

## Experimental and Computational Vibration Study of Amino Acids

Musa E. Mohamed<sup>1,\*</sup>, Abdelhafeez M. A. Mohammed<sup>2</sup>

<sup>1</sup>Department of Chemistry, College of Applied and Industrial Sciences, University of Bahri, Sudan

<sup>2</sup>Department of Chemistry, Faculty of Education, Alzaiem Alazhari University, Sudan

\*E-mail address: [musa.elballa@gmail.com](mailto:musa.elballa@gmail.com)

### ABSTRACT

Vibrational studies of amino acids experimentally and theoretically have been performed. The Semi-empirical methods optimization by PM6 and RM1 on the *l*- and *d*-amino acids (alanine, phenylalanine, aspartic and glutamic acid), showed no difference in energy between *l*- and *d*-isomers. The vibrational frequencies were calculated by semi-empirical methods (PM6 and RM1) and *ab initio* methods (B3LYP/6-31+G(d)) and were scaled down by factors of 0.925 (RM1), 1.09 (PM6) and 0.89 (B3LYP/6-31+G(d)). The calculated and experimental vibrational frequencies have shown good general agreement.

**Keywords:** Vibrational studies, Amino Acids, semi-empirical methods (PM6 and RM1), *ab initio* methods (B3LYP/6-31+G(d)).

### 1. INTRODUCTION

Amino acids are the molecular building blocks of peptides and proteins. The unit structure adopted by amino acids, on condensation into larger molecules, effectively determines their secondary structure in crystalline samples.

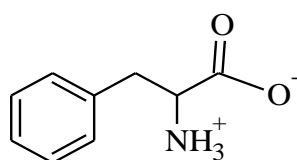
Amino acid molecules have a zwitterionic structure when they are in the liquid or solid phase. Indeed these two cases allow hydrogen bonds to be formed, stabilizing the ionic conformation  $\text{R-CH}(\text{COO}^-)\text{NH}_3^+$ . On the Contrary, the intermolecular bonds are not present in the gas phase and these molecules have a non-zwitterionic structure  $\text{R-CH}(\text{COOH})\text{NH}_2$ . This has been confirmed by experimental studies of the amino acids glycine [1-3] and alanine [4]. There are two long-standing problems encountered in the study of zwitterionic amino acids by infrared (IR) absorption spectroscopy and *ab initio* molecular orbital methods. The first concerns the inability of standard *ab initio* molecular orbital calculations (for isolated molecules) to provide optimized structures for the monomeric zwitterionic forms of the amino acids. A number of molecular orbital studies (self-consistent field (SCF) methods) [5-7] have shown that the zwitterionic amino acids do not exist in the gas phase, as isolated monomers.

This conclusion has been confirmed experimentally by matrix isolation (MI) studies [8-10], a technique in which the amino acids are first vaporized, then trapped and isolated in inert gas matrices at very low temperatures. The MI infrared spectra clearly show that amino acids are present in un-ionized neutral forms after evaporation. For the monomers, the structure optimizations started at the zwitterionic forms, with large basis sets, always converge to the un-ionized neutral molecules. For some smaller basis sets (e.g. 6-31G(d)), convergence to structures with strong intramolecular hydrogen bonding is obtained. These are not expected to be real structures but occur as a consequence of the relatively low theoretical level of the calculations.

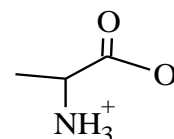
The second problem concerns the measurement of IR absorption spectra of monomers of the amino acids in their zwitterionic forms. In the gas phase the amino acids occur in their un-ionized forms. The zwitterions have to be stabilized by their local environment. This is achieved in polar solutions and in the solid state. Measurement of the IR absorption spectra of the solid (KBr pellet), and solution are not of the monomer but of tiny crystals in the case of KBr pellets, and of strong solute-solvent hydrogen bonding in the case of aqueous solutions.

Thus, with the existing standard sampling techniques IR absorption spectra can only be measured when the zwitterions are strongly perturbed, either by neighbouring zwitterions (crystals in KBr pellets), or in solution (strong H-bonding). Other traditional methods involving evaporation of sample (such as MI, molecular beam and gas phase) to obtain monomeric spectra are essentially inapplicable to studying zwitterionic amino acids because of the structural transformation, from zwitterionic to neutral un-ionized form.

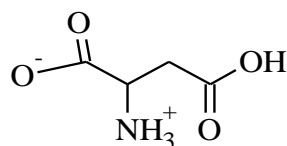
To carry out meaningful calculations on the zwitterionic forms of the amino acids, either solvation effects or specific intermolecular interactions must be included. Calculations including specific intermolecular interactions are usually impracticable, because of the number of molecules (large multimers or clusters) that must be included in the computation. However, the inclusion of a general solvation effect in the calculations can be readily undertaken. Self-consistent reaction field (SCRF) methods [11], which are implemented in Gaussian packages include the solvation effect.



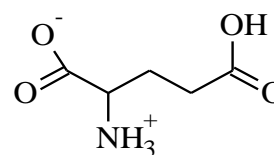
Phenylalanine



Alanine



Aspartic Acid



Glutamic Acid

**Figure 1.** Zwitterionic Structures of Amino Acids.

These methods model the solvent as a continuum of uniform dielectric constant ( $\epsilon$ ), the reaction field, which can interact with the solute molecules and lead to net stabilization. It should be noted that the solvent used in SCRF calculations is restricted to non-aqueous system [11], because as pointed out by Foresman, where intermolecular interactions are relatively large the continuum model is inappropriate, and specific interactions have also to be included. For aqueous solutions this would require the inclusion of a shell of water molecules inside the cavity in addition to the solute [12]. It was established for glycine in aqueous solution, two water molecules are sufficient to stabilize the glycine zwitterions [13].

Therefore, the use of the continuum model in which no specific intermolecular interactions are included in the calculations excludes comparison with the spectra of aqueous solution and crystal. Moreover, the IR and Raman spectra of amino acids in aqueous solution are usually broad, overlapped or incomplete as a result of strong solvent absorption and solute-solvent interaction. Even in the event that appropriately calculated spectra could be obtained, satisfactory and complete comparisons would be difficult to establish.

