

“DEVELOPMENT AND CHARACTERIZATION OF TELMISARTAN NANOCRYSTALS PREPARED BY VARIOUS METHODS”

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
**MASTER IN PHARMACY
PHARMACEUTICS**

By

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TOPIC APPROVAL PERFORMA

LIT (Pharmacy)/Department of Pharmaceutical Sciences

Program : P570-NN1::M.Pharm. (Pharmaceutics)

COURSE CODE : PHSS14 REGULAR/BACKLOG : Regular GROUP NUMBER : PHRRGD0012

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Qualification : _____ Research Experience : _____

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PROPOSED TOPIC : Development and characterization of nanocrystals of telmisartan prepared by different techniques

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	Parameter	Rating (out of 10)
1	Project Novelty: Potential of the project to create new knowledge	6.50
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	6.50
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	6.50
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	6.50
5	Social Applicability: Project work intends to solve a practical problem.	6.00
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	6.50

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Final Topic Approved by PAC: Development and characterization of nanocrystals of telmisartan prepared by different techniques

Overall Remarks: Approved

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Abstract

The nanocrystalline formulation is an effective way to increase the solubility of the poorly water soluble drug. Drug nanocrystals are the simple structure made up of two layers: a solid drug core and another is a layer of stabilizer which stabilize the nanocrystals. Selection of suitable stabilizers strongly effects the stability of nanocrystals. The present study is based on the formulation of Telmisartan nanocrystals by various methods. Telmisartan was identified as the direct acting Angiotensin II receptor antagonist, thus possess an essential role in the maintenance of high blood pressure. Telmisartan is a Biopharmaceutical Classification System class II drug with low aqueous solubility. The present study includes the enhancement of aqueous solubility as well as improved bioavailability of Telmisartan by preparing its nanocrystals. Telmisartan nanocrystals were prepared by two different methods, Solvent/Antisolvent precipitation method and other is antisolvent precipitation supplemented by Sonication technique using different polymers like PEG 6000, PEG 4000 and PVPK30 singly or in combination. The resultant nanocrystals were characterized by particle size, polydispersity index, zeta potential, transmission electron microscopy, scanning electron microscopy, and XRD. The particle size of the best formulation was found to be 164.2nm with a polydispersity index of 0.288. The zeta potential of the formulation found to be -39mv. The dissolution study was performed between telmisartan pure drug and nanocrystals of telmisartan resulted in to 12 fold increase in bioavailability. Therefore resultant nanocrystals prepared by antisolvent evaporation followed by sonication method were effective in increasing the bioavailability as well as dissolution of the drug.

Keywords: Solubility, Drug carrier, Nanocrystals, Telmisartan, PEG 6000

ACKNOWLEDGEMENT

First and above all, I praise god, the almighty for providing me this opportunity and granting me the capability to proceed successfully. This thesis appears in its current form due to assistance and guidance of several people.

I owe my special thanks to **Mr. Sujit Bose Assistant Professor**, Lovely School of Pharmaceutical Sciences, Lovely Professional University for their valuable warm encouragement, thoughtful guidance, gracious attention and motivation for completing my project work. It gives me immense pleasure to submit my dissertation work as his student.

I want to express my deep thanks to my **Co-Guide “Mr. Rupesh Sharma”** for the trust, the insightful discussion, offering valuable advice, for your support during the whole period of the study, and especially for your patience and guidance during the writing process.

I am very obliged to **“Dr. S. Tamilvanan”** HOD Lovely School of Pharmaceutical Sciences, Lovely Professional University for giving his support to proceed my project work.

I am very grateful to **“Dr. Monica Gulati”** Senior Dean Lovely School of Applied Medical Sciences, Lovely Professional University whose continuous guidance helped me a lot to face many problems during my project work.

I would like to thank my parents **“Mr. Daljeet Chand”** and **“Mrs. Sunita Patial”** who have always been with me. It was due to my parent’s dream, ambition, and sacrifice that I get so much ability to face all challenges during my research work today with misty eyes and folded hands I am unable to express my hidden thoughts into words for my family. I would like to express my heartiest gratitude to my friends **“Arun Kumar, Rhythm Bassi, Pooja Sharma, Keerti Bhardwaj, Yusuf Nawaz Khan, Paras Famta, Deepak ghai and Nuni sagar”** as without their love and support it would not have been possible to complete the task.

Words will fall short to express my feelings for my parents. They are the soul of my energy and motivation. Their blessings have to lead me towards achieving my goals.

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The work described in this thesis authorizes “**Development and Characterization of Telmisartan Nanocrystals prepared by various methods**” have been carried out by **Ms. Swati Patial** under the guidance of **Mr. Sujit Bose**. I certify that this is her real work. The work described is original and has not been submitted before for purpose of getting a degree in any university. The candidate has performed the work with her dedication to the best of my satisfaction.

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LIST OF ABBREVIATIONS

Cm	Centimetre
cm-1	Centimetre inverse
COA	Certificate of Analysis
°C	Degree Centigrade
DSC	Differential Scanning Calorimetry
% EE	Percentage Entrapment Efficiency
<i>et al.</i>	And co-workers
FDA	Food and Drug Administration
Fig.	Figure
FTIR	Fourier Transform Infra-Red
G	Gram
HCl	Hydrochloric acid
Hrs	Hours
ICH	International Conference on Harmonization
KBr	Potassium Bromide
LOD	Limit of Detection
LOQ	Limit of Quantification
Λ	Lambda
Ltd.	Limited
Mg	Microgram
Mg	Milligram
Min	Minute
ml	Millilitre
N	Normal
Nm	Nanometer
% RSD	Percent Relative Standard Deviation
PI	Polydispersity Index
PEG	Poly ethyl glycol
PVP	Poly vinyl pyrrolidone
Rpm	Rotations per minute
SD	Standard Deviation
TEM	Transmission Electron Microscopy
TEL	Telmisartan
UV	Ultra Violet
WHO	World Health Organisation

CHAPTER- 1 INTRODUCTION

Hypertension is an enduring therapeutic state in which the blood pressure in arteries is chronically elevated. Pulse is compressed by two estimations, either systolic or diastolic, that rely on whether the muscles of the heart is shrinking (systole) or relaxing (diastole). At relaxing position the normal blood pressure of healthy human ranges within 100-140mmHg systolic to 60-90mmHg diastolic. High blood pressure supposed to exist if it is regular between 140/90mmHg. Hypertension or High Blood Pressure, defined as 140mmHg systolic and 90mmHg diastolic, however, the risk for the prevalence of disease appears to increase even above 120/80mmHg (Sinny Delacroix 1*, Ramesh G Chokka1, 2014). Hypertension is a modifiable risk factor for heart failure, stroke, end-stage renal disease and peripheral vascular disease. It is approximated that hypertension is concerned with 7.5 million death per year globally. In the year 2010, hypertension has been identified as the leading risk factor for death and disability. It has been estimated for 9.4 million death and 7 percent of disability. In India, High blood pressure is concerned with 57 percent of stroke deaths, 24 percent of CHD deaths and 10% of all deaths. It was also estimated that the number of hypertensive patients will double from 118 million in 2000 to 213 million in India by 2025. The pattern for the interpretation of hypertension was proposed by WHO (1959) and fourth US joint National committee. (JNC IV, 1988) (Midha, 2013).

Sign and symptoms:

Etiology of hypertension is unknown and its recognition is typically done by screening. The extent of individual report headache (especially at the back of the head and in the morning), further vertigo, tinnitus, changed vision and collapsing episodes

1.1) TYPES OF HYPERTENSION

1.1.1) Primary hypertension pressure:

Primary high blood pressure: Primary or essential hypertension is the most prevalent type of high blood pressure, related with 90-95% of all cases (Oparil et al., 2003). Although etiology of primary hypertension is unknown, it tends to be familial and often associated with increased salt intake or obesity. HTN is also associated with the problematic interplay of environmental factors or genes. Different usual genetic variation with little variation with massive effect on BP, however, the genetic origin of hypertension remain misunderstood (Sinny Delacroix 1*, Ramesh G Chokka1, 2014).

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1.1.2) Secondary hypertension:

Secondary blood pressure: Secondary hypertension affects 5% of antihypertensive patients and can be identified by various causes such as renal artery stenosis, chronic kidney disease, sleep apnoea and adrenal diseases. Renal sickness is the most common optional explanation for hypertension. Secondary hypertension is somehow related to endocrine conditions, such as Cushing's disorder, thyrotoxicosis, glandular disorder, acromegaly, glandular disease, and neoplasm.

A typical aspect in both cases is a failure of a number of mechanisms associated with the regulation of normal blood pressure as such, SNS, renin-angiotensin-aldosterone system, Na⁺ and water retention system has also been considered as factors in the development of disease (Sinny Delacroix 1*, Ramesh G Chokka1, 2014).

1.2) Classification of hypertension:

Table 1.1

Classification of hypertension proposed by Sixth Joint National Committee VI: (Howell et al., 2004)

Category	Systolic arterial pressure(mmHg)	Diastolic arterial pressure(mmHg)
Optimal	<120	<80
Normal	90-119	60-79
High normal	120-139	80-89
Hypertension		
Stage 1	140-159	90-99
Stage 2	160-179	100-109
Stage 3	≥180	≥110

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1.3) Pathophysiologic mechanism of hypertension:

In a large portion of the general population with setup fundamental hypertension, extended inflexibility to blood flow accounting for blood pressure whereas cardiac output rests usually. Hypertension is furthermore identified with little peripheral blood vessel which can increase blood vessel come, increment cardiovascular preload and at last cause pulse dysfunction. Pulse pressure is normally extended more established individuals with high blood pressure. This can imply whether the systolic pressure is unusually high, diastolic pressure might be normal or low where this condition is called as isolation pulsation high blood pressure. The increased pulse pressure in old age people with hypertension or confined systolic hypertension is clarified by extended blood vessel toughness, which generally goes along with aging and will be aggravated by hypertension.

Pathophysiologic mechanism of hypertension:

In a large portion of the general population with set up fundamental (primary) hypertension, extended inflexibility to blood flow (total peripheral resistance) accounting for great pressure whereas cardiac yield rests usually. There is a confirmation that few more youthful persons through pre-hypertension or 'marginal hypertension' have a great rate of the stream, a hoisted beat rate, and customary fringe resistance, termed hyperkinetic marginal hypertension. These men and women develop the general options of founded HTN in future lifestyles at their cardiac yield falls and peripheral conflict increases with age. Regardless of whether this example is normal of every one of the individuals who ultimately develop hypertension is debated. Hypertension is furthermore identified with little peripheral blood vessel which can increase blood vessel come, increment cardiovascular preload and, at last, cause pulse dysfunction. Regardless of whether enlarged dynamic vasoconstriction plays an occupation in set up, HTN is blurred.

Pulse pressure is normally extended to more established individuals with HTN. This can imply whether the systolic pressure is unusually high, however, diastolic pressure might be normal or low where this condition is as termed isolation pulsation hypertension. The high rhythm pressure in old people with hypertension or confined systolic HTN is clarified by extended blood vessel toughness, which generally goes along with aging and will be aggravated by hypertension (Oparil et al., 2003).

INTRODUCTION

Pathophysiologic mechanism of hypertension

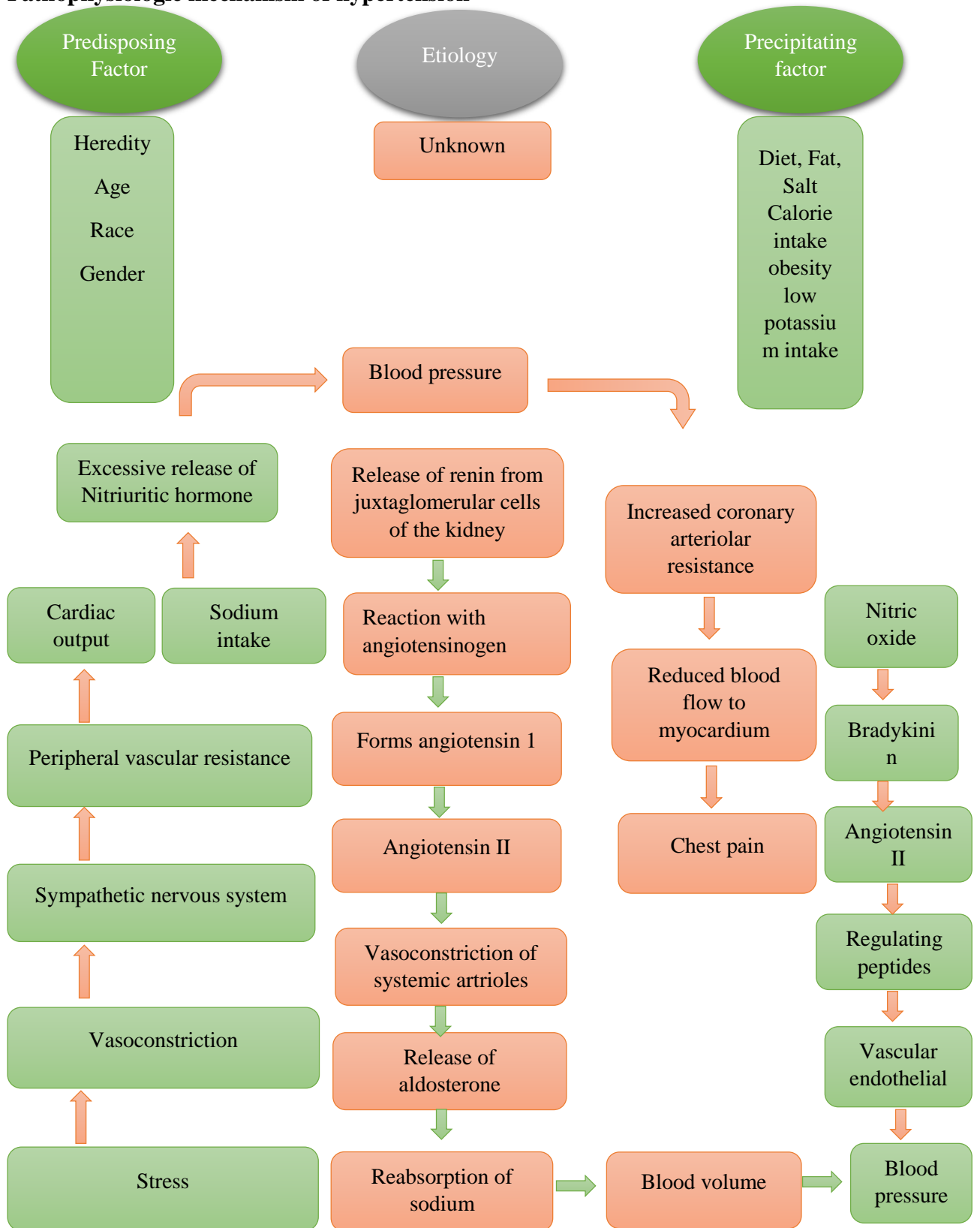


Figure 1.1 Diagrammatic representation of pathophysiology of hypertension

INTRODUCTION

Table 1.2

1.4) Pharmacotherapy for hypertension: (K.D Tripathi)

Class	Generic name/ brand name	Mode of action	Suitable for	Contraindicated
ACE inhibitors	Captopril, Enalapril , Lisinopril etc.	ACE inhibitors exert their effect by inhibiting the conversion of inactive angiotensin I to active angiotensin II.	All grades of essential hypertension as well as renovascular hypertension .	Contraindicated in patients with bilateral renal artery stenosis.
Angiotensin antagonists	Telmisartan, Losartan, Candesartan etc.	Angiotensin antagonists block angiotensin II from binding to its target receptor, therefore prevent vasoconstriction and fluid retention.	High renin cases or patients with low salt diet.	Pregnancy and hyperkalemia.
Calcium channel blockers	Verapamil, Diltiazam, Nifedepine etc.	CCBs lowers the blood pressure by blocking the entry of Ca ⁺ into vascular smooth muscles, which results into	Asthma and COPD patients. Raynauds and migraine patients.	Ischaemic heart disease Left ventricular hypertrophy

INTRODUCTION

		vasodilation and decreased blood pressure contractility.		
Diuretics	<p>Thiazides: Hydrochlorothiazide Chlorthalidone</p> <p>High ceiling: Furosemide</p> <p>K⁺ sparing: Spironolactone</p>	<p>Thiazides act by inhibiting the absorption of sodium and chloride in the distal convoluted tubule.</p> <p>High ceiling diuretics selectively inhibit the luminal Na⁺, K⁺, 2Cl⁻ symporter, therefore reduce the NaCl reabsorption.</p> <p>Potassium-sparing: acts on aldosterone receptor on distal and cortical tubule, thereby decrease the NaCl reabsorption.</p>	<p>Low renin hypertension</p> <p>Obese patient with volume overload</p> <p>Renal disease with Na⁺ retention.</p>	<p>Gout.</p> <p>Abnormal lipid profile.</p> <p>Pregnancy induced hypertension.</p>

1.4.1) Angiotensin antagonist:

INTRODUCTION

<p>β adrenergic blockers</p>	<p>Propranolol, Atenolol etc.</p>	<p>Beta-blockers lower the blood pressure by blocking beta 1-adrenergic receptor resulting in decreased cardiac contractility and reduced cardiac output.</p>	<p>Angina or post-MI patients. Non-obese, high renin hypertension.</p>	<p>Left ventricular failure Bradycardia Asthma, PVD</p>
<p>α Adrenergic blockers</p>	<p>Prazosin, terazosin</p>	<p>Alpha-adrenergic blockers lower blood pressure by blocking vasoconstricting α 1-adrenoreceptor on vascular smooth muscles.</p>	<p>Hypertension with benign prostate hypertrophy</p>	
<p>Central sympatholytic</p>	<p>Clonidine, methyldopa</p>	<p>Central sympatholytics act on α2 receptor present in the medulla, thereby increase sympathetic outflow and fall in BP.</p>		

INTRODUCTION

Vasodilators	Hydralazine, Minoxidil.	These are directly acting arteriolar vasodilators.		
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Drugs blocking type 1 angiotensin II receptor known as angiotensin II receptor blockers . The renin-angiotensin system plays an essential role in the progression of hypertension & coronary heart diseases. Blockage of angiotensin II receptors represents selective inhibition of pressor responses of the renin-angiotensin system. This action blocks vascular constriction, Na⁺ and water retention, activation of the SNS, constriction of efferent and afferent arterioles of kidney and activation of myocardial & vascular fibrosis (Wienen et al., 2000).

Angiotensin receptor blockers are the most important classes of drugs used in the treatment of high blood pressure, heart failure, and diabetic nephropathy. Angiotensin promotes the insulin resistance; thus any approach which decreases the concentration of angiotensin antagonist may result in improved insulin sensitivity.

Telmisartan was identified as the direct acting AngiotensinII receptor antagonist, thus possess an essential role in the maintenance of high blood pressure. Telmisartan shows significantly potent and sustains action on AngiotensinII-mediated pressor responses in animal models as well as human (Wienen et al., 2000)

Telmisartan is a whitish crystalline powder having Equivalent weight 514.6 and a melting point of 261 to 263°C. The Solubility of telmisartan in aqueous solution is strongly dependent on the pH of the solution and maximum solubility observed in high and low pH. Telmisartan is poorly soluble in the range of pH 3-9. Telmisartan belongs to class II drug from BCS (Biopharmaceutical Classification System). BCS categorized drugs in four classes according to their permeability and aqueous solubility. Drugs fall in class II and class VI faces solubility problems, where rate limiting step for their absorption is dissolution in an aqueous medium(Vimalson, 2016)

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Table 1.3 Classification of drugs as per BCS:

Class	Solubility	Permeability	Characteristics features
Class I	High	High	Excellent absorption orally.
Class II	Low	High	Irregular absorption pattern due to less solubility
Class III	High	Low	Irregular absorption due to permeability limitation.
Class IV	Low	Low	Minimum absorption due to solubility and permeability limitation

The Solubility of the drugs can be defined as the maximum amount of solute that can be dissolved in the given quantity of solvent at a particular temperature. Drugs having highest solubility show the highest bioavailability. Solubility can be described as:

Table 1.4 Solubility Profile of drugs: (S.V. Kadam et al., 2013)

Descriptive terms	Parts of solvent required as per part of solute
Very soluble	Less than 1
Freely soluble	From 1-10
Soluble	From 10-30
Sparingly soluble	From 30-100
Slightly soluble	From 100-1000
Very slightly soluble	From 1000-10000
Insoluble	More than 10000

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1.5) The solubility of the drugs can be enhanced by various methods such as:

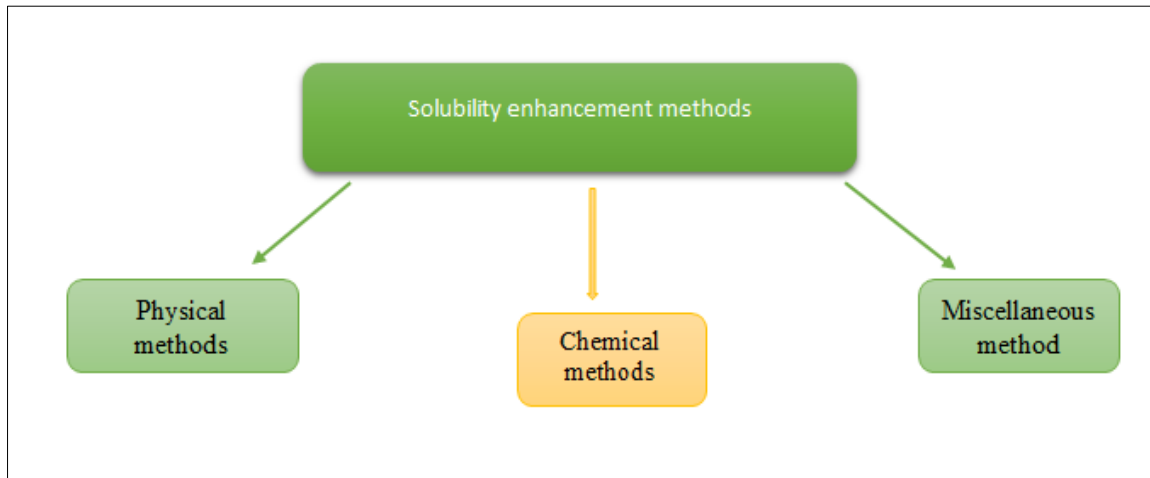


Figure1.2. Diagrammatic representation of Solubility enhancement methods

1.5.1) **Physical methods:** physical methods include reduction of particles size by physical forces so as to increase the effective surface area, which further increases the dissolution rate. It includes various techniques such as:

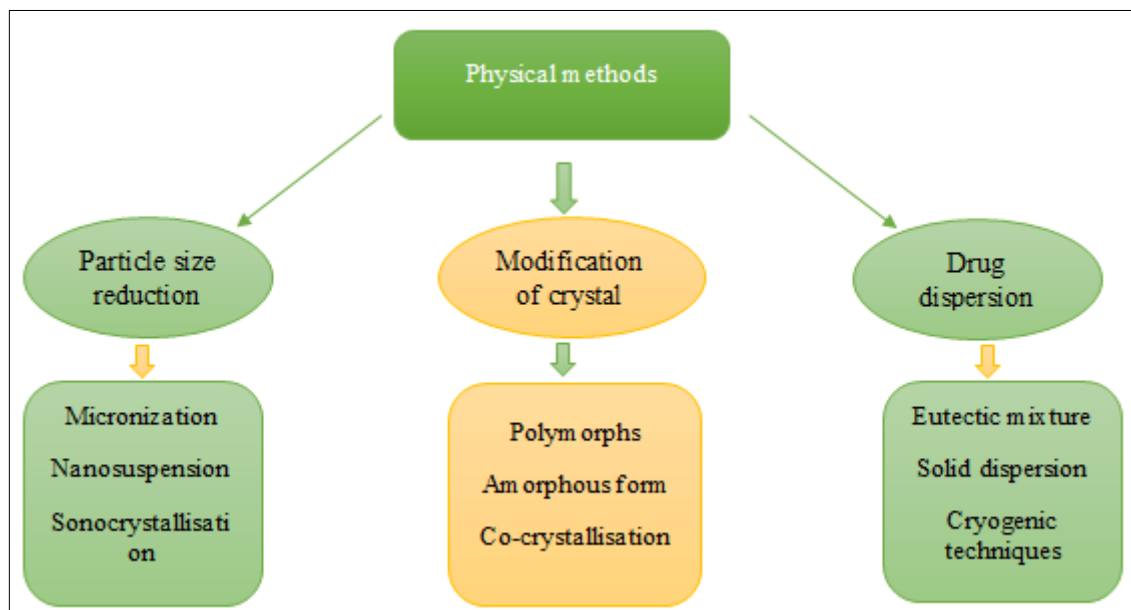


Figure 1.3.Diagrammatic representation of physical methods for solubility enhancement (Kadam et al., 2013)

1.5.1.1) **Particle size reduction:**

The particle size of the drug is directly related to its solubility; as the particle size decreases its effective surface area to volume ratio increases. The smaller particles allow large interaction with the solvent which leads to increased solubility. Size reduction can be obtained by

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traditional methods like spray drying and comminution which depends on mechanical stress and new techniques like micronization and nanonization (A. Kumar et al., 2014)

1.5.1.1.1) Micronization:

The oral bioavailability of drugs given as solid dosage form depends on the size, size distribution, and morphology of the drug particles. Micronization is a technique which increases the effective surface area of various drugs by decreasing its size leads to enhanced solubility of the drugs. Micronization is achieved by milling technique using the Jet mill, rotor-stator and colloid mills (Kesarwani and Pradesh, 2014)

1.5.1.1.2) Nanosuspension:

Nanosuspensions are submicron colloidal dispersion consisting of Nano-sized drug particles, which are stabilized by surfactants. Nanosuspension increases the dissolution rate of the drug because of enhanced surface area exposed and reduction in Ostwald ripening effect. Nanonization can be used to increase the solubility of drug particles which are insoluble in both aqueous as well as lipid media (Kadam et al., 2015)

Technologies used for the preparation of nanosuspensions are:

Precipitation methods (Bottom-up methods)

Dispersion methods (Top-down techniques)

Combination methods (Sheetal Z Godse and Patil, Swapnil M Kothavade, 2013).

