Drug-Cyclodextrin Complexes: An Approach to Enhance the Solubility and Dissolution Properties of Poorly Soluble Drugs

By

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DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not concurrently being submitted for any degree other than that of Doctor of Philosophy being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised the work of others.

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and my sweet family.

ABSTRACT

The main objective of this study was to investigate different manufacturing processes claimed to promote inclusion complexation between different drugs and cyclodextrins (econazole and α-cyclodextrin; indomethacin and methyl-β-cyclodextrin; olanzapine and methyl-β-cyclodextrin; flurbiprofen and methyl-β-cyclodextrin) in order to enhance the apparent solubility and dissolution properties of drugs. Specifically, the effectiveness of supercritical carbon dioxide processing for the preparation of solid drug-cyclodextrin inclusion complexes was investigated and compared to other preparation methods. Nitrate, besylate, sulfosalicylate dihydrate and maleate salts of econazole were synthesised. The solid drug-cyclodextrin inclusion complexes were prepared by physical mixing, freeze drying from aqueous solution and processing with supercritical carbon dioxide. The complexes were evaluated by scanning electron microscopy, differential scanning calorimetry, X-ray powder diffraction, ^{1}H-nuclear magnetic resonance (nuclear Overhauser effect correlation spectroscopy and inversion recovery T_1 measurement experiments), and dissolution rate studies. Inclusion yield (%) studies of econazole base into α- and methyl-β-cyclodextrin were conducted in supercritical carbon dioxide to investigate the influence of pressure, temperature and contact time on the inclusion. All the working parameters (pressure, temperature and contact time) played a significant role in the inclusion of econazole base into cyclodextrins. Isothermal titration calorimetric studies of econazole besylate and sulfosalicylate dihydrate salts and α-cyclodextrin confirmed the formation of complexes between the salts and α-cyclodextrin in a 1:1 stoichiometry. Different degrees of crystallinity were observed in the analyses of products prepared by various methods, suggesting the possibility of drug-cyclodextrin interactions of different efficiencies, which may give rise to different degrees of inclusion formation and/or crystallinity of the sample. Nevertheless, products obtained by the freeze-drying and supercritical carbon dioxide-inclusion methods were among the ones showing the highest interaction between the drug and the cyclodextrin. All systems based on α-cyclodextrin and methyl-β-cyclodextrin exhibited greater drug release profiles than the drug alone. Solid state complexation using supercritical carbon dioxide processing proved to be useful complexation method for econazole and its salts into α-cyclodextrin; indomethacin, olanzapine and flurbiprofen into methyl-β-cyclodextrin. The freeze drying method produced highly amorphous and rapid dissolving complexes; however, it was characterised by long, energy-intensive processing steps. Supercritical carbon dioxide inclusion method was shown to be an efficient approach for the preparation of solid-state inclusion complexes. It is an efficient and economic process that allows the formation of solid complexes based in strong intermolecular forces in high yield in a single step avoiding the use of organic solvents and the problems associated with their residues.

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Original Research Articles

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Rudrangi, S.R.S., Trivedi, V., Mitchell, J.C., Alexander, B.D., Wicks, S.R., 2015b. Preparation of Olanzapine and Methyl-β-Cyclodextrin Complexes Using a Single-Step, Organic Solvent-free Supercritical Fluid Process: An Approach to Enhance the Solubility and Dissolution Properties. Int. J. Pharm. 494(1), 408-416.

Original Research Articles Ready for Submission

Rudrangi, S.R.S., Trivedi, V., Mitchell, J.C., Alexander, B.D. and Wicks, S.R., 2015. Econazole-Cyclodextrin Complexes: An Approach to Enhance the Solubility and Dissolution Properties of a Poorly Soluble Drug

Rudrangi, S.R.S., Trivedi, V., Mitchell, J.C., Alexander, B.D. and Wicks, S.R., 2015. The Evaluation of Supercritical Fluid Technology as a Preparative Technique for the Preparation of Flurbiprofen-Methyl-β-Cyclodextrin Complexes.

Conference Proceedings (Selected)

Poster: Rudrangi, S.R.S., Al-Ashmouny, E., Alexander, B.D., and Wicks, S.R., 2014. Preparation and Characterisation of Econazole Besylate-α-Cyclodextrin Complexes: An Approach to Improve Solubility and Dissolution Rate. UK PharmSci-2014, Hertfordshire, UK.

Poster: Rudrangi, S.R.S., Al-Ashmouny, E., Alexander, B.D., and Wicks, S.R., 2014. Preparation and Characterisation of Econazole Sulfosalicylate Dihydrate-α-Cyclodextrin Complexes: An Approach to Improve Solubility and Dissolution Rate. UK PharmSci-2014, Hertfordshire, UK.

Podium and Poster: Rudrangi, S.R.S., Trivedi, V., Alexander, B.D., and Wicks, S.R., 2014. Preparation of Econazole-α-Cyclodextrin Complexes Using a Single-Step, Organic Solvent-Free Supercritical Fluid Process. 17th International Cyclodextrin Symposium (ICS-17), Saarbrücken, Germany.

Podium and Poster: Rudrangi, S.R.S., Trivedi, V., Alexander, B.D., and Wicks, S.R., 2013. Solid State Econazole-Cyclodextrin Complexes Prepared By Supercritical Carbon Dioxide Processing. 3rd European Conference on Cyclodextrins, Antalya, Turkey.

Podium and Poster: Rudrangi, S.R.S., Trivedi, V., Alexander, B.D., and Wicks, S.R., 2013. The Evaluation of Supercritical Fluid Technology as a Preparative Technique for the Manufacture of Econazole-Cyclodextrin Complexes: A Comparison with Conventional Methods. UK PharmSci-2013, Edinburgh, UK.

Poster: Rudrangi, S.R.S., Trivedi, V., Alexander, B.D., and Wicks, S.R., 2013. Influence of the Preparation Method on the Physicochemical Properties of Binary Systems of Econazole with Cyclodextrins. UOG-AAPS Student Chapter Conference, London, UK.

Chapter1: Introduction

1.0 Background

The poor water solubility and the resulting low oral bioavailability of drugs are two of the major challenges encountered by drug discovery and development scientists (Lipinski, C.A. *et al*., 1997; Lipinski, C.A., 2000). Drug solubility, permeability, lipophilicity, pK_a, and stability are five physicochemical parameters that are mainly considered in the early screening of new compounds. Owing to poor water solubility, nearly 40% of new chemical entities fail to reach the market in spite of exhibiting useful pharmacodynamic activities (Lipinski, C.A., 2002). Nearly 50% of drugs that are orally administered encounter formulation problems owing to their high lipophilicity (Gursoy, R.N., Benita, S., 2004). Poorly water soluble drugs also exhibit slow drug dissolution, which ultimately leads to insufficient and erratic bioavailability (Amidon, G. *et al*., 1995; Leuner, C., Dressman, J., 2000).

Formulation approaches to enhance water solubility are therefore very important. The main approaches used by the pharmaceutical industry to enhance the solubility and dissolution rate of drugs include: physical modifications, *e.g.* micronisation, nanosuspension and engineering solid dispersions; modification of the crystal habit, *e.g.* polymorphs, amorphous forms and co-crystallisation; chemical modifications, *e.g.* salt formation and inclusion complexation (Rasenack, N., Müller, B., 2002; Pandya, P. *et al*., 2008; Shiraki, K. *et al*., 2008; Thommes, M. *et al*., 2011; Ober, C., Gupta, R., 2012; Dahan, A. *et al*., 2013; Gao, L. *et al*., 2013; Rudrangi, S.R.S. *et al*., 2015). These approaches are not always commercially useful as they have serious limitations including, physical instability on storage, the risk of precipitation from the solid amorphous state, chemical degradation induced by mechanical stress caused by milling and grinding and difficulties associated

with the removal of toxic organic solvents (Serajuddin, A.T.M., 1999). Salt formation and inclusion complexation of drug molecules with cyclodextrins are the two main commercial approaches for improving solubility and dissolution rate of drugs (Loftsson, T. *et al*., 2005).

1.1 A Brief History of Cyclodextrins

Cyclodextrins were first isolated in 1891 by the French scientist A.Villiers as degradation products of starch. They were originally named as "cellulosines" (Villiers, A., 1891; Eastburn, S.D., and Tao, B.Y., 1994; Appel, E.A. *et al*., 2012). The foundations of cyclodextrin chemistry were laid by the Austrian microbiologist Franz Schardinger between 1903 and 1911 when he discovered both α- and β-cyclodextrins (French, D. *et al*., 1949). It was not until 1938 that Karl Freudenberg suggested the existence of even larger cyclodextrins on the occasion of the discovery of γ-cyclodextrin (Freudenberg, K., and Meyer-Delius, M., 1938). Freudenberg and his co-workers reported in the late 1930s that cyclodextrins were cyclic oligosaccharides composed of glucose units (Freudenberg, K., and Jacobi, R., 1935). The ability of cyclodextrins to form inclusion complexes was later described by Cramer and his co-workers (Freudenberg, K., and Cramer, F., 1948).

By the early 1950s the chemical structures of α-, β- and γ-cyclodextrins were elucidated, and their ability to solubilise and stabilise drugs was discovered by Freudenberg and his co-workers. Freudenberg, Cramer and Plieninger obtained the first cyclodextrin-related patent entitled "Method for preparation of inclusion compounds of physiologically active organic compounds" in 1953 (Freudenberg, K. *et al*., 1953).

In 1976, the world's first natural cyclodextrin-containing pharmaceutical product, prostaglandin-E2/β-cyclodextrin (Prostarmon ETM® sublingual tablets), was marketed in Japan by Ono Pharmaceutical Co. Later Brauns and Mueller (Mueller, B.W., Brauns, U.,

1985), and Pitha (Pitha, J., 1988) filed patents on the modified cyclodextrin (2-hydroxypropyl)-β-cyclodextrin that had been adapted for use in parenteral formulations between 1983 and 1985. Stella and Rajewski (Stella, V., Rajewski, R., 1992) patented sulphobutylether-β-cyclodextrin in 1992 which became the second pharmaceutically important modified cyclodextrin for use in parenteral formulations (Del Valle, E.M.M., 2004; Kurkov, S.V., Loftsson, T., 2013).

By the late 1990s many cyclodextrin-containing pharmaceutical products appeared on the European and the US markets. A century after their discovery, cyclodextrins are still regarded as physicochemically enigmatic molecules with unexplored potential and many novel cyclodextrin-based drug delivery technologies continue to be developed.

1.2 Cyclodextrin Structure

1.2.1 The primary chemical structure

Cyclodextrins, also known as cyclomaltoses, cycloamyloses and Schardinger dextrins, are macrocyclic oligomers of α-D-glucose with a hydrophilic exterior and a lipophilic central cavity (Villiers, A., 1891; French, D. *et al*., 1949; Eastburn, S.D., and Tao, B.Y., 1994; Del Valle, E.M.M., 2004; Appel, E.A. *et al*., 2012). Cyclodextrins are composed of α-D-glucopyranose units (Figure 1.1) which are bound via α-1,4-glycosidic linkages (Figure 1.2) (Del Valle, E.M.M., 2004).

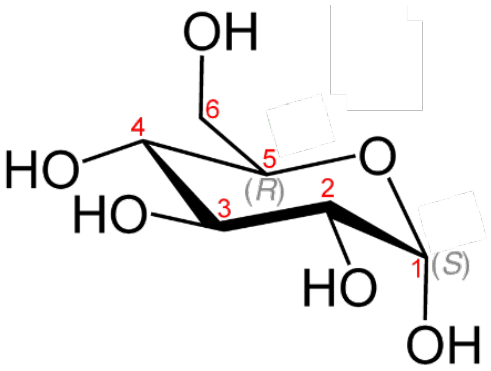

Figure 1.1: The structure and numbering convention of α-D-glucopyranose. Chemical modification is achieved by ether bridging of the hydroxyl groups in the 2, 3 and 6 positions. Hydroxyl groups in the 1 and 4 positions form the α-1,4-glycosidic linkage (Del Valle, E.M.M., 2004).

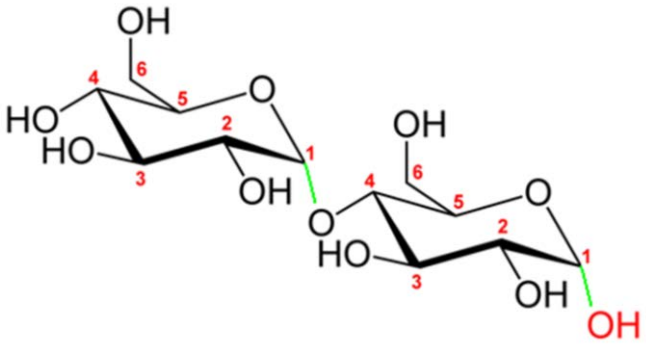

Figure 1.2: The structure and numbering convention of two α-D-glucopyranose molecules linked by an α-1,4-glycosidic linkage (Del Valle, E.M.M., 2004).

They are naturally occurring rigid and bucket shaped structures, obtained via an intramolecular transglycosylation reaction where starch is degraded by the enzyme cyclodextrin glucanotransferase, which is obtained from the bacterium *Bacillus macerans* (Van der Veen, B.A. *et al*., 2000; Van der Maarel, M.J.E. *et al*., 2002; Del Valle, E.M.M., 2004; Loftsson, T., *et al*., 2005; Kurkov, S.V., Loftsson, T., 2013).

1.2.2 The nanomolecular structure of cyclodextrins

Cyclodextrins are cyclic oligomers and consist of six, seven or eight α-D-glucopyranose units (α, β and γ, respectively). Figure 1.3 shows the structure of the six-membered ring

that can be viewed as the general structure of higher-order cyclodextrins, *i.e.* β and γ (Pinho, E., *et al*., 2014).

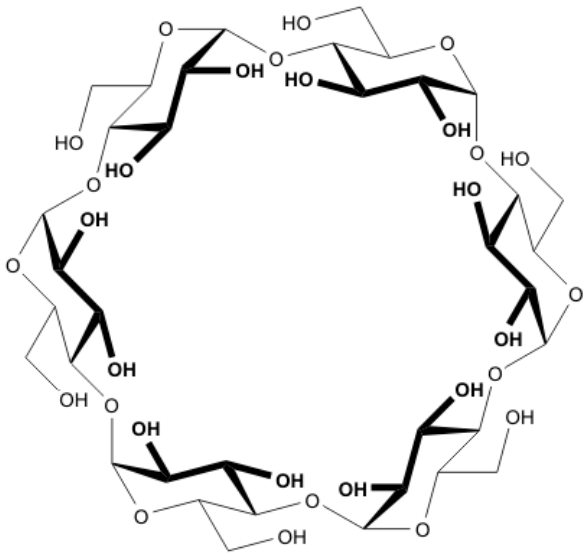

Figure 1.3: The structure of α-cyclodextrin showing the cyclic oligomeric structure achieved by the linkage of the six component α-D-glucopyranose molecules fused by α-1,4-glycosidic linkages (Loftsson, T., *et al*., 2005).

Cyclodextrins have a polar (hydrophilic) exterior and a relatively non-polar (hydrophobic) interior cavity (Figure 1.4) (Szejtli, J., 1998; Dodziuk, H., 2006).

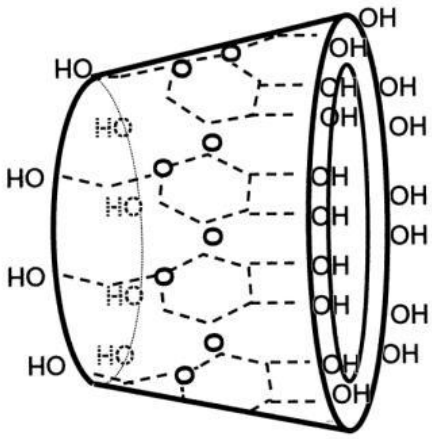

Figure 1.4: The structure of γ-cyclodextrin showing the toroidal nanostructure. The hydroxyl groups in the 6-position of the α-D-glucopyranose molecules are positioned on the narrower "rim"; those at the 2- and 3- positions are situated on the wider "rim" (Loftsson, T., *et al*., 2005).

The interior cavity of cyclodextrin can harbour a poorly water soluble drug, while the exterior increases its apparent water solubility (Bodor, N.S., 1993; Rudrangi, S.R.S. *et al*., 2015). The $^4C_1$-chair conformation of the glucose monomers gives rise to "molecular bucket or cage" like structures (Gregoriadis, G., and McCormack, B., 1995; Hierlemann, A. *et al*., 1999; Mati, S.S. *et al*., 2013). The oligomers of glucose form into a hollow truncated cone shape with the primary (6-) hydroxyl groups located on the narrow rim of the torus and the secondary (2- and 3-) hydroxyl groups on the wider rim (Szejtli, J. and Osa, T., 1996) as shown in the Figure 1.4. The non-bonding electron pairs of the glycosidic oxygen bridges are directed towards the centre of the cavity, producing a high electron density and lending the cyclodextrin a Lewis base character (Skinner, P.J. 1999). The physico-chemical properties of the natural cyclodextrins are shown in Table 1.1 (Li, S., Purdy, W.C., 1992; Sabadini, E., *et al*., 2006; Zhang, J., Ma, P.X., 2010).

Table 1.1: Physicochemical and nanostructural properties of α, β and γ-cyclodextrins

Property	**α-Cyclodextrin**	**β-Cyclodextrin**	**γ-Cyclodextrin**
Glucopyranose units	6	7	8
Cavity depth (nm)	0.79	0.79	0.79
Cavity diameter (nm)	0.47-0.53	0.60-0.65	0.75-0.83
Molecular weight (Dalton)	972.84	1134.98	1297.12
Solubility (g l^{-1})	129.5 ± 0.7	18.4 ± 0.2	249.2 ± 0.2
Melting temperature range (°C)	250-260	255-265	240-245

1.2.3 Natural and modified cyclodextrins

Cyclodextrins may be classified into two types: natural and modified. Modified cyclodextrins are also referred to as substituted or derivatised cyclodextrins. Natural cyclodextrins consist of six to thirteen α-D-glucopyranose units. The most commonly used

pharmaceutical cyclodextrin excipients are α, β and γ-cyclodextrins. Natural cyclodextrins have limited aqueous solubility because of strong intermolecular hydrogen bonding in the crystalline state. It is now well known that the disruption of hydrogen bonding via molecular substitution gives rise to enhanced water solubility. This semi-synthetic approach has given rise to a wide range of modified cyclodextrins (Davis, M.E. and Brewster, M.E., 2004; Hakkarainen, B., *et al*., 2005).

Modified cyclodextrins are the derivatives of natural cyclodextrins, resulting from the reaction of functional groups such as methyl, dimethyl, hydroxypropyl, sulfobutylether with the 2-, 3- and 6-hydroxyl groups of the α-D-glucopyranose units. Derivatisation interrupts the strong intermolecular hydrogen bonding of natural cyclodextrins eliminating crystallinity and enhancing the aqueous solubility. Modified cyclodextrins are typically amorphous in nature (Hakkarainen, B., *et al*., 2005). Many chemically modified cyclodextrins have been prepared and the cyclodextrin derivatives of biomedical and pharmaceutical interest are listed in Table 1.2 (Rajewski, R.A, Stella, V.J., 1996; Davis, M.E. and Brewster, M.E., 2004; Loftsson, T., *et al*., 2005).

Table 1.2: Cyclodextrins and their derivatives of pharmaceutical interest.

Cyclodextrin	**Common abbreviation**	**Type**
α-Cyclodextrin	α-CD	Natural
β-Cyclodextrin	β-CD	Natural
γ-Cyclodextrin	γ-CD	Natural
(2-Hydroxypropyl)-α-cyclodextrin	HP-α-CD	Derivatised
(2-Hydroxypropyl)-β-cyclodextrin	HP-β-CD	Derivatised
(2-Hydroxypropyl)-γ-cyclodextrin	HP-γ-CD	Derivatised
Methyl-β-cyclodextrin	M-β-CD	Derivatised
Dimethyl-β-cyclodextrin	DM-β-CD	Derivatised

Sulfobutylether-β-cyclodextrin	SBE-β-CD	Derivatised

1.2.4 The pharmaceutical uses of cyclodextrins

Cyclodextrins are mainly employed as complexing agents in the pharmaceutical industry to enhance the aqueous solubility of poorly water-soluble drugs and to improve drug stability and bioavailability (Miyake, K. *et al*., 1999; Siefert, B. *et al*., 1999; Kim, C. and Park, J., 2004; Avdeef, A. *et al*., 2007; Brewster, M.E. and Loftsson, T., 2007; Rudrangi, S.R.S. *et al*., 2015). Clinically and pharmaceutically, they also prevent or reduce ocular and gastrointestinal irritation, eliminate or reduce taste or odour (Szejtli, J., Szente, L., 2005; Lantz, A.W. *et al*., 2006), prevent drug-drug or drug-additive interactions (Brewster, M.E. and Loftsson, T., 2007), in addition to converting liquid drugs and oils into amorphous or microcrystalline powders (Hirayama, F. and Uekama, K., 1999).

Cyclodextrins improve the water solubility of drugs by forming non-covalent, dynamic, water-soluble inclusion complexes. They eliminate the crystallinity of drugs by specific inclusion physical bonding or by non-specific vitrification to form solid dispersions (Londhe, V. and Nagarsenker, M., 1999). The ability of cyclodextrins to form inclusion complexes during a mutual dissolution process can result in enhanced drug dissolution even if there occurs no specific complexation in the solid state (Becket, G. *et al*., 1999). A substantial increase in apparent dissolution rate has been reported for drugs such as piroxicam, ibuprofen and cinnarizine after inclusion in β-cyclodextrin (Tokumura, T. *et al*., 1985; Woodcock, B.G. *et al*., 1993), for benzodiazepines and digoxin included in γ-cyclodextrin (Dressman, J.B. *et al*., 1990) and for indomethacin after inclusion in methyl-β-cyclodextrin (Rudrangi, S.R.S. *et al*., 2015). Table 1.3 lists the examples of the use of cyclodextrins to enhance drug solubility and dissolution rate.

Table 1.3: Examples of drugs where cyclodextrin enhanced apparent drug solubility and dissolution rate has proven to be clinically useful. See Table 1.2 for the key to common abbreviations.

Cyclodextrin	Drug	Reference
α-CD	Praziquantel	(Becket, G. *et al*., 1999)
β-CD	Nimesulide	(Chowdary, K.P.R. and Nalluri, B.N., 2000)
	Sulfamethizole	(Pose-Vilarnovo, B. *et al*., 2001)
	Lorazepam	(Sanghavi, N.M. *et al*., 1993)
	Ketoprofen	(Ann, H.J. *et al*., 1997)
	Piroxicam	(Cavallari, C. *et al*., 2002)
	Itraconazole	(Chowdary, K.P.R. and Rao, S.S., 2001)
	Ibuprofen	(Ghorab, M.K. and Adeyeye, M.C., 2001)
	Praziquantel	(Becket, G. *et al*., 1999; Arias, M.J. *et al*., 2000)
γ-CD	Praziquantel	(Becket, G. *et al*., 1999)
	Omeprazole	(Arias, M.J. *et al*., 2000)
	Digoxin	(Uekama, K. *et al*., 1983)
HP-β-CD	Albendazole	(Castillo, J.A. *et al*., 1999)
	DY-9760e	(Nagase, Y. *et al*., 2001)
	Itraconazole	(Chowdary, K.P.R. and Rao, S.S., 2001)
	Carbamazepine	(Brewster, M.E. *et al*., 1991)
	Zolpidem	(Trapani, G. *et al*., 2000)
	Phenytoin	(Latrofa, A. *et al*., 2001)
	Rutin	(Miyake, K. *et al*., 2000)
M-β-CD	Indomethacin	(Rudrangi, S.R.S. *et al*., 2015)
DM-β-CD	Naproxen	(Bettinetti, G. *et al*., 1992)
	Camptothecin	(Kang, J. *et al*., 2002)
SBE-β-CD	Danazol	(Jain, A.C. and Adeyeye, M.C., 2001)
	Fluasterone	(Zhao, L. *et al*., 1999)
	Spironolactone	(Kaukonen, A.M. *et al*., 1998)

The utility of natural cyclodextrins as drug carriers is restricted by their low aqueous solubility. Hydroxyalkylated derivatives of cyclodextrins such as (2-hydroxypropyl)-α-cyclodextrin, (2-hydroxypropyl)-β-cyclodextrin and (2-hydroxypropyl)-γ-cyclodextrin have a higher aqueous solubility than the parent natural cyclodextrins and can produce more soluble complexes with drugs.

Improving the apparent solubility of poorly soluble drugs using inclusion complexes generally enhances bioavailability (Miyake, K. *et al*., 1999; Kim, C. and Park, J., 2004). Whether the enhancement in dissolution rate leads to an enhancement in bioavailability depends on whether the rate and/or extent of absorption is limited predominantly by dissolution when the oral dosage form is administered. When administered in an oral capsule with (2-hydroxypropyl)-β-cyclodextrin, the antihistamine cinnarizine showed a significant enhancement in bioavailability in beagle dogs, probably due to the more rapid rate of dissolution (Brewster, M.E. *et al*., 1991). It was demonstrated by Miyake and co-workers (Tinwalla, A. *et al*., 1995; Järvinen, T. *et al*., 1995) that dimethyl-β-cyclodextrin significantly improved the aqueous solubility and oral bioavailability of the immunosuppressant drug cyclosporine.

Inclusion complexes with cyclodextrins are known to improve the chemical and physical stability of drugs both in solution and in the solid state (Saenger, W., 1980; Szejtli, J. (Ed.), 1982; Uekama, K. and Otagiri, M., 1987; Szejtli, J. (Ed.), 1988; Brewster, M.E., 1991; Fromming, K.H. and Szejtli, J. (Eds.), 1994; Loftsson, T., 1995).

Cyclodextrins can protect against hydrolysis, degradation, photodecomposition and oxidation and hence increase the shelf life of drugs. Matsuda and Arima reported that cyclodextrins may enhance the stability of drugs by inhibiting the interaction of drug with formulation vehicles and by inhibiting bioconversion of the drug at the site of absorption (Matsuda, H. and Arima, H., 1999). Cyclodextrins shield the unstable drugs by

encapsulating them at the molecular level and therefore insulate them against several degradation processes. Table 1.4 lists examples of cyclodextrin-enhanced drug stability.

Table 1.4: Examples of cyclodextrin-enhanced drug stability

Stabilisation	Drug	Cyclodextrin	Reference
Thermal (solid state)	Diclofenac sodium	β-CD	(Cwiertnia, B. *et al*., 1999)
Photo	Flutamide	β-CD	(Sortino, S. *et al*., 2001)
Photo	DY-9760e	SBE-β-CD	(Nagase, Y. *et al*., 2001)
	Promethazine	HP-β-CD, DM-β-CD	(CyDex Inc Web site)
Acid hydrolysis/photo	Doxorubicin	HP-β-CD, HP-γ-CD	(Brewster, M.E. *et al*., 1992)
Hydrolysis	Digoxin	γ-CD	(Uekama, K. *et al*., 1983)
	Rutin	HP-β-CD	(Miyake, K. *et al*., 2000)
	Camptothecin	RDM-β-CD	(Kang, J. *et al*., 2002)
	Melphalan	SBE-β-CD, HP-β-CD	(Ma, D.Q. *et al*., 2002)
	Carmustine	SBE-β-CD, HP-β-CD	(Ma, D.Q. *et al*., 2002)
Degradation by	Spironolactone	SBE-α-CD, SBE-β-CD,	(Jarho, P. *et al*., 2000)
deacetylation		HP-β-CD, γ-CD, β-CD	(Jarho, P. *et al*., 2000)
Intramolecular cyclisation	Quinapril	β-CD, HP-β-CD	(Li, J. *et al*., 2002)
(solid state)			

1.3 Ternary Complexes or the Interaction of Cyclodextrins with Pharmaceutical Salts

The formation of ternary complexes is reported to enhance the complexation efficiency of drugs and cyclodextrins. The addition of small amounts of a third component (a conjugate acid/base) into the system has been studied (Loftsson, T., *et al*., 1994). The apparent enhancement in solubilisation efficiency, and the consequent increased dissolution rate of the drug, is attributed to a beneficial stabilisation of the primary interaction between the drug and cyclodextrin. The formation of ternary complexes has the clinical and economic

benefit that it allows a reduction in the amount of cyclodextrin needed for inclusion and hence used in formulations (Loftsson, T., *et al*., 1994).

The formation of ternary complexes or interaction of cyclodextrins with pharmaceutical salts will form a major part of this PhD program. Therefore, a detailed discussion of this topic is given in the introduction section of Chapter III.

1.4 Processes Used in the Preparation of Drug-Cyclodextrin Inclusion Complexes

Drug-cyclodextrin inclusion complexes that form part of a solution are prepared by adding solid drug to an aqueous solution of the cyclodextrin whilst continuously stirring at ambient temperature (Mura, P. *et al*., 1999). An increase in apparent solubility results and this can be used to make useful medicines from poorly water soluble drugs. Solid-state inclusion complexes are prepared using the following processes: physical mixing (Mura, P. *et al*., 1999; Reddy, M.N. *et al*., 2004), kneading (Reddy, M.N. *et al*., 2004; Cirri, M. *et al*., 2005), solvent evaporation (Al-Marzouqi, A.H. *et al*., 2009), freeze drying (Cao, F. *et al*., 2005; Bankar, P.V. and Mahatma, O., 2012), spray drying (Vozone, C. and Marques, H.C., 2002) and supercritical fluid processing (Van Hees, T. *et al*., 2002; Charoenchaitrakool, M. *et al*., 2002; Bandi, N. *et al*., 2004; Perrut, M. *et al*., 2005; Rodier, E. *et al*., 2005; Shehatta, I. *et al*., 2005; Al-Marzouqi, A.H. *et al*., 2006; Al-Marzouqi, A.H. *et al*., 2007a; Al-Marzouqi, A.H. *et al*., 2007b; Türk, M. *et al*., 2007; Rudrangi, S.R.S. *et al*., 2015).

1.4.1 Physical mixing

A physical blend or mixture of the solid forms of a cyclodextrin and a drug can be prepared by mechanical trituration or tumble mixing (Mura, P. *et al*., 1999; Reddy, M.N. *et al*., 2004). On the laboratory scale, physical blends are prepared by triturating the pure components together in a mortar then passing the blend through a suitable sieve.

1.4.2 Freeze drying

Freeze drying, also known as lyophilisation, is a suitable technique to get porous, amorphous drug products with high degree of interaction between drug and cyclodextrin (Cao, F. *et al*., 2005). The required quantity of drug is added to an aqueous solution of cyclodextrin while stirring. Water is removed through primary freezing and subsequent secondary drying of a solution containing both drug and cyclodextrin at reduced pressure. Thermolabile drugs can be processed using freeze drying techniques to avoid exposure of the drug to high temperatures. Long processing times and poor flow properties of the obtained complexes are limitations of this method of preparation. The work of Bankar and Mahatma is typical of many reports on dissolution enhancement, in this case describing leflunomide freeze-dried with (2-hydroxypropyl)-β-cyclodextrin (Bankar, P.V. and Mahatma, O., 2012).

1.4.3 Supercritical fluid processing

Inclusion complex formation techniques employing a supercritical fluid, most commonly carbon dioxide, as the primary processing solvent are emerging as an alternative to the use of organic solvents with the potential for high inclusion efficiencies (Van Hees, T. *et al*., 1999; Banchero, M. and Manna, L., 2011).

A supercritical fluid is a substance that exists above the critical temperature (T_C) and pressure (P_C). The critical temperature of a substance is the maximum temperature at which a gas can be converted into a liquid by an increase in pressure while the critical pressure of a substance is the maximum pressure at which a liquid can be converted into a gas (vapour) by an increase in the liquid temperature (Deshpande, P.B. *et al*., 2011). Under these conditions, no phase boundaries exist (Figure 1.5).

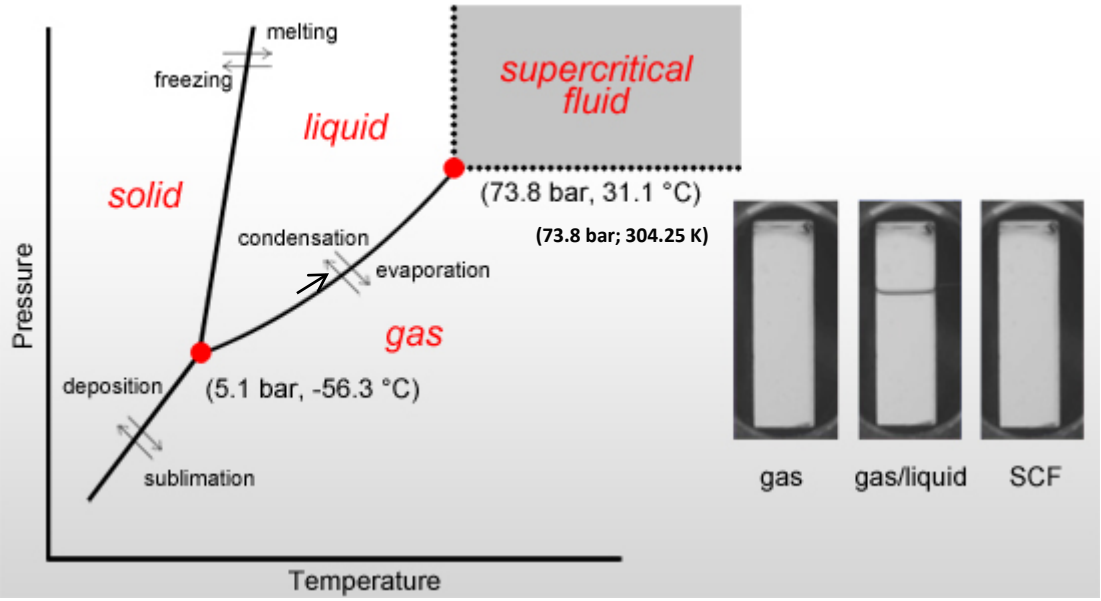

Figure 1.5: Carbon dioxide pressure-temperature phase diagram (Clemson University Restoration Institute, Warren Lasch Conservation Center).

In the supercritical region, the physicochemical properties of the substance including, viscosity, density, diffusion coefficient, and heat conductivity values are transitional between those of the liquid and the gas. A supercritical fluid can behave either as a liquid or as a gas *e.g.* it can diffuse through solid substances like a gas and dissolve them like a liquid. Small changes in temperature or pressure above the critical point can result in significant changes in density and modifies the physicochemical properties of the supercritical fluid.

Supercritical fluid technology has several commercial advantages. Rapid single-step processing is possible under mild and efficient processing conditions (temperature and pressure) and it is possible to reduce the particle size of the drug and other materials (Wong, J.M., Johnston, K.P., 1986; Young, J.L. and Simone, J.M., 2000; Cansell, F. *et al*., 2003; Dos Santos, I.R. *et al*., 2003; Thies, C. *et al*., 2003; Byrappa, K. *et al*., 2008; Lin, Y.-C., Jang, S.-M., 2008; Erkey, C., 2009). Organic solvents can be replaced by supercritical fluids in a wide range of laboratory and industrial processes (Sone, M. *et al*., 2013).

Removal of organic solvents from the drug product to the levels approved by the Food and Drug Administration is very challenging and therefore conventional techniques used for the preparation of inclusion complexes (co-evaporation, spray drying and kneading) involve several drying steps for considerable time, which may also affect the drug stability (Al-Marzouqi et al., 2006). Hence, it is highly recommended to eliminate the use of organic solvents in the preparation of drugs or drug-cyclodextrin complexes.

1.4.3.1 Supercritical carbon dioxide

Carbon dioxide behaves as a supercritical fluid above its critical temperature (31.25°C) and critical pressure (73.8 bar). Supercritical carbon dioxide is an odourless, colourless, non-hazardous, non-toxic, non-combustible, inexpensive, chemically stable solvent that can be readily removed from the drugs or drug-cyclodextrin complex particles by simple evaporation (Rudrangi, S.R.S. *et al*., 2015). Its non-toxic and non-combustible properties make it environmentally acceptable. Due to its very low critical point, carbon dioxide has been used extensively for chemical and pharmaceutical research. Supercritical carbon dioxide can enhance the apparent solubility and dissolution rate of poorly water soluble drugs by micronisation of drug particles (Van Hees, T. *et al*., 2002; Charoenchaitrakool, M. *et al*., 2002; Bandi, N. *et al*., 2004; Perrut, M. *et al*., 2005; Rodier, E. *et al*., 2005; Shehatta, I. *et al*., 2005; Al-Marzouqi, A.H. *et al*., 2006; Al-Marzouqi, A.H. *et al*., 2007a; Al-Marzouqi, A.H. *et al*., 2007b; Türk, M. *et al*., 2007; Lin, Y.-C., Jang, S.-M., 2008).

Many polar or semi-polar drugs of pharmaceutical interest are not soluble in supercritical carbon dioxide. Non-polar, lipophilic molecules tend to show better solubility. An approach to improve the solvation power of supercritical carbon dioxide and reduce miscibility pressures is the addition of a cosolvent. The addition of just a small quantity of cosolvent, which shall be discussed in the next section, increases the solubilising power of

the supercritical carbon dioxide-cosolvent binary solution, making it possible to dissolve polar drug molecules of clinical interest (Pöhler, H. and Kiran, E., 1997a; Pöhler, H. and Kiran, E., 1997b).

1.4.3.2 The solubilisation of drugs in supercritical carbon dioxide

Solvents that are Generally Recognized as Safe (GRAS solvents) by the US Food and Drug Administration are recommended for use in pharmaceutical applications as their residues are considered to be non-toxic. Ethanol, acetone and ethyl acetate are some examples of GRAS solvents used in supercritical carbon dioxide processing (Grandelli, H.E. *et al*., 2012). The rational use of cosolvents in supercritical carbon dioxide processing has not been widely investigated. Grandelli *et al*. explored the use of the acetone, ethanol and ethyl acetate in cyclodextrin-drug complexation (Grandelli, H.E. *et al*., 2012; Grandelli, H.E. *et al*., 2013; Grandelli, H.E. and Kiran, E., 2013).

Most drugs do not exhibit good solubility in supercritical carbon dioxide, and therefore a large number of studies employed carbon dioxide as an antisolvent. Mueller and Fisher first patented an antisolvent process where the antihypertensive drug clonidine hydrochloride and the polymer DL-polylactic acid in dichloromethane were sprayed into a vessel containing supercritical carbon dioxide. The diffusion of dichloromethane into supercritical carbon dioxide caused the polymeric drug molecules to precipitate entrapping the drug (Mueller, B.W. and Fisher, W., 1989).

Jun *et al*. prepared complexes between simvastatin and (2-hydroxypropyl)-β-cyclodextrin also using the supercritical antisolvent process (Jun, S.W. *et al*., 2007). A drug/cyclodextrin solution was prepared by dissolving drug and cyclodextrin (1:1 molar ratio) in a mixture of dichloromethane and ethanol (1:2% v/v). The supercritical process was performed at 40°C and 120 bar. The solution of the complex was sprayed into the

particle formation vessel containing supercritical carbon dioxide. The diffusion of dichloromethane and ethanol into supercritical carbon dioxide caused drug-cyclodextrin complexes to precipitate. The aqueous solubility and dissolution rate increased in the inclusion complex, compared with the physical mixture and the crystalline drug. The authors concluded that the supercritical antisolvent process is a useful method for the preparation of the complexes.

The use of supercritical carbon dioxide for the preparation of solid-state drug-cyclodextrin inclusion complexes has been investigated. Complexes have been manufactured with the cyclodextrin in suspension (α- or β-cyclodextrin) or dispersed above its melting point (methyl-β-cyclodextrin). Drug and cyclodextrin particles are placed into a reaction vessel with supercritical carbon dioxide at pre-set temperatures and pressures. The reaction conditions are maintained for pre-determined times after which the solid complex is recovered by simple depressurisation. The critical processing parameters for this method of producing solid-state drug cyclodextrin complexes are poorly understood and have not been the subject of a systematic study. The key features of studies reported in the literature are summarised in Table 1.5.

Table 1.5: Investigation of the use of supercritical carbon dioxide for the preparation of solid drug-cyclodextrin inclusion complexes[a].

			Effect of physical condition on Inclusion Yield					
Researchers	**API**	**Cyclodextrin**	**Pressure**	**Temperature**	**Time**	**API state (in $ScCO_2$)**	**Cyclodextrin (in $ScCO_2$)**	**Complex (Physical form)**
Van Hees, T. *et al.*, 2002.	Piroxicam	β-Cyclodextrin	Decreases	Increases	Increases	Solid - Below melting point	Solid - Below melting point	Crystalline
Charoenchaitrakool *et al.*, 2002	Ibuprofen	Methyl-β-cyclodextrin	Increases	Not reported	No effect	Solid - Below melting point	Molten - Above melting point	Amorphous
Bandi *et al.*, 2004	Budesonide	(2-Hydroxypropyl)-β-cyclodextrin	Not reported	Not reported	Not reported	Solid - Below melting point	Solid - Below melting point	Amorphous
Bandi *et al.*, 2004	Indomethacin	(2-Hydroxypropyl)-β-cyclodextrin	Not reported	Not reported	Not reported	Solid - Below melting point	Solid - Below melting point	Amorphous
Al-Marzouqi, A.H. *et al.*, 2007b.	Econazole nitrate	β-Cyclodextrin	No effect	Increases	Not controlled	Solid - Below melting point	Solid - Below melting point	Amorphous
Türk, M. *et al.*, 2007 [CPD]	Ibuprofen	β-Cyclodextrin	No effect	Positive	Not controlled	Solid - Below melting point[b]	Solid - Below melting point	Amorphous[c]
Rudrangi, S.R.S. *et al.*, 2015	Indomethacin	Methyl-β-cyclodextrin	Not reported	Not reported	Not reported	Solid - Below melting point	Molten - Above melting point	Amorphous

[a]M.P. = Melting Point; $ScCO_2$ = Supercritical carbon dioxide; CPD = Controlled Particle Deposition technique

[b] - The drug (Ibuprofen) was solubilized in supercritical carbon dioxide

[c] - Disappearance of X-Ray diffraction patterns (peaks) of Ibuprofen in the complex may indicate either the transformation of drug from the crystalline into the amorphous state or by the formation of an inclusion complex in which drug is embedded in the β-cyclodextrin cavity.

The majority of reported supercritical inclusion complex formation techniques, while effective, are limited by the drug solubility in carbon dioxide (Charoenchaitrakool, M. *et al.*, 2002)

1.4.3.3 Drug cyclodextrin complex formation in cosolvent-free supercritical carbon dioxide

Whilst much effort has been expended to solubilise drug molecules in supercritical carbon dioxide, *e.g.* with the use of cosolvents, there is growing evidence in the literature that this is unnecessary. Evidence now exists for the formation of drug and cyclodextrin complexes where either the drug or cyclodextrin, or both, remain suspended in supercritical carbon dioxide.

Rudrangi *et al.* (Rudrangi, S.R.S. *et al.*, 2015) investigated the effectiveness of a single-step, organic solvent-free supercritical carbon dioxide process for preparing inclusion complexes between indomethacin and methyl-β-cyclodextrin and compared this to other conventional methods. The drug-cyclodextrin mixtures were processed at different working conditions [35°C/100 bar, 35°C/200 bar, 45°C/100 bar and 45°C/200 bar] in order to study the influence of pressure and temperature on the inclusion complex formation. The mixtures were heated to the desired temperature and held for 1 hour before recovering the solid complex by depressurisation at a rate of 7-8 bar min^{-1}. The resulting complexes were highly amorphous and exhibited significantly higher dissolution rates in phosphate buffer (pH 7.4) than that of the drug alone and the complexes prepared by freeze drying and physical mixing.

Bandi *et al.* (Bandi, N. *et al.*, 2004) used a supercritical fluid carbon dioxide process to prepare budesonide and indomethacin complexes with (2-hydroxypropyl)-β-cyclodextrin. Drug-cyclodextrin mixtures were dispersed in supercritical carbon dioxide at a pressure of 211 bar and the contents heated to 30°C for 20 hours. The resulting complexes were assessed for crystallinity and dissolution rate. Complexes formed by using supercritical carbon dioxide were less crystalline compared with the physical mixtures with an

improved dissolution rate. The authors concluded that the changes in crystallinity and enhancement of dissolution rate indicated the formation of inclusion complexes.

Ali *et al.* (Al-Marzouqi, A.H. *et al.*, 2007c) investigated the influence of the supercritical carbon dioxide processing on the physicochemical properties of econazole complexes with β-cyclodextrin and compared with the conventional methods of physical mixing, co-evaporation and annealing at high temperature. Complexes with high amorphous content were produced when the physical mixtures were processed with supercritical carbon dioxide by heating to 130°C for 3 hours. Pressure changes (between 100 bar and 450 bar) made no difference to inclusion.

Türk *et al.* (Türk, M. *et al.*, 2007) used a process referred to as Controlled Particle Deposition (CPD), a technique intended for a controlled deposition of drug particles in porous carriers, to form complexes of ibuprofen and β-cyclodextrin using supercritical carbon dioxide. Ibuprofen first dissolves in supercritical carbon dioxide followed by permeation of the supercritical solution into the pores of the carrier and complexation of the drug inside the pores. The authors investigated the phase behaviour of the (RS±)-ibuprofen and R(-)-ibuprofen in carbon dioxide and observed that a solid-fluid two-phase equilibrium existed in the case of the (RS±)-ibuprofen in the temperature range from 31 to 47°C for each pressure while the melting points of the carbon dioxide/R(-)-ibuprofen system are below the vapour pressure of pure carbon dioxide. As a result, for R(-)-ibuprofen a solid-fluid two-phase equilibrium exists only at relatively low temperatures and pressures. Based on the phase behaviour studies, the authors performed the inclusion experiments at 30°C and at pressures of either 250 or 300 bar to ensure that only a solid-fluid two-phase equilibrium exists for each pressure. The drug content analyses revealed that, when the drug and cyclodextrin were loaded into separate cartridges and processed with supercritical carbon dioxide, about 88 % of the drug was included into the cavity of

cyclodextrin whereas, only 60.5 % of the drug was included into the cyclodextrin when the physical mixture was processed with supercritical carbon dioxide.

1.5 Characterisation of Inclusion Complexes

1.5.1 Differential scanning calorimetry

Differential scanning calorimetry is a thermoanalytical technique widely used to determine the melting point and other thermal transitions such as glass transition and crystallisation. The technique can also provide an estimate of the energetics of the solid-state transformations. This technique has been widely used to characterise drug-cyclodextrin complexes. The disappearance of the drug melting peak is more usually observed if a drug-cyclodextrin inclusion complex has been formed (Singh, R. *et al*., 2010). This is attributable to the inability of the drug to crystallize when included in the cyclodextrin cavities. The absence or disappearance of the drug melting peak indicates that the drug has become completely amorphous and does not ultimately indicate that a drug-cyclodextrin inclusion complex is the cause for drug amorphisation. As a result, differential scanning calorimetry alone is not capable of providing definitive proof of complex formation.

Manosroi *et al.* (Manosroi, J. *et al*., 2005) have employed differential scanning calorimetry for characterising the binary mixtures of azelaic acid with (2-hydroxypropyl)-β-cyclodextrin prepared by physical mixing, co-evaporation and freeze drying. Crystalline azelaic acid was characterized by a sharp melting endotherm at 105°C which corresponds to the melting point of the drug. (2-hydroxypropyl)-β-cyclodextrin is an amorphous oligomer and lacks a distinct thermal event. The physical mixture demonstrated the melting endotherm of azelaic acid, representing that an inclusion complex was not obtained by simple mixing of the pure components, whereas, the binary system formed by

a co-evaporation process had no distinct drug melting endotherm, suggesting that azelaic acid was incorporated in the cavity of (2-hydroxypropyl)-β-cyclodextrin.

1.5.2 Powder X-ray Diffraction

Powder X-ray diffraction is an analytical technique widely used in the solid-state characterisation of compounds. It provides information on the degree of crystallinity of a compound and the unit cell dimensions of the crystalline lattice. Changes in the crystallinity of drug or/and cyclodextrin may be observed in the diffraction pattern if a drug-cyclodextrin inclusion complex is formed. Complexation is not readily distinguishable from any polymorphic transformations that may occur in the drug molecules during the complex formation process.

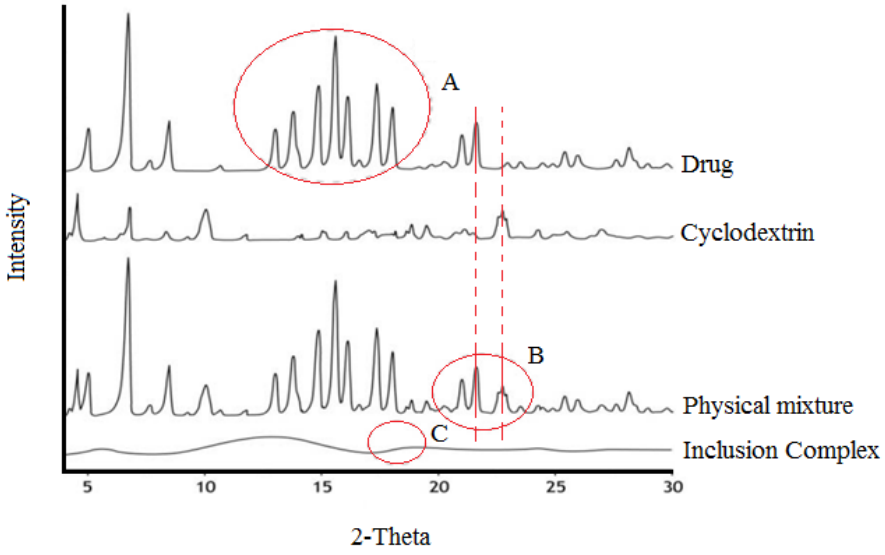

Figure 1.6: Representative typical X-ray diffractogram model of a pure drug, cyclodextrin, the physical mixture and the inclusion complex. **A** represents well-defined, narrow peaks of a crystalline component, **B** represents the overlap of the patterns of the drug and cyclodextrin, and **C** represents a region in the pattern devoid of peaks, which is characteristic of an amorphous complex (Takahashi, A.I., *et al*., 2011).

The diffraction pattern of a physical mixture is usually a sum of the patterns of the individual components (Singh, R. *et al*., 2010) whereas diffractograms of inclusion complexes shows new characteristics different from those observed for the individual components. A smooth, broad peak is usually observed in the diffraction pattern of an inclusion complex which suggests the presence of an amorphous compound that is devoid of crystallinity. In general this is considered as an indication of a true complex. Most reports consider X-ray diffraction as a definitive proof of complex formation.

1.5.3 Scanning Electron Microscopy

Scanning electron microscopy is used to study the microscopic aspects of the components and to qualitatively define the formation of inclusion complexes. The morphology, size, shape and porosity of the materials can be observed using scanning electron microscopy.

The inclusion complexes are morphologically compared with the drug, cyclodextrin and physical mixture. The photomicrographs of the physical mixture usually shows two distinct components (drug and cyclodextrin), whereas the inclusion complexes show a new morphology which may include loss of original shape, crystallinity or/and formation of a single phase (Zhang, X. *et al*., 2009). However, this technique is not adequate to confirm the formation of inclusion complexes (Didja, A. *et al*., 1989; Al-Marzouqi, A.H. *et al*., 2006; Jadhav, G.S. and Vavia, P.R., 2008). Recent investigations featuring the use of this technique to evaluate the formation of inclusion complexes are presented in Table 1.6**.**

Table 1.6: Examples of recent investigations featuring the use of scanning electron microscopy in the characterisation of inclusion complexes.

Drug	Cyclodextrin	Evidence of complexation	Reference
Bupivacaine	β-CD	Loss of the crystallinity	(Jug, M. *et al*., 2010)
Omeprazole	β-CD and Me-β-CD	Loss of original shape and formation of a single phase	(Zhang, X. *et al*., 2009)
Piroxicam	HP-β-CD	Loss of original shape	(Ungaro, F. *et al*., 2011)
Ropivacaine	HP-β-CD	Formation of a single phase	(De Araujo, D.R. *et al*., 2008)

1.5.4 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy is used to determine the formation of inclusion complexes because of its ability to reveal structure by identifying the functional groups in the drug molecule included in the cavity of the cyclodextrin. Proton-nuclear magnetic resonance spectroscopy gives the most direct evidence for the inclusion of a drug into the cavity of cyclodextrin in solution (Sinha, V.R. *et al*., 2005; Jadhav, G.S. and Vavia, P.R., 2008; Franco, C. *et al*., 2009). The direction in which drug molecule penetrates into the cavity of cyclodextrin can also be determined by this technique (Uekama, K. *et al*., 1983; Rao B.P. *et al*., 2006). There are six protons in a glucopyranose unit of the cyclodextrin; three (H-1, H-2 and H-4) located on the exterior surface, two (H-3 and H-5) directed towards the interior of the cavity, H-3 near the wider exit and H-5 near the narrow side. The last proton, H-6, is closer to the narrow exit of the cavity. These six protons are investigated in the ^{1}H-nuclear magnetic resonance spectroscopy studies. Proton signal changes take place in the spectra of cyclodextrin-drug molecule complexes (Nagarsenker, M.S. and Joshi, M.S., 2005; Borodi, G. *et al*., 2008) because of the formation of interactions, when inclusion complexation occurs between the drug and cyclodextrin.

Aithal *et al.* (Aithal, K.S. *et al*., 1995) employed ^{1}H-nuclear magnetic resonance technique for investigating the inclusion of bropirimine with β-cyclodextrin formed by freeze drying and co-precipitation techniques. The signals of H-3 and H-5 atoms of β-cyclodextrin were shifted upfield more compared to the signals of H-1, H-2 and H-4 atoms. This was an indication of inclusion of the drug in the cyclodextrin cavity.

1.5.5 *In vitro* Dissolution Studies

In vitro dissolution studies are used to evaluate the occurrence of inclusion complexation between the drug and cyclodextrin, where the aim of complex formation is to enhance the aqueous solubility of poorly soluble drugs. In some studies the dissolution profiles are obtained using powders of the drug, the physical mixture or the complex, without compression (Hussein, K. *et al*., 2007), and in others they are obtained from tablets (Dahiya, S., Pathak, K., 2007). The formed complexes are evaluated by comparing the amount of drug dissolved within certain time (10, 30 or 45 minutes) and the dissolution efficiency. A higher rate of dissolution is expected for the complexes when compared to that of either the drug or the physical mixture, which in turn occurs because of the enhanced solubility achieved by the complexation of the drug (Jun, S.W. *et al*., 2007). Recent investigations featuring the use of this technique to characterise inclusion complexes are presented in Table 1.7.

Table 1.7: Examples of recent investigations featuring the use of dissolution studies for the characterisation of inclusion complexes.

Drug	Cyclodextrin	Evidence of complexation	Reference
Bicalutamide	β-CD	Time required to release 90% of drug	(Patil, A.L., 2008)
Celecoxib	HP-β-CD	Percentage dissolved in 10 minutes	(Cappello, B. *et al*., 2007)
Lovastatin	HP-β-CD	Time required to release 50% of drug	(Corti, G. *et al*., 2007)

1.5.6 Isothermal titration calorimetric Studies

Isothermal titration calorimetry is a powerful technique used in the investigation of the drug-cyclodextrin interaction mechanisms (Bouchemal, K., 2008). It is based on the measurement of the heat released or absorbed during the interaction between two molecules (the drug and cyclodextrin in this context). Isothermal titration calorimetry is a highly sensitive technique and has become an indispensable tool for determining the energetics (the affinity constant (*K*) also known as association or stability constant), stoichiometry (*N*), the changes in enthalpy (ΔH), the changes in entropy (ΔS) and the Gibbs free energy (ΔG)), thereby providing complete thermodynamic profile of the molecular interactions between drug and cyclodextrins (Bouchemal, K., 2008; Bouchemal, K. *et al*., 2009; Daoud-Mahammed, S. *et al*., 2009; Daoud-Mahammed, S. *et al*., 2010; Rekharsky, M.V. and Inoue, Y., 2008).

Several mechanisms concomitantly contribute to the formation of inclusion complexes which include, but are not limited to: (a) van der Waals or hydrophobic interactions (Barone, G. *et al*., 1986; Hallen, D. *et al*., 1992); or (b) steric effects and hydrogen bonding (Tong, W.-Q. *et al*., 1991b; Gelb, R.I. *et al*., 1995); or (c) changes (loss or gain) in entropy or enthalpy attributable to rearrangement, addition and removal of water of solvation for both the drug and cyclodextrin molecules (Inoue, Y. *et al*., 1993; Linert, W. *et al*., 1989; Loftsson, T. *et al*., 1996; Ross, P.D. and Rekharsky, M.V., 1996; Tong, W.-Q. *et al*., 1991a).

