CHAPTER – IV

AIM AND OBJECTIVE

4.1 AIM

The present research is aimed at **"Design and Characterization of Drug Delivery System using Hot Melt Coating Technique."** This involves the application of Hot Melt Coating (HMC) for

A. Taste Masking of Tenofovir Disoproxil Fumarate (TDF)
B. Improvement in Stability of Sitagliptin Phosphate Monohydrate (SPM)

4.2 Objectives

The important objectives of the proposed research work were,

1. Characterization of Tenofovir Disoproxil Fumarate (TDF) and Sitagliptin Phosphate Monohydrate (SPM) API.
2. Designing the manufacturing process for a pre-determined aim.
3. Selection of raw materials.
4. Optimization and evaluation of Manufacturing Process Parameters.
5. Evaluation of the final product of Tenofovir Disoproxil Fumarate (TDF) for Taste Masking and Stability Improvement for Sitagliptin Phosphate Monohydrate (SPM).
6. Comparative Dissolution Assessment.
7. Stability Evaluation of Optimized Dosage Forms.

4.3 Quality Target Product Profile (QTPP)

Part A:

The QTPP for Tenofovir Disoproxil Fumarate Pellets and Tablets is outlined in the table below. The Quality Attributes identified as Critical Quality Attributes (CQAs) are also provided. The goal is to develop solid oral dosage form pellets and tablets. The objective is to investigate the potential of the Hot Melt Coating technique for Taste-masking when formulating either Pellets or Tablets. Taste masking for the pellets is particularly important for pediatric and elderly patients who have difficulty swallowing large tablets.[22, 23]

Table 4.1 : Quality Target Product Profile (QTPP) for TDF Pellets and Tablets

QTPP Elements	Target (Pellets)	Target (Tablets)
Dosage form	Pellets (in Sachets)	Tablets (in Bottles)
Dosage design	Immediate Release	Immediate Release
Route of administration	Oral	Oral
Dosage Strength	300 mg	300 mg
Stability	At least 24 months at room temperature	At least 24 months at room temperature
Drug product Quality attributes	Physical attributes	Physical attributes
	Taste-masking (Bitterness)	Taste-masking (Bitterness)
	Assay	Assay
	Dissolution	Dissolution
Container closure system	Suitable for product stability	Suitable for product stability

Part B:

The QTPP for Sitagliptin Phosphate Tablets 50mg (SPM)is defined in the following table. The quality attributes that were identified as drug product Critical Quality Attributes (CQAs) are also given below. The target is to formulate a Hot Melt Coated Tablets. The aim is to explore the potential of the hot melt coating technique for stability enhancement.[22,23]

Table 4.2 : Quality Target Product Profile (QTPP) for SPM Tablets

QTPP Elements	Target
Dosage form	Hot Melt Coated Tablets
Dosage design	Immediate Release
Route of administration	Oral
Dosage Strength	50 mg
Stability	At least 24 months at Room Temperature
Drug product Quality attributes	Physical attributes
	Moisture uptake

QTPP Elements	Target
	Assay
	Dissolution
Container closure system	Suitable for product stability

4.4 Critical quality attributes

The Drug Product Critical Quality attributes of the drug products were identified[24] as below-

Part A:

Table 4.3 : Critical Quality Attributes of TDF Pellets

Drug Product Quality Attributes		Target	Is this CQA?	Justification
Physical Attributes	Appearance	White to off-white coated pellets	No	For better patient acceptance and compliance with treatment regimens, the target for pellet size is set. Formulation and process variables do not impact appearance. The size of pellets is controlled by fixing the screen sizes. Hence, it is not critical.
	Size	Easily swallowable	No	
	Taste	No Bitter taste	Yes	Affects patient acceptability, hence it is Critical.
Assay		95-105%	Yes	Assay variability will affect safety and efficacy and is influenced by process variables.
Dissolution		NLT 80% (Q) in 30 minutes	Yes	Failure to meet the dissolution specification may impact on therapeutic efficacy. Both formulation and process variables may affect dissolution.

Table 4.4 : Critical Quality Attributes of TDF Tablets

Drug Product Quality Attributes		**Target**	**Is this CQA?**	**Justification**
Physical Attributes	Appearance	White to off white tablets	No	Similar to marketed products. Formulation and process variables do not impact appearance and size. Hence, it is not critical.
	Size	Similar to marketed	No	
	Taste	No Bitter taste	Yes	Affects patient acceptability, hence CQA.
Assay		95-105%	Yes	Assay variability will affect safety and efficacy and is influenced by process variables.
Dissolution		NLT 80%(Q) in 30 minutes	Yes	Failure to meet the dissolution specification may impact on therapeutic efficacy. Both formulation and process variables may affect dissolution.

Part B:

Table 4.5 : Critical Quality Attributes of Sitagliptin Phosphate Monohydrate Tablets

Drug Product Quality Attributes		**Target**	**Is this CQA?**	**Justification**
Physical Attributes	Appearance	White to off-white tablets	No	Similar to marketed products. Formulation and process variables do not impact appearance and size. Hence,
	Size	Similar to	No	

Drug Product Quality Attributes	Target	Is this CQA?	Justification
	marketed		it is not critical.
Assay	90-110%	Yes	Assay variability will affect safety and efficacy and is influenced by process variables.
Moisture uptake	NMT 2%	Yes	Moisture uptake by the drug will result in degradation and loss of assay.
Dissolution	NLT 80% (Q) in 30 minutes	Yes	Failure to meet the dissolution specification may impact on therapeutic efficacy. Both formulation and process variables affect dissolution.

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CHAPTER – V

MATERIALS AND METHODS

PART A:

5.1 Drug Profile: Tenofovir Disoproxil Fumarate (TDF)

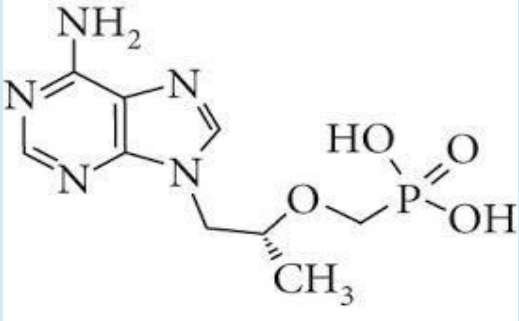
Tenofovir Disoproxil Fumarate is a tenofovir prodrug that is the fumaric acid salt of tenofovir's bis-isopropoxycarbonyloxymethyl ester derivative. In vivo, TDF is transformed into tenofovir, an acyclic nucleoside phosphonate (nucleotide) analogue of adenosine 5'-monophosphate. Tenofovir has action against HIV-1 reverse transcriptase. It is used in conjunction with other antiretroviral medications to treat human immunodeficiency virus type 1 infection in adults and paediatric patients aged two years and up who weigh at least ten kilogrammes. It is used to treat chronic hepatitis B virus (HBV) in adults and children aged two and above who weigh at least ten kilogrammes.[1]

Category: Nucleoside Reverse Transcriptase Inhibitor (NRTIs) namely HIV-1 reverse transcriptase inhibitor and an HBV reverse transcriptase inhibitor (HBV RTI)

Mechanism of Action: It is an acyclic nucleoside phosphonate diester, similar to adenosine monophosphate. It requires diester hydrolysis to become tenofovir, which is subsequently phosphorylated by cellular enzymes to become tenofovir diphosphate (TFV-DP), an essential chain terminator. Tenofovir diphosphate inhibits HIV-1 and HBV reverse transcriptase (RT) activity by competing with the natural substrate deoxyadenosine 5'-triphosphate and, if incorporated into DNA, ending the chain. Tenofovir diphosphate is a mild inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ.[2]

Table 5.1 : Characteristics of Tenofovir Disoproxil Fumarate

Parameter	Tenofovir Disoproxil Fumarate
CAS No.	202138-50-9
Chemical Name	Tenofovir disoproxil fumarate is 9-[(R)-2[bis[[(isopropoxycarbonyl)oxy]-methoxy] phosphinyl] methoxy] propyl] adenine fumarate (1:1}
Empirical Formula	$C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$
Molecular Weight	635.52
Description	White to off-white bitter crystalline powder with

Parameter	Tenofovir Disoproxil Fumarate
	characteristic odor
Melting point	114-118°C
Structure	
Solubility	About 13.4 mg/mL in distilled water at 25 °C. In ethanol and DMSO solubility is about 100 mg/ml. Solubility in 0.1N hydrochloric acid is about 78.2 mg/ml and in methanol is about 96.3 mg/ml.
(log P)	1.25 at 25 °C
Peak plasma time (T_{max}):	1-1.4 hours
Metabolism	Metabolized by CYP enzymes.
Excretion	About 70-80% tenofovir is recovered in urine unchanged in 72 hours
Half-life	Approximately 17 hours
Dosage forms & Strengths	Tablets, Oral Powder 300 mg
Storage	Store tenofovir tablets or tenofovir powder dosage form at 20 to 25°C. The bulk powder of tenofovir should be below 4°C. Store in tightly closed container. Keep away the tenofovir tablets or tenofovir powder dosage form from the reach of children.

5.1.1 Determination of Organoleptic Properties (TDF)

The organoleptic properties of Tenofovir disoproxil fumarate were determined by sensory evaluation like color, odor, and taste.

Table 5.2 : Organoleptic Properties of Tenofovir Disoproxil Fumarate

Parameter	Observed
Color	White to off-white
Odor	Characteristic
Taste	Bitter

Inference: The taste of Tenofovir Disoproxil Fumarate was found to have bitter characteristics.

5.1.2 Particle Size Distribution (TDF):

The particle size distribution of Drug substance referred from Vendor COA. The method of testing is Malvern Master sizer.[3]

Inference: The Particle size distribution of Tenofovir disoproxil fumarate API: d (0.9) – 190 µm.

5.1.3 Density and Flow Properties (TDF):

Bulk density is the ratio of bulk weight to bulk volume. Fifty g of API was precisely weighed and carefully poured via a glass funnel into a 100 ml calibrated measuring cylinder. The surface was carefully levelled with no pressure.[4] The volume occupied by pellets was utilised to calculate the bulk density (g/ml) using the equation,

Bulk density = Weight of powder ÷ $Bulk\ volume\ of\ pellets$…….(5.1)

Tapped density is ratio of bulk weight and tapped volume. Tapped density was estimated in a similar way to that of bulk density. However, final volume was measured after tapping the cylinder from 3 inches until constant volume was obtained using Electrolab tapped density apparatus.[4] The volume occupied by pellets after tapping was noted and tapped density (g/ml) was calculated using the equation,

Tapped density = Weight of powder ÷ Tapped $volume\ of\ pellets$…….(5.2)

Carrs's Index is calculated using bulk density and tapped density data.[5]

$Carr's\ Index = (Tapped\ density - Bulk\ density) \div Tapped\ density \times 100$ …….(5.3)

Density and flow properties of Tenofovir disoproxil fumarate API was evaluated and the results are given in the following table,

Table 5.3 : Density and Flow Properties of TDF

Bulk density (g/mL)	Tapped density (g/mL)	Carr's Index (%)
0.35	0.51	30.85

Inference: Tenofovir disoproxil fumarate API showed poor to very poor flow characteristic.

5.1.4 Solubility of Tenofovir Disoproxil Fumarate

The aqueous solubility of the drug is significant in drug absorption. When the drug is administered orally, the composition of GI fluid changes with the position of the dosage form. Therefore, to simulate the conditions, the solubility of the drug in 0.1N HCl, distilled water, and phosphate buffer pH 6.8 was determined. Also, the solubility was determined in ethanol, methanol, and DMSO.[6]

Technique: Equilibrium solubility method was used.

Procedure: It is determined by placing 1 g of TDS in 100 ml of distilled water on a rotatory shaker for 24 hr at 37°C.After 24 hours the solution was filtered using 0.45 µ member filter. The amount dissolved in the filtrate was determined using UV- Visible spectrophotometer at 260 nm.[6,7]

Table 5.4 : Solubility of TDF

Solvent	Solubility (mg/ml)
0.1 N HCl	77.86 ± 2.06
Distilled water	13.27 ± 0.45
pH 6.8 phosphate buffer	68.33 ± 1.96
Ethanol	97.42 ± 3.57
Methanol	80.09 ± 2.81
DMSO	101.55 ± 3.32

Inference: The highest solubility among aqueous media is in 0.1 N acid and the lowest in distilled water. Among the organic solvents, highest solubility is found in DMSO.

5.1.5 Melting point of TDF:

The melting point of Tenofovir disoproxil fumarate API was evaluated using two different methods, Capillary technique using mineral oil and Differential Scanning Calorimetry (DSC)[8]

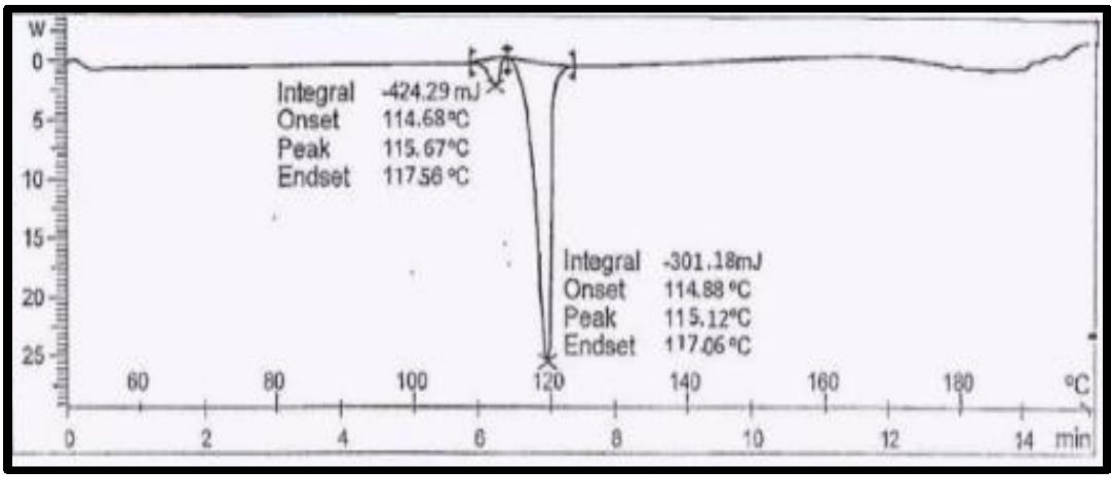


Fig. 5.1 : DSC Thermogram of Tenofovir Disoproxil Fumarate

Inference: Melting point of Tenofovir disoproxil fumarate API found to be between 115-118°C (by Capillary technique) and 115.67°C (by DSC).

5.1.6 Loss on Drying of TDF

The Loss on drying is determined using Hot air oven technique. The 1 g of TDF at 105±2°C was kept in hot air oven till constant weight was observed.

Inference: The % Loss on Drying for TDF sample was found to be 1.23%.

5.2 Risk Assessment for Drug Substance Attributes of TDF

A risk evaluation of the drug substance attributes was conducted to determine the potential influence of each attribute on the drug product Critical Quality Attributes. The assessment results and related reasoning are summarised here. The relative risk of each trait was rated as high, medium, or low. The high-risk features deserved additional research, but the low-risk attributes did not. According to current

understanding, a medium risk is tolerable. Further examination of medium risk may be required to lessen the danger.[9]

Table 5.5 : Overview of Relative Risk Ranking System

Low	Broadly acceptable risk. No further investigation is needed.
Medium	Risk is accepted. Investigation may be needed in order to reduce the risk.
High	Risk is unacceptable. Investigation is needed to reduce the risk.

The risk assessment of drug substance qualities that affect drug product CQAs is provided below based on the drug substance's physicochemical parameters.

Table 5.6 : Risk Assessment of the Drug Substance Attributes for TDF

Drug product CQA	Tenofovir drug substance attributes					
	Organoleptic properties	**Particle size**	**Solubility**	**Melting point**	**Loss on drying**	**Flow properties**
Taste	High	Low	Low	Low	Low	Low
Content uniformity	Low	Low	Low	Low	Low	Low
Assay	Low	Low	Low	Low	Low	Low
Dissolution	Low	Medium	Medium	Low	Low	Low

Table 5.7

Drug Substance Attributes	Drug Product CQAs	Justification
Organoleptic Properties (Color, Odor, Taste & nature)	Taste	High risk as the taste of API is bitter, so it impacts patients' acceptance. It may induce nausea.
	Content Uniformity	Low risk as organoleptic characteristics has no direct impact on Content uniformity, assay and dissolution.
	Assay	
	Dissolution	

Particle size	Taste	Low risk as Particle size has no direct impact on taste
	Content Uniformity	Particle size may have impact on flow properties and hence, assay and content uniformity. However, API is being granulated. Hence risk is low.
	Assay	
	Dissolution	Particle size may have impact on dissolution hence risk is medium.
Melting Point	Taste	Low risk as Melting Point has no direct impact on taste, Content uniformity, assay, and dissolution
	Content Uniformity	
	Assay	
	Dissolution	
Solubility	Taste	Low risk as Solubility has no direct impact on taste, content uniformity, assay and dissolution
	Content Uniformity	
	Assay	
	Dissolution	The solubility of drug substance in dissolution medium has impact on dissolution. The risk is medium.
Loss on Drying	Taste	Low risk as Loss on drying has no direct impact on taste, content uniformity, assay, and dissolution.
	Content Uniformity	
	Assay	
	Dissolution	
Flow Properties	Taste	Low risk as Flow properties has no direct impact on taste.
	Content Uniformity	Tenofovir disoproxil fumarate has very

	Assay	poor flow property. However, the drug substance would be subjected to dry granulation process to facilitate blend flow. Hence, impact of flow of Tenofovir disoproxil fumarate on content uniformity and assay is low.
	Dissolution	Flow of Tenofovir disoproxil fumarate is unlikely to impact dissolution. The risk is low.

5.3 Excipients for TDF:

The excipients were selected based on literature search, the requirement of immediate-release tablets and pre-formulation studies. Excipients were selected based on their functionality. For development of tablet dosage form, the grades suitable for wet granulation process and hot melt coating were selected.

5.3.1 Gelucire 43/01

A glyceride with intermediate melting point used as a matrix agent for sensitive APIs and a viscosity-increasing agent in oral and topical formulations. It is composed of mono-, di- and triglyceride esters of fatty acids (C_8 to C_{18}), the triester fraction being predominant. It is available in pellet form. It is a blend of saturated triglycerides of different fatty acids, viz., C8 - 3%, C10 - 2%, C12 - 29%, C14 - 2%, C16 - 17%, and C18 - 36%. Gelucire® 43/01 is ideal for API protection and capsule filling. Matrix-former for protection of APIs sensitive to oxidation, humidity, or light. It is composed of PEG-esters, a small glyceride fraction and free PEG. It is a solid at ambient temperature making it suitable for capsule filling, melt granulation and extrusion. It is available in pellet form.[10]

Synonyms (USP/NF/JPE/EP): Hard fats

Preferred FDA Name: Fat, Hard

CAS Number: 157710-38-8

Melting point: 42-46°C

Category: Protective, Lubricant, 3D printing drug carrier, emulsifier, viscosity modifier, solubilizes.

