RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2025.051

ScienceAsia 51 (4): 2025: ID 2025051: **1–5**

The effects of microwave inducement towards inclusion complex formation by nanoencapsulation of linalool

Nantina Moonprasith[a], Purin Sathirathai[a], Sasimas Katanyutanon[a], Masayuki Yamaguchi[b], Luckhana Lawtrakul[a,*], Pisanu Toochinda[a,*]

[a] School of Bio-Chemical Engineering and Technology, Sirindhorn International Institute of Technology, Thammasat University, Pathum Thani 12120 Thailand

[b] School of Materials Science, Japan Advanced Institute of Science and Technology, Ishikawa 923-1292 Japan

*Corresponding authors, e-mail: luckhana@siit.tu.ac.th, pisanu@siit.tu.ac.th

Received 27 Aug 2024, Accepted 2 Jun 2025
Available online 13 Jul 2025

ABSTRACT: Linalool, a chiral alcohol terpene, possesses distinctive odor profiles due to its enantiomers which are lavender-like (*R*)-linalool and herbaceous (*S*)-linalool. It is commonly found and used in wine, cosmetics, and aromatic products. However, products containing linalool may suffer from short shelf life due to its high volatility. Improving the stability and creating an ability to control the enantiomeric ratio of linalool can enhance the shelf life and alter the odor profile of the final products. For the stability improvement, nanoencapsulation with beta-cyclodextrin (β-CD) can be introduced with the added benefit of altering the enantiomeric ratio of linalool. In this study, microwave inducement at 300 W for 5 s was introduced during the complex formation process to induce rotational motions of linalool. The results showed that microwave inducement improved the encapsulation efficiency from 50.70% to 59.42%. This is probably due to the induced rotation of linalool molecules which increases the probability of proper alignments of the host and guest molecules. In addition, results from GC-FID showed that microwave inducement increased the composition of (*R*)-linalool in the inclusion complex from 49.87% to 52.10%. Results from semi-empirical PM7 calculations with 2:2 host:guest ratio showed that the *RR*/Dimer had lower complexation energy and was more balanced in terms of charge distribution. The increase in stability of *RR*/Dimer suggests that the binding between (*R*)-linalool dimers and β-CD is preferred, which leads to the higher enantiomeric selectivity towards (*R*)-linalool in the inclusion complex.

KEYWORDS: enantiomeric selectivity, inclusion complex, linalool, microwave inducement, nanoencapsulation

INTRODUCTION

Linalool, a chiral compound found in aromatic plants, is known for its strong flavors and odors as well as medical properties, such as anticarcinogenic, antidiabetic, antioxidant, and antimicrobial effects [1, 2]. Linalool exists in two enantiomeric forms: (3*R*)-(−)-linalool with a lavender scent and (3*S*)-(+)-linalool with an herbaceous scent [3]. These enantiomers have different detection-threshold values, with the threshold for (*S*)-linalool 10-fold of that of (*R*)-linalool [4]. Therefore, the enantiomeric ratio of linalool tremendously influences the odor profiles of final products. The ability to control the enantiomeric ratio of linalool can be used to modify the products' aroma; therefore, an affordable and easy technique to improve the enantiomer is yet to be found due to their similar chemical properties. Moreover, linalool is a terpene alcohol with low aqueous solubility and high volatility, which limits its shelf life in commercial products [5].

To address these limitations, nanoencapsulation with β-cyclodextrin (β-CD) has been proposed. β-CD, a cyclic oligosaccharide, is widely used as an encapsulating agent because it improves the stability and aqueous solubility of various chemical compounds and pharmaceutical agents through the formation of inclusion complexes [6, 7]. However, the encapsulation methods often require complicated and costly processes, such as crosslinking β-CD or strict control of process temperatures to optimize the encapsulation efficiency [8–10]. Microwave-assisted synthesis has emerged as an efficient alternative due to its ability to enhance molecular interactions, reduce processing time, and improve complexation yield [11, 12]. Microwave has been used as the source of heating in encapsulation of other essential oils with cyclodextrin derivatives and found to increase encapsulation efficiency [13, 14].

It should also be noted that the application of β-CD for enantiomeric selection of linalool has not been widely investigated. Computational simulations and molecular docking calculations from previous work of our group suggested the complex formation at 1:1 host-guest ratio between β-CD and linalool and indicated that the inclusion complexes of (*R*)-linalool and β-CD have lower average complexation energy compared to the complexes of (*S*)-linalool and β-CD [15]. The difference in complexation energy implies that enantiomeric selectivity can be achieved through inclusion complex formation.

