REFRACTIVE INDEX FOR ALANINE, GLUTAMIC ACID AND HISTIDINE IN AQUEOUS NaCl SOLUTIONS AT DIFFERENT TEMPERATURES

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Experimental refractive index data for L-alanine, L-histidine and L-glutamic acid in aqueous NaCl solutions at different temperatures (293.15, 298.15, 303.15, 313.15 and 323.15 K) and ambient pressure are reported. The obtained data were used to calculate molar refraction and overall polarizability of the solutions. Moreover, the Lorentz-Lorenz empirical equation was employed to correlate density previous data with refractive index for binary and ternary systems containing the investigated amino acids, water and NaCl. Results are interpreted in terms of interactions in these mixtures. Additionally, a computational investigation of dipole moment and molecular polarizability by density functional theory (DFT) at wB97XD/6-31 G+(d,p) level is presented.

Keywords: amino acids, refractive index, molar refractivity, DFT computations

1. Introduction

Amino acids are key components of peptides and proteins that due to their dual ionic structure dictate their interactions in physiological media and ligand-target protein complexation. The outside terminal hydroxyl- or amino- functional groups or other substituents of constitutive amino acids at the protein surface interact directly with small molecules or ligands within the active binding site. Thus, the binding process and consequently the biological response, are dictated by the nature and strength of molecular interactions occurring between amino acid residues of the biological target and ligand or drug.

Moreover, the anions of electrolytes from physiological media, such as chloride, influence also the nature of molecular forces between existing anions and cations in biological fluids, influencing protein structure by affecting properties (e.g. solubility, denaturation or activity of enzymes). Thus, strong electrolyte solutions are intensively studied in literature to predict volumetric, transport and other related properties of solutes such as amino acids in pseudo

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physiological media [1-4], but more detailed investigations on thermodynamic and physical-chemical properties are still needed.

Although there is a great interest of researchers for the volumetric and transport properties of amino acids in electrolyte solutions [5-8], the refractive index behavior is less studied, even they are very expeditious and employ small amount of sample. As refractive indices are usually reported together with density, they are studied jointly and the derived properties such as molar refraction, R_M, and polarizability parameter, α, can provide information about the volumetric behavior of the system. Use of R_M and α has become increasingly important in the study of drug interactions. Moreover, the magnitude of R_M and α are discussed in terms of intermolecular forces between solutes (amino acids) and its surroundings (solvent molecules, electrolytes, macromolecular assemblies exposed area).

To improve our understanding of the behavior of L-histidine (His), L-alanine (Ala) and L-glutamic acid (Glu) in aqueous NaCl solutions, the refractive index data have been reported. The temperature range covers the physiologically relevant range 293.15–323.15 K Therefore, we investigated the corresponding binary and ternary systems of amino acids on large concentration range (within their solubility domains) in aqueous solutions which goes untill 1.5 mol·kg^{-1} NaCl.

The refractive index data for aqueous solutions of L-alanine are reported in literature by Shekaari and co-workers [9-11] and for their aqueous NaCl solutions, by Rodrıguez *et al.* [12]. For L-histidine aqueous solutions there is only one study in literature, of Deosarkar et al. [13], with metformin hydrochloride. To our knowledge, data for aqueous solutions of L-histidine and L-glutamic acid are lacking in the literature. From refractive index data, molar refraction and overall polarizability of aqueous solutions on wide ranges of temperature and NaCl concentrations were calculated and discussed in terms of possible interactions occurring in these solutions.

Molecular descriptors such as molecular polarizability and dipole moment, which are indicators for interactions in solutions, have also been investigated to understand the behaviour of these amino acids in aqueous NaCl solutions.

This article is a continuation of our work on the systematic measurements of thermophysical properties of aqueous electrolyte solutions containing amino acids [14-18].

2. Experimental

2.1. Chemicals

Specifications of investigated amino acids and salt are given in Table 1.

Table 1

Specifications of used chemicals

Name of chemical	Purity (mass fraction %)	Source
L-alanine	>99	Sigma-Aldrich
L-glutamic acid	>99	Sigma-Aldrich
L-histidine	>99	Sigma-Aldrich
NaCl	>99.8	Merck
Water	Doubly distilled, deionized and degassed water with a specific conductance of $5 \cdot 10^{-5}$ S·m^{-1} at 298.15 K	

2.2 Preparation method

The mixtures of the desired composition were freshly prepared by mass using an analytical balance HR-120 (A&D Japan) with a precision of $\pm 10^{-4}$ g. Solutions were prepared by molality according to previous reported procedure [14].

