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High Frequency Characterization of Amino Acids for Synthesis of Proteins

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Abstract—*Amino acids and proteins are the important biomolecules present in all living species. They are also essential constituents of food and medicines. To understand the synthesis and functioning of proteins, characterization of amino acids is critical. There are various techniques for characterization of amino acids, and microwave-based analysis is one of them. Various studies have shown that microwave-based analysis is useful in characterization of various biomolecules in different frequency ranges. However, current literature lacks the characterization of these biomolecules at super high frequency ranges (above 20 GHz). In this study, we have reported the microwave-based analysis of three amino acids which are L-Glutamic acid, L-Tryptophan acid, and O-Benzyl-L-serine and their combinations with each other in the frequency range from 18 GHz to 140 GHz. The dipole moment and polarization were the key parameters for the characterization of the amino acids. These parameters were estimated by calculating the EM power intensity. Our results show that the dipole moment of these amino acids lie between 0.01-0.1 Debye and the dipole moment of the combination of any two amino acids is less than the pure amino acids, which shows the collective power intensity of the samples decreases. This study can be useful in understanding the physical and chemical properties of amino acids and can be helpful in the synthesis of proteins.*

INTRODUCTION

It is a well-known fact that Amino acids are the basic building blocks of proteins. These are organic molecules consisting of basic amino group (-NH_2), and acidic carboxylic group (-COOH). Each amino acid has its set of side chains that differ from one another. They also play an important role in the growth and health of all living organisms [1], [2]. Furthermore, amino acids are important in pharmaceutical and cosmetic industries due to their good chelating properties [3]. Proteins are the chains of amino acids that assemble peptide linkage, different groups of Amino acids determine the uniqueness of each protein [4]. They are the most versatile molecular entities present in all living system and play a pivotal role in all biological processes like, providing structural and functional support to cells, encoding RNA and DNA, catalyzing chemical reactions, and transporting metabolites [5],[6]. The use of microwaves in spectroscopy essentially became possible during World War II, due to the development of microwave technology for RADAR. Microwave spectroscopy is the study of the absorption and emission of electromagnetic waves of frequency range 300 MHz to 300 GHz by any sample [7]. Emission takes place when a molecule loses energy in form of radiation and absorption is the reverse process [8]. Microwave spectroscopy is possible only for the molecules which possess dipole moment [7]. It is useful in finding the bond length, dipole moment, and in the study of weakly bound complexes [9]. Studies have shown various biological effects of microwaves and their applications in medical field such as power absorption in human body, interaction with the proteins, interaction with nervous system [10], and their molecular effects. Moreover, absorption and emission of electromagnetic waves by any sample depends upon the dielectric properties of that sample [11]. This article reports microwave-based characterization of L-Glutamic acid, L-Tryptophan acid, and O-Benzyl-L-serine. The dipole moment and polarization were the key parameters for the characterization of the amino acids, which were estimated by calculating the absorbed EM power intensity. These studies can help understand the physical and chemical properties of amino acids and the underlying electrical interactions in the synthesis of proteins.

MATERIALS AND METHODS

Preparation of amino acid samples

For high frequency characterization, three amino acids, L-Glutamic acid (LG), L-Tryptophan (LT), O-Benzyl-L-serine (OB) were purchased from Sigma Aldrich. These amino acids were separately dissolved in 0.5 M HCl at concentration of 25 mg/ml. Samples of combination of amino acids, Tryptophan + Glutamic acid (TG), Tryptophan + Benzyl (TB), Benzyl + Glutamic acid (BG) and combination of all three (TBG) were prepared by equal volume of the individual amino acid.

Experimental Set-up

Since the frequency range chosen for the experiment was from 18 GHz to 140 GHz, a single module does not support this wide range, rather different microwave multipliers and mixers corresponding to the suitable operation range were used. To achieve this, the frequency range was divided into three parts, 18 GHz to 44 GHz, 60 GHz to 90 GHz and, 90 GHz to 140

GHz. It is to be noted that due to the instrumentation limit frequency range from 44GHz to 60 GHz could not be covered. The experimental set-ups for the above-mentioned frequency ranges are briefly discussed below:

- For 18 GHz to 44 GHz, a 3D printed sample holder as shown in Figure-1 was used, two launchers were used to transmit and receive the signal in which one was connected to signal generator (Keysight PSGE8267D Vector Signal Generator) with the help of 1m long low loss coaxial cable to generate the signal of frequency range 500 MHz to 44GHz. Another launcher was connected to signal analyzer (Keysight N9030B PXA Signal Analyzer) with the help of low-loss coaxial cables. The complete process of frequency sweeping, and data acquisition was automated through a Raspberry Pi based automation framework.

