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Physicochemical Characterization of Safranal-β-Cyclodextrin Inclusion Complexes Prepared By Supercritical Carbon Dioxide and Conventional Methods.

Sanaz Abbaszadegan

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PHYSICOCHEMICAL CHARACTERIZATION OF SAFRANAL-β-CYCLODEXTRIN INCLUSION COMPLEXES PREPARED BY SUPERCRITICAL CARBON DIOXIDE AND CONVENTIONAL METHODS

Sanaz Abbaszadegan

This thesis is submitted in partial fulfillment of the requirements for the Master of Science in Chemical Engineering degree
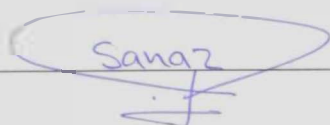
Under the direction of Dr. Ali H. Al-Marzouqi

September 2013

DECLARATION OF ORIGINAL WORK

I, Sanaz Abbaszadegan, the undersigned, a graduate student at the United Arab Emirates University (UAEU) and the author of the thesis titled "Physicochemical Characterization of Safranal- β -Cyclodextrin Inclusion Complexes Prepared by Supercritical Carbon Dioxide and Conventional Methods" hereby solemnly declare that this thesis is an original work done and prepared by me under the guidance of Dr. Ali H. Al-Marzouqi, in the College of Engineering at UAEU. This work has not been previously formed as the basis for the award of any degree/diploma or similar title at this or any other university. The materials borrowed from other sources and included in my thesis have been properly acknowledged.

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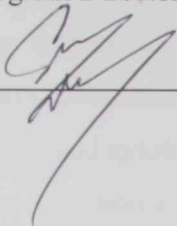
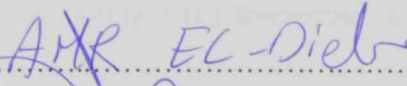   Department .... _Chemical Engineering_ ....

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ABSTRACT

Saffron (*Crocus sativus.* Linn) has attracted much attention over the last decade because it has a large number of potent and biologically active compounds such as crocin, crocetin, picrocrocin and safranal. Researchers have shown that safranal has high antioxidant and cytotoxicity activities against several types of tumour cells (e.g., hepatocellular carcinoma) both *in-vitro* and *in-vivo*. However, the low aqueous solubility of safranal prevents using it as a therapeutic or preventive agent.

β-cyclodextrin (β-CD) inclusion complexes are being used in pharmaceutical applications to alter physicochemical properties (e.g., solubility, volatility, stability, chemical reactivity, and bio-availability) of poorly water soluble drugs. Thus the aim of this work is to investigate the potential of different methods for the preparation of safranal-β-CD inclusion complexes in order to enhance the poor solubility and dissolution rate of safranal in aqueous solutions.

Inclusion complexes having a molar ratio of 1:2 (safranal-β-CD) were prepared using different methods such as kneading (KN), co-evaporation (COE), sealed-heating (SH), and supercritical CO_2 (SC-CO_2). Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and proton nuclear magnetic resonance spectroscopy (H-NMR) were used to identify the physicochemical properties of inclusion complexes. Phase solubility and dissolution measurements were also studied. Effects of temperature (35 and 55°C) and pressure (100 and 300 bar) on the inclusion complexes prepared using the SC-CO_2 method were also investigated.

The results indicated that the formation of safranal-β-CD inclusion complexes was affected by the preparation method being used, and the SC-CO_2 method proved to be more effective than conventional techniques. FT-IR and H-NMR results indicated the formation of inclusion between β-CD and safranal in the complex prepared by different methods. A" B_s" type solubility with an apparent solubility constant (K_s) of 51.48 M^{-1} for safranal was obtained from the initial slope of phase solubility diagram. The intrinsic solubility of safranal was increased from 3.852 mM to 5.217 mM in the presence of 10.00 mM of β-CD. Therefore, the initial phase solubility of safranal was enhanced by about 35% in water solution. Dissolution rate studies showed that inclusion complexes might dissolve faster than pure safranal or even physical mixture

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of safranal-β-CD. Hence, β-CD could be useful for solid safranal formulations. The solvent-free product prepared by SC-CO$_2$ showed high aqueous solubility and may provide minimal side effects for human use.

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Last but not least, I would like to express my greatest and deepest sincere appreciation to my devoting family members who were always encouraging me to complete my M.Sc. degree prosperously.

DEDICATION

My thesis is dedicated to my beloved parents, who nurtured my educational interest and dreams to look forward. Without their love, none of this would have happened.

To my affable husband, *Iman*, for his remarkable patience, unwavering love, and support over my postgraduate course.

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LIST OF ABBREVIATIONS/NOMENCLATURE/SYMBOLS

B_s	A type of solubility
S_0	Initial substrate concentration, mg/L
CD	Cyclodextrin
COE	Co-evaporation
D_2O	Deuterium oxide
K_{ow}	Octanol-Water Partition Coefficient, M^{-1}
K_s	Apparent solubility constant, M^{-1}
KN	Kneading
P	Pressure, bar
P_c	Critical pressure, bar
PM	Physical mixture
ppm	Parts per million
$SC\text{-}CO_2$	Supercritical carbon dioxide
SCF	Supercritical fluid
SFE	Supercritical extraction
SH	Sealed-heating
T	Temperature, ^oC
T_c	Critical temperature, ^oC
δ	Singlet signal, ppm
$\Delta\delta$	Signal changes, ppm

1 INTRODUCTION

1.1 Overview

Phytochemical compounds represent good sources for developing therapeutic agents because of their limited toxicity. Safranal, the most potent bioactive compound in saffron, has drawn a considerable number of scientific studies to investigate its biological-pharmaceutical properties. Safranal has shown to have high antioxidant activity and high cytotoxicity against several cancer cells *in-vitro* and *in-vivo*. However, the low aqueous solubility of safranal has limited its use as a therapeutic or preventive agent. Therefore, the purpose of this study was to improve safranal physicochemical properties by forming inclusion complexes with β-cyclodextrin (β-CD) using the supercritical carbon dioxide technique and to compare with those similar ones of safranal-β-CD complexes prepared by conventional methods (kneading, sealed-heating and co-evaporation). The effect of experimental operating conditions used in the SC-CO_2 method (temperature and pressure) on the prepared inclusion complexes has also been evaluated. The solubility constant was determined by Higuchi and Connors method and calculated from the phase solubility diagram. The physicochemical properties of the solid complexes prepared by different methods were determined by Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), scanning electron microscope (SEM) and proton nuclear magnetic resonance (H-NMR) spectroscopy and compared with those of the conventional methods. The dissolution properties of the various inclusion complexes were evaluated according to the dispersed amount method. A brief summary of thesis's organization is provided in this section. There are four chapters besides the abstract.

Chapter one, INTRODUCTION, includes: Overview, Statement of the Problem, Relevant Literature and Potential Contributions and Limitations of the Study. Promising effects of phytochemical compounds like safranal and their limitations as therapeutic agents and potential solutions by forming inclusion complexes between drug and cyclodextrins to enhance physicochemical properties of drugs are mentioned. In addition, the main reason for utilizing the recent novel technology (supercritical CO_2) to form drug-β-cyclodextrin inclusion complex is presented. Relevant literature shows a comprehensive study of the previous research works done

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on safranal, β-CD and different inclusion complex formation methods, with emphasis on supercritical CO_2 preparation method.

Chapter two, METHODS, highlights the materials and techniques used in this research. This includes a complete explanation of used chemicals and applied experimental methods for sample preparation and analytical methods to characterize the products.

Chapter three, RESULTS AND DISCUSSION, is dedicated to the discussion of the most important generated results. Firstly, the influence of different preparation methods such as kneading (KN), co-evaporation (COE), sealed-heating (SH) and supercritical CO_2 (SC-CO_2) on the formation of safranal-β-CD inclusion complex are discussed using results obtained by analytical techniques, including Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and proton nuclear magnetic resonance (H-NMR) spectroscopy. Secondly, the results obtained from the phase solubility and dissolution measurements for different preparation methods are discussed.

Chapter four, CONCLUSION, presents the conclusions of this study and the scope of the future research works.

1.2   Statement of the Problem

Safranal is a flavouring and aromatic chemical with high antioxidant and cytotoxic properties that has potential effect against specific type of cancer cells. However, the low aqueous solubility of safranal prevents using it as a therapeutic or preventive agent. The water solubility of safranal at 25°C, estimated from Log K_{ow} (Octanol-Water Partition Coefficient), is about 134.2 mg/L. Safranal is highly soluble in methanol, ethanol, petroleum ether and glacial acetic acid.

Cyclodextrins are a set of cyclic oligosaccharides composed of six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) subunits of α- (1,4) linked glucopyranose. Inclusion complexes are formed by "host-guest" intramolecular interactions resulting in significantly modified properties of the guest molecules. Inclusion compounds have different physicochemical properties, such as altered solubility, reduced volatility, reduced/enhanced stability, modified chemical reactivity and altered bioavailability as compared to the free molecule. Cyclodextrins also have

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negligible cytotoxic effects; therefore, they are widely used in pharmaceutical applications as a vehicle for drug delivery of poorly water-soluble drugs.

Conventional methods for preparing inclusion complexes between cyclodextrins and drugs include kneading (KN), high-energy co-grinding (GR), co-evaporation (COE), freeze-drying, sealed-heating (SH) and spray drying (SD). To evaluate the most satisfactory method for a specific drug–cyclodextrin system, several factors such as good yield, simplicity, rapidity, ease of scaling up, low cost and performance of the obtained product should be carefully considered. In fact, it has been verified that the preparation method can significantly influence the characteristics of the end products.

A more recently utilized method to form inclusion complexes between cyclodextrins and drugs is the use of supercritical fluids. In order to enhance aqueous solubility and dissolution rate of various drugs, a number of studies have used supercritical carbon dioxide to form inclusion complexes between drugs and cyclodextrins and compared to the results with the inclusion complexes prepared by conventional methods. While the supercritical fluid extraction (SFE) method was conducted as a non-destructive method for isolating safranal as the major volatile component of saffron, supercritical fluids can also be used for inclusion formation between safranal and β-cyclodextrin (β-CD). Supercritical fluids method allows the elimination of organic solvents and the expensive post-processing of the extracts for solvent removal. The use of carbon dioxide as supercritical fluid has the additional advantages of being non-toxic, non-flammable, inert, cheap and easily removable from the product by decompression of the system.

The principle objectives of this research are:

- To investigate the effects of different preparation methods on the formation of safranal-β-CD inclusion complexes.
- To test the solubility and dissolution rate of safranal-β-CD inclusion complexes prepared by different methods.

To achieve the first objective, prepared inclusion complexes were characterized by various analytical techniques, such as Fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and proton nuclear magnetic resonance (H-NMR) spectroscopy. To gain the second goal, phase solubility based on Higuchi-Connors method and dissolution measurements

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were studied. The effects of temperature and pressure on the properties of complexes prepared by SC-CO$_2$ method were also studied.

1.3 Relevant Litrature

This section summarizes the previous relevant works about safranal as a potent bioactive component, β-cyclodextrin as a media for improving the properties of the drug, and several techniques that have been utilized in the formation of the inclusion complex, such as kneading (KN), co-evaporation (COE), sealed heating (SH), and supercritical carbon dioxide (SC-CO$_2$). The first part discusses the drug of interest (safranal) with its properties (e.g. poor solubility in water), uses, and production methods. The second part covers the properties, toxicological considerations, inclusion complex formation, complexation techniques, and applications of cyclodextrins (CDs) with special focus on β-cyclodextrin and its abilities to improve the solubility of pharmaceutical substances. The third part discusses the applications of supercritical fluids (SCFs) in the pharmaceutical field focusing on the inclusion complex formation between drug and CDs using supercritical carbon dioxide, and providing a critical review of the recent advances in comparison with conventional methods.

1.3.1 SAFRANAL

1.3.1.1 Background

Saffron, a member of Iridaceae (iris) family, scientifically known as "*Crocus. Sativus, Linn*" has four crucial bioactive compounds namely, crocin, crocetin, picrocrocin, and safranal. Crocin (mono-glycosyl or di-glycosylpolyene esters) constitutes about 6-16% of saffron's dry matter with high aqueous solubility. Crocetin is a natural carotenoid dicarboxylic acid precursor of crocin. Picrocrocin (mono-terpene glycoside precursor of safranal and product of zeaxanthin degradation) is responsible for the actual taste of saffron (1%-13% of saffron's dry matter). Safranal, a cyclical terpenic aldehyde, represents approximately 30-70% of the essential oil and 0.001-0.006% of dry matter, subscribing not only to the sensory profile of saffron (color, taste, and aroma), but also to its health-promoting properties. In fact, safranal is the main substance responsible for the bitter taste and the actual color of saffron (Alonso et al., 1996; Melnyk et al., 2010; Tarantilis & Polissiou, 1997).

1.3.1.2 Physicochemical Properties of Safranal

Safranal (2, 6, 6-trimethyl-1, 3-cyclohexadien-1-carboxaldehyde or $C_{10}H_{14}O$) (Figure 1) has shown to have high antioxidant potential as well as cytotoxicity towards certain cancer cells in vitro. However, the low aqueous solubility of safranal prevents using it as a therapeutic or preventive agent. The water solubility of safranal at 25°C, estimated from Log K_{ow} (Octanol-Water Partition Coefficient), is about 134.2 mg/L. Safranal is highly soluble in methanol, ethanol, petroleum ether and glacial acetic acid (Melnyk et al., 2010; Rezaee & Hosseinzadeh, 2012).
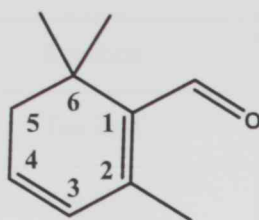
Figure 1. Safranal chemical structure

Some physical and chemical properties of safranal based on *Sigma Aldrich Safety Data Sheet* are summarized in Table 1 (Aldrich, 2012). Safranal is a clear yellowish liquid with a low molecular weight of 150.22 (g/mole). It's density at 25 °C is approximately 1 (0.9734 g/cm^3). It has a boiling point of 70 °C at 1 mmHg, which means it has a very high boiling point (T_b) at atmospheric pressure like other oily liquids. Safranal is very soluble in methanol, ethanol, petroleum ether and glacial acetic acid.