2.3. Measurements of refractive index

Refractive indices of the binary and ternary mixtures at the sodium D-line were measured with a thermostated Abbe refractometer after calibrating it with doubly distilled, Millipore water. The precision of the measurements was ±0.0001. The temperature of the solutions was maintained in an electronically controlled water bath having a precision of ±0.05 K. An average of triplicate measurements was performed for each sample.

2.4. Computational details

Spartan'14 software package from Wavefunction, Inc., Irvine, CA, U.S.A [19] was used for parameters computations on the optimized geometries, of the investigated amino acids. Calculations of dipole moment and molecular polarizability were carried out in vacuum and in water, for equilibrium geometry at ground state, using Density Functional Theory with wB97XD functional in conjunction and 6-31+G(d,p) basis set [20]. Graphical map's representation of molecular electrostatic potential along with dipole vector were generated for the lowest energy conformers of the investigated amino acids in the above – mentioned conditions.

3. Results

The measured refractive indices, n_D, of aqueous NaCl solutions of L-histidine, L-alanine and L-glutamic acid over the temperature range (293.15–323.15) K are presented in Table 2. The refractive indices of all mixtures decrease with an increase in temperature and increase with increasing concentrations of both amino acid and salt. This increasing implies that the packing of amino acids

molecules forms more compact structures with enhanced interactions between amino acids and aqueous NaCl.

From Fig. 1 it can be seen that n_D linearly increases with the molality of amino acids, *m*. The parameters of the linear fit (eq. 1) are given in Table 3.

$$n_D = n_0 + mS_n \quad (1)$$

Table 2

Values of refractive index (n_D) and molar refraction (R_M) of the solutions of L-alanine, L-histidine and L-glutamic acid in aqueous NaCl at different temperatures

m	n_D					R_M ($cm^3 \cdot mol^{-1}$)				
	T (K)									
(mol·kg^{-1})	293.15	298.15	303.13	313.15	323.15	293.15	298.15	303.13	313.15	323.15
L-Alanine in water										
0.0000	1.3330	1.3326	1.3320	1.3310	1.3300	3.709	3.709	3.709	3.711	3.717
0.1028	1.3353	1.3346	1.3338	1.3326	1.3310	3.748	3.746	3.743	3.744	3.743
0.2100	1.3374	1.3367	1.3354	1.3341	1.3320	3.787	3.784	3.776	3.777	3.771
0.2917	1.3384	1.3377	1.3365	1.3351	1.3332	3.810	3.808	3.801	3.800	3.797
0.4350	1.3400	1.3389	1.3378	1.3367	1.3350	3.850	3.843	3.837	3.840	3.838
0.6907	1.3442	1.3429	1.3419	1.3405	1.3386	3.933	3.925	3.920	3.920	3.917
0.9967	1.3482	1.3472	1.3462	1.3445	1.3424	4.023	4.018	4.014	4.011	4.007
L-Alanine in 0.5027 mol·kg^{-1} aqueous NaCl										
0.0000	1.3374	1.3369	1.3363	1.3352	1.3343	3.753	3.753	3.753	3.755	3.762
0.0514	1.3386	1.3381	1.3375	1.3362	1.3350	3.773	3.774	3.773	3.774	3.778
0.1992	1.3403	1.3400	1.3398	1.3381	1.3368	3.816	3.819	3.823	3.820	3.823
0.4036	1.3439	1.3435	1.3426	1.3411	1.3400	3.888	3.889	3.886	3.885	3.891
0.7050	1.3474	1.3473	1.3466	1.3450	1.3433	3.975	3.980	3.979	3.977	3.977
1.3353	1.3558	1.3552	1.3549	1.3535	1.3520	4.172	4.172	4.176	4.177	4.179
1.5302	1.3582	1.3579	1.3571	1.3559	1.3541	4.230	4.234	4.232	4.236	4.235
L-Alanine in 1.0000 mol·kg^{-1} aqueous NaCl										
0.0000	1.3428	1.3423	1.3411	1.3400	1.3386	3.808	3.809	3.803	3.807	3.810
0.1072	1.3445	1.3439	1.3431	1.3419	1.3402	3.845	3.845	3.843	3.846	3.846
0.4262	1.3489	1.3485	1.3473	1.3460	1.3452	3.948	3.950	3.944	3.946	3.956
0.7371	1.3512	1.3520	1.3511	1.3500	1.3485	4.028	4.043	4.041	4.045	4.048
1.3676	1.3609	1.3600	1.3591	1.3576	1.3561	4.244	4.242	4.240	4.241	4.243
1.5648	1.3628	1.3621	1.3612	1.3599	1.3581	4.299	4.299	4.297	4.301	4.300
L-Alanine in 1.4970 mol·kg^{-1} aqueous NaCl										
0.0000	1.3478	1.3468	1.3460	1.3444	1.3433	3.861	3.857	3.856	3.856	3.862
0.1003	1.3490	1.3481	1.3473	1.3460	1.3448	3.892	3.889	3.888	3.891	3.896
0.3020	1.3520	1.3514	1.3505	1.3488	1.3469	3.961	3.962	3.960	3.958	3.957
0.6770	1.3570	1.3555	1.3548	1.3530	1.3511	4.085	4.076	4.076	4.074	4.073
0.7889	1.3584	1.3569	1.3560	1.3545	1.3529	4.120	4.112	4.110	4.111	4.113
0.9521	1.3601	1.3589	1.3580	1.3563	1.3549	4.171	4.165	4.164	4.163	4.167
L-Histidine in water										
0.0000	1.3330	1.3325	1.3320	1.3310	1.3300	3.709	3.708	3.709	3.711	3.717
0.0511	1.3356	1.3348	1.3341	1.3334	1.3320	3.751	3.747	3.745	3.751	3.753
0.1003	1.3368	1.3361	1.3356	1.3347	1.3329	3.778	3.775	3.776	3.780	3.778
0.1985	1.3397	1.3389	1.3381	1.3368	1.3351	3.837	3.834	3.831	3.832	3.831

