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Collective mode frequency shifts in L-serine and a series of isotopologues in the terahertz regime

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ABSTRACT

Terahertz (THz) time-domain spectroscopy was used to monitor collective mode shifts in L-serine, L-serine-2,3,3-d_3, L-serine-d_4, and L-serine-d_7 at both room and liquid nitrogen (LN_2) temperatures. Increasing the molecular mass by deuteration caused an expected absorbance red-shift; however, the magnitude of the displacement could not be predicted using normal mode analysis. Both modes at 67.8 cm^{-1} and 91.4 cm^{-1} demonstrated a greater peak shift upon deuterium substitution at non-hydrogen bonding sites than at sites that participated in hydrogen bonding. This is evident in the larger peak shifts observed in L-serine-d_3 than in L-serine-d_4, despite a smaller increase in mass. This leads to the conclusion that both peaks present in the room temperature spectra of L-serine likely arise primarily from other intermolecular interactions with <50% contribution from hydrogen bonding. This goes against the prediction that peaks in the THz spectra of amino acids are predominantly due to the hydrogen bonding network that makes up the crystal lattice.

1. Introduction

THz spectroscopy has seen an increase in research over the last few decades, due in part to more accessible instrumentation. In the past, liquid nitrogen-cooled detectors, frequency mixed sources, and complicated optical setups were required to access the THz regime. In the case of time-domain instruments the introduction of photoconductive antenna sources/detectors allowed the first commercial instrumentation to be developed, providing more access to researchers. This is of particular importance, because it allows more research time to be devoted to applications of THz spectroscopy, in lieu of instrumentation advancement.

THz radiation lies between microwaves and infrared energy in the electromagnetic spectrum and as such shares characteristics with both neighboring regions. Much like microwave spectroscopy, gaseous samples absorb THz frequencies that are resonant with rotational transitions. THz absorbance by solid samples is due to vibrational transitions, as with infrared spectroscopy; however, instead of intramolecular interactions, THz radiation excites intermolecular bonds. Thus, the network of bonds that compose a crystal lattice is uniquely sampled with THz radiation.

Currently, there are many interesting application areas for THz research. Pharmaceutical applications include polymorphic disambiguation [1], which takes advantage of the intermolecular interactions to distinguish among different crystal structures of a given drug, or tablet surface (coating) analysis [2]. Since the THz radiation is nondestructive, homeland security is interested in THz research because it is an efficient, safe way to perform full body scanning [3]. In addition, there is the possibility that THz could be used for standoff detection of drugs or explosives [4–6]. Biological applications are centered on tissue imaging and DNA or protein studies.

There have been many published articles that focus on THz spectra obtained from DNA, proteins, and their building blocks, amino and nucleic acids [7–16]. In the case of amino acids, it has been shown that computer modeling of a single amino acid is internally consistent with itself and similar to experimental data [17,18]. However, once two, or greater, amino acids are chained together, the theoretical spectra start to disagree, calling into question the initial model used for lone molecules [19,20]. It is clear that there is still a lack of understanding about THz absorbance phenomena.

Previous work postulated that increasing the molecular mass of an analyte via deuterium substitution led to calculable red shifts in THz absorbance peaks proportional to the square root of the reduced mass of the molecule [13]. Additional work demonstrated that not only does this trend not always hold true, but that each collective mode shifts independently based on the particular groups of heavy atoms and the intermolecular forces which they do

