VIBRATIONAL CIRCULAR DICHROISM

APPLICATION NOTE

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Infrared absorption spectroscopy (IR) is the principal tool available for the study of molecular vibrations. Vibrational circular dichroism (VCD) is the differential absorption of left and right circularly polarized infrared light by vibrating molecules. The two techniques are often combined to create the spectroscopic measurement technique that will be described here, FTIR-VCD.

FTIR-VCD is used to study the subtle differences in vibrational spectra that result from molecules that differ only in their three dimensional geometry. The technique has been shown to be particularly useful for the study of the conformational characteristics of large biological samples such as proteins and nucleic acids and smaller molecules like chiral pharmaceuticals. It can be used in conjunction with *ab initio* calculations to determine the absolute configuration of newly synthesized molecules, and can be used to determine enantiomeric purity in molecules whose absolute configuration is already known.

The use of an interferometer, like a Michelson interferometer, to modulate the IR signal before it enters a sample makes vibrational spectroscopy much faster and easier to do. The information obtained by this method must be decoded by a computer. The decoding (or demodulating) process is called a Fourier Transform, hence the acronym FTIR. In FTIR-VCD the infrared light emerging from the Michelson interferometer is polarization modulated using a photoelastic modulator (PEM). The light oscillates between left and right circularly polarized at the PEM frequency, and this is how information about the differential absorption of the two polarizations is obtained. The VCD signal is four to six orders of magnitude smaller than the normal IR absorption signal. The use of PEM modulation gives VCD an ac detection advantage as well as a dynamic range advantage.

Figure 1 is a block diagram of a typical instrument that used for FTIR-VCD. The infrared light is modulated by the Michelson interferometer and then enters the VCD portion of the set up. By passing through a linear polarizer and a PEM, the Fourier modulated IR beam is further modulated at the PEM frequency. To obtain a VCD spectrum the doubly modulated signal is first demodulated at the PEM frequency by a lock-in amplifier, then Fourier transformed by a computer. Figure 2 is a block diagram outlining the steps in the demodulation of the IR signal.

The output from the detector is typically amplified by an ac-coupled detector preamplifier and is then processed through two independent paths to obtain the average and differential IR signals. The low pass filter allows the FTIR signal to pass through to the computer and is the source of a conventional IR spectrum. The high pass filter allows information at the PEM frequency (usually

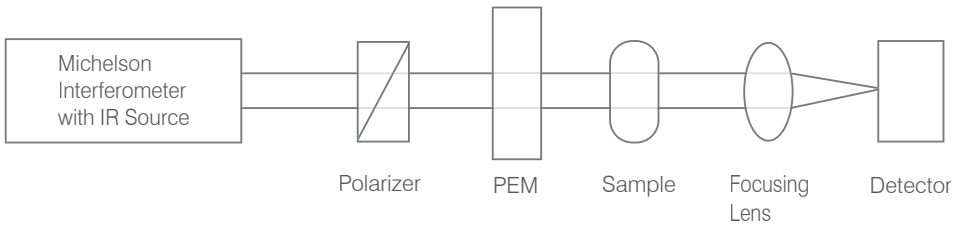

Figure 1: A block diagram of an FTIR-VCD set-up

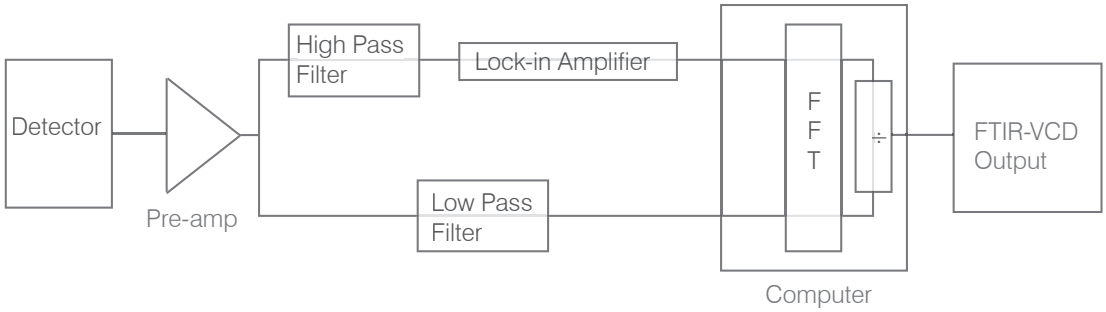

Figure 2: An electronic block diagram for a typical FTIR-VCD set-up

20-60 kHz) to pass to the lock-in amplifier. The lock-in is referenced to the PEM frequency and this is how the VCD spectrum is generated. The two signals are recombined at the computer where the FTIR and VCD signals are ratioed to give the final, complete FTIR-VCD spectrum.

