BINARY AND TERNARY COMPLEXES OF β-CYCLODEXTRIN WITH ISONIAZID AND ETHAMBUTOL: CHARACTERIZATION AND MOLECULAR MODELING STUDIES

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by

GOH SOEN QENG

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LIST OF SYMBOLS

mg	Milligram
kg	Kilogram
α	Alpha
β	Beta
γ	Gamma
K	Stability constant
^{1}H	Hydrogen-1
^{13}C	Carbon-13
δ	Chemical shift
Δδ	Induced shift
ΔH	Enthalpy change
ΔS	Entropy change
MHz	Megahertz
ppm	Parts per million
kV	Kilovolt
mA	Milliampere
nm	Nanometre
kJ mol^{-1}	Kilojoule per mole
fs	Femtosecond
ps	Picosecond
atm	Atmosphere
E	Energy
Å	Angstrom

kcal/mol	Kilocalories per mole

LIST OF ABBREVIATIONS

TB	Tuberculosis
MTB	Mycobacterium tuberculosis
WHO	World Health Organisation
INH	Isoniazid
ETB	Ethambutol
RFP	Rifampicin
PZA	Pyrazinamide
CDs	Cyclodextrins
FT-IR	Fourier transform infrared
NMR	Nuclear magnetic resonance
2D NOESY	2-dimensional nuclear Overhauser effect spectroscopy
2D ROESY	2-dimensional rotating-frame Overhauser effect spectroscopy
TGA	Thermogravimetric analysis
DSC	Differential scanning calorimetry
SEM	Scanning electron microscopy
XRD	X-ray diffraction
MD	Molecular dynamics
DXH	Duloxetine hydrochloride
BCS	Biopharmaceutical classification system
MDD	Major depressive disorder
HP	Hydroxypropyl
pH	Potential of hydrogen
ARG	L-arginine

HPMC	Hydroxypropyl methylcellulose
PVP/PVP30	Polyvinylpyrrolidone K30/polyvinyl propylene
POLO	Poloxamer 188
DAP	Dapsone
M	Methyl
UV	Ultraviolet
MIC	Minimum inhibitory concentration
ZOI	Zone of inhibition
RMSD	Root-mean-square deviation
RMSF	Root-mean-square fluctuation
AR	Analytical research
DMSO-d_6	Dimethyl sulfoxide-d_6
KBr	Potassium bromide
N_2	Nitrogen
GROMACS	Groningen machine for chemical simulations
ATB	Automated Topology Builder
SPC	Simple point charge
PME	Particle mesh Ewald
LINCS	Linear constraint solver
NVT	Canonical ensemble
NPT	Isobaric-isothermal ensemble
RDF	Radial distribution function

KOMPLEKS BINARI DAN TERNARI β-SIKLODEKSTRIN DENGAN ISONIAZID DAN ETAMBUTOL: PENCIRIAN DAN KAJIAN PEMODELAN MOLEKUL

ABSTRAK

Pengambilan ubat antituberkulosis jangka panjang akan membawa kepada tindak balas buruk yang serius yang akhirnya akan menjejaskan kesihatan dan kesejahteraan pesakit. Pada masa ini, diketahui bahawa kitaran makro supramolekul seperti siklodekstrin (CDs) amat berguna untuk meningkatkan keterlarutan ubat dan mekanisme penyasaran sambil mengurangkan dos ubat serta memberikan banyak faedah terapeutik. Dengan itu, kemungkinan pembentukan kompleks inklusi ternari CDs-ubat diterokai, di mana β-siklodekstrin (β-CD) dipilih sebagai pembawa perumah supramolekul untuk menampung dua jenis molekul ubat tamu antituberkulosis barisan pertama secara serentak, iaitu isoniazid (INH) dan etambutol (ETB). Kompleks ternari β-CD/INH/ETB telah disediakan menggunakan kaedah penyejatan pelarut. Spektroskopi inframerah transformasi Fourier (FT-IR), spektroskopi resonans magnetik nuklear proton (^{1}H-NMR), dan spektroskopi kesan Overhauser nuklear 2 dimensi (2D-NOESY) NMR telah digunakan untuk menyiasat kumpulan fungsi dan struktur kompleks. Pengimbasan mikroskop elektron (SEM) dan pembelauan sinar-X (XRD) digunakan untuk mengkaji morfologi permukaan dan perubahan kehabluran semasa pembentukan kompleks. Analisis termogravimetrik (TGA) telah dilakukan untuk menyiasat sifat terma kompleks. Keputusan FT-IR dan kedua-dua jenis NMR telah mendedahkan kejayaan penembusan kedua-dua molekul ubat ke dalam rongga β-CD. Imej SEM dan spektrum XRD telah menunjukkan perubahan drastik dalam struktur permukaan dan pengurangan kehabluran semasa pembentukan kompleks,

yang telah menunjukkan kejayaan pembentukan sebatian baru. TGA telah membuktikan bahawa kompleks ternari mempunyai kestabilan terma yang lebih tinggi berbanding dengan komponen tulen dan kompleks binari (jumlah kehilangan berat: β-CD/INH/ETB: 83.52%, β-CD: 97.88%, INH: 99.45%, ETB: 99.19%, β-CD/INH: 95.81%, β-CD/ETB: 92.00%). Simulasi dinamik molekul (MD) juga dilakukan dalam usaha untuk memahami interaksi molekul antara β-CD, INH dan ETB. Nilai anjakan kimia NMR telah disokong oleh ikatan teori yang diperoleh daripada simulasi MD. Tenaga interaksi yang dikira untuk β-CD/INH/ETB ialah -132.8123 kcal/mol, iaitu lebih negatif berbanding dengan β-CD/INH (-36.4236 kcal/mol) dan β-CD/ETB (-36.3997 kcal/mol), yang telah menunjukkan bahawa kompleks ternari yang disintesis adalah lebih stabil daripada kedua-dua kompleks binari.

