A stable ondansetron hydrochloride nanosuspension for improved dissolution: Development, optimization and *invitro* evaluation

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CERTIFICATES

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CERTIFICATE

This is to certify that the dissertation entitled **"A STABLE ONDANSETRON HYDROCHLORIDE NANOSUSPENSION FOR IMPROVED DISSOLUTION: DEVELOPMENT, OPTIMIZATION AND *INVITRO* EVALUATION"** is a bonafied research work done by Mr. **DEEPAK M (Reg. No: 261911401)** in partial fulfillment for the award of degree of Master of Pharmacy in Pharmaceutics. The Research work was carried out in Department of Pharmaceutics, Karpagam College of Pharmacy and submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai under the supervision and guidance of **Dr. S. MOHAN, M. Pharm., Ph.D.,** during the academic year 2020-2021. The results embodied in this dissertation have not been submitted to any other university or institute for the award of any degree or diploma.

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I hereby declare that this dissertation work entitled **"A STABLE ONDANSETRON HYDROCHLORIDE NANOSUSPENSION FOR IMPROVED DISSOLUTION: DEVELOPMENT, OPTIMIZATION AND *INVITRO* EVALUATION"** submitted by me, in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutics to The Tamilnadu Dr.M.G.R. Medical University, Chennai is the result of my original and independent research work carried out under the guidance of **Dr. S. MOHAN, M.Pharm., Ph.D.,** Department of Pharmaceutics, Karpagam College of Pharmacy, Coimbatore during the academic year 2020-2021. The work is original and the dissertation either in part or full has not been submitted by me or any other person to any University/Institute in any part of thesis/ dissertation/ monograph.

I hereby further declare that the Department of Pharmaceutics, Karpagam College of Pharmacy, Coimbatore shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic or research purpose.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"A STABLE ONDANSETRON HYDROCHLORIDE NANOSUSPENSION FOR IMPROVED DISSOLUTION: DEVELOPMENT, OPTIMIZATION AND *INVITRO* EVALUATION"** submitted by **Mr. DEEPAK M (Reg. No: 261911401)** to The Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment for the Degree of Master of Pharmacy in Pharmaceutics is a bonafied work carried out during the academic year 2020-2021 by the candidate at the Department of Pharmaceutics, Karpagam College of Pharmacy, Coimbatore and was evaluated by us.

EXAMINATION CENTRE:

DATE:

INTERNAL EXAMINER **EXTERNAL EXAMINER**

Dedicated to

My

Beloved

Parents, Teachers

and

Society

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By,

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LIST OF ABBREVIATIONS

BCS	Biopharmaceutical classification system
OND HCl	Ondansetron Hydrochloride
5-HT3	5- Hydroxy tryptamine 3
DDS	Drug delivery system
nm	Nanometer
rpm	Rotation per minute
mv	Millivolts
μm	Micro meter
PDI	Polydispersity index
UV	Ultra violet
FT IR	Fourier transform infrared
TEM	Transmission electron microscopy

INTRODUCTION

SOLUBILITY:

Solubility, the phenomenon of dissolution of solute in solvent to give a homogenous system, is one of the important parameters to achieve desired concentration of drug in systemic circulation for desired (anticipated) pharmacological response.

The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, presystemic metabolism, and susceptibility to efflux mechanisms. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability. [1]

IMPORTANCE OF SOLUBILITY:

Solubility, dissolution and gastrointestinal permeability are basic parameters that control the rate and extent of drug absorption and its bioavailability. The aqueous solubility of the drug plays an important role in drug absorption after oral administration. Inadequate aqueous solubility of active pharmaceutical ingredients is very challenging in the development process of new drug products. The poorly water-soluble drug belongs to BCS class II. Dr. Gordon Amidon categorized this class as low solubility, high permeability candidate. When dissolution increases slightly, it produces a significant effect on bioavailability. Various methods that enhance the solubility of poorly water-soluble drug include hydrotropic, complexation, solid dispersion, salt formation, emulsification, co-crystallization and nano-crystal.

SOLUBILITY CHALLENGES OF ONDANSETRON HCl:

The drug is poorly water soluble and possesses intermediate value of log P (i.e., 2.4) [2]. Ondansetron exhibits low (i.e., 45%) and inconsistent bioavailability, potentially due to high hepatic first-pass metabolism and high P-gp efflux, besides the inadequate aqueous solubility [3,4]. The myriad drug delivery systems of ONH developed so far have yielded limited fruition in oral bioavailability enhancement [5].

METHODS TO OVERCOME SOLUBILITY: [6]

A number of methodologies can be adapted to improve solubilization of poor water-soluble drugs and further to improve its bioavailability.

- ✓ Particle size reduction
- ✓ Nanosuspension technology
- ✓ Surfactant
- ✓ Salt formation
- ✓ pH adjustment
- ✓ Hydrotrophy
- ✓ Solid dispersion.

SIGNIFICANCE OF OND HCl IN CHEMOTHERAPHY INDUCED NAUSEA AND VOMITTING: [7]

Comparisons of ondansetron with metoclopramide in patients treated with various types of chemotherapy have shown better response rates with ondansetron. Ondansetron has also been shown to be effective in controlling nausea and vomiting in patients receiving cyclophosphamide with an anthracycline and in patients receiving combination therapy with cyclophosphamide, methotrexate, and fluorouracil. Adverse effects appear to be mild and include headache, constipation, diarrhea and transient abnormalities in liver function tests. The dose of ondansetron (as the hydrochloride salt) for the prophylaxis of chemotherapy-induced nausea and vomiting in adults is 0.15 mg/kg i.v. every four hours for three doses, beginning 30 minutes before antineoplastic therapy. The efficacy of ondansetron is comparable to that of metoclopramide, and the adverse-effect profile is much less problematic.

ONDANSETRON HYDROCHLORIDE [8]

Ondansetron hydrochloride is a serotonin receptor 5-HT_3 (Hydroxy tryptamine) antagonist which is used as anti-emetic agent.

Nausea [9]

Nausea is an unpleasant sensation of wanting to vomit, and is often associated with cold sweat, pallor, salivation, loss of gastric tone, duodenal contraction, and the reflux of intestinal

contents into the stomach. Nausea generally proceeds vomiting, but can occur by itself. The system that brings about the loss of gastric tone, gastric relaxation, is the efferent part of the long loop intestinal reflex that relaxes the gut during food intake.

Retching

Retching is a strong involuntary effort to vomit, and usually follows nausea. During retching, the abdominal muscles, chest wall and diaphragm all contract without any expulsion of gastric contents.

Vomiting

Vomiting is the forceful expulsion of the contents of the gastrointestinal system out through the mouth. From an evolutionary perspective, it is thought to have evolved as a defense mechanism of the body, serving a protective function to rid the body of noxious substances that have been ingested, rather than allowing them to be retained and absorbed by the intestine. Contrary to popular belief, the stomach itself does not actively expel its contents during vomiting. The stomach, oesophagus, and their relevant sphincters are all in fact relaxed during vomiting. Most of the force that expels the contents arises from the contraction of the diaphragm, which is the major respiratory muscle, and the abdominal muscles, which are the muscles involved in active expiration. [10]

Ondansetron hydrochloride acts both centrally and peripherally to prevent and treat nausea and vomiting. Central effects are mediated by the antagonism of $5HT_3$ serotonin receptors in the area postrema. The area postrema is located on the "chemoreceptor trigger zone". This zone senses neurotransmitters like serotonin, toxins and other signals, and plays a role in mediating the sensation of nausea and subsequent vomiting. Ondansetron also has effects peripherally by acting on the vagus nerve. It acts on the 5-HT3 receptors that can be found at the vagus nerve terminals. Within the GI tract, the vagus nerve can sense nausea and vomiting triggers, such as stomach irritants, and it forms synapses within the nucleus tractus solitarius of the brainstem, another region important in vomiting. The peripheral actions of ondansetron are thought to be the predominant mechanism for its antiemetic effects. It is metabolized primarily by the cytochrome P450 system of the liver [7].

Ondansetron hydrochloride is primarily used as a first - line drug for the management of nausea and vomiting associated with

1. Cancer chemotherapy,
2. Chronic medical illness,
3. Gastroenteritis,
4. Post-operative,
5. Radiation therapy and bone marrow transplantation [7, 11].

The $5\text{-}HT_3$ receptors are located centrally in the chemoreceptor trigger zone and the vagal afferent nerve terminals, are key receptors in nausea and vomiting caused after surgery, radiotherapy, chemotherapy. Ondansetron hydrochloride has shown success in the treatment of acute emesis and not induced undesirable side effects such as extrapyramidal reactions compared with metochlopramide and prochlorperazine. The drug is poorly water soluble and exhibits low (i.e., 45%) and inconsistent bioavailability, potentially due to high hepatic first-pass metabolism and inadequate aqueous Solubility [7]. The shorter biological half-life and frequent dosing in chemotherapy-induced nausea and vomiting make it as an ideal candidate for formulating into nanosuspension[9].

Commercial formulations of ondansetron hydrochloride are oral tablets, orodispersible tablets, mouth dissolving film, parenterals or solid suppositories. Orally administered ondansetron rapidly absorbed, however, it is extensively metabolized by the liver. Moreover, it tastes bitter,

and tends to be discharged by vomiting. [10] On the contrary, intravenous administration of ondansetron shows rapid effects, but the rapid onset of effects causes some side effects such as headache, constipation, or diarrhea. In addition, applicability of injection is limited by local pain, early inactivation of the drug, and difficulty of repeated dosing. Ondansetron solid suppositories also have some disadvantages such as leakage out from rectum, low patient compliance, relatively slow absorption, and low bioavailability [9].

INFLUENCE OF SURFACTANTS TO INHIBIT P-GP EFFLUX:

To overcome these problems, various P-gp inhibitors have been widely studied. They include small molecule drugs (active pharmaceutical ingredients (APIs) and new chemical entities (NCEs), natural constituents, and pharmaceutically inert excipients. In general, P-gp inhibitors can block drug-binding sites competitively, non-competitively, or allosterically, interfere with ATP hydrolysis, and alter the integrity of cell membranes. In particular, for oral drug delivery, these inhibitors improve intestinal absorption, tissue distribution, and reduce substrate metabolism and elimination, resulting in enhanced pharmacokinetic properties and oral bioavailability.

Pharmaceutical excipients can interact with lipid bilayers and indirectly or nonspecifically modulate P-gp activity. Generally, pharmaceutical excipients exhibit a low nonspecific pharmacological activity and thus do not cause serious side effects compared with APIs and NCEs. Surfactants that can inhibit P-gp consist of polysorbates (e.g., polysorbate 80, 20), sucrose esters (e.g., sucrose monolaurate), tocopheryl esters (e.g., TPGS), PEG esters (e.g., PEG-35 castor oil).

NANOSUSPENSION:

Nanoparticulate systems provide enhanced absorption due to the slower elimination rate of these particle [14]. Currently more than 40% of new chemical entities are reported to be lipophilic compounds and one-third of drugs recognized by United States Pharmacopeia are poorly soluble. During the process of drug exploration and investigation, more and more new drug candidates have been found to be insoluble or poorly soluble in aqueous medium or organic solvent. Poor solubility may trigger issues, such as poor bioavailability due to uncontrollable precipitation and an erratic absorption profile. Bioavailability refers to the percentage of drug that has been incorporated into circulation of human body. Therefore, developing new formulating strategies for drug molecules with poor solubility to attain an adequate bioavailability has become a challenge for the scientific and industrial professionals. Several methods have been developed, such as

complexation by surfactants or cyclodextrins, solid dispersions, Solubilization using co-solvents, salt formation, screening of soluble drug analogs or prodrugs and application of permeation enhancers, oily solutions, and surfactant dispersions. Even though that methods have achieved some reasonable success, their limitation, such as a large amount of additives that may induce stability and toxicity issues. Therefore, a unique strategy is urgently needed that can resolve the formulation-related challenges so as to improve their clinical efficacy and maximally optimize the pharmaco- economics [12].

The nanosuspension drug delivery system (DDS) was firstly reported in 1994 and a lot of research efforts were devoted to it so as to generate a general formulation for the poorly soluble drugs. Nanosuspension is a colloidal dispersion of submicron drug particles. A pharmaceutical nanosuspension is usually defined as very finely dispersed and biphasic colloid containing solid drug particles of a size less than 1 μm. The suspension contains no matrix material and is stabilized by surfactants and polymers. It is prepared via suitable routes as DDS for the application of oral topical, parenteral, ocular, and pulmonary administration. Nanosuspensions with the drug particle reduced to nano-size exhibit excellent advantages. Reducing the particle size can greatly increase the solubility, thus improving the bioavailability. Additionally, there are other distinguished advantages, such as easy formulation, ease of scale-up, narrow size distribution of drug particle, controllable drug quantity, and little batch-to-batch variation. Moreover, this strategy can be generally applied to drugs with poor solubility in both aqueous and nonaqueous media. The nanosuspension DDS also makes it possible for the drug to be applied as a liquid dosage form or transformed into solid dosage form, such as tablet or capsule. Therefore, the drug can be administrated via oral, pulmonary, ocular, dermal, and intravenous routes. A decent number of nanosuspension DDS products have been marketed [13]

PREPARATION OF LIQUID NANOSUSPENSION DDS

Liquid nanosuspension DDS is a liquid colloidal dispersion of crystalline or amorphous drug nanoparticles with average size below 1000 nm, stabilizers and liquid dissolution medium. Stabilizers are polymer surfactants utilized to maintain the stability of a nanosuspension. The liquid dissolution medium is water, aqueous solution, non aqueous solution, or organic solvent. A variety of factors, such as the crystalline structure and particle size of the drug nanoparticle can affect its saturation solubility.[14] Other factors, such as dissolution medium and temperature also play a

role. According to the Kelvin and the Ostwald-Freundlich equations, the saturation solubility increases with the decrease of particle size when the size of the drug particles falls below the size of 1 μm. Drug particle size reduction also results in an increase in surface area, thus accelerating the rate of dissolution and improving the bioavailability. Many fabrication techniques have been developed to prepare the liquid nanosuspension DDS by research laboratories and pharmaceutical professionals. Those methods are generally classified into three categories as (1) top-down technology, (2) bottom-up technology, and (3) combination technol- ogy. Other preparation techniques, such as supercritical fluid technology, emulsification-solvent evaporation method, and melt emulsification method exhibit some excellent advantages and will be discussed as well in the following section [15]

TOP-DOWN TECHNOLOGY

In top-down technology, large drug crystals decrease into micrometer range and then go down to nanodimension in a stabilizer solution. The principle methods utilized in the top-down technology include the high-energy process called high-pressure homogenization and the low-energy process called media milling. [16]

High-Pressure Homogenization:

High-pressure homogenization is commonly used for preparation of nanosuspensions of poorly soluble drugs. There are two types of high-pressure homogenization that are categorized as dissocubes and nanopure. Homogenization process of dissocubes occurs in aqueous media, whereas homogenization process of nanopure occurs in water mixtures or nonaqueous media. In the dissocubes process, a suspension is forced through a small gap when a pressure as high as 1500 bar is applied. The resulting decrease in the static pressure and increase in the dynamic pressure can cause the water to boil at room temperature. Water boiling at room temperature will generate a lot of gas bubbles. The gas bubbles will lead to cavitation phenomenon in which the gas bubbles implode after the suspension departs the gap and the pressure goes back to atmospheric level. The implosion combined with collisions and high shear causes the drug particles to fragment into nano-size. Factors including drug particle hardness, number of homogenization cycles, homogenization pressure applied, and temperature may influence the physical characteristics of the resulting nanosuspensions, such as the particle size distribution.

Media Milling:

The media milling method was discovered by Liversidge et al. In this method, nanosuspension is generated by pearl mills or high-shear media mills. During the shearing process, the drug particles are broken down into nanoparticles and because of its connection to the recirculating chamber, continuous production is maintained. Both batch operation and continuous operation can be performed through media milling, and the particle size can be reduced to smaller than 200 nm in 30–60 min. The milling media or balls are usually made of polystyrene resin with hardness or ceramic-sintered aluminum oxide. The aqueous suspension of the drug and stabilizer is filled in the milling chamber and the milling media or pearls rotate at a very high shear rate, thus

forming drug nanoparticles from friction and collisions. Shearing can generate a lot of heat, and this procedure can be undertaken with controlled temperature. Additionally, the main advantages of media milling are reduce batch-to-batch variation and facile scale-up. However, this method may cause the erosion of pearls that might contaminate drug nanoparticle product

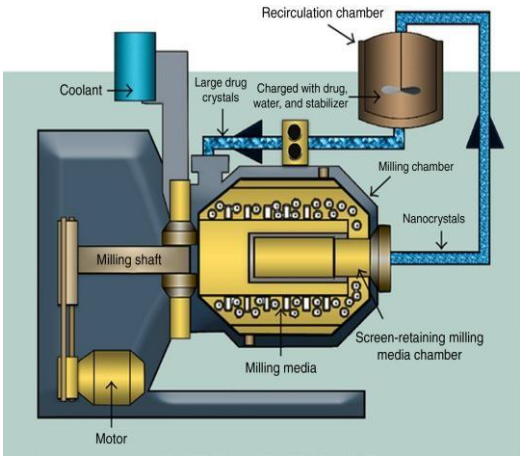


Fig-1: Media Milling Process

Bottom-up Technology:[17]

In the bottom-up process, the drug is usually dissolved in an organic solvent and an antisolvent is added to form precipitation in the present of a stabilizer. The commonly utilized bottom-up techniques are antisolvent precipitation and precipitation- ultrasonication.

