STABILIZATION OF THE AMORPHOUS FORM OF POORLY SOLUBLE DRUGS USING ETHYL CELLULOSE IN SOLID DISPERSIONS

A Thesis

Presented to the Graduate faculty of the University of the Sciences
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By
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ABSTRACT

The aims of this research was to evaluate the partial crystallinity of two BCS class II drugs (Ketoconazole and Chlorpropamide) when prepared as solid-dispersions with three viscosity grades of Ethyl -Cellulose (EC7, EC45, or EC100 cP). Two processes were explored for preparing the dispersions; namely, spray drying, and, co-precipitation induced by non-solvent addition. The partial crystallinity of the formulations was evaluated using Differential Scanning Calorimetry, with crystal enthalpy values of 103.4 J/G for Ketoconazole and 98.8 J/G for Chlorpropamide (from literature). Both formulation processes yielded free-flowing white powders with a size range of 3 to 49 μm. Control formulations with no EC content showed crystal enthalpies ranging from 83-97%, suggesting practically no amorphous stabilization in the absence of Ethyl Cellulose. The co-precipitation process was ineffective in preparing amorphous formulations for Ketoconazole, as crystal enthalpies ranged from 82-100%. For Chlorpropamide, however, the efficiency of the co-precipitation process improved with the viscosity grade of the Ethyl Cellulose, with EC7, EC45, and EC100 showing % crystalline enthalpies of 98, 76, and 51% respectively. The progressive decrease in crystal enthalpies suggested a corresponding increase in the amorphous forms of these drugs. Spray-dried formulations prepared with EC7 showed practically no crystalline enthalpies for both Ketoconazole and Chlorpropamide, suggesting that these drugs were almost entirely trapped in their amorphous state, and would expect to show higher solubility upon dissolution. Overall, this research shows that spray-drying with low viscosity grade Ethyl Cellulose such as EC7 is an optimal approach for the preparation of amorphous solid-dispersions of BCS Class II drugs.

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This is to certify that the thesis prepared by

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AMORPHOUS STABILIZATION OF POORLY SOLUBLE DRUGS USING ETHYL CELLULOSE SOLID DISPERSIONS AS A STABILIZER

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I Preliminary Section

LIST OF ABBREVIATIONS AND SYMBOLS

ΔH – Change in Enthalpy

BCS – Biopharmaceutics Classification System

cP – Centipoise

Cs – Solubility

DSC – Differential Scanning Calorimetry

EC - Ethyl Cellulose

F MAS NMR – F MAS Nuclear Magnetic Resonance Spectroscopy

FDA – Food and Drug Administration

IR – Infrared

LE – Loading Efficiency

PVP – Polyvinylpyrrolidone

RH – Relative Humidity

SEDDS – Self-Emulsifying Drug Delivery System

SEM – Scanning Electron Microscopy

SLS – Sodium Lauryl Sulfate

T_c – Crystallization Temperature

T_g – Glass Transition Temperature

T_m – Melting Point

XRPD – Xray Powder Diffractometry

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1. INTRODUCTION

The aqueous solubility of drug molecules is an essential prerequisite for drug action following oral administration. For systemic drug action to occur, a solid chemical entity has to first dissolve in bodily fluids, and then partition through biological membranes to reach the site of intended action [1]. For many new chemical entities, however, this constitutes a major problem. Approximately 40% of marketed drugs and 70-90% of drugs in clinical trials are believed to be poorly water-soluble[2, 3].

A small increase in the dissolution rate can also lead to a substantial increases in bioavailability [4]. If the dissolution rates of a drug is substantially slower than its transit rate through the intestines, the process results in low oral bioavailability and an incomplete absorption [5].

1.1 The Biopharmaceutical Classification System

The Biopharmaceutical Classification System (BCS) was introduced in the mid-1990s as a scientific framework to characterize drugs by their aqueous solubility and partition coefficient relative to the therapeutic dose [6]. The classification system is applied in most aspects of formulation design, such as the choice of a dosage form, route of administration, excipient selection, and eventually the final drug dose in the formulation.

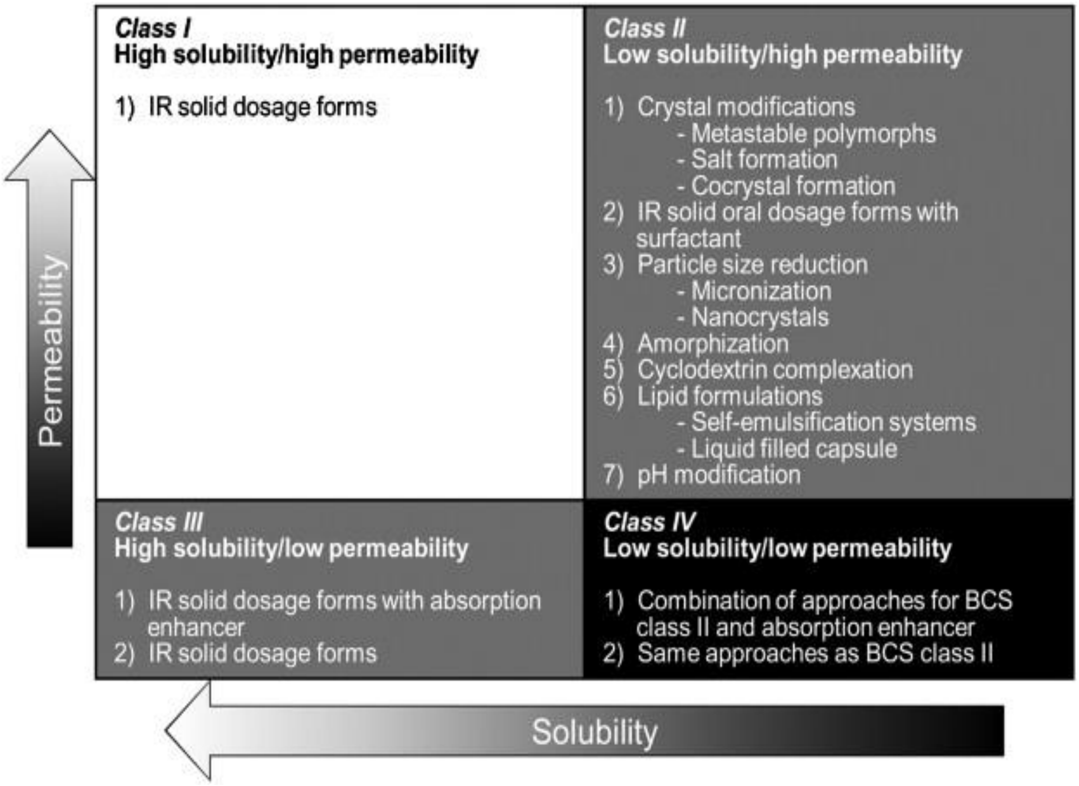
The first major criterion in the BCS classification system is aqueous solubility relative to therapeutic drug dosage. A drug must dissolve in several aqueous biological fluids, including gastrointestinal, extra- and intra-cellular fluids, and blood, prior to reaching its systemic target site. Limited aqueous solubility can result in insufficient drug concentration at the site of action, thereby resulting in lowered or negligible therapeutic effect. As per the Food and Drug Administration (FDA), a drug is considered to be highly soluble if its highest dose strength is readily soluble in 250 ml or less of aqueous media with a pH range of 1-7.5 at 37°C [7]. Also, a drug product is considered to exhibit a rapid dissolution rate when 85% or more of the drug dose dissolves in the dissolution medium within 30 minutes [7]. The second criterion in the BCS system, the partition coefficient, is also a key factor that influences the drug's ability to cross biological membranes. The value of the partition coefficient allows the prediction of *in vivo* pharmacokinetic factors, such as bioavailability. A drug is considered highly permeable when 90% or more of the orally administered dose is absorbed compared to an intravenous reference dose [7].

1.1.1 Categories of BCS compounds:

According to BCS, drugs are classified into four main groups[7].

1. <u>Class I drugs</u> exhibit high permeability and high solubility, and, therefore, do not pose significant challenges to drug formulation or performance of the drug product. Examples of this are Verapamil, Propanolol, Carbamezapine, and Ketoprofen [7].
2. <u>Class II drugs</u> exhibit high permeability but low solubility and are, therefore, the most amenable to amelioration by appropriate formulations [7]. Examples include Danazol, Phenytoin, Ketoconazole, Nifedipine, and Nicardipine.
3. <u>Class III drugs</u> have low permeability and high solubility. These drugs could benefit from drug delivery strategies, such as nanoparticle formulations and absorption enhancement strategies. Examples of Class III drugs are Acyclovir, Captopril, and Cimetidine.
4. <u>Class IV drugs</u> have low permeability and low solubility [7]. Since Class IV drugs come with a host of problems with respect to administration and poor bioavailability,

they have substantial difficulty in reaching the market and are considered to be poor drug candidates. Examples include Taxol, Furosemide, and Tobramycin [7]. Figure 1 shows the four classes in the BCS classification system, along with drug properties and relevant methods for formulation.



Class I **High solubility/high permeability** 1) IR solid dosage forms	***Class II*** **Low solubility/high permeability** 1) Crystal modifications - Metastable polymorphs - Salt formation - Cocrystal formation 2) IR solid oral dosage forms with surfactant 3) Particle size reduction - Micronization - Nanocrystals 4) Amorphization 5) Cyclodextrin complexation 6) Lipid formulations - Self-emulsification systems - Liquid filled capsule 7) pH modification
Class III **High solubility/low permeability** 1) IR solid dosage forms with absorption enhancer 2) IR solid dosage forms	***Class IV*** **Low solubility/low permeability** 1) Combination of approaches for BCS class II and absorption enhancer 2) Same approaches as BCS class II

Figure 1. Categories of the BCS and formulation techniques [8].

1.1.2 BCS Class II (Solubility limiting) drugs

The concentration (C) of a drug in a fluid at any particular time is a function of its solubility (C_s) and the rate of dissolution (dC/dt). This relationship is described by the Noyes-Whitney equation [9]:

$$\frac{dC}{dt} = k(C_s - C) \qquad (1)$$

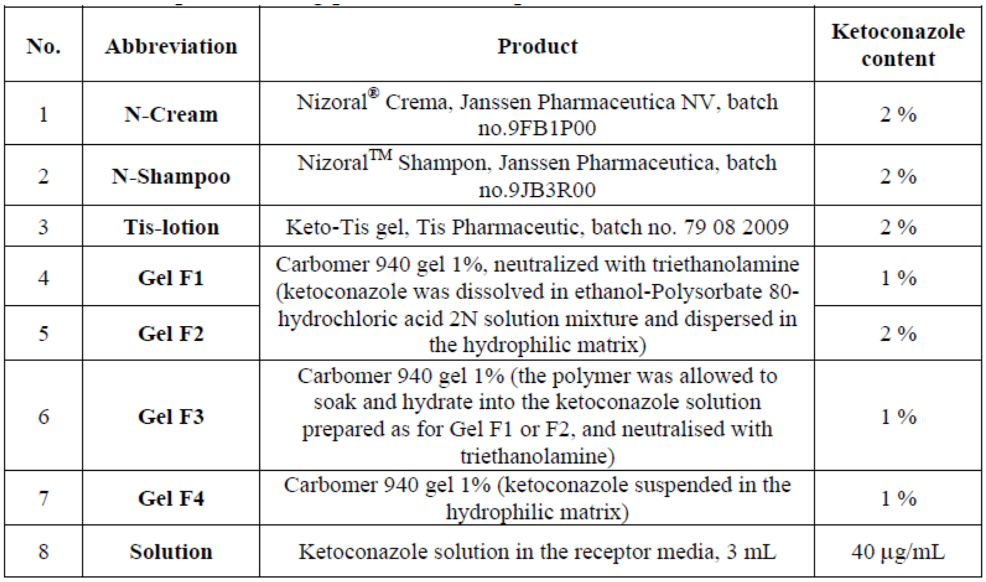
; where the constant, k, is the dissolution rate constant. BCS Class II drugs are characterized by poor aqueous solubility that often results in slow dissolution rates and incomplete absorption. The drugs are typically weak acids with low pKa values of ≤ 4.5 and intrinsic solubility < 0.01 mg/ml [10]. Intrinsic solubility is defined as the solubility of a fully unionized drug.

