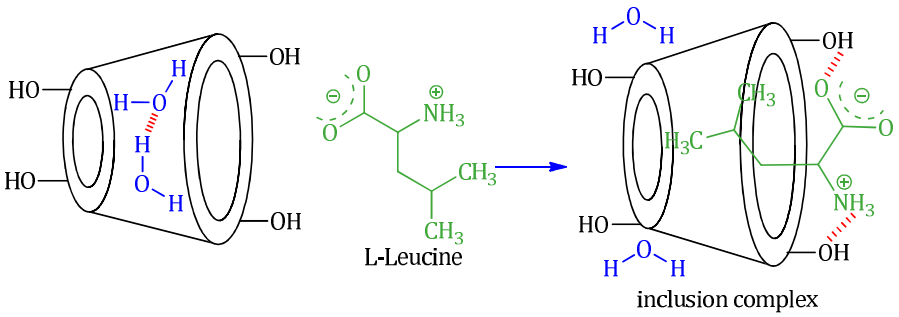
CHAPTER IV

Probing inclusion complexes of cyclodextrins with amino acids by physicochemical approach

Formations of host-guest inclusion complexes of two natural amino acids, *viz.*, L-Leucine and L-Isoleucine as guests with α and β-cyclodextrins have been investigated which include diverse applications in modern science such as controlled delivery in the field of pharmaceuticals, food processing etc. Surface tension and conductivity studies establish the formation of inclusion complexes with 1:1 stoichiometry. The interactions of cyclodextrins with amino acids have been supported by density, viscosity, refractive index, hydration and solvation number measurements indicating higher degree of inclusion in case of α-cyclodextrin. L-Leucine interacts more with the hydrophobic cavity of cyclodextrin than its isomer. With the help of stability constant by NMR titration, hydrophobic effect, H-bonds and structural effects the formations of inclusion complexes have been explained.



IV. 1. Introduction

Cyclodextrins (CD) are the members of cyclic oligosaccharide family containing six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units, which are bound by α-(1–4) linkages [1, 2]. CDs have a torus-shaped ring structure with polar hydrophilic rims and relatively hydrophobic inner cavity (Scheme IV.1) [3]. Due to this type of unique structure they can build up host–guest inclusion complexes (ICs) with various small molecules having hydrophobic moiety, e.g., vitamins, amino acids, ionic liquids etc [4, 5]. The hydrophobic part of the guest molecule is accommodated into the hydrophobic cavity of CD whereas the polar part of the guest (if present) makes association with the polar rims resulting stabilisation of the IC [6]. For this reasons CDs have vast applications in the field of pharmaceuticals, pesticides, foodstuffs, toilet articles, textile processing industry, supramolecular host-guest chemistry, molecular encapsulation etc [7, 8]. CDs form stable host-guest ICs with essential amino acids e.g., arginine, histidine, lysine, phenyl alanine, glutamic acid [9, 10] ionic liquids e.g., 1-butyl-4-methylpyridinium iodide [11, 12], RNA nucleosides [13] etc. as guest molecules.

In the present study we have attempt to ascertain the nature of formation of ICs of α and β-CD with two natural α-amino acids, *i.e.*, L-Leucine (L-Leu) and L-Isoleucine (L-Ile) in 0.001, 0.003, 0.005 mass fractions of α and β-cyclodextrins in aqueous media. Aim of this work is to explore the formation, carrying and controlled release of the two essential amino acids by forming IC with host CDs without chemical & biological modification of the guests.

L-Leu is used in the biosynthesis of proteins and is essential in humans, *i.e.*, our body cannot synthesize it and thus it must be incorporated from outside, which may be done using α and β-CD as carriers [14, 15]. It is a major component of the subunits in ferritin, astacin etc. proteins and L-Leu is an activator of mTOR; it is the only dietary amino acid that has the capacity to directly stimulate muscle protein synthesis [16, 17]. On the other hand L-Ile is used in the biosynthesis of proteins and it is also essential in humans. L-Ile is synthesized from pyruvate

employing leucine biosynthesis enzymes in other organisms such as bacteria [18, 17].

Here, the nature of the ICs and their interactions have been studied by surface tension, conductivity, 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 two amino acids. NMR titrations have also been done by ^{1}H NMR spectroscopy to confirm the inclusion phenomenon and the binding constants have been calculated from the titration by using Benesi–Hildebrand method [19].

IV. 2. Experimental Section

IV. 2. 1. Source and Purity of Samples

The above mentioned two amino acids and CDs of puriss grade were purchased from Sigma-Aldrich, Germany and used as it was. The mass fraction purity of L-Leu, L-Ile, α-CD and β-CD were ≥ 0.98, 0.98, 0.98 and 0.98 respectively.

IV. 2. 2. Apparatus and Procedure

Solubilities of the two CDs and that of the above two α-amino acids in aqueous CDs have been verified in triply distilled, deionized and degassed water. It was detected that these were quite soluble in aqueous CDs. All the stock solutions of L-Leu and L-Ile were prepared by mass (Mettler Toledo AG-285 with uncertainty 0.0001 g) and the working solutions were got by mass dilution at 298.15 K. Changes of molarity to molality were done using the densities of the solutions [20]. Sufficient precautions were made to decrease the evaporation during mixing.

pH values were measured by Mettler Toledo Seven Multi pH meter having uncertainty ±0.001. It was studied in a water bath with thermostat maintaining the temperature at 298.15 K, having uncertainty in temperature ±0.01 K.

Surface tensions of the solutions were determined by platinum ring detachment technique using a Tensiometer (K9, KRŰSS; Germany) at 298.15 K. Accuracy of the study was ±0.1 mN m ^{1}. Temperature of the system was maintained by circulating thermostated water through a double-wall glass vessel holding the solution.

Conductivities of the solutions were studied by Mettler Toledo Seven Multi conductivity meter having uncertainty 1.0 μSm^{-1}. The study was carried out in a thermostated water bath at 298.15K with uncertainty ±0.01K. HPLC grade water was used with specific conductance 6.0 μS m^{-1}. The conductivity cell was calibrated using 0.01M aqueous KCl solution.

The densities (ρ) of the solutions were studied by vibrating *U*-tube Anton Paar digital density meter (DMA 4500M) having precision ±0.00005 g cm^{-3} and uncertainty in temperature was ±0.01K. The density meter was calibrated by standard method [20].

Viscosities (η) were determined by Brookfield DV-III Ultra Programmable Rheometer with spindle size 42. The detail has already been depicted before [20].

Refractive indexes of the solutions were studied with a Digital Refractometer from Mettler Toledo having uncertainty ±0.0002 units. The detail has already been described before [20].

^{1}H NMR spectra were recorded in D_2O at 300 MHz using Bruker ADVANCE 300 MHz instrument at 298 K. 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. In each titration initially 0.5 mL 1.0 mM amino acid solution was taken and then 10 µL 10 mM CD solution was added into it at five several times.

IV. 3. Result and Discussion

IV. 3.1. Surface tension study illustrates the inclusion and the stoichiometric ratio of the inclusion complexes

Surface tension (γ) measurement can be used to obtain valuable information about the formation of inclusion complex in CDs [9, 21]. It is known that γ for aqueous solutions of pure *α* and *β*–CD don't show any remarkable change with increasing concentrations [13]. The pH data of both these amino acids show the existence of NH_3^+ and COO^- in their zwitterionic forms. Thus the side groups being nonpolar, both the amino acids show surfactant like behavior, *i.e.*, there is considerable decrease in surface tension of their aqueous solutions. Therefore while amino acids make ICs with CDs remarkable change in surface tension should be observed [10].

Here γ of aqueous amino acids has been measured with increasing concentration of α and *β*-CD at 298.15K (Table IV.1-IV.4). Both L-Leu and L-Ile showed increasing trend of γ with increasing concentration of α and *β*-CD (Figure. IV.1) may be because of removal of the amino acid molecules (surface active) from the surface of the solution into the hydrophobic cavity of α and *β*-CD forming host-guest inclusion complexes (Scheme IV. 2) [22]. Each plot also indicates that there is single break point at certain concentrations. Finding of break point in surface tension curve not only indicates formation of IC but also gives information about its stoichiometry, *i.e.*, appearance of single, double and so on break point in the plot indicates 1:1, 1:2 and so on stoichiometry of host:guest ICs (Scheme IV.3) [23, 8].

The values of γ with corresponding concentrations of α and *β*-CD and the concentration of amino acid at each break have been listed in table IV.5 and the overall variation of γ value is shown in table IV.1-IV.4, which clearly reveal that the breaks have been found at certain concentrations of amino acids and CD where their concentration ratio in the solution was almost 1:1, thus this study proves formation of 1:1 ICs of the studied amino acids with both α and *β*-CD.

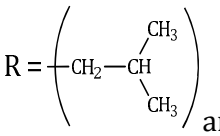
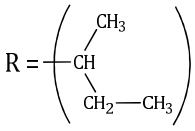
IV. 3.2. Conductivity study explains the inclusion process and their stoichiometric ratio

With the help of conductivity (κ) study the inclusion phenomenon can be confirmed [24, 25]. It also suggests the stoichiometry of the inclusion complex formed [10]. As found from pH data the amino acids exist in ionic form in aqueous solution, thus show considerable value of κ. As aqueous CD solution was added to the aqueous solution of an amino acid (table IV.1-IV.4), the κ was observed to show decreasing trend probably because of encapsulation of the amino acid molecules inside the cavity of CD (Scheme IV. 2). At certain concentrations of both CD and amino acid single break was found in each of the conductivity curve (Figure. IV.2), which indicates the formation of ICs. The values of κ and corresponding concentrations of both α and *β*-CD with the concentration of each amino acid at each break have been listed in table IV.5, which reveal that the ratio of the concentrations of each amino acid and CD at the break point was found to be approximately 1:1, suggesting the host-guest ratio to be 1:1 [10]. In this study of all the four cases of L-Leu and L-Ile with *α* and *β*-CD similar results are found, but the conductivity near the break is found to be a little lower for *α*-CD than *β*-CD, which is might be due to the former is better host for the two studied guests than the later.

IV. 3.4. Density study: group contributions and interactions between amino acids and cyclodextrins

The interactions between amino acids and cyclodextrins can be studied from the apparent molar volume (ϕ_v) and limiting apparent molar volume (ϕ_v^o) [9]. The apparent molar volume is the measure of the sum of geometric volume of the central solute molecule and changes in the solvent volume due to the interactions with the solute around the co-sphere [26]. ϕ_v can be calculated from the density of the solutions at 298.15 K (Table IV.8) using the provided equation. The magnitude of ϕ_v is found to be positive for both the two amino acids in both aqueous α and β-CD and this suggests strong solute–solvent interactions. ϕ_v varies linearly with the square root of molal concentration ($\sqrt{m}$) and is fitted to

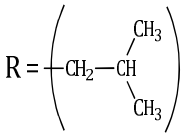
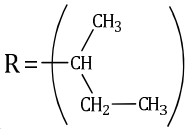
the masson equation, from where ϕ_v^o has been determined (Table IV.11, Figure. IV.3) [27]. The values of ϕ_v^o increase with the increase of mass fractions of α and β-CD for both L–Leu and L-Ile indicating that the ion-hydrophilic group interactions are stronger than ion-hydrophobic group interactions. In the present ternary system (amino acid + aq. CD) the interactions of the charged groups (COO^- and NH_3^+) of the amino acids is localized with -OH groups of cyclodextrins. Due to this interaction the electostriction of water results an increase in volume. In this study the ϕ_v^o are measured for glycine, L–Leu and L-Ile at 298.15K for different mass fractions (0.001, 0.003, 0.005 M) of α and β-CD. From Figure. IV.3 it can be observed that ϕ_v^o for L-Leu is greater than that of L-Ile. This can be explained on the basis of their structures. The side group of L-Ile is relatively more spherical than that of L-Leu, creating less surface area of the former than the later. Thus, because of more surface area of the side hydrophobic group of L-Leu, it interacts better with the hydrophobic cavity of CD, which is reflected in the ϕ_v^o values in figure IV.3.

The structures of L-Leu and L-Ile can be obtained by replacing of the H atom of glycine by  and  groups respectively. Due to the similarity in structure of the two amino acids there must be a correlation in their ϕ_v^o values. The contributions of different groups present in L-Leu and L-Ile towards ϕ_v^o in different mass fractions of α and β-CD have been estimated (Table IV.12) [28, 26].The contribution of zwitterionic group (NH_3^+,COO^-) is in the range of 25.36–26.88× 10^{-6} m^3 mol^{-1} and 25.68–27.06 × 10^{-6} m^3 mol^{-1} for α and β-CD respectively. The higher value in case of β-CD suggests that the interaction of the zwitterionic groups of two amino acids is more with β-CD than α-CD, probably because of more number of –OH groups in β-CD than α-CD. The contribution of the hydrophobic side group (R) of both L-Leu and L-Ile is found grater for α-CD than that for β-CD, which suggests that the inclusion phenomenon is better in α-CD than that in β-CD. This may be explained on the

basis of more compact structure of the IC in case of α-CD than that in β-CD due to smaller cavity size of the former than the later [10].

IV. 3.5. Viscosity study: group contributions

The viscosity of aqueous cyclodextrin solution increases with increasing mass fraction of *α* or *β*-CD (Table IV.6) due to structure making contribution of cyclodextrins with water molecules. For the ternary system (amino acid+aq. CD) the viscosity of the solution increases with the increasing molarity of amino acids (Table IV.7). The viscosity *B*-coefficient indicates the solute-solvent interactions, which are found to be all positive and increase with the increasing concentrations of *α* and *β*-CD (Table IV.11, Figure. IV.4). This is considered to arise due to increasing amino acid–CD interaction [9]. The viscosity *B*-coefficients for L-Leu is higher than L-Ile because in case of the former the solute solvent interaction is more than the later.

In the present study the contributions of the zwitterionic groups and the side groups towards the viscosity *B*-coefficient for the two amino acids L–Leu and L-Ile of different mass fractions of α and β-CD have been determined (Table IV.12) [26]. The contributions of the zwitterionic group (NH_3^+, COO^-) 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  and  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. The contribution of viscosity *B*-coefficient for the side group (R) is found to be grater for L–Leu than that for L-Ile, which may be explained on the basis of more surface area of the former than the later, thereby making grater hydrophobic interactions with the hydrophobic inner surface of CD.

IV. 3.6. pH measurement proves the ionic states of the amino acids

Existence of zwitterionic forms of amino acids in aqueous solution can be understood with the help of pH measurement [10]. The values of pH for L-Leu in aqueous α and β-CD ranges from 5.89 to 5.14 and 5.75 to 5.13 respectively at 298.15 K, while for L-Ile it ranges from 6.14 to 5.39 and 5.85 to 5.38 for α and β-CD respectively at the same temperature (Table IV.6, IV.7). The pH value decreases with the increasing concentration of the respective amino acids and also with the increase of concentration of α and β-CD for both the two amino acids. These values clearly show the variation in their zwitterionic forms, *i.e.*, the amine and carboxylic acid groups exist in ionic forms $-NH_3^+$ and $-COO^-$ respectively.

IV. 3.7. Refractive index show the compactness of the inclusion complexes

The molecular interactions taking place in solution systems can be studied with the help of refractive index (n_D) and molar refraction (R_M) [9]. The higher value of R_M and the limiting molar refraction (R^0_M) indicate that the medium is more compact and dense (Table IV.11) [10]. In this study the R^0_M value increases with increasing concentration of α and β-CD. It is evident from fig. IV.5 that the ICs of L-Leu with both α and β-CD are denser or closely packed than those of L-Ile, may be due to greater hydrophobic interaction between L-Leu and both the cyclodextrins. These data obtained from refractive index study are in good agreement which have been found from density and viscosity measurements.

IV. 3.8. Hydration number and solvation number: solvation by cyclodextrin molecule

In the present ternary system effect of the solvent is a key feature. Here, water act only as the medium for the amino acids and CDs to interact between them. Thus, CDs have crucial role and it is termed as the co-solvent. The effect of this co-solvent is studied in terms of solvation number (S_n), which is the measure

of the solvation taking place between the primary or secondary hydroxyl groups of cyclodextrins and zwitterionic groups of amino acids; and the order of encapsulation of the side group by CD molecule [9, 13].The hydration number (n_H) is the order of hydration by water molecules surrounding the amino acids in the free state. It can be observed from table IV.13 that the values of hydration number decreases whereas the solvation number increases with increasing mass fraction of α and β-CD for both L-Leu and L-Ile. The trend in the S_n and n_H value suggests that in the ternary solution system the electrostriction of water diminishes with increasing mass fraction of CD [26]. In this study the solvation number is more for L-Leu than L-Ile due to more hydrophobic part of the former than the later. L–Leu is more solvated, *i.e.*, the encapsulation of hydrophobic part inside the cavity of CD is greater. Lower hydration numbers as well as higher the solvation numbers in α-CD than β-CD for the two studied amino acids further suggests that α-CD is more fascinated for solvation than β-CD.

IV. 3.9. ^{1}H NMR titration: determination of binding constant

Inclusion of a guest molecule into the cavity of CD results in the chemical shift of the guest and CD in the ^{1}H NMR spectra due to the interaction of the CD with the guest molecule [19]. In the structure of CD the H3 and H5 hydrogens are situated inside the conical cavity, particularly, the H3 are placed near the wider rim while H5 are placed near the narrower rim, the other H1, H2 and H4 hydrogens are located at the exterior of the CD molecule (Scheme IV.1) [29,30]. Here, we have titrated the amino acid molecules by adding CD and observed the chemical shift changes (Δδ) of the concerned protons [31, 32]. The chemical shift (δ) of the H3 of CD was found to be shifted in each of the four cases, indicating the guest amino acid molecules enter into the cavity of CD through the wider rim [33]. But for simplicity and to find the binding constant with the help of reliable Benesi–Hildebrand method for 1:1 host-guest ICs (equation 1), [19, 34]. we used the Δδ of the CH_3 groups which are situated at the terminal of the hydrophobic moiety of the amino acids (Scheme IV.1).

$$\frac{1}{\delta} = \frac{1}{k'[AA]K_b} \quad \frac{1}{[CD]} + \frac{1}{k'[AA]}$$

(1)

Fig. IV.6 and IV. 7 shows the titration spectra of L-Leu and L-Ile with α-CD respectively (Figure. IV.8 and Figure. IV.9 for β-CD). Each titration spectra clearly shows the shift of signals arising from H3 of CD and that of the CH_3 of the amino acids. The details of the titration and finding out of binding constant (K_b) are shown in table IV.14-IV.17. The values of K_b for each of the ICs were evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot (Figure. IV.10), [35]. The binding constants indicate that L-Leu forms stronger IC than L-Ile and α-CD forms stronger IC than β-CD (Table IV.13).

IV. 3.10. Structural influence of cyclodextrins

Cyclodextrin is extensively used as host molecules because of its hydrophobic cavity and hydrophilic rims which provide an appropriate environment for the apolar part of a guest molecule to reside inside the cavity and the polar part makes association with the polar rims, thereby stabilizing the whole IC [1,7]. The lipophilic cavity diameter of *α* and *β*-CD is 4.7-5.3Å and 6.0-6.5Å respectively [16]. The two studied amino acid molecules L-Leu and L-Ile have the diameter within this range and can be encapsulated inside the cavity of CD [34]. No covalent bonds are broken or formed during formation of the ICs. Another factor responsible for the formation of ICs is that polar water molecules are present inside the slightly apolar cavity of cyclodextrin. This is generally energetically unfavoured [4].So, the water molecules are readily substituted by the hydrophobic moiety of the amino acids. This results in a more stable lower energy state. The stoichiometry of the inclusion complex is found as 1:1, which is supported by both surface tension and conductivity measurements. This may be explained as after inclusion of one amino acid molecule the zwitterionic part blocks the rim, so, it would be difficult for a second molecule to be inserted into the cavity [16]. Insertion of the guest amino acid molecule was found through the

wider rim of the CD, so as to make maximum contact of the hydrophobic side groups of the amino acids with the hydrophobic cavity of *α* and *β*-CD [19].

IV. 4. Conclusion

The two amino acids *viz.*, L-Leu and L-Ile form host-guest inclusion complexes with both *α* and *β*-CD with 1:1 stoichiometry which is confirmed by NMR, surface tension and conductivity measurement. The amino acid-CD interactions in the solution have been interpreted by density, viscosity, refractive index and solvation number measurements. Thus the present work adds a dimension in the field of contemporary science of controlled delivery of these two amino acids by using *α* and *β*-CD.

Tables:

Table IV. 1. Data for surface tension and conductivity study of aqueous L-Leucine-*α*-CD system at 298.15K[a]

Volm of *α*-CD (mL)	Total volm (mL)	Conc of L-Leucine (mM)	Conc of *α*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (μS m^{-1})
0	10	10.000	0.000	65.0	170.2
1	11	9.091	0.909	66.0	163.8
2	12	8.333	1.667	66.9	159.5
3	13	7.692	2.308	67.6	156.1
4	14	7.143	2.857	68.3	153.1
5	15	6.667	3.333	68.9	150.6
6	16	6.250	3.750	69.4	148.4
7	17	5.882	4.118	69.9	146.2
8	18	5.556	4.444	70.3	144.8
9	19	5.263	4.737	70.7	143.7
10	20	5.000	5.000	71.1	142.7
11	21	4.762	5.238	71.2	142.4
12	22	4.545	5.455	71.3	142.1
13	23	4.348	5.652	71.4	141.7
14	24	4.167	5.833	71.5	141.5
15	25	4.000	6.000	71.6	141.2
16	26	3.846	6.154	71.7	140.9
17	27	3.704	6.296	71.8	140.7
18	28	3.571	6.429	71.9	140.4
19	29	3.448	6.552	71.9	140.3
20	30	3.333	6.667	71.9	140.1

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

Table IV. 2. Data for surface tension and conductivity study of aqueous L-Leucine-*β*-CD system at 298.15K[a]

Volm of *β*-CD (mL)	Total volm (mL)	Conc of L-Leucine (mM)	Conc of *β*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (μS m^{-1})
0	10	10.000	0.000	65.0	170.1
1	11	9.091	0.909	65.9	164.7
2	12	8.333	1.667	66.8	161.3
3	13	7.692	2.308	67.5	158.5
4	14	7.143	2.857	68.2	155.7
5	15	6.667	3.333	68.8	153.7
6	16	6.250	3.750	69.3	151.6
7	17	5.882	4.118	69.8	149.9
8	18	5.556	4.444	70.2	148.9
9	19	5.263	4.737	70.5	147.7
10	20	5.000	5.000	70.9	146.7
11	21	4.762	5.238	71.2	146.2
12	22	4.545	5.455	71.4	145.8
13	23	4.348	5.652	71.5	145.4
14	24	4.167	5.833	71.6	145.1
15	25	4.000	6.000	71.7	144.8
16	26	3.846	6.154	71.8	144.6
17	27	3.704	6.296	71.8	144.3
18	28	3.571	6.429	71.9	144.0
19	29	3.448	6.552	71.9	143.7
20	30	3.333	6.667	71.9	143.5

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

Table IV. 3. Data for surface tension and conductivity study of aqueous L-Isoleucine-*α*-CD system at 298.15K[a]

Volm of *α*-CD (mL)	Total volm (mL)	Conc of L-Isoleucine (mM)	Conc of *α*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (μS m^{-1})
0	10	10.000	0.000	65.2	152.3
1	11	9.091	0.909	66.3	147.2

2	12	8.333	1.667	67.2	142.9
3	13	7.692	2.308	67.9	138.4
4	14	7.143	2.857	68.6	135.0
5	15	6.667	3.333	69.1	131.8
6	16	6.250	3.750	69.6	129.1
7	17	5.882	4.118	70.1	126.2
8	18	5.556	4.444	70.5	123.7
9	19	5.263	4.737	70.9	121.8
10	20	5.000	5.000	71.2	120.1
11	21	4.762	5.238	71.3	119.6
12	22	4.545	5.455	71.4	119.3
13	23	4.348	5.652	71.5	119.0
14	24	4.167	5.833	71.6	118.6
15	25	4.000	6.000	71.7	118.4
16	26	3.846	6.154	71.7	118.0
17	27	3.704	6.296	71.8	117.7
18	28	3.571	6.429	71.8	117.5
19	29	3.448	6.552	71.9	117.3
20	30	3.333	6.667	71.9	117.0

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

Table IV. 4. Data for surface tension and conductivity study of aqueous L-Isoleucine-*β*-CD system at 298.15K[a]

Volm of *β*-CD (mL)	Total volm (mL)	Conc of L-Isoleucine (mM)	Conc of *β*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (μS m^{-1})
0	10	10.000	0.000	65.2	152.1
1	11	9.091	0.909	66.2	146.8
2	12	8.333	1.667	67.1	142.8
3	13	7.692	2.308	67.8	139.5
4	14	7.143	2.857	68.6	137.0
5	15	6.667	3.333	69.1	134.4
6	16	6.250	3.750	69.6	132.0
7	17	5.882	4.118	70.0	129.9
8	18	5.556	4.444	70.5	128.0
9	19	5.263	4.737	70.8	126.5

10	20	5.000	5.000	71.2	125.0
11	21	4.762	5.238	71.3	124.7
12	22	4.545	5.455	71.4	124.2
13	23	4.348	5.652	71.5	123.8
14	24	4.167	5.833	71.6	123.5
15	25	4.000	6.000	71.6	123.2
16	26	3.846	6.154	71.7	122.9
17	27	3.704	6.296	71.7	122.6
18	28	3.571	6.429	71.8	122.3
19	29	3.448	6.552	71.8	122.0
20	30	3.333	6.667	71.8	121.8

Table IV. 5. Values of surface tension (γ) at the break point with corresponding concentrations of cyclodextrins and amino acids and values of conductivity (κ) at the break point with corresponding concentrations of cyclodextrins and amino acids at 298.15 K [a]

	Conc of α-CD /mM	Conc of amino acid /mM	γ [a] /mN·m^{-1}
L-Leucine	5.01	4.99	71.1
L-Isoleucine	5.06	4.94	71.2
	Conc of β-CD /mM	Conc of amino acid /mM	γ [a] /mN·m^{-1}
L-Leucine	5.13	4.87	71.2
L-Isoleucine	5.17	4.83	71.3
	Conc of α-CD /mM	Conc of amino acid /mM	κ [a] /μS·m^{-1}
L-Leucine	4.78	5.22	143
L-Isoleucine	5.01	4.99	120
	Conc of β-CD /mM	Conc of amino acid /mM	κ [a] /μS·m^{-1}
L-Leucine	4.82	5.18	147
L-Isoleucine	5.03	4.97	125

[a] Standard uncertainties (u): temperature: u(T) = ±0.01 K, surface tension: $u(\gamma)$ = ±0.1 mN m ^{1} , conductivity: $u(\kappa)$ = ±1.0 μS·m^{-1}.

