HOST-GUEST INTERACTIONS BETWEEN BETA-CYCLODEXTRIN AND SELECTED FLUOROQUINOLONES: A MOLECULAR MODELLING STUDY

by

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LIST OF ABBREVIATIONS

AD	Autodock
ADT	Autodocktools
AG	Autogrid
AM1	Austin model 1
ASA=Δ	Accessible surface area
ASC	Apparent surface charge
B3LYP	Becke, Three parameters, Lee Yang Parr method
CDs	Cyclodextrins
CE	Capillary electrophoresis method
CM-β-CD	Carboxymethyl-beta-cyclodextrin
DFT	Density functional theory
DLG	Docking log file
DM-β-CD	Dimethyl-beta-cyclodextrin
DPF	Docking parameter file
FTIR	Fourier transform infrared spectroscopy
G03	Gaussian 03
GA	Genetic algorithm
GAFF	General Amber force field
GB	Generalized born model
GLG	Grid log file
GPF	Grid parameter file
GROMACS	GROningen MAchine for Chemical Simulation
GROMOS	Groningen molecular simulation (force field, molecular simulation package
H-Bond, HB	Hydrogen bond

HCCC	High-counter current chromatography
HF	Hartree –Fock theory
HPLC	High-performance liquid chromatography
HP-β-CD	Hydroxypropyl- beta-cyclodextrin
HP-γ-CD	Hydroxypropyl- gamma-cyclodextrin
IEF-PCM	Integral equation formalism-PCM
LGA	Lamarckian genetic algorithm
LINCS	LINear Constraint Solver
MGL	Molecular Graphic Laboratory
MSD	Mean square displacement
M-β-CD	Methyl-beta-cyclodextrin
NBO	Natural-bond order
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear overhauser effect Spectroscopy
NPT	Isothermal-isobaric ensemble, Systems of constant particle Number , Pressure and Temperature
P3M	Particle-particle mesh
PBC	Periodic boundary conditions
PCM	Polarizable Continuum Model
PDB	Protein Data Bank format
PDBQT	Protein data bank, partial charge and atom type
PM3	Parameterized Model number 3
PME	Particle Mesh Ewald
PMV	Python Molecular Viewer
RDF	Radial Distribution Function
Rg (x)	Radius of gyration for (x)

RMSD	Root-Mean Square Deviation
RMSF	Root mean square fluctuation
RNA	Ribonucleic acid
ROESY	Rotating frame nuclear overhauser effect spectroscopy
SAS	Solvent accessible surface
SASA	Solvent-accessible surface area
SBE-β-CD	Sulfobutylether-β-CD
SCRF	Self-consistent reaction field
SES	Solvent excluding surface
SPC	Simple point charge model
SPC/E	Extended simple point charge model
S-β-CD	Sulphur-beta-cyclodextrin
vdW	van der Waals
VMD	Visual Molecular Dynamics
VWS	van der Waals-surface
α-CD	Alpha-cyclodextrin
β-CD	Beta-cyclodextrin
γ-CD	Gamma-cyclodextrin

LIST OF SYMBOLS

D	Debye unit
E_{bond}	Bond energy
$E_{complex}$	FQ/β-CD complex energy
$E_{complexation}$	Complexation energy
E_{coul}	Coulomb energy
$E_{desolvation}$	Desolvation energy
$E_{dihedral}$	Dihedral angle energy
E_{docked}	Docking energy
$E_{electrostatics}$	Electrostatic energy
$E_{free\ energy\ binding}$	Lowest binding free energy
E_{FQ}	Fluoroquinolone energy
$E_{FQ/β\text{-}CD}$	FQ/β-CD energy
$E_{H\text{-}bond}=E_{h\text{-}bond}$	Hydrogen bond energy
E_{HOMO}	Highest occupied molecular orbital
$E_{interaction}$	Interaction energy
$E_{intermolecular}$	Intermolecular energy
$E_{internal}$	Internal energy
$E_{kinetic}=E_{k}$	Kinetic energy
E_{LJ}	Lennard –Jones energy
E_{LUMO}	Lowest unoccupied molecular orbital
E_{opt}	Optimization energy
$E_{potential}=Ep$	Potential energy
$E_{solvation}$	Solvation energy

$E_{torsional}$	Torsional energy
$E_{Total}=E_T$	Total energy
$E_{unbond}=E_{non-bond}$	Non-bonding energy
E_{vdw}	van der Waals energy
$E_{\beta-CD}$	β-cyclodextrin energy
F	Force
Fs	Femtosecond
G_{cav}	Cavitation free energy
G_{sol}	Gibbs free energy of solvation
G_{dr}	Dispersion – repulsion free energy
G_{es}	Electrostatic free energy
K_i	Inhibition constant
N_{tor}	Number of torsions angle in molecule
pKi	Logarithms inhibition constant
RAM	Random-access memory
RT	Gas constant.Temperature
x, y, z	Coordinates
ε	Dielectric constant
ε_{in}	Dielectric constant of solute
ε_{out}	Dielectric constant of solvent
Ψ_{glu}, ϕ_{glu}	Torsion angle between glucose in maltose