Table 1. Physical and chemical properties of safranal

Appearance	Clear, liquid, yellow
Molecular weight	150.22 (g/mole)
Density	0.9734 (g/cm^3) at 25 °C
Boiling point	70 °C at 1 mmHg
Flash point	86 °C
Reflective index	1.5210-1.5300
Soluble in:	Methanol, ethanol, petroleum ether, glacial acetic acid

1.3.1.3   Natural Sources of Safranal

As shown in Table 2, safranal could be detected in several botanical sources such as saffron, rooibos, tea leaf, fig leaf, wolfberry and cumin seed. It is believed that the geographical origins can make a great difference in the safranal content of saffron (Maggi et al., 2011).

Table 2. Natural sources of safranal

Scientific name	Common name	Growing location
Crocus stivus	Saffron	Greece, Southwest Asia
Aspalathuslinearis	Rooibos	South Africa
Camellia sinensis	Tea leaf	East, South and Southeast Asia
Ficuscarica	Fig leaf	Middle East, West Asia
Lyciumchinense	Wolfberry	China
Cuminumcyminum	Cumin seed	East Mediterranean to India

1.3.1.4   Safranal Extraction

Several laboratory techniques have been used for the extraction of volatile compounds including safranal from saffron. These methods include microsimultaneous hydrodistillation extraction (Kanakis et al., 2004), supercritical fluid extraction (Lozano et al., 2000), liquid extraction with organic solvents (Caballero-Ortega et al., 2007; Li et al., 1999; Lozano et al., 1999; Tarantilis et al., 1995), ultrasound-assisted extraction (Kanakis et al., 2004; Maggi et al., 2011). It is believed that greater hydrophobic extracting solvents would result in better safranal extraction (Lozano et al., 2000).

1.3.1.5   Safranal Pharmacological and Biological Effects

Various scientific studies have been performed to investigate biological-pharmaceutical effects of safranal. The potential effects of safranal include its flavouring and odorant property (Toro-Sánchez et al., 2006), antioxidant property (Assimopoulou et al., 2005), and protective property against gastric ulcers

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(Kianbakhsh & Mozaffari, 2009) and cancer cells (Escribano et al., 1996). These promising properties make safranal as a potential therapeutic agent. However, a great deal of clinical trials and toxicological studies are still needed to approve safranal as a drug. Major biological effects of safranal have extensively been studied. The effects of aqueous or ethanolic extracts of safranal on the human central nervous system (CNS) such as its antidepressant effects (Hosseinzadeh et al., 2004), protective effects (Hosseinzadeh & Sadeghnia, 2005), and effects on memory (Hosseinzadeh & Ziaei, 2006) have been studied. Safranal has also shown antidiabetic (Kianbakhsh & Hajiaghaee, 2011), anti-hypertensive (Imenshahidi & Hosseinzadeh, 2010), antimicrobial (Oroojalian et al., 2010; Pintado et al., 2011), and antioxidant (Hoseeinzadeh et al., 2009; Assimopoulou et al., 2005) effects. In addition, safranal has shown to have sunscreen properties (Golmohammadzadeh at al., 2011). Pathological assessment of heart, liver and spleen, showed no abnormal effects due to the use of safranal, but histological evaluations showed abnormalities and toxic effects of safranal, especially at the dose of 0.5 ml/kg or higher in kidney and lung (Hosseinzadeh et al., 2011).

1.3.2 CYCLODEXTRINS

1.3.2.1 Background

Cyclodextrins (CDs) were first discovered by Villiers in 1891 (Villiers, 1891). Very small amounts of crystalline materials (about 3 g/kg of starch) were formed from starch digest by *Bacillus amylobacter* and were named as 'cellulosine'. In 1930, Schardinger (Eastburn & Tao, 1994) succeeded in isolating two crystalline products, namely dextrins A and B, according to their lack of reducing power. In 1931, the newly crystalline products renamed as 'crystallised dextrin α' and 'crystallised dextrin β', which were produced in large amounts (25-30%) from starch by *Bacillus maceransstrain. γ* dextrin was isolated in 1935. X-ray crystallography enabled the scientists to determine the chemical structures of α and β-cyclodextrin in the early 1940s (Buschmann & Schollmeyer, 2002). In 1948, the X-ray structure of γ-cyclodextrin showed that the CDs could form inclusion complexes (Loftsson & Brewster, 1996). Gradually, it was proved that cyclodextrins exhibit a strong ability to form inclusion complexes with several compounds (Loftsson & Brewster, 1996). As a result of an apolar cavity providing a hydrophobic matrix, cyclodextrins are able to

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form inclusion complexes with a wide variety of hydrophobic guest molecules (Szetjil, 1998). One or two guest molecules can be entrapped by one, two or three cyclodextrins (Hirayama & Uekama, 1999).

1.3.2.2 Properties of Cyclodextrins

Cyclodextrins are also known as cycloamyloses, cyclomaltoses and schardingerdextrins (Eastburn & Tao, 1994; Szetjil, 1998). In general, cyclodextrins are produced from degradation of starch by cyclodextrin-glucano-transferase (CGTase) enzyme (Eastburn & Tao, 1994; Szetjil, 1998). Six, seven and eight α-(1,4)-linked glycosyl units constitute α-, β- and γ-cyclodextrins, respectively (Figure 2). β-cyclodextrin (Figure 3) is the most available, least expensive and on average the most useful (Dass & Jessup, 2000). The main properties of cyclodextrins including their molecular weight, solubility, and dimensions are mentioned in Table 3.
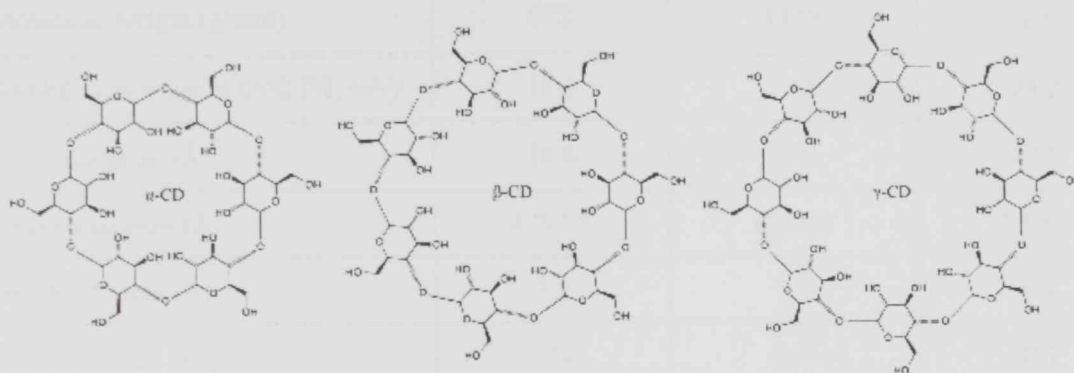
Figure 2. Chemical structure of α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin
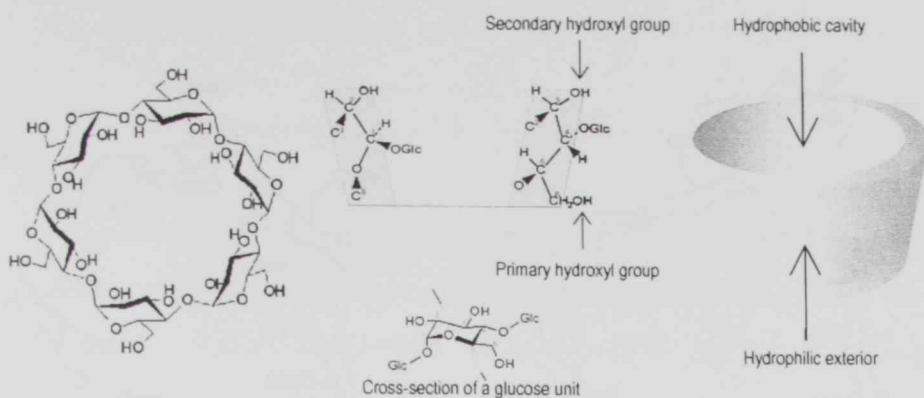
Figure 3. Chemical structure of β-cyclodextrin and inclusion complex formation

Table 3. Properties of cyclodextrins (Del Valle, 2004)

Property	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Number of glucopyranose units	6	7	8
Molecular weight (g/mol)	972	1135	1297
Solubility in water at 25°C (%, w/v)	14.5	1.85	23.2
Outer diameter (Å)	14.6	15.4	17.5
Cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
Height of torus (Å)	7.9	7.9	7.9
Cavity volume (Å^3)	174	262	427

Several cyclodextrin derivatives such as methyl-β-cyclodextrin (M-β-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD), and 2-hydroxylpropyl-β-cyclodextrin (HP-β-CD) (Figure 4) could be synthesized by aminations, esterifications or etherifications of primary and secondary hydroxyl groups of cyclodextrins. These modifications could improve the solubility and stability against the light or oxygen effects, as well as assist in controlling the chemical activity of the guest molecules by making significant changes in hydrophobic cavity and derivatives volume. Depending on the substituent, the solubility of the cyclodextrin derivatives is usually different from that of their parent cyclodextrins (Eastburn & Tao, 1994).

(a)   (b)

(c)
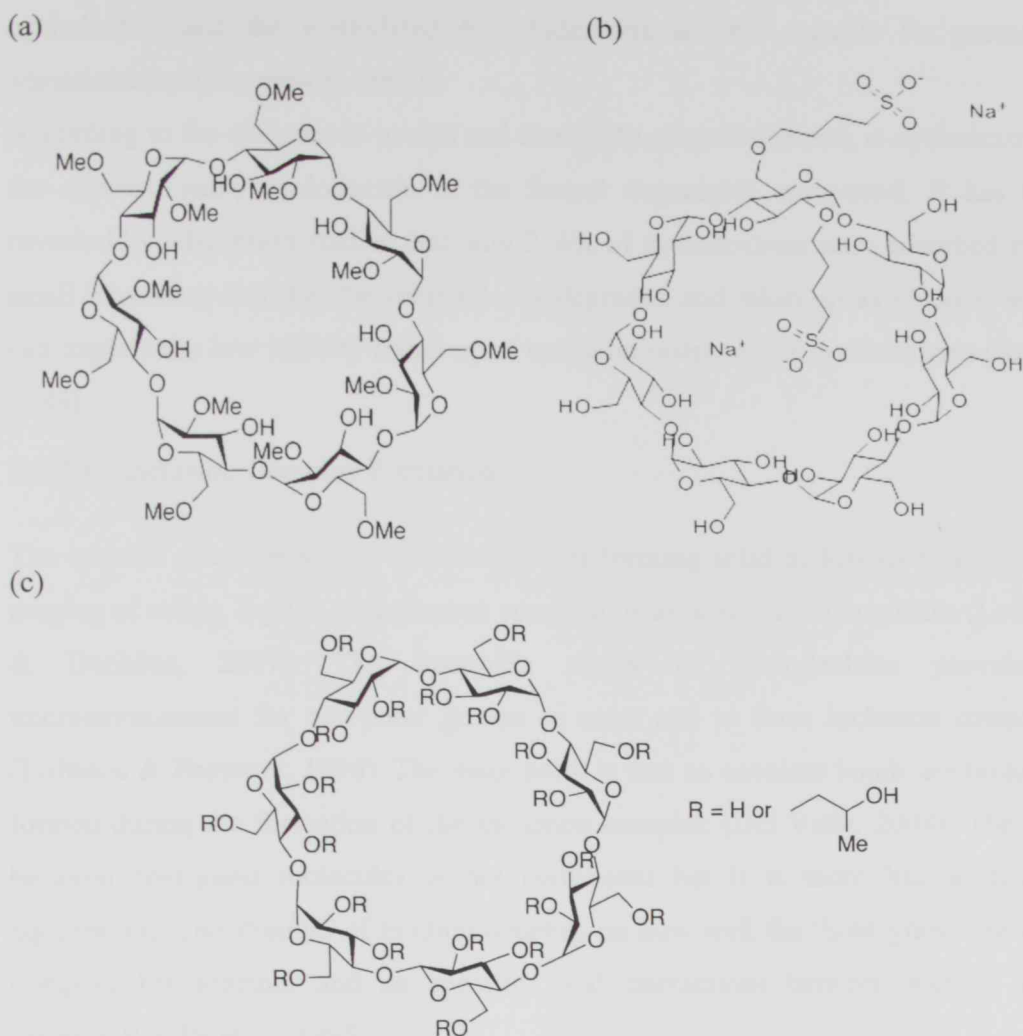
Figure 4. β-cyclodextrin derivatives: methyl-β-cyclodextrin (a), sulfobutylether-β-
cyclodextrin (b), and 2-hydroxylpropyl-β-cyclodextrin (c).

1.3.2.3   Toxicological Concerns

The safety of three common natural cyclodextrins and some of their derivatives were
investigated in many research works (Irie & Uekama, 1997). All the toxicity studies
have indicated that orally administered cyclodextrins are non-toxic, due to lack of
absorption from the gastrointestinal tract (Irie & Uekama, 1997). Moreover, the
majority of the safety assessments proved that γ-cyclodextrin, 2-hydroxypropyl-β-
cyclodextrin (HP-β-CD), sulphobutylether-β-cyclodextrin (SBE-β-CD), sulphated β-
cyclodextrin and maltosyl β-cyclodextrin are safe even when administered
parenterally. However, toxicological studies have also shown that the parent α- and β-

cyclodextrin and the methylated β-cyclodextrins are not suitable for parenteral administration (Thompson, 1997).

