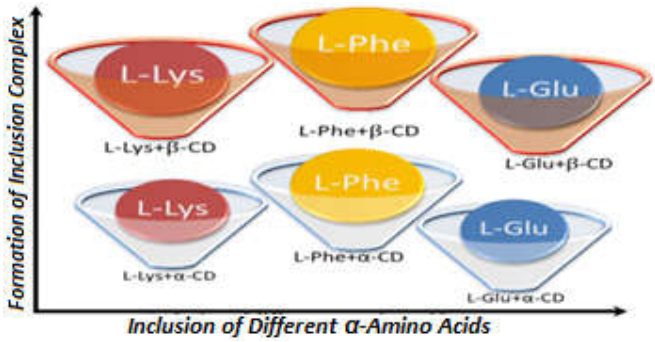
CHAPTER VIII

Host–guest inclusion complexes of α and β-cyclodextrins with α-amino acids

Studies of molecular inclusions of a congener series of guest amino acid molecules into the host cavity of α and β-cyclodextrins in aqueous solution have been focused on modern research gaining far reaching effect. With both the α and β-cyclodextrins, it is found that 1 : 1 host–guest inclusion complexes are formed with all the guest molecules at both low and high pH. The variation of the thermodynamic parameters with guest size and state are used to draw inferences about contributions to the overall binding from the driving forces, namely, hydrophobic effect, van der Waals forces, H-bonds, electrostatic forces, structural effect and configurational theory. The formation and comparative study of inclusion complexes have been analyzed by available data supplemented with surface tension, pH, density, viscosity and refractive index.



VIII.1. Introduction

There has been an increasing interest in the use of cyclodextrins as a tool for controlled release of active compounds due to their outstanding ability to form molecular inclusion complexes with hydrophobic guest molecules. Cyclodextrins are formed from the enzymatic degradation of starch by bacteria. They are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units, which are bound together by α-(1–4) linkages forming a torus-shaped ring structure (scheme 1). Due to their unique property, i.e., polar hydrophilic outer shell and relatively hydrophobic inner cavity, they can build host–guest complexes by inclusion of the suitable hydrophobic moiety of guest molecules (e.g., α-amino acid).[1] Formation of these complexes have significant applications

in stabilization, carry and controlled delivery and are directed by packing effect, solubility and the interactions of the guest with host molecules without any chemical modification.[2]

To the best of our knowledge, there has been no previous study on these ternary solution systems. In the present study, we attempt to ascertain the nature of the formation of inclusion complexes inside into α and β-cyclodextrins with three α-amino acids, namely, L-lysine, L-phenylalanine and L-glutamic acid in 0.001, 0.003, 0.005 mass fractions of α and β-cyclodextrins in aqueous media (scheme 1).

VIII.2. Experimental section

VIII.2.1. Source and purity of samples

The studied compounds, namely, amino acids and cyclodextrins of puriss grade, were purchased from Sigma-Aldrich, Germany and used as purchased. The mass fraction purity of L-Lys, L-Phe, L-Glu, α-cyclodextrin and β-cyclodextrin were ≥ 0.98, 0.98, 0.99, 0.98, and 0.98, respectively.

VIII.2.2. Apparatus and procedure

Solubility of the chosen cyclodextrins in water (deionized, triply distilled, degassed water with a specific conductance of 1×10^{-6} S cm^{-1}) and titled compounds namely, amino acids in aqueous cyclodextrin, were precisely checked prior to the start of the experimental work, and the selected amino acids were freely soluble in all proportions of aqueous cyclodextrin. All the stock solutions of the amino acids were prepared 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. The conversions of molality into molarity were performed using density values. Adequate precautions were made to reduce evaporation losses during mixing.

The surface tension experiments were performed by platinum ring detachment method using a Tensiometer (K9, KRUSS, Germany) at the experimental temperature. The accuracy of the measurement was within ±0.1 mN m^{-1}. Temperature of the system was maintained by circulating auto-thermostat water through a double-wall glass vessel containing the solution.

pH values of the experimental solutions were measured by Mettler Toledo Seven Multi pH meter with uncertainty 0.009. The measurements were made in a thermostated water bath maintaining the temperature at 298.15 K. The uncertainty in temperature was 0.01 K.

The densities (ρ) of the solvents were measured by means of vibrating a U-tube Anton Paar digital density meter (DMA 4500M) with a precision of 0.00005 g cm^{-3} maintained at 0.01 K of the desired temperature. It was calibrated by passing de-ionized, doubly distilled, degassed water and dry air.

The viscosities (η) were measured using a Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42.

Refractive index was measured with the help of a Digital Refractometer Mettler Toledo. The light source was LED, λ=589.3 nm. The refractometer was calibrated twice using distilled water, and calibration was checked after every few measurements. The uncertainty of refractive index measurement was 0.0002 units.

VIII.3. Result and discussion

VIII.3.1. Surface tension study

Surface tension (γ) measurement can be used to obtain valuable clues about the formation of the inclusion complex in cyclodextrins. It is found that γ for aqueous solutions of pure α and β-CD don't show any remarkable change with increasing concentration (table 1), whereas the aqueous solutions of L-Lys, L-Phe and L-Glu show considerable variations (figure 1).[3] The pH data of aq. solution of L-Lys (8.95–9.00) indicates the existence of an $-NH_3^+$ group in both the zwitterion and at the end of the butylamine side chain. Due to this charged structure, the ionic interaction might occur by NH_3^+, COO^- and the end $-NH_3^+$ group of L-Lys, which reflects an increase in γ value with increasing concentration. Similar variation has been observed in the surface tension of the aq. solution of L-Glu, i.e., γ values gradually increase with increasing concentration due to the presence of a negatively charged deprotonated carboxylate ($-COO^-$) group at the side chain, as evident from pH = 4.10–4.25, which is responsible for interaction in solution. However, the situation is different in case of L-Phe, as the variation of γ in aqueous solution shows a considerable decrease with increasing concentration (figure 1). This is because of the appreciable hydrophobic nature of

the benzyl (–CH_2Ph) group at the side chain, as well as hydrophilic zwitterionic group present, which can behave like a surfactant, resulting in a decrease in the surface tension.

The surface tensions (γ) with corresponding concentration of amino acid in different mass fractions of aq. α and β-CD have been observed for three α-amino acids (figure 1). In each case, the trends of the curves in surface tensions (γ) against concentration (molality) are similar to that of aq. amino acids, but each curve clearly shows a break point in surface tension at a certain concentration, i.e., the γ values increase (L-Lys and LGlu) or decrease (L-Phe) with corresponding concentration, reaching a certain point (break point), and then become approximately steady, which obviously indicates the formation of the inclusion complex. The formation of inclusion complexes is responsible for the insertion of the hydrophobic (aliphatic or aromatic) group of the chosen amino acid inside into the cavity of α and β-CD. There is a possibility that the inclusion complex may have different stoichiometries, like 1 : 1, 1 : 2, 2 : 1, 2 : 2 (scheme 2) ratios of CD and amino acid respectively. Single break, double break and so on in the curve of surface tension are indications of 1 : 1, 1 : 2, and so on inclusion complexes by cyclodextrin.[4] In figure 1, each curve shows a single break point, which further suggests that 1 : 1 inclusion complexes are formed. Two intersecting straight lines have been drawn for determination of the value of γ and the corresponding concentration at the break point of the respective amino acid (table 2). For each amino acid, the change in γ is suppressed with the increasing mass fraction of aq. α and β-CD compared to aq. amino acid, i.e., the break point comes at the lower concentration of the respective amino acids, and the γ value comes closer to that of aq. CDs, which suggests that inclusion becomes more feasible when increasing the amount of CD is present in solution. If we compare aq. α-CD and β-CD, both the values of γ and concentration at the break point are lower in the case of aq. β-CD than that of aq. α-CD for L-Lys and L-Glu; however, for L-Phe, γ is higher at the lower concentration in aq. β-CD than α-CD. This is obviously due to the fact that β-CD provides a more viable feature (cavity diameter and volume) for the formation of feasible inclusion complex than α-CD. The studied amino acids, thus, form soluble 1 : 1 complexes with both the cyclodextrins, in which we visualize the nonpolar tail group of the amino acid to be inserted via the wider rim, so as to make maximum contact with the cyclodextrin cavity (scheme 3), while the charged polar head residue remains in the wider rim of cyclodextrin or in bulk solution. This is also in correlation with the data from density and viscosity measurements, which undoubtedly

establishes that β-CD is more efficient than α-CD in the formation of the inclusion complexes with the above three selected α-amino acids.

