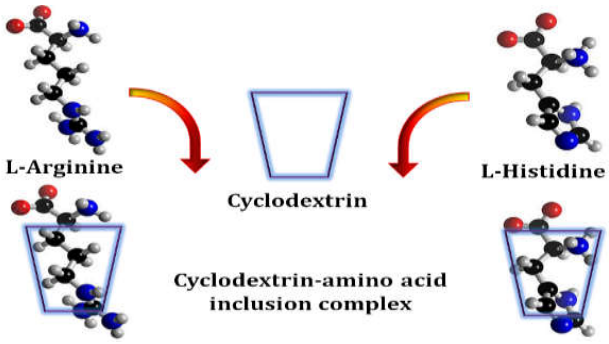
CHAPTER XI

NMR, surface tension and conductivity studies to determine the inclusion mechanism: thermodynamics of host-guest inclusion complexes of natural amino acids in aqueous cyclodextrins

Assembly of two natural amino acids (namely, L-Arginine and L-Histidine) as guests with α and β-cyclodextrins as hosts to form inclusion complexes in aqueous medium has been demonstrated which are highly suitable for diverse applications in modern bio-medical sciences. ^{1}H NMR study establishes the formation of inclusion complexes, while surface tension and conductivity studies confirm that the inclusion complexes have been formed with 1:1 stoichiometry. Nature of the complexes has been established by thermodynamic parameters, based on density, viscosity, and refractive index measurements. Contributions of different groups of the guest molecules towards the limiting apparent molar volume and viscosity-*B* coefficient are determined and solvation numbers are calculated. All the parameters support the formation of the inclusion complexes, which are explained basing upon hydrophobic effect, H-bonds, electrostatic forces and structural effects.



XI.1. Introduction

In the modern science cyclodextrins are extensively used for controlled release of compounds due to their exceptional capability to form inclusion complexes with a variety of guest molecules, thus it is an important task to follow whether a molecule forms inclusion complex with cyclodextrins.[1,2] Cyclodextrins are cyclic oligosaccharides containing six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units, which are

bound by α-(1–4) linkages forming a truncated conical structure having the hydrophobic interior and hydrophilic rims containing primary and secondary –OH groups (scheme 1).

Hence, because of having exceptional structure, they can build up stable host-guest inclusion complexes by accommodating the non-polar component of the guest molecule into its hydrophobic cavity and stabilizing the polar part of the guest molecule by the polar rims.[3,4] This explains about the modern-day interest for cyclodextrins in controlled release of bio-active molecules (*e.g.*, drugs, vitamins, amino acids etc.), food flavours, deodorisers, paint ingredients etc. as well as removal of toxic materials, waste products and pollutants without any chemical modification.[5]

In the present work two natural amino acids (namely, L-Arginine and L-Histidine) (scheme 1) have been studied with α and β-cyclodextrins to observe whether they form host-guest inclusion complex by the study of ^{1}H NMR, surface tension and conductivity. Nature of the inclusion complexes are established by density, viscosity and refractive index measurements by calculating the contributions towards the limiting apparent molar volume and viscosity-B coefficient of different groups of the guest molecules, solvation number and limiting molar refraction by taking 0.001, 0.003, 0.005 mass fractions of α and β-cyclodextrins in aqueous medium.

XI.2. Experimental Section

XI.2.1. Source and Purity of Samples

The selected amino acids and cyclodextrins of puriss grade were bought from Sigma-Aldrich, Germany and used as purchased. The mass fraction purity of L-Arg, L-His, α-cyclodextrin and β-cyclodextrin were ≥ 0.98, 0.99, 0.98 and 0.98 respectively.

XI.2.2. Apparatus and Procedure

The solubility of the selected cyclodextrins and that of the selected amino acids in aqueous cyclodextrins have been precisely checked in triply distilled, deionized and degassed water and observed that these were freely soluble in all proportion of aqueous cyclodextrins. All the stock solutions of the amino acids were prepared by mass (measured

using Mettler Toledo AG-285 with uncertainty 0.0001 *g*), and the working solutions were obtained by mass dilution at 298.15 K. The conversions of molarity to molality have been done using density values.[6] Adequate precautions were taken to reduce evaporation losses during mixing.

NMR spectra were recorded in D_2O unless otherwise stated. ^1H NMR spectra were recorded at 400 MHz and 500 MHz using Bruker Avance 400 MHz and Bruker Avance 500 MHz instruments respectively at 298.15K. Signals are quoted as δ values in ppm using residual protonated solvent signals as internal standard (D_2O : δ 4.79 ppm). Data are reported as chemical shift.

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

Specific conductance values of the experimental solutions were measured by Mettler Toledo Seven Multi conductivity meter with uncertainty 1.0 μS m^{-1}. The measurements were made in a thermostated water bath maintaining the temperature at 298.15 K and using the HPLC grade water with specific conductance of 6.0 μS m^{-1}. The cell was calibrated using a 0.01M aqueous KCl solution. The uncertainty in temperature was 0.01 K.

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 *U*-tube Anton Paar digital density meter (DMA 4500M) with a precision of ±0.00005 g cm^{-3} maintained at ±0.01K of the desired temperature. It was calibrated by passing triply 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 indexes were measured with the help of a Digital Refractometer Mettler Toledo. The light source was LED, λ=589.3nm. The refractometer has been calibrated twice using distilled water and calibration has been checked after every few measurements. The uncertainty of refractive index measurement was ±0.0002 units.

XI.3. Result and Discussion

XI.3.1. ^{1}H NMR study confirms inclusion phenomenon

Insertion of a guest molecule into the hydrophobic cavity of α and β-CD results in the chemical shift of the protons of the cyclodextrin molecule in the ^{1}H NMR spectra, which is due to the interaction of the host cyclodextrin with the guest molecule.[7] In the structure of cyclodextrin the H3 and H5 hydrogens are located inside the conical cavity, particularly, the H3 are placed near the wider rim while H5 are placed near the narrower rim of cyclodextrin molecule. The other H1, H2 and H4 hydrogens are situated at the exterior of the cyclodextrin molecule (scheme 2).[8]

Thus when a guest molecule enters into the cavity of cyclodextrin it interacts with the H3 and H5 protons, resulting in the upfield chemical shift of these protons. As the H3 is located near the wider rim of cyclodextrin, through which usually the guest enters, the shift is higher for it than that for the H5 proton which is situated near the narrower rim at the interior of cyclodextrin. The other H1, H2 and H4 hydrogens also show upfield chemical shift, but it is less compared to that of the interior protons.[9]

In the present work the molecular interactions of L-Arg and L-His with α and β-cyclodextrin have been studied by ^{1}H NMR spectra by taking 1:1 molar ratio of the amino acid and α or β-cyclodextrin in D_2O at 298.15K (table 1). It has been found that there are considerable upfield shifts ($\Delta\delta$) of interior H3 and H5 protons, little shifts of exterior H1, H2 and H4 protons of cyclodextrin, as well as that of the interacting protons of the amino acids (figure 1-4).

This establishes that inclusion phenomenon has occurred between the chosen host and guest molecules. Upon inclusion the upfield chemical shift values (Δδ) of the H3 and H5 protons of α and β-cyclodextrin have been listed in table 2, which show that the interaction of the guest amino acids with H3 is more than that with H5, suggesting the inclusion has taken place through the wider rim of α and β-cyclodextrin.

XI.3.2. Surface tension study explains the inclusion as well as stoichiometric ratio of the inclusion complexes

Amino acid molecules being exist in zwitterionic structures show considerable increase in surface tension of their aqueous solution, while aqueous cyclodextrin solution does not show any remarkable change in surface tension compared to pure aqueous solvent.[10] Thus while amino acids make inclusion complexes with cyclodextrins remarkable change in surface tension should be observed, also getting single, double, etc. break in the surface tension curve indicate 1:1, 1:2, etc. stoichiometries of the host and guest in the formed inclusion complex (scheme 3).[11,12]

In the present study the guest amino acid molecules exist as zwitterionic forms and also contain basic side groups, thereby having charge in their molecules, thus there might be some ionic interactions between the charged groups resulting an increase in surface tension of the aqueous solution, which would be distinctly affected in presence of α or β-CD. Here a set of solutions have been prepared having 10 $mmolL^{-1}$ concentration of L-Arg or L-His with increasing concentration of α or β-CD and the surface tension is measured at 298.15K. The trend of the surface tension curve is found to be progressively falling with increased concentration of α and β-CD, which may be attributed due to the formation of the inclusion complex (figure 5).

The curves for both the amino acids are similar, but the slope of L-Arg is higher than that of L-His, which may be due to greater number of L-Arg molecules are present in the charged structure than that of L-His, both of which are encapsulated in the cyclodextrin cavity as inclusion is occurred. Single discernible breaks at about 10 $mmolL^{-1}$ concentration of both α and β-CD are found for all the possible four cases indicating 1:1 stoichiometric ratio for each of the inclusion complexes formed (table 3).