INTERAKSI PERUMAH-TETAMU ANTARA BETA-SIKLODEKSTRIN DAN FLUOROKUINOLON TERPILIH: SUATU KAJIAN PEMODELAN MOLEKUL

ABSTRAK

Fluorokuinolon (FQs) ialah satu kumpulan antibiotik penting yang terdiri daripada gelang kuinolon hidrofobik dan gelang piperazina hidrofilik. Beta-siklodekstrin (β-CD) dan terbitannya kini digunakan secara meluas dalam sistem penghantaran ubat, kerana rongga hidrofobiknya mampu membentuk kompleks rangkuman dengan pelbagai molekul organik. Dalam kajian ini, interaksi perumah tetamu antara FQs dan β-CD menggunakan tiga teknik permodelan molekul iaitu mekanik kuantum, pengedokan molekul dan dinamik molekul telah dijalankan. Rangkuman molekul FQ ke dalam rongga β-CD boleh berlaku melalui dua orientasi iaitu dengan gelang kuinolon (orientasi A) atan gelang piperazina (orientasi C) menghadap rongga β-CD. Kesan pelarut dimodelkan secara implisit menggunakan model kontinuum berkutub (PCM) dalam pengiraan mekanik kuantum dan dengan menggunakan model caj titik mudah (SPC) dalam simulasi dinamik molekul 100 ps. Pengiraan mekanik kuantum menunjukkan rangkuman FQ ke dalam rongga β-CD berlaku melalui gelang piperazina didapati lebih stabil sebanyak 230-241 kJ/mol. Dalam simulasi pengedokan molekul, kestabilan kompleks rangkuman yang mempunyai bilangan molekul H_2O yang disingkirkan dari rongga β-CD adalah mempengaruhi kepadanan molekul FQ ke dalam rongga β-CD. Penurunan tenaga pengikatan bersama-sama 8-11 molekul air yang disesarkan dari rongga β-CD diperhatikan. Dalam simulasi dinamik molekul, kesan pelarut pada perubahan konformasi β-CD dan pergerakan molekul FQ di dalam dan di luar rongganya telah dikaji menggunakan simulasi dinamik molekul ensembel NPT.

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ABSTRACT

Fluoroquinolones (FQs) is an important group of antibiotics made up of a hydrophobic quinolone and a hydrophilic piperazine rings. Beta-cyclodextrin (β-CD) and its derivatives are currently being widely used in drug delivery systems, owing to its hydrophobic cavities which are capable of forming inclusion complexes with a variety of organic molecules. In this work, the host-guest interactions between FQs and β-CD were investigated using three molecular modelling techniques, i.e. quantum mechanics, molecular docking and molecular dynamics. The penetration of FQ molecules into the β-CD cavity take place via two orientations which are A-orientation with the quinolone ring or C-orientation with the piperazine ring facing towards the β-CD cavity. The effect of solvent was modeled implicitly using polarizable continuum model (PCM) in the quantum mechanics calculation and by simple point charge water model (SPC) in a 100 ps molecular dynamics simulation. The quantum mechanics calculations show the inclusion of FQs into the β-CD cavity occur via the piperazine ring and is 230-241 kJ/mol more favourable than via the quinolone ring facing in. In the molecular docking simulation, the stability of the inclusion complexes with varying number of H_2O displaced from the β-CD cavity influenced the fitting of the FQ molecule into the β-CD's cavity. In molecular dynamics simulation, the effect of solvent on the conformational change of β-CD and the movement of the FQ molecule inside and outside the cavity has been investigated using the NPT ensemble.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Computer simulation is about finding solutions for mathematical models using a computer. It involves several stages beginning from choosing a model for a particular physical system, implementing the model in computer program, calculating the required parameters related to the system and investigating the results of the simulation. Computer simulation is applied in various fields such as mathematics, physics, biology and chemistry. It is also applied in engineering, medicine, agriculture and even sewage water disposal.

Choosing a model and the degree of system representation is related to the objectives of simulation and features being monitored. The degree of model representation range from macroscopic to microscopic and molecular scale systems, where the latter is usually referred as molecular modeling. Molecular modeling for chemical systems is usually based on either of two theories namely molecular mechanics (MM) or quantum mechanics (QM). Although MM modeling requires lower computational power, the QM modeling provides more precision concerning the system's behavior. Due to the availability of computational power, QM modeling is now ubiquitous where computationally intensive *ab initio* methods are employed besides the cheaper semi-empirical and empirical algorithms.

One of the classes of compounds that found its way in many practical applications is cyclodextrin (CD). CDs are deemed important because of several reasons (Szejtli & Szente, 2005), among others, CDs are seminatural products

produced from starch, a renewable and natural material via a relatively simple enzymatic conversion, they are produced in bulk using environmentally friendly technologies and their ability in acting as inclusion complexes make them significant for the alternation of toxic effects of some organic ligand. CDs are also non-toxic and their derivatives are often consumed as ingredients in drugs, food (Szente & Szejtli, 2004) and cosmetics. In this work, the molecular modeling of complexes of CDs with fluoroquinolone base compounds is carried out. Fluoroquinolone (FQ) based compounds are important and effective antibacterial drug agents. Researchers have divided the FQ molecules on the basis of their antibacterial drug spectrum (Tilloston, 1996). The second, third, and fourth-generation of FQ drugs have been removed from the clinical practice either because of severe toxicity issues or discontinued by their manufacturers, depending on improved structure and increase substituted functional group (Zhanel et al., 1999).

The molecular modelling of CD is usually hindered by some limitations related to the flexibility, ionization state and hydration water molecules. Frequently, the exact representation of solvent molecules is ignored due to computational cost. Therefore, an important aim of this study is to understand computational simulation by applying explicit solvent representation. When water molecules are in the cavity of cyclodextrins (CDs), they have the ability to interact with the guest (G) (ligand) molecule as well as with the CD molecule. Water molecules can form hydrogen bond networks that stabilize the G/CD complexes in solution. The considerable factors affecting the interaction and conformation of the G/CD system include H-bonding, van der Waals and electrostatic forces (Liu & Guo, 2002; Szejtli, 1998). In this work, fluoroquinolone based compounds act as the guest molecule.

Two types of water molecules are involved in the G/CD interaction namely internal and external water molecules that have the ability to make shells, surrounding the CD inclusion complexes. In general, higher effect on interaction is exerted by comparing the first and the second shell of water molecules. Internal water molecules are seen to play essential structural and functional roles. They can satisfy the hydrogen bonding potential within the CD structure and influences its stability as well as flexibility. The treatment of water molecules in G/CD complex has remained a challenge. Only a few studies have been carried out on water molecules located within the cavity which are involved in the interactions between G molecule and the CD molecule. Consequently, the position of water molecules and their influence on the stability of CD complexes are explored in this study.

1.2 Problem Statement

Fluoroquinolones (FQs) are medicinally important compounds used as antibiotics. Some FQ compounds such as sparfloxacin are sensitive to temperature and undergo photodissociation (Pierfitte et al., 2000; Salgado et al., 2005). By binding with CDs it has been shown that the solubility of FQ compounds such as ofloxacin molecule is enhanced (Hayakawa et al., 1986; Sato et al., 1986). Moreover, CDs are widely used in drug delivery systems to promote better tissue distribution (Loftsson et al., 2007). It is also known that water molecules are involved in the binding of FQ compounds and CDs, however, the available studies which investigate such interaction are limited. This study attempts to investigate the effects of water on FQs/CDs inclusion complexes using molecular modelling.

