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Experimental and Computational Vibration Study of Amino Acids

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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-emprical 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.

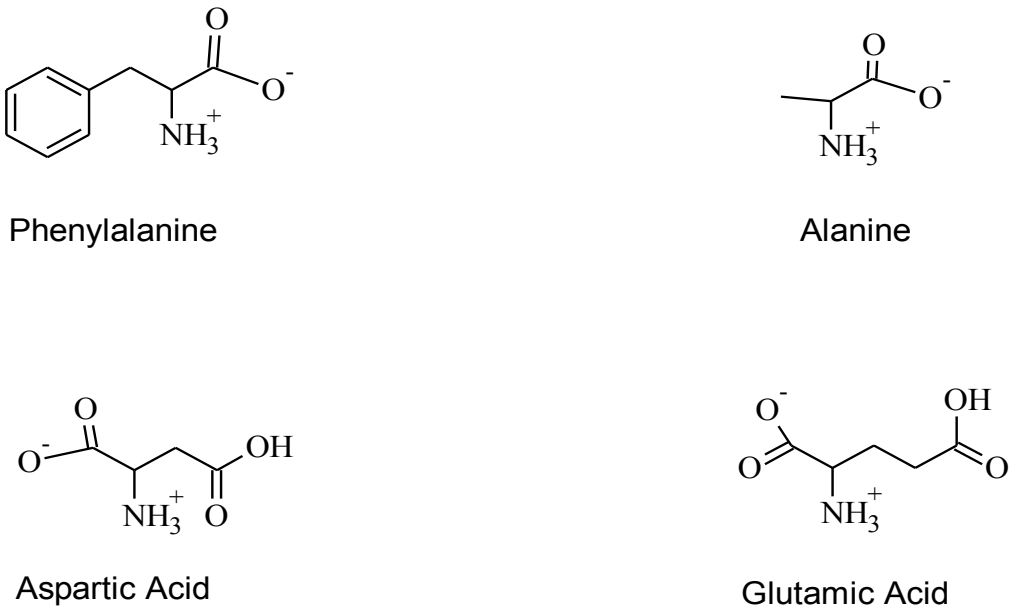

Figure 1. Zwitterionic Structures of Amino Acids.

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–^{15}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^{-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. Semiemprical Methods

The Semi empirical computational methods RM1and 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 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
l-alanine	-92.7728	-98.13294
d-alanine	-92.7728	-98.13295
l-phenylalanine	-69.7187	-73.25132
d-phenylalanine	-71.9725	-75.59622
l-aspartic	-184.2430	-189.38191
d-aspartic	-184.2430	-189.38191
l-glutamic	-185.8963	-187.89090
d-glutamic	-185.8963	-187.89090

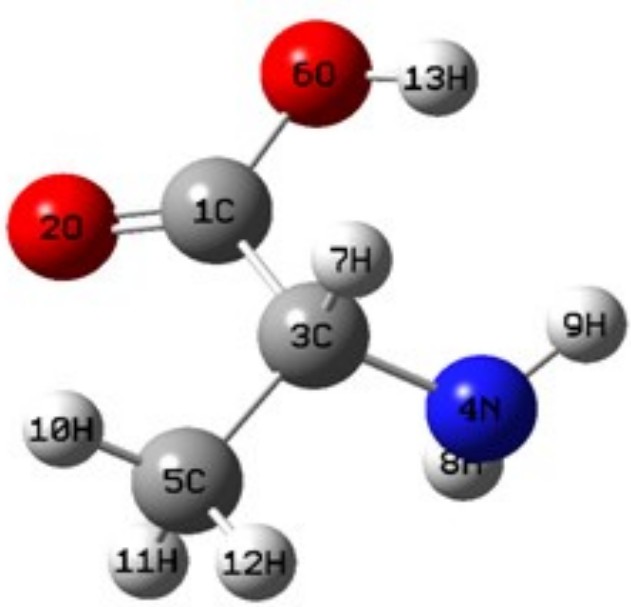

Figure 2. Molecular Structure of *l*-Alanine as Predicted by the *ab initio* Method, with the Atomic Numbering Used in This Work.

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 CH_3 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 NH_3 group. The corresponding calculated frequencies are 2691 cm^{-1}, 2633 cm^{-1} and 2748 cm^{-1} by *ab initio*, RM1 and PM6 repectively. 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 NH_2 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
ν_1	3099	3085	3082	3310	Asym. NH_2 stretching
ν_2		3071	3041	3167	CH stretching in CH_3
ν_3	2926	3065	3035	3096	Asm.CH stretching in CH_3
ν_4		2853	2936	2796	Puckering of CH_3
ν_5		2801	2924	2774	CH stretching
ν_6		2798	2903	2717	CH strething in CH_3 group
ν_7	2604	2633	2748	2691	NH stretchingin NH_2
ν_8		1809	1979	1643	Asym. COO- stretching + NH bend.
ν_9	1595	1514	1755	1522	NH_2 bending
ν_{10}	1354	1410	1475	1354	NH_2 scissoring
ν_{11}	1307	1256	1328	1343	NH_3 puckering
ν_{12}		1245	1285	1276	CH_3 deformation
ν_{13}		1226	1248	1262	CH_3 deformation
ν_{14}		1198	1246	1195	CH_3 puckering
ν_{15}		1194	1230	1150	Mixed vibration (NH_2puck + CH_3 def
ν_{16}		1182	1184	1133	CH bend. + NH bend.
ν_{17}	1149	1104	1176	1044	CH_2 bend. + CH bend. + NH_2 bend
ν_{18}	1114	1062	1165	1005	C-C stretch + CH bend. + NH bend.
ν_{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*-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^{-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 CH_2 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 CH_2 stretching and symmetric CH_2 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.

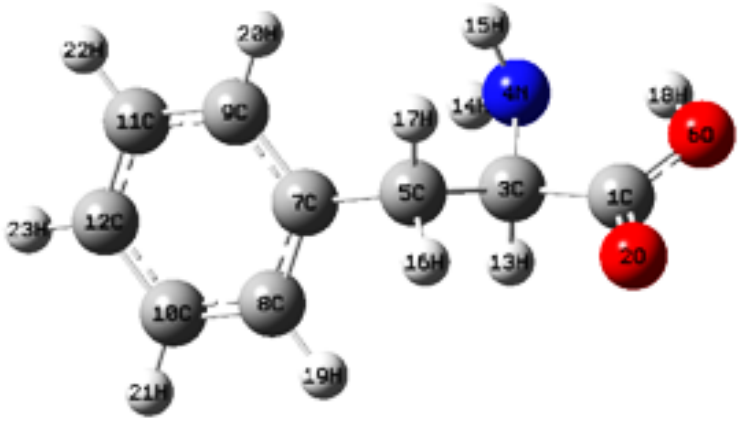

Figure 3. Molecular Structure of *l*-Phenylalanine.

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

ν_{20}		1239	1216	1251	NH symmetric bending
ν_{21}		1231	1208	1227	COO^- symmetric stretching
ν_{22}	1218	1222	1191	1213	CH_2 bending
ν_{23}		1190	1170	1201	CH bending in phenyl ring
ν_{24}	1161	1174	1168	1157	C-C stretching
ν_{25}	1130	1154	1155	1119	CH_2 bending
ν_{26}	1097	1142	1144	1095	Phenyl ring Stretching
ν_{27}	1081	1125	1133	1084	CH bending in phenyl ring
ν_{28}		1109	1122	1078	C-C stretching in phenyl ring
ν_{29}	1040	1091	1090	1059	CH bending in phenyl ring
ν_{30}		1058	1079	1019	CH bending in phenyl ring
ν_{31}		1048	1061	995	NH rocking
ν_{32}		1041	1052	980	CH rocking in phenyl ring
ν_{33}		1011	1035	938	Out-of- plane CH stretching in phenyl
ν_{34}		993	984	924	NH rocking
ν_{35}		970	965	903	CH bending in phenyl ring
ν_{36}		925	941	892	Out-of- plane CH stretching in phenyl
ν_{37}	879	907	922	871	Out-of- plane CH stretching in phenyl
ν_{38}	859	885	909	835	Phenyl ring Stretching
ν_{39}		881	875	827	Out-of- plane CH stretching in phenyl
ν_{40}		850	853	792	CH_2 rocking
ν_{41}		814	834	776	$C\text{-}COO^-$ stretching
ν_{42}		804	823	770	Out-of- plane CH stretching in phenyl
ν_{43}	744	742	791	763	CH_2 rocking
ν_{44}	703	737	742	725	C-N stretching
ν_{45}		666	681	677	Out-of- plane CH stretching in phenyl
ν_{46}	642	613	647	645	COO^- bending
ν_{47}		575	597	632	Out-of –plane ring deformation
ν_{48}	579	555	573	564	COO^- waging
ν_{49}		529	557	549	In plane ring deformation
ν_{50}	480	472	529	510	In-plane ring deformation
ν_{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.

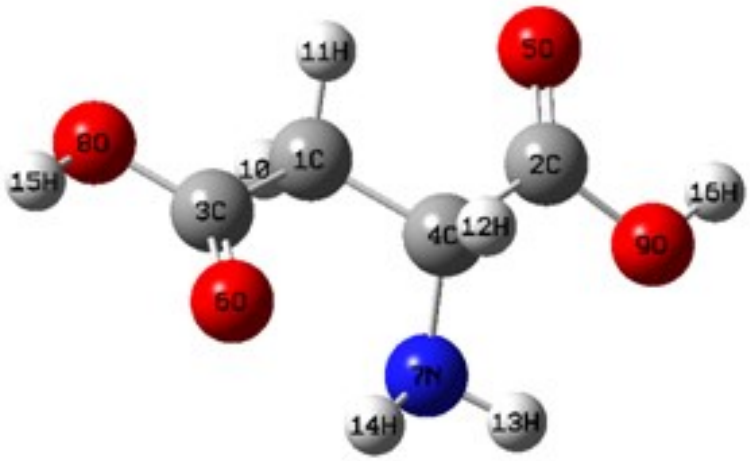

Figure 4. Molecular Structure of *l*-Aspartic Acid.

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

ν_5	2974	2774	2904	2770	CH_2 asymmetric stretching
ν_6	2965	2763	2884	2758	CH_2 symmetric stretching
ν_7	2935	2710	2876	2736	CH_2 symmetric stretching
ν_8		2706	2767	2721	NHN bending + C=O stretching
ν_9		2621	2618	2709	NHN in-plane bending
ν_{10}	1781	1820	1806	1610	C=O stretching
ν_{11}	1635	1799	1783	1589	CH bending + HCH bending
ν_{12}	1405	1511	1502	1512	CH in-plane bending
ν_{13}	1386	1429	1348	1398	CO stretching + CH_2 torsion
ν_{14}	1337	1413	1340	1319	C-C stretching + CO stretching
ν_{15}	1301	1266	1328	1277	CH_2 torsion
ν_{16}	1279	1243	1281	1263	CH_2 torsion + NH_2 in-plane bending
ν_{17}		1235	1269	1236	C-OH asymmetric stretch. in COOH
ν_{18}	1234	1218	1248	1221	CN stretching
ν_{19}		1195	1237	1197	C—CH_2-C bending
ν_{20}		1190	1223	1170	C-C stretching
ν_{21}	1139	1177	1130	1138	OH bending + CH_2 torsion + CN stretching
ν_{22}	1124	1165	1120	1125	OH in-plane bending
ν_{23}	1108	1131	1097	1091	CN stretching + OH in-plane bending
ν_{24}	1092	1063	1091	1060	C-C stretching
ν_{25}	1024	1089	1066	1043	C-C stretching + CH_2 rocking + CO stretch.
ν_{26}		1072	1052	997	CC stretching + NH_2 in-plane bending
ν_{27}		1012	1031	946	CH_2 rocking + CC stretching
ν_{28}		994	991	920	OH rocking
ν_{29}	886	951	932	889	CC stretching + CO stretching
ν_{30}	814	877	908	826	HOCC out-of-plane bending + CH_2 rock.
ν_{31}		867	874	803	OH wagging + CH_2 wagging
ν_{32}	754	790	837	752	CH_2 rocking
ν_{33}		662	740	705	NH_2-C-COOH bending
ν_{34}	668	590	660	689	HOCC out-of-plane bending
ν_{35}	646	577	607	640	NH_2 out-of-plane bending
ν_{36}	612	559	598	607	COOH in-plane bending

ν_{37}	570	528	577	568	OH out-of-plane bending
ν_{38}	553	522	538	510	OH out-of-plane bending
ν_{39}		481	505	496	COOH in-plane bending
ν_{40}		459	499	471	COOH in-plane bending
ν_{41}		409	452	420	CCC in-plane bending + CNN in-plane ben
ν_{42}		331	337	322	CCC in-plane bending
ν_{43}		320	277	275	CCN in-plane bendin
ν_{44}		282	266	265	NH_2 torsion
ν_{45}		235	238	248	CCC in-plane bending

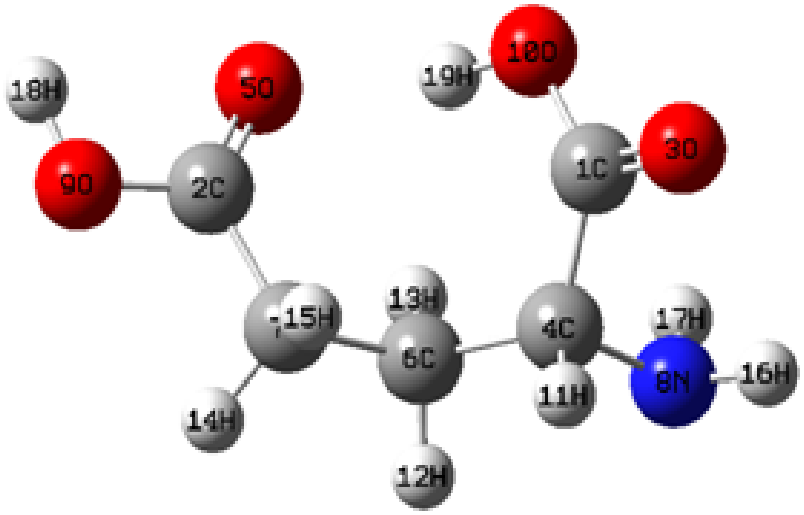

Figure 5. Molecular Structure of *l*-Glutamic Acid.

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 vibrationa 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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