THESIS/DISSERTATION APPROVED BY

August 4, 2015

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PREPARATION, CHARACTERIZATION AND DISSOLUTION STUDY FOR CURCUMIN-RESVERATROL-CYCLODEXTRIN AMORPHOUS TERNARY SYSTEM

By

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A THESIS

Submitted to the faculty of the Graduate School of the Creighton University in Partial fulfillment of the Requirements for the Degree of Master of Science in the Department of Pharmacy Sciences

Omaha, NE

(June, 24, 2015)
ABSTRACT

Curcumin and resveratrol are naturally occurring polyphenolic compounds, showing synergistic potential in treatment of several chronic disorders. However, their poor oral bioavailability, which is primarily due to limited aqueous solubility, is a major hurdle in tapping their synergistic potential for therapeutic purposes. Cyclodextrins are oligosaccharides which shield hydrophobic functionalities and enhance aqueous solubility of compounds. The objective of present research was to prepare and characterize curcumin-resveratrol-cyclodextrin amorphous ternary systems, for simultaneous dissolution enhancement of both drugs.

Ultraviolet-Visible (UV-Vis) spectroscopy method was developed for simultaneous quantification of drugs. Alpha(α)-cyclodextrin, beta(β)–cyclodextrin, gamma(γ)–cyclodextrin and hydroxyl propyl beta(HPβ)–cyclodextrin were screened for their apparent aqueous solubility enhancement ability for curcumin and resveratrol. HPβ–cyclodextrin was found to be most effective. Solid dispersions in molar ratios of 1:1:2 (curcumin:resveratrol:each cyclodextrin), were prepared using solvent evaporation method and characterized by light microscopy, scanning electron microscopy(SEM), thermogravimetric analysis(TGA), modulated differential scanning calorimetry(MDSC), X-ray powder diffraction(XRPD), infrared(IR) spectroscopy, and Raman spectroscopy. Solid dispersions appeared to have irregular morphology and optimum thermal stability. Both drugs showed sharp melting peaks which disappeared in HPβ-cyclodextrin based solid dispersion, indicating reduction in their crystallinity. XRPD study showed HPβ-cyclodextrin based solid dispersion was amorphous in nature. IR spectroscopy indicated H-bonding between drugs and cyclodextrin, Raman spectroscopy indicated shielding of drugs by cyclodextrin cavity.

Dissolution of HPβ-cyclodextrin based solvent evaporated solid dispersion and corresponding physical mixture was carried out in USP type II apparatus with phosphate buffer pH 7.4. Within 30 minutes, dissolution enhancement of 2.4 fold was achieved for curcumin via solid dispersion.
compared to physical mixture, 55.3% of resveratrol was released from solid dispersion compared to 23.3% from physical mixture.

Solid dispersions of curcumin and/or resveratrol and HPβ-cyclodextrin were prepared by freeze drying using acetone:water, water solvent systems, and characterized. Dissolution study showed that within 5 minutes, 98% of curcumin and 100% of resveratrol were released from acetone:water based solid dispersion.

Thus, HPβ-cyclodextrin based solid dispersion appeared effective for simultaneous dissolution enhancement of curcumin and resveratrol. Freeze drying emerged as more suitable method owing to limited use of organic solvents. In-vitro efficacy of solid dispersions should be explored in future.
PREPARATION, CHARACTERIZATION AND DISSOLUTION STUDY FOR CURCUMIN-RESVERATROL-CYCODEXTRIN AMORPHOUS TERNARY SYSTEM

Graphical representation of the abstract.
PREFACE

Posters:

- **Gala U**, Chauhan H. Preparation and Characterization of Novel Curcumin-Resveratrol-Hydroxy Propyl Beta Cyclodextrin Freeze Dried System. AAPS Annual Meeting and Exposition, Orlando, 2015. [Accepted]


- Meng F, **Gala U**, Prasad D, Chauhan H. Characterization of Binary (Drug-Polymer) and Ternary (Drug-Drug-Polymer) Solid Dispersion: Role of Low Polymer Concentration on their Stabilization. AAPS Annual Meeting and Exposition, San Diego, 2014.


Research Articles:

- Gala U, Chauhan H. Dissolution Enhancement of Curcumin and Resveratrol Using Cyclodextrins. [In preparation]


Review Articles:


Editorial:

Dedicated to my family
ACKNOWLEDGEMENTS

I would like to express my earnest gratefulness to my advisor and mentor, Dr. Harsh Chauhan for his constant encouragement, support and guidance during the entire course of the program. I would like to extend my gratitude to my committee members, Dr. Somnath Singh and Dr. Jeffrey North for their valuable suggestions in my research project and preparation of this manuscript. I sincerely acknowledge, once again Dr. Somnath Singh and Dr. Manzoor Khan, my current and past program directors, for their guidance throughout the entire program.

I would like to specifically thank my lab members- Fan Meng, Anne Trivino and Jonathan Bernick for their help and all the scientific discussions during the lab meetings. I am grateful to Mr. Daniel Munt, Mr. Fujita Jiro, Mrs. Barabara Fegley and Dr. Shah Valloppilly for their assistance with my experimental studies. I would also like to thank Dawn Trojanowski and the entire department of Pharmacy Sciences for their support during the past two years. I am thankful to the interfaith prayer service team’14, Creighton graduate student government’14 and health sciences library staff for making my journey at Creighton a wonderful one! My friends Mansi Shah and Suleman Hussain deserve a special mention for being there whenever I needed them.

Most importantly I thank my family and I owe all my achievements to them. My Father Mr. Hasmukhlal Gala has shown constant confidence in me, my mother Mrs. Ranjanben Gala has bestowed me with immense love. My sisters Dr. Mita Gala and Mrs. Kanan Chheda have been my pillars of strength. I am lucky to have a second set of parents in form of my in-laws Mr. Narendra Savla and Mrs. Nisha Savla who constantly encourage me to achieve the best. I am also thankful to my brother in laws Mr. Dhiren Chheda and Mihir for boosting me up during my hard times. My niece Prisha deserves a big thanks for adding that extra happiness in my life. Finally, I thank God and my husband Romil, whose love, care and faith in me inspires me to be a better person every day and achieve my dreams.
LIST OF ABBREVIATIONS

µg    Microgram
µm    Micrometer
ATR   Attenuated Total Reflectance
C     Celsius
CAS   Chemical Abstracts Service
CD    Cyclodextrin
Cur   Curcumin
DSC   Differential Scanning Calorimetry
FDA   Food and Drug Administration
FT-IR Fourier Transform Infrared Spectroscopy
gm    Gram
HP    Hydroxy Propyl
hr    Hour
ID    Identification
IUPAC International Union of Pure and Applied Chemistry
M     Molar
mbar  Millibar
MDSC  Modulated Differential Scanning Calorimetry
mg    Milligram
min   Minute
ml  Milliliter
MOE  Molecular Operating Environment
mol  Mole
MT  Milli Torr
ng  Nanogram
nmol  Nanomole
pKa  Acid dissociation constant
PM  Physical Mixture
Res  Resveratrol (Trans form)
rpm  Rotations per Minute
SD  Solid Dispersion
SEM  Scanning Electron Microscopy
TGA  Thermo Gravimetric Analysis
UV  Ultraviolet
Vis  Visible
XRPD  X-ray Powder Diffraction
α  Alpha
β  Beta
γ  Gamma
°  Degree
Θ  Theta
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Chapter 1

1. Introduction

1.1 Chronic diseases and phytochemicals’ need

Chronic diseases such as cancer, diabetes, cardiovascular and kidney diseases pose biggest challenge to public healthcare. Rates of chronic disease continue to increase in all countries (industrialized, middle income, low income), surpassing infections as a disease burden among adults [1]. World Health Organization (WHO) estimates that by 2020, chronic diseases will account for three fourth of all deaths worldwide [2].

The global response to chronic diseases related research remains inadequate due to economic constrains, despite of growing evidence of epidemiological impact of chronic diseases [3]. For chronic diseases, that can’t be treated with lifestyle solutions, medications are mostly prescribed. These medications, often come with associated risks of side-effects and toxicities [4]. Also, owing to complexities associated with chronic diseases, their treatment modalities usually include combination of medications, which may lead to adverse drug interactions [5]. Thus, there exist combination of economic and medication associated hurdles in treatment of chronic diseases.

Phytochemicals emerge as a potential solution for problems associated with treatment of chronic diseases. Synthetic drug development involves processes such as drug design/discovery and studying the associated physiological effects of those drugs, which could be expensive [6]. On the other hand phytochemical drug development process could be comparatively inexpensive, due to benefits such as deciphering already existing phytochemical structure, thereby designing the drug and a large pool of knowledge of long term physiological effects of phytochemicals due to their human consumption [6]. On
similar lines, due to pre-existing knowledge of human consumption of fruits and vegetables, the phytochemicals derived from them, could be expected to have better safety profile. Thus, there is growing interest in phytochemicals as a source of new drugs [7]. According to a study in 2012-13, ~15 % of drug interventions in ClinicalTrials.gov were potentially plant related [8].

1.2 Benefits of phytochemicals, synergism and bioavailability

Craig WJ. illustrates phytochemicals as guardians of our health. He states that, consumption of plant foods, provides several phytochemicals, nonnutritive substances in plants that possess health-protective benefits [9]. There is strong evidence that consumption of fruits and vegetables is inversely related to risks of several chronic diseases such as cancer and cardiovascular diseases [10,11]. Rudolf Virchow, a German physician in nineteenth century suggested a link between inflammation and cancer, cardiovascular diseases, diabetes, pulmonary diseases, neurological diseases and other chronic diseases [12]. Antioxidants play a key role in treatment of inflammation. Fruits, grains and vegetables contain several phenolic compounds, terpenoids, pigments, and other natural antioxidants that have been related with guarding from and/or treatment of inflammation and thereby chronic diseases [13]. Additionally, these phytochemicals can provide preventive advantage by regulating enzymes important in metabolizing xenobiotics and carcinogens, by modifying nuclear receptors and cellular signaling of proliferation and apoptosis, and by acting indirectly through antioxidant actions that reduce proliferation and protect DNA from damage [14]. Phytochemicals such as capsaicin, gingerol,
epigallocatechins, quercetin etc. have shown potential in treatment of chronic diseases [15].

Chen et al. and his team analyzed results of over 100 herbal medicine based clinical trials and concluded that the effectiveness of phytochemicals/nutraceuticals lies in their synergistic potential and they excluded the notion of placebo effect of phytochemicals [16]. For example, Liu and group found out that, vitamin C in apples with skin accounts for only 0.4% of the total antioxidant activity, indicating that most of the antioxidant activity may come from synergy between phenolics and flavonoids in apples [17]. Liu RH also suggested potential synergy between phytochemicals leads to their cancer preventive effect [18]. For instance, binary combination of ginger’s biophenolics such as 6-gingerol, 8-gingerol, 10-gingerol and 6-shogoal exhibit synergy to inhibit prostate cancer cell proliferation [19].

Although pharmacological benefits of several phytochemicals are well proven, yet there exist a hurdle of their poor oral bioavailability in tapping their complete potential. Several reasons such as poor aqueous solubility, extensive pre-systemic metabolism, poor absorption, gastric instability etc. underlie poor oral bioavailability of phytochemicals [20]. For example, genistein and biochanin A, which, structurally should have good absorptive properties, are excreted by an efflux mechanism into the gut at a high rate that limits their oral bioavailability [21]. Sometimes, highly water-soluble phytochemicals, which exhibit excellent dissolution profiles, have poor oral bioavailability, owing to their conjugation with natural sugars (glycosides), which may impede their absorption across lipophilic cell membranes via simple passive diffusion processes [20]. Quercetin is unstable during gastric and pancreatic digestions because quercetin is easily degraded at high pH which
occurs due to digestion process and thus that limits its oral bioavailability [22]. However, amongst all of these reasons, one of the major cause of poor oral bioavailability of phytochemicals is poor aqueous solubility. Catechin is a phytochemical found in green tea. It has health benefits corroborated in animal studies for cancer chemoprevention, hypercholesterolemia, atherosclerosis, and so forth. However, its poor aqueous solubility limits its tapping of complete pharmacological potential [23]. Ellagic acid induces vasorelaxation, oxygen free radical scavenging, hypolipidemic, anti-inflammatory and anti-carcinogenic activities. It is poorly water soluble and thus extensive research is being done on this molecule to enhance its aqueous solubility [24].

Amongst the discussed phytochemicals, research on polyphenols curcumin and resveratrol has gained high momentum, due to wide therapeutic applications of these molecules. For instance, the percent publications between 2000 and 2011 being ~95% of the total publications on curcumin [25]. Also, curcumin is one of the most investigated phytochemicals with over 3,770 hits on using curcumin as the search string on Pubmed with about 1,200 hits in the last few years alone [26]. Resveratrol has also attracted great interest in the research community, with 4064 publications referenced on the U.S. National Library of Medicine's PubMed service between 1978 and 2011, of which 96% were between 2000 and 2011 [27].

1.3 Curcumin

Curcumin was discovered by Vogel and Pelletier, who reported the isolation of yellow coloring-matter from the rhizomes of *Curcuma longa* (turmeric) [28]. Later the matter was found to be a mixture of resin and turmeric oil. In 1910, Milobedzka and Lampe identified
the chemical structure of curcumin as diferuloylmethane [28]. The description of curcumin is illustrated in table 1.1 [29].

Table 1.1 Description of curcumin [29]

<table>
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Curcumin has two isomers, enol form and diketone form, owing to occurrence of intramolecular hydrogen atoms transfer at the β-diketone chain of curcumin (Table 1.1). A predominant keto form is seen in acidic and neutral solutions whereas a more stable enol form is observed in alkaline medium [30]. Experimentally observed pKa values of 8.54, 9.30 and 10.69 have been attributed to the dissociation of the enolic proton and the two phenolic protons, respectively [31]. The aqueous solubility of curcumin is ~11 ng/ml, rendering it as practically insoluble in water [32]. Curcumin is soluble in ethanol, methanol, acetic acid and several other organic solvents [29]. Curcumin is sometimes, referred to as combination of curcumin and curcuminoids demethoxycurcumin and
bisdemethoxycurcumin which too have pharmacological potential owing to their antioxidant capability [33]. Curcumin is considered hydrophobic, based on predicted values of log P ranging from 2.56 to 3.29 [34]. It has poor oral bioavailability, for instance 3.6 g of oral dose to human leads to a plasma level of 11.1± 0.6 nmol/ml [30]. Major reasons contributing to the low plasma and tissue levels of curcumin appear to be due to poor absorption, rapid metabolism, and rapid systemic elimination [30]. The main reason of poor absorption is its poor aqueous solubility. Toxicologically, curcumin is relatively inert and does not appear toxicity to either animal or humans even at high does [31]. It has been found to be non-toxic to humans up to the dose of 12 g/day [30]. Curcumin has been reported to be light sensitive [35].

1.4 Resveratrol

The non-flavonoid phytoalexin resveratrol and its various derivatives are widely distributed in gymnosperms and dicotyledons including groundnuts. Resveratrol was discovered in 1976 in the grape vine, *vitis vinifera*, as a response to fungal infection or injury [36]. The description of resveratrol is illustrated in table 1.2 [37]. Resveratrol has two forms, cis and trans of which trans resveratrol is more stable (Table 1.2) [36]. Hereafter, trans-resveratrol would be referred to as resveratrol in the experiments. The trans-resveratrol is stable for months, except in high-pH buffers, when protected from light and cis-resveratrol is stable only near pH neutrality [36]. Experimentally observed pKa values of 8.8, 9.8 and 11.4 have been attributed to the dissociation of the three phenolic protons, the first two being on polyphenolic ring and the other being on phenolic ring [38].The water solubility of resveratrol is 0.03 mg/ml and thus it is practically insoluble in
Resveratrol is soluble in ethanol, methanol, dimethyl sulfoxide and other organic solvents [37].

**Table 1.2 Description of resveratrol [37]**

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<td>IUPAC Name</td>
<td>5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol</td>
</tr>
<tr>
<td>Chemical Formula</td>
<td>C\textsubscript{14}H\textsubscript{12}O\textsubscript{3}</td>
</tr>
<tr>
<td>Molecular mass</td>
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Resveratrol is a hydrophobic molecule and about 75% of dissolved resveratrol can get absorbed in humans. Yet it has poor oral bioavailability owing to poor aqueous solubility and first pass metabolism [39,40]. About 25 mg of resveratrol given orally to humans, leads to a plasma concentration of 7.1 µg/L [41]. Resveratrol is considered to be safe with no marked toxicity. In a multiple dose study for resveratrol - 25 mg, 50 mg, 100 mg, 150 mg, or placebo, given every 4 hr for 48 hr, the most frequent adverse event was frontal headache in 3 out of 40 cases with no direct relation to ingested resveratrol dose [41].
1.5 Synergistic activity of curcumin and resveratrol

Curcumin and resveratrol both have been reported to possess radio protective property and are useful in treatment of radiation induced cell damage [42]. Both these compounds, have also been shown effective in treatment of Alzheimer’s diseases and eye keratitis [43,44]. Nuclear factor kappa β-mediated cytokine expression in adipocytes can be inhibited by both curcumin and resveratrol, thereby both being effective in treatment of type 2 diabetes mellitus [45]. Curcumin and resveratrol both have been found to be effective in treatment of colon cancer cells, colorectal cancer and hematologic malignancies [46-48]. Aristotle and his co-workers showed that curcumin and resveratrol can induce apoptosis in pediatric neuroblastoma [49]. Additionally, Curcumin is known to possess wound healing and anti-human immunodeficiency virus property [50-52]. Resveratrol shows neuroprotective property [53]. Resveratrol is famously associated with French paradox (i.e. low occurrence of cardio-vascular diseases in French people in spite of high consumption of fatty diet) due to its cardio-vascular protective action [54-56].

Apart from the above mentioned unique and common pharmacological effects, curcumin and resveratrol have also shown synergistic potential in their various pharmacological effects mediated in varied chronic diseases.

