

## PHOTOPHYSICAL EVALUATION OF $\beta$ -CARYOPHYLLENE CYCLODEXTRIN INCLUSION COMPLEXES THROUGH BENESI-HILDEBRAND ANALYSIS

<sup>1</sup>Devi S.K

<sup>2</sup>Dr. Prema Kumari J

<sup>1</sup>Research Scholar, Reg. No: 22213162032013, Department of Chemistry, Scott Christian College (Autonomous), Nagercoil-629003, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India-627012.

<sup>2</sup>Research Supervisor, Associate Professor, Department of Chemistry, Scott Christian College (Autonomous), Nagercoil-629003, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India-627012.

\*Corresponding author E-mail : [premaisaac67@gmail.com](mailto:premaisaac67@gmail.com)

**How to cite this article:** Devi S.K, Dr. Prema Kumari J, (2023). Photophysical Evaluation Of  $\beta$ -Caryophyllene Cyclodextrin Inclusion Complexes Through Benesi-Hildebrand Analysis, Library Progress International, 43(2), 2356-2366.

**Abstract:** Host-guest complexation is a powerful molecular strategy to enhance the physicochemical behavior of natural bioactives with limited aqueous solubility. In this study,  $\beta$  caryophyllene (BCP), a pharmacologically active sesquiterpene isolated from *Aegle marmelos* leaves, was investigated for its inclusion interactions with  $\alpha$ -cyclodextrin ( $\alpha$ -CD) and  $\beta$  cyclodextrin ( $\beta$ -CD). Absorption and fluorescence emission spectroscopy were employed to monitor spectral variations of BCP in the presence of increasing cyclodextrin concentrations. Quantitative analysis was performed using the Benesi-Hildebrand method, where linear plots confirmed 1:1 stoichiometry of the inclusion complexes. The stability constants obtained demonstrated notable differences between BCP: $\alpha$ -CD and BCP: $\beta$ -CD systems, underscoring the influence of cavity dimensions and molecular fit on host-guest affinity. These findings provide spectroscopic evidence of differential binding efficiency and establish a framework for evaluating cyclodextrin assisted modulation of bioactive natural products.

**Keywords:**  $\beta$ -Caryophyllene,  $\alpha$ -Cyclodextrin,  $\beta$ -Cyclodextrin, Benesi-Hildebrand analysis, Stability constant, Host-guest interaction.

### 1. INTRODUCTION

Natural products have historically served as indispensable sources of therapeutic agents, with many clinically approved drugs being directly derived from or inspired by them<sup>1</sup>. Despite their pharmacological promise, most phytoconstituents face challenges such as low solubility, poor bioavailability, volatility, and instability, restricting their clinical utility<sup>2</sup>.

*Aegle marmelos* (Bael), a Rutaceae family plant widely used in Ayurveda and Unani medicine, is traditionally applied in gastrointestinal, diabetic, inflammatory, and infectious conditions<sup>3</sup>. Phytochemical studies of its leaves report alkaloids, flavonoids, phenolics, and terpenoids<sup>4</sup>, among which  $\beta$ -caryophyllene (BCP) is particularly significant. Found in *A. marmelos*, clove, and *Cannabis sativa*, BCP is a non-toxic sesquiterpene approved as a flavoring agent<sup>5</sup>. It exhibits broad pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, analgesic, and anticancer effects<sup>6,7</sup>. Its role as a selective agonist of the CB2 receptor further highlights its therapeutic potential in immune regulation, neuroprotection, and metabolic disorders<sup>8</sup>. However, its clinical application is constrained by poor aqueous solubility, high

volatility, and limited bioavailability<sup>9</sup>.

Cyclodextrins (CDs), cyclic oligosaccharides of six to eight glucopyranose units, are well established as carrier systems that enhance solubility, stability, and delivery of lipophilic molecules<sup>10–12</sup>. Host–guest compatibility is dictated by cavity size:  $\alpha$ -CD (4.7–5.3 Å) is suited to smaller molecules, while  $\beta$ -CD (6.0–6.5 Å) is preferred for bulkier terpenes<sup>13,14</sup>.

Spectroscopic methods, particularly UV–visible absorption and fluorescence emission, provide sensitive tools for probing inclusion complexes<sup>15</sup>. The Benesi–Hildebrand (B-H) method remains a robust quantitative approach for determining complex stoichiometry and stability, offering valuable insights into natural product–CD interactions<sup>16–19</sup>.

Accordingly, This study explores the formation of inclusion complexes between  $\beta$ -caryophyllene (BCP), isolated from *Aegle marmelos* leaves, and  $\alpha$ - and  $\beta$ -cyclodextrins through absorption and fluorescence spectroscopy. The Benesi–Hildebrand approach was employed to determine the complex stoichiometry and calculate stability constants, providing a quantitative assessment of host–guest interactions and cavity compatibility.