XI.3.3. Conductivity study illustrates inclusion process and their stoichiometric ratio

Measurement of conductivity of a solution is an important tool to elucidate the inclusion phenomenon. Study of conductivity is not only a very common method to illuminate the host-guest inclusion event but also to identify the stoichiometry of the inclusion complex formed.[13-15] As the amino acid molecule enters into the hydrophobic cavity of α or β-CD the conductivity of the solution decreases gradually, *i.e.*, the conductivity of the solution is markedly affected by the inclusion phenomenon. In this study the conductivities of a series of solutions having 10 $mmolL^{-1}$ concentration of aqueous L-Arg or L-His with increasing concentration of either of the two cyclodextrins have been measured, the trend of the conductivity is found to be regularly declining which is obvious due to the formation of inclusion complex between cyclodextrin and amino acid (figure 6).

A noticeable break is found in the conductivity curve at around 10 $mmolL^{-1}$ concentration for both α and β-CD, suggesting that the stoichiometry of the amino acid-cyclodextrin inclusion complex is equimolar, *i.e.*, 1:1 host-guest inclusion complex is formed (scheme 3). Complex or more number of breaks in the conductivity curve suggests different stoichiometry *e.g.*, 1:2, 2:1, 2:2 etc. for the inclusion complex. In this study of all the four cases of L-Arg and L-His with α and β-CD similar results are found, but the conductivity near the break is found to be a little lower for β-CD than α-CD, which is might be due to the former is better host for the two studied guests than the later (table 3).

XI.3.4. pH study confirms ionic states of the amino acids

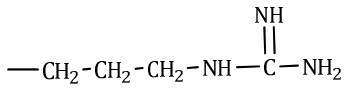
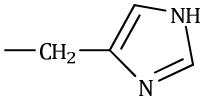
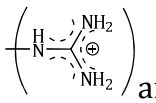
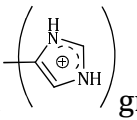
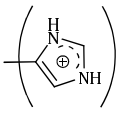
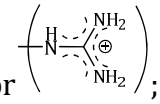
pH measurement is a handy tool for understanding the structures of the zwitterionic forms of amino acids in aqueous solution.[16] The values of pH for L-Arg and L-His in both the aqueous α and β-CD increase with increasing concentration of the respective amino acids (table 4, 6). The range of the pH values at 298.15K for the two selected amino acids in the aqueous α and β-CD solutions under experimental consideration are 7.38-7.56 and 9.80-10.73 for L-His and L-Arg respectively. These values

clearly show the variation in their zwitterionic forms, *i.e.*, besides existing as $-NH_3^+$ and $-COO^-$ of the amine and carboxylic acid groups, the side chain of the amino acids be present as cationic groups by acquiring a proton from the aqueous solution, thus by increasing the pH value of the solution.

XI.3.5. Density study: group contributions and interactions between amino acids and cyclodextrins

Apparent molar volume (ϕ_v) and limiting apparent molar volume (ϕ_v^o) are considered as sensitive tools for understanding the interactions taking place in solutions. The apparent molar volume is the measure of 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. Here ϕ_v has been determined from the measured density of the solutions at 298.15K (table 4, 6) and by using the suitable equation, the magnitude of which is found to be positive for all the systems under study, indicating strong solute–solvent interactions.[10,17] ϕ_v varies linearly with the square root of molal concentration (table 8) and is fitted to Masson equation, from where the limiting apparent molar volume (ϕ_v^o) has been determined (table 9).[18] The values of ϕ_v^o increases with the increase of mass fractions of α and β-CD for both L-Arg and L-His indicating the ion–hydrophilic group interactions are stronger than ion–hydrophobic group interactions. In the present ternary system interactions are taking place between the zwitterionic groups and the side chain ionic group of the amino acids with the localized hydroxyl groups of cyclodextrins, which the electrostriction of water resulting in an increase in volume. The ϕ_v^o values for both the amino acids and cyclodextrins at different mass fractions are represented in figure 7, which suggests that ϕ_v^o of L-Arg is greater than that of L-His due to more electrostriction which is further due to presence of additional methylene groups (that lengthens the chain increasing the hydrophobic interaction) and the guanidine group (that interacts better than the imidazole group with the –OH groups of cyclodextrin) that provide an enforcing tendency in L-Arg resulting a net increase in volume.

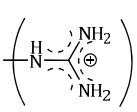
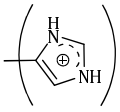
The consequences for the selected amino acids can be recognized on the basis of the limiting partial molar volume which is found to be higher for L-Arg than L-His. In this

study the values of ϕ_v^o are measured for glycine, L-Arg and L-His at 298.15K for different mass fractions of α and β-CD (table 5, 7). If one H from the side chain of glycine is replaced by  and  side groups L-Arg and L-His are found respectively, so there should be a correlation among the structures of the amino acids as well as in the values of ϕ_v^o, which greatly affect the inclusion complexes taking place in the solution systems.[10,15,17] The variations of ϕ_v^o for different groups present in L-Arg and L-His with different mass fractions of α and β-CD have been estimated (table 10). It is observed that the contribution of zwitterionic group (NH_3^+,COO^-) is in the range of 23.24–25.76 × 10^{-6} m^3 mol^{-1} and 25.32–27.48 × 10^{-6} m^3 mol^{-1} for α and β-CD respectively, which indicates that the interactions between the –OH groups of cyclodextrins and the polar head groups (NH_3^+,COO^-) of amino acids are strong, but it is stronger for β-CD than α-CD. The contributions of hydrophobic (CH), (CH_2) groups decrease and that of the hydrophilic  and  groups increase with the increase of mass fractions of both the cyclodextrins, which suggest that the ion-dipolar interactions increase over the hydrophobic interactions for the two selected amino acids when the mass fractions of both the cyclodextrins are increased in the solution. It is observed that the contribution to ϕ_v^o for  is more than that for  ; but as the contributions of the hydrophobic alkyl groups are summed the overall ϕ_v^o for L-Arg is found to be greater than that for L-His with both the aqueous cyclodextrin solutions.

XI.3.6. Viscosity: group contribution and Solvation Number: degree of solvation by cyclodextrin molecule

The viscosity of aqueous cyclodextrin solution increases with increasing mass fraction of α or β-CD (table 4) due to structure making contribution of cyclodextrins with water molecules. In the studied ternary systems viscosity is found to be increasing with increasing molality of amino acids (table 6). The viscosity *B*-coefficients (table 9), which are the indication of solute–solvent interactions, are found to be all positive (figure 8), and

increase with the increasing concentration of α and β-CD, which is considered to arise because of increasing amino acid–cyclodextrin interaction as well as increase in the salvation.[19,20]

The contributions of different groups of the amino acids to the viscosity *B*-coefficient have been derived (table 10).[10,15,17] The contributions of the zwitterionic group (NH_3^+,COO^-) and the polar groups  and  increase with increasing mass fractions of α and β-CD, suggesting the grater solvation of the ionic groups with the –OH groups of cyclodextrin molecules, while that of the hydrophobic (CH), (CH_2) groups are found as increasing demonstrating the increased solvation of the hydrophobic part of the amino acids inside the hydrophobic cavity of α and β-CD.

In the present study solvation number is the measure of the interaction taking place between the primary or secondary hydroxyl groups of cyclodextrins and the zwitterionic or the polar side groups of amino acids, this is because as the mass fraction of the cyclodextrins increases in the ternary solution system the electrostriction of water diminishes resulting an increase in the solvation number (table 11), which is found to be higher for L-Arg than L-His, this may be explained as L-Arg contains longer alkyl chain and the more basic guanidine group than L-His, the encapsulation of the hydrophobic part inside the cavity of cyclodextrin is grater as well as the guanidine group interacts better with the –OH groups of cyclodextrin replacing the surrounded water molecules.[10,21]

The solvation number in case of β-CD is observed higher than that of α-CD, this is probably the more number of –OH groups (primary and secondary) coordinate with the proper phase of interaction with the zwitterionic and polar side groups replacing the water molecules around the amino acid molecules.

XI.3.7. Refractive Index demonstrates compactness of inclusion complexes

The refractive index (n_D) and molar refraction (R_M) are considered as valuable tools for investigating the molecular interactions taking place in solution systems (table 4, 6, 8). As the interaction between the solute and solvent (here formation of inclusion complex

between amino acids and cyclodextrins) increases the medium becomes more compact, resulting the higher value of limiting molar refraction (R^0_M) (table 9).[10,22] Therefore, it is evident from figure 9 that the inclusion complexes of L-Arg with both the α and β-CD are more dense or closely packed than those of L-His, which may be explained as due to greater hydrophilic as well as hydrophobic interaction between L-Arg and both the cyclodextrins. These findings are in good agreement which have been found from density and viscosity measurements.

XI.3.8. Structural Influence of Cyclodextrins

Formation of host-guest inclusion complex depends on the size of the guest molecule as well as the cavity diameter of the host molecule, thus it is a dimensional suitability between these two species.[10] The uniqueness of cyclodextrin molecule is the hydrophobic cavity and hydrophilic rims, which provide an appropriate environment for the apolar part of a molecule to reside inside the cavity, while the polar part makes association with the polar rims, thereby stabilizing the whole inclusion complex.[1,23] Another driving force for the formation of the inclusion complex is the release of the water molecules from the hydrophobic cavity into the bulk, which is a entropy driven process.[15] The stoichiometry of the inclusion complex is found as 1:1 (scheme 3) from conductivity and surface tension measurements, which may be explained on the basis of the fact that after inclusion of one amino acid molecule it would be difficult for a second molecule to be inserted into the cavity because the zwitterionic part and the ionic side group make some kind of blockage at the wider rim of the host molecule.[10] The insertion of the guest amino acid molecule is expected from the wider rim of the cyclodextrin molecule, so as to make maximum contact of the alkyl groups with the cyclodextrin cavity (scheme 4), which is also supported by NMR data.

