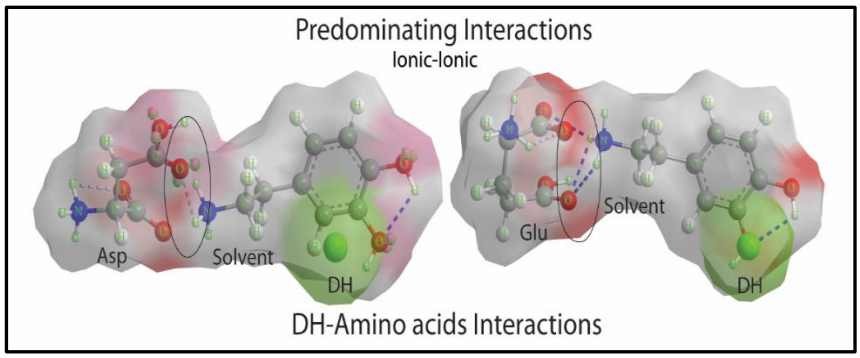
CHAPTER-IV

Investigation of Diverse Interactions of Amino Acids (Asp and Glu) in Aqueous Dopamine Hydrochloride with the Manifestation of the Catecholamine Molecule Recognition Tool in Solution Phase



ABSTRACT

The density (ρ), viscosity (η) and molar conductivities (Λ) of Aspartic Acid and Glutamic Acid were measured in aqueous solution of Dopamine Hydrochloride at three different temperature having an interval of 10K under atmospheric pressure. Spectroscopic methods like UV–Visible, Nuclear Magnetic Resonance spectroscopy and Raman spectroscopy were also applied to ensure the nature of drug–amino acid interaction in solution phase. was used to We have also calculated the binding constants for both amino acids in the ternary system by the Benesi-Hildebrand method. The Gibbs free energy change of the system was calculated indicating spontaneity of the systems. From viscosity study, structure making behaviours of both amino acids were detected in aqueous Dopamine Hydrochloride. All the spectroscopic measurements and calculated thermophysical parameters indicate towards the greater extent of interaction in the Glutamic Acid- Dopamine Hydrochloride system than the Aspartic Acid one.

Keywords: Aspartic Acid (Asp); Dopamine Hydrochloride (DH); Glutamic Acid (Glu); Gibbs free energy(ΔG^0); Association constant (K_a)

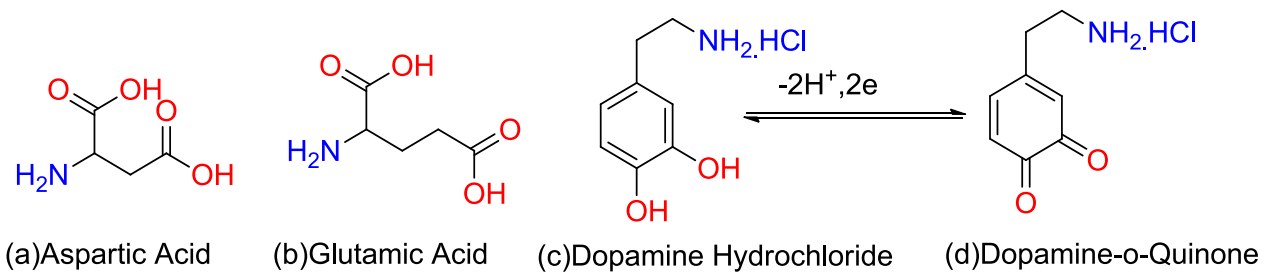
1. INTRODUCTION

2-(3,4-Dihydroxyphenyl) ethylamine hydrochloride commonly known as Dopamine Hydrochloride(DH) is one of the most significant catecholamine neurotransmitters in the mammalian central nervous system (CNS) [122] [123] [124] [125] [100] [126]. It undergoes

oxidation easily and ubiquitously in the human body [124] [127] [125] [128] [129] [130] [131] e.g., DH-related oxidative stress and neuromelanin synthesis in CNS. DH shows influences on the pathogenesis of some neurodegenerative disorders such as Alzheimer and Parkinson diseases [132] [133] [134] [135]. An understanding of the interaction of DH with amino acids is thus of great importance in elucidating it's *in vivo* oxidation [136] [137, 138].

l-glutamic acid (Glu) is an important non-essential amino acid that acts as an excitatory neurotransmitter in the central nervous system of mammals [139]. It can also be served as a precursor molecule for the synthesis of various metabolites including N-acetyl-*L*-glutamate, δ-1-Pyrroline-5-carboxylate, β-citryl glutamate, *l*-γ-glutamyl-*l*-cysteine [121, 140-143] [144]. Aspartic acid(Asp) is one of the 20 natural proteinogenic amino acids which are the building blocks of proteins [145, 146].This amino acid has an important role in the urea cycle and DNA metabolism. This is a major excitatory neurotransmitter, which is sometimes found to be increased in epileptic and stroke patients. It is decreased in depressed patients and in patients suffering from brain atrophy. In this article, we have reported the nature of the interaction between two amino acids and aqueous Dopamine Hydrochloride (DH) on the basis of spectroscopic studies and physicochemical approach. Moreover, a wide temperature range (293.15–313.15 K), which also covers our body temperature, that is, 310.15 K, has been chosen for the present study to acquire more relevant information regarding the hydration behaviour of these biologically important molecules in presence of Dopamine Hydrochloride.

The structure of both amino acids and Dopamine Hydrochloride are shown in **Scheme1.**



Scheme1. Chemical structure of (a) Aspartic Acid (b) Glutamic acid, (c) Dopamine Hydrochloride and (d) Dopamine-o-Quinone.

2. EXPERIMENTAL SECTION

2.1. Materials used

Source and Purity of Samples

Dopamine hydrochloride of puriss grade ≥98.5% was collected from Sigma-Aldrich, Germany and was used without further purification. *l*- Aspartic acid and *l*-Glutamic acid of puriss grade were purchased from Sigma-Aldrich, Germany and used as it was. The mass fraction purity of the amino acids was ≥0.99.

Table 1: Sample Description

Name of chemicals	Source	CAS no	Purification method	Mass purity
L-Aspartic Acid	Sigma-Aldrich, Germany	56-84-8	Used as purchased	≥0.99%
L-Glutamic Acid	Sigma-Aldrich, Germany	56-86-0	Used as purchased	≥0.99%
Dopamine Hydrochloride	Sigma-Aldrich, Germany	62-31-7	Used as purchased	≥98.5%
Deuterium oxide	Sigma-Aldrich, Germany	7789-20-0	Used as purchased	99.9%

3. RESULTS AND DISCUSSION

3.1. Apparent molar volume

The physical properties of binary mixtures in different mass fractions (w_1 = 0.0001, 0.0003, 0.0005) of aqueous dopamine hydrochloride solutions at 293.15 K, 303.15 K and 313.15 K are reported in Table. 2.

The apparent molar volume can be determined (Table. 3) using the equation stated below

$$\phi_V = M/\rho - (\rho - \rho_o)/m\rho_o\rho \tag{1}$$

Where M denotes the molar mass of the amino acids (kg·mol^{-1}), ρ^0 and ρ are the densities of the solvent and solution (kg·m^{-3}) respectively and m stands for the molality (mol·kg^{-1}) of the experimental solution. The large and positive ϕ_V values suggest strong solute-solvent interactions (Table. S1). From Table. S1. It is observed that the apparent molar volume increases with a rise in temperature during the experiment.

This indicates few water molecules in the vicinity of the amino acids might get released to the bulk water in presence of DH. The interactions taking place between the amino acids and drug molecule can be classified as- (a) ionic-hydrophilic interactions between $-NH_3^+$ and $-COO^-$ groups of Asp and Glu and –OH group of DH (b) hydrophilic-hydrophilic interactions between $-CH_2COOH$ group of Asp and –OH group of DH and CH_2CH_2COOH of Glu and –OH of DH, (c) ionic-hydrophobic interactions between $-NH_3^+$ and $-COO^-$ groups of Asp and Glu and alkyl group of DH, (d) hydrophilic-hydrophobic interactions between –OH group of DH and alkyl group of Aspartic acid, Glutamic acid and (e) hydrophobic-hydrophobic interactions between alkyl group of Asp and the alkyl group of DH.

The increase in apparent molar volume takes place to a greater extent in case of glutamic acid as shown in Table. S1. On comparing the structural context, it is observed that due to the presence of an extra $-CH_2$-($-CH_2CH_2COOH$), such a tendency becomes higher in glutamic acid. With increasing concentration of DH, the probability of formation of hydrogen bond increases, leading to the better ionic-hydrophilic interaction between $-NH_3^+$ of DH and $-COO^-$ of glutamic acid.

Limiting apparent molar volume (ϕ_V^0) and the experimental slopes (S_V^*) can be obtained by employing the least square fitting of apparent molar volume values to the linear Eq. (2) (Masson equation) [73].

$$\phi_V = \phi_V^0 + S_V^* \cdot \sqrt{m} \tag{2}$$

Where S_V^* is the experimental slope and it indicates the nature of the solute-solute interaction. ϕ_V^0 refers to the extent of solute-solvent interactions. Table. 5 shows that on increasing concentration of DH and temperature, ϕ_V^0 values follow an increasing pattern (Fig. 1). From the quantitative comparison of values between S_V^* and ϕ_V^0, it is revealed that the magnitudes of ϕ_V^0 values are higher than those of S_V^* and it indicates predominant solute-solvent interactions over the solute-solute interactions at all temperatures[71]. The values of S_V^* are negative and their decreasing trend with increasing temperature diminishes the possibility of solute-solute interactions. From this, we can easily conclude that stronger drug-amino acid interaction takes place at a higher concentration of DH and their interaction increases with increase in temperature [147, 148]

Table. 2. Values of density (ρ) and viscosity (η) of aqueous Dopamine Hydrochloride with Standard Deviation (SD) and Average Absolute Deviation (AAD) in different mass fraction (w_1) at T= (293.15-313.15) K[a] and (p = 0.1MPa) respectively.

Mass fraction of aq. DH (w_1)	Temp. (K[a])	ρ·10^{-3} /kg·m^{-3}	SD	AAD	η/ mPa·s	SD	AAD
	293.15	0.99823	0.00001	0.000005	0.962	0.001	0.0005
0.001	303.15	0.99561	0.00001	0.000005	0.946	0.002	0.0010
	313.15	0.99220	0.00001	0.000005	0.922	0.001	0.0005
	293.15	0.99901	0.00002	0.000010	0.942	0.001	0.0005
0.003	303.15	0.99622	0.00001	0.000005	0.924	0.002	0.0010
	313.15	0.99343	0.00003	0.000010	0.899	0.002	0.0010
	293.15	1.00204	0.00002	0.000010	0.921	0.001	0.0005
0.005	303.15	0.99892	0.00001	0.000005	0.905	0.001	0.0005
	313.15	0.99481	0.00001	0.000005	0.883	0.003	0.0010

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]w_1 is the mass fraction of aqueous dopamine hydrochloride.

Table. 3. Experimental values of density (ρ) of amino acids in different mass fraction of aqueous Dopamine Hydrochloride with Standard Deviation (SD) and Average Absolute Deviation (AAD) at T= (293.15 to 313.15) K[a] and (p = 0.1MPa) respectively.