## 2. MATERIALS AND METHODS

### 2.1. Reagents Used

$\beta$ -Caryophyllene (BCP) was isolated from fresh leaves of *Aegle marmelos* using Soxhlet extraction with ethanol, followed by chromatographic purification.  $\alpha$ -Cyclodextrin ( $\alpha$ -CD) and  $\beta$ -cyclodextrin ( $\beta$ -CD) of analytical grade were purchased from Sigma-Aldrich and used without further purification. Distilled water and ethanol were used as solvents for all spectroscopic experiments. All other chemicals and reagents were of analytical grade and used as received.

### 2.2. Preparation of Inclusion Complexes

Stock solutions of BCP were prepared by dissolving the purified compound in ethanol at a known concentration. Aqueous solutions of  $\alpha$ -CD and  $\beta$ -CD at varying concentrations were prepared to study host–guest interactions. Inclusion complexes were formed by mixing a fixed volume of BCP solution with cyclodextrin solutions at different concentrations, The mixtures were then undergoes spectroscopic measurements immediately.

### 2.3. Spectroscopic Measurements

#### 2.3.1. UV-Visible Absorption Spectroscopy

The absorption spectra of BCP in the presence of varying concentrations of  $\alpha$ -CD and  $\beta$ -CD were recorded using a UV-Visible spectrophotometer (Systronic Double Beam Spectrophotometer-2203) over the wavelength range of 200–400 nm. The maximum absorption wavelength ( $\lambda_{\text{max}}$ ) was identified, and changes in absorbance were used for Benesi–Hildebrand analysis.

#### 2.3.2. Fluorescence Emission Spectroscopy

Fluorescence emission spectra of BCP: $\alpha$ -CD and BCP: $\beta$ -CD complexes were recorded using a fluorescence spectrophotometer (JASCO Spectrofluorometer FP-8200). Emission intensities were recorded, and the enhancement in fluorescence was analyzed to assess complex formation.

### 2.4. Determination of Stoichiometry and Stability Constants

The Benesi–Hildebrand (B–H) method was employed to determine the stoichiometry and stability constants of BCP: $\alpha$ -CD and BCP: $\beta$ -CD. This method is based on the linear relationship

between the reciprocal change in absorbance (or fluorescence intensity) of the guest molecule and the reciprocal of cyclodextrin concentration, assuming a 1:1 host–guest complex formation.

For absorption measurements, the B–H equation for a 1:1 complex was used:

$$\frac{1}{\Delta A} = \frac{1}{K \Delta A_{\max} [\text{CD}]} + \frac{1}{\Delta A_{\max}}$$

where  $\Delta A$  is the change in absorbance at  $\lambda_{\max}$  upon addition of cyclodextrin,  $\Delta A_{\max}$  is the maximum change in absorbance,  $[\text{CD}]$  is the molar concentration of cyclodextrin, and  $K$  is the stability constant.

For fluorescence measurements, the equation is similar:

$$\frac{1}{\Delta F} = \frac{1}{K \Delta F_{\max} [\text{CD}]} + \frac{1}{\Delta F_{\max}}$$

where  $\Delta F$  is the change in fluorescence intensity, and  $\Delta F_{\max}$  is the maximum change observed upon complex formation.

In practice, a fixed concentration of BCP was mixed with varying concentrations of cyclodextrin, and the absorbance or emission spectra were recorded. The reciprocal of the spectral change ( $1/\Delta A$  or  $1/\Delta F$ ) was plotted against the reciprocal of cyclodextrin concentration ( $1/[\text{CD}]$ ). The slope and intercept of the resulting straight line were used to calculate the stability constant ( $K$ ) and to confirm 1:1 stoichiometry of the inclusion complex.