The charged terminal groups are projected toward the aqueous environment and can make H-bonds with the –OH groups at the both rims of the cyclodextrin molecule (scheme 5). Thus the stabilizing factors for the formation of the inclusion complexes are firstly, the displacement of polar water molecules from the apolar cavity of cyclodextrin and return to the bulk of the water, making large number of H-bonds; secondly, decrease

of the repulsive forces between the hydrophobic alkyl groups of amino acids in the aqueous environment and increase in the hydrophobic interactions as the inclusion takes place in the apolar cavity of cyclodextrin; and finally, the inclusion complex is stabilized by the formation of H-bonds between the polar groups of amino acids and the primary as well as the secondary –OH groups at both the rims of α and β-CD (scheme 5).

XI.4. Conclusion

The present study concludes that the two essential amino acids, namely, L-Arg and L-His form host-guest inclusion complexes with α and β-CD. NMR study confirms the inclusion phenomenon while surface tension and conductivity studies reveal that 1:1 inclusion complexes have been formed. Density, viscosity and refractive index measurements are used to characterize the formed inclusion complexes by determining the group contributions of the limiting apparent molar volume and viscosity-B coefficient, as well as solvation number and limiting molar refraction. All the findings support the formation of the inclusion complexes and thus the current work describes its appropriateness towards miscellaneous applications as controlled delivery system in the field of modern bio-medical sciences.

Tables

Table 1. ^{1}H NMR data of α-CD, β-CD, L-Arg, L-His and inclusion complexes at 298 K

α-Cyclodextrin (500 MHz, Solv: D_2O) δ /ppm
3.48-3.51 (6H, t, *J* = 9.00 Hz), 3.53-3.56 (6H, dd, *J* = 10.00, 3.00 Hz), 3.74-3.83 (18H, m), 3.87-3.91 (6H, t, *J* = 9 Hz), 4.97-4.96 (6H, d, *J* = 3 Hz)
β-Cyclodextrin (400 MHz, Solv: D_2O) δ /ppm
3.49-3.54 (7H, t, *J* = 9.2 Hz), 3.57-3.60 (7H, dd, *J* = 9.6, 3.2 Hz), 3.79-3.84 (21H, m), 3.87-3.92 (7H, t, *J* = 9.2 Hz), 5.00-5.01 (7H, d, *J* = 3.6 Hz)
L-Arginine (500 MHz, Solv: D_2O) δ /ppm
1.48-1.53 (4H, m), 3.07-3.08 (2H, m), 3.13-3.14 (1H, m)
L-Histidine (500 MHz, Solv: D_2O)

δ /ppm
3.01-3.16 (2H, m), 3.85-3.86 (1H, m), 6.97 (1H, s), 7.67 (1H, s)
α-CD+L-Arg (500 MHz, Solv: D_2O) δ /ppm
1.39-1.43 (4H, m), 2.97-2.98 (2H, t, *J* = 6.5 Hz), 3.03-3.04 (1H, t, *J* = 5.5 Hz), 3.43-3.46 (6H, t, *J* = 9.00 Hz), 3.49-3.52 (6H, dd, *J* = 10.00, 3.00 Hz), 3.59-3.63 (6H, t, *J* = 9 Hz), 3.66-3.75 (18H, m), 4.97-4.96 (6H, d, *J* = 3 Hz)
α-CD+L-His (400 MHz, Solv: D_2O) δ /ppm
3.00-3.16 (2H, m), 3.84-3.85 (1H, m), 6.89 (1H, s), 7.59 (1H, s), 3.46-3.49 (6H, t, *J* = 9.00 Hz), 3.51-3.54 (6H, dd, *J* = 10.00, 3.00 Hz), 3.66-3.70 (6H, t, *J* = 9 Hz), 3.69-3.78 (18H, m), 4.97-4.96 (6H, d, *J* = 3 Hz)
β-CD+L-Arg (400 MHz, Solv: D_2O) δ /ppm
1.38-1.42 (4H, m), 2.96-2.97 (2H, t, *J* = 6.5 Hz), 3.02-3.03 (1H, t, *J* = 5.5 Hz), 3.44-3.49 (7H, t, *J* = 9.2 Hz), 3.53-3.56 (7H, dd, *J* = 9.6, 3.2 Hz), 3.59-3.64 (7H, t, *J* = 9.2 Hz), 3.69-3.74 (21H, m), 5.00-5.01 (7H, d, *J* = 3.6 Hz)
β-CD+L-His (400 MHz, Solv: D_2O) δ /ppm
2.97-3.13 (2H, m), 3.84-3.85 (1H, m), 6.88 (1H, s), 7.58 (1H, s), 3.45-3.50 (7H, t, *J* = 9.2 Hz), 3.54-3.57 (7H, dd, *J* = 9.6, 3.2 Hz), 3.62-3.67 (7H, t, *J* = 9.2 Hz), 3.73-3.78 (21H, m), 5.00-5.01 (7H, d, *J* = 3.6 Hz)

Table 2. Change in chemical shifts of the H3 and H5 protons of cyclodextrin host molecules when complexed with amino acid guest molecules in D_2O at 298.15K[a]

	Δδ /ppm			
	L-Arginine		L-Histidine	
	H3	H5	H3	H5
α-cyclodextrin	0.278	0.088	0.208	0.052
β-cyclodextrin	0.283	0.099	0.252	0.062

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

Table 3. Values of surface tension (γ) and conductivity (κ) at the break point with corresponding concentration of aqueous α and β-cyclodextrin at 298.15 K [a]

	L-Arginine		L-Histidine	
	Surface tension			
	Conc /mM	γ /mN·m^{-1}	Conc /mM	γ /mN·m^{-1}
α-cyclodextrin	9.9	76.5	9.8	74.5
β-cyclodextrin	9.9	76.0	9.8	74.0
	Conductivity			
	Conc /mM	κ /μS·m^{-1}	Conc /mM	κ /μS·m^{-1}
α-cyclodextrin	10.1	176	10.5	65
β-cyclodextrin	10.0	170	11.0	64

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

Table 4. Experimental values of density (ρ), viscosity (η), refractive index (n_D) and pH of different mass fractions of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

Aq. solvent mixture	$\rho\times10^{-3}$ /kg·m^{-3}	η /mP·s	n_D	pH
	aq. α-CD			
w_1 = 0.001	0.99737	1.29	1.3329	6.65
w_1 = 0.003	0.99800	1.30	1.3332	6.60
w_1 = 0.005	0.99865	1.31	1.3335	6.56
	aq. β-CD			
w_2 = 0.001	0.99752	1.30	1.3330	6.58
w_2 = 0.003	0.99817	1.31	1.3333	6.53
w_2 = 0.005	0.99893	1.32	1.3336	6.50

[a] Standard uncertainties u are: $u(\rho)$ = 5×10^{-5} g·cm^{-3}, $u(\eta)$ = 0.003 mP·s, $u(n_D)$ =0.0002, u(pH) =0.01, and u(T) =0.01K.

Table 5. Experimental values of density (ρ) and viscosity (η) of glycine in different mass fractions of aqueous *α* and *β*-cyclodextrin mixtures at 298.15 K[a]

molality /mol·kg^{-1}	$\rho\times10^{-3}$ /kg·m^{-3}	η /mP·s	molality /mol·kg^{-1}	$\rho\times10^{-3}$ /kg·m^{-3}	η /mP·s
Glycine					
	w_1 = 0.001[b]			w_2 = 0.001[b]	
0.0100	0.99767	1.31	0.0100	0.99779	1.31
0.0251	0.99820	1.31	0.0251	0.99835	1.32
0.0402	0.99874	1.32	0.0402	0.99891	1.33
0.0553	0.99930	1.32	0.0553	0.99946	1.33
0.0704	0.99983	1.33	0.0704	1.00002	1.34
0.0855	1.00041	1.33	0.0855	1.00060	1.35
	w_1 = 0.003[b]			w_2 = 0.003[b]	
0.0100	0.99825	1.32	0.0100	0.99851	1.33
0.0251	0.99880	1.33	0.0251	0.99904	1.34
0.0401	0.99932	1.34	0.0401	0.99955	1.35
0.0552	0.99988	1.34	0.0552	1.00012	1.35
0.0703	1.00045	1.35	0.0703	1.00070	1.36
0.0855	1.00107	1.35	0.0854	1.00133	1.37
	w_1 = 0.005[b]			w_2 = 0.005[b]	
0.0100	0.99892	1.33	0.0100	0.99924	1.34
0.0251	0.99948	1.34	0.0251	0.99979	1.35
0.0401	1.00005	1.35	0.0401	1.00036	1.36
0.0552	1.00062	1.36	0.0552	1.00090	1.37
0.0703	1.00121	1.37	0.0703	1.00153	1.38
0.0854	1.00181	1.38	0.0854	1.00213	1.39

[a] Standard uncertainties *u* are: $u(\rho)$ = 5×10^{-5} kg·m^{-3}, $u(\eta)$ =0.003 mP·s and u(T) =0.01K.
[b] w_1 and w_2 are mass fractions of *α* and *β*-cyclodextrin in aqueous mixture respectively.