293.15 K[a]				303.15 K[a]			313.15 K[a]		
[c]m /mol kg^{-1}	[d]ρ·10^{-3} /kg·m^{-3}	SD	AAD	[d]ρ·10^{-3} /kg·m^{-3}	SD	AAD	[d]ρ·10^{-3} /kg·m^{-3}	SD	AAD
w_1=0.001[b](Asp+DH)									

0.001	0.99826	0.00003	0.000010	0.99565	0.00003	0.000010	0.99225	0.00003	0.000010
0.003	0.99840	0.00002	0.000010	0.99578	0.00002	0.000010	0.99236	0.00003	0.000010
0.005	0.99855	0.00003	0.000010	0.99592	0.00002	0.000010	0.99249	0.00002	0.000010
0.007	0.99872	0.00002	0.000010	0.99608	0.00003	0.000010	0.99265	0.00001	0.000005
0.009	0.99890	0.00003	0.000010	0.99626	0.00003	0.000010	0.99281	0.00001	0.000005
w_1=0.003^b(Asp+DH)									
0.001	0.99907	0.00002	0.000010	0.99624	0.00003	0.000010	0.99344	0.00001	0.000005
0.003	0.99919	0.00001	0.000005	0.99635	0.00001	0.000005	0.99354	0.00003	0.000010
0.005	0.99933	0.00003	0.000010	0.99648	0.00001	0.000005	0.99366	0.00001	0.000005
0.007	0.99949	0.00002	0.000010	0.99663	0.00001	0.000005	0.99380	0.00001	0.000005
0.009	0.99967	0.00001	0.000005	0.99680	0.00003	0.000010	0.99396	0.00003	0.000010
w_1=0.005^b(Asp+DH)									
0.001	1.00208	0.00002	0.000010	0.99895	0.00001	0.000005	0.99483	0.00003	0.000010
0.003	1.00218	0.00001	0.000005	0.99904	0.00003	0.000010	0.99491	0.00002	0.000010
0.005	1.00230	0.00001	0.000005	0.99915	0.00003	0.000010	0.99501	0.00002	0.000010
0.007	1.00244	0.00002	0.000010	0.99928	0.00001	0.000005	0.99512	0.00001	0.000005
0.009	1.00260	0.00002	0.000010	0.99943	0.00001	0.000005	0.99526	0.00003	0.000005
w_1=0.001^b(Glu+DH)									
0.001	0.99827	0.00001	0.000005	0.99567	0.00001	0.000005	0.99226	0.00002	0.000010
0.003	0.99844	0.00002	0.000010	0.99581	0.00003	0.000010	0.99239	0.00001	0.000005
0.005	0.99862	0.00002	0.000010	0.99599	0.00001	0.000005	0.99255	0.00003	0.000010
0.007	0.99881	0.00002	0.000010	0.99616	0.00001	0.000005	0.99272	0.00003	0.000010
0.009	0.99902	0.00003	0.000010	0.99636	0.00003	0.000010	0.99290	0.00003	0.000010
w_1=0.003^b(Glu+DH)									
0.001	0.99907	0.00002	0.000010	0.99624	0.00001	0.000005	0.99344	0.00001	0.000005
0.003	0.99921	0.00002	0.000010	0.99635	0.00001	0.000005	0.99354	0.00002	0.000009
0.005	0.99937	0.00001	0.000005	0.99649	0.00001	0.000005	0.99367	0.00001	0.000005
0.007	0.99953	0.00001	0.000005	0.99664	0.00002	0.000009	0.99382	0.00001	0.000005
0.009	0.99972	0.00003	0.000010	0.99682	0.00001	0.000005	0.993980	0.00001	0.000005
w_1=0.005^b(Glu+DH)									
0.001	1.00208	0.00003	0.000010	0.99895	0.00003	0.000010	0.99483	0.00001	0.000005
0.003	1.00217	0.00002	0.000010	0.99903	0.00002	0.000010	0.99490	0.00001	0.000005
0.005	1.00229	0.00003	0.000010	0.99914	0.00002	0.000010	0.99501	0.00002	0.000010
0.007	1.00243	0.00001	0.000005	0.99925	0.00002	0.000010	0.99513	0.00002	0.000010
0.009	1.00259	0.00001	0.000005	0.99682	0.00003	0.000010	0.99528	0.00003	0.000010

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]w_1 is the mass fraction of aqueous dopamine hydrochloride.

[c]m is the molarity of amino acid in water and water + dopamine hydrochloride solvent systems. Standard uncertainties, u, in molarity is u ([c]m) = 0.0002 mol·kg^{-1}, in density values u ([d]ρ)= ±0.00001 kg m^{-3}

3.2 Temperature dependent limiting the apparent molar volume

The temperature dependence of values can be expressed by the general polynomial equation given below,

$$\phi_V^0 = a_0 + a_1 T + a_2 T^2 \tag{3}$$

Where, a_0, a_1 and a_2 are the empirical coefficients and the values of these coefficients are evaluated by the least-squares fitting of apparent molar volume at different temperatures (Table. S2). The limiting apparent molar expansibilities, ϕ_E^0, can be obtained by the following equation,

$$\phi_E^0 = \left(\delta\phi_V^0/\delta T\right)_P = a_1 + 2a_2 T \tag{4}$$

Differentiation of Eq. 4 with respect to temperature gives the values of the limiting apparent molar expansibilities (ϕ_E^0) (Table. 6).

These values are also employed in the interpretation of the structure-making or breaking properties of numerous solutes [71, 73, 149] [147, 150] [151, 152] [153]The value of $\left(\delta\phi_E^0/\delta T\right)_P$ is evaluated with the help of the following thermodynamic expression by Hepler [154]

$$\left(\delta\phi_E^0/\delta T\right)_P = \left(\delta^2\phi_V^0/\delta T^2\right)_P = 2a_2 \tag{5}$$

If the sign of the second derivatives of the limiting apparent molar volume with respect to the temperature $\left(\delta\phi_E^0/\delta T\right)_P$ is positive or a small negative, the molecule is said to be a structure maker; otherwise, it is a structure breaker.

. As is evident from **Table.6.**, the $\left(\delta\phi_E^0/\delta T\right)_P$ values for all the complexes are positive i.e. amino acids are predominantly structure makers in all of the cases and this tendency gets enhanced with increasing DH concentration.

Figures:

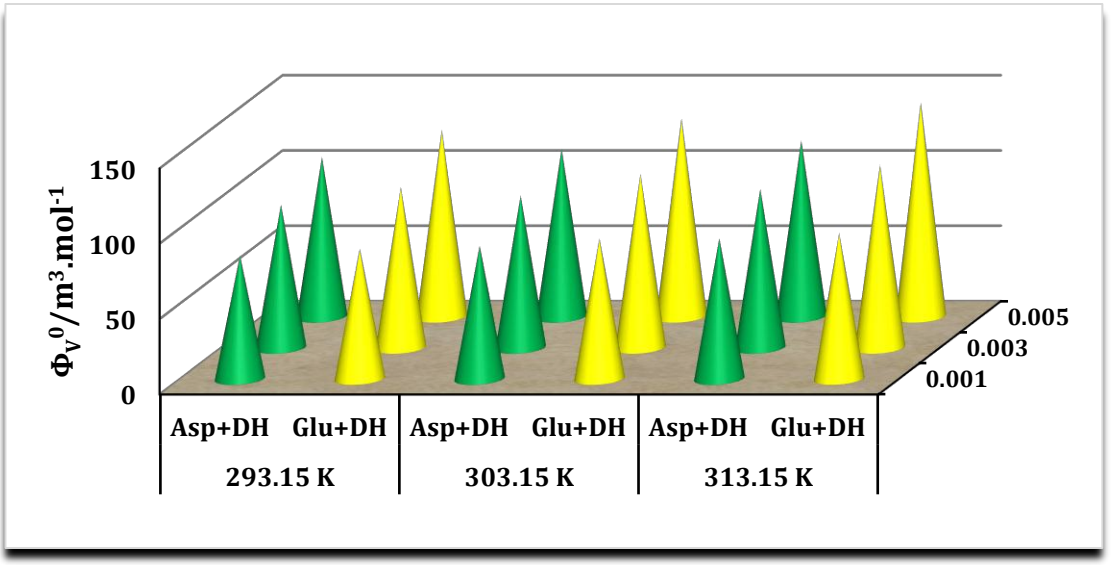


Fig.1. Plot of limiting apparent molar volume (ϕ_V^0) of Asp and Glu in mass fractions (a) 0.001, (b) 0.003, (c) 0.005 (w_1) of aqueous DH at T= (293.15 to 313.15) K and (p = 0.1MPa) respectively.

3.3 Viscometric Measurements

The experimental viscosity (η) values for L-Glutamic acid and L-Aspartic acid in aqueous solutions of Dopamine Hydrochloride at three different temperatures are listed in Table. 4. The viscosity data are analyzed on the basis of A- and B- coefficients of viscosity using the Jones-Dole equation [75].

$$(\eta/\eta_o - 1)/\sqrt{m} = (\eta_r - 1)/\sqrt{m} = A + B\sqrt{m} \quad (6)$$

Where, $\eta_r = \eta/\eta_0$ is the relative viscosity of the solution, η and η_0 denotes the viscosities of the solution and the solvent, respectively, and m defines molality of amino acids. The Falkenhagen coefficient, A, accounts for the solute-solute interactions, while the Jones-Dole coefficient, B, is a measure of structural modifications induced by the solute-solvent interaction [75][78]. The intercepts and slopes of the linear regression of $(\eta_r-1)/\sqrt{m}$ vs. $\sqrt{m}$ plots give the values of A- and B-coefficients, respectively. From **Table.5.**, it is observed that the values of B-coefficients of the studied homologous series of α-amino acids vary linearly with the number of carbon atoms on their alkyl chains at given temperatures [155].

[156]. A same increasing trend is observed for the *A*-coefficients also and the fact may be attributed to the increasing concentration of solute in DH solution which leads to higher charge accumulation and hence solute-solute interaction increases.

The values of *B*-coefficients increase (i) when the water is replaced by DH and (ii) with increasing concentration of DH. The strong interaction immobilizes the solvent molecules, preventing the viscous flow of solution, thereby, increasing the viscosity. Thus, the *B*-coefficients values given in **Table.5.** are suggesting strong solute-solvent interactions. The solute-solvent interaction becomes more effective when it comes to the matter of interaction between L-glutamic acid and dopamine hydrochloride, as reflected from *B* coefficients compared to that of L-aspartic acid.

(d*B*/dT) is a measure of the structure making or structure breaking ability of solute in the solvent. In general, the temperature derivative of *B*-coefficient (d*B*/dT) offers negative and positive value for structure-maker and structure-breaker solutes in solution respectively [157] [76]. The (d*B*/dT) values reported in **Table.7.** are positive, which indicates that both of the amino acids act as structure maker in aqueous-DH and such tendency seems to be higher in case of Glutamic acid.

