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Label-free and Dilution-free Detection and Analysis of Molecular Chirality via Terahertz Spectrometry

White paper **July 8, 2010**

1. Why chirality analysis important

The analysis of chiral molecules is of central importance to modern day chemistry, biochemistry [1] and material science [2-4]. Enantiomeric purity is as paramount as chemical purity to the pharmaceutical industry [5]. Related to this is the optical purity of chiral catalysts and the resulting enantiomeric excess measurements necessary to evaluate synthetic transformations. Analytical techniques capable of surveying chirality have also been used to elucidate reaction mechanisms [1]. More recently materials including polymers [6] as well as supramolecular assemblies and switches [7-10] employing chiral elements have given rise to differential behavior with matter and energy. These initial reports may pave the way for molecular machines and data storage devices that use chirality. On a macromolecular scale, discrete proteins, DNA, RNA, polysaccharides and complexes of these biomolecules are routinely analyzed using chiral optical techniques to gain insight into their three-dimensional structural features, most notably their helicity [1].

2. Established Methods for Chiral Analysis

Traditional methods for small organic and inorganic molecules include optical rotation (OR) and optical rotary dispersion (ORD). The measurements rely on optical refraction at fixed and variable wavelength of the UV-Vis spectrum, respectively. Their use in characterization of chiral small molecules is extensive, but limited to descriptions of rotating linearly polarized light in one of two directions with a given magnitude. Additionally, several measurements (e.g. multiple concentrations) are often required to determine whether a compound is dextrorotatory or levorotatory and other complications arise with solutions of chiral compounds in solvents. These effects can even include a reversal of sign in the optical rotation as a function of solvent [1].

Larger biomolecules and synthetic macromolecules give the same information when using OR and ORD, but three-dimensional information (e.g. helical content) has been extracted using circularly polarized light through circular dichroism (CD). CD is an absorption technique that requires an accessible chromophore. For small molecules containing few chiral centers the CD spectra often has a small electronic transitions making analysis challenging. Small molecules containing other forms of chirality (such as helixes as found in helicenes), however give large electronic transitions, though these chiral elements are rare in small molecules such as

pharmceuticals. Biomolecules on the other hand typically have helical content that gives rise to CD spectra with appreciable magnitude.

All existing optical techniques require dilute solutions of known concentration. The solvent effects mentioned above can invert the results, or even result in a *null* measurement if the wrong solvent is selected. Additionally, achiral sample impurities can diminish or in some instances enhance the magnitude of the measured signal if they can order over a short range (e.g. through hydrogen bonding) about the chiral material and affect either refraction (OR), or absorption (CD). Small amounts of analyte also can't be easily analyzed if the resulting solution is too dilute. Despite the physical laws that govern optical techniques, they still hold only over narrow concentration ranges and are highly solvent dependent. Temperature also can play a role in these measurements. More recently vibrational techniques have been developed including vibrational optical rotatory dispersion, vibrational circular dichroism and Raman optical activity. These techniques use IR radiation that induce vibrational modes of different functional groups and have several advantages over optical techniques.

Other methods including Mass Spectrometry, Fluorescence and Nuclear Magnetic Resonance (NMR) have also been employed [11–12]. NMR has been widely employed in the study of small molecules in particular enatiomeric excess determination (ee). This technique is very powerful, but severely limited to molecules that can be covalently modified with other, often times expensive enatiopure reagents. In other instances non-covalent coordination is used with expensive chiral shift reagents that have narrow scope by being drastically limited to interaction with molecules containing very specific functional groups [13].

3. Advantages of Terahertz Spectroscopy

Terahertz spectrometry offers several advantages when compared to current methods. First, the terahertz region of the electromagnetic spectrum results in vibrational resonance, thus making terahertz a general method to study molecules including those lacking an optical chromophore. The sample amount necessary required to obtain a terahertz spectra is quite small, on the order of 20 micro liters and can be done neat without the need for dilution, thorough direct application to a glass slide. Terahertz vibrational modes tend to involve all the atoms of the molecule and would be expected to give rise to unique spectra for each unique molecule, while retaining characteristic vibrational modes for certain functional groups. Terahertz spectroscopy is also sensitive to intermolecular interactions and may be capable of studying the interaction of multiple chiral molecules – if these modes can be deconvoluted. This may have distinct advantages to NMR methods employing chiral shift reagents, or advantages to material science and interfacial interactions. The resulting data gives rise to many distinct bands that can be used to

evaluate chirality.