HLB: 1-2

Drop point: 53-57°C

Mean particle size: 50 μ

Formulations & Processes: Melt processes: granulation and capsule molding. Topical lotions, emulsion and ointment.

Pharmacopoeial compliance: Yes

Solubility: It is hydrophobic grades and insoluble in water.

Applications: Diltiazem hydrochloride (melt granulation) as matrix carrier in multi-unit floating drug delivery system, Metoprolol succinate (melt granulation) in sustained release floating drug delivery system, Cefuroxime axetile (melt granulation filled in capsule) in enhancement of bioavailability, Famotidine in floating tablet, Metformin hydrochloride (melt granulation) in enhancement of bioavailability, Tramadol HCL mouth dissolving tablets for taste masking, and increase drug release on aging from matrices using Gelucire® 43/01. It is used as a lipid binder in melt techniques so that the physicochemical properties and plasticity of the lipid agglomerate offers high resistance to fracture, useful for flash melt and chewable tablets.[10]

Safety: Safety of use is supported by toxicological data and food additive status.

Storage: Store in cool and dry place in moisture resistant plastic containers.

5.3.2 Precirol ATO 5

A glyceride with an intermediate melting point that is utilised as a lubricant and flow aid in powder mixes for capsule filling, as well as a coating agent for flavour masking. It is an organic molecule classified as long-chain fatty acid. These are fatty acids having an aliphatic tail that comprises 13 to 21 carbon atoms. Stearic acid is also commonly used as a solid lipid in the manufacture of NLCs. Aadhunik Industries is India's leading manufacturer of Glyceryl Palmitostearate or Glycerol Palmitostearate, Speciality Chemicals, Pharmaceutical Excipients, Food Fragrance and Flavour chemicals. It is made via direct esterification of stearic and palmitic acids with glycerine in the absence of a catalyst. FDA recommended as excipient in non-parenteral formulations.[11,12]

Synonym: Glycerin Palmitostearate; Glycerol Palmitostearate; 2-[(1-oxo-hexadecyl)-oxyl]-pxy]-1,3-Propanediyl Dicotadecanoate and 1,2,3-Propane Triol; Glyceryl Distearate; Glyceryl Distearate (Type I); Precirol® ATO 5

CAS Number: 8067-32-1

CAS name: Precirol ATO 5

Pharmacopoeial Compliance: USP-NF; Ph.Eur

IUPAC Name: Hexadecanoic acid; octadecanoic acid; propane-1,2,3-triol

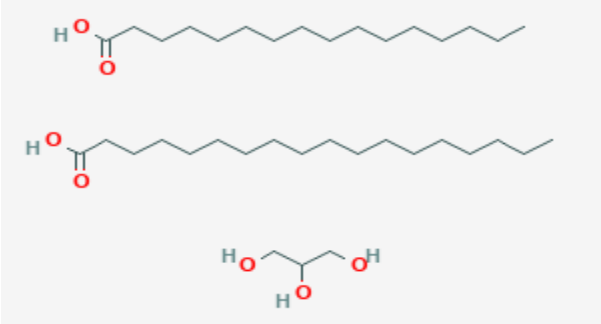


Fig. 5.2: Structure of Precirol ATO 5

Appearance: Fine white waxy powder.

Solubility: Freely soluble in chloroform and dichloromethane but practically insoluble in water, mineral oil and alcohol.

Category: Lubricant and flowing aid for capsules, coating for protection and taste masking, lipid matrix for sustained release and SLN/NLC, viscosity modification. It works as stabilizer, non-ionic emulsifier, emollient, gelling agent and as plasticizer in pharmaceutical formulations.

Molecular formula: $C_{37}H_{76}O_7$

Molecular weight: 633.0 g/mol

Melting point: 50-60 °C

Boiling point: 200°C

Drop point: 53-57°C

Flash point: 162 °C

Heavy metal: 10 ppm maximum.

Dosage forms: Oral and topical formulation

Particle size: About 50-60 µ

Hydrogen bond donor: 5

Hydrogen bond acceptor: 7

Covalently bonded unit: 3

Acid value: < 6

Iodine value: < 3

Peroxide value: < 3

Hydroxy value: 60-115

Saponification value: 175-195

Water content: < 1%

Free glycerine content: < 3%

1- Monoglyceride content: < 8-11%

Unsaponified matter: < 1%

Sulphated ash: < 0.1%

HLB: 1-2

Median lethal dose, LD_{50} (Rat oral dose): > 6 g/kg

GHS Hazards: Not classified. Food and Drug Administration (FDA) added to food substances (Document 184.1329)

Applications: In solid dosage forms as lubricant, matrix former in sustained release dosage forms using melt granulation or hot melt coating technique. It is used in immediate release formulation. It is used as polymer in microcapsules preparations and converting into tablet or capsule unit dosage form. It is used as biodegradable injectable gel.

Storage: It should be stored at temperature not exceeding 35 °C.

Containers: Air tight container, protected from light and moisture.

Incompatibilities: Ketoprofen and naproxen

5.3.3 Microcrystalline Cellulose (Avicel PH 101)

Synonyms: Avicel PH; Cellets; Celex; cellulose gel; hellulosum microcristallinum; Celphere; Ceolus KG; crystalline cellulose; E460; Emcocel Ethispheres; Fibrocel; MCC Sanaq; Pharmacel; Tabulose; Vivapur.

Molecular Formula: $(C_6H_{10}O_5)_n$, $n \approx 200$

Molecular weight: 36000g/mol

CAS Number: 9004-34-6

Structural Formula:

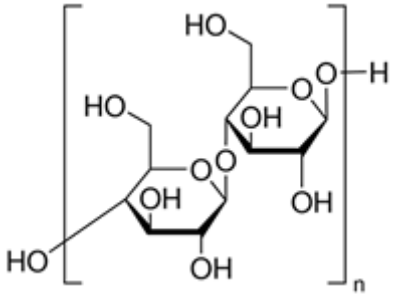


Fig. 5.3 : Structure of Microcrystalline Cellulose

Functional Category: Adsorbent; suspending agent; tablet and capsule diluent; tablet disintegrates.

Description: It is a purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles.

Solubility: Slightly soluble in 5% w/v sodium hydroxide solution; practically insoluble in water, dilute acids, and most organic solvents.

pH: pH 5.0-7.5

Melting point: Chars at 260–270°C.

Angle of repose: 33-49°

Bulk density: 0.337 g/ml

Tapped density: 0.478 g/ml

True density: 1.512- 1.668 g/ml

Moisture content: < 5%

Particle size: 20-200µ

Specific surface area: 0.78- 1.30 m^2/g

Incompatibilities: Microcrystalline cellulose is incompatible with strong oxidizing agents.

Stability & Storage condition: Microcrystalline cellulose is a stable though hygroscopic material. The bulk material should be stored in a well-closed container in a cool, dry place.

Applications: Microcrystalline cellulose is widely utilised in medicines, particularly as a binder/diluent in oral tablet and capsule formulations using both wet-granulation and direct-compression processes. In addition to its function as a binder/diluent, microcrystalline cellulose possesses lubricating and disintegrating qualities that make

it valuable in tableting. Microcrystalline cellulose is also utilised in cosmetics and culinary goods.[13]

Incompatibilities: It is incompatible with strong oxidizing agents.

Handling precaution: It may be irritant to the eyes. Use of gloves, eye protection aid, and dust mask is recommended.

Stability: Stable although it is hygroscopic material.

Storage: Store in well closed container in cool and dry place.

5.3.4 Spray Dried Lactose (DCL 11)[14]

Synonyms: Lactopress Spray-Dried, FlowLac 100, NF Lactose–316 Fast Flo, Pharmatose DCL 11, Pharmatose DCL 14, Super-Tab Spray-Dried, NF Lactose–315, Flowlac, Granulac, Microfine, Pharmatose, Prismalac, milk sugar, HMS, Sorbolac, Super-Tab, Tabletosse, Wyndale, Zeparox, Lactochem, Inhalac, Capsulac, Fastflo.

Chemical name: It is a mixture of α-and-β-lactose, and O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate. O-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose anhydrous

CAS registry number: 63-42-3 and 64044-51-5.

Molecular formula: $C_{12}H_{22}O_{11}$ and $C_{12}H_{22}O_{11}.H_2O$

Molecular weight: 342.30 (anhydrous) and 360.31 (monohydrate)

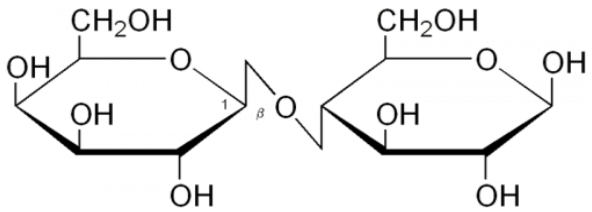


Fig. 5.4: Structure of Lactose

Category: Diluent or filler directly compressible tablet and capsule, binder. Diluent in dry powder inhaler.

Description: It is white or off-white crystalline odorless powder with sweet in taste. It is directly compressible and normally composed of 80–90% of α-lactose monohydrate and 10–20% of amorphous lactose. The α- lactose is about 15% sweet in comparison with sucrose. The β- lactose is sweeter than α- lactose.

Solubility: It is water-soluble, insoluble in ether and chloroform and having slight solubility in ethanol.

pH (10% solution): 4.5 to 7.5

Optical rotation: + 55.4 °

Melting point: 202–205 °C

Flash point: 358–360 °C

Bulk density: 0.57 – 0.62 g/ml

Tapped density: 0.67 – 0.78 g/ml

Moisture content: Maximum 6

Loss on drying: 0.3 – 0.6%

Osmolarity: 9.75% is osmolar with serum

Pharmaceutical applications: As a binder, filler-binder, spray-dried lactose is majorly preferred in direct compression of tablets.

Storage: Containers must be firmly sealed stored in a well-closed container in a cool, dry place.

Incompatibilities: Spray dried lactose is incompatible with agents with primary amino group, with amino acids, aminophylline, amphetamines, and also with lisinopril. Maillard reaction may occurs between lactose and these compounds to produce brown or yellow brown-coloured products.

Safety: Spray dried lactose is employed as diluents in oral solid medicaments. It is also preferred in parenteral. Lactose shows adverse reactions due to its intolerance, majorly in persons with deficient of enzyme lactase.

Handling precautions: Normal precautions as per the conditions and extent of material being handled need to be observed. During handling extreme creation of dust and inhalation of dust must be avoided.

5.3.5 Polyvinyl Pyrrolidone K-30[15]

Povidones are a family of water-soluble polymers based on N-vinylpyrrolidone that combine a unique set of properties for application in a wide variety of dosage forms. These are prepared by synthetic reaction. They contain linear chain of 1-vinyl 2-pyrrolidone with varying degree of polymerization. Based on polymerization molecular weight of polymer changes. The viscosity of these polymers is based on K-value, which is range from 10-120 corresponding to molecular weight range from 2,500 to 30,00,000. It manufactured by spray drying spherical form. They are commonly used as binders for the development of tablet formulations, whether

manufactured by wet granulation, dry granulation, or direct compression. The special grade pyrogen free povidone grades are available for parenteral preparation. These polymers are used in solid dispersion formulations to enhance the solubility of active pharmaceutical ingredients and increase bioavailability. Their various grades are also used to inhibit recrystallization in liquid soft gels. It was firstly used as plasma expander in 1940, now it was replaced by dextran. It is not absorbed by GIT and mucous membrane. It does not cause irritation to skin and mucous membrane and hence included in GRAS list.

Synonyms: Kollidone; Povidone; Plasdone; Poly[1-(2-oxo-1-pyrrolidinyl) ethylene];

Non-proprietary Names: BP, JP, USP: Povidone, and PhEur: Povidonum

Chemical Name: 1-Ethenyl-2-pyrrolidinone homopolymer

CAS Registry Number: 9003-39-8

Molecular formula: $(C_6H_9NO)_n$

Molecular weight: 50, 000

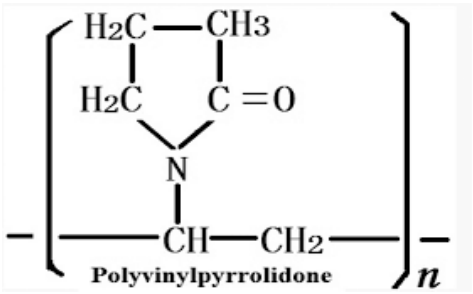


Fig. 5.5 : Structure of Polyvinyl Pyrrolidone

Description: Colourless to almost colourless, fine, white to creamy amorphous hygroscopic powder. It is pH stable and form transparent film.

Odour: Odourless

Melting point: 150° C

Residue on ignition: ≤ 0.05 %

Water content: ≤ 5.00 %

Sulphated ash: ≤ 0.10 %

pH of solution (5% at 25° C in water): 3.0-5.0

Viscosity of solution (5% at 25° C in water): 2.4 cP

K-value: 27-32.4

Residual monomer content: 0.8%

Peroxide content: ≤ 400 ppm

Hydrazine content: ≤ 1 ppm

Lead content (USP): ≤ 10 ppm

Nitrogen content: 11.5-12.8%

Bulk density: 0.29-0.39 g/ml

Tapped density: 0.39-0.54 g/ml

True density: 1.180 g/ml

Solubility: Freely soluble in acids, chloroform, ethanol Practically insoluble in ether. Soluble in water (0.5g/ 10 ml).

Functional category: Disintegrant; dissolution enhancer; emulsion stabilizer in creams & lotions, dispersant, suspending agent; tablet binder, viscosity modifier & coating agent.

Storage and Precautions: It darkens on heating at 150°C and reduce aqueous solubility. It is stable heat exposure around 110– 130°C for short cycle.

Incompatibilities: It forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital, tannin, and other compounds. Handling precautions: Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection, gloves, and a dust mask are recommended.

Regulatory status: Accepted as a food additive in Europe. Listed in the FDA Inactive Ingredients Database (IM and IV injections; ophthalmic preparations; oral capsules, drops, granules, suspension tablets; sublingual tablets; topical and vaginal preparations).

Applications: Used in adhesives, inks, glue stick, synthetic fiber and porous membrane manufacture. Dispersant in ceramics.

Storage: Store in tightly closed in a dry, cool and well-ventilated place. The containers should be resealed immediately after use and kept upright to prevent leakage.

Incompatibilities: The efficacy of preservatives like thiomersal was affected adversely by complexation when used with povidone.

Precautions: Avoid skin, and eye contact. Avoid inhalation of vapour or mist. Normal measures to be taken for preventive fire protection. Use protective aids.

Consult a physician in any case of following-

1. If inhaled or breathed in, move person into fresh air.
2. If not breathing, give artificial respiration.
3. In case of skin contact, wash off with soap and plenty of water.
4. In case of eye contact, rinse thoroughly with plenty of water for at least 15 minutes
5. If swallowed, never give anything by mouth to an unconscious person. Rinse mouth with water.

5.3.6 Alpha- Tocopherol[16]

Alpha-Tocopherol is a naturally occurring fat-soluble fatty molecule with varied levels of powerful antioxidant action, often known as vitamin E. It is an amphipathic chemical present in plant tissues, although it may also be produced synthetically. RRR-alpha-tocopherol acetate is a relatively stable version of vitamin E that is frequently employed as a food ingredient when necessary. It has the capacity to neutralise endogenous free radicals. Vitamin E naturally occurs in eight fat-soluble isoforms: α-, β-, γ-, and δ-tocopherol, as well as α-, β-, γ-, and δ-tocotrienol. Supplementing with α-tocopherol is the only way to correct symptoms of vitamin E insufficiency, as the body preferentially utilises it. Tocopherol additionally protects the skin from the sun's damaging UV radiation. It is used in a broad range of goods, including sunscreens and moisturisers, cosmetics, hair styling products, and more. It also acts as a preservative, keeping the items fresh. It is used as a moisturising and conditioning agent. It has anti-inflammatory qualities that moisturise and heal damaged, brittle hair. Tocopherol's antioxidant capabilities encourage healthy hair.

Synonym and trade name: Tocopherol; α-Tocopherol; Vitamin E; alpha-Tocopherol; Copherol Fl300; Vitamin E; all-rac- α-Tocopherol; DL-α-Tocopherol; α-Tocopherolum; E307; RRR-α-Tocopherol; Synthetic alpha Tocopherol; all-rac-α-Tocopherol;

Molecular formula: $C_{29}H_{50}O_2$

Molecular weight: 430.71

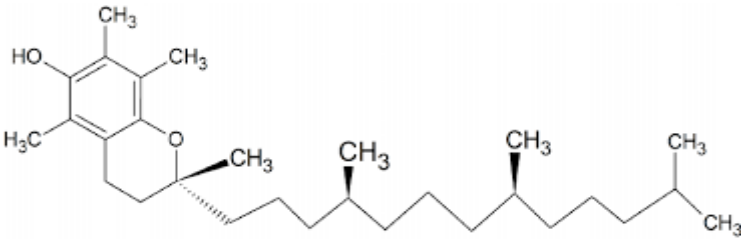


Fig. 5.6 : Structure of Alpha-tocopherol

IUPAC Name: 2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol, (±)-3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-2H-1 Benzopyran-6-ol; 5,7,8-trimethyltocol

CAS Number: 59-02-9

Category: Antioxidant; Fatting Agent; and Therapeutic Agent

Description: Alpha Tocopherol is supplied as a clear, colourless or yellowish-brown, viscous oily liquid.

Solubility: Soluble in DMSO (100 mg/ mL) and ethanol (100 mg/ mL) require ultrasonication. Practically insoluble in water. Freely soluble in ethanol, ether, acetone, and vegetable oils.

Melting point: 2.5-3.5°C

Boiling point: 200-220°C

Acid value: ≤ 2

Optical rotation: -0.01- + 0.01°

Heavy metals: < 20 ppm

Refractive index: 1.503-1.507

Specific gravity: 0.947-0.955

Density: 0.947-0.955 g/ml

Flash point: 240°C

Indications: Vitamin E, known for its antioxidant effects, has been demonstrated to protect against cardiovascular disease and some forms of cancer while also boosting the immune system. It may be of some use to persons with asthma or rheumatoid arthritis. It may help with a variety of neurological diseases, including Alzheimer's, ocular issues including cataracts, diabetes, and premenstrual syndrome. It may also help protect skin from UV rays, however claims that it reverses skin ageing, increases male fertility, and enhances exercise performance are unfounded. It may help relieve

muscle cramps. Because of vitamin E's biologic action, there is ongoing interest and study into whether its antioxidant characteristics may be used to help prevent or treat a range of conditions, including cardiovascular disease, eye issues, diabetes, cancer, and others. However, there is presently insufficient official data and evidence to suggest any novel uses for vitamin E.

