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RESEARCH ARTICLE

HOLLOW TORUS BASED ON INCLUSION OF BIOACTIVE MOLECULE INVESTIGATED BY SURFACE TENSION, CONDUCTANCE AND NMR

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ABSTRACT

The host –guest interaction of an amino acid (L-cysteine) as guest with α and β cyclodextrines have been investigated which have significant applications in in the field of medicine such as controlled drug delivery. The ^{1}H NMR study confirms the formation of inclusion complex while surface tension and conductivity studies support the formation inclusion complex with 1:1 stoichiometry. The host-guest interaction has been explained on the basis of hydrogen bonding, Vanderwaal's force and exceptional structure of cyclodextrin.

INTRODUCTION

In modern day cyclodextrin (CD) are used for the controlled release of various compounds and drugs (Roy *et al*., 2016). Due to the exceptional truncated conical shape it has the capability to form inclusion complex with a variety of guest molecules including drug, vitamins, ionic liquids, neurotransmitters etc (Yang *et al*., 2013). Cyclodextrin is a cyclic oligosaccharides having glucopyranose units six (α-CD), seven (β-CD) and eight (γ-CD) linked by α-(1-4) bonds (Szejtli, 1998) (scheme 2) The conical structure of CD have hydrophobic interior and hydrophilic rim with primary and secondary –OH groups. These hydroxyl groups are responsible of forming hydrogen bonding with guest molecules (Szejtli, 1996) CD is considered safe for human body. In order to be biologically active a molecule should retain its integrity and should be able to cross the lipophilic membrane. CD has the ability to encapsulate the guest without any chemical modification of it. Sometimes it also increases the solubity of guest. The controlled release of CD is also used in food cosmetic, paint industry and removal of different toxic materials, pollutants, waste products without any chemical change (Connors, 1997). L-Cysteine is an amino acid which is building block of protein. (Scheme 1) It is a powerful anti oxidant. It is also used to metabolize of lipid, boosting the immune system. L-cysteine increase malie fertility, reduce inflamination and combarts decrease osteoporosis. In the body, cysteine is also used to produce the amino acid taurine as well as coenzyme A, biotine and heparin. Cysteine is component in beta karatin and it is proved that it preserve skin elasticity. It also protects the lining of digestive system (Grunberger *et al*., 2007; Safarinejad, 2009). In the present study we investigate the nature of formation and stoichiometry of inclusion complex of α and β-CD with natural amino acid L- cysteine in aqueous media. Aim of this work is the formation, carrying and controlled release of L- cysteine by forming inclusion complex with host cyclodextrin molecules without chemical and biological modification of the guests.

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Experimental Section

Source and Purity of Samples

The amino acid L-cysteine and CDs of purists grade were purchased from Sigma-Aldrich, Germany. The mass fraction purity of L-cysteine, α-CD and β-CD were 0.97,0.98,0.98 respectively.

Apparatus and Procedure

Conductance measurement were carried out in Mettler toledo seven multi conductivity meter having uncertainty 1.0 μSm^{-1}.The conductivity of solution were studied in a thermo

stated water bath at 298.15K with accuracy 0.001K.HPLC grade water was used with specific conductance $10\mu Sm^{-1}$. The 0.01M aqueous KCL solution using for calibrated of the conductivity cell. Surface tension of the solution were studied by platinum ring detachment technique using a tensiometer (K9, KRUSS; Germany) at 298.15K with uncertainty $0.1mN.m^{-1}$. The temperature of the system was maintained by circulating thermo stated water through a double- wall glass vessel holding the solution. NMR spectra were recorded using D_2O as a solvent. ^1H NMR spectra were recorded at 298.15K in 400 MHz and 500 MHz respectively using Bruker ADVANCE 400MHz and 500MHz instrument. Residual protonated solvent Signals are quoted as δ values in ppm using internal standard (D2O:δ 4.79ppm). Data are reported as chemical shifts.