Table 6. Experimental values of density (*ρ*), viscosity (*η*), refractive index (n_D) and pH of selected amino acids in different mass fractions of aqueous *α* and *β*-cyclodextrin mixtures at 298.15 K[a]

molality /mol·kg^{-1}	$\rho\times10^{-3}$ /kg·m^{-3}	η /mP·s	n_D	pH	molality /mol·kg^{-1}	$\rho\times10^{-3}$ /kg·m^{-3}	η /mP·s	n_D	pH

L-Arginine									
w_1 = 0.001[b]					w_2 = 0.001[b]				
0.010038	0.99792	1.31	1.3330	10.32	0.010038	0.99800	1.33	1.3332	10.25
0.025140	0.99878	1.33	1.3332	10.44	0.025141	0.99875	1.34	1.3334	10.35
0.040295	0.99965	1.34	1.3333	10.54	0.040300	0.99953	1.36	1.3335	10.43
0.055503	1.00052	1.36	1.3335	10.63	0.055514	1.00033	1.39	1.3337	10.54
0.070763	1.00141	1.37	1.3336	10.69	0.070782	1.00114	1.40	1.3339	10.61
0.086075	1.00232	1.39	1.3339	10.73	0.086105	1.00197	1.42	1.3341	10.69
w_1 = 0.003[b]					w_2 = 0.003[b]				
0.010032	0.99851	1.32	1.3334	10.30	0.010031	0.99862	1.34	1.3335	10.12
0.025127	0.99932	1.34	1.3335	10.42	0.025126	0.99934	1.36	1.3336	10.25
0.040275	1.00013	1.36	1.3336	10.55	0.040277	1.00010	1.38	1.3337	10.36
0.055478	1.00097	1.37	1.3338	10.62	0.055485	1.00084	1.40	1.3339	10.44
0.070735	1.00181	1.39	1.3340	10.66	0.070747	1.00164	1.42	1.3341	10.55
0.086043	1.00268	1.40	1.3342	10.70	0.086063	1.00245	1.44	1.3343	10.64
w_1 = 0.005[b]					w_2 = 0.005[b]				
0.010026	0.99911	1.34	1.3337	9.99	0.010024	0.99935	1.35	1.3338	9.80
0.025113	0.99985	1.35	1.3338	10.12	0.025108	1.00004	1.37	1.3340	9.95
0.040256	1.00062	1.37	1.3339	10.27	0.040249	1.00077	1.40	1.3341	10.17
0.055453	1.00141	1.39	1.3341	10.40	0.055448	1.00150	1.42	1.3342	10.32
0.070708	1.00218	1.41	1.3342	10.52	0.070702	1.00226	1.43	1.3344	10.46
0.086017	1.00298	1.42	1.3344	10.61	0.086013	1.00303	1.45	1.3346	10.60
L-Histidine									
w_1 = 0.001[b]					w_2 = 0.001[b]				
0.010037	0.99791	1.30	1.3330	7.45	0.010036	0.99801	1.320	1.3331	7.44
0.025128	0.99878	1.32	1.3331	7.48	0.025126	0.99885	1.330	1.3332	7.46
0.040262	0.99969	1.33	1.3333	7.51	0.040259	0.99977	1.340	1.3334	7.48
0.055437	1.00065	1.34	1.3334	7.53	0.055431	1.00075	1.360	1.3336	7.50
0.070655	1.00159	1.35	1.3335	7.55	0.070644	1.00175	1.370	1.3338	7.51
0.085911	1.00258	1.36	1.3336	7.56	0.085893	1.00279	1.400	1.3339	7.52
w_1 = 0.003[b]					w_2 = 0.003[b]				
0.010031	0.99850	1.32	1.3333	7.44	0.010029	0.99863	1.34	1.3334	7.45
0.025114	0.99932	1.33	1.3334	7.47	0.025112	0.99943	1.35	1.3335	7.46

0.040242	1.00020	1.35	1.3335	7.49	0.040237	1.00032	1.36	1.3337	7.47
0.055412	1.00109	1.37	1.3337	7.51	0.055402	1.00127	1.39	1.3338	7.48
0.070629	1.00196	1.38	1.3338	7.53	0.070609	1.00223	1.40	1.3340	7.50
0.085882	1.00292	1.39	1.3339	7.55	0.085854	1.00324	1.44	1.3341	7.52
		w_1 = 0.005[b]					w_2 = 0.005[b]		
0.010024	0.99911	1.33	1.3336	7.40	0.010022	0.99936	1.35	1.3337	7.38
0.025100	0.99989	1.35	1.3338	7.45	0.025094	1.00012	1.37	1.3338	7.40
0.040221	1.00072	1.36	1.3339	7.47	0.040210	1.00098	1.39	1.3339	7.42
0.055386	1.00156	1.39	1.3341	7.49	0.055368	1.00189	1.41	1.3341	7.44
0.070597	1.00240	1.39	1.3342	7.51	0.070566	1.00284	1.43	1.3342	7.47
0.085852	1.00326	1.41	1.3343	7.53	0.085806	1.00379	1.45	1.3343	7.49

[a] Standard uncertainties u are: $u(\rho)$ = 5×10^{-5} 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 7. Apparent molar volume (ϕ_V) and (η_r-1)/$\sqrt{m}$ of glycine in different mass fractions of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

molality /mol·kg^{-1}	ϕ_V ×10^6 / m^3 mol^{-1}	(η_r-1)/$\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$	molality /mol·kg^{-1}	ϕ_V ×10^6 / m^3 mol^{-1}	(η_r-1)/$\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$
		Glycine			
	w_1 = 0.001[b]			w_2 = 0.001[b]	
0.0100	41.20	0.070	0.0100	41.19	0.082
0.0251	40.39	0.079	0.0251	40.35	0.093
0.0402	39.95	0.083	0.0402	39.65	0.100
0.0553	39.55	0.087	0.0553	39.38	0.105
0.0704	39.19	0.094	0.0704	38.90	0.106
0.0855	38.95	0.096	0.0855	38.61	0.108
	w_1 = 0.003[b]			w_2 = 0.003[b]	
0.0100	41.17	0.091	0.0100	41.17	0.115
0.0251	40.36	0.102	0.0251	40.36	0.119
0.0401	39.67	0.106	0.0401	39.63	0.130
0.0552	39.17	0.113	0.0552	38.95	0.132

0.0703	38.73	0.116	0.0703	38.45	0.136
0.0855	38.36	0.119	0.0854	37.94	0.143
w_1 = 0.005[b]			w_2 = 0.005[b]		
0.0100	41.15	0.123	0.0100	41.14	0.129
0.0251	39.95	0.130	0.0251	39.93	0.137
0.0401	39.10	0.134	0.0401	39.10	0.145
0.0552	38.59	0.137	0.0552	38.36	0.150
0.0703	38.00	0.144	0.0703	37.67	0.153
0.0854	37.52	0.150	0.0854	37.13	0.159

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

molality /mol·kg^{-1}	$\phi_V \times 10^6$ / m^3 mol^{-1}	$(\eta_r\text{-}1)/\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$	$R_M \times 10^6$ /m^3 mol^{-1}	molality /mol·kg^{-1}	$\phi_V \times 10^6$ / m^3 mol^{-1}	$(\eta_r\text{-}1)/\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$	$R_M \times 10^6$ /m^3 mol^{-1}
L-Arginine							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.010038	119.51	0.155	35.9068	0.010038	126.51	0.230	35.9235
0.025140	118.11	0.196	35.8955	0.025141	125.31	0.194	35.9161
0.040295	117.51	0.193	35.8740	0.040300	124.26	0.230	35.8979
0.055503	117.24	0.230	35.8623	0.055514	123.42	0.294	35.8887
0.070763	116.79	0.233	35.8402	0.070782	122.79	0.289	35.8791
0.086075	116.27	0.264	35.8369	0.086105	122.15	0.315	35.8689
w_1 = 0.003[b]				w_2 = 0.003[b]			
0.010032	123.45	0.154	35.9247	0.010031	129.44	0.229	35.9306
0.025127	121.64	0.194	35.9054	0.025126	127.63	0.241	35.9144
0.040275	121.19	0.230	35.8861	0.040277	126.18	0.266	35.8969
0.055478	120.44	0.229	35.8755	0.055485	125.88	0.292	35.8899
0.070735	120.01	0.246	35.8649	0.070747	124.86	0.316	35.8807
0.086043	119.38	0.262	35.8532	0.086063	124.07	0.338	35.8712
w_1 = 0.005[b]				w_2 = 0.005[b]			