### 3. RESULTS

#### 3.1. Benesi-Hildebrand Analysis of Absorption Spectra

##### 3.1.1. BCP: $\alpha$ -CD Absorption B-H Plot

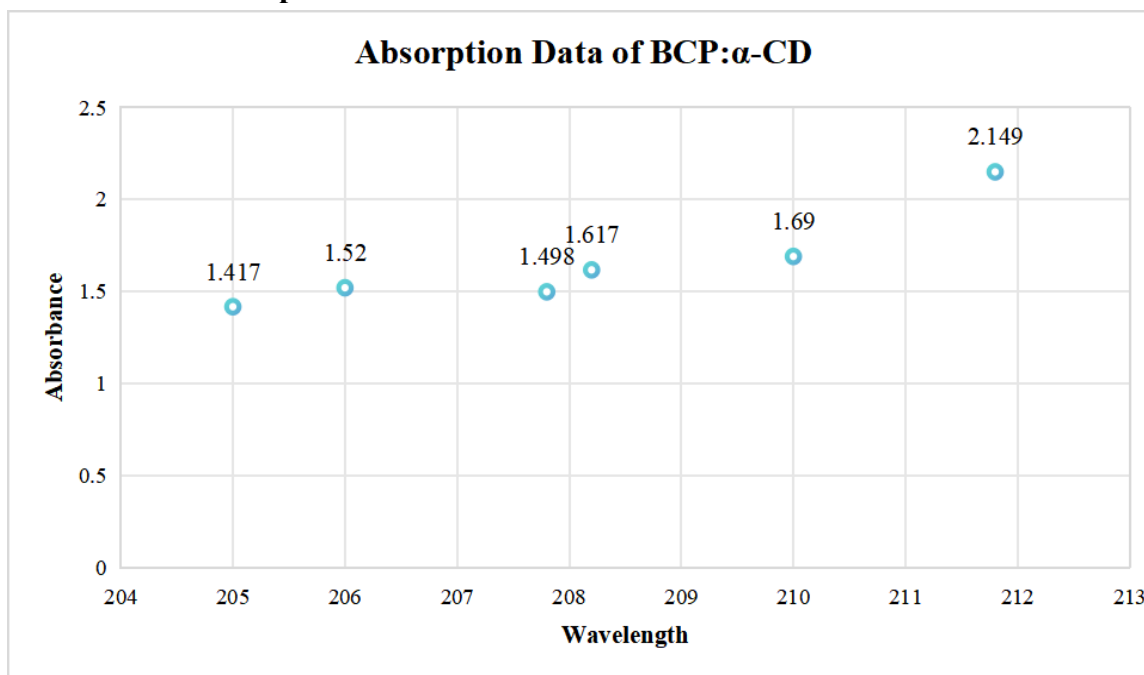
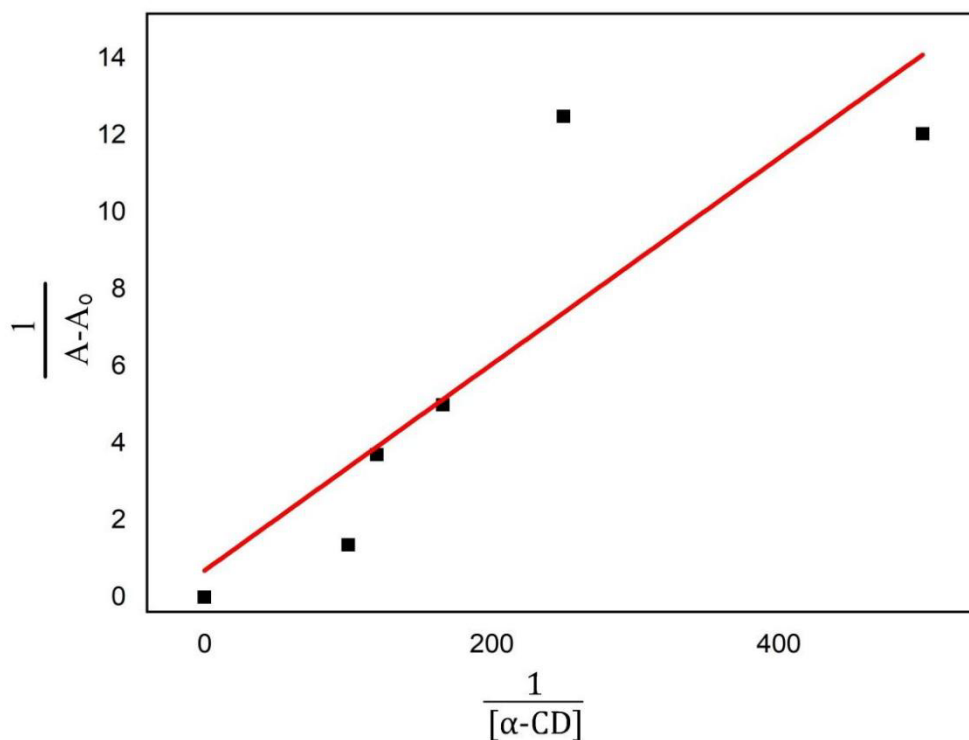


Figure 1: Absorption Analysis of BCP: $\alpha$ -CD Inclusion Complex

Table 1: Absorption Parameters of BCP: $\alpha$ -CD Complex

Con. of $\alpha$ -CD	$\lambda_{\max}$	A	A-A <sub>0</sub>	$\frac{1}{A - A_0}$	$\frac{1}{[\alpha - \text{CD}]}$
0	205	1.417	0	0	0