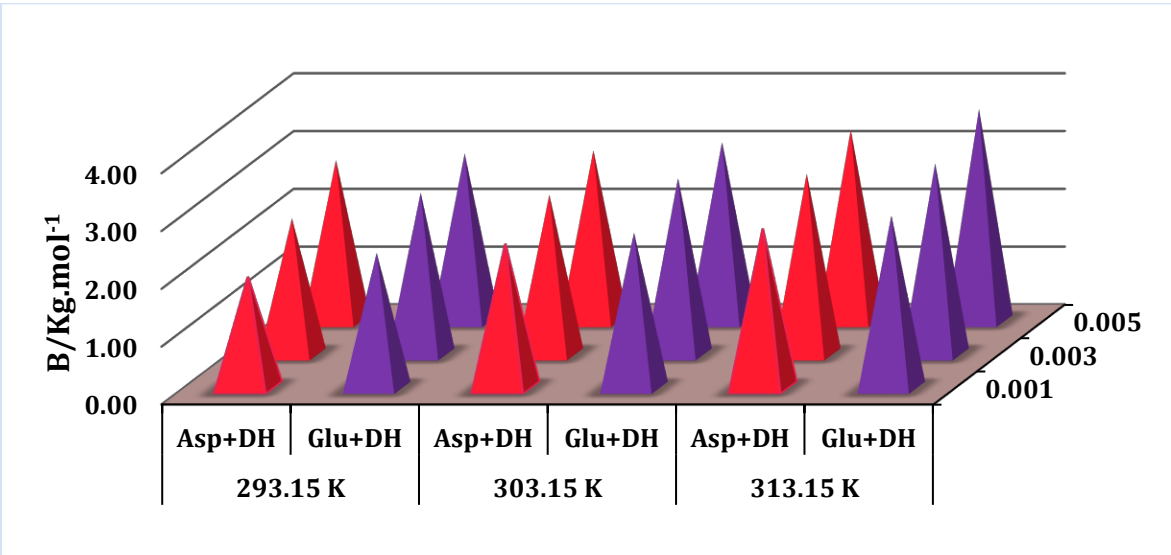


Fig.2. Plot of viscosity *B*-coefficient of Asp and Glu in mass fractions (w_1) (a) 0.001, (b) 0.003, (c) 0.005 of aqueous DH at *T*= (293.15 to 313.15)K and (*p* = 0.1MPa) respectively.

Table. 4. Experimental values of viscosity (η) of amino acids in different mass fraction of aqueous Dopamine Hydrochloride with Standard Deviation (SD) and Average Absolute Deviation (AAD) at T= (293.15 to 313.15) K[a] and (p = 0.1MPa) respectively.

	293.15 K[a]			303.15 K[a]			313.5 K[a]		
[c]m /mol kg^{-1}	[e]η /mPa·s	SD	AAD	[e]η /mPa·s	SD	AAD	[e]η /mPa·s	SD	AAD
				w_1=0.001[b](Asp+DH)					
0.001	0.966	0.001	0.0005	0.952	0.003	0.0010	0.928	0.001	0.0005
0.003	0.971	0.002	0.0010	0.959	0.001	0.0005	0.934	0.001	0.0005
0.005	0.976	0.001	0.0005	0.964	0.002	0.0010	0.941	0.001	0.0005
0.007	0.980	0.002	0.0010	0.971	0.001	0.0005	0.947	0.002	0.0010
0.009	0.984	0.003	0.0010	0.976	0.001	0.0005	0.954	0.001	0.0005
				w_1=0.003[b](Asp+DH)					
0.001	0.948	0.002	0.0010	0.930	0.001	0.0005	0.906	0.002	0.0010
0.003	0.954	0.001	0.0005	0.938	0.002	0.0010	0.915	0.001	0.0005
0.005	0.960	0.003	0.0010	0.944	0.002	0.0010	0.922	0.001	0.0005
0.007	0.966	0.002	0.0010	0.951	0.001	0.0005	0.928	0.003	0.0010
0.009	0.972	0.001	0.0005	0.957	0.001	0.0005	0.936	0.001	0.0005
				w_1=0.005[b](Asp+DH)					
0.001	0.927	0.002	0.0010	0.912	0.002	0.0010	0.890	0.001	0.0005
0.003	0.935	0.001	0.0005	0.919	0.001	0.0005	0.898	0.002	0.0010
0.005	0.941	0.001	0.0005	0.926	0.001	0.0005	0.907	0.001	0.0005
0.007	0.948	0.002	0.0010	0.933	0.001	0.0005	0.913	0.003	0.0010
0.009	0.954	0.002	0.0010	0.941	0.002	0.0010	0.921	0.001	0.0005
				w_1=0.001[b](Glu+DH)					
0.001	0.968	0.001	0.0005	0.948	0.001	0.0005	0.929	0.002	0.0010
0.003	0.974	0.001	0.0005	0.955	0.001	0.0005	0.936	0.001	0.0005
0.005	0.980	0.002	0.0010	0.962	0.001	0.0005	0.943	0.002	0.0010
0.007	0.986	0.001	0.0005	0.968	0.002	0.0010	0.950	0.003	0.0010

0.009	0.992	0.002	0.0010	0.975	0.002	0.0010	0.958	0.002	0.0010
				w_1=0.003[b](Glu+DH)					
0.001	0.949	0.002	0.0010	0.931	0.001	0.0005	0.891	0.001	0.0005
0.003	0.956	0.002	0.0010	0.939	0.003	0.0010	0.898	0.002	0.0010
0.005	0.963	0.001	0.0005	0.947	0.002	0.0010	0.907	0.003	0.0010
0.007	0.969	0.001	0.0005	0.953	0.001	0.0005	0.913	0.001	0.0005
0.009	0.976	0.002	0.0010	0.960	0.002	0.0010	0.921	0.002	0.0010
				w_1=0.005[b](Glu+DH)					
0.001	0.928	0.001	0.0005	0.912	0.003	0.0010	0.892	0.001	0.0005
0.003	0.936	0.002	0.0010	0.920	0.002	0.0010	0.899	0.001	0.0005
0.005	0.942	0.001	0.0005	0.928	0.002	0.0010	0.908	0.002	0.0010
0.007	0.950	0.001	0.0005	0.935	0.002	0.0010	0.916	0.002	0.0010
0.009	0.957	0.002	0.0010	0.942	0.001	0.0005	0.924	0.001	0.0005

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]w_1 is the mass fraction of aqueous dopamine hydrochloride.

[c]m is the molarity of amino acid in water and water + dopamine hydrochloride solvent systems. Standard uncertainties, u, in molarity is u ([c]m) = 0.0002 mol·kg^{-1}, in viscosity values u ([e]η) =±0.003 mPa.s

Table. 5. Limiting apparent molar volume (ϕ_V^0), experimental slopes (S_v^*) and viscosity A and *B*-coefficient of amino acids in different mass fraction of aqueous Dopamine Hydrochloride at *T*= (293.15 to 313.15) K[a] and (*p* = 0.1MPa) respectively.

Temp/K[a]	$\phi_V^0 \cdot 10^6$ /m^3·mol^{-1}	$S_V^* \cdot 10^6$ /m^3·mol$^{-3/2}$·kg$^{1/2}$	*B* /kg$^{1/2}$·mol$^{-1/2}$	*A* /kg$^{1/2}$·mol$^{-1/2}$
(Asp+DH)		w_1 = 0.001[b]		
293.15	81.66	-274.83	1.9240	0.0565
303.15	88.24	-289.43	2.3534	0.1054

313.15	93.34	-295.83	2.7853	0.1116
(Asp+DH)		w_1 = 0.003[b]		
293.15	94.97	-348.57	2.4937	0.0855
303.15	101.05	-350.96	2.7636	0.1105
313.15	105.41	-353.75	2.9558	0.1187
(Asp+DH)		w_1 = 0.005[b]		
293.15	106.01	-364.05	2.7582	0.0944
303.15	111.36	-359.37	3.1294	0.1217
313.15	116.96	-353.25	3.3094	0.1283
(Glu+DH)		w_1 = 0.001[b]		
293.15	86.33	-317.24	2.3323	0.1011
303.15	93.06	-310.92	2.8024	0.1114
313.15	96.73	-297.24	2.8989	0.1284
(Glu+DH)		w_1 = 0.003[b]		
293.15	106.72	-394.11	2.6824	0.1063
303.15	115.57	-385.47	3.0513	0.1207
313.15	121.30	-401.50	3.0998	0.1221
(Glu+DH)		w_1 = 0.005[b]		
293.15	124.02	-391.75	2,9813	0.1113
303.15	131.57	-419.77	3.3037	0.1250
313.15	142.22	-488.50	3.6705	0.1344

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]*w_1 is* the mass fraction of the aqueous dopamine hydrochloride

Table. 6. Limiting apparent molar expansibilities (ϕ_E^0) of amino acids in different mass fraction of aqueous Dopamine Hydrochloride at *T*= (293.15 to 313.15) K[a] and (*p* = 0.1MPa) respectively**.**

.Mass fraction	$\phi_E^0 \cdot 10^6$ /m³·mol⁻¹·K⁻¹			$\left(\partial\phi_E^0/\partial T\right)_P \cdot 10^6$ /m³·mol⁻¹·K⁻²
		(Asp + DH)		
T/K[a]	293.15	303.15	313.15	
w_1 = 0.001[b]	0.738	0.590	0.442	-0.015
w_1 = 0.003[b]	0.666	0.494	0.322	-0.017
w_1 = 0.005[b]	0.552	0.578	0.604	0.003
		(Glu + DH)		
T/K[a]	293.15	303.15	313.15	
w_1 = 0.001[b]	0.828	0.523	0.218	-0.031
w_1 = 0.003[b]	1.041	0.729	0.417	-0.031
w_1 = 0.005[b]	0.600	0.910	1.220	0.031

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]w_1 is the mass fraction of aqueous dopamine hydrochloride.

Table.7. Values of *dB/dT* for Aspartic Acid and Glutamic Acid in different mass fraction of aqueous Dopamine Hydrochloride (w_1) at *T*= (293.15 to 313.15) K[a] and (*p* = 0.1MPa) respectively.

Mass fraction	$\frac{dB}{dT}$ /kg^1/2·mol^1/2·K⁻¹	
	(Asp + DH)	(Glu + DH)
w_1 = 0.001[b]	0.042	0.065
w_1 = 0.003[b]	0.039	0.050
w_1 = 0.005[b]	0.026	0.077

[b]w_1 is the mass fraction of the aqueous dopamine hydrochloride.

3.4 Refractive Index calculation

The molar refraction R_M was evaluated using the Lorentz-Lorenz relation [158], as the value is directly proportional to molecular polarizability [159], this quantity is a measure of the ability of the molecular orbitals to be impaired under an electrical field applied

$$R_M = \left\{(n_D^2-1)/(n_D^2+2)\right\}(M/\rho) \tag{7}$$

Where R_M, n_D, M and ρ are the molar refraction, the refractive index, the molar mass and the density of the solution respectively. The values of measured n_D are tabulated in **Table. S3**. From the values of R_M, it might be concluded that in aqueous medium Dopamine reacts with Glutamic acid more profoundly than Aspartic acid, which is further supported by other experimental evidence.