0.3021	1.3432	1.3420	1.3416	1.3404	1.3388	3.904	3.898	3.899	3.901	3.901
L-Histidine in 0.4998 mol·kg^{-1} aqueous NaCl										
0.0000	1.3374	1.3369	1.3363	1.3352	1.3343	3.755	3.753	3.752	3.758	3.764
0.0504	1.3395	1.3390	1.3385	1.3371	1.3358	3.792	3.790	3.790	3.794	3.796
0.0969	1.3412	1.3406	1.3401	1.3389	1.3375	3.824	3.821	3.822	3.827	3.829
0.1962	1.3441	1.3438	1.3433	1.3418	1.3399	3.885	3.886	3.886	3.889	3.884
0.2869	1.3467	1.3462	1.3458	1.3445	1.3430	3.940	3.939	3.941	3.946	3.946
L-Histidine in 0.9993 mol·kg^{-1} aqueous NaCl										
0.0000	1.3428	1.3423	1.3411	1.3400	1.3386	3.811	3.810	3.805	3.809	3.813
0.0519	1.3447	1.3439	1.3432	1.3418	1.3401	3.847	3.843	3.843	3.844	3.845
0.0935	1.3464	1.3457	1.3445	1.3430	1.3417	3.878	3.875	3.870	3.870	3.876
0.1869	1.3488	1.3480	1.3472	1.3459	1.3441	3.933	3.929	3.929	3.931	3.932
0.2803	1.3516	1.3509	1.3498	1.3484	1.3469	3.993	3.990	3.987	3.988	3.992
L-Histidine in 1.4972 mol·kg^{-1} aqueous NaCl										
0.0000	1.3478	1.3468	1.3460	1.3444	1.3433	3.861	3.857	3.858	3.857	3.864
0.0509	1.3490	1.3482	1.3479	1.3464	1.3445	3.891	3.889	3.893	3.895	3.894
0.0881	1.3502	1.3495	1.3488	1.3473	1.3460	3.916	3.915	3.916	3.917	3.923
0.1793	1.3531	1.3525	1.3518	1.3500	1.3480	3.977	3.977	3.978	3.976	3.975
0.2723	1.3566	1.3559	1.3550	1.3531	1.3515	4.045	4.045	4.043	4.041	4.045
L-Glutamic acid in water										
0.0000	1.3330	1.3325	1.3320	1.3310	1.3300	3.709	3.708	3.709	3.711	3.717
0.0115	1.3331	1.3327	1.3321	1.3311	1.3302	3.713	3.713	3.713	3.715	3.722
0.0240	1.3333	1.3328	1.3323	1.3313	1.3304	3.719	3.718	3.718	3.721	3.727
0.0301	1.3334	1.3330	1.3325	1.3315	1.3306	3.721	3.721	3.722	3.724	3.731
0.0445	1.3338	1.3333	1.3328	1.3318	1.3308	3.729	3.728	3.728	3.731	3.737
0.0669	1.3342	1.3336	1.3330	1.3320	1.3310	3.739	3.737	3.737	3.739	3.745
L-Glutamic acid in 0.5027 mol·kg^{-1} aqueous NaCl										
0.0000	1.3374	1.3369	1.3362	1.3352	1.3343	3.753	3.753	3.752	3.755	3.763
0.0105	1.3378	1.3372	1.3366	1.3355	1.3345	3.760	3.759	3.759	3.761	3.768
0.0201	1.3379	1.3373	1.3366	1.3356	1.3346	3.764	3.763	3.761	3.765	3.772
0.0301	1.3380	1.3376	1.3369	1.3359	1.3348	3.768	3.769	3.767	3.771	3.776
0.0470	1.3384	1.3380	1.3373	1.3362	1.3352	3.776	3.778	3.776	3.779	3.785
0.0572	1.3387	1.3381	1.3375	1.3365	1.3355	3.782	3.781	3.781	3.785	3.791
L-Glutamic acid in 1.0000 mol·kg^{-1} aqueous NaCl										
0.0000	1.3428	1.3422	1.3412	1.3400	1.3386	3.808	3.808	3.804	3.807	3.810
0.0097	1.3429	1.3424	1.3413	1.3400	1.3387	3.812	3.813	3.808	3.810	3.814
0.0199	1.3431	1.3425	1.3417	1.3402	1.3390	3.817	3.817	3.815	3.815	3.820
0.0294	1.3430	1.3427	1.3418	1.3403	1.3391	3.819	3.822	3.819	3.819	3.824
0.0404	1.3432	1.3427	1.3419	1.3405	1.3392	3.824	3.825	3.823	3.824	3.828
0.0461	1.3433	1.3427	1.3420	1.3408	1.3393	3.827	3.827	3.826	3.829	3.831
0.0606	1.3436	1.3430	1.3422	1.3410	1.3396	3.834	3.834	3.833	3.835	3.839

