COMPUTATIONAL METHODS FOR THE INTERACTION BETWEEN CYCLODEXTRINS AND NATURAL COMPOUNDS

BY

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ABSTRACT

Molecular interactions of Cannabidiol (CBD) and Delta-9-Tetrahydrocannabinol (THC9) with Beta-Cyclodextrins (BCD) are investigated by computer modelling methods. The results revealed that the interactions between BCD/CBD and BCD/THC9 are possible and form multiple hosts with one guest inclusion complexes (1:1) and (2:1). In one-to-one inclusion complex results suggested that there are two conformations for BCD/CBD and one conformation for BCD/THC9. It is also recommended that the more intermolecular hydrogen bonding between host and guest. The stability of the hosts (BCD and 2BCD) and guests (CBD and THC9) inclusion complexes are determined by the complexation energies and the HOMO and LUMO gaps which suggested that 2:1 inclusion complex (-83.53 to -135.36 kcal/mol) are more energy favorable than the 1:1 inclusion complex (-30.00 to -34.92 kcal/mol). However, the deformation energies of the host and the guest components in the 2:1 inclusion complex (37.47-96.91 kcal/mol) are much higher than those in the 1:1 inclusion complex (3.49-8.69 kcal/mol) which means the formation processes of the 2:1 inclusion complex are more difficult than 1:1 inclusion complex. The result of this study is supported by the experimental results that the complexation constant of 1:1 BCD/CBD (K_s = 300 M^{-1}) is greater than that of 2:1 2BCD/CBD (K_{ss} = 0.833 M^{-1}).

Keywords: Cannabis, Cannabidiol, Tetrahydrocannabinol, Cyclodextrins, Inclusion Complex

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Nat Triamchaisri

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LIST OF SYMBOLS/ABBREVIATIONS

Symbols/Abbreviations	Terms
ACD	Alpha-Cyclodextrin
B3LYP	"Becke, 3-parameter, Lee–Yang–Parr" Hybrid Function
BCD	Beta-Cyclodextrin
CBD	Cannabidiol
CD	Cyclodextrin
Conf.	Conformation
ΔE	Complexation Energy
ΔG	Free Binding Energy
DFT	Density Function Theory
DMBCD	2,6-Dimethyl-Beta-Cyclodextrin
E_{DEF}	Deformation Energy
GCD	Gamma-Cyclodextrin
HH	Head-to-Head Position
HPBCD	2-Hydroxypropyl-Beta-Cyclodextrin
Ks	Complexation Constant of 1:1 Complex
Kss	Complexation Constant of 2:1 Complex
TH	Tail-to-Head Position
THC	Tetrahydrocannabinol
THC8	Delta-8-Tetrahydrocannabinol
THC9	Delta-9-Tetrahydrocannabinol
TT	Tail-to-Tail Position
μ	Electric Dipole Moment
μ_{sp} (component)	Electric Dipole Moment value of a component at a single point of the optimized geometry

CHAPTER 1
INTRODUCTION

1.1 Natural Compounds Interested

Cannabis, also known as marijuana, is a psychoactive drug that has been used for thousands of years for various purposes. The plant has a complex history and has been used for medicinal, recreational, and spiritual purposes throughout many cultures and civilizations. The cannabis plant contains more than 100 active compounds, known as cannabinoids, with the most well-known being THC (tetrahydrocannabinol) and CBD (cannabidiol). THC is the psychoactive compound that produces the "high" associated with marijuana use, while CBD has medicinal properties and does not produce a high. Cannabis has a long history of use for medicinal purposes, dating back to ancient China and India, where it was used to treat a variety of ailments such as pain, nausea, and anxiety. In the Western world, cannabis was introduced as a medicinal treatment in the 1800s, and it was widely prescribed by doctors for a range of conditions. However, in the early 20th century, cannabis became stigmatized due to concerns about its psychoactive effects and potential for abuse. In the United States, cannabis was criminalized in the 1930s under the Marihuana Tax Act, which imposed heavy taxes and regulations on cannabis-related activities. This law effectively banned the use and distribution of cannabis and led to a decline in its medicinal use.

In recent years, there has been growing interest in the medicinal properties of cannabis, particularly in the treatment of chronic pain, nausea, and other conditions. The legalization of medical cannabis has been approved in many states and countries, and research has shown promising results in the treatment of various conditions. However, the use and legalization of cannabis remain controversial issues, with proponents arguing that it has numerous medical benefits and should be legalized for recreational use, while opponents cite concerns about the potential for abuse, addiction, and negative health effects. Cannabis has a rich history of use for medicinal, recreational, and spiritual purposes, and its active compounds have been studied extensively for their potential therapeutic benefits. While the debate over its use and

legalization continues, research and advancements in the field of cannabis science are likely to shed more light on the potential benefits and risks associated with its use.

Cannabidiol, or CBD, is a compound found in the cannabis plant that has garnered significant attention in recent years for its potential health benefits. Unlike its psychoactive counterpart, THC, CBD does not produce a “high” and is legal in many countries. CBD is one of over 100 cannabinoids found in the cannabis plant, and it is thought to interact with the body's endocannabinoid system (ECS), which regulates various bodily functions such as mood, appetite, and sleep. The ECS consists of receptors located throughout the body, and CBD is believed to bind to these receptors, potentially producing various therapeutic effects.

One of the most widely recognized benefits of CBD is its ability to reduce anxiety and stress. A 2019 study published in the Journal of Clinical Psychology found that CBD significantly reduced anxiety levels in patients with social anxiety disorder. Another study published in 2019 in the Permanente Journal found that CBD was effective in reducing anxiety and improving sleep in patients with anxiety and sleep disorders. CBD has also been studied for its potential anti-inflammatory properties. A 2018 review of existing research found that CBD may have therapeutic potential for treating various inflammatory conditions, such as rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis. In addition to its potential therapeutic benefits, CBD has also been investigated for its safety and tolerability. A 2018 report from the World Health Organization found that CBD was generally well-tolerated and had a good safety profile, with no evidence of abuse potential or harm to public health. Despite its potential benefits and safety profile, the legality of CBD remains a complex issue in many countries. In the United States, CBD extracted from hemp plants containing less than 0.3% THC is legal at the federal level, but regulations vary by state. In other countries, the legality of CBD can vary widely, with some countries allowing the use of CBD only for medical purposes, while others have more permissive laws. CBD is a compound found in the cannabis plant that has shown promise in treating a variety of conditions, including anxiety, inflammation, and sleep disorders. While more research is needed to fully understand its therapeutic potential, CBD has a good safety profile and is legal in many countries. As interest in CBD continues to grow, it is likely to become an increasingly popular and widely used therapeutic option.

Delta-9-tetrahydrocannabinol, or THC9, is the primary psychoactive compound found in the cannabis plant. It is responsible for the “high” associated with marijuana use and is one of the most well-known cannabinoids found in the plant. THC9 works by binding to cannabinoid receptors in the brain and nervous system, activating the release of dopamine, a neurotransmitter associated with pleasure and reward. This produces the characteristic euphoric effects of marijuana use. In addition to its psychoactive effects, THC9 has been studied for its potential therapeutic benefits. One of the most widely recognized benefits of THC9 is its ability to reduce pain and inflammation. A 2018 review of existing research found that THC9 was effective in treating chronic pain and neuropathic pain, and it may also have anti-inflammatory properties. THC9 has also been studied for its potential use in treating various medical conditions, such as nausea and vomiting associated with chemotherapy, muscle spasms associated with multiple sclerosis, and loss of appetite and weight loss associated with HIV/AIDS. In many countries, medical marijuana containing THC9 is legal for these and other medical conditions.

However, the use of THC9 is not without its risks. Heavy use of marijuana containing high levels of THC9 has been associated with various negative health effects, such as impaired memory and cognition, increased risk of psychosis, and respiratory problems. Additionally, the use of marijuana containing THC9 can lead to addiction and withdrawal symptoms in some individuals. The legality of THC9 varies widely by country and jurisdiction. In some countries, such as the Netherlands, the use of marijuana containing THC9 is legal for recreational use. In other countries, such as the United States, marijuana containing THC9 is legal for medical use in some states, but still illegal at the federal level. THC9 is the primary psychoactive compound found in the cannabis plant and is responsible for the “high” associated with marijuana use. While it has been studied for its potential therapeutic benefits, heavy use of marijuana containing high levels of THC9 can have negative health effects and can lead to addiction. The legality of THC9 varies widely by country, and regulations surrounding its use and distribution continue to be the subject of ongoing debate and discussion.

1.2 Statement of the Problem

One of the main challenges associated with using CBD as a therapeutic compound is its poor water solubility. CBD is hydrophobic, meaning it does not readily dissolve in water. This can make it difficult to incorporate into water-based products, such as beverages or creams. The low water solubility of CBD can also impact its bioavailability, or the amount of CBD that is absorbed and utilized by the body. When CBD is ingested, much of it can be lost during digestion and metabolism before it can reach its target receptors. Improving the water solubility of CBD has the potential to increase its bioavailability and improve its therapeutic effectiveness.

Like CBD, THC9 also faces the challenge of poor water solubility. THC9 is hydrophobic and does not readily dissolve in water, which makes it difficult to incorporate into water-based products. This can limit the effectiveness of THC9 when used in products such as beverages, which are a popular method for consuming marijuana. THC9 that is not water-soluble may not be fully absorbed by the body, which can reduce its therapeutic effects and potency. Improving the water solubility of THC9 has the potential to improve the effectiveness and safety of marijuana-based products. The water solubility of CBD is about 0.0126 mg/mL, and THC9 is about 0.0028 mg/mL, indicating low bioavailability. Recent studies show that the inclusion complex of cyclodextrins with lipophilicity substances can increase water solubility and improve stability.

Molecular modelling techniques have been widely used in chemistry and pharmacology to obtain insight into information at the molecular level of systems of interest. The computational results help to explain the molecular interactions and suggest the mechanisms that govern the processes when experimental techniques are insufficient. The calculation models can predict and screen the results when varying the compounds or the system conditions prior to the traditional experimental methods.

1.3 The Objective of the Study

Molecular modelling of inclusion complexes between CBD and THC9 with natural cyclodextrins is performed and compared with the laboratory results to identify whether the computational method can predict the trends along with the laboratory results or not. Hence, the objective of the study will be as follows,

- Find the fitting parameters to determine the reliability of complex formation by computational methods.
- Find the possibilities of 1:1 and 2:1 of beta-cyclodextrins with CBD and THC9 inclusion complexes computationally. This also includes inclusion mechanisms with the relationship of reactants and products in chemical reactions.

1.4 Scope of the Study

The computational molecular docking results of CBD and THC9 with beta-cyclodextrins will be examined and compared with laboratory results of prior studies in order to show that how the reliability of computational methods.