It is very important to have an understanding of the energetics (*e.g.* the thermodynamic parameters and the binding constants) of the interaction between a drug and cyclodextrin in order to understand the phenomena associated to molecular recognition (Bouchemal, K. and Mazzaferro, S., 2012). The understanding of the thermodynamics of drug-cyclodextrin

complex formation can provide information on these mechanisms or driving forces for inclusion and hence plays a crucial role in determining formulation potential and the stability of the product and therefore the *in vivo* drug release. All of these consequently improve the efficacy of the pharmaceutical formulation (De Namor, A.F.D. *et al*., 1992; Todorova, N.A. and Schwarz, F.P., 2007). Isothermal titration calorimetry has wide applications in cyclodextrin chemistry, cellular biology, polymer chemistry, and biochemistry (Arnaud, A. and Bouteiller, L., 2004; Cooper, M.A., 2003; Ladbury, J.E. and Chowdhry, B.Z., 1996; Obert, E. *et al*., 2007; Othman, M. *et al*., 2009; Segura-Sanchez, F. *et al*., 2009; Xing, S. *et al*., 2009). These experiments take just few hours and require small amounts of sample when compared to conventional methods.

Owing to the wealth of information that isothermal titration calorimetry provides, several researchers have employed the technique to characterise different drug-cyclodextrin complexes (Aki, H. *et al*., 2001a; Rodriguez-perez, A.I. *et al*., 2006). The technique has been earlier utilised to study the inclusion complexation between barbiturates and cyclodextrins (Aki, H. *et al*., 1998; Aki, H. *et al*., 2001a; Aki, H. *et al*., 2001b), to determine the thermodynamic parameters for formation of the inclusion complexes of natural (α-, β- and γ-) cyclodextrins with ibuprofen in Tris-HCl buffer solutions (pH 7.0) (Xing, S. *et al*., 2009) and to determine the energetics of the interactions between sertaconazole and hydroxypropyl-β-cyclodextrin (Rodriguez-perez, A.I. *et al*., 2006).

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Chapter 2: The Evaluation of Supercritical Fluid Technology as a Preparative Technique for the Manufacture of Econazole-α-Cyclodextrin Complexes: A Comparison with Conventional Methods

AIMS

- To investigate different manufacturing processes claimed to promote inclusion complexation between econazole and cyclodextrins in order to enhance the apparent solubility and dissolution properties of econazole.
- Especially, to evaluate the effectiveness of a single-step, organic solvent-free supercritical carbon dioxide process for preparing econazole-α-cyclodextrin inclusion complexes and compare to other preparation methods such as physical mixing and freeze-drying.

2.1 Introduction

Econazole is an imidazole antifungal drug indicated in the treatment of mycotic infections. It has a very poor aqueous solubility (about 3 μg mL^{-1} at 25°C) and a dissolution rate which limits both its therapeutic application and efficacy (Heel, R.C. *et al*., 1978; Dellenbach, P. *et al*., 2000; Ahmad, Z. *et al*., 2005; Ahmad, Z. *et al*., 2006; Ahmad, Z. *et al*., 2007; Ahmad, Z. *et al*., 2008; Kinazaki, A. *et al*., 2009). Hence, there is a need to enhance the aqueous solubility and dissolution rate of econazole (Figure 2.1). It has been reported that conventional complexation with α- and β-cyclodextrins (Mura, P. *et al*., 1992; Pedersen, M. *et al*., 1993) and formation of salts with various hydroxy-acids significantly

improved both the water solubility and dissolution rate of econazole (Mura, P. *et al*., 2001).

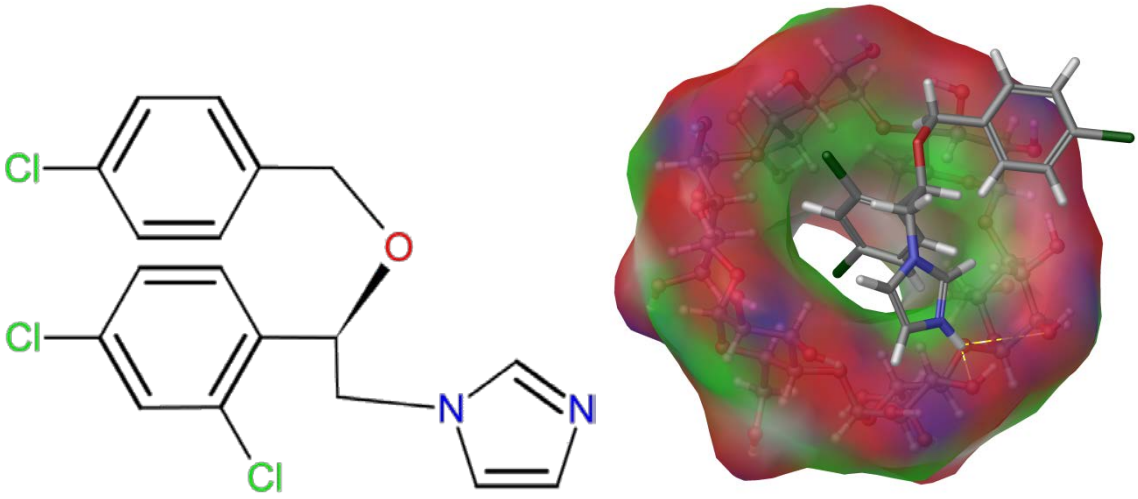


Figure 2.1: Structure of econazole base and econazole docked in α-cyclodextrin.

The favourable effect of cyclodextrin complexation on both the physicochemical properties and biological efficacy of econazole has also been reported (Al-Marzouqi, A.H. *et al*., 2007c). Econazole has an affinity for various acids and forms salts, and forms inclusion complexes with various cyclodextrins; hence, econazole has the ideal attributes of a model drug.

The aim of the study was to investigate different manufacturing processes claimed to promote inclusion complexation between econazole and cyclodextrins in order to enhance the apparent solubility and dissolution properties of econazole. Especially, the effectiveness of a single-step, organic solvent-free supercritical carbon dioxide process for preparing econazole-α-cyclodextrin inclusion complexes was evaluated and compared to other preparation methods.

2.2 Materials and Methods

2.2.1 Materials

Econazole nitrate (≥98%, molecular weight: 444.70 g mol^{-1}), α-cyclodextrin (≥98%, molecular weight: 972.84 g mol^{-1}) and sodium hydroxide (1 N) were purchased from Sigma-Aldrich, Gillingham, Dorset, UK while dichloromethane (>99%) was obtained from Fisher Scientific, UK.

2.2.2 Preparation of Econazole Free Base (Stripping the Nitrate Salt from the Base)

Econazole nitrate (25 g, 65 mmol) was added to water (150 mL) and continuously stirred for one hour. A saturated solution of sodium hydroxide (1 N, 12.5 mL) was added drop-wise to the mixture and stirred constantly until a basic pH (9.0) was obtained. Dichloromethane (250 mL) was added to the mixture and the solution was stirred for one hour. The organic layer was separated, then dried with anhydrous magnesium sulphate (15 g), filtered and concentrated under reduced pressure at 50°C to give a white, dry powder. The product was recrystallized twice using ethanol to produce 19.35 g (90% yield) of white crystalline econazole free base.

2.3 Preparation of Binary Mixtures of Econazole with α-Cyclodextrin

All the binary mixtures were prepared in a 1:1 molar ratio of econazole to α-cyclodextrin.

2.3.1 The physical mixing process

The required amounts of econazole (0.50 g, 1.3 mmol) and α-cyclodextrin (1.27 g, 1.3 mmol) were weighed and tumble-mixed for about 15 minutes; the mixtures were passed through 0.150 mm aperture sieve and stored in a desiccator over fused calcium chloride.

2.3.2 The freeze-drying process

Econazole (0.50 g, 1.3 mmol) was added to an aqueous solution of α-cyclodextrin (1.27 g, 1.3 mmol) while mixing with a magnetic stirrer. After equilibrating in a horizontal shaker at room temperature for 48 hours, the resulting solution was frozen at -60°C and was dried in a freeze-dryer (ScanVac CoolSafe, UK) at 25°C for 48 hours and then sieved through 0.150 mm aperture sieve. The samples were stored in a desiccator.

2.3.3 Supercritical carbon dioxide inclusion method

The complex was prepared using an extraction apparatus (Thar Process Inc., Pittsburgh, PA). The physical mixture of econazole (0.50 g, 1.3 mmol) and α-cyclodextrin (1.27 g, 1.3 mmol) was placed in a sample cell as shown in the Figure 2.2. Carbon dioxide was pumped from a cylinder via a cooling unit into the sample cell. 200 bar pressure was created by pumping against an automated back pressure regulator. The sample cell in the reaction vessel was heated to 60°C and held for 3 h before recovering the solid complex by depressurisation at a rate of 7-8 bar min^{-1}. The product was then homogenized in a mortar prior to further analysis.

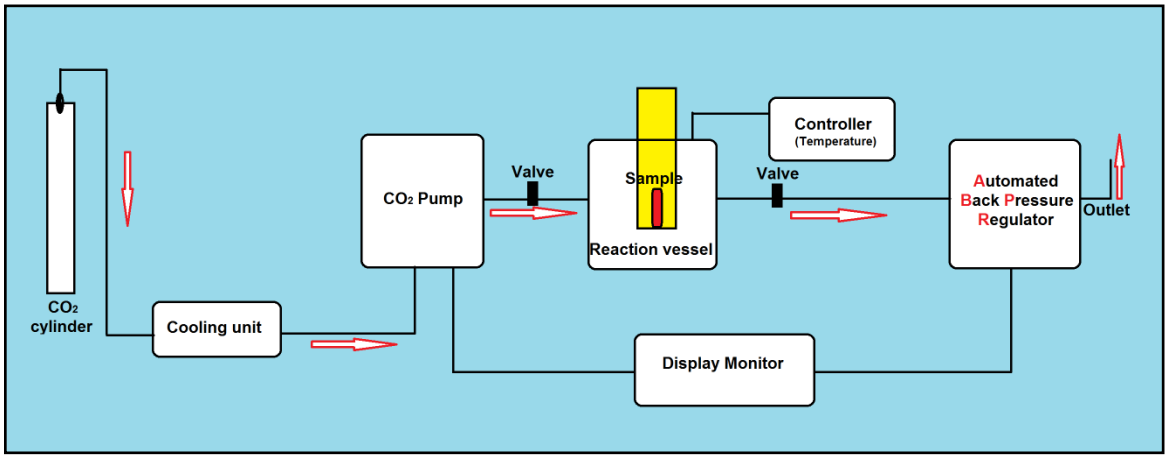


Figure 2.2: Schematics of supercritical carbon dioxide processing using the extraction apparatus supplied by Thar process Inc., USA.

2.4 Analysis of the Prepared Binary Mixtures

Having manufactured these model complexes, the next aim is to compare the physical properties with those of complexes prepared using the other more conventional techniques.

2.4.1 Differential Scanning Calorimetric Analysis

Differential scanning calorimetry of individual components and the complex systems was carried out using a FP-90 central processor (Mettler-Toledo, LLC, UK) in air. Calibration was carried out with indium as a reference standard. 5-6 mg samples were accurately weighed using a digital analytical balance. The samples were transferred to aluminium pans which were then closed and heated in the instrument at a scan rate of 10°C min^{-1} over the temperature range from 50 to 200°C.

2.4.2 Powder X-ray Diffraction Analysis

The X-ray powder diffractograms were obtained on a D8 Advance X-ray Diffractometer (Bruker, Germany) in theta-theta Bragg-Brentano geometry using reflection mode. Data was collected between 2-50° 2θ, with a step size of 0.006° and a counting time of 0.5 s per step using Cu Kα radiation.

The degree of crystallinity (% Crystallinity) is defined as the percentage by weight of crystalline drug in a sample containing drug in both the amorphous and crystalline states (Suryanarayanan, R. and Mitchell, A.G., 1985) and was determined using the amorphous subtraction method. The area under the curve of the crystalline peaks was first measured; later the total area of peaks (crystalline and amorphous) was measured. An amorphous background was modelled using the DIFFRAC-SUITE TOPAS 4.2 software (Bruker, USA). The amorphous background was subtracted from the total area of peaks to leave just the crystalline area.

The degree of crystallinity of the mixtures was estimated by the following relationship:

% Crystallinity = (Area of crystalline peaks / Total area of all peaks) x 100

The crystalline nature of a sample is displayed by sharp peaks in the powder X-ray diffraction patterns. A decrease in the crystallinity (reductions in peak intensity), shifts in peak position and disappearance of peaks, appearance of new diffraction peaks, or a complete diffuse pattern were considered to be related to possible drug amorphisation and/or complexation.

2.4.3 Scanning Electron Microscopy Analysis

The surface morphology of the raw materials and of the binary systems was examined by means of Hitachi SU-8030 scanning electron microscope (Tokyo, Japan). The powders were securely fixed on an aluminium stub using double-sided adhesive tape and then were made electrically conductive by coating in vacuum with a thin layer of chromium (~300 Å), for 30 s and at 30 W. The photomicrographs were taken at an excitation voltage of 2.0 kV and a magnification of ×350.

2.4.4 ^{1}H-Nuclear Magnetic Resonance Studies

^{1}H-nuclear magnetic resonance spectra (500 MHz Jeol FT NMR, with pulsed field gradients, Tokyo, Japan) of pure components and their equimolar combinations were taken in deuterated water (99.9 atom % D) by adding the minimum amount of deuterated-hydrochloric acid to dissolve econazole (pH 2.5). The concentration of each component was 0.026 M. The samples were degassed to avoid the interference of dissolved oxygen and the spectra were recorded with a 5 mm tube at 25°C. Samples were referenced with respect to the solvent. Chemical shifts (δ) were measured relative to the peak at 4.65 ppm, due to the deuterium oxide. Chemical shift changes ($\Delta\delta$) were calculated by using the formula: $\Delta\delta = \delta(\text{complex}) - \delta(\text{free})$.

2.4.5 2D-Rotating-frame Nuclear Overhauser Effect Correlation Spectroscopy

2D ^{1}H-^{1}H ROESY experiments were performed in spin-lock conditions. The spectrum of econazole:α-cyclodextrin complex was acquired using 4096 data-points, 256 increments, 16 scans for each increment and 500 ms mixing time.

2.4.6 Relaxation time (T_1)-measurement Inversion Recovery Experiments

The relaxation time (T_1) measurement experiments were recorded for all samples using a standard inverse-recovery experiment. The samples were degassed to avoid the interference of dissolved oxygen and the experiments were recorded at 25°C. For ^{1}H NMR, a 90° pulse was typically of 10-15 μs, and the recycling time was set to three times the largest value of T_1, *i.e.* 15 s.

2.4.7 Dissolution Studies

Dissolution studies of econazole from all drug-carrier binary systems, and for econazole alone, were conducted using USP Type II paddle method (Hanson G2 Vision® Classic 6, Chatsworth, CA) with deionised water as the dissolution medium. The samples, corresponding to 40 mg of drug were dispersed into 600 ml of the dissolution media maintained at 37 ± 0.5°C and stirred at 100 rpm. Suitable aliquots were withdrawn and filtered (0.45 μm filter pore size, Sartorius, UK) at the specified times and the drug concentration was determined by UV spectroscopy (Cary Bio 100 UV-Vis, Agilent Technologies, Santa Clara, CA 95051) using 10 mm quartz cuvettes. The same volume of fresh medium was added to the beaker and the correction for the cumulative dilution was calculated. Each test was performed in triplicate. The parameters used to characterize the dissolution curves were the percentage of drug dissolved at 10 min and 30 min.

2.5 Results and Discussion

2.5.1 Differential Scanning Calorimetric Analysis

The differential scanning calorimetry thermograms for pure econazole, freeze-dried econazole, supercritical carbon dioxide processed econazole, pure α-cyclodextrin, freeze-dried α-cyclodextrin and supercritical carbon dioxide processed α-cyclodextrin are presented in Figure 2.3.

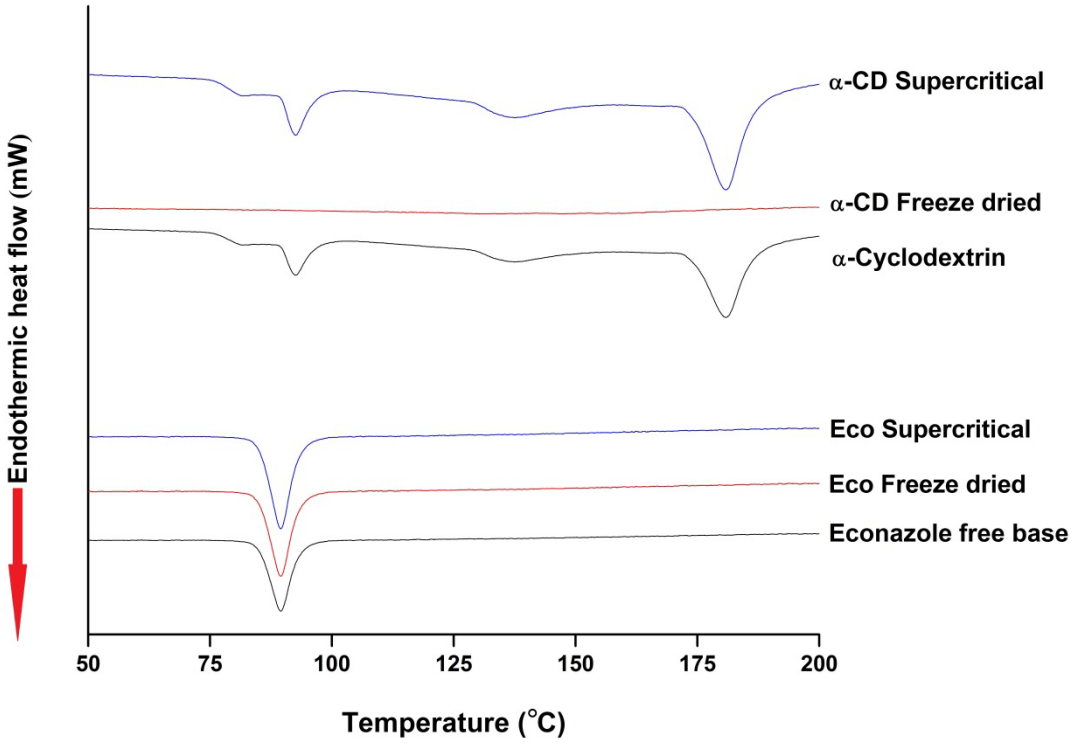


Figure 2.3: Differential scanning calorimetry thermograms of econazole, freeze-dried econazole, supercritical carbon dioxide processed econazole, α-cyclodextrin, freeze-dried α-cyclodextrin and supercritical carbon dioxide processed α-cyclodextrin (Supercritical carbon dioxide processing conditions: 60°C and 200 bar).

As shown in the figure, pure econazole, freeze-dried econazole and supercritical carbon dioxide processed econazole exhibited a sharp melting endotherm at 92°C, indicating a crystal form. The results indicate that neither of the processes altered the melting behaviour or crystallinity of the drug.

Pure α-cyclodextrin and α-cyclodextrin processed with supercritical carbon dioxide displayed phase transitions from 70°C -80°C, 85°C-100°C and 160°C-180°C owing to the loss of water molecules from the cyclodextrin. However, a complete disappearance of the peaks was observed for the freeze-dried α-cyclodextrin indicating its amorphisation. This indicates that freeze-drying has altered the crystallinity and phase behaviour of the cyclodextrin.

The differential scanning calorimetry thermograms for econazole-α-cyclodextrin 1:1 molar binary systems (physical mixing, freeze-drying and supercritical carbon dioxide inclusion) are presented in Figure 2.4.

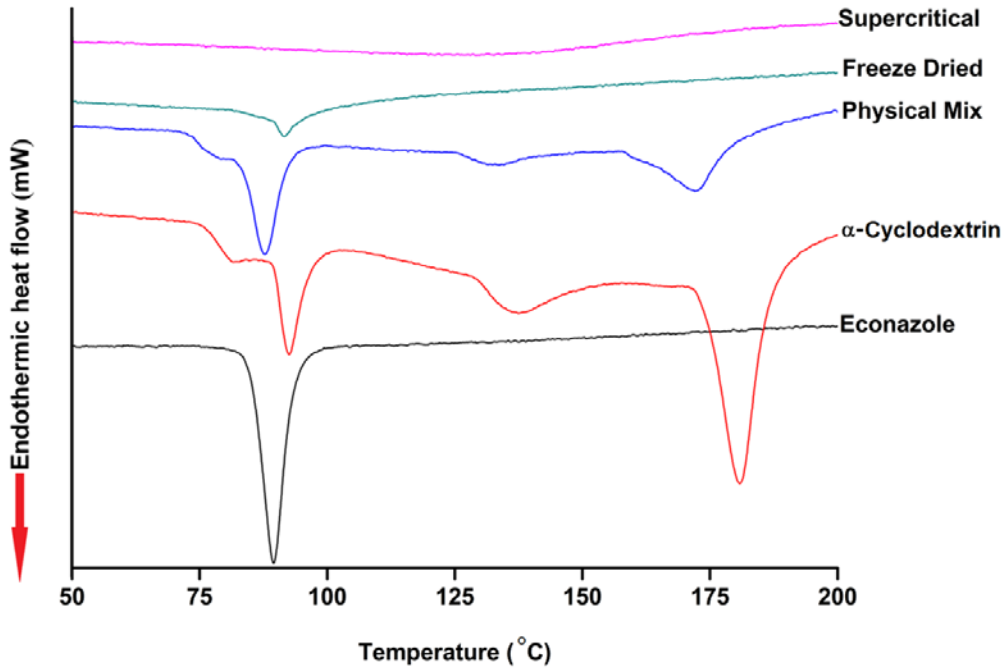


Figure 2.4: Differential scanning calorimetry thermograms of econazole, α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 60°C and 200 bar).

The thermogram for the physical mixture showed the presence of both pure components with slight shifts, suggesting that no inclusion occurred when drug and cyclodextrin were

simply mixed together (Wang, X. *et al.*, 2014 and Zhou, S.-Y. *et al.*, 2014). A shallow drug endothermic peak was observed in the case of freeze-dried product, which shifted to higher temperature. However, a complete disappearance of the drug peak was observed for the supercritical carbon dioxide inclusion system. The reduced intensity of drug endothermic peak suggests an incomplete inclusion of econazole in the cavity of α-cyclodextrin while the complete disappearance of the melt peak indicates interactions between econazole and α-cyclodextrin and the formation of inclusion complex with loss of crystallinity (Charoenchaitrakool, M. *et al.*, 2002; Kfoury, M. *et al.*, 2014 and Nieddu, M. *et al.*, 2014).

Cabral Marques, H.M. *et al.*, (Marques, H.M.C. et al., 1990) have explained that the melting point of the guest molecules shift to a different temperature or disappear within the temperature range where the cyclodextrin lattice is decomposed, when they are included in the crystal lattice or in the cyclodextrin cavity.

2.5.2 X-ray Powder Diffraction Analysis

Figure 2.5 shows the X-ray powder diffractograms of pure econazole, freeze-dried and supercritical carbon dioxide processed econazole, pure α-cyclodextrin, freeze-dried and supercritical carbon dioxide processed α-cyclodextrin. The X-ray powder diffraction patterns of pure econazole, freeze-dried and supercritical carbon dioxide processed econazole displayed sharp peaks and flat background indicative of its crystalline nature. The results indicated that neither of the processes altered the crystallinity of the drug.

Pure α-cyclodextrin and α-cyclodextrin processed with supercritical carbon dioxide displayed sharp peaks and flat background indicative of its crystalline nature. However, the diffraction pattern of freeze-dried α-cyclodextrin displayed several broad amorphous halos. The loss of crystallinity was most prominent for the cyclodextrin processed by freeze drying, suggesting an almost complete drug amorphisation in agreement with differential

scanning calorimetry analysis. This indicated that freeze-drying has altered the crystallinity of the cyclodextrin.

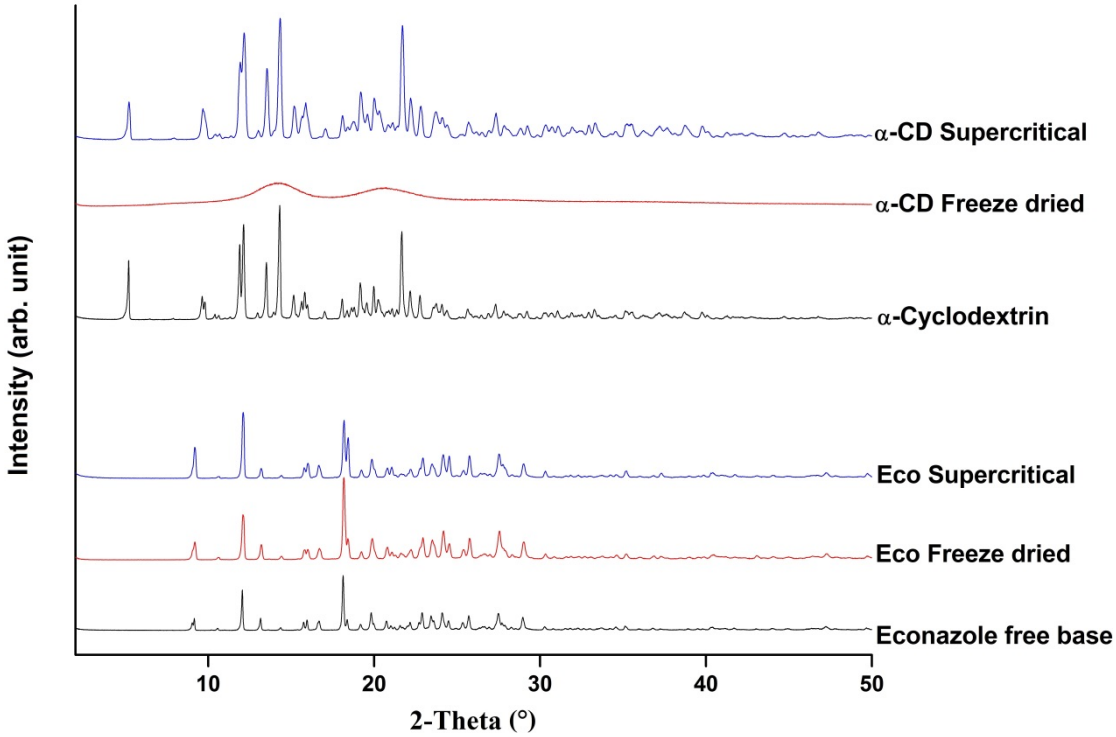


Figure 2.5: X-ray powder diffractograms of econazole, freeze-dried econazole, supercritical carbon dioxide processed econazole, α-cyclodextrin, freeze-dried α-cyclodextrin and supercritical carbon dioxide processed α-cyclodextrin (supercritical carbon dioxide processing conditions: 60°C and 200 bar).

Figure 2.6 shows the powder X-ray diffraction patterns of drug-cyclodextrin 1:1 molar binary systems obtained by different preparation methods.

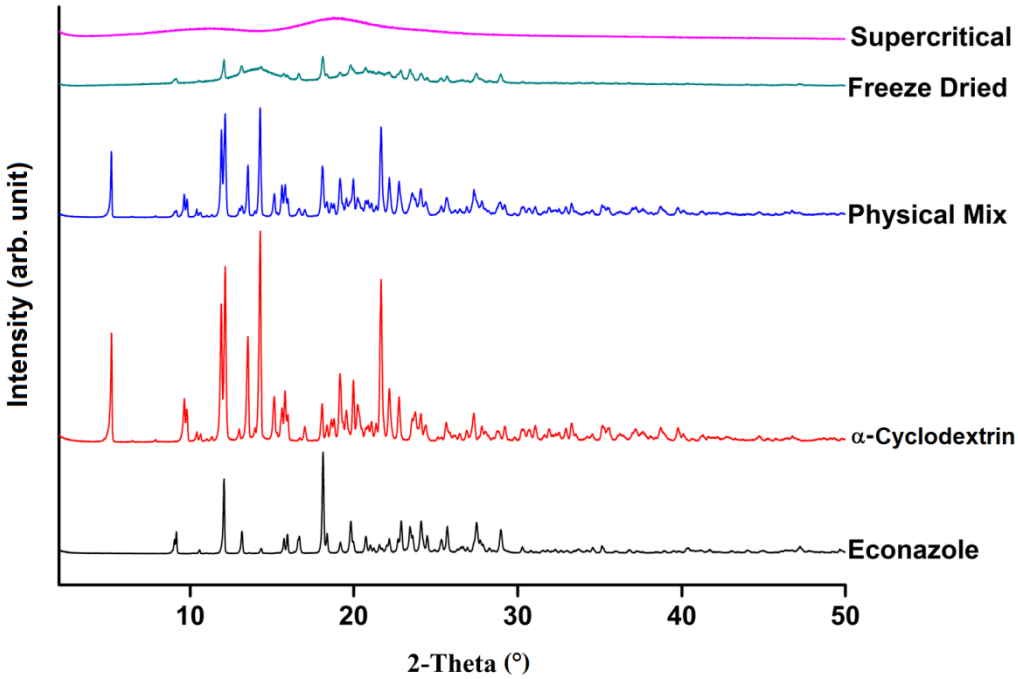


Figure 2.6: X-ray powder diffractograms of econazole, α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 60°C and 200 bar).

The physical mixture of econazole and cyclodextrin showed a similar diffraction pattern to that of the respective individual components, but with some changes in the size of several peaks in the binary sample. The presence of sharp peaks in the diffraction pattern of the physical mixture indicates the retention of the crystalline structure of econazole. In contrast, the freeze-dried system exhibited considerable diminution of the diffraction peaks in agreement with the differential scanning calorimetry results, suggesting that it is less crystalline than the physical mixture.

The reduction in crystallinity attributed to the freeze-drying treatment is clearly evident for pure α-cyclodextrin, while econazole does not show this effect. The freeze-dried system was found to be 20% crystalline. The diffraction pattern of supercritical carbon dioxide-

inclusion system was devoid of drug peaks and displayed broad amorphous halos, suggesting an almost complete drug amorphisation and/or complexation in agreement with differential scanning calorimetry analysis (Spamer, E. *et al.*, 2002 and Banchero, M. *et al.*, 2013). Disappearance of drug peaks indicates the strong interaction between drug and α-cyclodextrin, suggesting formation of stable hydrogen bonds as explained by Figueiras, A. *et al.*, (2007) and Hirlekar, R.S. *et al.*, (2009).

The increase in the amorphous content of the drug was observed in the following order: physical mixing < freeze-drying < supercritical carbon dioxide inclusion.

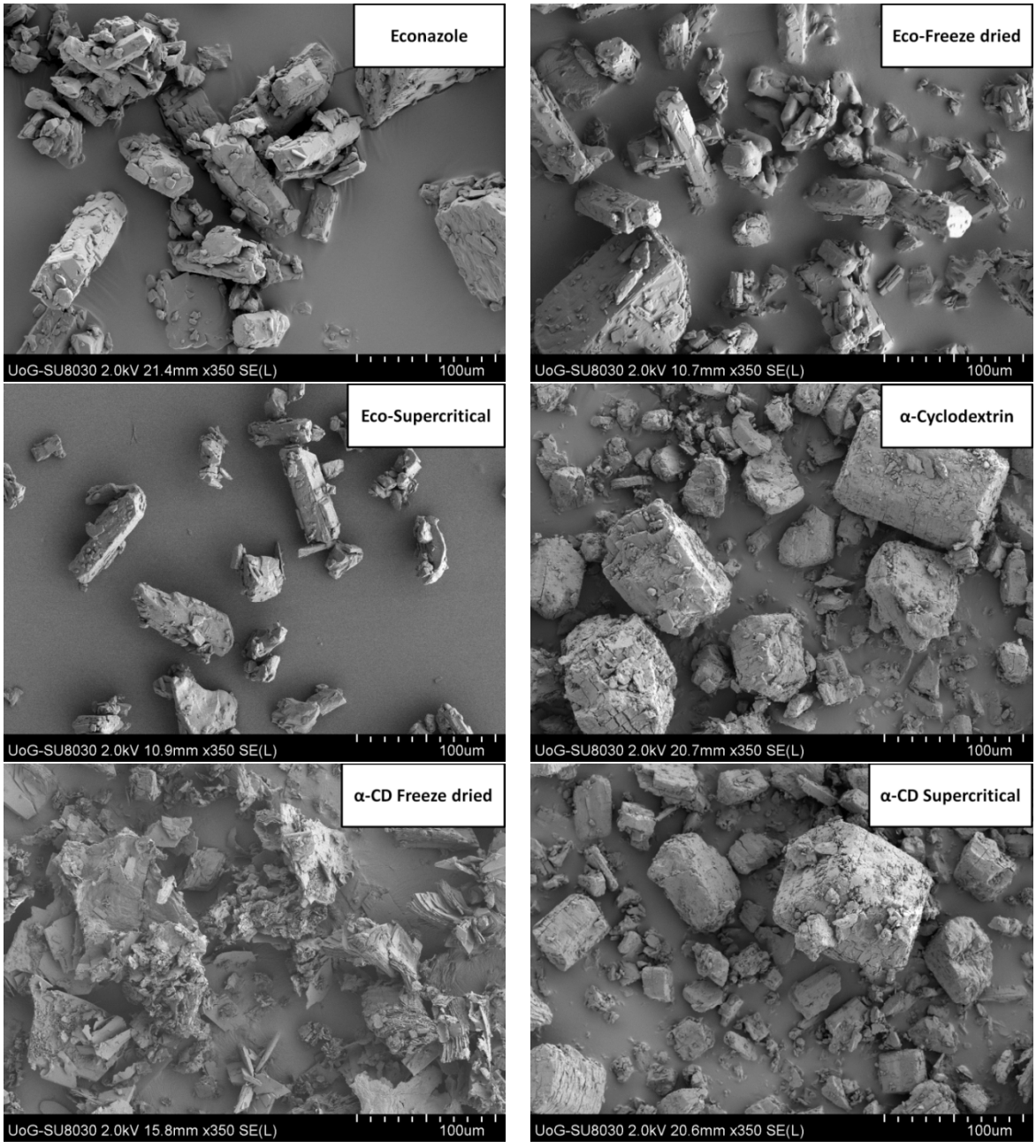
2.5.3 Scanning Electron Microscopy Analysis

The scanning electron photomicrographs of pure econazole, freeze-dried and supercritical carbon dioxide processed econazole, pure cyclodextrin, freeze-dried and supercritical carbon dioxide processed cyclodextrin and binary mixtures are illustrated in Figure 2.7.

From scanning electron microscopy analysis, econazole appeared as small to large crystals (8-350 µm) with a tendency to self-agglomerate. The larger crystals were prismatic and columnar whereas, the smaller crystals were both rods and flat plates. Pure econazole, econazole processed with supercritical carbon dioxide and freeze-dried econazole showed no discernible differences in the morphology.

Pure α-cyclodextrin and α-cyclodextrin processed with supercritical carbon dioxide was prismatic and appeared as the fragments of larger crystals (20-115 µm). The larger crystals were rectangular in shape. However, freeze-dried α-cyclodextrin exhibited drastic changes in morphology. The photomicrographs suggest that supercritical carbon dioxide processing does not alter the morphology of the cyclodextrin.

Physical mixture of drug and cyclodextrin appeared as rectangular crystals (108-180 µm). The comparable morphology of the physical mixture with that of the pure components could reveal that apparently no econazole-α-cyclodextrin interaction has taken place during processing. The freeze-dried product afforded large glassy shards and sharp-edged irregular fragments with the largest particle at the size of 500 µm. Supercritical carbon dioxide inclusion system appeared as massive and very fine indistinct grains (0.3-55 µm).



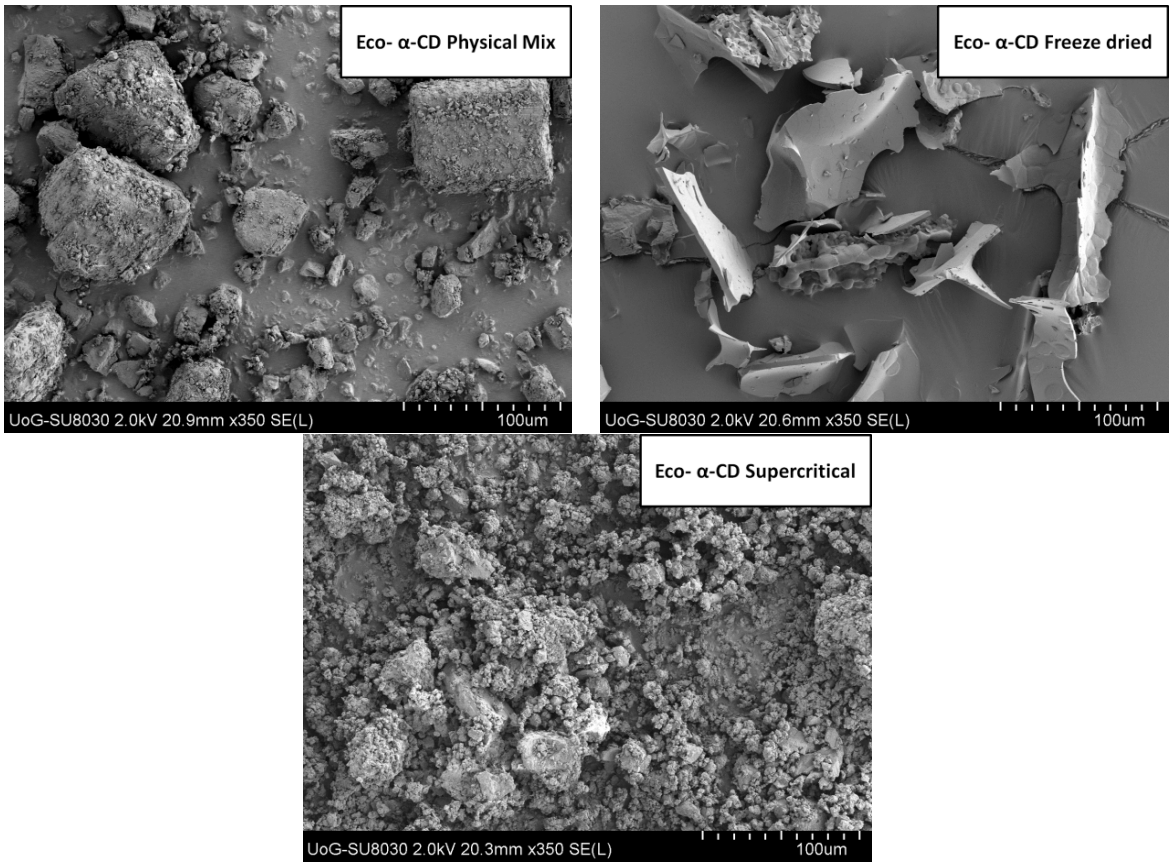


Figure 2.7: Scanning electron photomicrographs of econazole, freeze-dried and supercritical carbon dioxide processed econazole, pure α-cyclodextrin, freeze-dried and supercritical carbon dioxide processed α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze drying and supercritical carbon dioxide processing at ×350 magnification (supercritical carbon dioxide processing conditions: 60°C and 200 bar).

In the freeze-dried and supercritical carbon dioxide processed products, the original morphology of the raw materials disappeared, and it was not possible to differentiate between the two components. They appeared as agglomerates. The drastic change of the shapes and aspect of the particles was indicative of the presence of a new solid phase, suggesting the existence of a single phase, as explained by Ribeiro, L.S.S. *et al.*, (2003); Naidu, N.B. *et al.*, (2004) and Figueiras, A. *et al.*, (2007). These observations are in good agreement with the powder X-ray diffraction studies.

As explained by Arias, M.J. *et al.*, (2000) and Figueiras, A. *et al.*, (2007), scanning electron microscopy analysis is not decisive for evaluating the formation of an inclusion complex in the solid state; however, it can be advantageous to prove the homogeneity of the solid phases.

2.5.4 ^{1}H-Nuclear Magnetic Resonance Studies

^{1}H-NMR spectroscopy studies can be employed to monitor changes in molecular chemical environments of the drug-cyclodextrin complexes by studying the chemical shift of NMR signals of the drug and the carrier. ^{1}H-NMR spectroscopy studies of econazole base, α-cyclodextrin and econazole-α-cyclodextrin 1:1 molar binary systems were undertaken to monitor the inclusion of econazole inside the cavity of α-cyclodextrin. The structures of econazole base and α-cyclodextrin are presented in Figure 2.8 with their protons numbered in red and green, respectively.

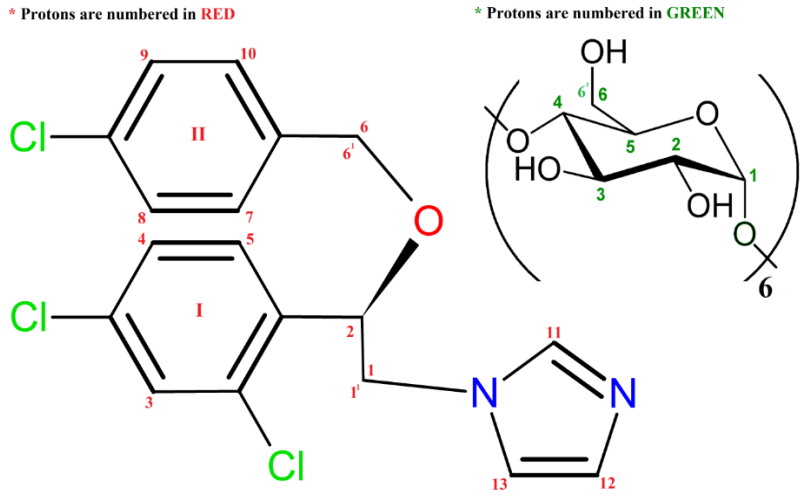


Figure 2.8: Structures of econazole base and α-cyclodextrin with their protons numbered in red and green, respectively.

Figures 2.9 and 2.10 present the ^{1}H-nuclear magnetic resonance spectra of econazole and α-cyclodextrin. As shown in the figures, the peak at 4.65 ppm corresponds to the solvent (D_2O).

The ^{1}H-nuclear magnetic resonance spectra of econazole, α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems are shown in Figures 2.9 (a-d).

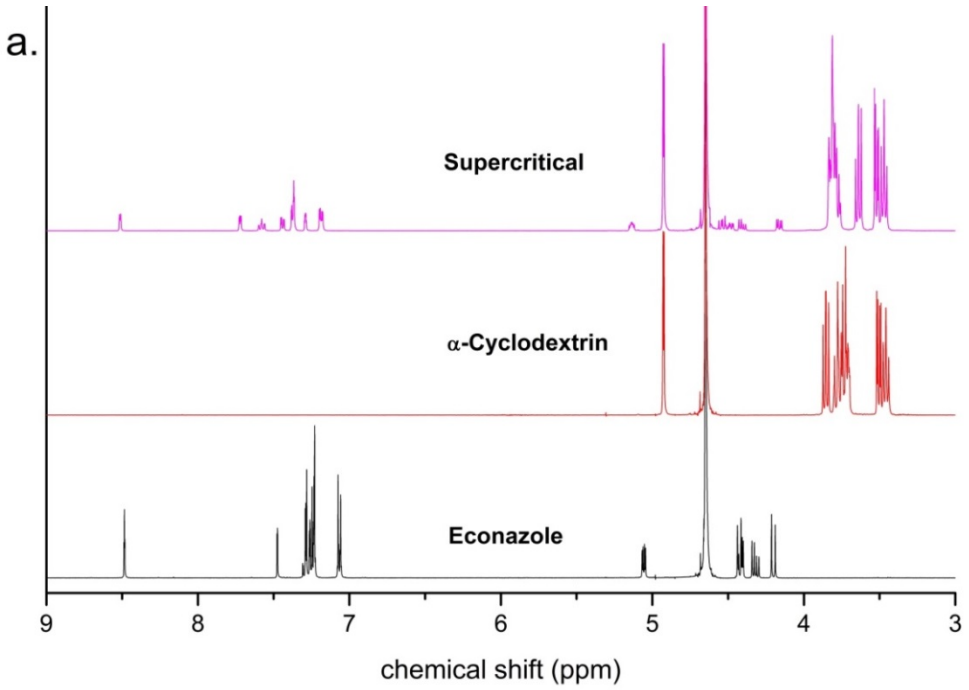


Figure 2.9a: ^{1}H-nuclear magnetic resonance spectra of econazole, α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems prepared by supercritical carbon dioxide inclusion method.

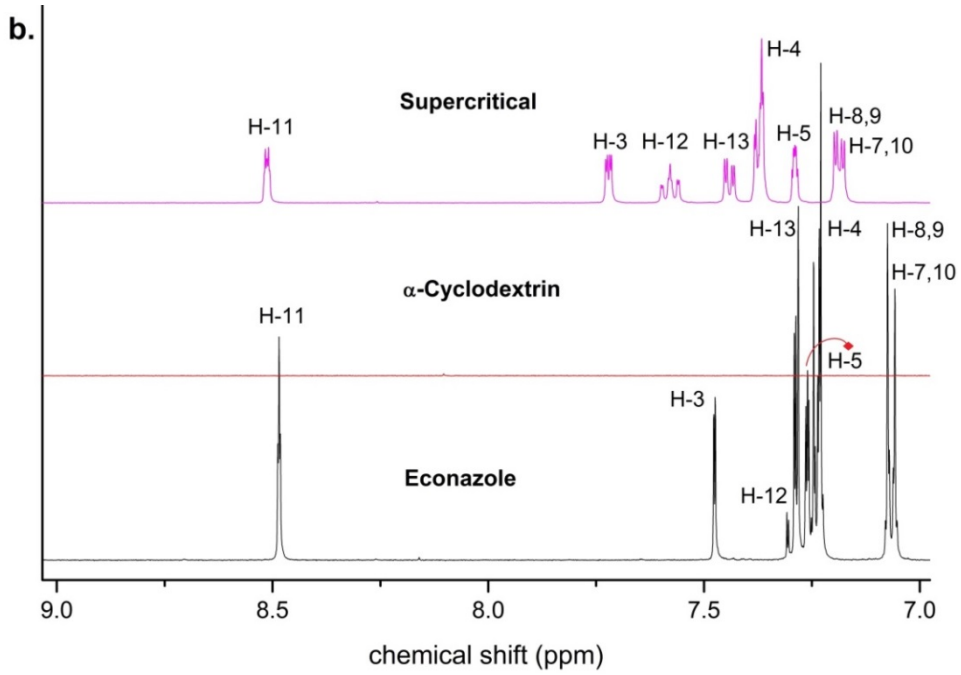


Figure 2.9b: Enlarged ^{1}H-nuclear magnetic resonance spectra of econazole, α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems prepared by supercritical carbon dioxide inclusion method from 9.0-7.0 ppm.

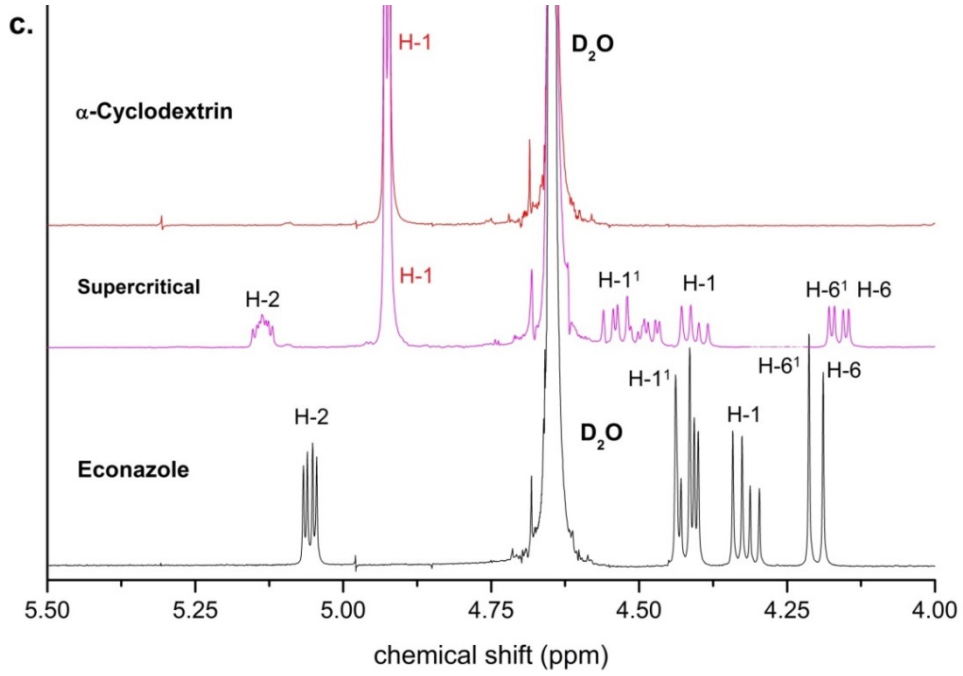


Figure 2.9c: Enlarged ^{1}H-nuclear magnetic resonance spectra of econazole, α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems prepared by supercritical carbon dioxide inclusion method from 5.5-4.0 ppm.

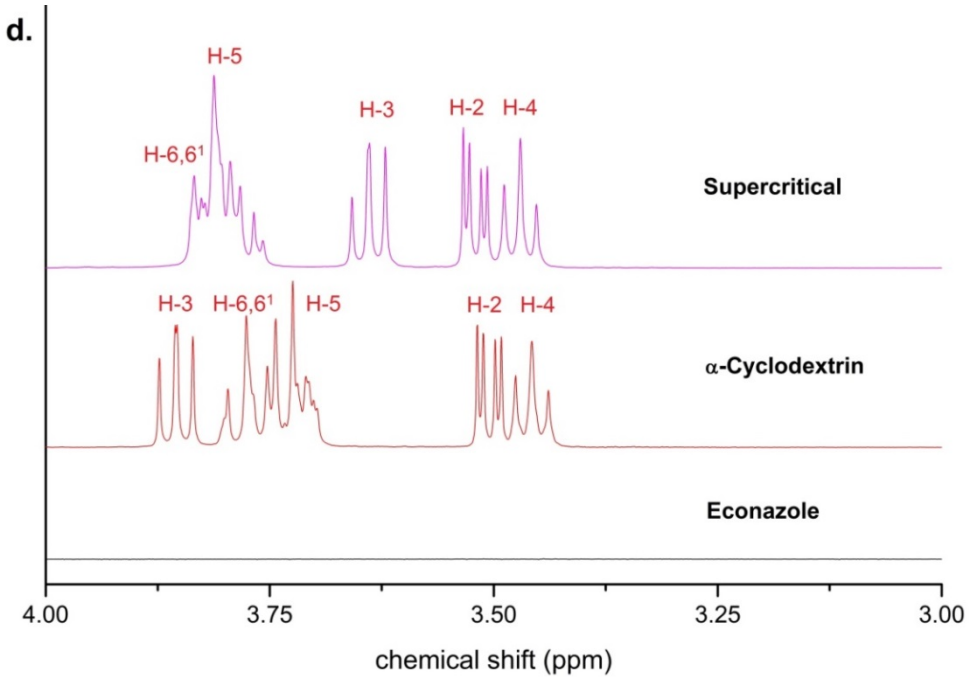


Figure 2.9d: Enlarged ^{1}H-nuclear magnetic resonance spectra of econazole, α-cyclodextrin and econazole-α-cyclodextrin (1:1 molar) systems prepared by supercritical carbon dioxide inclusion method from 4.0-3.0 ppm.

Schneider, H.J. *et al.,* (1998) suggested the change in the chemical shift of the protons caused by the host-guest interaction as the first evidence of the guest inclusion in the cyclodextrin cavity. Chemical shift (δ) locations of econazole and α-cyclodextrin proton signals were identified and changes in the chemical shift (Δδ) values were calculated after comparison with the spectra obtained for the econazole-α-cyclodextrin complexes. Econazole aromatic signals displayed significant changes in the presence of α-cyclodextrin ascribed to the anisotropic effect of the aromatic ring inside the cyclodextrin cavity (Thakkar, A.L. and Demarco, P.V., 1971). Table 2.1 summarises the changes in the chemical shifts induced on proton signals of econazole and α-cyclodextrin in the binary systems, respectively.

Table 2.1: Changes in the chemical shifts (δ ppm) for econazole and α-cyclodextrin protons in the binary mixture [a, b]

Proton (Eco)	δ (ppm)	Δδ (±α-CD)	Proton (α-CD)	δ (ppm)	Δδ (±Eco)
H-1	4.33	0.10	H-1	4.93	0.00
H-1[1]	4.43	0.10	H-2	3.50	0.00
H-2	5.05	0.09	H-3	3.86	-0.24
H-6	4.19	-0.03	H-4	3.46	0.00
H-6[1]	4.21	-0.03	H-5	3.71	-0.08
H-7,10	7.06	0.13	H-6,6[1]	3.77	0.04
H-8,9	7.07	0.13			
H-3	7.47	0.26			
H-5	7.26	0.11			
H-4	7.23	0.16			
H-11	8.49	0.02			
H-12	7.31	0.27			
H-13	7.29	0.15			

[a]Protons with the same number are on the same carbon atom but are magnetically unequivalent and hence have different chemical shifts.

[b]A positive sign of Δδ ppm shows a downfield displacement while the negative sign shows an upfield displacement.

As shown in the Table 2.1, protons of both phenyl rings of econazole (H-3, H-4, H-5, H-7, H-8, H-9 and H-10) have experienced a downfield displacement, suggesting their involvement in hydrophobic interactions with α-cyclodextrin. H-12, H-13 protons of the imidazole ring have also experienced significant downfield displacement while H-11 has shown very less effect. The downfield displacement of the protons may be ascribed to a

variation of local polarity when the drug is inside the cavity as suggested by Djedaini, F. *et al.*, (1990).

In case of α-cyclodextrin, the H-3 and H-5 protons (directed to the core of the cavity) experienced diagnostic upfield displacements, while the H-1, H-2 and H-4 protons (located on the exterior of the cavity) were unaffected; confirming the inclusion of drug into cyclodextrin cavity as explained by Singh, R. *et al.*, (2010), Kfoury, M. *et al.*, (2014) and Kfoury, M. *et al.*, (2015). The upfield displacement of the protons may be ascribed to magnetic anisotropy effects inside the cyclodextrin cavity that are induced by the cloud of π-electrons as suggested by Veiga, F.J.B. *et al.*, (2003).

To summarise, the chemical shift changes observed for econazole protons could indicate that either of the phenyl rings and the imidazole ring that can be inserted into the α-cyclodextrin cavity. Furthermore, the chemical shift changes observed for H-3 and H-5 protons of α-cyclodextrin could indicate that the inclusion of the drug can occur from either of the hydroxyl sides of the cavity. ^{1}H-nuclear magnetic resonance spectroscopy studies confirm that econazole interacts with the inside of the cyclodextrin cavity and thus that inclusion really occurs with α-cyclodextrin. Our observations are mostly in agreement with the work of Mura and co-workers (Faucci, M.T. et al., 2000); however the authors have reported the absence of involvement of the imidazole moiety in the inclusion of drug into cyclodextrin.

2.5.5 2D-^{1}H–^{1}H ROESY Studies

2D ^{1}H-^{1}H ROESY experiments were then performed to further investigate the interactions between the components in solution. The experimental data provides the evidence for the spatial proximity between drug and cyclodextrin atoms following the observation of intermolecular dipolar cross-correlations. ROESY experiments provide information about

the part of the drug included inside the cyclodextrin cavity, the mode of penetration, the depth of penetration and orientation of the guest molecule (Pinto, L.M.A. *et al*., 2005).

Figure 2.10 shows an expanded section of the contour plot of the 2D ^{1}H-^{1}H ROESY spectrum of econazole-α-cyclodextrin (1:1 molar) systems prepared by supercritical carbon dioxide inclusion method.

