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CHARACTERIZATION OF DIFFERENT HYDROPHILIC POLYMERS AND THEIR APPLICABILITY IN HOT MELT EXTRUSION TECHNOLOGY

A Dissertation

Presented in partial fulfillment of requirements

for the degree of Doctor of Philosophy in the Department of Pharmaceutics

The University of Mississippi

by

Sindhuri Maddineni

April 2013

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ABSTRACT

Research in the pharmaceutical field involves investigation of a new drug, delivery route, a delivery system, a technology to design the delivery system, or a combination thereof. Scientists have explored several delivery routes such as oral, pulmonary, nasal, injection/implant, transdermal/dermal, transmucosal etc. for their potential to transport a variety of small and large drug molecules. In the present research work, oral route, a widely accepted route of administration, for the delivery of products with a major market share, has been considered for the delivery of poorly soluble actives. The use of appropriate carrier matrices (polymers) and excipients help in incorporating these drugs and developing a dosage form/drug delivery system with desired properties. The oral delivery systems are most popular, convenient for administration, and mainly include conventional solid dosage forms such as pellets, tablets, and/ or milled material filled in capsules. Since most of the actives studied under current research have low aqueous solubility, it was necessary to utilize a novel technology such as hot melt extrusion (HME) in combination with hydrophilic polymers to obtain tailored drug release.

Over recent years HME technology has found widespread application as a viable drug delivery option in the drug development process. Some of the HME applications include taste masking, solid-state stability enhancement, solubility enhancement etc. Solubility enhancement in the HME process occurs through the dispersion of a poorly soluble drug in a polymeric carrier matrix essentially forming a solid dispersion. While this technology can help in producing amorphous or crystalline solid dispersions depending upon several factors, solubility

enhancement applications are centered on generating amorphous dispersions, primarily because of the free energy benefits they offer. Amorphous solid dispersions result when melt extruded drug-polymer is cooled at a rate that does not allow the drug to recrystallize, or processed at temperatures where drug melts but remains immiscible with the carrier. Such processing results in kinetic entrapment of the drug in its amorphous state. These dispersions also provide maximum specific surface area and higher saturation solubility, which ultimately increase drug solubility. Although these types of systems exhibit increased rate of dissolution due to high thermodynamic activity, they have a potential to revert to the more stable crystalline form. Thorough understanding of the physicochemical properties of amorphous solid dispersions and their corresponding in vivo behavior is required for the realization of their true potential in the pharmaceutical industry.

In the research projects outlined in this dissertation, the focus has been to characterize the different hydrophilic polymeric extrudates produced utilizing HME technology, and emphasize their pharmaceutical applications. HME, in conjunction with suitable polymers, has been demonstrated as a viable approach to develop a novel pellet dosage form with potential abuse deterrent properties. In addition, its application for solubility enhancement aims at generating amorphous solid dispersions utilizing novel hydrophilic polymers, followed by the in-depth characterization of the produced melt extrudates. Moreover, the effect of various formulation variables and process parameters has also been investigated. This underlying research also facilitates the development of a wide-ranging stable solid oral dosage forms with modulated drug release.

The key objectives of the chapters in the dissertation are: (1) To develop an abuse-deterrent (AD) platform technology in the formulation development utilizing HME technique; (2) To investigate the effect of process variables and formulation factors on characteristics of hot melt extrudates containing hydrophilic vinylpyrrolidone/vinyl acetate copolymer (Kollidon® VA 64); (3) To investigate the feasibility of producing stable drug-loaded Soluplus® extrudates utilizing HME technology, and to study the influence of formulation and processing parameters such as drug-load and heating duration, respectively, on the drug-polymer miscibility as well as the release from melt extrudates; (4) To explore the feasibility of producing soluble Soluplus®-Curcumin extrudates utilizing HME technology, including the drug-polymer miscibility studies, and the influence of surfactants on dissolution rate of this poorly water-soluble model drug.

DEDICATION

This dissertation is dedicated to everyone who helped me and guided me through my own times of stress and anxiety. In particular, I thank my beloved parents Mr. Krishnaiah Maddineni and Mrs. Sandhya Rani Maddineni for teaching me the values of simplicity, patience and perseverance, and my lovely sister, Dr. Hima Bindu Maddineni, for her affection and unyielding support. Also, I would like to specially thank Dr. Sunil Kumar Battu for his constant support and encouragement during hard times. They all have made me what I am today with their unending encouragement and guidance in every aspect of my life.

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CHAPTER - 1

Hot Melt Extrusion: A Promising Technique to Produce a Novel Abuse-Deterrent/Tamper-Resistant Formulation

1.1.Introduction

In recent years, prescription drug abuse has been identified as source of rapidly growing concern in the United States. Of all the available drugs, Opioids, CNS depressants and stimulants are the three most established drug classes that are abused, next to marijuana (1). Pain, being a potentially debilitating symptom associated with a variety of chronic medical conditions, has necessitated the application of numerous opioids, which have a high potential for abuse. The number of prescriptions for these drugs has also increased considerably over the past two decades (2). Moreover, as per one of the national surveys, more focus on the non-medical use of these opioids has been noticed in all of the different age groups, teenagers being a major constituent amongst those abusers (3, 4). In addition, the number of emergency visits as well as the death rates due to overdose of these medications has increased dramatically over the past few years (5). These drugs, being reasonably accessible to the population, have been largely misused or abused via physical or chemical manipulation in order to obtain high drug concentration in the body to produce euphoric effects, or a significant "high".

The majority of the opioids/ controlled substances that have shown potential for abuse are available in the form of immediate release (IR) and extended release (ER) tablets and capsule dosage forms, trans-dermal patches, trans-mucosal lozenges etc. The consumption of more

number of IR units via oral route results in higher concentrations of the drug in the systemic circulation, producing the desired “high” (6). In addition, there has been a marked interest in manipulating the ER dosage forms due to the large dose of the drug per unit dosage form (7). Apart from the oral route, alternative delivery routes have been explored by the abusers to achieve a similar psychological feel in a short period or with a limited amount of material in hand (8). Since these conventional formulations are not hard enough to resist crushing, the obtained powder-like fine material is easily snorted via nasal route. In the same scenario, if the active is highly soluble in the aqueous medium, it could be extracted in a small volume and injected directly into the blood stream to get an instantaneous “high.” Some of the other drugs with higher vapor pressures are abused or misused by smoking or volatilization (9). An epidemiologic study conducted on the subjects who were addicted to Oxycontin (Oxycodone HCl), have reported that majority of the users have started taking the drugs orally, but over the time have advanced to administering the API through different routes: 62% reported snorting, 26% through injecting, and only 14% by oral route (10).

Due to this alarming issue, the recent interest of several pharmaceutical industries has been shifted towards developing a platform technology, which either prevents or minimizes the abuse of, or tampering with, the prescription opioids. Currently, there are surprisingly few formulations that are either under development or commercially available in the United States that demonstrate such properties (11). Some of the marketed products that are currently approved by United States Food and Drug Administration (FDA) are Oxycontin® OP ER tablets, Oxecta IR tablets, Suboxone sublingual tablets, Nucynta ER tablets, and Opana ER tablets. These emerging opioid containing formulations were produced by novel approaches such as

incorporation of gelling agents, nasal irritants, antagonists etc., in the formulations. Thus, they are not only designed to discourage the abusers by reducing the risk of misuse and/or abuse, but are also dually beneficial to physicians in meeting their goals of maximizing pain relief in afflicted populations. However, none of these formulations can guarantee an absolute abuse deterrence/tamper proof. In addition, the majority of these formulations have been produced via conventional methodologies involving several processing steps such as wet/dry granulation, blending, compression, curing, coating, etc., which adds longer time and higher cost to the development process.

Although research in this particular area has gained enormous attention due to the pressure from the law makers and the FDA, there is not much literature published/available, especially on the TR formulations developed utilizing novel hot melt extrusion (HME) technology. In a recent study by Bartholomaeus et al. the authors produced an ER opioid formulation with crush-resistant features using a combination of a planetary-gear extruder and a tablet press on a laboratory scale, which involved few processing steps (12). However, their primary objective was to stabilize the formulation and be bioequivalent to the marketed ER formulation. In addition, the rationale for their formulation selection does not involve DOE and necessitates further discussion.

The objective of this current research is two-fold. Firstly, utilizing a DOE approach and employing HME processing in identifying and producing an optimal formulation. Secondly, to produce TR formulations that is more resistant to crushing/grinding/milling (to avoid insufflation), and to minimize the drug extraction (to avoid abuse via injection). HME is a novel and viable technique that has been effectively utilized in the pharmaceutical industry to improve

the bioavailability of the actives, especially those having low aqueous solubility, by formation of molecular dispersions (13-15). This technique is also time-efficient and cost-effective, due to the involvement of fewer processing steps, and utilization of limited excipients. In this study, we have made a sincere attempt to demonstrate the capability of single-step HME technology in producing a TR formulation utilizing appropriate commonly used excipients (16, 17). It should be noted that the terms 'TR' and 'AD' may be used interchangeably throughout this paper.

1.2.Materials and Methods

1.2.1. Materials

Lidocaine HCl (LID) and Vitamin E Succinate (VES) were purchased from spectrum chemicals (Decatur, AL, USA). PolyOx™ WSR 301 (PEO-301), and PolyOx™ N80 (PEO-N80) were kindly gifted from Colorcon (West point, PA). Benecel™ K4M (HPMC K4M), and Benecel™ K15M (HPMC K15M) were kindly gifted from Ashland Inc. (Wilmington, DE). Eudragit® RSPO (EUD) and Carbopol 71G (CBP) were kindly gifted by Evonik (Piscataway, NJ), and Lubrizol (Cleveland, Ohio), respectively. Dibasic potassium phosphate and tribasic sodium phosphate were purchased from sigma-Aldrich (St. Louis, MO). High performance liquid chromatography (HPLC) grade water was freshly prepared in the laboratory by Nanopure systems (Barnstead, Dubuque, IA, USA). All solvents utilized in the study were of analytical grade and obtained from Fisher Scientific (Fair Lawn, NJ, USA).

1.2.2. Analytical Method

An in-house reverse phase high performance liquid chromatography (HPLC) based analytical method was developed for the determination/quantification of LID. This method was validated according to ICH and FDA guidelines for chromatographic methods. An HPLC equipped with a

UV detector, Waters Symmetry shield 5µ C18 column (250×4.6 mm), and an isocratic mode of elution with the mobile phase consisting of 20mM ammonium acetate with 30mM TFA, acetonitrile, and methanol (70:10:20) at a flow rate of 1.2 mL/min were employed to quantify the drug at a wavelength (λmax) of 254 nm. The acquired data was processed using Empower 2 build 2154 software (Waters Inc., Mount Holly, NJ, USA).

1.2.3. Preparation of Physical Mixtures/ Blends

Prior to extrusion, the required materials were sieved through a USP # 30 (600µM) mesh, accurately weighed, and transferred to a twin-shell V-blender (The Patterson-Kelly co., Inc. East Stroudsburg, PA). The materials were then mixed at 25rpm for 10 minutes in the V-blender to obtain a homogeneous blend.

1.2.4. Preparation of Hot Melt Extrudates

1.2.4.1.HME: Initial Screening

In order to identify a suitable carrier matrix to produce an AD/TR formulation, different polymers such as HPMC K4M, HPMC K15M, PEO-301, and EUD were evaluated for extrudability at barrel temperatures ranging from 110°C-180°C and a screw speed of 50 rpm in a twin-screw compounder (MiniLab II HAAKE Rheomex CTW5, ThermoFisher Scientific). The polymeric matrices selected from those tested, (i.e. PEO-301 & EUD, and their blends mixed in ratios of 1:1, 2:1, and 1:2 PEO-301 and EUD respectively) were extruded with LID at barrel temperatures ranging from 110˚C-130˚C and a screw speed of 50 rpm utilizing a lab scale twin-screw Hot Melt extruder (Prism 16mm EuroLab, Thermo Fisher Scientific) with 3mm diameter circular die. All of the extrudates contained 10% w/w VES as a processing aid and 20% w/w LID. The obtained extrudates were cut to a length of 3mm using a bench-top pelletizer (Thermo

Fisher Scientific) and stored appropriately until further testing.

1.2.4.2.HME: Box-Behnken DOE

Based on the results from the initial screening, PEO-301 was chosen as the carrier matrix, while HPMC K15M and CBP were employed as gelling agents; each of these were designated as a formulation factor. Box-Behnken design (Table 1-1) was used to determine the effects of the three formulation factors, each at three levels, on TR characteristics of the melt-extruded pellets. A response surface methodology was utilized to identify the optimized formulation. A total of 14 formulations, including two center points, generated by the design were extruded at temperatures ranging from 110˚C-130˚C and a screw speed of 50 rpm using a twin-screw hot melt extruder (Prism 16mm Eurolab ThermoFisher Scientific). The final extrudates were then cut to 3mm in length using a bench-top pelletizer (ThermoFisher Scientific) and stored until further testing.

Table 1-1: Box-Behnken Design for the Three Formulation Factors (PEO-301; HPMC K15M; and CBP) Under Study

Std Order	Run Order	Pt Type	Blocks	PEO-301	HPMC K15M	CBP
2	1	2	1	40	10	6
13	2	0	1	30	15	6
5	3	2	1	20	15	2
8	4	2	1	40	15	10
14	5	0	1	30	15	6
10	6	2	1	30	20	2
6	7	2	1	40	15	2
12	8	2	1	30	20	10
1	9	2	1	20	10	6
11	10	2	1	30	10	10
9	11	2	1	30	10	2
3	12	2	1	20	20	6
7	13	2	1	20	15	10
4	14	2	1	40	20	6

The experimental design and data analysis were conducted using Minitab® 15.0. The current response surface methodology DOE was analyzed using multiple regression statistics. A full quadratic model was fitted to the collected responses, and 'P' values for each of the factors were used to determine their significance. Highest order, most insignificant terms were sequentially removed until the significant factors were identified. Residual analysis was performed on the final reduced model, and the developed model was validated by executing and analyzing a confirmation run.

1.2.5. Weight Variation

Twenty pellets from a 100g batch were randomly selected from each of the DOE formulations and weighed using a Sartorius lab scale digital balance. The values were recorded, and the mean ± standard deviation (SD) was calculated.

1.2.6. Hardness and Friability Testing

The diametric compression strength testing was performed on ten randomly picked pellets per formulation using the Varian tester, and the hardness values were recorded.

The friability testing was performed on a sample of approximately 6.6g of extruded pellets utilizing a Varian friabilator. Pre-weighed pellets were placed in a friabilator and rotated at a speed of 25rpm for 4min. The pellets were then de-dusted, reweighed, and percentage weight loss (friability) was calculated for all of the DOE formulations.

1.2.7. Fine particle reduction

The obtained pellets equivalent to 200mg of weight from each of these formulations were evaluated for particle size reduction. The pellets were initially subjected to a low-shear grinding in a mortar and pestle for about 5min, followed by milling using a coffee grinder for an

additional 5min. The obtained particles (n=3) were passed through a USP # 100 (150 µm) mesh, and the fraction retained on the sieve was recorded.

1.2.8. Drug Extraction in Water

200mg of extruded pellets (equivalent to 40mg of LID) were added to 5ml of de-ionized water in a 20mL scintillation vial. Each of these formulations was vortexed for 2 minutes and kept aside for 30 minutes. At the end point, the samples were further mixed, and an aliquot of 1mL was collected, further diluted with methanol, filtered using 0.2 µm, 13mm PTFE membrane filters (Whatman, Piscataway, NJ). These solutions were analyzed using an HPLC at a wavelength of 254 nm. The test was performed in triplicate and the mean ± SD were recorded. Additionally, these resultant solutions/dispersions have been qualitatively assessed for an increase in the viscosity or by visual observation for gel formation.

1.2.9. Drug Extraction in Alcohol

The extruded pellets (200mg) with a dose containing approximately 40mg drug were taken and dispersed in 60mL of 50% v/v absolute alcohol (simulating one large shot of whiskey). Each of these formulations was vortexed for 2 minutes and kept aside for 30 minutes. At the end point, the samples were further mixed, and an aliquot of 1mL was collected, diluted with methanol, filtered using a 0.2 µm, 13mm PTFE membrane filters (Whatman, Piscataway, NJ), and analyzed utilizing an HPLC at a wavelength of 254 nm. The test was performed in triplicate and the mean ± SD were recorded.

1.2.10. Drug Release Studies

The *in vitro* dissolution studies were carried out on the extruded pellets containing PEO, EUD, and their blends as polymeric matrices with approximately 40mg of LID. The pellets are

weighed accurately and filled in size#1 gelatin capsules. Dissolution testing (USP XXXI, Apparatus II) was performed utilizing a Hanson SR8-plus™ dissolution test station (Hanson Research Corporation, Chatsworth, CA) operated at 100-rpm paddle speed. The dissolution medium consisted of 750mL of 0.1N hydrochloric acid (pH 1.2), preheated to 37°C. After 2hrs, 250mL of 0.2M tribasic sodium phosphate was added to the existing medium in the dissolution vessel and the pH of the entire medium in the dissolution vessel was adjusted to pH 6.8 by adding either 2N HCl or NaOH. An aliquot of 1.5mL was collected at pre-determined time intervals, filtered, and analyzed using an HPLC at a λmax of 254nm. Drug concentrations were calculated from a standard calibration plot and expressed as cumulative percentage drug dissolved. The release studies were also performed in triplicate and the mean values were compared.

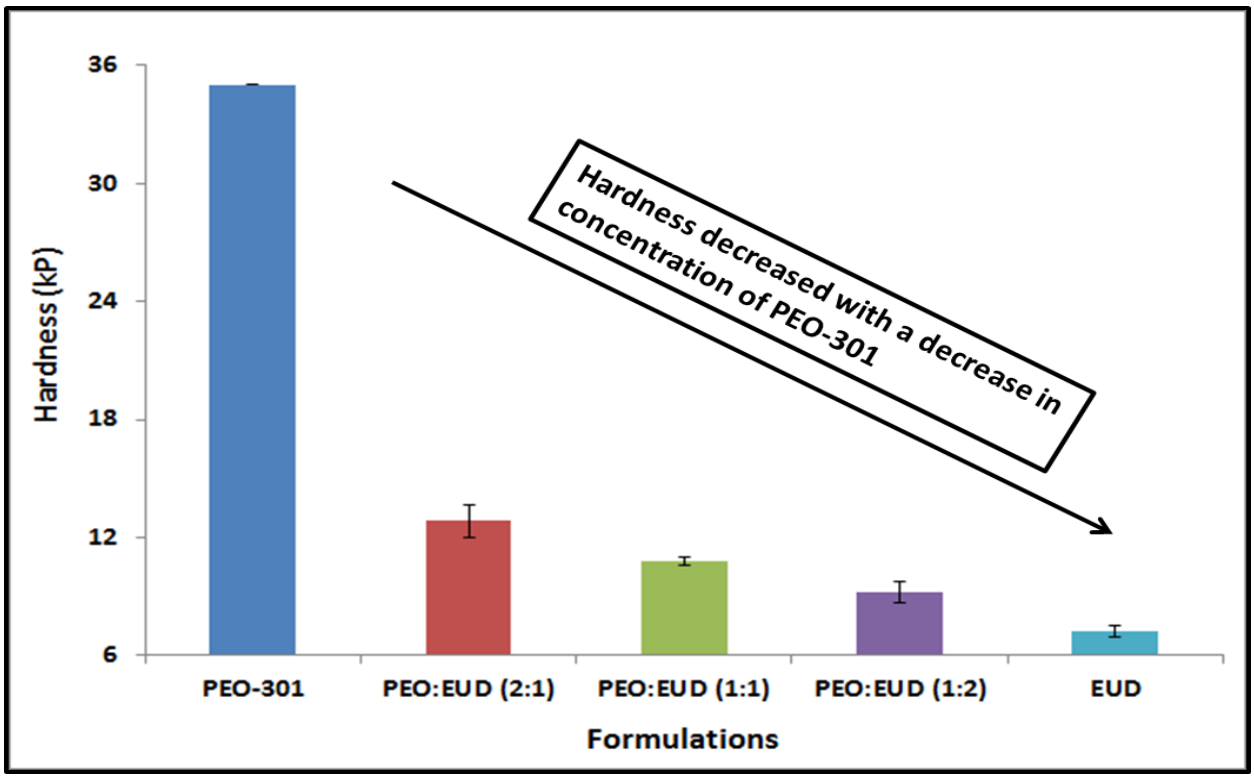
1.3. Results

1.3.1. Analytical Method

The validation of the HPLC method employed in this study was carried out as per the ICH and FDA guidelines for chromatographic methods. The linear calibration range for the detection of LID was found to be 5-200 µg/mL, with a coefficient of determination (R^2) of 0.999. The limit of detection and quantitation for the drug were 0.3 and 1.0 µg respectively, and the retention time for LID was 8.9 minutes. The percentage relative standard deviation (RSD) within replicates (n=3) was less than 2.0%, which demonstrates the reproducibility of the method. Precision was tested by injecting a single drug concentration (20µg/mL) 10 times, and the peak area was recorded and evaluated. A precision of less than 1.0% of RSD was observed.

1.3.2. Weight Variation, Hardness and Friability Testing

For all of the formulations, the weight and length of the pellets were within 28mg±10% and 3mm±5%, respectively. The hardness of the extruded pellets increased as the concentration of PEO-301 in the extruded pellets increased. This seemed especially true when the concentration of PEO-301 exceeded 50% of the total polymer weight. Moreover, the pellets made of pure PEO-301 could not be broken, but could only be deformed by the apparatus. A maximum hardness of 35kP was displayed on the instrument. This indicates a plastic behavior as a result of thermoplastic nature of the PEO-301 (Figure 1-1). Friability values were less than 0.1% in all of the DOE batches produced.



Note: The extrudates prepared from PEO-301 (pure PolyOx™ WSR 301) could only be deformed, but not broken during the hardness evaluation.

Figure 1-1: Hardness Evaluation of the Preliminary Melt-Extruded Pellet Formulations

1.3.3. Fine particle reduction

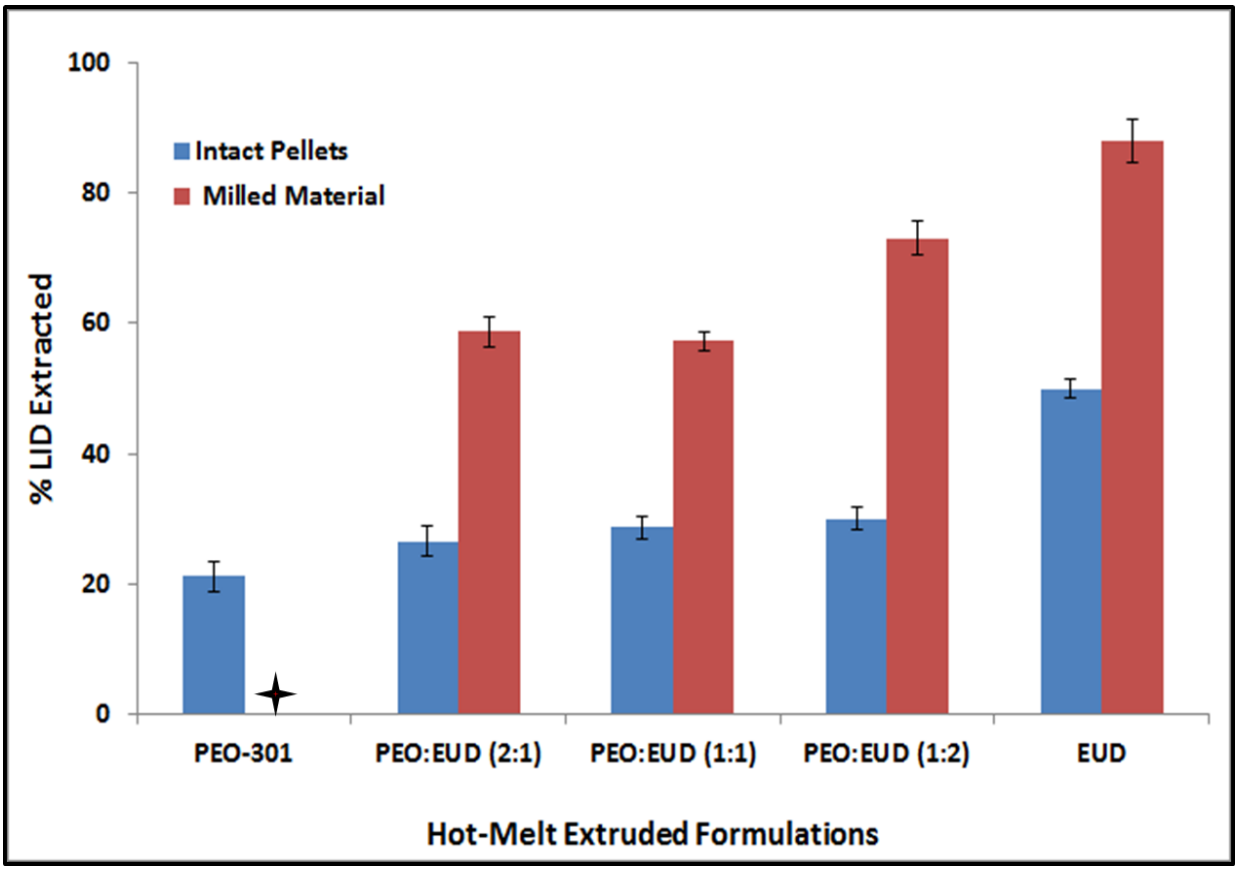
All of the particles obtained from grinding the extruded pellets in a mortar and pestle, followed by milling in a coffee grinder, were completely retained on the USP # 100 mesh screen (>150μ).

1.3.4. Drug Extraction in Water

The intact pellet formulation with pure PEO-301 had 27.4% of the drug extracted in water (approximately 11mg out of 40mg of LID) over 30 minutes, whereas all of the other formulations have shown higher drug extraction (>50%) over the same period (data not presented). The formulation with pure PEO-301 visually demonstrated a superior gel formation when compared to the other formulations.

1.3.5. Drug Extraction in Alcohol

The observed drug extraction in alcohol (ethanol) was only 21.3% from the intact pellets containing pure/unblended PEO-301 in comparison to those made with pure EUD, which was approximately 50% of LID loading. The percentages of drug extracted in alcohol from all of the other intact pellet formulations were found to be between that of the pure polymer compositions. In addition, milled pellets exhibited significantly higher drug extraction compared to the intact pellets as depicted in Figure 1-2.



✦The pellets made of PEO-301 could not be milled into fine powder. Hence, this sample was not tested for drug extraction in alcohol from the Hot Melt extruded pellets.