Dhawan et al. treated mice with beno(a)pyrene and thereby induced lung carcinogenesis in mice. They studied the effect of curcumin and resveratrol alone and in combination and found that curcumin and resveratrol individually resulted in a decrease in the micronuclei
formation; however, it was not statistically significant. Whereas, combination of curcumin and resveratrol resulted in a statistically significant decrease in micronuclei formation and thereby treated lung carcinoma [57]. In another study the same group, concluded that combined treatment with curcumin and resveratrol maintains adequate zinc levels and regulates inflammation by cox-2 and cell cycle arrest by p21 during lung carcinogenesis in mice and thereby helps in controlling lung carcinogenesis [58]. In a recent study, Zhang et al., observed that, curcumin and resveratrol in combination regulate drug-metabolizing enzymes as well as antioxidant enzymes during lung carcinogenesis in mice [59]. Dhawan et al. evaluated molecular mechanics behind synergistic chemo-preventive effects of curcumin and resveratrol during lung carcinogenesis and found out that synergism involved modulation of p53 hyper-phosphorylation, regulation of caspases and cellular metabolism enzymes [60]. The group also found that ultrahistoarchitecture during lung cancer significantly improves on combined treatment of curcumin and resveratrol [61]. Thus, there exist plethora of evidence on synergistic effect of curcumin and resveratrol in treatment of lung cancer.

The most important pharmacological activity of curcumin is its antioxidant ability. Thus, a group studied the antioxidant effect of curcumin in comparison and combination with other phytochemicals. They found out that same concentration of resveratrol had half the antioxidant activity of curcumin, but curcumin-resveratrol combination resulted in a synergistic antioxidant effect, $15.5 \pm 1.7\%$ greater than an average of individual activities. Also, this synergy was significantly greater (p < 0.05; about 4-fold) than that of curcumin together with the quercetin [62]. Group from Egypt, observed that combination of curcumin and resveratrol produced a higher anti-obesity, anti-atherogenic, anti-diabetic
and antioxidant activities on experimental obese diabetic rats than their individual influences [63]. Sarkar et al. treated colon cancer cell lines using curcumin, resveratrol and their combination and observed that the combination affected greater number of cells than either agent alone [64]. Buhrmann et al. found that combining curcumin and resveratrol activates extracellular signal-regulated kinase 1/2 signaling, a pathway that is involved in the maintenance of chondrocyte differentiation and survival and thereby helps in treatment of rheumatoid arthritis [65]. A group prepared targeted immunoliposomes against breast cancer cell line and found curcumin and resveratrol combination to be effective [66]. Another group prepared liposomes loaded with curcumin and resveratrol and observed that the combination was effective against prostate-specific phosphatase and tensin homolog knockout mice [67]. Agrewala et al. found that curcumin and resveratrol combination is effective in immune suppression [68]. This is advantageous in treatment of autoimmune diseases and even allergic disorders [68]. Nagar et al. observed antiproliferative activity of curcumin and resveratrol in treatment of colorectal cancer [69]. A research group based in Italy, found that resveratrol potentiates the in vitro as well as in vivo anti-tumoral effects of curcumin in head and neck carcinomas [70]. Wei et al. showed that curcumin and resveratrol significantly inhibited the proliferation of Hepa1-6 cells in a dose- and time-dependent manner and their combination elicited a synergistic antiproliferative effect in Hepa1-6 cells. Thus, the combination has great potential in treatment of liver cancer [71]. Shakebaei et al. observed synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes, thereby making the combination useful in treatment of osteoarthritis [72]. Figure 1.1 illustrates various diseases/disorders/conditions in which curcumin and resveratrol combination has been effective and synergistic.
Figure 1.1 Synergistic effect of curcumin and resveratrol in various diseases/disorders/conditions.

1.6 Solubility enhancement for curcumin and resveratrol

A vast amount of research has been done on solubility and dissolution enhancement of curcumin. The majority of work seems to be focused on using polymers for solubility enhancement of curcumin. Xu et al. prepared solid dispersion systems of curcumin in polyvinylpyrrolidione K30, at various weight ratios by co-evaporation of curcumin and polyvinylpyrrolidione K30 in ethanol solution. They found that compared to curcumin, the solubility of curcumin in solid dispersion system had increased by several folds [73]. Amin et al. used kollidon® VA 64 i.e. vinylpyrrolidone-vinyl acetate copolymers, for solubility and dissolution rate enhancement of curcumin [74]. They observed a 100% drug release in first 60 minutes from solid dispersion of curcumin and kollidon® VA 64 [74]. Patel et al.
prepared binary dispersions of curcumin and polyethylene glycol 4000 and 6000, and observed that the solubility of curcumin increased linearly with the increase in polymer concentration [75]. Kadu and Waghmare et al. observed similar relationship between curcumin and hydroxypropyl methyl cellulose concentration [76]. Tran and group combined curcumin with various combination of cellulosic and glycolic polymers and observed improvement in curcumin dissolution rate [77]. In another study, Tran and group found best curcumin release when they combined curcumin with polyethylene 6000: hydroxypropyl methyl cellulose 4000 in weight ratio 1:8:4 [78]. Uriyapongson et al. enhanced solubility of curcumin from turmeric oleoresin by using polyethylene glycol 400 and magnesium oxide at ratio of 1:1:3 [79]. Edgar et al. prepared amorphous solid dispersions of curcumin in cellulose derivative matrices such as carboxymethylcellulose acetate butyrate, hydroxypropylmethylcellulose acetate succinate and cellulose acetate adipate propionate. They concluded hydroxypropylmethylcellulose acetate succinate based dispersions were useful for pH-triggered release profile, chemical stabilization, and strong enhancement of curcumin solution concentration [80]. Tan et al. prepared sustained-release solid dispersion of curcumin by employing water-insoluble carrier cellulose acetate and thereby improved curcumin’s oral bioavailability [81]. Baboota and group prepared nanoglobules of curcumin with labrafac lipophile, unitop, polyethylene glycol 400 and distilled water as an oil, surfactant, co-surfactant and aqueous phase respectively using aqueous titration method and observed 96.21% of curcumin released within 6hr [82]. Moosavi-Movahedi et al. used beta casein-micelle as a nano vehicle for solubility enhancement of curcumin [83]. A research group prepared novel nanocarrier system, so-called curcuemulsomes, where they encapsulated curcumin inside the solid core of
emulsomes and found an increased in curcumin bioavailability [84]. Li and group tried solubility enhancement of curcumin using supercritical CO₂ based silk fibroin carrier [85]. Lu et al. used solubilizing properties of ruboside to enhance curcumin solubility [86]. Hydroxy propyl beta cyclodextrin has also been used for solubility enhancement of curcumin [87].

Solubility enhancement of resveratrol too has been explored. Similar to curcumin, Edgar et al. used cellulose matrices for solution concentration enhancement of resveratrol. They prepared resveratrol and carboxymethylcellulose acetate butyrate, hydroxypropylmethylcellulose acetate succinate and cellulose acetate adipate propionate solid dispersions and concluded that cellulose esters are attractive candidates for reveratrol bioavailability enhancement [88]. Cyclodextrins too have been explored for resveratrol’s solubility enhancement [89]. Cavalli et al. prepared beta cyclodextrin based nanopsponges and characterized them for stability, in vitro release, cytotoxicity and permeation ability. They found that in comparison to pure resveratrol, the nanosponges showed improved stability, in vitro release, cytotoxicity and permeation ability for resveratrol [90]. Ng et al. examined the impact of the impact of aqueous solubility and dose manipulation on the pharmacokinetics of resveratrol. For aqueous solubility enhancement they used hydroxyl propyl beta cyclodextrin and randomly methylated beta cyclodextrin, which were able to enhance aqueous solubility of resveratrol. On pharmacokinetic study in mice, they concluded that aqueous solubility barrier might affect the speed but not the extent of resveratrol absorption [91]. Pai et al. synthesized nanoparticles of resveratrol using copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups. They studied the pharmacokinetics of resveratrol
nanoparticles in rats and concluded that in addition to several factors, aqueous solubility of resveratrol does play a role in enhancing its oral bioavailability [92]. Tan et al. prepared resveratrol nanoparticles by temperature-controlled antisolvent precipitation method with hydroxypropyl methylcellulose as the stabilizer. The nanoparticles significantly enhanced saturation solubility and dissolution rate of resveratrol and they concluded that the oral bioavailability of resveratrol would be expected to be enhanced [93]. Other delivery systems such as liposomes, solid lipid nanoparticles etc. have been explored for resveratrol delivery [94]. Specific efforts towards developing a combined curcumin resveratrol system for solubility enhancement of both curcumin and resveratrol are lacking. However, attempts to deliver both the drugs together have been made [66, 67, 95-97].

1.7 Cyclodextrins

Cyclodextrins are a group of cyclic oligosaccharides consisting of (α-1,4)-linked α-D-glucopyranose units. They contain a somewhat lipophilic central cavity and a hydrophilic outer surface (Figure 1.2). The natural α-, β- and γ-cyclodextrin consist of six, seven, and eight glucopyranose units, respectively [98].
Figure 1.2 General structure of cyclodextrin.

The most common cyclodextrins and their description is listed in table 1.3 [99]. Hydroxy propyl β cyclodextrin seems to have highest solubility in water. In our study we used hydroxyl propyl beta cyclodextrin with molecular weight of ~1540.

Table 1.3 Commonly used cyclodextrins and their description

<table>
<thead>
<tr>
<th>Cyclodextrin</th>
<th>N</th>
<th>Substitutiona</th>
<th>R</th>
<th>MWb</th>
<th>Solubility in water (mg/ml)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Cyclodextrin</td>
<td>6</td>
<td>-</td>
<td>-H</td>
<td>972.00</td>
<td>145.00</td>
</tr>
<tr>
<td>β-Cyclodextrin (βCD)</td>
<td>7</td>
<td>-</td>
<td>-H</td>
<td>1135.00</td>
<td>18.50</td>
</tr>
<tr>
<td>2-Hydroxypropyl-β-cyclodextrin</td>
<td>7</td>
<td>0.65</td>
<td>-CH₂CHOHCH₃</td>
<td>1400.00</td>
<td>&gt;600.00</td>
</tr>
<tr>
<td>Randomly methylated β-cyclodextrin</td>
<td>7</td>
<td>1.80</td>
<td>-CH₃</td>
<td>1312.00</td>
<td>&gt;500.00</td>
</tr>
<tr>
<td>β-CD sulfobutyl ether sodium salt</td>
<td>7</td>
<td>0.90</td>
<td>-(CH₂)₆SO₃⁻Na⁺</td>
<td>2163.00</td>
<td>&gt;500.00</td>
</tr>
<tr>
<td>γ-Cyclodextrin</td>
<td>8</td>
<td>-</td>
<td>-H</td>
<td>1297.00</td>
<td>232.00</td>
</tr>
<tr>
<td>2-Hydroxypropyl-γ-cyclodextrin</td>
<td>8</td>
<td>0.60</td>
<td>-CH₂CHOHCH₃</td>
<td>1576.00</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

N No. of glucopyranose units; a Average number of substituents per glucopyranose repeat unit; b MW in Daltons; c Solubility in pure water at approx. 25°C.

Cyclodextrins have been used for solubility enhancement of several drugs [100]. More than 30 cyclodextrin/drug products are available in market [101]. Thus, cyclodextrins are promising candidates for solubility enhancement of curcumin and resveratrol.
1.8 Solid dispersions

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug [102]. Development of solid dispersion has become an established solubilization technology for poorly water soluble drugs [103]. Amorphous solid dispersions are most promising candidates for solubility enhancement, since the drug is presented in an amorphous form [104]. Thus, we intend to develop amorphous solid dispersion system.

1.9 References


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Chapter 2

2. Research Rationale

2.1 Need for curcumin-resveratrol formulation development

Several clinical trials involving oral delivery of curcumin, resveratrol have been conducted [1-7]. The common drawback seen in almost all these trials was limited oral bioavailability of curcumin and resveratrol. Diverse attempts to formulate and deliver these drugs and thereby enhance their bioavailability have been done [8,9]. Curcumin nanoparticles using polylactic glycolic acid based polymers have been formulated [8-11]. Similarly, nanoformulations of curcumin such as micelles and emulsions containing cyclodextrins, modified starch, hyaluronic acid, chitosan, dextran, medium chain triglycerides, cremophor EL, β-lactoglobulin etc. have been made [12-19]. Resveratrol nanoformulations containing lecithin, hydroxyl propyl methyl cellulose, polyvinyl pyrrolidone, polystyrene, pectin, cyclodextrins etc. have been formulated [20-29]. A few attempts to combine curcumin and resveratrol have also been done [30-32].

None of the aforementioned attempts have given extremely promising results, due to low aqueous solubility, stability issues, high metabolism and low bioavailability of curcumin and resveratrol [33-35]. Hence, there arises a need of robust, stable formulation that enhances the apparent aqueous solubility of both curcumin and resveratrol, thereby their bioavailability and thus harness their synergistic potential.

2.2 Significance, contribution and long term goal

This research is significant because it will pave the way for development of curcumin and resveratrol combination’s oral formulation. For treatment of chronic diseases, especially
cancer there is a need of potent and safe molecules and curcumin-resveratrol combination has been found to be effective. Currently, curcumin and resveratrol formulations available in market are unregulated by FDA and very little is known about their potential in terms in-vitro and in-vivo drug release [36-39]. Our research would help develop a foundation for development of curcumin and resveratrol formulation which could be approved by FDA. This could significantly boost the treatment modality for chronic diseases such as cancer. Moreover, since we are working on enhancing drugs aqueous solubility and thereby oral formulation development, this would translate into patient friendly treatment. Also, the excipients and processes we have explored are cost-effective during scale-up stages. This again would mean an affordable treatment modality for chronic diseases.

The main focus of the research is aqueous solubility enhancement. Although, there is a wide pool of aqueous solubility enhancement techniques that have been explored. Yet no technique tailors to wide spectrum of drugs and our research would open an arena for the same. One of the key challenges associated with formulation development of polyphenolic compounds is poor aqueous solubility [40]. Thus, the knowledge obtained from this research would be useful for formulating other similar polyphenolic compounds.

Additionally, our research would broaden the understanding of solid dispersion development using cyclodextrins. We intended to use complimentary characterization techniques in our study, to characterize physical, thermal and chemical features of solid dispersions. This would include using Raman spectroscopy and Infrared spectroscopy to detect any chemical changes in the system. Very few attempts have been made in this direction, and our learnings would be helpful for exploration of application of these techniques.
Hence, we believe our research would contribute to the domain of combination formulation development. The outcomes of our research would help directing the future work by extrapolation in aspects of polyphenolic compounds formulation development, aqueous solubility enhancement, and formulation and characterization.

**Our long term goal is to be able to draw the curcumin and resveratrol combination from bench side to bedside.** Also, we would explore our formulation excipient(s) and technique(s) for other poorly aqueous soluble drugs.

### 2.3 Innovation

We explored cyclodextrins as an excipient for our formulation development. Thus, we initiated a novel and innovative attempt to combine two poorly aqueous soluble polyphenolic drugs by using cyclodextrins and thereby create stable ternary solid dispersion. Most ternary solid dispersions prepared, utilize high molecular weight polymers. However, we developed ternary solid dispersion using a comparatively low molecular weight polymer. Moreover, cyclodextrins traditionally have been explored for complexation with single drug. But in our study, we explored combination drug loading into cyclodextrin. Overall, we believe our research is innovative because it showed a new perspective for aqueous solubility enhancement of polyphenolic drugs. More so, we explored solid dispersions using cyclodextrin with higher drug loading. Thus, not only we employed an innovative strategy but also explored innovative applications of already established excipients and techniques.

### 2.4 Hypothesis

Solid dispersion of curcumin and resveratrol with cyclodextrin leads to dissolution enhancement of curcumin and resveratrol.
2.5 Strategy

Solid dispersions is a well-known strategy which has been used to enhance aqueous solubility of drugs [41]. Pharmaceutical solid dispersions refer to a system wherein one or more active ingredient is dispersed in an inert carrier in a solid state [41,42]. Cyclodextrins are cyclic oligosaccharides which approximate a truncated cone or torus, generating a hydrophilic exterior surface and a nonpolar cavity in the interior. As such, cyclodextrins can interact with appropriately sized molecules to result in the formation of inclusion complexes. These noncovalent complexes offer a variety of physicochemical advantages over the unmanipulated drugs including the possibility for increased water solubility and solution stability [43-45]. This is because cyclodextrins shield hydrophobic groups of compounds. Thus, we employed the strategy of dispersing drugs in cyclodextrin matrix, thereby leading to dissolution enhancement and stabilization of drugs.

2.6 Research plan

For the proposed strategy, we adopted the research plan outlined in figure 2.1. According to the plan, we first explored the feasibility of our strategy. In the feasibility domain, we explored the ability of cyclodextrins to enhance the aqueous solubility of the drugs curcumin and resveratrol. Also, using simple technique for solid dispersion preparation, such as solvent evaporation, we explored the likelihood of solid dispersion formation using cyclodextrins with curcumin and resveratrol. Based on this information, we selected the most suitable cyclodextrin for our purpose.
In the next stage, of our plan, we developed the solid dispersion. For that, we first optimized the selected cyclodextrin content and thenceforth we used a scalable technique, such as freeze drying, for solid dispersion preparation.

Once, our formulation was developed, we evaluated several aspects of it, ranging from thermal properties to physical nature. Also, the interactions between components of solid dispersions were studied. The performance of solid dispersions, in terms of dissolution enhancement of curcumin and resveratrol was examined. And also, the stability of the prepared solid dispersions was evaluated. It’s important to note that evaluation techniques were implemented throughout the research.

**Figure 2.1** Research plan for the strategy adopted. This research explored the domains of feasibility, development and evaluation for the project.

### 2.7 Specific Aims

Based on the research plan, the following were the specific aims of the study:
Specific Aim 1:
To select a suitable cyclodextrin for dissolution enhancement of curcumin and resveratrol.

