Amplified vibrational circular dichroism

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EXPERIMENTAL METHODS

In this chapter we describe the experimental methodologies used in the acquisition of VCD spectra. The chapter is organized in two parts: the first part contains an overview of the functioning of a Fourier-Transform VCD setup with the fundamentals of Fourier-transform spectroscopy and the polarization modulation approach required for the measurement of VCD signals. The second part reports on the design of an optically-transparent thin-layer electrochemical cell that was specifically developed for VCD measurements of chiral redox-active molecular systems.

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3.1 VIBRATIONAL CIRCULAR DICHRÖISM MEASUREMENTS

3.1.1 Fourier Transform Spectroscopy

All infrared absorption and VCD measurements reported in this thesis were performed using a Fourier-Transform (FT) VCD setup (Bruker) consisting on FT-IR spectrometer (Vertex 70) and a polarization modulation module (PMA 50). The first design of a FT-VCD apparatus was reported by Nafie et al., in 1979 [31]. The incorporation of FT-IR instead of conventional dispersive technology led to the first series of VCD measurements showing an unprecedented combination of signal-to-noise ratio and spectral resolution (Nafie et al., 1982[32]).

Before we describe the experimental details of a FT-VCD measurement (see section 3.1.2), we will briefly visit the basic principles of FT-IR spectroscopy. Fig. 4 shows the layout of a Michelson interferometer. Incoming light from an IR source (S) hits a beam splitter (BS) of 50% transmittance, which splits the light into two identical beams. Both beams are reflected on two distinct mirrors and recombined again by the same BS and sent to the detector (D). One of the mirrors is fixed (FM) while the other is movable (MM). When both mirrors are at equal distance from the BS they create a zero path difference (ZPD). However, if the MM is out of the

Figure 4: Optical layout of the Michelson interferometer. S: IR source; BS: beam splitter; FM: fixed mirror; MM: movable mirror; D: detector. The dashed line represents the ZPD (zero path difference).
ZPD point, the second beam has to travel an additional distance \( \delta = 2L \) (called retardation), the optical path difference between the two beams. The interference pattern generated by all wavelengths (called interferogram) is recorded at the detector as intensity as a function of retardation, \( I(\delta) \):

\[
I(\delta) = \int_0^\infty I(\nu) \cos [2\pi \nu \delta + \theta(\nu)] \, d\nu
\]

where \( \nu \) represents the frequency values in wavenumbers, and \( \theta(\nu) \) is a phase factor. At the ZPD, the retardation, \( \delta \), is zero and all wavelengths interfere constructively forming a center burst. The transmission spectrum can be obtained by performing a Fourier transform of the interferogram:

\[
I(\nu) = \text{FT}[I(\delta)] = \frac{1}{2\pi} \int_0^\infty I(\delta) \cos [2\pi \nu \delta] \, d\delta
\]

### 3.1.2 Fourier Transform VCD Spectroscopy

Fig. 5 shows a block diagram of the optical-electronic layout of a Fourier-Transform VCD spectrometer. An IR source emits light which is directed through an interferometer that encodes each spectral point with a Fourier frequency. The output radiation from the interferometer passes through an optical filter (F) and is linearly polarized (P) to define a single state of polarization. The optical filter (F) limits the range of frequencies that passes through the setup. In this way one can have higher light intensity for a specific range without saturating the detector (D). The polarizer (P) selects incoming light linearly polarized \( 45^\circ \) with respect to the stress axes of the photoelastic modulator (PEM).

#### 3.1.2.1 Photoelastic modulator (PEM)

The PEM is the dynamic polarization device that is used to modulate the polarization of the IR light, crucial for the VCD measurement. It makes use of the photo-elastic effect to produce alternating left (LCP) and right
circularly polarized (RCP) light. To this purpose, a periodic voltage is
applied to a piezoelectric transducer which applies mechanical stress
onto the photo-elastic material (ZnSe for infrared light). The ZnSe bar is
isotropic in the absence of stress, but becomes birefringent when stress
in applied. The bar vibrates at a frequency determined by its length and
the speed of a longitudinal sound wave. The piezoelectric transducer is
tuned to the resonance frequency of the ZnSe, which is the PEM mod-
ulation frequency. The modulation amplitude of the PEM is linearly de-
pendent on the current on the piezoelectric transducer. Therefore, the
oscillating birefringent effect is at its maximum and generates fully cir-
cularly polarized light, at maximum compression and stretching of the
photo-elastic material (Fig. 6).