CHAPTER 2
LITERATURE REVIEW

2.1 Cannabis

The cannabinoid is described as a psychoactive substance that can be extracted from cannabis or marijuana plant which can be divided into three species that are Sativa, Indica, and Ruderalis depending on the concentration of one of the main components called tetrahydrocannabinol (Hall & Solowij, 1998). Nowadays, benefits from cannabis are being used in many fields, such as ritualism, medicine, and recreational purposes, but there are some unwanted adverse effects from it as well. Cannabis can be used in various ways; the simplest way is by smoking, vaporizing, and oral route. Most of the cannabis can be transformed into cannabis products such as eye drops and aerosols (Baggio et al., 2014). These products can be taken by sublingual, ingesting edibles, and rectal administration. Compounds that can be found in cannabis are classified into several chemical classes such as hydrocarbon, nitrogenous compound, amino acids, cannabinoids, proteins, sugar, alcohols, aldehydes, ketones, acids, fatty acids, esters, steroids, terpenes, non-cannabinoid phenol, vitamins, pigments, and elements (Nascimento et al., 2015). The most important compound that is present in cannabis is the cannabinoid, which is composed of 21 carbons, as shown in **Figure 2.1**.

There are many different types of cannabinoids, and they can be classified into Tetrahydrocannabinol (THC), Tetrahydrocannabinol acid (THCA), Cannabidiol (CBD), Cannabidiolic acid (CBDA), Cannabigerol (CBG), Cannabinol (CBN), Cannabichromene (CBC), Tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV), etc. Two important chemicals in cannabis that are used for medical therapy are delta-9-tetrahydrocannabinol (THC9) and cannabidiol (CBD) (National Academies of Sciences, 2017), their chemical structures are shown in **Figure 2.2** and **Figure 2.3**, respectively. When THC9 is transferred into the human brain, it binds with both forms of cannabinoid receptor (CB) that are CB1 and CB2 (Mackie, 2008; World Health, 1993). The effects of this binding are euphoria and hallucination or delusion. For CBD, it has fewer effects on the nervous system than THC9 (Tortoriello et al., 2014). CBD

binds to CB2 in the immune system and peripheral nerves more than CB1(Mechoulam & Hanus, 2002). This CB2 acts as an antinociception to control immune responsibility and cytokines by reducing T-lymphocyte and secretion of cytokines such as Interferon-gamma or Interleukin-12 which helps to reduce pain and aching in the nervous system (Nagarkatti, Pandey, Rieder, Hegde, & Nagarkatti, 2009). Therefore, cannabis is used to treat continuous pain, numbness, and muscle pain dealt with multiple abnormal hardening in tissue. In addition, using cannabis as a drug reduces nausea and vomiting in patients who suffer from cancer that is treated with chemotherapy and increases orexin in AIDS patients that have significant muscle losses (Nagarkatti et al., 2009). However, there are many side effects from specific chemicals in cannabis which has to be reduced in order to obtain fewer adverse effects such as nervousness disorder, sudden uncontrollable fear, bipolar disorder, depressive disorder, schizophrenia, and insomnia (Freeman, Hindocha, Green, & Bloomfield, 2019).

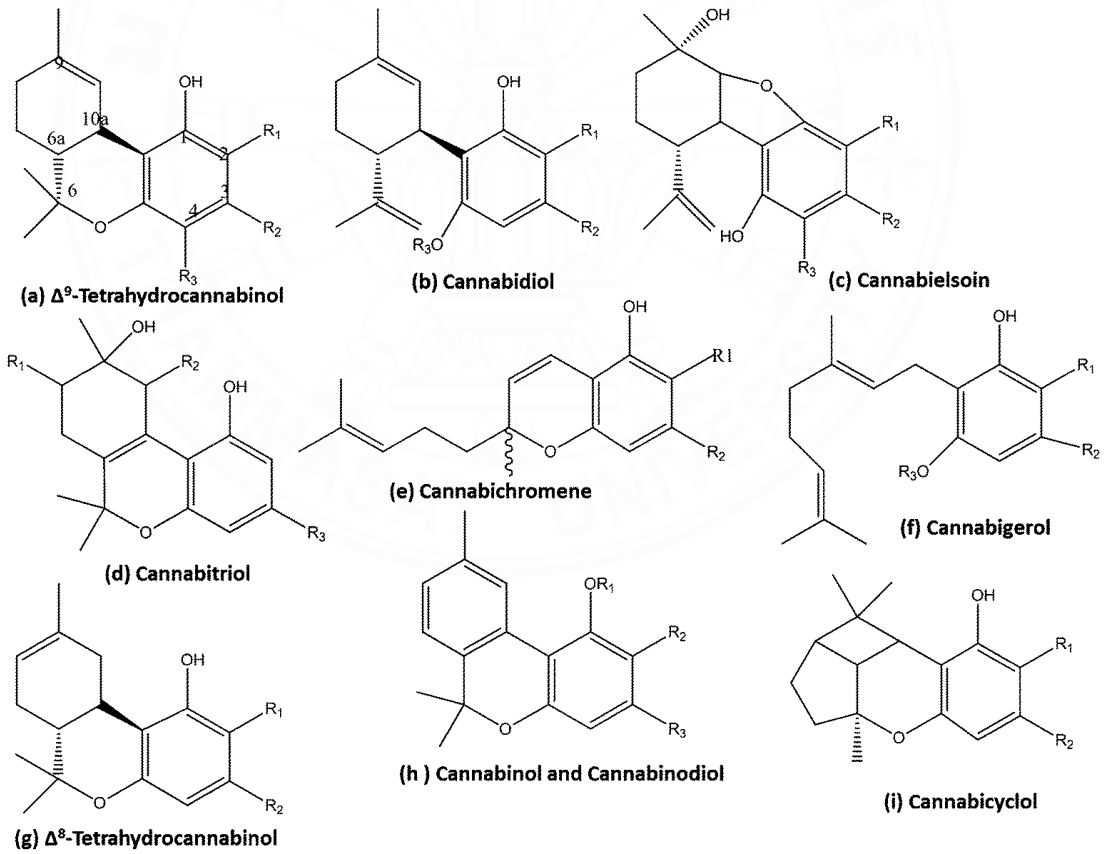


Figure 2.1 Chemical structures of nine cannabinoids, from (Nascimento et al., 2015)

Under laws and regulations in Thailand, cannabis is used as a drug in an appropriate amount in the medical field. Drugs are chemical compounds including

plants that affect the body and mind in a significant way when taken into the body. For example, the amount increases after each use, giving anxiousness when not taking it, so cannabis has been approved by Thailand's law. It is stated that cannabis cannot be planted, sold, and taken in possession when there are more than 10 kilograms of it, and is considered illegal. However, the medical department has publicly claimed about medical cannabis that it can be divided into three groups:

1. Cannabis extract is useful for treatment where it has academic data to support in nausea and vomiting in patients receiving chemotherapy.
2. Cannabis extract may have benefits in controlling symptoms where it has the additional research information on safety and effectiveness issues to support the use in Parkinson's disease, Alzheimer's disease, and anxiety disorder.
3. Cannabis extract that is still lacking of data from supporting research where it can be helpful in treatment but is not clear enough for safety and effectiveness which requires research in the tube and animal experiments to conduct research in humans such as the treatment of various types of cancer.

Nowadays, Thailand has drug law version 7 B.E. 2562 which is modified from drug law B.E. 2522 about advantages of making research on cannabis extraction, which has medical benefits, which many countries have accepted widely and revised the law for medical use as well to open the opportunity allowing people to take benefits from cannabis to cure diseases and use in medical, but under the guidance of a licensed physician according to academic principles legally, to strengthen the country's pharmaceutical security and to prevent the occurrence of monopoly in medicine. It also includes giving the opportunity to make research about cannabis, developing for medical benefits, and using it to treat the diseases under observation and control of doctors.

2.2 Cannabidiol (CBD)

The IUPAC name of CBD is 2-[(1R,6R)-3-methyl-6-prop-1-en-2-ylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol and its molecular formula is $C_{21}H_{30}O_2$ with the molecular weight of 314.5g/mol (Hanuš, Meyer, Muñoz, Taglialatela-Scafati, & Appendino, 2016). CBD is known to be a non-psychoactive substance that is mainly used for medicinal purposes which can be employed by inhalation, oral ingestion,

sublingual, and dermal absorption. But dermal absorption might take more time to absorb than others. It can be exerted through receptors that can be found in the brain, peripheral and central nervous system, muscle, lipid, and immune system. Cannabidiol is mainly used for medicinal purposes such as medical treatment of pain and anxiety and is used as an anticonvulsant for neurologic disorders. FDA also approved CBD-based drugs to treat seizure disorders.

Its mechanism includes binding to a receptor that gives an effect to calcium level within the cell. It also binds to serotonin receptors that can regulate our mood balance giving happiness by preventing the death of brain cells and reducing anxiety. A normal dosage taken per day is 300 mg and a high dose is about 1200 to 1500 mg (Shah, 1988). However, the amount of dosage taken by each person depends on the cases and ways. Advantages that are found in this component are reduction of the side effect of THC, and treatment of gynecologic cancers in women in the form of oil.

However, CBD still lacks some pharmacokinetic profiles which are poor solubility, its solubility is less than 0.0126 mg/mL of water at 25°C, low bioavailability, and high lipophilicity (Birnbaum et al., 2019). The chemical structure of CBD is shown in **Figure 2.2**.

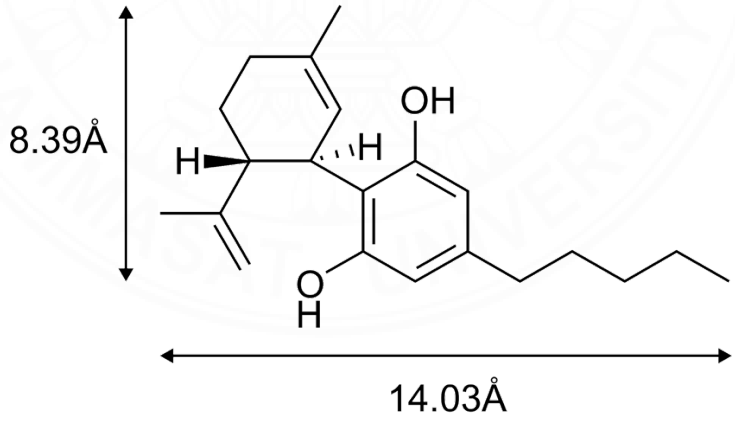


Figure 2.2 Chemical structure of CBD.

2.3 Delta-9-Tetrahydrocannabinol (THC9)

The IUPAC name is (6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol and its molecular formula is $C_{21}H_{30}O_2$ with the molecular weight of 314.5 g/mol (Hanuš et al., 2016). Delta-9-Tetrahydrocannabinol

(THC9) is one of the main components in cannabis. People who take this component via cannabis tend to feel “high” as a definition feeling very happy, excited, and enthusiastic because it gives effects to the central and peripheral nervous system.

The mechanism of action of THC exerted in the body is more complicated than CBD. There are many types of THC where each type has different conformations. The type of THC that is often being used is delta-9-tetrahydrocannabinol (THC9) because it can be easily found in the cannabis plant. There are 2 chiral atoms in THC9 which in on carbon number 6 and number 10, giving us 4 stereoisomers where the naturally occurring one is (-)-trans- Δ9-THC. It takes only minutes for it to reach the highest concentration in blood via smoking. Taking it at low doses (4-20 mg) may result in soft calm or hallucinating at high doses (50-210 mg). The advantages of THC9 are known to be pain relief and relaxation, dizziness reduction, inhibition of cancer cells, and induction in euphoria and elation. Its solubility is 0.28 mg/100mL in water at 25°C which is said to be poor (Garrett & Hunt, 1974). There are some disadvantages as well such as induction in hallucination and anxiety, delusion, and changing of thoughts, causing short-term memory (Ashton, 1999).