The slope of the plot, S_n, is a constant which depends on physico-chemical properties of amino acid (molecular weight) and temperature, and n_0 is the refractive index at infinite dilution. Even if the data for Glu are limited to the amino acid concentration range of 0.0-0.067 mol·kg^{-1}, it seems that S_n values for ternary solutions follows the order: His > Glu ≈Ala. As other authors mentioned in literature [21] in aqueous solutions the higher value of slope of the plot

refractive index *vs.* amino acid concentration the stronger interaction between solute and solvent molecules. Having in mind the structural properties of the investigated amino acids: imidazole aromatic ring in L-histidine, negative charge in L-glutamic acid, and non-polar side chain in L-alanine, we can explain the above-mentioned order of variation. The biggest refractive index values for His solutions are due to both its higher molar mass and π-electron delocalization across the imidazole ring.

From Table 3 it can be observed that linear fit (R^2 values) is better for Ala solutions (R^2>0.993) followed by His (R^2>0.986) and Glu (R^2>0.91). Refractive index at infinite dilution increases with the amount of salt in solutions and decreases with temperature. These data can be used both in industry and research. Similar linear dependencies are found in literature for drug + aqueous-salt solutions [22,23].

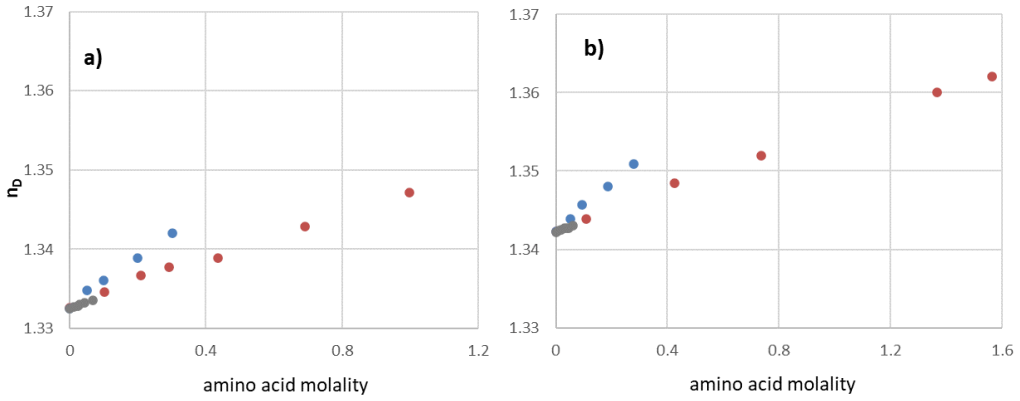


Fig. 1. Variation in refractive index at 298.15 K with concentration of amino acids: L-alanine (●), L-glutamic acid (●), and L-histidine (●) in: a) aqueous and b) aqueous 1.0 mol·kg^{-1} NaCl solutions