Interactions: Orlistat may limit the absorption of D-alpha-Tocopherol acetate, resulting in a lower serum concentration and perhaps a reduction in effectiveness. A variety of cholesterol-lowering medicines (such as cholestyramine and colestipol), as well as orlistat, sucralfate, mineral oil, and the fat replacement olestra, which inhibit fat absorption, may theoretically reduce the absorption of fat-soluble vitamins, including vitamin E.

Storage: Below 4°C, protect from light, stored under inert environment

Shelf life: Below -20°C as powder for 3 years, below 4°C as powder, 2 years, in solvent below -80°C for 6 months, in solvent below -20°C for 1 month. Avoid freeze-thaw cycle.

Incompatibilities: Incompatible with metal ion and peroxides. It is absorbed by plastics.

5.3.7 Magnesium Stearate[17]

Molecular Formula: $[CH_3 (CH_2)_{16}COO]$ 2Mgs

Synonyms: Dibasic magnesium stearate; Magnesium Distearate

Structural formula:

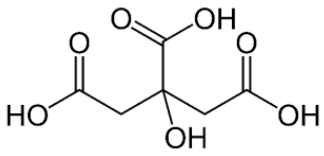


Fig. 5.7 : Structure of Magnesium Stearate

Synonyms: Dibasic magnesium stearate; Magnesium distearate; Magnesiistearas; Magnesium octadecanoate; Octadecanoic acid, magnesium salt; Stearic acid; Magnesium salt; Synpro90.

Empirical formula: $C_{36}H_{70}MgO_4$

Molecular weight: 591.24 g/mol

Description: It is a very fine, light white, precipitated or milled powder with a low bulk density, a mild stearic acid odour, and a distinct flavour. The powder feels oily to the touch and easily sticks to the skin.

Functional categories: Tablet and capsule lubricant.

Solubility: Practically insoluble in ethanol, ethanol (95%), ether and water; slightly soluble in warm benzene and warm ethanol (95%).

Melting point: 117–150˚C

Density: 1.092 g/cm^3

Loss on drying: 46.0%

Stability and storage conditions: Magnesium stearate is stable and should be stored in a well-closed container in a cool, dry place.

Incompatibilities: Incompatible with strong acids, alkalis, and iron salts. Avoid mixing with strong oxidizing materials.

Applications: It is primarily used as a lubricant in capsule and tablet manufacture.

5.3.8 Talc[18]

Non-proprietary names:

BP: Purified talc

JP and USP: Talc

Synonyms: Magsilosmanthus; Magsil Star; Powdered talc; Purified French chalk; Purtalc.

Empirical formula: $Mg_6 (Si_2O_5)_4(OH)_4$

Molecular Formula: $Mg_6 (Si_2O_5)_4(OH)_4$

Structure:

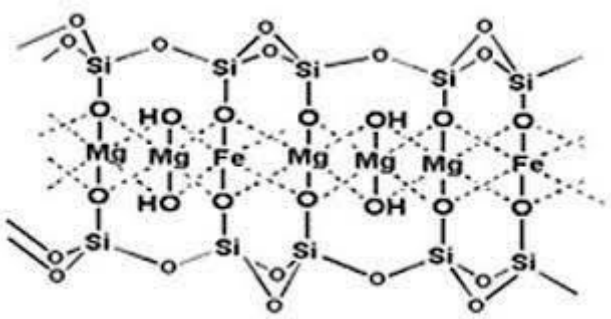


Fig. 5.8 : Structure of Talc

Description: Talc is a very fine; white to greyish-white, odourless, impalpable, unctuous, Crystalline powder. It adheres readily to the skin and is soft to the touch and free from grittiness.

Functional categories: Anticaking agent; glidant; tablet and capsule diluent; tablet and capsule lubricant.

Solubility: Practically insoluble in dilute acids and alkalis, organic solvents, and water.

Melting point: 150˚C

Handling Precautions: Talc is irritant if inhaled and prolonged excessive exposure may cause Pneumoconiosis. Eye protection, gloves and respirator is recommended.

Stability and storage conditions: Talc is a stable material and may be sterilized by heating at 160°C for not less than 1 hour. It may also be sterilized by exposure to ethylene oxide or gamma irradiation. Talc should be stored in a well-closed container in a cool, dry place.

Incompatibilities: Incompatible with quaternary ammonium compounds.

Applications:

1. It is used as a diluent, lubricant in tablet formulations.
2. In a novel powder coating for extended-release pellets and as an adsorbent.
3. In topical preparations, it is used as a dusting powder, used to clarify liquids.
4. It is also used in cosmetics and food products.

5.3.9 Crospovidone (CP)[19]

Non-proprietary Names

BP: Crospovidone

Ph Eur: Crospovidonum

USP/NF: Crospovidone

Synonyms: Crosslinked povidone; Kollidon CL; E1202; Kollidon CL-M; 1-vinyl-2-pyrrolidinone homopolymer; Polyplasdone XL.

Chemical Name: 1-Ethenyl-2-pyrrolidinone homopolymer

Empirical Formula: $(C_6H_9NO)_n$

Molecular weight: >1 000 000

Structural Formula

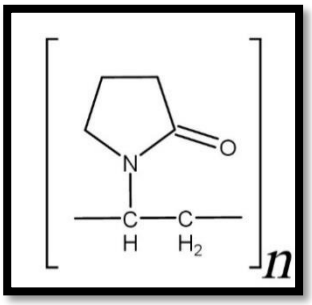


Fig. 5.9 : Structure of Crospovidone

Functional Category: Tablet disintegrant.

Description: Crospovidone is a hygroscopic powder that is white to creamy-white, free-flowing, almost tasteless, odorless or nearly odorless.

Typical properties

Acidity/alkalinity: pH 5.0–8.0 (aqueous slurry of 1% w/v)

Density: 1.22 g/cm^3

Solubility: In water and most typical organic solvents are almost insoluble.

Applications in pharmaceutical preparation or technology

Crospovidone is a water-insoluble tablet disintegrant and dissolving agent that is used at a rate of 2-5 percent in tablets manufactured by direct compression or wet and dry granulation. It has a strong capillary action and a high hydration capacity, with a low tendency to create gels. According to study, the crystal structure of crospovidone influences the breakdown of analgesic tablets. Larger molecules dissolve more quickly than smaller ones. Crospovidone can be used as a solubility enhancer. Crospovidone can be used in the co-evaporation method to increase the solubility of weakly water-soluble medications. In the presence of enough solvent, the medication is deposited on crospovidone, which is subsequently vaporised. This approach produces a faster rate of disintegration.

Storage Conditions and Stabilization: Crospovidone should be kept in a tightly sealed container in a cold, dry location since it is hygroscopic.

Incompatibilities: Most organic and inorganic medicinal compounds are compatible with crospovidone. When subjected to a specific water level, crospovidone may form molecular intermediates with some compounds.

Safety: Crospovidone is a safe and non-irritant substance that is utilised in oral medicinal formulations. Short-term animal toxicology tests have revealed no negative impacts associated with crospovidone.

Handling Precautions: Follow standard safety procedures that are suitable for the conditions and amount of material being handled. Eye protection, gloves, and a dust mask are recommended.

5.3.10 Silicified Microcrystalline Cellulose (PROSOLV)[20]

Non-proprietary Names

Synonyms: Silicified Microcrystalline Cellulose; SMCC; Microcrystalline Cellulose, Silicified; Comprecel® SMCC; PROSOLV® SMCC; Avicel® SMCC

Empirical Formula: (C6H10O5)n

Molecular weight: Approx. 36 000

Structural Formula

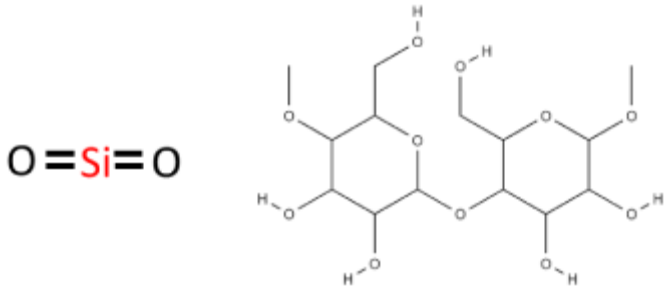


Fig. 5.10 : Structure of Silicified Microcrystalline Cellulose

Functional Category: Filler, Cushioning agent.

Description: It is a co-processed pharmaceutical excipient made up of two functional excipients: microcrystalline cellulose and colloidal silicon dioxide. The two component materials are not covalently bonded but quite stable.

Typical properties

Appearance: White fibrous powder with high flowability

pH value: 5.0-7.5 (10% w/v aqueous suspension)

Density: 1.58 g/cm

Applications in pharmaceutical preparation or technology

Silicified MCC has superior flowability and lower cohesion than non-silicified Microcrystalline cellulose grades. In pharmaceutical goods, silicified microcrystalline

cellulose is used as a filler-diluent in direct compression or capsule filling procedures. It can also be added to wet granulated powder mixtures (extra-granularly) to improve formulation compaction qualities, especially in cases when normal Microcrystalline cellulose is ineffective.

Storage Conditions and Stabilization: Should be kept in a tightly sealed container in a cold, dry location since it is hygroscopic.

Incompatibilities: Most organic and inorganic medicinal compounds are compatible

Safety: It is a safe and non-irritant substance that is utilised in oral medicinal formulations. Short-term animal toxicology tests have revealed no negative impacts associated with silicified MCC.

Handling Precautions: Follow standard safety procedures that are suitable for the conditions and amount of material being handled. Eye protection, gloves, and a dust mask are recommended.

5.3.11 Mannitol [21]

Non-proprietary Names

Synonyms: D-mannitol

Chemical Name: (2R,3R,4R,5R)-hexane-1,2,3,4,5,6-hexol

Empirical Formula: C6H14O6

Molecular weight: 182.17 g/mol

Structural Formula

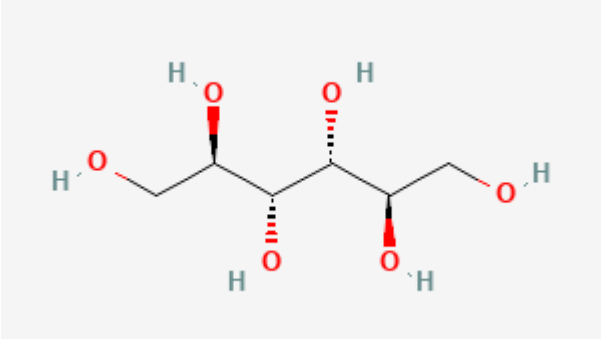


Fig. 5.11 : Structure of Mannitol

Functional Category: Filler

Description: white odourless crystalline solid having a sweet taste

Typical properties

Appearance: White powder

Flowability: Highly flowable

Density: 1.52 g/cm

Applications in pharmaceutical preparation or technology

Mannitol is generally used in pharmaceutical formulations and offers several benefits. It is known to contribute to the overall efficiency and safety of medications. Here are some key benefits of Mannitol in pharmaceutical formulations:

Enhanced Stability and Shelf Life: Mannitol is highly stable, making it an appropriate excipient for medicinal formulations. Its capacity to resist crystallisation contributes to the stability of medicinal formulations and guarantees that the product's quality remains consistent throughout time. This improved stability also helps to lengthen shelf life. As a result, it decreases the possibility of deterioration and guarantees that the drug stays effective for the prescribed storage term.

Osmotic Properties for Drug Delivery: Mannitol's osmotic qualities make it useful in medication delivery systems. It is often utilised in osmotic-controlled release formulations, which allow for regulated medication release over time. Mannitol's osmotic pressure may be modified to provide various release patterns, resulting in regulated and sustained distribution of the active pharmaceutical ingredient (API).

Compatibility with Active Ingredients: Mannitol is well-known for being compatible with a wide range of active medicinal substances. Its inert properties and lack of reactivity with diverse medicinal components make it an appealing choice as a filler or diluent in pharmaceutical formulation. This compatibility means that Mannitol does not interfere with the chemical stability or efficacy of the active components, hence contributing to the medication's overall trustworthiness.

Patient Safety and Tolerability: Mannitol is typically well tolerated by patients, with very few adverse events reported. Its safety profile makes it appropriate for use in formulations designed for a variety of patient groups. Mannitol is often used in oral dose forms including pills and capsules. Here, its sweetness improves the medication's palatability. Mannitol's low caloric content and non-cariogenic characteristics make it an ideal ingredient for sugar-free recipes. As a result, it caters to the demands of diabetics and those on a tight diet.

PART B

5.4 Drug Profile: Sitagliptin Phosphate Monohydrate[1-5]

Sitagliptin phosphate is used type II diabetes in which body cannot produce sufficient insulin or insulin produced cannot work appropriately. It is prescribed to patient who doing regular exercise and intake-controlled diet regularly. It increases the production of insulin and decrease glucagon preparation in patient body. It is available in market in tablet form alone or in combination with metformin or ertugliflozin. It is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor used for the management of type 2 diabetes mellitus. It is FDA approved drug (16th October 2006).

Insulin is a molecule generated by the human body that helps remove sugar from the bloodstream and transport it to cells, where it may be utilised for energy. Incretins are hormones in the body that control insulin synthesis and release. Sitagliptin works by keeping incretin hormones from breaking down too rapidly. This improves insulin sensitivity and decreases blood sugar. Controlling high blood sugar levels helps avoid kidney disease, blindness, nerve difficulties, limb loss, and sexual dysfunction.

Category: Antidiabetic Agents

Mechanism of Action: Sitagliptin inhibits DPP-4, which delays the inactivation of incretins such as GLP-1 and GIP. Incretins are produced throughout the day and increased in response to meals to maintain glucose homeostasis. Reduced inhibition of incretins boosts insulin production and decreases glucagon release in a glucose concentration-dependent way. These effects result in improved blood glucose management, as seen by lower glycosylated haemoglobin (HbA1c).

Groups: Approved, Investigational

BCS Class: BSC Class III (low solubility and high permeability)

Mode of action: Inhibits DPP-4, increasing levels of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)

Molecular weight: 407.11

Molecular formula: $C_{16}H_{15}F_6N_5O$

CAS Number: 486460-32-6

IUPAC Name: (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4] triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one

IUPAC Name: (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4] triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one

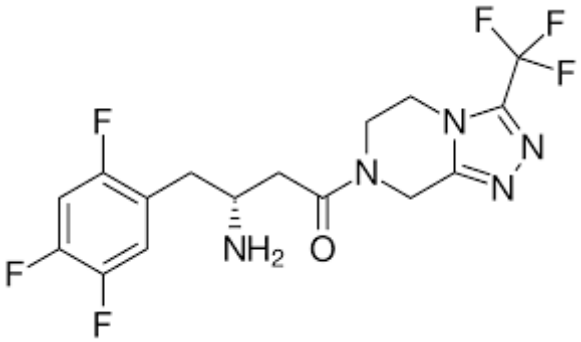


Fig. 5.12: Structure of Sitagliptin

Description: White to off-white crystalline non-hygroscopic solid with distinct odour and bitter taste

Solubility: Soluble in water and DMSO, slightly soluble in methanol and very slightly soluble in acetone, acetonitrile and ethanol. Insoluble in isopropanol and isopropyl acetate.

Water content: NMT 0.5%

Melting point: Melting point of Sitagliptin base, Sitagliptin phosphate monohydrate and Sitagliptin phosphate anhydrous are 120.29°C, 206.37°C and 214.92°C respectively.

Dissociation constant (pKa): 8.78

Nature: It is basic compound can accept H+ ion and became positively charged. Due to presence tertiary amine, it is categories under weak acid.

Partition coefficient: 1.8 for octanol/ water system

Peak time: 2 hr

Half-life: 11-12 hr

Drug absorption: Drug absorbed throughout intestine

Oral bioavailability: Before or after meal 87%

Volume of distribution: 198 L

Protein binding: 38%

Excretion: Approximately 79% of the dosage was eliminated unaltered via urine. Minor metabolites produced by CYP P450, CYP 3A4, and, to a lesser extent, CYP 2C8. Sitagliptin is a substrate for the human organic anion transporter-3 (hOAT-3),

which may play a role in its renal clearance. The clinical significance of hOAT-3 in sitagliptin transport has not been determined. Sitagliptin is also a p-glycoprotein substrate, which may play a role in sitagliptin's renal clearance. Cyclosporine, a p-glycoprotein inhibitor, did not alter sitagliptin's renal clearance.

Elimination: 87% of administered dose excreted through urine and remaining through faces.

Clearance: 350 ml/ min

Safety and efficacy: Safe to use throughout pregnancy and breastfeeding. There is no safety and efficacy data for paediatrics. There was no difference in reported safety or efficacy data when comparing to the elderly. Patient reaction did not vary significantly by gender, age, race, ethnicity, or BMI. When prescribing to a patient with renal impairment, use caution.

Side effects: Stomach upset and pain, diarrhoea, vomiting, upper respiratory infection, runny nose, sore throat, headache, lower sugar, skin rashes, swelling, hiving, etc.

Indication: Indicated as an adjunct to diet and exercise to improve glycaemic control in adults with type 2 diabetes mellitus. It is not used to treat type 1 diabetes or patients with a history of pancreatitis.

Warning: It increases the risk of pancreatitis and joint pain. If patient with gall stone, alcoholism, kidney issues, pancreatitis, smoking and high triglyceride levels should inform the patient before starting treatment.

Dose and Dosage form: Tablets of 25, 50 and 100 mg. Typical dose 100 mg once daily.

Storage: Store at temperature between 20-25°C. Protect from light and moisture. Stable if stored as directed under conditions directed. Avoid storage with oxidizing agents.

Stability: Sitagliptin phosphate monohydrate and Sitagliptin phosphate anhydrous are stable than Sitagliptin base.