THC9 is well known as the main psychoactive substance of cannabis (Birnbaum et al., 2019) that has a tri-cyclic 21-carbon structure without nitrogen and with two chiral centers in trans configurations. Moreover, THC9 is a fragile substance that can be decomposed when exposed to air due to auto-oxidation and also heat or light (Shah, 1988). THC9 can be employed by inhalation, oral ingestion, and sublingual. But will be absorbed more quickly if taken by inhalation or known as smoking and vaporizing. Due to smoking, THC9 is quickly passed through the lung into the bloodstream, and blood will carry THC9 to the brain. Moreover, after taking THC9, it will produce a lot of psychoactive effects including feeling more relaxed, calm, and energetic, the headache had no more pain, paranoia, anxiety, perceptual alterations, and cognitive deficits, and exacerbates psychotic symptoms in patients with schizophrenia. However, THC9 is extremely low solubility in water and high lipid solubility (Shah, 1988), its water solubility is 0.0028 mg/mL (Garrett & Hunt, 1974). The chemical structure of THC9 is shown in **Figure 2.3**.

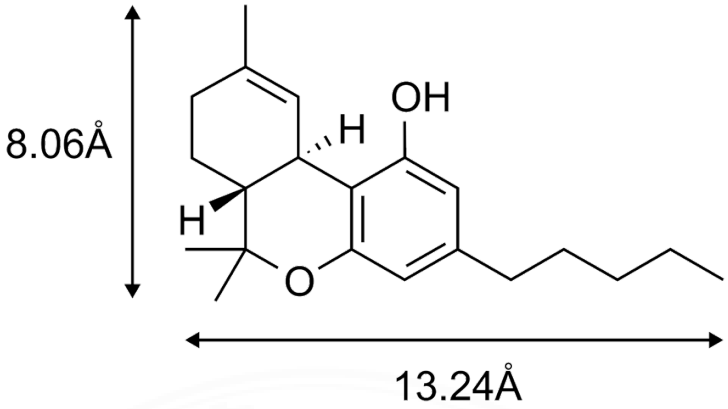


Figure 2.3 Chemical structure of THC9.

2.4 Cyclodextrins (CD)

As shown in **Figure 2.4**, cyclodextrin is a macrocyclic ring of glucose subunits joined by Alpha-1, 4 glycosidic bonds, and those consisting of 6, 7, and 8 glucose units for Alpha-cyclodextrin (ACD), Beta-cyclodextrin (BCD), and Gamma-cyclodextrin (GCD), respectively. They have the shape of a truncated cone with a hydrophilic outer surface and lipophilic cavity. All the secondary hydroxyl groups (corresponding to the C2 and C3 carbon atoms of the glucose units) are at one of the edges of the cavity (wide rim), whereas the primary hydroxyls are in the other end of the cavity (narrow rim). Cavity size is the major determinant as to which CD is used in complexation with hydrophobic molecules.

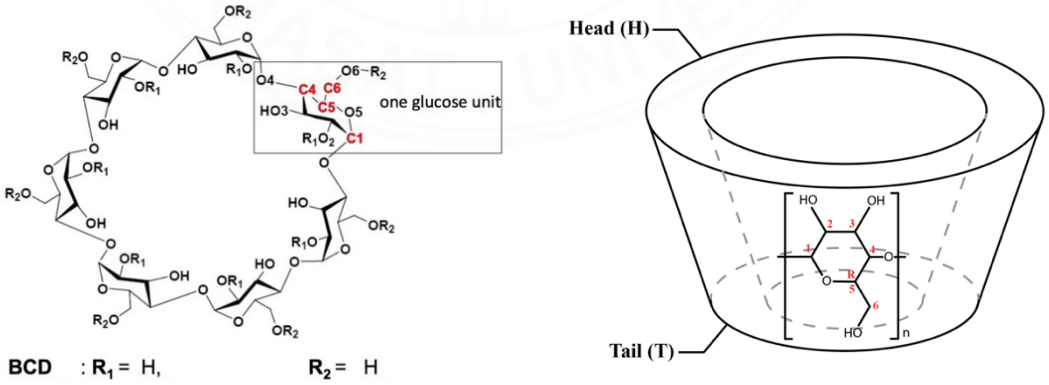


Figure 2.4 Chemical structure of Beta-cyclodextrin (BCD).

The solubility of natural CD is rather poor therefore chemical substitutions at the 2-, 3-, and 6-hydroxyl sites would greatly increase solubility. Recently, the inclusion complex between CBD and three native cyclodextrins have found to improve

the aqueous solubility and thermal stability of CBD compounds. Moreover, these inclusion complexes exhibit greater in vitro against human cancer lines Hep G2 and A549 (Lv et al., 2019). Unfortunately, there is no information related to the conformation of inclusion complexes, which is important for the assessment of the inclusion mechanism of CBD encapsulated with CDs.

(Hatziagapiou et al., 2022a; Li et al., 2022) Additionally, the molecular dynamics simulation offers a thorough knowledge of the chemical processes and interactions that take place between the molecules of cyclodextrin and the poorly water-soluble cannabinoids.

2.5 Density functional theory (DFT)

Density functional theory (DFT) is a computational method used in materials science and quantum chemistry to calculate the electronic structure of molecules and solids. It is based on the principle that the total energy of a system can be expressed as a functional of the electronic density.

In DFT, the behavior of electrons is described by their density rather than their individual positions and momenta. This allows for a simpler and more efficient calculation of the electronic structure of a system, as it avoids the need to calculate the complex wave function of the system.

DFT can be used to calculate a wide range of properties, such as the electronic structure, binding energy, and geometry of molecules and solids. It has been used to study a diverse range of systems, including catalysts, semiconductors, and biomolecules. DFT is widely used in both academia and industry for its ability to provide accurate predictions of the properties of materials and molecules. It is particularly useful for predicting the properties of large and complex systems, where traditional quantum mechanical methods are not practical.

2.6 M062X/6-31g(d,p)

In computational chemistry, the notation "M062X/6-31g(d,p)" refers to a specific computational method and basis set used in density functional theory (DFT) calculations.

The "M062X" refers to the specific exchange-correlation functional used in the DFT calculation. This particular functional is a modification of the commonly used B3LYP functional and is known for its accuracy in describing noncovalent interactions.

The "6-31g(d,p)" refers to the basis set used to describe the electronic wave function of the system being studied. This particular basis set includes six Gaussian functions for each atom in the molecule or material, with different exponents and contraction coefficients for each function. The "d" and "p" indicate that polarization functions have been added to the basis set, which allows for better description of the electronic structure of the system.

In summary, the notation "M062X/6-31g(d,p)" represents a specific combination of DFT functional and basis set used in computational chemistry calculations. This particular combination is commonly used in studies of small molecules and materials, and is known for its accuracy in describing noncovalent interactions.

2.7 Semi-empirical methods

Semi-empirical methods are a type of computational chemistry method that is commonly used in the study of molecules and materials. These methods aim to calculate the electronic structure of a system using a combination of quantum mechanics and experimental data.

Unlike ab initio methods, which rely solely on theoretical calculations of the electronic structure, semi-empirical methods incorporate experimentally determined parameters into the calculations. This makes them more computationally efficient than ab initio methods, while still providing a reasonable level of accuracy for many systems.

Semi-empirical methods are typically used to study large and complex systems, such as proteins, polymers, and large molecules, where traditional quantum mechanical methods are not practical. They are also used to explore reaction mechanisms and properties of materials.

Semi-empirical methods are based on the Hartree-Fock theory but use a simplified form of the molecular orbital theory. They are often used in combination with other computational chemistry methods, such as molecular dynamics.

While semi-empirical methods offer several advantages, such as speed and efficiency, they also have limitations. For example, they are less accurate than ab initio methods, and may not be suitable for studying certain types of systems. However, semi-empirical methods continue to be widely used in the field of computational chemistry due to their speed, efficiency, and ability to provide useful insights into complex systems.

2.8 PM7

PM7 is a semi-empirical computational chemistry method developed by the group of Professor J. J. P. Stewart at Queen's University in Canada. It is a member of the PM family of methods, which includes earlier versions such as PM3 and PM6.

Like other semi-empirical methods, PM7 combines theoretical calculations with experimental data to calculate the electronic structure of molecules and materials. Specifically, PM7 uses a modified version of the Hartree-Fock theory, which includes empirical corrections for various types of interactions and effects that are not fully accounted for in the theoretical calculations.

The empirical corrections used in PM7 were determined through a combination of quantum mechanical calculations and experimental data. These corrections improve the accuracy of the method compared to earlier versions of the PM family of methods.

PM7 has been shown to be a useful method for studying a wide range of molecular and materials systems, including biomolecules, materials for energy storage and conversion, and catalysts. It has been implemented in several computational chemistry software packages, including MOPAC, GAMESS-US, and Gaussian 16.

Overall, PM7 is a semi-empirical computational chemistry method that combines theoretical calculations with experimental data to provide accurate and efficient calculations of the electronic structure of molecules and materials.

2.9 Comparisons between DFT and Semi-empirical methods

Density functional theory (DFT) and semi-empirical methods are two commonly used computational chemistry methods, but they differ in their approaches to calculating the electronic structure of molecules and materials.

Methodology: DFT is based on the principle that the total energy of a system can be expressed as a functional of the electronic density. It uses the electronic density as the primary variable to describe the behavior of electrons, which allows for a simpler and more efficient calculation of the electronic structure of a system. On the other hand, semi-empirical methods use experimentally determined parameters to supplement theoretical calculations, which makes them more computationally efficient than ab initio methods.

Accuracy: DFT is generally considered to be more accurate than semi-empirical methods, especially for systems that involve strong electron correlation or highly delocalized electrons. Semi-empirical methods are often less accurate than ab initio methods, but they can provide a reasonable level of accuracy for many systems, especially for large and complex systems.

Applicability: DFT is suitable for a wide range of systems, including solids, molecules, and surfaces, while semi-empirical methods are typically used to study large and complex systems, such as proteins, polymers, and large molecules, where traditional quantum mechanical methods are not practical.

Speed: Semi-empirical methods are generally faster than DFT, as they require less computational resources. This makes them useful for studying large and complex systems, where DFT calculations would be impractical.

Limitations: Both DFT and semi-empirical methods have their limitations. DFT relies on approximations to calculate the electronic structure, and some of these approximations can lead to inaccuracies in certain systems. Semi-empirical methods are less accurate than ab initio methods and may not be suitable for studying certain types of systems.

In summary, while both DFT and semi-empirical methods have their strengths and weaknesses, they are both important tools in the field of computational chemistry. The choice of method depends on the specific system being studied and the resources available for the calculations.

CHAPTER 3
METHODOLOGY

When anticipating the behavior of molecules and ions, molecular modeling techniques offer an affordable way that sheds light on the atomistic mechanics underlying interactions between molecules. The outcomes of molecular modeling can be utilized as a template for a real experiment. According to (Freeman et al., 2019), performing the molecular docking algorithms can find the most favorable conformation of host and guest inclusion complex by several docking programs that are available such as AutoDock, GOLD, Glide, and FlexX. This approach can also be used to describe experimental phenomena that are challenging for measurement equipment to pick up on. Geometry optimization and molecular dynamics make up the simulation methodologies. The simulation can be used to determine the equilibrium structure and energy. The energy calculation within an atomic interaction, which is directly computed by quantum mechanics or empirical interaction parameters in accordance with the rules of statistical mechanics, forms the basis of the simulation modeling technique. However, the molecular modeling approach still has some limitations. It does not provide any hardware requirement and when performing the simulation, it also does not tell the user how long it will take to process. The research design schematic diagram is illustrated in **Figure 3.1**.