1.5.1.2) Modification of crystal:

1.5.1.2.1) Polymorphs/amorphous form:

Polymorphism is the property of the compound or element to crystallize in more than one crystalline form. Diff polymorphic forms of drugs have same chemical properties but exhibit different physicochemical properties such as solubility, melting point, density, texture, and stability. Polymorphs are classified into various forms according to their thermodynamic properties (Arun et al., 2012)

Enantiotropes: In this system, one polymorphic form can be reversibly interchanged into another form at definite transition temperature below its melting point

Monotropes: Reversibility in the transition is not dependent in case of monotropes

Metastable form: These are a generally unstable system, having high energy and high solubility, thus possess high surface area, bioavailability, and efficacy

Amorphous form is associated with high energy and high surface area thus suited more as compare to crystalline form(Sheetal Z Godse and Patil, Swapnil M Kothavade, 2013).

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1.5.1.2.2) Hydrates/solvates:

A solvate is a chemical compound which contains a solvent as part of its crystal structure. Solvates exhibit different crystalline known as pseudo-polymorphs and phenomenon is called as pseudomorphism. In molecular adduct, in which water molecules are assimilated into the crystal lattice of solid is known as hydrates. Hydrates are less soluble as compare to solvate (Vimalson, 2016).

1.5.1.2) Drug dispersion:

1.5.1.3.1) Eutectic mixtures: These mixtures are prepared when both drug and polymers are mixed in a molten state but on cooling they crystallize as two different constituents having slight solubility. Both drug and polymer exhibit reduced particle size thus leads to enhanced effective surface area and increased dissolution profile of active drug (Vimalson, 2016)

1.5.1.3.2) Solid dispersion: Solid dispersion is characterized as an aggregation of solid products consists of two or more different compounds; hydrophobic drug and hydrophilic matrix. In solid dispersion technique, hydrophobic drugs dispersed into highly soluble hydrophilic polymers which increase the solubility of the drug (Parve et al 2014). Widely used the hydrophilic carrier for solid dispersion are Polyvinylpyrrolidone (Povidone, PVP), Polyethylene glycol (PEGs), Plasdone S630. Surfactants such as Sodium lauryl sulfate (SLS), Tween 80 and Docusate sodium (Kumar and Singh, 2016)

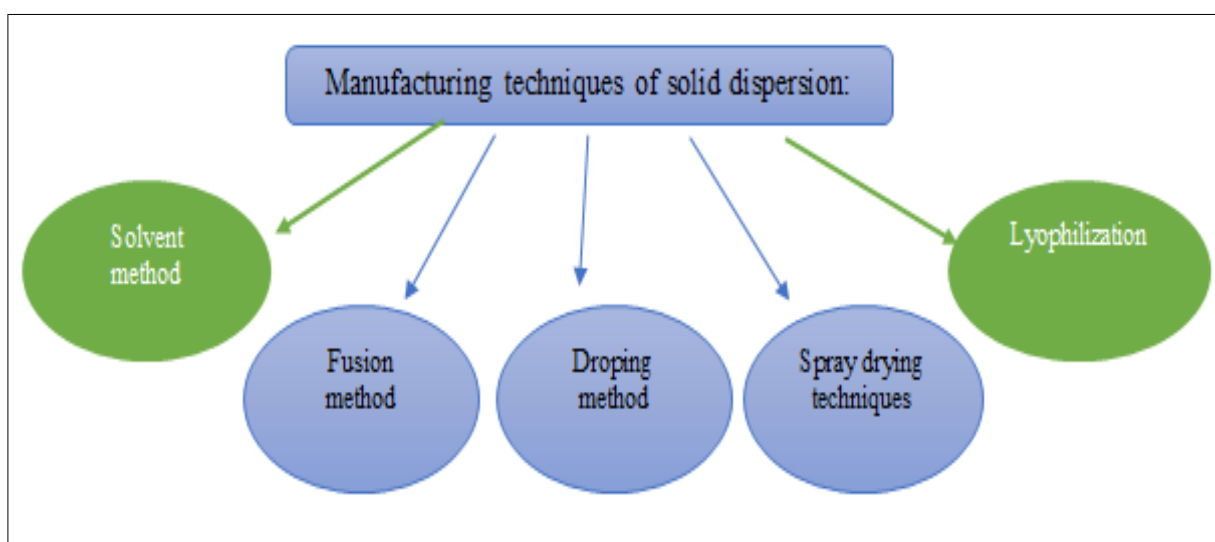


Figure 1.4.Diagrammatic representation of manufacturing techniques of solid dispersion

1.5.1.2) Cryogenic technique: cryogenic techniques produces nano range amorphous drug particles with high porosity at very low temperature thus enhances the solubility of the drug. Factors which affect the cryogenic inventions are type of injection device (capillary, rotatory,

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pneumatic and ultrasonic nozzle), location of nozzle (above or under the liquid solvent), and the composition of cryogenic liquid (hydro fluoroalkanes, nitrogen, argon, oxygen and organic solvent). After the completion of the process, drying can be done by various techniques: spray freeze drying, atmosphere freeze drying, vacuum freeze drying and lyophilization (Savjani et al., 2012).

Types of cryogenic techniques:

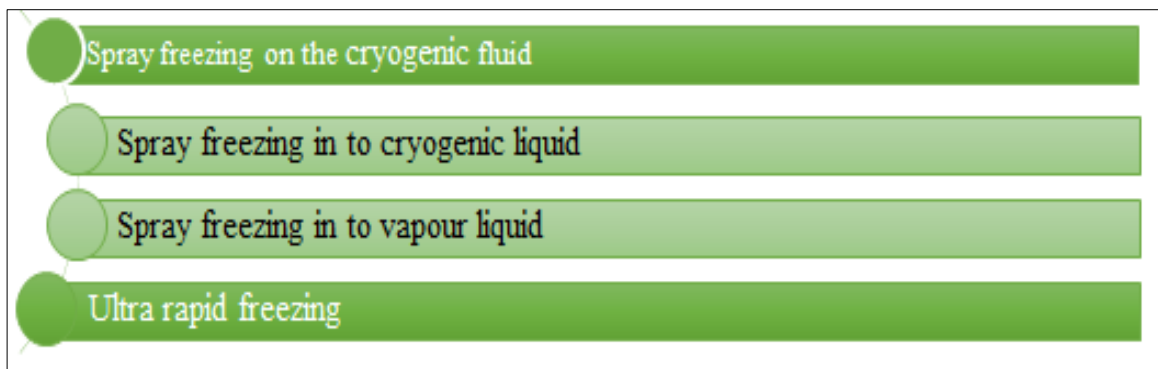


Figure 1.5. Diagrammatic representation of cryogenic techniques

1.5.2) Chemical method: Chemical methods include:

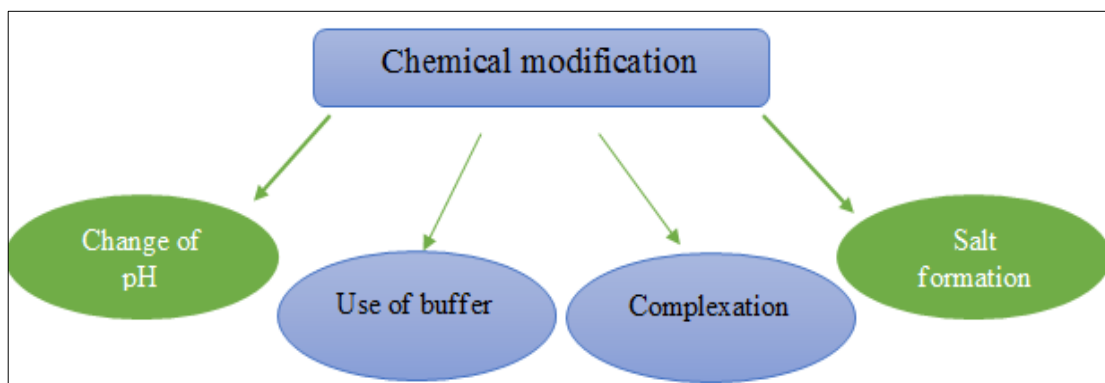


Figure 1.6 Diagrammatic representation of chemical method of solubility enhancement

1.5.2.1) Change of pH: The absorption of the drug within gastrointestinal tract is largely dependent on its pKa value and pH value of individual regions. By this method, the hydrophobic drug may potentially dissolve in water by protonating or deprotonating the molecule. For weakly acidic drugs with lesser pKa value and weakly basic drugs with higher pKa value, it is the simplest and most adequate technique of increasing their solubility. There is a very little effect pH adjustment on nonionizable drugs (Vadlamudi and Dhanaraj, 2016)

1.5.2.2) Complexation: Complexation is defined as the interaction between two or more than two molecules which give rise to a nonbonded moiety with distinct stoichiometry. It involves

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comparatively weak forces like hydrophobic interaction, London forces, and hydrogen bonding (Hiral et al., 2012)

Table 1.5 Various types of complexing agents used in the pharmaceutical formulations are:

S/No.	Types of complexation agents	Examples
1.	Inorganic type	I _B ⁻
2.	Coordination type	Hexamine Cobalt(III) Chloride
3.	Chelates	EGTA, EDTA
4.	Metal olefin	Ferrocene
5.	Inclusion	Choleic acid, Cyclodextrins.
6.	Molecular complexes	Polymers

Two types of the complex:

1.5.2.2.1) Stacking complex: Stacking complexes are formed by the association of nonpolar part of a drug and a complexing agent which leads to the elimination of water molecule from the nonpolar part. This causes a reduction in total energy of the system. These complexes can be homogenous or heterogeneous. Examples of compounds that form stacking complexes are Nicotinamide, pyrene, anthracene, salicylic acid, and benzoic acid.

1.5.2.2.2) Inclusion complexes: Inclusion complexes are an association of nonpolar part of one molecule into the cavity of another molecule. It is non-bonded entity does not include forces so called as non-bonded complexes. Cyclodextrin and their derivatives are widely used in complexation. Cyclodextrin forms complex with drug and enhance its solubility and bioavailability. Most common derivatives of cyclodextrin used in pharmaceutical formulations are R- cyclodextrin, and hydroxyl propyl-R-cyclodextrin HP-R-CD (Kadam et al., 2013)

Inclusion complexes are prepared by:

1.5.2.2.2.1) Kneading method: (Vemula et al., 2010)

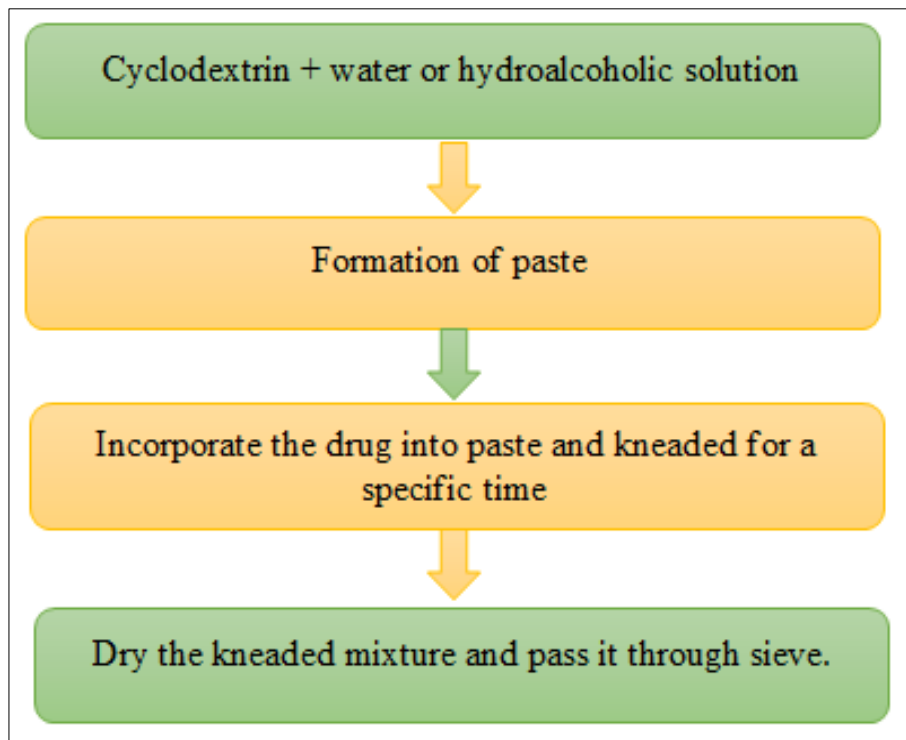


Figure1.7. Diagrammatic representation of kneading method

1.5.2.2.2.2) Neutralization precipitation: (Chaudhary and Patel 2013)

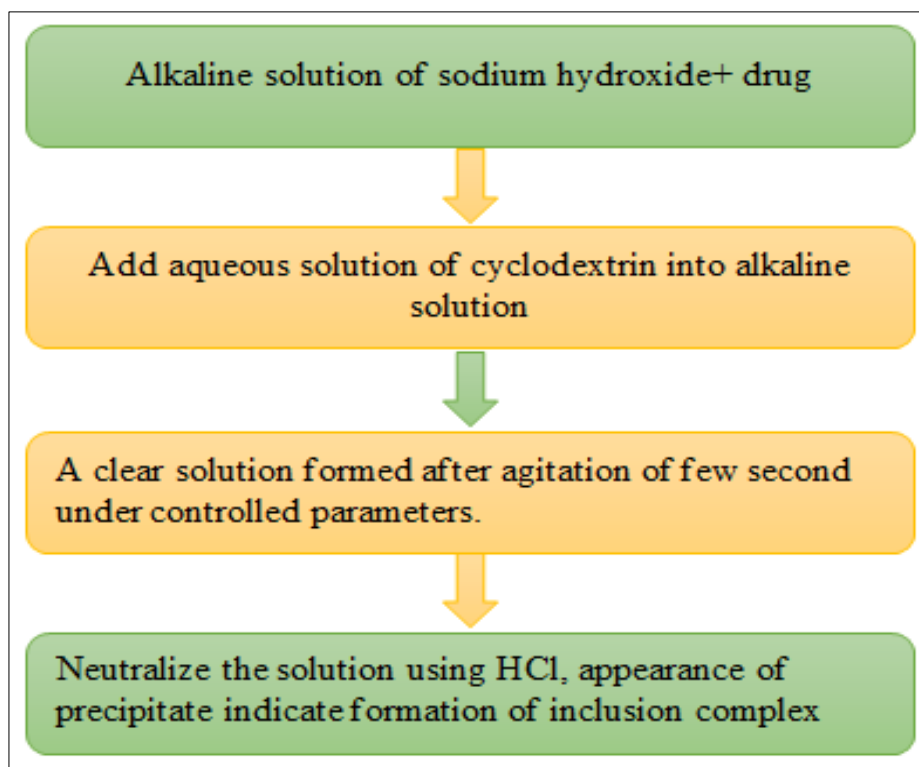


Figure1.8 Diagrammatic representation of neutralization method.

1.5.2.2.2.3) Co-grinding Method: (Kadam et al., 2013)

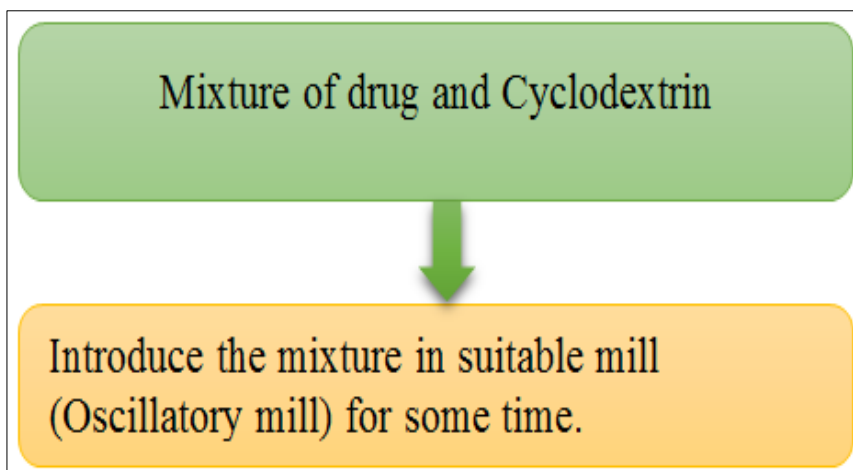


Figure1.9 Diagrammatic representation of Co-grinding method

1.5.2.2.2.4) Spray-Drying Method: (Kumar and Singh, 2013)

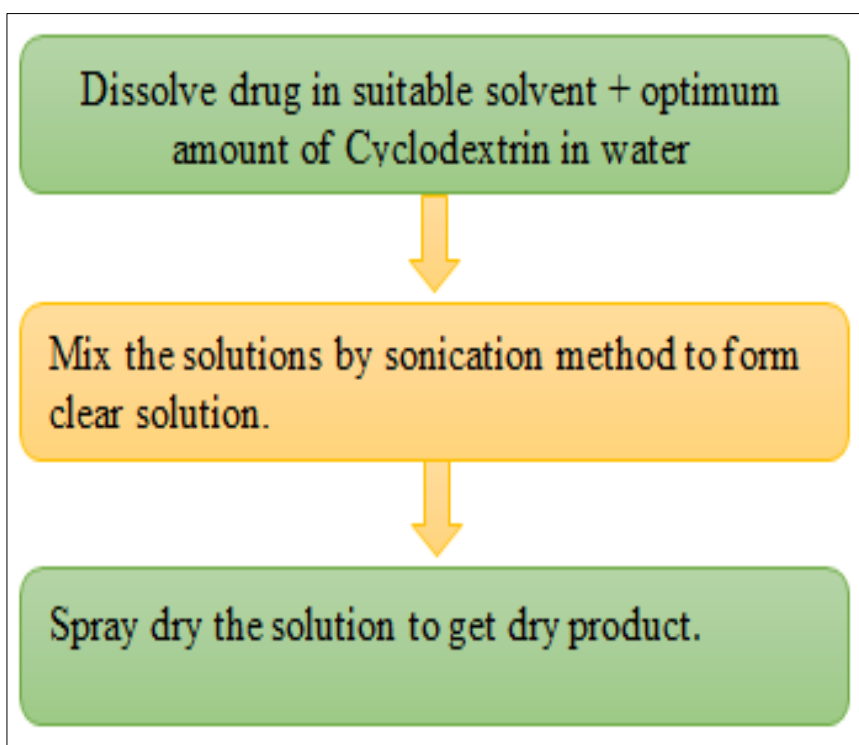


Figure1.10 Diagrammatic representation of Spray-drying method

1.5.2.2.2.5) Microwave irradiation Method: (Patil et al., 2010)

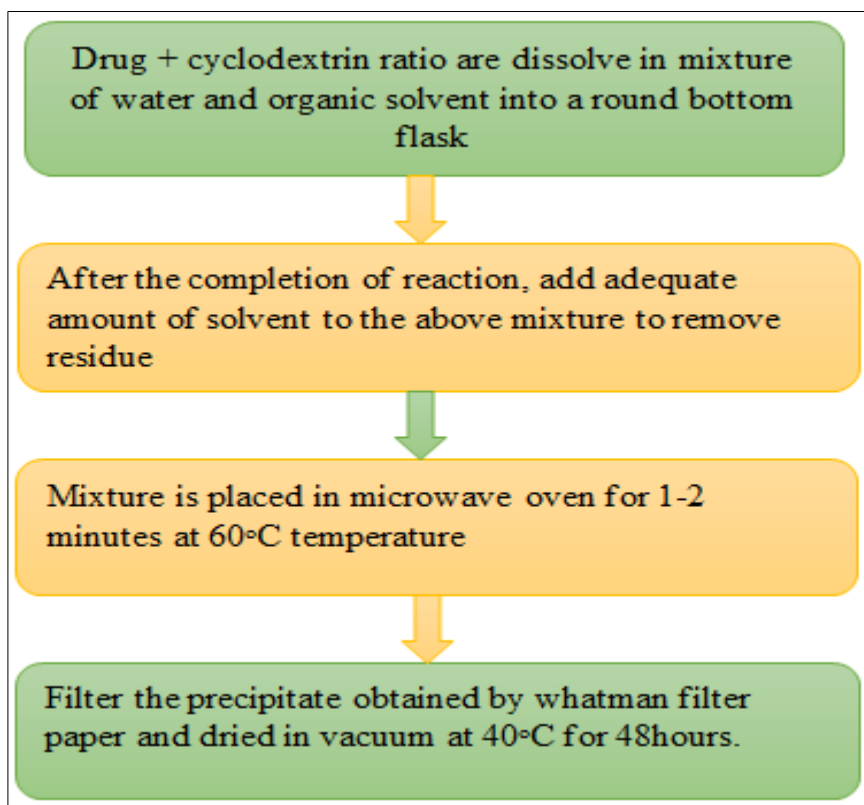


Figure1.11 Diagrammatic representation of Microwave irradiation method

1.5.2.2.2.6) Freeze drying technique: Suitable for thermolabile drugs (Chaudhary and Patel, 2013)

