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Novel Approaches to Optical Activity Measurements



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Introduction

1.1 Life is chiral

The term chirality has its origin in the Greek word *kheir*, which means hand. It was brought into general use by Lord Kelvin, who stated in 1894 [1]:

“I call any geometrical figure, or any group of points, chiral, and say that it has chirality, if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself.”

Perhaps the most prominent chiral objects are our left and right hands. A molecule is called chiral when it possesses handedness, i.e. it can be found in two geometrical configurations of its atoms with mirror-image symmetry. These mirror images are called enantiomers when referring to molecules, while the word enantiomorphs is usually still reserved for larger objects like crystals. Many organic and natural molecules are chiral, especially those affecting our daily life like peptides, enzymes, amino acids (except glycine) or proteins. But the reason why these molecules exist primarily just in one configuration is still unclear.

The two enantiomers of a chiral molecule possess similar physical and chemical properties in a non-chiral environment but will act differently in the interaction with other chiral objects. This may lead to very different biochemical and physiological effects. Referring to pharmaceutical active compounds this difference is of great interest. While one of the configurations possesses the desired properties the other one may have limited activity or unwanted side effects. A prominent example is the molecule thalidomide (Fig. 1.1), which was sold as a sedative drug (Contergan) in the late 1950s. It was prescribed to treat morning sickness, but caused severe birth defects in the newborn babies. Studies soon indicated that the (*S*)-(-)-enantiomer could be responsible for the fetal damage due to the interaction with the embryo's prenatal DNA [2]. Since both thalidomide enantiomers racemize quickly

in the human body it is not sufficient to take just the (*R*)-conformer. Although the complete picture turned out to be not that simple, the thalidomide case raised awareness for the discrimination of enantiomers of chiral pharmaceuticals to prevent side effects, and the determination of the enantiomeric excess (or purity) is now a requirement demanded by the FDA (U.S. Food and Drug Administration).

1.2 Probing chirality

Optical methods provide a direct means of distinguishing the enantiomers of chiral molecules. Most of these methods are based on the differential interaction of the molecules with circularly polarized light, which is also chiral. It is macroscopically described by a difference in the complex refractive index $\delta\tilde{n} = \delta n + i \delta\kappa$ to left- and right- circularly polarized light, which is referred to as *Optical Activity*. It was first discovered by Jean-Baptiste Biot, Augustin Fresnel, and Aimé Cotton in the early 19th century [3,4]. The usually small differences ($< 10^{-6}$) result in tiny effects, which in turn are experimentally demanding to observe. Nevertheless, a number of optical methods have been developed.

The difference in the real part of the index, called *Circular Birefringence (CB)*, is detectable in refraction, diffraction or scattering. If linearly polarized light traverses an optically active medium, the Circular Birefringence will cause the rotation of the plane of polarization, known as *optical rotation*. In the polarimetry the rotation is detected for the determination of the handedness and/or the concentration of optically active solutions [3]. The variation in the rotation angle as a function of the wavelength is known as *Optical Rotary Dispersion (ORD)*. Since the rotation angle is also proportional to the pathlength

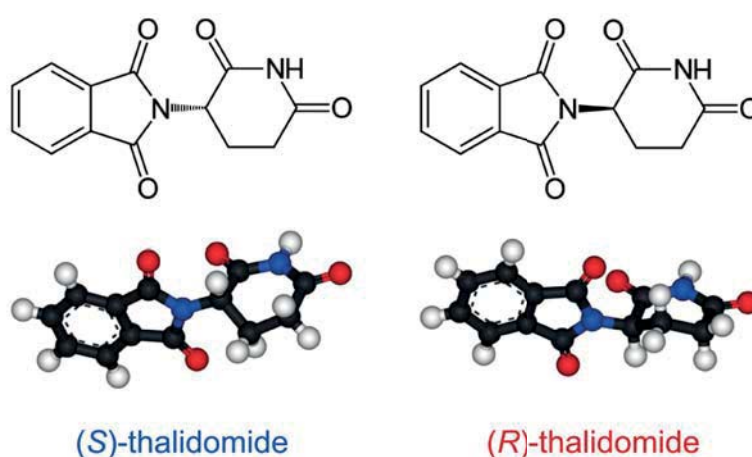


Figure 1.1: Both enantiomers of thalidomide, the chiral molecule that caused the Contergan scandal in the late 1950s. Where the (*R*)-enantiomer seems to act as a sedative the other enantiomer turned out to cause dramatic birth defects. (adapted from Wikimedia Commons)