RESULTS AND DISCUSSION

^1H NMR study establishes inclusion

^1H-NMR study confirms the inclusion phenomenon between the host CDs and the above mentioned amino acid cysteine (Roy *et al*., 2016; Roy *et al*., 2016) In the present work the molecular interactions of L-cysteine with α and β-cyclodextrins have been studied using the ^1H NMR spectra by taking a 1 : 1 molar ratio of the amino acid and CDs in D2O at 298.15 K. Insertion of L- cysteine in the hydrophobic cavity of CDs results chemical shifts of both the acid and CDs due to interaction between them. From scheme 3 it can be observed that the H3 and H5 protons of CD are located inside the cavity

Table 1. Data for surface tension study of aqueous L-Cystein with *α*-CD and β-CD system at 298.15K[a]

Volm of CD (mL)	Total volm (mL)	Conc of L-cysteine (mM)	Conc of CD (mM)	Surface tension in α-CD (mN m^{-1})	Surface tension in β-CD (mN m^{-1})
0	10	10.000	0.000	64.2	64.2
1	11	9.091	0.909	65.3	65.3
2	12	8.333	1.667	66.2	66.2
3	13	7.692	2.308	67.0	66.9
4	14	7.143	2.857	67.7	67.6
5	15	6.667	3.333	68.3	68.2
6	16	6.250	3.750	68.8	68.7
7	17	5.882	4.118	69.3	69.1
8	18	5.556	4.444	69.7	69.5
9	19	5.263	4.737	70.1	69.9
10	20	5.000	5.000	70.6	70.1
11	21	4.762	5.238	70.8	70.2
12	22	4.545	5.455	70.9	70.3
13	23	4.348	5.652	71.0	70.4
14	24	4.167	5.833	71.2	70.5
15	25	4.000	6.000	71.3	70.6
16	26	3.846	6.154	71.4	70.7
17	27	3.704	6.296	71.5	70.8
18	28	3.571	6.429	71.7	70.9
19	29	3.448	6.552	71.8	71.0
20	30	3.333	6.667	71.9	71.1

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

Table 2. Data for conductivity study of aqueous L-cysteine with α and *β*-CD system at 298.15K[a]

Volm of *β*-CD (mL)	Total volm (mL)	Conc of L-Leucine (mM)	Conc of –CD (mM)	Surface tension (mN m^{-1})	Surface tension (mN m^{-1})
0	10	10.000	0.000	130	130
1	11	9.091	0.909	127	128
2	12	8.333	1.667	124	125
3	13	7.692	2.308	122	123
4	14	7.143	2.857	120	122
5	15	6.667	3.333	118	120
6	16	6.250	3.750	116	119
7	17	5.882	4.118	115	118
8	18	5.556	4.444	114	117
9	19	5.263	4.737	113	116
10	20	5.000	5.000	112	115
11	21	4.762	5.238	112	115
12	22	4.545	5.455	112	114
13	23	4.348	5.652	111	114
14	24	4.167	5.833	111	114
15	25	4.000	6.000	111	114
16	26	3.846	6.154	111	114
17	27	3.704	6.296	111	114
18	28	3.571	6.429	110	114
19	29	3.448	6.552	110	114
20	30	3.333	6.667	110	114

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

Table 3. Values of surface tension (γ) at the break point with corresponding concentrations of cyclodextrins and amino acids and values of conductivity () at the break point with corresponding concentrations of cyclodextrins and amino acids at 298.15 K