One of the most commonly used formulation strategies for BCS class II drugs is to prepare them in physical forms with higher intrinsic solubility (C_s). For instance, amorphous drug forms have higher intrinsic solubility compared to their crystalline polymorphs, and are therefore preferred over their crystalline counterparts. However, amorphous drugs are thermodynamically unstable, and will eventually revert to more stable crystalline polymorphs given sufficient time. Stabilization of amorphous drug therefore constitutes a key component in the formulation of BCS class II drugs.

1.1.3 Choice of drugs:

Two BCS Class II drugs, Ketoconazole and Chlorpropamide were selected for this study. These drugs are highly permeable across the gastric mucosa, yet exhibit poor solubility in water, resulting in a dissolution rate-limited oral absorption and bioavailability. Ketoconazole is an imidazole antifungal agent that can be administered orally or prescribed for topical application. It is traditionally used to prevent and treat fungal infections of the skin and nails that can spread through the bloodstream, such as candidiasis, histoplasmosis, systemic blastomycosis, coccidiodomycosis, and tinea [11]. It has recently been used for the clinical treatment of Cushing's disease, since it is a potent inhibitor of gonadal and adrenal steroidogenesis [12]. It is marketed commercially as an antidandruff shampoo, topical cream, and oral tablet [13]. The U.S. brand names for Ketoconazole are Extina, Nizoral, Xolegel and Nizoral A-D.

Table 1. Description of products and formulations of Ketoconazole [13].



No.	Abbreviation	Product	Ketoconazole content
1	**N-Cream**	Nizoral® Crema, Janssen Pharmaceutica NV, batch no.9FB1P00	2 %
2	**N-Shampoo**	Nizoral™ Shampon, Janssen Pharmaceutica, batch no.9JB3R00	2 %
3	**Tis-lotion**	Keto-Tis gel, Tis Pharmaceutic, batch no. 79 08 2009	2 %
4	**Gel F1**	Carbomer 940 gel 1%, neutralized with triethanolamine (ketoconazole was dissolved in ethanol-Polysorbate 80-hydrochloric acid 2N solution mixture and dispersed in the hydrophilic matrix)	1 %
5	**Gel F2**		2 %
6	**Gel F3**	Carbomer 940 gel 1% (the polymer was allowed to soak and hydrate into the ketoconazole solution prepared as for Gel F1 or F2, and neutralised with triethanolamine)	1 %
7	**Gel F4**	Carbomer 940 gel 1% (ketoconazole suspended in the hydrophilic matrix)	1 %
8	**Solution**	Ketoconazole solution in the receptor media, 3 mL	40 μg/mL

Ketoconazole has a molecular weight of 531.43 g/mol and a melting range of 148.1-150.2 °C. The amorphous form of the drug has a glass transition temperature (T_g) of 42.9 °C [14]. With an aqueous solubility of 8.38 μg/ml at 30 °C, it is practically insoluble at physiological pH [15]. The drug is dibasic with pKa values of 2.94 and 6.51 [16]. The structure of Ketoconazole is shown in Figure 2.

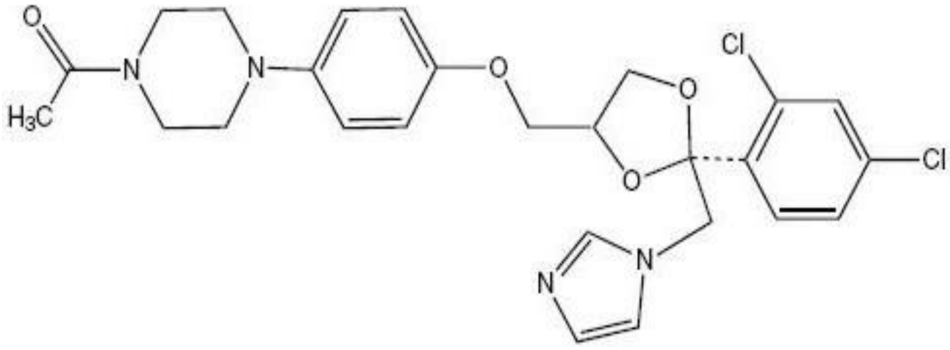


Figure 2. Chemical structure of Ketoconazole [11].

Chlorpropamide belongs to a class of medications called sulfonylureas [17]. It is marketed as an oral antidiabetic drug. It is used to treat type II diabetes mellitus by stimulating the pancreas to produce insulin [18]. It is commercially available with the brand names Diabinese and Glucamide. Chlorpropamide has a molecular weight of 276.74 g/mol and a melting range of 126.0-129.0 °C. It is weakly acidic with a pKa of 5.0. The T_g for amorphous Chlorpropamide is 16 °C [19]. Chlorpropamide, USP, is a white crystalline powder with no characteristic odor. It is practically insoluble in water with the solubility being < 0.5 mg/ml [20] with a solubility at pH 6 of 2.1–2.3 mg/ml [21]. The structure of Chlorpropamide is shown in Figure 3 below.

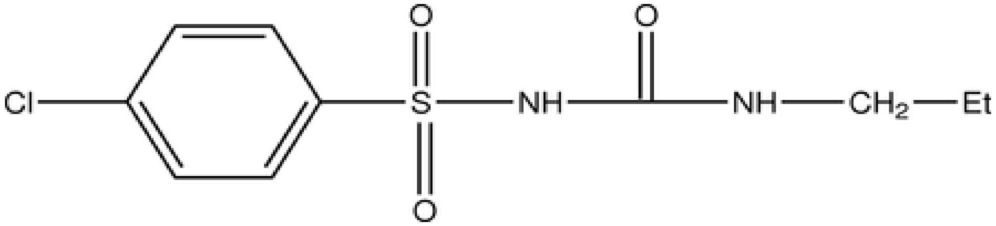


Figure 3. Chemical structure of Chlorpropamide [22].

1.2 Solubility enhancement strategies for BCS Class II drugs

1.2.1 pH modification

The formation of salts of weak acids and weak bases has been previously reported to enhance solubility for insoluble drugs [23]. The ionized form of the drug, represented by the salt of a weak acid or a weak base, is more soluble compared to the unionized drug. Solubilizing excipients may further increase the micro-environmental pH in a weak acidic drug dosage form to a range higher than its pKa, resulting in higher immediate solubility that enhances the dissolution rate. Likewise, acidifying agents can be used to maintain a low internal dosage form pH for weakly basic drugs, thereby increasing solubility. However, converting high molecular weight lipophilic drugs and neutral compounds to a salt form might not always be feasible[24]. The salts of weak acids and bases could also revert to their original unionized, poorly soluble forms leading to aggregation and polymorphic changes upon storage [25].

1.2.2 Self Emulsification:

Poorly soluble drugs may be emulsified into lipid-based formulations to enhance solubility and dissolution rate [23]. These oil-based solutions, emulsions or self-emulsifying drug delivery systems (SEDDS) form stable micellar solutions or dispersions that allow rapid partitioning of even highly lipophilic drugs into the aqueous medium[2]. Lipid composition and the type of lipid formulation can improve both drug solubilization and the lipid digestion rate, thereby improving drug absorption [26]. Natural surfactants such as sodium lauryl sulfate (SLS), and synthetic/ semi-synthetic surfactants such as Polyoxyethyl enesorbitan monolaurate (Tween) & Polyoxyl castor oil (Cremophor) may also be used to solubilize poorly water-soluble drugs in aqueous media [27]. These techniques tend to be highly empirical in nature. Moreover, as the solubility of the drug remains unchanged, drug precipitation is frequently encountered upon loss or modification of the lipid component.

1.2.3 Complexation:

Complexation with bile salts or cyclodextrins can facilitate rapid dissolution of hydrophobic drugs. Cyclodextrins are hydrophilic oligosaccharides with a hydrophobic central cavity [28]. Inclusion of drugs, such as Piroxicam [29] or Ibuprofen [30] in βcyclodextrin, and digoxin [31] in γ-cyclodextrin, has resulted in markedly increased dissolution rates.

1.2.4 Particle Size Reduction

Micronization or nanonization of solid drug particles results in substantial increases in surface area, resulting in greater dissolution rates. Ball milling is an efficient size reduction method that can reduce particle size to 50 μm, while wet milling can achieve sizes smaller than 50 μm [23]. To reduce the risk of heat generation and recrystallization during the size reduction, cyrogenic milling may be pursued because any heat generated should be carried away, for example, by liquid nitrogen [32]. The use of air jet mills helps to further reduce particle size to about 2-5 μm.

A study by Li et al. [33] showed increased dissolution rates for nifedipine upon particle size reduction by wet milling in ball mills. They found that this process results in a high surface energy, thereby causing irreversible particle aggregation [33]. To prevent such instability, surfactants, dispersing agents, or polymers are needed to minimize recrystallization of the amorphous domains during such processes.

1.2.5 Cocrystal Formation:

Cocrystal formation is a practical approach to improve the dissolution rate of drugs that are not ionized at physiological conditions [34]. As opposed to a pure crystal, a cocrystal consists of two or more components that exhibit long-range order in the solid state.

1.2.6 Amorphization:

Amorphization involves the preparation of a reduced crystallinity or an absence of crystallinity in the drug that allows faster dissolution in comparison to its crystalline form [23]. Amorphous structures lack the well-defined close packing exhibited by crystalline structure, and there require less energy to solubilize when exposed to dissolution media. For indomethacin, the solubility enhancement ratio for the amorphous to crystalline form was experimentally determined to be 4.9 [35]. Theoretically, the value could be as high as 29 when the effects of ionization and water sorption are ignored [35].

A limitation to the use of formulations involving amorphous drug is the inherent thermodynamic instability in these formulations [36]. Amorphous forms could revert to a more stable crystalline structure during long-term storage [37]. The crystallization tendency of amorphous drug results from thermodynamic forces that lead to free energy differences between these forms [36].

