A new method was developed to produce an anti-fungal drug, itraconazole, 
(C_{35}H_{38}C_{12}N_{8}O_{4}), using supercritical carbon dioxide. Different operating conditions 
(five levels of temperature, four levels of pressure, four different treatment times, 
flow, cooling and mixing) were tested in order to produce a desired itraconazole 
product which does not degrade during the product formation and has the highest 
dissolution rate in gastric fluid. An intrinsic mathematical model was also 
developed to predict some of the products.

The optimum treatment condition was found to be at 135 °C, 300 atm and 30 
minutes of SC CO₂ flow through itraconazole solution. The dissolution profiles of 
itraconazole in gastric fluid at different treatment conditions predicted by the 
mathematical model agreed with experimental data.
Itraconazole Formation Using Supercritical Carbon Dioxide

by

Yi-Min Tang

A Thesis Submitted
to
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the requirement for the
degree of
Master of Science

Presented March 28, 2000
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorize release of my thesis to any reader upon request.
ACKNOWLEDGMENT

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I would like to give my special thanks to my dear parents for their love and encouragement. Thanks to my many friends here and in Taiwan for their thoughtful support. Also I want to thank all the professors in the Chemical Engineering Department for their technical knowledge and support.
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NOMENCLATURE

A  Interfacial area of drug, cm$^2$
C  Concentration in gastric fluid at any time, mg/L
k  Convective- mass transfer coefficient, cm/min
M  Mass of drug substance remaining to be dissolved, mg
P  Treatment pressure, atm
R$^2$  R-square value
SEE  Sum of the squares of the errors
SST  Sum of the squares of the deviations
T  Treatment temperature, °C
t  Time

Greek letter

$\alpha$  Constant in Eqn. (6.4), kA/V, min$^{-1}$

Subscripts

i  Experiment identifier
c  Critical property
s  Property at saturation condition

Superscripts

Exp  Experimental determined parameter
Cal  Calculated parameter

Symbol

-  Average
ITRACONAZOLE FORMATION USING SUPERCRITICAL CARBON DIOXIDE

CHAPTER 1
INTRODUCTION

Supercritical Fluid science and technology have given new directions in research and applications in the last few years. Supercritical Fluid technology is growing rapidly because of the unique physical properties of such fluids. They can simply change from gas-like to liquid-like by adjusting the pressure and temperature. Because of these special characteristics, supercritical fluids are used in extractions, separations, chemical reactions, impregnation, polymer processing, food processing, environmental remediation and pharmaceutical production (Kiran and Brennecke, 1993).

Supercritical carbon dioxide has been chosen in the new technology because of its several advantages: cheapness, non-toxicity and nonflammable properties, and its availability. It is also easy to operate because of the convenient critical temperature and pressure of carbon dioxide ($T_c = 31.3 \, ^\circ C$, $P_c = 72.9 \, \text{atm}$).

One important application of SCFs is in drug formation. The Food and Drug Administration (FDA) examines new product ingredients, manufacturing processes, toxicological studies on animals, therapeutic claim, and clinical trials on human beings very strictly (Ansel, Popvich and Allen, jr, 1995). The FDA has strict requirements for the use of organic solvents and surfactants in drugs because many organic solvents and surfactants are not safe
for humans or the environment. Organic solvents and detergents are used in some conventional drug formulations to produce a homogeneous solution. Therefore the FDA requires that most solvents and detergents need to be removed from the solution before the drug can be used for human use. Removal of such chemicals usually involves a high temperature process (spray drying and melt pressing), which may cause drug degradation. A comparison between conventional drug formation methods and supercritical carbon dioxide method is shown in Table 1.1.

Itraconazole is an anti-fungal agent, which has therapeutic effects for patients who have fungal diseases, for example, AIDS (Acquired Immune Deficiency Syndrome) patients. It is poorly soluble in aqueous solutions and since it is to be dissolved in gastric or intestine fluids, it is desirable to increase its solubility in aqueous solutions.

In order to reduce drug blood level fluctuations, dosage frequency and health care cost, controlled-release drugs have been developed rapidly. Some drugs, such as itraconazole, need to have rapid oral absorption. Drug particles from conventional formulation methods are larger and less porous resulting in smaller surface area, so the drug takes longer to dissolve in the fluids of human bodies. Sometimes the efficacy of the drug can be severely limited by its poor aqueous solubility, and some side effects of certain drugs are due to poor solubility. Therefore, increasing drug dissolution rate would be highly valuable.
Table 1.1  Comparison of conventional method and supercritical carbon dioxide method.

<table>
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<th>Conventional Method</th>
<th>Supercritical Carbon Dioxide Method</th>
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<tr>
<td>Several steps, such as dissolving drug in solvent, removal of solvent, coating and</td>
<td>Fewer steps. No need for solvent removal, and drying.</td>
</tr>
<tr>
<td>milling or grinding.</td>
<td></td>
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<tr>
<td>Organic solvent and surfactant are needed</td>
<td>SC carbon dioxide instead of organic solvent. CO$_2$ is safe (nonflammable and nontoxic).</td>
</tr>
<tr>
<td>High temperature operation. Degradation or denaturation caused by heat or oxygen in</td>
<td>Mild temperature condition.</td>
</tr>
<tr>
<td>the process.</td>
<td></td>
</tr>
<tr>
<td>High cost of solvent disposal.</td>
<td>Lower cost of solvent disposal (CO$_2$ is safe).</td>
</tr>
<tr>
<td>Size reduction techniques (i.e. milling and grinding) are needed and such techniques</td>
<td>Fine particles can be produced.</td>
</tr>
<tr>
<td>usually affect the crystallinity and chemical stability of pharmaceuticals.</td>
<td></td>
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Supercritical fluid technology has the potential to solve some of the problems associated with conventional drug formulations. The focus of this research is forming itraconazole into a successful drug product formulation using supercritical fluids. Itraconazole and all other ingredients are mixed with supercritical carbon dioxide instead of an organic solvent. Higher porous itraconazole particles can be produced in supercritical carbon dioxide. The larger surface area of itraconazole particles should result in faster dissolution rate of the drug in gastric fluid. Since no or little organic solvent needs to be used in the process, the solvent-handling fee is expected to be lower.