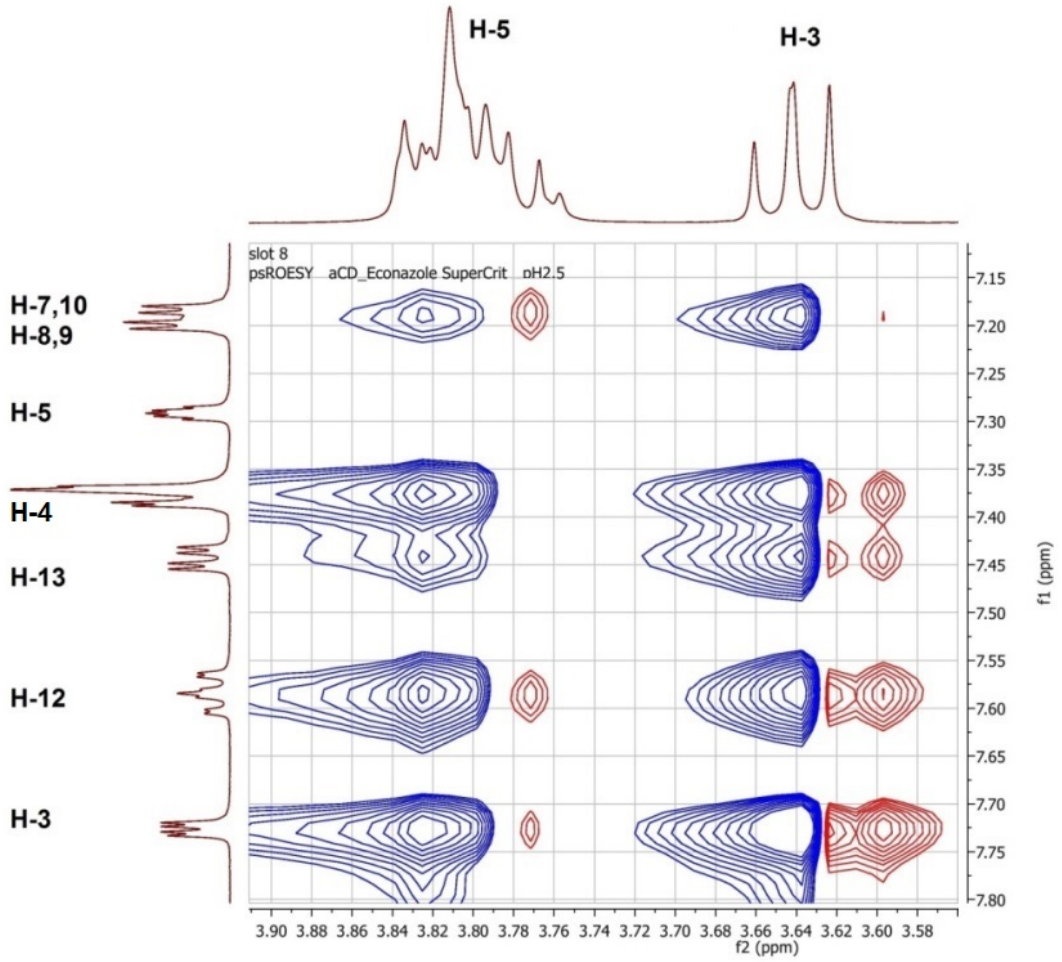


Figure 2.10: Expanded region of 2D ^{1}H-^{1}H ROESY (500 MHz) spectrum of econazole-α-cyclodextrin (1:1 molar) systems prepared by supercritical carbon dioxide inclusion method showing the ^{1}H-^{1}H nOes between protons of α-cyclodextrin and econazole.

As shown in the figure, the ROESY spectrum showed cross-correlation between the H-7, H-8, H-9 and H-10 protons (located on the mono-chloro phenyl moiety (II)) of econazole

with H-3 proton of α-cyclodextrin; H-5 and H-13 protons of econazole with H-5 proton of α-cyclodextrin; H-3 and H-12 protons of econazole with H-3 and H-5 protons of α-cyclodextrin.

The 2D-^{1}H-^{1}H ROESY observations were in good agreement with results obtained from the ^{1}H-NMR spectroscopic studies and suggest that the complex formed between econazole and α-cyclodextrin involves the phenyl rings and imidazole moiety of econazole entering the α-cyclodextrin cavity. Similar observations were reported by Fernandes and co-workers (Veiga, F.J.B. *et al.*, 2003) for nicardipine hydrochloride and β-cyclodextrin in unbuffered deuterated water (D_2O).

2.5.6 Relaxation time (T_1)-measurement Inversion Recovery Experiments

Longitudinal relaxation times (T_1) give information about the nucleus mobility in solution and the interaction between host and guest molecules by a qualitative analysis of the decreasing of T_1 values in the complexes (Joseph, B.L. and Eugene, P.M., 2004). Further evidence for the inclusion complexation of econazole with α-cyclodextrin was obtained through analysis of the spin lattice relaxation times (T_1) of their protons. Table 2.2 shows the T_1 variation of NMR signals of econazole and α-cyclodextrin protons in the binary mixture.

Table 2.2: Longitudinal relaxation times (T_1, s) of econazole and α-cyclodextrin protons in the binary mixture

Proton (Eco)	**$T_{1\ Eco}$ (s)**	**$T_{1\ Eco:\alpha\text{-}CD}$ (s)**	**Proton (α-CD)**	**$T_{1\ \alpha\text{-}CD}$ (s)**	**$T_{1\ \alpha\text{-}CD:Eco}$ (s)**
H-1	0.59	0.48	H-1	0.65	0.22
H-1[1]	0.65	0.28	H-2	1.09	0.78
H-2	1.30	1.28	H-3	1.07	0.47
H-6	0.83	0.73	H-4	0.64	0.61
H-6[1]	0.31	0.18	H-5	0.45	0.32
H-7,10	2.01	1.62	H-6,6[1]	0.33	0.27
H-8,9	2.16	1.33			
H-3	1.88	0.64			
H-5	2.66	1.21			
H-4	2.54	1.32			
H-11	3.68	1.82			
H-12	1.97	0.74			
H-13	1.98	1.04			

T_1 lowering of econazole protons was indicative of the change in the movement of the drug molecule in the presence of α-cyclodextrin and a strong host-guest interaction. As explained by Grillo, R. *et al*., (2007), this may be ascribed to the restricted rotation of econazole protons as a result of its complexation in the α-cyclodextrin cavity. A marked T_1 lowering was detected for H-7, H-8, H-9 and H-10 protons (located on the mono-chloro phenyl moiety (II)); H-3, H-4 and H-5 protons (located on the di-chloro phenyl moiety (I)); H-11, H-12 and H-13 protons (located on the imidazole moiety) suggesting that the drug penetrated the α-cyclodextrin cavity through different modes. Grillo, R. *et al*., (2007) showed the T_1 lowering of the hydroxymethylnitrofurazone and dimethyl-β-cyclodextrin system as a strong evidence of interactions between both molecules.

To summarise the trends observed in the nuclear magnetic resonance studies,

1. The evidence for the formation of inclusion complexes between econazole and α-cyclodextrin was obtained through the nuclear magnetic resonance studies (^{1}H-NMR, 2D- ^{1}H–^{1}H ROESY studies and relaxation time (T_1)-measurement inversion recovery experiments).

2. The studies indicated the encapsulation of the phenyl rings and imidazole moiety of econazole inside the α-cyclodextrin cavity through multiple modes.

3. Lowering of spin lattice relaxation times ($T_{1)}$ was noticed for all the econazole protons indicating the change in the movement of econazole in the presence of α-cyclodextrin and a strong host-guest interaction.

2.5.7 Dissolution Studies

The mean dissolution curves of econazole from various binary systems with α-cyclodextrin are presented in Figure 2.11.

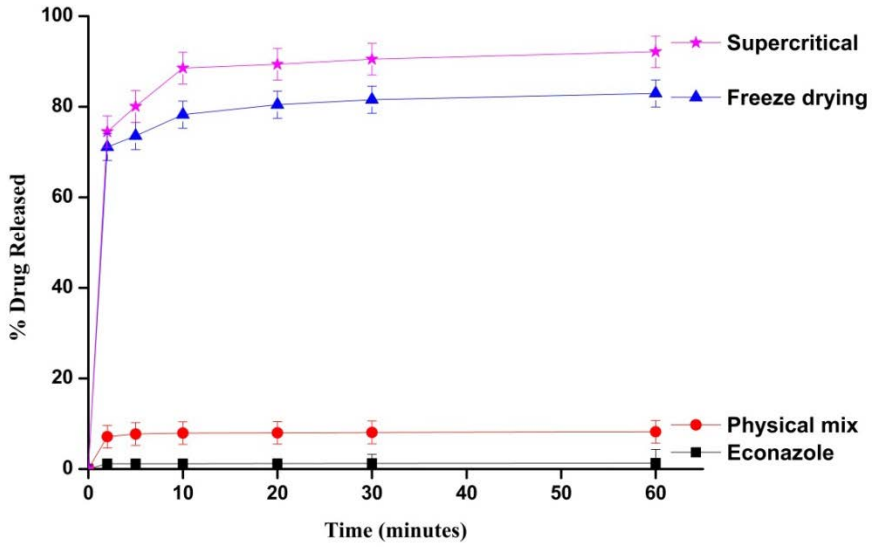


Figure 2.11: Dissolution profiles of econazole and econazole-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 60°C and 200 bar).

The results in terms of percent of active ingredient dissolved at 10 min and 30 min are collected in Table 2.3.

Table 2.3: Percent drug dissolved of the total amount added from econazole and econazole-α-cyclodextrin (1:1 molar) systems prepared by various methods.

Sample	**% drug dissolved** (10 min)	**% drug dissolved** (30 min)
Econazole	1.2	1.3
Physical mixing	7.9	8.2
Freeze drying	78.2	82.9
Supercritical carbon dioxide inclusion	88.5	90.5

All the systems with cyclodextrins exhibited higher dissolution properties than drug alone. As for the influence of the preparation method, an analogous trend was observed with all binary mixtures: the greatest improvement of the drug dissolution properties was obtained with supercritical carbon dioxide inclusion product, followed by freeze-dried product and finally by physical mixture. The improved dissolution characteristics of the physical mixture may be attributable to improvements in drug wettability as suggested by Charoenchaitrakool and co-workers (Charoenchaitrakool, M. *et al.*, 2002) there is no evidence of the *in situ* formation of readily soluble complexes in the medium as suggested by Banchero and co-workers (Banchero, M. *et al.*, 2013). The further enhanced dissolution of the freeze-dried and supercritical carbon dioxide inclusion products may be attributed to the reduced particle size, the high energetic amorphous state and the formation of inclusion

complexes between drug and cyclodextrin in the solid state as suggested by Hancock, B.C. and Zografi, G., (1997); El-Badry, M. *et al.*, (2009) and Khadka, P. *et al.*, (2014).

2.6 Conclusion

Solid systems of econazole with α-cyclodextrin in the 1:1 molar ratio were prepared by supercritical carbon dioxide-inclusion method and compared to products obtained using physical mixing and freeze drying. The results obtained by different characterisation techniques suggest complete complexation or amorphisation of econazole and cyclodextrin binary samples prepared by supercritical carbon dioxide-inclusion method. Different degrees of modification were observed in the analyses of products prepared by various methods, suggesting the possibility of drug-cyclodextrin interactions of different strengths, which may give rise to different degrees of inclusion formation and/or amorphisation of the sample. Nevertheless, products obtained by the supercritical carbon dioxide-inclusion method were among the ones showing the highest interaction between the drug and the cyclodextrin. All the systems with cyclodextrins exhibited better dissolution properties than drug alone. The greatest improvement of the drug dissolution properties was obtained with supercritical carbon dioxide-inclusion, followed by freeze drying and finally by physical mixing. Therefore, a solid inclusion method using supercritical carbon dioxide carrier proved to be a novel and useful complexation method for econazole into α-cyclodextrin. Furthermore, since this method has no toxic solvent residue, products obtained by this method should provide minimal side effects in humans, compared to those obtained by techniques, which require the use of organic solvents. The preliminary data allows hoping that the complexation of econazole with cyclodextrins will lend an ample credence for better therapeutic efficacy.

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Chapter 3: The Evaluation of Supercritical Fluid Technology as a Preparative Technique for the Manufacture of Econazole Salts and α-Cyclodextrin Complexes: A Comparison with Conventional Methods

Aims

- To study the interaction of cyclodextrins with different salts of econazole and then investigate the influence of different manufacturing processes (employed in Chapter 2) on the physicochemical properties of ternary econazole salt-α-cyclodextrin inclusion complexes
- To determine the effect of salt on the improvement in the solubility and dissolution rate of econazole.
- To gain an insight into the thermodynamics of complexation in addition to the strength of the interaction between econazole besylate and sulfosalicylate dihydrate salts and α-cyclodextrin using isothermal titration calorimetry.

3.1 Introduction

The formation of ternary complexes is reported to enhance the complexation efficiency of drugs and cyclodextrins. The addition of small amounts of a third component (a conjugate acid/base) into the system has been studied (Loftsson, T., *et al*., 1994). The apparent enhancement in solubilisation efficiency, and the consequent increased dissolution rate of

drugs, is attributed to a stabilization of the main interaction between the drug and cyclodextrin. The formation of ternary complexes has the clinical and economic benefit that it allows a reduction in the amount of cyclodextrin needed for inclusion and hence used in formulations (Loftsson, T., *et al*., 1994).

Different research groups have proposed that the solubilisation effect of hydroxy-acids such as lactic acid, malic acid, tartaric acid relies on their ability to modify the intramolecular hydrogen bond system that involves the secondary hydroxyl groups of cyclodextrins. (Germain, P. *et al*., 1995; Fenyvesi, E. *et al*., 1999). It has been reported that the drugs that elicit the most significant enhancement in solubility are intensely bound to the cyclodextrin cavity despite the fact they are in the ionised form.

Mura and co-workers (Mura, P. *et al*., 1999) studied the effects of various hydroxy-acids and cyclodextrins on the aqueous solubility of econazole and found that the solubilisation could be best achieved by the concurrent use of α-cyclodextrin and lactic acid in a 1:1:2.5 molar ratio (econazole : α-cyclodextrin : lactic acid).

Kim and co-workers (Kim, Y. *et al*., 1998) investigated the effects of formation of the tartarate, esylate and mesylate salts on the aqueous solubility of ziprasidone. They found that the solubility of ziprasidone was enhanced by 775-, 1600- and 3000-fold, respectively. The solubility was unexpectedly enhanced by 88,000-, 54,300- and 146,666-fold, respectively in a 40% w/v sulphobutylether β-cyclodextrin solution. They attributed the differential solubility to ion-pair formation. The authors suggested that the hydroxy-acid interacts more effectively with the external hydrogen bond system of cyclodextrins with a stronger ion pair. Their results indicated that the use of ziprasidone mesylate in combination with sulfobutylether β-cyclodextrin was found to have a commercially significant improvement in apparent solubility.

Szemán and co-workers (Szemán, J. *et al.*, 1994) investigated the effects of tartaric acid on the aqueous solubility of astemizole-β-cyclodextrin complexes and found the solubility of astemizole was significantly enhanced when compared to the drug itself and to the drug-cyclodextrin complex, respectively.

In this study econazole (a basic drug), chosen as a model can form salts with acids and form ternary complexes with cyclodextrins. Now that the effectiveness of the supercritical carbon dioxide process for preparing econazole-α-cyclodextrin inclusion complexes has been evaluated, the next aim was to study the interaction of cyclodextrins with salts of econazole and then investigate the influence of different manufacturing processes (employed earlier) on the physicochemical properties of econazole salt-α-cyclodextrin inclusion complexes, as an approach to improve the solubility and dissolution rate of econazole.

To our knowledge, there have been no reports of the direct investigation of the inclusion complexes of econazole or its salts with α-cyclodextrin in phosphate buffer (pH 4.5) using isothermal titration calorimetry. Hence, the purpose of this study was to gain an insight into the energetics and mechanisms of complexation in addition to the strength of the interaction between econazole salts and α-cyclodextrin using isothermal titration calorimetry.

3.2 Materials and Methods

3.2.1 Materials

α-Cyclodextrin (≥98%, molecular weight: 972.84 g mol^{-1}), nitric acid (≥99.0%, molecular weight: 63.01 g mol^{-1}), benzenesulfonic acid (98.0%, molecular weight: 158.18 g mol^{-1}) and 5-sulfosalicylic acid dihydrate (≥99.0%, molecular weight: 254.21 g mol^{-1}) and

potassium dihydrogen phosphate (≥98%) were purchased from Sigma-Aldrich, Gillingham, Dorset, UK. Ethanol (≥99.0%) was obtained from Fisher Scientific, UK. Deionised water was used for the preparation of phosphate buffer (0.05 M pH 4.5).

3.2.2 Synthesis of Econazole Besylate Salt

Benzenesulfonic acid (2.07 g, 13.08 mmol) was dissolved in ethanol (50 mL) and then econazole free base (5 g, 13.08 mmol) was added to the mixture. The solution was continuously stirred for 30 minutes until a homogeneous solution was obtained. The solution was then concentrated under reduced pressure at 70°C to give a white, dry powder. The obtained product was recrystallized twice using ethanol to produce 6.50 g (92% yield) of white crystalline econazole besylate salt (Figure 3.1).

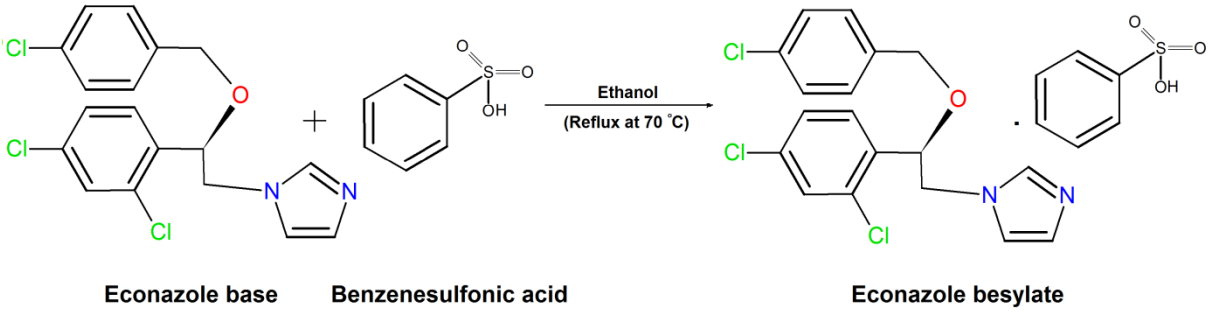

Figure 3.1: Synthesis of econazole besylate

3.2.3 Synthesis of Econazole 5-Sulfosalicylate Dihydrate Salt

5-Sulfosalicylic acid dihydrate (3.32 g, 13.08 mmol) was dissolved in ethanol (50 mL) and then econazole free base (5 g, 13.08 mmol) was added to the mixture. The solution was continuously agitated for 30 minutes until a homogeneous solution was obtained. The solution was then concentrated under reduced pressure at 70°C to give a white, dry powder. The obtained product was recrystallized twice using ethanol to produce 7.57 g (91% yield) of white crystalline econazole sulfosalicylate dihydrate salt (Figure 3.2).

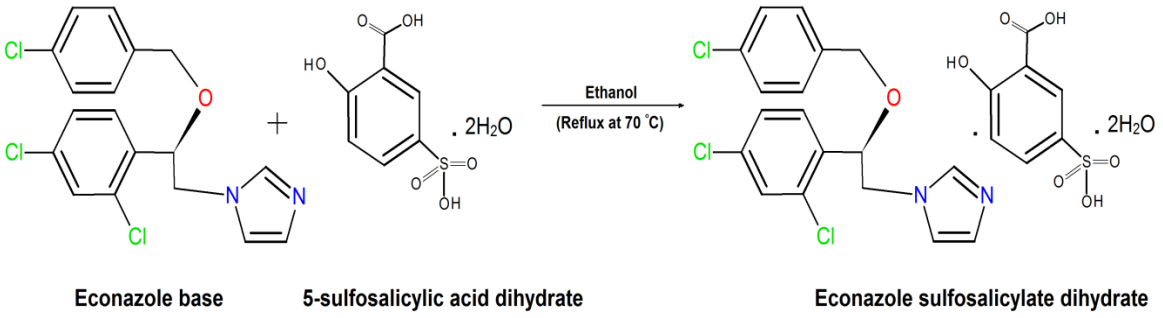

Figure 3.2: Synthesis of econazole sulfosalicylate dihydrate

3.2.4 Synthesis of Econazole Maleate Salt

Maleic acid (1.52 g, 13.08 mmol) was dissolved in ethanol (50 mL) and then econazole free base (5 g, 13.08 mmol) was added to the mixture. The solution was continuously agitated for 30 minutes until a homogeneous solution was obtained. The solution was then concentrated under reduced pressure at 70°C to give a white, dry powder. The obtained product was recrystallized twice using ethanol to produce 5.87 g (90% yield) of white crystalline econazole maleate salt (Figure 3.3).

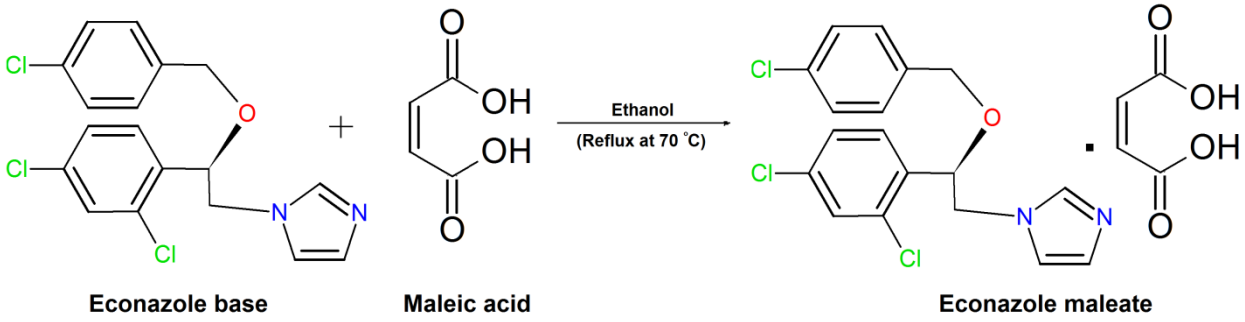

Figure 3.3: Synthesis of econazole maleate

3.3 Preparation of Binary Mixtures of Econazole with α-Cyclodextrin

All the binary mixtures were prepared in a 1:1 molar ratio of econazole salts to α-cyclodextrin. The masses of the salts and cyclodextrin used are as follows: econazole nitrate (1.00 g, 2.25 mmol) and α-cyclodextrin (2.19 g, 2.25 mmol); econazole besylate

(1.00 g, 1.85 mmol) and α-cyclodextrin (1.80 g, 1.85 mmol); econazole sulfosalicylate dihydrate (1.00 g, 1.57 mmol) and α-cyclodextrin (1.53 g, 1.57 mmol); econazole maleate (1.00 g, 2.00 mmol) and α-cyclodextrin (1.95 g, 2.00 mmol).

3.3.1 The physical mixing process

The required amounts of econazole salts and α-cyclodextrin were accurately weighed and tumble-mixed for about 15 minutes; the mixtures were passed through 0.150 mm aperture sieve and stored in a desiccator over fused calcium chloride.

3.3.2 The freeze-drying process

Econazole salts were added to aqueous solutions of α-cyclodextrin while mixing with a magnetic stirrer. After equilibrating in a horizontal shaker at room temperature for 48 hours, the resulting solutions were frozen at -60°C and were dried in a freeze-dryer (ScanVac CoolSafe, UK) at 25°C for 48 hours and then sieved through 0.150 mm aperture sieve. The obtained products were then labelled and stored in sealed containers for characterisation.

3.3.3 Supercritical carbon dioxide inclusion method

The complexes were prepared using an extraction apparatus (Thar Process Inc., Pittsburgh, PA). The physical mixtures of econazole salts and α-cyclodextrin were placed in a sample cell. Carbon dioxide was pumped from a cylinder via a cooling unit into the sample cell. 250 bar pressure was created by pumping against an automated back pressure regulator. The sample cell in the reaction vessel was heated to 100°C and held for 3 h before recovering the solid complexes by depressurisation. The obtained products were then homogenized (in a mortar), labelled and stored in sealed containers prior to further analysis.

3.4 Solubility Studies of Econazole Salts Alone and in the Presence of α-Cyclodextrin

Solubility measurements of econazole salts (nitrate, besylate, sulfosalicylate dihydrate and maleate) alone and with varying molar quantities of α-cyclodextrin (1:1, 1:2 and 1:3) were performed by adding drug salt (20 mg) or drug salt-α-cyclodextrin mixture equivalent to 20 mg of the drug salt to 10 mL of deionised water in sealed glass containers. The solutions were agitated for 48 hours at 25°C and 37°C (to determine the significance of temperature on the solubility of the salts). The solutions were then filtered (0.45 µm filter pore size, Sartorius, UK) and the econazole nitrate, besylate, sulfosalicylate dihydrate and maleate salts were assayed for drug concentration by ultra-violet spectroscopy (Cary 100 Bio UV-Vis, Agilent Technologies, USA) at 271nm, 264 nm, 299 nm and 273 nm, respectively. The UV spectrum of α-cyclodextrin was recorded and was found to have a negligible absorbance in the 220-350 nm range covered by the absorbance of econazole salts. Furthermore, it was noted that the wavelength of maximum absorbance of econazole salts did not change when in the presence of the α-cyclodextrin.

3.5 Analysis of the Prepared Binary Mixtures

3.5.1 Differential Scanning Calorimetric Analysis

Differential scanning calorimetry of individual components and the complex systems was carried out using a FP-90 central processor (Mettler-Toledo, LLC, UK). The instrument was calibrated with indium as a reference standard. 5-6 mg samples were accurately weighed and transferred to aluminium pans which were then closed and heated in the instrument at a scan rate of 10°C min^{-1} over the temperature range from 50 to 200°C.

3.5.2 Powder X-ray Diffraction Analysis

The X-ray powder diffractograms were obtained on a D8 Advance X-ray Diffractometer (Bruker, Germany) in theta-theta Bragg-Brentano geometry using reflection mode. Data was collected between 2-50° 2θ, with a step size of 0.006° and a counting time of 0.5 s per step using Cu Kα radiation.

3.5.3 Scanning Electron Microscopy Analysis

The surface morphology of econazole salts and the complex systems was examined by means of Hitachi SU-8030 scanning electron microscope (Tokyo, Japan). The powders were securely fixed on an aluminium stub using double-sided adhesive tape and then were made electrically conductive by coating in vacuum with a thin layer of chromium (~300 Å), for 30 s and at 30 W. Secondary electron images were taken at an excitation voltage of 2.0 kV and a magnification of ×350.

3.5.4 Dissolution Studies

Dissolution studies of econazole salts from all drug-carrier binary systems (nitrate, besylate, sulfosalicylate dihydrate and maleate salts of econazole and α-cyclodextrin), and for econazole salts alone, were conducted using USP Type II paddle method (Hanson G2 Vision® Classic 6, Chatsworth, CA) with deionised water as the dissolution medium. The samples, corresponding to 100 mg of econazole nitrate, sulfosalicylate dihydrate and maleate and 200 mg of econazole besylate were dispersed into 600 ml of the dissolution media maintained at 37 ± 0.5°C and stirred at 100 rpm. Suitable aliquots were withdrawn with a filter-syringe (pore size 0.45 μm) at the specified times and the drug concentration was determined by UV spectroscopy (Cary Bio 100 UV-Vis, Agilent Technologies, Santa Clara, CA 95051). The same volume of fresh medium was added to the beaker and each

test was performed in triplicate. The parameters used to characterize the dissolution curves were the percentage of drug dissolved at 10 min and 30 min.

3.5.5 Isothermal Titration Calorimetric Studies

The energetics of the interactions between econazole besylate and sulfosalicylate salts and α-cyclodextrin was evaluated by MicroCal VP-ITC MicroCalorimeter equipped with a Thermovac-2 sample degasser and thermostat (MicroCal, LLC, Northampton, MA). The calorimeter setup consists of a sample cell (with a fill volume and working volume of 1.8 mL and 1.4 mL, respectively) and a reference cell, enclosed in an adiabatic jacket as shown in the Figure 3.4. Both the cells were completely filled (1.4 mL) during all the experiments.

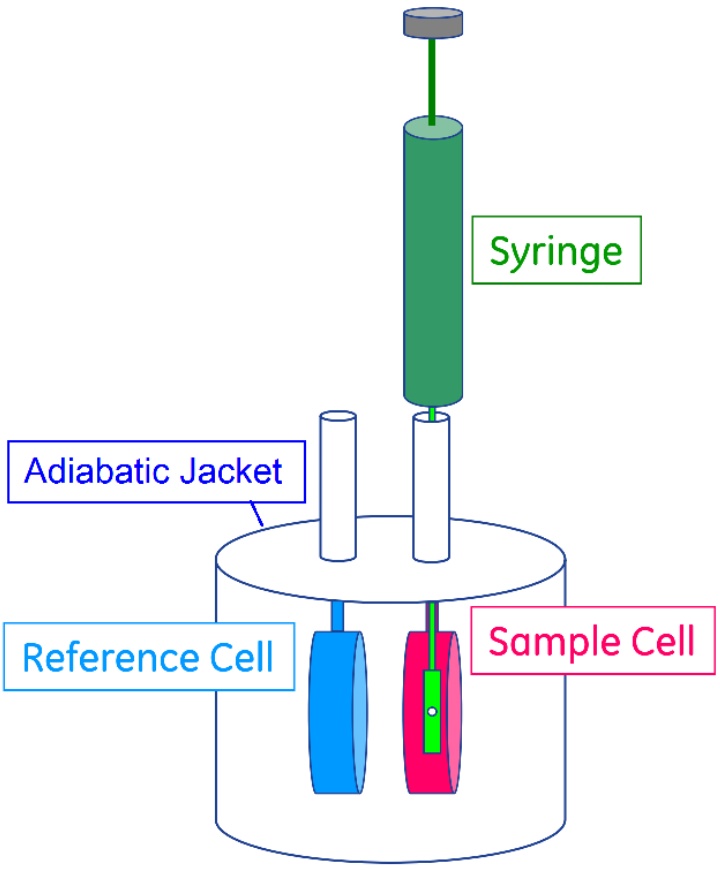

Figure 3.4: Schematics of Isothermal titration calorimeter supplied by MicroCal, LLC, Northampton, MA.

Firstly, a primary temperature equilibration was carried out for the sample in the cell, followed by a secondary equilibration with the syringe in place. A chemical calibration based on a titration of aqueous barium chloride into 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) was carried out to confirm the validity of the data produced in this work. The instrumental parameters for the studies used in this work are presented in Table 3.1.

Table 3.1: Instrumental settings (experimental and injection parameters) used in the isothermal titration calorimetric studies for the binding of econazole besylate and sulfosalicylate dihydrate salts with α-cyclodextrin in phosphate buffer (pH 4.5).

Parameter	**Value**
Cell Temp (^oC)	25
Reference power (µcal sec^{-1})	30
Initial delay (sec.)	100
Syringe concentration (mmol)	80
Cell concentration (mmol)	5
Stirring speed (rpm)	307
Total number of injections	29
Volume of injection (µL)	10
Duration of injection (sec.)	20
Spacing between injections (sec.)	240
Filter period (sec.)	2

The experiments were carried out in triplicate at 25°C in phosphate buffer (pH 4.5); titrating the α-cyclodextrin solution into the drug (econazole besylate/sulfosalicylate dihydrate) solution. A degassed aliquot (1.436 mL) of 1 mmol econazole besylate or sulfosalicylate dihydrate solution was placed in the sample cell while the same volume of phosphate buffer (pH 4.5) was placed in the reference cell. 290 µL of degassed α-cyclodextrin (10 mmol for titration against econazole besylate and 20 mmol for titration against econazole sulfosalicylate dihydrate) solution were loaded in the titration syringe.

The experiment involved 29 consecutive injections of small aliquots (10 µL each) of this α-cyclodextrin solution in the sample cell while stirring continuously at 307 rpm. Delay between each injection was 240 s. The amount of heat released or absorbed was recorded

following each injection. Difference in temperature (ΔT) between the sample and reference cells was monitored and power was supplied to both cells to maintain a constant difference in temperature. The output in the study is presented as a plot of the power released (or absorbed) *versus* time for the sample cell. Experiments were conducted under similar conditions to obtain the heats of dilution and mixing involved in: (i) injection of α-cyclodextrin solution (290 µL) into the pH 4.5 phosphate buffer (1.436 mL) and (ii) addition of buffer (290 µL) to the econazole besylate/sulfosalicylate dihydrate solution (1.436 mL). All experiments were performed in triplicate with the contributions from heats of dilution subtracted from all isotherms.

VPViewer 2000 software was used to control the operation of the calorimeter and set up the parameters (number of injections, volume of injection, and time interval between each injection) while the VP-ITC Origin 8.5 software was employed to analyse the data using a standard (non-linear least squares) fit model to compute the energetics of the reaction. The change in Gibbs free energy (ΔG) was determined using the affinity constant ($K_{b)}$ while the change in entropy (ΔS) was determined by the van't Hoff isotherm. χ^2 minimization was performed repeatedly to obtain the best fit parameters.

3.6 Results and Discussion

3.6.1 Solubility Studies

Cyclodextrins improve the water solubility of drugs by forming non-covalent, dynamic, water-soluble inclusion complexes (Londhe, V. and Nagarsenker, M., 1999). The effect of cyclodextrins on the solubility of drugs has been discussed earlier in section 1.2.4. The examples of the use of cyclodextrins to enhance drug solubility and dissolution rate can be seen in Table 1.3.

Solubility studies of econazole nitrate, besylate, sulfosalicylate dihydrate and maleate were carried out in deionised water at 25°C and 37°C alone and with varying ratios of α-cyclodextrin (1:1, 1:2 and 1:3). All the solubility studies were performed in triplicate and the relative increase in solubility was compared with that of the solubility of free base. The solubility data for econazole nitrate, besylate, sulfosalicylate dihydrate and maleate alone and with varying ratios of α-cyclodextrin are presented in Tables 3.2, 3.3, 3.4 and 3.5, respectively.

The solubility of econazole nitrate alone and in the presence of α-cyclodextrin (1:1 molar ratio) at 25°C and 37°C was found to be 0.42 mg ml^{-1} and 1.03 mg ml^{-1}; 0.88 mg ml^{-1} and 1.48 mg ml^{-1}, respectively. The solubility of econazole besylate alone and in the presence of α-cyclodextrin (1:1 molar ratio) at 25°C and 37°C was found to be 1.34 mg ml^{-1} and 2.09 mg ml^{-1}; 1.81 mg ml^{-1} and 2.32 mg ml^{-1}, respectively. The solubility of econazole sulfosalicylate dihydrate alone and in the presence of α-cyclodextrin (1:1 molar ratio) at 25°C and 37°C was found to be 0.54 mg ml^{-1} and 0.87 mg ml^{-1}; 1.18 mg ml^{-1} and 1.56 mg ml^{-1}, respectively. The solubility of econazole maleate alone and in the presence of α-cyclodextrin (1:1 molar ratio) at 25°C and 37°C was found to be 0.71 mg ml^{-1} and 1.37 mg ml^{-1}; 1.16 mg ml^{-1} and 1.98 mg ml^{-1}, respectively.

The increase in temperature had a positive effect on the solubility of all the salts of econazole alone and in the presence of cyclodextrin. Moreover, the solubility of the drug increased with increasing quantities of cyclodextrin as observed by Cabral Marques, H.M. *et al.*, (1990); Mura, P. *et al.*, (2001) and Ribeiro, L.S.S., *et al.*, (2003).

Mura and co-workers (Mura, P. *et al*., 1999) studied the effects of various hydroxy-acids (nitric, malic, citric, gluconic, lactic and tartaric acid) and α-, β-, γ- and hydroxypropyl-β-cyclodextrins on the aqueous solubility of econazole and found that the solubilisation could be best achieved by the concurrent use of α-cyclodextrin and malic acid in a 1:1:1 molar ratio (econazole: α-cyclodextrin: malic acid). The authors reported that a synergistic effect was found in drug-acid-cyclodextrin ternary systems which were more effective than corresponding salts and drug-cyclodextrin complexes. The authors reported the solubility of econazole base in the presence of nitric acid (binary mixture) at 25°C as 0.48 mg ml^{-1}; while it increased to 5.6 mg ml^{-1} in the presence of nitric acid and α-cyclodextrin (ternary mixture)

Chiesi and co-workers (Chiesi, P. *et al*., 1993) investigated the effects of tartaric or citric acid on the aqueous solubility of cis-ketoconazole-β-cyclodextrin complexes and found that the solubility of *cis*-ketoconazole was enhanced by 2200- and 80-fold when compared to the drug itself and to the drug-cyclodextrin complex, respectively.

Vikmon and co-workers (Vikmon, M. et al., 1994) investigated the effects of citric, tartaric, or ascorbic acid and β-cyclodextrin simultaneously, on the aqueous solubility of terfenadine and found that the solubility of terfenadine was enhanced by 3,000-5,000-fold and 400-600-fold in comparison to the drug itself and to the drug-cyclodextrin complex, respectively.

Table 3.2: Solubility data for econazole nitrate in deionised water with varying ratios of α-cyclodextrin (1:1, 1:2 and 1:3) at 25°C and 37°C

Temp (°C)	Drug or Drug-CD mixture	Solubility (mg ml^{-1}) of Eco Nitrate	Relative Increase*
25	Econazole Nitrate	0.42	120
25	Eco Nitrate + α-CD (1:1 molar)	1.03	300
37	Econazole Nitrate	0.88	250
37	Eco Nitrate + α-CD (1:1 molar)	1.48	420
37	Eco Nitrate + α-CD (1:2 molar)	1.80	510
37	Eco Nitrate + α-CD (1:3 molar)	2.19	630

Table 3.3: Solubility data for econazole besylate in deionised water with varying ratios of α-cyclodextrin (1:1, 1:2 and 1:3) at 25°C and 37°C

Temp (°C)	Drug or Drug-CD mixture	Solubility (mg ml^{-1}) of Eco Besylate	Relative Increase*
25	Econazole Besylate	1.34	320
25	Eco Besylate + α-CD (1:1 molar)	2.09	490
37	Econazole Besylate	1.81	430
37	Eco Besylate + α-CD (1:1 molar)	2.32	550
37	Eco Besylate + α-CD (1:2 molar)	4.72	1110
37	Eco Besylate + α-CD (1:3 molar)	4.79	1130

***Relative Increase** = Ratio between drug solubility in binary or ternary system and that of econazole base. The solubility of econazole base at 25°C and 37°C was found to be 3µg ml^{-1}

Table 3.4: Solubility data for econazole sulfosalicylate dihydrate with varying ratios of α-cyclodextrin (1:1, 1:2 and 1:3) at 25°C and 37°C

Temp (°C)	Drug or Drug-CD mixture	Solubility (mg ml^{-1}) of Eco Sulfosalicylate	Relative Increase*
25	Eco Sulfosalicylate dihydrate	0.54	110
25	Eco SSal + α-CD (1:1 molar)	0.87	170
37	Eco Sulfosalicylate dihydrate	1.18	240
37	Eco SSal+ α-CD (1:1 molar)	1.56	310
37	Eco SSal + α-CD (1:2 molar)	3.83	770
37	Eco SSal + α-CD (1:3 molar)	4.89	980

Table 3.5: Solubility data for econazole maleate in deionised water with varying ratios of α-cyclodextrin (1:1, 1:2 and 1:3) at 25°C and 37°C

Temp (°C)	Drug or Drug-CD mixture	Solubility (mg ml^{-1}) of Eco Maleate	Relative Increase*
25	Econazole Maleate	0.71	180
25	Eco Mal + α-CD (1:1 molar)	1.37	350
37	Econazole Maleate	1.16	297
37	Eco Mal + α-CD (1:1 molar)	1.98	507
37	Eco Mal + α-CD (1:2 molar)	2.91	745
37	Eco Mal + α-CD (1:3 molar)	3.90	997

***Relative Increase** = Ratio between drug solubility in binary or ternary system and that of econazole base. The solubility of econazole base at 25°C and 37°C was found to be 3µg ml^{-1}

3.7 Analysis of the Prepared Econazole Salt-α-Cyclodextrin Binary Systems

3.7.1 Differential Scanning Calorimetric Analysis:

Differential scanning calorimetry has commonly been employed in the characterization of solid-state interactions between drugs and cyclodextrins (Reddy, M.N., *et al.*, 2004). The drug–cyclodextrin interactions can be evaluated through the appearance, shift, or disappearance of endothermal or exothermal signals (Verma, R.K., Garg, S., 2004). The thermal behaviour of econazole salt-α-cyclodextrin mixtures was studied using differential scanning calorimetry to confirm the formation of the inclusion complexes.

The differential scanning calorimetry thermograms for econazole nitrate, α-cyclodextrin, and econazole nitrate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.5.

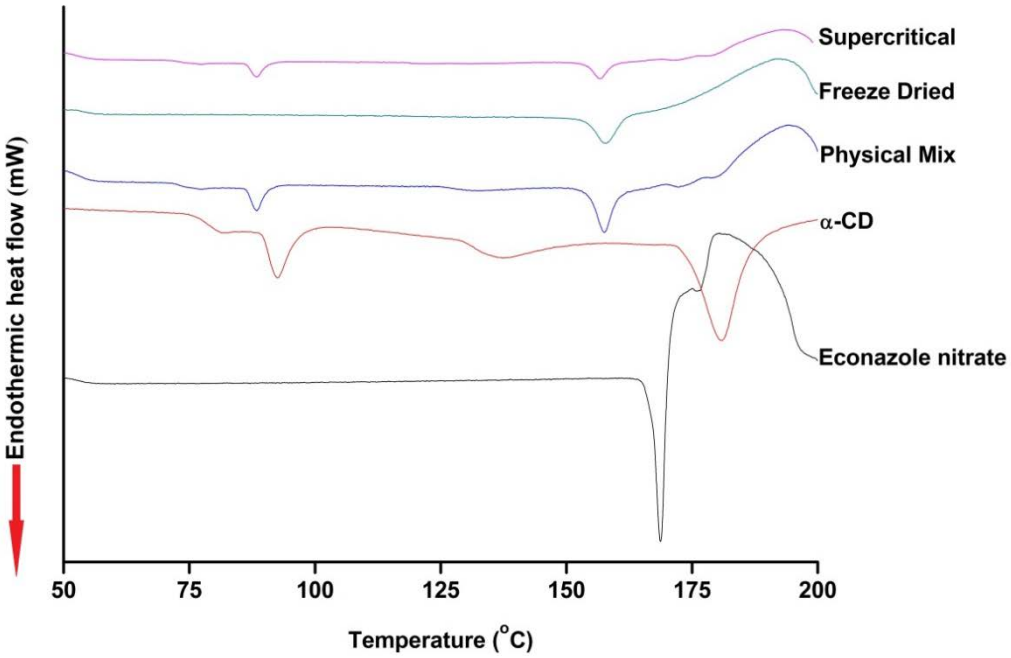

Figure 3.5: Differential scanning calorimetry thermograms of econazole nitrate, α-cyclodextrin and econazole nitrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

As shown in the figure, econazole nitrate exhibited a sharp melting endotherm at 168.7°C, indicating a crystal form. α-Cyclodextrin displayed broad phase transitions from 80°C - 100°C and 160°C - 180°C owing to the loss of water molecules from the cyclodextrin as suggested by Giordano, F. *et al.,* (2001).

The thermogram for all the binary mixtures showed the persistence of the endothermic peak of econazole nitrate. A small decrease in the intensity of the drug peak was observed in all the binary mixtures, indicating a small degree of interaction between drug and cyclodextrin (Erden, N., Çelebi, N., 1988). The characteristic endothermic effect of cyclodextrin slightly shifted to lower temperature for the binary mixtures prepared by physical mixing and supercritical carbon dioxide inclusion. The shift of the α-cyclodextrin peak to lower temperature suggests a disorder in the water molecules inside cyclodextrin cavity as explained by Valsami and group (Vertzoni, M. *et al.*, 2006). For the binary mixtures prepared by freeze-drying and supercritical carbon dioxide inclusion, the cyclodextrin peak was very small; however, a complete disappearance of the cyclodextrin peak was observed for the freeze-dried system indicating strong interactions between drug and α-cyclodextrin. The effect of cyclodextrin on the thermogram of econazole nitrate was observed as broadening and decrease in the intensity of the peak. Furthermore, the characteristic endothermic effect of the drug shifted to lower temperature from 168.7°C to *ca.* 157°C for all the binary mixtures indicating possible interactions between econazole nitrate and α-cyclodextrin. The broadening, reduced intensity and shift of the drug endothermic peak to lower temperature could be attributed to the interaction between drug and cyclodextrin (Patil, A.L. *et al.*, 2008; Wesolowski, M., Rojek, B., 2013 and Senthil, K., Vijaya, C., 2015).

The differential scanning calorimetry thermograms for econazole besylate, α-cyclodextrin and econazole besylate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.6.

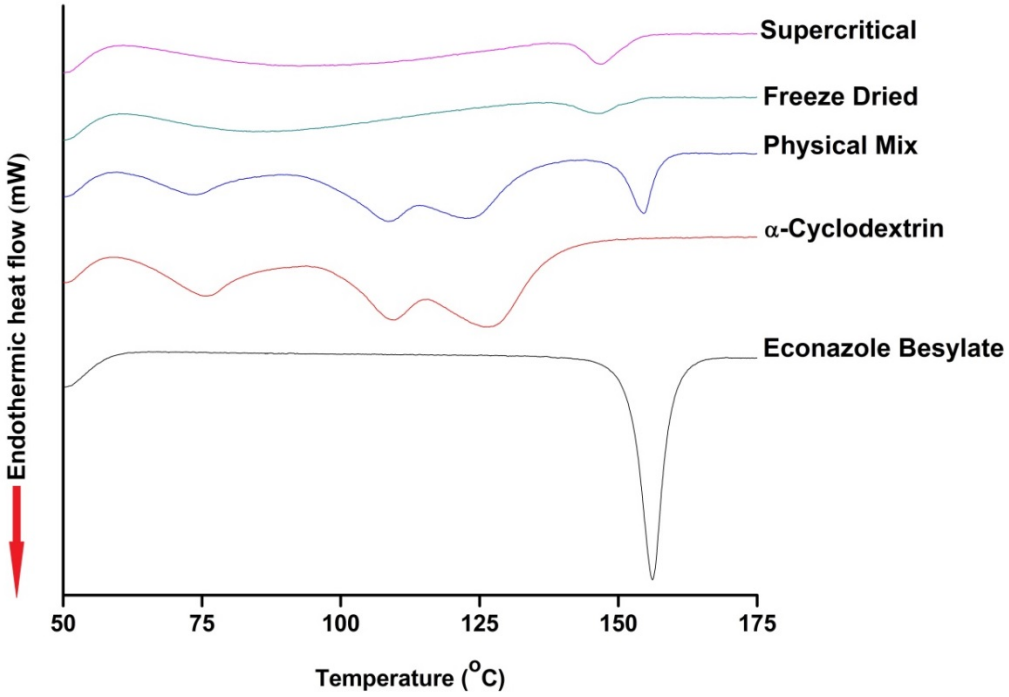

Figure 3.6: Differential scanning calorimetry thermograms of econazole besylate, α-cyclodextrin and econazole besylate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

As shown in the figure, econazole besylate exhibited a sharp melting endotherm at 156.2°C, indicating a crystalline form. The thermogram of physical mixture displayed peaks resulting from the superimposition of econazole besylate and α-cyclodextrin. The thermogram for all the binary mixtures showed the persistence of the endothermic peak of econazole besylate suggesting that the drug has not completely become amorphous or the existence of free guest molecule (Al-Marzouqi, A.H. *et al*., 2007a). The drug endothermic peak for the freeze-dried and supercritical carbon dioxide inclusion systems shifted to lower temperature *ca.* 147.9°C and 147.5°C, respectively and appeared to be very broad

and weak with reduced intensity indicating interactions between the drug and cyclodextrin as suggested by Li, N. *et al.*, (2005).

The differential scanning calorimetry thermograms for econazole sulfosalicylate dihydrate, α-cyclodextrin and econazole sulfosalicylate dihydrate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.7.

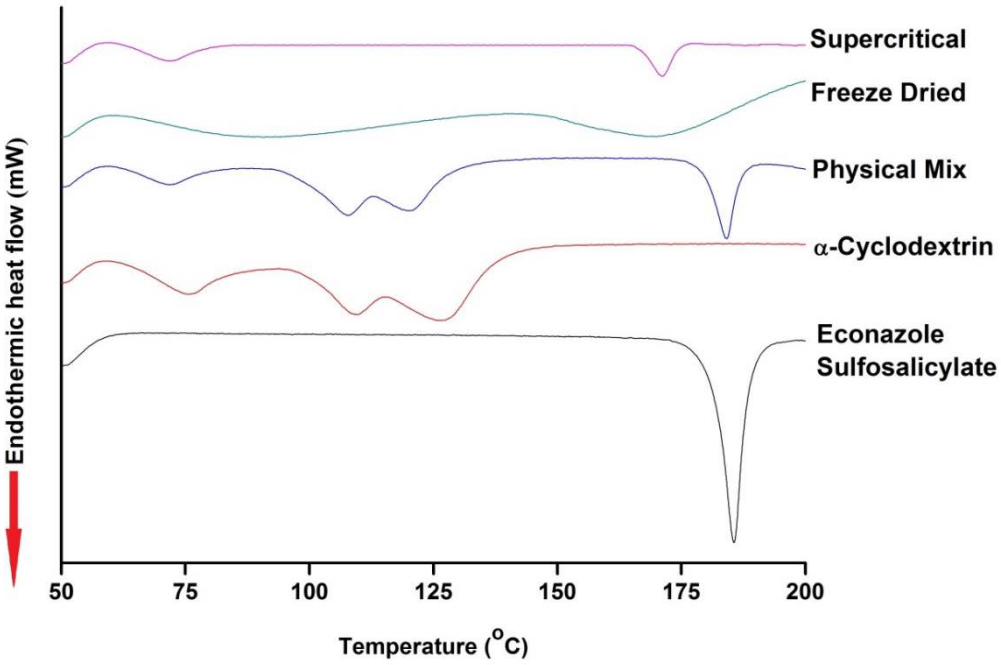

Figure 3.7: Differential scanning calorimetry thermograms of econazole sulfosalicylate dihydrate, α-cyclodextrin and econazole sulfosalicylate dihydrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

Econazole sulfosalicylate dihydrate exhibited a sharp melting endotherm at 185.6°C, indicating a crystalline form. The thermogram of physical mixture displayed peaks resulting from the superimposition of econazole sulfosalicylate dihydrate and α-cyclodextrin, suggesting that no inclusion occurred when drug and cyclodextrin were simply mixed together (Wang, X. *et al.*, 2014 and Zhou, S.-Y. *et al.*, 2014). The thermogram for the physical mixture and the supercritical carbon dioxide inclusion

systems showed the persistence of the endothermic peak of econazole sulfosalicylate dihydrate. A decrease in the intensity of the drug peak was observed in the supercritical carbon dioxide inclusion system while a broad endotherm was observed for the freeze-dried system. The endotherms corresponding to cyclodextrin disappeared completely for the freeze-dried system; however, supercritical carbon dioxide inclusion system displayed some endothermic effect of cyclodextrin. Furthermore, the characteristic endothermic effect of drug shifted to lower temperature (*ca.* 173°C) for these systems indicating interactions between drug and the cyclodextrin (Li, N. *et al.*, 2005) and suggests the inclusion of drug in the cyclodextrin cavity (Cabral Marques, H.M. *et al.*, 1990).

The differential scanning calorimetry thermograms for econazole maleate, α-cyclodextrin and econazole maleate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.8. As shown in the figure, econazole maleate exhibited a sharp melting endotherm at 153.1°C, indicating a crystalline form.

The thermogram of physical mixture displayed peaks resulting from the superimposition of econazole maleate and α-cyclodextrin. The thermogram for all the binary mixtures showed the persistence of the endothermic peak of econazole maleate. A decrease in the intensity of the drug peak was observed in the freeze-dried and supercritical carbon dioxide inclusion systems. The endotherms corresponding to cyclodextrin disappeared completely for the freeze-dried system; however, supercritical carbon dioxide inclusion system displayed some endothermic effect of cyclodextrin. Furthermore, the characteristic endothermic effect of drug shifted to higher temperature for the freeze-dried (*ca.* 157.2°C) and supercritical carbon dioxide inclusion systems (*ca.* 156.4°C) indicating interactions between drug and the cyclodextrin and suggests formation of inclusion complexes (Cabral Marques, H.M. *et al.*, 1990).

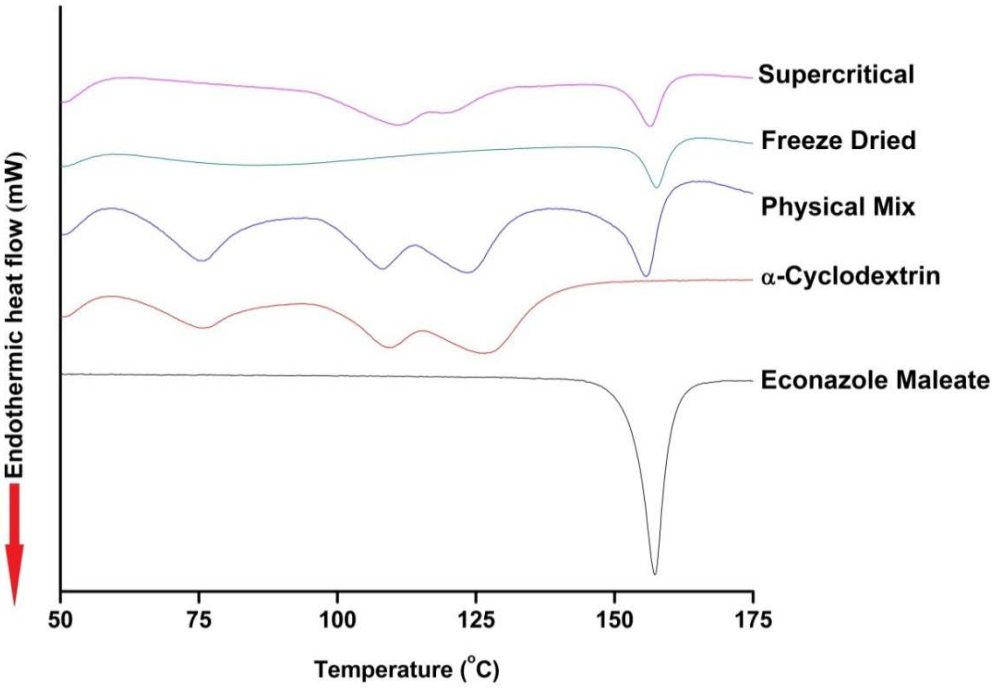

Figure 3.8: Differential scanning calorimetry thermograms of econazole maleate, α-cyclodextrin and econazole maleate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

Comparing the trends observed throughout the differential scanning calorimetric analysis of different econazole salt-α-cyclodextrin inclusion complexes, some general trends can be elucidated:

1. The thermogram of the physical mixtures of the besylate, sulfosalicylate dihydrate and maleate salts of econazole and α-cyclodextrin displayed peaks resulting from the superimposition of pure components.
2. The freeze-dried and supercritical carbon dioxide inclusion systems exhibited considerable broadening and reduction in the intensity of the drug endothermic peak indicating interactions between the drug and α-cyclodextrin.
3. The characteristic endothermic effect of drug showed no shifts for the inclusion systems of α-cyclodextrin with econazole base. However, the endothermic peak of

drug shifted to lower temperature for the freeze-dried and supercritical carbon dioxide inclusion systems of α-cyclodextrin with nitrate, besylate and sulfosalicylate dihydrate salts of econazole. Contrary to these results, the characteristic endothermic effect of drug shifted to higher temperature for the freeze-dried and supercritical carbon dioxide inclusion systems of α-cyclodextrin with econazole maleate.

3.7.2 Powder X-ray Diffraction Analysis:

Powder X-ray diffraction analysis was carried out to obtain further supporting evidence for the formation of inclusion complexes between econazole salts and α-cyclodextrin. The X-ray powder diffractograms of econazole nitrate, α-cyclodextrin, and econazole nitrate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.9.