1.3 Methods for preparing amorphous solid dispersions:

1.3.1 Melting (or fusion) method:

The process consists of melting a physical mixture of the drug and polymer followed by controlled cooling, followed by size reduction via pulverization or milling [38]. Controlled cooling may be achieved by flash cooling in liquid nitrogen [39] or fast cooling in an ice bath [40] or circulating cold water (< 4°C) [41]. Spray congealing can also be used to solidify the molten drug-polymer mixture [42].

An alternate approach to processing a hot melt is to extrude it. The process, termed hot melt extrusion or hot stage extrusion, might also use carbon dioxide as a plasticizer to broaden the use of this technique for thermolabile compounds [43]. The total duration of exposure to high temperature can also be limited to as little as one minute [44]. Solid dispersions can also be prepared by heating a binder, drug, and excipient to a temperature above the melting point of the one of the three components if the other components dissolve in the melt. This can be accomplished using conventional high shear mixers. The use of a rotary processor is another alternative that produces stable solid dispersions with a higher binder content [45].

One of the major limitations of the hot melt methods is the high temperature necessary for these processes. Such temperatures can cause the drug to oxidize, otherwise decompose, or evaporate leading to loss of its function [46]. Oxidative degradation can be overcome by melting the mixture in a vacuum or in the presence of an inert gas or antioxidant [44].

1.3.2 Supercritical fluid extraction:

Solid dispersions can be obtained by the use of supercritical fluids **(**SCFs**)**[38]. Carbon dioxide is commonly used as the SCF of choice because it is relatively inert, non-toxic, and has a critical point of 31°C when pressure exceeds 73 atmospheres. A mixture of the drug in solvent is sprayed through a nozzle into an expansion vessel, where the solution is extracted by the SCF, resulting in precipitates that are the solid dispersion particles [38]. Major drawbacks of this process are the limited choices for supercritical fluids and its high cost [47].

1.3.3 Solvent Evaporation methods

This approach consists of dissolving the drug and carrier in a solvent, followed by solvent removal to prepare a solid dispersion [46]. This process avoids excessive heating and might, therefore, be applied to thermolabile drugs. A limitation to this process is the difficulty of finding solvents where drugs achieve acceptable solubility and finding a solvent that has a good safety profile. High residual solvent levels may be expected in the final product [48] and typically these residual solvent levels must be dramatically reduced.

1.3.3.1 Co-precipitation:

Co-precipitation, or phase separation, is a process in which a drug and carrier are dissolved in a common solvent. A miscible non-solvent is added slowly, with continuous stirring, to precipitate the dispersion [49]. Eventually any residual solvent and non-solvent must be evaporated to “dry” the product. A disadvantage to this process is the variability in particle size in the final product causing varying dissolution rates. A schematic representation of this process is shown in Figure 4.

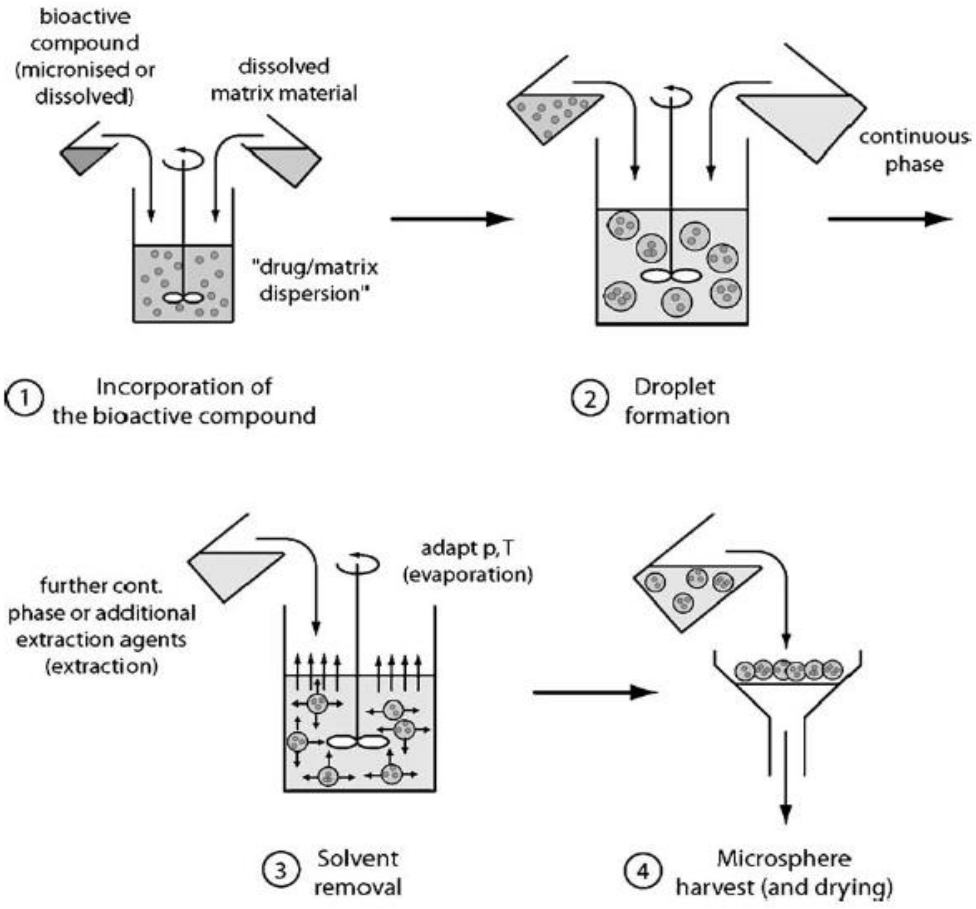


Figure 4. Microsphere preparation by phase separation [49].

1.3.3.2 Spray Drying

Spray drying consists of either dissolving [50] or suspending [51] the drug and excipient in a solvent followed by spraying the mixture into a chamber of flowing heated air to facilitate solvent removal. A schematic of the spray-drying process is shown in Figure 5 below.

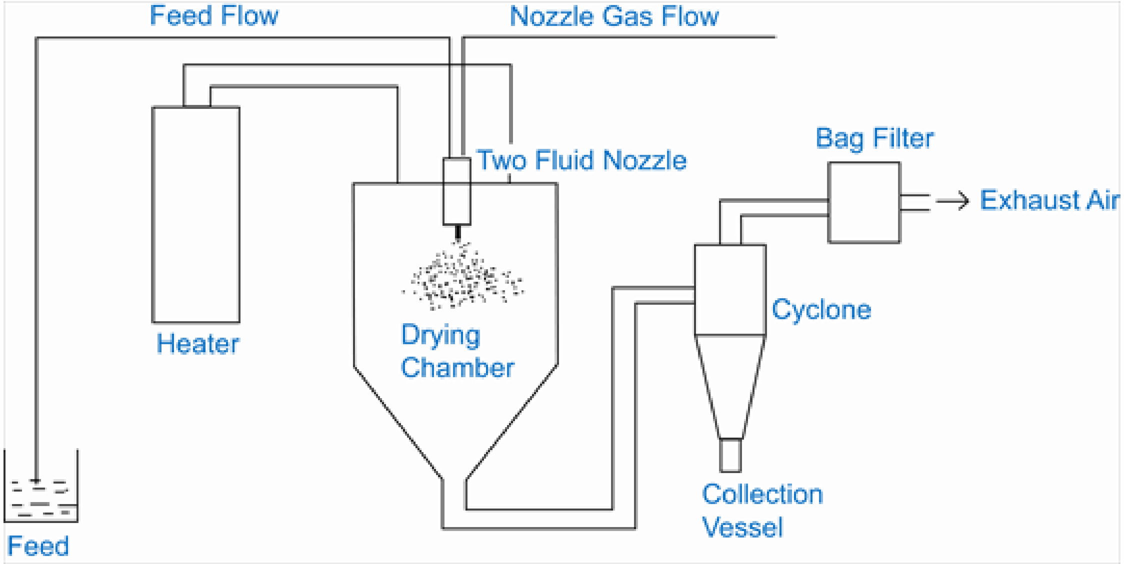


Figure 5. Process of spray drying [52].

This method yields particles with a uniform size distribution and minimal batch to batch variability [53]. The physical and chemical stability of the drug is preserved, and residual solvent levels tend to be low[54]. Ambike, et. al., showed that amorphous solid dispersions of simvastatin showed higher dissolution rates when prepared by spray drying [53]. The final product existed in a supercooled, rubbery state if the outlet temperature could be maintained above the T_g of Simvastatin[53]. A study by Patel et. al.. showed that the solubility of Tenoxicam could also be enhanced by spray-drying with PVP [55].

1.4 Excipient selection:

Polymeric excipients have been used to stabilize amorphous drugs in solid dispersions by increasing the overall T_g, and therefore reducing drug mobility at ambient storage temperatures [38]. This antiplasticization effect is dependent on the nature and concentration of the polymer used for the process [56]. Maximum stabilization occurs when the drug and polymer are mixed at a molecular level to form a solid solution rather than a two-phase dispersion [57]. For instance, the antiplasticization of amorphous Indomethacin (T_g of 42°C) with PVP (T_g of 177°C) was shown to prevent drug crystallization [58].

Polymers such as Polyethylene glycol (PEGs), and low molecular weight substances such as sugars, have also been used to stabilize amorphous drugs [41]. Water-insoluble matrix formers include some members of the Eudagrit family (esters of Acrylic and Methacrylic acid) and examples of amphiphilic materials are the Gelucires (PolyEthyl ene glycol glycerides)[59].

Ethyl Cellulose is a polymer used extensively as an excipient in oral formulations typically for controlled release solid dosage forms. The polymer is hydrophobic, and is soluble in a wide range of organic solvents. It also has a high glass transition temperature (T_g 130-133 °C), enabling its use as an antiplasticization polymer in amorphous stabilization of the drugs selected in this study.

1.5 Methods for characterizing amorphous dispersions:

Several techniques can determine the crystalline content relative to amorphous content in a solid dispersion. Commonly employed techniques include ^{19}F MAS (Magic Angle

Spinning) Nuclear Magnetic Resonance Spectroscopy (^{19}F MAS-NMR), Infrared Spectroscopy (IR), Differential Scanning Calorimetry (DSC), X-ray Powder Diffraction (XRPD) and Scanning Electron Microscopy (SEM) [60]. Spectroscopic techniques, such as Raman and IR spectroscopy, include a detailed intramolecular level of analysis, whereas thermal analysis and diffraction techniques study properties dependent on macroscopic properties [61]. Solid state spectroscopic techniques, such as Raman spectroscopy, have an advantage over diffraction techniques for nearly pure samples because individual crystalline and amorphous components can be detected over a broader spectrum of materials undergoing conformational changes [62]. Hedoux et al. showed the applicability of Raman spectroscopy in the characterization of indomethacin polymorphs [63].

Diffraction techniques offer value in evaluating partially crystalline solids due to effectiveness in quantifying molecular order in such systems. X-ray powder diffraction provides a quantitative fingerprint of the crystallinity in a solid phase [60]. For an amorphous sample having no regularity in its three-dimensional molecular arrangement, the diffracted beams have destructive interference in all directions. The amorphous content also exhibits greater free volume between molecules. The result is a broad, irregular diffraction pattern commonly labeled a “halo”.