Specific Aim 2:
To optimize, prepare and characterize solid dispersions of curcumin and resveratrol with selected cyclodextrin.

Specific Aim 3:
To evaluate the amorphous stability of prepared solid dispersions.

2.8 References


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Chapter 3

3. Cyclodextrin Selection

3.1 Materials and instrumentation

Following materials such as drugs, excipients and solvents have been used in the experiments (Table 3.1):

Table 3.1 Materials used in the study

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<tr>
<th>Name</th>
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<th>Lot. No./Batch No.</th>
<th>CAS No.</th>
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<td>A0298306</td>
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<tr>
<td>Resveratrol</td>
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<td>FR094121301</td>
<td>501-36-0</td>
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<td>Cavamax W6 Pharma</td>
<td>Ashland</td>
<td>601019</td>
<td>10016-20-3</td>
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<tr>
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<td>Cavamax W7 Pharma</td>
<td>Ashland</td>
<td>701118</td>
<td>7585-39-9</td>
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<tr>
<td>(Beta Cyclodextrin)</td>
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<tr>
<td>Cavamax W8 Pharma</td>
<td>Ashland</td>
<td>80P244</td>
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</tr>
<tr>
<td>(Gamma Cyclodextrin)</td>
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<td></td>
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<tr>
<td>Kleptose HPB-</td>
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<td>(Parentral Grade)</td>
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<tr>
<td>(Hydroxy Propyl Beta)</td>
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<tr>
<td>Cyclodextrin)</td>
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</tr>
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<td>Name</td>
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<td>Lot. No./Batch No.</td>
<td>CAS No.</td>
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</tr>
<tr>
<td>Sodium Phosphate</td>
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</tr>
<tr>
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<tr>
<td>Sodium Phosphate</td>
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<tr>
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<td>Fisher Scientific</td>
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<td>67-56-1</td>
</tr>
</tbody>
</table>

Following instruments/machines have been used in the experiments (Table 3.2):

**Table 3.2** Instruments/machines used in the study

<table>
<thead>
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<th>Instrument/Machine</th>
<th>Make/Company</th>
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<tr>
<td>Weighing Balance</td>
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<tr>
<td>UV-Vis spectrophotometer</td>
<td>Denver Instrument Company TR-104</td>
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<tr>
<td>Magnetic stirrer</td>
<td>BioTek Synergy H1 Hybrid Reader</td>
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<tr>
<td>Sonication Bath</td>
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<tr>
<td>Solvent Evaporator</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Vacuum assembly</td>
<td>BUCHI Rotavapor R-215</td>
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<tr>
<td>Light microscope</td>
<td>BUCHI Vacuum Pump V-700</td>
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<tr>
<td>Aluminum mount</td>
<td>OLYMPUS BX51M</td>
</tr>
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</table>

Hitachi M4 style aluminum mount
### Instrument Machine

<table>
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<th>Instrument Machine</th>
<th>Make/Company</th>
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</thead>
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</tr>
<tr>
<td>Thermogravimetric analyzer</td>
<td>Shimadzu DTG 60</td>
</tr>
<tr>
<td>Modulated differential scanning calorimeter</td>
<td>DSC Q-2000, TA Instrument</td>
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<tr>
<td>X-ray diffractometer</td>
<td>PANalytical Empyrean Diffractometer</td>
</tr>
<tr>
<td>IR instrument</td>
<td>Thermo-Nicolet Avatar 370 DTGS, ATR-Pike</td>
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<td>Raman microspectrometer</td>
<td>Bruker-Senterra</td>
</tr>
<tr>
<td>Dissolution apparatus</td>
<td>Vision Class 6 Hanson</td>
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</tbody>
</table>

#### 3.2 Analytical method development for concurrent quantification of curcumin and resveratrol

**3.2.1 Rationale:**

UV-Visible spectroscopy is one of the most commonly used techniques in pharmaceutical analysis. It measures the amount of ultraviolet or visible radiation absorbed by a substance in solution [1]. Both, curcumin and resveratrol have been quantified using UV-Vis spectroscopy [2,3]. UV-Vis spectroscopy has been used for simultaneous analysis of multi-component mixtures as well [4,5]. However, a UV-Vis spectroscopic method for simultaneous quantification of curcumin and resveratrol has not been developed yet. Thus, we developed UV-Vis spectroscopy along with simultaneous equation method for concurrent quantification of curcumin and resveratrol.
3.2.2 Methods:

**UV-Vis Scans:**
Stock solutions of curcumin and resveratrol were prepared separately in methanol (10 µg/ml). The solutions and blank were scanned in triplicates for their wavelength of maximum UV-Vis absorbance, in range of 250-700 nm (n=3). UV-Vis spectrophotometer with plate reader arrangement was used. Concentrated solutions of α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and HPβ-cyclodextrin in deionized water and blank were scanned in triplicates to check their UV-Vis absorbance, in range of 250-700 nm.

**Standard Plots:**
Solutions of curcumin and resveratrol (in methanol) in concentration range of 0.05 µg/ml to 25.00 µg/ml and blank were prepared separately and then scanned in triplicates at wavelength of its maximum absorbance.
The 10 µg/ml stock solutions of curcumin and resveratrol were serially diluted in concentration range of 0.6-10.0 µg/ml. The solutions and blank were then scanned in triplicates at wavelength of maximum absorbance of curcumin and resveratrol (n=3). Standard curves for both drugs were developed at wavelength of maximum absorbance of curcumin and resveratrol (n=3).

**Simultaneous Equations:**
Using standard curve equations, simultaneous equations were developed. Simultaneous equation method was then used to develop equations for concurrent quantification of
curcumin and resveratrol. The equations were tested for standard known concentrations (5 µg/ml each) of curcumin and resveratrol mixture in triplicates (n=3).

**Note:** Wherever applicable, pure drug(s) based samples were covered with aluminum foil or amber colored apparatus were used.

### 3.2.3 Results and Discussion:

**UV-Vis Scans:**

![UV-Vis spectral scans of curcumin and resveratrol](image)

**Figure 3.1** UV-Vis spectral scans of curcumin and resveratrol. Curcumin showed its maximum absorbance at a wavelength of 424 nm and that for resveratrol was 306 nm.
Figure 3.2 UV-Vis spectral scans of cyclodextrins. Cyclodextrins used in the study showed an UV-Vis absorbance of less than 0.05 at 306 nm and 424 nm.

UV-Vis scan of curcumin showed its maximum absorbance at a wavelength of 424 nm and that for resveratrol was 306 nm (Figure 3.1). Mandal et al. and Verkman et al. also used a wavelength of 424 nm for determination of curcumin [6,7]. Walle et al. and Kristl et al. used a wavelength of 306 nm for determination of trans-resveratrol (resveratrol) [8,9]. Concentrated solutions of all cyclodextrins used in the study showed an UV-Vis absorbance of less than 0.05 at 306 nm and 424 nm (Figure 3.2). Thus, presence of cyclodextrins was less likely to affect the UV-Vis absorbance of curcumin and resveratrol.

Standard Plots:
Curcumin and resveratrol both obeyed Beer-Lambert’s law in concentration range of 0.05 µg/ml to 25.00 µg/ml at 424 nm and 306 nm respectively. Pawar et al. reported linearity range of 1 mg/ml to 7 mg/ml for curcumin determination in a cream formulation at a
wavelength of 422 nm and Ramaiah et al. reported linearity range of 5 µg/ml to 25 µg/ml for curcumin determination in a nano-formulation at a wavelength of 421 nm [10,11].

![Graphs](image)

**Figure 3.3** Standard curves of (a) Curcumin at 424 nm; (b) Curcumin at 306 nm; (c) Resveratrol at 306 nm and (d) Resveratrol at 424 nm.

Bonnefont-Rousselot et al. observed a linearity range of 0-300 µM for resveratrol at a wavelength of 304 nm [3]. The coefficient of determination for curcumin at 424 nm and 306 nm, and for resveratrol at 306 nm was found to be 0.99 (Figure 3.3). Thereby, indicating acceptable linearity in the method. Resveratrol showed coefficient of determination of ~0.00 at 424 nm and with absorbance of less than 0.027 at all the evaluated concentrations (Figure 3.3).
Simultaneous Equations:

**Equations derived from Standard Curve:**

\[
\text{Curcumin}_{424} \ y = 0.0978x + 0.016 \quad \ldots \ldots (1)
\]

\[
\text{Resveratrol}_{424} \ y = (1E-18)x + 0.027 \quad \ldots \ldots (2)
\]

1E-18 can be considered to be negligible and thus zero.

\[
\text{Curcumin}_{306} \ y = 0.0102x + 0.0474 \quad \ldots \ldots (3)
\]

\[
\text{Resveratrol}_{306} \ y = 0.1873x + 0.0267 \quad \ldots \ldots (4)
\]

For a mixture of Curcumin and Resveratrol:

At wavelength of 424 nm,

\[
\text{Absorbance}_{424} = (K_{424\text{Cur}} \times C_{\text{Cur}}) + (K_{424\text{Res}} \times C_{\text{Res}}) \ldots \ldots (5)
\]

At wavelength of 306 nm,

\[
\text{Absorbance}_{306} = (K_{306\text{Cur}} \times C_{\text{Cur}}) + (K_{306\text{Res}} \times C_{\text{Res}}) \ldots \ldots (6)
\]

Thus,

\[
C_{\text{Cur}} = \frac{\text{Absorbance}_{424}}{K_{424\text{Cur}}} \ldots \ldots (7) \quad \text{(From 1,2 and 5)}
\]

\[
C_{\text{Res}} = \frac{[\text{Absorbance}_{306\text{Cur}} - (K_{306\text{Cur}} \times C_{\text{Cur}})]}{K_{306\text{Res}}} \quad \text{(From 3,4,6 and 7)}
\]

\[K=\text{slope, C= Concentration, Cur=Curcumin, R=Resveratrol}\]

**Figure 3.4** Simultaneous equations for curcumin and resveratrol concurrent analysis, by UV-Vis spectroscopy.
Using, simultaneous equation method, suitable equations were developed for concurrent determination of both curcumin and resveratrol. These equations were found to be acceptable on determination of known standard concentrations of curcumin and resveratrol with a standard deviation of less than 2%. Hence, we were able to develop a new and suitable analytical method for concurrent quantification of curcumin and resveratrol.

3.3 Solubility studies to check effectiveness of cyclodextrins in solubility enhancement of curcumin and resveratrol

3.3.1 Rationale:

Solubility studies are one of the prerequisites for cyclodextrin selection. In several studies, variety of cyclodextrins are screened for their solubility enhancement capability for drug under study and then a suitable cyclodextrin is chosen [12,13]. In order to investigate the ability of various cyclodextrins for solubility enhancement of curcumin and resveratrol we screened them individually with curcumin as well as resveratrol, separately. More so, we carried out the solubility studies for a period of seven days, in order to investigate the likelihood of crashing out of drugs i.e. curcumin and resveratrol post solubilization.

3.3.2 Methods:

1 g of α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and HPβ-cyclodextrin were dissolved separately in 100 ml of deionized water, in two sets. In one set 100 mg of curcumin and in another set 100 mg of resveratrol was added. Also, 100 mg of curcumin and 100 mg of resveratrol were added separately in 100 ml of deionized water. All the conical flasks were placed on magnetic stirrer and stirred at the motion speed of 5 units
for a period of seven days. The entire assembly was covered with cardboard. At time points of 2 hr, 4 hr, 6 hr, 8 hr, 12 hr, 24 hr, 48 hr, 72 hr, 96 hr and 168 hr, 1 ml of sample was withdrawn from each flask and filtered using 0.45 µm syringe filter and diluted with 1 ml methanol. 1 ml of deionized water was added in respective flask after sample withdrawal. The curcumin based samples were then analyzed at a wavelength of 424 nm and resveratrol based samples were analyzed at a wavelength of 306 nm in triplicates with blanks using UV-Vis spectroscopy. Pictorial representation of solubility studies is depicted in figure 3.5.

Note: Wherever applicable, pure drug(s) based samples were covered with aluminum foil or amber colored apparatus were used.
Figure 3.5 Solubility study design for (a) curcumin and (b) resveratrol. Solubility studies were carried out separately for curcumin and resveratrol in α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, HPβ-cyclodextrin aqueous solutions.
3.3.3 Results and Discussion:

Figure 3.6 Amount of (a) Curcumin and (b) Resveratrol dissolved in presence and absence of several cyclodextrins over a week. HPβ-CD was found to be effective in enhancing the apparent aqueous solubility of both curcumin and resveratrol, as compared to α, β, γ-CD.

All cyclodextrins under study, enhanced the apparent aqueous solubility of curcumin as compared to only curcumin i.e. in absence of cyclodextrins. α-cyclodextrin showed a
sharp increase in apparent aqueous solubility of curcumin, between 12-24 hr, however, there was sharp decrease in amount of curcumin dissolved by 48 hr. β-cyclodextrin, showed least solubility enhancement for curcumin as compared to α-cyclodextrin, γ-cyclodextrin and HPβ-cyclodextrin. γ-cyclodextrin showed increase in solubility enhancement of curcumin until 12 hr, followed by slight increase from 48 hr to 96 hr. A slight decrease in solubility of curcumin was seen after 96 hr in presence of γ-cyclodextrin. Of all cyclodextrins under study, HPβ-cyclodextrin showed highest solubility enhancement for curcumin with least tendency of curcumin crashing out. Loftsson et al. screened HPα-cyclodextrin, HPβ-cyclodextrin, HPγ-cyclodextrin, randomly methylated β-cyclodextrin, sulfobutylether β-cyclodextrin and hydroxytrimethylammoniumpropyl β-cyclodextrin for solubility enhancement of curcumin [14]. They observed highest concentration, approximately 8X10^{-4} M or approximately 290 µg/ml of curcumin, in 11 % solution of randomly methylated β-cyclodextrin. They also observed that the affinity of curcumin was highest for the relatively hydrophobic cavity of randomly methylated β-cyclodextrin and the large cavity of HPγ-cyclodextrin. Yadav et al. evaluated β-cyclodextrin, γ-cyclodextrin, HPβ-cyclodextrin and methyl β-cyclodextrin for curcumin solubility enhancement [15]. They concluded that cavity size of methyl β-cyclodextrin and HPβ-cyclodextrin was optimal for entrapment of the curcumin molecules and provided the greatest solubilization effect. Several attempts to enhance the solubility of curcumin with β-cyclodextrin and lipophilic derivatives of α-cyclodextrin and γ-cyclodextrin have been done [16-18]. All cyclodextrins under study, enhanced the apparent aqueous solubility of resveratrol as compared to only resveratrol i.e. in absence of cyclodextrins. For all cyclodextrins under
study, the apparent aqueous solubility enhancement for resveratrol increased up to 12 hr and an approximate steady phase was observed thenceforth. The likelihood of resveratrol crashing out of system was minimal for all cyclodextrins under study. α-cyclodextrin and β-cyclodextrin showed similar solubility enhancement for resveratrol. γ-cyclodextrin showed least solubility enhancement for resveratrol. As seen for curcumin, HPβ-cyclodextrin showed highest solubility enhancement for resveratrol. Nunez-Delicado et al. demonstrated that cyclodextrins can be used as resveratrol complexation agent, to increase total resveratrol concentration in aqueous solution [19]. Zou et al. investigated the solubility enhancement of resveratrol by β-cyclodextrin and HPβ-cyclodextrin and observed high solubility enhancement and inclusion of resveratrol by HPβ-cyclodextrin [20]. Attempt to enhance the solubility of resveratrol with β-cyclodextrin based nanosponge has been carried out [21].

3.4 Preparation and characterization of solvent evaporated curcumin-resveratrol-cyclodextrin solid dispersions

3.4.1 Rationale:

Solubility studies cannot serve as a standalone method for selection of cyclodextrins. It is important to analyze the feasibility of preparation of solid dispersions using various cyclodextrins and also the physico-chemical properties of solid dispersions so obtained. Solvent evaporation technique has been used to prepare solid dispersions of drug with cyclodextrin and has been found to be effective [22,23]. Solvent evaporation is also a quick technique and thus can be used for initial screening purposes. Techniques such as light microscopy and scanning electron microscopy have been used to analyze the morphology
of solid dispersions [24,25]. Thermogravimetric analysis and differential scanning calorimetry have been used to analyze the thermal behavior of solid dispersion [25,26]. X-ray diffraction has been used to study the state of drug in solid dispersion [25,26]. Infrared spectroscopy and Raman spectroscopy have been employed to characterize interactions between drugs and excipients in solid dispersions [25,27]. Dissolution study determines the performance of solid dispersion [28]. Thus, we designed thorough preparation and physico-chemical characterization techniques and evaluated the cyclodextrin based solid dispersions and selected suitable cyclodextrin.

3.4.2 Methods:

Solid dispersion preparation:

Preparation of α-cyclodextrin based solid dispersions:

189.10 mg of curcumin, 121.65 mg of resveratrol and 1000 mg of α-cyclodextrin were weighed. Curcumin and resveratrol were added to 30 ml of methanol and sonicated for 5 min. α-cyclodextrin was added to 20 ml of deionized water and sonicated for 5 min. Both the solutions were mixed and placed in a rotary solvent evaporator. The bath temperature set was 70 °C. The pressure was kept between 150-230 mbar. The rotation speed was set to 80 rpm. The total run time was 65 min. The residue (solid dispersion) was then dried overnight in a vacuum chamber at 150 mbar pressure and collected.

Preparation of β-cyclodextrin based solid dispersions:

162 mg of curcumin, 100 mg of resveratrol and 1000 mg of β-cyclodextrin were weighed. Curcumin and resveratrol were added to 30 ml of methanol and sonicated for 5 min. β-cyclodextrin was added to 20 ml of deionized water and sonicated for 5 min. Both the
solutions were mixed and placed in a rotary solvent evaporator. The bath temperature set was 70 °C. The pressure was kept between 150-230 mbar. The rotation speed was set to 80rpm. The total run time was 60 min. The residue (solid dispersion) was then dried overnight in a vacuum chamber at 150 mbar pressure and collected.