Antisolvent Precipitation Method: [18]

Antisolvent precipitation has been utilized for years to prepare drug particles in micro-and nano- size. Generally, the drug is first dissolved in a solvent, to which the antisolvent is then added quickly in the presence of a surfactant. The rapid formation of the drug precipitate results in the sudden super saturation of drug in the mixed solution, thus forming the ultrafine crystalline or amorphous drug nanoparticles. This process involves two phases: nuclei formation and crystal growth. The nanocrystals grown under supersaturation condition usually have a broad particle size distribution. In order to prepare nanosuspension with suitable particle size distribution factors, such as the ratio of organic solvent and antisolvent, the anti-solvent addition rate, the drug precursor concentration, the temperature of the nanoparticle formation, the stabilizer utilized should be taken into account. In order to obtain a more stable nanosuspension, the

stabilizer utilized should exhibit excellent affinity to the particle surface and a high diffusivity so as to rapidly cover the newly generated surface. Besides, there should be enough stabilizers to completely cover the surface of the particles. Temperature has an effect on the formation of nanoparticle, thus changing the size distribution. Generally, the solubility of drugs increases and the level of super saturation decreases with the increase of temperature, and the available crystallization particles are reduced. The resulting increase of solute molecules will benefit the rapid growth of the nanocrystal. In comparison to size reduction, the antisolvent precipitation method is quite suitable for particle size reduction. The antisolvent is added into a drug solution to precipitate drugs. The major disadvantage of this method is to maintain uniform particle size. Large particle sizes increase the broad particle size frequency. The researcher introduces the sonication of the solution for restricting fast nucleation and crystallization. The ultra-sonication have the advantages of feasible mixing with mass transfer and accelerate molecular diffusion. [15]

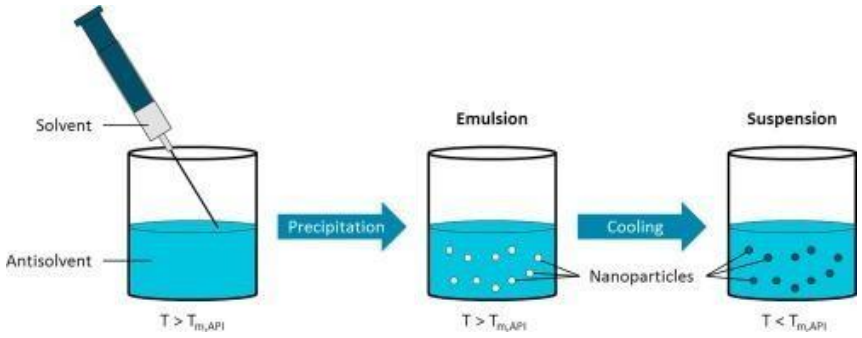


Fig-2: Antisolvent Precipitation Method

Precipitation-Ultrasonication Method:

In recent years, ultrasonication as an effective strategy for controlling the crystallization process has been developed due to the fact that ultrasound irradiation can promote the molecular diffusion and mass transfer. It was found that the crystal size can be reduced by simply increasing the applied ultrasonic power. Nevertheless, when the ultrasonic power input is between 400 and 580 W, no significant change in the particle size was observed. Therefore, 400 W power input is enough to achieve the optimal particle size reduction. In addition, the time that the drug particle is exposed to ultrasonication has an effect on the nanoparticles size. It was found that the particle size is significantly decreased if the ultrasonication time was extended to 15 min.. Besides, polydispersity index the nanoparticle quality can be significantly improved with the application of ultrasonication

Flash Nanoprecipitate:[19]

Recently, flash nanoprecipitation (FNP) as a bottom-up process has been developed to prepare the organic nanoparticles. Confined impinging jet mixers (CIJMs) and multi-inlet vortex mixers (MIVMs) are designed to effectively mix all components in the solvent mixture. The rapid precipitation of solutes and a higher supersaturation level in flash nanoprecipitation generally accelerate the nanoparticle formation.[20] Usually CIJM, utilized as a tool to achieve FNP, can efficiently promote the product conversion. However, in this method identical momentum and equivalent volumetric flow rates of the antisolvent streams are required. This requirement has limited the build-up of the supersaturation level inside the mixer. [21]

In order to resolve the limitation of CIJM, MIVM is designed for FNP to achieve rapid micromixing and ease of utilization and scaling up. The self-sustained micromixing of MIVMs is driven by mixing each stream with the momentum. The mixing of streams with unequal volumetric flows is allowed in the four-stream MIVM. And the supersaturation and solvent composition can be tuned by changing the content and flow velocity of each individual stream. Therefore it is still possible to achieve good micromixing of streams, even with one or more at high flow volume and the rest at low flow volume. Hence, the condition of rapid micromixing and high-supersaturation levels can be utilized to prepare nanoparticles with controlled size distribution. [22]

COMBINATION TECHNOLOGY:

The combined technology generally undergoes a bottom-up process, such as precipitation, which is followed by top-down process, such as high-pressure homogenization. A combination of precipitation and homogenization techniques usually gives a better particle size distribution and better stability. Water-miscible solvents including methanol, ethanol, and isopropanol are used to perform the precipitation. It is necessary to remove the solvents left over in the precipitate even though trace amount of solvent is allowed in the formulation process. An evaporation step is included to yield solvent-free starting material, which is further processed by high-pressure homogenization. NANOEDGE technology is a commonly utilized integrated technology and is a registered trademark by Baxter International. NANOEDGE is designed to enable water-insoluble drugs to be useful medications. The application of NANOEDGE technology can overcome the drawback of both the precipitation technique and the homogenization technology. In the

NANOEDGE method, the precipitated suspension is further homogenized, thus causing the particle size to decrease and further crystal growth to be impeded. [23]

OTHER TECHNOLOGIES:

Supercritical Fluid Technology:

Supercritical fluid technology is commonly utilized to prepared nanoparticles from drug solutions. The various methods including the supercritical solution process, supercritical antisolvent process and precipitation with compressed antisolvent process (PCA) can be utilized. In this method, the drug solution is expanded in supercritical fluid with a nozzle, which results in the loss of solvent power of the supercritical fluid and precipitates the drug as fine particles. It was reported that cyclosporine nanoparticles in the size range of 400-700 nm were prepared by using this method. In the PCA method, the drug solution is atomized into a chamber with com- pressed carbon dioxide.[24] Fine crystals precipitate out with the solution getting supersaturated after the solvent is removed. In the supercritical antisolvent process, a supercritical fluid in which a drug is poorly soluble is used. The drug is dissolved into a solvent that is miscible with the supercritical fluid. After injecting the drug solution into the supercritical fluid, the supercritical fluid extracts all the solvent and the drug solution turns supersaturated. Then the drug will precipitate as fine crystals. Compared with other techniques, the major drawback of the discussed methods is that most of the solvents utilized are hazardous. Also, large amount of surfactants and stabilizers are used during the preparation process.[25]

Emulsification-Solvent Evaporation Technique:

In the emulsification-solvent evaporation technique, the solution of a drug is first prepared. Then, the emulsification was performed in another liquid in which the drug was poorly soluble. After evaporating all the solvent, the drug nanocrystals precipitate out. The high-speed stirrer can be utilized to regulate the crystal growth and particle aggregation by creating high-shear forces.[26]

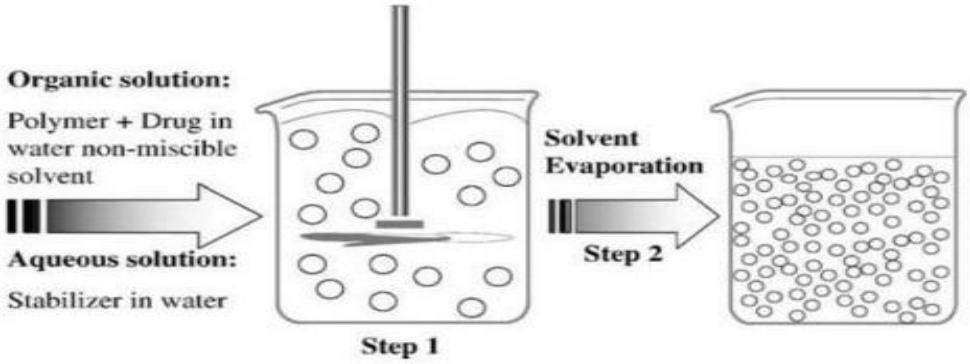


Fig-3:Solvent Evaporation Process

Emulsion Diffusion Method:

The emulsion cannot only be utilized as a drug-delivering vehicle but also can be used as templates to prepare nanosuspensions. For the drugs that are soluble in volatile organic solvent and partially water-miscible organic, the emulsion diffusion method can be used. The drug particles are dissolved in the organic solvent or the mixture of solvents. Then, the organic solution is dispersed in the aqueous phase containing suitable surfactants with stirring to form an emulsion. High-pressure homogenization is commonly applied to further homogenize the emulsion after the emulsion was obtained. After homogenization, the emulsion was diluted with water and homogenized by homogenizer to diffuse the organic solvent, which was followed by conversion of the droplets into solid particles. The particle size of the nanosuspension can be tuned by controlling the size of the emulsion droplet since one particle is formed in each emulsion droplet. In the emulsion diffusion method, methanol, ethanol, ethyl acetate, and chloroform are commonly used as the organic solvents. The advantage of the emulsion diffusion method is that no specialized equipment is required. Particle size can be easily be tuned by controlling the emulsion droplet size. Also, the formulation is easy to scale up. However, the major drawback for the emulsion-diffusion method is that nanosuspensions of drugs with poor solubility in both aqueous and organic media cannot be prepared via this method.[27] Besides, hazardous solvents are used in the preparation process, which will cause safety concerns. Di- ultrafiltration was required for the purification of the drug nanosuspension, which will elevate the cost of the whole process. A large amount of surfactants and stabilizers are required to be utilized in the fabrication process. All these disadvantages may limit its widespread application.

Melt Emulsification Method:

In the melt emulsification method, the drug material is firstly dispersed in the aqueous solution with stabilizer. Then an emulsion was obtained after the solution was heated to a temperature above the melting point of the drug and homogenized. During this process, the emulsion was maintained at a temperature above the melting point of the drug. Then the drug emulsion was cooled to room temperature slowly or with an ice bath. Compared with the previously discussed solvent-diffusion method, the advantage of the melt-emulsification method is that organic solvents are not necessary in the preparation process. It was reported that ibuprofen nanosuspension prepared from the melt-emulsification method exhibits better dissolution rate than the nanosuspension formulated with the solvent-diffusion method. [28]

Lipid Emulsion/Micro-Emulsion Template:

Another way to prepare nanosuspensions is by dispersing the drug particles in a partially water miscible solvent to form an emulsion and diluting the emulsion. Microemulsions can also be used as templates to prepare nanosuspensions. Thermodynamically stable and isotropically clear microemulsions can be prepared by mixing two immiscible liquids, such as oil and water in the presence of an interfacial film of surfactant and co-surfactant. The drug can be dispersed in the preformed micro-emulsion or loaded into the internal phase. After appropriate dilution of the micro-emulsion, the drug nanosuspension will be obtained. The advantage for the lipid emulsions as templates for nanosuspension is that the particle size of the nanosuspension is controllable. Also, the preparation is easy to scale up. However, the major drawback is the utilization of organic solvents that may cause toxicity. Additionally, a large amount of surfactants and stabilizers are required to be used. [29]

Nanojet Technology:

Nanojet technology is an opposite stream technique. In this technology, a chamber is utilized in which the stream of suspension is separated into two or more parts and the particles colloid with each other at high pressure. The high shear force generated from the high-pressure process can help reduce the particle size. Commonly utilized microfluidizers M110L and M110S (microfluidics) are based on this principle. The major disadvantage of this technique is that a

large portion of microparticles may be formed with a large mass passing through the microfluidizer. [30]

FORMULATION CONSIDERATIONS: [31]

1. Temperature.

2. Stabilizer.

3. Solvents.

4. Stirring speed.

The formulation of nanosuspension is a complicated process and many factors affecting the formulation should be considered.

Temperature:

It is important to maintain the optimal temperature while formulating the nanosuspension. In the emulsion technique, homogenization process occurs at a lower temperature after the drug-containing organic solution is added to the aqueous surfactant solution. Maintaining a low temperature is an important precaution to be taken because of the fact that organic solvents are involved in the formulation and a higher temperature will result in irregular particles formation because of rapid removal of organic solvent. On the other hand, if a lower temperature is maintained during formulation, the solvent diffuses out of the system at a slow rate and the formation of spherical and uniform nanoparticles is favoured.

Stabilizer and Other Additives: [32]

During the formulation process of nanosuspension, the stabilizer plays a critical role. The stabilizer is able to wet the nanoparticles completely and supply steric or ionic barriers to impede the Ostwald's ripening and agglomeration of nanosuspensions, thus producing a stable formulation. In some formulation processes, a mixture of stabilizers is required and the drug-to-stabilizer ratio in the formulation usually varies from 1:20 to 20:1. Specific nanosuspension requires specific investigation. Povidones, cellulosics, polysorbates, poloxamers, and lecithin are the most widely investigated stabilizers. To better formulate nanosuspensions, other additives,

such as cosurfactants, buffers, salts, and polyols are added. However, the administration route of the formulated drug or the properties of the drug particles should be considered when choosing the additives. Similarly, different cosurfactants will be used when different formulation methods were chosen to prepare nanosuspensions from emulsions. It was observed that cosurfactants have an effect on the phase behaviour, the emulsion composition, and the drug loading into the nanosuspension. However, transcutol, glycofurol, ethanol, and isopropanol can still be used as safe cosurfactants when the nanosuspension was formulated via emulsion method.

Organic Solvents:

In the formulation process of nanosuspensions, sometimes organic solvents must be utilized. Therefore, potential toxicity and the ease of their removal from the formulation should be thoroughly considered when picking the organic solvent. Generally the water-miscible solvents, such as ethanol and isopropanol, and partially water-miscible solvents, such as ethyl formate, propylene carbonate, triacetin, butyl lactate, and benzyl alcohol are considered to be pharmaceutically acceptable and less hazardous. Those solvents are preferred over the conventional hazardous halogenated solvents, such as chloroform and dichloromethane in the formulation process.

Stirring Speed:

It is easy to neglect the stirring speed as a key variable in the nanosuspension formulation. High- pressure homogenization (HPH) or high shear homogenization (HSH) of nanosuspensions can result in suspensions with smaller nanoparticles. Increasing the speed of stirring in the HSH process or increasing the number of cycles in the HPH process can easily obtain the nanosized range of drug particles. However, a huge amount of foam in the suspension will be generated with the instruments operating at a high speed. This can lower the efficacy in size reduction and the formation of uniform nanoparticle. Therefore, it is important that the instruments run at a recommended speed and with an optimal cycle number. [33]

Characterization of Nanosuspensions:

Nanosuspensions are characterized in similar ways as those used for conventional suspensions such as appearance, color, odor, assay, related impurities, etc. Apart from the mentioned parameters, the nanosuspensions should be evaluated for their particle size, zeta potential, crystalline status, dissolution studies and *in vivo* studies. [34]

Particle Size Distribution:

Particle size distribution determines the physiochemical behaviour of the formulation, such as saturation solubility, dissolution velocity, physical stability, etc. The particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) and coulter counter multisizer. The PCS method can measure particles in the size range of 3 nm to 3 μm and the LD method has a measuring range of 0.05-80 μm. The coulter counter multisizer gives the absolute number of particles, in contrast to the LD method, which gives only a relative size distribution. For IV use, particles should be less than 5 μm, considering that the smallest size of the capillaries is 5-6 μm and hence a higher particle size can lead to capillary blockade and embolism.[35]

Zeta Potential:

Zeta potential is an indication of the stability of the suspension. For a stable suspension stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is required whereas in case of a combined electrostatic and steric stabilizer, a zeta potential of ±20 mV would be sufficient.

Crystal Morphology:

To characterize the polymorphic changes due to the impact of high-pressure homogenization in the crystalline structure of the drug, techniques like X-ray diffraction analysis in combination with differential scanning colourimetry or differential thermal analysis can be utilized. Nanosuspensions can undergo a change in the crystalline structure, which may be due to an amorphous form or to other polymorphic forms because of high-pressure homogenization.[36]

Dissolution Velocity and Saturation Solubility:

Nanosuspensions have an important advantage over other techniques, that it can increase the dissolution velocity as well as the saturation solubility. These two parameters should be determined in various physiological solutions. The assessment of saturation solubility and dissolution velocity helps in determining the *in vitro* behaviour of the formulation. Size reduction leads to an increase in the dissolution pressure. An increase in solubility that occurs with relatively low particle size reduction may be mainly due to a change in the surface tension leading to increased saturation solubility. The energy introduced during the particle size reduction process leads to an increase in the surface tension and an associated increase in the dissolution pressure. [37]

Stability of Nanosuspension:

The high surface energy of nanosized particles induces agglomeration of the drug crystals. The main function of the stabilizer is to wet the drug particles thoroughly to prevent Ostwald ripening and agglomeration of the nanosuspension and form a physically stable formulation by providing a steric or an ionic barrier. Typical examples of stabilizers used in nanosuspensions are cellulosics, poloxamer, polysorbates, lecithin, polyoleate and povidones. Lecithin may be preferred in developing parenteral nanosuspensions.