BINARY AND TERNARY COMPLEXES OF β-CYCLODEXTRIN WITH ISONIAZID AND ETHAMBUTOL: CHARACTERIZATION AND MOLECULAR MODELING STUDIES

ABSTRACT

Long term intake of antituberculosis drugs will lead to severe adverse reactions that will ultimately deteriorate a patient's health and well-being. Presently, it is known that supramolecular macrocycles such as cyclodextrins (CDs) are useful to enhance drug solubility and targeting mechanism while reducing drug dosage by providing many therapeutic benefits. With that, the possibility of formation of drug-CDs ternary inclusion complex was explored, whereby β-cyclodextrin (β-CD) was chosen as the supramolecular host carrier to accommodate two kinds of first-line antituberculosis guest drug molecules simultaneously, namely isoniazid (INH) and ethambutol (ETB). The inclusion complex of β-CD/INH/ETB was prepared using solvent evaporation method. Fourier transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), and 2-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY) NMR were used to investigate the functional groups and structure of the complex. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) were employed to study the surface morphology and crystallinity changes during complex formation. Thermogravimetric analysis (TGA) was performed to investigate the thermal properties of the complex. FT-IR and both types of NMR results had revealed the successful penetration of both the drug molecules into the β-CD cavity. SEM images and XRD spectra had shown a drastic change in surface structure and reduction in crystallinity during complex formation, which had indicated successful formation of a new compound. TGA had proven that the ternary inclusion

complex had significantly greater thermal stability as compared to the pure components and the binary inclusion complexes (total weight loss of β-CD/INH/ETB: 83.52%, β-CD: 97.88%, INH: 99.45%, ETB: 99.19%, β-CD/INH: 95.81%, β-CD/ETB: 92.00%). Molecular dynamics (MD) simulations were also performed to understand the molecular interaction between β-CD, INH and ETB. The theoretical bonds obtained from MD simulation had supported the NMR chemical shift values. The interaction energy calculated for β-CD/INH/ETB was -132.8123 kcal/mol, which was more negative than β-CD/INH (-36.4236 kcal/mol) and β-CD/ETB (-36.3997 kcal/mol), which had indicated that the synthesised ternary inclusion complex was more stable than both binary inclusion complexes.

CHAPTER 1

INTRODUCTION

1.1 General background of research

Tuberculosis (TB) is one of the most fatal transmissible diseases widespread all over the world. It is caused by a type of bacteria known as mycobacterium tuberculosis (MTB), which is highly contagious through airborne transmission, that includes tiny droplets produced by coughs and sneezes. Although the bacteria mainly target the lungs, there are high possibilities of them attacking other parts of the body if left untreated (Jnawali and Ryoo 2013). According to the annual report from World Health Organisation (WHO) for year 2019, TB was classified as one of the top 10 causes of death in the world, where around 10 million people were infected, and 14% of the people did not manage to survive (Geneva: World Health Organization 2020).

Among the first-line treatment for combating TB include the use of combination of various drugs as shown in Figure 1.1, namely rifampicin (RFP/$C_{43}H_{58}N_4O_{12}$), isoniazid (INH/$C_6H_7N_3O$), pyrazinamide (PZA/$C_5H_5N_3O$) and ethambutol (ETB/$C_{10}H_{24}N_2O_2$) in certain dosage, as to effectively get rid of the bacteria while preventing antibiotic resistance to a particular drug (Butov et al. 2020). However, the main emphasis of this research is specifically on INH and ETB, as they are commonly prescribed together for prolonged treatment of TB even in the initial phase. To simplify, INH helps to reduce the division of MTB in the body by inhibiting the synthesis of mycolic acid, while ETB works by hindering the production of an enzyme called arabinosyl transferase, where both components mentioned are essential

elements required in the biosynthesis process of bacteria cell wall (Wheeler and Anderson 1996; Goude et al. 2009). Generally, the recommended dosage for both INH and ETB are around 10-25 mg/kg of human weight per day, which varies according to the severity of infection (Sundell et al. 2020; Kiser et al. 2012). Since the dosage are considered relatively high, long-term intake of these drugs may lead to adverse drug reactions and side effects, which include hepatitis, dermatitis, and neurotoxicity related effects such as loss of vision and colour blindness (Kass and Shandera 2010). Hence, it is necessary to improve drug delivery systems for prolonged TB treatment, to minimise the occurrence of harmful side effects that contribute to increased deterioration of the patient's body.

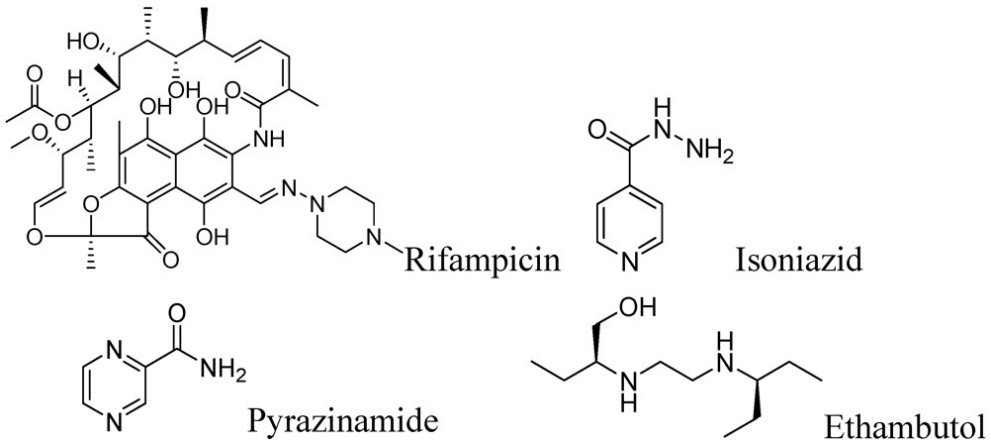

Figure 1.1: First line anti-TB drugs (Shakya et al., 2012).