Table IV.6. 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]

Aqueous solvent mixture	$\rho\times10^{-3}$ /kg m^{-3}	η /mP s	n_D	pH
aq. *α*-CD				
w_1 = 0.001	0.99735	1.29	1.3329	6.65
w_1 = 0.003	0.99802	1.30	1.3332	6.61
w_1 = 0.005	0.99868	1.31	1.3335	6.56
aq. *β*-CD				
w_2 = 0.001	0.99755	1.30	1.3328	6.57
w_2 = 0.003	0.99819	1.31	1.3331	6.53
w_2 = 0.005	0.99895	1.32	1.3334	6.51

[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 IV. 7. 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-Leucine									
w_1 = 0.001[b]					w_2 = 0.001[b]				
0.010037	0.99766	1.30	1.3336	5.89	0.010036	0.99776	1.320	1.3334	5.90
0.025121	0.99845	1.31	1.3337	5.83	0.025130	0.99809	1.330	1.3335	5.83
0.040241	0.99925	1.32	1.3339	5.73	0.040274	0.99844	1.350	1.3336	5.72
0.055384	1.00028	1.34	1.3341	5.62	0.055467	0.99879	1.360	1.3337	5.61
0.070550	1.00138	1.35	1.3343	5.52	0.070709	0.99916	1.380	1.3338	5.51
0.085742	1.00250	1.36	1.3346	5.41	0.086000	0.99952	1.400	1.3339	5.41
w_1 = 0.003[b]					w_2 = 0.003[b]				
0.010030	0.99830	1.32	1.3339	5.77	0.010030	0.99837	1.33	1.3338	5.76

0.025105	0.99910	1.33	1.3340	5.65	0.025116	0.99867	1.36	1.3339	5.61
0.040203	1.00020	1.35	1.3343	5.53	0.040251	0.99901	1.38	1.3340	5.56
0.055334	1.00118	1.36	1.3345	5.44	0.055434	0.99938	1.38	1.3341	5.41
0.070482	1.00235	1.39	1.3348	5.39	0.070667	0.99975	1.40	1.3342	5.37
0.085660	1.00345	1.40	1.3349	5.34	0.085948	1.00012	1.43	1.3343	5.32
	w_1 = 0.005[b]					w_2 = 0.005[b]			
0.010024	0.99890	1.33	1.3339	5.58	0.010021	0.99917	1.35	1.3339	5.58
0.025089	0.99975	1.34	1.3342	5.47	0.025091	0.99964	1.37	1.3340	5.43
0.040182	1.00072	1.36	1.3345	5.35	0.040203	1.00020	1.39	1.3341	5.32
0.055313	1.00156	1.38	1.3347	5.28	0.055344	1.00100	1.41	1.3342	5.26
0.070453	1.00275	1.40	1.3345	5.19	0.070519	1.00182	1.44	1.3343	5.12
0.085616	1.00395	1.41	1.3347	5.15	0.085724	1.00270	1.45	1.3344	5.14
					L-Isoleucine				
	w_1 = 0.001[b]					w_2 = 0.001[b]			
0.010036	0.99770	1.31	1.3330	5.82	0.010035	0.99778	1.33	1.3330	6.19
0.025120	0.99850	1.33	1.3332	5.80	0.025129	0.99813	1.35	1.3331	5.97
0.040234	0.99944	1.35	1.3334	5.76	0.040273	0.99848	1.36	1.3332	5.91
0.055367	1.00058	1.36	1.3338	5.71	0.055464	0.99884	1.39	1.3333	5.88
0.070533	1.00162	1.38	1.3341	5.69	0.070706	0.99920	1.40	1.3334	5.85
0.085724	1.00270	1.39	1.3345	5.64	0.085997	0.99956	1.43	1.3335	5.74
	w_1 = 0.003[b]					w_2 = 0.003[b]			
0.010030	0.99835	1.32	1.3335	5.82	0.010029	0.99839	1.34	1.3335	5.86
0.025104	0.99914	1.34	1.3336	5.80	0.025115	0.99871	1.37	1.3336	5.82
0.040207	1.00009	1.36	1.3339	5.76	0.040249	0.99905	1.39	1.3337	5.79
0.055338	1.00110	1.38	1.3341	5.71	0.055433	0.99940	1.41	1.3338	5.72
0.070471	1.00250	1.39	1.3347	5.69	0.070664	0.99978	1.43	1.3339	5.67
0.085642	1.00365	1.40	1.3350	5.64	0.085945	1.00015	1.44	1.3340	5.59
	w_1 = 0.005[b]					w_2 = 0.005[b]			
0.010024	0.99891	1.34	1.3337	5.63	0.010021	0.99924	1.35	1.3336	5.62
0.025087	0.99982	1.36	1.3340	5.61	0.025080	1.00010	1.37	1.3338	5.65
0.040184	1.00068	1.38	1.3342	5.59	0.040152	1.00145	1.38	1.3341	5.57
0.055308	1.00165	1.40	1.3345	5.55	0.055258	1.00254	1.41	1.3344	5.53
0.070453	1.00275	1.43	1.3347	5.49	0.070382	1.00375	1.43	1.3346	5.47
0.085625	1.00385	1.44	1.3349	5.40	0.085536	1.00488	1.45	1.3349	5.39

[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 IV.8. 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	0.99765	1.31	0.0100	0.99778	1.31
0.0252	0.99821	1.31	0.0252	0.99835	1.32
0.0402	0.99875	1.32	0.0402	0.99890	1.33
0.0553	0.99929	1.32	0.0553	0.99946	1.33
0.0705	0.99985	1.33	0.0705	1.00003	1.34
0.0855	1.00042	1.33	0.0855	1.00061	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	0.99881	1.33	0.0251	0.99905	1.34
0.0401	0.99932	1.34	0.0401	0.99955	1.35
0.0552	0.99988	1.34	0.0552	1.00011	1.35
0.0703	1.00047	1.35	0.0703	1.00070	1.36
0.0855	1.00108	1.35	0.0854	1.00132	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	0.99950	1.34	0.0251	0.99980	1.35
0.0401	1.00005	1.35	0.0401	1.00036	1.36
0.0552	1.00063	1.36	0.0552	1.00091	1.37
0.0703	1.00121	1.37	0.0703	1.00153	1.38
0.0854	1.00182	1.38	0.0854	1.00214	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 IV. 9. 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 /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 /m^3 mol^{-1}
L-Leucine							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.010037	100.44	0.077	27.0886	0.010036	110.44	0.154	27.0711
0.025121	87.40	0.098	27.0745	0.025130	109.84	0.146	27.0695
0.040241	83.89	0.116	27.0675	0.040274	109.19	0.192	27.0674
0.055384	78.10	0.165	27.0544	0.055467	108.89	0.196	27.0653
0.070550	73.79	0.175	27.0393	0.070709	108.44	0.231	27.0626
0.085742	70.77	0.185	27.0311	0.086000	108.26	0.262	27.0602
w_1 = 0.003[b]				w_2 = 0.003[b]			
0.010030	103.37	0.154	27.0933	0.010030	113.38	0.152	27.0840
0.025105	88.14	0.146	27.0790	0.025116	112.17	0.241	27.0833
0.040203	76.82	0.192	27.0712	0.040251	110.87	0.266	27.0814
0.055334	73.86	0.196	27.0594	0.055434	109.73	0.227	27.0787
0.070482	69.45	0.261	27.0498	0.070667	109.08	0.258	27.0761
0.085660	67.42	0.263	27.0275	0.085948	108.66	0.312	27.0734
w_1 = 0.005[b]				w_2 = 0.005[b]			
0.010024	109.31	0.152	27.0770	0.010021	109.28	0.227	27.0697
0.025089	88.49	0.145	27.0761	0.025091	103.68	0.239	27.0643
0.040182	80.28	0.190	27.0718	0.040203	100.03	0.264	27.0565
0.055313	78.91	0.227	27.0638	0.055344	94.00	0.290	27.0423
0.070453	73.12	0.259	27.0170	0.070519	90.26	0.342	27.0275
0.085616	69.26	0.261	26.9994	0.085724	87.14	0.336	27.0111
L-Isoleucine							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.010036	96.43	0.155	27.0433	0.010035	108.44	0.230	27.0411
0.025120	85.40	0.196	27.0363	0.025129	108.24	0.243	27.0390
0.040234	79.13	0.232	27.0256	0.040273	108.19	0.230	27.0369
0.055367	72.64	0.231	27.0242	0.055464	107.98	0.294	27.0345
0.070533	70.36	0.263	27.0182	0.070706	107.86	0.289	27.0321
0.085724	68.41	0.265	27.0184	0.085997	107.79	0.341	27.0297
w_1 = 0.003[b]				w_2 = 0.003[b]			

0.010030	98.36	0.154	27.0625	0.010029	111.37	0.229	27.0614
0.025104	86.54	0.194	27.0485	0.025115	110.57	0.289	27.0601
0.040207	79.58	0.230	27.0448	0.040249	109.87	0.304	27.0583
0.055338	75.32	0.262	27.0322	0.055433	109.37	0.324	27.0561
0.070471	67.30	0.261	27.0384	0.070664	108.65	0.345	27.0532
0.085642	65.06	0.263	27.0294	0.085945	108.31	0.339	27.0505
	w_1 = 0.005[b]				w_2 = 0.005[b]		
0.010024	108.31	0.229	27.0620	0.010021	102.28	0.227	27.0457
0.025087	85.68	0.241	27.0595	0.025080	85.26	0.239	27.0372
0.040184	81.28	0.267	27.0509	0.040152	68.74	0.227	27.0228
0.055308	77.27	0.292	27.0467	0.055258	65.97	0.290	27.0154
0.070453	73.12	0.345	27.0317	0.070382	62.66	0.314	26.9974
0.085625	70.44	0.339	27.0167	0.085536	61.47	0.337	26.9890

[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 IV.10. Apparent molar volume (ϕ_V) and $(\eta_r\text{-}1)/\sqrt{m}$ of glycine in different mass fractions of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

molality /mol kg^{-1}	$\phi_V \times 10^6$ / m^3 mol^{-1}	$(\eta_r\text{-}1)/\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$	molality /mol kg^{-1}	$\phi_V \times 10^6$ / m^3 mol^{-1}	$(\eta_r\text{-}1)/\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$
		Glycine			
	w_1 = 0.001[b]			w_2 = 0.001[b]	
0.0100	41.20	0.071	0.0100	41.17	0.081
0.0252	40.38	0.079	0.0252	40.35	0.093
0.0402	39.95	0.082	0.0402	39.66	0.101
0.0553	39.56	0.087	0.0553	39.38	0.105
0.0705	39.20	0.093	0.0705	38.91	0.107
0.0855	38.94	0.097	0.0855	38.60	0.109
	w_1 = 0.003[b]			w_2 = 0.003[b]	
0.0100	41.17	0.091	0.0100	41.17	0.115
0.0251	40.37	0.101	0.0251	40.37	0.120
0.0401	39.65	0.107	0.0401	39.63	0.131
0.0552	39.15	0.112	0.0552	38.97	0.133
0.0703	38.75	0.115	0.0703	38.46	0.136
0.0855	38.37	0.120	0.0854	37.95	0.144
	w_1 = 0.005[b]			w_2 = 0.005[b]	

0.0100	41.14	0.123	0.0100	41.14	0.129
0.0251	39.95	0.131	0.0251	39.94	0.138
0.0401	39.11	0.134	0.0401	39.10	0.145
0.0552	38.59	0.139	0.0552	38.37	0.151
0.0703	38.02	0.143	0.0703	37.67	0.153
0.0854	37.54	0.149	0.0854	37.12	0.160

[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.

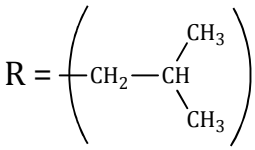
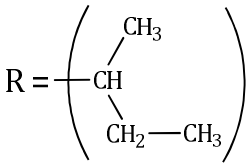
Table IV.11. Limiting apparent molar volume (ϕ_V^o), experimental slope (S_V*), viscosity *A* and *B*-coefficient and limiting molar refraction (R_M^o) of amino acids in different mass fractions of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

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^O /m^3 mol^{-1}
		Glycine			
w_1 = 0.001[b]	41.25	-9.32	0.150	0.0028	
w_1 = 0.003[b]	41.69	-10.85	0.160	0.0030	
w_1 = 0.005[b]	42.13	-12.05	0.170	0.0032	
		L-Leucine			
w_1 = 0.001[b]	113.3	-149.8	0.613	0.0079	27.122
w_1 = 0.003[b]	118.8	-186.9	0.649	0.0663	27.129
w_1 = 0.005[b]	123.8	-194.6	0.678	0.0642	27.135
		L-Isoleucine			
w_1 = 0.001[b]	109.7	-148.3	0.578	0.1026	27.057
w_1 = 0.003[b]	115.0	-174.3	0.608	0.1001	27.076
w_1 = 0.005[b]	120.8	-182.9	0.655	0.1486	27.092
		Glycine			
w_2 = 0.001[b]	41.81	-10.26	0.160	0.0030	
w_2 = 0.003[b]	42.08	-12.07	0.170	0.0033	
w_2 = 0.005[b]	42.31	-14.49	0.180	0.0035	
		L-Leucine			
w_2 = 0.001[b]	111.6	-111.7	0.580	0.0754	27.078
w_2 = 0.003[b]	116.0	-125.8	0.630	0.1112	27.091
w_2 = 0.005[b]	121.9	-117.6	0.648	0.1477	27.108
		L-Isoleucine			
w_2 = 0.001[b]	108.7	-113.4	0.539	0.1586	27.048
w_2 = 0.003[b]	113.0	-116.2	0.576	0.1844	27.068

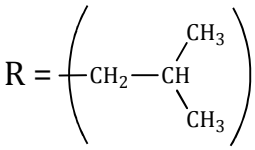
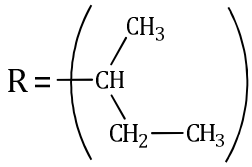
w_2 = 0.005[b]	119.7	-117.1	0.600	0.1471	27.081

[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 IV. 12. Contributions of zwitter ionic group (NH_3^+), (COO); (CH) and side group (R) to the limiting apparent molar volume (ϕ^0_V) and viscosity *B*-coefficient for the 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}					
	w_1=0.001[b]	w_1=0.003[b]	w_1=0.005[b]	w_2=0.001[b]	w_2=0.003[b]	w_2=0.005[b]
(NH_3^+), (COO)	25.36	26.16	26.88	25.68	26.52	27.06
(CH)	7.95	7.77	7.63	8.07	7.78	7.63
R = 	80.00	84.88	89.30	77.86	81.70	87.22
R = 	76.40	81.08	86.30	74.96	78.70	85.02

Groups	*B* / kg mol^{-1}					
	w_1=0.001[b]	w_1=0.003[b]	w_1=0.005[b]	w_2=0.001[b]	w_2=0.003[b]	w_2=0.005[b]
(NH_3^+), (COO)	0.098	0.101	0.104	0.100	0.103	0.106
(CH)	0.026	0.030	0.033	0.030	0.034	0.037

R = 	0.489	0.519	0.541	0.450	0.494	0.505
R = 	0.454	0.478	0.518	0.409	0.440	0.457

[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 IV.13. Hydration number (n_H), solvation number (S_n) of the amino acids at different mass fraction of aqueous α and β-cyclodextrin respectively and binding constant (K_b) of different amino acid-cyclodextrin inclusion complexes at 298.15 K[a]

	n_H			S_n			$K_b^E \times 10^{-3}$
	0.001	0.003	0.005	0.001	0.003	0.005	/M^{-1}
Aqueous α-CD (w_1)[b]							
L-Leucine	5.24	5.02	4.83	5.41	5.46	5.48	0.56
L-Isoleucine	5.31	5.13	4.91	5.27	5.29	5.32	0.54
Aqueous β-CD (w_2)[b]							
L-Leucine	5.34	5.16	4.93	5.20	5.32	5.43	0.52
L-Isoleucine	5.44	5.21	4.99	4.96	5.01	5.10	0.50

[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. [E] Mean error in K_b = ±0.01×10^{-3} M^{-1}

Table IV.14. Data for the Benesi-Hildebrand double reciprocal plot performed by ^{1}H NMR spectroscopy for aqueous L-Leucine-*α*-CD system at 298 K (concentration of L-Leucine = 500 μM)

[α-CD] /μM	δo	δ	Δδ	1/[α-CD] /M^{-1}	1/Δδ	Intercept	Slope	K_b^E /M^{-1}
196		0.9682	0.0108	5100	92.6			
385		0.9760	0.0186	2600	53.8			
566	0.9574	0.9829	0.0255	1767	39.2	9.2349886	0.0164781	560.44
741		0.9908	0.0334	1350	29.9			
909		0.9944	0.0370	1100	27.0			

^{E} Mean error in K_b = ±0.01 M^{-1}

Table IV.15. Data for the Benesi-Hildebrand double reciprocal plot performed by ^{1}H NMR spectroscopy for aqueous L-Leucine-*β*-CD system at 298 K (concentration of L-Leucine = 500 μM)

[β-CD] /μM	δo	δ	Δδ	1/[β-CD] /M^{-1}	1/Δδ	Intercept	Slope	K_b^E /M^{-1}
196		0.9668	0.0094	5100	106.4			
385		0.9720	0.0146	2600	68.5			
566	0.9574	0.9801	0.0227	1767	44.1	10.143918	0.0194092	522.63
741		0.9868	0.0294	1350	34.0			
909		0.9918	0.0344	1100	29.1			

^{E} Mean error in K_b = ±0.01 M^{-1}

Table IV.16. Data for the Benesi-Hildebrand double reciprocal plot performed by ^{1}H NMR spectroscopy for aqueous L-Isoleucine-*α*-CD system at 298 K (concentration of L-Isoleucine = 500 μM)

[α-CD] /μM	δo	δ	Δδ	1/[α-CD] /M^{-1}	1/Δδ	Intercept	Slope	K_b^E /M^{-1}
196	0.9385	0.9456	0.0071	5100	140.8	13.404553	0.0249846	536.51

385		0.9512	0.0127	2600	78.7			
566		0.9561	0.0176	1767	56.8			
741		0.9600	0.0215	1350	46.5			
909		0.9624	0.0239	1100	41.8			

[E] Mean error in K_b = ±0.01 M^{-1}

Table IV. 17. Data for the Benesi-Hildebrand double reciprocal plot performed by ^{1}H NMR spectroscopy for aqueous L-Isoleucine-*β*-CD system at 298 K (concentration of L-Isoleucine = 500 μM)

[β-CD] /μM	δo	δ	Δδ	1/[β-CD] /M^{-1}	1/Δδ	Intercept	Slope	K_b^E /M^{-1}
196		0.9444	0.0059	5100	169.5			
385		0.9489	0.0104	2600	96.2			
566	0.9385	0.9526	0.0141	1767	70.9	15.332567	0.0304047	504.28
741		0.9566	0.0181	1350	55.2			
909		0.9597	0.0212	1100	47.2			

[E] Mean error in K_b = ±0.01 M^{-1}

Figures:

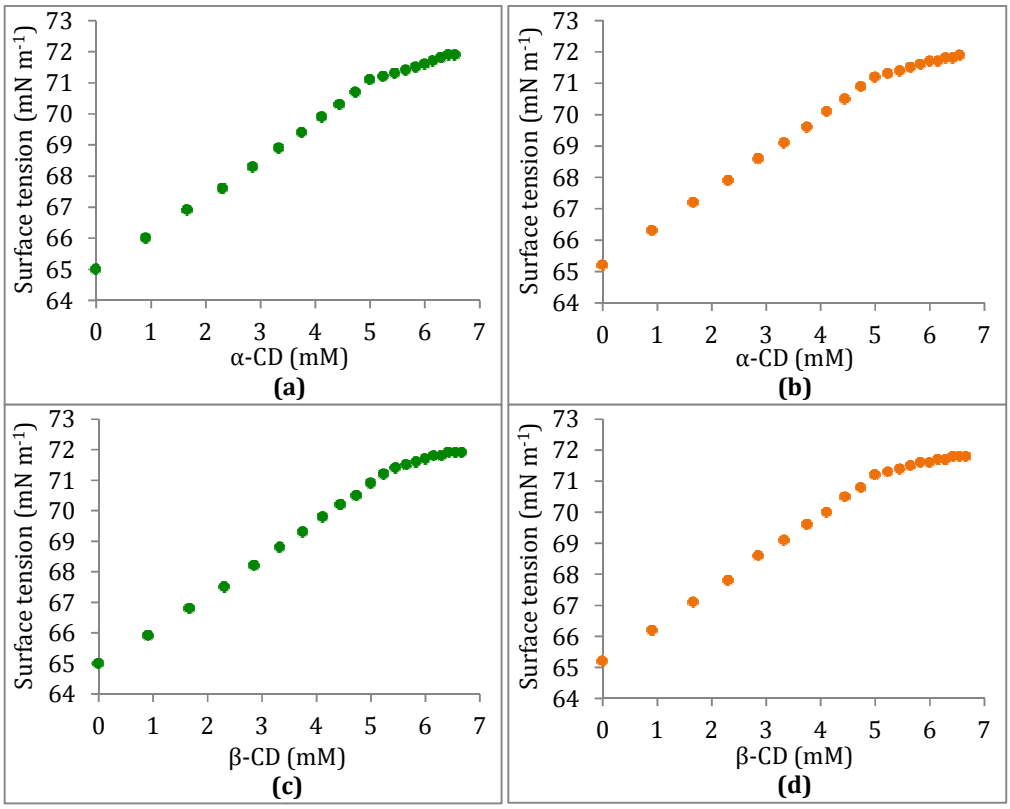
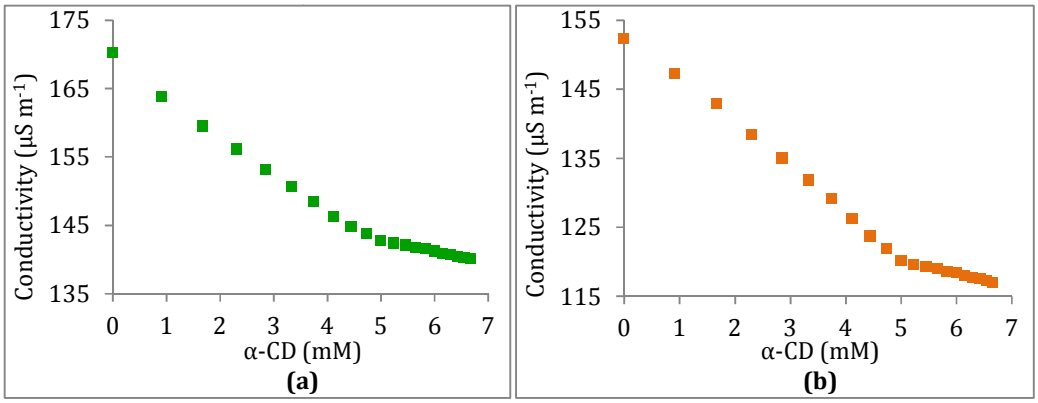


Figure IV. 1. Variation of surface tension of aqueous (a) L-Leu-α-CD, (b) L-Ile-α-CD, (c) L-Leu-β-CD and (d) L-Ile-β-CD systems respectively at 298.15 K.



