UNIVERSITY OF NAIROBI

DEPARTMENT OF PHARMACEUTICS AND PHARMACY PRACTICE

FORMULATION DEVELOPMENT OF SUSTAINED RELEASE ZIDOVUDINE-LAMIVUDINE FIXED DOSE COMBINATION PAEDIATRIC MINI MATRICES BY HOT MELT EXTRUSION

BY
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A Thesis submitted in the fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmaceutics of the University of Nairobi.

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DECLARATION

This thesis is my original work and has not been presented in any other institution for degree award or other qualification.

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DEDICATION

This piece of work is dedicated to my parents Mr. Mahindra Maru & Mrs Nalini Maru, my sisters, and my loving son Aryan

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PUBLICATIONS AND POSTER PRESENTATION AT CONFERENCES

- Shital M. Maru, Marcel De Matas, Adrian Kelly, Anant Paradkar, K.A.M. Kuria, I.O. Kibwage, G.O.Kokwaro. Characterization of Hot Melt Extruded Sustained Release Mini Matrices of Zidovudine and Lamivudine with Kollidon®SR. Accepted for poster presentation at AAPS Annual meeting and Exposition 2012, Chicago, USA. Poster # W5041.

- Shital M.Maru, Marcel DeMatas, Adrian Kelly, Anant Paradkar. Characterization of rheological and thermal properties of Zidovudine, Lamivudine and plastic blends with Ethylcellulose to assess their suitability for hot melt extrusion. European Journal of Pharmaceutical Sciences. 44 (2011) 471-478.

- Shital M.Maru, Marcel DeMatas, Adrian Kelly, Anant Paradkar. Development of sustained release mini matrices of zidovudine and lamivudine with ethylcellulose and PEO by hot melt extrusion. AAPS PharmSci Suppl. (2010) 12 (S2) SA 8121, T.2192. PWSC and AAPS Annual Meeting and Exposition 2010, New Orleans, USA.

- Shital M.Maru, Marcel DeMatas, Adrian Kelly, Anant Paradkar. Study of thermal and rheological properties of Kollidon SR®, Zidovudine, Lamivudine blends to assess their suitability for hot melt extrusion. AAPS PharmSci. Suppl. (2010) 12 (S2) W4195, SA8126. PWSC and AAPS Annual Meeting and Exposition 2010, New Orleans, USA.

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ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
API	Active pharmaceutical ingredient
ART	Antiretroviral therapy
ARVs	Antiretrovirals
AZT	Zidovudine
AZT-TP	Zidovudine triphospate
3TC	Lamivudine
Cmax	Maximum concentration
CD_4 count	Measurement of how many functional CD4 T-cells are circulating in the blood
C- t profile	*In- vivo* blood drug concentration over time profile
DSC	Differential Scanning Calorimetry
DNA	Dinucleic acid
D4T	Stavudine
DTZ	Diltiazam
EFV	Efavirenz
EMA	European Medicine Agency
Ethocel	Ethylcellulose
FDC	Fixed dose combination
FDA	Food and Drug Administration
FTC	Emticitabine
FT-IR	Fourier transform infrared spectroscopy

G´	Elastic modulus or storage modulus
G˝	Viscous modulus or loss modulus
GIT	Gastro intestinal tract
GSM	Gylceryl monostearate
HAART	Highly Active Antiretroviral Treatment
HDPE	High density polyethylene
HIV	Human Immunodeficiency Virus
HME	Hot melt extrusion
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxy propyl methyl cellulose
ID_{50}	Median infective dose
IUC	Information unit for conventions
LPV	Lopinavir
L/D	Length (L) of the screw divided by the diameter (D)
MEC	Minimum Effective Concentration
MIC	Minimum Inhibitory Concentration
min	Minute
ml	Millilitre
MTCT	Mother to child transmission
n	Number
NFV	Nelfinavir
NIR	Near infrared
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitors

NUCA 3001	North American randomized, double blind on clinical trial protocol comparing safety and efficacy
NVP	Nevirapine
PAT	Process analytical technology
PEG	Polyethylene Glycol
PEO	Polyethylene Oxide
pH	Log (base 10) of hydrogen ion concentration
PIs	Protease inhibitors
PVP	Polyvinyl pyrollidone
PVP/VA	Polyvinyl pyrollidone/Vinyl acetate
QbD	Quality by design
µg/l	Micrograms per litre
UNAIDS	United Nations programs for AIDS
US FDA	United States Food and Drug Agency
UNICEF	United Nations Children's Fund
USP	United States Pharmacopeia
S.D	Standard Deviation
SIV	Simian Immunodeficiency Virus
RNA	Ribonucleic acid
RPM	Rotation per Minute
RSD	Relative standard deviation
RTV	Retonavir
TEC	Triethylcitrate
Tg	Glass transition temperature

Tg_{mix}	Glass transition temperature of mixture
TGA	Thermo gravimetric analysis
Tm	Melting temperature
Tmax	Time taken to reach maximum concentration
TP	Triphosphate
VA	Vinyl acetate
WHO	World Health Organization
XRPD	X-Ray Powder Diffraction

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ABSTRACT

HIV/AIDS has become one of the major and deadliest pandemics in the world today. Besides concerns about cost and accessibility, a major concern for current therapies in most developing countries is the lack of age appropriate antiretroviral formulations which might expose children to “homemade” formulations with significant risks of variable dosing, poor and erratic absorption and inappropriate pharmacokinetic profiles. In this light and with the several campaigns driven by the World Health Organisation (WHO), UNICEF and the European Medicines Agency (EMA) in mind, the potential for developing alternative presentations for paediatric use has been raised. Flexible options such as granules, pellets, mini- matrices which children are able to swallow or which can be dispersed on the tongue or in water have been identified as the presentations of choice in some of these WHO led discussions. It is therefore critical that feasibility assessments are undertaken to demonstrate the likelihood of formulating essential medicines in this manner. In this regard, two antiretroviral drugs have been identified for this study. Oral zidovudine (AZT) and lamivudine (3TC) are soluble drugs with short elimination half-lives and moderate bioavailability. Frequent high doses are therefore required to achieve and maintain therapeutic blood levels. As a result, dose-dependent toxic side effects are frequently observed. One way to avoid dose dependent side effects is by formulating the dosage forms as sustained release formulations intended to optimize a therapeutic regimen by providing slow and continuous drug delivery over the entire dosing giving reduced side effects, whilst also providing greater patient compliance and convenience. To have access and availability of paediatric fixed dose combination mini matrices at low cost, a fast, flexible and efficient manufacturing process which can be adapted by local manufacturers in HIV/AIDS endemic regions such as Sub-Saharan African is required. One such process that could be adapted and scaled up for production easily is hot melt extrusion (HME).

This study was therefore designed to investigate the physicochemical properties of zidovudine and lamivudine and their influence on interactions with matrix forming polymers such as ethylcellulose, Polyvinylpyrrolidone/Vinyl acetate (Kollidon® SR) and polyethylene oxide (PEO) during hot melt extrusion. Furthermore, mini-matrix formulations of fixed dose combinations of zidovudine and lamivudine were developed followed by evaluation of key quality attributes including *in-vitro* dissolution and accelerated stability studies. The dissolution data were used to make estimations of *in-vivo* performance using a method described in the literature.

The results showed that Zidovudine and Lamivudine were thermally stable and miscible (Van Krevelen's solubility parameter calculations) with polymers such as ethylcellulose, Kollidon® SR and PEO enabling extrusion by hot melt extrusion. Thermal and crystalline characteristics were studied using Differential Scanning Calorimetry (DSC) and X-ray powder diffraction studies (XRPD) which showed that the drugs were transformed from the crystalline to the amorphous state. Rheological studies showed that addition of Zidovudine decreased the melt viscosity with all the polymers while Lamivudine seemed to saturate the polymer at 40%w/w concentration at which point there was a marked increase in melt viscosity. Triethyl citrate (TEC) was used as plasticizer during hot melt extrusion, whilst polyethylene oxide (PEO) was added to modulate the drug release profile. For some formulation variants, extensive drug release (98%) was observed over a period of 24 hrs, particularly for mini-matrices of zidovidine and lamivudine formulated with Kollidon® SR. The release was related to the increases in percentage of plasticizer TEC which may have caused strong coalescence between the drug and polymer particles. Accelerated stability studies done using mini matrices in open and closed high density polyethylene (HDPE) bottles for a period of three month in a hot oven chamber at 40º C and 75% Relative Humidity (RH) as well as at 60ºC in

closed HDPE bottles for 15 days and the results showed that the both zidovudine and lamivudine did not recrystallized and the matrices were stable after 3 months. The data obtained from the predicted *in vivo* drug blood concentration profiles for ethylcellulose and Kollidon® SR formulations showed that both zidovudine and lamivudine not only sustained drug release for over 16 hrs but also maintained drug concentration within the effective drug concentrations (therapeutic window) over the same period which suggests that side effects caused by frequent dosing of the drugs could be avoided.

In conclusion, it is feasible to produce stable fixed dose combination sustained release mini matrices of zidovudine/lamivudine using HME with ethylcellulose, Kollidon® SR and PEO. This platform therefore provides potential to be used a standard method for producing sustained release formulations of antiretroviral drugs for use in the paediatric population.

CHAPTER ONE: HUMAN IMMUNO DEFICIENCY VIRUS (HIV) AND ACQUIRED IMMUNO-DEFICIENCY SYNDROME (AIDS): A GLOBAL EPIDEMIC

1.0 Introduction

The Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome (HIV/AIDS) has become a major global Health problem in recent times where children are also affected. According to United Nations program on HIV/AIDS (UNAIDS) about 60 million people have been infected with HIV since the beginning of the epidemic, out of which 25 million have died due to HIV related causes. Currently about 33.3 million people are living with HIV worldwide with 67% (22.4 million) living in Sub-Saharan Africa and 91% of all new infections are in children living in Sub Sahara (UNAIDS 2010).

Overall, in 2009 only about 4 million people in the world living with HIV/AIDS in low and middle income countries had access to HIV treatment (UNAIDS Report on Global AIDS epidemic, 2010). Antiretroviral therapy (ART) is the gold standard used to treat HIV/AIDS infection. By Dec, 2009, in Sub–Saharan Africa, only about 2.9 million people received ART which accounted for a 44% total coverage for the treatment. In 2009, 2.3 million children aged between 0-15years, in Sub-Saharan Africa were estimated to be living with HIV/AIDS infection out of 2.5 million children living with HIV, globally (UNAIDS, 2010). Only 35% of these children infected with HIV/AIDS are receiving the ART therapy in Sub-Saharan Africa (UNAIDS, 2010).

The major concern for children not receiving antiretroviral therapy is the lack of appropriate and efficacious formulations that may deny access to appropriate paediatric antiretroviral formulations. This chapter introduces to HIV/AIDS, the use of antiretroviral therapy and the factors that relate to limited access and availability of efficacious and stable paediatric antiretroviral formulations.

1.1 Discovery of HIV

The Acquired Immunodeficiency syndrome (AIDS) was first described by Gottlieb in 1981 (Gottlieb et al, 1981). The Human immunodeficiency virus (HIV) was identified as the Causative agent in 1983 by Francoise Barré-Sinoussi (Barre-Sinoussi et al, 1983) and Luc Montaigner and later confirmed by Robert Gallo.

HIV is a retrovirus that is grouped into two types: HIV-1 and HIV-2. HIV-1 occurs worldwide whereas HIV-2 is mainly observed in West Africa and India. HIV-2 is closely related to the Simian Immunodeficiency Virus (SIV) that is carried by African green monkeys. SIV causes AIDS like in captive rhesus monkeys thus supporting the theory that at some stage HIV-1 and HIV-2 may have been transmitted from primates to Humans (Mortimer, 1988).

1.1.2 Transmission of HIV

The most common mode of transmission of the virus throughout the world is through unprotected sexual intercourse with multiple partners, whether it is through vaginal or anal (Natural treatment for AIDS, 2010).

The other methods of transmission are:

- Through the receipt of infected blood or blood products and donated organs. Donating blood does not risk of disease contraction since the needle used for such purposes are compulsorily and always sterile.
- Mother to child which occurs in utero and at birth
- Through breast milk of an infected mother
- By reuse and sharing of contaminated and unsterile needles used by drug users
- Through unsterile therapeutics procedure, e.g. surgery

Mother to child transmission (MTCT) is responsible for more than 90% of paediatric infections worldwide. Children can be infected during pregnancy, in uterus, during delivery or postnatally through breast feeding. In the developed countries, before the prophylactic era, the transmission rate from mother to child ranged from 15- 25% provided the mothers did not breast feed (Msellati P. *et al*, 1995).

1.2 Life cycle of the HIVvirus

HIV can be called "immunosuppressive" because it infects the cells of the immune system that leads to their demolition (Beucrley & Sattentau, 1993). HIV is an RNA (ribonuclease) retrovirus that infects CD4 Lymphocytes, dendritic cells and macrophages . The reproduction of HI-virus in the human body can be divided into six steps (Raffanti and Haas. 2001) as shown in Figure 1.1.

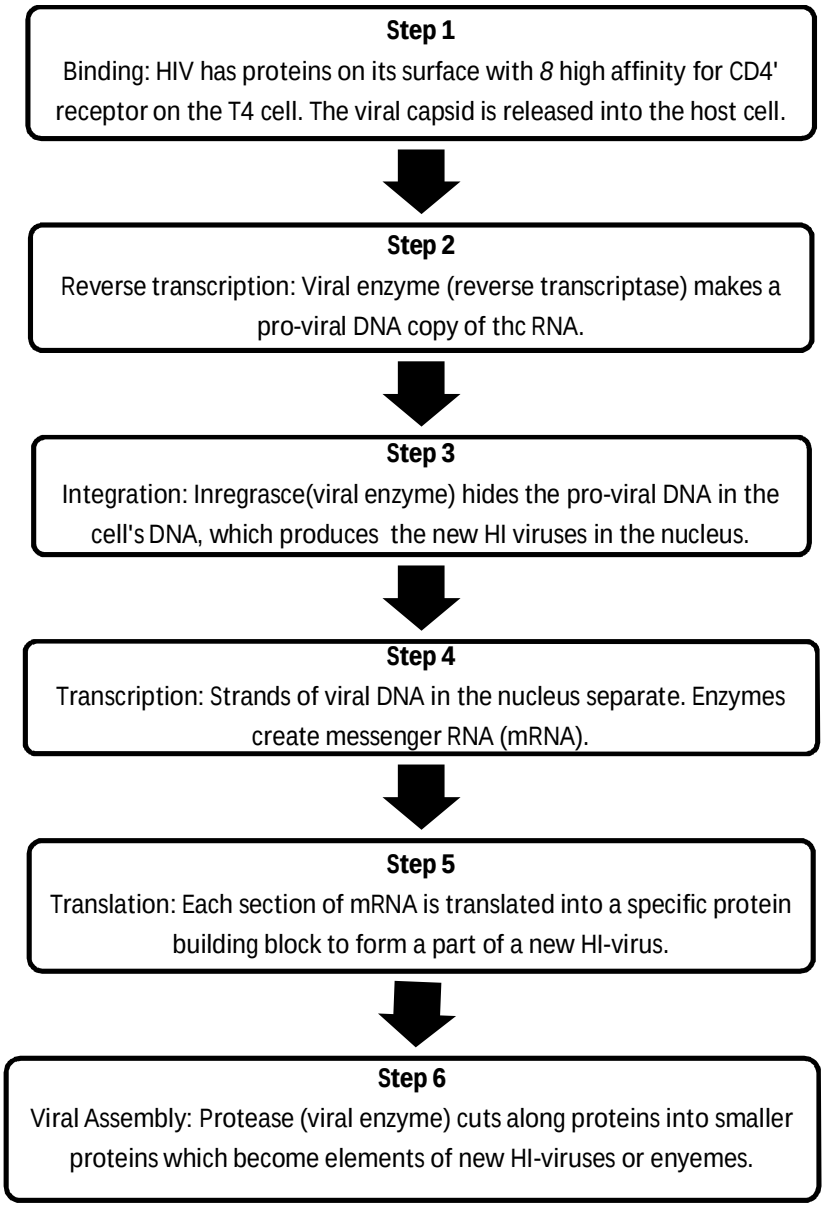

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Figure 1.1: Reproduction of the HI virus in the human body.

Once the new viral units are assembled they leave the host cell and create new viruses that are able to infect new cells and undergo reproduction (Raffanti and Haas, 2001).

1.2.1 Pathology of HIV

A clinical problem is triggered by HIV and it snowballs into the development of the disease from the time of sero-conversion (primary HIV) to AIDS and then death (Mindel et al, 2001)

HIV disease is classified based on the presence of clinical symptoms and signs, CD4'count, investigative signs and the availability of HIV screening.

The World Health Organization (WHO) staging system for HIV infection in adult and adolescent patients is divided into four stages whose symptoms are:

- Stage 1: Asymptomatic
- Stage 2: < 10% loss of body weight, minor mucotaneous manifestations, re current upper respiratory tract infection
- Stage 3: > 10% loss of body weight, Chronic diarrhoea for more than a month, oral candidiaces, prolonged fever for more than a month, pulmonary TB, Bacterial infections
- Stage 4: HIV wasting syndrome: weight loss of > 10% body weight, chronic diarrhoea and prolonged fever, Pneumocystis carinii pneumonia, Kaposi's sarcoma.

1.2.2 Classification of adult patients based on the WHO staging system:

a) Those without laboratory evidence of an HIV infection, but with positive diagnosis of an indicator disease.

b) Those with laboratory evidence of the presence of an HIV infection, regardless of the presence of other causes of immunodeficiency, or any of the specified indicator diseases whether diagnosed presumptively or definitively.

c) Those with laboratory evidence against an HIV infection. AIDS is diagnosed in this group only when all the other major causes of immunodeficiency have been excluded e.g.

A high dose of any long term systematic corticosteroid or other immunosuppressive cytotoxic disease and the patient has had unequivocal Pneumocystis carinii pneumonia or any other disease indicative of AIDS and a CD4 (T- helper lymphocytes) count of ≤ 350 cells/mm^3.

1.2.3 Children infected with HIV are defined differently from adults.

The WHO classifies the age terminologies of paediatric patients with HIV as described in Table 1.1.

Table 1.1: WHO classification of age terminologies of paediatric patients with HIV

Term		Definition
Infant	=	<12 months of age
Under 12 months of age	=	<12 months of age
12 months of age or older	=	≥12 months of age
Age 5 and over	=	>59 months of age

Furthermore, upon diagnosis of the below symptoms children are classified as having HIV

a) In children, recurrent or multiple serious bacterial infections and lymphoid interstitial pneumonitis or pulmonary lymphoid hypoplasia are taken as symptoms indicative of AIDS.

b) More strict criteria are used for children who are less than five months old and whose mothers are thought to have been infected with HIV during the child's perinatal period. (Adler, 1993).

WHO, 2010 has now revised the treatment guidelines and has now started to test infants who are less than six weeks old and who have been thought to have been infected by their mothers during post partum or perinatal period and has highly recommended the start of antiretroviral treatment (ART).

1.3 WHO guidelines for paediatric antiretroviral therapy

Infants and children can be infected with HIV during pregnancy, during delivery and post partum, through breastfeeding, or through sexual or parenteral exposure (WHO, 2010).

The most efficient and cost-effective way to tackle paediatric HIV globally is to reduce mother-to-child transmission (MTCT). However, every day there are nearly 1500 new infections in children under 15 years of age, more than 90% of them occurring in the developing world and most being associated with MTCT (UNAIDS, 2010) There is thus a critical need to provide antiretroviral therapy (ART) for infants and children who become infected despite the efforts being made to prevent such infections (WHO, 2010).

Antiretroviral (ARV) treatment in HIV-infected children is necessary to allow a similar rapid scale-up of treatment as it is being done in adults. Recommendations targeting at developing countries take into account realities in terms of:

- Health care infrastructure
- Availability of human resources
- Socioeconomic context and
- Currently available drug formulations

To facilitate ARV therapy, standardized and simplified ARV regimens have been formulated. These regimens consist of first line treatment and the second line of treatment regimens for individuals who cannot tolerate or fail to respond to the first line treatment regimen (WHO, 2010). The use of three ARV medications is currently the standard treatment for HIV infection in order to achieve the best possible suppression of viral replication and to arrest the progression of HIV disease. It is important to maximize the durability and efficacy of any first-line regimen by incorporating approaches to support adherence (WHO, 2010).

According to WHO, the following age terminologies are used for ART initiation, Infants are below 12 months old and children are classified into three classifications: children under 12 months of age, children of 12 months of age or older and children of age 5years and over (> 59 months of age). ART is for infant is recommended to start as soon as possible when clinically diagnosed of HIV infection.

To facilitate ARV therapy, standardized and simplified ARV regimens have been formulated. This treatment regimen is known as the Highly Active Antiretroviral Treatment (HAART). These regimens consist of first line treatment and the second line of treatment regimens for individuals who cannot tolerate or fail to respond to the first line treatment regimen (WHO, 2010). The use of three ARV medications is currently the standard treatment for HIV infection in order to achieve the best possible suppression of viral replication and to arrest the progression of HIV disease. It is important to maximize the durability and efficacy of any first-line regimen by incorporating approaches to support adherence (WHO, 2010). The preferred option when choosing a first-line regimen for infants and children is two (2) nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI). These drugs prevent HIV replication by inhibition of the action of reverse transcriptase, the enzyme that HIV uses to make a DNA copy of its RNA.

The technical reference group based this decision on available evidence, clinical experience and programmatic feasibility for the wider introduction of ART to infants and children in resource-limited settings. NRTI/NNRTI-based regimens are efficacious and generally less expensive; generic formulations are more often available and a cold chain is not required. In addition, they preserve a potent new class (i.e. protease inhibitors [PIs]) for the second line (WHO, 2010). The NRTIs include drugs from three different drug analogues:

1. Thymidine analogue: zidovudine (AZT) and stavudine (D4T)
2. Cytidine analogue: lamivudine (3TC)
3. Guanosine analogue: abacavir (ABC) (WHO, 2006)

NNRTIs drugs commonly used are Efiverenz (EFV) and niverapine (NVP).

Protease inhibitors (PIs) are not usually recommended as the first line treatment in children. However PIs can be used along with the NRTI in the first line of treatment if the infant or child has been previously exposed to NVP or other NRTIs either directly or via the mother who is on ARV treatment during pregnancy, during labour or during breastfeeding. Examples of PIs commonly used are Lopinavir (LPV), Retonavir (RTV) and Nelfinavir (NFV).

1.4 Fixed dose combination (FDC) formulations of arvs for paediatric use

Fixed dose combination formulations contain two or three ARV drugs combined together in the form of usually a tablet. Fixed dose combination for of ARV drugs are commonly used in adults and a few in children either in the form of tablets or syrups. The advantages of FDCs over single ARV drug administration is that FDCs are easier to administer as these are combined in the form of one tablet. FDCs also provide accurate and harmonized dosing and

thus improve therapeutic efficacy. FDCs also in turn improve drug adherence and scale up for HIV care for children (WHO, 2006).

The WHO recommends pharmaceutical manufacturers to urgently develop new AZT based paediatric FDCs in the form of granules or scored tablets. Granules and scored tablets are easier to store and handle unlike liquid formulations which are bulky and difficult to transport and store (Table 1.2).

Table 1.2: WHO Priority and dosing strengths requirement for required FDCs products of ARVs for Paediatrics (WHO, 2006).

Degree of Priority	Product	Strength (mg)
Urgent	AZT/3TC/NVP	60:30:55
	AZT/3TC	60:30
	D4T/3TC	7:30
	D4T/3TC/NVP	7:30:55
	Lopinavir/ritonavir(Lop/r)	90:22.5
High	ABC/3TC	60:30
	AZT/3TC/ABC	60:30:60
Important	EFV/FTC	100:35

1.4.1 Lack of paediatric ARVs

WHO and other international and government organizations around the world have been trying to scale up the accessibility of ARVs especially in developing regions of the world.

Paediatric ARVs accessibility in poor resource settings has had constraints despite many efforts and declaration by both international and government bodies around the world to scale

up universal access. There are various reasons for the limited access of paediatric ARV formulations; the major reasons have been discussed as below:

1. Cost

Though there are significant efforts made by World Health Organisation (WHO) along with government bodies to have cheaper paediatric ARV formulations available. These paediatric formulations as compared to adult formulations still remain costly. An adult fixed dose ARV treatment costs about $140 per year while first line fixed dose ARV treatment for children costs almost four to eight times more per year (UNICEF, 2006). This means that more adult ARV treatments could be funded for the same amount of monies. It is a well known fact that most of the population in Sub-Saharan Africa live on less than a dollar a day, thus buying paediatric ARV treatment would be very expensive. Most pharmaceutical companies manufacturing generic drugs rely on economies of scale because of small profit margins and therefore without a large market it is hard to compete (Dianinso, 2007).

2. Limited number for available paediatric formulations

The numbers of fixed dose combinations of ARVs for paediatrics available are very few comparatively. Splitting of available adult tablets is inappropriate as it may cause under dosing or over dosing in children. The lack of appropriate and efficacious formulations may deny access to appropriately dosed formulations and thus expose children to "homemade" formulations. The tablets/capsules intended for adults may not be available in appropriate doses for children. However, liquid formulations may require cold chain during distribution (e.g., d4T liquid) and be difficult to store especially in the tropical countries like Kenya where half of the population live below the poverty line and cannot

therefore afford refrigeration. Splitting of adult tablets, while suboptimal, has been the only way to provide the ARVs to an ill child in some parts of this country. Splitting tablets more than once is felt too inaccurate and not recommended.

3. Dosing complications

Paediatric ARV dosing is based on child's body weight. Having the Fixed dose combination formulations complicate paediatric dosing especially when adult tablets are used and split several times. Splitting tablets more than once is felt too inaccurate and not recommended. This in turn may result in adverse effects due to overdosing or treatment failure due to under dosing as the splitting may not have been done by an expert and the dose being not titrated according to weight of the child.

4. Labelling issues

Some of the available ARVs have not been labelled for paediatric use especially for infants. Nahata (1999) stated that only 20% of the drugs marketed in United Stated have labelling for paediatric use and out of the 80 drugs approved for paediatric use, only five are used in newborns and infants. There are currently 22 antiretroviral drugs that have been approved by the United States Food and Drug Administration (US FDA) for use in treatment of HIV/AIDS infected adults but out of which only 12 drugs have been approved for use in children (Guiaquinto et al, 2008).

1.4.2 Conclusion: The way towards better availability of paediatric ARV formulations

HIV/AIDS is a major global concern. There are concerns regarding the growing numbers of paediatric HIV/AIDS infections, especially in the developing world and in particularly with the Sub-Saharan Africa where the number of paediatric infections are the largest as compared

to the other parts of the world. Most of these paediatric HIV infections are perinatal infections in nature and thus this means that diagnosis and paediatric ARV treatment needs to be initiated as early as possible. One way forward to scale up the availability of ARV paediatric formulation is to introduce appropriate fixed dose combination generic formulations that are in the form of granules, mini matrices or dispersible solid dosage forms which have appropriate taste or are taste masked. These formulations would also be easy to administer the right dosage and have a long shelf life which would require no refrigeration. Production of these effective paediatric ARVs could be done locally in an intention to reduce the cost of importation and the overall cost of treatments. Producing locally would mean local pharmaceutical companies based in Sub-Saharan Africa to have cheaper, one step, continuous and effective methods of processing the above mentioned paediatric dosage forms and thereby be able to meet the needs of the patients locally.

1.5 Active Pharmaceutical Ingredients (API): Zidovudine and Lamivudine used in fixed dose combination formulations.

1.5.1 Zidovudine

Zidovudine (also known as 3'-azido-3'-deoxythymidine, AZT or ZDV), is a nucleoside reverse transcriptase inhibitor (NRTI), a synthetic thymidine analogue that has antiretroviral activity against HIV-1, HIV2 and Human T-cell lymphotrophic virus (HTLV) (Hardman et al, 2001). Zidovudine was the first anti-HIV compound approved for clinical use, is still widely used for antiretroviral (ARV) therapy, either alone or in combination with other ARV agents according to the first line regimen as described in Table 1.1. The drug's selectivity of action against HIV is due to its high affinity as a substrate for the enzyme viral reverse

transcriptase compared to the mammalian cellular DNA polymerase (Collins and Unadkat, 1989).

1.5.1.1 Chemical Properties

AZT is a crystalline, odourless, white to off- white powder

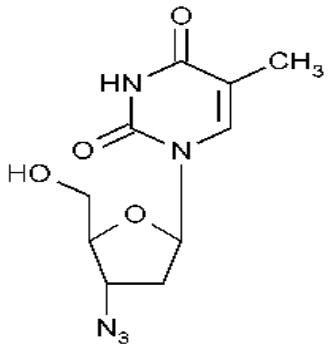

Fig 1.2: Molecular structure of AZT

- IUC Name: Thymidine, 3´-azido-3´-deoxy-. 3´-Azido-3´-deoxythymidine
- Molecular formula: $C_{10}H_{13}N_5O_4$
- Molecular weight: 267.24
- Melting point range: 121- 128ºC
- Solubility: soluble in ethanol and sparingly soluble in water (25mg/ml) (Merck, 1999).

1.5.1.2 Stability

Thermal analysis of AZT was undertaken by Araujo et al. (2003) and the thermal decomposition was shown to occur in three stages. The results showed that the product originated from the first thermal decomposition stage corresponds to the cleavage followed

by elimination of the azide group and consequent formation of thymine. The second event corresponds to thermal decomposition of thymine (Araujo et al, 2003).

1.5.1.3 Pharmacokinetic profile

The bioavailability of AZT after oral administration is only 60% owing to an extensive hepatic first-pass metabolism, which necessitates frequent administration of a high dose of the drug (Douglas, R.D., 1990). The half-life of the drug is approximately 1.1 h (Klecker et al, 1987). Despite its moderate bioavailability and short half-life, zidovudine is rapidly absorbed from the gastrointestinal tract and exhibits plasma concentration of 1.2µg/ml at 0.8hr. Zidovudine is absorbed throughout the GIT and is freely soluble in any pH.

In the systemic circulation, it is first converted to zidovudine- triphosphate which is pharmacologically active. The biological half life ($t_{1/2}$) of zidovudine-triphosphate is 4hrs thus necessitating frequent administration i.e. up to 3 to 4 times a day to maintain therapeutic drug levels. As a result, dose-dependent toxic side effects are frequently observed (Merigan and Skowron, 1990) and (Kieburtz et al, 1992). With current dosing regimens, which employ twice- or thrice-daily administration, plasma AZT concentrations are below optimal antiretroviral concentrations (1 µM) for more than half of the dosing interval (Balis et al,1989 and Balis et al 1989]. Although the active intracellular metabolite, AZT-triphosphate (AZT-TP), has a longer $t_{1/2}$ (4 h) than the parent drug, the phosphorylation of AZT to AZT-TP is inefficient and is saturatable at doses exceeding 100 mg (Balis et al 1989). AZT-TP accounts for only 10% of total intracellular AZT nucleotides, and peak AZT-TP levels are similar after doses of 100 and 300 mg, suggesting that higher doses of AZT administered less frequently may be optimal.

AZT is metabolised in the liver by enzyme glucoronidase to the major inactive metabolite 3'-azido- 3' deoxy-5-0-β-D-glucopyranosyl thymidine which is excreted in the urine as metabolite (65-75%) and as unchanged drug (15-17%) (Gibbon, 2003). AZT also shows good penetration into the central nervous system and demonstrates plasma protein binding of approximately 30% (Gibbon, 2003).

Furthermore, the pharmacokinetic profile and intracellular metabolism of AZT provides a strong rationale for the development of a sustained-release formulation.

1.5.1.4 Adverse reactions

The common adverse effects reported with the use of AZT are anaemia, leucopenia and neutropenia (Gibbon, 2003).

Other adverse reactions include headache, nausea, insomnia, myalgia, seizures, confusion, hepatotoxicity and on occasion lactic acidosis (Takemoto et al, 2000). AZT should be used with caution in patients with bone marrow suppression and anaemia (Gibbon, 2003 and Takemoto et al, 2000). AZT may also cause muscle damage by possibly inhibiting mitochondrial DNA polymerase-γ, associated with reduced amounts of mitochondrial DNA (Rafanti and Haas, 2001).

1.5.1.5 Adult and Paediatric dose

The oral adult dose is 300mg to be taken once every 12 hours. Mother to child transmission prevention requires an oral dose of 300 mg every 12 hours for the last four weeks of pregnancy, to be increased to another 300mg every 3 hours from the onset of labour to delivery (Gibbon, 2001). After birth, the oral target dose is 4mg/kg every 12 hours starting within 12 hrs after birth and continuing up to 6 weeks of age. The intravenous target dose for

AZT is 1.5mg/kg infused over 30 minutes every 6 hours until oral dosing is possible (WHO, 2010). Paediatric dosing of AZT for solid oral formulations is based on weight of the child as proposed by WHO. The target dose for liquid oral formulations is 10mg/ml every 8hrs for children more than 6 weeks old (WHO, 2010).

1.5.2 Lamivudine

Lamivudine ((L-2', 3'-dideoxy-3'-thiacytidine, 3TC), is a nucleoside reverse transcriptase inhibitor (NRTI). It is a cytosine nucleoside analog that shows activity against HIV-1, HIV-2 as well as hepatitis B virus (Raffanti and Hass, 2001).

3TC has two chiral centres (Yuen et al, 1995) and was found to have a negative enantiomer: 2'-deoxy-3'thiacytidine which is less toxic but demonstrates greater antiretroviral activity (Raffanti and Hass, 2001) than the positive enantiomer which demonstrates extreme toxicity. 3TC undergoes anabolic phosphorylation by intercellular kinases to form an active metabolite lamivudine 5'- triphosphate which prevents HIV-1 replication (Johnson et al, 1991)

1.5.2.1 Chemical properties

3TC is a white to off-white crystalline solid powder (GlaxoSmithKline, 2010).

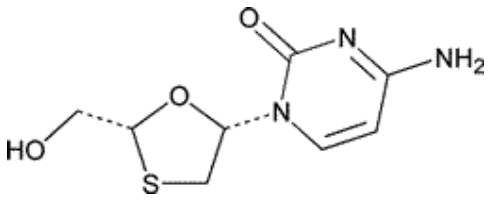

Figure 1.3: Molecular structure of 3TC

- IUC Name: 2(1*H*)-Pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-, (2*R-cis*)-. (–)-1-[(2*R*, 5S)-2-(Hydroxymethyl)-1, 3-oxathiolan-5-yl] cytosine
- Molecular formula: $C_8H_{11}N_3O_3S$
- Molecular weight: 229.26
- Melting range: 172-178 ºC
- Solubility: 70mg/ml in water at 20ºC (GlaxoSmithKline, 2007). It is soluble in water and sparingly soluble in methanol and practically insoluble in acetone.

1.5.2.2 Stability

The chemical stability of 3TC in oral liquid formulation is mainly influenced by the pH and it has been shown that 3TC stability is improved when the pH is increased from 4.5 to 7.5(Nguyen et al, 1995).

1.5.2.3 Pharmacokinetic profile

- Following oral administration, 3TC is rapidly absorbed and extensively distributed. It reaches peak plasma levels in approximately one hour and has an oral bioavailability of 80% both in the presence and absence of food. Elimination half life is 6-9 hr.
- After multiple-dose oral administration of 3TC 300 mg tablets once daily for 7 days in healthy subjects, steady-state C_{max} was 2.04mcg/ml and the 24-hour, steady-state AUC was 8.87 mcg (hr)/ml (GlaxoSmithKline, 2006)
- Binding to plasma protein is low and approximately 70% of an IV dose of 3TC is recovered unchanged in urine with metabolism being a minor route of elimination (GlaxoSmithKline, 2006).

1.5.2.4 Adverse reaction

In general 3TC tends to cause fewer adverse effects than most other NRTIs and is therefore frequently used in combination with other ARVs when treating paediatric patients.

The following adverse effects commonly observed when using 3TC

- Peripheral neuropathy, pancreatitis
- Vomiting, nausea and upper abdominal pains
- Headache, fatigue, fever
- Rash, pruritis and sweating

1.5.2.5 Adult and Paediatric Dose

The adult dose is 150mg to be taken every 12 hours. In patients weighing less than 50 kg, a dose of 2mg/kg taken every 12 hours is typically used (Gibbon, 2001).

Yeun et al, 2004 investigated on the equivalent steady state pharmacokinetics of lamivudine in plasma and lamivudine triphosphate within cells following administration of lamivudine at 300 milligrams once daily and 150 milligrams twice daily and found out once daily dosing of lamivudine was pharmacokinetically equivalent to twice daily dosing (based on AUC and other related parameters) and the once daily dosing was well tolerated. Other long term phase I and II trials confirmed that the safety profile of lamivudine did not differ over the dose range of 0.5 to 12 mg/kg/day (~37.5 to 900 mg/day), administered for 24 weeks or longer (van Leeuwen et al, 1995). Furthermore an NUCA3001 study, which compared lamivudine at 300 mg twice daily (twice the daily dose administered in this study) with 150 mg twice daily

in combination with zidovudine over 24 weeks, no differences in type or incidence of adverse events were noted(Eron et al, 1995).

Neonates less than 30 days old are given doses of 2mg/kg twice daily, whilst children between 3 months and 12 years of age receive 4mg/kg twice daily with a maximum of 150 mg per day (Fletcher et al, 2004).

Clearance in children less than 3 years old is elevated and minimal observed toxicity allows for higher dosing in younger children to up to 5 mg/kg twice daily. Pharmacokinetic data on once daily dosing suggests acceptable troughs and that the overall troughs are similar to twice daily dosing (Bergshoeff et al, 2005). Once daily dosing is however not recommended in children under 3 years (Burger et al, 2005).

1.6 Adequate sustained release oral dosage form for paediatrics

The selection of a route of administration and dosage form is majorly governed by the therapeutic considerations, properties of the drug substance, technical feasibility and patients needs (Stoltenberg et al, 2010). The oral route is one of the most preferred routes of administration for children. Recently, there have been many changes and new regulations introduced with major medicines regulatory bodies around the world, in relation to paediatric medicines. The main aim with regard to paediatric regulation changes was to improve on the availability of child appropriate drugs and dosage forms. Liquid formulations are the most preferred drug dosage forms though they have several disadvantages such as physical, chemical and microbial instability, unpleasant taste, issues with taste masking and they lack controlled release properties (Breitkreutz, 1999). WHO stated that solid dosage form is the preferred route of administration for children for a number of reasons including better

stability, ease of dose adjusment according to the body weight of the child, better taste masking properties, high content uniformity, non toxic excipients, low cost of production and the ability to have control release characteristics (Breitkreutz, 1999). Furthermore many research groups have undertaken studies on the acceptability of solid dosage forms by children and whether children under the age of 2 years would be able to swallow mini matrices/mini tablets/ mini pellets of about 2mm.

During ARV therapy, it is crucial to maintain the systemic drug concentration(s) within the therapeutic level(s) throughout the treatment course (Chien et al, 1989). Since AZT and 3TC act as metabolic antagonists of thymidine and its antiviral effects are time dependent, an adequate sustained release delivery of both AZT and 3TC would be desirable. Furthermore, the side effects of both drugs have been primarily attributed to repeated drug dosing (Colson et al, 1991) and thus AZT and 3TC could potentially benefit from delivery using a sustained release dosage forms. Not only would efficacy be enhanced through improved patient compliance but also the reduced frequency of dosing would also limit the impact of oscillations in plasma drug levels minimising the side effects associated with frequent dosing that will increase adherence to ARV regimen.

Reports on studies of extended release formulations of AZT were found where matrix tablets were formulated using a combination of hydrophilic polymer (Acrylic) and a hydrophobic polymer (Ethylcellulose) and AZT release was extended up to 12hrs (Kuksal et al, 2006). Benghuzzi and co-workers formulated and studied the in-vitro release from a sustained release ceramic capsule of AZT that was formulated using tricalcium phosphate and alumino-calcium-phosphorous oxide (Benghuzzi et al, 1990). Benghuzzi and his team further also investigated the *in vivo* release of AZT from a long term sustained delivery system which was

formulated using hydroxyapatite and tricalcium-phosphate ceramic implants (Benghuzzi et al, 2000).

Similarly, controlled release oral tablets of 3TC were designed and studied by Ravi and co-workers who designed the matrices using a release retardant, Hydroxypropyl methylcellulose (HPMC) of various viscosity, proportion and studied the effect of compression force during compression on the release of 3TC (Ravi et al, 2007).

However, limited literature is available on studies of sustained release oral paediatric fixed dose combination formulations of AZT and 3TC. The ability to develop such a preparation could reduce the frequency of dosing, ensuring better patient adherence with associated improvements in health outcomes. The aim of this thesis is to formulate and evaluate an approach for producing age appropriate formulations of AZT and 3TC in a sustained release presentation.

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CHAPTER TWO: HOT MELT EXTRUSION TECHNOLOGY FOR PRODUCTION OF SOLID ORAL SUSTAINED RELEASE DOSAGE FORMS.

2.0 Introduction

Hot melt extrusion (HME) has been widely used in the plastic industry to manufacture various items from insulated wires and pipes to plastic sheets and injection syringes since the early 1930s. First applications of HME as a manufacturing tool in the pharmaceutical industry were realized by Doelker and his co-workers in 1971 (Doelker, 1971). HME is a technique where solid dispersions are commonly produced by elevating temperatures of the polymer and the drug component during manufacture to facilitate dispersion. Some of the other technologies used for the production of solid dispersions are: spray drying (Jung et al, 1999), co-precipitation and co-evaporation (Sekikwa et al, 1978), freeze drying (Sekikwa et al, 1983), Roll mixing (Nozwa et al, 1986), melt evaporation method (Goldberg et al, 1966), melt agglomeration (Vilhelmsen, 2005) and Super critical fluid technology(SCF) (Muhrer et al, 2006). Hot melt extrusion technique has recently gained popularity in the pharmaceutical industry to prepare pharmaceutical dosage forms like pellets (Follonier et al, 1994 and Young et al, 2002), granules (Follonier et al, 1995), transdermal and transmuscosal drug delivery systems, sustained release tablets (Zhang et al, 1999 and McGinity et al, 1997) and implants (Rothen-Weinhold, 2000).

Hot melt extrusion has also been widely used in particular for formulation of poor water soluble drugs. Foster et al, 2001, worked on selection of excipients for poor water soluble drugs for hot melt extrusion. Chokshi, 2004 used hot melt extrusion to improve the dissolution rates of poor water soluble drugs. Molecular dispersions for oral delivery of itraconazole (Verreck et al, 2003), nifedipine (Li et al, 2006), indomethacin (Chokshi et al, 2008) and nimodipine (Zheng et al, 2007) have successfully been produced by using HME technology.

During HME, the molten plastic mass containing polymer and other suitable additives such as plasticizers is melted and forced at high temperature through a die by the action of an Archimedian screw rotating in a heated barrel (Paradkar et al, 2008). The molten polymers during the extrusion process can function as thermal binders and act as drug depots and/ or drug release retardants upon cooling and solidification.

HME has many advantages over conventional pharmaceutical processes (Crowley, 2003).It is a solvent free process whereby solvents are not needed to bind and granulate and thereby reducing the number of processing steps thus giving higher production output. Moreover HME is not only a more efficient process but it also improves the quality and efficacy of the products manufactured (Ghebre-Sellassie and Martin, 2003). HME is a dust free process where dust free pellets and other dosage forms can be produced efficiently. During HME the polymer and the drugs are mixed at molecular level while in their molten state by an extruder thereby forming a solid dispersion or solid solution. The short residence time and low temperatures during HME ensure that the polymer and the drugs are gently compounded without degradation. This technique is especially used to improve the solubility of poor water soluble drugs and increase their bioavailability. HME has been widely used to produce controlled release or sustained release dosage forms by using polymers that can be able to provide sustained release drug profile. Taste masking of poor tasting active pharmaceutical ingredients (API) has been successfully done by using HME. The polymers used for HME should be of pharmaceutical grade, thermoplastic and not necessarily be compressible like the polymers used for direct compression. The components used for HME should be thermally stable at processing temperatures during short heat processing time. HME is a robust manufacturing process which can be run practically in any pharmaceutical factory (Kotler et al, 2010) and high production rates can be obtained using small scale HME machines. Installation of HME equipment would require low investment since it is a one step process as compared to

conventional pharmaceutical process for production of tablets or other solid dosage forms. Table 2.1 gives an overview of the advantages of HME over conventional pharmaceutical processes.

The few disadvantages of HME are that APIs that are heat sensitive like proteins cannot be used for this process, the polymers used should be free from moisture and the processing and handling activities should be done by trained personnel since the equipment used is not traditionally like the ones used in pharmaceutical manufacturing.

HME has received considerable attention from both academia and pharmaceutical industry as a drug delivery technology. There are more than 100 publications that have been published in scientific literature and the number of patents in this area has increased steadily (Crowley, 2003).

Table 2.1: Advantages of HME over conventional pharmaceutical manufacturing

Conventional pharmaceutical processing	Hot melt extrusion advantages
• Require organic solvents or water for various processes	• It is completely a solvent free process
• Require many steps to produce a dosage form e.g. granulation, drying, compression, coating, polishing etc	• One step continuous process
• Takes longer processing times due to many processing steps	• Shorter and more efficient processing time
• Require more labour and equipment to process dosage form	• Require less labour and equipment
• Poor bioavailability due to poor drug solubility	• Produce solid dispersions which lead to improved and drug dissolution and bioavailability
• Poor taste masking	• Complete taste masking
• Poor unreliable sustained release profile	• Reliable sustained release profile
• Difficult process for manufacturing films	• Films and transdermal films manufacturing easier.

2.1 Equipment, process and principles of hot melt extrusion technology

2.1.1 Hot melt extrusion Equipment

The extrusion process is done using the hot melt extruder. Pharmaceutical hot melt extruders are designed and adapted for mixing drugs with polymers (carriers) in various dosage forms (Kotler et al, 2010). There are two types of extruders used for the extrusion process: ram extruders and screw extruders. Ram extruders basically work on displacement mechanism, where the components in the extruder are displaced through a die orifice and thereby generating high pressure (Crowley, 2003). A screw extruder fundamentally consists of a platform that supports a drive system, an extrusion barrel; a rotating screw arranged on a screw shaft and an extrusion die for defining product shape. The process parameters are controlled via a centrally controlled electronic unit and the extrusion drive system generally comprises motor, gearbox, linkage and thrust bearings, whereas the barrel and screw is commonly utilized in a modular configuration (Whelan and Dunning, 1996).