APPLICATION OF NANOSUSPENSION:

Due to the particle-size reduction and surface-area increase of nanosuspension, it has revealed the potential to solve the problems associated with the delivery of poorly water-soluble and poorly lipid-soluble drugs. The nanosuspension DDS has found wide application in the area of oral pulmonary, parenteral and ocular drug delivery and they have exhibited excellent advantage over traditional drug administration route. The application of nanosuspension DDS in different areas will be discussed in the following section. [38]

Oral drug administration:

The oral administration route is preferred over the various other administration routes of drug delivery due to the many advantages it exhibits. These advantages include safety, good patient compliance, ease of ingestion, pain avoidance, and versatility to accommodate various types of drugs. Oral administration of nanosuspension DDS improvig the bioavailability of poorly soluble drugs. The mai advantages of nanosuspension DDS for oral administration, such as enhanced dissolution rate and solubility of poorly soluble drugs, high adhesiveness of drug nanocrystals on the epithelial gut wall, prolonged absorption time of drug nanocrystals due to long gastrointesti-nal tract, and reduced variability caused by food. Both liquid dosage form and solid dosage form, such as powder, tablet, pellet, capsule, and film can be utilized in oral administration. The prepared liquid nanosuspensions can be directly utilized in liquid dosage form for oral administration.

Parenteral administration:

Parenteral administration is preferred at times over other drug-administrations routes, such as in emergency situations of cardiac arrest and anaphylactic shock. This type of administration route exhibits several advantages, such as first-pass metabolism avoidance, better bioavailability, and reliable dosage. Compared with oral administration, parenteral administration has control over the dose and rate, thus generating more predictable pharmaco-dynamic and pharmacokinetic profiles. Generally, to avoid capillary blockage, the size of drug particles administered should be below 5 μm. In a study on the investigation of oridonin nanosuspension has the ability to inhibit tumor growth, it was observed that Oridonin in the form of nanosuspension can greatly elevate the rate of tumor inhibition by almost 20% when compared with the conventional form of Oridonin. The efficiency of therapy is improved while the cost is greatly reduced with the aid of nanosuspension.

Pulmonary drug delivery:

Pulmonary drug administration is very effective in solving several respiratory problems, such as chronic obstructive pulmonary diseases and asthma. Compared to mentioned oral and parenteral drug administrations, pulmonary drug administration possesses its own advantages. In pulmonary drug administration, the drug is directly delivered to the site of action. A decreased

dosage is required and side effects are improved. Conventional pulmonary drug administration has a lot of problems, such as poor drug residence time, rapid drug release, and a lack of selectivity. By using the nanosuspension strategy, the problems of poor drug solubility and a lack of selectivity can be resolved by delivering the drug directly to affected pulmonary cells. Nanosuspensions have a stronger adhesiveness to mucosal surfaces, which will results in prolonged residence time at target site, thus improving the selectivity and maximally reducing the drug loss. Pulmonary administration routes usually lead to an increased bioavailability because the nanosuspensions can enhance drug dissolution and diffusion rate and thus impede undesirable drug deposition in the pharynx and mouth. [39]

Ocular administration:

Traditional methods in ocular therapy have some disadvantages, such as bad drug solubility in lachrymal fluids, many side effects generated from systematic drug absorption, as well as repeated instillation. These limitations posed by the conventional administration route can be overcome with the application of nanosuspensions. This nanosuspension strategy exhibits many advantages, such as prolonging the ocular drug residence time and excellent improvement of bio-availability. In addition, positively charged nanoparticles in nanosuspension have strong adhesion to negatively charged mucin and the drug release can be extended. For example, in ocular drug delivery, chitosan as a mucoadhesive cationic polymer is utilized to adhere to negatively charged mucin and thus the drug residence time is greatly prolonged. This natural adhesiveness of drug nanoparticles can decrease the amount of drug loss.

Dermal delivery:

Due to an increased penetration of nanocrystals into a membrane, the nanocrystals exhibit improved permeation and bio-adhesiveness. In order to develop intravenous formulations, the injectability and fast dissolution needs to be investigated. However, for years researchers failed to take advantage of the use of adhesion, fast dissolution, or increased penetration for dermal and mucosal applications. The concentration of the poorly soluble drug was increased and this may result in an increased concentration gradient between the formulation and the skin, thus increasing the penetration of drug nanocrystal. In investigation of a penetration barrier, it was observed that the penetration ability of lutein nanocrystals through cellulose nitrate membranes

are fourteen times better than that of raw powder. However, these lutein nanocrystals stayed in the skin of pig ear after its entry because of its lipophilicity.

Targeted drug delivery:

The uptake in vivo behavior of drug nanoparticles can be tuned by changing the particle size. The change in particle size results in the change of surface properties. Thus, the nanosuspension system can be used as a targeted delivery system. Smart crystal such drug particles below 100 nm can be used in the DDS to avoid the phagocytotic uptake of nanocrystals. Development of nanosuspension for targeted drug delivery is feasible due to its simplicity. The organ distribution of the drug particles depends on the surface properties of the drug particles, such as surface hydrophobicity, charge, and concentration of certain functional groups. Therefore it is surprising to see that tween 80-coated nanocrystals can be used for brain targeting. The application of atovaquone nanocrystals coated with tween 80 to treat toxoplasmosis is a good example.

Bioavailability enhancement:

Factors including the poor solubility, the poor permeability, and poor stability of the drugs in the gastrointestinal tract (GIT) may lead to poor bioavailability. By transforming the drug particles into nanosuspensions, the problem of poor solubility and poor permeability across the membrane can be resolved. For example, Oral administration of the danazol nanosuspension as gonadotrophin inhibitor can give an absolute bioavailability of 82.3% compared with the only 5.2% of conventional dispersion. The nanosuspension of amphotericin B showed a significant improvement in its oral absorption compared with the conventional formulation. Oleanolic acid as a hepatoprotective agent is poorly soluble and bioavailability was improved with the use of a nanosuspension. The therapeutic effect was greatly elevate, which indicated elevated bioavail-ability. The nanosuspension exhibits faster dissolution (90% in 20 min) compared with the dissolution from a coarse powder (15% in 20 min).

REVIEW OF LITERATURES

2. LITERATURE REVIEW

Bhamare et al., (2021) reviewed regarding the major barriers in the development of oral dosage form as poor solubility. Poor water solubility obstructs drug bioavailability and decreases the pharmaceutical development. Etodolac is nonsteroidal anti-inflammatory drug having a wide spectrum of activities but belongs to BCS class II. They attempted to improve solubility by forming ternary inclusion complexes of Etodolac with PVP K30 and β-Cyclodextrins. Based on the observations and results, one can easily conclude about the usefulness of the complexation techniquee for the enhancement of solubility [54]

Sarwar Beg et al., (2012) aimed to prepared the solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride (ONH) to enhance its oral bioavailability by improving its aqueous solubility and facilitating its absorption though lymphatic pathways. The prepared liquid SNEDDS formulations were characterized for viscosity, refractive index, droplet size and zeta potential. The TEM study confirmed the formation of nanoemulsion following dilution of liquid SNEDDS. In vivo pharmacokinetic studies in Wistar rats observed significant increase in Cmax (3.01-fold) and AUC (5.34-fold) using SSNEGs compared to pure drug, whereas no significant difference ($p > 0.1$) was observed with the liquid SNEDDS. Thus, the present studies ratify the bioavailability enhancement potential of SSNEGs of ONH prepared using porous carriers.[55]

Rajwat et al., (2019) reviewed pertaining to the physicochemical, pharmaceutical, and pharmacokinetic properties of ondansetron hydrochloride dihydrate arrive at a decision on whether a marketing authorization of an immediate release (IR) solid oral dosage form can be approved based on a Biopharmaceutics Classification System (BCS)-based biowaiver. It is a weak base and thus exhibits pH-dependent solubility. However, it is able to meet the criteria of "high solubility" as well as "high permeability" and can therefore be classified as a BCS class I drug. But the free base of ondansetron was practically insoluble in water. Based on its favorable physicochemical properties, pharmacokinetic data and the minimal risks associated with an incorrect bioequivalence decision, the BCS-based biowaiver procedure can be recommended for ondansetron hydrochloride dihydrate IR tablets. [56]

Kathpalia *et al.*, (2019) aimed to design a novel pH based precipitation method for formulation of atovaquone nanosuspension to enhance the bioavailaility of the drug and the study compared efficiency of two bottom-up methods namely pH and anti-solvent based precipitation. Microfluidiazation was used as top-down technology. The anti-solvent precipitation method uses tetrahydrofuran as the solvent and pH based precipitation method includes sodium hydroxide as basifying agent. pH based precipitation showed enhanced solubility and drug release for atovaquone when compared to anti solvent based precipitation method. [57]

Dharmendrasinh V *et al.*, (2017) carried out a study to formulate and characterize oral fast dissolving film containing nanosuspension of Cilnidipine. Initially Cilnidipine nanosuspension was prepared by high pressure homogenization (HPH) and optimized for Stabilizers. Nanosuspension was optimized by two factor-three level full factorial design. The dependent variables measured as a response were particle size and Zeta potential. The Optimized nanosuspension was further transformed in fast dissolving film by solvent casting method utilizing HPMC E15, Hydroxy ethyl Cellulose, PVP K-30 and HPMC E5 as a film forming polymers. The effect of plasticizer and their concentration were tested for physicomechanical properties of casted films. The optimized formulation selected from overlay plot containing HPMC E15 and Triethylcitrate showed greater drug dissolution. The stability study of optimized formulation for 3 month showed no appreciable change in drug content and *in vitro* drug release. [58]

Zhang *et al.*, (2017) review article elaborately explains about Nanosuspension drug delivery system. The preparation of nanosuspension are top- down and bottom- up technology. Temperature, stabilizer, stirring speed and time are formulation consideration. The characterization of nanosuspension involves particle size, PDI, surface charge, morphology, stability, saturation solubility, in vivo biological performance. It also explains post processing, dosage form of nanosuspension and application of nanosuspension. [59]

Barkat *et al.*, (2017) focuses on the development and characterization of nanosuspension of a poorly soluble drug, silver sulfadiazine (SSD) incorporated in Aloe vera gel (AV-gel) for improving its therapeutic efficacy. The SSD solution in ammonia was subjected for nanoprecipitation in surfactant solution and particle size was optimized by varying concentration

of surfactant. Optimized formulation constituted of 5.5% (w/v) Span 20 and 5.5% (w/v) Tween 80 as a dispersing agent and 0.5% (w/v) Poloxamer 188 as a co-surfactant. The prepared nanosuspension was evaluated for particle size, polydispersity index, surface morphology, and x-ray diffraction study. The optimized nanosuspension was incorporated into nanogel with the addition of 1% AV-gel and 0.5% Carbopol 940 for topical delivery of nanosized SSD. In vitro drug release reported that significant enhancement in release rate of the drug from developed nanogel formulation (77.16 ± 3.241%) in comparison to marketed formulation (42.81 ± 1.452%) after 48 h. [59]

Kalvakuntla *et al.*, (2016) The study compared the particle size of nanosuspensions prepared by the first generation approach and H96 approach and to evaluate the effectiveness of H96 approach. Aprepitant nanosuspension was prepared by HPH (high pressure homogenization) and H96 (Lyophilization + HPH) approach. The prepared nanosuspensions were characterized for their particle size, PDI and zeta potential. The optimized nanosuspension having combination of tween 80 and poloxamer 188 as stabilizer reported that particle size of 35.82 nm with improved solubility and dissolution profile. The author reported that lyophilization prior to high pressure homogenization shows efficient particle size reduction yielding smaller particles than first generation preparation technique. [60]

Shahtalebi *et al.*, (2015) formulated and evaluated an orally disintegrating tablet containing Ondansetron by using semi-synthetic and natural superdisintegrants. Orodispersible tablets were prepared by direct compression using natural superdisintegrant (Karaya gum) and semi-synthetic superdisintegrant (croscarmellose). The prepared tablets were evaluated for hardness, friability, thickness, drug content uniformity, wetting time and water absorption ratio. According to the results of optimized batches, the best concentrations of superdisintegrants (7.88 mg karaya gum and 7.78 mg croscarmellose) gave rapid disintegration in 31 seconds and showed 99% drug release within 5 minutes. The study concludes that karaya gum, a natural superdisintegrant gives rapid disintegration and high release when used with synthetic superdisintegrant in the formulation of ODT. [61]

Taneja *et al.*, (2015) formulated efavirenz nanosuspension employing the antisolvent precipitation-ultrasonication method, and to enhance its solubility by reducing particle size to the

nanometer range. Efavirenz is a non-nucleoside reverse transcriptase inhibitor, and is classified as BCS Class II drugs. Its erratic oral absorption and poor bioavailability make it a potential candidate for being formulated as a nanosuspension.. The effects of different process parameters were studied and optimized with respect to particle size and poly dispersity index (PDI). The optimized formulation was also subjected to lyophilization, to further increase the solubility and stability, and the technology is potentially suited to a range of poorly water-soluble compounds. In vitro dissolution study reports that 90% of drug released within 2 hours which is quite effective than the pure drug. [62]

Deshpande *et al.*, (2014) Formulated and evaluated Ondansetron hydrochloride orally disintegrating tablet which is prepared by sublimation technique. The objective is to mask the bitter taste of the drug and to formulate into ODT for enhancing the palatability of the drug. Polyethylene glycol (PEG) is used as taste masking agent, cross povidone as superdisintegrant and camphor is used as sublimating agent. Disintegration and dissolution of formulated tablets shows best results than the marketed tablets. Hence the method was used to enhance the bioavailability of drug. [63]

Kharb *et al.*, (2014) targeted to mask the bitter taste of ondansetron hydrochloride by lipid matrix granules. Glyceryl monostearate (geleol pellets) is used as lipid substance and can act as release retarding agent. Hot melt fusion (HMF), solventless method is used. *In vitro* drug release decreased with increasing the amount of lipid carrier, and the bitter taste perception of drug has been effectively masked by the lipid based formulation. Hence higher amount of Glyceryl monostearate were capable of suppressing the bitterness. [64]

Thadkala *et al.*, (2014) Prepared and investigated better and stable amorphous ezetimibe nanosuspensions for oral bioavailability enhancement. Nanosuspensions of ezetimibe were prepared by solvent-antisolvent precipitation technique using the surfactant Tween 80 as stabilizer. The formulation was optimized for particle size by investigating two factors that is, solvent:antisolvent ratio and surfactant concentration, at three levels. The formulations were characterized for particle size, surface morphology, crystallinity, zeta potential, saturation solubility, in vitro drug release and in vivo drug absorption study. The author reported that ezetimibe nanosuspensions increased the saturation solubility to an extent of 4-times which dissolved in the dissolution medium within 1 hour.

Sahu *et al.*, (2013) developed and evaluated nanosuspension of felodipine, a poorly water soluble antihypertensive drug. Felodipine nanosuspension were produced by precipitation–ultrasonnication technique. The prepared nanosuspensions were characterised for particle size, zeta potential, polydispersity index, Scanning electron microscopy (SEM), Differential scanning calorimetry (DSC), X-ray diffraction (XRD) pattern and release behavior. The average particle size of felodipine Nanoparticles reported was in the range of 60– 330 nm. It was further confirmed by SEM photograph. The particle size varies with increase in concentration of drug and stabilizer. The preparations showed negative zeta potential and polydispersity index in the range of 0.3–0.8. DSC and XRD studies indicated that the crystallinity of precipitated felodipine nanoparticles was markedly lowered than the pure drug. The in vitro drug release of the formulation reported was 79.67% release in 4 h. Author concluded that the nanoprecipitation with ultrasonnication have the potential to formulate homogenous nanosuspensions with uniform-sized stable nanoparticles of felodipine. [65]

L.Shid *et al.*, (2013) Review explained about nanosuspension. Nanosuspension technology solved the problem of drugs which are poorly aqueous soluble and less bioavailability. Stability and bioavailability of the drugs can be improved by the Nanosuspension technology. Preparation of Nanosuspension is simple and applicable to all drugs which are aqueous insoluble. It explains about preparation techniques such as wet mill, high pressure homogenizer, emulsion solvent evaporation, melt emulsification method and super critical fluid techniques. Nanosuspension can be prepared using stabilizers, organic solvents and other additives such as buffers, salts, polyols, osmogent and cryoprotectant. Nanosuspensions can also be used for targeted drug delivery when incorporated in the ocular inserts and mucoadhesive hydrogels. [66]

Sarwar beg *et al.*, (2013) Prepared the solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride (ONH) to enhance its oral bioavailability by improving its aqueous solubility and facilitating its absorption though lymphatic pathways. The study includes screening of excipients for solubility and pseudo ternary phase diagrams suggested the suitability of capmul MCM as lipid, labrasol as surfactant, and tween 20 as cosurfactant for preparation of self-emulsifying formulations. The optimized liquid SNEDDS were transformed into free flowing

granules by adsorption on the porous carriers like Sylysia (350, 550, and 730) and Neusilin. The porous carriers (Sylysia 350, 550, 730) were found to be suitable in transforming the SNEDDS into SSNEGs. The study reports that bioavailability enhancement potential of SSNEGs of OH prepared using porous carriers.