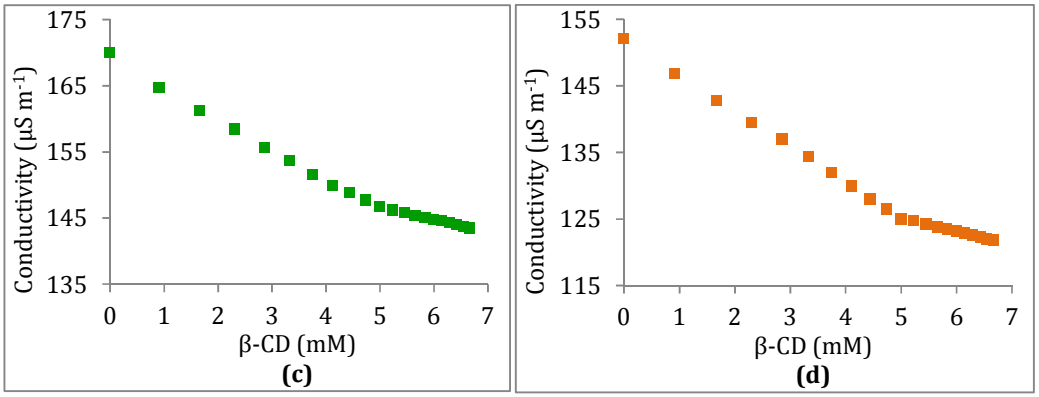


Figure IV.2. Variation of conductivity of aqueous (a) L-Leu-α-CD, (b) L-Ile-α-CD, (c) L-Leu-β-CD and (d) L-Ile-β-CD systems respectively at 298.15 K.

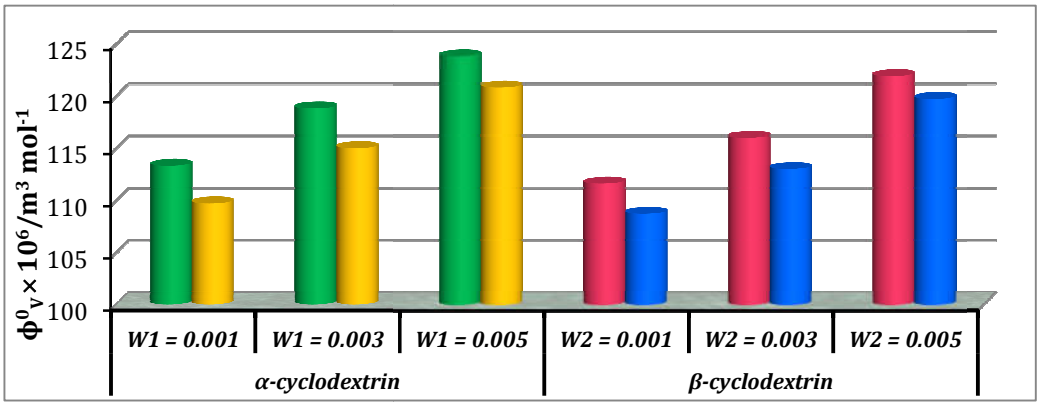


Figure IV.3. Plot of limiting molar volume (ϕ^0_V) for L-Leu (green & red) and L-Ile (yellow & blue) in different mass fractions (w) of aqueous α-CD and aqueous β-CD respectively at 298.15 K.

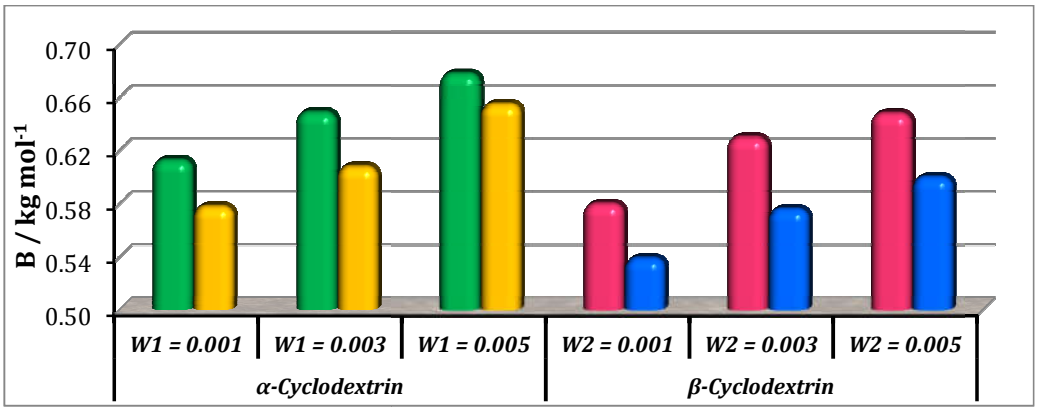


Figure IV. 4. Plot of viscosity *B*-coefficientL-Leu (green & red) and L-Ile (yellow & blue) in different mass fractions (w) of aqueous α-CD and aqueous β-CD respectively at 298.15 K.

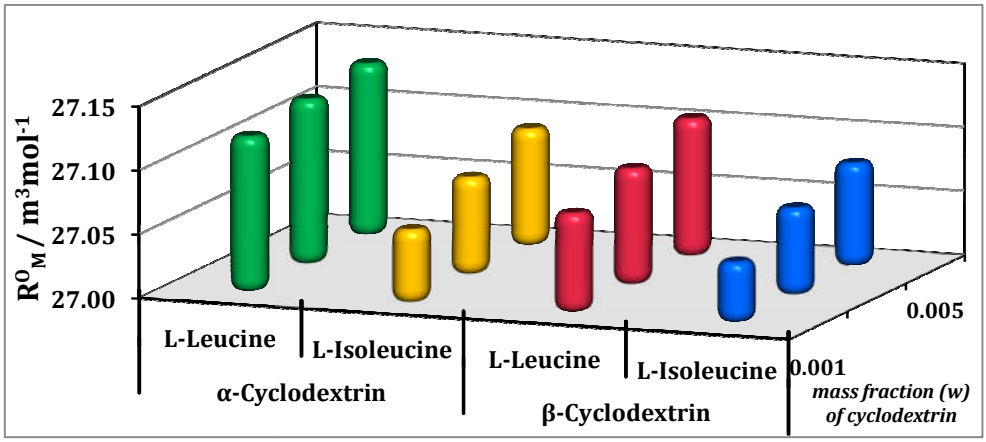


Figure IV. 5. Plot of limiting molar refraction (R^0_M) for L-Leu (green & red) and L-Ile (yellow & blue) in different mass fractions (w) of aqueous α-CD and aqueous β-CD respectively at 298.15 K.

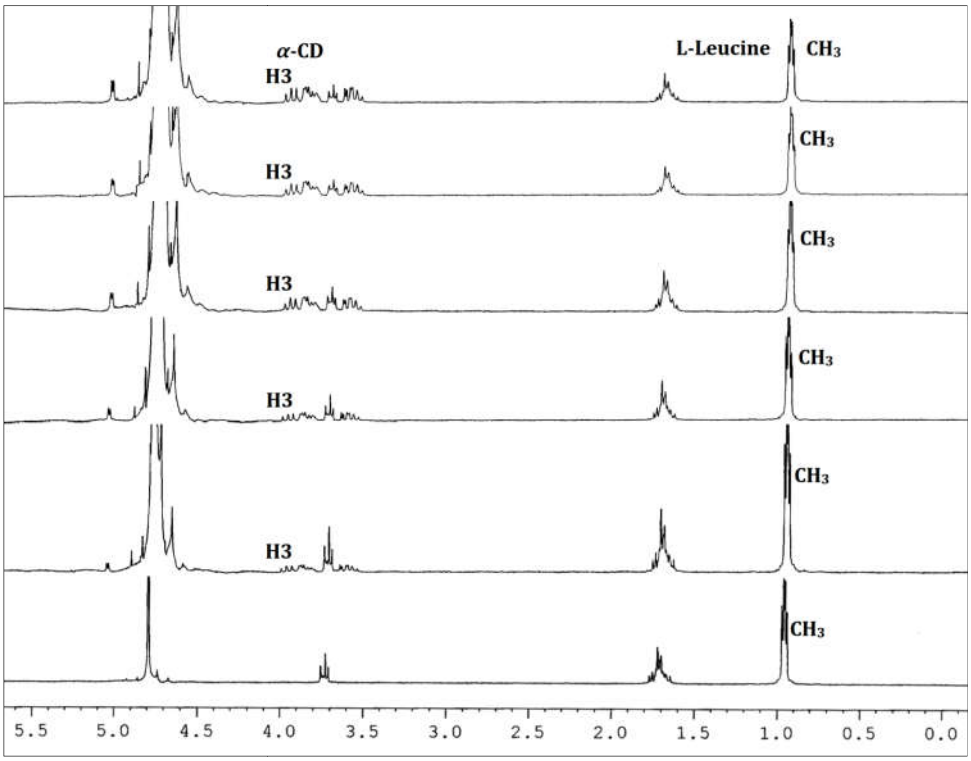


Figure IV.6. ^1H NMR titration spectra of L-Leucine with α-CD in D_2O at 300 MHz at 298 K.

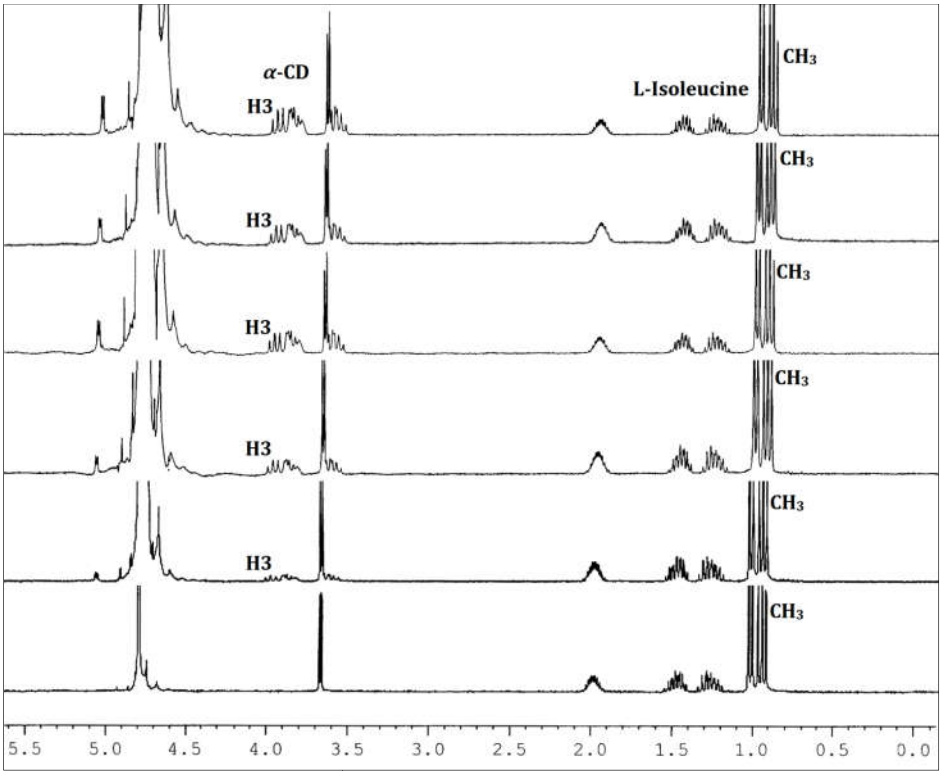


Figure IV.7. ^{1}H NMR titration spectra of L-Isoleucine with α-CD in D_2O at 300 MHz at 298 K.

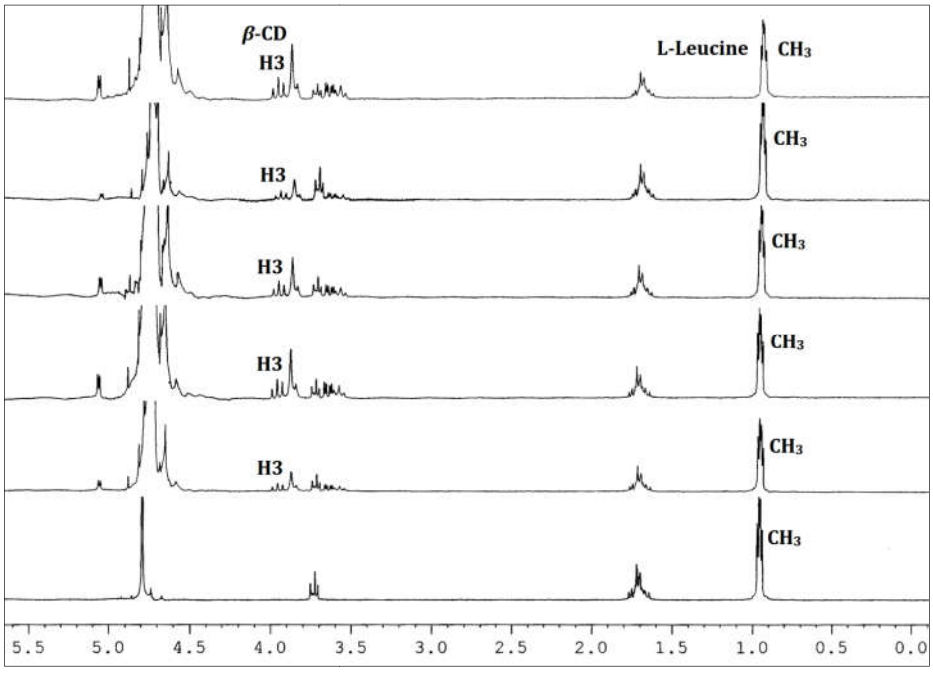


Fig. IV.8. ^{1}H NMR titration spectra of L-Leucine with β-CD in D_2O at 300 MHz at 298 K.

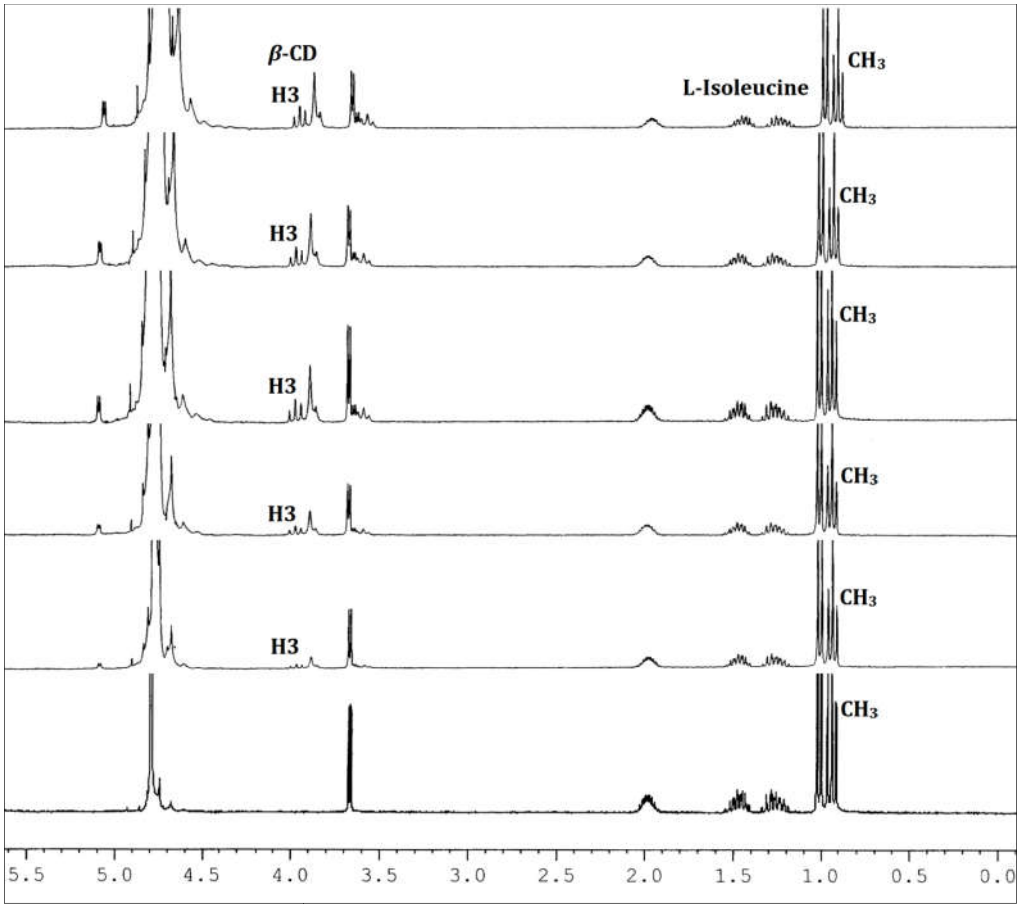
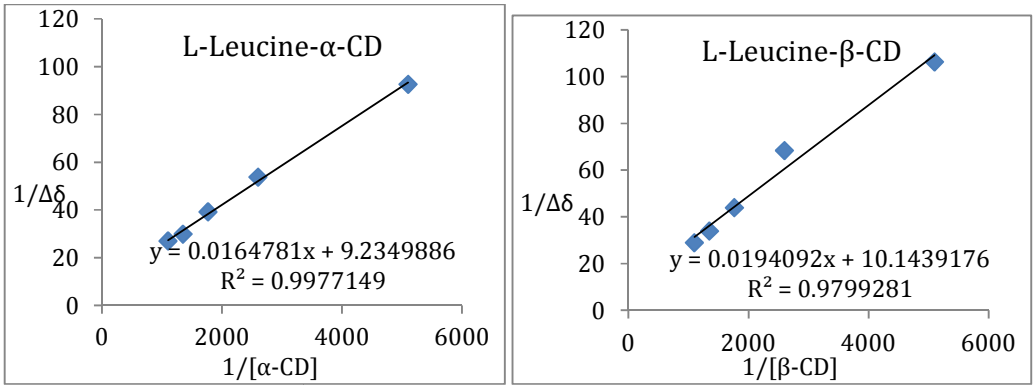


Fig. IV. 9 ^{1}H NMR titration spectra of L-Isoleucine with β-CD in D_2O at 300 MHz at 298 K.



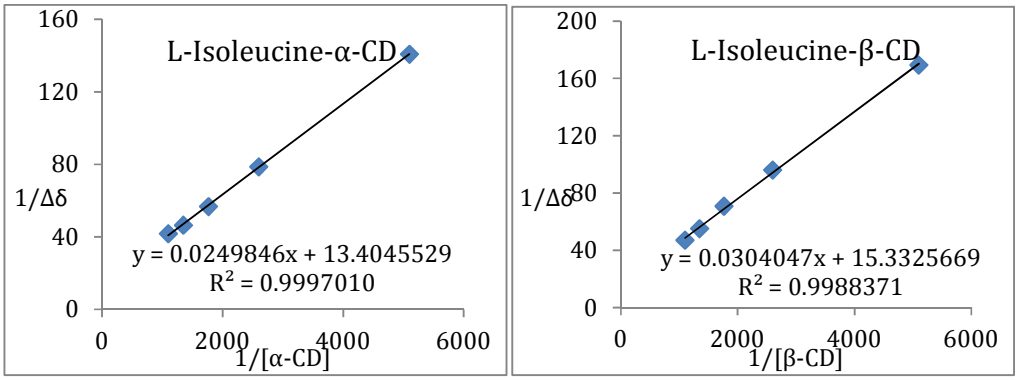
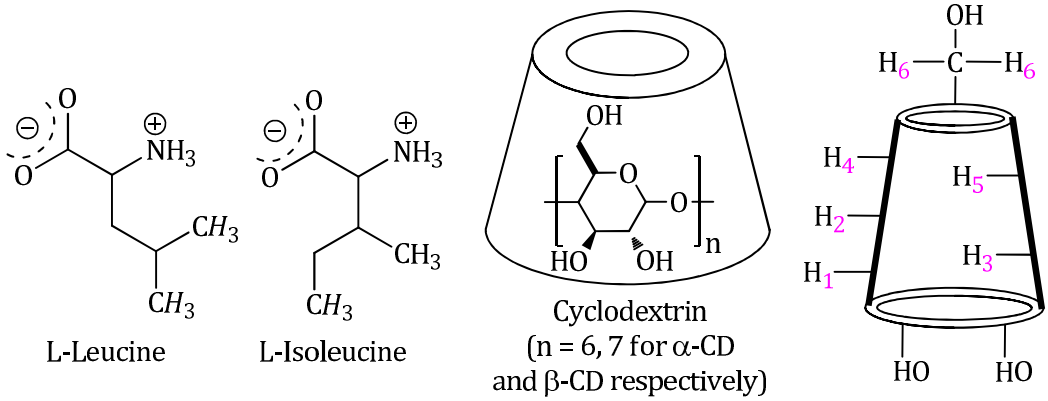
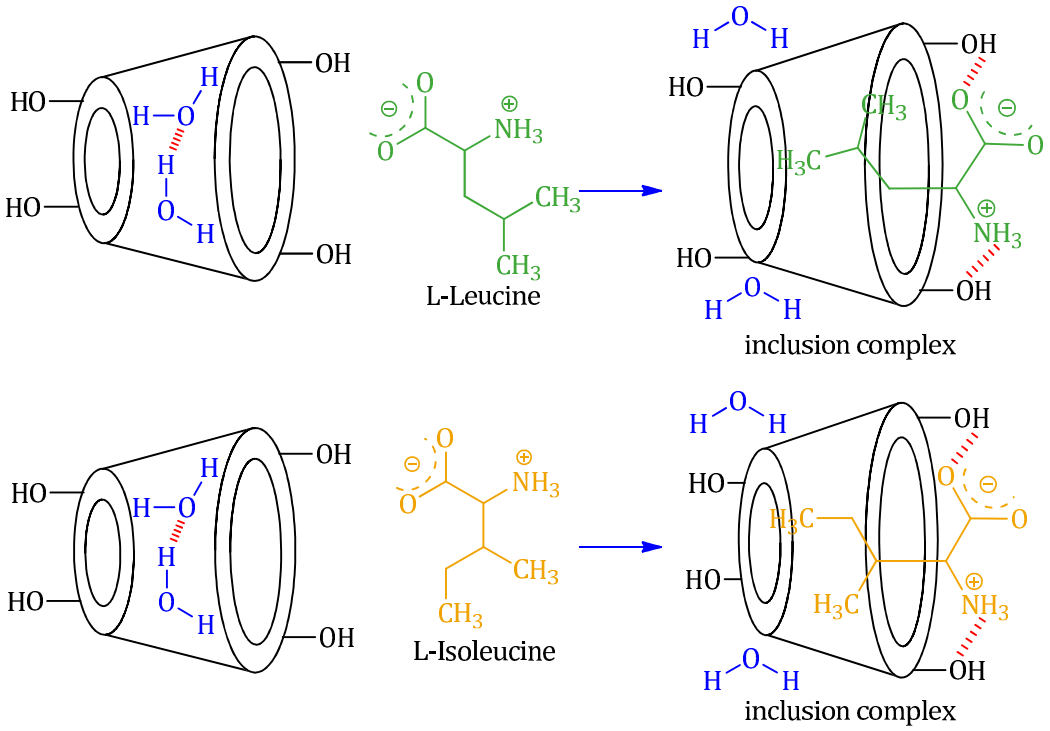


Fig IV.10. Benesi-Hildebrand double reciprocal plot for the effect of α and *β*-CD on the chemical shift of L-Leucine and L-Isoleucine.