DSC is a useful thermal analysis tool for the characterization of amorphous and crystalline materials. It is also commonly used due to ease of analysis and small sample size requirement, typically ranging from 3-15 mg. DSC measures temperatures and heat flow associated with thermal transitions in materials. Examples of transitions observed in DSC include the glass transition temperature, an enthalpy change, a melting point, the degree of crystallinity, and a heat capacity change. Figure 6 shows a plot of enthalpy or volume versus temperature for a crystalline solid[64].

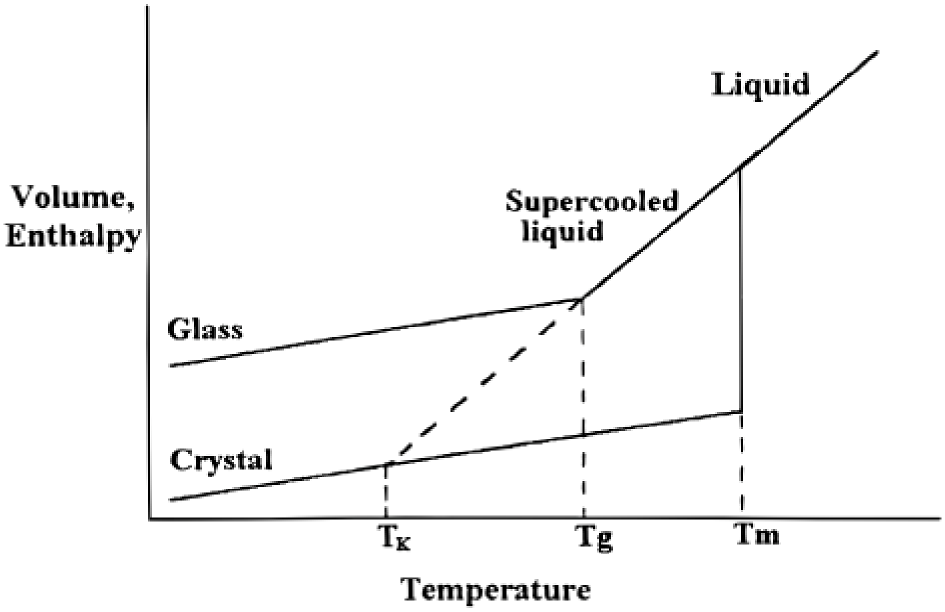


Figure 6.Changes in volume/enthalpy with regards to temperature [64].

The glass transition temperature, T_g, represents a temperature below which molecular mobility of a supercooled liquid is reduced substantially, and is a characteristic of the amorphous domains in the solid dispersion. The crystalline domains are characterized by the melting point (T_m). The percent crystallinity in the sample can be calculated using equation 2 below:

$$X_c\ (\%) = 100\times\underline{\Delta H/}\Delta H_0 \qquad (2)$$

where X_c = the weight percent of crystalline material

ΔH = observed enthalpy of fusion of the sample being tested

$\Delta H_{0\,=}$ enthalpy of fusion of the 100% crystalline material.

1.6 Research goal and specific aims:

The goal of this research is to evaluate and compare two common methods of preparing amorphous solid dispersions for BCS class II drugs: (1) spray drying, and, (2) precipitation by non-solvent addition. To ensure stability of the amorphous precipitate, the effects of co-precipitation and or/spray-drying with three different grades of Ethyl Cellulose (7, 45, and 100 cP) were also explored.

The two specific aims of this proposal are as follows:

1. To study the effect of spray drying and non-solvent induced precipitation on the percent crystallinity of two BCS Class II drugs, Ketoconazole (KZ) and Chlorpropamide (CP).
2. To study whether the inclusion of Ethyl Cellulose as a formulation excipient will enable: (a) the preparation, and (b) the stability of KZ and CP with higher % amorphous content. Three grades of Ethyl Cellulose of intrinsic viscosity 7, 45, and 100 cP were evaluated.

1.7 Significance

Ketoconazole and Chlorpropamide are BCS Class II drugs. They have stable crystalline forms with very limited solubility. While the amorphous forms of these two drugs have a substantially higher solubility, the preparation and formulation of amorphous Ketoconazole and Chlorpropamide are limited by the low stability of the high-energy amorphous structures. This research will elucidate whether co-precipitation with Ethyl Cellulose can lower the energy of the solid dispersion and, thereby, eliminate or at least reduce the rate of transformation of amorphous Ketoconazole or Chlorpropamide into their corresponding crystalline structures. This study will also examine how the amorphous structures of these two drugs and their physical stability vary as a function of the method of preparation and the molecular weight of the excipient (Ethyl Cellulose) used in the production of the solid dispersions. Overall, the results of this research will improve the formulation of amorphous solid dispersions for BCS Class II drugs. This research will also confirm the value of differential scanning calorimetry as a tool for studying formulation variables in amorphous solid dispersions.

2. MATERIALS AND METHODS

2.1 Materials

Ethyl Cellulose (7, 45, and 100 cP) was donated by Colorcon (West Point, PA). The glass transition temperature of Ethyl Cellulose ranges from 106-133 °C and its melting point 160-210 °C [65]. A model drug, Ketoconazole (1-[4-(4-{[(2R,4S)-2-(2,4-Dichlorophenyl)-2-(1H-imidazol-1-ylmEthyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]ethan-1-one; MW 531.43 g/mol; melting range 148.1-150.2 °C) was purchased from VMR (New Brunswick, NJ). A second drug, Chlorpropamide (1-(p-chlorobenzenesulfonyl)-3-propylurea; MW 276.74 g/mol; melting range 126.0-129.0 °C) was purchased from Sigma-Aldrich (Saint Louis, MO). The solvents, analytical grade dichloromethane and n-hexane, were obtained from Sigma-Aldrich and used without further purification.

2.2 Methods

2.2.1 Spray drying:

A 1:1 mixture of Ethyl Cellulose (7, 45, or 100 cP) and drug (KZ or CP) was dissolved to produce a solution of 5.0% (w/w) in dichloromethane. Controls were prepared using pure EC or drug at a 5% (w/w) concentration in dichloromethane. The solutions were spray dried using the bench top Mini Spray Dryer B-290 Buchi shown in Figure 7. The instrument is equipped with a two fluid-nozzle system operated in a co-current mode in which the feeding solution and drying-air flow are in the same direction. The nozzle is operated by compressed air that disperses the solution into fine droplets which then allow the solvent to evaporate resulting in the formation of solid particles. The parameters that were set for the operation included the following: an inlet temperature of 70 °C, aspirator setting of 100%, and pump rate at 30% with the Q-flow at 40 mm. Spray-dried particles were collected from the cyclone separator and stored in closed vials in a desiccator at room temperature.

2.2.2 Non-solvent induced co-precipitation:

The solutions using dichloromethane as a solvent was prepared for the phase separation/co-precipitation technique were similar to those described in section 2.2.1. Approximately 350-450 ml of the mixture was placed into a 1000 ml beaker and the non-solvent n-hexane added at the rate of 1 ml/min under constant stirring (1100 rpm) with the aid of a magnetic stir bar to induce phase separation/co-precipitation. The formation of the precipitate was confirmed by the visual detection of turbidity. Following the addition of n-hexane of approximately 100 ml, the particles in the solution was allowed to settle for an additional 2-4 hours after which the supernatant was removed. The resulting particles were washed with n-hexane and allowed to dry for an additional 24-48 h in a glass Petri dish under a vertical laminar flow hood. The dried particles were transferred to 20 ml scintillation vials and stored in a desiccator at room temperature.

Figure 7. Buchi Mini Spray Dryer B-290 with specific settings.

2.2.3 Particle Size analysis

Particle images are obtained using a biological science light microscope (Olympus IX-ILL100LH) (40-1000X). Particle size analysis was performed using ImageJ software.

2.2.4. Thermal Analysis:

<u>Differential Scanning Calorimetry</u>: A Mettler Toledo DSC822e differential scanning calorimeter was used in these studies. Each of the spray dried and precipitated products, in combination with EC 7 cP, EC 45 cP or EC 100 cP, individually processed with Ketoconazole and Chlorpropamide as the active was evaluated. The controls consisted of

pure polymer and pure drug. About 3-6 mg samples were placed in crimped aluminum pans with pierced lids and subjected to heating at 10 °C/min in the presence of nitrogen with a flow rate of 20 ml/min. The samples were subjected to two heating cycles with an intermittent cooling cycle across a temperature range of 0-250 °C. The thermal parameters evaluated were the glass transition temperature (T_g), melting point (T_m), crystallization temperature (T_c) and their corresponding changes in enthalpy (ΔH). The data were analyzed with STARe software.

Thermogravimetric analysis: To calculate the amount of residual solvent or water in the dispersions, thermogravimetric analyses were performed using a Mettler Toledo TGA/SDTA 851e. About 3-10 mg of the sample was heated over the temperature range 25-250°C at 10°C/min in an atmosphere of nitrogen with a flow rate of 20 ml/min. The weight loss of the sample was recorded as a function of temperature. The rate, extent, and temperature range of the weight loss were evaluated to quantitatively determine residual solvent and moisture levels in the samples as well as to examine if the samples were thermally degrading.

2.2.5 Determination of drug load

Ketoconazole content: To establish a calibration curve for Ketoconazole, a stock solution of the drug (pKa = 6.51) [16] was prepared at a concentration of 1000 μg/ml in 0.1 N hydrochloric acid. Serial dilutions were made to yield concentrations of 100, 200, 400, 600, and 800 μg/ml. An absorbance for each dilution was measured at a wavelength of 270 nm using a UV-1800 UV Spectrophotometer (Shimadzu, Japan). The wavelength of 270 nm was selected based on literature[66]. Quartz cells with a 1 cm pathlength were used for absorbance measurements. The absorbance by the blank (0.1 N HCl) was subtracted and the net absorbance was plotted against drug concentration to obtain a calibration curve.

To determine drug content within the spray-dried or precipitated products, an accurately weighed mass of about 7.5 mg of each sample was dissolved in 15 mL of dichloromethane and equilibrated for 1 hour to ensure dissolution. Controls were prepared by accurately weighing 3.75 mg each of Ethyl Cellulose (7, 45, or 100 cP) and Ketoconazole, followed by dissolution in dichloromethane. Samples were made for each of the solutions to ensure statistical validity. The drug was extracted from 15 mL of dichloromethane solution by adding 5 mL 0.1 N hydrochloric acid with vigorous agitation. The mixture was then subjected to high-speed centrifugation at 6000 rpm for about 10 min which resulted in two clear layers. The aqueous phase was withdrawn and the entire process was repeated two more times to maximize drug extraction.