At present, it is a known fact that supramolecular macrocycles are useful mediums used in drug delivery systems to enhance drug stability, solubility, targeting, and stimuli responsiveness, with the goal of reducing dosage and dosing frequency by upgrading the therapeutic benefits (Saji 2022a). This unique method is more often introduced as host-guest chemistry, whereby desired drugs are accommodated by

hydrophilic macrocycle hosts, for instance, crown ethers, porphyrins, pillar[n]arenes, calix[n]arenes, cucurbiturils, cyclodextrins, and rotaxanes (Braegelman and Webber 2019). With that said, cyclodextrins (CDs) inclusion complexes have proven their usefulness across wide variety of industries, with greater dominance in food processing and pharmaceutical industries (Del Valle 2004). With regards to drug delivery systems, complexation of drugs with CDs reduces the hydrophobicity of poorly soluble drugs, thus increasing the dissolution rate and boosting the absorption by human body. As this positive pharmacological effect happens, the local concentration of drugs administered can be lowered, which is the main purpose of modifying it (Tiwari, Tiwari and A. K. Rai 2010).

As seen in Figure 1.2, typical CDs ($C_{42}H_{70}O_{35}$) can be classified into D-(+)-glucopyranose monomers ranging from six to eight units in a ring interconnected by α-1,4-linkages, which are labelled α-, β-, and γ-CD, respectively. However, α-CD has limited cavity space which is deemed unfitting to accommodate most drugs while γ-CD is relatively pricy, which is not a cost-effective option for mass production. As a result, β-CD has always been the top pick when it comes to modification of drug delivery systems (Sharma and Baldi 2016). Although the field of CDs-drug inclusion complexes has been explored since decades ago, it is mostly revolving around binary systems, where only a type of desired drug undergoes complexation with the host CDs molecule. This situation has gained the interest of researchers nowadays in exploring similar complexes, but with ternary systems instead. However, the ternary components used are usually additives, natural or synthetic polymers, and solubilising agents which aid to improve the CDs-drug complexation or the drug-release process, without being

directly involved in the host-guest reaction itself (Rajesh Jagtap and Shrinivas Mohite 2019).

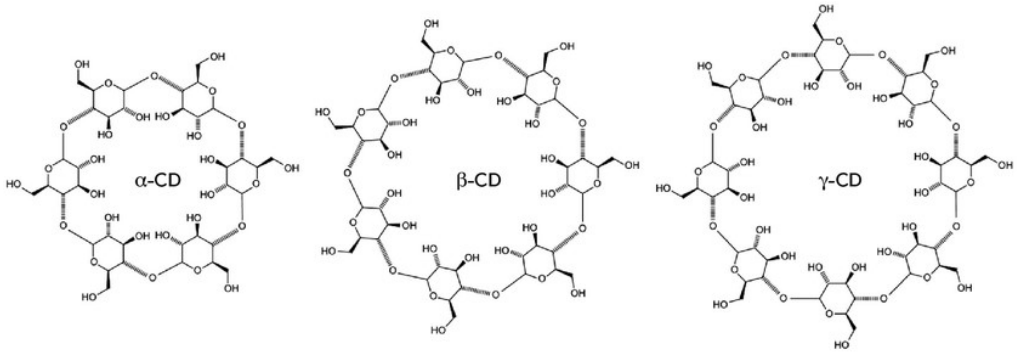

Figure 1.2: Structures of α-, β-, and γ-CD (Nikitenko & Prassolov, 2013).

The purpose of this research work was to perform complexation of host β-CD molecule with two kinds of first-line TB drugs simultaneously, which are INH and ETB. The synthesised β-CD/INH/ETB ternary inclusion complex was characterized using various advance spectroscopic methods to confirm the penetration of both drug molecules into β-CD cavity, and to investigate its thermal properties, crystallinity, as well as changes in surface morphology. Additionally, theoretical simulations using computer software were also performed to illustrate the molecular interactions involved between β-CD, INH, and ETB during complexation process.

Based on the above literature reviews, cyclodextrins play an important role in the development of inclusion complexes with considerable boost in the chemical and biological properties of the encapsulated active guest drug component. For similar reasons, this research is vital to explore and investigate such improvements on the fixed-dose combination (FDC) of more than one anti-TB drugs rather than improving an individual drug, as to improve the drug formulation for the ultimate purpose of

increasing bioavailability and reducing drug dosage for the prevention and reduction of side effects led by long-term drug consumption. The combination of INH and ETB was chosen in this research work, with the main reason being both drugs have the smallest molecular size among the four first line anti-TB drugs along with many bonding sites, which would most likely be successfully encapsulated in the limited cavity space of cyclodextrin at the same time. Furthermore, it was shown in past research that INH and ETB possess synergistic molecular mechanism in terms of countering the production of MTB cells, where the mycobactericidal effect of INH was significantly boosted by the suppression action of inhibin subunit alpha (inhA) gene by ETB (Zhu et al. 2019). Besides, WHO Model List of Essential Drugs has recommended the use of two-drug formulations in the treatment of TB, which includes the combination of INH and ETB in a single tablet (Albanna et al. 2013).

1.2 Objectives of the research

The specific objectives of this research are as follows:

a) To synthesise binary and ternary inclusion complexes of β-cyclodextrin with isoniazid and ethambutol using solvent evaporation method.
b) To characterize the synthesised binary and ternary inclusion complexes using FT-IR, ^{1}H-NMR, 2D NOESY NMR, TGA, SEM, and XRD.
c) To evaluate the interaction between β-cyclodextrin with isoniazid and ethambutol using spectroscopic and molecular dynamics (MD) simulation techniques.

1.3 Scope and limitations of the research

The main goal of this research is to spark the interest on synthesising β-CD ternary inclusion complex containing two guest drug molecules at the same time. To the best of our knowledge, there are little to no literatures available on such complex at the time this thesis is written. This study reports the stepwise synthesis of β-CD/INH and β-CD/ETB binary inclusion complexes and β-CD/INH/ETB ternary inclusion complex using the method of choice – solvent evaporation, which is a convenient method to be carried out in small scale laboratories, though other methods can also be employed. The synthesis of the inclusion complexes was done in natural temperature and conditions, not in a closed and controlled environment.