To facilitate the formation of inclusion complexes, proper alignment of host and guest molecules is crucial. In this study, microwave inducement was introduced to promote rotational motion of the host and guest molecules. Its effects on encapsulation

efficiency and enantiomeric selectivity of linalool were investigated, and a simulation was conducted on both (*R*)- and (*S*)-linalool dimers to explain the effects of microwave on nanoencapsulation of linalool enantiomers.

MATERIALS AND METHODS

Linalool- β-CD inclusion complex formation and collection

Solutions of the host molecules were prepared by dissolving 8 mM of β-CD (98%, Tokyo Chemical Industry, Tokyo, Japan) in 20 ml solution comprising 30% ethanol (99.9%, RCI Labscan, Bangkok, Thailand) by volume in RO water to optimize encapsulation efficiency [16]. Subsequently, the solutions underwent magnetic stirring for 3 min. The guest molecule, racemic linalool (97%, Sigma Aldrich, Missouri, USA), was introduced into each sample with a 1:1 molar ratio with β-CD. The samples were then separated into 2 groups: the control group prepared "without microwave" and the test group prepared "with microwave". Samples in the latter group underwent 3 cycles of microwave treatment in a MARS 6 microwave oven (CEM, North Carolina, USA) at a constant temperature of 30 °C and power of 300 W. Each cycle of microwave treatment lasted for 5 s with 1-min intervals for cooling down. Once all the microwave cycles were finished, the samples were incubated in an incubator shaker operating at 25 °C and 150 rpm for 1 h. After incubation, the samples were placed in an ice bath set at −4 °C for 10 min to facilitate additional precipitation of the inclusion complexes. The resulting solid inclusion complexes were subsequently filtered using filter papers and a vacuum pump, followed by rinsing with cold ethanol to remove any non-encapsulated linalool. The solid inclusion complexes were then allowed to air-dry at room temperature for 4 h and subsequently stored at 4 °C.

Encapsulation efficiency evaluation with high performance liquid chromatography (HPLC)

HPLC was performed to determine the amount of encapsulated linalool. Encapsulation efficiency was calculated using Eq. (1). A mobile phase consisting of 55% acetonitrile solution by volume (99.9%, RCI Labscan) in Type 1 deionized water was used. The solid inclusion complexes, after being dried, were mixed with 10 ml of ethanol each, followed by a 30-min sonication (Crest Ultrasonics, New Jersey, USA). The samples prepared were then injected into the 1260 Infinity II LC system (Agilent Technologies, California, USA). The stationary phase used was a C18 column (Luna 5 µm C18(2) 100 Å, 250 mm × 4.6 mm column, Phenomenex, California, USA), set at 25 °C with a flow rate of 1 ml/min and injection volume of 5 µl. UV detection was performed at 215 nm, with the detector sensitivity set at 0.005 absorbance units full scale and the chart speed of 5 mm/min.

$$\text{Encapsulation efficiency} = \frac{\text{Amount of encapsulated linalool}}{\text{Initial amount of linalool}} \times 100\% \quad (1)$$

Enantiomeric analysis with gas chromatography with flame ionization detection (GC-FID)

The enantiomeric analysis of linalool/β-CD inclusion complexes was conducted using GC-FID (Clarus 580, PerkinElmer, Massachusetts, USA). Prior to injection, the encapsulated linalool was extracted with ethanol and underwent filtration with a 0.22 µm syringe filter. The chiral capillary column used was MEGA-DEX DET BETA (30 m × 0.32 mm, 0.25 µm film thickness). The injection volume was set at 1 µl, with a split flow of 60 ml/min and a temperature of 250 °C. The oven temperature was programmed to increase from 60 °C to 100 °C at a rate of 2 °C/min, followed by another increase from 100 °C to 200 °C at a rate of 30 °C/min, then held for 2 min. The detector operated at 250 °C, and helium was used as the carrier gas at a flow rate of 1 ml/min. The percentage of (*R*)-linalool in the analyzed sample was calculated using Eq. (2), and it was subtracted from 100% to obtain the percentage of (*S*)-linalool.