In other words, the lack of detailed structural information at the molecular level in the interaction of a FQ/β-CD is one of the main problems in the development

better FQ properties. Thus, it is hoped that with a better understanding of β-CD inclusion complexes, the problems faced in some FQ compounds, for example, problems of photodissociation, can be solved. In this study, molecular modeling simulations were employed as a computational tool to complement experimental methods in the investigation of the dynamics and stability of the FQs/CDs systems.

1.3 Research Questions

This study is conducted to provide answers to the research questions of what are the roles of solvent (water molecules) in CDs inclusion complexes and the types of driving forces that contribute to the stability of the complexes. In addition, the objective of this study is to determine the importance of simulation time on accuracy of predicted results. And try to answer of how about the effect of the solvation method on the binding conformation property.

1.4 Research Objectives

The objectives of this study are:

1. To model the structure of several fluoroquinolones (FQs) and β-cyclodextrin (β-CD) inclusion complexes and verity the results with available experimental data.
2. To determine the effects of water molecules and the influence of solvation method on the binding conformations of FQs /β-CD inclusion complexes.
3. To investigate the stability of inclusion complexes determined in FQs /β-CD using quantum mechanics, molecular docking and molecular dynamic simulations.

4. To determine the lowest energy conformations for the inclusion complexes using quantum mechanics and molecular mechanical calculations.

1.5 Thesis Outline

This thesis is arranged as follows: Chapter 1 contains introduction, problem statement, research questions and objectives. Chapter 2 contains an introduction to the research topic involving the CD, FQ and its complexes (FQ/CD) and molecular modelling theory, and lists the review of the key studies on theoretical and experimental CD inclusion complexes. Chapter 3 describes the methodology, including the method for treating water as solvents with CD and FQ, using quantum mechanics calculation, molecular docking, and molecular dynamics simulation. Chapter 4 describes the major results of the quantum mechanics calculations, molecular docking, molecular dynamics simulations, and the theoretical analysis of the inclusion of FQ based molecules within the CD cavity. Finally, the last chapter summarizes the main results of this work, including the recommendations for future research.

CHAPTER 2

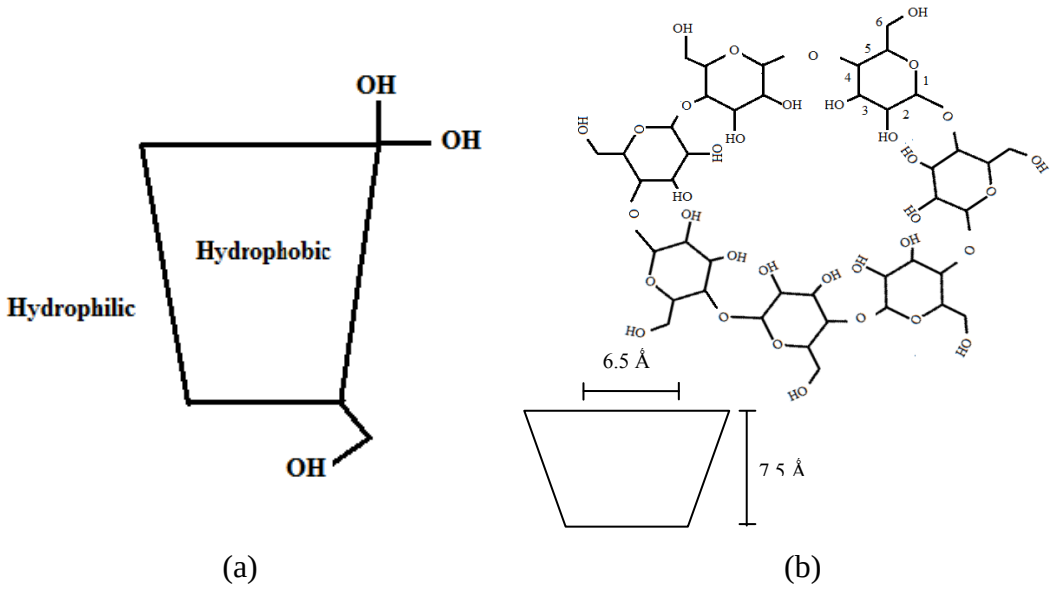
LITERATURE REVIEW

2.0 Introduction

In this chapter, a literature review of various works that had been carried out on cyclodextrin (CD) based compounds, fluoroquinolones (FQs), their interactions, molecular modelling of FQs/CD interaction, the effects of solvents on their interactions and some experimental work related to their interactions is attempted. The discussion will begin with a short introduction to CD, their structures and uses. Then, the discussion will continue with FQs and a thorough discussion on the effects of solvents on the FQs/CD interactions. These will be discussed in the spirit of computer models and experimental work.

2.1 Cyclodextrins (Host)

Generally, cyclodextrins (CDs) are cyclic oligosaccharides comprising of α-cyclodextrins (α-CD) with 6 glucopyranose units, beta-cyclodextrin (β-CD) with 7 glucopyranose units and γ-cyclodextrins (γ-CD) with 8 glucopyranose units linked by α-(1, 4) bonds. Cycloamyloses, cyclomaltoses, or schrodinger dextrins are other names for CDs. They generate a hydrophilic exterior surface and a non-polar interior cavity, as shown in Figure 2.1. (a). These hydrophilic cyclic oligosaccharides have lipophilic central cavities (through the formation of water-soluble inclusion complexes). Thus, CDs are able to solubilize several hydrophobic drugs in aqueous solutions (Uekama et al., 1998).



(a) (b)

Figure 2.1. Schematic representation of (a) the hydrophobic and hydrophilic regions of β-CD cylinder (b) 2D-structure of β-CD and its numbering scheme (Szejtli, 1998).

