

PREPARATION OF NANOPARTICLES OF OXCARBAZEPINE BY HIGH PRESSURE HOMOGENIZATION TECHNIQUE

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ABSTRACT: A majority of drugs developed and used today suffer from poor bioavailability due to poor aqueous solubility or low dissolution rate. One of the main focus of pharmaceutical technology is developing novel strategies to enhance the solubility of poorly water soluble drugs. Nano suspensions are a promising strategy for improving the dissolution rate and oral bioavailability of poorly water soluble drugs by reducing the particle size or transforming drugs from crystalline to an amorphous state. In the present time an increasing attention has focused on nanosuspensions considering these general advantage of nanotechnology, the main objective of the present investigation was to prepare Nanosuspension of Oxcarbazepine which is widely used as antiepileptic drug belongs to BCS class II (low solubility, high permeability). Nanosuspension of Oxcarbazepine were produced by combining nanoprecipitation and high pressure homogenization technique with using polymer like poloxamer 188, soluplus, PLGA etc. All the suspension prepared with the different drug : polymer ratio showed a significant decrease in particle size with increase in the number of homogenization cycles and pressure. Nanosuspension were prepared and analyzed for particle size, zeta potential, entrapment efficacy and *in vitro* drug release study. Batch (PLG2) drug : polymer:stabilizer (1:0.5:0.5), shows particle size (229.1nm), P.I.(0.411), zeta potential (-0.2), % entrapment efficacy (73.86) and better drug release. Oxcarbazepine nanoparticles were produced by converting nanosuspension into dry powder by lyophilization technique. The physicochemical properties of drug and its nanoparticles were characterized by SEM, XRD, FT-IR, DSC, *in-vitro* drug dissolution and saturation solubility. The DSC and XRD results indicated that nanoprecipitation led to amorphization of oxcarbazepine. An increase in the stability of nanoparticles was also assured by sufficient adsorption of the polymer on to the surface.

Keywords: Oxcarbazepine, Nanoparticles, Polymers, Homogenization, Lyophilization, Nanosuspension.

INTRODUCTION

Nanotechnology is the term to design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel/superior characteristic or property. Nanoscience and nanotechnology give an expanding research area, which involves structures, devices, and systems with novel properties and functions due to the arrangement of their atoms on the 1–100 nm scale. The nanotechnology field was subject to a growing public awareness and controversy in the early 2000s, and in turn, the beginnings of commercial applications of nanotechnology. Nanotechnologies contribute to almost every field of science, including physics, materials science, chemistry, biology, computer science, and engineering.

In recent years nanotechnologies have been applied to human health with promising results, especially in the field of cancer treatment. This review concludes the progress and main principles of nanoscience and nanotechnology and represents the pre-modern as well as modern timeline era of discoveries and milestones in these fields. The prefix ‘nano’ is referred to a Greek prefix meaning ‘dwarf’ or something very small and depicts one thousand millionth of a meter (10^{-9} m). We should distinguish between nanoscience, and nanotechnology. Nanoscience is the study of structures and molecules on the scales of nanometers ranging between 1 and 100 nm, and the technology that utilizes it in practical applications such as devices etc. is called nanotechnology.^[1] A Nanoparticles (NPs) is a small particle that ranges between 1 to 100 nanometers in size. These NPs have been investigated to improve the BA, solubility, permeability, stability and therapeutics efficacy of drugs. While convert in NPs decrease in size of particles which increases surface area of particles and this in turn increases the dissolution rate by the Noyes Whitney equation. Preparation of NPs by various methods which are solvent evaporation technique, nano-precipitation technique, solvent diffusion technique dialysis technique, high pressure homogenization technique (HPH). PolymericNPs (PNPs) are particles within the size range from 1 to 1000 nm and can be loaded with active compounds entrapped within or surface-adsorbed onto the polymeric core.

1.1. Drug delivery system developed using nanotechnology principle are:

- Solid lipid NPs
- Polymeric NPs
- Nanoemulsion
- Nanosuspension
- Nanocrystals

1.2. NPs mediated drug delivery system:

NPs can offer significant advantages over the traditional delivery mechanisms in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release possibility to use in different types of drug administration and the capability to transport both hydrophilic and hydrophobic molecules. The drugs may be enclosed inside the sphere of the NPs or linked to the surface. Once they are at the target site, the drug payload may be released from the NPs by diffusion, swelling, erosion or degradation. Active systems are also possible, e.g. drug release in response to the input of external energy such as targeted ultrasound, light or magnetic field. A few types of NPs including Lps, polymeric NPs, solid lipid NPs and dendrimers have been widely investigated as antimicrobial drug delivery platforms, of which several products have been introduced into pharmaceutical market.

The advantages of using NPs as a drug delivery system include the following:

1. Controlled and sustained release of the drug during the transportation and at the site of localization, altering organ distribution of drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficiency and reduction in side effects.
2. Drug can be incorporated in to the system without any chemical reaction; this is an important factor for preserving the drug.
3. Controlled release and drug degradation characteristics can be readily modulated.
4. There is no wastage of drug and thus enhanced BA of drug at specific site in right proportion for prolonged period of time.
5. It improve the solubility of poorly water soluble drugs, prolong half life of drug systemic circulation by reducing immunogenicity, release drug at sustained rate and lower the frequency of administration.
6. It provides comfort and compliance to the patient and yet improves the therapeutic performance of the drug over conventional systems.

1.3. Liposomes (Lps) for drug delivery: Lps are spherical lipid vesicles with a bi layered membrane structure consisting of amphiphilic lipid molecules. It is used as drug delivery NPs. It can be made of either natural or synthetic lipids. Currently, Lps are the most widely used antimicrobial drug delivery system. One of the distinguishing features of Lps is its lipid bilayer structure, which mimics cell membranes and can readily fuse with infectious microbes. By directly fusing with bacterial membranes, the drug payloads of Lps can be released to the cell membranes or the interior of the bacteria . It carries both hydrophobic and hydrophilic compounds without chemical modification.

1.4.PNPs for drug delivery: Biocompatible and biodegradable polymers are used extensively for controlled drug release. Polymeric NPs are structurally stable and properties such as size, zeta potentials and drug release profiles can be tuned by selecting different polymer lengths, surfactants and organic solvents during the synthesis. The surface of polymeric NPs typically contains functional groups that can be chemically modified with targeting ligands . The drugs can be either absorbed to the nano capsules during the polymerization process or covalently conjugated to the surface of the NPs after they are formed.

1.5.Dendrimers (Dend) for drug delivery: Dend are highly ordered and regularly branched globular macromolecules. It consists of three regions: a core, layers of branched repeat units emerging from the core, and functional end groups on the outer layer of repeat units. The highly-branched nature of dend provides large surface area to size ratio and allows great reactivity with microorganisms in vivo . Both hydrophobic and hydrophilic agents can be loaded into dend. Hydrophobic drugs can be loaded inside the cavity in the hydrophobic core, and hydrophilic drugs can be attached to the multivalent surfaces of dend through covalent conjugation or electrostatic interaction.

1.6.1Ceramic NPs: CNPs are inorganic systems with porous characteristics. Because these particles can be easily engineered with the desired size and porosity, growing interest has recently emerged to utilize CNPs as drug vehicles. Most of the research has been exploring typical biocompatible CNPs such as silica, titania, alumina etc. In cancer therapy because of their physiological stability, hybridizing CNPs with DNA offers a potential therapy to target liver cancer cells.

1.6.2 PNPs:They are solid colloidal particles ranging in size from 10 to 1000 nm (1µm). Drug may be dissolved, entrapped, encapsulated or attached to a NPs matrix . Because these systems have very high surface areas, drugs may also be adsorbed on their surface. Polymer-based NPs effectively carry drugs, proteins and DNA to target cells and organs. Depending upon Method of Preparation Polymeric NPs can be divided into

- 1.Nanospheres: They are the systems in which the drug is confined to a cavity surrounded by a unique polymer membrane.
2. Nanocapsules: They are the matrix systems in which the drug is physically and uniformly dispersed

PNPs advantages:

1. Increases the stability of any volatile agents & can be easily and cheaply fabricated in large quantities by a multi methods.
2. Has significant advantages over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
3. Delivers a higher concentration of pharmaceutical agent.
4. The choice of polymer and the ability to modify drug release from polymeric NPs have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
5. Targeted Drug Delivery System. polymeric NPs can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

PNPs disadvantages:

1. Very costly formulation with no low yield
2. Productivity is more difficult.
3. As a industrial applications, Technology transfer to commercial production is very difficult.
4. Reduced ability to adjust the dose
5. Highly sophisticated technology
6. Requires skills to manufacture.
7. Stability of dosage form is big issue owing to its nano size

Characterization of PNP's

1. Particle size and size distribution
2. Surface area, surface chemistry, surface coating and porosity
3. Hydrophilicity and surface charge density
4. Purity and quality
5. Stability (on shelf and upon administration)
6. Drug release parameters and bioequivalence testing considerations
7. % Entrapment efficiency
8. % PNP Yield
9. Size evaluation :Done by number of methods with/without Freeze fracture like
 - a. Photon correlation spectroscopy,
 - b. Transmission electron microscopy(TEM)
 - c. Scanning electron microscopy(SEM)
10. Surface Charge, Molecular Wt, Structure& Crystallinity :
 - Surface Charge: Measuring particle velocity in electric field
 - Molecule charge: Gel permeation
 - For Structure/crystallinity different thermal methods are used DSC , DTA ,TGA
11. In vitro Drug release :Ideally Franz-diffusion cell

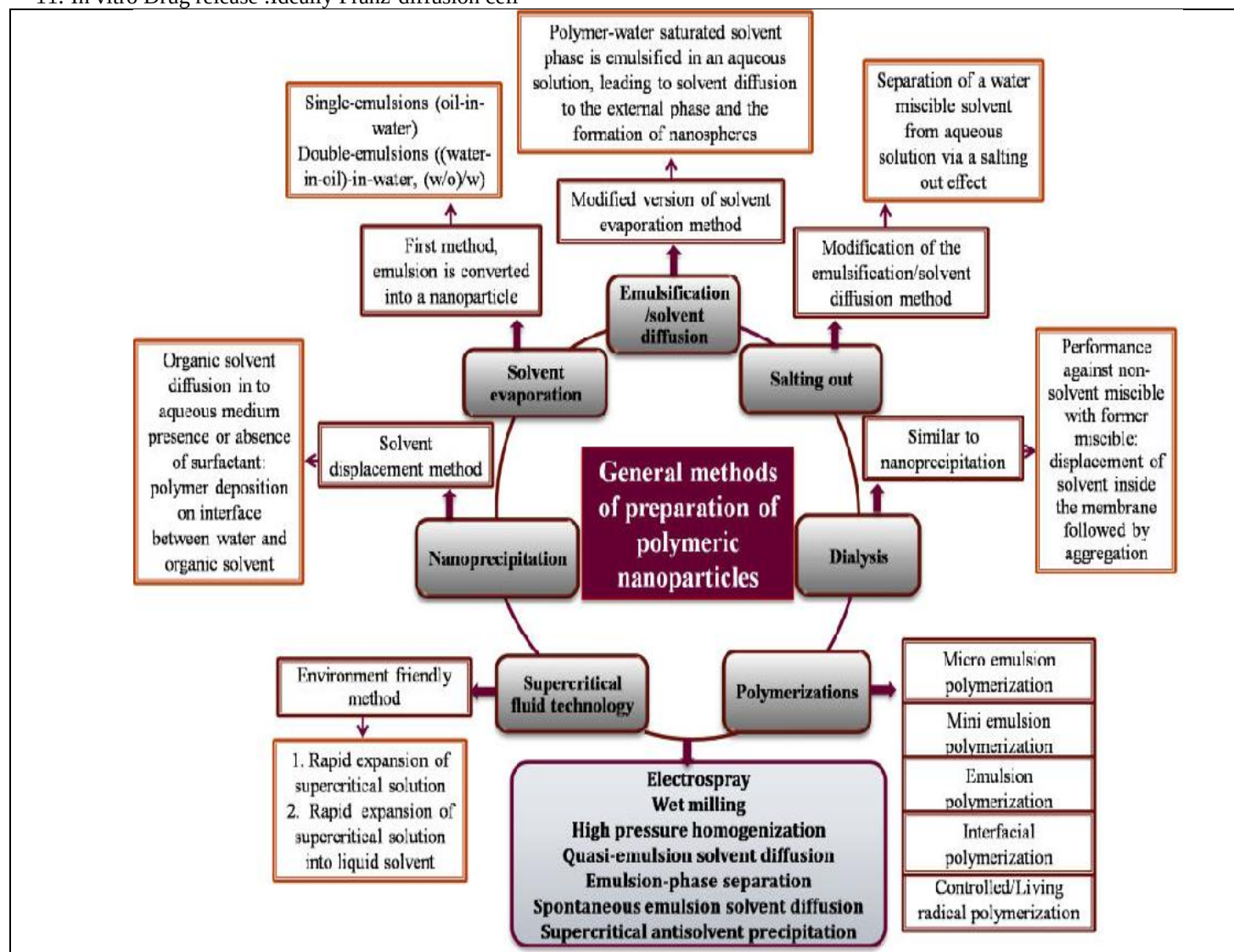


Fig 1.1 General methods for preparation of polymeric nanoparticles

1.7. Methods to preparation of PNPs:

1.7.1. Antisolvent crystallization method : Anti-solvent crystallization is the separation and purification method which is used as an effective way to prepare micro to nano-size drug particles . This technique produces crystals from solutions and controls the crystalline properties such as particle size and their morphology. The use of the antisolvent in crystallization reduces the solubility of a solute in the solution and to induce rapid crystallization.