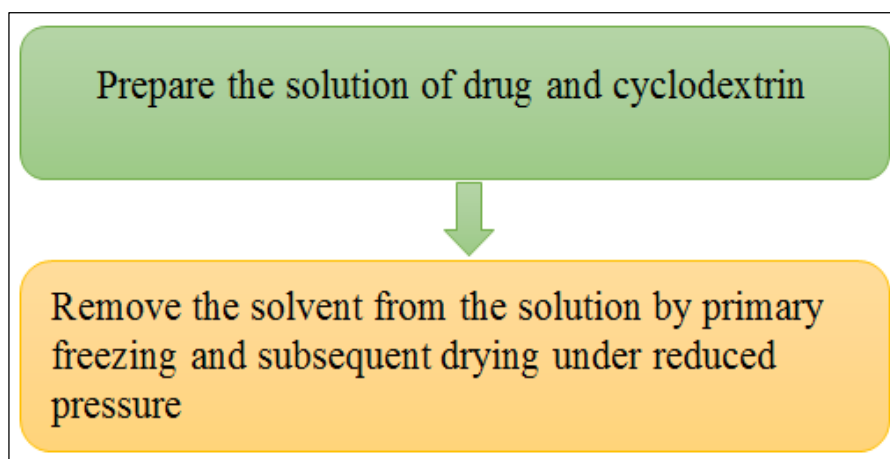


Figure1.12 Diagrammatic representation of Freeze drying technique

1.5.2.3) Salt formation: Almost 75 % of all drugs are weak base, 20 % are weak acids, and remaining 5 % are non-ionic, amphoteric, and alcohols in nature. Salt formation is the simplest and most efficient method to enhance the solubility of the acidic and basic drugs. Improvement

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in solubility of these drugs can be achieved by selection of suitable salt. Weak acid and weak base can be used to form a salt of these drugs (Sikarra et al., 2012)

1.5.3) Miscellaneous methods: It includes various methods:

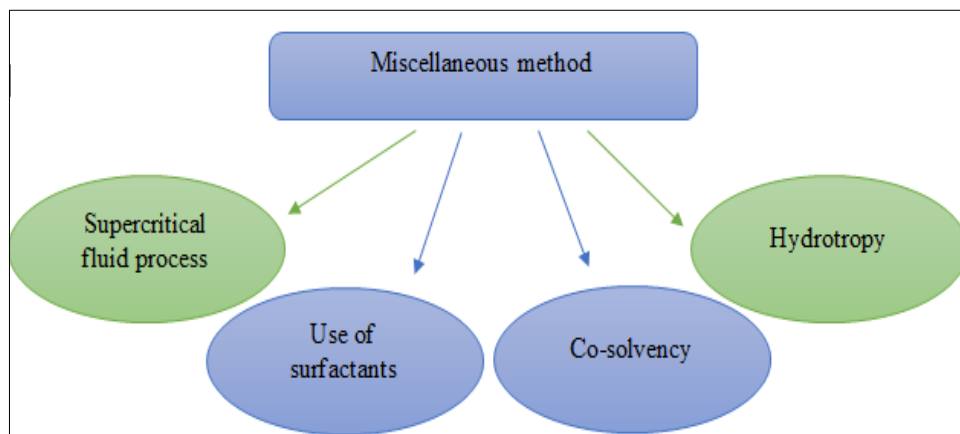


Figure1.13 Diagrammatic representation of miscellaneous methods

1.5.3.1 Supercritical fluid process: Supercritical fluid can be described as dense non-condensable fluid whose temperature and pressure are greater than its critical temperature and critical pressure having properties of both liquid and gas. It is the novel approach of solubility enhancement and nanosizing which increase the particle size reduction by supercritical fluid processes. Carbon-dioxide is widely used in supercritical fluid processes either as a solvent or as an antisolvent. SCF processes reduce the size of particle up to 5-2000nm in diameter (Thakkar et al., 2009)

Two basic techniques in Supercritical fluid technology are:

1.5.3.1.1) RESS: In this technique, a supercritical solvent which is completely saturated with solute molecules is allowed to flow at a rapid rate, leads to precipitation of solute molecules. Rapid expansion is acquired by passing the solute from the nozzle at supersonic speed cause super-saturation of solute molecules. This process is called supercritical fluid nucleation (Sheetal Z Godse and Patil, Swapnil M Kothavade, 2013).

1.5.3.1.2) Supercritical antisolvent technique: Supercritical fluids as an antisolvent includes Gaseous Antisolvent (GAS), Supercritical Antisolvent (SAS), Aerosol Solvent Extraction System (ASES), and Particles by Compressed Antisolvent (PCA). The process is based on the principle that when the solution is accurately expanded by a gas, the solute phase becomes insoluble in solvent and precipitation occurs. The highly useful for hydrophilic drugs (Parhi and Suresh, 2013)

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1.5.3.2) Use of surfactants: Use of surfactants to increase the solubility of the drug is the conventional approach which reduces the interfacial tension between the surface of solvent and solute particles leads to increased wetting and salvation interaction. Various surfactants used are Polyglycolized glyceride, Span, Tween, polyoxyethylene stearates and synthetic copolymers like poloxamer, poly (caprolactone)-b-poly (ethylene oxide) (Parve et al., 2014).

1.5.3.3) Colsolvency: The solubility of hydrophobic drugs can be enhanced by adding water miscible solvent in which drug show good solubility called co-solvent. Cosolvent is mixtures of one or more water-miscible solvent and water which increases the solubility of the drugs. The various solvents which are used as co-solvent are PEG 300, ethanol or propylene glycol. Co-solvent increases the water solubility of the drug several times as compare to the solubility of drug alone. The technique can be used for both oral and parenteral formulation. Examples of co-solvent used for parenteral formulation are ethanol, propylene glycol, polyethylene glycol, glycerine, Dimethylsulphoxide and, Dimethylacetamide (Parve et al., 2014).

1.5.3.4) Hydrotropy: Hydrotropy is defined as the process to enhance the solubility of hydrophobic drugs by the addition of a lot of additives. Hydrotropic agents are ionic organic salts includes ethanol, aromatic alcohol, urea, a- or b-naphthols, catechol and salicylates and nicotinic ionic surfactants like dodecylated oxibenzene, diacids, and solute contains alkali metal salts of different organic acids. A Concentrated solution of sodium benzoate, sodium-p-hydroxybenzoate, sodium-o-hydroxy benzoate, nicotinamide, urea, sodium citrate and sodium acetate are used to enhance the aqueous solubility of poorly soluble drugs. The enhancement of solubility is due to salt-in effect. The principle on which it works is similar to complexation which involves a weak bonding between the solute and hydrotropic reagents (Kadam et al., 2013).

1.5.4) Nanotechnology approaches to increase solubility:

Nanotechnology is defined as the investigation and utilization of material and structure at nano-range of approximately 100 nanometres or less (Patel et al., 2012).

1.5.4.1) Nanocrystals:

Nanocrystal is a crystalline solid having proportion in nanorange; or nanoparticle crystalline structure. Nano-crystallisation is the technique of converting drug particles to nano range of 1-1000 nanometres. The advantage of this method is that it can be used for any drug. There are two different methods that can be applied to produce nanocrystals; top down and bottom up method. The top-down method includes milling and high-pressure homogenization and

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Bottom-up method include precipitation and cryo-vacuum method (Junghanns and Müller, 2008)

1.5.4.1.1) Milling:

The wet-milling method can be used for the preparation of nanocrystals. In ball mill reduction in particle size is done by attrition and impact forces. Widely used mills are tumbling ball mill and stirred media mill. The major disadvantages of this method are mill surfaces degradation and frequent suspension contamination (Khadka et al., 2014)

1.5.4.1.2) High-pressure homogenization:

In this technique, a colloidal aqueous dispersion containing crystalline drug is passed through a narrow gap with high velocity and high pressure. Dissociates formed if homogenization is done in water or Nanopure in non-aqueous medium. Particle size reduced by shear and cavitation forces. In liquid high pressure exerted causes the liquid to boil leads to the formation of gas bubbles. The factors which effects the particle size obtained from homogenization is nature of the drug, the number of homogenization cycles and applied pressure (Hui et al., 2015)

1.5.4.1.3) Precipitation:

In precipitation method, a firstly dilute solution of the drug is prepared by dissolving it in an appropriate solvent. Then the solution of the drug is injected into water. At the time of injection, the water is vigorously stirred so as to precipitate drug into nanocrystals. Nanocrystals are filtered from the solution and then air dried (Patel et al., 2012).

1.5.4.1.4) Cryo-vacuum method:

In this method, the drug is firstly dissolved in water to gain quasi-saturated solution. The principle step of the method is immediate cooling of above solvent by immersing it into liquid nitrogen at -196°C . Rapid cooling leads to a sudden rise in the degree of saturation based on decreased solubility and formation of ice crystals when the temperature falls below 0°C . It leads to fast nucleation of dissolved drugs. This method leads to the formation of pure crystals without using harmful reagents (Patel et al., 2012).

1.5.4.1.5) Crystallization method for preparation of nanocrystals:

Crystallization method for preparation of nanocrystals followed dissolution, nucleation, the growth of crystals, filtration, and drying. Crystallization method minimizes the requirement of mechanical energy. Crystallization methods involve conventional crystallization techniques as well as supercritical fluid, ultrasonication, high gravity, microemulsion and cryogenic techniques (Gao et al., 2015).

1.5.4.1.5.1) Reactive crystallization:

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This method follows instant chemical reaction as well as rapid nucleation. In this method, the particle size distribution and distribution of super-saturation is effected by mixing process. This method has not been widely used due to the limitation of reaction speed. Acid-base neutralization has been used for the preparation of nanocrystals of azithromycin and itraconazole with a diameter of 413nanometers and 279.3nanometers. The method is appropriate for hydrophobic drugs having weakly basic or acidic property should be soluble in strong acid (HCl) or basic solution (NaOH). Neutralization of solution is done by addition of acid or base proceed to decrease of solubility further, induces supersaturation. Nanocrystals produced by this method are stabilized with help of stabilizers

1.5.4.1.5.2) Anti-solvent crystallization:

In this method, the super saturation which is induced by mixing the solution with an anti-solvent leads to crystal nucleation and growth by two processes coagulation and condensation. The drug must be sufficiently soluble in the solvent but insoluble in antisolvent. This method is further classified by various methods (Sadeghi et al., 2016)

1.5.4.1.5.3) Hydrosol crystallization:

This approach is used for hydrophobic API which is soluble in the water-miscible organic solvent. From this method, nanocrystals can be directly obtained by mixing organic drug solution with the liquid phase. The only limitation of this method is difficult to control the mixing condition. Mostly amorphous nanoparticles are obtained from this process (Chan and Kwok, 2011)

1.5.4.1.5.4) Ultrasonic crystallization:

Ultrasonic crystallization can be simply done by dipping a sonicator probe into a reaction vessel which further kept on stirring to mix the solution with antisolvent. The ultrasound irradiation leads to a reduction of particle size and increases the micro-mixing. The particle size can be controlled by various parameters such as depth of horn immersion, horn length and sonicator duration, intensity and frequency. Nanocrystals of nitrendipine have been successfully prepared by this method with an average diameter of 200nm (Sander et al., 2014)

1.5.4.1.5.5) Flash nanocrystallization:

Flash nanocrystallization is a process, using rapid micromixing to prepare high supersaturation and kinetically controlled aggregates of poorly water soluble drugs. This technique is further classified as confined liquid impinging jets (CLIJ) and multi-inlet vortex mixer (MIVM) by two different types of mixers. (Singh et al., 2016)

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In confined liquid impinging jets, crystallization process occurs in a region of intense mixing and extreme turbulence which is created by a jet of drug solution impinging a stream of antisolvent coming through two alternate nozzles mounted on a chamber. Micro-mixing time which controls the particle size must be shorter than the formation time of nanocrystals.

The process of multi-inlet vortex mixer, the organic solute, amphiphilic block copolymers and inorganic nanostructures are dissolved in a water-miscible organic solvent. Rapid mixing of organic solvent with water leads to high super-saturation in short period of time to start instant nucleation. Peptide nanocrystals have been reported to be produced by this method successfully.

1.5.4.1.5.6) Microfluidic crystallization:

In this method, the streams of anti-solvent and drug solution are mixed in a microfluidic reactor which generates one or more flow pattern by changing the inlet angles or diameter. The two fluid coming in reactor keep laminar flow without turbulence leads to molecule diffusion across the interface. When mixing and diffusion occurred in the diffusion layer, the super-saturation occurred which leads to growth and crystal nucleation. Hydrocortisone crystals are successfully prepared by this method with an average size of 80-450nm

1.5.4.1.5.7) High-gravity controlled precipitation:

This method is performed in high gravity conditions. The particles obtained by this method have a narrow size distribution. The method involves impinging of two streams in rotating packed bed which is then subjected to high gravity condition, offers to flow the mixture through packing before leaving the reactor vessel. This technique can be categorized into antisolvent type and reactive type.

1.5.4.1.5.8) High gravity antisolvent precipitation:

In this method, one of stream contains drug solution and other contains anti-solvent. The rapid mixing of two solution leads to nucleation and growth of drug particles. The nanoparticles of cefuroxime axetil with an average size of 300nm have been prepared by this method.

1.5.4.1.5.9) High gravity reactive precipitation:

In HGRP method, the two streams of reactants are fed into distributors. The instant mixing leads to the formation of nanoparticles due to chemical reaction. The nanoparticles of azithromycin with a size range between 300 and 500nm have been produced by this method.

1.5.4.1.5.10) Evaporative crystallization: (Gao et al., 2015).

Spray drying:

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It is a most widely used method, involves the use of colloidal or sol particles as precursors. After that, the fine droplets are evaporated in hot air to form dry particles. The method is widely used for the preparation of solid lipid nanoparticles and microparticles. The nanocrystals prepared by this method are amorphous in nature (Patel, 2015)

Electrospraying:

In this method, nanoparticles are prepared by flowing the liquid through the capillary nozzle under the influence of electric current which evaporates the solution to form dry nanoparticles. The size and charge of the droplets can be controlled by adjusting the voltage applied and flow rate of the nozzle. The nanoparticles prepared by this method are highly charged that prevent aggregation and helps in self-dispersion. The nanoparticles obtained from this method are amorphous in nature and the method is not suitable for liquid with a surface tension greater than 50mN/m or conductivity more than 10^{-4} m/S. insulin nanocrystals have been prepared by this method with a size range of 88-117nm.

1.5.4.1.5.11) Evaporative precipitation into aqueous solution:

In this process the, the heated organic solution of the drug is atomized into an aqueous phase which causes rapid evaporation leads to fast nucleation results in nanoparticle suspension. The suspension then dried by lyophilization and spray drying. Amorphous nanocrystals of cyclosporine A has been prepared by using this method.

1.5.4.1.6) Combination methods: (Gao et al., 2015).

Combination techniques are new methods to prepare the nanocrystals, which couple crystallization process with mechanical processes. Mechanical energy can be supplied by various methods such as high-pressure homogenization, milling, high energy mixing and ultrasonication. High-pressure homogenization is most commonly employed process. HPH involves impact forces to prepare drug nanocrystals. The approach has been used to prepare fenofibrate (Triglide) nanocrystals.

1.5.4.1.6.1) Combination of microprecipitation with high-pressure homogenization:

In this process, the first drug is dissolved in an organic phase. Then the resulting solution is mixed with an antisolvent which is aqueous in nature. The mixing leads to the formation of crystals by precipitation. Now, HPH is the use of further reduces the size of the crystals.

1.5.4.1.6.2) Combination of nonaqueous freeze drying with HPH:

It is a most effective method for particle size reduction. The processing of freeze dried powder with HPH leads to the formation of smaller particles of average size 100nm with only few homogenization cycles.

CHAPTER- 2

LITERATURE REVIEW

A Literature review is to establish the theoretical framework for the topic. With the help of literature review, the research scholar can follow the chronological method to describe the topic.

Work done on Nanocrystals:

Drug nanocrystals are defined as crystals with the size range of nanometer. They are also known as nanoparticles with crystalline nature. Nano-crystallisation is the technique of converting drug particles to nano range of 1-1000 nanometres. Nanocrystals increase the bioavailability of the drug for both parenteral as well as oral route.

2.1.1: Amrita et al., 2012 the research article provides the detailed information about the development of telmisartan nanocrystals. In this article, the nanocrystals were prepared by Evaporative Antisolvent Technique using different stabilizers such as PEG 6000, poloxamer 188, PVP K30, and TPGS in combination or singly. The nanosuspension prepared was characterized by its particle size, poly dispersibility index, Zeta potential, DSC, and SEM. The resultant nanocrystals were found to be in nano range with average particle size of 82.63nm. Nanosuspension of telmisartan were prepared which showed a tenfold increase in bioavailability as well as stable formulation

2.1.2: Sheetal Z Godse et al., 2013 the review article provides the detailed information about the different techniques for improvement in solubility of poorly water soluble drug to enhance its wettability and dissolution rate. These techniques can be used to enhance the solubility Biopharmaceutical classification System II drugs. Most of the drug from this class are highly lipophilic and poor water solubility. In this article, Various methods such as physical modification, chemical modification, and other miscellaneous techniques have been described to improve solubility

2.1.4: Gao et al., 2015 it gives the overview of various methods of crystallization for the preparation of nanocrystals. Crystallization is the simplest and most efficient method used to prepare nanocrystals. Nanocrystals prepared by crystallization method are both crystalline and amorphous in nature. In this article, various methods such as traditional method and new methods for preparation of nanocrystals has been described. Traditional methods include precipitation crystallization and reactive crystallization whereas new methods contain high gravity controlled precipitation and supercritical fluid crystallization to prepare

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nanocrystals. All the methods for preparation have been described in the article with its merits and demerits

2.1.5: Gajanan Shinde et al., 2014 the article provides the detailed procedure about the formation of Repaglinide nanocrystals. Repaglinide is the BCS class II drug which is used for diabetes. To improve its solubility its crystals were prepared by high-pressure homogenization. Different batches were prepared by optimizing the type of polymer, percentage polymer concentration, a number of cycles and HPH pressure for the formulation of nanocrystals. The resultant nanocrystals were characterized for its particle size, poly dispersibility index, zeta potential, DSC, X-ray diffraction and SEM. Average particle size of the formulation was found to be 187nm

2.1.6: Basavaraj K. Nanjwade et al., 2014 The article is based on the preparation of nanocrystal of lovastatin by precipitation method. Lovastatin is BCS class II drug which possesses high lipophilicity but poor water solubility. This study has been carried out to increase its solubility and dissolution rate. Precipitation method was used to prepare crystals of Lovastatin. The method involves two steps: Preparation of drug solution in organic solvent and Addition of drug solution in water. The various formulation has been prepared by changing the stabilizer, surfactants and solvent system. The nanocrystals result in enhanced solubility, Dissolution rate and increased bioavailability in the systemic circulation

2.1.7: Arun Kute et al., 2014 The article reveals complete information about the design, development and complete evaluation of valsartan nanocrystal for solubility enhancement. Valsartan is an antihypertensive drug used in the management of essential hypertension. Valsartan is highly lipophilic and poorly water soluble drug. To increase its solubility its nanocrystals has been prepared. The nanocrystals were prepared by using antisolvent precipitation method using polymer like PVP K30, and PEG -4000 as a cryoprotectant and acetone as solvent. Nine formulations have been prepared by changing the concentration of polymers. The resultant nanocrystals are characterized by particle size, zeta potential, and poly dispersibility index and many another parameter such as Differential Scanning calorimetry , Scanning Electron Microscopy and stability studies. nanocrystals with drug polymer ratio found to be stable with least particle size

2.1.8: Sunny Shah et al., 2015 the article demonstrates the investigation carried out to design, optimize and evaluation of Lurasidone Hydrochloride Nanocrystals for dissolution characteristics and solubility enhancement. Nanocrystals are prepared by media milling method by using Zirconium oxide beads. Various stabilizers used for stabilizing the preparation

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of nanocrystals are PVP K30, SLS, Poloxamer 188, HPMC and PVP-S 630. Resultant nanocrystals obtained are crystalline in nature

2.1.9: Annika Tuomela et al., 2016 the article describes the stabilizing agents for drug nanoparticles for solubility enhancement. The article gives the complete description of the polymers and surfaces active agents that have been utilized for the preparation of nanocrystals. These Stabilizing agents are used to stabilize the nanocrystals formulation by surrounding the drug core. Typical stabilizers are amphiphilic in nature so as they enhance the wettability and dissolution of the nanocrystals. Stabilizing agents are basically divided into various categories such as ionic surfactants, non-ionic surfactants, polymeric stabilizer and a miscellaneous category. Some examples are Poloxamers, Celluloses, Vitamin E TPGS, Soluplus, etc

2.1.10: Simeng Mu et al., 2016 in this article Spironolactone nanocrystals were prepared by wet milling method for oral use. The milling was done by using PM planetary ball mill By changing various polymers such as HPMC-E5, F68, and F127. Lyophilization was done to obtain the powder form of nanocrystals. further Characterization was done by like Differential scanning calorimetry, X-Ray diffraction, and Raman spectroscopy. In vitro dissolution testing has been done to check the release of the drug which shows improved bioavailability

2.1.11: Jan P. Moschwitz et al., 2012 the article provides the detailed information about the drug nanocrystals in the pharmaceutical development process. The two top-down processes wet ball milling and high-pressure homogenization are widely used at industrial scale. The article provides the detailed information about the commercial drug nanocrystals, their method of preparation. More than 50 nanocrystals formulation are present in the market which are prepared by two methods one is High pressure homogenization and Wet ball Milling

2.1.12: Qiang Fu et al., 2016 the article is based on nisoldipine nanocrystals for improvement of oral bioavailability. Nisoldipine is a poorly water soluble drug which is used for management of hypertension. Nisoldipine nanocrystals are prepared by a media-milling method in different stabilizer solution such as PVP K30, HPMC-E5, and SDS. Then the formulation is checked for various characterization parameters like Scanning Electron Microscopy, Differential Scanning Calorimetry, Powder X-ray Diffraction, FT-IR, Dissolution and animal testing. Average size of the nanocrystals was found to be less than 300nm

2.1.13: Mauludin et al., 2012 the article illustrates the formulation approaches for fast dissolving Ibuprofen Nanocrystals. it is a nonsteroidal anti-inflammatory drug which is used in the treatment of inflammatory rheumatoid arthritis. In this study, the nanocrystals were prepared by High-Pressure Homogenization Technique incorporated pellet and effervescent

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formulation. The nanocrystals were obtained by Lyophilization and spray drying of the nanosuspension. The resultant nanocrystals have shown excellent dissolution in .1N HCl within 15 minutes

