CHAPTER I

INTRODUCTION

1.1 Fragrances / Flavors

Fragrance chemicals and flavor chemicals are usually one and the same. The use determines, whether they are referred to as a fragrance material or a flavoring material. Fragrance is not a single material of clearly defined properties but rather a mixture of individual chemicals. Important properties of fragrance chemicals included volatility, polarity, surface activity and stability (http://www.fragrance.htm, 2006).

1.1.1 Sensation of fragrances

Fragrances are added in consumer products to give the products a pleasant odor during use. Fragrances are found in cleaners, cosmetics, fabric softeners and detergents. The sensation of fragrance can be occurred through numerous routes. Skin contact and absorption occurs when fragranced products are applied. Ingestion is a common route and olfaction plays an important part in taste (http://www.fragrance.htm, 2006).

1.1.2 Classification of fragrances

The vast majority of fragrance materials in use now are either naturals or synthetics. Naturals are obtained from plants or animals as a source of essential oil and aroma compounds. These aromatics are usually secondary metabolites produced by plants as protection against herbivores, infection, as well as to attract pollinators (http://en.wikipedia.org/wiki/fragrance).

Plant sources :

The sources may be derived from various parts of a plant. A plant can offer more than one source of aromatics as follows (http://en.wikipedia.org/wiki/fragrance).

1. Flowers and blossoms
2. Leaves and twigs
3. Roots , rhizomes and bulbs
4. Seeds
5. Fruits
6. Woods
7. Bark

***Animal sources* :**

1. Musk
2. Civet
3. Castoreum
4. Ambergris
5. Honeycomb

Synthetics are usually derived from chemical reactions using crude oil or turpentine oil as the starting material. Synthetics are usually single molecules that are very different than the complex mixtures found in nature. Many synthetics are not found in nature at all. There are trace impurities leftover from the reaction process, and these impurities affect odor quality and safety of the synthetic material.

Fixolide

In this study, we are interested in fixolide, a synthetic fragrance which is one of the favorable fragrance in personal care and household products in Thailand and other countries. The chemical structure of fixolide, a naphthalene derivative, is as shown in Figure 1.

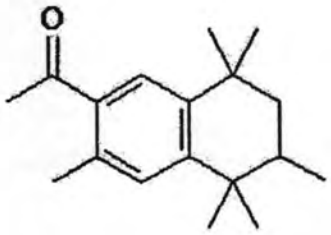

Figure 1. Structure of fixolide

Physical properties of fixolide (http://www.petitemarie.com)

Empirical formular	: $C_{18}H_{26}O$
Molecular weight	: 258
Chemical name	: 7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN)
Description	: A white to off-white, powerful , warm, radient or sweet fruity musk odor
Melting point	: > 54 ℃
Boiling point	: 180 ℃
Solubility	: 1.25 mg in 1 L of water

AHTN, commercially known as fixolide or tonalide, is a synthetic fragrance musk and known as the polycyclic musk that is used in place of more expensive natural musks. AHTN is produced in one plant in the Netherlands in an annual volume of 1000 to 5000 tons (Human and Environment Risk Assessment on Ingredient of Household Cleaning Products, 2006). It is a widely used ingredient of fragrance formulations added in soaps, detergents, fabric softeners, and other household products as well as in cosmetic and fined perfumes. Polycyclic musks are important ingredients for the fragrance industry not only because of their typical and unique smell, which determines the odor of a product to a great extent. The level of AHTN in such products is typically at a level of several percents (usually 0.1-12 %). The principal exposure to AHTN from household products can be considered to be via the skin.

AHTN is not a skin or eye irritant and shows no photoxicity potential on humans at concentrations significantly higher than would be encountered from the use of fragranced household products. AHTN is a non-genotoxic substance (Human and Environment Risk Assessment on Ingredient of Household Cleaning Products, 2006). In recent years, polycyclic musks have become the most important commercial synthetic musks due in part to concern about the environment distribution and toxicological effects which lead to subsequent reduction in use of the nitro musks (e.g. musk xylene and musk ketone). In 1996, the worldwide production of polycyclic musks, 95 % of which were AHTN and HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-haxamethylcyclopenta-γ-2-benzopyran) (Rimkus, 1999). AHTN is semivolatile and is degraded under light exposure and high temperature (Sanchez-Prado, 2004). Its water solubility at 25°C is only 1.25 mg/L.

1.2 Cyclodextrins

Cyclodextrins (CDs), also known as Schardinger dextrins, cycloamyloses and cycloglucans,comprise a family of cyclic oligosaccharides. CDs are formed by the action of the cyclodextrin glycosyltransferases (CGTase) on a solution containing starch. Because of the helical structure of the starch molecules, the primary product of the cleavage by the CGTase undergoes an intramolecular reaction and α-1,4-linked cyclic products are formed, called CDs (Bekers *et al.*, 1991) (Figure 2).