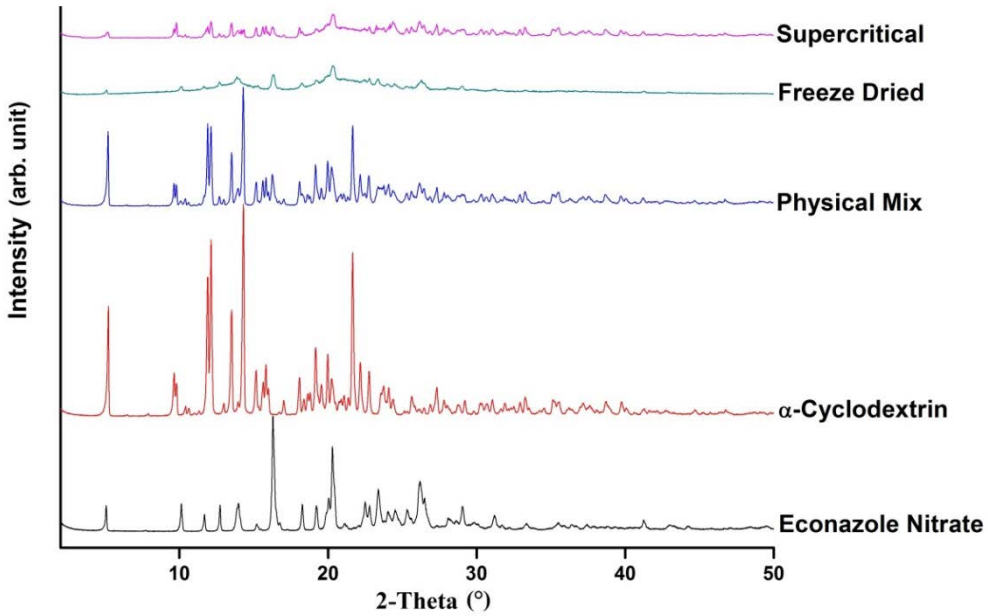

Figure 3.9: X-ray powder diffractograms of econazole nitrate, α-cyclodextrin and econazole nitrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The diffraction pattern of the econazole nitrate displayed sharp peaks at 2θ equal to 5.07°, 10.13°, 11.68°, 12.73°, 13.97°, 16.29°, 18.27°, 19.22°, 20.04°, 20.29°, 22.50°, 22.80°, 23.38°, 24.51°, 25.32°, 26.19° and 29.05° confirming its crystallinity in agreement with the differential scanning calorimetric analysis. α-Cyclodextrin displayed sharp peaks at 2θ equal to 5.21°, 9.64°, 9.79°, 11.90°, 12.13°, 13.51°, 14.31°, 15.16°, 15.62°, 15.82°, 18.07°, 19.17°, 19.55°, 19.98°, 20.24°, 22.18°, 22.77°, 23.75°, 24.10° and 27.33° with a flat background indicating its crystalline nature.

The degree of crystallinity (% crystallinity) of econazole nitrate-α-cyclodextrin systems produced by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods was found to be 99%, 11% and 15%, respectively. The binary mixture prepared by physical mixing showed a similar diffraction pattern to that of the respective individual components. In contrast, the freeze-dried and supercritical carbon dioxide inclusion systems exhibited considerable diminution of the diffraction peaks; though several sharp peaks corresponding to α-cyclodextrin with reduced intensity were observed. It can be observed from the diffractogram that the crystalline nature of econazole nitrate was still maintained in these systems; however the relative reduction of diffraction intensity of econazole nitrate suggests that the quality of the crystals were reduced (Barzegar-Jalali, M., *et al.*, 2010 and Singh, R. *et al*., 2010).

The X-ray powder diffractograms of econazole besylate, α-cyclodextrin and econazole besylate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.10. The diffraction pattern of the pure econazole besylate displayed sharp peaks at 2θ equal to 6.04°, 8.86°, 15.18°, 15.96°, 16.45°, 17.03°, 18.24°, 18.67°, 20.30°, 21.19°, 22.61°, 23.49°, 24.19°, 24.71°, 25.06°, 25.72°, 26.76°, 27.12°, 27.71° and 28.19° confirming its crystallinity in agreement with the differential scanning calorimetric analysis.

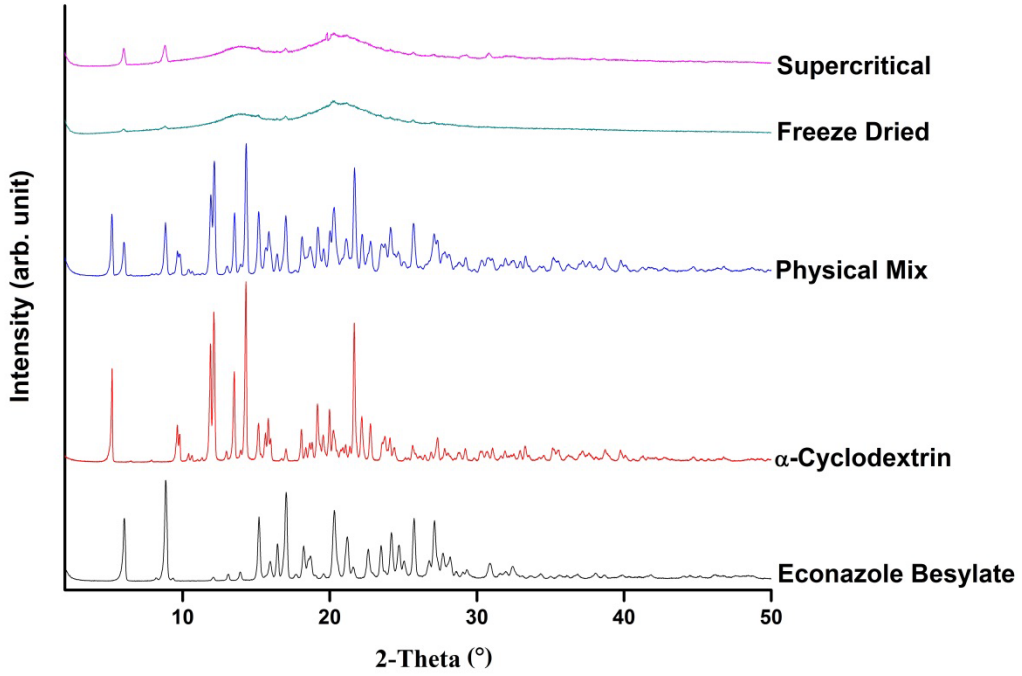

Figure 3.10: X-ray powder diffractograms of econazole besylate, α-cyclodextrin and econazole besylate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The degree of crystallinity (% crystallinity) of econazole besylate-α-cyclodextrin systems produced by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods was found to be 98%, 3% and 6%, respectively. The binary mixture prepared by physical mixing showed a similar diffraction pattern to that of the respective individual components. The supercritical carbon dioxide inclusion system showed a great reduction in the degree of crystallinity with broad weak peaks; however, it displayed two sharp peaks at 2θ equal to 6.04°, 8.86° corresponding to econazole besylate. The loss of crystallinity was most prominent for the freeze-dried system with several broad halos and broad weak peaks suggesting drug amorphisation and/or high interaction between drug and cyclodextrin. Williams III, R.O., *et al.*, (1998) and Spamer, E. *et al.*, (2002) suggested the loss of crystallinity as an added evidence for the formation of inclusion complexes.

The X-ray powder diffractograms of econazole sulfosalicylate dihydrate, α-cyclodextrin and econazole sulfosalicylate dihydrate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.11. The diffraction pattern of the pure econazole sulfosalicylate dihydrate displayed sharp peaks at 2θ equal to 7.06°, 8.48°, 10.38°, 12.66°, 13.16°, 14.62°, 16.84°, 17.40°, 19.11°, 19.56°, 20.34°, 20.84°, 21.19°, 21.50°, 22.34°, 23.51°, 24.66°, 25.47°, 27.77°, 28.37°, 28.64° and 30.84° confirming its crystallinity in agreement with the differential scanning calorimetry analysis.

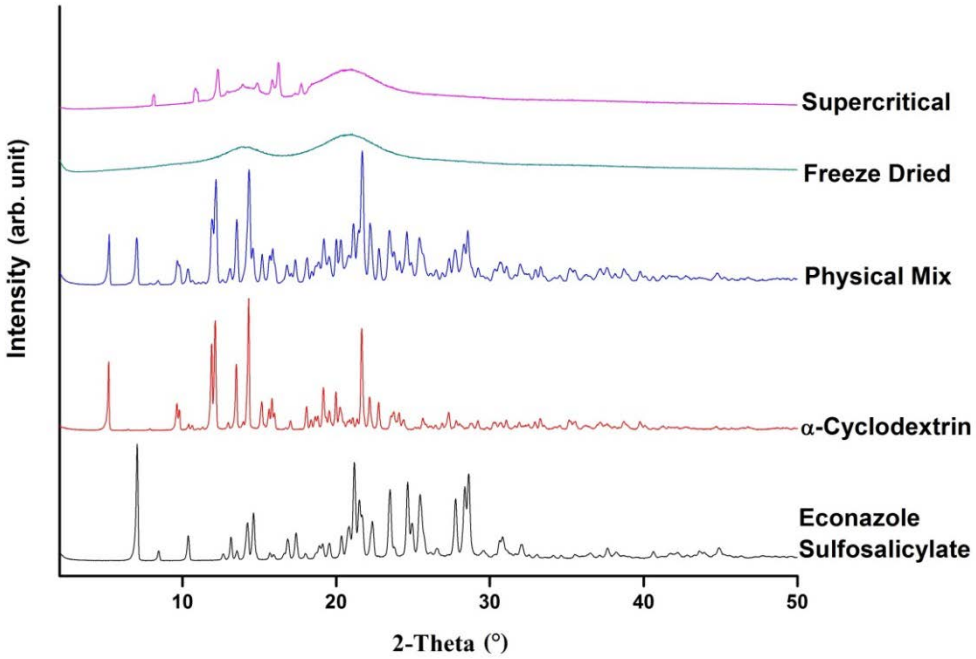

Figure 3.11: X-ray powder diffractograms of econazole sulfosalicylate dihydrate, α-cyclodextrin and econazole sulfosalicylate dihydrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The degree of crystallinity (% crystallinity) of econazole sulfosalicylate dihydrate-α-cyclodextrin systems produced by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods was found to be 100%, 0% and 11%, respectively. The binary mixture prepared by physical mixing and supercritical carbon dioxide inclusion showed a

similar diffraction pattern to that of the respective individual components. The freeze-dried system displayed several broad amorphous halos, suggesting complete drug amorphisation and/or high interaction between drug and cyclodextrin. Similar to the diffraction pattern of the econazole besylate-α-cyclodextrin supercritical carbon dioxide inclusion system, the supercritical carbon dioxide inclusion system exhibited considerable diminution of diffraction peaks. However, the diffraction pattern of freeze-dried system was devoid of drug peaks and displayed broad amorphous halos. The disappearance of the drug peaks clearly indicates the absence of the crystalline drug; however this does not allow distinguishing between drug amorphisation and its inclusion into the cyclodextrin cavity (Banchero, M. *et al.*, 2013).

The X-ray powder diffractograms of econazole maleate, α-cyclodextrin and econazole maleate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.12.

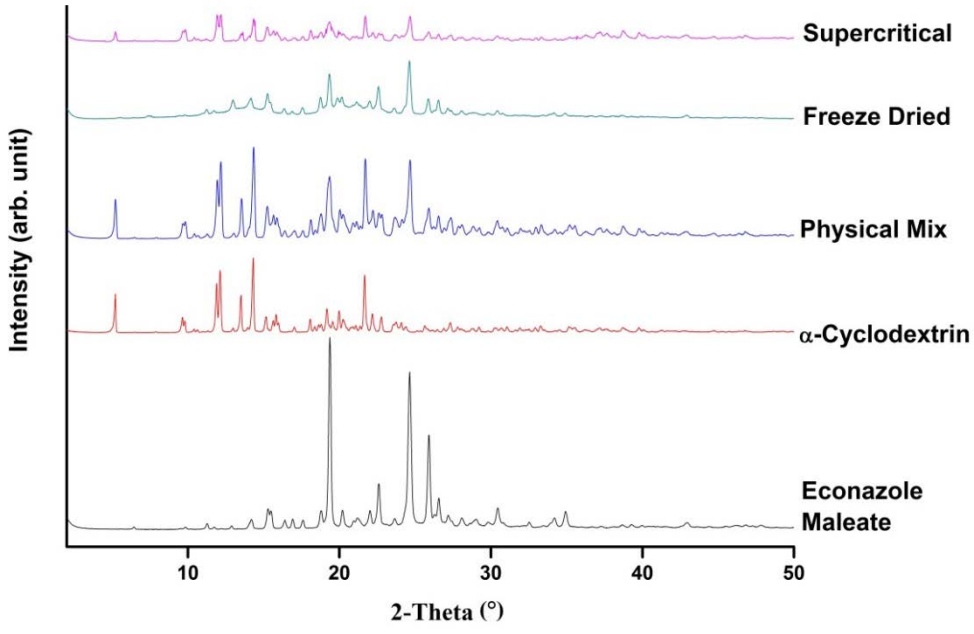

Figure 3.12: X-ray powder diffractograms of econazole maleate, α-cyclodextrin and econazole maleate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The diffraction pattern of the econazole maleate displayed sharp peaks at 2θ equal to 6.43°, 9.84°, 11.26°, 12.89°, 14.19°, 15.28°, 15.48°, 16.41°, 16.92°, 17.60°, 18.78°, 19.37°, 20.20°, 21.19°, 22.01°, 22.61°, 23.66°, 24.64°, 25.92°, 26.56°, 27.18°, 28.10°, 30.45°, 34.18°, and 34.92° confirming its crystallinity in agreement with the differential scanning calorimetric analysis.

The degree of crystallinity (% crystallinity) of econazole maleate-α-cyclodextrin systems produced by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods was found to be 98%, 11% and 16%, respectively. The binary mixture prepared by physical mixing showed a similar diffraction pattern to that of the respective individual components. Similar to the diffraction patterns of the binary mixtures of econazole nitrate and α-cyclodextrin, all the binary mixtures of econazole maleate displayed the drug peaks but with reduced intensity.

Taking the trends observed throughout the X-ray diffraction analysis of the inclusion complexes formed with the different salts presented above, it is possible to discern some general trends. These can be summarised as:

1. The diffraction patterns of the physical mixtures of all the drug salts and α-cyclodextrin are simply the superimposition of the patterns of the respective pure components.
2. The freeze-dried and supercritical carbon dioxide inclusion systems exhibited considerable diminution of the diffraction peaks indicating interactions between the drug and α-cyclodextrin.
3. The increase in the amorphous content of the drug in the binary systems of α-cyclodextrin with econazole free base, nitrate, besylate and sulfosalicylate dihydrate salts of econazole was observed in the following order: physical mixing <

supercritical carbon dioxide processing < freeze-drying. However, contrary to these results, the increase in the amorphous content of the econazole maleate in the binary systems was observed in the following order: physical mixing < freeze-drying < supercritical carbon dioxide processing.

4. Conversely, the loss of the crystallinity of the freeze-dried products can be a consequence of the freeze-drying process and hence powder X-ray diffraction analysis could not discriminate if the obtained product was a true inclusion complex or an amorphous mixture.

3.7.3 Scanning Electron Microscopy Analysis:

Scanning electron microscopy analysis was used to study the morphological aspects of all the salts and their binary mixtures. The technique doesn't provide an adequate evidence to confirm the inclusion complexation; however it assists to assess the existence of a single phase in the obtained mixtures (Duchêne, D., 1987). Examples of recent investigations featuring the use of scanning electron microscopy in the characterisation of inclusion complexes can be seen in Table 1.6.

The scanning electron photomicrographs of econazole nitrate, α-cyclodextrin, and econazole nitrate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.13. From scanning electron microscopy analysis, econazole nitrate appeared as small-to-large crystals (5-50 µm). Similar to the free base, the larger crystals were prismatic and columnar whereas, the smaller crystals were both rods and flat plates. Pure econazole nitrate and econazole nitrate processed with supercritical carbon dioxide showed no differences in the morphology.

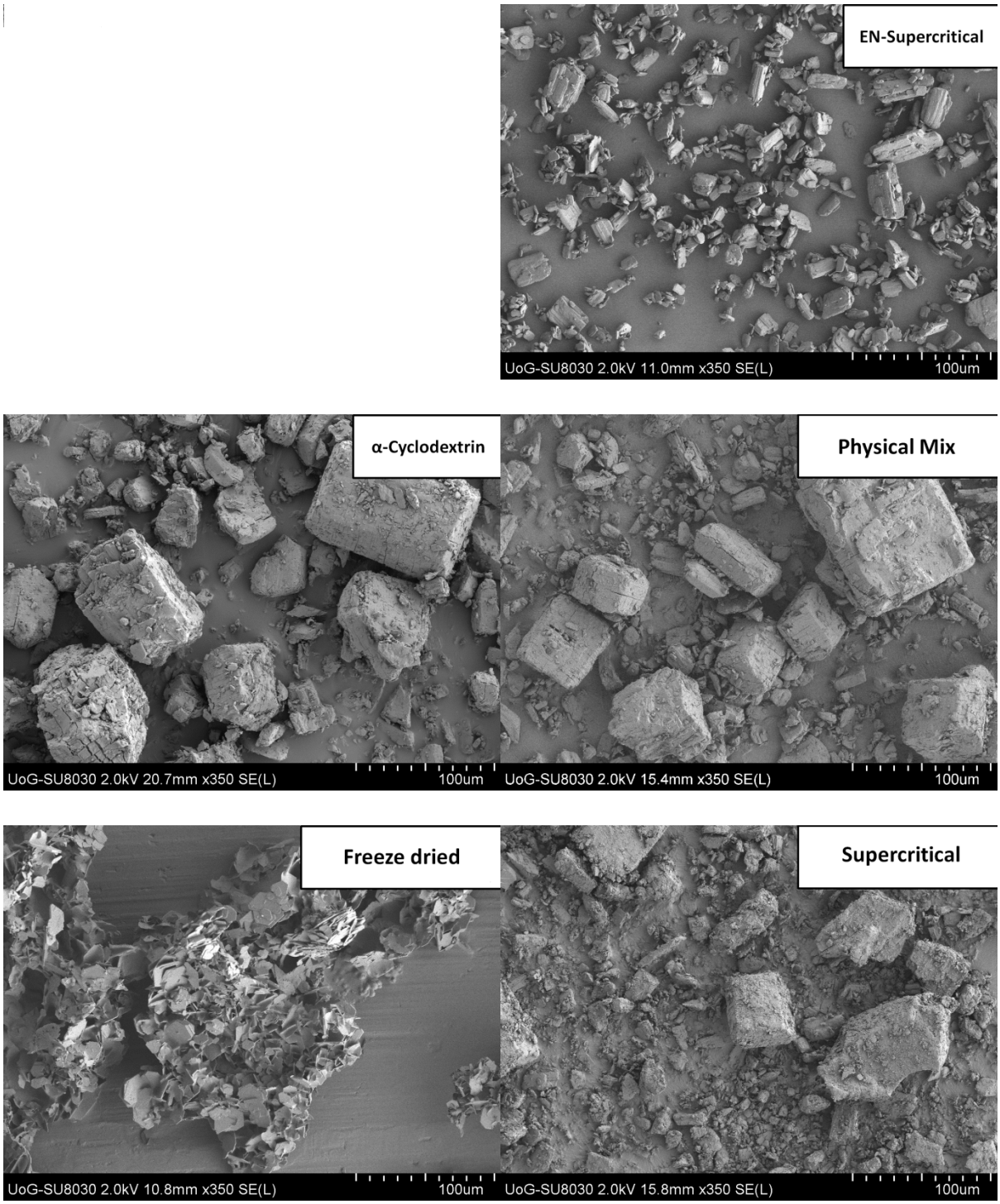

Figure 3.13: Scanning electron photomicrographs of econazole nitrate (unprocessed) and supercritical carbon dioxide processed econazole nitrate, α-cyclodextrin, and econazole nitrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide processing at ×350 magnification (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The physical mixture of drug and cyclodextrin appeared as rectangular crystals (10-100 µm). The photomicrograph of the physical mixture showed clearly the characteristic econazole nitrate crystals, mixed with or adhered to the surface of the cyclodextrin crystals, confirming the presence of crystalline drug. Freeze-dried system appeared as small irregular fragments with the largest particle at the size of 50 µm. Supercritical carbon dioxide inclusion system appeared as a mixture of larger and much smaller grains (5-65 µm). In the freeze-dried product, the original morphology of the raw materials disappeared, and it was not possible to differentiate between the two components. It appeared as agglomerates. The drastic change of the shapes of the particles and their aspect was indicative of the presence of a new solid phase, leading to the suggestion of the existence of a single phase, thus corroborating the powder X-ray diffraction observations (Zhang, X. *et al.*, 2009).

The scanning electron photomicrographs of econazole besylate, α-cyclodextrin and econazole besylate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.14. From scanning electron microscopy analysis, econazole besylate appeared as small columnar crystals (5-10 µm) with a tendency to self-agglomerate. The photomicrograph of physical mixture showed clearly the mixture of drug and cyclodextrin crystals. Freeze-dried system appeared as irregular flaky fragments while the supercritical carbon dioxide inclusion system appeared as large indistinct lumps (20-70 µm) with network like voids. In the freeze-dried and supercritical carbon dioxide processed products, the original morphology of the raw materials disappeared, and it was not possible to differentiate between the two components. They appeared as agglomerates. It was suggested by Naidu and co-workers (Naidu, N.B. *et al.*, 2004) that modification in the shape of drug particles indicates the formation of a new solid phase.

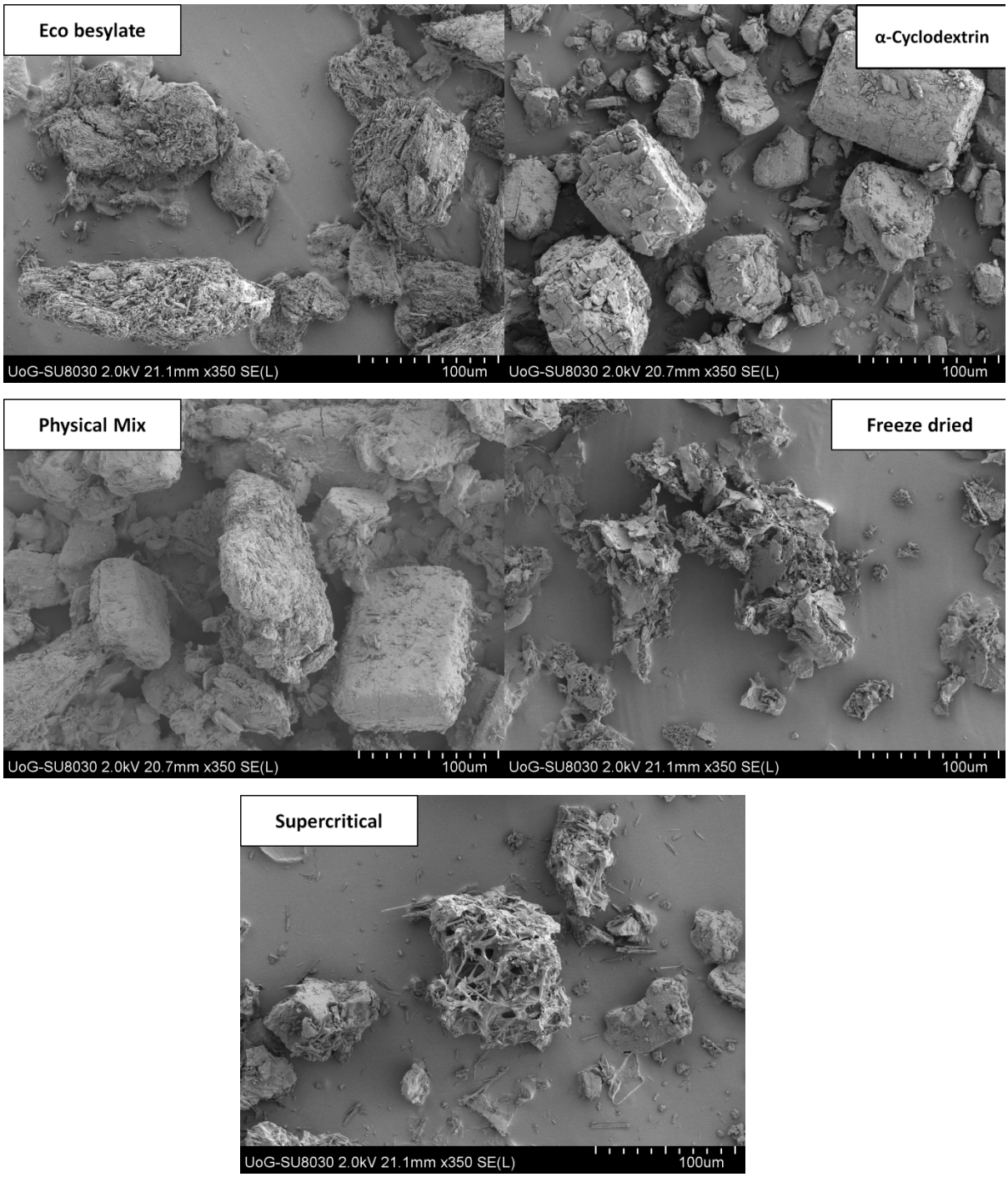

Figure 3.14: Scanning electron photomicrographs of econazole besylate, α-cyclodextrin and econazole besylate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods at ×350 magnification (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The scanning electron photomicrographs of econazole sulfosalicylate dihydrate, α-cyclodextrin and econazole sulfosalicylate dihydrate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.15.

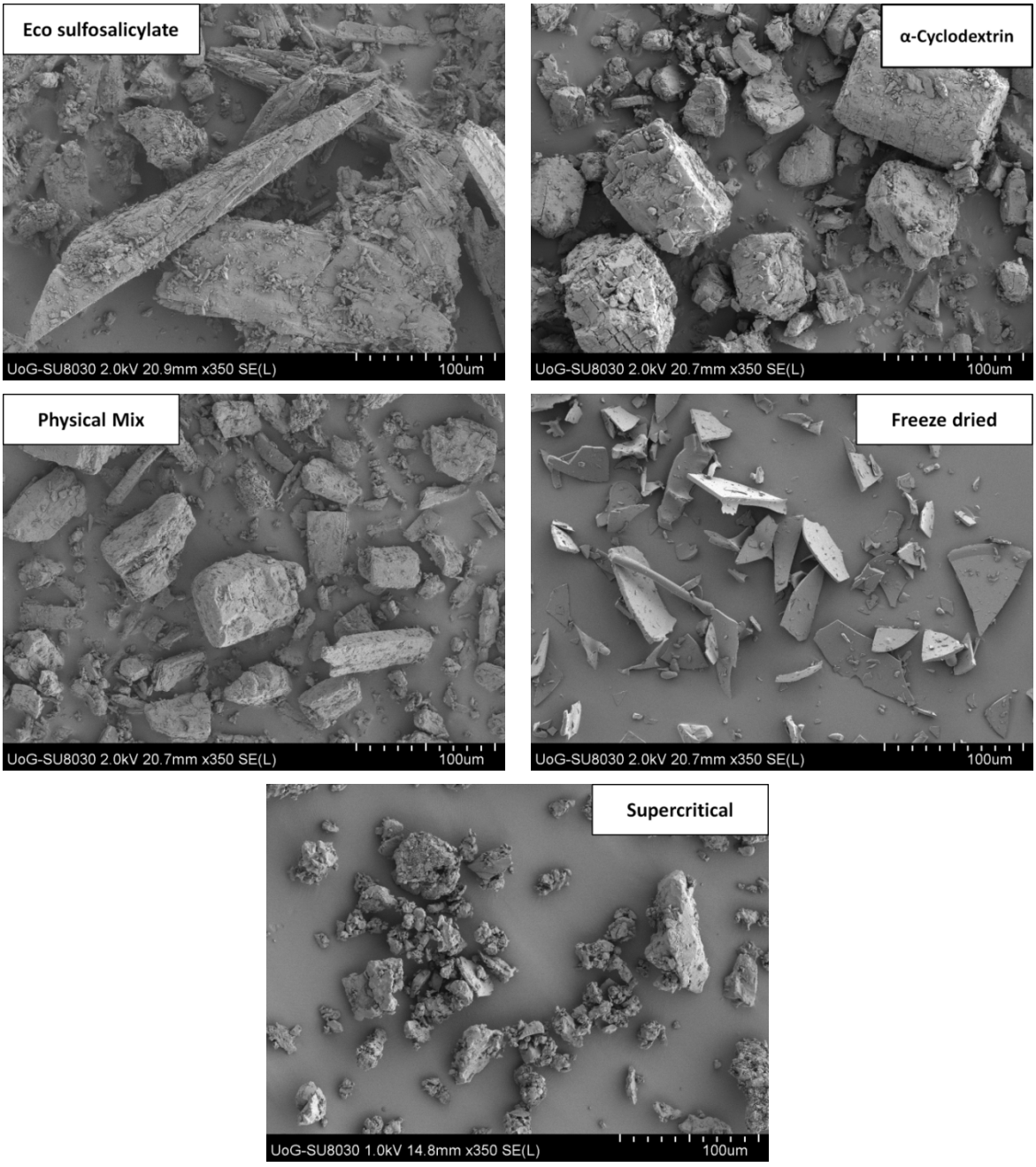

Figure 3.15: Scanning electron photomicrographs of econazole sulfosalicylate dihydrate, α-cyclodextrin and econazole sulfosalicylate dihydrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods at ×350 magnification (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

From scanning electron microscopy analysis, econazole sulfosalicylate dihydrate appeared as small to very large sharp crystals (10-250 µm) with a tendency to self-agglomerate. Crystalline structure of the drug is in agreement with the differential scanning calorimetry and powder X-ray diffraction analysis. Freeze-dried system appeared as large glassy shards and sharp-edged irregular fragments with the largest particle at the size of 500 µm. Supercritical carbon dioxide inclusion system appeared as a mixture of massive and very fine indistinct grains (3-80 µm).

The scanning electron photomicrographs of econazole maleate, α-cyclodextrin and econazole maleate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.16. From scanning electron microscopy analysis, econazole maleate appeared as small to large crystals (10-150 µm) with a tendency to self-agglomerate. Crystalline structure of the drug is in agreement with the differential scanning calorimetry and powder X-ray diffraction analysis. The supercritical carbon dioxide inclusion system appeared as a mixture of small to large indistinct lumps (5-65 µm). Freeze-dried system appeared as agglomerates of irregular shaped fragments. The original morphology of the raw materials disappeared, and it was not possible to differentiate between the two components.

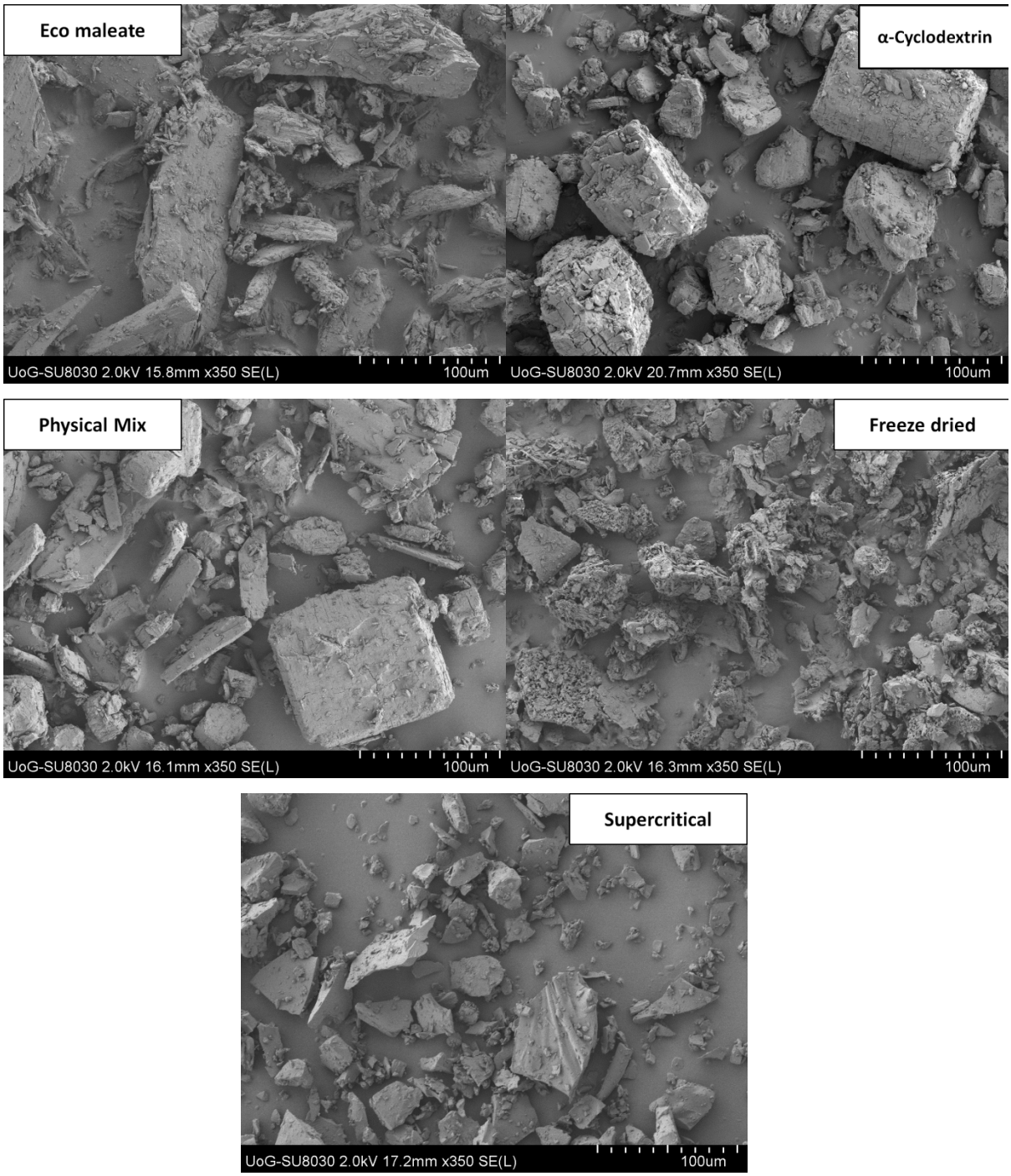

Figure 3.16: Scanning electron photomicrographs of econazole maleate, α-cyclodextrin and econazole maleate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods at ×350 magnification (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

As for the results of the other analytical techniques, it is worthwhile in highlighting succinctly the general trends observed across each of the econazole salt-cyclodextrin inclusion complexes:

1. The photomicrographs of the physical mixtures of all the drug salts and α-cyclodextrin showed clearly the characteristic salt crystals, mixed with or adhered to the surface of the cyclodextrin crystals, confirming the presence of crystalline drug.
2. The comparable morphology of the physical mixtures with pure components could reveal that apparently no drug salt-α-cyclodextrin interaction has taken place in the solid state.
3. The original morphology of the raw materials disappeared in the freeze-dried and supercritical carbon dioxide inclusion products and it was not possible to differentiate between the two components.

3.7.4 Dissolution Studies:

The dissolution studies of econazole salts and their binary mixtures with α-cyclodextrin were carried out in triplicate to investigate the extent of drug release from the binary mixtures. The dissolution profiles of econazole nitrate from drug alone and econazole nitrate-α-cyclodextrin 1:1 molar binary systems are presented in Figure 3.17.

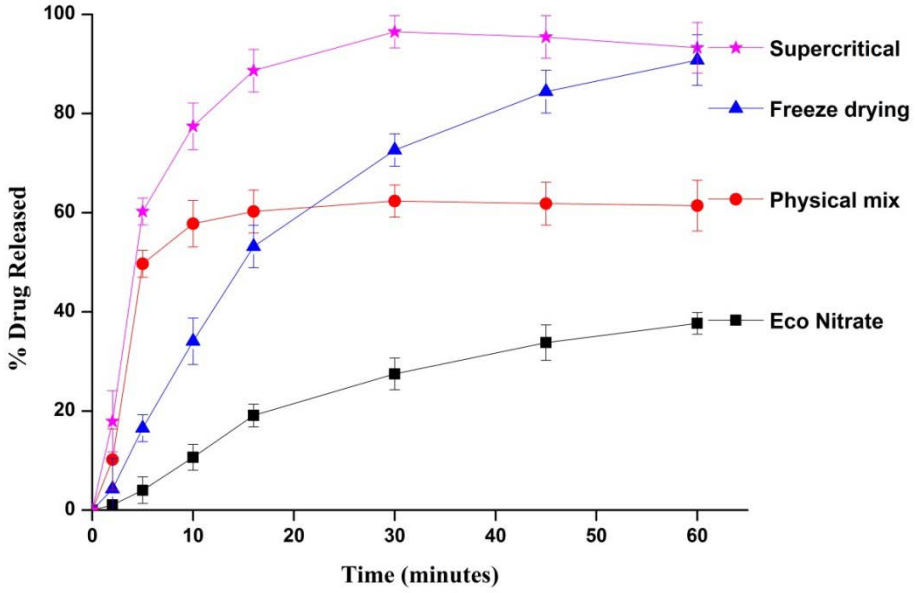

Figure 3.17: Dissolution rate profiles of econazole nitrate and econazole nitrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The results in terms of percent of active ingredient dissolved at 10 min and 30 min are collected in Table 3.6.

Table 3.6: Percent econazole nitrate dissolved at 10 and 30 min from econazole nitrate and econazole nitrate-α-cyclodextrin systems.

Sample	**% drug dissolved** (10 min)	**% drug dissolved** (30 min)
Econazole nitrate	8.7	27.5
Physical mixing	57.8	62.4
Freeze-drying	34.1	72.6
Supercritical carbon dioxide inclusion	77.4	96.5

All the systems with cyclodextrins exhibited greater dissolution properties than drug alone. The greatest improvement of the drug dissolution was obtained with the binary mixture

prepared by supercritical carbon dioxide inclusion, followed by freeze-drying and physical mixing. The amount of econazole nitrate released from drug alone was low with 8.7 % dissolving within 10 minutes. The binary mixtures prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion exhibited an increased drug release with 57.8 %, 34.1 % and 77.4 % of econazole nitrate dissolved within 10 minutes, respectively. As shown in Figure 3.16, the release of the drug from the freeze-dried product was initially slower than the physical mixture but increased gradually with time. The binary mixture prepared by supercritical carbon dioxide inclusion exhibited a drug release of more than 90 % within 30 minutes.

The dissolution profiles of econazole besylate from drug alone and econazole besylate-α-cyclodextrin (1:1 molar) binary systems are presented in Figure 3.18.

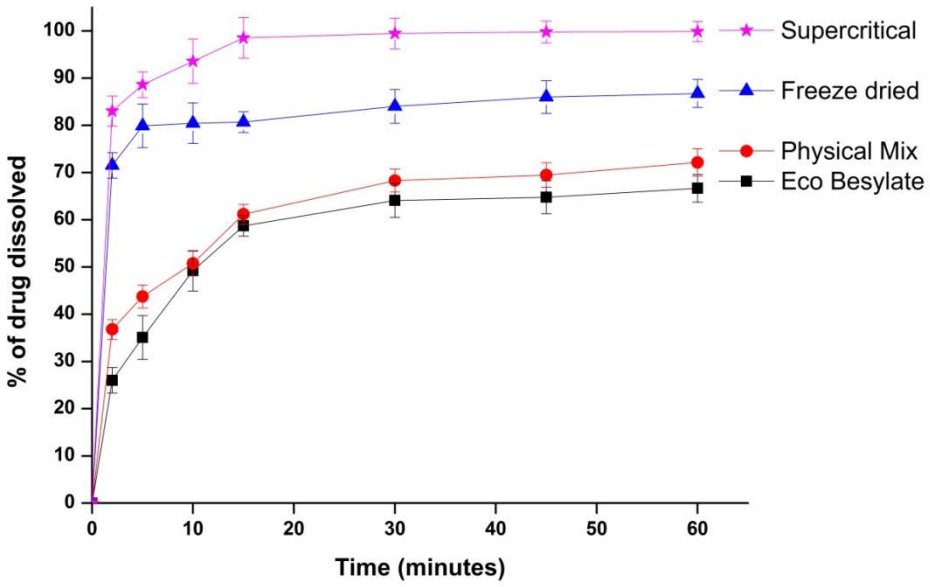

Figure 3.18: Dissolution rate profiles of econazole besylate and econazole besylate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The results in terms of percent of active ingredient dissolved at 10 min and 30 min are collected in Table 3.7.

Table 3.7: Percent econazole besylate dissolved at 10 and 30 min from econazole besylate and econazole besylate-α-cyclodextrin systems.

Sample	**% drug dissolved** (10 min)	**% drug dissolved** (30 min)
Econazole besylate	49.2	64.1
Physical mixing	50.8	68.3
Freeze-drying	80.4	84.1
Supercritical carbon dioxide inclusion	93.6	99.5

The amount of econazole besylate released from drug alone was 49.2 % dissolving within 10 minutes. The binary mixtures prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion exhibited an increased drug release with 50.8 %, 80.4 % and 93.6 % of econazole besylate dissolved within 10 minutes, respectively. The binary mixtures prepared by freeze-drying and supercritical carbon dioxide inclusion exhibited a drug release of more than 80 % within 10 minutes. The binary mixture prepared by supercritical carbon dioxide inclusion exhibited complete drug release within 30 minutes.

The dissolution profiles of econazole sulfosalicylate dihydrate from drug alone and econazole sulfosalicylate dihydrate-α-cyclodextrin (1:1 molar) binary systems are presented in Figure 3.19.

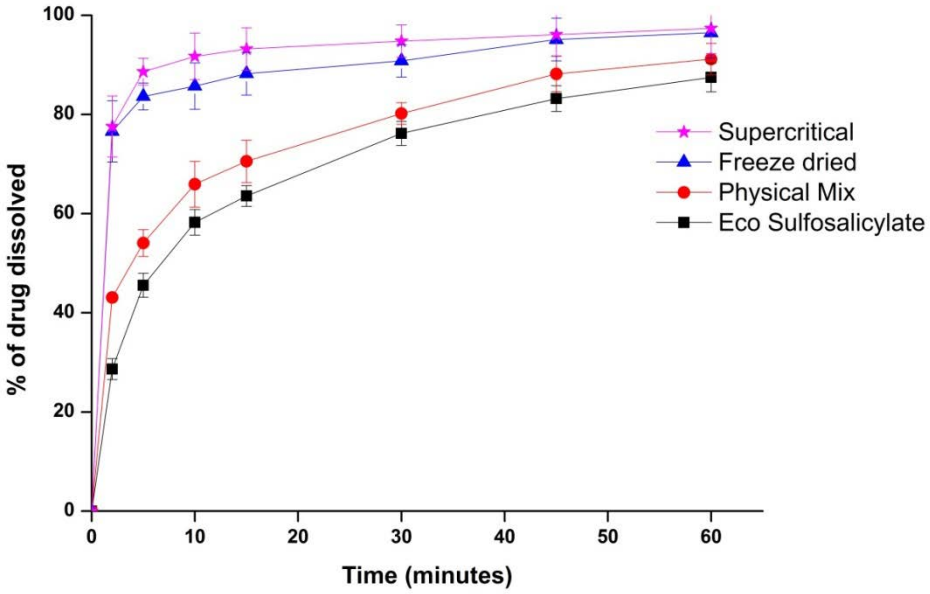

Figure 3.19: Dissolution rate profiles of econazole sulfosalicylate dihydrate, and econazole sulfosalicylate dihydrate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

The results in terms of percent of active ingredient dissolved at 10 min and 30 min are collected in Table 3.8.

Table 3.8: Percent econazole sulfosalicylate dihydrate dissolved at 10 and 30 min from econazole sulfosalicylate dihydrate and econazole sulfosalicylate dihydrate-α-cyclodextrin systems.

Sample	**% drug dissolved** (10 min)	**% drug dissolved** (30 min)
Econazole sulfosalicylate dihydrate	58.2	76.2
Physical mixing	65.9	80.1
Freeze-drying	85.7	89.8
Supercritical carbon dioxide inclusion	91.6	94.8

The amount of econazole sulfosalicylate dihydrate released from drug alone was 58.2 % dissolving within 10 minutes. The binary mixtures prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion exhibited an increased drug release with 65.9 %, 85.7 % and 91.6 % of econazole sulfosalicylate dihydrate dissolved within 10 minutes, respectively. The binary mixtures prepared by freeze-drying and supercritical carbon dioxide inclusion exhibited a drug release of more than 85 % within 10 minutes.

The dissolution profiles of econazole maleate from drug alone and econazole maleate-α-cyclodextrin (1:1 molar) binary systems are presented in Figure 3.20.

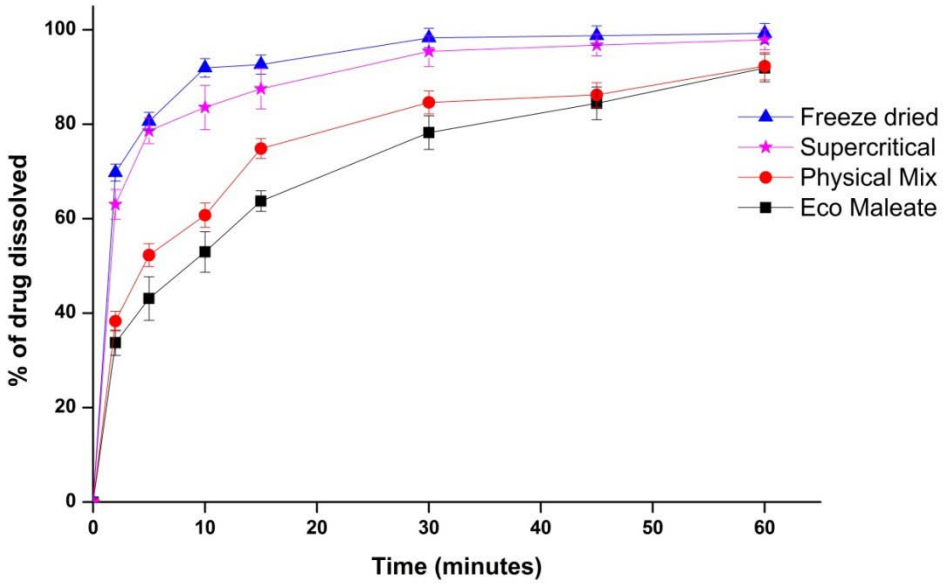

Figure 3.20: Dissolution rate profiles of econazole maleate and econazole maleate-α-cyclodextrin (1:1 molar) systems prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion methods (supercritical carbon dioxide processing conditions: 100°C and 250 bar).

In case of the drug release profiles of econazole maleate-α-cyclodextrin systems, it is worthwhile in highlighting that the greatest improvement of the drug dissolution was obtained with the binary mixture prepared by freeze-drying, followed by supercritical carbon

dioxide inclusion and physical mixing. The results in terms of percent of active ingredient dissolved at 10 min and 30 min are collected in Table 3.9.

Table 3.9: Percent econazole maleate dissolved at 10 and 30 min from econazole maleate and econazole maleate-α-cyclodextrin systems

Sample	**% drug dissolved** (10 min)	**% drug dissolved** (30 min)
Econazole maleate	52.9	78.2
Physical mixing	60.8	84.6
Freeze-drying	91.9	98.3
Supercritical carbon dioxide inclusion	83.6	95.5

The amount of econazole maleate released from drug alone was 52.9 % dissolving within 10 minutes. The binary mixtures prepared by physical mixing, freeze-drying and supercritical carbon dioxide inclusion exhibited an increased drug release with 60.8 %, 91.9 % and 83.6 % of econazole maleate dissolved within 10 minutes, respectively. The binary mixtures prepared by freeze-drying and supercritical carbon dioxide inclusion exhibited a drug release of more than 80 % within 10 minutes and more than 90 % within 30 minutes.

Examples of recent investigations featuring the use of dissolution studies for the characterisation of inclusion complexes can be seen in Table 1.7.

Comparing across the different econazole salt-α-cyclodextrin inclusion complexes, some general trends from the data can be elucidated:

1. All the systems with α-cyclodextrin exhibited better drug release profiles than the drug salts alone. The improved dissolution characteristics of the physical mixture may be attributable to improvements in drug wettability as suggested by

Charoenchaitrakool and co-workers (Charoenchaitrakool, M. *et al.*, 2002); there is no evidence of the *in situ* formation of readily soluble complexes in the medium as suggested by Banchero and co-workers (Banchero, M. *et al.*, 2013).

2. The improvement in the drug release from the binary systems of α-cyclodextrin with nitrate, besylate and sulfosalicylate dihydrate salts of econazole was observed in the following order: physical mixture < freeze-drying < supercritical carbon dioxide processing. However, contrary to these results, the improvement in the release of econazole maleate from the binary systems was observed in the following order: physical mixing < supercritical carbon dioxide processing < freeze-drying.
3. The enhanced dissolution of the freeze-dried and supercritical carbon dioxide inclusion products may be attributed to the reduced particle size, the high energetic amorphous state and the formation of inclusion complexes between drug and cyclodextrin in the solid state as suggested by Hancock, B.C. and Zografi, G., (1997); El-Badry, M. *et al.*, (2009) and Khadka, P. *et al.*, (2014).

3.7.5 Isothermal Titration Calorimetric Studies:

3.7.5.1 Control Experiment: Isothermal Titration Calorimetric Studies of Barium Chloride with 1,4,7,10,13,26-hexaoxacyclooctadecane (18-Crown-6)

Control experiments were carried out to calibrate the isothermal titration calorimeter. Figure 3.21a represents the titration curve for the binding of barium chloride with 1,4,7,10,13,26-hexaoxacyclooctadecane (18-crown-6) in phosphate buffer (pH 4.5) at 25°C while Figure 3.21b represents the binding isotherm from which the energetics was determined. The binding isotherm produced is a plot of differential heat output on addition of barium chloride versus the molar ratio of barium chloride added to 18-crown-6. The

stoichiometry of the interaction between barium chloride and 18-crown-6 was found to be 1:1 while the binding affinity (K_b), enthalpy (ΔH) and Gibbs free energy (ΔG) were found to be 8.31 ± 0.33 x 10^3 dm^3 mol^{-1}, -30.29 ± 0.29 kJ mol^{-1} and -22.662 kJ mol^{-1}, respectively. The standard formation enthalpy for the interaction demonstrated a large negative value, indicating an exothermic process and strong energy release during complexes formation as suggested by Stojanov and co-workers (Stojanov, M. *et al.*, 2011).

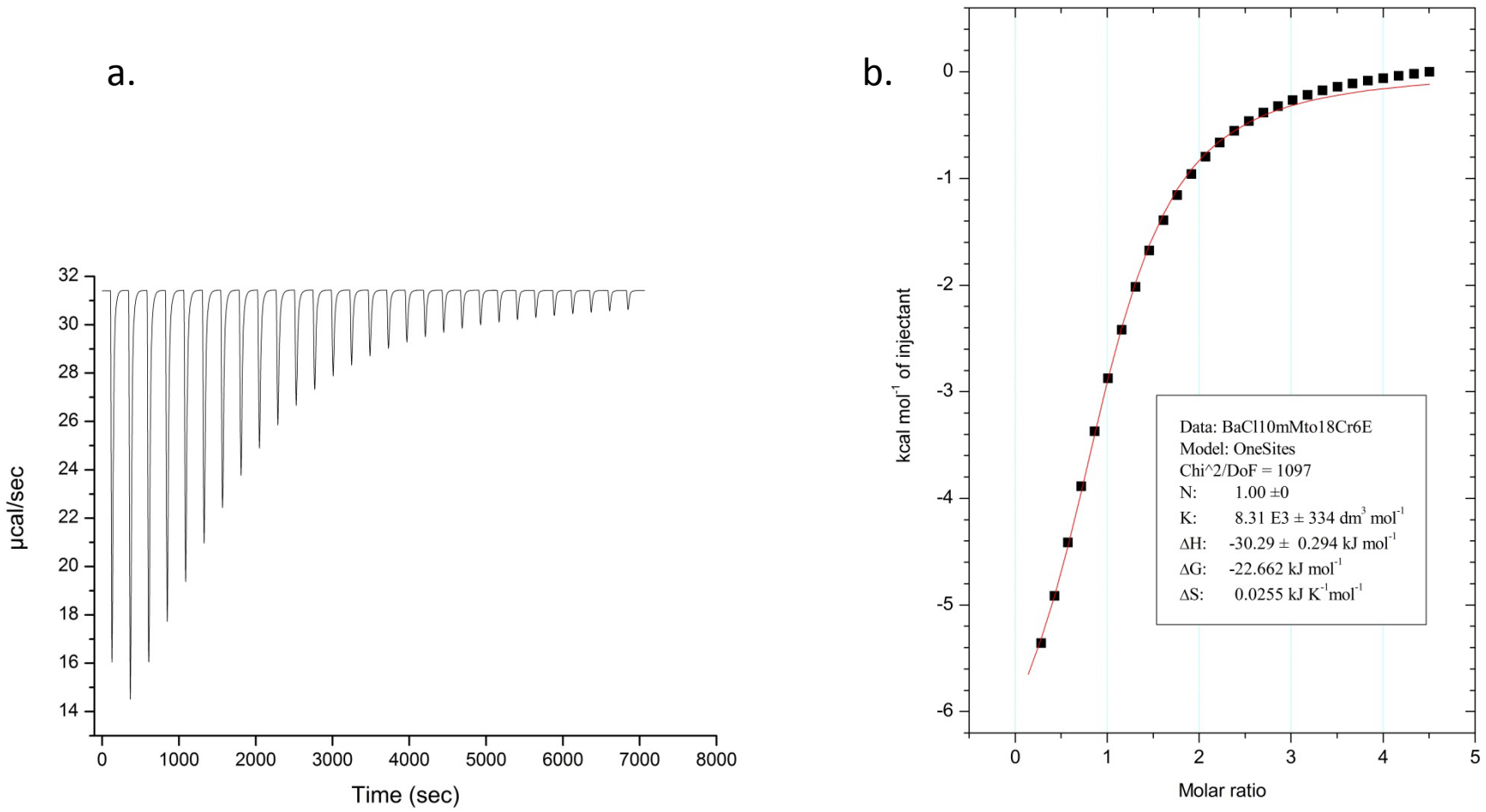

Figure 3.21: Experimental data acquired using isothermal titration calorimetry for the periodic calibration study *i.e.*, for the binding interaction of barium chloride with 1,4,7,10,13,26-hexaoxacyclooctadecane (18-crown-6) at 25°C in phosphate buffer (pH 4.5). (a) Shows exothermic heat release upon injection of 10 µL aliquots of barium chloride into 18-crown-6. (b) Shows integrated heat data, giving a corresponding binding isotherm.

The complex formation between econazole besylate/sulfosalicylate dihydrate salts and α-cyclodextrin was examined using isothermal titration calorimetry in phosphate buffer (pH 4.5). The thermodynamic data for the binding of econazole besylate and sulfosalicylate dihydrate salts with α-cyclodextrin are collected in Table 3.10.

3.7.5.2 Isothermal Titration Calorimetric Studies of Econazole Besylate and Econazole Sulfosalicylate Dihydrate with α-Cyclodextrin

Figure 3.22a represents the titration curve for the binding of econazole besylate (1 mmol) with α-cyclodextrin (10 mmol) in phosphate buffer (pH 4.5) at 25°C while Figure 3.22b represents the binding isotherm from which the energetics was determined. The binding isotherm produced is a plot of differential heat output on addition of α-cyclodextrin versus the molar ratio of α-cyclodextrin added to econazole besylate. A negative enthalpy value (ΔH) of -10.76 ± 0.67 kJ mol^{-1} was obtained for the interaction of econazole besylate and α-cyclodextrin indicating that the process was exothermic. The stoichiometry of the interaction was found to be 1.14:1. The binding affinity (K_b) and Gibbs free energy (ΔG) were found to be 8.55 ± 0.74 x 10^3 dm^3 mol^{-1} and -18.89 kJ mol^{-1}, respectively.

Figure 3.23a represents the titration curve for the binding of econazole sulfosalicylate dihydrate (1 mmol) with α-cyclodextrin (20 mmol) in phosphate buffer (pH 4.5) at 25°C while Figure 3.23b represents the binding isotherm from which the energetics was determined. The binding isotherm produced is a plot of differential heat output on addition of α-cyclodextrin versus the molar ratio of α-cyclodextrin added to econazole sulfosalicylate dihydrate. A negative enthalpy value (ΔH) of -10.31 ± 0.59 kJ mol^{-1} was obtained for the interaction of econazole sulfosalicylate dihydrate and α-cyclodextrin indicating an exothermic process. The stoichiometry of the interaction was found to be 1.11:1. The binding affinity (K_b) and Gibbs free energy (ΔG) were found to be 9.09 ± 1.08 x 10^3 dm^3 mol^{-1} and -19.01 kJ mol^{-1}, respectively.

