



Theoretical studies on the encapsulation of Paracetamol in the α , β and γ Cyclodextrins

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ABSTRACT

Cyclodextrins are cyclic oligosaccharides which have recently been recognized as useful pharmaceutical excipients. The molecular structure of these glucose derivatives, which approximates a truncated cone or torus, generates a hydrophilic exterior surface and a non polar cavity interior. As such, cyclodextrins can interact with appropriately sized molecules to result in the formation of inclusion complexes. These non-covalent complexes offer a variety of physicochemical advantages over the un manipulated drugs including the possibility for increased water solubility and solution stability. Further, chemical modification to the parent cyclodextrin can result in an increase in the extent of drug complexation and interaction. In this short article, the effects of substitution of paracetamol in α , β & γ cyclodextrin and the forces involved in the drug-cyclodextrin complex formation are discussed. Some general observations are made which predict that γ complexes are most stable than β complexes which are more stable than α complex.

Keywords: Cyclodextrin, Complex, Paracetamol, ab initio methods, Host guest complex, Solubility, Inclusion and Drug delivery.

INTRODUCTION

Although cyclodextrins are frequently regarded as a new group of pharmaceutical excipients, they have been known for over 100 years [1]. The foundations of cyclodextrin chemistry were laid down in the first part of this century [2, 3] and the first patent on cyclodextrins and their complexes was registered in 1953[4]. However, until 1970 only small amounts of cyclodextrins could be produced and high production costs prevented their widespread usage in pharmaceutical formulations. Recent biotechnological advancements have resulted in dramatic improvements in cyclodextrin production, which has lowered their production costs. This has led to the availability of highly purified cyclodextrins and cyclodextrin derivatives which are well suited as pharmaceutical excipients. These carbohydrates are mainly used to increase the aqueous solubility, stability, and bioavailability of drugs, but they can also, for example, be used to convert liquid drugs into microcrystalline powders, prevent drug-drug or drug additive interactions, reduce gastrointestinal or ocular irritation, and reduce or eliminate unpleasant taste and smell. The following is a short review of the effects of cyclodextrins on the solubility and stability of drugs in aqueous solutions with emphasis on the more recent developments. For further information on cyclodextrins and their physicochemical properties the reader is referred to several excellent books and reviews published in recent years [5-13].

Computational studies on Host-Guest complexes

Modern computational chemistry has proven to be a useful tool in a variety of chemical problems and have gained popularity to an extent such that the inherent empirical nature of chemistry seemed to be lost in the recent years. These computational chemical methods find a variety of applications in structure, spectroscopy and dynamics. These methods are classified as (a) Molecular mechanics methods (b) *ab initio* methods (c) Semi-empirical methods and (d) density functional methods. The ability of cyclodextrin to form inclusion complex with a guest molecule make them useful for catalysis [14], chiral separation [15, 16] and in drug delivery which is a function of two key factors:

- Steric: Relative size of cyclodextrin to guest molecule should be comparable, if the guest is wrong size, it will not fit to cyclodextrin.
- Thermodynamic interaction: There must be favorable net energetic driving force that pulls the guest into the cyclodextrin.
- α cyclodextrin can form low molecular complex with aliphatic side chain, β will form complex with aromatic and heterocycles and γ cyclodextrin can accommodate larger molecules such as macrocycles and steroids.

Inclusion complexation with cyclodextrin is like a “host-guest interaction”. In this interaction cyclodextrin act as host molecule and the drug molecule to be entrapped in host cavity act as guest molecule. Comparing to other encapsulation methods, which involve entrapment of more than one guest, cyclodextrin complexation involve entrapment of one molecule of guest in cyclodextrin cavity. For formation of complex with cyclodextrin, variety of non-covalent forces like Vander wall forces, hydrophobic interaction, and dipole movement are responsible. In majority of cases only a single guest molecules is entrapped in the cavity. For High molecular weight molecules, more than one molecule of cyclodextrins can bind to the guest.

Structure and physicochemical properties

Cyclodextrins (CDs) are cyclic oligomers of alpha-D-glucose formed by the action of the glucosyltransferase enzyme on starch. The Cyclodextrins has a specific coupling of glucose monomers in cyclic manner giving a rigid conical molecular structure with a hydrophilic exterior and hollow hydrophobic interior of a specific volume. This internal cavity is capable of accommodating wide range of guest molecules including polar as well as non-polar aliphatic and aromatic compounds. The inside cavity of appropriate dimension is conducive to binding to various guest molecules to form an inclusion complexes. CDs' chemistry has been reviewed many times through book-length treatment [17-21] and several shorter and more specialized articles [22-42]. There exist a significant number of computational studies over the past fifteen years that have been led to a deeper understanding of the structure, dynamics and chemical behavior of CDs'. Two excellent review articles covering theoretical aspects of CDs and their inclusion-occlusion complexes have been published [43-44]. The last article covers up to 1998, and since then a meaningful number of computational studies have appeared further describing the detailed, atomic level behavior of these molecules.