To find the optimum treatment temperature and pressure of supercritical carbon dioxide and the treatment time to produce itraconazole, different experiments at different conditions were performed in this study. The dissolution profiles of itraconazole products produced in this study were measured for each sample in order to determine the dissolution rate of itraconazole in gastric fluid. Dissolution studies were carried out in simulated gastric fluids at 37.5 °C and the results compared with the commercial itraconazole product, Sporanox, as a reference.

Chapter 2 presents an introduction to itraconazole, conventional methods to produce the itraconazole, formulation Sporanox®, and the problems, an introduction to supercritical fluids and a literature review of drug delivery systems, and the controlled release of drugs. The objectives of this work are given in Chapter 3. Chapter 4 discusses experimental materials and methods. The results are presented in Chapter 5. Finally conclusions, and future work are included in Chapter 6.
CHAPTER 2
BACKGROUND AND LITERATURE REVIEW

Itraconazole formulation development includes several steps: solubility study in SC CO₂, formulation study, drug formation methods, drug delivery system and dissolution study. Then there will be some clinical investigations and drug regulatory evaluation before going to market. Those steps are shown in the flow chart (Fig 2.1). Formulation study has been done in the Pharmacy Department. This study focused on supercritical fluids technology and dissolution study. Drug delivery systems development will be studied in the future. SCF technology is introduced in Chapter 2.1. Chapter 2.2 discusses drug delivery systems. Chapter 2.3 is the introduction of itraconazole. Chapter 2.4 includes conventional methods to produce itraconazole and its problems.
Figure 2.1 Flow chart of itraconazole development.
2.1 Supercritical Fluids

A pure supercritical fluid is a substance, which is heated and compressed above its critical point (including its critical temperature \( T_c \) and critical pressure \( P_c \)). Above its critical point, it is neither a liquid nor a gas, but a fluid changing continuously from liquid-like to gas-like as its pressure decreases. The phase diagram of a single substance is shown in Figure 2.2. In the diagram, \( C \) represents the critical point, and \( T \) represents the triple point. Curve TC means the gas-liquid coexistence curve when liquid and gas are in equilibrium. As both the temperature and pressure are increased, curve TC tends towards \( C \). The liquid becomes less dense because of the effect of thermal expansion and the gas becomes denser due to the increasing pressure. SCFs can have better mass transfer through a porous matrix due to the higher diffusivities than normal liquids (Saad and Gulari, 1984). Because the densities of SCFs are higher than normal gases, SCFs can facilitate higher solubility than gases. The densities of liquid and gas phases become equal (called critical density, \( \rho_c \)) at the critical point and the substance becomes a supercritical fluid. The values of densities, solubility and other physical properties in the supercritical region are between those of typical liquids and gases. A comparison of SCF and typical gas and liquid is presented in Table 2.1.

Supercritical Fluids Technology offers a convenient way to change solvating properties from gas-like to liquid-like without changing chemical structure (McNally and Bright, 1992). Therefore, Supercritical Fluids Technology has grown rapidly.
Table 2.2 presents the critical parameters (critical temperatures, critical-pressure and critical densities) of some compounds as supercritical fluids. The most popular compound today is carbon dioxide because of its low critical temperature, non-toxicity, non-flammable property and cheapness. The carbon dioxide molecule is non-polar so it is a non-polar solvent. However, it has some affinity with polar solutes due to its large molecular quadrupole (Westwood, 1993). Therefore, pure carbon dioxide can be used to dissolve many large organic solutes. The most popular application currently is the extraction and recovery of polar organic compounds from aqueous media. In industries, supercritical carbon dioxide technology is used for separation of natural components, such as caffeine from coffee beans and rose oil from roses. Table 2.3 shows some applications. Drug product formulation has caught much attention in application of SCFs Technologies in past years.
Figure 2.2 Phase diagram of a single substance.

Table 2.1 A comparison of physical properties of SCFs, a typical gas and liquid (Westwood, 1993).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Gas</th>
<th>SCF</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion Coefficient (cm$^2$/s)</td>
<td>$10^{-1}$</td>
<td>$10^{-3}$</td>
<td>$10^{-5}$</td>
</tr>
<tr>
<td>Density (g/cm$^3$)</td>
<td>$10^{-3}$</td>
<td>0.2-0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Viscosity (mPa s)</td>
<td>$10^{-2}$</td>
<td>$10^{-2}$-$10^{-1}$</td>
<td>1.0</td>
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Table 2.2  Critical parameters of some useful compounds as SCFs (Anderson, 1993).

<table>
<thead>
<tr>
<th>Substance</th>
<th>$T_c$ (°C)</th>
<th>$P_c$ (MPa)</th>
<th>$\rho_c$ ($10^3$ Kg/m$^3$)</th>
</tr>
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<tr>
<td>CO$_2$</td>
<td>31.30</td>
<td>7.39</td>
<td>0.47</td>
</tr>
<tr>
<td>N$_2$O</td>
<td>36.50</td>
<td>7.35</td>
<td>0.45</td>
</tr>
<tr>
<td>SF$_6$</td>
<td>45.50</td>
<td>3.76</td>
<td>0.74</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>132.50</td>
<td>11.40</td>
<td>0.24</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>374.00</td>
<td>23.00</td>
<td>0.34</td>
</tr>
<tr>
<td>n-C$<em>4$H$</em>{10}$</td>
<td>152.00</td>
<td>3.80</td>
<td>0.23</td>
</tr>
<tr>
<td>n-C$<em>5$H$</em>{12}$</td>
<td>197.00</td>
<td>3.78</td>
<td>0.23</td>
</tr>
<tr>
<td>Xe</td>
<td>16.60</td>
<td>5.92</td>
<td>1.10</td>
</tr>
<tr>
<td>CC$_{12}$F$_2$</td>
<td>112.00</td>
<td>4.13</td>
<td>0.56</td>
</tr>
<tr>
<td>CHF$_3$</td>
<td>25.90</td>
<td>4.75</td>
<td>0.52</td>
</tr>
</tbody>
</table>
Table 2.3  Commercial-scale supercritical CO₂ extraction processes (Anonymous, 1995).