VIII.3.2. pH study

The pH values of the three amino acids, e.g., L-Lys, L-Phe and LGlu, clearly show the existence and variation in their zwitterionic forms (scheme 4). The pH of L-Lys in both aq. α and β-CD is 9.76–10.12, suggesting the existence of an $-NH_3^+$ group in the butylamine side chain. The value of pH increases with the increasing concentration of L-Lys as well as with the increase in concentration of α and β-CD (table 1, 3), indicating that after inclusion, the end $-NH_3^+$ group interacts with the –OH group of cyclodextrin by making an H-bond (scheme 5). The existence of a side chain carboxylate $-COO^-$ and zwitterionic group of L-Glu is confirmed by shifts from pH = 4.10–4.25 in aq. solution to 3.16–3.40 in α and β-CD. Note that the lower pH is due to release of an H^+ ion from carboxylic acid (–COOH) group in the side chain. However, the case of L-Phe is different; the pH range lies within 5.10–6.36 in both aq. α and β-CD, which shows the simple zwitterionic structure and the rest of the part are hydrophobic groups. Thus, L-Phe acts as a surfactant and is very suitable for the formation of the inclusion complex with the apolar cavity of CDs.

VIII.3.3. Density study

Volumetric properties, such as apparent molar volume (ϕ_v) and limiting apparent molar volume (ϕ_v^o) are regarded as sensitive tools for understanding the interactions that take place in solutions. The apparent molar volume can be considered to be the sum of the geometric volume of the central solute molecule and changes in the solvent volume due to its interaction with the solute around the co-sphere. Thus, ϕ_v has been determined from the solution density using the suitable equation (table 1, 3).[5] The magnitude of ϕ_v (table 4) is found to be large and positive for all the studied systems, suggesting strong solute–solvent interactions.[6] The ϕ_v values decrease with increasing molarity (m) of amino acid in both the aq. α and β-CD, respectively, for all the amino acids under study. ϕ_v varies linearly with m and could be least-square fitted to the Masson equation from where limiting molar volume, ϕ_v^o (partial molar volume at infinite dilution), have been estimated.[5] If the variation of ϕ_v with m showed considerable scatter, ϕ_v^o can be determined either graphically or taken as the average of the ϕ_v values when the slope tends to zero, within the range of R^2 = 0.9989 to 0.9999 in linear regression coefficients. The values have been represented in

table 5. The trend of variation of ϕ_v^o of selected amino acids follows the order: Glu < Lys < Phe.

The increase of ϕ_v^o for amino acids with increasing mass fraction and the increasing positive transfer volumes suggests that the ion–ion and ion–hydrophilic group interactions are stronger than ion–hydrophobic group interactions. In the present ternary system (amino acid + aq. cyclodextrin), the interactions of head groups (COO^- and NH_3^+) of amino acids with the cyclodextrin is localized at the –OH groups. Due to these interactions, the electrostriction of water caused by the charged centers of the amino acid will be reduced, which results in an increase in volume.

It should be noted (figure 2) that ϕ_v^o of L-Glu is less than that of L-Lys owing to greater electrostriction. This is because the additional methylene groups (with increasing chain length) provide an increasing structure, enforcing tendency in L-Lys, and as a result, the water in the overlapping co-spheres is more structured than in the bulk. When this water relaxes to the bulk, there is a decrease in volume. However, in amino acids, the interactions increase with the addition of $–CH_2$ groups, and consequently there is a net increase in volume.

The results on the chosen amino acids can be rationalized on the basis that the partial molar volume is observed to increase with the increasing molar mass and size of the amino acid (table 5, 6). The ϕ_v^o for glycine, L-alanine, L-valine was studied earlier.[6] When one H of L-Ala is replaced by $–(CH_2)_4NH_2$ (L-Lys), $–CH_2C_6H_5$ (L-Phe), $–(CH_2)_2COOH$ (L-Glu) groups, there is a huge change in ϕ_v^o, this should increase by virtue of its increased chain length as well as size. L-Glu has a hydrophobic $–(CH_2)_2$ and hydrophilic acid (–COOH) group, because the ϕ_v^o of which is less compatible to L-Lys [containing a hydrophobic $–(CH_2)_4$ and hydrophilic ($–NH_2$) group], though they have almost same molar mass. When a $–CH_2$ group (L-Ala) is replaced by a hydrophobic $–(CH_2)_2$ group (L-Glu), ϕ_v^o increases because of the structure-enhancing behavior of the alkyl group. If $–(CH_2)_2$ group of (L-Glu) is replaced by another hydrophobic $–(CH_2)_2$ group (L-Lys), the increase in the partial molar volume should be greater, relative to L-Glu owing to greater hydrophobicity of the side chain, as it observed. On the other hand, L-Phe has the maximum value of ϕ_v^o in the series of studied amino acids, which can be attributed to its great effect of both the zwitterions and benzyl ($–CH_2C_6H_5$) group as well as its largest size and mass.

The entire groups present in the studied amino acids (L-Lys, L-Phe, and L-Glu) greatly affect the inclusion complexes occurring in the solution systems. Variations of ϕ_v^o and viscosity *B*-coefficient with the number of carbon atoms (n_c) in the alkyl chain for basic, neutral and acidic amino acids in presence of α and β-CD have been estimated (table 6). It is observed that for chosen amino acids, while the slopes are the volume contributions by the CH_2 group found within the range of 7.34-20.11 × 10^{-6} m^3 mol^{-1} for ϕ_v^o, which is of the order reported for amino acids in water (16 × 10^{-6} m^3 mol^{-1}) and the intercept, the volume contribution by polar head groups stay in the range of 23.11-34.94 × 10^{-6} m^3 mol^{-1}.[7] For L-Lys the contribution of (NH_3^+, COO^-) to ϕ_v^o is larger than that of the –CH_2– group and decreases with the increase in the mass fraction (w_n) for both the α and β-CD, which indicates that the interactions between the –OH group (primary or secondary) of CD and the polar head groups (NH_3^+, COO^-) of amino acids are strong, but they decrease with increasing mass fraction of α and β-CD. The contribution of –CH_2 group increases, which suggests that –CH_2 group exerts the +I effect. For a particular mass fraction (e.g., w_n = 0.001), contribution of (NH_3^+, COO^-) groups for L-Lys is less significant than L-Phe, which in turn become less than L-Glu, which indicates that the (NH_3^+, COO^-) group contribution is effective for L-Glu. However, the –CH_2 group contribution has been found to possess the opposite trend, i.e., contribution of the –CH_2 group is greater for L-Lys than L-Phe, which in turn is greater than L-Glu. This suggests that the –CH_2 group exerts the +I effect; as a result of increasing the number of –CH_2 groups interactions are more intense. If we consider the other group for L-Lys, the contribution of the –$(CH_2)_4NH_2$ group increases with increasing the mass fraction of both the α and β-CD, but, for the –NH_2 group, it decreases. This indicates that the effect of the hydrophilic end –NH_2 group is very poor, whereas –$(CH_2)_4NH_2$ is more effective due to the greater number of –CH_2 groups and also the greater +I effect. For L-Phe, contributions of both $PhCH_2$– and –Ph to ϕ_v^o are found to be greater and increase with mass fraction of CD, which indicates that both the groups strongly affect the hydrophobic solvation as well as side chain phenyl group effect. For L-Glu, both the –(CH_2)COOH and end –COOH group contribution increase with the rising amount of mass of CD in solution, which indicates that both groups are contributed in similar fashion. Between these two, the contribution of –COOH is higher than –CH_2, suggesting the hydrophilic end –COOH is stronger.