Figure 1-2: Drug Extraction in Alcohol from the Preliminary Melt-Extruded Pellet Formulations

1.3.6. Drug Release Studies

All of the extruded formulations, irrespective of their polymeric compositions (varying PEO: EUD ratios), have demonstrated similarity factor values (f_2) of greater than 50. The pellets composed of PEO-301 alone with 20% LID served as the reference (Figure 1-3).

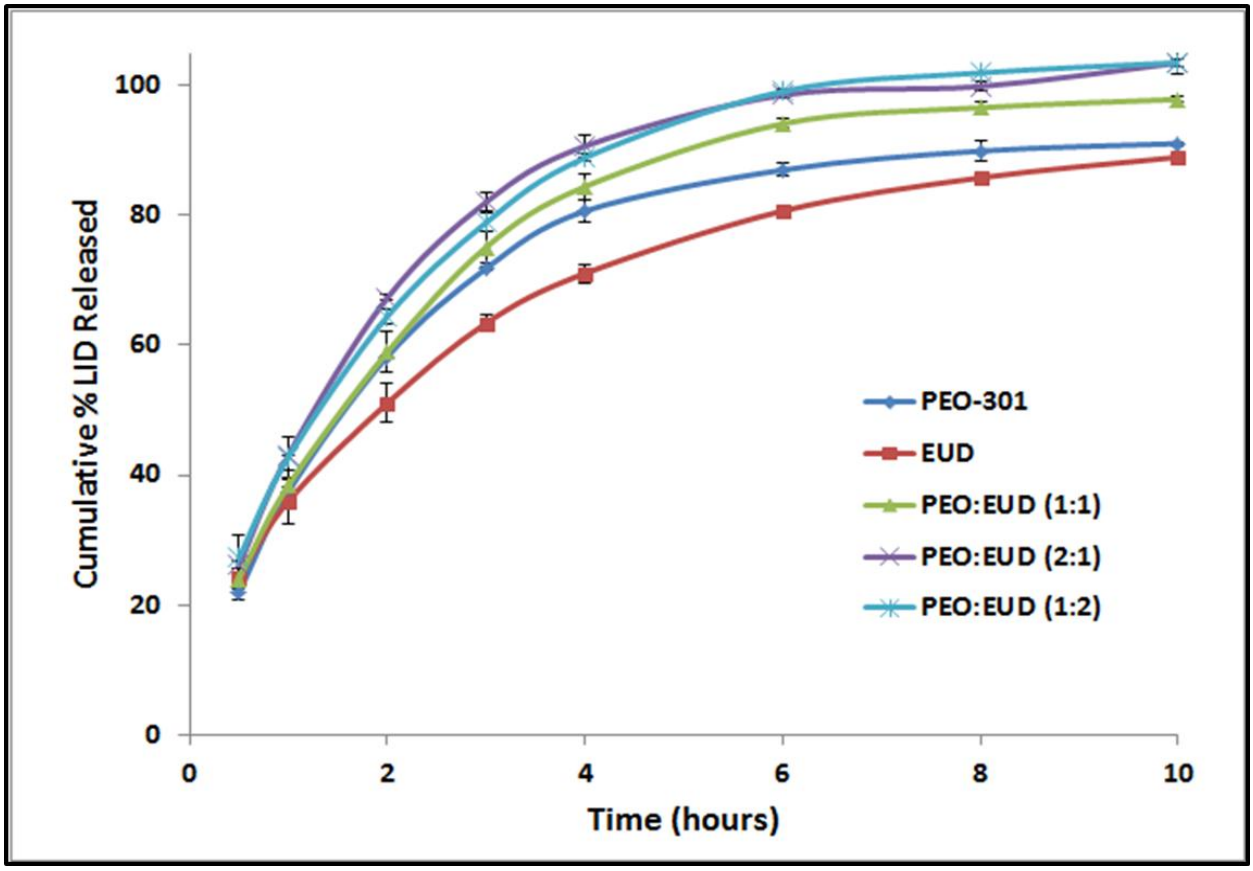


Figure 1-3: Drug Release from the Preliminary Melt-Extruded Pellet Formulations

1.4. Discussion

Due to controlled and restricted access to opioids, an active with high water and alcohol solubility (LID) was chosen as a model drug in the current study. Different carrier matrices such as HPMC K4M, HPMC K15M, PEO-301, and EUD were initially screened to identify suitable carriers that exhibit AD/TR properties. When attempting to extrude neat polymers at 90% w/w polymer level, formulations containing HPMC could not be extruded even at temperatures as high as 180°C. Irrespective of its molecular weight, HPMC could not be plasticized under the employed extrusion conditions, and consequently was not pursued further as a carrier matrix. On the other hand, PEO-301 and EUD were easily extruded at a much lower temperature range of

125-140°C. The next level of screening involved studying the effects of chosen polymeric carriers (PEO-301 and EUD) and their combinations at three different ratios to produce formulations with AD/TR features. All of these five formulations could be extruded relatively easily, and without incident. These formulations were subjected to hardness, crushing, extraction and dissolution testing to choose the best carrier among those tested, that is, the one with the most favorable AD/TR properties.

Snorting/insufflation is one of the most convenient and predominant modes of drug abuse, where the tablet formulations are finely crushed and inhaled or "snorted" via nasal route to get rapid onset of action. When inhaled intra-nasally, drugs could reach both the brain (by-passing the blood-brain barrier), and the systemic circulation (by-passing intestinal and hepatic metabolism). Targeting the drugs to the brain would be the most effective approach to get an instant "high" if the particles could get deposited in the olfactory region (required particle size <5 μm), which facilitates the drug's entrance into the cerebrospinal space (18). However, this small particle size could also result in the drug deposition in the lower airways. Conventional nasal formulations, on the other hand, maintain a particle size of 20-30 μm to facilitate their permeation across the highly vascularized nasal mucosal epithelium (18). However, because of the low volume availability in the nasal environment, the rate-limiting step for drug absorption would be drug dissolution. Therefore, to produce a formulation with TR properties, it is required to alter the formulation such that the particle size could not be minimized to an appreciable extent. This results in a decreased surface area and ensures slower drug dissolution in the available low volume nasal cavity. It has been demonstrated in this study that all of the pellet formulations obtained from either pure EUD or its combination with PEO-301 could be broken,

but not reduced into very fine particles as was attempted by the milling process in a coffee grinder (Figure 1-4).

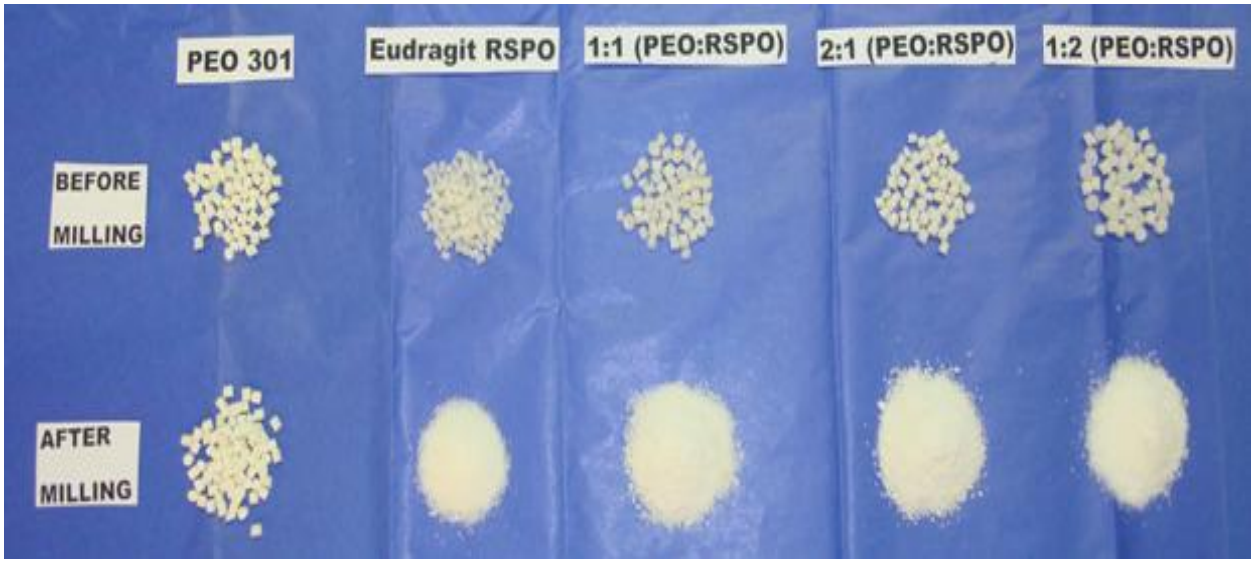


Figure 1-4: Visual Comparison of Different HME Pellet Formulations before and after Particle Size Reduction

Another route of abuse that has gained considerable attention, and is highly favored among abusers, is through extraction of the drug into aqueous solvent and injecting it directly into the blood stream. To prevent this, a TR formulation should be able to gel sufficiently when in contact with the aqueous media in order to minimize drug extraction. All of the extruded pellet formulations, except the one containing pure EUD, exhibited a reasonable gelling tendency in water. Moreover, the formulation extruded with pure PEO-301 has demonstrated increased swelling and produced a superior viscous gel relative to the other formulations. Although the high molecular weight PEO-301 does hydrate and subsequently forms an extraction preventative gel layer, polymer hydration tends to occur somewhat more slowly than small molecule dissolution. The coupling of these two events could have resulted in the drug's escape prior to

the gel formation event. On the other hand, EUD being a copolymer of ethyl acrylate and methyl methacrylate, and having a low content of the methacrylic acid ester with a quaternary ammonium group, it is less permeable and practically insoluble in water and might have undergone erosion. Hence, as the EUD concentration in the formulation increased, the drug extraction from the intact pellets might also have increased due to both high drug solubility leading to diffusion through any formed channels, and lack of EUD gelling in the aqueous phase.

Apart from the aqueous media, extraction studies were also carried out in hydro-alcoholic solutions. This increased drug extraction from EUD pellets was attributed to the higher solubility of both the EUD polymer and drug in alcohol. Apart from testing the whole pellets, all of the extrudates were subjected to milling and the milled portions were also exposed to alcohol for LID extraction. As expected, due to an increase in surface area as a result of milling, all of these formulations under investigation have shown much greater drug release in alcohol (60-90%) when compared to the intact pellets (21-50%). The percent drug extracted between intact and milled pellets for all of the tested formulations was statistically significantly different ($p<0.05$). However, the drug extraction from the milled PEO-301 pellets could not be performed as the pure PEO compacts were very plastic in nature and, as previously mentioned, very difficult to crush.

The obtained dissolution data was fitted to different release models, and the drug release mechanism from all of these formulations was established using the Krosmeyer-Peppas model. The high coefficients of determination (R^2) values were observed when plotted as per the Higuchi equation, which indicates that, the regression line fits the data well. Additionally, the calculated ‘n’ values for all of these five preliminary formulations were in the range of 0.28-0.37,

which indicates that the drug's release is mainly by fickian diffusion.

PEO-301, being a hydrophilic polymer, demonstrates rapid hydration and swelling of the polymer when in contact with the aqueous media resulting in the formation of a uniform gel layer on the surface of the matrix. Since the model drug under investigation is highly water-soluble, it quickly gets dissolved in the media and diffuses out through the channels formed due to polymer swelling (19). However, the molecular weight of the polymer under discussion, PEO-301, is relatively high (4 million), resulting in a slower gel erosion and a prolonged drug release behavior, which translates to reduced extractability. On the other hand, although the EUD matrices hydrate to a lesser extent, the drug release follows a similar mechanism of diffusion. This could be attributed to the high aqueous solubility of the LID that diffuses through the channels formed due to EUD erosion, or dissolution of other soluble formulation components. The study results were in accordance with the previously reported data in the literature (20).

Based on the preliminary results obtained from the above studies, it has been concluded that the pure PEO-301 extrudates exhibited the most suitable properties as an AD/TR carrier matrix when processed by HME. However, these pellets need further improvement to minimize the drug's extraction in aqueous media, thereby preventing intravenous drug abuse. In order to improve the formulation further, excipients such as CBP and HPMC K15M were employed as gelling agents, and Vitamin E Succinate (VES) was used a processing aid in all of the formulations. Both of these polymers, each at three different levels, were studied as gelling agents to increase the viscosity of the formulation when in contact with water.

CBP was studied in a broad range of concentrations (2-10% w/w). In general, if the concentration of CBP in the formulation is beyond 3%, it could potentially exhibit pH dependent

drug release, which is not desirable. However, the grade of CBP chosen is a granular form, and requires a high concentration (~10% w/w or higher) in the formulation to demonstrate gelling and pH dependency. For CBP to act as a gelling agent, an alkaline environment is required to ionize the polymeric chains and induce swelling for gel formation. Therefore, K_2HPO_4 was added as a buffering agent in the formulation. Furthermore, researchers have utilized HPMC up to 30% w/w in controlled release tablet formulations to provide sufficient gelling. However, when used at high levels, there is a potential for the development of a brittle formulation that could be crushed easily and compromise the insufflation inhibiting feature. When a minimal amount of HPMC is used, there is a possibility of insufficient gel formation in water leading to enhance drug extraction. As our primary intent was to improve the extraction in water (learning from the preliminary experiments), 10-20% w/w range was assumed to be reasonable to study. With several ingredients present in the formulation, a DOE was performed to optimize the levels of PEO-301, HPMC K15M and CBP to produce a formulation with better TR/AD features keeping all of the other components unchanged except for PEO-N80, which was used as filler. The base formulation composition utilized for DOE and its excipient functionality is presented in Table 1-2.

Table 1-2: Composition and Functionality of Hot Melt Extruded Base Pellet Formulation Utilized in Design of Experiments

Formulation Components	Concentration (% w/w)	Functionality
Lidocaine HCl (LID)	20	Active
PEO-301	20 – 40	Gelling Polymeric Matrix
HPMC K15M	10 – 20	Gelling Polymeric Matrix
Carbopol 71G (CBP)	2 – 10	Gelling Agent
Vitamin E Succinate	10	Process Aid
K_2HPO_4	1	Base/Gel Promoter
PEO-N80	3 – 33	Polymeric Filler

All of the extruded formulations produced under DOE were evaluated for various TR tests and the obtained results were considered responses for optimization. The TR tests performed were: (a) hardness testing using a Varian hardness tester; (b) fine particle reduction using a mortar & pestle and a coffee grinder, followed by passing the milled extrudates through a 100 mesh screen; (c) extraction of the active pharmaceutical ingredient (API) in water; and (d) extraction of the API in 60mL of 50% v/v absolute ethanol.

A total of 14 formulations produced under the Box-Behnken design, including two center points, were easily extruded at the employed conditions. This design is a more efficient DOE compared to a full factorial or a central composite design with an equal number of factors. The Box-Behnken design uses edge midpoints; the design is run at a condition halfway between two crners rather than at a corner. Moreover, this design does not use axial points. Hence, all of the design runs will be within the initial design space. This feature is useful when it is necessary to

ensure that all design runs are in defined or “safe” operating space.

Hardness testing revealed that all of the produced formulations were plastic in nature and deformed. A maximum hardness value of 35kP was recorded on the instrument. None of the pellets subjected to testing could be broken. LID pellet formulations demonstrated good post-processing drug content (approximately 99.0% and a relative standard deviation of 2.0%). In addition, a low-shear pulverization using mortar & pestle, followed by milling in a coffee grinder generated particles with reduced size in all of the DOE formulations. However, none of these formulations passed through mesh # 100 when subjected to sieve analysis, and the particle size of all of the milled portions were larger than 150µ, which is indicative of the formulations’ resistance to snorting/insufflation. As mentioned earlier, the larger the particle size, the smaller the surface area, which could lead to a relatively slower dissolution and lower absorption across the nasal environment.

All of the formulations exhibited an appreciable gelling tendency in water with only 8-12% of drug being extracted. Good gelling behavior in water is attributed to the water uptake potential of the employed hydrophilic polymers present in the formulation and their ionizable groups, due to the micro-environmental pH, resulting in an increase in the viscosity of the aqueous medium. Studies in the literature have reported that HPMC in combination with CBP demonstrated a synergistic effect with a stronger cross linking between the polymers leading to the formation of a more firm gel structure because of the hydrogen bonding between the anionic CBP and the HPMC. Additionally, interaction between non-ionic polymers has also been well established in the literature. Some reports have mentioned that PEO in combination with HPMC might be favorable when slower initial release is required from the matrices. Both PEO and

HPMC, being hydrophilic and non-ionic polymers, are dually advantageous in that they can facilitate a faster hydration of PEO-301, and could also result in the formation of a stronger gel layer. This ultimately results in minimizing the drug diffusion from the swollen matrix while simultaneously decreasing the erosion rate of the formed gel layer (21). Similar behavior was also reported with HPMC in the presence of non-ionic hydrophilic polymer, hydroxylpropyl cellulose (HPC).

When analyzing the percentage of the API extracted in water, the level of HPMC K15M was the only factor that demonstrated a significant influence on the response ($P<0.05$). The R-Sq (adjusted) value in the final reduced model indicated that the model explained 35% of the data variability (Figure 1-5). In addition, the P value for 'Lack-of-Fit' was found to be 0.136 ($P>0.05$). Therefore, the null hypothesis was accepted i.e., the model developed is not different from the proper fitting model. The reduced final model for this response can be provided as follows:

Percentage API extracted in water = 8.085 + 0.172 (HPMC).....**Equation 1**

The drug extraction in alcohol was another response that ranged from 17-45%, amongst different formulations tested. Similar results were observed in a study conducted by Robert et al. who studied the release of aspirin from HPMC matrix in hydro-alcoholic media (22). The authors noticed an initial sudden burst release of the drug without causing any dose dumping, which was attributed to the delayed hydration of HPMC in the alcoholic medium. Therefore, in the present study both PEO and HPMC being insoluble in alcohol, high drug extraction in alcohol was accounted for by the high solubility of LID as well as the delayed hydration of the HPMC polymeric matrix in the presence of ethanol.

Response Surface Regression: %API extracted in Water versus HPMC

Final reduced Model: The analysis was done using uncoded units.

Estimated Regression Coefficients for %API extracted in Water

Term	Coef	SE Coef	T	P
Constant	8.0852	0.94401	8.565	0.000
HPMC	0.1718	0.06103	2.814	0.016

S = 0.863045 PRESS = 12.8902
R-Sq = 39.76% R-Sq(pred) = 13.13% R-Sq(adj) = 34.74%

Analysis of Variance for %API extracted in Water

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	1	5.900	5.900	5.8996	7.92	0.016
Linear	1	5.900	5.900	5.8996	7.92	0.016
HPMC	1	5.900	5.900	5.8996	7.92	0.016
Residual Error	12	8.938	8.938	0.7448		
Lack-of-Fit	1	1.706	1.706	1.7061	2.59	0.136
Pure Error	11	7.232	7.232	0.6575		
Total	13	14.838				

Figure 1-5: Response Surface Regression after Model Reduction Obtained Using "Percent API Extracted in Water" as a Response

'Percentage API extracted in alcohol' was also significantly influenced (P<0.05) by the level of HPMC K15M employed in the formulation. When the obtained data was fitted to the model, the R-Sq (adjusted) value in the final reduced model indicated that the model explained 34% of the data variability (Figure 1-6**)**. In addition, the P value for 'Lack-of-Fit' was found to be 0.377 (P>0.05). Hence, the null hypothesis was accepted i.e., the model developed is not different from the proper fitting model. The reduced final model for this response can be provided as follows:

Percentage API extracted in alcohol = 6.787 + 1.343 (HPMC)…….**Equation 2**

Response Surface Regression: Alcohol Gelling versus HPMC

Final reduced Model: The analysis was done using uncoded units.

Estimated Regression Coefficients for Alcohol Gelling

Term	Coef	SE Coef	T	P
Constant	6.787	7.5083	0.904	0.384
HPMC	1.343	0.4854	2.766	0.017

S = 6.86435 PRESS = 797.327
R-Sq = 38.94% R-Sq(pred) = 13.90% R-Sq(adj) = 33.85%

Analysis of Variance for Alcohol Gelling

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	1	360.60	360.60	360.60	7.65	0.017
Linear	1	360.60	360.60	360.60	7.65	0.017
HPMC	1	360.60	360.60	360.60	7.65	0.017
Residual Error	12	565.43	565.43	47.12		
Lack-of-Fit	1	40.52	40.52	40.52	0.85	0.377
Pure Error	11	524.91	524.91	47.72		
Total	13	926.03				

Figure 1-6: Response Surface Regression after Model Reduction Obtained Using "Percent API Extracted in Alcohol" as a Response

The standardized residual plots (Figures 1-7A and 1-7B) of the final reduced models for the responses 'percentage API extracted in water' and 'percentage API extracted in alcohol' meet all of the basic assumptions of a good model fit. The figures indicate that the residuals are normally distributed around approximately the mean of zero. Moreover, the residuals are homoscedastic, also known as homogeneity of variance (residuals have constant variability and are independent of fitted value), and do not show any trending (independent of observation order). Response optimization plots (Figure 1-8A and 1-8B) indicated that lower extraction of

API in water (9.8%) and alcohol (20.2%) could be achieved by using HPMC even at lower concentrations (10% w/w level), when other factors are utilized at appropriate concentrations in the formulation.

In order to validate the developed model, a confirmation batch utilizing PEO-301, HPMC K15M, and CBP at 20%, 10% and 6% w/w, respectively, was produced and analyzed. The predicted values obtained from the response optimizer were found to be similar to the observed values of 9.4% and 19.5% respectively, for percentage API extracted in water, and percentage API extracted in alcohol demonstrating the validity of the developed model. This final formulation was studied for drug release along with other TR properties and found that almost 60-70% of the drug was released in 2-3hrs (data not presented). Although extended release polymers such as PEO-301 and HPMC K15M were used as carrier matrices in the present study, the observed faster dissolution rate in the initial hours could be attributed to the high water solubility of the LID, as well as utilization of lower molecular weight PEO (PEO-N80) as a filler in the formulation, which does not swell to a great extent and compromises the integrity of the gel network relatively quickly as opposed to high molecular weight grade PEO polymers.

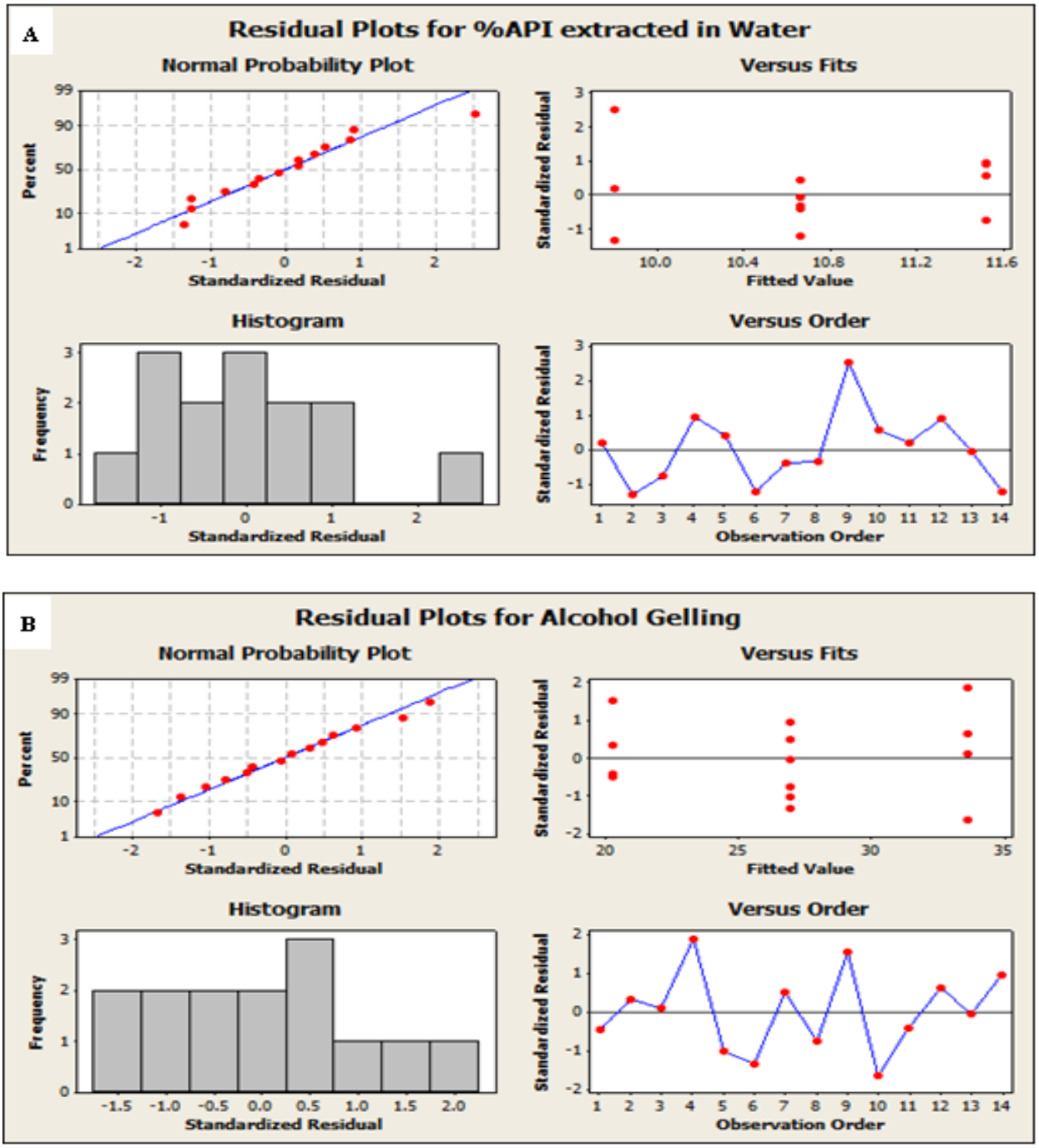


Figure 1-7: Standardized Residual Plots on the Final Reduced Models for the Responses: (A) Percent API Extracted in Water; (B) Percent API Extracted in Alcohol