<table>
<thead>
<tr>
<th>Process</th>
<th>Plant Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee Decaffeination</td>
<td>Bremen, Germany (two plants), Italy, Poszzillo, Houston and Texas</td>
</tr>
<tr>
<td>Tea Decaffeination</td>
<td>Germany and Munchmuenster</td>
</tr>
<tr>
<td>Fatty Acids from Spent Barley</td>
<td>Dusseldorf and Germany</td>
</tr>
<tr>
<td>Nicotine Extraction</td>
<td>Hopewell and Virginia</td>
</tr>
<tr>
<td>Rose-Residual Oil SCE</td>
<td>Oklahoma City</td>
</tr>
<tr>
<td>CO₂ Refining of Extracted Pyrethrum</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>Hops Extraction and Spices</td>
<td>Australia, Germany (two plants), Melbourne, Munchmuenster, Reigat, United Kingdom, Wolnzach, Nebraska, Washington (two plants) and Yakima</td>
</tr>
<tr>
<td>Flavors Extraction</td>
<td>France and Grasse</td>
</tr>
<tr>
<td>Flavors/Aromas</td>
<td>Germany and Rehlingen</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>Japan</td>
</tr>
<tr>
<td>Color Extraction-Red Pepper</td>
<td>Japan (six plants)</td>
</tr>
</tbody>
</table>
2.2 Drug Delivery Systems

After producing effective drugs, the next step will be to develop the best drug delivery system for the specific drug and control drug release at the desired rate, which can enhance the efficacy of therapeutic agents through controlled release. Drug delivery systems refer to the technology utilized to deliver drugs to the desired site inside the body for drug release and absorption. The difference between the approach of the classical or traditional drug discovery process and new ones is that screening systems now are much more selective, involving the use of in vitro receptor binding techniques and enzyme inhibition assays. In order to develop the successful dosage forms, it is convenient to use delivery route as a meaning of classifying drug delivery issues. There are site-specific, implantable, ocular, transdermal, intranasal, sublingual, buccal, intravenous, intramuscular, subcutaneous, inhalation, and oral delivery systems, etc. The site-specific delivery systems deliver drugs directly to affected tissues. Implantable drug delivery systems are placed completely under the skin-usually in a convenient but inconspicuous location. They are small solid dosage forms containing concentrated drug and they continuously release their medication over prolonged periods without repeated insertion of needles. Ocular drug delivery includes drug-impregnated membranes. Transdermal supports the passage of drug substances from the surface of the skin through its various layers and into the systemic circulation. Drug materials applied topically to the nasal conjunctiva will enter the nose through the nasolacrimal duct just beneath the anterior end of the inferior turbinate. Sublingual drug delivery systems are to put drugs under tongue. Buccal drug delivery is to place drug tablets at the
sides inside mouth. Intravenous drug delivery is to inject drugs directly into blood. Intramuscular delivery systems are to inject drugs directly into muscles. Subcutaneous drug delivery systems deliver drug into skin by injection. Inhalation drug delivery is to spray drugs through mouth. Oral is, and will be the most convenient and commonly employed route of drug delivery systems. There are many technologies available for oral drug delivery systems from liquids, capsules and tablets through various sustained-release systems to highly sophisticated osmotic devices (Gardner, Sharp, Laboratories and Point, 1987). The oral drug delivery system is also the system chosen in this study.

2.3 Itraconazole

Itraconazole (C_{35}H_{38}C_{12}N_{8}O_{4}, molecular weight = 705.64) is a synthetic triazole anti-fungal drug (Figure 2.3). Itraconazole is a white to slightly yellowish powder. It consists of a 1:1:1:1 racemic mixture of four diastereomers, each of which has three chiral centers. Itraconazole is highly lipophilic so it is barely soluble in aqueous solutions (less than 100 mcg /100 mg in aqueous media within PH = 1-12.7). It is most soluble in dichloromethane (25 mg /100 mg). The solubility of itraconazole in various solvents and in aqueous media is shown in Table 2.4. Itraconazole is a weak base (pKa = 3.7) and has a log C (n-octanol/water) partition coefficient of 5.66 when PH = 8.11 (Kapsi, 1998). It can distribute extensively in tissues and especially is found in higher-level tissues, such as in brain. The half-life of itraconazole, the time required for the drug’s blood or plasma concentration to decrease by one half is very long (about 20 hours). Itraconazole can inhibit cytochrome
P-450-dependent metabolism of ergosterol, which is a vital component of fungal cell membranes, and then interfere in sterol biosynthesis causing the death of cells inside the membranes of cells (Kapsi, 1998). Clinical use of itraconazole is treatment of fungal infections in immunocompromised and non-immunocompromised patients. Patients who have blastomycosis, pulmonary, extrapulmonary, histoplasmosis and aspergillosis diseases can take itraconazole. Itraconazole is also used for patients with onychomycosis of the toenail with or without fingernail involvement due to dermatophytes (marketed itraconazole, Sporanox).

