecular frame to the susceptibility in the laboratory frame.

In isotropic media one might expect that only the susceptibilities of eWM processes support WM since the polarization underlying eWM requires orientational averaging over an *even* number of direction cosines. Indeed there are numerous examples of eWM processes in isotropic liquids and gases where oWM processes are absent. Examples of eWM processes that may be seen in any isotropic medium include one-photon absorption and emission (examples of 2WM), and many 4WM processes including two-photon absorption, coherent and incoherent Raman scattering, the Kerr effect, and photon echoes or free-induction decays.

However, odd-wave mixing is also possible in isotropic media, since it turns out that an *odd*-number of direction cosines also possess a non-vanishing isotropic average. In fact there is one rotationally invariant element at second-order (3WM) and six such elements at fourth-order (5WM). These tensor elements are, however, only supported if the medium is non-centrosymmetric. Such is the case for isotropic optically active systems.

The possibility of such chirality-based oWM was recognized by Giordmaine as early as 1965[1] and the first sum-frequency generation (SFG) experiments (a 3WM process) were reported a year later.[2] Apart from one brief report[3] that supports the findings of Rentzepis et al.,[2] no work on 3WM from chiral liquids has appeared until recently.

A recent effort to reproduce the early SFG experiments in aqueous solutions of arabinose has failed to detect any SFG.[4] It suggests a probably artifactual basis of one of the reports (Ref. 3). With the aid of quantum chemical calculations,[4] the authors find that any true off-resonant electronic SFG from solutions of small chiral molecules, such as arabinose, would be considerably weaker than previously thought.

The second order susceptibility of an isotropic medium is the basis of SFG and difference frequency generation (DFG) from gases, liquids, or amorphous solids. It relates directly to the one rotationally invariant component, $\bar{\beta}$, of the microscopic first hyperpolarizability tensor. Thus if the number density of a given enantiomer is N we have

$$\chi^{(2)} = \frac{N}{\varepsilon_0}\bar{\beta} \tag{1}$$

where the appropriate rotational averaging of the full beta tensor leaves only

$$\bar{\beta} = \frac{1}{6}(\beta_{xyz} - \beta_{xzy} + \beta_{yzx} - \beta_{yxz} + \beta_{zxy} - \beta_{zyx}) \tag{2}$$

This tensor element is a pseudoscalar having unusual properties.[5] When all 8 possible Liouville paths for the density matrix evolution to second order are properly incorporated and rotationally averaged, one finds a tensor element composed of two parts. The two components consist of the fully antisymmetrized triplet of transition moments and an energy factor (built of two complex energy denominator poles specific to each of the eight Liouville paths). We write

$$\bar{\beta} = \sum_{m,n} \bar{\mu}^{(3)}(m,n)\boldsymbol{F}(m,n) \tag{3}$$

The sum is twice independently over the full basis set. Now it turns out that

$$\bar{\mu}^{(3)}(m,n) = \frac{1}{6}\vec{\mu}_{gm}\cdot\left(\vec{\mu}_{mn} \times \vec{\mu}_{ng}\right) = \frac{1}{6}\varepsilon_{\alpha\beta\gamma}\mu_{gm_\alpha}\mu_{mn_\beta}\mu_{ng_\gamma} \tag{3a}$$

The energy factor may be algebraically reduced to

$$\begin{aligned}\boldsymbol{F}(m,n) = {} & \frac{\omega_2 - \omega_1}{\hbar^2} \\ & \times \Bigg[\frac{1}{(\omega_1 - \omega_{mg} + i\Gamma_{mg})(\omega_2 - \omega_{mg} + i\Gamma_{mg})(\omega_1 + \omega_2 - \omega_{mn} + i\Gamma_{mn})} \\ & + \frac{1}{(\omega_1 + \omega_{mg} + i\Gamma_{mg})(\omega_2 + \omega_{mg} + i\Gamma_{mg})(\omega_1 + \omega_2 + \omega_{mn} + i\Gamma_{mn})} \\ & + \frac{1}{(\omega_1 - \omega_{mg} + i\Gamma_{mg})(\omega_2 - \omega_{mg} + i\Gamma_{mg})(\omega_1 + \omega_2 - \omega_{ng} + i\Gamma_{ng})} \\ & + \frac{1}{(\omega_1 + \omega_{mg} + i\Gamma_{mg})(\omega_2 + \omega_{mg} + i\Gamma_{mg})(\omega_1 + \omega_2 + \omega_{ng} + i\Gamma_{ng})}\Bigg]\end{aligned} \tag{3b}$$