The basic components of an extruder are: the feeder hopper, temperature controlled barrel for heating or cooling, the die (different designs and configurations are available) and a down streaming processor (pelletizer, cutter, cooling line etc). Screw extruders provide better product homogeneity than ram extrusion. The major drawback of ram extruders is limited melting capacity which causes poor temperature uniformity in the extrudate (Crowley, 2003).

2.1.1.1 Types of Screw extruders

There are two types of screw extruders: single screw extruders and twin screw extruders. The single screw extruders are simple screw extruders where one set of screw, rotating in one direction are used to extrude while building pressure on the polymer melt and then allowing extrusion through the die (Figure 2.1). Single screw extruders are mechanical, simple to operate and are low cost investment. Twin screw extruders have two similar screws that are closely configured next to each other and either rotate in the same (co-rotating) or in the opposite direction (counter rotating) (Figure 2.2). Counter rotating screw designs are utilized where very high shear forces are needed during extrusion. The materials are exposed to high shear in between the gap where the two counter rotating screws meet during rotation. This screw layout is beneficial for dispersing the particles in a blend although the main disadvantage of this layout is high air entrapment during extrusion, low maximum speeds and output. Co-rotating screws however are preferred since they can be operated at high speeds thereby giving high output as well as provide good mixing and conveying characteristics.

Twin screw extruders have many advantages over the single screw extruders. During extrusion the drugs, polymers and additives are dispersed and mixed thus this involves breaking of minor particle aggregates. In order to achieve this, a critical amount of force is required which is achieved by using twin screw extruders (Douglas et al, 2010). The other advantages are easier material feeding

- High kneading potential
- High dispersing capacities
- Shorter and constant residence time

- Less tendency of overheating (important for heat sensitive APIs), (Crowley et al, 2007).

Twin screws used in the extruder could be either of intermeshing or non- intermeshing as shown in figure 2.3. The intermeshing screw type is preferred due to greater degree of conveying is achievable and shorter residence time. In practice co-rotating intermeshing screws are a preference (Kotler et al, 2010).

2.1.1.2 Conveying and kneading elements of the Screw

Most pharmaceutical extruders have been designed in a way that the screws can be configured according to the need and materials to be extruded or to achieve high or low shear rates during extrusion. Figures 2.1 and 2.2A also show the various processes that occur on the different sections of the screws. These sections of the screws are: feeding, compression and metering sections respectively. The length of the screw is defined by term L/D (length (L) of screw divided by the diameter (D) of the screw) (Kotler et al, 2010). The common L/D screws used in extruders have L/D 40 and 24. The specific screw features are as shown in Figure 2.3. The axial channel depth is the distance from the screw roots to the inner barrel surface, the perpendicular flight width is the distance between the screw flight and the inner barrel surface, the channel width is the distance between two neighbouring flights, and the helix angle is the angle between the flight and the direction perpendicular to the screw axis.

The depth and/or pitch of the screw flights differ within each zone generating variable pressure along the screw length (Figure 2.4). Due to the large screw flight depth and pitch, the pressure within the feed zone is very low allowing for consistent feeding from the hopper and gentle mixing of API and excipients. At this stage of the process the pressure within the

extruder is very low. The subsequent compression zone imparts a high degree of mixing and compression to the material by decreasing the screw pitch and/or the flight depth, resulting in a gradual increase in pressure along the length of the compression zone (Breitenbach 2002).

The screw configuration can be varied using different conveying and kneading screw elements. The kneading elements can be aligned in different angles of 30º, 60º or 90º and are used for plasticizing, mixing and dispersing respectively. The kneading elements are overall used to introduce shear energy to the extruded material (Kotler et al, 2010) while the conveying elements are used in the open chambers of the extruder for feeding, melt exchange and degassing while in the closed chamber of the extruder the conveying elements are placed in front of the kneading elements for conveying the materials as well as for building high pressure during extrusion process. Figure 2.5 shows conveying and kneading elements of the screw.

Studies done by Nakamichi et al, (2002) showed that the configuration of the screw plays an important role in changing the crystallinity and the dissolution properties of a solid dispersion. In their study they investigated the how the kneading elements and its configuration advance angle can influence the crystallinity and dissolution rate profiles of the drugs and the dissolution rate increased in comparison to the physical mixture, but no super-saturation occurred.

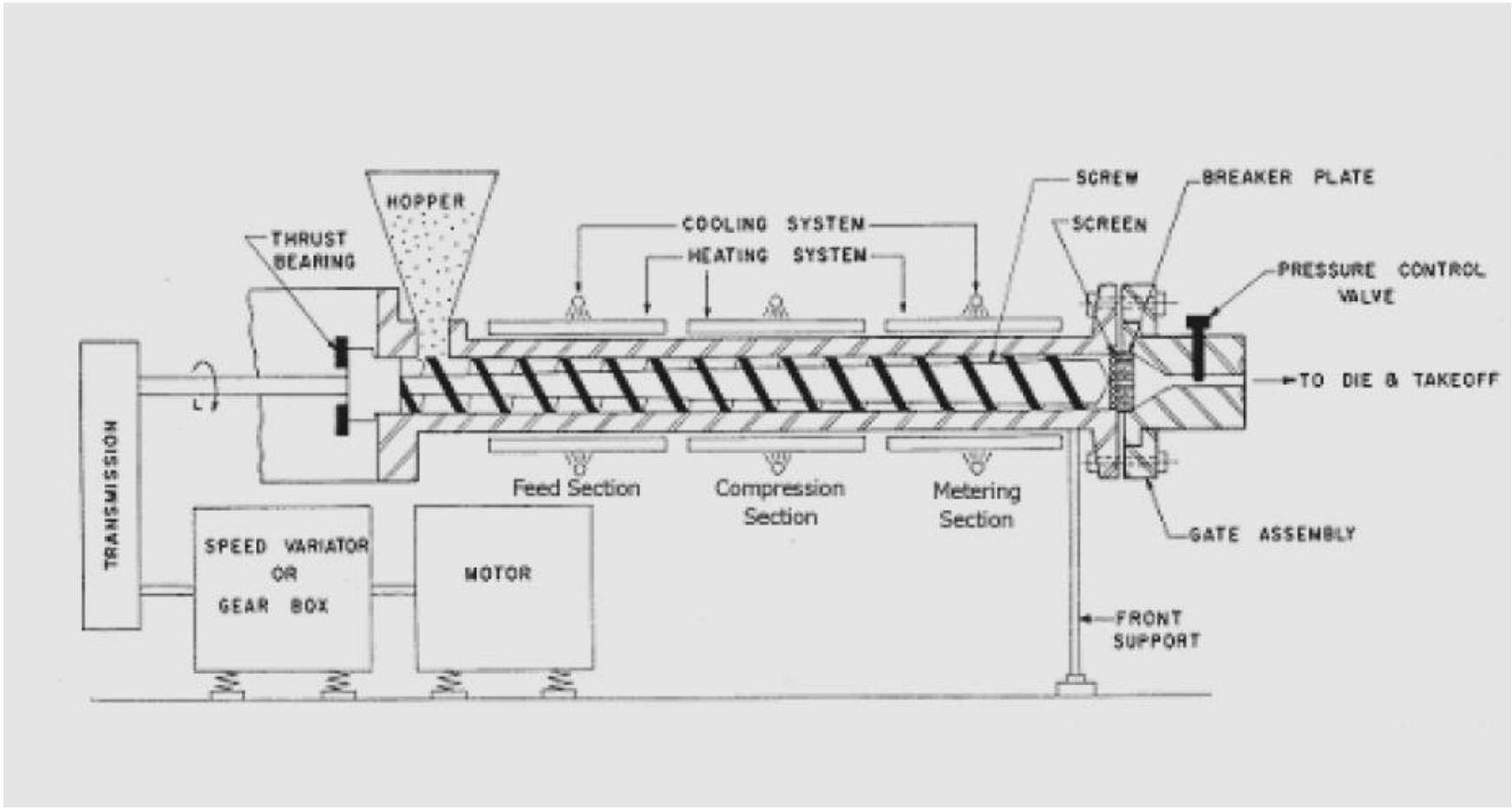
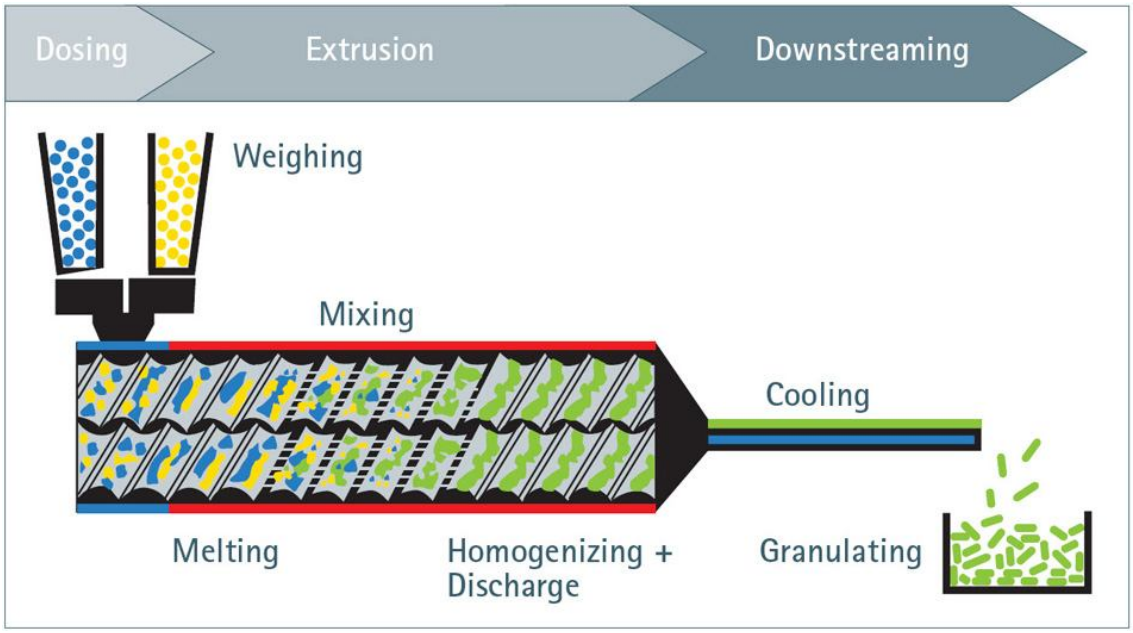


Figure 2.1: Diagram of single screw extruder (Crowley, 2003)

A



B

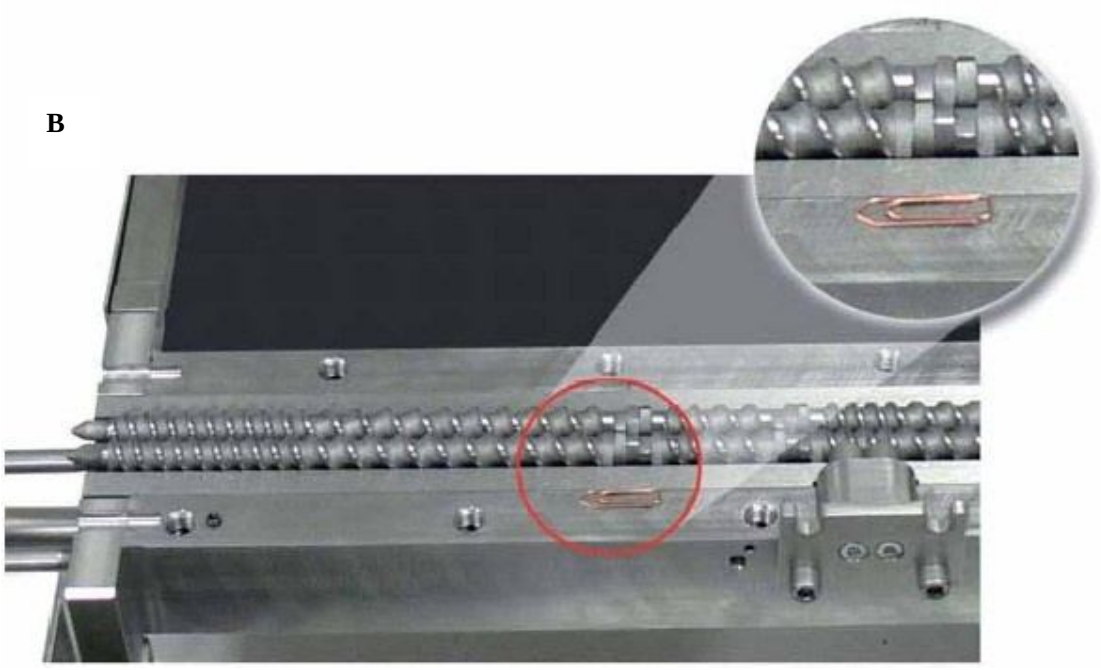


Figure 2.2: (A) Diagram showing twin screw extruder (B) Picture inset of twin screws with kneading elements configuration in a pharmaceutical hot melt extruder

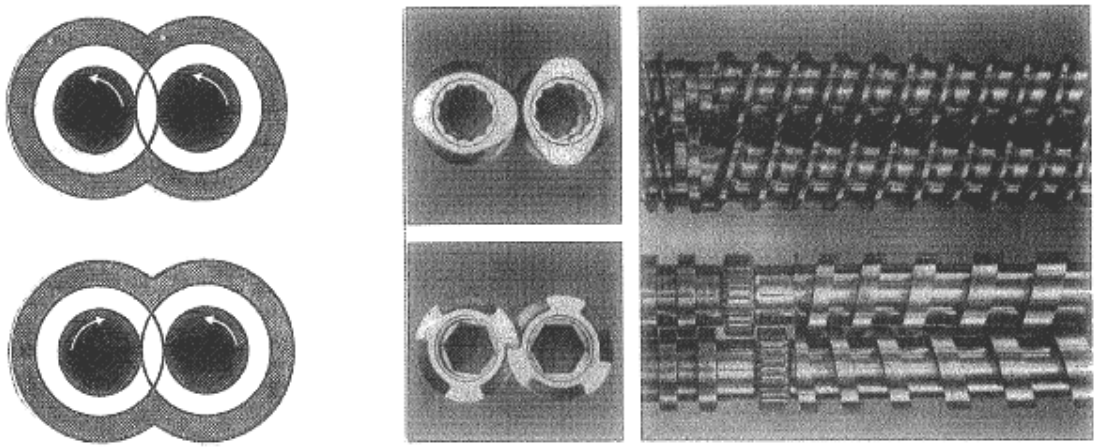


Figure 2.3: Twin-screw configurations: (top) intermeshing co-rotating twin-screw extruder, (bottom) intermeshing counter-rotating twin-screw extruder (Mollen, 2003).

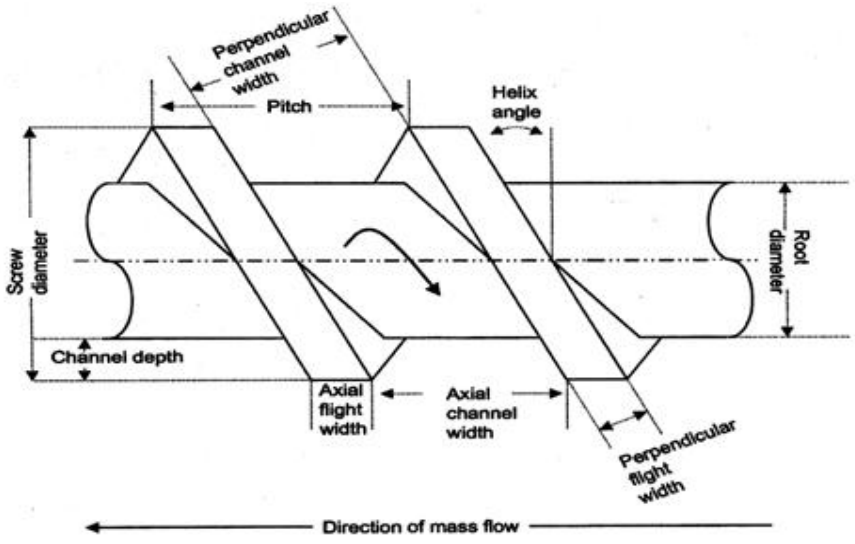


Figure 2.4: Features of screw element (Chokshi and Zia, 2004)

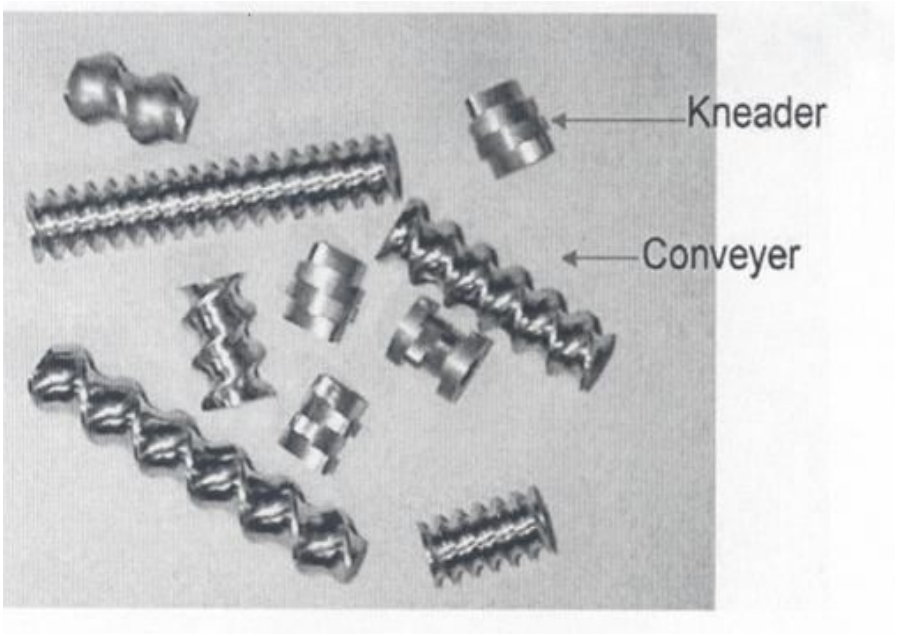


Figure 2.5: Conveying and kneading elements of the screw (Chokshi and Zia, 2004)

Extrudates produced by HME. Furthermore, they examined the influence of the screw revolution speed in combination with the configuration of the screw. They thus found from the studies that samples prepared with kneading elements with an advance angle of 60° were transparent and exhibited super saturation on dissolution testing. Detaching the kneading elements from the screw and operating only feed screw elements, the crystallinity could be reduced and the dissolution rate increased in comparison to the physical mixture, but no super saturation occurred.

Hot melt extruder screws used for pharmaceutical applications are made of stainless steel for ease of cleaning, reduce friction and chemical reactions.

2.1.1.3 Feeders

The primary functions of the feeder are to regulate the mass transfer properties of the process, ensure constant material throughput, maintain formulation consistency, and introduce ingredients in proper order, and data collection and acquisition to enable process validation and lot traceability. Multiple feeders are used to proportion multiple ingredients based on weight. Running materials simultaneously in the proper ratio essentially promotes homogeneous mixing within the hot melt extrusion device. Residence time of the mass in the extruder may vary depending on the size of the extruder, Gravimetric and volumetric feeders are used for this purpose. During gravimetric feeding the screw rotation speed is adjusted such that the mass flow rate remains constant (weight per unit of time). This method is the preferred method of feeding in pharmaceutical HME according to US Food and Drug Administration's process analytical technology (PAT) initiative for designing, analyzing, and controlling the manufacturing process through quality control measurements during processing (Martin, 2008). Volumetric feeding discharges a certain volume of material from the feeder as a function of time. Volumetric feeding is usually used to refill feeding devices during gravimetric feeding so as to maintain a constant flow of materials. The accuracy achieved in volumetric feeding is about 2-5% but however can become inaccurate if the bulk density of the material used varies. Volumetric feeders do not recognise the weight variations as do gravimetric feeders since they discharge certain volume of material per unit time.

2.1.1.4 Die

At the end of the barrel a die is attached. The Die used could be of different designs and configuration according to the shape or type of dosage form required. It is the die that determines the shape of the extrudate. Dies should supply the melt in uniform pressure,

temperature and viscosity till exit of the material. Thermoplastic polymer molecules are made of long chains which take up a randomly coiled configuration when at rest. When the polymers in their molten state are forced through the die orifice the molecules align/orientate themselves to the direction of flow and the long chains straighten themselves. When molten polymer emerges from a die many of its molecules will have been oriented in a direction parallel to the axis of the die orifice. When no longer constrained in the direction of extrusion the cross section of the extrudate expands. This phenomenon is known as die swell. Die swell usually occurs when the extrusion rate is high. Die swells could be avoided by reducing the rate of extrusion or by increasing the extrusion temperature while keeping the extrusion rate constant, to name a few.

2.1.1.5 Down streaming equipment

The extruders are often attached to a down streaming equipment/ processor or system. The down streaming equipment is used to finish, shape and analyse the extrudates (Kotler et al, 2010). The extrudates are usually extruded in the form of long spaghetti like strands or in the form of ribbons depending on the die used during extrusion thus cooling and cutting/ pelletizing is done using downstream processing equipment. Other down streaming equipment like Chill roller flaker is used to cool and chill the strands extruding from the extruder and cut them into flakes. Process Analytical tools can be attached as a down streaming equipment to carry out in process analysis to check homogeneity of the exrudates. Equipment for NIR (Near infrared spectroscopy), FT-IR (Infrared) analysis is usually attached. Figure 5 shows a pelletizer used to cut extrudates in to defined size after extrusion.

2.1.1.6 Monitoring gauges

The extruder has monitoring gauges on it. These are:

- Pressure gauges
- Temperature gauge
- Extrusion torque monitor
- Screw speed controller

The monitor gauges are used to control and monitor various conditions during extrusion.

2.1.2 Hot melt extrusion process

Hot melt extrusion process follows the following steps:

- Feeding the extruder using the feed hopper
- Conveying and kneading of mass by the screw
- Flow of molten mass through the die
- Exit from the die and downstream processing

A complete set up of pharmaceutical HME is shown in figure 2.6 below.

Figure 2.6: Thermo Scientific Pharma HME 16 extruder set up with air cooled conveyor and pelletizer (Thermoscientific, 2008)

Before extrusion, the different zones of the barrel are set to specific temperatures that are required during extrusion. The starting materials (feed stock) are fed into the hopper. This section has deeper flights or greater pitch of the conveying elements of the screws that are fitted in the feeding hopper. This screw geometry enables the materials to fall easily on to the heated screws of the barrel at the feeding section and conveyed further. The helix angle and the pitch determine the through put at constant rotation speed of the screws. The feed stock must have good flow properties. The angle of the feed hopper should exceed the angle of repose of the feed material for the feed stock to flow appropriately or else the feed stock will form a bridge at the throat of the hopper and will cause erratic flow (Crowley, 2003). A constant feed rate and screw speed will ensure there is a constant amount of material in the

extruder and thus the shear rate and residence time of the material in the extruder will also remain constant. The feed stock once on the barrel is conveyed as a solid plug on to the heated barrel. The heating is generated partly by the friction due to the shearing of the rotating screws as well as the electrical heating of the barrel. The temperature in the various sections in the barrel is monitored by a thermocouple. For efficient mass flow to occur in the feeding section; there should be low friction at the screw interface and high friction along the barrel. Apart from that, particle shape, bulk density and compression properties of the feed stock also contribute to the feeding efficiency (Crowley, 2003). Temperature at the feeding section can be adjusted accordingly such that there is efficient flow of materials by having optimum friction at the surface of the barrel as well as a rise in pressure to enable flow of materials so as to have an efficient output of extrudates from the die. Temperatures should also be adjusted so that the viscosity of the melt flowing is low enough for the melt to be conveyed along the barrel while keeping temperatures low enough to avoid any thermal degradation of the materials of the melt. Temperature increase can contribute to the depolymerisation of polymer chains where polymer chain scission may occur due to the shearing effect of the screws (McGinity et al, 2001). Inconsistent mass flow in the feeding section can cause ‘‘surge’’ phenomenon that will cause cyclical variations in the output rate, head pressure, and product quality (Swarbick, 2004).

The solid plug reaches a transition section (in between the feeding section and the compression section) where the materials of the feed stock are mixed and melting of these materials start. Upon reaching the compression section of the extruder, the materials start melting. The temperature along the compression zone is usually set at 30 °C to 60 ° C above the glass transition temperature of amorphous polymers or above melting point of crystalline material used in the feed stock (Swarbick, 2004). Compression occurs within this section by

decreasing the flight depth and maintaining a constant thread pitch (Martin, 2001). This would result in increased pressure and the materials to move along the barrel. The melt in the barrel moves by circulation in the helical path by means of transverse flow, drag flow, pressure flow and leakages (Crowley et al, 2007). The flow of materials could be reversed along the barrel by using a screw design that has kneading elements: reverse flighted 30° elements. The space between the screw diameter and the width of the barrel is normally about 0.1mm to 0.2mm (Martin, 2001). The molten material in the barrel from the compression section reaches the metering section in the form of a homogeneous plastic melt that is ready for extrusion through the die. Uniform extrudates are obtained by ensuring flow of the molten mass is uniform and that there is no stagnation at the different zones up to the die cavity.

The primary function of the melt metering section is to convey a homogenous melt to an appropriate die. Momentum and mass balance across the metering section indicate that the metering rate of an extruder can be increased by increasing the channel depth, decreasing the screw flight surface area, lowering the pressure drop across this section and by increasing the polymer viscosity.

While the factors that drive the three sections of the screw in the barrel of an extruder have been well characterized, the output of the extruder is dependent not only on the dynamics of each of the three sections but also on the interaction between them.

The temperature settings in all the three sections can be accurately controlled independently from low to high temperatures and degradation of materials can be minimized. Usually the residence time of the materials in the extruder is about 2 to 5 minutes depending on the screw speed and the feed rate (Crowley et al, 2004).

Kotler et al, (2010) have categorized the process variables of twin screw extruder as: Continuous and step changes. Continuous changes are modifications made during the running process while step changes are those made off line. The continuous changes include parameters such as feed rate, screw speed and temperature of the barrel while step changes include parameters such as screw design, die design and the barrel layout. The continuous changes are important changes that influence the output of the extrudates, residence time of the materials, mechanical energy and the temperature of the material (Kotler et al, 2010).

There is a relationship between the parameters: Screw speed, feed rate and temperature on the influence of residence time of the material in the extruder and the torque during extrusion as shown in Table 2.2.

	Residence time	**Torque**
Feed rate	↓	↑
Screw speed	↓	↑
Temperature	↓	↓

Table 2.2: Influence of screw speed, feed rate and temperature on the residence time of the material and the torque (Kotler et al, 2010).

2.1.3 Materials used for HME

Some of the properties of materials used for hot melt extrusion are:

- Must be of same purity and safety as the materials used in conventional pharmaceutical applications
- Must be able to deform and melt in the extruder and solidify upon exit from extruder
- Should be thermally stable at relevant processing temperatures though it may not necessarily tie down to thermo-labile compounds since the residence time in the extruder is relatively short.

Most materials used in pharmaceutical hot melt extrusion have been used for the production of tablets, capsules, pellets, granules and transdermals using the conventional manufacturing methods (Crowley, 2003).

The primary materials used for any hot melt extruded formulation are polymers (matrix carrier), drug and plasticizers. The other excipients that could be used in HME formulations are drug release modifying agents, bulking agents, thickening agents and lubricants if required.

2.1.3.1 Polymers

Polymers that are used in hot melt extruded formulations are important functional excipients when the drug is embedded in the polymer matrix. Polymers are selected on the basis of drug to polymer miscibility/solubility as well as the final dosage form to be extruded and the stability of the polymer when formulated into a final dosage form (Chokshi and Zia, 2004). The polymers used for hot melt extrusion must possess

thermoplastic characteristics and should be stable at temperatures used for extrusion. The other characteristics are that they should have suitable glass transition temperature (*Tg*) and melting temperature (*Tm*). The extrudability of polymers mainly depends on the *Tg* and *Tm* and melt viscosity. The *Tg* and Tm of polymers used for HME, should usually be between 50°C-180°C. Polymers with high *Tg* or Tm require high extrusion temperatures and can degrade the heat sensitive drugs at very high temperatures. Other features include low hygroscopicity; non toxic since large amounts of the polymers is to be used (Kotler et al, 2010). Polymers with high capacity of solubilisation are preferred since a larger drug load can be solubilised in the polymer. Lipophilicity, hydrogen bonding donors and acceptors (Foster et al, 2001) and amide groups are basic necessity for high solubilisation capacity of polymers (Kotler et al, 2010). Polymers of high molecular weight generate high viscosity melts and are difficult to melt. Most polymers however show thixotropic behaviour which means that with increase in shear stress, the viscosity decreases.

There are various polymers that were studied for the purpose of producing dosage forms by HME technique. Granules of diclofenac sodium were formulated with caranauba wax (Miyagawa et al, 1996, Sato et al, 1997, Miyagawa et al, 1999). Granules of diclofenac sodium were also formulated using agar, microcrystalline cellulose, polyethylene oxide, Eudragit® L 100 (Lyons et al, 2006). Diltiazem HCl pellets have been formulated using ethylcellulose, cellulose acetate butyrate, Eudragit® RS PM and poly (ethylene-co-vinyl-acetate) (Li et al, 2006 and Verhoeven et al, 2006). Besides pellets, HME was used to formulate tablets using various polymer systems. Chlorpheniramine maleate using polyethylene oxide (Crowley et al, 2002), Eudragit® RS PO (Zhu et al, 2002a and b).

The drug release kinetics from hot melt extruded formulations highly depend on the selection of polymers used as carriers during hot melt extrusion.

Polymers are water soluble, water insoluble and waxes. Examples of water soluble polymers used are: hydroxylpropyl cellulose, poly (vinyl) pyrollidone (PVP) and polyethylene oxide (PEO), xanthan gum, methylcellulose, hydroxy propy methylcellulose (HPMC).

Some examples of water insoluble or hydrophobic polymers are: Ethylcellulose, poly (ethylene-co-vinyl acetate). Example of a wax commonly used is carnauba wax.

Drug release depends on the type of polymer used as a carrier. Water soluble polymers release drug by diffusion (Keleb et al, 2010) and erosion mechanism (Repka et al, 2001), while drug release from water insoluble polymers is diffusion controlled (Crowley et al, 2007).

In order to have a zero order drug release or site specific drug delivery along the gastro intestinal tract; ionic or pH dependent polymers are used. A few commonly used pH dependent polymers are methacrylate copolymers such as Eudragit® L, Eudragit® FS, Eudragit® S, Eudragit® E, Carbopol®, highly deacetylated chitosan and xanthan gum. Commonly used pH independent polymers of poly methacrylate co-polymers used for controlled drug release are Eudragit® RS, Eudragit RL and Eudragit® NE. Table 2.3 give a list of polymers commonly used for pharmaceutical HME.

Chemical Name	Trade Name	Tg (°C)	Tm (°C)	Reference
Hydroxypropyl cellulose	Klucel®	130	-	(Sato et al, 1997; Repka et al, 1999, Repka et al, 2000a; Repka et al, 2000b; Repka et al, 2001a; Repka et al, 2001b, Repka et al, 2004)
Poly (ethylene oxide)	Polyox WSR	-67	65-80	(McGinity et al, 1997; Zhang et al, 1999; Repka et al, 1999; Repka et al, 2000a; Repka et al, 2000b; Crowley et al, 2002,
Poly(Vinyl pyrrolidone)	Kollidon®	168	-	(Follonier et al, 1995; Hamaura et al, 1999, Foster et al, 2001, Hulsman et al, 2001; Keleb et al, 2001.)
Polyethylene	-	-125	140	(Aitken-Nichol et al, 1996, Sprockel et al, 1997)
Xanthan gum	-	-	-	(Fukuda et al,2006)
Hydroxy propyl Methylcellulose (HPMC or Hypromellose)	Methocel	175	-	(Liu et al, 2001; Verreck et al, 2003, Six et al, 2003; Mehuys et al 2004)
Hydroxy propyl Methylcellulose Acetate Succinate	Aqoat-AS	-	-	(Nakamichi et al, 2001)
Hydroxypropyl Methylcellulose Phthalate	-	137	150	(Follonier et al, 1995; Nakamichi et al, 2002)
Polyvinyl Alcohol	Evanol			(Follonier et al, 1995)
Ammonium methacrylate copolymer	Eudragit® RS/RL	64	-	Follonier et al, 1994, Kidokoro et al, 2001, Zhu et al,2002 a&b, Crowley et al, 2007
Poly(dimethylaminoethylmethacrylate-co-methacrylic esters	Eudragit E	50		Aiteken-Nichol et al, 1996; Repka et al, 200, Repka et al, 2001
N-vinylpyrrolidone/ polyvinylacetate (60/40)	Kollidon VA 64	101		Feng et al, 2011
Polyvinylpyrrolidone/ polyvinylacetate	Kollidon® SR	39 and 152		Özgüney et al, 2009
Polyvinylcaprolactum-polyvinylacetate-polyethylene glycol graft copolymer	Soluplus®	70	-	Hardung et al, 2010
Metharcylic acid/ ethylacrylate	Kolicoat MAE	114		Kotler, 2010
Ethylene oxide/propylene oxide	Lutrol Poloxamer 407	57 -	 -	Karl et al, 2011 -
Macrogolgylcerol hydrostearate 40	Cremophor			Kotler, 2010

Table 2.3: Commonly used polymers for pharmaceutical HME

2.1.3.2 Plasticizers

Plasticizers are important ingredients used during HME to facilitate processing and modify the properties of polymers. They are usually low molecular weight compounds that soften polymers to make them more flexible and improve workability (Crowley et al, 2007). Plasticizers reduce the glass transition temperature (Tg) and melt viscosity of polymers. These plasticizers occupy the sites along the polymer chain, reduce polymer-polymer chain secondary bonding and provide more mobility for macromolecules of the polymer and thereby resulting in softer more easily deformable mass (Rahman and Brazel, 2004, Verreck e al, 2003) for easier processing. Plasticizers are commonly used with polymers that have high glass transition temperatures or in blend with polymers that can thermally degrade at higher temperatures. During HME process it is important for the polymers to have low melt viscosity since this will maximize output and yield; and minimize residence time of the materials in the heated barrel (Schilling, 2009) and will require less torque.

There are three theories of how the mechanism of plasticization of polymers occurs: the lubricity theory, the gel theory, the free volume theory and the polarity theory.

The lubricity theory: the plasticizer positions itself between the polymer chains so that attractive forces between the macromolecules are lowered and the gliding of the planes is facilitated during plastic flow (Schilling, 2009). The gel theory describes a polymer as a three-dimensional network structure that is stabilized by temporary points of attachment between the polymeric chains similar to a physically cross-linked gel.

Plasticizers reduce the number of contacts by solvation of the chains, resulting in a less rigid network of higher elasticity (Schilling, 2009). The free volume theory is based on the theory and principles of thermodynamics. According to this theory, the polymer volume is the sum

of occupied sites and non occupied sites or free volume. The free volume is the space between the atoms and molecules of the polymer in chain and is created by the movement of the polymer chain ends, side chains and the main chain or increasing the number of the polymer end groups. This movement is impacted by the molecular weight and structure of the polymer and temperature. Above Tg or in the presence of plasticizer, the molecules have enough energy to move, bend or even rotate thus the free volume increases and therefore the polymer chains have greater mobility (Wypych, 2004).

According to the polarity theory, the intermolecular forces between the plasticizer molecules, the polymer molecules and the polymer/plasticizer molecules must be well balanced to ensure that the gel is stable. Therefore, plasticizers contain one or more of both polar and nonpolar groups which must match the polarity of the polymer. The polarity of a plasticizer molecule depends on the presence of groups containing oxygen, phosphorus and sulphur. In terms of chemical structure, a plasticizer is a polar aromatic compound comprised of polar, polarizable and nonpolar portions (Zhu, 2002).

The polymer- plasticizer compatibility is defined as the ability of the plasticizer to form a homogeneous phase with the polymer without exudation of liquid-state plasticizers or crystallization of solid-state plasticizers (Wypych, 2004). In practice, semi empirical correlations of mutual solubility of substance and similar parameters of physical characteristics have been used. It is however important to know the solubility of plasticizer in polymer because the plasticizer needs to remain solubilised in the polymer at a molecular level during storage temperature. High compatible polymer-plasticizer blends show complete solubility during storage. Plasticizers should have similar solubility parameters with polymers to maximize compatibility. Compatible polymer- plasticizer blends should have similar solubility parameters with a difference or not more than 2 (Wypych, 2004).

Crowley et al, 2007 have given some characteristics of plasticizers used for the preparation of pharmaceutical dosage forms:

- Should have good efficiency
- Should have good stability
- Should be compatible with the polymer and other materials used during HME
- Should have good permanence

Plasticizers can be in solid or liquid state. Solid state plasticizers have an advantage over liquid plasticizers since they are easier to handle and process during extrusion. They can be dry blended with the drug and polymer without forming tacky pastes with poor flow properties during processing as well as they produce highly homogeneous blends with good flow properties. As compared to liquid plasticizers, solid plasticizers are less volatile and the evaporation loss during extrusion is minimized giving a homogeneously blended extrudates as the final product that has good output during extrusion.

Triacetin, citrate esters and low molecular weight polyethylene glycols, fatty acid esters and sebacate esters are some of the plasticizers commonly used for pharmaceutical hot melt extrusion. Some of the drugs used along with the polymers act as plasticizers by lowering the Tg of the polymer. Ibuprofen, guaiphenesin, ketoprofen and preservatives like methylparaben lower the Tg of the polymer and thus the processing temperature of the polymers since the polymers are miscible with them and are termed as non traditional solid state plasticizers (DeBarbender et al, 2002; Wu and McGinity, 2003; Crowley et al, 2004). Composites of 60/40 wt % ibuprofen/ethyl cellulose have been extruded at temperatures as low as 60°C, although the Tg of ethyl cellulose exceeds 130°C (DeBranbander, 2002).

Seibold, 2005 used supercritical carbon dioxide (CO_2) to extrude composites of 4-amino salicylic acid where the supercritical carbon dioxide at such temperature and pressure conditions exhibit gas-like transport properties but also demonstrate liquid-like solvent capacities and densities thus act like temporary plasticizer. The extruded composites showed better drug dissolution when extruded with super critical CO_2

Plasticizers have an influence on the physical, mechanical and drug release rate of pharmaceutical dosage forms. The effect of plasticizer on drug release was studied by Bodmeir et al, 1990 where propranolol HCL beads were coated with Eudragit® RS 30D containing Triethyl citrate (TEC). A U-shape drug release was found. The release rate constant was high at low plasticizer levels, then went through a minimum plateau, and increased at higher plasticizer concentrations. The U-shaped curve could be explained that at low plasticizer levels, the latex particles were insufficiently plasticized. This interfered with the coalescence or fusion of the latex particles and resulted in the faster release. At high levels of TEC, the increase in release rate constant could be explained with the leaching out of the TEC.

Zhu et al, 2002 researched on the influence of plasticization of Eudragit® RS PO chlorpheniramine meleate and TEC. With increase in percentage of Chlorpheniramine meleate, Diltiazem (DTZ) and TEC drug release rate increased.

Mehta et al, 2001 prepared multi-unit controlled release system of a poorly water soluble thiazole based leukotriene D4 antagonist containing Eudragit® L 100 55 and Eudragit® S 100 by an extrusion/spheronization technique (44). When 15% triethyl citrate, based on the Eudragit® weight, was incorporated into the pellet formulation with 1:1 and 1:3 ratios of Eudragit® L 100 55: Eudragit® S 100 respectively, enhanced drug release from the pellets

was observed compared with the corresponding pellets without the plasticizer. The authors attributed this result to the increased dissolution rate of the plasticized polymer.

Plasticizers help to improve the physical-mechanical properties of hot melt extrudates. Addition of plasticizers in transdermal films improves film flexibility (Aitken-Nichol et al, 1996; Repka et al, 1999a). Plasticizers have an influence on the product's tensile strength and elastic modulus by increasing the film coalescence.

2.1.3.3 Other processing Aids

Other functional excipients or processing aids used during HME may include plasticizers, glidants or thermal lubricants, stability enhancers (antioxidants and pigments) or release-modifying agents (pore-formers, gelling polymers, super disintegrants, retardants or electrolytes for pH modification, solubilising agents or surfactants).

During hot melt extrusion, polymers; whether unplasticized or unplasticized may undergo degradation at very high temperatures and cause their instability. The stability of the polymers can be improved by addition of antioxidants which can be classified as either preventive antioxidants or chain breaking antioxidants. The preventive antioxidants are materials that prevent initiation of free radical chain reactions (e.g. edetate disodium and citric acid) while reducing agents interfere with the autoxidation in a preventive manner since these materials preferentially undergo oxidation. Example of reducing agent is ascorbic acid. Hindered phenols and aromatic amines are used as chain breaking antioxidants that inhibit free radical chain reaction (Crowley et al, 2007).

Other examples of commonly used antioxidants in hot melt extrudates are Vitamin E, butylated hydroxyl toluene and butylated hydroxyanisole.

Thermal lubricants are used to facilitate extrusion. Examples of thermal lubricants used during extrusion are waxy materials like gylceryl monostearate (GSM). Thermal lubricants lower the melt viscosity during extrusion process and not necessarily lower the Tg temperature.

2.1.3.4 Drugs

Active pharmaceutical ingredients often limit processing and formulation options available to formulate dosage forms. Hot melt extrusion offers a number of advantages over traditional formulation processes. It is a solvent free process thus avoids any potential hydrolytic degradation caused by water or hydro alcoholic solvents used during traditional processes. Poorly compactable drugs can be easily processed into tablets or pellets using HME (Crowley et al, 2007). The drugs must be thermally stable to be used for hot melt extrusion. A thermal, physical and chemical assessment of the drugs must always be done before hot melt extruding. Depending on the unique properties of the drug substance and the other excipients in the formulation, the drug may be present as un dissolved i.e. solid dispersion or completely dissolved in polymer i.e. solid solution in the final dosage form. The state of the drug in the dosage form may have profound impact on the processibility and stability of the product (Crowley, 2003). Oxprenolol hydrochloride was shown to melt under the hot-melt extrusion processing conditions thus decreasing the viscosity of the extrudate to yield a material with poor handling properties (Follonier et al, 1994). Lidocaine was shown to lower the glass transition temperature of Eudragit® E/HDPE films (Aitken-Nichol et al, 1996). Ibuprofen acts like a non traditional plasticizer when extruded with Ethylcellulose where composites were

extruded at temperatures as low as 60 °C although the Tg of Ethylcellulose exceeds 130 °C (DeBrabender et al, 2000). Examples of drugs that have commonly been used along with carriers for hot melt extrusion are: Iburofen (DeBrabender et al, 2000, and 2002), Nifedipine (Foster et al, 2001), Indomethacin (Foster et al, 2001), Chlorpheneramine maleate (Zhang et al, 1999; Repka et al, 2000), Theophylline (Henrist and Remon, 1999; Young et al, 2002), Dilitiazem Hydrochloride (Follonier et al, 1995), Carbamezapine (Perisutti et al, 2002), Acetoaminophen (Ndindayino et al, 2002), Metoprolol tartrate (Verhoeven et al, 2009), Beclutamide (Andrews, 2010), Naproxen and Frusemide (Kindermann et al, 2011).

2.2 Solid dispersions

Solid dispersion technology is the science of dispersing one or more active ingredients in an inert matrix in the solid state in order to achieve altered solid-state properties such as increased solubility and dissolution rate, sustained drug release and improved stability (Habib, 2001).

By definition, solid dispersions and solid solutions can be differentiated based on the molecular state of the drug in the carrier matrix (polymer). If the drug is dissolved at molecular level, i.e. the drug forms one phase system with polymer, it is referred as a solid solution; whereas, if the drug is in a two phase system with polymer and forms a microcrystalline dispersion, it is generally referred to as a solid dispersion (Chokshi & Zia, 2004).

The drug can be molecularly dispersed in the polymer, in amorphous form or in crystalline form. Therefore, based on the polymer and drug molecular arrangement, different types of solid dispersions can be distinguished. Regarding pharmaceutical application, 3 sub-classifications have gained more importance: solid crystalline suspension, solid glassy

suspension and solid glassy solution (also mentioned as solid solution) (Van Doorge et al, 2006). Classification of Solid dispersions is shown in Figure 2.7 and the characteristics of solid dispersions are shown in Table 2.4.

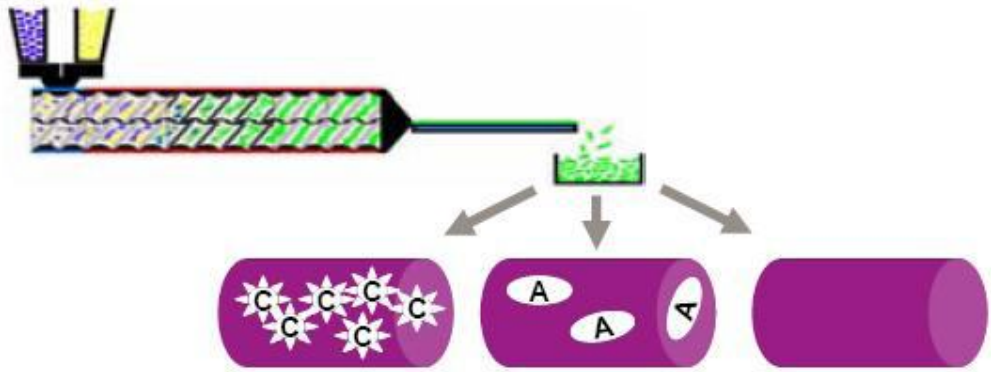


Figure 2.7: Schematic representation of three sub- classifications of solid dispersions. From Left to right: solid crystalline suspension, solid glassy suspension, and solid glassy solution. Key: A is Amorphous drug phase and C is crystalline drug phase.