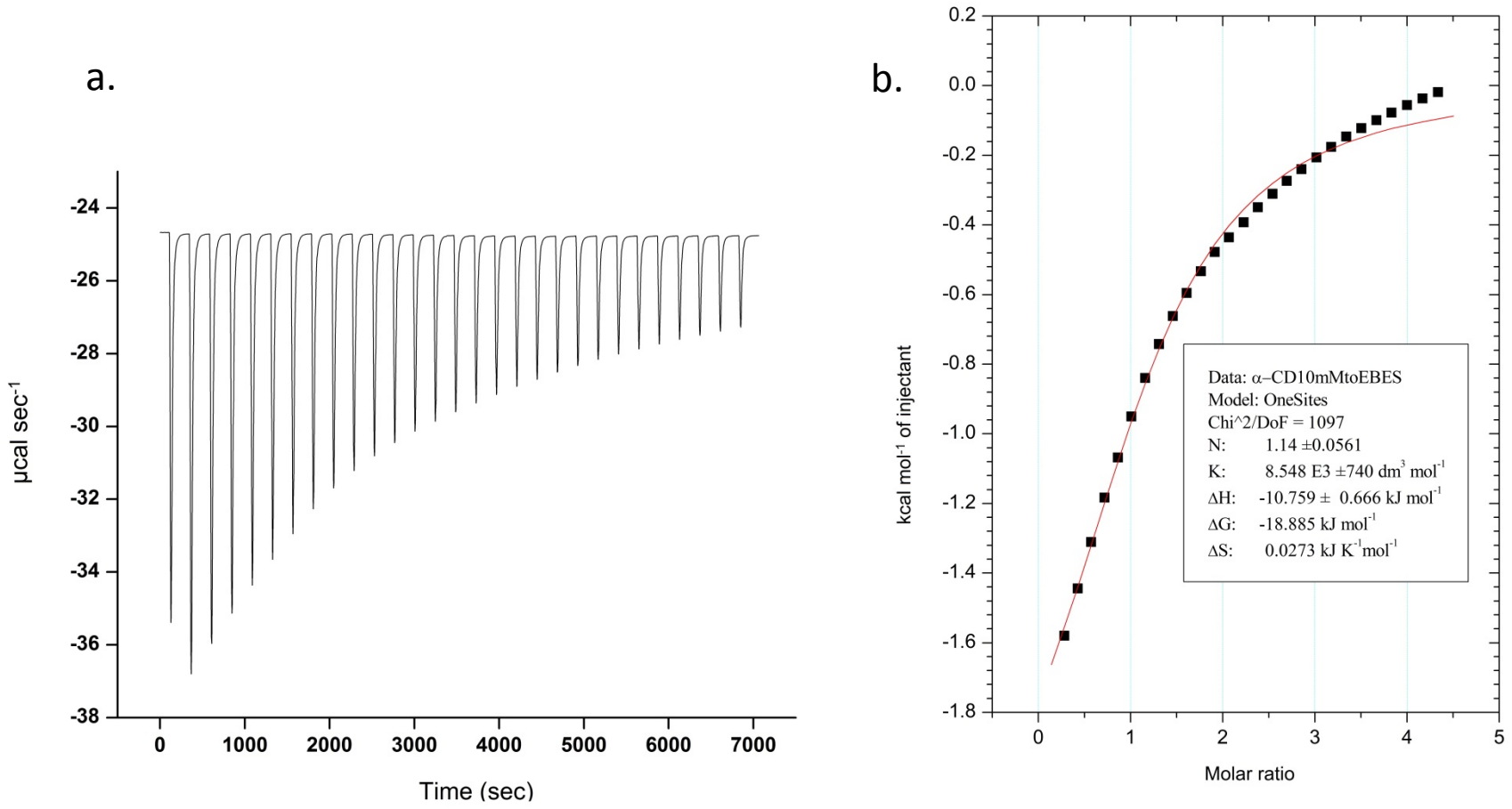

Figure 3.22: Experimental data acquired using isothermal titration calorimetry for the binding interaction of econazole besylate with α-cyclodextrin at 25°C in phosphate buffer (pH 4.5). (a) Shows exothermic heat release upon injection of 10 µL aliquots of α-cyclodextrin (10 mmol) into econazole besylate (1 mmol) solution. (b) Shows integrated heat data, giving a corresponding binding isotherm.

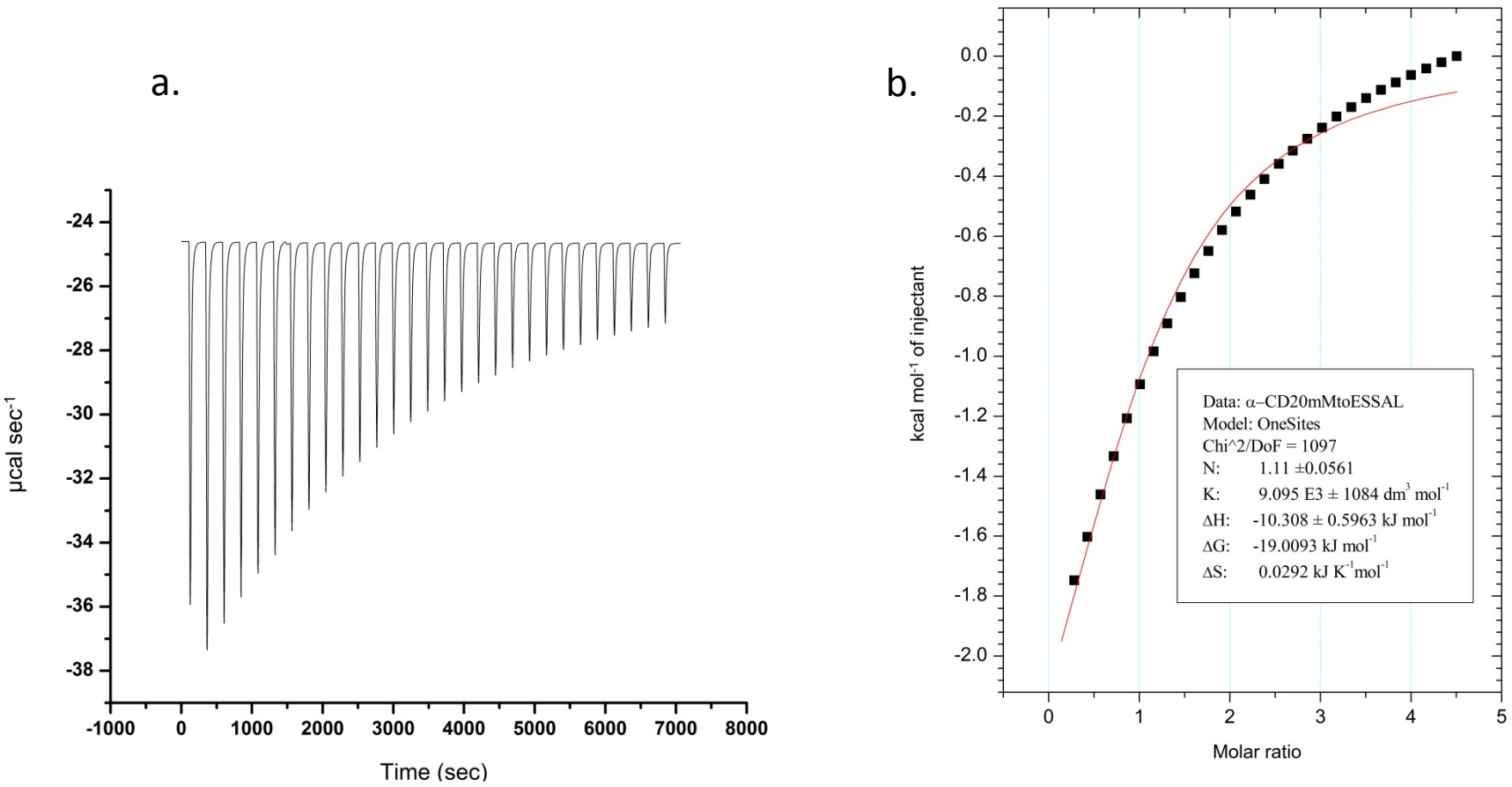

Figure 3.23: Experimental data acquired using isothermal titration calorimetry for the binding interaction of econazole sulfosalicylate dihydrate with α-cyclodextrin at 25°C in phosphate buffer (pH 4.5). (a) Shows exothermic heat release upon injection of 10 µL aliquots of α-cyclodextrin (20 mmol) into econazole sulfosalicylate dihydrate (1 mmol) solution. (b) Shows integrated heat data, giving a corresponding binding isotherm.

Table 3.10: Thermodynamic data acquired using isothermal titration calorimetric studies for the binding of econazole besylate and sulfosalicylate dihydrate salts with α-cyclodextrin at 25°C in pH 4.5 phosphate buffer.

Econazole salt	Temperature (K)	Stoichiometry (N)	10^3K_b (dm^3 mol^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (kJ K^{-1} mol^{-1})
	298	1.19:1	8.792 ± 0.988	-11.007 ± 0.687	-18.963	0.0267
Besylate	298	1.23:1	8.876 ± 1.034	-10.764 ± 0.670	-18.988	0.0276
	298	1.14:1	7.978 ± 0.199	-10.508 ± 0.642	-18.703	0.0275
Mean			8.548 ± 0.741	-10.759 ± 0.666	-18.885	0.0273
	298	1.04:1	9.334 ± 1.125	-10.137 ± 0.585	-19.106	0.0301
Sulfosalicylate	298	1.11:1	9.037 ± 1.1045	-10.359 ±0.636	-19.031	0.0291
	298	1.19:1	8.918 ± 1.024	-10.427 ±0.568	-18.890	0.0284
Mean			9.095 ± 1.084	-10.308 ± 0.596	-19.009	0.0292

The negative Gibbs free energy value in both the cases suggests that the complexes have less free energy than the free econazole besylate and sulfosalicylate dihydrate salts and α-cyclodextrin. Moreover, the negative values for enthalpy and Gibbs free energy suggest that the binding is favoured and this promotes the formation of complexes. In both the cases, the reaction was more enthalpically driven as the internal energy of the system was the influencing factor and not the entropy.

The negative enthalpy value in both cases indicates an exothermic process which may be ascribed to the release of the cavity-bound water molecules to the bulk water, a solvophobic effect due to the aromatic rings of the econazole and van der Waals interactions between econazole besylate and sulfosalicylate dihydrate salts and the cavity of the α-cyclodextrin as suggested by Connors, K.A., (1997), Rekharsky, M.V. and Inoue, Y., (1998) and Stojanov, M. *et al.*, (2011).

Isothermal titration calorimetry has been earlier utilised to determine the thermodynamic parameters for formation of the inclusion complexes of α-cyclodextrin with cetirizine in aqueous solution (Stojanov, M. *et al.*, (2011)). The enthalpy (ΔH) and Gibbs free energy (ΔG) values of this interaction were found to be -5514 kJ mol^{-1} and -4029 kJ mol^{-1}, respectively. The large negative enthalpy values indicate an exothermic process and strong energy release during complexes formation.

Xing, S. et al., (2009) carried out the isothermal titration calorimetry studies of α-cyclodextrin with ibuprofen in Tris-HCl buffer solutions (pH 7.0) at 25°C and. The interaction was driven by enthalpy and entropy; the authors reported the stoichiometry (N), binding affinity (K_b), enthalpy (ΔH) and Gibbs free energy (ΔG) values of 1:1, 650 dm^3 mol^{-1}, -3.20 kJ mol^{-1} and -16.00 kJ mol^{-1}, respectively.

Segura-Sanchez, F. *et al.*, (2009) employed isothermal titration calorimetry studies to investigate the complexation mechanism between (+)-usnic acid and α-, β- and γ-,

cyclodextrins. However, the authors reported that (+)-usnic acid did not interact with α-cyclodextrin and tended to interact more favourably with γ- cyclodextrin (stoichiometry (N) = 1:1, binding affinity (K_b) = 1.03 x 10^3 dm^3 mol^{-1}, Gibbs free energy (ΔG) = -17.18 kJ mol^{-1}) than β- cyclodextrin (stoichiometry (N) = 1:1, binding affinity (K_b) = 1.53 x 10^3 dm^3 mol^{-1}, Gibbs free energy (ΔG) = -12.46 kJ mol^{-1}). The solubilizing effect of natural cyclodextrins (1.5% w/w concentration) was compared on (+)-usnic acid and the apparent solubility of (+)-usnic acid was observed to be the highest with γ-cyclodextrin when compared to β-cyclodextrin and almost nil for α-cyclodextrin. The authors suggested that the intensity of molecular interactions between (+)-usnic acid and the cyclodextrins employed had a direct consequence on their solubilization efficiency.

3.8 Conclusions

Solid systems of nitrate, besylate, sulfosalicylate dihydrate and maleate salts of econazole with α-cyclodextrin in the 1:1 molar ratio were prepared by supercritical carbon dioxide inclusion method and compared to products obtained using physical mixing and freeze drying. Different degrees of modification were observed in the analyses of products prepared by various methods, suggesting the possibility of drug-cyclodextrin interactions of different strengths, which may give rise to different degrees of inclusion formation and/or amorphisation of the sample. Nevertheless, products obtained by the freeze drying method were among the ones showing the highest loss of crystallinity suggesting high interaction between the drug and the cyclodextrin. All the systems with cyclodextrins exhibited better dissolution properties than drug alone. The greatest improvement of the drug dissolution properties was obtained with supercritical carbon dioxide-inclusion, followed by freeze drying and finally by physical mixing. Solid state complexation using supercritical carbon dioxide processing and freeze drying proved to be useful complexation

methods for econazole salts into α-cyclodextrin. The freeze drying method produced highly amorphous and rapid dissolving complexes; however, it was characterised by long, energy-intensive processing steps. Furthermore, supercritical carbon dioxide-inclusion method has no toxic solvent residue; products obtained by this method should provide minimal side effects in humans, compared to those obtained by techniques that require the use of organic solvents. Isothermal titration calorimetric studies confirmed that the complexes between econazole besylate and sulfosalicylate dihydrate salts and α-cyclodextrin in pH 4.5 phosphate buffer at 25°C formed in a 1:1 stoichiometry. All the complexes exhibited a decrease in Gibbs free energy upon complexation and therefore were thermodynamically favourable. With regards to changes in enthalpy it can be realised that a significantly negative value was observed for both the complexation events and therefore denotes that the reactions were exothermic and enthalpically driven. In conclusion, isothermal titration calorimetry proved to be useful for getting insights into the thermodynamics of the complexes between econazole salts and α-cyclodextrin.

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Chapter 4: Phase Behaviour of Econazole Base and its Salts in Supercritical Carbon Dioxide

Aims

- To study the phase behaviour of econazole free base (and its salts), in supercritical carbon dioxide in order to decide the most suitable conditions for forming inclusion complexes with different cyclodextrins.
- To investigate the influence of pressure, temperature and contact time on the inclusion yield (%) of econazole free base into α- and methyl-β-cyclodextrin.

4.1 Introduction

Most drugs are thermally instable at their melting temperature. Grandelli and co-workers (Grandelli, H.E. *et al*., 2012) reported melting point depression of drugs as a conceivable approach to avoid thermal degradation upon melting and thus allowing the use of their melt during processing for the formation of complexes with cyclodextrins. Fischer and co-workers (Fischer, K. *et al*., 2003) have earlier reported that gases (such as carbon dioxide, xenon and ethylene) with high solubilising potential can depress melting points significantly, while the gases (such as nitrogen and helium) with low solubilising potential result in increased melting points of some compounds (such as naphthalene and phenanthrene) . The sorption of carbon dioxide into the matrix of the solute in conjunction with solute-solvent intermolecular interactions, results in the melting point depression of a solute, thereby, resulting in weaker attractions between the solute segments within the matrix (Sze Tu, L., 2000).

Melting point depression of drugs such as naproxen (Türk, M. and Kraska, T., 2009), ibuprofen (Uchida, H. *et al*., 2004; Türk, M. *et al*., 2006) and piroxicam (Grandelli, H.E. *et*

al., 2012) in supercritical carbon dioxide has been earlier reported in the literature. In these studies, melting temperatures of naproxen, ibuprofen and piroxicam were shown to be pressure dependent. Türk *et al.* (2006) reported that the melting temperature of ibuprofen decreased in a linear fashion from 76°C to 48°C (*i.e.*, a depression of 18°C) with increasing pressure up to 100 bar. No further reduction in the melting temperature was observed for ibuprofen by increasing the pressure above 100 bar. Türk *et al.* (2009) reported that the melting temperature of naproxen in supercritical carbon dioxide decreased in a linear fashion from 154°C to 140°C (*i.e.*, a depression of 14°C) with increasing pressure up to 300 bar.

To our knowledge, phase behaviour *e.g.* melting of econazole free base in supercritical carbon dioxide has not been reported in literature. The main aim of this study was to establish the phase behaviour of econazole free base (and its salts), in supercritical carbon dioxide in order to decide the most suitable conditions for forming inclusion complexes with different cyclodextrins.

4.2 Materials and Methods

4.2.1 Materials

Econazole nitrate (≥98%, molecular weight: 444.7 g mol^{-1}), α-cyclodextrin (≥98%, molecular weight: 972.84 g mol^{-1}) and methyl-β-cyclodextrin (average molecular weight: 1310 g mol^{-1}, extent of labeling: 1.6–2.0 mol CH_3 per unit anhydroglucose) were purchased from Sigma-Aldrich, Gillingham, Dorset, UK. The besylate, sulfosalicylate dihydrate and maleate salts of econazole were synthesised in our laboratory from econazole free base which was obtained by stripping the econazole nitrate as described in Section 2.2.2.

4.2.2 Phase Behaviour Studies of Econazole Free Base and Salts with Nitrate, Besylate, Sulfosalicylate Dihydrate, Maleate Complexes with α-Cyclodextrin in Supercritical Carbon Dioxide

The phase behaviours of econazole free base and its salts with nitrate, besylate, sulfosalicylate dihydrate, maleate and their inclusion complexes with α-cyclodextrin were studied at pressure ranging from 100 to 320 bar using a phase monitor (Supercritical Fluid Technologies, Inc., Newark, Delaware, USA). Figure 4.1 shows the schematics of the phase monitor. The setup of the instrument is as follows: a manually controlled syringe pump is attached to a view cell (volume = 30 mL). The interior of the view cell is monitored by a CCD (charge-coupled device) camera and illuminated by a fibre optic light source. The view cell also contains an internal magnetic stirrer for effective mixing of the carbon dioxide. Heating of the view cell is controlled and monitored by an internally mounted resistance temperature detector (resistance thermometer) with temperature ranging from ambient to 150°C. Samples can be placed in a glass capillary tube mounted on the sample holder in the optimal viewing position.

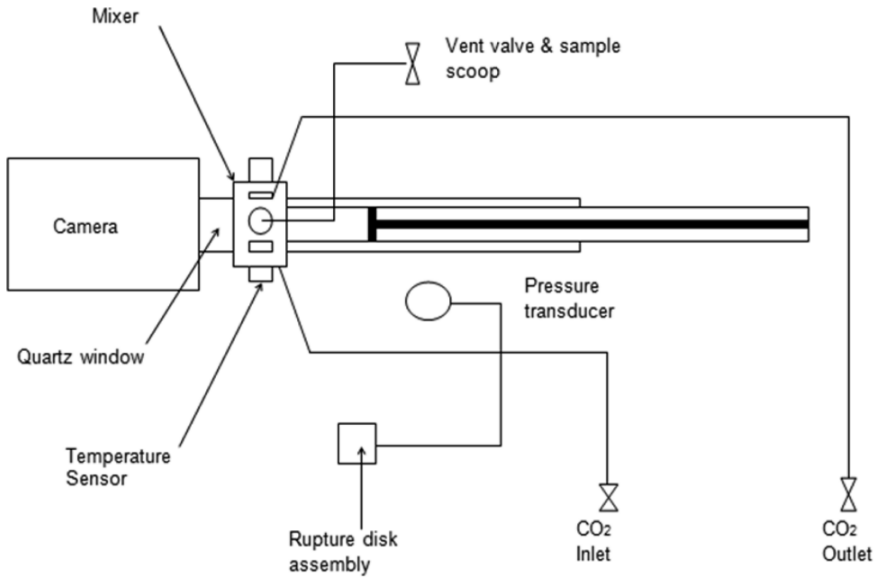


Figure 4.1: Schematics of phase monitor supplied by Supercritical Fluid Technologies, Inc., Newark, Delaware, USA.

The phase monitor was calibrated using naphthalene as standard prior to the experiments. In general, *ca.* 2-3 mg of sample (econazole base, econazole nitrate, -besylate, -sulfosalicylate dihydrate, -maleate, α-cyclodextrin and methyl-β-cyclodextrin) was well packed into the melting point capillary tube mounted on the sample holder in the reaction chamber. Carbon dioxide was introduced through the cylinder into the cell until a desired pressure was achieved. This pressure was then kept constant during the experiment by manually rotating the piston. The temperature was increased by 1-2.5°C increments until the phase of the sample was changed (*e.g.* melted).

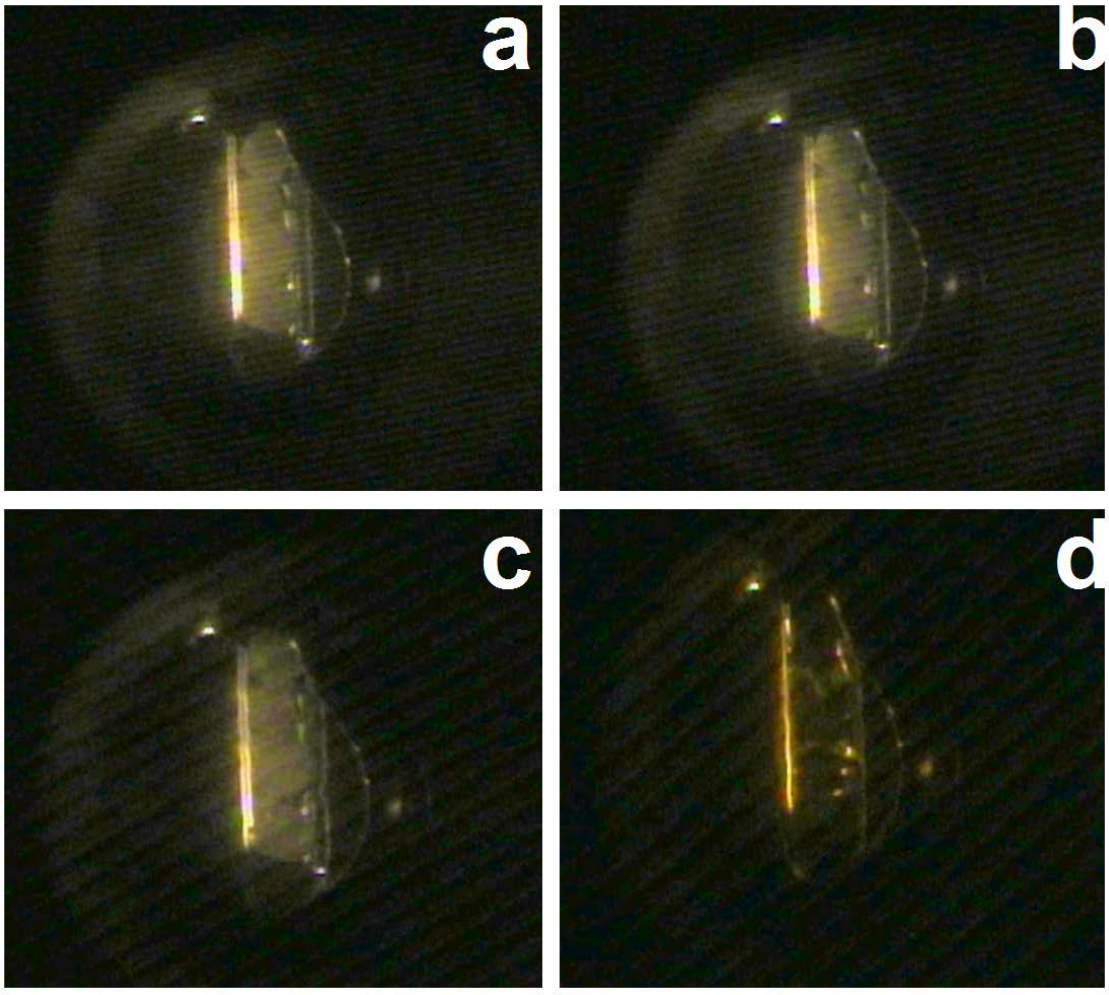


Figure 4.2: Phase changes observed for econazole free base: (a) at the beginning of the experiment, (b) after the introduction of carbon dioxide; (c) at the initiation of melting; (d) and after complete melting had occurred (processing conditions: 60°C and 200 bar).

The phase behaviour of all the samples was monitored through a quartz window *via* the charge-coupled device camera attached to the cell. The experiments were conducted at various pressures and the data were collected in triplicate. Figure 4.2 presents an example of econazole base at different stages of the experiment. The melting point of econazole base was noted as the temperature at which the entire sample was in the liquid phase (point d).

4.2.3 Inclusion Yield Studies of Econazole Base-α-Cyclodextrin Complexes Prepared by Supercritical Carbon Dioxide Inclusion

The influence of pressure, temperature and contact time on the inclusion yield (%) of econazole base into α-cyclodextrin was investigated in the temperature range of 40-65°C at constant pressure of 200 bar and at constant temperature of 60°C in the pressure range of 100-225 bar and two levels of contact time (1 h and 3 h). A physical mixture of econazole base-α-cyclodextrin (1:1 molar) was used for the inclusion yield experiments as the same molar ratio was used in previous studies (Chapter 2).

4.2.3.1. Evaluation of Inclusion Yields for Econazole Base into α-Cyclodextrin

The inclusion yields (%) for econazole into α-cyclodextrin were evaluated according to the differential solubility method reported by Van Hees and co-workers (Van Hees, T. *et al*., 2002).

The method consisted of two steps:

i. **Determination of the total econazole base content:**

   An accurately weighed sample (10 mg) of econazole-α-cyclodextrin complex was dispersed in a hydroalcoholic (50:50 v/v - ethanol/water) solution (10 mL) in which both the drug and cyclodextrin are soluble. The mixture was agitated (100 rpm) at

37°C for 30 minutes and the resultant solution was filtered (0.45 μm filter pore size) and assayed for the concentration of total econazole base by UV spectroscopy (Cary 100 UV-Vis, Agilent Technologies, Santa Clara, CA 95051). Determination of the total econazole base content in the complexes allows the verification that there is no loss of econazole base during the (complex) preparation process.

ii. Determination of the free econazole base content:

An accurately weighed sample (10 mg) of econazole-α-cyclodextrin complex was dispersed in ethanol (10 mL) in which α-cyclodextrin is not soluble and in which the included econazole base remains encapsulated. The mixture was agitated (100 rpm) at 37°C for 30 minutes and the resultant solution was filtered and assayed for the concentration of free econazole base as described earlier.

The inclusion yield (%) of the econazole base into cyclodextrin was determined by using the following equation:

$$\textbf{Inclusion yield}\ (\%) = \left[\frac{\text{Total econazole base content} - \text{Free econazole base content}}{\text{Total econazole base content}}\right] \text{x } 100$$

4.2.4 Inclusion Yield Studies of Econazole Base-Methyl-β-Cyclodextrin Complexes Prepared by Supercritical Carbon Dioxide Inclusion

The influence of pressure, temperature and contact time on the inclusion yield (%) of econazole base into methyl-β-cyclodextrin was investigated in the temperature range of 35-65°C at constant pressure of 150 bar and at constant temperature of 40°C in the pressure range of 100-200 bar and a contact time of 1 h.

4.2.4.1. Evaluation of Inclusion Yields for Econazole Base into Methyl-β-Cyclodextrin

The inclusion yields (%) for econazole into methyl-β-cyclodextrin were evaluated by the method discussed earlier modified to use different solvents to accommodate the solubility of methyl-β-cyclodextrin.

The method consisted of two steps:

i. **Determination of the total econazole base content:**

   An accurately weighed sample (10 mg) of econazole-methyl-β-cyclodextrin complex was dispersed in ethanol (10 mL) in which both the drug and cyclodextrin are soluble. The mixture was agitated (100 rpm) at 37°C for 30 minutes and the resultant solution was filtered (0.45 μm filter pore size) and assayed for the concentration of total econazole base.

ii. **Determination of the free econazole base content:**

   An accurately weighed sample (10 mg) of econazole-methyl-β-cyclodextrin complex was dispersed in diethyl ether (10 mL) in which methyl-β-cyclodextrin is insoluble and in which the included econazole base remains encapsulated. The mixture was agitated (100 rpm) at 37°C for 30 minutes and the resultant solution was filtered and assayed for the concentration of free econazole base.

It is to be noted that diethyl ether erodes plastic syringe filters; therefore, a special filtration setup was used in this study. The filtration was performed using a glass syringe equipped with 0.2 μm polycarbonate filter fitted into stainless steel syringe type membrane holder (13 mm) supplied by GE Healthcare Whatman, UK.

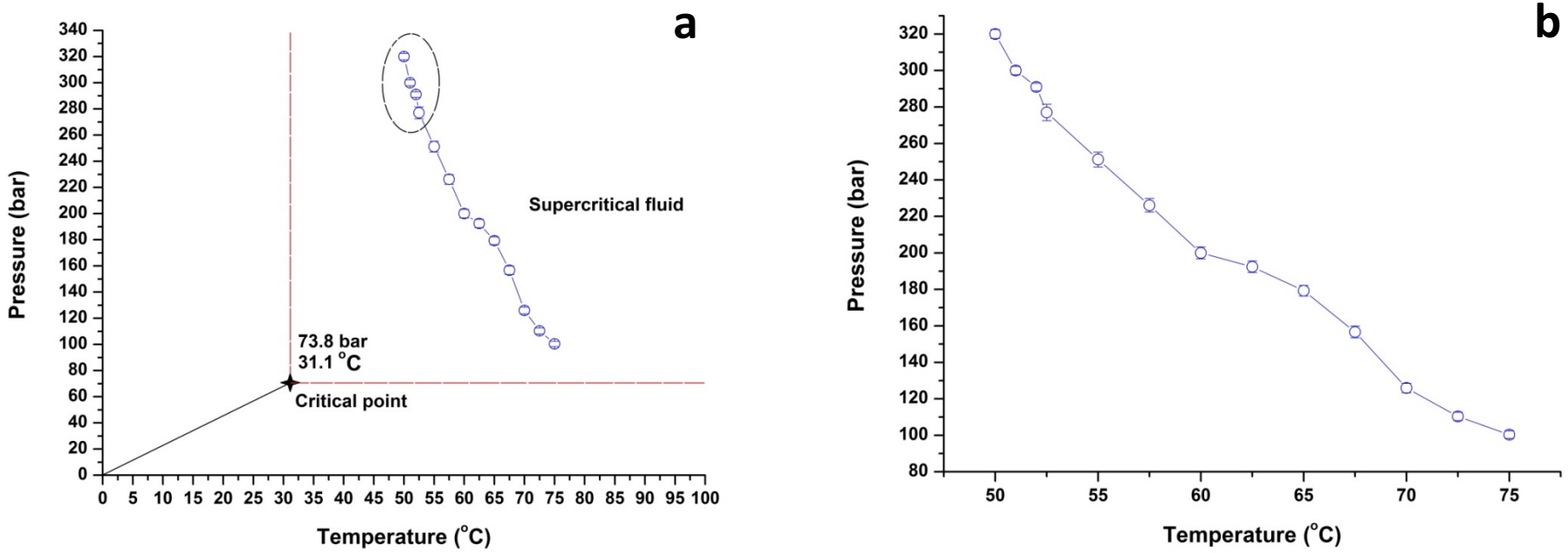
4.3 Results and Discussion

4.3.1 Phase Behaviour Studies

Phase behaviour studies of all of the samples (econazole base and the econazole salts with nitrate, besylate, sulfosalicylate dihydrate, maleate, α- and methyl-β-cyclodextrin) were carried out with pure carbon dioxide as the solvent. Melting temperatures of econazole base at different pressure conditions in carbon dioxide are shown in Figure 4.3 and are collected in Table 4.1.

Table 4.1: Experimental pressure-temperature data of the solid-liquid-gas curve for the binary system of econazole base and carbon dioxide (melting conditions for econazole base in carbon dioxide).

Temperature (°C)	**Pressure (Bar)**	**Temperature (°C)**	**Pressure (Bar)**
75	100.4 ± 2.1	57.5	226.1 ± 3.7
72.5	110.3 ± 2.3	55	251.2 ± 4.1
70	125.9 ± 2.6	52.5	277.0 ± 4.5
67.5	156.7 ± 3.2	52	291.1 ± 2.1
65	179.3 ± 2.9	51	300.1 ± 2.2
62.5	192.4 ± 3.1	50	320.2 ± 2.5
60	200.1 ± 3.3		



Figures 4.3: (a) Shows the melting temperatures of econazole base at different pressure conditions in carbon dioxide (b) Shows an enlarged version of a. where the liquid-gas phase boundary has been shown for clarity.

In this study, supercritical carbon dioxide was used to depress the melting point of econazole base and the viscosity of molten econazole. The melting temperature of econazole base is depressed in supercritical carbon dioxide and the melted econazole base can then be dispersed in supercritical carbon dioxide and distributed into the pores of α-cyclodextrin. This process deals with the premise that pressurized gas can diffuse into a solute, lowering both its melting point and viscosity. It is feasible to prepare a formulation with increased drug encapsulation as this process does not affect the solubility of a drug in supercritical fluid.

As discussed earlier in Chapter 2, econazole base melted at 92°C at atmospheric pressure. The melting point of econazole base was depressed by 17°C in supercritical carbon dioxide at 100.37 (± 2.06) bar; while, the greatest melting point depression of 42°C was observed at 320.24 (± 2.47) bar. Grandelli *et al.* (2012) demonstrated the melting point depression of non-steroidal anti-inflammatory drug piroxicam in supercritical carbon dioxide in addition to the binary mixtures of carbon dioxide and co-solvent (ethanol or acetone). The authors reported that the melting temperature of piroxicam reduced in pure supercritical carbon dioxide from 200°C to 183°C (*i.e.*, a depression of 17°C) at 350 bar. The melting point of piroxicam was further reduced to 163°C at 290 bar with a 90:10 wt% carbon dioxide-ethanol mixture, while a 90:10 wt% carbon dioxide-acetone mixture reduced the melting temperature to a lesser extent to 172°C at 230 bar.

Pure α-cyclodextrin and the econazole salts with nitrate, besylate, sulfosalicylate dihydrate, and maleate were individually placed in the cell and the system was gradually pressurised up to 320 bar at 100°C. No visually noticeable quantity of the samples was dissolved and no melting of the samples was observed when processed with supercritical carbon dioxide. Hence, it was inferred that the samples were in solid state and nearly insoluble in supercritical carbon dioxide under the (pressure and temperature) conditions of study.

Melting temperatures of methyl-β-cyclodextrin at different pressure conditions in carbon dioxide are collected in Table 4.3. At atmospheric pressure, methyl-β-cyclodextrin melted at 181°C. The melting point of methyl-β-cyclodextrin was depressed by 116°C (i.e., to 65°C), in supercritical carbon dioxide at 81.3 (±2.9) bar; while, the greatest melting point depression of 146°C (i.e., to 35°C), was observed at 174.9 (± 2.6) bar.

Table 4.3: Experimental pressure-temperature data of the solid-liquid-gas curve for the binary system of methyl-β-cyclodextrin and carbon dioxide (melting conditions for methyl-β-cyclodextrin in carbon dioxide).

Temperature (°C)	Pressure (Bar)
65	81.3 ± 2.9
60	88.6 ± 3.1
55	94.7 ± 3.2
50	103.5 ± 3.7
45	121.2 ± 3.2
40	158.4 ± 2.3
35	174.9 ± 2.6

The phase behaviour of methyl-β-cyclodextrin in carbon dioxide has been earlier studied. Charoenchaitrakool *et al.* (2002) studied the phase behaviour of methyl-β-cyclodextrin in carbon dioxide and the authors reported that the melting point of cyclodextrin was depressed by 146°C (*i.e.*, to 35°C), 141°C (*i.e.*, to 40°C) and 136°C (*i.e.*, to 45°C) when contacted with carbon dioxide at 147, 143 and 94 bar, respectively. Banchero *et al.* (2013) studied the phase behaviour of methyl-β-cyclodextrin in carbon dioxide and the authors reported that the melting point of cyclodextrin was depressed by 141°C (*i.e.*, to 40°C) in supercritical carbon dioxide at 200 bar. In both the cases (Charoenchaitrakool and Banchero), it was reported that the melting of methyl-β-cyclodextrin in carbon dioxide

favours the complexation of drug and methyl-β-cyclodextrin without any addition of water or auxiliary agents.

4.3.2 Inclusion Yield Studies of Econazole Base with α- and Methyl-β-Cyclodextrin Complexes Prepared by Supercritical Carbon Dioxide Processing

After studying the phase behaviour of econazole base and α-cyclodextrin in supercritical carbon dioxide, inclusion yield (%) studies were carried out at different temperature and pressure conditions to study the influence of pressure, temperature and contact time in supercritical carbon dioxide on the inclusion of econazole base into α- and methyl-β-cyclodextrins.

4.3.2.1 Effect of Pressure and Temperature on the Inclusion Yield (%) of Econazole Base-α-Cyclodextrin Complexes

The influence of temperature, pressure and contact time in supercritical carbon dioxide on the inclusion yield (%) of econazole base into α-cyclodextrin is illustrated in Figures 4.4 and 4.5.

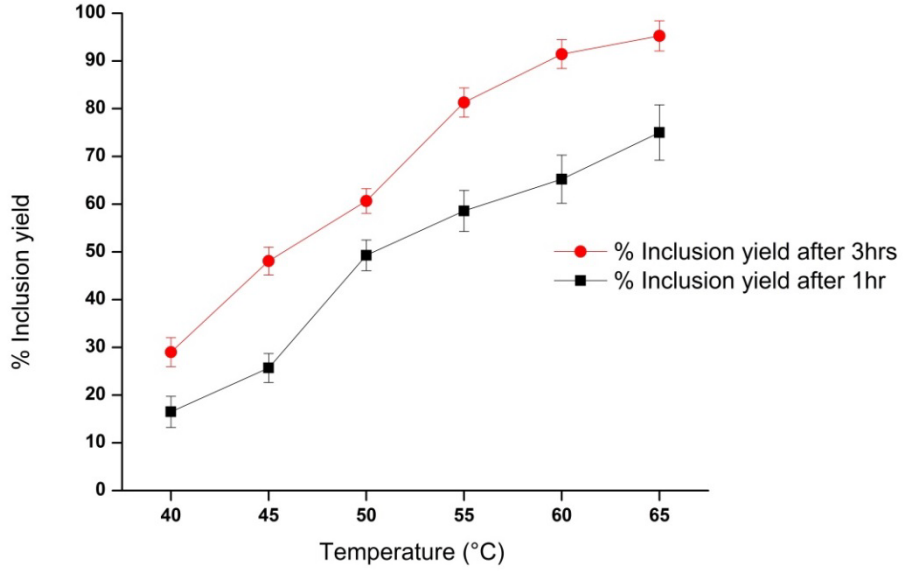


Figure 4.4: Inclusion yield (%) of econazole base into α-cyclodextrin as function of temperature and contact time (1 h and 3 h) in supercritical carbon dioxide at a constant pressure of 200 bar.

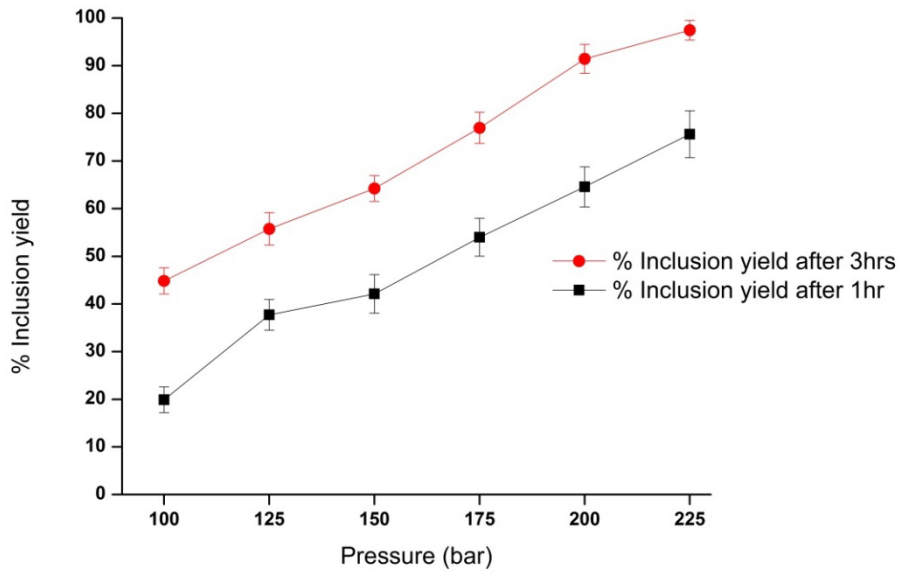


Figure 4.5: Inclusion yield (%) of econazole base into α-cyclodextrin as function of pressure and contact time (1 h and 3 h) in supercritical carbon dioxide at a constant temperature of 60°C.

The inclusion yield (%) data of econazole base into α-cyclodextrin in supercritical carbon dioxide at various working conditions are collected in Table A2 (Appendix)**.** As illustrated in Figure 4.4, increasing the temperature at a constant pressure of 200 bar resulted in a higher included econazole base content in the complex. At 40°C and 200 bar, the inclusion yield was 16.50 (± 3.27) % and 28.99 ± (3.04) % after 1 h and 3 h, respectively. The maximum inclusion yield (95.26 ± 3.16 %) was obtained after 3 h at 65°C and 200 bar.

As illustrated in Figure 4.5, increasing the pressure at a constant temperature of 60°C resulted in a higher included econazole base content in the complex. At 60°C and 100 bar, the inclusion yield was 19.91 (± 2.70) % and 44.84 (± 2.74) % after 1 h and 3 h, respectively. The maximum inclusion yield (97.44 ± 2.06 %) was obtained after 3 h at 60°C and 225 bar.

All the parameters (temperature, pressure and contact time) had a positive influence on the inclusion yield (%) and played a significant role in the inclusion of econazole base into α-cyclodextrin.

Van Hees *et al.* (2002) produced inclusion complexes with piroxicam and β-cyclodextrin using supercritical carbon dioxide and investigated the influence of the pressure, temperature and contact time on the inclusion of the drug into cyclodextrin. Complexes prepared by supercritical carbon dioxide processing exhibited significantly higher dissolution rates in water than that of the drug alone and the physical mixtures. The authors concluded from their studies that as the temperature and contact time increases drug inclusion increases; however, increasing pressure in the system reduced drug inclusion.

4.3.2.2 Effect of Temperature and Pressure on the Inclusion Yield (%) of Econazole Base-Methyl-β-Cyclodextrin Complexes

The influence of temperature and pressure in supercritical carbon dioxide on the inclusion yield (%) of econazole base into methyl-β-cyclodextrin is illustrated in Figures 4.6 and 4.7. The inclusion yield (%) data of econazole base into methyl-β-cyclodextrin in supercritical carbon dioxide at various working conditions are collected in Table A3 (Appendix).

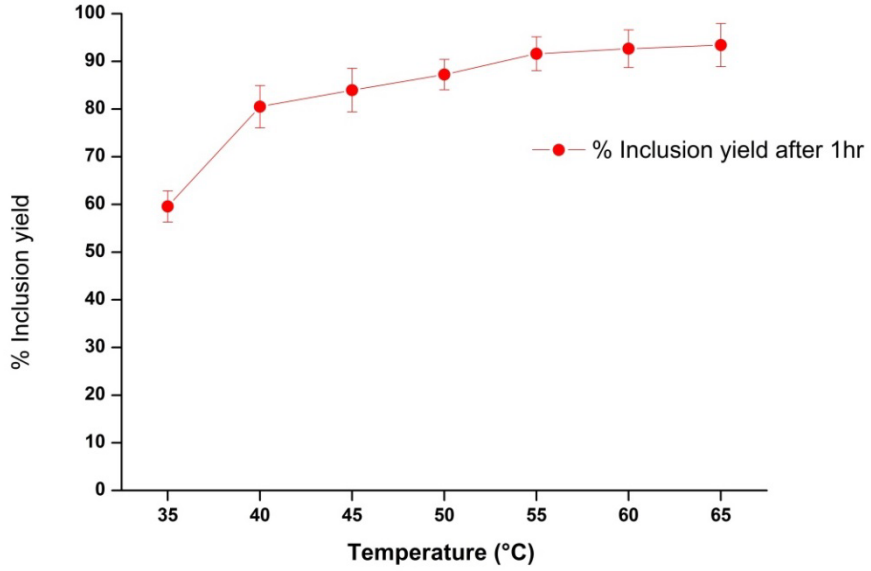


Figure 4.6: Inclusion yield (%) of econazole base into methyl-β-cyclodextrin as function of temperature in supercritical carbon dioxide at a constant pressure of 150 bar, and a contact time of 1 h.

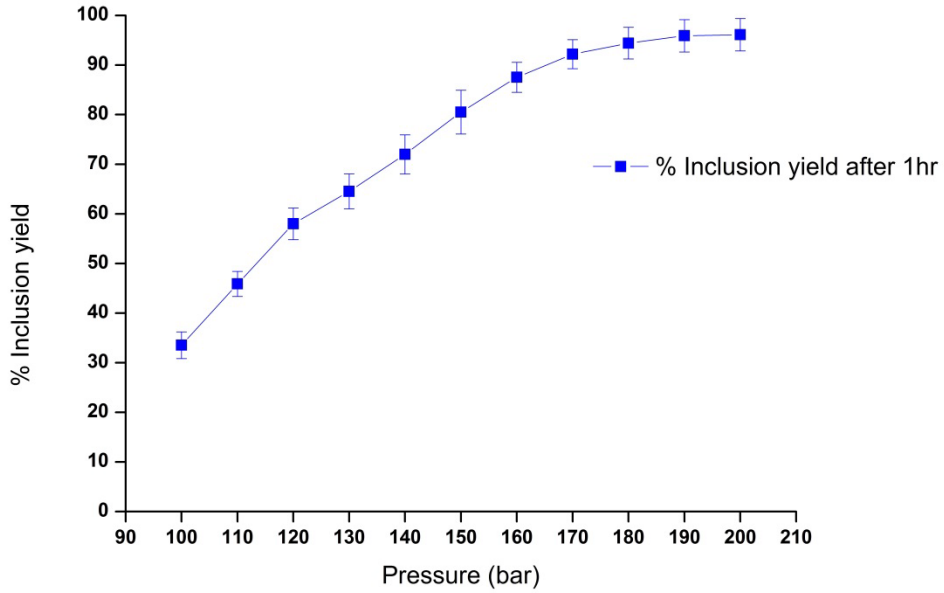


Figure 4.7: Inclusion yield (%) of econazole base into methyl-β-cyclodextrin in function of pressure (100-200 bar) and contact time (1 h) in supercritical carbon dioxide at a constant temperature of 40°C, and a contact time of 1 h.

As illustrated in Figure 4.6, increasing the temperature at a constant pressure of 150 bar resulted in a higher included econazole base content in the complex. At 35°C and 150 bar, the inclusion yield was 59.59 (± 3.27) %; while a maximum yield of 93.42 (± 4.41) % was

obtained when the temperature was increased to 65°C. As illustrated in Figure 4.7, increasing the pressure at a constant temperature of 40°C resulted in a higher included econazole base content in the complex. At 40°C and 100 bar, the inclusion yield was 33.53 (± 2.69) %; while a maximum yield of 96.12 (± 3.26) % was obtained when the pressure was increased to 200 bar.

Both the parameters (temperature and pressure) had a positive influence on the inclusion yield (%) and played a significant role in the inclusion of econazole base into α-cyclodextrin; however, the influence of pressure was greater on the inclusion when compared to the temperature as it showed a much wider change in inclusion yield.

Charoenchaitrakool et al. (2002) investigated the use of a continuous, dynamic supercritical carbon dioxide process for producing inclusion complexes with ibuprofen and methyl-β-cyclodextrin. In their study, the methyl-β-cyclodextrin melted in the supercritical carbon dioxide and later passed ibuprofen-laden supercritical carbon dioxide through a packed bed of methyl-β-cyclodextrin for 2 h. The resulting complexes were highly amorphous and showed extremely high apparent dissolution rates. In contrast to Van Hees *et al.* (2002), these authors reported that higher drug content in the complex was achieved with an increase in pressure; increasing processing time (between 1 and 24 h) had no impact on inclusion efficiency. It is to be noted that two different processes were used, *i.e.* a batch process (Van Hees) and a continuous flow process (Charoenchaitrakool).

4.4 Conclusions

Phase behaviour studies of econazole base and the econazole salts with nitrate, besylate, sulfosalicylate dihydrate, maleate and α-cyclodextrin were carried out with pure carbon dioxide as the solvent. The melting point of econazole base was significantly depressed from 92°C to 50°C at 320.24 (± 2.47) bar. α-Cyclodextrin and the salts of econazole showed no

changes in the phase behaviour in supercritical carbon dioxide under the pressure and temperature conditions of this study. Inclusion yield (%) studies of econazole base into α-and methyl-β-cyclodextrin were conducted in supercritical carbon dioxide to investigate the influence of pressure, temperature and contact time on the inclusion. All the working parameters (pressure, temperature and contact time) played a significant role in the inclusion of econazole base into cyclodextrins. To conclude, melting point depression of econazole base provides a new pathway for efficient formation of inclusion complexes between econazole base and α-cyclodextrin; promoted by the melting of econazole base.

4.6 References

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Chapter 5: Influence of the Preparation Method on the Physicochemical Properties of Indomethacin and Methyl-β-Cyclodextrin Complexes

ABSTRACT

The main objective of this study was to investigate different manufacturing processes claimed to promote inclusion complexation between indomethacin and cyclodextrins in order to enhance the apparent solubility and dissolution properties of indomethacin. Especially, the effectiveness of supercritical carbon dioxide processing for preparing solid drug-cyclodextrin inclusion complexes was investigated and compared to other preparation methods. The complexes were prepared by physical mixing, co-evaporation, freeze-drying from aqueous solution, spray-drying and supercritical carbon dioxide processing methods. The prepared complexes were then evaluated by scanning electron microscopy, differential scanning calorimetry, X-ray powder diffraction, solubility and dissolution studies. The method of preparation of the inclusion complexes was shown to influence the physicochemical properties of the formed complexes. Indomethacin exists in a highly crystalline solid form. Physical mixing of indomethacin and methyl-β-cyclodextrin appeared not to reduce the degree of crystallinity of the drug. The co-evaporated and freeze-dried complexes had a lower degree of crystallinity than the physical mix; however the lowest degree of crystallinity was achieved in complexes prepared by spray-drying and supercritical carbon dioxide processing methods. All systems based on methyl-β-cyclodextrin exhibited better dissolution properties than the drug alone. The greatest improvement in drug dissolution properties was obtained from complexes prepared using supercritical carbon dioxide processing, thereafter by spray-drying, freeze-drying, co-evaporation and finally by physical mixing. Supercritical carbon dioxide processing is well known as an energy efficient alternative to other pharmaceutical processes and may have application for the preparation of solid-state drug-cyclodextrin inclusion complexes. It is an effective and economic method that allows the formation of solid complexes with a high yield, without the use of organic solvents and problems associated with their residues.

5.1 Introduction

Poor water solubility and the resulting low oral bioavailability of drugs is one of the major challenges encountered by drug discovery and development scientists (Lipinski, C.A., 2000; Leuner and Dressman., 2000). Various approaches to enhance the solubility and dissolution rate of drugs have been reported (Strickley, R.G., 2004; Vasconcelos, T. et al., 2007). Inclusion complexation of drug molecules with cyclodextrins has been extensively used to improve the solubility and dissolution rate of drugs (Mura, P. et al., 1999; Nagase, Y. et al., 2001; Jain, A.C. and Adeyeye, M.C., 2001; Bandi, N. et al., 2004; Jambhekar, S. et al., 2004). Cyclodextrins are macrocyclic oligomers of α-D-glucose with a hydrophilic exterior and a lipophilic central cavity. The interior cavity of cyclodextrin can harbour a poorly water soluble drug, whilst the hydrophilic exterior increases its apparent solubility (Bodor, N.S., 1993).

Several processing methods have been developed to prepare drug-cyclodextrin inclusion complexes in the solid-state, *e.g.* grinding (Mura, P. et al., 2001), kneading (Moyano, J.R. et al., 1995; Salústio, P.J. et al., 2009), roll mixing (Nozawa, Y. et al., 1997), ultrasound compaction (Fini, M. et al., 1997), co-precipitation from various solvents (Montassier, P. et al., 1997), freeze-drying (Pose-Vilarnovo, B. et al., 2001) and spray-drying (Moyano, J.R. et al., 1997). Most of these processing methods require either a comparatively high energy input, employ organic solvents, or both. Supercritical carbon dioxide (SC-CO_2) has also been recently studied by various researchers for the preparation of drug-cyclodextrin complexes.

Carbon dioxide becomes supercritical above its critical temperature (31.25°C) and critical pressure (73.8 bar). Supercritical carbon dioxide is a non-toxic, inexpensive, chemically stable, environmentally acceptable solvent that can be readily removed from the drugs or drug-cyclodextrin complexes after processing. It can be an attractive alternative to many of the methods currently used to produce drug-cyclodextrin complexes in the solid-state as it avoids the use of organic solvents (Van Hees, T. et al., 2002; Bandi, N. et al., 2004; Perrut,

M. et al., 2005; Rodier, E. et al., 2005; Al-Marzouqi, A.H. et al., 2007; Al-Marzouqi, A.H. et al., 2007a; Al-Marzouqi, A.H. et al., 2006; Shehatta, I. et al., 2005; Charoenchaitrakool, M. et al., 2002 and Türk, M. et al., 2007). Furthermore, the solvation properties of supercritical carbon dioxide can be beneficially tailored to dissolve a diverse range of solutes by simply regulating the temperature and pressure (Hassan, H.A. et al., 2007).

The use of the supercritical carbon dioxide process in the preparation of inclusion complexes between ibuprofen and methyl-β-cyclodextrin has already been investigated (Charoenchaitrakool, M. et al., 2002). This work involved passing ibuprofen-laden supercritical carbon dioxide through the packed bed of molten methyl-β-cyclodextrin for 2 hours. The resulting complexes were highly amorphous and showed extremely high apparent dissolution rates. Similarly, complexes of ketoprofen with methyl-β-cyclodextrin prepared by supercritical carbon dioxide processing at 40°C and 200 bar significantly improved the dissolution rate of the drug (Banchero, M. et al., 2013). It was found that the melting of methyl-β-cyclodextrin in supercritical carbon dioxide favours the complexation of drug and methyl-β-cyclodextrin without any addition of water or auxiliary agents.

Indomethacin is a non-steroidal anti-inflammatory drug indicated in the treatment of osteoarthritis, rheumatoid arthritis, tendinitis, gout and ankylosing spondylitis (Sweetman, S.C., 2005). It has poor aqueous solubility and a low dissolution rate which limits both its therapeutic application and efficacy (Lobenberg, R. and Amidon, G.L., 2000; Hirasawa, N. et al., 2003). Various approaches have been employed to address low solubility and dissolution rate: engineering a reduction in crystallinity by co-milling and supercritical co-precipitation processing (Lim, R.T.Y. et al., 2013); solid dispersions (El-Badry, M. et al., 2009); liquid-solid compacts (Nokhodchi, A. et al., 2005); inclusion complexation (Jambhekar, S. et al., 2004).

Indomethacin has an affinity for various cyclodextrins and the favourable effect of natural cyclodextrins (Wulff, M. et al., 2002; Salústio, P.J., 2009) and their derivatives, such as

hydroxypropyl-β- and hydroxyethyl-β-cyclodextrin (Jambhekar, S. et al., 2004) and 2,6-di-O-methyl-β-cyclodextrin (Iohara, D. et al., 2008) on its pharmaceutical properties has been previously reported; the complexation behaviour of indomethacin with methyl-β-cyclodextrin has not.

The aim of this study was to investigate the influence of the preparation methods on the physicochemical properties of indomethacin and methyl-β-cyclodextrin complexes. Inclusion complexes between indomethacin and methyl-β-cyclodextrin were prepared by physical mixing, co-evaporation, freeze-drying from aqueous solution, spray-drying and supercritical carbon dioxide processing methods. Binary systems were characterised by scanning electron microscopy, differential scanning calorimetry, X-ray powder diffraction, solubility and dissolution studies.

5.2 Materials and methods

5.2.1 Materials

Indomethacin (≥ 99 %, molecular weight: 357.79) and methyl-β-cyclodextrin (average molecular weight: 1310, extent of labeling: 1.6-2.0 mol CH_3 per unit anhydroglucose) were purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Hydrochloric acid and ethanol were obtained from Fisher Scientific (Loughborough, UK). Carbon dioxide (99.9 %) was obtained from BOC Ltd (Guildford, Surrey, UK). All reagents were used as received.