The physical and chemical properties of the antisolvent can alter the rate of mixing with the solutions and thereby affect the rate of nucleation and crystal growth of the crystallizing compounds. Additionally, parameters of crystallization experiments strongly influence the mechanism of particle formation and govern the form of crystal size and its distribution.

1.7.2.Solvent Evaporation: Solvent evaporation was the first method developed to prepare polymeric NPs from a preformed polymer. In this method, the preparation of an oil-in-water (o/w) emulsion is initially required, leading to nanospheres production. Firstly, an organic phase is prepared, consisting of a polar organic solvent in which the polymer is dissolved, and the active ingredient (e.g., drug) is included by dissolution or dispersion. Dichloromethane and chloroform have been widely used, although more often in the past. Due to their toxicity, they have been replaced by ethyl acetate, which displays a better toxicological profile, and therefore, it is more suitable for biomedical applications.

An aqueous phase, which contains a surfactant (e.g., polyvinyl acetate; PVA), has also been prepared frequently. The organic solution is emulsified in the aqueous phase with a surfactant, and then it is typically processed by using high-speed homogenization or ultrasonication, yielding a dispersion of nanodroplets. A suspension of NPs is formed by evaporation of the polymer solvent, which is allowed to diffuse through the continuous phase of the emulsion.

The solvent is evaporated either by continuous magnetic stirring at room temperature (in case of more polar solvents) or in a slow process of reduced pressure (as happens when using e.g., dichloromethane and chloroform). After the solvent has evaporated, the solidified NPs can be washed and collected by centrifugation, followed by freeze-drying for long-term storage.

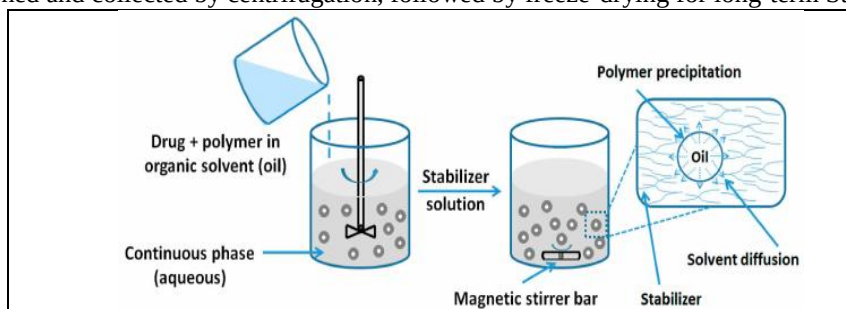


Fig.1.2 Schematic representation of the solvent evaporation method

1.7.3. Emulsification/Solvent Diffusion : This method consists on the formation of an o/w emulsion between a partially water-miscible solvent containing polymer and drug, and an aqueous solution with a surfactant. The internal phase of this emulsion consists of a partially hydro-miscible organic solvent, such as benzyl alcohol or ethyl acetate, which is previously saturated with water in order to ensure an initial thermodynamic balance of both phases at room temperature. The subsequent dilution with a large amount of water induces solvent diffusion from the dispersed droplets into the external phase, resulting in the formation of colloidal particles.

Generally, this is the method used to produce nanospheres, but nano capsules can also be obtained if a small amount of oil (such as triglycerides: C6, C8, C10, C12) is added to the organic phase. Finally, depending on the boiling point of the organic solvent, this latter stage can be eliminated by evaporation or by filtration. This method is schematically shown in Figure 3. At the end, it is possible to obtain NPs with dimension ranging from 80 to 900 nm. This method is frequently applied for polymeric NPs production, despite the requirement of a high volume of the aqueous phase, which must be eliminated from the colloidal dispersion, and despite the risk of diffusion of the hydrophilic drug into the aqueous phase.

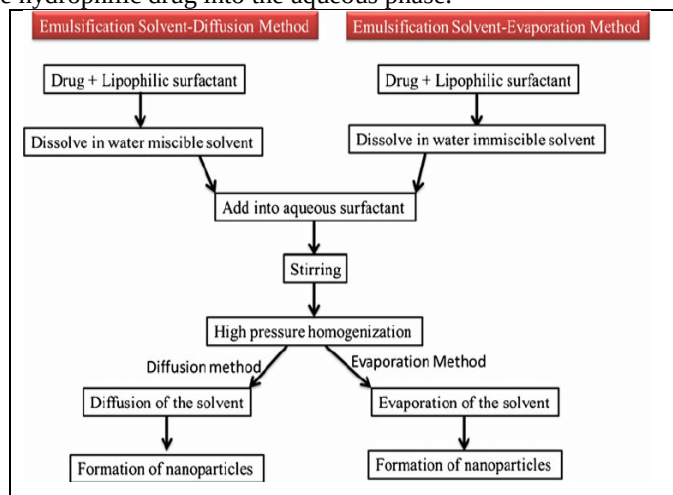


Fig 1.3 Schematic representation of the emulsification/solvent diffusion method

1.7.4. Emulsification/Reverse Salting-Out : The above described emulsification/solvent diffusion method can be considered as a modification of the emulsification/reverse salting-out method. The salting-out method is based on the separation of a hydro-miscible solvent from an aqueous solution, through a salting-out effect that may result in the formations of nanospheres. The main difference is the composition of the o/w emulsion, which is formulated from a water-miscible polymer solvent, such as acetone or ethanol, and the aqueous phase contains a gel, the salting-out agent and a colloidal stabilizer. Examples of suitable salting-out agents include electrolytes, such as magnesium chloride (MgCl_2), calcium chloride (CaCl_2) or magnesium acetate [$\text{Mg}(\text{CH}_3\text{COO})_2$], as well as non-electrolytes e.g., sucrose.

The miscibility of acetone and water is reduced by saturating the aqueous phase, which allows the formation of an o/w emulsion from the other miscible phases. The o/w emulsion is prepared, under intense stirring, at room temperature. Then, the emulsion is diluted using an appropriate volume of deionized water or of an aqueous solution in order to allow the diffusion of the organic

solvent to the external phase, the precipitation of the polymer, and consequently, the formation of nanospheres. The remaining solvent and salting-out agent are eliminated by cross-flow filtration. The condition of complete miscibility between the organic solvent and water is not essential but it simplifies the execution process. This method is schematically presented in Figure 4. The dimensions of the nanospheres obtained by this method vary between 170 and 900 nm. The average size can be adjusted to values between 200 and 500 nm, by varying polymer concentration of the internal phase/volume of the external phase.

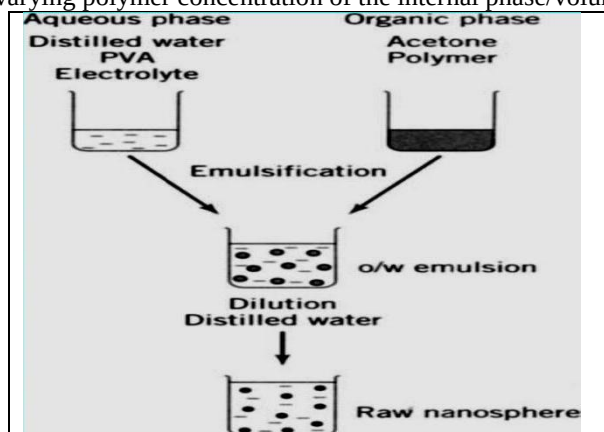


Fig 1.4 Schematic representation of the emulsification/solvent diffusion method

1.7.5. Nanoprecipitation: This method, also designated as solvent displacement method, requires two miscible solvents (Figure 5). The internal phase consists of a polymer dissolved in a miscible organic solvent, such as acetone or acetonitrile. Because of immiscibility in water, they can be easily removed by evaporation. The principle of this technique relies on the interfacial deposition of a polymer after the displacement of the organic solvent from a lipophilic solution to the aqueous phase. The polymer is dissolved in a water-miscible solvent of intermediate polarity, and this solution is added stepwise into an aqueous solution under stirring (in a dropwise way), or by controlled addition rate. Due to the fast spontaneous diffusion of the polymer solution into the aqueous phase, the NPs form instantaneously in an attempt to avoid the water molecules. As the solvent diffuses out from the nanodroplets, the polymer precipitates in the form of nano capsules or nanospheres. In general, the organic phase is added to the aqueous phase, but the protocol can also be reversed without compromising the NPs formation.

Usually, surfactants can be included in the process to guarantee the stability of the colloidal suspension, although their presence is not required to ensure the formation of NPs. The obtained NPs are typically characterized by a well-defined size and a narrow size distribution, which are better than those produced by the emulsification solvent evaporation procedure.

Nanoprecipitation is a method frequently used for the preparation of polymeric NPs with around 170 nm dimensions, but it also allows the acquisition of nanospheres or nano capsules. Nanospheres are obtained when the active principle is dissolved or dispersed in the polymeric solution. Nano capsules are obtained when the drug is previously dissolved in an oil, which is then emulsified in the organic polymeric solution before the internal phase is dispersed in the external phase of the emulsion.

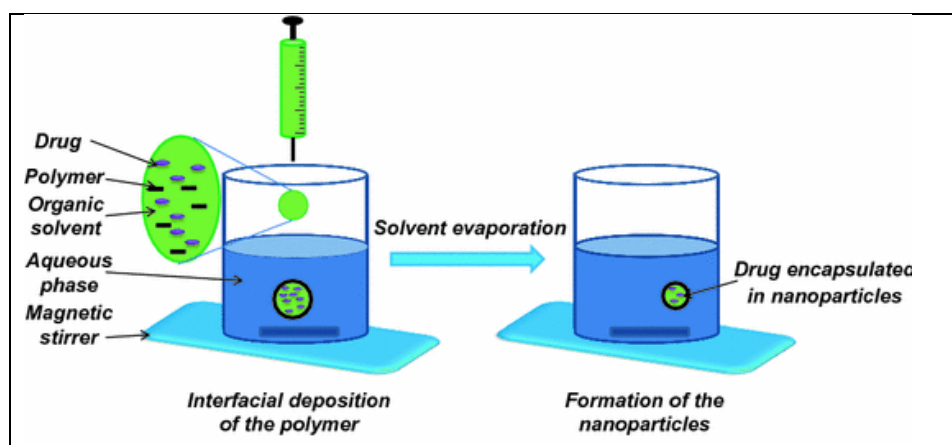


Fig 1.5 Schematic representation of the Nanoprecipitation

1.8. HPH:

HPH in pharmaceutical industry is specifically used for size reduction, mixing, and stabilization of dispersions or droplets. In HPH, liquid is passed through a narrow gap under high pressure where the different processing parameters lead to changes in globule/particle size. HPH is a non-thermal technology. HPH has the distinct advantage of being one of the most versatile and scalable processing methods for the preparation of different vesicular and non vesicular lipid based nanosystems such as nanoemulsion, solid lipid NPs, nanostructured lipid carriers, nanocrystals. The principle of HPH Technique based on cavitation in the aqueous phase. The cavitation forces between particles are high and to convert drug micro particle into NPs. As per principle of HPH method BA and dissolution rate of low soluble drug example OXZ may be improved by reducing their particle size by HPH technique. So OXZ NPs prepared by HPH technique.

Homogenization is method of producing homogeneity throughout a product by change in particle size. HPH combined pressure and mechanical forces to achieve a uniform and consistent product. HPH can accomplish using either cold or hot temperatures.

HPH gives narrow particle size besides other equipment.^[2] High-pressure homogenization has been used to prepare nanosuspension of many poorly water soluble drugs. In this method, the suspension of a drug and surfactant is forced under pressure through a nanosized aperture valve of a high pressure homogenizer. The principle of this method is based on cavitation in the aqueous phase. The cavitations forces within the particles are sufficiently high to convert the drug microparticles into NPs. The concern with this method is the need for small sample particles before loading and the fact that many cycles of homogenization are required. It is most widely used method for preparing nanosuspensions of many poorly aqueous soluble drugs.

It involves three steps:

1. Firstly drug powders are dispersed in stabilizer solution to form pre-suspensions.
2. Secondly the pre-suspension is homogenized in high pressure homogenizer at a low pressure for premilling.
3. Finally homogenized at high pressure for 10 to 25 cycles until the nano-suspensions of desired size are formed.

The operating parameters which effect the efficiency of HPH are as follows:

- Pressure
- Temperature
- Number of passes
- Valve and impingement design
- Flow rate

That HPH technique is unique from other mixing equipment. These forces produce fine particle size which confers increased NPs surface area and BA. HPH allows for an even dispersion of ingredients within a medium; this is incredibly important for the pharmaceutical industry, where active ingredients need to be evenly dispersed within a pill, tablet, capsule, or syrup and within the cosmetic industry, where customers expect their lotions and creams to have an even texture and appearance.