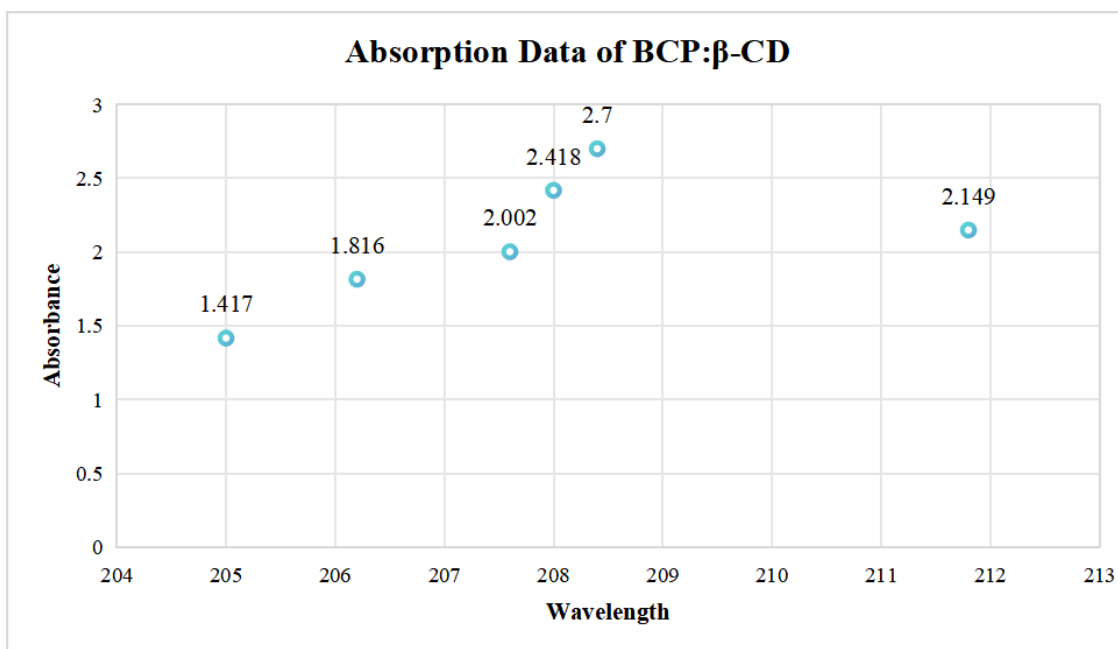
0.002	206	1.520	0.083	12.04	500
0.004	207.8	1.498	0.08	12.5	250
0.006	208.2	1.617	0.2	5	166
0.008	210	1.690	0.27	3.70	125
0.01	211.8	2.149	0.73	1.36	100



**Figure 2: Absorption-based binding analysis of BCP:α-CD**

Figure 1 presents the UV–Vis profile of BCP:α-CD and Figure 2 and Table 1 shows the absorption study of the BCP:α-CD inclusion complex using the Benesi-Hildebrand method. The linear plot of  $1/(A-A_0)$  versus  $1/[\alpha\text{-CD}]$  indicates the formation of a 1:1 stoichiometric complex. From the slope and intercept of the plot, the binding constant (K) for the BCP:α-CD complex was calculated as **227 M<sup>-1</sup>**.

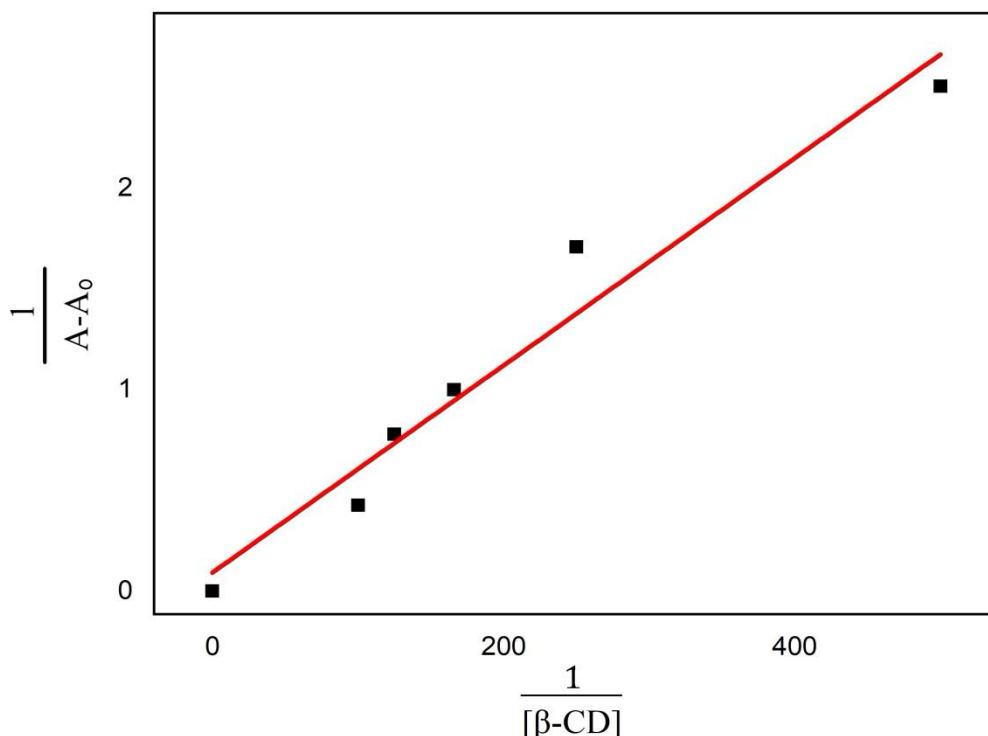
### 3.1.2. BCP:β-CD Absorption B-H Plot



**Figure 3: Absorption Analysis of BCP:β-CD Inclusion Complex**

**Table 2: Absorption Parameters of BCP:β-CD Complex**

Con. of β-CD	$\lambda_{\max}$	A	A-A <sub>0</sub>	$\frac{1}{A - A_0}$	$\frac{1}{[\beta - CD]}$
0	205	1.417	0	0	0
0.002	206.2	1.816	0.399	2.506	500
0.004	207.6	2.002	0.585	1.709	250
0.006	208	2.418	1.001	0.999	166
0.008	208.4	2.700	1.283	0.779	125
0.01	209	3.762	2.345	0.426	100