Preparation of γ-cyclodextrin based solid dispersions:

142 mg of curcumin, 88 mg of resveratrol and 1000 mg of γ-cyclodextrin were weighed. Curcumin and resveratrol were added to 20 ml of methanol and sonicated for 5 min. γ-cyclodextrin was added to 10 ml of deionized water and sonicated for 5 min. Both the solutions were mixed and placed in a rotary solvent evaporator. The bath temperature set was 70 °C. The pressure was kept between 150-230 mbar. The rotation speed was set to 80 rpm. The total run time was 40 min. The residue (solid dispersion) was then dried overnight in a vacuum chamber at 150 mbar pressure and collected.

Preparation of HPβ-cyclodextrin based solid dispersions:

119 mg of curcumin, 74 mg of resveratrol and 1000 mg of HPβ-cyclodextrin were weighed. Curcumin and resveratrol were added to 20 ml of methanol and sonicated for 5 min. HPβ-cyclodextrin was added to 10 ml of methanol and sonicated for 5 min. Both the solutions were mixed and placed in a rotary solvent evaporator. The bath temperature set was 70 °C. The pressure was kept between 50-150 mbar. The rotation speed was set to 150 rpm. The total run time was 30 min. The residue (solid dispersion) was then dried overnight in a vacuum chamber at 150 mbar pressure and collected.

Thus, solid dispersions in 1:1:2 M molar ratios of curcumin:resveratrol:cyclodextrin were prepared. Figure 3.7 depicts pictorial representation of solvent evaporated solid dispersion
preparation. Similar physical mixtures of all cyclodextrins with curcumin and resveratrol were prepared.

Figure 3.7 Method of preparation of solvent evaporated solid dispersions. The solid dispersions in 1:1:2 M molar ratios of curcumin:resveratrol:cyclodextrin were prepared.

Physico-chemical characterization of solid dispersion:

*Light Microscopy:*

The pure drugs, cyclodextrins, physical mixtures and corresponding solid dispersions were mounted on glass slides. The samples were then observed under a light microscope with 20 X objective. The images were captured using OPUS software.

*Scanning electron microscopy (SEM):*

An aluminum mount was coated with an adhesive using an adhesive Tab. The HPβ-cyclodextrin based solid dispersion was placed on the adhesive surface, excess sample that did not adhere to the surface was gently blown away leaving a single layer of particles on the surface of the mount. The sample was then placed in an EMS/Quorum ES R sputter coater and coated with gold/palladium to render it conductive. Sample was viewed in a
scanning electron microscope at 15 kilovolts voltage. Images were captured as TIFF’s using a Quartz PCI system.

Thermogravimetric analysis (TGA):
Approximately 5-10 mg of pure drugs, cyclodextrins, physical mixtures and corresponding solid dispersions were placed in open aluminum pans and heated at a rate of 10 °C/min, up to 300 °C/500 °C, with a nitrogen purge, in a thermogravimetric analyzer.

Modulated differential scanning calorimetry (MDSC):
Approximately 3-10 mg of pure drugs, cyclodextrins, physical mixtures and corresponding solid dispersions were placed in aluminum pans and hermetically sealed with a lid/ lid with pinhole. The samples pans along with reference pan were subjected to heating-cooling heating cycles, at a rate of 2 °C/min, from -40 °C to 200 °C/350 °C, with a nitrogen purge, in a modulated differential scanning calorimeter.

X-ray diffractometry (XRPD):
X-Ray powder diffraction patterns were obtained for pure drugs, cyclodextrins, physical mixtures and corresponding solid dispersions using X-ray diffractometer. The data in the 2θ range 5–60 ° was collected in focusing geometry using X-ray Diffractometer, operated with Cu Kα radiation at 40 kilovolts and 45 milliampere. A mask of 20 mm and a divergence slit of 1/4 °were used on the incident beam path. Thin layer of powder sample was placed on a zero background Si plate and the sample holder was continuously spun at the rate of 90 °/second during the measurement. Solid state PIXcel3D detector was scanned at a rate of 0.135 °/second to collect data and a diffracted beam monochromator for the PIXcel detector was utilized to improve signal to noise ratio.

Infrared spectroscopy (IR):
The pure drugs, cyclodextrins, physical mixtures and corresponding solid dispersions were placed in IR instrument sample holder and subjected to IR spectroscopy. The IR instrument had ATR setup with ZnSe crystal. 32 scans were taken for each sample and the resolution was set to 4 units and IR spectra were collected. Background spectra were taken every 30min.

_Raman spectroscopy:_

The pure drugs, cyclodextrins, physical mixtures and corresponding solid dispersions were mounted on glass slides. The samples were placed on stage of Raman microspectrometer and subjected to Raman light. The aperture of 50×100 µm and the object lens of 20 X size were used to focus on samples. The samples were subjected to a laser beam having a wavelength of 785 nm and spectral data in the range of 0-2000 cm⁻¹ wavenumber was collected. A resolution level of approximately 3.5 cm⁻¹ was set in the instrument.

_Dissolution studies:_

Dissolution studies using USP type II paddle apparatus, phosphate buffer pH 7.4-100 ml, paddle speed of 150 rpm at 37±0.5 °C was carried for curcumin:resveratrol:HPβ-cyclodextrin solid dispersion and corresponding physical mixture [n=3]. At time points of 0 min, 5 min, 10 min, 30 min, 60 min, 120 min, 240 min, 360 min, 480 min and 720 min, 1 ml of sample was withdrawn from each dissolution vessel and filtered using 0.45 µm syringe filter and diluted with 1 ml methanol. 1ml of phosphate buffer pH 7.4 was added in respective vessel after sample withdrawal. The samples were then analyzed at a wavelength of 306 nm and 424 nm in triplicates with blanks using UV-Vis spectroscopy and amounts of curcumin and resveratrol dissolved were calculated using simultaneous equation method.
Preparation and characterization of binary system:

In order to supplement the understanding of curcumin:resveratrol:cyclodextrin ternary systems, curcumin and resveratrol binary system were developed. Solid dispersion of curcumin and resveratrol in 1:1 M ratio was prepared by solvent evaporation, using methanol as solvent. A corresponding physical mixture was prepared. Both the samples were then analyzed by modulated differential scanning calorimetry, X-ray diffraction and Raman spectroscopy.

Note: Wherever applicable, pure drug(s) based samples were covered with aluminum foil or amber colored apparatus were used. The molar ratios or concentrations mentioned are with respect to solid state combination of drug(s) and/or excipients.

3.4.3 Results and Discussion:

Solid dispersion preparation:

Successful solid dispersions of curcumin, resveratrol and cyclodextrins were prepared, using solvent evaporation method in a rotary evaporator. The α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin based solid dispersions, required water as one of the solvent. Thus, they had a higher run time, during solvent evaporation. The HPβ-cyclodextrin based solid dispersion, did not require water, as HPβ-cyclodextrin is soluble in methanol. Thus, it had a comparatively lower run time during solvent evaporation. Similarly, Elhady et al. prepared simvastatin, hydroxybutyl-β-cyclodextrin solid dispersion using methanol as solvent and solvent evaporation technique [29]. Setyanwan
et al. prepared complexes of curcumin and HPβ-cyclodextrin using alcohol as solvent and solvent evaporation technique in a rotary evaporator [30]. The use of alcohol as a solvent in both the cases led preparation of optimum solvent evaporated dried masses and similar observation was made in HPβ-cyclodextrin based solid dispersion. Sinico et al. prepared inclusion complexes between resveratrol and cyclodextrins using wet technologies, due to speculation of resveratrol instability in rotary evaporation process [31]. However, Taylor et al. prepared solid dispersions of resveratrol with various polymers using rotary evaporation [32]. All solid dispersions that were prepared appeared uniform in color, indicating uniform dispersion of drugs. The α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin based solid dispersions lacked free flowing property and the probable cause could be use of water as one of the solvent. The HPβ-cyclodextrin based solid dispersion was free flowing.

**Physico-chemical characterization:**

*Light Microscopy:*

Light microscopy or optical microscopy gave a preliminary view of appearance and morphology of drugs, cyclodextrins, physical mixtures, and solid dispersions (Figure 3.8). Curcumin was orange-yellow in color and had fine particulate appearance. Resveratrol was off-white in color and appeared as agglomerated powder. All cyclodextrins were fine white particles in appearance. Although, physical mixtures had uniform appearance, but under the microscope, regions of drugs and cyclodextrin were evident. This indicated no apparent morphological changes of drugs and excipients on mixing. All solid dispersions were uniform in appearance.
The clear regions of drugs and cyclodextrin were not evident in solid dispersions as seen in physical mixtures. This indicated morphological changes between drugs and excipients on solvent evaporation. Similar attempts to use optical microscopy to understand solid dispersions appearance have been made. Chowdhury et al. used optical microscopy to examine atorvastatin and its binary solvent evaporated solid dispersions with poloxamer 188, in their in vitro dissolution study [33].
**Scanning electron microscopy:**

*Cur: Res: Hydroxy-propyl Beta Cyclodextrin*

![SEM Images of HPβ-cyclodextrin based solvent evaporated solid dispersion](image)

**Figure 3.9** SEM Images of HPβ-cyclodextrin based solvent evaporated solid dispersion; Above-100 X magnification, Below-500 X magnification.

The SEM image of HPβ-cyclodextrin based solid dispersion was taken. At 100 X magnification, the solid dispersions appeared as irregular chunks. On higher magnification of 500 X the solid dispersion appeared to have large chunk with small particles dispersed in it. Similarly, Khanam et al. studied carvedilol solid dispersion with HPβ-cyclodextrin using SEM [25].
Thermogravimetric analysis:

Table 3.3 TGA data of drugs, cyclodextrins, their respective physical mixtures and solvent evaporated solid dispersions

<table>
<thead>
<tr>
<th>% Weight Loss</th>
<th>Curcumin</th>
<th>Resveratrol</th>
<th>Alpha Cyclodextrin system</th>
<th>Beta Cyclodextrin system</th>
<th>Gamma Cyclodextrin system</th>
<th>Hydroxypropyl Beta Cyclodextrin system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>CD</td>
<td>PM</td>
<td>SD</td>
<td>CD</td>
<td>PM</td>
</tr>
<tr>
<td>0±0.5</td>
<td>16.81</td>
<td>16.83</td>
<td>21.03</td>
<td>17.87</td>
<td>16.54</td>
<td>17.41</td>
</tr>
<tr>
<td>5±0.5</td>
<td>275.54</td>
<td>280.97</td>
<td>81.85</td>
<td>82.58</td>
<td>45.55</td>
<td>79.44</td>
</tr>
<tr>
<td>10±0.5</td>
<td>288.49</td>
<td>299.42</td>
<td>273.47</td>
<td>262.66</td>
<td>54.16</td>
<td>96.23</td>
</tr>
</tbody>
</table>

Curcumin and resveratrol were significantly thermostable. About 5% weight loss for curcumin was observed at ~275 °C and that for resveratrol was observed at ~280 °C (Table 3.3). Chauhan et al. observed similar trend in thermostability for curcumin [16]. Roldughin et al. corresponded the weight loss of resveratrol after 250 °C to its melting and sublimation, in an argon atmosphere [34]. Owing to their moisture content, cyclodextrins showed initial weight loss at lower temperatures of less than 100 °C (Table 3.3). Moyano et al. illustrated several thermal properties of cyclodextrins [35]. They classified thermal decomposition of α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin as a two-step process, wherein the initial step is due to dehydration and the later step is due to thermal degradation. Catenacci et al. observed a weight loss of 3.55% by weight for HPβ-cyclodextrin and corresponded it to loss due to water content [36]. In most cases, solid dispersions were slightly less thermostable than their corresponding physical mixtures. This might be because in physical mixture the components retained their inherent thermal behavior, however solvent evaporation might have led to physical changes in the components. Except α-cyclodextrin based solid dispersion, all other solid dispersions showed ~10% weight loss until 250 °C and were significantly thermostable (Table 3.3).
Modulated differential scanning calorimetry:

The sharp melting peak of curcumin was seen at 175.77 °C and thus indicated crystalline nature of curcumin (Figure 3.10). At a heating rate of 10 °C/min Yadav et al., Masson et al. and Mahadik et al. observed the melting peak for curcumin at 180 °C, thus the difference between our observed values and these reported values could be due to our low heating rate which could capture precise melting event [15,37,38]. However, at a heating rate of 10 °C/min from 25 °C-210 °C Joseph et al. found the melting peak of curcumin at 175.26 °C similar to our observation [39]. Nangia et al. described a polymorph of curcumin form 2, having a melting point of 175.12 °C [40]. The resveratrol melted at 261.20 °C with a sharp melting endotherm, thereby indicating resveratrol crystallinity (Figure 3.10). Srimornsak et al. observed the melting of resveratrol as two peak events, one at 254 °C and the other at 269 °C [41]. Ibold and Kumpugdee-Vollrath reported resveratrol melting at ~254 °C [42]. Cavalli et al. and Sinico et al. observed melting of resveratrol at 267 °C [21,31]. Zaho et al. observed melting event for resveratrol at 265.6 °C [43]. Melting of resveratrol between 261-263 °C has also been seen [44]. Thus, the reported temperatures for resveratrol melting ranged from 254 °C to 269 °C and in our study resveratrol melted at 261.20 °C. These variations could be attributed to extraction/preparation solvents used for resveratrol and differences in heating rates used to study resveratrol melting.

The thermogram of α-cyclodextrin showed enothermic events at 85.74 °C, 132.83 °C, a sharp melting event at 168.66°C and 181.52 °C (Figure 3.10). The initial two events may be attributed to water loss, however, the cause of later events needs to be explored. Sorrenti et al. thermally characterized several commercial brands of α-cyclodextrin and attributed a
few thermal events seen in α-cyclodextrin thermogram to trace(s) of aromatic or chlorinated solvents used in the preparation and separation of α-cyclodextrin [45].

Figure 3.10 MDSC thermograms of drugs, cyclodextrins, their respective physical mixtures and solvent evaporated solid dispersions. The melting endotherm for both drugs
is absent in HPβ-cyclodextrin solid dispersion, indicating possible decrease in drugs crystallinity.

The α-cyclodextrin based physical mixture showed thermal events similar to α-cyclodextrin thermogram and also a melting peak at 204.19 °C, which could be resveratrol in crystalline form. The α-cyclodextrin based solid dispersion showed thermal events similar to α-cyclodextrin thermogram. There were no peaks indicative of curcumin and resveratrol seen in α-cyclodextrin based solid dispersion thermogram.

The β-cyclodextrin thermogram showed melting event at 120.86 °C, which could be attributed to water loss (Figure 3.10). Similar observations, were made by Moyano et al. in their compilation of thermal properties of cyclodextrins [35]. The thermogram of β-cyclodextrin based physical mixture and solid dispersion showed two events indicative of β-cyclodextrin water loss and curcumin melting respectively. No event indicative of resveratrol was seen. Thus, curcumin may be retaining its crystallinity in the β-cyclodextrin based solid dispersion.

The γ-cyclodextrin thermogram showed melting event at 87.12 °C, which could be attributed to water loss (Figure 3.10). Similar observations, were made by Moyano et al. in their compilation of thermal properties of cyclodextrins [35]. The thermogram of γ-cyclodextrin based physical mixture showed two events indicative of γ-cyclodextrin water loss and curcumin melting respectively. The thermogram of γ-cyclodextrin based solid dispersion did not show melting peaks for either curcumin or resveratrol thereby, indicating their loss of crystallinity.

The HPβ-cyclodextrin thermogram, its physical mixture and solid dispersion showed thermal event indicative of water loss. Similar observation in DSC curve of HPβ-
cyclodextrin was made by Catenacci et al. [36]. A sharp melting peak at 172.72 °C indicative of curcumin melting was seen in HPβ-cyclodextrin based physical mixture thermogram (Figure 3.10). The thermogram of HPβ-cyclodextrin based solid dispersion did not show melting peaks for either curcumin or resveratrol thereby, indicating their loss of crystallinity. The second and third MDSC cycles did not show significant events for all samples that were examined.

**X-ray diffractometry:**

The XRPD pattern of curcumin showed that it is crystalline in nature (Figure 3.11). Mahadik et al. and Matioli et al. observed similar crystalline pattern for curcumin [38,47]. Resveratrol showed sharp diffraction peaks indicating its crystalline nature (Figure 3.11), which is corroborated by literature reports as well as our MDSC results [41,31]. The β-cyclodextrin and its physical mixture both showed sharp diffraction peaks, indicating their crystallinity (Figure 3.11). Sorrenti et al. characterized the commercially available brands of β-cyclodextrin and found them to be crystalline in nature [45]. The β-cyclodextrin based solid dispersion had fewer diffraction peaks as compared to its physical mixture. However, presence of drug melting peak in β-cyclodextrin based solid dispersion thermogram (Figure 3.10) and diffraction peaks in its XRPD pattern indicated some amount of drug(s) remained crystalline (Figure 3.11).
Figure 3.11 XRPD pattern of drugs, cyclodextrins, their respective physical mixtures and solvent evaporated solid dispersions. Both drugs are crystalline in nature. HPβ-cyclodextrin solid dispersion is amorphous in nature.
Similar, to α-cyclodextrin and β-cyclodextrin, γ-cyclodextrin too showed diffraction peaks indicating crystallinity (Figure 3.11). Sorrenti et al. analyzed the commercially available brands of γ-cyclodextrin and found them to be crystalline in nature [45]. The γ-cyclodextrin based physical mixture, showed diffraction peaks and so did its solid dispersion XRPD pattern (Figure 3.11). Thus, even though no melting peaks indicative of curcumin and resveratrol were seen in γ-cyclodextrin based solid dispersion thermogram (Figure 3.10), yet solid dispersion was crystalline in nature.

