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UV-Vis Spectroscopic Characterization of β-Cyclodextrin-Vanillin Inclusion Complex

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Abstract: Cyclodextrin molecules can form inclusion complexes with various compounds of appropriate shape and size. The complexation can enhance the solubility and stability of the inclusion guest compound. The stoichiometry and stability constant of the host-guest complex are highly important for physical, chemical, biological, and environmental studies. A simple and rapid spectroscopic method investigated the inclusion of vanillin and β-cyclodextrin (β-CD). The continuous variation technique was used to estimate the stoichiometry of the inclusion complex. The association constant of vanillin with β-CD was determined by using Benesi-Hildebrand and Scott's methods which were calculated to be 179 and 187 $M^{-1,}$ respectively, with the stoichiometry ratio, was 1:1 for the inclusion complex of β-CD with vanillin.

Keywords: Vanillin; Cyclodextrin; Inclusion complex; Association constant; UV-Vis spectroscopy.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucose units obtained from the enzymatic degradation of starch [1-3]. The most common forms of CDs consist of 6, 7, and 8 glucose units linked by α-1,4 glycosidic bonds and are named α, β and γ CD, respectively (Figure 1a) [4,5]. CD is known to have a truncated cone shape with the characteristic of having a hydrophobic inner cavity and hydrophilic outer surface [2,5,6] (Figure 1b). The most significant feature of CDs is their lipophilic cavity which provides a microenvironment to a wide variety of appropriately sized hydrophobic compounds to form inclusion complexes [7-11] through the release of enthalpy-rich water molecules [12-15].

The formation and stability of the host-guest complex depend on various factors, including the cavity size of CD and the guest. The second critical factor is the thermodynamic interactions between the three components, guest, CD and solvent, to form a more favorable driving force that causes the guest to enter into the CD cavity [1,15]. No covalent bonds are formed or broken during the complex formation, and the guest molecules in the complex are in equilibrium with free molecules in the media surrounding the CD [16,17]. The main forces for the complex formation of the host with the guest are; van der Waals interaction, hydrophobic interactions, hydrogen bonding, and electrostatic attraction [1,4,18-20]. As a result of this complex formation, the physical and chemical properties of the guest molecules are improved, such as water solubility, reduced volatility, suppression of unpleasant odors or tastes, and stability against light, heat, or oxidation. [21-26]. Therefore, cyclodextrins have been applied in food, cosmetics, and pharmaceutical preparations [27-30].

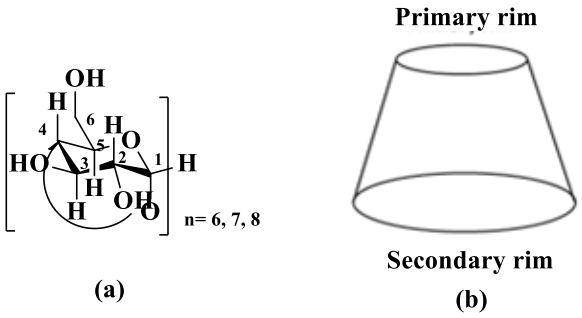

Figure 1. Chemical structure of (a) α, β and γ CDs (composed of 6, 7 and 8 glucose units respectively) and (b) the truncated cone shape of CD.

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Currently, the inclusion interactions of CDs with guests have been widely investigated by various analytical techniques [7,8,31,32]. The most common analytical methods used to study the solid state of the complex are Differential scanning calorimetry (DSC) [33-35], X-ray Powder Diffractometry (XRD) [8,36,37], scanning electron microscopy (SEM) and Fourier Transform Infrared spectroscopy (FT-IR) [8,37].

Solution studies of the complex can be achieved using sound velocity [38], surface tension [39], potentiometry [40], Nuclear Magnetic Resonance (NMR) [37,41,42], chromatographic methods [43-46], high-performance liquid chromatography (HPLC) [47], fluorescence [48,49] and Ultraviolet-Visible (UV-Vis) spectroscopy [37,50-52].