$$\text{Percentage of } (R)\text{-linalool} = \frac{\text{Amount of } (R)\text{-linalool}}{\text{Amount of } (R)\text{-linalool} + \text{Amount of } (S)\text{-linalool}} \times 100\% \quad (2)$$

Method of calculation for molecular simulation

The crystal structure of (*R*)-linalool with β-CD in a molecular ratio of 2:2 was downloaded from the Cambridge Crystallographic Data Centre with the deposition number of 1439675 [17]. The modification of the X-ray structures for atom elimination, atom addition, and chirality alteration was performed in Discovery Studio 2020 Client program [18]. Geometry optimization of all the investigated molecular structures was performed with the semi-empirical PM7 [19] in gas phase using the Gaussian 16 molecular modeling package [20].

The interaction between the linalool and the β-CD molecules can be quantified by the complexation energy (ΔE) in Eq. (3):

$$\Delta E = E_{cpx}^{opt} - \left(E_{host}^{opt} + E_{guest}^{opt}\right) \quad (3)$$

where E_{cpx}^{opt}, E_{host}^{opt}, and E_{guest}^{opt} are the optimized energies of the inclusion complex (four molecules), the isolated host molecules (β-CD dimer), and the isolated guest molecules (two linalool; *RR* or *SS*), respectively. The lower the complexation energy value, the more stable the inclusion complex.

RESULTS AND DISCUSSION

Effect of microwave on encapsulation efficiency of linalool with β-CD

In this study, the encapsulation step of linalool with β-CD was performed with and without microwave

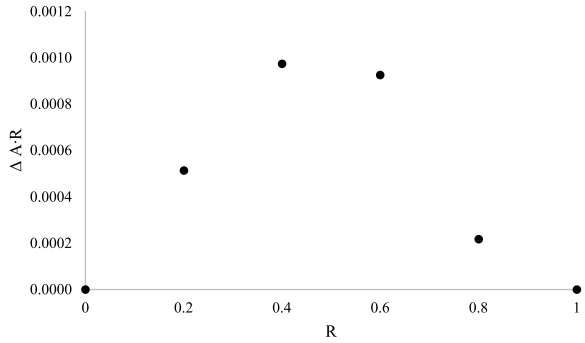

Fig. 1 Job plot of linalool and β-CD from the method of continuous variation, where R represents the molar ratio of linalool to β-CD and $\Delta A \cdot R$ is the change in absorbance multiplied by the molar ratio.

Table 1 Encapsulation efficiency of linalool/β-CD inclusion complexes prepared without and with microwave.

Sample	Encapsulation efficiency (%)
Without microwave	50.70 ± 0.26
With microwave	59.42 ± 0.14

inducement. The formation of inclusion complex between linalool and β-CD using the same encapsulation steps had been confirmed using FT-IR and TGA analyses in our previous publication [16], and the stoichiometry of the complex was verified to be 1:1 using the method of continuous variation, as shown in Fig. 1. The encapsulation efficiency in each case was calculated via Equation 1, based on the amount of encapsulated linalool measured by HPLC. The average encapsulation efficiency of the linalool/β-CD inclusion complexes prepared without and with microwave from 5 trials together with the standard deviations are shown in Table 1. The linalool/β-CD inclusion complex prepared with microwave showed 8.72% improvement in encapsulation efficiency compared to its non-microwave counterpart. The result suggests that microwave can promote the encapsulation process of linalool as expected, and it can also be utilized to improve the stability of linalool in other applications. This improvement is likely due to the induced rotational motions of linalool and β-CD which create a higher probability of proper alignment of the host and guest molecules.

Effect of microwave on enantiomeric selectivity in linalool/β-CD inclusion complex

In addition to the effect on encapsulation efficiency, the effect of microwave inducement on the enantiomeric selectivity of linalool in the inclusion complex was also investigated with GC-FID using a chiral column. The percentages of each linalool enantiomer in the inclusion complexes prepared with and without microwave are presented and compared with the enantiomeric percentages of a linalool racemic standard in Table 2. The inclusion complex without microwave increased the percentage of (*R*)-linalool from 49.24% to 49.87% compared to linalool racemic standard. It shows that (*R*)-linalool is slightly more preferred than (*S*)-linalool during the encapsulation process with β-CD. On the other hand, when microwave was applied during encapsulation, the proportion of (*R*)-linalool increased from 49.24% to 52.10%. The application of microwave during encapsulation yielded an increase in the (*R*)-linalool percentage compared to encapsulation without microwave. This improvement can be utilized to modify the odor profile of linalool-based products. The reasons for this improvement will be investigated using a molecular simulation on dimers of (*R*)- and (*S*)-linalool.