5.3 Preparation of Binary Mixtures of Indomethacin with Methyl-β-Cyclodextrin

All binary mixtures were prepared in a 1:1 molar ratio of indomethacin to methyl-β-cyclodextrin. After processing, the samples were stored in a desiccator over solid calcium chloride until submitted for analysis.

5.3.1 Physical Mixing

An equimolar mixture of indomethacin and methyl-β-cyclodextrin was accurately weighed and tumble-mixed for 15 minutes.

5.3.2 Co-evaporation

Indomethacin (300.01 mg) and methyl-β-cyclodextrin (1097.99 mg) were dissolved in ethanol (50 mL) and distilled water (10 mL), respectively, and mixed. The mixture was agitated in an orbital shaker for 24 hours until a clear solution was obtained. The solvents were then evaporated under reduced pressure which resulted in the isolation of a pale yellowish, dry powder.

5.3.3 Freeze-Drying

Indomethacin (300.00 mg) was added to the aqueous solution of methyl-β-cyclodextrin (1098.02 mg) while mixing with a magnetic stirrer. After equilibrating the mixture in an orbital shaker at room temperature for 48 hours, the resulting solution was held at -60°C and was dried in a freeze-dryer (ScanVac CoolSafe, UK) for 48 hours and then sieved through 0.150 mm sieve.

5.3.4 Spray-Drying

Ethanolic indomethacin (300.02 mg dissolved in 50 mL) and aqueous methyl-β-cyclodextrin (1098.01 mg dissolved in 10 mL) solutions were mixed and agitated until a clear solution was obtained. The final clear solution was spray-dried in a LabPlant SD-05, under the following conditions: inlet temperature: 150°C, outlet temperature: 77°C, flow rate of the solution 400 mL h^{-1}, air flow rate 40–50 $m^3 h^{-1}$, and atomising air pressure 1.5 bar.

5.3.5 Supercritical Carbon Dioxide Process

The supercritical carbon dioxide process was carried out in the static mode to achieve the inclusion of indomethacin in the methyl-β-cyclodextrin. The complexes were prepared using an extraction apparatus supplied by Thar Process Inc., USA.

The accurately weighed physical mixtures of indomethacin and methyl-β-cyclodextrin (300.02 mg and 1098.02 mg; 300.00 mg and 1098.03 mg; 300.01 mg and 1097.98 mg; 299.99 mg and 1098.00 mg, respectively) were placed in a sample cell as shown in the Figure 5.1.

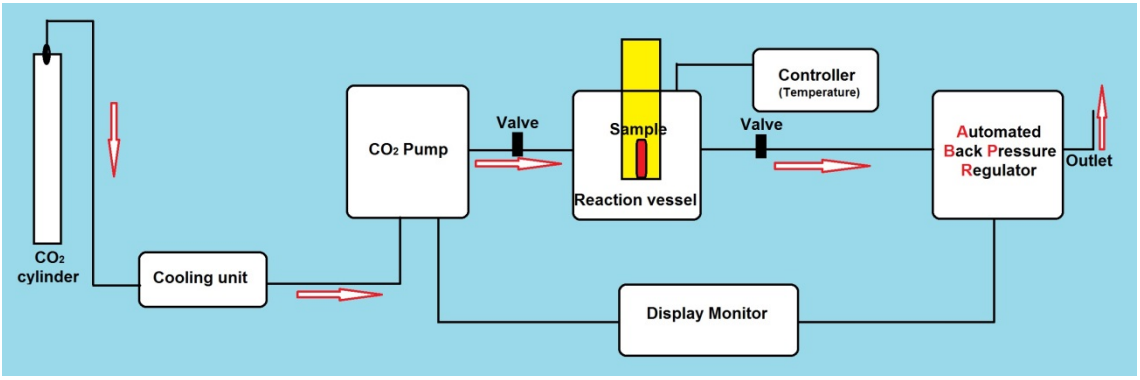


Figure 5.1: Schematics of supercritical carbon dioxide processing using the extraction apparatus supplied by Thar Process Inc., USA.

Carbon dioxide was pumped from a cylinder *via* a cooling unit into the sample cell. The physical mixtures were processed at four different working conditions [35°C/100 bar, 35°C/200 bar, 45°C/100 bar and 45°C/200 bar] in order to study the influence of pressure and temperature on the inclusion complex formation.

The required pressure was achieved by pumping carbon dioxide against an automated back-pressure regulator. The sample cell in the reaction vessel was heated to the desired temperature and held for 1 hr before recovering the solid complex by depressurisation at a rate of 7-8 bar min^{-1}. The product was then homogenised in a mortar prior to further analysis.

5.4 Analysis of the Prepared Binary Mixtures

5.4.1 Differential Scanning Calorimetry (DSC)

DSC analysis of individual components and the complex systems was carried out using a FP-90 central processor (Mettler-Toledo, LLC, UK). Calibration was carried out with indium as reference material. For each sample 5 mg was accurately weighed and hermetically sealed in aluminium pans and heated at a rate of 10°C min^{-1}. The thermogram was collected over the temperature range from 50 to 200°C.

5.4.2 X-Ray Powder Diffraction (XRPD)

X-ray powder diffractograms of individual components and the complex systems were obtained on a D8 Advance X-ray Diffractometer (Bruker, Germany) in theta-theta Bragg-Brentano geometry using reflection mode. Data was collected between 2-40° 2θ, with a step size of 0.006° and a counting time of 0.5 seconds per step using Cu Kα radiation.

The degree of crystallinity (% Crystallinity) is defined as the percentage by weight of crystalline drug in a sample containing drug in both the amorphous and crystalline states and was determined using the amorphous subtraction method (Suryanarayanan, R. and Mitchell, A.G., 1985). The area under the curve of the crystalline peaks was first measured; later the total area of peaks (crystalline and amorphous) was measured. An amorphous background was modelled using the DIFFRAC-SUITE TOPAS 4.2 software (Bruker, USA). The amorphous background was subtracted from the total area to leave just the crystalline area. The degree of crystallinity of the mixtures was estimated by the following relationship:

$$\% \text{ Crystallinity } = \left[\frac{\text{Area of crystalline peaks}}{\text{Total area of all the peaks}} \right] * 100$$

5.4.3 Scanning Electron Microscopy Analysis

The surface morphology of the raw materials and of the binary systems was examined by means of Hitachi SU-8030 scanning electron microscope (Tokyo, Japan). The samples were securely fixed on an aluminium stub using double-sided adhesive tape and then were made electrically conductive by coating in vacuum with a thin layer of chromium (~300 Å) at 30 W for 30 seconds. The photomicrographs were taken at an excitation voltage of 2.0 KV and a magnification of ×350.

5.4.4 Practical Yield

Determination of the practical yield is an important measure to know the efficiency of any process and to select the most effective method for the production of inclusion complexes. Percentage practical yield of all the binary systems was determined by the following equation:

$$\text{Practical yield (\%)} = \left[\frac{\text{Practical weight of the binary mixture}}{\text{Theoretical weight (Drug + Cyclodextrin)}}\right] * 100$$

5.4.5. Solubility Studies

Phase solubility studies were performed in triplicate according to the method reported by Higuchi and Connors (1965). Samples were prepared by adding excess indomethacin (in amount above its solubility), to 10 ml phosphate buffer (pH 7.4) solutions containing successively increasing concentrations (0, 3, 6, 9, 12, 15, 18 and 21 × 10^{-3} M) of methyl-β-cyclodextrin, in sealed glass containers. The solutions were agitated (100 rpm) at 37°C for 48 hours. Following equilibrium, the solutions were then filtered (0.45 µm filter pore size) and assayed for drug concentration by ultra-violet spectroscopy (Cary 100 UV-Vis, Agilent Technologies, USA). Phase-solubility diagrams were represented as the concentration of total dissolved indomethacin against the concentration of methyl-β-cyclodextrin. The binding

constant ($K_{1:1}$) for indomethacin-methyl-β-cyclodextrin complexes was calculated from the slope of the initial straight portion of the curve.

The UV spectrum of methyl-β-cyclodextrin was recorded and was found to have absorbance levels within the instrumental noise range in the 220-350 nm range covered by the absorbance of indomethacin. Scans and calibration curves of dilute solutions of indomethacin, alone and in the presence of methyl-β-cyclodextrin, showed no change in λ_{max} and molar extinction coefficient. This methodology was therefore considered valid for the determination of the total indomethacin in the various systems studied.

5.4.6 Dissolution Studies

The extent of dissolution of indomethacin from all drug-carrier binary systems, and for indomethacin alone, was conducted using USP Type II paddle method (Hanson G2 Vision® Classic 6, Chatsworth, CA) with phosphate buffer (pH 7.4) as the dissolution medium (El-Badry, M. et al., 2009). The samples, corresponding to 50 mg of indomethacin were dispersed into 900 ml of the dissolution media maintained at 37 $\pm$ 0.5°C and stirred at 100 rpm. 5 ml aliquots were withdrawn and filtered at the specified time intervals (5, 10, 15, 30, 45 and 60 minutes) and the drug concentration was determined by UV spectroscopy. The same volume of fresh medium was added to the dissolution vessel and the correction for the cumulative dilution was calculated. Each test was performed in triplicate. The parameters used to characterise the dissolution curves were the percentage of drug dissolved at 30 minutes and the dissolution efficiency at 30 and 60 minutes (calculated according to Khan, K.A., 1975).

5.4.7 Computational Details

Molecular docking calculations were conducted using the Glide (grid-based ligand docking) application implemented in the Maestro 9.3 software package (Schrodinger, LLC, New York, 2012). The methyl-β-cyclodextrin structure was prepared by adding hydrogens, followed by an energy minimisation to a convergence of RMSD 0.30Å using OPLS_2005 as force field.

Indomethacin was energetically minimised and ionisation considered at pH 7 using the ioniser subprogram of LigPrep 2.6. The 'Generate grid' sub application of the Glide tool was utilised for the generation of grid by selecting the whole methyl-β-cyclodextrin structure as a receptor site to locate coordinates of the receptor centre. The generated grid was then utilised as a receptor for docking of indomethacin using the 'standard precision' (SP) flexible docking method, located in the Glide tool. Docked cyclodextrin and ligand complexes were visualised and molecular surface complex pictures were generated using Maestro.

5.5 Results and Discussion

5.5.1 Differential Scanning Calorimetry Analysis

Thermograms for indomethacin, methyl-β-cyclodextrin and indomethacin-methyl-β-cyclodextrin binary systems (physical mix, co-evaporated, freeze-dried, spray-dried and supercritical carbon dioxide processed) are presented in Figure 5.2.

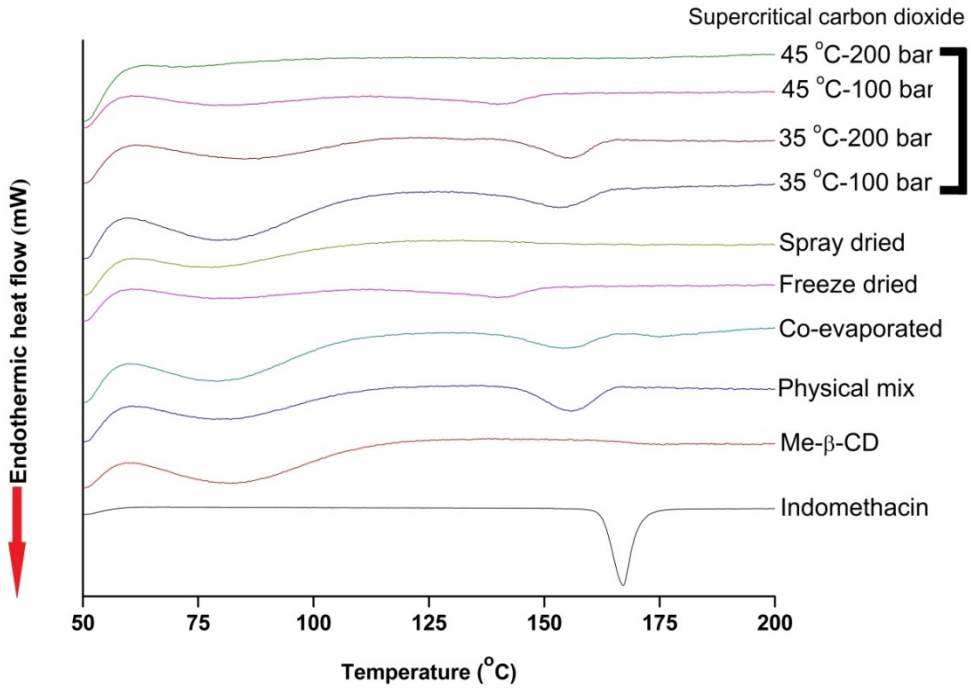


Figure 5.2: DSC thermograms of indomethacin, methyl-β-cyclodextrin and indomethacin-methyl-β-cyclodextrin (1:1 molar) systems prepared by various processing methods.

Indomethacin exhibited a sharp melting endotherm at 162°C confirming the crystalline nature of the starting material. This corresponds to the γ-crystalline polymorph form of indomethacin (Atef, E. et al., 2012). Methyl-β-cyclodextrin showed only a broad endothermic event between 60°C and 120°C ascribed to the loss of water as explained by Banchero, M. et al., 2013.

An endothermic peak, reduced in intensity compared with the melting endotherm of indomethacin, was observed for the samples prepared by physical mixing, co-evaporation, freeze-drying and supercritical carbon dioxide processing at 35°C/100 bar, 35°C/200 bar and 45°C/100 bar, indicating an incomplete inclusion of the drug in the methyl-β-cyclodextrin cavity. Inclusion complexes prepared by spray-drying and supercritical carbon dioxide processing at 45°C/200 bar showed an absence of the indomethacin melt peak indicating interactions between drug and methyl-β-cyclodextrin and the formation of an inclusion complex with loss of crystallinity as suggested by Charoenchaitrakool et al., 2002.

Table 5.1: Enthalpy values of indomethacin and indomethacin-methyl-β-cyclodextrin systems obtained from DSC thermograms.

Sample	**Enthalpy values[a] of endothermic events (mJ)**
Indomethacin	605.00[b]
Physical mixing	79.26
Co-evaporation	57.36
Freeze-drying	46.31
Spray-drying	0.00
Supercritical carbon dioxide processing	
35°C/100 bar	56.80
35°C/200 bar	46.69
45°C/100 bar	19.94
45°C/200 bar	0.00

[a]Enthalpy values are corrected for weight of the cyclodextrin; [b]Attributed to the melting endotherm of the γ–polymorph.

The enthalpy values of the endothermic events in Figure 5.2 are presented in Table 5.1 as calculated by integration of peak area and comparison with the melt event of indomethacin. They show a decrease in enthalpy for the samples containing methyl-β-cyclodextrin and were zero for the complexes prepared by spray-drying and supercritical carbon dioxide processing at 45°C/ 200 bar.

5.5.2 X-Ray Powder Diffraction Analysis

The X-ray powder diffractograms of pure indomethacin, methyl-β-cyclodextrin and binary systems are presented in Figure 5.3.

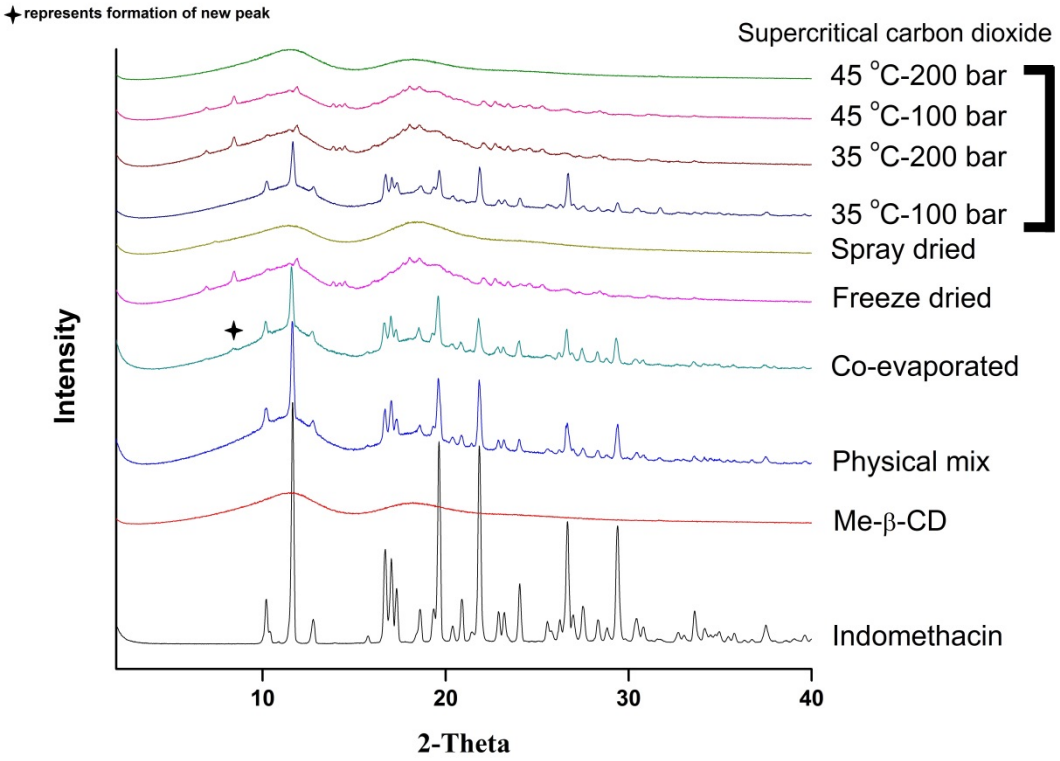


Figure 5.3: X-ray powder diffractograms of indomethacin, methyl-β-cyclodextrin and indomethacin-methyl-β-cyclodextrin (1:1 molar) systems prepared by various processing methods.

Sharp peaks on the diffractogram of pure indomethacin confirm its crystalline nature. Indomethacin showed characteristic peaks at 2θ equal to 10.20°, 11.64°, 12.77°, 15.75°, 16.70°, 17.03°, 17.32°, 18.60°, 19.34°, 19.64°, 20.90°, 21.83°, 22.88°, 23.21°, 24.05°, 26.65°,

27.49° and 29.38°. This corresponds to the γ-crystalline polymorph form of indomethacin (Lin, S.Y., 1992). The diffractogram of methyl-β-cyclodextrin displayed no sharp peaks except two broad halos at 11° and 18° indicating its amorphous nature.

The complexes prepared by physical mixing, co-evaporation and supercritical carbon dioxide processing at 35°C/100 bar showed a similar diffraction pattern to that of the respective individual components but with a reduction in the peak heights. The co-evaporated product showed a new peak (as represented by an asterisk in the figure) indicating the formation of a new crystalline phase (Iacovino, R. et al., 2012). In contrast, the complexes prepared by freeze-drying and supercritical carbon dioxide processing at 35°C/200 bar; and 45°C/100 bar exhibited considerable diminution of the diffraction peaks and exhibited shift in the peaks at 2θ equal to 10.20°, 11.64°, 12.77°, 15.75°, 16.70°, 17.03°, and 17.32° to lower 2θ angles. These results suggest that incomplete complexation occurred between indomethacin and methyl-β-cyclodextrin under these processing conditions.

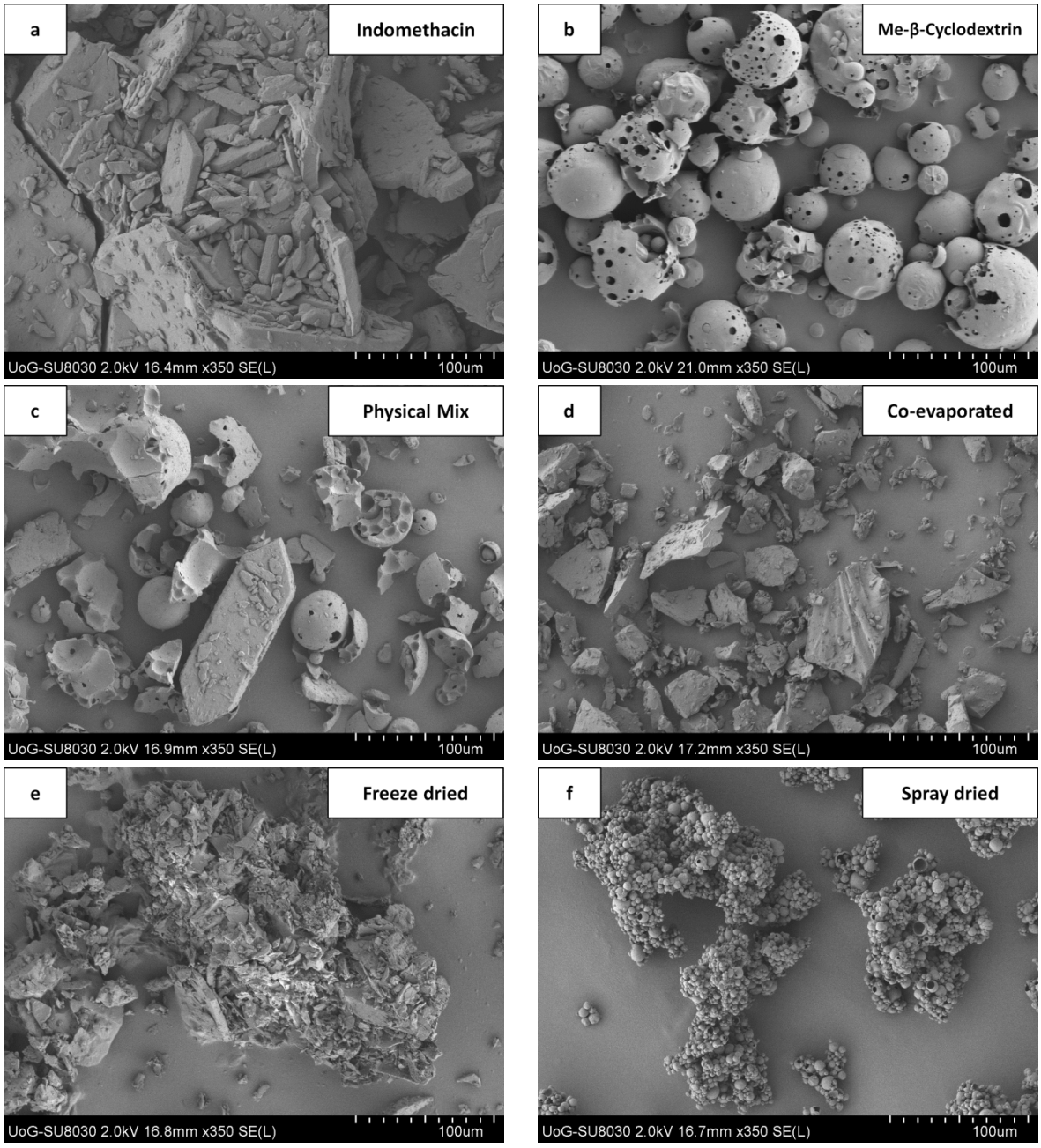
The diffraction pattern of the complexes prepared by spray-drying and supercritical carbon dioxide processing at 45°C/200 bar displayed two broad amorphous halos similar to that of the pure methyl-β-cyclodextrin. The absence of any peaks corresponding to indomethacin suggested complete complexation between indomethacin and methyl-β-cyclodextrin.

The decrease in the degree of crystallinity of the drug was observed in the following order: supercritical carbon dioxide processing at 45°C/200 bar (0% crystalline) ≈ spray-drying (0% crystalline) > supercritical carbon dioxide processing at 45°C/100 bar (11.23% crystalline) > freeze-drying (11.57% crystalline) > supercritical carbon dioxide processing at 35°C/200 bar (11.68% crystalline) > supercritical carbon dioxide processing at 35°C/100 bar (16.04% crystalline) > co-evaporation (16.91% crystalline) > physical mixing (18.02% crystalline). Decrease in the crystallinity indicated by reductions in peak intensity, shifts and appearance

of new peaks/ disappearance of peaks or a complete diffuse pattern might be related to possible loss of drug crystallinity and/or complexation.

5.5.3 Scanning Electron Microscopy

Figure 5.4 presents the photomicrographs of indomethacin, methyl-β-cyclodextrin and indomethacin-methyl-β-cyclodextrin binary systems prepared by various processing methods.



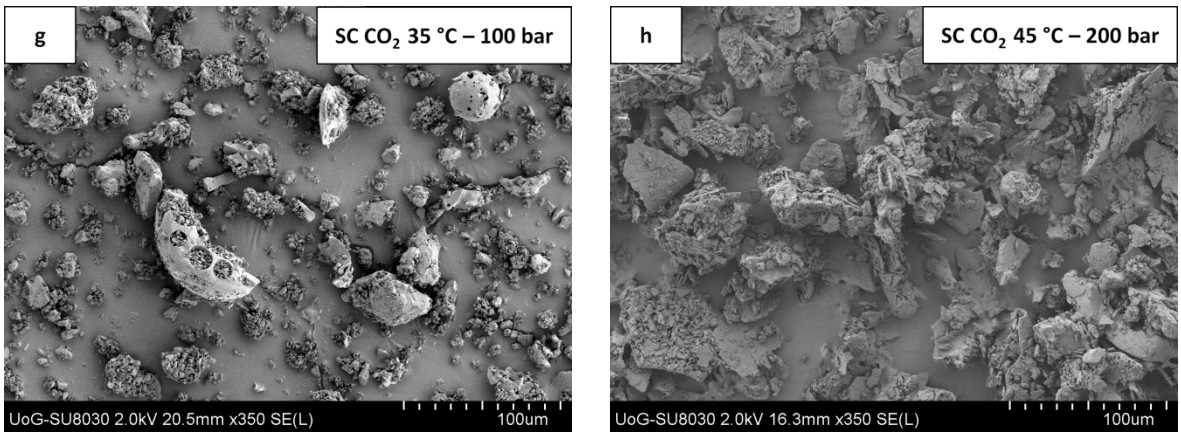


Figure 5.4: SEM photomicrographs of indomethacin (a), methyl-β-cyclodextrin (b) and indomethacin-methyl-β-cyclodextrin binary systems prepared by physical mixing (c), co-evaporation (d), freeze-drying (e), spray-drying (f), supercritical carbon dioxide processing at 35 °C/100 bar (g) and 45 °C/200 bar (h).

From SEM analysis, pure indomethacin (Figure 5.4a) appeared as small to large irregularly sized and shaped crystals (8-340 µm) with a tendency to self-agglomerate. Pure methyl-β-cyclodextrin (Figure 5.4b) appeared as perforated hollow spheres in the range of 10-90 µm. The product of the physical mixture (Figure 5.4c) appeared as a mixture of large crystals (100-150 µm) and broken hollow spheres (50-75 µm). Comparable morphology of the complexes prepared by physical mixing and supercritical carbon dioxide processing at 35°C/100 bar (Figure 5.4g) suggests that the two components were not taken into solution as the hollow sphere methyl-β-cyclodextrin morphology is retained. In the binary mixtures prepared by co-evaporation (Figure 5.4d), freeze-drying (Figure 5.4e) and supercritical carbon dioxide processing at 45°C/200 bar (Figure 5.4h) the original morphology of the raw materials disappeared, and it was not possible to differentiate between the two components. The spray-dried system (Figure 5.4f) showed homogenous aggregates of spherical particles (2-10 µm). Under high magnification they appeared as spherical particles with an uneven surface unlike the complexes prepared by supercritical carbon dioxide processing which had an irregular

morphology. The primary particle size of the spray-dried system was smaller than that of the products formed by other methods.

5.5.4 Practical Yield

The percentage practical yield of all the binary mixtures is tabulated in Table 5.2. The results reveal that the practical yield of the binary mixtures prepared by various methods was in the following order: physical mixing > all supercritical carbon dioxide processing > freeze-drying > co-evaporation > spray-drying.

Table 5.2: Percentage practical yield of indomethacin-methyl-β-cyclodextrin binary systems

Method	**Practical yield (%)**
Physical mixing	99.60
Co-evaporation	82.07
Freeze-drying	90.80
Spray-drying	60.33
Supercritical carbon dioxide processing	
35°C/100 bar	93.21
35°C/200 bar	92.76
45°C/100 bar	93.74
45°C/200 bar	92.14

All inclusion methods resulted in the loss of mass of the complexes to some extent. The co-evaporation method required the use of large volumes of organic solvent and additional energy-intensive processing steps for the removal of the residual solvent. Freeze-drying produced highly amorphous complexes but with lower yields which may be ascribed to the loss (of mass) of the water from methyl-β-cyclodextrin. Recovery of the spray-dried product was very difficult

due to electrostatic deposition of the complex on the internal chamber wall resulting in a very low product yield.

5.5.5 Solubility Studies

The phase solubility diagram of indomethacin with various concentrations of methyl-β-cyclodextrin in phosphate buffer medium (pH 7.4) is presented in Figure 5.5.

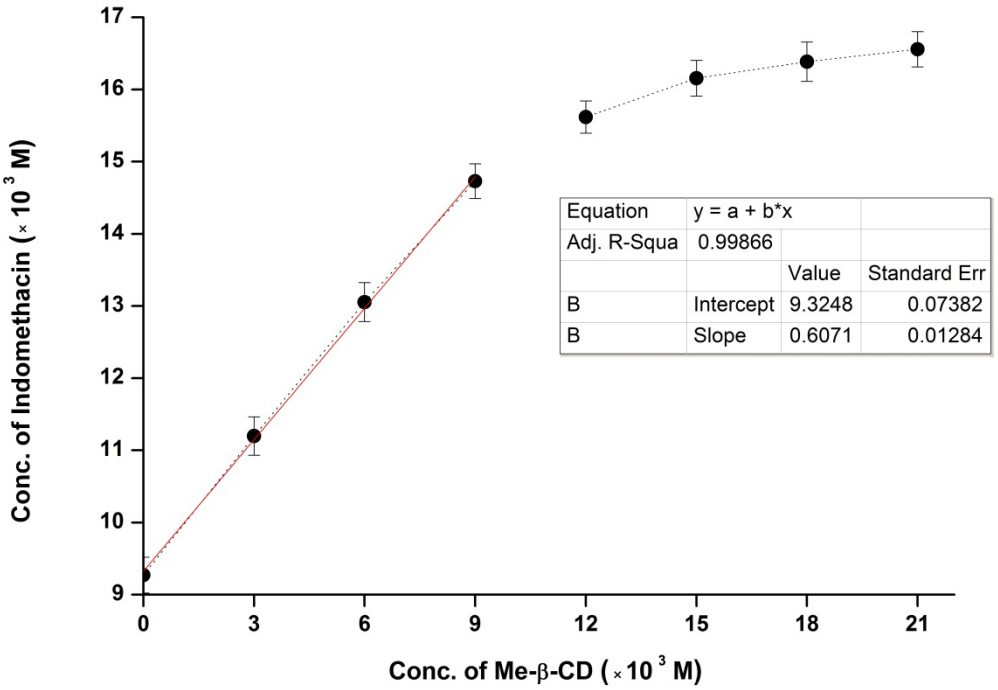


Figure 5.5: Phase solubility studies of indomethacin with increasing concentrations of methyl-β-cyclodextrin at 37°C and in pH 7.4 phosphate buffer.

Methyl-β-cyclodextrin formed A_N-subtype complexes with indomethacin. The solubility of indomethacin increased with the concentration of methyl-β-cyclodextrin, but the curve showed a negative curvature at higher cyclodextrin concentrations (12 to 21 mM) suggesting that methyl-β-cyclodextrin was less effective at higher concentrations. Similar phase solubility profiles (curvature shapes) were reported by Buchanan, C.M. et al. (2006) for some antifungal drugs and hydroxybutenyl-β-cyclodextrin in some buffers and by Mihajlovic, T. et al. (2012) for aripiprazole and 2-hydroxypropyl-β-cyclodextrin. The binding constant of the

complexes in this study ($K_{1:1}$ = 167 M^{-1}) was calculated from the slope of the initial straight portion of the curve. Phase solubility studies of indomethacin-β-CD complexes were carried out by Salústio, P.J. et al. (2009) in water and the authors reported a stability constant of $K_{1:1}$ = 366 M^{-1}. Jambhekar, S. et al. (2004) previously reported the stability constant values in the range of $K_{1:1}$ = 1,000-1,100 M^{-1} for indomethacin with β-, hydroxyethyl-β-, and hydroxypropyl-β-cyclodextrins in aqueous solutions containing ammonia.

5.5.6 Dissolution Studies

Figure 5.6 presents the dissolution profiles of indomethacin from various binary systems.

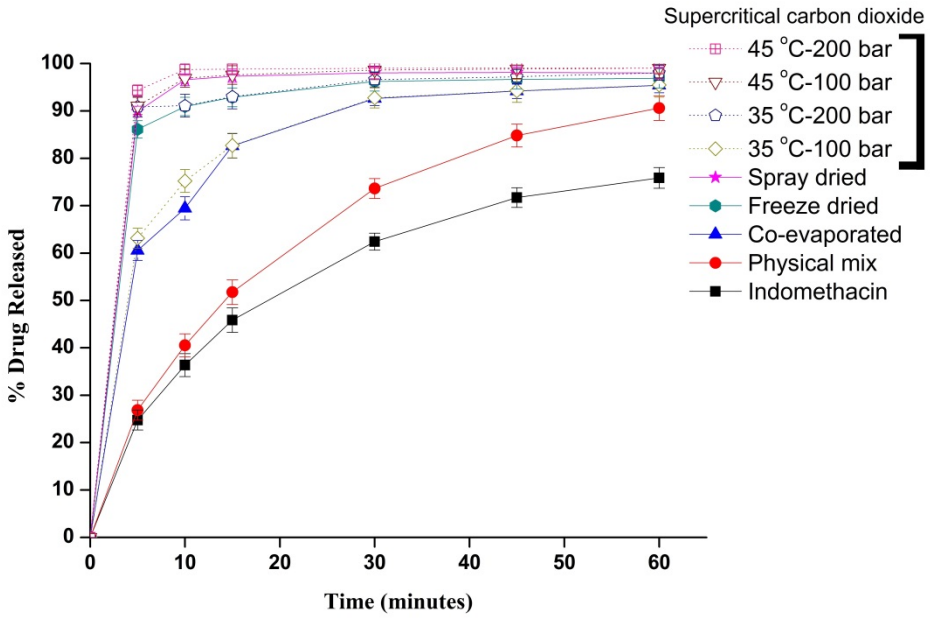


Figure 5.6: Dissolution profiles of indomethacin and indomethacin-methyl-β-cyclodextrin binary systems prepared by various processing methods at 37°C and in pH 7.4 phosphate buffer.

It is evident that all the binary systems exhibited a more rapid release and a greater extent of dissolution than the drug alone. The greatest improvement of the drug dissolution properties was obtained with the binary mixture prepared by supercritical carbon dioxide processing at 45°C/200 bar, followed by supercritical carbon dioxide processing at 45°C/100 bar, spray-drying, freeze-drying, co-evaporation and physical mixing. The results in terms of percent of

active ingredient dissolved at 30 min and the dissolution efficiency at 30 and 60 minutes are presented in Table 5.3.

Table 5.3: Percent indomethacin dissolved (DP) at 30 minutes and Dissolution Efficiency (DE)[b] at 30 and 60 minutes from indomethacin and indomethacin-methyl-β-cyclodextrin binary systems prepared by various methods (USP Apparatus II; pH 7.4 phosphate buffer; 37°C; 100 rpm).

Sample (Method)	**DP_{30}**	**DE_{30}**	**DE_{60}**
Indomethacin	62.43 ± 1.80	41.08 ± 1.42	55.76 ± 1.81
Physical mixing	73.64 ± 2.10	46.89 ± 1.51	65.18 ± 1.92
Co-evaporation	92.65 ± 1.54	72.38 ± 1.69	83.25 ± 1.96
Freeze-drying	96.23 ± 2.03	84.52 ± 1.78	90.57 ± 2.03
Spray-drying	98.05 ± 1.24	88.02 ± 1.94	93.05 ± 2.34
Supercritical carbon dioxide processing			
35 °C at 100 bar	92.76 ± 2.10	73.82 ± 1.92	84.01 ± 2.04
35 °C at 200 bar	96.61 ± 2.03	85.49 ± 2.03	91.39 ± 2.10
45 °C at 100 bar	98.65 ± 1.54	88.53 ± 1.91	93.70 ± 2.42
45 °C at 200 bar	99.01 ± 1.05	89.89 ± 1.96	94.47 ± 2.18

[b] DE_{30} and DE_{60} were calculated from the area under the dissolution curve at 30 and 60 minutes respectively and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time of the total amount added.

The amount of indomethacin released from drug alone was low with 36.36 ± 2.42 % dissolving within 10 minutes. The binary mixtures prepared by physical mixing, co-evaporation and supercritical carbon dioxide processing at 35°C/100 bar exhibited an increased drug release with 40.53 ± 2.19 %, 69.46 ± 2.42 % and 75.23 ± 2.67 % of indomethacin dissolved within 10 minutes. The binary mixtures prepared by freeze-drying, spray-drying and supercritical carbon dioxide processing at 35°C/200 bar, 45°C/100 bar and 45°C/200 bar exhibited a drug release of more than 90 % within 10 minutes.

The improved dissolution characteristics of the physical mixture may be attributable to improvements in drug wettability as suggested by Charoenchaitrakool and co-workers (Charoenchaitrakool et al., 2002) there is no evidence of the *in situ* formation of readily soluble complexes in the medium as suggested by Banchero and co-workers (Banchero, M. et al., 2013). Based on the SEM analysis (Figure 4f), the enhancement in the dissolution of the spray-dried product may be ascribed to a reduction in particle size. The greater improvement obtained with all other binary mixtures could also be explained by complex formation in the solution state prior to drying of the drug and methyl-β-cyclodextrin (Lin, S.Y. and Kao, Y.H., 1989; Guyot, M. et al., 1995). Furthermore, the reduced crystallinity of indomethacin in the binary mixtures could be considered as an important factor in the enhancement of its dissolution as the amorphous form of a drug is more readily soluble and exhibits an improved dissolution profile compared with the crystalline form (Hancock, B.C. and Zografi, G., 1997; El-Badry, M. et al., 2009) owing to the higher Gibbs free energy in the amorphous form (Khadka, P. et al., 2014).

5.5.7 Docking Studies

Indomethacin docked in the cavity of methyl-β-cyclodextrin is represented in Figure 5.7. The indomethacin structure is depicted in stick form with a mesh representing the limited of the calculated molecular surface (grey with reddish tint in the cavity); the hydrophilic area (cyan mesh); the hydrophobic area (orange mesh at the mid cavity depth of methyl-β-cyclodextrin). The figure shows the binding of indomethacin in the cavity of methyl-β-cyclodextrin primarily through anchorage by the phenyl group presumably due to its hydrophobicity. Inclusion in other orientations may not be favoured due to steric hindrance associated with the substituted indole moiety. The interactions responsible for the inclusion seem to be purely hydrophobic in nature, as no indications of hydrogen bonding can be found in the optimal complex geometries.

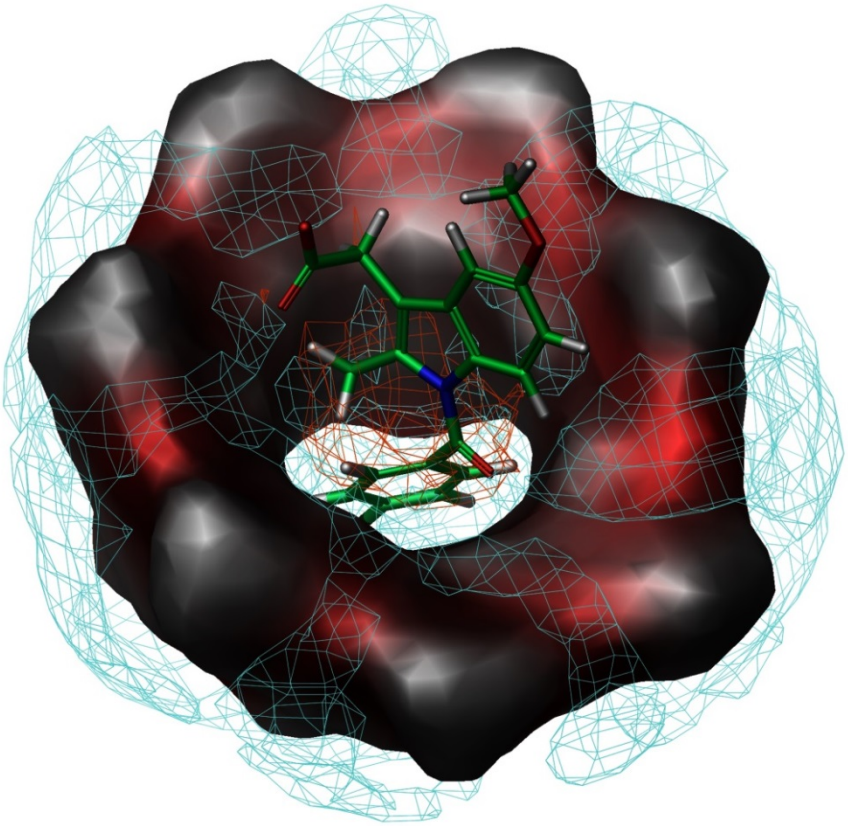


Figure 5.7: Representation of the complex between methyl-β-cyclodextrin (orange surface) and indomethacin (stick representation with surface meshes) obtained by computational molecular docking (1:1 stoichiometry).

The binding affinity, van der Waals energy and docking score values for inclusion of indomethacin in the cavity of methyl-β-cyclodextrin are 27.880 kcal mol^{-1}, -28.941 kcal mol^{-1} and -4.882 kcal mol^{-1}, respectively.

5.6 Conclusion

Solid systems of indomethacin with methyl-β-cyclodextrin in the 1:1 molar ratio were prepared using physical mixing, co-evaporation, freeze-drying, spray-drying and supercritical carbon dioxide processing methods. The results obtained by different characterisation techniques suggest complete complexation of indomethacin and methyl-β-cyclodextrin prepared by spray-drying and supercritical carbon dioxide processing methods. Molecular-

docking studies support the formation of stable molecular inclusion complexation of indomethacin with methyl-β-cyclodextrin. Different degrees of crystallinity were observed in the analyses of products prepared by various methods, suggesting the possibility of drug-methyl-β-cyclodextrin interactions of different efficiencies, which may give rise to different degrees of inclusion formation and/or crystallinity of the sample. All indomethacin-methyl-β-cyclodextrin complexes exhibited a faster and greater extent of drug dissolution than the drug alone. Complexes obtained by the supercritical carbon dioxide processing and spray-drying showed the greatest improvement in drug dissolution properties. This study has demonstrated that the processing conditions used in the preparation of solid-state inclusion complexes of indomethacin and methyl-β-cyclodextrin critically affects morphology and physical performance.

The co-evaporation method required the use of organic solvent and additional processing steps for its removal. Freeze-drying produced highly amorphous complexes but was characterised by long, energy-intensive processing steps. Spray-drying, from an aqueous ethanolic solution, was a comparatively rapid process and produced highly amorphous, free flowing agglomerates of microspheres; however, usage of the organic solvent and low product yields presents practical challenges. Supercritical carbon dioxide processing was shown to be an efficient approach for the preparation of solid-state inclusion complexes. It is an efficient and economic process that allows the formation of solid complexes based in strong intermolecular forces in high yield in a single step avoiding the use of organic solvents and the problems associated with their residues.

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Chapter 6: Preparation of Olanzapine and Methyl-β-Cyclodextrin Complexes Using a Single-Step, Organic Solvent-Free Supercritical Fluid Process: An Approach to Enhance the Solubility and Dissolution Properties.

ABSTRACT

The purpose of this study was to evaluate a single-step, organic solvent-free supercritical fluid process for the preparation of olanzapine-methyl-β-cyclodextrin complexes with an express goal to enhance the dissolution properties of olanzapine. The complexes were prepared by supercritical carbon dioxide processing, co-evaporation, freeze drying and physical mixing. The prepared complexes were then analysed by differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy, solubility and dissolution studies. Computational molecular docking studies were performed to study the formation of molecular inclusion complexation of olanzapine with methyl-β-cyclodextrin. All the binary mixtures of olanzapine with methyl-β-cyclodextrin, except physical mixture, exhibited a faster and greater extent of drug dissolution than the drug alone. Products obtained by the supercritical carbon dioxide processing method exhibited the highest apparent drug dissolution. The characterisation by different analytical techniques suggests complete complexation or amorphisation of olanzapine and methyl-β-cyclodextrin complexes prepared by supercritical carbon dioxide processing method. Therefore, organic solvent-free supercritical carbon dioxide processing method proved to be novel and efficient for the preparation of solid inclusion complexes of olanzapine with methyl-β-cyclodextrin. The preliminary data also suggests that the complexes of olanzapine with methyl-β-cyclodextrin will lead to better therapeutic efficacy due to better solubility and dissolution properties.

6.1 Introduction

Olanzapine is a second-generation atypical neuroleptic drug approved by the Food and Drug Administration as a first-line therapy for the treatment of schizophrenia and mania associated with bipolar disorder (Abdelbary and Tadros, 2013). It suffers from poor aqueous solubility (12-44 µg mL^{-1}) and a low dissolution rate leading to an erratic bioavailability (Kulkarni *et al*., 2010; Dixit *et al*., 2011; Raman *et al*., 2013). Moreover, the drug undergoes extensive hepatic first-pass metabolism and is required in high doses (Sood *et al*., 2013).

Several approaches have been reported to enhance the solubility and dissolution rate of olanzapine, *e.g.* solid-dispersions (Krishnamoorthy *et al*., 2011), nano-emulsions (Raman *et al*., 2013), solid-lipid nanoparticles (Sood *et al*., 2013), freeze dried tablets (Dixit *et al*., 2011) and inclusion complexation with cyclodextrins (Kulkarni *et al*., 2010; de Freitas *et al*., 2012).

Cyclodextrins, also known as cyclomaltoses, cycloamyloses and Schardinger dextrins, are macrocyclic oligomers of α-D-glucose with a hydrophilic exterior and a relatively non polar central cavity (Villiers, 1891; French *et al*., 1949; Eastburn and Tao, 1994; Del Valle, 2004; Appel *et al*., 2012; Kfoury *et al*., 2014; Kfoury *et al*., 2015; Rudrangi *et al*., 2015). Cyclodextrins can form inclusion complexes by taking up the entire or a part of lipophilic drug molecule in its hydrophobic interior cavity (Loftsson and Duchêne, 2007; Salústio *et al*., 2009). Through formation of inclusion complexes, cyclodextrins are known to enhance the aqueous solubility and dissolution rate of poorly soluble drugs (Trapani *et al*., 2000. Pose-Vilarnovo *et al*., 2001; Latrofa *et al*., 2001; Jain and Adeyeye, 2001; Riekes *et al*., 2010).

The study published by de Freitas *et al*. (2012) reported that olanzapine and methyl-β-cyclodextrin (Me-β-CD) complexes prepared in the 1:1 molar ratio using rotary evaporation exhibit a higher dissolution profile than the active alone or in a state of physical mixture. Despite their success, the complex preparation by the stated method required an organic

solvent which is not desirable. Removal of environmentally harmful organic solvents from the drug product to the levels approved by the Food and Drug Administration is very challenging and therefore conventional techniques used for the preparation of inclusion complexes (co-evaporation, spray drying and kneading) involve several drying steps for considerable time, which may also affect the drug stability (Al-Marzouqi *et al*., 2006). Hence, it is highly recommended to eliminate the use of organic solvents in the preparation of drugs or drug-cyclodextrin complexes.

The aim of the present study was to produce olanzapine-methyl-β-cyclodextrin complexes in the same stoichiometric ratios (1:1 molar) without using organic solvents or auxiliary agents. Therefore, application of supercritical fluid processing was studied as an alternative to conventional methods in the current work.

A supercritical fluid is defined as a substance that exists above its critical pressure and temperature. Supercritical fluids feature densities like liquids and viscosities and diffusivities like gases and hence offer excellent mass transfer and solubilising properties (York, 1999; Kompella and Koushik, 2001; Sunkara and Kompella, 2002; Bandi *et al*., 2004). Carbon dioxide becomes supercritical above 31.25°C and 73.8 bar. Supercritical carbon dioxide (SC-CO_2) is environmentally benign and is considered to be green. Supercritical carbon dioxide is a non-combustible, non-toxic, recyclable and environment-friendly solvent (Palakodaty and York, 1999; Lang and Wai, 2001; Lee *et al*., 2008; Deshpande *et al*., 2011; Girotra *et al*., 2013; Rudrangi *et al*., 2015) and has provided an appealing alternative to toxic organic solvents or conventional complexation media. Supercritical carbon dioxide has been successfully employed in the preparation of inclusion complexes between various drugs and cyclodextrins in dynamic or static modes (Table 6.1).

Table 6.1: Drug-cyclodextrin complexes prepared by supercritical carbon dioxide processing in static or dynamic modes.

Drug	Cyclodextrin	Mode	Reference
Acetaminophen	β-CD	Not reported	Giordano *et al*., 1996.
Benzocaine	β-CD	Static	Al-Marzouqi *et al*., 2007a
		Static	Al-Marzouqi *et al*., 2007b
Budesonide	HP-β-CD[a]	Static	Bandi *et al*., 2004
	γ-CD	Static	Toropainen *et al*., 2006
Bupivacaine	β-CD	Static	Al-Marzouqi *et al*., 2007b
Econazole	β-CD	Static	Al-Marzouqi *et al*., 2007c
		Static	Al-Marzouqi *et al*., 2009
Eflucimibe	γ-CD	Not reported	Papet *et al*., 2003
	γ-CD	Static	Rodier *et al*., 2005
Fluconazole	β-CD	Static	Al-Marzouqi *et al*., 2009
Flurbiprofen	TMe-β-CD[b]	Not reported	Moribe *et al*., 2007
Ibuprofen	Me-β-CD	Static	Charoenchaitrakool *et al*., 2002
	β-CD	Static	Türk *et al*., 2007
		Static	Hussein *et al*., 2007
Imazalil	β-CD	Static	Lai *et al*., 2003
Indomethacin	HP-β-CD	Static	Bandi *et al*., 2004
	Me-β-CD	Static	Rudrangi *et al*., 2015
Itraconazole	β-CD	Static	Al-Marzouqi *et al*., 2006
		Static	Al-Marzouqi *et al*., 2009
		Static	Hassan *et al*., 2007
Ketoprofen	Me-β-CD	Static	Banchero *et al*., 2013

Mepivacaine	β-CD	Static	Al-Marzouqi *et al*., 2007b
Miconazole	β-CD	Static	Van Hees *et al*., 2002
	HP-γ-CD[c]	Static	Barillaro and Bertholet, 2004.
Naproxen	TMe-β-CD	Not reported	Moribe *et al*., 2007
	β-CD	Dynamic	Junco *et al*., 2002
Piroxicam	β-CD	Static	Van Hees *et al*., 1999
		Static	Van Hees *et al*., 2002
		Static	Grandelli *et al*., 2012
Simvastatin	HP-β-CD	Dynamic	Jun *et al*., 2007

*In static mode, the contents of the cell are exposed to carbon dioxide, pressurized and allowed to equilibrate; while carbon dioxide is circulated continuously through the cell in the dynamic mode.

[a]HP-β-CD: Hydroxypropyl-β-cyclodextrin; [b]TMe-β-CD: Trimethyl-β-cyclodextrin; [c]HP-γ-CD: Hydroxypropyl-γ-cyclodextrin.

The use of the supercritical carbon dioxide processing has already been investigated in the preparation of drug-methyl-β-cyclodextrin inclusion complexes (Charoenchaitrakool *et al*., 2002; Banchero *et al*., 2013; Rudrangi *et al*., 2015). A significant improvement in the dissolution rate of drug was observed in all cases. It was suggested by Banchero and co-workers (Banchero *et al*., 2013) that the liquefaction of methyl-β-cyclodextrin in supercritical carbon dioxide favours the complexation of drug and cyclodextrin without any addition of water or auxiliary agents as the drug molecules would better reach the cavity of the cyclodextrin in the molten or liquid state.

The effect of supercritical carbon dioxide processing on the preparation of olanzapine-methyl-β-cyclodextrin complexes has not yet been reported. Inclusion complexes were prepared by physical mixing, freeze drying, co-evaporation and supercritical carbon dioxide processing at various working (temperature and pressure) conditions. The prepared complexes were then

characterized by solubility studies, differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy and dissolution studies.

6.2 Materials and methods

6.2.1. Materials

Olanzapine (≥99%, molecular weight: 312.44, CAS number: 132539-06-1) was obtained from Dr. Reddy's Laboratories Ltd. (Hyderabad, Telangana, India). Methyl-β-cyclodextrin (average molecular weight: 1310, CAS number: 128446-36-6, extent of labeling: 1.6–2.0 mol CH_3 per unit anhydroglucose) was purchased from Sigma–Aldrich (Gillingham, Dorset, UK). Carbon dioxide (99.9%) was obtained from BOC Ltd. (Guildford, Surrey, UK). All chemicals were used as received without further purification.

6.3 Preparation of Binary Mixtures of Olanzapine with Methyl-B-Cyclodextrin

All binary mixtures of olanzapine with methyl-β-cyclodextrin were prepared in a 1:1 molar ratio. The processed samples were stored in a desiccator over solid calcium chloride until submitted for analysis.

6.3.1 Physical Mixing

Physical mixture was obtained by tumble-mixing an accurately weighed equimolar mixture of olanzapine and methyl-β-cyclodextrin for 15 minutes.

6.3.2 Freeze-Drying

Olanzapine was added to the aqueous solution of methyl-β-cyclodextrin under constant stirring. The mixture was agitated in an orbital shaker at room temperature until equilibrium was attained (48 h). The resultant suspension was frozen at −60°C and then lyophilized in a

freeze-dryer (ScanVac CoolSafe, UK) for 48 h. The obtained product was sieved through 0.150 mm sieve.

6.3.3 Co-evaporation

An ethanolic solution of olanzapine was added to an aqueous solution of methyl-β-cyclodextrin and the mixture was agitated in an orbital shaker for 24 h. The solvents were then evaporated under reduced pressure to yield a pale yellowish, dry powder.

6.3.4 Supercritical Carbon Dioxide Process

The complexes were prepared using an extraction apparatus supplied by Thar Process Inc., USA in the static mode. The schematics of supercritical carbon dioxide processing have been previously described in detail (Rudrangi *et al*., 2015).

The physical mixtures of olanzapine and methyl-β-cyclodextrin (100.01 mg and 412.28 mg; 100.03 mg and 412.27 mg; 100.02 mg and 412.28 mg; 100.01 mg and 412.30 mg, respectively) were placed in a sample cell. Carbon dioxide was pumped from a cylinder via a cooling unit into the sample cell. The physical mixtures were processed at four different working conditions [45°C-100 bar, 45°C-200 bar, 55°C-100 bar and 55°C-200 bar] in order to study the influence of pressure and temperature on the formation of inclusion complexes.

The desired pressure was achieved by pumping carbon dioxide against an automated back-pressure regulator. The sample cell in the reaction vessel was heated to the desired temperature and held for 1 h before recovering the solid complex by depressurisation at a rate of 7–8 bar min^{-1}. The product was then homogenised in a mortar prior to further analysis.

6.4 Analysis of the Prepared Binary Mixtures

6.4.1 Differential Scanning Calorimetry Analysis (DSC)

Thermal analysis of pure materials and the binary mixtures was carried out using a differential scanning calorimeter (Mettler-Toledo, LLC, UK). The equipment was periodically calibrated with indium. Accurately weighed samples (5 mg) were hermetically sealed in aluminium pans and heated at a rate of 10°C min^{-1} from 50°C to 200°C.

6.4.2 X-Ray Powder Diffraction Analysis (XRPD)

X-ray powder diffraction analysis of pure materials and the binary mixtures was carried out at room temperature using a D8 Advance X-ray Diffractometer (Bruker, Germany) in theta–theta Bragg–Brentano geometry using reflection mode. The diffractograms were collected between 2–40° 2θ, with a step size of 0.006° and a counting time of 0.5 s per step using Cu Kα radiation. The degree of crystallinity (% Crystallinity) was determined using the amorphous subtraction method (Suryanarayanan, R. and Mitchell, A.G., 1985).

6.4.3 Scanning Electron Microscopy Analysis (SEM)

Micrographs of pure materials and the binary systems were collected using a Hitachi SU-8030 scanning electron microscope. The samples were securely mounted on aluminium stubs using double-sided adhesive tape and made electrically conductive by coating in vacuum with a thin layer of chromium (~300Å) at 30 W for 30 s. The photomicrographs were obtained at an excitation voltage of 2.0 kV and a magnification of ×350.