In general, β-CD molecule is widely used commercially and is often employed as a drug carrier, microvessel reactor, protein mimic, etc, (Del Valle, 2004; Kurkov & Loftsson, 2012; Loftsson & Duchene, 2007). The structure of β-CD molecule has a complete secondary belt, which is formed due to a series of hydrogen bonds (H-bonds) between the C2-OH group of one glucopyranoside unit and the C3-OH group of the adjacent glucopyranose unit, resulting a rather rigid structure (Figure 2.1.b) (Szejtli, 1998). This intramolecular H-bond formation is responsible for the lower solubility of the β-CD molecule in water. By contrast, the H-bond belt is incomplete in the α-CD molecule because of the distortion and rotation in the position of one glucopyranose unit. Consequently, instead of six possible hydrogen bonds, only four can be simultaneously established. The γ-CD forms less intramolecular hydrogen bonding and possesses a more flexible structure; therefore, it is the most soluble, as compared to the α and β forms.

Recently, β-CD has received a lot of interest in the field of host-guest supramolecular complexes because of its numerous applications in various fields. The individual glucose units are held in a C-1 chair conformation and joined together by α – 1 and 4 glycosidic linkages to form a cyclic structure. Inside the cavity, three rings of H-atoms bind to C-5 glycosidic O-atoms (Figure 2.1.b). H-atoms are at C-3 position. The glycosidic oxygen bridges produce high electron density. Thus, the inner cavity is hydrophobic, which is incompatible with water. With the existence of the hydroxyl group, the inside groups and the external forces show hydrophilic interaction. Accordingly, there is a low possibility of H-bond formation. Among the three kinds of (-OH) groups, primary C6-OH groups are the most basic and nucleophilic. The C2-OH groups are acidic, while C3-OH groups are the most inaccessible (inside cavity). Thus, electrophilic reagents will initially attack the C6-OH groups and the more reactive reagents will further react with C2-OH and C3-OH groups. The electrophilic attack not only depends on the position of the hydroxyl groups of CD, but also the size of the CD cavity (Szejtli, 1998).

β-CD forms inclusion complexes with other biologically active agents and compounds such as crown ethers and calixarenes where they act as the enabling excipient for products such as drugs (Loftsson & Duchene, 2007), foodstuff (Szejtli & Szente, 2005) and cosmetics (Buschmann & Schollmeyer, 2002).

The CD encapsulation of a lipophilic, water-insoluble drug molecule can have an effect on several physicochemical properties of cyclodextrin. For instance, it can increase the aqueous solubility and stability of the drug, without changing the chemical structure or basic pharmacological activity (Szejtli, 1998). CD complexation enhances the overall pharmacological activity of the drug by

increasing its availability at the target site (Loftsson & Olafsson, 1998). For four basic practical uses of CDs classified as follows; as carriers (solubilizers, stabilizers) for biologically active substances, enzyme models, catalysts and additives of separating agents for activity or batch processes. Finally, the CD-derivatives can be synthesized using one-pot reaction and these CD derivatives can be used as non-toxic materials in the industries owing to their complex forming capacity (Szejtli, 1998).

The improvement of chemical and physical properties for guest as CD inclusion complexes has exhibit certain benefits such as stabilization of light or oxygen-sensitive substances, modification of the chemical reactivity of the guest molecules, fixation of volatile substances, improvement in solubility of drugs, modification of liquid substances to powder, protection against drug degradation caused by microorganisms (Szejtli, 1989), masking of the nauseating smell (Hedges, 1998), taste and pigment of the drugs and catalytic activity (Breslow & Dong, 1998; Easton, 2005) of CDs with the guest molecules.

CD can be used in the stationary phase or as solution additives. The shape and size selectivity of cyclodextrins provide the basis for the separation of compounds owing to the binding constants of various molecules with cyclodextrins (Schneiderman & Stalcup, 2000). Thus, molecular discrimination can be achieved. The characteristics of CDs and their derivatives make them appropriate for application in analytical chemistry, agriculture, pharmaceuticals, food and industrial materials (Singh et al., 2002). In analytical chemistry, cyclodextrins are mainly used in gas chromatography, high performance liquid chromatography and capillary zone electrophoresis to separate drug molecules (especially chiral isomers).

CDs are capable of forming inclusion complexes with many kinds of drug molecules by taking part either partially or as a whole molecule into the cavity. No covalent bonds are formed or broken throughout the complex formation. In aqueous solution, the complexes are readily dissociated and free drug molecules remain in equilibrium with bound molecules within the CD cavity. Two critical factors determine the formation of the inclusion complexes between CDs and guest molecules (Loftsson & Duchene, 2007). The first factor is the relative size of the CD cavity to the size of the guest molecule or to certain key functional groups within the guest. If the guest is not of the appropriate size, it will not properly fit into the CD cavity. The second factor encompasses the thermodynamic interactions between different compounds of the system (CD, guest and solvents). There must be a favourable net energetic driving force that pulls the guest into the CD cavity. CD inclusion complexes are mainly formed by the substitution of included water molecules with appropriate guest species. The release of high-energy water molecules decreases the energy of the system. Other factors that contribute to the formation of CD inclusion complexes are hydrogen bonding, vdW and hydrophobic interactions (Loftsson & Duchene, 2007).

There are four energetically favourable interactions which help to shift the equilibrium to form the inclusion complexes; the first is displacement of polar water molecules from the non-polar CD cavity. Second, the increased number of H-bonds formed by the displacement of water returning for the bulk solvent and a decrease in the repulsive interactions (increase in the attraction interactions) between hydrophobic guest and aqueous environment. Finally, an increase in the hydrophobic interactions as the guest inserts itself into the non-polar CD cavity (Pela, 2007).

Table 2.1 summarizes some of the chemical and physical properties of some CDs. They usually have open cylinder shapes with hydrophobic inner sides and the hydrophilic outer sides (as shown in Figure 2.1).

