

Experimental and Computational Vibration Study of Amino Acids

Musa E. Mohamed^{1,*}, Abdelhafeez M. A. Mohammed²

¹Department of Chemistry, College of Applied and Industrial Sciences, University of Bahri, Sudan

²Department of Chemistry, Faculty of Education, Alzaiem Alazhari University, Sudan

*E-mail address: 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 $R-CH(COO^-)NH_3^+$. On the Contrary, the intermolecular bonds are not present in the gas phase and these molecules have a non-zwitterionic structure $R-CH(COOH)NH_2$. 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.

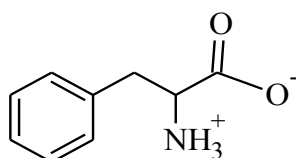
This conclusion has been confirmed experimentally by matrix isolation (MI) studies [8-10], a technique in which the amino acids are first vaporized, then trapped and isolated in inert gas matrices at very low temperatures. The MI infrared spectra clearly show that amino

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.

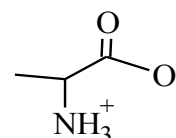
The second problem concerns the measurement of IR absorption spectra of monomers of the amino acids in their zwitterionic forms. In the gas phase the amino acids occur in their un-ionized forms. The zwitterions have to be stabilized by their local environment. This is achieved in polar solutions and 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.

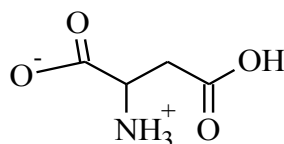
To carry out meaningful calculations on the zwitterionic forms of the amino acids, either solvation effects or specific intermolecular interactions must be included. Calculations including specific intermolecular interactions are usually impracticable, because of the number of molecules (large multimers 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.



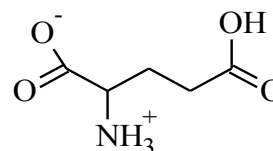
Phenylalanine



Alanine



Aspartic Acid



Glutamic Acid

Figure 1. Zwitterionic Structures of Amino Acids.

These methods model the solvent as a continuum of uniform dielectric constant (ϵ), 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 α - and β -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-d4 acid, and *l*-aspartic-¹⁵N 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^{-1} -4000 cm^{-1} [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 ^{15}N and d_4 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 Hartree-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 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)

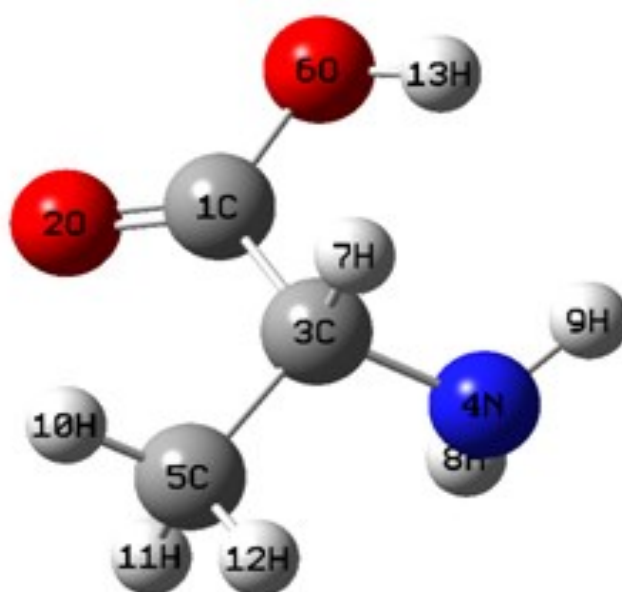
The optimized semiempirical RM1, PM6 and *ab initio* B3LYP/6-31+G(d) geometries were used as starting point to carry force field calculations for non-zwitterionic, *l*-alanine, *l*-phenylalanine, *l*-aspartic and *l*-glutamic acid. To minimize the systematic errors of these calculations, scaling factors 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 cm^{-1} is assigned to the asymmetric NH stretching mode.