A successfully homogenized product contains particles that are so small and fine that they do not fall out of solution. This is incredibly important to the food and beverage industry the last thing a customer wants is a bottle of juice that has particles visibly falling out of solution.

Homogenization process is classified in three types ultrasonic pressure homogenization, pressure homogenization, mechanical homogenization. In HPH the suspension of drug and surfactant is forced hole pressure through nano size hole valve of HPH.

HPH consists of forcing a pressurized fluid to flow through a minute gap, which not only homogenizes the fluid but also increases the fluid's temperature. By manipulating initial temperature and applied pressure, HPH treatment can be used to pasteurize or sterilize the product.



Fig 1.6 High pressure homogenizer

Advantages of HPH :

1. Low risk of product contamination.
2. Allows aseptic production of nano- suspensions for parenteral administration.
3. Particle size may reduced upto 1 nm.

Disadvantages of HPH :

1. Prerequisite of micronized drug particles.
2. Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.
3. Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.

1.9.Solubility : Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for achieving required pharmacological response. Drug absorption depends on the lipid solubility of the drug, its formulation and the route of administration. A drug needs to be lipid soluble to penetrate membranes unless there is an active transport system or it is so small that it can pass through the aqueous channels in the membrane. Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for desired (anticipated) pharmacological response. Low aqueous solubility is the major problem encountered with formulation development of new chemical entities as well as for the generic development. More than 40% new chemical entities developed in pharmaceutical industry are practically insoluble in water.

Table No.1.1 Biopharmaceutical classification system with characteristics of drug

BCS CLASS	Solubility	Permeability
I	High	High

II	Low	High
III	High	Low
IV	Low	Low

The poor solubility and low dissolution rate of poorly water soluble drugs in the aqueous gastrointestinal fluids often cause insufficient BA. Especially for class II (low solubility and high permeability) substances according to the BCS, the BA may be enhanced by increasing the solubility and dissolution rate of the drug in the gastro-intestinal fluids. As for BCS class II drugs rate limiting step is drug release from the dosage form and solubility in the gastric fluid and not the absorption, so increasing the solubility in turn increases the BA for BCS class II drugs.

Techniques for Solubility Enhancement:

Following techniques are used to enhance solubility

1. Physical Modifications

Particle size reduction like micronization and nanosuspension, modification of the crystal habit like polymorphs, amorphous form and cocrystallization, drug dispersion in carriers like eutectic mixtures, solid dispersions, solid solutions and cryogenic techniques.

2. Chemical Modifications

Change of pH, use of buffer, derivatization, complexation, and salt formation.

3. Miscellaneous Methods

Supercritical fluid process, use of adjuvant like surfactant, solubilizers, cosolvency, hydrotrophy, and novel excipients.

1.10. Polymers used in preparation of NPs :

Polymers have been used to develop devices for controlling drug delivery or for replacing failing natural organs. In oral delivery, polymers are used as coatings, binders, taste maskers, protective agents, drug carriers, and release controlling agents. Synthetic and natural-based polymers have found their way into the pharmaceutical and biomedical industries and their applications are growing at a fast pace. The word “polymer” means “many parts.” A polymer is a large molecule made up of many small repeating units. Polymers are complex and giant molecules usually with carbons building the backbone, different from low molecular weight compounds. The small individual repeating units/molecules are known as monomers (means single part). A polymer with two different monomers is known as a copolymer or homopolymer.

Characteristics of ideal polymer

1. Low Density.
2. Low coefficient of friction.
3. Good corrosion resistance.
4. Good mould ability.
5. Excellent surface finish can be obtained.
6. Can be produced with close dimensional tolerances.
7. Economical.
8. Poor tensile strength.
9. Low mechanical properties.
10. Poor temperature resistance.
11. Can be produced transparent or in different color.

Classification of polymers:

1. classification based on source
2. classification based on structure
3. classification based on polymerization
4. classification based on molecular force

1.11. GENERAL MECHANISM OF DRUG RELEASE FROM POLYMER :

Three primary mechanism for drug release namely:

- Diffusion
- Degradation
- Water penetration (Swelling)

Any of these mechanism can occur in a given release system Drug release from polymer by diffusion, Rate limiting step is diffusion of drug through inert water insoluble membrane barrier.

There are two types a) Reservoir b) Matrix

1. Diffusion:

1. Reservoir diffusion system: In membrane-controlled reservoir devices, the drug is contained in a core, which is surrounded by a polymer membrane, and it is released by diffusion through this rate-controlling membrane e.g. Poly(N-vinyl pyrrolidone), Poly(ethylene-co-vinyl acetate).

2. Matrix diffusion system: In these devices, the drug is released either by passing through the pores or between polymer chains, and these are the processes that control the release rate.

2. Degradation : The drug molecules, which are initially dispersed in the polymer, are released as the polymer starts eroding or degrading. The four most commonly used biodegradable polymers in drug delivery systems are poly(lactic acid), poly(lactic-co-glycolic acid), polyanhydrides, poly(ortho esters), and poly(phosphoesters).

3. Water penetration (swelling) This type of systems are initially dry and when placed in body, absorb water or other fluid and it swells. Swelling increases aqueous solvent content within the formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into external environment.

1.12. Nanosuspension:

Nanosuspension is new carrier free colloidal drug delivery system with nano sized particles below 1000 nm and considered as a great drug delivery method to enhance drug dissolution and solubility. Drug nanosuspensions emerged as one solution to delivering such hydrophobic drugs. Scaling down to NPs enhances drug aqueous solubility and BA by increasing drug surface area.

Production Methods for Preparing Nanosuspension

- Media Milling
- High Pressure Homogenization
- Microprecipitation-High Pressure Homogenization (Nanoedge Technology)
- Emulsion Diffusion Method
- Melt Emulsification Method

Nanosuspensions have been demonstrated to have a number of advantages compared to traditional forms of drug delivery.

These advantages are summarised below:

- (i) solving the problem of poor aqueous solubility and poor BA of the drugs from biopharmaceutical classification systems (BCS) II and IV. For example, nanosizing of azithromycin has proven to increase considerably its dissolution rate; that is, more than 65% was dissolved after 5 hours compared to just 20% that was dissolved from micro-sized system
- (ii) increased drug BA
- (iii) applicability to most drugs which are poorly soluble in both aqueous and organic media
- (iv) simple production methods
- (v) possibility to incorporate nanosuspensions in various dosage formats such as tablets, pellets, and capsules following standard manufacturing techniques. For example, ketoprofen nanosuspension has been successfully transformed into pellets

1.13. Lyophilization (Lyop) technique:

Freeze drying is the process of removing moisture from a frozen product using vacuum. Lyop means freeze drying. The term “lyop” defines a process to produce a product that “loves the dry state”. A stabilizing process in which a substance is first frozen and then the quantity of the solvent is reduced, first by sublimation (primary drying stage) and then desorption (secondary drying stage) to values that will no longer support biological activity or chemical reactions. Lyop or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (desorption).

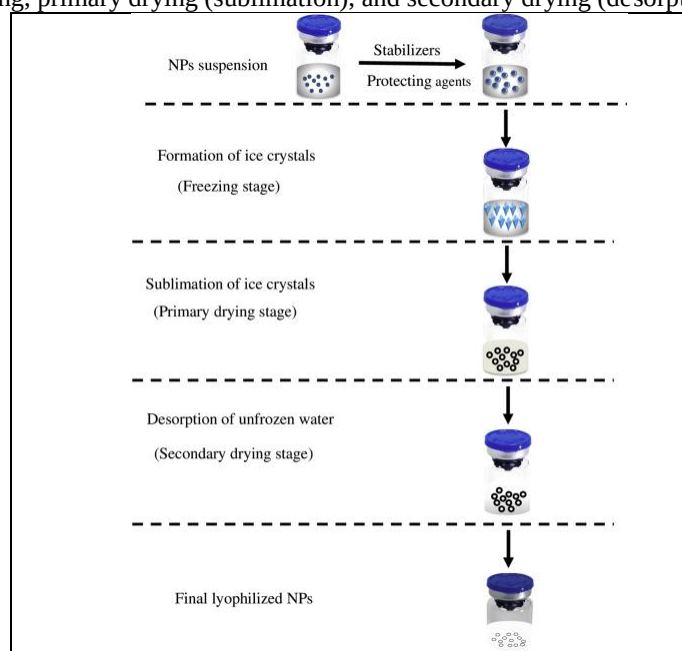


Fig 1.7 Schematic representation of the lyophilization technique

Objectives of lyoprocess

1. To preserve the biological activity of a product.
2. To reduce the product weight to lower the transportation cost.
3. To extend the shelf life or stability.
4. To dry thermolabile materials.
5. To eliminate the need for refrigerated storage.
6. To get accurate, sterile dosing into the final product container.

Advantages of lyop include:

1. Ease of processing a liquid, which simplifies aseptic handling
2. Enhanced stability of a dry powder

3. Removal of water without excessive heating of the product
4. Enhanced product stability in a dry state
5. Rapid and easy dissolution of reconstituted product

Disadvantages of lyop include:

1. Increased handling and processing time
2. Need for sterile diluent upon reconstitution
3. Cost and complexity of equipment

Although lyop has many advantages compared to other drying and preserving techniques it has quite a few disadvantages. It is a long and cost intensive process, requires sterile diluents for reconstitution, it should only be used when product is unstable and heat-labile and the limited amount of vials processed in each run restricts the overall production capacity.¹²

The lyoprocess generally includes the following steps:

1. Dissolving the drug and excipients in a suitable solvent, generally water for injection (WFI).
2. Sterilizing the bulk solution by passing it through a 0.22 micron bacteria-retentive filter.
3. Filling into individual sterile containers and partially stoppering the containers under aseptic conditions.
4. Transporting the partially stoppered containers to the lyophilizer and loading into the chamber under aseptic conditions.
5. Freezing the solution by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber or pre-freezing in another chamber.
6. Applying a vacuum to the chamber and heating the shelves in order to evaporate the water from the frozen state.
7. Complete stoppering of the vials usually by hydraulic or screw rod stoppering mechanisms installed in the lyophilizer.

There are many new parenteral products, including anti-infectives, biotechnology derived products, and in-vitro diagnostics which are manufactured as lyophilized products. Additionally, inspections have disclosed potency, sterility and stability problems associated with the manufacture and control of lyophilized products. In order to provide guidance and information to investigators, some industry procedures and deficiencies associated with lyophilized products are identified in this Inspection Guide. It is recognized that there is complex technology associated with the manufacture and control of a lyophilized pharmaceutical dosage form. Some of the important aspects of these operations include: the formulation of solutions; filling of vials and validation of the filling operation; sterilization and engineering aspects of the lyophilizer; scale-up and validation of the lyop cycle; and testing of the end product. This discussion will address some of the problems associated with the manufacture and control of a lyophilized dosage form.

1.14. OXZ NPs by HPH technique significance:

- BA of OXZ is dissolution rate limited.
- OXZ is high permeability and now focus on solubility enhancement. Now researchers have been investigating several formulation approaches like micronization, complexation, NPs etc.
- As (BCS) class II drug having high lipophilicity and poor aqueous solubility.
- OXZ is a highly lipophilic molecules having very low dissolution in the gastrointestinal fluids.
- Its BA is highly variable.
- Polymeric NPs can increase the rate and extent of oral absorption of a low-solubility, high-permeability drug.
- By improving water solubility the absorption rate increases and also increase in BA.

NEED OF INVESTIGATION

Bioavailability of BCS class II drugs is likely to be dissolution rate limited. But due to their high permeability the drugs have been on focus for solubility enhancement. In the recent times researchers have been investigated several formulation approaches like micronization, complexation, NPs etc. NPs brings some advantages to the oral drug delivery field. Polymer is the key ingredient in the nanoparticle system. The choice of polymer provide considerable design flexibility by facilitate modulation of physiochemical parameters such as size, surface charge hydrophobicity and drug release properties. OXZ is Biopharmaceutical Classification System (BCS) class second drug which have low solubility, high permeability. Oxcarbazepine is a keto analogue of carbamazepine and, like the parent drug, is a potent anticonvulsant used alone or in combination with other agents in the therapy of partial seizures. It is reported to be practically insoluble in water and has low to variable BA. This solubility problem may be overcome by NPs formulation.

AIM & OBJECTIVE

The aim of present work was to increase the solubility of OXZ, a poorly watersoluble drug by Nanosuspension technology.

1. Pre-formulation study of OXZ.
2. Better polymer and their ratios will be find out to promote the solubility of OXZ.
3. Preparation of nanosuspension: OXZ nanosuspension were produced by combining nanoprecipitation and high pressure homogenization technique with the polymers such as Poloxamer 188, Soluplus, PLGA etc.
4. Optimization of nanosuspension for maximum drug loading and minimum particle size by varying formulation and process variables.
5. Characterization of prepared nanosuspension for:
 - Particle size
 - Size distribution
 - % Encapsulation efficiency
 - % Cumulative drug release
6. Conversion of liquid nanosuspension into solid form and its characterization as FTIR, DSC, XRD, SEM.
7. Stability study

PLAN OF WORK

- Exhaustive literature survey
- Selection of suitable drug candidate and excipients
- Procurements of materials

5.1. Preformulation study of pure drug:

1. Organoleptic properties
2. Melting Point
3. Spectroscopic evaluation
 - a. Determination of absorption maxima
 - b. Preparation of standard curve by UV-Visible spectroscopy.
 - c. Interpretation of IR spectrum of drug, XRD, DSC, SEM.
 - d. Determination of saturation solubility of drug.