Motivated by the need to solve the above problems of (i) measuring the absorption spectra of monomers of zwitterions of amino acids and (ii) undertaking appropriate *ab initio* calculations; Jensen, J. H. and Gordon, M. S. have developed and optimized new IR sampling technique, and have carried out non-aqueous SCRF molecular orbital calculations. The IR absorption spectra of glycine [14] and alanine [15] have previously been measured with this technique, and the SCRF calculated spectra for these zwitterions were shown to be in good agreement with the measured spectra.

*L*-alanine is the smallest naturally occurring chiral amino acid. The assignment of its fundamental vibrations is of importance in modeling amino acids and the nature of the mechanism of their inversion.

The vibrational spectra of  $\alpha$ - and  $\beta$ -alanine molecules in both zwitterionic and neutral forms are studied by FT-IR, Raman and MI-IR spectroscopy with the aid of results from theoretical SCF-MO *ab initio* calculations. The spectroscopic data obtained under the various experimental conditions (crystalline phase; low temperature matrix isolated molecules) made it possible to undertake a detailed assignment of the vibration frequencies [16].

The infrared spectra of two conformers of the nonionized alanine have been analyzed and assigned using DFT/B3LYP/aug-cc-pVDZ and MP2/aug-cc-pVDZ for geometry and frequency theoretical calculations. These methods yields vibrational frequencies in excellent agreement with experimental data [17].

The infrared spectra of the alanine molecule also have been studied in solid as well as in aqueous solution [18]. The vibration frequencies for the fundamental modes of alanine in neutral and zwitterionic form have been calculated using AM1, RHF, and DFT method with different basis sets [18]. RHF/6-31G, DFT/6-31G, 6-31+G\* and 6-311++G\*\* calculations for vibrational frequencies of both *l*- and *d*-alanine and zwitterionic alanine (zala) have been performed in both gas phase and in aqueous solution [18].

It was concluded that while there is no significant difference between the corresponding frequencies of *l*- and *d*-alanine in gas phase, the situation is not the same for zala and alanine in water [18]. A solvation model (PCM) for neutral alanine and zala at DFT/6-31+G\* and 6-311++G\*\* level has also been performed. Gas phase and salvation using Polarized Continuum (PCM) model calculations for alanine and zala reveal that neutral alanine is more stable in gas phase while the reverse is true in aqueous medium. A comparison between the experimentally observed IR spectra of alanine in solid and water solution does not show much variation in corresponding frequencies but theoretically some differences are predicted.

The infrared spectra of *l*-aspartic acid, *l*-aspartic-d<sub>4</sub> acid, and *l*-aspartic-<sup>15</sup>N acid as solid samples [19] data was used to propose a general assignment of the fundamental modes in the basis of the isotopic shifts measured.

The infrared spectrum and molecular structure of zwitterionic *l*-β-phenylalanine were studied by means of matrix isolation method and *ab initio* molecular orbital calculation. The self-consistent reaction field calculations at HF/6-(311G(d,p)) level were carried out on zwitterionic phenylalanine present in a continuum of KBr. Good agreement in the terms of both frequencies and intensities was found between the calculated and observed full mid-IR spectra [20].

The Fourier transform infrared spectra of the grown *l*-phenylalanine crystals were recorded in the frequency region 450 cm<sup>-1</sup>-4000 cm<sup>-1</sup> [21], the recorded FTIR spectra was compared with the standard spectra of the functional groups. The presence of all the functional groups occurring in *l*-phenylalanine was confirmed.

The MNDO-scaled harmonic force field of glutamic acid was obtained in the space of a set of non-redundant local-symmetrized internal coordinates [22] by a set of scaling factors transferred from aspartic acid. The theoretical vibration frequencies for the fundamentals were successfully compared with the observed values, and the frequencies of the <sup>15</sup>N and d<sub>4</sub> isotopic derivatives were also calculated and compared with the experiments [22]. These results and the description of the normal modes followed by means of the potential energy distribution, agree with the previous assignments proposed for most of the observed bands, showing an extensive coupling among the bending and skeletal stretching coordinates [22].

The molecular structure of glutamic acid in the non-zwitterionic form has been optimized by using the semiempirical (MNDO, AM1) methods and *ab initio* calculation at the 4-31G level [23]. The results were compared with previous reported data for other amino acids. The *ab initio* optimized structure was used as the starting point for a further force field calculation, and vibration frequencies and theoretical assignments were thus obtained. In order to compare these results with experiments, the FT-IR spectrum of glutamic acid in an argon matrix was recorded and the measured frequencies were successfully compared with theoretical values, which were previously scaled by using a set of scaling factors [23].

According to the reasons mentioned above in the introduction, most molecular orbital calculations on amino acids are performed on the un-ionized species. In this work we calculated the IR spectra of our amino acids in the non-zwitterionic form using semiempirical and *ab initio* force field calculations, and compared the results with experimental spectra.

## 2. METHODS OF CALCULATION

### 2. 1. Semiempirical Methods

The Semi empirical computational methods RM1 and PM6 were used as implemented in MOPAC2007 package, on a personal computer. In this procedure, we first searched for the energy minima on the potential energy surface of the selected amino acids (*l*-alanine, *l*-phenylalanine, *l*-aspartic and *l*-glutamic acid). The molecular structures corresponding to the global minima in the potential energy surface for the non-zwitterionic amino acids were predicted in the four cases starting from a structure with standard parameters, which were optimized simultaneously employing very restricted convergence criteria in the last steps of the computations.

The optimized RM1 and PM6 geometries were used as the reference geometry to calculate the vibrational frequencies of the amino acids (*l*-alanine, *l*-phenylalanine, *l*-aspartic

and *l*-glutamic acid). It is possible to find a local minimum of the potential energy surface of the amino acids, where all the calculated frequencies were positive, thus allowing the comparison with experimental results. A scaling factor of 0.925 for RM1 common to all frequencies is used to adjust the theoretical frequencies before it was compared with experimental [24], and 1.09 factor was used for PM6.