Table 2.4: Characteristics of solid dispersions (Kolter et al, 2010)

Extrudate	Solid crystalline suspension	Solid glassy suspension	Solid glassy solution
Polymer phase	Amorphous	Amorphous	Amorphous
Drug phase	Crystalline	Amorphous	Amorphous/molecularly dissolved
Appearance	Opaque	Translucent	Translucent
A DSC will find	Glass transition (Tg) and Melting peak (Tm)	2 x Glass transition (Tg)	1 x Glass transition (Tg)
Thermodynamic stability	Almost stable	Unstable (Kinetically controlled)	Stable (drug below saturation solubility)

In a solid glassy solution, the drug is molecularly dispersed in the polymer phase, resulting in a single phase solid system. The drug and the polymer are both in the amorphous state and as a consequence there is only one Tg that can be distinguished on a DSC thermogram. In a solid glassy suspension, the drug still exists in an amorphous form in the polymer. However, it is not molecularly dispersed (there are no chemical interactions between drug and polymer) resulting in a two phase solid system. In this case, two Tg's can be distinguished on the DSC thermogram, confirming that there are still two distinct phases present in the extrudates. In a solid crystalline suspension, the drug is in the crystalline form, giving the extrudate an opaque appearance. On a DSC thermogram just one Tg is detected (polymer Tg) and one or more melting peaks, corresponding to the drug crystalline phase. A solid glassy suspension is thermodynamically less stable than a solid crystalline suspension. For these reasons the drug can recrystallize as a result of heat or humidity (Goldberg et al., 1965).

For poor water soluble drugs, solid dispersions (particularly solid glassy suspensions or solid glassy solutions) have several advantages including provision of drug in at reduced particle size and increased surface area which can result in increased dissolution rates, with an improved bioavailability. For glassy solid dispersions, the drug is presented in a high energy amorphous state demonstrating markedly improved solubility and dissolution properties. The wettability of the drug can also be improved through homogeneous dispersion in a matrix constructed from a hydrophilic polymer, leading to enhanced dissolution rate. Similar, greater porosity of drug particles can also be achieved, which might also, result in a higher dissolution rate (Dhirendra et al., 2009). In the case of highly water soluble drugs, a crystalline suspension (thermodynamically more stable) of the drug in a matrix created by a hydrophobic polymer, can retard drug release opening up the potential to produce sustained release preparations.

Improvement in bioavailability of poorly soluble compounds (Biopharmaceutical Classification System (BCS): Class II) with these systems is primarily based on improved dissolution rates. In the case of solid dispersions, this is achieved by a combination of particle size reduction, improvements in wetting behaviour in addition to deagglomeration and micellization of the drug with hydrophilic polymers. In case of solid solutions, improvement in dissolution rate is due to high energy amorphous nature of the drug. Thermodynamically, solid solutions are more unstable compared to solid dispersions because in the solid solution the drug exists in a high energy amorphous form, which is prone to precipitation or crystallization under environmental stress such as moisture and heat during processing and storage (Chokshi and Zia, 2004).

As described above, by judicious choice of the polymers it is possible to delay or slow down the release of drug by formulating it as a solid dispersion. A wide variety of polymers that are poorly soluble or which swell under aqueous conditions have potential as carriers for controlled release dosage forms (Leuner & Dressman, 2000). Insoluble or hydrophobic polymer carriers are also used to produce controlled-release pharmaceutical formulations. This approach therefore provides an accessible means by which to modify the release of drugs using techniques such as hot melt extrusion.

2.3 Controlled release oral dosage forms for Zidovudine and Lamivudine

The oral route of administration in today's day and age represents as one of the most preferable and predominantly used routes of administration for HIV/AIDS paediatric patients on antiretroviral medications. Unlike the other dosage forms administration such as parenteral dosage forms, oral dosage forms allow easy and convenient drug administration with no need for intervention from a health care practitioner. Oral drug delivery systems (ODDS) are classified into immediate release systems and extended release systems. The immediate release dosage forms disintegrate fast and release drugs for immediate action.

Dosage forms with controlled release properties provide numerous advantages over conventional, immediate release systems (Ghebre-Sellassie, 1994).

The advantages of controlled release dosage forms are:

i. Fewer and more effective doses - which results in improved patient compliance, a more constant drug plasma level through zero-order release thus improved drug efficacy and safety ratio,
ii. The reduced frequency of administration with better effectiveness would result in more cost effective treatment.
iii. A lower amount of drug necessary for the same effect, thus fewer drugs induced side effects (Huang & Brazel, 2001).
iv. The materials used must be biocompatible with body tissues during the prolonged contact, non-toxic and non-degradable.

When using an immediate release formulation, the blood level of the drug rises after each administration to decrease until the next administration. As some fluctuations are expected, it is necessary that the blood level of the drug remains between a maximum value – (the maximum tolerated concentration) and a minimum value (below which the drug has no further therapeutic effect) otherwise termed as therapeutic window. The drugs used for modified release dosage forms should maintain a constant plasma level over a prolonged period as well as have a broad therapeutic window to avoid any health hazard to the patient in case of undesirable burst release of the initial loading dose (Hoichman et al, 2004). Figure 2.8 below illustrates the drug levels in blood with both traditional and controlled release formulations.

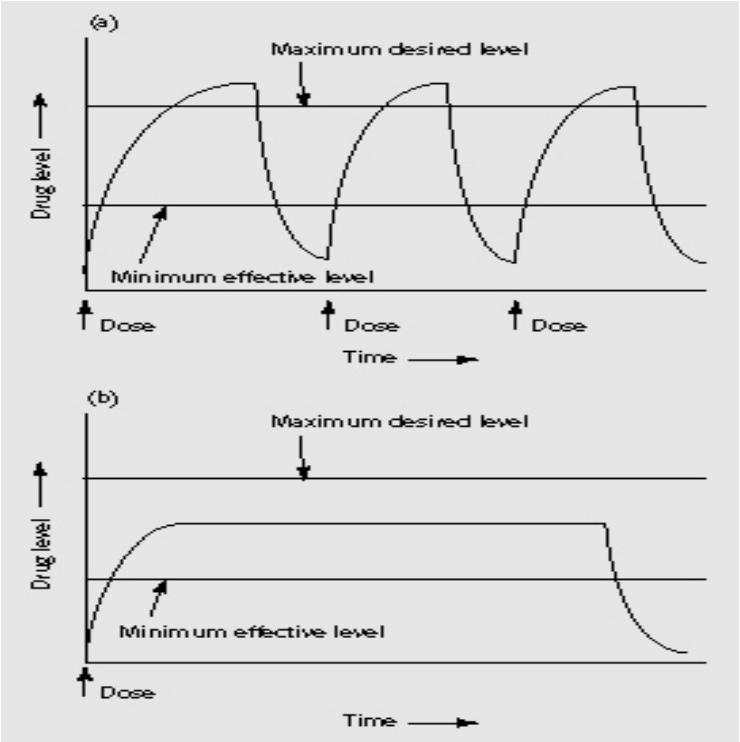


Figure 2.8: Drug concentration in blood in a) traditional formulation b) controlled release/modified release formulation.

Sustained release systems, are characterized by their ability to either control the rate or the area of drug release so as to improve the pharmacokinetic profile of the drug and improve patient compliance as well as reducing side effects (Sansom, 1999 and Getsios et al, 2004). With respect to oral drug delivery, the release can either be sustained during the transit of the dosage form in the gastro-intestinal tract or delayed to regions subsequent to the stomach including the small intestine or the colon.

Various chemical and physical approaches have been used to achieve controlled release profile of the drug for oral dosage forms and thereby have control of drug absorption into the systemic circulation (Qiu and Zhang, 2000). The three main principles used to achieve extended release profile are: 1) modification of the biological system (e.g. inhibition of the

renal clearance by probenicid) 2) modification of the drugs (e.g. crystal modifications, drug solubility modifications, chelation products, prodrugs or probiotic use) are in practice not often applied (Remon & Vervaet, 2002; Varshosaz et al, 2006) and 3) modification of dosage form which is the most common and efficient methods to achieve sustained release. It is used frequently and based mainly on the following mechanisms: (1) diffusion of the drug species from or through the system; (2) a chemical or enzymatic reaction leading to degradation of the system, or cleaving of the drug from the system; and (3) solvent activation, either through osmosis or swelling of the system. A combination of mechanisms is possible (Langer, 1998).

Most oral sustained release technologies are based on polymeric systems. These polymeric systems are broadly categorised into the following: i) membrane controlled or reservoir system ii) osmotic systems and iii) matrix systems (Qui, 2009).

The reservoir or membrane system is a system where the drug is entrapped in a polymeric shell or coat. This system requires coating of the drug core with insoluble, pH independent polymer. Such systems consist of dosage forms like capsules, pellets and coated tablets. Tablet coating is an expensive and time consuming process and moreover has problems like drug release reproducibility, dose dumping and no release of large molecules or drugs of ionic species (Langer, 1998; De Brabander et al., 2003). Examples of polymers used for reservoir systems are: ethyl cellulose, methyl cellulose and methacrylate esters copolymer (Ventakraman et al, 2000).

The osmotic system is a system that employs osmotic pressure as a driving force to generate a constant drug release provided a constant osmotic pressure is maintained while a few other physical systems are constrained (Remingtons, 2006). The pressure applied to the higher-concentration side to inhibit solvent flow is called the osmotic pressure. The system contains

an osmotically active drug embedded in a tablet core or an osmotically inactive drug with an osmotically active salt surrounded by a semi permeable membrane containing a small orifice. The semi permeable membrane allows free diffusion of water but not the drug. Upon contact with the dissolution media in the body water will flow into the tablet core and set up an osmotic gradient that controls the release of the drug. Most osmotic systems are able to achieve a zero order release rate which can be predicted by modulating the release rate parameters.

Sustained release matrix systems can be classified into either single unit also known as monoliths (tablets, coated tablets) or multi unit. Multiple units (pellets, granules, microparticles, nanoparticles) with controlled release properties tend to provide better pharmacokinetic properties and formulation flexibility (Ghebre-Sellassie, 1994). Particles small enough to pass through the stomach pylorus spread more rapidly and homogeneously in the intestine independently of gastric emptying and feeding state. The drug absorption can be maximized while plasma level fluctuations and intra- and inter-subject variations are reduced. In contrast to monolithic dosage forms including film-coated tablets, high local concentrations and dose dumping are avoided (Schilling, 2010). Multi particulates like pellets can be loaded with different drugs and can be blended and formulated in a single dosage form. This allows the administration of two or more types of drugs that may or not be chemically compatible, at the same or different sites within the gastro-intestinal tract. Furthermore, pellets with different release rates from the same drug can be combined in a single unit dosage form in order to achieve the desired drug release profile (Pearnchob, 2002).

Matrix systems involve the drug embedded homogeneously throughout polymer/carrier. Natural, semi-synthetic and synthetic polymers are used as matrix formers and carriers to

provide solid dosage forms with the desired controlled release rates. Matrix systems are most widely used for extended drug delivery due to the following reasons:

1. It is a versatile and effective drug delivery system to achieve extended drug release.
2. It is easy to manufacture using conventional processes and equipment
3. It is cost effective.
4. This system can accommodate both high and low drug loading with significant control on the tablet size which increases the formulation options.
5. It can also accommodate drugs with low and high solubility.
6. Design flexibility. Single or combination of polymers can be used to formulate the system. Polymers with different characteristics can be combined in a system to achieve unique advantages of the system.

Hydrophilic and hydrophobic polymers are used either alone or in combination to formulate matrix devices so as to achieve the desired drug release rates.

The hydrophilic polymers are water soluble in nature. They hydrate and swell when in contact with water, forming a gel on the surface of the system (Qui and Zhou, 2011). The following three modes occur with hydrophilic polymers: swelling, diffusion and dissolution and are explained as follows (Colombo et al, 2000):

i. Water concentration gradients are formed at polymer-water interface. This results in imbibition of the water into the matrix. This process depends on the physical status of the system. In dry systems the diffusion coefficient is low, where as in swollen gels it is similar to that of pure water. Water acts as a plasticizer and reduces the glass transition temperature of the polymer. Once the concentration of water is high enough

to reduce the glass transition temperature to the temperature of the system, the polymer chains undergo transition from glass to rubbery state.

ii. Water imbibition causes polymer swelling and results in dimensional changes of the system. Geometric dimensions of the system increase as a result of swelling.

iii. Contact of water with the drug in the system aid the dissolution of the drug and the dissolved drug diffuses out of the system.

iv. Continuous water penetration increases water content in the system. Increasing water content substantially increases the diffusion coefficient.

v. Poorly soluble drugs do not dissolve freely in the available water in the system. Thus, coexistence of dissolved and un-dissolved species of drug can be observed. However, non – dissolved drug does not diffuse out.

vi. Systems containing high loading of freely soluble drugs undergo structural changes as drug diffuses out. As drug diffuses out the matrix becomes more porous and offers less resistance to the remaining drug in the matrix.

vii. Depending on the polymer type, extent of cross linking and degree of substitution, the polymer dissolves slowly or rapidly. If the entire drug is released before any significant reduction in polymer content due to its dissolution, then this phenomenon is less important.

The systems described are termed as fronts in the matrix device as shown in Figure 2.9.

Overall, during the diffusion stages in the gel phase, drug release is dependent on drug solubility and its loading. The rate of diffusion in the gel phase is dependent on the rate of dissolution of the drug in the gel.

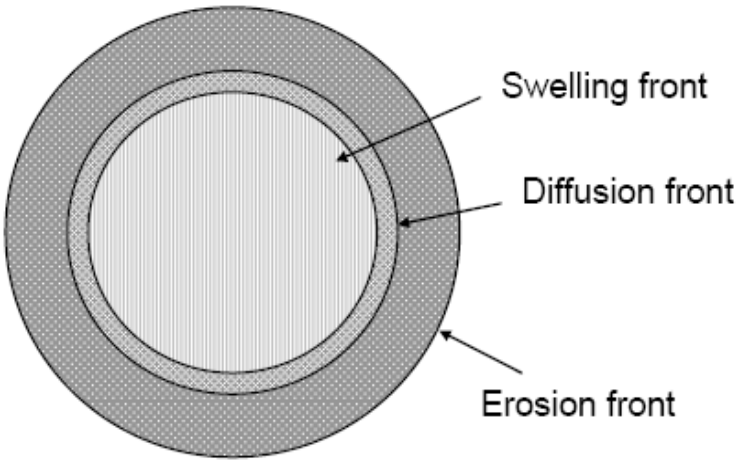


Figure 2.9: The various fronts in a hydrophilic matrix system

Hydrophobic polymers are insoluble in water and the major release rate controlling mechanism is the insoluble matrix properties. Unlike hydrophilic matrix systems, in presence of water there is negligible or no increase in surface or dimensions of the system. Drug release is mainly by dissolution and diffusion. The reason for attenuation of drug release rate is based on the following release mechanism. When a matrix system consisting of a solid dispersion of a drug in an insoluble matrix is placed in the dissolution medium, the initial drug release occurs from the tablet's superficial layers and, consequently, the release rate is relatively fast. As time passes the external layers of the matrix become depleted of the drug and water molecules must travel through long, tortuous channels to reach the drug remaining in the deeper layers of the matrix. Similarly, the drug solution that is formed within the matrix must diffuse through long capillaries to reach the external dissolution medium. The primary reason for the continuously decreasing rate of drug release is the increasing distance

that must be traversed by water and drug molecules into, and out of, the matrix, respectively (Islam et al, 2010).

Downstream processing of monolithic, multiparticulate systems is important so as to improve patient compliance and dose accuracy. Multiparticulate systems are commonly filled in capsules for ease of administration or compressed into tablets or simply pelletized into mini tablets or mini matrices.

A promising technique to produce sustained release mini matrices is hot melt extrusion. Follonier and co-workers (1995) researched on producing sustained release diltiazem pellets using four different polymers ethylcellulose, cellulose acetate butyrate poly(ethylene-co-vinyl acetate) and Eudragit® RS PM. Drug loading of 30- 70% were used in this research and the results showed that the rate and extent of drug release were due to the carrier polymer used, drug loading and the pellet particle size. De Brabender and co-workers (2003) developed sustained release ibuprofen mini matrices with ethylcellulose using hot melt extrusion. Drug release was very slow despite high drug loadings and therefore hydrophilic polymers like hydroxyl propyl methyl cellulose was added which increased the drug release by great extent though initial burst effect of the drug during release was observed. Xanthan gum was then included in the formulations to eliminate the initial burst effect and achieve close to zero order drug release profile. Miyagawa and co-workers (1996) used twin screw extruder to produce cylindrical sustained release pellets of ibuprofen using carnauba wax. Their investigations showed that carnauba wax could be extruded well below its melting temperature and have high mechanical strength. The drug release from the wax matrix pellets were influenced by the formulation. Özgüney and co-workers (2009) developed and characterized Kollidon® SR extended mini matrices of Ibuprofen and Theophylline, on a poor water soluble drug and the other a highly water soluble drug. Ibuprofen had a plasticizing

effect on Kollidon® SR while theophylline had no such effect on Kollidon® SR and both drugs showed that release rates were dependent on drug loading. In case of Theophylline however, increase in temperature during extrusion resulted in decrease in drug release.

2.4 HME marketed Products

Hot melt extrusion is a rapidly growing technique for the production of solid oral dosage forms. U.S. and Germany so far hold about 56% of all the issued patents for HME based pharmaceutical products. Recently, a pharmaceutical company (SOLIQS) has developed a proprietary Meltrex® formulation and redeveloped a protease inhibitor (retinovir/ lopinavir) combination product, Kaletra, for the treatment of HIV. Hot melt extruded Kaletra tablets were shown to have significant advantages of patient compliance over the soft gel capsules due to reduced dosing frequency and improved stability.

2.5 Objectives of the Study

The overall objective of these studies was to explore the interactions and rheological behaviour of AZT/3TC and rate modifying polymers under conditions typically experienced during HME and so predict the optimal qualitative compositions for processing. On the basis of this understanding, the primary aim was to evaluate and select the appropriate processing conditions and quantities of inactive ingredients predicted to give desirable rate and extent of exposure in humans for each of the model drug compounds from a mini-matrix combination formulation with the associated aim of determining the relationship between composition and mechanism of drug release.

The specific objectives of the study were :

1. Pre-formulation studies to characterize the thermal characteristics, rheological properties and miscibility of AZT and 3TC with plasticizers and rate modifying polymers including ethylcellulose, Kollidon® SR and PEO.
2. Determination of their suitability for hot melt extrusion and prediction of the most appropriate qualitative compositions for producing sustained release mini-matrix formulations.
3. To determine a suitable quantitative composition giving desirable release for both drugs whilst determining the impact of composition on the mechanism of drug release and predicting the influence on likely in-vivo performance.

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CHAPTER THREE

PRE FORMULATION STUDIES: THERMAL, RHEOLOGICAL AND MISCIBILITY CHARACTERIZATION OF ZIDOVUDINE/LAMIVUDINE, PLASTICIZER AND POLYMER PHYSICAL MIXTURES TO ASSESS THEIR SUITABILITY FOR HOT MELT EXTRUSION.

Abstract

The influence of antiretroviral drugs and plasticizers on the rheological and thermal characteristics of ethyl cellulose, Kollidon® SR and Polyethylene oxide (PEO) formulations intended for hot melt extrusion have been investigated. Antiretroviral drugs used were Zidovudine (AZT) and Lamivudine (3TC), whilst plasticizers included triethylcitrate (TEC) and polyethylene glycol (PEG-6000). Physical mixtures containing polymers with varying concentrations of drugs and plasticizers were characterized using differential scanning calorimetry (DSC) and parallel plate oscillatory rheometry. Similar studies were done with Physical mixtures of Kollidon® SR and drugs at varying concentrations.

The viscosity of physical mixtures containing both drugs was lower than observed for pure ethyl cellulose, indicating that the drugs had a plasticising effect. This was confirmed by lowering of the glass transition temperature (Tg) of ethyl cellulose. At the highest loading of 40% by weight, lamivudine appeared to become saturated within the polymer, causing an increase in viscosity and showing evidence of recrystallization upon cooling. In the case of Kollidon® SR viscosity decreased with increase in the percentages of AZT, 3TC and TEC compared to pure Kollidon® SR. TEC at concentrations of 5% and 10%w/w were shown to

give mixtures with adequate rheological behaviour for extrusion. Formulations with highest concentrations of TEC however, showed phase separation

Both TEC and PEG-6000 were found to lower the Tg of ethyl cellulose, although PEG-6000 recrystallized upon cooling which makes it unsuitable for use in the proposed controlled release formulations. Both plasticizers were also shown to reduce the viscosity of ethyl cellulose, more significantly than for TEC. The results indicate that formulations containing ethyl cellulose, zidovudine and lamivudine mixed with TEC are suitable for processing by hot melt extrusion. Both drugs were completely soluble in Kollidon® SR while formed a solid crystalline dispersion with PEO

3.0 Introduction

Hot melt extrusion (HME) is a solvent free processing technique used to produce a range of formulations including monolithic systems. During HME, the molten plastic mass containing polymer and other suitable additives such as plasticizers is melted by the action of an Archimedean screw rotating in a heated barrel and forced at high temperature through a die (Paradkar et al, 2008). Opportunities therefore exist for thermal degradation of the active compounds and the carrier, due to the high processing temperatures used. This poses a limitation for thermally sensitive active compounds and carriers.

The thermal and mechanical degradation of formulation components during polymer extrusion have been reported (Capone et al, 2007 and Murphy et al, 2008). These studies provide information on the temperatures which can be used successfully to provide solid dispersions of appropriate quality.

Pharmaceutical polymers widely used in HME include synthetic cellulose derivatives, methacrylate polymers, polyethylene oxide (PEO), polyvinyl pyrrolidone and pyrrolidone-co-vinyl acetate (Crowley et al, 2007). Plasticizers are commonly incorporated alongside these polymers during HME to improve the workability and flexibility of the formulation by increasing intermolecular separation and include triethyl citrate (TEC), propylene glycol, polyethylene glycols (PEG) and diethyl phthalate. Wang et al, 1997 and Ghebremeskel et al, 2007 reported that plasticizers facilitate reductions in elastic modulus, tensile strength, polymer melt viscosity and glass transition temperature (Tg).

Solubility parameters have previously been used as a pre-formulation tool to predict the miscibility between active pharmaceutical ingredients and the polymers used in medicinal products (Hancock et al, 1997, Foster et al, 2001, Chokshi et al., 2005 and Greenlagh et al., 1999).

Modulated temperature DSC enables determination of the glass transition temperature (Tg) of polymers and their respective mixtures, which provide an indication of relaxation time (t) associated with molecular motion that can vary with heating and cooling rates. Temperature dependence of molecular motion determines the physical properties of materials (Hancock and Zografi, 1997) and so other measures of physical characteristics are required beyond the thermal properties of these systems. As an example, the viscosity of a polymer melt (i.e. its resistance to flow) decreases with increasing temperature due to higher levels of molecular motion and inter-molecular separation, which effectively determines the ability to process materials by extrusion.

Knowledge of polymer melt rheology is therefore an important element in HME for determining the suitability of polymer formulations for processing as well as giving

information on the likely quality of finished products, where melt viscosity may be affected by the drug loading at a given shear rate. For the mass of melt to flow through the extruder via the screws, viscosity of the melt must be appropriate along with the temperature so that the torque required to rotate the screw of the extruder is not overloaded. Perisutti et al, 2002 evaluated the rheological properties of carabamezapine and PEG 4000 as a potential rapid release dosage form using a capillary extrusion rheometer. Verreck et al, 2006, studied the extrusion torque and die head pressure as indicators of melt viscosity during extrusion of ethyl cellulose with p-amino salicylic acid using carbon dioxide as a plasticizer.

Ultimately the rheological behaviour of the formulation components determines the ability to extrude, which is dictated by properties of both drugs and polymers. High viscosity formulations can cause excessively high torque levels, high residence times in the extruder and lead to degradation. (Lyons et al, 2007). Temperatures encountered during extrusion should not approach the degradation temperature of the active ingredient.

Oscillatory rheometry is a sensitive technique used to characterise the viscoelastic flow behaviour of polymer melts and other soft solids at a range of processing rates and set temperatures, for which their rheological properties of depend greatly upon molecular weight and structure (Suwardie et al, 2011).

In this study, Zidovudine (AZT) and lamivudine (3TC) have been evaluated for use in HME process for the production of sustained release mini-matrices.

Ethyl cellulose is a polymer with a history of use in modified release formulations for active pharmaceutical ingredients and is compatible with HME. There have been several investigations recently on the use of Kollidon® SR for production of sustained release matrices using HME (Özgüney, 2009). High molecular weight Polyethylene Oxide polymers

are often used in modified release formulations either alone or as pore formers along with hydrophobic polymers. An evaluation of the suitability of the ethylcellulose, Kollidon® SR and PEO formulations containing both drugs and a suitable plasticizer is however required.

Despite numerous investigations of the physicochemical and rheological characteristics of polymer melts with drugs, no reports of ethyl cellulose, Kollidon® SR and PEO behaviour when processed with Zidovudine and Lamivudine have been published. The objective of this study was therefore to evaluate the influence of Zidovudine, Lamivudine and plasticizers on the rheological and thermal properties of ethyl cellulose and assess their suitability for HME to produce controlled release matrices.

3.1 Materials and Methods

3.1.1 Materials

Ethylcellulose STD 10 premium with viscosity of 10.3 mPa.s (cps) ethoxyl content of 49.4% and Polyethylene Oxide (POLYOX™ PEO-WSR N-80) with approximate molecular weight of 200,000 Da. were donated by Colorcon, Dartford, UK. Kollidon® SR was donated by BASF SE, Ludwigshafen, Germany. Zidovudine (AZT) and lamivudine (3TC) were gifts from Cosmos Pharmaceuticals, Nairobi Kenya, whilst triethyl citrate (TEC) and polyethylene glycol (PEG 6000) were supplied by Sigma-Aldrich, UK.

3.1.1.1 Ethylcellulose

Ethylcellulose is an ethyl ether of cellulose and long chain polymer of β- anhydroglucose units (Figure 3.1). Ethylcellulose ethers are a family of inert hydrophobic polymers (organosoluble thermoplastics) that are essentially tasteless, odourless, colourless, non-caloric and physiologically inert. Ethylcellulose is useful in a variety of pharmaceutical applications: as rate-controlling polymer in matrix tablets, as tablet binder and cushioning agent, as viscosity- increasing agent, as coating polymer, as granulation binder and as matrix for solid dispersions (Rowe *et al*, 2003). Ethylcellulose is insoluble and provides pH independent drug release in oral dosage forms. Ethylcellulose in this study was used as a modified release/sustained release agent.

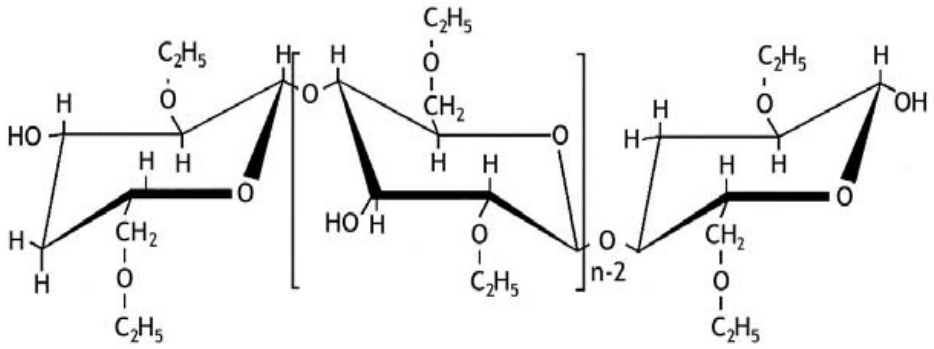


Figure 3.1: Molecular structure of Ethylcellulose

3.1.1.2 Kollidon® SR

Kollidon® SR is a relatively new sustained release polymer. It is physical mixture of 80% poly vinyl acetate and 20% polyvinyl pyrrolidone (povidone) as shown in Figure 3.2 and is stabilized by with 0.8% sodium lauryl sulphate and 0.2% colloidal silica. It appears as a white slightly yellowish free flowing powder and is insoluble in water (Kotler et al, 2010).It has excellent free flowing characteristics.

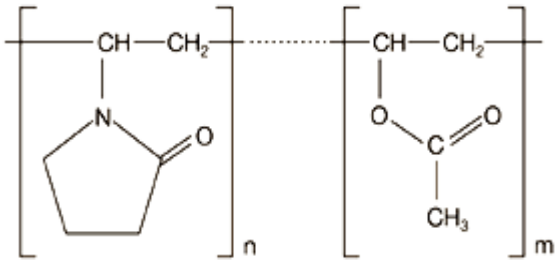


Figure 3.2: Chemical structure of polyvinyl pyrrolidone (Povidone) and polyvinyl acetate.

3.1.1.3 Polyethylene oxide or PEO (POLYOX™ WSR N-80)

Polyethylene oxide (PEO) is water soluble resin that is non-ionic, linear and high molecular weight, thermoplastic homo polymer manufactured by heterogeneous catalytic polymerization of ethylene oxide monomer (Crowley et al, 2002). The chemical structure of Polyethylene oxide is shown in figure 3.3. PEO (POLYOX™) are white semi crystalline, free flowing powders and are available in a wide variety of molecular weight grades that range from 100,000 thousand to 8,000,000 Da (Rowe et al, 2003) and have a melting temperature range of 57-73°C. The molecular weight of POLYOX™ WSR N-80 is approximately 200,000 Da. It is miscible in water in all ratios due to the hydration of ether oxygen (Crowley et al, 2002). PEO with smaller particle sizes have lower molecular weight

as well lower melting temperature and those with larger particle sizes have higher molecular weight and higher melting temperature.

PEO has commonly been used in the pharmaceutical industry in: controlled release and modified release solid dose matrix (Kim, 1995), as tablet binder (Rowe et al, 2003), transdermal delivery systems, mucosal bioadhesives, nanoparticles (Zweers et al, 2004), hydrogel (Dhawan et al, 2005), solid dispersion (Abu- Diak et al, 2011) and hot melt extruded dosage forms like (Crowley et al, 2002, Crowley et at, 2004, Prodduturi et al, 2005).

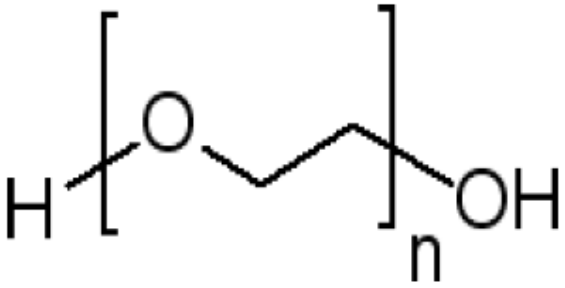


Figure 3.3: Molecular structure of Polyethylene oxide

3.1.2 Methods

3.1.2.1 True density Measurements

Approximately 5mg of AZT, 3TC and the polymers was weighed and a helium pycnometer (Accupyc 1330, Micromeritics, Norcross, GA) was used to determine the true density in triplicate. The true density obtained was used to calculate the glass transition temperature (Tg) using the Gordon Taylor equation described in section 3.1.2.5.

3.1.2.2 Preparation of Physical mixtures

Polymer: drug physical mixtures were produced by dry mixing the component powders in the appropriate ratios using a mortar and pestle for 10-15 min. Polymer: drug mixtures were prepared at ratios 80:20, 70:30, 60: 40% w/w. Physical mixtures of polymers with TEC and PEG 6000 were prepared at ratios of 95:5, 90:10, 80:20, 70:30, and 60:40% w/w using a similar process.

3.1.2.3 Solubility Parameter Calculation

The solubility or miscibility of drugs or Active Pharmaceutical ingredients (APIs) with polymer during formulation of solid dispersions can be predicted by using the solubility parameter calculations (Nair et al, 2001, Chokshi et al, 2005, Ghebremeskel et al, 2007, Maniruzzaman et al, 2011, Liu et al, 2011). The solubility parameter (δ) is one way to quantify the cohesive energy of a material, which in turn determines many of the critical physico-chemical properties (e.g. solubility, melting point, incompatibility) of drugs and excipients. There are group contribution methods, such as the Hansen's approach, by which solubility parameters can be calculated.

The Hansen solubility parameters of the drugs and the polymer were calculated from the chemical structure using the group contribution method described by Hofyzer/Van Krevelen (Foster et al, 2001) given in equations 1 and 2.

$$\delta^2 = \delta^2_d + \delta^2_p + \delta^2_h \quad (1)$$

Where

$$\delta_d = \Sigma F_{di}/V \quad \delta_P = \sqrt{\Sigma F^2_{pi}}/V \quad \delta_h = \sqrt{\Sigma E_{hi}}/V \quad (2)$$

δ is the total solubility parameter; δ_d is contribution from dispersion forces; δ_P is the contribution from polar interactions; δ_h is the contribution factor from hydrogen bonding, V is the molar volume; F_{di} is the molar attraction constant due to dispersion component; F_{pi} is the molar attraction constant due to polar component; E_{hi} is the hydrogen bond energy and V is the molar volume.

Solubility parameters have been used by many authors to predict the miscibility of drugs in polymers (Foster et al., 2001; Hancock et al., 1997; Chokshi et al., 2005; Greenlagh et al., 1999.). Foster et al., 2001 further suggested that interactions between polar (δ_P) and hydrogen bonding groups $E_{hi,}$ of the component drug and excipients may also affect solubility.

The solubility parameters for zidovudine and lamivudine were calculated using knowledge of molecular structure (Figure 3.4).

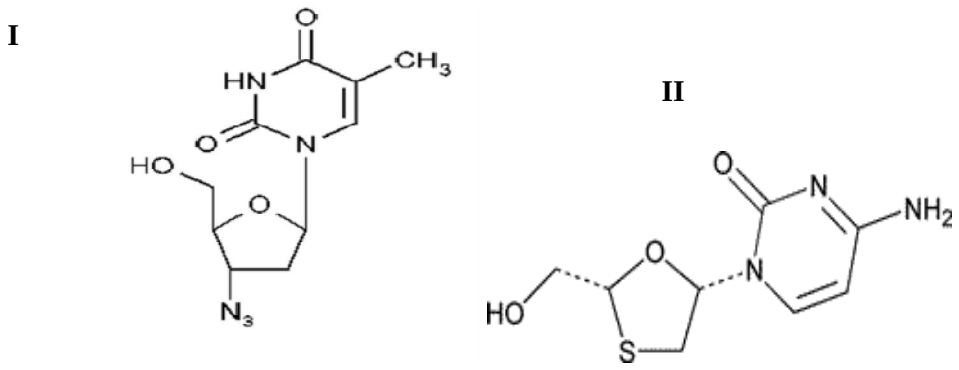


Figure 3.4: Molecular structure of zidovudine (I) and Lamivudine (II)

3.1.2.4 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) measures the amount and rate of change in weight of the samples as a function of time or temperature. The measurements are used primarily to to predict the thermal stability of materials at temperatures as high as 1000ºC. TG is used to characterize samples that exhibit weight loss or weight gain due to oxidation, dehydration or decomposition.

The main components of a TGA system are; the electronic microbalance and a controller, a furnace, temperature sensors, programmer and recording device (Figure 3.5).

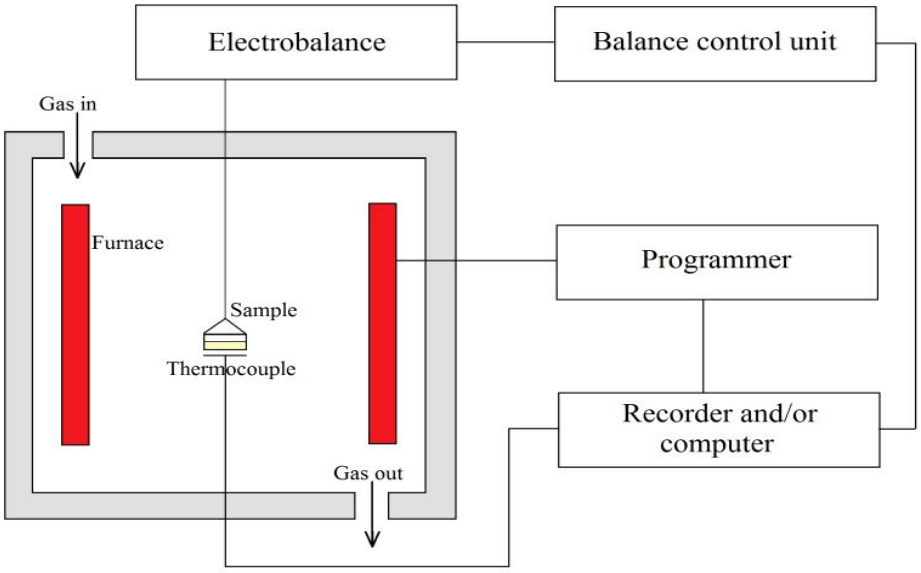


Figure 3.5: The main elements in a TGA instrument (Richardson and Charsley, 1998)

The balance used in many systems is a form of electronic microbalance with a typical resolution of 1µg (Haines, 1995). With such high sensitivity freedom from vibration is essential. A gas purge is also often used (typically nitrogen) as corrosive oxidising gases near

the balance are undesirable; baffles or a suitably designed furnace then compensate for the noise of convective effects.

In this study, all formulation constituents (polymers, drug and plasticizers) were analysed in triplicate by TGA to determine their degradation temperatures using the TA instrument Q5000 (TA instruments, NJ, USA) with Universal explorer software. Samples of approximately 2 mg were weighed into aluminium pans and weight loss was monitored at a heating rate of 5°C/min across the temperature range of 25°C to 250°C for the drug substances, Kollidon® SR and PEO and 25°C to 300°C for ethylcellulose.

3.1.2.5 Differential Scanning Calorimetry analysis (DSC)

Differential scanning calorimetry (DSC) is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and a reference are measured as a function of temperature (Figure 3.6). Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures being studied. The basic principle underlying this technique is that, when the sample undergoes a physical transformation such as phase transitions, heat will preferentially flow to or from the sample to maintain it at the same temperature as the reference. Heat flow to or from the sample is dependent on whether the process is exothermic or endothermic. For example, as a solid sample melts to a liquid, greater heat flow to the sample is required to increase its temperature at the same rate as that of the reference. This is due to the absorption of heat by the samples as it undergoes the

endothermic phase transition from solid to liquid. Likewise, as the sample undergoes exothermic processes (such as crystallisation), less heat is required to raise the sample temperature. By observing the difference in heat flow between the sample and reference, DSC is able to measure the amount of heat absorbed or released during such transitions.

A baseline is obtained which is used as a background in sample analysis. Any deviation from this baseline relates to thermal event, and the area under this deviation is proportional to the enthalpy change for that event.

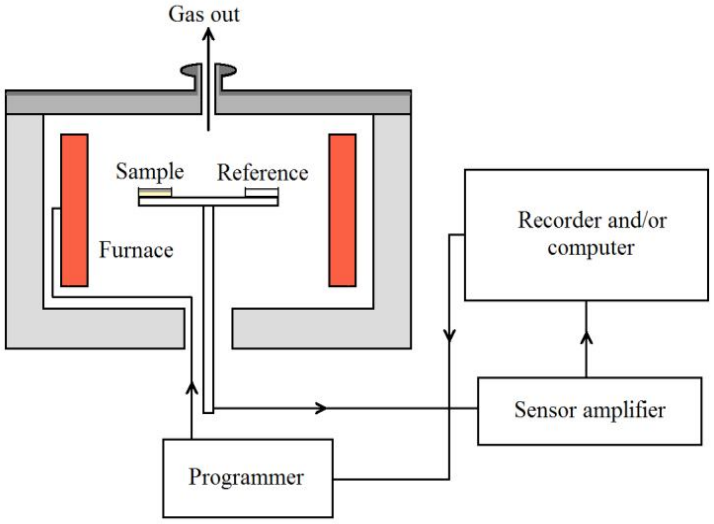


Figure 3.6: General DSC set up

The thermal properties of materials (physical mixtures and individual components) were assessed by modulated temperature Differential Scanning Calorimetric (MT-DSC) using Q 2000 DSC (TA instrument, NJ) under nitrogen atmosphere. Samples (~ 5mg) were prepared in sealed aluminium pans and a customised heat-cool-heat cycle was applied to determine glass transition temperature (Tg) of the physical mixtures. A heating rate of 5°C/min was

used to heat the samples from 25°C to 150°C. A cooling rate of 20°C/min was then used to reduce the temperature of samples to 0°C with subsequent reheating of samples at 1°C/min to 150°C. Triplicate samples were used for DSC studies. Glass transition temperature (Tg) and melting temperature (Tm) were obtained from the thermograms using the Universal explorer software. The Gordon Taylor theory (Gordon et al, 1952) (Equation 3) was applied to compare the theoretical glass transition temperature for the multi-component systems with the experimental glass transition temperatures (Tg).

Gordon Taylor equation:

$$Tg_{mix} = (w_1 Tg_1 + K w_2 Tg_2)/w_1 K w_2 \qquad K = Tg_1 \rho_1 / Tg_2 \rho_2 \qquad (3)$$

Where Tg is the glass transition temperature w_1 and w_2 are weight fractions of components, K is a constant calculated from the densities ρ and Tg of the components.

3.1.2.6 Rheological Analysis

Rheological parameters were obtained using the Anton Paar Physica MCR 301 Rheometer (Anton Paar, Germany GmbH.) using 25mm parallel plates with a gap distance of 1mm in oscillation mode. Frequency sweep test was performed at angular frequency range from 100 to 0.1 rad/s at strain amplitude of 0.5% and readings were taken every 5 seconds (Figure 3.7). Temperatures used in these experiments were based on the thermal characteristics of the components of the physical mixtures measured by TGA and DSC. The test temperature was set prior to adding the test material and the run was commenced when the test material was in a flowable state. All measurements were conducted in duplicate. Set temperature was 150°C for formulations containing ethyl cellulose and AZT, PEG and TEC, and 165°C for ethyl

cellulose with 3TC since during the test runs it was difficult to run a frequency sweep test at 150°C and there temperature was raised to 165°C.

The power law fit was used in the linear shear thinning region of the rheological data obtained:

$$\eta = K\,\gamma^{n-1} \quad \text{(Power law equation)} \qquad (4)$$

Where η is the viscosity, γ is the shear rate; K is the consistency coefficient which describes the overall range of viscosities across the measured shear rate range. n is the Power Law Index. For shear-thinning polymers $0< n <1$. The more shear thinning the polymer the closer n is to zero.

Figure 3.7: Anton Paar MCR 301 Rheometer

3.1.2.7 Hot Stage microscopy

Many of the transitions which can be monitored thermally for a given material are also accompanied by a physical transformation which alters the material's interaction with polarised light. This polarisation is usually achieved by passing light first through a first polar filter, then the sample, then a second filter set at 90° to the first (cross polarisation). If the sample is isotropic in all directions, such as a liquid or a cubic crystal, then the polarisation of the light will not be changed as it passes through the sample and no colour will be observed;

this will appear black under the microscope as all light has been blocked out. Liquid crystals or solids of other crystallographic systems will lead to characteristic interference colours due to differences in the refractive index in the different crystallographic directions (birefringence). When a transition takes place the observed colour and light intensity will change as the material's physical properties and refractive index alter. These changes can then be monitored visually or recorded. Polarized light is used to detect birefringence in small amounts of sample help decide if they are crystalline, amorphous or a mixture of both (Storey and Ymén, 2011). Polarized light microscopy has been used by several researchers to investigate how crystalline APIs interact with polymers in mixtures at elevated temperatures (Llyod et al. 1997 and Lakshman et al. 2008).

The miscibility of the model drugs with ethylcellulose was observed using the Carl Zeiss microscope (Carl Zeiss Inc. Thornwood, NY) using a temperature controlled hot stage (Linkam Inc.). Approximately 1 mg of each physical mixture was placed on an open glass slide and a temperature ramp of 5°C/min from room temperature to 180°C was applied with rapid cooling at a rate of 20°C/min back down to room temperature (25°C). The sample was observed under polarized light and images were captured at 5X magnification at 30 second intervals using Axioplan imaging software. Hot stage microscopy studies were only done for ethylcellulose blends due to unavailability of the machine during the study period.

3.2 Results and Discussion

3.2.1 Solubility Parameter as an indicator for miscibility of drugs and plasticizers in Polymer mixtures

The solubility parameters were calculated using the group contribution method of Hofyzer/Van Krevelen described by Foster et al, 2001. The calculated solubility parameters (δ/Mpa$^{1/2}$) are shown in Table 3.1 and Table 3.2 with compounds showing similar solubility parameters having marked potential to demonstrate miscibility (Greenlalgh et al 1999). It is noted that compounds with differences in solubility parameter ($\Delta\delta$) < 7Mpa$^{1/2}$ are more likely to be miscible than compounds with a $\Delta\delta$ > 10Mpa$^{1/2}$ which are likely to be immiscible (Greenlalgh et al, 1999). The solubility parameter for Zidovudine is 20.3while TEC (Hancock et al 1996) and PEG 6000 have solubility parameters of 20.4Mpa$^{1/2}$ (Vesey et al, 2005) and 19.7Mpa$^{1/2}$(Barton, 2000) respectively. The calculated solubility parameters (δ/Mpa$^{1/2}$) for zidovudine (AZT) and lamivudine (3TC) are 20.3 Mpa$^{1/2}$ and 20.5 Mpa$^{1/2}$ respectively. The difference in solubility parameters between ethylcellulose and AZT and 3TC is <7Mpa$^{1/2}$ suggesting that both the drugs are likely to be miscible with the polymers. The calculated solubility parameters (δ/Mpa$^{1/2}$) for Zidovudine (AZT) and Lamivudine (3TC) are also similar to that of the three polymers and thus concluding that these compounds are miscible (Table 3.3). The difference in solubility parameters between ethylcellulose and PEG-6000 is $\Delta\delta$ 2.4 Mpa$^{1/2}$, which suggests also that this combination of polymer and plasticizer will also be miscible.