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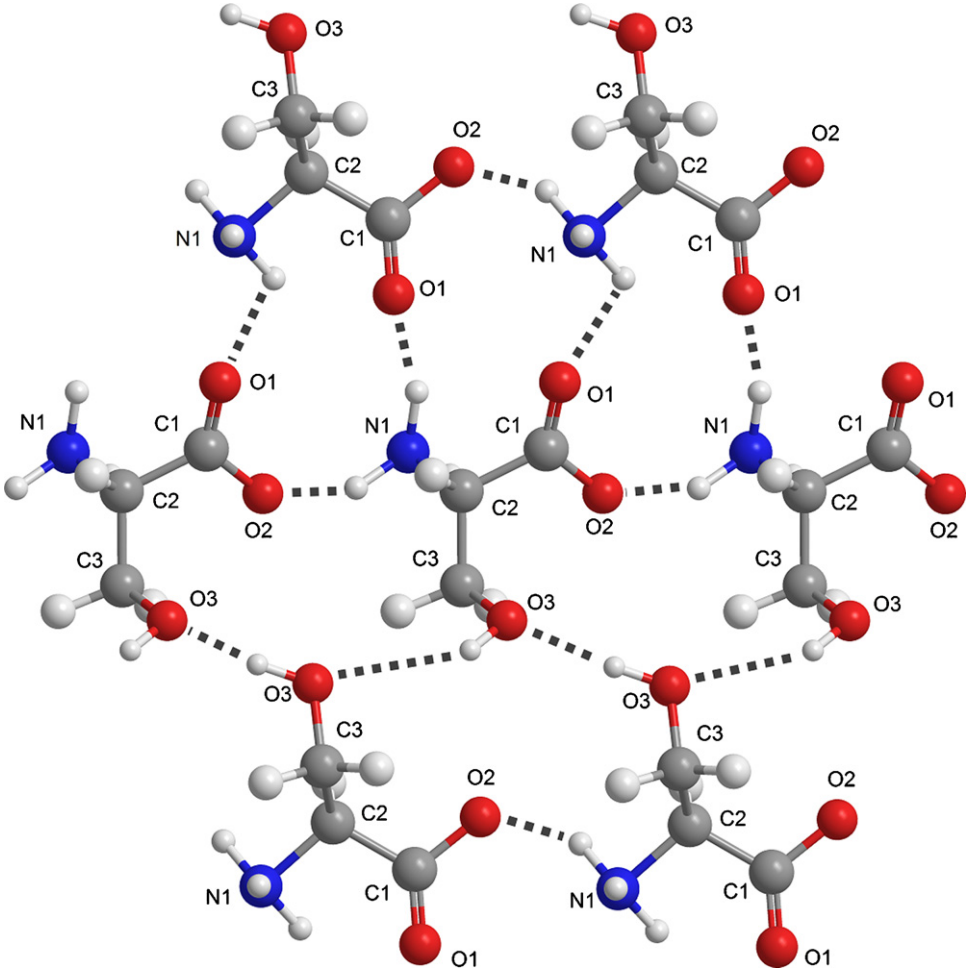

Fig. 1. L-Serine (I) crystal structure 1 along the *a* axis, reproduced from Parsons et al. [25]. The pellets used in this study were formed at 134 MPa, which is significantly less than the 4.8 GPa that is required to make the transition to L-serine (II). Not seen in this picture are the hydrogen bonds from the third hydrogen on the nitrogen atom and the hydrogen on the hydroxyl group of the amino acid side chain.

(or do not) participate [9]. Deuteration of anything less than all of the hydrogens in L-alanine led to a peak shift that differed from the theoretical shift. Intermediate deuterations caused either a greater or lesser shift in peak position compared to theoretical. Given the position of each hydrogen in the unit cell and its role in hydrogen bonding, this allowed the hydrogen bond character of each peak to be assessed. This relationship agrees with work done by Jepsen et al. on polycrystalline saccharides, where it was found that the observed vibrational modes had contributions due to both van der Waals forces and hydrogen bonding [21]. This could account for the difference in peak shifts observed in the deuterated L-alanine study.

Thus, observation of which absorbing mode(s) red shift in the THz spectra can be correlated to the role and location of heavy atoms in the crystal structure to make THz spectral assignments. This research attempts to look into this further with THz analysis of L-serine. L-Serine differs from L-alanine by the addition of a single –OH group on its side chain, which greatly changes the crystal structure (Fig. 1), as is evident in the THz spectra. This study expands on previous work by examining the effect of temperature on the THz spectra of L-serine and three of its isotopologues.

2. Materials and methods

Crystalline L-serine was purchased from Sigma Aldrich at a purity of ≥99%. L-Serine-2,3,3-d_3 and L-serine-d_7 were purchased from CDN isotopes at 98% deuterium purity. L-Serine-d_4 was synthesized by mixing 200 mg of L-serine with 20 mL of deuterated water (D_2O), which allows the 4 exchangeable amino and hydroxyl hydrogens on serine to be deuterated. Exchange was confirmed by direct infusion mass spectrometry on a Waters Acquity UPLC–MS (Fig. 2).