**Figure 2.3** Chemical structure of itraconazole.
Table 2.4  Itraconazole solubility in different solvents and aqueous media (Kapsi, 1998).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility in g/100ml Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (pH = 7.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hexane</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>0.002</td>
</tr>
<tr>
<td>2-propanol</td>
<td>0.008</td>
</tr>
<tr>
<td>Polyethylene glycol 400</td>
<td>0.013</td>
</tr>
<tr>
<td>Tetrachloroethane</td>
<td>0.013</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.026</td>
</tr>
<tr>
<td>4-methyl 2-pentanone</td>
<td>0.058</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.061</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.11</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.12</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.16</td>
</tr>
<tr>
<td>Polyethylene glycol 400</td>
<td>0.19</td>
</tr>
<tr>
<td>2-propanone</td>
<td>0.20</td>
</tr>
<tr>
<td>2-butanone</td>
<td>0.23</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>1.1</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>2.2</td>
</tr>
<tr>
<td>N,N-dimethyl formamide</td>
<td>3.5</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>25</td>
</tr>
</tbody>
</table>
2.4 Conventional Method and Problems of Producing Itraconazole and Formulations

From Table 2.4, it is seen that itraconazole is most soluble in dichloromethane (methylene chloride); however, it is poorly soluble in water, and other aqueous or organic solvents. In today’s pharmaceutical industry, the existing formulation of itraconazole is Sporanox produced by Janssen Pharmaceutica. A solution was prepared by adding itraconazole powder and hydroxypropyl methylcellulose (1:1.5) into dichloromethane and denatured ethanol (60:40) while stirring. Another seal-coating spraying solution with dichloromethane and polyethylene glycol 20000 (5.4:1) were prepared while stirring, denatured ethanol was added, and the solution was stirred until homogeneous. The itraconazole solution was sprayed to the spheres of sugar beads and then those sugar beads were dried to remove dichloromethane at a temperature of about 80 °C and at a pressure of about 200-300 mbar (20-30 kPa). The prepared seal-coating solution was then sprayed to the itraconazole containing beads from the former step, and dried again at 50-55°C. Because itraconazole has very low solubility in other aqueous or organic solvents, sticks together easily after the process, and precipitates in gastric fluids, HPMC was added as a hydrophilic agent as well as to avoid precipitating, and polyethylene glycol 20000 (PEG) was added to prevent the drug loaded beads from sticking together. The biggest problem here is the use of dichloromethane because the FDA has very strict requirements for minimum presence of dichloromethane (less than 0.005 %). Therefore a drug delivery system without the use of highly regulated organic solvents needs to be developed. Another problem is too many steps in this process. In addition, it is known that itraconazole needs a
faster release in gastric fluids because it is only 56% bioavailable. Using SCF technology has solved those problems.
CHAPTER 3
OBJECTIVES OF THIS RESEARCH

The goal of this study is to develop an immediate release delivery system for itraconazole. There are three main objectives summarized below:

Objective 1
Avoid the use of organic solvents to develop a product of the water-insoluble anti-fungal agent, itraconazole while is bioequivalent to Sporanox. Carbon dioxide will be used instead of methylene chloride.

Objective 2
Find the optimum operating conditions in this itraconazole formulation production process, including temperature, pressure, treatment time, mixing and flow.

Objective 3
Meet the itraconazole dissolution requirement: higher than 80% drug dissolution at 45 minutes in simulated gastric fluid at 37.5 °C, 100 rpm.
CHAPTER 4
EXPERIMENTAL MATERIALS AND METHODS

4.1 Introduction

This research was designed to produce an itraconazole formulation, which has high dissolution in simulated gastric fluid (higher than 85% at 45 minutes). The optimum extraction pressure and temperature were found during SCF extraction of itraconazole. In this chapter, the experimental apparatus, methods and materials used in this study to form what itraconazole are discussed. Section 4.2 discusses the itraconazole formulation used in this study. Section 4.3 describes the equipment used for the treatment of itraconazole formulation with SC CO2. Experimental method is discussed in section 4.4 and the last section of this chapter explains the chemicals used in this study.

4.2 Formulation of Itraconazole Formation

Ingredients used in the itraconazole formulation have been studied by Shivakumar Kapsi in 1998 in the college of Pharmacy at Oregon State University. The formulation includes five ingredients: itraconazole, polyethylene glycol 20000 (PEG), a hydrophilic polymer, a super disintegrated sodium starch, and a wetting agent. PEG 20000 was selected between PEG 3350, 8000 and 20000 because itraconazole dissolution in gastric fluid improved considerably (10%) as the molecular weight of PEG increased. The hydrophilic polymer was found to prevent precipitation of the drug during dissolution. The sodium starch was added to speed up drug release in gastric
fluid. A wetting agent has been chosen to enhance the solubility of the drug (Kapsi, 1998). The problem of this method was the difficulty of controlling treatment temperature and high temperature may cause degradation of drug. In this study, treatment temperature was well controlled by the SCF Extraction system. SC CO₂ is added to the above formulation in order to increase drug dissolution and enhance drug release in gastric fluid.

4.3 Supercritical Fluid Extraction System

The schematic diagram of experimental apparatus is shown in Figure 4.1. This equipment consists of a SCF Extractor (SFX™ 2-10) and a syringe pump and controller system (ISCO 2601). Supercritical Fluid Extractor is made for high-temperature and high-pressure usage. It consists of an oven for heating, a temperature controller and two 10-ml stainless steel cells. The syringe pump from ISCO was used to feed carbon dioxide to the extractor. The pump system consisted of a control panel to set the pressure or flowrate. The pressure range was 10 to 7,500 psi and the pressure accuracy was ±2 % of full scale (ISCO manual). A vial was connected to the extractor to collect samples.
Figure 4.1 Schematic diagram of experimental equipment.
4.4 Experimental Method

A simple one-step extraction method was used in this study. There was no secondary solvent removal step required using this method. Therefore, all waste disposal fees were eliminated. First a desired amount of sample including all ingredients (itraconazole, PEG 20000, the hydrophilic polymer, the sodium starch and glycerol) was loaded into the cell and mixed before introducing the treatment system. The syringe pump was then filled with carbon dioxide from a supply cylinder. Parameters, such as cell temperature and pump pressure, were set to the desired values. Carbon dioxide at supercritical conditions was added to the cell. After treatment equilibrium was reached (about 15 minutes), supercritical solution was allowed to flow into the vial. To generate mixing in the cell, flow rates of 0.5 to 2.0 ml/min were used in all experiments. After a desired period of time, the pressure in the cell was suddenly dropped to atmospheric pressure.