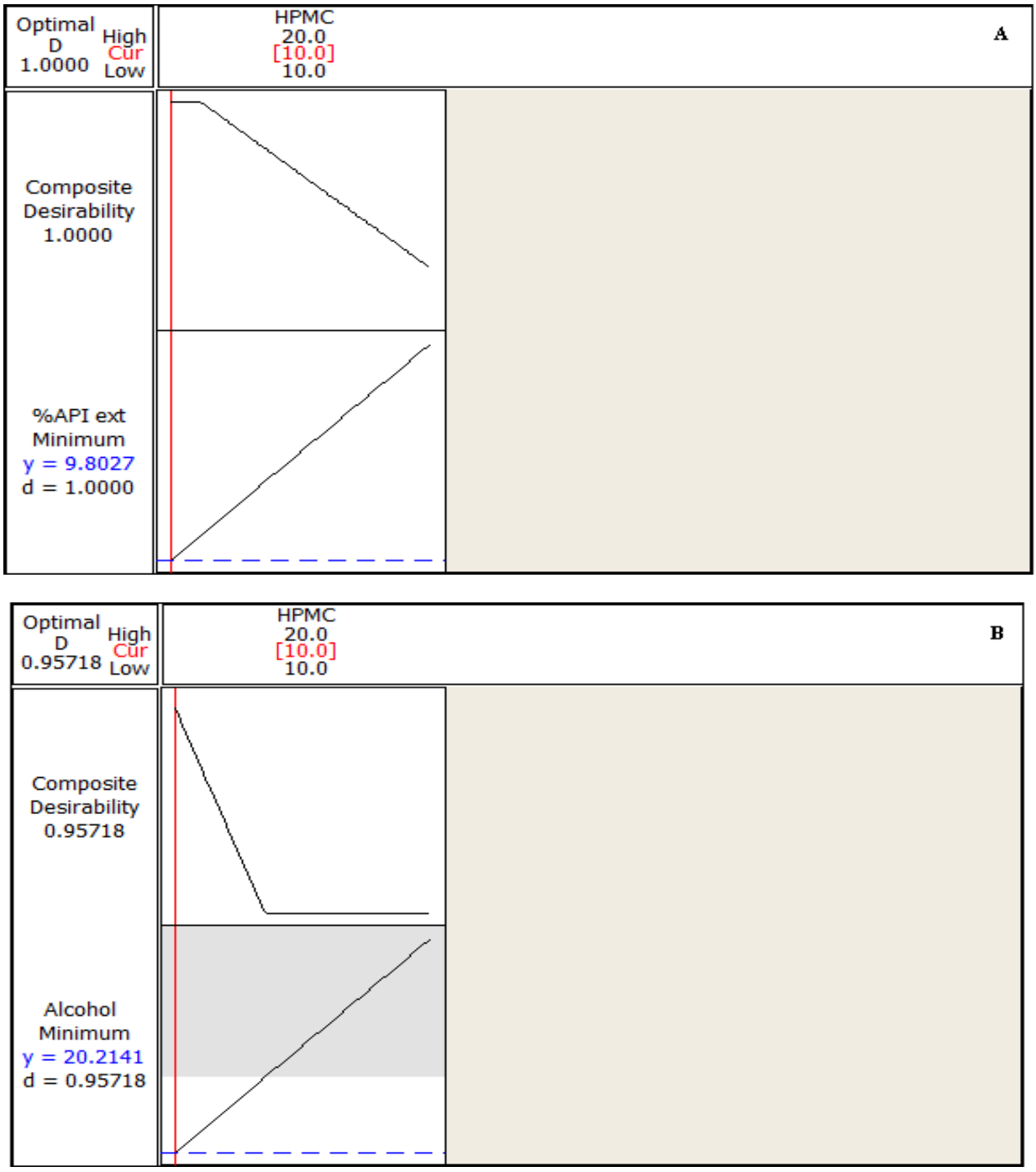


Figure 1-8: Response Optimization Plot for the HPMC (the only Significant Factor) on the Responses: (A) Percent API Extracted in Water; (B) Percent API Extracted in Alcohol

1.5. Conclusion

HME was demonstrated as a viable technique for the development of much needed abuse-deterrent formulations. Moreover, high molecular weight grade polyethylene oxide was shown to be utilized as a TR matrix, while HPMC K15M in combination with CBP significantly improved the gelling characteristics in water and alcohol to prevent API extraction. The optimized response models created for 'percentage API extracted in water' and 'percentage API extracted in alcohol' also correlate well with the data generated, confirming its validity. Our future studies will be focused to work with controlled substances as model drugs, and evaluate newer excipients in conjunction with appropriate processing conditions to further improve the so far developed formulation and fully characterize its dissolution and stability characteristics. Although these formulations are termed as AD/TR dosage forms, these can never be completely abuse-free as none can stop a determined person from abusing the drugs. Still, these developed formulations would most likely serve the purpose as the abusers might definitely need to perform an enormous amount of work, or exhibit uncharacteristic patience, to use them in an unintended way. These formulations could also significantly reduce the deaths due to overdose, as well as decrease the percentage of young children and patients with acute or chronic pain who might convert into potential abusers.

1.6. Acknowledgements

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the authors and do not necessarily represent the official view of NIGMS or NIH. In addition, the authors would also like to thank Ashland Inc., the Dow Chemical Company, Evonik, and Lubrizol for their generous supply of polymers.

CHAPTER - 2

Characterization of Kollidon® VA 64 Hot Melt Extrudates: Influence of Formulation and Process Parameters on Dissolution and Stability Characteristics

2.1.Introduction

In the recent years, several therapeutic compounds of complex nature were produced as a result of high throughput screening and combinatorial chemistry. It has been estimated that more than 40% of all of these produced new molecular entities suffer from solubility-limited bioavailability. Oral delivery of such compounds presents one of the most frequent and formidable challenges to formulation scientists. Myriad techniques such as spray drying (23), solvent-evaporation (24), nanocrystal formation (25), complexation (26), and micronization of API (27) etc., have been widely utilized in the industry to demonstrate the improved solubility and/or dissolution rate of such BCS Class II drugs, thereby enhancing their bioavailability. In addition, over the past couple of decades, HME technique has gained enormous interest amongst researchers in improving the bioavailability of drug substances, especially those having low water solubility, by formation of molecular dispersions (13, 14, 16, 17). Compared to other techniques HME offers several advantages such as being solvent free and involving fewer processing steps.

HME is the process of pumping raw materials with a rotating screw under elevated temperature through a die into a product of uniform shape. These final extrudates obtained

utilizing thermal energy may be in the form of films or rods that can be cut as tablets or pellets. To date, several research papers have been published by scientists that focused on the preparation of solid dispersions/solutions of numerous model drugs with low solubility such as Clotrimazole (CTZ), Indomethacin and Nifedipine (NIF) etc. utilizing HME technique. Shibata et al. produced solid solution of Indomethacin and crospovidone using twin-screw extruder, and demonstrated that the parameters such as residence time, screw speed and heating temperature play a significant role in the extrusion process (28). On the contrary, in a study by Verhoeven et al., the effect of process parameters (screw design, feed rate and screw speed) on Metoprolol tartarate and ethyl cellulose mini matrices were studied, and revealed that the process parameters had no significant influence on drug quality as well as drug release, indicating robustness of HME technique (29). In addition, the role of kneading paddle elements and operating conditions of twin screw extruder on NIF and HPMCP extrudates were studied by Nakamichi et al. (30), and concluded that all of these factors played an important role in obtaining ideal solid dispersions. Most of these previous studies reveal that the influence of process parameters primarily depends on multiple factors such as the API involved, the carrier matrix utilized, and the processing conditions employed during the HME process.

In the current study, NIF (a dihydro pyridine calcium channel antagonist) was employed as a model drug and Kollidon® VA 64 as the polymeric matrix. NIF is a light sensitive yellow powder, practically insoluble in water, with a melting point of 172-174°C. It is a calcium channel blocker used in the treatment of hypertension and angina. NIF is currently available as both immediate and extended release tablet dosage forms given at a dose of 10-30 mg t.i.d. and 30-60 mg once daily, respectively, depending on the patient's condition. The utilized polymer matrix,

Kollidon® VA 64, is a vinyl pyrrolidone/vinyl acetate copolymer in 6:4 ratios. It is a hygroscopic white powder, soluble both in water and alcohols and has a glass transition temperature around ~ 106°C. It has several pharmaceutical applications such as a binder in tablets, a granulating agent and a film former. The properties such as poor API oral-bioavailability of 45-56% due to solubility limitations, and the soluble nature of the polymer, including its lower glass transition temperature make them an appropriate choice as the drug and the carrier for HME processing at relatively lower temperatures.

The nature of Kollidon® VA 64 as a matrix polymer in HME has been previously studied by the researchers to some extent. Rapidly disintegrating tablets containing Indomethacin and Kollidon® VA 64 extrudates were prepared by Dinunzio et al., and the authors studied the use of Ceolus(™) microcrystalline cellulose in producing these tablets (31). In addition, Jijun et al. has prepared the solid dispersion tablets of Nimodipine with a mixture of Eudragit® EPO and Kollidon® VA 64 by combining HME with direct compression, to improve its dissolution behavior in different media (32). However, a further in-depth characterization of the Kollidon® VA 64 hot melt extrudates involving the processing and formulation parameters is warranted for a thorough understanding of the formed dispersion systems utilizing this polymer. Therefore, in the current study, we have extensively studied the influence of various process parameters such as screw speed and extrusion temperature, and formulation factors such as drug load, plasticizer type and concentration on extrudability, drug release, and stability. In addition, the effect of moisture on content uniformity and dissolution, and effect of extrusion on powder flow characteristics have also been studied.

2.2.Materials and Methods

2.2.1. Materials

Nifedipine USP was purchased from LETCO Medical (Decatur, AL, USA); polyethylene glycol 3350, triethyl citrate, stearic acid, vitamin E succinate, propyl paraben, sodium lauryl sulfate (SLS), potassium phosphate monobasic, and sodium hydroxide were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Kollidon® VA 64 was kindly gifted by BASF Corporation. High performance liquid chromatography (HPLC) grade water was freshly prepared in the laboratory by Nanopure systems (Barnstead, Dubuque, IA, USA). All solvents utilized in the study were of analytical grade and obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2.2. Analytical Method

In-house reverse phase high performance liquid chromatography (HPLC) based analytical method was developed for determination/quantification of NIF, and validated according to ICH and FDA guidelines for chromatographic method. An HPLC equipped with UV detector, Waters Symmetry shield 5µ C18 column (250×4.6 mm), and an isocratic mode of elution with mobile phase containing acetonitrile and water (55:45) at a flow rate of 1.0 mL/min were employed to quantify the drug at a wavelength (λmax) of 235 nm. The acquired data was processed using Empower 2 build 2154 software (Waters Inc., Mount Holly, NJ, USA).

2.2.3. Solubility Parameter Calculations

Hansen solubility parameters were obtained from the literature, and for materials not referenced in the literature were calculated based on their molecular structure and melting point using Molecular Modeling Pro, v6.2.8 (ChemSW, Fairfield, CA).

2.2.4. Thermal Gravimetric Analysis (TGA)

The thermal stability of the NIF, Kollidon® VA 64, their physical mixture and the melt extruded samples was determined as a function of weight loss. The analysis was performed on the samples (4-5 mg approximately) using a Perkin-Elmer Pyris 1 thermo-gravimetric analyzer (Norwalk, CT) operated a ramp rate of 20°C/min from a temperature of 50°C to 250°C. The % weight loss for all of the samples tested was recorded using the Pyris 1 TGA software. All of the TGA runs were performed in an open pan with purge and protective nitrogen gas flow at 40 mL/min.

2.2.5. Differential Scanning Calorimetry (DSC) Analysis

DSC was used to characterize the miscibility of NIF in a polymeric carrier, Kollidon® VA 64, utilizing a Perkin-Elmer Diamond DSC instrument (Shelton, CT). A 2-3 mg sample of the pure drug and the polymer, and their physical mixtures with varying drug concentrations (5, 10, 20, 40, 60, and 80%) were weighed, sealed in aluminum crimped pans (Kit 0219-0062, Perkin-Elmer Instruments, Shelton, CT), and heated from 40 to 245°C at a ramp rate of 20°C/min under nitrogen purge at a flow rate of 20 mL/min. The hot melt extrudates were prepared by varying the drug concentrations as mentioned above, and subjected to an initial heat-cool cycle (by heating to 140°C at the rate of 20°C/min and held for 5 min followed by cooling) to remove the thermal history of the samples. A second heat cycle was initiated wherein the samples were heated from 40 to 230°C at a ramp rate of 20°C/min under nitrogen purge at a flow rate of 20 mL/min.

2.2.6. Hot Melt Extrusion (HME)

NIF and Kollidon® VA 64 were used as a model drug and carrier matrix, respectively.

Prior to processing, required materials previously sieved through mesh # 40 were accurately weighed, premixed in a mortar and pestle to get a homogeneous mixture. The resultant blend was further mixed in a twin-shell dry V-blender (The Patterson-Kelly co., Inc. East Stroudsburg, PA) at a speed of 25rpm for 10 minutes. Each of these resultant drug-polymer binary mixtures containing 25% of the drug were extruded at three different temperature profiles (115, 135, and 155°C) utilizing a bench top co-rotating twin-screw hot melt extruder (Prism 16mm EuroLab, Thermo Electron Corporation, Newington, NH) using 8 mm round opening die. Also, at each of the temperature profiles three different screw speeds of 25, 50, and 100 rpm were employed and a total of nine hot melt extrudates of the same formulation were obtained to study the effect of process parameters. In addition, effect of processing aids and extrudates containing 10%/90% w/w and 40%/60% w/w drug/polymer mixtures were produced utilizing HME to study the effect of drug load. All of these final extrudates obtained were stored in foil-lined polyethylene bags and stored in a refrigerator until further analysis.

2.2.6.1.Post-Processing Drug Content

A portion of all of these extruded formulations were crushed into fine powder using mortar and pestle and stored in amber colored glass bottles. These processed and powdered extrudates were analyzed for the drug content immediately after extrusion through the hot melt extruder. A known amount of the extrudates and physical mixture were dissolved in 3:2 methanol: water, diluted and filtered using 0.2 µm, 13 mm PTFE membrane filters (Whatman, Piscataway, NJ) and analyzed utilizing a HPLC at a wavelength of 235 nm.

2.2.7. X-Ray Diffraction (XRD) Testing

XRD testing was conducted using a Philips Model 1710 X-ray diffractometer (Philips

Electronic Instruments Inc., Mahwah, NJ) to assess crystallinity in conjunction with thermal techniques. Powders of the pure drug sample (NIF), the polymer (Kollidon® VA 64), physical mixture, and the hot melt extrudates were passed through a # 40 mesh screen prior to the testing. Physical mixtures were prepared by pre-mixing NIF and Kollidon® VA 64 in the appropriate ratio (25% drug/75% polymer). Instrument was operated at an accelerating voltage of 40 kV and 30 mA. Samples of powder were placed into channeled stages and the diffraction profile was measured from 3.0^{o} to 50.0^{o} using a 2θ step size of 0.02^{o} and dwell time of 3s.

2.2.8. Moisture Absorption Study

In order to investigate the extent of moisture absorption by the drug, polymer, (1:3; drug: polymer) physical mixture, and the hot melt extruded binary samples in the ratios of 1:9, 1:3, and 2:3 of drug: polymer produced by the previously described methods, each of the samples was placed in a 40°C/75%RH environment, in opened amber colored borosilicate glass bottles for 7 days. In addition, the HME samples were also compared before and after moisture absorption for content uniformity.

The moisture absorbed by the samples is reported in terms of their % weight gain and was calculated by the following procedure. A sample of approximately 200 mg was weighed and placed in opened amber glass bottles which were equilibrated at 40°C/75%RH. The % weight gain (G) of samples was calculated using equation 3 provided below:

——— ---------- **Equation 3**

Where is the weight of the sample before moisture absorption, is the initial weight of a glass bottle with sample (before moisture absorption) and is the final weight of a glass bottle with sample (after moisture absorption). All measurements were performed in triplicate and were

compared with the initial data.

2.2.9. Flow Properties

The pure polymer, drug-polymer physical mixtures with varying drug loads (10, 25 & 40% w/w), and their corresponding extrudates have been assessed for their flow characteristics using traditional “angle of repose” measurement. In this experiment, a funnel was set to a fixed height using a clamp such that the distance between the lower tip of the funnel and the surface of a graph paper placed below is constant. The powder was slowly poured through the funnel on to the paper until the bulk material forms a conical pile, with its tip touching the base of the funnel. The angle of repose for each of these powders was then measured by the equation 4 provided below:

— --------- Equation 4

In the above equation, ‘h’ is the height of the pile of powder, and‘d’ is the diameter of the surface covered by the powder.

2.2.10. *In vitro* Dissolution Testing

Dissolution testing (USP XXXI, Apparatus I) was performed utilizing a Hanson SR8-plus™ dissolution test station (Hanson Research Corporation, Chatsworth, CA) operated at 50 rpm paddle speed. Drug release from the formulations extruded by varying the process parameters were evaluated for their release. Moreover, the effect of drug loaded samples (10%, 25%, and 40% w/w) were compared before and after moisture absorption for drug dissolution. An amount equivalent to 25.0 mg NIF (theoretical drug loading) from the physical mixture and the hot melt extrudates previously ground in mortar and pestle was accurately weighed and filled in gelatin capsule # 3. These capsules were added to the dissolution vessel containing 900mL of

pH 6.8 phosphate buffer with 1% w/v sodium lauryl sulfate (SLS) preheated to 37°C. During testing 1.5mL samples were removed from the dissolution vessels at pre-determined time intervals and replaced with an equal volume of fresh dissolution medium. Samples were immediately filtered using 13 mm PTFE membrane filters (Whatman, Piscataway, NJ) with a pore size of 0.2 µm and analyzed using HPLC at a λ_{max} of 235nm. Drug concentration was calculated from a standard calibration plot and expressed as cumulative % drug dissolved. The release studies were also performed in triplicate and the mean values were compared.

2.2.11. Stability Studies

A portion of NIF-Kollidon® VA 64 extrudates produced were stored in amber colored borosilicate glass bottles at 3 different temperatures of 4, 25 and 40°C and analyzed at pre-determined time intervals for the amount of NIF present using HPLC. The stability of these extrudates was then compared to the physical mixture. The results of the stability studies are expressed as a percentage of NIF degraded at various time intervals.

2.2.12. Statistical analysis

To compare between different formulations, statistical analysis was performed utilizing one way analysis of variance (ANOVA). A statistically significant difference was considered when $P<0.05$.

2.3.Results and Discussion

2.3.1. Analytical Method

Validation was carried out as per the ICH and FDA guidelines for the chromatographic method development. The linear calibration range for the detection of NIF was found to be 5-200 µg/mL, with the co-efficient of determination (R^2) of 0.999. The limit of detection and

quantitation for the drug were 0.2 and 1.0 µg/mL, and the retention time for the NIF was ~7.8 minutes. The % relative standard deviation (RSD) within replicates (n=3) was less than 2%, demonstrating reproducibility of the method. Precision was tested by injecting a single drug concentration of 20µg/mL, and the peak area (n=10) was recorded. A superior precision was observed with less than 0.5% of RSD.

2.3.2. Solubility Parameter Calculations

A three dimensional Hansen solubility parameter obtained from either literature or Molecular Modeling Pro™ software represents dispersive, polar and hydrogen bonding interactions of a molecule, and it could be used to estimate the interactions amongst the components in a formulation (33). From a pharmaceutical applications standpoint, this parameter is a very useful tool in predicting the API morphology and drug-polymer miscibility in the solid dispersions and the drug-excipient interactions in the development of solid oral dosage forms. The effective solubility parameter obtained from Hansen method for NIF and Kollidon® VA 64 were 17.9 $MPa^{1/2}$ and 21.1 $MPa^{1/2}$, respectively. Theoretically, in order to observe at least partial miscibility between the drug and the polymer, the solubility parameter difference ($\Delta\delta$) between them is required to be at least less than 7.0 $MPa^{1/2}$ (34, 35). In the present scenario, $\Delta\delta$ is 3.2, which is significantly less than 7.0 $MPa^{1/2}$, indicating the NIF miscibility in the utilized carrier matrix to form a solid dispersion.

2.3.3. Thermal Gravimetric Analysis

It is very crucial to study the thermal profiles of the active and the individual excipients present in a formulation, prior to melt extrusion. In order to evaluate the active and excipients processability and stability during extrusion, the materials were subjected to heat ramp and identified that both, pure components and their physical mixture, exhibited less than 2% weight

loss throughout the temperature range studied (Figure 2-1). Therefore, it has been concluded that the processing temperatures under study are suitable to evaluate; however, it should be noted that heat ramp was very quick in case of TGA study and the outcome of prolonged exposure of the components to such high temperatures is not yet clear and requires further investigation.

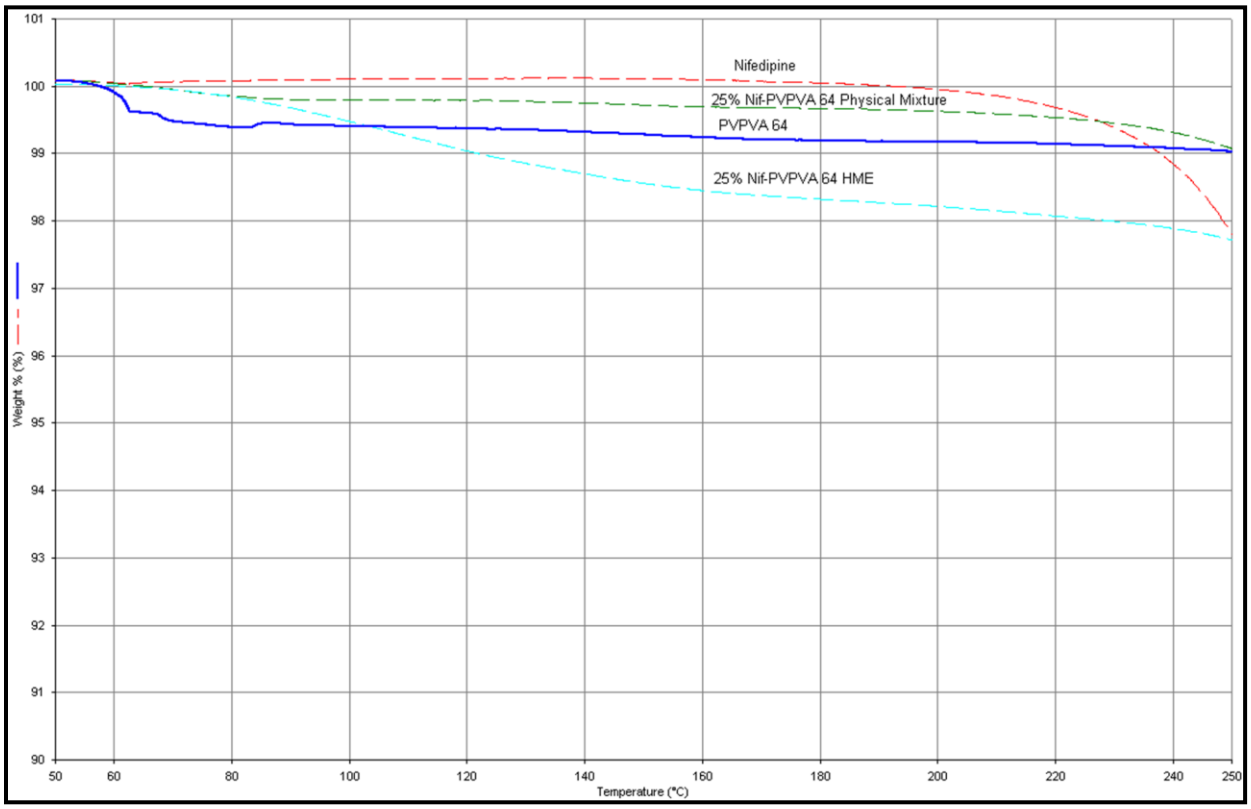


Figure 2-1: An Overlay of Thermogravimetric Analysis Profiles of Nifedipine, Kollidon® VA 64, 25% Nifedipine – 75% Kollidon® VA 64 Physical Mixture, and their Corresponding Hot Melt Extrudates

2.3.4. Differential Scanning Calorimetry Analysis

Solid dispersion has been one of the promising approaches utilized to enhance the dissolution rate of the poor-soluble actives. An important prerequisite to attain the solid

dispersion formation is miscibility or interaction between the API and polymeric components in a formulation. In the present study, the miscibility of NIF at various concentrations in Kollidon® VA 64 carrier matrix was investigated using Perkin-Elmer diamond DSC. In the thermograms shown in Figures 2-2A and 2-2B, temperature is plotted on the X-axis and heat flow (Endotherm up) on the Y-axis. The DSC thermogram of the pure drug revealed a characteristic melting endotherm of NIF at 172-174°C (36), while the physical mixtures exhibited a slight melting point depression of the drug in presence of the polymer, indicating its miscibility in the carrier matrix. NIF was completely miscible only at a concentration of 5% w/w in the physical mixtures, whereas the hot melt extrudates demonstrated good miscibility of the drug (up to 40% w/w) in the polymeric matrix. The peak corresponding to the melting of NIF was clearly evident in the DSC thermogram of the physical mixtures at 40% w/w of the active, whereas it disappeared in the melt extrudates (loss of crystallinity) at same drug load. This could be attributed to the formation of an amorphous solid dispersion of NIF in the Kollidon® VA 64 matrix. However, NIF at a concentration of 60% and 80% w/w in the melt extrudates does not seem to be completely miscible.