0.010026	128.37	0.191	35.9325	0.010024	132.34	0.227	35.9336
0.025113	126.37	0.193	35.9157	0.025108	129.94	0.239	35.9284
0.040256	125.12	0.228	35.8978	0.040249	128.34	0.302	35.9119
0.055453	124.19	0.259	35.8889	0.055448	127.61	0.322	35.8955
0.070708	123.94	0.287	35.8711	0.070702	126.76	0.313	35.8877
0.086017	123.43	0.286	35.8620	0.086013	126.10	0.336	35.8796
L-Histidine							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.010037	101.42	0.077	31.9805	0.010036	106.41	0.154	31.9860
0.025128	99.01	0.147	31.9613	0.025126	102.20	0.146	31.9678
0.040262	97.41	0.155	31.9497	0.040259	99.15	0.153	31.9558
0.055437	95.77	0.165	31.9277	0.055431	96.66	0.196	31.9419
0.070655	95.11	0.175	31.9064	0.070644	94.96	0.203	31.9274
0.085911	94.10	0.185	31.8836	0.085893	93.38	0.262	31.9029
w_1 = 0.003[b]				w_2 = 0.003[b]			
0.010031	105.36	0.154	31.9877	0.010029	109.35	0.229	31.9923
0.025114	102.56	0.146	31.9702	0.025112	104.94	0.193	31.9754
0.040242	100.35	0.192	31.9508	0.040237	101.59	0.190	31.9643
0.055412	99.17	0.229	31.9397	0.055402	98.97	0.259	31.9427
0.070629	98.78	0.232	31.9207	0.070609	97.33	0.259	31.9294
0.085882	97.46	0.236	31.8988	0.085854	95.68	0.339	31.9059
w_1 = 0.005[b]				w_2 = 0.005[b]			
0.010024	109.30	0.152	31.9943	0.010022	112.27	0.227	31.9950
0.025100	105.69	0.193	31.9867	0.025094	107.67	0.239	31.9794
0.040221	103.54	0.190	31.9689	0.040210	104.01	0.264	31.9606
0.055386	102.38	0.259	31.9595	0.055368	101.44	0.290	31.9489
0.070597	101.72	0.230	31.9413	0.070566	99.40	0.314	31.9273
0.085852	101.05	0.261	31.9226	0.085806	98.08	0.336	31.9058

[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.

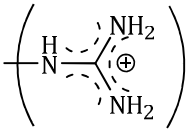
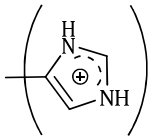
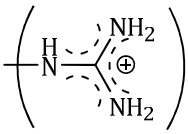
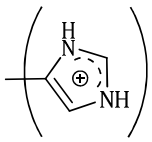
Table 9. Limiting apparent molar volume (ϕ_V^0), experimental slope (S_V^*), viscosity *A* & *B*-coefficient and limiting molar refraction (R_M^0) of amino acids in different mass fractions of aqueous *α* and *β*-cyclodextrin mixtures at 298.15 K[a]

Aq. solvent mixture	$\phi^0_V \times 10^6$ / $m^3\ mol^{-1}$	$S^*_V \times 10^6$ / $m^3 mol^{-3/2} kg^{1/2}$	*B* / $kg\ mol^{-1}$	*A* / $kg^{1/2}\ mol^{-1/2}$	$R_M^0 \times 10^6$ / $m^3\ mol^{-1}$
Glycine					
$w_1 = 0.001$[b]	41.22	-9.33	0.152	0.0028	–
$w_1 = 0.003$[b]	41.64	-10.85	0.156	0.0030	–
$w_1 = 0.005$[b]	42.08	-12.06	0.160	0.0032	–
L-Arginine					
$w_1 = 0.001$[b]	120.86	-15.71	0.522	0.102	35.95
$w_1 = 0.003$[b]	125.18	-19.90	0.538	0.106	35.96
$w_1 = 0.005$[b]	130.58	-25.55	0.586	0.118	35.97
L-Histidine					
$w_1 = 0.001$[b]	105.08	-38.05	0.502	0.045	32.01
$w_1 = 0.003$[b]	108.98	-40.06	0.528	0.087	32.02
$w_1 = 0.005$[b]	112.80	-42.37	0.548	0.099	32.03
Glycine					
$w_2 = 0.001$[b]	41.88	-10.25	0.152	0.0030	–
$w_2 = 0.003$[b]	42.12	-12.08	0.158	0.0033	–
$w_2 = 0.005$[b]	42.36	-14.50	0.164	0.0035	–
L-Arginine					
$w_2 = 0.001$[b]	128.84	-22.80	0.546	0.144	35.97
$w_2 = 0.003$[b]	131.98	-26.97	0.584	0.157	35.98
$w_2 = 0.005$[b]	135.18	-31.90	0.602	0.164	35.99
L-Histidine					
$w_2 = 0.001$[b]	113.02	-68.10	0.528	0.075	32.03
$w_2 = 0.003$[b]	116.28	-71.60	0.546	0.130	32.04
$w_2 = 0.005$[b]	119.50	-75.14	0.584	0.156	32.05

[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 10. Contributions of zwitterionic group (NH_3^+, COO^-), (CH), (CH_2) groups and end group 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 298.15 K [a]

Groups	$\phi^0_V \times 10^6$ / $m^3\ mol^{-1}$			*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]
(NH_3^+), (COO^-)	23.24	24.88	25.76	0.096	0.098	0.100
(CH)	8.99	8.38	8.16	0.028	0.029	0.030
(CH_2)	17.98	16.76	16.32	0.056	0.058	0.060
$(CH_2)_3$	53.94	50.28	48.96	0.168	0.174	0.180
	34.69	41.64	47.70	0.230	0.237	0.276
	54.87	58.96	62.56	0.322	0.343	0.358
	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]
(NH_3^+), (COO^-)	25.32	26.82	27.48	0.100	0.104	0.108
(CH)	8.28	7.65	7.44	0.026	0.027	0.028
(CH_2)	16.56	15.30	14.88	0.052	0.054	0.056
$(CH_2)_3$	49.68	45.90	44.64	0.156	0.162	0.168
	45.56	51.61	55.62	0.264	0.291	0.298
	62.86	66.51	69.70	0.350	0.361	0.392

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

Table 11. Solvation number (S_n) of the amino acids at different mass fractions of aqueous α and β-cyclodextrin respectively at 298.15 K[a]

	S_n		
	w_1=0.001 [b]	w_1=0.003 [b]	w_1=0.005 [b]
L-Arginine	4.59	4.62	4.66
L-Histidine	4.50	4.53	4.58
	w_2=0.001 [b]	w_2=0.003 [b]	w_2=0.005 [b]
L-Arginine	4.65	4.68	4.72
L-Histidine	4.56	4.60	4.65

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

Figures

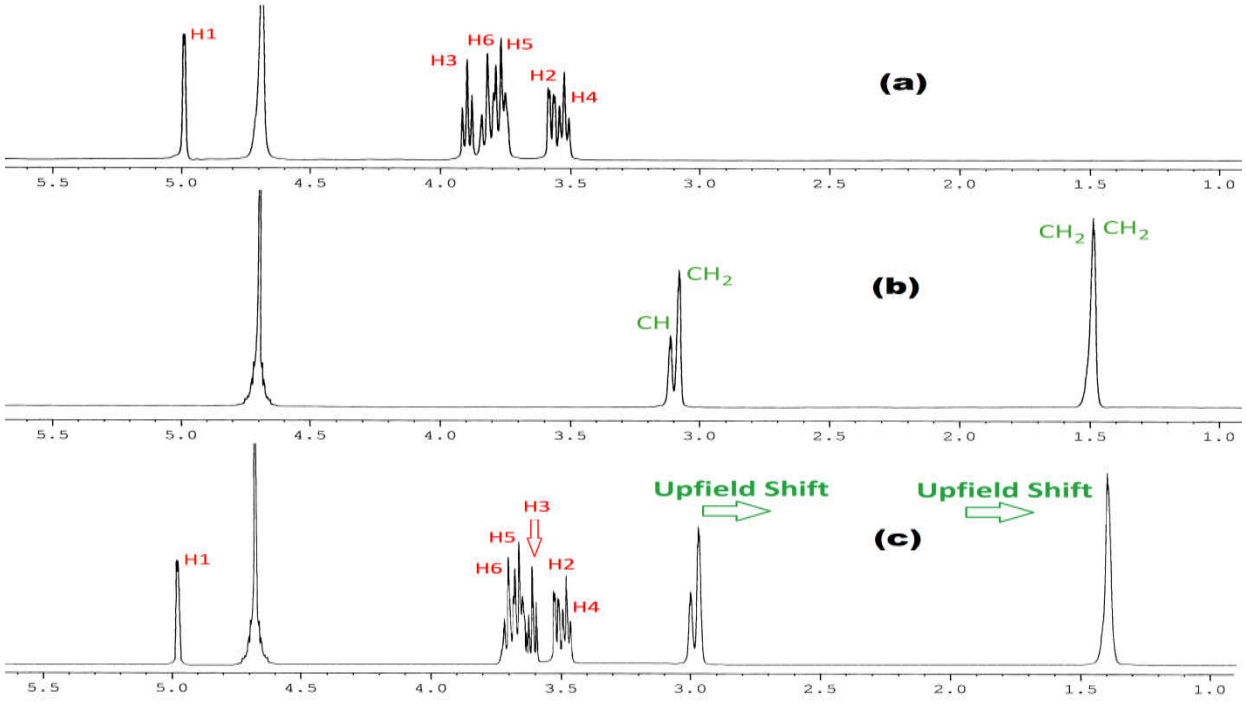


Figure 1. ^{1}H NMR spectra of (a) *α*-CD, (b) arginine and (c) 1:1 molar ratio of *α*-CD & arginine in D_2O at 298.15K.