To further characterize the synthesised complexes, this research has included ^{1}H-NMR, FT-IR, and 2D-NMR studies, due to the effectiveness of structure elucidation using these methods. TGA spectroscopy results were reported as they can be supporting evidence to show the changes in thermal properties of complex upon successful synthesis. To gain more convincing proofs, the surface structure and crystallinity changes were also studied using XRD and SEM, as the captured images will clearly show how the complexes differ from the individual components.

Additionally, MD simulations were performed to verify the accuracy of the results obtained from solid state characterization, as the theoretical most feasible binding modes of every component in the complex will be displayed. However, the main use of MD simulation in this study was to obtain the binding energies of the synthesised complexes, as a reliable indication of the complex stabilities. Other MD

calculations, such as those that are involved in electronic configurations and molecular orbitals were not of our main concern and hence were not investigated.

CHAPTER 2

LITERATURE REVIEW

2.1 Host-guest chemistry

Host-guest chemistry is a division under supramolecular chemistry, whereby a host molecule accommodates a desired guest molecule or ion (as shown in Figure 2.1). Interestingly, the host and the guest molecules are held together by non-covalent bonds in which electron sharing does not occur, for instance hydrogen bonding, hydrophobic interaction, and van der Waals forces (Sambasevam et al. 2013). They key difference between hydrophobic interaction and van der Waals forces is that hydrophobic interaction arises from disruption of hydrogen bonds between water molecules which increases the tendency of non-polar substance to aggregate due to repulsive force in aqueous environment, whereas van der Waals forces are the results of instantaneous fluctuating induced dipole between polar or non-polar molecules, which can be attractive or repulsive force (Ho-Min 2017).

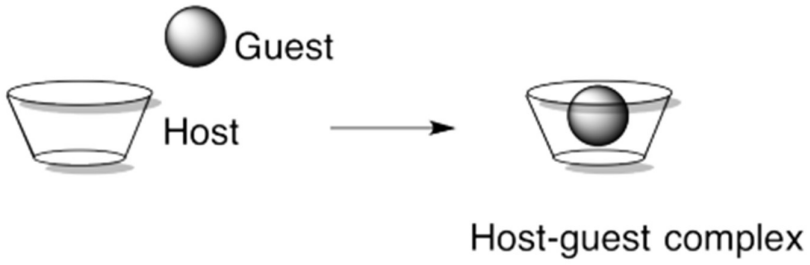

Figure 2.1: Simplified diagram of host-guest interaction.

Historically, host-guest chemistry was an idea developed from the phrase ‘like a key to a lock’ by Emil Fischer in 1894. According to his hypothesis, an enzyme is rather specific in its biochemical actions, where its geometry can be described as a

'lock', and will naturally complement the substrate, which he termed as a 'key', in which catalytic reactions will take place if both were to meet. Inspired by this model, it is proposed by researchers that, host molecule resembles the 'lock', and the guest molecule acts as the 'key' (Yu et al. 2020).

In 1967, Charles Pedersen reported the synthesis of the first macrocyclic host molecule – a family of crown ethers that can undergo complexation with alkali metal cations (Pedersen 1967). Since then, several other types of host molecules have reportedly been synthesised, such as carcerands and cryptands that are responsive receptors to metal atoms or ions, and cyclodextrins which are able to form complexes with guest drug molecules (Wenz 2012). Hence, the future directions of macrocyclic host molecule synthesis are pointing towards achieving highly specific recognition properties for desired guest molecules, which can be further explored in various practical applications, for example biochemical sensing (Pinalli et al. 2018), biomimetic catalysis (Marchetti and Levine 2011), and drug delivery systems (Webber and Langer 2017).

2.2 Supramolecules in drug delivery systems

In recent decades, the design of drug delivery systems with relation to supramolecular chemistry has gained significant interest towards the approach of improving physicochemical and pharmacological effects of drugs. Supramolecules allow for composition control of drugs at molecular levels, enhanced pathway for incorporating drugs, and most importantly, creation of better delivery systems which are responsive to a wide selection of physiological indicators (Karim et al. 2016).

When it comes to this topic, supramolecular nanoscale drug delivery system is one of the most explored categories. Generally, the rationale for the employment of nanoscale drug carriers is to overcome the common disadvantages of consumer drugs, namely poor solubility, low cellular uptake, and stability, as well as excessive toxicity (Saji 2022b). The nanoscale drug carriers that have been vastly explored over the years include inorganic carriers such as gold (Farooq et al. 2018) and silica (Karaman and Kettiger 2018) nanoparticles, and organic carriers like liposomes (Xing et al. 2016) and proteins (Kariduraganavar et al. 2019). The main benefits of nanoscale carriers in drug delivery are specific targeting mechanism, improved drug solubility, and limited accumulation of off-site drug (Jin et al. 2019).

On the other hand, there has been an increasing interest among researchers to synthesise cyclodextrin based supramolecular drug delivery systems. Due to their high flexibility of forming inclusion complexes with wide selection of molecules, which include organic, inorganic, organometallic, charged and neutral molecules, cyclodextrins are playing a significant role in the synthesis of supramolecular host for guest drug accommodation. Furthermore, the attempt to modify and improve cyclodextrin-drug complexes have seen a great amount of success, where in vivo solubility and drug activity of the complexes have experienced obvious enhancements (Laza-Knoerr et al. 2010). Through intensive clinical testing, it was found that cyclodextrin based drug delivery stands out from other supramolecular host in terms of ease of administration, protection of encapsulated drugs, manipulation of delivery rate, and specific cell interactions (Simões et al. 2015).