A decrease in the hydration number (n_H) and increase in solvation number (S_n) on addition of α and β-CD (table 7) is due to the decrease in the electrostriction of water. L-Lys with a larger charge separation, greater hydrophobicity and +I effect, than L-Glu has a larger

solvation number value, which is consistent with the results of Ogawa et al.[8] Due to the large electrostriction and greater effect of the $-CH_2C_6H_5$ group in the case of L-Phe, solvation numbers are found to be higher than in the other amino acids studied. Table 7 also shows that L-Lys is more solvated by CD than L-Glu. If we consider individual cyclodextrin, initially, in aqueous mixture, all or maximum of –OH (primary or secondary) groups interact with the water molecules present in the bulk solution. After addition of chosen amino acids (separately), they coordinate the –OH of cyclodextrins by replacing the water molecules, with the proper phase of interaction, such as zwitterionic groups, the end $-NH_3^+$ group of butylamine of L-Lys, the side chain $-COO^-$ group of L-Glu, and the benzyl group ($-CH_2C_6H_5$) of L-Phe, and therefore there is a net increase in solvation number. Lower hydration numbers as well as higher solvation numbers in β-CD than α-CD for studied amino acids further suggest that β-CD are more useful for solvation than α-CD.

VIII.3.4. Viscosity study

The viscosity of aq. CD increases with an increase in employed mass fraction w = 0.001, 0.003 and 0.005 (table 1), attributed to the structure-making influence between CD and water by breaking the H-bonded structure of water in its vicinity. For the ternary system (amino acid + aq. CD), at a given concentration of CD, the viscosity of the solution increases with the increasing molarity of amino acids (table 3). The viscosity *B*-coefficient is known to depend on the size and shape of the solute molecules, which indicate the solute–solvent interactions.[9] The *B*-coefficients of all the studied amino acids were positive (figure 3) and increase with the increasing concentration of CD, which may be considered to arise from the increasing amino acid–cyclodextrin interaction as well as increasing solvation.

The group contributions of the amino acids to viscosity *B*-coefficient have been derived and shown in table 6, which illustrate that B(NH_3^+, COO^-) for L-Lys and L-Glu decreases, and B($-CH_2$) values decrease with increasing mass fraction (w_n) of both CD, suggesting that the effect of (NH_3^+, COO^-) groups are diminished and that of $-CH_2$ groups are enhanced in the structure for solute–solvent interactions in solutions.[6] The side chain contribution shows positive and greater for L-Phe than L-Lys, which in turn is greater than L-Glu. This fact is due to the structure making propensity, and these findings are found to be the same as the trend discussed in group contribution to volume.

VIII.3.5. Refractive index study

The refractive index and molar refraction is also a valuable tool for investigating the molecular interaction in solution. More refractive index indicates that the velocity of light becomes lower through the medium; in other words, more molar refraction (R_M) as well as limiting molar refraction (R_M^0) (table 4, 5) indicate that the medium is denser or more compact.[10,11] Therefore, from figure 4 it is evident that the inclusion complex of L-Phe with both the α and β-CD is more closely packed than that of L-Lys and L-Glu. This may be explained because of the greater hydrophobic interaction between the $-CH_2Ph$ group of L-Phe and the hydrophobic cavity of α and β-CD than that of the $-(CH_2)_n-$ group present in L-Lys and L-Glu. This reflects that the inclusion complexes formed by L-Phe are denser and stronger, which is in good agreement with the data found from surface tension, density and viscosity.

VIII.3.6. Structural influence of cyclodextrins

Complex formation is a dimensional fit between the host cavity of CD and the amino acid molecule. The most notable feature of the cyclodextrin molecule (lipophilic cavity diameter of α and β-CD is 4.7–5.3 Å and 6.0–6.5 Å respectively) provides a micro environment into which appropriately sized non-polar moiety enters and forms strong inclusion complexes (scheme 5).[12] However, no covalent bonds are broken or formed during formation of the inclusion complex.[13] The main driving force in aqueous solution is that the slightly apolar cyclodextrin cavity is occupied by water molecules, which are energetically unfavoured (polar–apolar interaction) and therefore can be readily substituted by more hydrophobic side chain groups of α-amino acid molecules, which are less polar than water, to attain an apolar–apolar association and decrease cyclodextrin ring strain resulting in a more stable, lower energy state.[3,14] One or two cyclodextrin molecules can entrap one or more amino acid molecules; therefore, the plausible host : guest ratio of the inclusion is 1 : 1, 1 : 2, 2 : 1, and 2 : 2, or an even more complicated association complex and higher order of equilibrium can exist simultaneously (scheme 2). However, the simplest and most frequent case of host : guest ratio is 1 : 1 because of molecular encapsulation by α and β-CD. Moreover, it is difficult for a second amino acid molecule to be trapped by the cavity of the cyclodextrin after inclusion of a molecule. This is because, the cavity size (scheme 1) and volume allow a single molecule to accommodate through the wider or secondary rim, and both the narrow and wider rims are blocked (scheme 3, 5). The inclusion result states that the binding strength of

L-Phe–CD complex is well fit together on specific local interactions between surface atoms and forms stronger inclusion than L-Lys–CD and L-Glu–CD.

Based on these dimensions of α and β-cyclodextrins, the selected amino acids can typically complex with aliphatic, aromatics side chains. Hence, the positive interactions occurred to form the inclusion complex by:

- displacement of polar water molecules from the apolar cavity of cyclodextrin,
- increased number of H-bonds formed as the substituted water, which returns to the larger pool,
- reduction of the repulsive interactions between the hydrophobic group of amino acid and the aqueous environment,
- an increase in the hydrophobic–hydrophobic interactions as the inclusion of amino acid takes place in the apolar cavity of cyclodextrin.

VIII.4. Conclusion

The extensive study concludes the formation of inclusion complexes for all the titled α-amino acids in the apolar cavity of both α and β-cyclodextrins. Surface tension study confirms that 1 : 1 inclusion complex was formed. All the derived parameters obtained from the supplementary data of density, viscosity and refractive index strongly support the formation of the inclusion complex as well as solute–solvent interaction taking place in the studied solution systems. The order of interaction for selected α-amino acid inside into α and β-CD is as follows: L-Glu < L-Lys < L-Phe. Hence, the findings discussed and explained in this paper illustrate the advancement of the work and demonstrate the suitability for diverse applications.

Tables

Table 1. Experimental values of density (ρ), viscosity (η), refractive index (n_D), surface tension (γ) and pH in deferent mass fraction of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

Aq. solvent mixture	ρ×10^{-3} /kg·m^{-3}	η /mP·s	n_D	γ /mN·m^{-1}	pH
		aq. α-CD			
w_1 = 0.001	0.99732	1.300	1.3328	71.62	6.44
w_1 = 0.003	0.99792	1.310	1.3331	71.49	6.39
w_1 = 0.005	0.99859	1.321	1.3334	71.45	6.32
		aq. β-CD			
w_2 = 0.001	0.99747	1.304	1.3329	71.71	6.65
w_2 = 0.003	0.99815	1.313	1.3332	71.61	6.10
w_2 = 0.005	0.99890	1.323	1.3336	71.57	5.57

[a] Standard uncertainties u are: $u(\rho)$ =2×10^{-6} kg·m^{-3}, $u(\eta)$ =0.003 mP·s, $u(n_D)$ =0.0002, $u(\gamma)$ =0.03 mN·m^{-1}, u(pH) =0.01, and $u(T)$ =0.01K

Table 2. Values of surface tension at the break point (γ) with corresponding concentration of amino acids in different mass fraction of aqueous α and β-cyclodextrin respectively at 298.15K[a]

mass fraction (w)	conc (m)	γ /mNm^{-1}	conc (m)	γ/mNm^{-1}	conc (m)	γ/mNm^{-1}
	L-Lys		*L*-Phe		*L*-Glu	
w_1=0.001[b]	0.0441	77.81	0.0389	62.37	0.0403	77.67
w_1=0.003[b]	0.0427	77.11	0.0385	65.06	0.0396	76.62
w_1=0.005[b]	0.0421	76.26	0.0378	65.66	0.0389	75.56
w_2=0.001[b]	0.0413	77.67	0.0364	62.83	0.0387	77.61
w_2=0.003[b]	0.0405	77.06	0.0356	65.24	0.0361	76.29
w_2=0.005[b]	0.0381	76.15	0.0341	66.22	0.0337	75.49

[a] Standard uncertainties u are: $u(T)$ =0.01K.

[b] w_1 and w_2 are mass fractions of α and β-cyclodextrin in aqueous mixture respectively.