5.5 Risk Assessment for Drug Substance Attributes (SPM)

A risk assessment of the drug substance attributes was performed to evaluate the impact that each attribute could have on the drug product Critical Quality Attributes. The outcome of the assessment and the accompanying justification is provided as a summary below. The relative risk that each attribute presents was ranked as high, medium or low. The high-risk attributes warranted further investigation whereas the low-risk attributes required no further investigation. The medium risk is considered acceptable based on current knowledge. Further investigation for medium risk may be needed in order to reduce the risk.[6]

Based upon the physicochemical properties of the drug substance, the risk assessment of drug substance attributes that impact the drug product CQAs is given below.[7]

Table 5.8 : Risk Assessment of the Drug Substance Attributes for SPM

Drug product CQA	Sitagliptin drug substance attributes				
	Particle size	Solubility	Melting point	Water content	Flow properties
Moisture uptake	Low	Low	Low	Medium	Low
Drug Content	Low	Low	Low	Medium	Low
Dissolution	Low	Low	Low	Low	Low

Table 5.9: Justification of Risk Assessment of Drug Substance Attributes of SPM

Drug Substance Attributes	Drug Product CQAs	Justification
Particle size	Moisture uptake	Low risk as Particle size has no direct impact on Moisture uptake. The API has good flow characteristics, thus does not have any impact on drug content of final product. This is BCS class III drug thus particle size has no impact on dissolution.
	Drug Content	
	Dissolution	
Solubility	Moisture uptake	Low risk as solubility has no direct impact on

Drug Substance Attributes	Drug Product CQAs	Justification
	Drug Content	Moisture uptake and drug content. This is BCS class I drug thus particle size has no impact on dissolution.
	Dissolution	
Melting point	Moisture uptake	Low risk as Melting point has no direct impact on Moisture uptake, drug content and dissolution
	Drug Content	
	Dissolution	
Water content	Moisture uptake	Medium Risk as Sitagliptin is affected by moisture and leads to degradation at higher rate. API has controlled water content about NMT 0.5%.
	Drug Content	
	Dissolution	Low risk as Water content has no direct impact on dissolution
Flow Properties	Moisture uptake	Low risk as Melting point has no direct impact on Moisture uptake, drug content and dissolution
	Drug Content	
	Dissolution	

5.6 Coating Agent Profile:

5.6.1 Palmitic Acid[8]

The majority of animal and vegetable fats include palmitic acid (35 to 45% of palm oil). It is the most abundant fatty acid found in animals, plants, and microbes. It is the primary component of palm tree oil (palm oil, palm kernel oil, and coconut oil), although it may also be found in meat, cheese, butter, and dairy products. It is composed of sixteen carbon atoms and contains long-chain saturated fatty acids. It is biochemically safe for use in food labelling and dietary advice. Excess carbs in the body are turned into palmitic acid. It is the first fatty acid created during fatty acid synthesis and serves as a precursor for longer fatty acids. Palmitate has a negative

feedback loop with acetyl CoA carboxylase (ACC), which converts acetyl-CoA to malonyl-CoA, which is then added to the expanding acyl chain, limiting further palmitate production.

Palmitoylation is a process that involves adding a palmitoyl group to some proteins. Palmitoylation plays a key role in the membrane localisation of several proteins. Palmitic acid is produced by processing fats and oils with water at high pressure and temperature (over 200°C), resulting in the hydrolysis of triglycerides. The combined mixture is then distilled.

Synonym: n-Hexadecanoic acid, 1-Pentadecanecarboxylic acid, Cetylic acid, Hexadecylic acid, palmitate, Palmitinic acid, palmitinsaeure, cetyl acid, n-hexadecoate, pentadecane carboxylate, 1-hexyldecocanoate, n-hexadecoate, palmic acid, Acid, palmitic

Occurrence: Animals, plants and microorganisms

Dietary sources: Meat, dairy products, palm oil, coconut oil, and breast milk

Chemical name: Palmitic acid

Molecular formula: CH3 (CH2)14COOH

Molecular weight: 256.42

Description: White or off-white, odourless or slight characteristic odour and oily taste large crystalline powder

Melting point: 59 to 63°C

Boiling point: 351.5°C

Acid value: 217 to 220

Iodine value: NMT 1.5

Saponification value: 208 to 222

Refractive index (nD80) = 1.4 to 1.6

Log P: 6.4

CD ratio: 16

Specific gravity: 0.849 to 0.851

Density: 0.8527 g/ml

Specific surface area: 0.51 to 0.53 m2/g

Polar surface area: 37.3 $A^{\circ 2}$

Viscosity: 7.80 mPa.sec (cP) at 62°C

pH: 2.7

Solubility: It is insoluble in water, freely soluble in chloroform, ether, isopropyl alcohol, hot ethanol (95%). It is soluble in amyl acetate, carbon tetrachloride, ethanol, acetone, and benzene. Miscible with diethyl ether.

Distribution coefficient: log (oil/water) = 8.6

Applications: Palmitic acid is commonly utilised as a lubricant and addition in industrial processes. It is used to produce metallic stearates, medicines, soaps, cosmetics, and food packaging. It functions as a softener, accelerator, activator, and dispersion agent in rubbers. It functions as a coating agent in modified release formulations. It is utilised as an emollient and emulsifier.

Physiological role: Crucial for membrane physical properties, protein palmitoylation, and surfactant activity

Stability: Palmitic acid is stable under ordinary conditions. Anti-oxidant may be added in the container.

Storage: Store in a tightly closed container in a cool, dry and well-ventilated area.

Keep away from sources of ignition and incompatible substances.

5.6.2 Stearic Acid[8,9]

Stearic acid is an eighteen-carbon, long-chain saturated fatty acid. It is biochemically safe for use in food labelling and dietary guidelines. However, studies from over 30 years ago shows that, in terms of diet and heart disease concerns involving saturated fatty acids, stearic acid may act differently than other saturated fatty acids found in considerable quantities in the diet. The predominant long-chain saturated fatty acids in the diet include lauric (C12), myristic (C14), palmitic (C16), and stearic (C18).

Stearic acid is found in a variety of foods, including meat and fat-containing dairy products. Stearic acid, as a proportion of total fat calories, is relatively consistent in beef, hog, lamb, and veal at 9 to 12%, whereas poultry has a lower percentage at 6 to 7%. Common cooking oils contain just 2-4% stearic acid, while hydrogenation of vegetable oils for shortening and margarine can raise the percentage. Of all

commercially available fats, cocoa butter has the highest amount of stearic acid. Stearic acid accounts for around 3 to 4% of total calories in the United States diet.

Occurrence: Found in many animal and vegetable fats

Dietary sources: Meat, poultry, fish, eggs, dairy products, fats, beef tallow, lard, butterfat, cocoa butter, and shea butter

Benefits: Moisturizing and anti-inflammatory properties

Synonym: Octadecanoic acid, 1-Heptadecane carboxylic acid, Stearophanic acid, n-Octadecanoic acid

Chemical name: Stearic acid

Molecular formula: $C_{18}H_{36}O_2$

Molecular weight: 284.48

Description: White to faintly yellowish glossy crystalline solid or white to yellowish white powder with slight tallow-like taste.

Melting point: 68 to 70°C

Acid value: 200 to 212

Iodine value: Less than 4

Saponification value: 200 to 220

Refractive index (n_D^{80}) = 1.4299

Specific gravity: 0.940 to 0.941 (Water = 1)

Specific surface area: 0.51 to 0.53 m^2/g

Density: 0.847 g/cm^3 (20°C)

Boiling point: 232°C (450°F; 505 K) at 15 mmHg

Solubility: It is easily soluble in benzene, diethyl ether, acetone, chloroform, carbon disulfide, carbon tetrachloride, amyl acetate, hexane, propylene glycol and toluene. It is insoluble in cold water, hot water, and slightly soluble in ethanol.

Distribution coefficient: The product is more soluble in oil and log (oil/water) = 8.2.

Applications: Its applications include food, medicines, and cosmetics. It is widely utilised as a lubricant, binder, and coating agent. It serves as a medication carrier with prolonged release. Stearic acid is commonly employed as an emulsifying and solubilising agent. It also serves as a hardening agent in glycerin suppositories.

Precautions: Do not ingest or breathe dust

Incompatibilities: It is not compatible with most metal hydroxides or oxidising agents. Many metals react to generate insoluble stearates; when combined with calcium and zinc salts, ointment base becomes lumpy. The compatibility of stearic acid with medicines was examined using Differential Scanning Calorimetry (DSC). Stearic acid induces pitting in aqueous tablet film coating, which is determined by its melting point.

Stability: Stearic acid is stable under ordinary conditions. Anti-oxidant may be added in the container.

Storage: Store in tightly closed container in a cool, dry and well-ventilated area. Keep away from sources of ignition.

5.7 Excipient Profile:

5.7.1 Dicalcium Phosphate[10, 11,12]

Dicalcium phosphate dibasic (DP) is an inorganic, insoluble diluent used in tablet and capsule production. In pharmaceutical development, two hydration forms of dicalcium phosphate are used: anhydrous (DPA) and dihydrate. The anhydrous form is a triclinic crystal, whereas the dihydrate generates a monoclinic structure. Dicalcium phosphate dihydrate has excellent flow characteristics and minimal hygroscopicity. However, depending on temperature (40-50 °C) and humidity (32-75% relative humidity), it tends to lose water of hydration, which may induce chemical instability of APIs in dosage forms. The anhydrous version offers an option without sacrificing medication stability.

The two forms have distinct porosities due to their hydration. Because there is no water in the crystal structure, the anhydrous form has a larger porosity, allowing for better compressibility and quicker disintegration. It is chemically made up of calcium ions (Ca^{2+}) and phosphate anions in a 1:1 molar ratio. Dibasic calcium phosphate is one of a family of eleven mineral compounds known as calcium phosphate. They have considerable uses in the medical, geological, construction, and dentistry industries. The materials created vary in function, composition, structure, physical qualities, and applications depending on the manufacturing processes used.

In the pharmaceutical industry, the three most significant minerals from this family are dibasic calcium phosphate anhydrous, dibasic calcium phosphate dihydrate, and

tricalcium phosphate, which are employed as diluents and fillers in solid dosage formulation. Dibasic calcium phosphate dihydrate has the empirical formula $CaHPO_4.2H_2O$ and so occurs as a dihydrate. The "di" prefix in the popular name comes from the fact that the HPO_4^{2-} anion is formed by removing two protons from phosphoric acid (H_3PO_4).

Synonyms: Dibasic calcium phosphate, calcium monohydrogen phosphate, monohydrogen calcium phosphate, phosphoric acid calcium salt, calcium phosphate, calcium phosphate dibasic Dibasic Calcium Phosphate Hydrate; Calcium Hydrogen Phosphate; Calcium Hydrogen Phosphate Dihydrate; Calcium Hydrogen Orthophosphate dihydrate; Calcium Monohydrogen Phosphate Dihydrate; Di-Cafos; Dicalcium Orthophosphate; DI-TAB; E341; Emcompress.

Pharmacopoeial Compliance: USP-NF; Ph. Eur; IP; J.P; FCC

Molecular Formula: $CaHPO_4$

Molecular weight: 172.09 g/mol (dihydrate), 136.057 g/mol (anhydrous)

CAS Number: 7757-93-9 (Calcium hydrogen phosphate) and 7789-77-7 (dicalcium phosphate)

EC Number: 231-826-1

UNII Code: O7TSZ97GEP

Structural Formula:

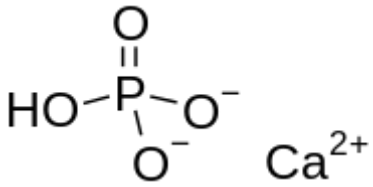


Fig. 5.13: Structure of Dicalcium Phosphate

IUPAC Name: Calcium hydrogen phosphate

Functional Category: Tablet and capsule diluent. Aid in tooth paste as polishing agent.

Description: White triclinic crystalline odourless taste less powder.

Solubility: Insoluble below pH 4.8 (dihydrate and anhydrous forms).

In water 0.02 g/100 mL (anhydrous) and 0.02 g/100 mL (dihydrate)

Practically insoluble in ethanol, ether, and water; soluble in dilute acids.

Structure: Insoluble below pH 4.8 (dihydrate and anhydrous forms)

Angle of repose: 28.3°

pH: pH 12.3-12.5

Melting point: 128°C.

Angle of repose: 33-49°

Bulk density: 0.915 g/ml

Tapped density: 1.17 g/ml

True density: 2.929 g/cm^3 (anhydrous); 2.31 g/cm^3 (dihydrate)

Flash point: Standard state at 25°C, 77°F, and 100 kPa (Non- inflammable)

Specific surface area: 0.44 – 0.46 m^2/g

Moisture content: < 5%

Loss on ignition: 24.5 -26.5%

Assay: 98.0 -105.0% (≥ 98.0%)

Regulatory Status: Dibasic calcium phosphate dihydrate is a permitted pharmaceutical excipient and food ingredient in Europe. It is currently registered with the USP-NF, Ph.Eur, and JP. It is also GRAS-listed and appears in the FDA Inactive Ingredients Database (for oral capsules and tablets). Dibasic calcium phosphate dihydrate is widely used in oral pharmaceuticals, food items, and toothpastes because to its acceptance as a reasonably harmless and non-irritant substance.

Stability & Storage condition: Microcrystalline cellulose is a stable though hygroscopic material. The bulk material should be stored in a well-closed container in a cool, dry place.

Applications: Dibasic calcium phosphate dehydrate is used in medicinal formulations because to its superior compactability and flow characteristics. Brittle fracture is the primary deformation mechanism of coarse grades, which considerably lowers strain-rate sensitivity and the potential for tablets to laminate. This is extremely useful during formulation scale-up in new product development, which is less difficult. The sole disadvantage is that dibasic calcium phosphate dihydrate is abrasive, significantly reducing tooling life. As a result, significant quantities of lubricants are necessary in formulations (often greater than 1% w/w for magnesium stearate or sodium stearyl fumarate).

Commercial grades of Dibasic calcium phosphate dihydrate include a milled grade that is commonly used in wet-granulation or roller-compaction, as well as a coarse grade designed for direct-compression applications. Dibasic calcium phosphate dihydrate does not absorb moisture from the environment and remains stable under normal settings. However, if the material is exposed to certain circumstances (high temperature and humidity), it may lose its water of crystallisation. This phenomena has the potential to affect high-temperature operations (such as packing and aqueous film coating) as well as the majority of moisture-sensitive actives.

Incompatibilities: It is incompatible with chelating agents

Handling precaution: Workers should take the necessary measures when working with Dibasic calcium phosphate dihydrate, taking into account the conditions and quantity of material involved. Because the milling grades might produce dust, a respirator or dust mask is required. It may cause irritation to the eyes. Gloves, eye protection, and a dust mask are suggested.

Stability and Storage: Dibasic calcium phosphate dihydrate is a non-hygroscopic, reasonably stable compound. However, if kept or treated wrongly, it might lose its water of crystallisation, affecting the excipient's stability and future dosage form processing. As a result, the bulk material should be properly kept (in a well sealed container in a cold, dry location away from direct heat or moisture).

5.7.2 Colloidal Silicon Dioxide (Aerosil)[13]

Aerosil(R) 200, also known as fumed silica, is used as an anticaking agent in powders and a thickening agent in solutions. Ungraded products supplied in a grade suitable for general industrial use or research purposes and typically are not suitable for human consumption or therapeutic use.

CAS Number: 7631-86-9

EC Number: 231-545-4

Molecular weight: 60.08

Molecular formula: SiO_2

Structural formula:

O = Si = O

Description: White odorless and tasteless crystalline as well as amorphous solid

Solubility: Almost insoluble in water, common acid, can be dissolved in hydrofluoric acid to generate silicon fluoride gas, slowly with the heat of concentrated phosphoric acid.

Melting point: 1,700 °C

Solubility in Water: > 1 mg/l

Density: Approximate 2.2 g/cm^3 (20 °C)

Specific surface area: 450m^2/g

Loss on drying: Less than 1.5% when sample dried at 105 °C

Weight loss on ignition: Not more than 8.5%

SiO2 content based on ignition: >99.8%

Stability: Stable under recommended storage conditions. The physical and chemical properties are stable, easy to form, inert, the melt is layered, and the expansion coefficient is small when heated.

Applications: Pharmaceutical excipients, glidants and suspending agents.

Precaution: Avoid contact with skin and eyes.

Shelf life: 5 years

5.7.3 Polyethylene Glycol 4000[14]

Polyethylene glycol, or PEG, is a hydrophilic polymer. It is easily synthesised from ethylene oxide via anionic ring opening polymerisation into a wide range of molecular weights and end groups. When PEG is crosslinked into networks, it can have a high water content and produce "hydrogels". PEG is an ideal substance for biological applications since it does not elicit an immune response.

Synonyms: Carbowaxes.

IUPAC Name: Polyethylene glycol

CAS Number: 25322-68-3

Chemical Formula: $C_{2n}H_{4n+2}O_{n+1}$, n = 91 units

Chemical Name: Alpha-Hydro-omega-hydroxypoly(oxy-1,2-ethanediyl)

Molecular weight: 4000 (3100-4010)

Structural Formula

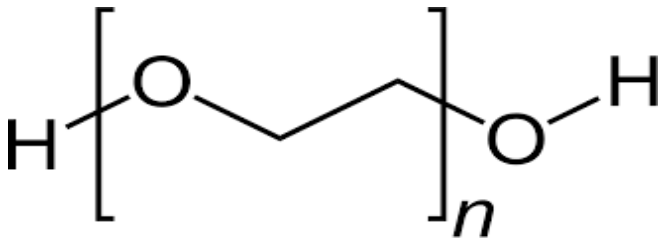


Fig 5.14: Structure of Polyethylene Glycol 4000

Description: White, odourless and slightly sweet flakes or powdered solid

Molar Mass: average molecular weight is about 4,000

Density: 1. 2 g/cm^3

Solubility: Soluble in water, acetone, alcohols, benzene, glycerin, glycols and aromatic hydrocarbons and slightly soluble in aliphatic hydrocarbons.

Melting Point: 53-58°C

pH: 5 – 7

Moisture content: NMT 1%

Flash Point: 138.6°C

Viscosity: 90.0 cSt at 25 °C, 7.3 cSt at 99 °C

Specific gravity: 1.11

Chemical Properties:

1. It is strongly hydrophilic.
2. The partition coefficient of PEG 400 between Hexane and Water is 0.00015.

Uses:

1. Pharmaceuticals: As a solvent, excipient, and coating agent in tablets, capsules, and ointments.
2. Cosmetics: As a humectant, emollient, and thickener in skincare products, hair care products, and makeup.
3. Food: As a food additive, texture modifier, and moisture-retaining agent in beverages, baked goods, and confectionery.
4. Biotechnology: As a precipitant, stabilizer, and protectant in biological samples and biopharmaceuticals.

5. Industrial: As a lubricant, binder, and dispersant in various industrial applications.
6. Polyethylene glycol can be used in medication formulations to increase dissolution rate and oral bioavailability. PEG may be utilised to effectively isolate edible nanoparticles (ENPs), which have powerful anti-cancer and anti-inflammatory properties. PEG acts as a crowding agent, forming a net-like mesh that traps and precipitates nanovesicles. For example, ginger-derived ENPs are separated utilising a cost-effective PEG-based approach rather than ultracentrifugation. PEG can be utilised as a precursor to create degradable hydrogels for the controlled release of hydrophilic and high-molecular-weight medicines.

Benefits: High structural flexibility, low viscosity, solubility in water and many solvents, biocompatible, amphiphilic, high hydration capacity, devoid of steric hinderance, ability to hold moisture avoid drying out, compatibility with many materials and stable.

