Cross-Polarization Detection Enables Fast Measurement of Vibrational Circular Dichroism

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As a powerful tool in the hands of structural chemists, vibrational optical activity (VOA) suffers from poor sensitivity translating to rather time-demanding experimental spectrum acquisition. The heterodyned Fourier-transform spectral interferometry\(^2\) and a cross-polarization detection scheme are set to remove this handicap, as the new experimental setup already provided encouraging results. The future is difficult to predict, but vibrational circular dichroism (VCD) and vibrational optical rotatory dispersion (VORD) spectra of limonene, a simple chiral compound, successfully obtained by the heterodyne detection promise to open new horizons in chiral vibrational spectroscopy.\(^2\)

Utilization of chirality for the determination of molecular structure and intermolecular interactions has a rich history and is intertwined with many fields of science. In the early years of the nineteenth century, rotation of the polarization plane of linearly polarized light by a chiral medium was observed. Later this effect was explained at a molecular level by Louis Pasteur.\(^3\) The reason why optical rotation is sensitive to chirality is that the circularly polarized light is inherently chiral itself, a property that was better grasped a century later when quantum electrodynamics described the dualistic wave–particle character of the photons.\(^4\)

About fifty years ago, optical rotatory dispersion (ORD)\(^5\) and later ultra-violet circular dichroism (UVCD, measuring the difference in the absorption of right- and left-circularly polarized light)\(^6\) became standard analytical tools widely used for a convenient monitoring of absolute configuration and conformational changes in chemistry and molecular biology. In visible and ultra-violet regions the CD signal is relatively strong (\(~10^{-3}\) of the total absorption), and as conventional optical elements may be used, the construction of the spectrometers is fairly straightforward.

It was soon realized that utilizing a similar phenomenon in the vibrational region could provide more detailed and reliable stereochemical information, because the vibrational spectrum contains many more bands sensitive to minute features of the molecular structure.\(^7,8\)

The vibrational region was successfully tackled in 1973 when the circular-polarization dependence of vibrational Raman scattering was first measured with visible laser light.\(^9\) In 1974 this success was followed by a report on VCD.\(^10\) Ever since then, VOA has been applied to a broad scale of problems ranging from small chiral molecules to proteins, nucleic acids, and viruses.\(^11–17\)

Nevertheless, the technique remained confined to specialized laboratories, predominantly in the United States, for a long time. As the light wavelength is much larger than the molecular dimensions, the VOA signal is very weak, typically mere \(10^{-7}–10^{-1}\) of the total scattering or absorption. Accurate measurements required advanced instrumentation, high stability of the optics and light sources, and special detectors coupled with dedicated electronics, such as lock-in amplifiers. First commercial attempts to sell the VCD instruments failed in the eighties. The instrumentation problems, however, were eventually solved and this vibrational optical activity was reintroduced to the market around 1997, this time successfully. A few years later, an ROA spectrometer became commercially available as well.

Similar to conventional spectrometers, the new detection technique utilizes the familiar phenomenon of linearly polarized light being rotated and partially depolarized by an optically active medium, as shown diagrammatically in Figure 1. In a traditional VCD measurement, a piezoelectric photoelastic modulator is used to periodically yield the left- and right-circularly polarized (LCP, RCP) light. This produces a varying signal at the detector, which is electronically weighted against the total (non-polarized) absorption.\(^18\)

However, as shown by Rhee et al.,\(^2\) in a cross-polarization detection one can also utilize the perpendicular component \(\mathbf{E}_\perp\). It is related to the complex optical activity susceptibility \(\Delta \chi(\omega)\), given by Equation (1):

\[
\mathbf{E}_\perp(\omega) \sim \Delta \chi(\omega)\mathbf{E}_\| (\omega),
\]

where \(\mathbf{E}_\|\) is the complex transmitted electric field spectrum resulting from an interference between the input field and the induced electric-dipole-allowed optical free induction decay (FID) field. The imaginary and real part of \(\Delta \chi(\omega)\) corresponds to the CD and ORD spectra, respectively.

The \(\mathbf{E}_\perp\) component is very weak, but it can nevertheless be amplified and measured by heterodyned Fourier-transform spectral interferometry (Figure 2). A major problem in the experimental setup was the separation of the parallel and perpendicular components. This was achieved by extremely selective calcite plate polarizers. Although they work in a limited spectral region only, the technique successfully yielded reproducible VOA (CD and ORD) signals of the CH stretching fundamental vibrations of limonene.