According to the differences in size and flexibility of cyclodextrins, α-cyclodextrin is the slowest, and γ-cyclodextrin is the fastest degradable compound. It has been revealed by adsorption studies that only 2–4% of cyclodextrins were adsorbed in the small intestines, and that the remainder is degraded and taken up as glucose, which can explain the low toxicity found upon oral administration of cyclodextrins (Szejtli, 1989).

1.3.2.4 Inclusion Complex Formation

The specific characteristic of cyclodextrins is forming solid inclusions from a wide-ranging of solids, liquids, and gaseous compounds as host–guest complexes (Loftsson & Duchêne, 2007). The lipophilic cavity of cyclodextrins provides a microenvironment for non-polar groups to enter and to form inclusion complexes (Loftsson & Brewster, 1996). The main point is that no covalent bonds are broken or formed during the formation of the inclusion complex (Del Valle, 2004). The bond between host-guest molecules is not permanent but it is more like a dynamic equilibrium. The strength of binding depends on how well the 'host–guest' inclusion complex fits together and on specific local interactions between surface atoms (Muñoz-Botella et al., 1995).

Inclusion complexes could be formed either in solution or in the crystalline state. Although water is a commonly used solvent, but a co-solvent system or any non-aqueous solvent can also be utilized as a solvent (Connors, 1997). The architecture of cyclodextrins makes them markedly different from non-cyclic carbohydrates in the same molecular weight range. In fact, this structure can lock or cage guest molecules within its host cavity and give a significant rise to beneficial modifications of guest molecules, which are not achievable otherwise (Szetjil, 1998). The greatest driving force during the inclusion formation is the release of enthalpy-rich water molecules from the cavity, while water molecules are displaced by more hydrophobic guest molecules present in the solution to reach an apolar-apolar bond and decrease of cyclodextrin ring strain resulting in a more stable lower energy state (Szetjli, 1989).

The obvious properties of cyclodextrins are 1) solubility enhancement of highly insoluble guests, 2) stabilization of labile guests against the degradative effects of

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oxidation, and visible or UV light and heat, 3) control of volatility and sublimation, 4) physical isolation of incompatible compounds, 5) chromatographic separations, 6) taste modification by masking off flavours and unpleasant odours, and 7) controlled release of drugs and flavours. Therefore, cyclodextrins are used in food (Gomes et al., 2013), pharmaceuticals (Al-Marzouqi et al., 2009), cosmetics (Buschmann & Schollmeyer, 2002), environment protection (López-de-Dicastillo et al., 2010), bioconversion (Rao & Ravishankar, 1999), packing and the textile industry (Piercey et al., 2012).

Straight or branched chain of compounds such as aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and polar compounds; such as halogens, oxyacid and amines can form an inclusion complex with cyclodextrins (Del Valle, 2004). The applications of cyclodextrins could be expanded by modification means substituting various functional compounds on the primary and/or secondary face of the molecule. The functionality of modified cyclodextrins is greatly increased, due to the availability of multiple reactive hydroxyl groups (Eastburn & Tao, 1994; Lewis & Sumpter, 1996; Nazi et al,. 2012).

Two key factors are important in the formation of inclusion complexes. The first is related to the spatial arrangement of atoms in a guest molecule either the relative size of cyclodextrin to the size of the guest molecule or certain key functional groups within the guest. The second critical factor is the thermodynamic interactions between the different components of the system (cyclodextrin, guest, solvent). To form an inclusion, there must be an appropriate net energetic driving force that pulls the guest into the cyclodextrin (Forgo et al,. 2003; Nguyen et al., 2013).

The cavity height in all three kinds of cyclodextrins is the same; so, the number of glucose units determines the internal diameter and its volume. Only low molecular weight molecules or compounds with aliphatic side chains can enter the α-cyclodextrin. β-cyclodextrin usually complexes with aromatic and heterocycles and γ-cyclodextrin can interact with larger molecules such as macrocycles and steroids (Singh et al., 2002).

A variety of techniques can be used to form inclusion complexes, but their use depends on the properties of the active material, the equilibrium kinetics, the other formulation ingredients and processes and the final dosage form desired (Muñoz-

Botella et al., 1995). Each of these processes usually uses a small amount of water to help drive the thermodynamics (Liu et al., 2012).

Cyclodextrin inclusion complex formation is a stoichiometric molecular phenomenon. A collection of non-covalent forces, such as van der Waals forces, hydrophobic interactions and other forces, are responsible for the formation of inclusion complex. Generally, one guest molecule is placed in one cyclodextrin 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 cyclodextrin molecule 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 molar ratios are not always achieved, especially with high or low molecular weight guests (Kurkov & Loftsson, 2013; Loftsson & Duchêne, 2007).

Four different aspects are influential in the formation of cyclodextrin inclusion complex: solution dynamics, temperature, solvents and water (Brewster & Loftsson, 2007; Kurkov & Loftsson, 2013; Loftsson & Duchêne, 2007; Singh et al., 2002).

o Solution dynamics:

The possibility of complex formation is more in solution than in crystalline form since in solid phase only the surface molecules of the cyclodextrin crystal are available for complexation. Inclusion complexes are formed more rapidly when the guest molecules are either in soluble form or as dispersed fine particles.

o Effects of temperature:

Temperature has more than one effect on the formation of inclusion complexes. Generally, heating increases the solubility of the complex as well as that of the guest, and it causes higher probability of complex formation. But, at the same time temperature also destabilizes the complex. Most complexes start to decompose at 50–60 °C, while some complexes are stable at higher temperatures, especially if the guest is strongly bounded or the complex is highly insoluble.

o Use of solvents:

The more soluble the cyclodextrin in the solvent, the more molecules are available for interaction. If the solvent interacts with the cyclodextrin molecules, the guest must be able to displace the solvent from the cyclodextrin cavity. The solvent must easily be

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removed if solvent-free complexes are needed. Water is very easily replaced and it is the most commonly used solvent in which complexation reactions are performed. In the case of multi-component guests, one of the components may act as a solvent and be included as a guest. Usually, not all the guests have enough solubility in water, so inclusion formation is either very slow or impossible. In such cases, using an organic solvent to dissolve the guest is desirable. The solvent should not form a complex well with cyclodextrin and be easily removed by evaporation. For example, ethanol and diethyl ether are such solvents.

o Effects of water:

In a solution, as the amount of water increases, the more cyclodextrin and guest molecules dissolve, leading to a more afficient complex formation. However, as the amount of water is further increased, the concentration of cyclodextrin and guest molecules may be so low that they would not get in contact as easily as they would in a more concentrated solution. Therefore, it is desirable to keep the amount of water sufficiently low to ensure a complexation occurs at a sufficiently fast rate.

Some high molecular weight compounds such as oils have a tendency to associate with themselves rather than interacting with cyclodextrin. In such cases, using more water in addition to good mixing will allow better dispersion and separation of the oil molecules or isolation of the oil molecules from each other. When the oil molecules come into contact with cyclodextrin, they form a more stable complex than they would if less water were present. It is important to note that volatile guests can be lost during inclusion complex formation, especially if heat is used. Using a sealed reactor and refluxing the volatile guests back to the mixing vessel prevent the loss of volatile guests during the complexation.

1.3.2.5 Equilibrium Constants

Measurements of stability or equilibrium constant (K_c), and the dissociation constant ($K_d = 1/K_c$) of the drug–cyclodextrin complexes are two evidences of changes in the physicochemical properties of a compound upon inclusion. The K-values are determined based on changes in the physicochemical properties of the guest molecule upon intraction with CDs by measuring the aqueous solubility (Hirayama & Uekama, 1987), phase solubility (Liu et al,. 1992), and chemical shifts of the product and

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comparing to those of the original molecules (Suzuki et al,. 1993). While it is possible to use both guests or host changes to generate equilibrium constants, guest properties are usually most easily assessed and therefore used for determination of K-values.

1.3.2.6   Phase Solubility

The phase–solubility method described by Higuchi and Connors is the most useful and widely applied analytical approach to the equilibrium subject (Higuchi & Connors, 1965). It is defined as "an examination of the effect of a solubilizer (cyclodextrin) on the drug being solubilized (substrate)". A phase–solubility diagram is constructed by plotting the total molar concentration of the drug on the y-axis and the total molar concentration of cyclodextrin on the x-axis (Figure 5).
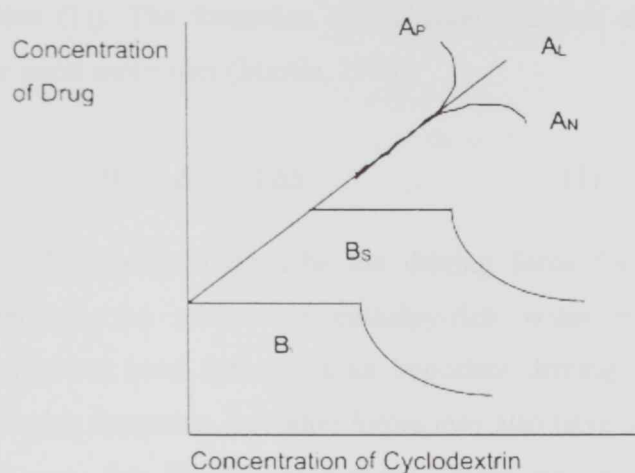
Figure 5. Phase-solubility relationships (Higuchi & Connors, 1965)

Higuchi and Connors divided the phase–solubility diagrams into two main categories, A- and B-types. A-type curves indicate the formation of soluble inclusion complexes while B-type behaviour suggests the formation of inclusion complexes of poor solubility. A B_S-type response shows the formation of inclusion complexes with limited solubility and a B_i-curve is indicative of insoluble complexes. The A-curves are subdivided into three main groups: A_L (Linear increases of drug solubility as a function of cyclodextrin concentration), A_P (positively deviating isotherm) and A_N (Negatively deviating isotherms).

Among the cyclodextrins, β-cyclodextrin often gives rise to B-type curves due to its poor water solubility. The chemically modified forms of β-CDs including 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulphobutylether-β-cyclodextrin (SBE-β-CD) usually indicate the formation of soluble inclusion complexes (i.e. A-type systems) (Del Valle, 2004). The phase solubility curve can not only verify the inclusion complex formation qualitatively but also can be used to determine equilibrium constants.

1.3.2.7 Thermodynamic Parameters

Inclusion complex formation is usually correlated with a negative ΔG (the standard Gibbs free energy change) caused by a large negative ΔH (the standard enthalpy change) and a ΔS (the standard entropy change), which can either be negative or positive (Equation (1)). The formation of inclusion complex also depends on the properties of the guest molecules (Martin, 1993).

$$\Delta G = \Delta H - T\Delta S \qquad (1)$$

There is no simple theory to describe the driving force for inclusion complex formation. Although, the release of enthalpy-rich water molecules from the cyclodextrin cavity has been defined as an important driving force for the guest-cyclodextrin complex formation, but other forces may also have important roles. Such forces include van der Waals interactions, hydrogen bonding, hydrophobic interactions, release of ring strain in the cyclodextrin molecule and changes in solvent-surface tensions (Bergeron, 1984; Connors, 1997; Loftsson & Brewster, 1996).

1.3.2.8 Complexation Techniques

Several techniques, namely: co-precipitation (co-evaporation), slurry complexation, paste complexation (kneading), damp mixing and heating (sealed-heating), extrusion and dry mixing (physical mixing) are used to form inclusion complexes. Table 4 shows a summary of advantages and disadvantages of these techniques. In all methods, the amount of time required is dependent on the guest.

In co-precipitation method, cyclodextrin is dissolved in water and the guest is added while stirring the cyclodextrin solution. In many cases, the solution of cyclodextrin

and guest must be cooled while stirring before a precipitate is formed. The precipitate may be washed with a small amount of water or other water-miscible solvent such as ethyl alcohol, methanol or acetone. In the case of using hydrophobic drugs, another similar method, namely co-evaporation is used. In the co-evaporation method, cyclodextrin and the guest are compeletly dissolved in water and in an orgnic solvent (i.e, ethanol), respectively. The solutions of cyclodextrin and guest are then added together to make a clear solution of cyclodextrin and guest. The precipitate is formed while heating the solution in a rotaty evaporator. These are the most widely used methods in the laboratory.

In slurry complexation method, it is not necessary to dissolve the cyclodextrin completely to form a complex. Cyclodextrin can be added to water as solids and stirred to make a saturated solution. Guest molecules are then added, forming complex with the cyclodextrin in solution. As the inclusion complex saturates the water phase, the complex will crystallise or precipitate out of the aqueous phase. The cyclodextrin crystals will dissolve and continue to saturate the aqueous phase to form the complex and precipitate or crystallise out of the aqueous phase.

In paste complexation method, only a small amount of water is added to the physical mixture of cyclodextrin and guest to form a paste by mixing in a mortar and pestle. The resulting complex can be dried directly or washed with a small amount of water and collected by filtration or centrifugation. In another similar method, namely kneading, an organic solvent is used instead of water to make a paste from a physical mixture of cyclodextrin and guest. Mixing is continued until solvent is completely removed.

In damp mixing and heating method, the guest and cyclodextrin are thoroughly mixed and placed in a sealed container. The sealed container and its contents are heated to about 100 °C and then the contents are removed and dried. This method is also known as sealed-heating.

In extrusion method, cyclodextrin, guest and water are premixed and added to the extruder. The extudate containing the inclusion complex is then dried.

In dry mixing or physical mixing, guests are complexed by simply adding guest to the cyclodextrin and mixing them together. This works best with oils or liquid guests, since no water needs to be added.