The three volumes of the aqueous solutions were combined and the absorbance was measured at 270 nm wavelength to calculate the Ketoconazole content using the calibration curve. The blank consisted of 0.1 N HCl exposed to and then separated from 5 mL of pure dichloromethane three times and combined to yield a total volume of 15 mL. The absorbance measurements were used to determine Ketoconazole concentration in the 15 mL of extracted volume. This concentration was compared to that of the control samples to determine the percent loading efficiency (LE%) shown in equation 3, which was used subsequently as a correction factor to determine the drug content in all spray dried and precipitated samples.

$$\text{LE (\%)} = 100\,\frac{C_{sp}}{C_t} \qquad (3)$$

where; C_{sp} is the drug concentration determined from spectroscopic measurements and C_t is the total drug concentration determined from actual amount used in the control.

Chlorpropamide content: The analysis of Chlorpropamide concentration was performed in a manner similar to that for Ketoconazole, except that 0.1 N sodium hydroxide (NaOH) was used. The pKa of Chlorpropamide is 5.0 [67]. Briefly, triplicate stock solutions of Chlorpropamide were prepared in 0.1 N NaOH solution at 100 μg/ml. Dilutions were made to yield concentrations of 5, 10, 20, 30, 40 and 50 μg/ml and the absorbance of each solution was measured at 230 nm [68] to obtain a calibration curve for Chlorpropamide.

The drug content and LE (%) were calculated by a procedure similar to that described for Ketoconazole except that 15 mL of dichloromethane and 0.1 N NaOH were used for extraction. The total combined volume for the 3 extracts was 45 ml of NaOH.

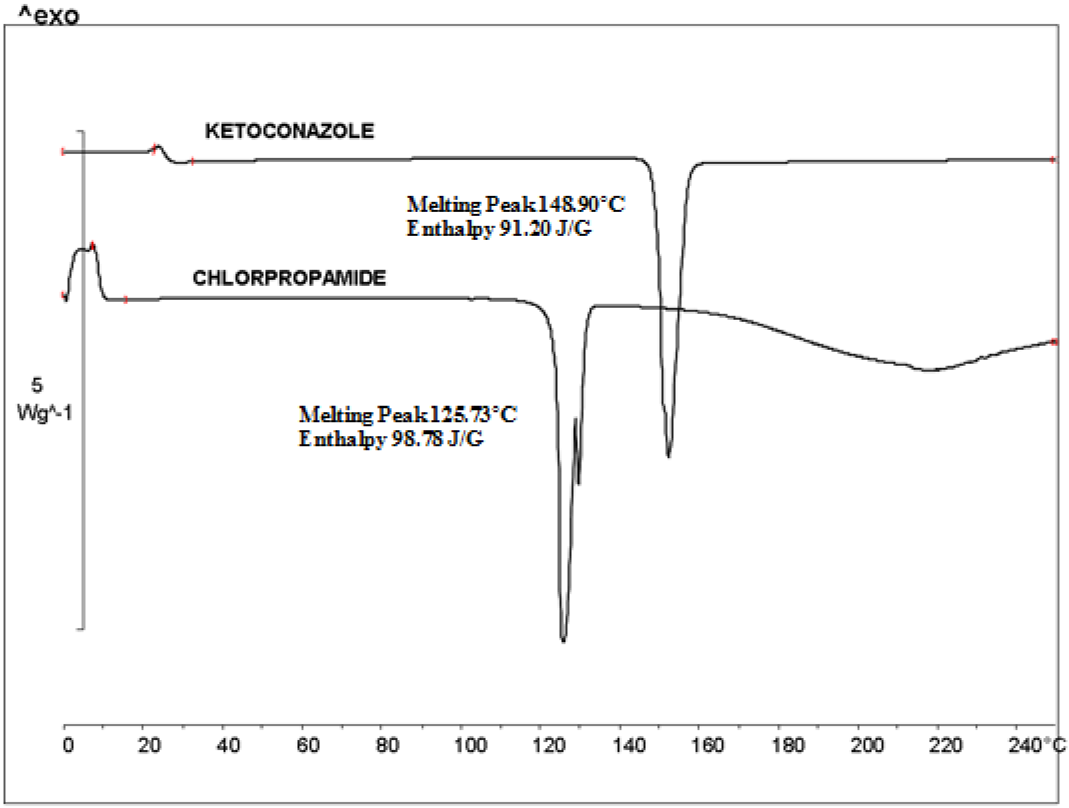
3. RESULTS

3.1 Partial Crystallinity of unprocessed Ketoconazole and Chlorpropamide

Figure 8 shows representative DSC thermograms of unprocessed Ketoconazole and Chlorpropamide. The DSC thermogram for Ketoconazole showed a distinct endothermic transition with a peak at 148.9°C. This transition corresponds to the melting transition (T_m) of Ketoconazole, which is reported to be around 148.1 – 150.2°C [69]. The melting enthalpy (ΔH_m) was 91.20 J/G. This value corresponds to a 88% crystallinity, using a reported literature reference of 103.4 J/G [70]. A glass transition (T_g) was also observed for Ketoconazole at about 25°C, corresponding to the roughly 12% amorphous Ketoconazole. Similarly, DSC thermograms for Chlorpropamide showed an endothermic transition at 125.7°C, which was consistent with a Chlorpropamide melting range from

126.0-129.0°C [69]. The melting enthalpy was 98.8 J/G comparable with existing literature values at 100.2 J/G [17]). The roughly 99% crystallinity in Chlorpropamide was consistent with the complete absence of T_g in this sample. The dual characteristics of the melting endotherm for Chlorpropamide suggested the possibility of polymorphism within this drug, and will be addressed later in the discussion section of this thesis.

Figure 8. Representative DSC thermograms of unprocessed Ketoconazole and Chlorpropamide.



Overall, the DSC thermograms showed that unprocessed Ketoconazole and Chlorpropamide have high levels of partial crystallinity, ranging from 88-99%. In their unprocessed form, these drugs are almost entirely crystalline, and therefore make good candidates for amorphous stabilization studies.

3.1 Partial Crystallinity of processed Ketoconazole and Chlorpropamide

Table 2 shows the effect of two processing methods, nonsolvent-induced co-precipitation and spray-drying, on the amorphous content in Ketoconazole and Chlorpropamide. Short-term stability studies were also performed to assess the stability of the amorphous form during a 2-week period, and stored in a desiccator at room temperature and pressure.

Table 2. The effect of processing on the thermal properties of Ketoconazole and Chlorpropamide.

KETOCONAZOLE			
Physical Property	**Unprocessed**	**Precipitated**	**Spray Dried**
Glass Transition, T_g (°C)	ND	35.0 ± 10.9	29.0 ± 12.7
Melting Peak, T_m (°C)	148.9	149.0 ± 0.6	147.8 ± 2.0
Enthalpy of Melting (J/G)	91.2	94.3 ± 2.4	85.8 ± 2.9
% Crystallinity	88.2%	91.2% ± 2.3	83% ± 2.8
CHLORPROPAMIDE			
Physical Property	**Unprocessed**	**Precipitated**	**Spray Dried**
Glass Transition T_g (°C)	ND	ND	ND
Melting Peak T_m (°C)	125.7	126.9 ± 0.6	129.8 ± 0.2
Enthalpy (J/G)	98.8	89.0 ± 5.8	87.7 ± 6.6
% Crystallinity	99%	88.9% ± 5.8	87.5% ± 6.6

The results shown in Table 2 indicate no substantial differences between the thermal properties of the unprocessed versus processed BCS Class II drugs. For Ketoconazole, the spray-dried formulation showed slightly higher amorphous content, whereas the crystallinity in the co-precipitated formulation was actually higher than the unprocessed drug. For Chlorpropamide however, both processed formulations showed a roughly 10% lowering in crystallinity. Table 3 shows the DSC results of the short-term stability studies for both Ketoconazole and Chlorpropamide. Although minor variations were observed in the percent crystallinity of both drugs after days 1, 7, and 14, all differences were within the margin of error, and were therefore not considered significant.

The results shown in tables 2 suggest that the two processing methods, namely, nonsolvent-induced co-precipitation, and spray-drying, have no practical value in the preparation and/or stabilization of amorphous Ketoconazole and Chlorpropamide. The need for additional stabilizing excipients such as Ethyl Cellulose is strongly indicated, especially if significant lowering of crystallinity is desired in these BCS Class II compounds.

Table 3. The effect of time on the stability of processed Ketoconazole and Chlorpropamide measured using Differential Scanning Calorimetry.

KETOCONAZOLE						
	Precipitated Sample			**Spray Dried Sample**		
Physical Property	**Day 1**	**Day 7**	**Day 14**	**Day 1**	**Day 7**	**Day 14**
T_g (°C)	35.0 ± 10.9	18.0 ± 1.1	ND	29.0± 12.7	26.7 ± 5.9	28.6 ± 8.0
Melting Peak (°C)	149.0 ± 0.6	148.1 ± 2.7	146.2 ± 4.7	147.7 ± 2.0	148.3 ± 1.0	147.3 ± 1.2
Enthalpy of Melting (J/G)	94.3 ± 2.4	94.6 ± 2.8	97.1 ± 3.9	85.8 ± 2.9	83.3 ± 5.8	89.0 ± 1.4
% Crystallinity	91.5 ± 2.3	91.8 ± 2.7	94.3 ± 3.8	83.3 ± 2.8	80.9 ± 5.6	86.4 ± 1.3
CHLORPROPAMIDE						
	Precipitated Sample			**Spray Dried Sample**		
Physical Property	**Day 1**	**Day 7**	**Day 14**	**Day 1**	**Day 7**	**Day 14**
T_g (°C)	ND	ND	ND	ND	ND	ND
Melting Peak (°C)	126.9 ± 0.6	126.8 ± 0.8	127.6 ± 0.5	129.8 ± 0.2	129.9 ± 0.9	129.8 ± 0.6
Enthalpy of Melting (J/G)	89.0 ± 5.8	88.4 ± 5.0	85.8 ± 4.4	87.7 ± 6.6	84.5 ± 2.7	82.4 ± 7.1
% Crystallinity	88.9% ± 5.8	88.3% ± 5.0	85.7% ± 4.4	87.5% ± 6.6	84.4% ± 2.7	82.2% ± 7.1

3.1 Effect of processing with Ethyl Cellulose as excipient:

3.1.1 Particle size:

Three grades of Ethyl Cellulose were used as excipients. Formulations were either co-precipitated or spray-dried. The particle size range of the formulations, along with associated standard deviations is shown in Table 4.

