NMR Spectroscopic characterization of β-cyclodextrin inclusion complex with vanillin

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Abstract. The inclusion of vanillin by β-cyclodextrin was investigated by $^1$H NMR. The continuous variation technique was used to evidence the formation of soluble 1:1 complex in aqueous solution. The association constant of vanillin with β-cyclodextrin has been obtained at 298 K by fitting the experimental chemical shifts differences, $\Delta \delta_{\text{obs}} = \delta_{\text{free}} - \delta_{\text{obs}}$ of the observed guest and host protons, with a non-linear regression method. Besides the effective association constant, the fitting procedure allows a precise determination of all chemical shift parameters characterizing the pure complex. They can by used for an analysis of the geometry of the molecular complex in solution.

1. Introduction
Vanillin, on 4-hydroxy-3-methoxybenzaldehyde is the primary component of the extract of the vanilla bean. Natural “vanilla extract” is a mixture of several hundred different compounds in addition to vanillin. Artificial vanilla is a solution of pure vanillin, usually of synthetic origin. Because of the scarcity and expense of natural vanilla extract, there has long been interest in the synthetic preparation of its predominant component. Today, most artificial vanillin is produced from the petrochemical raw material guaiacol. The largest use of vanillin is as flavouring, usually in sweet foods. The ice cream and chocolate industries together comprises 75% of the market of vanillin as flavouring. Vanillin displays antioxidant and antimicrobial properties and may be used as a food preservative. It is also used in the fragrance industry, in perfumes, and the mask unpleasant odours or tastes of drugs. Vanillin has been encapsulated in oxidized starches and in β-cyclodextrin in order to enhance the protection towards oxidation.

Cyclodextrins (CDs) are cyclic oligosaccharides containing six ($\alpha$-CD), seven ($\beta$-CD) or eight ($\gamma$-CD) α-1,4-linked glucopyranose units with a hydrophilic hydroxyl group on their outer surface and a hydrophobic cavity in the center. The hydrophilic exterior of the CD molecules can make them water soluble, but the hydrophobic cavity provides an environment for appropriate sized non-polar molecules. In aqueous solution CDs are capable of forming inclusion complex with many molecules by taking up a whole molecule or some part of it, into the cavity. These non-covalent complexes offer a variety of physicochemical advantages over uncomplexed molecules including increased water solubility and stability. In this paper, we report a $^1$H NMR study of the inclusion complex formed between vanillin (figure 1a) and β-cyclodextrin (figure 1b), in aqueous medium. Analysis of our data by the continuous variation method confirms that the inclusion occurs and the complex has 1:1 stoichiometry. The association constant was calculated by a non-linear least squares regression.
analysis of the observed chemical shift changes of the vanillin and β-CD protons as a function of β-CD concentration.

\[
\begin{align*}
& (\alpha) \quad \text{C} = O \\
& (\beta) \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H} \\
& (\gamma) \quad \text{OH} \quad \text{OH} \\
& (\delta) \quad \text{CH}_3
\end{align*}
\]

(a) (b)

**Figure 1.** Chemical structure of (a) vanillin and (b) β-CD.

2. **Experimental**

2.1. **Materials**

β-cyclodextrin (water content 8 mol/mol) was purchased from Sigma Chemie GmbH Germany and vanillin from Alfa Aesar GmbH&CoKG, Germany. Both chemicals were used without any further purification. Deuterium oxide (99.7% D) was obtained from Heavy Water Plant Romag - Prod, Romania.

2.2. **Apparatus**

The $^1$H NMR experiments were performed at 500 MHz on a Bruker Avance III, spectrometer. The NMR spectra were recorded in D$_2$O solution at 298 K and all chemical shifts were measured relative to TMS. Typical conditions were as follows: 32 K data points, sweep width 3500 Hz giving a digital resolution of 0.1 Hz point. The $90^\circ$ pulse width was 10 µs and the spectra were collected by co-addition of 16 to 32 scans.

2.3. **Procedure**

In order to study the complexation process between vanillin and β-CD in solution, two stock solutions in D$_2$O, both having 10 mM were prepared. Based on these two equimolar solutions, a series of nine samples ($i = 1 - 9$) containing both the vanillin and β-CD molecules were prepared. This was accomplished by mixing the two solutions to constant volume at varying proportions, so that a complete range ($0 < r < 1$) of the ratio $r = [X] / ([H] + [G])$ was sampled. $X = G$ or $H$ and $[H]$ and $[G]$ are the total concentrations of the host (β-CD) and guest (vanillin), respectively. Thus the total concentration $[H] + [G] = [M] = 10$ mM was kept constant for each solution. The same set of samples was used both for the determination of stoichiometry and association constant.

3. **Results and discussion**

NMR is a technique which provides the most evidence for the inclusion of a guest molecule into the hydrophobic CD cavity in solution. The inclusion of vanillin in β-CD is shown by the change in the chemical shift of some guest vanillin and host (β-CD) protons in the complex, in comparison with the chemical shifts of the same protons in the free components. Partial $^1$H NMR spectra of pure components and vanillin: β-CD mixture in a 1:1 molar ratio is presented in figures 2 and 3.