Dandan Liu *et al.*, (2012) aimed to prepare carvedilol nanosuspension using the anti-solvent precipitation–ultrasonication technique to improve its dissolution rate and oral bioavailability. Sodium dodecyl sulfate (SDS) as stabilizer and Alpha-tocopherol succinate was used as co-stabilizer in the formulation. Response surface methodology based on central composite design was utilized to evaluate the formulation factors that affect the size of nanosuspensions. The nanosuspensions exhibited markedly enhanced dissolution rates compared with the raw drug and the commercial tablet. And alpha-tocopherol succinate is an efficient co-stabilizer for fabrication of stable aqueous nanosuspensions via the anti-solvent precipitation–ultrasonication technique. [67]

Sheshala *et al.*, (2011) Formulated taste-masked orally disintegrating tablets of Ondansetron by wet granulation technique. Microcrystalline cellulose (Avicel) as diluent and disintegrant in addition to aspartame as a sweetener were used in all formulations. The prepared tablets were evaluated for weight variation, thickness, hardness, friability, drug content, water content, *in vitro* disintegration time and *in vitro* drug release.. All formulations disintegrated rapidly within 5.83 to 33.0 seconds. The optimized formulation containing 15% Polyplasdone XL-10 releases more than 90% of drug within 5 minutes and the release was comparable to that of a commercial product. In human volunteers, optimized formulation was found to have a pleasant taste and mouth feel and they disintegrated in the oral cavity within 12 seconds. The stability results were also found to be satisfactory. A pharmacokinetic study with the optimized formulation was performed in comparison with the marketed product and they were found to be bioequivalent. [68]

Patel *et al.*, (2011) reviewed on different types of preparation techniques, Characterization and Pharmaceutical applications of Nanosuspension. The review includes preparative techniques of nanosuspension such as precipitation, high pressure homogenization, media milling, liquid emulsion, micro emulsion, melt emulsification methods. The Characterization involves particle

size, particle size distribution, morphology, surface charge. [69]

Bhoyar *et al.,* (2010) Targeted to mask the taste of ondansetron hydrochloride and to formulate into a patient-friendly dosage form. Complexation technique using indion 234 and an ion-exchange resin was used to mask the bitter taste and it was formulated into an orodispersible tablet (ODT). The drug loading onto the ion-exchange resin was optimized for mixing time, activation, effect of pH, mode of mixing, ratio of drug to resin and temperature. The formulated tablets were evaluated for hardness, friability, drug content, weight variation, content uniformity, water absorption ratio, *in vitro* and *in vivo* disintegration tests and *in vitro* drug release. The obtained results revealed that ondansetron HCl has been successfully taste masked and formulated into an ODT as a suitable alternative to the conventional tablets. [70]

Avani Gosai *et al.,* (2008) formulated and evaluated oro dispersible tablets of Ondansteron hydrochloride by direct compression method. The tablets were prepared by using sodium starch glycolate and croscarmellose sodium as superdisintegrants. Microcrystalline cellulose was used as diluent. Mannitol, mint flavor, sodium saccharin were used to enhance the organoleptic properties of tablets. The tablets were evaluated for post compression parameters such as weight variation, friability, hardness, *in vitro* disintegration time, wetting time and drug release characteristics. All the parameters were found within the U.S.P limits. Hardness and friability data indicated good mechanical strength of tablets. Dissolution study revealed faster drug release rate of Ondansetron hydrochloride from the tablets as compared with marketed conventional tablet of Ondansetron hydrochloride. The study concluded that batch S2C2 showed 98.63% drug release at the end of 30 minutes and emerged as best formulation. [71]

Attia Shafie MA *et al.,* (2020) studied the physical stability of BSP loaded chitosan-alginate nanoparticles and evaluated after storage for 3 months under different temperature conditions. Betamethasone sodium phosphate nanoparticles (F12 andF3C) were stored in polyethylene plastic bottles with droppers and placed at 25° ± 2°C or at 40° ± 2°C away from light. He added Benzalkonium chloride (0.02%) to each sample as preservative to prevent the microbial growth during the storage period. At 1, 2 and 3 months, samples were withdrawn and tested for drug content, pH, viscosity and particle size. The encapsulating efficiency was tested after 3 months. The results of physicochemical properties of F3C and F12 upon storage showed good stability as

the drug content was within the accepted range, the pH was close to that of tear fluid (5-7). [72]

Amit A. Patel *et al*., (2020) stated that in comparison to size reduction, the antisolvent precipitation method is quite suitable for particle size reduction. The antisolvent is added into a drug solution to precipitate drugs. The major disadvantage of this method is to maintain uniform particle size. Large particle sizes increase the broad particle size frequency. He introduced the sonication of the solution for restricting fast nucleation and crystallization. The ultra-sonication have the advantages of feasible mixing with mass transfer and accelerate molecular diffusion. [69]

AIM AND OBJECTIVES

AIM AND OBJECTIVE

AIM

The research is aimed to fabricate Ondansetran nanosuspension to manage chemotherapy induced nausea and vomiting with enhanced solubility and bioavailability for rapid onset of action by bypassing the P-gp efflux.

OBJECTIVES

- To formulate ondansetron hydrochloride nanosuspension by nano precipitation method using ultra sonication technique.
- To Optimize the process parameters and product concentrations by using Design expert software (130.9.0) .
- To Characterize and evaluate formulated nanosuspension.
- To improve *in vitro* dissolution behavior and bioavailability of the ondansetron hydrochloride and to compare with marketed formulation (Emeset) and API.

PLAN OF WORK

PLAN OF WORK

PHASE I - Preformulation Studies

- Determination of Melting point
- Determination of λmax
- Construction of calibration curve
- Compatibility studies.

PHASE II - Formulation of Ondansetron Hydrochloride Nanosuspension

- Preparation of Ondansetron hydrochloride nanosuspension by Solvent evaporation technique.
- Optimization of Ondansetron-NS by 2^3 full factorial design

PHASE III – Evaluation of Nanosuspension

- Particle size and Polydispersity index (PDI)
- Zeta potential
- Drug content
- Morphology by TEM analysis

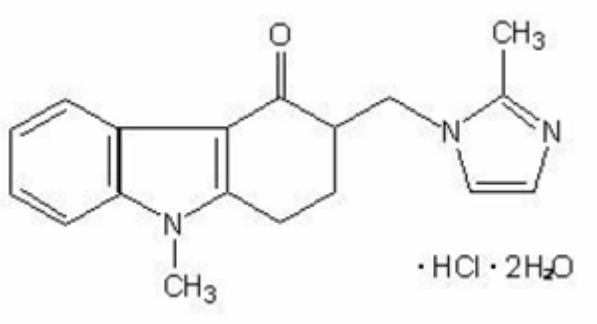
PHASE IV - I*n vitro* dissolution study

- Comparison of drug release data with formulated nanosuspension, API and marketed oral solution.
- Release kinetics

DRUG & EXCIPIENT PROFILE

DRUG PROFILE

ONDANSETRON HYDROCHLORIDE [26]

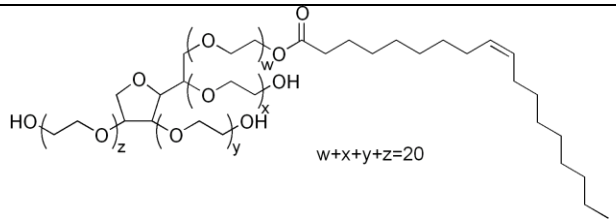
Chemical structure	
Description	Ondansetron Hydrochloride is the hydrochloride salt of the racemic form of ondansetron, a carbazole derivative and it is a selective competitive serotonin 5-hydroxytryptamine type 3 (5-HT3) receptor antagonist with antiemetic activity.
Chemical name	9-methyl-3-[(2-methylimidazol-1-yl)methyl]-2,3-dihydro-1*H*-carbazol-4-one;hydrochloride.
Category	A competitive serotonin 5-HT_3 receptor antagonist.
pH	4.5-4.6.
Molecular formula	$C_{18}H_{20}ClN_3O$
Molecular weight	329.8 g/mol
CAS number	99614-01-4

Hydrogen bond acceptor	1
Hydrogen bond donor	2
Solubility	Sparingly soluble in water , ethanol and soluble in methanol.
Melting point	231-232°C
BCS Class	Class- II
Log p	2.56
Log S	-3.1
pKa (Strongest Acidic)	15.39
pKa (Strongest Basic)	7.34
Mechanism of action	Ondansetron appears to competitively block the action of serotonin at $5HT_3$ receptors peripherally in the gastrointestinal tract as well as centrally in the area of the CNS, where the chemoreceptor trigger zone (CTZ) for vomiting is located, resulting in the suppression of chemotherapy- and radiotherapy-induced nausea and vomiting.
Absorption	Ondansetron is absorbed from the gastrointestinal tract and undergoes some limited first-pass metabolism. Mean bioavailability was 50% to 60%. Bioavailability is also slightly enhanced by the presence of food.

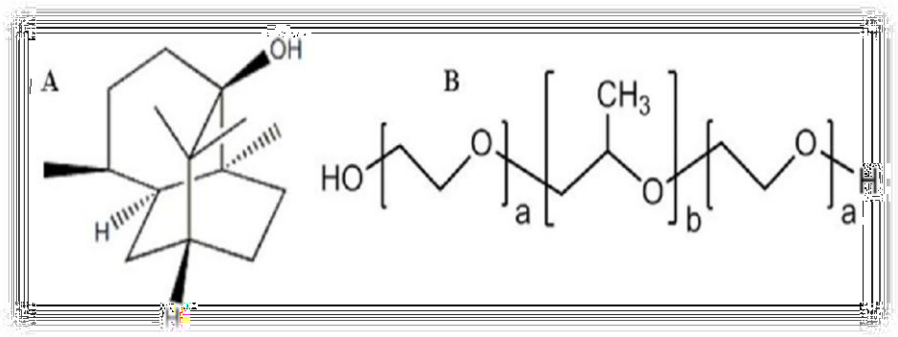
Distribution	70% to 76% protein binding.
Metabolism	Extensively metabolized by hydroxylation on the indole ring, followed by glucuronide or sulfate conjugation.
Elimination	Elimination in Urine and Faeces and 5% recovered in urine as parent compound.
Half life	3.5 to 6 hours
Dose	4mg and 8mg tablet , 2mg/ ml injection
Dosage forms	Tablets, oro dispersible tablets, mouth dissolving film, injection.
Drug interactions	CYP3A4 inducers (eg, Phenytoin, Carbamazepine and Rifampicin) may reduce the plasma levels of Ondansetron thereby decreasing the antiemetic effect. Concomitant use of Tramadol may result in reduced analgesic activity of Tramadol.
Marketed products	Avetron-MD 4mg, Egatron-4 4mg, Vomicard-MD 4mg, Estaset 4 mg, Ondaris syrup, Northstar oral solution, Nvest syrup, Vondan injection, Ondax injection.

EXCIPIENT PROFILE

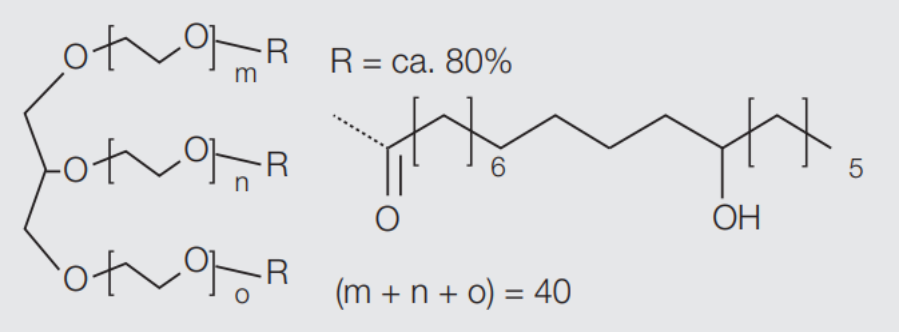
TWEEN– 80 [28]

Synonym	Polysorbate 80
Structure	
Molecular Formula	$C_{64}H_{124}O_{26}$
Molecular Weight	1310 g/mol
Boiling point	More than 100°C
Melting Point	-20°C
Colour	Amber coloured liquid
Solubility	Soluble in water and ethanol
Uses	Non- ionic surfactant, emulsifier
Storage	Stored in a well closed container, protecting from light , cool and dry place

POLOXAMER 188

Synonyms	Poloxalene, pluronic F127
Structure	
Molecular Formula	$C_5H_{10}O_2$
Molecular Weight	102.133 g/mol
Description	Poloxamer is an epoxide
Solubility	Soluble in water
Odour	Odourless
Uses	Surfactant, stabilizer

KOLLIPHOR RH40

Synonyms	Polyoxyl 40 Hydrogenated Castor Oil
Structure	
Molecular Formula	$C_4H_7Cl_2NO_2$
Molecular Weight	172.08 g/mol
Description	Kolliphor® RH 40 is a solubilizer, emulsifier and primary surfactant used in a multitude of pharmaceutical formulations.
Solubility	Kolliphor® RH 40 forms clear solutions in water, ethanol, 2-propanol, n-propanol, ethyl acetate, chloroform, carbon tetrachloride, toluene and xylene.
Odour	Very little odor and in aqueous solutions is almost tasteless.
Uses	Surfactant, stabilizer

Materials used

Table-1: List of materials used

S.No	Ingradients	Vendor
1	Ondansetron Hydrochloride	Gift Sample from Medopharm, Hosur
2	Poloxamer- 188	Sigma Aldrich
3	Tween- 80	Loba chemie, Mumbai
4	Kolliphor RH 40	BASF Chemicals
5	PEG 4000	Lobachem Limited

Instruments Used

Table-2: List of Instruments Used

S.No	Instruments	Model
1	Digital Balance	Shimadzu, AY 220
2	Digital Melting point Apparatus	Selec TC 303
3	Magnetic Stirrer	Remi Equipments LTD, 1 MLH
4	Bath Sonicator	Labman Scientific Instruments
5	Digital pH Meter	LI- 120, ElCO
6	UV Visible Spectrophotometer	UV 1650 PC Shimadzu
7	FT- IR Spectroscopy	Thermo Fisher Scientific
8	Zeta Sizer	Nano ZS 90,Malvern
9	Centrifuge	REMI RC500
10	Homogenizer	Remi Equipments LTD
11	Probe sonicator	Labman Scientific Instruments

METHODOLOGY

METHODOLOGY

PREFORMUALTION STUDIES: [40]

Preformulation studies involve physical, chemical and biological characterization of new drug substances in order to develop stable, safe and effective dosage form. Preformulation testing encompasses all studies enacted on a drug compound in order to produce useful information for subsequent formulation of a stable and bio-pharmaceutically suitable drug dosage form.

Melting Point: [41]

The digital melting point apparatus was used to determine the melting point of drug. A capillary tube was taken and fused at one side with the help of a Bunsen burner. The drug ondansetron hydrochloride was introduced into the capillary tube through the unsealed end and then placed in a melting point viewer. Then the temperature at which drug starts melting was considered as the melting point of the drug.

Determination of λ max: [40]

10 mg of accurately weighed Ondansetron hydrochloride was dissolved in 10 ml of 1.2 pH buffer in a 100 ml volumetric flask. It was made up to 100 ml by using distilled water to get a concentration of 100 µg/ml (Stock A). From the above stock solution A, concentration ranges from 2µg/ml to 10 µg/ml was prepared by pipetting 0.2 ml to 1 ml. It was made up to 10 ml using distilled water. The higher and lower concentration from the above solution was taken and scanned by using double beam UV visible spectrophotometer between the wavelength ranges of 200nm to 400 nm.

Standard Curve: [41]

10 mg of accurately weighed Ondansetron hydrochloride was dissolved in 10 ml of 1.2 pH and 7.4 pH buffer in a 100 ml volumetric flask. It was made up to 100 ml by using distilled water to get a concentration of 100 µg/ml (Stock A). From the above stock solution A, concentration ranges from 0.2µg/ml to 1.0 µg/ml was prepared by pipetting 0.2 ml to 1 ml. It was made up to 10 ml using distilled water. The absorbance of each concentration was analyzed in the UV visible double beam Spectrophotometer at 248 nm. A calibration curve was plotted with concentration on the x-axis and absorbance on the Y-axis. The correlation coefficient (r2) was determined from the graph.

FT-IR Studies (Drug- Polymer Compatibility): [45]

Drug polymer compatibility was determined by ATR plate method using Fourier Transform Infrared Spectrophotometer. The samples were prepared by using ATR plate method and it was scanned between 400-4000 cm^{-1}.

Preparation of Ondansetron Hydrochloride Nanosuspension: [50]

Ondansetron hydrochloride nanosupension was prepared by Nano precipitation method. Aqueous solutions of stabilizers was prepared by dissolving poloxamer 188, tween 80,PEG 4000 and kolliphor RH 40 in water using magnetic stirrer. HPMC was added to aqueous phase as crystal growth inhibitors. Ondansetron hydrochloride was suspended in the stabilizer solution. The organic phase containing drug solution was slowly injected into aqueous phase using syringe. Then the mixture was subjected to probe sonication for 15 min.