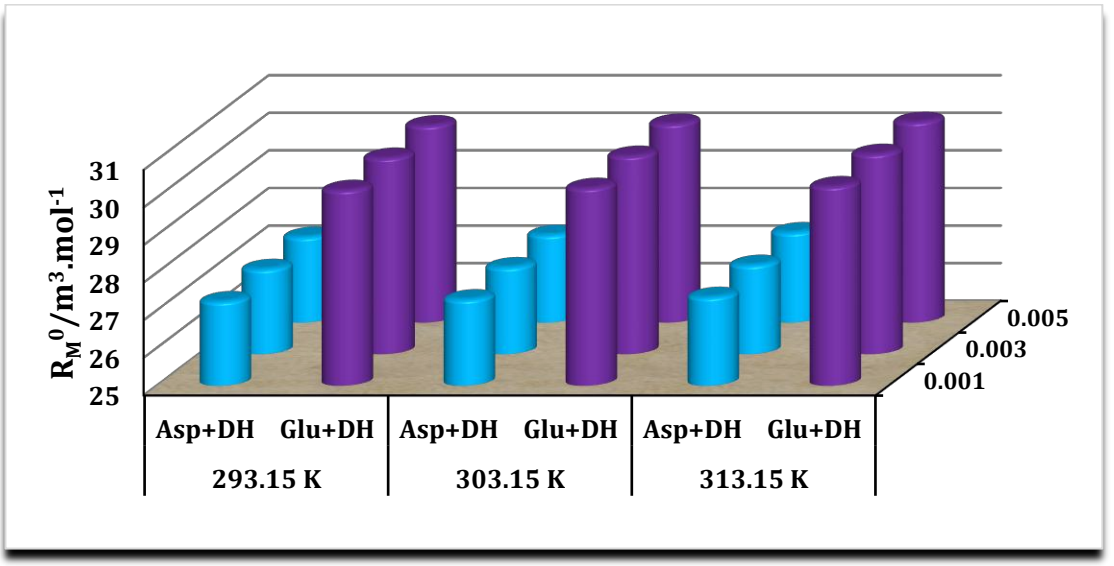


Fig.3. Plot of limiting molar refraction (R_M^o) of Asp and Glu in mass fractions(w_1) (a) 0.001, (b) 0.003, (c) 0.005 of aqueous DH at T= (293.15 to 313.15)K and (p = 0.1MPa) respectively.

Table. 8. Limiting molar refractions (R_M^0) values of amino acids in different mass fraction of aqueous Dopamine Hydrochloride at *T*= (293.15 to 313.15) K[a] and (*p* = 0.1MPa) respectively.

Mass fraction	R_M^0 /m^3·mol^{-1}		
(Asp+DH)	293.15 K[a]	303.15 K[a]	313.15 K[a]
w_1 = 0.001[b]	27.25	27.29	27.36
w_1 = 0.003[b]	27.26	27.31	27.37
w_1 = 0.005[b]	27.28	27.33	27.40
(Glu+DH)	293.15 K[a]	303.15 K[a]	313.15 K[a]
w_1 = 0.001[b]	30.21	30.26	30.31
w_1 = 0.003[b]	30.22	30.28	30.33
w_1 = 0.005[b]	30.25	30.29	30.34

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]w_1 is the mass fraction of aqueous Dopamine Hydrochloride.

3.5 Conductance

The molar conductance (Λ, Sm cm^{-1}) for all studied systems was evaluated using the following equation, Λ =1000κ/C, where C is the molar concentration and κ is the measured specific conductance of the studied systems (DH+Asp) and (DH+Glu) [160]. The conductance curves (Λ versus c) for the above systems were found to be gradually decreasing and depicted in Fig.4 and 5.

The molar conductance (Λ) was measured at three different temperature i.e. 293.15K – 313.15K with an interval of 10K and the variation was performed with respect to the concentration of dopamine hydrochloride, following usual conductance technique (**Table.9.**). The dependence of Λ on DH concentration at different temperatures is shown in **Fig.4.** and **Fig.5.**

On increasing the concentration of DH in amino acid solution, the decrease of Λ was observed [161] Such observation can be attributed to the following factors- (i) The interaction due to the

hydrophobic effect operating along all hydrocarbon chains of amino acids and alkyl chain of the drug molecule itself,(ii) the structural change of dopamine on increasing temperature. Dopamine experiences aerial oxidation, which leads the molecule to achieve the Quinone structure from the Benzenoid form and (iii) Dopamine-*o*-Quinone (DQ) being less reactive than the Benzenoid one, facilitates the intermolecular hydrogen bonding interaction ($-NH_3^+$.....$-COO^-$) between Dopamine ($-NH_2.HCl$) and amino acids (-COOH).

Table.9. Conductivity values for the Aspartic Acid and Glutamic Acid in aqueous Dopamine Hydrochloride at three different temperature (K[a]) and (p = 0.1MPa).

Conc. of amino acids (mM)	Conc. of DH (mM)	Molar conductance of Aspartic Acid ($S\ cm^2\ mol^{-1}$)			Molar conductance of Glutamic Acid ($S\ cm^2\ mol^{-1}$)		
		293.15K[a]	303.15K[a]	313.15K[a]	293.15K[a]	303.15K[a]	313.15K[a]
10	0	41.71	42.03	43.02	27.72	28.82	30.36
9.091	0.909	25.74	26.92	28.46	18.48	19.94	20.80
8.333	1.667	20.45	22.10	23.27	16.07	16.83	17.55
7.692	2.308	18.41	19.37	20.48	14.28	15.13	15.91
7.143	2.857	16.14	17.83	19.13	13.44	14.42	15.07
6.667	3.333	15.15	16.76	18.05	12.98	13.70	14.30
6.250	3.750	14.55	15.98	17.25	12.50	13.20	12.90
5.882	4.118	14.08	15.22	16.64	12.19	12.89	13.48
5.556	4.444	13.68	14.84	16.12	11.99	12.59	13.24
5.263	4.737	13.38	14.19	15.72	11.74	12.30	12.90
5.000	5.000	13.03	13.88	15.32	11.51	12.12	12.77
4.762	5.238	12.89	13.77	15.18	11.38	11.95	12.56
4.545	5.455	12.73	13.60	14.91	11.21	11.82	12.44
4.348	5.652	12.51	13.40	14.78	11.05	11.69	12.29
4.167	5.833	12.33	13.20	14.60	11.01	11.60	12.25
4.000	6.000	12.30	13.15	14.43	10.92	11.53	12.16
3.846	6.154	12.24	13.10	14.29	10.83	11.44	12.16
3.704	6.296	12.05	13.01	14.19	10.76	11.37	11.92
3.571	6.429	11.94	12.83	14.08	10.69	11.27	11.82
3.448	6.552	11.85	12.76	14.01	10.62	11.22	11.75
3.333	6.667	10.55	10.66	10.69	10.55	11.16	11.69

[a] Standard uncertainties in temperature (T)=0.01K

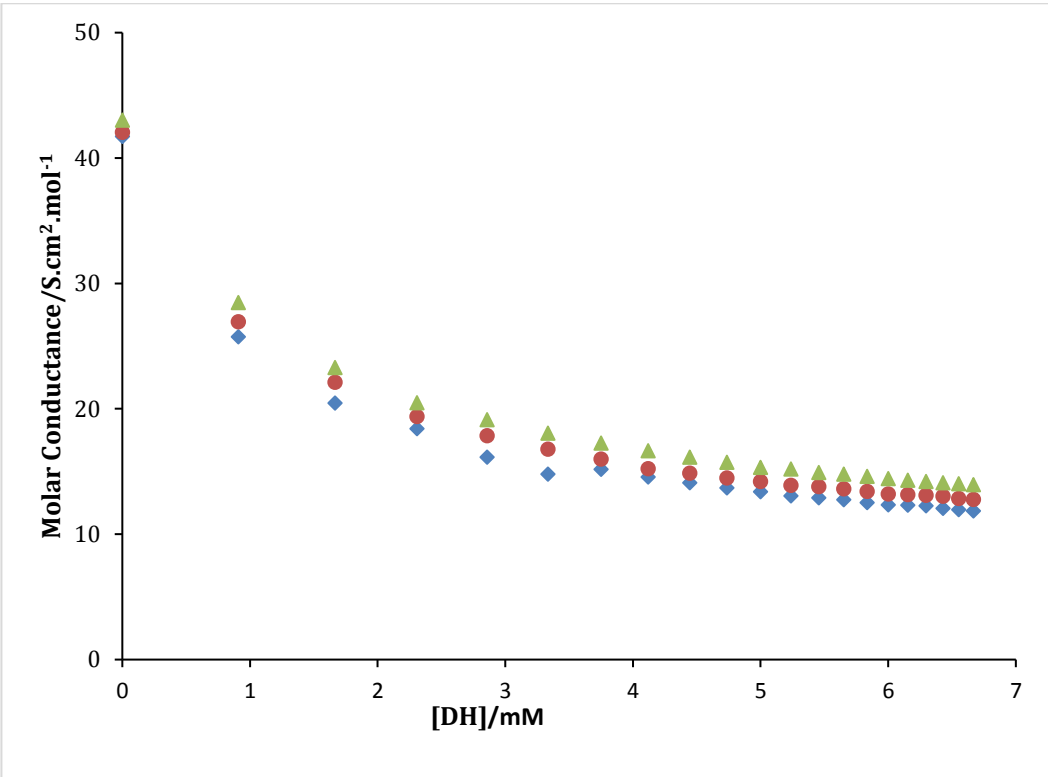


Fig.4. Molar conductivity of Aspartic Acid in aqueous solution with Dopamine Hydrochloride at T=293.15K (♦), 303.15K (●), 313.15K (▲) and (p = 0.1MPa) respectively.

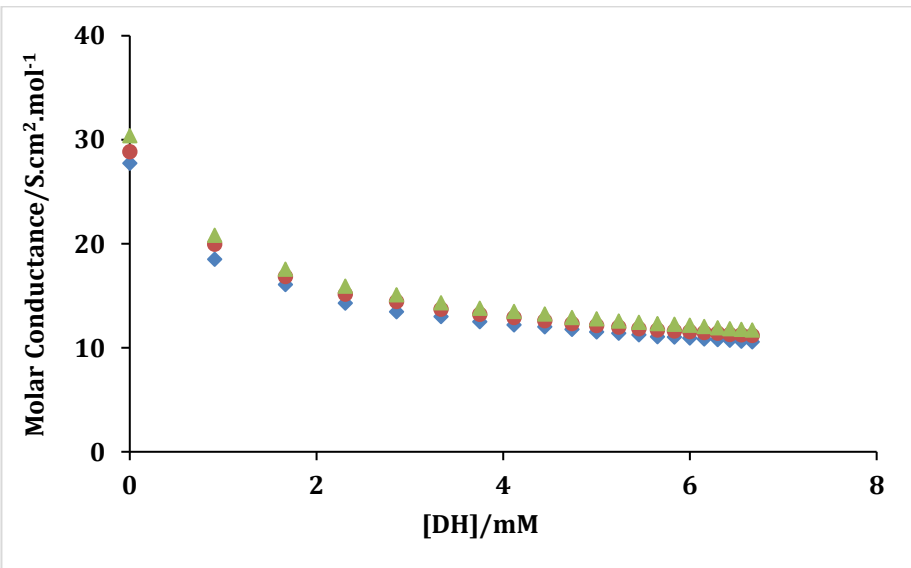


Fig.5. Molar conductivity of Glutamic Acid in aqueous solution with Dopamine Hydrochloride at T= 293.15K (♦), 303.15K (●), 313.15K (▲) and (p = 0.1MPa) respectively.

3.6 UV–Visible spectroscopy

The absorption maxima of the amino acids were recorded by varying concentration of drug molecule (DH) in aqueous solution are shown in **Fig.6a.**and **Fig.7a.** The strong absorption peaks for both cases were observed at ~264 nm (λ_{max}), which move towards longer wavelength on increasing concentration of DH. This, in turn, indicates the possibility of Red Shift in the medium [66] [65].From **Fig.6a-7a**, a CONCLUSION might be drawn that at a fixed composition of *l*-aspartic acid and *l*-glutamic acid, the absorption maximum increases with increase in the concentration of Dopamine hydrochloride

3.6.1 Association constant and Thermodynamic parameters

The association constants (K_a) for both the system (Asp+DH) and (Glu+DH) were calculated using the modified Benesi-Hildebrand equation, Eq. (8) [162], (Fig. 6b-7b) based on UV-visible spectroscopy and the values are presented in Table. 12.

$$\frac{1}{A-A_0}=\frac{1}{\Delta\varepsilon[AA]K_a}.\frac{1}{[DH]}+\frac{1}{\Delta\varepsilon[AA]} \tag{8}$$

Where [DH] and [AA] refers to the total concentration of the drug (DH) and amino acids respectively, Δϵ is the change in molar extinction coefficient between the free and amino acids bound with DH. ΔA stands for the absorption changes of amino acids on the addition of DH. The values of K_a for each of the system were evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot (**Table.10. and 11**).