From the values of experimental refractive index, molar refraction of the investigated solution, R_M, was calculated using the well-known relation proposed by Lorentz–Lorenz [24,25]:

$$R_M = \frac{n_D^2 - 1}{n_D^2 + 2} \times \sum \frac{x_i M_i}{\rho} \qquad (2)$$

where x_i is the mole fraction of i^{th} component in the ternary (water+NaCl+amino acid) solutions, M_i is the molar mass of i^{th} component in such mixtures, ρ is the density of the solution. We used our previously reported density data for the same amino acid and NaCl concentrations [14-17].

The calculated values of molar refraction of the solutions are also given in Table 2. It is known that the molar refraction is primarily dependent on composition and is less affected by temperature. From Table 2 it can be observed that there is not much variation of R_M with temperature.

Table 3

Fitting parameters of the plot representing refractive index as a function of amino acid molality (Eq.1) along with correlation coefficient, R^2, and standard error, SD, at different temperatures

Parameter	T (K)				
	293.15	298.15	303.13	313.15	323.15
L-Alanine in water					
n_0 ($m^3 \cdot mol^{-1}$)	1.3337	1.3331	1.3322	1.3311	1.3296
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0148	0.0142	0.0140	0.0134	0.0127
R^2	0.9930	0.9934	0.9971	0.9990	0.9973
SD	0.00048	0.00044	0.00029	0.00016	0.00025
L-Alanine in 0.5027 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3378	1.3373	1.3369	1.3354	1.3343
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0134	0.0135	0.0134	0.0135	0.0130
R^2	0.9983	0.9978	0.9984	0.9996	0.9991
SD	0.00037	0.00042	0.00037	0.00017	0.00026
L-Alanine in 1.000 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3429	1.3426	1.3416	1.3404	1.3391
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0128	0.0126	0.0127	0.0126	0.0124
R^2	0.9945	0.9987	0.9987	0.9989	0.9963
SD	0.00069	0.00034	0.00034	0.00031	0.00055
L-Alanine in 1.4970 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3478	1.3470	1.3462	1.3447	1.3434
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0132	0.0126	0.0125	0.0123	0.0119
R^2	0.9983	0.9964	0.9969	0.9977	0.9979
SD	0.00024	0.00033	0.00030	0.00026	0.00024
L-Histidine in water					
n_0 ($m^3 \cdot mol^{-1}$)	1.3334	1.3329	1.3323	1.3314	1.3302
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0324	0.0305	0.0307	0.0299	0.0276
R^2	0.9920	0.9943	0.9950	0.9856	0.9869
SD	0.00040	0.00032	0.00030	0.00049	0.00044
L-Histidine in 0.4998 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3378	1.3372	1.3367	1.3355	1.3343
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0318	0.0322	0.0327	0.0320	0.0298
R^2	0.9941	0.9941	0.9939	0.9962	0.9964
SD	0.00033	0.00033	0.00034	0.00026	0.00024
L-Histidine in 0.9993 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3431	1.3424	1.3414	1.3402	1.3387
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0308	0.0303	0.0304	0.0299	0.0294
R^2	0.9940	0.9951	0.9951	0.9982	0.9978
SD	0.00031	0.00027	0.00028	0.00016	0.00018
L-Histidine in 1.4972 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3475	1.3466	1.3460	1.3446	1.3432
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0326	0.0336	0.0326	0.0312	0.0296
R^2	0.9949	0.9982	0.9986	0.9975	0.9904
SD	0.00029	0.00018	0.00015	0.00020	0.00036
L-Glutamic acid in water					
n_0 ($m^3 \cdot mol^{-1}$)	1.3329	1.3325	1.3320	1.3310	1.3300