Cyclodextrins as Hosts

Cyclodextrins are the cyclic oligosaccharides made up of 6 or more α – D glucopyranose units linked by 1→ 4 glycosidic linkages. These were firstly described by A .Villers in 1891[1].

There are mainly three types of cyclodextrins.

- α cyclodextrins = made of six glucose molecules linked in ring.
- β cyclodextrins = made of seven glucose molecules linked in ring.
- γ cyclodextrins = made of eight glucose molecules linked in ring.

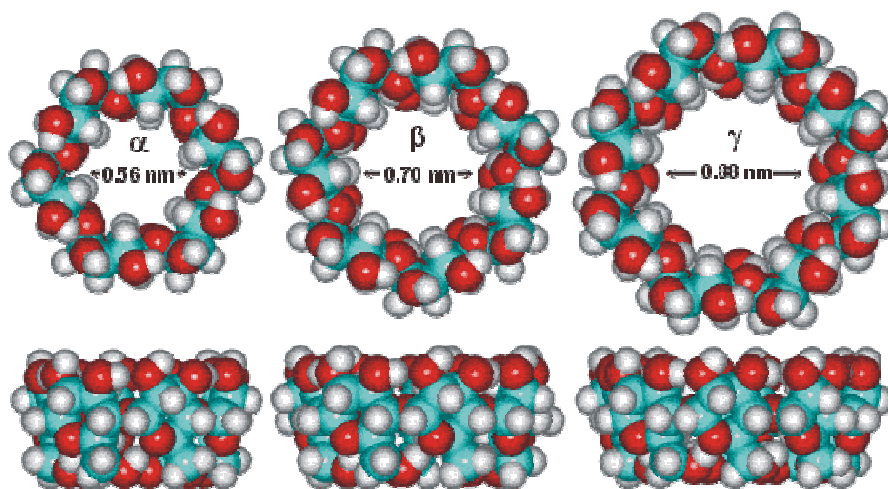


Figure 1. structure of α , β , and γ cyclodextrins

Cyclodextrin consist of 6,7,or 8 (α , β , and γ) D – glucopyranose units connected by alpha (1 \rightarrow 4) glycosidic linkages. The most stable three-dimensional molecular configuration for these non – reducing cyclic oligosaccharides takes the form of a toroid with the upper (larger) and lower (smaller) opening of the toroid presenting secondary and primary hydroxyl groups respectively to solvent environment. The interior of the toroid is hydrophobic as a result of the electron rich environment provided in large part of the glycosidic oxygen atom.

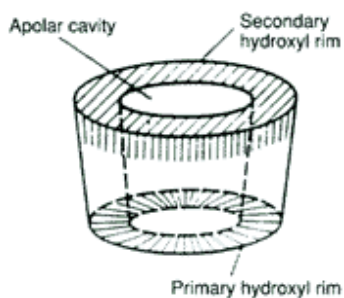


Figure 2. Structure of toroid

Each glucose unit contain two secondary hydroxyl groups at C 2 and C 3 position and primary hydroxyl group at C 6 position produces 18 to 24 sites for chemical modification and derivatization. Cyclodextrins are synthesized from starch by easily available enzyme glucosyltransferase (CGT), along with α amylases. Starch is liquefied by heat treatment or using α – amylase, then CGT is added for the enzymatic conversion. The product gives the mixture of three main types of cyclic molecule.

Properties

- Formation of host – guest inclusion compounds with lipophilic compounds.
- Solubility: - soluble in water (low), DMF, DMSO, Pyridine.
- Low toxicity.
- Biodegradable.