The HPβ-cyclodextrin showed no diffraction peaks and was thus amorphous in nature (Figure 3.11). Catenacci et al. too found HPβ-cyclodextrin to be amorphous in nature [36]. The HPβ-cyclodextrin based physical mixture showed diffraction peaks, thereby indicating crystallinity of drug(s). The HPβ-cyclodextrin based solid dispersion showed neither melting peaks of drugs in thermogram (Figure 3.10) nor any diffraction peak in its XRPD pattern (Figure 3.11), thereby indicating that drugs have been converted to their amorphous form.

Thus, of all solid dispersions under study, the HPβ-cyclodextrin based solid dispersion was found to be amorphous in nature.

**Infrared spectroscopy:**

The IR spectrum of curcumin is shown in figure 3.12. IR spectrum of curcumin showed a sharp peak at 3507 cm\(^{-1}\) indicating the presence of –OH group. There were peaks seen at 2923 cm\(^{-1}\) and 2856 cm\(^{-1}\) indicating –C-H stretch. A peak at 1741 cm\(^{-1}\) indicating –C=O characteristic is also seen.
Figure 3.12 IR Spectra of drugs, cyclodextrins, their respective physical mixtures and solvent evaporated solid dispersions. The peak at 3507 cm$^{-1}$ and the broad peak at 3293 cm$^{-1}$ of curcumin and resveratrol respectively, indicating phenolic hydroxyl groups are
significantly depressed in IR spectra of all solid dispersion, thereby suggesting possible H-bonding between drugs and cyclodextrins.

The peak at 1626 cm\(^{-1}\) indicates \(\nu(C=\pi C)\) and \(\nu(C=\pi O)\) of the inter-ring chain. Another peak at 1600 cm\(^{-1}\) can be attributed to the symmetric aromatic ring stretching vibrations \(\nu(C=\pi C_{\text{ring}})\). The 1509 cm\(^{-1}\) peak can be designated to the \(\nu(C=\pi O)\). The peak at 1430 cm\(^{-1}\) signifies \(\delta(\pi C\pi C)\), \(\delta(\pi C\pi H)\) and \(\delta(C=\pi OH)\) of aromatic rings. The peak at 1272 cm\(^{-1}\) can be assigned to enol –C-O. The 1149 cm\(^{-1}\) peak indicates \(\delta(C\pi CH)\) of aromatic rings and \(\delta(C=\pi OH)\) of the enolic group coupled to \(\delta(C=\pi CH)\) in the inter-ring chain. The peak at 1022 cm\(^{-1}\) is indicative of –C-O-C stretch, the peak at 960 cm\(^{-1}\) shows benzoate trans-CH vibration and peak at 713 cm\(^{-1}\) indicates cis CH vibration of aromatic ring. Herein, vibrational mode \(\delta\) indicates in plane bending and \(\nu\) indicates stretching. Joseph et al. and Spiteller et al. observed similar peaks in their attempt to understand vibrational spectra of curcumin [39,46].

The IR spectrum of resveratrol is shown in figure 3.12. The IR spectrum of resveratrol showed a strong peak at 3293 cm\(^{-1}\) indicating free O–H stretching vibrations. It also showed peaks at 1608 cm\(^{-1}\), 1587 cm\(^{-1}\), 1513 cm\(^{-1}\) and 1463 cm\(^{-1}\) which were all indicative of aromatic ring vibrations. There were peaks at 2919 cm\(^{-1}\) and 1380 cm\(^{-1}\), indicating –C-H stretch. A peak at 1145 cm\(^{-1}\) signifying –OH stretch was seen. Peaks at 987 cm\(^{-1}\) and 966 cm\(^{-1}\) indicating bending vibration of C = C-H were seen. Also, a peak at 829 cm\(^{-1}\) indicating phenyl ring substitution was observed. These results were in accordance to those observed by Sinico et al. and Zaho et al. [31,43].

All cyclodextrins, showed peak at 3405 cm\(^{-1}\) (±1 cm\(^{-1}\)) indicative of O–H stretching vibrations, a peak at 2932 cm\(^{-1}\) (±1 cm\(^{-1}\)) which can be attributed to C–H stretching
vibrations and peaks at 1375 cm\(^{-1}\) (±1 cm\(^{-1}\)), 1035 cm\(^{-1}\) (±1 cm\(^{-1}\)) indicating C–H, C–O stretching vibrations.

All physical mixtures spectra were summation of spectra of their components (Figure 3.12). Certain characteristics, for instance the curcumin peak at 3508 cm\(^{-1}\) indicative of –OH stretch was present in β-cyclodextrin based physical mixture spectrum. However, peaks of curcumin at 3508 cm\(^{-1}\) and that of resveratrol at 3293 cm\(^{-1}\) indicative of their –OH stretch were absent in solid dispersion spectra. This could be most likely because of H-bonding between drugs and cyclodextrins. However, it can be argued that these peaks are probably depressed or masked by those of cyclodextrins. Understanding of peak shifts in region of 750 cm\(^{-1}\) -2000 cm\(^{-1}\) was difficult owing to presence of cyclodextrin’s peaks in the same region. Thus, a complimentary spectroscopic technique such as Raman spectroscopy would help to yield a better understanding of interactions within the solid dispersion.

**Raman spectroscopy:**

The curcumin Raman spectra showed prominent peaks at 964 cm\(^{-1}\), 1151 cm\(^{-1}\), 1184 cm\(^{-1}\), 1250 cm\(^{-1}\), 1430 cm\(^{-1}\), 1600 cm\(^{-1}\) and 1627 cm\(^{-1}\) (Figure 3.17). The peak at 964 cm\(^{-1}\) is indicative of ν(C–O) and δ(C–OH) of enolic group; 1151 cm\(^{-1}\) peak indicates δ(CCH) of aromatic rings and δ(C–OH) of the enolic group coupled to δ(C=CH) in the inter-ring chain; 1184 cm\(^{-1}\) peak signifies δ(CH\(_3\)) of methoxy group and δ(CCH) of chain between keto group and aromatic ring; peak at 1250 cm\(^{-1}\) denotes δ(CH) of the aromatic rings which is combined to ν(C–O) of the ether groups linked to these rings; peak at 1430 cm\(^{-1}\) signify δ(CCC), δ(CCH) and δ(C–OH) of aromatic rings; 1600 cm\(^{-1}\) peak indicates ν(C=С) of
aromatic rings and peak at 1627 cm\(^{-1}\) indicates \(\nu(C=C)\) and \(\nu(C=O)\) of the inter-ring chain [46,47]. Herein, vibrational mode \(\delta\) indicates in plane bending and \(\nu\) indicates stretching.

The resveratrol Raman spectra showed prominent peaks at 996 cm\(^{-1}\), 1155 cm\(^{-1}\), 1169 cm\(^{-1}\), 1349 cm\(^{-1}\), 1425 cm\(^{-1}\), 1458 cm\(^{-1}\), 1604 cm\(^{-1}\), 1628 cm\(^{-1}\) and 1871 cm\(^{-1}\) (Figure 3.17). The peak at 996 cm\(^{-1}\) indicates \(\nu(C-O)\) of enolic group, \(\nu(C=C)\) of the inter-ring chain, \(\delta(C-H)\) of aromatic ring; peak at 1155 cm\(^{-1}\) denotes \(\delta(C-H)\) of the aromatic ring, \(\nu(C-O), \delta(C-OH)\) of enolic group; 1169 cm\(^{-1}\) peak signifies \(\delta(C-H)\) of aromatic ring; 1349 cm\(^{-1}\) peak indicates \(\delta(C-H)\) of aromatic ring, \(\delta(C-OH)\) of enolic group; group assignments for peaks at 1425 cm\(^{-1}\) and 1458 cm\(^{-1}\) are related to aromatic ring vibrations; 1604 cm\(^{-1}\) peak indicates \(\nu(C=C)\) of aromatic rings and peak at 1628 cm\(^{-1}\) indicates \(\nu(C=C)\) and \(\delta(C-H)\) of aromatic rings [48]. Assignment for peak at 1871 cm\(^{-1}\) was not certain.

Most peaks in Raman spectra of physical mixtures were similar to those seen in curcumin, resveratrol and respective cyclodextrin Raman spectrum (Figure 3.13). The Raman spectra of \(\alpha\)-cyclodextrin based solid dispersion, \(\beta\)-cyclodextrin based solid dispersion are similar to their physical mixture. However the spectra of \(\gamma\)-cyclodextrin based solid dispersion and HP\(\beta\)-cyclodextrin based solid dispersion show significant peak shift at 1627/1626 cm\(^{-1}\) and comparative depression of peak at 1603/1601 cm\(^{-1}\), thereby indicating H-bonding of phenolic hydroxyl groups of drugs with respective cyclodextrins and shielding of aromatic ring of drugs by cyclodextrin cavity. These changes are most prominent for HP\(\beta\)-cyclodextrin solid dispersion. Thus, Raman spectroscopy indicated H-bonding between drugs and cyclodextrin and shielding of drug’s hydrophobic regions by cyclodextrin.
Figure 3.13 Raman Spectra of drugs, cyclodextrins, their respective physical mixtures and solvent evaporated solid dispersions. Significant peak shifts were observed in HPβ-cyclodextrin based solid dispersion, thereby indicating interactions between drugs and HPβ-cyclodextrin in the solid dispersion.
Dissolution studies:

For curcumin, within first 30 min, 4.35 % of curcumin was released from HPβ-cyclodextrin based solid dispersion as compared to 1.27 % of curcumin release from HPβ-cyclodextrin based physical mixture. At 12 hr the curcumin released from HPβ-cyclodextrin based solid dispersion was approximately 3.08 %. Thus, almost 2.4 folds of solubility enhancement was achieved for curcumin within first 30 min itself, by solid dispersion as compared to the physical mixture. Yadav et al. observed 25.14 % higher curcumin release within first 1 hr by its HPβ-cyclodextrin complex as compared to the pure curcumin [15].

For resveratrol, within first 30 min 55.27 % of resveratrol was released from HPβ-cyclodextrin based solid dispersion as compared to 23.26 % of resveratrol release from HPβ-cyclodextrin based physical mixture. At 8 hr the resveratrol released from HPβ-cyclodextrin based solid dispersion was approximately 58.12 %. Thus, almost 32.03 % higher resveratrol release was achieved within first 30 min by solid dispersion as compared to the physical mixture. Zaho et al. showed solubility increase upto 24.94 mg/ml of resveratrol in its physical mixture with HPβ-cyclodextrin and solubility increase upto 25.13 mg/ml of resveratrol in its complex with HPβ-cyclodextrin [43].

Thus, considering solid dispersion of both drugs combined i.e. curcumin and resveratrol in HPβ-cyclodextrin, an optimum dissolution enhancement was achieved for both the drugs.
Figure 3.14 Percentage of (a) curcumin and (b) resveratrol released from physical mixture and HPβ-cyclodextrin based solvent evaporated solid dispersion. Dissolution of curcumin and resveratrol was higher from cyclodextrin solid dispersion as compared to physical mixture.
Preparation and characterization of binary system:

*Modulated differential scanning calorimetry:*

![MDSC thermograms of drugs, their physical mixture and solvent evaporated solid dispersion.](image)

**Figure 3.15** MDSC thermograms of drugs, their physical mixture and solvent evaporated solid dispersion. The melting endotherm for both drugs is present in their solid dispersion. The sharp melting peak of curcumin was seen at 175.77 °C and thus indicated crystalline nature of curcumin (Figure 3.15). The resveratrol melted at 261.20 °C with a sharp melting endotherm, thereby indicating resveratrol crystallinity (Figure 3.15). The physical mixture showed melting endotherms for both curcumin and resveratrol, but at lower temperatures i.e. 172.47 °C and 224.25 °C respectively (Figure 3.15). Nangia et al. has reported melting of curcumin-form3 at 172.85 °C, which is similar to the melting point of curcumin observed in the physical mixture [40]. Additionally, Vishwanatha and Mukerjee observed melting of pure curcumin at 172 °C [49]. There are no reports for resveratrol melting at
224.25 °C. However, it can be assumed that resveratrol and curcumin mixing leads to melting point depression of resveratrol. From, physical mixture of curcumin and resveratrol it was evident that both drugs retained their crystallinity. The thermogram for solid dispersion of curcumin and resveratrol showed unidentified crystallization peak followed by melting endotherms at 169.89 °C and 232.49 °C, representing curcumin and resveratrol respectively (Figure 3.15). Nangia et al. observed the onset of curcumin-form 3 melting at 168.29 °C, similar to that observed in solid dispersion thermogram for curcumin [40]. Resveratrol melting at 232.49 °C in solid dispersion, could again be attributed to its melting point depression. Existence of melting peaks for curcumin and resveratrol in their solid dispersion indicated their crystalline nature retention. No significant events were observed in second and third MDSC cycles.

X-ray diffractometry:

![XRPD pattern of drugs, their physical mixture and solvent evaporated solid dispersion](image)

Figure 3.16 XRPD pattern of drugs, their physical mixture and solvent evaporated solid dispersion. The drugs, their physical mixture and solvent evaporated solid dispersion were all found to be crystalline in nature.
The XRPD pattern of curcumin and resveratrol showed that they are crystalline in nature (Figure 3.16). The physical mixture of curcumin and resveratrol showed diffraction peaks similar to those of curcumin and resveratrol indicating their presence in crystalline form (Figure 3.16). Similar, observation was made in MDSC thermogram, wherein, melting peaks indicative of both curcumin and resveratrol were present in physical mixture thermogram. The X-ray diffraction pattern for solid dispersion showed sharp peaks, thereby indicating its crystalline form (Figure 3.16). Several peaks of resveratrol were evident in the XRPD pattern of solid dispersion and a few curcumin peaks were also seen. Similar observation, of drugs in crystalline form was made in MDSC study. Thus, the drugs remain crystalline on mixing or solvent evaporation.

Raman spectroscopy:

![Raman spectra of drugs, their physical mixture and solvent evaporated solid dispersion](image)

**Figure 3.17** Raman spectra of drugs, their physical mixture and solvent evaporated solid dispersion. No significant peaks shifts were observed, thereby indicating no strong interaction between the drugs.
The curcumin and resveratrol Raman spectra are explained in the previous section. The Raman spectrum for physical mixture showed peaks indicative of curcumin and resveratrol peaks (Figure 3.17). There were no significant peak shifts observed for Raman spectrum of physical mixture, thereby indicating no strong interactions between curcumin and resveratrol. The Raman spectrum of solid dispersion was essentially similar to that of physical mixture (Figure 3.17). However, there was a significant peak shift at 1633 cm\(^{-1}\) which indicates the conjugation of \(\nu(C=C)\) and \(\nu(C-O)\) in rings has reduced and there are weak interactions between the –OH and –OCH\(_3\) groups on aromatic rings of curcumin and resveratrol.

### 3.5 Molecular modeling studies

#### 3.5.1 Rationale:

Molecular modeling involves theoretical methods and computational techniques which are employed to model or simulate variety of molecular interactions [50]. Molecular modeling has been widely employed for the process of drug discovery, however it’s applications are now being explored for drug delivery systems’ development [51]. Douroumis et al. used molecular modeling to estimate drug polymer interactions and thereby assist solid dispersion development [52]. According to a survey of publications until 2013, docking studies rank second amongst number of papers published on drug-cyclodextrin based molecular modeling studies [51]. Herein, we used molecular modeling so as to understand the lowest energy orientations of drugs and cyclodextrin, specifically HPβ-cyclodextrin and the possible interactions amongst them. This helped us modify the levels of solid dispersion components, if the need be.
3.5.2 Methods:

The software Molecular Operating Environment (MOE), developed by the Chemical Computing Group (Montreal, Canada, 2013) was used. The structures of curcumin and resveratrol (trans-resveratrol), were built in MOE. The carbon atoms for curcumin and resveratrol were depicted in yellow and white respectively. The hydrogen and oxygen atoms were shown in light grey and red respectively. For curcumin:resveratrol interaction study, a conformational search was performed using the MMFF94x forcefield to identify the lowest energy curcumin:resveratrol configuration. The 2-hydroxypropyl-β-cyclodextrin structure was generated by alkylating the seven primary alcohol groups on β-cyclodextrin (βCD, downloaded from the protein data bank, PDB ID = 1GVI) with 2-hydroxy propyl groups. The partial charges were then assigned to the structure using the MMFF94x forcefield. A conformational search was performed and the lowest energy conformer was subsequently used in docking studies.

For, curcumin:HPβ-cyclodextrin and resveratrol:HPβ-cyclodextrin interactions, curcumin and resveratrol were individually docked into the 2-hydroxypropyl-β-cyclodextrin structure. Amongst the complexes so obtained, lowest energy complexes were selected for curcumin:HPβ-cyclodextrin and resveratrol:HPβ-cyclodextrin and were further energy minimized using the MMFF94x forcefield. For, curcumin:resveratrol:HPβ-cyclodextrin interaction, the energy minimized curcumin:HPβ-cyclodextrin and resveratrol:HPβ-cyclodextrin complexes, were further energy minimized in presence of each other using the MMFF94x forcefield.
3.5.3 Results and Discussion:

![Images of molecular structures](image)

**Figure 3.18** The computationally modelled structures of curcumin, resveratrol, HPβ-
cyclodextrin, curcumin:resveratrol, curcumin:HPβ-cyclodextrin, resveratrol:HPβ-cyclodextrin and curcumin:resveratrol:HPβ-cyclodextrin. The molecular modeling studies suggested that HPβ-cyclodextrin was likely to enhance the apparent aqueous solubility of curcumin and resveratrol by shielding their hydrophobic regions.

The modelled structures of curcumin, resveratrol and HPβ-cyclodextrin appeared optimum. There are intra-molecular H-bonding in HPβ-cyclodextrin. A possible H-bond between keto group of curcumin and phenol group of resveratrol, was observed.