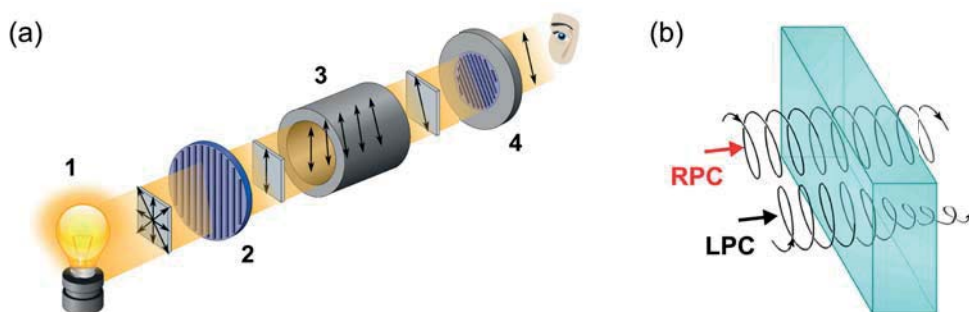


Figure 1.2: (a) Schematic of a polarimeter for the detection of optical rotation. Unpolarized light (1) is linearly polarized (2) before passing the optical active sample (3). The rotation is detected with a second rotatable polarizer (4). (b) Circular dichroism (CD) manifests itself in the differential absorption between left- and right-circularly polarized light. (adapted from Wikimedia Commons)

the light travels inside the sample, longer pathlength cells are needed if small differences δn are to be detected. But this is difficult if only small volumes are available, as is often the case in high-throughput screenings or if new compounds are synthesized. Polarimetry in microfluidic volumes is therefore generally not possible. In this thesis a scheme to measure optical activity in sub-micro liter volumes is presented.

Circular Dichroism (CD) refers to the difference to left- and right-circularly polarized light, $\delta\kappa$, in the imaginary part of the complex refractive index. It is equivalent to a differential absorption of the two circular polarization states, which is measured with a CD spectrometer. Nowadays CD spectroscopy has a wide range of applications depending on the spectral region of interest. UV CD, for example, is used to examine the secondary structure of proteins or charge-transfer processes in chiral metallic complexes [5]. Vibrational Circular Dichroism (VCD) observed across the absorption bands of vibrational molecular transitions extends the CD studies to the middle infrared (MIR) region. Being sensitive to the mutual orientation of distinct functional groups, VCD provides structural information about chiral molecules. Together with *ab initio* calculations VCD has become a powerful tool for the determination of the absolute conformation of small molecules. It is also used for structural studies of small organic molecules, or proteins and DNA [5]. One difficulty, however, is often the strong absorption of the solvent itself, especially in aqueous (polar) solvents. This often restricts traditional VCD instrumentation to selected spectral regions and special solvents. In this thesis it is shown how the use of MIR lasers permit VCD studies in aqueous solutions.

Raman-Optical-Activity (ROA) is an alternate form of Vibrational Optical Activity (VOA), measuring the differential Raman scattering intensities of the circular polarizations. It is a complementary technique to VCD spectroscopy. Because ROA spectroscopy uses laser in the transparent (visible) spectral region of the solution, ROA does not suffer from solvent

absorption. It can be observed in a number of forms defined by the polarization of the incident and scattered light [3, 6]. In scattered circular polarization (SCP) experiments, for example, is the incident light linearly polarized and differences in circular polarization components of the scattered light are measured. In dual circular polarization (DCP) ROA the incident as well as the scattered light are circularly polarized. Nevertheless, ROA instrumentations are experimentally and technically more demanding compared to VCD instrumentation and due to the weakness of the observed ROA signals.