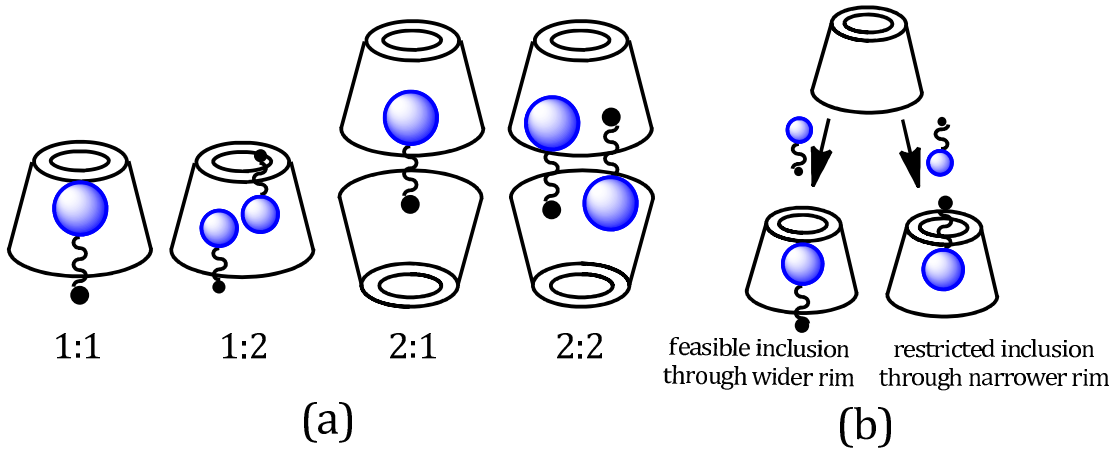
Schemes



Scheme IV. 1. Molecular structure of L-Leu, L-Ile in aqueous solution and cyclodextrin molecule with interior and exterior protons.



Scheme IV. 2. Schematic representation of mechanism for the formation of 1 : 1 inclusion complex of L-Leu and L-Ile with both α and β-cyclodextrin.

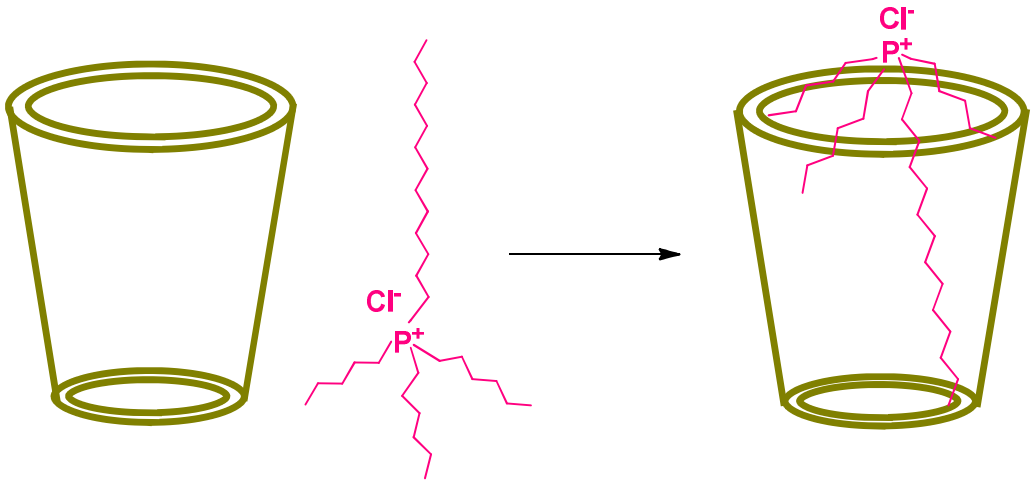


Scheme. IV. 3. (a) Proposed schemes of different possibilities of host-guest ratio for inclusion complex. (b) Proposed schemes of feasible and restricted inclusion of the guest into the host molecule.

CHAPTER V

Cage to cage study of Ionic liquid and cyclic oligosachharides to form inclusion complexes

The inclusion complexation behaviour, charecterization and binding ability of Trihexyltetradecylphosphonium chloride with α and β-Cyclodextrin have been investigated both in aqueous and solid state by means of ^{1}H-NMR, surface tension, conductivity, density, viscosity,refractive index, FT-IR,HRMS study. The shifts in the NMR spectra reveals that part of the ionic liquid is inserted in the cyclodextrin molecules. Surface tension and conductivity study approves the 1:1 stoichiometry of the inclusion complex while density,viscosity and refractive index shows the interaction of the ionic liquid with host molecules. FT-IR and HRMS confirms the inclusion phenomenon. Binding constants have been evaluated using a non linear programme indicating a higher degree of encapsulation in case of β-cyclodextrin compared to α-cyclodextrin.



Published in RSC Advances, 2017, 7, 40803-40812

V. 1. Introduction

The field of Ionic liquids draws the attention of modern chemists because of their unusual properties such as low vapour pressure, high thermal and chemical stability, large liquid temperature range and high solvation ability towards inorganic, organic, complex salts and biopolymers.[1] These properties make them completely different from molecular liquids. Ionic liquids are generally constituted with a large organic cataion and a small anion.[2] They have vast applications in various chemical industries because of their green nature. They produce less hazardous compounds during their use.[3] Phosphonium based ionic liquids are less toxic and more thermally stable than nitrogen based ionic liquids.[1] In this research article the phosphonium based hydrophobic ionic liquid Trihexyltetradecylphosphonium chloride(TTP)(Scheme 1)has been used. This ionic liquid is highly used in separation of different dyes including methylene blue from aqueous media. This has also application as additives to improve the yield of essential oils in the hydrodistillation process.[4]

Cyclodextrin(CD), a cyclic oligosachharide, is well known in supramolecular chemistry as molecular host because of their ability of inclusion of a range of guest molecules through non covalent interaction in their hydrophobic cavity.[5] α, β and γ cyclodextrins consists of 6,7 and 8 glucose units respectively linked by α-1,4 glucosidic linkage.[6] They can be described as a shallow truncated cone having primary and secondary hydroxyl groups in the rims (Scheme 2).[5] They can form stable inclusion complex with drugs,vitamins,ionic liquids, amino acids, neurotransmitters etc guest molecules.[7] The weak intermolecular forces acting between the guest and host includes Vanderwaal's force, dipole-dipole interaction, electrostatic and hydrogen bonding interactions.[8] CD's are non-toxic and considered safe to humans. Formation of inclusion complex is the best method to improve the physicochemical properties of the guest molecule. Due to the enormous application of the inclusion complexes formed by them they are used in cosmetic, food and pharmaceutical industeries.[9]

In the present study we attempt to ascertain the formation and nature of IC of α and β-CD with TTP in aqueous environment by spectroscopic and physicochemical studies. Our aim is to explore the formation, carrying and controlled release of this ionic liquid by forming IC with CD without any chemical and biological modification of the guest molecule. Thus it will find better utility in the dye industry and also in the hydrodistillation process of oils. To the best of our knowledge no theoretical investigations concerning inclusion complex formation between α and β-CD and TTP have been perform so far.

V. 2. Experimental Section

V.2.1. Source and Purity of Samples

Trihexyltetradecylphosphoniumchloride, α-cyclodextrin and β-cyclodextrin of puriss grade were bought from SigmaAldrich, Germany. The chemicals are used in the experiment in the same condition as purchased. The mass fraction purity of TTP, α-cyclodextrin and β-cyclodextrin ≥ 0.98, ≥ 0.99 and ≥ 0.98 respectively.

V.2.2. Apparatus and Procedure

The above mentioned ionic liquid trihexyltetradecylphosphonium chloride and the two CDs are freely soluble in triply distilled, deionized and degassed water. The stock solutions of TTP and aqueous CD were prepared by mass at 298.15 K. Mettler Toledo AG-285(uncertainty 0.0001 g) was used for weighing.

^{1}H NMR spectra were taken in D_2O at 300 MHz with help of Bruker Advance instrument at

298.15 K. Signals are mentioned as δ values in ppm. The internal standard is D_2O (protonated signal at 4.79ppm). Data are cited as chemical shift.

The surface tension study was performed with platinum ring detachment technique usinga Tensiometer (K9, KRSS; Germany). The temperature is maintained at 298.15 K by circulation of thermostated water through a double

wall glass vessel containing the solution. The accuracy of the instrument is about ±0.1mN m^{-1}.

The conductivity study was carried out using a Mettler Toledo Seven Multi conductivity meter (uncertainty ±1.0 μSm^{-1}) in a thermostated waterbath at 298.15 K. HPLC grade water was used with a specific conductance of 6.0mSm^{-1}. Calibration of the conductivity cell was done with 0.01 M aqueous KCl solution. Uncertainty of temperature was ±0.01 K.

The densities (r) of the series of solutions were measured by vibrating U-tube Anton Paar digital density meter (DMA 4500 M) (precision ±0.00005 g cm^{-3}). Calibration of the density meter was carried out by standard method. Uncertainty of temperature was ±0.01 K.

The viscosities (η) of the solutions are measured with Brookfield DV-III Ultra Programmable Rheometer(spindle size 42). Other informations have already mentioned.

Digital Refractometer from Mettler Toledo has been used to measure the refractive index of the solutions (uncertainty ±0.0002 units). Other informations have already mentioned.

The two inclusion complexes of the ionic liquid TTP with both CD molecules (TTP+α-CD, TTP+β-CD) have been prepared in 1:1 molar ratio. 1.0mmol α-CD and 1.0mmol TTP were separately dissolved in 30 ml water. The two solutions were separately stirred for 4 hours. Then the aqueous solution of the TTP was dropwise added to aqueous α-CD solution. The mixture is stirred for 72 hours at about 60° c. The solution is filtered at 60° c and allowed to cool to 10° c. It was kept for 12 hours. After that the suspension was filtered and white crystalline powder was found. It was washed with ethanol and dried in air.

The solid inclusion complexes are dissolved in methanol. HRMS spectra were recorded with a Q-TOF high resolution instrument by positive mode electro-spray ionization.

FT-IR spectra were taken by Perkin Elmer FT-IR spectrometer by KBr disk technique. For preparation of KBr disk 1mg of the solid inclusion complex and 100mg KBr were mixed. The scanning range of the spectra is 4000–400 cm^{-1} at room temperature.

V. 3. Result and discussion

V.3.1. ^{1}H-NMR spectra

^{1}H-NMR study confirms the formation of inclusion complex of TTP with α and β-CD.[9,10] Insertion of ionic liquid molecule into the hydrophobic cavity of CD molecules consequences the chemical shift of both the guest and host molecule. The TTP molecule results dimagnetic shielding of the protons as a result of interaction with the CD protons after inclusion. The position of different protons in the CD molecules are shown in scheme 3. The H3 and H5 protons are situated inside the cavity near the wider rim and narrower rim respectively. The other protons H1,H2 and H4 are situated outside the CD molecule.[11,12] The respective δ values of the ionic liquid TTP, α-CD, β-CD and inclusion complexes are reported in table V.1. The protons of CD and TTP show considarable upfield shift in 1:1 inclusion complex of the ionic liquid and CD (Figure 1,2). It can be concluded from chemical shift that the protons of the hydrocarbon chain of TTP interacts more with the H3 protons than H5 suggesting the TTP molecule enters in the hydrophobic cavity from wider end. The shift in δ value of both the CD perhaps due to change of environment after inclusion complex formation. The H6 proton of α and β-CD remain uneffected after inclusion which again supports the fact that the guest molecule inserts from wider end.

V.3.2. Surface Tension Study

The formation and stoichiometry of the inclusion complexes can be interpreted with surface tension study.[13,14] Adding CD to water does not change the surface tension (γ)of water as it is hydrophobic in nature. This fact also illustrates that CD is surface inactive compounds.[15] The ionic liquid TTP

contains many long hydrocarbon chains and acts as strong surface active agent. The γ value of TTP is lower than pure water. Here the γ value of a series of solutions of TTP with increasing concentration of α-CD and β-CD have been measured at 298.15K (Table V.2-V.3). The γ value is found to increase for both the CD molecules. This is probably due to insertion of the hydrocarbon chain of the ionic liquid from the solution to the hydrophobic cavity of the host CD molecules. A single distinguisable break appears in the two surface tension plots depicted in figure V.3 which suggests the formation of IC.[16,17] The concentrations of TTP and the CD molecules at the break point (Table V.4)is approximately 1:1 which further confirms the stoichiometric ratio of the two ICs as 1:1. More number of breaks in the plot suggests complex stoichiometry of the complex such as 1:2,2:1,2:2 etc. the surface tension at the break point is slightly higher for β-CD indicating it a better host compared to α-CD.

V.3.3. Conductivity Study

Conductivity study also supports the formation and stoichiometry of the two inclusion complexes formed.[18,19] The aqueous solution of the ionic liquid TTP shows considarable conductivity as it exsists as a charged structure. In this study the conductivity of a series of solutions of TTP with increasing concentration of α-CD and β-CD have been measured at 298.15K (Table V.2-V.3). The conductivity value shows regular decrease and after a sharp break point the conductivity value almost becomes constant. Similar results obtained in case of both host CD molecules. The decrease in the κ value probably due to the encapsulation of the long hydrocarbon chain of the guest TTP molecules in the hydrophobic cavity of CD. The values of κ and corresponding concentration of the host CD molecules are reported in table 1 which suggests that the ratio of concentration of TTP and the CD at the break point is almost 1:1.The appearance of sharp break(Figure V.4) points suggest the formation of inclusion complex and also the stoichiometry as 1:1.[20]

The break point indicates certain concentration where maximum number of TTP molecules are inserted in CD molecule ever before. [11]A dynamic equilibrium exsists between the guest ionic liquid and host CD molecules.

Ionic liquid + cyclodextrin Inclusion complex

Maximum inclusion takes place at break point, after it the concentration of CD is higher than the concentration of TTP and the equilibrium shifts more towards the formation of IC.

V.3.4. Density study: illustrates the interaction

The interaction between the ionic liquid and the host molecules can be nicely explained with the help of density study. The apparent molar volume (φ_v) and limiting aparent molar volume (φ_v°) have been calculated to explain the interaction. φ_v can be defined as the summation of volume of the central solute molecule and changes in the solvent volume as a result of interaction of the solute around its co-sphere.[21] The TTP forms a ternary solution system with aqueous CD molecules. Here TTP acts as solute and the CD plays the role of co-solvent. φ_v° illustrates the interaction between them in the following system. φ_v values have been determined from the solvent density (measured at 298.15K)(Table V.6) using Masson Equation (Table V.7). φ_v° values were calculated by applying least square treatments to the plots of φ_v versus $\sqrt{m}$ with the help of Masson Eqn.[22,23] The limiting aparent molar volumes are depicted in figure V.5. φ_v and φ_v° values shows decreaseing and increasing trend respectively for TTP with the increase of concentration of CD molecules. This trend clearly indicates that for this ionic liquid the ion-hydrophilic group interactions are more than ion –hydrophobic interactions. The limiting aparent molar volume increases regularly with increasing mass fraction of CD molecules. The value is slightly higher for β-CD indicating that it is a better host for TTP. The probable reason behind the assumption is that the hydrocarbon chains are encapsulated in the hydrophobic cavity of host CD and the positively charged P atoms interacts with the hydrophilic –OH groups of the CD present in the rim.

The larger cavity of β-CD helps to form more stable inclusion complexes with TTP as it contains more number of polar –OH groups.

V.3.5. Viscosity

The inclusion of of the ionic liquid TTP in the CD molecule can be also explained with the help of viscosity study.[24,25] The viscosity of the solution increases with the increase of the molarity of TTP in this ternary system due to structure making contribution of CD with water molecules (Table V.6). The viscosity B coefficient have been determined (table V.7) which explains the solute solvent interactions based upon the size and shape of solvent molecules. This parameter is found to be positive and depicted in the figure 6 . The rising value of B signifies the increasing interaction of TTP with CD and higher solvation.[21] The long hydrophobic decyl chain is encapsulated in the CD cavity. Again the B value is higher for β-CD than α-CD as the former is better host due to larger diameter than the latter. The viscosity result shows similarity with that of density study and it can be concluded that the structure of CDs and TTP are responsible for this kind of interaction.

V.3.6. Refractive Index

The refractive index(η_D) also explains the interaction between the ionic liquid TTP with CD molecules.[20,21] It also supports the data obtained from density and viscosity data.The η_D values for a series of solutions are measured(Table V.6) with increasing molarity of TTP. The molar refraction (R_M) and limiting molar refraction ($R_M\circ$) of the solutions were also determined (V.7 and V.8). The plot (Figure V.7) shows that the $R_M\circ$ value increases with the increase of mass fraction of TTP. The increasing values of both R_M and $R_M\circ$ signify the ternary solution becomes more compact and dense. This means the inclusion complex of TTP with both the CD molecules are closely packed than TTP probably due to greater hydrophobic and ion-hydrophilic interactions. The higher $R_M\circ$ value for β-CD illustrates that it can better accommodate the ionic liquid in comparison to α-CD.

V.3.7. FT-IR spectra

The encapsulation of TTP in the hydrophobic cavity of CD was confirmed from FT-IR spectroscopy as the band resulted from the inserted part of TTP showed a shift or their intensities are changed.[26,27] FT-IR spectra of TTP, α-CD, β-CD and the inclusion complexes were represented in figure V.8 and V.9. The various frequencies of the above mentioned compounds are reported in table V.8. The IR spectrum of the ionic liquid can be characterized by principal absorption peaks at 3372.75(Symmetrical Stretching of –C-H from CH_3), 2941.63(Symmetrical Stretching of -C-H from $–CH_2$), 1454.58(Stretching of P-CH_2-) etc. The broad –O-H stretching frequency for α-CD and β-CD was observed at 3412.10 and 3349.84 cm^{-1} respectively. In the two IC's the –O-H frequency shifted to lower regeion i.e.; 3378.08 and 3335.76 cm^{-1} for α-CD and β-CD respectively. The reason behind the fact is the involvement of the –O-H groups of both the CDs in hydrogen bonding with the guest TTP molecule.[28,29] The prominent peaks of the ionic liquid for –P-CH_2, -CH_3,-CH_2- of the hydrocarbon chains are shifted in both the IC's. The changes in the FT-IR spectra of TTP are due to the restriction of the vibration of free TTP molecules as the hydrocarbon chains are inserted in the cavity of CD molecules. No additional peaks are obtained in the spectra of IC's. This fact again suggests that only non covalent interaction exsists between the CDand TTP, only Vanderwaal's interaction are present. [30,31]

V.3.8. Mass Spectra

The charecterisation of the two inclusion complexes can also be done by ESI-MS study.[32,33] Although it is difficult to interpret sometimes but m/z value helps to charecterise the inclusion complexes formed. Figure V.9 and V.10 shows the MS spectra of the two inclusion complexes TTP+α-CD, TTP+β-CD respectively. The intense peaks at m/z 1491.80 and 1653.85 indicates the proton adduct of TTP-α-CD IC and TTP-β-CD IC. No other significant peaks are observed at higher values. This study confirms the formation of two inclusion complexes with 1:1 stoichiometry. [34, 35]

V.3.9. Binding Constants: Non linear isotherms

Association constants(K_b) of the two inclusion complexes have been determined from conductivity study.[25] The insertion of the ionic liquid molecule into the hydrophobic cavity of the two CD molecule results the change in conductivity of the aqueous solution. A non linear programme was used to determine the binding constants depending upon this fact.[36,37] There exsists an equilibrium between guest TTP and host CD molecules leading to the formation of 1:1 inclusion complex. The equilibrium can be represented as

$$TTP_f + CD_f \overset{K_b}{} IC \qquad (1)$$

The expression of the binding constant can be obtained from the above equation as

$$K_b = \frac{[IC]}{[TTP]_f[CD]_f} \qquad (2)$$

In the above equation, [IC],$[TTP]_f$ and $[CD]_f$ expresse the equilibrium concentration of the inclusion complex, free TTP and cyclodextrin molecule respectively. The binding constant K_b can be expressed in terms of conductivity κ as

$$K_b = \frac{[IC]}{[TTP]_f[CD]_f} = \frac{(\kappa_{obs}-\kappa_o)}{(\kappa-\kappa_{obs})[CD]_f} \qquad (3)$$

$$\text{where, } [CD]_f = [CD]_{ad} \quad \frac{[TTP]_{ad}(\kappa_{obs}-\kappa_o)}{(\kappa-\kappa_o)} \qquad (4)$$

Where κ_o, $\kappa_{obs}$and κ represent the conductivity of TTP initially, during addition of host and final state respectively. $[TTP]_{ad}$ and $[CD]_{ad}$ are the concentrations of IL and the added CD respectively. Application of the non linear programme to the binding isotherms gives the value of K_b (Table V.10). The association constant for TTP is slightly higher in case of β-CD probably due to the reason that it can

better accommodate the ionic liquid due to its more larger dimension compared to α-CD.

V.4. Conclusion

The present article confirms that the above mentioned ionic liquid Trihexyltetradecylphosphonium chloride forms inclusion complex with both α and β-CD in aqueous medium and in solid state. These two IC's can be used for controlled release of this ionic liquid. ^{1}H-NMR study confirms the inclusion phenomenon whereas surface tension and conductivity study reveal the 1:1 stoichiometry of the complexes. Density ,viscosity and refractive index study show the interaction between the guest and host CD's. FT-IR spectra and mass spectra also supported the formation of IC. The binding constants for the formation of the two IC's have been evaluated from non linear isotherms using conductivity study. It is found to be higher for β-CD. These two IC's have application in various industrial processes to make them greener.

.