Sample preparation procedures described in Heuser and Taulbee were followed [22,23]. Briefly, samples were prepared by combining 15 mg of serine with 345 mg of polyethylene (PE) powder. In order to minimize scattering, the sample particle size was reduced to ≤100 μm, corresponding to the shortest wavelength in the usable bandwidth of the spectroscopy system. Using a simple mortar and pestle, the size of most serine crystals was reduced to <20 μm [24]. The pellets were pressed at a pressure of 2 tons, have a thickness of approximately 3 mm, and diameter of 13 mm. Six pellets were prepared for each compound. All pellets were stored in a dessicator to minimize water absorption, which could adversely affect the liquid nitrogen (LN_2) spectra.

Spectra were obtained using a Teraview TPS Spectra 2000 THz time-domain spectrometer in transmission mode. 18,000 time-domain sweeps were averaged for each sample spectrum, providing a very high signal-to-noise ratio. Each time-domain sweep is acquired at 30 Hz, resulting in an acquisition time of 10 min for 18,000 scans. All spectra were collected while purging the sample chamber with nitrogen gas at a flow rate of 10 L/min. The spectra were acquired at a resolution of approximately 1 cm^{-1}

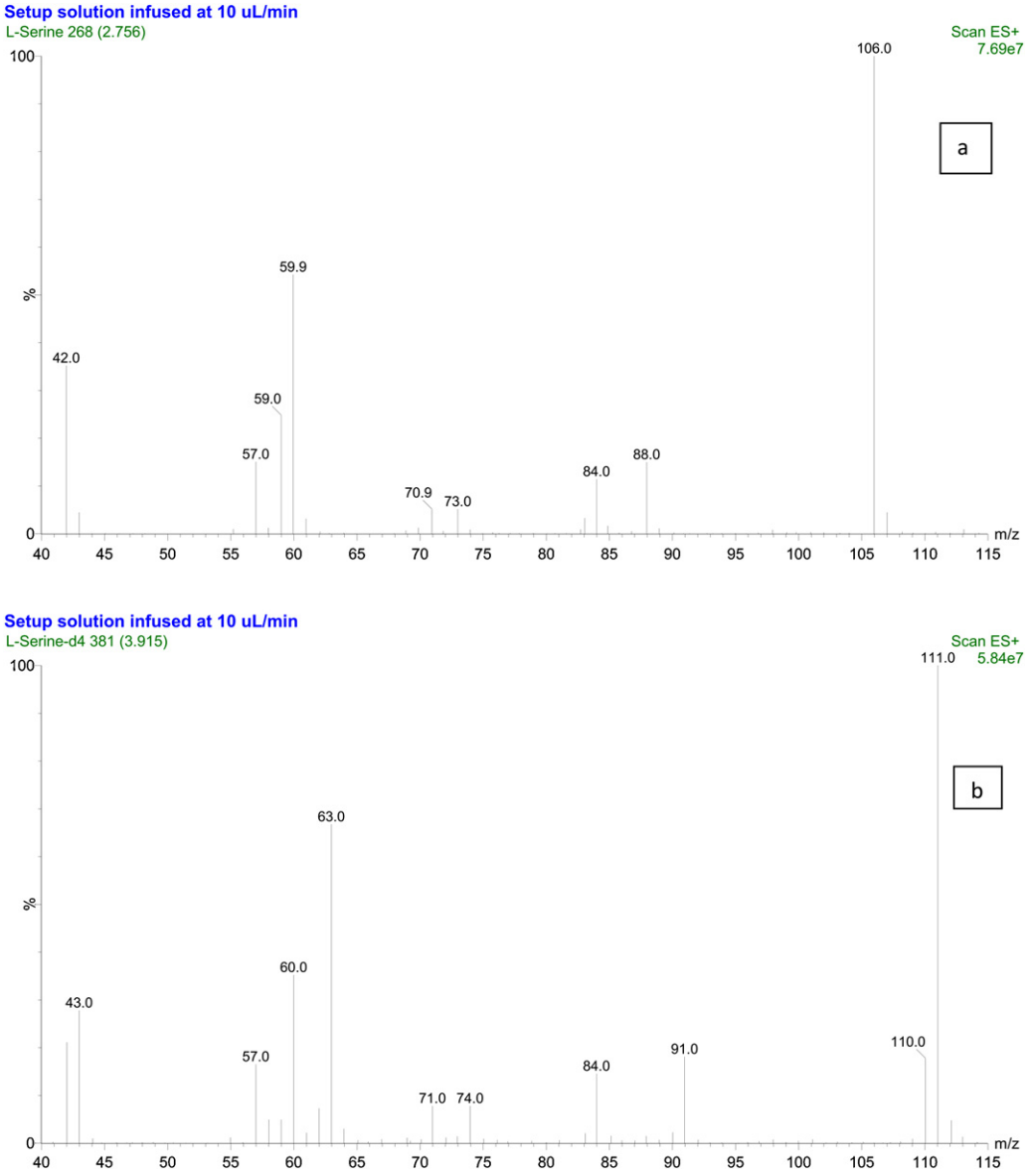

Fig. 2. Mass traces for L-serine and the deuterium exchange of L-serine in D_2O as obtained by direct infusion mass spectrometry. (a) L-Serine dissolved in H_2O – the molecular ion at 106 amu which corresponds to the MW + 1 is observed. (b) L-Serine-d_4 in D_2O – the expected mass of this molecule is 109. The peak at 1 1 1 corresponds to the MW + 2, since the molecule will take on a deuteron instead of a proton.