S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0188	0.0169	0.0162	0.0162	0.0154
R^2	0.9786	0.9842	0.9689	0.9689	0.9691
SD	0.00007	0.00006	0.00008	0.00008	0.00007
L-Glutamic acid in 0.5027 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3375	1.3369	1.3362	1.3352	1.3342
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0208	0.0215	0.0220	0.0219	0.0206
R^2	0.9713	0.9871	0.9783	0.9913	0.9824
SD	0.00009	0.00006	0.00008	0.00005	0.00007
L-Glutamic acid in 1.000 $mol \cdot kg^{-1}$ aqueous NaCl					
n_0 ($m^3 \cdot mol^{-1}$)	1.3428	1.3423	1.3412	1.3399	1.3386
S_n ($m^3 \cdot mol^{-2} \cdot kg$)	0.0121	0.0117	0.0167	0.0177	0.0160
R^2	0.9116	0.9367	0.9547	0.9441	0.9789
SD	0.00009	0.00007	0.00008	0.00010	0.00005

The variation with amino acid molality is presented in Fig. 2, where it can be observed that the order of variation of R_M with amino acid molality is: His > Glu >≈ Ala; also, higher R_M values of amino acids in the presence of NaCl.

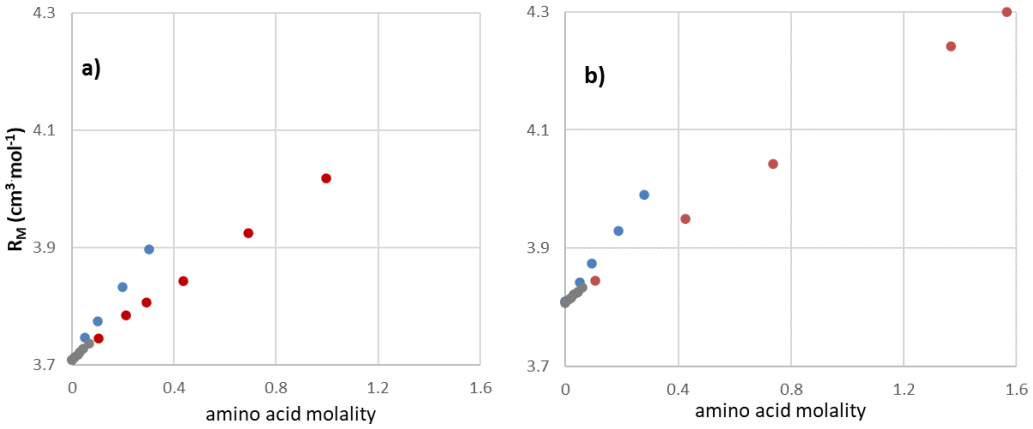


Fig. 2. Plots of molar refraction (R_M) at 298.15 K for L-alanine (●), L-glutamic acid (●), and L-histidine (●) for a) aqueous and b) aqueous 1.0 $mol \cdot kg^{-1}$ NaCl solutions

It is known that when the structure of a molecule becomes more complex, its electron cloud becomes more decentralized, and the polarizability of the molecule increases [9]. It is evident that the mixtures of L-histidine (imidazole ring) with water or aqueous NaCl are denser or closely packed than those of L-glutamic acid (ionic side chain) and L-alanine (hydrophobic side chain). These findings are in good agreement with the results found from density and viscosity measurements. Thus, the order of variation of transfer volumes in NaCl solutions: Glu > Ala > His, which is opposite to the one above, can be interpreted by predominance of hydrophobic interactions in His and Ala solutions and of

ionic/hydrophilic ones in Glu solutions. The obtained R_M values are similar with those found in literature for aqueous L-alanine [10] and L-histidine [13].

The experimental values of refractive index and density of the solutions [14-16] were correlated thru Lorentz–Lorenz equation with an empirical equation [26]:

$$f(n) = (n_D^2 - 1)/(n_D^2 + 2) = k\rho \tag{3}$$

where the *k* values were obtained by fitting the data with a least-squares algorithm by minimizing the standard deviations (*SD*) given below.

$$SD = \left(\frac{\sum|f(n)^{exp} - f(n)^{calc}|^2}{n}\right)^{0.5} \tag{4}$$

The obtained parameters are given in Table 4 and indicate that *k* decreases with increasing NaCl concentration for all three systems and is no variation with temperature, as expected. The very small standard deviations (*SD*) values indicate a very good correlation of $f(n)$ with density. Therefore, by using *k* values and n_D for mixtures, we can estimate the density of the mixture and *vice versa*.