Table 3.1: Calculation of Solubility parameter using Hofyzer/Van Krevelen group contribution method for Zidovudine

Groups	**F_{di}**	**F^2_{pi}**	**E_{hi}**	**$\Sigma^z V/cm^3\ mol^{-1}$**
(1)CH_3	420	0	0	33.5
(2)CH_2	540	0	0	32.2
(1)NH	160	210	3100	4.5
(3)CH	600	0	0	-3.0
(2)C=O	0	0	4000	21.6
(2)C=	140	0	0	-11.0
(1)N-	20	800	5000	-9.0
(3)N=	60	2400	15000	15.0
(2)Rings	380	-	-	32
(1) –O-	100	400	3000	3.8
(1) OH	210	500	20000	13.0
Σ	**2630**	**4310**	**48100**	**132.6**
	$\delta_{d=}$ 2630/132.6	δ_P =√4310/132.6	δ_h = √48100/132.6	
	$\sqrt{19.8^2}$ +	32.5^2	362.75^2	
Σ	**20.3**			

Table 3.2: Calculation of Solubility parameter using Hoyer/Van Krevelen group contribution method for lamivudine

Groups	**F_{di}**	**F^2_{pi}**	**E_{hi}**	**$\Sigma^z V/cm^3\ mol^{-1}$**
(1)NH_2	280	-	8400	19.2
(1)CH_2	270	0	0	16.1
(1)NH	160	210	3100	4.5
(1)CH	80	0	0	-1.0
(2)-C=O	290	770	2000	10.8
(3)-C=	210	0	0	-11.0
(1)N-	20	800	5000	-9.0
(1)N=	-	-	-	5
(2)Rings	380	-	-	32
(1) –O-	100	400	3000	3.8
(1) OH	210	500	20000	13.0
(1)-S-	440	-	-	12.0
Σ	**2630**	**2470**	**38400**	**103.9**
	$\delta_{d=}$ 2630/103.9	δ_P =√2470/103.9	δ_h = √38400/103.9	
	$\sqrt{25.3^2}$ +	23.77^2 +	369.6^2	
Σ	**20.5**			

Table 3.3: Solubility Parameters using group contribution method by Hofyzer/Van Krevelen of the compounds used for hot melt extrusion

Compound	Solubility parameter (δ/Mpa$^{1/2}$)
Ethylcellulose	20.0
Kollidon® SR	20.3
POLYOX WSR N-80 (PEO)	22.2
Zidovudine	20.3
Lamivudine	20.5
Triethyl citrate (TEC)	19.7
Polyethylene glycol (PEG)- 6000	22.4

3.2.2 True density measurements

The true average densities of AZT and 3TC were 1. 3571 g/cm^3(S.D ± 0.0045g/cm^3) and 1.7262 g/cm^3(S.D ± 0.0021g/cm^3) respectively while the true average density measured for ethylcellulose, Kollidon SR and PEO were 1.1671g/cm^3 (S.D ± 0.0017g/cm^3), 1.3008 g/cm^3 (S.D ± 0.0044g/cm^3) and 1.26 g/cm^3 (S.D ± 0.0031g/cm^3) respectively.

3.2.3 Thermal Analysis

The onset temperatures of degradation for AZT and 3TC determined by TGA were 197°C and 200°C respectively, whilst ethyl cellulose degraded with onset temperature of 300°C, Kollidon® SR at around 198°C and POLYOX WSR N80 at 280°C. On the basis of these TGA data, it was evident that the extrusion temperatures during HME should be set at levels

markedly below 190°C to minimise the thermal degradation of formulation constituents as shown in Figure 3.8.

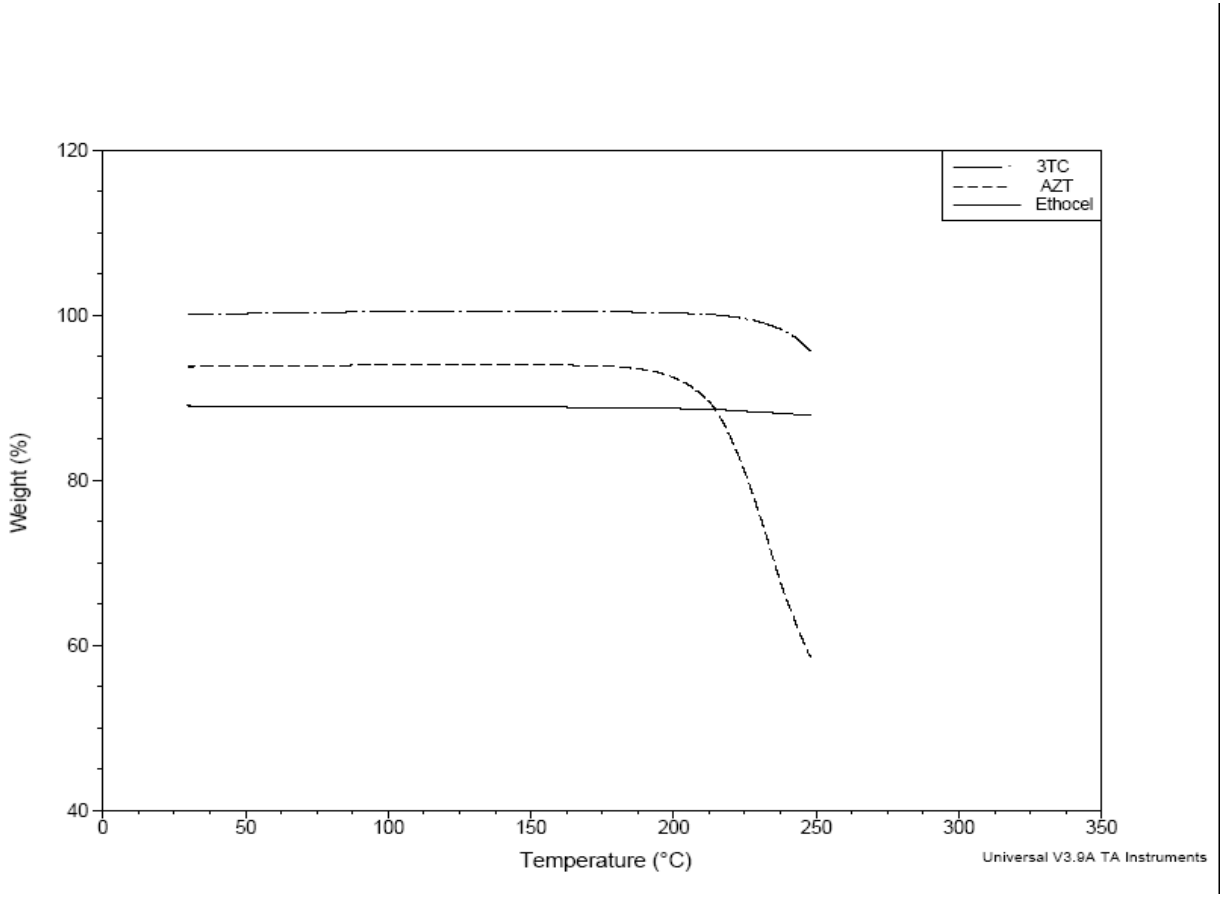


Figure 3.8: TGA thermograms of Ethylcellulose, AZT and 3TC

3.2.3.1 Ethylcellulose

DSC studies were performed to assess whether a solid dispersion of polymer and drug would be formed during HME and predict the extrusion temperatures that could be used to process the material effectively. The glass transition temperature (Tg) and melting temperatures (Tm) of the individual components were obtained using the Universal Analyzer as shown in Table 3.2. The Tm of individual components in the physical mixtures with AZT were observed on the thermograms during the first heating cycle but were not observed during the second heat cycle, which indicates that the drugs had become amorphous following cooling of the co-formulated systems (Figure 3.9). For 3TC a shallow peak was observed during the cooling cycle. On the second heat cycle a very shallow peak was also observed close to the Tm of 3TC (Figure 3.10).

The experimental Tg values of the blends, obtained by DSC analysis during the second heat cycle were compared with theoretical Tg values calculated using the Gordon Taylor equation as shown in Table 3.4. Calculated values were typically lower than experimental Tg measurements. The calculated Tg for the polymer and AZT mixture at 80%w/w: 20%w/w ratio was 86.2°C, whilst the experimentally derived parameter was 117°C. It was however difficult to establish the Tg from the second heat cycle since the Tg of ethyl cellulose was within the same range of the AZT Tm. The experimental Tg for samples containing ethyl cellulose with both 30%w/w and 40%w/w AZT was 115°C. The Tg did not appear to be affected with increasing AZT load as shown in figure 3.4. During the second heat cycle, AZT formulations with ethyl cellulose showed an absence of Tm regardless of drug loading. These results indicate that super cooled fluids in the amorphous state have been formed following melting and cooling of the samples, which supports the utility of HME to produce amorphous solid dispersions of these compounds.

Thermograms of the physical mixtures of ethyl cellulose with 3TC at all three concentrations showed a small re crystallization peak at approximately 169 ± 0.6°C during the fast cooling cycle. The second heat cycle showed two glass transition (Tg) temperatures, the first one at 70 ± 0.2°C which was close to the Tg for 3TC, with the second Tg at 127°C being the Tg for ethyl cellulose as shown in figure 3.10. The 3TC with ethyl cellulose results indicate that after the heat- cool cycle, solid dispersions of 3TC and ethyl cellulose are formed.

The plasticizers reduce the thermal processing temperature of the polymer during HME and allow the polymer to flow through the extruder. The plasticizer increases the intermolecular separation of the polymer molecules (Wang et al 1997) and reduces elastic modulus, tensile strength, melt viscosity and glass transition temperature of the polymer (Ghebremeskel et al 2007). With increases in the percentage of TEC in the physical mixtures, the Tg of ethyl cellulose formulations was shown to be lower than that of the pure polymer (Table 3.4).

Formulations containing equivalent levels of PEG-6000 showed glass transition temperatures for ethyl cellulose formulations at markedly lower levels than those containing TEC as the plasticizer. Signs of crystalline PEG-6000 were however observed upon re-evaluation of the cooled samples, which makes the polymeric plasticizer less attractive as a constituent of solid dispersion formulations.

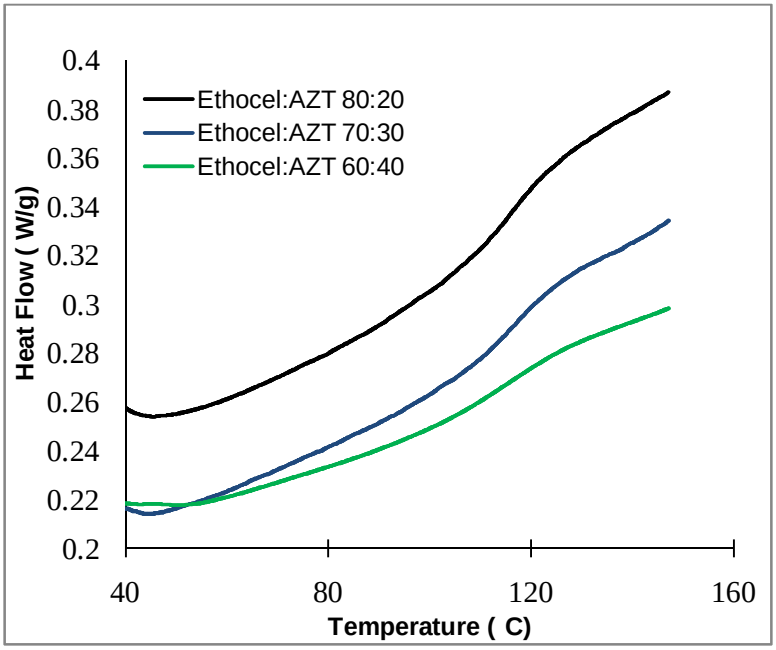


Figure 3.9: Glass transition temperatures (Tg) of physical mixtures of ethylcellulose: zidovudine (AZT) at concentrations of 80:20%, 70:30% and 60:40%.

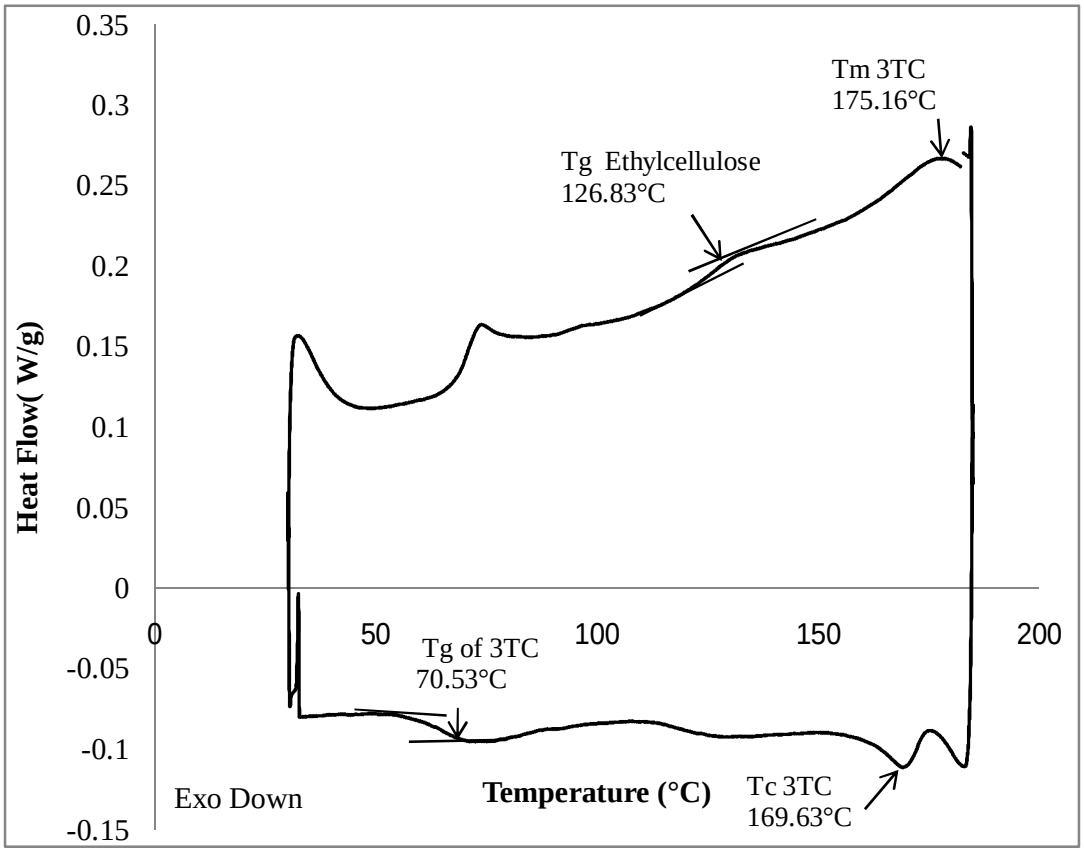


Figure 3.10: Glass transition temperatures and re crystallization peak seen during the cool cycle and second heat cycle of physical mixtures of ethyl cellulose and 3TC at 60:40% concentrations

	***T*m °C** **(melting point temperature)**	***T*g °C** **(glass transition temperature)**	**Calculated Tg_{mix}°C using Gordon Taylor equation**
Ethyl cellulose	179 ±0.5	127 ±0.2	-
Zidovudine (AZT)	127 ±0.5	32 ±0.2	-
Lamivudine (3TC)	176±0.8	64 ±0.5	-
Ethyl cellulose: AZT 80:20	-	117 ±0.2	86.2
70:30	-	115 ±0.2	73.5
60:40	-	115 ±0.2	63.6
Ethyl cellulose: 3TC 80:20	-	70± 0.3 and128 ±0.1	110.3
70:30	-	70± 0.1 and 127 ±0.1	102.2
60:40	-	70±0.2 and127 ±0.1	85.6

Table 3.4: Melting temperature(Tm°C), glass transition temperature (Tg°C) and glass transition temperature of physical mixtures (Tgmix°C) of ethyl cellulose, AZT, 3TC and physical mixtures. (± Standard deviation, n=3)

Table 3.5: Comparison of melting and glass transition temperatures from DSC measurements of ethylcellulose, TEC and PEG-6000 physical mixtures.

(± Standard deviation, n=3)

	Tm°C (melting temperature)	**Tg°C (glass transition temperature)**
Ethylcellulose	179±0.1	127±0.2
Ethylcellulose: TEC		
95:5	-	125±0.3
90:10	-	110±0.6
80:20	-	76±0.6
70:30	-	63.5±0.5
60:40	-	62±0.4
Ethylcellulose: PEG6000		
0:100	60±0.3	-
95:5	59±0.5	117±0.4
90:10	58±0.8	85±0.5
80:20	58±0.8	69±0.6
70:30	58±0.8	63±0.4
60:40	58±0.8	58±0.2

3.2.3.2 Kollidon® SR

The DSC of pure Kollidon® SR exhibited Tg at 38.8°C (S.D ± 0.5°C) (Figure 3.11 and Figure 3.12). The DSC curve for the all the blends of AZT and 3TC with Kollidon® SR showed the absence of melting endotherm in the second heat cycle (Figure 3.11). During the cooling cycle there were no signs of AZT recrystallization. This showed that the AZT and 3TC are miscible in polymer, providing an amorphous dispersion. Tg of the blends with the drugs did not however change compared to the Tg of pure polymer (Figure 3.11). Tg of Kollidon® SR with AZT 20%, 30% and 40% w/w were 38.6°C(S.D ± 0.5 °C), 37.8°C (S.D ± 0.2°C) and 37°C (S.D± 0.2°C) respectively. With increase in the percentage of TEC, the Tg of Kollidon® SR in the physical mixture was lower than that of pure Kollidon® SR. 20% TEC in

the blend reduced the Tg of pure Kollidon® SR to about 40% lower in temperature (Figure 3.12). PEG 6000 lowered the Tg of Kollidon® SR much lower that TEC however PEG showed signs of re-crystallization on the cooling cycle as well as the second heat cycle which would be a less attractive plasticizer to use due to the re-crystallization as it would in future render the final product unstable.

3.2.3.3 PEO

Pure PEO showed a sharp endothermic peak of 65.9°C (S.D ± 0.3°C) corresponding to its melting point (Tm). Tg of PEO could not be obtained though references obtained indicated Tg of -65°C (Dow, 2010). AZT in presence of PEO showed a broad endothermic peak 123.6°C during the first heat cycle for all the physical mixtures but did not appear on the cooling cycle or the second heat cycle. All the physical mixtures indicated the presence of a sharp exothermic peak during the cooling cycle and an endothermic peak during the second heat cycle. Bothe the endothermic and exothermic peaks were in close proximity to the Tm of PEO (Figure 3.13). It was also observed that as the proportion of AZT increased in the physical mixture both the exothermic peaks and endothermic peaks temperature lowered in relation to the Tm of PEO (Figure 3.13).

During the first heat cycle, the physical mixtures of PEO and 3TC showed two sharp endothermic peaks at 65.9°C (S.D ± 0.5°C) and a second close to 176.6°C (S.D ± 0.6°C) which were melting points of PEO and 3TC respectively. During the cool cycle, 3TC did appear as a very broad yet small exothermic crystalline peak at 167.8°C (S.D ± 0.5°C), which meant that it crystallised back and was partially miscible with PEO. A sharp exothermic peak 34.8°C was also observed during the cooling cycle that was close to the re-crystallization

peak of PEO. PEO endothermic peak was observed at around 60.8°C (S.D ± 0.3°C) during the second heat cycle. Increase in the proportion of 3TC in the physical mixture did not have any effect on the Tm of PEO neither did 3TC re-crystallize during the cooling cycle. This therefore suggests that 3TC co formulated with PEO and did not re-crystallize upon cooling.

As the percentage of TEC increased in the physical mixture Tm of PEO was reduced (Figure 3.14). The Endothermic peaks of PEO were observed to become broader as the percentage of TEC was increases which indicated PEO molecule separation. PEG 6000 Tm was observed to be 60.3°C (S.D ± 0.4°C). PEG however caused no shifts in Tm of PEO, which suggests that there was no interaction between PEG and PEO. PEO and PEG 6000 are both used in combination as hydrophilic carriers for the formulation of mini matrices by HME (Verhoeven, 2008)

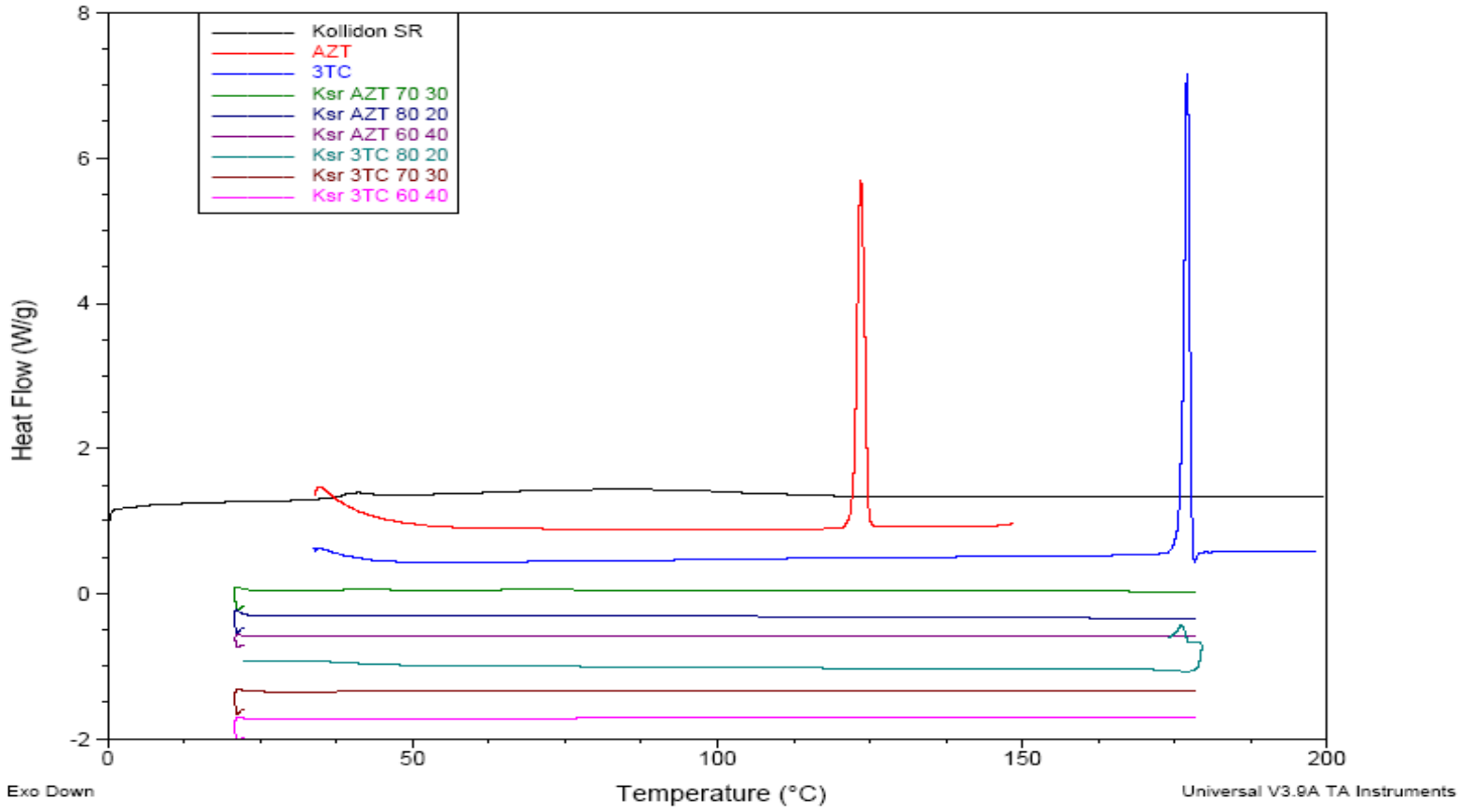


Figure 3.11: DSC measurements of Kollidon® SR, AZT and 3TC blends.

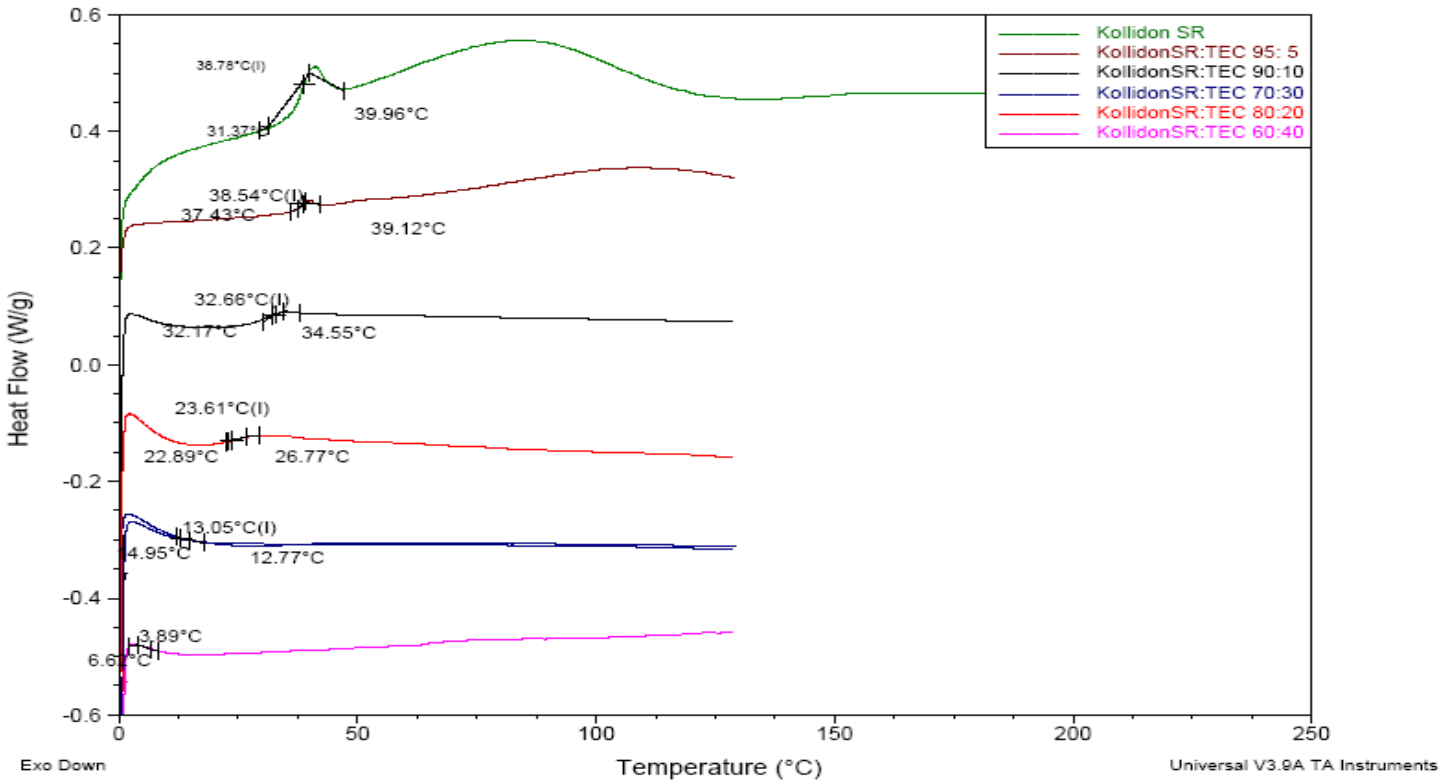


Figure 3.12: Comparison of melting and glass transition temperatures from DSC measurements of Kollidon® SR, TEC blends.

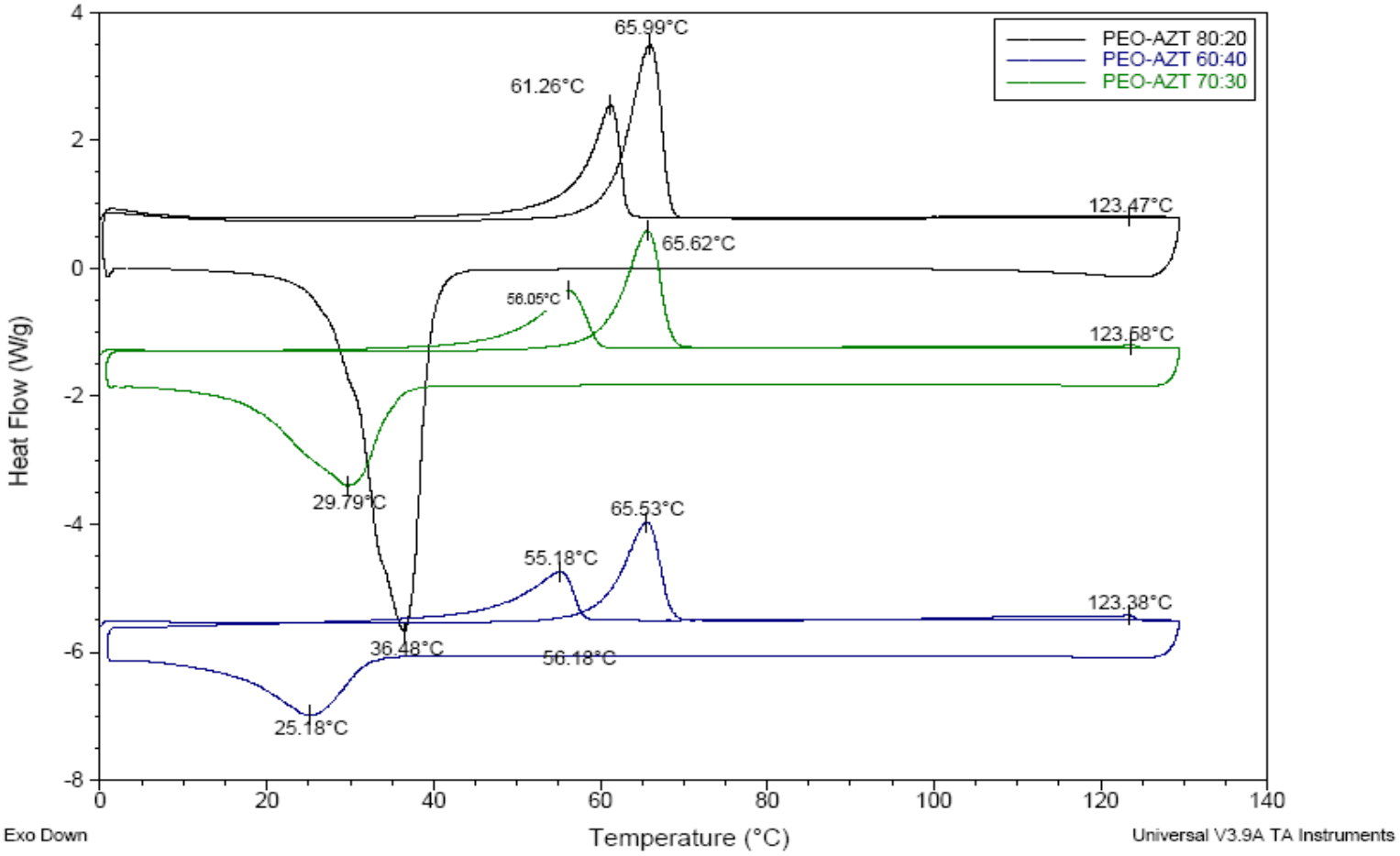


Figure 3.13: Endothermic and Exothermic peaks of PEO and AZT during heat/cool/ heat cycles

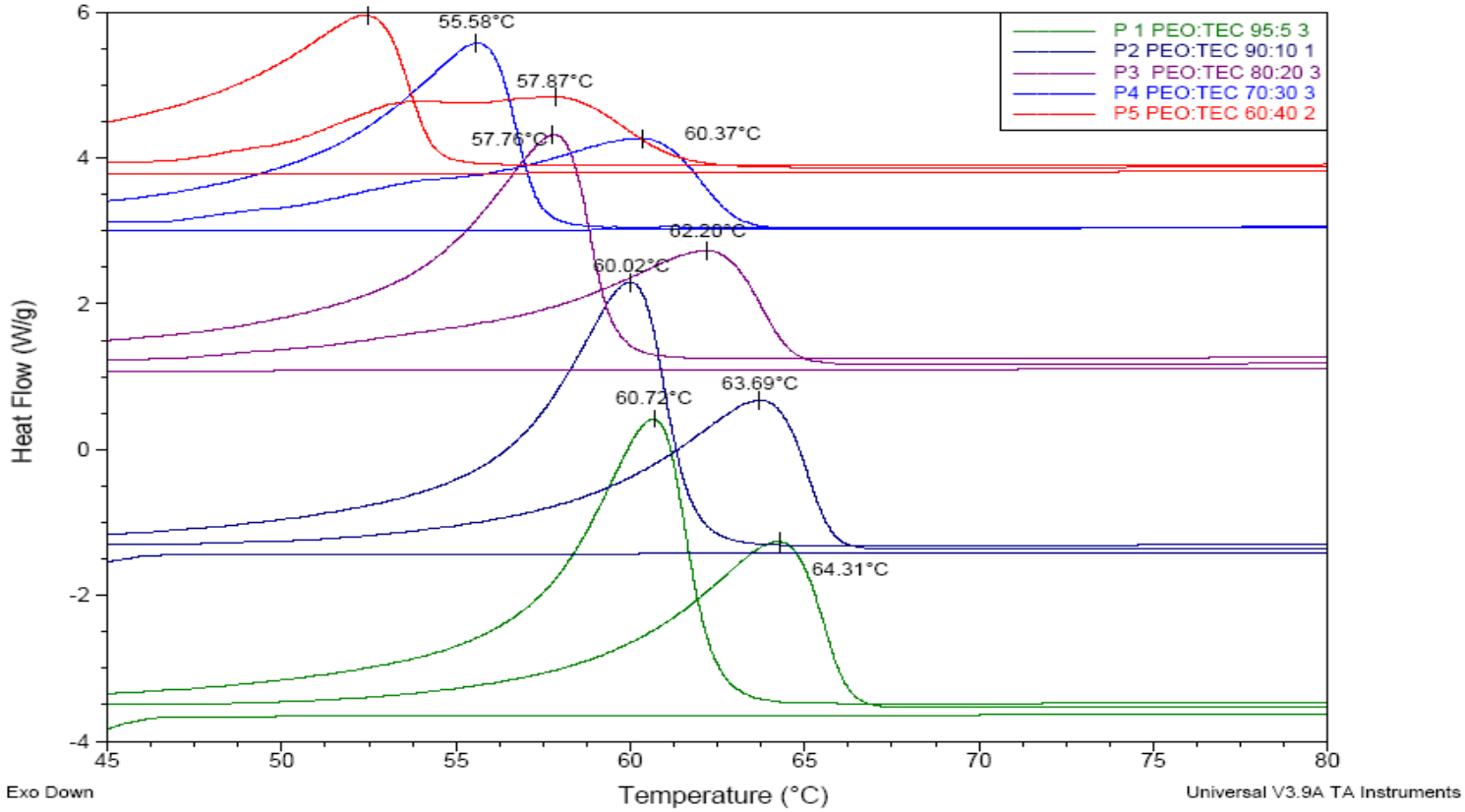


Figure 3.14: Effect of TEC on melting temperature (Tm) of PEO

3.3 Rheological characteristics

3.3.1 Ethylcellulose

Complex viscosity (η^*) of a polymer is a frequency dependent material property which can be measured during forced harmonic oscillation between two parallel plates. Measured complex viscosity of formulations of ethyl cellulose, drugs and plasticisers are shown in figures 3.15 a, b, c and d at an angular frequency range of between 0.1 and 100s^{-1}. The viscosity of ethyl cellulose in this region was found to follow shear-thinning behaviour typical of many polymer melts, i.e. its viscosity decreased as angular frequency (indicative of processing speed) increased. The viscosity was also found to be highly temperature dependent, with significant reduction between set temperatures of 150°C and 165°C

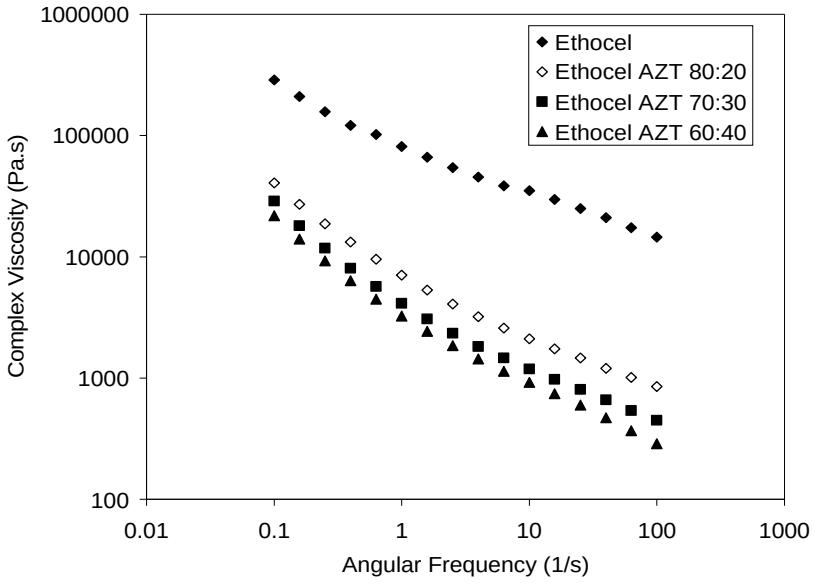
Incorporation of 20-40% by weight of AZT in ethyl cellulose (figure 3.15a) was found to have a plasticising effect, i.e. decreased viscosity. This effect has been quantified by fitting power law indices 'n' and 'K' from equation 3 to the measured data, as shown in Table 3.4. The consistency index K is a measure of a material's resistance to flow at low rate, the power law index n is a measure of rate dependency, lower values of n indicating greater levels of shear thinning. The power law shear thinning index commonly ranges from 0.25 to 0.9 for most polymer melts (Crowley, 2003). It can be seen that increased loadings of AZT caused a significant decrease in both n and K, as reflected by a downward shift and steeper gradient of the viscosity curves as shown in Table 3.6 and figure 15a respectively. Rheological characterization of formulations containing 3TC at 165°C (figure 3.15b) showed that drug had a plasticising effect up to a level of 30%, but then a reinforcing effect on further increases to 40%. These results suggest that the polymer was probably saturated with dissolved drug at the 40% loading. At higher drug loadings, an 'upturn' in the viscosity curve was also

noticeable at higher angular frequencies. This suggests that at the start of the test ($100s^{-1}$) the drug may not have been fully miscible with the polymer, gradually mixing in during the first few oscillatory stages of the test.

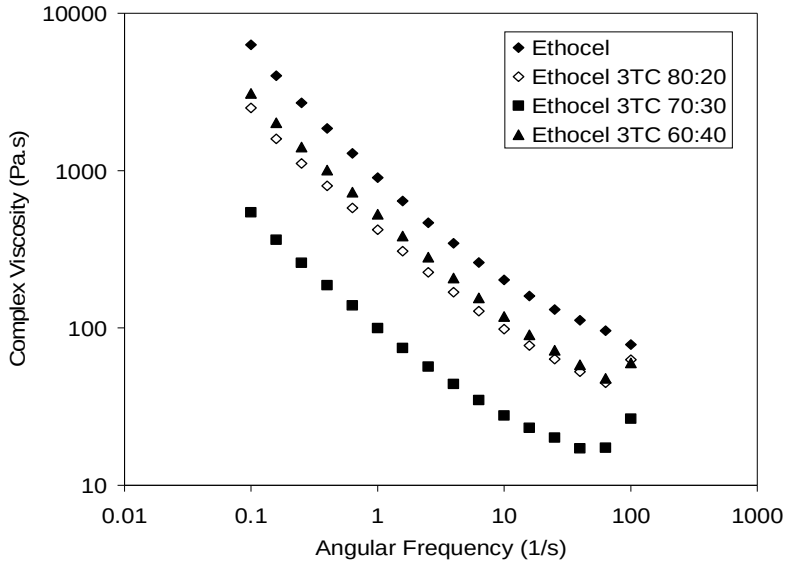
Incorporation of TEC (figure 3.15c) was found to have a significant plasticising effect on the viscosity of ethyl cellulose, as indicated by large reductions in fitted K values. At higher loadings of 30 and 40% by weight, the viscosity curve deviated from the linear power law fit and demonstrated a marked bi-modal shape, with steeper shear thinning behaviour at low rates and less rate dependence at high rates. This may indicate a lubricating effect of the plasticiser causing slip between the surface of the polymer and the rheometer plate at high rates. Complex viscosities of ethyl cellulose and PEG-6000 blends (figure 3.15d) also decreased with increased concentration of PEG 6000 up to a level of 40% by weight. Comparison of the consistency indices in table 3.6 showed that PEG had a less significant plasticising effect than TEC.

A comparison of the effect of drug and plasticizer loading on complex viscosity at an angular frequency of $10s^{-1}$ (representative of rates encountered in the extrusion process) is shown in figure 3.16. The plasticising effect of each additive can clearly be seen, with the exception of 3TC at 40% by weight. At 150°C the viscosity of ethyl cellulose with AZT was found to be comparable to that of TEC at the same loadings.

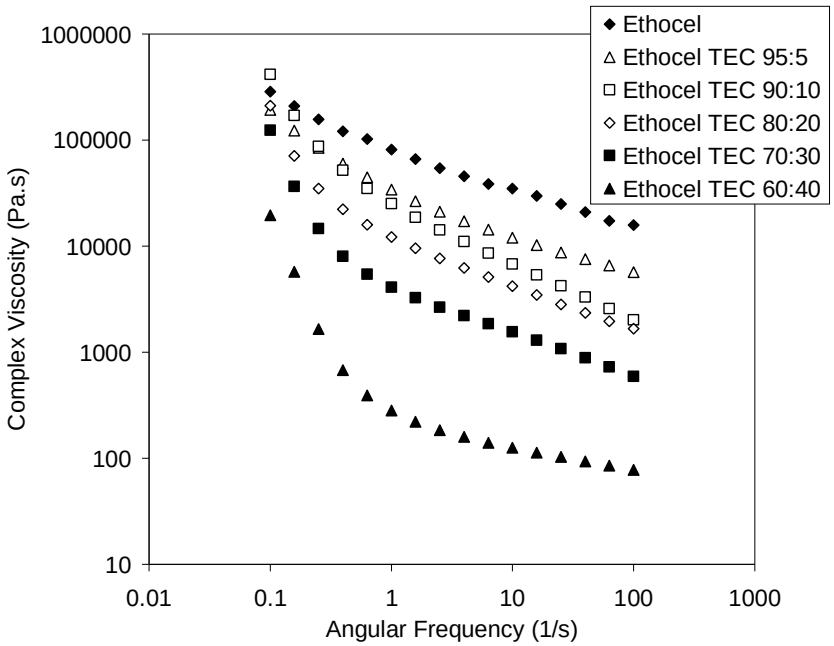
(a)

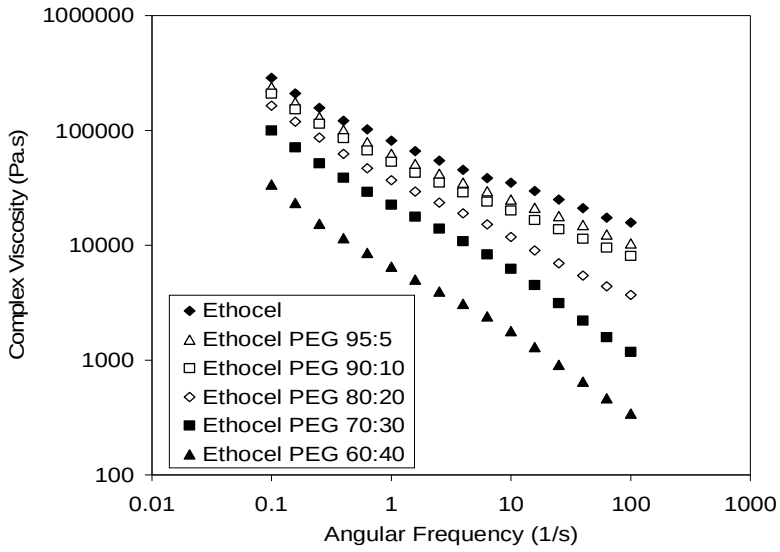

1000000
100000
10000
1000
100
0.01
0.1
1
10
100
1000
Complex Viscosity (Pa.s)
Angular Frequency (1/s)
Ethocel
Ethocel AZT 80:20
Ethocel AZT 70:30
Ethocel AZT 60:40

(b)


10000
1000
100
10
0.01
0.1
1
10
100
1000
Complex Viscosity (Pa.s)
Angular Frequency (1/s)
Ethocel
Ethocel 3TC 80:20
Ethocel 3TC 70:30
Ethocel 3TC 60:40

(c)





(d)

Figure 3.15: Complex viscosity of (A) ethyl cellulose and zidovudine (AZT) blends at 150°C (B)ethyl cellulose and lamivudine (3TC) blends at 165°C (C) ethyl cellulose and TEC blends at 150°C (D) ethyl cellulose and PEG-6000 blends at 150°C.