Table 1. Relative Stability of Amino Acids (ΔE) 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 cm^{-1} , 3085 cm^{-1} and 3082 cm^{-1} , respectively, literature work at 3389 cm^{-1} (*ab initio* [18]), 3085 cm^{-1} (AM1 [18]), which shows semi-empirical methods give closed value to the experimental methods than *ab initio*.

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

attributed to the asymmetric CH stretching in the CH₃ group, since the corresponding theoretical values are 3096 cm⁻¹, 3065 cm⁻¹ and 3035 cm⁻¹ for *ab initio*, RM1 and PM6 respectively, in the literature 2898 cm⁻¹ by *ab initio* [18] and 2758 cm⁻¹ for Am1 [18].

The peak observed at 2604 cm⁻¹ in the IR spectrum is assigned to the NH stretching in the NH₃ group. The corresponding calculated frequencies are 2691 cm⁻¹, 2633 cm⁻¹ and 2748 cm⁻¹ by *ab initio*, RM1 and PM6 respectively. The frequency of 2631 cm⁻¹ was calculated by *ab initio* [18].

The calculated peaks at 1643 cm⁻¹, 1809 cm⁻¹ and 1979 cm⁻¹ for the asym. COO⁻ 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⁻¹ in IR spectrum. The peak at 1595 cm⁻¹ in the IR spectrum is assigned to the NH₂ scissoring mode, according to calculated values at 1522 cm⁻¹, 1514 cm⁻¹ and 1755 cm⁻¹.

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

Table 2. Calculated Vibrational Frequencies cm⁻¹ for *l*-Alanine Compared with Experimental.

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

| | | | | | |
|------------|-----|-----|-----|-----|--------------------------------------|
| ν_{20} | | 965 | 984 | 913 | H-C-C-H bend. + NH ₂ def. |
| ν_{21} | 918 | 930 | 935 | 821 | CNH bend |
| ν_{22} | 850 | 826 | 893 | 816 | CN stretch. + C-C stretching |
| ν_{23} | 769 | 677 | 840 | 706 | HNC + CCH bending |
| ν_{24} | 646 | 657 | 676 | 654 | CCC + COO bending |
| ν_{25} | 543 | 570 | 558 | 569 | OCOH + NH ₂ bending |
| ν_{26} | 412 | 486 | 472 | 441 | OH bending |
| ν_{27} | | 378 | 426 | 432 | NCC bending + CCCO |

4. 2. Assignment of *l*-Phenylalanine

The Non-zwitterionic structure of *l*-phenyl-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⁻¹ due to NH asymmetric stretching, confirmed the existence of amino group, the corresponding calculated IR spectrum are 3187 cm⁻¹, 3083 cm⁻¹ and 3084 cm⁻¹ at B3LYP/6-31+G(d), RM1 and PM6 respectively, the same peak at 3150 cm⁻¹ was predicted by SCRF calculation [20] and assigned to NH asymmetric stretching frequency.

The peak calculated at 3106 cm⁻¹, 3065 cm⁻¹ and 3040 cm⁻¹ 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⁻¹ establishes the presence of CH₂ asymmetric stretching, as the corresponding theoretical values of this work at 3030 cm⁻¹, 3009 cm⁻¹ and 3016 cm⁻¹, and calculated by SCRF at 3001 cm⁻¹ [20] are very near.

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

The calculated values at 2843 cm⁻¹, 2835 cm⁻¹, 2821 cm⁻¹, 2818 cm⁻¹ at B3LYP/6-31+G(d), 2836 cm⁻¹, 2831 cm⁻¹, 2824 cm⁻¹, 2821 cm⁻¹ at RM1, and 3002 cm⁻¹, 2997 cm⁻¹, 2992 cm⁻¹, 2983 cm⁻¹ 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⁻¹, 2776 cm⁻¹ and 2907 cm⁻¹ are assigned to CH stretching, since the corresponding band in the literature calculated by *ab initio* (SCRF) [20] is at 2930 cm⁻¹.