3.1 Computational Hardware

Asus ROG Zephyrus S17 GX701LXS-HG042T workstation laptop is used in this research. The computer has 16 CPU Cores of Intel Core i7-10875H (2.30 GHz, 16 MB L3 Cache, up to 5.10 GHz) with upgraded RAM to 48GB DDR4 3,200 MHz and 4TB CORSAIR MP400 Gen3 PCIe x4 NVMe M.2 SSD that has the speed of reading at 3,400 MB/s and speed of writing at 3,000 MB/s. Dual boot system of Microsoft Windows 10 and Ubuntu Desktop 22.04 LTS installed in a separate partition for flexibility and compatibility with computational software.

3.2 Molecular Construction

The crystallographic structure of the guests; CBD and THC9, and the host; BCD are downloaded from The Cambridge Crystallographic Data Centre (CCDC) website at https://www.ccdc.cam.ac.uk/structures/ in .mol extension

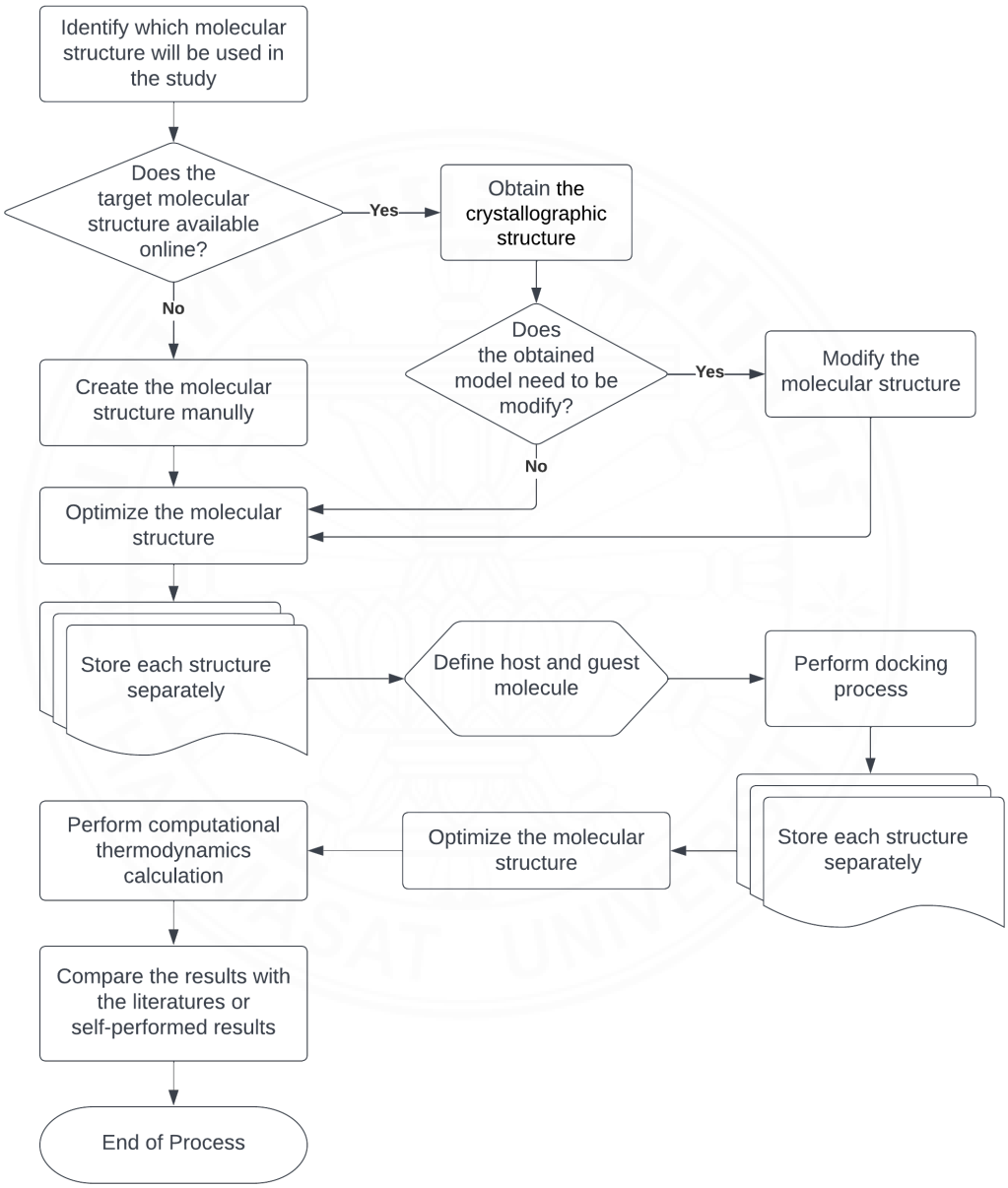


Figure 3.1 Schematic diagram of research workflows of the study.

(Deposition number: 1533487 [CBD] , 702456 [THC9], 1107192 [BCD], respectively). The structures are cleaned by removal of the unwanted structure and water molecules from the download structure by using Web Lab Viewer Pro software. Lastly, hydrogen

atoms are added to the structure by clicking on the Hydrogen icon and then save the clean structure back to the .mol extension file.

3.3 Molecular Docking Calculation

AutoDock 4.2.6 and AutoDock Tools 1.5.7 (ADT) (Morris et al., 2009) are used for molecular docking by following these steps.

1. Prepare the guest (THC9 or CBD) and save it to the .pdbqt extension file.
2. Prepare the host (BCD) and save it to the .pdbqt extension file.
3. Prepare the grid parameter file with the grid space of 0.375 and the grid size of 48x48x48 and save it to the .gpf extension file.
4. Prepare the docking parameter file by fixing the host to be a rigid body and adding a guest into the system. Set the search parameter to run 100 times in the Genetic Algorithm. Then set the output to Lamarckian GA as a .dpf extension file.
5. Run the AutoGrid with the .gpf extension file, then run the AutoDock for the docking process with the .dpf extension file.
6. If the docking process is successful, there will be no error. If the error occurs, there are two possibilities. First, the preparation of the host or the guest is wrong. Or second, the host and guest cannot interact with each other.
7. For successful docking, the AutoDock output file with the .dlg extension will be generated, then check for Conformations.

3.4 Molecular Structure Conformation Analysis

AutoDock Tools (ADT) are used to identify the conformation of each structure that ADT processed by the following steps.

1. Use ADT to open the .dlg extension file.
2. Open the macromolecule (.pdbqt extension file) with the Analyze menu in ADT.
3. Load conformations from the Analyze menu.
4. Select (double click) each conformation and look at the structure. Identify molecular structure into conformation. Each conformation has a unique position

between the host and the guest. Save the output separately to the .pdb extension file.

3.5 Preparation for Complexation Energy Calculation

Discovery Studio 2020 Client (DSC) and GaussView 6.0 are used to put the host and the guest back together into one molecular structure file. Then generate the complexation energy calculation file by the following steps.

1. Open the DSC.
2. Open all conformation files (.pdb extension).
3. Add Hydrogens to each conformation from the menu Chemistry > Hydrogens > Add
4. Open the host file (.pdb extension).
5. Copy the host structure (Edit > Select All) and paste it into every conformation.
6. Save each file to .mol2 extension file
7. Open GaussView 6.0
8. Open each conformation (.mol2 extension file) one by one.
9. Setup the optimization method and save the prep-file into the .gjf extension.
10. Open the prep-file (.gjf extension) with Notepad.
11. Change the head file to

    %mem = 1GB

    # opt m062x/6-31g(d,p) geom=connectivity

    Note: %CPU=0-3 option may be added into the second line for handling multiple calculation jobs at the same time.
12. Save the file. Now the prep file is ready for optimization by Gaussian Software

3.6 Complexation Energy Calculation

Gaussian 16.C.01.AVX for Linux on Ubuntu Desktop 22.04 LTS (Frisch et al., 2016) are used for complexation energy calculation. Recent research found that the M062X method based on the density functional theory (DFT) is suitable for exploring microscopic reaction mechanisms. In this work, M062X functional along with 6-31g(d,p) basis set is employed to optimize geometric structures of reactants, transition states, and intermediates involved in the investigated reactions.

3.7 Interpretation of Results

The first results are provided by the AutoDock calculations. The essential results are in .dlg file from the docking process. The data provided within the file should include the free binding energy of the inclusion complexes (ΔG), the conformations, and the frequencies of each rank and cluster. The .dlg results also contain the guest's coordinates and structures that can be extracted to the .pdb file. These structures and coordinates are combined with its original host and formed an initial structure of the inclusion complex and will be saved to .mol extension as a new molecule group. These initial structures are used to identify the conformation formed.

The representative of each conformation is used for deeper analysis with Gaussian 16 software package. Gaussian 16 uses .mol file from each conformation to calculate for the optimized molecular energy ($E_{component}$), the electric dipole moment (μ), the optimized geometrical parameters, the highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO) energies, thermodynamic properties, and other related molecular properties which obtained from M062X/6-31g(d,p) calculations.

The HOMO, LUMO molecular orbital energies and their energy gaps (Δ|HOMO-LUMO|) manifests the stabilization of the inclusion complex. To interpret the Δ|HOMO-LUMO| results, it could be said that the higher Δ|HOMO-LUMO| energy gap tend to have higher stability.

The complexation energy is identified for each host. If the calculated energy is high, it is unlikely to form the inclusion complex because high energy makes unstable conformation. If the calculated energy is low, it is likely to form a stable inclusion complex conformation. And the conformation with the lowest complexation energy is the most stable inclusion complex conformation.

The complexation energy (ΔE) between host(s) and guest in the minimized geometries are evaluated by the following equation:

$$\Delta E = E_{inclusion\ complex} - (E_{host} + E_{guest}) \quad (3.1)$$

The deformation energy (E_{DEF}) for each host and guest component throughout the formation of the inclusion complex was defined as in Equation (2):

$$E_{DEF}(component) = E(component)_{sp}^{opt} - E(component)^{opt} \quad (3.2)$$

where $E(component)_{sp}^{opt}$ is the single-point energy of the component taken from the optimized complex and $E(component)^{opt}$ is the energy of the optimized geometry of each free component.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 One-to-one Inclusion Complexes of BCD with CBD and THC9

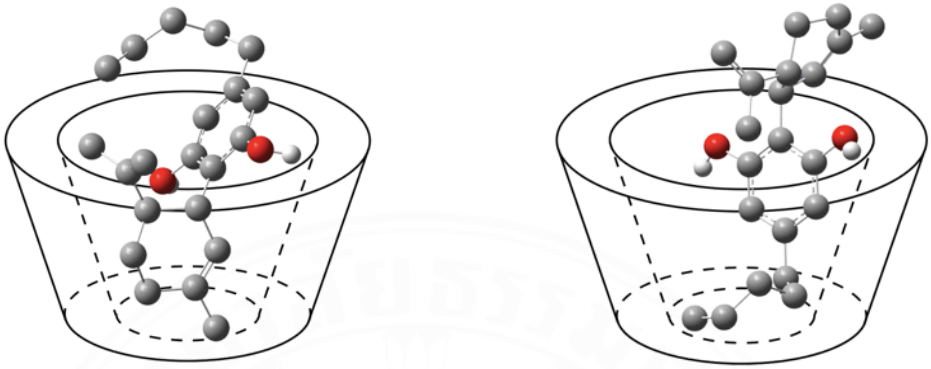
The results from molecular docking by AutoDock are shown in Table 4.1. Two conformations are present in the inclusion complexes of BCD with CBD and THC9 in the 1:1 host-guest molecular ratio. While BCD/THC9 inclusion complex presented only in conformation II with the highest binding energy at -6.73 kcal/mol, the BCD/CBD inclusion complex presented conformation I at 99% frequency at lower binding energy at -6.57 kcal/mol on average and conformation II at 1% with higher binding energy at -6.07 kcal/mol on average.