Table 3. Experimental values of density (ρ), viscosity (η), refractive index (n_D) and pH of selected amino acids in deferent mass fraction of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

molality /mol·kg^{-1}	ρ×10^{-3} /kg·m^{-3}	η /mP·s	n_D	pH	molality /mol·kg^{-1}	ρ×10^{-3} /kg·m^{-3}	η /mP·s	n_D	pH
Glysine					L-Lysine				
w_1 = 0.001^b					w_1 = 0.001^b				
0.0100	0.99766	1.309	-	-	0.0100	0.99773	1.338	1.3330	9.76
0.0251	0.99819	1.316	-	-	0.0251	0.99841	1.363	1.3334	9.81
0.0402	0.99873	1.322	-	-	0.0403	0.99913	1.384	1.3337	9.89
0.0553	0.99928	1.327	-	-	0.0555	0.99989	1.403	1.3341	9.94
0.0704	0.99984	1.332	-	-	0.0707	1.00068	1.421	1.3345	9.97
0.0855	1.00040	1.336	-	-	0.0859	1.00148	1.437	1.3350	9.99
w_1 = 0.003^b					w_1 = 0.003^b				
0.0100	0.99826	1.322	-	-	0.0100	0.99828	1.349	1.3333	9.79
0.0251	0.99879	1.331	-	-	0.0251	0.99889	1.377	1.3337	9.84
0.0401	0.99934	1.338	-	-	0.0403	0.99956	1.400	1.3340	9.92
0.0552	0.99990	1.345	-	-	0.0554	1.00027	1.422	1.3344	9.98
0.0703	1.00047	1.350	-	-	0.0707	1.00101	1.442	1.3347	10.01
0.0855	1.00105	1.355	-	-	0.0859	1.00180	1.460	1.3351	10.05
w_1 = 0.005^b					w_1 = 0.005^b				
0.0100	0.99893	1.337	-	-	0.0100	0.99890	1.362	1.3336	9.82
0.0251	0.99947	1.348	-	-	0.0251	0.99945	1.393	1.3339	9.89
0.0401	1.00003	1.357	-	-	0.0402	1.00007	1.419	1.3342	9.96
0.0552	1.00060	1.364	-	-	0.0554	1.00074	1.443	1.3345	10.01
0.0703	1.00119	1.372	-	-	0.0706	1.00147	1.466	1.3348	10.07
0.0854	1.00179	1.378	-	-	0.0859	1.00222	1.487	1.3352	10.12
L-Phenylalanine					L-Glutamic acid				
w_1 = 0.001^b					w_1 = 0.001^b				
0.0100	0.99777	1.347	1.3330	6.26	0.0100	0.99795	1.328	1.3330	3.35
0.0251	0.99847	1.381	1.3335	6.12	0.0251	0.99898	1.346	1.3334	3.28
0.0403	0.99919	1.409	1.3339	6.01	0.0402	1.00007	1.361	1.3337	3.24
0.0555	0.99992	1.435	1.3343	5.91	0.0554	1.00121	1.375	1.3341	3.23
0.0708	1.00066	1.459	1.3347	5.82	0.0706	1.00239	1.388	1.3345	3.22
0.0861	1.00142	1.482	1.3351	5.74	0.0858	1.00362	1.400	1.3349	3.21
w_1 = 0.003^b					w_1 = 0.003^b				

0.0100	0.99831	1.358	1.3333	6.21	0.0100	0.99850	1.339	1.3334	3.34
0.0251	0.99894	1.395	1.3337	6.08	0.0251	0.99946	1.359	1.3337	3.28
0.0403	0.99961	1.426	1.3341	5.95	0.0402	1.00051	1.376	1.3340	3.25
0.0555	1.00031	1.454	1.3344	5.84	0.0554	1.00162	1.392	1.3343	3.23
0.0707	1.00103	1.481	1.3348	5.76	0.0705	1.00277	1.406	1.3346	3.22
0.0861	1.00178	1.506	1.3352	5.70	0.0857	1.00398	1.419	1.3349	3.21
	w_1 = 0.005[b]					w_1 = 0.005[b]			
0.0100	0.99891	1.372	1.3337	6.10	0.0100	0.99912	1.351	1.3336	3.32
0.0251	0.99947	1.412	1.3341	5.95	0.0251	1.00002	1.373	1.3339	3.27
0.0403	1.00008	1.447	1.3345	5.83	0.0402	1.00102	1.392	1.3342	3.25
0.0555	1.00074	1.479	1.3348	5.73	0.0553	1.00207	1.409	1.3345	3.23
0.0707	1.00146	1.509	1.3351	5.68	0.0705	1.00319	1.425	1.3348	3.22
0.0860	1.00219	1.538	1.3354	5.64	0.0857	1.00438	1.441	1.3351	3.21
	Glysine					L-Lysine			
	w_2 = 0.001[b]					w_2 = 0.001[b]			
0.0100	0.99781	1.315	-	-	0.0100	0.99786	1.322	1.3331	9.81
0.0251	0.99834	1.323	-	-	0.0251	0.99851	1.338	1.3334	9.88
0.0402	0.99889	1.330	-	-	0.0403	0.99920	1.352	1.3338	9.93
0.0553	0.99944	1.336	-	-	0.0555	0.99992	1.364	1.3342	9.97
0.0704	1.00001	1.341	-	-	0.0707	1.00067	1.375	1.3346	9.99
0.0855	1.00058	1.346	-	-	0.0859	1.00143	1.386	1.3350	10.01
	w_2 = 0.003[b]					w_2 = 0.003[b]			
0.0100	0.99849	1.328	-	-	0.0100	0.99848	1.319	1.3334	9.84
0.0251	0.99902	1.338	-	-	0.0251	0.99906	1.334	1.3337	9.91
0.0401	0.99957	1.347	-	-	0.0402	0.99970	1.351	1.3341	9.95
0.0552	1.00014	1.354	-	-	0.0554	1.00039	1.368	1.3345	9.99
0.0703	1.00072	1.360	-	-	0.0706	1.00111	1.385	1.3349	10.02
0.0854	1.00131	1.367	-	-	0.0859	1.00186	1.403	1.3353	10.04
	w_2 = 0.005[b]					w_2 = 0.005[b]			
0.0100	0.99924	1.340	-	-	0.0100	0.99918	1.327	1.3339	9.87
0.0251	0.99978	1.352	-	-	0.0251	0.99969	1.348	1.3342	9.94
0.0401	1.00034	1.361	-	-	0.0402	1.00028	1.372	1.3345	9.99
0.0552	1.00092	1.369	-	-	0.0554	1.00093	1.396	1.3349	10.02

0.0703	1.00152	1.376	-	-	0.0706	1.00163	1.424	1.3352	10.05
0.0854	1.00213	1.384	-	-	0.0859	1.00238	1.453	1.3356	10.08
L-Phenylalanine					L-Glutamic acid				
w_2 = 0.001[b]					w_2 = 0.001[b]				
0.0100	0.99788	1.318	1.3326	6.36	0.0100	0.99808	1.323	1.3332	3.40
0.0251	0.99854	1.333	1.3330	6.18	0.0251	0.99908	1.339	1.3336	3.32
0.0403	0.99924	1.345	1.3333	6.02	0.0402	1.00016	1.352	1.3339	3.26
0.0555	0.99996	1.357	1.3335	5.88	0.0554	1.00129	1.364	1.3343	3.23
0.0708	1.00070	1.368	1.3338	5.76	0.0706	1.00248	1.375	1.3346	3.21
0.0861	1.00146	1.379	1.3341	5.67	0.0858	1.00371	1.386	1.3349	3.20
w_2 = 0.003[b]					w_2 = 0.003[b]				
0.0100	0.99849	1.321	1.3334	6.01	0.0100	0.99870	1.325	1.3335	3.32
0.0251	0.99907	1.339	1.3338	5.90	0.0251	0.99963	1.343	1.3338	3.25
0.0403	0.99969	1.358	1.3342	5.82	0.0402	1.00064	1.360	1.3341	3.21
0.0555	1.00036	1.378	1.3347	5.73	0.0554	1.00173	1.376	1.3344	3.20
0.0707	1.00106	1.398	1.3352	5.65	0.0705	1.00286	1.393	1.3347	3.18
0.0861	1.00178	1.420	1.3357	5.61	0.0857	1.00407	1.410	1.3350	3.17
w_2 = 0.005[b]					w_2 = 0.005[b]				
0.0100	0.99916	1.328	1.3338	5.49	0.0100	0.99939	1.329	1.3338	3.30
0.0251	0.99965	1.355	1.3342	5.40	0.0251	1.00024	1.349	1.3341	3.24
0.0403	1.00020	1.384	1.3346	5.31	0.0402	1.00119	1.370	1.3344	3.21
0.0555	1.00083	1.413	1.3350	5.23	0.0553	1.00223	1.392	1.3347	3.19
0.0707	1.00149	1.443	1.3354	5.15	0.0705	1.00337	1.415	1.3350	3.18
0.0860	1.00222	1.474	1.3359	5.10	0.0857	1.00454	1.439	1.3352	3.16

[a] Standard uncertainties u are: $u(\rho)$ =2×10^{-6} kg·m^{-3}, $u(\eta)$ =0.003 mP·s, $u(n_D)$ =0.0002, u(pH) =0.01, and $u(T)$ =0.01K

[b] w_1 and w_2 are mass fractions of α- and β-cyclodextrin in aqueous mixture respectively.