CDs are able to form inclusion complexes with various molecules, thus altering their physical/chemical properties. Three natural CDs are readily available: α-,β-, and γ-cyclodextrin (α-, β-, and γ-CD) formed by six , seven, and eight D-glucose units, respectively (Figure 3). CDs with fewer than six glucose residues are too strained to exist, whereas those with more than eight residues are difficult to isolate (Loftsson and Brewster, 1996; Connors, 1997). Of these large-ring CDs only δ-CD (containing nine glucose units) has been well characterized (Miyazawa, 1995). The major applications of CDs include its use in the field of pharmaceutics, cosmetics, textile, food and flavors by improving solubility and/or stability of various guest molecules (Szejtli, 1998).

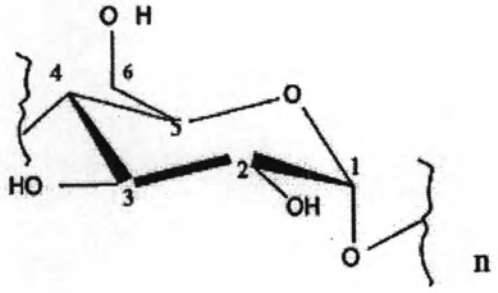

Figure 2. Cyclodextrin with n-glucopyranose units (n = 6, 7 or 8)

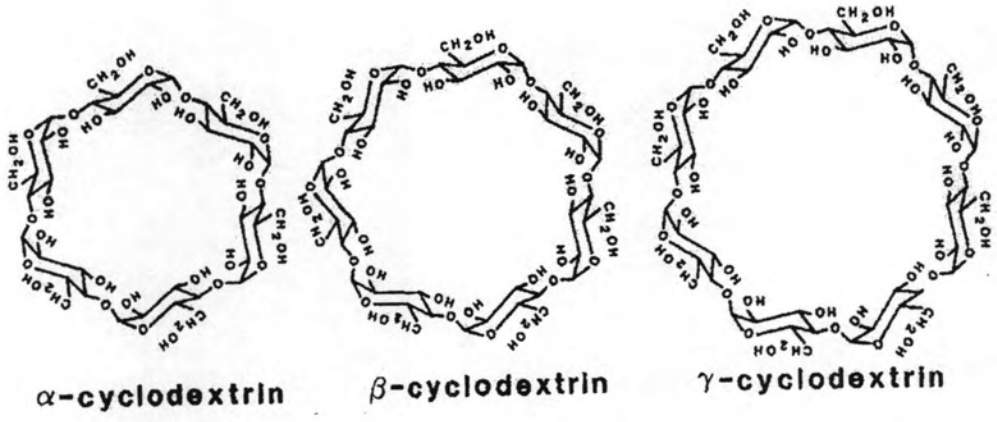

Figure 3. Structure of α-,β-, γ-cyclodextrin

1.2.1 Structure and Physicochemical Properties

The natural CDs are linked by α-1,4-glycosidic bonds, as a consequence of the $^4C_1$ conformation of the glucopyranose unit and the lack of free rotation around glycosidic bonds, the CDs are not perfectly cylindrical molecules but are toroidal or cone shaped. The secondary hydroxyl groups (on the C-2 and C-3 atoms of the glucose units) are situated on one edge of the ring and all primary hydroxyls on the other. The secondary hydroxyl side is wider than the primary hydroxyl side (Figure 4).

The cavity of the torus consists of a ring C3-H groups, C5-H groups and a ring of glucosidic oxygen. For this reason, the cavity of the torus is non-polar (compare to water). This makes CDs exterior decidedly hydrophilic whereas the interior of the cavity is rather hydrophobic. Free rotation of primary hydroxyls reduces the effective diameter of the cavity on the side they occur, while the secondary hydroxyl groups on the relative rigid chains cannot rotate (Szejtli, 1982; Bekers *et al.*, 1991).

The dimensions of the CDs alter with the number of glucose units. Because of their different internal cavity diameters, each CD shows a different capability of complex formation with guest molecules of different size and polarity. The complex formed is called the "inclusion complex" (Bekers *et al.*, 1991). Table 1 lists some of the important physicochemical properties of CDs and some of their derivatives.