Tables

Table V.1. ^{1}H NMR data of TTP, α-CD, β-CD and inclusion complexes

α-Cyclodextrin: (500 MHz, Solv: D_2O) δ=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), 4.96-4.97 (6H, d, *J* = 3 Hz)
β-Cyclodextrin: (500 MHz, Solv: D_2O) δ=3.49-3.54 (6H, t, *J* = 9.2 Hz), 3.57-3.60 (6H, dd, *J* = 9.6, 3.2 Hz), 3.79-3.84 (18H, m), 3.87-3.92 (6H,t, *J* = 9.2 Hz), 5.00-5.01 (6H, d, *J* = 3.6 Hz)
TTP: (400 MHz, Solv: D_2O) δ=0.71-0.77(9H,d,*J*=), 1.0-1.61(48H,m), 2.0-2.07(2H,d,*J*=), 2.29(6H,s)
TTP-α-CD: (1:1 molar ratio, 300 MHz, Solv: D_2O) : δ= 0.90-1.22(54H,m), 3.22-3.26(6H, t, *J*= Hz),3.30-3.32(6H, dd,J=)3.47-3.57(18H, m),3.59-3.62(6H, t, *J* = Hz)
TTP-β-CD: (1:1 molar ratio, 300 MHz, Solv: D_2O) : 0.88-1.20(54H,m),1.35-1.37(2H,d,*J*=),3.18-3.22(6H, t, *J*=),3.28-3.30(6H, dd,J=), 3.42-3.52(18H, m), 3.52-3.67(6H, t, *J* = Hz)

Table V.2. Data for surface tension and conductivity study of aqueous TTP-*α*-CD system at 298.15K[a]

Volm of *α*-CD (mL)	Total volm (mL)	Conc of TTP (mM)	Conc of *α*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (mS m^{-1})
0	10	10.000	0.000	25.5	5.81
1	11	9.091	0.909	28.4	5.29
2	12	8.333	1.667	31.3	4.77
3	13	7.692	2.308	33.6	4.34
4	14	7.143	2.857	35.9	3.98
5	15	6.667	3.333	37.7	3.64
6	16	6.250	3.750	39.5	3.35

7	17	5.882	4.118	40.8	3.14
8	18	5.556	4.444	42.1	2.93
9	19	5.263	4.737	43.5	2.75
10	20	5.000	5.000	45.0	2.60
11	21	4.762	5.238	45.4	2.54
12	22	4.545	5.455	45.9	2.51
13	23	4.348	5.652	46.2	2.48
14	24	4.167	5.833	46.5	2.45
15	25	4.000	6.000	46.8	2.43
16	26	3.846	6.154	47.0	2.41
17	27	3.704	6.296	47.2	2.39
18	28	3.571	6.429	47.4	2.37
19	29	3.448	6.552	47.6	2.35
20	30	3.333	6.667	47.8	2.33

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

Table V.3. Data for surface tension and conductivity of aqueous TTP-*β*-CD system at 298.15K[a]

Volm of *β*-CD (mL)	Total volm (mL)	Conc of TTP (mM)	Conc of *β*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (mS m^{-1})
0	10	10.000	0.000	25.5	5.81
1	11	9.091	0.909	28.8	5.27
2	12	8.333	1.667	31.7	4.74
3	13	7.692	2.308	34.1	4.31
4	14	7.143	2.857	36.4	3.95
5	15	6.667	3.333	38.3	3.61
6	16	6.250	3.750	39.9	3.31

7	17	5.882	4.118	41.2	3.10
8	18	5.556	4.444	42.5	2.89
9	19	5.263	4.737	43.9	2.71
10	20	5.000	5.000	45.4	2.56
11	21	4.762	5.238	45.8	2.51
12	22	4.545	5.455	46.2	2.48
13	23	4.348	5.652	46.6	2.45
14	24	4.167	5.833	46.9	2.42
15	25	4.000	6.000	47.2	2.40
16	26	3.846	6.154	47.4	2.38
17	27	3.704	6.296	47.6	2.36
18	28	3.571	6.429	47.8	2.34
19	29	3.448	6.552	48.0	2.32
20	30	3.333	6.667	48.2	2.30

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

Table V.4.Values of Surface Tension (γ) and Conductance (κ) data the break point with corresponding concentration of α and β-CD at 298.15K.

TTP			
α-CD		**β-CD**	
Surface Tension γ mNm^{-1}	45.57	Surface Tension γ mNm^{-1}	45.84
Concentration(mM)	5.30	Concentration(mM)	5.24

Conductivity Concentration(mM)	κ mSm^{-1}	Conductivity Concentration(mM)	κ mSm^{-1}
4.97	2.59	4.95	2.56

Table V.5. Experimental values of density (ρ), viscosity (η) and refractive index (n_D) of different mass fractions of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

Aqueous solvent mixture	$\rho\times10^{-3}$ /kg m^{-3}	η /mP s	n_D
	aq. α-CD		
w_1 = 0.001	0.99735	1.29	1.3329
w_1 = 0.003	0.99802	1.30	1.3332
w_1 = 0.005	0.99868	1.31	1.3335
	aq. β-CD		
w_2 = 0.001	0.99755	1.30	1.3328
w_2 = 0.003	0.99819	1.31	1.3331
w_2 = 0.005	0.99895	1.32	1.3334

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

Table V.6. Experimental values of density (*ρ*), viscosity (*η*) and refractive index (*n*D) of selected ionic liquid 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	molality /mol kg^{-1}	$\rho\times10^{-3}$ /kg m^{-3}	η /mP s	n_D
TTP							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.010073	0.99791	1.31	1.3335	0.010073	0.99795	1.33	1.3341
0.025352	0.99911	1.33	1.3336	0.025361	0.99876	1.35	1.3342
0.040821	1.00066	1.34	1.3338	0.040859	0.99974	1.37	1.3343
0.056485	1.00228	1.36	1.3339	0.056567	1.00086	1.39	1.3344
0.072328	1.00416	1.37	1.3340	0.072482	1.00211	1.41	1.3345
0.088371	1.00599	1.38	1.3342	0.088605	1.00345	1.43	1.3347
w_1 = 0.003[b]				w_2 = 0.003[b]			
0.010068	0.99841	1.33	1.3346	0.010066	0.99859	1.34	1.3349
0.025349	0.99921	1.35	1.3347	0.025340	0.99955	1.37	1.3350
0.040841	1.00017	1.37	1.3348	0.040816	1.00077	1.39	1.3352
0.056546	1.00122	1.39	1.3349	0.056491	1.00216	1.41	1.3354
0.072462	1.00237	1.40	1.3350	0.072362	1.00371	1.43	1.3356
0.088593	1.00358	1.42	1.3351	0.088440	1.00524	1.45	1.3359
w_1 = 0.005[b]				w_2 = 0.005[b]			
0.010062	0.99902	1.341	1.3353	0.010060	0.99925	1.35	1.3357
0.025335	0.99975	1.365	1.3354	0.025328	1.00003	1.38	1.3358
0.040821	1.00065	1.384	1.3355	0.040807	1.00099	1.40	1.3359
0.056526	1.00156	1.403	1.3356	0.056492	1.00215	1.43	1.3362
0.072434	1.00275	1.421	1.3358	0.072387	1.00337	1.44	1.3364
0.088559	1.00395	1.440	1.3360	0.088500	1.00459	1.46	1.3367

Table V.7. Apparent molar volume (ϕ_V), $(\eta_r\text{-}1)/\sqrt{m}$ and molar refraction (R_M) of selected ionic liquid TTP 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$^{-1/}$	$R_M \times 10^6$ /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$^{-1/}$	$R_M \times 10^6$ /m^3 mol^{-1}
TTP							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.010073	464.54	0.162	106.5855	0.010073	480.49	0.230	107.3600
0.025352	450.10	0.180	107.1893	0.025361	472.07	0.261	107.3020
0.040821	437.72	0.200	107.0897	0.040859	465.70	0.278	107.2260
0.056485	430.82	0.212	106.9820	0.056567	460.26	0.294	107.1350
0.072328	423.15	0.225	106.8381	0.072482	455.28	0.314	107.0304
0.088371	418.77	0.235	106.6671	0.088605	451.00	0.328	106.9455
w_1 = 0.003[b]				w_2 = 0.003[b]			
0.010068	481.26	0.222	107.4476	0.010066	480.18	0.251	107.5027
0.025349	472.65	0.246	107.3874	0.025340	465.75	0.293	107.4167
0.040841	466.48	0.259	107.3167	0.040816	455.63	0.310	107.3450
0.056546	462.04	0.278	107.2364	0.056491	447.94	0.331	107.2659
0.072462	458.07	0.294	107.1488	0.072362	441.25	0.346	107.1774
0.088593	454.80	0.305	107.0592	0.088440	437.16	0.359	107.1095
w_1 = 0.005[b]				w_2 = 0.005[b]			
0.010062	485.95	0.236	107.5943	0.010060	489.82	0.249	107.6730
0.025335	477.14	0.264	107.5448	0.025328	476.61	0.286	107.6149
0.040821	470.68	0.280	107.4772	0.040807	468.80	0.315	107.5428
0.056526	467.56	0.299	107.4085	0.056492	461.61	0.338	107.5129
0.072434	461.78	0.315	107.3390	0.072387	456.65	0.349	107.4454
0.088559	457.91	0.333	107.2686	0.088500	453.43	0.367	107.4049

[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 V.8. Limiting apparent molar volume (ϕ_V^o), experimental slope (S_V^*), viscosity *A* and *B*-coefficient and limiting molar refraction (R_M^o) of ionic liquid TTP 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* / $kg^{1/2}\ mol^{-1/2}$	$R_M^o \times 10^6$ / $m^3\ mol^{-1}$
TTP					
$w_1 = 0.001^b$	487.4	-236.5	0.376	0.122	107.59
$w_1 = 0.003^b$	494.2	-134.3	0.423	0.178	107.68
$w_1 = 0.005^b$	499.7	-140.2	0.483	0.185	107.79
TTP					
$w_2 = 0.001^b$	495.7	-149.9	0.492	0.180	107.61
$w_2 = 0.003^b$	501.3	-221.3	0.540	0.201	107.72
$w_2 = 0.005^b$	507.1	-186.3	0.596	0.191	107.82

Table V.9. Frequencies at FTIR spectra of PB,α-CD, β-CD and solid inclusion complexes

	Wave Number / cm^{-1}	**Group**
TTP	3372.75	Symmetrical Stretching of –C-H from CH_3
	2941.63	Symmetrical Stretching of -C-H from $–CH_2$
	1624.43	Bending of -C-H from $–CH_2$
	1454.58	Stretching of P-CH_2-
	1215.23	Bending of P-CH_2-

	1111.34	Weak Stretching of -C-C-
	989.76	Weak bending of -C-C-
α-Cyclodextrin	3412.10	stretching of O-H
	2930.79	stretching of –C-H from –CH_2
	1406.76	bending of –C-H from –CH_2 and bending of O-H
	1154.39	bending of C-O-C
	1030.39	stretching of C-C-O
	952.36	skeletal vibration involving α-1,4linkage
β-Cyclodextrin	3349.84	stretching of O-H
	2921.52	stretching of –C-H from –CH_2
	1412.36	bending of –C-H from –CH_2 and bending of O-H
	1157.57	bending of C-O-C
	1033.51	stretching of C-C-O
	938.53	skeletal vibration involving α-1,4linkage
TTP+α-CD	3378.08	Stretching of –O-H of α-CD
	2927.03	Stretching of –C-H from –CH_2 of TTP
	2364.96	Stretching of –C-H from –CH_2 of TTP
	1626.46	Bendingof –C-H from –CH_2 of TTP
	1148.51	Bending of –C-O-C of α-CD
	1017.64	Stretching of –C-C-O of α-CD
TTP+β-CD	3335.76	Stretching of –O-H of β-CD

	2916.86	Stretching of –C-H from –CH_2 of TTP
	1646.96	Bending of –C-H from –CH_2 of TTP
	1385.73	Stretching of P-CH_2 from TTP
	1156.87	Bending of –C-O-C- from β-CD
	1080.34	Weak stretching of C-C of TTP
	1021.32	Stretching of –C-C-O of β-CD

Table V.10. Binding constants (Kb) of various ionic liquid–cyclodextrin inclusion complexes

Binding constant[b] $K_b \times 10^{-3}/M^{-1}$		
Temperature [a]/K	TTP-α-CD	TTP-β-CD
293.15	2.35	2.47
298.15	2.14	2.27
303.15	1.98	2.02

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

[b] Mean errors in $K_b=\pm 0.01\times 10^{-3}M^{-1}$

Figures

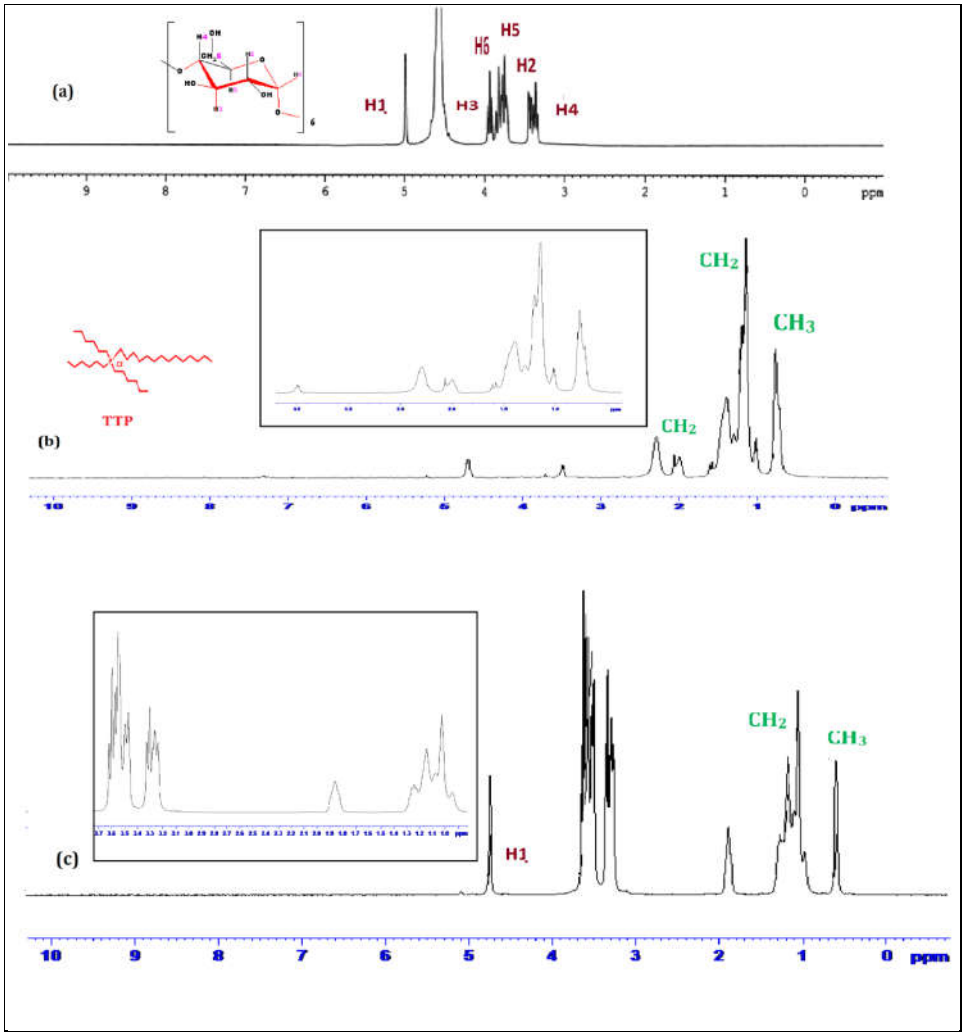


Figure V.1. ^{1}H NMR Spectra of (a) α-CD (b) TTP and (c) 1:1 molar ratio of α-CD + TTP in D_2O in 298.15 K.

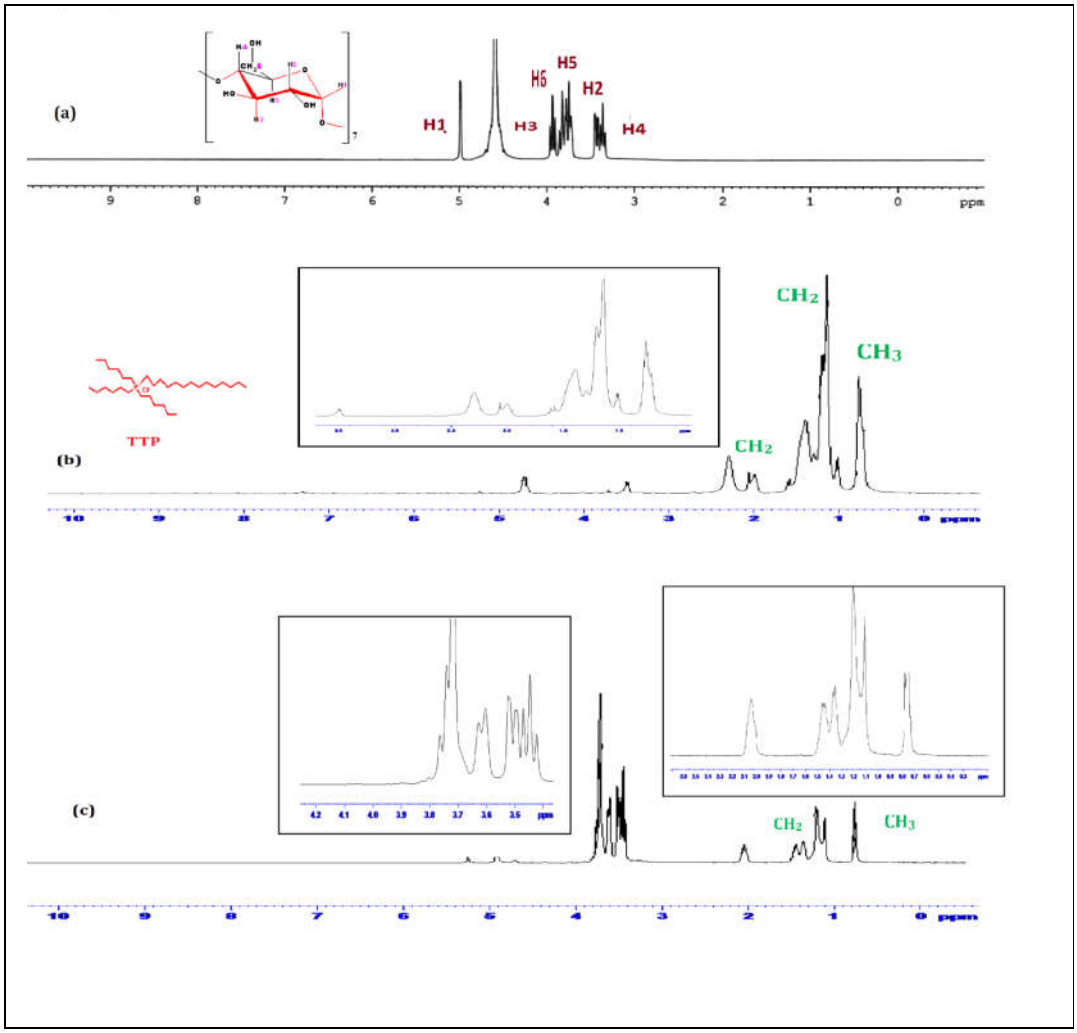


Figure V.2. ^{1}H NMR Spectra of (a) β-CD (b) TTP and (c) 1:1 molar ratio of β-CD + TTP in D_2O in 298.15 K.

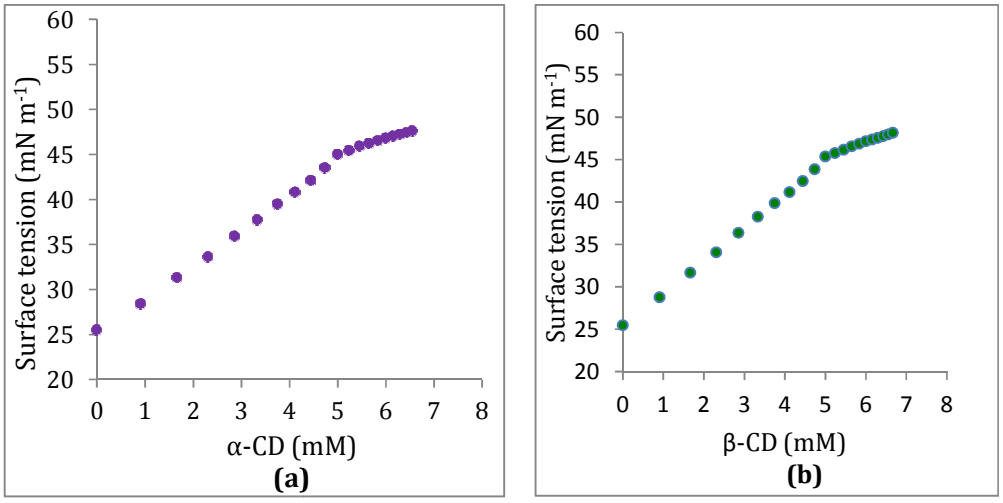


Fig V.3. variation of surface tension of aqueous TTP solution with increasing concentration of (a) α-CD (b)β-CD respectively at 298.15K.

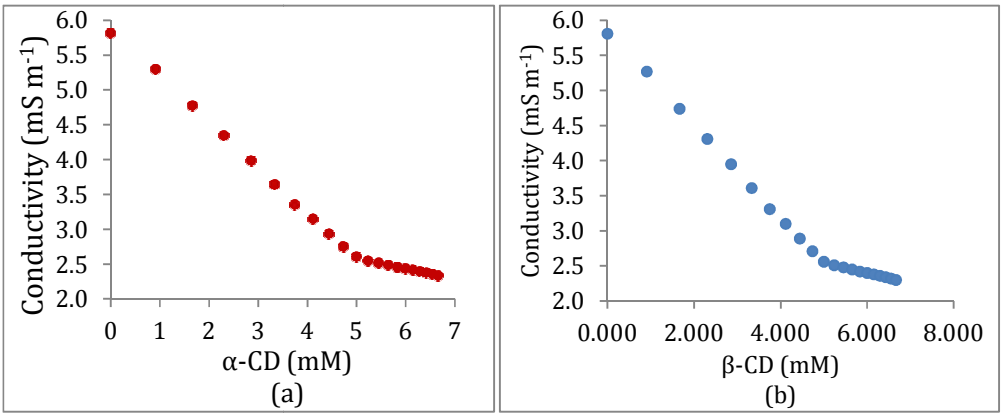


Fig V.4. variation of conductivity of aqueous TTPsolution with increasing concentration of (a) α-CD (b)β-CD respectively at 298.15K.