Table 4: Particle size range for co-precipitated and spray-dried formulations

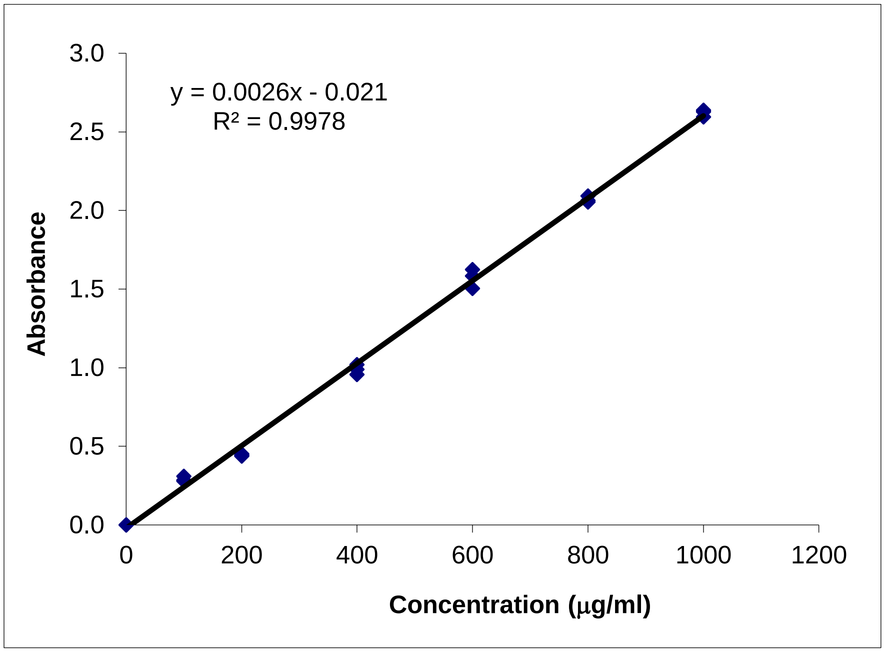
Sample	Co-precipitated (µm)	Spray-dried (µm)
KE	3.5 ± 1.3	3.1 ± 1.6
CH	15.3 ± 6.7	8.7 ± 3.7
EC 7 + KE	7.1 ± 4.8	6.4 ± 2.4
EC 45 + KE	5.3 ± 3.2	4.2 ± 2.3
EC 100 + KE	49.3 ± 3.4	18.4 ± 7.6
EC 7 + CH	5.9 ± 5.8	11.3 ± 9.4
EC 45 + CH	8.9 ± 6.2	6.5 ± 5.0
EC 100 + CH	22 ± 13	7.2 ± 3.7

The particle size of the co-precipitated formulation was in the range of ~ 3 to 49 µm. The range for the spray-dried formulation was much narrower, and ranged from ~ 3 to 18 µm. The standard deviations were relatively high, suggesting that the particles lacked uniformity within the size range.

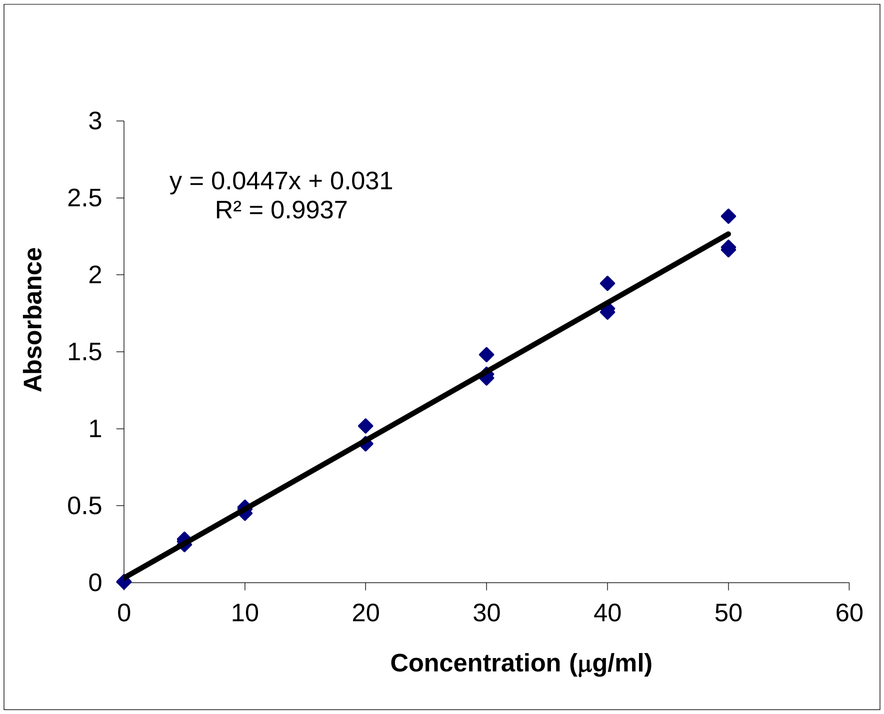
3.1.2 Drug content and loading efficiency

The figures 9 (a) and 9 (b) show the calibration curves that were used to determine the concentration of Ketoconazole and Chlorpropamide in the co-precipitated and spray-dried formulations. Both calibration curves showed a linear dependence of UV absorbance on the drug concentration, with an R^2 value > 0.99.

Figures 9 (a): Calibration curve for the determination of Ketoconazole concentration determination.



Figures 9 (b): Calibration curve for the determination of Chlorpropamide concentration determination.



The drug content calculated for each dispersion is listed below in Table 5. Table 5 shows that both formulation processes were successful in entrapping roughly 50% of the drug.

Table 5. The drug content in Ketoconazole and Chlorpropamide dispersions with Ethyl Cellulose of different molecular weights.

Sample	Co-Precipitated (%)	Spray-Dried (%)
EC 7 + KE	55.1	48.9
EC 45 + KE	49.4	48.6
EC 100 + KE	53.9	51.0
EC 7 + CH	49.5	50.2
EC 45 +CH	58.0	54.0
EC 100 + CH	43.7	55.4

3.1.3 Partial crystallinity of EC formulations:

3.1.3.1: Thermal Transitions in Pure Ethyl Cellulose:

Figure 10 shows the DSC Thermograms of the three grades of Ethyl Cellulose used in this study. The thermograms in all cases showed a broad endothermic transition in the temperature range of 30 – 100°C. The temperature range indicates the possibility that the endotherm corresponds to moisture loss in the Ethyl Cellulose sample.

Following the endothermic transition, a glass transition (T_g) was also detected in the thermograms for all Ethyl Cellulose samples. The T_g appeared to be a function of the viscosity grade of the Ethyl Cellulose sample, with EC 100 showing the highest value. These results are consistent with reported values for T_g, which are in the range of 106-133°C [71]. A final melting endotherm, or T_m, was also observed in these samples. Reported T_m values for Ethyl Cellulose are between 160-210°C [69]. Table 6 shows the specific values of the glass transition temperature, melting peak, and associated melting enthalpy for all the Ethyl Cellulose grades evaluated in this study.

Figure 10. DCS thermograms of pure Ethyl Cellulose, of grades 7, 45, and 100 cP.

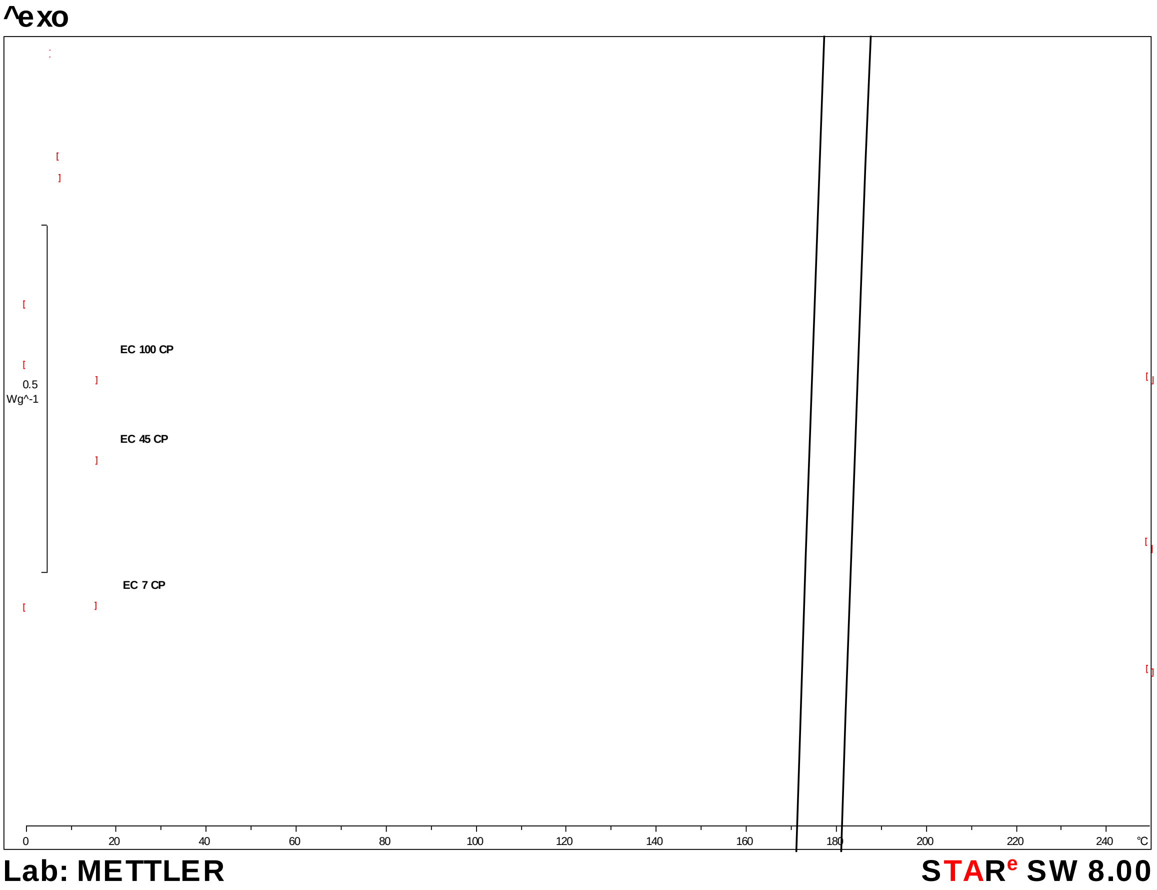


Table 6. Thermal transition in pure Ethyl Cellulose

Physical Property	EC 7	EC 45	EC 100
Glass Transition T_g (°C)	125.8	129.2	136.9
Melting Peak T_m (°C)	154.3	176.1	189.3
Enthalpy (J/G)	4.3	5.6	6.0

These results confirm the semi-crystalline nature of Ethyl -Cellulose. Semi-crystalline polymers simultaneously possess amorphous as well as crystalline domains within their solid-state structure. The amorphous portions of the polymer are responsible for the glass transition, whereas the crystalline portions show melting behavior in DSC thermograms. Instability in semi-crystalline materials is a consequence of the amorphous domains transitioning into more crystalline fractions. The enthalpy of melting (ΔH_m) is a quantitative measure of the crystalline fraction in a semi-crystalline polymer, and can be accurately measured by the DSC. Evaluation of changes in ΔH_m are therefore a quantitative measure of the stability of semi-crystalline polymers.

Table 7 shows the stability profile of pure Ethyl Cellulose samples, when subjected to the formulation processes of co-precipitation or spray-drying.

Table 7. Effect of formulation on the stability of pure Ethyl Cellulose. The numbers below show melting enthalpies (ΔH_m) of Ethyl Cellulose in J/G.