Table 4. Apparent molar volume (ϕ_V), $(\eta_r-1)/\sqrt{m}$ and molar refraction (R_M) of selected amino acids in different mass fraction of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

Aq. solvent mixture	ϕ_V ×10^6 /m^3 mol^{-1}	$(\eta_r-1)/\sqrt{m}$ /kg$^{1/2}$mol^{-}	R_M ×10^6 /m^3 mol^{-1}	Aq. solvent mixture	ϕ_V ×10^6 / m^3 mol^{-1}	$(\eta_r-1)/\sqrt{m}$ /kg$^{1/2}$mol^{-}	R_M ×10^6 /m^3 mol^{-}

		1/2				1/2	1
Glysine				L-Lysine			
$w_1 = 0.001^b$				$w_1 = 0.001^b$			
0.0100	41.18	0.069	-	0.0100	105.47	0.223	30.1369
0.0251	40.38	0.078	-	0.0251	102.87	0.243	30.1580
0.0402	39.93	0.084	-	0.0403	101.21	0.261	30.1763
0.0553	39.54	0.088	-	0.0555	99.73	0.274	30.1909
0.0704	39.17	0.093	-	0.0707	98.45	0.289	30.2047
0.0855	38.94	0.095	-	0.0859	97.51	0.299	30.2163
$w_1 = 0.003^b$				$w_1 = 0.003^b$			
0.0100	41.16	0.092	-	0.0100	110.42	0.236	30.1522
0.0251	40.35	0.101	-	0.0251	107.61	0.260	30.1639
0.0401	39.65	0.107	-	0.0403	105.41	0.282	30.1710
0.0552	39.15	0.114	-	0.0554	103.68	0.298	30.1766
0.0703	38.72	0.115	-	0.0707	102.26	0.316	30.1827
0.0855	38.33	0.117	-	0.0859	100.75	0.331	30.1871
$w_1 = 0.005^b$				$w_1 = 0.005^b$			
0.0100	41.13	0.121	-	0.0100	115.35	0.242	30.1364
0.0251	39.93	0.129	-	0.0251	111.95	0.277	30.1546
0.0401	39.13	0.136	-	0.0402	109.34	0.298	30.1662
0.0552	38.58	0.139	-	0.0554	107.25	0.319	30.1757
0.0703	37.98	0.146	-	0.0706	105.20	0.339	30.1849
0.0854	37.48	0.148	-	0.0859	103.63	0.359	30.1947
L-Phenylalanine				L-Glutamic acid			
$w_1 = 0.001^b$				$w_1 = 0.001^b$			
0.0100	120.51	0.299	34.0527	0.0100	84.36	0.169	30.3277
0.0251	119.51	0.335	34.0763	0.0251	80.95	0.189	30.3340
0.0403	118.76	0.356	34.0922	0.0402	78.59	0.199	30.3390
0.0555	118.23	0.385	34.1060	0.0554	76.61	0.209	30.3435
0.0708	117.79	0.399	34.1208	0.0706	74.90	0.220	30.3476
0.0861	117.27	0.419	34.1322	0.0858	73.21	0.229	30.3508
$w_1 = 0.003^b$				$w_1 = 0.003^b$			

0.0100	126.45	0.320	34.0659	0.0100	89.32	0.175	30.3401
0.0251	124.65	0.366	34.0846	0.0251	85.71	0.202	30.3354
0.0403	123.20	0.392	34.0988	0.0402	82.55	0.217	30.3319
0.0555	121.99	0.421	34.1098	0.0554	80.02	0.227	30.3287
0.0707	121.01	0.442	34.1209	0.0705	78.01	0.239	30.3261
0.0861	120.03	0.463	34.1330	0.0857	75.99	0.245	30.3232
$w_1 = 0.005^b$				$w_1 = 0.005^b$			
0.0100	133.38	0.340	34.0791	0.0100	94.26	0.189	30.3350
0.0251	130.17	0.392	34.0943	0.0251	90.06	0.215	30.3424
0.0403	128.12	0.426	34.1064	0.0402	86.50	0.230	30.3468
0.0555	126.28	0.456	34.1158	0.0553	83.98	0.245	30.3512
0.0707	124.37	0.481	34.1237	0.0705	81.53	0.259	30.3547
0.0860	123.01	0.509	34.1300	0.0857	79.12	0.269	30.3588
Glysine				L-Lysine			
$w_2 = 0.001^b$				$w_2 = 0.001^b$			
0.0100	41.17	0.084	-	0.0100	107.46	0.260	30.1270
0.0251	40.37	0.092	-	0.0251	104.86	0.285	30.1510
0.0402	39.67	0.099	-	0.0403	103.20	0.306	30.1694
0.0553	39.35	0.104	-	0.0555	101.90	0.322	30.1852
0.0704	38.88	0.107	-	0.0707	100.73	0.338	30.2002
0.0855	38.58	0.110	-	0.0859	99.85	0.348	30.2148
$w_2 = 0.003^b$				$w_2 = 0.003^b$			
0.0100	41.15	0.114	-	0.0100	113.40	0.274	30.1275
0.0251	40.34	0.120	-	0.0251	109.99	0.308	30.1536
0.0401	39.64	0.129	-	0.0402	107.64	0.330	30.1713
0.0552	38.96	0.133	-	0.0554	105.66	0.353	30.1866
0.0703	38.43	0.135	-	0.0706	104.09	0.370	30.2015
0.0854	37.96	0.141	-	0.0859	102.73	0.382	30.2137
$w_2 = 0.005^b$				$w_2 = 0.005^b$			
0.0100	41.12	0.128	-	0.0100	118.32	0.294	30.1594
0.0251	39.91	0.138	-	0.0251	114.72	0.334	30.1765
0.0401	39.11	0.143	-	0.0402	111.81	0.362	30.1884
0.0552	38.38	0.148	-	0.0554	109.40	0.385	30.1997

0.0703	37.68	0.151	-	0.0706	107.31	0.407	30.2091
0.0854	37.11	0.158	-	0.0859	105.36	0.423	30.2171
L-Phenylalanine				L-Glutamic acid			
w_2 = 0.001[b]				w_2 = 0.001[b]			
0.0100	124.51	0.329	34.0132	0.0100	86.35	0.184	30.3372
0.0251	122.70	0.372	34.0265	0.0251	82.94	0.203	30.3432
0.0403	121.25	0.401	34.0373	0.0402	80.08	0.218	30.3473
0.0555	120.22	0.426	34.0448	0.0554	77.87	0.231	30.3508
0.0708	119.35	0.447	34.0519	0.0706	75.75	0.243	30.3537
0.0861	118.55	0.465	34.0588	0.0858	73.91	0.251	30.3566
w_2 = 0.003[b]				w_2 = 0.003[b]			
0.0100	131.43	0.342	34.0499	0.0100	92.30	0.198	30.3413
0.0251	128.63	0.394	34.0802	0.0251	88.09	0.221	30.3406
0.0403	126.92	0.429	34.1019	0.0402	85.04	0.239	30.3401
0.0555	125.24	0.456	34.1215	0.0554	82.19	0.256	30.3397
0.0707	123.85	0.481	34.1377	0.0705	79.99	0.267	30.3394
0.0861	122.71	0.501	34.1535	0.0857	77.63	0.276	30.3390
w_2 = 0.005[b]				w_2 = 0.005[b]			
0.0100	139.34	0.370	34.0696	0.0100	98.24	0.211	30.3476
0.0251	135.34	0.424	34.0930	0.0251	93.63	0.239	30.3495
0.0403	132.84	0.467	34.1106	0.0402	89.98	0.260	30.3509
0.0555	130.24	0.501	34.1247	0.0553	86.68	0.276	30.3522
0.0707	128.33	0.529	34.1381	0.0705	83.36	0.290	30.3533
0.0860	126.27	0.554	34.1498	0.0857	80.87	0.304	30.3541

[a] Standard uncertainties u are: $u(T)$ =0.01K

[b] w_1 and w_2 are mass fractions of α- and β-cyclodextrin in aqueous mixture respectively.