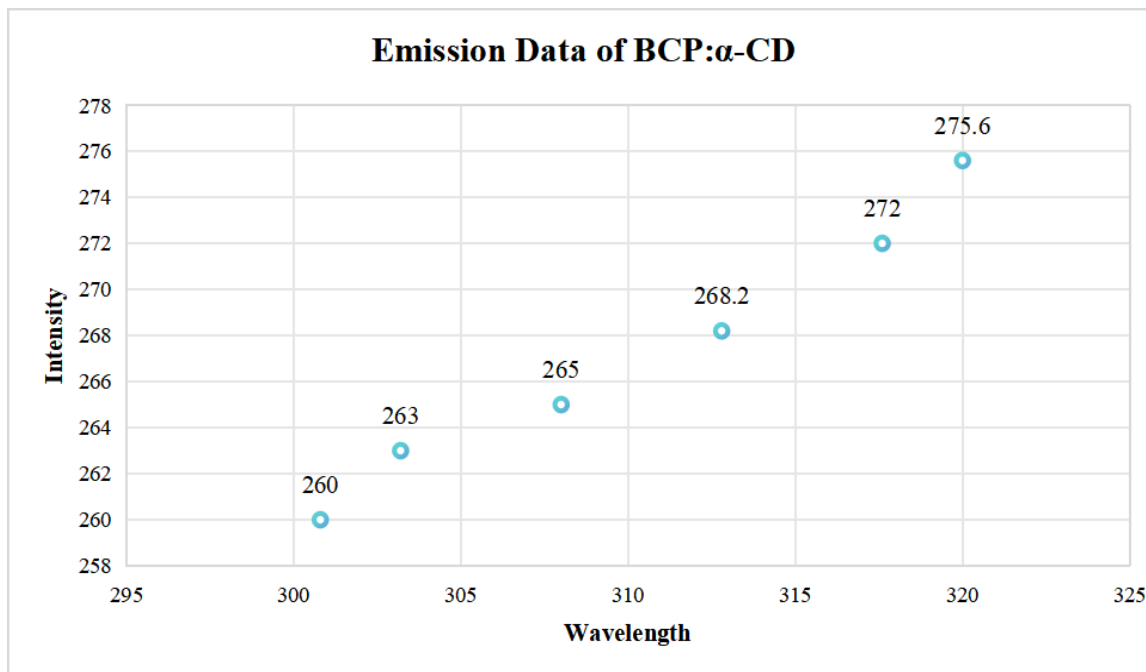


**Figure 4: Absorption-based binding analysis of BCP:β-CD**

Figure 3 shows the absorption spectra of BCP:β-CD. The BCP:β-CD inclusion complex (Figure 4 & Table 2) exhibited a Benesi-Hildebrand binding constant ( $K$ ) of  $435 \text{ M}^{-1}$ , indicating a relatively strong interaction between β-caryophyllene and β-cyclodextrin in solution. This stability constant further suggests that β-CD provides an optimal hydrophobic environment.