5.2. Experimental study:

1. Preparation of nanosuspension by high pressure homogenization method.
2. Optimize the formulation by performing the Characterization test of nanosuspension.
 - a. Particle size
 - b. Zeta potential
3. Preparation of NPs by converting nanosuspension into dry powder by LPH technique.
4. Characterization test of NPs
 - a. Drug content
 - b. Saturation solubility
 - c. FTIR, XRD, DSC, SEM
5. Stability study

DRUG AND EXCIPIENT PROFILE**6.1 DRUG PROFILE :****OXZ:**

Synonym	:	Oxcarbamazepine, Oxcarbazepina, Oxcarbazepine, Oxcarbazepinum
IUPAC name	:	5-oxo-6H-benzo[b][1]benzazepine-11-carboxamide
Chemical Formula	:	C ₁₅ H ₁₂ N ₂ O ₂
CAS Registry Number	:	28721-07-5
Molecular Weight	:	252.268 g/mol
Melting point	:	215.5 °C
Description	:	White to faintly orange crystalline powder
Solubility	:	Water in soluble (0.07mg/mL), soluble in Methanol, DMSO, Acetone, SLS .
Storage	:	Store at room temperature
Applications	:	Anticonvulsant

Pharmacology :

Oxcarbazepine is a prodrug, which is largely metabolized to its pharmacologically active 10-monohydroxy derivative licarbazepine (sometimes abbreviated MHD).^{[2][18]} Oxcarbazepine and MHD exert their action by blocking voltage-sensitive sodium channels, thus leading to the stabilization of hyper-excited neural membranes, suppression of repetitive neuronal firing and diminishment propagation of synaptic impulses.^[2] Furthermore, anticonvulsant effects of these compounds could be attributed to enhanced potassium conductance and modulation of high-voltage activated calcium channels.^[2]

Pharmacodynamics:

Both oxcarbazepine and licarbazepine were found to show anticonvulsant properties in seizure models done on animals. These compounds had protective functions whenever tonic extension seizures were induced electrically, but such protection was less apparent whenever seizures were induced chemically. There was no observable tolerance during a four weeks course of treatment with daily administration of oxcarbazepine or licarbazepine in electroshock test on mice and rats. Most of the antiepileptic activity

can be attributed to licarbazepine. Aside from its reduction in side effects, it is presumed to have the same main mechanism as carbamazepine, sodium channel inhibition, and is generally used to treat the same conditions.

Mechanism of action : The exact mechanism through which oxcarbazepine and its active metabolite, MHD, exert their anti-epileptic effects is unclear, but is thought to primarily involve the blockade of voltage-gated sodium channels. The opening and closing of sodium channels allows for the propagation of action potentials along neurons - in epilepsy, these action potentials can occur in excess of that required for normal function, and the repetitive and pathological firing of these action potentials leads to seizure activity. Both oxcarbazepine and MHD are thought to inhibit seizure activity by binding to the inactive state of voltage-gated sodium channels, thus prolonging the period in which the receptor is unavailable for action potential propagation. This helps to stabilize hyperexcited neuronal membranes, inhibit repetitive neuron firing, and prevent the spread of seizure activity within the CNS without affecting normal neuronal transmission. Increased potassium conductance and modulation of voltage-activated calcium channels is also thought to play a role in the anti-seizure activity of oxcarbazepine. Inhibition of glutamatergic activity was thought to contribute to oxcarbazepine's activity, but this effect could not be replicated in vivo.

Bioavailability : The bioavailability of the oral formulation of oxcarbazepine is **high (>95%)**. It is rapidly absorbed after oral administration, reaching peak concentrations within about 1-3 hours after a single dose, whereas the peak of MHD occurs within 4-12 hours.

Metabolism: Oxcarbazepine is rapidly and extensively metabolized to its primary metabolite, MHD, which is responsible for the bulk of its anti-epileptic activity and exists in much higher concentrations in the plasma than the parent drug.^{10,11} MHD is formed via reduction by several members of the aldo-keto reductase family of cytosolic liver enzymes and exists as a racemate in plasma in an approximate ratio of 80% (S)-MHD to 20% (R)-MHD.¹ MHD is further metabolized to glucuronide conjugate metabolites for excretion, and small amounts are oxidized to 10-,11-dihydro-10,11-dihydroxycarbamazepine (DHD) which is pharmacologically inactive.^{10,11,7} Only 10% of an administered dose of oxcarbazepine will remain as either the parent drug or glucuronide conjugates of the parent drug.

Excretion

Plasma clearance of oxcarbazepine has been estimated to be approximately 84.9 L/h, whereas plasma clearance of its active metabolite, MHD, was estimated to be 2.0 L/h.² Rapid metabolic clearance appears to be the main pathway for oxcarbazepine, while clearance of its metabolites occurs mainly via renal excretion.

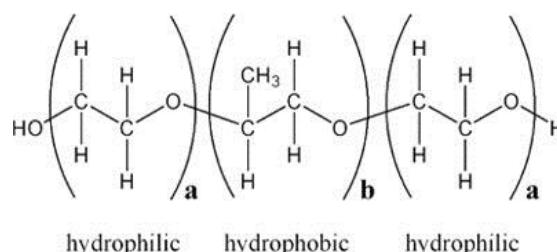
Adverse reaction : The most common adverse effects of oxcarbazepine are usually related to the central nervous system and digestive system, including fatigue, drowsiness, diplopia, dizziness, nausea and vomit. The common skin adverse reaction is rash. Long-term use of oxcarbazepine may also cause hyponatremia.

Therapeutic uses: Oxcarbazepine is an antiepileptic medication that works in the brain to prevent and control seizures. It is approved for the treatment of partial seizures.

6.2. EXCIPIENT PROFILE :

6.2.1. Poloxamer 188 :

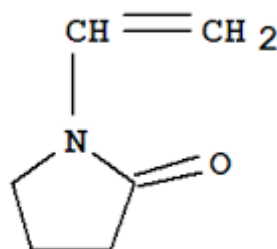
Structure:



Synonyms	: Kolliphor 188, Pluronic F68, Lutrol F68, Polyetene Polypropylene glycol, Polyoxyethylene - Polyoxypropylene Block Copolymer, Poly(Ethylen oxide-co-Polypropylene oxide)
Empirical formula	: $\text{-HO}(\text{CaH}_4\text{O})_a(\text{C}_3\text{H}_7\text{O})_b(\text{C}_2\text{H}_4\text{O})_a\text{H}$
Chemical name	: 2-Methyloxirane; oxirane
Chemical Formula	: $\text{C}_5\text{H}_{10}\text{O}_2$
Functional category	: Surfactant
CAS Registry Number	: 9003-11-6
Molecular Weight	: 102.133 g/mol
Melting point	: 52 °C
Bulk density	: 1050 kg/m ³
HLB value	: 24
Description	: Poloxamers generally Occur as white, waxy, free flowing granules, or as cast solids. They are practically odorless and tasteless. At room temperature, Poloxamer 188 occurs as a colorless liquid.
Functional Category	: Dispersing agent; emulsifying and co emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

6.2.2. Polyvinylpyrrolidone (PVP K30):

Structure :

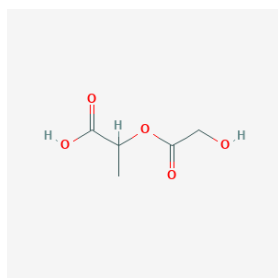


Synonyms : PVP, Povidone, Polyvidone, Plasdone
Chemical formula : $(C_6H_9NO)_n$
Functional category : Suspending and dispersing agent and vehicle for pharmaceuticals
CAS Registry number : 9003-39-8
Molecular Wt. : 40000
Melting point : 150 to 180 °C
Description : Polyvinylpyrrolidone (PVP), also commercially known as K30, is a water soluble polymer. It has a hygroscopic nature with good adhesive properties. It has a stable pH and has the ability to form transparent films.

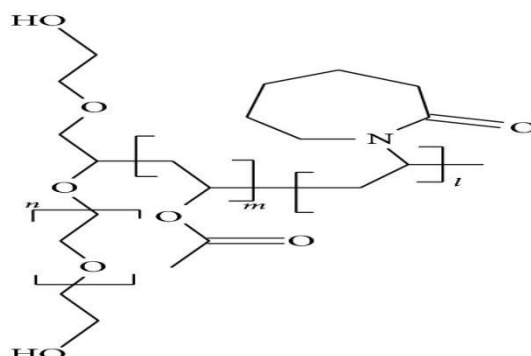
Solubility: PVP is soluble in water and other polar solvents. It is soluble in various alcohols, such as methanol and ethanol.

6.2.3. Poly lactic co glycolic acid (PLGA):

Structure :



Synonym : Poly(D,L-lactide-co-glycolide)
Chemical formula : $C_5H_8O_5$
Functional category : Polymer
CAS Registry number : 34346-01-5
Molecular Wt : 148.11
Melting point : 240°C
Description : vary from fully amorphous to fully crystalline depending on block structure and molar ratio.
Solubility: Insoluble in water. It is soluble in chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate.
6.2.4. Soluplus:
Structure :



Synonym : Polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer
CAS Registry number : 402932-23-4
Molecular Wt : 140,000 g/mol
Appearance : white or almost white, crystalline powder
Solubility : Soluble in water.
Functional category : Novel Carrier Polymer

Sr. No.	Materials	Source
1.	OXZ	Titan laboratories Mahad
2.	Poloxamer-188	Molychem Lab, Mumbai
3.	Soluplus	Molychem Lab, Mumbai
4.	PLGA	Molychem Lab, Mumbai
5.	Sodium Hydroxide	Fine Chemical, Mumbai
6.	SLS	Fine Chemical, Mumbai
7.	Methanol	Fine Chemical, Mumbai
8.	Acetone	Fine Chemical, Mumbai
9.	Distilled water	EDATE Brothers, Kolhapur

MATERIAL AND METHOD

The drug ,excipients and chemicals or reagent used for various experiments are enlisted as follows:

Table No. 7.1. List of materials used for research work

Table No. 7.2. List of instrument and equipment used for research

Sr.No.	Instruments/ Equipment's	Source
1.	Electronic Balance	Shimadzu AUW 220D
2.	Bath sonicator	Bio techniques India
3.	Magnetic Stirrer	Remi Equipment t. Ltd.
4.	UV-Visible Spectrophotometer	Agilent Technology Carry 60 UV-vis UV-1800 Shimadzu, Japan
5.	FTIR	Agilent
6.	Dissolution Test Apparatus	Veego
7.	Dialysis Bag	Highmedia, Indla
8.	Micropipettes	Swastik Instrument Private Ltd. Mumbai
9.	Ultrasonicator	Quality Instruments, Mumbai
10.	High Pressure Homogenizes	GEA NIRO SOAVI panda plus
11.	Lyophilizer	LABCONCO. Mettle Toledo, Switzerland
12.	DSC	Mettle Toledo, Switzerland
13.	XRD	Philips, Netherland
14.	Centrifuge	Remi Motors Ltd, Mumbai

15.	Particle Size analyser	Malvern Zetasizer, Malvern instruments Ltd
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7.1 Selection of drug material:

Several novel drug delivery system are available but the major problem associated is Stability. Additionally there are many drug which belong to BCS Class-II having low solubility which is the major limitation for formulation this can be overcome by formulation of such drug with polymeric nanoparticle Nano suspension. The drug delivery system in the form of Nano suspension exhibit better stability & increase solubility being converted from micro to nano size. Because of nano size drug will exhibit the improved physical properties like higher solubility due to higher surface area, so rate of dissolution will be enhanced. Even formulation in polymeric nanoparticle will exhibit better solubility.

7.2. Selection of excipients:

Polymers viz. Poloxamer were selected for the formulation of Nanosuspension as they following characteristics.

- Positive surface charged which will help in Nanosuspension stability
- Excellent drug entrapment,
- Non-toxicity and non-irritancy
- Stability and compatibility with drug

7.3. Selection of method :

The HPH technique was used in the preparation of the OXZ nanosuspension. Polymers were added to nanosuspension formulations to diminish the free energy of the system by diminishing the interfacial tension as well as to prevent the NPs aggregation through the electrostatic/steric stabilization. A pre-milling procedure was performed to reduce the large crystals in the suspension prior to subjecting them to high pressure homogenization. This prevents the large crystals from blocking the homogenization gap present in the piston gap homogenizer. The pressure for the high-pressure homogenizer was set at 1500 bar and the sample was passed through for 30 cycles.

It has been shown that increasing the pressure beyond 2000 bar has little effect in further decreasing the particle size; thus 1500 bar up to 30 homogenization cycles were chosen for this study. Cycle 20 was chosen as the optimized cycle as it yielded the lowest particle size and PDI compared with the other cycles. Further cycles were not needed as the particle size difference was not significant.