The absolute configuration of chiral compounds has been historically difficult to determine. The technique most often used was Xray crystallography which required that the sample be a single crystal. Many organic and bio-organic compounds are difficult to crystallize, so an alternative technique for making the determination of absolute configuration was needed. This need increased in1992 after the FDA required that the absolute configuration of chiral pharmaceuticals be determined before they were introduced.

Gossypol is a naturally occurring compound that has been investigated as a male fertility-inhibiting compound.

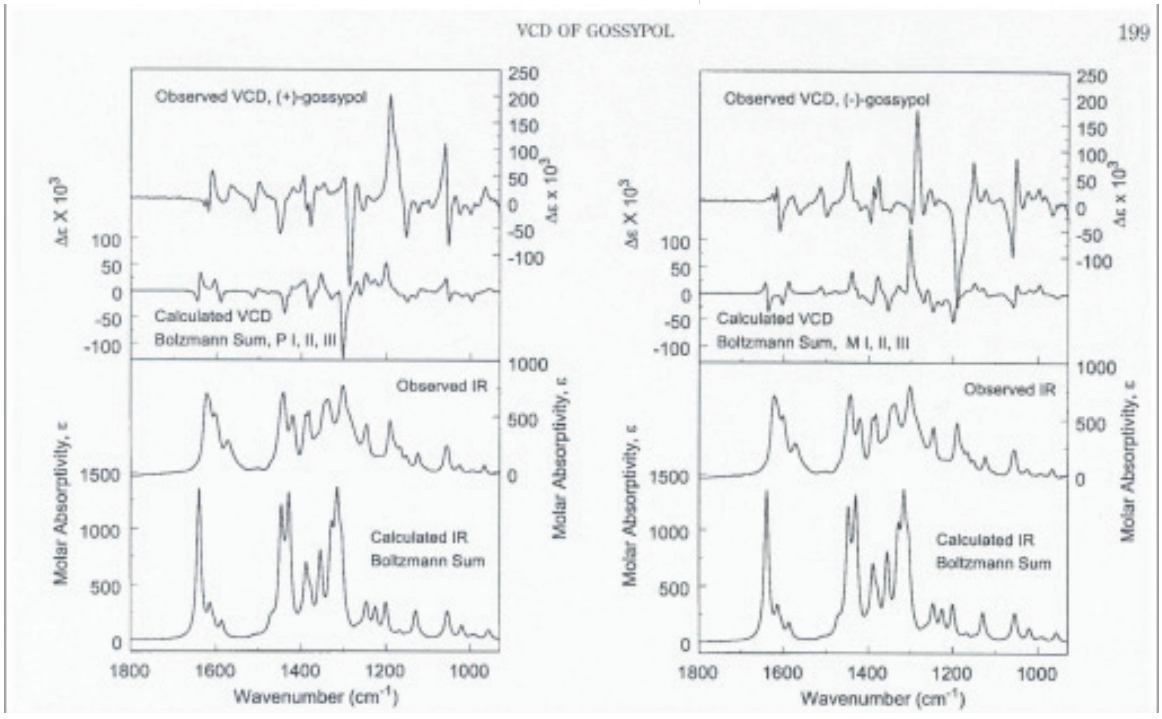

Figure 3: Comparison of IR And VCD spectra of (+) and (-) gossypol with calculated spectra

[1] T.B. Freedman, Xiaolin Cao, R.V. Oliveira, Q.B. Cass, and L.A. Nafie, CHIRALITY 15: 196-200 (2003)

It is a chiral compound, but its absolute configuration has been difficult to determine since it is an oil at room temperature and therefore difficult to crystallize. Teresa Freedman and coworkers used FTIR-VCD to do this determination in conjunction with *ab initio* calculations that predict the VCD spectrum of each configuration.[1] Their work is included here with the permission of Laurence A. Nafie.

Figure 3 shows a comparison of (+) and (-) gossypol's VCD spectra and the spectra predicted by calculations using Gaussian 98. This data provided the information necessary to determine which configuration is attributable to the (+) and (-) forms of gossypol.

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FURTHER READING

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