Sample	**Un-processed (J/G)**	**Co-Precipitated (J/G)**			**Spray Dried (J/G)**		
		Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
EC 7 cP	4.3	9.1 ± 6.8	11.3 ± 9.5	17.4 ± 17.0	3.7 ± 0.5	3.7	6.6 ± 2.0
EC 45 cP	5.6	7.9 ± 1.2	7.9 ± 1.2	7.3 ± 1.0	6.7 ± 1.5	11.6	7.3 ± 0.6
EC 100 cP	6.0	4.8 ± 0.1	7.8 ± 1.0	6.8 ± 1.2	8.4	7.0	7.0 ± 1.3

Based on the results shown in Table 7, EC 7 appeared to be a poor choice for co-precipitated formulations. The standard deviations for the co-precipitated EC 7 were almost 100% of the mean, indicating poor homogeneity in the amorphous/crystalline ratios in these polymers. The crystalline enthalpy values also roughly doubled between day 1 and day 14 for EC 7 in both the processed formulations, indicating poor stability in the amorphous domains of EC 7 upon storage.

It may also be noted that the effect of EC molecular weight on % crystallinity appears to show the opposite trend for co-precipitated and spray-dried formulations. For co-precipitated Ethyl Cellulose, % crystallinity decreases with molecular weight, whereas % crystallinity increases with MW for the spray-dried Ethyl Cellulose.

3.1.3.2: Drug-loaded Ethyl Cellulose formulations:

Table 8: Effect of Ethyl Cellulose grade and formulation process on the thermal properties of Ketoconazole and Chlorpropamide formulations.

Physical Property	**EC 7 + KE**		**EC 45 + KE**		**EC 100 + KE**	
	Coprecipitated	Spray-dried	Coprecipitated	Spray-dried	Coprecipitated	Spray-dried
Glass Transition T_g (°C)	ND	ND	ND	ND	ND	ND
Melting Peak (T_m), (°C)	151.5	140.6	152.9	149.7	152.8	150.0
Enthalpy (J/G)	48.2	1.3	41.9	2.2	51.6	3.3
% Crystallinity	93.5	2.6	81.3	4.3	100.1	6.5
Physical Property	**EC 7 + CH**		**EC 45 + CH**		**EC 100 + CH**	
Glass Transition T_g (°C)	ND	ND	ND	ND	51.4	24.8
Melting Peak (T_m), (°C)	125.9	ND	125.3	125	125.9	124.4
Enthalpy (J/G)	53.4	0	35.3	29.0	31.8	29.3
% Crystallinity	97.9	0	75.7	61.2	51.4	63.5

For Ketoconazole, the spray-dried formulation process resulted in a dramatic reduction in the percent crystallinity to almost insignificant values. The lowest values of crystalline enthalpy were obtained for spray-dried EC 7. These were expected to correspond to the formulation with the highest degree of "amorphization" for Ketoconazole. For all EC-Chlorpropamide coprecipitated formulation, a consistent decline in % crystallinity was observed with the increasing viscosity grade of Ethyl Cellulose. The spray-dried formulation prepared with EC 7 showed no melting peak in the expected melting range of Chlorpropamide. The possible appearance of a glass transition was masked by a broad endotherm in the range of 10-100°C. Overall, these studies suggest that spray dried formulations prepared with Ethyl Cellulose of viscosity grade 7 cP yield formulations that are almost completely amorphous, and are therefore expected to show the highest possible solubility.

3.1.3.2: Stability studies:

Figures 11 a, b and c shows the effect of time on the amorphous drug content of the co-precipitated and spray-dried formulations, three samples evaluated over a 14 day period. For Ketoconazole formulations containing Ethyl Cellulose 7 or 45 cP, the spray-drying process is successful in preparation of stable amorphous formulations. For spray-dried Ketoconazole formulations prepared with EC 100 cP (figure 11c), a steady decline by as much as 10% was observed in the amorphous content during the 2 week period. The co-precipitation process was ineffective in preparing, and therefore stabilizing amorphous formulations of Ketoconazole.

For Chlorpropamide, the results were comparable to Ketoconazole for both spray-dried and co-precipitated formulations prepared with EC 7 cP. The spray-dried formulations were practically completely amorphous and stable, while the co-precipitated formulations were completely crystalline.

Figure 11 (a). Effect of Ethyl Cellulose 7 cP on the stability of amorphous Ketoconazole and Chlropropamide prepared by two formulation processes.

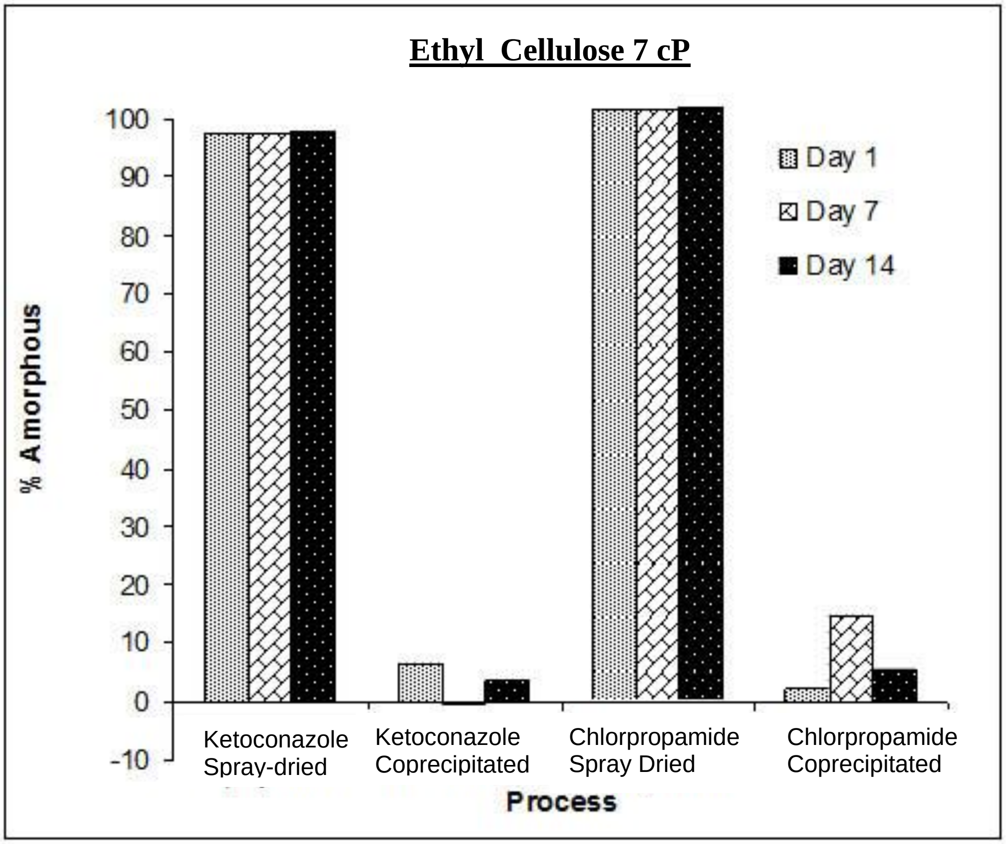


Figure 11 (b) shows that the spray-drying process was less effective for the amorphization of Chlorpropamide compared to Ketoconazole. For spray-dried formulations with EC 45 cP, only about 40% was calculated to be amorphous, and this value decreased by about 5-10% during the 2 week period. Despite the low degree of amorphization, the spray-drying process was still superior to the co-precipitation process for Chlorpropamide formulations prepared with EC 45 cP.

Figure 11 (b). Effect of Ethyl Cellulose 45 cP on the stability of amorphous Ketoconazole and Chlorpropamide prepared by two formulation processes.

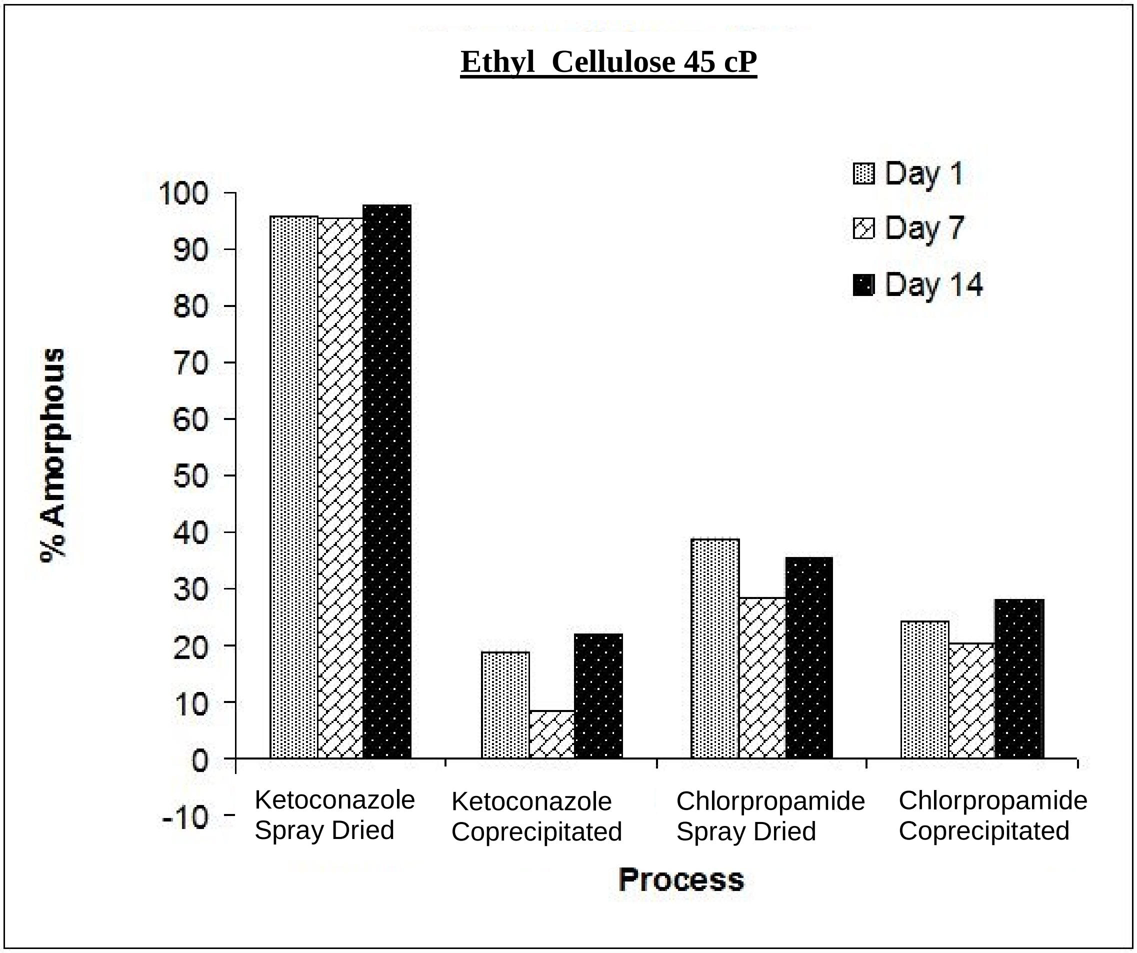
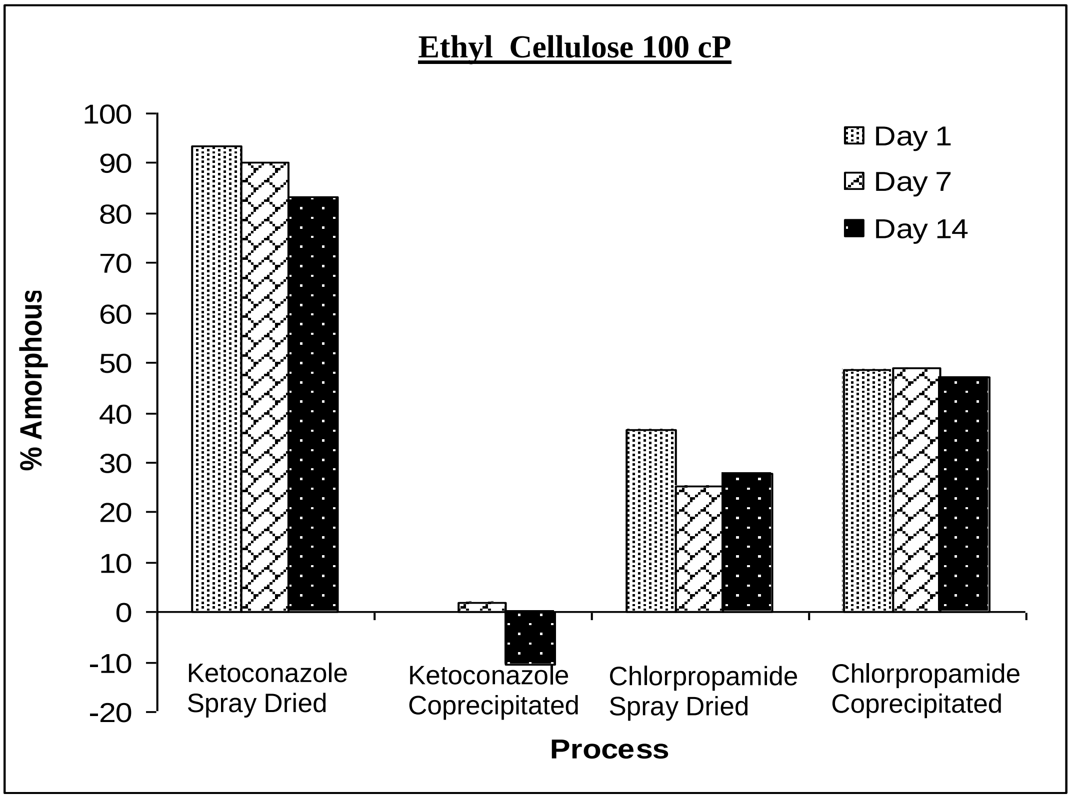