7.4. Preformulation study of pure drug:(OXZ)

7.4.1. Experimental method:

Preformulation testing is one of the primary steps in the rational development of dosage forms of a drug. It is nothing but testing of materials before the formulation of dosage forms. Simply it is defined as an investigation of physical and chemical properties of drug substance, alone and when combined with excipients. Hence, the Preformulation study on the procured sample of drug includes Physical tests and compatibility studies.

7.4.1.1. Identification tests:

The OXZ was studied for its identity, purity and physicochemical nature by way of certain identification tests likewise; Infrared spectrum, U.V. spectra, melting point and few other physicochemical tests were performed.

7.4.1.2. Organoleptic properties:

The sample of OXZ was evaluated visually for its physical state, color and odor.

7.4.1.3. Melting Point:

Melting Point of OXZ was determined by open Capillary tube method. Briefly, OXZ was placed in a capillary tube whose one end was sealed and attached to the thermometer. Then the whole assembly was kept in liquid paraffin oil and heated. The progress in temperature was monitored. The point at which drug started to melt was noted as the melting point.

7.4.2. Analytical method:

7.4.2.1. UV Spectroscopy:

The UV spectroscopy was used to determine the wavelength maxima of drug and to determine the concentration of drug present in the solution.

7.4.2.2. Determination of λ_{max}

Single beam UV-visible spectrophotometer (Agilent 1800) was used with matched quartz cells of 1 cm in width. Accurately weighed 10 mg of OXZ transferred to 10 ml volumetric flask containing methanol and volume was made to 10 ml. This solution was treated as stock solution and diluted with methanol up to 10 ml to obtain 10 ($\mu\text{g/ml}$) concentrations. The standard solution of OXZ 10 ($\mu\text{g/ml}$) was scanned in the range of 400-200 nm in 1.0 cm cell against blank solvent and spectra were recorded. The wavelength of maximum absorbance was considered for further studies.

7.4.2.3. Development of calibration curve of OXZ using UV-Visible Spectrophotometry:

10mg OXZ was dissolved in 10 ml methanol (1000 ppm). Then by using this stock solution other solutions of 20 ppm and 2 ppm were prepared. By using the stock solutions of 20 ppm and 2 ppm further dilutions were made to obtain concentrations of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 ppm and were analyzed by using Single beam UV-Visible spectrophotometer (Agilent 1800) with matched quartz cells of 1 cm in width. The standard wavelength was fixed for carrying out then calibration. Finally the absorbance values were plotted against the concentration in MS-excel and equation of line and regression coefficient value was determined.

7.4.2.4. Saturation Solubility study:

Saturation solubility is a compound-specific constant only depending on the temperature and the properties of the dissolution medium. However, below a size of approximately 1 -2 μm . Saturation solubility is also a function of the particle size. Saturation solubility of Plain drug OXZ were carried out in distilled water and phosphate buffer pH 6.8 for which 5 mg of drug (weigh equivalent to 5 mg of drug) in 2 ml distilled water were taken separately and were allowed to be stirred in an isothermal shaker

(37.0 $\pm$ 1.0°C) for 24h. The stirred sample were further taken in test tubes and centrifuged (Remi) at 10000 rpm for 15 minutes. The samples were then filtered, diluted suitably and analyzed spectrophotometric ally at 376 nm.

7.4.2.5. Fourier Transform Infrared (FTIR) Spectrophotometer study:

The pure drug OXZ analyzes with IR grade KBr. The FTIR spectrum of OXZ was recorded using FTIR (Cary-630 Agilent technology). The spectrum was recorded over the range of wave number 4000 to 400 cm^{-1}

7.4.2.6. X-Ray Diffraction Studies:

X-ray powder diffraction measurements were carried out on drug using a diffractometer (X'Pert MPD Model, Phillips, Holland). The results were recorded over a range of 0-40°. using the Cu-target X-ray tube and Xe-filled detector. The operating conditions were: voltage 40 kV; current 30 mA; scanning speed 1/min. The X-ray diffraction procedure to estimate the degree of crystallinity was based upon the measurement of the total scattering and the Scattering from the crystalline region of pure drug.

7.4.2.7. Differential Scanning Colorimetric study (DSC):

DSC scans of the powdered sample of OXZ drug were studied using DSC- Shimadzu 60 with TDA trend line software. The temperature rate at a Scanning rate of 20°C/min under dry air flow (100 ml/min) between 50°C and 300°C. The minimum purity required for this method is approximately 98%. therefore, the DSC method is rarely used to warrant purity.

7.4.2.8. Scanning electron microscopy (SEM):

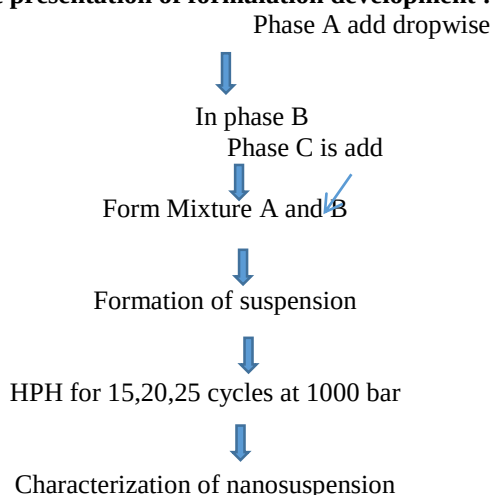
The average size and size distribution of OXZ were determined by SEM (Model INCA wave), in which the samples were mounted rigidly on the surface of a bronze-specimen holder called a specimen stub using a double-sided adhesive tape and coated with an ultrathin coating of electrically-conducting material, gold, deposited on the sample either by low vacuum Sputter coating or by high vacuum evaporation with gold and observed under suitable magnification.

7.5 METHOD OF PREPARATION

7.5.1. Formulation development

Phase	Ingredients	Process / Equipment
A	OXZ + Methanol	1. Dissolve OXZ in methanol 2. Complete dissolution of OXZ in methanol
B	PLGA + Acetone	1. Dissolve PLGA in acetone 2. Complete dissolution of PLGA in acetone
C	PVP K 30 + distilled water	1. Dissolve PVP K 30 in distilled water 2. Complete dissolution of PVP K 30 in distilled water

General schematic presentation of formulation development :



7.5.1.2. Screening of Polymer for LOX Nanoparticles Development:

Four different surfactants were initially used for formulation development namely Poloxamer 188, PLGA, Soluplus. For each polymer batch of 1% concentration is prepared at stirring speed of 1200 rpm. From these, PLGA was selected based on resultant particle size and entrapment efficiency.

7.5.1.3. Composition for formulation batches :

Batch code	OXZ (mg)	PLGA (%)	PVP K 30 (%)	HPH cycles at 1000 bar
F1	100	1	1	25
F2	100	0.5	0.5	25
F3	200	1	1	25

7.6 formulation of nanosuspension :

7.6.1. General step for preparation of nanosuspension:

- 1) Nanoprecipitation
- 2) High-pressure homogenization

Preparation of OXZ Nanosuspension:

Briefly, accurately weighed OXZ(100mg) was dissolved in 10 ml Methanol to form organic solution of drug. Specified quantities of polymer PLGA (1-3%) were dissolved in 10 ml acetone to form an polymer solution. Also PVP K 30 were dissolved in 10 ml distilled water. All solutions were passed through a 0.45 µm filter (Gelman Laboratory, Mumbai, India). The treatment of aqueous solution was done with an ultrasonic probe sonicator (FS-5, Frontline limited, Mumbai, India.) for 10 min. The batches were coded with 'S' to stand for sonicated batch. Addition of organic solvents by means of a syringe positioned with the needle directly into polymer containing water. Organic solvents were left to evaporate off under continuous stirring of the Nanosuspension at room temperature for 2 hours. Further, the sonicated OXZ suspensions were homogenized by HPH (high pressure homogenizer), using an (GEA Niro Soavi Panda Plus) homogenizer at 1000 bar for 25 cycles to obtain the final product. The homogenized nanosuspension was evaluated for the particle size distribution, Zeta potential, %CDR, % EE, etc. This was done to assure the need of incorporation of homogenization stage in the preparation of nanosuspension.

7.7. Characterization of Liquid state (Nanosuspension):

7.7.1. Particle size and Polydispersity index analysis:

The size of particles and their distribution were determined using Zetasizer (Nano ZS Malvern Instruments, UK) using a process called dynamic light scattering (DLS). Samples were examined to determine the mean particle size, size distribution, and polydispersity index (PD). This technique measures the time-dependent fluctuations in the intensity of scattered light, which occurs because the particles are under Brownian motion. Analysis of these intensity fluctuations enables the determination of the diffusion coefficient of the particles, which are converted into the size distribution. The average particle size and PDI of the nanosuspension samples were determined at 25°C. The results are represented as an average diameter of the nanosuspension (Z-average mean) with the PDI. The particle size distribution (PSD) was characterized using PDI, which is a measure of the width of size distribution.

7.7.2. Zeta Potential analysis:

The Zeta Potential is a measure of the electric charge at the surface of the particles, indicating the physical stability of colloidal systems. The zeta potential values higher than indicate long-term electrostatic stability of aqueous dispersions. An increase in the absolute value of zeta potential is correlated with a lesser tendency to aggregate/flocculate and is indicative of colloidal stability of the nanosuspension. To obtain a nanosuspension exhibit good stability. The zeta potential of Nanosuspension was analyzed using Malvern Zeta analyzer (Serial Number: MAL1098084, Zetasizer Ver. 7.12). The sample was placed in cuvette with necessary dilutions and it was then kept in analyzer to determine the zeta potential.

7.7.3. Percentage entrapment efficiency:

The encapsulation efficiency of nanoparticles was determined by first separating nanoparticles formed from the aqueous medium by centrifugation at 10,000 rpm for 20 min at room temperature. The amount of free drug OXZ in the supernatant was measured by UV spectrophotometry after dilution with Methanol. %EE was calculated by following equation

$$\% \text{ Entrapment efficiency} = \frac{(T_p - T_f) \times 100}{T_p}$$

Where,

T_p is the total OXZ used to prepare the nanoparticles and T_f is the free OXZ in the Supernatant.

The following calibration curve was used for determination of entrapment efficiency,

$$Y = mx + C$$

7.7.4. Drug release study (in vitro):

Vitro drug release of the suspensions was carried out using Dialysis bag with USP-type I dissolution apparatus (basket type). The volume was 900 ml of dissolution medium Phosphate buffer solution pH 6.8). The temperature of the medium was maintained at $37 \pm 0.5^\circ$. The apparatus was allowed to run for 75 rpm. A liquid of 5 ml samples were withdrawn at various intervals. The samples were filtered through Whatman filter. The fresh dissolution medium (Phosphate buffer solution pH 6.8) was replaced every time with the same quantity of the sample. Collected samples were analyzed at λ_{max} of drug 254nm phosphate buffer solution pH 6.8.

7.8 Conversion of the liquid Suspension into solid form (Preparation of NPs by lyophilization)

Different methods are available like lyophilization, spray drying, vacuum drying. LYOP because it requires less time and energy compared to spray drying. Solid state formulations are having long term stability compared to liquid state. So, stable batches of preliminary trial were tried to convert in a powder by LYOP technology. was used to convert OXZ liquid Nano suspensions into dry powders. before lyop starch added as a cryoprotectant.

7.9 . Characterization of Solid state: (Nanoparticles)

NPs was analyzed for FTIR, XRD, DSC and SEM as per procedure of pure drug. (Refer procedure: 7.4.2.5.-7.4.2.8.)

7.10. Drug content:

The percent drug Content of OXZ NPs was estimated by dissolving appropriate quantity individual NPs equivalent to 150 mg in Methanol. The samples were mixed thoroughly dissolve the drug in methanol. The sample was sonicated using Ultrasonicator for 15 min and analyzed using UV spectrophotometer and absorbance was recorded.

7.11. Stability Study of Nanoparticles:

The OXZ PLGA loaded polymeric NPs were stored at 2-8°C and at room temperature for one month. After every week the formulations were analyzed for % drug content using UV spectroscopy as an analytical method.

8.0 RESULTS AND DISCUSSION

In the present work a successful attempt was made to achieve a rapid dissolution of OXZ by preparing the formulation by with different ratios. Effect of polymer and their molar ratios on in vitro dissolution of drug was also carefully deliberated. Optimized batch was characterized for FTIR, DSC, XRD & SEM studies. The formulated formulations of optimized batches were evaluated for in vitro dissolution & for stability study (short term).

8.1 Preformulation Study of pure drug (OXZ):

8.1.1 Identification Test:

The identification tests of OXZ were performed by the respective methods. A results are mentioned in table and values observed are within the range. From the results it is clear that the OXZ is in the pure form.

Table 8.1 Identification tests of OXZ

Sr. No.	Physical properties and test	Methods	Description
1.	Physical state	Visual observation	Solid
2.	Colour	Visual observation	White to faintly orange crystalline powder
3.	Odour	Smelling by nose	Odourless
4.	Melting point	Capillary Method	215.5 °c-230°C
5.	U.V. Spectra	U.V visible Spectrophotometer	UV spectra were obtained at standard condition and it shows max of 254 nm in methanol
6.	Infra-Red Spectra	IR Spectroscopy	The infra-red absorption spectrum is concordant with the reference spectrum

8.1.2 Solubility:

OXZ was found to be freely soluble in methanol, slightly soluble in ethanol and practically insoluble in water.