For this specific purpose, $V_{\text{PEM(max)}}$ is set such that the PEM works
periodically as a quarter-wave retardation plate. The incoming lineally
polarized light is set to 45° with respect to the optical axis of the PEM.
In case of compression, the horizontal component is retarded with respect to the vertical component. Stretching results in retardation of the vertical component with respect to the horizontal. Depending which of the components is retarded, LCP or RCP light is generated when \( V_{\text{PEM}} = V_{\text{PEM(max)}} \).

After passing through the PEM, the beam travels through the sample (S) and is focused into a nitrogen-cooled Mercury Cadmium Telluride (HgCdTe or MCT) detector. The beam at the detector is doubly modulated. In addition to the Fourier (low frequency) modulation from the FTIR source, the beam is modulated between left and right polarization states at the PEM modulation frequency, which is typically tens of kilohertz. The signal then follows two independent electronic pathways (Fig. 5) in order to obtain the differential (AC) signal and the average (DC) signal.

The DC interferogram, \( I_{\text{DC}}(\delta) \), is obtained by passing the signal through a low-pass filter that removes the high-frequency modulation and results in the standard FT-IR signal modulated at the Fourier frequencies, \( \omega_F \), constant with respect to the polarization modulation. To obtain the AC interferogram, \( I_{\text{AC}}(\delta) \), a high-pass filter attenuates the low-frequency component in the detector signal. Demodulation of the high frequency component is achieved by passing the signal through a lock-in amplifier (LIA) (Fig. 5) which is referenced to \( \omega_M \) and returns the AC interferogram as an output, now only modulated at the Fourier frequencies.
frequencies, $\omega_F$. Both interferograms are then phase corrected (see section 3.1.3) and Fourier transformed to yield DC and AC transmission intensities, $I_{DC}(\nu)$ and $I_{AC}(\nu)$, respectively.

3.1.2.2 Relationship between the AC/DC signals and VCD spectra

The differential absorption between LCP and RCP light, i.e., the VCD spectrum, is obtained from the ratio of the Fourier-transformed AC and DC interferograms as follows: [33]

$$\Delta A(\nu) = A_L(\nu) - A_R(\nu) = \frac{0.8685}{2J_1[\psi_M^0(\nu)]} \left[ \frac{I_{AC}(\nu)}{I_{DC}(\nu)} \right]$$

where, $J_1[\psi_M^0(\nu)]$ is the first-order Bessel function and $\psi_M^0(\nu)$ is the maximum retardation induced by the PEM for a certain frequency $\nu$. The ratio is taken in order to normalize out any dependencies on the source intensity and instrument transmission characteristics. In order to remove the spectral dependence of the Bessel function, $J_1[\psi_M^0(\nu)]$, an additional calibration measurement is required (see section 3.1.3).

3.1.3 Calibration Measurement

Phase corrections for both AC and DC interferograms have to be determined before the Fourier transforms can be performed ($\theta(\nu)$ in Eq. 48). The two interferograms pass through different electronic pathways. Therefore, the Fourier components of the two interferograms experience different phase shifts for the same Fourier frequency. The lock-in amplifier time constant and the phase factor introduced to process the AC signal give rise to this mismatch.

Calibration of the DC interferogram can be done using the intensity maximum of the interferogram at the ZPD point. This is done automatically by software that controls the FT-IR spectrometer using an algorithm that determines the phase correction function. However, the algorithm assumes that all detector intensities are positive, which is not the case for the AC interferogram. The AC interferogram requires a more delicate procedure due to its differential nature. Since the intensities of $I_{AC}(\nu)$...
can be either positive or negative, an auxiliary calibration measurement is required to determine the phase correction function when only positive intensities are present. After that, the phase correction is transferred to the sample measurement of $I_{AC}(\nu)$.