Table 5. Limiting apparent molar volume (ϕ_V^0), experimental slope (S_V^*), viscosity *A* & *B*-coefficient and limiting molar refraction (R_M^0) of selected amino acids in different mass fraction of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

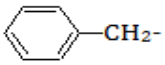
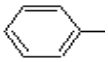
sol. mix	$\phi_V^0 \times 10^6$ /m³ mol⁻¹	$S_V^* \times 10^6$ /m³·mol⁻³/²·kg¹/²	*B* /kg·mol⁻¹	*A* / kg¹/²·mol⁻¹/²	$R_M^0 \times 10^6$ /m³ mol⁻¹	sol. mix	$\phi_V^0 \times 10^6$ /m³ mol⁻¹	$S_V^* \times 10^6$ /m³·mol⁻³/²·kg¹/²	*B* /kg·mol⁻¹	*A* /kg¹/²·mol⁻¹/²	$R_M^0 \times 10^6$ /m³ mol⁻¹
L-Lysine											

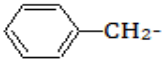
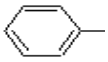
w_1 = 0.001[b]	109.53	-41.41	0.403	0.180	30.09	w_2 = 0.001[b]	111.23	-39.40	0.462	0.213	30.08
w_1 = 0.003[b]	115.46	-49.99	0.493	0.184	30.13	w_2 = 0.003[b]	118.84	-55.45	0.568	0.217	30.08
w_1 = 0.005[b]	121.56	-61.16	0.599	0.181	30.10	w_2 = 0.005[b]	125.24	-67.44	0.671	0.227	30.12
L-Phenylalanine											
w_1 = 0.001[b]	122.15	-16.59	0.619	0.236	34.01	w_2 = 0.001[b]	127.57	-30.98	0.703	0.259	33.98
w_1 = 0.003[b]	129.86	-33.35	0.737	0.247	34.03	w_2 = 0.003[b]	135.89	-45.12	0.822	0.262	33.99
w_1 = 0.005[b]	138.77	-53.66	0.862	0.254	34.05	w_2 = 0.005[b]	146.09	-67.14	0.960	0.274	34.02
L-Glutamic acid											
w_1 = 0.001[b]	90.10	-57.46	0.303	0.139	30.31	w_2 = 0.001[b]	93.04	-64.91	0.355	0.148	30.32
w_1 = 0.003[b]	96.49	-69.70	0.359	0.143	30.34	w_2 = 0.003[b]	100.06	-75.91	0.412	0.157	30.34
w_1 = 0.005[b]	102.28	-78.44	0.415	0.148	30.32	w_2 = 0.005[b]	107.79	-90.99	0.482	0.163	30.34

[a] Standard uncertainties u are: $u(T)$ =0.01K

[b] w_1 and w_2 are mass fractions of α- and β-cyclodextrin in aqueous mixture respectively.

Table 6. Contributions of zwitterionic group (NH_3^+, COO^-), CH_2 group, end group and the other alkyl chains to the limiting apparent molar volume (ϕ_V^0) and viscosity B-coefficient for amino acids in different mass fraction of aqueous α and β-cyclodextrin respectively at 298.15K[a]

Groups	$\phi_V^0 \times 10^6$/ m^3 mol^{-1}			B/ kg mol^{-1}		
	w_1=0.001[b]	w_1=0.003[b]	w_1=0.005[b]	w_1=0.001[b]	w_1=0.003[b]	w_1=0.005[b]
L-Lys						
NH_3^+, COO^-	27.57	25.99	24.24	0.098	0.068	0.032
-CH_2-	14.71	16.66	18.71	0.037	0.069	0.107
-$(CH_2)_4NH_2$	67.25	72.81	78.61	0.268	0.356	0.460
end –NH_2 gr	8.41	6.17	3.77	0.120	0.080	0.032
L-Phe						
NH_3^+, COO^-	30.16	28.59	26.58	0.052	0.108	0.168
-CH_2-	12.12	14.06	16.37	0.083	0.029	-0.029
	79.87	87.21	95.82	0.484	0.600	0.665
	67.75	73.15	79.45	0.401	0.571	0.636
L-Glu						
NH_3^+, COO^-	34.94	33.23	31.61	0.107	0.082	0.056
-CH_2-	7.34	9.42	11.34	0.028	0.055	0.083
-$(CH_2)_2COOH$	47.82	53.84	59.33	0.168	0.222	0.276
end -COOH	33.14	35.00	36.65	0.112	0.112	0.110
	w_2=0.001[b]	w_2=0.003[b]	w_2=0.005[b]	w_2=0.001[b]	w_2=0.003[b]	w_2=0.005[b]
L-Lys						
NH_3^+, COO^-	27.13	24.98	23.11	0.079	0.044	0.010
-CH_2-	15.37	17.94	20.11	0.058	0.096	0.133
-$(CH_2)_4NH_2$	68.73	75.92	82.02	0.235	0.428	0.528

end –NH_2 gr	7.25	4.16	1.58	0.093	0.044	-0.004
L-Phe						
NH_3^+, COO^-	28.83	27.12	24.76	0.094	0.151	0.218
-CH_2-	13.67	15.80	18.46	0.041	-0.014	-0.079
	85.07	92.97	102.87	0.568	0.657	0.663
	71.40	77.17	84.41	0.527	0.643	0.584
L-Glu						
NH_3^+, COO^-	34.06	33.16	29.85	0.081	0.055	0.023
-CH_2-	8.44	9.76	13.37	0.054	0.082	0.116
-$(CH_2)_2COOH$	50.54	57.14	64.57	0.220	0.275	0.343
end -COOH	33.66	37.62	37.83	0.112	0.111	0.111

[a] Standard uncertainties u are: $u(T) = 0.01K$
[b] w_1 and w_2 are mass fractions of α and β-cyclodextrin in aqueous mixture respectively.

Table 7. Hydration number (n_H) and solvation number (S_n) of the amino acids in deferent mass fraction of aqueous α and β-cyclodextrin respectively at 298.15K[a]

	n_H			S_n		
Aq. α-CD (w_1)[b]	0.001	0.003	0.005	0.001	0.003	0.005
L-Lys	6.98	5.01	2.97	3.68	4.27	4.93
L-Phe	5.52	2.95	-0.02	5.07	5.68	6.21
L-Glu	4.54	2.41	0.48	3.36	3.72	4.06
Aq. β -CD (w_2)[b]						
L-Lys	6.42	3.88	1.75	4.15	4.78	5.36
L-Phe	3.71	0.94	-2.46	5.51	6.05	6.57
L-Glu	3.56	1.22	-1.35	3.82	4.12	4.47

[a] Standard uncertainties u are: $u(T) = 0.01K$
[b] w_1 and w_2 are mass fractions of α and β-cyclodextrin in aqueous mixture respectively.