or 30 GHz. Rather than performing a step scan along the entire delay line, data were collected using rapid scan, which obtains the THz pulse by sampling at fixed time intervals while the delay line oscillates. The absorbance data were extracted from the THz pulse by applying a Fast Fourier Transform and dividing by the reference (360 mg PE pellet) spectrum.

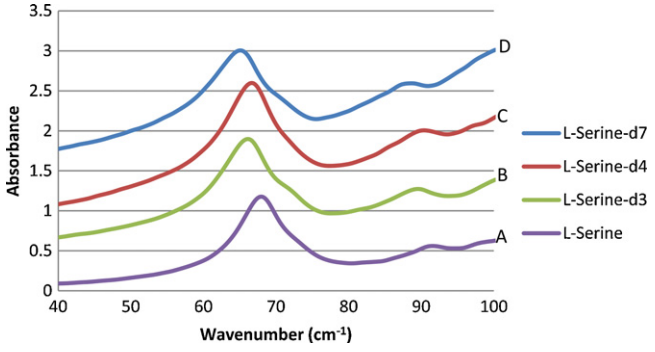

Fig. 3. Room temperature spectra of all L-serine samples (a) L-serine, (b) L-serine-2,3,3-d_3, (c) L-serine-d_4, (d) L-serine-d_7; absorbance offset for clarity.

Spectra were obtained at both room temperature (293 K) and LN_2 temperature (77 K). Room temperature spectra were obtained under normal operating conditions. In order to obtain LN_2 spectra, it was necessary to construct a container capable of holding LN_2 and the sample, while still allowing the THz radiation to pass through. A simple stainless steel container capable of holding ≈150 mL of LN_2 was designed and used. The pellet rests in a sample compartment (cylinder) that traverses the middle of the box and is penetrated by the THz beam at normal incidence.

3. Results and discussion

In order to ensure the pellet preparation process was reproducible, two sets of three L-serine pellets were prepared on different days. It was found using Student's *t*-test that there were no significant differences in the peak maxima of the sets of pellets made on different days (*t*-calc = 2.23, *t*-crit = 2.78). In addition, one

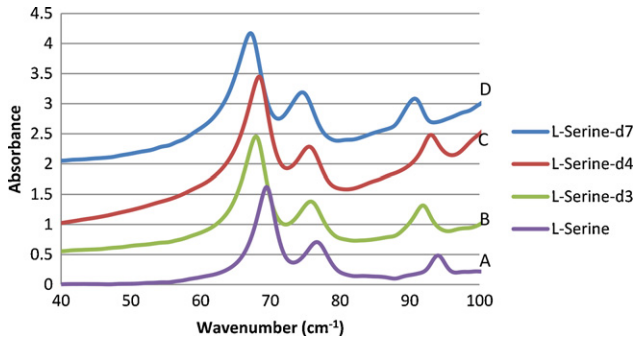

Fig. 4. LN_2 temperature spectra of all L-serine samples (a) L-serine, (b) L-serine-2,3,3-d_3, (c) L-serine-d_4, (d) L-serine-d_7; absorbance offset for clarity.

