

PEM-BASED VIBRATIONAL CIRCULAR DICHROISM

Vibrational circular dichroism (VCD) is the differential absorption between left and right circularly polarized light (usually) in the infrared (IR) region. It is a measurement of vibrational optical activity for chiral molecules. VCD spectra contain unique and important information about molecular structure for organic molecules of different sizes and biochemical samples such as peptides, proteins and nucleic acids.¹ As a result, VCD is becoming an important tool for studying different levels of molecular structure.

Experimental VCD measurements were first reported in the 1970s.² Prof. Philip Stephens' group at the University of Southern California made perhaps the most significant contribution to the VCD instrumentation using a PEM-based dispersive spectrometer.³ In 1979, Prof. Laurence Nafie and co-workers designed and built the first FTIR-VCD spectrometer at Syracuse University.⁴ Prof. Timothy Keiderling at the University of Illinois at Chicago reported the first magnetic VCD in 1981.⁵ Over the years, important improvements have been made by different research groups for both dispersive and FTIR VCD measurements.

In a VCD experiment, polarization modulation is normally achieved by use of a photoelastic modulator (PEM).⁶ Since VCD signals are typically 4-6 orders of magnitude smaller than normal IR absorption signals, polarization modulation allows one to obtain the differential signal directly. This gives VCD the AC detection advantage and a dynamic range advantage⁴ over the traditional method of subtracting the two spectra measured for left and right circularly polarized light, respectively.

DISPERSIVE-VCD

In a typical dispersive-VCD spectrometer, the IR beam from a broad band light source is resolved in a narrow band pass of wavelengths by a monochromator. The "monochromatic" light is then focused onto a sample. Before the sample, there is a PEM following a linear polarizer. After passing through the sample, the IR light beam is focused onto a detector.

In a dispersive-VCD instrument, a double modulation scheme is employed. The first modulation (an intensity modulation) is obtained by a mechanical chopper at a frequency on the order of 100 Hz. The second modulation (a polarization modulation of 30-100 kHz) is accomplished by a combination of the PEM and a linear polarizer. In such an experiment, the chopped IR light is modulated between left and right circular polarization states. The double-modulated signal reaching the detector is processed with lock-in amplifiers to obtain the average signal $((A_L + A_R)/2)$ and the differential signal $(A_L - A_R)$.⁷

RAPID-SCAN FTIR-VCD

A basic FTIR spectrometer has several advantages over a dispersive IR spectrometer, and all of them are applicable to the VCD realm. In an FTIR-VCD spectrometer, the IR beam from a light source first passes through an

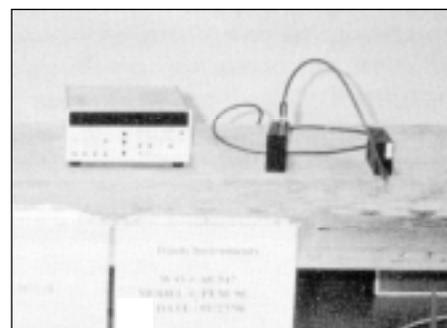
MORE ABOUT VCD

VCD is one of several techniques that measure optical activity. Of these various measurements, VCD in particular has grown rapidly over the last twenty years.

An excellent overview of the history, development and value of the two forms of vibrational optical activity – VCD and ROA (Raman optical activity) – is available in an article published in *Chemical & Engineering News* (Jan. 16, 1995, pages 27-34).

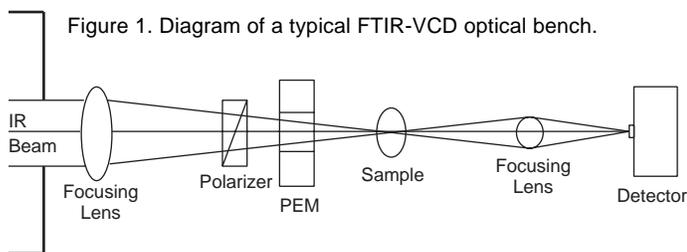
As noted in the article, VCD is "a totally viable and very sensitive technique" for applications in biochemistry.

Both VCD and ROA "can provide important new information on the absolute stereochemistry and conformation of biological molecules in aqueous solution."



PEM-90 Gains Certification

The PEM-90 photoelastic modulator is tested in the emissions chamber at CKC Laboratories for CE Mark and FCC certification. See story page 4.



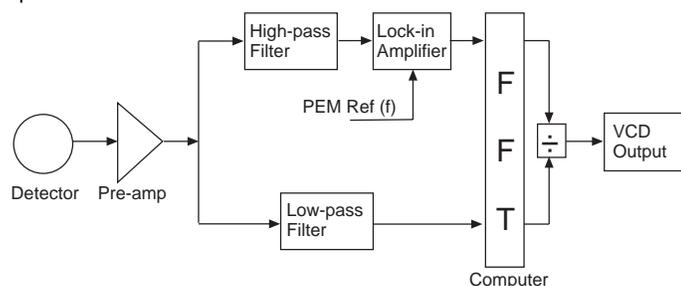
interferometer and then goes to a VCD optical bench as shown in Figure 1.