Table 4

Correlation parameter *k* (Eq. 3) for L-alanine, L-histidine and L-glutamic acid in aqueous NaCl solutions along with standard deviations *SD* at different temperatures

Parameter	*T* (K)				
	293.15	298.15	303.13	313.15	323.15
L-Alanine in water					
k	0.208	0.207	0.207	0.207	0.207
SD	0.00091	0.00080	0.00077	0.00069	0.00060
L-Alanine in 0.5027 mol·kg^{-1} aqueous NaCl					
k	0.206	0.206	0.206	0.206	0.206
SD	0.00149	0.00153	0.00153	0.00156	0.00144
L-Alanine in 1.0000 mol·kg^{-1} aqueous NaCl					
k	0.205	0.205	0.205	0.205	0.206
SD	0.00158	0.00151	0.00156	0.00153	0.00149
L-Alanine in 1.4970 mol·kg^{-1} aqueous NaCl					
k	0.204	0.204	0.204	0.204	0.204
SD	0.00109	0.00099	0.00099	0.00096	0.00089
L-Histidine in water					
k	0.207	0.207	0.207	0.207	0.207
SD	0.00075	0.00065	0.00067	0.00062	0.00054
L-Histidine in 0.4998 mol·kg^{-1} aqueous NaCl					
k	0.206	0.206	0.206	0.206	0.206
SD	0.00072	0.00075	0.00079	0.00076	0.00064
L-Histidine in 0.9993 mol·kg^{-1} aqueous NaCl					
k	0.205	0.204	0.204	0.204	0.205
SD	0.00068	0.00066	0.00067	0.00065	0.00063
L-Histidine in 1.4972 mol·kg^{-1} aqueous NaCl					
k	0.203	0.203	0.203	0.203	0.203

SD	0.00079	0.00086	0.00079	0.00074	0.00070
L-Glutamic acid in water					
k	0.206	0.206	0.206	0.206	0.206
SD	0.00004	0.00006	0.00007	0.00006	0.00006
L-Glutamic acid in 0.5027 mol·kg^{-1} aqueous NaCl					
k	0.204	0.204	0.204	0.205	0.205
SD	0.00004	0.00003	0.00004	0.00003	0.00003
L-Glutamic acid in 1.0000 mol·kg^{-1} aqueous NaCl					
k	0.203	0.203	0.203	0.203	0.204
SD	0.00009	0.00009	0.00004	0.00005	0.00004

The overall polarizability of the studied solutions, α, was calculated from the molar refraction of the solutions, R_M, with the following equation:

$$\alpha = 3R_M/4\pi N_A \quad (5)$$

where N_A =Avogadro's constant ($6.023 \cdot 10^{23}$ mol^{-1}).

The obtained α values are shown in Fig. 3, where it can be observed the order of variation of overall polarizability with amino acid molality is: His > Glu >≈ Ala. As is mentioned in literature, in systems with delocalized π-electrons, such as His, polarizability significantly increases when subjected to an electric field because the electronic cloud becomes more decentralized [9,27]. The overall polarizability of the systems also increases with increasing amount of amino acids and NaCl in the mixture (Fig. 3). Similarly, Sawale *et al.* [22] observed that the addition of drug in binary aqueous-salt solution introduced stronger polarizability to solution due to interactions between polar parts of the drug and water dipoles. Also, the variation trend is slightly influenced by temperature. The obtained α values are very similar with those found in literature for aqueous His [13,28].

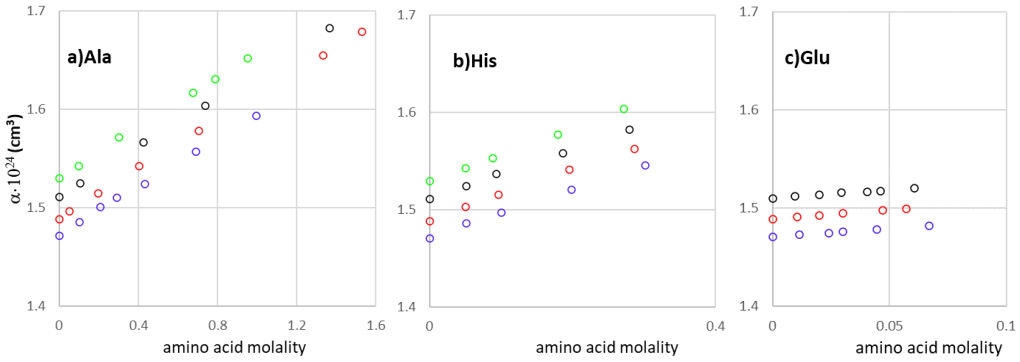


Fig. 3. Plots of overall polarizability ($\alpha \cdot 10^{24}$) at 298.15 K for the aqueous solutions of a) L-alanine, b) L-histidine and c) L-glutamic acid: (o) 0.0 mol·kg^{-1}, (o) 0.5 mol·kg^{-1}, (o) 1.0 mol·kg^{-1}, (o) 1.5 mol·kg^{-1} NaCl