Table 2.1. *The properties of cyclodextrins derivatives (Del Valle, 2004 and Uekama et al., 1998*)

Property	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Crystal water wt.%	10.2	13.2-14.5	8.13-17.7
Number of water inside the cavity	6	11	17
Number of OH (down/ up)	(6/12)	(7/14)	(8/16)
Approximate cavity volume ($Å^3$)	174	262	427
Diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
Outer diameter (Å)	14.6 ± 0.4	15.4 ± 0.4	17.5 ± 0.4
Solubility in water at 25 ˚ C (%,w/v)	14.5	1.85	23.2
Molecular weight (g/mol)	972	1135	1297
Number of glucopyranose units	6	7	8

CD derivatives molecules can be classified according to the type, polarity, and the size of the substituents. The use of CD derivatives is limited because they are usually obtained through a complex synthesis, which increases the price of the new products. For pharmaceutical applications, the optimum CD derivative to be used as a parental drug carrier should be cheap, highly soluble in water, available in a high purity and non-toxic (Stella & He, 2008). In addition, they should be stable during sterilization so that they can be stored in aqueous solutions and do not react with cholesterol, phospholipids, or other cell-membrane compounds (Loftsson et al., 2007). At the moment, these ideal cyclodextrin derivatives do not exist. CDs derivatives molecules have the capability to remove the toxic compounds in the human body as well as in the environment. Additionally, they can separate chiral compounds, isomers, enantiomers and tautomers (Eastburn & Tao, 1994).

The other important application of CD derivatives molecules is that they can be used as a parental drug carrier because of their high solubility in water at different temperatures, their low cost and their availability in pure form. CD derivatives are found to be non-toxic in chronic treatments even at high doses. They act as a powerful solubilizer in various lipophilic drugs. They are also found to be highly stable for heat sterilization and for open storage in solutions (Davis & Brewster, 2004). Additionally, these CDs are non-reactive with cholesterol, phospholipids and other alternative cell membrane components. Moreover, CD is biodegradable within the circulatory system and can be excluded as small molecular metabolites (Szente & Szejtli, 1999). In this work, the interaction of CD with FQs is studied. It is thus imperative that we review the work that had been carried out on FQ and related compounds.

2.2 Fluoroquinolones (Guests)

Fluoroquinolone is an important group of synthetic antibiotics with antibacterial drug activities and are divided into structural F-atom and quinolone ring. Quinolones have position C-3 attached to a carboxylic acid group (Figure 2.2), and are frequently referred to as 4-quinolones (Andriole, 2005). The addition of 6-fluoro- and 7-piperazinyl groups to the quinolone increase the antibacterial activity. This group of fluoroquinolones shows a higher antibacterial drug activity compared to its parent compounds. The piperazine ring has subsequently showed the increase in the ability of fluoroquinolones to penetrate the bacterial cell walls and enhance the activity of quinolones (Tillotson, 1996).

The introduction of a cyclopropyl side chain at C-1 position, in the ciprofloxacin molecule, marks an improvement in the activity. Ofloxacin molecule

and its L-isomer of levofloxacin molecule, showed another structural modification in the form of a bridging ring between the N-1 and C-8, which also provide an improvement to its activity (Appelbaum & Hunter, 2000). The structural substituent groups of ciprofloxacin, ofloxacin and sparfloxacin compounds, used in this study are shown in Table 2.2.

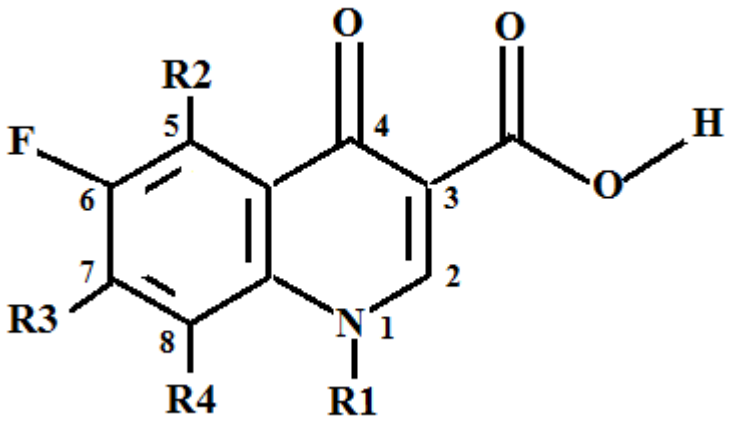
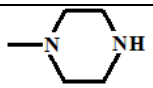
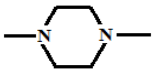
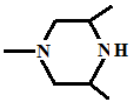
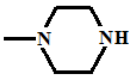
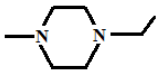
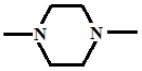
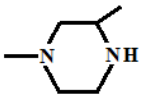
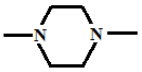
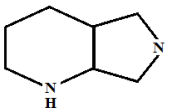
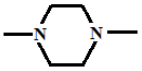


Figure 2.2. The general structure of fluoroquinolones and its numbering scheme.

FQs molecules were found very effective against both Gram-negative and Gram-positive pathogens than to other antibacterial. Beside their wider antimicrobial spectrum, FQs molecules are also characterized by their greater antimicrobial potency, better pharmacokinetic profiles and metabolic properties, as compared to their progenitor antibacterial drug (Appelbaum & Hunter, 2000; Bertino & Fish, 2000; Blondeau, 2004). FQs have good tissue penetration together with a long half-life (30-70% bioavailability) which resulted in a once-per-day dosage (Wise & Honeybourne, 1999). When a FQ molecule binds with a DNA-topoisomerase complex, it results the ternary complex which inhibits the cell growth and DNA replication (Hooper & Wolfson, 1985).

Table 2.2. *Examples of some fluoroquinolones (Appelbaum & Hunter, 2000; Hooper & Wolfson, 1985; Zhanel et al., 1999*)

Compound	R1	R2	R3	R4
Ciprofloxacin	 (Cyclopropyl)	H	 (Piperazine)	H
Ofloxacin	O-fused ring	H		O-fused ring
Sparfloxacin		NH_2		F
Norfloxacin	$-CH_2CH_3$	H		H
Enrofloxacin		H		H
Levofloxacin	O-fused ring	H		O-fused ring
Lomefloxacin	$-CH_2CH_3$	H		F
Pefloxacin	$-CH_2CH_3$	H		H
Moxifloxacin		H		$-OCH_3$
Rufloxacin	S-fused ring	H		S-fused ring

The driving forces within the formation of host-guest inclusion complexes between the β-CD and FQ molecules in aqueous mediums can be attributed to many factors. However, the primary driving force (Liu & Guo, 2002) is the hydrophobic interaction between the guest and water molecules (Barr et al., 2004; Hapiot et al., 2006). The repulsive forces between the included water molecules and the non-polar CD cavity (Szejtli, 1998); and those between bulk water and the non-polar guest molecule are many driving forces.