The peaks calculated at 2723 cm⁻¹, 2706 cm⁻¹ by B3LYP/6-31+G(d), at 2717 cm⁻¹, 2628 cm⁻¹ by RM1 and at 2889 cm⁻¹, 2711 cm⁻¹ by PM6 for the asymmetric CH₂ stretching and symmetric CH₂ stretching respectively, are not experimentally observed.

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

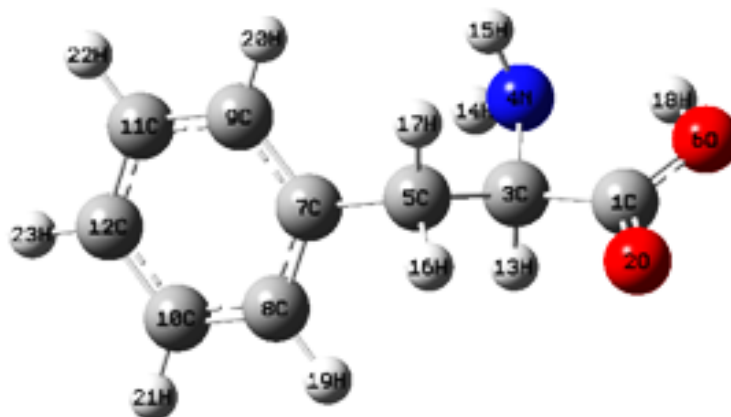


Figure 3. Molecular Structure of *l*-Phenylalanine.

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

| Mode | Obs. | RM1 | PM6 | Ab initio | Assignment |
|------------|------|------|------|-----------|--------------------------------------|
| ν_1 | 3436 | 3083 | 3084 | 3187 | NH asymmetric stretching |
| ν_2 | | 3065 | 3040 | 3106 | NH symmetric stretching |
| ν_3 | 3028 | 3009 | 3016 | 3030 | CH_2 asymmetric stretch. |
| ν_4 | 2918 | 2847 | 3006 | 2854 | CH stretching in phenyl Ring |
| ν_5 | | 2836 | 3002 | 2843 | CH stretching in phenyl Ring |
| ν_6 | | 2831 | 2997 | 2835 | CH stretching in phenyl Ring |
| ν_7 | | 2824 | 2992 | 2821 | CH stretching in phenyl Ring |
| ν_8 | | 2821 | 2983 | 2818 | CH stretching in phenyl Ring |
| ν_9 | | 2776 | 2907 | 2759 | CH stretching |
| ν_{10} | | 2717 | 2889 | 2723 | CH_2 asymmetric stretching |
| ν_{11} | | 2628 | 2711 | 2706 | CH_2 symmetric stretching |
| ν_{12} | 1683 | 1809 | 1688 | 1637 | COO^- asymmetric stretching |
| ν_{13} | 1596 | 1607 | 1539 | 1510 | NH asymmetric bending |
| ν_{14} | 1478 | 1601 | 1534 | 1472 | NH asymmetric bending |
| ν_{15} | 1449 | 1508 | 1500 | 1454 | Phenyl ring Stretching |
| ν_{16} | 1393 | 1457 | 1422 | 1369 | Phenyl ring Stretching |
| ν_{17} | 1358 | 1418 | 1384 | 1343 | Phenyl ring Stretching |
| ν_{18} | 1332 | 1401 | 1343 | 1329 | CH_2 bending |
| ν_{19} | | 1282 | 1238 | 1267 | NH symmetric bending |

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

| | | | | |
|------------|-----|-----|-----|-------------------------------|
| ν_{52} | 412 | 429 | 432 | Out-of-plane ring deformation |
| ν_{53} | 396 | 412 | 370 | Out-of-plane ring deformation |
| ν_{54} | 329 | 350 | 369 | C-C deformation |
| ν_{55} | 319 | 328 | 315 | C-N deformation |
| ν_{56} | 298 | 296 | 299 | C-C deformation |
| ν_{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 cm^{-1} and 3250 cm^{-1} due to OH stretching, have the corresponding calculated IR spectrum at 3280 cm^{-1} , 3275 cm^{-1} at B3LYP/6-31+G(d), 3090 cm^{-1} , 3079 cm^{-1} at RM1, and 3058 cm^{-1} , 3018 cm^{-1} at PM6 respectively.