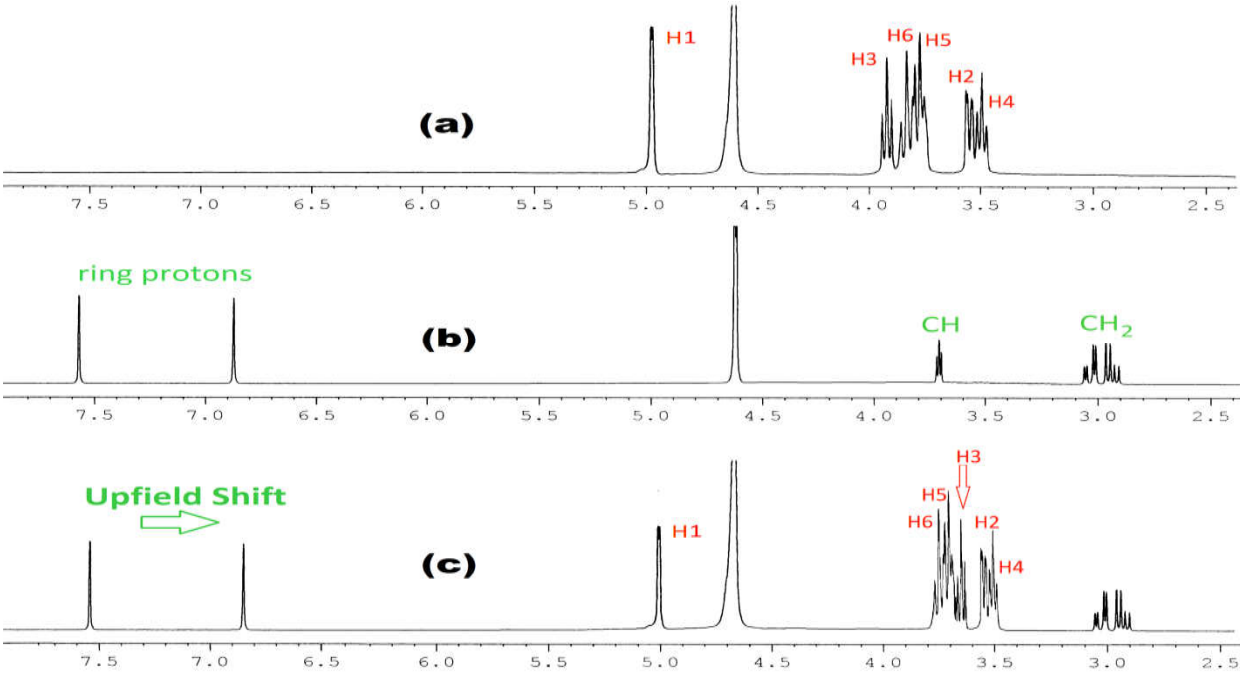


Figure 2. ^{1}H NMR spectra of (a) α-CD, (b) histidine and (c) 1:1 molar ratio of α-CD & histidine in D_2O at 298.15K.

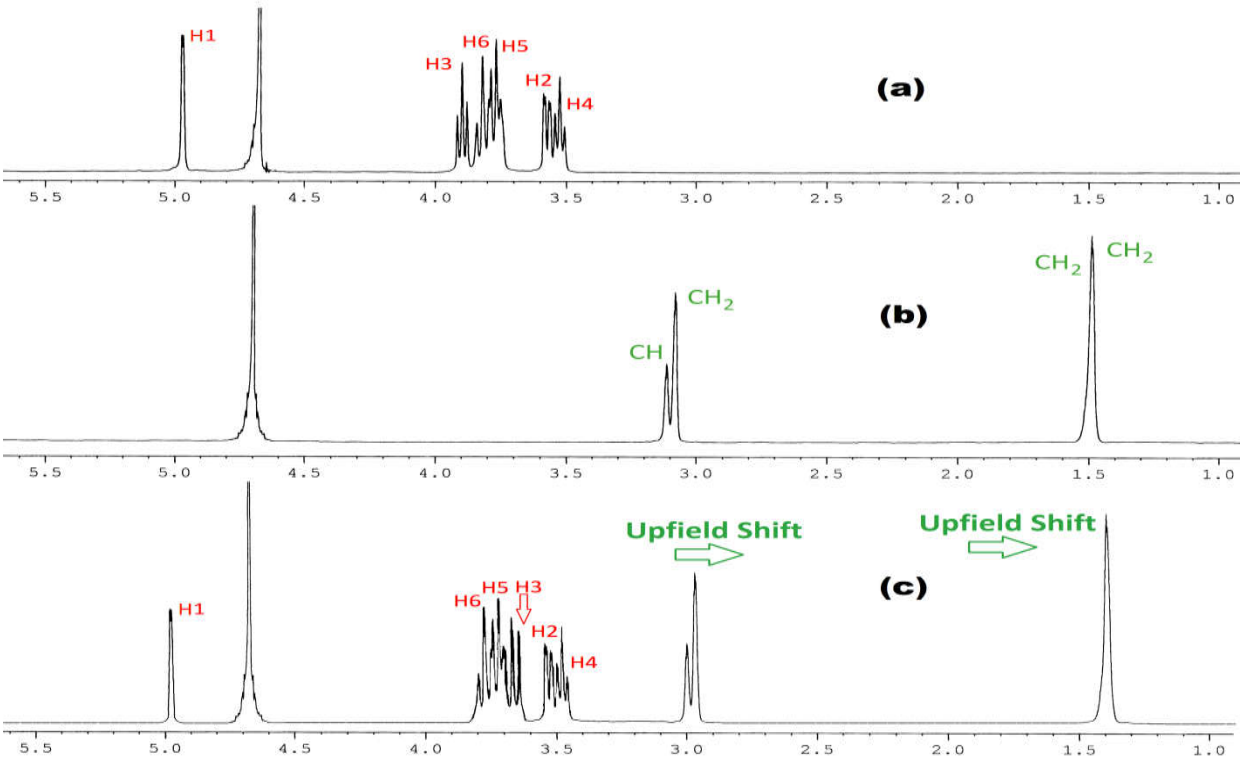


Figure 3. ^{1}H NMR spectra of (a) β-CD, (b) arginine and (c) 1:1 molar ratio of β-CD & arginine in D_2O at 298.15K.

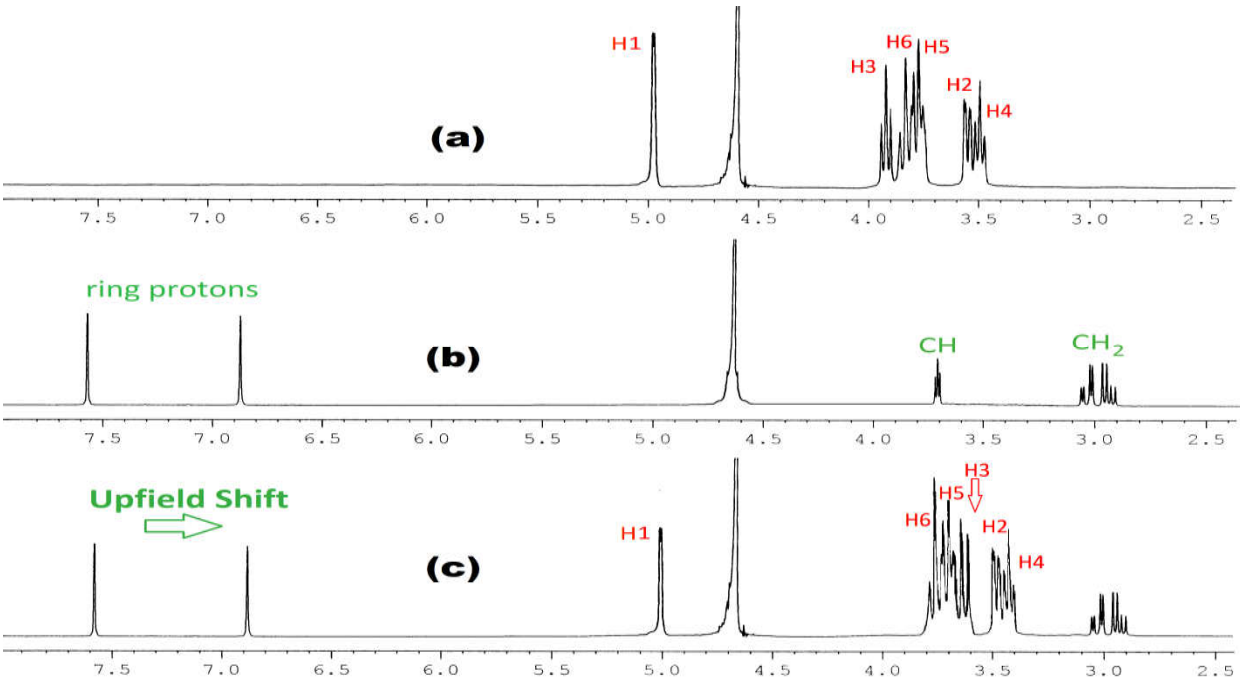


Figure 4. ^{1}H NMR spectra of (a) β-CD, (b) histidine and (c) 1:1 molar ratio of β-CD & histidine in D_2O at 298.15K.