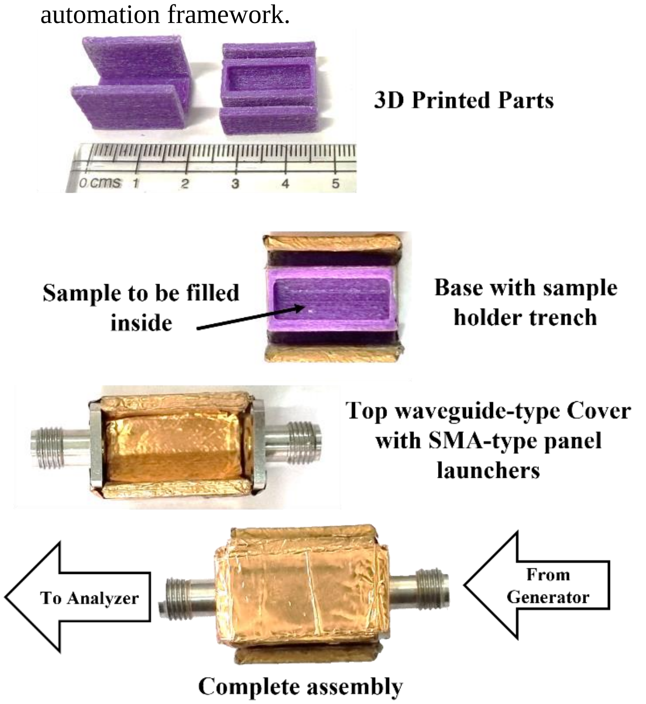

Figure 1 3D printed waveguide-cum sample holder design for experiments in frequency range 18 GHz to 44 GHz.

- In the second part of the experiment frequency range was covered from 60 GHz to 90 GHz. The setup is shown in Figure 2. A radio frequency multiplier (VDI WR-12.0, 60-90 GHz) was connected with a signal generator to generate the signal of frequency 60 to 90 GHz. Horn antenna (OML WR-12) was connected to multiplier. To receive the signal, another horn antenna was aligned in front of the first one as shown in Figure-1. Polypropylene Microtube (Tarsons) containing solution was placed between the two horn antennas. To analyse the signal, Signal analyzer was connected to the second Horn Antenna. For high frequency signals, RF mixer (Wave Guide Harmonic mixer 55 - 90 GHz) was connected between the second horn antenna and signal analyzer to demodulate the signal without changing its characteristics.
- In the third part of experiment frequency range was covered from 90 to 140 GHz. in this part RF mixer was replaced with WR-8 OML Harmonic mixer from KEYSIGHT. Otherwise, whole apparatus was same as discussed above. For the scanning of samples, power in the signal generator was fixed at 10 dBm. The distance between the horn antennas was fixed at 8.2 inches (for 60 to 90 GHz) and 3.1 inches for (90 to 140 GHz). A microtube filled with solution was placed near the receiver antenna.

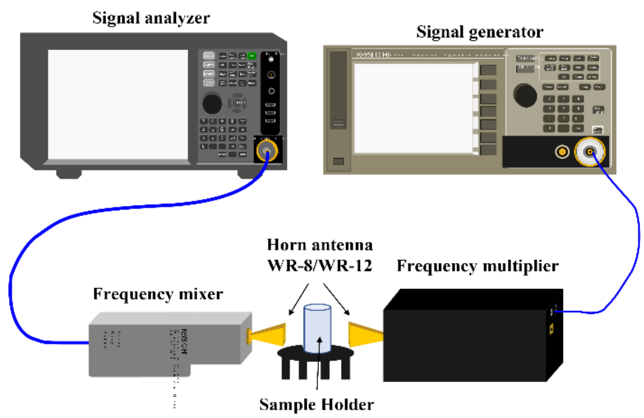

Figure 2: Experimental setup for microwaves scanning of samples

MEASUREMENTS OF ELECTRIC FIELD, POLARIZATION, DIPOLE MOMENT

To understand the dielectric properties of amino acids in the frequency range of 500 MHz to 140 GHz, following equations were taken into consideration.