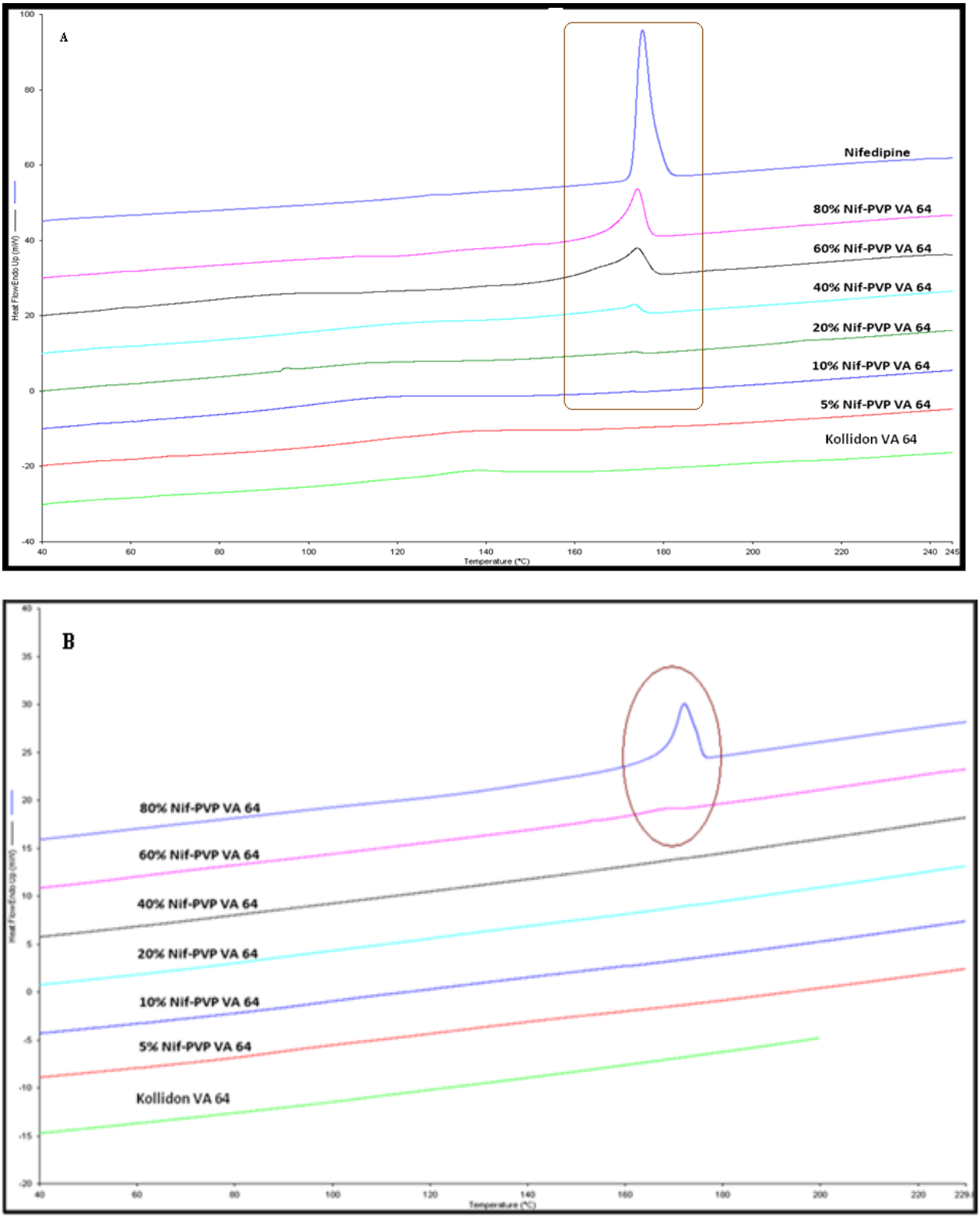


Figure 2-2: DSC Thermograms of (A) Pure Nifedipine, Kollidon® VA 64 and the

Nifedipine/Kollidon® VA 64 Physical Mixtures with Varying Drug Loads, and (B) Pure Nifedipine, Kollidon® VA 64 and the Nifedipine/Kollidon® VA 64 Hot Melt Extrudates with Varying Drug Loads

2.3.5. Hot Melt Extrusion (HME)

2.3.5.1.Effect of processing parameters (Temperature and Screw speed)

The process parameters employed in the study are provided in Table 2-1. All of the formulations exhibited a maximum die pressure of 6-7 bars, and the material feed rate was consistent in each case. The extrudates processed at lower barrel temperatures (115 and 135°C), irrespective of screw speeds, exhibited higher post-processing drug content (97-100%).

Table 2-1: Various Processing Parameters Employed, and Responses Observed During HME

Extrusion Temperatures (deg C)	115	135	155
Extrusion Speeds (rpm)	25	50	100
Residence Time (min)	6 to 7	3 to 4	1 to 2
Observed Max Die Pressure	6 to7 bars		
Torque Maintained	50%-60% - (12 - 15 Nm)		

However, those processed at a higher extrusion temperature of 155°C, the post-processing drug content decreased with decrease in screw speed from 100 to 25rpm (Figure 2-3). In particular, the formulation produced at a higher temperature (155°C) and lower screw speed

(25rpm) exhibited only 45% drug remaining, post-process. The lower screw speed of 25rpm displayed relatively longer material residence time in the extruder barrel (approximately 6-7 minutes) in comparison to the higher screw speeds, and the exposure of the active at 155°C for such a long period might have caused drug degradation. The results were in accordance with the findings of Caira et al., where the authors identified an existence of a solvated crystal form of NIF using single-crystal X-ray technique, which undergoes de-solvation leading to a complete disruption of crystal structure and conversion into a thermo-sensitive monoclinic polymorph at relatively high temperatures of 150-153°C (36). Both of the processing parameters, temperature and screw speed, were found to have a significant impact on the post-processing drug content of the final extrudates.

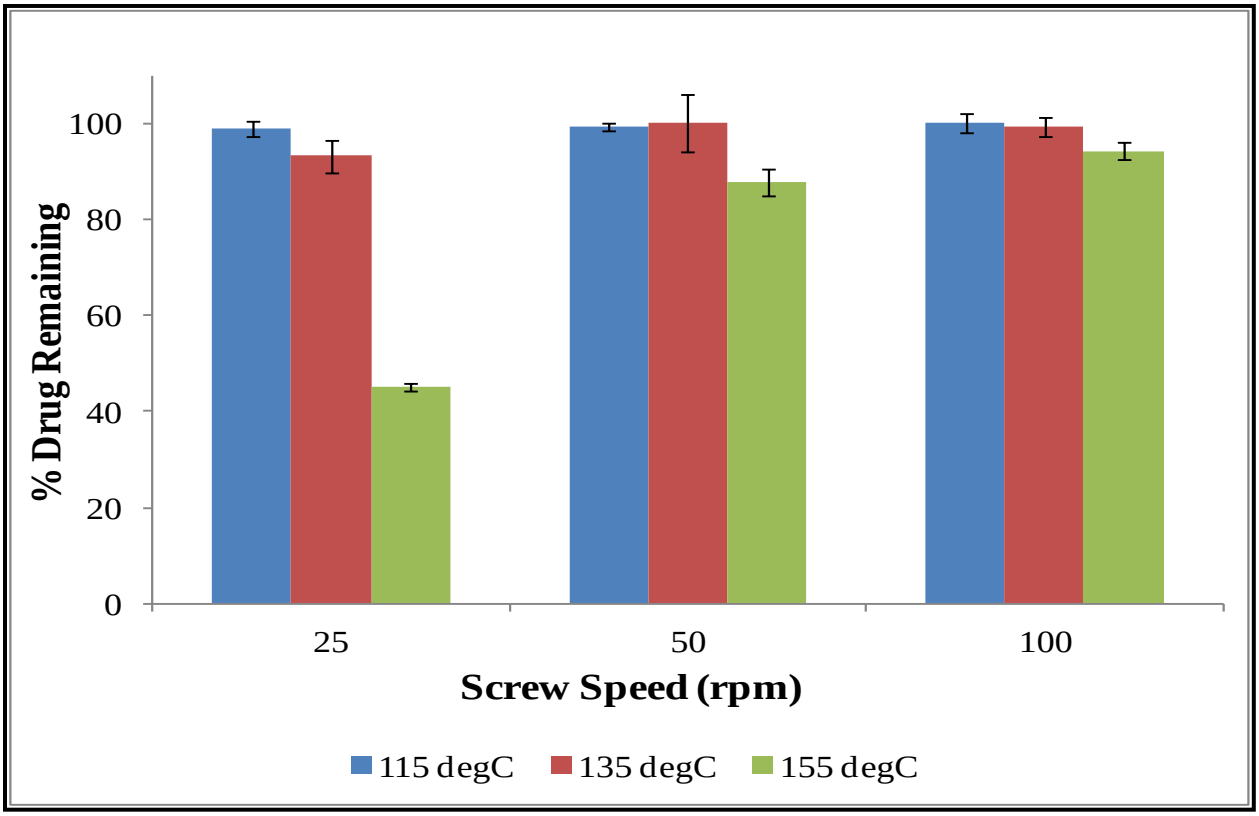


Figure 2-3: Effect of Processing Parameters on Post-Processing Drug Content in

Nifedipine/Kollidon® VA 64 Hot Melt Extrudates. Data Represent Mean ± S.D (n=6)

2.3.5.2.Effect of formulation factors: Drug load and Process aids

It was identified from the process evaluation that use of lower temperatures was suitable to produce reasonable extrudates with minimal drug degradation. Consequently, 10%, 25%, and 40% w/w NIF formulations were extruded at 135°C and 50rpm screw speed, which resulted in formulations with excellent post-processing drug content (99-100%) in the formulations, with no sign of drug degradation. The drug concentrations in the formulations were chosen based on the results obtained from preliminary DSC studies that supported formation of solid dispersions in mixtures comprising up to 40% w/w drug.

Furthermore, to produce a flexible formulation with greater ease and at lower temperatures, processing aids are very essential. Kollidon® VA 64, as mentioned earlier, softens above 100°C and may necessitate the use of a processing aid in order to produce a uniform melt at relatively low process temperatures. For this purpose, several hydrophilic and hydrophobic additives such as polyethylene glycol 3350, triethyl citrate, stearic acid, vitamin E succinate, and propyl paraben, each at two different concentrations (2.5% and 5% w/w), were investigated as process aids to evaluate their miscibility in drug-loaded Kollidon® VA 64 blend. Each of these physical mixtures, in the preliminary set of experiments, containing 25% w/w drug, 70-72.5% w/w of the polymer, and the processing aids was prepared into polymeric patches via casting method on a heated brass plate at 130°C using a punch and 8 mm round die, and their properties were observed visually for flexibility and texture. None of the chemical processing aids utilized in the formulation exhibited flexibility in the melt casted films of the polymeric carrier. Moreover, they produced either glassy or sticky products at the concentrations employed.

Nevertheless, Kollidon® VA 64 was extrudable at 90% w/w, with only a model drug in the formulation.

2.3.6. X-Ray Diffraction (XRD) Testing

DSC studies indicated the drug-polymer miscibility in the hot melt extruded formulations (data not shown), which has been further confirmed by the XRD analysis. The diffraction pattern of pure NIF exhibited sharp and highly intense peaks at 2θ degrees of 8.0, 11.9, 19.5, and 24.0, indicating crystalline nature of the drug (37). However, the physical mixtures containing drug and polymer at 1:3 ratios, when subjected to X-ray diffraction, exhibited relatively less intense and more diffused peaks, but the 2θ angles of the peaks remained unaffected (Figure 2-4). These changes in peak intensity could be attributed to the dilution of the active in the Kollidon® VA 64 matrix, and the unchanged 2θ angles of the peaks indicate existence of original crystalline form of the drug in the physical mixture. On the contrary, XRD pattern of melt extruded formulations produced using different process parameters did not demonstrate any sign of crystallinity or characteristic peaks at specific 2θ angles, indicating formation of an amorphous solid dispersion in those formulations. This data further strengthens the DSC results discussed earlier, where the active was completely miscible in the melt extrudates up to 40%w/w with no sign of characteristic melting endotherm of NIF.

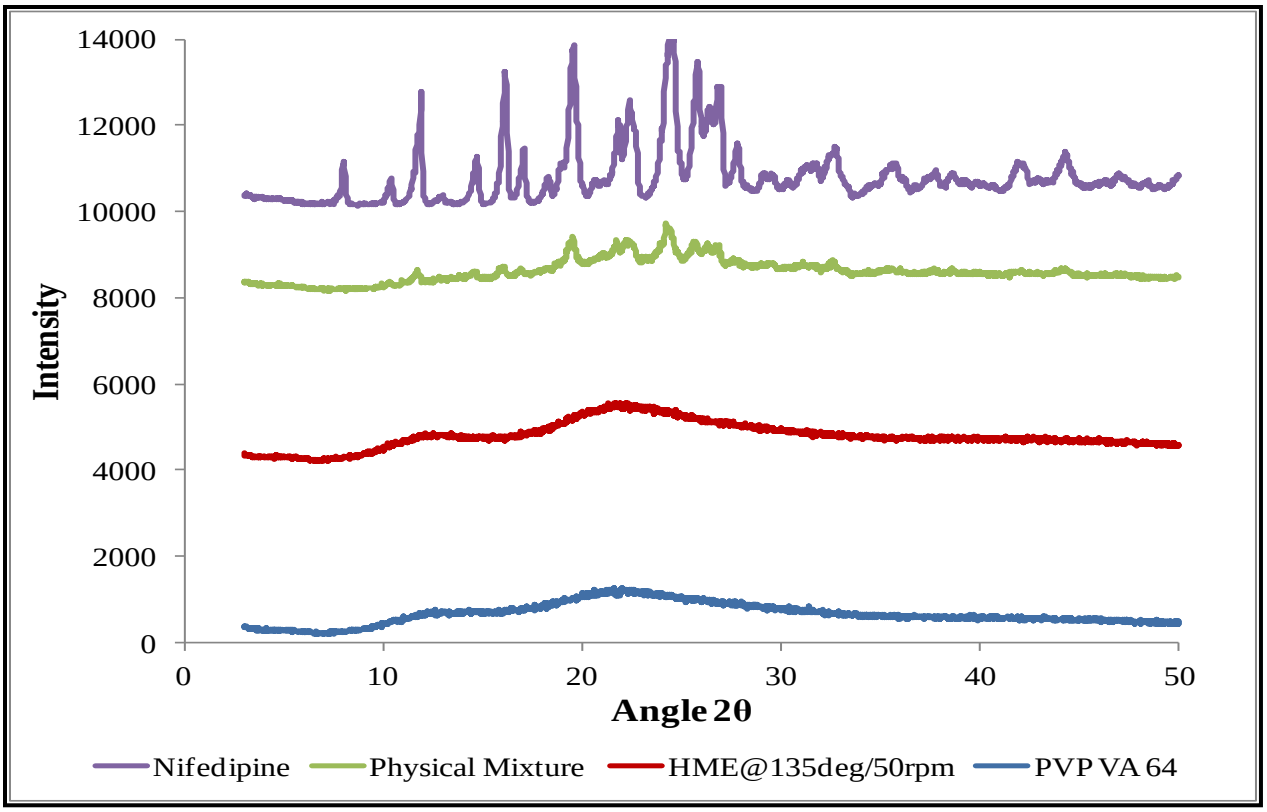


Figure 2-4: X-ray Diffraction Patterns of Nifedipine, Kollidon® VA 64, Drug/Polymer Physical Mixture (1:3), and their Corresponding Melt Extruded Sample Produced at 135°C and 50 rpm Screw Speed

2.3.7. Moisture Absorption Study

It was hypothesized that variability in the intimacy of mixing in different powder samples would be explained by investigating the extent of moisture absorption, which is directly proportional to the amount of hygroscopic surface area of the material being tested (38). Thus extent of moisture absorption could be an indicative of the intimate mixing of powders, and high moisture content in the powder mixtures could lead to inconsistency in the drug content and drug dissolution. In the present study, the extent of moisture absorption is estimated by percentage weight gained by the powder mixtures, when subjected to high relative humidity conditions.

Although mixtures were exposed to high humidity conditions for 7 days, all of the samples under test reached equilibrium within 24 hours. The hot melt extrudates (at 25% w/w drug loading) exhibited 15-20% decrease in weight gain compared to their corresponding physical mixture, and about 30% decrease in weight gain in comparison to the pure polymer, when subjected to the same conditions. Amongst the hot melt extrudates, the formulations produced utilizing different process temperatures and screw speeds did not demonstrate any significant difference in % moisture absorbed (data not presented), indicated by unchanged percentage weight gain of the mixtures. However, the weight gain in the milled extrudates decreased from 20% to 45% as the drug concentration increased from 10% to 40% w/w, in comparison to the pure polymer (Figure 2-5). This decrease in the % weight gain or reduced moisture uptake could be attributed to the presence of low concentrations of hygroscopic polymer in the formulations with higher drug load.

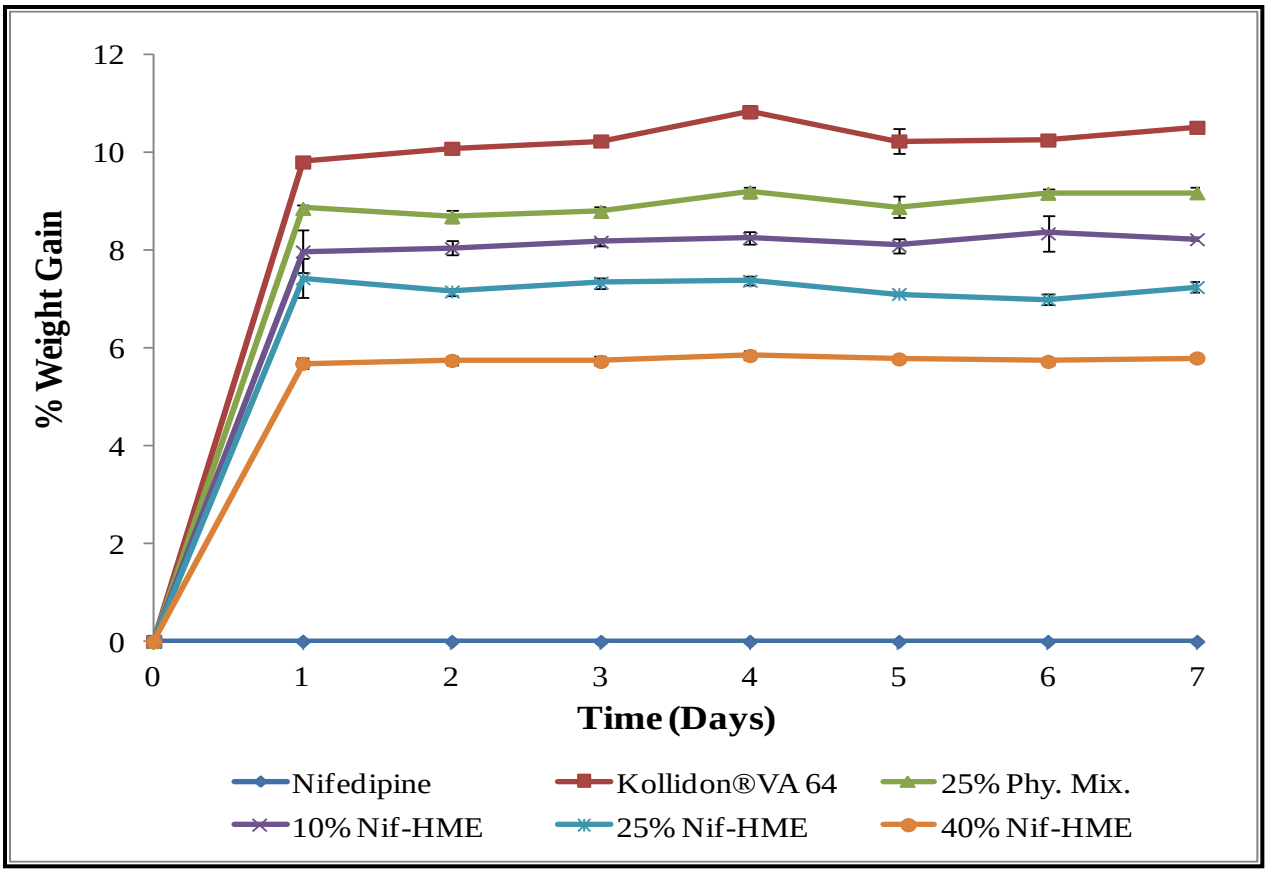


Figure 2-5: Moisture Absorption Study of Nifedipine, Kollidon® VA 64, Drug/Polymer Physical Mixture (1:3), and the Milled Extrudate Samples Produced at Varying Drug Loads. Data Represent Mean ± S.D (n=3)

Additionally, content uniformity of NIF in the milled hot melt extrudates containing 10%, 25% and 40% w/w drug loads, prior to and after exposure to high humidity conditions for a period of 7 days was evaluated. From the data presented in Figure 2-6, it is obvious that the milled extrudate samples did not show any great change in the drug content uniformity before and after being exposed to high moisture environment, irrespective of their drug concentrations. Although the % weight gain varied amongst milled extrudates with different drug loading, the drug distribution within the polymeric mixtures was not affected by the presence of moisture.

This could be due to the drug being uniformly distributed at a molecular level in the polymeric matrix, during HME process, which provides intimate contact of both the components in a formulation.

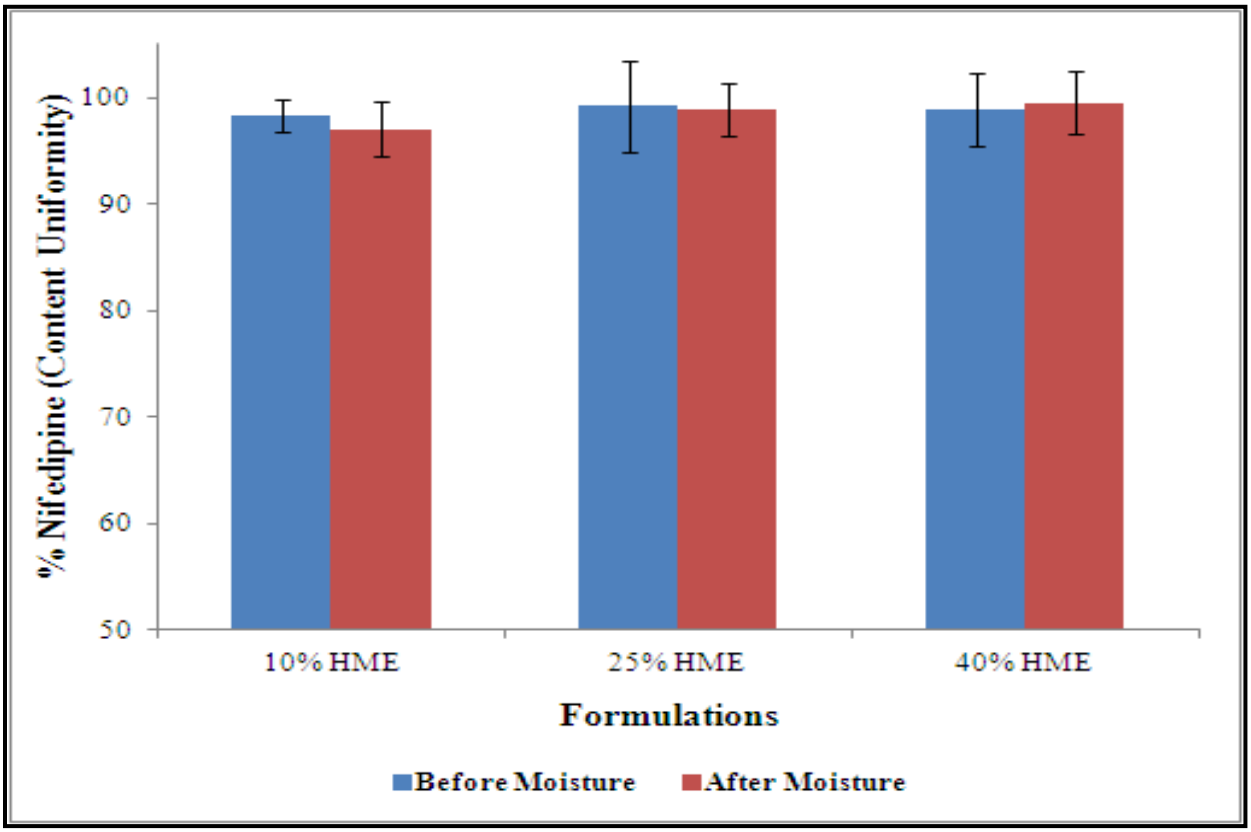


Figure 2-6: Content Uniformity of Nifedipine in the Milled Hot Melt Extrudates Containing 10%, 25% and 40% W/W Drug Loads and in 25% Drug-75% Polymer Physical Mixture Prior to, and After Exposure to High Humidity Conditions. Data Represent Mean ± S.D (n=3)

2.3.8. Flow Properties

Manufacturing dosage forms such as tablets and capsules is very simple and cost-effective approach that has been in practice over many decades. In order to produce such

formulation with good content uniformity, it is essential to consider the powder characteristics such as particle shape, density, coefficient of friction of the material etc. Angle of repose, utilized to measure the flow properties, is also related to these attributes mentioned above. Smaller angles correspond to wide spread of the bulk material, indicating better flow. From the study results presented in Table 2-2, Kollidon® VA 64 demonstrated superior flow by itself. However, an increase in the drug load from 10% w/w to 40% w/w in the physical mixtures resulted in larger angles that indicate poor flow characteristics. This behavior could be attributed to the difference in particle sizes between the API and the polymer, and to the cohesive nature of API diluting the free flowing polymer.

Table 2-2: Flow Properties of the Drug-Polymer Mixtures before and after HME

Samples	Angle of Repose (θ)	
	Prior to Extrusion	**Post Extrusion**
PVPVA 64	**21.95**	--
10% Drug Load	**30.66**	**23.85**
25% Drug Load	**37.88**	**24.32**
40% Drug Load	**39.21**	**23.50**

On the contrary, similar binary physical mixtures when processed utilizing hot melt extruder followed by milling into fine powder, demonstrated superior powder flow characteristics independent of drug loading. A possible explanation for this observation could be formation of a solid dispersion post-extrusion, as previously confirmed by DSC and XRD studies, where drug dissolves at a molecular level in the hydrophilic polymer matrix resulting in

changing the API physical properties. Based on the results, it is obvious that HME technique could be utilized to enhance the flow characteristics of the bulk material that could potentially minimize the content uniformity issues in conventional dosage forms. Furthermore, morphology and physical properties of the active and other excipients chosen in a formulation would be important in governing the flow characteristics.