2.1.14: Balamarkonda CH et al., 2013 the article explains the significance of nanocrystals in drug delivery system. It also gives the information regarding the detailed procedure for preparation of nanocrystals which includes Bottom up, top down, combination technique, spray drying and other methods used in the preparation of nanocrystals. Various Characterization methods such as determination of drug content, Scanning electron microscopy, Differential Scanning calorimetry, dissolution testing and stability studies has been explained. Pharmaceutical application of nanocrystals in drug delivery such as oral administration, parenteral administration, Ophthalmic delivery system, targeted drug delivery and dermal drug delivery were also mentioned in the article

2.1.15: Smet et al., 2012 prepared paclitaxel nanocrystals by wet milling technique using stabilizer which is Pluronic F127 and F68 for hyperthermic intraperitoneal chemotherapy. paclitaxel is used for the treatment of ovarian cancer. the prepared nanocrystals were Characterized mean particle size, zeta potential, poly dispersibility and Scanning electron microscopy. They evaluated the effect of Paclitaxel nanocrystal for the treatment of HIPEC by checking the cytotoxicity of both the stabilizer and the formulation. Moreover, the effect of nanocrystalline formulation on tumor growth was evaluated by MRI at the interval of 7days in rats

2.1.16: Wang et al., 2012 the article reveal the complete information about the formulation and evaluation of puerarin nanocrystals and further assessment of pharmacokinetic parameters of the developed formulation. In this article, nanocrystals have been prepared by two different techniques such as the top-down and bottom-up method by changing the concentration of stabilizers and surfactants. The nanocrystals obtained were crystalline in nature with average particle size of 423.6nm and polydispersity index 0.13. The resultant nanocrystals exhibited reduced Cmax and clearance but increased half-life and mean resistance time as compare to the puerarin solution

2.1.17: Oner et al., 2010 the article summarizes the preparation of ezetimibe nanocrystals to increase the solubility and dissolution rate. Nanocrystals were prepared by wet milling and high-pressure homogenization method by using pluronic F127 as a stabilizer. Nanocrystals of the formulation were obtained by lyophilisation for 72hrs at the -55°C temperature at

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0.01mmhg pressure. after drying, tablets were prepared from nanocrystals. Characterization of the formulation was done by FTIR, differential Scanning Calorimetry, X-Ray diffraction and in vitro dissolution testing. On evaluation, it was noticed that the dissolution rate of nanocrystal formulation is more as a comparison to the tablet formulation

2.1.18: Amighi et al., 2005 the article provide the detailed description of nifedipine nanocrystals for the treatment of high blood pressure. Nifedipine belongs to the category of Calcium channel blockers and can also be used to treat angina pectoris. High-pressure homogenization was used to prepare nanocrystals of nifedipine along with HPMC. Homogenization was done in two steps first is milling low-pressure homogenization and then 20 cycles with high-pressure homogenization. The formulation was evaluated for in-vitro and in-vivo studies results into 6 fold enhancement of permeation rate across the different in-vitro models

2.1.19: Cui et al., 2011 formulated niterdipine nanocrystals using tandem precipitation homogenization method followed by spray drying. The resultant nanocrystals found to be crystalline in nature and characterized by different parameters such as differential scanning calorimetry, X-ray diffraction and Scanning Electron Microscopy. The in-vitro dissolution profile of niterdipine nanocrystals was found to increase as compare to commercial tablet and physical mixture. The in-vivo testing states that the maximum concentration of nanocrystals was increased up to 15 fold and 10 fold more than the commercial tablet and physical mixture

2.1.20: Zuo et al., 2013 prepared fenofibrate nanocrystals by using bead milling method. They analyzed five type of hydrophilic excipient in combination with sodium dodecyl sulfate. Spray dried nanocrystals were prepared by using mannitol and sodium dodecyl sulfate. They found that the size of nanocrystals strongly influenced by the inlet temperature, mannitol and weight ratio of fenofibrate. Dissolution profile obtained from the spray dried nanocrystals was quite similar to the commercial nanocrystal formulation but faster than the micronized formulation

2.1.21: Kumar et al., 2014 illustrate the formulation and Characterization of lecithin complexed glibenclamide nanocrystals. They analyzed the effect of lecithin and PEG 20000 on the properties such as stability and particle size reduction of the nanocrystals. Studies state that the average particle size of pure glibenclamide is 1551nm where the size of nanocrystals was between 155-842nm. Finally, they found that the lecithin complexed glibenclamide nanocrystals exhibit enhanced stability profile as well as surface properties and can be used for the preparation of highly stabilizing formulation

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2.1.22: Nayak et al., 2015 prepared nelfinavir mesylate nanocrystals to improve pharmacokinetic properties of the drug using ball milling. Nelfinavir is used to treat HIV infection but due to its poor water solubility it precipitates in the intestine and shows poor bioavailability. They prepared nanocrystals by top-down technique and finally dried by lyophilization using mannitol as a cryoprotectant. Nanocrystals with PVA as stabilizer shows high dissolution behavior as compared to the nanocrystals prepared with Poloxamer 407. SEM and DSC showed that there was a slight decrease in crystalline nature of the drug as compare to the raw drug

2.1.23: Nakarani et al., 2010 prepared itraconazole nanosuspension to increase the aqueous solubility and the formulation related parameters such as saturation solubility, dissolution rate and oral bioavailability of the drug. They used pearl milling technique using zirconium oxide beads, glycerol as a wetting agent and poloxamer as a stabilizer. Optimization of the formulation was done by changing various parameters such as size of the beads and timing of stirring. On drying the mean particle diameter was found to be 294nm. SEM and DSC showed that there is no change in crystalline behavior and melting point of the drug. Hence the formulation is found to be stable with high drug content

2.1.24: Ravichandran et al., 2014 formulated capsule of curcumin nanocrystals and compare it dissolution profile with the marketed capsule in different media. The aim of the study is to obtain the stable nanocrystals drug capsule with greater dissolution velocity and increased saturation stability. Result demonstrates that the nanocrystals formulation leads to better bioavailability as compare to poorly soluble drug

2.1.25: Du et al., 2013 formulated glimepiride nanocrystals to increase the dissolution properties of the drug. The in-vitro dissolution testing showed increase dissolution rate of nanocrystals loaded capsule of glimepiride as compare to micronized and marketed capsule. In-vivo studies showed an evident increase in bioavailability of nanocrystals loaded capsule of glimepiride as compared to the marketed formulation

2.1.26: Abdul Hasan Sathali A et al., 2013 formulated and evaluated nanocrystals of Paliperidone. Nanocrystals have been developed to enhance the solubility and dissolution rate of the drug. In this article, nanocrystals were prepared by nanoprecipitation method by using polymer at different concentration and various stabilizing agents poloxamer 188, poloxamer 407 and PVP K30. The dried nanocrystals obtained by lyophilisation. Characterization was done on the basis of Particle size, Infrared spectroscopy, Differential Scanning Calorimetry, Determination of entrapment efficiency, and in-vitro dissolution study, drug release pattern,

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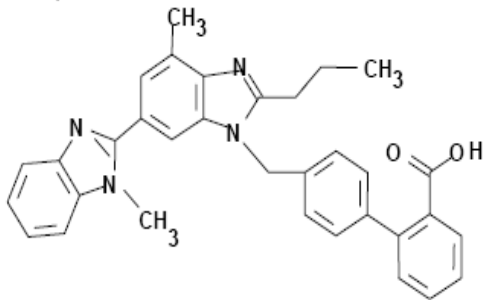
stability studies data and X-ray diffraction. The particle size found in the range of 100-200nm and poly dispersibility index in the range of 0.1 to 0.2

2.1.27: Mansouri et al., 2011 prepared nanocrystals of ibuprofen by solvent/ antisolvent precipitation method. Ibuprofen Nanocrystals.it is a nonsteroidal anti-inflammatory drug which is used in the treatment of inflammatory rheumatoid arthritis and moderate pain. The aim of the study is to increase the dissolution rate of the drug in water solvent. The preparation was done by using only two polymer SLS and TEA which acts as stabilizing agents. The characterization was done on the basis of particle size, Infrared spectroscopy, Differential Scanning Calorimetry, Scanning Electron Microscopy, and In-vitro dissolution study. The nanocrystals were prepared in nano range of 300-400nm. The dissolution rate of prepared nanoparticles is increased 2.33 times in comparison to the raw drug

2.1.28: Y.Lu et al., 2014 prepared and evaluated formulated nanocrystals of paclitaxel stabilized by transferrin. Clinical trials demonstrated that paclitaxel is highly effective in tumor due to high affinity for microtubules. Due to low solubility paclitaxel, it has been formulated as microparticles, nanoparticles, liposomes and use of cyclodextrins and cosolvents. The formulation was prepared by antisolvent precipitation followed by sonication method. In this method, trials were done by using various solvent, polymers. Various parameters which effect the size of nanocrystals are a time of stirring, type of solvent, the concentration of paclitaxel, stabilizer and antisolvent-solvent ratio. The formulation was stable over the period of 3 months

2.2 Drug Profile

Table 2.2

Parameter	Description
Drug	Telmisartan
Chemical structure	 <p>The chemical structure of Telmisartan is shown. It features a central benzimidazole ring system. One nitrogen atom of the benzimidazole is substituted with a methyl group (CH₃). The 2-position of the benzimidazole is substituted with a propyl group (CH₂CH₂CH₃). The 6-position of the benzimidazole is substituted with a methyl group (CH₃). The 1-position of the benzimidazole is substituted with a (1,4-biphenyl)-2-carboxylic acid group, which consists of a biphenyl system with a carboxylic acid group (-COOH) at the 2-position of the second phenyl ring.</p>
Molecular Weight	514.6
Chemical formula	C ₃₃ H ₃₀ N ₄ O ₂
IUPAC Name	[, 1 -biphenyl]-2-carboxylic acid, 1-[(1, 4 dimethyl - 2-propyl [, 6 -bi-1H-benzimidazol]-1-yl) methyl]
Appearance	White Crystalline Powder
Pharmacology	Treatment of Hypertension
Pharmacodynamics	Telmisartan inhibits the Angiotensin II receptor subtype this blocks vasoconstriction and aldosterone secretion
Oral Absorption	50%
Protein binding	90%
Metabolism	Hepatic
Rate of elimination	>98% excreted in unchanged form through faeces and urinary excretion < 1%
Mean plasma half-life	24hrs
Dosage form	Tablet 20mg, 40mg, 80mg
Melting range	261-263° C

2.3 Excipient Profile (Rowe C Raymond et al., 2009)

Surfactants:

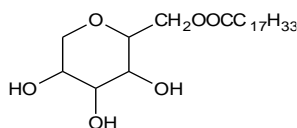
Surface active agents are the pharmaceutical chemical substances which help in reducing the surface tension/interfacial tension between two immiscible liquids.

2.3.1 SPAN-80

Span-80/polysorbate-80: - it is type of nonionic surfactant

Colour: - yellow (viscous)

Molecular structure: -



Span 80

Molecular formula: - C₂₄H₄₄O₆

Molecular weight: - 428.61

Solubility: Span 80 is soluble in ethanol, isopropyl alcohol, mineral oil and vegetable oil etc

Mechanism of action: - span-80 helps in solubilization of hydrophobic drugs by the formation of micelles.formation of micelles takes place in different steps: Firstly dissolve surfactant in water phase in a very small concentration then a fraction of surfactant will be adsorbed at air-water interface whereas remaining will be in bulk. When more of surfactant will be added then air-water interface becomes saturated and the surfactant is forced into the bulk of the liquid. at high concentration, the molecule or ion of the surfactant aggregate to form particles of a colloidal size which are known as micelles. the concentration at which micelles formation takes place is known as critical micelles concentration (CMC).

HLB value: - 4.3

Other names: - sorbitan oleate, emulsifier S 80

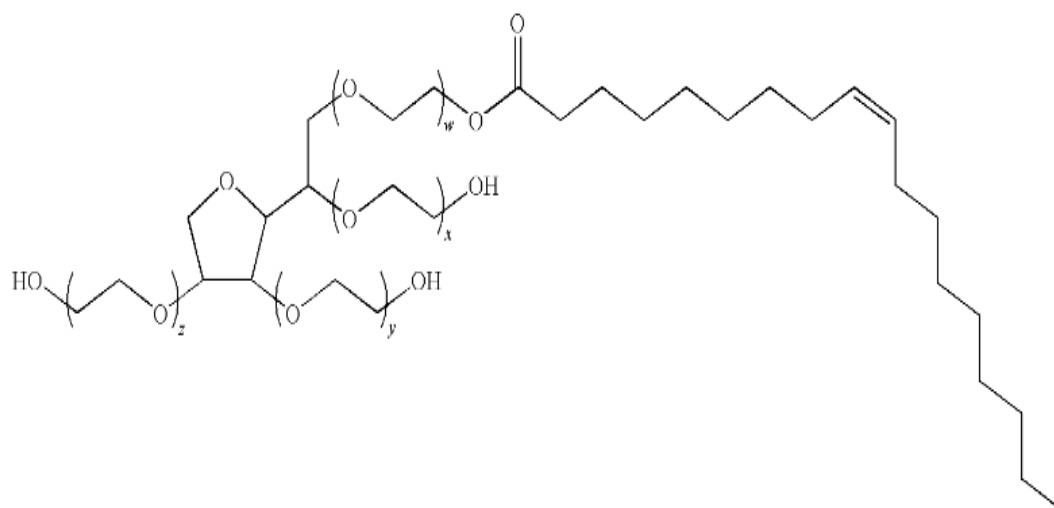
Uses: -Span 80 is used as solubilizer, emulsifier, stabilizer.

2.3.2 TWEEN-80

It's a type of nonionic surfactant

Colour: - yellow (viscous)

Molecular structure: -



Molecular formula: - $C_{64}H_{124}O_{26}$

Molar mass: - 1,310 g/mol

Solubility: - Soluble in Water, Ethan oil, vegetable oil and insoluble in mineral oil

HLB value: - 15.0

Other names: - Emulsifier T 80

Uses: - used as emulsifier, wetting agent, penetrating agent

Polymers:

The polymer word indicates 'many parts'. Polymers are long chain molecules made up of smaller repeating units called monomer.

2.3.3 Polyethylene glycol 6000

Non-proprietary name: Macrogol 6000

Synonym: Macrogol, PEG, Pluriol E, Polyethylene glycol

Chemical Name: α -hydroxy- ω -hydroxypoly (oxy-1,2-ethanediyl)

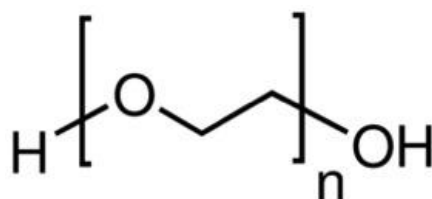
Empirical formula and Molecular formula:

$HOCH_2(CH_2OCH_2)_mCH_2OH$, where m is equivalent to the no. of ethyl groups present.

Colour: White or off-White

Odor: sweet odor

Structural formula:



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Distinctive properties:

Melting point: 55-63°C

Moisture content: hygroscopic in nature, where hygroscopicity decreases with increase in 'molecular weight.

Solubility profile:

In water: form gelly substance

In organic solvent: soluble

In oils: insoluble

Uses: lubricants, a Drug carrier, and stabilizing agent.

2.3.4 Polyethyleneglycol(PEG)-400

Synonyms: Carbowax, Macrogels

Chemical name: α -hydro- ω -hydroxypoly(oxy-1,22-ethanediyl)

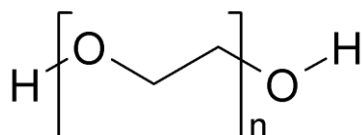
Colour: Colourless

Odor: Odorless

Molecular formula: $C_{2n}H_{4n+2}O_{n+1}$, The value of n lies between 8.2 to 9.1

Molecular weight: 380-420

Molecular structure:



Solubility:

- PEG 400 is soluble in acetone, alcohol, benzene, glycerine and water.
- And slightly soluble in aliphatic hydrocarbons.

Melting point: 4-8°C

Uses:

- Used as a plasticizer, solvent.

Lubricant for tablets and capsules.

2.3.5 Polyvinyl pyrrolidone K30:

Synonyms: PVP, Povidone, Co-povidone, crospovidone, PVPP

Chemical name: Poly[1-(2-oxo-1-pyrrolidiny)ethylene]

Colour: White or off white in color

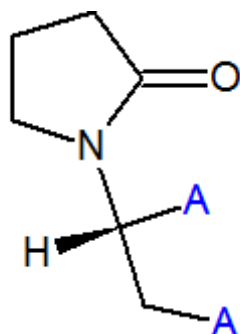
Odor: Odorless

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Molecular formula: $(-\text{CH}(\text{NCH}_2\text{CH}_2\text{CH}_2\text{CO})\text{CH}_2-)_n$

Molecular weight: 35000- 50000

Molecular structure:



Solubility:

PVP K30 is soluble in water, methanol, alcohol, ethanol, chloroform and glycerol, acetic acid. It is insoluble in dimethyl ether, acetone, toluene, ethyl acetate, xylene, and carbon tetrachloride

Melting point: 150- 180°C

Uses:

It is used as the polymer in many pharmaceutical preparations as binder, stabilizing agent and emulsifier.

RESEARCH ENVISAGED AND PLAN OF WORK

CHAPTER 3

RESEARCH ENVISAGED AND PLAN OF WORK

3.1 Rationale

Telmisartan is a lipophilic compound having antihypertensive activity. Oral administration of Telmisartan leads to only 50% of absorption which is the major drawback of the drug. However, the bioavailability of the drug is only 42% which is dose dependent and increases to 58% with an increase in dose from 40mg to 160mg. Moreover, 99% of the drug binds plasma proteins albumin and α - globulins.

This angiotensin –converting enzyme inhibitors can promote the dose-related insulin resistance, thus by reducing the dose insulin sensitivity can be increased (Wienen et al., 2000). In recent years the Nanocrystals have been widely used to increase the bioavailability of the drug. Nanocrystals presumed to enhance the solubility of the drug as well as decrease the dose of the drug. Therefore, an attempt is being made in the present study to develop the nanocrystals of telmisartan, which may offer enhanced bioavailability and dissolution rate.

3.2 Aim and objectives:

3.2.1 Aim of work

The aim of the presented work was “ Development and characterization of nanocrystals of Telmisartan by various methods”

3.2.2 Objectives

- To prepare the nanocrystals of highly lipophilic drug Telmisartan and improve the solubility and dissolution rate of the drug
- To enhance insulin sensitivity in patients suffering from hypertension and hyperglycemia
- To decrease dose-related side effect of the drug

3.3 Comprehensive plan of the work

- Selection of excipients like polymers and stabilizers
- Screening of methods for formulation of nanocrystals
- Preformulation study: compatibility study, solubility analysis, partition coefficient, pre-screening study for development of formulation
- Development of nanocrystals containing drug by optimizing various polymers and physical variable by optimization technique

RESEARCH ENVISAGED AND PLAN OF WORK

- Physical and chemical characterization of nanocrystals, particle size analysis, zeta potential, transmission electron microscopy, scanning electron microscopy, X-ray diffraction, stability study and in-vitro drug release

MATERIAL AND METHODS

CHAPTER 4

MATERIAL AND METHODS

4.1 List of material and equipment used during the study

Table 4.1 List of material used in study

S.No.	Chemical/Material	Source/Manufacturer
1	Acetone	Loba Chemie Pvt. Ltd. Mumbai, India
2	Ethanol	Loba Chemie Pvt. Ltd. Mumbai, India
3	Isopropanol	Loba Chemie Pvt. Ltd. Mumbai, India
4	Dichloromethane	Loba Chemie Pvt. Ltd. Mumbai, India
5	PVP K30	Loba Chemie Pvt. Ltd. Mumbai, India
6	PEG 6000	Loba Chemie Pvt. Ltd. Mumbai, India
7	Tween 80	Loba Chemie Pvt. Ltd. Mumbai, India
8	Hydrochloric acid	Thermo fisher Scientific Pvt. Ltd Mumbai, India
9	Potassium dihydrogen phosphate	Loba Chemie Pvt. Ltd. Mumbai, India
10	Sodium hydroxide	Loba Chemie Pvt. Ltd. Mumbai, India
11	Sodium dodecyl sulfate	Loba Chemie Pvt. Ltd. Mumbai, India

MATERIAL AND METHODS

Table 4.2 List of Equipment and software used in the study:

S. No	Equipment	Model and Manufacturer
1	Bath sonicator	Athena technology, ATS-02
2	Dessicator	Tarsons Products Pvt. Ltd. Kolkata, India 5 L
3	Electronic weighing balance	Shimadzu Co.Ltd., Japan, CY360
4	Eppendorf tubes	
5	FTIR spectrophotometer	Shimadzu Co.Ltd., Japan Spectrum 400
6	Hot air oven	Cadmach Drying Oven, Cadmach Machinery Ltd., Ahmadabad, India
7	Magnetic stirrer	Remi, Pvt. Ltd. Mumbai, India Q-5247
8	Spray dryer	Spray mate, JISL, India.
9	Particle size analyser	Beckman coulter RQ-121/D
10	pH meter	Systronic, μ pH system, India
11	UV spectrophotometer	Shimadzu Co.Ltd., Japan 2M9F365001
12	Transmission Electron microscope	Field Electron and Ion Co. TECNAI G ² F-20
13	Trinocular microscope	Kyowa getner, Japan 10390
14	Stability Chamber	REMI Electrotechnik Pvt Ltd. vasai, Mumbai, India
15	Borosilicate Type-I glass	<u>Tarsons</u> Products Pvt. Ltd. Kolkata, India
16	Syringe and needle	
18	Dissolution Apparatus	DS 8000 LABINDIA, Thane West, Maharashtra, India

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19	Scanning Electron Microscope	Japan Electron Optics Laboratory Company, Limited
20	X-ray Diffractometer	Philips Inovation Labs
21	Centrifuge	Remi, Pvt. Ltd. Mumbai, India CM-12PLUS
22	Zeta analyser	Beckman coulter

EXPERIMENTAL WORK

CHAPTER-5 EXPERIMENTAL WORK

5.1 Physicochemical characterization and identification of Telmisartan

5.1.1 Physical appearance test

Telmisartan was characterized for different organoleptic properties such as color, odor, and appearance.

5.1.2 Melting point

The melting point of the telmisartan was determined by the capillary method. In this method, the drug was filled into capillary tube sealed at one end at the height of 3mm from the closed end. The capillary was introduced into the digital melting point apparatus and the melting point was noted by recording the temperature at which the drug starts melting, till the entire sample get melted. Thus a range of melting point was noted.

5.1.3 Infrared spectral analysis

The IR spectral of telmisartan was observed by preparing potassium bromide disk. The finally ground telmisartan powder was mixed with potassium bromide and pressed with specific hydraulic compression. The prepared KBr pellet was then observed under FTIR and the spectrum was recorded. The FTIR spectrum obtained was compared with spectrum obtained with telmisartan given.

5.2 Determination of absorbance maxima (λ_{\max})

10mg of telmisartan was accurately weighed from calibrated digital weighing balance and was dissolved in a small quantity of 0,1 N HCl. The solution was then transferred to 10ml of volumetric flask and sonicated for next 15min. The volume was made up to 10ml to give a stock solution of 1mg/ml or 1000 μ g/ml. 1ml of the stock solution was then transferred to 10ml volumetric flask and volume was makeup to 10 ml with 0.1N HCl. The resulting dilution was 100 μ g/ml. 5ml of the sample was taken from 100 μ g/ml dilution and transfer to volumetric flask and volume was made up to 10ml. the resulting dilution was 50 μ g/ml. both the samples are scanned under double beam UV spectrophotometer. The wavelength at which maximum absorbance was shown by both the dilutions, that was recorded as λ_{\max} for the telmisartan.