Table 1. Physical properties of α , β , and γ cyclodextrins

Properties	Alpha	Beta	Gamma
No. Of glucose units	6	7	8
Molecular wt	972	1135	1297
Solubility in water (g/cc)	14.5	1.85	23.2
pKa at 25°C	12.33	12.2	12.08
Inner diameter (nm)	0.45 – 0.57	0.62 – 0.78	0.79 – 0.95
Outer diameter (nm)	1.37	1.53	1.69
Height (nm)	7.9	7.9	7.9
Cavity volume	0.174	0.262	0.472
Crystal form	Hexagonal plate	Monoclinic parallelogram	Quadratic prism

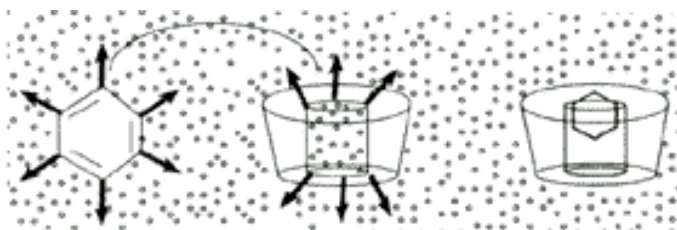
Complex formation of guest molecule with α , β & γ Cyclodextrins occurs in the stoichiometric ratio (1:1,1:2,2:1). Complexation is substrate unspecific. Complexes with lower alcohols, amines or with compound containing heteroatom is easy and is stable in water solution. Complexation improves molecules solubility and stability.

Bonding in cyclodextrins

Cyclodextrins binds to guest molecule with bonding other than covalent bond. It is the interplay of atomic (vanderwalls forces), thermodynamic (hydrogen bonding), and solvent (hydrophobic) forces that count for the stable complexes, which may be formed with chemical substances in the non polar environment of the cyclodextrin cavity. The complex exists in equilibrium depend on the concentration of the cyclodextrin, the guest and water. The bonding may break up with change in pH and temperature of solution, leading to cleavage of hydrogen or ionic bonds between host and guest. Cyclodextrins can release the drug in the body, hence acts as a drug carrier. Cyclodextrin can be used in environment protection, in food industry for preparation of cholesterol free products, to stabilize the volatile or unstable compounds and the reduction of unwanted taste and odor, to remove bed smell by forming complex with fragrances, solubilization of hydrophobic compounds, separation of chiral compound and stabilization of reactive compounds, In pharmaceuticals, they are used to enhance the rate of dissociation of drug, in faster absorption of drug after oral application, enhancement in drug absorption independent of the acidity of the gastric juice, reduced side effects, skin irritation, muscular tissue damage, kidney damage and activity period of drug is increased.

Mechanism of drug capture by cyclodextrins

Cyclodextrins form complex with drug rapidly because even the most lipophilic compounds are solvated by water to some extent and these discrete hydrated molecular particles are better able to get part of the hydrophilic hydroxyl group at the entrance of the cyclodextrin cavity.

**Figure 3. Mechanism of drug capture of cyclodextrin**

The hydrating molecules of water can actively interact with the hydroxyl group on the rim of cyclodextrin toroid or they may simply shield the hydrophobic drug molecule from being repelled by the hydroxyl group. Once, the part of the hydroxyl rim, the hydrating molecule of water is driven from the hydrophobic cavity leaving the drug molecule to find its most stable resting place.

In the case of extremely water insoluble substances, the true equilibrium may not be achieved for hours or days because molecular separation due to hydration occurs extremely slowly.

Dissociation is an equally rapid process, most often caused by the sudden increase in the number of water molecules outside the cavity, even though there may be an initial energy barrier to dissociation, the concentration gradient created becomes excess and the chemical (drug) is displaced.

Paracetamol as guest

The paracetamol is a polar molecule which is also known as acetaminophen. The width of molecule is 0.43nm and height is around 0.78nm. The molecule can be accommodated in all the three CDs. The complex of Paracetamol and CDs is stable due to hydrogen bonding and hydrophobic interactions. Paracetamol is a minor analgesic that is given orally for relief of mild to moderate pain. It also has antipyretic effect and is commonly used in children for this action. It is also known as Acetaminophen. Paracetamol consists of a benzene ring, substituted by one hydroxy group and the acetamide group in the para position. It is extensively conjugated system, as the lone pair of the hydroxyl oxygen, the benzene pi cloud, the nitrogen lone pair, and the lone pair on the carbonyl oxygen are all conjugated.