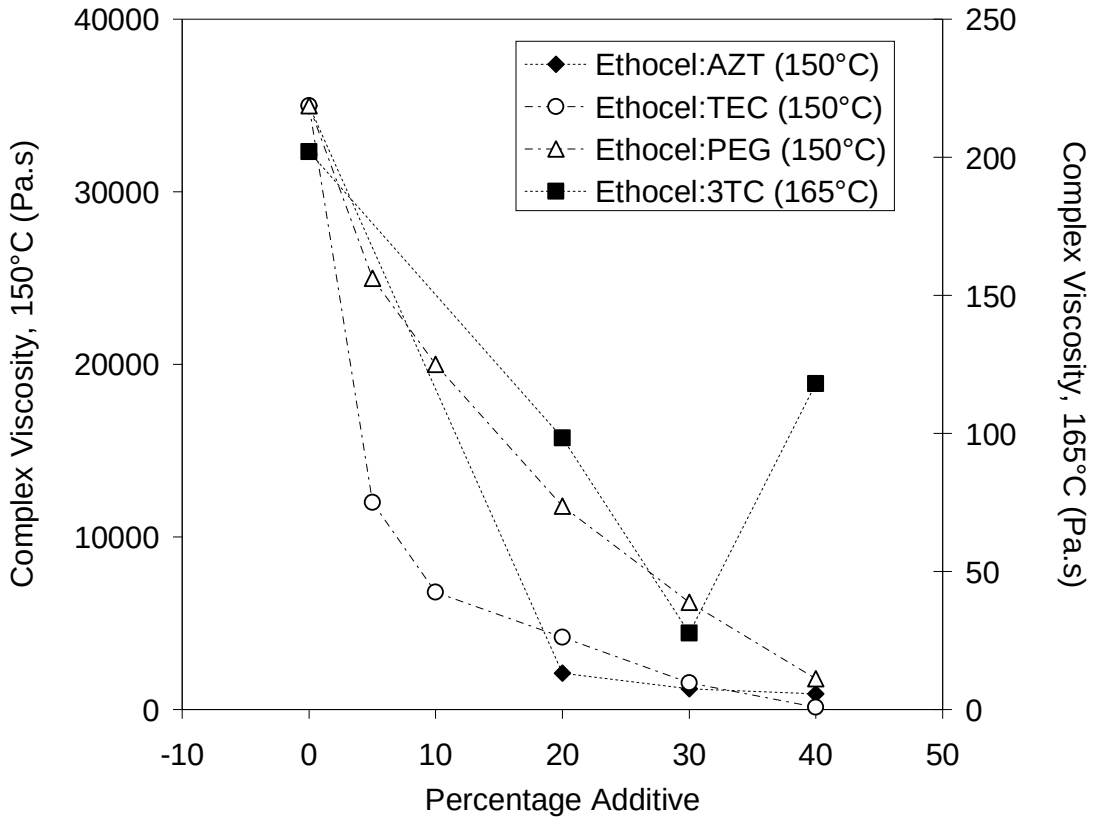


Figure 3.16: Effect of drug and plasticizer content on measured complex viscosity at an angular frequency of 10 s^{-1}; AZT, TEC and PEG measured at 150°C, 3TC measured at 165°C

Ethylcellulose and Zidovudine (AZT) at 150°C		
Ethocel : AZT	n	K ($Pa.s^n$)
100:0	0.60	89125
80:20	0.45	8128
70:30	0.42	5012
60:40	0.40	3890
Ethylcellulose and Lamivudine (3TC) at 165°C		
Ethocel : 3TC	**n**	**K ($Pa.s^n$)**
100:0	0.26	978
80:20	0.31	442
70:30	0.36	108
60:40	0.30	550
Ethylcellulose and Triethyl Citrate (TEC) at 150°C		
Ethocel: TEC	**n**	**K ($Pa.s^n$)**
100:0	0.60	89125
95:5	0.51	40794
90:10	0.31	35310
80:20	0.41	17685
70:30	0.37	7040
60:40	0.36	665
Ethylcellulose and Polyethylene Glycol (PEG) at 150°C		
Ethocel: PEG	**n**	**K ($Pa.s^n$)**
100:0	0.60	89125
95:5	0.56	71450
90:10	0.54	58748
80:20	0.46	40345
70:30	0.38	23222
60:40	0.36	6968

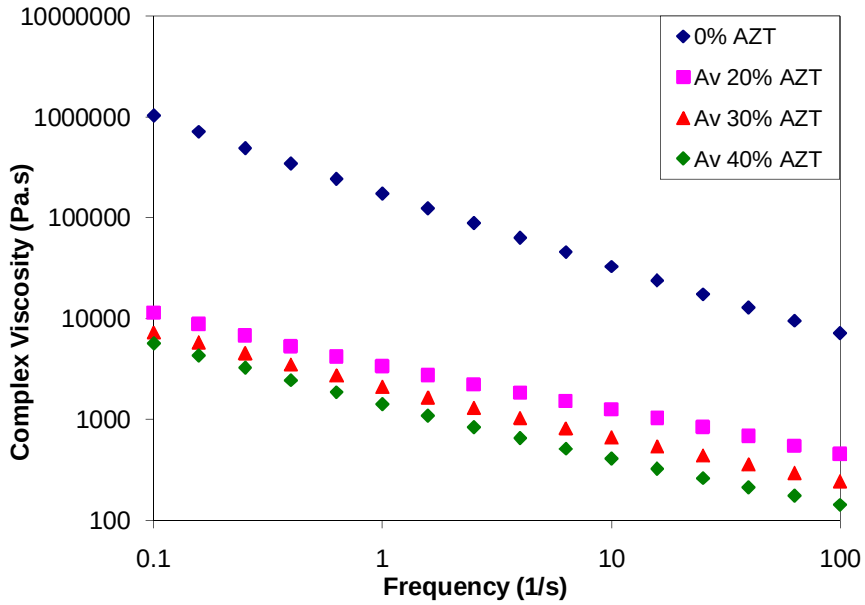
Table 3.6: Fitted rheological parameters using the power law equation for ethylcellulose, AZT, 3TC, TEC and PEG-6000 blends at different concentrations (% w/w).

3.3.2 Kollidon® SR

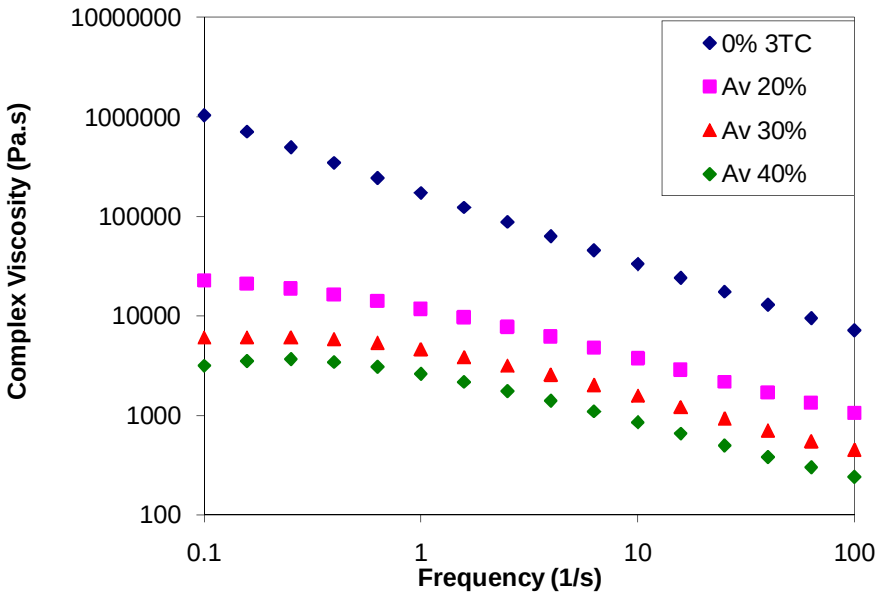
Temperature settings during the oscillatory rheometry studies were kept at 150°C while other settings were kept the same as mentioned above. Temperatures above 150°C gave a foul burning odour and the melt turned light brown in colour.

Viscosity decreased with increase in percentage of AZT, 3TC and TEC compared to the viscosity of pure Kollidon® SR (Figures 3.17 a, b, c, d). AZT and 3TC had plasticizing effect on Kollidon® SR as the percentage of drug loading increased. A maximum drug load of 40% for both AZT and 3TC could be used with Kollidon® SR to give mixtures with adequate rheological characteristics during the actual extrusion process. Power Law shear thinning indices, n, and consistency indices, K, were calculated for AZT, 3TC, TEC and PEG-6000 in various percentages used in the physical mixture with Kollidon® SR (Table 3.6). Consistency index K reduced with each loading of the drugs and plasticizers. TEC at concentrations of 5%w/w and 10%w/w were shown to give mixtures with adequate rheological behaviour for extrusion. Formulations with highest concentrations of TEC however, showed phase separation during the frequency sweep test.

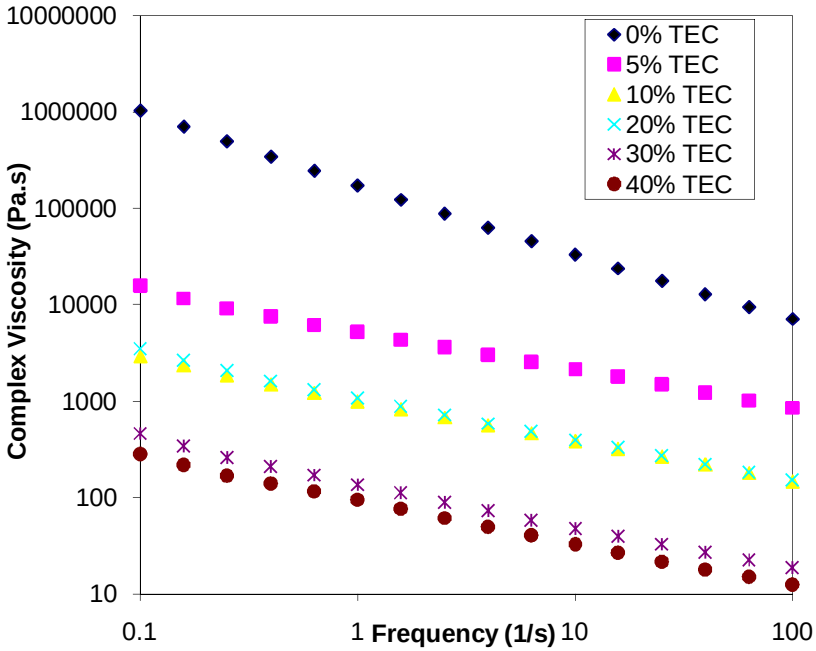
a)


10000000
1000000
100000
10000
1000
100
Complex Viscosity (Pa.s)
0.1
1
10
100
Frequency (1/s)
0% AZT
Av 20% AZT
Av 30% AZT
Av 40% AZT

b)


10000000
1000000
100000
10000
1000
100
Complex Viscosity (Pa.s)
0.1
1
10
100
Frequency (1/s)
0% 3TC
Av 20%
Av 30%
Av 40%

c)



d)

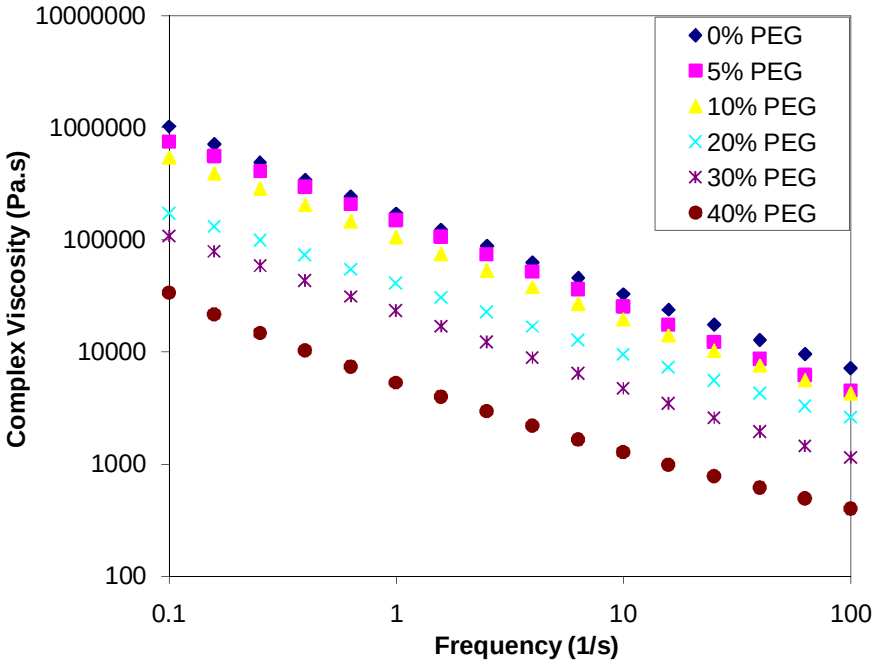


Figure 3.17: Complex viscosity of Kollidon® SR and a) Zidovudine (AZT) blends

(b) Lamivudine (3TC) blends c) TEC blends (d) PEG-6000 blends at 150°C.

Kollidon®SR and Zidovudine (AZT) at 150°C		
Kollidon®SR: AZT	n	K (Pa.s^n)
100:0	0.74	131816
80:20	0.48	3695
70:30	0.55	1590
60:40	0.47	216
Kollidon® SR and Lamivudine (3TC) at 150°C		
Kollidon®SR: 3TC	n	K (Pa.s^n)
100:0	0.74	131816
80:20	0.42	8102
70:30	0.416	3064
60:40	0.44	2568
Kollidon®SR and Triethyl Citrate (TEC) at 150°C		
Kollidon®SR: TEC	n	K (Pa.s^n)
100:0	0.74	131816
95:5	0.41	5386
90:10	0.43	1028
80:20	0.44	1096
70:30	0.46	140
60:40	0.45	93
Kollidon®SR and Polyethylene Glycol (PEG) at 150°C		
Kollidon® SR : PEG	n	K (Pa.s^n)
100:0	0.74	131816
95:5	0.70	134547
90:10	0.71	104471
80:20	0.61	41093
70:30	0.67	22906
60:40	0.63	5957

Table 3.7: Fitted rheological parameters using the power law equation for Kollidon® SR, AZT, 3TC, TEC and PEG-6000 blends at different concentrations (% w/w).

3.3.3 PEO

The investigation temperature range in the case of PEO was kept from 80°C to 150°C for pure PEO samples. Figure 3.18 shows the complex viscosities of pure PEO from 80°C to 150°C.

The temperature settings were set so as to simulate the processing temperatures during HME. The temperatures were set above the glass temperature of the pure polymer, plasticizer and APIs. Complex viscosity decreased with increase in percentage of AZT loading. Complex viscosity in the case of 3TC loading decreased with increase in percentage of 3TC with PEO, though 40% 3TC loading had slightly higher viscosities than 30% 3TC load. These results suggest that the polymer was probably saturated with dissolved drug at the 40% loading. Power law indices n and K were calculated and are shown in Table 3.7. The indices reduced with increase in percentage load of AZT, TEC and PEG while with 3TC K increased at 40% 3TC load. Data obtained was useful to set the ideal temperature for PEO/Drugs and PEO/Plasticizer physical mixtures. Ideal viscosity range for HME process is between 700 Pas and 10 kPas (Kotler, 2010). Temperature was set 150°C for frequency sweep test for PEO/Plasticizer and PEO/Drugs physical mixtures. Figure 3.19 a, b, c and d show the complex viscosities of physical mixtures of PEO/AZT, PEO/3TC, PEO/TEC, PEO/PEG.

Dynamic frequency sweeps are commonly done to determine the visco-elastic properties of the polymers and the effects of drug/ plasticizers can easily be determined at a particular temperature. Visco-elastic materials have two components: G´ (elastic modulus or storage modulus) and G˝ (viscous modulus or loss modulus). A typical response for a polymer melt is to exhibit elastic dominated behaviour at high frequencies and viscous dominated behaviour at low frequencies. This means that there is a critical frequency at which the two responses are equal. This is obviously a well defined point and conveniently this “cross-over” frequency and modulus has been shown to depend on the molecular weight and molecular

weight distribution of some linear polymers. A potential advantage of utilising this point as a quality control tool is that the cross-over of elastic and viscous moduli occurs at significantly higher frequencies than the point at which a constant value of shear viscosity occurs (Malvern, 2005). The elastic and loss modulus of a polymer represents the Figure 3.20 shows that at 80°C, the G″ is higher than G´ during the initial frequencies and then cross over during higher frequencies. This describes that PEO is initially solid but has visco-elastic properties thus it starts to flow like a liquid once the cross over point is reached. The cross over frequency increases with increase in temperature (as in the case of with most visco-elastic materials) as shown in Table 3.8. The data in Table 3.8 suggests that HME temperatures should be set between 100°C to 15°C when using PEO. Frequency sweep tests were done at 150°C with PEO/drug physical mixtures. There were no cross-over points seen with AZT and 3TC loading at 150°C which means that addition of the AZT and 3TC gave the polymer melt liquid like properties which are ideal characteristics when extruding.

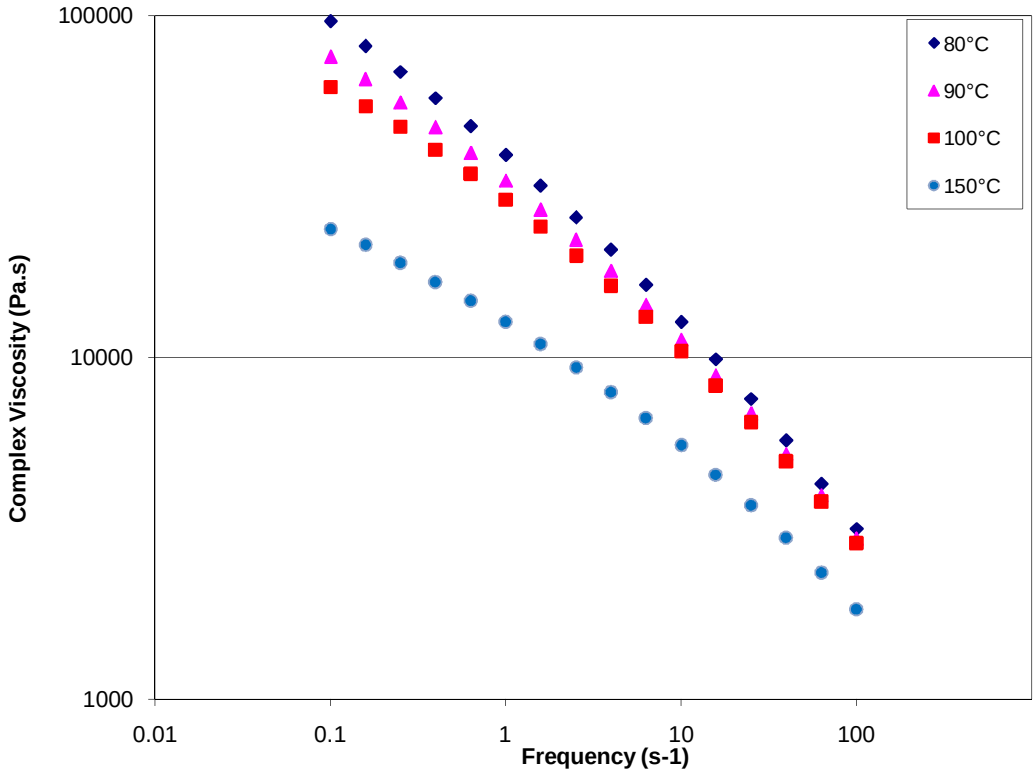
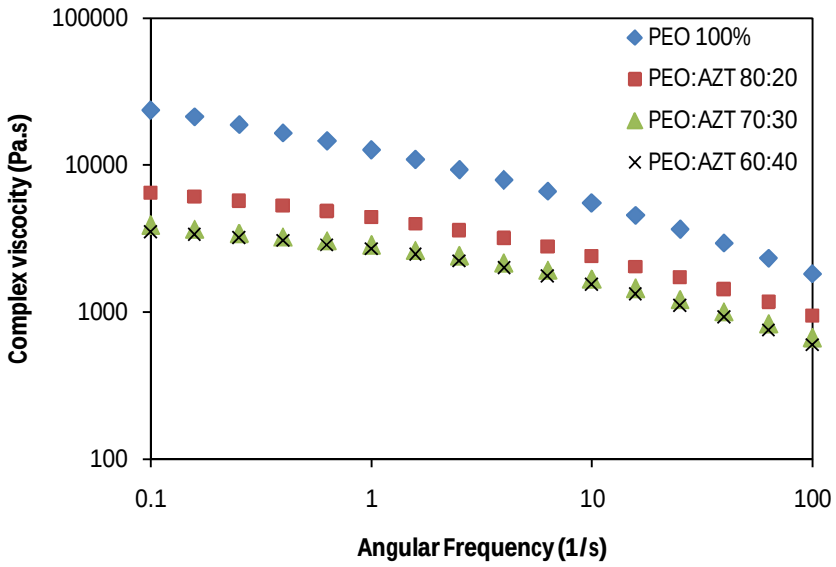
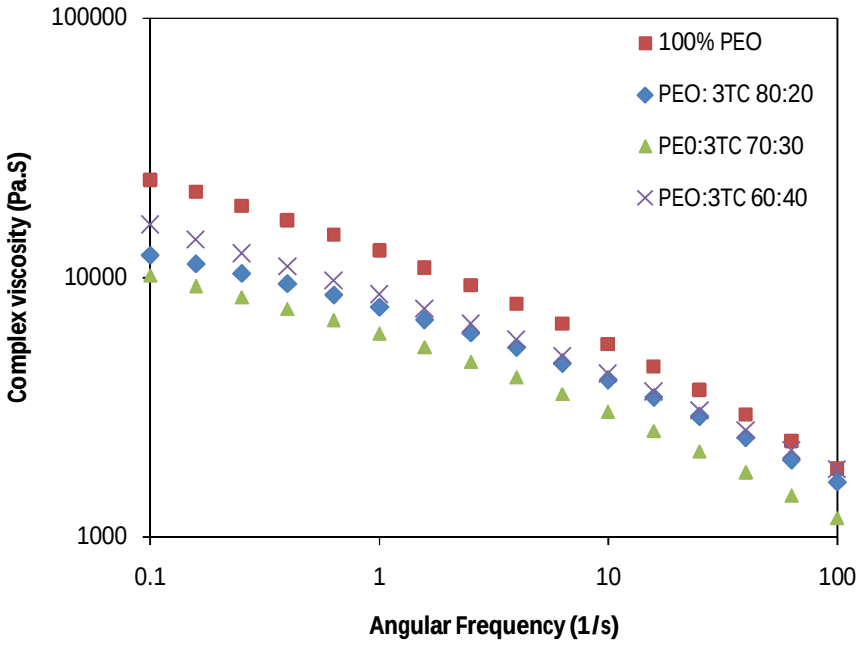


Figure 3.18: Complex viscosity of pure PEO at different temperatures

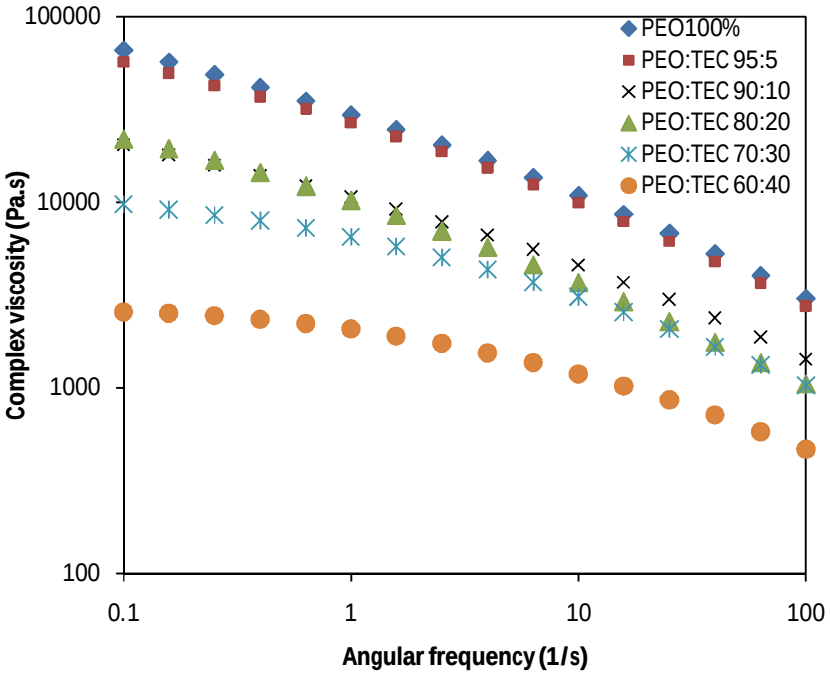
A)


100000
10000
1000
100
0.1
1
10
100
Complex viscocity (Pa.s)
Angular Frequency (1/s)
PEO 100%
PEO:AZT 80:20
PEO:AZT 70:30
PEO:AZT 60:40

B)


100000
10000
1000
0.1
1
10
100
Complex viscosity (Pa.S)
Angular Frequency (1/s)
100% PEO
PEO: 3TC 80:20
PE0:3TC 70:30
PEO:3TC 60:40

C)



D)

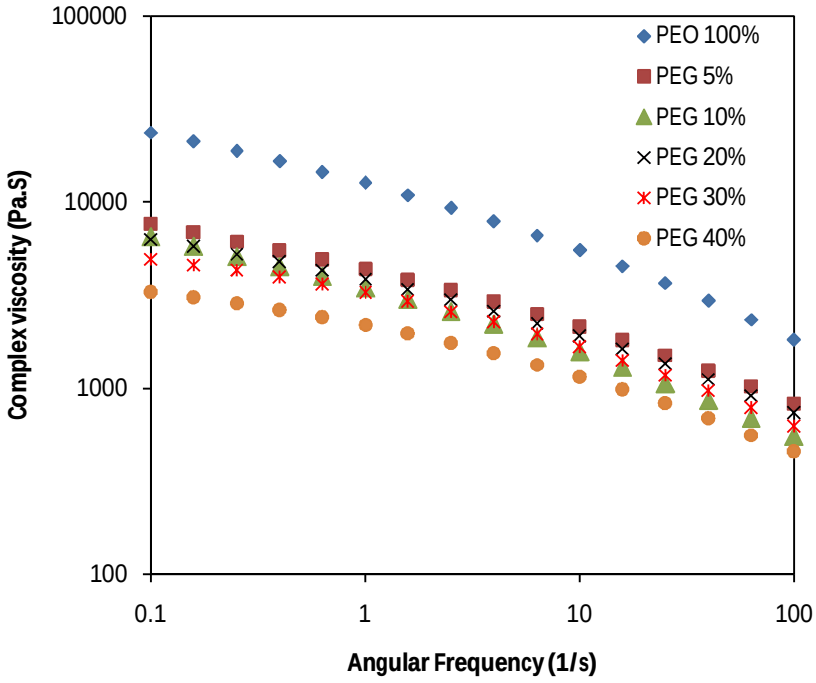


Figure 3.19: Complex viscosity of PEO and A) Zidovudine (AZT) blends (B) lamivudine (3TC) blends C) TEC blends (D) PEG-6000 blends at 150°C.

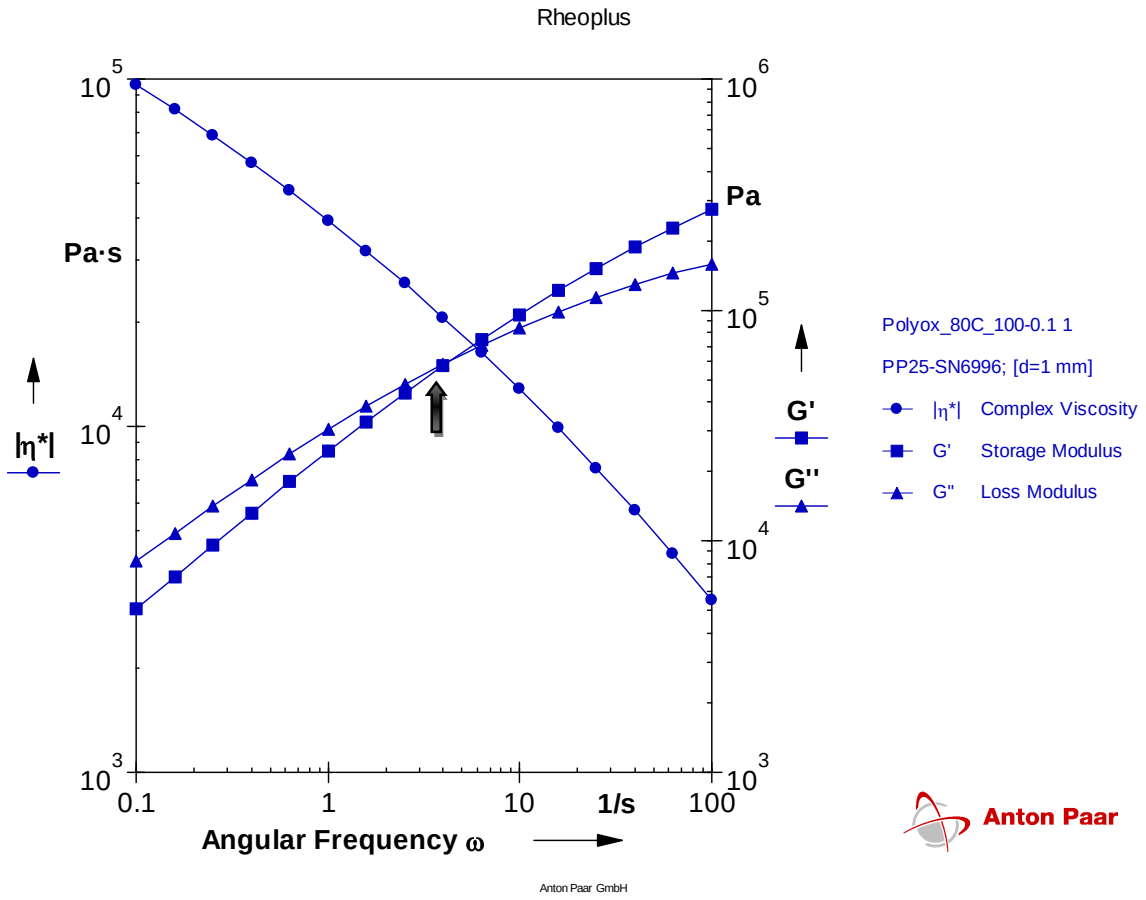


Figure 3.20: Cross over frequency (rad/s) of Pure PEO at 80°C where G□ (storage modulus) crosses over G□ (loss modulus).

Table 3.8: Cross over frequency (G□/G□) of pure PEO at different temperatures

Temperature (□C)	Cross over frequency (rad/s)
80	3.6
90	6.3
100	9.9
150	39.8

3.3.4 Hot stage microscopy

When the individual components were heated gradually at a rate of 5°C/min over the temperature range of 20°C to 180°C, it was observed that ethylcellulose became tacky or started to liquefy at 128°C and melted at 180°C. These observations were linked to the melting temperature (Tm) of ethyl cellulose, AZT and 3TC. AZT melted at approximately 126°C to 127°C when evaluated as a single substance and also when blended with ethyl cellulose (Figure 3.20 A (b)). The crystals of AZT were clearly observed under polarized light and were shown to melt when the temperature reached approximately 126°C to 127°C. Upon rapid cooling and reheating, the crystals of AZT were no longer observed which indicates that both drug and polymer were miscible and had formed a solid solution. 3TC which melted at 175.8°C when in crystalline form was also shown to form a solid dispersion on rapid cooling of the 30% w/w drug loaded sample with no melting transition for crystallites observed on reheating. The ethyl cellulose and 3TC blend with 40% w/w concentration however showed the presence of crystals (figure 3.20 B(c)) which suggests that the solution of drug in polymer becomes saturated above a loading of 30% w/w. This phenomenon supports the findings of the rheological studies which showed higher complex viscosities for blends with a drug loading exceeding 30% w/w of 3TC.

A **B**

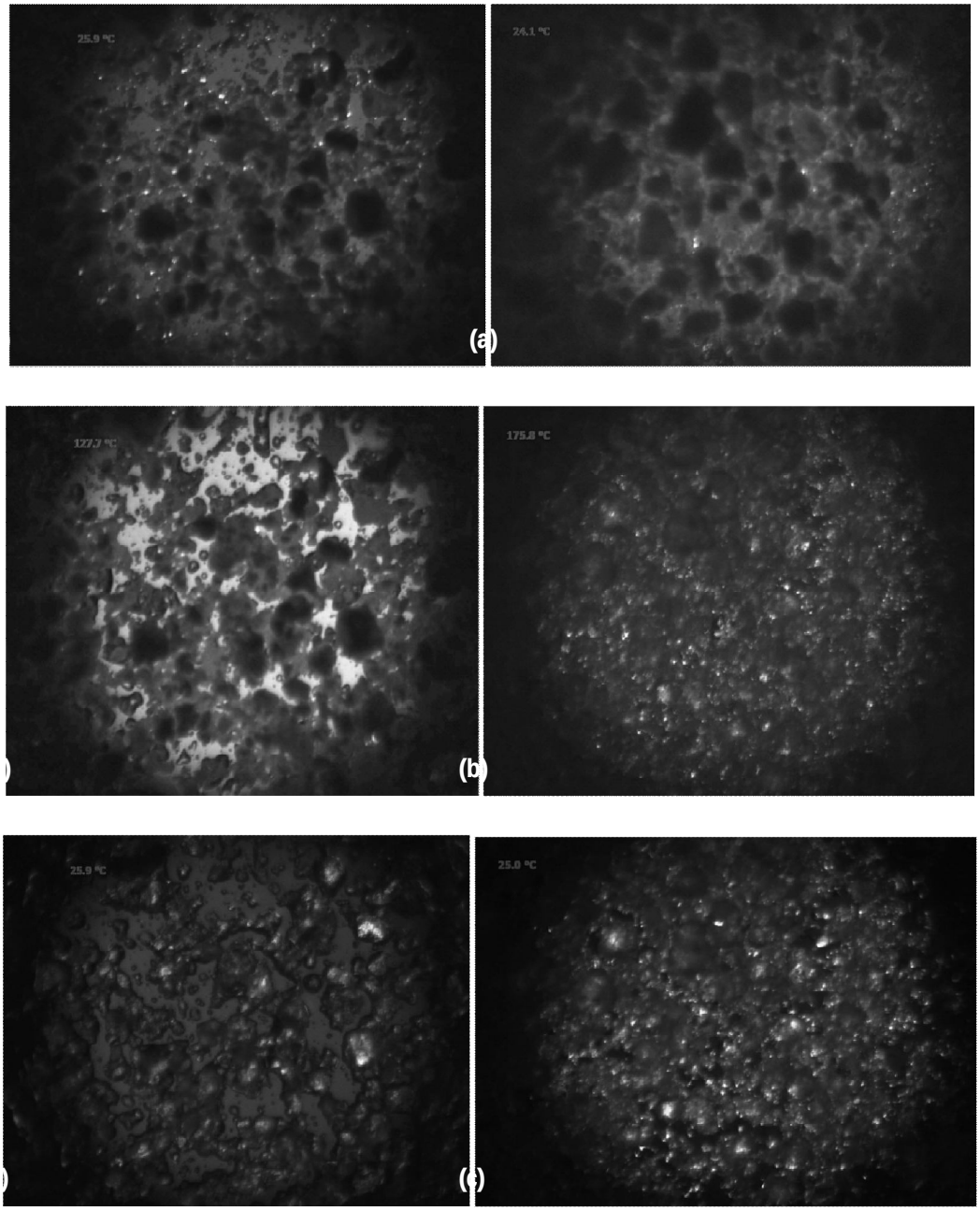


Figure 3.21 : A) Hot stage microscopy under polarised light of ethylcellulose: AZT(60: 40) during the heating cycle at heating rate and rapid cooling cycle at (a) 25.9°C (b)127.7°C (under un polarised light) (c) 25.9°C during cooling cycle. B) : Hot stage microscopy under polarised light of ethylcellulose: 3TC (60: 40) during the heating cycle at 5°C/min and rapid cooling cycle at 20°C/min at (a)24.1°C (b)175.4°C (c)25°C during cooling cycle.

3.4 Conclusion

Two anti-retroviral drugs, AZT and 3TC showed good compatibility with ethyl cellulose, Kollidon® SR and PEO. The results obtained suggested that the formulations studied are suitable for further exploration using hot melt extrusion. Thermal analysis showed that AZT forms an amorphous solid solution with the three polymers at all loadings studied and had a plasticizing effect on the flow properties of ethyl cellulose. 3TC appeared to become saturated within the polymer (Ethylcellulose and PEO) at drug loadings above 30% by weight, forming a solid dispersion with slightly crystalline structure. TEC appeared to be most suitable plasticizer to aid processing during hot melt extrusion at loadings of 5 to 10% by weight. PEG-6000 was found to re-crystallize upon cooling and thereby may render the formulations unstable during storage. Melt extrusion temperatures of between 130ºC and 165ºC are suggested, with a possibility of degradation occurring above a temperature of 180ºC.

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CHAPTER FOUR: FORMULATION AND CHARACTERIZATION OF ETHYLCELLULOSE SUSTAINED RELEASE, FIXED DOSE COMBINATION MINI MATRICES OF ZIDOVUDINE AND LAMIVUDINE

4.0 Introduction

A combination of ethylcellulose with a hydrophilic polymer such as PEO has been shown to modify the release rate of drugs (Vervaet et al, 2009). In this regard, high molecular weight PEO has been successfully applied in the development of oral controlled release dosage forms owing to its ability to control the rate of swelling, its muco-adhesive properties and erosion behaviour that allows reproducible control of release rates (API).

In the present study, sustained- release mini matrices were developed by hot melt extrusion for Zidovudine (AZT) and Lamivudine (3TC) with ethylcellulose. PEO was added to tailor drug release with the impact of PEO levels on *in vitro* release characteristics evaluated mechanistically using a range of standard equations. Furthermore a pharmacokinetic modelling approach was used to predict *in vivo* plasma drug concentration- time profiles and bioavailability outcomes for each drug from these *in vitro* dissolution data. In this regard, BCS biowaiver guidelines were introduced in 2000 by the FDA and in 2002 by EMEA (FDA guidance, 2000; EMEA, 2002). *In vivo* bio-equivalency studies may be replaced by *in vitro* studies for BCS I drugs if the test and the reference have similar dissolution profiles.

Although modified release Zidovudine and Lamivudine formulations are not available in the market, the *in vivo* pharmacokinetic data of immediate release formulations and controlled release obtained from various studies by Chokephaibulkit et al, 2011, Abu Izza et al, 1997, Sood and Panchagula, 2003, Kayitare et al, 2003 were considered for prediction of *in vivo* blood drug concentration profiles through simulation of in-vitro dissolution data.

The *in vivo* data obtained from the prediction by simulation could be useful for future human or animal *in vivo* and bioequivalence testing of modified/sustained release FDC zidovudine and lamivudine oral formulations.

4.1 Materials and Methods

4.1.1 Materials

Ethylcellulose STD 10 premium (Ethocel) with viscosity of 10.3 mPa.s (cps) ethoxyl content of 49.4% and polyethylene oxide (POLYOX™ or PEO-WSR N-80) of approximate molecular weight of 200,000 Da. were donated by Colorcon, Dartford, UK. Zidovudine (AZT) and lamivudine (3TC) were gifts from Cosmos Pharmaceuticals, Nairobi Kenya, while triethyl citrate (TEC) was supplied by Sigma-Aldrich, UK.

4.1.2 Methods

4.1.2.1 Composition of Physical Mixtures

Polymers: drug physical mixtures were produced by dry mixing the component powders in the appropriate ratios using a mortar and pestle for 10-15 min. The proportion of Zidovudine (AZT) and Lamivudine (3TC) drug content in the physical mixture was kept at 2:1 in all formulations. TEC was then blended in slowly in small portions for about 15-20 min until all was uniformly blended with the rest of the dry powder.

4.1.2.2 Production of mini matrices

A 16mm co-rotating twin screw extruder (Pharmalab, Thermo Scientific, UK, as shown in Figure 4.1) was used with standard screw configuration having length diameter ratio of 40:1, consisting of forward feeding elements, a mixing section and a discharge section.

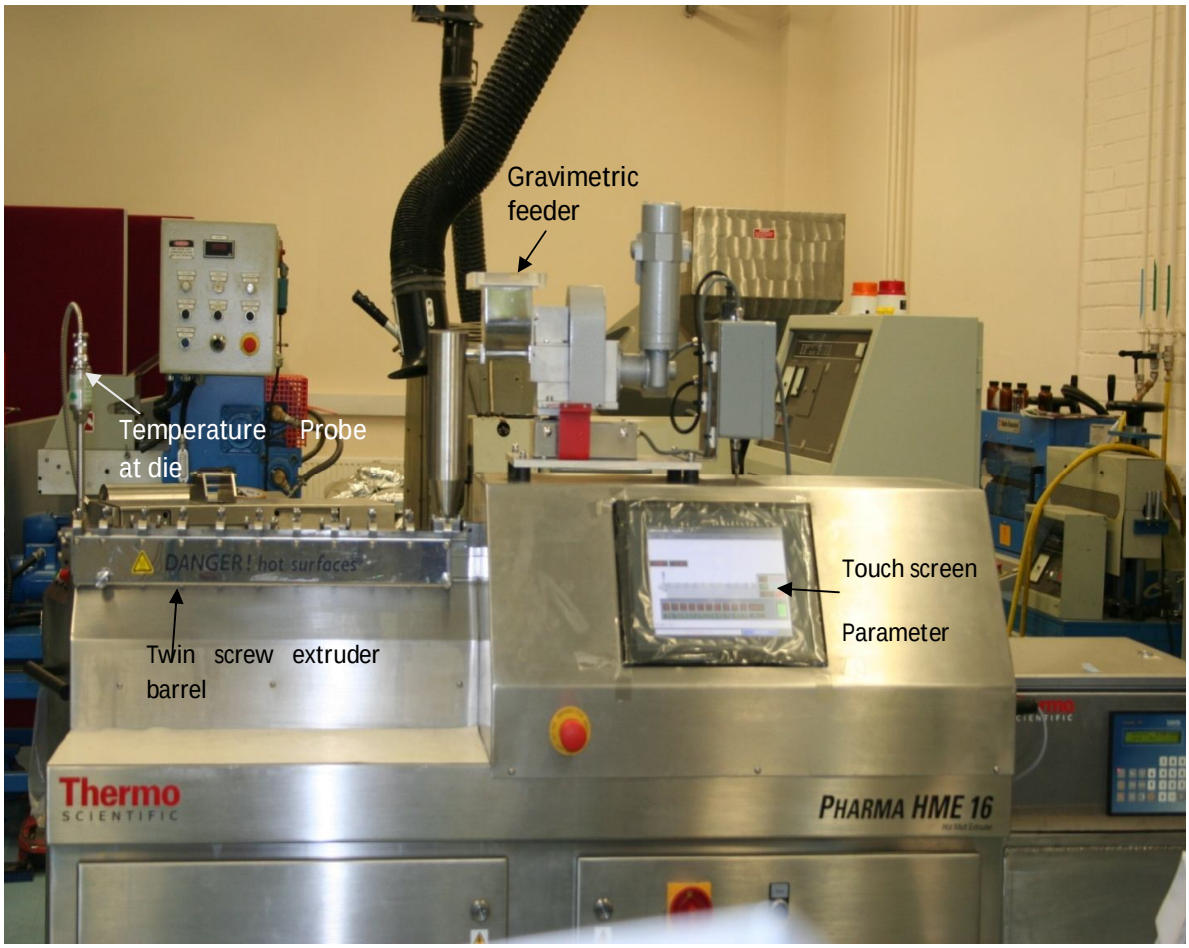


Figure 4.1: Thermo Scientific Pharma HME 16 extruder

The feeding section consisted of transport elements with a double helix to convey the materials along the screw length. The mixing section consisted of bi-lobal kneading blocks of 4mm thickness arranged to effectively blend the materials. The mixing section (28 screw diameters from the feed end) consisted of 18 mixing paddles with increasing staggering

angle: 9 elements at 30º, 5 elements at 60º and 4 elements at 90º. The discharge element which has a length of 24mm was placed at the end of the screw. Figure 4.2 shows the screw configuration used during extrusion

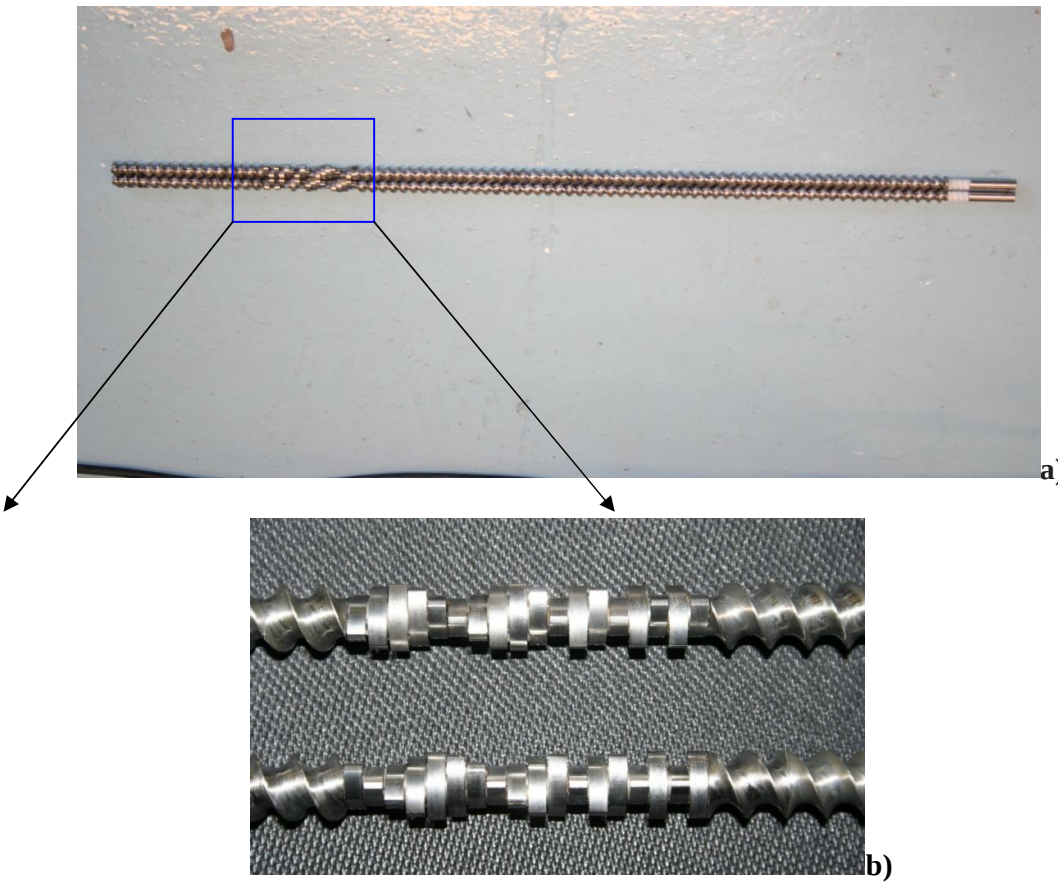


Figure 4.2: a) Stainless steel Twin screws with standard screw configuration setting b) close up of the mixing elements used in standard screw configuration for extrusion.

The extruder was attached to a Brabender gravimetric twin screw powder feeder. The extruder temperatures along the 10 screw zones (from Feeder to Die) of the extruder were set between 80-180ºC for the various formulations (Table 4.1). Once the temperatures were stable in the extruder, extrusion was carried out at a powder feed rate of 20g/min (1.2Kg/h) and a screw speed of 60 rpm. The extrudates were air cooled and extrudates strand attached

to a laboratory pelletizer (Eurolab, Varicut, UK) which pelletized the extrudates according to the thickness required i.e. from 1mm length pellets. The pelletizer was set to cut at 1mm. The pellets were then sieved through sieve no. 10 (2mm), 14 (1.4mm), 18 (1mm) and sieve no.35 (0.5mm) to exclude any large particles and small particles. The pellets/matrices that were retained on sieve no.18 (1mm) were used for further investigation. All the samples were then stored in a PVP glass vials at room temperature.