Table 4. Advantages and disadvantages of complexation techniques (Del Valle, 2004)

Technique	Advantages	Disadvantages
Co-precipitation (Co-evaporation)	✓ Most widly used method.	✓ Not easy to scale-up. ✓ Large volumes of water needed. ✓ Large tank capacity needed. ✓ Time and energy for heating and cooling is large. ✓ Treatment and disposal of the mother liquor needed. ✓ High temperature needed.
Slurry complexation	✓ Performed at ambient temperatures.	✓ Large volumes of water needed. ✓ Large tank capacity needed. ✓ For certain guests, some heat may be required to increase the rate of complexation.
Paste complexation (kneading)	✓ Only a small amount of water is needed.	✓ Pastes will sometimes dry forming a hard mass instead of a fine powder.
Damp mixing and heating (sealed-heating)	✓ Uses little or no water.	✓ The amount of water added, the degree of mixing and the heating time must be optimized for each guest.
Extrusion	✓ A continuous process ✓ Uses very little water. ✓ Degree of mixing, amount of heating and time can be controlled.	✓ Because of the heat generated, some heat-labile guests may decompose.
Dry mixing (Physical mixing)	✓ Performed at ambient temperature.	✓ The risk of caking on scale-up. ✓ Mixing may not be sufficiently thorough leading to incomplete complexation. ✓ Long time required.

1.3.2.9 Drying of Inclusion Complexes

In some complexation methods, inclusion complexes should be dried at the end of the process for different reasons e.g. to remove traces of solvent or to reduce the guests' losses. Care has to be taken that the complex is not destroyed during the drying process. Depending on the guest, type of dryer (oven, fluid bed dryer, or other type) has to be chosen. For highly volatile guests (T_b <100 °C) a lower temperature must be used to minimize the lose of guests during drying. Complexes can also be spray-dried. Spray drying is not a viable means of drying highly volatile and heat-labile guests. Moreover, a desiccator or freeze dryer can also be used to dry complexes. Freeze-

drying is especially useful for heat-labile guests and soluble complexes such as hydroxy-propylated cyclodextrin complexes (Singh et al., 2002).

1.3.2.10 Release of Guest

Under dry conditions, an inclusion complex is very stable, displaying long shelf life at ambient temperatures. Displacement of the inclusion complex by another guest requires heating or, in many cases, placing in water. When an inclusion complex is placed in water, the complex is dissolved first and then the release of the complexed guest starts when water molecules displace it. Finally between all, free and complexed cyclodextrin, the guest and the dissolved and undissolved complex, equilibrium is established.

In multiple guest or cyclodextrin type cases of complexes, guest molecules are not necessarily released in the same proportion like in the original guest mixture. Solubility and rate of release from the complex may differ in each guest complex. If release rates are different for each component, it is possible to obtain an intended release pattern by alteration of the guest formulation (Del Valle, 2004; Kurkov & Loftsson, 2013).

1.3.2.11 Applications of Cyclodextrins

From a microscopic point of view, each guest molecule, which is individually surrounded by a cyclodextrin or any of its derivatives is a micro-encapsulated molecule. A micro-encapsulated molecule has more advantages due to its altered chemical and physical properties than the original guest molecules. Some advantages are as follows:

- Improvement of solubility of substances.
- Modification of liquid substances to powders.
- Stabilization of light- or oxygen-sensitive substances.
- Modification of the chemical reactivity of guest molecules.
- Fixation of very volatile substances.
- Masking of bad smell and taste.
- Masking pigments or the colour of substances.
- Catalytic activity of cyclodextrins with guest molecules.

- Protection against degradation of substances by micro- organisms.

These properties of cyclodextrins or their derivatives make them suitable for applications in the pharmaceutical field, analytical chemistry, agriculture, and food (Singh et al., 2002). Below, only the advantages of cyclodextrin in "Pharmaceutical field" have been summarized.

1.3.2.12 Pharmaceutical Applications

Only a small number of pharmaceutical active agents have sufficient solubility in water. The unique characteristic of micro-encapsulated molecules with cyclodextrins is their potential use in drug delivery through biological membranes. Like all drug substances, micro-encapsulated molecules not only have a certain level of water solubility to be easily delivered to the cellular membrane, but also they are hydrophobic enough to cross the membrane (Loftsson & Duchêne, 2007). Cyclodextrins keep the hydrophobic drug molecules in solution and deliver them to the surface of biological membranes just like true carriers. The relatively lipophilic membrane has a low affinity for the hydrophilic CD molecules, thus keeping the CDs in the aqueous membrane exterior. Therefore, cyclodextrins increase drug availability at the surface of the biological barrier. On the other hand, CDs act as penetration enhancers. For example, cyclodextrins have been used successfully in aqueous dermal formulations (Hirayama & Uekama, 1999), aqueous mouthwash solutions (Kristmundsdóttir et al., 1996), nasal drug delivery systems (Kublik et al., 1996) and several eye drop solutions (Loftsson & Stefánsson, 1997).

There are numerous pharmaceutical applications of cyclodextrins; for example, α- or β-cyclodextrin inclusion complexes increase the water solubility of many poorly water-soluble substances and in some cases, improve bioavailability as well as pharmacological effect that can allow a reduction in the dose of the drug administered (Carrier et al., 2007). In addition, inclusion complexes can also improve the handling of volatile products that can lead to a different way of drug administering, e.g. in the form of tablets (Hirayama & Uekama, 1999). Moreover, cyclodextrins are used to facilitate the stability of substances to increase their resistance to hydrolysis, oxidation, heat, light and metal salts (Irie & Uekama, 1999). The inclusion of irritating products in cyclodextrins can also protect the gastric mucosa for the oral

route, and reduce skin damage for the dermal route. Furthermore, cyclodextrins can be applied to reduce the effects of bitter or irritant tasting and bad smelling drugs (Brewster & Loftsson, 2007).

1.3.3 SUPERCRITICAL FLUIDS

1.3.3.1 Background

In 1822, Baron Cagniard discovered supercritical fluids and observed that the boundary between a gas and a liquid disappears with increase in temperature. However, it was not clear until 1869 when Andrews claimed the existence of this new state of matter (Manivannan et al.,1998).

A fluid reaches the supercritical region when heated and pressurized, above its critical pressure (P_c) and temperature (T_c). At such condition, the fluid is called a supercritical fluid (SCF). The critical point represents the end of the vaporization curve in the PT phase diagram (Figure 6). As shown in the figure, three single phases (solid, liquid and gas) of a substance may exist depending on the system temperature and pressure. If a mixture of two or more phases exists in these states, a separation between the phases is distinct because of the differences in the properties of each individual phase. The solid lines between phases indicating the coexistence of two phases as solid-gas, solid-liquid and liquid-gas curves corresponding to sublimation, melting, and vaporization, respectively. The intersection of these three curves is a triple point where the three phases are coexisting (Manivannan et al., 1998; Pasquali & Bettini, 2008).
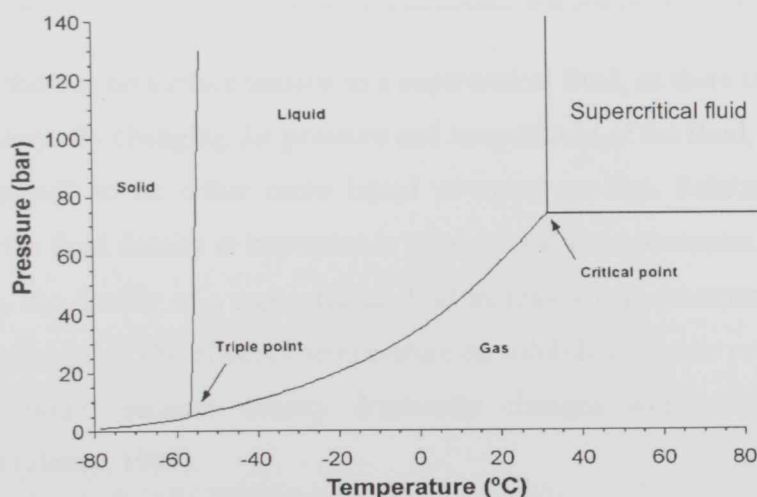

Figure 6. CO_2 phase diagram (Pasquali & Bettini, 2008)

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The supercritical status corresponds to a region where the physicochemical properties of the compound are intermediate between those of the liquid and the gas. So neither liquefaction will be taken place as a result of pressure increase, nor gas will be formed as a result of temperature rise. The macroscopic appearance of a fluid or solvent at the critical point is of a homogeneous and an opalescent system without apparent phase separation because, at this point, the density of the gas and liquid are identical (Pasquali & Bettini, 2008).

Table 5 shows a general comparison between the physical properties of gases, liquids and SCFs (Henry, 1997). Like a gas the SCF shows lower viscosity and higher diffusivity relative to the liquid. These properties facilitate mass transfer during an extraction or impregnation process. Like a liquid, the SCF shows a density value high enough for exerting solvation effects (Manivannan et al., 1998). A SCF is dense but highly compressible, thus, any pressure change results in density alteration and, consequently, in solvent power variation (Brunner, 1994). In the vicinity of the critical point, the compressibility is high, and a small pressure change yields a great density modification.

Table 5. Physical properties of a typical gas, liquids and SCF

Property	Gas	SCF	Liquid
Density (g/cm^3)	10^{-3}	0.3	1
Diffusivity (cm^2/sec)	0.2	10^{-3}	10^{-5}
Dynamic Viscosity (g/cm.sec)	10^{-4}	10^{-4}	10^{-2}

In addition, there is no surface tension in a supercritical fluid, as there is no liquid/gas phase boundary. By changing the pressure and temperature of the fluid, the properties could be "tuned" to be either more liquid or more gas-like. Solubility, which is affected by the fluid density is important in supercritical fluid processes. At a constant temperature, the density of a supercritical fluid increases with pressure, thus causing solubility to increase. The effect of temperature on solubility is more pronounced near the critical point, because density drastically changes with small changes in temperature (Henry, 1997).

All fluids become supercritical above their critical coordinates, although in some cases, extremely high pressure and temperature might be required. Supercritical carbon dioxide (SC-CO_2) is the most widely used SCF for pharmaceutical applications owing to its low critical temperature (31.2 °C) and pressure (72.8 bar), and being nontoxic, nonhazardous, non-flammable, chemically stable, inexpensive, recyclable, and environmentally acceptable (Mukhopadhyay, 2000). In addition, near the critical point, CO_2 is a good solvent for non-polar to slightly polar solutes with low molecular weight. Moreover, using SC-CO_2 as a solvent leaves no solvent residue in the extract since CO_2 is in gas phase at ambient condition and naturally seperates from the product. Table 6 lists the critical properties of some common solvents used as SCFs (Dean, 1998; Henry, 1997).

Table 6. Critical properties of some common solvents used as SCFs

Solvents	Chemical Formula	Critical Density (g/cm^3)	Critical Temp. (°C)	Critical Pressure (bar)
Carbon dioxide	CO_2	0.469	31.2	72.8
Water	H_2O	0.322	374.4	221.4
Methanol	CH_3OH	0.272	240.1	82.0
Ethanol	C_2H_5OH	0.276	240.75	60.6
Acetone	C_3H_6O	0.278	234.95	46.4
Methane	CH_4	0.162	-82.75	45.4
Ethane	C_2H_6	0.203	32.4	49.5
Propane	C_3H_8	0.217	96.65	41.9
Ethylene	C_2H_4	0.215	9.25	49.7
Propylene	C_3H_6	0.232	91.75	45.4

Water with high critical temperature (374.4 °C) and high critical pressure (221.4 bar) is not suitable for SCF applications dealing with thermally labile compounds. Other organic solvents (e.g., methanol, ethanol and acetone), in addition to their high critical points, are toxic chemicals. Methane has a very low critical temperature (-82.75 °C) requiring high amount of energy for attaining SC condition. Ethane, propane, ethylene

and propylene with acceptable critical condition, are very expensive and hazardous chemicals.

1.3.3.2 Applications of Supercritical Fluids

In the last 30 years, SCF technology has been extensively used in extraction applications including the extraction of caffeine from coffee and tea, flavours from hops, cholesterol and fat from eggs, acetone from antibiotics and organics from water (Henry, 1997; Mukhopadhyay, 2000; Reverchon & De Marco, 2006). Supercritical fluid technology is also used for a wide range of pharmaceutical applications such as cyclodextrin inclusion complex formation, particle and crystal engineering, drug coating, foaming and tissue engineering, liposome preparation, sterilization and solvent removal (Cocero et al., 2009; Pasquali & Bettini, 2008). Ability of SC-CO_2 to form inclusion complex depends on the compound functional group, molecular weight and polarity (Al-Marzouqi et al., 2007; Sauceau et al., 2008).

1.3.3.3 Cyclodextrin Inclusion Complex Formation using SC-CO_2

As explained earlier, the specific characteristic of cyclodextrins (CDs) is their ability to form inclusion complexes from a wide range of solids, liquids and gaseous compounds. The required time to achieve the successful inclusion complex formation depends on the host–guest equilibrium time. Moreover, the strength of binding depends on how well the 'host–guest' inclusion complex fits together and on specific local interactions between surface atoms (Del Valle, 2004). Inclusion complexation improves the physicochemical properties of guest molecules such as solubility, dissolution rate, chemical stability, absorption and bioavailability of poorly water-soluble guests (Al-Marzouqi et al., 2007; Uekama et al., 2003).

Conventional techniques present some drawbacks such as long inclusion formation time, high solvent consumption, labour intensive, difficult to automate, use of toxic solvents and often requiring post cleanup steps (Al-Marzouqi et al., 2007; Brewster & Loftsson, 2007). Most of these drawbacks are overcome by SC-CO_2, mainly because SC-CO_2 is a nontoxic, non-hazardous and environmentally acceptable solvent and can easily be separated from the product.