Amino acid	Conc. of α-CD/mM	Conc. of amino acid/mM	γ^a/mNm^{-1}
Cystine	5.27	4.73	70.77
	Conc. of β-CD/mM	Conc. of amino acid/mM	γ^a/mNm^{-1}
	4.79	5.20	69.91
	Conc. of α-CD/mM	Conc. of amino acid/mM	$\kappa^a/\mu Sm^{-1}$
	4.72	5.28	112.76
	Conc. of β-CD/mM	Conc. of amino acid/mM	$\kappa^a/\mu Sm^{-1}$
	5.20	4.8	114.52

whereas H1, H2 and H4 are situated in the exterior of CD molecules. Among H3and H5, H3 are near to the wider rim and H5 are closer to the narrower rim (Caso *et al*., 2015). As most of the guest molecules are inserted through the wider rim, the H3 proton is more shifted compared to H5. In the present study the ^{1}H-NMR spectra of both the CD, L-cysteine inclusion complexes, the H3 and H5 protons of CD and the protons of acid show considerable shift. (Figure 1 and Figure 2) The other protons of CD show little shift in the spectra. This fact is in agreement with the formation of inclusion complex (Wang *et al*., 2014). As indicated by chemical shift data the interaction of the H3 proton with cysteine is much higher than H5 probably due to insertion of the amino acid through the wider rim.

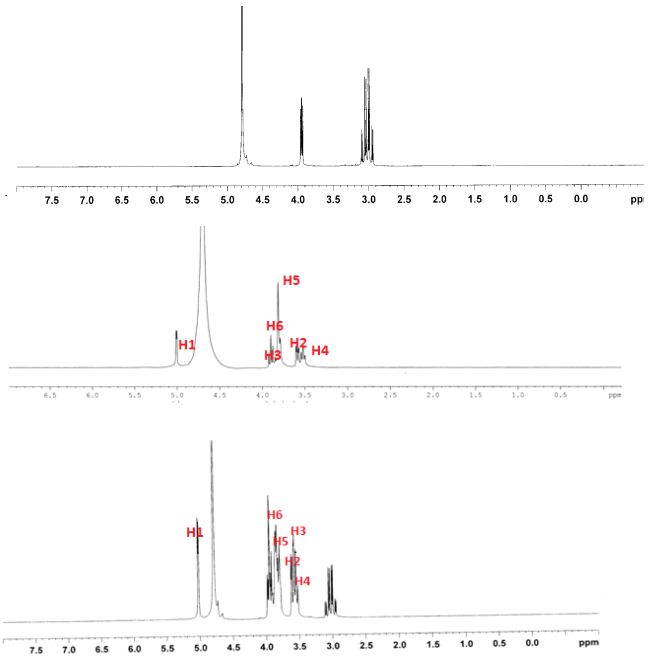


Figure 1. ^{1}H NMR spectra of (a)cysteine (b) α-CD and (c) 1:1 M ratio of α-CD & cysteine in D_2O at 298.15 K

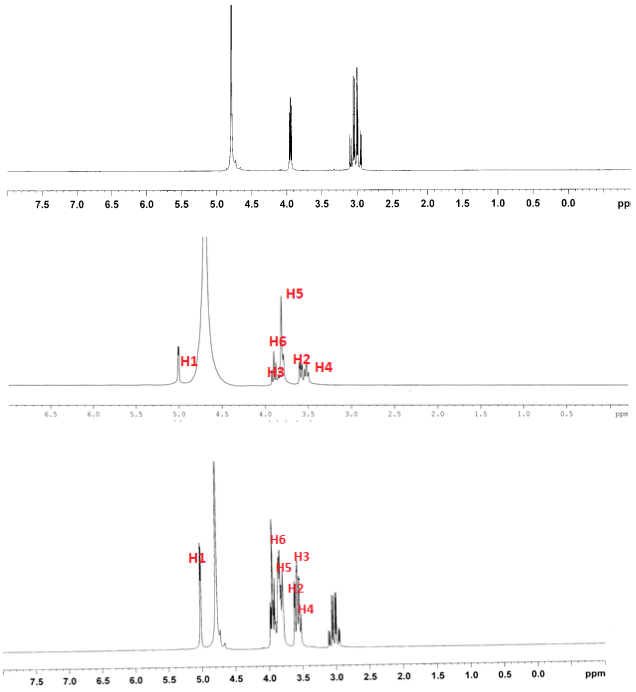


Figure 2. ^{1}H NMR spectra of (a)cysteine (b) β-CD and (c) 1:1 M ratio of β-CD & cysteine in D_2O at 298.15 K