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

The bands at 2942 cm^{-1} , 2853 cm^{-1} establish the presence of CH asymmetric stretching in the CH_2 , as the corresponding theoretical values at 2775 cm^{-1} , 2738 cm^{-1} at B3LYP/6-31+G(d), 2764 cm^{-1} , 2709 cm^{-1} at RM1, and 2865 cm^{-1} , 2771 cm^{-1} at PM6, and in the literature the peak assigned at 3103 cm^{-1} by ab initio [27]. The peak at 1620 cm^{-1} is assigned to C=O asymmetric stretching, the corresponding calculated values are at 1607 cm^{-1} , 1826 and 1821 cm^{-1} respectively. In the literature C=O asymmetric stretching is calculated at 1764 cm^{-1} by *ab initio* [27].

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

| Mode | Obs. | RM1 | PM6 | Ab initio | Assignment |
|---------|------|------|------|-----------|--------------------------------|
| ν_1 | 3420 | 3090 | 3058 | 3280 | OH stretching |
| ν_2 | 3250 | 3079 | 3018 | 3275 | OH stretching |
| ν_3 | 3141 | 3017 | 2966 | 3189 | NH asymmetric stretching |
| ν_4 | 3011 | 3016 | 2887 | 3114 | NH symmetric stretching |
| ν_5 | 2942 | 2764 | 2865 | 2775 | CH stretching in CH_2 |
| ν_6 | 2853 | 2709 | 2771 | 2738 | CH stretching in CH_2 |
| ν_7 | | 2605 | 2767 | 2623 | CH stretching in CH_2 |
| ν_8 | 1692 | 1831 | 1832 | 1619 | C=O stretching + NHN bending |

| | | | | | |
|------------|------|------|------|------|--|
| ν_9 | 1620 | 1826 | 1821 | 1607 | C=O asymmetric stretching |
| ν_{10} | 1538 | 1528 | 1500 | 1504 | NHN bending + C=O stretching |
| ν_{11} | 1398 | 1417 | 1480 | 1305 | CH bending + HCH bending |
| ν_{12} | 1377 | 1399 | 1336 | 1293 | CH bending |
| ν_{13} | 1292 | 1262 | 1298 | 1243 | HCH bending + CC stretching + CN str. |
| ν_{14} | 1213 | 1241 | 1267 | 1217 | C-C stretching + C-N stretching |
| ν_{15} | | 1233 | 1242 | 1181 | NH ₂ bending + CN stretching |
| ν_{16} | 1142 | 1214 | 1202 | 1149 | C-OH symmetric stretching in COOH |
| ν_{17} | 1117 | 1186 | 1188 | 1132 | C-OH asymmetric stretch. in COOH |
| ν_{18} | 1073 | 1164 | 1178 | 1082 | CH ₂ rocking + C-C stretching |
| ν_{19} | | 1108 | 1131 | 1050 | C—CH ₂ -C bending |
| ν_{20} | | 1082 | 1110 | 1029 | C-C stretching |
| ν_{21} | 987 | 1055 | 1022 | 1011 | C-C stretching + C-N stretching |
| ν_{22} | 895 | 1017 | 1011 | 937 | NH ₂ wagging |
| ν_{23} | 854 | 947 | 978 | 859 | C-C stretching + CO stretching + NH ₂ bend. |
| ν_{24} | | 918 | 920 | 803 | C-C-C wagging |
| ν_{25} | | 865 | 861 | 788 | C-C-C bending |
| ν_{26} | | 680 | 846 | 761 | OH rocking |
| ν_{27} | 693 | 635 | 637 | 654 | OH rocking |
| ν_{28} | 642 | 589 | 572 | 597 | OH rocking |
| ν_{29} | 580 | 535 | 542 | 578 | COO ⁻ bending |
| ν_{30} | 525 | 523 | 516 | 546 | COO ⁻ bending |
| ν_{31} | | 499 | 492 | 511 | OH wagging + CH ₂ wagging |
| ν_{32} | 478 | 466 | 474 | 474 | NH ₂ twisting |
| ν_{33} | | 456 | 440 | 461 | NH ₂ -C-COOH bending |
| ν_{34} | 397 | 355 | 347 | 361 | NH ₂ wagging |
| ν_{35} | | 325 | 317 | 320 | Torsion COO ⁻ |
| ν_{36} | 294 | 306 | 287 | 295 | Torsion COO ⁻ |