1.2.2 Cyclodextrin Derivatives

From Table 1, the parent CDs, in particular β-CD has the lowest solubility in water compared with α-CD and γ-CD. The C2-OH group of one glucose unit can form a hydrogen bond with the C3-OH group of the adjacent glucose unit. In the β-CD molecule, these intramolecular hydrogen bondings are completely formed, which gives a hydrogen bond belt, that detracts from hydrogen bond formation with surrounding water molecules; this results in the least aqueous solubility. On the other hand, in the α-CD molecule, the hydrogen bond belt is incomplete because one

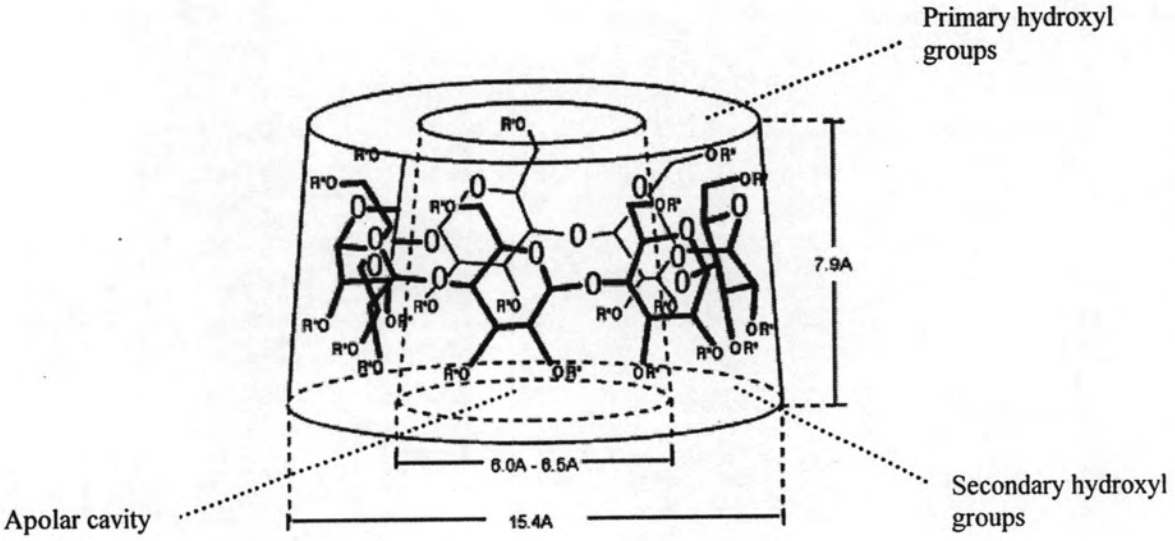

Figure 4. Functional structure scheme of β-cyclodextrin

Table 1. Physical properties of the CDs and some derivatives (Bekers *et al.*, 1991)

	α	β	γ	DM-β[1)]	HP-β[2)]
*Number of glucose residues:	6	7	8	7	7
*Cavity dimensions(A°)					
- Cavity diameter:	5	6	8	6	6
- Height of torus	7.9	7.9	7.9	10.0	
- Diameter of periphery:	14.6	15.4	17.5		
* Molecular weight:	973	1135	1297	1331	±1300
*Aqueous solubility [3)]	14.5	1.85	23.2	57	>50
* Melting point (°C):	275	280	275	295-300	
* pKa [4)] :	12.3	12.2	12.1		
* Half-life of ring opening [5)] (hr):	6.2	5.4	3.0	8.5	
* enzymatic Hydrolysis [6)]:	negligible	slow	rapid		

[1)] heptakis-2,6-di-O-methyl-β-CD

[2)] 2-hydroxypropyl-β-CD

[3)] in grams per 100 ml water at ambient temperature

[4)] pKa: by potentiometry at 25 ^{0}C

[5)] Half-life of ring opening: in 1 N HCl at 60 ^{0}C

[6)] by *Aspergillus oryzae* α-amylase

glucopyranose unit is in a distorted position. Consequently, instead of the six possible hydrogen bonds, only four can be established fully. The γ-CD is a non coplanar, more flexible structure, therefore it is the most soluble of the three CDs (Szejtli, 1988).

The natural CDs can be modified for many different purposes, for example, to improve the low aqueous solubility of β-CD or to decrease the toxicity in parenteral applications. The hydroxyl groups of CDs are available as starting points of structural modifications and various functional groups have been incorporated into the CD molecules (Figure 5). For example, alkylated CDs and hydroxyalkylated CDs are prepared to obtain better properties than natural CDs (Bekers et al., 1991). These derivatives usually are produced by aminations, esterifications, or etherifications of primary and secondary hydroxyl groups of the CDs. Virtually all derivatives have a changed hydrophobic cavity volume and also these modifications can improve solubility, stability against light or oxygen and help control the chemical activity of guest molecules. There are many types of derivatives as shown in Table 2.