The problems associated with optical techniques are significant. Lack of a suitable chromophore and solvent effects are two major issues that make chiral analysis complicated for many systems. Moving beyond the visible spectra to the terahertz region provides new opportunities to study whole molecule phenomena, or chirality around functional groups that do not have strong optical or IR activity. Terahertz interaction with macromolecules is sensitive to the vibrational modes of the entire macromolecule. This characteristic has been successfully deployed to characterize systems such as non-ionic detergents in biopharmaceuticals [14], transdermal drug delivery [15], single nucleotide polymorphism [16], and DNA hybridization [17].

4. Preliminary data and supporting evidences: Terahertz Spectrometry of (+) and (-) Limonene

Terahertz Spectroscopy (TS) was used to determine if the interactions are sensitive to discern between different chirality of known chiral molecules. In particular, terahertz measurements were conducted on (R)-(+)-LIMONENE and (S)-(-)-LIMONENE (Aldrich). A transmission-mode terahertz spectrometer (TeraSpectra, Applied Research & Photonics, Harrisburg, PA) was used to characterize the specimens. Fig. 1 shows the spectrometer and the experimental arrangement.

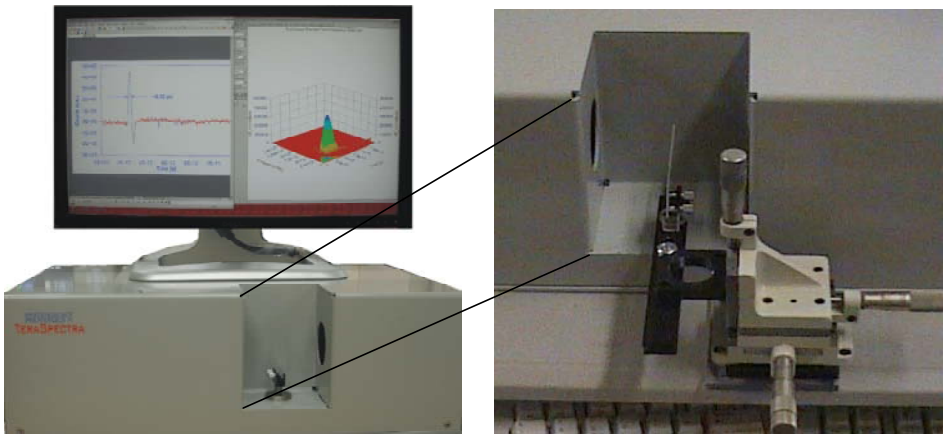

Fig. 1: The terahertz spectrometer (TeraSpectra, left) and sample on a glass slide mounted on a XYZ-stage to place in the beam path (right). Samples in solid, liquid and gaseous forms may be measured.

4.1. Sample preparation:

Chemicals were used as received. 20 µL of each compound was dispensed on a glass slide. The samples were spread evenly to make a uniform coating on respective glass slides. The slides were mounted on a XYZ stage (Fig. 1) one at a

time and their spectra were acquired.

4.2. Results and discussion

Fig. 2 shows the time domain temporal signal (an interferogram or terahertz pulse) of both samples. Fig. 3 shows the Fourier transform frequency spectra of both samples obtained from the time domain signal of Figure 2. As can be identified from Figure 3, at several frequencies the signal shows opposing peaks in the two samples. This initial result indicates that there is differential polarized terahertz absorption as a function of handedness.