5.3 Method validation for Telmisartan in pH 0.1N HCl solution

5.3.1 Calibration plot of Telmisartan in 0.1N HCl buffer solution

EXPERIMENTAL WORK

10mg of Telmisartan was accurately weighed from celebrated digital weighing balance and was dissolved in a small quantity of 0.1N HCl solution. The solution was then transferred to 10ml of volumetric flask. the volume was made to 10ml to give a stock solution of 1mg/ml and then sonicated for 15min. this 10ml of the solution was transferred to 100ml volumetric flask and volume was made up to the mark with 0.1N HCl solution. Thus, the dilutions were prepared of 2, 4, 6, 8, 10, 12µg/ml. then the dilution was scanned on a double beam UV-visible spectrophotometer. The wavelength at which maximum absorbance was shown by the dilution, that was recorded as the maximum wavelength of Telmisartan and samples were scanned on maximum wavelength. The analysis was carried out triplicate.

5.3.2 Linearity and Range

Linearity is the ability of the method to elicit the result of test samples that are directly proportional to analyte concentration within a given range. The range is the interval between upper and lower level of analytes that can be determined with accuracy, precision, and linearity. The accepted criteria for linearity is that the correlation coefficient (R^2) should not be less than 0.990 for a least square method of analysis of the line. Different aliquots of stock solution were sufficiently diluted to get a solution in concentration ranging 2 to 10µg/ml in triplicate. The calibration plot was obtained by plotting graphs between absorbance versus concentration data and linear regression analysis was carried out for the same.

5.3.3 Accuracy

It represents the closeness of agreement between the value which is accepted either as a conventional true value and the value obtained from a test sample (ICH, Q2 (R1) guidelines 2005). Accuracy was determined by performing recovery studies. It is performed by preparing different concentration level (2,6,10) µg/ml. The study was carried out by preparing three sample solution at each recovery level. Absorbance was analyzed on a UV Spectrophotometer. Percentage mean recovery along with the percentage R.S.D was calculated.

5.3.4 Precision

The precision expresses the closeness of agreement between the series of measurement obtained from multiple sampling of the homogenous sample under the prescribed condition (ICH, Q2 (R1) guidelines 2005). The precision of proposed method was determined for five concentration (2, 4, 6, 8,10) covering the whole linearity range by interday (repeatability) and interday studies (intermediate precision) Intraday precision was determined by examining the concentration (2,4,6,8,10µg/ml) at three different time points on the same day and interday

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precision is determined by analysing the solution at three distinctive time points on different days. For analyzing the precision, percentage R.S.D was calculated for intraday and interday precision studies.

5.3.5 Robustness

It is the measure of the capacity to remain unaffected by small, however deliberate variation in the method parameters and gives an indication of its reliability during the normal usage (ICH, Q2 (R1) guidelines, 2005). The robustness of proposed method is assessed by evaluating the influence of temperature (18°C and 24°C). The percentage R.S.D was determined for solutions (2,6,10µg/ml) at a different temperature.

5.3.6 Limit of detection (LOD) and Limit of Quantification(LOQ):

Limit of detection is the minimal measure of analyte in a sample which can be distinguished but not necessarily quantified as an exact value (ICH, Q2 (R1) guidelines, 2005).

The limit of Quantification of an individual analytical procedure is the most minimal amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. Estimation of L.O.D and L.O.Q was based on the R.S.D and the slope of the calibration of the standard curve.

$$\text{L.O.D} = 3.3\sigma/S \quad \dots\text{Equation 5.1}$$

(σ = Standard deviation of the intercept of linear regression equation)

(S= Slope of the regression equation)

$$\text{L.O.Q} = 10\sigma/S \quad \dots\text{Equation 5.2}$$

(σ = Standard deviation of the intercept of linear regression equation)

(S= Slope of the regression equation)

5.4 Preformulation Studies:

5.4.1.1 Drug Excipient Compatibility

Compatibility was carried out for the pure drug, Excipient, and Drug: Excipient mixture in a ratio of 1:1. The above mixture was placed in an amber color bottle and stored in accelerated storage condition ($40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$ & $60 \pm 2^\circ\text{C}/80 \pm 5\% \text{ RH}$) in which one bottle is closed with an aluminum foil and another container in an open condition. After 2-4 weeks the samples are observed for any physical changes.

5.4.1.2 Chemical characterization of drug Excipient mixture:

Chemical compatibility of drug and Excipient mixture was checked after performing FTIR analysis of the drug with and without the Excipient. The peak of telmisartan along with the Excipient was observed. the effect of the Excipient on the major peaks of the Telmisartan was

EXPERIMENTAL WORK

observed to find out the compatibility of the drug with the Excipient. The undistributed peaks of the drug states compatibility of the drug with Excipient.

5.4.2 Solubility studies:

The solubility study of Telmisartan was done by using various solvents and variable pH to understand the solubility profile of drug (IP, 2007). Standard plot of telmisartan was observed by using various solvents such as DCM, Di-ethyl ether, methanol, iso-propyl alcohol, 0.1N HCl solution. The samples of drug solution in different solvents were taken and diluted suitably to observe the absorbance of the drug by using U.V spectrophotometer at λ_{max} 290.50 nm. The drug concentration in each solvent was calculated from the standard plot and the graph was plotted between the concentrations vs. absorbance.

5.4.3 Partition coefficient:

The partition coefficient study was performed by using octanol and water. Both the solvents (20 ml) were filled in a separating funnel to which 10mg of the drug was added. The mixture was allowed to shake for 24 hr at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The funnel was kept undisturbed to separate the two layers. The aqueous, organic layer was collected separately and the concentration of drug was found using U.V spectrophotometer (Pokhariya et al., 2016)

5.5 Screening study:

5.5.1 Screening of the method for the preparation of nanocrystals:

METHOD 1: Nanocrystals were prepared by antisolvent evaporation followed by sonication technique. An appropriate amount of drug was taken and dissolved in an antisolvent which is immiscible with water. Then, a 1ml solution of telmisartan was injected into deionised water with or without polymers or surfactants at 4°C with rapid stirring at 1200 rpm followed by intense sonication (Lu et al., 2014)

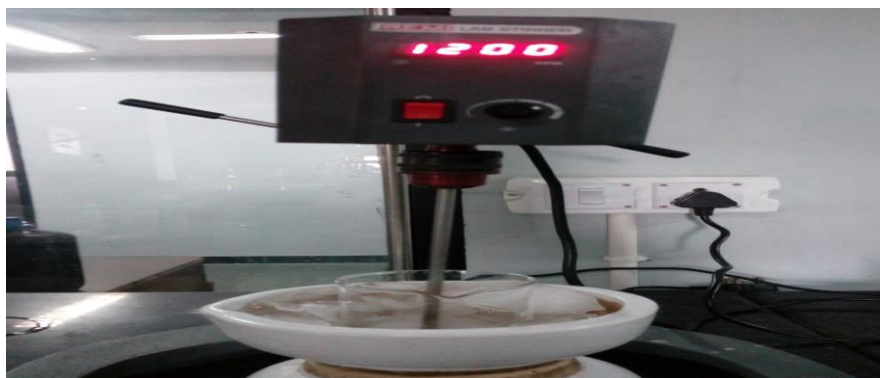


Fig 5.1 Representing preparation of Nanocrystals by Method 1

EXPERIMENTAL WORK

Method 2: Nanocrystals are prepared by solvent/antisolvent precipitation technique. In this technique, the drug solution was injected into water containing surfactants such as SDS and stabilizer PVPK30, and PEG 6000 single or in combination under stirring at 1000-1200 rpm. Precipitation of nanocrystals occurs immediately upon mixing (Shelar et al., 2013)

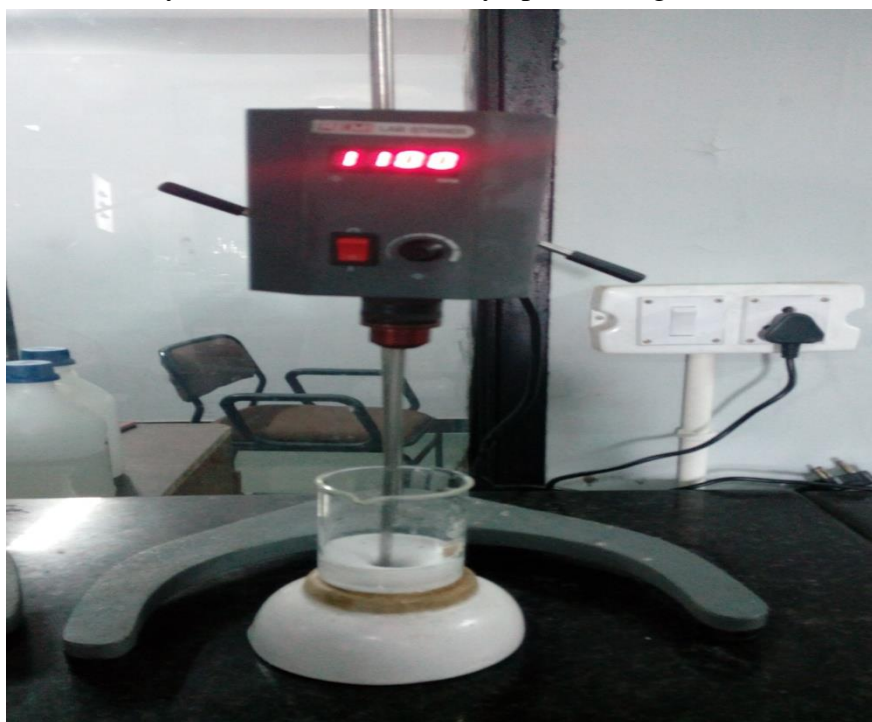


Fig 5.2 Representing preparation of Nanocrystals by Method 2

To check the effect of polymers and Quantity of polymers on nanocrystals, different batches of nanocrystals was prepared by using the above-mentioned method for preparation of nanocrystals. 12 batches with different polymers and their quantity was prepared. The effect of ratio was analyzed by observing the shape and size of the crystals on optical microscope

5.5.2 Effect of preparation technique on the nanocrystals formulation:

Nanocrystals formulations prepared by using antisolvent precipitation followed by sonication technique (Y.Lu et al., 2013). Telmisartan was weighed properly and 1ml solution of Telmisartan was injected into deionised water at 4°C temperature with continuous stirring at 1200 rpm and intense sonication. Various solvents are evaluated such as methanol, ethanol, isopropyl alcohol, and Dichloromethane. Polymers and surfactants were chosen from PEG 4000, PEG 6000, and PVP K30. Processing condition was evaluated for their ability to prepare nanocrystals.

EXPERIMENTAL WORK

Table 5.3 Screening the ratio of components for formulation by antisolvent evaporation followed by sonication.

Sr. NO.	Batch code	Components	Ratio
1	T1	TEL: PEG 6000	1:10
2	T2	TEL: PEG 6000	1:20
3	T3	TEL: PEG 6000	1:30
4	T4	TEL: PVP K30	1:10
5	T5	TEL: PVP K30	1:20
6	T6	TEL: PVP K30	1:30
7	T7	TEL: PEG 4000	1:10
8	T8	TEL: PEG 4000	1:20
9	T9	TEL: PEG 4000	1:30
10	T10	TEL:PEG6000:PVPK30	1:10:10
11	T11	TEL:PEG6000:PEG4000	1:10:10
12	T12	TEL:PEG4000:PVPK30	1:10:10

Table5.4 Preparation of nanocrystals by antisolvent evaporation supplemented by sonication technique

S No.	Component	Range
1	Telmisartan	1-3mg/ml
2	Polymer	10-30mg/ml
3	Water	20-40ml
4	Antisolvent	1ml

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Table 5.5 Preparation of nanocrystals by solvent-antisolvent evaporation technique

Sr. NO.	Batch code	Drug: polymer	Ratio
1	TI	Telmisartan: PEG 6000	1:10
2	TII	Telmisartan: PEG 6000	1:20
3	TIII	Telmisartan: PVP K30	1:10
4	TIV	Telmisartan: PVP K30	1:20
5	TV	Telmisartan: PEG 400	1:10
6	TVI	Telmisartan: PEG 400	1:20
7	TVII	Telmisartan: SLS	1:10
8	TVIII	Telmisartan: SLS	1:20

5.6 Characterization and Evaluation of Nanocrystals:

5.6.1 Optical Microscopy:

Optical microscopy was done by optical Microscope at 10 X and 40X using the optical lens for viewing the abundance of crystalline structure and physical appearance. The morphological characteristics were studied for nanocrystals by optical microscope

5.6.2 Particle size and zeta potential analysis: The nanocrystals prepared by antisolvent precipitation supplemented by sonication were subjected to particle size analysis using particle size analyser (Beckman coulter). Average particle size, polydispersity index, and zeta potential were determined by deionised water (Chogale et al., 2016)

5.6.3 Transmission electron microscopy (TEM):

A drop of a sample was placed onto a carbon-coated grid and allowed to dry. The grid containing the sample was observed under the transmission electron microscope with an accelerating voltage of 120 kV. The nanocrystals were observed by focusing the lens. The images were then obtained after focusing the microscope with different magnifications of 120000-400000 X (Gao et al., 2008)

5.6.4 Morphological studies of nanocrystals:

Morphological studies of the nanocrystalline formulation were done by Scanning Electron Microscopy (Yarraguntla et al., 2016) The sample was analyzed by mounting it on metal stubs

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and later the stub was coated with conductive gold with a stupper coater which as attached to the instrument by using electron potential of 15kV at 25mA for 10 minutes.

5.6.5 X-ray Diffraction:

The powder Xray diffraction studies were carried out. The spectra were recorded by using X-ray diffractometer using copper as anode material having voltage current of 40mA, 45 kV. The sample was analyzed at a 2θ angle range of 25° . Step time was 110.67 and time of scanning is 30minutes (Dinnebier et al., 2000)

5.6.6 In Vitro dissolution studies:

An accurately weighed amount of Telmisartan nanocrystals was taken for in-vitro dissolution studies. The studies were carried out in triplicate using USP type I dissolution test apparatus. The study was carried out in 900ml .1N HCl and 6.8 Phosphate buffer at 50rpm and temperature $37\pm 0.5^\circ\text{C}$. aliquots of 5ml were taken at a regular interval of time and analyzed spectrophotometrically. The cumulative amount of released drug at each time point was calculated (Bajaj et al., 2012)

5.6.7 Stability studies: The stability study for best formulation was carried out at 4°C and 40°C to check the stability over wide range of temperature. The formulation was assessed for percentage entrapment efficiency and zeta potential (Soma et al., 2013)

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RESULTS AND DISCUSSION

6.1 Identification and characterization of Telmisartan

6.1.1 Physical description

The sample of telmisartan was identified and characterized as per requirements of COA (certificate of analysis) issued by the manufacturer and (USP 30 NF 25, 2007). Results are shown in table 6.1.

Table 6.1 Identification and characterization of telmisartan

Parameters	Specifications as per COA	Observation
Physical state	Solid	Solid
Colour	White	White
Odor	Odorless	Odorless

6.1.2 Melting point analysis

The observed experimental melting point by capillary method complies with the reported melting point as shown in table 6.2 (Wienen et al., 2000)

Table 6.2 Melting Point of telmisartan

Parameter	Specification as per COA	Observation
Melting range	261-263	260-262

6.1.3 Identification of the drug telmisartan by FTIR spectra

FTIR analysis provides a complete description of physiochemical properties of the drug. The FTIR spectra of the given sample showed comparable major absorption bands with that of a reference standard of telmisartan. The structure of telmisartan is presented in Fig.6.1. These matching for characteristic peaks of the drug with that of reference standard confirmed the purity of drug (Fig.6.2). The characteristic peaks represented the functional groups present along with the wave numbers which ensured the absorption.

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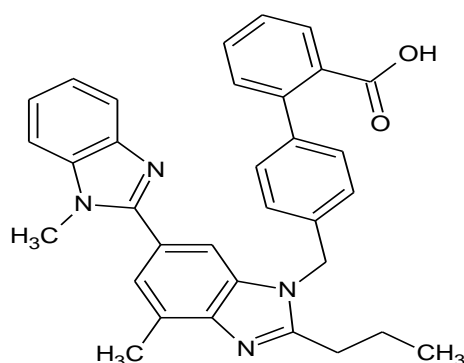


Fig.6.1 Structure of Telmisartan (Amrinder Singh*, K.K. Jha, Anuj mittal, 2013)

The FTIR spectra of pure drug Telmisartan showed several absorption bands at 3392cm^{-1} (N-H stretching vibration), 3059.2cm^{-1} (aromatic C-H stretching vibration), 2958.9cm^{-1} (aliphatic C-H stretching vibration), 1697.4cm^{-1} (shows presence of carbonyl group), 1599.04cm^{-1} (aromatic C=C bending and stretching vibration) and 1456cm^{-1} (shows presence of C=C aromatic group). Thus, presence of above- mentioned group confirmed the identity of the drug (Chella et al., 2014)

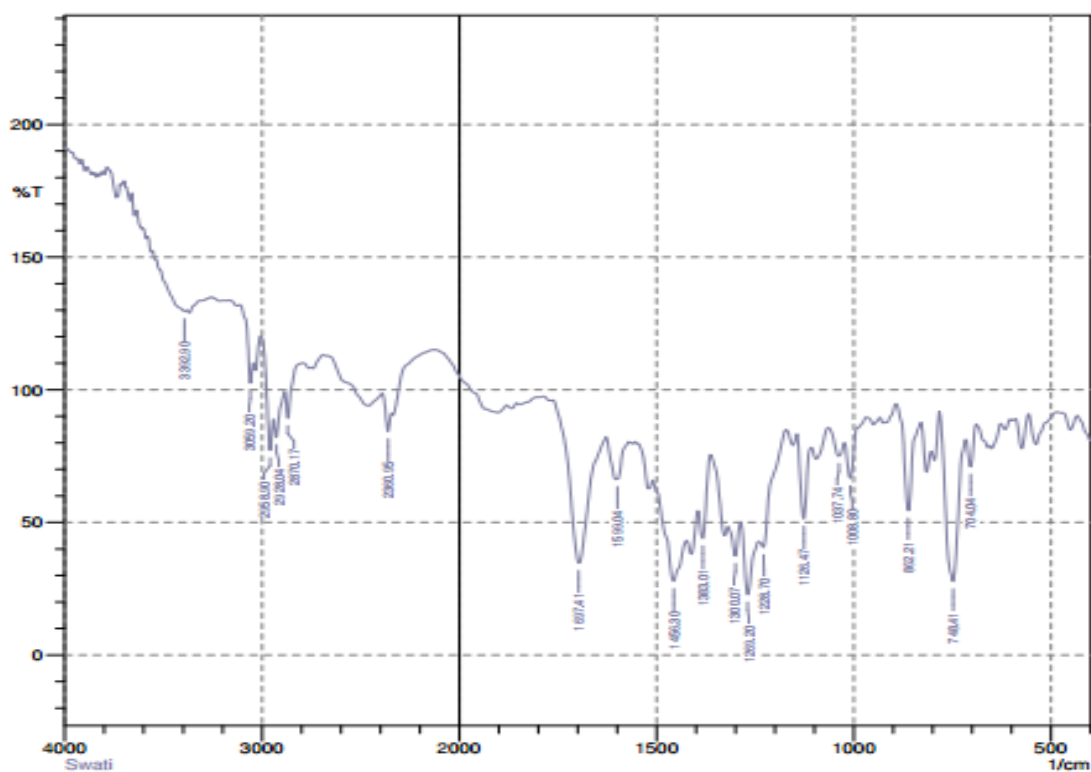


Fig 6.2 FTIR spectrum of Telmisartan on Shimadzu 1800S FTIR

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6.2 Determination of absorption maxima (λ max) of telmisartan

The λ max of telmisartan was found to be 290.5nm in 0.1N HCl solution. The scanning of the drug was done in the range (200-400 nm) as shown in the Fig.6.3.

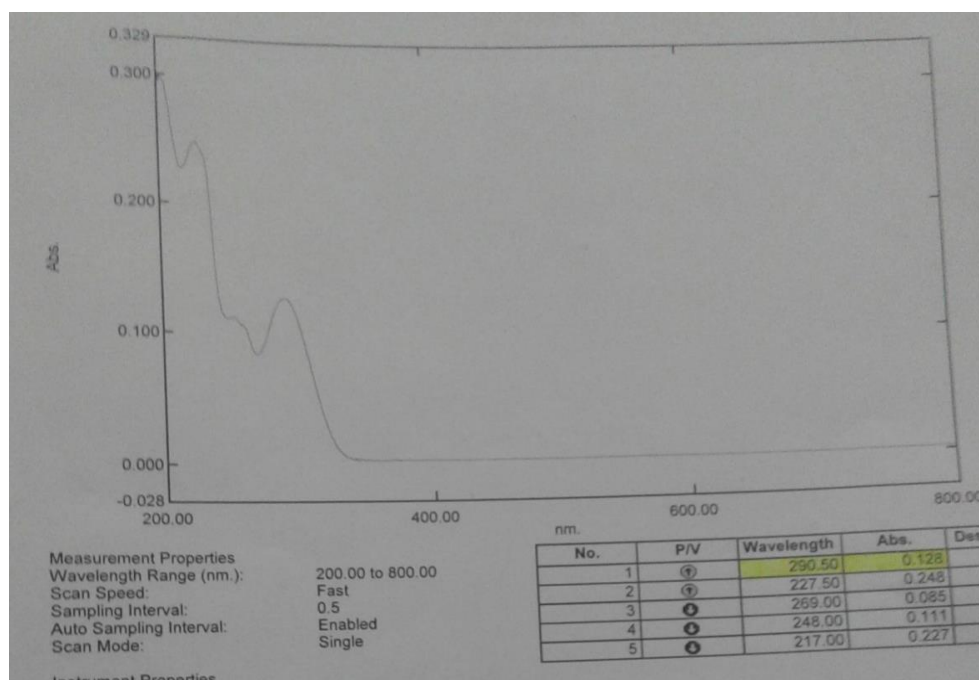


Fig 6.3 Representing absorption maxima of telmisartan

6.3 Analytical method validation of Telmisartan in 0.1N HCl solution.

The U.V spectroscopic method was validated to check the suitability for the purpose prescribed (ICH, Q2 (R1) guidelines, 2005). The process of validation depicts whether the method is good for its intended purpose or not. The proposed method was validated according to ICH guidelines with respect to linearity, accuracy, precision, LOD, LOQ and robustness. The λ max selected was 290.50 nm and the linearity was established in the range of 2-12 $\mu\text{g/ml}$ with correlation coefficient, $R^2 = 0.9991$. The validity of the proposed method was further assessed by recovery studies. The characteristic parameters are shown in table 6.8.