Although, SCFs were first observed more than a century ago (in 1822), studies on the utilization of SC-CO_2 as a novel technology to form cyclodextrin inclusion complexes with drugs in the solid-state started in 1999 when a successful complexation (94%

inclusion yield) between piroxicam and β-cyclodextrin was obtained (Van Hees et al.,1999). Miconazole-cyclodextrin inclusion complexes were also produced by SC-CO_2 and compared with conventional methods of physical mixing, spray drying and freeze drying (Van Hees et al., 2002).

By using a similar method, Moneghini and others have tried to include nimesulide into β-CD. The obtained results indicated that the drug was only partially included. However, the physicochemical characterization of the obtained product pointed out the existence of interactions between drug and carrier that led to an increased *in vitro* drug dissolution rate (Moneghini et al., 2004). Inclusion complex between methyl-β-cyclodextrin and ibuprofen prepared by SC-CO_2 showed a significant enhancement of dissolution profiles due to the amorphous character and improved wettability of the product (Charoenchaitrakool et al., 2002). Türk and his coworkers developed a process to prepare ibuprofen-β-CD complexes using SC-CO_2, which resulted in higher dissolution rates than the physical mixture of ibuprofen and β-CD (Türk et al., 2007).

Inclusion complexes between itraconazole and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) prepared by SC-CO_2 showed significantly higher solubility of itraconazole in aqueous solutions compared to pure drug (Peeters et al., 2002). Other researchers also showed that itraconazole-cyclodextrin inclusion complexes prepared by SC-CO_2 could improve the solubility of itraconazole in aqueous solutions. However, inclusion yield of itraconazole-cyclodextrin complex prepared by SC-CO_2 was relatively low, which was probably due to the large molecular weight of itraconazole and its low solubility in SC-CO_2 (Al-Marzouqi et al., 2006; Al-Marzouqi et al., 2007). Another drug from the same triazole group (econazole) was also studied to test the SC-CO_2 technique with a lower molecular weight antifungal drug (Al-Marzouqi et al., 2007). In that study, econazole-cyclodextrin inclusion complexes were prepared using SC-CO_2, as well as conventional methods (i.e., kneading, coevaporation, sealed-heating, physical mixing) and significant enhancement in aqueous solubility of econazole was observed.

1.4   Potential Contributions and Limitations of the Study

The preparations of safranal−β-CD inclusion complexes by supercritical carbon dioxide and by conventional techniques have not been studied yet. Also the influence

of different preparation methods on safranal—β-cyclodextrin inclusion complexes to enhance the solubility and dissolution rate has never been investigated.

Results from this study may provide novel formulations for safranal with enhanced solubility, dissolution rate, bioavailability and efficacy. Therefore, this investigation should pave the way for ultimate human utilization. However, efficacy, safety and pharmacokinetics of the current inclusion complexes obtained by different methods need future investigations both *in-vitro* and *in-vivo* before being approved as a therapeutic drug for human use.

The limitation of this study may arise from insufficient interaction between safranal and β-CD, thus leading to relatively low inclusion yield. This limitation may be overcome by using other CDs with different cavity size (i.e. α and γ) or β-CD derivatives having functional groups (i.e. M-β-CD, HP-β-CD, and SBE-β-CD).

2 MATERIALS AND METHODS

2.1 Materials

Safranal of 88% minimum purity, β-cyclodextrin hydrate (99%) and ethanol with 99.8% purity were purchased from Sigma Aldrich, USA. All the time, the sample was stored at room temperature. Liquefied CO_2 with a purity of 99.95% was obtained from Abu-Dhabi Oxygen company, UAE. All the materials were stored at room temperature until used. All reagents and solvents were of analytical grade. All solutions were prepared using deionized double distilled water and used within 24 h.

2.2 Experimental Methods

2.2.1 Physical Mixture (PM)

A 1:2 (molar ratio) safranal−β-CD physical mixture was prepared by mixing safranal and β-CD in a mortar. The mixture was thoroughly homogenized by spatula for 15 min.

2.2.2 Kneading (KN)

The kneaded products were prepared by adding a droplet of ethanol to the safranal−β-CD physical mixture of 1:2 molar ratio. The sample was pounded thoroughly with a pestle to obtain a homogenous mixture and pounding was continued until the solvent was completely removed. The sample was kept in a desiccator overnight to remove traces of solvent.

2.2.3 Co-evaporation (COE)

Co-evaporated products were obtained by dissolving β-CD in distilled water at 25°C and safranal (giving the 1:2 safranal: β-CD molar ratio) in ethanol at the same temperature. The solutions were added together after obtaining clear solutions. The solvents were removed using a rotary evaporator at 75°C while safranal and β-CD were precipitated respectively by gradually evaporating ethanol and water. The sample was kept in a desiccator overnight to remove traces of solvents.

2.2.4   Sealed-heating (SH)

Sealed-heated products were prepared by placing the safranal$-\beta$-CD physical mixture of 1:2 molar ratio in a glass container, adding 10 μl distilled water and then sealing the glass container using a flame. The sample was kept in an oven for 3 h at 75°C, after which the sample was removed and kept in a desiccator overnight to remove traces of water.

2.2.5   Supercritical Carbon Dioxide (SC-CO$_2$)

The supercritical carbon dioxide (SC-CO$_2$) experimental apparatus (Figure 7) is composed of a CO$_2$ cylinder with a dip-tube high pressure syringe pump with a maximum operating pressure of 500 bars and a controller system (Model 260D, ISCO, USA), and SFE extraction unit (ISCO, SFX 220, USA). The dip-tube allows only liquefied CO$_2$ to be transferred to the pump as the liquid resides in the bottom of the cylinder whereas the gaseous CO$_2$ is at the top. The extraction unit consists of a dual-chamber extraction module with two 10 ml stainless steel cells and a temperature controller with a maximum operating temperature of 150 °C.

The pump controller has a control panel displaying time, pressure, CO$_2$ flow rate as well as volume of CO$_2$ passed. Pressure within the chamber was measured and controlled by the system whereas the temperature was measured and controlled by the temperature controller. The precision of the temperature measurements of the extraction system was $\pm$ 0.1°C. A micrometering valve (HIP 15-12AF1-V) was used to facilitate a good control of the flow rate. Moreover, water bath with heating coil surrounded the CO$_2$ cylinder to provide higher CO$_2$ pressure in the cylinder for better transfer of CO$_2$ to the pump. A thermocouple was connected to a temperature controller (Omega CN9000A) to control the temperature on the surface of the micrometering valve.
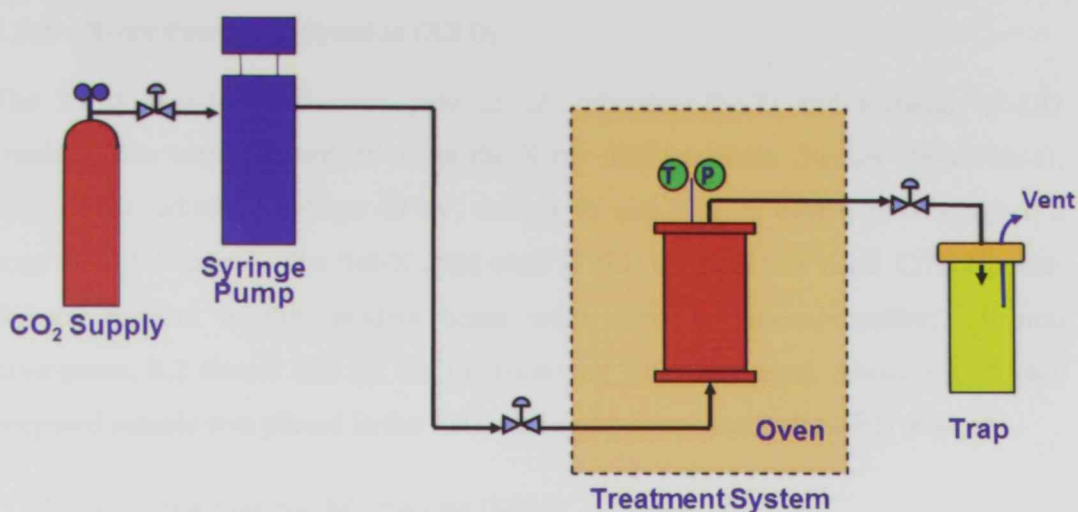
Figure 7. Supercritical carbon dioxide experimental apparatus

A 10 ml stainless steel cell was filled with physical mixture of safranal−β-CD at a 1:2 molar ratio. Cell filters with 5/8-inch diameter were placed at the top and bottom of the cell. The extraction cell was placed in the extraction chamber. CO_2 was then pressurized to the desired pressure in the high pressure syringe pump. The system was held in static mode for 180 min after which the pressure in the cell was dropped to atmospheric pressure within 15 min. The powder obtained was ground and homogenized in a mortar and collected in a vial. After each run, the system was flushed first using about 25 ml ethanol followed by about 50 ml SC-CO_2. The above procedure was repeated at different temperatures (35 °C and 55 °C) and pressures (100 and 300 bar).

2.3   Analytical Methods

2.3.1   Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra (Perkin-Elmer Mod. 1600) of individual safranal, β-CD and safranal−β-CD inclusion comlexes were obtained as Nujol dispersion in the wave range number of 4000-400 cm^{-1} at a resolution of 2 cm^{-1}. Small amount of Potassium Bromide (KBr) was added to about 3 mg of the prepared sample to reach the weight of 10 mg in order to dilute the sample.

2.3.2 X-ray Powder Diffraction (XRD)

The X-ray powder diffraction patterns of individual β-CD and safranal$-\beta$-CD combinations were determined using the X-ray diffractometer (Bruker D8-advance), with CuKα radiation, voltage 40 kV, current 40 mA, and 2θ over a 2-60° range at a scan rate of 1°min^{-1}. The Sol-X solid-state Si (Li) detector was used. C/Ni Goeble-Spiegel mirrors in the incident beam were used as monochromator; 1.0 mm divergence, 0.2 scatter and 0.1 for the receiving slits were used. About 1 g of each prepared sample was placed in the XRD cell and became ready for XRD analysis.

2.3.3 Scanning Electron Microscope (SEM)

The morphology of the pure β-CD and safranal$-\beta$-CD inclusion complexes was examined using a Jeol JSM 5600 SEM on samples pretreated by the deposition of a thin layer of sputtered gold at room temperature using a sputter coater before the examination.

2.3.4 Proton Nuclear Magnetic Resonance (H-NMR) Spectroscopy

H-NMR spectra of individual safranal, β-CD and safranal $-\beta$-CD inclusion complexes were recorded on a Varian 400-MHz spectrometer at 25 °C. The solvent was D_2O for all the samples.

2.3.5 Phase Solubility Studies

The solubility studies were performed according to the Higuchi and Connors method [Higuchi & Connors,1965]. An excess amount of safranal (about 1 ml) was added to 10.00 ml of aqueous phosphate buffer solutions (pH=7.4) containing various concentrations of β-CD. The suspensions formed were sonicated for 15 min and were shaken for 3 days in a water bath at 40 °C. After equilibrium was achieved, an aliquot part was filtered through a Millipore membrane (0.45 μl). The filtered solutions were 25 times diluted in water and the amount of dissolved safranal was determined using a Shimadzu UV/ VIS -1800, scanning spectrometer at 310 nm.

2.3.6 Dissolution Studies

Dissolution rates of safranal, both pure and from the different safranal-carrier inclusion complexes, were determined in water at 37 ± 0.5°C according to the dispersed amount method, by adding 50 mg of safranal or safranal-equivalent to 500

ml of water, in a 600 ml beaker coated by a heating jacket. A stainless steel two-blade propeller was immersed in the beaker 25 mm from the bottom and rotated (f=100 min^{-1}). Suitable aliquots were withdrawn with a filter-syringe (pore size 0.45 μm) at the specified times and the safranal concentration was spectrometrically analysed (UV/VIS 1800 Shimadzu). The same volume of fresh medium was added to the beaker and the correction for the cumulative dilution was calculated. Dissolution was characterized as the percent of safranal dissolved after 10 min, representing the index of the rate of dissolution.

3 RESULTS AND DISCUSSION

Safranal−β-CD inclusion complexes prepared by different methods were investigated using FT-IR, XRD, SEM and H-NMR to evaluate the potential and effectiveness of these preparation methods in complex formation. In addition, phase solubility evaluation was performed and solubility constant was calculated. The effects of varying experimental conditions such as temperature and pressure on the complex formation were also investigated.

3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of pure safranal, pure β-CD, and safranal−β-CD (1:2) inclusion complexes prepared by physical mixing, kneading, co-evaporation, sealed-heating and SC-CO_2 at different temperatures and pressures, are shown in Figure 8 and 9. Changes in spectra upon complexation, such as peaks shift and reduction in intensity, depended on the preparation method, suggesting different degrees of interaction and/or amorphization in the different products. These changes could easily be comparing the appearance or nonexistence of characteristic peaks of group frequencies of safranal (Table 7). In the pure safranal spectrum (Figure 8), the bands appearing with variable and strong intensity at 1634 and 1664 cm^{-1} are attributed to the two stretching vibrations of alkenes and carboxyl groups in pure safranal respectively.

Although the band at 1664 cm^{-1} was not maintained exactly in all products spectrum, it caused significant shifts for the band at 1634 cm^{-1} to appear at 1650 cm^{-1} in most of the samples. Therefore, the FT-IR spectrum of safranal−β-CD physical mixture freshly prepared at 25°C and of other safranal−β-CD products obtained by kneading, sealed-heating, co-evaporation and SC-CO_2 showed significant differences in group frequencies range with respect to the pure safranal spectrum, probably attributed to the extent of the formation of safranal−β-CD inclusions. This reveals a modification of the environment of safranal and thus indicates safranal−β-CD interactions.