The solution in the cell was cooled by a cooling system Dionex SFE 703 or in dry ice. The product was ground in a blender (Waring commercial laboratory blender) and particle ranging in size between 0.2-1 mm were sieved through two different meshes. Dissolution profiles of itraconazole release were obtained using the United States Pharmacopoeia (USP) XXII apparatus II (VK 7000®, Vankel Industries, Inc., Edison, NJ). The dissolution measurement was a paddle stirring method as shown in Figure 4. The equipment of VK 7000 is shown in Figure 4.2. Dissolution media consisted of 900 ml of enzyme-free simulated gastric fluid (PH = 1.4 ± 0.1) maintained at 37 °C. The solution was mixed at 100 rpm for 60 minutes. 3 ml dissolution samples were collected at 5,
diluted to 10 ml with simulated gastric fluid. Finally, the 10 ml diluted solution was taken for UV analysis at a fixed wavelength of 226 nm using a Beckman DU-600 spectrometer.
Figure 4.2 Schematic diagram of dissolution apparatus.
4.5 Source of Materials

Grade 2.8-carbon dioxide was purchased from Industrial Welding Supply (Albany, Oregon). Itraconazole powder was provided by College of Pharmacy, Oregon State University. Polyethylene glycol 20000 was purchased from Sigma Chemical Co, St. Louis. Sodium starch was purchased from Edward Mendell Co. The hydrophilic polymer was from Dow Chemical Co, Midland, and the wetting agent was provided by Sigma Chemical Co, St. Louis.
CHAPTER 5
RESULTS AND DISCUSSION

The dissolution profile of innovator itraconazole formulation (Sporanox®) is shown in Figure 5.1. 90% itraconazole was dissolved in gastric fluid after 60 minutes. Better products, which have higher dissolution and faster drug release than Sporanox® were produced. This chapter presents in this study important results obtained from scanning electron microscopic pictures and dissolution measurements is divided into five sections. The first section presents two different ways to show the effect of carbon dioxide on the product formed and its dissolution profiles; the particle morphology and solvent power. The second section shows the effect of treatment temperature. The effect of treatment time is explained in the third section. The next section discusses how mixing during the treatment process affects the dissolution profiles of itraconazole. The last section shows the effect of two cooling methods on the dissolution results after the drug was formed. The solubility of itraconazole in SC CO₂ is low because no drug was found in the collecting vial. However, SC CO₂ is soluble in drug solution because it was visually seen CO₂ coming out of the drug solution when dropping the pressure after treatment. The solubility of SC CO₂ in drug solution is a function of treatment temperatures, treatment pressures, treatment time and mixing. The products from SC CO₂ method are white and porous solid. 96% of the chemicals were recovered. Yellowish solid would be formed due to high treatment temperature (>140 °C) or long treatment time (> 30 minutes).
Figure 5.1 Dissolution profile of itraconazole from innovator product, Sporanox.
5.1 Effect of Carbon Dioxide

Supercritical carbon dioxide has significant effect on the itraconazole and its dissolution profile. This fact can be proved from two ways: the particle morphology, and solvent power.

5.1.1 Particle Morphology of Itraconazole Products

Scanning electron microscopic (SEM) photomicrographs of two different samples are shown in Figures 5.2 and 5.3. The sample in Fig. 5.2 was without supercritical carbon dioxide and that in Fig. 5.3 was treated with carbon dioxide at 300 atm. The one without SC CO₂ results in a solid network consisting of intertwining aggregated cubes. The morphology in Fig. 5.3 consists of many thin layers with small pores inside. The different morphology in Fig. 5.3 was due to the effect of CO₂ during the treatment and depressurization. The expansion of the drug solution by depressurizing SC CO₂ produced more porous products. BET surface area measurement is needed to quantify the actual surface area of these samples.
Figure 5.2  SEM photomicrograph of itraconazole formed at the treatment condition: without SC CO$_2$, $T = 135$ °C and $t = 10$ minutes.
Figure 5.3 SEM photomicrograph of itraconazole formed at the treatment condition: with SC CO₂ (P = 300 atm), T = 135 °C and t = 10 minutes.

To see the effect of CO₂ on dissolution profile, experiments were performed with and without CO₂ at different conditions and the results are presented in Figures 5.4, 5.5 and 5.6. These figures show that the amount of dissolved drug in gastric fluid increases significantly (23-40 %) in one-hour dissolution experiment with CO₂ at 300 atm compared to runs without CO₂. Results from dissolution measurement and SEM photomicrographs of the product prove that CO₂ has significant effects on the morphology of the drug and its dissolution profile. Figures 5.4, 5.5 and 5.6 are all the same P, T, treatment
time but they differ in whether explotab was added at the end (Fig. 5.4) and if there was mixing during heating process (Fig. 5.6). Figure 5.6 is without mixing during heating process and explotab was added at the beginning with other ingredients. Fig. 5.4 shows that the drug precipitated after 20 minutes.

Product, that precipitate in gastric fluid are not desirable and thus explotab was added to the ingredients at the beginning of the process in all other experiments. From Figures 5.5 and 5.6, it can be concluded that mixing during heating process did not have significant effect on the products, and thus the other experiments are wither with mixing or without mixing during the heating process.