6.3.1 Calibration curve of Telmisartan in 0.1NHCl solution

The calibration plot of Telmisartan was prepared by taking 2, 4, 6, 8, 10,12 $\mu\text{g/ml}$ (table 6.3) concentrations of Telmisartan in 0.1N HCl solution as shown in table 6.4. The experiments were performed in triplicate to find the standard deviation and percentage relative standard deviation. Absorbance range was found to be 0.130 to 0.655. The regression coefficient (R^2 value) was 0.9991 which showed linearity between 2-12 $\mu\text{g/ml}$ concentrations. The Lambert-

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Beer law was obeyed within the linearity range. The standard regression equation was found to be $y = 0.0541x + 0.0114$

Table 6.3 The absorbance of Telmisartan in 0.1N HCl solution at 290.50 nm.

Concentration ($\mu\text{g/ml}$)	Mean Absorbance \pm S.D (n= 3)	% Relative Standard deviation(RSD)
0	0	0
2	0.130 \pm 0.001	0.632
4	0.232 \pm 0.005	0.256
6	0.339 \pm 0.001	0.304
8	0.447 \pm 0.005	0.138
10	0.556 \pm 0.006	0.103
12	0.655 \pm 0.005	0.089

Linear Regression (R²) **0.9991**

6.3.2 Linearity and Range

Table 6.3 shows concentration and absorbance at 290.50 nm. Linearity was observed in the range of 2-12 $\mu\text{g/ml}$ at 290.50 nm with a significant higher value of correlation coefficient, $r^2 = 0.999$ thus, follow Beer-Lamberts law in this range as shown in Fig. 6.4.

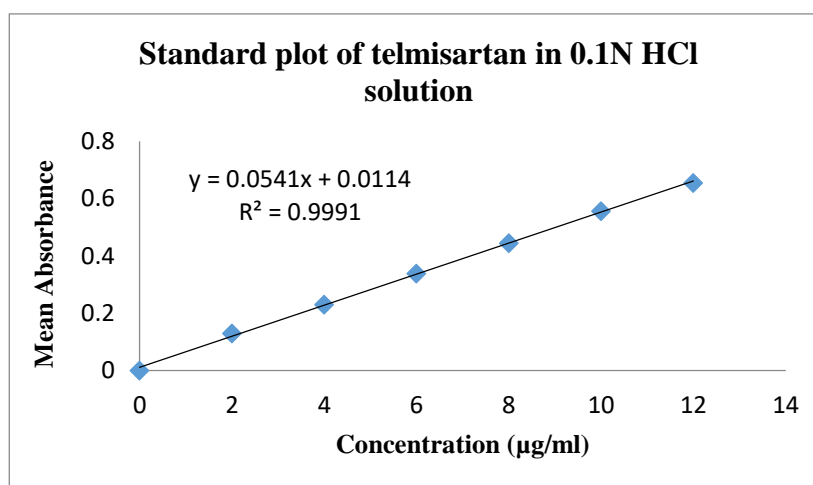


Fig. 6.4. Calibration curve of Telmisartan in 0.1HCl solution

6.3.3 Accuracy: Accuracy results as shown in table 6.4 displayed good reproducibility with RSD value below 2. The method was found to be accurate as percentage recovery was found to be within the range of 94.83 to 99.07. These results proved that the method is accurate.

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Table 6.4 Result of accuracy of Telmisartan in 0.1HCl solution

Concentration ($\mu\text{g/ml}$)	Mean Absorbance \pm S.D (n= 3)	% Mean recovery	% Relative Standard deviation(RSD)
2	1.897 \pm 0.025	94.83	1.32
6	5.880 \pm 0.029	98.00	0.50
10	9.907 \pm 0.027	99.07	0.29

6.3.4 Precision

The results of intraday, interday repeatability and reproducibility have been summarized in table 6.5 and 6.6 respectively. The results were found to show good reproducibility with % RSD below 2. The results were very close to the true value. There was negligible variation in intraday and interday precision.

Table 6.5 Result of intraday precision of Telmisartan in 0.1HCl solution

Concentration ($\mu\text{g/ml}$)	Mean Absorbance \pm S.D (n= 3)	% Mean recovery	% Relative Standard deviation(RSD)
2	1.990 \pm 0.029	99.50	1.48
6	5.917 \pm 0.009	98.61	0.16
10	9.847 \pm 0.029	98.47	0.29

Table 6.6 Result of interday precision of Telmisartan in 0.1HCl solution

Concentration ($\mu\text{g/ml}$)	Mean Absorbance \pm S.D (n= 3)	% Mean recovery	% Relative Standard deviation(RSD)
2	1.977 \pm 0.034	98.83	1.72
6	5.850 \pm 0.071	97.50	1.22
10	9.667 \pm 0.033	96.67	0.34

6.3.5 Robustness

Telmisartan solution was analysed six times at two different temperature to determine robustness of the method. The result is indicated as %RSD

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Table 6.7 Result of robustness of Telmisartan in 0.1HCl solution

Concentration ($\mu\text{g/ml}$)	Mean Absorbance \pm S.D (n= 3)	% Mean recovery	% Relative Standard deviation (RSD)
2	1.963 \pm 0.021	98.17	1.05
6	5.880 \pm 0.029	98.00	0.50
10	9.763 \pm 0.037	97.63	0.38

6.3.6 Limit of Detection and Limit of Quantification

Estimation of LOD and LOQ were determined by the standard deviation of response (s) and slope of the calibration curve (m). The standard deviation of Y intercepts of the regression line was used as standard deviation.

$$\text{LOD} = 3.3 \text{ s/m}$$

$$\text{LOQ} = 10 \text{ s/m}$$

The LOD and LOQ were found to be 0.693 $\mu\text{g/ml}$ and 2.1 $\mu\text{g/ml}$ respectively as given in table 6.8. These results demonstrate that the method is sensitive and can detect the drug in the above-mentioned concentration range.

Observed values of LOD and LOQ

Standard Deviation of response (s)	0.0114
Slope (m)	0.0541
Ratio (s/m)	0.210
LOD	0.693
LOQ	2.1

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Table 6.8 Characteristics for Telmisartan in 0.1N HCl solution

S. No.	Parameter Result	
1.	Absorption Maxima	290.50nm
2.	Beers law range	2-12µg/ml
3.	Correlation coefficient	0.9991
4.	Regression equation	$y = 0.0541x + 0.0114$
5.	Slope	0.0541
6.	Intercept	0.0114
7.	Accuracy	99.07
8.	Precision, Intraday, and Interday	98.47, 98.83
9.	LOD µg/ml	0.693
10.	LOQ µg/ml	2.1

6.4 Preformulation studies

6.4.1 Drug excipients compatibility

The results obtained from compatibility study of telmisartan with various excipients showed no physical incompatibility between telmisartan and excipients under stress conditions. However, there was no change in color, appearance.

Table 6.9 Drug and excipients in 1:1 ratio for compatibility studies

S.No.	Ingredients	Color	Appearance	State	Lumps
1	Telmisartan	White	Crystalline	Solid	Absent
2	PEG 6000	White	Crystalline	Solid	Absent
3	SLS	White	Crystalline	Solid	Absent
4	PVP K30	White	Crystalline	Solid	Absent
5	Telmisartan: PEG 6000	White	Crystalline	Solid	Absent
6	Telmisartan: PVP K30	White	Crystalline	Solid	Absent
7	Telmisartan: SLS	White	Crystalline	Solid	Absent
8	Telmisartan: PEG 6000: PVP K30	White	Crystalline	Solid	Absent

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Table 6.10 Drug and excipients in 1:1 ratio at different time intervals

Ingredients	1 st Day	2 nd Day	3 rd Day	10 th Day	15 th Day
Telmisartan					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
PEG 6000					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
PVP K30					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
SLS					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Telmisartan: PEG 6000					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Telmisartan: PVP K30					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√

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Lumps	√	√	√	√	√
Telmisartan: SLS					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√
Telmisartan: PEG 6000: PVP K30					
Color	√	√	√	√	√
Appearance	√	√	√	√	√
State	√	√	√	√	√
Lumps	√	√	√	√	√

√ shows no change in drug Excipient mixture.

6.4.2 Solubility analysis of telmisartan

The solubility data was obtained for telmisartan at 42°C using an ultraviolet absorption assay method to determine the concentration of drug present in the saturated solutions (IP, 2007). The solubility profile of drug with the buffers was helpful to determine that whether the drug was dispersed or solubilized in the buffer systems. The solubility profile in the decreasing order of solubility was found to be as follows: 0.1 N HCl > pH 7.4 phosphate buffer > pH 6.8 phosphate buffer > ethanol > water. The pH solubility profile of telmisartan was generated and was reported and shown in table 6.10. The solubility profile signifies that the drug gets sparingly solubilized in the DCM so it can be dispersed in the DCM during the formulation. Thus, the solubility profile helped to generate the supportive information regarding the final formulation.

Table 6.11 Solubility telmisartan in various buffers and solvent system (IP, 2007)

S.No.	Solvent (variable pH)	Solubility (mg/ml)	Solubility Profile
1.	Water	10000	Insoluble
2.	Ethanol	110	Soluble
3.	0.1N HCl	9	Freely soluble

Solubility: Soluble in ethanol, insoluble in water, highly soluble DCM. Telmisartan is supplied as crystalline solid. Telmisartan is soluble in organic solvents such as DCM and dimethyl formamide. The solubility of telmisartan in these solvents is >10mg/ml. It is slightly soluble in

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methanol, sparingly soluble in IPA; very slightly soluble in acetone; practically insoluble in water. The solubility of telmisartan in water is less than 1 µg/ml.

6.4.3 Partition coefficient of telmisartan

The partition coefficient of telmisartan between octanol and water (log P) was determined (Pokhariya et al., 2016). The study indicated that Telmisartan has a log P value equals to 3.58. This value is quite low as compared to the most sartan.

6.4.4 Prescreening study for selection of ratio of components

Prescreening study was done to select the levels for the design of the experiment. For this, the formulations with suitable ratios of drug and polymers were selected (Abdul Hasan Sathali et al., 2013). Levels were decided on the basis of literature. The nanocrystals were prepared by the antisolvent evaporation method followed by sonication technique and solvent-antisolvent evaporation method and were evaluated for various evaluation parameters. The ratio of the components was screened by optical microscopy as shown in table 6.12.

Table 6.12 screening the ratio of components in Antisolvent precipitation followed by precipitation technique.

Batch code	Drug: Polymer	Ratio	Nanocrystals
T1	Telmisartan: PEG 6000	1:10	Present
T2	Telmisartan: PEG 6000	1:20	Present
T3	Telmisartan: PEG 6000	1:30	Present
T4	Telmisartan: PVP K30	1:10	Present
T5	Telmisartan: PVP K30	1:20	Present
T6	Telmisartan: PVP K30	1:30	Present
T7	Telmisartan: PEG 400	1:10	Present
T8	Telmisartan: PEG 400	1:20	Present
T9	Telmisartan: PEG 400	1:30	Present
T10	Telmisartan: PEG 6000: PVP K30	1:10:10	Present
T11	Telmisartan: PVP K30: PEG 400	1:10:10	Present
T12	Telmisartan: PEG 400: PEG 6000	1:10:10	Present

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Table 6.13 Representing ratio of components and process parameter for preparation of formulation T1, T2 and T3 by Method 1

Name of contents	T1	T2	T3
Telmisartan(mg)	1	1	1
Polymer(mg)	10	20	30
Water(ml)	40	40	40
Homogenization(rpm)	1200	1200	1200



Fig 6.5 Representing T1,T2 and T3 formulations prepared by Method 1

Table 6.14 Representing ratio of components and process parameter for preparation of formulation T4, T5 and T6 by Method 1

Name of contents	T4	T5	T6
Telmisartan(mg)	1	1	1
Polymer (mg)	10	20	30
Water(ml)	40	40	40
Homogenization(rpm)	1200	1200	1200

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Fig 6.6 Representing T4, T5 and T6 formulation prepared by Method 1

Table 6.15 Representing ratio of components and process parameter for preparation of formulation T7, T8 and T9 by Method 1

Name of contents	T7	T8	T9
Telmisartan(mg)	1	1	1
Polymer (mg)	10	20	30
Water(ml)	40	40	40
Homogenization(rpm)	1200	1200	1200



Fig 6.7 Representing T7, T8 and T9 formulation prepared by Method 1

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Table 6.16 Representing ratio of components and process parameter for preparation of formulation T10, T11 and T12 by Method 1

Name of contents (ml)	T10	T11	T12
Telmisartan(mg)	1	1	1
Polymer (mg)	10	20	30
Water(ml)	40	40	40
Homogenization(rpm)	1200	1200	1200



Fig 6.8 Representing T10, T11 and T12 formulation prepared by Method 1

Table 6.17 Screening the ration of components for preparation of nanocrystals by antisolvent precipitation technique.

Batch code	Drug: polymer	Ratio	Nanocrystals
TI	Telmisartan: PEG 6000	1:10	Present
TII	Telmisartan: PEG 6000	1:20	Present
TIII	Telmisartan: PVP K30	1:10	Present
TIV	Telmisartan: PVP K30	1:20	Present
TV	Telmisartan: PEG 400	1:10	Present
TVI	Telmisartan: PEG 400	1:20	Present
TVII	Telmisartan: SLS	1:10	Present
TVIII	Telmisartan: SLS	1:20	Present

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Table 6.18 Representing ratio of components and process parameter for preparation of formulation TI, and TII by Method 2

Name of contents	TI	TII
Telmisartan(mg)	1	1
Polymer(mg)	10	20
Water(ml)	50	50
Homogenization(rpm)	1100	1100



Fig 6.9 Representing TI and TII formulation prepared by Method 2.

Table 6.19 Representing ratio of components and process parameter for preparation of formulation TIII, and TIV by Method 2

Name of contents	TIII	TIV
Telmisartan(mg)	1	1
Polymer(mg)	10	20
Water(ml)	50	50
Homogenization(rpm)	1100	1100

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Fig 6.10 Representing TIII and TIV formulation prepared by Method 2.

Table 6.20 Representing ratio of components and process parameter for preparation of formulation TV, and TVI by Method 2

Name of contents	TV	TVI
Telmisartan(mg)	1	1
Polymer(mg)	10	20
Water(ml)	50	50
Homogenization(rpm)	1100	1100



Fig 6.11 Representing TV and TVI formulation prepared by Method 2

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Table 6.21 Representing ratio of components and process parameter for preparation of formulation TVII, and TVIII by Method 2

Name of contents	TVII	TVIII
Telmisartan(mg)	1	1
Polymer(mg)	10	20
Water(ml)	50	50
Homogenization(rpm)	1100	1100



Fig 6.12Representing TVII and TVIII formulation prepared by Method 2

6.5 Formulation Development trials

6.5.1 Optimization of nanocrystals formulations prepared by Method I

The design of optimization contained two independent variables (X1, X2) and one dependent variable (Y1). The X variables were a drug (% w/w) and polymer (% w/w) respectively, whereas, the Y variable was percentage entrapment efficiency. According to the reference, 12 formulations were suggested. Each of them was formulated and analyzed for entrapment efficiency.

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Table 6.22 Factors combination and responses in formulation prepared by Method 1

Run no.	Drug (mg)	Polymer (mg)	Homogenization (rpm)	Entrapment efficiency (%)
T1	1	10	1200	92
T2	1	20	1200	93.5
T3	1	30	1200	96
T4	1	10	1200	76
T5	1	20	1200	83.3
T6	1	30	1200	84
T7	1	10	1200	72
T8	1	20	1200	78.8
T9	1	30	1200	88
T10	1	20	1200	71.2
T11	1	20	1200	78.2
T12	1	20	1200	78.8

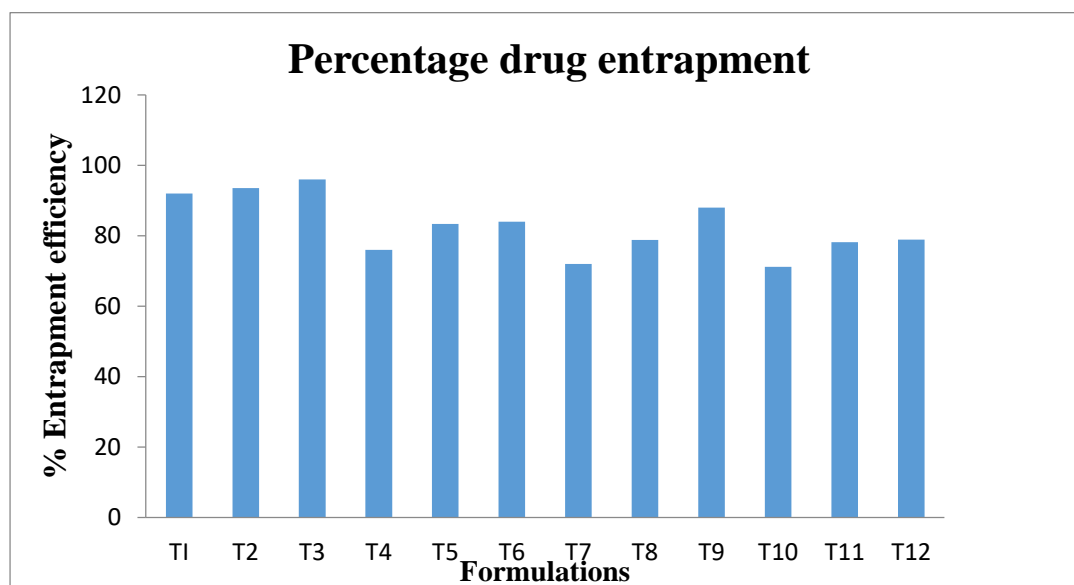


Fig 6.13 Percentage drug entrapment of various nanocrystals formulations prepared by method 1

6.5.2 Optimization of nanocrystals formulations prepared by Method II The design of optimization contained two independent variables (X1, X2) and two dependent variables (Y1). The X variables were a drug (% w/w) and polymer (% w/w) respectively, whereas, the Y

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variable was percentage entrapment efficiency. According to the literature review, 8 formulations were suggested. Each of them was formulated and analyzed for entrapment efficiency.

Table 6.23 Factors combination and responses in formulation prepared by Method II

Run no.	Drug (mg)	Polymer (mg)	Homogenization (rpm)	Entrapment efficiency (%)
TI	1	10	1100	91.08
TII	1	20	1100	94.04
TIII	1	10	1100	75.55
TIV	1	20	1100	83.33
TV	1	10	1100	76.44
TVI	1	20	1100	82.87
TVII	1	10	1100	75.33
TVIII	1	20	1100	69.04

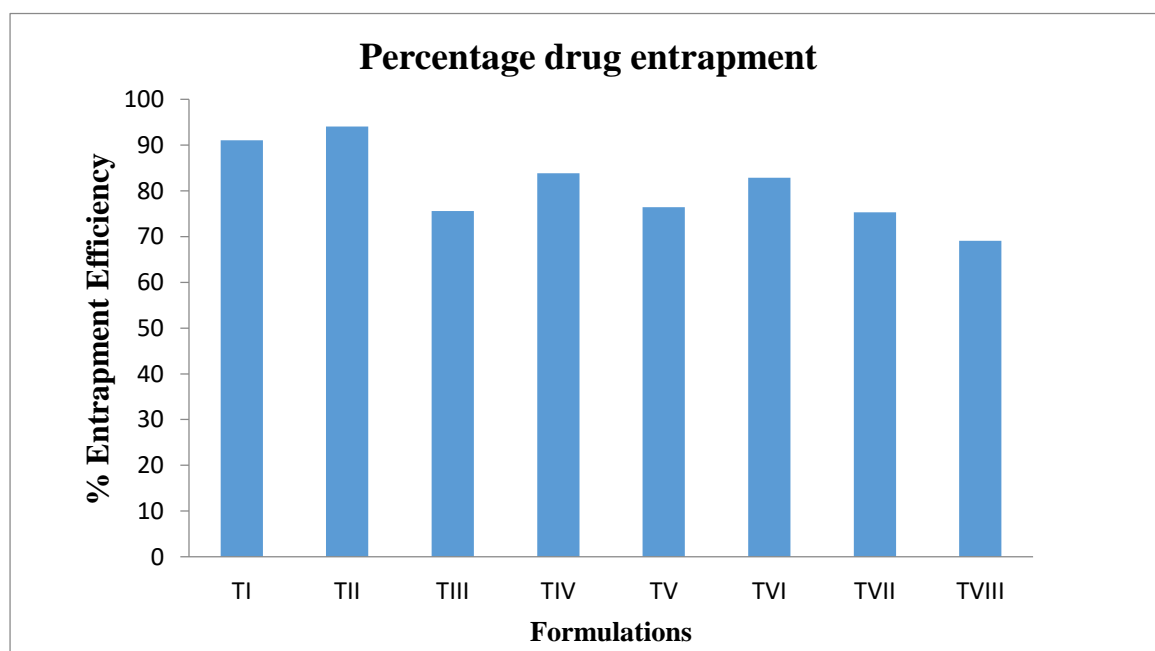


Fig 6.14 Percentage drug entrapment of various nanocrystals formulations prepared by method

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6.6 Characterization and Evaluation of nanocrystals

6.6.1 Optical microscopy

The formulations prepared by two methods were examined for optical microscopy as shown in Fig.6.7 and Fig.6.8. Optical microscopy showed that the nanocrystals were observed in formulations studied at 100 X. The micrographs of nanocrystals revealed the presence of crystalline structures which were nano in appearance.

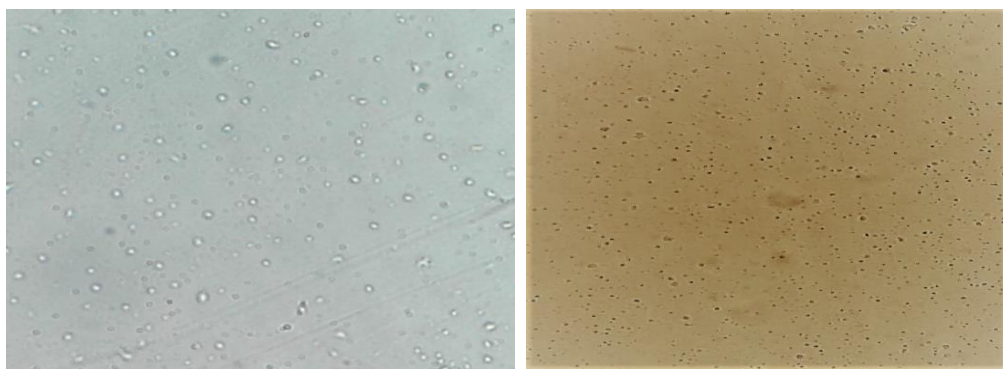


Fig no. 6.15 Optical photomicrographs of nanocrystals prepared by Method I



Fig no.6.16 Optical photomicrographs of nanocrystals formulations prepared by method II

6.6.2 Particle size and Zeta potential Analysis

The mean particle size and Polydispersity Index (PI) of nanocrystals are presented in table 6.13. The differences in the nanocrystalline formulations prepared with variable ratios of polymer, stabilizer, and drug were utilized to find the optimized formulation. The nanocrystals prepared by two methods are shown in fig. 6.12, fig. 6.14 and fig no. 6.16. In general, nano and microcarriers with Polydispersity Index (PI) value higher than 0.3 shows large size distributions and have the tendency to aggregate (Chogale et al., 2016). The optimized formulation prepared by the method I showed average particle size of 164.2 nm with PI of 0.288 whereas average particle size of nanocrystals prepared by method II is 2.9 μ with PI of

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1.041. This shows that the optimized nanocrystals formulations prepared by the method I are homogeneous with uniform distribution.