FTIR-VCD can be considered to be a double modulation experiment. The FTIR's interferometer creates the Fourier modulation. Each optical wavelength is modulated at a different Fourier frequency. At a medium scan speed, the interferometer modulates the mid-IR light at a Fourier frequency below several kilohertz. The polarization modulation, which is obtained in the same way as in a dispersive-VCD spectrometer, is about 10 times faster than the highest Fourier frequency. The resulting signal at the detector is processed separately to obtain the average and differential spectra. The average signal is computed according to the conventional method used in the standard FTIR systems.

To obtain the differential signal, the pre-amplified detector signal is first passed through a high pass filter in order to attenuate the Fourier modulated signals, and is then output to a lock-in amplifier referenced to the PEM frequency. The time constant of the lock-in amplifier should be set short enough to pass the Fourier frequencies efficiently for the rapid-scan FTIR-VCD experiments. The demodulated signal from the lock-in amplifier is then sent to the FTIR standard electronics for fast Fourier transform. After phase correction, the final VCD spectrum is obtained from the ratio of the differential spectrum to the average spectrum and the calibration of the VCD intensity. A typical electronic block diagram for processing FTIR-VCD data is shown in Figure 2.

Since FTIR-VCD is a double modulation spectroscopy, it is generally necessary that the two modulation frequencies be different by a factor of 10 or more. This is difficult to achieve in the near IR region with a rapid-scan instrument. When these two frequencies do not spread wide enough, several negative effects may appear, such as overloading of the lock-in, increasing the phase error

Figure 2. The electronic block diagram for a typical FTIR-VCD spectrometer.



and limiting FTIR scanning speeds.⁷ However, some of those problems can be reduced or even eliminated by use of a step-scan FTIR.

STEP-SCAN FTIR-VCD

In the step-scan mode, data is collected while the moving mirror in an interferometer is stopped (or effectively stopped) at a digitizing position. Due to the poor DC sensitivity of the IR detector/preamplifiers, the average IR signal needs to be detected by phase modulation. Phase modulation is normally achieved by oscillating one of the two mirrors in the interferometer at a frequency on the order of 100 Hz. The phase modulated normal IR signal can be demodulated using a lock-in amplifier (or an equivalent demodulator) before fast Fourier transform.

In a PEM-based VCD experiment, the IR beam is modulated by the PEM at a frequency on the order of 10 kHz. The differential signal can be better measured without phase modulation.⁸ In this case, the detector output can be processed through the same lock-in as in the rapid-scan mode, except that a much longer time constant can now be used, affording more dynamic range and less noise.

It is worth noting that VCD artifacts (the deviation from a correct VCD spectrum) are a problem with both dispersive and FTIR-VCD instruments. They are probably a more serious problem with an FTIR-VCD spectrometer. Dealing with artifacts requires careful attention to experimental detail, understanding of instrumental principles, and ingenuity. When the artifacts are reduced or eliminated, excellent VCD results, as shown in the next section, can be obtained using both dispersive and FTIR spectrometers.

SELECTED VCD EXAMPLES

It is beyond the scope of this article to discuss either the variety of samples that can be studied using this technique or the importance of the information that can be obtained from this technique. Future issues of this newsletter are planned which will address selected VCD subjects in more detail. For now, a limited sampling of VCD spectra are shown here as examples.

Figure 3 depicts two VCD spectra obtained using FTIR-VCD systems in the mid-IR region. Figure 3a is a raw VCD spectrum of (-)- α -pinene between ~ 1350 and ~ 900 cm^{-1} , which was collected in just under two minutes.⁹ This spectrum, when calibrated in intensity, would have a $\Delta A/A$ on the order of 1×10^{-4} . Figure 3b displays the carbonyl-stretching absorbance (bottom) and VCD (top) spectra for a tripodal peptide, a precursor to a synthetic ion carrier that is a biomimetic analog of the siderophore entero-bactin.¹⁰ The VCD data, interpreted with a theory based on coupled oscillators, was used to determine the solution conformation of this tripodal peptide. The VCD data was found to be consistent with a C_3 symmetry structure when the inter-chain hydrogen-bonding network forms a right-handed propeller with the oxygen ends of

Figure 3 (a). Raw VCD spectrum of (-)- α -pinene in CCl_4 ; 100 AC and 10 DC scans (data acquisition time: ~1.5 minutes); (b). carbonyl-stretching absorbance (bottom) and VCD (top) spectra for L,L,L-tris(N-Boc-leucylamidoethyl)amine in C_2Cl_4 . (Reprinted with permission from references 9 and 1c.)