2.3 Cyclodextrins (CDs)-based drug delivery systems

The CDs-based drug delivery system is a research field established decades ago, where the first phase had emphasised on synthesis of simple inclusion complexes and preliminary study using various techniques. Up to the present, the field of study had developed into discovery of derivatives for drug delivery, exploration of α-, β-, and γ-CD, as well as polymeric derivatives and relation with nano properties (Singh et al. 2022). As a summary, CDs serve to enhance drug delivery systems not just by the oral route, but also parenteral, ophthalmic, nasal, rectal, colon specific, peptide and protein, oligonucleotide, topical, and brain targeting delivery routes (Chordiya Mayur and Senthilkumaran 2012). Even though it is a proven fact that the use of CDs in drug delivery systems is beneficial, it is sensible to identify CDs based drug delivery systems in terms of their useful features which are crucial factors of consideration for further research in this branch of study.

One of the major advantages of using CDs in drug delivery systems is the enhancement of solubility. CDs possess the ability to hide most of the hydrophobic functionalities of poorly-soluble drugs within its cavity while exposing the hydrophilic hydroxyl groups on the external surface (Tiwari, Tiwari and A. Rai 2010). For drug delivery interests, a variety of CDs derivatives were also studied to determine whether the substituents have great influence on the solubilising capability, and it was found that methylating any secondary OH group gave the best outcome in terms of solubility improvement (Yousaf et al. 2023). This situation was attributed to the great reduction of drug crystallinity and inhibition of drug crystallisation by methylated CDs, which ultimately increases the dissolution rate of the drug (Liu et al. 2023).

Apart from solubility, CDs aid in drug delivery systems by increasing the permeability of drugs across biological membranes such as gastrointestinal membrane or nasal membrane. However, excessive use of CDs may result in decrease of drug permeability (Másson et al. 1999). To diffuse through biological membranes, the active drug must possess sufficient lipophilicity while having high level of aqueous solubility in the unstirred water level (UWL) to partition into a lipophilic membrane. With the use of CDs, the effective permeability of the drug through UWL is increased due to reduction in its apparent thickness, if and only if the chemical potential of the said drug is equal on both sides of the biological membrane. This has indicated that CDs used should only be sufficient to solubilise the desired drug, exceeding a certain threshold amount would give net loss in permeability efficiency, thus highlighting the importance of deciding the correct molarity of CDs used in inclusion complexation (Beig et al. 2013).

Additionally, one of the key roles of CDs in drug delivery systems include the protection of guest drug molecules by avoiding unnecessary reactions until the drug reaches its desired biological target (Karthic et al. 2022). For instance, CDs-based targeting and intelligent drug carrier was reported to had delivered antitumor agents to desired biological sites very efficiently, while the in vitro drug release test had given positive outcomes by having the antitumor agents released in ideal quantity over a set amount of time (Zhang et al. 2019). With the help of CDs, the drug release behaviour could also be modified into immediate release, delayed release, or prolonged release by altering the derivatives used, such as alkylated or acylated CDs retarding the release of water-soluble drugs (Salústio et al. 2011). This is particularly useful in obtaining the desired mode of drug-release for clinical applications in prolonged treatment.

Besides, another important feature of CDs-based drug delivery system is the influence of CDs on physical and chemical stability of drug. Generally, exposure to oxygen, water, sunlight radiation and chemical reactions are among the conditions for an active drug molecule to undergo degradation. Upon encapsulation in CDs inner cavity, these reactants are prevented from encountering the protected drug molecule, thus increasing its molecular stability (Yousaf et al. 2023). However, a few recent studies had shown that certain drugs are stabilised by CDs in solid dosage forms but are destabilised by the same host carrier in aqueous forms, for example β-lactam antibiotics undergo catalysed degradation with the presence of CDs in aqueous solutions (Aiassa et al. 2023). The workaround solution for such issues would be employing different derivatives of CDs on the same drug until desired results are obtained (Popielec and Loftsson 2017). Therefore, the effect of CDs on various drugs should be verified in the final drug formulation before the drug delivery system can be deemed successful in improving the physical and chemical stability of the drug.

Safety and toxicity are important criteria when it comes to the usage of CDs in drug delivery systems. A study had shown that CDs were not absorbed from gastrointestinal tract when administered through the oral route due to their bulky and natural hydrophilic characteristic, after which the consumed CDs would disappear rapidly from circulation and renally excreted (Gidwani and Vyas 2015). For more specific examples, α-CD and β-CD have high resistance towards human amylase but are almost immediately metabolised when passing through the gastrointestinal tract and excreted in the form of faeces, while γ-CD is readily digested by the same enzyme (Muankaew and Loftsson 2018). Through animal test studies, β-CD releases negligible toxicity when consumed through oral route with a daily dosage of less than 600 mg/kg

but may cause certain harm, such as decrease in body weight and nephrotoxicity if excessively taken (Gould and Scott 2005). With the in-depth studying of in-vivo and in-vitro toxicity profile of CDs, the United States Food and Drug Administration (US FDA) has classified natural CDs as non-toxic food additives (Gidwani and Vyas 2015).

2.4 Application of cyclodextrins (CDs) in pharmaceutical drugs

To date, a great number of research have reported the use of CDs in modification of drug compounds. CDs are known for possessing unique structure of hydrophobic centre cavity and hydrophilic exterior, which allows for the accommodation of various lipophilic drugs within their cavities. Drugs which have undergone inclusion complexation with CDs have greater water solubility, physical and chemical stability, reduced drug odour and side effects when compared to the unmodified drugs (Loftsson and Brewster 2010). As shown in Figure 2.2, the mechanism of inclusion complexation revolves around the removal of water molecules from the lipophilic cavity of CDs, which causes the decrease of hydrogen bonds formed that leads to the increase of repulsive interaction between the guest drug and the aqueous environment, causing the guest drug molecule to exert itself in the CDs cavity when the compatibility of size and polarity are met (Yousaf et al. 2023).