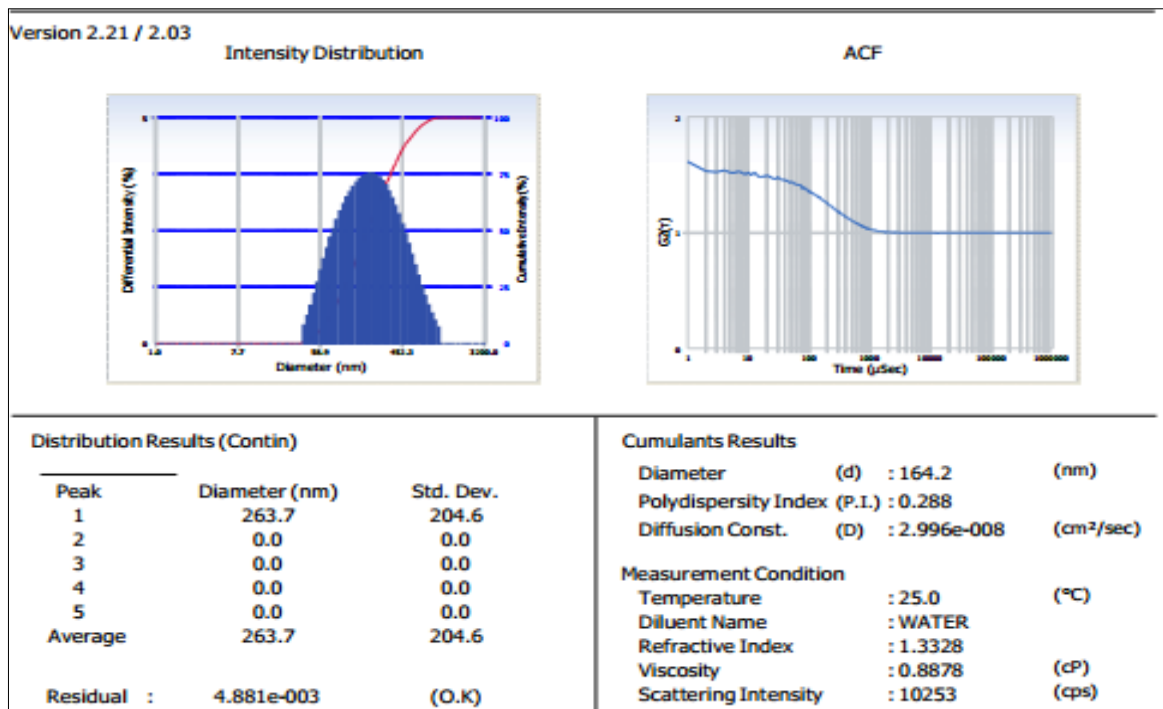


Fig. 6.17 Particle size of nanocrystals formulation (T1) prepared by method I

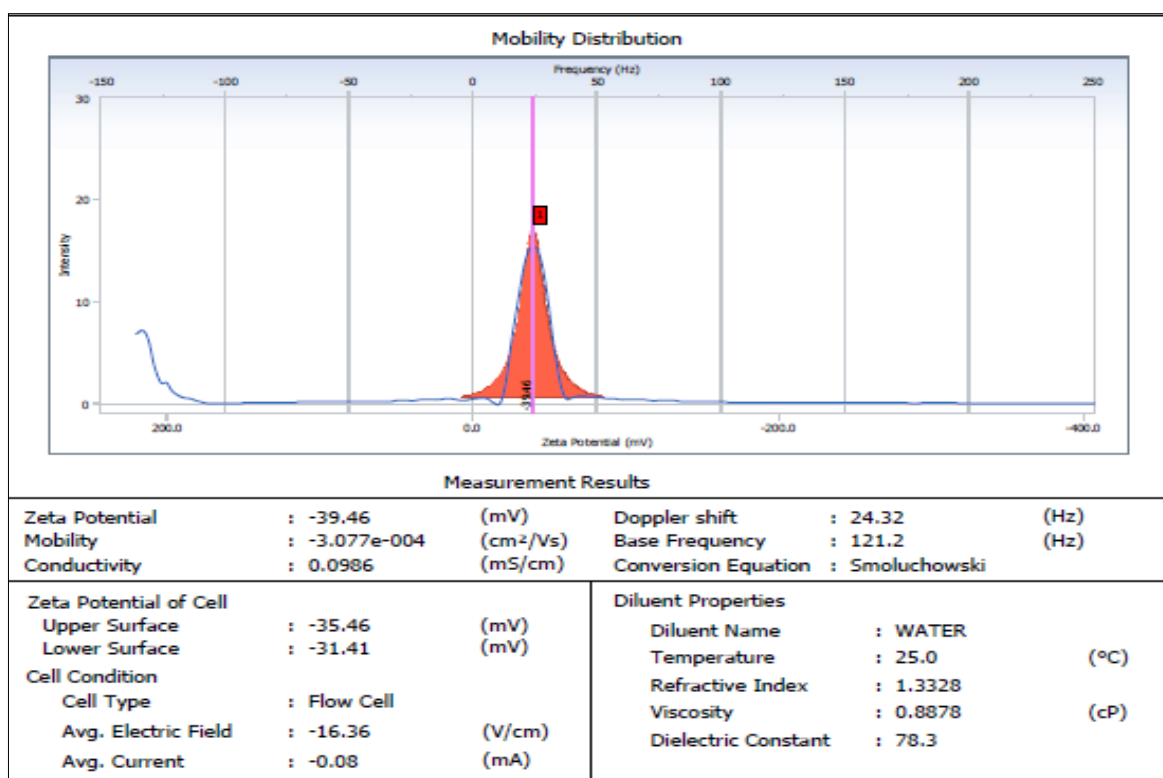


Fig no. 6.18 Zeta potential of nanocrystals formulation (T1) prepared by method I

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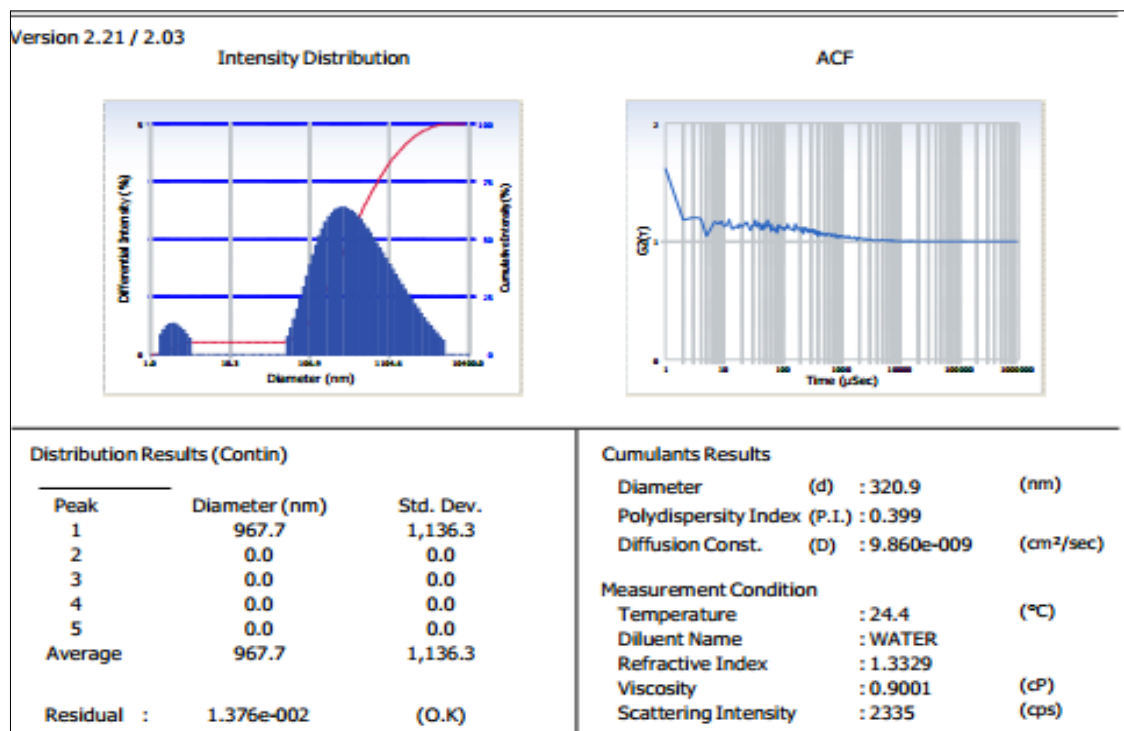


Fig. 6.19 Particle size of nanocrystals formulation (T5) prepared by method I

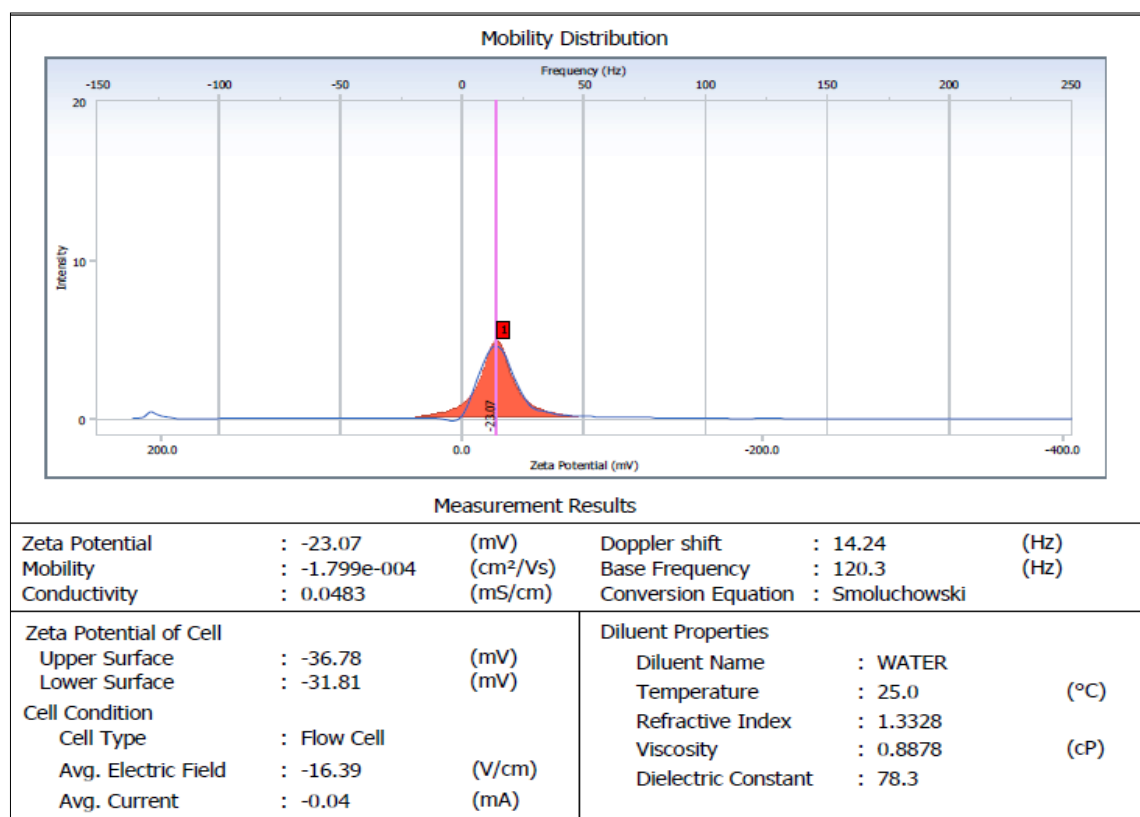


Fig no. 6.20 Zeta potential of nanocrystals formulation (T5) prepared by method I.

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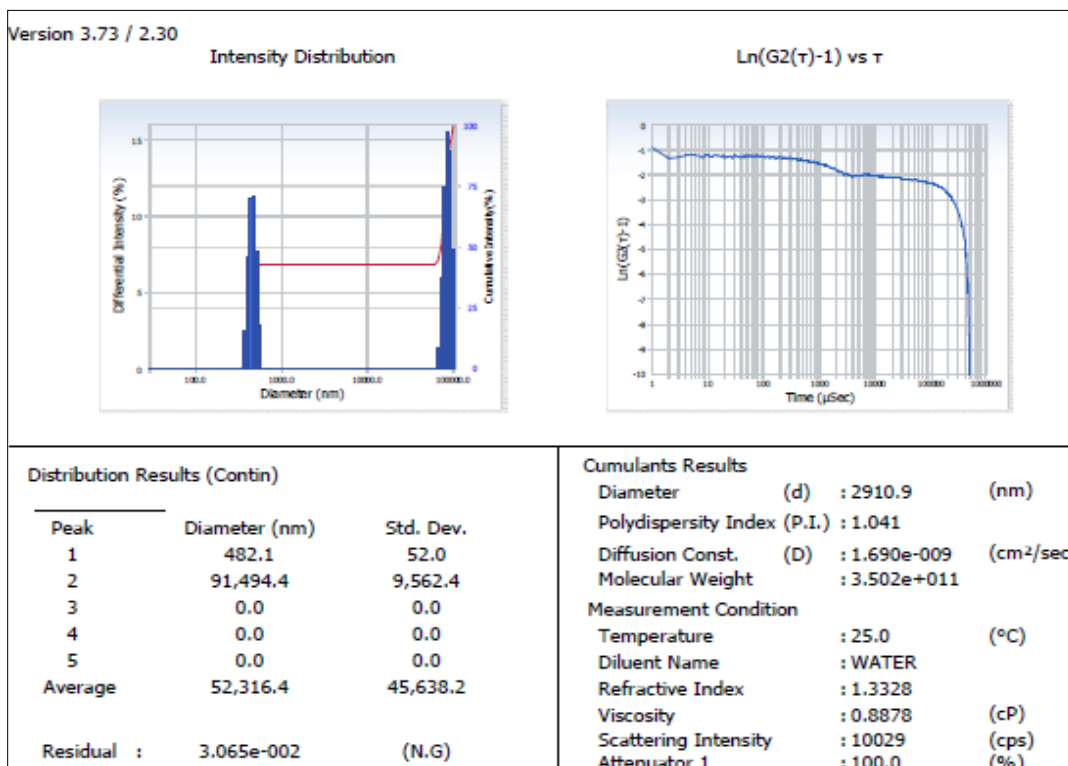


Fig no. 6.21 Particle size of nanocrystals formulation (T1) prepared by method II

6.6.3 Transmission electron microscopy (TEM)

TEM photomicrographs of some representable nanocrystals are shown in Fig 6.12-6.14. The grid containing the sample was observed under the transmission electron microscope with an accelerating voltage of 120 kV with magnification between 190000 X – 500000 X. Nanocrystals were discrete, uniform and rod-shaped. The diameter was found to be within the range of 11-100 nm.

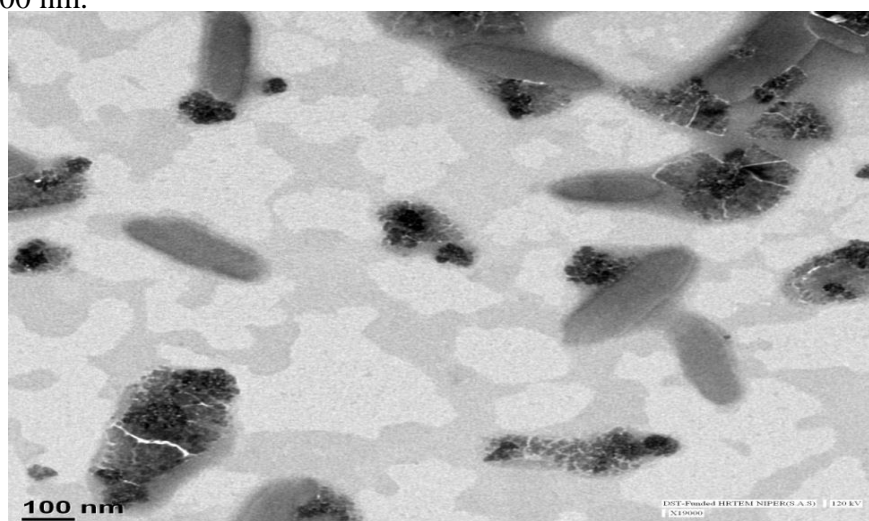


Fig. 6.22 Transmission electron micrograph of (T1) nanocrystals with magnification 190000 X

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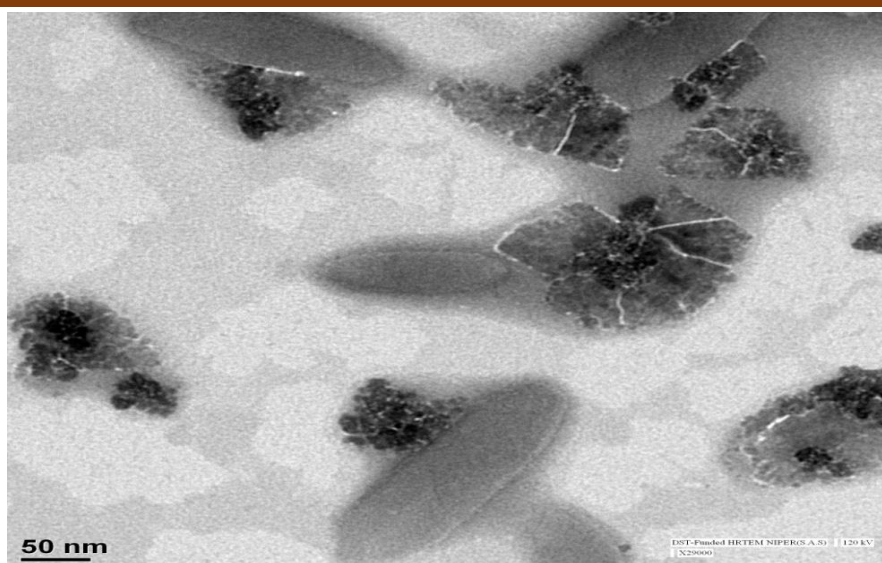


Fig. 6.23 Transmission electron micrograph of (T1) nanocrystals with magnification 290000 X



Fig. 6.24 Transmission electron micrograph of nanocrystals(T1) with magnification 500000 X showing diffraction.

6.6.4: Scanning Electron Microscopy: Morphology of spray dried nanocrystals were determined by Scanning electron microscopy. SEM micrographs revealed spherical crystals with smooth surface within size range of 50-500nm

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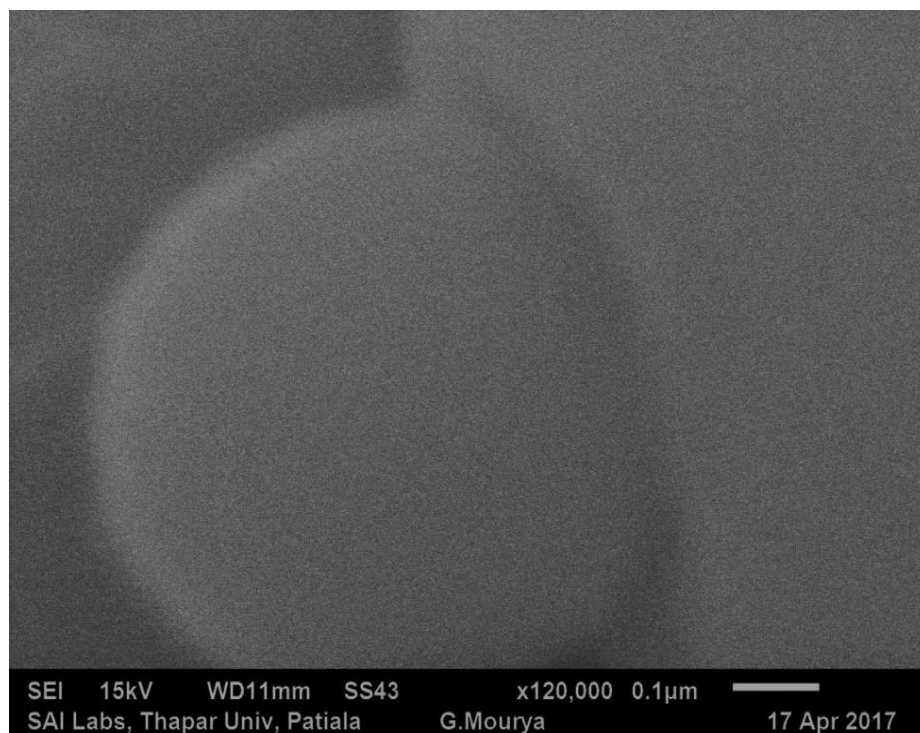


Fig. 6.25 Scanning electron micrograph of nanocrystals (T1) with magnification 120,000X

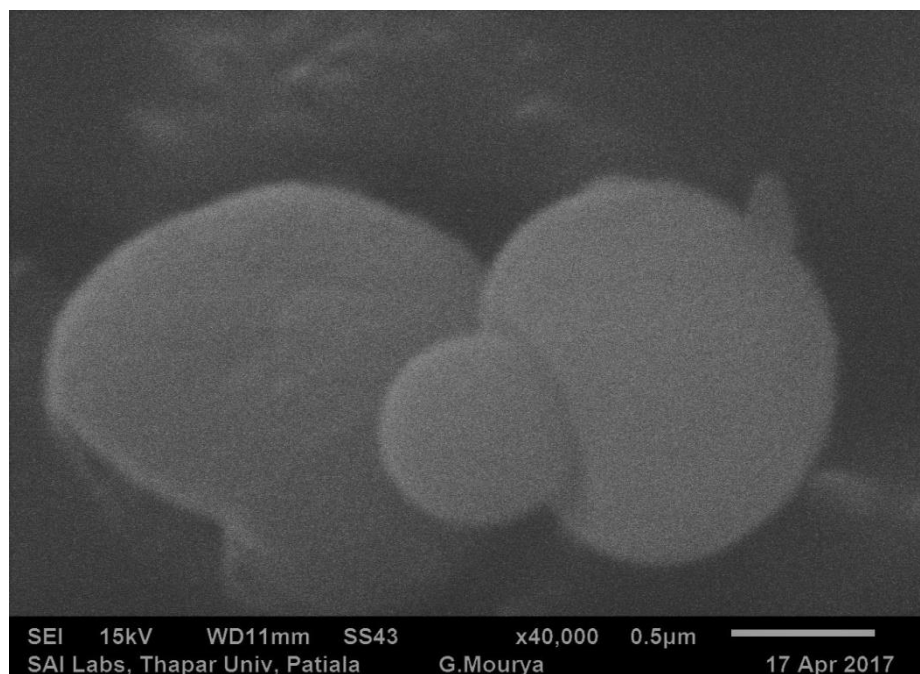


Fig. 6.26 Scanning electron micrograph of nanocrystals (T1) with magnification 40,000X

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6.6.5 X-Ray diffraction: The XRD spectra of nanocrystals, shows that there is no detectable change in the crystalline nature of the drug. Where the sharp peaks in the graph show crystallinity of the drug

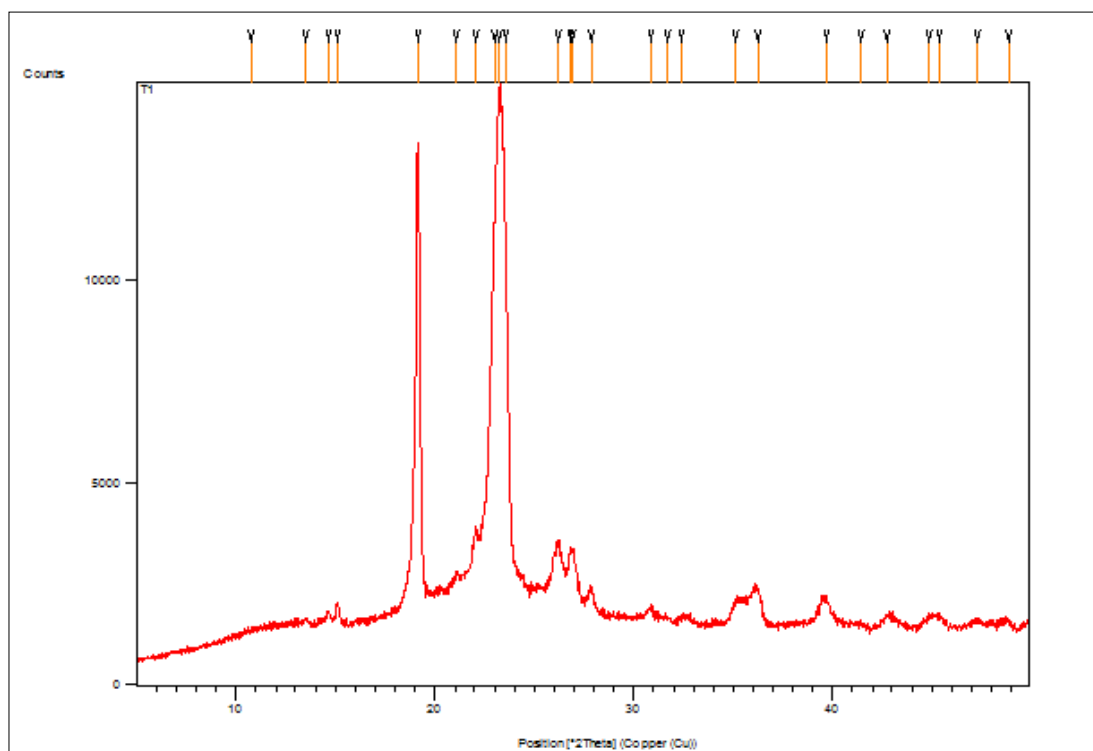


Fig no. 6.27 Represents XRD spectra of Telmisartan nanocrystals

6.6.6 In Vitro dissolution studies

In- Vitro drug release studies were performed in Dissolution test apparatus (LABINDIA D5 000) in 1.2 pH HCl buffer and 6.8 pH phosphate buffer. The dissolution study was performed between telmisartan pure drug and nanocrystals of telmisartan. In 1.2 pH HCl buffer, the drug release of pure drug after 50min was found to be 6% whereas in the case of nanocrystals formulation the percentage drug release was 70.5%. And in 6.8 pH phosphate buffer drug release of pure drug was 2.5% and 70.07% of telmisartan nanocrystals.