We see from Eq. 3a that $\bar{\mu}^{(3)}(m,n) = -\bar{\mu}^{(3)}(n,m)$ to reveal anti-Hermitian properties of the transition moment factor. This allows one to reduce the independent double sum to a one-sided double sum:

$$\bar{\beta} = \sum_{n>m}\bar{\mu}^{(3)}(m,n)(\boldsymbol{F}(m,n) - \boldsymbol{F}(n,m)) \tag{4}$$

Clearly $\bar{\beta}$ must vanish for the diagonal component ($m=n$). This is a novel feature not encountered in the many eWM spectroscopies. In eWM processes the diagonal (single state) contributions are a major feature in resonance phenomena.

In the present 3WM spectroscopy such contributions vanish and resonances can only appear when two nondegenerate excited states are engaged. In general, two types of complex energy poles appear. One represents a one-photon resonance with one of the two excited states (with either incident field), the other is a two-photon resonance with the second excited state at the sum frequency (SF). These two types of poles may be seen in the reduced form of the energy factor (Eq. 3b).

The frequency difference pre-factor in $\boldsymbol{F}(m,n)$ (Eq. 3b) immediately shows that second harmonic generation is not possible. Thus all SFG or DFG must be two-color in nature. Furthermore in the full static limit there can be no signal. Less obviously this turns out to be true even if one frequency is set to zero (there can be no Pockels effect in an isotropic medium).

These many novel features of $\bar{\beta}$ include the property that even if all fields are dynamic once all resonances are remote (as in the few previous SFG studies of optically active sugars) Kleinman symmetry becomes effective and SFG or DFG are at best extremely weak. Put differently, the presence of resonances, either one or both of them, will dramatically amplify these 3WM processes. When *both* the one and two-photon resonances are active, calculations indicate that amplifications of the SFG intensity are expected to reach eight to twelve orders of magnitude above SFG signals driven by truly off-resonant fields.[5b,5c,6,7]

Finally, within the last year there have been two reports of chirality based partially resonant SFG from Professor Shen's laboratory.[8,9] In the first infrared/visible SFG is seen from solutions of optically active limonene in which the IR field establishes a one-photon vibrational resonance while the two-photon resonance at the SF is absent. In the second paper[9] solutions of the chiral molecule 1,1′-bi-2-naphthol (BN) in tetrahydrofuran (THF) are shown to produce chirality-based SFG in the near UV region (using the YAG fundamental at (1064 nm) and a second field, somewhat tunable, near its second harmonic). Here the only resonance is the two-photon one at the SF in the near-UV band of BN. $\bar{\beta}$ having only such partial resonance has been modeled theoretically,[5b,5c] and found to be magnified by about four orders of magnitude above a reference non-resonant situation regardless of whether the resonance is vibrational or electronic. (The inherently weaker transition moment associated with the vibrational pole is compensated for by the narrowness of that pole.) In both cases, each only singly resonant, the SFG signal is readily detectable at eight orders of magnitude greater than that predicted for any as yet unseen SFG from the fully nonresonant reference system.

Our preliminary experimental efforts to study fully resonantly enhanced SFG use the Ti:sapphire fundamental at 800 nm and its second harmonic at 400 nm. It turns out that chiral carotenoids offer just the right state pairs that align with these two fields to achieve both the one-photon resonance at 400 nm as well as two-photon resonances at the 266 nm SF. Quantum chemical calculations show how the chiral carotenoid astaxanthin should be an ideal candidate for such studies.