In Figures 5.4, 5.5 and 5.6, the itraconazole dissolution in gastric fluid were much higher when CO₂ was used during the treatment conditions compared to runs without CO₂. This shows that the use of CO₂ enhances the dissolution rate of the product in gastric fluid. Another way of showing the importance of CO₂ is to look at the temperature needed to obtain the same dissolution rates with and without CO₂ (Fig. 5.7). As shown in the figure, almost the same dissolution profiles are obtained for drugs produced with and without CO₂. However, the drug produced with CO₂ requires 15 °C lower temperature than that produced without CO₂. As higher temperatures (> 140 °C) may result in drug decomposition, the product produced with CO₂ is more favorable. The time delay in Fig. 5.7 is due to the fact that the capsule dissolved about 1–4 minutes slower than the other samples after it was placed in gastric fluid.
Figure 5.4 Dissolved amount of itraconazole as a function of time. Treatment conditions: with CO₂ (P = 300 atm) or without CO₂, T = 130 °C, t = 10 minutes and add explotab at the end.
Figure 5.5 Dissolved amount of itraconazole as a function of time. Treatment conditions: with CO$_2$ (P = 300 atm) or without CO$_2$, T = 130 °C and t = 10 minutes and without mixing during heating process.
Figure 5.6 Dissolved amount of itraconazole as a function of time. Treatment conditions: with CO$_2$ (P = 300 atm) or without CO$_2$, T = 130 °C, t = 10 minutes and with mixing during heating process.
Figure 5.7  Dissolved amount of itraconazole as a function of time. Treatment conditions: with CO\(_2\) (P = 300 atm, T = 135 °C) or without CO\(_2\) (T = 150 °C), t = 10 minutes and with mixing during heating process.

5.1.2 Solvent Power

Solvent power is the strength of solvent for dissolving solutes. A SEM photomicrograph of the product formed at a subcritical pressure (P = 30 atm) is presented in Figure 5.8. The picture shows two kinds of morphologies. On the right side is a network of aggregated cubes similar to Figure 5.2. The left side consists of some large pieces with a few pores inside. Fig. 5.8 seems to be a combination of Fig. 5.2 and Fig. 5.3. The significantly different morphologies observed in Figures 5.8 and 5.3 may due to the different solvent powers at the to pressures.
Figures 5.9, 5.10 and 5.11 present the dissolved drug as a function of time at different treatment pressures. Higher operating pressures give higher dissolution rates because the solvent strength increases and more SC CO$_2$ gets into the drug solution as pressure increases. Results from these figures show that CO$_2$ and its solvent power are important in producing a product with a high dissolution rate in gastric fluid. From Figures 5.9 and 5.10, the drugs that were conditioned to a pressure of 400 atm precipitated in gastric fluid. Therefore, pressures above 300 atm were not used in proceeding experiments.

Fig. 5.9 shows a much lower (about 30-60 %) dissolution than Figures 5.10 and 5.11 due to the lower temperature used in Figure 5.8 (120 °C compared to 130 and 135 °C). Temperature was an important parameter on dissolution of the product in gastric fluid because solubility of itraconazole in the solution is a strong function of temperature. Effect of temperature on dissolution profile is discussed in section 5.2.
Figure 5.8  SEM photomicrograph of itraconazole formed at the treatment condition: with CO₂ (P = 30 atm), T = 135 °C, t = 30 minutes.
Figure 5.9  Dissolved amount of itraconazole as a function of time. Treatment conditions: P = 200, 300 and 400 atm, T = 120 °C, t = 10 minutes, without mixing during heating process and with flow.
**Figure 5.10** Dissolved amount of itraconazole as a function of time. Treatment conditions: $P = 200$, 300 and 400 atm, $T = 130^\circ C$, $t = 10$ minutes, without mixing during heating process and with flow.
Figure 5.11 Dissolved amount of itraconazole as a function of time. Treatment conditions: $P = 30, 200$ and $300$ atm, $T = 135$ °C, $t = 30$ minutes, with mixing during heating process and with flow.
5.2 Effect of Temperature

Two SEM photomicrographs of samples formed at two different treatment temperatures are shown in Figure 5.12 and 5.3. The product formed at the lower temperature (120 °C) (Figure 5.12) consists of intertwining solid pieces, which may have lower porosity and surface area. Dissolved itraconazole as a function of time at various treatment temperatures are shown in Figures 5.14, 5.15 and 5.16. From Figure 5.14, the dissolution profile of the product at 140 °C resulted in precipitation of itraconazole in gastric fluid; therefore, this temperature has been eliminated in later experiments. Higher treatment temperatures showed higher dissolution of drug in gastric fluid due to the fact that more itraconazole would dissolve in PEG solution at high temperatures. Of course temperatures of 140 °C and higher may result in decomposition or degradation of drug. The optimum temperature was found to be 135 °C.
Figure 5.12  SEM photomicrograph of itraconazole formed at treatment condition: \( P = 300 \text{ atm}, \ T = 120 \, ^\circ\text{C} \) and \( t = 10 \text{ minutes} \).
Figure 5.13  SEM photomicrograph of itraconazole formed at treatment condition: $P = 300$ atm, $T = 135 \, ^\circ C$ and $t = 30$ minutes.
Figure 5.14  Dissolved amount of itraconazole as a function of time. Treatment conditions: T = 110, 120, 130 and 140 °C, P = 200 atm, t = 10 minutes, without mixing during heating heating process and with flow.
Figure 5.15 Dissolved amount of itraconazole as a function of time. Treatment conditions: $T = 110$, 120 and 130 °C, $P = 300$ atm, $t = 10$ minutes, without mixing during heating process and with flow.
Figure 5.16 Dissolved amount of itraconazole as a function of time. Treatment conditions: T = 120, 130 and 135 °C, P = 300 atm, t = 10 minutes, with mixing during heating process and with flow.

5.3 Effect of Treatment Time

Figures 5.3 and 5.13 present the different morphology of the products produced at different treatment times. No notified difference in morphologies was observed. Effect of treatment time on dissolution profile of itraconazole in
gastric fluid is shown in Figures 5.17 and 5.18. The amount of dissolved itraconazole in gastric fluid increased with the increase of treatment time, except for one-hour treatment time. One-hour treatment time may cause decomposition of drug due to a long exposure of drug to a high temperature and formed yellowish product. Therefore, the optimum treatment time was found to be 30 minutes.