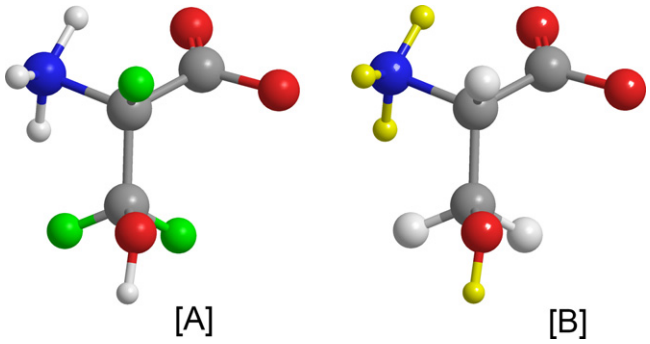

Fig. 5. 3-D representation of L-serine-d_3 and L-serine-d_4. (A) L-Serine-d_3 with deuterated positions colored green. (B) L-Serine-d_4 with deuterated positions shown in yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Room temperature (≈293 K) absorbance maximum ν (cm^{-1}) for the two collective modes present in the L-serine and L-serine deuterated species. As expected, peak red-shifting occurs as the mass is increased. Each peak is statistically different from the next closest peak, as determined by Student's t-test at the 95% confidence level.

RT	L-Ser	L-Ser-d_3	L-Ser-d_4	L-Ser-d_7
Peak 1	67.8 ± 0.1	66.0 ± 0.1	66.8 ± 0.1	65.2 ± 0.1
Peak 2	91.4 ± 0.1	89.4 ± 0.1	90.1 ± 0.1	89.0 ± 0.3

Table 2
LN_2 temperature (77 K), absorbance maximum ν (cm^{-1}) for the three collective modes present in the L-serine species studied. Values for peaks 1 and 3, which correspond to 1 and 2 in the room temperature spectra, all experienced significant blue shifting due to the decreased temperature. Peak 2, which is only present as a shoulder at room temperature, is resolved from peak 1. All peaks were determined to be significantly different using Student's t-test at the 95% confidence level.

LN2	L-Ser	L-Ser-d_3	L-Ser-d_4	L-Ser-d_7
Peak 1	69.4 ± 0.1	67.9 ± 0.0	68.3 ± 0.0	67.2 ± 0.1
Peak 2	76.5 ± 0.1	75.8 ± 0.1	75.6 ± 0.1	74.5 ± 0.1
Peak 3	94.0 ± 0.0	91.8 ± 0.1	92.9 ± 0.1	90.7 ± 0.1

set of pellets was examined on two different days, to ensure that signal fluctuation would not affect the spectra. As before, Student's t-test was used to show that there was no significant difference in the peak maxima of the two sets of samples (t-calc = 0.57, t-crit = 2.23).

Fig. 3 shows the two observable collective mode vibrations of crystalline L-serine at room temperature in the THz regime with peak maxima at approximately 68 and 91.5 cm^{-1}. There is also a third, unresolved peak at approximately 72 cm^{-1} that appears as a shoulder of the peak at 68 cm^{-1}. At LN_2 temperatures, broadening is alleviated and this shoulder is almost baseline resolved from the peak at 68 cm^{-1}. Fig. 4 shows the three peaks in the LN_2 temperature spectra of L-serine at approximately 69.5, 76.5, and 94 cm^{-1}. Compiled peak data for room and LN_2 temperature spectra can be seen in Tables 1 and 2, respectively. It should be noted that transition from room to LN_2 temperature and back again is reversible, but it is not without complications. There is a high probability that small, invisible cracks that may traverse the entire diameter of the pellet will be introduced if the sample is not carefully removed from the LN_2 container. If the sample is re-examined and the beam should happen to strike one of these cracks at an off angle, false absorbance peaks will appear. This is not an issue during initial cooling, but care should be taken when reanalyzing any sample after significant cooling has taken place.