Table 4.1 The lowest and the average values of free energy of binding (ΔG) of CBD and THC9 with BCD inclusion complexes (1:1 host-guest ratio) and the frequency of conformations in a cluster were obtained from molecular docking calculations at 298.15 K. The starting geometry of the host and guest molecules was calculated by the M062X/6-31g(d,p) method.

Host/Guest	Cluster	Conformation	Frequency (%)	ΔG (kcal/mol)	
				Lowest	Average
BCD/CBD	1	I	41	-6.87	-6.57
	2	I	46	-6.81	-6.62
	3	I	12	-6.73	-6.49
	4	II	1	-6.07	-6.07
BCD/THC9	1	II	44	-6.77	-6.73
	2	II	26	-6.72	-6.64
	3	II	25	-6.71	-6.68
	4	II	4	-6.41	-6.35

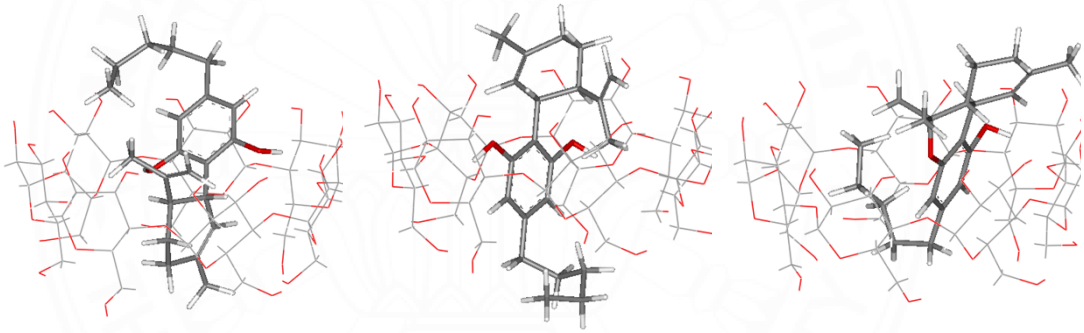
In term of conformations, the results show that in conformation I, the five-carbons chain of CBD was placed outside of the BCD cavity near the wider side (which will be referred to as the head) but stay close to the rim as shown in **Figure 4.2** (a). In conformation II, the five-carbon chains of CBD were placed inside the BCD cavity near the narrow side (which will be referred to as the tail) while the methylcyclohexene group was placed outside the BCD cavity near the wider rim as shown in **Figure 4.1**.

It is also worth noted that the molecular structure of the BCD barely changes even after optimization by Gaussian 16 with M062X/6-31g(d,p) basis as illustrated in **Figure 4.2**.



Conformation I Conformation II

Figure 4.1 Schematic representation of two conformations of the inclusion complex.



(a) BCD/CBD Conf. I (b) BCD/CBD Conf. II (c) BCD/THC9 Conf. II

Figure 4.2 The 3D structure of host/guest (BCD/CBD and THC9) after optimization

The complexation energies of one-to-one between BCD and CBD, THC9 were calculated by Gaussian 16 with M062X/6-31g(d,p) basis giving us more insight about the inclusion complexes (the lower complexation energy, the better inclusion complex formed). After we used the results from **Table 4.2** to calculate for complexation energies, it shows that the inclusion complex between BCD and CBD in conformation I has the lowest energy at -0.047802 Hartree followed by the inclusion complex of BCD and CBD in conformation II with the complexation energy at -0.051476 Hartree and the lowest complexation energy was found with the inclusion complex of BCD and THC9 in conformation II at -0.055649 Hartree

Table 4.2 The optimized energies and electric dipole moment values calculated by Gaussian 16 with M062X/6-31g(d,p) and PM7 basis set of isolated molecules and one-to-one inclusion complexes of BCD with CBD and THC9.

	M062X/6-31g(d,p)		PM7	
	E (Hartree)	μ (debye)	E (Hartree)	μ (debye)
Isolated molecule				
CBD	-968.341449	1.751573	-0.167722	1.565129
THC9	-968.365367	1.061717	-0.182331	0.858517
BCD	-4273.837301	3.533151	-2.718975	3.087480
Inclusion Complex				
BCD/CBD Conf. I	-5242.226552	4.765104	-2.820625	6.852157
BCD/CBD Conf. II	-5242.230226	3.576586	-2.810944	5.877669
BCD/THC9 Conf. II	-5242.258317	3.187291	-2.827520	5.907508

Table 4.3 The calculated complexation energies from M062X/6-31g(d,p) and PM7 basis set of one-to-one inclusion complexes of BCD with CBD and THC9 in conformation I and II.

	ΔE (Hartree)	
	M062X/6-31g(d,p)	PM7
BCD/CBD Conf. I	-0.047802	0.066072
BCD/CBD Conf. II	-0.051476	0.075753
BCD/THC9 Conf. II	-0.055649	0.073786

This complexation is also supported by the deeper analysis of hydrogen bond interaction between host and guest. For BCD/CBD conformation I with the highest energy, there are 3 hydrogen bonds that occur on the $O4_{(BCD)n}$, $O2_{(BCD)n+1}$ and $O4_{(BCD)n+2}$, which means the CBD inside the BCD cavity leans on one side of the BCD and therefore, the electric dipole moment is rather high (as shown in **Table 4.2**). For BCD/CBD conformation II with lower complexation energy, it can be explained by the hydrogen bonds that occurred at $O4_{(BCD)n} \cdots H_{(O1H\text{-}CBD)}$ and at $O4_{(BCD)n+3} \cdots H_{(O3H\text{-}CBD)}$, which makes the bond between BCD and THC9 stronger and more comfortable because of the occurred site at n and n+3 of BCD is in the opposite site, therefore, CBD would rather not moving and resulted in the lower dipole moment at 3.576586 debye. The lowest complexation energy was found with BCD/THC9 inclusion complex at -0.055649 Hartree and the lowest dipole moment at 3.187291 debye. This can be explained further with the three hydrogen bonds that occurred at the two opposite sites

of BCD (n and n+3) with one additional hydrogen bond at $O_{(THC9)} \cdots H_{(O3H\text{-}BCD)n-2}$ which could explain why the BCD/THC9 has the lowest complexation energy when compared with the other two inclusion complexes.

Table 4.4 Distance of hydrogen bonds between host (BCD) and guest (CBD and THC), obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

		Distance (Å)
BCD/CBD Conf. I	$O4_{(BCD)n} \cdots H_{(O1H\text{-}CBD)}$	1.837
	$O2_{(BCD)n+1} \cdots H_{(O3H\text{-}CBD)}$	1.992
	$O4_{(BCD)n+2} \cdots H_{(O3H\text{-}CBD)}$	2.499
BCD/CBD Conf. II	$O4_{(BCD)n} \cdots H_{(O1H\text{-}CBD)}$	1.901
	$O4_{(BCD)n+3} \cdots H_{(O3H\text{-}CBD)}$	1.918
BCD/THC9 Conf. II	$O4_{(BCD)n} \cdots H_{(O1H\text{-}THC9)}$	2.039
	$O2_{(BCD)n+1} \cdots H_{(O1H\text{-}THC9)}$	2.243
	$O_{(THC9)} \cdots H_{(O3H\text{-}BCD)n-2}$	2.847

A dipole moment is a measure of the separation of positive and negative electrical charges in a system, typically a molecule. It arises when there is an uneven distribution of electrons within a molecule, leading to a separation of charge. This separation creates a positive end and a negative end, similar to the poles of a magnet. In this case, the dipole moment values of BCD/CBD and BCD/THC9 systems (4.7651, 3.5766, and 3.4526 debye, respectively) are closer to the dipole moment of free BCD component (3.5332 debye) than those of free CBD or THC, which means that the guest molecules are likely to be encapsulated inside the hydrophobic cavity of BCD.

Table 4.5 Dipole moment (μ) of host (CBD) and guests (CBD and THC9) obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

	μ (debye)	μ_{sp} (guest) (debye)	μ_{sp} (host) (debye)
BCD	3.5332		
CBD	1.7515		
THC9	1.0617		
BCD/CBD Conf. I	4.7651	3.0744	3.2881
BCD/CBD Conf. II	3.5766	3.3216	3.4526
BCD/THC9 Conf. II	3.4526	1.0512	3.5170

The |HOMO-LUMO| gap is used for further investigation in the stability of the 1:1 inclusion complex. It has been found that the |HOMO-LUMO| gap is quite large (7.9174 to 8.2080 eV) compared to the stabilized BCD at 10.9860 eV. This indicated that all inclusion complexes are chemically stable in both free host and guest molecules.

Table 4.6 HOMO, LUMO, and energy gaps Δ|HOMO-LUMO| of host (CBD) and guests (CBD and THC9) obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

	HOMO (eV)	LUMO (eV)	Δ|HOMO-LUMO| (eV)
BCD	-8.7310	2.2550	10.9860
CBD	-6.9408	1.2640	8.2048
THC9	-6.9302	1.2583	8.1884
BCD/CBD Conf. I	-7.2456	0.9625	8.2080
BCD/CBD Conf. II	-7.1514	0.8041	7.9555
BCD/THC9 Conf. II	-7.1133	0.8041	7.9174

For the complexation energy (ΔE) results, it shows that the values are negative for all inclusion complexes (-29.9962, -32.3017, and -34.9203 kcal/mol, respectively) which suggests that their inclusion complex formations are energetically favorable. The deformation energy (E_{DEF}) is the energy required to distort each monomer into the structure it adopts within the complex. The deformation energy of CBD molecule is higher than that of BCD in both Conf. I and Conf. II, which indicates that the flexibility of the CBD structure plays an important role in stabilizing the whole system upon complexation. In the BCD/THC9 inclusion complex, both components exhibit low value of deformation energies, which means that the complex formation is very favorable. This information is supported by small changes of μ_{sp} (component) values compared to the dipole moments of isolated guest and host molecules. According to the value of ΔE and E_{DEF}, the stability of 1:1 inclusion complex is in the following order: BCD/THC9 Conf. II > BCD/CBD Conf. II > BCD/CBD Conf. I.