6.6.6.1 Standard curve of telmisartan in 1.2 pH HCl buffer

A standard curve of telmisartan in 0.1N HCl was prepared by dissolving the 10mg drug into 10ml 0.1N HCl. Then the 100ppm solution was prepared from above stock solution by taking 1ml into 10ml volumetric flask and make up the volume up to 10ml with 1.2 pH HCl buffer.

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Further dilutions were prepared by taking 2,4,6,8,10 ml from 100ppm solution and make up the volume up to 10ml.

Table 6.24 Standard curve of telmisartan in 1.2 pH HCl buffer at λ_{\max} 290.5

S. No	Concentration($\mu\text{g/ml}$)	Absorbance
1	0	0
2	2	0.238
3	4	0.421
4	6	0.623
5	8	0.841
6	10	0.976

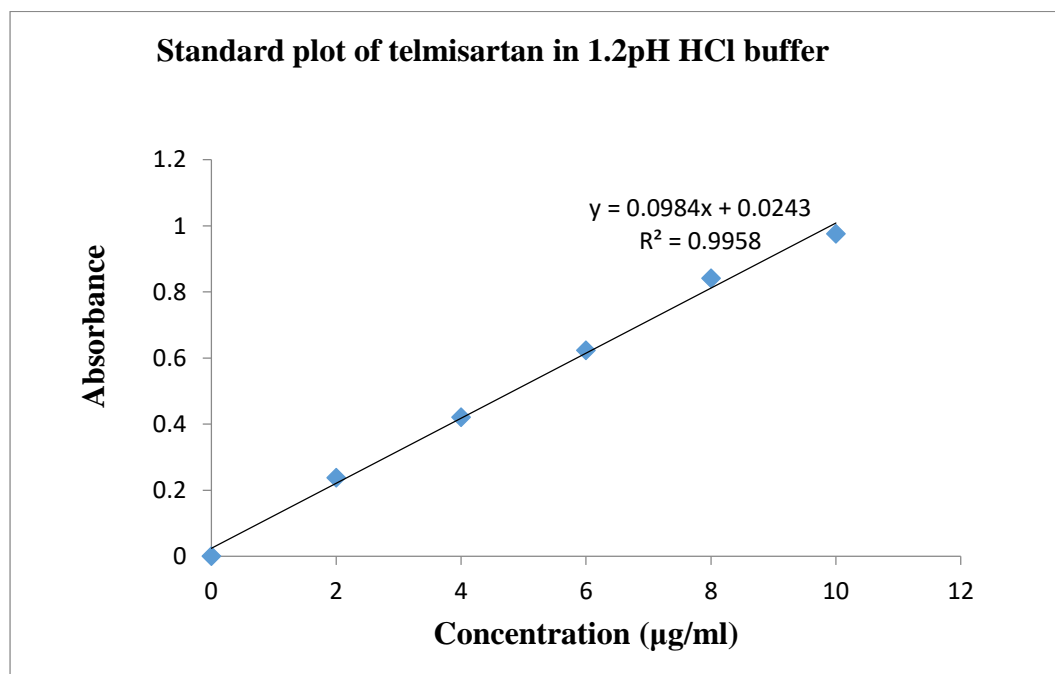


Figure 6.28 Calibration curve of telmisartan in 1.2 pH HCl buffer

Table 6.25 In-Vitro Dissolution release of pure telmisartan in 1.2 pH HCl buffer

S. No	Time(min)	Absorbance	Conc. ($\mu\text{g/ml}$)	Cum. Drug release	% DR of pure drug
0	0	0	0	0	0
1	10	0.625	6.104	1.22	3.05
2	20	0.93	9.2	1.87	4.67
3	30	0.124	1.013	2.06	5.16
4	40	0.131	1.087	2.21	5.52
5	50	0.136	1.138	2.27	5.68

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Table 6.26 In-Vitro Dissolution release of telmisartan nanocrystals in 1.2 pH HCl buffer

S. No	Time(min)	Absorbance	Conc. ($\mu\text{g/ml}$)	Cum. Drug release	% DR of formulation
0	0	0	0	0	0
1	10	0.5994	5.868	1.16	29
2	20	0.985	9.804	1.98	49.5
3	30	0.126	1.03	2.1	52.5
4	40	0.156	1.34	2.73	68.25
5	50	0.16	1.38	2.82	70.5

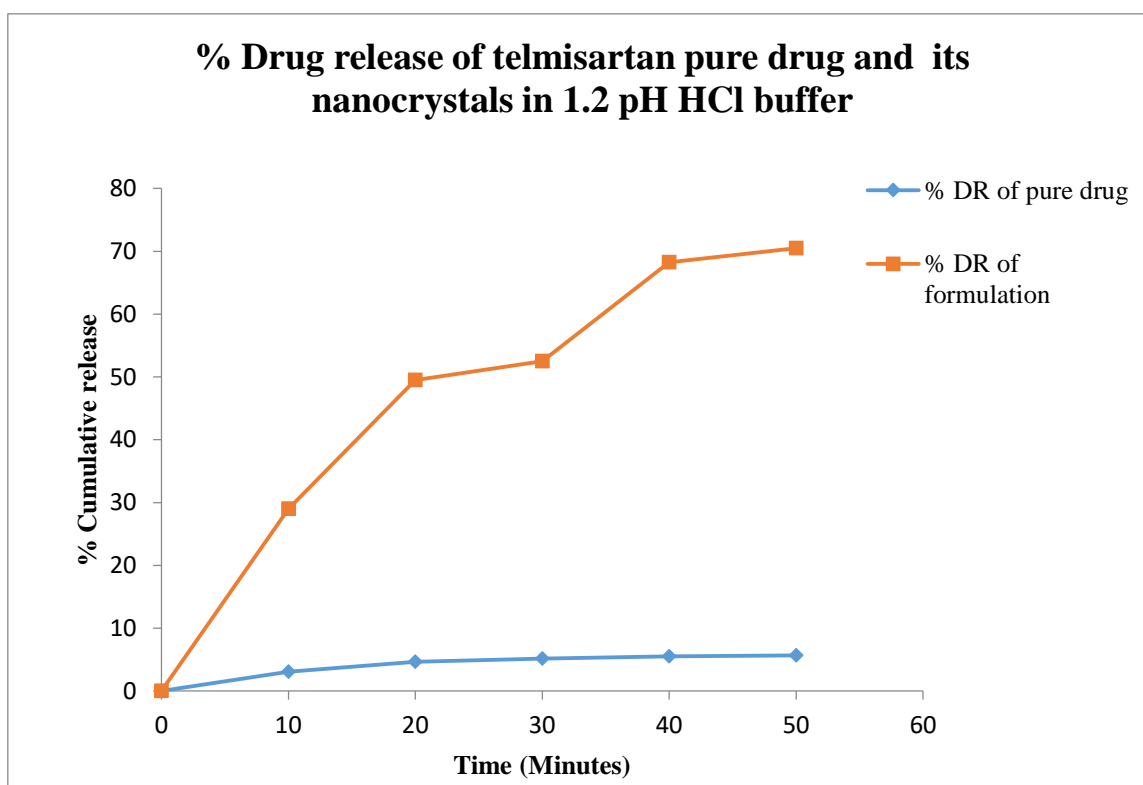


Fig: 6.29 Percentage cumulative release of Telmisartan pure drug and its nanocrystals in 1.2 pH HCl buffer.

6.6.6.2 Standard curve of telmisartan in 6.8 pH phosphate buffer

A standard curve of telmisartan in 6.8 pH phosphate buffer was prepared by dissolving the 10mg drug into 10ml 6.8 pH phosphate buffer. Then the 100ppm solution was prepared from above stock solution by taking 1ml into 10ml volumetric flask and make up the volume up to 10ml with 6.8 pH phosphate buffer. Further dilutions were prepared by taking 2,4,6,8,10 ml from 100ppm solution and make up the volume up to 10ml with 6.8 pH phosphate buffer.

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Table 6.27 Standard curve of telmisartan in 6.8 pH phosphate buffer at λ_{\max} 296

S. No	Concentration($\mu\text{g/ml}$)	Absorbance
0	0	0
1	2	0.116
2	4	0.241
3	6	0.326
4	8	0.433
5	10	0.515

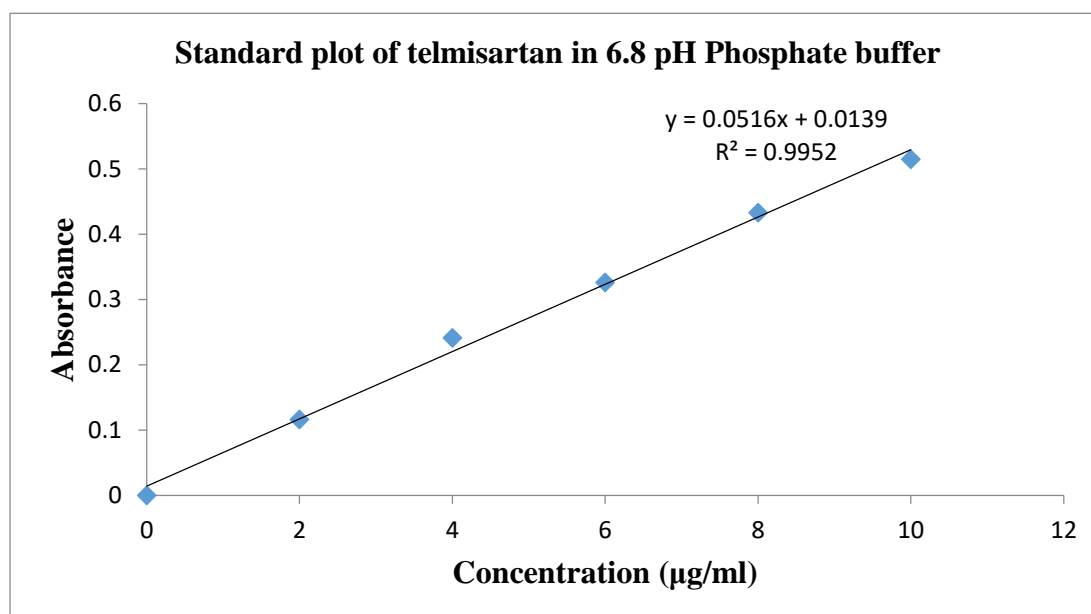


Figure 6.30 Calibration curve of telmisartan in 6.8 pH phosphate buffer

Table 6.28 In-Vitro Dissolution release of pure telmisartan in 6.8 pH phosphate buffer

S. No	Time (minutes)	Absorbance	Concentration ($\mu\text{g/ml}$)	Cumulative Drug release	% DR of pure drug
0	0	0	0	0	0
1	10	0.0519	0.736	0.662	1.65
2	20	0.0532	0.761	0.685	1.71
3	30	0.0566	0.827	0.744	1.86
4	40	0.0696	1.079	0.967	2.41
5	50	0.0719	1.124	1.013	2.53

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Table 6.29 In-Vitro Dissolution release of telmisartan nanocrystals in 6.8 pH phosphate buffer

S.No	Time(min)	Absorbance	Concentration (µg/ml)	Cumulative Drug release	% DR of formulation
0	0	0	0	0	0
1	10	0.0684	1.056	0.9	22.5
2	20	0.1162	1.98	1.787	44.68
3	30	0.1432	2.25	2.261	56.47
4	40	0.1562	2.77	2.442	61.05
5	50	0.1741	3.12	2.803	70.07

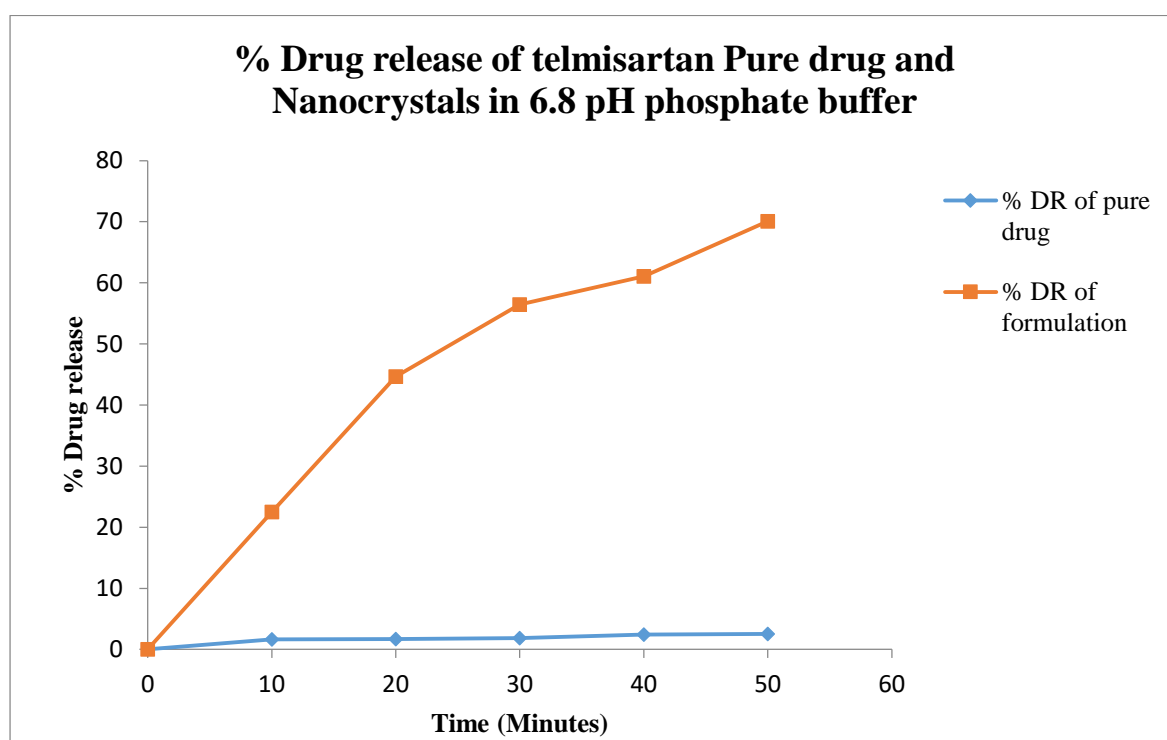


Fig: 6.31 Percentage cumulative release of Telmisartan pure drug and its nanocrystals in 6.8 pH phosphate buffer

6.6.6.3 Analysis of release mechanism of optimized formulation by kinetic modeling

The in-Vitro drug release data obtained from dissolution testing were fitted into several release kinetic models such as zero order, first order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell model. These kinetic models help to understand the release mechanism of the formulation. The pharmaceutical formulation that does not disintegrate fast and releases the drug slowly follows

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zero order kinetics. The dosage form in which dissolution rate depends on the concentration of the drug follows first order kinetics. Higuchi models are used for those formulations that contain poorly water soluble and water soluble drugs entrapped in solid and semisolid matrixes. For tablets in which dissolution occurs in planes, which is parallel to the drug surface Hixson-Crowell model is applied. Whereas Korsmeyer-Peppas model is used for the formulation containing polymers in which the release mechanism is not well known and more than one release pattern involved (Ahuja et al., 2007). The R² and k values are mentioned in the table. the model in which the R² value is found to be near 1.000 was considered as a best-fit model for the formulation. In Korsmeyer -Peppas model if the value of n is equal or greater than 0.5 it means release mechanism follows fickian transport and if it is less than 0.5 that means the formulation follows anomalous transport.

Table 6.30 Representing the release kinetic of pure telmisartan and telmisartan nanocrystals in 1.2 PH HCl buffer

S.No	Formulation	Zero order	First order	Higuchi model	Korsmeyer-Peppas model	Hixson-Crowell model
1	Pure TEL	K= 0.142 r ² = 0.628	K= 0.001 r ² = 0.647	K= 0.892 r ² = 0.958	K=1.536 r ² = 0.985 n= 0.345	K= 0.00 r ² = 0.64
2	Nanocrystals TEL	K=1.656 r ² = 0.834	K= 0.028 r ² = 0.969	K= 10.205 r ² = 0.984	K= 9.625 r ² = 0.985 n= 0.517	K= 0.008 r ² = 0.943

Here K represents the slope except for first order where k = slope × 2.303

RESULTS AND DISCUSSION

Table 6.31 Representing the release kinetic of pure telmisartan and telmisartan nanocrystals in 6.8 PH Phosphate buffer

S.No	Formulation	Zero order	First order	Higuchi model	Korsmeyer-Peppas model	Hixson-Crowell model
1	Pure TEL	K= 0.060 r ² = 0.613	K= 0.001 r ² = 0.620	K= 0.375 r ² = 0.934	K= 0.720 r ² = 0.970 n= 0.313	K= 0.00 r ² = 0.618
2	Nanocrystal TEL	K= 1.592 r ² = 0.891	K= 0.026 r ² = 0.987	K= 9.745 r ² = 0.976	K= 6.827 r ² = 0.984 n= 0.601	K= 0.007 r ² = 0.9717

Here K represents the slope except for first order where $k = \text{slope} \times 2.303$

Korsmeyer-Peppas model is the most befitting model for the nanocrystals formulation. From the value of n, it was concluded that the formulations followed fickian diffusion.

6.6.7 Stability study of best formulation T1 prepared by method 1 stored at 4°C and 40°C/RH

The best formulation T1 containing 1mg active ingredient and the 10mg polymer is analyzed for its stability at 4°C temperature. The stability was analyzed on the basis of one parameters: Percentage entrapment efficiency (K. Nanjwade et al., 2011)

Table 6.32 Stability studies of nanocrystals at 4°C temperature

Evaluation parameters	Temperature of storage	0 day	30 days	60 days
% Entrapment efficiency	4°C	92	91.41	90.65

The values of drug entrapment efficiency states that there were slight changes observed in entrapment efficiency and the formulation was stable for a period of 2 months

SUMMARY AND CONCLUSION

CHAPTER 7

SUMMARY AND CONCLUSION

Hypertension is a modifiable risk factor for heart failure, stroke, end-stage renal disease and peripheral vascular disease. Almost 50% of urban and 50% of rural population of India suffers from high blood pressure and takes medication for the treatment of the disease. In order to improve the bioavailability and to reduce side effects of the conventional dosage, form nanocrystals have been prepared.

Telmisartan was selected as a model drug for research work. The characterization of telmisartan is done by Infrared spectroscopy and melting point analysis. The nature of the drug is analyzed by its partition coefficient and solubility analysis. Analytical method validation of telmisartan in 0.1N HCl solution was carried out to establish a simple and reproducible analytical method for estimation of telmisartan. Two different methods are selected for preparation of nanocrystals of telmisartan. Prescreening studies were carried out to check the range and ratio of polymers. 12 formulations were prepared by method 1 and 8 formulations were prepared by method 2 according to the reference. The formulation was analyzed to check entrapment efficiency and optical microscopy. 3 optimized batches from two different methods were selected to analyze particle size and zeta potential. On the basis of results of particle size and zeta potential one best formulation was selected for further characterization. The best formulation was studied for its morphology by TEM which ensures the formation of nanocrystals with a size range of 164.2nm. The best-optimised formulation was prepared in bulk and spray dried. The powder characteristics were analyzed by Scanning electron microscopy which reveals the spherical structures and crystalline property of the drug are analyzed by XRD. The spray dried powder and active drug then transformed into tablet dosage form. The in-Vitro drug release studies were carried out by dissolution testing to compare release pattern of prepared nanocrystals and pure telmisartan. Drug release from pure drug in 1.2pH HCl buffer and 6.8pH Phosphate buffer was found to be 5.68% and 2.53% whereas the drug release from prepared nanocrystals in 1.2pH HCl buffer and 6.8pH Phosphate buffer was found to be 70.5% and 70.07%.

From the studies carried out between prepared nanocrystals and pure drug, it has been concluded that there is almost 12 fold increase in solubility of telmisartan. Therefore the

SUMMARY AND CONCLUSION

nanocrystals prepared by antisolvent evaporation followed by sonication method proved to be a most effective method to increase the solubility and dissolution rate of the drug.

FUTURE ASPECTS:

The present study has provided information regarding the preparation of nanocrystals of telmisartan to improve solubility and dissolution rate. The sincere efforts have been devoted to exploring all the possible outcomes related to the development, validation, and evaluation of the system. However, there is always a scope for a researcher to proceed further. The future aspects of the study involve the Ex-vivo study of the nanocrystals to treat hypertension as well as hyperglycemia. With the advancement and researches carried, nanocrystals have emerged as a potential delivery system to increase the solubility and bioavailability of pharmaceutical dosage form.

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