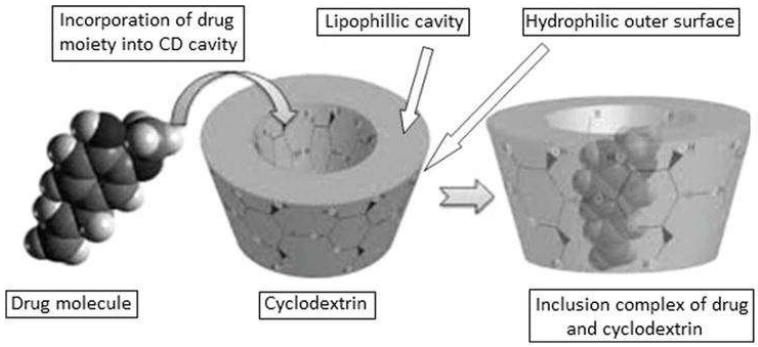

Figure 2.2: Interaction between drug molecule and CDs (Yousaf et al. 2023).

As mentioned earlier, the β-cyclodextrin (β-CD) variant is the most used type of CDs in the modification of drugs. Hence, several related studies were reviewed throughout the commencement of this research work. Known for their antitumor properties, azomethine compounds have always been a suitable candidate for cancer treatment. However, azomethines have limited solubility in organic solvents, which has restricted their wide usage in pharmaceutical industry. Hence, Nepal et al. and Sambasevam et al. had perform complexation of azomethine related compounds with β-CD, where their solubility and processability were dramatically increased for further applications (Nepal et al. 2003; Sambasevam et al. 2013). Within the same scope, Pandya et al. had formulated duloxetine hydrocholoride (DXH) inclusion complex using β-CD, as this Biopharmaceutics Classification System (BCS) class-II drug has poor aqueous solubility which leads to poor bioavailability and drug performance when consumed by patients who experienced major depressive disorder (MDD). The solubility and dissolution rate of β-CD/DXH was observed to be much higher when compared to pure DXH (Pandya et al. 2015). Furthermore, curcumin which is known for its anti-inflammatory, antioxidant, immunomodulatory properties, as well as chemoprevention agent towards colon and cervical cancer has always been limited in pharmaceutical applications due to its poor bioavailability and solubility while being chemically unstable. Hence, Ja'Far et al. had discovered a potential drug delivery system by encapsulating curcumin in β-CD cavity, which had enhanced its delivery efficiency and anti-proliferative effect (Ja'Far et al., 2018).

With regards to tuberculosis related drugs, Q. K. Anjani et al. had reported the inclusion complexation of rifampicin (RIF) with different types of CDs, namely β-CD, hydroxypropyl-β-CD (HPβ-CD), γ-CD, and hydroxypropyl-γ-CD (HPγ-CD), to find

out which host gives better improvements on the drug. Having assessed the dissolution profiles at different pH values and in vitro antibacterial activities of the four types of inclusion complexes, it was found that both β-CD and γ-CD based complexes had 60% improved drug release efficiency for RIF and possessed greater antibacterial activities, which had signified that reduction of RIF daily dosage is highly possible with inclusion complexation (Anjani et al. 2022). Besides, Razak et al. had found a reliable way of protecting another first line antituberculosis drug, isoniazid (INH) from its weakness of easy degradation when exposed to sunlight or UV light, which was complexation with β-CD (Razak et al. 2014). Additionally, β-CD/pyrazinamide (PZA) inclusion complex was synthesised by Ribeiro et al., where therapeutic effects of PZA had been increased and drug administration time had largely reduced (Ribeiro et al. 2015).

The findings from the research mentioned above were particularly important, as overdose of most medicinal drugs will give rise to irreversible bacterial resistance and various side effects, which are bound to complicate the treatment process. Since it is not possible to list every kind of drugs containing CDs, Figure 2.3 compiles a few examples of such drugs which are available for sale in the market, as a source of reference to display how CDs are widely used in the pharmaceutical industry today (Loftsson and Brewster 2010).

Drug/cyclodextrin	Trade name	Formulation	Company (country)
α-Cyclodextrin (αCD)			
Alprostadil	Caverject Dual	Intravenous solution	Pfizer (Europe)
Cefotiam-hexetil HCl	Pansporin T	Tablet	Takeda (Japan)
Limaprost	Opalmon	Tablet	Ono (Japan)
PGE1	Prostavastin	Parenteral solution	Ono (Japan); Schwarz (Europe)
β-Cyclodextrin (βCD)			
Benexate HCl	Ulgut, Lonmiel	Capsule	Teikoku (Japan); Shionogi (Japan)
Cephalosporin	Meiact	Tablet	Meiji Seika (Japan)
Cetirzine	Cetrizin	Chewable tablet	Losan Pharma (Germany)
Chlordiazepoxide	Transillium	Tablet	Gador (Argentina)
Dexamethasone	Glymesason	Ointment, tablet	Fujinaga (Japan)
Dextromethorphan	Rynathisol	Synthelabo (Europe)	
Diphenhydramine and chlortheophylline	Stada-Travel	Chewable tablet	Stada (Europe)
Ethinylestradiol and drospirenone	Yaz	Tablet	Bayer (Europe, USA)
Iodine	Mena-Gargle	Solution	Kyushin (Japan)
Meloxicam	Mobitil	Tablet and suppository	Medical Union (Egypt)
Nicotine	Nicorette	Sublingual tablet	Pfizer (Europe)
Nimesulide	Nimedex	Tablets	Novartis (Europe)
Nitroglycerin	Nitropen	Sublingual tablet	Nihon Kayaku (Japan)
Omeprazole	Omebeta	Tablet	Betafarm (Europe)
PGE2	Prostarmon E	Sublingual tablet	Ono (Japan)
Piroxicam	Brexin, Flogene, Cicladon	Tablet, suppository	Chiesi (Europe); Aché (Brazil)
Tiaprofenic acid	Surgamyl	Tablet	Roussel-Maestrelli (Europe)
2-Hydroxypropyl-β-cyclodextrin (HPβCD)			
Cisapride	Propulsid	Suppository	Janssen (Europe)
Indometacin	Indocid	Eye drop solution	Chauvin (Europe)
Itraconazole	Sporanox	Oral and intravenous solution	Janssen (Europe, USA)
Mitomycin	MitoExtra, Mitozytrex	Intravenous infusion	Novartis (Europe)
Sulfobutylether β-cyclodextrin sodium salt (SBEβCD)			
Aripiprazole	Abilify	Intramuscular solution	Bristol-Myers Squibb (USA); Otsuka Pharm. (USA)
Maropitant	Cerenia	Parenteral solution	Pfizer Animal Health (USA)
Voriconazole	Vfend	Intravenous solution	Pfizer (USA, Europe, Japan)
Ziprasidone mesylate	Geodon, Zeldox	Intramuscular solution	Pfizer (USA, Europe)
Randomly methylated β-cyclodextrin (RMβCD)			
17β-Estradiol	Aerodiol	Nasal spray	Servier (Europe)
Chloramphenicol	Clorocil	Eye drop solution	Oftalder (Europe)
γ-Cyclodextrin (γCD)			
Tc-99 Teboroxime[a]	CardioTec	Intravenous solution	Squibb Diagnostics (USA)
2-Hydroxypropyl-γ-cyclodextrin (HPγCD)			
Diclofenac sodium salt	Voltaren Ophtha	Eye drop solution	Novartis (Europe)
Tc-99 Teboroxime[a]	CardioTec	Intravenous solution	Bracco (USA)