Figures

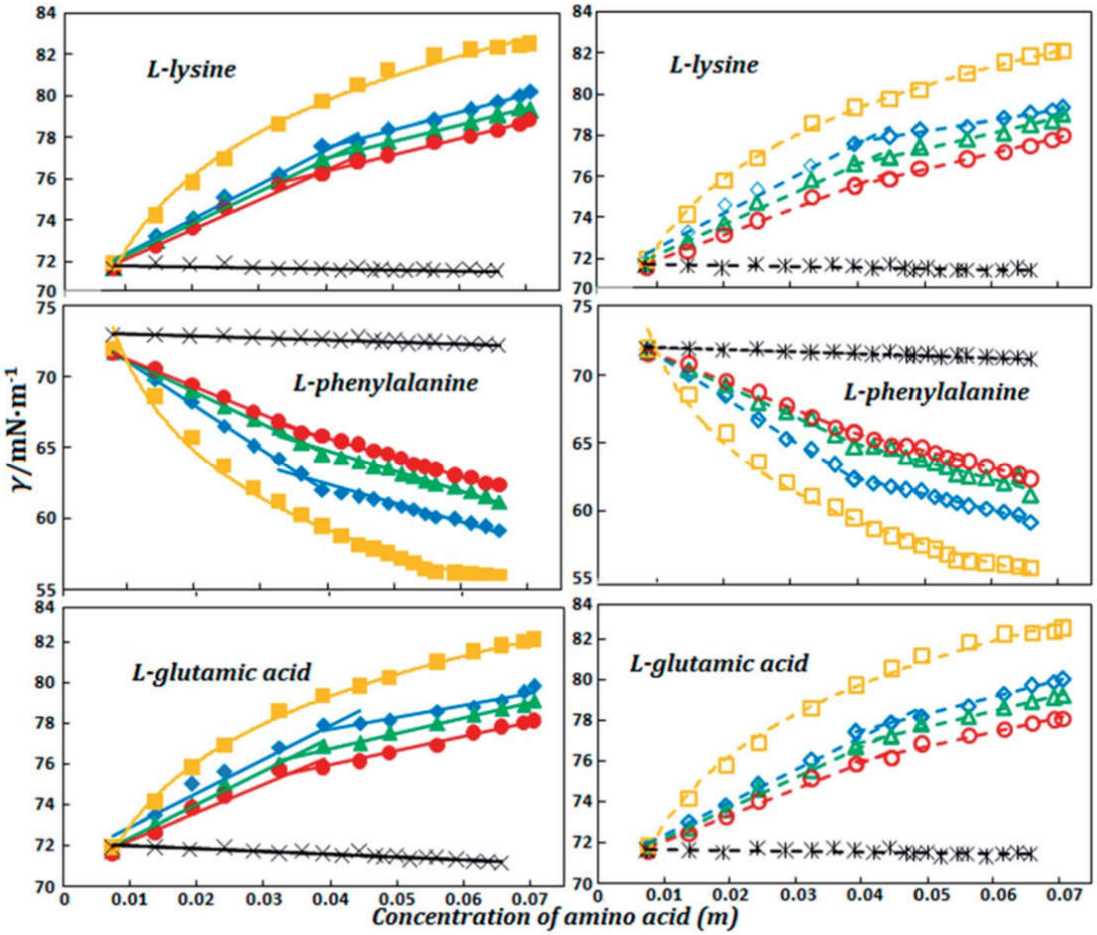


Figure 1. Plot of surface tension (γ) against concentration of amino acids (m) in pure α-CD (×), w_1=0.000(□), w_1=0.001(♦), w_1=0.003(▲), w_1=0.005(●) mass fraction of α-CD and in pure β-CD (✳), w_2=0.000(■) w_2=0.001(◊), w_2=0.003(Δ), w_2=0.005(○) mass fraction of β-CD respectively.

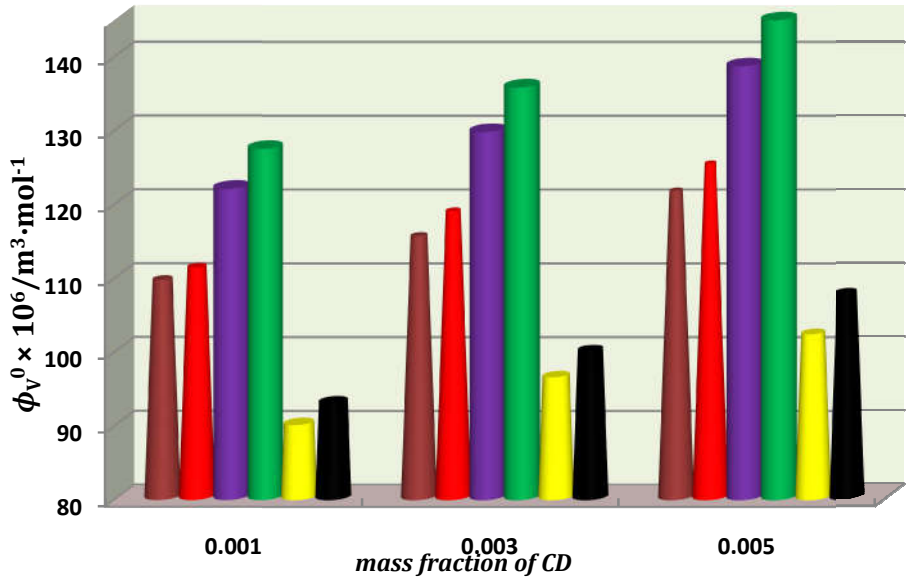


Figure 2. Plot of limiting molar volume (ϕ_V^0) vs mass fraction for L-Lys (brown), L-Phe (blue), L-Glu (yellow) in aqueous α-CD and for L-Lys (red), L-Phe (green), L-Glu (black) in aqueous β-CD respectively.

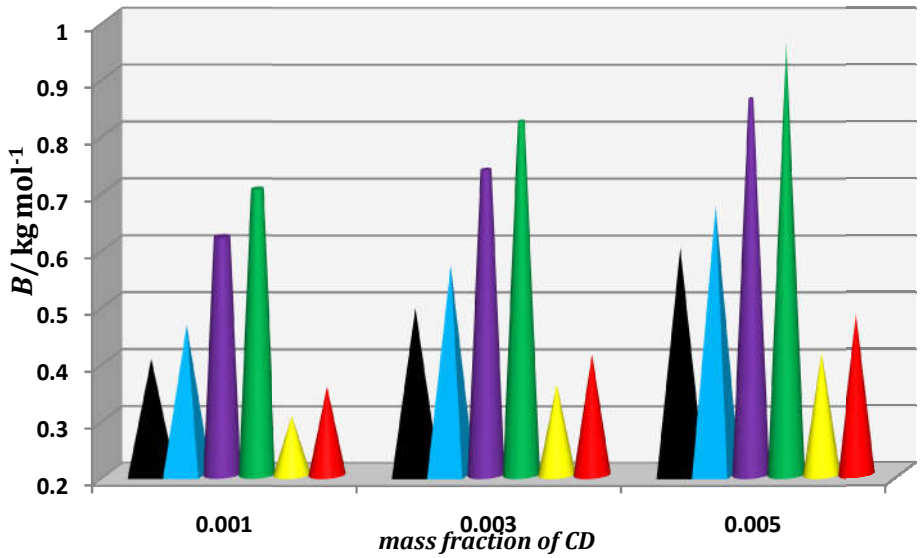


Figure 3. Plot of viscosity B-coefficient vs mass fraction for L-Lys (black), L-Phe (blue), L-Glu (yellow) in aqueous α-CD and for L-Lys (indigo), L-Phe (green), L-Glu (red) in aqueous β-CD respectively.