It is known that CD and FQ can interact as a host-guest interaction. It is also known from various works that this host-guest interaction is affected by solvents. It is thus imperative that a discussion on solvent effects be included in this review.

2.3 Solvent Models

Water is the most abundant compound on the earth. Water also has melting (0°C), boiling (100°C) and critical (374°C) temperatures (C) and a large dielectric constant (ε =78.39). The amount of water in most living species varies from 50 to 97% and the most important constituent of the body fluids and acts as a solvent for biomolecules. Water plays an important role in biological processes, such as molecular transport, biopolymer folding and aggregation, enzyme-substrate binding and catalysis. However, the effect of solvents in the computer simulations of biomolecules are modelled either implicit or explicit and cannot be neglected.

Solvent molecules effects can be incorporated into the simulation in two ways. The solvents can either be treated with their atomic details, known as an implicit solvent model or they can be represented as a dielectric continuum medium (Allen & Tildesley, 1989). The first approach is called an implicit solvent model. Explicit models are relatively less accurate, but the computational cost is smaller than the implicit model. This model enables proteins to be studied with relatively larger molar mass. The solute of interest is represented in its atomic detail, whereas the surrounding medium (solvent) is characterized by a polarizable continuum model, interacting primarily through polarization, dispersion, repulsion and cavitation effects (Tomasi et al., 2005).

2.3.1 Implicit Solvent Model

Implicit solvation model is generally used to represent solvents as a continuous medium instead of explicit solvent model. It is frequently applied in MD simulations and in other applications of MM model. The model is commonly applied to estimate the amount of total energy in solute-solvent interactions in the structural and chemical processes. Some examples of these processes are the folding or conformational transitions of polysaccharides, RNA, DNA and proteins, the association of biological macromolecules with ligands and the transport of drugs across biological membranes (Brewster & Loftsson, 2007; Loftsson et al., 2006 and 2007).

Over the years, various models have been developed under the implicit solvent model scheme. Among others are the Born Model (1920), Generalized Born Model, Onsager model (Onsager, 1936), Poisson Boltzmann Model and Polarizable Continuum Model (PCM) (Barone et al., 1997). In this work the polarizable continuum model (PCM) model will be used.

2.3.1.1 Polarizable Continuum Model

In this model, the condensed section is treated like a classical electronic medium represented by the solvent dielectric constant (for water $\varepsilon = 78.39$). The solute is placed into a virtual cavity with a suitable shape to enclose the full molecule. The polarizable continuum model (PCM) is commonly used in computational field to model solvation effects. If it were important to consider each solvent as a separate molecule, the computational cost of modelling a solvent-mediated chemical reaction would grow expensive high.

In PCM, the solvent is modelled as a continuum. An infinite, homogeneous, and isotropic dielectric medium were characterized by a dielectric constant (ε) (Barone et al., 1997). In this model, molecule was placed into a cavity and modelled after its real shape. The solute-solvent electrostatic interaction was calculated by introducing an *apparent surface charge distribution* spread on the cavity surface. The difference between the polarizable continuum model and Onsager model is the use of a molecular shaped cavity without a spherical cavity. In addition, in PCM, the solute is described by its electronic wave function (Tomasi et al., 2005).

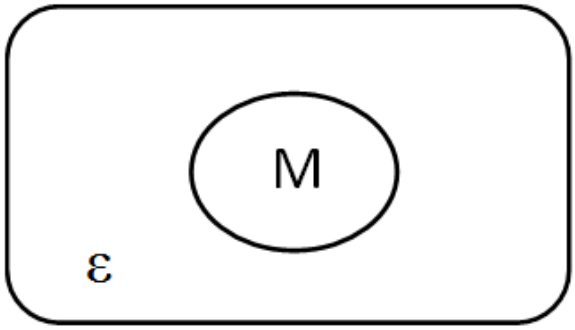


Figure 2.3. The reaction field model. Solute M is placed in a cavity in the solvent, which is treated as a polarizable continuum with a dielectric constant (ε).

The surface of the solute is one of the most useful theoretical tools for interpreting chemical processes in the solution. Surface integration relates to both the electrostatic energy and the dispersion-repulsion interaction energies between the solute and solvent molecules. The solute molecule (or ions) is placed in a cavity embedded in the infinitely polarizable dielectric medium of the solvent. In the polarizable continuum model method, the cavity is represented as a system of interlocking spheres. The solute molecule is represented by a charge distribution inside the cavity, as shown in Figure 2.3. The initial distribution is usually taken from gas phase calculations, geometry optimization, population analysis …etc.

Through the electrostatic interaction, the net charges are induced on the surface of the cavity (equation 2.1). The induced surface charges react and change the charge distribution in the solute. This mutual interaction takes place until equilibrium. An electrical field in the cavity by the contribution of solute charge distribution and the induced surface charges can be seen in Figure 2.4.