Table 2 Percentages of linalool enantiomers in racemic standard mixture and the inclusion complexes prepared without and with microwave.

Sample	(*R*)-linalool (%)	(*S*)-linalool (%)
Linalool racemic standard	49.24 ± 0.35	50.76 ± 0.35
Inclusion complex:		
-without microwave	49.87 ± 0.33	50.13 ± 0.33
-with microwave	52.10 ± 0.21	47.90 ± 0.21

Simulation results of linalool dimers

The semiempirical PM7 energy-optimized structures of linalool enantiomers complexed with β-CD molecules, in 1:1 and 2:2 guest-host stoichiometries, are shown in Fig. 2. The calculations were conducted in the gas phase, meaning that solvent molecules do not influence the binding of β-CD and linalool. In all complex systems studied, the dimethyl group of the linalool molecule extends toward the wider rim of the β-CD, while its hydroxyl group points downward into the cavity, and its vinyl group is positioned at the narrower rim of the β-CD.

The formation of inclusion complexes in both the 1:1 and 2:2 guest:host ratios is energetically favourable and stable, as indicated by the negative complexation energy values (ΔE) and the large |HOMO–LUMO| gap values, as shown in Table 3. β-CD can form 1:1 inclusion complex with both linalool enantiomers with comparable energy levels ($\Delta E = -29.34$ and -30.88 kcal/mol). However, the ΔE values for the 2:2 guest:host ratio (*RR*/Dimer, *SS*/Dimer, and *RS*/Dimer) are much lower than those for the 1:1 ratio (*R*/β-CD and *S*/β-CD), suggesting that the complex with 2:2 guest:host ratio is more stable and more likely to form. The lowest complexation energy, found in the *RR*/Dimer ($\Delta E = -83.03$ kcal/mol), indicates that it forms the most stable complex. Additionally, *RR*/Dimer had the lowest dipole moment ($\mu = 5.82$ D),