Methylated Cyclodextrins

The methyl substituent to the parent β-CD improves the water solubility of the derivatives versus the parent β-CD. The aqueous solubility of methylated cyclodextrins increases as the number of methyl groups reaches around 13-14 (2/3 of all hydroxyls), and then decreases as methylation approaches 21 methoxy per β-CD molecule. For example, heptakis(2,6-di-*O*-methyl)-β-CD (DIMEB), heptakis(2,3,6-tri-*O*-methyl)- β-CD (TRIMEB) and randomly methylated-β-CD (RAMEB), see Table 2. A particular methylated β-CD, the randomly methylated-β-CD is the most appropriate form as commercially viable for drug formulation, it has a very good aqueous solubility, high binding capacities for most of poorly soluble drugs and available in constant quality of a reasonable price (Szente and Szejtli, 1999).

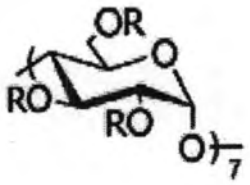

Figure 5. General structure of β-cyclodextrin and major derivatives

Table 2. Examples of cyclodextrins and their major derivatives with pharmaceutical and other applications

Cyclodextrin	Abbreviation	R	n
α-cyclodextrin	α-CD	H	6
β-cyclodextrin	β-CD	H	7
γ-cyclodextrin	γ-CD	H	8
6-O-Glycosyl-β-cyclodextrin	G-β-CD	Glucosyl or H	7
6-O-Maltosyl-β-cyclodextrin	G_2-β-CD	Maltosyl or H	7
Dimethylated-β-cyclodextrin	DIMEB	CH_3 or H	7
Trimethylated-β-cyclodextrin	TRIMEB	CH_3 or H	7
Randomlymethyl-β-cyclodextrin	RAMEB	CH_3 or H	7
2-Hydroxypropyl-β-cyclodextrin	HP-β-CD	$CH_2CHOHCH_3$ or H	7
Hydroxybutenyl-β-cyclodextrin	Hben-β-CD	$CH_2CHOHCHCH_3$ or H	7
Sulfobutylether-β-cyclodextrin	SBE-β-CD	$(CH_2)_4SO_3Na$ or H	7

n is the number of glucose units which comprise the cyclodextrin molecule.

The derivatives may have different degree of substitution of the 2nd, 3rd, or 6th position of the glucose units (www.cyclodex.com)

Hydroxyalkylated Cyclodextrins

The hydroxyalkylated cyclodextrins are amorphous material with high water solubility. They are prepared by a chemical reaction between reactive alkylene oxide and the free hydroxyl groups (C2, C3 and C6) on each glucose unit. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) is mostly utilized in the series of hydroxyalkylated-β-CD derivatives because of its high water solubility. HP-β-CD is prepared by condensation of β-CD with propylene oxide in a sodium hydroxide solution. In this reaction, hydroxyls of glucose residues, which are located on the outside of the torus, are etherified with 2-hydroxypropyl. The multicomponent character prevents any crystallization and thus makes it on average 27 times more soluble in water than β-CD (Szente and Szejtli, 1999). The extent of CD modification is given by the degree of substitution which is the number of substituents per molecule

1.2.3 Inclusion Complex Formation

Inclusion complexes are molecular compounds having the characteristic structure of an adduct, in which one of the compound (host molecule) spatially encloses another. The enclosed compound (guest molecule) is situated in the cavity of the host molecule without affecting the structure of the host (Szejtli, 1982).

Cyclodextrins are able to interact with a very wide range of compounds and the resulting inclusion compounds belong to the type of host-guest complexes. In these complexes (Figure 6), a guest molecule is held within the cavity of the cyclodextrin host molecule. Complex formation is a dimensional fit between host cavity and guest molecule. The hydrophobic cavity of cyclodextrin molecule provides a microenvironment into which appropriately size non-polar moieties can enter to form inclusion complexes. No covalent bonds are formed during formation of the inclusion complex and the binding of guest molecule within the host cyclodextrin is not fixed or permanent (Martin, 2004).

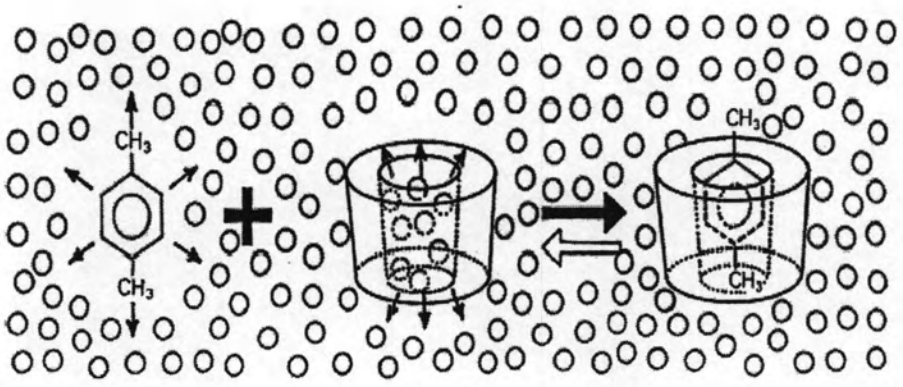

Figure 6. Schematic representation of the formation of cyclodextrin inclusion complexes