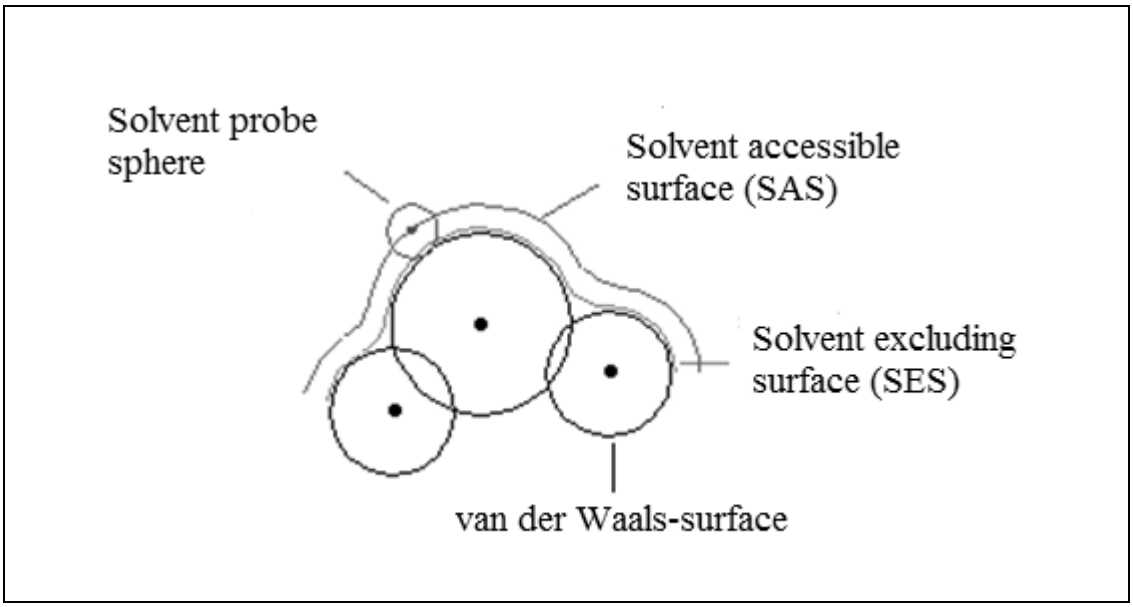


Figure 2.4. The cavity around atoms through the polarizable continuum model theory.

The molecular free energy of solvation is computed as the sum of three terms:

$$G_{sol} = G_{es} + G_{dr} + G_{cav} \quad (2.1)$$

where

G_{es} is the free energy of electrostatic solvent,

G_{dr} is the free energy of dispersion-repulsion for solute-solvent interaction, and

G_{cav} is free energy of the cavitation solute molecule.

In the polarizable continuum model, the molecule (solute) is located inside a cavity, surrounded by a homogeneous dielectric solvent. The solute-solvent interactions between the charge distributions, is composed of the solute molecule and

the solvent, (Miertus et al., 1981). The cavity is generally constructed by interlocking spheres that lead to the vdW surface or the solvent accessible surface (SAS). A more elaborate and accurate description employs the Connolly surface to represent the solvent excluded surface (SES) by rolling a sphere over the cavity (Connolly, 1983), as shown in Figure 2.4. The PCM is based on an approximate treatment of the solvent as a continuum medium, which is characterized by its dielectric constant (ε) and interaction with the solute molecule. The solute can be described either at the quantum mechanical level or the classical collection of point charges (Scalmani et al., 2004). They can be computed in the space around its location. Moreover, an empty cavity can be carved inside the medium to occupy host molecules, which point out the solute-solvent interface in the cavity. The electrostatic problem of solvent mutual polarization theory and continuum method can be reduced by using boundary conditions at the cavity surface, leading to significant simplifications in the associated equations.

In this model, PCM, a subsystem is enclosed in a cavity as an envelope of spheres (atoms), which are surrounded by the solvent. The reaction field of the solvent can be expressed in terms of the electrostatic potential, generated by suitable polarization charges (q) on small elements such as tessera (mentioned in Figure 2.5). Since the number of tessera linearly increases along with the dimensions of the solute. Polarizable continuum model strongly depends on the size of the system and the construction of the cavity, which consist of the calculation of the electrostatic reaction potential and polarization charges (Tomasi, 2004). In the PCM method, the solute molecule studied is located inside a molecular cavity surrounded by a dielectric medium (solvent), as shown in Figure 2.5.

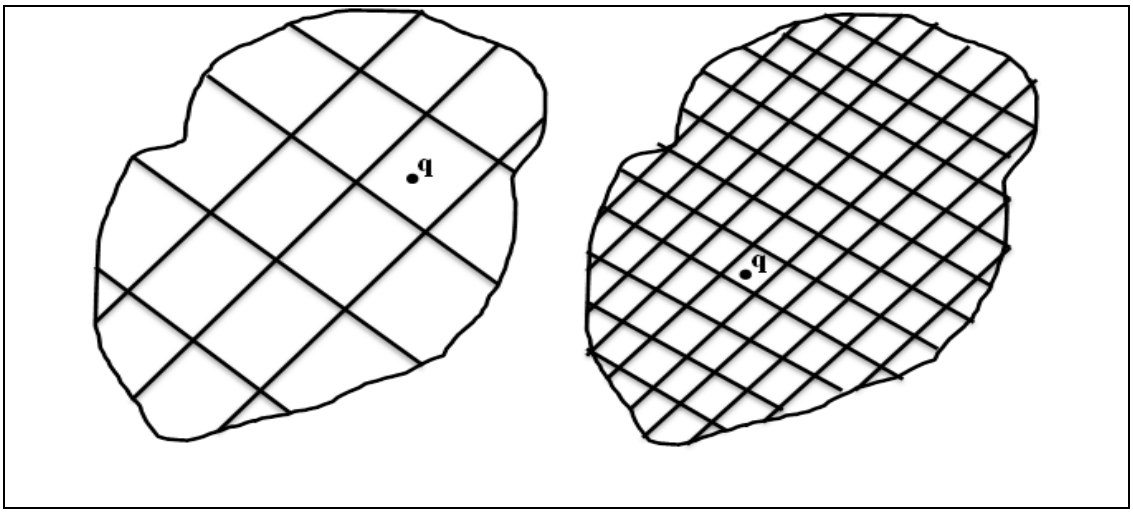


Figure 2.5. Graphical representation of the different tessera sizes

Under PCM, all the complex effects occur due to the mutual solute/solvent electrostatic interactions that are modelled by replacing the solvent with a continuum dielectric constant (ε) set equal to the experimental value. This electrostatic problem was solved by using the binary element method (Pomelli & Tomasi, 2001).