Table 7. Group frequencies of Safranal

Bond	Type	F Rang (cm^{-1})	Frequency (cm^{-1})	Intensity
C-H	Alkanes	2850-2970	2924.9	Strong
		1340-1470	1458.0	Strong
C-H	Alkenes	3010-3095	3038.5	Medium
		675-995	721.0	Strong
C=C	Alkenes	1610-1680	1634.3	Variable
C=O	Carboxylic acids	1660-1760	1664.6	Strong

The effect of varying temperature (35 or 55°C) and pressure (100 or 300 bar) on the products obtained by SC-CO$_2$ method is illustrated in Figure 9. Table 8 shows the significant changes ($\Delta\delta$) in frequencies appeared in the FT-IR spectrum of the product treated with SC-CO$_2$ at different operating conditions. The frequencies for β-CD observed at 3370.72 cm^{-1}, 2928.53 cm^{-1}, 1157.84 cm^{-1}, and 1029.24 cm^{-1}, correspond to the symmetric and asymmetric stretching of ν_{OH}, ν_{CH2}, ν_{C-C} and bending vibration of ν_{O-H} respectively.

Table 8. Changes in IR frequencies of selected β-CD functional groups upon complexation with safranal using SC-CO$_2$ at different temperature and pressures.

β –CD		Inclusion Complexes			
Bond	Frequency(cm^{-1})	100 bar	$\Delta\delta$	300 bar	$\Delta\delta$
At 35 °C					
OH	3306.1	3255	-51.1	3314.3	+8.2
CH2	2925.4	2927.1	+1.7	2927.1	+1.7
C–C	1157.1	1157	-0.1	1157.3	+0.2
O–H	1031.5	-----*	-----	1028.2	-3.3
At 55 °C					
OH	3306.1	3352.2	+46.1	3346.9	+40.8
CH2	2925.4	2926.2	+0.8	2928.1	+2.7
C–C	1157.1	1157.4	+0.3	1157.2	+0.1
O–H	1031.5	1031.8	+0.3	1032.9	+1.4

* The peak is completely disappeared.

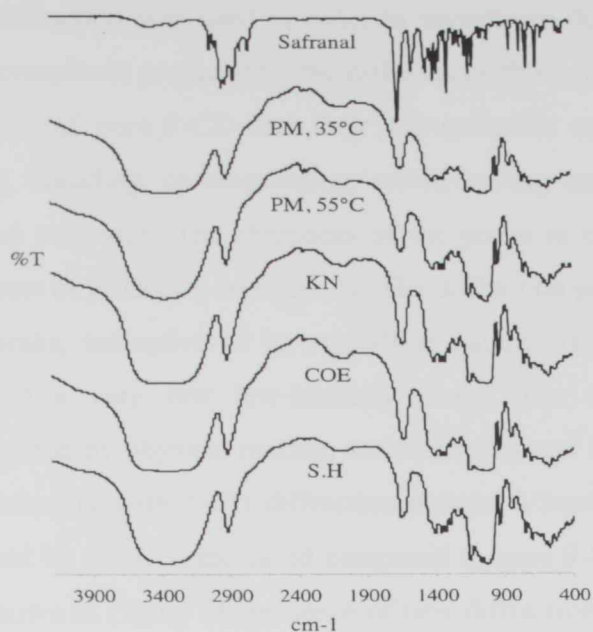$\Delta\delta$ = IR frequency of inclusion complex minus that of pure β-CD

Figure 8. FT-IR spectrum of pure Safranal, and safranal–β-CD (1:2) inclusion complex products prepared by physical mixing, kneading, co-evaporation, sealed-heating
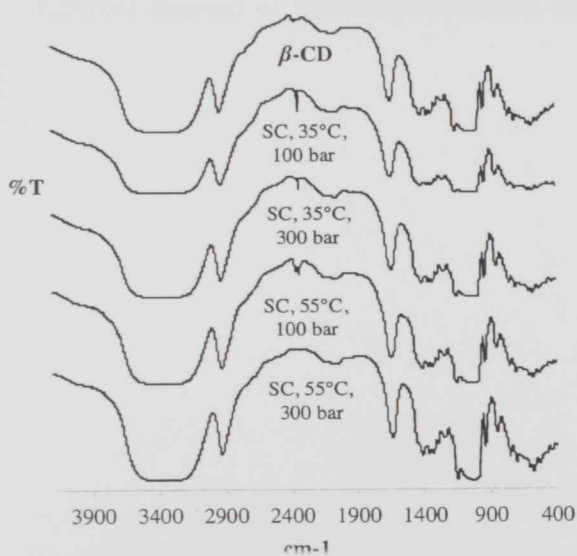

Figure 9. FT-IR spectra of pure β-CD and safranal–β-CD (1:2) inclusion complex products prepared by SC-CO$_2$ at different temperatures and pressures

3.2   X-Ray Powder Diffraction (XRD)

X-ray powder diffraction was used in order to investigate the crystalline nature of safranal–β-CD complexes prepared by the different methods. Figure 10 and 11 show the XRD patterns of pure β-CD and their 1:2 inclusion complexes prepared by physical mixing, kneading, co-evaporation, sealed-heating and SC-CO_2 at different temperatures and pressures. The sharpness of the peaks in the diffraction patterns indicates the extent of product's crystallinity. The diffraction pattern of β-CD showed several sharp peaks, indicative of its crystalline nature. The product obtained by kneading showed a very few low-intensity peaks. The crystalline patterns of components prepared by physical mixing, sealed-heating and co-evaporation showed similarities in intensity with β-CD diffraction pattern. Visually, the crystallinity of products obtained by SC-CO_2 increased compared to pure β-CD diffraction pattern. Moreover, as shown in Figure 11, presence of new diffraction peaks was detected in samples prepared by SC-CO_2 methods. These new peaks were attributed to the formation of a new solid phase induced by complexation of safranal with β-CD. The XRD patterns of the examined samples showed crystalline or partially amorphous products, indicating the possibility of safranal–CD interactions of different strengths, which may lead to different degrees of inclusion formation and/or amorphization of the sample.
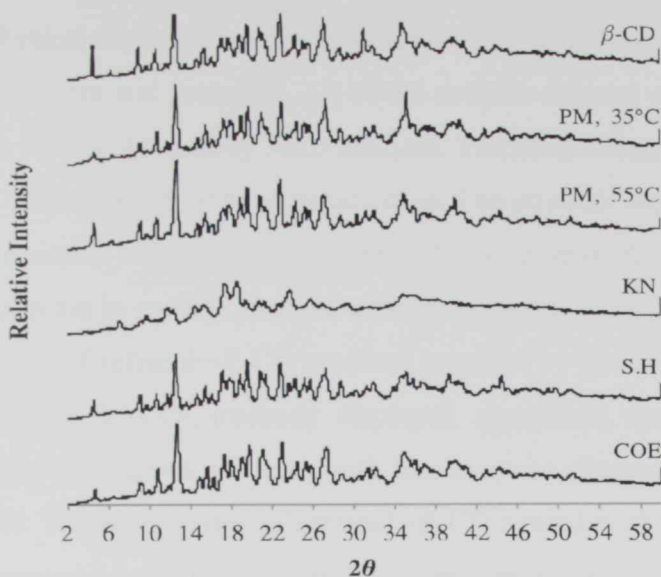
Figure 10. XRD patterns of pure β-CD and safranal–β-CD (1:2) inclusion complexes prepared by physical mixing, kneading, sealed-heating, co-evaporation

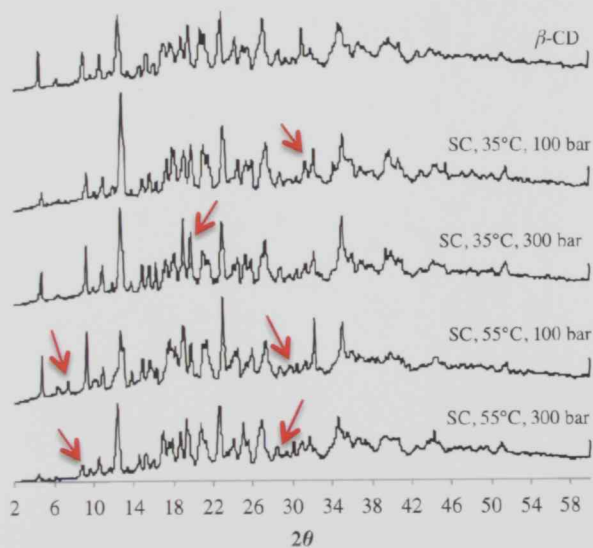

Figure 11. XRD patterns of pure β-CD and safranal–β-CD (1:2) inclusion complexes prepared by SC-CO$_2$ method at different temperatures and pressures

3.3 Scanning Electron Microscope (SEM)

Figure 12 shows the SEM images of pure β-CD and the 1:2 inclusion complexes prepared by physical mixing, kneading, co-evaporation, sealed-heating and SC-CO$_2$ at different temperatures and pressures. All of the samples showed crystalline structure, confirming the results obtained by XRD analysis. The morphology of untreated β-CD consisted of a solid network of aggregated cubes. The physical mixture exposed to 35 or 55 °C gave a similar morphology consisting of a solid network of aggregated cubes with a slight increase in particle size and a minimal decrease in particle density. SEM photomicrographs of safranal−β-CD products prepared by kneading, co-evaporation, sealed-heating and SC-CO$_2$ methods displayed significant changes, resulting in smaller or larger aggregated particles with less or more sharpness or tenacity than untreated β-CD. The morphology of safranal−β-CD treated with SC-CO$_2$ at different conditions consisted of a solid network of bigger aggregated cubes with patches of very small dot-like particles, which was not seen in the SEM photomicrographs of the other samples. This unique morphology observed for inclusion complexes prepared by SC-CO$_2$ showed the effect of this method on the safranal−β-CD mixture.
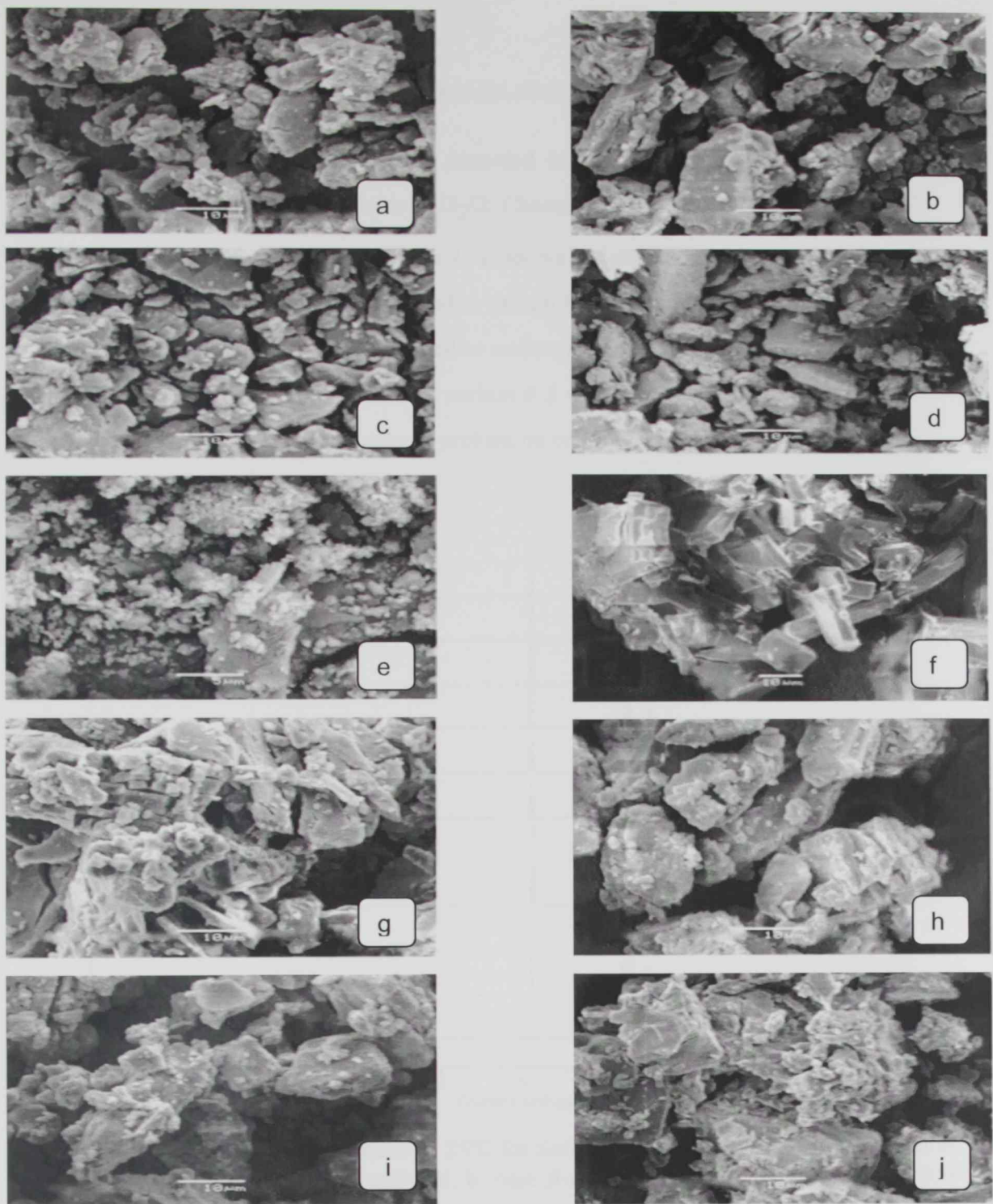
Figure 12. SEM photomicrographs of pure β-CD (a) and safranal–β-CD (1:2) inclusion complexes prepared by sealed-heating (b), physical mixing (at 35°C (c) and 55°C (d)), kneading (e), co-evaporation (f) and SC-CO_2 method at different temperatures and pressures. (SC-CO_2, 35°C, 100 bar (g); SC-CO_2, 35°C, 300 bar (h); SC-CO_2, 55°C, 100 bar (i); SC-CO_2, 55°C, 300 bar (j))

3.4   Proton Nuclear Magnetic Resonance (H-NMR) Spectroscopy

Figure 13 shows the H-NMR spectra recorded in 25°C for safranal, β-CD and 1:2 safranal-β-CD inclusion complexes in D_2O. Chemical shifts are reported in ppm (δ).