## 2. 2. *Ab initio* Methods

The *ab initio* molecular orbital calculations were carried out employing the Gaussian 03 program. In this procedure we first searched for the energy minima on the potential energy surface of the amino acids corresponding to the lowest energy conformer and then calculated infrared frequencies using harmonic approximations. Initially, geometry was optimized using Hartee-Fock level of theory with 6-31+G(d) basis set (RHF/6-31+G(d)) and less effective methods of Density Functional Theory (DFT) at B3LYP/6-31+G(d) which consider electron correlations with three-parameters hybrid functions combined with the Lee, Yang and Parr-Correlation function [25]. The optimized geometry of amino acids (*l*-alanine, *l*-phenylalanine, *l*-aspartic and *l*-glutamic acid) was used to calculate the vibrational frequencies. The calculated frequencies values were scaled down by a single factor of 0.89 before it were compared with experimental data.

## 3. EXPERIMENTAL

The FTIR spectra of *l*-amino acids (alanine, phenylalanine, aspartic and glutamic acid) were recorded with the Fourier Transform Infrared spectrometer model 8400 S, in the frequency region 400-4000  $\text{cm}^{-1}$ . Samples in the solid states were measured in KBr matrix. Pellets were obtained with a hydraulic press.

## 4. RESULTS AND DISCUSSION

Taking into account the geometries of *l*- and *d*-amino acids (alanine, phenylalanine, aspartic and glutamic acid), no difference in energy minima was observed for the two isomers by semiempirical methods (RM1 and PM6), see Table (1)

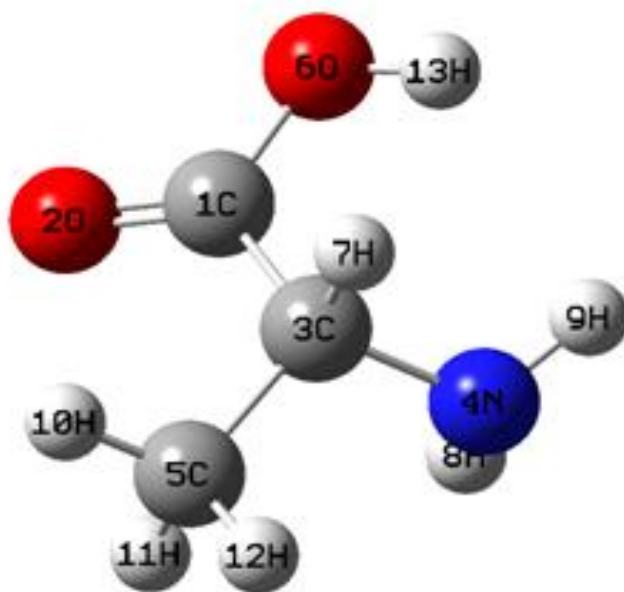
The optimized semiempirical RM1, PM6 and *ab initio* B3LYP/6-31+G(d) geometries were used as starting point to carry force field calculations for non-zwitterionic, *l*-alanine, *l*-phenylalanine, *l*-aspartic and *l*-glutamic acid. To minimize the systematic errors of these calculations, scaling factors of 0.89, 0.925 and 1.09 were applied for *ab initio*, RM1 and PM6 respectively. In Tables 2-5, we have listed the scaled calculated vibrational frequencies of non-zwitterionic, *l*-alanine, *l*-phenylalanine, *l*-aspartic and *l*-glutamic acid respectively. We have also proposed assignments for the observed frequencies.

### 4. 1. Assignment of *l*-alanine

The non-zwitterionic structure of *l*-alanine is shown in Figure 2. The assignment for calculated vibrational frequencies and those observed in the IR spectra are shown in Table 2. The calculated and experimental vibrational frequencies show a good general agreement. The frequencies observed at 3099  $\text{cm}^{-1}$  is assigned to the asymmetric NH stretching mode.

**Table 1.** Relative Stability of Amino Acids ( $\Delta E$ ) at Different Semiempirical Methods RM1 and PM6.

Heat of formation in Kcal/mol		
Amino acids	PM6	RM1
<i>l</i> -alanine	-92.7728	-98.13294
<i>d</i> -alanine	-92.7728	-98.13295
<i>l</i> -phenylalanine	-69.7187	-73.25132
<i>d</i> -phenylalanine	-71.9725	-75.59622
<i>l</i> -aspartic	-184.2430	-189.38191
<i>d</i> -aspartic	-184.2430	-189.38191
<i>l</i> -glutamic	-185.8963	-187.89090
<i>d</i> -glutamic	-185.8963	-187.89090

**Figure 2.** Molecular Structure of *l*-Alanine as Predicted by the *ab initio* Method, with the Atomic Numbering Used in This Work.

The corresponding theoretical values at B3LYP/6-31+G(d), RM1 and the PM6 level are  $3310\text{ cm}^{-1}$ ,  $3085\text{ cm}^{-1}$  and  $3082\text{ cm}^{-1}$ , respectively, literature work at  $3389\text{ cm}^{-1}$  (*ab initio* [18]),  $3085\text{ cm}^{-1}$  (AM1 [18]), which shows semi-empirical methods give closed value to the experimental methods than *ab initio*.

The peak calculated at  $3167\text{ cm}^{-1}$ ,  $3071\text{ cm}^{-1}$  and  $3041\text{ cm}^{-1}$  having no counterpart in the IR spectrum refers to the CH stretching mode. The peak observed at  $2926\text{ cm}^{-1}$  in the IR is

attributed to the asymmetric CH stretching in the CH<sub>3</sub> group, since the corresponding theoretical values are 3096 cm<sup>-1</sup>, 3065 cm<sup>-1</sup> and 3035 cm<sup>-1</sup> for *ab initio*, RM1 and PM6 respectively, in the literature 2898 cm<sup>-1</sup> by *ab initio* [18] and 2758 cm<sup>-1</sup> for Am1 [18].