The calibration procedure consists of replacing the sample (S) with a CdS multiple waveplate (MWP) and a second polarizer (P2) placed behind it (see Fig. 7). The polarizers P and P2 are rotated parallel to each other. The waveplate is a birefringent ($\lambda/4$) crystal with the fast axis parallel to the modular axis of the PEM. In practice, this means that the calibration spectrum will exhibit maxima when the CdS plate retards the component of the circularly-polarized light that was not retarded in the PEM. Minima will occur when both CdS plate and PEM retard the same linear component, which results in rotation of the linear polarization by $\pi$ and consequent blockage of all the light by polarizer P2. The dependence of Eq. 50 on the Bessel function $J_1[\psi_M(\nu)]$ relies on the properties of the CdS plate and it is determined from the calibration measurement as well as all the gain factors introduced by electronics processing. The resulting calibration function $(0.8685/2J_1[\delta_M(\nu)])$ is used to divide the uncalibrated VCD spectrum to obtain the final calibrated VCD spectrum.

Figure 7: Block-diagram for the VCD calibration measurement. The sample (S) is replaced by a multi waveplate (MWP) and a second polarizer (P2).

### 3.1.4 Baseline correction

Once the VCD spectrum has been acquired, a baseline correction has to be applied in order to compensate for small systematic errors introduced by the VCD instrumentation that return a baseline which do not repre-
sent the true zero VCD signal. There are many ways to perform this correction. The most rigorous one consists of the measurement of the opposite enantiomeric form of the sample under exactly the same experimental conditions (concentration and optical pathlength). Subtraction of opposite enantiomers divided by two eliminates all possible artifacts, yielding a baseline corrected VCD spectrum. In case the enantiomer is not available, one may use a racemic mixture of the compound. However, subtraction of the spectrum of the test compound by its racemic mixture has a lower signal-to-noise ratio in comparison with the previous option. Finally, and perhaps the more commonly used method, uses the solvent VCD spectrum for the baseline correction. The solvent has a zero VCD spectrum, but in most cases will contain all possible artifacts. Subtraction of the solvent VCD spectrum from the VCD spectrum of the test compound will remove existing artifacts. This last option is used more frequently since many of the target molecular systems that are investigated with VCD are biological molecules such as enzymes and proteins, which are not available in racemic or enantiomeric forms.
3.2 AN OPTICALLY TRANSPARENT THIN-LAYER ELECTROCHEMICAL CELL FOR THE STUDY OF VCD OF CHIRAL REDOX-ACTIVE MOLECULES

For the experiments described in chapters 5 and 8, an optically transparent thin-layer electrochemical (OTTLE) cell with a locally extended optical path has been developed to perform vibrational circular dichroism (VCD) spectroscopy on chiral molecules prepared in specific oxidation states by means of electrochemical reduction or oxidation. The new design of the electrochemical cell successfully addresses technical challenges involved in achieving sufficient infrared absorption. The VCD-OTTLE cell proves to be a valuable tool for the investigation of chiral redox-active molecules.

3.2.1 Introduction

Electrochemical cells that enable secondary chemical reactions to be monitored in situ under controlled conditions (e.g., temperature, pressure) are very useful for the selective detection, identification and characterization of short-lived redox species and detailed investigation of redox paths. [34] Chiral molecules that exhibit distinct chiroptical signatures upon electrochemical generation of radical ions in solution have been investigated with electronic circular dichroism (ECD) spectroscopy.[35, 36, 37] The high ECD signal intensities generally observed for organic or inorganic compounds make CD spectroelectrochemistry a suitable tool to determine chiroptical and stereochemical properties of natural and synthetic optically active compounds. In situ ECD spectra of electrochemically generated target species have appeared in several reports on chiroptical activity of redox-active molecular systems in the UV-Vis spectral region. [35, 36, 37] However, such studies are limited to species featuring an optically active chromophore.