Figure 5.17 Dissolved amount of itraconazole as a function of time. Treatment conditions: \( t = 10 \) or 30 minutes, \( T = 130 \, ^\circ\text{C} \), \( P = 300 \, \text{atm} \), without mixing during heating process and with flow.
Figure 5.18 Dissolved amount of itraconazole as a function of time. Treatment conditions: $t = 10, 20, 30$ and 60 minutes, $T = 135 \, ^\circ C$, $P = 300 \, \text{atm}$, with mixing during heating process and with flow.
5.4 Effect of Mixing during Treatment

Dissolution profile of itraconazole formed with CO₂ flow (0.5-2 ml/min) or without CO₂ flow during the treatment process is shown in Figure 5.19. Having a small CO₂ flow rate (≤ 2 ml/min) resulted in higher dissolution rates. Flow of SC CO₂ through the treatment cell may generate a better mixing of the ingredients leading to a product, which contains more itraconazole and has a higher dissolution rate in gastric fluid.

Figure 5.19 Dissolved amount of itraconazole as a function of time. Treatment conditions: with flow (0.5-2 ml/min) or without flow, T = 135 °C, P = 300 atm, t = 30 minutes and with mixing during heating process.
5.5 Effect of Cooling

When the treatment process is completed, the solution must be rapidly cooled to entrap the drug in solution in the interstitial spaces of PEG 20000. To see the effect of cooling rate on dissolution profile of itraconazole in gastric fluid, two different cooling methods were used. Cooling in Dionex (-6 °C) or dry ice (-57 °C) did not have a considerable effect (about 3 %) on the dissolution of drug in gastric fluid (Fig. 5.20). Cooling in Dionex system was selected for all other experiments.

Figure 5.20 Dissolved amount of itraconazole as a function of time. Treatment conditions: cooled in dry ice or Dionex, T = 130 °C, P = 300 atm, t = 10 minutes, without mixing during heating process and with flow.
CHAPTER 6

MODEL DEVELOPMENT FOR ITRACONAZOLE RELEASE

6.1 Introduction

It is well known that drug availability in the market is usually determined by the dissolution rate of the drug from the dosage form. The release of drug from a drug product may be influenced by the physical and chemical properties of the drug dosage form. Also, the release of a drug from its physical structure is determined by the rate at which it dissolves in the surrounding medium (Banakar, 1992). The rate of dissolution of a drug is defined as the amount of the drug substance going into the solution from a solid state per unit time in standard conditions. In this study, a simple but clear intrinsic dissolution model was developed to define itraconazole dissolution in the gastric fluids.

6.2 Mathematical Model Used for Dissolution Profile

The intrinsic dissolution model was chosen in this study. Dissolution rate involves some physical parameters, such as interfacial area, shape and the substance and the solubility of the substance in the solution. Dissolution can be described as a heterogeneous system resulting in a net effect of mass transfer from the escape from the surface of small drug particles to solution. It can be expressed mathematically as

\[
\frac{dM}{dt} = kA(C_s - C)
\]  

(6.1)
or

\[ V \frac{dC}{dt} = kA(C_s - C) \quad (6.2) \]

where \( V \) is the volume of the solution; \( M \) is the mass of drug substance in the solution; \( A \) is the interfacial surface area of drug; \( C_s \) is the saturation concentration usually referred to as solubility in the medium; \( C \) is the concentration dissolved into the solution at any time \( t \); and \( k \) is the convective-mass transfer coefficient.

In this model, the capacity coefficient, \( kA \) was assumed to be constant during the dissolution process because the solution was a very dilute system as the amount of dissolved drug into the solution medium was small compared to the 900-ml gastric fluid. Therefore, the volume of the solution was also assumed to be constant.

The initial and final conditions in this study were \( C = 0 \) at \( t = 0 \) and \( C = C_s \) at \( t = \infty \).

Rearranging equation (6.2) gives

\[ \frac{dC}{dt} = \alpha(C_s - C) \quad (6.3) \]

where \( \alpha = k(A/V) \)

Integrating equation (6.3) and applying the initial and final conditions give

\[ C = C_s(1 - e^{-\alpha t}) \quad (6.4) \]
was experimentally determined by keeping the itraconazole formulation for about two days in gastric fluid. Two products were used to determine $C_i$. One product was obtained at the best treatment condition ($T = 130\,^\circ C$, $P = 300\,\text{atm}$, $t = 30$ minutes, with mixing during heating process and with flow during treatment), and the other product was obtained without $CO_2$ ($T = 150\,^\circ C$) to obtain the same dissolution profile. The average of $C_i$ of two products was $140.3744\,\text{mg/L}$. The R-square fitting method was used to find the parameters, $\alpha$. The equation is expressed mathematically as

$$R^2 = 1 - \frac{\text{SSE}}{\text{SST}}$$

(6.5)

where SSE is the sum of the squares of the errors, and SST is the sum of the squares of deviations.

The mathematical definition for SSE and SST are as follows

$$\text{SSE} = \sum_i (C_i^{Exp} - C_i^{Cal})^2$$

(6.6)

$$\text{SST} = \sum_i (C_i^{Exp} - \overline{C}^{Exp})^2$$

(6.7)

where $C_i^{Exp}$ is the experimental concentration at time $t$ and $C_i^{Cal}$ is the calculated concentration at the same time. $\overline{C}^{Exp}$ is the average experimental concentration.
6.3 Results and Discussion

The time delay happened sometimes in dissolution test due to the fact that capsules dissolved in gastric fluid slower (1-4 minutes). Those dissolution profiles have been shifted to correct the time delay before model fitting.