Quantum Chemical Calculations of $\bar{\boldsymbol{\beta}}$[7]

We have applied a configuration interaction singles sum-over-states (CIS-SOS) approach to calculate $\bar{\beta}$ for several chiral molecules including a representative chiral carotenoid, astaxanthin, over a range of realistic two-color excitation conditions. We have also carried out similar calculations of $\bar{\beta}$ for BN. This molecule was used to obtain chiral specific SHG from surfaces[10] and, as noted, just this year to demonstrate the partially electronically resonant SFG from the bulk of a solution.[9]

The excitation energies and dipole transition moments are calculated for fully optimized geometries (AM1 Hamiltonian) using the semi-empirical ZINDO package in Gaussian-98.[11] The SOS is computed with the first 100 excited states of astaxanthin and the first 120 excited states of BN.

A generic value of 750 cm^{-1} is assigned to each of the poles (corresponding to a FWHW Lorentzian of 1500 cm^{-1}), to partly account for electronic bands that are broad by virtue of their vibrational substructure — the nuclear coordinate space having been otherwise neglected in the purely electronic basis set. $\bar{\beta}$ is then obtained as a function of the two incident colors by performing the SOS indicated in Eq. 4. The dispersive properties of $|\bar{\beta}|$ are displayed in density plots with the frequencies of the two incident fields as variables. This leads to unusual 2-D SFG spectra which feature a true zero along the diagonal ($\omega_1 = \omega_2$) and extremely dramatic peaks and valleys in the (symmetric) off-diagonal regions. Such plots are shown in Fig. 1a for astaxanthin and Fig. 1b for BN. To expose $\bar{\beta}$ itself more quantitatively, and in a manner directly applicable to experiments in which ω_2 is always the harmonic of ω_1 (often a matter of convenience), we show in Figs. 2a and 2b plots of $\bar{\beta}$ in atomic units

$$\left(\frac{(ea_\mathrm{o})^3}{E_H^2} = \frac{(4\pi\varepsilon_\mathrm{o})^2 a_\mathrm{o}^5}{e}\right)$$

as a function of the fundamental frequency ω_1 for each molecule and its enantiomer. Calculated traces for both the R form