The band at 1538 cm⁻¹ is assigned to NHN bending + C=O stretching, calculated values at 1504 cm⁻¹, 1528 cm⁻¹, 1500 cm⁻¹ 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 cm^{-1} , between experimental and calculated by *ab initio* and RM1 but in the case of PM6 it is reasonable, i.e the CH_2 symmetric and asymmetric stretching frequencies bands observed at 2974 cm^{-1} , 2965 cm^{-1} and 2935 cm^{-1} , while the corresponding calculated frequencies by *ab initio* and semiempirical RM1 methods are 2770 cm^{-1} , 2758 cm^{-1} , 2736 cm^{-1} and 2774 cm^{-1} , 2763 cm^{-1} , 2710 cm^{-1} respectively.

The same calculated peaks by PM6 are at 2904 cm^{-1} , 2884 cm^{-1} and 2876 cm^{-1} . The calculated CH_2 spectra in the literature by *ab initio* 4-31G is 2972 cm^{-1} [23]. The peak observed at 1781 cm^{-1} is assigned to C=O stretching frequency, the calculated frequencies are 1610 cm^{-1} , 1820 cm^{-1} and 1806 cm^{-1} by *ab initio*, RM1 and PM6. The calculated one in the literature is assigned at 1765 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 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 cm^{-1} for *l*-Glutamic Acid Compared with Experimental.

| Mode | Obs. | RM1 | PM6 | Ab initio | Assignment |
|---------|------|------|------|-----------|--------------------------------|
| ν_1 | | 3101 | 3077 | 3268 | OH stretching |
| ν_2 | | 3093 | 3044 | 3170 | OH stretching |
| ν_3 | 3020 | 3004 | 2978 | 3130 | NH asymmetric stretching |
| ν_4 | 2994 | 2886 | 2975 | 3089 | CH stretching in CH_2 |

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

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

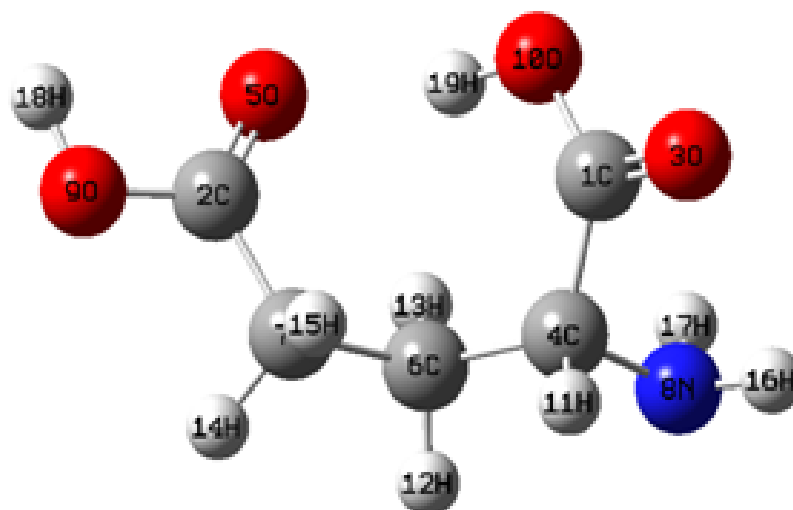


Figure 5. Molecular Structure of *l*-Glutamic Acid.