1. Surfactant
2. The polymer functions as a lubricating coating surface.
3. It is employed as a stationary phase in chromatography.
4. It is employed as a solvent, humectant, lubricant and solubilizer.
5. It is added as a precipitant in Plasmid DNA isolation & Protein crystallization.
6. It is also utilizing microbiology for concentrating viruses by PEG precipitation.
7. It is employed in Gene therapy.
8. In blood bank it is applied as a potentiator for determination of antigen and antibodies.
9. It is employed in toothpaste as a dispersing agent, in food and drinks employed as a foaming agent.
10. It is a part of many lubricants.

Safety:

PEG is regarded as safe and non-hazardous by the FDA. Sometime sensitization and intolerance reactions have been reported in humans exposed to PEG 400 in topically. It was of very low acute oral toxicity in a range of animal species, causing impairment to the digestive tract and diarrhoea in high doses.

Storage: Stored in well closed plastic container in cool well-ventilated place at 25-40 °C

Stability: Stable for more than 2 years when stored at 25 °C in moisture resistant conditions

Precaution: Precaution during handling (protect eyes, avoid inhalation and skin contact).

CHAPTER – VI

EXPERIMENTAL WORK

6. Materials and Methods for Tenofovir Disoproxil Fumarate (TDF)

6.1 Materials and Equipment for TDF

Table 6.1: List of Material and Suppliers for TDF

Sr. No.	Name of material	Supplier
1.	Tenofovir Disoproxil Fumarate	Mylan Laboratories Ltd., Aurangabad (MS), India
2.	Mannitol	S. D. Fine Chemicals, Mumbai, India
3.	Polyvinyl pyrrolidone (K-30)	S.D. Fine Chemicals, Mumbai, India
4.	Hydroxypropyl Cellulose (HPC-L)	S.D. Fine Chemicals, Mumbai, India
5.	Microcrystalline Cellulose (Avicel PH 101)	S.D. Fine Chemicals, Mumbai, India
6.	Silicified Microcrystalline Cellulose (Prosolve)	S.D. Fine Chemicals, Mumbai, India
7.	Spray dried lactose (DCL-11)	S.D. Fine Chemicals, Mumbai, India
8.	α- Tocopherol	S.D. Fine Chemicals, Mumbai, India
9.	Magnesium stearate	S.D. Fine Chemicals, Mumbai, India
10.	Talc	S.D. Fine Chemicals, Mumbai, India
11.	Hydrochloric acid	S.D. Fine Chemicals, Mumbai, India
12.	Methanol	S.D. Fine Chemicals, Mumbai, India
13.	Ethanol	S.D. Fine Chemicals, Mumbai, India
14.	Sulphuric acid	S.D. Fine Chemicals, Mumbai, India
15.	Potassium Dihydrogen Phosphate	S.D. Fine Chemicals, Mumbai, India
16.	Dimethyl Sulfoxide (DMSO)	S.D. Fine Chemicals, Mumbai, India
17.	p-Dimethylamino cinnamaldehyde	S.D. Fine Chemicals, Mumbai, India
18.	Gelucire®43/01	Gattefose SAS, 69804 Saint-Prist Cedex, France

Sr. No.	Name of material	Supplier
19.	Precirol® ATO 5	Gattefose SAS, 69804 Saint-Prist Cedex, France

All other chemicals were of laboratory grade and used as received.

Table 6.2: List of Equipment and Make Used for TDF

Sr. No.	Name of Equipment	Make
1.	Double cone blender	Dolphin India Pvt. Ltd., India
2.	Extruder- Spheronizer	Umang Pharmatek, India
3.	Pan coater	Adinath International, India
4.	Sieve shaker	Dolphin India Pvt. Ltd., India
5.	Tray dryer	Remi Laboratory Instrument, India
6.	Pizer tester	Veego Instruments Corporation, India
7.	Bulk Density apparatus	Electrolab India Pvt Ltd., India
8.	Friability test apparatus	Veego Instruments Corporation, India
9.	Disintegration test apparatus	Electrolab India Pvt Ltd., India
10.	Dissolution test apparatus	Electrolab India Pvt Ltd., India
11.	Ultrasonicator	ISP Technologies, India
12.	UV spectrophotometer	UV1800, Shimadzu, Japan
13.	Stability chamber	Remi Laboratory Instrument, India
14.	Tablet punching machine	Riddhi Pvt. Ltd., India
15.	pH Meter	Global electronics, India
16.	Potentiometer	ISP Technologies, India
17.	Differential Scanning Calorimeter (DSC 2500)	Water TA Instruments, New Castle, DE
18.	Analytical balance	Denver instrument, Germany

All the chemicals used were of laboratory and analytical grade. They were used as received.

6.2 Methods for TDF

6.2.1 Preformulation Studies for TDF

6.2.1.1 Organoleptic Properties of TDF

The organoleptic properties of TDF like colour, odour and taste were recorded.

6.2.1.2 Density and Flow Properties

Bulk density is ratio of bulk weight and bulk volume. Accurately weighed 50 g of API was poured gently through glass funnel into 100 ml calibrated measuring cylinder. The surface was cautiously levelled with null pressure.[4] The volume occupied by pellets was used for calculation of bulk density (g/ml) using the equation,

Bulk density = Weight of powder ÷ $Bulk\ volume\ of\ pellets$ ……. (6.1)

Tapped density is ratio of bulk weight and tapped volume. Tapped density was estimated in a similar way to that of bulk density. However, final volume was measured after tapping the cylinder from 3 inches until constant volume was obtained using Electrolab tapped density apparatus.[4] The volume occupied by pellets after tapping was noted and tapped density (g/ml) was calculated using the equation,

Tapped density = Weight of powder ÷ Tapped $volume\ of\ pellets$……. (6.2)

Carrs's Index is calculated using bulk density and tapped density data.

$Carr$'s $Index$= $(Tapped\ density - Bulk\ density) \div Tapped\ density \times 100$ …….(6.3)

6.2.1.3 Solubility

The equilibrium solubility of TDF was determined by placing 1 g of TDF in 100 ml of distilled water on a rotatory shaker for 24 hr at 37°C. After 24 hours, the TDF solution was filtered using 0.45 µ member filter and the amount dissolved in filtrate was determined using UV- Visible spectrophotometer at 260 nm. Similarly, the solubility was determined in 0.1N hydrochloric acid and phosphate buffer pH 6.8. The values of TDF solubility in various solvent were reported.[6]

6.2.1.4 Melting Point

The determination of melting point of TDF is preliminary test for the determination of purity of the drug. The lowering or widening of melting point range indicates

presence of impurity in the pure drug. The melting point of TDF was determined using capillary technique and compared with the reference value.

The more accurate, precise reproducible and reliable method for estimation of melting point is differential scanning calorimetry. Using DSC technique, the sample was previous dried in desiccator and sieved from 80 number sieve to assure uniform size fine particles. Accurately weighed about 2 mg of sample was placed in pan such a way that it covered bottom of the aluminum pan. The pan was crimped without applying excessive pressure.

The DSC thermogram of TDF sample was taken and melting point was recorded using nitrogen as purged gas at 50 ml/ min purge rate for maintaining inert atmosphere using Differential Scanning Calorimeter (DSC 2500, Water TA Instruments, New Castle, DE) in a temperature range 25-600°C. DSC cell was calibrated with indium (m.p. 156.6 °C; ΔHm = 28.54 J/g). An empty pan was covered cap used as reference. The baseline was taken for 2-3 min. The care was taken to avoid decomposition of sample in DSC cell. The sample was heated at 2°C/min for good resolution, as faster heating rate may decrease the resolution.[8]

6.2.1.5 UV-Visible Spectrophotometric Method Development

Instrument: Instrument used UV-Visible double beam spectrophotometer, Shimadzu Corporation (Japan), Model UV-1800 with a bandwidth of 0.5nm and a pair of 1cm matched quartz cells. Analytical balance (Denver instrument, Germany) and sonicator (Electro quip Ultra sonicator, Texas), pH meter (Global electronics, India) was used in the study. Calibrated glass wares used throughout the work.

Chemicals:

The drug (Tenofovir Disoproxil Fumarate) supplied by Mylan was used. The chemicals used were Methanol, Hydrochloric acid, p-Dimethylamino cinnamaldehyde, Sulphuric acid (AR Grade, S.D. Fine Chemicals, Mumbai, India) and distilled water.

Method:

Standard graphs of TDF

Accurately weighed 10 mg of TDF was taken in 100 ml volumetric flask and dissolved in 5 ml of methanol. The volume was made up to 100 ml with 0.1N

hydrochloric acid, which constitute stock solution of 100 µg/ml concentration. The stock solution was suitably diluted with 0.1N hydrochloric acid to prepare the working solutions of 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml. Suitability diluted sample was scanned between 200-400 nm to determine the absorption maxima of TDF. The working solutions were analyzed for their absorbance using UV-Visible spectrophotometer, at λmax about 260 and a standard graph was plotted. The procedure repeated to construct the standard graph of TDF in deionized water and phosphate buffer pH 6.8 (USP).[22]

Standard stock solution was prepared by dissolving 10 mg of Tenofovir in distilled water. The volume was made up to 10 ml with distilled water to get a concentration of 1000 mcg/ml. This was further diluted to get the working standard solution of 20 µg/ml.

Aliquots of standard drug solution of Tenofovir 0.5 – 3.5 ml. About 20 µg/ml TDF solution was taken and transferred into series of 10 ml graduated test tubes. To each test tube 2 ml of methanolic p-Dimethylamino cinnamaldehyde (3% w/v) (PDAC) and 0.5 ml of 0.1N sulphuric acid solution were added. After thorough shaking, the test tubes were set aside for 10 mins, for the reaction to complete. The volumes in each test tube were adjusted to 10 ml with methanol. The absorbances of the solutions were measured at 530 nm against reagent blank, and the calibration curve was plotted. Similarly, the absorbance of the sample solution was measured, and the amount of Tenofovir was determined by referring to the calibration curve.[23,24]

6.2.1.5 Loss on Drying

The percent loss on drying was estimated by keeping the 1 g of TDF at 105±2°C in hot air oven till constant weight was observed. The acceptance criteria as per Indian Pharmacopoeia is not more than 3.5%.[25]

6.2.1.7 Excipient Compatibility Studies for TDF

To evaluate the compatibility of selected excipients with Tenofovir disoproxil fumarate API, binary mixture of API and excipients were prepared with 1:1 ratio and exposed at 50 ± 2°C in glass vials closed with rubber stopper with aluminum cap sealing for 7 days. The samples were evaluated for physical characteristics (physical appearance).[26]

Table 6.3 : Excipient Compatibility Studies- Binary Mixture of TDF

Binary mixture
Tenofovir Disoproxil Fumarate (API)
Tenofovir Disoproxil Fumarate + Microcrystalline cellulose (Avicel PH101)
Tenofovir Disoproxil Fumarate + Spray-dried Lactose (DCL-11)
Tenofovir Disoproxil Fumarate + Mannitol
Tenofovir Disoproxil Fumarate + Polyvinyl Pyrrolidone (K-30)
Tenofovir Disoproxil Fumarate + Hydroxypropyl cellulose (HPC-L)
Tenofovir Disoproxil Fumarate + Magnesium stearate
Tenofovir Disoproxil Fumarate + Talc
Tenofovir Disoproxil Fumarate + Gelucire
Tenofovir Disoproxil Fumarate + Precirol ATO 5
Tenofovir Disoproxil Fumarate + Silicified Microcrystalline Cellulose (PROSOLV)
Tenofovir Disoproxil Fumarate + Cross-povidone
Tenofovir Disoproxil Fumarate + Alpha-tocopherol
Mixture of Tenofovir Disoproxil Fumarate + All above excipients

6.3 Formulation Development for TDF

Process Selection

Tenofovir disoproxil fumarate API has very poor flow characteristics, hence direct compression method was not chosen. Due to poor flow characteristics, wet mass followed by an extrusion-Spheronization process has been selected.

Initial Risk Assessment of the Process Variables

The initial risk assessment of the formulation variables and justification for the risk assessment are presented in table below.

Table 6.4 : Initial Risk Assessment of the Process Variables for TDF

Drug Product CQAs	Process Variables								
	Blending	Granulation (Wet mass)	Screening to form Extrudes	Spheronization	Drying (Oven)	Screening	HMC and Drying	Blending	Compression
Taste	Low	Low	Medium	Medium	Medium	Medium	High	Low	Medium
Content uniformity	Low	Low	Low	Low	Low	Low	Low	Low	Low
Assay	Low	Low	Low	Low	Low	Low	Low	Low	Low
Dissolution	Low	Low	Medium	Medium	Medium	Medium	High	Low	Low

Table 6.5 : Justification for the Initial Risk Assessment of the Process Variables for TDF

Process Variables	Drug Products CQAs	Justification
Blending	Taste	The blending stage has no direct impact on Taste of final product. Moreover, there is low risk for assay, content uniformity, and dissolution due to high drug content (More than 80% w/w)
	Content uniformity	
	Assay	
	Dissolution	
Granulation (Wet mass)	Taste	The granulation process has no control for taste masking, it will be controlled through hot melt coating, hence Low risk applicable. Risk of granulation for assay, content uniformity, and dissolution is Low due to high drug content (More than 80% w/w)
	Content uniformity	
	Assay	
	Dissolution	
Screening to	Taste	Medium risk as the attribute of Pellets strength

Process Variables	**Drug Products CQAs**	**Justification**
form Extrudes, Spheronization and Drying of Pellets	Dissolution	can be affected, which may impact on dissolution. Moreover, the strength of Pellets is critical for the further process of taste masking (Hot melt coating). Thus, this step is considered as Medium risk.
	Content uniformity	Low risk for assay due to high drug content (More than 80% w/w)
	Assay	
Screening	Taste	Screening of Pellets is critical for further process of taste masking (Hot melt coating, Lower or higher size of pellets may affect hot melt coating uniformity as well as dissolution consistency. Thus, Medium risk.
	Dissolution	
	Content uniformity	Low risk for assay due to high drug content (More than 80% w/w)
	Assay	
Hot melt coating and Drying	Taste	High risk for physical attribute –Taste, as it impacts on patient acceptability, and may side effect like nausea due to sever bitterness. Dissolution attribute may be impacted due to hot melt coating, as coating materials are waxy in nature i.e. hydrophobic. Thus, hot melt coating process considered as High-risk process variable.
	Dissolution	
	Content uniformity	Low risk for assay due to high drug content (More than 80% w/w)
	Assay	
Blending	Taste	Low risk as intragranular proportion is about 82% and drug is also more than 80%, hence impact of final blending of pellets with
	Dissolution	
	Content uniformity	

Process Variables	Drug Products CQAs	Justification
	Assay	extragranular material is minimal. As pellets are already coated, there is no direct impact of blending process over taste and dissolution
Compression	Taste	Medium risk as due to compression there may be rupturing of taste masking layer of hot melt coating of pellets, which leads to bitterness on oral administration.
	Dissolution	Low risk for assay due to high drug content (More than 80% w/w)
	Content uniformity	
	Assay	

6.3.1 Preparation of Drug Pellets for TDF

TDF and excipients were blended in a double-cone blender for 5 min. The polyvinyl pyrrolidone solution (2% w/v) was poured slowly over the powder blend.[26]

6.3.1.1 Selection of Filler

Three different fillers were evaluated for suitability. Granulation was performed using Microcrystalline Cellulose, Spray Dried Lactose, and Mannitol as filler. Polyvinyl Pyrrolidone solution of 2% concentration was used as a granulating agent.

Table 6.6 : Formulation of TDF Batches- Selection of Filler

Ingredients	Formulation code		
	F1	F5	F9
Tenofovir disoproxil fumarate	300	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	--	--
Spray-dried Lactose (DCL-11)	--	25	--
Mannitol	--	--	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)	q. s.	q. s.	q. s.

Note: Formulation code F2, F3, F4, F6, F7, and F8 are reproducibility batches of F1, and F5. Hence not captured in the table. The trial F9 was not satisfactory hence, not further considered.

Brief Manufacturing Process:

1. Tenofovir disoproxil fumarate and filler (Microcrystalline Cellulose (Avicel PH 101) or Spray dried lactose (DCL-11) or Mannitol) were sifted together through # 20 mesh.
2. Polyvinyl Pyrrolidone (K-30) was dissolved in purified water under stirring to form (2%w/w) solution.
3. Step 2. Binder solution was sprinkled over the sifted material of step 1 in the bowl with simultaneous manual mixing, till the wet mass (dough) formed.
4. The wet mass (dough) was squeezed through #16 mesh to form extrudes.
5. The wet extrudates charged into the extruder- spheronizer (Umang Pharmatek, India) and the spheronizer with cross-hatch plate of 1.2 mm was operated for 5 min at 850 rpm to produce TDF pellets.
6. The pellets were dried at 60°C for 3 h.
7. The dried pellets were sifted to collect 16-20 mesh fractions. The suitability is determined based on the % yield of good pellets fraction.

6.3.1.2 Selection of Binder

Two different binding agents were evaluated for suitability. Optimization was done using formulations of Microcrystalline Cellulose and Spray Dried Lactose. A 2% binder solution was prepared using Polyvinyl Pyrrolidone (K30) and Hydroxypropyl Cellulose (HPC-L). Granulation was performed at a fluid uptake of 8%.

Table 6.7 : Formulation of TDF Batches- Selection of Binder

Ingredients	Formulation code			
	F1A	F1B	F5A	F5B
Tenofovir disoproxil fumarate	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	25	--	--
Spray-dried Lactose (DCL-11)	--		25	25
Polyvinyl Pyrrolidone (K 30) (2% w/v)	q. s.	--	q. s.	--
Hydroxypropyl Cellulose (HPC-L) (2% w/v)	--	q.s.	--	q.s.

Brief Manufacturing Process:

1. Tenofovir disoproxil fumarate and filler (Microcrystalline Cellulose (Avicel PH 101) or Spray dried lactose (DCL-11)) were sifted together through # 20 mesh.
2. Polyvinyl Pyrrolidone (K-30) was dissolved in purified water under stirring to form (2%w/w) solution.
3. Hydroxypropyl Cellulose (HPC-L) was dissolved in purified water under stirring to form (2%w/w) solution.
4. Step 3 or 4 Binder solution was sprinkled over the sifted material of step 1 in bowl with simultaneous manual mixing, till the wet mass (dough) formed.
5. The wet mass (dough) was squeezed through #16 mesh to form extrudes.
6. The wet extrudates charged into the extruder- spheronizer (Umang Pharmatek, India) and the Spheronizer with a cross-hatch plate of 1.2 mm was operated for 5 min at 850 rpm to produce TDF pellets.
7. The pellets were dried at 60°C for 3 h.
8. The dried pellets were sifted to collect 16-20 mesh fractions. The suitability is determined based on the % yield of good pellets fraction.

6.3.1.3 Optimization of Fluid uptake

Two different fluid uptake levels were evaluated using Polyvinyl Pyrrolidone (K30) solution (2% w/v). Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11).