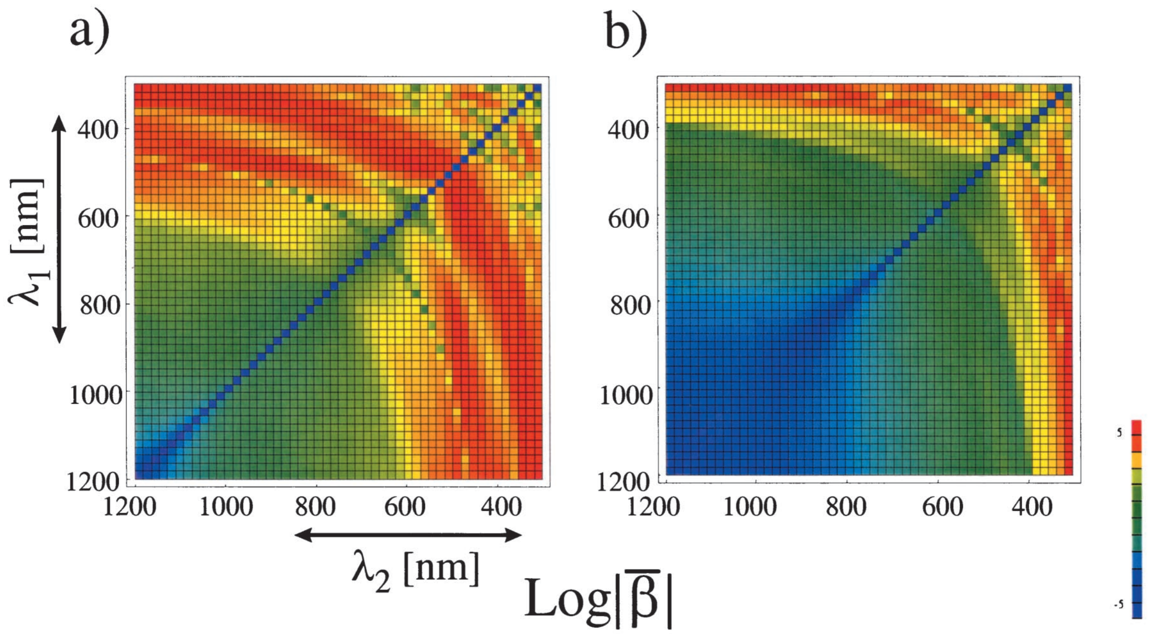

Fig. 1. Log|$\bar{\beta}(\omega_{SF} = \omega_1 + \omega_2)$|is shown for chiral a) astaxanthin and b) 1, 1′-bi-2-naphthol as a function of the wavelengths ($\lambda = 2\pi c/\omega$) of the incident beams. The 2-D SFG spectra are symmetric about the diagonal (where they are identically zero and where Log|$\bar{\beta}$| $\rightarrow -\infty$). The graph has a cutoff at -5, and extends 11 orders of magnitude, from -5 (dark blue) to 0 (green) to $+5$ (red). Corresponding homodyned SFG intensities would span 22 orders of magnitude.

and the S forms confirm that $\bar{\beta}_R = -\bar{\beta}_S$, as required. We see that for our Ti : sapphire based experiments with the fundamental at 800 nm, the calculated |$\bar{\beta}$| of astaxanthin is ~2000 au. That for BN is ~200 au. We find that the ZINDO CIS-SOS calculations tend to over-estimate linear and nonlinear polarizabilities, especially for larger molecules where CPU requirements further restrict the number of excited states that can be computed. The calculations thus give at best an order of magnitude estimate of the true $\bar{\beta}$, and especially so for astaxanthin which with 96 atoms is large for quantum chemical computations. As yet there are no measured values for astaxanthin (see below). However, the maximum value of |$\bar{\beta}$| for BN reported by Belkin et al.[9] is about 9 au for their set of two colors. From our own measurements in the 800 nm, 400 nm two-color configuration we estimate (with a quartz reference crystal) that |$\bar{\beta}$| is ~40 au. In our experiment the two-photon resonance at 266 nm falls into a vibronically broadened absorption of BN in the quartz UV. Were we to subsume such vibrationally broadened resonances in this UV band by choosing a larger than generic effective linewidth for the two-photon resonance the calculated value of |$\bar{\beta}$| is reduced to ~140 au.

For the Nd : YAG-based studies of SFG from BN[9] the generic choice of 750 cm^{-1} for the linewidths happens to be quite suited (according to the one-photon spectrum) to the two-photon resonant BN band in the SF region of the near-UV. The quantum chemical |$\bar{\beta}$| for BN under the Nd : YAG-based conditions comes to about 35 au. In contrast similar calculations for much smaller molecules, such as pinene and propylene oxide with the fundamental at 1064 nm and without available resonances predict[6] a $\bar{\beta}$ of $< 10^{-4}$ (au) (see also Ref. 5c). At the signal level $\left(|\bar{\beta}|^2\right)$ we see that the fully resonant astaxanthin could show up to $> 10^{12}$ amplification of SFG signal when both one- and two-photon resonances are active. Similar enhancements can be predicted for a fully resonant BN experiment.

We now turn to our preliminary search for SFG in solutions of astaxanthin and then show how chirality-based SFG from much more concentrated solutions of BN is readily observed.[9,14]

1. Search for Ti : Sapphire Based SFG from Astaxanthin in Chloroform. The strength of SFG from a chiral solution depends both on the strength of |$\bar{\beta}$| at the frequencies of the laser(s) and the concentration of the solution. If one works in liquid cells with path lengths that are comparable to the coherence length of the SFG process, typically 1 to 2 microns, then concentrations exceeding saturation could be tolerated even in the presence of strong absorption (resonance) at the incident and signal frequency.