6.4.4 Solubility Studies

Saturation solubility of olanzapine was measured in triplicate by adding excess amounts of the drug to 10 mL of deionised water in sealed glass containers. The solutions were agitated for 72 hours at 37 ± 0.5°C. The solutions were then filtered (0.45 µm filter pore size) and

assayed for drug concentration by ultra-violet spectroscopy (Cary 100 UV-vis, Agilent Technologies, USA) after dilution in 10 mm quartz cuvettes.

Phase-solubility studies were carried out in triplicate as described by Higuchi and Connors (1965). Excess amounts of olanzapine (*i.e.* in amounts above its solubility limit) were added to 10 mL of de-ionised water (pH 7.1) containing successively increasing concentrations (0, 5, 10, 15, 20, 25, 37.5 and 50 × 10^{-3} M) of methyl-β-cyclodextrin in sealed glass containers. The resultant solutions were agitated (100 rpm) at 37 ± 0.5°C until equilibrium was attained (72 h). The suspensions were then filtered and assayed for drug concentration as described above. The apparent stability constant ($K_{1:1}$) for olanzapine-methyl-β-cyclodextrin complexes was calculated from the slope of the linear portion of the phase solubility diagram.

6.4.5 Dissolution Studies

Dissolution studies of olanzapine from all binary systems and olanzapine alone were performed in triplicate using USP Type II paddle method (Hanson G2 Vision® Classic 6, Chatsworth, CA). Accurately weighed samples of drug or binary mixtures, equivalent to 10 mg of olanzapine were dispersed into 900 mL of deionised water (pH 7.1) at 37 ± 0.5°C and stirred at 50 rpm. At predetermined time points (2, 5, 10, 20, 45, and 60 min); 5 ml aliquots of the samples were drawn, filtered (0.45 µm filter pore size) and assayed for drug concentration by UV spectroscopy. The dissolution curves were characterized by the percentage of drug dissolved and the dissolution efficiency at 30 minutes. Dissolution efficiency was evaluated according to the method reported by Khan (1975).

6.4.6 Computational Details

Molecular docking calculations were conducted using Glide (grid-based ligand docking) application implemented in the Maestro 9.3 software package (Schrodinger, LLC, New York, 2012). The methyl-β-cyclodextrin structure was prepared by adding hydrogens, followed by

an energy minimisation to a convergence of RMSD 0.30Å using OPLS_2005 as force field. Olanzapine was energetically minimised and ionization considered at pH 7 using ionizer subprogram of LigPrep 2.6. The 'Generate grid' sub application of the Glide tool was utilised for the generation of grid by selecting the whole methyl-β-cyclodextrin structure as a receptor site to locate coordinates of the receptor centre. The generated grid was then utilised as a receptor for docking of olanzapine using the 'standard precision' (SP) flexible docking method, located in the Glide tool. Docked methyl-β-cyclodextrin and olanzapine complexes were visualised and molecular surface complex pictures were generated using Maestro.

6.5 Results and Discussion

6.5.1 Differential Scanning Calorimetry Analysis

Figure 6.1a presents the DSC thermograms of olanzapine (unprocessed) and olanzapine processed with supercritical carbon dioxide at 55°C-200 bar. The thermograms of olanzapine exhibited a sharp melting endotherm at ~198°C, indicating the crystalline nature of the drug.

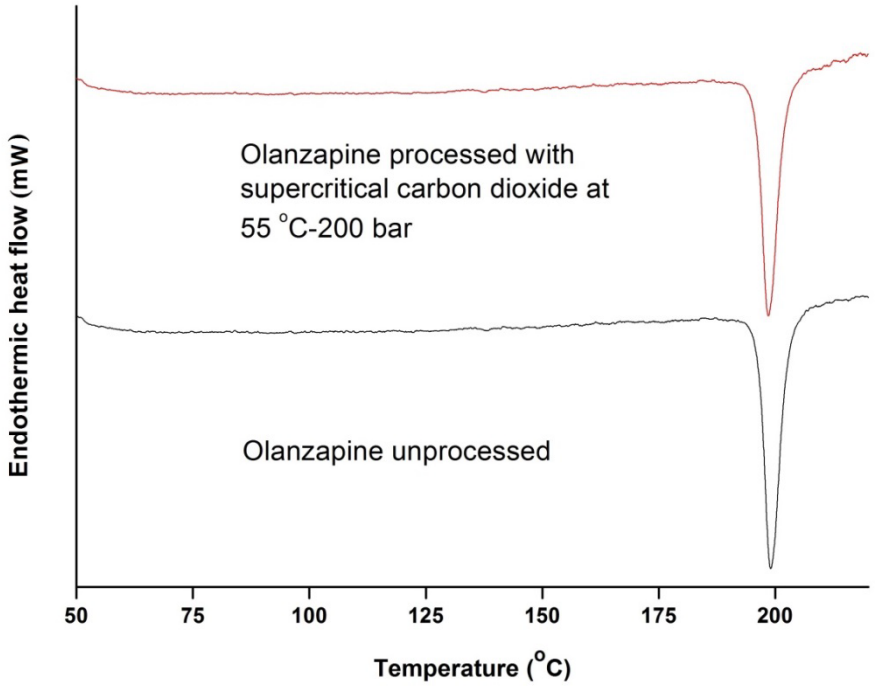

Figure 6.1a: Control study: DSC thermograms of olanzapine (unprocessed) and olanzapine processed with supercritical carbon dioxide at 55°C-200 bar.

DSC studies also indicated that processing the drug with supercritical carbon dioxide has not altered the crystallinity of the drug. Similar results were reported by Rudrangi *et al.* (2015) for indomethacin when processed with supercritical carbon dioxide.

Figure 6.1b presents the DSC thermograms of olanzapine, methyl-β-cyclodextrin and olanzapine-methyl-β-cyclodextrin binary systems prepared by various processing methods. Thermogram of methyl-β-cyclodextrin revealed a broad endothermic peak between 60°C and 120°C attributed to the release of water molecules as explained by Banchero *et al.* (2013).

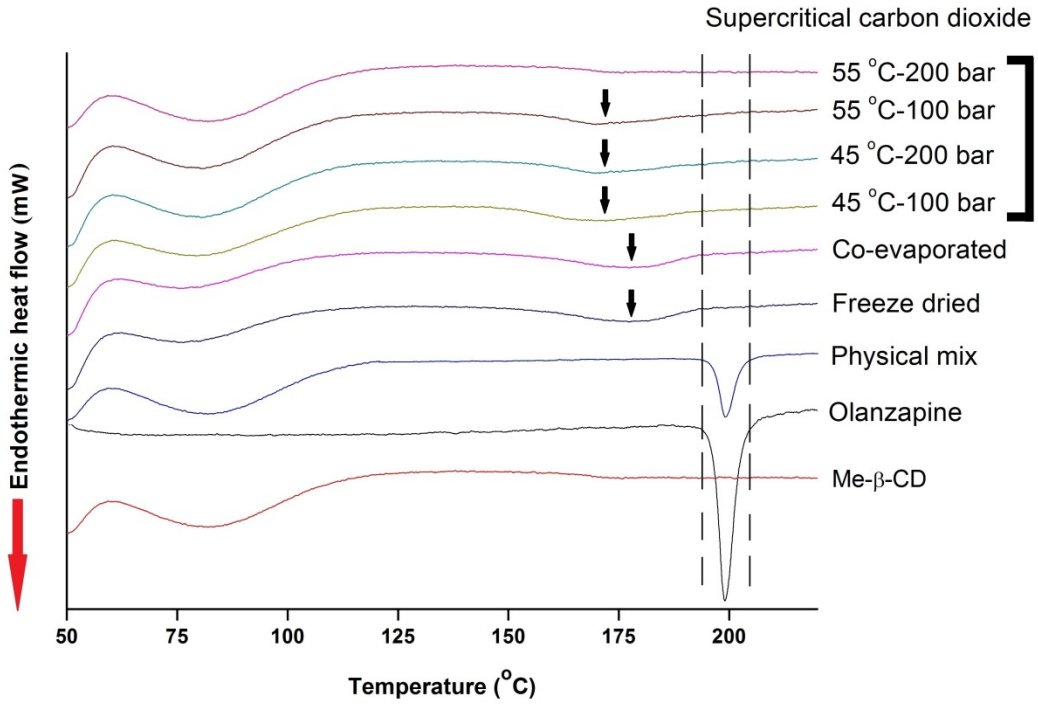

Figure 6.1b: DSC thermograms of olanzapine, methyl-β-cyclodextrin and olanzapine-methyl-β-cyclodextrin binary systems prepared by various processing methods

Thermograms of the binary mixture prepared by physical mixing showed a similar diffraction pattern to that of the respective drug and cyclodextrin. It displayed the melting endotherm of olanzapine, indicating the retention of the crystalline structure of the drug and suggests the absence of interaction between the drug and the cyclodextrin.

Thermograms of the binary mixtures prepared by freeze drying, co-evaporation and supercritical carbon dioxide processing at 45°C-100 bar, 45°C-200 bar and 55°C-100 bar displayed broad endothermic peaks of reduced intensity (as represented by arrows) compared with the melting endotherm of olanzapine and shifted to lower temperature. This may be ascribed to the partial inclusion of the drug into cyclodextrin as explained by Marques *et al.* (1990). On the other hand, thermogram of the complexes prepared by supercritical carbon dioxide processing at 55°C-200 bar revealed a complete disappearance of the drug endotherm which may be ascribed to the transformation of drug from crystalline to an amorphous state, or the formation of inclusion complexes (Charoenchaitrakool *et al.*,2002).

6.5.2 X-Ray Powder Diffraction Analysis

Figure 6.2a presents the X-ray powder diffraction patterns of pure olanzapine (unprocessed) and olanzapine processed with supercritical carbon dioxide at 55°C-200 bar.

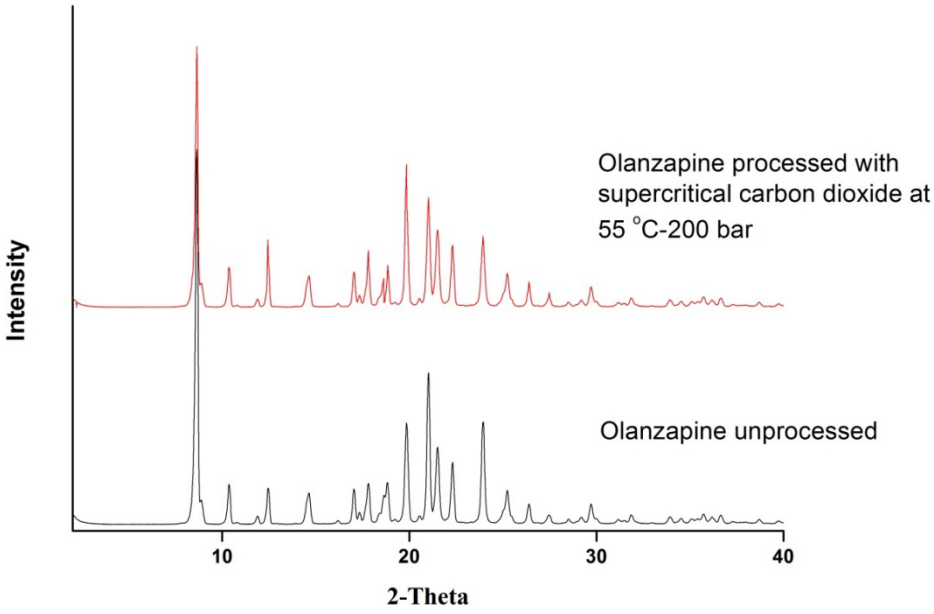

Figure 6.2a: Control study: X-ray powder diffractograms of olanzapine (unprocessed) and olanzapine processed with SC-CO_2 at 55°C-200 bar.

The diffraction pattern of olanzapine (pure and supercritical carbon dioxide processed) displayed sharp and intense characteristic peaks at a diffraction angle of 2θ equal to 8.63°, 10.36°, 12.44°, 14.64°, 17.03°, 17.81°, 18.84°, 19.85°, 21.02°, 21.50°, 22.32°, 23.94°, 25.24°, 26.39° and 29.71° confirming the crystalline nature of drug. In agreement with DSC analysis, XRPD analysis also indicated that supercritical carbon dioxide process had not altered the crystallinity of the drug.

Figure 6.2b presents the X-ray powder diffraction patterns of pure olanzapine, methyl-β-cyclodextrin and olanzapine-methyl-β-cyclodextrin binary systems prepared by various processing methods.

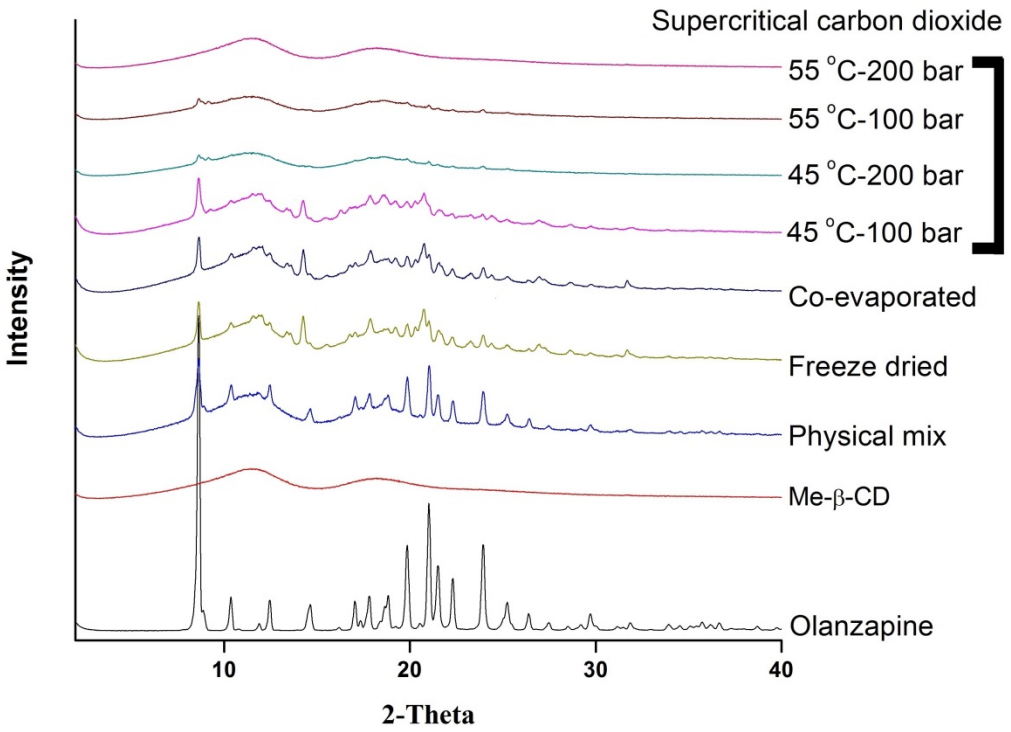

Figure 6.2b: X-ray powder diffractograms of olanzapine, methyl-β-cyclodextrin and olanzapine-methyl-β-cyclodextrin binary systems prepared by various processing methods

The diffraction pattern of methyl-β-cyclodextrin showed two broad halos at 2θ equal to 11° and 18° confirming its amorphous nature. Diffraction pattern of the binary mixture prepared by physical mixing showed a similar diffraction pattern to that of the respective drug and

cyclodextrin. It displayed all the principal peaks of olanzapine, indicating the retention of the crystalline structure of drug and suggests the absence of interaction between the drug and the cyclodextrin.

In agreement with the DSC results, the diffraction patterns of the complexes prepared by supercritical carbon dioxide processing at 45°C-200 bar and 55°C-100 bar revealed significant diminution of the diffraction peaks suggesting the interactions between drug and methyl-β-cyclodextrin. The diffraction pattern of the complexes prepared by supercritical carbon dioxide processing at 55°C-200 bar was characterised by the complete disappearance of the drug peaks. However, it displayed two broad features similar to that of the pure methyl-β-cyclodextrin suggesting the formation of inclusion complexes in which the drug was entrapped in the cavity of cyclodextrin (Charoenchaitrakool *et al*., 2002; Banchero *et al*,. 2013; Rudrangi *et al*., 2015).

The increase in the amorphous content of the drug was observed in the following order: supercritical carbon dioxide processing at 55 °C-200 bar (0% crystalline) > 55 °C-100 bar (4.34% crystalline) ≈ 45 °C-200 bar (6.49% crystalline) > 45 °C-100 bar (6.49% crystalline) > Co-evaporation (78.79% crystalline) > Freeze drying (80.17% crystalline) > Physical mixing (89.41% crystalline).

6.5.3 Scanning Electron Microscopy Analysis

Morphology of olanzapine, methyl-β-cyclodextrin and olanzapine-methyl-β-cyclodextrin binary systems prepared by physical mixing, freeze drying, co-evaporation and supercritical carbon dioxide processing was analyzed by SEM and is presented in Figure 6.3.

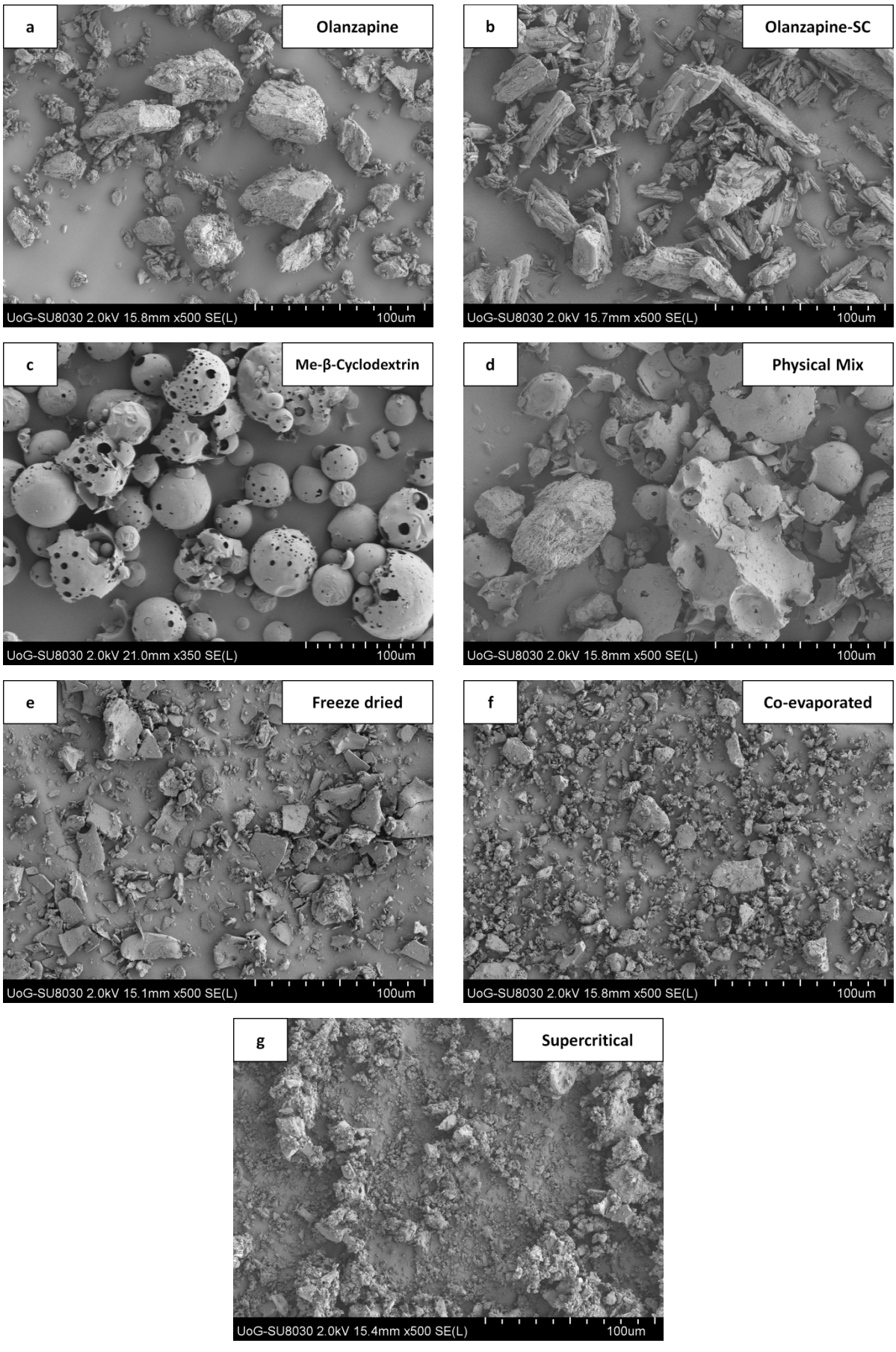
a
Olanzapine
UoG-SU8030 2.0kV 15.8mm x500 SE(L)
100um
b
Olanzapine-SC
UoG-SU8030 2.0kV 15.7mm x500 SE(L)
100um
c
Me-β-Cyclodextrin
UoG-SU8030 2.0kV 21.0mm x350 SE(L)
100um
d
Physical Mix
UoG-SU8030 2.0kV 15.8mm x500 SE(L)
100um
e
Freeze dried
UoG-SU8030 2.0kV 15.1mm x500 SE(L)
100um
f
Co-evaporated
UoG-SU8030 2.0kV 15.8mm x500 SE(L)
100um
g
Supercritical
UoG-SU8030 2.0kV 15.4mm x500 SE(L)
100um

Figure 6.3: SEM photomicrographs of olanzapine-unprocessed (a), olanzapine processed with supercritical carbon dioxide at 55°C-200 bar (b), methyl-β-cyclodextrin (c) and olanzapine-methyl-β-cyclodextrin binary systems prepared by physical mixing (d), freeze drying (e), co-evaporation (f) and supercritical carbon dioxide processing at 55°C-200 bar (g).

From SEM analysis, pure olanzapine (Figure 6.3a) and olanzapine processed with supercritical carbon dioxide at 55°C-200 bar (Figure 6.3b) appeared as small to large irregularly sized and shaped crystals with a tendency to self-agglomerate while pure methyl-β-cyclodextrin (Figure 6.3c) appeared as perforated hollow spheres. In agreement with DSC and XRPD analyses, SEM analysis also indicated that supercritical carbon dioxide process had not altered the crystallinity of the drug.

Photomicrograph of the physical mixture (Figure 6.3d) revealed the presence of olanzapine crystals, mixed with, or adhered to the surface of broken hollow spheres of methyl-β-cyclodextrin. In agreement with the XRPD analysis, the complexes prepared by freeze drying (Figure 6.3e) and co-evaporation (Figure 6.3f) showed the presence of crystalline olanzapine. Co-evaporated product appeared as aggregates of drug particles and broken spheres. Photomicrograph of the complexes prepared by supercritical carbon dioxide processing at 55°C-200 bar (Figure 6.3g) revealed the disappearance of the original morphology of the drug and methyl-β-cyclodextrin. The product appeared as heterogeneous aggregates and it was not possible to differentiate between the raw materials.

6.5.4 Solubility Studies

Aqueous solubility of olanzapine at 37 ± 0.5°C was found to be 1.96 ± 0.24 µg mL^{-1} and 23.49 ± 1.53 µg mL^{-1} at the end of 1 h and 72 h, respectively. No further improvement was observed in the drug solubility after 72 h. Kulkarni *et al*. (2010), Dixit *et al*. (2011) and

Raman *et al*. (2013) reported a drug solubility of 13.13 ± 1.6 µg mL^{-1}, 34.3 ± 11.1 µg mL^{-1} and 43.4 ± 1.74 µg mL^{-1} in distilled water at 37 °C, respectively.

Figure 6.4 presents the phase solubility diagram for the complex formation between olanzapine and methyl-β-cyclodextrin in deionized water at 37 ± 0.5°C. The studies revealed that the apparent solubility of olanzapine increased linearly as a function of methyl-β-cyclodextrin concentration over the entire concentration range studied. Linearity is a characteristic of A_L-subtype system, suggesting the formation of water soluble complexes in solution as explained by Higuchi and Connors (1965). Moreover, the linear (olanzapine and methyl-β-cyclodextrin) correlation with slope of less than 1 (0.0223) suggested the formation of 1:1 complexes over the concentration range (0–50 mM) investigated. The apparent stability constant ($K_{1:1}$), obtained from the slope of the linear phase solubility diagram was 304 M^{-1}. Similar phase solubility profile was reported for olanzapine-methyl-β-cyclodextrin complexes in distilled water by de Freitas *et al*. (2012).

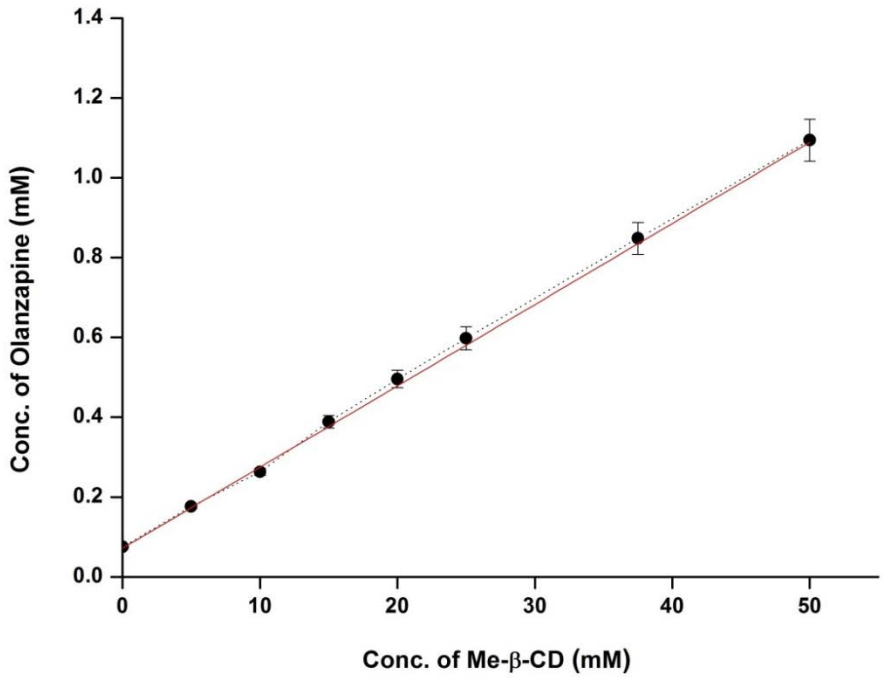

Figure 6.4: Phase solubility studies of olanzapine with increasing concentrations of methyl-β-cyclodextrin at 37 ± 0.5°C and in deionized water-pH 7.1.

Phase solubility studies of olanzapine-hydroxypropyl-β-cyclodextrin complexes were carried out by Kulkarni *et al*. (2010) in distilled water and the authors reported a stability constant of $K_{1:1}$ = 242 M^{-1}. Phase solubility studies of indomethacin-methyl-β-cyclodextrin complexes were carried out by Rudrangi *et al.* (2015) in phosphate buffer medium (pH 7.4) and the authors reported an A_N-subtype phase solubility diagram with a stability constant of $K_{1:1}$ = 167 M^{-1}.

6.5.5 Dissolution Studies

Figure 6.5 presents the dissolution profiles of olanzapine from drug alone and from drug-methyl-β-cyclodextrin binary systems in deionised water at 37 ± 0.5°C. All binary systems other than the physical mixture exhibited better dissolution profiles than the drug alone. The results in terms of percent of olanzapine dissolved at 30 min and the dissolution efficiency at 30 min are presented in Table 6.2.

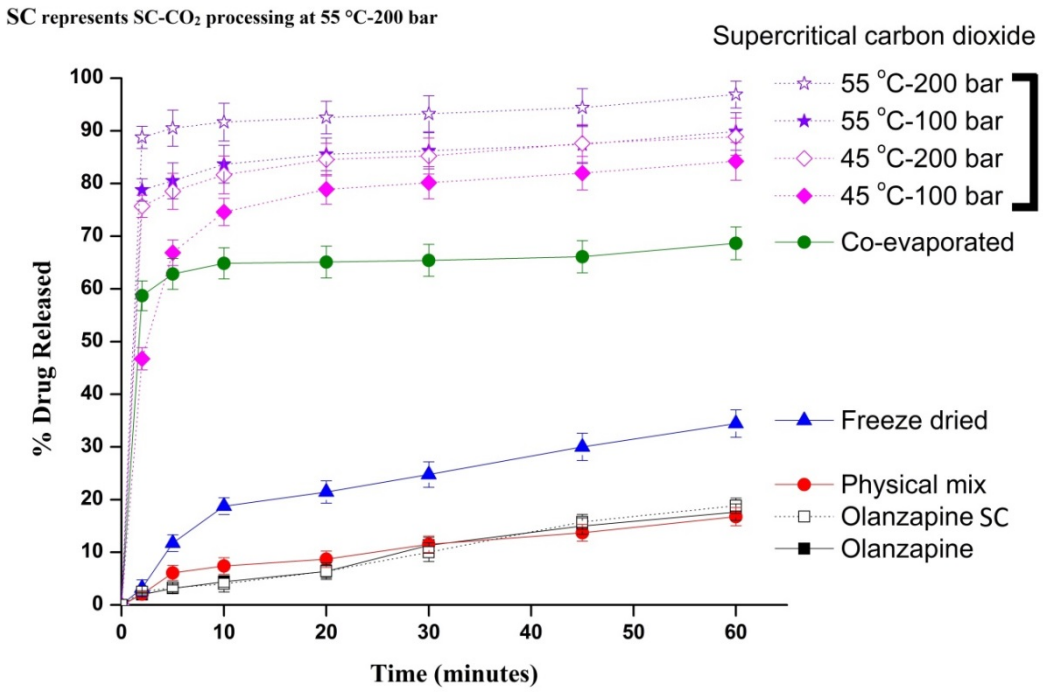

Figure 6.5: Dissolution profiles of olanzapine and olanzapine-methyl-β-cyclodextrin binary systems prepared by various processing methods at 37 ± 0.5°C and in deionised water (*n*=3).

The increase in the dissolution properties of olanzapine was observed in the following order: supercritical carbon dioxide processing at 55°C-200 bar > 55°C-100 bar ≈ 45°C-200 bar > 45°C-100 bar > co-evaporation > freeze drying > physical mixing.

The amount of olanzapine dissolved from unprocessed drug alone and drug processed with supercritical carbon dioxide at 55°C-200 bar was very low with 17.62 ± 1.59% and 18.84 ± 1.44% dissolving at the end of 60 min, respectively. The dissolution studies confirmed that supercritical carbon dioxide processing alone had not affected the dissolution of the drug.

The binary mixture obtained by physical mixing exhibited no increase in the drug dissolution with 16.75 ± 1.72% of olanzapine dissolving at the end of 60 min. Similar dissolution profile was reported for olanzapine-methyl-β-cyclodextrin physical mixture in distilled water by de Freitas *et al*. (2012).

Table 6.2: Percent olanzapine dissolved (DP) at 30 min and dissolution efficiency (DE)* at 30 min from olanzapine and olanzapine-methyl-β-cyclodextrin binary systems prepared by various processing methods (USP Apparatus II; deionised water-pH 7.1; 37 ± 0.5°C; 100 rpm; n=3).

Sample (Method)	**DP_{30}**	**DE_{30}**
Olanzapine	11.31 ± 1.52	5.7
Physical mixing	11.51 ± 1.57	7.6
Freeze drying	21.75 ± 2.42	17.3
Co-evaporation	65.42 ± 3.04	62.1
Supercritical carbon dioxide processing		
45°C-100 bar	80.15 ± 3.06	71.1
45°C-200 bar	86.23 ± 3.42	80.8
55°C-100 bar	85.47 ± 3.18	79.6
55°C-200 bar	93.28 ± 2.79	88.8

*DE_{30} was calculated from the area under the dissolution curve at 30 min and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time of the total amount added.

Improvement in the drug dissolution was found to be dependent both on the processing method and the processing conditions used for the preparation of complexes. The binary mixtures prepared by freeze drying and co-evaporation exhibited an increased drug release with 34.44 ± 2.61% and 68.67 ± 3.11% of olanzapine dissolved after 60 min, respectively. On the other hand, binary mixtures prepared by supercritical carbon dioxide processing resulted in more than 80% of drug dissolution within the first 30 min irrespective of the temperature and pressure employed. However, the binary mixtures prepared by supercritical carbon dioxide processing at 55°C and 200 bar showed highest drug dissolution with more than 90% of the drug dissolved within the first 10 min. These results suggested that both the temperature and pressure have influence on the formation of the inclusion complexes in supercritical carbon dioxide at studied parameters.

The improved dissolution characteristics of the binary mixtures can be attributed to the improved in drug wettability, high aqueous solubility (greater than 2000 mg mL^{-1}) and surfactant-like properties of methyl-β-cyclodextrin as suggested by Banchero *et al*. (2013), Cirri *et al*. (2005) and Guyot *et al*. (1995). Moreover, the greater improvement obtained with all the supercritical carbon dioxide processed systems could also be a result of reduced crystallinity of the binary mixtures (El-Badry, M. *et al*., 2009) and the formation of inclusion complexes between olanzapine and Me-β-CD in the solid state (Rudrangi *et al*., 2015).

6.5.6 Docking Studies

Computational molecular docking studies were conducted to study the possibility of molecular arrangement of olanzapine and methyl-β-cyclodextrin inclusion complexes. Figure 6.6 presents the best pose of olanzapine docked in the cavity of methyl-β-cyclodextrin.

The three-dimensional structure of olanzapine is depicted in stick form with a mesh representing the molecular surface (grey with reddish tint in the cavity); the hydrophilic area

(cyan mesh); the hydrophobic area (orange mesh at the mid cavity depth of methyl-β-cyclodextrin). The figure shows the binding of olanzapine in the cavity of methyl-β-cyclodextrin through anchorage by the methyl group of the piperazine due to its hydrophobicity. The interactions responsible for the inclusion seem to be purely hydrophobic in nature, as no indications of hydrogen bonding can be found in the optimal complex geometries. The binding affinity (GLIDE energy), van der Waals energy and docking score for inclusion of olanzapine in methyl-β-cyclodextrin are -24.13 kcal mol^{-1}, -21.57 kcal mol^{-1} and -3.09 kcal mol^{-1}, respectively.

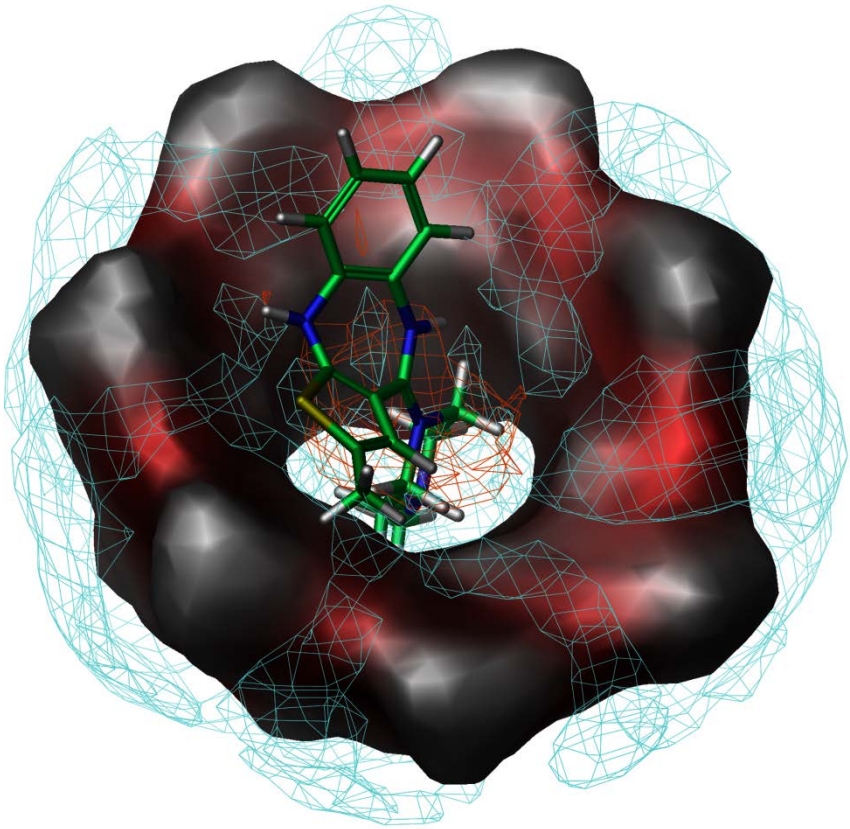

Figure 6.6: Representation of the complex between methyl-β-cyclodextrin (orange) and olanzapine (stick representation with surface meshes) obtained by computational molecular docking (1:1 stoichiometry).

Computational molecular docking studies of indomethacin with methyl-β-cyclodextrin were carried out by Rudrangi *et al.* (2015) using the Glide application and the authors reported the

binding affinity, van der Waals energy and docking score of 27.880 kcal mol^{-1}, -28.941 kcal mol^{-1} and -4.882 kcal mol^{-1} respectively.

6.6 Conclusions

Complexation of olanzapine with methyl-β-cyclodextrin was accomplished successfully using a single-step, organic solvent-free supercritical fluid process. The phase solubility diagram with methyl-β-cyclodextrin in de-ionised water was classified as A_L–subtype, indicating the formation of 1:1 stoichiometric inclusion complexes. All the binary mixtures with methyl-β-cyclodextrin, except physical mixture, exhibited a faster and greater extent of drug dissolution than the drug alone.

Information obtained from the differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy and dissolution studies suggest complete complexation or amorphisation of olanzapine and methyl-β-cyclodextrin prepared by supercritical carbon dioxide processing method.

Different degrees of crystallinity and dissolution rate were observed in the products processed by supercritical carbon dioxide at various processing conditions, suggesting the possibility of olanzapine-methyl-β-cyclodextrin interactions of different efficiencies in the solid state. Products obtained by the supercritical carbon dioxide processing method exhibited the highest apparent drug dissolution followed by co-evaporation, freeze drying and physical mixing. Therefore, a solid inclusion method using supercritical carbon dioxide proved to be a novel and efficient complexation method for olanzapine into methyl-β-cyclodextrin. Furthermore, since this method has no toxic solvent residue, products obtained by this method should provide minimal side effects in humans, compared to those obtained by techniques, which require the use of organic solvents.

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Chapter 7: Conclusions and Future Work

7.1 Conclusions

Solid systems of α-cyclodextrin with econazole and its nitrate, besylate, sulfosalicylate dihydrate and maleate salts in the 1:1 molar ratio were prepared by supercritical carbon dioxide-inclusion method and compared to products obtained using physical mixing and freeze-drying.

Nuclear magnetic resonance spectroscopy studies (^{1}H-NMR, 2D-^{1}H–^{1}H ROESY studies and relaxation time (T_1)-measurement inversion recovery experiments) indicated the interactions of econazole with α-cyclodextrin and confirmed the inclusion of econazole inside the cyclodextrin cavity. Isothermal titration calorimetric studies of econazole besylate and sulfosalicylate dihydrate salts and α-cyclodextrin conducted in pH 4.5 phosphate buffer at 25°C confirmed the formation of complexes between the salts and α-cyclodextrin in a 1:1 stoichiometry. The complexes were thermodynamically favourable and the reactions were exothermic and enthalpically driven.

Phase behaviour studies of econazole base, econazole salts with nitrate, besylate, sulfosalicylate dihydrate, maleate, α- and methyl-β-cyclodextrin were carried out with pure carbon dioxide as the solvent. The melting point of econazole base and methyl-β-cyclodextrin was significantly depressed while α-cyclodextrin and the salts of econazole showed no changes in the phase behaviour in supercritical carbon dioxide under the pressure and temperature conditions of this study.

Inclusion yield (%) studies of econazole base into α- and methyl-β-cyclodextrin were conducted in supercritical carbon dioxide to investigate the influence of pressure, temperature and contact time on the inclusion. All the working parameters (pressure, temperature and contact time) played a significant role in the inclusion of econazole base into cyclodextrins.

Different degrees of crystallinity were observed in the analyses of products prepared by various methods, suggesting the possibility of drug-cyclodextrin interactions of different efficiencies, which may give rise to different degrees of inclusion formation and/or crystallinity of the sample. Nevertheless, products obtained by the freeze-drying and supercritical carbon dioxide inclusion methods were among the ones showing the highest interaction between the drug and the cyclodextrin.

All the systems with cyclodextrins exhibited better dissolution properties than drug alone. The greatest improvement of the drug dissolution properties was obtained with supercritical carbon dioxide inclusion for econazole and the nitrate, besylate and sulfosalicylate dihydrate salts of econazole; however, freeze-drying showed the greatest improvement in drug dissolution for econazole maleate.

Freeze-drying and supercritical carbon dioxide inclusion produced highly amorphous and rapid dissolving complexes and proved to be useful complexation methods for econazole and its salts into α-cyclodextrin. The freeze-drying method is expensive and was characterised by long, energy-intensive processing steps (72-96 h). Supercritical carbon dioxide inclusion method is energy efficient and allows the formation of solid drug-cyclodextrin inclusion complexes in 3-4 h with a high yield, without the use of organic solvents and problems associated with their residues.

The preliminary data suggests that the complexes of econazole and its salts with α-cyclodextrin will lead to better therapeutic efficacy due to better solubility and dissolution properties.

7.2 Future Work

It was observed that the supercritical carbon dioxide inclusion method offered greater or similar improvement of the drug dissolution properties than the freeze-dried products which were almost amorphous. This may be attributed to the high level of interactions between econazole or its salts and α-cyclodextrin. Studies must be conducted to investigate the reason behind the faster release of supercritical carbon dioxide included complexes and interactions between the drug and cyclodextrin.

Isothermal titration calorimetric studies must be conducted to gain an insight into the thermodynamics of complexation in addition to the strength of the interaction between econazole base, its salts and different cyclodextrins.

Inclusion yield (%) studies of econazole and its salts into different cyclodextrin must be conducted to gain insights into level of interactions/inclusion between drug and cyclodextrin; the influence of pressure, temperature and contact time on the inclusion yield (%) must be investigated. The changes in inclusion yield (%) around the boundary of the p-T curve and the influence of contact time on the inclusion yield (%) of econazole- methyl-β-cyclodextrin complexes must be investigated.

Phase-solubility studies of econazole base and its salts with α-cyclodextrin must be conducted in order to understand the pattern of drug solubilisation, to evaluate the solubilizing efficiency of α-cyclodextrin and to determine the stability constant (*K*s) and complexation efficiency.

Molecular dynamics and nuclear magnetic resonance studies of econazole salts and α-cyclodextrin must be conducted in order to investigate the interaction of salts in the complex and the modes of penetration of the salts into the cyclodextrin cavity.

It was observed in Chapter 4 that the melting temperatures of econazole base and methyl-β-cyclodextrin were highly depressed in supercritical carbon dioxide. The mechanism of the melting temperature depression of econazole base and methyl-β-cyclodextrin in carbon dioxide must be investigated.

APPENDIX

The Evaluation of Supercritical Fluid Technology as a Preparative Technique for the Preparation of Flurbiprofen-Methyl-β-Cyclodextrin Inclusion Complexes: An Approach to Enhance the Solubility and Dissolution Properties.

ABSTRACT

The aim of this study was to enhance the apparent solubility and dissolution properties of flurbiprofen through inclusion complexation with cyclodextrins. Especially, the efficacy of supercritical fluid technology as a preparative technique for the preparation of flurbiprofen-methyl-β-cyclodextrin inclusion complexes was evaluated. The complexes were prepared by supercritical carbon dioxide processing and were evaluated by solubility, differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy, practical yield, drug content estimation and *in vitro* dissolution studies. Computational molecular docking studies were conducted to study the possibility of molecular arrangement of inclusion complexes between flurbiprofen and methyl-β-cyclodextrin. The studies support the formation of stable molecular inclusion complexes between the drug and cyclodextrin in a 1:1 stoichiometry. *In vitro* dissolution studies showed that the dissolution properties of flurbiprofen were significantly enhanced by the binary mixtures prepared by supercritical carbon dioxide processing. The amount of flurbiprofen released from drug alone was very low with 1.11 ± 0.09% dissolving at the end of 60 min while the binary mixtures processed by supercritical carbon dioxide at 45°C and 200 bar released 99.39 ± 2.34% of the drug at the end of 30 min. All the binary mixtures processed by supercritical carbon dioxide at 45°C exhibited a drug release of more than 80% within the first 10 min irrespective of the pressure employed. The study demonstrated the single step, organic solvent-free supercritical carbon dioxide process as a promising approach for the preparation of inclusion complexes between flurbiprofen and methyl-β-cyclodextrin in solid state.

1. Introduction

The water solubility of a drug is a key indicator for the solubility of drug molecules in the intestinal fluids and its contribution to bioavailability. Success of a formulation relies on how effectively it makes the drug molecules available at the site of absorption. However, poor water solubility and the resulting oral bioavailability of drugs has long been a problem to the pharmaceutical scientists as the dissolution is quite often the rate-limiting process in the absorption of a drug (Charman and Stella, 1991; Takagi *et al.*, 2006). The ability to increase water solubility can thus be very important to enhancing the therapeutic efficacy of drugs. Several strategies to improve the water solubility and dissolution rate of drugs have been reported (Gupta *et al.*, 1997; Ambade *et al.*, 2008; Tokumura *et al.*, 2009; Oh *et al.*, 2011; Rudrangi *et al.*, 2015a; Rudrangi *et al.*, 2015b). One of the most promising approaches for enhancing the bioavailability of poorly water soluble drugs is the inclusion complexation with cyclodextrins.

Cyclodextrins are a family of non-reducing cyclic oligosaccharides composed of (α-1,4)-linked α-D-glucopyranose units. They possess a hydrophobic cavity and a hydrophilic exterior (Kfoury *et al.*, 2015). The hydrophobic cavity accommodates a poorly water soluble drug, whilst the hydrophilic exterior facilitates high water solubility. Cyclodextrins have been reported to enhance the solubility and dissolution rate of several poorly soluble drugs (Jain and Adeyeye, 2001; Bandi *et al.*, 2004; Cirri *et al.*, 2005; Vega *et al.*, 2013; Rudrangi *et al.*, 2015a; Rudrangi *et al.*, 2015b).

Flurbiprofen [2-(2-fluoro-4-biphenylyl)propionic acid] (Figure A1) is a chiral non-steroidal anti-inflammatory drug approved by the Food and Drug Administration for the treatment of rheumatoid arthritis and osteoarthritis (Davies, 1995). It also has profound analgesic effects and is indicated in the treatment of gingivitis and alveolar bone resorption in periodontitis (Heasman, P.A. *et al.*, 1990). However, it has poor water solubility (5-13 µg mL^{-1}) and

dissolution rate (Anderson and Conradi, 1985) which limits both its therapeutic application and efficacy. Moreover, the drug suffers from a short terminal phase elimination half-life (3-6 h) and therefore requires frequent dosing (Heyneman *et al.*, 2000). Hence, there is a need to enhance the water solubility and dissolution rate of flurbiprofen.

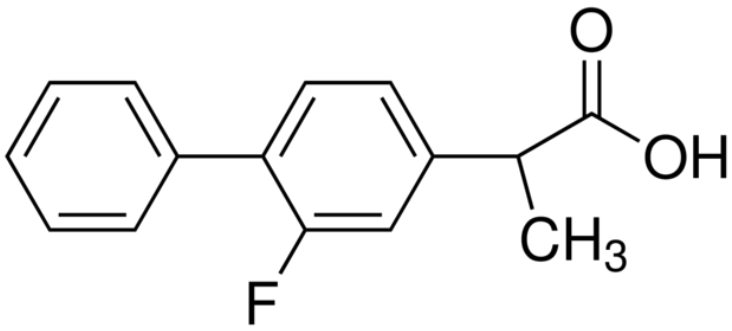


Figure A1. Structure of flurbiprofen

Several formulations that include dry elixir (Kim and Yoon, 1995), solid dispersion (Habib *et al.*, 1998; Oh *et al.*, 2011), salt formation (Gupta *et al.*, 1997), microemulsion (Park *et al.*, 1997; Ambade *et al.*, 2008), inclusion complex with cyclodextrins (Tokumura *et al.*, 2009; Li *et al.*, 2010) and cycloamyloses (Baek *et al.*, 2011) have been reported to improve the solubility and dissolution rate of flurbiprofen.

Flurbiprofen has an affinity for different cyclodextrins forming inclusion complexes. The favourable effect of natural β-cyclodextrin (Muraoka *et al.*, 2004; Cirri *et al.*, 2005; Tokumura *et al.*, 2009; Li *et al.*, 2010; Baek *et al.*, 2011) and its derivatives, such as hydroxypropyl-β-cyclodextrin (Govindarajan and Nagarsenker, 2005; Vega *et al.*, 2013), hydroxyethyl-β-cyclodextrin and methyl-β-cyclodextrin (Cirri *et al.*, 2005) on its pharmaceutical properties has been previously reported.

The study published by Cirri *et al.* (2005) reported that flurbiprofen and methyl-β-cyclodextrin complexes prepared in the 1:1 molar ratio using kneading and co-evaporation

techniques exhibit higher solubility and dissolution profiles than flurbiprofen alone or in a state of physical mixture. In spite of their success, the complexes prepared by the co-evaporation and kneading methods required an organic solvent. Organic solvents are known for their high dissolving properties; however, they are perilous and their presence in the final product (complexes) is highly undesirable and often requires energy-intensive drying steps involving great deal of time to remove the residual solvent (Al-Marzouqi *et al*., 2007; Al-Marzouqi *et al*., 2009). Hence, there is a need to avoid the use of organic solvents in the preparation of drug-cyclodextrin complexes.

Pharmaceutical scientists relentlessly try to avoid the usage of toxic organic solvents and exchange those to more environmentally friendly alternatives with similar properties. One of those effective alternatives is supercritical fluids. A supercritical fluid is a substance that exists above its critical point, where the phase boundaries diminish. Carbon dioxide is the most commonly employed supercritical fluid because of its low critical parameters. Supercritical carbon dioxide (SC-CO_2) has provided an excellent green alternative to harmful organic solvents and the use of supercritical carbon dioxide processing technique in the preparation of drug-cyclodextrin complexes has been reported (York, 1999; Kompella and Koushik, 2001; Sunkara and Kompella, 2002; Türk *et al*., 2007; Lee *et al*., 2008; Rudrangi *et al*., 2015a; Rudrangi *et al*., 2015b).

The application of supercritical carbon dioxide processing in the preparation of flurbiprofen-methyl-β-cyclodextrin complexes has not yet been reported. The aim of this study was to evaluate supercritical fluid technology as a preparative technique for the preparation of flurbiprofen-methyl-β-cyclodextrin inclusion complexes in solid state. Inclusion complexes were prepared by physical mixing and supercritical carbon dioxide processing at various working (temperature and pressure) conditions. The prepared complexes were then characterized by solubility studies, differential scanning calorimetry (DSC), X-ray powder

diffraction (XRD), scanning electron microscopy (SEM), practical yield, drug content evaluation and dissolution studies.

2. Materials and methods

2.1. Materials

Flurbiprofen (≥ 98.5%, molecular weight: 244.26) and methyl-β-cyclodextrin (average molecular weight: 1310, extent of labeling: 1.6-2.0 mol CH_3 per unit anhydroglucose) were purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Ethanol was obtained from Fisher Scientific (Loughborough, UK). Carbon dioxide (99.9%) was obtained from BOC Ltd (Guildford, Surrey, UK). All reagents were used as received.

2.2. Preparation of Binary Mixtures of Flurbiprofen with Methyl-β-Cyclodextrin

All binary mixtures were prepared in a 1:1 molar ratio of flurbiprofen to methyl-β-cyclodextrin. The processed samples were stored in desiccators until further use.

2.2.1. Physical Mixing

Required quantities of flurbiprofen and methyl-β-cyclodextrin were accurately weighed and tumble-mixed at 100 rpm for 15 min using a TURBULA® T2F mixer (Willy A. Bachofen AG – Maschinenfabrik, Muttenz, Switzerland).

2.2.2. Supercritical Carbon Dioxide Process

The supercritical carbon dioxide process was carried out in the static mode to achieve the inclusion of flurbiprofen in the methyl-β-cyclodextrin. The complexes were prepared using an extraction apparatus supplied by Thar Process Inc., USA.

The physical mixtures of flurbiprofen and methyl-β-cyclodextrin were placed in a sample cell as shown in the Figure A2. Carbon dioxide was pumped from a cylinder *via* a cooling unit into the sample cell. The physical mixtures were processed at six different working conditions [35°C/100 bar, 35°C/150 bar, 35°C/200 bar, 45°C/100 bar, 45°C/150 bar and 45°C/200 bar] in order to study the influence of temperature and pressure on the inclusion complex formation.

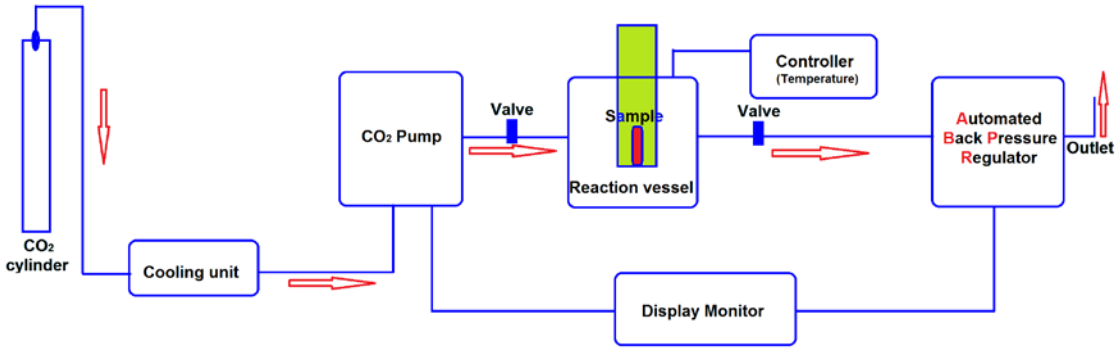


Figure A2. Schematics of supercritical carbon dioxide processing

The desired pressure was achieved by pumping carbon dioxide against an automated back pressure regulator (ABPR). The sample cell in the reaction vessel was heated to the desired temperature and held for 1 h before recovering the solid complex by depressurisation at a rate of 7-8 bar min^{-1}. The product was then homogenized prior to further use.

2.3. Analysis of the Prepared Binary Mixtures

Having prepared these model complexes by supercritical carbon dioxide processing, the next aim is to compare the physical properties with those of complexes prepared using physical mixing.

2.3.1. Solubility Studies

Saturation solubility of flurbiprofen was measured in triplicate by adding excess amounts of the drug to 10 mL of deionised water in sealed glass containers. The solutions were agitated

for 72 hours at 25°C. The solutions were then filtered (0.45 μm filter pore size) and assayed for drug concentration by ultra-violet spectroscopy (Cary 100 UV-vis, Agilent Technologies, USA) after dilution.

Phase solubility diagram was obtained according to the method reported by Higuchi and Connors (1965). Samples were prepared by adding excess flurbiprofen (in amount above its solubility), to 10 ml deionised water containing successively increasing concentrations (0, 2.5, 5, 10, 12.5, 15, 20 and 25×10^{-3} M) of methyl-β-cyclodextrin, in sealed glass containers. The solutions were agitated (200 rpm) at 25°C for 72 hours. Following equilibrium, the solutions were then filtered and assayed for drug concentration by ultra-violet spectroscopy as described above. Phase-solubility diagram was represented as the concentration of total dissolved flurbiprofen against the concentration of methyl-β-cyclodextrin. The binding constant ($K_{1:1}$) for flurbiprofen-methyl-β-cyclodextrin complexes was calculated from the slope of the curve.

2.3.2. Differential Scanning Calorimetry (DSC)

DSC analysis of flurbiprofen, Me-β-CD and the flurbiprofen-methyl-β-cyclodextrin complex systems was carried out using a FP-90 central processor (Mettler-Toledo, LLC, UK). The instrument was calibrated using indium as reference material. For each sample 5 mg was accurately weighed and hermetically sealed in aluminium pans and heated at a rate of 10°C min^{-1}. The thermogram was collected over the temperature range from 50 to 150°C. A control study was carried out to investigate the effect of supercritical carbon dioxide processing on flurbiprofen alone.