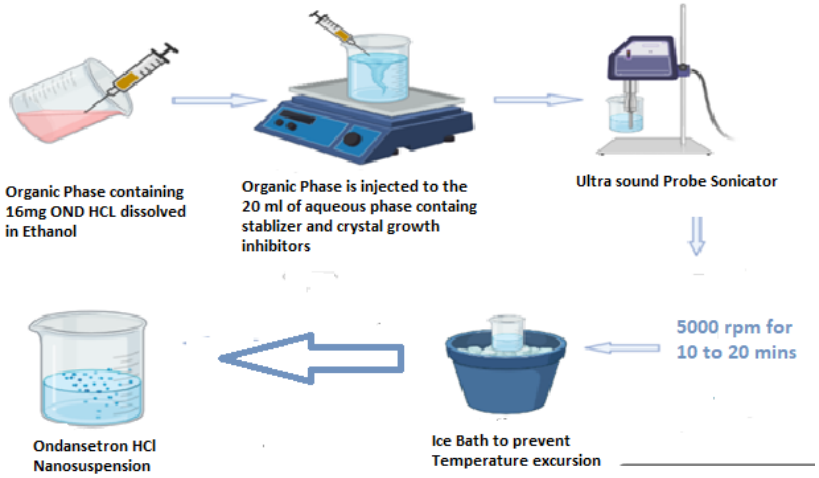


Figure 4: Preparation of OND HCl Nanosuspension by nanoprecipitation method

Table: 3 Composition of various formulations of OND HCl nanosuspension

FORMULATION CODE	ONDANSETRON	HPMC %	TWEEN 80 %	POLAXAMER 188 %	PVP 4000 %	KOLLIPHOR RH 40 %	ETHANOL in ml	WATER in ml
T1	4mg	1.25	2.5	–	–	–	2.5	20
T2	4mg	1.25	–	0.5	–	–	2.5	20
T3	4mg	1.25	–	–	5	–	2.5	20
T4	4mg	1.25	–	–	–	1	2.5	20

Optimization of Nanosuspension [47]

Optimization using 2^3 factorial design [39] [47]

In this study, a 2^3 factorial design was used to optimize the formulation variables of OND-NS containing 3 factors and evaluated at 2 levels. The concentration of Surfactant (HPMC K15) (X1), concentration of stabilizer (tween 80) (X2), and time of sonication (X3) were selected as independent variables based on preliminary studies and the mean particle size (Y1) and % drug content (Y2) as dependent factors. The independent factors and their range levels used in the study were determined. The experiments were designed by using DOE software (Version 13.0.9.0, Stat-Ease Inc., Minneapolis, MN, USA). A total of 8 formulations were designed by the software. The DOE software was used to give information not only on the critical values required to achieve the desired response but also the possible interactions of the selected independent variables on the dependent variables. The response surface method normally approximates the correlation function as a full quadratic equation. To perform the statistical data analysis, the Design-Expert Program 13.0 software was utilized and analysis of variance (ANOVA) was used to know the significance of the factors and their interactions.

Effect of stabilizer and its concentration:

The formulation was prepared using combination of poloxamer 188, PEG, kolliphor RH40 and tween 80 as stabilizers with varying concentration to obtain nanosuspension with optimum particle size.

Effect of sonication:

The effect of crucial parameters such as time and speed of sonication on nanosuspension was studied. The impact of sonication on particle size were characterized at various stages.

Table: 4 Design layout with actual values for 2^3 factorial design

S.No	Formulation code	HPMC %	Tween 80 %	Sonication Time in Mins
1	F1	2	3	20
2	F2	1	2	10
3	F3	2	2	20
4	F4	2	3	10
5	F5	1	3	20
6	F6	1	3	10
7	F7	1	2	20
8	F8	2	2	10

Characterization of Ondansetron hydrochloride Nanosuspension:

Particle size: [43]

The average particle size of prepared Nanosuspension was determined using dynamic light scattering using Malvern Zetasizer (Nano ZS90, Malvern instruments) at 25°C. The samples were kept in polystyrene cuvette and the readings were measured at a fixed angle.

Polydispersity index (PDI): [41]

Mean particle size and Polydispersity index (PDI) of prepared Nanosuspension were obtained using Malvern Zetasizer (Nano ZS90, Malvern instruments). After suitable dilution, prepared nanosuspension was added to the sample cell and determination was carried out. PDI values give idea about uniformity of size distribution.

Zeta potential: [41]

The zeta potential of prepared Nanosuspension was measured using Malvern Zetasizer (Nano ZS90, Malvern instruments) at 25°C. The samples were measured by zeta dip cell kept in polystyrene cuvette.

Drug Content: [42]

Drug content was determined by ultra centrifugation technique**.** Nanosuspension was dissolved in centrifuge tube containing 2 ml of distilled water. The solution was centrifuged at 12,000 rpm for 10 minutes. It was filtered and supernatant solution was analyzed using UV visible spectrophotometer at 248 nm. TDC = Vol. total / Vol. Aliquot drug × amount in aliquot × 100

% TDC = TDC / TA×100

Dissolution study: dialysis sac method [46] [48]

4–5 cm long portion of the dialysis tubing was made into a dialysis sac by folding and tying up one end of the tubing with thread. The sac was filled with 2 ml of the oral marketed solution of Ondansetron hydrochloride and formulated nanosuspension of Ondansetron hydrochloride. The sacs were suspended in the glass beakers containing 500 ml pH 0.1 and the glass beaker was stirred on magnetic stirred at 100 rpm. A 1 ml sample was taken until 2 h at the time interval of 5, 10, 15, 30, 45, 60, 90, and 120 min. An equal amount of fresh dissolution media was added after the withdrawal of each sample.

***In-vitro* Drug release kinetic:**

The release kinetic models for the *in-vitro* drug release profile were established using DD solver software. The release data of the optimized formulations were run in the software for various kinetic models. The model showing the best fit with respect to the regression coefficient (R2) was chosen to determine the release pattern of the drug from the Nanosuspension.

Morphological characterization by TEM:

Morphological examination of the optimized batch was carried out using a transmission electron microscope (ZEOL 100, Japan). Samples were stained with a solution of 2 % (w/v) osmium tetraoxide and finely spread over a slab for capturing images.

Stability study of Optimized ondansetron Nanosuspension:

Stability study of optimized ondansetron nanosuspension was carried out by placing formulation in glass vials at different temperature conditions for 3 months at room temperature (25ºC), refrigerator (4ºC). After 3 months samples were visually noted and change in particle size distribution, PDI and Zeta potential was observed.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Melting Point:

Melting point of Ondasetron hydrochloride was determined by capillary tube method and it was found to be 180°C respectively, which confirms the purity of the drug.

Determination of absorption maxima:

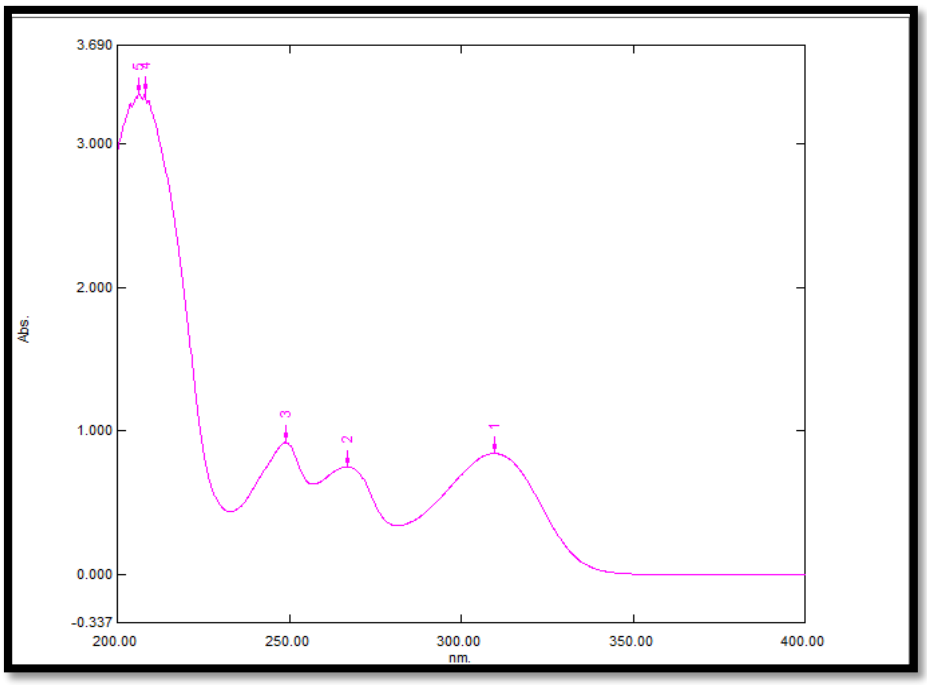


Figure: 5 λ max of ondansetron hydrochloride

Peak Pick

No.	P/V	Wavelength nm.	Abs.
1		309.80	0.842
2		266.80	0.750
3		249.00	0.919
4		208.00	3.354
5		206.40	3.350

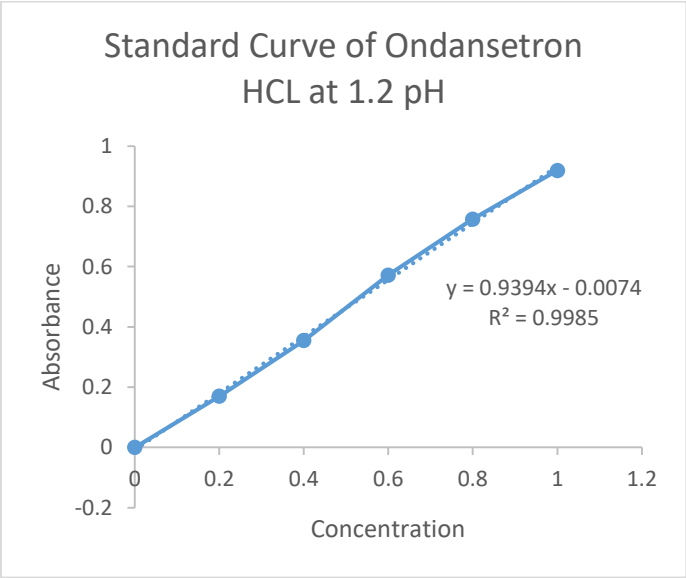
Figure: 6 λ max data of ondansetron hydrochloride

The stock solution containing 1.0 µg/ml was taken for detection of absorption maxima. 1ml of the stock solution was analyzed in the range of 200-400nm in UV spectroscopy. The maximum absorptionwas found at λ max 249nm. [51]

CALIBRATION CURVE OF OND HCL:

Table:5 Calibration curve of ondansetron hydrochloride

S.No	Concentration (µg/ml)	Absorbance
1.	0	0
2.	0.2	0.17
3.	0.4	0.355
4.	0.6	0.572
5.	0.8	0.758
6.	1	0.919



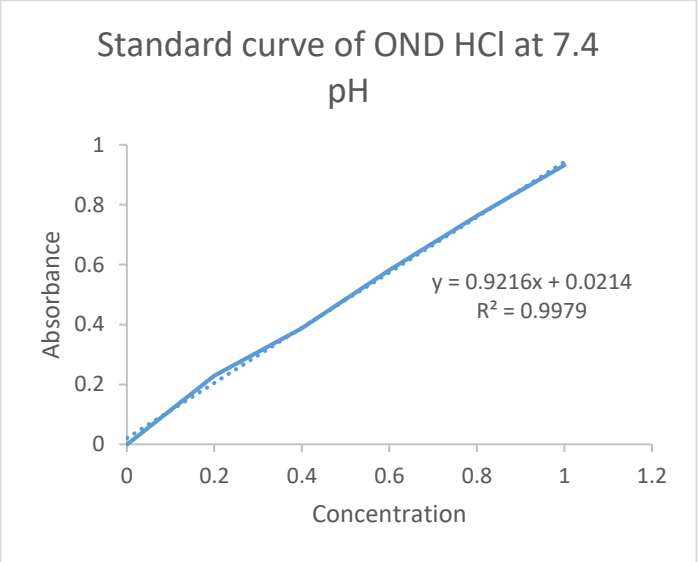


Figure: 7 Calibration graph of ondansetron hydrochloride at pH 1.2 and pH 7.4

Standard graph was constructed with concentration range of 0.2 to 1.0 µg/ml. The absorbance was determined corresponding to their concentration were shown in Table 5. & Fig 7. Correlation coefficient r^2 was found to be 0.9985 with a linear plot which indicates that Ond Hcl obey Beer lamberts law at the concentration range of 0.2 to 1.0 µg/ml

Compatibilty study:

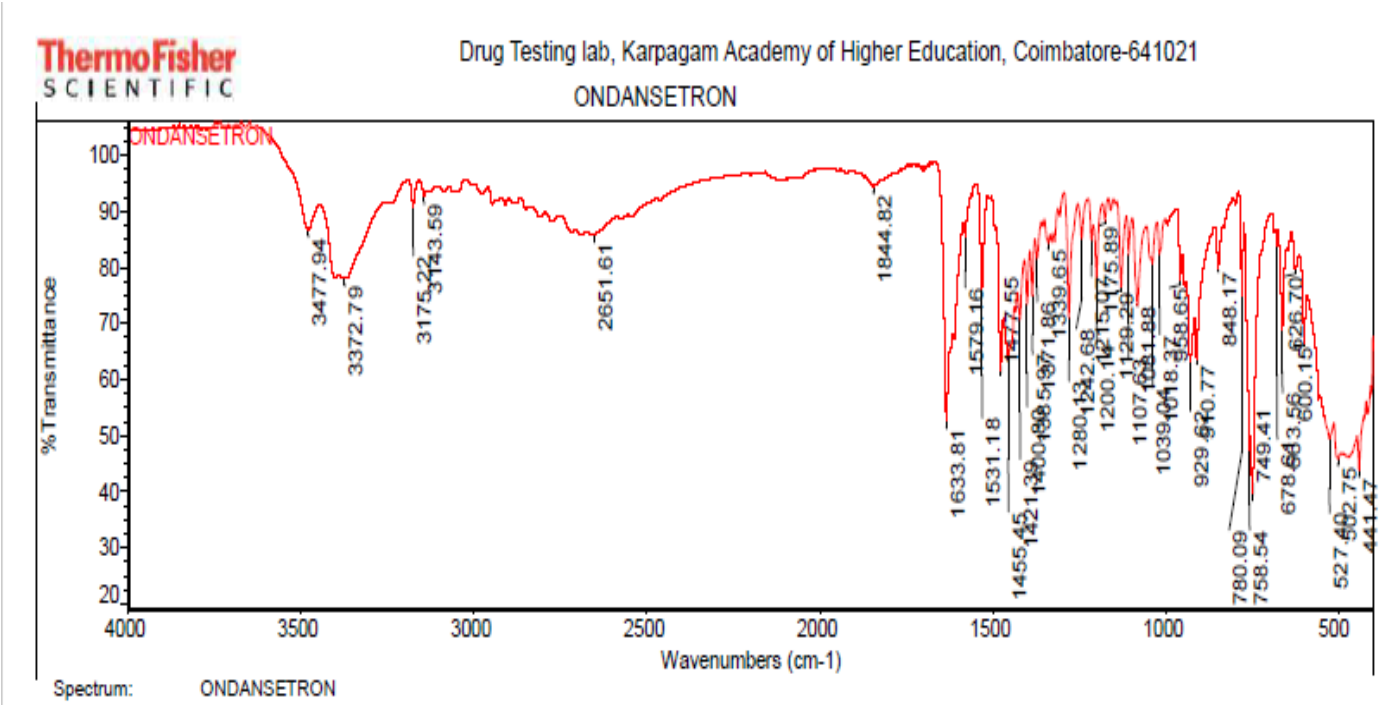


Figure 8: FT-IR spectrum of Ondansetron hydrochloride

Table 6: FT- IR data of Ondansetron hydrochloride

S.No	Wave number cm^{-1}	Wave range cm^{-1}	Assignment
1	1633.81	1690 - 1630	C=N stretching
2	1579.16	1650 - 1550	C=C stretching
3	3372.79	3400 - 3250	CH_3 angular

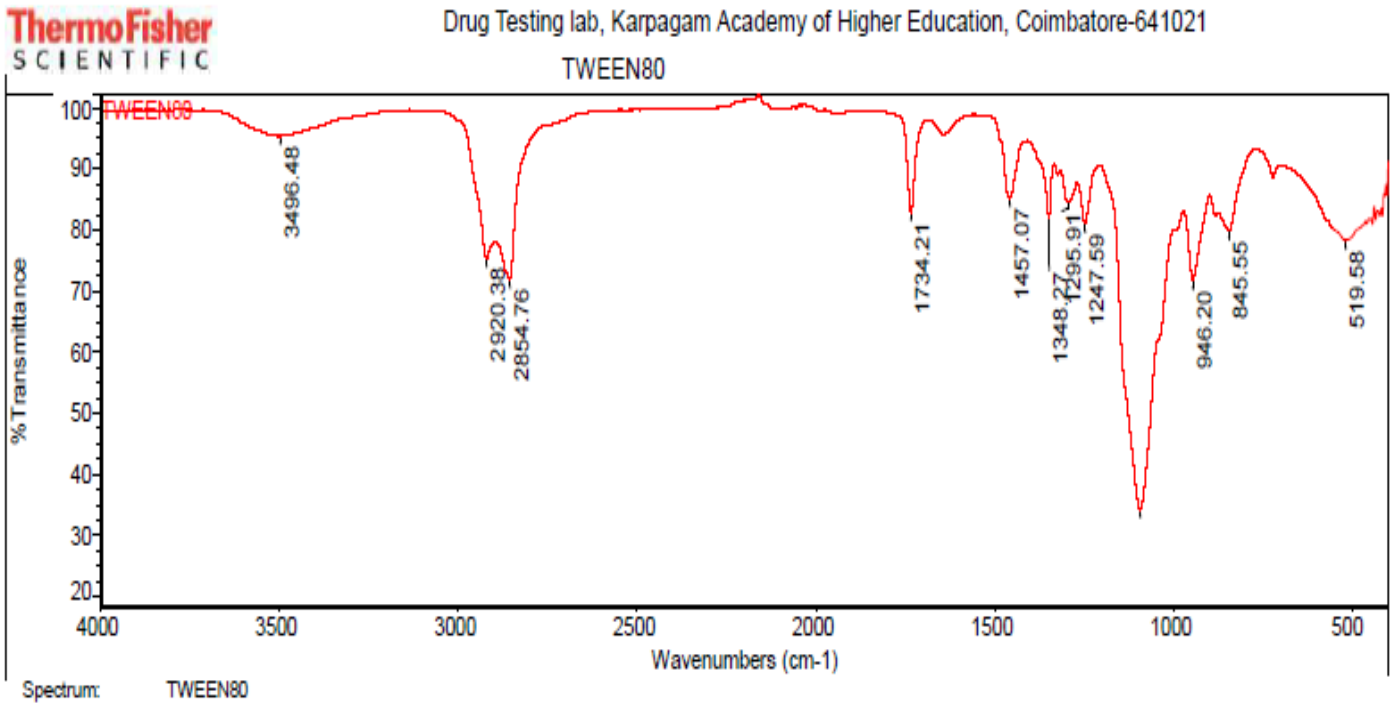


Figure 9: FT-IR spectrum of Tween 80

Table 7: FT- IR data of Tween 80

S. No	Wave number cm^{-1}	Wave range cm^{-1}	Assignment
1	3496.48	3550 – 3200	OH stretching
2	1734.21	1750 – 1680	C=O stretching
3	2920.38	3000 – 2850	CH stretching

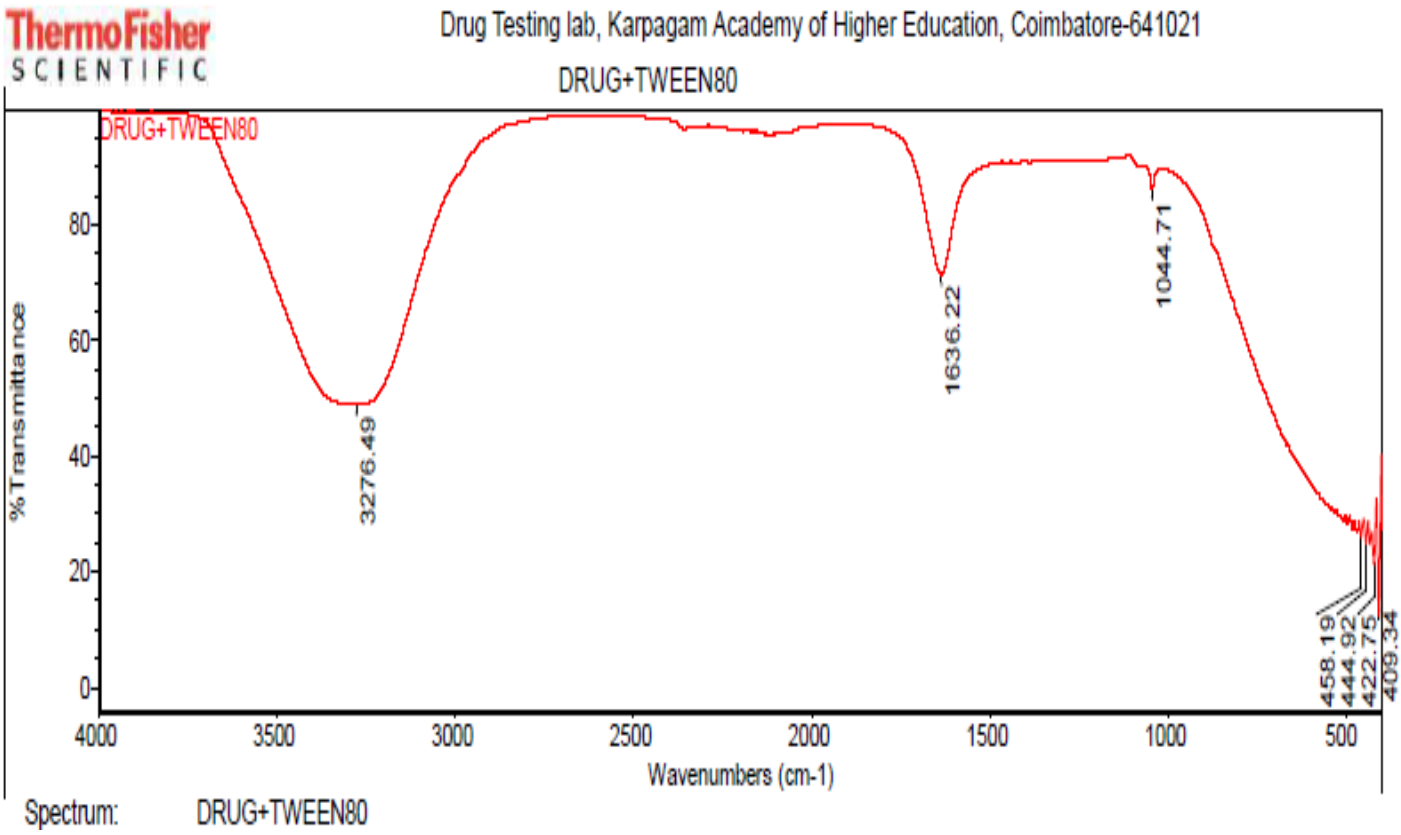


Figure 10: FT-IR spectrum of Ondansetron hydrochloride + tween 80

Table 8: FT-IR data of Ondansetron hydrochloride + tween 80

S.No	Wave number cm^{-1}	Wave range cm^{-1}	Assignment
1	1636.22	1690 – 1630	C=N stretching
2	3276.49	3550 – 3200	OH stretching
3	1044.71	1100 – 800	C – H bending

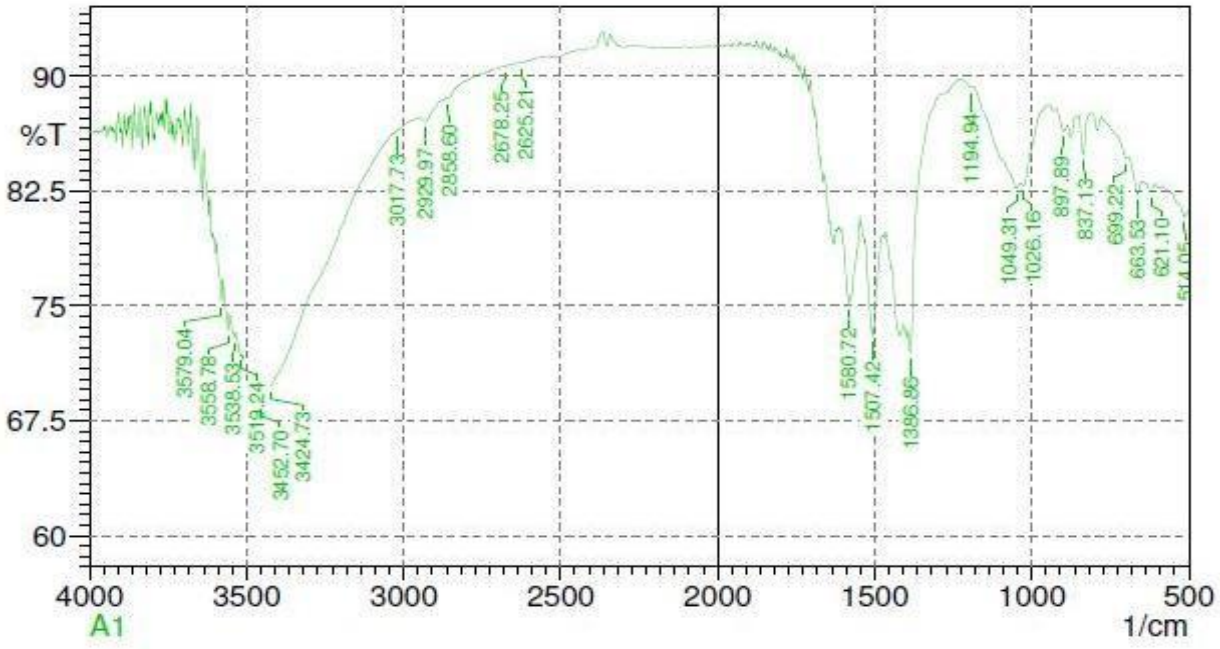


Figure 11: FT-IR spectrum of Physical mixture containing drug and excipients

Table 9: FT-IR data of Physical mixture containing drug and excipients

S.No	Wave number cm^{-1}	Assignment
1	3538.53	OH stretching
2	1580.72	C=N stretching
3	1386.86	C=O stretching
4	2929.97	CH stretching

Fourier Transform Infrared Spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. It is used to identify the functional group involved in the complex formation of Ondansetron hydrochloride + tween 80. Ondansetron spectrum shows absorption peak at 3372.79 cm^{-1}, 1633 cm^{-1}, 1579 cm^{-1} giving functional groups CH stretching, C=N stretching and C=C stretching respectively. The IR spectrum of Tween 80 has functional groups like OH, C=O and CH stretching. The complex compound Ondansetron hydrochloride + Tween 80 produces functional groups C=N stretching, OH stretching and C – H bending absorption peak at 1636.22 cm^{-1}, 3276.49 cm^{-1} and 1044.71 cm^{-1} respectively. There is a slight deviation in the peaks of C=O stretching in the complex compounds. The complex formed is compatible with the combined compounds, which can produce best combinational formulation.

Solubility studies:

Amongst the surfactants and cosurfactants investigated for equilibrium solubility studies viz. HPMC, tween 80, PVP 4000, kolliphor RH 40 and polaxamer 188, the highest solubility of ONH was observed in Tween 80(32 ± 3.2 mg/mL), HPMC (31.1 ± 1.8 mg/mL), kolliphor RH 40 (35.3 ± 2.3 mg/mL) and polaxamer 188 (28.5 ± 2.0 mg/mL). Hence, they were selected for further studies

Formulation of OND HCl nanosuspension:

OND HCl nanosuspension were formulated using Tween 80 (2.5%), Polaxamer (0.5%), PVP (5%) and Kolliphor RH 40 (1%) as stabilizers with HPMC (1.25%) as crystal growth inhibitors using probe sonicator at sonication time of 15 mins. The formulated suspension were investigated for its particle size, zeta potential and PDI to identify ideal stabilizer.

Figure: 12 Nanosuspension of ondansetron hydrochloride

Drug Content:

Table: 10 Drug content of formulations T1 to T4

S.No	FORMULATION CODE	DRUG CONTENT (AVG±SD)
1	T1	93.63±1.6226
2	T2	89.63±1.9561
3	T3	93.70±1.3856
4	T4	87.86±1.8013

Particle size and Zeta Potential:

Table: 11 Particle size and Zeta potential of formulations T1 to T4

S.No	FORMULATION CODE	PARTICLE SIZE	PDI	ZETA POTENTIAL
1.	T1	287.3	0.385	-7.82
2.	T2	523.1	0.756	-10.7
3.	T3	1163	1.000	-13.1
4.	T4	844.9	0.993	-17.4

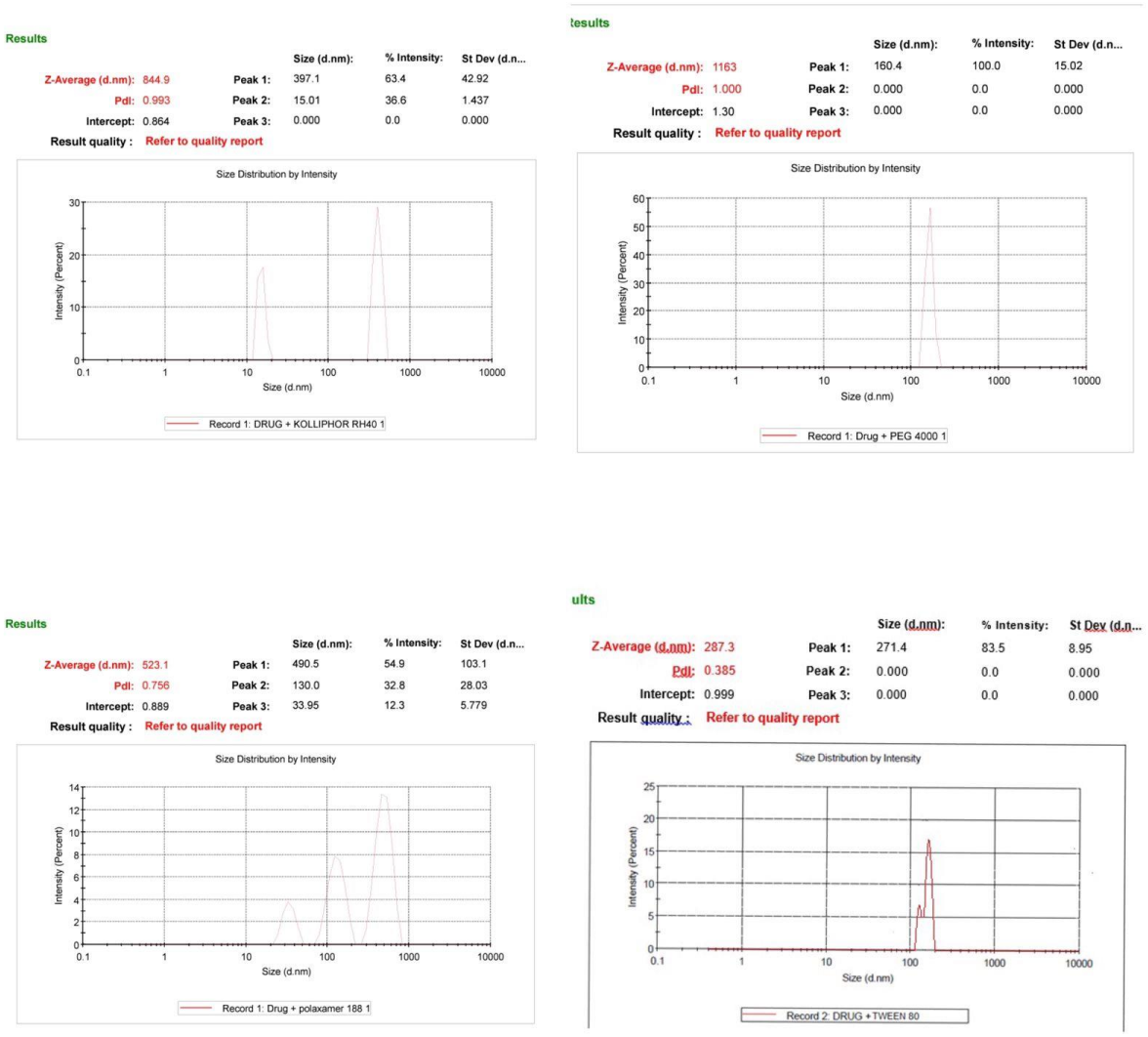


Figure: 13 Particle size and PDI of the formulations T1 to T4

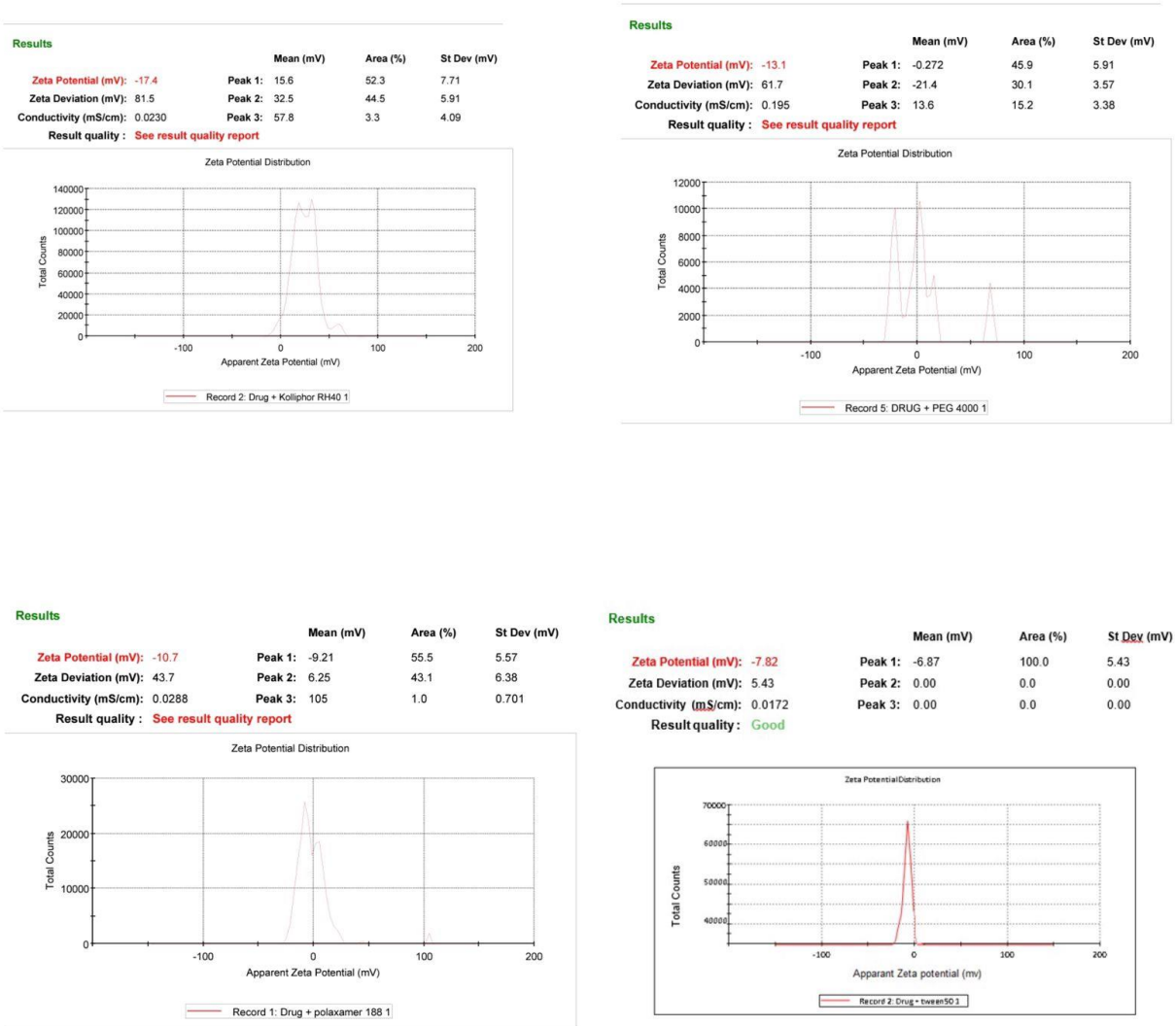


Figure: 14 Zeta potential of the formulations T1 to T4

The results of particle size, PDI and zeta potential for the formulations T1 to T4 were represented in table 11 & fig 13,14. The particle size of formulation T1 containing Tween 80 (2.5%) was 287.3nm, PDI 0.385 and zeta potential -7.82 were found to be significant. Whereas the other formulations were not within the optimum range.

From the results of particle size distribution tween 80 was selected for further optimization using 2^3 factorial design to identify the ideal formulation attributes to develop a stable nanosuspension.[52]

OPTIMIZATION BY USING 2^3 FACTORIAL DESIGN:

Table:12 Independent variables with their levels

Independent Variables	Variable level	
	Low level	**High level**
Coded Value	-1	+1
X1 – Concentration of HPMC	1	2
X2 – Concentration of Tween 80	2	3
X3- Sonication Time	10	20
Dependent Variables		
Y1 – Particle size (nm)		
Y2- % Drug Content		

The optimization of independent variables for the selected stabilizer Tween 80, HPMC and sonication time was done using 2^3 factorial design. Table 11 shows the variables used for the optimization of the formulation. The two level three factor (2^3) design was generated using Design expert 13 software. Each factor was evenly act at low and high levels. The response of the independent variables on dependent variables like particle size and drug content were evaluated. The formulation containing optimum variables were identified and the formulation with optimum range of variables were selected for the formulation of nanosuspension and further evaluated for cumulative drug release.

Table: 13 Evaluation of the batches F1 to F8 by 2^3 factorial design

S.No	Formulation Code	Particle Size (nm)	PDI	Zeta Potential (mv)	Drug Content (%)
1.	F1	290.2	0.392	-13.2	82.48
2.	F2	340.7	0.432	-11.8	92.89
3.	F3	350.4	0.441	-17.2	86.96
4.	F4	335.6	0.398	-16.5	91.42
5.	F5	228.2	0.376	-6.87	84.89
6.	F6	310.2	0.410	-12.0	90.32
7.	F7	305.9	0.421	-10.9	88.78
8.	F8	430.4	0.457	-18.2	92.32

Effect on particle size:

It was observed that all the independent parameters; Concentration of HPMC K 15, the concentration of Tween 80, and the sonication time of the system significantly affects the particle size. It can be concluded that the Concentration of HPMC K 15 (A), concentration of Tween 80 (B) and time of sonication (C) has a positive effect on particle size. It means, HPMC K 15, Tween 80 concentration and time of sonication changed, particle size decreased. When changes occurred in combination of two variables, particle size was decreased. The interaction factor AB, BC, and AC were negatively related to the particle size. HPMC K15 has a higher effect on the particle size because it has a higher coefficient value 427.1 than the coefficient value 162.8 of Concentration of Tween 80 and Sonication time coefficient value.

POLYNOMIAL EQUATION

PS= -85 +427.1 A +162.8 B+ 26.84 C – 146.1AB – 12.9 BC -20.88 AC +8.18 ABC

Effect on Drug content:

It was observed that all the independent parameters; Concentration of HPMC K 15, the concentration of Tween 80, and the Sonication time of the system significantly affect the drug content. It can be concluded that the HPMC K 15 concentration (A), concentration of Tween 80 (B) and sonication time (C) has a positive effect on drug content. It means as the concentration of

HPMC K15, Tween 80 and Sonication time increases from a lower level to a higher level, drug content increased. The interaction factor AB, BC, AC were negatively related to the drug content. Concentration of HPMC K15 has a higher effect on the drug content because it has a higher coefficient value 17.72 than the coefficient value 8.13 of Concentration of Tween 80 and Sonication time coefficient value 4.306.

POLYNOMIAL EQUATION

DC= 36.1 +17.72 A +8.13B +4.306C -3.290 AB -0.899BC -1.412AC +0.275ABC

ANOVA for selected factorial model

Response 1: PARTICLE SIZE

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	22130.21	3	7376.74	25.90	0.0044	significant
A-HPMC	6138.32	1	6138.32	21.55	0.0097	
B-SURFACTANT(TWEEN 80)	8659.28	1	8659.28	30.40	0.0053	
C-SONICATION TIME	7332.61	1	7332.61	25.74	0.0071	
Residual	1139.27	4	284.82			
Cor Total	23269.48	7				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

Figure: 15 ANOVA for Particle size

The model F-value of 25.90 implies the model is significant. There is only a 0.44% chance that an F-value could occur large due to noise.

Fit Statistics

Std. Dev.	16.88	R^2	0.9510
Mean	323.95	Adjusted R^2	0.9143
C.V. %	5.21	Predicted R^2	0.8042
		Adeq Precision	15.2302

Figure: 16 Fit Statistics for Particle size

The predicted R^2 of 0.8042 is in reasonable agreement with the Adjusted R^2 of 0.9143; i.e, the difference is less than 0.2.

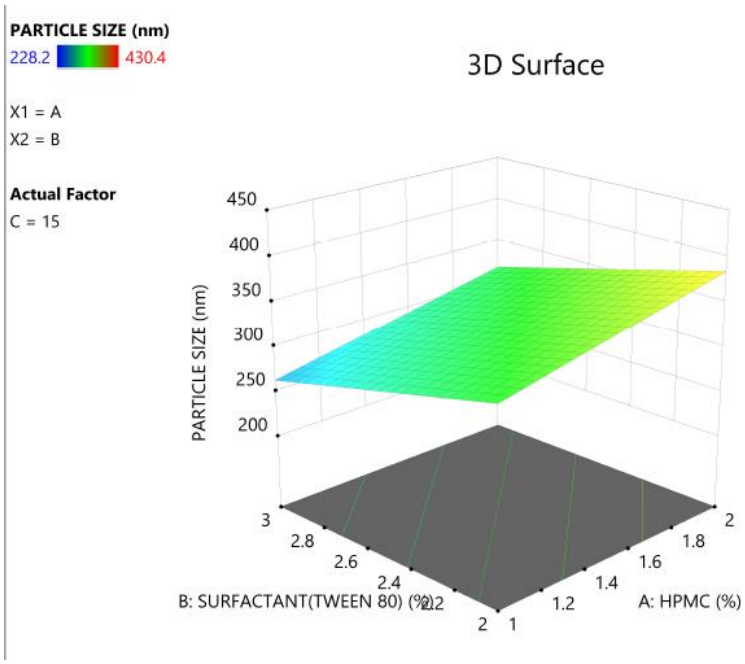


Figure: 17 Response surface plot for the effect of independent variables; (A) effect of concentration of HPMC K 15, (B) effect of Concentration of Tween 80 and (C) Sonication time on particle size.

ANOVA for selected factorial model

Response 2: Drug Content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	188.86	1	188.86	14.75	0.0085	significant
A-HPMC	188.86	1	188.86	14.75	0.0085	
Residual	76.80	6	12.80			
Cor Total	265.66	7				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

Figure: 18 ANOVA for Drug Content

The Model F-value of 14.75 implies the model is significant. There is only a 0.85% chance that an F-value could occur this large due to noise.

Fit Statistics

Std. Dev.	3.58	R^2	0.7109
Mean	85.24	Adjusted R^2	0.6627
C.V. %	4.20	Predicted R^2	0.4861
		Adeq Precision	5.4322

Figure: 19 Fit Statistics for Particle size

The Predicted R^2 of 0.4861 is in reasonable agreement with the Adjusted R^2 of 0.6627; i.e. the difference is less than 0.2.

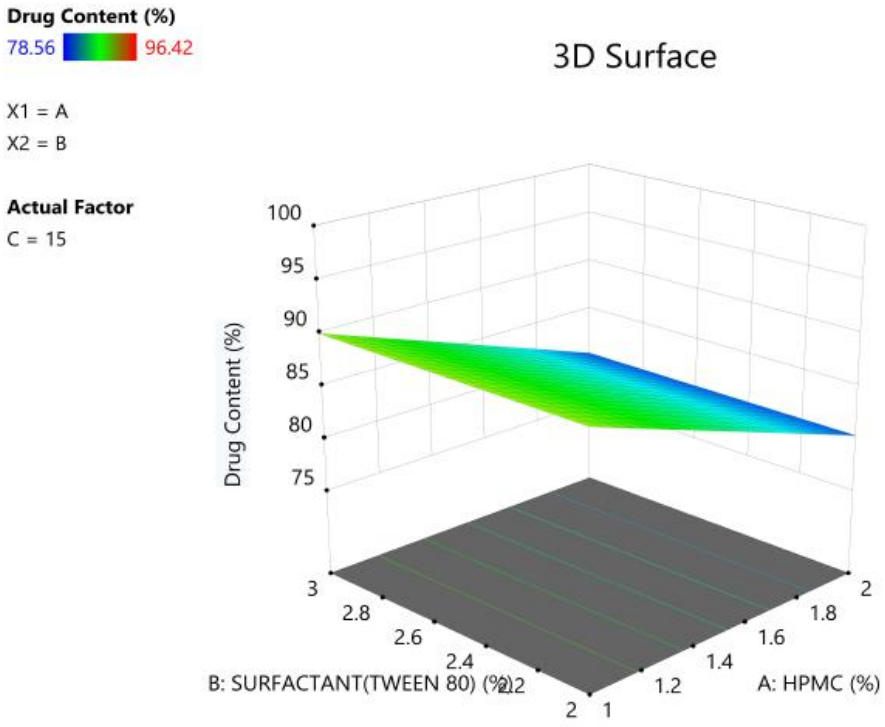


Figure: 20 Response surface plot for the effect of independent variables; (A) effect of concentration of HPMC K 15, (B) effect of Concentration of Tween 80 and (C) Sonication time on Drug content.

Optimization and validation

The main criteria for getting an optimum formulation of OND-NS was based on getting minimum particle size and good zeta potential with the help of the design optimization method of the Design-Expert-13 software. The composition of the optimized formulation was 1.5% of HPMC, 2.999% of surfactant (Tween 80) and 19.999 mins of sonication time. The optimized composition was used to prepare OND-NS with 16 mg/20ml of ondansetron. The observed values of particle size, zeta potential and encapsulation efficiency for the optimized formulations suggested that the obtained values are in good agreement with the predicted values (Table 14), (Fig. 21). The formulations prepared after optimization (OND-NS) were used for the in-vitro studies.

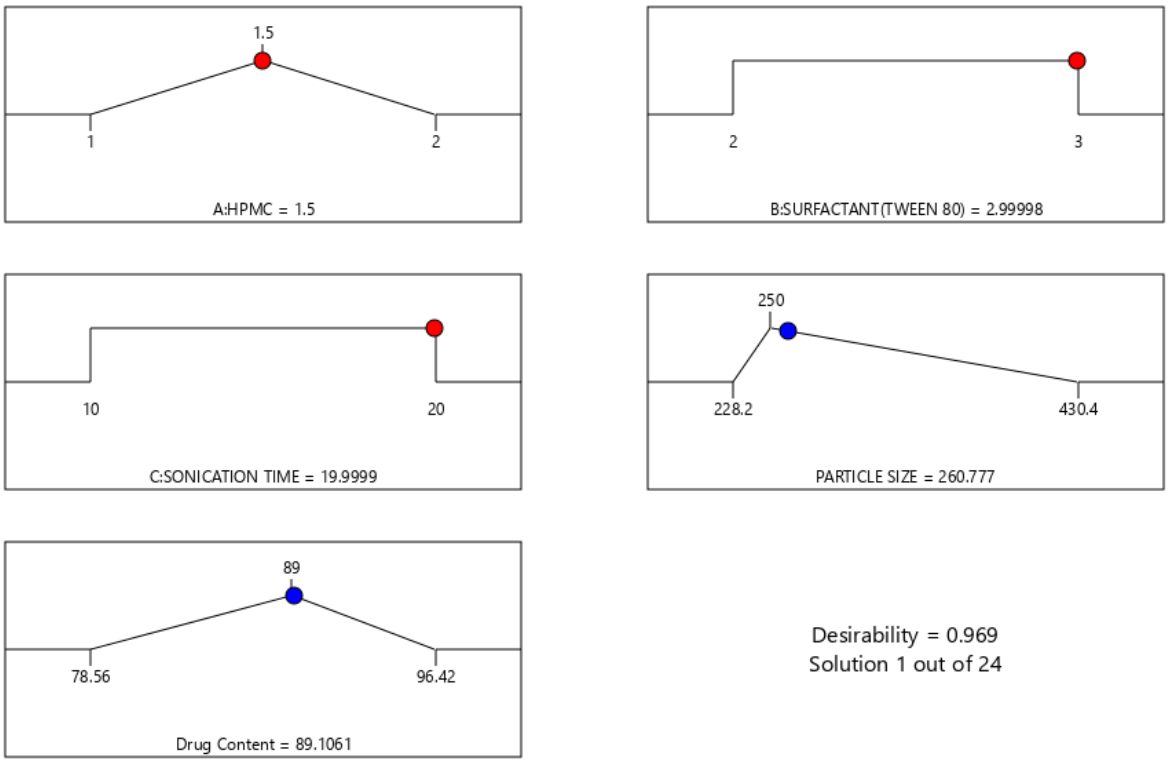


Figure: 21 Optimization plots for 2^3 design showing optimal values. The red color dots in figure A, B and C indicate the current factor settings and the blue color dot in figure D and E indicate the response to the current settings.

Table: 14 Physico-chemical characterization of optimized formulation OND-NS

Formulation	Particle size	Drug content
Optimized	250	89.01
Obtained	267.3	87.71

Particle size, PDI and Zeta potential:

The optimized batch showed the particle size 267.3 nm with PDI of 0.326.The drug content was found to be 87.71. The low value of PDI suggested that narrow particle size distribution and homogeneity of the formulation.

The zeta potential value confirm the physical stability of the formulation. Physical stability of the nanosuspension solely depends on the electrostatic repulsion force called zeta potential. Zeta potential depends on the ion nature of the polymer, surfactant and drug. HPMC and Tween 80 are non ionic in nature. A low level concentration of HPMC and high level concentration of surfactant.

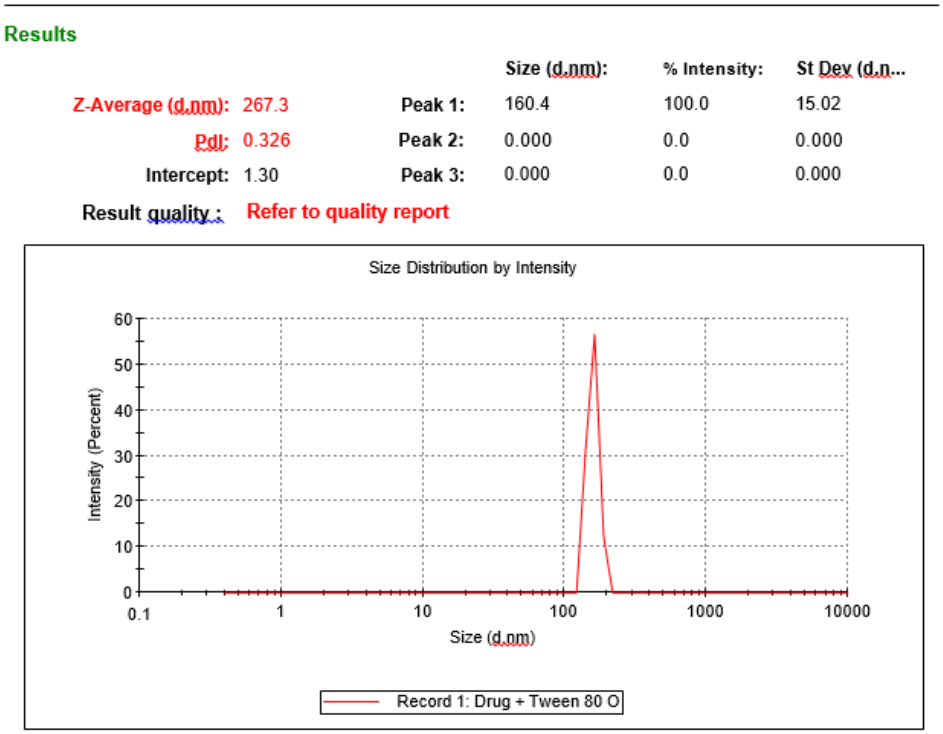


Figure: 22 Particle size of the optimized formulation

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -6.87	Peak 1:	-6.87	100.0	5.43
Zeta Deviation (mV): 5.43	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0172	Peak 3:	0.00	0.0	0.00
Result quality : Good				

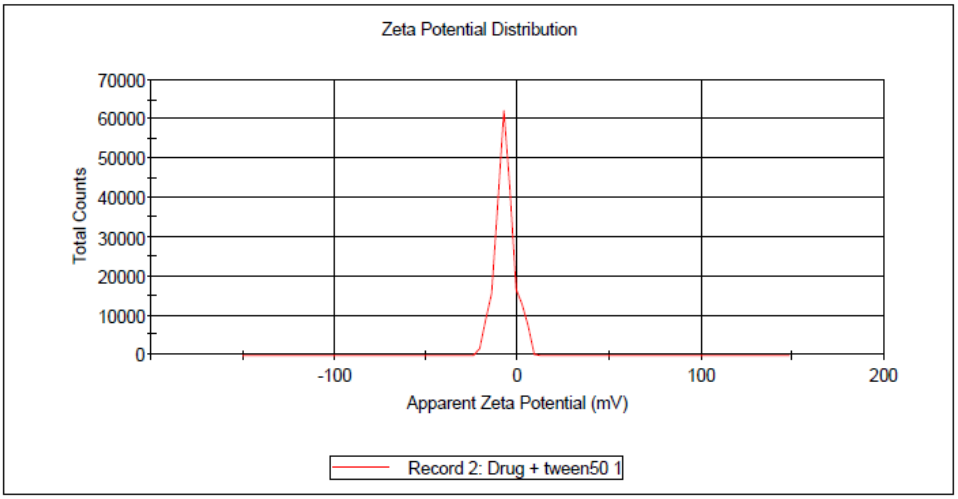


Figure: 23 Zeta potential of the optimized formulation

Table: 15 Cumulative dissolution data of test formulation, market formulation and pure drug at pH 1.2

S.No	Time in Mins	Pure drug	Marketed oral Formulation	Optimized Nanosuspension
1.	0	0±0	0±0	0±0
2.	5	4.8±0.56	6.7±0.65	40.2±0.59
3.	10	13.98±0.67	16.2±0.39	74.9±0.87
4.	20	18.56±0.32	22.7±0.51	96.5±0.73
5.	30	26.87±0.78	34.6±0.60	95.4±0.69
6.	40	37.65±0.59	43.2±0.49	92.3±0.49

±S.D:3

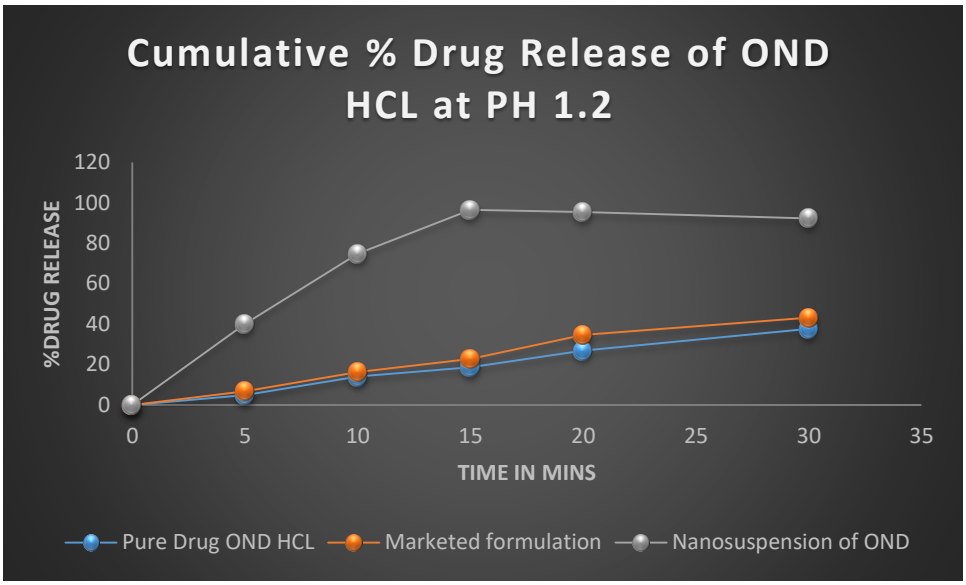


Figure: 24 Comparison of dissolution study of test formulation, market formulation and pure drug at pH 1.2

Table: 16 Cumulative dissolution data of test formulation, market formulation and pure drug at pH 7.4

S.No	Time in Mins	Pure drug	Marketed oral Formulation	Optimized Nanosuspension
1.	0	0±0	0±0	0±0
2.	5	7.2±0.34	8.3±0.59	59.2±0.88
3.	10	14.2±0.72	16.2±0.83	81.2±0.49
4.	20	20.3±0.61	24.8±0.51	98.9±0.29
5.	30	35.45±0.84	41.3±0.73	97.2±0.58
6.	40	42.65±0.48	49.9±0.29	95.2±0.49

±S.D:3

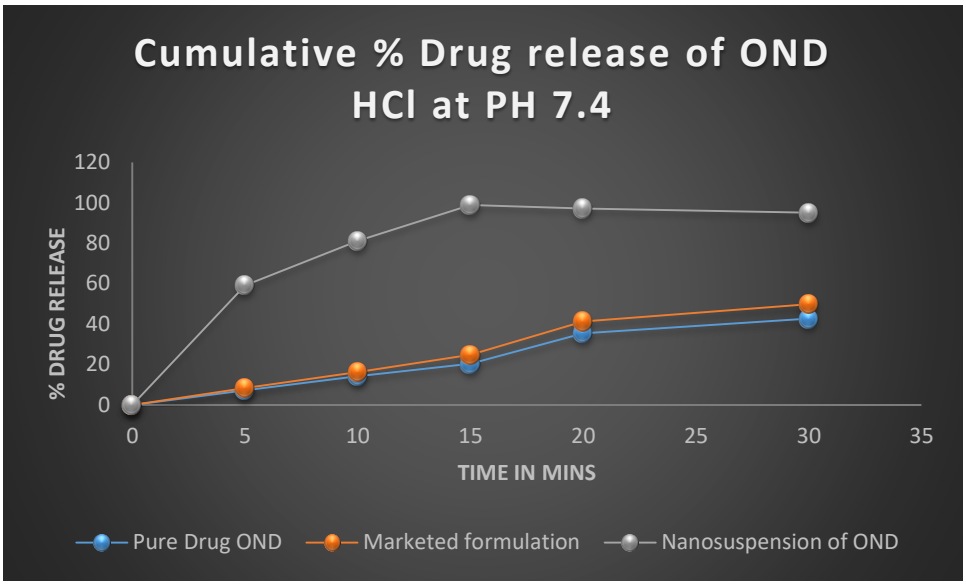


Figure: 25 Comparison of dissolution study of test formulation, market formulation and pure drug at pH 7.4

Cumulative Drug Release: [51]

The comparative dissolution profile of the optimized nanosuspension, marketed formulation and pure drug at PH 1.2 and 7.4 were performed and results were represented in table no:15 & 16 and figure no: 24 & 25 . The cumulative percentage drug release for the optimized formulation on 15 minutes at PH 1.2 and 7.4 were found to be 96.5% and 98.9% respectively. Whereas the drug release profile for marketed formulation at 40 minutes were 43.2% and 49.9% and for pure drug it was found to be 37.6% and 42.65%. The results of the invitro dissolution profile of the optimized formulation shows a significant increase when compared with the marketed oral formulation and pure drug. The increase in the dissolution rate of the nanosuspension may be due to the change in surface area with the presence of surfactants. The excellent disintegration property of Tween 80 and high surface area of the nanoparticles enchanced the rate of dissolution

Table 17: Corelation coefficient r^2 value of API, Marketed formlation and optimized nanosuspension at pH 7.4

S.No		Zero order	First order	Higuci	Kosermayer Peppas	Hixson Croxwell
1.	API	0.9698	0.9662	0.8469	**0.9701**	0.9700
2.	Marketed formulation	0.9728	0.9665	0.8503	**0.9732**	0.9724
3.	Optimized Nanosuspension	0.2798	**0.9924**	0.8565	0.9624	0.9829

Table 18: Corelation coefficient r^2 value of API, Marketed formlation and optimized nanosuspension at pH 1.2

S.No		Zero order	First order	Higuci	Kosarmayer Peppas	Hixson Croxwell
1.	API	**0.9933**	0.9885	0.8613	0.9933	0.9918
2.	Marketed formulation	0.9814	0.9851	0.8764	0.9842	**0.9869**
3.	Optimized Nanosuspension	0.4965	0.9718	0.8895	0.9161	**0.9832**

In-vitro Drug release kinetics

The release kinetic modeling was performed using the parameters that provide the closest fit between experimental observations and the nonlinear function. The best fits model was selected as per the obtained correlation coefficient (R^2) values. The model, which gives highest R^2 value, is considered as the best fit of release data. According to the obtained R^2 values, the release data is well fitted to the first order kinetic model for release at pH 7.4 and Hixson croxwell model at pH 1.2.

Based on the obtained *n* values for optimized nanosuspension at pH 7.4 the formulations undergone First order / anamolus / non-fickian diffusion drug release. The drug was released in a time dependent manner by diffusion and swelling.

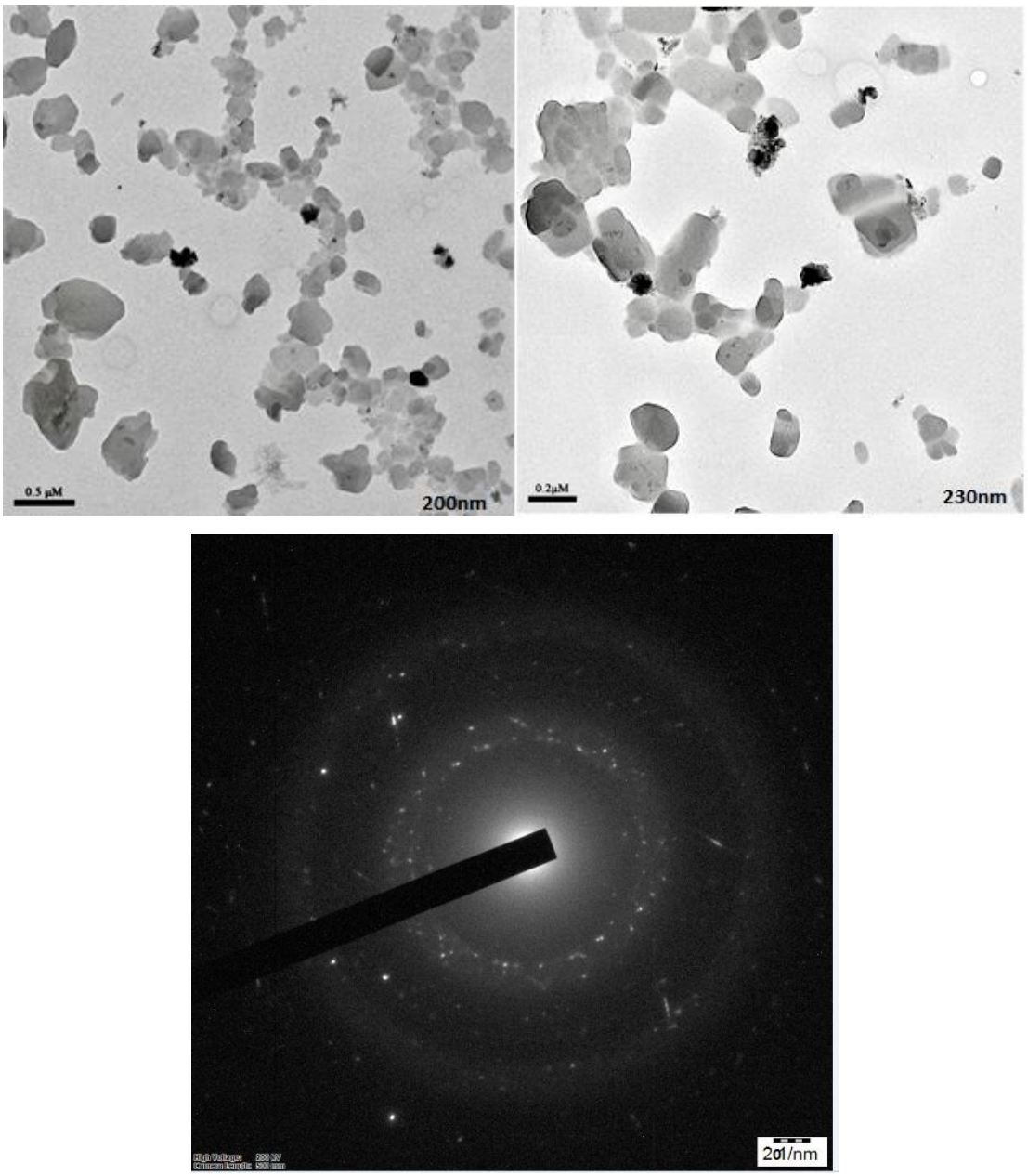


Figure: 26 TEM images of optimized Ondansetron hydrochloride nanosuspension

Morphology:

The morphological study of OND HCl nanosuspension at optimm condition was done by TEM. Figure. showed the TEM image of nanosuspension. They revealed that particles are spherical, having a smooth surface without having rough pores. The size of the particles were ranged from 200nm to 250nm.

Table 19: Stability data of optimized ondansetron nanosuspension at 4 ºC

S.no	Course of Study	Physical appearance	Particle size	PDI	Zeta potential
1.	Time of formulation	Clear	267.3	0.326	-6.87
2.	1 month	Clear	274.2	0.339	-6.72
3.	3 month	Clear	276.4	0.361	-7.22

Table 20: Stability data of optimized ondansetron nanosuspension at room temperature

S.no	Course of Study	Physical appearance	Particle size	PDI	Zeta potential
1.	Time of formulation	Clear	267.3	0.326	-6.87
2.	1 month	Clear	278.5	0.341	-6.81
3.	3 month	Clear	281.3	0.382	-7.41

There was no change in the physical appearance of the optimized formulation up to 3 months (at 4ºC and room temperature). The average particle size were 281.3 nm and 276.4 when stored at room temperature and refrigerator (4ºC) respectively. Before performing the stability study the particle size for optimized formulation was observed as 267.3nm, from this it cleared that the prepared nanosuspension was stable during the stability study period.

SUMMARY AND CONCLUSION

SUMMARY

- The present study demonstrated that ondansetron HCl loaded nanosuspension were successfully developed.
- Four formulations were formulated with different stablizers and surfactants.
- Out of four formulations, the formulation with the stabilizer and surfactant that resulted in low value of particle size and PDI were selected for further optimization. (T1- PS 287.3 and PDI 0.385)
- The formulation with different concentration of HPMC, stabilizer (Tween 80) and sonication time were further optimized using 2^3factorial design.
- Characterization was done for particle size, PDI and zeta potential to find out the best concentration of variables that fits the formulation.
- The particle size, PDI and zeta potential was found to be in the range of 228.2 to 430.4nm, 0.392 to 0.457 and -6.87mv to -18.2mv respectively.
- From the results of factorial design, the concentration of variables required to formulate the predicted particle size of 250nm was developed.
- With the predicted concentration of HPMC, tween 80 and sonication time, optimized nanosuspension of OND HCl was formulated and evaluated for particle size.
- The obtained particle size, PDI and zeta potential was found to be 228.2nm, 0.376 and -7.8mv respectively.
- Invitro dissolution studies revealed that formulation F5 has uplifted drug release of 92.5% and 98.9% on 40 minutes at pH 1.2 and pH 6.8 respectively, whereas the marketed oral formulation released 43.2% and 49.9% on 40 minutes at pH 1.2 and pH 6.8.
- The API of Ondansetron HCl released only 37.65% and 42.65% on 40 minutes at pH 1.2 and pH 6.8 respectively.
- The drug release kinetics optimized nanosuspension at pH 7.4 revealed that the formulations undergone First order / anamolus / non-fickian diffusion drug release
- The TEM images of the optimized formulation (F5) visualized the spherical and smooth surface of the particles in the range of 200nm to 250nm.
- Hence from our study the nanosuspension of Ondansetron HCl showed faster drug release than the marketed oral formulation and API, so it is evident that formulating into nanosuspension results in improved stability, solubility and rapid drug release.

CONCLUSION

It may be concluded that the nanosuspension of poorly soluble drugs such as ondansetron are easy to prepare and represent a promising novel approach for oral drug delivery. It is evident that the obtained results of *invitro* dissolution studies of the formulation shows improved solubility and rapid drug release. Consequently nanosuspension represent a promising alternative delivery system for improving the physiochemical properties of BCS class II drugs.

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