The peak extinction coefficient in the carotenoids located at ~480 nm is ~10^5. The off-peak extinction coefficient at ~400 nm may be as low at 4×10^4. For ~50% transparency in a 2 micron path, the carotenoid concentration may be allowed to approach 0.1 M. Chlorinated hydrocarbons (like chloroform) appear to be good solvents for carotenoids such as astaxanthin, but concentrations of only ~20 mM appear to be possible.

An order-of-magnitude calculation suggests that, if the quantum chemical calculations of $\bar{\beta}$ for astaxanthin are to be believed, the SF signal from 20 mM solutions of astaxanthin in chloroform should be only ~50 times weaker than SFG seen under identical experimental conditions from a thin quartz crystal. A more careful analysis that includes important geometric factors and also a more skeptical view of the calculated value $\bar{\beta}$ for astaxanthin suggests that the expected SFG signal should be more like four orders of magnitude less than that

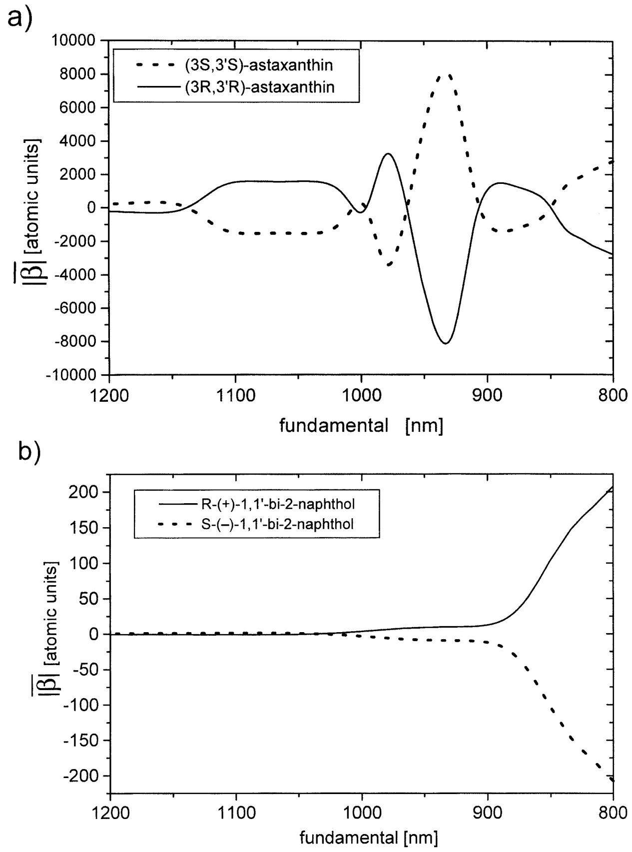

Fig. 2. The SFG $|\overline{\beta}(3\omega = 2\omega + \omega)|$ is computed using CIS-SOS (ZINDO) and shown in atomic units for the enantiomers of a) astaxanthin and b) 1,1′-bi-2-naphthol as a function of the wavelength of the incident fundamental ($\omega = 2\pi c/\lambda$). The imaginariy part of $\overline{\beta}$ for astaxanthin peaks at 960 nm which corresponds to a one-photon resonance of the harmonic with the absorption maximum of astaxanthin (at 480 nm). However, the real part of $\overline{\beta}$ favors shorter wavelengths and is thus asymmetric causing the $|\overline{\beta}|$ in a) to peak at around 930 nm.

from the reference crystalline quartz plate under identical illumination. This scaling would still place the expected SFG from the 20 mM astaxanthin well above the noise level of the experiment.

Several issues arise in absorbing media using regeneratively amplified high-peak power femtosecond laser pulses. In general, the resonantly excited molecules cannot return to their ground state within the pulse duration of a femtosecond pulse (say < 200 fs). Once excited, a given molecule is entirely removed from the ground state population until the next pulse arrives (for a repetition rate of 1 kHz the dark period is milliseconds). As one focuses the incident fields upon a sample of high optical density there is danger that nearly all molecules in the excitation volume are excited and removed from the ground state and thus no longer available for any (ground-state based) nonlinear optical processes.[12] Such "hole-burning" might easily quench the SFG expected from our astaxanthin solutions. Thus a single-pulse bleaching level of 10% or less may be regarded as acceptable.