Vanillin (4-hydroxy-3-methoxybenzaldehide) is the primary component of the extract of the vanilla bean (Figure 2a). It is artificially produced more cheaply from the petrochemical raw material guaiacol via chemical processes. Vanillin is significantly added as a flavoring agent in food and also in ice cream and chocolate industries. It can be used as a food preservative because of its antioxidant and antimicrobial properties [53].

Furthermore, it has been used as a catalyst in different polymerization reactions and as a chemical intermediate in cosmetics and pharmaceutical preparation [54]. The inclusion complexes offer great potential to protect and stabilize volatile flavors before use in foods [55-57]. As a result of improving the protection of vanillin toward oxidation in water after forming complexes with CDs, it can help solve its instability problem [53].

Therefore, much attention has been paid to investigating the interaction between vanillin and CDs. Divakar and Maheswaran studied the orientation of vanillin inside the CD cavity by detailed spectroscopic (NMR, UV-visible, fluorescence) and potentiometric studies [58] Karathanos et al. prepared the inclusion complexes of vanillin and β-CD (Figure 2b) by a freeze-drying method. Then, the complexes were analyzed in the solid state by DSC and in solution by NMR, whereas the solubility was determined by a phase solubility study [35]. Pîrnău et al. also investigated the inclusion of vanillin with β-CD by NMR method [53].

Computational study of the vanillin-β-CD inclusion complex was investigated using energy decomposition analysis, natural bond orbitals, non-covalent interactions-reduced density gradient, and independent gradient model to quantify the nature of non-covalent intermolecular between vanillin and β-CD suggesting the structure of the inclusion complex [59].

No key standard techniques can be applied to obtain in-depth information on the inclusion complex of CD with different guests. Therefore, investigation of the complex is usually performed using various analytical techniques for a better and complete understanding of the complex formation. NMR is a valuable technique that can be used to study inclusion complexes formed in solution. However, other spectroscopic techniques, such as fluorescence and UV spectroscopy in addition to HPLC, isothermal calorimetry, and capillary electrophoresis, can provide comprehensive information about the host and guest interaction inclusion complex [60]. UV-Vis spectroscopy is still widely used when sophisticated, expensive equipment is unavailable. It is well known to be a simple, easy, fast, and reliable technique for the determination of equilibrium constants in comparison to other analytical methods such as chromatography and HPLC. Accordingly, UV-Vis spectroscopic techniques were employed in this study to determine the binding constant for the vanillin – β-CD system using a linear mathematical model according to Benesi-Hildebrand and Scott's equations. Also, the complex stoichiometry was calculated using the continuous variation method based on the UV data.

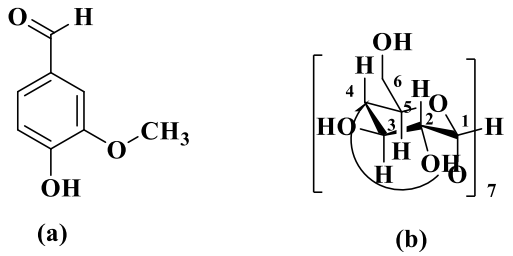

Figure 2. Chemical structure of (a) vanillin and (b) β-CD

2. Experimental

2.1. Materials

β-CD was purchased from SAFC, USA, and vanillin from Sigma-Aldrich, UK. Both chemicals were used as supplied without any further purification.

2.2. Methods

Two stock solutions of both vanillin and β-CD were prepared in water based on the continuous variation method with the original concentration of 5 mM. The series of nine samples from both the vanillin and β-CD were prepared by mixing the two solutions at

different proportions to constant volume at 10 mL so that a complete range ($0 < r < 1$) of the ratio $r = [X] / ([G] + [H])$ was sampled. X = G or H, and [G] and [H] are the total concentrations of the guest (vanillin) and the host (β-CD), respectively. The total concentration of both vanillin and β-CD was kept constant for each solution at 0.05 mM.