The free energy change (ΔG), was calculated from association constant by using the following equation

$$\Delta G = -RT \ln K_a \tag{9}$$

The ΔG values (**Table.12.**) for both the amino acid-dopamine system are negative which indicates the feasibility of the adduct formation by means of interactions.

The association constant for (Glu+DH) system was found to be higher than that of the (Asp+DH) system (**Table.12.**) which indicates higher interaction in (Glu+DH) system.

Table.10. Data for the Benesi-Hildebrand double reciprocal plot performed by UV-Vis spectroscopy for DH-Aspartic Acid system.

Temp /K[a]	[Asp] /μM	[DH] /μM	A_0	A	ΔA	1/[DH] /M^{-1}	1/ΔA	Intercept	Slope	K_a /M^{-1}
	50	10		0.57107	0.0702	50000.00	14.24501			
	50	20		0.62767	0.1268	25000.00	7.886435			
298.15	50	30		0.69371	0.19284	16666.70	5.185646			
	50	40		0.77844	0.27757	12500.00	3.602695			
	50	50	0.50087	0.82971	0.32884	10000.00	3.040993			
	50	60		0.91220	0.41133	8333.33	2.431138	0.0931	0.0011	3.10×10^2
	50	70		0.96366	0.46279	7142.86	2.160807			
	50	80		1.05985	0.55898	6250.00	1.788973			
	50	90		1.15249	0.65162	5555.56	1.534637			
	50	100		1.22085	0.71998	5000.00	1.388927			

[a] Standard uncertainties in temperature are: (T) = ±0.01 K.

Table.11. Data for the Benesi-Hildebrand double reciprocal plot performed by UV-Vis spectroscopy for DH-Glutamic Acid system.

Temp /K[a]	[Glu] /μM	[DH] /μM	A_0	A	ΔA	1/[DH] /M^{-1}	1/ΔA	Intercept	Slope	K_a /M^{-1}
	50	10		0.23670	0.09582	50000.00	10.43623			
	50	20		0.31460	0.17372	25000.00	5.756390			
298.15	50	30		0.39727	0.25639	16666.70	3.900308			
	50	40		0.53003	0.38915	12500.00	2.569703			
	50	50	0.14088	0.58770	0.44682	10000.00	2.238038	0.6184	0.0015	$9.45*10^2$
	50	60		0.67058	0.52970	8333.33	1.887861			
	50	70		0.70871	0.56783	7142.86	1.76109			
	50	80		0.82617	0.68529	6250.00	1.459236			
	50	90		0.92309	0.78221	5555.56	1.278429			
	50	100		1.02191	0.88103	5000.00	1.135035			

[a] Standard uncertainties in temperature are: (T) = ±0.01 K.

Table.12. Values of Association Constant (K_a) and free energy change (ΔG^0) of the two system (Asp+DH) and (Glu+DH).

	T/K[a]	K_a/ M^{-1}	ΔG^0/KJ mol^{-1}
(Asp+DH)		310.33	-14.22
	298.15		
(Glu+DH)		945.00	- 16.98

[a] Standard uncertainties in temperature are: (T) = ±0.01 K.

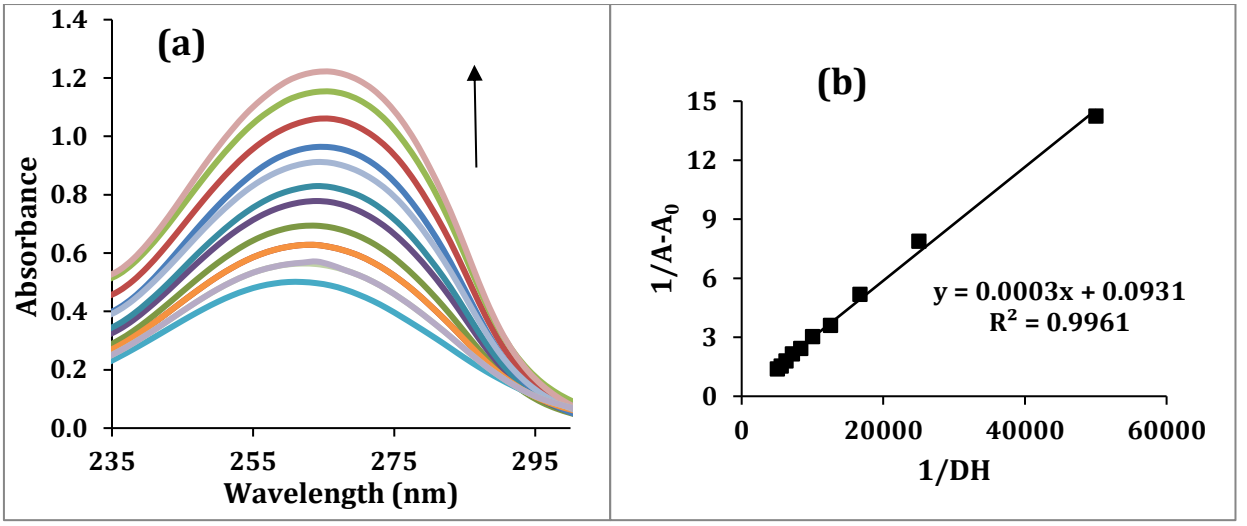


Fig.6.(a). Absorption spectra of Aspartic Acid (50 μM) in different concentrations of aq. DH (μM): (1) without DH, (2) 10 μM , (3) 20 μM, (4) 30 μM, (5) 40 μM, (6) 50 μM, (7) 60 μM , (8) 70 μM, (9) 80 μM, (10) 90 μM, (11) 100 μM. (b) Benesi–Hildebrand plot of $1/A–A_0$ vs. 1/[DH] for (Asp+DH) system.

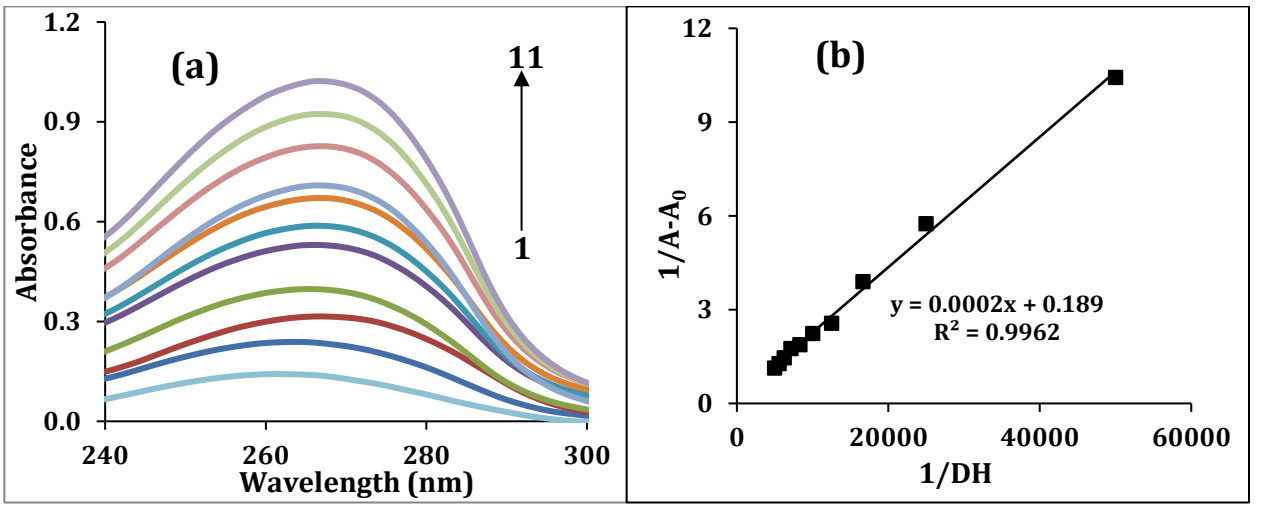


Fig.7.(a). Absorption spectra of Glutamic Acid (50 μM) in different concentrations of aq. DH (μM): (1) without DH, (2) 10 μM , (3) 20 μM, (4) 30 μM, (5) 40 μM, (6) 50 μM, (7) 60 μM , (8) 70 μM, (9) 80 μM, (10) 90 μM, (11) 100 μM. (b) Benesi–Hildebrand plot of $1/A–A_0$ vs. 1/[DH] for (Glu+DH),system.

3.7 Raman Spectroscopy

Because of the presence of the interaction in aqueous solution of DH with the amino acids studied, the structural alternation has led to the changes in the shifting of the corresponding peaks. The structures of the investigated molecules when they are hydrogen bonded to water are shown in **Scheme2a.** and **2b**. For DH and Aspartic acid N-H stretching frequency were observed at 3474cm^{-1} and 3456 cm^{-1}, whereas the N-H stretching frequency was detected at 3449 cm^{-1}. On the other hand, the N-H bond stretching frequency was found to be 3474 cm^{-1} and 3468cm^{-1} in case of DH and Glutamic acid respectively, but in case of (DH+Glu) solution, it was found to be 3453cm^{-1}, which was a clear indication for the existence of intermolecular H-bonding in both cases. Compared to the N-H free of hydrogen bonding, the interaction between N and H atoms for the DH is weaker due to the formation of the N-H---O structure, which will induce the redshift of N-H vibration.

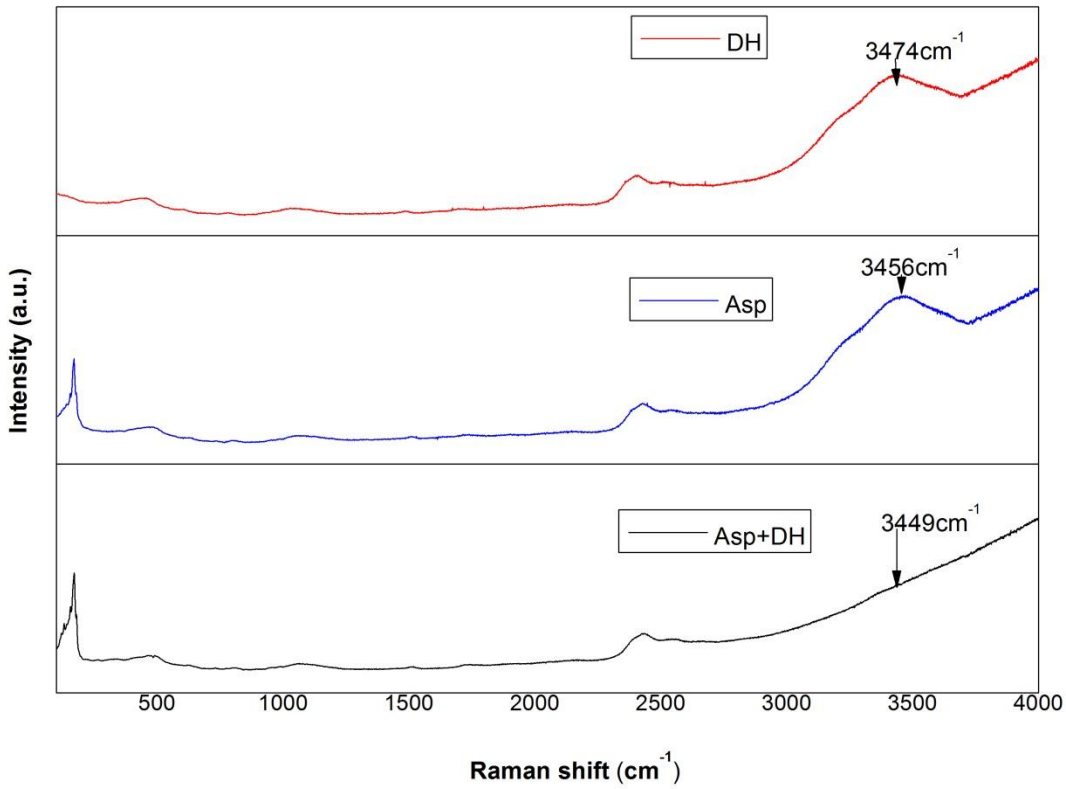


Fig.8. Raman Spectra of (a) Dopamine Hydrochloride, (b) Aspartic Acid and (c) (DH+Asp) solution