The minimum requirement for inclusion complex formation is that the guest molecule must fit, entirely, or at least partially into the cyclodextrin cavity. Stable complexes will not be formed with guest molecules which are too small because they will slip out the cavity. Complex formation is possible with molecules which are too bulky to penetrate into the cyclodextrin cavity if they possess certain groups or side chains of the bulky molecule which can penetrate into the cyclodextrin cavity (Szejtli, 1982).

The ability of a CDs to form an inclusion complex with guest molecule is a function of two key factors; the first is relative size of the CD to the size of the guest molecule, the second critical factor is the thermodynamic interactions between the different components of the system (CD, guest, solvent). For a complex to form, there must be a favourable net energetic driving force that pulls the guest into the CD (Martin, 2004).

1.2.4 Mechanism of Complex Formation

In general, native cyclodextrins can form inclusion complexes with differing amounts of water, if they do not contain another guest molecule there is usually at least one solvent molecule in their cavity. The complexes can be obtained both in solution and in the solid state. In solution, they exist in a rapidly exchanging equilibrium of the free cyclodextrin and guest molecule (Dodziuk, 2006).

The main driving force of complex formation is the release of enthalpy-rich water molecules from the cavity (the cavity-bound water molecules are at a higher energy, or in other words, they are "enthalpy-rich"). Water molecules in the cyclodextrin cavity cannot satisfy their hydrogen-bonding potentials, therefore they may be regarded as molecules of enhanced energy or enthalpy. The energy of the system is lowered when these enthalpy-rich water molecules are replaced by suitable guest molecules which are less polar than water. Although release of enthalpy-rich water from cyclodextrin cavity is probably an important driving force for guest-cyclodextrin complex formation, other forces may be important. These forces include

van der Waals interaction, hydrogen bonding, hydrophobic interaction and changes in solvent-surface tension (Loftsson and Brewster, 1996).

1.2.5 Determination of Inclusion Complex Stability Constants

Effects which can be obtained by cyclodextrin complex formation, such as enhancement of the solubility, bioavailability of a drug and volatilization of volatile compound, all depend on the stability and solubility of the complex. Consequently, the measurements of stability or equilibrium constants (K_c) or the dissociation constants (K_d) of the guest-CD complexes are important, since this is an index of changes in physicochemical properties of a compound upon inclusion (Martin, 2004).

Inclusion complex formation in solution is an equilibrium between complexed and non-complexed guest molecules. The general equation can be expressed as :

$$CD + Guest \Leftrightarrow CD\text{-}Guest$$

$$K_c = \frac{[CD-Guest]}{[CD][Guest]}$$

The stability constant (K_c) is better expressed as $K_{m:n}$ to indicate the stoichiometric ratio of the complex. It can be written :

$$m\,CD + n\,Guest \Leftrightarrow CD_mGuest_n$$

$$K_{m:n} = \frac{[CD_mGuest_n]}{[CD]^m[Guest]^n}$$

One of the most useful and widely applied analytical approaches in this context is the phase-solubility method described by Higuchi and Connors, 1965 (as mentioned in 1.4.1).

1.3 Preparation Methods of Inclusion Complexes

Various methods have been described for preparation of the inclusion complexes. However, each applied complexation method may give rise to differences in the complexation effectiveness. Types of guest and CD used and the mole ratio of guest-CD for complex formation are also important factors affecting the complexation effectiveness.

Generally, one guest molecule is included in one CD molecule, although in the case of some low molecular weight molecules, more than one guest molecule may fit into the cavity, and in the case of some high molecular weight molecules, more than one CD molecules may bind to the guest. In principle, only a portion of the molecule must fit into the cavity to form a complex. As a result, one-to-one mole ratio is not always achieved, especially with high or low molecular weight guests (Martin, 2004).

1.3.1 Co-precipitation (liquid medium*)*: CD is dissolved in water and the guest is added while stirring the CD solution. This solution is stirred for several hours or even days, until spontaneous precipitation of the inclusion is achieved. In some cases, the precipitation does not occur spontaneously, it is necessary to cool the medium at ambient or even lower temperature. The organic solvents can be used as a cosolvent. It may be better to work with a hot solution of cyclodextrin. After the precipitation step, a wash solvent and water, filtration and drying procedure is a pure solid dispersion. This method is the most widely used in the laboratory. It is performed with readily available equipment such as beaker, stirrer, and heat source. It has the advantage that the complex forming can be easily seen and the disappearance of the free guest was observed. However, the main disadvantage of this method is that it is not frequently used for large-scale formation of complex. Because of the size of

tanks required, wastewater disposal considerations and energy for heating and cooling may become important cost factors (Hedges, 1998).