[a]An older product contained γCD but has been replaced by HPγCD in the current product.

Figure 2.3: Pharmaceutical drugs containing CDs (Loftsson & Brewster, 2010).

2.5 From binary to ternary inclusion complex of CDs

In the early phase of synthesis for CDs inclusion complexes, it was usually revolving around binary systems, where only one host accommodates one desired drug molecule. To further improve the properties of synthesised complex and its synthesis process, researchers have been looking for suitable additives to catalyse the formation of binary inclusion complex, and this has led to the study of ternary inclusion complex.

As a solid example, Jadhav and Pore had synthesised hydroxypropyl-β-cyclodextrin (HPβ-CD)/Bosentan inclusion complex with and without the addition of an amino acid called L-arginine (ARG) and studied its physicochemical properties using various characterization methods. Although the synthesised complexes exhibit greater solubility and dissolution rate with and without ARG, it was found that complexation efficiency dramatically increased with the presence of ARG. It was concluded that introduction of ARG as the ternary component was useful to help reduce the amount of HPβ-CD used for synthesis, because excessive usage of CDs will obstruct drug absorption in the gastrointestinal tract (Jadhav and Pore 2017; Saokham et al. 2018).

Besides, Kulkarni et al. had investigated the effect of two types of CDs, which were β-CD and HPβ-CD, on their complexation with quercetin (QUN), an anti-inflammatory agent. To make the study more interesting, three types of hydrophilic polymers, namely hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone K30 (PVP) and poloxamer 188 (POLO) were incorporated separately into the synthesis of inclusion complexes as the ternary additive. With the presence of these polymers, the complexation efficiency and stability of synthesised complex showed obvious increment, especially the complexes with POLO. It was also proven that QUN/HPβ-CD/POLO ternary complex displayed the highest solubility and in vivo anti-inflammatory activity in comparison with the other binary and ternary complexes (Kulkarni et al. 2019).

Other than that, Grebogi et al. had performed encapsulation of dapsone (DAP), a synthetic sulfone drug with bacteriostatic properties into β-CD and 2-HPβ-CD, in the absence or presence of water-soluble polymers, namely polyvinylpyrrolidone (PVP30) and hydroxypropyl methylcellulose (HPMC). The finding in this research was slightly different from those mentioned above, which was the addition of water-soluble polymers into the binary complex to form ternary complex did not further enhance the stability of the complex nor the solubility of DAP. This research had concluded that 2-HPβ-CD was the more preferred than β-CD as the host molecule to form a more stable inclusion complex with significantly higher complexation efficiency, while the addition of ternary component was not necessary (Grebogi et al., 2012).

Furthermore, Chandha et al. had attempted to incorporate arteether, a drug used for the treatment of celebral malaria into a few derivations of β-CD, such as pure β-CD, methyl-β-CD (M-β-CD), and HPβ-CD as an approach to improve its antimalarial activity, as it had certain shortcomings and limitations upon application into treatment regimen. The major flaws include very poor solubility at room temperature and poor bioavailability where about 40% of the drug would undergo degradation upon reaching the stomach. The effects of addition of a ternary component, polyvinyl propylene (PVP) on the complexation and solubilising efficiency of the host molecules were also observed. Interestingly, higher value of stability constant (K) was obtained for pure β-CD in the presence of PVP, which had reflected a better complexation efficiency. However, the antimalarial pharmacological activity of the ternary complex did not manage to surpass that of binary complex using M-β-CD as the host molecule (Chadha et al., 2011).

Despite the great extent on research for ternary inclusion complexes of CDs, most of the ternary components added into synthesis were multi-functional polymer molecules that aid in improving speed and efficiency of inclusion complex formation. Unfortunately, there is not much of research info to be made as reference when it comes to synthesis of ternary inclusion complexes consisting of more than one drug in the CDs cavity simultaneously. This can possibly be a new direction in future research.

2.6 Synthesis methods of CDs-drug inclusion complex

It is known that the inner cavity of CDs is hydrophobic, while its surface is hydrophilic. Therefore, lipophilic drugs enter the interior of CDs most commonly via hydrophobic binding, although various other bonds such as dipole-dipole and van der Waals forces may be present during the host-guest binding process (Yuexian et al. 2005). Nevertheless, there are many methods that can be employed to synthesise CDs-drug inclusion complex, which is highly dependent on the physical and chemical characteristics of desired drug, as well as the type of CDs used.