Table 4.7 Complexation and deformation energies of host (CBD) and guests (CBD and THC9) obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

	ΔE (kcal/mol)	E_{DEF} (guest) (kcal/mol)	E_{DEF} (host) (kcal/mol)
BCD/CBD Conf. I	-29.9962	12.0319	8.6929
BCD/CBD Conf. II	-32.3017	11.6836	4.7126
BCD/THC9 Conf. II	-34.9203	2.9349	3.4940

4.2 Two-to-One Inclusion Complexes of BCD with CBD and THC9

For two-to-one hosts/guest inclusion complexes, there are three types of host (BCD) positions (configurations) which are head-to-head (HH), tail-to-tail (TT), and tail-to-head (TH) as illustrated in Figure 4.3 (b), (c), and (d), respectively.

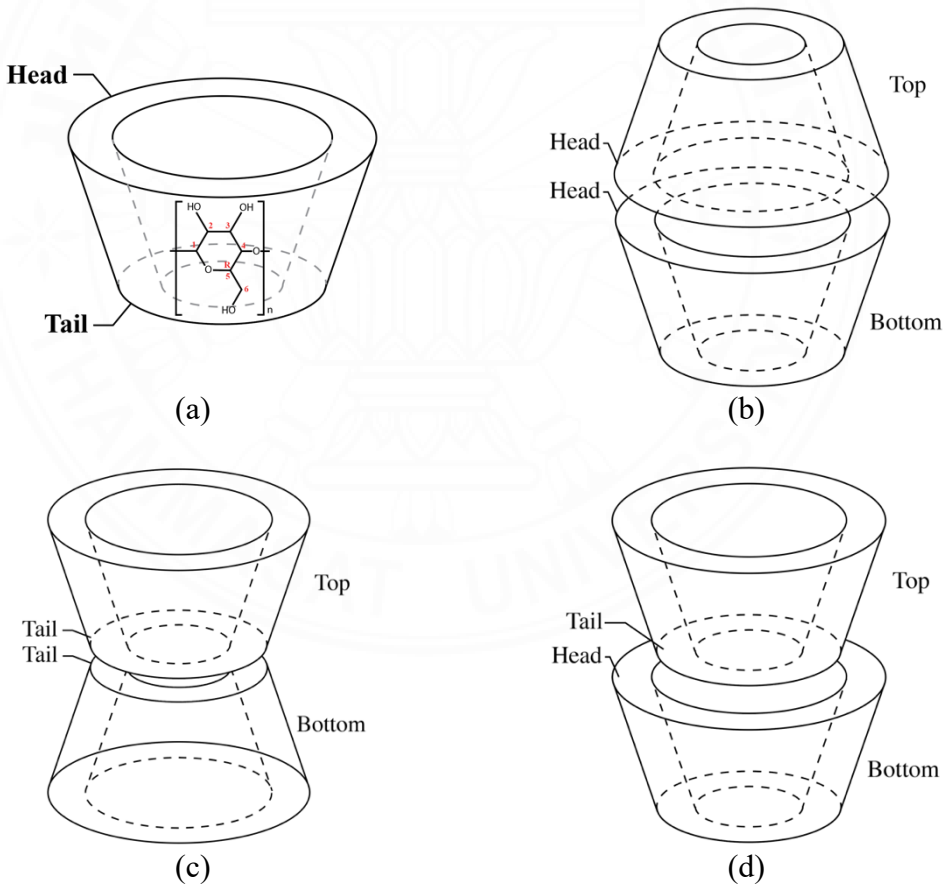


Figure 4.3 Schematic diagrams of (a) Beta-cyclodextrin with indication of head and tail position, (b) Two beta-cyclodextrins in the head-to-head (HH) position, (c) Two beta-cyclodextrins in the tail-to-tail (TT) position, (d) Two beta-cyclodextrins in the tail-to-head (TH) position.

The results of molecular docking by AutoDock suggested that there is only one conformation for each of all two-to-one inclusion complexes with two BCD in different positions and the CBD as a guest such as 2BCD/1CBD HH (conformation III) with an average binding energy of -8.63 kcal/mol, 2BCD/1CBD TT (conformation IV) with an average binding energy of -8.72 kcal/mol, 2BCD/1CBD TH (conformation V) with an average binding energy of -7.50 kcal/mol. The results from molecular docking also suggested similar results in two-to-one inclusion complexes with two BCD in different positions and the THC as a guest such as 2BCD/1THC9 HH with conformation III and an average binding energy of -9.51 kcal/mol, 2BCD/1THC9 TT with conformation IV and an average binding energy of -8.70 kcal/mol. However, in 2BCD/1THC9 TH, appeared to have 3 conformations, conformation V with an average binding energy of -8.12 kcal/mol, conformation VI with an average binding energy of -8.20 kcal/mol, and conformation VII with an average binding energy of -8.15 kcal/mol.

It is worth noted that the binding energy (ΔG) of the 1:1 complex is in the range of -6.07 to -6.87 kcal/mol, while ΔG of the 2:1 complex is in the range of -7.57 to -9.61 kcal/mol, which demonstrates that the 2:1 host-guest ratio complexation is more energy favorable than the 1:1 host-guest ratio.

Table 4.8 The lowest and the average values of free energy of binding (ΔG) of CBD and THC9 with 2BCD inclusion complexes (2:1 hosts-guest ratio) and the frequency of conformations in a cluster were obtained from molecular docking calculations at 298.15 K. The starting geometry of the host and guest molecules was calculated by the M062X/6-31g(d,p) method.

Host/Guest	Cluster	Conformation	Frequency (%)	ΔG (kcal/mol)	
				Lowest	Average
2BCD/1CBD HH	1	III	55	-8.97	-8.63
	2	III	16	-8.69	-8.48
	3	III	14	-8.66	-8.50
	4	III	10	-8.64	-8.44
	5	III	5	-8.61	-8.42
2BCD/1CBD TT	1	IV	38	-8.98	-8.72
	2	IV	61	-8.52	-8.12
	3	IV	1	-7.82	-7.82
2BCD/1CBD TH	1	V	91	-7.74	-7.50
	2	V	5	-7.65	-7.40

Host/Guest	Cluster	Conformation	Frequency (%)	ΔG (kcal/mol)	
				Lowest	Average
	3	V	4	-7.57	-7.38
2BCD/1THC9 HH	1	III	51	-9.61	-9.51
	2	III	43	-9.49	-9.41
	3	III	6	-9.12	-9.04
2BCD/1THC9 TT	1	IV	59	-8.77	-8.70
	2	IV	37	-8.67	-8.58
	3	IV	4	-8.23	-8.18
2BCD/1THC9 TH	1	V	41	-8.32	-8.12
	2	VI	16	-8.30	-8.20
	3	VII	4	-8.22	-8.15
	4	V	33	-8.11	-8.02
	5	V	6	-8.09	-8.03

In terms of conformations, conformation III is the first illustration of the two-to-one hosts and guest ratio where the two BCD are fixed with the head face to each other, and CBD or THC9 stays inside the cavity. In conformation IV, this is two-to-one hosts and guest ratios where the two BCD are fixed with the tail face to each other but different in guest positions whereas conformation IV has the methylcyclohexene group to the aromatic ring in the cavity of one BCD and the carbon chain point downward but still stay inside the cavity of another BCD.

For conformation V, conformation VI, and conformation VII, these are two-to-one hosts and guest ratios where the two BCD are fixed with the tail of one BCD face to the head of another BCD. In conformation V, CBD or THC9 stay inside the cavity of both BCDs where the methylcyclohexene group is close to the head of the top BCD and the aromatic ring is closer to the tail of the top BCD while the carbon chain stays in the cavity of the bottom BCD. In conformation VI, CBD or THC9 stay inside the cavity of both BCD where the methylcyclohexene group is close to the tail of the bottom BCD and the aromatic ring stay between both BCD and the carbon chain is in the cavity of the top BCD. In conformation VII, only occurred with THC9 where the methylcyclohexene group is in between the cavity of both BCD and the aromatic ring is close to the tail of the lower BCD while the carbon chain is outside the cavity but placed close to the tail's rim of the lower BCD. The schematic representation of five conformations is shown in **Figure 4.4**.

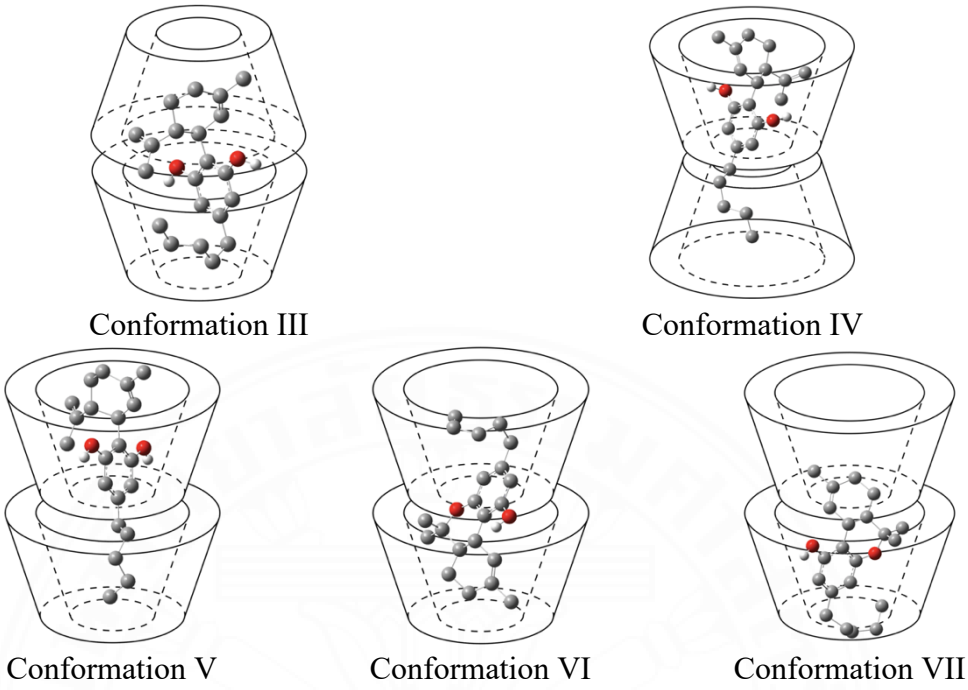


Figure 4.4 Schematic representation of five conformations of the inclusion complex.