Rapid photochemical destruction of the astaxanthin molecules would be a much more serious problem. Remarkably, a study of the photochemical destruction of astaxanthin in three different solvents — one being chloroform — has appeared.[13] Quantum yields for the (one-photon) destruction of astaxanthin are reported at several exciting wavelengths. They increase dramatically upon entering the near UV. At 405 nm the quantum yield in chloroform is 4.6×10^{-6} which suggests that astaxanthin is quite robust under such irradiation. Thus one molecule on average can endure $\sim 2 \times 10^5$ photocycles before being destroyed. With $\sim 10^{-1}$ cycles per femtosecond pulse (in our experiments) and the above reported quantum yield astaxanthin would need $\sim 2 \times 10^3$ s (or 1/2 hour) of exposure to decompose. In our experiments, typical exposure times are no more than a few minutes. Still the absence of any SF signal from chiral astaxanthin solutions remained a mystery. We finally checked carefully for any possible photochemical destruction of the astaxanthin in our experiments, and fully illuminated a 1 mm cuvette of an optically dense solution of (racemic) astaxanthin in chloroform using the identical 400 nm source. Bleaching (measured at the 495 nm absorption peak of the visible band) by the 400 nm source (25 mW to 50 mW average powers) is easily seen. Regardless of the source of the chloroform (different commercial grades, pre-treated with active carbon filters and basic alumina) we measure (linear) quantum yields for bleaching of about 1.6×10^{-4}, about 300 times larger than those reported. Similar values were found for the solvent chlorobenzene. This suggests, in the absence of any additional nonlinear photochemistry, that in our femtosecond SFG work the astaxanthin molecule should survive only about 6 seconds, not thirty minutes. In fact, under the focussed beam conditions of the SFG experiment, bleaching in less than a second is visually apparent with the 400 nm illumination. We also observe bleaching with 800 nm excitation where carotenoids exhibit no serious one-photon absorption. Nonlinear photochemistry appears to be intruding. Bleaching on such a short time scale is too rapid to be refreshed by diffusion from the un-illuminated regions of the micro-sample. Though the discrepancy between our one-photon quantum yield findings and those published[13] is not explained, both suggest that the photochemistry must be based on the intrinsic presence of the bulk chlorinated solvent and not related to some removable impurity. We have concluded that such photochemistry (and its nonlinear contribution) very likely explains the absence of the (fully resonant) chirality-based SFG from these solutions. Our plans are to search for "good" alternative solvents not so photochemically active or, if necessary, to devise a flow system.

2. Ti:Sapphire Based SFG in BN Solutions. Given the report of SFG from BN in THF based on the YAG fundamental[9] we turned our attention to this system using the identical Ti:sapphire based sources configured for the astaxanthin work. This time the expected chirality-based signals are easily seen. With ω_2 at 400 nm any one-photon resonance is still absent and one-photon photochemistry (if possible at all in BN/THF) cannot intrude. A two-photon resonance is available in the 266 nm region of the sum-frequency. We have performed an "enantiomeric excess titration" which takes the sample from all R to nearly all S (and vice versa) through its racemic mid-point, whilst the total concentration ([R] + [S]) is kept at 0.5 M. The SFG obeys the expected quadratic dependence on