Physical properties

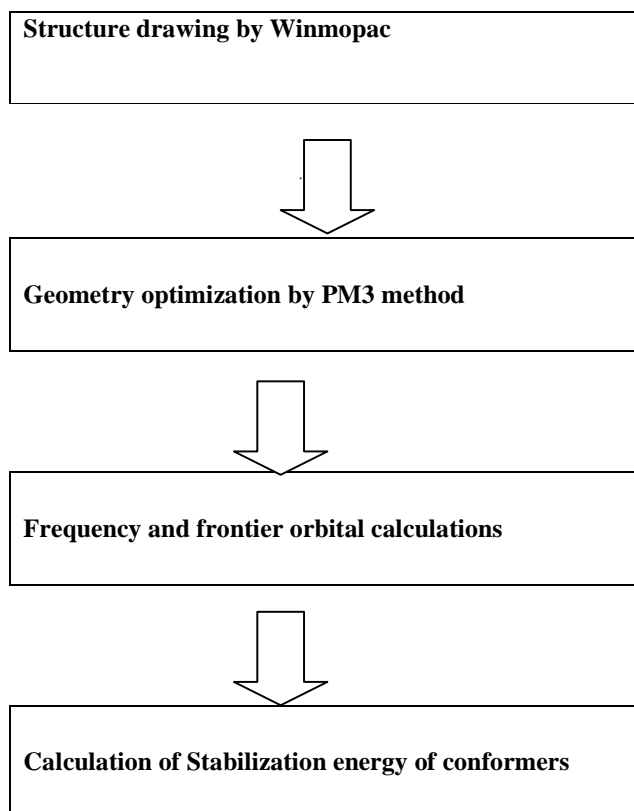
- Colour: White.
- State: Solid, Crystalline.
- Density: 1.263 g/ cc.
- Melting point: 169 °C.
- Solubility in water: 1.4 g/100ml at 20 °C.
- Solubility in organic solvents: soluble in methanol, ethanol, dimethylformamide, ethylene dichloride and insoluble in petroleum ether, pentane and benzene.
- Mol. Wt: 151.17 g/mol
- Bioavailability: almost 100%.
- Metabolism: 90 to 95 % Hepatic.
- Half-life: 1 to 4 hours.
- Excretion: Renal

Paracetamol is used to relieve mild to moderate pain, including instances of tension, headache, muscular aches, joint pain, general pain, and toothache. It is also used as an antipyretic that can reduce fever. It is suitable to most people including the elder and young children because it has low side effects. The recommended adult dose of Paracetamol is 500 mg tablets with four hours interval between two doses and not more than eight tablets recommended in 24 hours. There are no toxic effects even in prolonged or habitual use. It does not accumulate in the body, but overdose affects the liver leading to hepatotoxicity. While the precise mode of action of paracetamol is unclear, it is thought that like Aspirin, its effect is due to inhibition of prostaglandin synthesis. Paracetamol inhibits the enzyme cyclooxygenase responsible for the biosynthesis of prostaglandins. By reducing the synthesis of prostaglandin, paracetamol produces analgesia by elevating the pain threshold and reduces fever by resetting the hypothalamic heat-regulating center of the brain. Paracetamol is thought to act almost exclusively on the central nervous system with little peripheral effect. This explains why paracetamol even in higher doses, has limited anti-inflammatory effect and is associated with fewer gastrointestinal side effects.

The plasma half-life of paracetamol ranges from 1 to 4 hours. It is distributed throughout most fluids of the body and is metabolized primarily in the liver, and unmetabolized drug is excreted in the urine, but most metabolic products appear in the urine within 24 hours. Compared with other minor analgesics, such as aspirin, its onset of action is generally slower, since it is only absorbed once it has left the stomach and entered the duodenum.

EXPERIMENTAL SECTION

CD complexes were optimized by employing the semi-empirical PM3 method (parametric method 3). A parameterization scheme herein is based on the known data of heats of formation. It may be remarked here that the PM3 method yields good estimates of geometrical parameters of the stationary point geometries and relatively fast which make it useful for longer molecular systems containing, say 50 to few thousand atoms. GAUSSIAN 03 programs were used for obtaining the stationary point geometries. The geometries thus derived were confirmed to be local minima on the potential energy surface by calculating the vibrational frequencies. Since no vibrational frequency turns out to be imaginary these are confirmed to be local minima (one imaginary frequency implies a transition state and more than one imaginary frequencies lead to saddle point of order two or more on the potential energy surface.) A relative stabilization energies along with the free energy change associated with the inclusion phenomenon are reported for all the conformers of α , β and γ CDs. The results obtained using PM3 geometry optimization are reported as follows.

Computational Method**Calculations**

Stabilization energy of cyclodextrin complex with paracetamol is calculated as

$$1) \Delta E = E_{\text{complex}} - (E_{\text{cd}} + E_{\text{guest}}) * 2625.5 \text{ kJ mol}^{-1}$$

where, ΔE = stabilized energy.

E_{complex} = energy of complex.

E_{cd} = energy of host (cyclodextrin).