The peak observed at 2604 cm<sup>-1</sup> in the IR spectrum is assigned to the NH stretching in the NH<sub>3</sub> group. The corresponding calculated frequencies are 2691 cm<sup>-1</sup>, 2633 cm<sup>-1</sup> and 2748 cm<sup>-1</sup> by *ab initio*, RM1 and PM6 respectively. The frequency of 2631 cm<sup>-1</sup> was calculated by *ab initio* [18].

The calculated peaks at 1643 cm<sup>-1</sup>, 1809 cm<sup>-1</sup> and 1979 cm<sup>-1</sup> for the asym. COO<sup>-</sup> stretching coupled with a NH bending mode, does not appear in the IR spectrum. Barthes et. al. [26] have reported this frequency at 1630 cm<sup>-1</sup> in IR spectrum. The peak at 1595 cm<sup>-1</sup> in the IR spectrum is assigned to the NH<sub>2</sub> scissoring mode, according to calculated values at 1522 cm<sup>-1</sup>, 1514 cm<sup>-1</sup> and 1755 cm<sup>-1</sup>.

The calculation of this peak was reported at 1620 cm<sup>-1</sup> (*ab initio* [18]) and 1464 cm<sup>-1</sup> (Am1 [18]). Other vibrations in the finger print region (400-1354 cm<sup>-1</sup>) and the corresponding calculated frequencies by *ab initio*, RM1 and PM6 are listed in Table 2.

**Table 2.** Calculated Vibrational Frequencies cm<sup>-1</sup> for *l*-Alanine Compared with Experimental.

Mode	Obs.	RM1	PM6	Ab initio	Assignment
v <sub>1</sub>	3099	3085	3082	3310	Asym. NH <sub>2</sub> stretching
v <sub>2</sub>		3071	3041	3167	CH stretching in CH <sub>3</sub>
v <sub>3</sub>	2926	3065	3035	3096	Asm.CH stretching in CH <sub>3</sub>
v <sub>4</sub>		2853	2936	2796	Puckering of CH <sub>3</sub>
v <sub>5</sub>		2801	2924	2774	CH stretching
v <sub>6</sub>		2798	2903	2717	CH stretching in CH <sub>3</sub> group
v <sub>7</sub>	2604	2633	2748	2691	NH stretching in NH <sub>2</sub>
v <sub>8</sub>		1809	1979	1643	Asym. COO <sup>-</sup> stretching + NH bend.
v <sub>9</sub>	1595	1514	1755	1522	NH <sub>2</sub> bending
v <sub>10</sub>	1354	1410	1475	1354	NH <sub>2</sub> scissoring
v <sub>11</sub>	1307	1256	1328	1343	NH <sub>3</sub> puckering
v <sub>12</sub>		1245	1285	1276	CH <sub>3</sub> deformation
v <sub>13</sub>		1226	1248	1262	CH <sub>3</sub> deformation
v <sub>14</sub>		1198	1246	1195	CH <sub>3</sub> puckering
v <sub>15</sub>		1194	1230	1150	Mixed vibration (NH <sub>2</sub> puck + CH <sub>3</sub> def)
v <sub>16</sub>		1182	1184	1133	CH bend. + NH bend.
v <sub>17</sub>	1149	1104	1176	1044	CH <sub>2</sub> bend. + CH bend. + NH <sub>2</sub> bend
v <sub>18</sub>	1114	1062	1165	1005	C-C stretch + CH bend. + NH bend.
v <sub>19</sub>	1014	985	1073	966	H-N-C-C bend.

$\nu_{20}$	965	984	913	H-C-C-H bend. + NH <sub>2</sub> def.
$\nu_{21}$	918	930	935	CNH bend
$\nu_{22}$	850	826	893	CN stretch. + C-C stretching
$\nu_{23}$	769	677	840	HNC + CCH bending
$\nu_{24}$	646	657	676	CCC + COO bending
$\nu_{25}$	543	570	558	OCOH + NH <sub>2</sub> bending
$\nu_{26}$	412	486	472	OH bending
$\nu_{27}$	378	426	432	NCC bending + CCCO

#### 4. 2. Assignment of *l*-Phenylalanine

The Non-zwitterionic structure of *l*-phenly-alanine is shown in Figure 3. The assignment for calculated frequencies and those observed in the IR spectra are shown in Table 2. The characteristic bands at 3436 cm<sup>-1</sup> due to NH asymmetric stretching, confirmed the existence of amino group, the corresponding calculated IR spectrum are 3187 cm<sup>-1</sup>, 3083 cm<sup>-1</sup> and 3084 cm<sup>-1</sup> at B3LYP/6-31+G(d), RM1 and PM6 respectively, the same peak at 3150 cm<sup>-1</sup> was predicted by SCRF calculation [20] and assigned to NH asymmetric stretching frequency.

The peak calculated at 3106 cm<sup>-1</sup>, 3065 cm<sup>-1</sup> and 3040 cm<sup>-1</sup> not appearing in the experimental IR spectrum are for the NH symmetric stretching, the peak may have disappeared because of H-bonding.

The band at 3028 cm<sup>-1</sup> establishes the presence of CH<sub>2</sub> asymmetric stretching, as the corresponding theoretical values of this work at 3030 cm<sup>-1</sup>, 3009 cm<sup>-1</sup> and 3016 cm<sup>-1</sup>, and calculated by SCRF at 3001 cm<sup>-1</sup> [20] are very near.

The peak at 2918 cm<sup>-1</sup> is assigned to CH stretching in the phenyl ring, since the corresponding calculated values are at 2854 cm<sup>-1</sup>, 2847 cm<sup>-1</sup> and 3006 cm<sup>-1</sup> by B3LYP/6-31+G(d), RM1 and PM6 respectively, the calculation of this band in the literature is 2996 cm<sup>-1</sup> by *ab initio* (SCRF) [20].