$$I = \frac{Power\ (Watt)}{Area} = \frac{1}{2}\varepsilon c E^2 \quad (1)$$

$$E = \sqrt{\frac{2I}{\varepsilon c}} \quad (2)$$

$$P = \chi_e \varepsilon E = \sum \frac{p_i}{V} \quad (3)$$

$$\frac{PV}{N} = p \quad (4)$$

Where, I is intensity in (W/m^2), c is speed of light, ε is Permittivity of amino acids, χ is susceptibility of sample, V and N are volume of sample and number of molecules of amino acids present in that volume. Electric field was calculated by using the intensity of the sample from that electric field, polarization (P) was calculated. Using eq. (4), dipole moment (p) was obtained.

RESULTS AND DISCUSSION

From the studies done using above setup, We have observed that there was a power drop which was considered as power loss inside the sample from that power loss using above equations we have calculated other parameters such as Power Intensity(I) electric field (E), Polarization (P), and Dipole moment (D) which are given in Table-1. From the above results few observations were made, Polarization of Amino acids and their mixtures at high frequencies was between 0-1 C/m. Values of Dipole moment for amino acids lie between 0.01-0.1UNIT which shows that Dipole moment decreases at high frequencies. On comparing the Dipole moment and Polarization of pure Amino acids with their mixtures it was observed that Dipole moment and Polarization for combination of amino acids was decreased in comparison to their pure form.

Table 1: Results

Sample Amino Acids	Power Loss dBm	Intensity $\frac{mW}{mm^2}$	E-Field $\frac{V}{m}$	P $\frac{\mu C}{m^2}$	p $Cm \times 10^{-31}$	p $Debye$
LG	6.78	6.58	307.51	7.34	0.72	0.022
LT	6.12	5.94	241.38	12.45	1.71	0.051
OB	6.65	6.46	251.65	12.98	1.68	0.051
TB	5.75	5.58	231.90	12.37	1.65	0.049
TG	6.06	5.88	236.14	13.02	1.31	0.039
BG	5.63	5.46	237.44	11.04	1.23	0.037
TGB	6.08	5.90	238.57	12.73	1.55	0.047

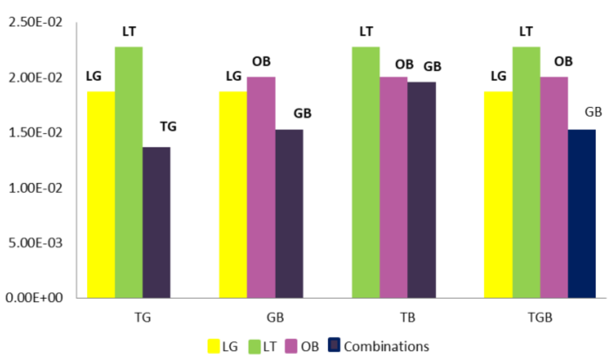

Figure 2: Comparison of dipole moment of pure amino acids with their mixture

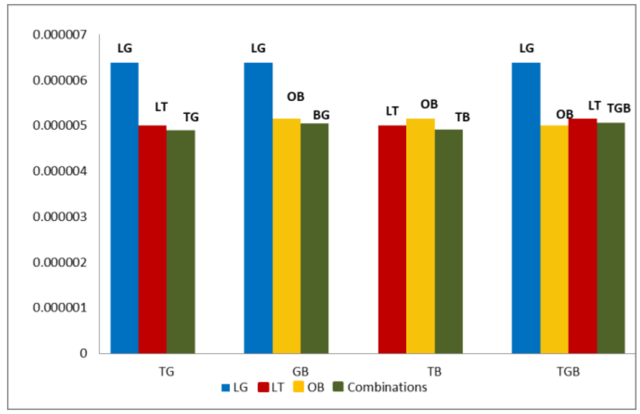

Figure 3: Comparison of polarization of pure amino acids with their mixtures

CONCLUSION

As amino acids are basic building blocks of proteins, and also play an important role in various other fields. Characterization of amino acids is important to study the protein structures and characteristics and also in the artificial synthesis of proteins. Although, amino acids have been well studied in the microwave spectrum, however, the range of frequencies for which they are studies is low. In our study, we have tried to characterize and calculated polarization and dipole moment of the amino acids and their combinations with each other with the help of microwave spectroscopy in the range 18 GHz to 140 GHz. Our results show that the dipole moment decreases for the combination of two samples.

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