To the best of our knowledge, the only VCD spectra of chiral organic radicals have been reported by Mori et al. [38]. However, the radical cations were obtained on addition of a chemical oxidant so that many
aspects of the chiroptical activity (e.g. the reversibility of the oxidation, and possible intermediate states) could not be investigated.

This section contains a technical description of the first practical design of an optically transparent thin-layer electrochemical (OTTLE)[39] cell applicable for VCD measurements on electrochemically generated radical ions, using a commercially available VCD spectrometer.

3.2.2 Practical considerations for spectroelectrochemical measurements

The combination of thin-layer cyclic voltammetry (TL-CV) and spectroelectrochemistry (SEC) [34] has practical challenges that need to be addressed. Acquiring acceptable mid-infrared absorption spectra of redox active species requires combination of an appropriate window material (e.g., CaF₂, BaF₂, NaCl, KBr, CsI), supporting electrolyte and aprotic solvent (e.g., acetonitrile, dichloromethane, tetrahydrofuran, including their deuterated forms) to create wide transparent spectral windows in the functional-group, fingerprint and aromatic regions showing characteristic vibrational modes of the redox couples. Often, target compounds require highly concentrated solutions in order to obtain sufficiently high optical density (≈ 1 OD) for a VCD measurement. Working with highly concentrated solutions (> 10 mM) may lead to complications such as formation of aggregates and irreversible chemical processes. Moreover, the combination of long measurement times (required to obtain a good VCD spectrum) and concentrated samples requires a balanced design for an optimum performance of the cell. For any design of the OTTLE[39] construction, the thickness of the electrolyte layer around the working electrode is a key geometrical detail that determines the electrolysis time.[34]

3.2.3 VCD Spectroelectrochemical Cell

Introducing a more sophisticated design of the working electrode to meet the practical requirements of a VCD measurement inevitably compromises the time for electrolysis to be completed. We have therefore adapted an OTTLE cell[39] by introducing a relatively long pathlength
Figure 8: Exploded view of the VCD-OTTLE cell: 1. metal cover plates (with filling ports); 2. rubber gasket; 3. CaF$_2$ window; 4. custom PTFE spacer (1 mm thick) with a circular aperture (4 mm diameter); 5. custom polyethylene-electrode spacer (see enhanced view in Fig. 32); 6. protective frame; 7. electrode plug.

(1.2 mm) in the probed area, which is customized to efficiently perform VCD measurements. Details of the cell construction are shown in Fig. 8. The new construction presents two principal modifications: a) a new non-planar shape of the working electrode melt-sealed into the polyethylene spacer together with the counter and pseudo-reference electrodes, and
Figure 9: Enhanced view of the PTFE spacer and the custom polyethylene-electrode spacer. WE - working electrode (shaped Pt minigrid), CE - counter electrode (flat Pt minigrid), RE - pseudo-reference electrode (Ag wire).

b) addition of an extra PTFE spacer with a customized geometry (see Fig. 32). In particular, a small cut has been made in the centre of the 5×6 mm Pt minigrid, and the cut wires have been folded outwards, resulting in a cylindrical protrusion in the centre of the working electrode and introducing a 3.5 mm–diameter hole in the centre of the Pt minigrid (see Fig. 32). A 1 mm thick PTFE spacer with a circular aperture of 4 mm diameter facing the Pt minigrid aperture (Fig. 32) is placed on top of the 0.2 mm PE spacer with melt-sealed electrodes. The PTFE spacer has four major functions: it (1) maintains the cylindrical shape of the working electrode when the upper CaF₂ window is put in place; (2) preserves the thin-layer geometry at the counter and pseudo-reference electrodes in order to avoid diffusion of products from the working electrode; (3) allows for a large volume of solution only at the probing site so as to increase the optical pathlength; (4) serves as a mask for the remaining thin-layer area of the electrode compartment. The total 1.2 mm optical pathlength
ensures sufficient infrared absorption intensity to measure VCD signals on solutions with concentrations as low as \(7 \times 10^{-3}\) mol dm\(^{-3}\). The protruding cylindrical shape of the minigrid electrode ensures a complete, although relatively slow (\(\approx 3\) min), electrochemical conversion along the whole optical path. Fig. 33 shows the reversible cyclic voltamogram of \(7 \times 10^{-3}\) M of (S)-methyl 2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propanoate in CD\(_3\)CN (0.1 M Bu\(_4\)NPF\(_6\)) recorded at the scan rate of 5 mVs\(^{-1}\).