For, curcumin:HPβ-cyclodextrin it was seen that, the hydrophobic regions of curcumin such as aromatic rings and inter ring chain were shielded by cyclodextrin ring. Such shielding effect was also interpreted in Raman spectrum of curcumin:resveratrol:HPβ-cyclodextrin solid dispersion (Figure 3.13). The methoxy and hydrophilic phenolic groups of curcumin were outside the cyclodextrin cavity and were thus exposed to solvent. Hence, the shielding effect of cyclodextrin, would thereby help in enhancing the apparent aqueous solubility of curcumin. Similarly, for resveratrol:HPβ-cyclodextrin, the hydrophobic regions of resveratrol were shielded by cyclodextrin ring. The hydrophilic phenolic group remained exposed to solvent. Thus, it’s likely that apparent aqueous solubility of resveratrol would be enhanced by HPβ-cyclodextrin. A similar configuration was observed by Zou et al. for resveratrol:HPβ-cyclodextrin complex [53].

When, both complexes were energy minimized in presence of each other the ligands i.e. curcumin, resveratrol and receptor i.e. HPβ-cyclodextrin maintained their original orientations and neither ligand seemed to be displaced out of the cyclodextrin cavity. Thus, molecular modeling yielded possible inclusion phenomena that may exist in the solid dispersions. Also, the 1:1:2 M ratio of curcumin:resveratrol:HPβ-cyclodextrin seemed
optimum. However, it’s important to note that the docking studies done herein, elucidate only the inclusion phenomena seen with drug-cyclodextrin. Thus, to encompass non-inclusion solubility enhancement ability of cyclodextrins, cyclodextrin levels would be modified in further studies.

3.6 Summary and conclusion

UV-Vis spectroscopy provided suitable method for simultaneous quantification of curcumin and resveratrol. The solubility studies elucidated the ability of apparent aqueous solubility enhancement of curcumin and resveratrol by all cyclodextrins under study. The solvent evaporation technique led to development of optimum solid dispersions. Microscopy studies gave a preliminary view of appearance of solid dispersions. TGA study showed optimum thermal stability of all solid dispersions. MDSC study and XRPD study indicated amorphous nature of HPβ-cyclodextrin based solid dispersion. The IR and Raman spectroscopy studies illustrated interactions between drugs and HPβ-cyclodextrin in their solid dispersion. The dissolution confirmed the potential of HPβ-cyclodextrin in dissolution enhancement of curcumin and resveratrol. The molecular modeling results, supported the ability of HPβ-cyclodextrin to enhance the apparent aqueous solubility of curcumin and resveratrol by shielding their hydrophobic regions. Thus, HPβ-cyclodextrin emerged as the most promising cyclodextrin for apparent aqueous solubility enhancement of curcumin and resveratrol. Hence, HPβ-cyclodextrin would be explored for further studies.
3.7 References


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42. Kumpugdee-Vollrath M, Ibold Y. Determination of trans resveratrol and different types of cyclodextrins by SAXS and WAXS at B1. Available at:


44. Resveratrol safety data sheet. Acros Organics. Available at:


Chapter 4

4. Solid Dispersion Development

4.1 Materials and instrumentation

Following materials such as drugs, excipients and solvents have been used in the experiments (Table 4.1):

Table 4.1 Materials used in the study

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Following instruments/machines have been used in the experiments (Table 4.2):

**Table 4.2 Instruments/machines used in the study**

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4.2 Optimization, preparation and characterization of freeze-dried curcumin and/or resveratrol-cyclodextrin solid dispersion

4.2.1 Rationale:

From the studies in Chapter 3, it was evident that HPβ-cyclodextrin was most suitable amongst the analyzed cyclodextrins for dissolution enhancement of both curcumin and
resveratrol. Thus, it was decided to carry out further experimentation with HPβ-cyclodextrin.

Cyclodextrins can aid or impede drug delivery through biological membranes, thus it’s extremely important to optimize cyclodextrin concentration in solid dispersions [1]. Brewster et al. suggested that pharmaceutical formulations, should contain as small an amount of cyclodextrins as possible, since they can decrease drug bioavailability and also preservative efficacy in a formulation [2]. Thus, in order to optimize cyclodextrin concentration in solid dispersion, three concentrations of HPβ-cyclodextrins were screened for their curcumin and resveratrol solubility enhancement ability and most appropriate concentration was chosen.

With optimized HPβ-cyclodextrin concentration, solid dispersions were prepared using freeze drying technique. Freeze drying was chosen because unlike solvent evaporation, freeze drying involves use of water and little to almost nil amount of organic solvents [3]. Freeze drying also is an industrially friendly process, leading to formation of uniform batches [4]. Zheng et al. found improved oral bioavailability of baicalein in solid dispersions prepared by freeze drying as oppose to those prepared by solvent evaporation [5]. The samples for freeze drying technique were optimized too. Solid dispersions of individual drugs and drug combination were prepared so as to develop better understanding of the system. The solid dispersions were characterized by light microscopy and scanning electron microscopy to analyze their morphology. Thermogravimetric analysis, differential scanning calorimetry and X-ray diffraction were used to analyze thermal behavior and state respectively of solid dispersions. Infrared spectroscopy and Raman spectroscopy were used to characterize the interactions in solid dispersions. Thus, we designed thorough
preparation and physico-chemical characterization techniques and evaluated the freeze
dried solid dispersions.

4.2.2 Methods:

Optimization of HPβ-cyclodextrin concentration:

HPβ-cyclodextrin fractions weighing 0 mg, 192.63 mg, 385.25 mg and 770.5 mg
corresponding to no cyclodextrin, 2 M, 4 M and 8 M ratio to that of 23 mg curcumin and
14.25 mg resveratrol were taken. The weighed HPβ-cyclodextrin fractions were then
dissolved in 20 ml deionized water separately and mixed. Once HPβ-cyclodextrin was
dissolved 23 mg curcumin and 14.25 mg resveratrol were added to only deionized water
and HPβ-cyclodextrin solutions. These mixtures were then sonicated for 10 min. Thus, the
mixtures contained 20 ml deionized water and 1:1:0 M, 1:1:2 M, 1:1:4 M and 1:1:8 M of
curcumin:resveratrol: HPβ-cyclodextrin. The mixtures were then stirred on a magnetic
stirred at a speed of 5 units. At 24 hr, 1 ml samples were withdrawn from each mixture and
filtered using 0.45 μm syringe filter and diluted with methanol. These samples were
analyzed for curcumin and resveratrol content in triplicates using
-Vis spectroscopy.

Preparation of solid dispersion:

Solid dispersions of curcumin: HPβ-cyclodextrin in molar ratio of 1:1, resveratrol: HPβ-
cyclodextrin in molar ratio of 1:1 and curcumin:resveratrol:HPβ-cyclodextrin in molar
ratio of 1:1:2 were prepared. Two types of solvent systems one with acetone and deionized
water and other with only deionized water were used to prepare solid dispersion samples
for freeze drying. Thus, following samples were prepared for freeze drying:
• 59.7 mg of curcumin was mixed in 2 ml of acetone, 250 mg HPβ-cyclodextrin and 10 ml of deionized water was added to it.

• 37 mg of resveratrol was mixed in 2 ml of acetone, 250 mg HPβ-cyclodextrin and 10 ml of deionized water was added to it.

• 59.7 mg of curcumin and 37 mg of resveratrol were mixed in 4 ml of acetone, 500 mg HPβ-cyclodextrin and 20 ml of deionized water was added to it.

• 59.7 mg of curcumin, 250 mg HPβ-cyclodextrin were mixed in 10 ml of deionized water.

• 37 mg of resveratrol, 250 mg HPβ-cyclodextrin were mixed in 10 ml of deionized water.

• 59.7 mg of curcumin, 37 mg of resveratrol and 500 mg HPβ-cyclodextrin were mixed 20 ml of deionized water.

All the above samples were sonicated for 5 min, thereafter placed on a magnetic stirrer and stirred for 7 days at a speed of 5 units. During stirring the acetone based sample vials were covered with foil and hole for acetone to escape was made. The acetone elimination post 7 days was confirmed by smelling the samples. The samples were filtered using 0.45 µm filter under a vacuum pressure of 150 mbar. The filtrates were analyzed for their curcumin and/or resveratrol content using UV-Vis spectroscopy. The filtrates were then transferred to petri plates and freeze dried. Following freeze-drying cycles were employed:

Freeze cycle-

Shelf setpoint (ºC): -50

Time (min): 240
Final freeze (ºC): -60
Extra freeze (min): 20
Pre vacuum start (MT): 200

Freeze cycle-
Shelf set point (ºC): -50
Time (min): 240
Final freeze (ºC): -60
Extra freeze (min): 20
Pre vacuum start (MT): 200

Primary dry cycle-

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<th>7</th>
<th>8</th>
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<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shelf setpoint (ºC)</td>
<td>-20</td>
<td>-20</td>
<td>-10</td>
<td>-10</td>
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<td>0</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Time (min)</td>
<td>25</td>
<td>240</td>
<td>30</td>
<td>240</td>
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<td>240</td>
<td>30</td>
<td>240</td>
<td>30</td>
<td>360</td>
</tr>
<tr>
<td>Vacuum setpoint (MT)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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</table>

Secondary dry cycle-
Shelf set point (ºC): 20
Time (min): 360
Vacuum setpoint (MT): 10
Final setpoint (ºC): 5

The freeze dried samples were then transferred to vials.
Physico-chemical characterization of solid dispersion:

Light Microscopy:
The solid dispersions were mounted on glass slides. The samples were then observed under a light microscope with 20 X objective. The images were captured using OPUS software.

Scanning electron microscopy (SEM):
The acetone:water based solid dispersions were analyzed. An aluminum mount was coated with an adhesive using an adhesive Tab. The solid dispersion was placed on the adhesive surface, excess sample that did not adhere to the surface was gently blown away leaving a single layer of particles on the surface of the mount. The sample was then placed in an EMS/Quorum ESR sputter coater and coated with gold/palladium to render it conductive. Sample was viewed in a scanning electron microscope at 15 kilovolts voltage. Images were captured as TIFF’s using a Quartz PCI system.

Thermogravimetric analysis (TGA):
Approximately 5-10 mg of solid dispersions were placed in open aluminum pans and heated at a rate of 10 °C/min, up to 300 °C/500 °C, with a nitrogen purge, in a thermogravimetric analyzer.

Modulated differential scanning calorimetry (MDSC):
Approximately 3-10 mg of solid dispersions were placed in aluminum pans and hermetically sealed with a lid/ lid with pinhole. The samples pans along with reference pan were subjected to heating-cooling heating cycles, at a rate of 2 °C/min, from -40 °C to 200 °C/350 °C, with a nitrogen purge, in a modulated differential scanning calorimeter.
**X-ray diffractometry (XRPD):**

X-Ray powder diffraction patterns were obtained for solid dispersions using X-ray diffractometer. The data in the 2θ range 5–60° was collected in focusing geometry using X-ray Diffractometer, operated with Cu Kα radiation at 40 kilovolts and 45 milliampere. A mask of 20 mm and a divergence slit of 1/4° were used on the incident beam path. Thin layer of powder sample was placed on a zero background Si plate and the sample holder was continuously spun at the rate of 90°/second during the measurement. Solid state PIXcel3D detector was scanned at a rate of 0.135°/second to collect data and a diffracted beam monochromator for the PIXcel detector was utilized to improve signal to noise ratio.

**Infrared spectroscopy (IR):**

The solid dispersions were placed in IR instrument sample holder and subjected to IR spectroscopy. The IR instrument had ATR setup with ZnSe crystal. 32 scans were taken for each sample and the resolution was set to 4 units and IR spectra were collected. Background spectra were taken every 30 min.

**Raman spectroscopy:**

The solid dispersions were mounted on glass slides. The samples were placed on stage of Raman microscpectrometer and subjected to Raman light. The aperture of 50×100 µm and the object lens of 20 X size were used to focus on samples. The samples were subjected to a laser beam having a wavelength of 785 nm and spectral data in the range of 0-2000 cm⁻¹ wavenumber was collected. A resolution level of approximately 3.5 cm⁻¹ was set in the instrument.
Note: Wherever applicable, pure drug(s) based samples were covered with aluminum foil or amber colored apparatus were used. The molar ratios or concentrations mentioned are with respect to solid state combination of drug(s) and/or excipients.

4.2.3 Results and Discussion:

Optimization of HPβ-cyclodextrin concentration:

Figure 4.1 Amount of curcumin dissolved by varying HPβ-cyclodextrin concentration.

Figure 4.2 Amount of resveratrol dissolved by varying HPβ-cyclodextrin concentration.
Sravanthi et al. suggested that for optimum formulation performance, cyclodextrin concentration should be optimized [1]. From, figure 4.1 we can see that with increase in HPβ-cyclodextrin concentration, amount of curcumin dissolved increased. From absence of HPβ-cyclodextrin, to approximately 2 M concentration of HPβ-cyclodextrin, there was an increase in amount of curcumin dissolved. From 2 M to 4 M concentration of HPβ-cyclodextrin, there was a slight increase in amount of curcumin dissolved and it negatively deviated from the linear profile. A plausible reason for this could be limited amount of HPβ-cyclodextrin availability and there may be some self-association of HPβ-cyclodextrin molecules. From 4 M to 8 M concentration of HPβ-cyclodextrin, there was a drastic increase in amount of curcumin dissolved and it positively deviated from the linear profile. This, may be due to increase of HPβ-cyclodextrin availability in spite of self-association, thereby leading to higher solubilization of curcumin. Brewster et al. has suggested the tendency of self-association of cyclodextrin molecules, leading to negative deviation on phase solubility plot [6].

From, figure 4.2 we can see the effect of increase in HPβ-cyclodextrin concentration, on amount of resveratrol dissolved. From absence of HPβ-cyclodextrin, to approximately 2 M concentration of HPβ-cyclodextrin, there was drastic increase in amount of resveratrol dissolved. From 2 M to 4 M concentration of HPβ-cyclodextrin, there was a slight increase in amount of resveratrol dissolved and in both cases there was a positive deviation from linear profile. However, at 8 M HPβ-cyclodextrin there was a comparative decrease in amount of resveratrol dissolved. This might be due to some dissociation between resveratrol and HPβ-cyclodextrin units. Also, it’s noteworthy that at 8 M concentration of HPβ-cyclodextrin, the amount of curcumin dissolved increases drastically and thus that
may be due to better association between curcumin and cyclodextrin. Also, there may be occurrence of some self-association between HPβ-cyclodextrin molecules. These reasons could be likely explanation of the solubility profiles seen. However, the exact causes and dependency of factors such as sonication time, stirring time, stirring speed and microenvironment related factors remain to be explored.

Masson et al. proposed that the most common stoichiometry of association between cyclodextrin and drug are 1:1 M [7]. Tonnesen et al. suggested occurrence of 1:1 M stoichiometry of association between HPβ-cyclodextrin and curcumin in buffered media [8]. He also observed existence of some 1:2 M stoichiometry of association between HPβ-cyclodextrin and curcumin. Nunez-Delicado et al. considered an assumption of 1:1 M stoichiometry of association between HPβ-cyclodextrin and resveratrol [9]. Considering these possibilities and the fact that at 2 M HPβ-cyclodextrin concentration, optimum amount of both curcumin and resveratrol were dissolved with no drastic negative deviation from linear profile, using 1:1:2 M ratio of curcumin:resveratrol: HPβ-cyclodextrin seems acceptable.

Preparation of solid dispersion:

Optimum freeze dried solid dispersions were prepared. Water based systems were difficult to filter as compared to acetone:water based systems. On analyzing the filtrate concentration, it was seen that higher amount of drugs were dissolved in acetone:water based system as compared to water based systems (Table 4.3).
### Table 4.3 Amount of drug(s) in filtrate

<table>
<thead>
<tr>
<th></th>
<th>Acetone:water based system</th>
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<tbody>
<tr>
<td></td>
<td>Curcumin:HPβCD SD</td>
<td>Resveratrol:HPβCD SD</td>
<td>Curcumin:resveratrol:HPβCD SD</td>
</tr>
<tr>
<td>Curcumin</td>
<td>63.13</td>
<td>-</td>
<td>133.99</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>-</td>
<td>2149.53</td>
<td>1275.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Water based system</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Curcumin:HPβCD SD</td>
<td>Resveratrol:HPβCD SD</td>
<td>Curcumin:resveratrol:HPβCD SD</td>
</tr>
<tr>
<td>Curcumin</td>
<td>20.92</td>
<td>-</td>
<td>157.18</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>-</td>
<td>1143.82</td>
<td>1028.08</td>
</tr>
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</table>

The solid dispersions so freeze dried were fluffy in nature. Joseph et al. successfully prepared curcumin:HPβ-cyclodextrin complexes using freeze drying technique [10]. For preparation curcumin:HPβ-cyclodextrin complexes, Wadu et al. compared common solvent evaporation, freeze drying and pH shift methods and found common solvent evaporation method to be most efficient [11]. However, for reasons discussed earlier, use of freeze drying technique was optimum for curcumin:resveratrol:HPβ-cyclodextrin solid dispersion preparation. Xia et al. successfully loaded resveratrol in HPβ-cyclodextrin using freeze dryer [12]. Thus, freeze drying yielded curcumin:HPβ-cyclodextrin solid dispersion, resveratrol:HPβ-cyclodextrin solid dispersion and curcumin:resveratrol:HPβ-cyclodextrin solid dispersion.
Physico-chemical characterization of solid dispersion:

*Light Microscopy:*

**Figure 4.3** Appearance of freeze dried solid dispersions under a light microscope.

Light microscopy gave initial knowledge of appearance of freeze dried solid dispersions (Figure 4.3). All solid dispersions appeared different from the corresponding pure drugs. The appearance of acetone:water based solid dispersions and corresponding solid dispersions was similar. The curcumin:HPβ-cyclodextrin solid dispersions had slight yellowish tinge in their light microscopy image. The resveratrol:HPβ-cyclodextrin solid dispersions appeared uniformly white in color. The curcumin:resveratrol:HPβ-cyclodextrin solid dispersions appeared white with yellowish tinge in color. All the light microscopy images had high light reflectance and thus, very little information could be yielded from them. Patila et al. characterized candesartan cilexetil solid dispersions made
by kneading and spray drying method using optical microscopy [13]. However, owing to flaky nature of candesartan cilexetil solid dispersions made by freeze drying method, they did not use optical microscopy for its characterization.