Surface tension study supports inclusion

Surface tension gives valuable information about the nature and formation of inclusion complex (Roy *et al*., 2014). The aqueous solution of CD do not show any considerable change of surface tension. The amino acid show the existence of $NH_3^+$and COO^- in their zwitterionic forms (Roy *et al*., 2015).Thus side group being non polar L- cysteine show surfactant like behavior and it has a tendency to decrease the surface tension of aqueous solutions like other surfactants (Pineiro, 2007). Here surface tension (γ) is measured for a series of solution with increasing concentration of both host α and β cyclodextrin at 298.15K. The γ values shows increasing trend in case of both the guests. (Table 1) Perhaps it is due to the formation of inclusion complex between L- cystein and CD because due to the removal of the surface active L- cysteine molecules from the surface of the solution into the hydrophobic cavity α and β cyclodextrin. In the two surface tension plots appearance of single break point indicates formation of inclusion complex (Figure 3). The values of surface tension with corresponding concentration of α and β cyclodextrin and concentration of cystine at each break has been listed in Table 1. Over all variation of γ and one beak point clearly show that at certain concentration of amino acid and CD where their concentration ratio in solution was almost 1:1, thus the study proves 1:1 ratio in both α and β CD (Gao 2006) (Table 3).

Conductivity study informs inclusion

The conductivity measurement also gives valuable information not only about the inclusion phenomena but also the stoichometry of the inclusion complex formed (Apelblat *et al*., 2007; Qian *et al*., 2013). If inclusion complex is formed by L-cystein with α and β CD, the conductivity of the solutions distinctly affected. The amino acid L- cystein exists as zwitterions and due to the existence of this charged structure, it shows considerable conductivity. With addition of both the host α and β-CD the conductivity gradually decrease indicating the amino acid molecule enters into the hydrophobic cavity of α or β CD.(Table 2) The conductivities of a series of solution having 10 $mmolL^{-1}$ concentration of aqueous solution of cystein with increasing concentration of cyclodextrins have been measured. The trend of conductivity regularly declining which indicates formation of the inclusion complex between CD and amino acid. A sharp single break is found in the conductivity curve in each case (Figure 4). This is again in agreement with the fact that1:1 host – guest inclusion complex is formed between L- cystine and CD's (Roy *et al*., 2016; Saha *et al*., 2016) a dynamic equilibrium is attained between the guest amino acid and host CD molecules. The break point is that at which maximum inclusion takes place.

Structural influence of cyclodextrin

Cyclodextrins are molecules with inner hydrophobic cavity and hydrophilic rims which provide an opportunity to act as as host molecules. The guest molecule's apolar part reside inside the cavity and polar part of the guest molecule makes association with the polar rims, thereby forming stable inclusion complex .The apolar cavity diameter of α -CD is 4.7-5.3Å and β- CD is6-6.5Årespectively (Saha *et al*., 2016) The size of natural amino acid L-Cysteine is within the range which can be easily encapsulated inside the cavity of CD.