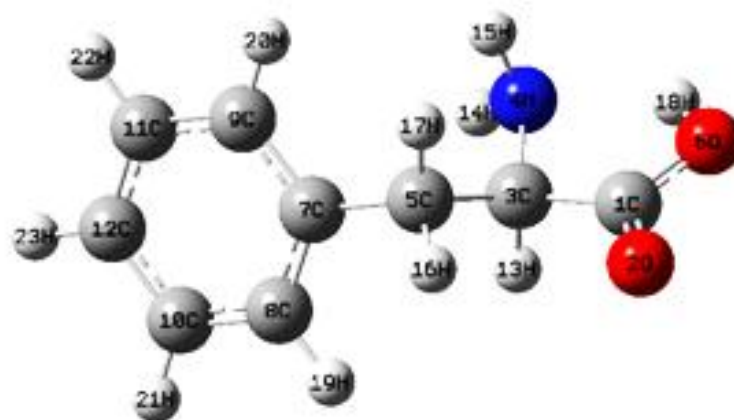
The calculated values at 2843 cm<sup>-1</sup>, 2835 cm<sup>-1</sup>, 2821 cm<sup>-1</sup>, 2818 cm<sup>-1</sup> at B3LYP/6-31+G(d), 2836 cm<sup>-1</sup>, 2831 cm<sup>-1</sup>, 2824 cm<sup>-1</sup>, 2821 cm<sup>-1</sup> at RM1, and 3002 cm<sup>-1</sup>, 2997 cm<sup>-1</sup>, 2992 cm<sup>-1</sup>, 2983 cm<sup>-1</sup> at PM6 also are for the CH stretchings in phenyl ring which are not observed in IR experimental spectrum.

The bands calculated at 2759 cm<sup>-1</sup>, 2776 cm<sup>-1</sup> and 2907 cm<sup>-1</sup> are assigned to CH stretching, since the corresponding band in the literature calculated by *ab initio* (SCRF) [20] is at 2930 cm<sup>-1</sup>.

The peaks calculated at 2723 cm<sup>-1</sup>, 2706 cm<sup>-1</sup> by B3LYP/6-31+G(d), at 2717 cm<sup>-1</sup>, 2628 cm<sup>-1</sup> by RM1 and at 2889 cm<sup>-1</sup>, 2711 cm<sup>-1</sup> by PM6 for the asymmetric CH<sub>2</sub> stretching and symmetric CH<sub>2</sub> stretching respectively, are not experimentally observed.

The band observed at 1683 cm<sup>-1</sup> is assigned to COO<sup>-</sup> asymmetric stretching, since the corresponding theoretical values are 1637 cm<sup>-1</sup>, 1809 cm<sup>-1</sup> and 1688 cm<sup>-1</sup> by *ab initio*, RM1 and PM6 respectively. Assignments of other vibrations from 1600-300 cm<sup>-1</sup> are listed in Table 3.





**Figure 3.** Molecular Structure of *l*-Phenylalanine.

**Table 3.** Calculated Vibrational Frequencies in  $\text{cm}^{-1}$  for *l*-Phenylalanine Compared with Experimental.

Mode	Obs.	RM1	PM6	Ab initio	Assignment
$\nu_1$	3436	3083	3084	3187	NH asymmetric stretching
$\nu_2$		3065	3040	3106	NH symmetric stretching
$\nu_3$	3028	3009	3016	3030	$\text{CH}_2$ asymmetric stretch.
$\nu_4$	2918	2847	3006	2854	CH stretching in phenyl Ring
$\nu_5$		2836	3002	2843	CH stretching in phenyl Ring
$\nu_6$		2831	2997	2835	CH stretching in phenyl Ring
$\nu_7$		2824	2992	2821	CH stretching in phenyl Ring
$\nu_8$		2821	2983	2818	CH stretching in phenyl Ring
$\nu_9$		2776	2907	2759	CH stretching
$\nu_{10}$		2717	2889	2723	$\text{CH}_2$ asymmetric stretching
$\nu_{11}$		2628	2711	2706	$\text{CH}_2$ symmetric stretching
$\nu_{12}$	1683	1809	1688	1637	$\text{COO}^-$ asymmetric stretching
$\nu_{13}$	1596	1607	1539	1510	NH asymmetric bending
$\nu_{14}$	1478	1601	1534	1472	NH asymmetric bending
$\nu_{15}$	1449	1508	1500	1454	Phenyl ring Stretching
$\nu_{16}$	1393	1457	1422	1369	Phenyl ring Stretching
$\nu_{17}$	1358	1418	1384	1343	Phenyl ring Stretching
$\nu_{18}$	1332	1401	1343	1329	$\text{CH}_2$ bending
$\nu_{19}$		1282	1238	1267	NH symmetric bending

v <sub>20</sub>		1239	1216	1251	NH symmetric bending
v <sub>21</sub>		1231	1208	1227	COO <sup>-</sup> symmetric stretching
v <sub>22</sub>	1218	1222	1191	1213	CH <sub>2</sub> bending
v <sub>23</sub>		1190	1170	1201	CH bending in phenyl ring
v <sub>24</sub>	1161	1174	1168	1157	C-C stretching
v <sub>25</sub>	1130	1154	1155	1119	CH <sub>2</sub> bending
v <sub>26</sub>	1097	1142	1144	1095	Phenyl ring Stretching
v <sub>27</sub>	1081	1125	1133	1084	CH bending in phenyl ring
v <sub>28</sub>		1109	1122	1078	C-C stretching in phenyl ring
v <sub>29</sub>	1040	1091	1090	1059	CH bending in phenyl ring
v <sub>30</sub>		1058	1079	1019	CH bending in phenyl ring
v <sub>31</sub>		1048	1061	995	NH rocking
v <sub>32</sub>		1041	1052	980	CH rocking in phenyl ring
v <sub>33</sub>		1011	1035	938	Out-of- plane CH stretching in phenyl
v <sub>34</sub>		993	984	924	NH rocking
v <sub>35</sub>		970	965	903	CH bending in phenyl ring
v <sub>36</sub>		925	941	892	Out-of- plane CH stretching in phenyl
v <sub>37</sub>	879	907	922	871	Out-of- plane CH stretching in phenyl
v <sub>38</sub>	859	885	909	835	Phenyl ring Stretching
v <sub>39</sub>		881	875	827	Out-of- plane CH stretching in phenyl
v <sub>40</sub>		850	853	792	CH <sub>2</sub> rocking
v <sub>41</sub>		814	834	776	C-COO <sup>-</sup> stretching
v <sub>42</sub>		804	823	770	Out-of- plane CH stretching in phenyl
v <sub>43</sub>	744	742	791	763	CH <sub>2</sub> rocking
v <sub>44</sub>	703	737	742	725	C-N stretching
v <sub>45</sub>		666	681	677	Out-of- plane CH stretching in phenyl
v <sub>46</sub>	642	613	647	645	COO <sup>-</sup> bending
v <sub>47</sub>		575	597	632	Out-of -plane ring deformation
v <sub>48</sub>	579	555	573	564	COO <sup>-</sup> waging
v <sub>49</sub>		529	557	549	In plane ring deformation
v <sub>50</sub>	480	472	529	510	In-plane ring deformation
v <sub>51</sub>		415	483	463	COO <sup>-</sup> rocking