### 3.2. Benesi-Hildebrand Analysis of Emission Spectra

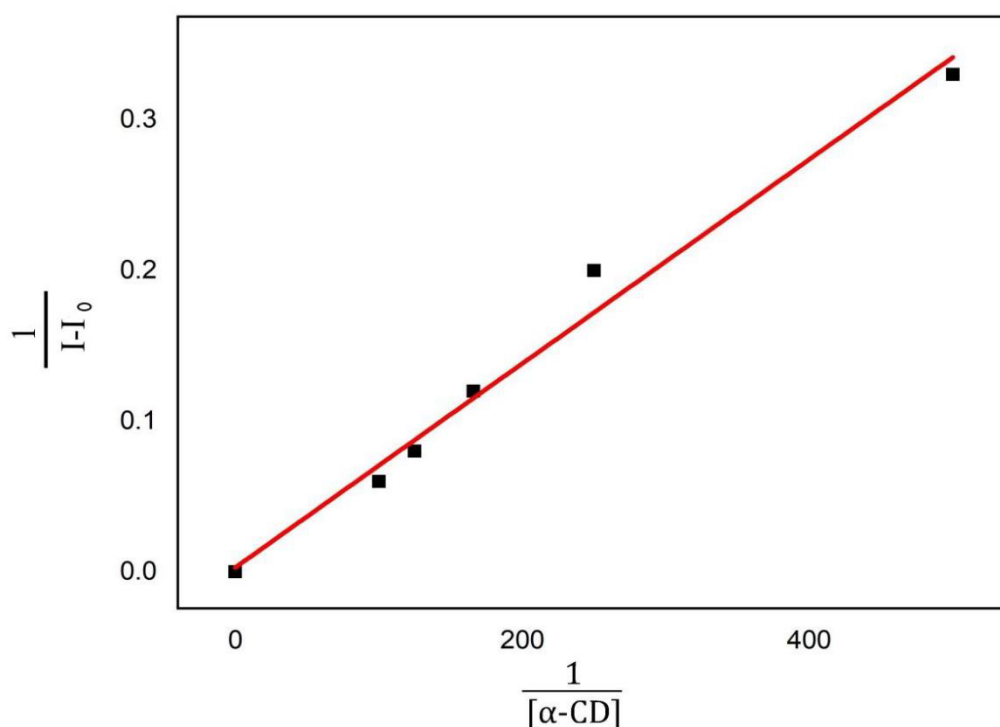
#### 3.2.1. BCP:α-CD Emission B-H Plot



**Figure 5: Emission Analysis of BCP:α-CD Inclusion Complex**

**Table 3: Emission Parameters of BCP:α-CD Complex**

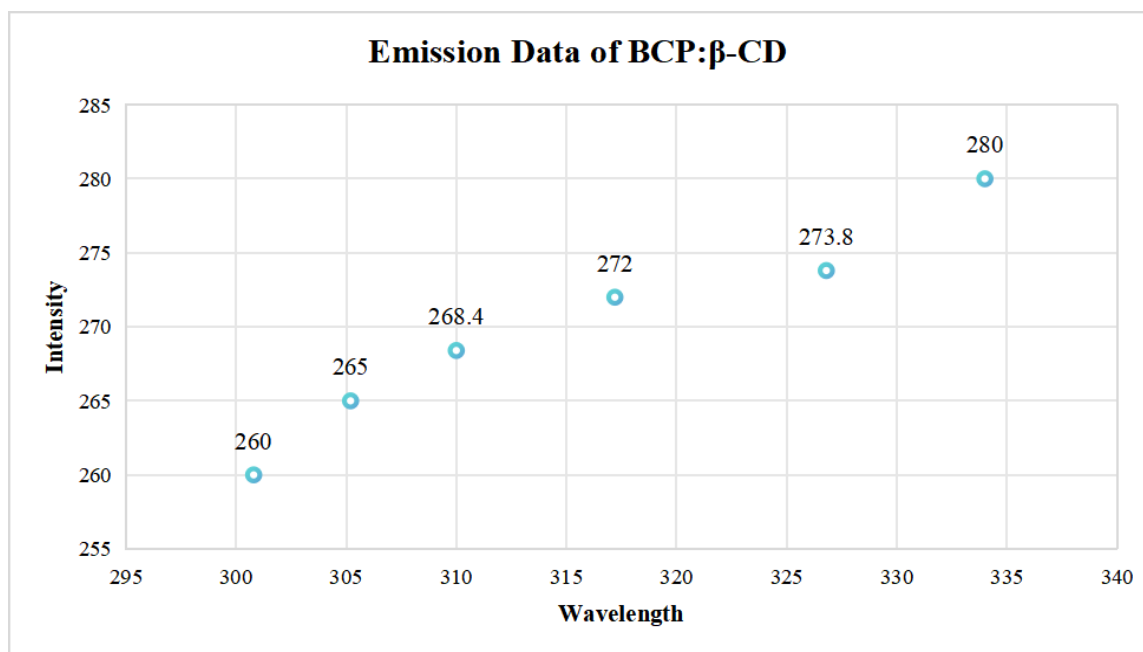
Con. of $\alpha$ -CD	$\lambda_{\text{max}}$	I	I-I <sub>0</sub>	$\frac{1}{I - I_0}$	$\frac{1}{[\alpha - \text{CD}]}$
0	300.8	260	0	0	0
0.002	303.2	263	3	0.33	500
0.004	308	265	5	0.2	250
0.006	312.8	268.2	8.2	0.12	166
0.008	317.6	272	12	0.08	125
0.01	320	275.6	15.6	0.06	100



**Figure 6: Emission-based binding analysis of BCP: $\alpha$ -CD**

Figure 5 depicts the emission data of the BCP: $\alpha$ -CD complex. The fluorescence emission spectral analysis of BCP in the presence of increasing concentrations of  $\alpha$ -cyclodextrin produced a linear B-H plot in Figure 6 & Table 3, consistent with the formation of a 1:1 inclusion complex. From the slope and intercept of the plot, the K value was determined to be 104 M<sup>-1</sup>, indicating a moderate affinity between BCP and  $\alpha$ -CD.