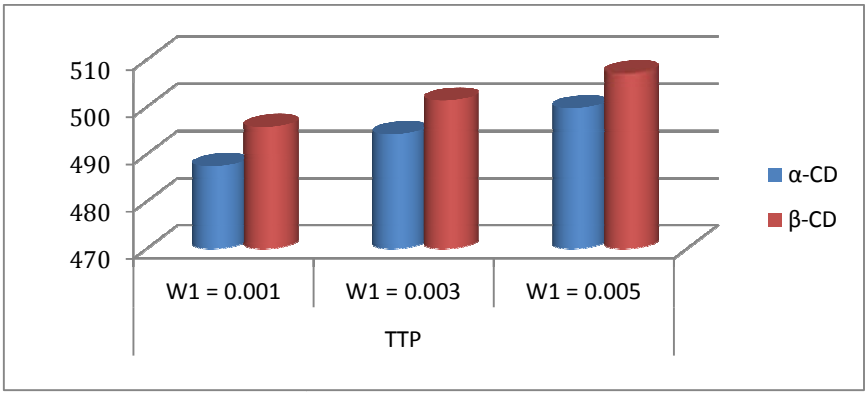


Fig V.5: Plot of limiting molar volume (φ_v°) against mass fraction (w) of aqueous α-CD and β-CD for TTP(α-CD Blue, β-CD Brown) at 298.15 K

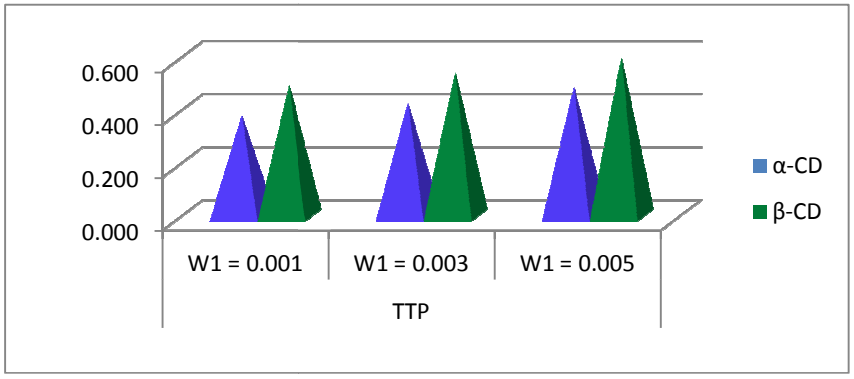


Fig V.6. Plot of viscosityB-coefficient against mass fraction(w) of aqueous α-CD and aqueous β-CD for TPP(blue and green respectively) at 298.15K

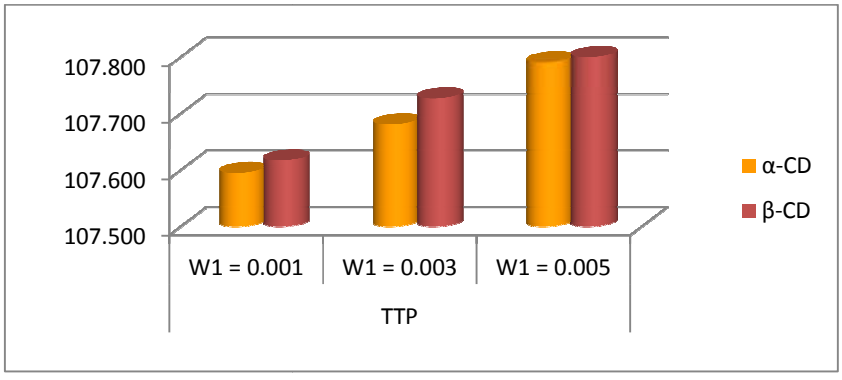


Fig V.7. Plot of limiting molar refraction(R_M°) for TPP in different mass fraction of aqueous α-CD and β-CD at 298.15K

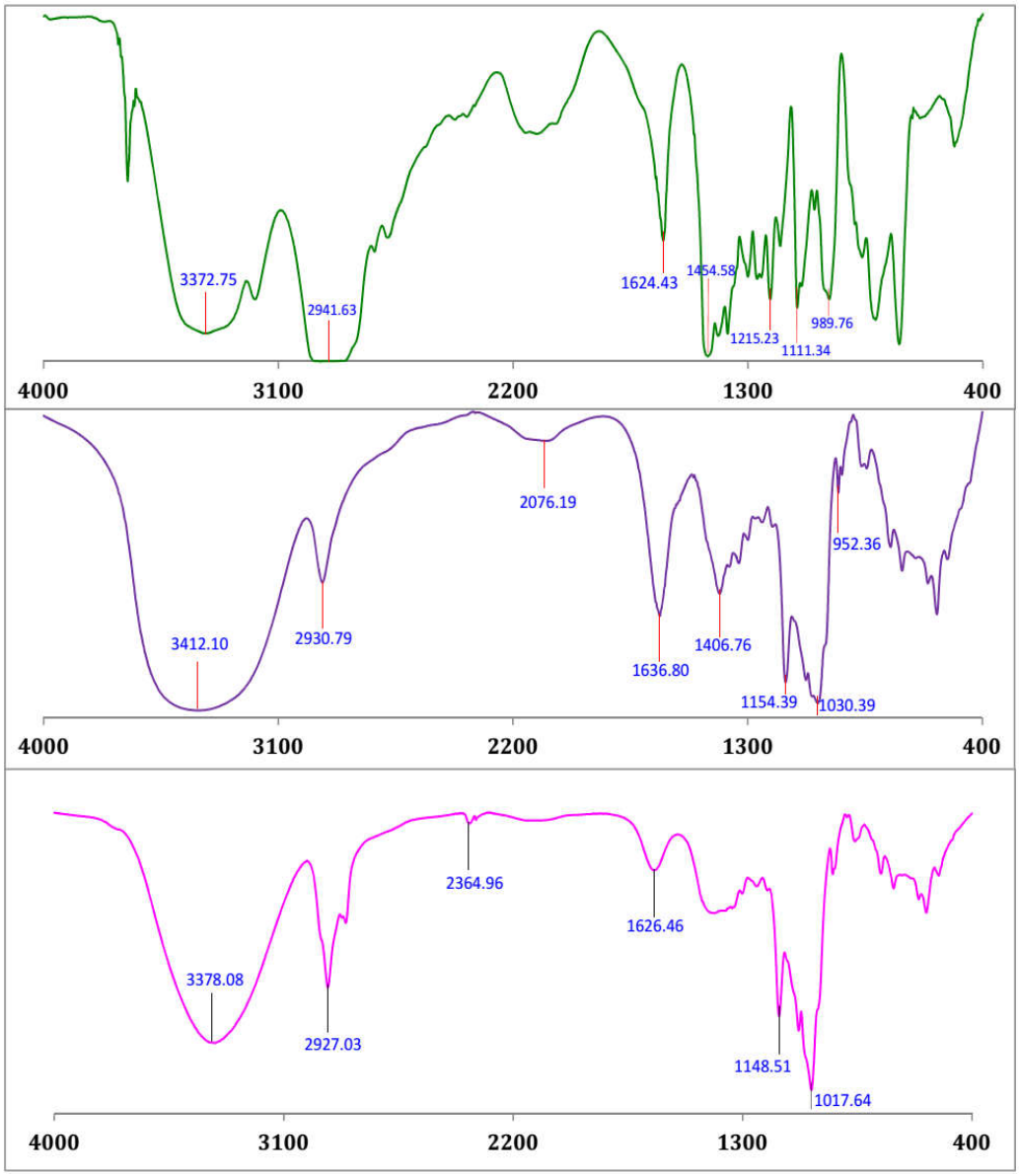


Fig V.8. FTIR spectra of (a) TTP (b) α-CD and (c) TTP-α-CD inclusion complex.

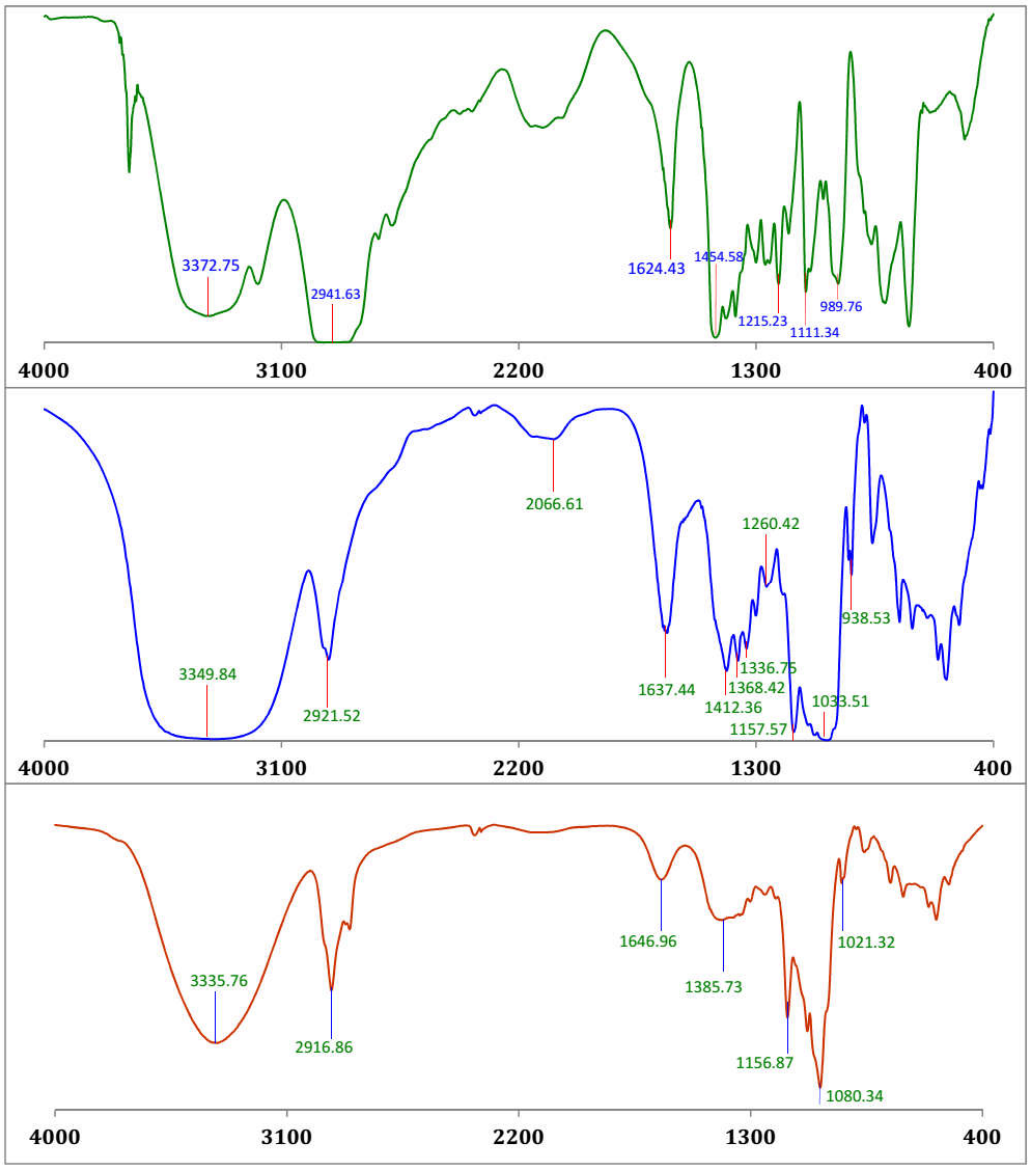


Fig V.9. FTIR spectra of (a) TTP (b) β-CD and (c) TTP-β-CD inclusion complex.

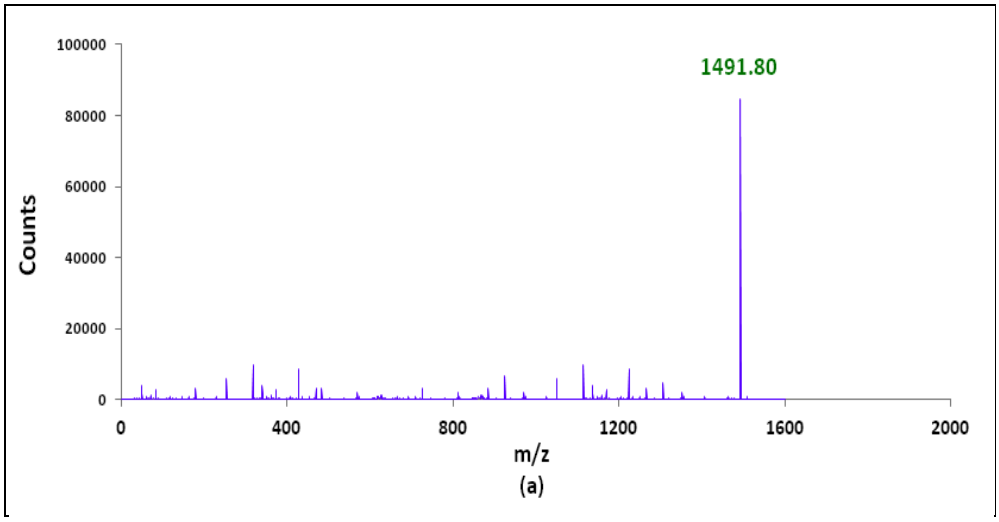


Fig V.10. ESI mass spectra of TTP-α-CD inclusion complex.

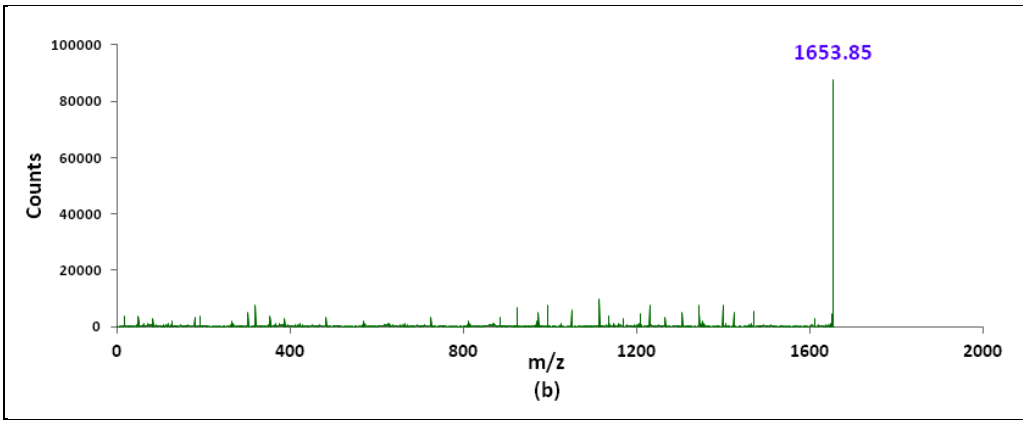
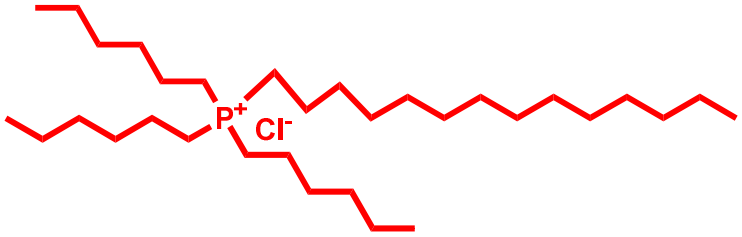
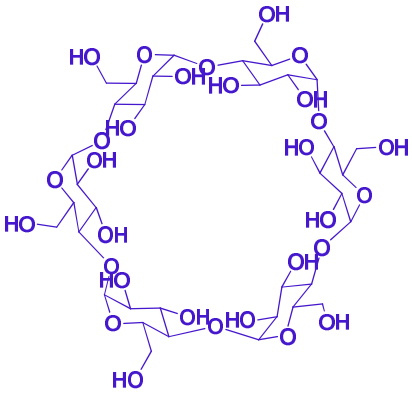


Fig V.11: ESI mass spectra of TTP-β-CD inclusion complex.

Schemes

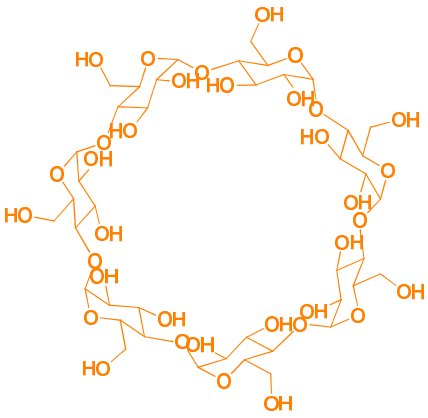


Scheme V.1: Two dimentional molecular structure of the ionic liquid Trihexyltetradecylphosphonium chloride.



α-CD

(a)

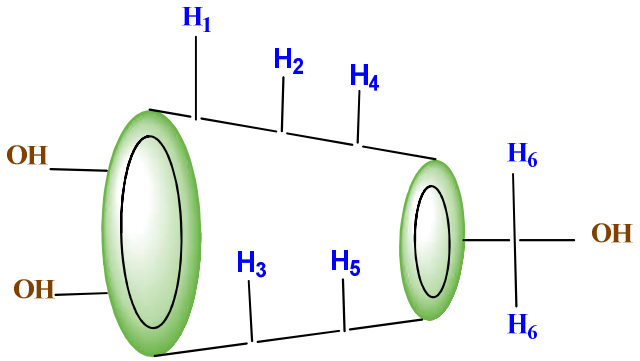


β-CD

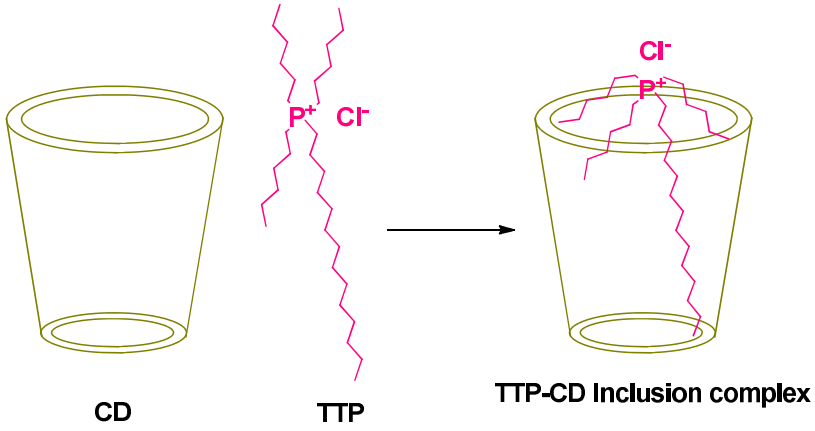
(b)

(c)

Scheme V.2: Structure of (a) α-CD (b) β-CD and (c) cone structure of cyclodextrin molecule



Scheme V.3. Location of different protons in truncated conical structure of α and β-cyclodextrin

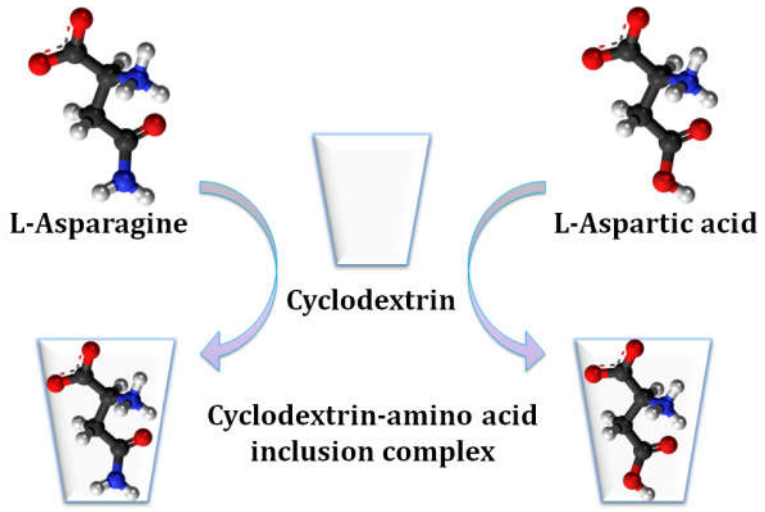


Scheme V.4. Plausible mechanism of formation of inclusion complex between TTP and CD molecule.

CHAPTER VI

Study to explore host-guest inclusion complexes of cyclodextrins with biologically active molecules in aqueous environment

Molecular inclusion of two natural amino acids, *viz.,* L-asparagine and L-aspartic acid as guest into the host cavity of α and β-cylodextrins in aqueous solution have been studied which have various applications in the field of present bio-medical science for controlled delivery of necessary amount of the guest at the targeted site for a period of time efficiently and precisely. Surface tension and conductivity studies establish the formation of inclusion complexes with 1:1 stoichiometry. The inclusion complexes have been characterized by various thermodynamic factors basing upon density and viscosity studies. Contributions of various groups of the guest amino acid molecules toward the limiting apparent molar volume and viscosity *B*-coefficient have been calculated, as well as the solvation and hydration numbers are determined to support the inclusion phenomenon. Formations of the inclusion complexes have been explained with the help of hydrophobic effect, H-bonding, electrostatic forces and structural effects.



VI.1. Introduction

Cyclodextrins (CDs) have enormous applications in the modern science for controlled release of various compounds due to their special ability to form inclusion complexes with diverse guest molecules [1]. They are cyclic oligosaccharides having six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units, bound together by α-(1-4) linkages [2]. CDs are formed during bacterial digestion of cellulose and have shape like a truncated cone rather than perfect cylinders. They have unique structural features, *i.e.*, polar hydrophilic rims having primary and secondary –OH groups and hydrophobic inner cavity (Scheme V.1) [3]. Due to this type of structure CDs can act as molecular hosts for various biological, pharmaceutical, organic and inorganic guest molecules by forming host–guest inclusion complexes [4, 5]. Advantageous changes in the physical properties of the guest molecules may occur after encapsulation by CD, which, hence, are used for stability, solubility, bioavailability and as carrier for the bio-active molecules through the formation of inclusion complexes [6, 7].

L-Asparagine (L-Asn) or 2-amino-3-carbamoylpropanoic acid and L-Aspartic acid (L-Asp) or 2-aminobutanedioic acid are two natural amino acids that are used in the biosynthesis of proteins (Scheme V.1). L-Asn is required for the development of brain and plays an important role in the synthesis of ammonia [8]. L-Asp is the precursor of many essential amino acids and participates in gluconeogenesis process in mammals [9]. In this present work we have attempted to ascertain the nature of formation of inclusion complexes of the above two α-amino acids *viz.* L-Asn and L-Asp in 0.001, 0.003, 0.005 mass fractions of α and β-CDs in aqueous media with the help of the various important properties such as surface tension, conductivity, density, viscosity and pH.

VI. 2. Experimental Section

VI. 2.1. Source and Purity of Samples

The above mentioned two amino acids and CDs of puriss grade were purchased from Sigma-Aldrich, Germany and used as it was. The mass fraction

purity of L-Asn, L-Asp, α-CD and β-CD were ≥ 0.99, 0.99, 0.98 and 0.98 respectively.

VI.2.2. Apparatus and Procedure

Solubilities of the two CDs and that of the above two α-amino acids in aqueous CDs have been verified in triply distilled, deionized and degassed water. It was detected that these were quite soluble in aqueous CDs. All the stock solutions of L-Asn and L-Asp were prepared by mass (Mettler Toledo AG-285 with uncertainty 0.0001 g) and the working solutions were got by mass dilution at 298.15 K. Changes of molarity to molality were done using the densities of the solutions [10]. Sufficient precautions were made to decrease the evaporation during mixing.

pH values were measured by Mettler Toledo Seven Multi pH meter having uncertainty ±0.001. It was studied in a water bath with thermostat maintaining the temperature at 298.15 K, having uncertainty in temperature ±0.01 K.

Surface tensions of the solutions were determined by platinum ring detachment technique using a Tensiometer (K9, KRŰSS; Germany) at 298.15 K. Accuracy of the study was ±0.1 mN m ^{1}. Temperature of the system was maintained by circulating thermostated water through a double-wall glass vessel holding the solution.

Conductivities of the solutions were studied by Mettler Toledo Seven Multi conductivity meter having uncertainty 1.0 μSm^{-1}. The study was carried out in a thermostated water bath at 298.15K with uncertainty ±0.01K. HPLC grade water was used with specific conductance 6.0 μS m^{-1}. The conductivity cell was calibrated using 0.01M aqueous KCl solution.

The densities (ρ) of the solutions were studied by vibrating *U*-tube Anton Paar digital density meter (DMA 4500M) having precision ±0.00005 g cm^{-3} and uncertainty in temperature was ±0.01K. The density meter was calibrated by standard method [10].

Viscosities (η) were determined by Brookfield DV-III Ultra Programmable Rheometer with spindle size 42. The detail has already been depicted before [10].

VI. 3. Result and Discussion

VI.3.1. pH measurement proves the ionic structures of the amino acids

pH study is an important technique to get clue about the existence of zwitterionic states of amino acids in aqueous media [11, 12]. The range of pH values for L-Asn in both aqueous α and β-CD was from 7.12 to 6.11 whereas for L-Asp it ranged from 3.53 to 2.74 in the same system at 298.15K. The pH value decreases with increasing concentration of the two amino acids, also with increasing concentration of α and β-CD respectively (Table V.8). These may be attributed as the –OH groups at the rims of CD molecules interact with the amino acids by making H-bonds or by ion-dipolar interactions, the proton releasing ability and proton accepting ability of –COOH and $-NH_2$ groups respectively vary in presence of different amount of CD in solution. As the β-CD has one more glucopyranose unit, it has more number of –OH groups, thus interaction with amino acids is more in case of β-CD than in case of α-CD. Consequently the pH values of the two amino acid solutions are different in α and β-CD. Hence, these pH values evidently illustrate the existence and difference in the zwitterionic states of the amino acids, *i.e.*, the amine and carboxylic acid groups exist in ionic forms $-NH_3^+$ and $-COO^-$ respectively (Scheme V.1) and the carboxylic acid group in the side chain of L-Asp exists as $-COO^-$. The lower pH in case of L-Asp is due to liberation of an H^+ ion from –COOH group at the side chain.