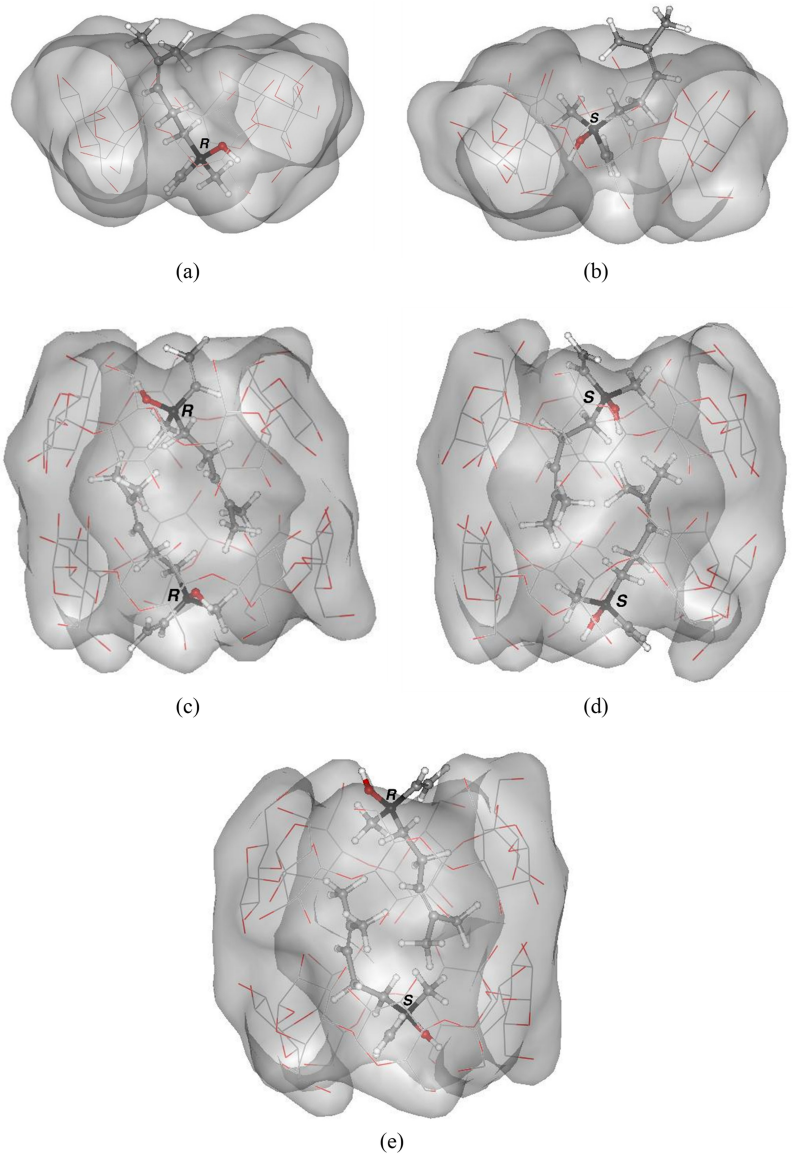

Fig. 2 PM7 energy-minimized structure of 1:1 and 2:2 guest-host stoichiometry complexes of (a) *R*/β-CD, (b) *S*/β-CD, (c) *RR*/Dimer, (d) *SS*/Dimer, and (e) *RS*/Dimer. Linalool molecules are presented as ball and stick models while β-CD molecules are presented as line models with their surface with the probe radius 1.4 Å. Hydrogen atoms of β-CD are omitted for simple clarification.

Table 3 Dipole moment (μ), LUMO and HOMO energies, and complexation energy (ΔE) of 1:1 and 2:2 guest-host stoichiometry complexes calculated by PM7 method.

	R/β-CD	*S*/β-CD	*RR*/Dimer	*SS*/Dimer	*RS*/Dimer
μ (D)	6.21	10.71	5.82	18.46	8.26
LUMO (eV)	0.76	0.59	0.55	0.32	0.62
HOMO (eV)	−9.30	−9.51	−8.96	−9.16	−9.22
|HOMO–LUMO| (eV)	10.06	10.10	9.51	9.49	9.85
ΔE (kcal/mol)	−29.34	−30.88	−83.03	−55.90	−67.55

which suggests a more balanced charge distribution compared to the *SS*/Dimer and *RS*/Dimer complexes. A higher dipole moment typically results in lower complex stability [20, 21], contributing to the preference for (*R*)-linalool in the 2:2 guest-host inclusion complex with β-CD.

CONCLUSION

This study explored the effect of microwave inducement during the encapsulation of linalool in β-CD with a 1:1 molar ratio. Results from HPLC demonstrated that microwave inducement could increase encapsulation efficiency from 50.70% to 59.42%. Microwave inducement could also influence the enantiomeric ratio of linalool by increasing the percentage of (*R*)-linalool in the inclusion complex from 49.87% to 52.10%. A semi-empirical PM7 molecular simulation was performed with a guest:host ratio of 2:2 and showed that the formation of complexes between (*R*)-linalool dimers and β-CD is preferred due to the lower complexation energy and lower dipole moment of the *RR*/Dimer complex. (*S*)-linalool dimers which showed higher dipole moments both in their free and complex forms may be more disturbed by the oscillating electromagnetic field from microwave and hindered from forming a complex with the β-CD host molecules. This study thus confirmed that microwave inducement can be applied to the encapsulation of linalool to improve its stability and control its enantiomeric ratio.

Acknowledgements: This work was supported by Thammasat University Research Fund, Contact No. TUFT 053/2565. The first author acknowledges the JAIST Scholarship awarded by Sirindhorn International Institute of Technology, Thammasat University.