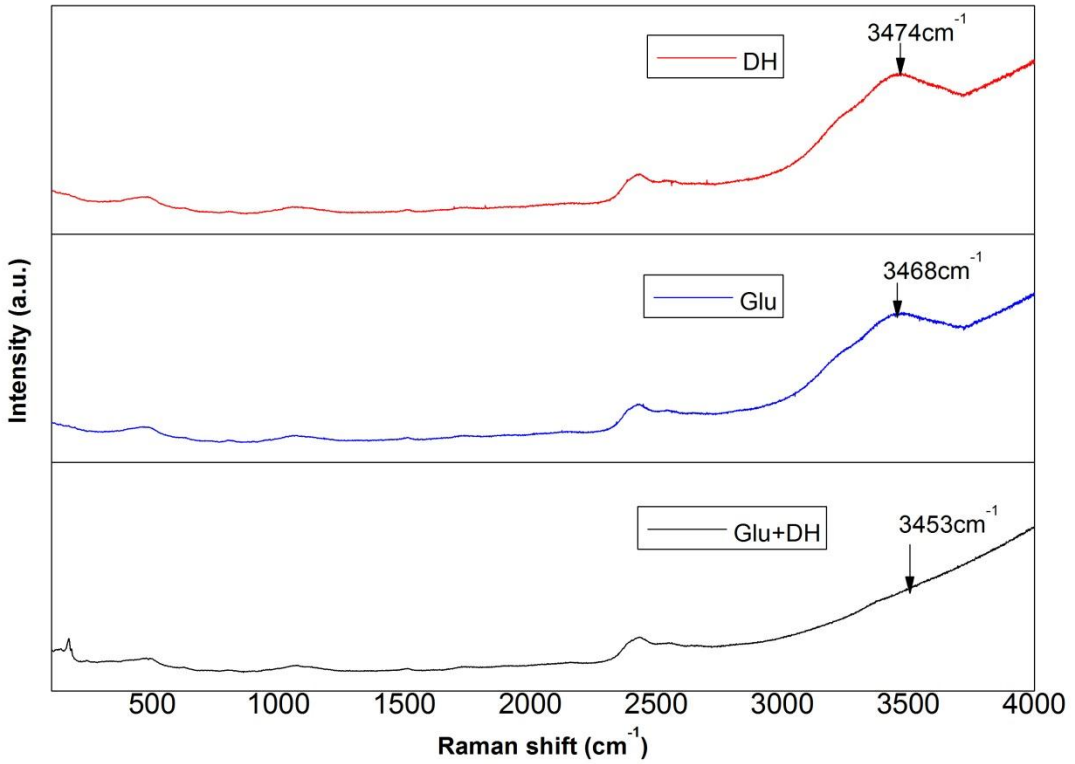
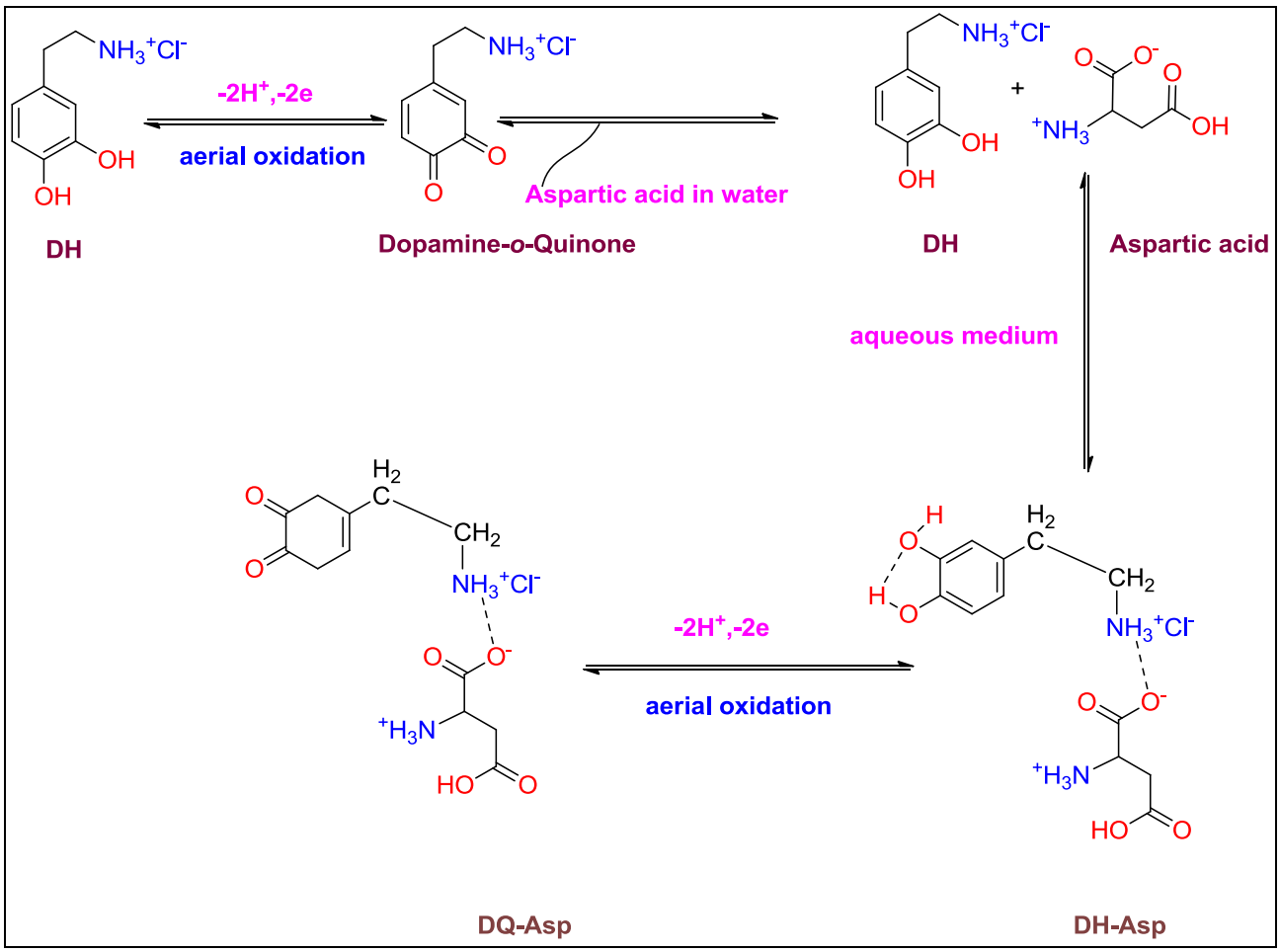


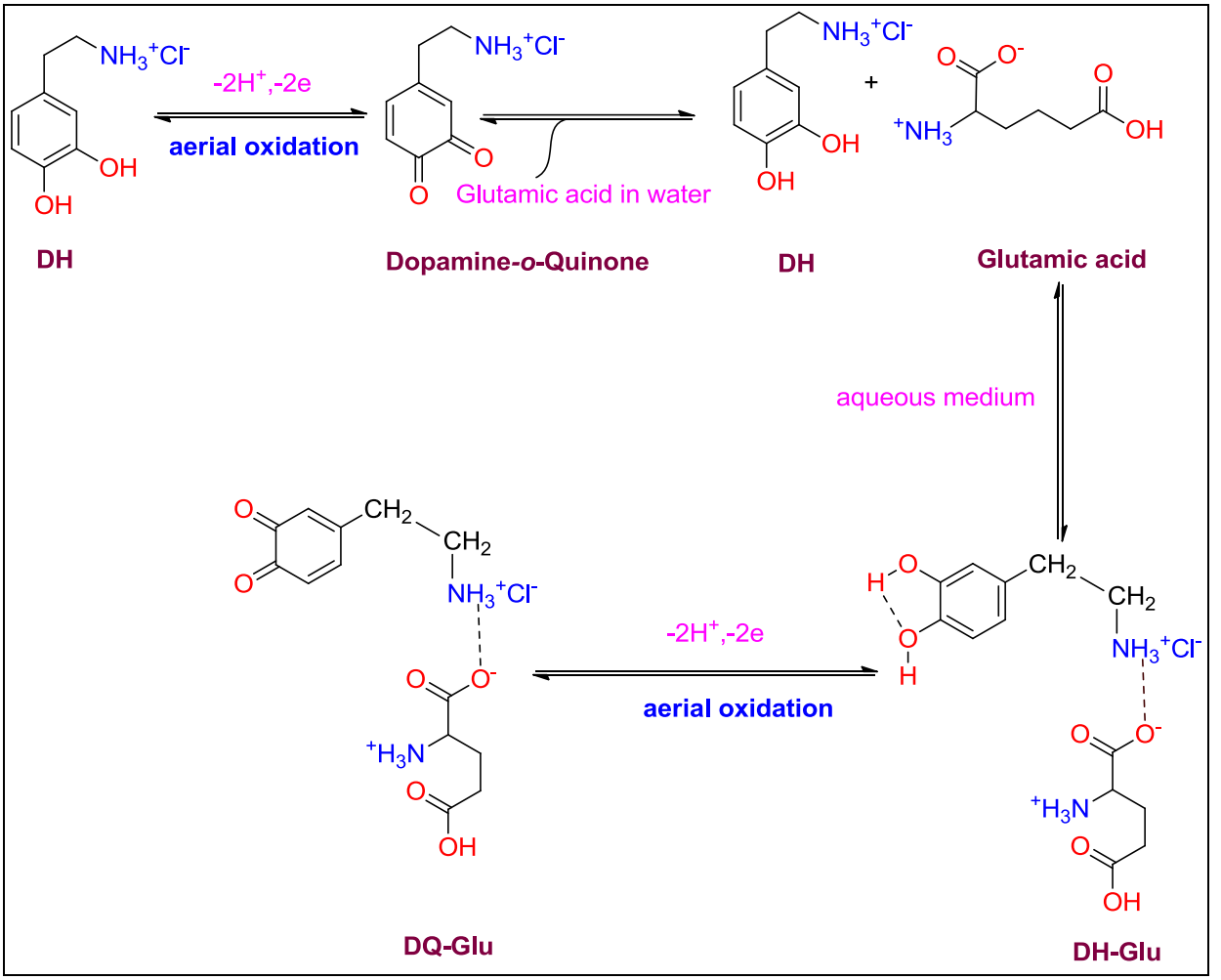
Fig.9. Raman Spectra of (a) Dopamine Hydrochloride, (b) Glutamic Acid and (c) (DH+Glu) solution

Table.13. Stretching frequency of N-H band in Raman Spectroscopy

Functional group/vibration	Region	Raman
υ(N-H)	3300 - 3500 cm^{-1}	medium



Scheme2a. Plausible interaction between Aspartic acid and Dopamine hydrochloride in aqueous medium.