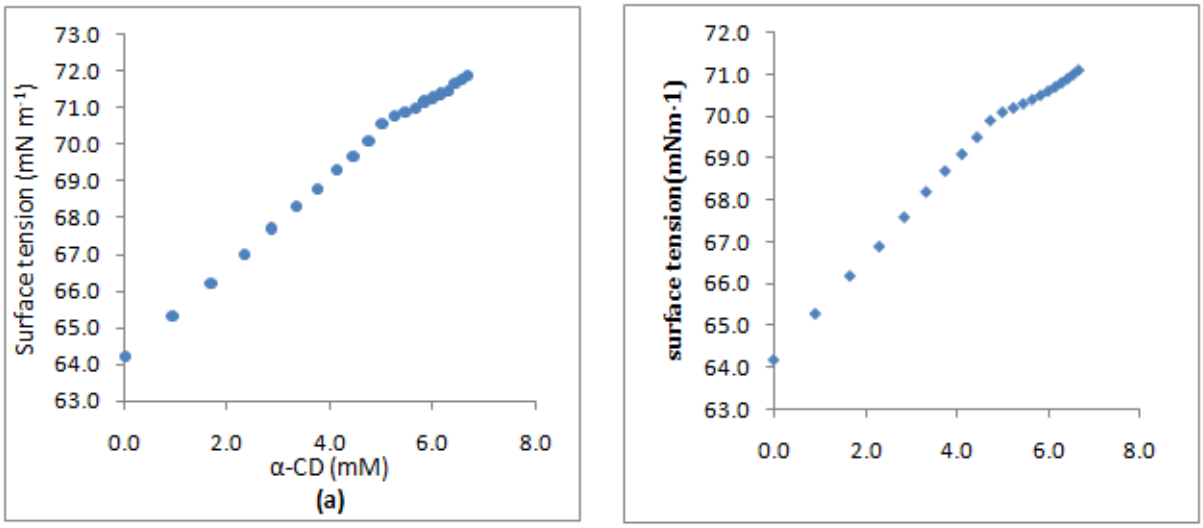


Figure 3. Variation of surface tension of aqueous (a) cysteine-α-CD and (b) cysteine-β-CD systems respectively at 298.15 K