For constant association determination, 0.5 mL aliquot of 1 mM stock solution of vanillin (0.05 mM) was transferred into 10 mL volumetric flasks containing increasing concentrations of β-CD (2, 4, 6 and 8 mM), and each flash was diluted to the final volume with water.

2.2. Instrumentation

The samples are analyzed in a glass cuvette and positioned in the Varian CARY 50 Probe UV-Visible spectroscopy using Cary WinUv software. The blank was also run between the solvent used for the prepared samples.

3. Results and discussion

3.1. Determination of the stoichiometry

Before proceeding with the calculation of the binding constants, it is important to study the stoichiometry of the vanillin-β-CD complex. One of the best methods used to explore the stoichiometry of the inclusion complex between the host and the guest is the continuous variation method or so-called Job's plot [61,62], which has been applied here using UV-Vis spectroscopy. The absorbance of the vanillin during the addition of the β-CD was measured at 280 nm. The change in the absorbance (ΔA) was calculated by measuring the absorbance of vanillin in the absence (A_0) and presence (A) of β-CD. The calculated quantities Δ A[van] was plotted versus r (= [G] / [G] + [H]), where [G] and [H] are the concentrations of vanillin and β-CD in the van- β-CD complex. The resulting plot showed a maximum value of r at 0.5 with a highly symmetrical shape (Figure 3), revealing a 1:1 stoichiometry ratio of the complexation.

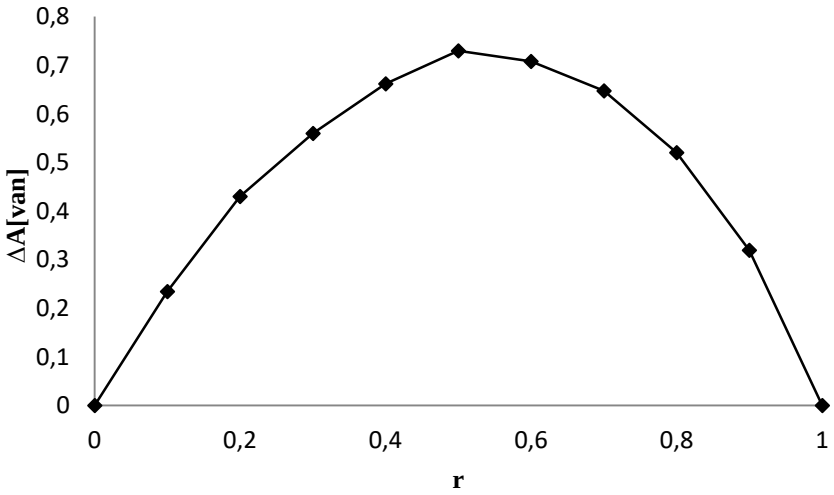

Figure 3. Job's plot of vanillin with β-CD inclusion complex showing 1:1 stoichiometric. Absorbance measurements were carried out at 280 nm

3.2. Determination of the association constant

The Benesi- Hildebrand method is a common way to study the inclusion complex between the host and the guest [63,64]. The UV absorption spectra of vanillin in the absence and presence of β-CD are shown in Figure 4. It can be observed that there were obvious bands at 205, 230, and 280 nm, which could be assigned to π-π* electronic transitions of the phenyl ring. In contrast, the band at 309 nm was assigned to n-π* transitions of the carbonyl group of the aldehyde substituent in the vanillin [65]. No absorption band was observed for β-CD due to the absence of either π-electrons or non-bonding electrons [66]. With the increase of β-CD concentration, the absorption bands showed a hyperchromic effect, although the concentration of vanillin was constant. The significant increase in the absorbance may be probably due to a partial shielding of the excitable chromophore electrons of vanillin by hydrophobic CD when vanillin was embedded into the CD cavity (evaluation) [67-70]. This change observed may also be attributed to the replacement of the solvation shell around the guest entirely or partially by the CD molecule, which creates a new environment for the interactions suggested by the formation of the inclusion complex [71]. Meryem et al. investigated the intermolecular interaction between vanillin and β-CD, suggesting that both H-bond and van der Waals interactions were the major contributions to stabilizing the inclusion complex and thus concluding the vanillin was totally embedded into the β-CD [59]. Other NMR studies showed that the complexation of vanillin induced the chemical shift of the vanillin protons suggesting the vanillin was oriented towards the β-CD cavity and thus confirming vanillin-β-CD complex formation [35,53,58].