2.3.9. *In vitro* Dissolution Testing

Dissolution testing of the melt extrudates *in vitro* is another important aspect that gives an idea of how a formulation behaves *in vivo* in terms of drug release. All of the extruded formulations produced at different screw speeds and temperatures had shown similar release profiles; in addition, more than 90% of the drug was released within 1 hour from all of the formulations tested (Figure 2-7A & 2-7B). Similarity factor 'f_2' was utilized to compare the dissolution profiles amongst different formulations (39), and was calculated using equation 5, where 'n' is the total number of sampling intervals and 'R_t' and 'T_t' are cumulative % drug released from reference and test formulations at any time interval 't.'

$$f_2 = 50 \times \log\{[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100\}$$ ---------- **Equation 5**

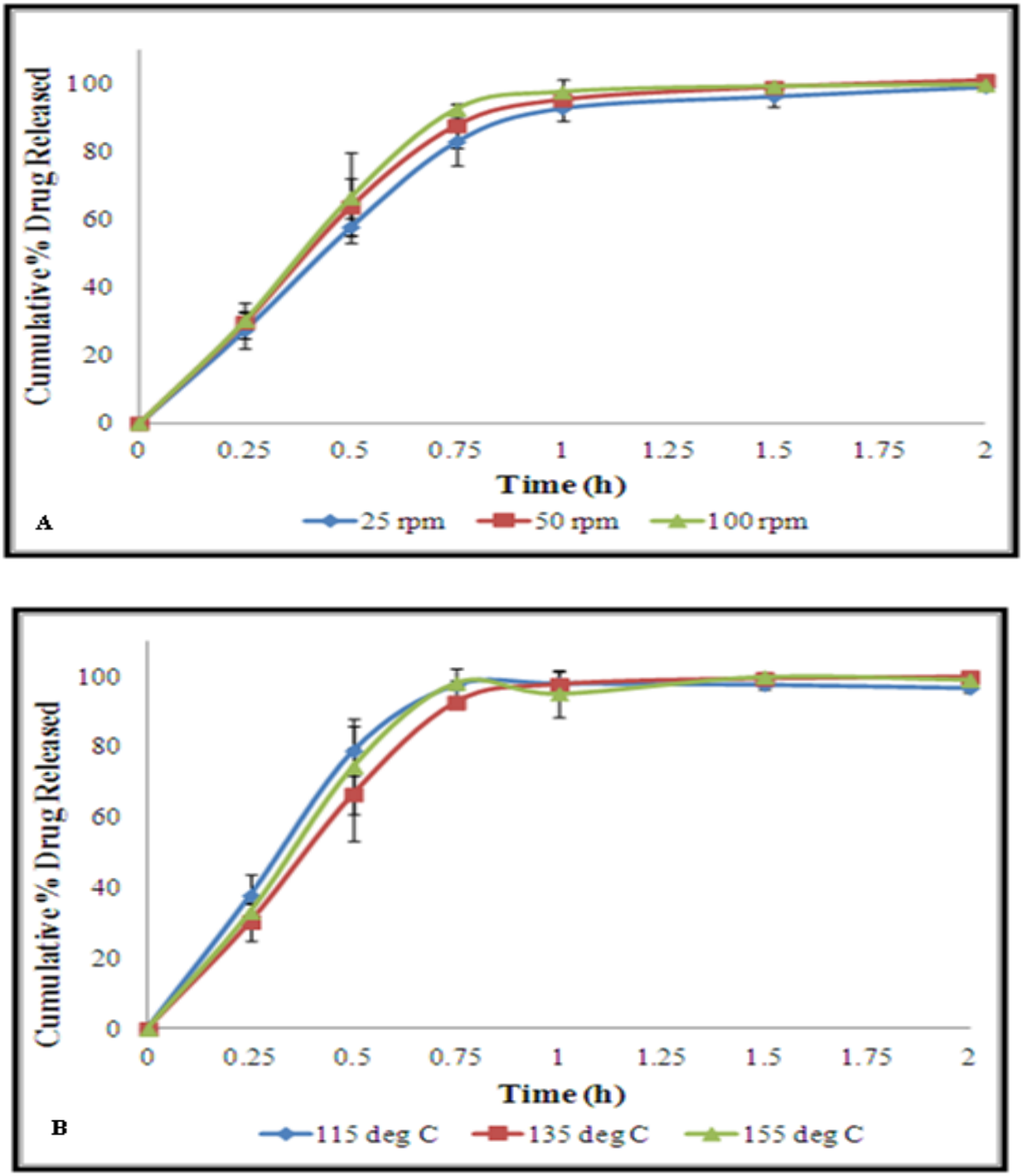


Figure 2-7: Dissolution Profiles of Nifedipine/Kollidon® VA 64 HME (A) Processed at 135°C- Effect of Processing Speeds; and (B) Processed at 100rpm Screw Speed- Effect of Processing Temperatures. Data Represent Mean ± S.D (n=3)

All of the obtained f_2 values, when compared amongst the dissolution profiles, were between 50 and 100 indicating that the drug release was similar for all of the formulations under investigation. Similarly, extrudates with varying drug loads of 10, 25 and 40% w/w were analyzed for the drug release, and their release patterns were presented in Figure 2-8.

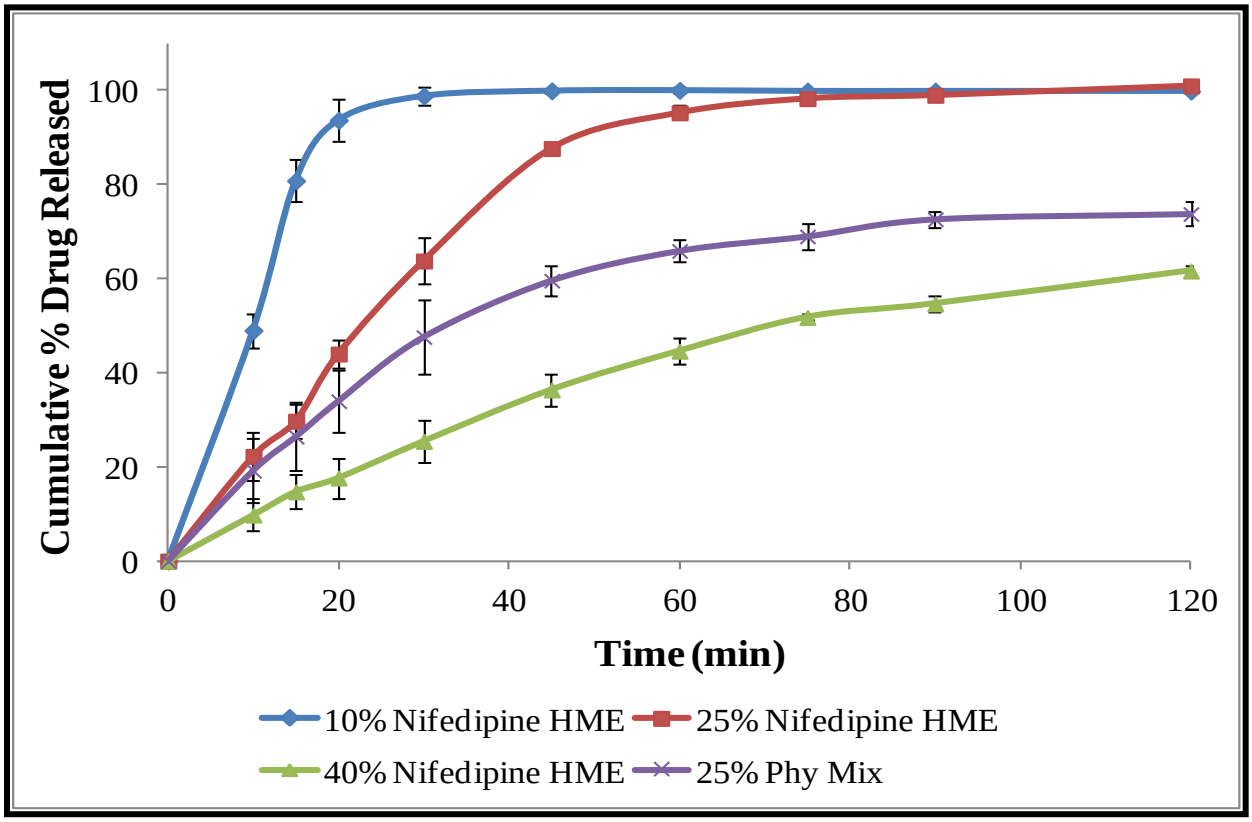


Figure 2-8: Drug Release from Nifedipine/Kollidon® VA 64 Hot Melt Extrudates Containing Varying Drug Loads Produced at 135°C and 50rpm Screw Speed. Data Represent Mean ± S.D (n=3)

The formulation containing 10% drug loading demonstrated a higher release rate compared to 25%, followed by 40% w/w drug load. The extrudates with lower drug concentration (10% w/w) behaved as an immediate release dosage form with more than 90% of the drug being released within 20 minutes, while those containing 25% w/w NIF loading

released the same within 60 minutes. Hence, it was obvious that the drug load has a greater impact on the release of the drug, and could be especially attributed to the presence of more amount of hydrophilic matrix, Kollidon® VA 64, in the solid dispersion formulations containing 10% w/w NIF. On the contrary, the hot melt extrudates containing 40% w/w NIF demonstrated slower drug dissolution in comparison to the extrudates with lower drug loading, which could be ascribed to the poor solubility and wettability of the active.

In addition, drug release from the extrudates with varying drug concentrations was studied before and after subjecting to the moisture absorption study. There was no significant difference observed in the release profiles (data not shown) before and after the moisture absorption, at each of the drug loads tested. Moreover, release of NIF from the 25% w/w extrudates was found to be different, and significantly superior (Figure 2-8) compared to its corresponding 25% w/w physical mixture (f_2=36.70). This is indicative of an improved drug dissolution rate from the extrudates in comparison to their corresponding physical mixtures, due to the formation of solid dispersion (previously evidenced by the DSC and XRD data) utilizing HME technique.

2.3.10. Stability Studies

Achieving a stable formulation with minimal or no API degradation throughout the product's shelf-life is of paramount importance for any given dosage form. The conventional dosage forms have several excipients incorporated in their formulations that lead to an increased possibility of drug-excipient incompatibilities, resulting in drug degradation. On the contrary, hot melt extrudates in the present study have only drug-polymer binary mixture, which minimizes the risk of drug instability in the formulation. Additionally, it is also well known that temperature

is a key factor that influences the reaction rates, and plays an important role in chemical degradation (40). As per Arrhenius equation a pharmaceutical system under investigation, when subjected to excessive thermal exposure undergoes accelerated degradation. However, Arrhenius assumption may be often invalid because of the unexpected phase changes or adsorbed moisture. Hence, it is advisable to use the lowest temperatures for the study that produces measurable failure in allocated time. Considering the fact, the chemical stability of the extruded formulations was studied by storing the samples at three different temperatures (4, 25 and 40°C) and analyzing them for % active remaining in the extrudates, which represents the scale on Y-axis (Figure 2-9). The API degradation in all of the formulations under study was found to be less than 5% for up to 3 months (period of study). These results indicate that the produced extrudates were chemically stable, and the processing conditions did not significantly impact the stability of NIF present in the extrudates.

This data was further corroborated with the physical stability results. As previously mentioned, elevated temperatures promote the reaction rate by lowering the activation energy. Typical elevated storage temperature under practice for accelerated degradation of a pharmaceutical system is 40°C. Therefore, the physical stability of the extrudates stored at this temperature for three months was assessed for change in the crystallinity or amorphous nature of the formed solid dispersions.

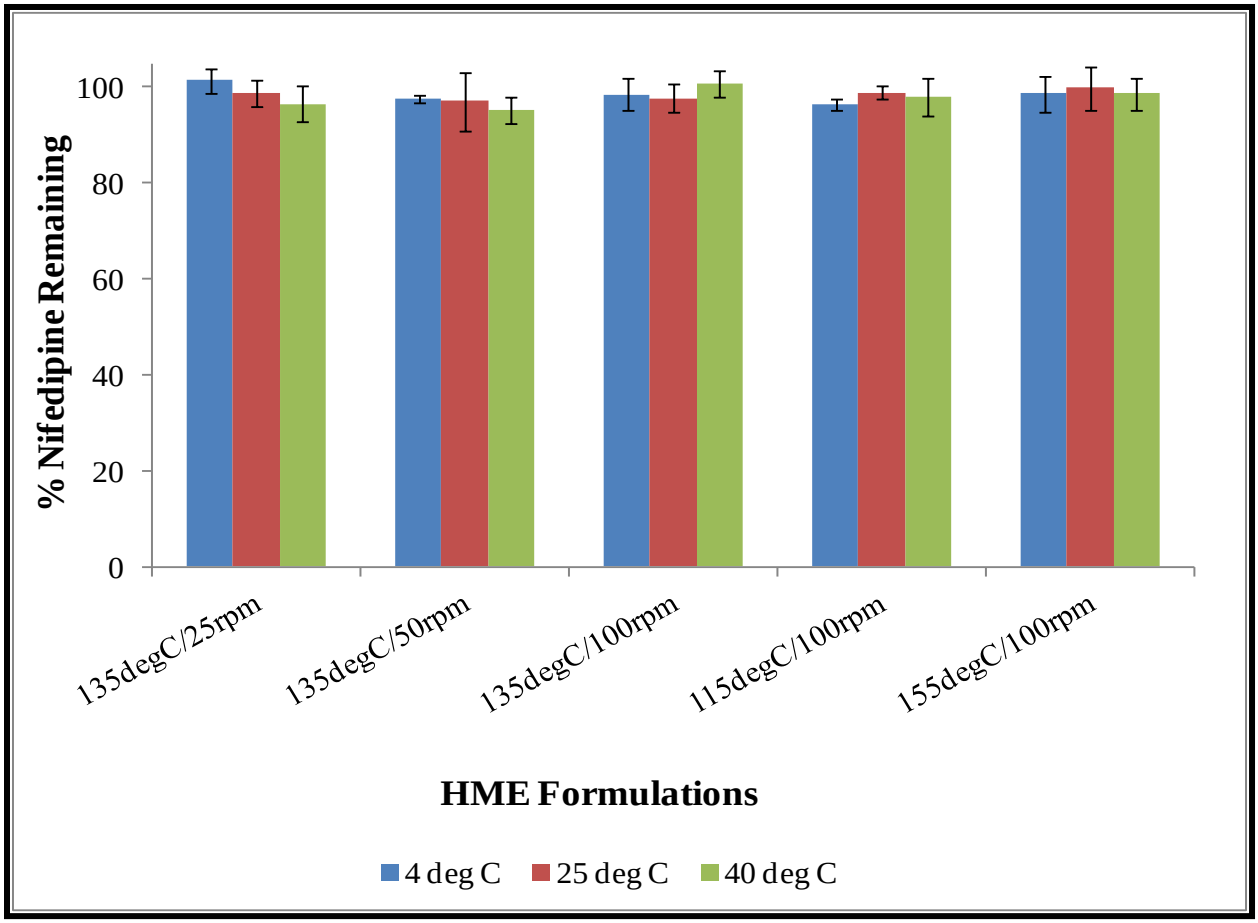


Figure 2-9: Chemical Stability of Nifedipine/Kollidon® VA 64 Hot Melt Extrudates Produced Utilizing Varying Processing Conditions, Stored at Three Different Temperatures for a Specific Period of Time. Data Represent Mean ± S.D (n=3)

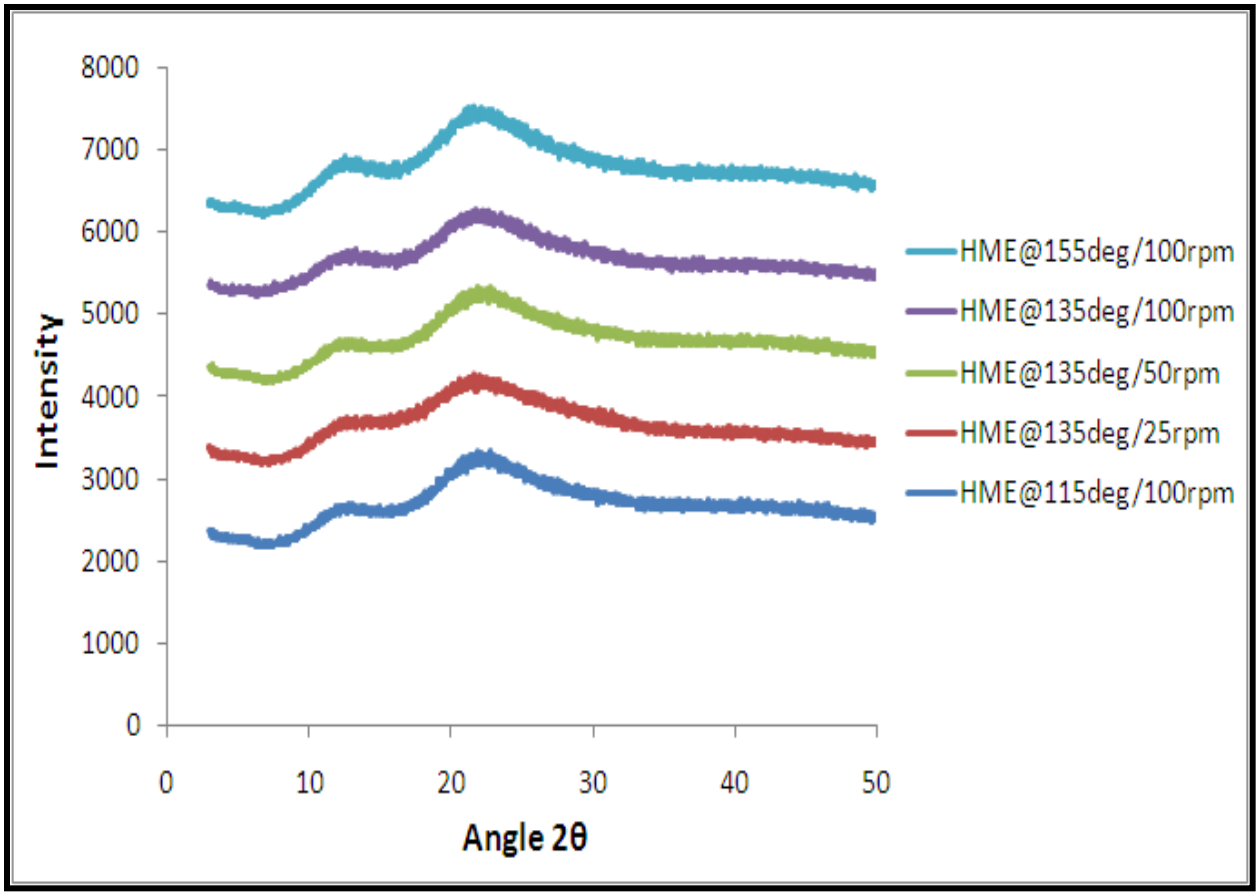


Figure 10: XRD Patterns Indicating Physical Stability of Nifedipine/Kollidon® VA 64 Hot Melt Extrudates Produced Utilizing Varying Processing Conditions, Stored at 40°C for a Specific Period of Time

XRD patterns (Figure 2-10) of the extrudates under investigation did not demonstrate any sign of crystallization after exposing to elevated temperature for such a long period, signifying their physical stability. In addition, all of the extruded formulations with 10%, 25% and 40% drug loading were also found to be physically and chemically stable up to 3 months (data not presented) under employed conditions.

2.4.Conclusions

In this study, melt extrusion technique in combination with Kollidon® VA 64 produced stable extrudates with higher drug loading and superior drug release. NIF was found to be miscible in Kollidon® VA 64 up to 40% w/w drug load, without having need of any processing aid for extrusion. Kollidon® VA 64, on the other hand, was extrudable at 90% w/w with only a model drug in the formulation. The processing parameters had a significant impact on the post-processing drug content, and the influence of drug load on release from extrudates was remarkable. These results demonstrate the importance of selecting appropriate carrier matrix for HME process, and utilizing suitable formulation and process conditions based on physicochemical properties of the active and the polymeric carrier under study, which could greatly influence the final properties of the produced extrudates.

2.5.Acknowledgements

This project was supported by Grant# P20RR021929 from the National Center for Research Resources (NIH/NCRR). The authors would also like to thank BASF Corporation for its generous supply of Kollidon® VA 64, as well as Dr. James W. McGinity for his assistance in conducting XRD studies.

CHAPTER - 3

Characterization of Soluplus® Hot Melt Extrudates: Effect of Formulation and Process Parameters on Miscibility, Dissolution and Stability Characteristics

3.1.Introduction

Advent of high throughput screening and combinatorial chemistry is resulting in more complex API (active pharmaceutical ingredient) structures. It has been estimated that 40-50% of all new molecular entities suffer from poor bioavailability due to low aqueous solubility (16). Formulation of such compounds for oral delivery presents one of the most frequent and formidable challenges to formulation scientists. Numerous techniques such as spray drying, solvent-evaporation, nano-crystal formation, complexation, and micronization of API etc., have been widely utilized in the industry to demonstrate the improved solubility and/or dissolution rate of such drugs, thereby enhancing their bioavailability (23-27). In addition, over the past couple of decades, HME technique has gained enormous interest amongst researchers in improving the bioavailability of drug substances, especially those having low water solubility, by formation of molecular dispersions (13, 14, 16, 17). HME offers several advantages such as being solvent free and involving fewer processing steps in comparison to other techniques.

As mentioned earlier, HME is the process of pumping raw materials with a rotating screw under elevated temperature through a die into a product of uniform shape. These final extrudates obtained utilizing thermal energy may be in the form of films or rods that can be cut as tablets or

pellets. To date, several research papers have been published by scientists that focused on the preparation of solid dispersions/solutions of numerous poorly aqueous soluble model drugs such as CTZ, indomethacin and NIF etc. utilizing HME technique. In addition, different grades of hydrophilic and hydrophobic polymers such as PolyOx™, Eudragit®, Kollidon®, Ethocel® were utilized to produce such dispersions, and were characterized in-depth using HME technique.

Soluplus®, a recently introduced polymer, is mainly employed as the carrier matrix in the current study. Soluplus® is a graft copolymer of PEG 6000/vinyl caprolactam/vinyl acetate copolymer in 13/57/30 ratios. It is white to yellowish free flowing granules with a glass transition temperature at ~70°C. The high flowability combined with its good extrudability characteristics demonstrate an excellent performance of the polymer in the formation of solid solutions, especially in HME processes. Due to the presence of PEG 6000, it is believed to have solubilization effect as well. Therefore, Soluplus® combines the benefits of solid solutions and solubilization which thereby increases solubility of poorly soluble drugs and hence enhance bioavailability. The properties mentioned above makes Soluplus® an attractive carrier matrix for HME processing, but to date, there is little research published on Soluplus® and needs further evaluation.

Lust et al. studied the formation of piroxicam solid dispersions using Soluplus® as the carrier matrix. The authors concluded that Soluplus® enhanced the dissolution as well as the oral bioavailability of piroxicam in rats (41). Solid dispersions of various drugs such as indomethacin, famotidine, camptothecin, and fenofibrate were also produced using this polymeric solubulizer, Soluplus® (42-44). It is also been used in the preparation of bicalutamide nano-crystals which are produced by anti-solvent precipitation method (45). In addition, it is also

used as a carrier matrix in the preparation of carbamazepine-nicotinamide co-crystals (46). Apart from being used as a carrier matrix in HME, Soluplus® has also been used in the electro-spinning and KinetiSol® dispersing techniques as well (47, 48).

In addition, many research scientists have also published the effect of process parameters during extrusion. Few examples are: Shibata et al. produced solid solution of Indomethacin and crospovidone using twin-screw extruder, and demonstrated that the parameters such as residence time, screw speed, and heating temperature play a significant role in the extrusion process (28). On the contrary, in a study by Verhoeven et al., the effect of process parameters (Screw design, feed rate and screw speed) on metoprolol tartarate and ethyl cellulose mini matrices were studied, and revealed that the process parameters had no significant influence on drug quality as well as drug release, indicating robustness of HME technique (29). In addition, the role of kneading paddle elements and operating conditions of twin screw extruder on NIF and HPMCP extrudates were studied by Nakamichi et al., and concluded that all of these factors played an important role in obtaining ideal solid dispersions (30). Most of these previous studies reveal that the influence of process parameters primarily depends on multiple factors such as the API involved, the carrier matrix utilized, and the processing conditions employed during the HME process.

Therefore, in the current study, CTZ was utilized as a model drug, which is a practically water-insoluble antifungal agent with a melting point of 146-147°C, and Soluplus® was used as a polymeric matrix to further investigate the feasibility of producing drug-loaded Soluplus® extrudates. Solid dispersions in the present study were produced using a MiniLab II HAAKE Rheomex CTW5 (conical twin-screw compounder) manufactured by ThermoFisher Scientific. In

early drug development stages, very less quantities of drug is available to conduct the feasibility studies. In such circumstances, this kind of equipment is well suited as the minimum quantity required for this extruder is only 5-10 gm. This manuscript illustrates the readers the effect of formulation and process parameters such as drug load and drug residence time on a much smaller scale, especially, when small amount of the drug is available. The influence of formulation and processing parameters such as drug-load and residence time, respectively, on the drug-polymer miscibility and drug release was also studied, including the physical and chemical stability of these extrudates.

3.2.Materials and Methods

3.2.1. Materials

Clotrimazole USP was purchased from LETCO Medical (Decatur, AL, USA); Hydrochloric acid was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Soluplus® was kindly gifted by BASF Corporation. High performance liquid chromatography (HPLC) grade water was freshly prepared in the laboratory by Nanopure systems (Barnstead, Dubuque, IA, USA). All solvents utilized in the study were of analytical grade and obtained from Fisher Scientific (Fair Lawn, NJ, USA).

3.2.2. HPLC Analysis

In-house reverse phase high performance liquid chromatography (HPLC) based analytical method was developed for determination/quantification of CTZ. An HPLC equipped with UV detector, Novapak phenyl column (150×4.6 mm) with particle size of 3µ, and an isocratic mode of elution with mobile phase containing methanol and 25mM KH_2PO_4 buffer (80:20) at a flow rate of 1.0 mL/min were employed to quantify the drug at a wavelength (λmax)

of 215 nm. All injections were performed by an autosampler, and the injection volume was 20µL. The acquired data was processed using Empower 2 build 2154 software (Waters Inc., Mount Holly, NJ, USA).

3.2.3. Thermal Gravimetric Analysis (TGA)

The thermal stability of the CTZ, soluplus®, and their physical mixture samples was determined as a function of weight loss. The analysis was performed on the samples (4-5 mg approximately) using a Perkin-Elmer Pyris 1 thermo-gravimetric analyzer (Norwalk, CT) operated at a ramp rate of 20°C/min from a temperature of 40°C to 250°C. The % weight loss for all of the samples tested was recorded using the Pyris 1 TGA software. All of the TGA runs were performed in an open pan with purge and protective nitrogen gas flow at 40 mL/min.

3.2.4. Differential Scanning Calorimetry (DSC) Analysis

DSC was used to characterize the miscibility of CTZ in a polymeric carrier, soluplus®, utilizing a Perkin-Elmer Diamond DSC instrument (Shelton, CT). A 2-3 mg sample of the drug, polymer, and physical mixtures with varying drug concentrations (10, 20, 30, 40, and 50%) were weighed, sealed in aluminum crimped pans (Kit 0219-0062, Perkin-Elmer Instruments, Shelton, CT), and heated from 30 to 210°C at a ramp rate of 20°C/min under nitrogen purge at a flow rate of 20 mL/min. The hot melt extruded samples were subjected to an initial heat-cool cycle (by heating up to 130°C at the rate of 20°C/min and held for 5 min followed by cooling) to remove the thermal history of the samples. A second heat cycle was initiated wherein the samples were heated from 40 to 210°C at a ramp rate of 20°C/min under nitrogen purge at a flow rate of 20 mL/min. In addition, the extruded samples with 30% drug load were subjected to prolonged heat exposure (varying residence time of 1, 5, and 10 min) at a barrel temperature of 130°C, and their

thermal characteristics were also studied.