VI.3.2. Surface tension measurement proves inclusion and shows the stoichiometry of the inclusion complexes

Surface tension (γ) study may be applied to acquire important information about the formation of inclusion complex inside CDs [13, 14]. Because of existence of polar groups in the side chain of the two studied amino

acid molecules, they show substantial increase in γ of their aqueous solutions, but there is no significant change in γ for aqueous CD solution compared to pure water [15]. In this work the two natural amino acids L-Asn and L-Asp exsist as zwitterionic forms and also contain polar side groups (-$CONH_2$ group in L-Asn and COO^- in L-Asp) hence there may be ionic interactions among the charged groups resulting an increase in γ of their solutions [16]. In presence of α and β-CD the surface tension is markedly affected. Here γ of aqueous amino acids has been measured with increasing concentrations of α and β-CD at 298.15K (Table V1-V4).

The surface tension values were declining regularly for both the two amino acids with increasing concentration of α and β-CD might be due to the formation of inclusion complexes inside the cavity of α and β-CD (Figure V.1). Similar curves are obtained for both L-Asn and L-Asp each with single noticeable break at a point where the concentration ratio of the host and the guest is about 1:1 for all the four cases indicating 1:1 stoichiometry of each inclusion complexes formed (Table V.5). More break points in the curve would imply complex stoichiometries (1:2, 2:1, 2:2 etc.) of the inclusion complexes (Scheme V.2) [17, 18]. The two amino acids, therefore, form 1:1 inclusion complexes with both CDs. The amino acids enter into the CDs via the wider rim to make highest contact with the CD cavity, increasing the hydrophobic interactions (Scheme V.3).

VI.3.3. Conductivity measurement explains the inclusion phenomenon and the stoichiometry

The formation of host–guest inclusion complex and also the stoichiometry of the inclusion complex can be established by conductivity study [19, 20]. From pH measurement it is obvious that the conductivity of solutions is due to the zwitterionic forms of amino acids. While the guest molecule goes into the hydrophobic cavity of CD, the conductivity of the solution goes on decreasing regularly. This is due to the decrease of mobility of amino acid molecules after inclusion in the cavity of cyclodextrin. Therefore the inclusion phenomenon has

great effect upon the conductivity of the solution. In the present study the conductivity of the solutions having 10 mmolL^{-1} conc of aqueous L-Asn and L-Asp have been measured with increasing conc of both the CDs (Table V.1-V.4). The conductivity of the solutions regularly decreases for both the two natural amino acids in both aqueous α and β-CD which is obviously for the formation of inclusion complex (Figure V.2).

In every curve of conductivity vs concentration of CD, a sharp break is found at such a point where the concentration ratio of the host and the guest is about 1:1, signifying that the stoichiometric ratio of amino acid-CD inclusion complex is equimolar, *i.e.*, 1:1 host-guest inclusion complexes have been formed (Table V.6) [11].

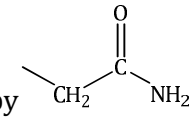
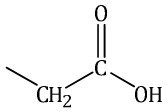
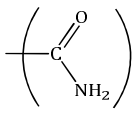
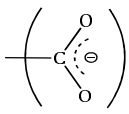
Scheme V.4 shows the plausible mechanism of formation of inclusion complexes which illustrate the hydrophobic hydrocarbon part is situated inside the cavity and the polar groups are situated just at both the rims of CD and get stabilized by making H-bonds with the rimmed –OH groups.

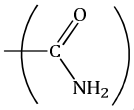
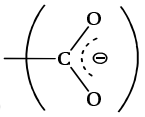
Different stiochiometry of the inclusion complexes such as 1:2, 2:1, 2:2 etc would be recognized by more break points in the conductivity curve (Scheme V.2). For both the amino acids the conductivity at the break was found slight lower for β-CD than α-CD, which may be due to β-CD is better host than α-CD for the above two amino acids.

VI.3.4. Density study: group contributions and interaction involving the host and the guest

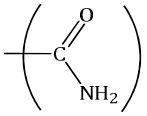
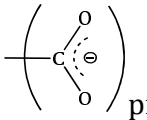
The characteristic behavior of interactions (here, inclusion) of solute can be obtained from apparent molar volume (ϕ_v) and limiting apparent molar volume (ϕ_v^o) [13]. ϕ_v is measure of the total geometric volume of the central solute and the changes in solvent volume due to the interactions with the solute surrounding the co-sphere [21]. ϕ_v^o signifies the solute-solvent interactions in the amino acids+aq. CD ternary solution systems. For this principle, ϕ_v have been calculated from the solution densities using the appropriate equation at 298.15 K. The magnitudes of ϕ_v (table V.10) are found to be large and positive for all

examined systems, indicating strong solute-solvent interactions (here, solute = amino acid and co-solvent = CD; thus, here ϕ_V indicates host-guest interaction). The limiting apparent molar volume (ϕ_v^o) has been determined from Masson equation (Table V.12) [11]. For the two amino acids, *i.e.*, L-Asn and L-Asp the ϕ_v values decrease with increase in the molality (m) of amino acid in both the aqueous CDs. ϕ_v varies linearly with $\sqrt{m}$ and may be fitted to the Masson equation from where ϕ_v^o have been estimated. The values of ϕ_v^o increases with the increase of conc of α and β-CD for both L-Asn and L-Asp signifying the ion–hydrophilic group interactions are stronger than ion–hydrophobic group interactions. ϕ_v^o for the two amino acids and CDs at different mass fractions have been shown in figure 3.

The ϕ_v^o for L-Asn is larger than L-Asp due to more electrostriction which is due to existence of $-CONH_2$ that interacts better than the $-COO^-$ group, by making H-bonds with the rimmed –OH groups of CD, resulting a net increase in the volume of L-Asn. If one H is replaced from the side chain of glycine by  and  the side groups L-Asn and L-Asp are found respectively. Due to this correlation of structure there should be a correlation in their ϕ_v^o values. Contribution of different groups present in the two amino acids to the limiting apparent molar volume (ϕ_v^o) has been estimated in table V.13 [21, 22]. The contribution of zwitterionic group (NH_3^+,COO^-) is found in the range of 22.51–23.21 × 10^{-6} m^3 mol^{-1} and 22.76–23.28 × 10^{-6} m^3 mol^{-1} for α and β-CD respectively, suggesting that the interactions among the –OH groups of CDs and the polar groups (NH_3^+,COO^-) of amino acids are strong and it is stronger for β-CD than α-CD. The contribution of hydrophobic (CH) and (CH_2) group and also the polar  group and  group increases with the increase in concentration of both the CDs, which suggest that the ion-dipolar interactions

increases for the two amino acids L-Asn and L-Asp. The contribution of  group is more compared to  , indicating the former group interacts better with the –OH groups at the rim of CDs than the later, which provide an enforcing affinity in L-Asn resulting a net increase in the volume.

VI.3.5. Viscosity study: group contributions

Viscosity study is another sensitive tool to interpret the interactions between amino acids and CDs. For the studied ternary system (amino acid+aq. CD), the viscosity of the solution increases with the increasing molarity of the amino acids (table V.8). The viscosity *B*-coefficients (Table V.12), are the indication of solute–solvent interactions (here, solute = amino acid and co-solvent = CD; thus, viscosity *B*-coefficients indicate host-guest interaction) which depend on the size and shape of the solute molecules. The *B*-coefficients of the two amino acids L-Asn and L-Asp are positive (Figure V.4) and increases with the increasing conc of both α and β-CD due to increase of amino acid–CD interaction as well as increasing solvation [13]. The contributions of different groups of the amino acids to the viscosity *B*-coefficient have been estimated (Table V.14). The contributions of the zwitterionic group (NH_3^+, COO^-) and the polar groups  and  present in the side chain increase with increasing mass fractions of α and β-CD due to better solvation of the ionic groups with the –OH groups of CD molecules [21]. The contributions of the hydrophobic (CH), (CH_2) groups also increases suggesting that the interaction with the hydrophobic cavity of the CD increases.

VI.3.6. Hydration number and solvation number: solvation by cyclodextrin molecule

Hydration number (n_H) is the order of hydration by water molecules surrounding the amino acids whereas the solvation number (S_n) expresses the

solvation of the two amino acids by the cyclodextrin molecule, *i.e.*, the interaction between the polar groups of the guest and the –OH groups at the primary and secondary rims of CD [13, 15]. S_n are evaluated from apparent molar volume and viscosity *B*-coefficient. n_H decreases and S_n increases with increasing concentration of both α and β-CD for the two amino acids, *i.e.*, L-Asn and L-Asp (Table V.15). The trend in the S_n and n_H value suggest that in the ternary solution system the electrostriction of water weakens with increasing conc of CD [21]. The solvation of L-Asn is higher than that of L-Asp which may be explained due to the presence of $-CONH_2$ group which makes grater association with the –OH groups at the rim of CDs than $-COO^-$ group and the encapsulation into the cavity of CD is higher in case of L-Asn. Low hydration numbers as well as high the solvation numbers in case of β-CD than α-CD for the above two amino acids again suggests that β-CD is more fascinated for solvation than α-CD.

VI.3.7. Structural influence of cyclodextrins

Formation of host-guest inclusion complexes of the two amino acids, *i.e.*, L-Asn and L-Asp with α and β-CD not only depends upon the cavity diameters of the CDs but also the size of the two chosen guest molecules. CD has an exceptional structure with hydrophobic cavity and hydrophilic rims that afford suitable environment for the non-polar part of a molecule to reside inside the cavity whereas the polar part of the guest interacts with the polar rims and stabilize the whole inclusion complex formed [1, 5, 23]. No covalent bonds are broken or formed in formation of the inclusion complex. In aqueous solution of CD, there are few water molecules inside the hydrophobic cavity, which is thermodynamically unfavourable [17]. So, the apolar part of the amino acids readily substitute the water molecules resulting a more stable lower energy state which is the other driving force for formation of the inclusion complex. The trapped water molecules are liberated in the bulk increasing the entropy of the system.

CD can accommodate one or more guest depending upon the size of amino acid molecules to form inclusion complexes with stoichiometry 1:1, 1:2, 2:1, and 2:2 and even more complicated complex but surface tension and conductivity studies suggest that here the stoichiometry is 1:1. The inclusion of the guest molecule is likely through the wider rim of the CD molecule, which allows the alkyl groups to make maximum contact with the cyclodextrin cavity (Scheme V.3) [11]. The charged end groups are projected in the direction of the aqueous environment making H-bonds with the –OH groups at the both rims of the CD molecule (Scheme V.4).

VI.4. Conclusion

The above mentioned studies conclude that the amino acids, *viz.,* L-Asn and L-Asp form host-guest inclusion complexes with both α and β-CD. Surface tension and conductivity measurement reveal that the inclusion complex formed with 1:1 stoichiometry. Density and viscosity studies were used to characterize the inclusion complexes through determination of the group contributions toward the limiting apparent molar volume and viscosity *B*-coefficient. The solvation number and hydration number also support the inclusion phenomenon. All the results demonstrate the formation of the inclusion complexes and thus the present work has diverse application in the field of controlled delivery of these two amino acids by using α and β-CD.

Tables

Table VI.1. Data for surface tension and conductivity study of aqueous L-Asparagine-α-CD system at 298.15K[a]

Volm of α-CD (mL)	Total volm (mL)	Conc of L-Asparagine (mM)	Conc of α-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (μS m^{-1})
0	10	10.000	0.000	82.2	190.0
1	11	9.091	0.909	80.6	181.0
2	12	8.333	1.667	79.4	171.5
3	13	7.692	2.308	78.5	164.1
4	14	7.143	2.857	77.6	157.2
5	15	6.667	3.333	76.9	151.5
6	16	6.250	3.750	76.2	145.5
7	17	5.882	4.118	75.6	141.6
8	18	5.556	4.444	75.1	136.0
9	19	5.263	4.737	74.6	133.4
10	20	5.000	5.000	74.3	130.0
11	21	4.762	5.238	74.1	129.1
12	22	4.545	5.455	74.0	128.2
13	23	4.348	5.652	73.9	127.4
14	24	4.167	5.833	73.8	126.5
15	25	4.000	6.000	73.7	125.7
16	26	3.846	6.154	73.6	124.9
17	27	3.704	6.296	73.5	123.2
18	28	3.571	6.429	73.4	122.8
19	29	3.448	6.552	73.3	122.1
20	30	3.333	6.667	73.1	121.5

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

Table VI.2. Data for surface tension and conductivity study of aqueous L-Asparagine-*β*-CD system at 298.15K[a]

Volm of *β*-CD (mL)	Total volm (mL)	Conc of L-Asparagine (mM)	Conc of *β*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (μS m^{-1})
0	10	10.000	0.000	82.0	188
1	11	9.091	0.909	80.9	176
2	12	8.333	1.667	79.7	165
3	13	7.692	2.308	78.7	157
4	14	7.143	2.857	77.8	150
5	15	6.667	3.333	77.0	144
6	16	6.250	3.750	76.3	138
7	17	5.882	4.118	75.7	134
8	18	5.556	4.444	75.1	130
9	19	5.263	4.737	74.6	127
10	20	5.000	5.000	74.2	124
11	21	4.762	5.238	73.9	123
12	22	4.545	5.455	73.8	122
13	23	4.348	5.652	73.7	121
14	24	4.167	5.833	73.6	120
15	25	4.000	6.000	73.5	119
16	26	3.846	6.154	73.4	118
17	27	3.704	6.296	73.3	117
18	28	3.571	6.429	73.2	116
19	29	3.448	6.552	73.1	115
20	30	3.333	6.667	73.0	115

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

Table VI.3. Data for surface tension and conductivity study of aqueous L-Aspartic acid-*α*-CD system at 298.15K[a]

Volm of *α*-CD (mL)	Total volm (mL)	Conc of L-Aspartic acid (mM)	Conc of *α*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (mS m^{-1})
0	10	10.000	0.000	80.0	3.10
1	11	9.091	0.909	78.8	2.83
2	12	8.333	1.667	77.9	2.66
3	13	7.692	2.308	77.1	2.48
4	14	7.143	2.857	76.5	2.36
5	15	6.667	3.333	75.9	2.24
6	16	6.250	3.750	75.3	2.14
7	17	5.882	4.118	74.8	2.03
8	18	5.556	4.444	74.5	1.96
9	19	5.263	4.737	74.2	1.88
10	20	5.000	5.000	74.0	1.82
11	21	4.762	5.238	73.9	1.80
12	22	4.545	5.455	73.8	1.78
13	23	4.348	5.652	73.7	1.75
14	24	4.167	5.833	73.6	1.72
15	25	4.000	6.000	73.5	1.70
16	26	3.846	6.154	73.5	1.69
17	27	3.704	6.296	73.4	1.67
18	28	3.571	6.429	73.4	1.66
19	29	3.448	6.552	73.3	1.64
20	30	3.333	6.667	73.3	1.63

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

Table VI.4. Data for surface tension and conductivity study of aqueous L-Aspartic acid-*β*-CD system at 298.15K[a]

Volm of *β*-CD (mL)	Total volm (mL)	Conc of L-Aspartic acid (mM)	Conc of *β*-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (mS m^{-1})
0	10	10.000	0.000	80.2	3.07
1	11	9.091	0.909	79.4	2.81
2	12	8.333	1.667	78.5	2.60
3	13	7.692	2.308	77.7	2.44
4	14	7.143	2.857	77.0	2.31
5	15	6.667	3.333	76.4	2.17
6	16	6.250	3.750	75.9	2.08
7	17	5.882	4.118	75.4	1.99
8	18	5.556	4.444	75.0	1.90
9	19	5.263	4.737	74.7	1.83
10	20	5.000	5.000	74.3	1.77
11	21	4.762	5.238	74.0	1.75
12	22	4.545	5.455	73.8	1.73
13	23	4.348	5.652	73.7	1.71
14	24	4.167	5.833	73.6	1.69
15	25	4.000	6.000	73.5	1.66
16	26	3.846	6.154	73.4	1.64
17	27	3.704	6.296	73.3	1.62
18	28	3.571	6.429	73.2	1.61
19	29	3.448	6.552	73.1	1.60
20	30	3.333	6.667	73.0	1.59

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

Table VI.5. Surface tension (γ) values at the break point with corresponding concentrations of cyclodextrins and amino acids at 298.15 K [a]

	Conc of α-CD /mM	Conc of amino acid /mM	γ [a] /mN·m^{-1}
L-Asparagine	4.94	5.06	74.33
L-Aspartic acid	4.99	5.01	74.43
	Conc of β-CD /mM	Conc of amino acid /mM	γ [a] /mN·m^{-1}
L-Asparagine	5.16	4.84	74.02
L-Aspartic acid	5.24	4.76	74.08

[a] Standard uncertainties (u): temperature: u(T) = ±0.01 K, surface tension: $u(\gamma)$ = ±0.1 mN m ^{1}

Table VI.6. Values of conductivity (κ) at the break point with corresponding concentrations of cyclodextrins and amino acids at 298.15 K [a]

	Conc of α-CD /mM	Conc of amino acid /mM	κ [a]
			μS·m^{-1}
L-Asparagine	4.92	5.08	131
			mS·m^{-1}
L-Aspartic acid	4.95	5.05	1.83
	Conc of β-CD /mM	Conc of amino acid /mM	κ [a]
			μS·m^{-1}
L-Asparagine	4.90	5.10	126
			mS·m^{-1}
L-Aspartic acid	5.00	5.00	1.75

[a] Standard uncertainties (u): temperature: u(T) = ±0.01 K, conductivity: $u(\kappa)$ = ±1.0 μS·m^{-1}.

Table VI.7. Experimental values of density (ρ), viscosity (η) and pH of different mass fractions of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

Aqueous solvent mixture	$\rho\times10^{-3}$ /kg m^{-3}	η /mP s	pH
aq. α-CD			
w_1 = 0.001	0.99735	1.29	6.64
w_1 = 0.003	0.99802	1.30	6.61
w_1 = 0.005	0.99868	1.31	6.55
aq. β-CD			
w_2 = 0.001	0.99755	1.30	6.57
w_2 = 0.003	0.99819	1.31	6.54
w_2 = 0.005	0.99895	1.32	6.52

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

Table VI.8. Experimental values of density (ρ), viscosity (η) 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	pH	molality /mol kg^{-1}	$\rho\times10^{-3}$ /kg m^{-3}	η /mP s	pH
L-Asparagine							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.003009	0.99748	1.321	7.12	0.003008	0.99768	1.329	6.61
0.007525	0.99770	1.333	7.08	0.007523	0.99795	1.347	6.57
0.012044	0.99792	1.349	7.01	0.012039	0.99832	1.361	6.42
0.016567	0.99816	1.360	6.97	0.016557	0.99873	1.372	6.37
0.021092	0.99842	1.370	6.96	0.021076	0.99916	1.383	6.29
0.025617	0.99879	1.388	6.92	0.025601	0.99942	1.394	6.26
w_1 = 0.003[b]				w_2 = 0.003[b]			
0.003007	0.99812	1.330	6.83	0.003006	0.99829	1.345	6.50
0.007520	0.99838	1.346	6.74	0.007518	0.99854	1.367	6.43
0.012035	0.99865	1.359	6.70	0.012034	0.99874	1.383	6.36
0.016554	0.99894	1.372	6.68	0.016552	0.99904	1.397	6.30
0.021076	0.99915	1.385	6.65	0.021071	0.99941	1.407	6.26

0.025601	0.99944	1.395	6.61	0.025587	0.99996	1.425	6.23
w_1 = 0.005[b]				w_2 = 0.005[b]			
0.003005	0.99879	1.339	6.52	0.003004	0.99905	1.361	6.39
0.007514	0.99915	1.356	6.49	0.007512	0.99938	1.385	6.31
0.012024	0.99960	1.370	6.44	0.012021	0.99980	1.404	6.22
0.016535	1.00005	1.380	6.41	0.016531	1.00030	1.421	6.19
0.021049	1.00047	1.392	6.38	0.021042	1.00080	1.436	6.15
0.025562	1.00095	1.406	6.34	0.025553	1.00128	1.450	6.11
L-Aspartic acid							
w_1 = 0.001[b]				w_2 = 0.001[b]			
0.003009	0.99750	1.320	3.53	0.003008	0.99767	1.341	3.01
0.007524	0.99779	1.338	3.15	0.007524	0.99787	1.365	2.94
0.012042	0.99808	1.353	3.00	0.012042	0.99809	1.384	2.87
0.016562	0.99843	1.365	2.94	0.016564	0.99833	1.398	2.83
0.021085	0.99877	1.371	2.88	0.021089	0.99859	1.413	2.80
0.025612	0.99902	1.386	2.86	0.025615	0.99890	1.428	2.78
w_1 = 0.003[b]				w_2 = 0.003[b]			
0.003007	0.99814	1.332	3.12	0.003006	0.99830	1.353	3.00
0.007519	0.99848	1.351	2.94	0.007519	0.99848	1.379	2.91
0.012033	0.99885	1.366	2.87	0.012034	0.99881	1.398	2.85
0.016550	0.99920	1.379	2.83	0.016551	0.99910	1.414	2.81
0.021070	0.99945	1.391	2.80	0.021072	0.99940	1.430	2.78
0.025591	0.99985	1.400	2.79	0.025595	0.99967	1.445	2.75
w_1 = 0.005[b]				w_2 = 0.005[b]			
0.003005	0.99879	1.348	3.09	0.003004	0.99906	1.360	2.98
0.007515	0.99901	1.370	2.87	0.007512	0.99943	1.383	2.90
0.012028	0.99928	1.388	2.85	0.012021	0.99988	1.401	2.83
0.016543	0.99960	1.404	2.82	0.016531	1.00035	1.417	2.80
0.021059	0.99997	1.416	2.79	0.021041	1.00087	1.431	2.76
0.025580	1.00025	1.429	2.76	0.025552	1.00134	1.447	2.74