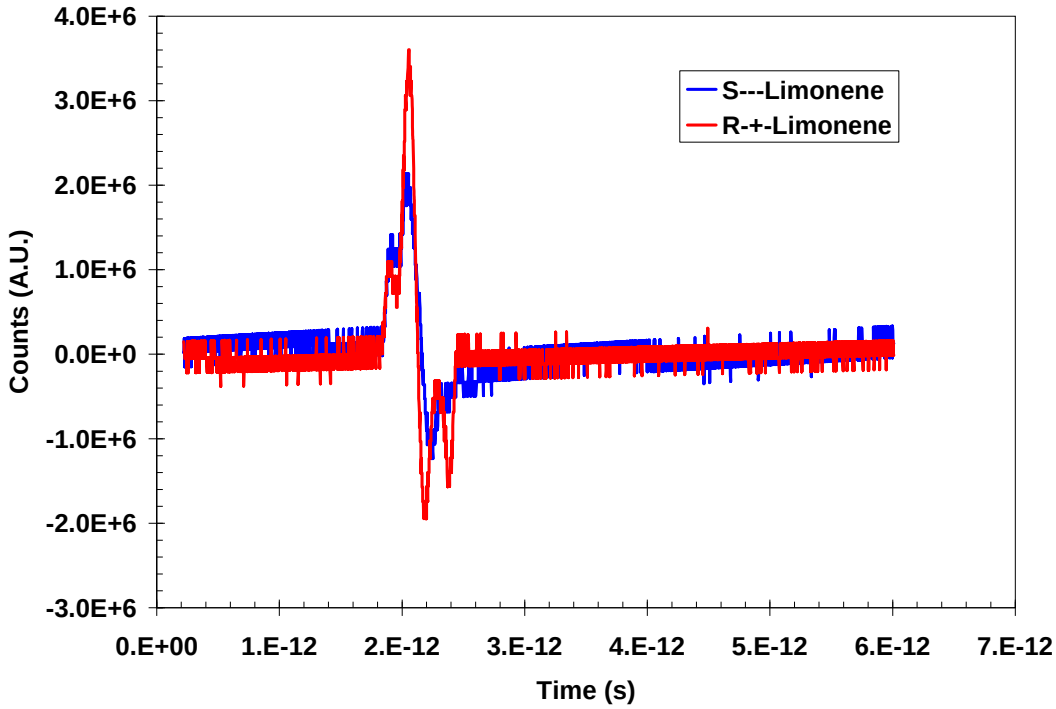

Fig. 2: Time-domain temporal signal (terahertz signal) obtained from(R)-(+)-LIMONENE and (S)-(–)-LIMONENE.

5. Commercialization and business potential

The development of commercial chiral terahertz instrumentation would be of high demand to industry and academia. Several problems with current technology leave significant gaps for a universal, straight forward and rapid method for chiral analyses (*vide supra)*. Polarized terahertz spectroscopy may have significant advantages to current optical techniques in several basic areas such as sample preparation and in significant areas where chiral molecules fail to give rise to spectroscopic differentiation using other methods.