Fig 8.1 Solubility study in different solvent system

Table No 8.2 Solubility of OXZ in different solvents

Sr. No.	SOLVENT	SOLUBILITY
1.	Methanol	Freely Soluble

2.	Ethanol	Practically insoluble
3.	Acetone	Slightly Soluble
4.	0.1 N HCL	Slightly Soluble
5.	Water	Practically insoluble
6.	SLS	Freely Soluble
7.	Chloroform	Slightly Soluble

8.1.3. Analytical Methods:

8.1.3.1. Determination of λ_{max}

The λ_{max} of OXZ by UV-spectrophotometer in methanol was found to be 254 nm and it is very close to standard λ_{max} . The UV-Visible spectrum of OXZ is shown in Fig.8.2, 8.3, 8.4, 8.5 and the value observed is shown in table 8.4 respectively.

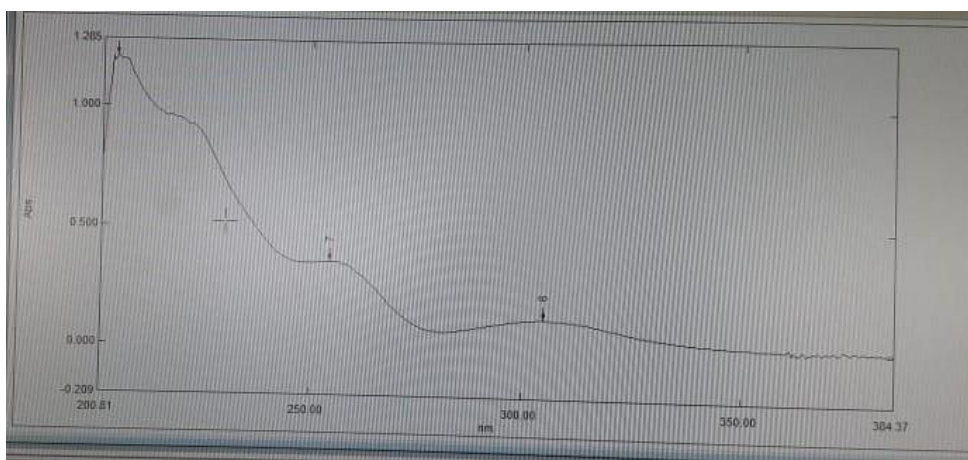


Fig 8.2. UV- Visible absorption spectrum of OXZ with Methanol

8.1.3.2. Preparation of calibration curve of OXZ

The graph of absorbance vs. Concentration for pure OXZ was found to be linear in concentration range 1 - 10 ($\mu\text{g/ml}$) at 254 nm. The drug obeys Beer's Lambert's law in the range 1 - 10 ($\mu\text{g/ml}$).

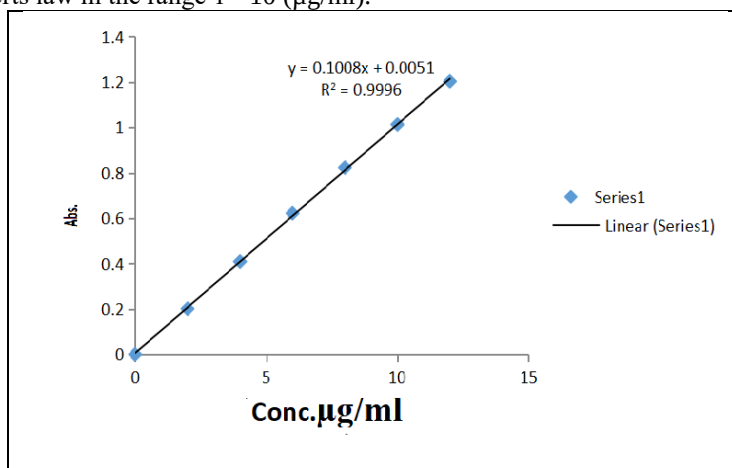


Fig 8.3 Calibration curve of OXZ in methanol

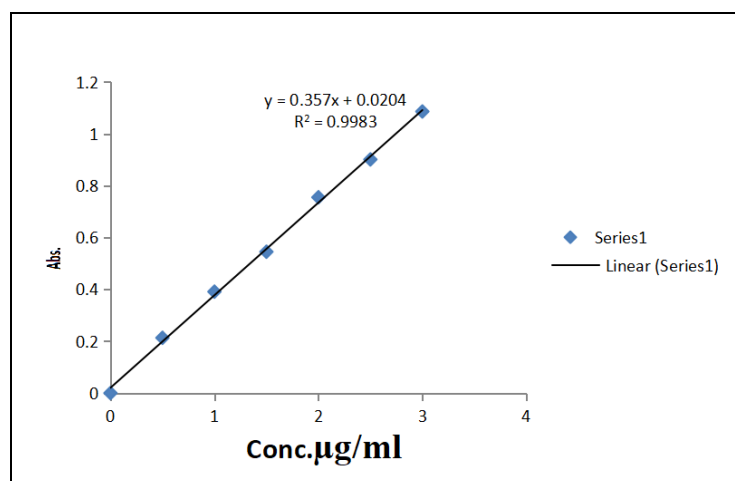


Fig.8.4 Calibration curve of OXZ in SLS and buffer 6.8

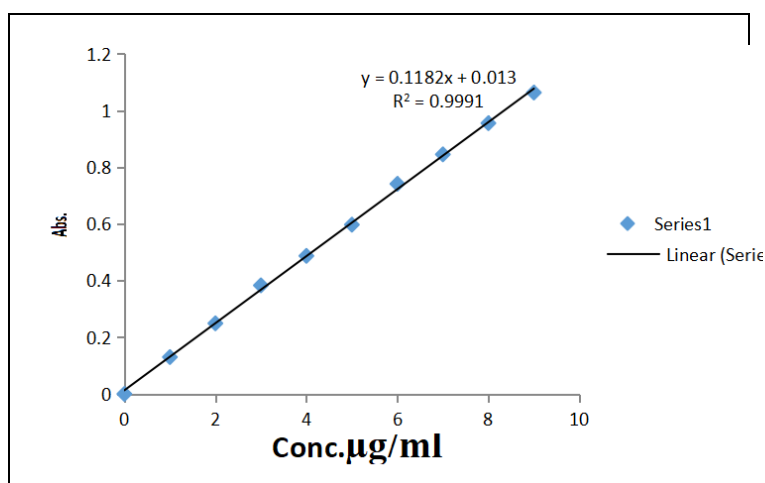


Fig 8.5 Calibration curve of OXZ in Distilled water and methanol

Table No 8.3 Calibration curve values of OXZ in different solvent

Sr.No.	Solvent	Slope	Intercept	R square value
1.	Methanol	0.10	0.005	0.999
2.	SLS	0.142	0.032	0.999
3.	Water and SLS	0.118	0.013	0.999
4.	Phosphate buffer 6.8 + Methanol	0.150	0.008	0.999
5.	Phosphate buffer 6.8 + SLS	0.372	0.04	0.979

The equations obtained from calibration curve of phosphate buffer pH 6.8, distilled water, SLS and methanol were used to calculate % CDR, saturation solubility, Drug content respectively.

8.1.3.3. Saturation solubility of OXZ in water

Saturation solubility is a compound-specific constant only depending on the temperature and the properties of the dissolution medium. Here saturation solubility of OXZ was performed in distilled water.

Saturation solubility of OXZ in water is **0.01827 (µg/ml)**



Fig 8.6 Saturation solubility of OXZ in water

8.2. Experimental Study:

8.2.1. Selection of Polymer & Its concentration:

Evaluate Polymers Poloxamer 188, PLGA, Soluplus on the basis of its Nano crystal size. For each polymer batch 1% concentrations is prepared at stirring speed of 1200 rpm on a magnetic stirrer and then homogenized on high-pressure homogenizer, 25 cycles at 1000 bar. Afterward, polymer and its concentration were optimized depending on the resultant particle size and entrapment efficiency of each batch. When PLGA was used, smallest particle size and higher entrapment were observed as compared to Poloxamer 188 and soluplus to better stabilization of droplets. So PLGA was used as polymer for further development of formulation.

8.2.2. Preparation of Nanosuspension by high pressure homogenization method:.

The dispersion contained in 3cm diameter cylinder; suddenly passes through a very narrow gap of 25 μm . The reduction in diameter from 3 cm to 25 μm leads to increase in dynamic pressure and decrease of static pressure below the boiling point of water at room temperature. Due to this, water starts boiling at room temperature and forms gas bubbles which implode when the suspension leaves the gap (called cavitation) and normal air pressure, are reached. (Refer 7.6.1. from Material and Method) Formation of Suspension.



Fig 8.7 Preparation of OXZ polymeric nanosuspension



Fig 8.8 Different ratios of batch F2

8.3. Characterization of liquid state (Nanosuspension)

8.3.1 Entrapment efficiency:

The encapsulation efficiency of NPs was determined by first separating the NPs formed from the aqueous medium by centrifugation at 10,000 rpm for 20 min at room temperature.

Table No 8.4 Percentage Entrapment efficiency of batches

Sr.No.	Batch No.	Ratio	% EE	Drug content
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1.	F1	1:1:1	65.58	95.2 $\pm$ 0.021
2.	F2	1:0.5:0.5	73.86	97.1 $\pm$ 0.09
3.	F3	2:1:1	58.30	94.7 $\pm$ 0.044

Mean $\pm$ S.D. ,n= 3

8.3.2. Particle Size and Size distribution analysis:

For the nanosuspension to be stable, apart from having a PDI less than 0.5, a uniform particle size distribution is vital. Having a broad size distribution in the system can result in the aggregation of particles and can dramatically affect its stability. A superior effect on particle size reduction was observed from F1 and F2 batches of nanosuspension, which showed mean particle size and PDI of 234.1 nm, 0.512 and 229.1, 0.411 respectively (Fig.8.5 & 8.6.). This results clearly shows that the usefulness of HPH method for nanosizing. This concludes that all the Polymeric Nanoparticles formed are monodispersed or of uniform size. The sizes of Polymeric NPs were found in nanometre;

therefore we could expect better drug release as compared to pure drug.

Table No 8.5 Z - average and PI value of Batch F1

Z-Average	234.1nm
PI	0.512

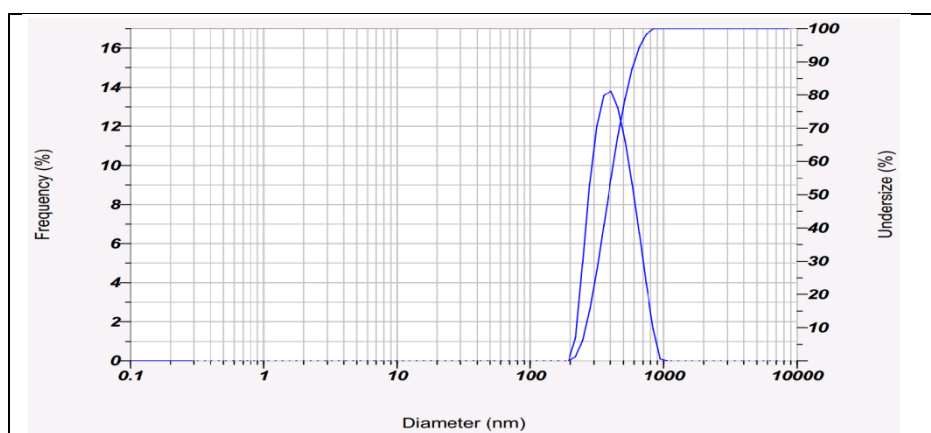


Fig 8.9 Particle size and size distribution analysis of OXZ loaded PLGA nanosuspension F1 batch

Table No 8.6 Z - average and PI value of Batch F2

Z-Average	229.1nm
PI	0.411

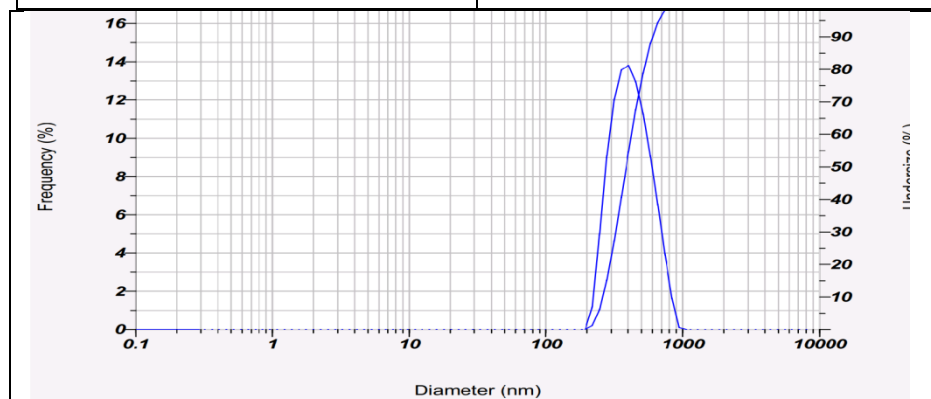


Fig 8.10 Particle size and size distribution analysis of OXZ loaded PLGA nanosuspension F2 batch

8.3.3. Zeta Potential analysis:

The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. It is used to predict the particle-particle interaction. The NPs possessing a zeta potential < -30 and $> +30$ mV are generally considered as stable. Fig.8.7. and 8.8. showed Batches F1 & F2 zeta potential as -1.1 and -0.2 respectively. The zeta potential values of the NPs were within the range indicating that the colloidal suspension is stable.