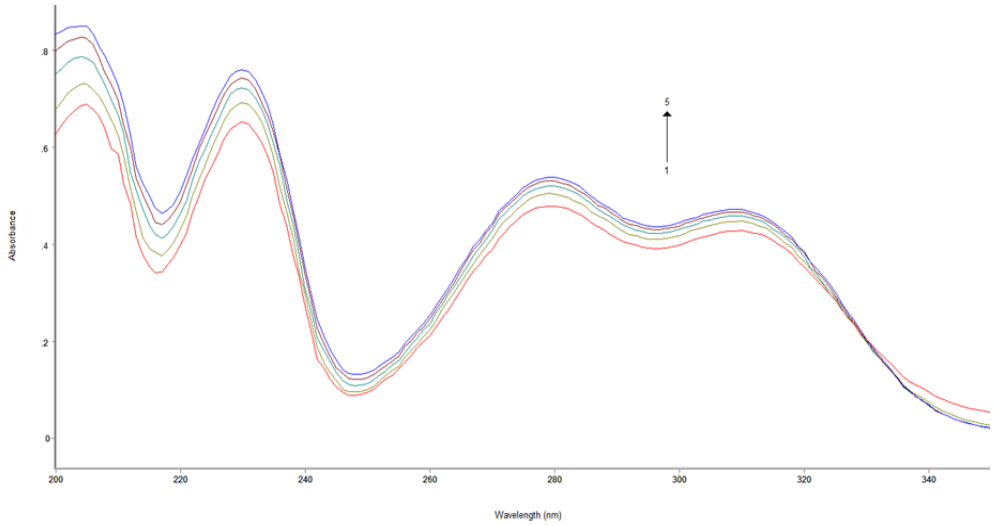

Figure 4. Absorption spectra of vanillin (0.05 mM) in the presence of β-CD. The concentrations of β-CD from 1 to 5 were 0, 2, 4, 6 and 8 mM, respectively

The stability of the complex can elucidate the degree of encapsulation of the guest molecule by βCD. The binding or stability constant (K) can be determined according to Benesi-Hildebrand Equation [72]:

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon\,[G]_0 K[CD]} + \frac{1}{\Delta\varepsilon\,[G]_0} \quad (1)$$

Where; ΔA and Δε are the absorbances and the molar absorptivity difference between the free and complexed vanillin, respectively. $[G]_0$ and [CD] are the initial vanillin and β-CD, respectively and K is the association constant.

The absorbance of vanillin solutions with the addition of β-CD was measured at 280 nm, and all measurements were made in triplicate (Table 1)

Table 1. Absorbance of vanillin-β-CD complex with various β-CD concentrations (0, 2, 4, 6, and 8 mM) was measured at 280 nm using UV-Vis spectroscopy.

β-CD (mM)	Absorbance at 280 nm			Mean	SD	% CV
	1	**2**	**3**			
0	0.482	0.476	0.480	0.479	0.003	0.637
2	0.511	0.504	0.508	0.508	0.004	0.692
4	0.529	0.520	0.524	0.524	0.005	0.860
6	0.538	0.530	0.534	0.534	0.004	0.749
8	0.545	0.538	0.543	0.542	0.004	0.665

The association constant can be calculated from the double reciprocal plot of 1/[β-CD] versus 1/ ΔA using Eq. 1 as shown in Table 2. A good linear relationship was obtained (Figure 5); thus, it proved the 1:1 stoichiometry of the inclusion complex in agreement with the Job's plot. The association constant was determined from the slope and the plot intercept, which was found to be 179 M^{-1}.