The NMR spectrum of safranal (Figure 13a) shows a signal at $\delta \approx 1.87$ ppm, which is attributed to the methyl protons attached to carbon # 2. The doublet at $\delta \approx 0.98$ ppm is attributed to the methyl protons attached to carbon # 6. The singlet at $\delta \approx 2.01$ ppm is attributed to the methylene protons on carbon # 5 while the doublets at $\delta \approx 6.15$ and 5.87 ppm are attributed to the methane protons on carbons # 3 and 4, respectively.
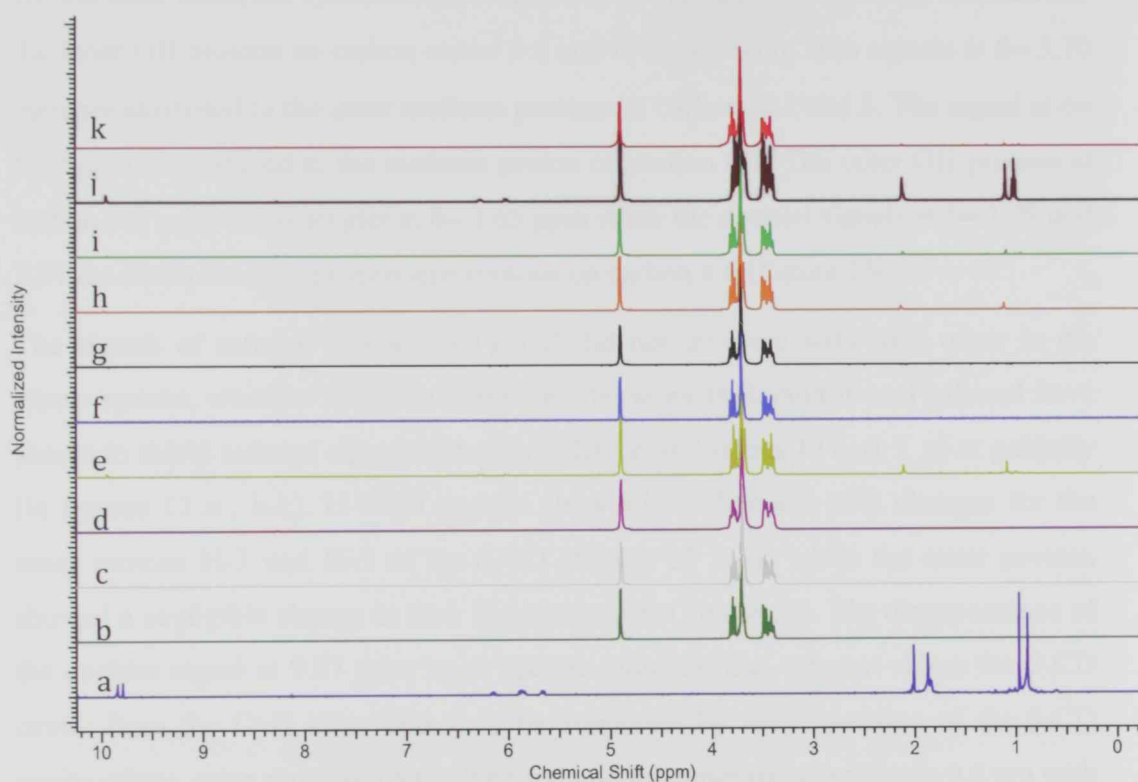

Figure 13. H-NMR spectra recorded at 25°C for Safranal, CD and its 1:2 β-CD inclusion complexes in D_2O. a: pure safranal; b: pure β-cyclodextrin; c: SC- CO_2, 35 °C , 300 bar; d: SC- CO_2, 55°C , 300 bar; e: SC- CO_2, 55°C , 100 bar; f: SC- CO_2, 35°C , 100 bar; g: co-evaporation; h: sealed heating; i: physical-mixing, 55°C; j: kneading; k: physical-mixing, 35°C.

The doublet shown at δ≈ 9.87 ppm is attributed to the methane proton attached to the carbonyl carbon. The appearance of this signal may indicate that safranal exists in two tautomeric forms as shown in Figure 14.

Figure 14. Safranal in two tautomeric forms

On the other hand, the cyclodextrin's signals at δ≈ 3.50 and 3.80 ppm are attributed to the inner OH protons on carbon atoms # 2 and 6, respectively. The signals at δ≈ 3.70 ppm are attributed to the inner methane protons on carbons # 2 and 3. The signal at δ≈ 5.00 ppm is attributed to the methane proton on carbon # 1. The outer OH protons at carbon # 6 is shown as singlet at δ≈ 3.65 ppm while the doublet signals at δ≈ 3.79 and 3.54 are attributed to the methylene protons on carbon # 6 (Figure 13b).

The signals of safranal protons and β-CD did not interfere with each other in the whole spectra, whereas inclusion complexes between cyclodextrin and safranal have shown to shield safranal signals either completely (in Figures 13 c, d, f, g) or partially (in figures 13 e , h-k). H-NMR spectra show slight chemical shift changes for the inner protons H-3 and H-5 of the β-CD (Figure 15 a, b) while the outer protons showed a negligible change in their chemical shifts (Figure 13). The disappearance of the doublet signal at 9.87 ppm in all spectra indicates that safranal enters the β-CD cavity from the C=O side. This may be supported by the dimension of the β-CD cavity whose outer rim diameter is 0.65 nm and the inner rim diameter is 0.6 nm with cavity volume of 0.14 ml/g, 6 inner cavity hydrated H_2O molecules and 3.6 externally hydrated water molecules.

Comparing different methods for inclusion complex formation based on changes in H-NMR spectra may indicate that supercritical method is more efficient compared to conventional methods used. The sequence of efficiency is supercritical > co-evaporation > PM > sealed heating > kneading. It is also observed that using 300 bar pressure is more efficient than 100 bar and 35°C temperature is more efficient than 55

°C for the formation of inclusion complexes using the supercritical method (Figure 13).

(a)   (b)
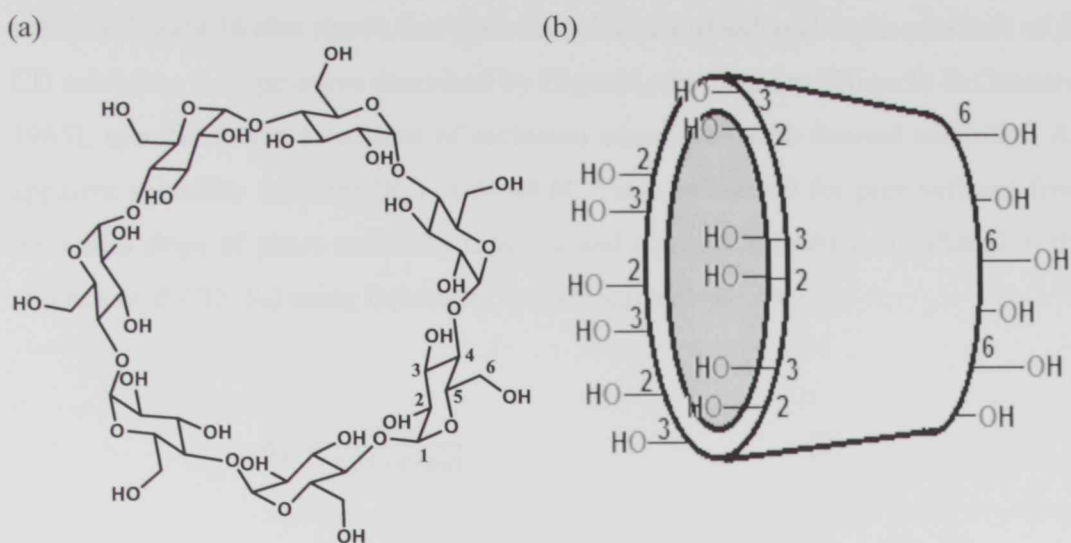
Figure 15. a and b. Chemical structure of β-CD.

3.5 Phase Solubility Studies

Cyclodextrin rings are amphiphilic (amphipathic) with the wider rim displaying the 2- and 3-OH groups and the narrower rim displaying 6-OH group on its flexible arm. These hydrophilic groups are on the outside of the molecule cavity whereas the inner surface is hydrophobic lined with the ether-like anomeric oxygen atoms and the C3-H and C5-H hydrogen atoms. The hydrophilic cyclodextrin molecules may bind non-polar suitably-sized aliphatic and aromatic compounds with molar ratio of 1:1, 2:1 or 1:2 ratios. The binding process is driven by the enthalpic and entropic gain and the release of water molecules from the cavity to the bulk phase. This is why cyclodextrins has been used to increase water solubility of normally hydrophobic compounds.

Effect of β-CD on the solubility of safranal in aqueous buffer solution was determined by measuring the equilibrium concentrations of safranal with changing the concentration of β-CD (Figure 16). As shown on the figure, the aqueous solubility of safranal in the phosphate buffer (pH=7.4) in the absence of β-CD was 3.85 mM equivalent to 0.5786 mg/mL. Addition of β-CD enhanced the poor aqueous solubility

of safranal by 35%. The maximum solubility of safranal (5.217 mM equivalent to 749.9 mg/L) was obtained at a 1:2 molar ratio of safranal: β-CD concentration. Further increase of β-CD concentration was found insignificant on the solubility of safranal. Figure 16 also shows that aqueous solubility of safranal in the presence of β-CD exhibits a B_s-type curve described by Higuchi and Connors [Higuchi & Connors, 1965], specific to the formation of inclusion complexes with limited solubility. An apparent solubility constant (K_s) of 51.48 M^{-1} was calculated for pure safranal from the initial slope of phase solubility diagram and aqueous solubility of safranal in the absence of β-CD (S_0) using Equation (2) (Waleczek et al., 2003).

$$K_s = \frac{slope}{S_0(1-slope)} \qquad (2)$$
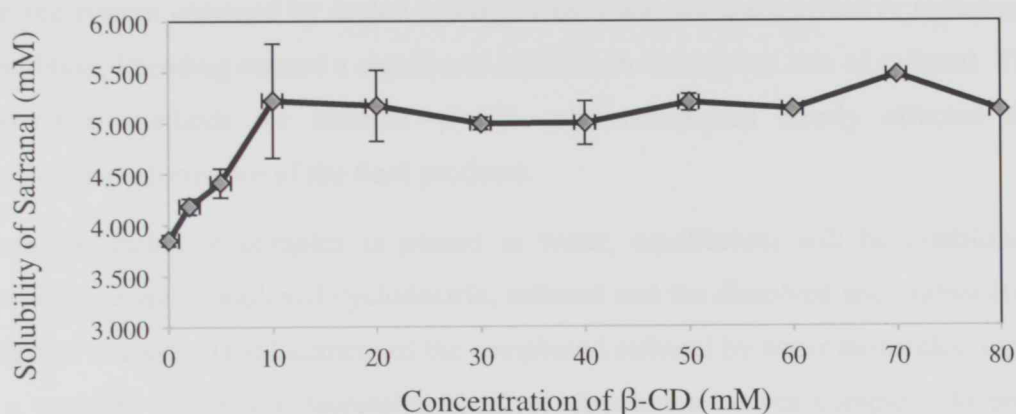
Figure 16. Effect of β-CD on phase solubility of safranal in phosphate buffer (pH=7.4) at 40°C

3.6   Dissolution Studies

Dissolution profiles of safranal–β-CD inclusion complexes obtained using different preparation methods were measured over time intervals up to 24 h (Figure 17, 18). Each test was repeated at least three times (coefficient of variation < 5%). The relative dissolution rates, calculated by dividing the concentration of safranal dissolved at any time by that obtained from pure safranal after the same time, show the performance of different preparation methods (Figure 19). Some products showed higher dissolution rate than safranal and they are in the order of SH > SC-CO₂ (at 55 °C, 100 bar) > KN

> PM (35 °C and 55 °C). While other products, namely, SC-CO$_2$ (at 35 °C, 100 bar; at 35 °C, 300 bar; at 55 °C, 300 bar) and COE resulted in lower dissolution rates than safranal. The reduction could be due to the strength of binding that depends on how well the 'host–guest' inclusion complex fits together. More significant changes ($\Delta\delta$) in IR frequencies using SC-CO$_2$ (at 35 °C, 100 bar; at 35 °C, 300 bar; at 55 °C, 300 bar) confirm the strength of binding and a good fit in inclusion complex. H-NMR also showed SC-CO$_2$ is the most effective method and pressure has more influence on the formation of inclusion complex. The increased dissolution rate of the physical mixture in comparison with safranal alone can be attributed to the improved powder wettability causing safranal to maintain contact with β-CD surface, as well as to the formation of readily soluble complexes in the dissolution medium [Al-Marzouqi et.al., 2007]. The best product obtained by using SC-CO$_2$ (55 °C, 100 bar) showed dissolution properties similar to those of the kneading product and only slightly lower than the system obtained by sealed-heating, which was the most effective technique. In addition, kneading caused a significant increase in dissolution rate of safranal. The preparation methods for safranal$-\beta$-CD solid complexes clearly affected the dissolution performance of the final products.