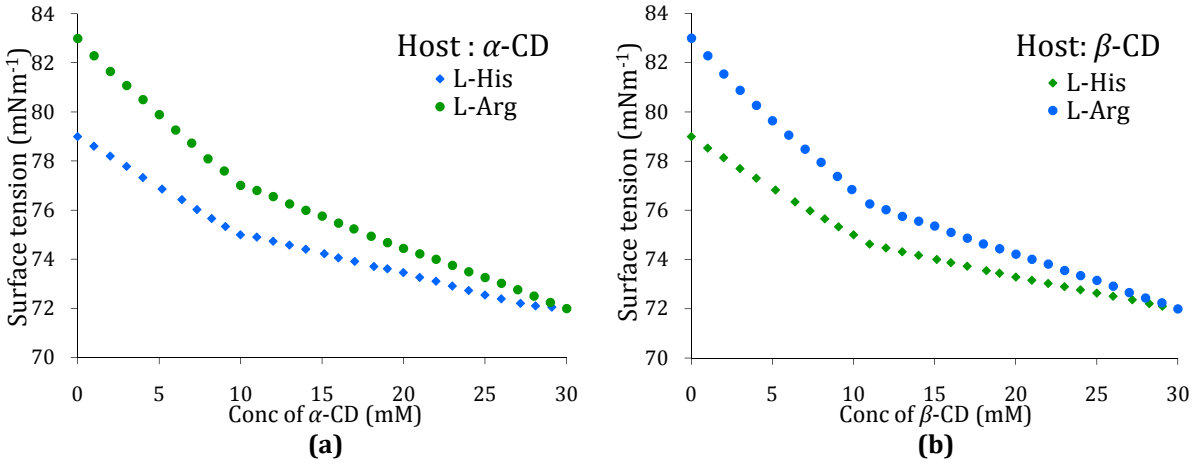


Figure 5. Variation of surface tension of L-arginine solution (10.0 mmol L^{-1}) and L-histidine solution (10.0 mmol L^{-1}) with increasing concentration of (a) α-cyclodextrin and (b) β-cyclodextrin respectively at 298.15 K.

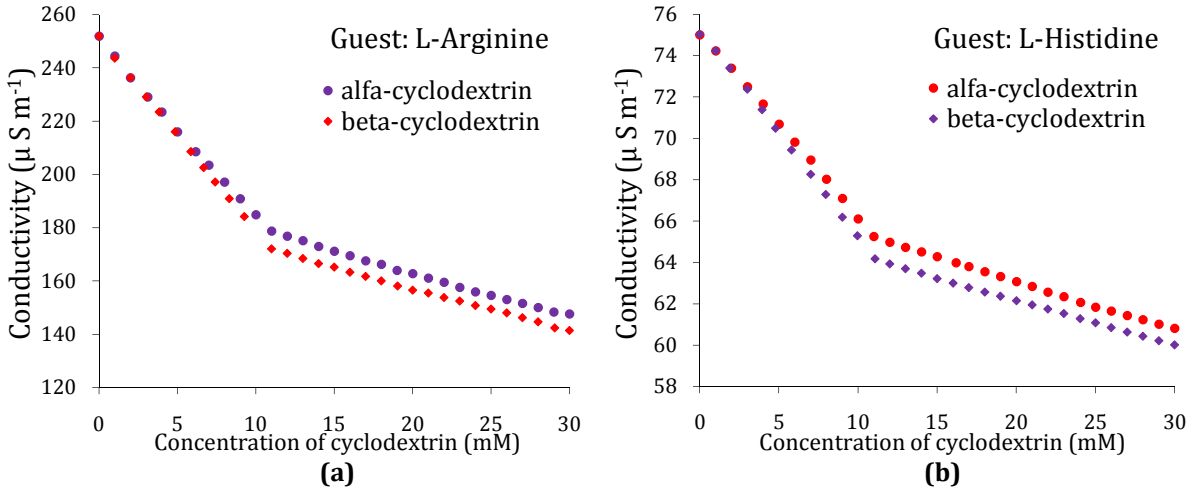


Figure 6. Variation of conductivity of aqueous (a) L-arginine solution (10.0 mmol L^{-1}) and (b) L-histidine solution (10.0 mmol L^{-1}) respectively with increasing concentration of *α* and *β*-cyclodextrin at 298.15 K.

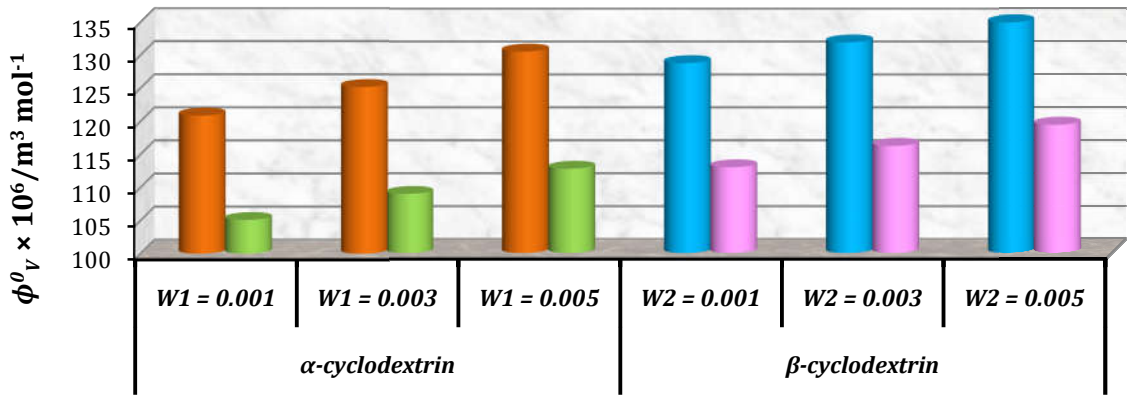


Figure 7. Plot of limiting molar volume (ϕ_V^0) against mass fraction (*w*) of aqueous *α*-CD and aqueous *β*-CD for L-arginine (orange & blue) and L-histidine (green & pink) respectively at 298.15 K.

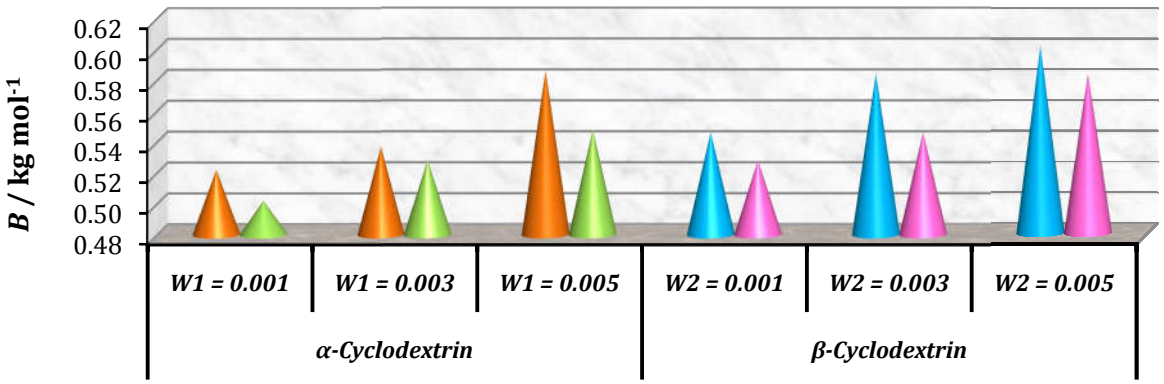


Figure 8. Plot of viscosity *B*-coefficient against mass fraction (*w*) of aqueous *α*-CD and aqueous *β*-CD for L-arginine (orange & blue) and L-histidine (green & pink) respectively at 298.15 K.