The absence of new peaks that could be assigned to the complex suggested that complexation is a dynamic process, the included vanillin being in a fast exchange between the free and bound states.
3.1. Determination of the stoichiometry

The stoichiometry of the complex must be calculated before proceeding with any association constant calculations. Determination of the stoichiometry of the vanillin-β-CD complex, by the continuous variation method was based on \(^1\)H NMR spectra obtained for vanillin and β-CD mixtures in which the initial concentrations of the two species were maintained constant and that ratio \(r\) varied between 0 and 1 (see section 2.3. Procedure). A physical parameter directly related to the concentration of the complex (in our case the chemical shift, \(\delta\)) is measured under these conditions and is plotted as a function of \(r\). The maximum value for this parameter well occur at \(r = n / (m + n)\), where \(m\) and \(n\) are, respectively, the proportions of vanillin and β-CD in the complex (vanillin)\(_{m}\)(β-CD)\(_{n}\). The calculated quantities \(\Delta\delta[βCD]\) are proportional to the concentration of the complex [1], and can be plotted against \(r\). The resulting continuous variation plots demonstrate that because \(r\) has a maximum value of 0.5 and highly symmetrical shapes (figure 4), the complex has 1:1 stoichiometry.
The induced shift, $\Delta \delta$ is defined as the difference in chemical shifts in the absence and in the presence of the other reactant for a given ratio $r$.

![Concentrations variation plot for H-5 proton of β-CD.](image)

Figure 4. Concentrations variation plot for H-5 proton of β-CD.

3.2. Determination of the association constant

In order to determine the extent of the intermolecular binding between vanillin and β-CD, the association constant has been evaluated. The association constant, $K$ for an 1:1 complex can be determined according to the following equation [2]:

$$
\Delta \delta^{(i,j)} = \frac{\Delta \delta^{(i,j)}_{c}}{2[X]} \left\{ [M] + \frac{1}{K} \left[ [M] + \frac{1}{K} \right]^2 - 4[H][G]\right\}^{1/2}
$$

(1)

where $i$ counts the sample number and $j$ the studied proton. If the studied proton belongs to the guest or host molecule, $X = G$ or $H$ respectively. $\Delta \delta^{(i,j)}_{c}$ represents the chemical shift difference (for a given proton) between the free component and the pure inclusion complex. Equation (1) involves no approximations and correlates the total concentrations of the guest and host molecules with the observed difference in the chemical shift:

$$
\Delta \delta^{(i,j)} = \delta^{(i,j)}_{\text{free}} - \delta^{(i,j)}_{\text{obs}}
$$

(2)

We used a computer programme [3] based on an iteration procedure following specific algorithms in order to fit the experimental values of $\Delta \delta^{(i,j)}$ to the appropriate equation. Each iteration sets up a quadratic programme to determine the direction of search and the loss function,

$$
E = \sum_{i,j} (\Delta \delta^{(i,j)}_{\text{obs}} - \Delta \delta^{(i,j)}_{\text{calc}})^2
$$

(3)

until the search converges.

The treatment of the whole set of protons studied yields one single $K$ value characterizing the inclusion process and a set of calculated $\Delta \delta^{(i,j)}_{c}$ values. The programme is quite flexible since it allows observing the chemical shift variation of the host, guest or both molecules as a function of variable guest or host concentrations.

In our case, we applied equation (1) for a set of protons consisting in H3 and H5 of β-CD and Ha, Hb and He of vanillin.
The association constant obtained using the above described procedure is \( K = 170.2 \text{ M}^{-1} \) with \( E = 8.29 \times 10^{-5} \) and a correlation factor \( r = 0.9995 \).

The complete set of chemical shifts in the free state and in pure complex is reported in table 1.

### Table 1. Chemical shifts of the proton of vanillin and \( \beta \)-CD in the free and complexed states.

| Proton | \( \delta_{\text{free}} \) (ppm) | \( \delta_{c} \) (ppm) |
|--------|---------------------------------|----------------------|
| H3     | 3.8907                          | 3.7774               |
| H5     | 3.7819                          | 3.6125               |
| Ha     | 7.4522                          | 7.5179               |
| Hb     | 6.9767                          | 7.0817               |
| He     | 3.8459                          | 3.9215               |

\( \delta_{c} = \delta_{\text{free}} - \Delta \delta_{c} \)

### 4. Conclusion

The vanillin-\( \beta \)-CD system in aqueous solution has been studies by \( ^1 \text{H} \) NMR. Analysis of our data by the continuous variation method indicates that the inclusion occurs and the complex has 1:1 stoichiometry.

The complexation-induced chemical shift of H3 and H5 protons of \( \beta \)-CD are those expected as a result of interaction with those protons of the vanillin (Ha, Hb and He) which are oriented towards the \( \beta \)-cyclodextrin cavity, thus confirming a vanillin / \( \beta \)-CD interaction.

The association constant \( K \) for the inclusion complex was evaluated from the observed difference in chemical shifts for vanillin and \( \beta \)-CD protons.

### References

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[2] Bogdan M, Caira M R and Farcas S I 2002 Supramol. Chem. 14 427
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