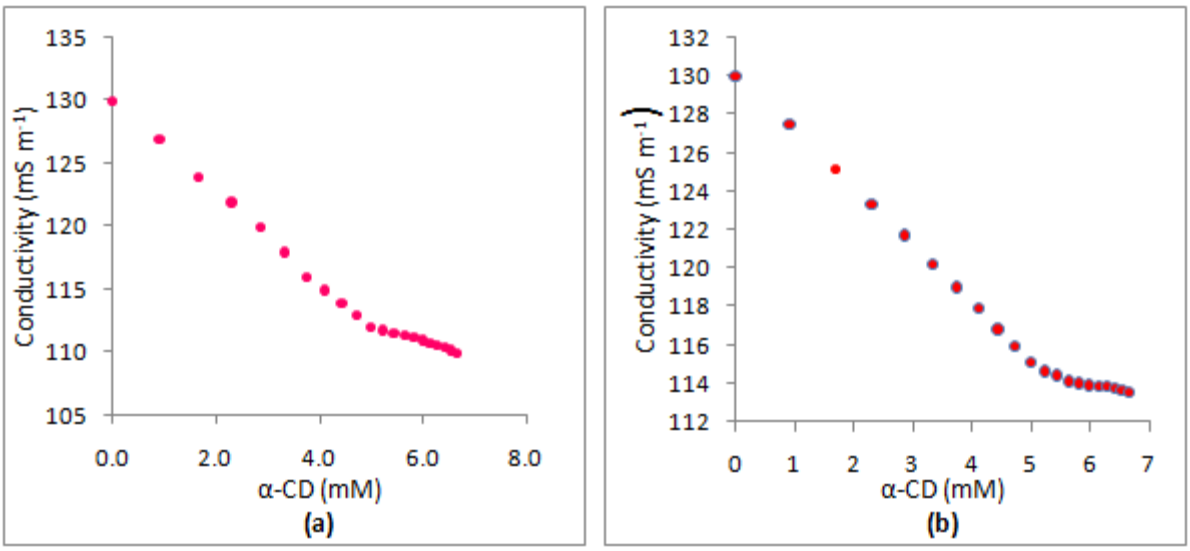
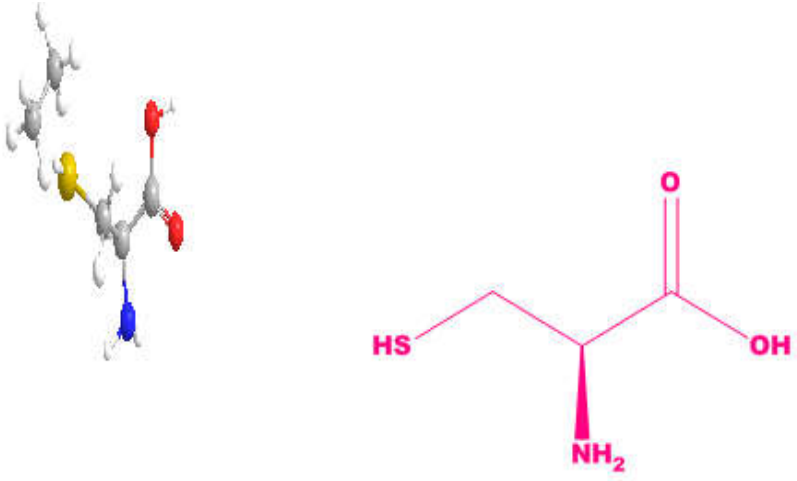
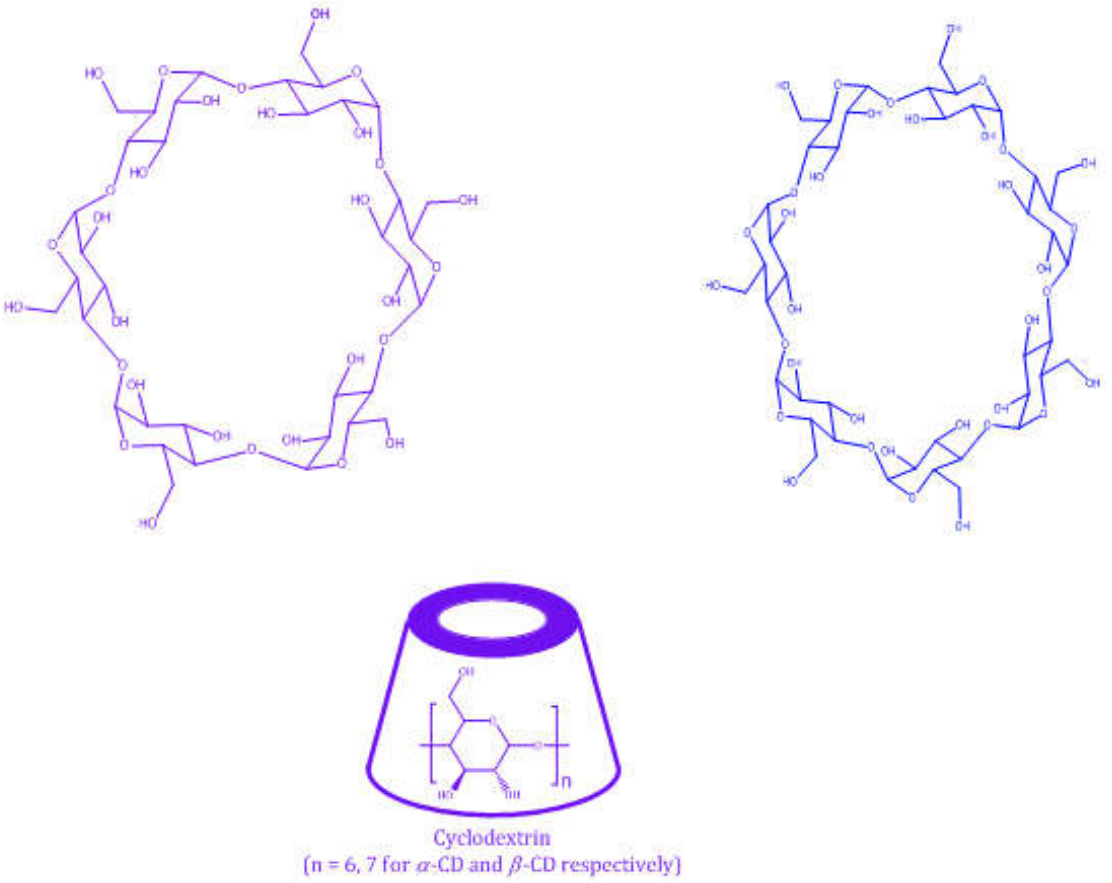