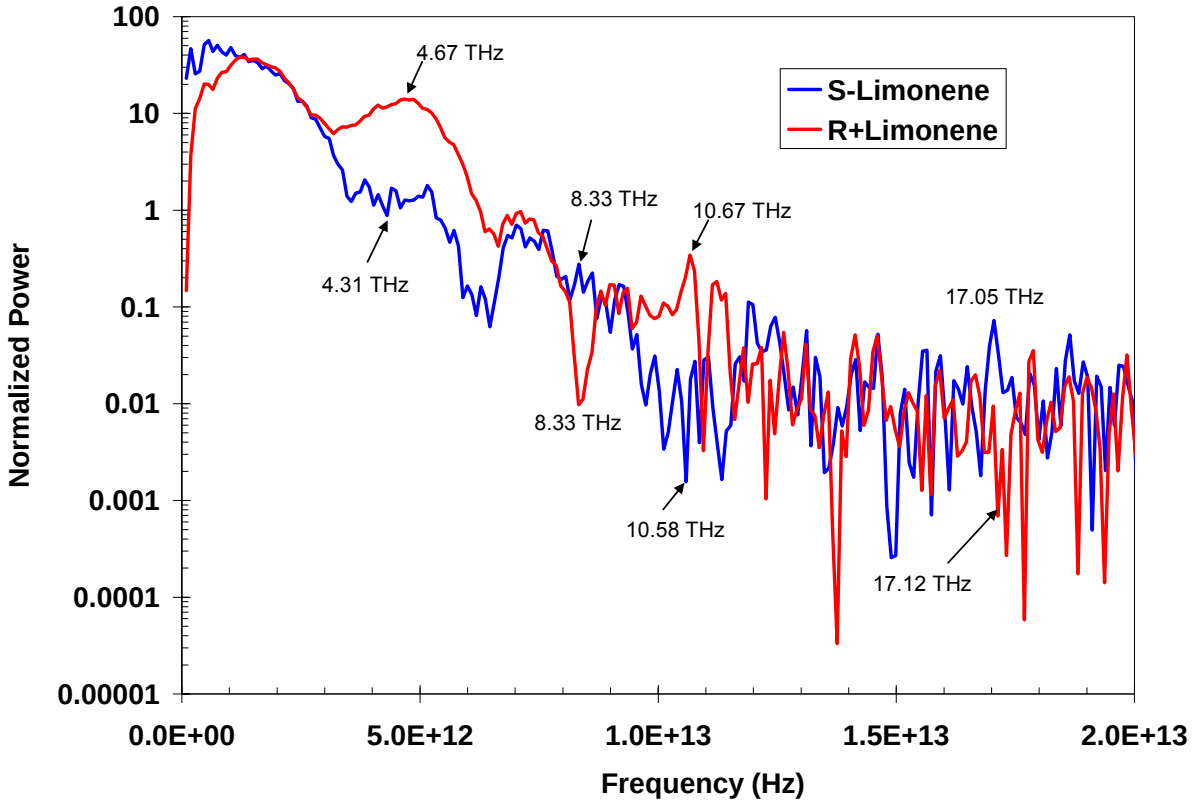

Fig. 3: Fourier transform (Lomb periodogram) frequency spectra of both specimen exhibits opposite peaks at several frequencies.

6. **Project Team, Capabilities, Facilities**

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California State University Long Beach

PROFESSIONAL PREPARATION:
S.U.N.Y. Onondaga Community College, Math and Science, A.S., highest honors, 1995
S.U.N.Y. College of Env. Sci. & Forestry, Environmental Chemistry, B.S., *magna cum laude*, 1998
The University of Chicago, Organic Chemistry, M.S., 2000
The University of Chicago, Organic Chemistry, Ph.D., 2005
The Scripps Research Institute, The Skaggs Institute for Chemical Biology, Supramolecular recognition, Post-doc, 2005-2007

APPOINTMENTS:

2007 – Present: Assistant Professor. Department of Chemistry and Biochemistry, California State University, Long Beach.

RECENT RELEVANT PUBLICATIONS:

1) Schramm, M. P. Restorp P., Zelder, F., Rebek J., Jr. (2008). Influence of Remote Asymmetric Centers in Reversible Encapsulation Complexes. J. Am. Chem. Soc. 130, 2450-2451. DOI: 10.1021/ja076162
2) Schramm, M. P., Rebek, J., Jr. (2008). Effects of Remote Chiral Centers on Encapsulated Molecules New. J. Chem. 32, 794-796.
3) Schramm, M. P., Hooley, R. J., Rebek, J., Jr. (2007) Guest Recognition with Micelle Bound Cavitands. J. Am. Chem. Soc. 129, 9773-9779. DOI: 10.1021/ja0723378
4) "Compounds with All-Carbon Functions: 1,3-dienes, Synthesis by Elimination Reactions" Schramm, M. P., (2009). Science of Synthesis, Houben-Weyl Methods of Molecular Transformation, Georg Thieme Verlag KG, 46.9.

EQUIPMENT, FACILITIES, STAFF:

1) Jasco Circular Dichrometer J-710
2) Jasco Polarimeter P-1010
3) 400 MHz Bruker SpectroSpin Magnet with Techmag Gradient Apollo Console with QNP and broadband Gradient Probes
4) 300 MHz Varian Unity Magnet with Dual Channel Probe
5) 1000 sq. ft. Modern Synthetic Organic Laboratory (Build in 2005)
6) One Undergraduate Researcher to aid in synthesis and experiment design

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PROFESSIONAL PREPARATION

Dr. Rahman is the founder and chief technology officer of ARP. ARP is one of the leading manufacturers of terahertz spectrometer based on its proprietary terahertz source fabricated from electro-optic dendrimer. ARP's TeraSpectra enjoys superior performance over other technologies in terms of wider terahertz range (~30 THz), higher source power (~5 mW, CW) and significantly higher sensitivity (~100 femto-molar).

Anis Rahman received MS (physics) and Ph.D. (EECE) from Marquette University and conducted postdoctoral research in (Chemistry/ChemE) at Columbia University under the mentorship of Nicholas J Turro.

RECENT RELEVANT PUBLICATIONS:

See http://arphotonics.net/technicalnotes.htm

Also refs. 14 through 17 below.
Awarded and pending patents.

EQUIPMENT:

ARP is manufacturer of several products around its terahertz technology. TeraSpectra is described above. In addition, TeraScan from ARP is a depth profiler of biological and non-metallic substrates via non-invasive method.

7. References

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