3.2.5. Hot Melt Extrusion (HME)

CTZ and soluplus® were used as a model drug and carrier matrix, respectively. Prior to processing, required materials previously sieved through mesh # 40 were accurately weighed and were further blended in a twin-shell dry V-blender (The Patterson-Kelly co., Inc. East Stroudsburg, PA) at a speed of 25rpm for 10 minutes. The CTZ-Soluplus® binary mixtures containing 10%, 20% and 30% of the drug load were extruded at 130°C and 70 rpm screw speed utilizing a conical twin screw compounder (MiniLab II HAAKE Rheomex CTW5, ThermoFisher Scientific). The torque (N-cm) and the pressure difference (ΔP) generated during the extrusion process were recorded.

In addition, to assess the effect of processing time, each of the above produced formulations were exposed at 130°C for 1, 5 and 10 minutes. All of these final extrudates obtained at each of the mentioned conditions were stored in foil-lined polyethylene bags and stored in a refrigerator until further analysis.

3.2.5.1.Post-processing Drug Content

A portion of all of these extruded formulations were crushed into fine powder using mortar and pestle and stored in amber colored glass bottles. These processed and powdered extrudates were analyzed for the drug content immediately after extrusion through the hot melt extruder. A known amount of the extrudates and physical mixture were dissolved in 4:1 methanol: water, diluted and filtered using 0.2 µm, 13 mm PTFE membrane filters (Whatman, Piscataway, NJ) and analyzed utilizing a HPLC at a wavelength of 215 nm.

3.2.6. Fourier-transform infrared spectroscopy

FTIR spectra for the drug, polymer, and their extrudates produced with varying drug loads were obtained using a Perkin Elmer FTIR spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA). A spectrum was collected for each sample within the wave number region 4,000-400 cm^{-1}. The spectra were analyzed for the absence or shift in the wave numbers of the characteristic peaks and reported.

3.2.7. *In vitro* Dissolution Testing

Dissolution testing (USP XXXI, Apparatus II) was performed utilizing a Hanson SR8-plus™ dissolution test station (Hanson Research Corporation, Chatsworth, CA) operated at 50 rpm paddle speed. Drug release from the formulations extruded at varying CTZ loads (10%, 20%, and 30% w/w) were evaluated for their release. Moreover, the effect of residence time (1, 5, and 10 minutes) on drug release from extrudates containing 30% CTZ was studied. A 60mg sample from each of the produced melt extruded formulations, previously ground in mortar and pestle, was accurately weighed and filled in gelatin capsule # 4. These capsules were added to the dissolution vessel containing 900mL of 0.1N hydrochloric acid (pH 1.2) preheated to 37°C. During testing 1.5mL samples were removed from the dissolution vessels at pre-determined time intervals and replaced with an equal volume of fresh dissolution medium. Samples were immediately filtered using 13 mm PTFE membrane filters (Whatman, Piscataway, NJ) with a pore size of 0.2 µm and analyzed using HPLC at a λmax of 215nm. Drug concentration was calculated from a standard calibration plot and expressed as cumulative % drug dissolved. The release studies were also performed in triplicate and the mean values were compared.

3.2.8. Stability Studies

A portion of CTZ-soluplus® extrudates produced were stored in amber colored

borosilicate glass bottles at 3 different temperatures of 4, 25 and 40°C and analyzed at pre-determined time intervals for the amount of CTZ present using HPLC. The chemical stability of these extrudates was then compared to the physical stability of the same assessed utilizing DSC after 3-month time point. The results of the chemical stability studies are expressed as a percentage of CTZ remaining at various conditions under investigation.

3.2.9. Statistical analysis

To compare between different formulations, statistical analysis was performed utilizing one-way analysis of variance (ANOVA). A statistically significant difference was considered when $P<0.05$.

3.3.Results and Discussion

CTZ is an antifungal compound that acts primarily by altering the permeability of the cell wall and inhibiting the synthesis of ergosterol, which is required for the production of fungal cell membrane. This active has very poor aqueous solubility characteristics with erratic oral absorption, and therefore presents itself as a good candidate for HME processing to demonstrate an enhancement in its dissolution characteristics. Soluplus®, on the other hand, is a hydrophilic polymer that also exhibit solubilization capacity of poorly soluble drugs.

As a part of pre-formulation studies, TGA was performed to demonstrate the heat stability and thermal processability of the drug and the polymer during melt extrusion process. The TGA studies revealed that Soluplus® and CTZ were found to be stable under employed extrusion conditions with about 2% weight change being observed (Figure 3-1), when heated up to 250°C. An initial small decrease in the weights of the pure polymer and its corresponding 10% drug physical mixture at about 100°C could be due to the loss of water from the Soluplus®.

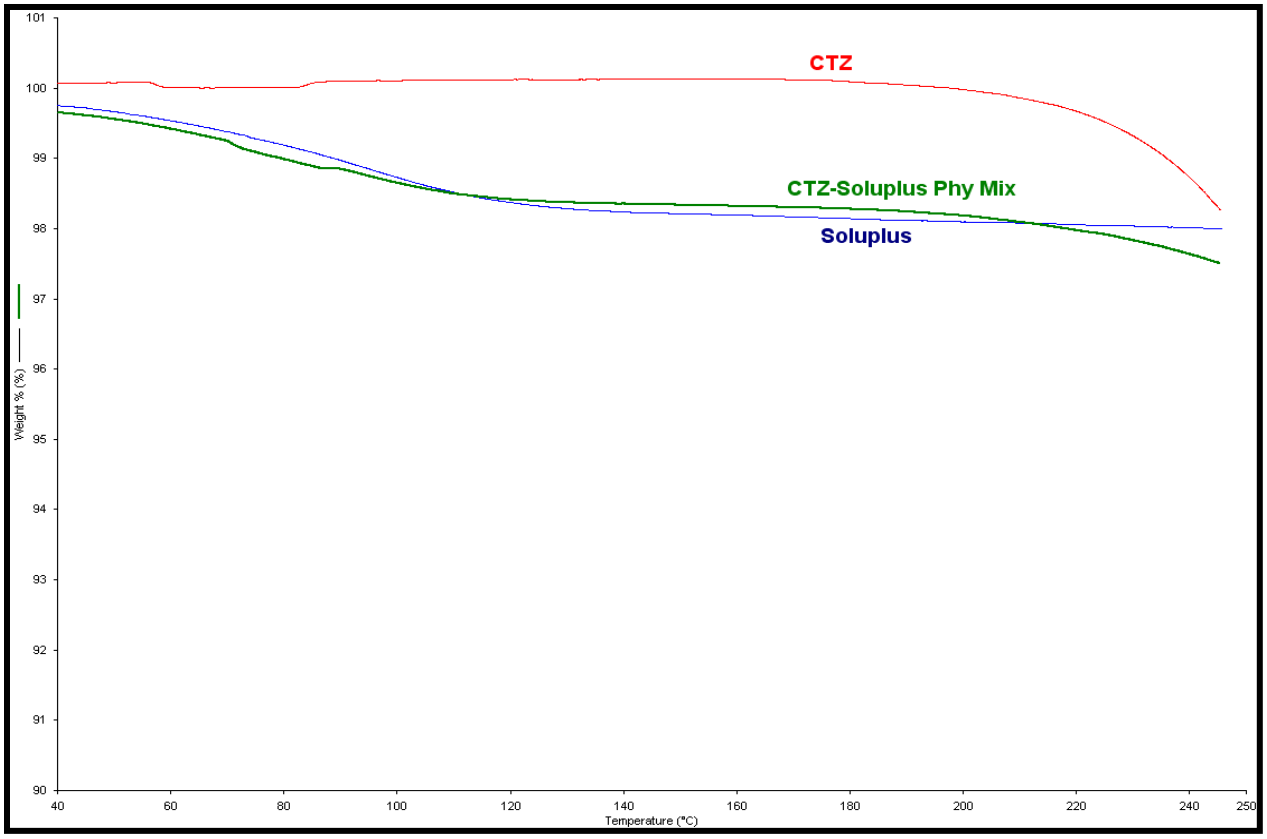


Figure 3-1: An Overlay of Thermogravimetric Analysis Profiles of Clotrimazole (CTZ), Soluplus®, and 10% CTZ – 90% Soluplus® Physical Mixture

It was evident from the DSC studies of the physical mixtures that CTZ exhibited a melting endotherm at all of the studied concentrations, which indicates that the drug's crystallinity is retained up on physical mixing with the polymer. However, a melting point depression of the drug was noticed in the presence of the polymer (Figure 3-2).

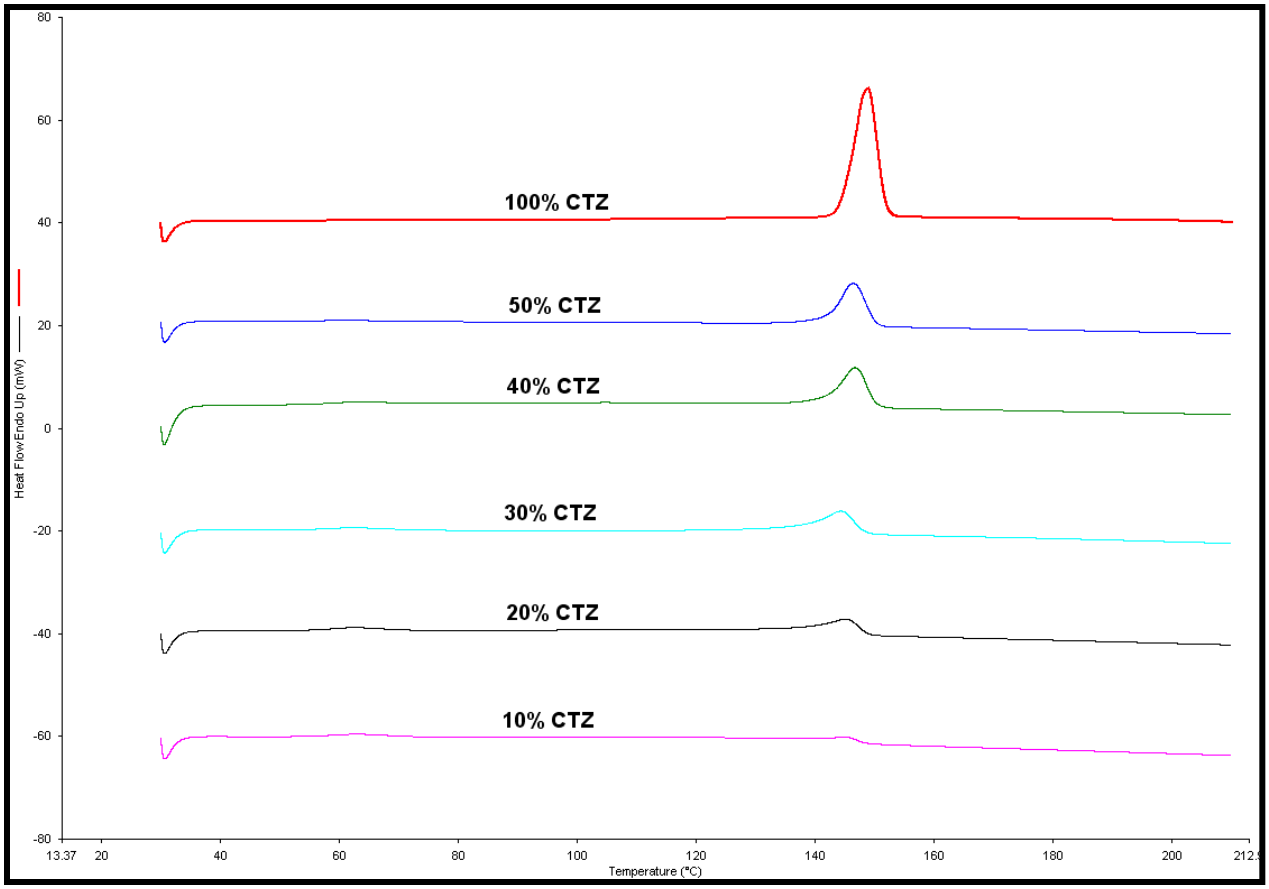


Figure 3-2: DSC Thermograms of Pure Clotrimazole (CTZ) and the CTZ/Soluplus® Physical Mixtures at Varying Drug Concentrations

In the present research, drug was extruded with polymer alone at different concentrations, without utilizing any of the processing aids. During the extrusion process both of the recorded responses, torque and pressure, decreased with an increase in the drug concentration. The data presented in Table 3-1 could be attributed to the plasticization effect of CTZ on Soluplus®. Additionally, the 30% w/w CTZ-soluplus® binary blends were subsequently exposed to barrel temperature for 1, 5, and 10 min, to further evaluate the effect of residence time. All of the produced extrudates exhibited very good post-processing drug content with a mean and relative standard deviation of 98.0% and 3.0%, respectively.

Table 3-1: Responses Observed During Melt Extrusion Processing of Clotrimazole at Different Drug Loads

Drug Load	Torque (N-cm)	ΔP (bar)
10%	72	37
20%	60	16
30%	37	8

The DSC thermograms of the extrudates demonstrated drug miscibility up to 20% w/w, when processed for one minute at a barrel temperature of 130°C. The higher drug loads demonstrated a characteristic melting peak of the drug around 144°C (Figure 3-3). Interestingly, the drug's melting peak disappeared in 30% w/w extrudates obtained by exposure at 130°C for 10 minutes in comparison to those produced by 1 and 5 minutes exposure at the same temperature conditions (data not shown). As the time of drug exposure to heat increased, complete drug-polymer miscibility was observed at 30% w/w drug load.

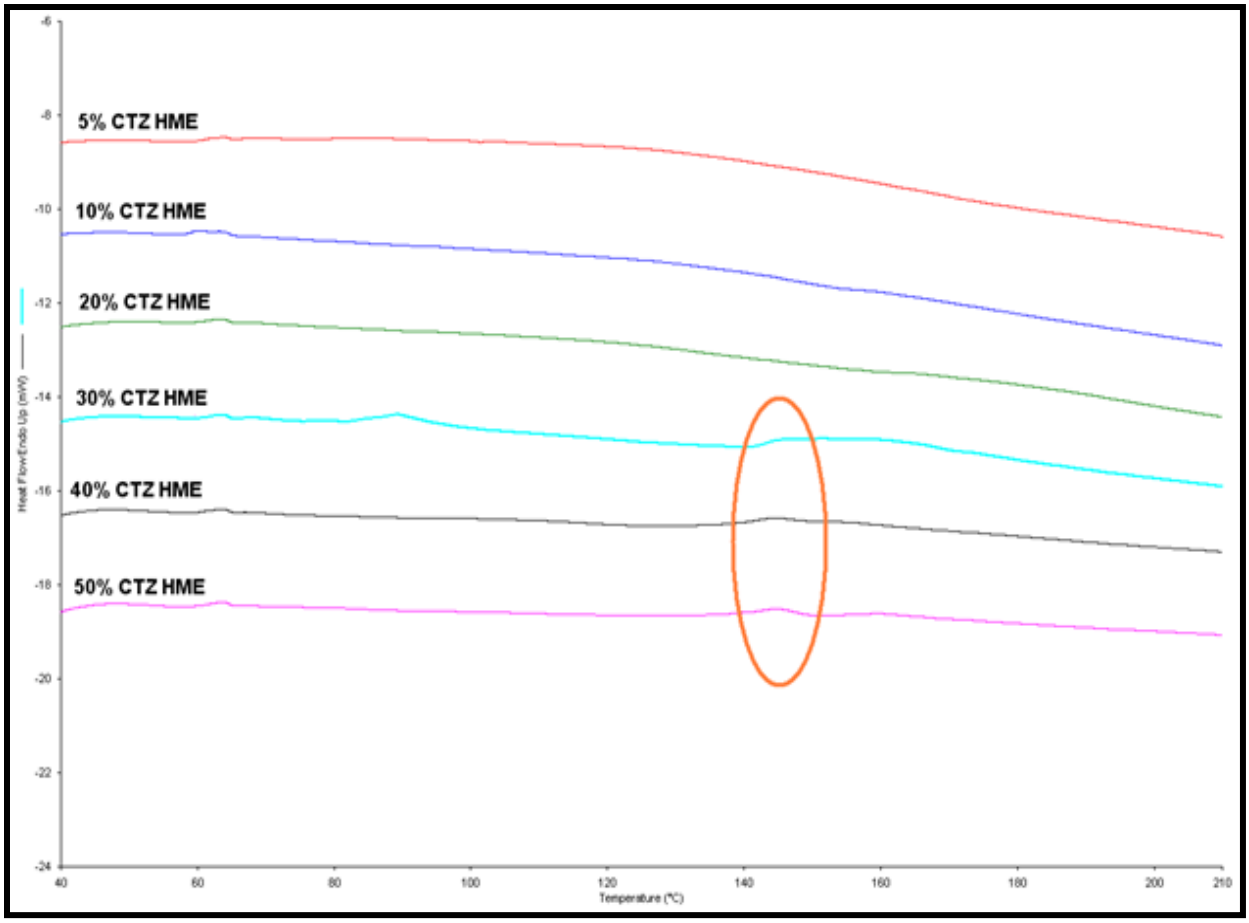


Figure 3-3: DSC Thermograms of CTZ/Soluplus® Hot Melt Extrudates: Effect of Drug Load

FT-IR spectroscopic analysis was performed on the extruded samples to study the drug-polymer interactions, and to corroborate the miscibility results obtained by DSC. The spectra of CTZ exhibit characteristic peaks at 3169 and 3042 cm^{-1} (aromatic C-H stretching), 1585, 1487 and 1305 cm^{-1} (benzene ring stretching), 1203 cm^{-1} (C-N stretching), and 1084 cm^{-1} (chlorobenzene stretching). The FTIR spectra of the extruded formulations presented in Table 3-2 and Figure 3-4 demonstrate the presence of CTZ characteristic peaks, indicating the absence of any chemical interactions between the drug and the polymer.

Table 3-2: Characteristic Peaks in the FTIR Spectra of Clotrimazole (CTZ) in Different Hot Melt Extruded Formulations

Formulations	Aromatic C-H Stretching (3169 & 3042 cm^{-1})	Benzene ring Stretching (1585, 1487 & 1305 cm^{-1})	C-N Stretching (1203 cm^{-1})	Chlorobenzene (1084 cm^{-1})
10% CTZ	√	√	√	√
20% CTZ	√	√	√	√
30% CTZ	√	√	√	√
30% CTZ-5min	√	√	√	√
30% CTZ-10min	√	√	√	√

Note: "√**" indicates presence of the characteristic peak**

Dissolution studies were conducted on the produced HME formulations to evaluate the enhanced drug release from the produced solid dispersions. Over 90% of CTZ was released in the first hour for the formulations containing 10% drug load. 10% CTZ-loaded matrices exhibited faster release followed by 20% and 30% w/w. This slower release could be attributed to the increasing concentration of hydrophobic drug from 10% to 30% in the extrudates. Drug load, however, did not have any significant impact on the release of formulations containing 10% and 20% CTZ (evident from 'f_2' values presented in Table 3-3) since the crystalline CTZ was completely converted into an amorphous form in 10% and 20% drug loaded extrudates.

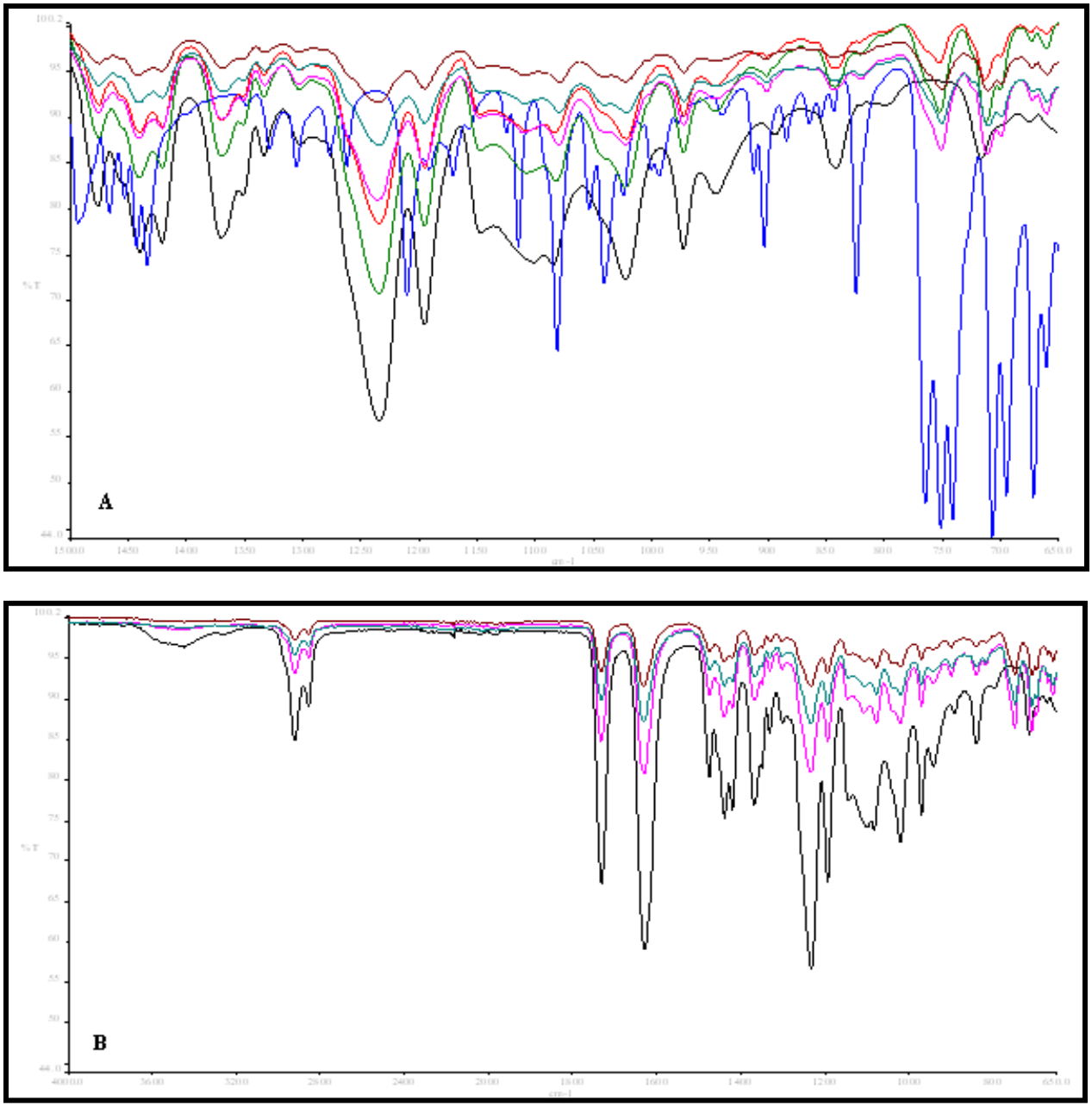


Figure 3-4: FTIR Spectra of Clotrimazole, Soluplus®, and Melt Extruded Formulations: (A) Effect of Drug Load; (B) Effect of Residence Time. The Colored Spectra are Represented as Follows: Black - Soluplus®; Blue – Clotrimazole (CTZ); Red – 10% CTZ HME; Green – 20% CTZ HME; Maroon – 30% CTZ-1min; Pink – 30% CTZ-5min; and Cyan – 30% CTZ-10min

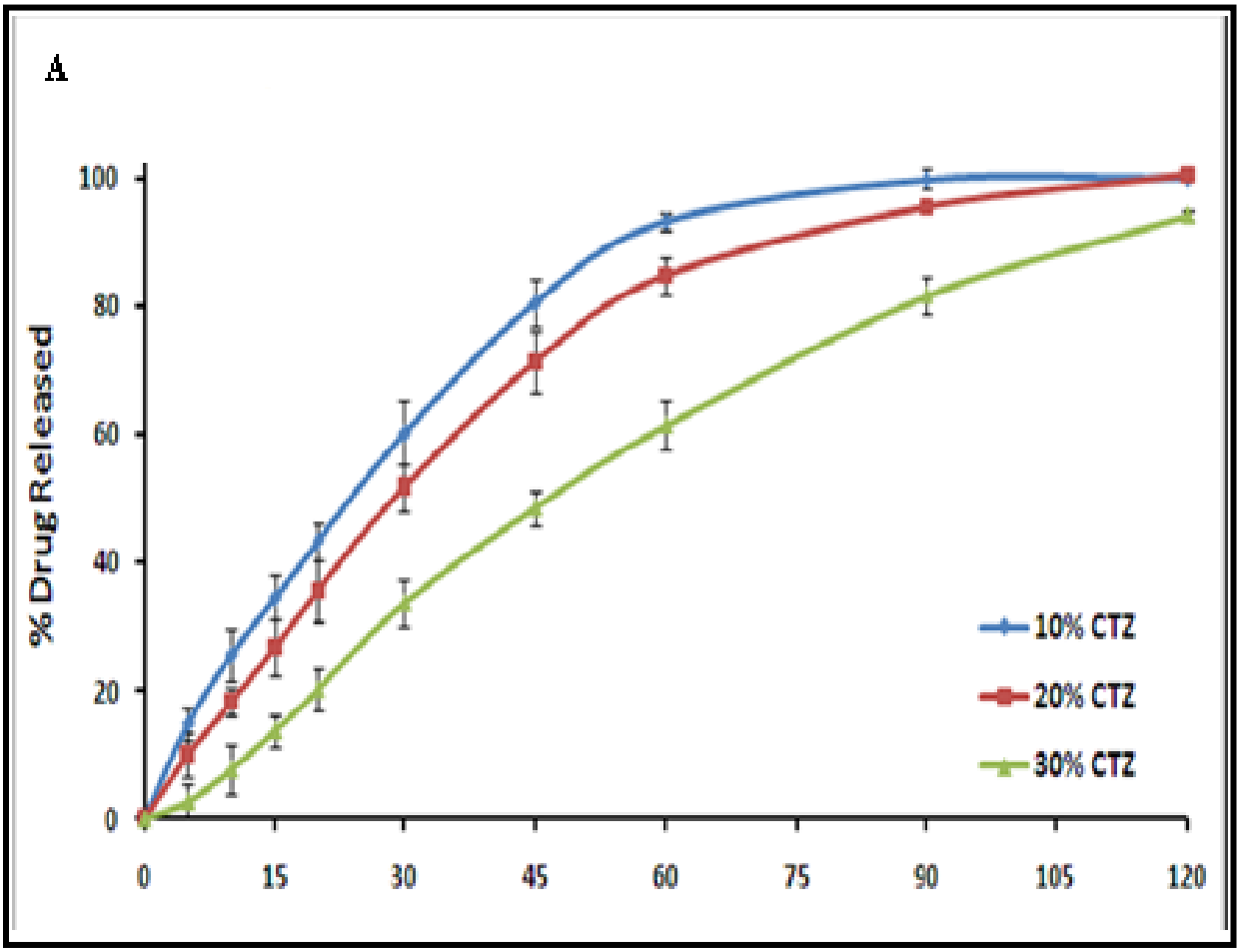
Table 3-3: Comparison of Dissolution Profiles Utilizing Calculated 'Similarity Factor' Values Between Different Melt Extruded Formulations

Formulations	'f_2' factor
Direct Flush - Drug Load Effect	
10%CTZ Vs 20%CTZ	56.1
10%CTZ Vs 30%CTZ	31.2
20%CTZ Vs 30%CTZ	39.2
30% CTZ - Residence Time Effect	
CTZ 1min Vs CTZ 5 min	55.6
CTZ 1min Vs CTZ 10 min	29.1
CTZ 5min Vs CTZ 10 min	36.2

Alternatively, the 30% drug loaded extrudates demonstrated a significantly slower release rate compared to 10% and 20% w/w drug loaded extrudates which can be attributed to the presence of crystalline drug in the higher drug loaded extrudates (**Figure 3-5A**). Moreover, the extrudates with 30% w/w drug load, when exposed at 130°C for 10 min in extrusion barrel did demonstrate a significantly faster release compared to the other formulations extruded after 1 and 5 min exposure times at 130°C (**Figure 3-5B**). This is due to the formation of amorphous CTZ in the extrudates produced (data supported by DSC), when exposed to the set temperature for a longer duration (10 min).