$v_{52}$	412	429	432	Out-of-plane ring deformation
$v_{53}$	396	412	370	Out-of-plane ring deformation
$v_{54}$	329	350	369	C-C deformation
$v_{55}$	319	328	315	C-N deformation
$v_{56}$	298	296	299	C-C deformation
$v_{57}$	233	255	258	N-H deformation

### 4. 3. Assignment of *l*-Aspartic Acid

The Non-zwitterionic structure of *l*-aspartic acid is shown in Figure 4. The calculated frequencies and the observed in the IR spectra are shown in Table 4. The calculated and experimental vibrational frequencies show a reasonable general agreement. The characteristic bands at  $3420\text{ cm}^{-1}$  and  $3250\text{ cm}^{-1}$  due to OH stretching, have the corresponding calculated IR spectrum at  $3280\text{ cm}^{-1}$ ,  $3275\text{ cm}^{-1}$  at B3LYP/6-31+G(d),  $3090\text{ cm}^{-1}$ ,  $3079\text{ cm}^{-1}$  at RM1, and  $3058\text{ cm}^{-1}$ ,  $3018\text{ cm}^{-1}$  at PM6 respectively.

The calculated IR spectra of OH stretching in the literature is at  $3481\text{ cm}^{-1}$  by B3lyp/3-21G [27]. The peak appearing at  $3141\text{ cm}^{-1}$ ,  $3011\text{ cm}^{-1}$  in observed IR spectrum is assigned to the NH asymmetric and symmetric stretching, since the corresponding calculated IR spectrum are  $3189\text{ cm}^{-1}$ ,  $3114\text{ cm}^{-1}$  at B3LYP/6-31+G(d),  $3017\text{ cm}^{-1}$ ,  $3016\text{ cm}^{-1}$  at RM1, and  $2966\text{ cm}^{-1}$ ,  $2887\text{ cm}^{-1}$  at PM6 respectively. The corresponding spectrum of NH in the literature is  $3400\text{ cm}^{-1}$  by B3lyp/3-21G [27].

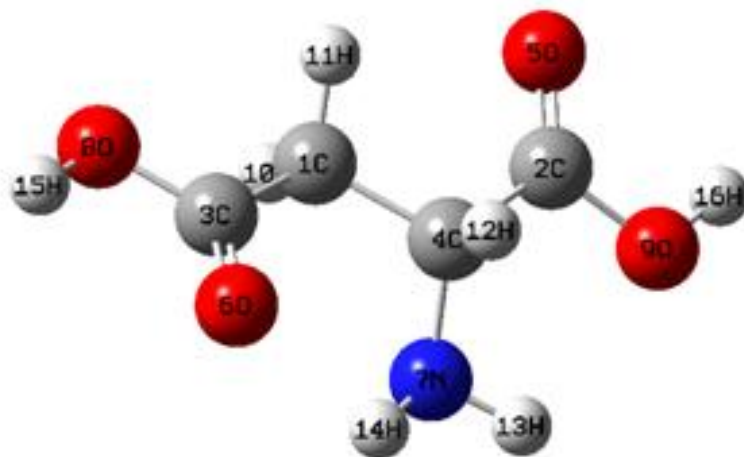
The bands at  $2942\text{ cm}^{-1}$ ,  $2853\text{ cm}^{-1}$  establish the presence of CH asymmetric stretching in the  $\text{CH}_2$ , as the corresponding theoretical values at  $2775\text{ cm}^{-1}$ ,  $2738\text{ cm}^{-1}$  at B3LYP/6-31+G(d),  $2764\text{ cm}^{-1}$ ,  $2709\text{ cm}^{-1}$  at RM1, and  $2865\text{ cm}^{-1}$ ,  $2771\text{ cm}^{-1}$  at PM6, and in the literature the peak assigned at  $3103\text{ cm}^{-1}$  by *ab initio* [27]. The peak at  $1620\text{ cm}^{-1}$  is assigned to C=O asymmetric stretching, the corresponding calculated values are at  $1607\text{ cm}^{-1}$ ,  $1826$  and  $1821\text{ cm}^{-1}$  respectively. In the literature C=O asymmetric stretching is calculated at  $1764\text{ cm}^{-1}$  by *ab initio* [27].