REFERENCES

1. Kamatou GPP, Viljoen AM (2008) Linalool: A review of a biologically active compound of commercial importance. *Nat Prod Commun* **3**, 1183–1192.
2. Bernhoft A (2008) Bioactive compounds in plants: Benefits and risks for man and animals. In: *Proceedings from a Symposium Held in Norwegian Academy of Science and Letters*, Novus Forlag, Oslo.
3. Özek T, Tabanca N, Demirci F, Wedge DE, Hüsnü Can Başer K (2010) Enantiomeric distribution of some linalool containing essential oils and their biological activities. *Rec Nat Prod* **4**, 180–192.
4. Padrayuttawat A, Yoshizawa T, Tamura H, Tokunaga T (1997) Optical isomers and odor thresholds of volatile constituents in citrus sudachi. *Food Sci Technol Int (Tokyo)* **3**, 402–408.
5. Turek C, Stintzing FC (2013) Stability of essential oils: A review. *Compr Rev Food Sci Food Saf* **12**, 40–53.
6. Somphon W, Loisruangsin A (2023) ZIF-8, chitosan and β-cyclodextrin-incorporated ZIF-8 nanohybrid for improving drug delivery. *ScienceAsia* **49**, 776–785.
7. Viernstein H, Wolschann P (2020) Cyclodextrin inclusion complexation and pharmaceutical applications. *ScienceAsia* **46**, 254–262.
8. Al-Nasiri G, Cran MJ, Smallridge AJ, Bigger SW (2018) Optimisation of β-cyclodextrin inclusion complexes with natural antimicrobial agents: thymol, carvacrol and linalool. *J Microencapsul* **35**, 26–35.
9. Ciobanu A, Mallard I, Landy D, Brabie G, Nistor D, Fourmentin S (2012) Inclusion interactions of cyclodextrins and crosslinked cyclodextrin polymers with linalool and camphor in lavandula angustifolia essential oil. *Carbohydr Polym* **87**, 1963–1970.
10. Aytac Z, Yildiz ZI, Kayaci-Senirmak F, Tekinay T, Uyar T (2017) Electrospinning of cyclodextrin/linalool-inclusion complex nanofibers: fast-dissolving nanofibrous web with prolonged release and antibacterial activity. *Food Chem* **231**, 192–201.
11. Rizkiana MF, Salsabila ZA, Aulia RI, Amini HW, Palupi B, Rahmawati I, Fachri BA (2024) Microwave-assisted synthesis of chitosan-hydroxyapatite composite from eggshells and its adsorption properties. *ScienceAsia* **50**, ID 2024067.
12. Chotpatiwetchkul W, Saengsawang M, Sriwong C (2022) Facile and green synthesis of AgNPs by microwave-assisted method using curcumin biomaterial for improving antibacterial activities of NR/Ag composite sheets. *ScienceAsia* **48**, 847–854.
13. Rodríguez-López MI, Mercader-Ros MT, López-Miranda S, Pellicer JA, Pérez-Garrido A, Pérez-Sánchez H, Núñez-Delicado E, Gabaldón JA (2019) Thorough characterization and stability of HP-β-cyclodextrin thymol inclusion complexes prepared by microwave technology: A required approach to a successful application in food industry. *J Sci Food Agric* **99**, 1322–1333.
14. Das S, Mohanty S, Maharana J, Jena SR, Nayak J, Subuddhi U (2020) Microwave-assisted β-cyclodextrin/chrysin inclusion complexation: an economical and green strategy for enhanced hemocompatibility and chemosensitivity *in vitro*. *J Mol Liq* **310**, 113257.
15. Poonphatanapricha T, Katanyutanon S, Jitapunkul K, Lawtrakul L, Toochinda P (2021) The preservation and enantiomeric selection of linalool by nanoencapsulation using cyclodextrins. *Sci Pharm* **89**, 42.
16. Ceborska M (2016) Structural investigation of the β-cyclodextrin complexes with linalool and isopinocampheol: Influence of monoterpenes cyclicity on the host-guest stoichiometry. *Chem Phys Lett* **651**, 192–197.
17. Brooks BR, Brooks III CL, Mackerell Jr AD, Nilsson L, Petrella RJ, Roux B, Won Y, Archontis G, et al (2009) CHARMM: the biomolecular simulation program. *J Comput Chem* **30**, 1545–1614.
18. Stewart JJP (2013) Optimization of parameters for semiempirical methods VI: more modifications to the NDDO approximations and re-optimization of parameters. *J Mol Model* **19**, 1–32.
19. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, et al (2016) *Gaussian 16*, revision c.01.
20. Petov GM, Matrosov EI, M I Kabachnik MI (1978) Determination of dipole moments and stability constants for molecular complexex. *Russ Chem Bull* **27**, 2020–2024.
21. Triamchaisri N, Toochinda P, Lawtrakul L (2023) Structural investigation of beta-cyclodextrin complexes with cannabidiol and delta-9-tetrahydrocannabinol in 1:1 and 2:1 host-guest stoichiometry: Molecular docking and density functional calculations. *Int J Mol Sci* **24**, 1525.