### 3.2.2. BCP: $\beta$ -CD Emission B-H Plot

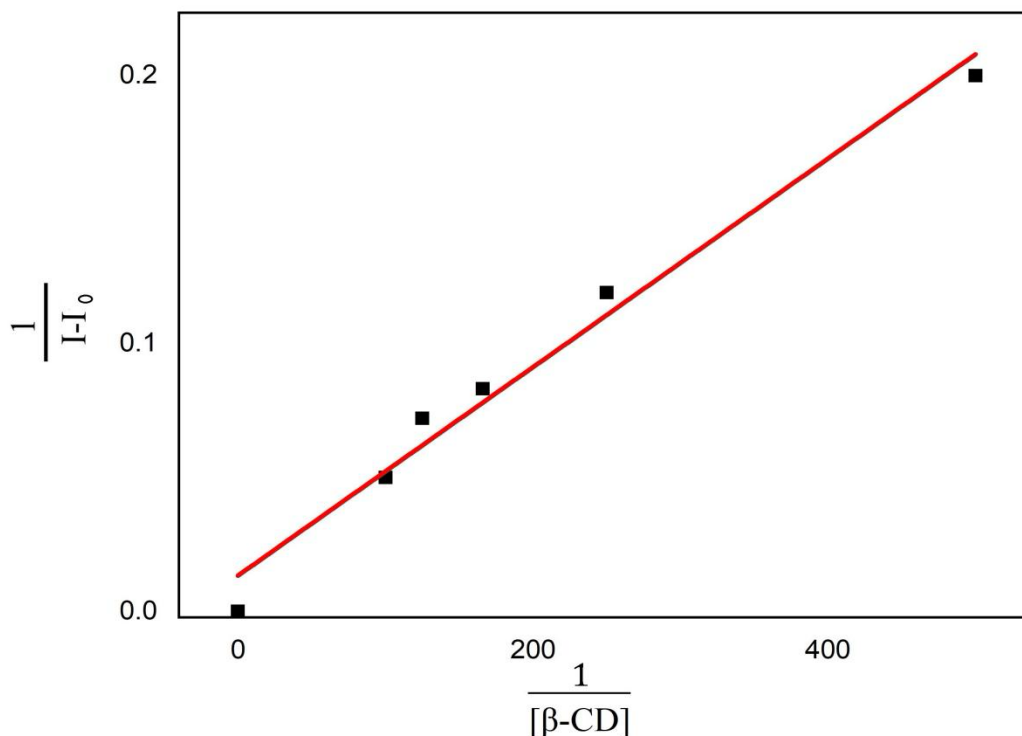


**Figure 7: Emission Analysis of BCP:β-CD Inclusion Complex**

**Table 4: Emission Parameters of BCP:β-CD Complex**

Con. of β-CD	$\lambda_{\text{max}}$	I	I-I <sub>0</sub>	$\frac{1}{I - I_0}$	$\frac{1}{[\beta - \text{CD}]}$
0	300.8	260	0	0	0
0.002	305.2	265	5	0.2	500
0.004	310	268.4	8.4	0.119	250
0.006	317.2	272	12	0.083	166
0.008	326.8	273.8	13.8	0.072	125
0.01	334	280	20	0.05	100





**Figure 8: Emission-based binding analysis of BCP:β-CD**

Figure 7 indicates the emission behavior of BCP:β-CD. Figure 8 & Table 4 indicate the fluorescence emission spectral analysis of BCP:β-CD produced a linear B-H plot, indicating the formation of a 1:1 inclusion complex. The binding constant (K) calculated from the plot was 151 M<sup>-1</sup>, suggesting good stability of the complex and confirming the effective encapsulation of BCP within the β-CD cavity.

## DISCUSSION

The inclusion complex formation between β-caryophyllene (BCP) and cyclodextrins (CDs) was systematically examined using absorption and fluorescence emission spectroscopy, with binding constants evaluated via the Benesi-Hildebrand method. Both α-cyclodextrin (α-CD) and β-cyclodextrin (β-CD) demonstrated the capacity to form 1:1 stoichiometric inclusion complexes with BCP, as evidenced by the linearity of their respective Benesi-Hildebrand plots.

The absorption studies revealed distinct differences in binding affinities between the two cyclodextrins. The BCP:α-CD complex exhibited a moderate binding constant of 227 M<sup>-1</sup>, indicating effective but comparatively weaker host-guest interactions. Conversely, the BCP:β-CD complex showed a significantly higher binding constant of 435 M<sup>-1</sup>, suggesting a stronger affinity for β-CD. This disparity can be attributed to the larger hydrophobic cavity of β-CD, which likely provides a more favorable microenvironment for encapsulating the hydrophobic BCP molecule, thereby stabilizing the complex more efficiently.

Fluorescence emission analysis corroborated these findings, further confirming the 1:1 stoichiometry of the complexes. The binding constants determined via fluorescence were somewhat lower than those obtained from absorption measurements 104 M<sup>-1</sup> for BCP:α-CD and 151 M<sup>-1</sup> for BCP:β-CD yet still indicated better interactions. The observed variations in binding constants between the two spectroscopic methods may reflect differences in the sensitivity of fluorescence and absorption techniques to local environmental changes around BCP upon complex formation.