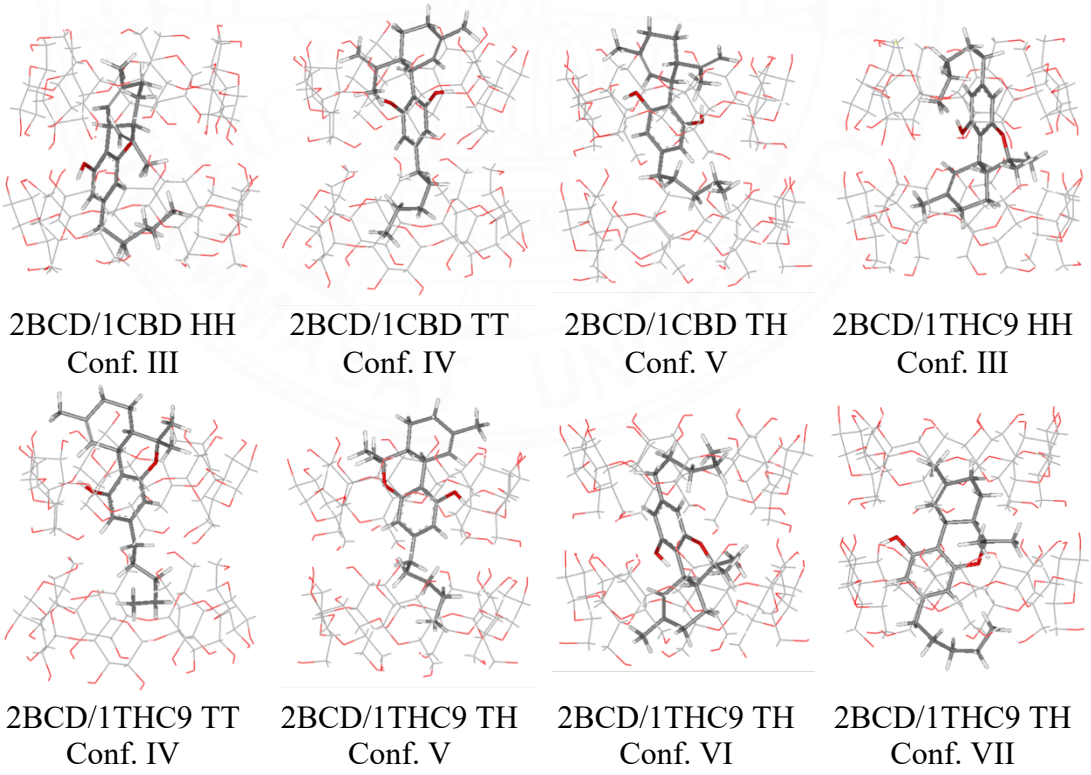


Figure 4.5 The 3D structure of host/guest (2BCD/1CBD and 1THC9) after optimization.

The complexation energies of two-to-one inclusion complexes between 2BCD and CBD, THC9 were calculated by Gaussian 16 with M062X/6-31g(d,p) basis giving us more insight into the inclusion complexes (the lower complexation energy, the better inclusion complex formed). The results from **Table 4.9** were further calculated to find the complexation energies as shown in **Table 4.10**, it shows that the inclusion complex of 2BCD/1THC9 TH in conformation V has the lowest energy at -0.215838 Hartree followed by the inclusion complex of 2BCD/1CBD TH in conformation V with the complexation energy at -0.205342 Hartree. The rest of the results are ranging between -0.215838 Hartree and -0.133195 Hartree where the highest complexation energy can be found with 2BCD/1CBD TT in conformation IV with the complexation energy of -0.133195 Hartree. It is worth noted that although the results are slightly different in each configuration, it can be observed that the lower complexation energy appears to frequently occur in conformation V and the higher complexation energy more frequently occurs in conformation IV.

Table 4.9 The optimized energies and electric dipole moment values calculated by Gaussian 16 with M062X/6-31g(d,p) and PM7 basis set of isolated molecules and two-to-one inclusion complexes of 2BCD with CBD and THC9

	M062X/6-31g(d,p)		PM7	
	E (Hartree)	μ (debye)	E (Hartree)	μ (debye)
Isolated molecule				
CBD	-968.341449	1.751573	-0.167722	1.565129
THC9	-968.365367	1.061717	-0.182331	0.858517
BCD	-4273.837301	3.533151	-2.718975	3.087480
Inclusion Complex				
2BCD/1CBD HH Conf. III	-9516.211815	4.940176	-5.521281	3.677899
2BCD/1CBD TT Conf. IV	-9516.149246	9.061154	-5.482219	8.792072
2BCD/1CBD TH Conf. V	-9516.221393	3.811498	-5.486602	5.405216
2BCD/1THC9 HH Conf. III	-9516.223869	1.850351	-5.525389	1.305490
2BCD/1THC9 TT Conf. IV	-9516.177678	8.416168	-5.506255	9.419340
2BCD/1THC9 TH Conf. V	-9516.255807	1.792509	-5.504941	4.341750
2BCD/1THC9 TH Conf. VI	-9516.240175	2.244000	-5.518611	4.434887
2BCD/1THC9 TH Conf. VII	-9516.228986	5.809793	-5.500000	6.244417

Table 4.10 The calculated complexation energies from M062X/6-31g(d,p) and PM7 basis set of two-to-one inclusion complexes of BCD with CBD and THC9 in various conformations

	ΔE (Hartree)	
	M062X/6-31g(d,p)	PM7
2BCD/1CBD HH Conf. III	-0.195764	0.084391
2BCD/1CBD TT Conf. IV	-0.133195	0.123453
2BCD/1CBD TH Conf. V	-0.205342	0.119070
2BCD/1THC9 HH Conf. III	-0.183900	0.094892
2BCD/1THC9 TT Conf. IV	-0.137709	0.114026
2BCD/1THC9 TH Conf. V	-0.215838	0.115340
2BCD/1THC9 TH Conf. VI	-0.200206	0.101670
2BCD/1THC9 TH Conf. VII	-0.189017	0.120281

The deeper analysis into intermolecular hydrogen bonding reveals that in two-to-one inclusion complexes, the hydrogen bonding usually occurs between $O4_{(Host)n} \cdots H_{(O1H\text{-}Guest)}$ ranging between 1.821Å to 3.099Å with only one binding site. Except for 2BCD/1CBD HH conformation III that has two hydrogen bonding at $O3_{(BCD\text{-}TOP)n} \cdots H_{(O1H\text{-}CBD)}$ with the distance of 1.992Å and at $O4_{(BCD\text{-}TOP)n} \cdots H_{(O1H\text{-}CBD)}$ with the distance of 3.099Å. And 2BCD/1CBD TT conformation IV appeared to have the different hydrogen bonding site at $O4_{(BCD\text{-}TOP)n} \cdots H_{(O3H\text{-}CBD)}$ with the distance of 1.855Å. The rest of the intermolecular hydrogen bonding between hosts and guest of two-to-one inclusion complexes is shown in **Table 4.11**.

Table 4.11 Distance of hydrogen bonds between host (2BCD) and guest (CBD and THC9), obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

		Distance (Å)
2BCD/1CBD HH Conf. III	$O3_{(BCD\text{-}TOP)n} \cdots H_{(O1H\text{-}CBD)}$	1.992
	$O4_{(BCD\text{-}TOP)n} \cdots H_{(O1H\text{-}CBD)}$	3.099
2BCD/1CBD TT Conf. IV	$O4_{(BCD\text{-}TOP)n} \cdots H_{(O3H\text{-}CBD)}$	1.855
2BCD/1CBD TH Conf. V	$O4_{(BCD\text{-}TOP)n} \cdots H_{(O1H\text{-}CBD)}$	1.821
2BCD/1THC9 HH Conf. III	$O4_{(BCD\text{-}BOTTOM)n} \cdots H_{(O1H\text{-}THC9)}$	2.779
2BCD/1THC9 TT Conf. IV	$O4_{(BCD\text{-}TOP)n} \cdots H_{(O1H\text{-}THC9)}$	2.082
2BCD/1THC9 TH Conf. V	$O4_{(BCD\text{-}TOP)n} \cdots H_{(O1H\text{-}THC9)}$	1.917
2BCD/1THC9 TH Conf. VI	$O4_{(BCD\text{-}BOTTOM)n} \cdots H_{(O1H\text{-}THC9)}$	2.417
2BCD/1THC9 TH Conf. VII	$O4_{(BCD\text{-}BOTTOM)n} \cdots H_{(O1H\text{-}THC9)}$	2.217

In terms of the electric dipole moment, it has a large ranging between 1.792509 debye to 9.061154 debye. It also is noticeable that the large electric dipole moment often occurs in conformation IV in TT configuration which is 2BCD/1CBD TT conformation IV with an electric dipole moment of 9.061154 debye, and 2BCD/1THC9 TT conformation IV with an electric dipole moment of 8.416168 debye. Whereas the lower electric dipole moment appeared to occur randomly in conformation V, III, and VI with 1.792509 debye from 2BCD/1THC9 TH, 1.850351 debye from 2BCD/1THC9 HH, and 2.244000 debye from 2BCD/1THC9 TH, respectively. All other electric dipole moment results can be observed in **Table 4.9** and **Table 4.12**.

Table 4.12 Dipole moment (μ) of host (2BCD) and guest (CBD and THC9), obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

	μ (debye)	μ_{sp} (guest) (debye)	μ_{sp} (host) (debye)
2BCD/1CBD HH Conf. III	4.9402	2.4524	2.6626
2BCD/1CBD TT Conf. IV	9.0612	0.9196	9.3617
2BCD/1CBD TH Conf. V	3.8115	2.6002	2.7540
2BCD/1THC9 HH Conf. III	1.8504	1.0526	3.0119
2BCD/1THC9 TT Conf. IV	8.4162	1.0390	9.4903
2BCD/1THC9 TH Conf. V	1.7925	1.0210	2.5470
2BCD/1THC9 TH Conf. VI	2.2440	3.0658	3.8716
2BCD/1THC9 TH Conf. VII	5.8098	2.0765	3.4883

As mentioned before, the |HOMO-LUMO| gap is used for further investigation in the stability of the 1:1 inclusion complex. For the 2:1 inclusion complex, it has been found that the |HOMO-LUMO| gap is quite large (7.8812 to 8.0616 eV) as well. This indicated that all inclusion complexes are chemically stable, as supported by the negative complexation energy values (ΔE) and the large |HOMO-LUMO| gaps.

Table 4.13 HOMO, LUMO, and energy gaps Δ|HOMO-LUMO| of host (2BCD) and guest (CBD and THC9), obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

	HOMO (eV)	LUMO (eV)	Δ|HOMO-LUMO| (eV)
2BCD/1CBD HH Conf. III	-7.3291	0.6705	7.9996
2BCD/1CBD TT Conf. IV	-7.1884	0.8346	8.0230
2BCD/1CBD TH Conf. V	-7.4964	0.4991	7.9955
2BCD/1THC9 HH Conf. III	-7.4328	0.4484	7.8812
2BCD/1THC9 TT Conf. IV	-7.1522	0.9094	8.0616
2BCD/1THC9 TH Conf. V	-7.3013	0.6318	7.9332
2BCD/1THC9 TH Conf. VI	-7.6812	0.2392	7.9204
2BCD/1THC9 TH Conf. VII	-7.6439	0.2963	7.9403

The complexation energy (ΔE) of 2:1 inclusion complex shows that 2BCD/1THC9 TH Conf. V has the lowest value followed by 2BCD/1CBD TH Conf. V, 2BCD/1THC9 TH Conf. VI, 2BCD/1CBD HH Conf. III, 2BCD/1THC9 TH Conf. VII, 2BCD/1THC9 HH Conf. III, 2BCD/1THC9 TT Conf. IV, and 2BCD/1CBD TT Conf. IV with the values of -135.4404, -128.8541, -125.6312, -122.8438, -118.6100, -115.3990, -86.4137, and -83.5811 kcal/mol, respectively. However, the deformation energy of the host molecule (E_{DEF} (host)), or 2BCD, of 2BCD/1THC9 TH Conf. VII (-87.8827 kcal/mol) is higher than 2BCD/1CBD HH Conf. III (-70.2202 kcal/mol) (negative sign referring to a change in energy, which means that complex formation should be difficult). Although the inclusion complexes with CBD and THC9 in 2BCD TT configuration exhibit low deformation energy (-37.4730 and -38.3873 kcal/mol, respectively), they have unbalanced charge distribution as indicated by the high dipole moment (9.0612 debye and 8.4162 debye for 2BCD/1CBD TT and 2BCD/1THC9 TT, respectively) as shown in Table 4.12, which ultimate lower the stability of compounds. That is the reason why 2BCD TT/guest systems have lower complexation energy than 2BCD HH/guest and 2BCD/guest TH guest systems. According to the values of ΔE, E_{DEF}, and μ the orders of 2:1 inclusion complex stability is 2BCD TH/guest Conf. V > 2BCD HH/guest Conf. III > 2BCD TT/guest Conf. IV.