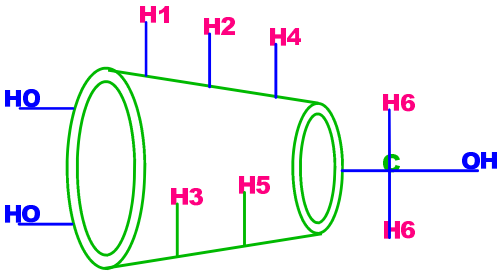
Figure 4. Variation of conductivity of aqueous (a) cysteine-α-CD and (b) cysteine-β-CD systems respectively at 298.15 K



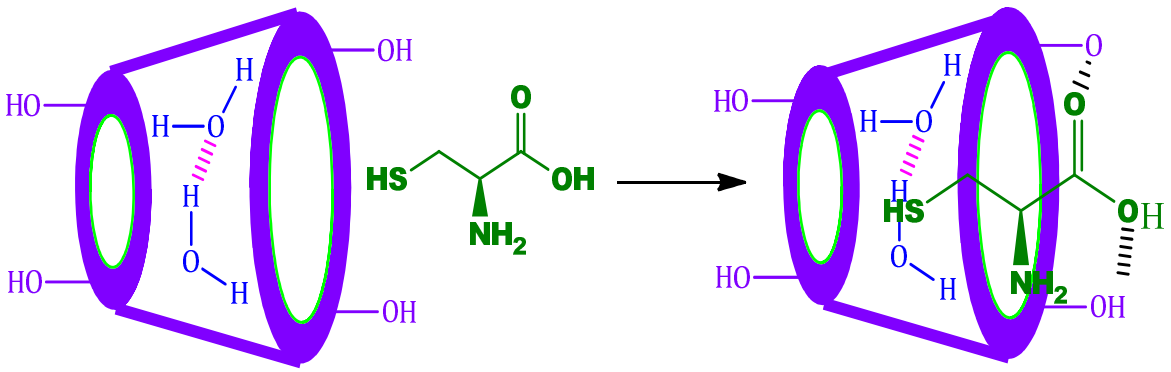
Scheme 1. Molecular (a) Three dimensional and (b) Two dimensional structure of amino acid cysteine



Scheme 2. Structure of cyclodextrin molecules



Scheme 3. Truncated conical structure of α and β-cyclodextrin



Scheme 4. Plausible Schematic representation of mechanism for the formation of (1 : 1) inclusion complex of cysteine with both *α* and *β*-cyclodextrin.

There is no covalent bond formation or breaking during the formation of inclusion complex (Saha *et al*., 2016). The polar water molecules are present inside the slightly apolar cavity of cyclodextrine. This is generally energetically unfavoured. So the polar water molecules are readily substituted by hydrophobic chains of the amino acids. This results a more stable energy state. The stoichiometry of the inclusion complex is found as 1:1, which is supported by conductivity and surface tension measurements. So after inclusion of one amino acid molecule the zwitterionic part blocks the rim by making hydrogen bonding with the rim –OH groups, so second molecule cannot enter. Hydrophobic part of L- cysteine was found to be inserted through the wider rim of cyclodextrin.

Conclusion

The ^{1}H-NMR spectra, surface tension and conductivity study shows that the natural amino acid L-cysteine forms host-guest inclusion complex with both the CDs. The surface tension and conductivity study suggests the inclusion complex formation and 1:1 stoichiometry of the complex while the NMR data confirms the inclusion. These two inclusion complexes have vast applications in the field of bio-chemistry.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: No human participation involved in this work.

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