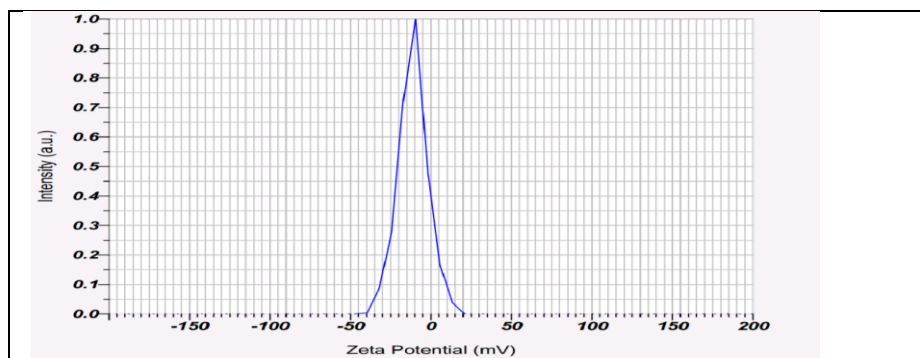


Fig 8.11 Zeta potential analysis of OXZ loaded PLGA nanosuspension batch F1 is -1.1

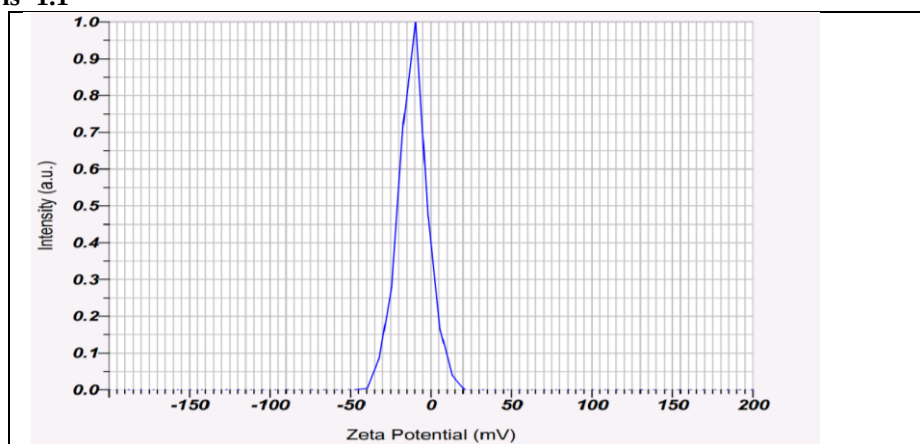


Fig 8.12 Zeta potential analysis of OXZ loaded PLGA nanosuspension batch F2 -0.2

8.4. Conversion of the Liquid Nanosuspension into a Solid Form:

Different methods are available like lyophilization, spray drying, vacuum drying, evaporation by heating but we are preferred lyophilization because it requires less time and energy. Solid state formulations are having long term stability compared to liquid state. So, stable batches of preliminary trial were tried to convert in a powder by lyophilization technology. A lyophilization was used to convert OXZ liquid Nano suspensions into dry powders.

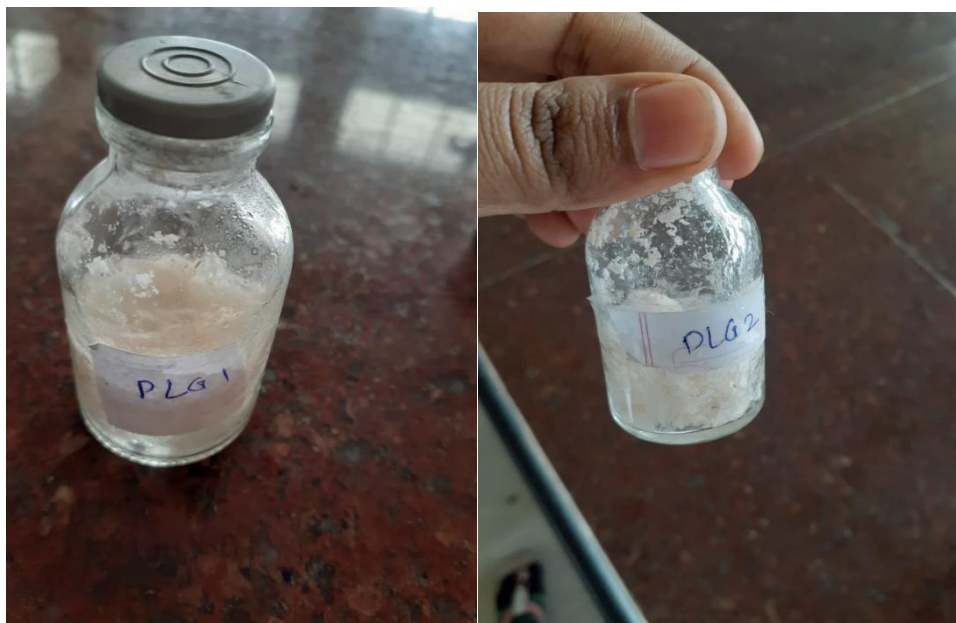


Fig.13 Lyophilized Powder of batch F1 and F2

8.5.Characterization of solid state (NPs)

8.5.1. Drug Content study:

The drug when loaded on polymer usually dispersed on the large surface area of the carrier at a molecular/supramolecular or particulate level. Further increase in carrier surface area may not lead to further subdivision of the drug due to increased cohesive forces/surface tension among the drug particles thus leading to the limiting value of effective surface area. This may reason drug content may increase with polymers on the basis of their concentrations.

8.5.2. Saturation solubility study:

Saturation solubility will increase if the particle size of the NPs is reduced below particular limit. Thus, nanonization might leads to increase in the saturation solubility of prepared NPs. The results of the solubility study indicated that pure OXZ possesses a very low solubility in water (0.01827 $\mu\text{g/ml}$). In this study, the aqueous solubility of OXZ NPs was improved greatly. The saturation solubility of nano- sized OXZ NPs (Batch F2-0.9069 $\mu\text{g/mL}$) was nine times greater than that of pure drug. The reduction of the particle size of OXZ to nano scale increased surface area and enhanced hydrophilicity were responsible for the significantly increased saturation solubility.



Fig 8.14 Saturation solubility process by Roatry Shaker
Table No 8.7 Saturation solubility in water of batch F2

Sr.No.	Batch No.	Ratio	Saturation solubility in water
1	F2	1:0.5:0.5	0.9069 $\pm$ 0.045
Mean $\pm$ S.D., n =3			

8.5.3 Fourier transform-infrared (FT-IR) analysis of OXC loaded PLGANPs

Fourier-transform infrared spectroscopy (FT-IR) gives information about the groups present in the structure of a material and the occurrence of chemical interactions. IR spectrum of OXZ, OXZ-loaded NPs were obtained.

Different peaks in IR spectra were interpreted for the presence of different groups. FT-IR spectrum of OXZ shows that the drug is in a crystalline form and the characteristic drug peaks are shown at 3466.55 cm^{-1} and 3339.34 cm^{-1} . The spectrum of OXZ -loaded PLGA NPs exhibited the same characteristic peaks of unloaded NPs, except for the decrease of the intensity of the carbonyl stretch together with a slight shift of OH stretching (3511 cm^{-1}). According to literature, a slight shift or modification in the intensity of the peaks is related to the possible overlapping between the characteristic polymer and drug peaks. Based on this consideration, the FT-IR spectra indirectly suggested the occurrence of a slight drug-polymer interaction, thus confirming that the drug was successfully encapsulated in PLGANPs.

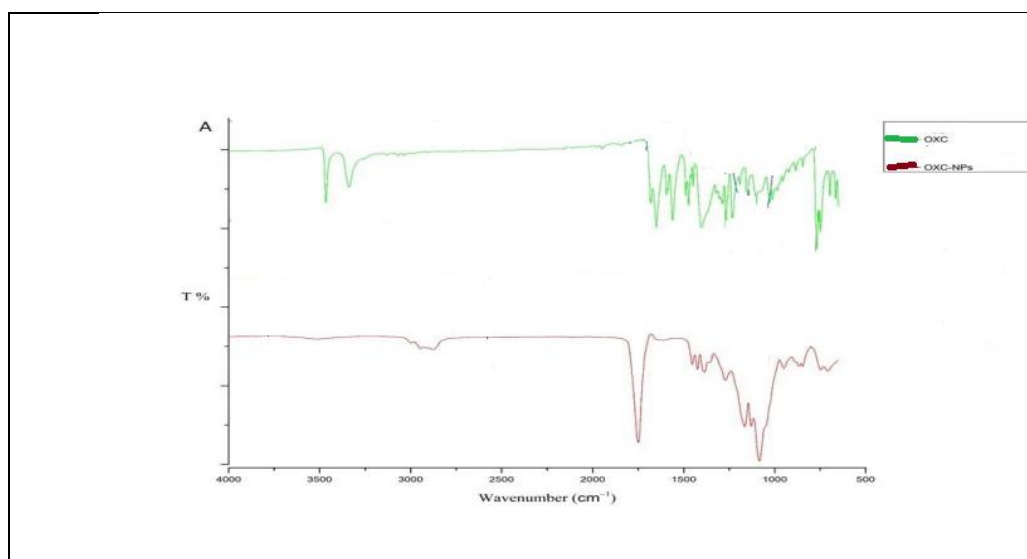


Fig 8.15 FTIR spectra of OXZ and OXZ loaded PLGA nanoparticles

8.5.4. In vitro Dissolution study :

Fig . indicate that dissolution profile of pure OXZ and batches From saturation solubility data it is found that OXZ is a typical poorly water soluble drug having pH dependent solubility. Dissolution Profile of pure OXZ manifesting 58.01 percent drug release up to 60 min. Dissolution enhancement of OXZ was elicited by polymers (PLGA).In nanoparticle dissolution improvement was might be because of increase in surface area by particle size reduction. In addition of, it was obvious that OXZ dissolution was enhanced with increase in molar ratios of polymers in prepared nanosuspension. From dissolution profiles of nanosuspension batches it has found that, to achieve 63.9% drug release batch F-2 had taken 30 min. and batch F-I had required 40 min. This specifies PLGA is able to give rapid dissolution of OXZ. This dissolution rate of the nanosuspension was distinctly superior compared to the plane drug, which might be attributed to increase in surface area, saturation solubility for nanoparticles



Fig 8.16 In vitro dissolution test apparatus

Table No.8.8 Drug release study

Time (min)	% cumulative drug release			
	OXZ	Batch F1	Batch F2	Batch F3
0	0	0	0	0
10	10.41±0.062	31.17±0.021	42.35±0.084	30.84±0.071
20	23.49±0.010	45.64±0.028	50.84±0.026	47.66±0.020
30	29.30±0.015	53.38±0.084	63.90±0.047	59.67±0.047
40	35.01±0.032	65.38±0.034	72.67±0.033	69.33±0.095
50	45.25±0.436	72.57±0.030	79.35±0.074	75.24±0.047
60	58.01±0.521	82.31±0.031	89.67±0.039	83.67±0.022
	Mean± S.D., n=3			

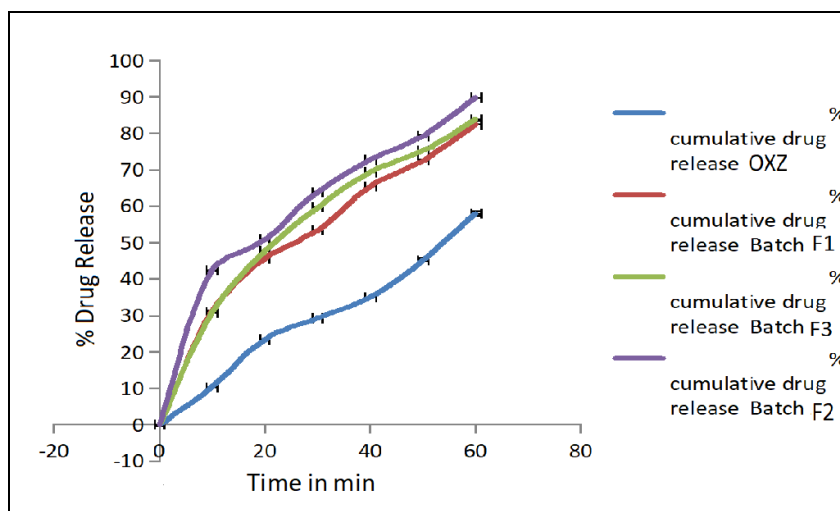


Fig 8.17 %Cumulative drug release of drug and batches

8.5.5. X-Ray Diffraction (PXRD):

XRD analysis was performed to analyze whether any potential changes happened in the inner structure of the OXZ NPs in comparison to the OXZ pure drug. The patterns obtained of pure OXZ, showed in Figure 8.26. The pure OXZ is crystalline and exhibited crystalline peaks 2θ values from 10 to 23°, indicating crystalline nature of OXZ. However, the degree of crystallinity, representing as intensity in XRD diagram, was significantly decreased after preparation of OXZ NPs by lyophilization process observed in the XRD pattern of batch (F2) Fig no.8.27 &8.28. However, the characteristic crystalline peaks disappeared in the pattern of prepared NPs producing a typical of an amorphous material revealing that the crystallinity of OXZ was decreased dramatically. Therefore, it can be assumed that the spray dried NPs will be physico-chemically stable during the storage. It can also be safely assumed that, better physicochemical properties such as observed; enhanced solubility and dissolution can be attributed to the particle size reduction and not to alterations in crystalline state.

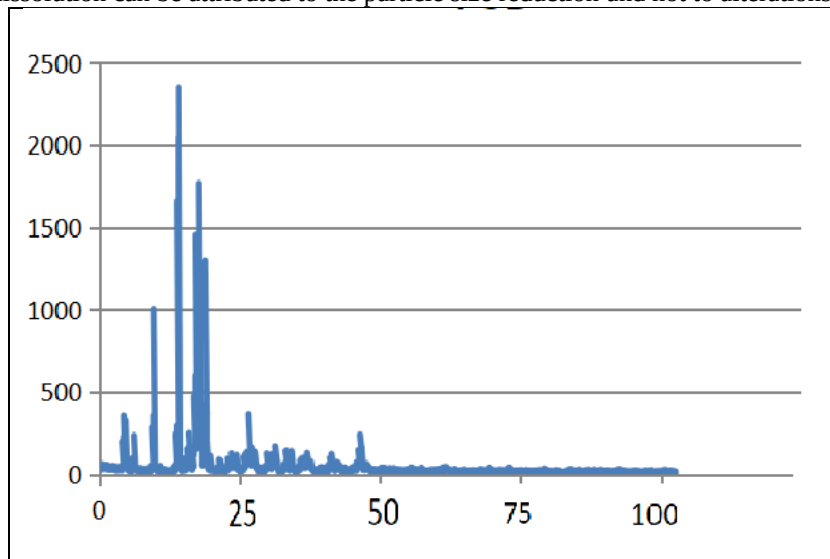


Fig 8.18 XRD pattern of OXZ

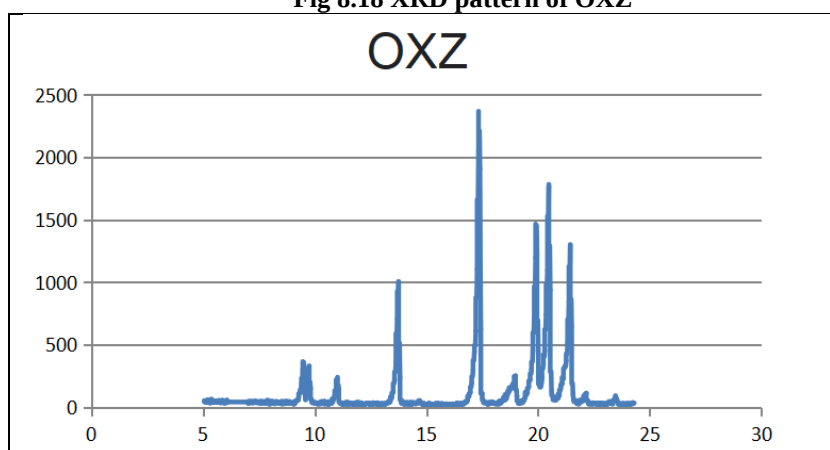


Fig 8.19 XRD pattern of Batch F2

8.5.6. Differential Scanning Calorimetry (DSC):

The DSC thermograms of the pure drug and NPs prepared using polymer (PLGA) are shown in Figure 8.29. & 8.30. DSC curves for the OXZ and OXZ NPs with a single sharp endothermic peak, attributing to the melting point of OXZ at 230°C, OXZ NPs (Batch F2) at 169.4°C.

There was no substantial change in the melting peak of drug in the NPs when compared to pure drug. The small shift to the lower melting point after precipitation process may attribute to the reduction of particle size to nanometer range. In other words, the nanosized small particles have a higher proportion of surface molecules with fewer nearest neighbors than larger particles, and thus are more weakly bound and less constrained in their thermal motion than molecules in the body of crystals, which is supposed to be responsible for the decrease of the melting point.

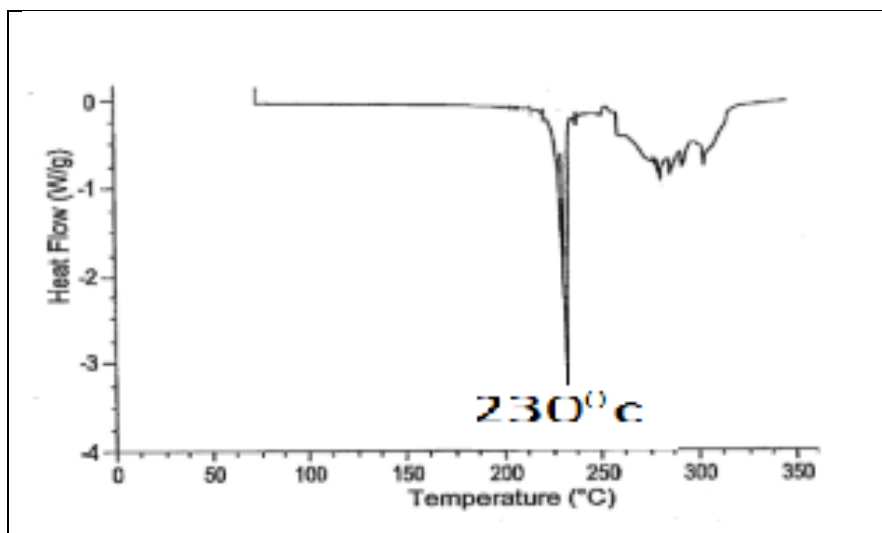


Fig 8.20 DSC pattern of OXZ

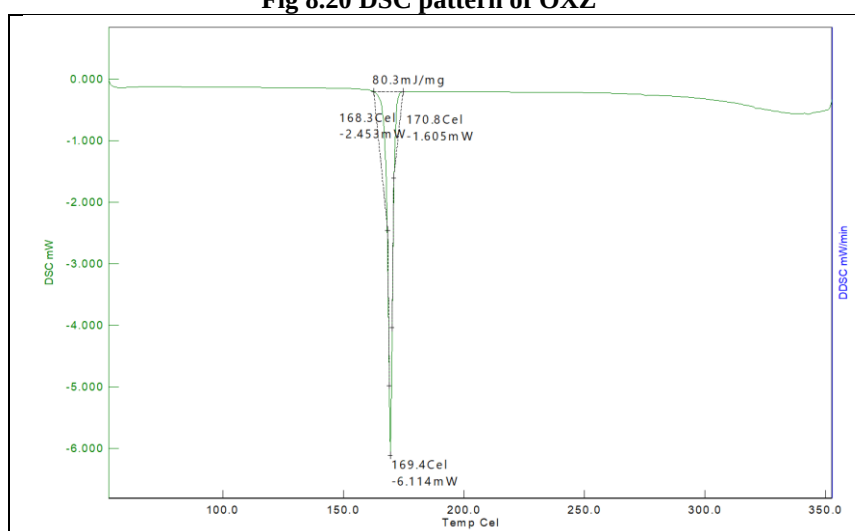


Fig 8.21 DSC pattern of batch F2

8.5.7 Scanning electron microscopy (SEM):

The SEM images are showed in Figure 8.15& 8.16

. It can be seen that the pure drug particles existed as micro crystals structure, However, OXZ NPs obtained by the combination of nano precipitation and HPH method followed drying by LYOP process were spherical with a narrow particle size distribution in the presence of a combination of PLGA. It was clearly seen that polymers were adsorbed onto the drug particle surface inhibiting particle growth and thus making the NPS stable. With the formation of spherical particles it can be expected to improve solubility of dried NPs of OXZ using nanosuspension LYOP technique.

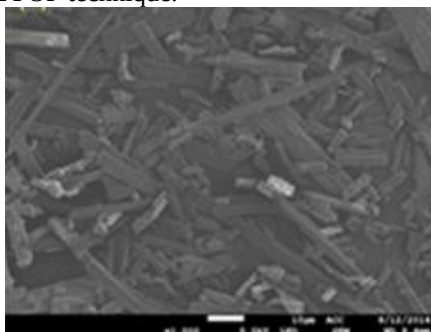


Fig 8.22 SEM of OXZ

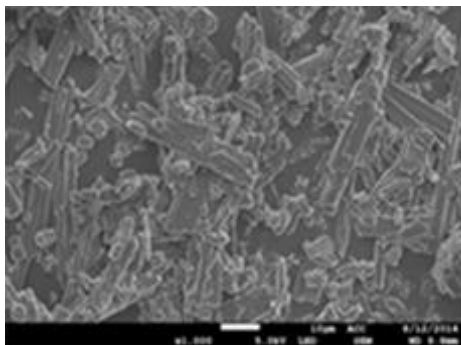


Fig 8.23 SEM of batch F2

8.5.8 Re-dispersibility in water

Amount of re-dispersibility of poorly water soluble drugs in water shows the accurate predictions of solubility and dissolution as the first step of absorption in gastrointestinal tract. In this test re-dispersion of samples recorded by camera after the powder was placed on the surface of distilled water in a beaker. As shown in fig 8.17 the OXZ did not re-dispersed in water. however LYOP NPs of OXZ by using PLGA polymer sample re-dispersed easily and became wet better than other sample of plane untreated OXZ the HPH samples prepared with PLGA POL re-dispersed in water easily. The white color of water illustrated this phenomenon as well. This test as visual study resulted the ability of HPH samples to be dispersed and wet in water. These results are in good agreement with saturation solubility and dissolution studies. The obtained result can be supported by the research work.

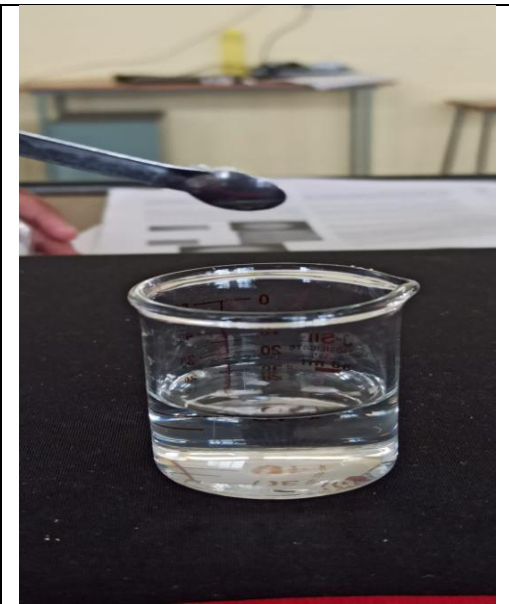


Fig 8.24 OXZ not Redispersible in water

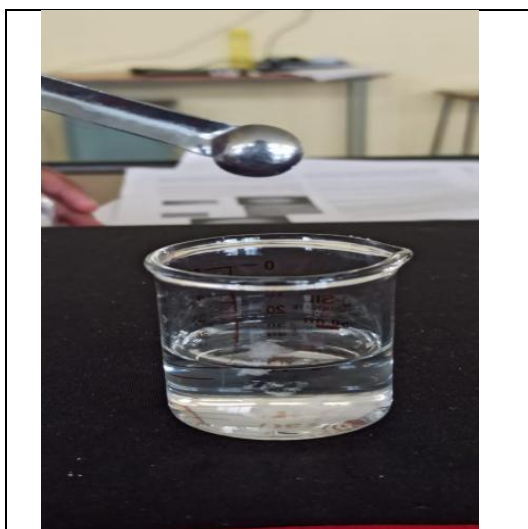


Fig 8.25 OXZ NPs Redispersible easily in water

SUMMARY & CONCLUSION

The characterization of OXZ was done with spectroscopy and found to encompass with the specification. The analytical method was used for construction of calibration curve of OXZ in methanol. Excipients were selected on the basis of nonirritating and toxic properties. The polymers PLGA was used to prepared polymeric nanoparticles with OXZ by High pressure homogenization method. Nanoparticles prepared by using different (drug: polymer) ratio (1:0.5), (F-2) Batches. The first prepared suspension of all batches containing different DRUG: Polymer ratio and that prepared suspension was sonicated by using Sonicator. Prepared suspension converted into nano size by using high pressure homogenizer (HPH).

The prepared Nanosuspension batches were analysed, and in vitro drug release study, all these characterization results shown that batch PLGA (1:0.5) had good results by compared with other batches ratio.

The prepared nanoparticle was characterized by the nanoparticles nature, With pure OXZ. In Vitro drug release study shown the good release of OXZ from loaded polymeric NPs compare with standard formulation. The release was better from Batch PLGA polymer compared to standard drug solution.