Stability is another important aspect of any formulation in the drug development cycle. In the current study, produced melt extruded formulations were subjected to both, physical (**Figure**

3-6A) and chemical stability (**Figure 3-6B**). The samples stored at three different temperatures were tested for crystallization of drug and found that the extruded formulations which formed amorphous dispersions initially have remained same and did not demonstrate any sign of re-crystallization during the storage period of three months. Similarly, the drug content of the initially formed extrudates remained between 96-103% at the end of three months, indicating chemical stability of the drug.



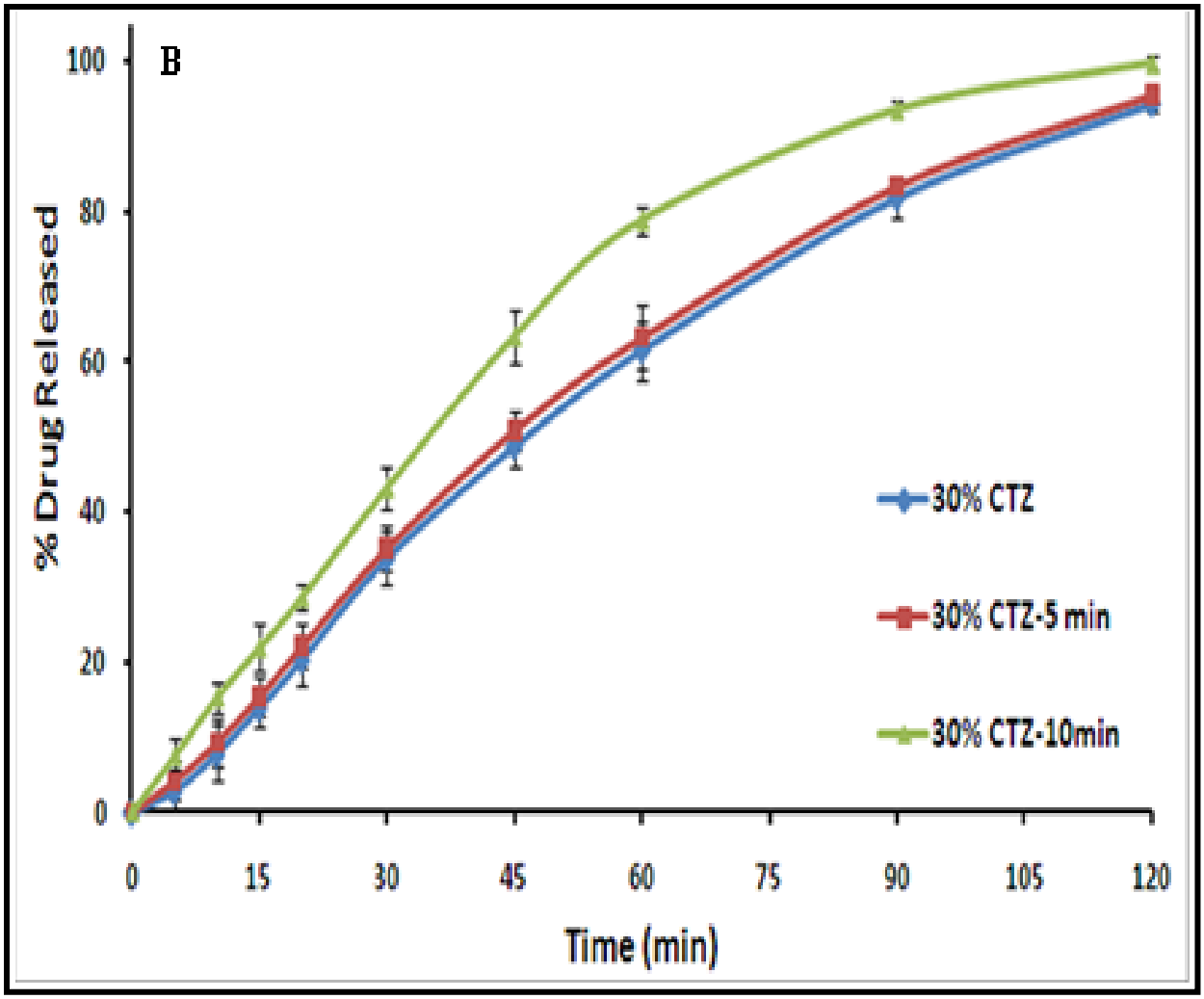


Figure 3-5: Clotrimazole Release from Hot Melt Extruded Formulations: (A) Effect of Drug Load; (B) Effect of Residence Time. Data Represent Mean ± S.D (n=3)

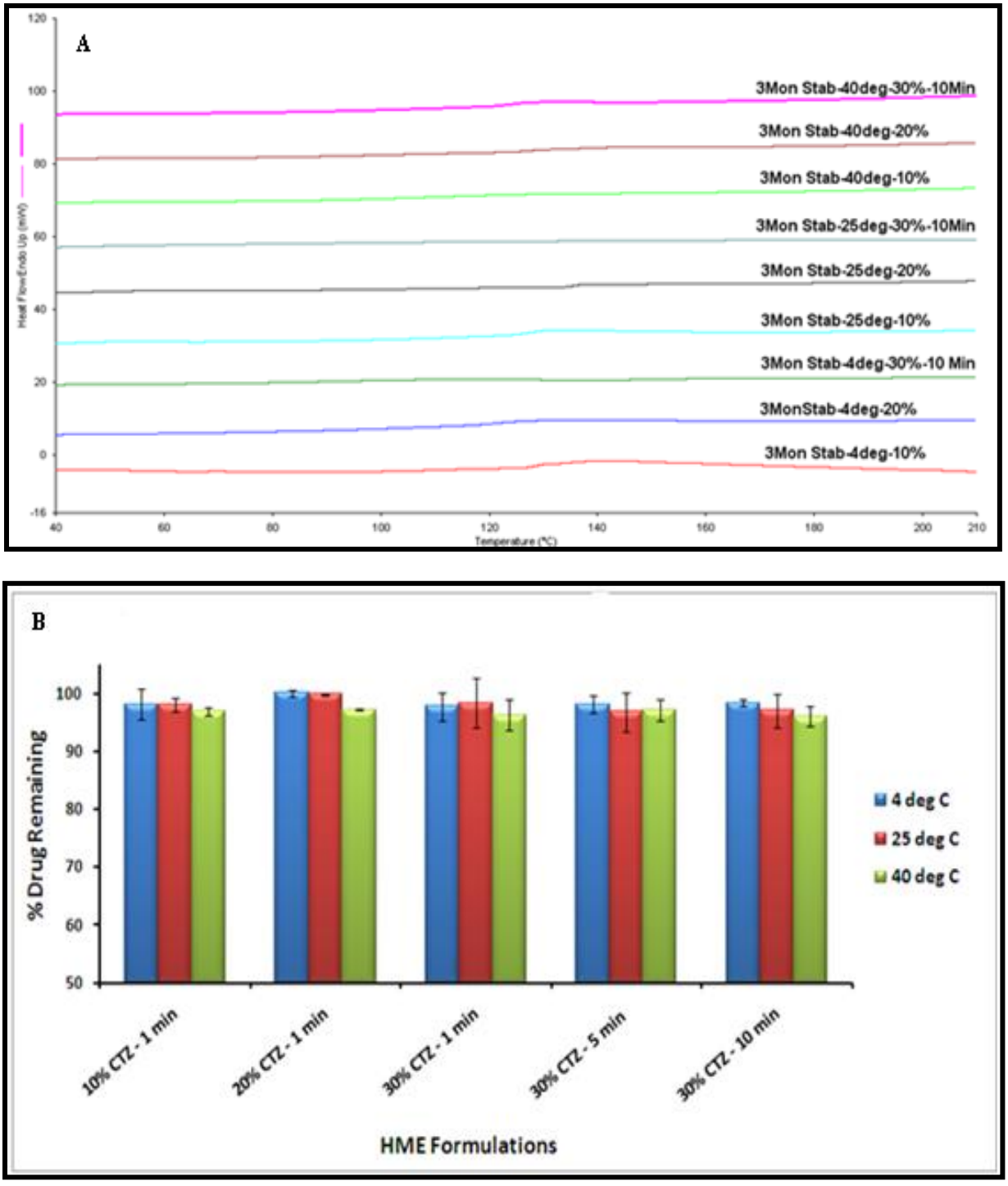


Figure 3-6: (A) Physical Stability Obtained from DSC Thermograms and; (B) Chemical Stability Obtained Utilizing HPLC Analysis of the Clotrimazole-Soluplus® Extrudates. Chemical Stability Data Represent Mean ± S.D (n=3)

3.4.Conclusion

CTZ was found to be miscible in Soluplus® up to 20% and 30% when processed for 1 min and 10min, respectively. Both formulation and process parameters, namely drug load and residence time of the binary mixture had a significant impact on the drug-polymer miscibility in the final extrudates which was confirmed by DSC and FTIR Studies. In addition, both, the drug load and residence time also had a significant impact on the CTZ release from the melt extrudates. At a relatively lower drug loads, complete solubilization of CTZ in Soluplus® was noticed. At the higher drug concentrations of CTZ (30% w/w drug load), an increase in the residence time of the formulation in the extrusion barrel aids in CTZ solubilization. The hydrophilic polymer, soluplus®, also combines the benefits of solid solutions and solubilization to further increase the dissolution rate of poorly soluble drugs. CTZ forms an amorphous solid dispersion within the soluplus® matrix, and the melt extrudates containing up to 30% drug load were found to be physically and chemically stable for a period of 3 months. Moreover, soluplus® was extrudable at 90% w/w with only a model drug in the formulation. The characteristics associated with this relatively new polymer such as hydrophilicity and low glass transition temperature, along with its ease of extrusion and additional solubilization capacity (due to polyethylene glycol) makes it more appealing for utilization as a carrier matrix with myriad actives in HME processing.

3.5.Acknowledgements

This project was supported by Grant# P20RR021929 from the National Center for Research Resources (NIH/NCRR). The authors would also like to thank BASF Corporation for its generous supply of Soluplus®.

CHAPTER - 4

Characterization of Soluplus® Hot Melt Extrudates: Effect of Formulation and Process Parameters on Miscibility, Dissolution and Stability Characteristics

4.1. Introduction

Curcumin is a safe, affordable and natural bioactive molecule of turmeric (Curcuma longa). It is a bright yellow colored natural product, practically insoluble in water at acidic and neutral pH, and soluble in alkali. This compound has gained considerable attention in recent years for its multiple pharmacological activities such as anti-inflammatory (49), anti-oxidant (50, 51), anti-microbial (52), anti-cancer (53), anti-diabetic (54), and as a neuroprotective agent (55, 56). In spite of its several potential therapeutic advantages, its optimum pharmaceutical potential has been limited by its lack of aqueous solubility and poor bioavailability (57, 58). To mitigate the above limitations research has been performed over the years to improve the solubility of curcumin utilizing nanocarriers, cyclodextrins (via complexation), carriers such as solutol® HS 15 (via solid dispersions) and surfactants (via micellar solubilization) (59-63).

Paradkar et al. has produced solid dispersion of curcumin and polyvinyl pyrrolidone utilizing spray drying technique (23). The authors demonstrated formation of a high energy amorphous phase and increased dissolution rate of curcumin from the produced solid dispersion when compared to their corresponding physical mixtures. In another study, Wu et al. utilized surfactants and appropriate solvents to produce self-microemulsifying drug delivery systems to

improve curcumin dissolution and bioavailability (64). Drug release from the formulation was completed within 10 minutes. The developed SMEDDS formulation improved the oral bioavailability of curcumin significantly, and the relative oral bioavailability of SMEDDS compared with curcumin suspension was 1213%. Recently, Bansal and co-workers produced curcumin implants using poly(ε-caprolactone) and HME technique (65). The data from the study showed that these implants were able to release curcumin for long duration and to modulate liver phase I and phase II enzymes, demonstrating curcumin's biological efficacy delivered via this delivery system.

Numerous research reports have been published on the use of hydrophilic polymers in producing solid dispersions to enhance dissolution rate of poorly soluble drugs. Soluplus® is one of the novel polymers comprising of PEG 6000, vinylcaprolactam and vinyl acetate in the ratios of 13, 57, and 30, respectively. It is white yellowish free flowing granules with a glass transition temperature of 69°C. When extruded, it combines the benefits of both solubilization and formation of solid solutions to enhance the bioavailability of poorly soluble drugs (66). Surfactants, on the other hand, are also widely used solubilizing agents in many pharmaceutical dosage forms. These surfactants when used at appropriate levels form the micellar systems in which the poorly soluble drugs are encapsulated and form a more soluble system. In this study, we chose three different types of surfactants mainly classified based on their charge: cationic (Cetyltrimethyl ammonium bromide-CTAB), anionic (Sodium lauryl sulfate-SLS) and non-ionic (Pluronic® F127) surfactants. Additionally, each of these surfactants was evaluated at three different concentrations to study their influence on drug release from the produced hot-melt extrudates.

Currently there are no available marketed formulations for curcumin as it exhibits solubility-limited poor oral bioavailability. In addition, there is no research reported utilizing combination of both, surfactants and HME. Therefore, in our current research, we attempted to formulate a solubilized curcumin oral dosage form with enhanced dissolution characteristics, and a high potential to demonstrate superior bioavailability utilizing a novel carrier, soluplus®, suitable surface active agents, and HME technology. The objective of this study was to investigate the feasibility of producing Soluplus®-Curcumin drug-loaded extrudates utilizing hot-melt extrusion (HME) technology. In addition, drug-polymer miscibility studies, including the influence of charge and concentration of the utilized surfactants on dissolution rate of this poorly water-soluble model drug were also investigated.

4.2. Materials & Methods

4.2.1. Materials

Curcumin, CTAB, SLS, and Pluronic® F127 were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Soluplus® was kindly gifted by BASF Corporation. High performance liquid chromatography (HPLC) grade water was freshly prepared in the laboratory by Nanopure systems (Barnstead, Dubuque, IA, USA). All other solvents utilized in the study were of analytical grade and obtained from Fisher Scientific (Fair Lawn, NJ, USA).

4.2.2. Analytical Method

In-house reverse phase high performance liquid chromatography (HPLC) based analytical method was developed for determination/quantification of curcumin, and validated according to ICH and FDA guidelines for chromatographic method. An HPLC equipped with UV detector, Waters symmetry shield 5µ C18 column (250×4.6 mm), and an isocratic mode of

elution with mobile phase containing acetonitrile and 2% acetic acid solution in water (48:52) at a flow rate of 2.0 mL/min were employed to quantify the drug at a wavelength (λmax) of 425 nm. The pH of the mobile phase is adjusted to 3.9, and the column temperature was maintained around 33°C. The acquired data was processed using Empower 2 build 2154 software (Waters Inc., Mount Holly, NJ, USA).

4.2.3. Thermal Gravimetric Analysis (TGA)

The thermal stability of the curcumin, and soluplus® samples was determined as a function of weight loss. The analysis was performed on the samples (4-5 mg approximately) using a Perkin-Elmer Pyris 1 thermo-gravimetric analyzer (Norwalk, CT) operated at a ramp rate of 20°C/min from a temperature of 40°C to 250°C. The % weight loss for both the samples tested was recorded using the Pyris 1 TGA software. All of the TGA runs were performed in an open pan with purge and protective nitrogen gas flow at 40 mL/min.

4.2.4. Differential Scanning Calorimetry (DSC) Analysis

DSC was used to characterize the miscibility of curcumin in a polymeric carrier, soluplus®, utilizing a Perkin-Elmer Diamond DSC instrument (Shelton, CT). A 2-3 mg sample of the drug, polymer, and physical mixtures with varying drug concentrations (10, 20, 30, 40, and 50%) were weighed and sealed in aluminum crimped pans (Kit 0219-0062, Perkin-Elmer Instruments, Shelton, CT). The physical mixture containing samples were subjected to an initial heat-cool cycle (by heating up to 230°C at the rate of 20°C/min, followed by immediate cooling) to remove the thermal history of the samples. A second heat cycle was initiated wherein the samples were reheated from 30 to 250°C at a ramp rate of 20°C/min under nitrogen purge at a flow rate of 20 mL/min. On the other hand, the melt extruded samples containing surfactants at

varying concentrations were subjected to a single heating cycle from 30 to 250°C at a ramp rate of 20°C/min.

4.2.5. Hot Melt Extrusion

Curcumin and soluplus® were used as a model drug and carrier matrix, respectively. Prior to processing, required materials previously sieved through mesh # 40 were accurately weighed and were further blended in a twin-shell dry V-blender (The Patterson-Kelly co., Inc. East Stroudsburg, PA) at a speed of 25rpm for 10 minutes. Each of the formulations contained 30% Curcumin (99% purity), Soluplus® (60 – 67.5%), and a surfactant (SLS, CTAB, and/or Pluronic® F127), each at three different concentrations - 2.5%, 5%, and 10% w/w. These formulations were extruded utilizing a conical twin-screw compounder (MiniLab II HAAKE Rheomex CTW5, ThermoFisher Scientific) at 155°C and 70rpm screw speed. All of these final extrudates obtained were stored in foil-lined polyethylene bags and stored in a refrigerator until further analysis.

4.2.5.1.Post-Processing Drug Content

A portion of all of these extruded formulations were crushed into fine powder using mortar and pestle and stored in amber colored glass bottles. These processed and powdered extrudates were analyzed for the drug content immediately after extrusion through the hot melt extruder. A known amount (20 mg) of the extrudates and physical mixture were dissolved in 100mL of acetonitrile and filtered using 0.2 µm, 13 mm PTFE membrane filters (Whatman, Piscataway, NJ) and analyzed utilizing a HPLC at a wavelength of 425 nm.

4.2.6. Drug Release Testing

Dissolution testing (USP XXXI, Apparatus II) was carried out utilizing a Hanson SR8-plus™ dissolution test station (Hanson Research Corporation, Chatsworth, CA) operated at 75

rpm paddle speed. The effect of surfactant type and concentration on drug release from extrudates containing 30% curcumin was studied. A sample weight containing curcumin equivalent to 100mg from each of the produced melt extruded formulations, previously ground in mortar and pestle, was accurately weighed and filled in gelatin capsule # 1. These capsules were added to the dissolution vessel containing 900mL of 0.5% w/w of SLS preheated to 37°C. During testing 1.5mL samples were removed from the dissolution vessels at pre-determined time intervals and replaced with an equal volume of fresh dissolution medium. The extruded formulation containing no surfactant, and the drug release from pure curcumin powder were utilized as controls. All of the samples were immediately filtered using 13 mm PTFE membrane filters (Whatman, Piscataway, NJ) with a pore size of 0.2 µm and analyzed using HPLC at a λmax of 425nm. Drug concentration was calculated from a standard calibration plot and expressed as cumulative % drug dissolved. The drug release from all of the melt extrudates and the pure drug were compared. The release studies were performed in triplicate and the mean values were compared.

4.3. Results & Discussion

The TGA studies (data not shown) revealed that Soluplus® and Curcumin were stable under employed extrusion temperatures. Hence, the formulations under study were extruded utilizing a conical twin-screw compounder (MiniLab II HAAKE Rheomex CTW5, ThermoFisher Scientific) at 155°C and 70rpm screw speed. Each of the formulations contained 30% curcumin (99% purity), soluplus® (60 – 67.5%), and a surfactant (SLS, CTAB, and/or Pluronic® F127), each at three different concentrations - 2.5%, 5%, and 10% w/w. The post-processing content results are presented in **Figure 4-1**. All of the produced formulations under

employed extrusion conditions exhibited a higher post-processing drug content (97-100%) with tighter standard deviation (<3%), indicating a robust formulation and process.

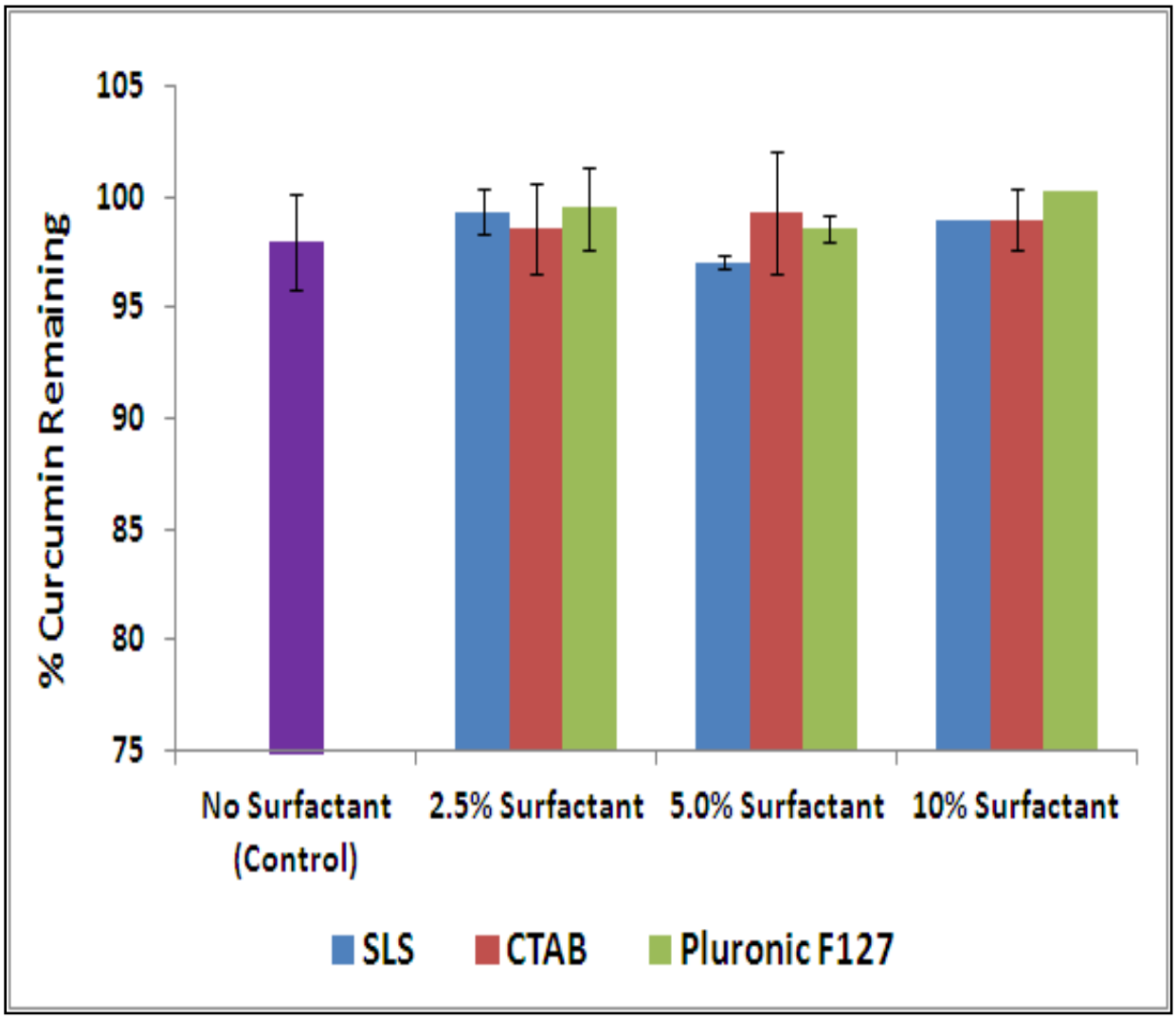
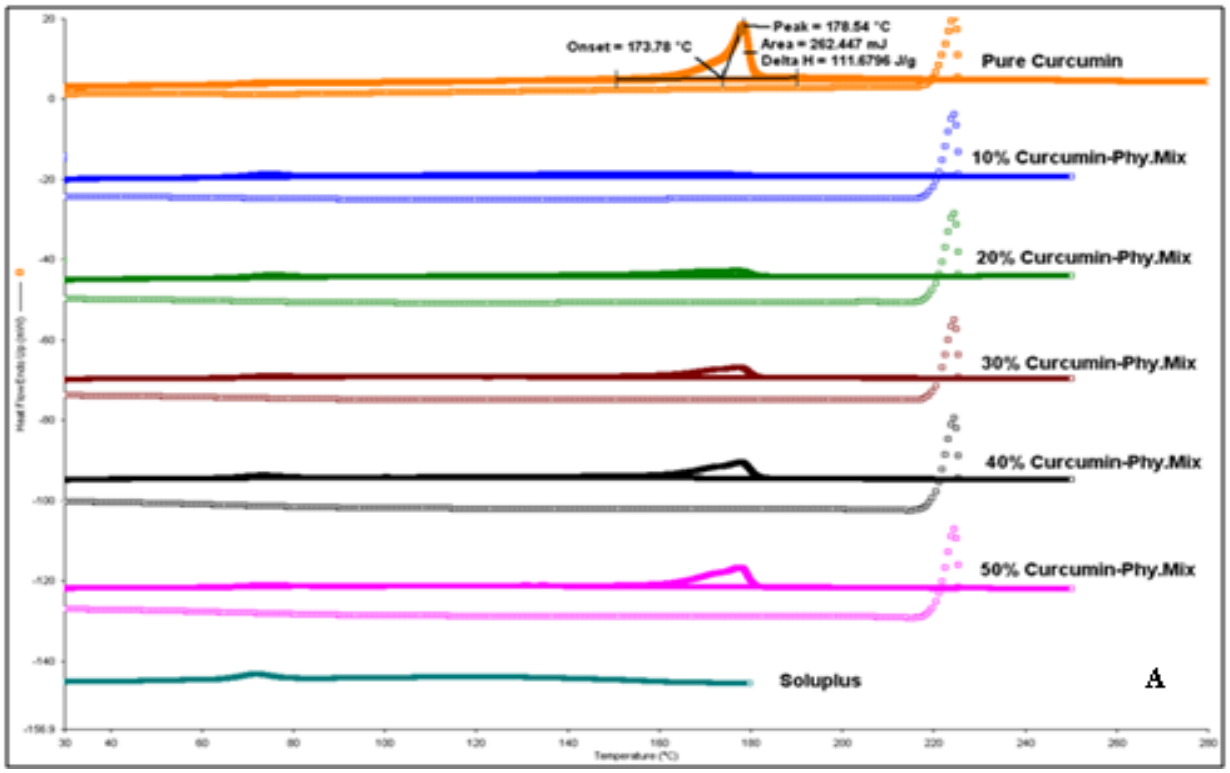
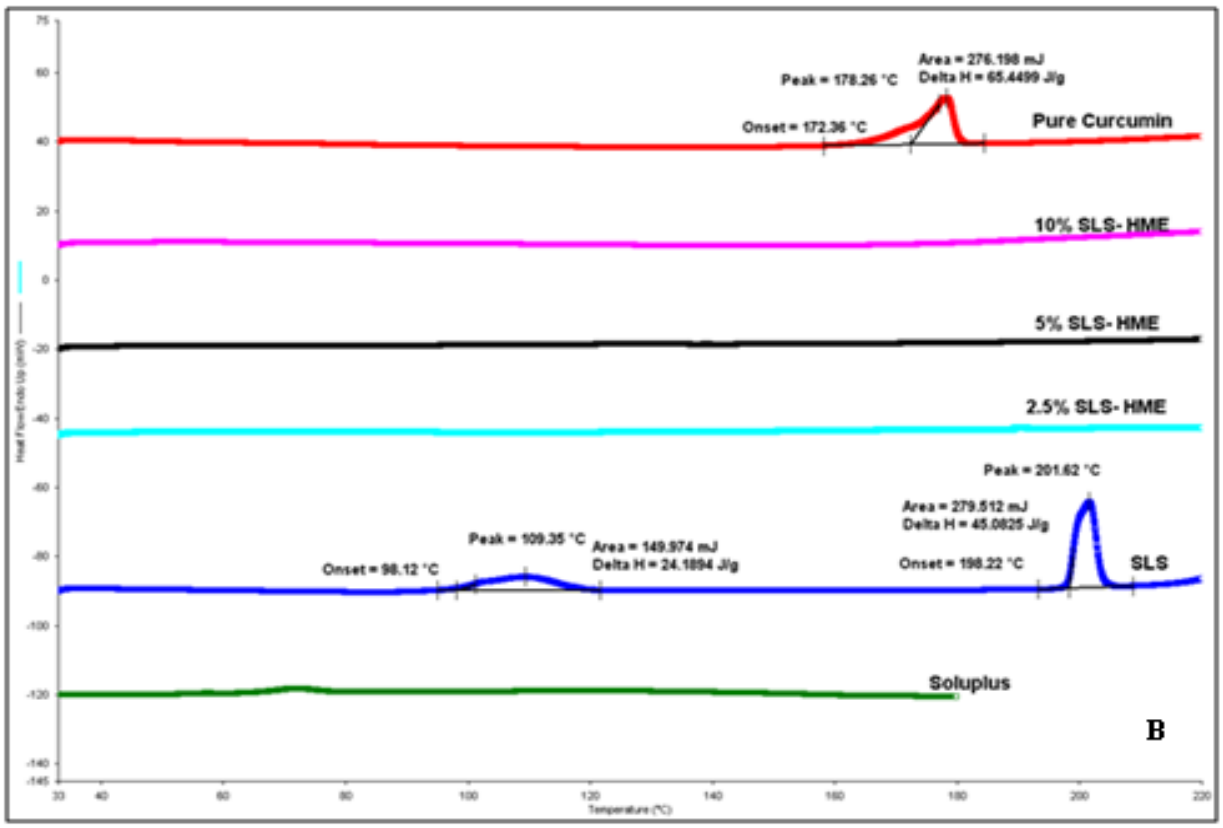