Table 2. Association constant determination of vanillin-β-CD inclusion complex based on Benesi-Hildebrand method.

1/ β-CD (mM^{-1})	Exp. 1 (A_0 = 0.482)			Exp. 2 (A_0 = 0.476)			Exp. 3 (A_0 = 0.480)			Mean
	A	**ΔA**	**1/ΔA**	**A**	**ΔA**	**1/ΔA**	**A**	**ΔA**	**1/ΔA**	
0.5	0.511	0.029	**34.5**	0.504	0.028	**35.7**	0.508	0.028	**35.7**	35.3
0.25	0.529	0.047	**21.3**	0.520	0.044	**22.7**	0.524	0.044	**22.7**	22.2
0.17	0.538	0.056	**17.9**	0.530	0.054	**18.5**	0.534	0.054	**18.5**	18.3
0.13	0.545	0.063	**15.9**	0.538	0.062	**16.1**	0.543	0.063	**15.9**	16.0
$A = A_{measured}$, $\Delta A = A - A_0$										

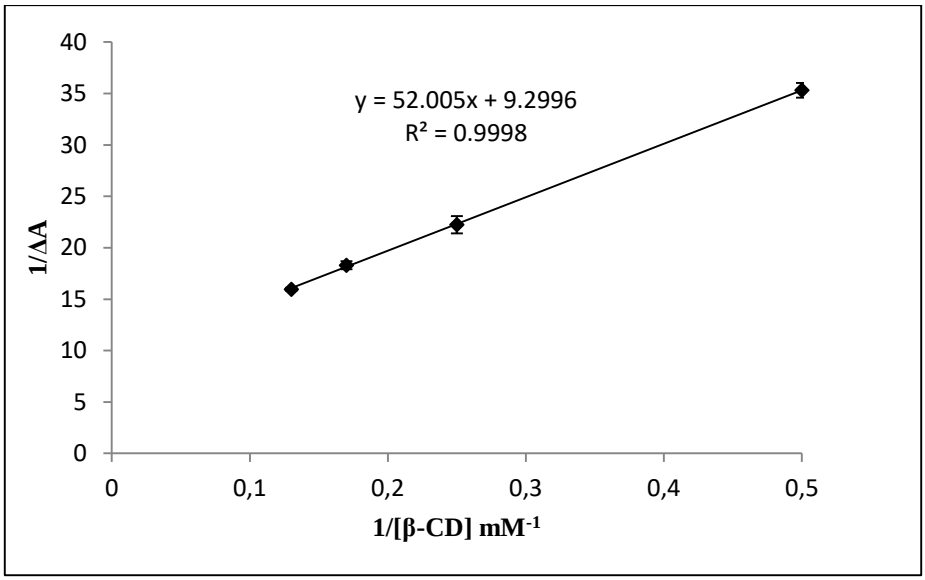

Figure 5. Double reciprocal plot of 1/ΔA vs. 1/[β-CD] of vanillin-β-CD complex (Mean ± SD, n=3).

The association constant of the vanillin-β-CD complex was also determined by Scott's method [73] as illustrated in Eq. 2: where ΔA is the absorbance difference of the vanillin in the absence and presence of β-CD and ΔA_{max} is the absorbance variation at saturation.

$$\frac{[CD]}{\Delta A} = \frac{[CD]}{\Delta A_{max}} + \frac{1}{K\,\Delta A_{max}} \quad (2)$$

The [β-CD]/ ΔA ratio was plotted as a function of [β-CD] as presented in Table 3, thus resulting in a linear fit confirming 1:1 stoichiometry for the inclusion complex (Figure 6) with the association constant was calculated to be 187 M^{-1}.

Table 3. Association constant determination of vanillin- β-CD inclusion complex based on Scott's method.