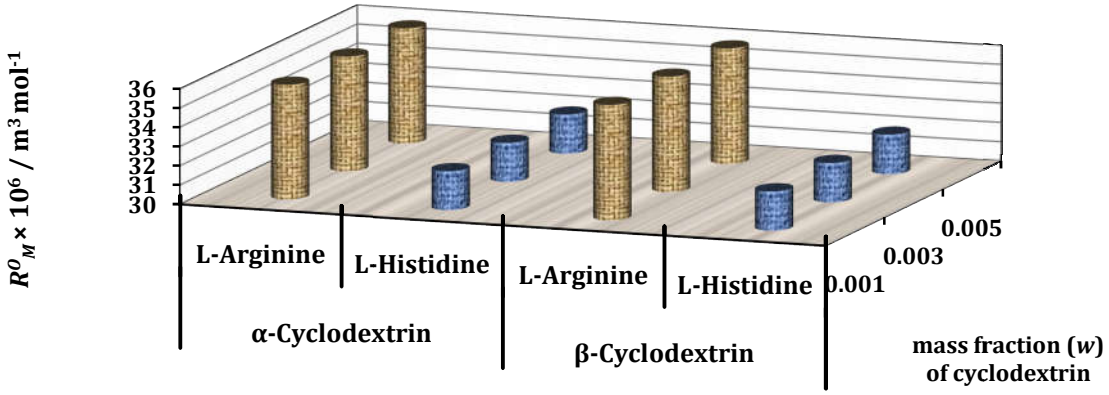
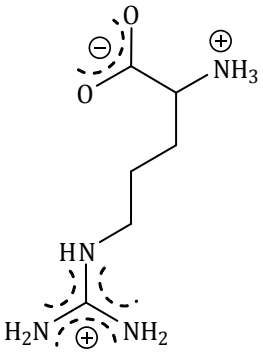
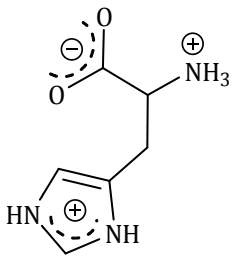


Figure 9. Plot of limiting molar refraction (R_M^0) for L-arginine and L-histidine in different mass fractions (*w*) of aqueous *α*-CD and aqueous *β*-CD respectively at 298.15 K.

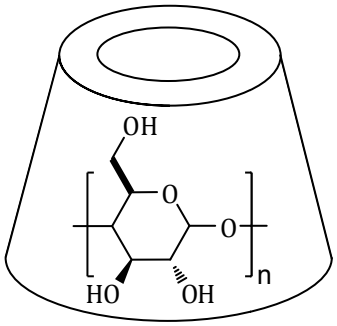
Schemes



L-Arginine

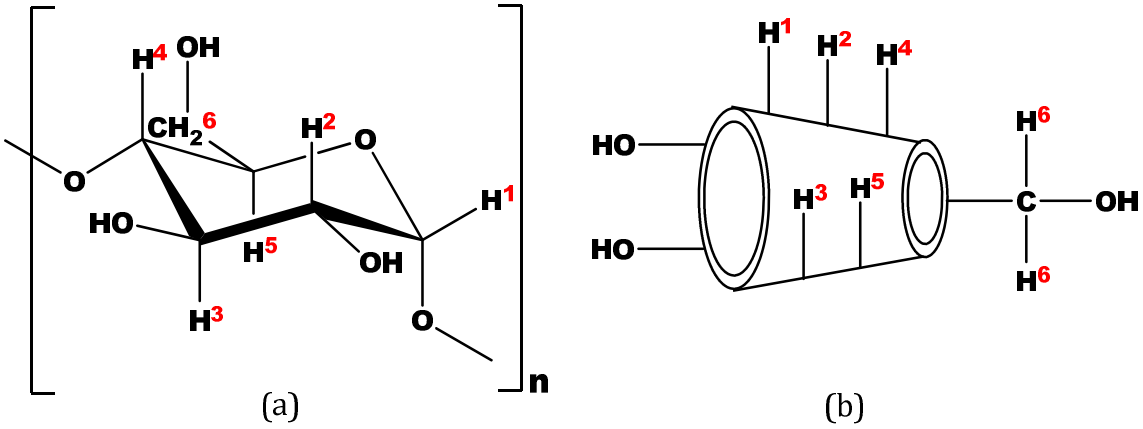


L-Histidine

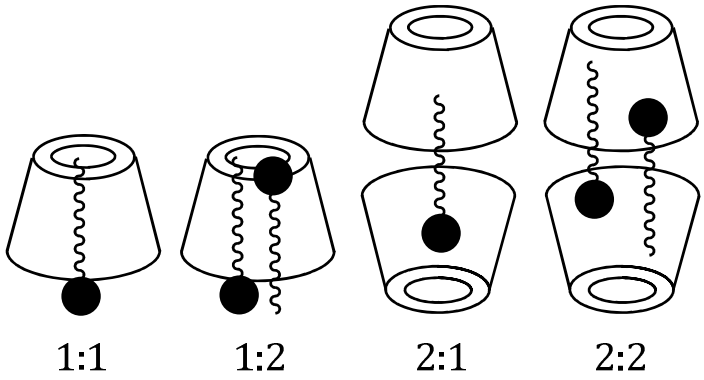


Cyclodextrin
(n = 6, 7 for *α*-CD and *β*-CD respectively)

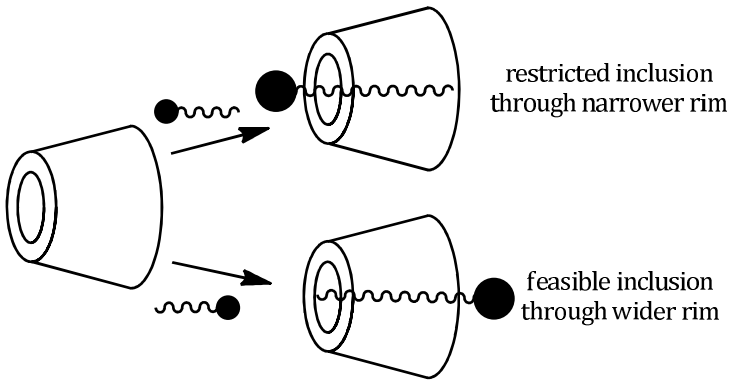
Scheme 1. Molecular structure of the *α*-amino acids in aqueous solution and the structure of cyclodextrin host molecule.



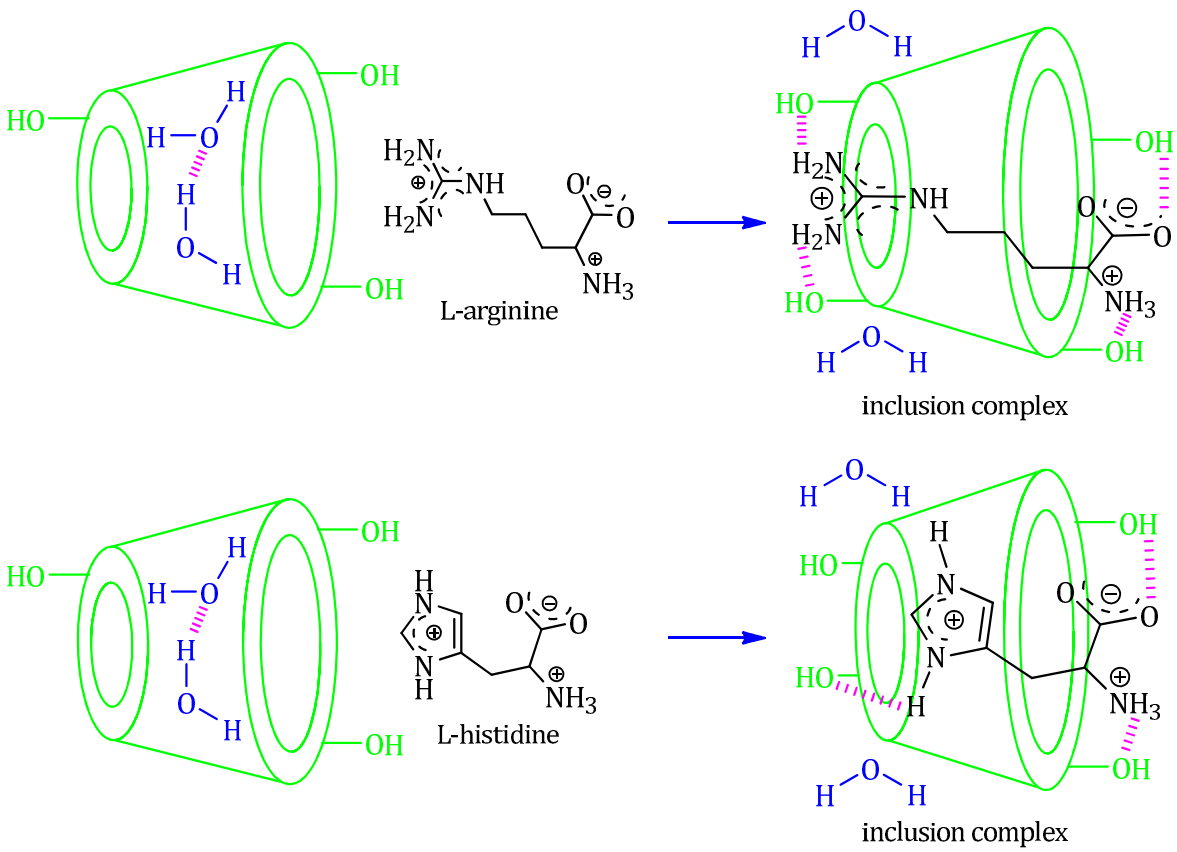
Scheme 2. (a) Stereo-chemical configuration and (b) truncated conical structure of *α* and *β*-cyclodextrins.



Scheme 3. Various possibilities of host-guest ratio for inclusion complex.



Scheme 4. Feasible and restricted inclusion of the guest into the host molecule.



Scheme 5. Schematic representation of mechanism for the formation of 1 : 1 inclusion complex of the α-amino acids with both α and β-cyclodextrin molecule.