Figure 10: CV of (S)-methyl 2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propanoate in CD\(_3\)CN (0.1 M Bu\(_4\)NPF\(_6\)) recorded at the scan rate of 5 mVs\(^{-1}\) with a PA\(_4\) Potentiostat (EKOM, Poln, Czech Republic).

The color change of this compound upon reduction can be used conveniently to assess whether the redox-active species is actually converted in the whole cylindrical volume at the probing site. In Fig. 11 we show photographs of the VCD-OTTLE cell containing the solution of the test compound. Upon electrochemical reduction, the colorless parent species is completely converted to the yellow-green radical anion species inside the cylindrical probing volume. The completion and reversibility of the electrolysis was confirmed by isosbestic UV-Vis spectral changes (see ref. [40]). The completion of the electrolysis in the cylindrical space is probably due to charge migration in the electric field. Importantly, the same remarkable effect, i.e., completed electrolysis, is observed for an exclusively thin-layer configuration of the electrode compartment, when the Pt minigrid working electrode features a 2-mm circular hole in the center. No electrolysis takes place at this distance from the outer edges of the working electrode.
Figure 11: Pictures of the VCD-OTTLE cell containing the neutral (left) and radical anion (right) forms of (S)-methyl 2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propanoate in CD$_3$CN (0.1 M Bu$_4$NPF$_6$). Inset: closeup of the cylindrical probing volume before and after the complete electrolysis.

3.2.4 VCD Spectroelectrochemical Assembly

The spectroelectrochemical VCD measurements were performed using a Bruker Vertex 70 FTIR spectrometer in combination with a Bruker PMA 50 VCD module. The new design of the OTTLE cell required adaptation of the PMA 50 sample compartment, since the resulting small aperture in the PTFE spacer would otherwise cause significant clipping of the circularly-polarized infrared light. We therefore assembled an optical (Kepler) telescope in the sample compartment, consisting of two ZnSe lenses of 50 and 38 mm focal lengths (see Fig. 12). The telescope allows the circularly-polarized infrared beam to focus into the small volume of solution where the electrochemical conversion occurs. The 3-mm beam waist is sufficiently small for all the infrared light to be transmitted through the 3.5-mm diameter hole in the grid of the working electrode.
3.2 OTTLE CELL FOR VCD

Figure 12: Adapted PMA50 sample compartment where the VCD-OTTLE cell is fixed in a sample holder arm between the two ZnSe lenses (f = 50 and 38 nm).

3.2.5 Concluding Remarks

The new design of an OTTLE cell enables in situ measurement of VCD spectra of radicals in solution for both organic and inorganic molecular systems. This VCD-OTTLE cell has been developed to overcome the problems encountered due to the inherently small VCD signals, which is the main topic of this dissertation. The first conclusions drawn from experiments performed with this cell are discussed in Chapter 5. Along with this specific purpose, the VCD-OTTLE cell design will be of general use by making it possible to investigate redox-active chiral compounds. Using the VCD-OTTLE cell, these compounds can be prepared in specific oxidation states in a controlled manner and simultaneously be investigated with VCD, allowing for a detailed determination of their configuration and conformation.

On a more general level, this cell is also very well suited for the application of spectroscopic techniques on redox-active species in which light scattering needs to be avoided. Examples include time-resolved laser spectroscopy, non-linear optical techniques and imaging.