1.3.2 Kneading: In this case even less water is used, and the CD is not dissolved, it is kneaded like a paste, either with a small amount of water to which the guest component has been added without a solvent, or a small amount of ethanol in which the guest has been suspended. The mixture is then thoroughly kneaded to obtain a paste and then dried. This method is very attractive due to its many advantages; the process is short, simple, inexpensive, solvent free, using a low temperature (useful for volatile or thermosensitive actives), low levels of water (useful in avoiding hydrolysis of some actives), excellent yield of solid dispersion, and no waste is produced (Alisara, 1999). There is the possibility of production on an industrial-scale, since various mixers such as an extruder, blade mixer or other kneading type of mixer can be used (Hedges, 1998).

1.3.3 Freeze-drying: This method can also be used for complex preparation when freeze the solution of cyclodextrin and guest at low temperature and dry under vacuum. Freeze-drying is carried out before any precipitation of the solid dispersion occurs. The main advantage of this process gives a product excellent solubility characteristics because the amorphous product is obtained. Therefore, most of freeze-dried products can be easily rehydrated. Heat and moisture-sensitive compounds retain their properties. This drying technique is less attractive than others because the costs of freeze drying are up to 50 times higher than spray drying and the storage and transport of particles produced is extremely expensive, the commercial applicability is also severely restricted by the long processing time (Desobry et al., 1997).

1.3.4 Spray drying: This method can also be used for complex preparation. Spray drying is a process describing the transformation of liquid feeds into dried particulate form by spray drying the feed into a hot drying. With volatile guests, some optimization of drying condition is required in order to reduce the losses. The advantage of this method is applicable to both heat sensitive and heat resistant materials. Another advantage of spray drying is the ability to control powder form

such as particle size, bulk density and moisture content. Disadvantages of spray drying are high cost both in equipment and operation, and poor thermal effectiveness when extremely high drying temperature is used and cause heat degradation (Dziezak, 1988). It is also not suitable for guests with very poor water solubility and has to solubilize in high percentage of alcohol or flammable solvents.

However, freeze drying and spray drying are possible to scale up since these methods were faster than the co-precipitation method, the yield of powder formed is higher and no waste product to process.

1.3.5 Physical mixing: The physical mixing or the dry mixing method involves mixing the cyclodextrin and the guest with no added water. The amount of mixing time required is variable and depends on the guest. This method is thus commonly used as the control condition for no or incomplete complexation at short time mixing of CDs with many guests. However, for some guest (e.g. lemon oil), this method may lead to complex formation. The main advantage is that no water need be added, unless a washing step is used. Its disadvantage is the risk of caking on scale-up, resulting in mixing not being sufficiently thorough leading to incomplete complexation, and with many guests, the length of time required (Martin, 2004).

1.4 Characterization of Inclusion Complex Formation

When the inclusion complexation of guest molecules into the CD cavity is formed, a guest molecule changes its physicochemical properties. These changes provide methods to detect whether guest molecules are really included in the CD cavity such as solubility technique, UV/VIS and FTIR spectroscopy, fluorescence, differential scanning calorimetry (DSC), thermogravimetry analysis (TGA), and nuclear magnetic resonance (NMR) (Bekers *et al.*, 1991). The inclusion complex formation can be detected in solution and in solid state.

1.4.1 Detection of inclusion of complex formation in solution

The most useful for detection of guest-CD complex in solution (soluble complex) is the phase solubility technique. A phase diagram is constructed by plotting, on the vertical axis, total molar concentration of substrate (guest molecule, S) found in the solution phase against the molar concentration of ligand or complexing agent (cyclodextrin, L) added to the system. The phase diagrams are observed to fall into two main classes: type A and type B diagrams.

The type A diagram, shown in Figure 7, is obtained when the complex formed is soluble and does not form a precipitate regardless of the amount of ligand added. This can be subdivided according to the nature of the obtained phase diagram. The A_L type, exhibiting a linear relationship between $[S]_t$ and $[L]_t$, is obtained when complexes are a first order dependence on $[L]_t$. The A_p type, showing a positive deviation from linearity, is obtained when the formed complexes contain more than one molecule of ligand. As the ligand concentration increases, the contribution of the higher order complexes increase. The remaining diagram, A_N type, exhibits a negative deviation which represents a decreasing dependence on ligand added at higher ligand concentrations. This may be explained on the basis of self-association of the ligand at high concentrations.