Table 6.8 : Formulation of TDF Batches- Optimization of Fluid Uptake

Ingredients / Parameter	Formulation code			
	F1C	F1D	F5C	F5D
Tenofovir Disoproxil Fumarate	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	25	--	--
Spray-dried Lactose (DCL-11)	--	--	25	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)	q. s.	q. s.	q. s.	q. s.
Fluid uptake level (%)	8	12	8	12

Brief Manufacturing Process:

1. Tenofovir disoproxil fumarate and filler (Microcrystalline Cellulose (Avicel PH 101) or Spray dried lactose (DCL-11)) were sifted together through # 20 mesh.
2. Polyvinyl Pyrrolidone (K-30) was dissolved in purified water under stirring to form (2%w/w) solution.
3. Step 2 Binder solution was sprinkled over the sifted material of step 1 in bowl with simultaneous manual mixing. The binder was added at the level of 8% and 12% fluid uptake.
4. The wet mass (dough) was squeezed through #16 mesh to form extrudes.
5. The wet extrudates were charged into the extruder- spheronizer (Umang Pharmatek, India) and the spheronizer with cross-hatch plate of 1.2 mm was operated for 5 min at 850 rpm to produce TDF pellets.
6. The pellets were dried at 60°C for 3 h.
7. The dried pellets were sifted to collect 16-20 mesh fractions. The suitability is determined based on the % yield of good pellets fraction.

6.3.1.4 Optimization of Screen Size for Preparation of Extrudes

Formulation of TDF batches

Two different sieve sizes were selected for optimization. The type of binder and Fluid uptake levels were kept constant. Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11).

Table 6.9 : Formulation of TDF Batches- Optimization of Screen Size for Preparation of Extrudes

Ingredients/Parameters	Formulation code			
	F1E	F1F	F5E	F5F
Tenofovir disoproxil fumarate	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	25	--	--
Spray-dried Lactose (DCL-11)	--	--	25	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)	q. s.	q. s.	q. s.	q. s.
Screen used for extrusion	#16	#20	#16	#20

Brief Manufacturing Process:

1. Tenofovir disoproxil fumarate and filler (Microcrystalline Cellulose (Avicel PH 101) / Spray dried Lactose (DCL-11)) were sifted together through # 20 mesh.
2. Polyvinyl Pyrrolidone (K-30) was dissolved in purified water under stirring to form (2%w/w) solution.
3. Step 2 Binder solution was sprinkled over the sifted material of step 1 in bowl with simultaneous manual mixing.
4. The wet mass (dough) was squeezed through #16 mesh / #20 mesh to form extrudes.
5. The wet extrudates were charged into the extruder- spheronizer (Umang Pharmatek, India) and the spheronizer with cross-hatch plate of 1.2 mm was operated for 5 min at 850 rpm to produce TDF pellets.
6. The pellets were dried at 60°C for 3 h.
7. The dried pellets were sifted to collect 16-20 mesh fractions. The suitability is determined based on the % yield of good pellets fraction.

6.3.1.5 Optimization of Spheronization Process

Formulation of TDF batches

Three different spheronizer cross-hatch plates of 1.0, 1.2, and 1.5 mm sizes were selected for optimization. The type of binder, Fluid uptake levels and extrusion screen were kept constant. Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11).

Table 6.10 : Formulation of TDF Batches- Optimization of Spheronization Process

Ingredients/Parameters	Formulation code					
	F1G	F1H	F1I	F5E	F5F	F5I
Tenofovir disoproxil fumarate	300	300	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	25	25	--	--	--
Spray dried lactose (DCL-11)	--	--	--	25	25	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
Cross-hatch plate size (mm)	1.0	1.2	1.5	1.0	1.2	1.5

Brief Manufacturing Process:

1. Tenofovir disoproxil fumarate and filler (Microcrystalline Cellulose (Avicel PH 101) or Spray dried lactose (DCL-11)) were sifted together through # 20 mesh.
2. Polyvinyl Pyrrolidone (K-30) was dissolved in purified water under stirring to form (2%w/w) solution.
3. Step 2 Binder solution was sprinkled over the sifted material of step 1 in bowl with simultaneous manual mixing.
4. The wet mass (dough) was squeezed through #16 mesh to form extrudes.
5. The wet extrudates were charged into the extruder- spheronizer (Umang Pharmatek, India) and the spheronizer with cross-hatch plate of 1.0, or 1.2, or 1.5 mm was operated for 5 min at 850 rpm to produce TDF pellets.
6. The pellets were dried at 60°C for 3 h.
7. The dried pellets were sifted to collect 16-20 mesh fractions. The suitability is determined based on the % yield of good pellets fraction.

6.3.1.6 Optimization of Drying Process for Pellets

Formulation of TDF batches

Two different drying times were selected for optimization. The type of binder, Fluid uptake levels, extrusion screen, and cross-hatch plate size were kept constant. Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11).

Table 6.11 : Formulation of TDF Batches- Optimization of Drying Process for Pellets

Ingredients/Parameters	Formulation code			
	F1J	F1K	F5J	F5K
Tenofovir disoproxil fumarate	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	25	--	--
Spray dried lactose (DCL-11)	--	--	25	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)	q. s.	q. s.	q. s.	q. s.
Drying time (hours)	1	3	1	3

Brief Manufacturing Process:

1. Tenofovir disoproxil fumarate and filler (Microcrystalline Cellulose (Avicel PH 101) or Spray dried Lactose (DCL-11)) were sifted together through # 20 mesh.
2. Polyvinyl Pyrrolidone (K-30) was dissolved in purified water under stirring to form (2%w/w) solution.
3. Step 2 Binder solution was sprinkled over the sifted material of step 1 in bowl with simultaneous manual mixing.
4. The wet mass (dough) was squeezed through #16 mesh to form extrudes.
5. The wet extrudates were charged into the extruder- spheronizer (Umang Pharmatek, India) and the Spheronizer with cross-hatch plate of 1.2 mm was operated for 5 min at 850 rpm to produce TDF pellets.
6. The pellets were dried at 60°C for 1 hour or 3 hours.
7. The dried pellets were sifted to collect 16-20 mesh fractions. The suitability is determined based on the % yield of good pellets fraction and Loss on Drying of pellets.

6.3.1.7 Optimization of Pellet Size/Screen Size

The marketed products (Vitamin D3 Sachet- Calcirol, Ibuprofen Effervescent Granules) were evaluated for their particle size by size analysis. Based on the observations, the 16 and 20 mesh fractions were found suitable.

6.3.2 Hot Melt Coating of Pellets:

Based on optimization studies, the optimized formula and process parameters of pellets manufacturing are as below,[27, 28]

Table 6.12 : Details Optimized Hot Melt Coating of TDF Pellets

Ingredients/Parameters	Formula/Parameter	
Tenofovir disoproxil fumarate	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	--
Spray dried Lactose (DCl-11)	--	25
Mannitol	--	--
Polyvinyl Pyrrolidone (K-30) (2% w/v)	q. s.	q. s.
Fluid uptake (%)	8	8

Ingredients/Parameters	Formula/Parameter	
Screen size (#)	#16	#16
Spheronizer cross-hatch plate (mm)	1.2	1.2
Drying time (hrs)	3	3
Pellets fractions (#)	16/20	16/20

The 20 mesh undersized and 16 mesh oversized pellets were rejected. TDF pellets were loaded into 10 inches diameter perforated coating pan equipped with 4 radially organized baffles and temperature regulation system.

The design of experiments was planned for optimization and identification of suitable hot melt coating ingredients and process parameters.

Table 6.13 : Hot Melt Coating Weight Build-up Parameters for TDF Pellets

Variables	Levels	
Hot melt coating agent	Gelucire® 43/01	Precirol® ATO 5
Coating build-up (%)	3,4,5	3,4,5
Pan Speed (rpm)	15,20	15,20
Coating temperature(ºC)	50,60	50,60

6.3.2.1 Optimization Hot Melt Coating Agent and Level of Coating:

The TDF pellets based on Microcrystalline Cellulose (Avicel PH 101) and Spray-dried lactose (DCL-11) were utilized for coating optimization. Two hot melt coating agents, Gelucire® 43/01 and Precirol® ATO 5 were used for coating evaluation, along with α- Tocopherol as an antioxidant for the coating agent. The experiments were performed with the below composition,

Table 6.14 : Optimization Hot Melt Coating Agent and Level of Coating for TDF

Ingredients	Formulation code											
	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21
Tenofovir disoproxil fumarate	300	300	300	300	300	300	300	300	300	300	300	300
Microcrystalline	25	25	25	25	25	25	--	--	--	--	--	--

Ingredients		Formulation code											
		F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21
Cellulose (Avicel PH 101)													
Spray dried lactose (DCL-11)		--	--	--	--	--	--	25	25	25	25	25	25
Polyvinyl Pyrrolidone(K-30) (2% w/v)		q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
Hot melt coating (%)	Gelucire® 43/01 (%)	2	3	4	--	--	--	2	3	4	--	--	--
	Precirol® ATO 5 (%)	--	--	--	2	3	4	--	--	--	2	3	4
	α-Tocopherol (%)	1	1	1	1	1	1	1	1	1	1	1	1

Brief Manufacturing Process:

1. The TDF pellets of Microcrystalline Cellulose (Avicel PH 101) and Spray dried Lactose (DCL-11) were formulated as per optimized procedure.
2. The drug pellets were loaded into the coating pan and rolled until pellet bed temperature of 60°C was attained.
3. The α- Tocopherol was added into the molten Gelucire® 43/01 or Precirol® ATO 5 under continuous stirring.
4. The molten coating mass was sprayed onto the rolling drug pellets in slow stream.
5. After the complete application of coating mass, the pellets were allowed to roll further for 10 min during which time the bed temperature was allowed to gradually come down. The pellets were then removed and cured in a dryer for 48 h. The parameters employed for HMC of Tenofovir pellets in the coating pan are given below,

Table 6.15: Process Parameters for Hot Melt Coating for Level of Coating for TDF

Parameter	Setting
Pellet weight	500 g
Pan speed	15 rpm
Coating level	3, 4 and 5% w/w
Pellet bed temperature	60°C
Relative humidity	40% RH
Coating time	30 min
Curing time	30°C for 48 h

6.3.2.2 Optimization of Coating Process Parameters: Pan Speed

The TDF pellets based on Microcrystalline Cellulose (Avicel PH 101) and Spray-dried Lactose (DCL-11) were utilized for coating optimization. Two hot melt coating agents, Gelucire® 43/01 and Precirol® ATO 5 were used for coating evaluation, along with α- Tocopherol. The experiments were conducted with two different pan speeds keeping coating weight build up constant.

Table 6.16: Optimization of Coating Process Parameters: Pan Speed

Ingredients/Parameters		Formulation code							
		F22	F23	F24	F25	F26	F27	F28	F29
Tenofovir disoproxil fumarate		300	300	300	300	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)		25	25	25	25	--	--	--	--
Spray-dried Lactose (DCL-11)		--	--	--	--	25	25	25	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)		q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
Hot melt coating (%)	Gelucire® 43/01 (%)	3	3	--	--	3	3	--	--
	Precirol® ATO 5 (%)	--	--	3	3	--	--	3	3

Ingredients/Parameters		Formulation code							
		F22	F23	F24	F25	F26	F27	F28	F29
	α- Tocopherol (%)	1	1	1	1	1	1	1	1
Pan Speed (RPM)		15	20	15	20	15	20	15	20

Brief Manufacturing Process:

1. The TDF pellets of Microcrystalline Cellulose (Avicel PH 101) and Spray-dried Lactose (DCL-11) were formulated as per optimized procedure.
2. The drug pellets were loaded into the coating pan and rolled until pellet bed temperature of 60°C was attained.
3. The α- Tocopherol was added into the molten Gelucire® 43/01 or Precirol® ATO 5 under continuous stirring.
4. The molten coating mass was sprayed onto the rolling drug pellets at pan speed 15 rpm or 20 rpm in slow stream.
5. After the complete application of coating mass, the pellets were allowed to roll further for 10 min during which time the bed temperature was allowed to gradually come down. The pellets were then removed and cured in a dryer for 48 h. The parameters employed for HMC of tenofovir pellets in coating pan are given below,

Table 6.17: Process Parameters for Hot Melt Coating for Optimization of Pan Speed

Parameter	Setting
Pellet weight	500 g
Pan speed	15 and 20 rpm
Coating level	4 % w/w
Pellet bed temperature	60°C
Relative humidity	40% RH
Coating time	30 min
Curing time	30°C for 48 h

6.3.2.3 Optimization of Coating Process Parameters: Temperature

The TDF pellets based on Microcrystalline Cellulose (Avicel PH 101) and Spray-dried Lactose (DCL11) were utilized for coating optimization. Two hot melt coating agents, Gelucire® 43/01 and Precirol® ATO 5 were used for coating evaluation, along with α- Tocopherol. The experiments were conducted with two different bed temperatures keeping pan rpm and coating weight build up constant.

Table 6.18: Optimization of Coating Process Parameters: Temperature

Ingredients/Parameters		Formulation code							
		F30	**F31**	**F32**	**F33**	**F34**	**F35**	**F36**	**F37**
Tenofovir disoproxil fumarate		300	300	300	300	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)		25	25	25	25	--	--	--	--
Spray-dried Lactose (DCL-11)		--	--	--	--	25	25	25	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)		q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
Hot melt coating (%)	Gelucire® 43/01 (%)	3	3	--	--	3	3	--	--
	Precirol® ATO 5 (%)	--	--	3	3	--	--	3	3
	α- Tocopherol (%)	1	1	1	1	1	1	1	1
Bed Temperature (ºC)		40	60	40	60	40	60	40	60

Brief Manufacturing Process:

1. The TDF pellets of Microcrystalline Cellulose (Avicel PH 101) and Spray-dried Lactose (DCL-11) were formulated as per optimized procedure.
2. The drug pellets were loaded into the coating pan and rolled until pellet bed temperature of 40°C or 60°C was attained.

3. The α- Tocopherol was added into the molten Gelucire® 43/01 or Precirol® ATO 5 under continuous stirring.
4. The molten coating mass was sprayed onto the rolling drug pellets at pan speed 20 rpm in slow stream.
5. After the complete application of coating mass, the pellets were allowed to roll further for 10 min during which time the bed temperature was allowed to gradually come down. The pellets were then removed and cured in a dryer for 48 h. The parameters employed for HMC of Tenofovir pellets in coating pan are given below,.

Table 6.19: Process Parameters for Hot Melt Coating for Optimization of Temperature

Parameter	Setting
Pellet weight	500 g
Pan speed	15 rpm
Coating level	4 % w/w
Pellet bed temperature	40°C and 60°C
Relative humidity	40% RH
Coating time	30 min
Curing time	30°C for 48 h

6.4 Evaluation of the Pellets

6.4.1 Pellet appearance

The coated and uncoated were snapped using digital microscope connected with personal computer.[29]

6.4.2 Mean Pellet Size (d_{mean})

The average size of pellets was carried out using sieving analysis technique. A sieve shaker and set of four active ASTM standard test sieves (#14, #16, #18 and #20) were used for the analysis. Accurately weighed 100 g of drug pellets were placed in sieve arranged over decreasing order of aperture size from top to bottom. The sieve shaker was operated for 5 min. The size distribution of pellets expresses the efficiency of the process of manufacture of the uniform size pellets.[29] The mean pellet size was calculated using equation.

$d_{mean} = \sum$ (% retained x Average aperture size)/100……. (6.4)

6.4.3 Bulk Density (ρ_b)

Bulk density is ratio of bulk weight and bulk volume. Accurately weighed 50 g of Tenofovir coated pellet fraction of 16/20 mesh were poured gently through glass funnel into 100 ml calibrated measuring cylinder. The surface was cautiously levelled with null pressure. The volume occupied by pellets was used for calculation of bulk density (g/ml) using equation[4]

$$\text{Bulk density} = \frac{\text{Weight of pellets}}{\text{Bulk volume of pellets}}$$

…… (6.5)

6.4.4 Tapped Density (ρ_t)

Bulk density is ratio of bulk weight and tapped volume. Tapped density was estimated in a similar way to that of bulk density. However, final volume was measured after tapping the cylinder from 3 inches until constant volume was obtained using Electrolab tapped density apparatus. The volume occupied by pellets after tapping was noted and tapped density (g/ml) was calculated using the equation[4]

$$\text{Tapped density} = \frac{\text{Weight of pellets}}{\text{Tapped volume of pellets}}$$ …… (6.6)

6.4.5 Carr Index (CI)

The external appearance of pellets and internal structure can alter material properties and porosity that greatly effect on pellet coating, flow and packing during tabletting or capsule filling. It also shows effect on drug release by affecting the capillary action of dissolved drug. Using bulk density and tapped density values of tenofovir coated pellets the compressibility index can be calculated using equation.

$$\text{Carr Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

……..… (6.7)

The flow ability of prepared pellets can be decided based on the standard values

Table 6.20 : Standard Carr's Index Flowability Chart

Carr's index	Flowability
10	Excellent
11-15	Good
16-20	Fair
21-25	Passable
26-31	Poor
32-37	Very poor
>38	Very very poor

6.4.6 Hardness and Friability

The hardness tenofovir pellets was determined by digital dial type hardness tester (Veego Instruments Corporation, India). For the friability study, accurately weighed 10.0 g of tenofovir coated pellets (initial weight) with 25 glass beads of 3 mm diameter were placed in the revolving drum of Roche's friabilator (Veego Instruments Corporation, India) for 100 revolutions operated at 25 rpm speed. The pellets were collected and placed on the sieve with 0.85 mm aperture and the smaller particles were allowed to pass through the sieve. The pellets were reweighed (Final weight) and % weight loss data were considered as %friability and calculated using equation [26]

$$\text{Friability (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

……. (6.8)

6.4.7 Drug Content

Accurately weighed 500 mg of hot-melt coated pellets were grind carefully in the mortar using pestle and sieved from 80 sieve. Accurately weighed 50 mg of this powder was transferred carefully to 100 ml volumetric flask and add 30 ml of methanol and ultrasonicated using laboratory sonicator (ISP Technologies, India) for 15 min to extract the tenofovir. Final volume was made with phosphate buffer pH 6.8 and diluted suitably. The diluted sample were scanned at 260 nm using double distilled water as blank using Ultraviolet- visible (UV) spectrophotometer (UV1800, Shimadzu, Japan). The drug content was calculated using standard calibration curve.[22]

6.4.8 In-vitro Drug Release Study

In-vitro drug release from Tenofovir pellets was carried out using United States Pharmacopoeia (USP) apparatus I (Basket Type), model Electrolab, 6 vessel assembly at 100 rpm. The 900 ml of 0.1N hydrochloric acid was used as dissolution medium since it can maintain sink condition during dissolution. Temperature of dissolution medium was maintained at 37 ± 0.5°C. The dissolution was conducted for an hour. Aliquots of 5 ml were withdrawn at predetermined intervals. An equivalent amount of fresh dissolution fluid equilibrated at the same temperature. Aliquots withdrawn were diluted suitably (1 ml to 20 ml), filtered and analyzed. All dissolution studies were conducted in triplicate and the mean values were plotted versus time with a standard deviation less than three indicating reproducibility of result. The percent cumulative drug release against time was plotted.[30, 31]