Table 4.1: Formulation composition and processing temperature range used for HME

Formulation	Composition (%w/w)	Processing Temperature range from feed to Die (□C)										Maximum Torque (%)	Melt pressure (Psi)	Extrudates Surface	Mini Matrices surface
F1	Ethocel/AZT+3TC/TEC (50/45/5)	80	100	120	130	140	150	160	170	175	180	84	39	Shark skin	cracks
F2	Ethocel/AZT+3TC/TEC (50/40/10)	80	100	120	130	140	150	160	170	175	180	46	14	Rough	cracks
F3	Ethocel/AZT+3TC/TEC (50/35/15)	80	100	110	120	130	140	150	160	165	170	38	8	smooth	smooth
F4	Ethocel/PEO/AZT+3TC/TEC (50/5/40/5)	80	90	100	110	120	130	140	150	160	165	82	16	Rough	Rough
F5	Ethocel/PEO/AZT+3TC/TEC (45/10/40/5)	80	90	100	110	120	130	140	150	160	165	42	8	smooth	Smooth
F6	Ethocel/PEO/AZT+3TC (40/30/30)	80	90	100	110	120	130	140	150	160	165	38	6	Smooth	smooth

4.1.2.3 Differential Scanning Calorimetry (DSC)

The thermal properties of matrices were assessed by modulated temperature Differential Scanning Calorimetric (MT-DSC) using Q2000 DSC (TA instrument, NJ) under nitrogen atmosphere. Samples (~ 5mg) were prepared in sealed aluminium pans and a customised heat-cool-heat cycle was applied to determine glass transition temperature (Tg) of the physical mixtures. A heating rate of 5°C/min was used to heat the samples from 25°C to 180°C. A cooling rate of 20°C/min was then used to reduce the temperature of samples to 0°C with subsequent reheating of samples at 1°C/min to 180°C. Triplicate samples were used for DSC studies.

4.1.2 X-Ray Powder Diffraction (XRPD) studies

The diffraction of x-rays by crystalline substances is of great analytical importance for characterising the solid state phase, since no two compounds would be expected to form crystals in which the three dimensional spacing of planes is totally identical in all directions. In most instances, a powdered sample in which particles are randomly oriented will present all possible crystal faces at a given interface and the diffraction from this powdered surface will therefore provide information on all possible atomic spacing relating to the crystal lattice. The powder pattern consists of a series of peaks detected at various scattering angles. These angles and their relative intensities are correlated with computed d-spacings to provide a full crystallographic characterisation of the powdered sample (Brittain et al., 1991) (Figure 4.3). This enables detection of crystal structure of drugs, polymers and plasticizers during pre- formulation studies and also helps to detect any changes which might occur during hot melt extrusion processing and stability studies. Relationships between d-spacing, the angle of

diffraction and the wavelength of the incident x-rays are described by the Bragg's law (Feeley, 1999) (Equation 1).

$$n\lambda = 2d \sin \Theta \qquad \textbf{(Equation 1)}$$

Where, n = order of the diffraction pattern

λ = wavelength of the incident beam

d = distance between the planes in the crystal

Θ = angle of beam diffraction

Structural analysis of the sample was carried out using a Bruker D-8 powder diffractometer (Bruker, Kahlsruhe, Germany), at room temperature. Powder samples were placed into a suitable sample holder and levelled using a glass cover slide, with scanning over 10-50 2θ using a 0.01 step width and a 1s time count and using a copper Kα radiation source of wavelength 1.542Å with 1mm slits. Samples were analysed in triplicate. The receiving slit was 1º and the scatter slit 0.2º. Calibration of the XRPD was performed using a carborundum (silicon carbide) standard.

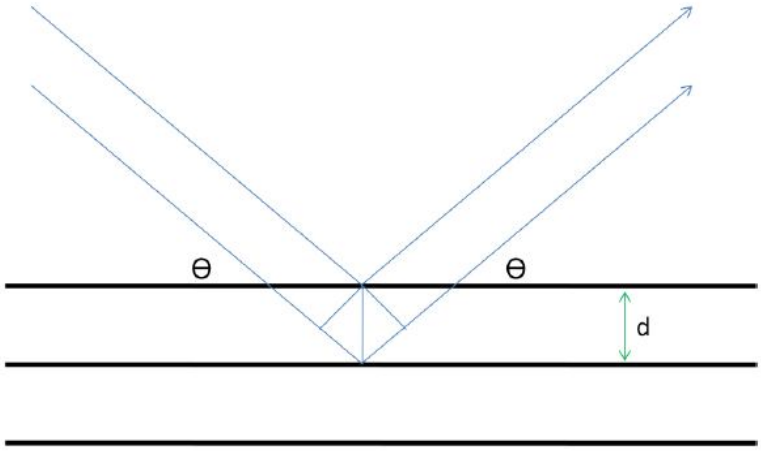


Figure 4.3: X-ray powder diffraction from two planes of atom.

4.1.2.5 Raman spectroscopy

Raman spectroscopy is a based on inelastic scattering of monochromatic light produced by a laser source. The sample molecules scatter photons from the incident laser light. The frequency of a small fraction of the scattered photons is changed (shift up or down); this is called the Raman Effect. The frequency shift is a parameter for vibrational, rotational and other low frequency transitions in the sample molecules. The scattered light is collected by a lens and sent to the detector, resulting in a Raman spectrum (Zhang, 2011).

For the spontaneous Raman Effect, the molecule will be excited from the ground state to a virtual energy state, and relax into a vibrational excited state, and which generates Stokes Raman scattering (McCreery, 2000). However, a small fraction of the molecules are in

vibrationally excited states and they are relaxed back to the ground state. The scattered photon appears at higher energy, as shown in Figure 4.4. This anti-Stokes Raman spectrum is always weaker than the Stokes-shifted spectrum. The Stokes and antistokes spectra contain the same frequency information.

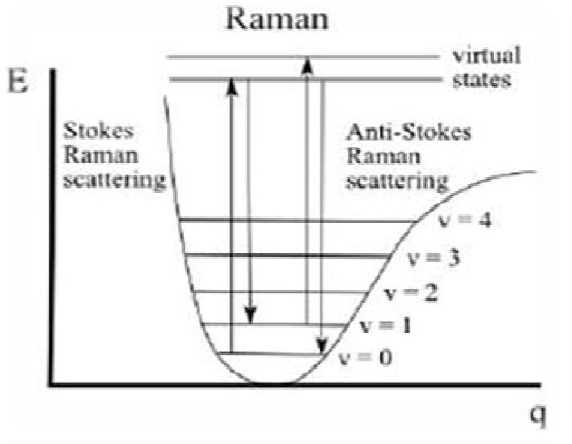


Figure 4.4: Vibrational energy state diagram for Raman. (Adapted from Siesler et al, 2002.) E is excitation energy, v is vibrational quantum number, q is vibrational coordinate, v = 0 is ground state, v = 1, v = 2, etc. are excited states.

Raman scattering occurs because a molecular vibration can change the polarisability of the molecule (McCreery, 2000). The polarisability measures the ease with which the electron cloud around a molecule can be distorted. A molecular polarisability change is required for the molecule to exhibit the Raman effect. In other words symmetric vibrations of non-polar groups are Raman active. The vibrations of a highly polar moiety, such as the O-H bond, are usually weak. Typical strong Raman scatterers are moieties with distributed electron clouds, such as carbon-carbon double bonds. Bending or stretching the bond changes the distribution of electron density substantially, and causes a large change in induced dipole moment.

Raman spectroscopy has been used quantify mixtures of polymorphs, utilizing distinct spectral peaks characteristic of each form or factor analysis techniques. The advantage of Raman spectroscopy over thermal analytical method like DSC is that Raman spectroscopy requires little or no sample preparation, it is a non-destructive and non intrusive analytical technique where the more labile sample structure (crystalline structure) can be studied without destroying the sample, there is minimum interference of water from the atmosphere. Raman spectroscopy was used in this project to investigate about the molecular information i.e. the crystalline and amorphous phases within the extrudates.

Typically, a sample is illuminated with a laser beam. Light from the illuminated spot is collected with a lens and sent through a monochromator. Wavelengths close to the laser line (due to elastic Rayleigh scattering) are filtered out and those in a certain spectral window away from the laser line are dispersed onto a detector, usually a CCD camera.

Raman spectroscopy was carried out using Renishaw InVia Raman spectrometer coupled with Leica DMLM microscope (Wotton-under-Edge, UK). Detector used was an air cooled CCD array detector (576 x 384 pixels). The sample was analysed using 785 nm (near infrared) and 514 nm (green) laser wavelengths, with ×10 and ×20 objective lenses, giving maximum spatial resolution of 1μm^2. 20 accumulations, each of 20 s exposure time were used to improve the signal-to-noise ratios of the recorded spectra.

4.1.2.6 In-vitro drug release studies

In vitro drug release studies were performed using USP 26 type II dissolution test apparatus (Copley Scientific, Nottingham, UK). Weighed samples of 275mg were placed in the dissolution vessel containing 900ml of pH 7.2 phosphate buffer (KH_2PO_4), maintained at 37 ± 0.5ºC and stirred at 50

rpm. 5ml aliquots were collected at predetermined time intervals of 30 min, 1hr, 2 hrs, 4hrs, 8hrs, 12hrs, 16hrs, 20hrs and 24hrs. The aliquots collected were filtered through 0.45µm filter paper and analysed by HPLC method described below.

4.1.2.7 Simultaneous Reverse Phase HPLC analysis

Dissolution samples were analysed simultaneously for AZT and 3TC using a reversed phase isocratic HPLC validated method (Beck et al, 2008) on a Waters 2695 LC system connected a pump, a Waters 2487 UV detector and an auto-sampler (Waters, Milford, MA, USA). A LiChrospher® 100 RP-18 5µm 250×4.6mm column (Merck, Darmstadt, Germany) was used which was maintained at a temperature of 20°C. The mobile phase consisted of 50:50 v/v ratio of methanol: acetate buffer (0.1M ammonium acetate in 0.5% glacial acetic acid). The pH of the mobile phase was adjusted to 6.5 using NaOH. The mobile phase was filtered through 0.45 µm membrane filter and degassed by sonication prior to use. The flow rate of the mobile phase was set at 1 ml/minute at ambient temperature. The run time interval was kept constant at 12 minutes for each run and the injection volume was 20µl. The wave length of detector was set at 270nm.

The concentration of AZT and 3TC samples was determined from the calibration curve constructed from a range of standard concentrations for AZT (100 to 400 µg/ml) and 3TC (50 to 250 µg/ml). The concentration of AZT and 3TC was calculated using the standard calibration curve which was obtained by plotting peak area of the drug against the concentration. Linear correlation was confirmed between 100.00 µg/ ml to 400.0 µg/ ml for AZT (R^2 = 0.9979) and 50 to 250µg/ml for 3TC (R^2 = 0.9973) and multiple injections yielded good reproducibility with RSD values between 0.08% (400.0µg/ ml) and 1.77% (100µg/ml)

for AZT and RSD values between 0.17% (250μg/ml) and 1.10% (50μg/ml) for 3TC. The batches were evaluated in triplicates and the average of the three profiles was plotted using cumulative % drug release vs. time in hours.

4.1.2.8 Drug content determination

Drug content was assessed for the mini matrices by weighing mini matrices having an equivalent of 100 mg of AZT and 50 mg of 3TC then transferring these samples into a 200 ml volumetric flask. Approximately 100 ml of mobile phase was added and the resultant sample was sonicated for 12 hrs. The sample was the adjusted to 100 ml and then diluted to final concentrations of 100 μg/ml and 50 μg/ml for AZT and 3TC respectively. Final solutions were filtered using 0.45 μm filter membrane and analysed using the HPLC method described above in 4.2.2.7. Drug content determinations were conducted in triplicate.

4.1.2.9 Modelling the drug release kinetics

The level and mechanism of drug release from the mini-matrices was determined by fitting the release rate data with the modelling equations: Zero order, first order, Hixson- Crowell, Koysmeyer- Peppa's equation and Higuchi matrix equations as described below. The data was analysed by PCP- Disso software (V3, Poona College of Pharmacy, Pune, India).

Zero order model equation:

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equations 1 and 2:

$$\mathbf{Q_0 - Q_t = K_0 t} \qquad \textbf{(Equation 1)}$$

Rearrangement of equation (1) yields:

$$\mathbf{Q_t = Q_0 + K_0 t} \qquad \textbf{(Equation 2)}$$

Where:

Q_t is the amount of drug dissolved in time *t*,

Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as cumulative amount of drug released *versus* time (Narashimhan et al 1999, Hadjiioannou et al, 1993).

Application: This relationship can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms and osmotic systems.

First Order model equation:

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation 3:

$$dC / dt = -Kc \qquad \textbf{(Equation 3)}$$

Where:

K is first order rate constant expressed in units of $time^{-1}$.

Equation (3) can be expressed as:

$$\log C = \log C_0 - Kt / 2.303 \qquad \textbf{(Equation 4)}$$

Where:

C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time.

The data obtained are plotted as log cumulative percentage of drug remaining *vs*. time which would yield a straight line with a slope of -K/2.303.

Application: This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices (Gibaldi et al,1967).

Hixson-Crowell model

Hixson and Crowell (1931) recognized that the particles regular area is proportional to the cube root of its volume. They derived the equation 5:

$$W_0^{1/3} - Wt^{1/3} = \kappa t \qquad \textbf{(Equation 5)}$$

Where:

W_0 is the initial amount of drug in the pharmaceutical dosage form, Wt is the remaining amount of drug in the pharmaceutical dosage form at time t and κ (kappa) is a constant incorporating the surface-volume relation. The equation describes the release from systems where there is a change in surface area and diameter of particles or tablets (Hixson and Crowell, 1931). To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as cube root of drug percentage remaining in matrix *versus* time.

Application: This expression applies to pharmaceutical dosage form such as tablets, where the dissolution occurs in planes that are parallel to the drug surface if the tablet dimensions diminish proportionally, in such a manner that the initial geometrical form keeps constant all the time. Hixson-Crowell equation overall indicates an erosion-dependent release mechanism in which the release rate is controlled by the dissolution rate of the drug particles.

Korsmeyer -Peppas model

Korsmeyer et al. (1983a) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer- Peppas model (Korsmeyer et al, 1983 b) given by Equation 6.

$$\mathbf{Mt / M\infty = Ktn} \qquad \textbf{(Equation 6)}$$

Where:

Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. For the case of cylindrical tablets, $0.45 \geq n$ corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super case II transport (Siepmann et al, 2001). To find out the exponent of *n* , the portion of the release curve, where Mt / M∞ < 0.6 should be used. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time.

Higuchi model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961. Initially conceived for planar systems, it was then extended to different geometrics and porous systems (Grassi et al, 2005). This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be

negligible); (iii) drug particles are much smaller than system thickness; (iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant; and (vi) perfect sink conditions are always attained in the release environment. Accordingly, model expression is given by the equation 7:

$$\mathbf{ft = Q = A\sqrt{D(2C - Cs)\,Cs\,t}} \qquad \textbf{(Equation 7)}$$

where:

Q is the amount of drug released in time t per unit area A, *C* is the drug initial concentration, *Cs* is the drug solubility in the matrix media and *D* is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance. This relation is valid during all the time, except when the total depletion of the drug in the therapeutic system is achieved. To study the dissolution from a planar heterogeneous matrix system, where the drug concentration in the matrix is lower than its solubility and the release occurs through pores in the matrix, the expression is given by equation (8):

$$\mathbf{ft = Q = \sqrt{D\delta/\tau\,(2C - \delta Cs)\,Cs\,t}} \qquad \textbf{(Equation 8)}$$

Where:

D is the diffusion coefficient of the drug molecule in the solvent, δ is the porosity of the matrix; τ is the tortuisity of the matrix and Q, A, Cs and t. have the meaning assigned above. Tortuisity is defined as the dimensions of radius and branching of the pores and canals in the matrix. In a general way it is possible to simplify the Higuchi model (Equation 9) as (generally known as the simplified Higuchi model):

$$\mathbf{f\,t = Q = K_H\,t^{1/2}} \qquad \textbf{(Equation 9)}$$

where, K_H is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.

Application: This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs usually a porous hydrophobic system where drug release occurs when the drug comes into contact with the dissolution media, subsequently dissolves and diffuses through media filled pores (Crowley et al, 2004).

Parameters such as T25%, T50%, T75%, T80% and T90% were obtained during data analysis for all the formulations. These are the time taken for 25%, 50%, 75%, 80% and 90% release of the drug content respectively.

4.1.2.10 Accelerated Stability studies

Accelerated stability studies were conducted to monitor the solid state stability of the formulations. Approximately 300mg of the mini matrices were dispensed in high density polyethylene (HDPE) bottles open and closed, for a period of three months in a hot oven chamber at 40ºC and 75% Relative Humidity (RH) as well as at 60ºC in closed HDPE bottles for 15 days. Within this chamber a beaker full of saturated sodium chloride solution which was placed to maintain the humidity conditions. Samples were removed at predetermined time intervals of 1 week for three months and tested for XRD and dissolution testing where the samples were tested using reverse phase HPLC method as described previously.

The comparison of dissolution release profile of mini matrices after accelerated stability testing were done with the dissolution release profile of mini matrices after hot melt extrusion using model independent method by calculating similarity factor f_2 value. The similarity factor f_2 value is a logarithmic reciprocal square root transformation of the sum of the squared differences in drug release percentage for the two delivery systems and is calculated as follows (FDA, 1997):

$$f_2 = 50\,\mathrm{Log}\left(\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right)$$

Where: *n* is the number of observations, *Rt* represent the percentage of drug released from the reference profile and *Tt* is the percentage of drug release from the test profile. Drug release profiles are judged to be when the *f2* is ≥ 50.

4.1.2.11 Predicting plasma drug concentration-time profile using in-vitro drug release

An attempt was made to predict the plasma drug concentration-time profiles using *in vitro* dissolution data and pharmacokinetic parameters of AZT and 3TC available from literature. The pharmacokinetics parameters for paediatrics were taken into account from literature for calculation and are as follows:

Pharmacokinetic Parameter	Volume of Distribution in L/Kg	Elimination rate (Ke) constant h^{-1}	Bioavailability (F) %	Half life $t_{1/2}$(hrs)	Dose in mg
AZT	1.4[a]	0.462	60[a]	1.5	300
3TC	1.3[a]	0.116	65[a]	6	150

[a] EMEA/H/C/0672/II/0011, 2007

Dose for sustained release used for the formulation was 300mg, AZT and 150 mg, 3TC for once daily use.

The *in vitro* release data was collated and analysed using Microsoft Excel software. The convolution approach of converting the in-vitro dissolution profile to *in vivo* / drug-concentration time profile was used stepwise as described by Quereshi, 2010. The following steps were used:

1. The *in vitro* dissolution results, % cumulative drug release Vs time obtained from section 4.1.2.6 are converted into discrete dosage segments where the amount release at each time interval is converted to amount released in milligrams (mg) by using the formula (% cumulative drug release x product strength/100).
2. The amount calculated in mg will not wholly be available in blood plasma since AZT and 3TC have 60% and 65% bioavailability (F) respectively. The amount then available in blood plasma is calculated using the formula (amount (mg) x F).
3. The amount calculated in step 2 will then be available for absorption and will be detected in blood plasma. After absorption, drug elimination begins thus the drug elimination rate is calculated for AZT and 3TC using the first order elimination rate formula which is calculated using the half life ($t_{1/2}$): $Ke = 0.693/t_{1/2}$

4. Using the single exponential equation the drug amount (mg) at each time interval is calculated. This amount of drug reflects to the total amount of drug available after absorption.
5. Lastly the drug concentration at each time interval is calculated from the total amount of drug available after absorption using the formula: Concentration (ng/ml) = amt. drug after absorption x 1000/ Vd x body wt.
6. The blood plasma concentration versus time profiles were then plotted on graph and the following parameters were obtained; Maximum concentration (Cmax) and time taken to reach Cmax (Tmax).

4.1.2.12 Statistical Analysis

Statistical analysis was computed using Miscrosoft Excel, 2007 (Miscrosoft®, USA). One-way ANOVA with Bonferroni post hoc test (student t-test) was used to determine the statiscal significant differences obtained between results. The results with p values < 0.05 were considered statistically significant ($\alpha = 0.05$).

To determine the effect of increasing TEC loading and PEO loading on drug release was statistical analysed using two-way ANOVA with Bonferroni post hoc test (student t-test) was used to determine the statiscal significant differences obtained between results. The results with p values < 0.05 were considered statistically significant ($\alpha = 0.05$).

4.2 Results and discussion

4.2.1 Processibility via hot melt extrusion

Ethylcellulose mini matrices were successfully prepared by HME with up to 40% w/w total of AZT and 3TC. The concentration of ethylcellulose was maintained at 50% w/w. Extrusion of ethylcellulose with the drugs alone was difficult at 180ºC and high torque values were observed during extrusion. Increasing temperatures above 180ºC degraded the drugs and the extrudates were observed to be a deep burnt colour. TEC was added to reduce the torque (motor load) during extrusion process. It was observed that with increase in TEC levels (up to 15%), there was a reduction in torque levels although the extrudates obtained with 15% TEC were transparent but too plastic and difficult to handle. Upon cooling the extrudates with 15% TEC turned brittle and were difficult to pelletize thus the maximum temperature during extrusion was reduced from 180ºC to 170ºC. The extrudates obtained at 170ºC were slightly less transparent, but less brittle and pelletizing into 2mm mini matrices was possible.

It was possible to easily extrude and process at different concentration mixtures of ethylcellulose, PEO and the drugs at 180ºC. The torque is the force of resistance on the screw to maintain a specific speed. The pressure gauge indicates the pressure at the location of the probe situated at the end of the barrel, just before the die. High torque values indicate greater resistance of the material on the screws during extrusion and when extruding through the die high pressure will be generated. The extrudates produced at 180ºC with PEO had a high viscosity and was difficult to extrude at the die with maximum torque per screw shaft of 2.1Nm (82%). The Exdrudate's surface had shark skin (melt fracture that has small spurt like protrusions on the surface of the extrudates) and was uneven/ rough throughout the surface of the extrudate (Figure 4.5 and Table 4.2).

Formulation	Processing Temp (°C)	Extrudate characteristics		AZT Dissolution at 24hrs in %	3TC Dissolution at 24 hrs in %
		DSC	XRPD		
F1	80-180	Amorphous	Amorphous	23	27
F2	80-180	Amorphous	Amorphous	36	38
F3	80-170	Amorphous	Amorphous	44	45
F4	80-165	Crystalline	Crystalline	82	83
F5	80-165	Crystalline	Crystalline	88	89
F6	80-165	Crystalline	Crystalline	98	99

Table 4.2: AZT and 3TC formulations with Ethylcellulose, PEO and TEC, processing temperatures, Extrudate characteristics and dissolution after hot melt extrusion trial.

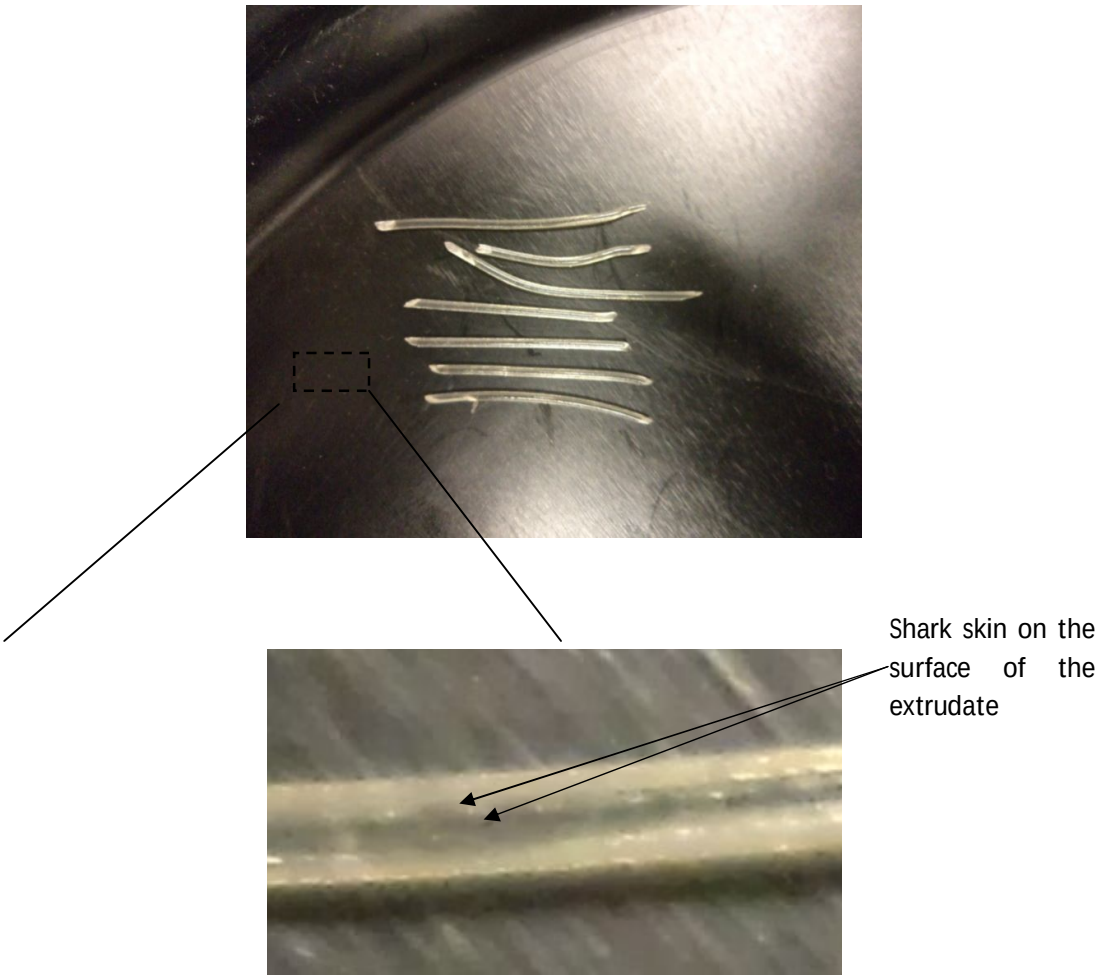


Figure 4.5: Extrudates surface to show shark skin on the surface of the extrudates after air cooling at room temperature

Extruding at higher temperatures would only result into degradation of the drugs and cause darkening of the polymers thus TEC 5% was added to the physical mixture and extruded at 180ºC. The extrudates were observed to collapse during extrusion and there was noticeable phase separation. The maximum temperature in the extruder zone was then reduced to 170ºC at which there was evidence of physical separation of the phases.

At 165ºC the extrusion was achievable at 50 rpm and low torque values. The extrudates were smooth with no shark skinning and could easily be pelletized. An increase in PEO concentration did not require the temperatures to be lowered any further than 165ºC since extrusion was difficult at temperatures lower than 165ºC. PEO at 30% w/w concentration plasticized ethylcellulose during extrusion and did not require TEC as a plasticizer. The physical mixture with 30% w/w of PEO was processible and smooth on the surface when the extrudates were pelletized. Figure 4.6 shows the extrduate strands after extrusion of F1, F3 and F6. It was observed that as the percentage of TEC increased the extrudates became more transparent in colour which is an overall sign that ethylcellulose has been plasticized. The addition of PEO to the blend for extrusion turned the extrudates opaque but as the quantity of PEO increased the surface of the extrudates became smoother and pelletization was much easier.

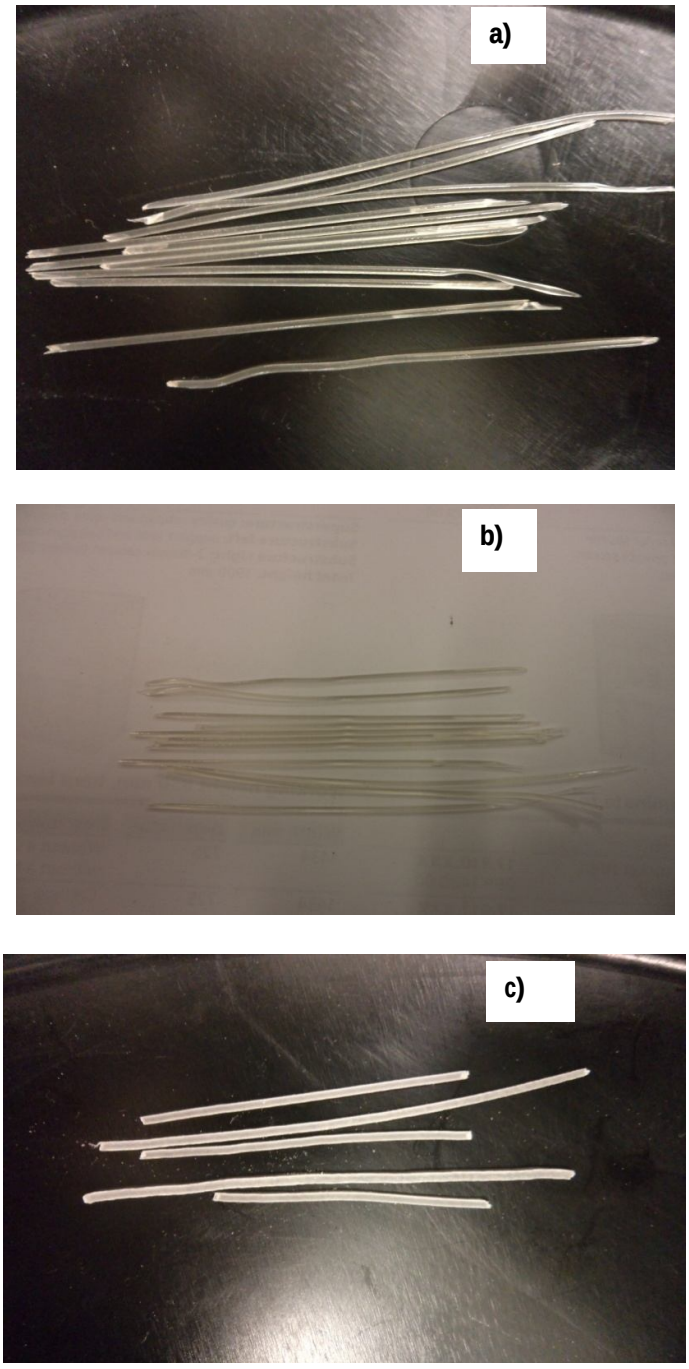


Figure 4.6: Extrudates of formulation a) F1: Ethocel/Drug/TEC (50/45/5%)

b) F3: Ethocel/Drug/TEC (50/35/15%) c) F6: Ethocel/Drug/TEC (40/30/30)

4.2.2 Physico-chemical analysis of the mini matrices

4.2.2.1 DSC studies

DSC studies were undertaken to investigate the physical state of the drugs within the polymer matrix after extrusion. The melting peak associated with AZT was not observed in the DSC curves of the first heating cycle for the extrudates of formulation F1 although a very broad endothermic Tm peak was detected at 151.3ºC (SD ± 0.4ºC) for 3TC. On cooling, a very small broad exothermic re-crystallization peak (Tc) relating to 3TC was observed 140.3ºC (SD ± 0.8ºC for all compositions suggesting that re-crystallization of amorphous drug has occurred (Figure 4.7). It is therefore observed that each of the drugs had been rendered predominantly amorphous during extrusion.

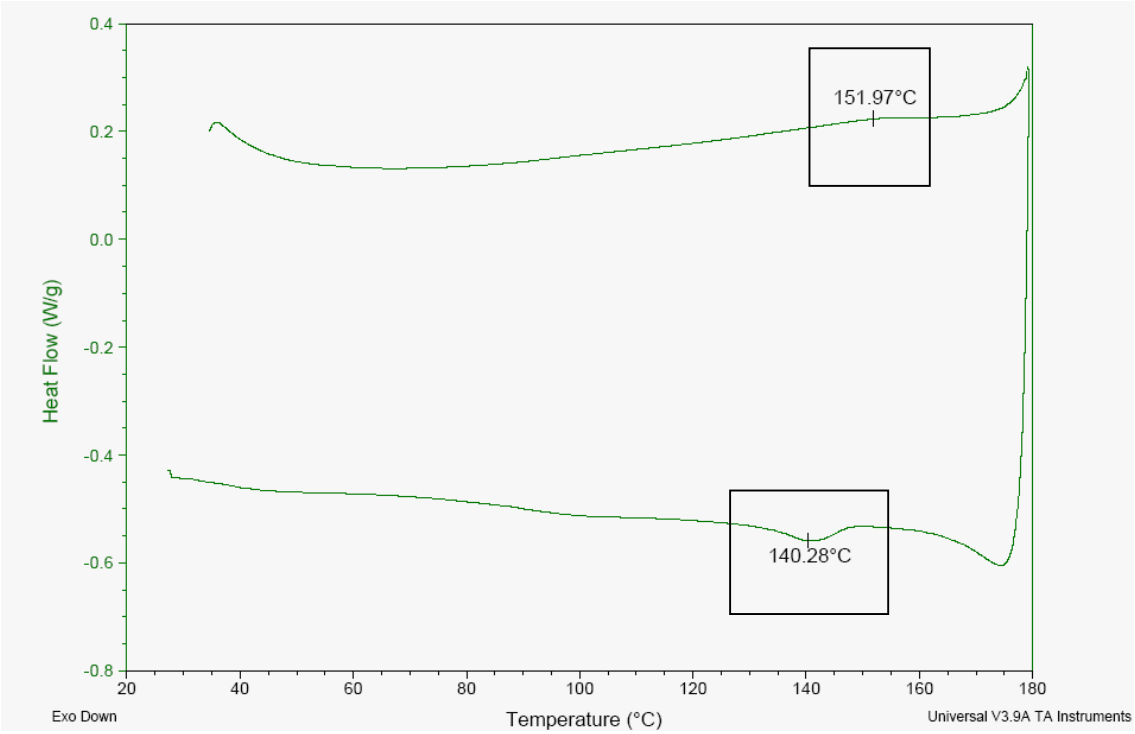


Figure 4.7: DSC curve of heat & cool cycle of formulation F1 where 3TC re-crystallization has been observed

As the percentage of TEC increased, the Tm of 3TC was shown to reduce resulting in the presence of a broad shallow peak. The associated re-crystallization peak was also shown to occur at lower temperature with similar broad and shallow shape which indicated that 3TC is partially miscible in ethylcellulose and PEO while AZT was fully soluble in the polymers. The Tm of pure PEO is 65.9°C (S.D ± 0.3°C). The Tm of PEO in the formulated extrudates was observed at 58.30º C for Formulation F6 (Ethocel/ PEO/ (AZT+3TC) at 40/30/30% w/w), whilst the Tg of the preparation was 103.2ºC (S.D ± 0.40ºC). In this example, TEC was not used since the relatively high level of PEO (30%w/w) was sufficient to plasticize ethylcellulose. It was therefore possible to manufacture the formulation with relative ease to produce smooth extrudates of acceptable appearance.

Tg of ethylcellulose was lowered to 86.3°C (S.D ± 0.8°C at 5% TEC (F1), 74.2°C (S.D ± 0.6°C) at 10% TEC (F2) and 70.3°C (S.D ± 0.8°C) at 15% TEC (F3). Addition of PEO at concentrations of 5 and 10%w/w to formulations F4 and F5 respectively which also contained 5%w/w TEC produced extrudates in which the Tm associated with PEO was observed as a broad endothermic peak, at and reduced temperature of 52.5°C (S.D ± 0.6°C) and 55.6°C (S.D ± 0.7°C) respectively relative to the Tm of pure PEO which occurs at 65.9°C (S.D ± 0.3°C). Formulation F4 contained PEO (5%w/w) and TEC (5%w/w) whilst F5 contained PEO and TEC at levels of 10%w/w and 5%w/w respectively. The results obtained were similar to the thermal data obtained for the physical mixtures in chapter three.

4.2.2.2 XRPD Studies

XRPD studies were done for the pure polymers, physical mixture drugs and formulations after extrusion and pelletization as shown in Figure 4.8. Unprocessed AZT, 3TC and PEO showed sharp diffraction peaks confirming the existence of highly crystalline materials. Pure unprocessed ethylcellulose however showed diffuse scatter with no discernible diffraction

peaks which clearly indicates that ethylcellulose is amorphous in nature. The crystalline nature of pure PEO was confirmed by presence of characteristics peaks at 2- theta angles of 19.3 and 23.4 degrees. The major crystalline peaks for AZT and 3TC were observed in the physical mixtures, but were absent in the extrudates indicating a complete loss of crystallinity after extrusion at all ratios and processing temperatures. This result suggests that an amorphous solid dispersion has been produced by HME.

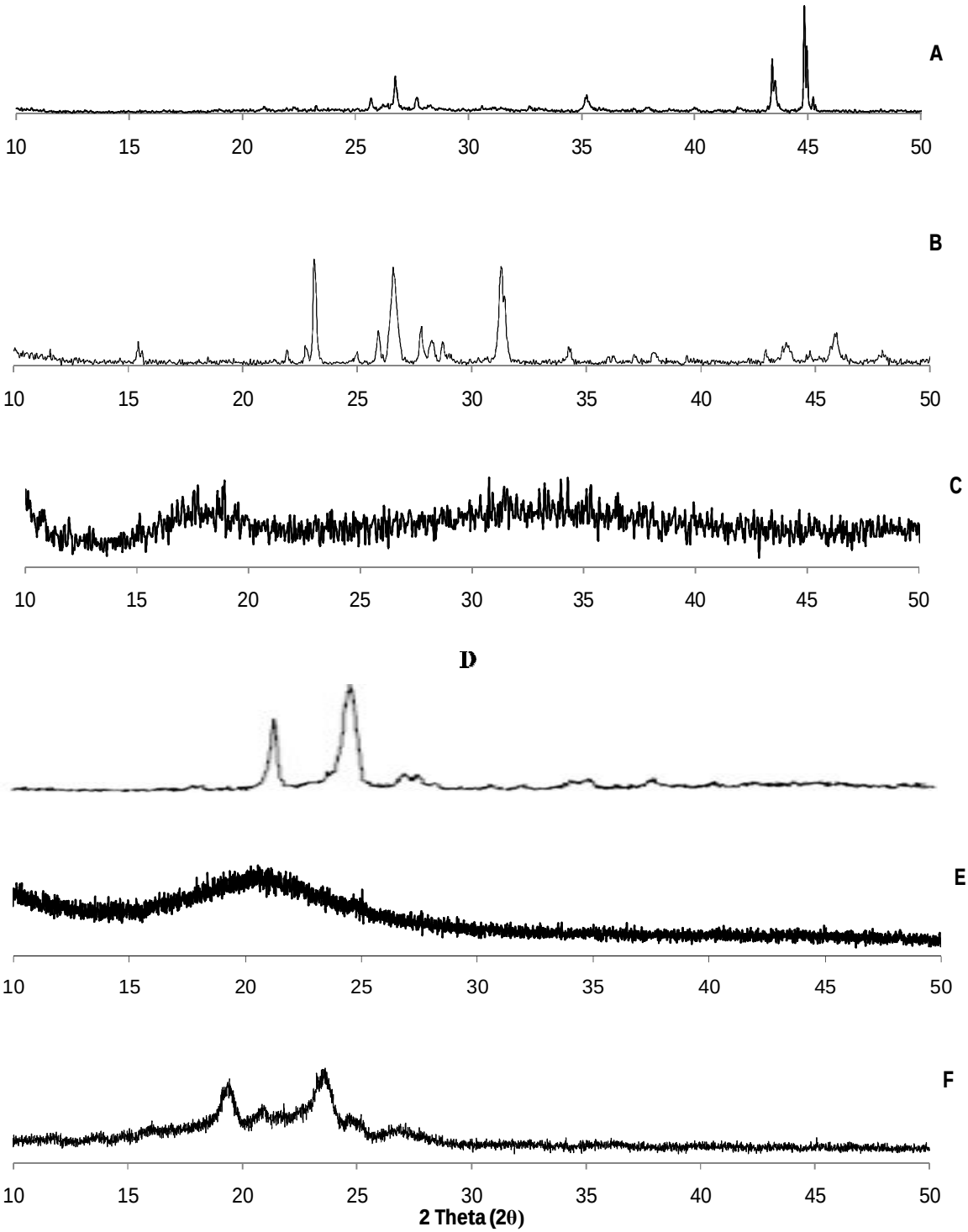


Figure 4.8: XRD patterns of A) AZT B) 3TC C) Ethylcellulose D) PEO E) Extrudate F1Ethocel/Drugs/TEC (50/45/5%w/w) F) Extrudate F6 ethylcellulose: PEO: drug (40/30/30 %w/w)

4.2.2.3 Raman spectroscopy studies

Vibrational spectroscopy is an excellent method for probing solid state interactions between molecules and more so Raman spectroscopy has been widely used as a process analytical tool to observe molecular interactions in the solid state. In this regard, Raman spectra for ethylcellulose, AZT, 3TC, physical mixtures of these constituents (50/45/5%w/w) and their resultant extrudates were obtained offline in wave number range 100 – 3200 cm^{-1} as shown in Figure 4.9. The focus of this study was determining the existence of specific intermolecular interactions in the extruded formulations produced by HME. The blue coloured boxes in Figure 4.9 show the ranges of greatest Raman activity for each of the analysed samples. Ethylcellulose was confirmed through presence of the Raman bands at 1360 cm^{-1} assigned to C- OH group and 881 a very weak band assigned to C_1- H bending. AZT signature peaks were observed at 2869 cm^{-1} assigned to C-H stretching, 2584 cm^{-1} and 2114 cm^{-1} assigned to N≡N and C= O stretching modes and 1643 cm^{-1} assigned to the C=C stretch of the pyrimidine ring. 3TC signature peaks were observed at 466, 774.cm^{-1}, 793 cm^{-1}, 1200 cm^{-1}, 1238 cm^{-1} and 1282cm^{-1} assigned to C-O-C stretching of the oxathiolane ring. The band observed at 1606 cm^{-1} was assigned to the cystedine nucleus. Most of the Raman bands observed for ethylcellulose and PEO were overlapping owing to the predominance of similar covalent bonding motifs in these structures. It was therefore difficult to identify one single peak that could be identified and monitored before and after extrusion to show changes in solid form. However it was observed that, after extrusion the intensity of peaks for AZT and 3TC were reduced and broadened relative to the peaks of the pure drugs and polymers. This was an indication that both AZT and 3TC had been transformed to the amorphous state by heating to temperatures in the range 165ºC to180ºC. It was further observed that the amount of drugs and percentage of TEC in the formulation markedly affected the degree of peak broadening and the reduction in the intensity of these bands. The vibrational

bands observed for Formulation F1 were slightly more intense than those for formulation F2 when both preparations were extruded at the same temperatures with other processing parameters also being similar (such as processing temperature, feed rate and screw configuration). This phenomenon was ascribed formulation F2 having a drug load of 40%w/w with TEC at 10%w/w facilitating the conversion to the amorphous state.

Raman bands for PEO were shown to be sharp and of high intensity which indicated that the polymer was crystalline in nature. The intensities of PEO peaks when extruded were decreased as were those of AZT and 3TC peaks. Figure 4.10 shows the Raman spectra of formulations F4 to F6 which were formulated with 5, 10 and 30%w/w of PEO respectively.

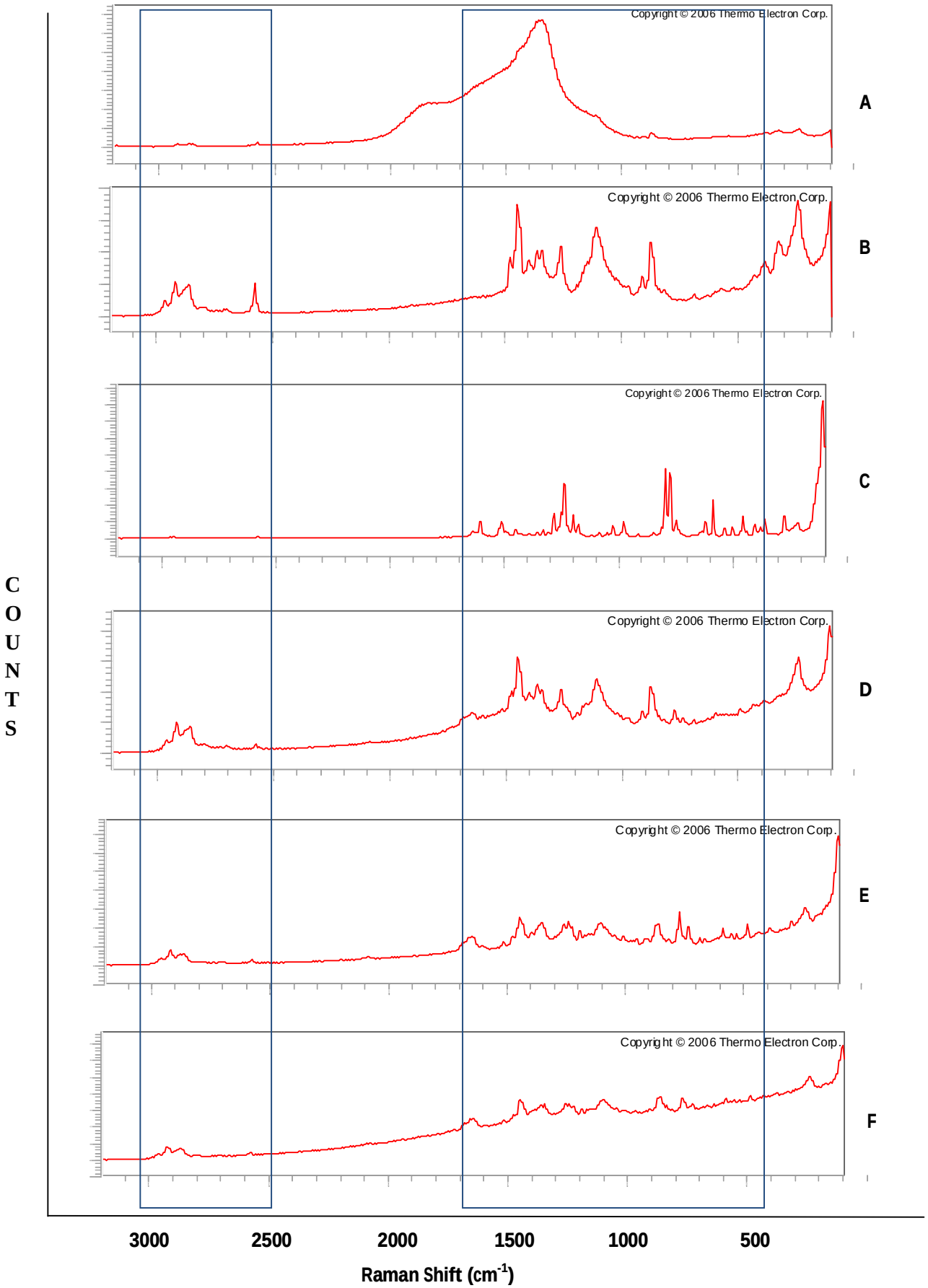


Figure 4.9: Raman spectra from 100-3200cm^{-1} of A)Ethylcellulose B)AZT C)3TC D) Physical mixture of ethylcellulose, Drugs, TEC (50:45:5%w/w) E) HME mini matrices of D at 180ºC F) HME mini matrices of ethylcellulose: drugs: TEC (50:40:10%w/w) at 180ºC.