The B type diagram, shown in Figure 8, results when the system develops the third phase consisting of the complex. If the complex exhibits some solubility, the diagram shows an initial rise in $[S]_t$ and the diagram is said to be a B_S diagram. If the complex is significantly soluble relative to the inherent solubility of the substrate, the system gives rise to the B_I diagram.

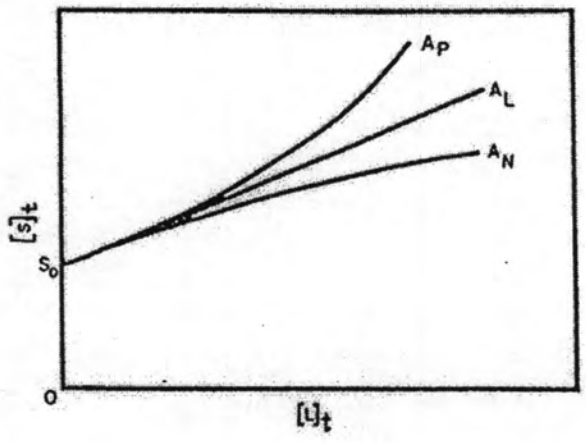

Figure 7. Phase solubility diagram of type A system

$[S]_t$ = the total molar concentration of dissolved substrate and

$[L]_t$ = the total molar concentration of ligand

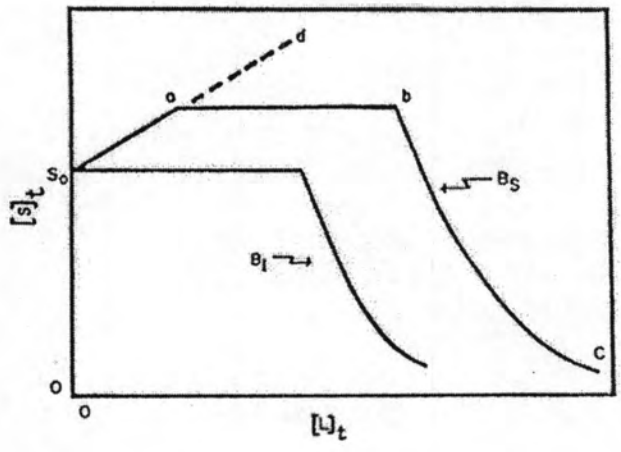

Figure 8. Phase solubility diagram of type B system

$[S]_t$ = the total molar concentration of dissolved substrate and

$[L]_t$ = the total molar concentration of ligand

source : *Adv. Anal.Chem. Instr.* 1965, 4:117-212.

1.4.2 Detection of inclusion of complex formation in solid state

Several methods can be applied for detection of the interaction of guest and CD molecules. Some useful techniques are discussed in this study.

- ***Differential Scanning Calorimetry (DSC)***

Thermoanalytical method, mainly DSC, is suitable to determine whether a particular product is a true complex. DSC is the measurement of the rate of heat evolved or absorbed by the sample, during a temperature program. When the guest molecules are included in the cyclodextrin cavity, the melting peak of the guest molecule reduced or disappeared, or shifted to a higher temperature before the thermic degradation of cyclodextrin (Szente and Szejtli, 1988).

- ***Fourier Transform Infrared Spectrometry (FTIR)***

IR spectroscopic technique contains informations about the vibrations of function groups in a solid state and is often site-specific in nature. This technique is commonly used for the characterization of solid substances. However, it is not generally suitable for detection of the inclusion complexes, since the CD bands change only slightly upon complex formation, and the fraction of the guest molecule contained in the complex is less than 25 %. Bands which can be assigned to the guest molecule are easily masked by the bands of the CD. However, with some guests, the shifts of absorbance bands, the changes in intensities and band widths could be observed (Szente and Szejtli, 1988).

1.5 Cyclodextrin in Flavor and Fragrance

The flavors and fragrances are used in several products. Many components of flavor and fragrance concentrates exhibit considerable sensitivity to air, light, or heat, which are difficult to work with in some applications. To overcome these disadvantages, CDs have been tested for use as flavor encapsulating agent. CD complexation represents a special way of encapsulation: entrapment of flavors/or fragrances, which is called "molecular encapsulation". In this process, every flavor constituent is surrounded by a CD ring which offers an almost perfect protection against damaging effects of the environment (Helena et al., 1995).

Several benefits to obtain by complexation with cyclodextrin can be summarized as follows (Hedges, 1998; Szejtli, 1982; Szente, and Szejtli, 1988):

1. CD can be used to stabilize compounds. The molecular entrapment of flavors reduces their equilibrium vapor pressure through binding with the cavity of a CD. The apolar-apolar interaction between the internal wall of the CD and the guest molecule results in remarkable heat resistence of volatiles, increase shelf-life and reduce loss of active ingredients owing to degradation or evaporation.