Spectra of the 4 L-serine species exhibited absorbance peaks at positions seen in Figs. 3 and 4 for room and LN_2 temperatures, respectively. When comparing absorbance spectra of L-serine in this work to previously published figures, peak positions are noticeably shifted, differing by 1–3 wavenumbers [17,18]. As this method has been statistically verified to produce consistent spectral results from several replicates and several days of analyses, these peak discrepancies in previous works could be attributable to sample preparation, environmental, or instrumental differences. The THz spectra of the three deuterated samples have not been previously published, but the trend of red-shifting with deuterium substitution has been reported in the literature for other amino acids. As mentioned above, it was previously hypothesized that mass-induced peak shifts could be proportional to the square root of the reduced mass for L-alanine [13]. However, it was determined that this hypothesis was false [9]. Based off the observed and calculated results with L-serine, it would appear that the simple model is not able to accurately predict any of the mode shifts. Tables 3 and 4 show the experimental and theoretical results for the collective mode shift at room and LN_2 temperature, respectively. Student's t-test was used to determine if the observed and theoretical peaks differed. Aside from peak 1 in L-serine-d_7 at LN_2 temperatures, each observed peak was statistically different from the predicted peak based off of the normal mode calculations.

This study offers unique experimental results that directly relate mass induced peak shifts to hydrogen/non-hydrogen bonding atoms. The two intermediate isotopologues studied, L-serine-d_3 and L-serine-d_4, represent deuteration of all hydrogens that are either uninvolved or involved in hydrogen bonding, respectively (Fig. 5). This allows the peak shifts based off the hydrogen bonding network to be examined and assists with correlating specific peaks to a type of atom in the molecule.

The two observable peaks in the room temperature spectra both exhibit larger shifts for L-serine-d_3 than L-serine-d_4. This indicates that the non-hydrogen bonding hydrogens play a greater role

Table 3
Observed values compared to theoretical calculations based off the reduced mass of the L-serine molecule being studied. Note that all observed peaks are statistically different from their corresponding theoretical peak as determined by Student's t-test at the 95% confidence level.

RT	L-Ser-d_3		L-Ser-d_4		L-Ser-d_7	
	Theoretical	Observed	Theoretical	Observed	Theoretical	Observed
Peak 1	66.0 ± 0.1	66.9	66.8 ± 0.1	66.5	65.2 ± 0.1	65.6
Peak 2	89.4 ± 0.1	90.1	90.1 ± 0.1	89.7	89.0 ± 0.3	88.5

Table 4
Observed and theoretical peak values for the deuterated L-serine molecules at 77 K. Other than peak 1 for L-serine-d_7 all observed peaks are statistically different than their theoretical predictions.

LN2	L-Ser-d_3		L-Ser-d_4		L-Ser-d_7	
	Theoretical	Observed	Theoretical	Observed	Theoretical	Observed
Peak 1	67.9 ± 0.0	68.4	68.3 ± 0.0	68.1	67.2 ± 0.1	67.2
Peak 2	75.8 ± 0.1	75.4	75.6 ± 0.1	75.1	74.5 ± 0.1	74.1
Peak 3	91.8 ± 0.1	92.7	92.9 ± 0.1	92.3	90.7 ± 0.1	91

in these two collective modes, leading to peak shifts of 1.8 and 2 cm^{-1} for peaks 1 and 2, respectively. These shifts are over 50% greater than what occurs due to the deuteration of the hydrogen bonding hydrogens in L-serine-d_4 (1.0 and 1.3 cm^{-1} for peaks 1 and 2).

Peaks 1 and 3 in the LN_2 temperature spectra of L-serine-d_3 and L-serine-d_4 follow the same trend; substituting the non-hydrogen atoms leads to a larger peak shift. However, the difference between peak 1 for the two intermediate compounds is smaller at 77 K (0.4 cm^{-1} vs. 0.8 cm^{-1}), which suggests that part of what accounts for this difference is minimized at lower temperatures. Peak 2, the shoulder that is resolved at lower temperatures, appears to have equal representation by all of the hydrogens present. Peak 2 is shifted 0.7 and 0.9 cm^{-1} by L-serine-d_3 and L-serine-d_4, respectively.

4. Conclusions

Terahertz spectra were recorded from 5 to 100 cm^{-1} for a series of L-serine compounds of increasing mass. The collective mode vibration shifts due to this increase in mass could be correlated, in part, to individual atoms in the molecules that make up the crystal lattice. It is interesting to note that both of the room temperature absorbance peaks in the L-serine THz spectra seem to have more of a contribution from the three hydrogens that are not involved in the H-bonding network. This method of analysis is possible due to the accuracy with which the peak assignments can be made with carefully prepared samples in moderately stable environmental conditions. Large biological molecules, like proteins and DNA, are still probably too complex to understand with THz radiation; however, relatively short peptides and small DNA strands may not be too complex, especially when both a theoretical and experimental approach are considered.

Acknowledgements

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