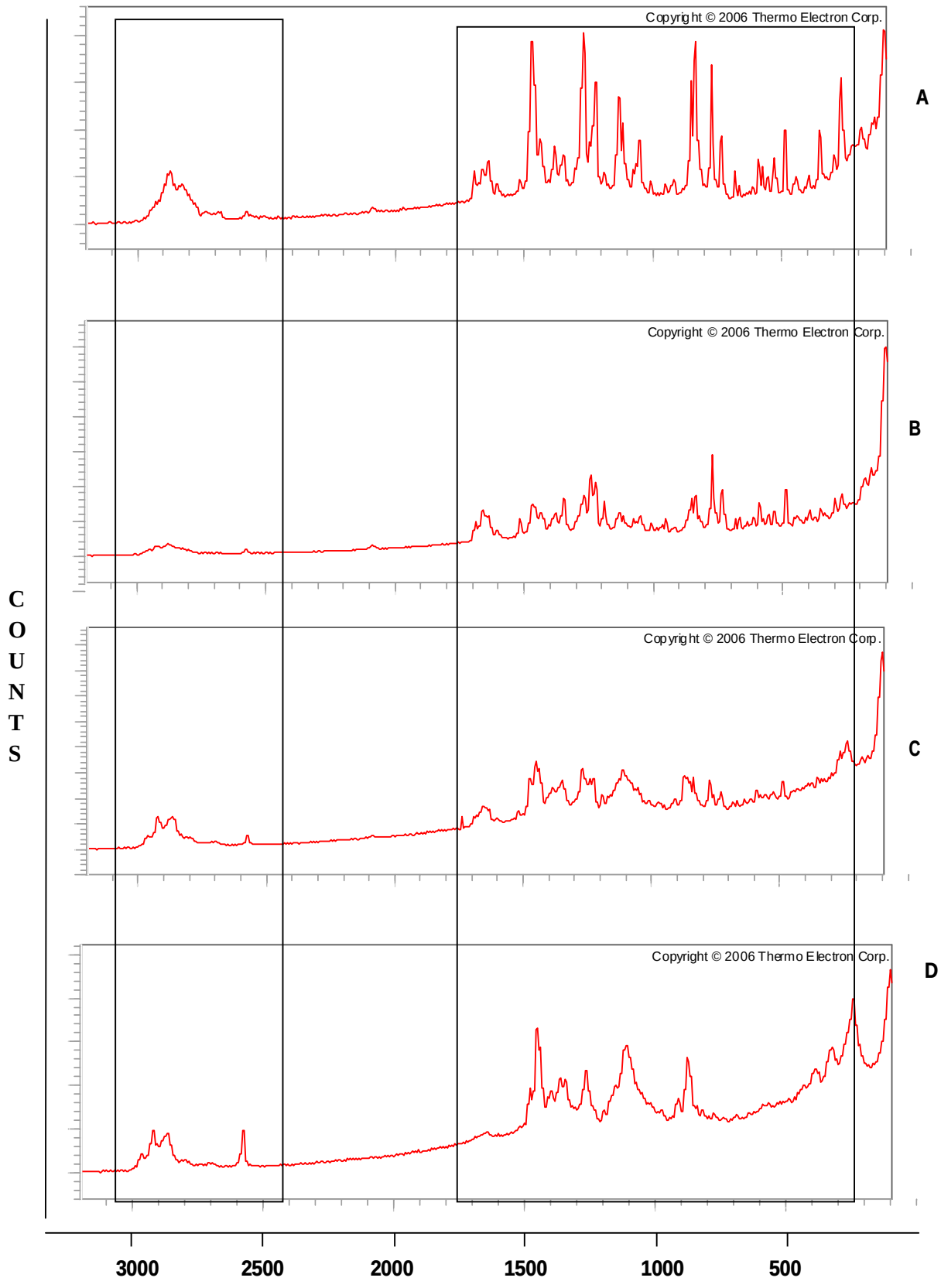


Figure 4.10: Raman spectra from 100-3200cm^{-1} of A) PEO B) F4 C) F5 D) F6

4.2.3 Drug release for Ethylcellulose matrices

HPLC analysis was used to determine the drug release and the drug content from the mini matrices. Standard drug calibration graph for AZT and 3TC are shown in Appendix 1 and 2 respectively. AZT and 3TC *In vitro* release from the formulations was in the order of F6> F5> F4> F3> F2> F1. Figure 4.11 shows the cumulative percentage drug release of AZT and 3TC from the mini matrices. Drug release from the matrices was significantly affected by the increase in percentage of plasticizer TEC and addition of PEO in case formulations of F4 to F6 (two- way ANOVA, $p < 0.001$).

The cumulative percentage of release of AZT and 3TC from formulations F1 to F6 is shown in Table 4.2 along with measured thermal and XRPD parameters of each formulation. The cumulative release of both AZT and 3TC significantly increased as the percentage of TEC was increased. At 5% w/w of TEC (F1) AZT and 3TC cumulative percentage drug release in 24 hrs was 23% and 27% respectively while at 15% w/w TEC (F3) the cumulative AZT and 3TC drug release in 24 hrs was 44% and 45% respectively. Zhu and co-workers demonstrated that the effect of the TEC level in melt extruded tablets was different from the film coated systems. While higher plasticizer levels promoted the film coalescence and decreased the drug release from the coated dosage form, the drug release increased at higher TEC levels for hot-melt extruded tablets due to pore formation in the matrix after TEC dissolution (Zhu et al, 2002). The porosity of the extrudates increased during dissolution testing and the soluble plasticizer functioned as a pore former when they leached out from the matrix to the surface of the extrudates on contact with the dissolution medium (Zhu et al, 2006).

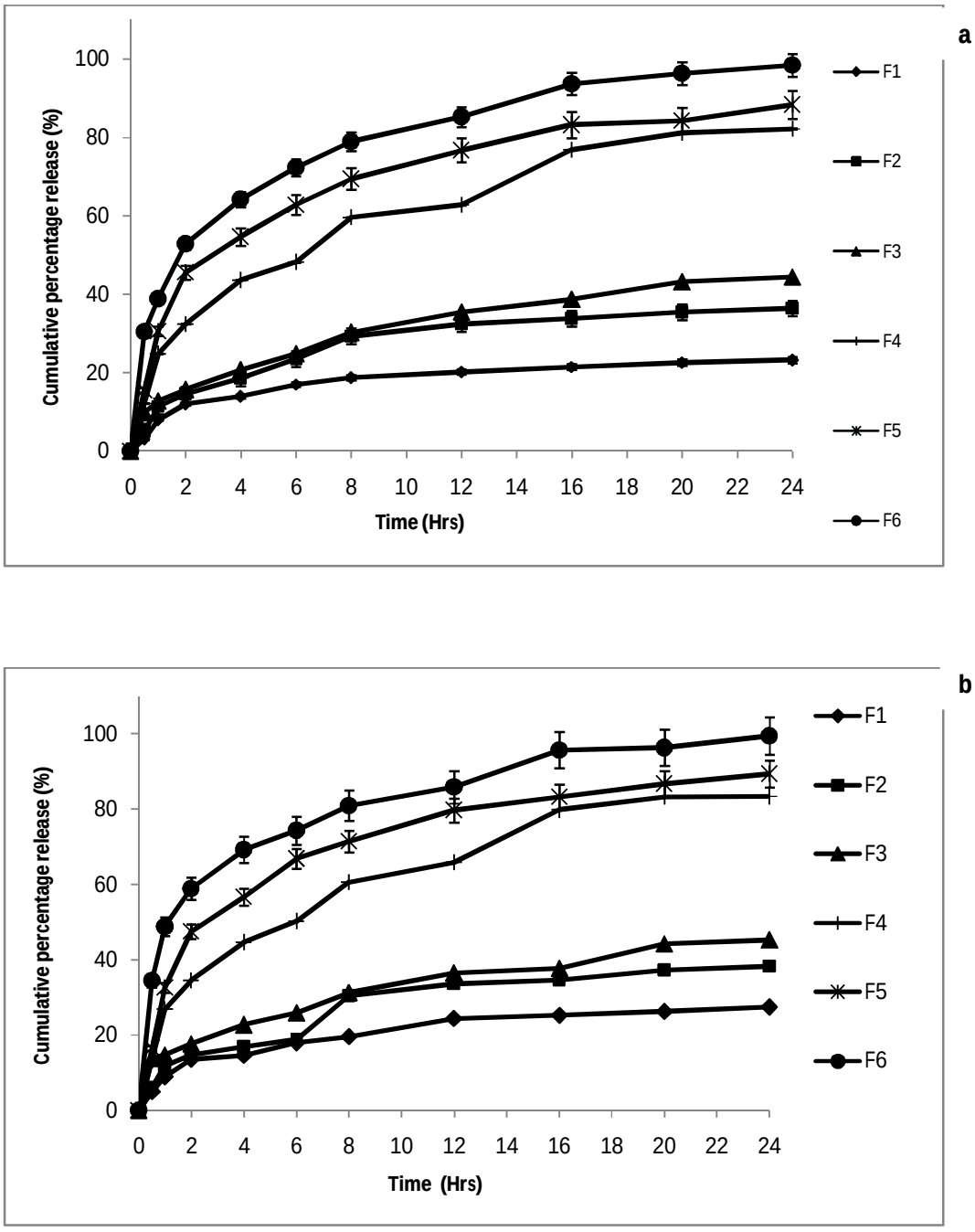


Figure 4.11: *In vitro* dissolution profile of a) AZT and b) 3TC from mini matrices formulations F1 to F6 over 24 hrs in Phosphate buffer at pH 7.2. (Error bars indicate ± Standard deviation, n=3,)

Drug release significantly increased further on addition of hydrophilic polymer PEO as in formulation F4, F5 and F6. Addition of 5% w/w PEO (F4) increased the cumulative percentage drug release of AZT and 3TC to 82% and 83% respectively. The amount of drug released was almost doubled on addition of PEO. Formulation F6 which contained PEO at 30% w/w and no TEC released AZT and 3TC at 98% and 99% respectively. PEO is a hydrophilic polymer which on contact with water becomes hydrated, with lowered viscosity to facilitate drug diffusion (Crowley et al, 200). PEO at 30% w/w in Formulation F6 containing PEO at a level of 30% w/w released AZT and 3TC at levels of 98% and 99% respectively, which is almost complete by 24hrs. The hydrogel formed upon contact with dissolution medium, dilated the lipophilic matrix structure of ethylcellulose and therefore promote enhanced drug release (Quinten et al, 2011).

It is well known that conventional fixed dose combination tablet formulations of AZT and 3TC cause dose dumping due to the initial burst release of the drugs, which leads to side effects. It would therefore be useful to develop sustained release formulation that would reduce the initial burst release and enable a reduction in the frequency of dosing so as to reduce side effects such as bone marrow suppression (Jain et al, 2006).

The results obtained from dissolution studies for formulation F1 to F6 showed that ethylcellulose retarded drug release for all the formulations and approximately 30% of both drugs were released within 1hr (Figure 4.11).

Formulation F1 with 50% ethylcellulose and 5% TEC released 8% and 8.9% of AZT and 3TC at 1 hr respectively while F2 with 50% ethylcellulose and 10% TEC released 11.4% and 11.8% of each drug respectively. Release of each active ingredient from Formulation F3 which contained 50% ethylcellulose and 15% TEC increased at 1hr with, 12.8% and 14.8%

of AZT and 3TC dissolved. Formulations F4 with the 50% ethylcellulose and 5% PEO had AZT and 3TC drug release at 24.8% and 26.8% respectively at the end of 1hr while F5 and F6 with 10% and 30% addition of PEO released both drugs just slightly above 30% at the end of 1hr. AZT and 3TC release at 1 hr for formulation F5 was 30.6%and 32.6% respectively while F6 release at the end of 1 hr was 38.8% and 48.8% for AZT and 3TC respectively.

The mini matrices formulations F1 to F6 formulated using a combination of ethylcellulose, TEC and PEO showed no burst release of the drugs during in-vitro dissolution tests which in turn show possibility of reduced dose dependent toxicities caused by the initial burst release of the drugs *In vivo* .

The range of kinetic models were applied to investigate on the best possible mechanisms of drug release from the hot melt extruded matrices F1 to F6.

The release rate kinetic data for formulations F1 to F6 is shown in Table 4.3.a and b for AZT and 3TC respectively. According to the data obtained F3 and F4 drug release did not fit zero order and first order order mechanism but showed linearity when modelled using the Higuchi's equation. For F3, AZT and 3TC the regression coefficients (R^2) were 0.99 and 0.98 respectively whilst for F4, the coefficients for each of the respective drugs were 0.98 and 0.97 respectively. Formulations F1 to F5 gave drug release for both active ingredients which fitted with the Korsmeyer- Peppas with (R^2) showed close linearity to 0.98 for both drugs except formulation F6. The n values for Korsmeyer- Peppas equation for formulation F1 and F2 for AZT were 0.46 and 0.46 respectively which relate to fickian (case 1) release while in the case of 3TC F1 and F2 n values were 0.46 and 0.51 respectively and these values are close to 0.5 ($n> 0.5 <1$) where the release if non fickian (anomalous) in characteristics. Non fickian (anomalous) release usually refers to a combination of diffusion and polymer relaxation or

erosion for controlling drug release. Formulations F3- F6 for both the drugs had n values more than 0.5 but less than 1 which was an indication of non-Fickian drug release mechanism.

Table 4.3: Drug release kinetic data for a) AZT b) 3TC

a)

Formulations	Zero Order		First Order		Higuchi		Korsmeyer-Peppas			Hixson- Crowell	
	R^2	k_o (h^{-1})	R^2	k_1(h^{-1})	R^2	k_H ($h^{-1/2}$)	R^2	n	K_{KP}(h^{-n})	R^2	k_{HC} ($h^{-1/3}$)
F1	0.7368	0.8179	0.4665	0.0353	0.9200	4.7444	0.9558	0.4566	0.0032	0.7654	0.0041
F2	0.8008	1.3685	0.4844	0.0396	0.9546	7.2552	0.9808	0.4560	0.0032	0.8397	0.0077
F3	0.8830	1.6410	0.4814	0.0392	0.9899	9.0192	0.9792	0.5044	0.1164	0.9287	0.0098
F4	0.8495	3.0790	0.4258	0.0436	0.9767	17.138	0.9667	0.5932	0.1591	0.9633	0.0309
F5	0.7458	3.0502	0.3572	0.0407	0.9285	17.667	0.9387	0.5964	0.2237	0.9366	0.0360
F6	0.7310	3.1978	0.3038	0.0378	0.9213	18.636	0.8935	0.5849	0.3272	0.9879	0.0676

b)

Formulations	Zero Order		First Order		Higuchi		Korsmeyer-Peppas			Hixson- Crowell	
	R^2	k_o (h^{-1})	R^2	k_1(h^{-1})	R^2	k_H ($h^{-1/2}$)	Rr^2	n	K_{KP}(h^{-n})	R^2	k_{HC} ($h^{-1/3}$)
F1	0.7991	0.9767	0.4767	0.0354	0.9549	5.5421	0.9815	0.4588	-0.0594	0.8313	0.0051
F2	0.8380	1.4601	0.5065	0.0401	0.9565	8.0973	0.9817	0.5035	-0.0564	0.8716	0.0083
F3	0.8618	1.5690	0.4246	0.0363	0.9763	8.6689	0.9464	0.4892	0.1832	0.9152	0.0096
F4	0.8408	3.1175	0.4133	0.0431	0.9735	17.413	0.9610	0.5928	0.1764	0.9583	0.0327
F5	0.7255	3.0506	0.3448	0.0402	0.9165	17.798	0.9320	0.5965	0.2385	0.9330	0.0378
F6	0.6793	2.9958	0.2690	0.0356	0.8820	17.720	0.8625	0.5758	0.3725	0.9540	0.0766

Formulation F6 did not fit well with zero order, first order, Higuchi or Korsmeyer- Peppa's equations but had good R^2 values when fitted with Hixson Crowell equation. The R^2 value calculated for Hixson Crowell equation fitting for AZT was 0.99 while for 3TC was 0.95 (Table 4.3 a & b). The cube root equation is applicable to the dissolution of monodisperse powder consisting of uniform sized particles. A plot of $(W_o)^{1/3}$- $(W_t)^{1/3}$ versus time will be linear when dissolution occurs from monodispersed particles of uniform size. This observation indicated that the drug dissolution from F6 (solid dispersion) is occurring from discretely suspended or deposited (monodisperse) particles. This might have also contributed to the enhanced dissolution rate of the solid dispersions in 24hrs as compared to formulations F1 to F5. All the six formulations when fitted with Higuchi's model equation and Korsmeyer-Peppas equation gave good coefficient of correlations which denoted that drug release for both AZT and 3TC was through diffusion mechanism.

Overall, the best model fitting was obtained from Korsmeyer- Peppa's equation for all the formulations F1-F6 were the coefficient correlation being the close to 0.99.

Table 4.4 and Table 4.5 give the T25% to T90% of formulations F1 to F6 for both AZT and 3TC respectively.

Parameter	F1	F2	F3	F4	F5	F6
T25%	24.90	7.57	5.56	1.11	0.41	0.15
T50%	197.14	45.41	29.88	6.19	3.56	1.83
T75%	661.28	129.53	79.91	16.94	12.38	7.80
T80%	801.77	153.05	93.46	19.89	15.10	9.82
T90%	1139.55	207.53	124.38	26.65	21.70	15.0

Table 4.4: T25%, T50%, T75%, T80% and T90% (hrs) parameters for formulations F1, F2, F3, F4, F5 and F6 formulations for AZT

Parameter	F1	F2	F3	F4	F5	F6
T25	16.29	7.58	4.56	0.96	0.34	0.06
T50	115.43	39.67	31.34	5.62	3.11	1.18
T75	362.94	104.49	96.79	15.84	11.38	6.51
T80	435.54	121.91	115.82	18.68	14.00	8.53
T90	607.51	161.51	160.70	25.24	20.40	14.00

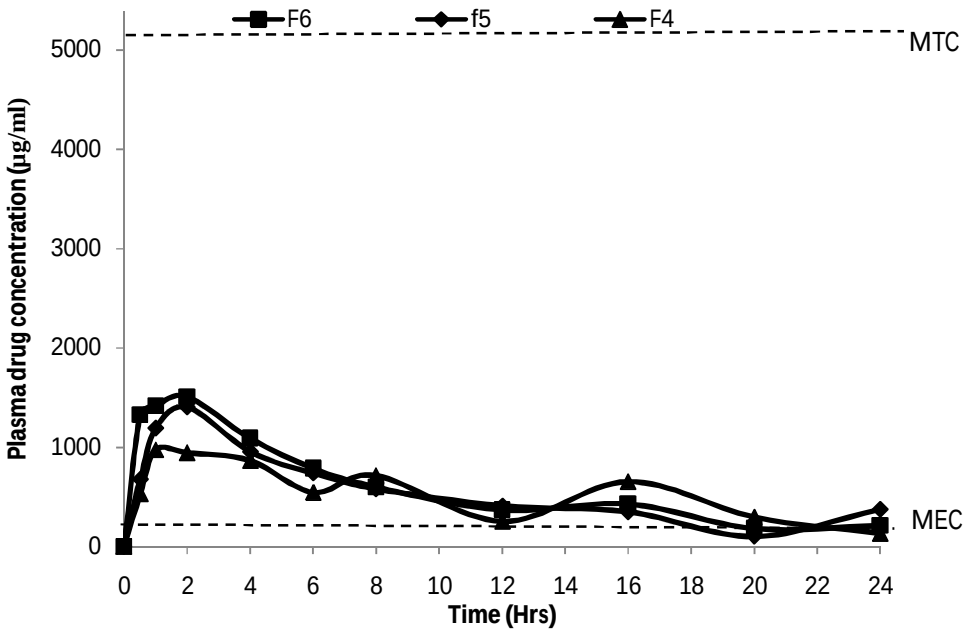
Table 4.5: T25%, T50%, T75%, T80% and T90% (hrs) parameters for formulations F1, F2, F3, F4, F5 and F6 formulations for 3TC

The T% parameter results obtained in table 4.3 and Table 4.4, show that the overall time taken for AZT release from all the six formulation was faster than for 3TC. The reason for faster AZT release from the matrix could be due to AZT being in an amorphous form after hot melt extrusion which provides a high energy state with relatively high solubility and rapid dissolution in aqueous media. On the other hand a very small portion of 3TC was shown to remain crystalline in nature as per the results shown in section 4.2.2.2, Figure 4.7 (thus resulting into a slightly slower release rate). Since crystalline phases are stable systems and possess lower surface energy, they usually have low dissolution tendency than amorphous phases resulting in a decreased dissolution rate when compared to non crystalline systems (Schilling, et al, 2009). Considering T50% and T80% Formulation F5 had a good sustained release profile with T50% typically occurring at an average of 4hrs and 3 hrs for AZT and 3TC respectively. Dissolution progressed thereafter with T80% observed at 15hrs and 14hrs for AZT and 3TC respectively. Formulation F6 released drug the fastest compared to the

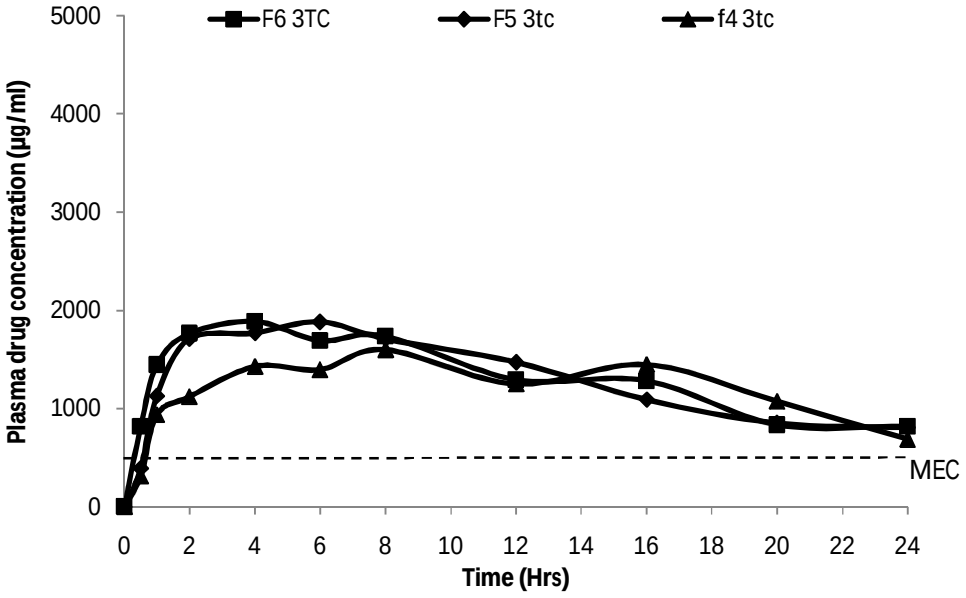
other formulations owing to its increased PEO loading, facilitating increase in drug diffusion due to increase in matrix porosity.

4.2.4 Prediction of *in vivo* blood drug concentration- time profiles

The mean *in-vitro* dissolution data obtained for formulations F4, F5 and F6 were used to simulate the *in vivo* blood drug concentration- time (C-t) profiles which would provide guidance as to whether or not *in vivo* drug release from the formulation would have sustained release mechanism as was in the case for *in vitro* release. F4, F5 and F6 showed good sustained release profiles taking into consideration the time taken for 25%, 50% and 80% drug release. The predicted blood drug concentration-time profiles for AZT and 3TC are shown in Figure 4.12a and b respectively. To evaluate these predicted C-t profiles, pharmacokinetic parameters such as Cmax (ng/ml) and Tmax (hr) were obtained as shown in Table 4.6.



a)



b)

Figure 4.12: Mean predicted In vivo blood drug concentration-time profile of formulations F4, F5 and F6 for a) AZT and b) 3TC (n=3). The dotted lines show minimum effective concentration (MEC and minimum toxic concentration (MTC). MTC concentration for 3TC is 300,000 µg/ml (not within scale).

Formulation	Cmax (ng/ml)		Tmax (hr)	
	AZT	3TC	AZT	3TC
F4	971	1598	2	8
F5	1402	1883	2	6
F6	1508	1886	2	4
conventional FDC AZT/3TC TAB 300/150mg**	3100	1500	0.6	1

Reported values are mean with relative SD (90% CI), ($p<0.05$)

** **Data obtained from Kayitare et al, 2009**

Table 4.6: Pharmacokinetic parameters of AZT and 3TC obtained from simulated In vivo blood concentration–time profiles of formulation F4, F5 and F6

The simulated In vivo blood drug- time profiles pharmacokinetic parameters were compared to those determined experimentally for the conventional FDC immediate release tablets containing AZT and 3TC owing to the provision of a prolonged release profile from the mini-matrix formulations. Lower Tmax was predicted for all the three formulations (F4, F5 and F6) with the data shown in Table 4.5. Formulation F4 had the lowest Cmax at 971 ng/ml (AZT) and 1598 ng/ml (3TC) as compared to formulations F5 (AZT 1402 ng/ml and 3TC 1883 ng/ml) and F6 (AZT 1508 ng/ml and 3TC 1500 ng/ml) as shown in Table 4.5. The Cmax for both AZT and 3TC in each of these formulations was markedly lower than observed for the conventional immediate release tablets developed for paediatrics which gave a Cmax of 3100 ng/ml (Kayitare et al, 2009). The Tmax of AZT was similar for all the three formulations remaining at 2hrs despite Cmax values changing for the same formulations. The Tmax for the conventional immediate release tablets of the same strength was 0.6hrs for AZT and 1hr for 3TC (Kayitare et al, 2009). . Thus Tmax for F4, F5 and F6 for 3TC was 8, 6 and 4hrs respectively as compared to the Tmax of 0.6 hrs observed for the

conventional FDC immediate release tablet, again showing the likely extension of the profile through a more sustained drug release.

The minimum inhibitory concentration (ID_{50}) for AZT is 1 µmol/L or 0.24 µg/ml (240 ng/ml) based on the in-vitro data (Abu-Izza et al, 1997). Literature data also suggest that AZT could be toxic at concentrations of 20-60 µM (5345 ng/ml-16034 ng/ml), whilst resistance might occur through prolonged exposure at concentrations in the range 10-25 µM (2672.4-6681 ng/ml) for periods of 38-48 days (Chiu and Duesberg, 1995). From the predicted blood concentrations for formulations F4, F5 and F6, concentrations likely to demonstrate toxicity were not reached. The results also indicate that the plasma concentration after administration for all the three formulations remained above the effective minimum concentration (MEC) of AZT for at least 16hrs, suggested that adequate efficacy could be achieved through use of these formulations above minimum effective concentration for a period up to up to 20 hrs, while for Formulations F5 and F6 these levels were maintained for a slightly shorter period of approximately 16 hrs.

The Cmax attained for all the three formulations for AZT were much lower than minimum toxic concentrations (MTC) as seen in Figure 4.15a. The reduction of Cmax for AZT is particularly significant knowing that the major toxicities of AZT such as myelo-suppression are concentration dependent. The reduction in AZT Cmax which is predicted to occur alongside plasma concentrations which are sustained above the minimum effective concentration (MEC) for extended periods (>16 hrs) might provide a valuable means to reduce known AZT toxicity which is typically associated with frequent dosing.

The 90% inhibitory (ID_{90}) concentration range for 3TC is between 87ng/ml - 464ng/ml (Bruno et al, 2001). Thus the 3TC plasma concentrations for all the three formulations were

maintained above ID_{90} for periods up to 24 hrs. The minimum toxic concentration for 3TC is 300 µg/ml (300,000ng/ml) which is approximately 150 times higher than the Cmax predicted for the three formulations. Formulation F4 had the lowest predicted peak plasma concentration of 3TC at 1598ng/ml while the Cmax values for F5 and F6 were very close despite F6 having lower drug loading than F5. As compared to the conventional FDC tablet of the same strength, formulations F4, F5 and F6 all had slightly higher Cmax but with a longer Tmax, which indicated that drug release was sustained for a prolonged period. Tmax for 3TC was predicted to diminish with increased PEO loading since PEO acted as a pore former which allowed for faster drug release from the matrix. Formulation F6 with PEO loading of 30% w/w was predicted to give a Tmax for 3TC of 4hrs, compared to formulation F4 (5% w/w PEO loading) with a Tmax of 8 hrs, despite the high drug loading of F4 (40% w/w). These findings suggest that PEO levels are critical to the rate and extent of drug release. In order to maintain appropriate plasma drug concentrations over a prolonged period, it is necessary to minimise the PEO loading and so for formulation F4 that (5% w/w PEO) drug release was sustained for a period exceeding 20 hrs and the drug plasma concentrations were maintained within the therapeutic window longer than for formulations F5 and F6, suggesting that for all formulations tested, the composition used for F4 was most optimal for enabling the safe and efficacious once daily delivery of both AZT and 3TC..

4.2.5 Drug content determination

Analysis of drug content by HPLC showed an average amount of AZT and 3TC in the weighed hot melt extruded mini matrices in all six formulation (F1 to F6) was between 99.3% (RSD1.4 %) and 99.9% (RSD 1.9%) of the declared value for AZT and 3TC respectively. This complied with USP34-NF-29 requirements stipulating a content ranging from 85.0% to

115.0% of the label claim with relative standard deviation less than or equal to 6.0% (USP34 NF-29, 2010). PEO was detected within 2.6 min with formulations F4, F5 and F6 while testing for drug content. Furthermore, there were no signs of impurities detected during drug content testing using HPLC. The drug content was good throughout the mini matrices in all the six formulation due to greater mixing capabilities of the twin screws during HME processing.

4.2.6 Accelerated stability analysis

Formulations F1 to F6 were kept for accelerated stability testing due to their good drug release profiles. XRPD patterns reflected no change in at all for Formulation F1 to F5 in open and closed bottles 40º C/75% RH while at. F6 showed a slight increase in PEO peak intensity at 40ºC/75% for the open bottles while the closed bottles did not show any statistical difference in intensity after 3 months (Figure 4.12). No crystalline peaks of AZT or 3TC were observed at the end of 1 month and 3 months in formulations F1 to F6 (Figure 4.13).

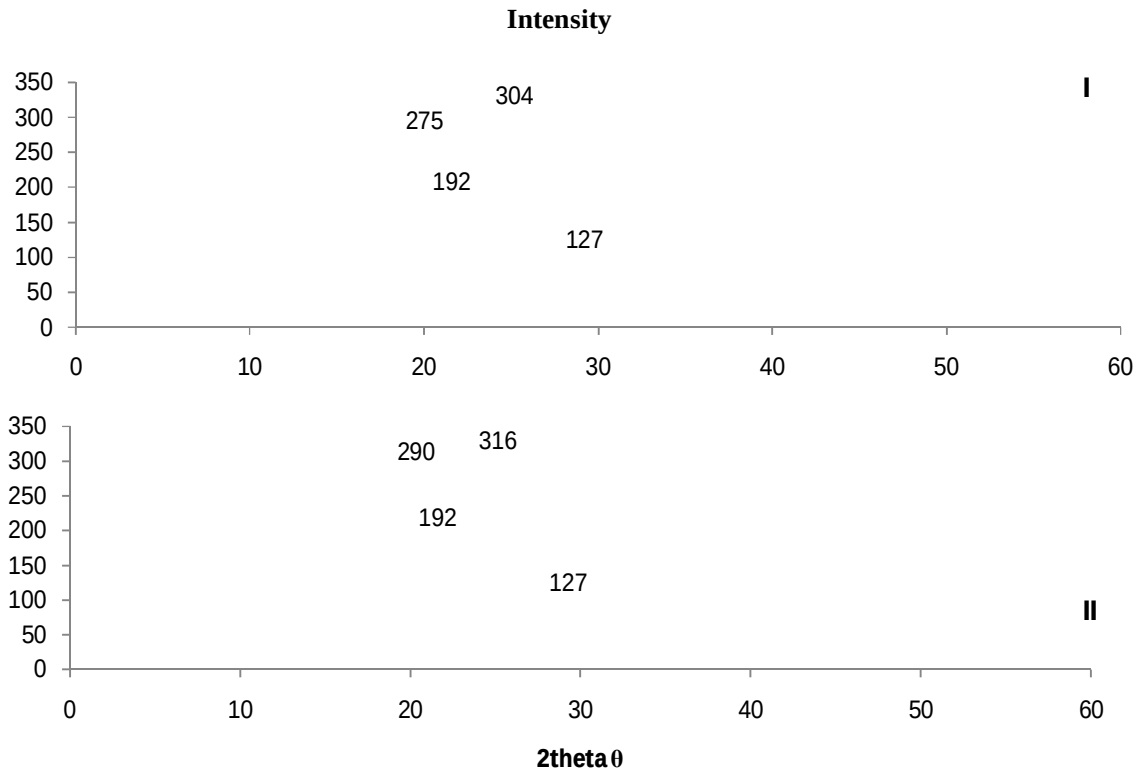


Figure 4.13: XRPD diffraction patterns for F6 intensity for I) before accelerated stability studies II) After 3 months accelerated stability studies showing increase in PEO peak intensities.

For samples at 60ºC tested for XRD after 15 days showed no difference in formulations F1 to F3 which had no PEO. The XRPD patterns did not reflect any peaks that correspond to either AZT or 3TC. The reason could be that AZT and 3TC formed a solid solution with ethylcellulose and the Tg of the solid solution formed after HME was higher than the storage temperature thus indicating no change in XRPD peaks. Formulation F4 to F6 showed slight increase in PEO peaks since the storage temperature was close to the Tm of PEO which could have caused it to melt but there were no physical signs of PEO melting. However there were no crystalline peaks of either the drug observed after 15 days which meant that both AZT and 3TC remained amorphous in the extrudates.

The dissolution profiles of F1 to F6 after storage at accelerated conditions at 40ºC/70% RH and 60ºC in closed container were compared with the dissolution profiles initially done prior to storage at accelerated conditions and the f_2 values were found to be ≥ 50, indicating that there was no statistically significant change in dissolution profiles (Table 4.7).

Table 4.7: Similarity f_2 values of AZT and 3TC release from formulations F1 to F6 before and after accelerated stability period in open bottles at 40ºC/75% RH.

Formulation	F1	F2	F3	F4	F5	F6
Similarity factor f_2 AZT release	69.9	79.8	97.1	95.5	94.8	70.5
Similarity factor f_2 3TC release	93.9	95.6	96.8	83.8	82.5	90.2

Stabilization of the drug release properties of polymeric matrices is challenging since such systems frequently exhibit changes in dimensional structure upon storage that can significantly decrease the drug release rate. This phenomenon is most frequently observed at storage conditions near or above the glass transition temperature of the matrix material, where stress relaxation and orientation of polymer chains is rapid (Omelczuk 1993). However, the accelerated stability studies at 40ºC/75% RH in open and closed bottle results showed that there was no apparent decrease in release of both the drug but rather a very slight but not significant increase in drug release in all the six formulations (one way ANOVA, p = 0.8). Formulations stored at 60ºC for 2 weeks did not show any significant difference in dissolution (one way ANOVA, p= 0.6). The f_2 values more than 50 compared between the drug release profile before and after accelerated stability period therefore indicate that the hot

melt extruded mini matrices remained stable during accelerated conditions. It is however to note that due to time constraints accelerated stability study period was short but for future work the accelerated stability studies should be carried out for a longer time period of one year since at the end of three months PEO showed slight increase in XRPD peak intensity. Prodduturi et al, 2005 observed during their research that due to PEO having low glass transition temperature (-57ºC), which is well below the storage temperature can cause re-crystallization of drugs.

4.3 Conclusion

Hot melt extrusion was used to produce stable homogeneous fixed dose combination mini matrices of AZT and 3TC that have potential to be formulated as sustained release dosage form using ethylcellulose (hydrophobic polymer) as a carrier. The results of this study demonstrate that zidovudine and lamivudine turned amorphous during hot melt extrusion process. XRPD studies suggested that zidovudine and lamivudine both formed solid solutions with ethylcellulose. PEO crystalline peaks were observed in the extrudates during XRPD. The drug release rate from the mini matrices can be manipulated using a specific amount of PEO (hydrophilic polymer) with TEC that acts as a pore former as well as plasticizers. Increase in PEO concentrations resulted in increase in drug release over time. Kinetic release analysis of dissolution profiles indicated that AZT and 3TC release from the HME mini matrices was mainly due to erosion and diffusion mechanisms. Most formulations had a coefficient of regression close to 0.99 in the case of Higuchi's constant which indicate that the drugs were homogeneously mixed with the polymer in the matrix.

The predicted *in vivo* blood drug concentration time profile showed that drug release from formulations with 50% w/w of ethylcellulose, 40% w/w drug loading, 5% w/w of PEO and

5% w/w of TEC sustained drug concentrations were within the effective therapeutic levels for 20 hrs thus avoiding the problem of toxicities related to frequent dosing. The formulations produced were stable under accelerated conditions for a period of 3 months.

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CHAPTER FIVE: FORMULATION AND CHARACTERIZATION OF KOLLIDON® SR SUSTAINED RELEASE, FIXED DOSE COMBINATION MINI MATRICES OF ZIDOVUDINE AND LAMIVUDINE

5.0 Introduction

Drug release in controlled release matrices are usually controlled by the type and concentration of the polymer and excipients (plasticizers) and processing conditions such as temperature (zyguney et al, 2009).

Kollidon® SR is a relatively new polymer known to have pH independent drug release characteristics and is commonly used for the production of sustained release tablets, pellets produced either by direct compression or by hot melt extrusion processing.

Zidovudine and Lamivudine require a high loading dose and using hydrophobic polymer like ethylcellulose necessitates the use of excipients like a hydrophilic polymer as a pore former to release of drugs through the hydrophobic matrix. The use of excipients thus increase the matrix size overall. Kollidon® SR contains 80% insoluble polyvinyl acetate and 19% soluble poly-vinyl-pyrrolidone. The soluble part could dissolve and leach out, resulting in porous polyvinyl acetate matrices. The use of additional excipients such as hydrophilic polymers/ matrix pore formers and high loadings of plasticizers therefore would not be necessary when using Kollidon® SR as a carrier.

In this study Kollidon® SR was used as a control release carrier for Zidovudine and Lamivudine and Triethyl Citrate (TEC) was used as a plasticizer for easy extrusion of the extrudates during hot melt extrusion process.

5.1 Materials and Methods

5.1.1 Materials

Kollidon® SR was donated by BASF SE, Ludwigshafen, Germany. Zidovudine (AZT) and lamivudine (3TC) were gifts from Cosmos Pharmaceuticals, Nairobi Kenya, whilst tri ethyl citrate (TEC) was bought from Sigma-Aldrich, UK.

5.1.2 Methods

The methods used in this study have been described in chapter four of this thesis, section 4.1.2.

5.2 Results and discussion

5.2.1 Processibility via hot melt extrusion

Kollidon® SR matrices of AZT and 3TC were successfully extruded using hot melt extrusion technique. It was difficult to extrude Kollidon® SR with the drugs alone therefore TEC was used as a plasticizer from 5% - 15% w/w in the formulations for ease of extrusion (Table 5.1). Extrusion was easier and faster when 5% TEC was added to the formulation. Increase in TEC more than 15% w/w resulted in phase separation during extrusion. Increasing temperatures above 150ºC resulted in the extrudates turning brown in colour. The extrudates obtained with maximum temperature kept at 150ºC were cream to very light brown in colour. It was difficult to extrude with higher than 50% drug loading which was due to higher torque values and increase in melt pressure caused by high amounts on dispersed crystalline drug

particles. The extrudates' surface appearance was smooth on addition of 5% w/w TEC (K1). Table 5.1 gives a brief description of extrudates' surface after extrusion. Formulation K4 with 50% w/w of drugs and 5% w/w of TEC appeared having a slightly rougher surface as compared to the other formulations after extrusion due to drug loading being more than the polymer loading which is an indication that some proportion of the drugs have remained in crystalline form after extrusion. Figure 5.1 is a picture of heterogeneous mix of Kollidon® SR mini- matrices of formulation K4 before sieving.

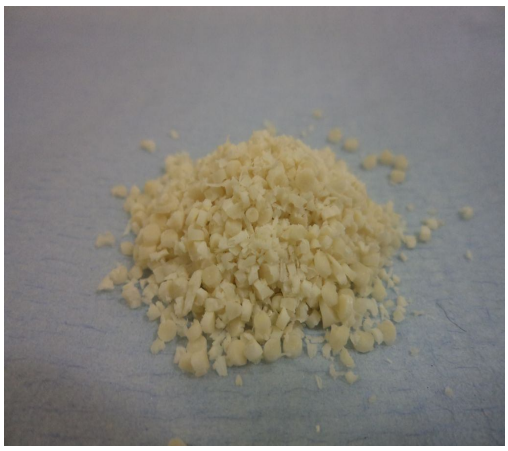


Figure 5.1: Heterogeneous mix of Kollidon mini matrices before sieving

5.2.2 Physico - chemical analysis of hot melt extruded matrices

MDSC studies were undertaken to investigate the physical state of the drugs within the polymer matrix after extrusion. Crystalline peaks were absent for all formulations (K1 to K4), indicating that both AZT and 3TC transitioned to amorphous state after extrusion. No crystalline peaks (Tc) were observed after the rapid cooling cycle for all formulations which indicated that both AZT and 3TC were completely miscible in Kollidon® SR as was suggested in Chapter 3 (section 3.2.2.2).

Table 5.1: Composition, processing parameters and extrudates' surface characteristics

Formulation	Composition (%w/w)	Processing Temperature range from feed to Die (□C)										Maximum Torque (Nm)	Melt pressure (Psi)	Extrudates Surface	Mini Matrices surface
K1	Kollidon® SR/AZT+3TC/TEC (45/50/5)	70	80	90	100	110	120	140	150	150	140	4.8	38	slightly rough	slightly rough
K2	Kollidon® SR/AZT+3TC/TEC (50/45/5)	70	80	90	100	110	120	140	150	130	120	4.6	34	smooth	smooth
K3	Kollidon® SR/AZT+3TC/TEC (50/40/10)	70	80	90	100	110	120	140	150	130	120	1.4	10	Smooth	smooth
K4	Kollidon® SR /AZT+3TC/TEC (50/35/15)	70	80	90	100	110	120	140	150	130	120	1.2	4	smooth	smooth

Note: The proportion of AZT to 3TC was kept at 2:1 used in all the formulations in Table 5.1

XRPD patterns for both AZT and 3TC (pure drug substance), their physical mixtures and the extrudates are presented in Figure 5.2. Both AZT and 3TC pure unprocessed drugs showed sharp diffraction peaks confirming the highly crystalline nature of the pure drugs. The peak positions conformed to the known patterns previously described in the literature (as mentioned in Chapter 4). Following hot melt extrusion, the intensity of both 3TC and AZT peaks were significantly reduced with substantial line broadening. The Diffraction peaks corresponding to AZT were totally absent indicating substantial loss of AZT crystallinity after extrusion at all studied formulation ratios and processing temperatures. 3TC was also shown to have transformed to the amorphous form which is illustrated for formulations K2 and K4. XRPD patterns for 3TC extrudates K1 and K4 however showed the presence of two small crystalline peaks at 16.08 and 22.04 2-theta (θ), which correspond to pure 3TC. It was however concluded that the peaks demonstrated much reduced intensity relative to the pure drug substance (Figure 5.1), which suggests that a proportion of the 3TC remains un-dissolved in the carrier.

Both XRPD and DSC results clearly demonstrated the loss of AZT and 3TC crystallinity following extrusion which suggests that Kollidon® SR has the ability to effectively dissolve both AZT and 3TC and form solid dispersions when extruded.