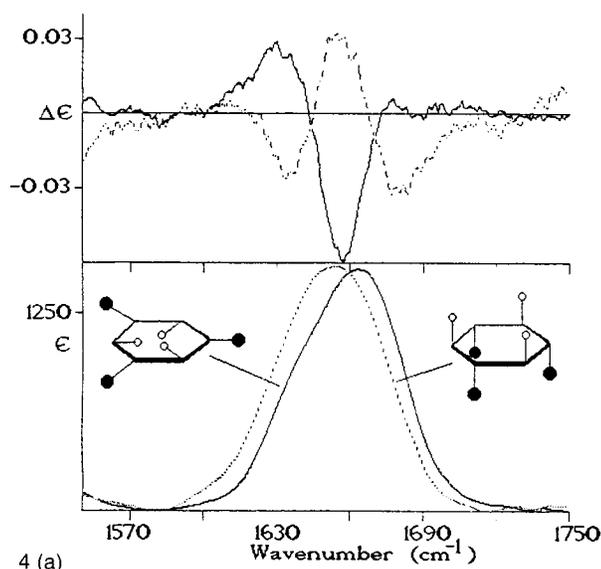
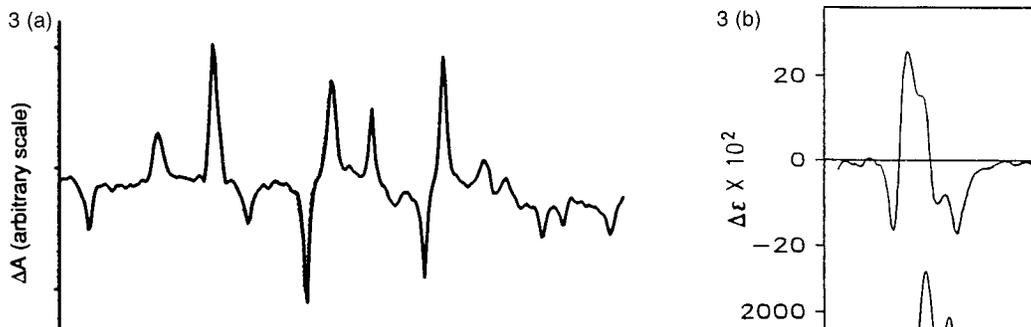
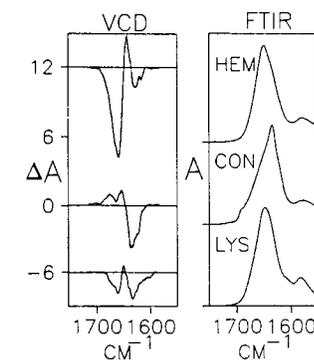


Figure 4 (a). Absorbance and VCD spectra of cyclo-(Pro-Gly)₃ in CDBr₃ (solid line) and CDBr₃/EtOD (dashed line); (b). Absorbance (right) and VCD (left, ΔA is on the order of 10^{-5}) spectra of hemoglobin (top, high α -helix), concanavalin A (middle, high β -sheet) and lysozyme (bottom, $\alpha+\beta$). (Reprinted with permission from references 1a and 1b.)



4 (b)

both the urethane and leucine sets of carbonyl dipoles directed upward along the C_3 axis.^{1c}

Several VCD spectra obtained using dispersive-VCD instruments are shown in Figure 4. Figure 4a shows the VCD spectra for two different conformations of a cyclic peptide, *cyclo*-(*-Pro-Gly*)₃, as caused by different solvents and the presence of metal ions.^{1a} The very different VCD spectra for these two conformations in solution demonstrate the high sensitivity of VCD for small structural changes. Figure 4b shows the absorbance and VCD spectra for three proteins – hemoglobin (high α -helix content in protein secondary structure), concanavalin A (high β -sheet) and lysozyme (mixed, $\alpha+\beta$).^{1b} Since the differential absorbance can be either positive or negative, VCD spectra have a significant enhancement in sensitivity to the secondary structure of proteins as compared to the absorbance (Figure 4b).

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Address correction requested.

INSIDE PEM

- PEM-Based Vibrational Circular Dichroism (VCD)
- CE, FCC Certification

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PEM-90 Receives CE Approval, FCC Certification

Hinds' PEM-90 photoelastic modulator systems have received both CE and FCC approval. The approvals were granted following extensive testing earlier this year.

The CE Mark

CE approval is now mandatory for a wide range of products marketed in the European Union. The "CE Mark" identifies a product as complying with the applicable health and safety requirements spelled out in about 40 different EU directives.

For Hinds, CE approval means that our PEM-90 systems meet the essential requirements of the Electromagnetic Compatibility (EMC) Directive. The EMC directive applies to most electronic equip-

ment. Under the directive, a product must not emit harmful emissions which might interfere with radio or telecommunications equipment operations, and the product itself must be immune from electromagnetic disturbances due to sources such as RF transmitters and other equipment.

The PEM-90 was certified as meeting both the emissions and immunity requirements.

FCC Certification

Hinds also is pleased to announce FCC certification for PEM-90 systems. "[FCC certification] gives our products a certain credibility," says Steve Varnum, electronic design engineer. "It lets the end user know that we go the extra mile to see how we comply with these regulations, even though they're not necessarily required. We wanted to

participate in domestic regulatory standards, as well as international ones."

By carrying these certifications, Varnum adds, "the customer is assured of the quality of the product. The additional testing will help us identify potential noise levels at frequencies that could interfere with customer applications, so we can address potential problems before they arise."

Additional Information

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