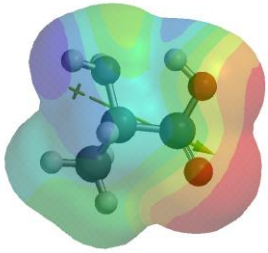
Computational results

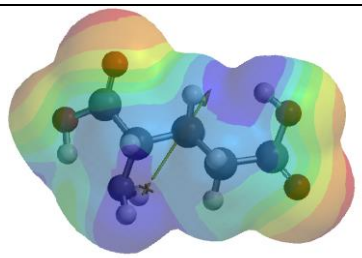
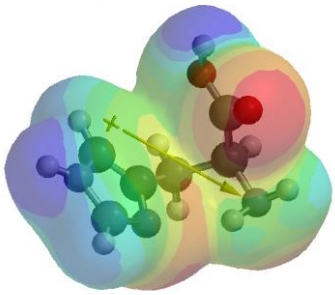
The geometry of amino acids was optimized by energy minimization and the stable configurations emerged. The obtained optimized structures are illustrated in Table 5, together with their 3D representations and directions of dipole vector in molecules, superposed with the electrostatic potential map. Also, the calculated values for molecular polarizability (by wB97X level of theory) of amino acids both in vacuum and water are shown in Table 5. Molecular polarizability of a molecule characterizes the capability of its electronic system to be distorted by the external field and it plays an important role in modeling many molecular properties and biological activities [29]. In literature it is known the performance of DFT methods to accurately predict the polarizability of amino acids and some of their aggregates when using appropriate exchange-correlation potentials and (large) basis sets [30,31].

From Table 5 it can be seen that the molecular polarizability of amino acids varies slightly in vacuum *vs.* water, while the dipole moment values vary significantly, which is also indicated in literature [32]. This is to be expected, because in the gas phase the most stable configuration of amino acids is the neutral one, while in solutions, the zwitterionic form has the most probable occurrence, influencing the optical properties. The order of variation of calculated molecular polarizability of amino acids both in vacuum and water is: His > Glu >≈ Ala, which matches the order resulting from the experiment, that is the overall polarizability of their aqueous NaCl solutions (Fig. 3/Table 2).

Table 5

Computed dipole moment and molecular polarizability for amino acids at equilibrium geometry in ground state in vacuum and in water using DFT/wB97X-D level of theory with /6-31 +(d, p) basis set

Compound	3D structure and dipole vector (optimized geometry)	Dipole moment (D) vacuum/water	Molecular polarizability (10^{-24} cm^3) vacuum/water
L-alanine		5.46 / 7.36	45.92 / 45.92

L-glutamic acid		3.92 / 6.24	49.74 / 49.79
L-histidine		5.29 / 6.58	50.86 / 50.95

6. Conclusions

The refractive indices for L-alanine, L-histidine and L-glutamic acid in aqueous NaCl solutions at different temperatures (293.15, 298.15, 303.15, 313.15 and 323.15 K) were reported. The refractive indices of all mixtures decrease with increasing temperature and increase with increasing concentrations of both amino acid and salt. The highest n_D values for His solutions are due to both its higher molar mass and π-electron delocalization across the imidazole ring. The correlation of refractive index with density previous data by Lorentz-Lorenz empirical equation indicate a very good correlation. The variation of both molar refraction and overall polarizability for the studied solutions is: His > Glu >≈ Ala. This indicate that the systems with delocalized π-electrons, such as His (imidazole ring), are denser or closely packed than those of Glu (ionic side chain) and Ala (hydrophobic side chain). The order of variation of calculated molecular polarizability (by DFT) of amino acids both in vacuum and water matches the order resulting from the experiment. The calculated dipole moments show that these values are enhanced in water than in vacuum.

R E F E R E N C E S

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