**Scanning electron microscopy (SEM):**

![SEM images of acetone:water based freeze dried solid dispersions; Above-100X magnification, Below-500X magnification.](image)

**Figure 4.4** SEM images of acetone:water based freeze dried solid dispersions; Above-100X magnification, Below-500X magnification.

Since, acetone:water based systems had higher drug content, they were evaluated by scanning electron microscopy (Figure 4.4). In 100 X magnification view, the curcumin:HPβ-cyclodextrin solid dispersion had clustered morphology. Diamond shaped cavities in the cluster were seen. On higher magnification of 500 X, these cavities seemed empty. The resveratrol:HPβ-cyclodextrin solid dispersion had irregular flaky morphology in 100 X as well as 500 X magnification. The curcumin:resveratrol:HPβ-cyclodextrin
solid dispersion was similar in appearance as that of resveratrol:HPβ-cyclodextrin solid dispersion. However, it had smaller irregular flakes which appeared scattered. Kale et al. studied quercetin:HPβ-cyclodextrin freeze dried complexes using scanning electron microscopy [14].

**Thermogravimetric analysis (TGA):**

**Table 4.4** TGA data of freeze dried solid dispersions

<table>
<thead>
<tr>
<th>% Weight Loss</th>
<th>Temperature (°C)</th>
<th>Acetone: Water based system</th>
<th>Water based system</th>
</tr>
</thead>
<tbody>
<tr>
<td>0±0.5</td>
<td>16.86</td>
<td>19.78</td>
<td>16.36</td>
</tr>
<tr>
<td>5±0.5</td>
<td>52.86</td>
<td>67.67</td>
<td>67.49</td>
</tr>
<tr>
<td>10±0.5</td>
<td>294.04</td>
<td>306.78</td>
<td>302.88</td>
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The freeze dried solid dispersions showed significant thermostability (Table 4.4). The acetone:water based solid dispersions were slightly more thermostable than water based solid dispersions. The initial weight loss of 5 %, for all solid dispersions occurred at 50 °C-70 °C and can be attributed to loss of HPβ-cyclodextrin moisture content. For all solid dispersions, 10 % weight loss occurred at a temperature >280 °C. The acetone:water based curcumin:HPβ-cyclodextrin solid dispersion showed weight loss of 10 % at 294.04 °C. Chauhan et al. observed similar weight loss at 300 °C for freeze dried curcumin:β-cyclodextrin complex [15]. The acetone:water based resveratrol:HPβ-cyclodextrin solid dispersion showed weight loss of 10 % at 306.78 °C and thus was more thermostable than acetone:water based curcumin:HPβ-cyclodextrin solid dispersion, the reverse was seen for water based systems.
**Modulated differential scanning calorimetry (MDSC):**

The sharp melting peak of curcumin was seen at 175.77 °C and thus indicated crystalline nature of curcumin (Figure 4.5). At a heating rate of 10 °C/min from 25 °C-210 °C Joseph et al. found the melting peak of curcumin at 175.26 °C similar to our observation [10]. The resveratrol melted at 261.20 °C with a sharp melting endotherm, thereby indicating resveratrol crystallinity (Figure 4.5). Melting of resveratrol between 261-263 °C has been seen [16].

The thermogram of acetone:water based curcumin:HPβ-cyclodextrin solid dispersion showed a broad melting endotherm at 85.78 °C(Figure 4.5). This could be attributed to loss of moisture content from HPβ-cyclodextrin. There was no peak corresponding to that of curcumin, seen in the thermogram, thereby by indicating conversion of crystalline curcumin to amorphous state. Chauhan et al. prepared freeze dried complexes of curcumin:β-cyclodextrin and characterized it DSC[15]. They observed that peaks belonging to curcumin at 172 °C had completely disappeared and also the β-cyclodextrin melting point peak was lowered to 86.5 °C compared to 99.5 °C, in curcumin:β-cyclodextrin complex thermogram. They concluded that, curcumin molecules would have completely replaced water molecules in β-cyclodextrin. The thermogram of water based curcumin:HPβ-cyclodextrin solid dispersion showed no peaks and hence the curcumin might have existed in amorphous state (Figure 4.5).

The thermogram of acetone:water based resveratrol:HPβ-cyclodextrin solid dispersion showed two thermal events, smaller one at 154.31 °C and a larger one at 215.71 °C, which can be assigned to resveratrol melting depression (Figure 4.5). However, the cause of thermal event at 154.31 °C remains to be explored. Cavalli et al. prepared β-cyclodextrin
nanosponges loaded with resveratrol using freeze drying technique and characterized it by DSC [17]. They observed partial suppression of melting endotherm of resveratrol in the thermogram and concluded partial protection of resveratrol due to the encapsulation of resveratrol with β-cyclodextrin. The thermogram of water based resveratrol:HPβ-cyclodextrin solid dispersion showed two thermal events, similar to those of acetone:water based resveratrol:HPβ-cyclodextrin solid dispersion.

The thermogram of acetone:water based curcumin:resveratrol:HPβ-cyclodextrin solid dispersion showed two thermal events. The smaller one at 162.21°C, which can be assigned to the peak seen for resveratrol:HPβ-cyclodextrin solid dispersion or melting depression of curcumin. However, since melting depression peak for curcumin wasn’t seen in curcumin:HPβ-cyclodextrin solid dispersion thermogram, its more likely that former is the indication for the peak at 162.21°C. And a larger one at 228.96 °C, which can be assigned to resveratrol melting depression. The thermogram of water based curcumin:resveratrol:HPβ-cyclodextrin solid dispersion showed two thermal events, similar to those of acetone:water based curcumin:resveratrol:HPβ-cyclodextrin solid dispersion.
Figure 4.5 MDSC thermograms of drugs and freeze dried solid dispersions.
**X-ray diffractometry (XRPD):**

The XRPD pattern of curcumin and resveratrol showed that they are crystalline in nature (Figure 4.6). Mahadik et al. and Sinico et al. both observed sharp diffraction peaks for curcumin and resveratrol respectively, thereby indicating their crystalline nature [18,19]. All freeze dried solid dispersions, showed no sharp diffraction peaks corresponding to either drugs, thereby indicating that the drugs are in amorphous state (Figure 4.6). Similarly, Joseph et al. observed hallow XRPD pattern for freeze-dried curcumin:HPγ-cyclodextrin complexes [10]. Cavalli et al. observed major hallow pattern for freeze dried resveratrol:β-cyclodextrin nanosponges [17].
Figure 4.6 XRPD pattern of drugs and freeze dried solid dispersions. Both drugs are crystalline in nature. All solid dispersions were amorphous in nature.
Infrared spectroscopy (IR):

**Figure 4.7** IR Spectra of drugs and freeze dried solid dispersions. The peak at 3507 cm$^{-1}$ and the broad peak at 3293 cm$^{-1}$ of curcumin and resveratrol respectively, indicating
phenolic hydroxyl groups are significantly depressed in IR spectras of respective solid dispersions, thereby suggesting possible H-bonding between drugs and cyclodextrins. The IR spectrum of curcumin and resveratrol are shown in figure 4.7. IR spectrum of curcumin showed a sharp peak at 3507 cm\(^{-1}\) indicating the presence of \(-\text{OH}\) group. The IR spectrum of resveratrol showed a strong peak at 3293 cm\(^{-1}\) indicating free O–H stretching vibrations. The IR spectra of acetone:water based solid dispersions were similar to corresponding water based solid dispersions (Figure 4.7). The peak of curcumin at 3507 cm\(^{-1}\) was absent in curcumin:HP\(\beta\)-cyclodextrin solid dispersion and also in curcumin:resveratrol:HP\(\beta\)-cyclodextrin solid dispersion. The peak of resveratrol at 3293 cm\(^{-1}\) was absent in resveratrol:HP\(\beta\)-cyclodextrin solid dispersion and also in curcumin:resveratrol:HP\(\beta\)-cyclodextrin solid dispersion. These changes are an indication of H-bonding between drugs and HP\(\beta\)-cyclodextrin.

Raman spectroscopy:

The curcumin and resveratrol Raman spectra showed prominent peaks indicative of their respective structures (Figure 4.8). The Raman spectra of all freeze dried solid dispersions showed significant noise, making it difficult to detect the Raman peaks (Figure 4.8). However, the peaks of interest in the region of 1600 cm\(^{-1}\) to 1640 cm\(^{-1}\) were well detectable for resveratrol:HP\(\beta\)-cyclodextrin and curcumin:resveratrol:HP\(\beta\)-cyclodextrin solid dispersions. The Raman spectra of acetone:water and water based curcumin:HP\(\beta\)-cyclodextrin solid dispersions showed no detectable peaks in the region of interest.
Figure 4.8 Raman Spectra of drugs and freeze dried solid dispersions. Significant peak
shifts were observed in curcumin:resveratrol:HPβ-cyclodextrin solid dispersions, thereby indicating interactions between drugs and HPβ-cyclodextrin in the solid dispersion. The Raman spectrum of acetone:water based resveratrol:HPβ-cyclodextrin solid dispersion showed a peak shift of resveratrol from 1604 cm$^{-1}$ to 1607 cm$^{-1}$ and 1628 cm$^{-1}$ to 1635 cm$^{-1}$, thereby indicating H-bonding of phenolic hydroxyl groups of resveratrol with hydroxyl groups of HPβ-cyclodextrin. Also, the peak at 1607 cm$^{-1}$ was relatively depressed, thereby indicating shielding of aromatic ring of resveratrol by HPβ-cyclodextrin cavity. The Raman spectrum of water based resveratrol:HPβ-cyclodextrin solid dispersion was similar to that of acetone:water based resveratrol:HPβ-cyclodextrin solid dispersion. But the relative peak depression at 1607 cm$^{-1}$ wasn’t seen and the peak shift was marginally less i.e. from 1628 cm$^{-1}$ to 1634 cm$^{-1}$. And thus, resveratrol may have not completely been shielded by HPβ-cyclodextrin cavity and less H-bonding may have occurred. Thus, acetone:water based system seemed to have caused better interaction between resveratrol and HPβ-cyclodextrin as compared to water based system. For the Raman spectra of curcumin:resveratrol:HPβ-cyclodextrin solid dispersions, the peak at 1607 cm$^{-1}$ seemed to be contributed by both curcumin and resveratrol peaks at 1600 cm$^{-1}$ and 1601 cm$^{-1}$ respectively. The Raman spectra of acetone:water and water based curcumin:resveratrol:HPβ-cyclodextrin solid dispersion did show relative peak depression at 1607 cm$^{-1}$ and peak shifts to 1635 cm$^{-1}$ and 1634 cm$^{-1}$, thereby indicating interactions between drugs and HPβ-cyclodextrin.

It’s important to note that, owing to filtration, prior to freeze drying, leads to low amount of curcumin in freeze dried samples. And thus, the results of characterization studies...
carried above for curcumin:resveratrol:HPβ-cyclodextrin solid dispersion may be more similar of those of resveratrol:HPβ-cyclodextrin solid dispersion.

4.3 Preparation and characterization of freeze dried curcumin-resveratrol-cyclodextrin solid dispersion

4.3.1 Rationale:

From, the studies carried out in section 4.3, we were able to optimize and prepare freeze dried curcumin:resveratrol:HPβ-cyclodextrin solid dispersion. Thus, now herein we created different batches of optimized formulation and tried understanding the same by a few characterization techniques. Based on section 4.3, we comprehended that 1:1:2 M ratio of curcumin:resveratrol:HPβ-cyclodextrin seems optimum. Thus, we employed 2 M concentration of HPβ-cyclodextrin. We also, comprehended that acetone:water based systems lead to better solid dispersions, whereby they enable higher interaction between drugs and HPβ-cyclodextrin. We utilized as low amount of acetone as possible to assure complete elimination of acetone. Thus, herein we employed acetone:water as solvent. We created batches of curcumin:resveratrol with filtration, curcumin:resveratrol:HPβ-cyclodextrin with filtration and curcumin:resveratrol:HPβ-cyclodextrin without filtration prior to freeze drying. This was done since, in section 4.3, less of curcumin was retained post filtration and thus creating an unfiltered batch would yield detailed understanding of the system. In section 4.3, we saw that light microscopy did not yield highly informative results. Thus, herein we used polarized light microscopy, which can yield information about typical birefringence, or double refraction and thus crystallographic sample content. Telang et al. also characterized binary solid dispersion systems composed of functional
excipients, using polarized light microscopy [20]. We characterized the solid dispersions with scanning electron microscopy post sieving, to know if better understanding of morphology could be obtained. Analysis of X-ray diffraction pattern to confirm the nature of solid dispersions was also done. Finally, to understand the performance of freeze dried solid dispersion, dissolution study was carried.

4.3.2 Methods:

Preparation of solid dispersion:

Solid dispersions of curcumin:resveratrol in molar ratio of 1:1 and curcumin:resveratrol:HPβ-cyclodextrin in molar ratio of 1:1:2 were prepared. A variable of filtration prior to freeze drying was introduced for curcumin:resveratrol:HPβ-cyclodextrin solid dispersion. Acetone:water was used as solvent system. Thus, following samples were prepared for freeze drying:

- 119.4 mg of curcumin and 73.9 mg of resveratrol were mixed in 5 ml of acetone, and 40 ml of deionized water was added to it and subjected to filtration at later stage.
- 119.4 mg of curcumin and 73.9 mg of resveratrol were mixed in 5 ml of acetone, 1000 mg HPβ-cyclodextrin and 40 ml of deionized water were added to it and subjected to filtration at later stage.
- 119.4 mg of curcumin and 73.9 mg of resveratrol were mixed in 5 ml of acetone, 1000 mg HPβ-cyclodextrin and 40 ml of deionized water were added to it and was not filtered at later stage.
All the above samples were sonicated for 5 min, thereafter placed on a magnetic stirrer and stirred for 7 days at a speed of 5 units. During stirring the acetone based sample vials were covered with foil and hole for acetone to escape was made. The acetone elimination post 7 days was confirmed by smelling the samples. The aforementioned samples were then filtered using 0.22 µm filter under a vacuum pressure of 150 mbar. The filtrates were analyzed for their curcumin and/or resveratrol content using UV-Vis spectroscopy. The filtrates and unfiltered sample were then transferred to petri plates in two batches, each and freeze dried. Following freeze-drying cycles were employed:

Freeze cycle-
Shelf setpoint (°C): -50
Time (min): 240
Final freeze (°C): -60
Extra freeze (min): 20
Pre vacuum start (MT): 200

Freeze cycle-
Shelf set point (°C): -50
Time (min): 240
Final freeze (°C): -60
Extra freeze (min): 20
Pre vacuum start (MT): 200

Primary dry cycle-
Secondary dry cycle-

Shelf set point (ºC): 20
Time (min): 360
Vacuum setpoint (MT): 10
Final setpoint (ºC): 5

The freeze dried samples were then transferred to vials.

Physico-chemical characterization of solid dispersion:

*Polarized Light Microscopy:*

The drugs and solid dispersions were mounted on glass slides. The samples were then observed under crossed polarized light with 20 X objective. The images were captured using Leica software.

*Scanning electron microscopy (SEM):*

The drugs, HPβ-cyclodextrin and all solid dispersions were analyzed. The solid dispersions of curcumin:resveratrol:HPβ-cyclodextrin, both filtration and no filtration type, were sieved via mesh no. 40, corresponding to 420 µm. An aluminum mount was coated with an adhesive using an adhesive Tab. The sample was placed on the adhesive surface, excess sample that did not adhere to the surface was gently blown away leaving a single layer of
particles on the surface of the mount. The sample was then placed in an EMS/Quorum ES R sputter coater and coated with gold/palladium to render it conductive. Sample was viewed in a scanning electron microscope at 15 kilovolt voltage. Images were captured as TIFF’s using a Quartz PCI system.

*X-ray diffractometry (XRPD):*

X-Ray powder diffraction patterns were obtained for curcumin:resveratrol:HPβ-cyclodextrin solid dispersions, both filtration and no filtration type using X-ray diffractometer. Owing to low recovery of curcumin:resveratrol solid dispersion, it wasn’t analyzed. The data in the 2θ range 5–60 ° was collected in focusing geometry using X-ray Diffractometer, operated with Cu Kα radiation at 40 kilovolts and 45 milliampere. A mask of 20 mm and a divergence slit of 1/4 ° were used on the incident beam path. Thin layer of powder sample was placed on a zero background Si plate and the sample holder was continuously spun at the rate of 90 °/second during the measurement. Solid state PIXcel3D detector was scanned at a rate of 0.135 °/second to collect data and a diffracted beam monochromator for the PIXcel detector was utilized to improve signal to noise ratio.

*Dissolution studies:*

Dissolution studies using USP type II paddle apparatus, phosphate buffer pH 7.4-100 ml, paddle speed of 150 rpm at 37±0.5 °C was carried for 200 mg of curcumin:resveratrol:HPβ-cyclodextrin solid dispersion filtered type and corresponding physical mixture [n=3]. The curcumin:resveratrol:HPβ-cyclodextrin solid dispersion filtered type sample was dissolved in methanol and drug content per mg of solid dispersion was found out to be 1.92 µg curcumin and 22.11 µg resveratrol, using UV-Vis spectroscopy. Thus, corresponding physical mixture was prepared. At time points of 0 min, 5 min, 10 min, 30 min, 60 min,
120 min, 240 min, 360 min, 480 min and 720 min, 1ml of sample was withdrawn from each dissolution vessel and filtered using 0.45 µm syringe filter and diluted with 1 ml methanol. 1ml of phosphate buffer pH 7.4 was added in respective vessel after sample withdrawal. The samples were then analyzed at a wavelength of 306 nm and 424 nm in triplicates with blanks using UV-Vis spectroscopy and amounts of curcumin and resveratrol dissolved were calculated using simultaneous equation method.