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.

References

- [1] Suenram, R. D., Lovas F. J., *J. Mol. Spectrosc.* 72 (1978) 372.
- [2] Suenram, R. D., Lovas F. G., *J. Am. Chem. Soc.* 102 (1980) 7180.
- [3] Iijima K., Tanaka K., Onuma S., *J. Mol. Struct.* 246 (1991) 257.
- [4] Iijima K., Beagley B., *J. Mol. Struct.* 248 (1991) 133.
- [5] Ding Y., Krogh-Jespersen K., *Chem. Phys. Lett.* 199 (1992) 261.
- [6] Sambrano J. R., de Sousa A. R., Queralt J. J., Andrès J., Longo E., *Chem. Phys. Lett.* 294 (1998) 1.
- [7] Császár A. G., *J. Phys. Chem.* 100 (1996) 3541.
- [8] Reva I. D., Plokhotnichenko A. M., Stepanian S. G., Ivanov A. Y., Radchenko E. D., Sheina G. G., Blagoi Y. P., *Chem. Phys. Lett.* 232 (1995) 141.
- [9] Rosado M. T. S., Duarte M. L. R. S., Fausto R., *J. Mol. Struct.* 410(1997) 343.
- [10] Reva I. D., Plokhotnichenko A. M., Stepanian S. G., Ivanov A. Y., Radchenko E. D., Sheina G. G., Blagoi Y. P., *J. Mol. Struct.* 318 (1994) 1.
- [11] Foreman J. B., Frisch Æ., *Exploring Chemistry with Electronic Structure Methods*, Second Edition, Gaussian, Pittsburgh, PA, (1996) 237.
- [12] Foresman J. B., *Private communication*, November 3 (1996).
- [13] Jensen J. H., Gordon M. S., *J. Am. Chem. Soc.* 117 (1995) 8159.
- [14] Cao X., Fischer G., in: *Proceedings of the 22nd Annual Meeting of Australian Society for Biophysics*, The Australian National University, Canberra, December, 1998.
- [15] Cao X., Fischer G., *Spectrochim. Acta Part A* 55 (1999) 2329-2342.
- [16] Rosado M. T. S., Duarte M. L. R. S., Fausto R., *J. Mol. Struct.* 410/411 (1997) 343-348.
- [17] Stepanian S. G., Reva I. D., Radchenko E. D., Adamowicz L., *J. Phys. Chem. A* 102 (1998) 4623-4629.
- [18] Santosh K., Amareshwar K. R., Rai S. B., Rai D. K., Singh A. N., Singh V. B., *J. Mol. Struct.* 791 (2006) 23-25.
- [19] Lopez N. J. T., Hernandez V., Ramirez F. J., *Biopolymers* 34 (2004)(8) 1065-1077.
- [20] Cao X., Fischer G., *J. Mol. Struct.* 519 (2000) 153-163.
- [21] Mahalakshmi R., Jesuraja S. X., Terome D. S., *Cryst. Res. Technol.* 41(8) (2006) 780-783.
- [22] Ramirez F. J., Lopez J. T. N., *Spectrochimica Acta. A* 51(2) (1995) 293-302.
- [23] Lopez Navarrete J. T., Bencivenni L., Ramondo F., Hernandez V., Ramiraz F. J., *J. Mol. Struct.* 330 (1995) 261-266.
- [24] Ohterski J. W., *Vibrational Analysis in Gaussian*, Gaussian Inc. Pittsburg, PA, 2000.
- [25] Lee C., Yang W., Parr R. G., *Phys. Rev. B* 37 (1988) 785.

- [26] Barthes M., Vik A. F., Spire A., Bordallo H. N., Eckert J., *J. Phys. Chem. A* 106 (2002) 5230.
- [27] Otavio V. C., Claudio A. T. S., Tiago G., Judith, F., *Spectrochimica Acta. A* 61(2) (2005) 337-345.

(Received 11 May 2013; accepted 15 May 2013)