Among the available methods today, spray drying occupies a significant role with high relevance in both research laboratories and the manufacturing industry (Patil, Pandya, et al. 2010). This method focuses on dissolving drug, CDs, and auxiliary substances (if any) simultaneously in a chosen organic solvent before which the solution is atomised into solid particles. The procedure of atomisation process revolves around exposing droplets of liquids to gaseous medium with high temperature, causing them to turn into completely fine and dry powder with no moisture content (Pokharkar et al. 2009). The benefits of spray drying include high reproducibility, working speed,

and generation of high purity end products (Jafar et al. 2018). However, a significant downside of using this method is the relatively low experimental yield of products (20 – 70%) when compared to other methods (Muzaffar 2015).

Next, kneading method is one of the most basic synthesis methods which is widely used in small scale laboratories. A fixed molar ratio of CDs and desired drug are mixed in a mortar, along with addition of droplets of ethanol-water solvent to produce a damp paste, where the paste will be grinded with a pestle until well-mixed, then it will be dried under vacuum to remove moisture (Cheirsilp and Rakmai 2017). In opposite to spray drying, this method gives high experimental yield and does not require high temperature, but the guest drug must have at least partial solubility in the solvent to form the mentioned paste-like mixture. However, the drug release level of complexes synthesised by this method is often not enough to achieve the minimum threshold to be applied in pharmaceutical industry (Khadka et al. 2014).

Besides, solvent evaporation method often becomes an alternative if researchers seek to overcome the disadvantages of kneading method, while spray drying equipment are not accessible, especially in laboratories which are low-cost established (Patil, Kadam, et al. 2010). Through this method, drug and CDs are dissolved in a common solvent, usually methanol or ethanol, and continuously stirred until completely dissolved. Then, the mixture solution is filtered and dried under vacuum to remove the solvent completely before the product is obtained (Somagoni et al. 2011). The edge of using solvent evaporation than other methods mentioned above is the prevention of thermal degradation of drug due to high temperature, and a good

dissolution profile attributed to minimal crystalline formation. However, there may be slight drawbacks of using this method, which are the presence of unevaporated solvent that can affect the nature of synthesised complex, and more difficult choice of organic solvent to dissolve both CDs and the desired drug for the synthesis process (Savjani et al. 2012).

Other than that, freeze-drying can be a viable method for the synthesis of CDs-drug inclusion complex. It has similar initial steps as solvent evaporation, but the CDs-drug mixture solution is frozen and freeze-dried before washing with organic solvent and dried under vacuum (Creteanu et al. 2019). This method produces end product that is highly amorphous with high stability of CDs-drug binding. Yet, it is not often a feasible choice, because long hours of freeze-drying are both time consuming and cost ineffective (Asbahr et al. 2009).

Although there are still many other methods that can be used to synthesise CDs-drug inclusion complex, for instance co-precipitation (Jiang et al. 2019) and milling (Jug and Mura 2018), the methods elaborated above are more commonly found in the literature. These methods are proven to give high efficiency during complexation process, and the products synthesised are deemed suitable for various characterization purposes.

2.7 Characterization of CDs-drug inclusion complex in solid state

The characterization of synthesised inclusion complex is a necessary step to confirm its successful formation. This is the preliminary procedure before moving

towards the application of the synthesised complex, as it is important to understand every aspect of its physical and chemical characteristics. With that said, there are several characterization methods commonly used for this purpose, and they are divided into two main categories – solid-state and liquid-state characterization.

For the solid-state characterization, the first method is Fourier transform infrared spectroscopy (FT-IR). This method is used to identify every functional group that are present in the synthesised inclusion complex, as every functional group has its own characteristic absorption band that will be retained even after complex formation (Berthomieu and Hienerwadel 2009). To explain it in simpler terms, say drug molecule A and host molecule B have their individual characteristic absorption bands, after the complex A-B is formed, the characteristic bands of drug A and host B will both appear in the FT-IR spectrum of complex A-B, which signifies that formation of the complex is a success. However, in the case of CDs-drug inclusion complex, the characteristic bands of CDs may be too dominant due to repeating D-glucopyranose units, causing some characteristic bands of the drug to disappear, which is why more characterization methods are needed (Rakmai et al. 2018).

As to study the thermal properties of synthesised inclusion complex in the solid state, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are two commonly used method. DSC is usually used to detect the melting, boiling, and sublimation points of the pure components in an inclusion complex, and slight observable changes in these points after complexation will give a strong clue on the formation of inclusion complex (Rani et al. 2019). As for TGA, it is a tool often used

to predict the thermal stability of an inclusion complex. Since the thermogram is plotted with weight percent against temperature, useful indicators such as decomposition temperature and total percentage weight loss can be calculated. As a rule of thumb, the greater the decomposition temperature, and the lower the total percentage weight loss over the same range of temperature, shows the higher the thermal stability of the inclusion complex (Prabu et al. 2020). Usually, the synthesised inclusion complex should have higher thermal stability when compared to the pure components due to the presence of non-covalent bonds within the complex (Salehi et al. 2021).

Next, scanning electron microscopy (SEM) is frequently used to characterize the surface morphology of synthesised inclusion complex. The key elements that are observed in the images obtained from SEM include changes in particle structure, dimensions, and crystallinity (Sneha and Chandrakant 2018). For example, the most common change for most CDs inclusion complexes upon complex formation is the appearance of more amorphous agglomerates and lesser bulky crystal structures (Alshehri et al. 2020). Although SEM image may not be sufficient to standalone and prove the formation of inclusion complex, it is very useful to tell whether a new solid compound is formed successfully. To solidify the predictions in SEM characterization, X-ray diffraction (XRD) is an accurate method to determine the changes in crystallinity of an inclusion complex upon complex formation. All pure components in the complex have its own characteristic diffraction peaks, which differ in angle and peak intensity. Generally, the stronger the peak intensity, the higher the degree of crystallinity of the molecule (Wang et al. 2014). After formation of complex, it is a common scenario that characteristic diffraction peaks of the pure components will