2. Compounds can be complexed with CDs to reduce their volatility. Interaction of the guest with the CD produces a higher energy barrier to be overcome to volatilize.

3. Fragrance and flavor are volatile substance, its easily volatile and/or labile compounds. The molecular encapsulation can be delayed and controlled release of compound by complexation with cyclodextrin. When the complexes are wetted or rewetted, a small amount of fragrance can escape from the complexes as a result of the equilibrium between the free and complexed states of the fragrance.

4. Cyclodextrin can increase the solubility of compounds which are pooly soluble in water. When the compound is surrounded by the molecule of cyclodextrin

as a result, the outer surface of the cyclodextrin contributes to the solubility of the complex.

5. The liquid compounds can be converted into the solid powder with a crystallized form or an amorphous form. It is the non-hygroscopic powder, which can facilitate mixing, storage, and transport.

Numerous published researches on the applications of cyclodextrin to fragrance or flavor were shown for improvement of these guests; e.g. stability and controlled-release. Examples will be mentioned in order to illustrate these applications.

The L-menthol which was added in food formulations was lost during the spray drying of liquid food materials, thus Liu *et al.* (2000) studied on application of β-cyclodextrin. They found that the retention of L-menthol increased with increasing the amount of β-CD. In 1986 Szente and Szejtli found that several hundred aroma components of coffee bean are chemically unstable during the preparation of coffee extracts, thus inclusion in cyclodextrin could be used to decrease a volatility and also covered the unpleasant bitterness of coffee drinks. In case of the preparation of food with high temperature, the fragrances such as benzaldehyde, citral, and vanillin were included with β-CD that gave the retention of these fragrances more than the liquid fragrance as a comparison (Reineccius *et al.*, 2004). Basquin *et al.* (1972) studied the peppermint-cyclodextrin complex, it can be easier blended with chewing gum than the liquid aroma. In this form, the peppermint remains longer in the chewing gum. The preparation of cosmetics and personal care products are another area which demands cyclodextrin use, mainly in volatily suppression of perfumes by stabilization, odor control, conversion of a liquid ingredient to a solid form and controlled release of fragrance from inclusion compound (Martin, 2004). Numanoglu (2005) reported that HP-βCD could be successfully used to control release of linalool and benzyl acetate in gel based moisturizing formulation. In addition to, the stability of these compounds can be increased by means of complexation. The possible irritant effect of fragrance such as eye irritation caused by the scent in a shampoo, can be reduced by using β-CD

(Szejtli, 1988). Wang *et al* (2005) found that the application of lavender oil was the most difficult task in textile aromatherapy. βCD was used to help improve by mixing with a lavender oil and fixed onto cotton. It was shown that inclusion compound could be formed. Cyclodextrin could transfer the aroma successfully to the cotton fabric and pose no skin irritation, skin sensibilization, and the rate of fragrance release decreased with time. The sustained-released of *d*-camphor and 3-*l*-methoxypropane 1,2-diol by 2-hydroxypropylated cyclodextrins (HP-βCD, HP-αCD, and HP-γCD) could be studied with circular dichroism and NMR spectroscopic method. Tanaka *et al* (1996) found that the release rate of both fragrance in three types of HP-CD decreased. Nevertheless, HP-βCD appeared to be the best.

The application of CDs can lead to certain benefits that result from the complexation. These include alteration of the solubility of fragrance, stabilization against the effects of light, heat and oxidation, reduction of volatility and others. To overcome the aforementioned problems of fixolide, a fragrance widely used in fabric softener, the formation of inclusion complex with CDs can be a feasible approach. In this research, βCD and derivatives (methyl- and hydroxypropyl-) are used in the study of complex formation. Inclusion complex in solid form will be prepared by different methods (co-precipitation, kneading and freeze-drying methods) with the aim of developing a formulation suitable for fixolide. To confirm the formation of true inclusion complex, DSC and FTIR will be chosen in this section. The properties of inclusion complexes in solid state such as thermostability, UV-stability and dissolution will be evaluated by comparison with a free form of fixolide. In addition, the application of solid complex for fabric softener will be also assessed.

1.6 The objective of this thesis

The aims of the present study are:

1. To investigate the ability of βCD and their derivatives in forming the inclusion complex with fixolide.
2. To determine the suitable method for preparation of inclusion complexes in solid state.
3. To study the thermostability, UV stability and dissolubility of the inclusion complexes.
4. To determine the degradation kinetics of fixolide and its inclusion complex.