[a] Standard uncertainties u are: $u(\rho)$ = 5×10^{-5} kg m^{-3}, $u(\eta)$ =0.003 mP s, 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 VI.9. 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	0.99765	1.31	0.0100	0.99780	1.31
0.0251	0.99821	1.31	0.0252	0.99833	1.32
0.0403	0.99873	1.32	0.0402	0.99890	1.33
0.0553	0.99931	1.32	0.0552	0.99944	1.33
0.0703	0.99980	1.33	0.0704	1.00004	1.34
0.0856	1.00042	1.33	0.0855	1.00061	1.35
	w_1 = 0.003[b]			w_2 = 0.003[b]	
0.0100	0.99826	1.32	0.0100	0.99851	1.33
0.0251	0.99880	1.33	0.0251	0.99906	1.34
0.0401	0.99933	1.34	0.0402	0.99955	1.35
0.0552	0.99988	1.34	0.0552	1.00014	1.35
0.0703	1.00045	1.35	0.0703	1.00070	1.36
0.0855	1.00109	1.35	0.0854	1.00132	1.37
	w_1 = 0.005[b]			w_2 = 0.005[b]	
0.0100	0.99892	1.33	0.0100	0.99923	1.34
0.0251	0.99949	1.34	0.0251	0.99979	1.35
0.0401	1.00005	1.35	0.0401	1.00036	1.36
0.0552	1.00063	1.36	0.0552	1.00091	1.37
0.0703	1.00121	1.37	0.0703	1.00153	1.38
0.0854	1.00182	1.38	0.0854	1.00215	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 VI.10. Apparent molar volume (ϕ_V) and $(\eta_r\text{-}1)/\sqrt{m}$ 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$^{-1/2}$	molality /mol kg^{-1}	$\phi_V \times 10^6$ / m^3 mol^{-1}	$(\eta_r\text{-}1)/\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$
L-Asparagine					
	w_1 = 0.001[b]			w_2 = 0.001[b]	
0.003009	89.02	0.438	0.003008	89.00	0.407
0.007525	85.68	0.384	0.007523	78.98	0.417
0.012044	84.84	0.417	0.012039	68.12	0.428
0.016567	83.25	0.422	0.016557	60.75	0.430
0.021092	81.38	0.427	0.021076	55.59	0.440
0.025617	75.85	0.475	0.025601	58.93	0.452
	w_1 = 0.003[b]			w_2 = 0.003[b]	
0.003007	98.98	0.421	0.003006	98.97	0.487
0.007520	84.29	0.408	0.007518	85.61	0.502
0.012035	79.78	0.414	0.012034	86.44	0.508
0.016554	76.51	0.430	0.016552	80.75	0.516
0.021076	78.47	0.450	0.021071	74.16	0.510
0.025601	76.59	0.457	0.025587	62.82	0.549
	w_1 = 0.005[b]			w_2 = 0.005[b]	
0.003005	95.58	0.404	0.003004	98.89	0.567
0.007514	69.55	0.405	0.007512	74.87	0.568
0.012024	55.53	0.418	0.012021	61.35	0.580
0.016535	49.15	0.416	0.016531	50.35	0.595
0.021049	46.94	0.431	0.021042	44.07	0.606
0.025562	43.16	0.458	0.025553	40.79	0.616
L-Aspartic acid					
	w_1 = 0.001[b]			w_2 = 0.001[b]	
0.003009	83.32	0.424	0.003008	93.33	0.575
0.007524	74.63	0.429	0.007524	90.66	0.576
0.012042	72.46	0.445	0.012042	88.32	0.589
0.016562	67.83	0.452	0.016564	86.04	0.586
0.021085	65.65	0.432	0.021089	83.78	0.599
0.025612	67.79	0.465	0.025615	80.36	0.615

	w_1 = 0.003[b]			w_2 = 0.003[b]	
0.003007	93.28	0.449	0.003006	96.61	0.599
0.007519	71.91	0.452	0.007519	94.60	0.607
0.012033	64.06	0.463	0.012034	81.58	0.612
0.016550	61.71	0.472	0.016551	78.09	0.617
0.021070	65.13	0.482	0.021072	75.62	0.631
0.025591	61.46	0.481	0.025595	75.20	0.644
	w_1 = 0.005[b]			w_2 = 0.005[b]	
0.003005	96.56	0.529	0.003004	96.53	0.553
0.007515	89.22	0.528	0.007512	69.17	0.551
0.012028	83.21	0.543	0.012021	55.66	0.560
0.016543	77.44	0.558	0.016531	48.30	0.572
0.021059	71.77	0.558	0.021041	41.72	0.580
0.025580	71.63	0.568	0.025552	39.42	0.602

[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 VI.11. Apparent molar volume (ϕ_V) and $(\eta_r\text{-}1)/\sqrt{m}$ of glycine in different mass fractions of aqueous α and β-cyclodextrin mixtures at 298.15 K[a]

molality /mol kg^{-1}	$\phi_V \times 10^6$ / m^3 mol^{-1}	$(\eta_r\text{-}1)/\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$	molality /mol kg^{-1}	$\phi_V \times 10^6$ / m^3 mol^{-1}	$(\eta_r\text{-}1)/\sqrt{m}$ /kg$^{1/2}$mol$^{-1/2}$
Glycine					
	w_1 = 0.001[b]			w_2 = 0.001[b]	
0.0100	41.20	0.070	0.0100	41.19	0.082
0.0252	40.38	0.079	0.0251	40.36	0.093
0.0402	39.95	0.083	0.0402	39.66	0.101
0.0553	39.56	0.088	0.0553	39.37	0.105
0.0704	39.20	0.094	0.0704	38.91	0.107
0.0855	38.95	0.097	0.0855	38.61	0.108
	w_1 = 0.003[b]			w_2 = 0.003[b]	
0.0100	41.17	0.091	0.0100	41.16	0.115
0.0251	40.37	0.102	0.0251	40.36	0.120
0.0401	39.67	0.106	0.0401	39.61	0.130
0.0552	39.18	0.113	0.0552	38.94	0.133
0.0703	38.73	0.116	0.0703	38.45	0.136
0.0855	38.37	0.119	0.0854	37.95	0.144
	w_1 = 0.005[b]			w_2 = 0.005[b]	
0.0100	41.15	0.123	0.0100	41.14	0.130
0.0251	39.96	0.130	0.0251	39.92	0.137
0.0401	39.10	0.135	0.0401	39.11	0.144
0.0552	38.60	0.137	0.0552	38.36	0.150
0.0703	38.01	0.143	0.0703	37.68	0.154
0.0854	37.53	0.151	0.0854	37.11	0.160

[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.

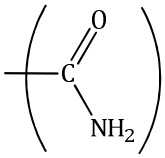
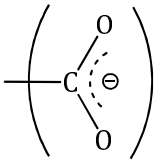
Table VI.12. Limiting apparent molar volume (ϕ_V^o), experimental slope (S_V^*), viscosity *A* and *B*-coefficient of amino acids in different mass fractions of aqueous *α* and *β*-cyclodextrin mixtures at 298.15 K[a]

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* /$kg^{1/2}\ mol^{-1/2}$
		Glycine		
w_1 = 0.001[b]	41.21	-9.31	0.148	0.0029
w_1 = 0.003[b]	41.62	-10.83	0.156	0.0030
w_1 = 0.005[b]	42.02	-12.02	0.168	0.0031
		L-Asparagine		
w_1 = 0.001[b]	95.61	-107.4	0.352	0.387
w_1 = 0.003[b]	105.0	-197.9	0.407	0.384
w_1 = 0.005[b]	115.6	-487.8	0.453	0.370
		L-Aspartic acid		
w_1 = 0.001[b]	90.0	-158.0	0.305	0.406
w_1 = 0.003[b]	100.8	-273.6	0.354	0.426
w_1 = 0.005[b]	110.7	-254.7	0.401	0.501
		Glycine		
w_2 = 0.001[b]	41.66	-10.22	0.158	0.0030
w_2 = 0.003[b]	41.92	-12.09	0.168	0.0033
w_2 = 0.005[b]	42.26	-14.52	0.180	0.0036
		L-Asparagine		
w_2 = 0.001[b]	105.6	-324.3	0.405	0.382
w_2 = 0.003[b]	115.6	-299.5	0.456	0.460
w_2 = 0.005[b]	125.6	-560.5	0.500	0.531
		L-Aspartic acid		
w_2 = 0.001[b]	100.6	-118.9	0.346	0.550
w_2 = 0.003[b]	110.4	-234.9	0.403	0.572
w_2 = 0.005[b]	120.0	-539.9	0.446	0.518

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

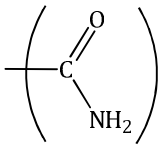
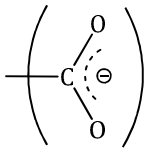
[b] w_1 and w_2 are mass fractions of *α* and *β*-cyclodextrin in aqueous mixture respectively.

Table VI.13. Contributions of the zwitterionic group (NH_3^+), (COO); (CH), (CH_2) and end group to the limiting apparent molar volume (ϕ^0_V) for the amino acids in different mass fraction of aqueous α and β-cyclodextrin respectively at 298.15 K [a]

Groups	$\phi^0_V \times 10^6$ / $m^3\ mol^{-1}$					
	w_1=0.001[b]	w_1=0.003[b]	w_1=0.005[b]	w_2=0.001[b]	w_2=0.003[b]	w_2=0.005[b]
(NH_3^+), (COO)	22.51	22.87	23.21	22.76	22.98	23.28
(CH)	9.35	9.38	9.41	9.45	9.47	9.49
(CH_2)	18.70	18.75	18.81	18.90	18.94	18.98
	45.05	54.01	64.18	54.49	64.21	73.85
	39.44	49.81	59.28	49.49	59.01	68.25

[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 VI.14. Contributions of zwitter ionic group (NH_3^+), (COO); (CH), (CH_2) and end group to the viscosity *B*-coefficient for amino acids in different mass fraction of aqueous *α* and *β*-cyclodextrin respectively at 298.15 K [a]

Groups	*B* / kg mol $^{-1}$					
	w_1=0.001[b]	w_1=0.003[b]	w_1=0.005[b]	w_2=0.001[b]	w_2=0.003[b]	w_2=0.005[b]
(NH_3^+), (COO)	0.092	0.094	0.098	0.094	0.098	0.100
(CH)	0.028	0.031	0.035	0.032	0.035	0.040
(CH_2)	0.056	0.062	0.070	0.064	0.070	0.080
	0.176	0.220	0.250	0.215	0.253	0.280
	0.129	0.167	0.198	0.156	0.200	0.226

[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 VI.15. Hydration number (n_H), and solvation number (S_n) of the amino acids at different mass fraction of aqueous α and β-cyclodextrin respectively at 298.15 K[a]

	n_H			S_n		
	0.001	0.003	0.005	0.001	0.003	0.005
Aqueous α-CD (w_1)[b]						
L-Asparagine	3.52	3.27	3.09	3.68	3.88	3.92
L-Aspartic acid	3.61	3.35	3.17	3.39	3.51	3.62
Aqueous β-CD (w_2)[b]						
L-Asparagine	3.41	3.18	2.97	3.84	3.94	3.98
L-Aspartic acid	3.49	3.21	3.04	3.44	3.65	3.72

[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

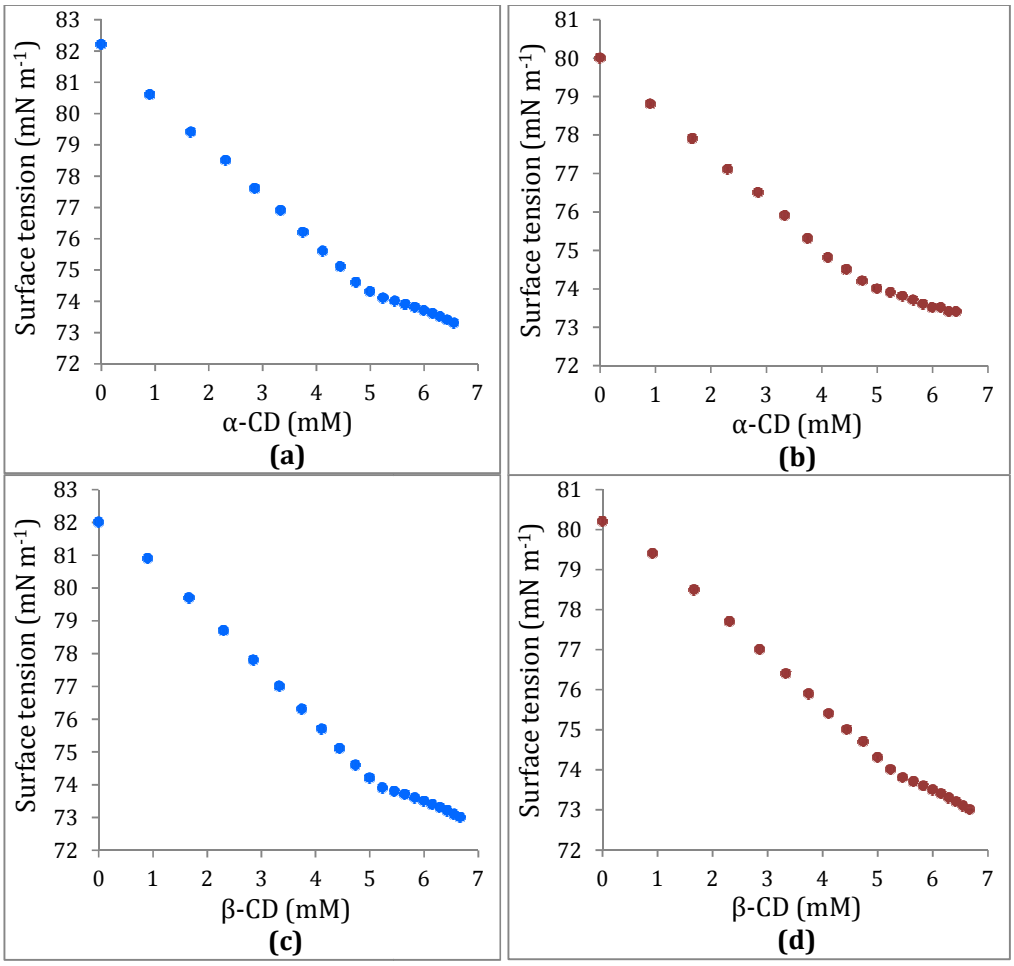


Figure VI.1. Variation of surface tension of aqueous (a) L-Asn-α-CD, (b) L-Asp-α-CD, (c) L-Asn-β-CD and (d) L-Asp-β-CD systems respectively at 298.15K.

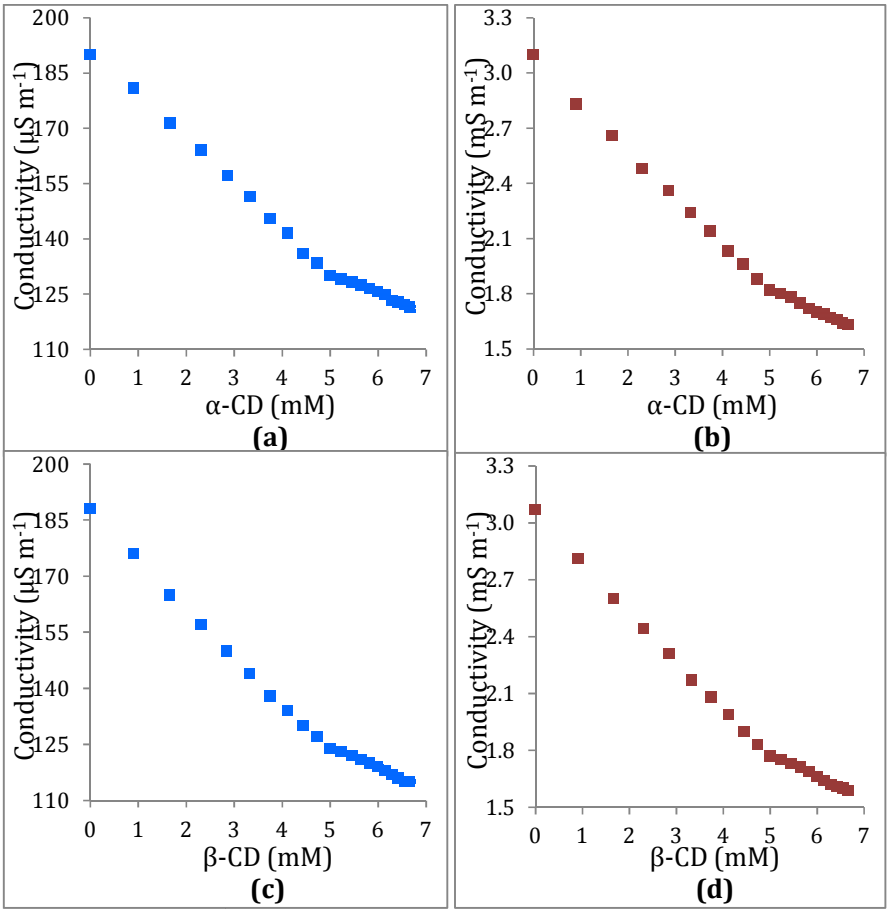


Figure VI.2. Variation of conductivity of aqueous (a) L-Asn-α-CD, (b) L-Asp-α-CD, (c) L-Asn-β-CD and (d) L-Asp-β-CD systems respectively at 298.15 K.

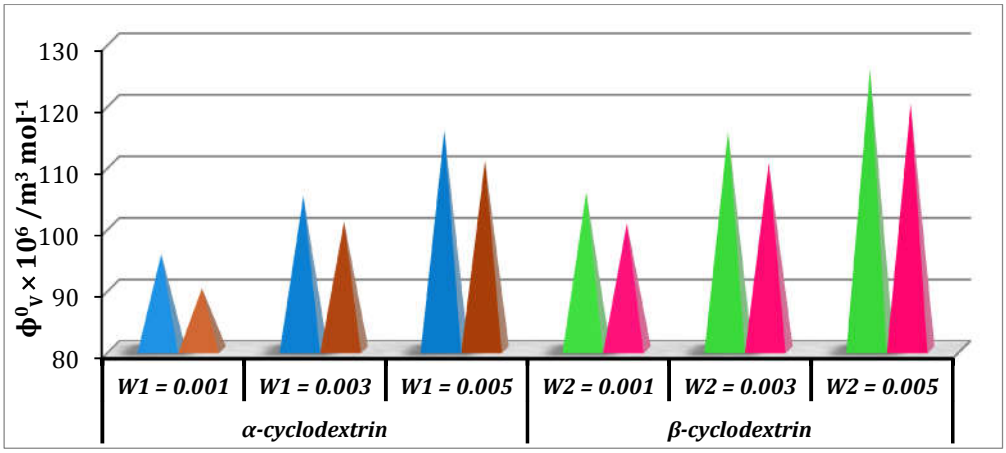
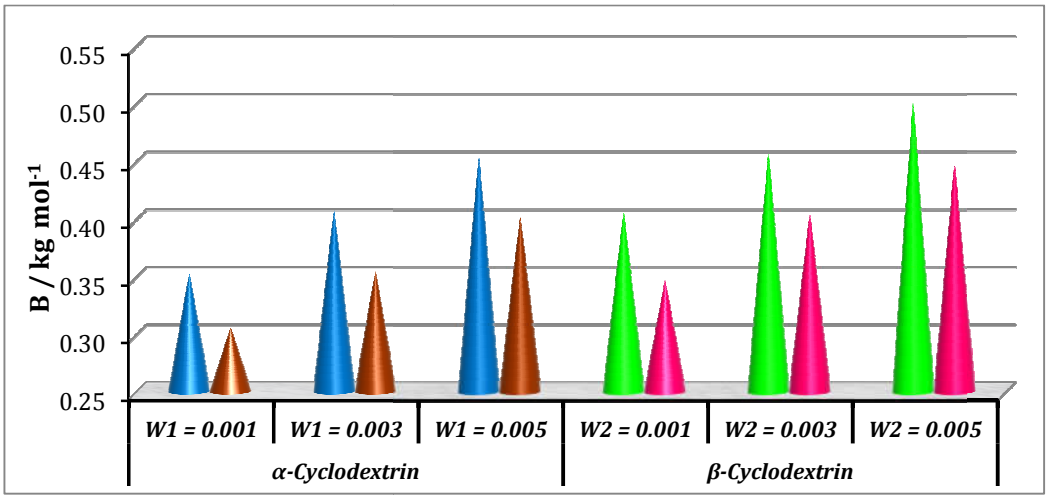
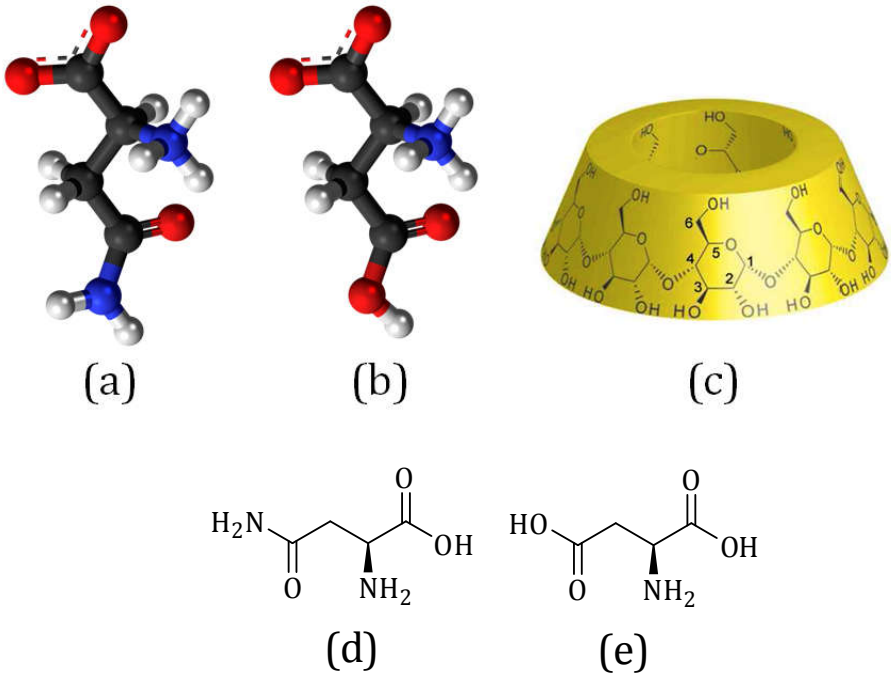


Figure VI.3. Limiting molar volume (ϕ^0_V) versus mass fraction (w) of aq. α-CD and aq. β-CD for L-Asn (blue & green) and L-Asp (brown & pink) respectively at 298.15 K.

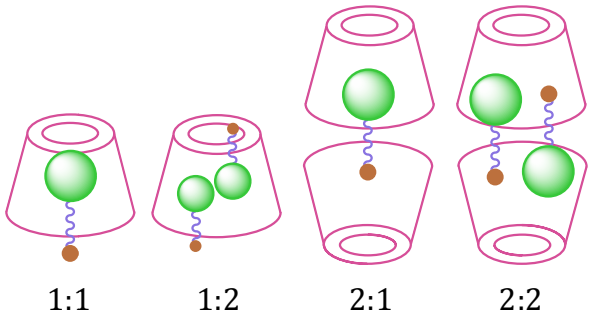


FigureVI. 4. Plot of viscosity B-coefficient against mass fraction (w) of aq. α-CD and aq. β-CD for L-Asn (blue & green) and L-Asp (brown & pink) respectively at 298.15 K.

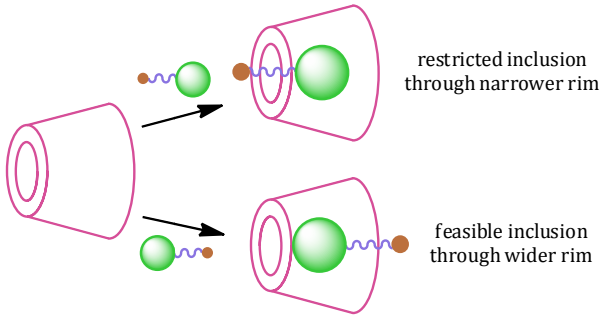
Schemes



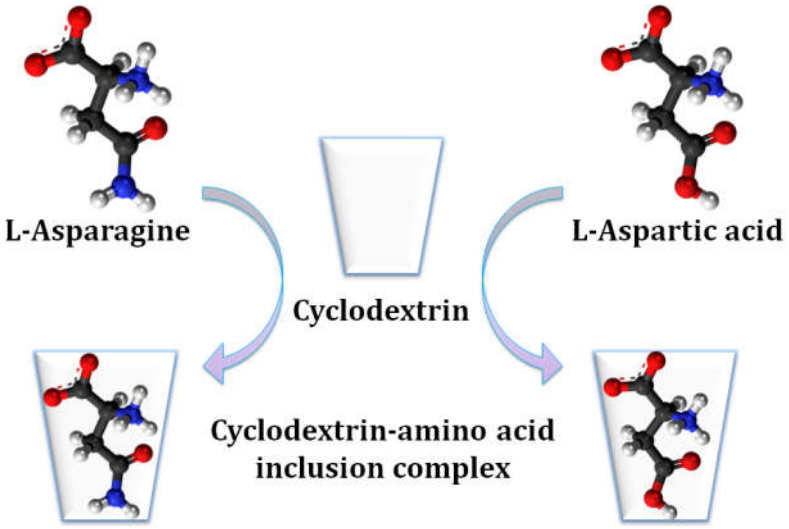
Scheme VI.1. 3D model of (a) L-Asn, (b) L-Asp (red: oxygen, blue: nitrogen, black: carbon, white: hydrogen), (c) cyclodextrin molecule and chemical structure of (d) L-Asn and (e) L-Asp.



Scheme VI.2. Proposal of various possibilities of host guest ratio of inclusion complex.



Scheme VI.3. Proposed possible and controlled inclusion of the guest into the host molecule.



Scheme VI.4. Plausible schematic presentation of the mechanism for formation of 1 : 1 inclusion complex of L-Asn and L-Asp with both α and β-cyclodextrin.