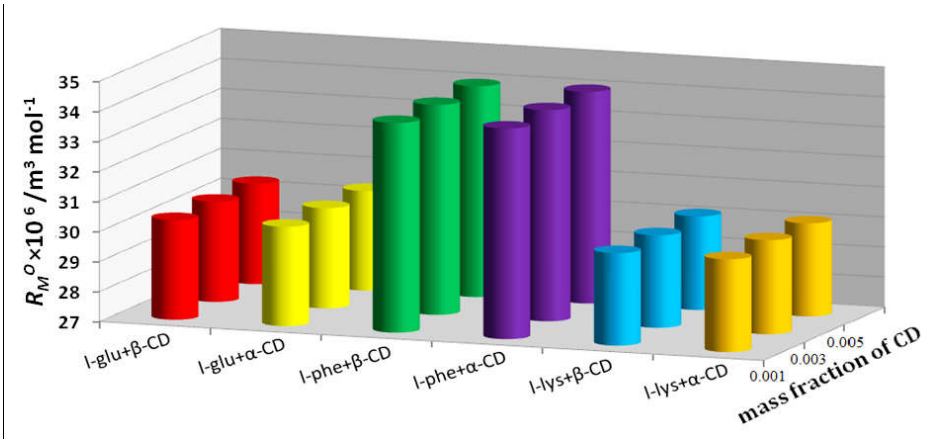
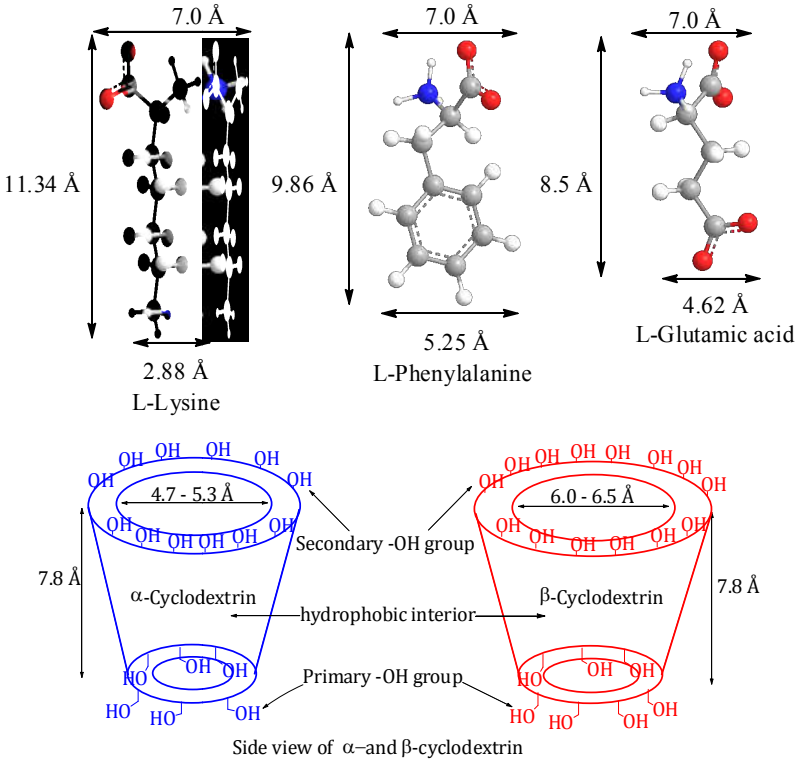
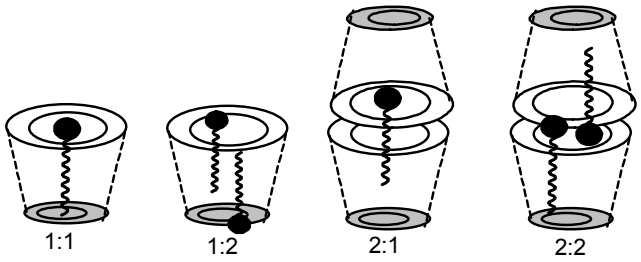


Figure 4. Plot of limiting molar refraction ($R_M{}^0$) for the amino acids in different mass fraction of aqueous α and β-CD respectively.

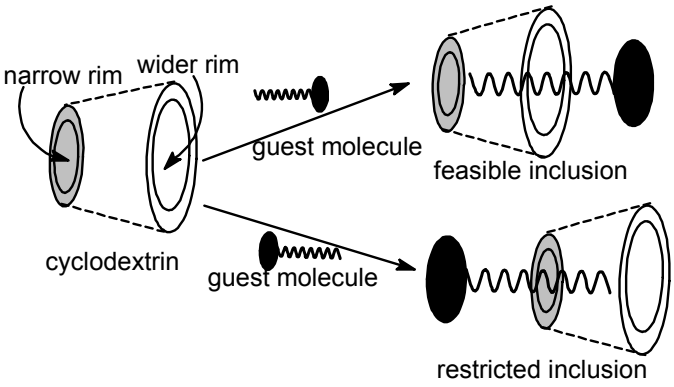
Schemes



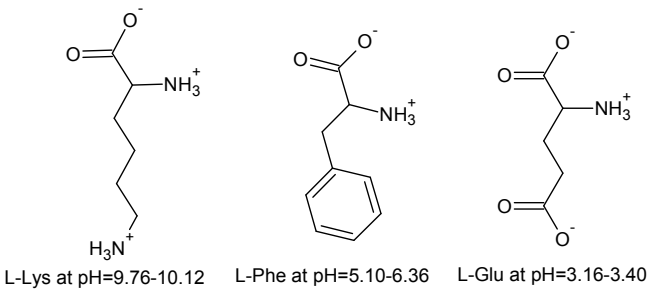
Scheme 1. Molecular structures of the α-amino acids and α, β-cyclodextrin (α-CD: 6 member and β-CD: 7 member sugar ring molecules).



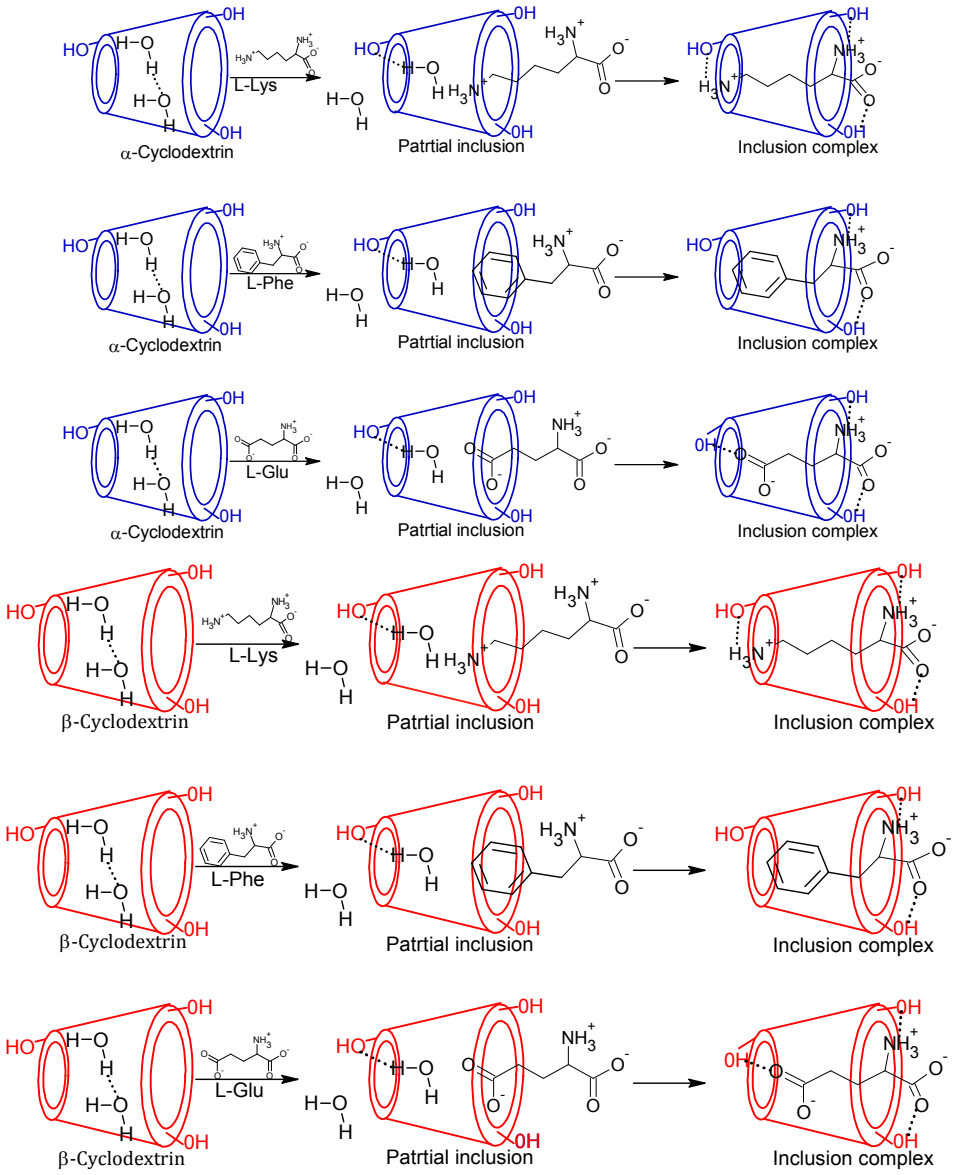
Scheme 2. Plausible stoichiometries for inclusion of host:guest molecule.



Scheme 3. Feasible and restricted inclusion of host:guest molecule.



Scheme 4. State of the amino acids in their respective pH range.



Scheme 5. Schematic representations of convincing mechanism for 1:1 inclusion complexes of the *α*-amino acids inside into *α* and *β*-cyclodextrin.