E_{guest} = energy of guest (paracetamol)

$$2) \Delta E_{\text{ref}} = E_{\text{most stable complex}} - E_{\text{complex}} * 2625.5 \text{ kJ mol}^{-1}$$

where, ΔE_{ref} = energy of complex with reference to most stable complex

Structure of complexes are given in Figure 4 and calculated energies of complexes are given in Table 2.

Table 2. energy of complex of α , β , and γ cyclodextrins with paracetamol

complex	ΔE , kJ mol ⁻¹	ΔE_{ref} , kJ mol ⁻¹	No of H-bonding	No of H-H interactions
Alpha 1	-66.7	0.00	1	3
Alpha 2	-64.88	02.6	1	4
Alpha 3	-54.4	11.03	2	2
Alpha 4	-52.0	13.1	1	4
Alpha 5	-53.1	13.1	1	3
Alpha 6	-43.8	23.6	0	3
Alpha 7	-39.1	26.3	0	3
Alpha 8	-39.4	39.4	0	3
Beta 1	-84.4	0.00	1	2
Beta 2	-80.1	05.3	1	2
Beta 3	-70.1	15.8	1	3
Beta 4	-64.4	21.0	0	3
Beta 5	-63.72	21.5	2	2
Beta 6	-61.5	23.6	1	3
Beta 7	-61.1	26.3	2	2
Beta 8	-55.6	31.5	0	1
Beta 9	-49.5	36.8	0	2
Beta 10	-50.10	36.8	0	3
Beta 11	+820.3	905.7	2	3
Gamma 1	-104.5	0.00	0	2
Gamma 2	-97.1	07.9	1	2
Gamma 3	-95.8	07.9	1	1
Gamma 4	-91.8	13.1	1	1
Gamma 5	-91.5	13.5	0	1
Gamma 6	-88.7	15.8	1	3
Gamma 7	-84.5	18.4	3	3
Gamma 8	-82.0	21.0	0	1
Gamma 9	-82.0	21.0	1	2
Gamma 10	-35.2	68.3	0	2

RESULTS AND DISCUSSION

The energy of the complex is less than the sum of energies of host (cyclodextrin) and guest (paracetamol).

2. Complex of α cyclodextrin with paracetamol:

The most stable complex is of energy -66.7 kJ mol⁻¹. The paracetamol molecule has width around 0.44 nm, and inner diameter of alpha cyclodextrin is about 0.57 nm. So paracetamol has to fix itself in guest straight up only. When methyl group is upward the hydrogen bonding may occur with three hydrogen atoms thus leading to more stability, the nitrogen lone pair and carbonyl oxygen may also interact with hydrogen of cyclodextrin. But, if hydroxyl group is upward then hydrogen bonding can occur only with one hydrogen and thus complex is not much stable. The Hydrogen – Hydrogen interactions are much strong in α complex than the hydrogen bonding. The extent of hydrogen bonding is less than the H – H interactions.

3. Complex of β cyclodextrin with paracetamol:

The most stable complex is of energy -84.4 kJ mol⁻¹. The inner diameter of beta cyclodextrin is 0.78 nm. Here the paracetamol molecule can be rotated and can also be kept slightly horizontal. The interaction can occur with primary as well as secondary hydroxyl group of cyclodextrin, leading to more stability. Thus, the position of hydroxyl or methyl group do not affect stability of complex. The extent of hydrogen bonding is greater in β complexes than H - H interactions.

4. Complex of γ cyclodextrin with paracetamol:

The most stable complex is of energy -104.5 kJ mol⁻¹. The inner diameter of gamma cyclodextrin is 0.95 nm. Here the paracetamol molecule can be rotated freely. The paracetamol entirely fits in to the of γ cyclodextrin, so the formation of hydrogen bonding and other interaction are easier thus leading to most stable complex of paracetamol. The position of hydroxyl or methyl group do not affect stability of complex. The of γ complex is stable due to balance of both H – bonding and H – H interactions.

CONCLUSION

- All the cyclodextrin i.e. (α , β & γ) forms stable inclusion complex with paracetamol.
- The γ cyclodextrin forms most stable complex while α forms least stable complex.
- The γ complexes are stable by 40 kJ mol^{-1} energy than α and by 20 kJ mol^{-1} energy than β .
- The β complexes are stable by 20 kJ mol^{-1} energy than α .
- The cavity of cyclodextrin and number of possible hydrogen bonding and other interactions determines the stability of the complex.
- The hydroxyl group of cyclodextrins and hetero atoms in guest are responsible for hydrogen bonding and complex stability.
- The position of methyl or hydroxyl group affects the α complex stability and not of β & γ complexes.
- The Hydrogen bonding as well as Hydrogen – Hydrogen interactions strongly govern the stability of complex.

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