2.3.3. X-Ray Powder Diffraction (XRPD)

X-ray powder diffractograms of flurbiprofen, methyl-β-cyclodextrin and the flurbiprofen-methyl-β-cyclodextrin complex systems were obtained on a D8 Advance X-ray Diffractometer (Bruker, Germany) in theta–theta Bragg–Brentano geometry using reflection

mode. Data were collected between 2-40° 2θ, with a step size of 0.006o and a time of 0.5 s per step using Cu Kα radiation. A control study was carried out to investigate the effect of supercritical carbon dioxide processing on flurbiprofen alone.

The degree of crystallinity (% crystallinity) of the flurbiprofen-methyl-β-cyclodextrin complex systems was determined using the amorphous subtraction method as reported in our earlier studies (Rudrangi *et al.*, 2015a).

2.3.4. Scanning Electron Microscopy (SEM) Analysis

The surface morphology of flurbiprofen, methyl-β-cyclodextrin and the flurbiprofen-methyl-β-cyclodextrin complex systems was examined by means of Hitachi SU-8030 scanning electron microscope (Tokyo, Japan). The powder samples were securely fixed on an aluminium stub using double-sided adhesive tape and then were made electrically conductive by coating in vacuum with a thin layer of chromium (~300 Å) at 30 W for 30 seconds. The photomicrographs were taken at an excitation voltage of 2.0 kV and a magnification of ×350.

2.3.5. Practical Yield

The efficiency of a method used for the preparation of inclusion complexes can be measured by determining the practical yield of the binary systems. Percentage practical yield of the binary systems was determined by the following equation:

$$\text{Practical yield (\%)} = \left[\frac{\text{Practical weight of the binary mixture}}{\text{Theoretical weight (Drug + Cyclodextrin)}}\right] * 100$$

2.3.6. Evaluation of the Flurbiprofen Content

Flurbiprofen-methyl-β-cyclodextrin binary system, equivalent to 10 mg of the drug, was accurately weighed and dispersed in ethanol (10 mL) in which both the drug and cyclodextrin are soluble. The mixture was agitated (100 rpm) at 25 ± 0.5°C for 30 min and the resultant solution was filtered (0.45 μm filter pore size) and assayed for the concentration of total

flurbiprofen by ultra-violet spectroscopy after dilution. Determination of the flurbiprofen content in the complexes allows the verification that there is no loss of flurbiprofen during the (complex) preparation process.

2.3.7. *In Vitro* Dissolution Studies

In Vitro dissolution studies of flurbiprofen from all drug-carrier binary systems, and for flurbiprofen alone, were conducted in triplicate using USP Type II paddle method (Hanson G2 Vision® Classic 6, Chatsworth, CA) with deionised water as the medium. The samples, corresponding to 1000 mg of flurbiprofen were dispersed into 900 ml of the dissolution media maintained at 25 $\pm$ 0.5°C and stirred at 100 rpm. 5ml aliquots were withdrawn and filtered (0.45 µm filter pore size) at the specified time intervals (2, 5, 10, 20, 30 and 60 min) and the drug concentration was determined by UV spectroscopy at λ_{max} = 246 nm. The same volume of fresh medium was added to the beaker and the correction for the cumulative dilution was calculated. The parameters used to characterize the dissolution curves were the percentage of flurbiprofen dissolved at 30 min, and the dissolution efficiency at 30 and 60 min. Dissolution efficiency was calculated according to Khan (1975).

2.3.8. Molecular Docking Studies

Molecular docking studies of flurbiprofen into methyl-β-cyclodextrin were conducted using Glide (grid-based ligand docking) application implemented in the Maestro 9.3 software package (Schrodinger, LLC, New York, USA, 2012) as reported earlier (Rudrangi et al., 2015a). Docked flurbiprofen and methyl-β-cyclodextrin complexes were visualised and molecular surface complex pictures were generated using Maestro.

3. Results and Discussion

3.1. Solubility Studies

Aqueous solubility of flurbiprofen at 25°C was found to be 12.29 ± 1.05 µg mL^{-1} and 30.68 ± 0.5 µg mL^{-1} at the end of 1 h and 24 h, respectively. No further improvement was observed in the drug solubility after 24 h.

Cirri *et al.* (2005), Li *et al*. (2010) and Baek *et al*. (2011) reported a drug solubility of 40 µg mL^{-1}, 10.45 ± 3.22 µg mL^{-1} and 5.12 ± 1.22 µg mL^{-1} in water at 25°C after 24 h, 120 h and 168 h, respectively. Herzfeldt and Kummel (1983) reported a drug solubility of 8 µg mL^{-1} and 31.2 µg mL^{-1} in pH 1.2 and 7.5 buffered solutions at 25°C after 24 h, respectively. Varma and Pandi (2005) reported a drug solubility of 48.2 µg mL^{-1} in water at 27°C after 72 h.

Phase solubility diagram of flurbiprofen with various concentrations of methyl-β-cyclodextrin in deionised water is presented in Figure A3.

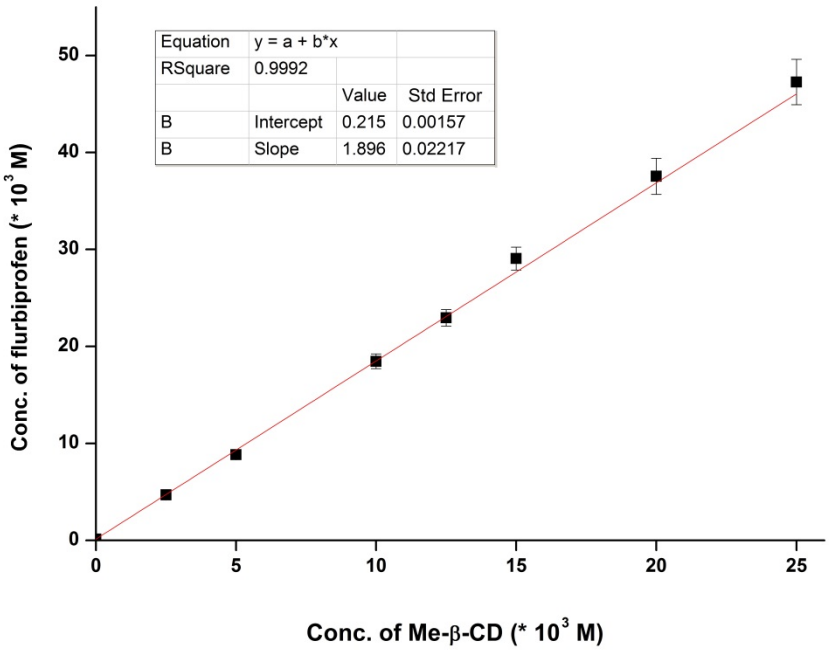


Figure A3. Phase solubility studies of flurbiprofen with increasing concentrations of methyl-β-cyclodextrin at 25°C and in deionised water.

The solubility of flurbiprofen increased in a linear fashion with the concentration of methyl-β-cyclodextrin, forming A_L-subtype complexes (presumably 1:1 stoichiometry). The binding constant of the complexes ($K_{1:1}$ = 16856 ± 1244 M^{-1}) was calculated from the slope of the curve.

Similar phase solubility profile was reported for flurbiprofen-methyl-β-cyclodextrin complexes in water by Cirri *et al*. (2005). The authors reported the stability constant values of $K_{1:1}$ = 11570 M^{-1} and 9660 M^{-1} in unbuffered water (pH ≈ 4.5) at 25°C and 37°C, respectively and the values of $K_{1:1}$ = 1480 M^{-1} and 1085 M^{-1} in phosphate buffer (pH 5.5) at 25°C and 37°C, respectively.

Rudrangi and co-workers carried out the phase solubility studies of methyl-β-cyclodextrin with indomethacin and olanzapine in phosphate buffer medium (pH 7.4) and de-ionised water (pH 7.1) at 37°C, respectively. Rudrangi *et al.* (2015a) reported an A_N-subtype phase solubility diagram with a stability constant of $K_{1:1}$ = 167 M^{-1} for the indomethacin-methyl-β-cyclodextrin complexes while Rudrangi *et al.* (2015b) reported an A_L-subtype phase solubility diagram with a stability constant of $K_{1:1}$ = 304 M^{-1} for the olanzapine-methyl-β-cyclodextrin complexes.

3.2. Differential Scanning Calorimetry Analysis

Figure A4a presents the DSC thermograms of flurbiprofen (pure) and flurbiprofen processed with supercritical carbon dioxide at 45°C/200 bar. Flurbiprofen (pure and supercritical carbon dioxide processed) exhibited a sharp melting endotherm at 118.26°C and 118.27°C, respectively confirming the crystalline nature of the starting material. DSC analysis indicates that supercritical carbon dioxide process has not altered the crystallinity of the drug. Similar results were reported by Rudrangi *et al.* (2015a and b) for indomethacin and olanzapine when processed with supercritical carbon dioxide.

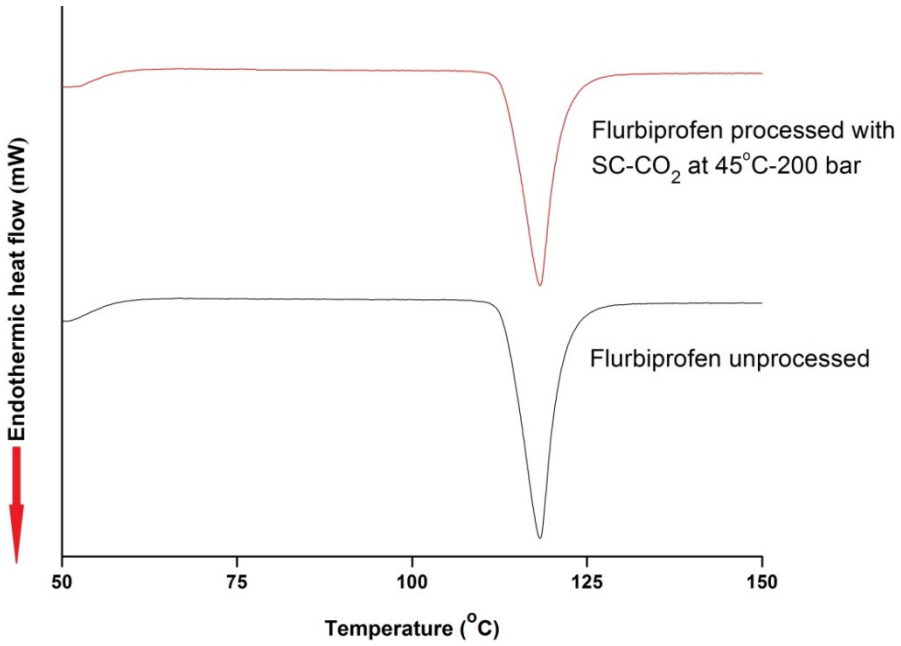


Figure A4a. Control study: DSC thermograms of flurbiprofen (unprocessed) and flurbiprofen processed with supercritical carbon dioxide at 45°C/200 bar.

DSC thermograms of flurbiprofen, methyl-β-cyclodextrin and flurbiprofen-methyl-β-cyclodextrin binary systems prepared by physical mixing and supercritical carbon dioxide processing are presented in Figure A4b. Methyl-β-cyclodextrin was characterized by a broad endothermic event between 60°C and 120°C ascribed to its dehydration as explained by Banchero *et al.* (2013) and Rudrangi *et al.* (2015a).

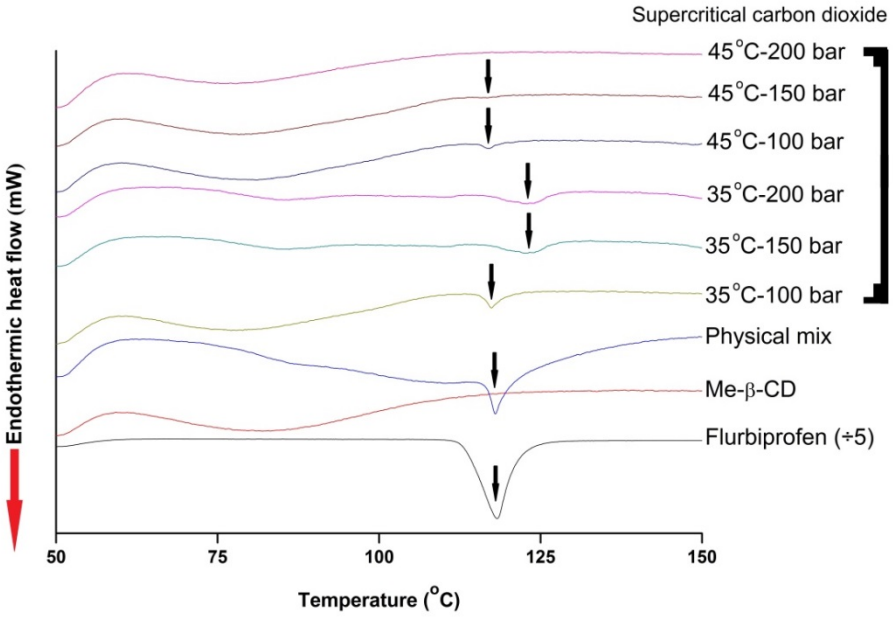


Figure A4b. DSC thermograms of flurbiprofen, methyl-β-cyclodextrin and flurbiprofen–methyl-β-cyclodextrin (1:1 molar) binary systems prepared by physical mixing and supercritical carbon dioxide processing (the flurbiprofen thermogram has been reduced by a factor of 5).

Thermograms of the binary mixtures prepared by physical mixing and supercritical carbon dioxide processing at 35°C/100 bar, 35°C/150 bar, 35°C/200 bar, 45°C/100 bar and 45°C/150 bar showed an endothermic peak of reduced intensity (as represented by arrows) corresponding to pure flurbiprofen. The reduced intensity of drug endothermic peak suggests an incomplete inclusion of flurbiprofen in the cavity of methyl-β-cyclodextrin. The binary mixtures prepared by supercritical carbon dioxide processing at 35°C/150 bar and 35°C/200 bar showed a broad endothermic peak of drug shifted to higher temperatures. Marques *et al.* (1990) have explained that the shift of the drug endothermic peak to a higher temperature may be attributed to the partial inclusion of the drug into cyclodextrin.

A complete disappearance of the flurbiprofen melt peak was observed in the inclusion complexes prepared by supercritical carbon dioxide processing at 45°C/200 bar indicating interactions between flurbiprofen and methyl-β-cyclodextrin and the formation of inclusion complex with loss of crystallinity as suggested by Charoenchaitrakool *et al.* (2002) and Rudrangi *et al.* (2015a and 2015b).

3.3. X-Ray Powder Diffraction Analysis

Figure A5a presents the X-ray powder diffractograms of flurbiprofen (pure) and flurbiprofen processed with supercritical carbon dioxide at 45°C/200 bar.

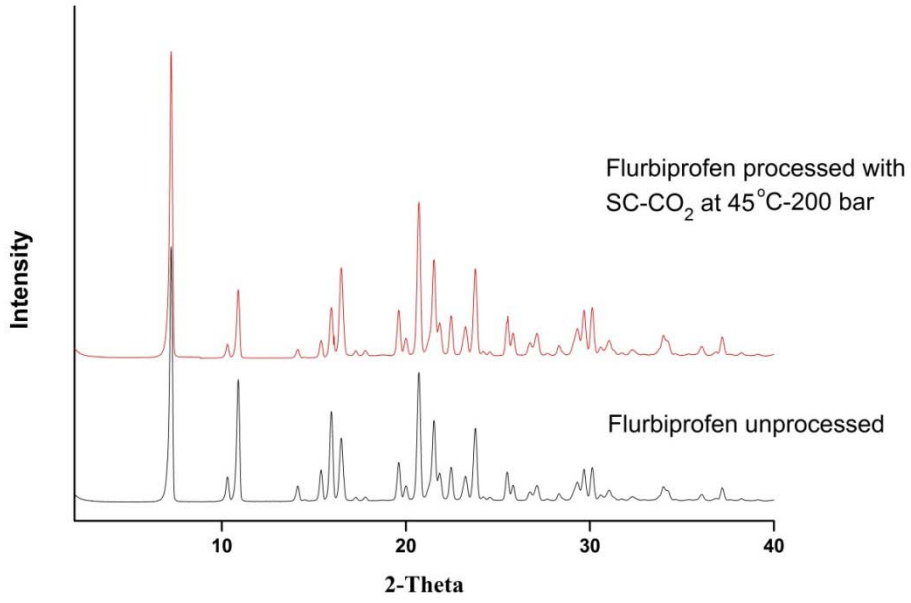


Figure A5a. Control study: X-ray powder diffractograms of flurbiprofen (unprocessed) and flurbiprofen processed with supercritical carbon dioxide at 45°C/200 bar.

The diffraction patterns of flurbiprofen (pure and supercritical carbon dioxide processed) showed sharp characteristic peaks at 2θ equal to 7.25°, 10.30°, 10.89°, 14.11°, 15.40°, 15.94°, 16.49°, 19.60°, 20.71°, 21.52°, 21.83°, 22.46°, 23.23°, 23.78°, 25.51°, 25.82°, 26.75°, 27.12°, 29.32°, 29.69°, 30.12°, 34.01° and 37.21° confirming the crystalline nature of the starting material. XRPD analysis indicates that supercritical carbon dioxide process has not altered the crystallinity of the drug in agreement with DSC analysis. Similar results were reported by Rudrangi *et al.* (2015a and b) for indomethacin and olanzapine when processed with supercritical carbon dioxide.

Figure A5b presents the X-ray powder diffractograms of flurbiprofen, methyl-β-cyclodextrin and flurbiprofen-methyl-β-cyclodextrin binary systems prepared by physical mixing and supercritical carbon dioxide processing at various working conditions. The diffraction pattern of methyl-β-cyclodextrin displayed two broad features at 11° and 18° indicating its amorphous nature.

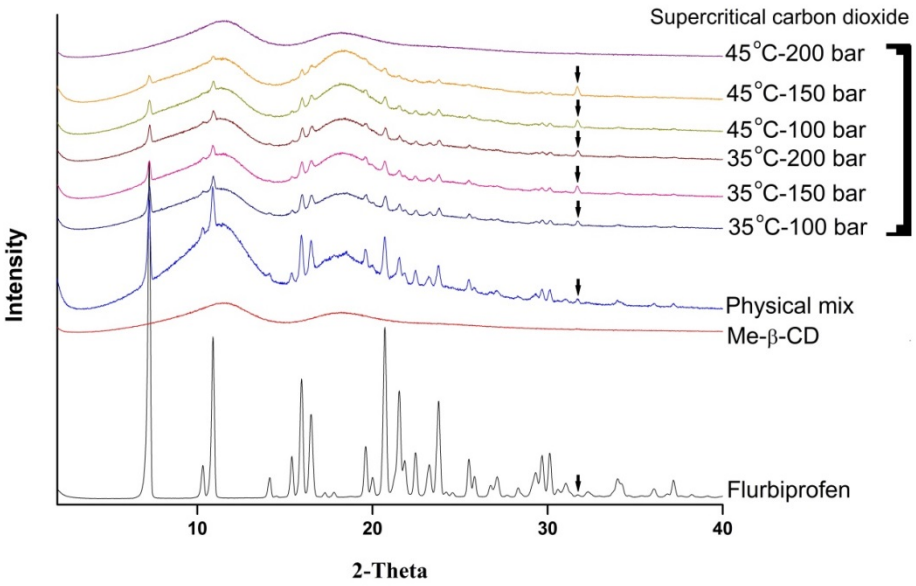


Figure A5b. X-ray powder diffractograms of flurbiprofen, methyl-β-cyclodextrin and flurbiprofen-methyl-β-cyclodextrin binary systems prepared by physical mixing and supercritical carbon dioxide processing.

The physical mixture of flurbiprofen and methyl-β-cyclodextrin showed a similar diffraction pattern to that of the respective individual components but with a reduction in the intensity of drug peaks. The presence of sharp peaks in the diffraction pattern of the physical mixture indicates the retention of the crystalline structure of flurbiprofen. In contrast, the complexes prepared by supercritical carbon dioxide processing at 35°C/100 bar, 35°C/150 bar, 35°C/200 bar, 45°C/100 bar and 45°C/150 bar exhibited considerable diminution of the diffraction peaks in agreement with the DSC results, suggesting the solid state interactions between drug and cyclodextrin under these processing conditions. A close analysis of the diffraction patterns of these samples and their comparison with that of the pure flurbiprofen showed an increase in the intensity of the drug peak at 2θ equal to 31.73°. This change could indicate a partial recrystallization of the drug during supercritical carbon dioxide processing or the formation of the inclusion complex between flurbiprofen and methyl-β-cyclodextrin as suggested by He and Li (2009) in the case of borneol and methyl-β-cyclodextrin complexes.

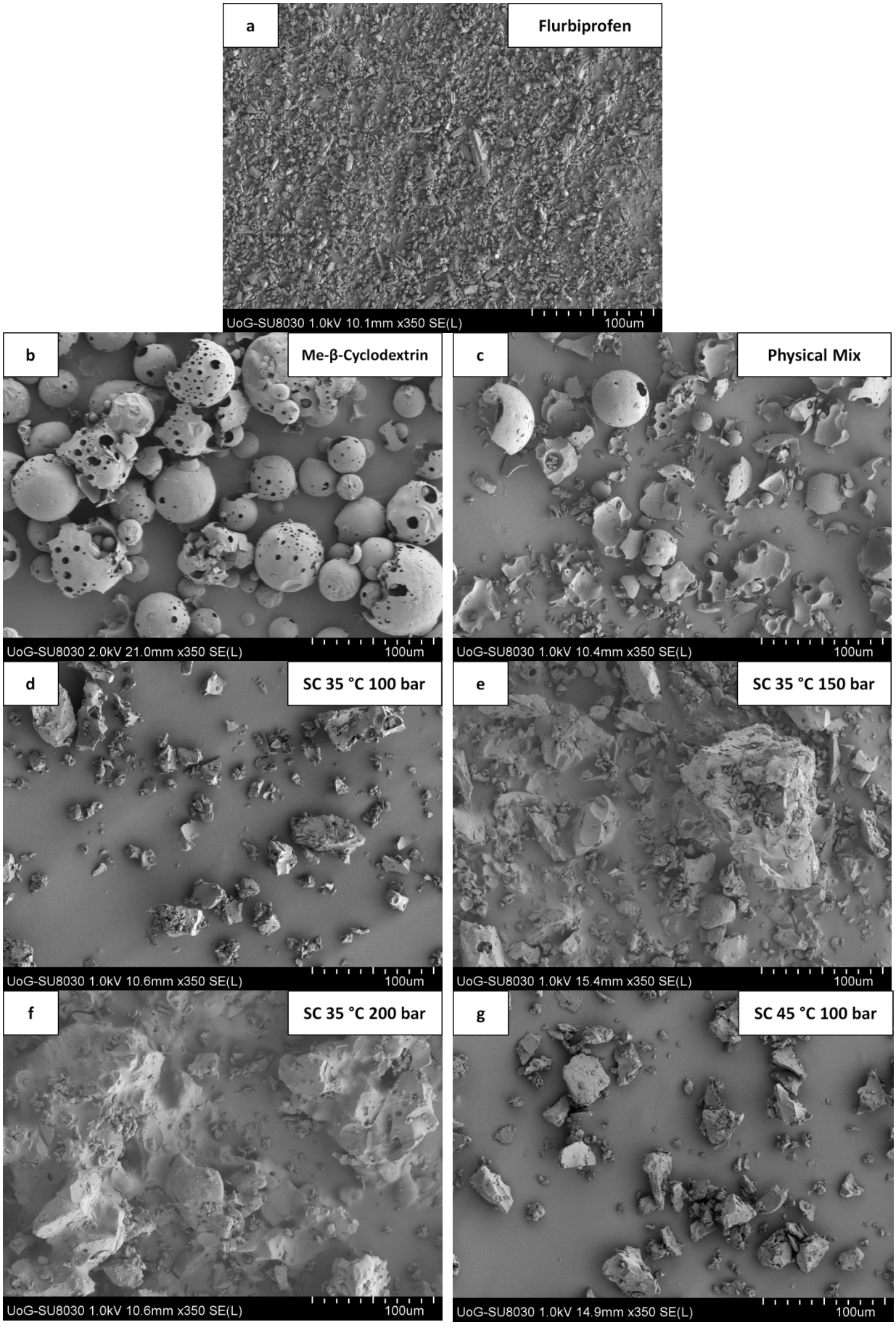
A complete disappearance of the drug peaks but the retention of two broad features similar to that of the pure methyl-β-cyclodextrin was observed in the diffraction pattern of the complexes prepared by supercritical carbon dioxide processing at 45°C/200 bar suggesting a complete complexation between flurbiprofen and methyl-β-cyclodextrin.

The decrease in the degree of crystallinity of the drug was observed in the following order: supercritical carbon dioxide processing at 45°C/200 bar (0% crystalline) > 45°C/150 bar (1.40% crystalline) > 45°C/100 bar (3.09% crystalline) > 35°C/200 bar (3.49% crystalline) > 35°C/150 bar (3.64% crystalline) > 35°C/100 bar (4.69% crystalline) > Physical mixing (8.99% crystalline).

3.4. Scanning Electron Microscopy Analysis

Figure A6 presents the photomicrographs of flurbiprofen, methyl-β-cyclodextrin and flurbiprofen-methyl-β-cyclodextrin binary systems prepared by physical mixing and supercritical carbon dioxide processing.

From SEM analysis, pure flurbiprofen (Figure A6a) appeared as small (8µm) to large (40 µm) columnar crystals with a smooth surface. Pure methyl-β-cyclodextrin (Figure A6b) appeared as perforated hollow spheres in the range of 10-90 µm. The product of the physical mixture (Figure A6c) appeared as a mixture of small columnar crystals (10-15 µm) and broken hollow spheres (50-75 µm). Comparable morphology of the complexes prepared by physical mixing and supercritical carbon dioxide processing at 35°C/100 bar, 45°C/100 bar and 45°C/150 bar (Figures A6d, A6g and A6h, respectively) with raw materials suggests that some flurbiprofen-methyl-β-cyclodextrin interaction had taken place in the solid state. In the complexes prepared by supercritical carbon dioxide processing at 35°C/150 bar, 35°C/200 bar and 45°C/200 bar (Figures A6e, A6f and A6i, respectively) the original morphology of the raw materials disappeared, and the products appeared as aggregates with an irregular morphology.


a
Flurbiprofen
UoG-SU8030 1.0kV 10.1mm x350 SE(L)
100um
b
Me-β-Cyclodextrin
UoG-SU8030 2.0kV 21.0mm x350 SE(L)
100um
c
Physical Mix
UoG-SU8030 1.0kV 10.4mm x350 SE(L)
100um
d
SC 35 °C 100 bar
UoG-SU8030 1.0kV 10.6mm x350 SE(L)
100um
e
SC 35 °C 150 bar
UoG-SU8030 1.0kV 15.4mm x350 SE(L)
100um
f
SC 35 °C 200 bar
UoG-SU8030 1.0kV 10.6mm x350 SE(L)
100um
g
SC 45 °C 100 bar
UoG-SU8030 1.0kV 14.9mm x350 SE(L)
100um

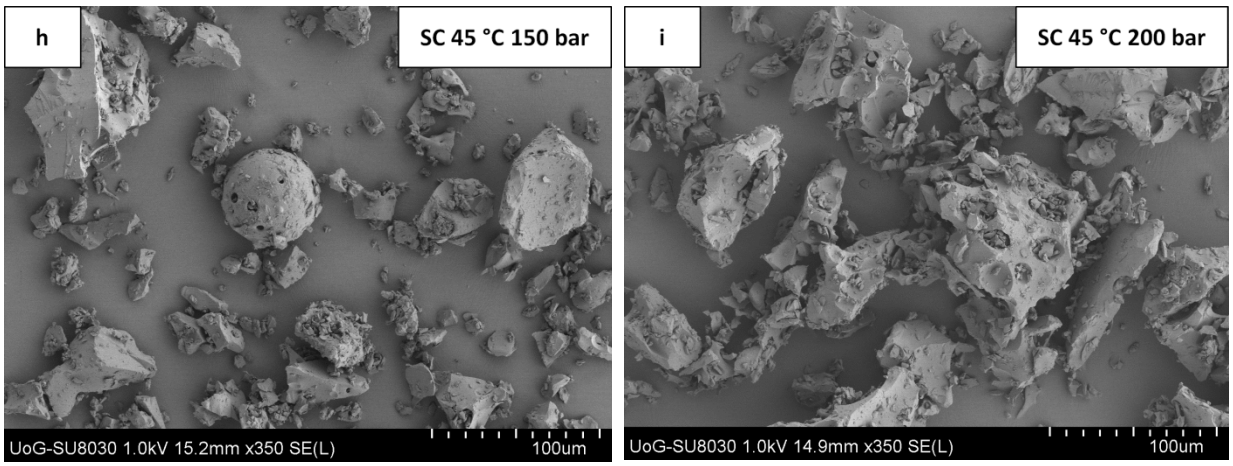


Figure A6. SEM photomicrographs of flurbiprofen (a), methyl-β-cyclodextrin (b) and flurbiprofen-methyl-β-cyclodextrin binary systems prepared by physical mixing (c), supercritical carbon dioxide processing at 35°C/100 bar (d), 35°C/150 bar (e), 35°C/200 bar (f), 45°C/100 bar (g), 45°C/150 bar (h) and 45°C/200 bar (i).

3.5. Practical Yield

The percentage practical yield of all the binary mixtures is tabulated in Table A1. It was observed that all the binary mixtures prepared by supercritical carbon dioxide processing resulted in the loss of mass of the complexes to some extent. The practical yield of the binary mixtures prepared by supercritical carbon dioxide processing ranged from 96.34 ± 0.14% to 98.41 ± 0.56%.

Table A1. Percentage practical yield of flurbiprofen-methyl-β-cyclodextrin binary systems

Method	**Practical yield (%)**
Physical mixing	99.49 ± 0.22
Supercritical carbon dioxide processing	
35°C/100 bar	98.37 ± 0.71
35°C/150 bar	97.76 ± 0.17
35°C/200 bar	98.41 ± 0.56
45°C/100 bar	97.63 ± 0.19
45°C/150 bar	96.34 ± 0.14
45°C/200 bar	97.89 ± 0.82

3.6. Evaluation of the Flurbiprofen Content

Flurbiprofen-methyl-β-cyclodextrin binary systems were subjected for evaluation of drug content in the binary systems and the data obtained are shown in Table A2. The percentage drug content the binary mixtures ranged from 94.59 ± 0.31% to 97.88 ± 0.18%.

Table A2. Drug content in flurbiprofen-methyl-β-cyclodextrin binary systems

Method	**Practical yield (%)**
Physical mixing	94.59 ± 0.31
Supercritical carbon dioxide processing	
35°C/100 bar	97.88 ± 0.18
35°C/150 bar	96.62 ± 0.21
35°C/200 bar	95.51 ± 0.37
45°C/100 bar	95.63 ± 0.42
45°C/150 bar	98.34 ± 0.16
45°C/200 bar	96.58 ± 0.63

3.7. Dissolution Studies

The mean dissolution curves of flurbiprofen from drug alone and from various binary systems with methyl-β-cyclodextrin are presented in Figure A7. All the binary systems exhibited better dissolution profiles than the drug alone. This enhancement may be attributed to the reduction of the crystallinity of the binary mixtures, the surfactant-like properties of cyclodextrin (Lin, and Kao, 1989; Guyot *et al*., 1995) and the formation of inclusion complexes between flurbiprofen and methyl-β-cyclodextrin in the solid state. The highest improvement of the drug dissolution properties was obtained with the binary mixture prepared by supercritical carbon dioxide processing at 45°C/200 bar, followed by the mixtures processed at 45°C/150 bar and 45°C/100 bar.

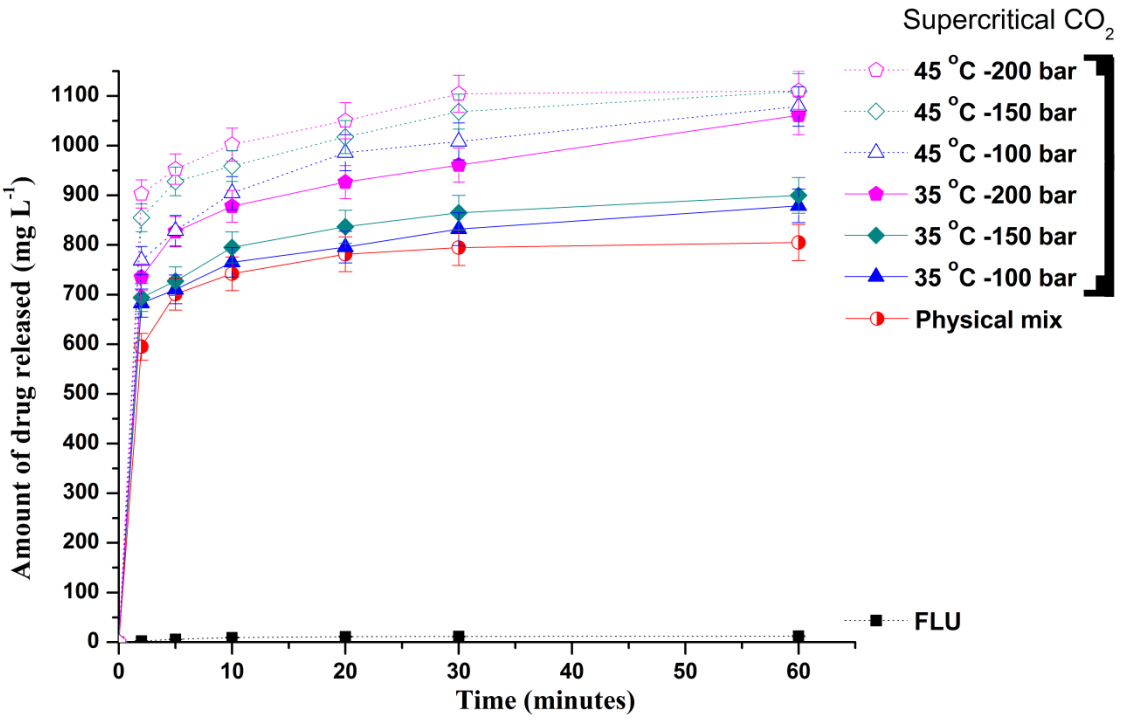


Figure A7. Dissolution rate profiles of flurbiprofen and flurbiprofen-methyl-β-cyclodextrin binary systems prepared by physical mixing and supercritical carbon dioxide processing.

The increase in the dissolution properties of flurbiprofen was observed in the following order: supercritical carbon dioxide processing at 45°C/200 bar > 45°C/150 bar > 45°C/100 bar > 35°C/200 bar > 35°C/150 bar > 35°C/100 bar > Physical mixing.

The results in terms of percent of active ingredient dissolved at 30 min and the dissolution efficiency at 30 min are presented in Table A3. The amount of flurbiprofen released from drug alone was very low with 1.11 ± 0.09% dissolving at the end of 60 min while the binary mixture obtained by physical mixing exhibited a significant increase in the dissolution with 72.47 ± 3.23% of flurbiprofen dissolved at the end of 30 min.

It is also evident from the results (Figure A7 and Table A3) that the degree of the improvement in the dissolution was found to be dependent not only on the method of preparation but also the processing conditions used for the preparation of complexes. All the supercritical carbon dioxide processed systems exhibited higher apparent drug dissolution than the physical mixture and the drug alone; however, considerable differences were observed in the degree of enhancement. The binary mixtures prepared by supercritical carbon dioxide processing at 35°C/100 bar exhibited a drug release of 74.88 ± 2.96%, while the mixtures processed at 45°C/200 bar released 99.39 ± 2.34% of the drug at the end of 30 min. All the binary mixtures prepared by supercritical carbon dioxide processing at 45°C exhibited a drug release of more than 80% within the first 10 min irrespective of the pressure employed. Both the temperature and pressure showed a positive influence on the formation of the inclusion complexes in the solid state.

Table A3. Percent flurbiprofen dissolved (DP) at 30 min and dissolution efficiency (DE)[a] at 30 min from flurbiprofen and flurbiprofen-methyl-β-cyclodextrin binary systems prepared by physical mixing and supercritical carbon dioxide processing.

Sample (Method)	**DP_{30}**	**DE_{30}**
Flurbiprofen	1.06 ± 0.09	0.82 ± 0.21
Physical mixing	72.47 ± 3.23	64.85 ± 2.68
Supercritical carbon dioxide processing		
35°C/100 bar	74.88 ± 2.96	65.19 ± 2.84
35°C/150 bar	77.84 ± 3.16	68.24 ± 2.78
35°C/200 bar	86.49 ± 3.11	76.50 ± 2.81
45°C/100 bar	90.78 ± 3.35	80.77 ± 2.98
45°C/150 bar	96.17 ± 3.16	85.53 ± 2.76
45°C/200 bar	99.39 ± 2.34	88.38 ± 2.83

[a]DE_{30} was calculated from the area under the dissolution curve at 30 min and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time of the total amount added.

The instantaneous dissolution properties offered by all the binary mixtures are attributable to improvement in drug wettability, high aqueous solubility (greater than 2000 mg mL^{-1} as reported by Banchero *et al*., 2013) and the superior complexing properties of methyl-β-cyclodextrin as suggested by Mura and co-workers (Cirri *et al*., 2005).

3.8. Docking Studies

Molecular docking studies were conducted to study the possibility of molecular arrangement of inclusion complexes between flurbiprofen and methyl-β-cyclodextrin. The single best pose of flurbiprofen docked in the cavity of methyl-β-cyclodextrin is presented in Figure A8.

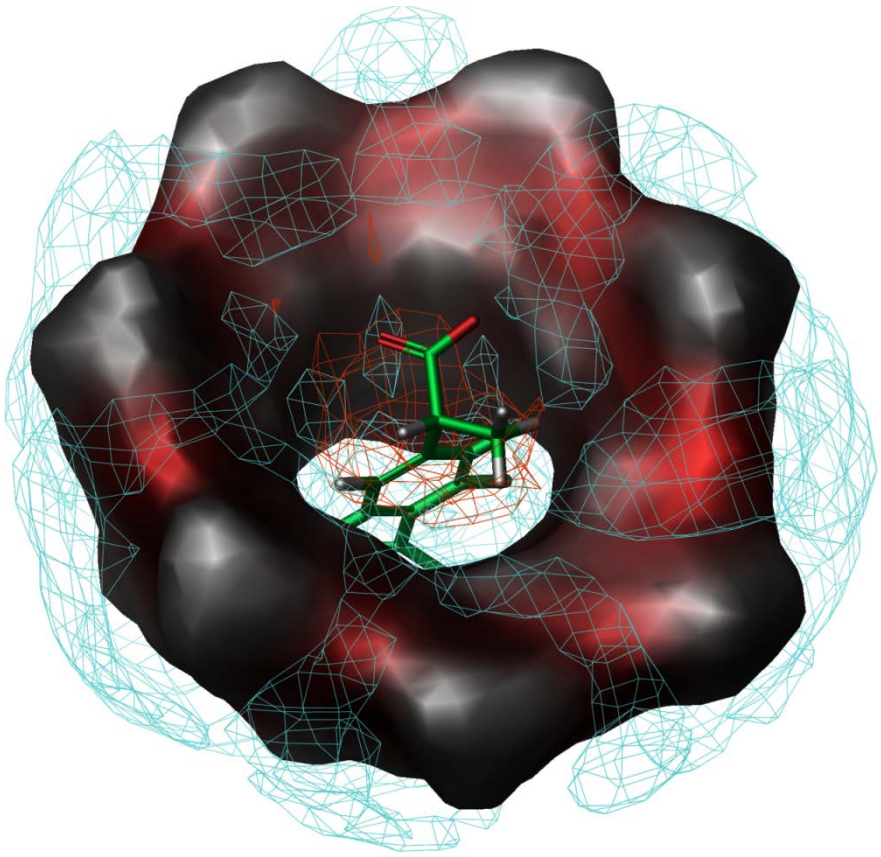


Figure A8. Representation of the complex between methyl-β-cyclodextrin (orange) and flurbiprofen (green) obtained by molecular docking (1:1 stoichiometry).

Flurbiprofen is coloured in green and the figure has a molecular surface (grey with reddish tint in the depths), mesh surface of hydrophilic area (cyan mesh) and the hydrophobic area (orange mesh at the mid depth of methyl-β-cyclodextrin). The figure shows the binding of flurbiprofen in the cavity of methyl-β-cyclodextrin through phenyl group presumably due to its hydrophobicity. Secondary hydrogen bonding exists between the oxygen atom of methyl-β-cyclodextrin and the hydrogen atom of flurbiprofen. The binding affinity (GLIDE energy), van der Waals energy and docking score for inclusion of flurbiprofen in methyl-β-cyclodextrin are -20.660 kcal mol^{-1}, -20.303 kcal mol^{-1} and -4.259 kcal mol^{-1} respectively.

Computational molecular docking studies of indomethacin and olanzapine with methyl-β-cyclodextrin were carried out by Rudrangi and co-workers using the Glide application. The

binding affinity (GLIDE energy), van der Waals energy and docking score for inclusion of indomethacin in methyl-β-cyclodextrin were found to be 27.880 kcal mol^{-1}, -28.941 kcal mol^{-1} and -4.882 kcal mol^{-1}, respectively (Rudrangi *et al.*, 2015a) while the binding affinity, van der Waals energy and docking score for inclusion of olanzapine in methyl-β-cyclodextrin were found to be -24.13 kcal mol^{-1}, -21.57 kcal mol^{-1} and -3.09 kcal mol^{-1}, respectively (Rudrangi *et al.*, 2015b).

4. Conclusions

Solid systems of flurbiprofen with methyl-β-cyclodextrin in an equimolar ratio were prepared by supercritical carbon dioxide processing and compared to products obtained by physical mixing. The results obtained by differential scanning calorimetry, X-ray powder diffraction analysis and dissolution studies suggest complete complexation or amorphisation of flurbiprofen and methyl-β-cyclodextrin binary samples prepared by supercritical carbon dioxide processing. Different degrees of modification (crystallinity and dissolution rate) were observed in the products processed by supercritical carbon dioxide at various processing conditions, suggesting the possibility of flurbiprofen-methyl-β-cyclodextrin interactions of different efficiencies in the solid state. Products obtained by the supercritical carbon dioxide processing exhibited the highest apparent drug dissolution followed by physical mixture and the drug alone. All the products processed by supercritical carbon dioxide at 45°C offered more than 80% drug release within the first 10 min irrespective of the pressure employed. Both the temperature and pressure showed a positive influence on the formation of the inclusion complexes in the solid state, with temperature playing even a higher role. Therefore, a solid inclusion method using supercritical carbon dioxide carrier proved to be a novel and useful complexation method for flurbiprofen into methyl-β-cyclodextrin. Furthermore, since this method has no toxic solvent residue, products obtained by this method should provide minimal side effects in humans, compared to those obtained by techniques, which require the

use of organic solvents. The preliminary data suggests that the complexation of flurbiprofen with methyl-β-cyclodextrin will lead to better therapeutic efficacy.

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APPENDIX

Table A4: List of approved and marketed pharmaceutical products containing cyclodextrins

Drug	CD	Trade name	Formulation	Company	Country
PGE_1	α-CD	Prostavasin	Intra-arterial infusion	Ono/Schwarz	Japan/Germany
PGE_1	α-CD	Viridal/Alprostadil/Edex/Caverject	Intra-cavernous injection	Schwarz	Germany
PGE_1	α-CD	Prostandin 500	Infusion	Ono	Japan
Aceclofenac	β-CD	Aceclofenac-β-Cyclodextrin	Tablet	Taj Pharm.	India
Benexate HCl	β-CD	Ulgut/Lonmiel	Capsule	Teikoku/Shionogi	Japan
Betahistine	β-CD	Betahist	Tablet	Geno Pharm.	India
Cefotiam-hexetil HCl	β-CD	Pansporin-T	Tablet	Takeda	Japan
Cephalosporin (E1207)	β-CD	Meiact	Tablet	Meiji	Japan
Cetirizine	β-CD	Zyrtec	Chewing tablet	Losan Pharma/	Europe/ USA
Cisapride	β-CD	Propulsid	Suppository	Janssen	Europe
Chlordiazepoxide	β-CD	Transillium	Tablet	Gabor	Austria
Dexamethasone	β-CD	Glymesason	Ointment	Fujinaga	Japan
Dextromethorphan	β-CD	Ryndthisol	Tablet	Synthelabo	Italy

Drug	CD	Trade name	Formulation	Company	Country
Diphenydramine	β-CD	Stada-Travel	Chewing tablet	Stada	Germany
Drospirenone/Ethinyl Estradiol/Levomefolate	β-CD	Safyral/Beyaz/Lorina	tablet	Bayer Healthcare/ Sandoz	Europe
Flunarizine	β-CD	Fluner	Tablet	Geno Pharm.	India
Iodine	β-CD	Mena-Gargle	solution	Kyushin	Japan
Meloxicam	β-CD	Mobitil	Tablet, suppository	Med. Union Pharm.	Egypt
Menthol/camphor	β-CD	Pain relief gel	Ointment	MMA Elite/ Doctor Hoy's	USA
Metronidazole	β-CD	Metrogel/Flagyl/Vandazol/Nidagel	Vaginal gel	Curatek/Fougera/Tolmar	USA/Canada
Minoxidil	β-CD	Alopexy	Solution	Pierre Fabre	Europe
Naphasoline HCl	β-CD	Clear eyes	Eye drop	Medtech	South Africa
Nicotine	β-CD	Nicorex/Nicorette	Tablet	Pierre Fabre	Europe
Nimesulide	β-CD	Nimedex	Tablet	Novartis, others	Europe
Nitroglycerin	β-CD	Nitropen	Sublingual tablet	Nihon Kayaku	Japan
Omeprazole	β-CD	Omebeta	Enteric caps.	Betafarm	Germany
PGE_1- OP-1206	γ-CD	Opalmon	Tablet	Ono	Japan
PGE_2	β-CD	Prostarmon E	Sublingual tablets	Ono	Japan
Piroxicam	β-CD	Cycladol/Brexin/Flamexin	Tablet	Chiesi	Europe

Drug	CD	Trade name	Formulation	Company	Country
Piroxicam	β-CD	Cycladol/Pyrodex/Medicam	Tablet	Ranbaxy/Sun/MMC	India
Piroxicam	β-CD	Brexin	Suppositories	Chiesi	Europe
Piroxicam	β-CD	Flogene	Paediatric liquid	Ache Laboratories	UK
Refocoxib	β-CD	Rofizgel	Tablet	Wockhardt	India
Salicylic acid	β-CD	Own Breakout Control	Antiacne Lotion	Own products	USA
Thiomersal	β-CD	Vitaseptol	Eye drop	Europhta	Monaco
Tiaprofenic acid	β-CD	Surgamyl	Tablet	Roussel-Maestrelli	Italy
Cisapride	HP-β-CD	Propulsid	Suppository	Janssen	Europe
Diclofenac	HP-β-CD	Dyolect	I.V. and I.M. Solution	Javelin Pharm.	Europe
Hydrocortisone	HP-β-CD	Dexacort	Mouth wash	Actavis	Europe
Indomethacin	HP-β-CD	Indocid/Indocyllir	Eye drop	Chauvin/Baush & Lomb	Europe
Itraconazole	HP-β-CD	Sporanox	Oral solution/I.V. solution	Janssen	Europe/USA
Perindopril tert.butylamine	HP-β-CD	Perindopril Erbumine	Tablets	Sandoz	Europe
Televancin	HP-β-CD	Vibativ	I.V. solution	Astellas Pharma	Europe
Voriconazole	HP-β-CD	Vorzu	Tablet	Ranbaxy	India
Diclofenac	HP-γ-CD	Voltaren Ophtha	Eye drop	Novartis	Europe

Drug	CD	Trade name	Formulation	Company	Country
Amiodarone	SBE-β-CD	Nexteron	I.V. solution	Hikma	UK
Aripiprazole	SBE-β-CD	Abilify	I.M. solution	BMS/Otuska	USA/Europe
Maropitant	SBE-β-CD	Cerenia	Parenteral solution	Pfizer Animal Health	USA
Voriconazole	SBE-β-CD	Vfend	I.V. solution	Pfizer	USA/Europe
Ziprazidone mesylate	SBE-β-CD	Geodon, Zeldox	I.M. solution	Pfizer	USA/Europe
Chloramphenicol	RM-β-CD	Clorocil	Eye drop solution	Oftalder	Poland
17-β-Estradiol	RM-β-CD	Aerodiol	Nasal spray	Servier	Europe

(*I.V. = Intravenous; I.M. = Intramuscular)

Table A5: Inclusion yield (%) data of econazole base into α-cyclodextrin in supercritical carbon dioxide at various levels of temperature, pressure and contact time.

Temperature (°C)[a]	Inclusion yield (%)			Pressure (Bar)[b]	Inclusion yield (%)	
	after 1 h	after 3 h			after 1 h	after 3 h
40	16.50 ± 3.27	28.99 ± 3.04		100	19.91 ± 2.70	44.84 ± 2.74
45	25.68 ± 3.03	48.09 ± 2.93		125	37.71 ± 3.22	55.76 ± 3.40
50	49.29 ± 3.21	60.67 ± 2.58		150	42.12 ± 4.05	64.24 ± 2.72
55	58.58 ± 4.31	81.31 ± 3.07		175	54.01 ± 3.97	76.95 ± 3.26
60	65.22 ± 5.03	91.44 ± 3.04		200	64.58 ± 4.20	91.44 ± 3.07
65	75.01 ± 5.79	95.26 ± 3.16		225	75.61 ± 4.92	97.44 ± 2.06

[a]The pressure was maintained constant at 200 bar; [b]Temperature was maintained constant at 60°C

Table A6: Inclusion yield (%) data of econazole base into methyl-β-cyclodextrin in supercritical carbon dioxide at various levels of temperature, pressure and contact time.

Temperature (°C)[a]	**Inclusion yield (%) after 1 h**	**Pressure (Bar)[b]**	**Inclusion yield (%) after 1 h**
35	59.59 ± 3.27	100	33.53 ± 2.69
40	80.50 ± 4.55	110	45.89 ± 2.51
45	83.98 ± 3.18	120	58.00 ± 3.18
50	87.24 ± 3.54	130	64.54 ± 3.54
55	91.61 ± 3.95	140	72.01 ± 3.92
60	92.65 ± 3.91	150	80.49 ± 4.32
65	93.42 ± 4.41	160	87.52 ± 3.04
		170	92.18 ± 2.93
		180	94.41 ± 3.18
		190	95.89 ± 3.27
		200	96.12 ± 3.26

[a]The pressure was maintained constant at 150 bar; [b]Temperature was maintained constant at 40°C

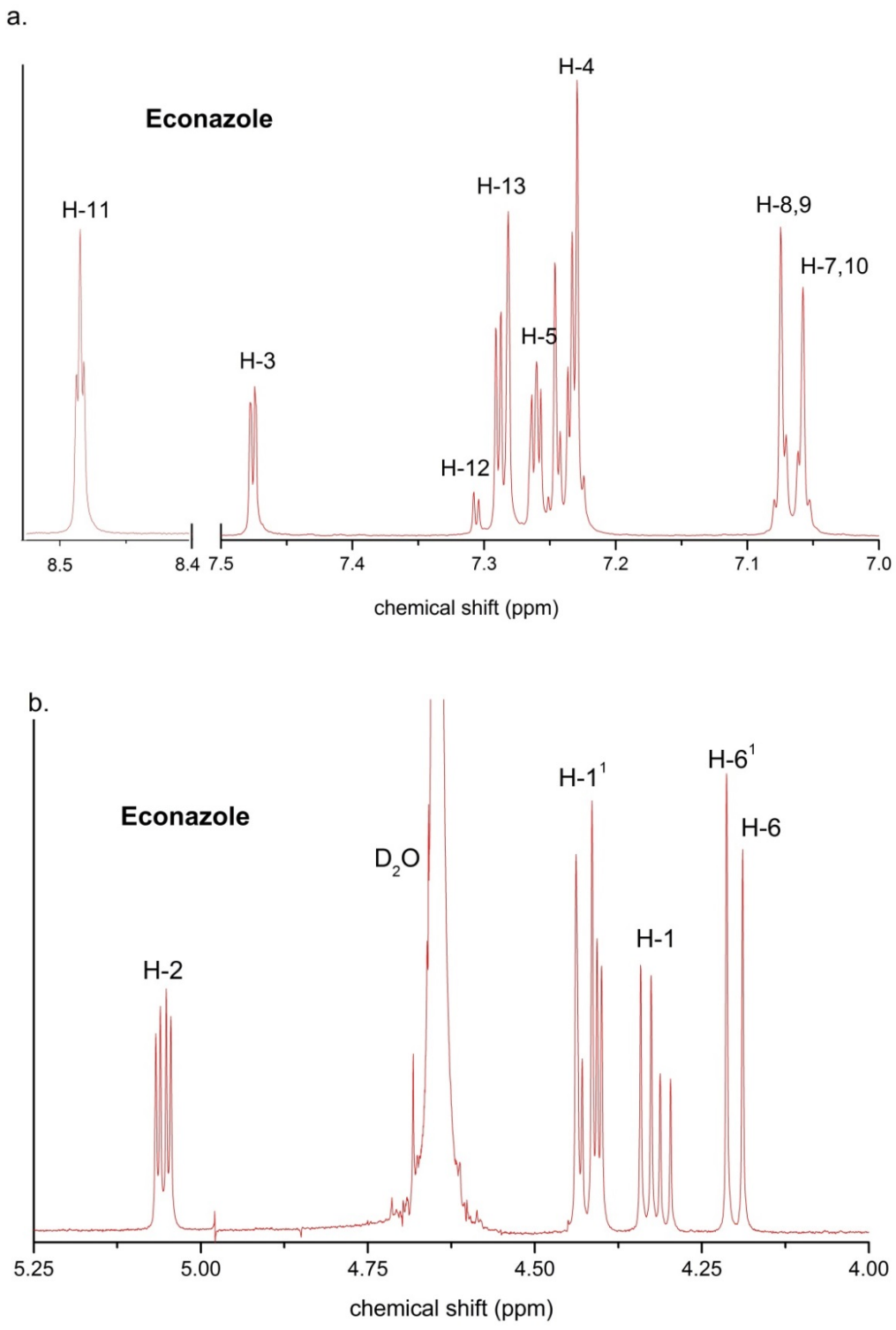

Figure A9: [1]H-nuclear magnetic resonance spectrum of econazole **a.** enlarged from 8.5-7.0 ppm and **b.** enlarged from 5.25-4.0 ppm.

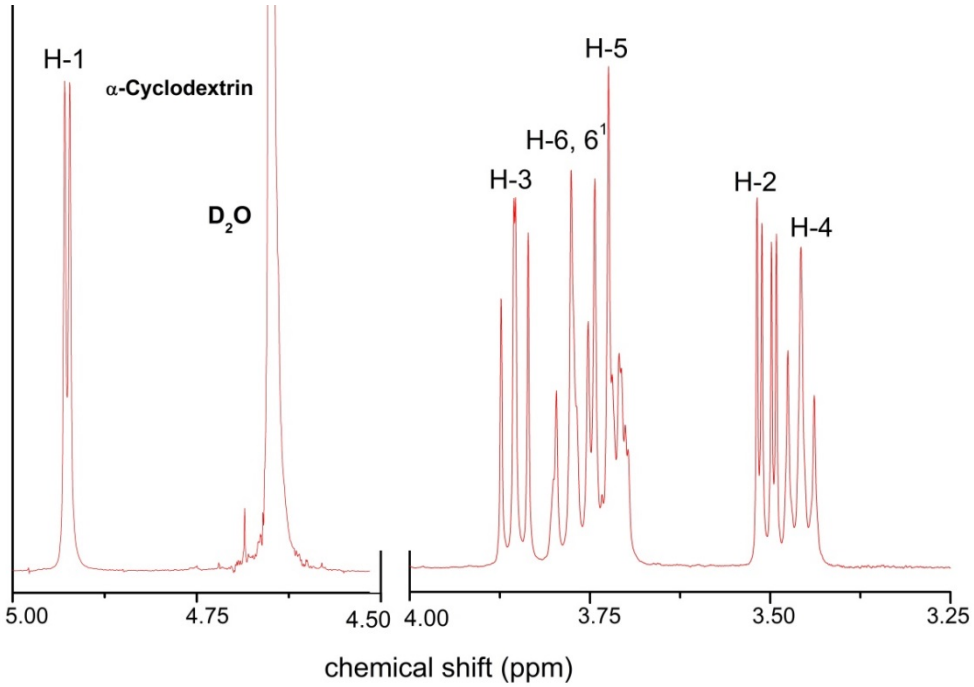

Figure A10: ^{1}H-nuclear magnetic resonance spectrum of α-cyclodextrin.