The direct, or at least alternate, detection of the chiral \(\mathbf{E}_\perp\) component might
thus be more advantageous than a subtraction of two large numbers corresponding to the LCP and RCP absorption in conventional spectrometers. A simultaneous recording of VORD represents yet another modality that may open new horizons, as this molecular property is rarely measured and interpreted. The biggest expectation of the new technique, that is, VOA measurements at femtosecond time scale, is yet to come. Currently, the technique provides the spectra in approximately the same time frame (a few minutes) as conventional VCD spectrometers. Given the tremendous ongoing improvements in the VOA instrumentation, however, a fast VCD technique is very likely to emerge soon.

The short optical pulses simultaneously produced static VCD and VORD spectra so far. To use the methodology to follow dynamical phenomena, such as real-time monitoring of protein folding, a recyclable system will have to be found, where a pump pulse triggers a conformational change, and a delayed pulse probes system relaxation at varying times. For IR spectroscopy, such a change could be represented by the temperature elevated by a short laser pulse in the “T-jump” experiments.[19] For a few microseconds, the peptide remains partially unfolded, which is reflected in a different absorption pattern of the carbonyl stretch. The IR changes are difficult to relate to the actual peptide conformation. Time-resolved VCD spectra thus might provide much-needed complementary information about the nature of the changes occurring in the sample. A suitable experimental system is critically important in this respect, as protein conformational changes, for example, occur at very different time scales and may overlap with faster localized internal motions.[20]

To some extent, the “classical” VOA spectra can already be utilized to follow the molecular dynamics. Inhomogeneous bandwidth of spectral bands carries information about the geometry dispersion and interaction with the environment.[21,22] For small systems, VOA provides conformational ratios with the same accuracy as the NMR technique.[23] Site-specific labeling with heavier stable isotopes and/or temperature-dependent data pro-

**Figure 1.** Left: Looking in the direction of the propagation, the oscillating electric field \( E_0 \) of an incident linearly (vertically) polarized light can be thought of as a sum of left- and right-circularly polarized components \( (E_L, E_R) \) rotating in opposite directions, but with the same angular frequencies \( (\omega_L = \omega_R) \). Right: While passing through a chiral medium, the two circular polarizations can be absorbed differently (e.g. \( |E_L| > |E_R| \)), which is referred to as circular dichroism. Additionally, they can propagate with different velocities (so that \( \omega_L \neq \omega_R \)) in which case the resultant vector \( E \) (polarization plane) rotates. The differential absorption and rotation result in an elliptical polarization. Conventionally, the polarized intensity components \( (|E_L|^2, |E_R|^2, |E|^2) \) are measured while recording VCD spectra. In the cross-polarization detection the perpendicular \( (E_L) \) and parallel \( (E_R) \) polarized components are utilized instead.

**Figure 2.** Top: Heterodyned detection of optical activity. The initial pulse is split at the first beamsplitter (BS1) to a reference signal \( E_0^{\text{ref}} \) and the one passing through the sample cell C \( E_0^{\text{FID}} \). Linear polarizers LP0 and LP1 select the required light components and the two signals are analyzed after the second beamsplitter. Bottom: VCD spectra of \( R \)- and \( S \)-limonene obtained by this technique; the third curve is a racemic baseline. Reprinted from ref. [2] with permission from the Nature Publishing Group.

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vide information on peptide unfolding that is spatially better resolved. We also attempted imposing geometry restrictions derived from optical spectra during molecular dynamics simulations.23 Linking the real time-dependence of the spectra to the structure would nevertheless pose new challenges, at least to the entire apparatus of computational chemistry. The time aspect of the molecular dynamics simulations, for example, is rarely verified experimentally due to the lack of suitable data. As the C–H stretching region appears particularly suitable for time-dependent measurements, simulations and interpretations of the spectra beyond the harmonic limit seem to be inevitable. Not only is the C/H covalent bond strongly anharmonic, but the C–H stretching modes are also coupled with other molecular motions via Fermi resonances.26,27 In a way, it is surprising that simple harmonic computations usually reproduce the main experimental features reasonably well in this frequency region, too.22 Fast-scale VOA recording might also provide an advantage over NMR spectroscopy, as the NMR magnetic response is much slower than the absorption of a photon.28 It is nevertheless interesting that all these techniques, so useful for chemists, explore the magnetic component of the radiation.

As in other fields, a combination of various techniques usually provides the most complete and objective picture of any process or phenomenon. The lively development of methods utilizing the vibrational optical activity of molecules significantly contribute to this knowledge. The recently demonstrated measurement of VCD and VORD by a cross-polarization detection scheme is a significant step forward in that it introduces an unprecedented time resolution to the spectra.

Keywords: chirality · femtochemistry · heterodyne detection · molecular dynamics · vibrational circular dichroism


Received: May 19, 2009
Published online on June 9, 2009