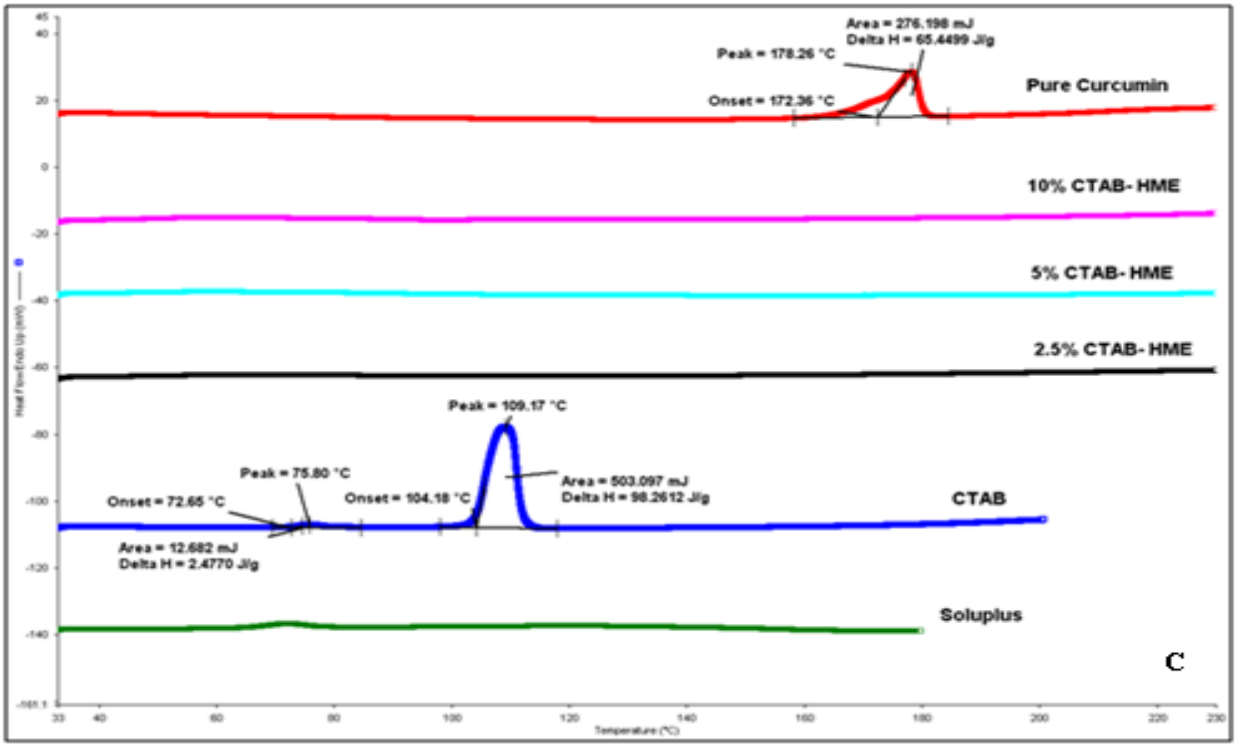
Figure 4-1: Post-Processing Drug Content of Curcumin Melt Extrudates

The DSC studies revealed that Curcumin exhibited an onset of melting endotherm at around 172°C. The drug's melting peak was visible in the physical mixtures (up to 50% w/w of drug) during the first heating cycle (**Figure 4-2A**), which disappeared following the second heating cycle. However, all of the produced extrudates did not demonstrate any melting

endotherm corresponding to curcumin, polymer, and/or the surfactants employed even during the first heating cycle. The thermograms of all of the extruded formulations (**Figures 4-2B to 4-2D**) demonstrated a single phase system, indicating an excellent miscibility of 30% Curcumin in the Soluplus® matrix.




Area = 276.198 mJ
Delta H = 65.4499 J/g
Peak = 178.26 °C
Onset = 172.36 °C
Pure Curcumin
10% SLS- HME
5% SLS- HME
2.5% SLS- HME
Peak = 201.62 °C
Area = 279.512 mJ
Delta H = 45.0825 J/g
Peak = 109.35 °C
Area = 149.974 mJ
Delta H = 24.1894 J/g
Onset = 98.12 °C
Onset = 198.22 °C
SLS
Soluplus
B


Area = 276.198 mJ
Delta H = 65.4499 J/g
Peak = 178.26 °C
Onset = 172.36 °C
Pure Curcumin
10% CTAB- HME
5% CTAB- HME
2.5% CTAB- HME
Peak = 109.17 °C
Peak = 75.80 °C
Onset = 72.65 °C
Onset = 104.18 °C
Area = 503.097 mJ
Delta H = 98.2612 J/g
CTAB
Area = 12.682 mJ
Delta H = 2.4770 J/g
Soluplus
C

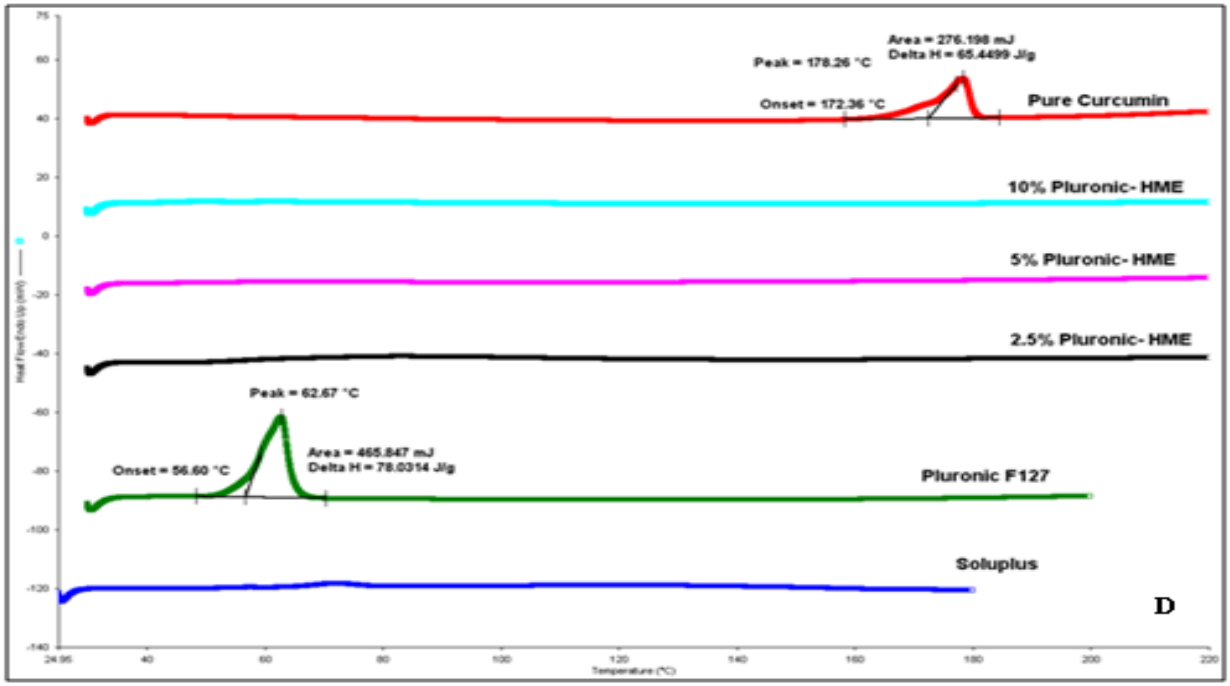


Figure 4-2: Curcumin-Soluplus® Miscibility Studies – (A) Comparative Study of the Drug-Polymer Physical Mixtures with Increased Drug Loading; (B) SLS HME Formulations with 30% Curcumin; (C) CTAB HME Formulations with 30% Curcumin; and (D) Pluronic® F127 HME Formulations with 30% Curcumin

It is also anticipated that the formed high-energy amorphous systems during extrusion would enhance the drug release from the extrudates. However, the impact of utilized surfactants in the formulation on the release of curcumin was not clearly understood. Therefore, drug release profiles obtained from all of the melt extrudates and the pure drug were compared. The interaction of curcumin with various charged aqueous surfactant solutions showed it exists in deprotonated enol form in surfactant solutions. The nitro and hydroxyl groups of o-nitrophenol interact with the carbonyl and hydroxyl groups of the enol form of curcumin by forming ground state complex through hydrogen bonds and offered interesting information about the nature of

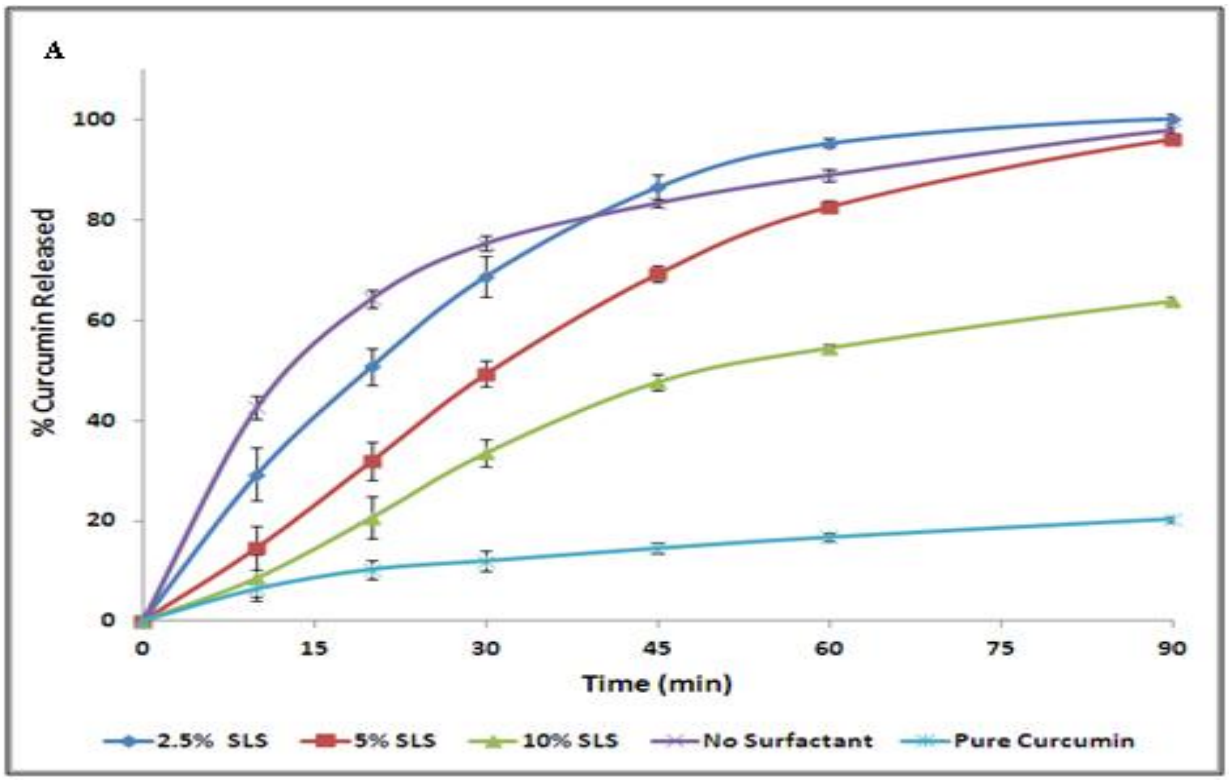
the interactions between the aqueous surfactant solutions and curcumin depending on charge of head group of the surfactant.

All of the extrudates released significantly higher amounts of curcumin (~64-100%) compared to that of the pure drug (~20%). Moreover, effect of charged surfactants and their concentrations on the release of curcumin was predominant. Increase in the SLS concentration from 2.5% to 10% decreased the drug release from 95.4% to 54.5% after 60 minutes (**Figure 4-3A**). This decrease in drug release could be attributed to the hydrolytic degradation of Curcumin in the alkaline pH (8.5-9.0) of dissolution medium (67). It has been reported that the Curcumin undergoes deprotonation in alkaline pH and exists as a negatively charged molecule (Cur^{3-}). The SLS micelles cannot prevent the degradation of the compound due to the electrostatic repulsions between the negatively charged head groups and Cur^{3-}, resulting in the dissociation of Cur^{3-} from the micelle followed by its alkaline hydrolysis in the aqueous phase of the solution **(68)**.

On the contrary, increased drug release was noticed with increasing concentration of CTAB from 2.5% to 10% (**Figure 4-3B**). This could be attributed to the improved stability of Curcumin due to its interaction with CTAB molecules. The cationic surfactants stabilize deprotonated Curcumin, Cur^{3-}, due to the attractive electrostatic interactions between the ionized drug and the positively charged CTAB head groups. No significant improvement in the drug dissolution was observed with increasing concentrations of non-ionic surfactant, Pluronic F127, when compared to the control formulation containing no surfactant, but only curcumin and soluplus® (**Figure 4-3C**).

All of these formulations were stored in accelerated conditions (40°C/75% RH) to study their physical stability. These stored formulations were subjected to DSC analysis at the end of

three months period and none of them exhibited recrystallization of curcumin in the extrudates (data not presented), demonstrating the physical stability of the formulations.



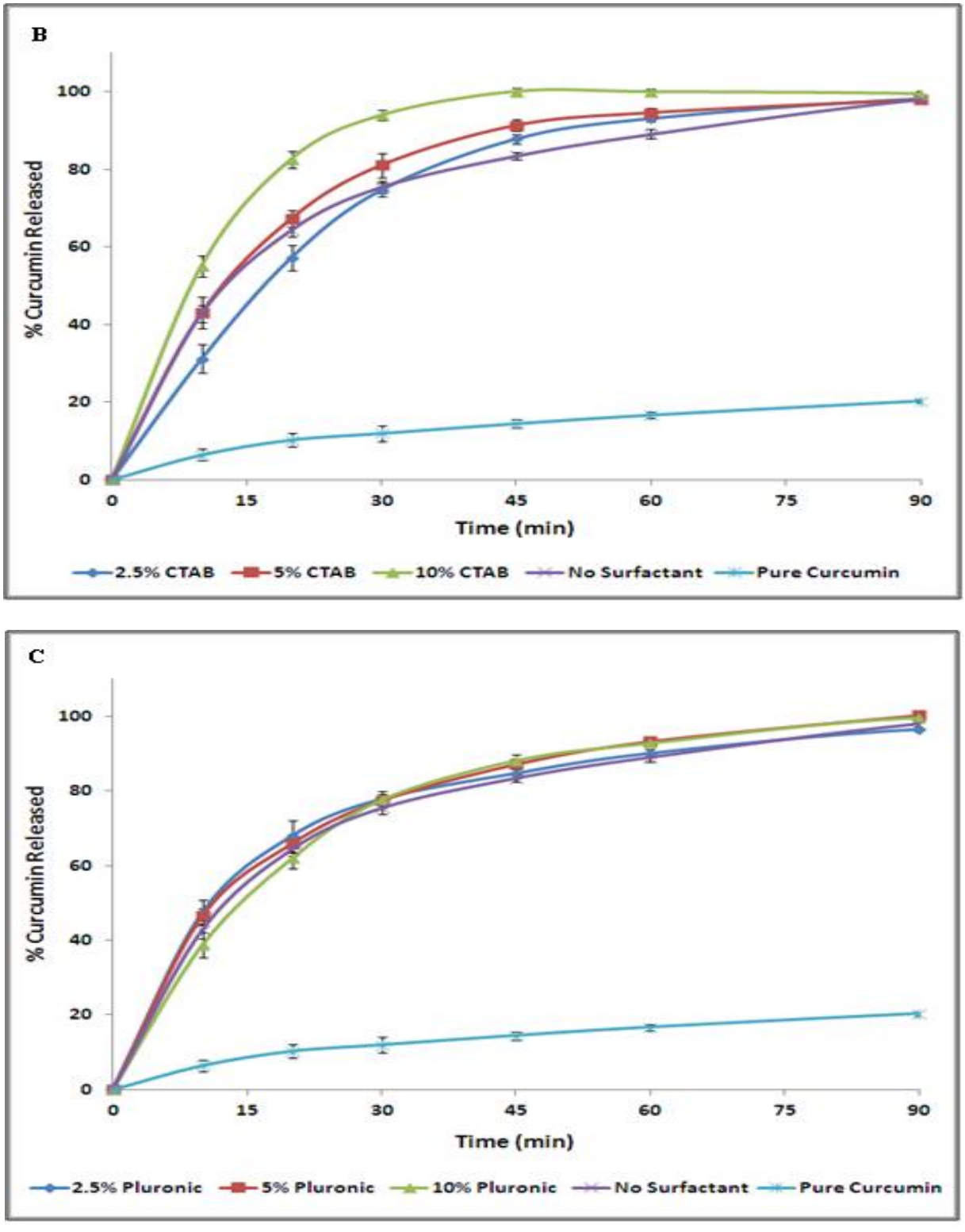


Figure 4-3: Effect of Surfactant Concentrations on Curcumin Dissolution from Hot-Melt

Extrudates – (A) SLS; (B) CTAB; (C) Pluronic F127

4.4. Conclusions

Curcumin was found to be miscible in soluplus® up to 50% drug load. Both, charge and the concentration of surfactant had a significant impact on the drug release from the HME extrudates. HME has been successfully utilized in conjunction with Soluplus®, and other functional excipients to improve the physical stability and the drug release. Soluplus® may provide a solution to increase solubility of poorly soluble drugs.

4.5. Acknowledgements

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CHAPTER - 5

Summary and Conclusions

5.1. Hot Melt Extrusion: A Promising Technique to Produce a Novel Abuse-Deterrent Formulation

- HME was demonstrated as a viable technique with a potential to develop novel abuse-deterrent formulations
- PEO 301 demonstrated its utility as a feasible TR matrix
- HPMC K15M in combination with CBP significantly improved the gelling characteristics in water and alcohol
- The optimized response models created for '%API extracted in water' and '% API extracted in alcohol' also correlated well with the data generated, validating the developed model.

5.2. Characterization of Kollidon® VA 64 Hot Melt Extrudates: Influence of Formulation and Process Parameters on Dissolution and Stability Characteristics

- Kollidon VA® 64 was extrudable at 75% w/w with only a model drug in the formulation.
- Both of the processing parameters, temperature and screw speed, had a significant impact on the post-processing drug content of the final extrudates.
- NIF forms a stable amorphous solid dispersion in Kollidon VA® 64 matrix.
- Drug release from all of the hot melt extrudates produced was similar. Moreover, release from the extrudates was found to be superior compared to the physical mixtures.

- NIF was found to be physically and chemically stable throughout the study period.

5.3. Characterization of Soluplus® Hot Melt Extrudates: Effect of Formulation and Process Parameters on Miscibility, Dissolution and Stability Characteristics

- At 10% and 20% drug load, there is complete solubilization of CTZ in Soluplus®. However, at higher drug loading of CTZ (30% drug load), the increase in residence time of the formulation in the extrusion barrel helps aid solubilization of CTZ.
- Drug load and heat exposure/residence time had a significant impact on the drug-polymer miscibility in the final extrudates, which was confirmed by DSC and FTIR Studies.
- Both, the drug load and residence time had a significant impact on the CTZ release from the melt extrudates.
- CTZ forms an amorphous solid dispersion within the Soluplus® matrix, and the melt extrudates containing up to 30% drug load were found to be physically and chemically stable for a period of 3 months.
- Moreover, Soluplus® was extrudable at 90% w/w with only a model drug in the formulation.
- Soluplus® combines the benefits of solid solutions and solubilization which thereby increases solubility of poorly soluble drugs.

5.4. Influence of Hot Melt Extrusion Technology, and Effect of Type and Concentration of Surfactants on Miscibility and Drug Release from Soluplus®-Curcumin Mixtures

- HME has been successfully utilized in conjunction with Soluplus®, and other functional excipients to improve the physical stability and the drug release
- Curcumin formed a single phase miscible system in soluplus® up to 50% drug load.

- Both, charge and the concentration of surfactant had a significant impact on the drug release from the HME extrudates.
- Extrudates were found to be physically stable throughout the storage period of three months under accelerated storage conditions.
- Soluplus®, in combination with appropriate surfactants may provide a solution to increase solubility and stability of the poorly soluble drugs.

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VITA

Sindhuri Maddineni, proud daughter of Mrs. Sandhya Maddineni and Mr. Krishnaiah Maddineni, was born in Andhra Pradesh, India on October 9, 1986. She attended Goutham Junior college in Vijayawada and she excelled in the state level competitive examination to get an entry in to one of the top universities of the state, University College of Pharmaceutical Sciences, Kakatiya University, India to pursue her Bachelors in Pharmacy. She graduated with a first class distinction in November 2007.

Immediately after completion of her Bachelors Program, she joined The University of Mississippi to earn her Doctoral Degree in Pharmaceutics. While continuing her education at the University of Mississippi, she also completed her internship in 2011at Abbott Pharmaceuticals (Waukegan, IL). Sindhuri gained vast knowledge in pre-formulation aspects of solid dosage forms, including the powder flow characterization by developing a predictive mathematical model during her internship.

Sindhu is a member of Honor Societies of Phi Kappa Phi, Sigma Xi, and Rho-Chi National Scholars Honorary Society. She is also a recipient of several awards including "Graduate Dissertation Fellowship" (University of Mississippi-2011), "Center of Research Excellence in Natural Products Neuroscience Fellowship" (funded by NIH for the year 2010 - 2011), "Graduate Travel Award" (Kakatiya University UCPS Alumni Association-USA chapter-2010 & 2011) and "Best Poster Presentation Award" (Southern Regional Discussion Group

(SRDG, Memphis–2011). As a graduate student, she served as the Senator for the Department of Pharmaceutics in Graduate Student Council (2010-2011), and Secretary (2010-2011) for the AAPS-University of Mississippi Student Chapter. She also served as a Graduate Teaching Assistant for Physical Pharmacy and Basic Pharmaceutics course (August 2008 – December 2009). Sindhu received the Doctor of Philosophy degree in Pharmaceutics in April 2013.