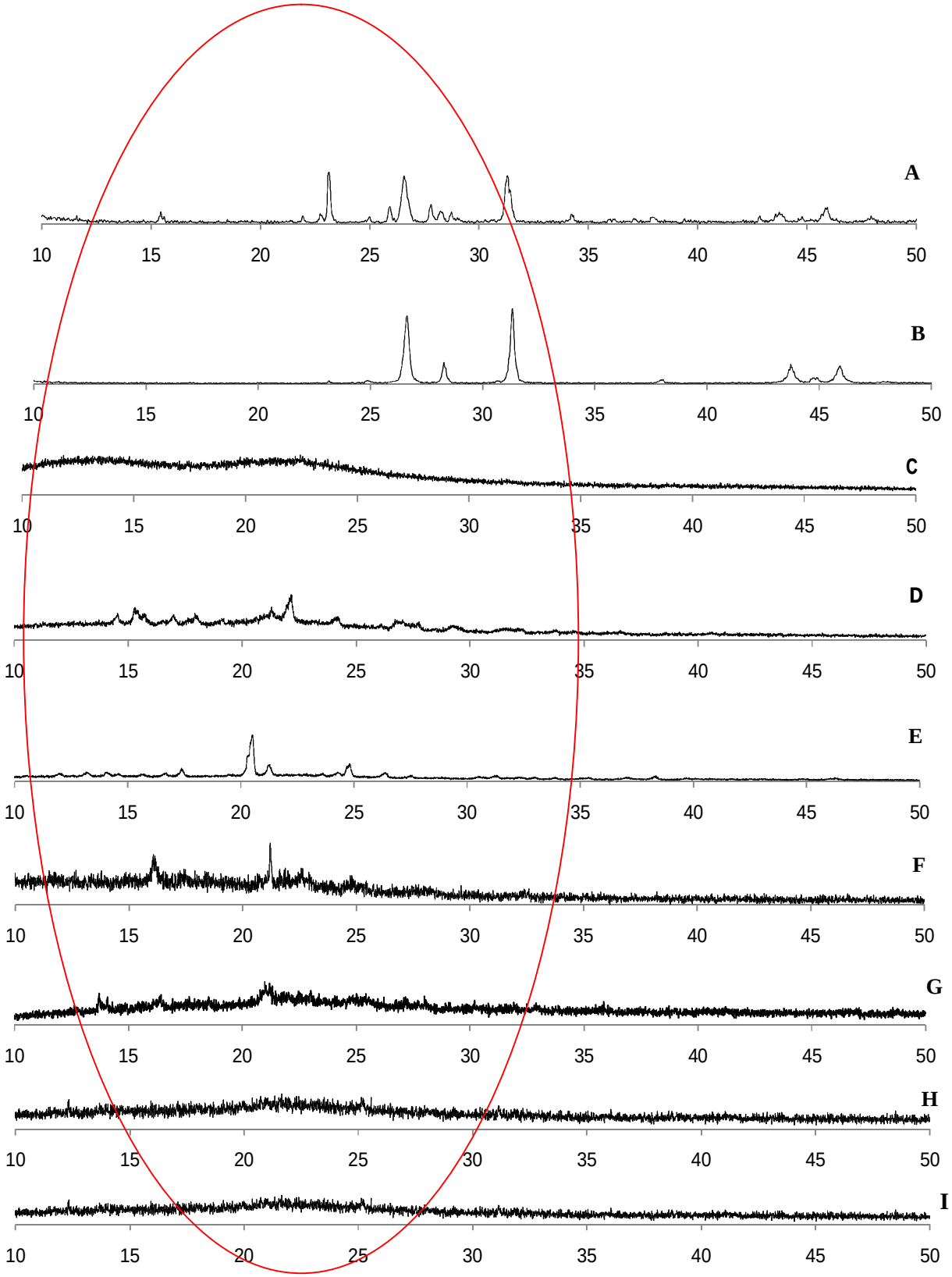


Figure 5.2: A) AZT pure drug B) 3TC pure drug C) Kollidon® SR D) Physical mixture of Kollidon® SR: 3TC (60:40%w/w) E) Physical mixture of Kollidon® SR : AZT (60:40%w/w) F) Extrudate K1 G) Extrudate K2 H) Extrudate K3 I) Extrudate K4

5.2.3 Analysis of drug content and *in-vitro* dissolution test

Analysis of drug content by HPLC showed an average amount of AZT and 3TC in the weighed sample of hot melt extruded mini matrices was between 101.4% (RSD=1.4 %) and 110.3% (RSD 3.1%) of the declared value for AZT and 3TC respectively in all four formulation K1 to K4. This complied with USP34-NF-29 requirements stipulating a content ranging from 90% to 110% of the label claim with relative standard deviation less than or equal to 6%. The drug content was good throughout the mini matrices in all the four formulations due to greater mixing capabilities of the twin screws during HME processing.

AZT and 3TC *in vitro* release from the formulations was in the order of K1> K2> K3> K4. Figure 5.3 shows the average cumulative percentage drug release of AZT and 3TC from the mini matrices. The average cumulative release of both AZT and 3TC increased as the percentage of the drug quantity was increased. At 45% w/w of drugs (K2) AZT and 3TC cumulative percentage drug release in 1hr was 21.9% and 33.7% and in 24 hrs was 88% and 89% respectively while at 50% w/w of drug loading (K1) the cumulative AZT and 3TC drug release in 1hr was 41.4% and 45.6% and 24 hrs was 98.1% and 99.3% respectively. The average cumulative percentage drug release of AZT and 3TC in 1hr, at drug loading 40%w/w (K3) was 36.7% and 54.9 84.5% while in 24hrs: 85.1% for AZT and 3TC respectively and at drug loading 35%w/w (K4) the average cumulative percentage of AZT and 3TC release over 24 hrs was 80% and 82% respectively.

Plasticizer TEC was used at in the formulations K1, K2, K3 and K4 at 5%w/w, 5%w/w, 10%w/w and 15%w/w respectively. Drug release was significantly sustained with increase in TEC loading. This is because TEC made the matrices denser and during extrusion increases inter particle cohesiveness. Similar observations were made by □zyguney and his co-

workers, when formulating mini matrices of theophylline using Kollidon® SR and TEC as plasticizer. TEC therefore had no effect on release of theophylline at higher drug loading where the dispersed particles (50%) probably formed a complete network allowing rapid drug release as in the case of formulation K1 (□zyguney et al, 2009).

It was observed from dissolution profiles of both AZT and 3TC that 3TC release was faster than AZT from the matrix. The probable reason being that AZT formed a solid solution with Kollidon® SR while a small percentage of un-dispersed amorphous 3TC that may have not fully solubilised with the carrier during HME and thus resulted in faster dissolution on contact with the dissolution medium. The matrices did not dissolve after 24 hrs and their shape was intact due to Kollidon® SR being made of water insoluble polyvinyl acetate.

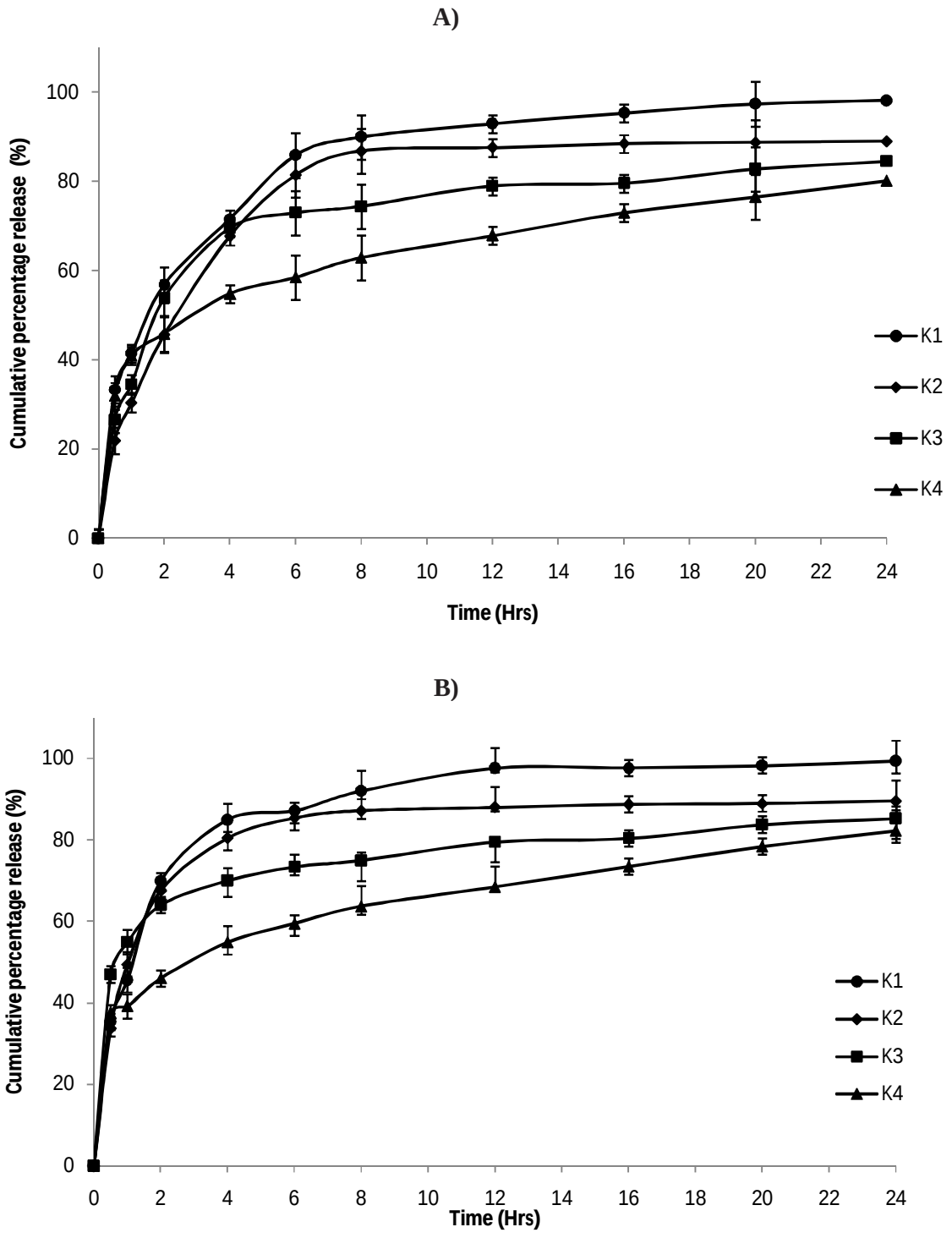


Figure 5.3: *In vitro* dissolution profile of A) AZT and B) 3TC from mini matrices formulations K1, K2, K3 and K4 over 24 hrs in Phosphate buffer at pH 7.2. (Error bars show mean ± SD, n=3).

5.2.4 Kinetic evaluation of drug release

The drug release mechanisms of the hot melt extruded mini matrices formulations K1, K2, K3 and K4 were evaluated by using the zero order, first order, Higuchi's equation, Koysmer-Peppa's equation and Hixson Crowell equation. Table 5.2 a) and b) give the regression coefficients (R^2) and the exponents for AZT and 3TC release respectively.

AZT release from Kollidon® SR matrix fitted best with first order equation where $R^2 > 0.98$ for all four formulations K1 to K4 while with 3TC release with K3 did not fit the first order equation and had $R^2 = 0.97$ but K1, K2 and K4 fitted first order equation with $R^2 > 0.98$. Formulation K3 for both AZT and 3TC release fitted Higuchi's equation where $R^2 > 0.98$ while for formulations K1, K2 and K4 where $R^2 < 0.98$. All the four formulations had high R^2 when fitted with Higuchi's equation which denoted a diffusion controlled mechanism for drug release. Formulation K3 also fitted well with the Korsmeyer- Peppa's equation with $R^2 < 0.98$ for AZT and 3TC release with n= 0.22 and n=0.22 respectively which shows that when n< 0.45 the drug release mechanism was by non-Fickian diffusion. 3TC release from formulation K2 fitted with Korsmeyer-Peppa's equation with $R^2 = 0.99$ ($R^2 < 0.98$). AZT release form formulation K2 did not fit Korsmeyer- Peppa's equation and the $R^2 = 0.94$. Formulation K2 did not fit with Hixson-Crowell equation for AZT only where $R^2 \geq 0.98$ while for 3TC release profile fitted Hixson- Crowell equation with formulations K2, K3 and K4 with $R^2 \geq 0.98$ as shown in Table 5.2. The coefficient correlations of AZT and 3TC for all the four formulations were quite high in the case of Hixson- Crowell equation thus indicating that drug release could be limited by drug particle dissolution rate where the drug particles' surface diminishes

over time during dissolution and not by diffusion as in the case of Korsmeyer- Peppa's equation.

The formulated matrices seemed to have a mixed drug release mechanism since the coefficients of correlation are quite high with respect to first order equation, Higuchi's equation, and Korsmeyer- Peppas and Hixson- Crowell equation.

Overall, the best equation fit for all the drug release profiles of AZT and 3TC from formulations K1, K2, K3 and K4 followed the first order release kinetic equation and Korsmeyer-Peppas equation. This indicated that the release of water soluble drugs from porous matrices where drug release from the matrices depended on the concentration of the drugs in the matrices over time and that drug release was diffusion controlled

Table 5.2: Drug release kinetic data for a) AZT b) 3TC

a)

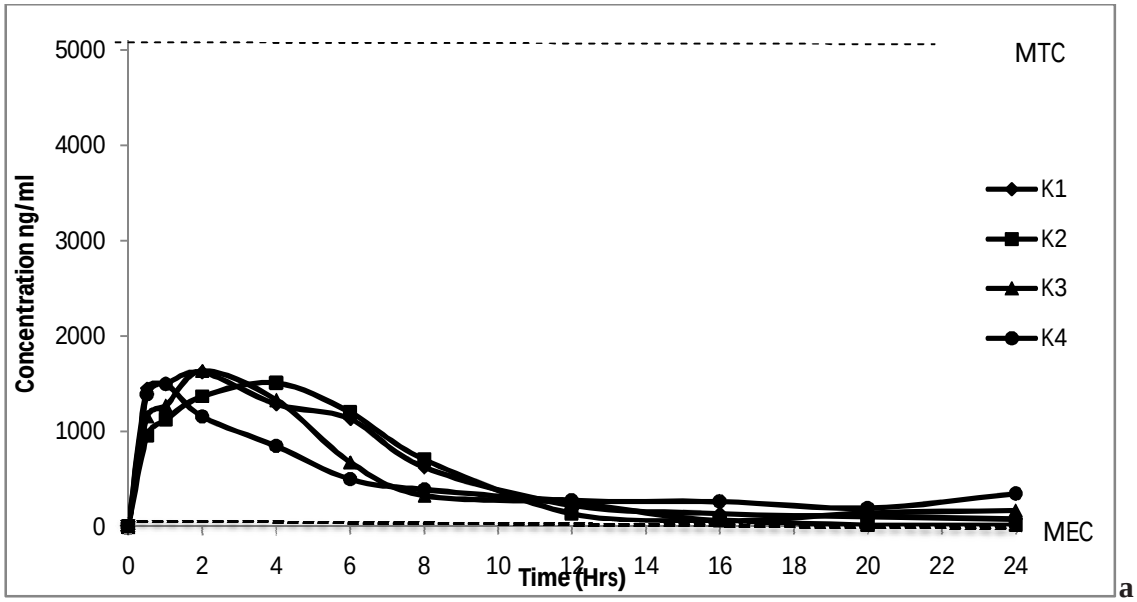
Formulations	Zero Order		First Order		Higuchi		Korsmeyer-Peppas			Hixson- Crowell	
	R^2	k_o (h^{-1})	R^2	k_1(h^{-1})	R^2	k_H ($h^{-1/2}$)	R^2	n	K_{KP}(h^{-n})	R^2	k_{HC} ($h^{-1/3}$)
K1	0.8277	5.767	0.9905	0.407	0.9229	25.371	0.9620	0.247	48.491	0.9883	0.064
K2	0.7781	5.318	0.9957	0.286	0.8872	23.318	0.9290	0.283	40.708	0.9767	0.059
K3	0.7941	4.921	0.9827	0.263	0.8951	21.743	0.9438	0.234	42.892	0.9585	0.056
K4	0.9360	4.445	0.9902	0.137	0.9860	19.467	0.9983	0.223	39.390	0.9857	0.037

b)

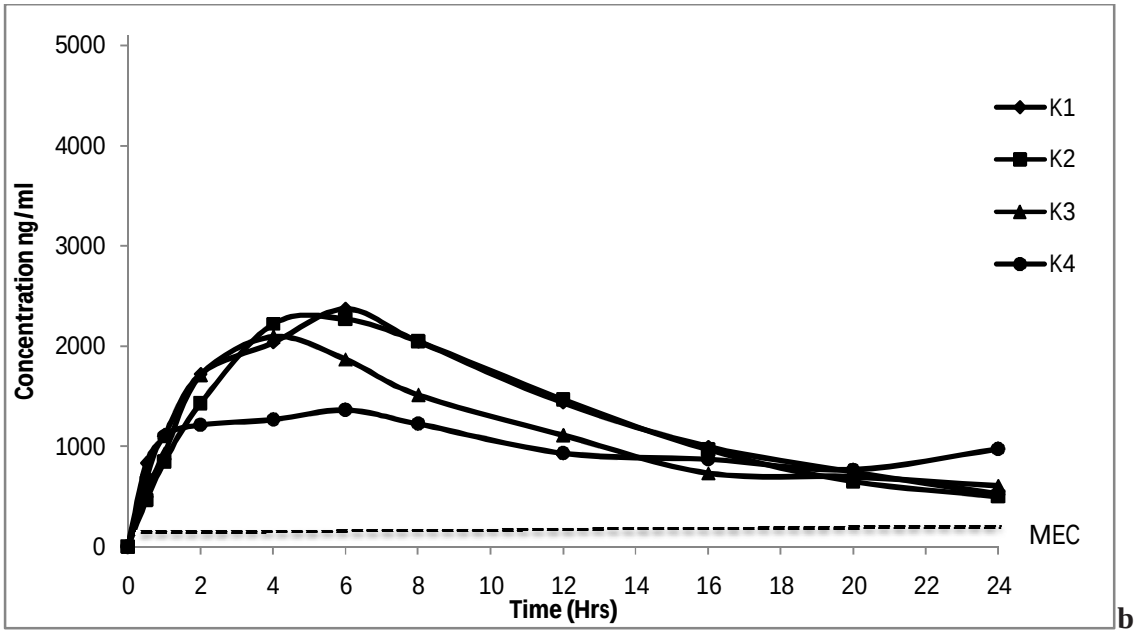
Formulations	Zero Order		First Order		Higuchi		Korsmeyer-Peppas			Hixson- Crowell	
	R^2	k_o (h^{-1})	R^2	k_1(h^{-1})	R^2	k_H ($h^{-1/2}$)	R^2	n	K_{KP}(h^{-n})	R^2	k_{HC} ($h^{-1/3}$)
K1	0.7687	5.933	0.9897	0.592	0.9450	26.387	0.9377	0.211	55.094	0.9543	0.066
K2	0.7091	5.433	0.9985	0.560	0.8308	24.398	0.9057	0.182	54.784	0.9286	0.063
K3	0.8682	4.999	0.9649	0.502	0.9460	22.445	0.9873	0.139	55.763	0.9762	0.059
K4	0.9552	4.523	0.9922	0.144	0.9942	19.786	0.9969	0.219	40.397	0.9904	0.039

5.2.5 Prediction of *In vivo* drug concentration in blood-time profiles

The mean In-vitro dissolution data obtained for formulations K1, K2, K3 and K4 were used to simulate the In vivo blood drug concentration- time (C-t) profiles. The predicted blood drug concentration-time profiles for AZT and 3TC are shown in Figure 5.4a and b respectively.



a



b

Figure 5.4: Predicted *In vivo* drug concentration in blood Vs time profiles of Kollidon® SR formulations for a) AZT and b) 3TC. The dotted line below indicates the minimum effective concentration (MEC) while the dotted line above indicates minimum toxic concentration (MTC). MTC concentration for 3TC is 300,000 ng/ml.

To evaluate these predicted C-t profiles, pharmacokinetic parameters such as Cmax (ng/ml) and Tmax (hr) were obtained as shown in Table 5.3.

Table 5.3: Pharmcokinetic parameters for Kollidon® SR matrices

Formulation	Cmax (ng/ml)		Tmax (hr)	
	AZT	**3TC**	**AZT**	**3TC**
K1	1619	2372	2	6
K2	1510	2270	4	6
K3	1638	2096	2	4
K4	1497	1365	1	6
Conventional FDC AZT/3TC TAB 300/150mg	3100	1500	0.6	1

Formulation K1 which had the highest drug loading achieved Cmax at 2 and 6hrs for AZT and 3TC respectively though AZT release was sustained till 12hrs while 3TC was sustained for 24hrs considering the fact that the drug release was sustained above the minimum effective concentration (MEC). The drug concentrations for K1 thus indicated that not all the drugs formed a solid dispersion with the polymer since the percentage of drug loading was higher than polymer loading. AZT and 3TC on extrusion converted into amorphous solids that dissolve faster than crystalline solids. There seemed to be a very small proportion of the drugs that may have remained crystalline after extrusion and forming a solid dispersion giving longer Tmax. Since Kollidon® SR is partially soluble, it is probable that a proportion of the excipient has dissolved in the dissolution medium and leached out resulting to form matrices of increasing porosity that provide a prolonged release of AZT leading to predicted plasma concentration above Minimum effective concentration (MEC) for periods up to 12hrs.

Formulation K2 achieved Cmax for AZT and 3TC at Tmax 4hrs and 6hrs respectively and predicted drug plasma concentrations were sustained and maintained above the minimum effective concentration (MEC) and below the minimum toxic concentration (MTC) for periods up to 10hrs and 24hrs for AZT and 3TC respectively

K3 had a low predicted Cmax for AZT and 3TC although Tmax for AZT was at 2hr with Tmax for 3TC predicted to be 4hrs. AZT and 3TC levels were maintained at therapeutic levels above MEC and below MTC for 12hrs and 24hrs respectively. The Tmax for both drugs was predicted to be reduced relative to formulation K2.

Formulation K4 had a drug loading of 35% w/w whilst the loading of TEC was 15%w/w. The Cmax for AZT was achieved by 1hr, whilst for this occurred at 6hr. AZT was shown to have sustained therapeutic concentrations for periods up to 16hrs whilst for 3TC, drug concentrations were maintained at therapeutic levels over 24hrs.

Formulation K4 demonstrated predicted therapeutic blood drug concentrations sustained over a prolonged period (16 hrs) although the Tmax of of AZT was predicted to be 1hr. It is therefore likely that the oral administration of formulation K4 would provide greater convenience than the current twice daily immediate release preparation.

5.2.6 Accelerated stability studies

Samples of formulations K1 to K4 were kept for accelerated stability testing. XRPD patterns reflected no change in at all for Formulation K1 to K3 in open and closed bottles 40 ºC/75% RH while K4 showed a slight increase in peak intensity that was close to at 40 ºC/75% for the

open bottles while the closed bottles did not show any difference in intensity after 3 months (Figure 5.4). No crystalline peaks of AZT were observed at 2-theta angles of 25.6, 27.76, 27.84, 35.12, 23.4, 43.44 and 44.84 degrees after 1 month and 3 months in formulations K1 to K3 though with K4 small peak with very low intensity, close to the 3TC peak at 2-theta angle 16.13 and 21.22 were observed at end of 3month storage at accelerated conditions (Figure 5.5).

For samples at 60ºC tested for XRPD after 15 days showed no difference in formulations K1 to K4. The XRPD patterns did not reflect any peaks that correspond to either AZT or 3TC. The reason could be that AZT and 3TC formed a solid solution with Kollidon® SR formed after HME thus indicating no change in XRPD peaks even after storage at high temperatures in closed containers. The formulations were tested for changes in dissolution profiles at 60ºC and there were no significant changes observed in dissolution profiles with all formulations K1, K2, K3, K4 having the similarity factors more than 50.

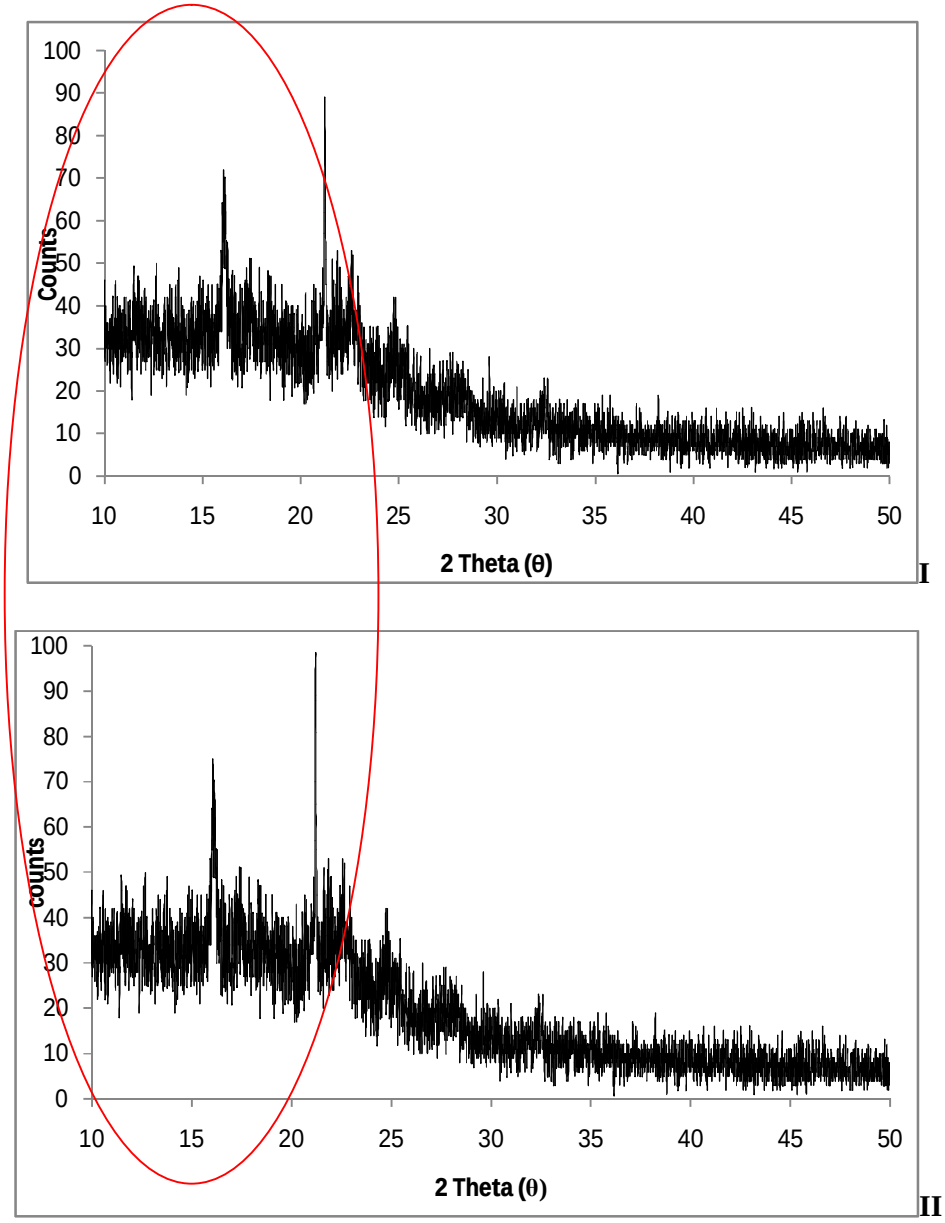
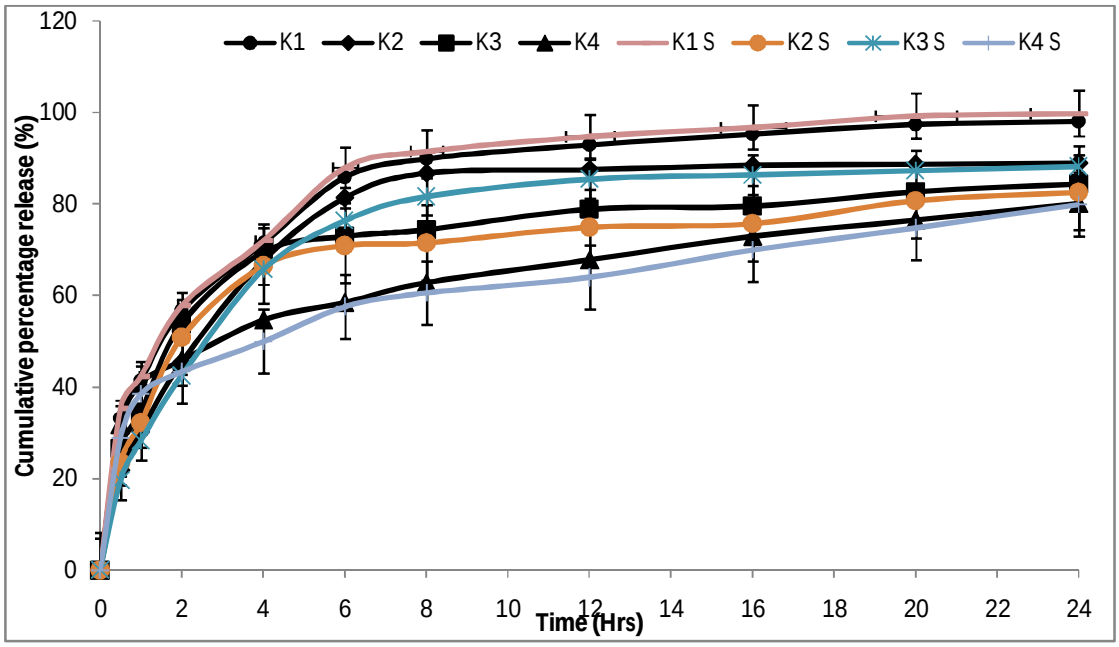


Figure 5.5: XRPD diffraction patterns for K4 intensity for I) before accelerated stability studies II) K4S after 3 months accelerated stability studies showing increase in 3TC peak intensities.

The dissolution profiles of K1 to K3 after storage at accelerated conditions at 40 ºC/70% RH in open container were found to have decreased over 3 months and the mini matrices of the formulations turned slightly yellowish in colour while there were no changes in dissolution in closed containers at the same accelerated conditions. The change in dissolution profile is usually an indication of polymer structural relaxation (Shao et al, 2001). Dissolution profile of K4 however has a very slight increase in drug release as compared to K1, K2 and K3 (Figure 5.6). The increase in drug release after accelerated storage could be due to the drugs transitioning to crystalline form after exposure to accelerated high temperature and humidity. This was also confirmed by a slight increase in peaks that were observed during XRPD studies.

a)



b)

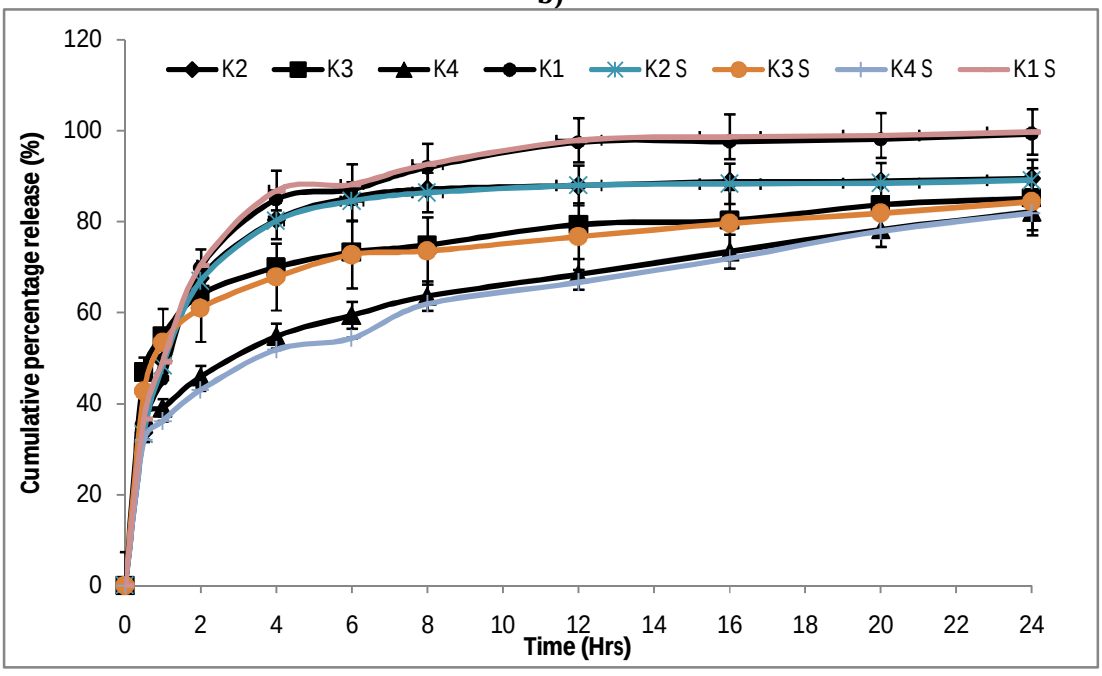


Figure 5.6: Comparisons of dissolution profiles before mini matrices formulations (K1, K2, K3 and K4 in black lines) and after accelerated stability (K1 S, K2 S, K3 S and K4S in coloured lines) of a) AZT b) 3TC (Error bars indicate mean ± SD, n=3).

The dissolution profiles were compared with the dissolution profiles initially done prior to storage at accelerated conditions and the f_2 values were found to be ≥ 50, indicating that there was no change in dissolution profiles (Table 5.4).

Table 5.4: Similarity f_2 values of AZT and 3TC release from formulations K1 to K4 before and after accelerated stability period in open bottles at 40°C/75% RH.

Formulation	K1	K2	K3	K4
a) Similarity factor f_2 AZT release	75.4	79.8	76.7	86.3
b) Similarity factor f_2 3TC release	96.8	81.2	75.1	87.0

5.3 Conclusion

Hot melt extrusion of thermoplastic formulations based on Kollidon® SR seems to be a technique that could be used to produce sustained release fixed dose combination AZT/3TC mini matrices for oral use. An overall observation made during hot melt extrusion using Kollidon® SR with the drugs was that processing and cleaning of the twin screws during and after extrusion was much easier and time saving as compared to extrusion using ethylcellulose which was difficult to clean since it is purely hydrophilic in nature. A total of 50% drug loading was possible to be hot melt extruded along with 5% w/w of TEC used as a plasticizer and *in vitro* drug release over 24hrs for both drugs was almost complete. XRPD and DSC studies of drug loading (Both AZT and 3TC) of up to 45% w/w in total showed that

the drugs were completely miscible in Kollidon® SR and formed a solid solution after extrusion. Drug load of 50% w/w formed a solid dispersion and from XRPD results demonstrated that a very small portion 3TC remained as crystalline and was un-dissolved in Kollidon® SR after extrusion and which may have caused the longer Tmax since crystalline drugs take longer time to dissolve than amorphous when in contact with water. Increase in TEC loading as plasticizer reduced release of the drugs due to densification of the matrices. From the predicted Blood drug concentration-time profiles, formulation K4 with 35% w/w drug loading and 15% w/w TEC was found to be having the best sustained release characteristics up to 16hrs and 24 hrs for AZT and 3TC respectively. The mini matrices remained stable after accelerated stability studies at 60ºC at normal humidity and at 40ºC/70% RH. XRPD and *in vitro* dissolution studies demonstrated relatively no significant change in properties as compared to properties before accelerated stability studies.

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CHAPTER SIX: GENERAL DISCUSSION AND CONCLUSION

The major concern today in most developing countries is the lack of appropriate and efficacious formulations that may deny access to appropriate paediatric antiretroviral formulations and expose children to “homemade” formulations. Most available dosage forms are not sufficiently flexible for dose adaptation according to the child’s body weight. The availability of the adult fixed dose combination (FDC) zidovudine/ lamivudine (300mg AZT/ 150mg 3TC) has forced many health care providers in resource limited settings to cut/split adult tablets several times to obtain a normal paediatric dose of 60mg AZT/30mg 3TC. This may cause under dosing or over dosing due to the incorrect manipulation of the commercially available dosage form. Liquid oral formulations commonly require refrigeration which is unavailable in most Sub-Saharan countries due to associated costs.

AZT and 3TC are freely soluble drugs that have short half life and low therapeutic index thus necessitating frequent oral dosing every 3-4hrs. This frequent dosing cause dose related haematological toxic effects that in turn reduces patient adherence to the ARV regimen. Both AZT and 3TC are freely soluble in any pH and hence a judicious selection of release retarding excipients is necessary to achieve constant sustained release in-vivo. The primary objectives of formulating sustained release drug delivery are to ensure safety and enhancement of efficacy of drug with improved patient compliance. So the use of these dosage forms is increasing in treatment of acute and chronic diseases as they maintain the concentration of drug in plasma above minimum effective concentration and below the minimum toxic level for extended period of time. Thus, sustained drug delivery results in

optimum drug therapy with reduced frequency of dosing and side effects and better health outcomes.

Pre-formulation studies were carried out to determine the influence of zidovudine, lamivudine and plasticizer content of polymeric formulations on their thermal, rheological and solubility characteristics with a view to assessing their suitability for HME. Dosage forms manufactured using HME are a typically a mixture of active pharmaceutical ingredients (APIs), polymers (matrix carriers) and other excipients. These ingredients need to be appropriately studied prior to extrusion so as to achieve excellent therapeutic and processing properties, a requirement for most pharmaceutical regulatory bodies and for cost effective manufacture.

In accordance with the objectives of this study to achieve sustained release of the drugs, the polymers that were chosen were known to have drug release retardant properties and were safe to be used in paediatric formulations. The polymers used for this study were ethylcellulose, Kollidon® SR and poly ethylene oxide (PEO).

Plasticizers used for the study were triethyl citrate (TEC) and polyethylene glycol (PEG-6000). Physical mixtures containing the polymers with varying concentrations of drugs and plasticizers were characterized using differential scanning calorimetry (DSC) and parallel plate oscillatory rheometry. Solubility parameter calculations were undertaken to predict the miscibility of the drugs and plasticizers in the polymers. The calculated solubility parameters (δ/Mpa$^{1/2}$) indicated that the polymers, drugs and plasticizers showed similar solubility parameters with differences of less than $\Delta\delta) < 7\text{Mpa}^{1/2}$, indicating compatibility between the different components. Thermal gravimetric analysis (TGA) indicated that both AZT and 3TC decomposed at temperatures above 200ºC which meant each of the APIs are suitable for processing by HME.

DSC studies of physical mixtures of ethylcellulose and the drug indicated the formation of solid solutions with AZT and 3TC although ethylcellulose was saturated once 3TC reached levels of 40%w/w of. AZT reduced the glass transition (Tg) temperature of ethylcellulose while 3TC did not seem to have effect since the melting temperatures of (Tm) of 3TC and ethylcellulose were very close.

Both AZT and 3TC were soluble in Kollidon® SR at various proportions indicated by absence of melting endotherms in DSC data and the presence of broad thermal profile typically associated with amorphous materials. Neither drug substance affected the glass transition temperature of Kollidon® SR. Rheological studies however showed that the addition of zidovudine decreased the melt viscosity of all the polymers while lamivudine seemed to saturate the polymer at a 40%w/w concentration at which point there was a marked increase in melt viscosity. Triethyl citrate (TEC) was chosen as plasticizer for hot melt extrusion since polyethylene glycol (PEG-6000) showed signs of re-crystallization upon cooling which was a sign of instability of the physical mixtures.

As the thermal and rheological studies showed that hot melt extrusion was possible for the studied mixtures of drug substances, polymers and plasticizers, experiments were undertaken to demonstrate the feasibility of developing sustained release mini matrices of zidovudine and lamivudine using ethylcellulose as a rate modifying polymer. The thermal and rheological results from the pre-formulation studies obtained in chapter three were used to determine the processing parameters for HME. Ethylcellulose alone could not be extruded with the drugs thus this warranted the inclusion of triethylcitrate (TEC) as plasticizer so as to lower the glass transition temperature of ethylcellulose and lower the extrusion temperatures to facilitate processing.

During these studies, it was observed that extrusion torque measured during processing was directly correlated with the plasticizer concentration, with increased levels giving greater torque measurements. *In vitro* dissolution studies undertaken using ethylcellulose based formulations across a range of drug loadings and plasticizer contents gave rise to low and incomplete release of both drugs over 24hr. The low and incomplete release of AZT and 3TC was attributed to the hydrophilic matrix of ethylcellulose having very low water permeability. Increasing the TEC concentration above 15% in the formulations resulted in very brittle matrices that were difficult to pelletize into the required size. Phase separation was also observed with higher TEC concentrations during extrusion. Polyethylene oxide (PEO) was then added as a pore former for 5%-15%w/w concentrations to increase drug release over time yet maintain the sustained release. Addition of 5%w/w of PEO to ethylcellulose resulted in a cumulative drug release of almost 80% over 24hrs. Increasing PEO concentration to 30%w/w resulted in almost complete release (100%) of both AZT and 3TC over 24 hrs with a sustained profile. DSC studies of mini matrices formulated with PEO indicated showed very broad melting peak (Tm) of PEO that was much lower than the Tm of pure PEO. The *in vitro* drug release results were used to model drug release using a range of modelling equations. The results of these studies indicated that drug release from the formulations occurred preferentially by diffusion although the coefficient of correlation for Higuchi's equation was also high which indicated that the drug release was through diffusion through a network of channels in the matrix and that the amorphous drugs were homogeneously distributed within in the polymer matrix forming solid solution. The Accelerated stability studies of formulations with PEO indicated no changes in the thermal profiles or XPRD patterns over a period of 3 months when stored at both high temperature and high humidity conditions in open and closed containers. Predicted in-vivo drug concentrations over 24hrs were predicted using the

convolution method described in the literature. The formulations with PEO had sustained drug levels that were within the therapeutic window. Higher PEO concentrations in the formulation resulted into complete but faster release of both drugs. PEO concentrations of 5% w/w were shown to give good sustained release of both drugs where the drug levels were maintained above the minimum therapeutic concentration for 20hrs.

As an alternative to ethylcellulose, the feasibility of developing mini-matrices using Kollidon-SR was evaluated. Kollidon® SR is a pre plasticized polymer that is made of 80% polyvinyl acetate and 20% polyvinyl pyrrolidone. This polymer does not need excipients during extrusion and is known to have a high drug loading capacity. Drug release is often influenced by the polymer type and permeability of the polymer. In this study, high drug loadings of 45%w/w could be extruded using Kollidon® SR although under these circumstances, the extrusion torque was comparatively very high thus 5%w/w of triethylcitrate (TEC) as plasticizer was added to ease extrusion. A total of four matrix formulations were prepared by hot melt extrusion containing varying proportions of drugs and TEC while keeping the proportion of Kollidon® SR fixed at 50%w/w. All other processing parameters were maintained throughout the study. X-ray powder diffraction (XRPD) studies indicated that AZT was predominantly amorphous whilst a low level of 3TC remained crystalline in the formulation with the highest drug loading and was evident in MDSC and rheology results obtained during preformulation studies in chapter three. The *In vitro* drug release studies undertaken on the four matrix formulations showed that drug release for both drugs was fastest in formulation with 50%w/w drug loading. Drug release slowed with increase in TEC levels since besides plasticization TEC may have caused strong coalescence between the drug and polymer particles which was evident from appearance that the matrices became denser on increase of TEC levels. The drug release mechanisms for all the four matrix formulations

were mixed with high coefficient of correlation for first order, Higuchi's equation, Korsmeyer-Peppa's equation and Hixson- Crowell equation. The First order and Korsmeyer-Peppa's equation gave best overall fit to the data, with the drug release probably occurring by anomalous transport which has contributions from non- Fickian diffusion and polymer relaxation or erosion that occurs when the matrix is hydrated in contact with the dissolution medium. The value obtained from Korsmeyer-Peppa's equation therefore indicates that drug release was controlled by more than one process and this could be due to matrix composition.

The predicted *in vivo* blood drug concentration versus time profile indicated that formulations with 50%w/w (high) drug loading could sustain both drugs for only 12hrs while formulations with lower drug loading but higher plasticizer level were able to sustain the release of both AZT and 3TC within the therapeutic levels for periods in the range 16-20hrs. The *in vivo* results obtained relates to the *in vitro* drug release from the formulation where drug release from matrices containing higher level of plasticizer gave sustained and complete release over 24hrs.

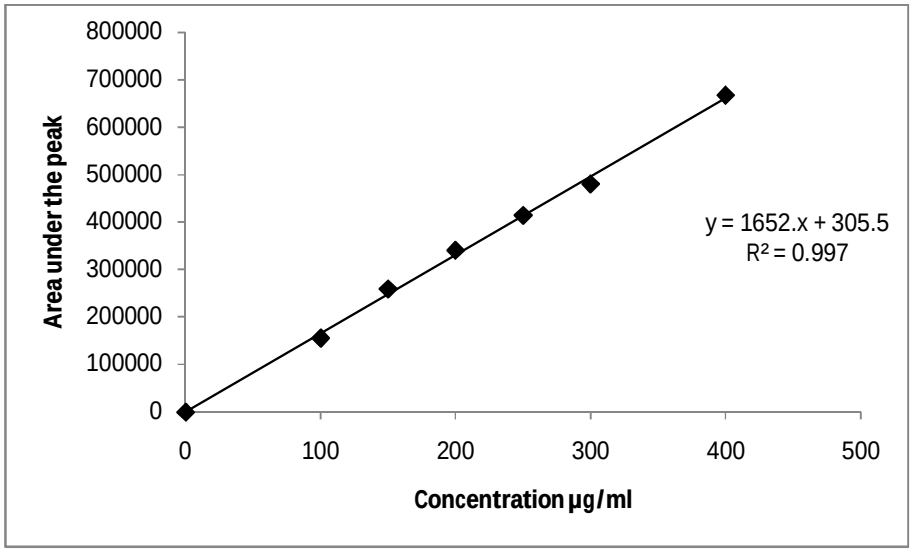
In conclusion, fixed dose zidovudine and lamivudine mini matrices with sustained release properties can successfully be produced using hot melt extrusion. Stable sustained release fixed dose combination mini matrices of zidovudine and lamivudine can be formulated using ethylcellulose and Kollidon® SR. The factors controlling the release rate from the formulations are the type of excipients, which not only impact processibility parameters during extrusion but also influence drug release mostly by diffusion and erosion mechanisms. The results presented in this thesis provide confidence that mini matrix formulations which can be tailored for use in children can be achieved through further optimisation of composition. In this regard, control of the level of pore forming agents and plasticizers which alter the viscosity of the extruded

matrices is most likely to enable the achievement of appropriate plasma drug concentrations, within the therapeutic window for periods up to 24hrs. This should allow development of a more convenient once daily dosage form for combinations of anti-retro-viral drugs, leading diminished toxicity, improved adherence and better health outcomes.

In future, *in vivo* bioavailability studies on hot melt extruded zidovudine/ lamivudine mini matrices are suggested so that *in vitro- in vivo* correlations (IVIVC) of drug dissolution data and drug bioavailability can be performed. Mini matrix formulation having good IVIVC can be further studied on optimization of mini matrix formulation to increase palatability and taste acceptance in children as well as to achieve dosing flexibility that is ideal for children under diverse age categories so as to maintain safety and accuracy of each dose administered to various age categories of children that could be done by dosing volumes and dose titration adjustment studies. Further studies to understand the interaction of AZT and 3TC with the polymers and plasticizers at molecular levels can be done using FT-IR studies to compliment and compare with the results obtained from Raman spectroscopy.

APPENDIX 1: Standard calibration curve used to calculate the concentration of zidovudine (AZT) in dissolution medium at fixed time intervals.

Concentration (µg/ml)	Area under the peak
0	0
100	155536
150	259109
200	340713
250	413816
300	479816
400	666804



APPENDIX 2: Standard calibration curve used to calculate the concentration of lamivudine (3TC) in dissolution medium at fixed time intervals.

Concentration (µg/ml)	Area under the peak
0	0
50	110429
75	156336
100	201200
125	234388
150	292725
200	371862
250	479806