Figure 11 (c) shows a reversal of effect of processing on Chlorpropamide. For Chlorpropamide formulations containing EC 100 cP, the co-precipitation process was much more effective compared to spray-drying. Almost 50% of Chlorpropamide was calculated to be trapped in a stable amorphous state in EC 100 cP.

Figure 11 (c). Effect of Ethyl Cellulose 100 cP on the stability of amorphous Ketoconazole and chlropropamide prepared by two formulation processes.



Overall, the results of this research indicate the following:

1. BCS Class II drugs can be trapped in stable amorphous states by spray drying with low viscosity grades (~ 7 cP) of Ethyl Cellulose.
2. The amorphization efficiency of the spray-drying process goes down significantly with increasing molecular weight (or viscosity grade) of the Ethyl Cellulose used.
3. Spray-dried amorphous formulations prepared with higher molecular weight Ethyl Cellulose (~ 100 cP) have poor stability.
4. The amorphization efficiency of the co-precipitation appears to improve with increasing molecular weights (or viscosity grade) of the Ethyl Cellulose used.

5. For EC 7 cP, both formulation processes show comparable levels of amorphous/crystalline content. For EC 45 and EC 100 cP, the ratios change significantly for Ketoconazole and Chlorpropamide. It therefore appears that the process efficiency is independent of the physicochemical drug characteristics at low Ethyl Cellulose molecular weight (or viscosity grade).

4. DISCUSSION

Ketoconazole and Chlorpropamide are classified as BCS Class II drugs, a class in which bioavailability and clinical efficacy are limited by drug solubility. As the solubility of amorphous drugs tends to be substantially higher than their corresponding crystalline polymorphs, these forms are preferred for BCS Class II compounds. Differential scanning calorimetry of unprocessed Ketoconazole and Chlorpropamide showed high degrees of crystallinity (figure 8), evidenced by distinct melting endotherms, and the absence of the glass transition. The percent crystallinity was calculated to be about 90% for both drugs being evaluated in this study. These results show that the drugs used in this research are good candidates for amophization studies, as they are present predominantly crystalline in their unprocessed form.

An evaluation of Figure 8 shows dual melting endothermal peaks for Chlorpropamide. Multiple endothermal peaks for pure compounds typically represent differences in crystal habit or morphology. The presence of dual endothermal melting peaks in Chlorpropamide has been reported previously[72]. Yeo, et. al., (2003) have reported such peaks for Chlorpropamide, prepared by a super-critical injection process carried out under slow and rapid injection process using acetone or Ethyl acetate as solvent. Yeo, et. al., (2003) suggest that such transitions are typical for different crystalline forms in the same specimen.

Figure: 12: DSC Thermograms of Chlorpropamide crystals: (a) unprocessed and processed, using acetone as solvent, and, (b) processed, using Ethyl acetate as solvent.[72]

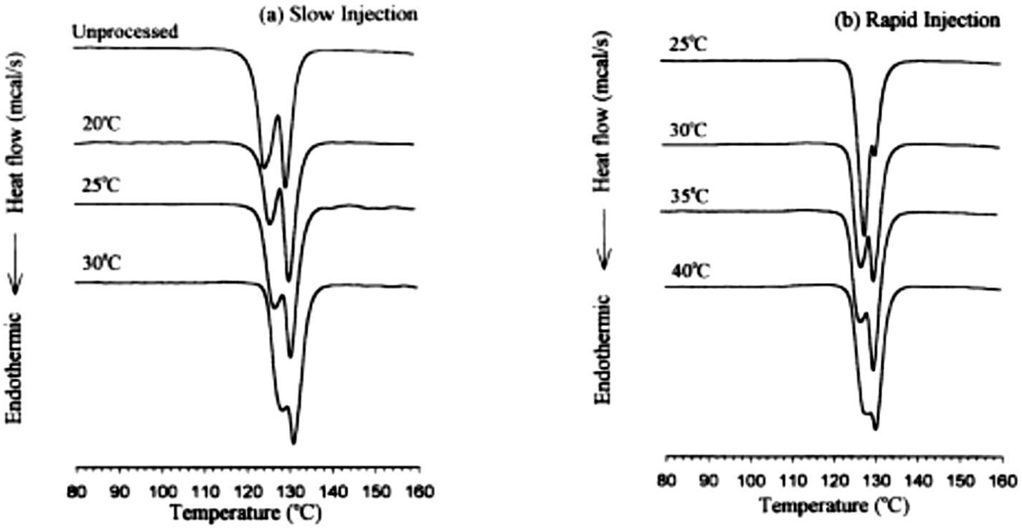


Figure 12 shows that height of each peak varies with processing temperature, with higher processing temperatures favoring the formation of crystalline structures with the higher melting peak.

To prepare amorphous formulations, two specific techniques for were evaluated, namely: non-solvent induced precipitation, and spray-drying. Both these techniques were relatively ineffective in preparing amorphous drug forms, as the % crystallinity values measured by crystalline enthalpies (DSC) were > 80% for both formulations. Consequently, the inclusion of the polymeric excipient, Ethyl Cellulose, was explored in this research for preparation of amorphous forms of Ketoconazole and Chlorpropamide. The use of such polymeric excipients is known to stabilize amorphous structures by fixing the drug molecules in place within the amorphous polymer matrix. Paradkar, et. al., suggest that the stability of drug in solid dispersions could be due to an increase in T_g (antiplasticization), resulting from the entrapment of drug in the polymer matrix during solvent evaporation[73]. In a study by van den Mooter et al., it was shown that antiplasticization is effective for Ketoconazole concentrations of up to 50% w/w [11]. The resulting dispersion exhibits a high viscosity at room temperature that impairs crystallization. Crystallization inhibition can also be due to the formation of hydrogen bonds between the polymer and the drug.

The high melting point of Ethyl Cellulose plays a key role in stabilizing an amorphous dispersion due to the large difference in melting point between the drug and the polymer [74]. Polymers with a higher T_g can increase the physical and chemical stability of amorphous drugs. In addition to T_g several other factors, such as entropy, enthalpy, hydrogen bonding, and moisture content, also affect recrystallization [75]. The molecular weight of a polymer has a bearing on the dissolution rate of the dispersion [41]. The dissolution rate for nortriptyline hydrochloride from its dispersions in PEG was found to be a log-linear function of the PEG molecular weight [77]. With the appropriate choice of polymer, polymer molecular weight, and the composition of the solid dispersions; solubility, and therefore, bioavailability can be greatly improved [76].

In this study, the three grades of unprocessed Ethyl Cellulose showed significant differences in their glass transition temperatures, melting temperatures, and melting enthalpies (table 6). As expected, all these values progressively increased with Ethyl Cellulose molecular weight.

An interesting finding of this research is that the effect of EC molecular weight in the preparation of amorphous formulations varies significantly with the formulation process. For the low viscosity grade Ethyl Cellulose (EC 7), complete amorphization was obtained for both drugs upon spray drying. This result was independent of the nature of the drug, as similar outcomes were obtained for both Ketoconazole and Chlorpropamide. The amorphous formulation obtained by spray drying with EC 7 was stable over a period of 2 weeks. The coacervation process was ineffective for both drugs when EC 7 was used as the excipient. Coprecipitated dispersions with higher molecular weights of Ethyl Cellulose showed higher amorphous content for Chlorpropamide, but not for Ketoconazole.

Mahlin, et. al., showed that the molecular structure of the pure drug profoundly dictates its conversion to its amorphous form than does the preparation methods [79]. Drugs such as Indomethacin and Itraconazole resulted in glassy forms with significant stability, whereas drugs like naproxen showed no amorphous content [79]. Our studies with Ketoconazole and Chlorpropamide showed that the effect of the drug's molecular structure is irrelevant for spray-dried formulations prepared with EC 7. The amorphization efficiency of spray-dried formulations is dictated by drug's molecular structure only at higher Ethyl Cellulose molecular weights. The reason for this molecular weight dependency in spray-dried formulations was unclear from these studies.

A major difference between the spray-drying and coacervation could be related to the solvent extraction time from the formulation. Spray-dried particles are produced and dried almost instantaneously, whereas the co-precipitated formulations are obtained over several hours.

5. CONCLUSION

The results of this research show that spray-drying with low molecular weight Ethyl Cellulose is a promising strategy to enhance the solubility of BCS Class II drugs such as Ketoconazole and Chlorpropamide.

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