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

Aditi Roy, Subhadeep Saha, Mahendra Nath Roy*

*Department of Chemistry, University of North Bengal, Darjeeling 734013, India*

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ABSTRACT

Molecular inclusion of two natural amino acids, *viz.*, L-asparagine and L-aspartic acid as guest into the host cavity of $\alpha$ and $\beta$-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.



## 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 ($\alpha$-CD), seven ($\beta$-CD) and eight ($\gamma$-CD) glucopyranose units, bound together by $\alpha$-(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 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 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 $\alpha$-amino acids *viz.* L-Asn and L-Asp in 0.001, 0.003, 0.005 mass fractions of $\alpha$ and $\beta$-CDs in aqueous media with the help of the various important properties such as surface tension, conductivity, density, viscosity and pH.

## 2. Experimental section

### 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, $\alpha$-CD and $\beta$-CD were $\geq$0.99, 0.99, 0.98 and 0.98 respectively.

### 2.2. Apparatus and procedure

Solubilities of the two CDs and that of the above two $\alpha$-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)

* Corresponding author.
*E-mail address:* mahendraroy2002@yahoo.co.in (M.N. Roy).

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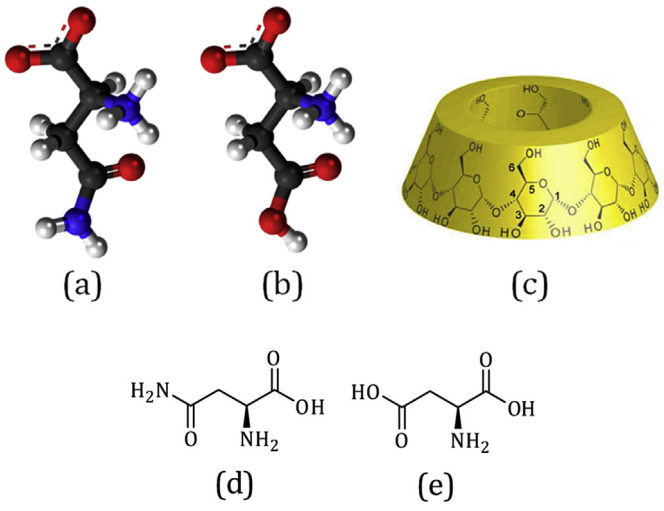


**Scheme 1.** 3D model of (a) L-Asn, (b) L-Asp (red: oxygen, blue: nitrogen, black: carbon, white: hydrogen), (c) cyclodextrin molecule and chemical structure of (d) L-Asn and (e) L-Asp.

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 $\mu$S $m^{-1}$. The study was carried out in a thermostated water bath at 298.15 K with uncertainty ±0.01 K. HPLC grade water was used with specific conductance 6.0 $\mu$S $m^{-1}$. The conductivity cell was calibrated using 0.01 M aqueous KCl solution.

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

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

## 3. Result and discussion

### 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 298.15 K. The pH value decreases with increasing concentration of the two amino acids, also with increasing concentration of $\alpha$ and $\beta$-CD respectively (Table S2). 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 $\beta$-CD has one more glucopyranose unit, it has more number of −OH groups, thus interaction with amino acids is more in case of $\beta$-CD than in case of $\alpha$-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 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.

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

Surface tension ($\gamma$) 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 $\gamma$ of their aqueous solutions, but there is no significant change in $\gamma$ 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 $\gamma$ of their solutions [16]. In presence of α and β-CD the surface tension is markedly affected. Here $\gamma$ of aqueous amino acids has been measured with increasing concentrations of $\alpha$ and $\beta$-CD at 298.15 K (Tables S4–S7).

The surface tension values were declining regularly for both the two amino acids with increasing concentration of α and $\beta$-CD might be due to the formation of inclusion complexes inside the cavity of α and $\beta$-CD (Fig. 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 1). More break points in the curve would imply complex stoichiometries (1:2, 2:1, 2:2 etc.) of the inclusion complexes (Scheme 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 3).

### 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 (Tables S4–S7). 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 (Fig. 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