According to the calculated the complexation energies (ΔE) (as shown in **Table 4.7** and **Table 4.14**), this indicate that the formation of the 2:1 host/guest ratio complex is chemically stable than the 1:1 host/guest ratio complex, the deformation energies of

the host and the guest components in the 2:1 complex are much higher than those in the 1:1 complex. This means the formation process of 2:1 is more difficult. This explains why the experimental complexation constant of 1:1 BCD/CBD (Ks = 300 M^{-1}) is greater than that of 2:1 2BCD/CBD (Kss = 0.833 M^{-1}) (Lv et al., 2019).

Table 4.14 Complexation and deformation energies of host (2BCD) and guest (CBD and THC9), obtained from M062X/6-31g(d,p) optimized inclusion complex structures.

	ΔE (kcal/mol)	E_{DEF} (guest) (kcal/mol)	E_{DEF} (host) (kcal/mol)
2BCD/1CBD HH Conf. III	-122.8438	10.0163	-70.2202
2BCD/1CBD TT Conf. IV	-83.5811	8.2348	-37.4730
2BCD/1CBD TH Conf. V	-128.8541	8.9426	-95.2610
2BCD/1THC9 HH Conf. III	-115.3990	7.4027	-72.5865
2BCD/1THC9 TT Conf. IV	-86.4137	4.7377	-38.3873
2BCD/1THC9 TH Conf. V	-135.4404	2.2892	-96.9119
2BCD/1THC9 TH Conf. VI	-125.6312	8.8366	-88.7794
2BCD/1THC9 TH Conf. VII	-118.6100	12.7880	-87.8827

CHAPTER 5
CONCLUSIONS

According to the docking results, BCD with CBD, and THC9 are possible to form both 1:1 host/guest inclusion complexes and 2:1 hosts/guest inclusion complexes whereas in 1:1 host/guest inclusion complexes of BCD and CBD produced two conformations, conformation I where the five-carbon chains of CBD pointing downward, and conformation II where the five-carbon chains of CBD pointing upward and the methylcyclohexene group stayed inside the cavity of BCD. While the 1:1 host/guest inclusion complexes of BCD and THC9 produced only conformation II result. The molecular structure of both inclusion complexes is optimized and found a reasonable intermolecular relationship of host and guest through the hydrogen bonds in both 1:1 and 2:1 inclusion complexes.

In 2:1 hosts/guest inclusion complexes, there are three configurations; HH, TT, TH of 2BCD. The results shown that there are five conformations occurs (conformation III to conformation VII) between the host (BCD) and the guests (CBD, and THC9). The stability of the hosts (BCD and 2BCD) and guests (CBD and THC9) inclusion complexes are determined by the complexation energies and the HOMO and LUMO gaps which suggested that 2:1 inclusion complex is more energy favorable than the 1:1 inclusion complex. According to the values of complexation energies, deformation energies, and dipole moments, the stability of 1:1 inclusion complex is in the following order: BCD/THC9 Conf. II > BCD/CBD Conf. II > BCD/CBD Conf. I and the stability of 2:1 inclusion complex is in the following order: 2BCD TH/guest Conf. V > 2BCD HH/guest Conf. III > 2BCD TT/guest Conf. IV. However, the deformation energies of 2BCD inclusion complexes were significantly higher than those of BCD inclusion complexes, which means the formation process with 2BCD complex is more difficult. Therefore, the inclusion complex of BCD with CBD and THC9 is more likely to be 1:1 inclusion complex than in 2:1 inclusion complex.

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APPENDICES

APPENDIX A

A REVIEW ON CBD AND THC INCLUSION COMPLEX WITH CYCLODEXTRINS

Cyclodextrin	Host : CBD	K	Reference
ACD	1 : 1	1.000 x 10^2 M^{-1} (-17.13 kJ/mol)	(Lv et al., 2019)
BCD	1 : 1	3.000 x 10^2 M^{-1} (-5.71 kJ/mol)	
	2 : 1	0.833 M^{-1}	
GCD	1 : 1	1.500 x 10^3 M^{-1} (-18.13 kJ/mol)	
	2 : 1	0.167 M^{-1}	
SACDD	1 : 1	3.353 x 10^6 M^{-1} (-37.25 kJ/mol)	(Chen et al., 2022)
TPACDD	1 : 1	7.540 x 10^5 M^{-1} (-33.55 kJ/mol)	
DMCD (T-T)	2 : 1	3.279 x 10^5 M^{-1} (-31.49 kJ/mol)	
DMBCD	1 : 1		(Li et al., 2022)
BCD	K(1:1) = 58.7		(Hatziagapiou et al., 2022a)
DMBCD	1 : 1 & 2 : 1		
TMBCD			
RMBCD	(DS~12)	K(1:1) = 1503.6	
HPBCD	(DS~4.5)	K(1:1) = 146.7	
HPBCD	1 : 1	CBD = 13800 M^{-1} THC9 = 4200 M^{-1}	(Mannila, Järvinen, Järvinen, Tarvainen, & Jarho, 2005)
RMBCD	1 : 1	CBD = 484100 M^{-1} THC9 = 19600 M^{-1}	
	2 : 1	CBD = 8 M^{-1} THC9 = 40 M^{-1}	
DMBCD	1 : 1	Ks = 3227.1	(Li et al., 2021)
BCD	1 : 1	Ks = 315.4	
ACD	1 : 1	Ks = 126.5	
GCD	1 : 1	Ks = 26.8	

Cyclodextrin	Host : THC	K	Reference
RMBCD	1 : 1	15000 M^{-1}	(Hazekamp & Verpoorte, 2006)

APPENDIX B

WORK DONE ON PRELIMINARY STRUCTURE

	CCDC		Optimized	Reference
	DI	DN		
CBD	CANDOM11	1533487	M062X/ 6-31g(d,p)	(Mayr, Grassl, Korber, Christoffel, & Bodensteiner, 2017)
THC9	MOFDIV	702456	M062X/ 6-31g(d,p)	(Gul, Carvalho, W.Berberich, Avery, & ElSohly, 2008)
BCD	BCDEXD03	1107192	M062X/ 6-31g(d,p)	(Steiner & Koellner, 2002)
SACDD	HAZKUQ	864041	M062X/ 6-31g(d,p)	(Chen et al., 2022)
TPACDD	HAZKUQ	864041	M062X/ 6-31g(d,p)	
DMBCD with CBD	SAVVOE	2098419	M062X/ 6-31g(d,p)	(Hatziagapiou et al., 2022c)
bis-TMBCD-HH with CBD	SAVVIY	2094890	M062X/ 6-31g(d,p)	(Hatziagapiou et al., 2022b)

APPENDIX C

GAUSSIAN 16 WITH M062X/6-31g(d,p) BASIS SET RESULTS

	M062X/6-31g(d,p)		PM7	
	E (Hartree)	μ (debye)	E (Hartree)	μ (debye)
Isolated molecule				
CBD	-968.341449	1.751573	-0.167722	1.565129
THC9	-968.365367	1.061717	-0.182331	0.858517
BCD	-4273.837301	3.533151	-2.718975	3.087480
SACDD	-9439.015766	11.309345	-5.283025	8.661610
TPACDD	-9930.711091	12.886879	-5.267471	5.579254
DMBCD	-4823.775386	1.807804	-2.465052	4.303073
HPBCD	-5625.372814	7.410162	-3.189054	3.637648
Inclusion Complex				
BCD/CBD Conf. I	-5242.226552	4.765104	-2.820625	6.852157
BCD/CBD Conf. II	-5242.230226	3.576586	-2.810944	5.877669
BCD/THC9 Conf. II	-5242.258317	3.187291	-2.827520	5.907508
2BCD/1CBD HH Conf. III	-9516.211815	4.940176	-5.521281	3.677899
2BCD/1CBD TT Conf. IV	-9516.149246	9.061154	-5.482219	8.792072
2BCD/1CBD TH Conf. V	-9516.221393	3.811498	-5.486602	5.405216
2BCD/1THC9 HH Conf. III	-9516.223869	1.850351	-5.525389	1.305490
2BCD/1THC9 TT Conf. IV	-9516.177678	8.416168	-5.506255	9.419340
2BCD/1THC9 TH Conf. V	-9516.255807	1.792509	-5.504941	4.341750
2BCD/1THC9 TH Conf. VI	-9516.240175	2.244000	-5.518611	4.434887
2BCD/1THC9 TH Conf. VII	-9516.228986	5.809793	-5.500000	6.244417
SACDD/CBD Conf. IV			-5.547313	10.512688
SACDD/CBD Conf. VIII	-9930.666419	9.910173	-5.514522	9.305232
SACDD/THC9 Conf. IV	-9930.710154	11.696687	-5.554849	9.440224
TPACDD/CBD Conf. IV	-10407.328668	4.762598	-5.561608	1.864537
TPACDD/CBD Conf. VIII	-10407.407902	6.343504	-5.544952	7.282442
TPACDD/THC9 Conf. IV	-10407.421765	10.017993	-5.584515	9.710209
DMBCD/CBD Conf. I	-5792.150722	7.351842	-2.722058	4.541426
DMBCD/CBD Conf. II	-5792.149561	5.489192	-2.719081	6.023806
DMBCD/THC9 Conf. I	-5792.172871	4.104914	-2.739383	3.976612
DMBCD/THC9 Conf. II	-5792.180840	3.927239	-2.727930	4.645649
2DMBCD/1CBD HH Conf. III			-5.317752	3.105140
2DMBCD/1CBD TT Conf. IV			-5.287245	4.826768
2DMBCD/1CBD TT Conf. VIII			-5.238476	5.070662
2DMBCD/1CBD TH Conf. I			-5.295274	6.430822
2DMBCD/1CBD TH Conf. V			-5.310843	9.023955
2DMBCD/1CBD TH Conf. VI			-5.302276	9.166799
2DMBCD/1CBD TH Conf. VII			-5.256702	10.159359
2DMBCD/1THC HH Conf. III			-5.297637	2.187606
2DMBCD/1THC TT Conf. IV			-5.288352	1.370048
2DMBCD/1THC TH Conf. V			-5.317147	6.904279
2DMBCD/1THC TH Conf. VI			-5.315405	8.799899
2DMBCD/1THC TH Conf. VII			-5.290756	7.389177
HPBCD/CBD Conf. I			-3.467401	3.079014
HPBCD/CBD Conf. II			-3.471222	4.847320
HPBCD/THC Conf. I			-3.482419	3.173593
HPBCD/THC Conf. II			-3.493727	8.237792

BIOGRAPHY

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Publications

Srihakulung, O., Triamchaisri, N., Toochinda, P., & Lawtrakul, L. (2020). Theoretical study on ferrocenyl hydrazones inclusion complexes with β-cyclodextrin and its three methylated derivatives. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*. doi:10.1007/s10847-020-01011-z

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