Scheme2b. Plausible interaction between Glutamic acid and Dopamine hydrochloride in aqueous medium.

3.8 1H NMR studies

Nuclear magnetic resonance (NMR) is an effective tool to study the changes of the electronic environment around the different protons of drug molecule (DH) in presence amino acids [163]. NMR samples were prepared by adding amino acids to dopamine hydrochloride in NMR tubes by mass. D_2O solvent was added with a syringe while the samples were kept under N_2 flow. NMR spectra of the solvent were also taken to avoid possibilities of contamination. All measurements were performed at 298.15 K using a 600 MHz spectrometer.

The chemical shifts of the amino acids and the drug studied depend on the electron density present in their surrounding area. Since H-bonding involves the transfer of electron cloud from hydrogen atoms to a neighbouring electronegative atom (O, N), the hydrogen experiences a net deshielding effect when there occurs an extensive hydrogen bonding and becomes less

deshielded when the possibility of such bonding is ruled out. Thus at higher concentration (strong H-bonding, strong deshielding) -OH,-NH protons appear at higher δ values compared to diluted one [164]. Considerable shifts for both δ values of alkyl protons of the benzene ring (in DH) and -NH_2 (in DH) proton occur after mixing with amino acids. It indicates the presence of intermolecular hydrogen bonding between these two compounds, leading to the decrease in electron density around the benzene ring.

The NMR data (given below) provide a CONCLUSION about the plausible contributing factors such as- (i) intermolecular H-bonding interactions between amino acids and DH (Scheme 2a and 2b), (ii) hydrophobic interactions [165] [166], which are in good agreement with the result obtained.

1H NMR Data:

Dopamine Hydrochloride (DH):

δ = 2.23 (-NH_2), 2.86 (2H, t, *J* = 7.2 Hz), 3.23 (2H, t, *J* = 7.2 Hz), 6.76 (1H, dd, *J* = 8.0 Hz), 6.85 (1H, d, *J* = 2Hz), 6.92 (1H, d, *J* = 8.4 Hz)

Aspartic Acid (Asp):

δ = 4.04-4.06 (1H, dd), 2.94-2.97 (2H, m)

Glutamic Acid (Glu):

δ = 2.15-2.21 (2H, m), 2.53-2.60 (2H, m), 3.81 (1H, m)

Dopamine Hydrochloride (DH) + Aspartic Acid (Asp):

δ = 2.26 (-NH_2), 2.92 (2H, t, *J* = 7.2 Hz), 3.25 (2H, t, *J* = 7.2 Hz), 6.80 (1H, dd, *J* = 8.0 Hz), 6.89 (1H, d, *J* = 2 Hz), 6.94 (1H, d, *J* = 8.4 Hz), 4.05-4.07 (1H, m), 2.95-2.98 (2H, m)

Dopamine hydrochloride (DH) + Glutamic acid (Glu):

δ = 2.30 (-NH_2), 2.94 (2H, t, *J* = 7.2 Hz), 3.27 (2H, t, *J* = 7.2 Hz), 6.82 (1H, dd, *J* = 8.0 Hz), 6.90 (1H, d, *J* = 2 Hz), 6.96 (1H, d, *J* = 8.4 Hz), 2.18-2.23 (2H, m), 2.56-2.62 (2H, m), 3.89 (1H, m)

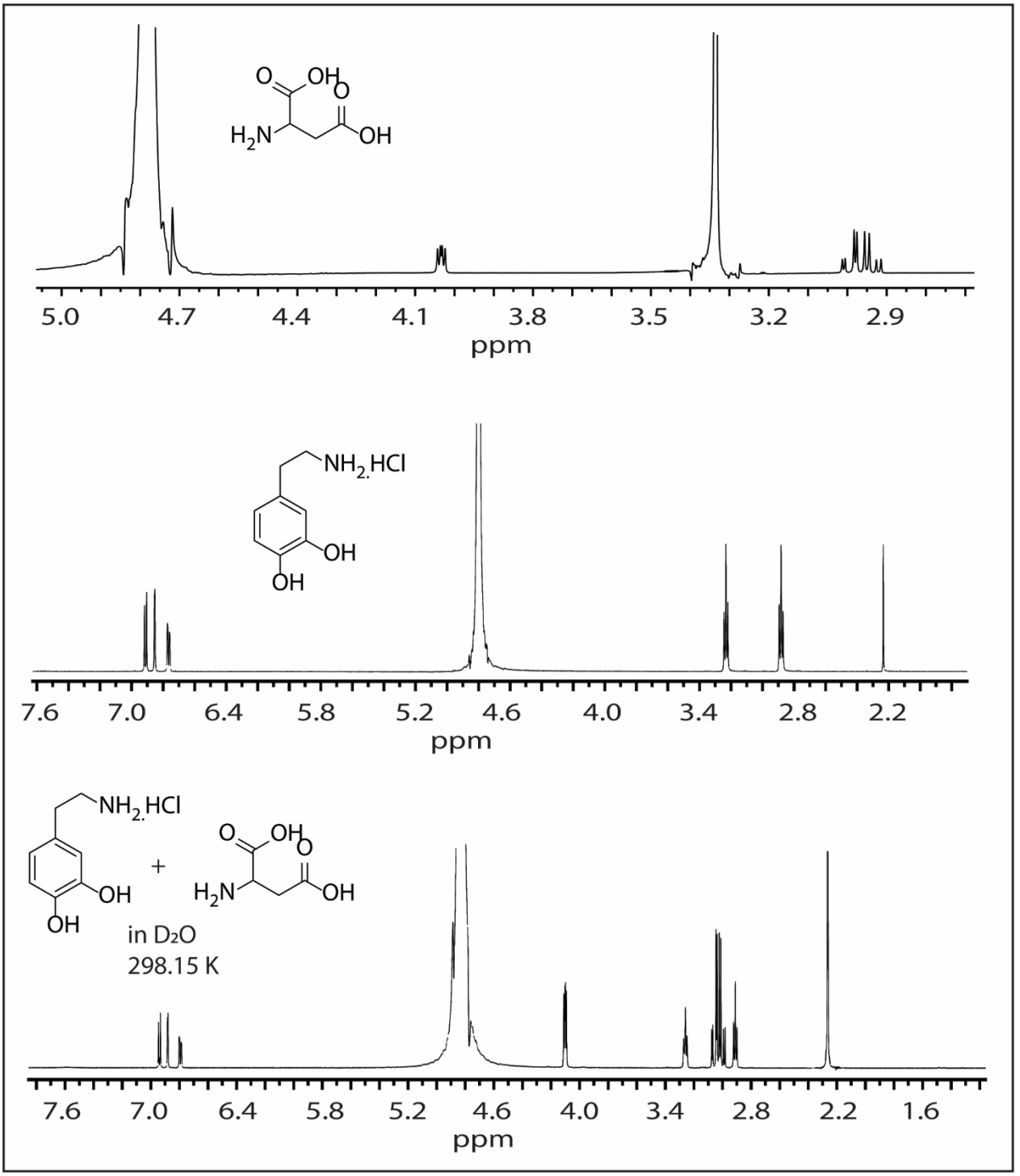


Fig.10. 1H NMR spectra of (a) Aspartic Acid (b) Dopamine Hydrochloride (c) (Dopamine Hydrochloride+ Aspartic Acid) solution in D_2O at *T*= 298.15K.

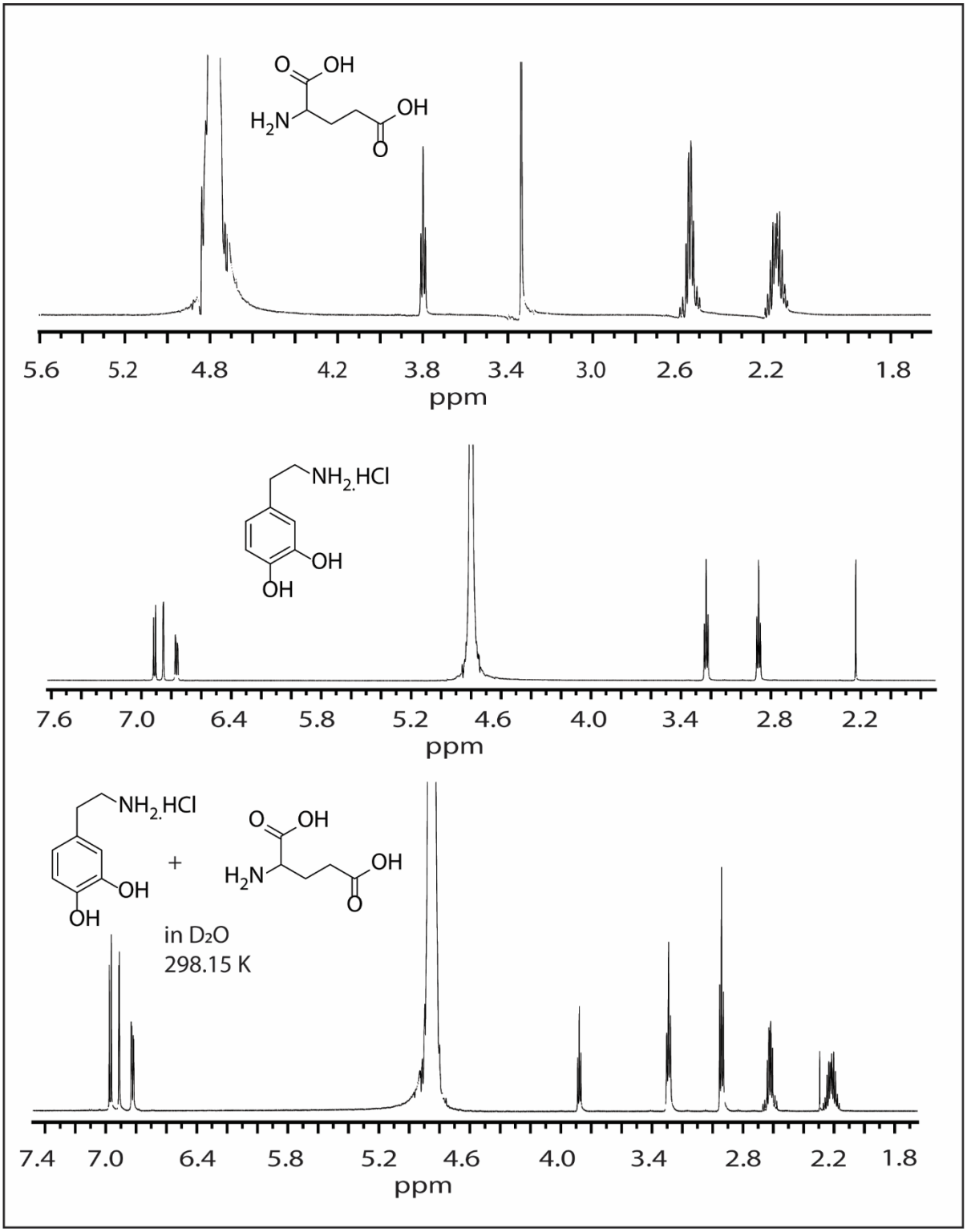


Fig.11. 1H NMR spectra of (a) Glutamic acid (b) Dopamine Hydrochloride (c) (Dopamine Hydrochloride+ Glutamic Acid) solution in D_2O at *T*=298.15K.