**Table 4.** Calculated Vibrational Frequencies in  $\text{cm}^{-1}$  for *l*-Aspartic Acid Compared with Experimental.

Mode	Obs.	RM1	PM6	Ab initio	Assignment
$v_1$	3420	3090	3058	3280	OH stretching
$v_2$	3250	3079	3018	3275	OH stretching
$v_3$	3141	3017	2966	3189	NH asymmetric stretching
$v_4$	3011	3016	2887	3114	NH symmetric stretching
$v_5$	2942	2764	2865	2775	CH stretching in $\text{CH}_2$
$v_6$	2853	2709	2771	2738	CH stretching in $\text{CH}_2$
$v_7$		2605	2767	2623	CH stretching in $\text{CH}_2$
$v_8$	1692	1831	1832	1619	C=O stretching + NHN bending

<b>v<sub>9</sub></b>	1620	1826	1821	1607	C=O asymmetric stretching
<b>v<sub>10</sub></b>	1538	1528	1500	1504	NHN bending + C=O stretching
<b>v<sub>11</sub></b>	1398	1417	1480	1305	CH bending + HCH bending
<b>v<sub>12</sub></b>	1377	1399	1336	1293	CH bending
<b>v<sub>13</sub></b>	1292	1262	1298	1243	HCH bending + CC stretching + CN str.
<b>v<sub>14</sub></b>	1213	1241	1267	1217	C-C stretching + C-N stretching
<b>v<sub>15</sub></b>		1233	1242	1181	NH <sub>2</sub> bending + CN stretching
<b>v<sub>16</sub></b>	1142	1214	1202	1149	C-OH symmetric stretching in COOH
<b>v<sub>17</sub></b>	1117	1186	1188	1132	C-OH asymmetric stretch. in COOH
<b>v<sub>18</sub></b>	1073	1164	1178	1082	CH <sub>2</sub> rocking + C-C stretching
<b>v<sub>19</sub></b>		1108	1131	1050	C—CH <sub>2</sub> -C bending
<b>v<sub>20</sub></b>		1082	1110	1029	C-C stretching
<b>v<sub>21</sub></b>	987	1055	1022	1011	C-C stretching + C-N stretching
<b>v<sub>22</sub></b>	895	1017	1011	937	NH <sub>2</sub> wagging
<b>v<sub>23</sub></b>	854	947	978	859	C-C stretching + CO stretching + NH <sub>2</sub> bend.
<b>v<sub>24</sub></b>		918	920	803	C-C-C wagging
<b>v<sub>25</sub></b>		865	861	788	C-C-C bending
<b>v<sub>26</sub></b>		680	846	761	OH rocking
<b>v<sub>27</sub></b>	693	635	637	654	OH rocking
<b>v<sub>28</sub></b>	642	589	572	597	OH rocking
<b>v<sub>29</sub></b>	580	535	542	578	COO- bending
<b>v<sub>30</sub></b>	525	523	516	546	COO- bending
<b>v<sub>31</sub></b>		499	492	511	OH wagging + CH <sub>2</sub> wagging
<b>v<sub>32</sub></b>	478	466	474	474	NH <sub>2</sub> twisting
<b>v<sub>33</sub></b>		456	440	461	NH <sub>2</sub> -C-COOH bending
<b>v<sub>34</sub></b>	397	355	347	361	NH <sub>2</sub> wagging
<b>v<sub>35</sub></b>		325	317	320	Torsion COO <sup>-</sup>
<b>v<sub>36</sub></b>	294	306	287	295	Torsion COO <sup>-</sup>

The band at 1538 cm<sup>-1</sup> is assigned to NHN bending + C=O stretching, calculated values at 1504 cm<sup>-1</sup>, 1528 cm<sup>-1</sup>, 1500 cm<sup>-1</sup> at B3LYP/6-31+G(d), RM1 and PM6 respectively. Assignments of other vibrations are listed in Table 4.



**Figure 4.** Molecular Structure of *l*-Aspartic Acid.

#### 4. 4. Assignment of *l*-Glutamic Acid

The Non-zwitterionic structure of *l*-glutamic acid is shown in Figure 5. The calculated frequencies and those observed in the IR spectra are shown in Table 5. There is a little increase in frequency value in the high-frequencies region between 2700-2900  $\text{cm}^{-1}$ , between experimental and calculated by *ab initio* and RM1 but in the case of PM6 it is reasonable, i.e the  $\text{CH}_2$  symmetric and asymmetric stretching frequencies bands observed at 2974  $\text{cm}^{-1}$ , 2965  $\text{cm}^{-1}$  and 2935  $\text{cm}^{-1}$ , while the corresponding calculated frequencies by *ab initio* and semiempirical RM1 methods are 2770  $\text{cm}^{-1}$ , 2758  $\text{cm}^{-1}$ , 2736  $\text{cm}^{-1}$  and 2774  $\text{cm}^{-1}$ , 2763  $\text{cm}^{-1}$ , 2710  $\text{cm}^{-1}$  respectively.

The same calculated peaks by PM6 are at 2904  $\text{cm}^{-1}$ , 2884  $\text{cm}^{-1}$  and 2876  $\text{cm}^{-1}$ . The calculated  $\text{CH}_2$  spectra in the literature by *ab initio* 4-31G is 2972  $\text{cm}^{-1}$  [23]. The peak observed at 1781  $\text{cm}^{-1}$  is assigned to C=O stretching frequency, the calculated frequencies are 1610  $\text{cm}^{-1}$ , 1820  $\text{cm}^{-1}$  and 1806  $\text{cm}^{-1}$  by *ab initio*, RM1 and PM6. The calculated one in the literature is assigned at 1765  $\text{cm}^{-1}$  by *ab initio* 4-31G [23]. There is also rise in the frequency value in IR bands in the case of semiempirical RM1 and PM6 methods in frequencies region 700-1000  $\text{cm}^{-1}$  when compared with experimental ones. The other assignments are show in Table 5. The only explanation of the departure of experimental values from calculated values is a possible intramolecular hydrogen bonding forming a cyclic structure, a situation not considered in theory.