Table 6.21 : In-vitro Dissolution Study Conditions for Tenofovir Pellets

Sr. No.	Parameters	Specifications
1.	Dissolution apparatus	USP Type I (Basket)
2.	Speed	100 rpm
3.	Temperature	37± 0.5°C
4.	Time	30 min
5.	Dissolution medium	0.1N hydrochloric acid
6.	Volume of dissolution medium	900 ml
7.	Sampling time	5, 10, 15, and 30 min

6.4.9 In-vitro Taste Evaluation

Hot melt coated pellets equivalent to 50 mg of drug were placed in test tube containing 10 ml of double distilled water maintained at 37±1°C, stirred gently to simulate conditions of mouth cavity. After every 30 sec aliquot of 1 ml was collected and replaced with fresh medium to maintain dissolution medium at 37±1°C. Each aliquot was diluted to 100 ml and the absorbance of diluted solution was recorded at 260 nm using UV-visible spectrophotometer. Taste evaluation was performed for 10 min.[32]

6.4.10 Determination of Threshold Bitter Taste

To taste the sensory bitter taste of drug twelve human volunteers were selected and coded. They were asked to thoroughly rinse the mouth cavity with purified water. The dilutions of drug concentration range 50-500 µg/ml were prepared. Each volunteer was informed to hold 5 ml solution for 10 sec and spat out. The volunteers were asked to rinse the mouth cavity with purified water after every treatment to avoid carryover effect of previous treatment. The score of bitterness given to each solution against the distilled water was recorded. The minimum concentration which was judged as bitter taste by volunteer was considered as bitter threshold.[33,34]

6.4.11 Taste Panel Method

To taste the bitter taste of and efficacy of hot melt coating for taste masking of drug twelve human volunteers were selected. They were asked to thoroughly rinse the mouth cavity with purified water. They were provided with the 50 mg of pellets over tongue for 10 sec. Taking the taste of pure drug solution as standard, the degree of bitterness was judged by volunteers according to bitterness scale.[35]

6.4.12 Stability Test

The pellets equivalent to unit dose were filled in self-sealing Aluminium pouch. They were stored at temperature 25±2°C & 60±5% RH and 40 ± 2°C & 75 ± 5% RH for 6 months in the stability chamber (Remi Laboratory Instrument, CHM-6). The pellets were evaluated for any changes in physical appearance and percent drug content after every month. The results obtained were compared with data obtained at zero time and pellets stored at 25±2°C and 60±5% RH. The pellets were evaluated for any changes in physical appearance, drug content and in-vitro drug release after every month. The results obtained were compared with data obtained at zero time and pellets stored at 25±2°C & 60±5% RH and 40 ± 2°C & 75 ± 5% RH[36,37].

6.5 Compression of Tablets

Each blend was prepared using two different fillers and two different hot melt-coated pellets. The compression was performed on three different hardness, low, medium and high hardness. Moreover, final blend of un-coated pellets was also compressed for comparative taste masking evaluation.

Table 6.22: Hot Melt Coated Pellets Using Gelucire® 43/01

Ingredients/Parameters		Formulation Code							
		F38	F39	F40	F41	F42	F43	F44	F45
Tenofovir Disoproxil Fumarate		300	300	300	300	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)		25	25	25	--	--	--	25	--
Spray-dried Lactose (DCL-11)		--	--	--	25	25	25	--	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)		q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
Hot melt coating (%)	Gelucire® 43/01 (%)	3	3	3	3	3	3	--	--
	α- Tocopherol (%)	1	1	1	1	1	1	--	--
Silicified MCC		60	60	60	60	60	60	60	60
Talc		8	8	8	8	8	8	8	8
Crospovidone		5	5	5	5	5	5	5	5
Magnesium Stearate		2	2	2	2	2	2	2	2
Hardness		L	M	H	L	M	H	M	M

L: Low Hardness, M: Medium Hardness, H: High Hardness

Table 6.23 : Hot Melt Coated Pellets Using Precirol® ATO 5

Ingredients/Parameters	Formulation Code							
	F46	F47	F48	F49	F50	F51	F52	F53
Tenofovir Disoproxil Fumarate	300	300	300	300	300	300	300	300
Microcrystalline Cellulose (Avicel PH 101)	25	25	25	--	--	--	25	--
Spray-dried Lactose(DCL-11)	--	--	--	25	25	25	--	25
Polyvinyl Pyrrolidone (K-30) (2% w/v)	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

Ingredients/Parameters		Formulation Code							
		F46	F47	F48	F49	F50	F51	F52	F53
Hot melt coating (%)	Precirol® ATO 5 (%)	3	3	3	3	3	3	--	--
	α- Tocopherol (%)	1	1	1	1	1	1	--	--
Silicified MCC		60	60	60	60	60	60	60	60
Talc		8	8	8	8	8	8	8	8
Crospovidone		5	5	5	5	5	5	5	5
Magnesium Stearate		2	2	2	2	2	2	2	2
Hardness		L	M	H	L	M	H	M	M

Brief Manufacturing Process:

1. The final blending of hot melt coated pellets of Gelucire® 43/01 or Precirol® ATO 5 or un-coated pellets were mixed with extragranular materials, Silicified Microcrystalline Cellulose (Cushioning agent), talc and magnesium stearate were mixed.
2. The compression of tablets using 10 station rotatory tablet punching (Riddhi Pvt. Ltd., India) using 8 mm circular biconcave punches by adjusting hardness. Tablets were compressed at Low, Medium and High hardness. Hardness levels 4±0.5 kg/cm^2 to 7±0.5 kg/cm^2 were evaluated.

6.6 Evaluation of Tablets

6.6.1 Organoleptic Properties

The prepared tablets were observed for appearance, colour, odour and taste.

6.6.2 Weight Variation Test

Twenty tablets were selected randomly and weighed. The average weight was determined. Then individual tablets were weighed and was compared with average weight. The comparison variation within the limits, prepared tablet batches pass the weight variation test.

Table 6.24: Standards for Uniformity of Weight

Sr. No.	Average weight	Percent deviation
1.	130 mg or less	10
2.	130 to 324 mg	7.5
3.	325 or More	5

Note: Limits as per US Pharmacopoeia

6.6.3 Tablet Hardness Test

The resistance of tablets to delivery or breakage under state of capacity, transportation and dealing with before utilization depends on its hardness. The hardness of tablet of every formulation can be estimate by Monsanto tester, Pfizer tester and Erweka Hardness Tester. The hardness was measured in terms of kg/cm^2. Tablet crushing strength (Fc) or hardness, the force required to break a tablet in a diametric compression, was measured using Monsanto tablet hardness tester. Randomly selected six tablets were used for evaluation from each batch. Each tablet was placed between anvil and the screw was rotated to apply pressure. The pressure required to break tablet was recorded on scale. The mean and standard deviation value were recorded.

6.6.4 Thickness

The thickness of individual tablets was measured using micrometer screw gauge, which permits accurate measurements and provides information of the variation between tablets.

6.6.5 Tablet Friability

The friability of the tablets was measured in a friability test apparatus. Tablets of a known weight (W0) (sample quantity approx. 6.5 g tablets) were dedusted in a drum for a fixed time (100 revolutions) and weighed (W) again. Percentage friability was calculated from the loss in weight as given in equation as below. The weight loss should not be more than 1 %. Determination was made in triplicate.

$$\text{Friability (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad \ldots (6.9)$$

6.6.6 Drug Content

Twenty tablets were weighed and taken in mortar and crushed to make powder. A quantity of powder weighing equivalent to 300 mg of TDF was taken in 1000 ml volumetric flask and 100 ml ethanol was added. Final volume was adjusted using distilled water with occasional shaking. The resultant solution was sonicated in Ultrasonicator (ISP Technologies, India) for 5 minutes. The solution was filtered using membrane filter 0.45µm and then the solutions absorbance was measured at 260 nm using UV visible spectrophotometer. Then the amount of drug present was calculated using standard graph.

6.6.7 In-vitro Disintegration Test

The disintegration test was carried out using 6 tablets using tablet disintegration test apparatus (Electrolab India Pvt Ltd., India). Distilled water was used as medium for test at 37ºC ± 2ºC. The time required for complete disintegration of the tablets was recorded. No palpable mass remain on mesh was recorded as disintegration time.

6.6.8 In-vitro Drug Release Study

In-vitro dissolution studies for prepared tablets and the marketed available tablets was carried out using USP apparatus I (Basket Type), model Electrolab, 6 vessel assembly at 100 rpm in 900 ml of distilled water as dissolution media, maintained at 37 ± 0.5°C. After predetermined interval 5 ml of sample was withdrawn from the dissolution medium and replaced with fresh medium. The solution was cooled down to room temperature filtered using membrane filter 0.45µm and then the solutions absorbance was measured at absorption maxima using UV visible spectrophotometer. The cumulative percentage of drug release was calculated and represent graphically. The similarity and difference factor were used to confirm the uniformity in release profile between Tablet B formulation and marketed formulation (Vired 300 Tablets).[29,30]

Table 6.25: In-vitro Dissolution Study Conditions for Tenofovir Tablets

Sr. No.	Parameters	Specifications
1.	Dissolution apparatus	USP Type I (Basket)
2.	Speed	100 rpm

Sr. No.	Parameters	Specifications
3.	Temperature	37± 0.5°C
4.	Time	30 min
5.	Dissolution medium	Distilled water
6.	Volume of dissolution medium	900 ml
7.	Sampling time	5, 10, 15 and 30 min

6.6.9 In-vitro Taste Evaluation

To taste the bitter taste of and efficacy of compressed tablets for taste masking of drug, twelve human volunteers were selected and coded them. They were asked to thoroughly rinse the mouth cavity with purified water. They were provided with one over tongue for 10 sec. Taking the taste of pure drug solution as standard, the degree of bitterness was judged by volunteers according to bitterness scale.[35]

6.6.10 Similarity Factor (f_2):

Different dissolution profiles were compared to establish the effect of formulation or process variables on the drug release as well as comparison of the test formulations to the theoretical release profile. The dissolution similarity was assessed using the Food and Drug Administration (FDA) recommended approach (f_2, similarity factor). The similarity factor is a logarithmic, reciprocal square root transformation of the sum of squared errors, and it serves as a measure of the similarity of two respective dissolution profiles. [38,39]

$$f_2 = 50.\ \text{Log}\ \{[1+ (1/n)\ \Sigma^{n}_{t=1}(R_t-T_t)^{2}]^{-0.5} \times 100\} \qquad (6.10)$$

Where, n = number of sample points,

R_t = percent of marketed product observed and

T_t = percent of test formulations release observed

FDA has set a published standard for similarity factor (f_2) value (50-100) for indication of similarity between two dissolution profiles. To use mean data, the coefficients of variation for mean dissolution profile of a single batch should be less than 10%. The average difference at any dissolution sampling point should not be greater than 15% between test and reference products. If the drug release is more than 85% in 15 minutes, the dissolution profiles are considered similar.[38,39]

6.6.11 Stability Test

The stability of the active ingredient is a major criterion in determining acceptance or rejection of batch. During stability study, the product was exposed to accelerated as well as long term stability conditions of temperature and humidity. The final formulation was filled in polycarbonate bottles sealed with Aluminium foil and kept in the humidity chamber maintained at 25ºC±2 ºC /60% ± 5%RH and 40ºC±2 ºC /75% ± 5%RH conditions for six months as per International Council of Harmonization (ICH) guidelines.[36,37]

Part B

6.7 Materials and Methods for Sitagliptin Phosphate Monohydrate

6.7.1 Materials and Equipment for SPM

Table 6.26: List of Material and Suppliers

Sr. No.	Chemical	Supplier
1.	Sitagliptin Phosphate Monohydrate	Mylan Laboratories Limited, India
2.	Stearic acid	S.D. Fine Chemicals, India
3.	Palmitic acid	S.D. Fine Chemicals, India
4.	Polyvinyl Pyrrolidone (K-30)	Thomas Baker Chemical Pvt. Ltd. India
5.	Dicalcium phosphate	Thomas Baker Chemical Pvt. Ltd., India
6.	Titanium dioxide	S.D. Fine Chemicals, India
7.	Polyethylene glycol-4000	Gem9 Envirotech Supplier, India
8.	Colloidal Silicon Dioxide (Aerosil)	S.D. Fine Chemicals, India
9.	Talc	Themis Laboratories, India
10.	Spray Dried Lactose (DCL-11)	S.D. Fine Chemicals, India
11.	Sodium Starch Glycolate	S.D. Fine Chemicals, India
12.	Microcrystalline Cellulose (Avicel PH 101)	S.D. Fine Chemicals, India

All the other chemicals were of analytical and laboratory grade used as procured.

Table 6.27: List of Equipment and Make

Sr. No.	Equipment	Make
1.	UV- Visible spectrophotometer	Schimadzu®, Japan
2.	USP dissolution test apparatus	Electrolab®, India
3.	Sieve shaker	Toshiwal and Company®, India
4.	Digital hardness tester	Veego Scientific®, India
5.	Roche friabilator	Veego Scientific®, India
6.	Ultrasonicator	ISP technologies®, India
7.	Stability chamber	Electrolab®, India
8.	Pan coater	Adinath International, India
9.	Bulk density apparatus	Electrolab India Pvt Ltd., India
10.	Friability test apparatus	Veego Instruments Corporation, India
11.	Disintegration test apparatus	Electrolab India Pvt Ltd., India
12.	Dissolution test apparatus	Electrolab India Pvt Ltd., India
13.	Tablet punching machine	Riddhi Pvt. Ltd., India
14.	pH Meter	Global electronics, India
15.	Analytical balance	Denver instrument, Germany
16.	Hardness tester	Veego Instruments Corporation, India

6.7.2 Methods for SPM

6.7.2.1 Preformulation Studies for SPM

6.7.2.1.1 Organoleptic Properties

The organoleptic properties of Sitagliptin Phosphate Monohydrate (SPM) like colour, odour and taste were determined. The appearance of the sample SPM was observed under microscope.

6.7.2.1.2 Melting Point:

Determination of melting point of drug is preliminary test for the determination of purity of the drug. Lowering or widening of melting point range indicates presence of impurity in the pure drug. The melting behavior was determined using a Mettler-Toledo MP70 melting point system (Greifensee, Switzerland). A capillary was used with a closed bottom, applying a heating rate of 10°C min^{-1} up to a temperature limit

of 400°C.[15]

The DSC analysis was performed in a Shimadzu® DSC-60 calorimeter (Kyoto, Japan). The samples were analyzed in an aluminum crucible containing around 2.0 mg of sample under dynamic synthetic air atmosphere (50 mL min−1) with a heating rate of 10°C min−1 and temperature range of 30°C to 400°C. The instrument was calibrated with indium and zinc (reference standards). The purity was determined using aluminum crucibles with approximately 2.0 mg of sample at a heating rate of 2 °C min−1 from 30°C to 400 °C. The purity of the sample was measured in triplicate using TASYS software (version 1.14, Shimadzu®), based on the Van't Hoff equation:

$$X2 = (To - Tm)\Delta Hf/RTo^{2} \quad \text{.....(6.11)}$$

Where, the purity is determined from the molar percentage of impurities present in the sample, X2 represents the mole fraction of impurities, Tm is the sample melting temperature, To is the melting point of the pure substance (°K), R is a gas constant and ΔHf is the heat of fusion of the main component (J mol−1).[16,17]

6.7.2.1.3 Identification (Fourier Transform Infrared (FTIR) Spectroscopy)

The infrared spectra were recorded on a Bruker Alpha-P FTIR spectrometer (Ettlingen, Germany) using attenuated total reflection (ATR) in the wavelength range of 3500 to 500 cm^{-1}, with a nominal resolution of 4 cm^{-1} and accumulation of 32 scans.[18]

6.7.2.1.4 Spectrophotometric Method for Measurement of Sitagliptin

Instrument: Instrument used UV-Visible double beam spectrophotometer, Shimadzu Corporation (Japan), Model UV-1800 with a bandwidth of 0.5nm and a pair of 1cm matched quartz cells. Analytical balance (Denver instrument, Germany) and sonicator (Electro quip Ultra sonicator, Texas), pH meter (Global electronics, India) was used in the study. Calibrated glass wares used throughout the work.

Chemicals:

The drug (Sitagliptin phosphate) supplied by Mylan was used. The chemicals used were Methanol (AR Grade, S.D. Fine Chemicals, Mumbai, India) and distilled water.

Method:

Standard Graphs of Sitagliptin

Preparation of Stock Solution: Accurately weighed 10 mg of sitagliptin phosphate was taken in a 100 ml volumetric flask and dissolved in 5 ml of methanol. The volume was made up to 100 ml with double distilled water, which constitute stock solution of 100 µg/ml concentration.

Preparation of Working Solution: The stock solution was suitably diluted with double distilled water to prepare the working solutions of 10, 20, 30, 40, 50, and 60 µg/ml.

Estimation of Absorption Maxima (λmax): A 100 µg/ml solution of sitagliptin phosphate was scanned between 200-400 nm to determine the absorption maxima (λmax) of sitagliptin.

Determination of Absorbance: The working solutions were analyzed for their absorbance using UV-Visible spectrophotometer, at λmax about 267 and a standard graph was plotted. The procedure repeated to construct the standard graph of sitagliptin in 0.01N hydrochloric acid, phosphate buffer pH 4.5 and phosphate buffer pH 8 (USP). [19-21]

6.7.2.1.5 Solubility Analysis:

Solubility is one of the most important parameters about achieving the desired concentration of the drug in systemic circulation to obtain the required pharmacological response. Poorly water-soluble drugs with a slow absorption, for instance, may show inadequate bioavailability.[22,23]

Solubility analysis of sitagliptin phosphate monohydrate was carried out by adding 257 mg SPM in different medium like 0.01N hydrochloric acid, 0.01M sodium citrate, 0.01 M sodium carbonate, acetate buffer (pH 4.5) and distilled water (pH 7.0) in volumetric flasks of 50 ml capacity. The overall solubility analysis was performed at 25°C. The buffer used were with pH not deviated than 0.5 units after addition of drug. The pH was recorded initially and after 24 hours shaking with wrist action shaker and the solutions were analyzed for dissolved drug substances. Solution was filtered through 0.45 µ membrane, and filtrate was analyzed spectrophotometrically. [24-26]

The solubility of SPM was also estimated in organic solvents like in acetone, acetonitrile, ethanol, methanol, isopropyl alcohol, isopropyl acetate, dimethyl sulfoxide and N, N-dimethyl formamide in the similar way.[27]

6.7.2.1.6 Partition Coefficient

Sitagliptin phosphate partition coefficient was determined in n-octanol-water system. A 500 mg of sitagliptin phosphate was transferred to a bottle containing 500 ml of n-octanol. The bottles were capped and agitated for 24h at room temperature to achieve complete equilibration. The phases were allowed to separate in a separating funnel. The aqueous layer containing the drug was analyzed at a maximum wavelength of 267 nm using UV-Visible spectrophotometer. The drug concentration in the aqueous layer was obtained from the calibration graph.[28] The partition coefficient of the drug was calculated using the equation:

$$K_{o/w} = C1\text{-}Cw/Cw\ (Vw\text{-}Vo) \quad \text{.....(6.12)}$$

Where, Ko/w = partition coefficient; C1 = total concentration of sitagliptin phosphate; Cw = concentration of sitagliptin phosphate in aqueous phase; Vw = volume of the aqueous phase; Vo = volume of the organic phase. The determination was done in triplicates.