Collectively, the data demonstrate that both  $\alpha$ - and  $\beta$ -cyclodextrins can effectively form inclusion complexes with BCP, with  $\beta$ -CD exhibiting a notably stronger binding affinity. These results emphasize the influence of cyclodextrin cavity size and hydrophobicity on the binding strength and stability of host-guest complexes. The formation of such complexes holds promise for enhancing the solubility, stability, and bioavailability of hydrophobic compounds like BCP in pharmaceutical and food applications.

## CONCLUSION

This study confirms that  $\beta$ -caryophyllene (BCP) forms stable 1:1 inclusion complexes with both  $\alpha$ - and  $\beta$ -cyclodextrins, as demonstrated by absorption and fluorescence spectral analyses using the Benesi-Hildebrand method. The binding constants reveal a stronger affinity of BCP for  $\beta$ -cyclodextrin compared to  $\alpha$ -cyclodextrin, likely due to the larger hydrophobic cavity of  $\beta$ -CD providing enhanced molecular encapsulation. These findings highlight the potential of cyclodextrins to improve the solubility and stability of hydrophobic compounds like BCP, paving the way for their effective application in pharmaceutical and food industries.

## REFERENCES

1. Cragg, G. M., & Newman, D. J. (2013). "Natural products: A continuing source of novel drug leads." *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1830(6), 3670–3695.
2. Ekor, M. (2014). "The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety." *Frontiers in Pharmacology*, 4(177), 1–10.
3. Arul, V., Miyazaki, S., & Dhananjayan, R. (2005). "Studies on the anti-inflammatory, antipyretic and analgesic properties of *Aegle marmelos* Corr. Serr. (Rutaceae) leaves in animal models." *Journal of Ethnopharmacology*, 96(1–2), 159–163.
4. Balakumar, R., Rajan, S., & Thirunalasundari, T. (2010). "Antifungal activity of *Aegle marmelos* leaf extract on dermatophytic fungi." *International Journal of Pharmaceutical Sciences Review and Research*, 3(1), 19–22.
5. Santos, P., Oliveira, R., & Almeida, S. (2022). "Therapeutic relevance of  $\beta$ -caryophyllene: Pharmacological applications and mechanisms of action." *Phytomedicine*, 104(154285), 1–13.
6. Fidy, K., Fiedorowicz, A., Strzdała, L., & Szumny, A. (2016). " $\beta$ -Caryophyllene and  $\beta$ -caryophyllene oxide—natural compounds of anticancer and analgesic properties." *Cancer Medicine*, 5(10), 3007–3017.
7. Bakır, B., Him, A., Öztürk, T., & Yesilada, E. (2008). "Evaluation of anti-inflammatory and antinociceptive effects of  $\beta$ -caryophyllene in rodents." *Journal of Ethnopharmacology*, 116(3), 530–536.
8. Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J. Z., Xie, X. Q., Altmann, K. H., Karsak, M., & Zimmer, A. (2008). "Beta-caryophyllene is a dietary cannabinoid." *Proceedings of the National Academy of Sciences USA*, 105(26), 9099–9104.
9. Younis, N. S., El-Nashar, H. A., & El-Tanbouly, G. S. (2020). "Pharmacological and therapeutic activities of  $\beta$ -caryophyllene: A review." *Frontiers in Pharmacology*, 11(564172), 1–18.
10. Del Valle, E. M. M. (2004). "Cyclodextrins and their uses: A review." *Process Biochemistry*, 39(9), 1033–1046.
11. Szejtli, J. (1998). "Introduction and general overview of cyclodextrin chemistry." *Chemical Reviews*, 98(5), 1743–1754.

12. Loftsson, T., & Brewster, M. E. (2012). "Cyclodextrins in the pharmaceutical sciences." *Journal of Pharmaceutical Sciences*, 101(9), 3019–3032.
13. Mura, P., Zoppi, A., & Maestrelli, F. (2023). "Cyclodextrin-based delivery systems for natural compounds: Design, characterization, and applications." *International Journal of Pharmaceutics*, 634(122685), 1–15.
14. Challa, R., Ahuja, A., Ali, J., & Khar, R. K. (2005). "Cyclodextrins in drug delivery: An updated review." *AAPS PharmSciTech*, 6(2), E329–E357.
15. Jin, X., Zhao, Y., & Li, Q. (2023). "Host–guest chemistry of cyclodextrins with bioactive molecules: Spectroscopic and computational approaches." *Journal of Molecular Liquids*, 387(122489), 1–11.
16. Benesi, H. A., & Hildebrand, J. H. (1949). "A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons." *Journal of the American Chemical Society*, 71(8), 2703–2707.
17. Connors, K. A. (1997). "The stability of cyclodextrin complexes in solution." *Chemical Reviews*, 97(5), 1325–1358.
18. Adeoye, O., & Cabral-Marques, H. M. (2017). "Cyclodextrin inclusion complexes: Effect on the physicochemical properties and bioactivity of essential oils." *Carbohydrate Polymers*, 159, 129–143.
19. Zhang, J., Li, Y., & Xu, Z. (2024). "Cyclodextrin-based nanocarriers for natural bioactive compounds." *International Journal of Biological Macromolecules*, 238(124056), 1–12.