Parameters $\alpha$ and $R^2$ for different treatment conditions are tabulated in Table 6.1. Experimental and predicted values of dissolved itraconazole in gastric fluid for different treatment times are shown in Figure 6.1. The model for 30-minute treatment condition fitted the data very well (the R-square = 0.9597). The model can fit better for longer treatment time. Experimental and calculated dissolution profile for different treatment temperatures are shown in Figure 6.2. The model for the highest treatment temperature ($T = 135^\circ$C) fitted the model best (R-square = 0.9458). The model can predict better fit for higher treatment temperature condition. A similar comparison plot was generated for the treatment pressures (Fig. 6.3). The model for the highest treatment pressure fitted the model very well (R-square = 0.9597).
Table 6.1  Calculated parameters of the intrinsic model

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<th>Parameters of the model</th>
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Figure 6.1 Experimental and calculated dissolved amount of itraconazole as a function of time. Treatment conditions: t = 30, 20 and 10 minutes, T = 135 °C, P = 300 atm, with mixing during heating process and with flow.
Figure 6.2 Experimental and calculated dissolved amount of itraconazole as a function of time. Treatment conditions: $T = 135, 130$ and $120 \degree C$, $P = 300$ atm, $t = 10$ minutes, with mixing during heating process and with flow.
Figure 6.3 Experimental and calculated dissolved amount of itraconazole as a function of time. Treatment conditions: $P = 300, 200$ and $30 \text{ atm}$, $T = 135 \, ^\circ\text{C}$, $t = 10 \text{ minutes}$, with mixing during heating process and with flow.
CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

A new method was developed to form a soluble anti-fungal agent, itraconazole, using pure supercritical carbon dioxide, instead of highly regulated organic solvents. This method was easy to operate because the treatment temperature and pressure were well controlled by ISCO SFE Extractor system.

All the samples were formed using the same formulation in this study, but the amounts of itraconazole dissolved in the drug solution at different treatment conditions were different and this explains why some products have lower dissolution rate than the others.

100 % itraconazole dissolution in one-hour was achieved at the treatment condition: T= 135 °C, P = 300 atm, t = 30 minutes, with mixing during heating process, with flow and cooling in Dionex system. SC CO₂ treatment improved dissolution of itraconazole in gastric fluid by 23-40 %. This increase in dissolution rate was due to the solvent power of CO₂ and possibly the higher porosity of the products. Higher pressure gives higher solvent power, but treatment pressures in excess of 300 atm caused precipitation of drug in gastric fluid. In order to get the same dissolution, the product without SC CO₂ treatment had to be produced under higher treatment temperature (15 °C higher).

Treatment temperature also has a significant effect on dissolution results. Temperatures higher than 140 °C may degrade the drug or cause precipitation in gastric fluid. Low temperatures (<120 °C) resulted in low dissolution rates of
itraconazole in gastric fluid. Therefore, the optimum treatment temperature was found to be 135 °C. Treatment time when used with a high temperature was proved to be an important factor on dissolution results. The product at 135 °C treated for 30 minutes obtained 25 % higher dissolution than one, which was treated for 10 minutes. Mixing during the treatment was also found to be an important effect to improve the dissolution. Due to the equipment limitations, mixing was generated by flow rate of the SC solution. The dissolution increased 20-25 % when mixing was generated. Rapid cooling can trap itraconazole in the solution in the interstitial spaces of PEG 20000. However, the dissolution was not significantly affected by the cooling rate.

An intrinsic dissolution model was developed with different parameters (α) at higher treatment temperature, pressure and longer treatment time and was tested to be able to predict the dissolution profiles of itraconazole in gastric fluid. The R-square values ranged from 0.1945 to 0.9597.

7.2 Recommendations

BET surface area measurements would be useful in correlating products’ surface area to dissolution profile.
BIBLIOGRAPHY


3. S. Westwood, Supercritical Fluid Extraction and Its Use in Chromatographic Sample Preparation (1993)


5. M. Paulaitis, J. Penninger, R. Gray, Jr., P. Davidson, Chemical Engineering at Supercritical Fluid Conditions (1983)


11. C. Hsu, “Kinetic Study of Si(NH₄)₂ Synthesis via Low Temperature Vapor Phase Reaction of SiCl₄ and NH₃ in a Fluidized Bed Reactor”, M.S. Thesis, Oregon State University, Corvallis, OR (1994)


17. K. Park, Controlled drug Delivery Challenges and Strategies, (1997)


APPENDICES
Appendix A: Standard Curve of Itraconazole

**Preparation**

20 mg raw itraconazole dissolved in 200 ml acetonitrile completely gave 100 % dissolution for 100 mg itraconazole in 1 L simulated gastric fluid. The solution was diluted in to 10, 20, 40, 60, and 80 %. Those solutions were tested for UV absorbance at fixed wavelength, 226 nm, using a Beckman DU-600 spectrophotometer.

**Standard Curve Data**

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<th>Standard Concentration (1ml drug /1L gastric fluid)</th>
<th>Abs 1</th>
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<th>Absorbance at 226nm.</th>
<th>Estimated Value</th>
<th>% Theory</th>
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Average 100.61951

Standard Deviation 1.19025

%C.V. 1.18293
Standard Curve of Itraconazole
Appendix B: Calculation for Dissolved Percent of Itraconazole in Gastric Fluid

Those dissolution experiments of 100 mg itraconazole were carried out in 900 ml gastric fluid so the standard data were converted into the same ratio. The absorbance correction of 100 % dissolution is below:

The 100 % standard absorbance in 900 ml gastric fluid = 0.45114 (the absorbance for 20 mg itraconazole in 200 ml acetonitrile) $\times$ 1000 ml/ 900 ml = 0.5012667

Those absorbance data were converted into percent of 100 mg itraconazole dissolved in gastric fluid by following formula:

Percent = (absorbance at any time / 0.5012667) $\times$ 100%
Appendix C: Data of Dissolution Study

Formulation: Itraconazole + PEG 20000 branched chain + Glycerol + HPMC (K15M) + Explotab

Device: ISCO cell
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*1 E is explotab.
Beginning means adding explotab at the beginning with other ingredients.
End means adding explotab after mixing other ingredients well until 120 °C.

*2 Dionex is the cooling system inside the SFE-703 Extraction System from Dionex.