The idea of molecular cavity is imperative in PCM. Molecular cavity is the portion of space within the surrounding medium occupied by the solute molecule. The easiest way to build the cavity is to use a sphere or an ellipsoid with the radius of axes dimensions. This very simple approach is still in use because of the availability of the exact analytical solutions of electrostatic equations. The most common way to define molecular cavities is to interlock a set of spheres, with each centred on the atoms of the solute. Once molecular cavity is formed, there are several ways to define the molecular surface. One of which is the van der Waals surface (vdWS). This is a molecular surface obtained by interlocking spheres centred on each atom and acquiring the corresponding vdW radius. This definition is particularly useful when the cavitation energy contribution has to be studied. In addition, the solvent-accessible surface (SAS) is an extension of the vdWS and it is defined as the surface

identified by a rolling spherical solvent probe on the vdWS. The probe dimension depends on the molecular solvent properties and nature. This method is usually utilized when short range interactions are to be computed (Tomasi et al., 2005).

The solvent excluded surface (SES) has similar characteristics to the SAS procedure, but it defines the surface, only as the contact point between the cavity and the probe. This method is defined as a smooth surface in which no solvent molecule can move. The volume enclosed in the SES, but not in the vdWS, is called the solvent-excluded volume.

Calculation of the electrostatic interaction between a continuous polarizable medium and the solute was based on Cances et al. (1997). The electrostatic problem of a charge distribution embedded in a cavity surrounded by a continuum dielectric strongly depends on the macroscopic structural characteristics of the dielectric itself. In other words, the derivation of the basic theoretical background cannot be made without defining what kind of system is being used. Within the continuum framework, the simplified expression of the average distribution function of the solvent molecules is usually exploited. This preliminary classification can be limited to form dielectric constant.

Some models also describe non-electrostatic solute-solvent interactions, which are normally defined as repulsion, dispersion and cavitation energies. Moreover, the geometric relaxation induced by solvent on solute often would not be neglected. So, an efficient solvation model must provide energy gradients and allow for the geometric optimization in the solution. In other words, it is desirable that direct (polarization), indirect and relaxation solvent effects be treated with the same level of accuracy.

Specifically, utilization of PCM in Gaussian 03 (Barone et al., 1997; Cossi et al., 2003) and the apparent polarization charges distributed on the cavity surface are determined by imposing the condition that the total electrostatic potential is zero on the surface. This binary condition is suited for cavities in the conducting media and can be described as solvation in polar liquids. It is computationally simpler, especially for the expression of energy gradients and thus can efficiently allow a geometrically optimized solution.

The unique property of water is that it has the ability to form H-bonds with other water molecules in three-dimensional networks. However, extensive hydrogen bonding shortens the existence of the local tetrahedral water structure (less than 0.1 ps), which continuously changes as a result of formation and breakage of hydrogen bonding. The dielectric constant of water is among the highest of all liquids; thus, it is an excellent solvent in which polar and ionic compounds, as well as biomolecules, such as proteins, nucleic acids and polymeric sugars easily dissolves. The internal dynamics and functions of protein structures are stabilized by using water.

Water not only helps to dissolve biologically relevant solutes, but also interacts with solvent-exposed solute groups. Therefore, it is very active in biochemical reactions that occur in aqueous solutions. Water interacts with solvent-exposed solute groups and alters its kinetic, structural and thermodynamic profiles relative to bulk water. This modified water is commonly known as water of hydration. The subtle changes to the properties of water near a particular solute group can vary which depends on the chemical nature of the group. Hydrating water molecules interact differently with the charge, polar and non-polar atomic groups of

a solute. The water molecules that hydrate charged or polar groups are structurally and thermodynamically distinct from those hydrating non-polar groups.

2.3.2 Explicit Solvent Models

Explicit solvation models are accurate but may be computational costly. Several models explicitly determine the solvent effects and they differ from one another depending on factors such as the number of points (atom and dummy sites), flexibility and rigidity and polarization effect inclusion in the model. Water models can be three-site, four-site, five-site, or six-site and computational cost increases with the number of interaction sites (Table 2.3). An interatomic distance of 9 degree of freedom is required for each pair of water molecules in a three-site model, whereas an interatomic distance of 10 degree of freedom is required in a four-site model. An interatomic distance of 17 and 26 degree of freedom are required in five- and six-site models, respectively.

Table 2.3. *Different water models and corresponding distances (Berendsen et al. 1981)*

Water model	Distances
3-site	9
4-site	10
5-site	17
6-site	26

Simple point charge model (SPC) (Berendsen et al., 1981) is a common water model example and the molecular dynamic (MD) package GROMACS uses SPC and extended-SPC (SPC/E) water models (Berendsen et al., 1987) in explicit solvation. The SPC/E water model integrates a polarization correction term into the potential energy function in equation (2.2):

$$E_{pol} = \frac{1}{2}\sum i \frac{\left(\mu - \mu^{\circ}\right)^2}{\alpha_i} \qquad (2.2)$$

Numerous water models with varying complexities have been generated and the most common of these models is the SPC model. Atoms are assumed to be in mid-charge in this model; each hydrogen atom has a charge of 0.41 e and oxygen has a charge of -0.82 e. This assumption produces an incorrect dipole moment; thus, the H-O-H angle is changed from the experimentally determined value between 104.45° and 109.42° to obtain the correct permanent dipole. As a result, SPC water that is lacking two lone pair electron diffuses faster than real water (Berendsen et al., 1981).

2.3.2.1 Simple Water Model

The simple water models treat the water molecule as rigid and rely only on non-bonded interactions. The electrostatic interaction is modelled using Coulomb's law and the repulsion and dispersion forces using the Lennard-Jones potential. The potential for models such as TIP3P and TIP4P (Jorgensen et al., 1983) are represented by:

$$E_{ab} = \sum_{i}^{na} \sum_{j}^{nb} \frac{k_C q_i q_j}{r_{ij}} + \frac{A}{r_{ij}^{12}} - \frac{B}{r_{ij}^{6}} \qquad (2.3)$$

where k_C, the electrostatic constant, has a value of 332.1 Å kcal/mol, q_i and q_j are the partial charges relative to the charge of the electron; r_{ij} is the distance between two atoms or charged sites; and A and B are the Lennard-Jones parameters. The charged sites may be on the atoms or on dummy sites (such as lone pairs). In most H_2O models, the Lennard-Jones term applies only to the interaction between the O-atoms.