β-CD	Exp. 1 (A_0 = 0.482)			Exp. 2 (A_0 = 0.476)			Exp. 3 (A_0 = 0.480)			Mean
mM	**A**	**ΔA**	**β-CD /ΔA**	**A**	**ΔA**	**β-CD /ΔA**	**A**	**ΔA**	**β-CD /ΔA**	
2	0.511	0.029	**69**	0.504	0.028	**71**	0.508	0.028	**71**	71
4	0.529	0.047	**85**	0.52	0.044	**91**	0.524	0.044	**91**	89
6	0.538	0.056	**107**	0.53	0.054	**111**	0.534	0.054	**111**	110
8	0.545	0.063	**127**	0.538	0.062	**129**	0.543	0.063	**127**	128
$A = A_{measured}$, $\Delta A = A - A_0$										

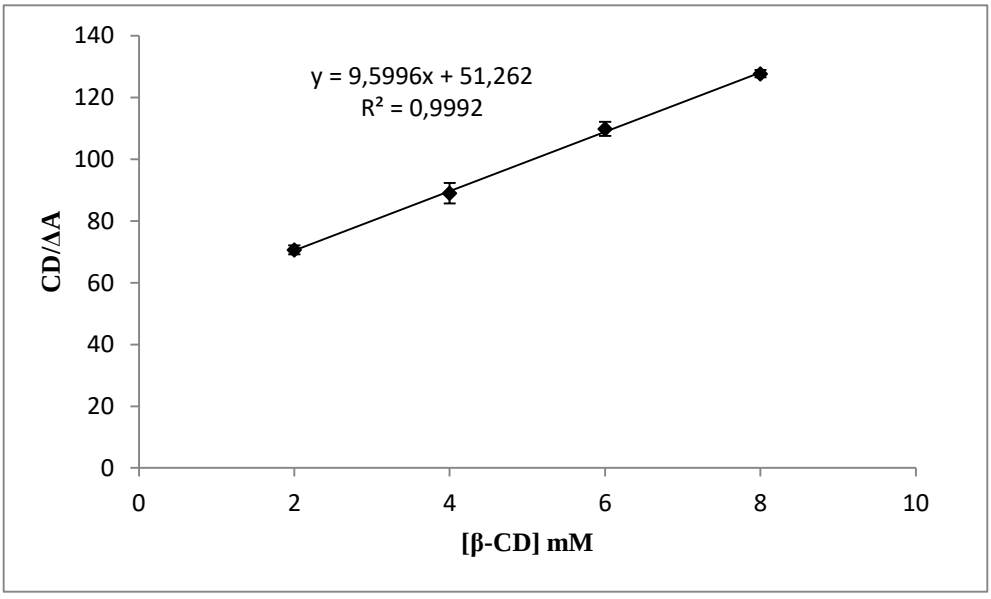

Figure 6. Scott's plot for vanillin-β-CD inclusion complex (Mean ± SD, n=3)

The constant association values for the inclusion complex obtained from Benesi-Hildebrand and Scott's methods were approximately similar (179 and 187 M^{-1}, respectively). Both calculation methods are well known and can be applied interchangeably to estimate the association constant of the complex. Numerous analytical methods, such as electrochemical, spectroscopic, or separation techniques, have been used to characterize host-guest complexes. Among these methods, NMR is the most widely used to provide detailed information about the complex, such as the orientation of the guest inside the CD cavity [74]. However, due to its simplicity, speed and usefulness, UV-Vis spectroscopy has broad applicability among the various reported techniques to determine the stoichiometry and the association constant of the inclusion complex.

4. Conclusion

The vanillin- β-CD system in an aqueous solution has been investigated by UV-Vis spectroscopy. Data analysis by the continuous variation method suggested that the inclusion complex has 1:1 stoichiometry. The association constant for the inclusion complex was estimated from the increasing absorbance of vanillin with the addition of β-CD, thus confirming a vanillin / β-CD interaction. The spectroscopic method is characterized by its simplicity, economy, speed, and availability to determine the stoichiometry and the association constant of the complex according to Benesi-Hildebrand and Scott's linear models. It may be concluded that the vanillin is embedded into the β-CD cavity forming the inclusion complex so that the vanillin can be protected from oxidation. Thus, it can be used as a food additive with higher stability.

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