When the inclusion complex is placed in water, equilibrium will be established between free and complexed cyclodextrin, safranal and the dissolved and undissolved inclusion complex. Displacement of the complexed safranal by water molecules might be a possible reason for decreasing trend of dissolution curves compared to pure safranal [Del Valle, 2004].

Figure 17. Mean dissolution curves of safranal from the 1:2 inclusion complexes with
β-CD obtained by different preparation methods first 60 minutes

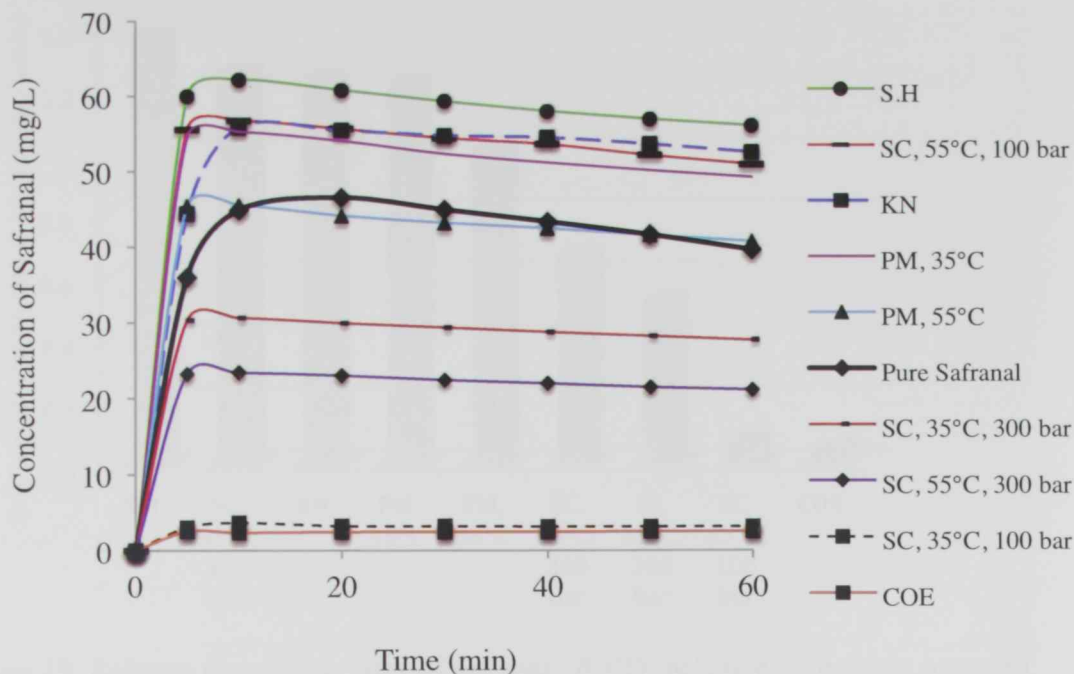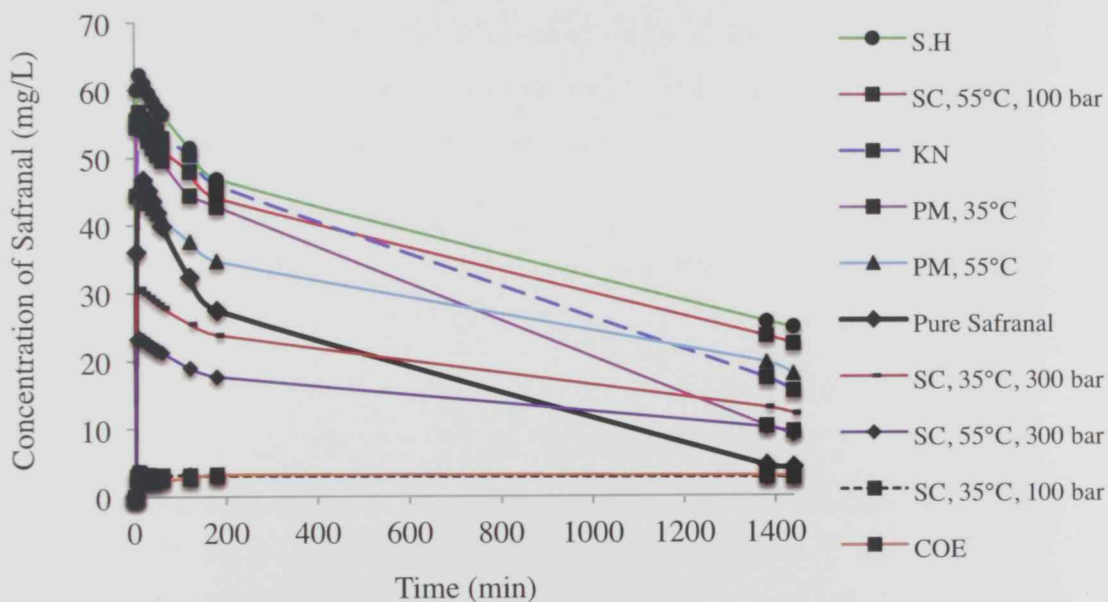Figure 18. Mean dissolution curves of safranal from the 1:2 inclusion complexes with
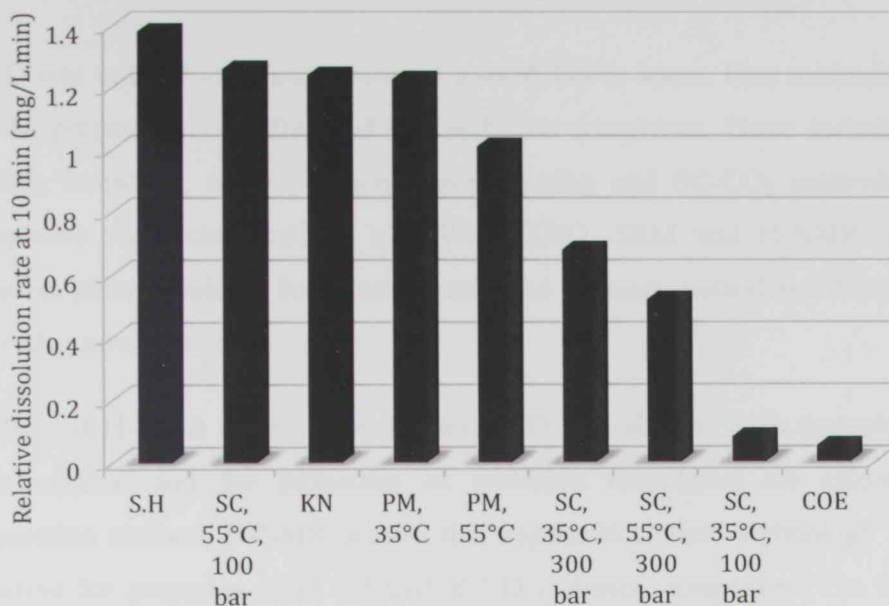β-CD obtained by different preparation methods a complete day (24 h)

Figure 19. Relative dissolution rates of safranal−β-CD inclusion complexes prepared by different methods after 10 min. Calculated by dividing the concentration of safranal dissolved at any time by that obtained from pure safranal after the same time

4 CONCLUSION

β-CD was utilized to enhance safranal's solubility in water. Five methods were used for the preparation of safranal–β-CD inclusion complexes. These included physical mixing, kneading, co-evaporation, sealed-heating and SC-CO_2 methods. Prepared complexes were characterized by FT-IR, XRD, SEM and H-NMR analysis. In addition, phase solubility based on Higuchi and Connors method and dissolution rates were also investigated.

FT-IR and H-NMR results showed that β-CD was able to form inclusion complex with safranal and the properties of inclusion complexes are affected by the preparation method. H-NMR proved that supercritical fluid technology is the most effective for preparing solid safranal–β-CD inclusion complexes. The influence of pressure on the formation of inclusion complexes was more than that of temperature for the products prepared by SC-CO_2.

According to the phase solubility diagram, β-CD gave rise to B_s-type curves with apparent solubility constant (K_s) of 51.48 M^{-1} indicating poor water solubility of safranal. Additionally β-CD up to 10.00 mM enhanced the phase solubility of safranal by about 35% in water solution. Further increase of β-CD concentration had insignificant effect on the aqueous solubility of safranal.

Dissolution studies showed safranal–β-CD inclusion products prepared by sealed-heating and SC-CO_2 (at 55 °C, 100 bar) had higher dissolution rate compared to pure safranal.

Effectiveness of SC-CO_2 method on the formation of safranal–β-CD inclusion complex is significant as shown by FT-IR and H-NMR analysis and dissolution performance of the final products. In comparison with other conventional methods, SC-CO_2 has advantages of avoiding the use of organic solvents, and therefore eliminates the need for removing the toxic solvent residuals. For the SC-CO_2 method, temperature and pressure had important roles on the formation of inclusion complexes. Pressure had more effect than temperature on the strength of inclusion complex formation. This may be due to the fact that as pressure increases the density of CO_2 increases, therefore, more safranal molecules may enter the β-CD cavity and

the formation of inclusion complexes may become easier and stronger with SC-CO$_2$ than with other conventional methods.

Efficacy, safety and pharmacokinetics of the current inclusion complexes obtained by different methods will be further investigated by both *in-vitro* and *in-vivo* experiments.

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الملخص

قد اجتذب الزعفران (Crocus. Sativus, Linn) الكثير من الاهتمام على مدى العقد الماضي بسبب وجود عدد كبير من المركبات النشطة بيولوجيا وألقوية مثل الكروسيتن (Crocetin) والبكروسين (Picrocrocin) و السفرنال (Safranal) وقد أظهرت الدراسات أن مادة السفرنال (Safranal) تتميز بقدرة عالية مضادة للأكسدة وللأنشطة السمية ضد عدة أنواع من الخلايا السرطانية (على سبيل المثال، سرطان الكبد) على حد سواء في المختبر وفي الجسم الحي. ومع ذلك، فإن ضعف قابلية الذوبان للسفرنال (Safranal) في المحاليل المائية قد قيدت بشكل واسع دراسة فاعليته و توافره البيولوجي في المسارات الدوائية في النظم البيولوجية.

تستخدم طريقة المجمعات المدرجة β-cyclodextrin (β-CD) في التطبيقات الصيدلانية لتغيير الخصائص الفيزيائية والكيميائية (مثل: الذوبان، التقلب، الاستقرار، التفاعلات الكيميائية والبيولوجية، والتوافر البيولوجي) للأدوية ضعيفة الذوبان في الماء.وبالتالي إن الهدف من هذا العمل التحقق من إمكانيات الأساليب المختلفة لإعداد المجمعات المدرجة (Safranal-β-CD) من أجل تعزيز قابلية الذوبان ومعدل الانحلال لمادة السفرنال (Safranal) في المحاليل المائية.

تم إعداد المجمعات المدرجة من (Safranal-β-CD) بتكوين قياسي متكافئ (01:02) باستخدام طرق مختلفة (مثل: العجن (KN)، التبخر المشترك (COE)، التدفئة المغلقة (SH)، وطريقة الحالة فوق الحرجة لثاني اوكسيد الكربون (SC-CO$_2$)). وتم تحديد الخصائص الفيزيائية للمجمعات المدرجة باستخدام طرق مختلفة (مثل: طريقة تحويل فورييه للطيف بالأشعة تحت الحمراء(FT-IR)، طريقة مسحوق الحيود للأشعة السينية (XRD)، وباستخدام المسح بالمجهر الإلكتروني (SEM) ,وطريقة البروتون النووي لتحليل الطيف بالرنين المغناطيسي (H-NMR)). وقد تمت دراسة خاصتي الذوبان المرحلي والانحلال القياسي. كما تم أيضا بحث تأثيرات درجة الحرارة (35°C, 55°C) والضغط (300bar, 100bar) على المجمعات المدرجة باستخدام أسلوب (SC-CO$_2$).

أشارت النتائج إلى أن تشكيل المجمعات المدرجة (Safranal-β-CD) قد تأثرت بطريقة الإعداد المستخدمة، وأثبتت أنّ استخدام (SC-CO$_2$) أكثر فعالية من التقنيات التقليدية. وأشار التحليل باستخدام (FT-IR) و (H-NMR) إلى تشكيل المجمعات المدرجة بين (β-CD) و (Safranal) باستخدام الطريق المختلفة. تم الحصول على نوعي الذوبان (A, B) مع ثابت ذوبان (K$_s$=51.48M^{-1}) لسفرنال (Safranal) من المنحدر الأولي من مخطط الذوبان المرحلي.

زادت قابلية الذوبان لسفرنال (Safranal) من 3.852ملم، إلى 5.217 ملم في وجود 10.00 ملم من (β-CD) لذلك تم تعزيز قابلية الذوبان للمرحلة الأولى لسفرنال (Safranal) إلى 35% في المحلول المائي. وأظهرت الدراسة أن معدل انحلال المجمعات المدرجة قد يكون أسرع منه في خليط نقي المادية من (Safranal) أو حتى في خليط يحتوي على (Safranal-β-CD). وبالتالي فانّ (β-CD) يمكن أن يكون من المفيد بالنسبة لتركيبات السفرنال الصلبة (Safranal). كما أظهرت الدراسة أن المنتجات الخالية من المذيبات التي تم إعدادها بطريقة (SC-CO$_2$) تتميز بذوبان مائي عالي ويمكن أن توفر الحد الأدنى من الآثار الجانبية للاستخدام البشري.

جامعة الامارات العربية المتحدة

كلية الهندسة

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دراسة الصفات الفيزيوكيميائية للمجمعات المدخلة لمادة السفرنال

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ساناز عباس زادكان

رسالة مقدمة لاستكمال متطلبات الحصول على درجة الماجستير في الهندسة الكيميائية

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بإشراف د. علي المرزوقي

سبتمبر ٢٠١٣

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