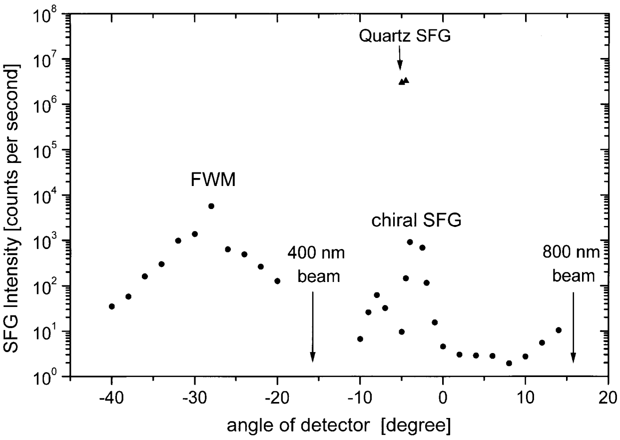

Fig. 3. The observed angular distribution of 266 nm photon (counted per second) is a convolution of the signals with the aperture of the detector (resolution of about 4 degree). The angles are shown relative to the bisector of the non-collinear incident beams at 400 and 800 nm. Both SFG ($3\omega = 2\omega + \omega$) and FWM ($3\omega = (2\omega) + (2\omega) - \omega$) from chiral 0.5 M solutions of 1,1′-bi-2-naphthol in tetrahydrofuran, as well as SFG from a reference quartz are shown.

the molefraction of enantiomeric excess.[14] In addition we have studied SFG as a function of the linear polarization of each of the two incident beams.[14] The results confirm the chirality-based origins of the SFG signal.

The coherent SF signal from any sample is expected with a *k*-vector along a unique direction determined by the momentum matching conditions in the dispersive medium. We illustrate in Fig. 3 the photon count (at 266 nm) measured as the detector sweeps an arc whose center is in the liquid cell. For the given non-collinear incidence of ω_1 and ω_2 (their *k*-vectors subtend an angle of about 30°). Fig. 3 shows the location of the SF signals, both from BN and a quartz reference crystal, relative to the location of the incident beams (that have traversed the sample). The signal from BN is seen to be about three-orders of magnitude weaker than that from the quartz reference. Also shown is a concomitant 4WM signal at $3\omega = (2\omega) + (2\omega) - \omega$ from BN/THF that is not chirality-specific and whose *k*-vector lies outside the *k*-vectors of the incident beams.

Though the calculated (and observed) singly resonant $|\bar{\beta}|$ of BN is much weaker than the calculated doubly resonant $|\bar{\beta}|$ of astaxanthin, the twenty-five fold concentration advantage (625 fold at the intensity level) of the 0.5 M BN solutions over the 20 mM astaxanthin solutions offers considerable compensation.

Summary

Three-wave mixing in isotropic media (whether SFG or DFG) requires the presence of chiral centers in enantiomeric excess. The one rotationally invariant microscopic first hyperpolarizability tensor element has special properties not normally seen in the many even-wave mixing spectroscopies. It forbids second harmonic generation. Resonances require the participation of a minimum of two excited states, not one. Quantum chemical calculations of this isotropic tensor element reveal 2-D 3WM spectra (with ω_1, ω_2 as variables) having the novel feature of zero-diagonal ($\omega_1 - \omega_2 = 0$, when $\omega_1 = \omega_2$). The off-diagonal features are exceptionally dramatic in their intensity contrast. The off-diagonal SFG strength — when both one- and two-photon resonances are active — may be enhanced by as much as twelve orders of magnitude over fully non-resonant SFG.

Using the Ti:sapphire fundamental and its second harmonic, preliminary efforts were undertaken to detect fully resonant SFG in 20 mM solution of the chiral carotenoid astaxanthin in chloroform. These were unsuccessful primarily because of solute/solvent photochemistry. On the other hand using the same laser source, SFG was easily seen in 0.5 M solutions of BN in THF. In these experiments only a two-photon resonance at the SF is active.

Quantum-chemical calculations of the isotropic tensor are within an order of magnitude of the observed 9 au (Nd:YAG) and ~40 au (Ti:sapphire).

It is hoped that a more suitable choice of solvent for the chiral carotenoids will permit a demonstration of fully resonant 3WM in an isotropic system.

We are grateful to Professor Frank Wise for letting us use his Ti:sapphire regenerative amplifier based femtosecond laser. We are indebted to Dr. H. Ernst (BASF) and Dr. R. Buchecker for providing us with enantiomerically pure samples of zeaxanthin and astaxanthin, respectively. One of us (PF) thanks NATO for a post-doctoral fellowship. And we both express our gratitude to the National Science Foundation for support (CHE-0095056).

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