In the field of nonlinear optics there are also forms of optical activity [7, 8], and observables exist at different orders of nonlinearity. The intensity of the generated light field in second-order nonlinear processes like sum-frequency (SFG) or second harmonic generation (SHG), for instance, can be used as a measure for the chirality of the sample. Beside these second order process additional optical activity observables are predicted to exist in higher-order processes like the (quartic) nonlinear Raman spectroscopy (BioCARS) with similar properties.

Beside the described methods there are also non-optical indirect tools for chiral structural analysis like nuclear magnetic resonance (NMR) spectroscopy or X-ray diffraction. Because both enantiomers show identical chemical shifts and spin-spin coupling constants, conventional NMR spectroscopy is unable to distinguish between them. This requires the use of chiral reagents typically leading to the formation of diastereomers showing different NMR signals. Ideas for a direct discrimination in chiral NMR have been published [9] as well as the use of achiral reagents [10]. The inelastic or resonant scattering of X-rays by optically active compounds in the solid state is still one of the key methods for the determination of absolute configuration. It has been first proposed and realized by Bijvoet in 1951 [11]. Since elastic X-ray scattering measures the interatomic distances which do not differ for a pair of enantiomers, the absolute configuration can not be determined. Using X-rays with a wavelength near the absorption of just one type of atoms the two crystallized enantiomers can be distinguished. This is due to the additional phase-lag of the radiation resonantly scattered at these atoms. This in turn leads to different scattering patterns for the crystal and its mirror image. Nevertheless, crystallization of optically active compounds with high purity and regularity is not always easy to accomplish, and the evaluation of scattering patterns of complex compounds gets difficult.

Despite the wide diversity of techniques detecting optical activity, this work is in part motivated by the challenge of detecting optical activity in small sample volumes or in difficult (strong absorbing) environments.

1.3 Motivation and outline

This work presents and investigates new optical methods for the analysis of chiral media based on different optical activity effects in refraction, absorption and scattering. It is

motivated by the following questions: How can optical activity (OA) be detected in small sample volumes as for example is the case in microfluidics? Can OA be analyzed using VCD in (aqueous) solutions despite the strong solvent absorption? Is there a possibility to observe OA in scattering and are the colloidal analogues chiral molecules?

The first method introduces a *chiral refractometer*. Here the refractive index difference δn (CB) is detected in refraction. At an interface between a chiral and an achiral medium both circular polarization components will refract with slightly different angles of refraction. Light which is initially collinear splits into two polarization components propagating in slightly different directions. This splitting is in the order of nanorad (10^{-9} rad) for millimolar (mM) solutions. Since the effect happens at the interface the method requires potentially much smaller fluid volumes ($\sim 1 \mu\text{l}$) than usual transmission based experiments (polarimetry). The sensitivity of the measurement scheme now depends on how small a beam displacement or separation can be detected. Therefore different beam position detection schemes are tested and compared with each other.

The second method which has been developed is a VCD spectrometer using quantum cascade lasers (QCL). These middle infrared (MIR) lasers provide orders of magnitude more optical power compared to thermal light sources. This allows optical activity measurements in strongly absorbing solutions. Results for different solutions recorded with the QCL-spectrometer are presented. These include the VCD spectra of aqueous solutions of the amino acid proline with an optical density of up to 3.5 in the examined spectral region. Despite their higher power levels and compact and rugged design QCLs suffer often from intensity fluctuations. This affects and limits the sensitivity in absorption measurements (for example VCD). Therefore a QCL based MIR refractometer is presented, which allows the detection of vibrational spectra without direct measurement of the QCL's intensity. The real and imaginary part (n and κ) of the refractive index are determined in an imaging setup. Changes in the beam profile at a total internal reflection interface are used to deduce simultaneously n and κ of the analyte.

Finally helical colloidal molecules with programmable shape and chirality are investigated. Glancing angle physical vapor deposition (GLAD) is used for the batch-production of large numbers of colloids. Suspensions are characterized in water by differential scattering of both circular polarization components. Scattering CD data of different chiral colloids (helices) are presented and compared to calculation based on a simple analytical model. It is shown that the differential scattering signals are sensitive to the chiral parameters of the colloidal scatterer.

1.4 Publication of main results

The main results of this thesis have been published in four peer-reviewed scientific journals and two conference proceedings, on which the following chapters (3,4,5,6) are based on: