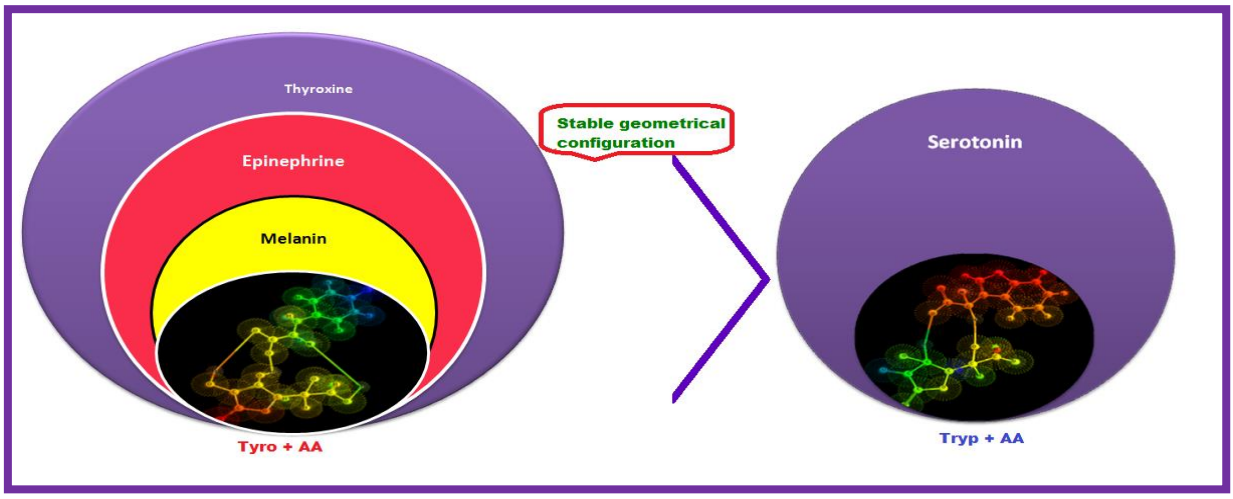
CHAPTER-IV

Assorted Interactions of Amino Acids Prevailing in Aqueous Vitamin C Solutions Probed by Physicochemical and Ab-Initio Contrivances

Highlights ⇒

- Geometry- optimised extended conformation obtained for amino acids prevailing in Aqueous (Vitamin C) Solutions.
- The solute – solvent interactions is maximum in Tyrosine.
- C-13 NMR spectra as well as Ab-initio are more reliable and supportive to study the Solute - Solvent interactions.
- Ascorbic acid acts as a co-enzyme which leads to the essential benefits of Tyro in presence of Ascorbic acid in various catabolism reactions in human body.
- The ion-solvent interactions dominate beyond the ion-ion interactions in studied solution.

Graphical Abstract ⇒



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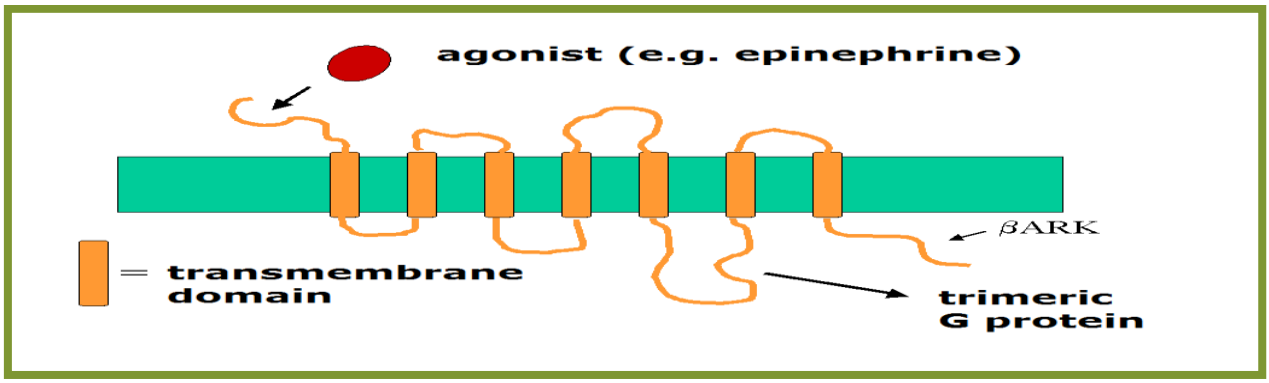
Abstract

Qualitative and quantitative analysis of molecular interaction prevailing in Tyrosine and Tryptophan in aqueous solution of Vitamin C have been probed by thermophysical properties.

The apparent molar volume (ϕ_V), viscosity *B*-coefficient, molal refraction (R_M) of tyrosine and tryptophan have been studied in aqueous vitamin C solutions at diverse temperatures via Masson equation which deduced solute-solvent and solute-solute interactions, respectively. Spectroscopic study along with physicochemical and computational techniques provides lots of interesting and highly significant insights of the model biological systems. The overall results established strong solute-solvent interactions between studied amino acids and vitamin C mixture in the ternary solutions.

1. Introduction

Amino acids, basic component of protein molecules particularly important in biochemistry are serious for life processes, and have countless functions in metabolism. Components of supreme significance of cell membranes are a variety of proteins. These membranes are thin, fluidic and highly flexible in nature which permits selective passage of materials from and into the cells plus permit lateral flow of membrane components in itself. A Transmembrane protein is a vital type of protein that spans total of the biological membrane to which it is permanently attached. Several Transmembrane proteins function as gateways to permit the transport of exact substances across the biological membrane. They habitually undergo significant conformational changes to shift a substance through the membrane. The Transmembrane proteins, viz., helical bundles, barrel proteins, localize aromatic amino acids (especially tyrosine, tryptophan) at the membrane/water interface where they form functionally noteworthy H-bonds with interfacial water.



A vitamin is an organic compound fundamental by an organism as a vital nutrient in restricted amounts. It is essential precursors for various coenzymes. These coenzymes are therefore required in almost each metabolic pathway [1]. Importantly ascorbic acid is also able to regenerate additional antioxidants as Vitamin E. Vitamin C is essential for the synthesis of

collagen, the intercellular “cement” which gives the configuration of muscles, vascular tissues, bones, and tendon. Vitamin C with Zn is also significant for healing wounds. It is also crucial for the metabolism of bile acids which may have implications for blood cholesterol levels as well as gallstones. Ascorbic acid and its sodium, potassium, and calcium salts are used as antioxidants food additives. It has an important role for synthesis of several chief peptide hormones, neurotransmitters and creatinine. It furthermore enhances the eye’s ability and delay the progression of advanced age linked muscular degeneration [2]. The molecular structures of the considered amino acids and vitamin C are represented in (scheme 1).In sight of the complexity and in extension of our earlier study [3-7]a simple model has been investigated in this paper to locate the nature of solute solvent interactions of aromatic π-system of tryptophan and tyrosine in 0.01, 0.03, and 0.05 mass fractions of aqueous ascorbic acid (AA), binary mixtures at different temperatures in order to understand the mechanisms involved in many vital events involving cell membranes. Spectroscopic study along with physicochemical and computational techniques provides scores of interesting and highly significant insights of the model biological systems.

2. Experimental Section

2.1 Source and purity of samples

The studied amino acids and co-solute vitamin were purchased from Sigma-Aldrich, Germany. The mass purity of salts was ≥ 0.99. The salts were preserved into a desiccator prior to use.

2.2 Apparatus and Procedure Prior to the start of experimental work solubility of amino acids and chosen vitamin C have been specifically checked in triply distilled water and observed that the selected aqueous Vitamin C were freely soluble in all proportion of amino acids . Every stock solutions of Vitamins were set by mass (weighed by Mettler Toledo AG-285 with uncertainty 0.0003 g), and then the working solutions were obtained by mass dilution at 298.15 K. Aqueous Vitamin binary solution used as solvent system. The uncertainty within molality of solutions evaluated to ±0.0001 mol kg^{-3}.Solution densities (ρ) were dignified by vibrating-u-tube Anton Paar digital density meter (DMA 4500M) by a precision of 0.00005 $g.cm^{-3}$ maintained at ±0.01K.

Viscosities measurements were done via a Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42. The viscosities were obtained using following equation

$$\eta = (100 / RPM) \times TK \times torque \times SMC \quad (1)$$

Where RPM, TK (0.09373),SMC (0.327) are speed, viscometer torque constant, spindle multiplier constant respectively. The instrument was calibrated against standard viscosity samples with the instrument provided, water and aqueous $CaCl_2$ solutions [8]. Temperature of the solution was maintained within ± 0.01°C with Brookfield Digital TC-500 thermostat bath.

Digital Refractometer Mettler Toledo was used to measure refractive index. The refractometer was rectified twice by distilled water and calibration was tested after each few measurements.

NMR spectra were taken in D_2O using Bruker AV-300 spectrometer operating for 1H at 300 MHz and for 13C at 75 MHz. Splitting patterns of protons were described as singlet, doublet, triplet, broad and multiplet. Chemical shifts (δ) were reported in parts per million (ppm) relative to TMS as internal standard (D_2O: δ 4.79 ppm).

Fluorescence spectra were noted via JASCO V-530 UV/VIS Spectrophotometer, with an uncertainty of wavelength resolution of ±2 nm. Measuring temperature was detained constant by a thermostat.

Ab-initio calculations are implemented through Gaussian 09W quantum chemical package [9].

3. Results and Discussion

3. 1. Apparent molar volume Physical properties of binary mixtures in different mass fractions (w_1=0.01, 0.03, 0.05) of aqueous ascorbic acid solutions at diverse temperatures are reported in (Table 1).(Table 2) describes experimental values of densities, viscosities, refractive indices, of studied amino acids for different mass fractions of aqueous AA mixture at different temperatures. For understanding of interactions volumetric properties, such as ϕ_V , ϕ_V^0 , observed as reflective tools in solutions. For this purpose, apparent molar volumes ϕ_V was calculated from the solutions densities with the help of the following equation and the values are specified in (Table 3).

$$\phi_V = M / \rho \; - \left(\rho - \rho_o\right) / m\rho_o\rho \quad (2)$$

Where M molar mass of the salt, m is the molarity of the solution, ρ and ρ_0 are density of the solution and aq. AA mixture respectively.

The positive values of ϕ_V in (Table 3) illustrates of strong solute-solvent interactions. There was a decrease in trend of apparent molar volumes ϕ_V with increase in molarity (*m*) of amino acid in aqueous AA. It indicates ion–solvent interactions increase with increase in concentration (w_1) of amino acids. The limiting apparent molar volumes ϕ_V^0 were obtained by a least-square method by plotting ϕ_V versus √*m* using Masson equation [10-13].

$$\phi_V = \phi_V^0 + S_V^* \cdot \sqrt{m} \qquad (3)$$

Where ϕ_V^0 represents apparent molar volume at infinite dilution, S_V^* is the experimental slope. ϕ_V^0 values were obtained by fitting dilute data (m< 0.1 mol·kg^{-1}) to equation 3. At infinite dilution, each monomer of solute is bordered only by solvent molecules, and being infinitely detached by other ones. It follows therefore, ϕ_V^0 is unaffected by solute-solute interaction and is a measure of solute-solvent interaction[14]. ϕ_V^0 data are often bounded with important information of solute–solvent interactions [15] happened in aqueous AA solution. Values of ϕ_V^0 and S_V^* are reported in (Table 4).A review of (Table 4) displays values of ϕ_V^0, positive for the amino acids for all the studied temperatures, proposing existence of strong solute–solvent interaction [16]. Besides, every temperature, values of ϕ_V^0 increase with increasing interacting centre from Tryptophan to Tyrosine. Banipal et al [17] also reports a comparable increase in ϕ_V^0 with increasing number of carbon atoms used for amino acids in aqueous glycerol, at 298.15 K. The performance of ϕ_V^0 for present systems can be described by means of co-sphere model, planned by Friedman and Krishnan [18], according to which consequence of overlap of hydration co spheres is destructive. Mishra et al. [19] using this model observed an overlap of co spheres of two ionic species causes an increase in volume, whereas an overlap of hydrophobic–hydrophobic groups, ion–hydrophobic groups grades in a net decrease in volume. Values of ϕ_V^0 and S_V^* for amino acids in pure water is implemented from the literature [20]. The parameter S_V^* is volumetric coefficient, and it describes the pair wise interaction of solute species in solution phase [21]. A quantitative assessment in (Table 4)between ϕ_V^0 and S_V^* values display the magnitude of ϕ_V^0 values is higher than S_V^*,

suggesting that ion-solvent interaction dominate above the ion-ion interactions in studied solution[22].

3.2 Temperature dependent limiting apparent molar volume: The deviation of ϕ_V^0 with the temperature of the amino acids in aqueous vitamin mixture has been confirmed by general polynomial equation as follows,

$$\phi_V^0 = a_0 + a_1 T + a_2 T^2 \qquad (4)$$

Where a_0, a_1, a_2 are the empirical coefficients depending on the solute, mass fraction (w_1) of the co-solute vitamin, and T is temperature in Kelvin. The coefficients values of the above equation for amino acids in AA mixtures are described in (Table 5).

The limiting apparent molar expansibilities, ϕ_E^0, are calculated by the following equation below,

$$\phi_E^0 = \left(\delta\phi_V^0/\delta T\right)_P = a_1 + 2a_2 T \qquad (5)$$

The values of ϕ_E^0 for diverse solutions of the investigated amino acids at (298.15, 303.15, and 308.15) K are reported in (Table 6). The table divulges that ϕ_E^0 is positive for all the vitamins in AA in studied temperatures. This fact can endorsed to the absence of caging or packing effect for the amino acids in solutions.

Throughout the past few years it has been highlighted by different workers that S_V^* is not the sole reason for determining the structure-making or -breaking nature of any solute. Hepler [23, 24] established a procedure of observing the sign of $\left(\delta\phi_E^0/\delta T\right)_P$ for the solute in terms of long-range structure-making and -breaking capacity of the solute in the AA using the following general thermodynamic equation,

$$\left(\delta\phi_E^0/\delta T\right)_P = \left(\delta^2\phi_V^0/\delta T^2\right)_P = 2a_2 \qquad (6)$$

It was found in [25] that if the sign of $\left(\delta\phi_E^0/\delta T\right)_P$ is positive, the molecule is a structure maker; otherwise, it is a structure breaker. From (Table 6) in our experiment, the $\left(\delta\phi_E^0/\delta T\right)_P$ values for both the amino acids are positive under investigation are predominantly structure makers in AA solutions.

3.3. Viscosity The relative viscosity (η_r) has been investigated by the Jones-Dole equation [26]. The viscosity records for the calculated systems are listed in (Table 2).

$$(\eta/\eta_o - 1)/\sqrt{m} = (\eta_r - 1)/\sqrt{m} = A + B\sqrt{m} \qquad (7)$$

Where $\eta_r = \eta/\eta_o$, η and η_0 are relative viscosities, the viscosities of the ternary solutions (amino acid + aq. AA) and binary aqueous AA mixture and *m* is the molarity of the ternary solutions. *A*and *B* are empirical constants known as viscosity *A*- and *B*-coefficients, which identifies to solute-solute, solute-solvent interactions respectively. The values of *A* and *B*-coefficients are reported in (Table 4).

In this experiment we found that the values of the *A*-coefficient increases marginally with the increase in mass of AA in the solvent mixture indicating the presence of very weak solute-solute interactions. These results support the agreement obtained from S_V^* values.
The viscosity *B*-coefficient value [27] gives suitable information concerning the solvation of the solvated solutes and their effects on the structure of the solvent in local vicinity of the solute molecules in the solutions. It is found that the values of the *B*-coefficient presented in (Table 4) are positive and much higher than *A*-coefficient, signifying the solute-solvent interactions are overriding over the solute-solute interactions.

(Table 4) in the manuscript displays that the values of the *B* coefficients of both amino acids marginally increase with increasing temperature, i.e., the dB/dT values are positive. Small positive dB/dT values in (Table 7) for the present amino acids perform nearly as structure-makers.

3.4. Refractive index The values of refractive index of the work are reported in (Table 2). The molar refraction R_M can be evaluated from Lorentz-Lorenz relation [28]

$$R_M = \left\{(n_D^2 - 1)/(n_D^2 + 2)\right\}(M/\rho) \qquad (8)$$

Where R_M, n_D, M and ρ are molar refraction, refractive index, molar mass and density of solution respectively. The ratio c_o/c represents refractive index of a substance where c is the speed of light in the medium and c_o the speed of light in vacuum. The refractive index of a compound describes its ability to refract light as it moves from one medium to another and thus, higher the refractive index of a compound, more the light is diverted [29]. According to

Deetlefs et al. [30] refractive index of a substance is higher when its molecules are more forcefully packed or in general when compound is denser. Hence, a survey of (Table 2 and 3) we found that the refractive index (n_D) and the molar refraction (R_M) respectively increases linearly with an increasing concentration of solutions.

3.5. Explanation for 1-H NMR- 300-MHz 1H N.M.R. spectra of the deuterated species of aromatic amino acids was analyzed. In case of 1H-NMR though we cannot compare the extent of H-bonding but we can conform the positioning of H-bonding, if we tally the both system's NMR then we can see that H4 and H5 protons in Tyro system, showing more upfield shift comparing in Tryp system, whereas in Tryp H4, H5, H6 peaks came all together in same position. On that account we can say that the OH gr in ascorbic acid adjacing with H4 and H5 proton for Tyro system is participating in H-bonding whereas in case of Tryp system, the OH-group which holds H6 in ascorbic acid, is participating in H-bonding. Since all the 1H-NMR were taken in D_2O system, we were unable to demonstrate the interaction between –OH and carboxylic acid groups; since those groups donot show any peak in NMR spectra[31, 32].

3.6. Explanation for 13-C NMR -From 13-C NMR we can easily say that interaction of ascorbic acid with tyrosine, is much more prominent than that of tryptophan because in Tryp there is only two H-bonding is possible that's why C7 and C5 carbon peaks are coming in NMR whereas other C's are missing in NMR (C4,C6 and C8) which confirms that two position H-bonding is happening but in case of tyrosine not only C5 and C7 but C8 also coming in NMR that means in tyrosine there, three H-bonding is happening[33, 34].The effect can be evaluated by constructing a two-dimensional energy diagram with respect to the orientations of the three –H bonds (Scheme 2).The evaluation of the above special effects is a significant computational challenge and left to a subsequent work [35-37].

3.7. Tryp/Tyro- fluorescence study- As reported in former studies for an excitation wavelength of 280 nm, both Tryp and Tyro will be excited. To selectively excite Tryp only, 295 nm wavelengths must be used. "Tryp + H_2O" was seen to be excited in the wavelength of 348 nm and similarly " Tyro + H_2O " was examined to be excited in the range of 303 nm the reason behind the shift is as the polarity of the solvent increases, the spectrum shifts to longer wavelengths and decreases in intensity. Vitamin-C does not fluoresce and so, derivatived ascorbic acid was prepared so that the chemical will fluoresce. This is done oxidizing ascorbic acid to dehydroascorbic acid in the presence of activated charcoal block which by itself along

with H_2O generate fluorescence within the range of 348 nm. The fluorescence intensity is proportional to the concentration of ascorbic acid [38-42].According to the present site, aqueous ascorbic acid concentration is kept fixed and the concentrations of amino acids were varied to generate fluorescence band. The fluorescent spectrophotometer then is applied to determine the fluorescence intensity of the fluorescent substances generated [fig 5, 6].

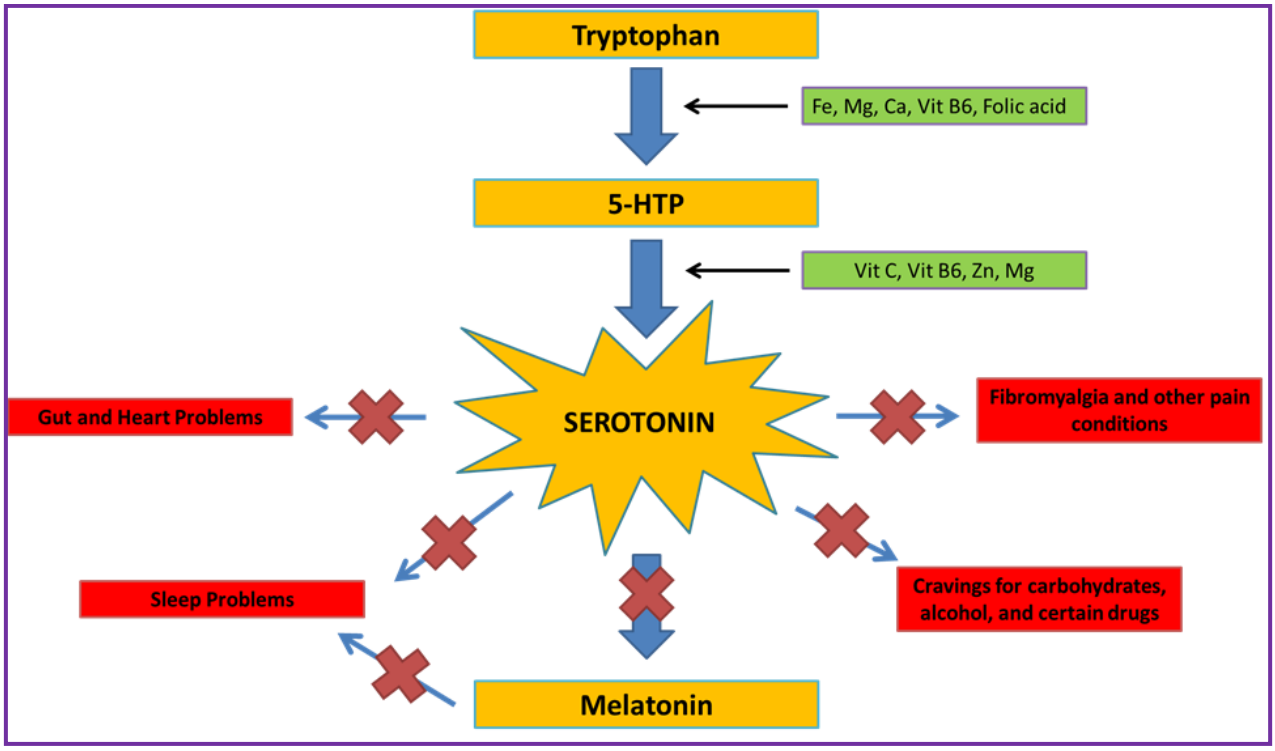
For tyrosine the excitation was affected at 275 nm and the emission spectra was observed at 305 nm and for the Tryp the excitation was affected at 295 nm and the emission spectra was observed at 355 nm, slide widths both set as 5 nm. From fluorescence study we can say that the extent of interaction between Tyro and ascorbic acid is much more prominent than that of Tryp because as interaction increases fluorescence intensity decreases and in Tyro as we increase the concentration, intensity gradually decreases and becoming low and low that means it is showing very good interaction which is not the case for tryptophan because FL intensity is not changing with gradual decrement. The Tyro emission is not sensitive to the polarity of environment as seen in the aforementioned studies[43-45] and thus has more probability to interact with ascorbic acid which could be explained even on the basis of dipole moment[46-48].[fig 7, 8].

3.8. Ab Initio Quantum Chemical Methodologies- This field is of great theoretical interest. Here there is no use of sequence alignments and no direct use of known structures. The basic idea is to erect empirical function that simulates real physical forces and potentials of chemical contacts. In this paper, numerical calculations have been performed using UB3LYP functional. Diffused basis functions have often been found to be effective in describing weak interaction among atoms. Hence we use 6-31+G(d) basis function for a correct description of weak interactions which may prevail in the transition structures. These calculations are implemented through Gaussian 09W quantum chemical package. The quantum chemical calculations estimate that the N…O-H, H…O-H,C=O…H-O weak H-bond interactions in tyrosine, in the same way C-H…O,O-H…C weak H-bond interactions in tryptophan predominantly exists as [Scheme 2] and [Scheme 3] clusters in the solvent sphere of aqueous ascorbic acid. Previous review works deliberated – Firstly, The weak hydrogen bond is an interaction wherein X–H···A wherein a hydrogen atom forms a bond between two structural moieties X and A, of which one or even both are of moderate to low electronegativity (1999) (Desiraju–Steiner definition). Secondly, any cohesive interaction X–H•••A where [H] carries a positive and [A] a negative (partial or full)

charge and the charge on X is more negative than on H (Steiner–Saenger definition). Thirdly, a hydrogen bond is said to exist when (a) there is evidence of a bond, and (b) there is evidence that this bond specifically involves a hydrogen atom already bonded to another atom(Pimentel–McClellan definition) (1960). Under certain conditions an atom of hydrogen is attracted by rather strong forces to two atoms instead of only one, so that it may be considered to be acting as a bond between them Pauling definition (1939). So, the aforesaid statements were well thought-out in our present work [49-57]. We have portrayed the existence of Tyro...AA and Tryp...AA complexes as clusters through quantum chemical calculations and we hereby compare calculated spectral values with the experimental spectral values in order to explain the shifts. Several estimated properties of assorted systems of Tyro (ascorbic acid)$_{aq}$ and Tryp(ascorbic acid)$_{aq}$ clusters are summarized in (Table 8).The stabilization of Tyro (ascorbic acid)$_{aq}$ clusters is revealed by the value of *E*, the optimization energy, which decreases in magnitude with the number of solute-solvent interactions up to three. From the calculation, Tyro (ascorbic acid)$_{aq}$ was found to be additional stable than other complexes as it has minimum value of *E*. For the complexation if more than three interactions are shown then value of *E* once more increases. Specifically, the optimum geometry would involve central Tyro is surrounded by aq-ascorbic acid molecules by the weaker H-bond interactions which can be explained on the basis of solution thermodynamics as solute-solvent interactions [58-63] [fig. 9(a), 9(b)].

Novelty behind the work When Tryp reacts in the presence of 5 moles of ascorbic acid, the product formed is serotonin. Obviously it is good quality for health, as it releases energy through TCA cycle. The beneficial task of Serotonin is not limited up to here. The significance arises when Serotonin is used as neurotransmitter and a vasoconstrictor. On the other hand it also has some adverse effects in a way such that if the concentration of Serotonin is increased than one have the tendency to get mad. In contrast Tyrosine leads to the catabolism in various pathways , in the former step it is transaminated to give Para –hydroxyphenylpyruvic acid by tyrosine transaminase in the presence of PLP(Pyridoxal phosphate (PLP, pyridoxal 5'-phosphate, P5P), the active form of vitamin B_6, is a coenzyme in a variety of enzymatic reactions.) It is induced by glucocorticoids. In subsequent step of the reaction a shifting of the side chain from para position to meta position takes place. Ascorbic acid is helpful in this reaction and thus acts as a co-enzyme of hydroxylase enzyme. Important specialized products from Tyro thus

obtained are Melanin, Catecholamines(epinephrine), Thyroxine .This leads to the essential benefits of Tyro in presence of AA in human body (Georg A. Sprenger, Aromatic Amino Acids; Paul M. Dewick. Medicinal Natural Products: A Biosynthetic Approach; Lehninger A. L., Lehninger Principles of Biochemistry).



Conclusion From the NMR data we can say that there is no peak for acid proton, so we can say that acid group is participating in H-bonding, the proton adjacent to $–NH_2$ group is also missing in the NMR. So, we can say may be some of the –OH group is also participating in H-bonding. In this work we have shown C-13 NMR spectra which are more reliable and supportive to study the Solute- Solvent interactions. We know from literature; Dipole fields of water molecules weakens the field amongst the cation and its corresponding anions. The weakening of this field and the associated weakening of the bonds of the cation to its corresponding anions is naturally stronger, the additional water molecules are attached, and the more their dipole moments are increased not only by the electrostatic but also by the covalent Solute-Solvent interactions. Imidazole ring is present in Tryp and so it is more polar and thus it has much more degrees of hydration, and more dipole moment (as calculated from the Ab-initio technique) i.e. why the shift is not like that of Tyro where only benzene ring is present in the co-ordination sphere of the solvation. So, it is confirmed from all the

experimental and theoretical calculations that the solute – solvent interactions is maximumin Tyrosine than that of the Tryptophan system.

Abbreviation: Tyrosine = Tyro, Tryptophan = Tryp, Fluorescence intensity = Fl intensity, Aqueous = aq, Aqueous ascorbic acid = (AA)

Keywords: Solute-solvent interaction, amino acids, Vitamin C, Fluorescence spectra and NMR Spectra.

Notes and Reference

1. Robinson, F. A.,(1951). The Vitamin B-Complexes: Chapter 4, Chapman&Hall: London.
2. M.N.Roy*, R K. Das, A.Bhattacharjee, Russian J. Phys. Chem A., 84, (2010) 2201.
3. D. Ekka,M.N. Roy*,J. Phys. Chem. B.,116,(2012)11687-11694.
4. D.Ekka,M.N.Roy*, Amino Acids.,45, (2013) 755-777.
5. M.N.Roy*, D.Ekka,S.Saha,M.C.Roy, RSC Adv.,4, (2014)42383-42390.
6. M.N.Roy*, T. Ray, M.C. Roy,B. Datta,RSC Adv., 4, (2014) 62244-62254.
7. M.N.Roy*,M.C.Roy, K. Roy, RSC Adv., 5, (2015)56717-56723.
8. I.M Abdulagatov, N.D Azizov.,Fluid Phase Equilibria240(2)**,**(2006)204.
9. D.O Masson,Phil. Mag. 8**,** (1929)218-223.
10. F. Millero,J. Chem. Rev.71**,** (1971) 147-176.
11. Y. Marcus, G.Hefter,Chem. Rev.104, (2004)3405.
12. Y.Marcus,J. Chem. Soc., Faraday Trans. 89, (1993) 713-718.
13. F.J.Millero, 1972. The partial molar volumes of electrolytes in aqueous solutions, in: R.A. Horne (Ed.), Water and Aqueous Solutions: Structure, Thermodynamics, and Transport Processes Wiley Interscience, New York.
14. K. Belibagli, E. Agranci,J. Solution Chem., 19, (1990) 480-498.
15. T.S. Banipal, G. Singh, B.S.Lark,J. Solution Chem., 30,(2001) 657-670.
16. H.L.Friedman, C.V.Krishnan,(1973).Water: A comprehensive Treatise; F. Franks, Ed., Vol. 3, Chapter 1. Plenum, New York.
17. A.K.Mishra, K.P.Prasad, J.C.Ahluwalia,Biopolymers.,22, (1983) 2397-2409.
18. F.J. Millero, A.L.Surdo, C.Shin,J. Phys. Chem.82, (1978) 784.
19. XuLi.,C.Ding, R. Lin,J. Solution Chem.35, (2006) 191.
20. R.K.Wadi, P.Ramasami,J. Chem. Soc. Faraday Trans.93, (1997) 243.
21. T.S. Banipal, D. Kaur, P.K. Banipal,.J. Chem.Eng.Data. 49, (2004) 1236.

22. M.N.Roy*,V.K.Dakua,B.Sinha,Int J Thermophys., 28, (2007) 1275.
23. M.N.Roy*, V.K. Dakua, B.Sinha,Int J Thermophys., 28, (2007) 1275.
24. G. Jones, D. Dole,J Am Chem Soc. 51, (1929**)**2950.
25. Z. Yan, J.Wang,J. Lu, Biophys. Chem.99, (2002) 199.
26. V.Minkin, O. Osipov,Y. Zhdanov,(1970). Dipole Momentsin Organic Chemistry. New York, Plenum Press.
27. M.Born,E.Wolf,(1999).Principles of Optics: Electromagnetic Theory of Propagation,Interference and Diffraction of Light (Cambridge University Press; London, 7th Ed.
28. M. Deetlefs, K. Seddon, M. Shara, Phys. Chem. Chem. Phys., 8, (2006) 642.
29. T. Loftsson, Cyclodextrins in drug deliveryExpert Opinion Drug Delivery, **2**(2), (2005)335.
30. K. Das, P. Bomzan, R.K.Das, B.Rajbanshi, M.N.Roy*,Chemical Physics Letters.671, (2017)7-14.
31. K. Das,M.C. Roy, and M.N.Roy*,. Journal of Advanced Chemical Sciences.3(1),(2017) 428-433.
32. T. Ray, and M.N.Roy*, RSC Adv.,5, (2015) 89431.
33. V. Singh, G. Sharma, R.L.Gardas,PLoS ONE; 10(5),(2015)e0126091.
34. S. Saha, T. Ray, S. Basak, andM.N. Roy*,New J. Chem., 40, (2016) 651.
35. R.E.Luna, S.R.Akabayov, J.J Ziarek and G. Wagner, FEBS Journal; 281,(2014) 1965–1973.
36. T. Kimura, N.Matubayasi, and M. Nakahara,Biophys J.; 86(2), (2004) 1124–1137.
37. J. Kobayashi, T. Higashijima, T. Miyazawa,Int J Pept Protein Res., 24(1), (1984) 40-7.
38. J. Poznański,A. Ejchart, K.L. Wierzchowski, M. Ciurak,Biopolymers., 33(5),(1993) 781-95.
39. A.T. Timpennan, K.E. Oldenburg, and J.V.Sweedler*Anal. Chem. 67, (1995)3421 – 3426.
40. Chen RF.,(1967). Fluorescence Quantum Yields of Tryptophan and Tyrosine Analytical Letters. Analytical Letters,volume 1:
41. J.T.Vivianand P.R.Callis,Biophysical Journal.,80,(2001) 2093–2109.
42. T.E. JrNeedham, A.N.Paruta, R.J.Gerraughty,Journal of Pharmaceutical Sciences.60,(1971) 565–567.

43. T.E. JrNeedham, A.N. Paruta, R.J. Gerraughty,Journal of Pharmaceutical Sciences.,60,(1971)258–260.
44. L.A. Munishkina, A.L.Fink,BiochimicaetBiophysicaActa., 1768, (2007)1862–1885.
45. Principles of Fluorescence Spectroscopy 2nd Edition (1999).Lakowicz, J.R.Editor, Kluwer Academic/Plenum Publishers, New York, New York.
46. A[1]Sillen, J.F Díaz.,Y. Engelborghs,Protein Sci., 9(1), (2000)158-69.
47. Stenesh J.,Biochemistry. (2013).
48. YauWai-Ming, C. William Wimley.,§,| Klaus Gawrisch.,‡ and Stephen H. White*., 37,(1998) 14713-14718.
49. J. ShoujunXu,Nilles.Michael, and Kit H. Bowen., Jr. a.Journal Of Chemical Physics.,119 ,(2003) 20 .
50. L.A. Munishkina, A.L.Fink,Biochimica et BiophysicaActa., 1768, (2007) 1862–1885.
51. J. Antosiewicz,Biophysical Journal. 69, (1995)1344-1354.
52. G.R.Desiraju, Acc. Chem. Res., 35,(2002) 565-573.
53. G.R.Desiraju, T.Steiner,(2001).The weak hydrogen bond: in structural chemistry and biology.
54. Jeffrey GA., Jeffrey GA.,(1997). Anintroduction to hydrogen bonding.
55. GR.,(2001).Chemistrybeyond the molecule – Nature. 412(6845), 397-400.
56. D[1]Das , G.R Desiraju,Chem Asian J., 1(1-2), (2006)231-44.
57. G.R Desiraju,ChemCommun ., (24), (2005)2995-3001.
58. P.J. Langley, J. Hulliger, R Thaimattam, G.R. Desiraju, *New J. Chem.*, *22*, (1998)1307-1309.
59. S.Sarkhel, G.R. Desiraju,.PROTEINS: Structure, Function, and Bioinformatics., 54 (2), (2004) 247-259.
60. S.K Panigrahi, G.R Desiraju,Proteins., 67(1), (2007)128-141.
61. G. A. Sprenger.,(2006). AromaticAmino Acids. Chapter,Amino Acid Biosynthesis ~ Pathways, Regulation and Metabolic Engineering, Volume 5 of the series Microbiology Monographs 93-127.
62. Paul M. Dewick ., Medicinal Natural Products: A Biosynthetic Approach.
63. Lehninger A. L., Nelson D. L., and Cox M. M.,Lehninger Principles of Biochemistry.

Tables:

Table 1.Values of density (ρ), viscosity (η), at 298.15, 303.15, 308.15 K and refractive index (n_D) at 298.15 K in different mass fraction (w_1) of aqueous Ascorbic Acid

Mass fraction of aqAA (w_1)	Temp/K	$\rho \cdot 10^{-3}$ /kg m^{-3}	η /mPa s	n_D
	298.15	0.99786	0.82	1.3324
w_1 = 0.01	303.15	0.99623	0.74	-
	308.15	0.99543	0.66	-
	298.15	0.99937	0.83	1.3330
w_1 = 0.03	303.15	0.99692	0.75	-
	308.15	0.99528	0.67	-
	298.15	1.00074	0.84	1.3338
w_1 = 0.05	303.15	0.99776	0.76	-
	308.15	0.99612	0.69	-

[a]uncertinity of the density $\boldsymbol{u(\rho)=0.00003}$x10^{-3} $kg.m^{-3}$; viscosity $\boldsymbol{u(\eta)= 0.01mPa\ s;}$ refractive index $\boldsymbol{u(n_D)= 0.0002;}$ temperature $\boldsymbol{u(T)= 0.01K}$

Table2. Experimental values of density (ρ) and viscosity (η) at 298.15 K, 303.15 K, 308.15 K and Refractive index (n_D) at 298.15 K of amino acidsin different mass fraction ofaqueous AA (w_1)

m /mol kg^{-1}	$\rho \cdot 10^{-3}$ /kgcm^{-3}	η /mPas	n_D	m / mol kg^{-1}	$\rho \cdot 10^{-3}$ /kg cm^{-3}	η /mPas	n_D
	w_1 = 0.01						
	Tryptophan+ aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	0.99841	0.84	1.3329	0.0555	0.99989	0.86	1.3341
0.0251	0.99879	0.85	1.3333	0.0707	1.00052	0.86	1.3346
0.0403	0.99933	0.85	1.3336	0.0860	1.00114	0.87	1.3352
T = 303.15 K				T = 303.15 K			
0.0101	0.99674	0.75		0.0557	0.99818	0.77	
0.0252	0.99721	0.76		0.0710	0.99877	0.77	
0.0404	0.99779	0.76		0.0863	0.99933	0.78	
T = 308.15 K				T = 308.15 K			
0.0101	0.99591	0.67		0.0559	0.99751	0.69	
0.0253	0.99643	0.68		0.0712	0.99792	0.70	
0.0406	0.99698	0.68		0.0866	0.99848	0.71	

	Tyrosine+ aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	0.99858	0.85	1.3330	0.0555	0.99998	0.87	1.3333
0.0251	0.99894	0.86	1.3331	0.0707	1.00061	0.87	1.3334
0.0403	0.99949	0.86	1.3332	0.0860	1.00133	0.88	1.3335
T = 303.15 K				T = 303.15 K			
0.0101	0.99689	0.77		0.0557	0.99835	0.79	
0.0252	0.99737	0.78		0.0710	0.99893	0.79	
0.0404	0.99794	0.78		0.0863	0.99945	0.80	
T = 308.15 K				T = 308.15 K			
0.0101	0.99614	0.68		0.0559	0.99764	0.70	
0.0253	0.99660	0.69		0.0713	0.99814	0.71	
0.0406	0.99718	0.69		0.0867	0.99862	0.72	
	w_1 = 0.03						
	Tryptophan + aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	0.99892	0.87	1.3338	0.0555	1.00054	0.89	1.3354
0.0251	0.99942	0.88	1.3345	0.0707	1.00097	0.89	1.3360
0.0403	0.99991	0.88	1.3349	0.0860	1.00151	0.90	1.3367
T = 303.15 K				T = 303.15 K			
0.0101	0.99751	0.78		0.0557	0.99847	0.80	
0.0252	0.99788	0.79		0.0710	0.99882	0.80	
0.0404	0.99833	0.79		0.0863	0.99931	0.81	
T = 308.15 K				T = 308.15 K			
0.0101	0.99622	0.69		0.0559	0.99791	0.71	
0.0253	0.99667	0.70		0.0712	0.99834	0.72	
0.0406	0.99743	0.70		0.0866	0.99875	0.72	
	Tyrosine+ aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	0.99909	0.88	1.3342	0.0555	1.00065	0.90	1.3358
0.0251	0.99959	0.89	1.3347	0.0707	1.00107	0.91	1.3363
0.0403	1.00017	0.89	1.3353	0.0860	1.00157	0.92	1.3369
T = 303.15 K				T = 303.15 K			
0.0101	0.99775	0.77		0.0557	0.99892	0.79	
0.0252	0.99813	0.78		0.0710	1.00045	0.80	

0.0404	0.99858	0.78		0.0863	1.00089	0.81	
T = 308.15 K				T = 308.15 K			
0.0101	0.99646	0.68		0.0559	0.99791	0.72	
0.0253	0.99691	0.69		0.0713	0.99845	0.72	
0.0406	0.99744	0.70		0.0867	0.99888	0.73	
	w_1 = 0.05						
	Tryptophan + aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	0.99938	0.89	1.3344	0.0555	1.00063	0.92	1.3361
0.0251	0.99978	0.91	1.3349	0.0707	1.00097	0.93	1.3366
0.0403	1.00022	0.91	1.3356	0.0860	1.00167	0.94	1.3372
T = 303.15 K				T = 303.15 K			
0.0101	0.99804	0.81		0.0557	0.99997	0.83	
0.0252	0.99839	0.82		0.0709	1.00039	0.84	
0.0404	0.99976	0.82		0.0863	1.00088	0.85	
T = 308.15 K				T = 308.15 K			
0.0101	0.99765	0.73		0.0559	0.99871	0.75	
0.0253	0.99789	0.74		0.0712	0.99912	0.76	
0.0406	0.99833	0.74		0.0866	0.99946	0.77	
	Tyrosine+ aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	0.99962	0.90	1.3349	0.0555	1.00163	0.92	1.3362
0.0251	1.00095	0.91	1.3353	0.0707	1.00197	0.93	1.3368
0.0403	1.00136	0.91	1.3357	0.0860	1.00239	0.95	1.3373
T = 303.15 K				T = 303.15 K			
0.0101	0.99819	0.83		0.0557	1.00020	0.86	
0.0252	0.99858	0.84		0.0710	1.00038	0.87	
0.0404	0.99994	0.84		0.0863	1.00058	0.88	
T = 308.15 K				T = 308.15 K			
0.0101	0.99779	0.74		0.0559	0.99879	0.77	
0.0253	0.99808	0.75		0.0712	0.99919	0.79	
0.0406	0.99819	0.76		0.0866	0.99948	0.80	

[a]uncertinity of the density $\boldsymbol{u(\rho)=0.00003}x10^{-3}$ $kg.m^{-3}$; viscosity $\boldsymbol{u(\eta)=0.02}$mPa s; refractive index $\boldsymbol{u(n_D)=0.0001}$; temperature $\boldsymbol{u(T)=0.02}$K.

Table3. Molality (*m*), apparent molar volume (ϕ_V) and $(\eta_r - 1)/\sqrt{m}$ at 298.15 K, 303.15 K, 308.15 K and molar refraction (R) at 298.15 K of amino acids in different mass fraction ofaqueous AA (w_1)

m /mol·kg^{-1}	$\phi_V \cdot 10^6$ m^3·mol^{-1}	$(\eta_r - 1)/\sqrt{m}$ kg$^{1/2}$·mol$^{-1/2}$	R	m /mol·kg^{-1}	$\phi_V \cdot 10^6$ m^3·mol^{-1}	$(\eta_r - 1)/\sqrt{m}$ kg$^{1/2}$·mol$^{-1/2}$	R
	w_1 = 0.01						
	Tyrosine + aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	132.49(±0.02)	0.082(±0.005)	35.009	0.0555	126.96(±0.02)	0.114(±0.002)	35.096
0.0251	130.46(±0.04)	0.096(±0.003)	35.055	0.0707	124.38(±0.02)	0.119(±0.005)	34.115
0.0403	129.25(±0.01)	0.099(±0.001)	35.079	0.0860	123.79(±0.04)	0.123(±0.001)	34.134
T = 303.15 K				T = 303.15 K			
0.0101	142.89(±0.03)	0.112(±0.005)		0.0101	135.85(±0.01)	0.183(±0.006)	
0.0252	138.05(±0.04)	0.145(±0.001)		0.0252	134.38(±0.02)	0.191(±0.007)	
0.0404	136.08(±0.01)	0.161(±0.003)		0.0404	132.87(±0.06)	0.198(±0.002)	
T = 308.15 K				T = 308.15 K			
0.0101	153.86(±0.01)	0.123(±0.002)		0.0559	144.78(±0.05)	0.165(±0.008)	
0.0253	149.26(±0.02)	0.133(±0.003)		0.0712	141.07(±0.04)	0.182(±0.002)	
0.0406	145.61(±0.01)	0.145(±0.002)		0.0866	140.58(±0.02)	0.198(±0.005)	
	Tryptophan+ aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	105.50(±0.02)	0.061(±0.005)	33.762	0.0555	97.83(±0.03)	0.086(±0.009)	34.126
0.0251	103.48(±0.08)	0.069(±0.002)	33.948	0.0707	95.88(±0.02)	0.097(±0.003)	34.159
0.0403	99.22(±0.06)	0.075(±0.002)	33.988	0.0860	94.68(±0.05)	0.112(±0.002)	34.187
T = 303.15 K				T = 303.15 K			
0.0101	114.92(±0.02)	0.139(±0.002)		0.0557	106.11(±0.03)	0.167(±0.009)	
0.0252	112.48(±0.05)	0.145(±0.002)		0.0710	105.82(±0.06)	0.176(±0.006)	
0.0404	108.86(±0.08)	0.155(±0.006)		0.0863	103.39(±0.04)	0.189(±0.001)	
T = 308.15 K				T = 308.15 K			
0.0101	121.47(±0.01)	0.154(±0.003)		0.0559	118.24(±0.03)	0.184(±0.005)	
0.0253	120.61(±0.02)	0.167(±0.004)		0.0713	117.81(±0.02)	0.197(±0.002)	
0.0406	119.61(±0.03)	0.171(±0.008)		0.0867	116.61(±0.01)	0.204(±0.009)	
	w_1 = 0.03						
	Tyrosine+ aq. AA						
T = 298.15 K				T = 298.15 K			

0.0100	129.35(±0.02)	0.085(±0.005)	36.034	0.0555	118.97(±0.01)	0.132(±0.004)	36.074
0.0251	123.99(±0.08)	0.108(±0.002)	36.049	0.0707	115.43(±0.04)	0.142(±0.006)	36.088
0.0403	121.60(±0.02)	0.121(±0.009)	36.065	0.0860	112.89(±0.03)	0.154(±0.002)	37.016
T = 303.15 K				T = 303.15 K			
0.0101	135.89(±0.02)	0.088(±0.007)		0.0557	129.20(±0.02)	0.159(±0.001)	
0.0252	133.46(±0.02)	0.117(±0.002)		0.0710	128.57(±0.07)	0.169(±0.002)	
0.0404	131.83(±0.09)	0.139(±0.002)		0.0863	125.93(±0.02)	0.177(±0.005)	
T = 308.15 K				T = 308.15 K			
0.0101	138.47(±0.01)	0.098(±0.002)		0.0559	134.76(±0.03)	0.151(±0.002)	
0.0253	137.99(±0.06)	0.118(±0.004)		0.0712	133.95(±0.02)	0.168(±0.009)	
0.0406	135.48(±0.02)	0.141(±0.002)		0.0866	131.25(±0.02)	0.187(±0.007)	
	Tryptophan+ aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	98.49(±0.03)	0.059(±0.005)	34.126	0.0555	89.37(±0.01)	0.095(±0.001)	34.152
0.0251	95.68(±0.03)	0.069(±0.002)	34.133	0.0707	88.31(±0.04)	0.106(±0.002)	34.166
0.0403	93.96(±0.02)	0.082(±0.009)	34.145	0.0860	87.86(±0.03)	0.117(±0.004)	34.188
T = 303.15 K				T = 303.15 K			
0.0101	101.29(±0.09)	0.079(±0.002)		0.0557	94.88(±0.02)	0.123(±0.003)	
0.0252	99.33(±0.07)	0.087(±0.004)		0.0710	92.56(±0.04)	0.143(±0.002)	
0.0404	96.34(±0.02)	0.095(±0.002)		0.0863	91.13(±0.01)	0.156(±0.002)	
T = 308.15 K				T = 308.15 K			
0.0101	106.12(±0.05)	0.086(±0.003)		0.0559	101.56(±0.02)	0.112(±0.002)	
0.0253	105.29(±0.06)	0.094(±0.002)		0.0713	98.33(±0.02)	0.123(±0.003)	
0.0406	103.75(±0.02)	0.102(±0.002)		0.0867	96.45(±0.05)	0.134(±0.002)	
	w_1 = 0.05						
	Tyrosine + aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	128.45(±0.08)	0.099(±0.002)	37.087	0.0555	125.88(±0.02)	0.123(±0.004)	37.161
0.0251	127.24(±0.02)	0.105(±0.002)	37.120	0.0707	124.30(±0.05)	0.133(±0.003)	37.177
0.0403	126.61(±0.02)	0.119(±0.001)	37.145	0.0860	123.65(±0.02)	0.145(±0.002)	37.193
T = 303.15 K				T = 303.15 K			
0.0101	137.46(±0.01)	0.119(±0.002)		0.0557	134.16(±0.02)	0.132(±0.002)	
0.0252	136.25(±0.02)	0.127(±0.003)		0.0709	133.56(±0.05)	0.141(±0.007)	
0.0404	135.82(±0.02)	0.130(±0.002)		0.0863	132.82(±0.02)	0.154(±0.007)	
T = 308.15 K				T = 308.15 K			

0.0101	143.47(±0.02)	0.121(±0.002)		0.0559	140.66(±0.06)	0.162(±0.004)	
0.0253	142.19(±0.02)	0.142(±0.001)		0.0712	139.85(±0.02)	0.171(±0.002)	
0.0406	141.43(±0.02)	0.145(±0.007)		0.0866	138.97(±0.06)	0.187(±0.002)	
	Tryptophan + aq. AA						
T = 298.15 K				T = 298.15 K			
0.0100	99.23(±0.02)	0.087(±0.004)	34.975	0.0555	95.82(±0.02)	0.127(±0.002)	35.008
0.0251	97.26(±0.02)	0.099(±0.002)	34.991	0.0707	92.53(±0.01)	0.139(±0.003)	35.017
0.0403	96.30(±0.02)	0.119(±0.004)	34.999	0.0860	89.19(±0.02)	0.148(±0.002)	35.027
T = 303.15 K				T = 303.15 K			
0.0101	105.22(±0.08)	0.077(±0.002)		0.0557	123.64(±0.03)	0.133(±0.002)	
0.0252	113.96(±0.02)	0.109(±0.002)		0.0710	128.51(±0.02)	0.141(±0.009)	
0.0404	119.09(±0.02)	0.129(±0.005)		0.0863	132.04(±0.02)	0.149(±0.002)	
T = 308.15 K				T = 308.15 K			
0.0101	112.53(±0.07)	0.085(±0.002)		0.0559	124.48(±0.02)	0.185(±0.002)	
0.0253	115.94(±0.02)	0.137(±0.004)		0.0712	128.64(±0.02)	0.199(±0.002)	
0.0406	119.27(±0.07)	0.159(±0.002)		0.0866	137.88(±0.07)	0.202(±0.001)	

Standard errors are given in the parenthesis.

Table4. Partial molar volumes (ϕ_V^0), experimental slopes (S_V^*), viscosity *A*- and *B*-coefficients of amino acids in different mass fraction ofaqueous AA (w_1) at 298.15 K, 303.15 K and 308.15 K respectively

Temp /K	$\phi_V^0 \cdot 10^6$ /$m^3 \cdot mol^{-1}$	$S_V^* \cdot 10^6$ /$m^3 \cdot mol^{-3/2} \cdot kg^{1/2}$	*B* / $kg \cdot mol^{-1}$	*A* / $kg^{1/2} \cdot mol^{-1/2}$
	w_1 = 0.01			
	Tyrosine+ aq. AA			
298.15	125.67(±0.03)	15.75(±0.02)	0.321(±0.009)	0.042(±0.009)
303.15	127.29(±0.06)	13.93(±0.06)	0.486(±0.002)	0.039(±0.001)
308.15	129.81(±0.02)	12.20(±0.04)	0.554(±0.003)	0.036(±0.002)
	Tryptophan + aq. AA			
298.15	96.58(±0.06)	24.19(±0.02)	0.233(±0.005)	0.021(±0.002)
303.15	97.04(±0.02)	21.38(±0.01)	0.343(±0.002)	0.015(±0.004)
308.15	99.99(±0.09)	19.27(±0.01)	0.467(±0.005)	0.011(±0.002)
	w_1 = 0.03			
	Tyrosine + aq. AA			
298.15	129.33(±0.06)	13.69(±0.02)	0.381(±0.002)	0.035(±0.004)

303.15	131.98(±0.02)	11.04(±0.06)	0.482(±0.007)	0.041(±0.001)
308.15	135.08(±0.03)	9.73(±0.02)	0.577(±0.007)	0.059(±0.002)
	Tryptophan + aq. AA			
298.15	99.17(±0.02)	12.18(±0.03)	0.391(±0.002)	0.018(±0.004)
303.15	102.93(±0.07)	11.54(±0.04)	0.469(±0.002)	0.015(±0.002)
308.15	103.51(±0.07)	10.01(±0.02)	0.587(±0.005)	0.012(±0.002)
	$w_1 = 0.05$			
	Tyrosine + aq. AA			
298.15	135.19(±0.05)	12.34(±0.02)	0.513(±0.002)	0.029(±0.004)
303.15	142.26(±0.02)	9.45(±0.03)	0.653(±0.001)	0.021(±0.002)
308.15	154.09(±0.02)	8.39(±0.02)	0.732(±0.002)	0.017(±0.002)
	Tryptophan + aq. AA			
298.15	101.65(±0.04)	11.19(±0.02)	0.402(±0.002)	0.017(±0.002)
303.15	103.76(±0.02)	8.76(±0.02)	0.456(±0.006)	0.015(±0.007)
308.15	105.01(±0.01)	6.12(±0.01)	0.508(±0.002)	0.011(±0.005)

Standard errors are given in the parenthesis.

Table5. Values of empirical coefficients (a_0, a_1, and a_2) of Equation 4 for amino acids in different mass fraction ofaqueous vitamin (w_1) at 298.15K to 308.15K respectively

solvent mixture	$a_0 \cdot 10^6$ /m^3·mol^{-1}	$a_1 \cdot 10^6$ /m^3·mol^{-1}·K^{-1}	$a_2 \cdot 10^6$ /m^3·mol^{-1}·K^{-2}
Tyrosine + aq. vitamin C			
w_1= 0.01	1213.00(±0.00)	-9.499(±0.004)	0.008(±0.002)
w_1= 0.03	1932.77(±0.02)	-11.881(±0.002)	0.009(±0.006)
w_1 = 0.05	2342.30(±0.01)	-12.752(±0.007)	0.013(±0.002)
Tryptophan + aq. vitamin C			
w_1 = 0.01	691.29(±0.06)	-7.600(±0.002)	0.0005(±0.0001)
w_1= 0.03	878.20(±0.02)	-8.648(±0.009)	0.0022(±0.0002)
w_1= 0.05	996.90(±0.04)	-9.514(±0.002)	0.0077(±0.0002)

Standard errors are given in the parenthesis.

Table6. Limiting apparent molal expansibilities (ϕ_E^0) for amino acids in different mass fraction of aqueous vitamin (w_1) at 298.15K to 308.15K respectively

solvent mixture	$\phi_E^0 \cdot 10^6$ /m^3·mol^{-1}·K^{-1}			$\left(\partial\phi_E^0/\partial T\right)_p \cdot 10^6$ /m^3·mol^{-1}·K^{-2}
Tyrosine + aq. vitamin C				
T/K	298.15	303.15	308.15	
w_1 = 0.01	0.154(±0.005)	0.284(±0.002)	0.464(±0.002)	0.046(±0.001)
w_1= 0.03	0.248(±0.001)	0.315(±0.002)	0.483(±0.008)	0.058(±0.008)
w_1= 0.05	0.321(±0.002)	0.449(±0.006)	0.571(±0.002)	0.067(±0.002)
Tryptophan + aq. vitamin C				
T/K	298.15	303.15	308.15	
w_1= 0.01	0.123(±0.004)	0.241(±0.002)	0.339(±0.002)	0.023(±0.005)
w_1= 0.03	0.157(±0.002)	0.257(±0.001)	0.385(±0.001)	0.042(±0.009)
w_1= 0.05	0.307(±0.001)	0.311(±0.005)	0.463(±0.002)	0.055(±0.002)

Standard errors are given in the parenthesis.

Table7. Values of *dB/dT* for the amino acids in different mass fraction of aqueous vitamin C (w_1) at 298.15 to 308.15K respectively

solvent mixture	*dB/dT*
Tyrosine+ aq. Vitamin C	

w_1= 0.01	0.0232 (±0.0001)
w_1 = 0.03	0.0246 (±0.0007)
w_1= 0.05	0.0269 (±0.0002)
Tryptophan+ aq. Vitamin C	
w_1= 0.01	0.0129 (±0.0008)
w_1= 0.03	0.0143 (±0.0009)
w_1= 0.05	0.0219 (±0.0002)

Standard errors are given in the parenthesis.

Table 8: Properties of Amino acids – Vitamin C Complexes calculated by the UB3LYP/6-31+g(d) method

Optimised Amino acids	**Optimised Amino acids in Vitamin C**
File Name = Tyrosine Calculation Method = UB3LYP Basis Set = 6-31G(d) E(UB3LYP) = -630.00433825 a.u. Dipole Moment = 2.5356 Debye	File Name = Ascorbic acid + Tyrosine Calculation Method = UB3LYP Basis Set = 6-31G(d) E(UB3LYP) = -1325.43514700 a.u. Dipole Moment = 5.4169 Debye
File Name = Tryptophan Calculation Method = UB3LYP Basis Set = 6-31G(d) E(UB3LYP) = -686.35576918 a.u. Dipole Moment = 3.4013 Debye	File Name = Ascorbic acid + Tryptophan Calculation Method = UB3LYP Basis Set = 6-31G(d) E(UB3LYP) = -1313.54700092 a.u. Dipole Moment = 7.1501 Debye

FIGURES

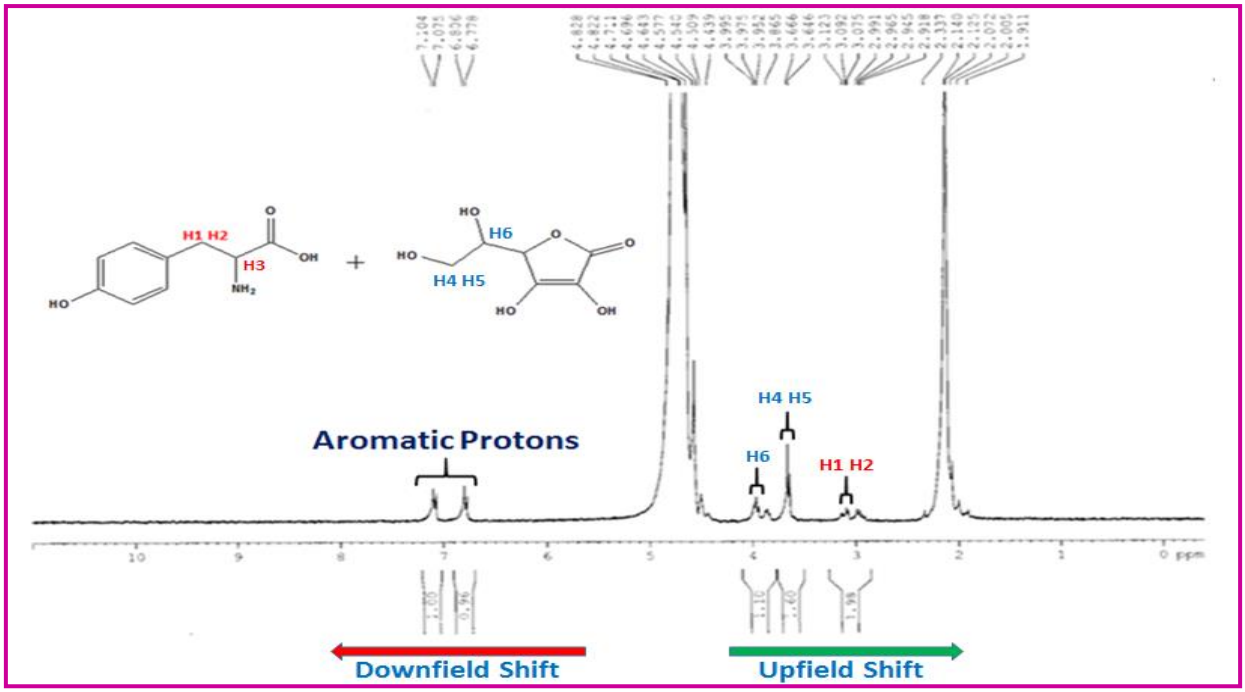


Figure 1.1H NMR spectra of Tyrosine along with Ascorbic acid in D_2O at 298.15 K. {1H-NMR (300 MHz, CDCl3): δ 7.10-7.6 (d, 2H), 6.83-6.77 (d, 2H), 3.99-3.95 (t, 1H), 3.66-3.64 (d, 2H), 3.12-3.07 (d, 2H)}.

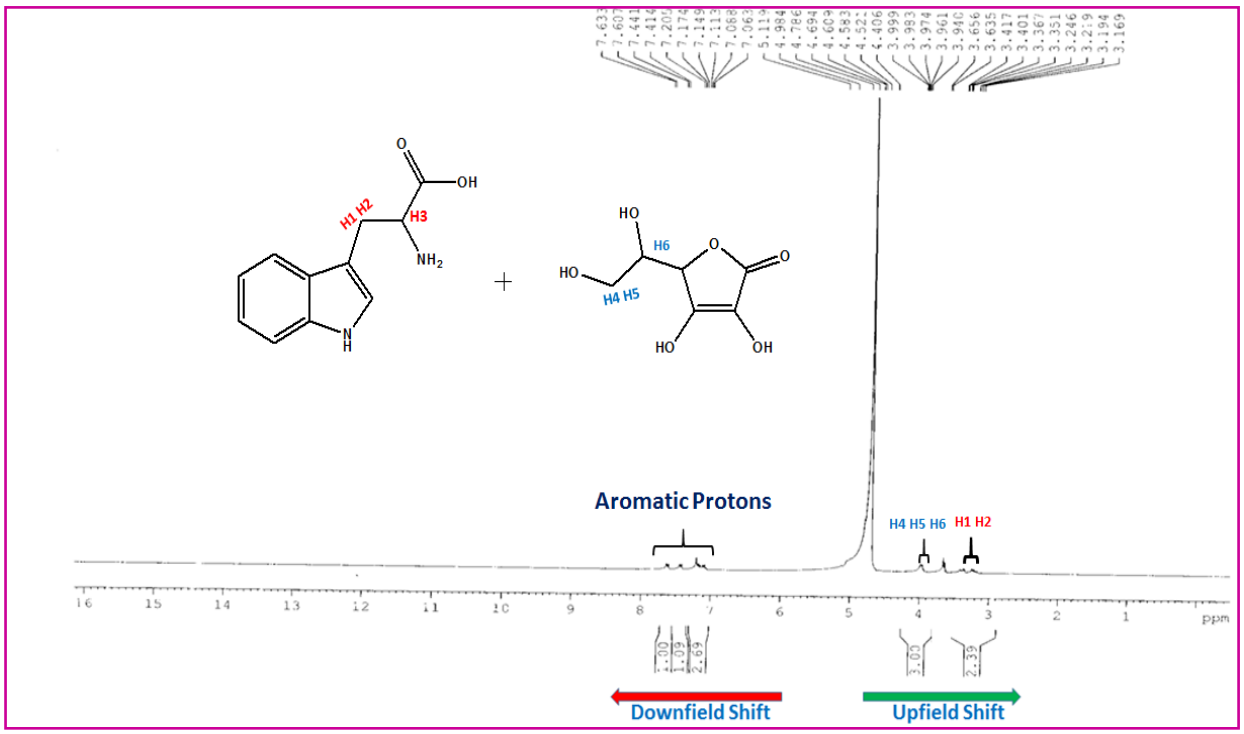


Figure 2.1H NMR spectra of Tryptophan along with Ascorbic acid in D_2O at 298.15 K. {1H-NMR (300 MHz, CDCl3): δ 7.63-7.60 (d, 2H), 7.44-7.41 (d, 2H), 7.20-7.06 (m, 2H), 3.99-3.94 (m, 3H), 3.24-3.19 (d, 2H)}.

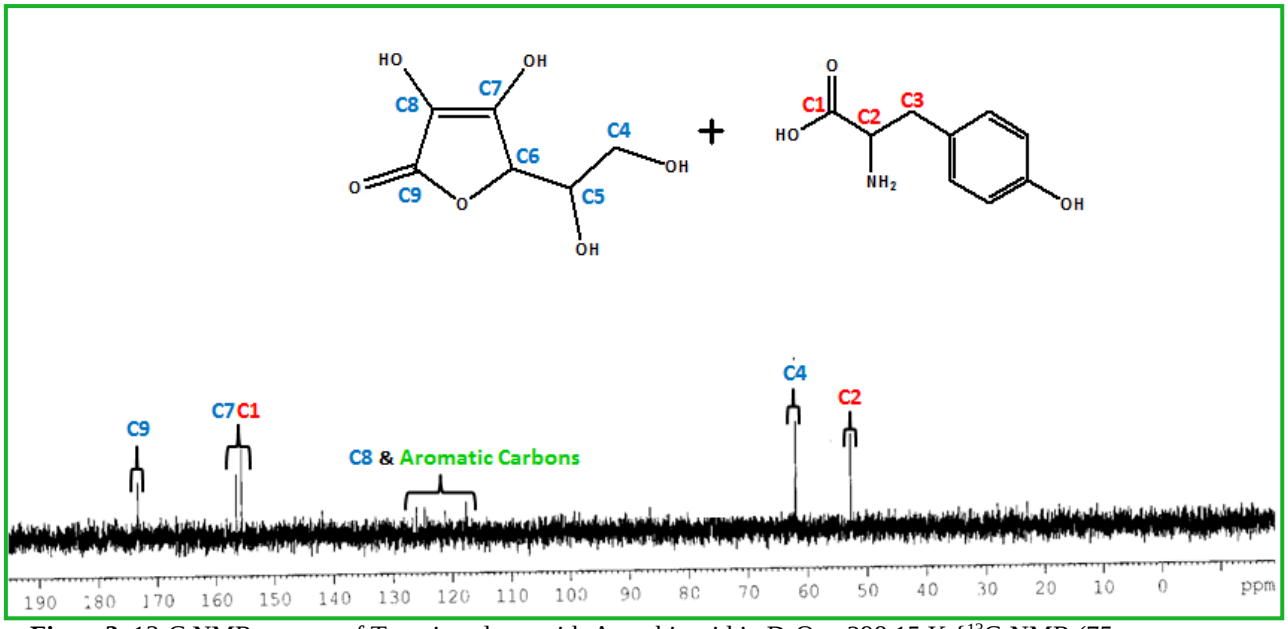


Figure3. 13-C NMR spectra of Tyrosine along with Ascorbicacid in D_2O at 298.15 K.{^{13}C-NMR (75 MHz, in D_2O): 174.50, 156.2, 155.8, 128.9, 127.3, 126.6, 126.4, 123.8, 63.9, 53.4}.

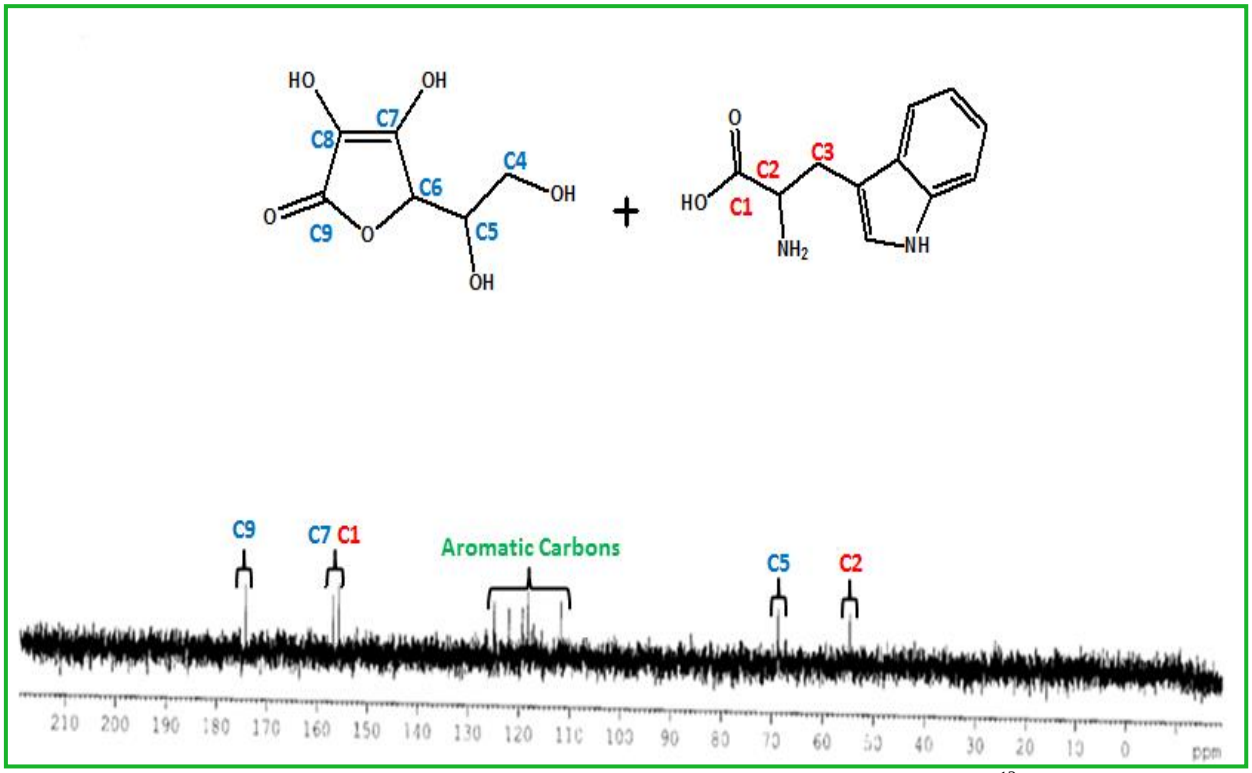


Figure 4.13-C NMR spectra of Tryptophan along with Ascorbic acid in D_2O at 298.15 K. {^{13}C-NMR (75 MHz, in D_2O): 174.40, 156.3, 155.8, 125.9, 123.3, 119.4, 118.3, 115.8, 114.2, 113.9, 113.2, 68.9, and 54.8}.

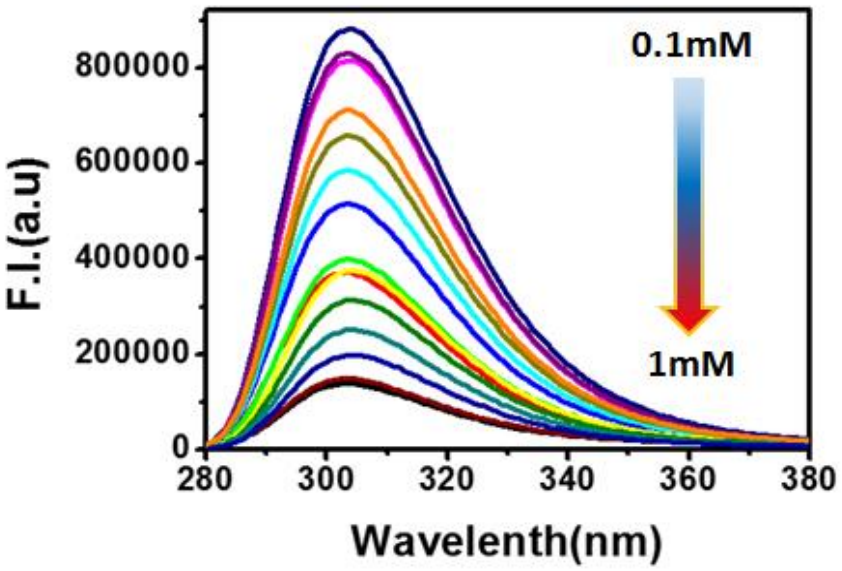


Figure 5.Fluorescence emission spectra of aqueous ascorbic acid in the presence of 0.1mM–1.0 mM of Tyrosine (λex =275 nm, slit =5/5)

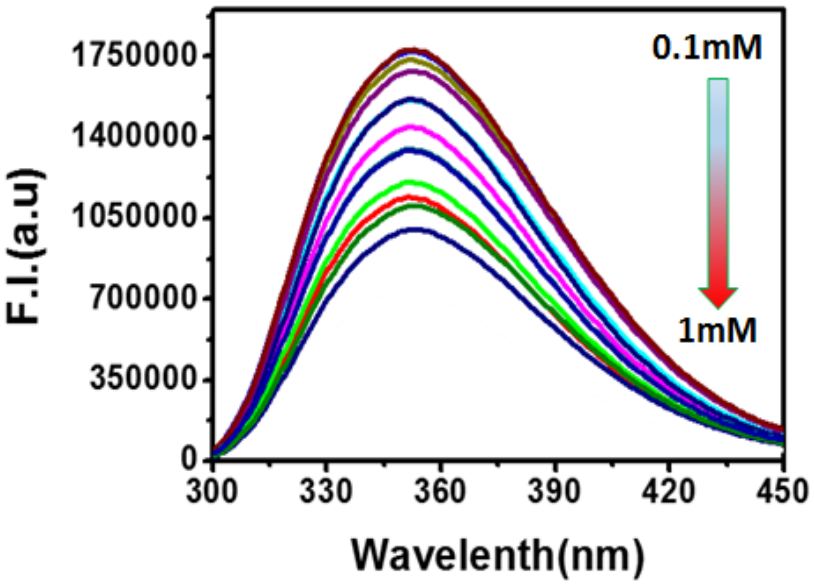


Figure 6.Fluorescence emission spectra of aqueous ascorbic acid in the presence of 0.1mM–1.0 mM ofTryptophan (λex =295 nm, slit =5/5)

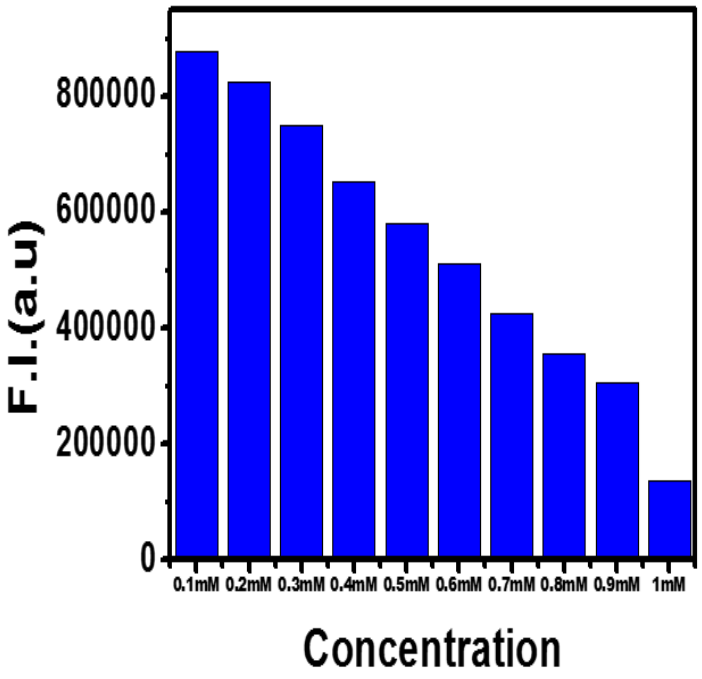


Figure 7. Bar diagram of the relative fluorescence intensity bar for the interaction of tyrosine using aqueous ascorbic acid.

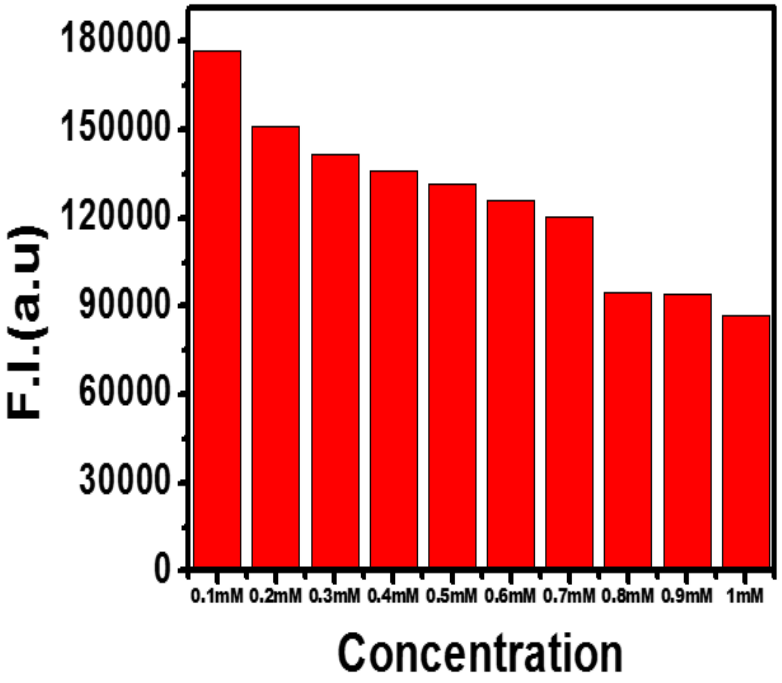


Figure 8. Bar diagram of the relative fluorescence intensity bar for the interaction of tryptophan using aqueous ascorbic acid.

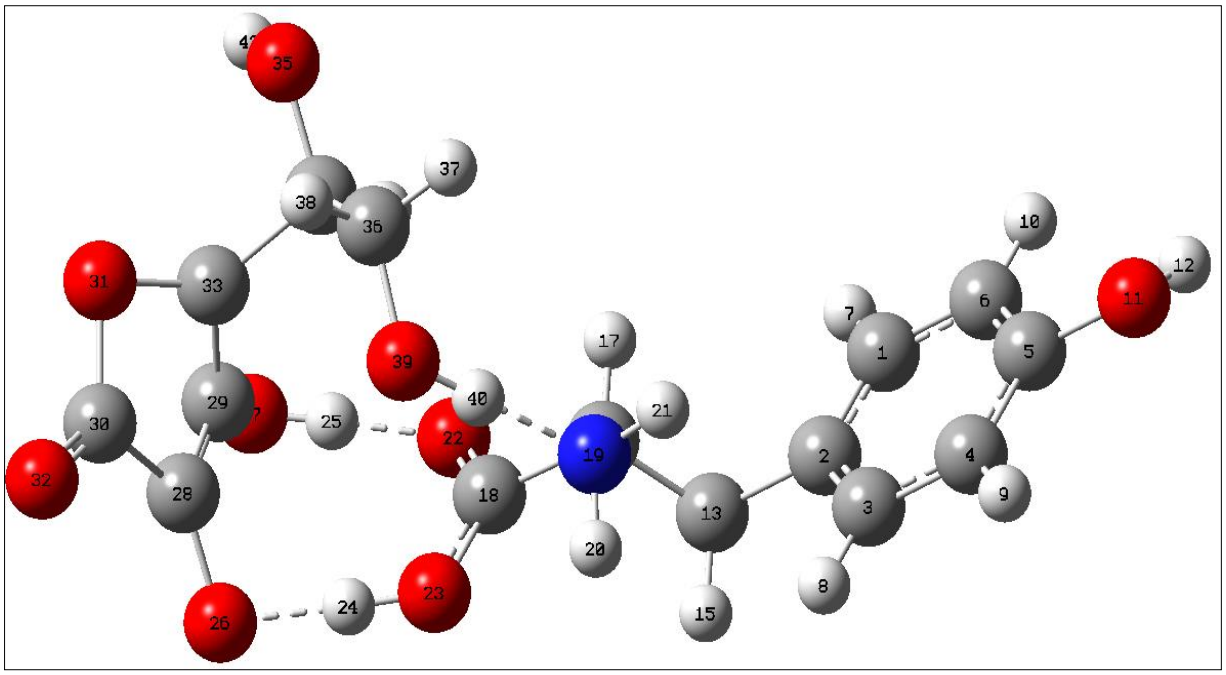


Figure 9(a). Optimum geometry for a complex consisting of a tyrosine encircled by ascorbic acid molecules involving three H-bonding (without Cartesian Co-ordinate).

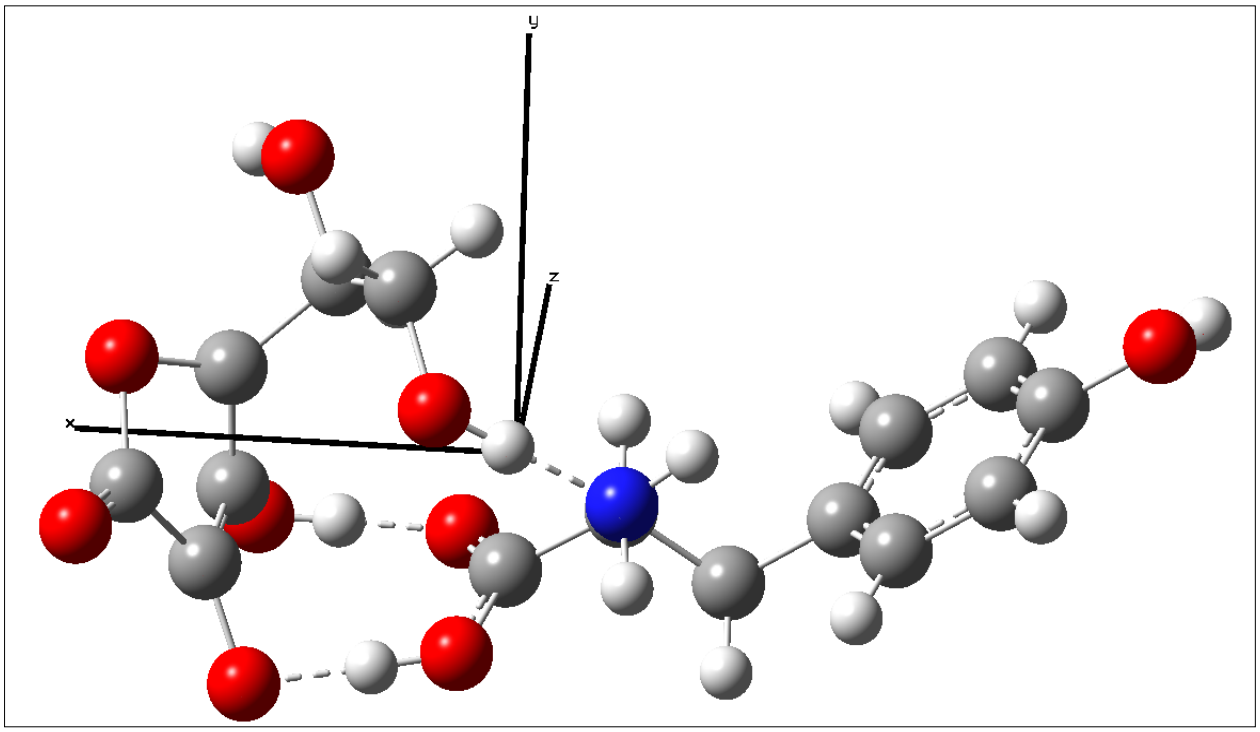
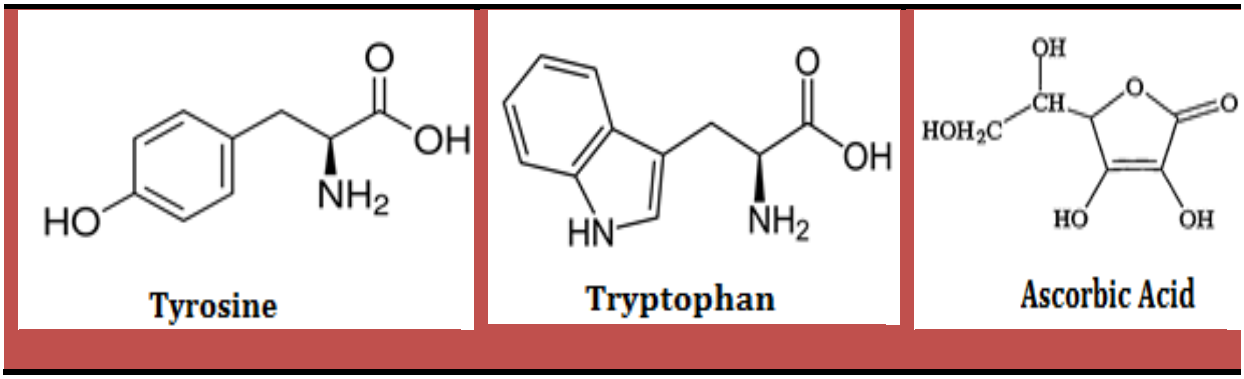
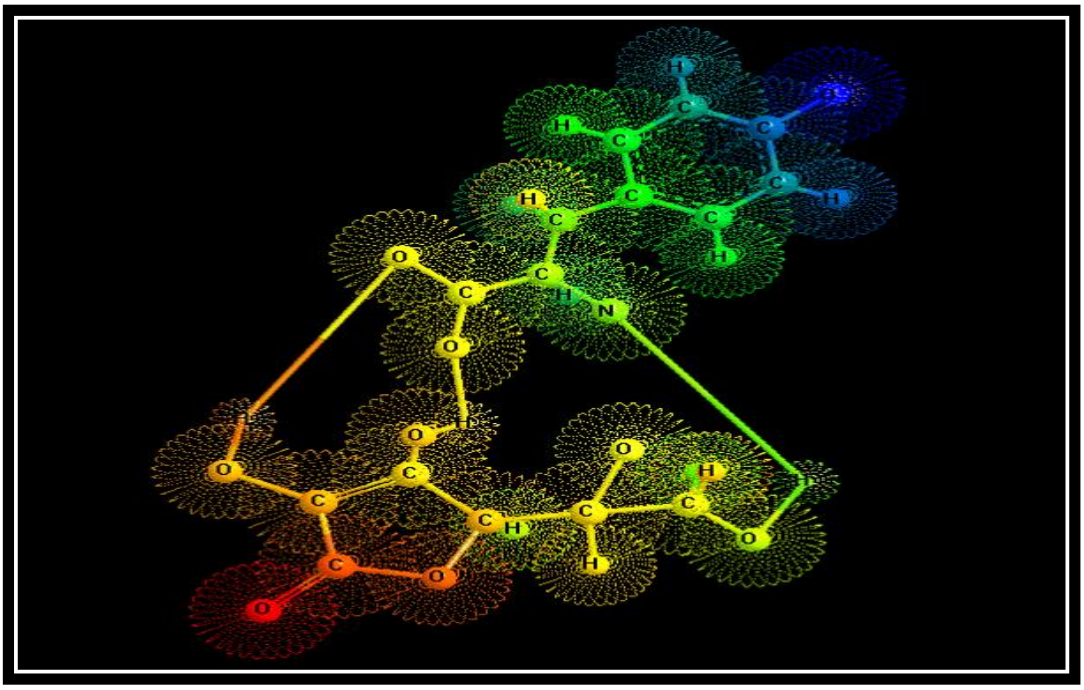


Figure 9(b). Optimum geometry for a complex consisting of a tyrosine encircled by ascorbic acid molecules involving three H-bonding (with Cartesian Co-ordinate).

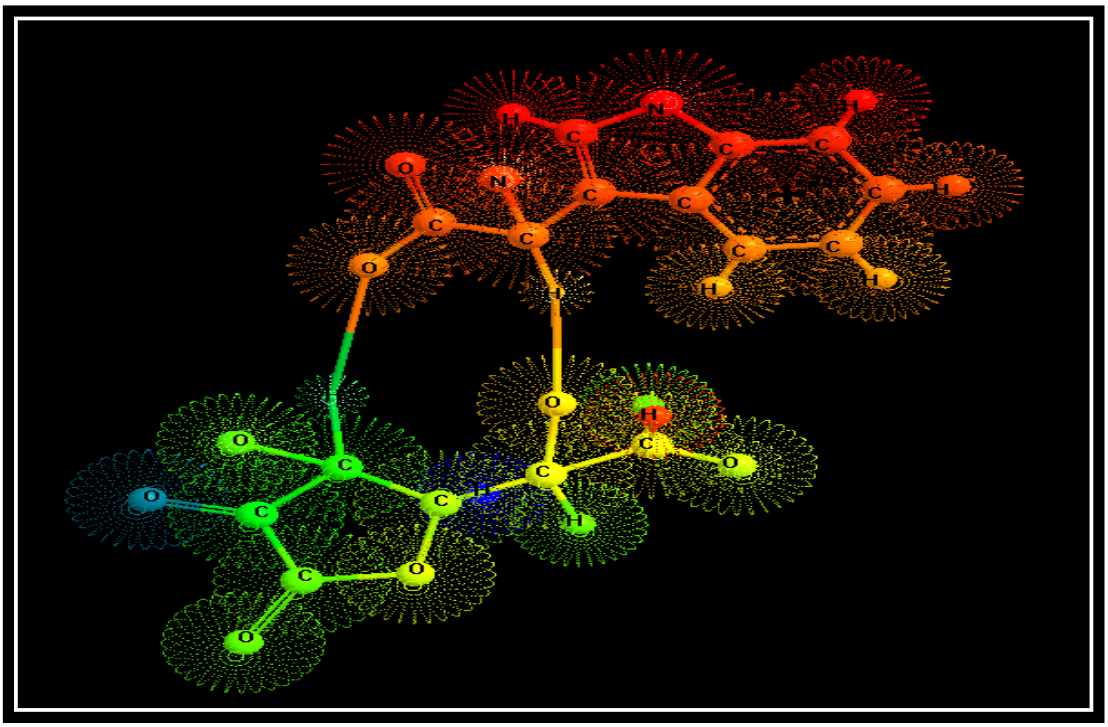
Schemes



Scheme 1: The molecular structures of Tyrosine, Tryptophan and Ascorbic acid.



Scheme 2. Diagrammatic representation of the Probable Geometrical Configurations involving tyrosine molecules surrounded by ascorbic acid molecules involving three H-bonding.



Scheme3. Diagrammatic representation of the Probable Geometrical Configurations involving tryptophan molecules surrounded by ascorbic acid molecules involving two H-bonding

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