4.3.3 Results and Discussion:

Preparation of solid dispersion:

<table>
<thead>
<tr>
<th>Amount of drug(s) in filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 4.5</strong> Amount of drug(s) in filtrate</td>
</tr>
<tr>
<td>Amount of drug dissolved (µg/ml)</td>
</tr>
<tr>
<td>Curcumin</td>
</tr>
<tr>
<td>Resveratrol</td>
</tr>
</tbody>
</table>

Optimum solid dispersions were prepared. The drug content in curcumin:resveratrol solid dispersion filtrate was extremely low. The drug content in curcumin:resveratrol:HPβ-cyclodextrin solid dispersion filtrate was low too. This may be due to smaller pore size for filtration. The solid dispersions of curcumin:resveratrol had almost nil recovery i.e. very negligible amount of solid dispersion was obtained post freeze drying. The curcumin:resveratrol: HPβ-cyclodextrin solid dispersions (both filtration and no filtration type), were fluffy in nature.
Physico-chemical characterization of solid dispersion:

Polarized Light Microscopy:

*Figure 4.9* Polarized light microscopy images of drugs, HPβ-cyclodextrin and freeze dried solid dispersions.

As discussed earlier, polarized light microscopy can generate contrast in birefringent specimens and helps in determining crystallographic nature of specimens. For pure curcumin, it was seen that polarized light underwent refraction and thus the sample appeared illuminated (*Figure 4.9*). Hence, curcumin seems to be crystalline in nature. Similarly, Yamada et al. observed intense birefringence for curcumin samples and concluded that curcumin was crystalline [21]. Resveratrol sample too, refracted polarized light and thus it can be assumed that resveratrol is crystalline in nature (*Figure 4.9*). Augustin et al. observed resveratrol crystals dispersed in water and found them as bright white spots [22]. The HPβ-cyclodextrin sample did not seem to refract light and appeared
opaque, thereby indicating its amorphous nature (Figure 4.9). The curcumin:resveratrol:HPβ-cyclodextrin solid dispersion (with filtration type), dint seem to show any birefringence and thus it can be assumed that it is amorphous in nature (Figure 4.9). Moreover, yellowish tinge of curcumin seemed uniformly dispersed in the image, thereby indicating that curcumin is uniformly dispersed in the solid dispersion. The curcumin:resveratrol:HPβ-cyclodextrin solid dispersion (without filtration type), also dint seem to show any high birefringence, however some illuminated spots were seen in the sample (Figure 4.9). Thus, it can be concluded that curcumin:resveratrol:HPβ-cyclodextrin solid dispersion (without filtration type) is amorphous in nature with some crystalline drug dispersed in it.

Scanning electron microscopy (SEM):

The SEM images of examined samples are shown in figure 4.10. The SEM image of curcumin showed, that it is irregular in shape. On higher magnification, some cylindrical curcumin particles were observed. Yadav et al. found SEM image of curcumin indicating similar irregular morphology [23]. Resveratrol appeared as irregular small scattered and aggregated particles. Chen et al. examined raw resveratrol and found it to have irregular block shaped morphology [24].
Figure 4.10 SEM images of drugs, HPβ-cyclodextrin, curcumin:resveratrol solid dispersion and solid dispersions of curcumin:resveratrol:HPβ-cyclodextrin, both filtration and no filtration type; Above-100 X magnification, Below-500 X magnification.
HPβ-cyclodextrin appeared to have some spherical and some cylindrical morphology with corrugated surface. Yadav et al. found HPβ-cyclodextrin to have similar spherical morphology [23]. The curcumin:resveratrol filtered freeze dried solid dispersion indicated irregular particles wherein, original morphology of both components had disappeared. The SEM images of solid dispersions of curcumin:resveratrol:HPβ-cyclodextrin, both filtration and no filtration type showed similar irregular flaky morphology. The curcumin and resveratrol particles appeared embedded in HPβ-cyclodextrin. Also, the spherical and some cylindrical morphology of HPβ-cyclodextrin appeared to be completely lost in the solid dispersions.

**X-ray diffractometry (XRPD):**

![XRPD pattern of freeze dried solid dispersions. All solid dispersions examined, were amorphous in nature.](image)

**Figure 4.11** XRPD pattern of freeze dried solid dispersions. All solid dispersions examined, were amorphous in nature.
The XRPD pattern of freeze dried solid dispersion is shown in figure 4.11. It was not possible to examine curcumin:resveratrol solid dispersion owing to its low recovery. The XRPD pattern of curcumin:resveratrol:HPβ-cyclodextrin solid dispersion, with and without filtration type, showed hallow pattern. Thus, both were amorphous in nature. These results are in accordance to the polarized light microscopy results. Except, that curcumin:resveratrol:HPβ-cyclodextrin solid dispersion without filtration type, showed some illumination in polarized light microscopy, thereby indicating presence of some crystalline drug. However, the concentration of crystalline drug might be too negligible to be detected in the X-ray diffractometrely. Thus, curcumin:resveratrol:HPβ-cyclodextrin solid dispersion, with and without filtration type, were both mostly amorphous in nature.

*Dissolution studies:*

Within, first 5min itself, about 8% of curcumin was released from physical mixture and 98% from its solid dispersion. The percent release of curcumin, at 30 min dropped to 7.8% from physical mixture and 76.8% from solid dispersion. Yet, the release of curcumin was almost 10 times higher from solid dispersion as compared to physical mixture. At later time points, greater than 100% but less than 120% of curcumin seemed to be released from solid dispersion. The reason, for this variability could be attributed to serial dilutions needed to analyze the solid dispersion’s samples. Usually a variability of up to +20% is acceptable.
Figure 4.12 Percentage of (a) curcumin and (b) resveratrol released from physical mixture and HP\(\beta\)-cyclodextrin based freeze dried filtered type, solid dispersion. Dissolution of curcumin and resveratrol was higher from solid dispersion as compared to physical mixture.
Thus, over a period of 12 hr almost 100 % curcumin was released from solid dispersion whereas >10 % curcumin was released from physical mixture. For curcumin release from solid dispersion analysis at time point 0min, 10min, 30min and 480min, outlier absorbances was observed and thus was not taken into account.

Initially, only 16 % of resveratrol was released from physical mixture and about 88% of resveratrol was released from solid dispersion. Thenceforth, almost 25 % of resveratrol was released from physical mixture and almost 100 % was released from solid dispersion. These levels were maintained for entire duration of dissolution study.

Thus, for both curcumin and resveratrol very high percentages were released from curcumin:resveratrol:HPβ-cyclodextrin based freeze dried filtered type, solid dispersion. Since, filtration step was involved in the solid dispersion preparation, thus only dissolved form of curcumin and resveratrol were present in final freeze dried solid dispersion and thus led to 100 % drug release. The relative standard deviation for curcumin and resveratrol release was less than 20 % for all three batches analyzed.

4.4 Amorphous stability study

4.4.1 Rationale:

From, the above studies we were able to develop amorphous solid dispersions. However, it’s critical that the amorphous nature is maintained throughout the shelf life of the solid dispersions. Thus, it’s important to check the amorphous stability of solid dispersions. Shengani et al. explains, that dissolution behavior of solid dispersions must remain unchanged during storage period and one of the best way to assure the same is to maintain the physical state of solid dispersions [25]. And additionally, the chemical stability is
indispensable. They further explain, that the crystalline particle if any in solid dispersion may act as a nuclei and may cause further crystallization, i.e. conversion of amorphous particles to crystalline state. Thus, in order to check the amorphous stability, we analyzed our solvent evaporated and freeze dried solid dispersions for a period of 6months/9months.

4.4.2 Methods:

The curcumin:resveratrol:HPβ-cyclodextrin, solvent evaporated solid dispersion, acetone:water based and water based curcumin:HPβ-cyclodextrin freeze dried solid dispersion, resveratrol:HPβ-cyclodextrin freeze dried solid dispersion and curcumin:resveratrol:HPβ-cyclodextrin freeze dried solid dispersion were evaluated for their amorphous stability. These sample were stored in amber colored vial and placed at normal room temperature. Initially, at 3month(±1 week), at 6month(±1 week) and/or at 9month(±1 week), time points the samples were withdrawn and analyzed by X-ray diffractometry. The data in the 2θ range 5–60° was collected in focusing geometry using X-ray diffractometer, operated with Cu Kα radiation at 40 kilovolt and 45 miliampere. A mask of 20 mm and a divergence slit of 1/4 ° were used on the incident beam path. Thin layer of powder sample was placed on a zero background Si plate and the sample holder was continuously spun at the rate of 90 °/second during the measurement. Solid state PIXcel3D detector was scanned at a rate of 0.135 °/second to collect data and a diffracted beam monochromator for the PIXcel detector was utilized to improve signal to noise ratio.
4.4.3 Results and Discussion:

Figure 4.13 XRPD pattern of solvent evaporated and freeze dried solid dispersions over a period of time. All solid dispersions at tested time points, were amorphous in nature and thus amorphously stable.
The curcumin:resveratrol:HPβ-cyclodextrin, solvent evaporated solid dispersion remained amorphous for the examined period (Figure 4.13). So was the case with, acetone:water based and water based curcumin:HPβ-cyclodextrin freeze dried solid dispersion, resveratrol:HPβ-cyclodextrin freeze dried solid dispersion and curcumin:resveratrol:HPβ-cyclodextrin freeze dried solid dispersion, which showed amorphous hallow pattern. Thus, it can be concluded, that the drug remain in amorphous state and do not crash out into crystalline form for the examined period. Thus, it would be likely that the solid dispersions would maintain their dissolution behavior.

4.5 Summary and conclusion

Optimization of HPβ-cyclodextrin content and solvent system, lead to preparation of optimum freeze dried solid dispersions. The prepared solid dispersions were found to be thermostable, amorphous in nature and showed inter-molecular interactions. Dissolution studies confirmed the functionality of freeze dried solid dispersions by leading to 100% drug release. Amorphous stability studies, indicated that both solvent evaporated and freeze dried solid dispersions remain amorphous for the analyzed period. Thus, HPβ-cyclodextrin seems to be promising for dissolution enhancement of curcumin, resveratrol and can be explored further for other poorly aqueous soluble drugs.

4.6 Auxiliary experiment- Establishment of R-HPLC method for concurrent quantification of curcumin and resveratrol
4.6.1 Rationale:

Reverse-phase high-performance liquid chromatography serves as a precise analytical technique for identification and quantification of components in a system. We decided to develop an R-HPLC method, which would be validated in future. Several chromatographic methods for curcumin determination in plasma, formulation etc. have been reported [26,27]. Similarly, chromatographic methods for resveratrol determination in plasma and nanoparticles, have been developed too [28,29]. There are reports for simultaneous quantification of both drugs in plasma and micellar formulation [30-32]. Thus, we used the method reported by Pillai et al. group and developed it further.

4.6.2 Methods:

Analysis was performed on a C18 column (3.9 mm in diameter, 300 mm in length, 10 µm pore size and 125 angstrom particle size). The mobile phase used was acetonitrile as organic phase and citric acid (pH=3.5) as aqueous phase (A) in ratio of 60:40 and flow rate 1 ml/min, in isocratic mode. The initial run time was 30 min. The analysis was performed at room temperature. The wavelength of determination for curcumin was 424 nm and resveratrol was 306 nm. In first development stage, blank-methanol, curcumin, resveratrol and combination samples (1:1 M ratio) prepared in methanol, were examined. Post results obtained, following modification were done in second development stage and combination sample was analyzed:

- Organic: aqueous phase- 70:30
- Organic: aqueous phase- 50:50
- Organic: aqueous phase- 40:60
- Organic: aqueous phase- 30:70

In third and final development stage, samples were prepared in organic: aqueous phase-60:40, flow rate was increased to 2 ml/min and gradient flow as follows was designed and combination sample was analyzed:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Organic Phase Concentration</th>
<th>Aqueous Phase Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>5.0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>8.0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>9.0</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

Standard curved for curcumin and resveratrol using combination samples were developed.

4.6.3 Results and Discussion:

Figure 4.14 First development stage chromatograms for (a) blank, (b) curcumin:resveratrol combination, (c) resveratrol and (d) curcumin.
Figure 4.15 Second development stage chromatograms for curcumin:resveratrol combination (a) organic:aqueous mobile phase ratio 70:30, (b) organic:aqueous mobile phase ratio 50:50, (c) organic:aqueous mobile phase ratio 40:60 and (d) organic:aqueous mobile phase ratio 30:70.

Figure 4.16 Third development stage chromatogram for curcumin:resveratrol combination with retention times highlighted.
Figure 4.17 Standard curves for (a) curcumin and (b) resveratrol, developed with R-HPLC method.

From the chromatograms in figure 4.14, we could see that the reported method with slight modification did not yield optimum chromatograms. The solvent peak and resveratrol peaks overlapped and the distance between resveratrol and curcumin peaks was small. Thus, in second development stage we decided to alter organic:aqueous mobile phase ratio and thereby alter the
hydrophobicity of the mobile phase. We could see in figure 4.15, that increasing the hydrophobicity of mobile phase i.e. making organic:aqueous mobile phase ratio 70:30 lead to decrease in distance between drug peaks. Then, decreasing the hydrophobicity of mobile phase i.e. making organic:aqueous mobile phase ratio 50:50 and so on, lead to increase in distance between drug peaks, but curcumin peak signal diminished (Figure 4.15). Also, in all chromatograms, the solvent peak interference was evident. Thus, in third development stage we altered the mobile phase with a gradient flow design, wherein initially we kept mobile phase more hydrophilic, then we made it more hydrophobic and then back to more hydrophilic. We prepared samples in mobile phase. This lead to optimum chromatogram (Figure 4.16). The retention time for resveratrol was seen to be 2.9 min and that for curcumin was 6.5 min. In figure 4.17 we can see standard curves for curcumin and resveratrol with the R-HPLC method developed and the curves had optimal linearity. Thus, a suitable R-HPLC method was developed for simultaneous detection of curcumin and resveratrol.

4.7 References


Chapter 5

5. Conclusion and Future Directions

5.1 Conclusion

Hydroxyl propyl beta cyclodextrin can significantly enhance the apparent aqueous solubility of both curcumin and resveratrol, as compared to alpha cyclodextrin, beta cyclodextrin and gamma cyclodextrin. Microscopic techniques like light microscopy, polarized light microscopy and scanning electron microscopy, can assist in understanding the morphology of solid dispersions. The thermal characterization techniques like thermogravimetric analysis, modulated differential scanning calorimetry coupled with X-ray diffraction analysis can yield detailed information about thermal and physical characteristics of drugs and solid dispersions. Molecular modeling, infrared spectroscopy along with Raman spectroscopy can help in comprehending interactions between components of solid dispersions. Overall, it can be concluded that solid dispersion of curcumin and resveratrol with hydroxyl propyl beta cyclodextrin, leads to dissolution enhancement of curcumin and resveratrol. Thus, hydroxyl propyl beta cyclodextrin solid dispersions can be explored for apparent aqueous solubility enhancement of other poorly water soluble polyphenolic compounds.

5.2 Future directions

5.2.1 Addendum to current study:

In solubility studies, other cyclodextrins such as randomly methylated beta cyclodextrin, hydroxyl propyl gamma cyclodextrin etc. should be explored. Factors
such as stirring speed, cyclodextrin solution concentration etc. should be taken into account.

For molecular modeling studies, in addition to docking, hydration properties of all cyclodextrins complexes used in this research should be studied, using molecular dynamics. This, would yield better knowledge about apparent aqueous solubility enhancement ability of the cyclodextrins and help in subsequent cycodextrin surface modifications, if need be.

For optimization of cyclodextrin content, additional ratios of curcumin:resveratrol:HPβ-cyclodextrin should be explored. Also, factors such as sonication time, stirring time, stirring speed and microenvironment related factors should be studied. Robust phase solubility plots should also be built.

For amorphous stability study, the international conference on harmonization’s stability guidelines should be followed. Also, qualitative and quantitative characterization of solid dispersions should be performed at each stability testing time point.

The reverse-phase high-performance liquid chromatography method developed, should be validated and then adopted for future quantification studies.

5.2.2 **Proceeding future studies:**

Research is an endless curiosity process that provides solutions to several problems as it proceeds. Thus, we suggest the following future studies that would carry forward the development of curcumin:resveratrol:HPβ-cyclodextrin solid dispersions and add to the pool of knowledge for solubilization techniques:
**Development of curcumin:resveratrol:HPβ-cyclodextrin solid dispersions:**

Once a robust curcumin:resveratrol:cyclodextrin solid dispersion has been developed, it should be tested for its therapeutic effectiveness on cell lines such as human colon cancer HCT-116 for colon cancer, hepa 1-6 carcinoma cells for hepatocellular carcinoma, IL-1β-stimulated human chondrocytes for osteoarthritis, Chlamydia pneumonia infected monocytes for atherosclerosis and so forth.

After particular therapeutic efficacy has been proven for the solid dispersion on the cell line, it should be tested on corresponding animal model. For example, for colon cancer, the effectiveness and pharmacokinetics of solid dispersion on colon cancer bearing, severe combined immunodeficiency mice model should be studied. The combinations dose should also be optimized.

Post, understanding of pharmacokinetics and therapeutic efficacy of solid dispersion and optimization of curcumin:resveratrol dose, its oral unit dosage form such as tablet or capsule should be developed. For this, the excipient concentration and tableting parameters/ capsule manufacturing parameters should be optimized.

On development of robust oral unit dosage forms of the solid dispersions, human clinical trials can be proposed. A subsequent new drug application could be filed.

Thus, **our long term goal - to be able to draw the curcumin and resveratrol combination from bench side to bedside,** would hopefully be achieved in future.
**Exploration of other avenues:**

The cyclodextrin based solid dispersion developed in the study should be explored for other poorly soluble polyphenolic drug combinations such as curcumin:quercetin, resveratrol:quercetin etc, whose synergistic pharmacological potential has been proven. Other forms of cyclodextrin formulations, such as nanoparticles, nanosponges, nanotubes and so forth should be explored.

The characterization techniques used in the study, should be explored for similar purposes for other poorly water soluble formulations and thereby yield an understanding of their chemical and physical properties.