6.7.2.1.7 pH Determination:

A 2% saturated solution of Sitagliptin Phosphate was prepared in distilled water and pH was measured by a digital pH meter.[29]

6.7.2.1.8 Loss on Drying (%)

Accurately weighed 2 g of SPM previously screened through sieve number 80 was placed in dry weighing bottle and transfer the bottle in hot air oven maintained below 10°C temperature than the melting point of SPM for 1-2 hours hot air oven till constant weight was observed. The acceptance criteria as per United States Pharmacopoeia is not more than 2%.[30]

6.7.2.2 Excipient Compatibility Studies

To evaluate the compatibility of selected excipients with sitagliptin phosphate monohydrate (SPM) API, a binary mixture of API and excipients were prepared with 1:1 ratio and exposed at 50 ± 2°C in glass vials closed with a rubber stopper with

aluminum cap sealing for 7 days. The samples were evaluated for physical characteristics (physical appearance).

Table 6.28 : Excipient Compatibility Studies- Binary Mixture

Binary mixture
Sitagliptin Phosphate Monohydrate (API)
Sitagliptin Phosphate Monohydrate + Stearic acid
Sitagliptin Phosphate Monohydrate + Palmitic acid
Sitagliptin Phosphate Monohydrate + Polyvinyl Pyrrolidone (K-30)
Sitagliptin Phosphate Monohydrate + Dicalcium Phosphate
Sitagliptin Phosphate Monohydrate + Titanium Dioxide
Sitagliptin Phosphate Monohydrate + Polyethylene lycol-4000
Sitagliptin Phosphate Monohydrate + Colloidal Silicon Dioxide (Aerosil)
Sitagliptin Phosphate Monohydrate + Talc
Mixture of Sitagliptin Phosphate Monohydrate + All above excipients

6.7.3 Formulation Development for Sitagliptin Phosphate Monohydrate

6.7.3.1 Process Selection

Sitagliptin phosphate monohydrate API has good flow characteristics, hence direct compression method was chosen.

6.7.3.1.1 Initial Risk Assessment of the Process Variables

The initial risk assessment of the formulation variables and justification for the risk assessment are presented in table below.

Table 6.29 : Initial Risk Assessment of the Process Variables

Drug Product CQAs	Process Variables		
	Blending	Compression	HMC and Drying
Moisture	Low	Low	Low
Assay	High	High	High
Dissolution	Low	High	High

Table 6.30 : Justification for the Initial Risk Assessment of the Process Variables

Process Variables	Drug Products CQAs	Justification
Blending	Moisture	The blending process has no direct impact on the Dissolution and Moisture of the final product.
	Dissolution	
	Assay	Blending process parameters such as blending time, blender speed are impacts on uniform mixing of API with other excipients. The uneven distribution impacts the assay of finished product. Hence, risk is considered as High.
Compression	Moisture	The compression stage has no direct impact on Moisture of the final product. Hence, risk is low.
	Assay	The compression process involves the conversion of final blend into unit dosage at a predefined target tablets weight. The individual tablets weight uniformity of tablets have impact on assay and the hardness has impact on the dissolution of tablets.
	Dissolution	
HMC and Drying	Moisture	The HMC coating process has no direct impact on moisture as it does not involve aqueous vehicle.
	Assay	HMC coating process involves heating of tablets and application of hot (molten) coating agent. The drug may degrade due to heat. Hence, risk is high.

Process Variables	Drug Products CQAs	Justification
	Dissolution	HMC coating process involves application of hydrophobic coating agents, which may impact on drug release. Hence, risk is high.

6.7.3.2 Selection of Filler:

Three different fillers were evaluated for suitability. Blending was performed using Microcrystalline Cellulose, Spray Dried Lactose, and Dicalcium phosphate as filler. All three final blends were compressed.

Table 6.31 : Formulation of Sitagliptin Batches- Selection of Filler

Ingredients	Formulation code		
	B1	B2	B3
Sitagliptin Phosphate Monohydrate	65	65	65
Dicalcium Phosphate	60	--	--
Microcrystalline Cellulose (Avicel PH101)	--	60	--
Spray Dried Lactose (DCL-11)	--	--	60
Talc	2	2	2
Croscarmellose Sodium	2	2	2
Colloidal Silicon Dioxide (Aerosil)	1	1	1

Brief Manufacturing Process:

1. Sitagliptin, Dicalcium phosphate or Microcrystalline Cellulose (Avicel PH 101) or Spray-dried Lactose (DCL-11) were sifted through #20 sieve.
2. The remaining materials Talc, Croscarmellose sodium, and Colloidal Silicon Dioxide (Aerosil) were sifted separately through #20 sieve.
3. Sifted materials of step 1 were loaded into a double cone blender and mixing was performed for 20 mins at 24 RPM.
4. Sifted materials of step 2 were loaded into a double cone blender and mixing was performed for 5 mins at 24 RPM.
5. Compressed the final blend (three blends of three different fillers) using a 10-station rotary tablet machine with 6-mm standard biconcave circular punches.

6. The tablets were tested for physical appearance, hardness, and disintegration time.

6.7.3.3 Selection of Disintegrant:

Two different Disintegrants were evaluated for suitability. Optimization was performed using Microcrystalline Cellulose (Avicel PH 101), as filler. Both the blends were compressed.

Table 6.32 : Formulation of Sitagliptin Batches- Selection of Disintegrant

Ingredients	Formulation code	
	B4	B5
Sitagliptin Phosphate Monohydrate	65	65
Microcrystalline Cellulose (Avicel PH101)	60	60
Talc	2	2
Sodium Starch Glycolate	2	--
Croscarmellose Sodium	--	2
Colloidal Silicon Dioxide (Aerosil)	1	1

Brief Manufacturing Process:

1. Sitagliptin, Microcrystalline Cellulose Avicel PH 101 were sifted through #20 sieve.
2. The remaining materials Talc, Croscarmellose sodium or Sodium Starch Glycolate, and Colloidal Silicon Dioxide (Aerosil) were sifted separately through #20 sieve.
3. Sifted materials of step 1 were loaded into a double cone blender and mixing was performed for 20 mins at 24 RPM.
4. Sifted materials of step 2 were loaded into a double cone blender and mixing was performed for 5 mins at 24 RPM.
5. Compressed the final blend (two blends of two different disintegrants) using a 10-station rotary tablet machine with 6-mm standard biconcave circular punches.
6. The tablets were tested for physical appearance, hardness, and disintegration time.

6.7.3.4 Selection of Glidant and Lubricant:

Colloidal Silicon Dioxide (Aerosil) was selected as a glidant, and Talc was selected as a lubricant in the above experiments for the selection of filler and disintegrant. The performance of the blend during compression was found satisfactory in all experiments. Hence, the same glidant and lubricants were identified as suitable for the formulation. The optimized formula for core tablets is as below:

Table 6.33 : Optimized Formulation of Sitagliptin Batches

Ingredient & Formulation code	mg/tablet
Sitagliptin Phosphate Monohydrate	65
Microcrystalline Cellulose (Avicel PH101)	60
Talc	2
Croscarmellose Sodium	2
Colloidal Silicon Dioxide (Aerosil)	1

6.7.3.5 Preparations of SPM core tablets:

Tablet containing 65 mg of Sitagliptin Phosphate Monohydrate equivalent to 50 mg of Sitagliptin were prepared. Compression was carried out using a 10-station rotary tablet machine with 6-mm standard biconcave circular punches.[31]

6.7.3.6 Selection of coating agents:

The suitability and performance of Gelucire® 43/01 and Precirol® ATO 5 as a hot melt coating agents were already evaluated for Tenofovir formulation; hence it was decided to assess alternate hot melt coating agents.

The experiments were designed to optimize and identify suitable hot melt coating ingredients and process parameters. The natural origin hot melt coating agents, stearic acid and palmitic acid were used for coating.[32]

Table 6.34 : Formulation of Hot Melt Coating Composition

Ingredients	Formulation Code							
	F1	F2	F3	F4	F5	F6	F7	F8
Stearic acid	4.00	3.75	3.5	3.25	--	--		
Palmitic acid	--	--	--	--	4.00	3.75	3.5	3.25
Polyethylene Glycol 4000	--	0.25	0.5	0.75	--	0.25	0.5	0.75
Titanium dioxide	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
α-tocopherol	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Brief Manufacturing Process:

1. Each batch of 400 SPM tablet cores equivalent 50 g of tablet were loaded into a 5-inch coating pan equipped with 4 radially organized baffles and a temperature regulation system.
2. The tablet cores were rolled at 40 rpm in the coating pan until tablet bed temperature was attained 60°C.
3. The hot melt coating agents, Stearic acid and palmitic acid were separately molt at 80°C.
4. Alpha-tocopherol was used as oil soluble antioxidant and Polyethylene Glycol 4000 (PEG 4000) was employed as pore former to achieve immediate release were added into the above molten hot melt coating solution. The Titanium dioxide were added into above solution under continuous stirring.
5. The molten coating mass was sprayed onto the rolling SPM tablets in slow stream by atomizing the coating solution (spray gun 100 ml S68; pilot at 15-psi pressure).
6. After the complete application of coating mass, the coated tablets were allowed to roll further for 15 min during which time the bed temperature was allowed to gradually come down.
7. The parameters employed for HMC of SPM in coating pan are given below table and followed same procedure for all formulations.[32]

Table 6.35 : Coating Variables for SPM Core

Sr. No.	Coating parameters	Tablets 400 (50 g)
1.	Spray rate	1.5 ml/min
2.	Atomizing air pressure	15 psig
3.	Tablet bed temperature	40-65°C
4.	Pan diameter	5 inches
5.	Pan speed	40-60 rpm
6.	Air flow	80-120 cfm
7.	Inlet air temperature	35- 45°C
8.	Outlet air temperature	40- 60°C
9.	Number of baffles	4
10.	Relative humidity	40%

6.7.3.7 Physico-Chemical Properties of Tablets:

6.7.3.7.1 Appearance:

Many pharmaceutical tablets use color as a vital means of rapid identification and consumer acceptance. But it must be uniform within a single tablet, from tablet to tablet and from lot to lot. The presence of an odor in a batch of tablets could indicate a stability problem e.g. the characteristic odor of acetic acid in degrading aspirin tablets or could be characteristic of the drugs e.g. vitamins have a characteristic odor. Taste is important in consumer acceptance of chewable tablets. The tablet cores were visually observed for any capping, chipping and lamination. The coated tablets were also checked for coating defects.[33]

6.7.3.7.2 Hardness:

For this test type of the Monsanto hardness tester to evaluate tablet hardness tester. The tester consists of a barrel containing a compressible spring held between two plungers. The lower plunger is placed in contact with the tablet and zero reading is taken. The upper plunger is then forced against a spring by turning a threaded bolt until the tablet fractures. As the spring is compressed, a pointer rides along a gauge in the barrel to indicate the force. The force of fracture is recorded in kilogram. For each formulation the hardness values for 6 tablets were determined using Monsanto hardness tester.[34]

6.7.3.7.3 Thickness:

The thickness of a tablet is the only dimensional variable related to the process. Thickness of individual tablets may be measured by a micrometer. Other techniques involve placing 5 tablets in a holding tray, where their total thickness may be measured by a sliding caliper scale. Tablet thickness should be controlled within a ± 5 % variation of a standard. Thickness must be controlled to facilitate packaging. It is expressed in mm. Average thickness was calculated.[34]

6.7.3.7.4 Percent Weight Gain:

Weight gain by tablet is one of the important parameters monitored during formulation, process and scale up development. Weight gain was calculated from the difference in the tablet weight before and after coating.[35]

6.7.3.7.5 Weight Variation Test:

This test examines uniformity in accordance with the formulation of each batch of tablets, which illustrates its content. In this study, we selected 20 tablets of SPM, which were weighed individually and collectively. Tablets were weighed individually. The average weight was calculated and compare the individual tablet weight. The tablet passes the U.S.P. test if not more than 2 tablets are outside the percentage limit and if the number of tablets differs by more than 2 times the percentage limit then test fails.[36]

The weight variation was calculated using the formula –

Weight variation (%) = (Initial weight - Average weight)/Average weight × 100…..(6.13)

It is meant to compare the USP limits and the data were recorded in table format.[37]

Table 6.36 : USP Limits for Weight Variation of Tablets

Sr. No.	Weight	Limit (%)
1	130 mg or less	10
2	130 mg to 324mg	7.5
3	More than 324 mg	5

6.7.3.7.6 Friability Test

As per USP standards, the friability test was performed using the Roche friabilator. It checks the tendency of the tablet to crumble, chip, or break upon abrasion or compression. It is important to check the friability of a tablet for complete dissolution in the gastrointestinal tract. The test checks the sturdiness of a tablet, and a loss of 1% tablet mass is acceptable during the process.[38]

6.7.3.7.7 Disintegration Test

As per USP standards, the SPM tablets are disintegrated into small granules to increase the surface area. It involves the disintegration of the tablets in a liquid medium as stated in the monograph under experimental conditions and recorded as disintegration time. The test is crucial, as it provides critical safety data in regard to the bioavailability of the solid dosage form of the drug. During this test, six tablets from batch were randomly selected and one tablet was placed in each tube with a mesh size of 10 basket as per USP standards. The basket was placed in a 1liter beaker containing phosphate buffer solution of pH 6.8 at 37°C. The apparatus was operated at 28-32 cycles/minutes and, simultaneously, a stopwatch was started. When all the particles passed from each test tube into the beaker, the finishing time was noted as the disintegration time. This disintegration test was a quantitative test, as time was measured during the test.[38]

6.7.3.7.8 In-vitro Dissolution Study of Marketed Products:

In-vitro dissolution of marketed film coated tablets of sitagliptin phosphate monohydrate was conducted to select the suitable dissolution medium for the study. It was used as yard sticks to prove the suitability of the dissolution conditions for the drug in study, whether sink conditions are maintained or not. In vitro dissolution of twelve marketed tablets.[39, 40]

Table 6.37 : In-vitro Dissolution Study Conditions for Marketed Products

Sr. No.	Parameters	Specifications
1.	Dissolution apparatus	USP Type II (Paddle)
2.	Paddle speed	100 rpm
3.	Temperature	37± 0.5°C

Sr. No.	Parameters	Specifications
4.	Time	1 hr
5.	Dissolution medium	Phosphate buffer pH 6.8
6.	Volume of dissolution medium	900 ml
7.	Sample volume	1 ml
8.	Sampling time	15, 30, 45 and 60 min
9.	Dilution factor	20

The sample solutions were filtered through Whatman filter paper (45 µm), from this filtered solution, 0.5 mL solution was taken into 10 mL volumetric flask and volume was made up with 6.8 pH buffer and solutions were analyzed at 267 nm by UV Spectrophotometer. The release in the dissolution medium was determined by software (PCP Disso v 2.08).

6.7.3.7.9 Drug Content:

Twenty tablets from each formulation were selected for the estimation of drug content. The tablet was weighed, triturated and transferred the powder to a 100 ml flask containing 50 ml of 0.1N HCL. The content of the flask was filtered through a filter, kept in a 100 ml volumetric flask. The residue was washed with another 40 ml of 0.1N HCL and the volume was made up to the mark. The sample was suitably diluted and analyzed spectrophotometrically against blank (0.1N HCL) at 267 nm using double beam UV- visible spectrophotometer.[41]

6.7.3.7.9.1 Procedure for Assay of SPM Formulations

Twenty tablets of SPM formulations were weighed and powdered. A quantity of tablet powder equivalent to 50 mg of SPM was transferred to 50 mL volumetric flask. Accurately measure 5 ml of methanol was transferred to 50 mL volumetric flask and ultrasonicated for 20 min and volume was made up to the mark with distilled water. The solution was then filtered through a Whatmann filter paper grade. The filtrate was appropriately diluted further. The absorbance of the resulting solution was measured at 267 nm and the amount of SPM was computed from its calibration plot.[42]

6.7.3.7.10 Moisture Uptake

A number of methods have been developed to test the moisture uptake of pharmaceutical dosages. The most commonly used approach is to measure the weight increase of the dosages at various constant temperature and humidity conditions. Typically, both coated and uncoated dosages are evaluated for their moisture uptake at these different humidity conditions created by a saturated salt solution, such as potassium chloride or sodium chloride. According to the United States Pharmacopeia (USP), for equilibrium moisture determinations, weighing should be carried out every hour until achievement of consecutive readings corresponding to a recorded mass change of less than 0.25%.[43]

6.7.3.7.11 Similarity Factor (f_2):

Different dissolution profiles were compared to establish the effect of formulation or process variables on the drug release as well as comparison of the test formulations to the theoretical release profile. The dissolution similarity was assessed using the Food and Drug Administration (FDA) recommended approach (f_2, similarity factor). The similarity factor is a logarithmic, reciprocal square root transformation of the sum of squared errors, and it serves as a measure of the similarity of two respective dissolution profiles.

$$f_2 = 50.\ \text{Log}\ \{[1+ (1/n)\ \Sigma^{n}_{t=1}(R_t-T_t)^{2}]^{-0.5} \times 100\} \ldots\ldots\ldots\ldots\ldots (6.14)$$

Where, n = number of sample points,

R_t = percent of marketed product observed and

T_t = percent of test formulations release observed

FDA has set a published standard for similarity factor (f_2) value (50-100) for indication of similarity between two dissolution profiles. To use mean data, the coefficients of variation for mean dissolution profile of a single batch should be less than 10%. The average difference at any dissolution sampling point should not be greater than 15% between test and reference products. If the drug release is more than 85% in 15 minutes, the dissolution profiles are considered similar.[44,45]

6.7.3.7.12 Stability Test

The stability of the active ingredient is a major criterion in determining acceptance or rejection of batch. During stability study, the product was exposed to accelerated as

well as long term stability conditions of temperature and humidity. The final formulation was packed in well- sealed Alu-alu package and kept in the humidity chamber maintained at 25ºC/60% RH and 40ºC/75% RH conditions for six months as per International Council of Harmonization (ICH) guidelines.[46]

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