**Table 5.** Calculated Vibrational Frequencies in  $\text{cm}^{-1}$  for *l*-Glutamic Acid Compared with Experimental.

Mode	Obs.	RM1	PM6	Ab initio	Assignment
$\nu_1$		3101	3077	3268	OH stretching
$\nu_2$		3093	3044	3170	OH stretching
$\nu_3$	3020	3004	2978	3130	NH asymmetric stretching
$\nu_4$	2994	2886	2975	3089	CH stretching in $\text{CH}_2$

<b>v<sub>5</sub></b>	2974	2774	2904	2770	CH <sub>2</sub> asymmetric stretching
<b>v<sub>6</sub></b>	2965	2763	2884	2758	CH <sub>2</sub> symmetric stretching
<b>v<sub>7</sub></b>	2935	2710	2876	2736	CH <sub>2</sub> symmetric stretching
<b>v<sub>8</sub></b>		2706	2767	2721	NHN bending + C=O stretching
<b>v<sub>9</sub></b>		2621	2618	2709	NHN in-plane bending
<b>v<sub>10</sub></b>	1781	1820	1806	1610	C=O stretching
<b>v<sub>11</sub></b>	1635	1799	1783	1589	CH bending + HCH bending
<b>v<sub>12</sub></b>	1405	1511	1502	1512	CH in-plane bending
<b>v<sub>13</sub></b>	1386	1429	1348	1398	CO stretching + CH <sub>2</sub> torsion
<b>v<sub>14</sub></b>	1337	1413	1340	1319	C-C stretching + CO stretching
<b>v<sub>15</sub></b>	1301	1266	1328	1277	CH <sub>2</sub> torsion
<b>v<sub>16</sub></b>	1279	1243	1281	1263	CH <sub>2</sub> torsion + NH <sub>2</sub> in-plane bending
<b>v<sub>17</sub></b>		1235	1269	1236	C-OH asymmetric stretch. in COOH
<b>v<sub>18</sub></b>	1234	1218	1248	1221	CN stretching
<b>v<sub>19</sub></b>		1195	1237	1197	C—CH <sub>2</sub> -C bending
<b>v<sub>20</sub></b>		1190	1223	1170	C-C stretching
<b>v<sub>21</sub></b>	1139	1177	1130	1138	OH bending + CH <sub>2</sub> torsion + CN stretching
<b>v<sub>22</sub></b>	1124	1165	1120	1125	OH in-plane bending
<b>v<sub>23</sub></b>	1108	1131	1097	1091	CN stretching + OH in-plane bending
<b>v<sub>24</sub></b>	1092	1063	1091	1060	C-C stretching
<b>v<sub>25</sub></b>	1024	1089	1066	1043	C-C stretching + CH <sub>2</sub> rocking + CO stretch.
<b>v<sub>26</sub></b>		1072	1052	997	CC stretching + NH <sub>2</sub> in-plane bending
<b>v<sub>27</sub></b>		1012	1031	946	CH <sub>2</sub> rocking + CC stretching
<b>v<sub>28</sub></b>		994	991	920	OH rocking
<b>v<sub>29</sub></b>	886	951	932	889	CC stretching + CO stretching
<b>v<sub>30</sub></b>	814	877	908	826	HOCC out-of-plane bending + CH <sub>2</sub> rock.
<b>v<sub>31</sub></b>		867	874	803	OH wagging + CH <sub>2</sub> wagging
<b>v<sub>32</sub></b>	754	790	837	752	CH <sub>2</sub> rocking
<b>v<sub>33</sub></b>		662	740	705	NH <sub>2</sub> -C-COOH bending
<b>v<sub>34</sub></b>	668	590	660	689	HOCC out-of-plane bending
<b>v<sub>35</sub></b>	646	577	607	640	NH <sub>2</sub> out-of-plane bending
<b>v<sub>36</sub></b>	612	559	598	607	COOH in-plane bending

V <sub>37</sub>	570	528	577	568	OH out-of-plane bending
V <sub>38</sub>	553	522	538	510	OH out-of-plane bending
V <sub>39</sub>		481	505	496	COOH in-plane bending
V <sub>40</sub>		459	499	471	COOH in-plane bending
V <sub>41</sub>		409	452	420	CCC in-plane bending + CNN in-plane ben
V <sub>42</sub>		331	337	322	CCC in-plane bending
V <sub>43</sub>		320	277	275	CCN in-plane bendin
V <sub>44</sub>		282	266	265	NH <sub>2</sub> torsion
V <sub>45</sub>		235	238	248	CCC in-plane bending

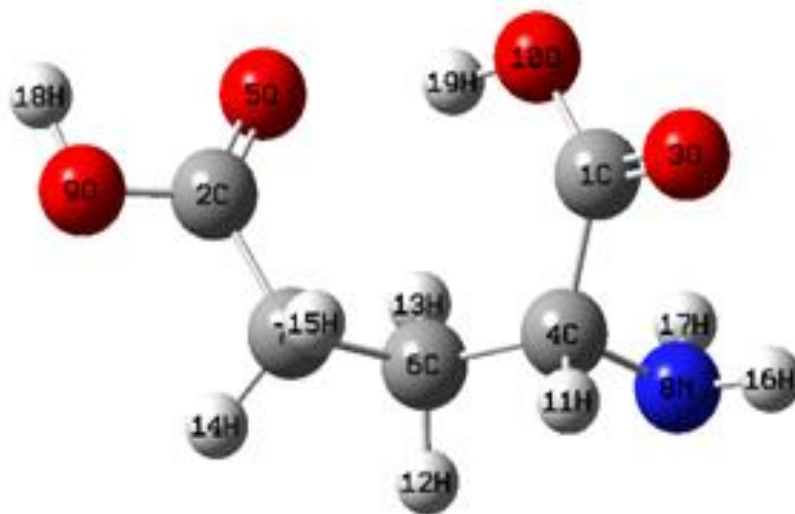


Figure 5. Molecular Structure of *l*-Glutamic Acid.

## 5. CONCLUSION

Assignments of amino acids experimentally and theoretically have been performed. New parameters of semi-empirical methods in MOPAC2007 MP6 and RM1 are used. Comparison of scaled theoretical and experimental vibrational frequencies exhibit good correlation confirming the reliability of the method employed here.

## SUPPLEMENTARY INFORMATIONS

The supplementary informations (output results of semi-empirical and *ab initio* methods calculations) are available as free of charge on request.

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