4. CONCLUSION

The results obtained from density, viscosity, and refractive index measurements were reported to establish the interaction between Dopamine Hydrochloride and amino acids in aqueous medium. The values of limiting apparent molar volume suggest the existence of stronger ion-ion interactions in case of *l*-Glutamic acid with Dopamine. The interactions were also anticipated by the UV-Visible absorption data. The higher value of association constant dictates greater interaction in Glu-DH system. The Raman spectra sensitively captured partial structure changes of the molecule [167]. From the thermophysical parameters and spectroscopic studies, it is observed that solute-solvent interactions increase from *l*-aspartic acid to *l*-glutamic acid. The experimental data also deliver a wealth of information on the structure and dynamics of such species. The observation not only provides new insight into the widely investigated interfacial chemical reaction of Dopamine, but it also defines a starting point for a detailed understanding of various macroscopic phenomena occurring in the system. Glutamate receptor agonists have been suggested as an alternative therapy to dopamine receptor antagonists in the treatment of schizophrenia [168]. Thus the extent of interaction between amino acids and DH also ensures its feasibility in the area of medicinal chemistry or biomedicine industry. Besides, the current work dictates a guideline for the catecholamine molecules recognition tool, a simple and fast method for identifying the type of interaction taking place in an aqueous environment.

Tables.

Table.S1. Apparent molar volume (ϕ_V) and $\frac{(\eta_r - 1)}{\sqrt{m}}$ for amino acids in different mass fraction of aqueous Dopamine Hydrochloride at different temperatures and (p = 0.1MPa).

^{c}m /mol·kg^{-1}	$\phi_V \cdot 10^6$ / m^3·mol^{-1}	$\frac{(\eta_r - 1)}{\sqrt{m}}$	^{c}m /mol·kg^{-1}	$\phi_V \cdot 10^6$ / m^3·mol^{-1}	$\frac{(\eta_r - 1)}{\sqrt{m}}$	^{c}m /mol·kg^{-1}	$\phi_V \cdot 10^6$ / m^3·mol^{-1}	$\frac{(\eta_r - 1)}{\sqrt{m}}$
				w_1=0.001^b				
(Asp+DH)	293.15 K^a			303.15 K^a			313.15 K^a	
0.001	72.2300	0.115	0.001	78.3447	0.164	0.001	83.3529	0.187
0.003	67.5549	0.161	0.003	73.1552	0.234	0.003	77.5879	0.237
0.005	62.6127	0.199	0.005	68.2804	0.257	0.005	73.3803	0.291

0.007	58.4910	0.217	0.007	64.0246	0.300	0.007	68.5245	0.321
0.009	55.0880	0.236	0.009	60.0420	0.324	0.009	64.5814	0.362
				w_1=0.003[b]				
0.001	83.1815	0.185	0.001	89.4399	0.195	0.001	93.4473	0.215
0.003	76.5083	0.229	0.003	81.7440	0.269	0.003	86.6833	0.303
0.005	71.1697	0.267	0.005	77.3941	0.300	0.005	81.2698	0.347
0.007	66.0218	0.302	0.007	71.9448	0.344	0.007	76.0490	0.373
0.009	60.9375	0.334	0.009	66.6867	0.372	0.009	70.8926	0.421
				w_1=0.005[b]				
0.001	93.9084	0.198	0.001	99.2071	0.220	0.001	105.4985	0.233
0.003	86.5900	0.269	0.003	92.1996	0.276	0.003	97.4072	0.308
0.005	80.9349	0.304	0.005	86.9940	0.317	0.005	92.9529	0.368
0.007	75.8025	0.347	0.007	81.4737	0.360	0.007	87.7181	0.398
0.009	70.7335	0.375	0.009	76.2156	0.412	0.009	82.6721	0.445
				w_1=0.001[b]				
(Glu+DH)	293.15 K[a]			303.15 K[a]			313.15 K[a]	
0.001	77.2691	0.181	0.001	82.4930	0.195	0.001	87.3343	0.208
0.003	67.2511	0.220	0.003	77.4709	0.250	0.003	80.5514	0.278
0.005	64.0453	0.263	0.005	70.2391	0.293	0.005	75.1229	0.312
0.007	59.9522	0.297	0.007	67.4267	0.327	0.007	72.7902	0.356
0.009	56.5652	0.327	0.009	63.0772	0.367	0.009	68.1038	0.403
				w_1=0.003[b]				
0.001	95.2233	0.198	0.001	102.5196	0.209	0.001	107.5699	0.238
0.003	83.8789	0.266	0.003	95.1583	0.297	0.003	100.8045	0.300
0.005	78.0064	0.315	0.005	89.0685	0.346	0.005	93.3622	0.360
0.007	74.4887	0.340	0.007	83.5905	0.370	0.007	87.2707	0.401
0.009	69.6427	0.378	0.009	77.8704	0.406	0.009	82.7556	0.440

w_1=0.005[b]								
0.001	110.9038	0.232	0.001	117.2566	0.220	0.001	125.6647	0.254
0.003	103.2527	0.273	0.003	109.4816	0.288	0.003	117.2362	0.327
0.005	97.1319	0.323	0.005	103.0413	0.345	0.005	107.4575	0.399
0.007	91.3722	0.373	0.007	96.3769	0.387	0.007	101.6724	0.441
0.009	86.0655	0.413	0.009	90.6724	0.411	0.009	95.0841	0.483

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]w_1 is the mass fraction of aqueous dopamine hydrochloride.

[c]m is the molarity of amino acid in water and water + dopamine hydrochloride solvent systems.

Standard uncertainties, u, in molality is u ([c]m) = 0.0002 mol·kg^{-1}.

Table.S2. Values of empirical coefficients (a_0, a_1, and a_2) of Equation 3 of amino acid in different mass fraction of aqueous Dopamine Hydrochloride at T= (293.15 to 313.15) K and (p = 0.1MPa).

Mass fraction	$a_0 \cdot 10^6$ /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}
		(Asp + DH)	
w_1 = 0.001[b]	-769.77	5.0767	-0.0074
w_1 = 0.003[b]	-843.26	5.7084	-0.0086
w_1 = 0.005[b]	60.26	-0.2104	0.0013
		(Glu + DH)	
w_1 = 0.001[b]	-1,468.33	9.7812	-0.0153
w_1 = 0.003[b]	-1,539.07	10.1872	-0.0156
w_1 = 0.005[b]	1,280.15	-8.4876	0.0155

[b]w_1 is the mass fraction of the aqueous dopamine hydrochloride.

Table.S3. Values of Refractive Index (n_D) and Molar Refraction (R_M) of Aspartic Acid and Glutamic Acid in different mass fraction of aqueous Dopamine Hydrochloride (w_1) at different temperatures (298.15 K[a], 303.15K[a] & 313.15K[a]) and (p = 0.1MPa) respectively.

Solution	Conc. (^{c}m /mol·kg^{-1})	n_D	R_M/m^3·mol^{-1}
	w_1 = 0.001[b]		
	293.15K[a]		
	0.001	1.3309	27.2687
	0.003	1.3311	27.2799
	0.005	1.3313	27.2906
	0.007	1.3315	27.3009
	0.009	1.3317	27.3110
	303.15K[a]		
	0.001	1.3309	27.2687
	0.003	1.3308	27.3291
(Asp+DH)	0.005	1.3310	27.3401
	0.007	1.3312	27.3507
	0.009	1.3314	27.3609
	313.15K[a]		
	0.001	1.3302	27.3811
	0.003	1.3305	27.4005
	0.005	1.3307	27.4119
	0.007	1.3309	27.4227
	0.009	1.3310	27.4257
	w_1 = 0.003[b]		
	293.15K[a]		
	0.001	1.3314	27.2840
	0.003	1.3316	27.2957
	0.005	1.3318	27.3068
	0.007	1.3320	27.3173
	0.009	1.3321	27.3199
	303.15K[a]		
	0.001	1.3310	27.3314
	0.003	1.3312	27.3433
(Asp+DH)	0.005	1.3314	27.3549

	0.007	1.3316	27.3658
	0.009	1.3317	27.3686
	313.15K[a]		
	0.001	1.3308	27.3935
	0.003	1.3310	27.4058
	0.005	1.3312	27.4175
	0.007	1.3314	27.4287
	0.009	1.3315	27.4318
	w_1 = 0.005[b]		
	293.15K[a]		
	0.001	1.3327	27.2989
	0.003	1.3329	27.3111
	0.005	1.3331	27.3227
	0.007	1.3333	27.3338
	0.009	1.3334	27.3368
	303.15K[a]		
	0.001	1.3323	27.3544
	0.003	1.3325	27.3669
(Asp+DH)	0.005	1.3327	27.3789
	0.007	1.3329	27.3903
	0.009	1.3330	27.3936
	313.15K[a]		
	0.001	1.3317	27.4226
	0.003	1.3319	27.4354
	0.005	1.3321	27.4478
	0.007	1.3323	27.4596
	0.009	1.3324	27.4633
	w_1 = 0.001[b]		
	293.15K[a]		
	0.001	1.3320	30.2338
	0.003	1.3323	30.2534
	0.005	1.3325	30.2646
	0.007	1.3327	30.2752
	0.009	1.3329	30.2855
	303.15K[a]		

	0.001	1.3317	30.2880
	0.003	1.3320	30.3084
(Glu+DH)	0.005	1.3322	30.3197
	0.007	1.3324	30.3309
	0.009	1.3326	30.3414
	313.15K[a]		
	0.001	1.3317	30.3337
	0.003	1.3320	30.3544
	0.005	1.3322	30.3661
	0.007	1.3324	30.3778
	0.009	1.3326	30.3887
	w_1 = 0.003[b]		
	298.15K[a]		
	0.001	1.3325	30.2508
	0.003	1.3328	30.2714
(Glu+DH)	0.005	1.3330	30.2832
	0.007	1.3332	30.2947
	0.009	1.3334	30.3055
	303.15K[a]		
	0.001	1.3321	30.3035
	0.003	1.3324	30.3249
	0.005	1.3326	30.3374
	0.007	1.3328	30.3492
	0.009	1.3330	30.3603
	313.15K[a]		
	0.001	1.3317	30.3558
	0.003	1.3320	30.3777
	0.005	1.3322	30.3903
	0.007	1.3324	30.4024
	0.009	1.3326	30.4141
	w_1 = 0.005[b]		
	293.15K[a]		
	0.001	1.3339	30.2753
	0.003	1.3342	30.2972

	0.005	1.3344	30.3100
	0.007	1.3346	30.3222
	0.009	1.3348	30.3339
	303.15K[a]		
	0.001	1.3333	30.3206
	0.003	1.3336	30.3428
(Glu+DH)	0.005	1.3338	30.3560
	0.007	1.3340	30.3684
	0.009	1.3342	30.3803
	303.15K[a]		
	0.001	1.3324	30.3714
	0.003	1.3327	30.3942
	0.005	1.3329	30.4074
	0.007	1.3331	30.4203
	0.009	1.3333	30.4323

[a]Standard uncertainties in temperature (T) = ±0.01 K.

[b]w_1 is the mass fraction of aqueous dopamine hydrochloride.

[c]m is the molality of amino acid in water and water + dopamine hydrochloride solvent systems. Standard uncertainties, u, in molality is u ([c]m) = 0.0002 mol·kg^{-1}.